Pyrimethanil

DOCUMENT M-CA, Section 1

IDENTITY OF THE ACTIVE SUBSTANCE

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Report: CA 5.1.1/1
[redacted] 2014a
14C-BAS 605 F - Study on the biokinetics in rats
2014/1092444
Guidelines: (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. L 142, OECD 417 (July 2010), EPA 870.7485, JMAFF Guidelines on the Compiling of Test Results on Toxicity - Tests on In Vivo Fate in Animals (2001)
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: Pyrimethanil (BAS 605 F)
Lot/Batch #: 1049-1010 (pyrimidinyl-2-^{14}C)
1050-1003 or 1050-0101 (pyrimidinyl-1,3-^{15}N)
L83-126 (unlabeled)
Purity: >98% (radiochemical; ^{14}C)
98.7% or 99.1% (radiochemical; ^{15}N)
99.6% (unlabeled)
CAS#: 53112-28-0
Development code: 12/0658-1 (^{14}C); 12/0655-1 or 12/0655-2 (^{15}N); 07/0077-3 (unlabeled) (236999)
Stability of test compound: Stable during dosing period

2. Vehicle and/or positive control:

Tap water containing 1% gum tragacanth

3. Test animals

Species:	Rat
Strain:	Sprague Dawley (Crl:CD(SD), Charles River, Sulzfeld, Germany)
Age:	7-13 weeks at start of acclimation
Sex:	Male and female
Number of animals:	Balance experiment: 14+1 high dose experiment: 10 animals 5 male rats; 4 male rats in evaluation 5 female rats; 4 female rats in evaluation (1 animal per sex was dosed with unlabeled test substance for 14 days additionally for potential substitution)
	Bile experiment: high dose: 36 animals 12 male rats; 4 male rats in evaluation 12 female rats for the first and 11 female rats for a second experiment; 12 female rats in evaluation. low dose: 24 animals 12 male rats; 6 male rats in evaluation 12 female rats; 5 female rats in evaluation (exclusions of animals from data evaluation are based on health status, insufficient bile flow and/or invalid results, e.g. invalid recoveries); total of 69 animals
Weight at dosing:	220-420 g (prior to first dosing)
Acclimation period:	At least 5 days
Diet:	Kliba lab diet (mouse/rat) either pelleted or as meal, *ad libitum*
Water:	Tap water *ad libitum*
Husbandry:	In an AAALAC-approved laboratory in accordance with the German Animal Welfare Act and the effective European Council Directive
Housing:	During acclimatization in groups in Macrolon cages, then individually in Type III Macrolon cages (unlabeled treatment and after surgery of biliary excretion experiments) or all-glass metabolism cages type Metabowl
Environmental conditions:	
Temperature:	20-24°C
Humidity:	30-70%
Air changes:	15 per hour
Photoperiod:	Alternating 12-hour light and dark cycles

4. Preparation of dosing solution

Balance/excretion: For the unlabeled test-substance preparation with a target concentration of 80 mg/mL the unlabeled test substance was taken and the required amount of the aqueous vehicle was added yielding in a nominal concentration of 80.1 mg/mL.

For the unlabeled test-substance preparation with a target concentration of 50 mg/mL appropriate amounts of the test-substance preparation with a target concentration of 80 mg/mL and aqueous vehicle were mixed yielding in a concentration of 50.0 mg/mL).

In order to achieve the required specific activity for the radiolabeled test-substance preparation, appropriate amounts of the radiolabeled test substance solution were taken and the organic solvent (toluene) was evaporated. Respective amounts of the unlabeled material, ^{15}N-labeled material and the aqueous vehicle were added. Based on the current data, the nominal concentration of the test substance was 50.0 mg/g test-substance preparation (corresponding to 50.0 mg/mL, assuming a density of 1 g/mL); the nominal specific activity was 3.41 MBq/g (corresponding to 3.41 MBq/mL).

Excretion via bile: In order to achieve the required specific activity for the radiolabeled test-substance preparation appropriate amounts of the radiolabeled test-substance solution were taken and the organic solvent was evaporated. Respective amounts of the unlabeled test substance, ^{15}N-labeled material and the aqueous vehicle were added.

Since the recovery of radioactivity in the test-substance preparation for the low dose experiment in male animals was insufficient, further radiolabeled test substance was added (for that purpose, a respective aliquot of the solution of the radiolabeled test substance was taken and evaporated to dryness). It was assumed that the insufficient recovery of radioactivity in this test-substance preparation was due to losses by absorption of radiolabeled test substance to the surface of the vessel. Therefore, the nominal concentration was calculated for the sum of weighed unlabeled and ^{15}N-labeled test substance added to the amounts of radiolabeled test substance recovered by LSC analysis. Based on these calculations, the nominal concentration of the test substance was 1.04 mg/g test-substance preparation (corresponding to 1.04 mg/mL, assuming a densitiy of 1 g/mL). The measured specific activity was 1.18 MBq/g (corresponding to 1.18 MBq/mL).

The recoveries of test substance and radioactivity in the test-substance preparation for the low dose experiment in female animals were insufficient. However, these losses were compensated by higher amounts of test substances used for the test-substance preparation. Therefore, the analyzed concentration is related to the target value instead of the nominal value. The target concentration of the test substance is 1.0 mg/g test-substance preparation (corresponding to 1.0 mg/mL, assuming a density of 1 g/mL). The measured specific activity is 1.18 MBq/g (corresponding 1.18 MBq/mL).

For the high dose groups, calculated nominal concentrations accounted to 80.56 mg/g (80.56 mg/mL) for male animals and 80.10 mg/g and 80.45 mg/g (80.10 mg/mL and 80.45 mg/mL) for female animals of run 1 and run 2. The nominal specific activities yielded in values of 3.99 MBq/g (3.99 MBq/mL) for the male rats and 5.32 MBq/g and 5.74 MBq/g (corresponding to 5.32 MBq/mL and 5.74 MBq/mL) for the female rats of run 1 and run 2.

The preparations were stirred in order to produce homogeneous preparations. Before start and at the end of the last administration, at least two samples were taken to determine the amount of radioactivity in the preparation and to demonstrate the correct concentration of the test substance, its homogeneity and its radiochemical purity.

B. STUDY DESIGN AND METHODS

1. Dates of work: October 09, 2012 – August 26, 2013

For mass balance, 4 male and 4 female animals were treated orally with test substance for 15 days (4 x unlabeled at 800 mg/kg bw, 10 x unlabeled at 500 mg/kg bw and 1 x labeled at 500 mg/kg bw at day 15). After the last administration, urine was collected after 6, 12 and 24 hours and subsequently in 24 hour time intervals up to 168 hours and feces was collected in 24 hour time intervals up to 168 hours.
For biliary excretion experiments, bile ducts of male (4 high dose, 6 low dose) and female rats (12 high dose, 5 low dose) were canulated in a surgery and after regeneration, animals were dosed orally with 800 mg/kg (high dose) or 10 mg/kg (low dose) ^{14}C-BAS 605 F and were placed in metabolism cages in order to collect bile in 3 hour intervals as well as urine and feces in 24 hour time intervals up to 72 hours, depending on the health state of the animals and the excretion rate.

Determination of total radioactivity in biological material:
After weighing, aliquots of liquid samples (bile, urine, plasma and cage wash) were mixed with scintillation cocktail (Hionic Fluor, Perkin Elmer) and analyzed for radioactivity without any additional treatment.
Soluene®-350 (Perkin Elmer) was added to blood cells. The samples were incubated followed by the addition of isopropanol. Then the samples were bleached by the addition of perhydrol solution (30%). After further incubation scintillation cocktail was added and the samples were measured by liquid scintillation counting.
Feces, contents of gut and stomach, carcass, lung, heart, spleen, kidney, testes, brain and liver were suspended in deionized water and were homogenized using a blender.
Aliquots of the suspensions of feces, contents of gut and stomach and carcass were dried by lyophylization, dissolved with Soluene®-350, filled up with isopropanol, bleached with perhydrol solution (30%) and scintillation cocktail was added before measurement of radioactivity by LSC.
To prepared aliquots of the suspensions of lung, heart, spleen, kidney, testes, brain and liver and to whole samples of muscle, adipose tissue, uterus/ovaries, thyroid glan, adrenal glan, pancreas, skin, bone marrow, stomach and gut Soluene®-350 and isopropanol was added, bleaching was performed with perhydrol solution (30%) and scintillation cocktail was added before measurement of radioactivity by LSC.
Bone samples were treated with hydrochloric acid (4M) and scintillation cocktail was added before measurement of radioactivity by LSC.

II. RESULTS AND DISCUSSION

Stability, homogeneity and concentration control analyses of the test substance preparations
The stability of the test substance in the test-substance preparations over the test period was verified by analyses for all test substance preparations. The concentrations and the homogeneous distribution of the test substance in the test-substance preparations were confirmed by analyses.
The nominal concentrations of the test substance preparations were in accordance with the target values. Differences between target doses and nominal doses were based on experimental variability. For the low dose experiment of bile excretion of female rats, the recovery of test substance in the test-substance preparation was insufficient, but compensated by higher amounts of test substances weighed in. Accordingly, concentration control was related directly to target concentration. The analytical concentrations were 100±10% of the target concentration/nominal concentrations and confirmed the theoretical values. A standard deviation below 5% confirmed the homogenous distribution of the test substance in the test substance preparations.

Kinetics
Balance/excretion (multiple high dose)
Mean values of excreted and residual radioactivity after 15 daily oral administration of BAS 605 F (4 x unlabeled at 800 mg/kg bw; 10 x unlabeled at 500 mg/kg bw and 1 x labeled at 500 mg/kg bw at day 15) to male and female rats are presented in Table 5.1.1-5.
Mean total recoveries of radioactivity were found to be 103.5% in males and 101.8% in females.
Within 48 hours after the (last) oral administration of ^{14}C-BAS 605 F, 89.89% and 86.69% of the administered radioactivity were found in urine of male and females rats, respectively. These data indicate fast excretion of BAS 605 F from the organism. Total excretion of radioactivity via urine after 168 hours was 91.00% for males and 88.29% for females indicating that dosed ^{14}C-BAS 605 F was excreted predominantly via urine. Within the first two days after administration of ^{14}C-BAS 605 F, means of 10.87% and 11.46% of the administered radioactivity were excreted via feces by males and females, respectively. Within 168 hours after dosing the radiolabeled test substance, the mean total amounts of radioactivity excreted via feces were found to be 11.52% for males and 12.23% for females.
Together with cage wash, mean total amounts of excreted radioactivity were found to be 102.9% of the administered radioactivity in males and 101.6% in females, reflecting more than 99% of the recovered radioactivity.
168 hours after administration of ^{14}C-BAS 605 F, small amounts of remaining radioactivity were found in carcass (0.05 and 0.07% of dose for males and females), liver (0.02% of dose in both sexes), skin (0.06 and 0.03% of dose for males and females), kidney (0.01% of dose for both sexes) and blood cells (0.01% of dose for both sexes).
Taken together, the balance data demonstrate that the major excretion of BAS 605 F dosed orally by gavage to rats occurred via the urine, accounting to about 90% of dose. Excretion via feces was a minor excretion pathway with mean values of about 12% of dose. Excretion was fast and occurred to a major extent already within two days after the last dosing.

Table 5.1.1-5: Mean excretion and retention of radioactivity after multiple doses

	Excretion of radioactivity (% of dose)	
Matrix / Time (h)	**Male**	**Female**
Urine		
0-6	34.93	23.90
6-12	26.31	24.50
12-24	24.91	31.83
24-48	3.74	6.46
48-72	0.41	0.65
72-96	0.27	0.37
96-120	0.17	0.23
120-144	0.11	0.17
144-168	0.16	0.18
Subtotal urine	91.00	88.29
Feces		
0-24	7.55	6.43
24-48	3.32	5.03
48-72	0.42	0.49
72-96	0.10	0.12
96-120	0.06	0.07
120-144	0.04	0.06
144-168	0.02	0.04
Subtotal feces	11.52	12.23
Cumulative excretion (total urine + feces)	**102.52**	**100.52**
Cage wash	0.38	1.12
Cumulative excretion (urine, feces + cage wash)	**102.90**	**101.64**
Blood cells	0.01	0.01
Plasma	0.00	0.00
Lung	0.00	0.00
Heart	0.00	0.00
Spleen	0.00	0.00
Kidney	0.01	0.01
Adrenals	0.00	0.00
Testes/ovaries	0.00	0.00
Uterus	N/A	0.00
Muscle	0.00	0.00
Brain	0.00	0.00
Adipose tissue	0.00	0.00
Bone	0.00	0.00
Bone marrow	0.00	0.00
Thyroid	0.00	0.00
Pancreas	0.00	0.00
Stomach content	0.00	0.00
Stomach	0.00	0.00
Gut content	0.01	0.02
Gut	0.00	0.00
Liver	0.02	0.02
Skin	0.06	0.03
Carcass	0.05	0.07
Total	**103.1**	**101.8**

N/A Not applicable

Excretion via bile

The mean biliary excretion of radioactivity after a single oral administration of ^{14}C-BAS 605 F to rats is presented in Table 5.1.1-6 and Table 5.1.1-7.

The bile excretion study was performed after bile catheterization as a balance experiment and bile, urine and feces were collected up to 72 hours from male and female rats that were dosed with ^{14}C-BAS 605 F at 800 mg/kg bw and 10 mg/kg bw. After the experiment, animals were sacrificed under isoflurane anesthesia, the GI-tract was removed and the remaining activity was measured in the carcass, the content of the stomach and the gut as well as in stomach and gut as well as in excreted urine, bile and feces samples. Based on the data of excreted radioactivity via bile and urine as well as on the remaining radioactive residues in carcass, the oral absorption of ^{14}C-BAS 605 F was calculated.

High dose (800 mg/kg bw)

Mean total recoveries of radioactivity were found to be 87.40% in males and 88.94% in females. Within 72 hours after administration of ^{14}C-BAS 605 F at a dose level of 800 mg/kg bw, mean excretion via bile was found to be 14.27% and 27.22% of the administered radioactivity in males and females, respectively. Total excretion of radioactivity via urine within 72 hours after the test substance administration was 68.94% of dose for males and 58.41% of dose for females. Within 72 hours after dosing, the mean total amount of radioactivity excreted via feces was found to be 2.75% for males and 1.12% for females. Total amounts of radioactivity in the GI tract were minor and accounted to a sum of 0.25% of dose in male and 0.50% of dose in female animals.

Low dose (10 mg/kg bw)

Mean total recoveries of radioactivity were found to be 86.34% in males and 90.01% in females. Within 72 hours after administration of ^{14}C-BAS 605 F at a dose level of 10 mg/kg bw, mean excretion via bile was found to be 19.35% and 24.97% of the administered radioactivity in males and females, respectively. Total excretion of radioactivity via urine within 72 hours after the test substance administration was 59.99% of dose for males and 62.32% of dose for females. Within 72 hours after dosing, the mean total amount of radioactivity excreted via feces was found to be 2.76% for males and 1.09% for females. Total amounts of radioactivity in the GI tract were minor and accounted to a sum of 0.07% of dose in male and 0.08% of dose in female animals.

Based on the mean amounts of radioactivity excreted via bile and urine, as well as the radioactive residues found in cage wash and carcass, absorption of ^{14}C-BAS 605 F in rats was calculated to be about 87% and 89% of the administered dose for male and female rats at a dose level of 800 mg/kg bw and about 84% and 89% of the administered dose for male and female rats at a dose level of 10 mg/kg bw. Taking the variability of individual animals into account, measured absorption of BAS 605 F within the performed bile excretion experiments can be assessed to be comparable between the sexes and the dose levels tested.

Table 5.1.1-6: Mean excretion and retention of radioactivity via urine, feces and bile after a single dose

	Excretion of radioactivity (% of dose)			
	800 mg/kg		10 mg/kg	
Matrix / Time (h)	**Male**	**Female**	**Male**	**Female**
Urine				
0-24	25.43	33.22	57.97	60.72
24-48	38.84	20.42	1.38	0.90
48-72	4.67	4.77	0.64	0.70
Subtotal urine	68.94	58.41	59.99	62.32
Feces				
0-24	0.93	0.31	1.72	0.88
24-48	1.27	0.58	0.83	0.15
48-72	0.54	0.24	0.21	0.07
Subtotal feces	2.75	1.12	2.76	1.09
Cumulative excretion (total urine + feces)	**71.69**	**59.53**	**62.75**	**63.41**
Cage wash	3.77	3.10	3.99	1.42
Cumulative excretion (urine, feces + cage wash)	**75.46**	**62.63**	**66.74**	**64.83**
Stomach content	0.03	0.36	0.01	0.00
Stomach	0.01	0.03	0.00	0.00
Gut content	0.18	0.09	0.06	0.07
Gut	0.03	0.02	0.00	0.01
Carcass	0.41	0.21	0.19	0.13
Subtotal bile	**14.27**	**27.22**	**19.35**	**24.97**
Total	**90.40**	**90.51**	**86.34**	**90.01**

Table 5.1.1-7: Mean excretion pattern of radioactivity via bile after a single dose

	Excretion of radioactivity (% of dose)			
	800 mg/kg		10 mg/kg	
Time (h)	**Male**	**Female**	**Male**	**Female**
0-3	0.40	0.38	8.06	9.83
3-6	0.93	1.20	6.78	9.16
6-9	2.49	2.45	2.37	4.06
9-12	2.01	3.66	0.76	1.01
12-15	0.44	2.66	0.46	0.36
15-18	0.33	3.31	0.36	0.17
18-21	0.47	2.83	0.17	0.12
21-24	1.13	3.79	0.08	0.08
24-27	1.29	3.81	0.06	0.05
27-30	1.23	2.81	0.05	0.03
30-33	1.11	0.62	0.03	0.03
33-36	0.96	0.57	0.03	0.04
36-39	0.67	0.75	0.02	0.02
39-42	0.28	0.51	0.01	0.01
42-45	0.13	0.18	0.01	0.01
45-48	0.28	0.40	0.01	0.02
48-51	0.23	0.36	0.01	0.02
51-54	0.09	0.13	0.01	0.01
54-57	0.05	0.04	0.01	0.01
57-60	0.05	0.03	0.01	0.01
60-63	0.03	0.02	0.01	0.00
63-66	0.02	0.01	0.01	0.00
66-69	0.02	0.02	0.01	0.00
69-72	0.03	0.01	0.02	0.01
Total	**14.27**	**27.22**	**19.35**	**24.97**

III. CONCLUSION

Absorption: After multiple administrations of BAS 605 F (4 x unlabeled at 800 mg/kg bw; 10 x unlabeled at 500 mg/kg bw and 1 x radiolabeled at 500 mg/kg bw at day 15) to rats, ^{14}C-BAS 605 F was rapidly and nearly completely absorbed from the gastrointestinal tract.
The bile excretion study was performed after bile catheterization as a balance experiment and bile, urine and feces were collected up to 72 hours from male and female rats that were dosed with ^{14}C-BAS 605 F at 800 mg/kg bw and 10 mg/kg bw. Based on these experiments, the absorbed fraction (calculated as the sum of radioactivity found in bile, urine, cage wash and carcass) of ^{14}C-BAS 605 F in male and female rats was calculated to be between 84% and 89% of the administered dose at dose levels of 800 and 10 mg/kg bw. Taking the variability of the individual animals into account, measured absorptions of BAS 605 F within the performed bile excretion experiments can be assessed to be comparable between the sexes and the dose levels tested.
Distribution: 168 hours after multiple administrations of ^{14}C-BAS 605 F, small amounts (0.01-0.07% of dose) of remaining radioactivity were found in carcass, liver, skin, kidney and blood cells.
Excretion: The main excretion after multiple administrations occurred via urine within two days after dosing (about 87 to 90% of administered radioactivity), additionally 1 to 2% of the administered radioactivity were excreted the following 5 days until 168 h. Minor parts of absorbed ^{14}C-BAS 605 F were excreted via feces (about 12% during the experiment of both sexes). The excretion data were comparable between the sexes.
The excretion of radioactivity after a single dose occurred within three days after dosing with higher excretions in urine than in feces. For the high dose, the ratio of biliary and urinary excretion was slightly different between the sexes: the bile excretion in males was lower (about 14% of dose) than in females (about 27% of dose), whereas the urinary excretion in males was higher (69% of dose) than in females (58% of dose). For the low dose level tested, the excretion pattern of the sexes was more or less comparable.

Report: CA 5.1.1/2
[redacted] 2015a
Excretion and metabolism of 14C-BAS 605 F after oral administration in rats
2014/1000804
Guidelines: EPA 870.7485, EPA 860.1000, JMAFF, OECD 417, EEC 87/302 B
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: Pyrimethanil (BAS 605 F)
Lot/Batch #: 1049-1010 (pyrimidinyl-2-^{14}C)
1050-0101 (pyrimidinyl-1,3-^{15}N; DX, V, W, C and R groups)
1050-1003 (pyrimidinyl-1,3-^{15}N; S groups)
L83-126 (unlabeled)
Purity: 99.7% (pyrimidinyl-2-^{14}C; radiochemical)
99.1% (pyrimidinyl-1,3-^{15}N; 1050-0101)
98.7% (pyrimidinyl-1,3-^{15}N; 1050-1003)
99.6% (unlabeled)
CAS#: 53112-28-0
Development code: 236999 (Reg. No)
Stability of test compound: Stable during dosing period

2. Vehicle and/or positive control: Water containing 1% gum tragacanth

3. Test animals

Species:	Rat
Strain:	Sprague Dawley (Crl:CD(SD), Charles River, Sulzfeld, Germany)
Age:	6-7 weeks (arrival) / 8 weeks (administration)
Sex:	Male and female
Number of animals:	36 (10 males + 10 females for DX, 4 males + 4 females for each V and W)
Weight at dosing:	201.1-373.7 g
Acclimation period:	8-9 days
Diet:	Kliba 3433 pellets, *ad libitum*
Water:	Tap water *ad libitum*
Husbandry:	
Housing:	During acclimatization in groups in Macrolon cages, then individually in plexiglass (DX groups) or Macrolon cages (V and W groups)
Environmental conditions:	
Temperature:	18-24°C
Humidity:	21-55%
Air changes:	Supply air: 31 changes/h, exhaust air: 42 changes/h
Photoperiod:	Alternating 12-hour light and dark cycles

4. Preparation of dosing solutions

For preparation of the application formulations for the dose groups DXM, DXF, VM, VF, WM and WF appropriate weights of ^{14}C-labeled BAS 605 F dissolved in toluene was concentrated to near dryness and ^{15}N-labelled and unlabeled BAS 605 F were added. The isotope ratios of ^{14}C : ^{15}N : ^{12}C were about 1 : 12 : 24 for dose groups DX and W and about 1 : 2 : 2 for dose groups V. Thereafter, the mixture of labeled and unlabeled BAS 605 F was taken up in gum tragacanth (1% in H_2O) and suspended using an Ultra Turrax blender.
The single oral doses were weighed into syringes and administered orally by gavage. The amount of the administered application formulation was calculated by weighing the syringes before and after application.

For groups CM, CF, RM, RF, SM and SF dosed in a related biokinetics study conducted at the Department of Experimental Toxicology and Ecology, BASF SE, 67056 Ludwigshafen, Germany, see BASF DocID 2014/1092444 summarized in this document.

B. STUDY DESIGN AND METHODS

1. Dates of work: February 18, 2013 - July 08, 2015

The excretion and metabolism of BAS 605 F (pyrimethanil) was investigated in male and female rats after oral application of a single oral dose of 10, 500 and 800 mg/kg body weight (bw) BAS 605 F, respectively. For all dose groups, mixtures of ^{14}C radiolabeled, ^{15}N-labeled and unlabeled test item were applied to facilitate metabolite identification by mass spectrometry and quantitative analysis using HPLC.

Urine, feces, tissue and plasma samples were collected from male and female rats of dose groups DXM and DXF (500 mg/kg bw), VF and VM (10 mg/kg bw) and WM and WF (800 mg/kg bw). In addition, urine, feces and bile samples from a biokinetics study (see BASF DocID 2014/1092444; summarized in this document) after oral administration of BAS 605 F were examined: Urine and feces samples were collected from male and female rats after multiple oral dosing with 500 mg/kg bw BAS 605 F (dose group CM and CF). Bile was collected from male and female rats with biliary-fistulae after single oral dosing of 10 mg/kg bw (dose group RM, RF) and 800 mg/kg bw BAS 605 F (dose group SM, SF), respectively.

Urine and feces were sampled 6 hours, 12 hours (6 and 12 hours only urine) and 24 hours post application and thereafter in daily intervals until 168 hours. Samples of several animals were combined for each time interval. Bile was collected in time intervals of 3 hours for up to 72 hours. Bile samples from the biokinetics study were also combined for several animals and urine and bile samples were further pooled to obtain samples from a longer time period. Reasonable efforts were made to identify a) all metabolites present at ≥1% of the administered dose or b) all metabolites present in tissue and plasma samples. Metabolites present at ≥5% of the administered dose were successfully identified.

Identification of metabolites is based on HPLC-MS/MS analysis of selected urine, feces and bile samples and on NMR analysis of isolated fractions from a urine sample and a feces extract. Residues in rat samples were quantified by radio HPLC. Peak assignment for components was based on HPLC-MS analysis using the same separation method and comparing the retention times and the chromatographic patterns and the respective ^{14}C radio-chromatogram of the HPLC-MS run with the chromatogram of the quantitative HPLC run of each particular sample (same column, solvent and gradient).

II. RESULTS AND DISCUSSION

Storage stability

The analytical investigations performed within the present study demonstrated the specific radioactivity, purity, stability and homogeneity of ^{14}C BAS 605 F in the vehicle for the performed experiments.

Distribution

Radioactive residues were investigated in liver, kidney, plasma and fat of male and female rats of dose groups VM, VF, WM and WF sacrificed 1 hour after dosing. This time interval corresponds to the maximum plasma levels described in a peer-reviewed biokinetics study (BASF DocID 1995/1001910).

In the low dose group VM and VF, the concentrations of detected radioactive residues were higher compared to the high dose group WM and WF, accounting for 0.38% and 0.63% (WF and WM) to 3.91% and 4.45% (VM and VF) of the administered dose in liver, 0.09% and 0.12% (WF and WM) to 1.53% and 2.14% (VF and VM) of the administered dose in kidney, 0.32% and 0.37% (WF and WM) to 1.66% and 1.78% (VM and VF) of the administered dose in fat and 0.02% and 0.03% (WF and WM) to 0.31% and 0.35% (VF and VM) of the administered dose in plasma. Blood was only analyzed in group W; the concentrations of detected radioactive residues accounted for 0.03% and 0.05% (WF and WM) of the administered dose.

No significant differences were found between the two genders.

Table 5.1.1-8: Tissue distribution and concentration level of parent equivalents in plasma and tissues at maximum plasma levels (t_{max}: 1 h) after a single oral dose BAS 605 F (mean values)

Matrix	Dose group (nominal dose)							
	V 10.0 mg/kg bw				W 800.0 mg/kg bw			
	Male (VM)		Female (VF)		Male (WM)		Female (WF)	
	% of dose	µg/g equiv	% of dose	µg/g equiv	% of dose	µg/g equiv	% of dose	µg/g equiv
Liver	3.91	10.40	4.45	12.38	0.63	154.49	0.38	94.22
Kidney	2.14	29.52	1.53	21.36	0.12	137.88	0.09	104.64
Blood	n.a.	n.a.	n.a.	n.a.	0.05	35.41	0.03	23.62
Plasma	0.35	4.23	0.31	4.64	0.03	43.52	0.02	27.58
Fat	1.66	12.62	1.78	23.22	0.37	235.81	0.32	312.10

n.a. Not analyzed

Excretion

The excretion of radioactive residues was rapid and comparable for both sexes. Over an observation period of about 168 hours, the majority of the radioactive residues (68-91% of the dose) were excreted via urine. Smaller portions were excreted via bile (11-31% of the dose) or feces (11-21% of the dose). Excretion via urine and feces or bile was nearly complete within 72 hours after dosing for all dose groups, with more than half of the administered dose being excreted within 24 hours for the multiple high dose groups CM / CF and within 48 hours for the single high dose groups DXM / DXF, respectively. For both sexes small portions of BAS 605 F (<0.9% of the dose) as the unchanged parent compound were only detected in feces. To a minor extent, the parent compound was found in tissue samples with the highest portions in fat extracts.

At maximum plasma level (approximately one hour after dosing), the concentrations of detected radioactive residues in tissues of low dose groups VM and VF and high dose groups WM and WF accounted for 0.38% and 0.63% (WF and WM) to 3.91% and 4.45% (VM and VF) of the administered dose in liver, 0.09% and 0.12% (WF and WM) to 1.53% and 2.14% (VF and VM) of the administered dose in kidney, 0.32% and 0.37 (WF and WM) to 1.66% and 1.78% (VM and VF) of the administered dose in fat and 0.02% and 0.03% (WF and WM) to 0.31% and 0.35% (VF and VM) of the administered dose in plasma.

Table 5.1.1-9: Excretion of radioactivity after single oral administration of ^{14}C-BAS 605 F to rats at a rate of nominally 500 mg/kg bw (group mean values)

Matrix	[% of the administered radioactivity]	
	Male (DXM)	Female (DXF)
Urine (h)		
0-6	11.30	2.19
6-12	16.24	15.10
12-24	25.07	26.87
24-48	19.07	20.15
48-72	1.23	2.01
72-96	0.75	0.93
96-120	0.36	0.63
120-144	0.27	0.43
144-168	0.31	0.41
Subtotal urine (0-168)	**74.59**	**68.73**
Feces (h)		
0-24	7.78	3.89
24-48	10.88	10.64
48-72	0.99	1.23
72-96	0.83	0.52
96-120	0.20	0.25
120-144	0.14	0.22
144-168	0.16	0.20
Subtotal feces (0-168)	**20.98**	**16.95**
Other sources		
Cage wash (after 12-13 days)	4.20	4.75
Liver (168 h)	n.a.	n.a.
Kidney (168 h)	n.a.	n.a.
Blood (168 h)	n.a.	n.a.
Plasma (168 h)	n.a.	n.a.
Carcass (168 h)	n.a.	n.a.
Total (0-168 h)	**99.78**	**90.43**

n.a. Not analyzed

Extractability

Extractability of radioactive residues of BAS 605 F in feces of male and female rats was moderate to high, with 54.1-88.0% of the total radioactive residues extracted. The main portions of radioactive residues were extracted with methanol (37.0-78.5% TRR), and lower portions were subsequently extracted with water (8.5-26.5% TRR).

Extractability of radioactive residues of BAS 605 F in liver and kidney of male and female rats was very high, with 95.2-118.1% of the total radioactive residues extracted. The main portions of radioactive residues were extracted with methanol (86.4-111.9% TRR), and lower portions were subsequently extracted with water (3.5-11.7% TRR).

Extractability of radioactive residues of BAS 605 F in fat of male and female rats was also very high, with 96.4-103.2% of the total radioactive residues extracted. The main portions of radioactive residues were extracted with acetonitrile (68.7-84.4% TRR), and lower portions were subsequently extracted with isohexane (15.9-34.4% TRR).

Table 5.1.1-10: Extractability of feces samples with solvents (methanol, water) after dosing of rats

Matrix	TRR combusted [mg/kg] [%TRR]	TRR calculated [mg/kg] [%TRR]	Solvent extract (ERR)		RRR	
			mg/kg	% TRR	mg/kg	% TRR
Single dose of 500 mg/kg bw; male rats (DXM)						
0-24 h	1605.64 100.0	1600.54 99.7	1131.28	70.5	469.27	29.2
24-48 h	1743.35 100.0	1729.43 99.2	1046.71	60.0	682.72	39.2
48-72 h	137.06 100.0	143.34 104.6	79.44	58.0	63.90	46.6
72-96 h	101.69 100.0	115.23 113.3	67.19	66.1	48.04	47.2
96-120 h	27.86 100.0	29.31 105.2	15.83	56.8	13.48	48.4
120-144 h	18.91 100.0	19.20 101.5	12.73	67.3	6.47	34.2
144-168 h	22.06 100.0	23.13 104.8	15.40	69.8	7.73	35.1
Single dose of 500 mg/kg bw; female rats (DXF)						
0-24 h	1060.89 100.0	1047.26 98.7	771.74	72.7	275.52	26.0
24-48 h	1351.92 100.0	1361.20 100.7	835.12	61.8	526.08	38.9
48-72 h	164.39 100.0	160.58 97.7	88.94	54.1	71.64	43.6
72-96 h	63.65 100.0	60.45 95.0	37.01	58.2	23.44	36.8
96-120 h	34.12 100.0	34.49 101.1	19.57	57.4	14.92	43.7
120-144 h	27.40 100.0	27.40 100.0	15.57	56.8	11.83	43.2
144-168 h	24.20 100.0	24.04 99.3	14.40	59.5	9.64	39.8
Repeated doses of 500 mg/kg bw; male rats (CM)						
0-24 h	333.68 100.0	363.23 108.9	259.75	77.8	103.48	31.0
24-48 h	162.26 100.0	176.71 108.9	118.18	72.8	58.53	36.1
Repeated doses of 500 mg/kg bw; female rats (CF)						
0-24 h	212.27 100.0	248.31 117.0	186.83	88.0	61.49	29.0
24-48 h	211.17 100.0	220.65 104.5	151.56	71.8	69.09	32.7

TRR: Total radioactive residue (sum of ERR + RRR)
ERR Extractable radioactive residue (solvents: methanol, water)
RRR: Residual radioactive residue after solvent extraction (solvents: methanol, water)

Metabolism

The major transformation steps in the metabolic pathway of BAS 605 F are

- Hydroxylations at the phenyl and/or pyrimidinyl ring and/or methyl group of BAS 605 F resulting in hydroxyl groups (Phase I), followed by
- Conjugation with glucuronic acid or sulfate of the mono- or dihydroxylated parent compound (Phase II).

Minor transformation steps were

- Cleavage of two C-N bonds in the pyrimidinyl ring of the parent compound with further hyydroxylation or
- Cleavage at the secondary amine bond of the parent compound and
- Glutathione conjugation with subsequent following degradation and cleavage processes to cysteine conjugates.

In urine of male and female rats of dose groups DXM, DXF, CM and CF, 16 (DXM), 21 (DXF), 18 (CM) or 20 (CF) metabolites and components were identified and ranged from 0.2% to 22% of the dose. The portions of radioactive residues of total identified metabolites and MS characterized components were between 59% of the dose (DXF) and 80% of the dose (CM). The metabolite patterns in urine were largely comparable for both sexes, but the amount of the major metabolites and components varied in between both genders. Differences existed also between the single high dose group DXM / DXF and the multiple high dose group CM / CF.

For dose group DXM / DXF, the most abundant metabolites in urine were metabolites M605F002 / M605F019 / M605F044 (M605F002 was not present in male rats), M605F026 / M605F017 (M605F017 was also not present in male rats), M605F018 and M605F021 / $C_{13}H_{15}N_3OS$. These metabolites are either glucuronide or sulfate conjugates, except for M605F002 which was generated by hydroxylation of the parent compound. Metabolites M605F019 / M605F044 were present at highest portions in male rats and accounted for 19% of the dose, whereas M605F002 / M605F019 / M605F044 only accounted for 11% of the dose for dose group DXF. The MS characterized component $C_{12}H_{13}N_3O_2S$ co eluting with metabolites M605F002 / M605F019 / M605F044 was only present for female rats. For the female rats of dose groups DXF the metabolites M605F026 / M605F017 (either conjugated with sulfate or hydroxylated) were present at highest portions (<15% of the dose), whereas M605F026 only accounted for 10% in male rats. Except for metabolite M605F042 (dose group DXM), metabolite M605F043 (dose group DXF) and M605F007 / M605F025 / $C_{10}H_{11}N_3O_3$ (dose group DXF) all detected metabolites and components were present at higher portions than 1% of the dose. Furthermore, except for M605F002, M605F017 and M605F007 / M605F025 / $C_{10}H_{11}N_3O_3$ (only present in female rats) all detected metabolites represent either glucuronide or sulfate conjugates and metabolites of the mono- or dihydroxylated parent compound.

For dose group CM / CF, the most abundant metabolites in urine were metabolites M605F002 / M605F019 / M605F044 and M605F024 / M605F023. These metabolites are either glucuronide or sulfate conjugates, except for M605F002 which was generated by hydroxylation of the parent compound. Metabolites M605F024 / M605F023 accounted for 21-22% of the dose for dose group CM and CF, respectively, whereas the metabolites M605F002 / M605F019 / M605F044 accounted for 22% of the dose for dose group CM and only for 12% of the dose for dose group CF. The MS characterized component $C_{12}H_{13}N_3O_2S$ co-eluting with metabolites M605F002 / M605F019 / M605F044 was also only present for female rats. Except for component $C_{13}H_{15}N_3O_4S_2$ (dose group CM) and metabolite M605F042 (dose group CF) all detected metabolites and components were present at higher portions than 1% of the dose. Furthermore, except for M605F003 (only present in male rats) all other detected metabolites represent either glucuronide or sulfate conjugates and metabolites of the mono- or dihydroxylated parent compound.
In contrast to the single high dose groups DXM and DXF, a higher amount of radioactive residues was excreted via urine for the multiple high dose groups CM and CF. Furthermore, a higher portion of metabolites conjugated with glucuronic acid than with sulfate was detected for dose groups CM and CF. This includes especially the metabolites M605F024 / M605F023 and M605F013, which amounted up to 5 times higher in dose groups CM and CF. In contrast, the sulfate conjugates M605F026, M605F018 and M605F021 / $C_{13}H_{15}N_3OS$ reached higher amounts in the dose groups DXM and DXF.

In feces, up to twelve metabolites, MS characterized components and parent compound were identified for male and female rats of dose groups DXM, DXF, CM and CF and ranged from 0.1% to 2.1% of the dose. The hydroxylated metabolite M605F002 was the most abundant component in feces of male and female rats of all dose groups (up to 1.5% of the dose for the single high dose group DX and up to 2.1% of the dose for the multiple high dose group CM, respectively). Small portions of parent compound (<0.9% of the dose for dose group DX and <0.3% of the dose for dose group C) were detected in both sexes and all dose groups. The residual metabolites accounted for less than 1.4% of the dose. Comparable metabolites and components were detected in the major peaks for both genders, but the amount of the major metabolites and components varied in between both genders.
In comparison to the single high dose groups DXM and DXF, comparable amounts of radioactive residues were excreted via feces for the multiple high dose groups CM and CF and no remarkable differences regarding the metabolite pattern was observed. Only with metabolite M605F042 and the components $C_{13}H_{15}N_3OS$ and $C_{10}H_{11}N_3O_5S$ sulfated metabolites and components (<1% of the dose) were detected, the residual fecal metabolites were cleaved or hydroxylated.

In bile, up to 18 metabolites and MS characterized components were identified for male and female rats of dose groups RM, RF, SM and SF and ranged from 0.01% to 13.1% of the dose. Up to seven metabolites were detected with portions higher than 1% of the dose. The metabolite patterns in bile were qualitatively comparable for both sexes, but showed some differences between the low dose group RM / RF and the high dose group SM / SF. The four main peaks of both genders of the low dose group RM / RF consisted of the metabolites M605F018, M605F019, M605F023 and M605F021 (up to 3.8%, 3.8%, 3.5% and 2.1% of the dose, respectively), whereas the main peak of both genders of the high dose group SM / SF consisted of the metabolites M605F024 / M605F023 (up to 13% of the dose for female rats). Some minor components were only detected in the dose group SM / SF.
For both, the low and high dose group, the amount of the major metabolites and components was higher for female rats. Metabolite M605F007 was only detected in male rats of dose groups RM and SM.
In accordance to urine, all metabolites except for metabolite M605F007 were excreted as glucuronide or sulfate or cysteine conjugates of the mono- or dihydroxylated parent compound in bile. In contrast to the low dose groups RM and RF, a higher portion of metabolites conjugated with glucuronic acid than with sulfate was detected for the high dose groups SM and SF. This includes especially the metabolites M605F024 / M605F023 and M605F013, which amounted up to 3.5 times higher in dose groups SM and SF. In addition, metabolite M605F024 was not present in the low dose groups RM and RF. In contrast, the sulfate conjugates M605F026, M605F018, M605F019 and M605F021 reached higher amounts in the dose groups RM and RF. In addition, M605F026 was not present in the high dose group SF.

Table 5.1.1-11: Identified metabolites and components in urine, feces and bile

Metabolite / Component	Urine DXM	Urine DXF	Urine CM	Urine CF	Feces DXM	Feces DXF	Feces CM	Feces CF	Bile RM	Bile RF	Bile SM	Bile SF
	Composition of radioactive residues in % of the dose											
BAS 605 F	-	-	-	-	0.9	0.6	0.2	0.3	-	-	-	-
M605F019[3]	19.3	11.4	22.3	12.2	-	-	-	-	2.1	3.8	0.4	2.9
M605F044[2]					-	-	-	-	-	-		
M605F002[1]	-				1.5	1.4	1.7	2.1	-	-	-	-
$C_{12}H_{13}N_3O_2S$	-		-		-	-	-	-	-	-	-	-
M605F021[3]	10.7	10.3	8.5	8.7	-	-	-	-	2.1	2.0	0.3	1.4
$C_{13}H_{15}N_3OS$					1.4	0.7	-	0.9	-	-	-	-
M605F003[1]	-	-		-			0.6	0.9	-	-	-	-
M605F004[1]	-	-	-	-	-	+	-	-	-	-	-	-
M605F007	-	0.3	-	-	0.2	0.2	-	0.1	0.6	-	+	-
M605F025	-		-	-			-	-	-	-	-	-
$C_{10}H_{11}N_3O_3$	-		-	-			-	-	-	-	-	-
M605F013[2]	2.4	2.8	6.1	10.2	-	-	-	-	-	1.2	0.7	2.4
M605F015	-	-	-	-	0.7	0.1	-	1.0	-	-	-	-
M605F031	-	-	-	-	0.8	1.4	0.6		-	-	+	-
M605F026[3] (two isomers)	9.6	14.6	4.5	7.6	-	-	-	-	0.4	2.0	0.1	-
M605F017[1]	-				0.7	0.4	0.4	0.2	-	-	-	-
$C_{18}H_{19}N_3O_8$	-	-	6.5	5.5	-	-	-	-	-	-	0.7	1.4
M605F018[3]	11.0	8.4			-	-	-	-	1.5	3.8		
M605F020[2]	1.2	1.4	5.7	4.9	-	-	-	-	1.0	0.9	1.0	4.0
$C_{18}H_{22}N_4O_5S$					-	-	-	-	-	-		
M605F022[2,4]	-	-	-	-	-	-	-	-	-	-	-	-
M605F024 - 33.6 min[4]	3.9	4.1	21.6	21.0	-	-	-	-	-	-	3.7	13.1
M605F023[2]					-	-	-	-	1.8	3.5		
M605F042	0.3	-	-	0.2	1.0	0.9	0.9	0.3	-	-	-	-
M605F043[3]	1.5	0.7	-	2.1	-	-	-	-	-	-	-	-
$C_{12}H_{13}N_3O_6S$	2.5	2.6	2.4	3.1	-	-	-	-	-	-	-	-
$C_{16}H_{20}N_4O_4S$	-	-	-	-	-	-	-	-	-	-	0.1	-
$C_9H_{11}N_3O$	-	-	-	-	-	0.1	-	-	-	-	-	-
$C_{12}H_{11}N_3O$	-	-	-	-	-	+	-	-	-	-	-	-
$C_{19}H_{23}N_3O_7S$	1.5	-	1.9	1.3	-	-	-	-	-	-	-	-
$C_{13}H_{15}N_3O_4S_2$	2.0	2.1	0.8	1.4	-	-	-	-	-	-	-	-
$C_{14}H_{12}N_4O_2S$	-	-	-	-	-	-	-	-	-	-	0.3	-
$C_{14}H_{14}N_4O_2S$	-	-	-	-	-	-	-	-	-	-		-
$C_{10}H_{11}N_3O_5S$	-	-	-	-	0.2	0.7	-	0.1	-	-	-	-
$C_{14}H_{14}N_4OS$	-	-	-	-	-	-	-	-	1.9	-	0.2	0.2
$C_{16}H_{15}N_5O_3S$	-	-	-	-	-	-	-	-	-	-	0.1	1.0
$C_{13}H_{12}N_4S$	-	-	-	-	-	-	-	-	1.7	-	-	-

1 Metabolites derived from hydroxylation of BAS 605 F
2 Metabolites derived from conjugation of mono- or dihydroxylated BAS 605 F with glucuronic acid
3 Metabolites derived from conjugation of mono- or dihydroxylated BAS 605 F with sulfate
4 Identified during structure elucidation but not quantified
+ Metabolite / component was assigned, but the % of dose value was below 0.1%

In tissues and plasma samples, up to six metabolites, MS characterized components and parent compound were identified for male and female rats of dose groups VM, VF, WM and WF and ranged from 0.001% to 1.5% of the dose. Only for the low dose groups VM and VF metabolite M605F018 and the parent compound were detected with portions higher than 1% of the dose. Beside the parent compound, the sulfate conjugate M605F018 (both ranging from 0.1% to 1.5% of the dose) was the most abundant component in liver, kidney and plasma of male and female rats of the low dose groups VM and VF with slightly lower portions in female rats. For the high dose groups WM and WF metabolite M605F018 was also a major component but was detected in minor portions (ranging from <0.01% to 0.1% of the dose) than the parent compound (ranging from 0.01% to 0.4% of the dose) in liver, kidney and plasma of male and female rats, also showing slightly lower portions in female rats. Except for male rats of dose group VM, only parent compound (up to 1.5% of the dose) was detected in fat extracts. Metabolite M605F004 was detected at small amounts (<0.1% of the dose) in plasma samples for all dose groups but only detected at small amounts (<0.1% of the dose) in liver and kidney samples of dose groups WM and WF. Metabolite M605F019 was only detected in kidney at small amounts <0.1% of the dose. The portions of radioactive residues of total identified metabolites and components were highest for liver (0.3-2.6% of the dose for dose group WF and VM, respectively) and lowest for plasma samples (0.01-0.2% of the dose for dose group WF and VM, respectively), whereby higher portions of radioactive residues were detected in all samples for the low dose groups VM and VF. The portions of radioactive residues of total identified metabolites and components in kidney accounted for 0.1-1.5% of the dose for dose group WF and VM, respectively, and in fat for 0.2-1.5% of the dose for dose group WF and VF, respectively. No gender specific differences were observed.

Table 5.1.1-12: Identified metabolites and components in tissues and plasma

Metabolite / Component	Liver				Kidney				Fat				Plasma			
	VM	VF	WM	WF	VM	VF	WM	WF	VM	VF	WM	WF	VM	VF	WM	WF
	Composition of radioactive residues in % of the dose															
BAS 605 F	0.9	1.1	0.4	0.3	0.2	0.3	0.1	+	1.5	1.6	0.3	0.2	0.1	0.1	+	+
M605F004[1]	-	-	+	+	-	-	+	+	-	-	-	-	+	+	+	+
M605F018[3]	1.5	1.2	0.1	+	1.1	0.6	+	+	+	-	-	-	0.1	0.1	+	+
M605F019[3]					+	-	+	+	-	-	-	-	-	-	+	-
M605F021[3]	0.1	0.1	+	-	0.2	0.2	+	+	-	-	-	-	+	+	+	-
M605F023[2]	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+
$C_{12}H_{11}N_3O$	-	0.1	-	+	-	-	+	-	-	-	-	-	+	+	-	-

1 Metabolites derived from hydroxylation of BAS 605 F
2 Metabolites derived from conjugation of mono- or dihydroxylated BAS 605 F with glucuronic acid
3 Metabolites derived from conjugation of mono- or dihydroxylated BAS 605 F with sulfate
+ Metabolite / component was assigned, but the % of dose value was below 0.1%

Table 5.1.1-13: Structures of metabolites identified in rat matrices

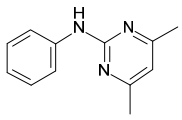
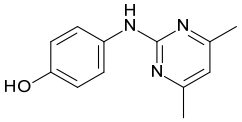
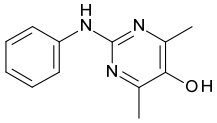
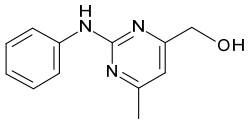
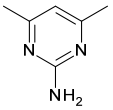
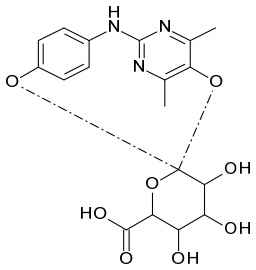
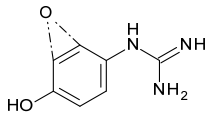
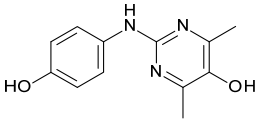
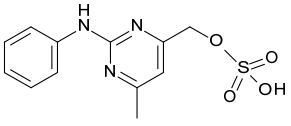
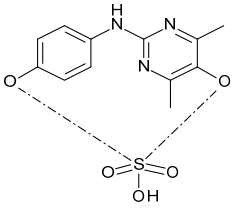
Metabolite designation				Structure/Name
BASF code	Synonym	Reg. No	CAS-No	
Pyrimethanil BAS 605 F M605F000	N/A	236999	53112-28-0	
M605F002	N/A	4739173	81261-84-9	
M605F003	N/A	5079484	-	
M605F004	N/A	5079485	-	
M605F007	N/A	40603	767-15-7	
M605F013	N/A	-	-	
M605F015	N/A	-	-	
M605F017	N/A	-	-	
M605F018	N/A	-	-	
M605F019	N/A	-	-	

Table 5.1.1-13: Structures of metabolites identified in rat matrices

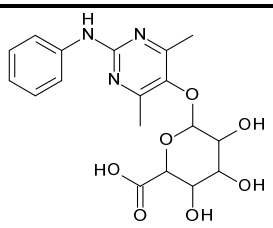
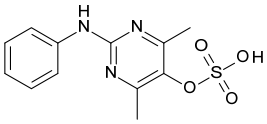
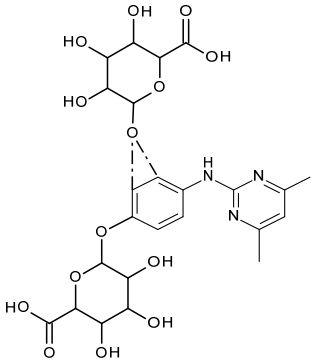
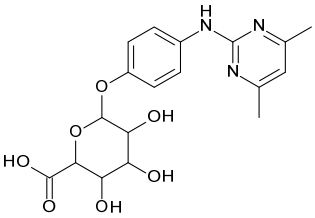
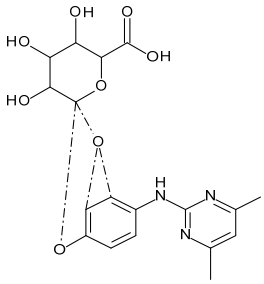
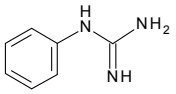
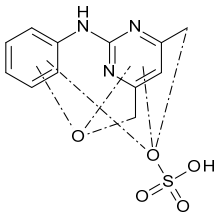
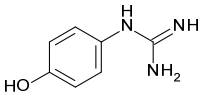
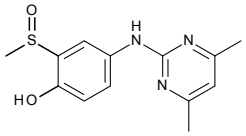
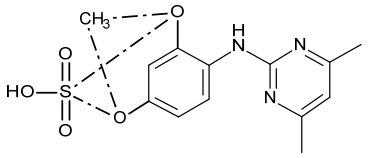
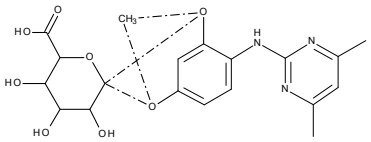
Metabolite designation				Structure/Name
BASF code	Synonym	Reg. No	CAS-No	
M605F020	N/A	-	-	
M605F021	N/A	-	-	
M605F022[1]	N/A	-	-	
M605F023	N/A	-	-	
M605F024	N/A	-	-	
M605F025	N/A	4182909	2002-16-6	
M605F026	N/A	-	-	
M605F031	N/A	-	-	

Table 5.1.1-13: Structures of metabolites identified in rat matrices

Metabolite designation				Structure/Name
BASF code	Synonym	Reg. No	CAS-No	
M605F042	N/A	-	-	
M605F043	N/A	-	-	
M605F044	N/A	-	-	

N/A Not applicable
1 Identified during structure elucidation but not quantified

III. CONCLUSION

In summary, comparable metabolite patterns of male and female rats in urine, feces, bile, tissue and plasma samples, respectively, were detected, but the amount of the major metabolites and components varied in between both genders. The slight differences in the quantity of metabolites of the two genders can be explained by variations in the kinetic behavior of metabolite generation and excretion.
Differences existed between the dose groups of the specific samples. Predominately glucuronide and sulfate conjugates of mono- or dihydroxylated BAS 605 F were excreted via urine and bile, whereas higher portions of sulfate conjugates were detected in the dose groups DXM / DXF and RM / RF, and higher portions of glucuronide conjugates were detected in the dose groups CM / CF and SM / SF. For urine and bile, the metabolites M605F024 / M605F023 and M605F013 (conjugated with glucuronic acid) were present in higher amounts for the dose groups CM / CF and SM / SF, whereas the metabolites M605F026, M605F018 and M605F021 / $C_{13}H_{15}N_3OS$ (conjugated with sulfate) reached higher amounts in the dose groups DXM / DXF and RM / RF. As the sulfate pool is limited, a higher capacity for glucuronidation than sulfation was observed for the high dose group SM / SF and the multiple high dose group CM / CF.
The experiments indicated that the major part of the radioactive residues was excreted via urine and only smaller portions via bile. The metabolite pattern for liver and kidney was comparable with slightly higher portions in liver. Metabolite M605F019 was only detected in kidney at small amounts <0.1% of the dose.

Figure 5.1.1-2: Proposed metabolic pathway of pyrimethanil in rats (part 1)

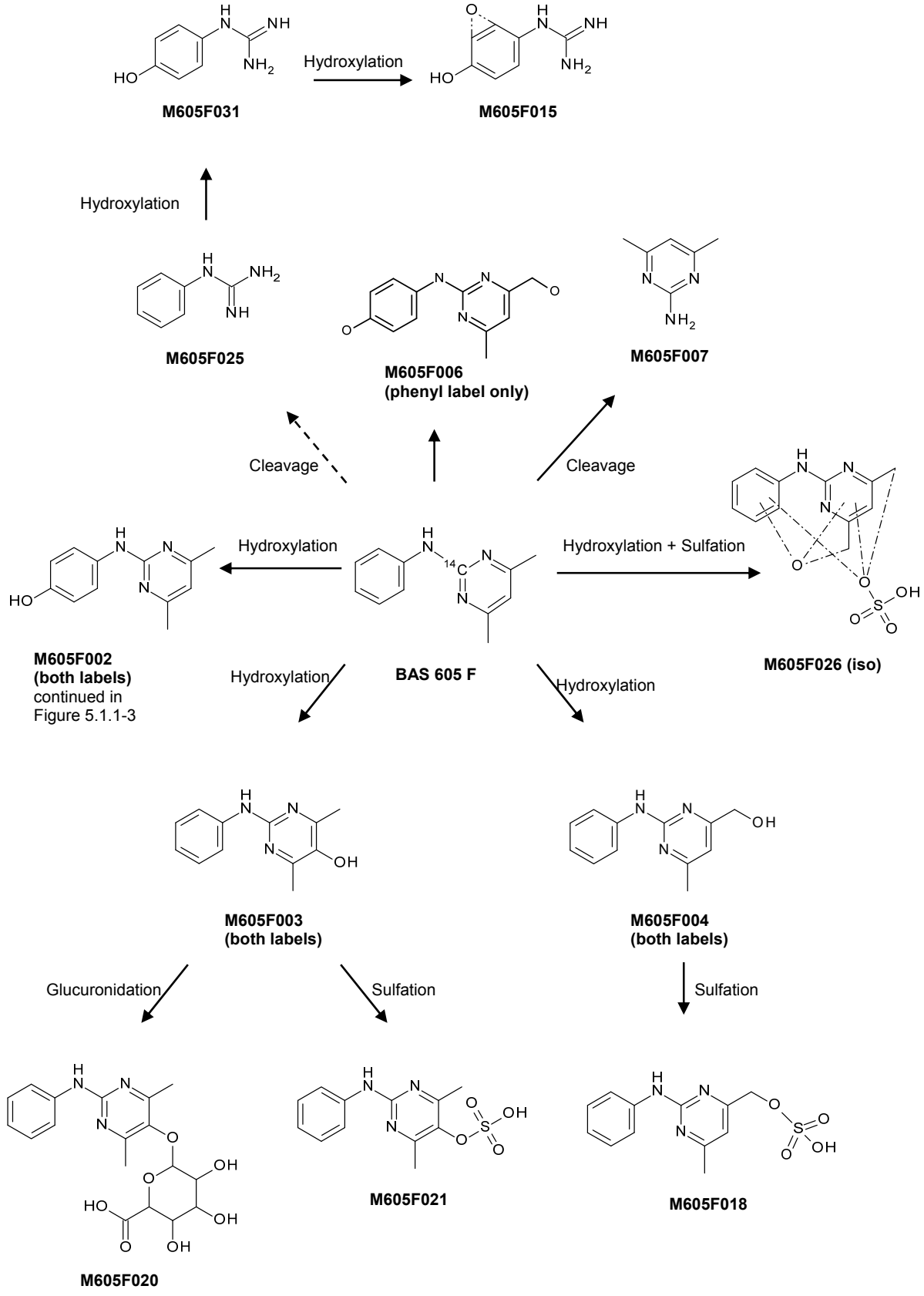
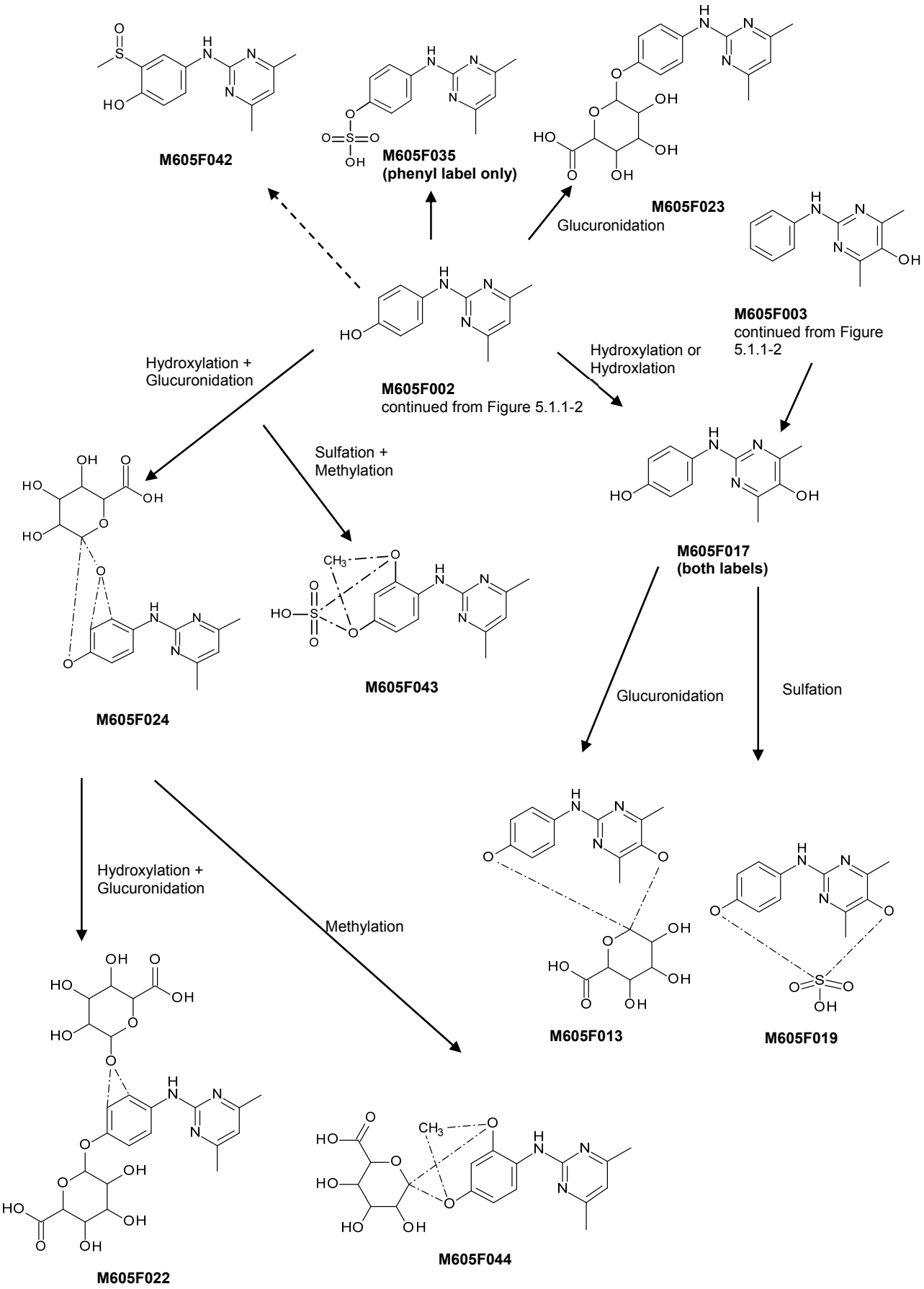


Figure 5.1.1-3: Proposed metabolic pathway of pyrimethanil in rats (part 2)



CA 5.1.2 Absorption, distribution, metabolism and excretion by other routes

According to the new data requirements for active ingredients of plant protection products as set out in Commission Regulation (EU) No. 283/2013 (1 March 2013, OJ L93, 1ff, 3.4.2013), "comparative *in vitro* metabolism studies shall be performed on animal species ... and on human material ...in order to determine the relevance of the toxicological animal data and to guide in the interpretation of findings and in further definition of the testing strategy..." (Section 5, Toxicological and metabolism studies, point 5.1.1., page 22).
~~In the absence of validated test method or guidance documents, and in agreement with the RMS this data requirement is waived in accordance to SANCO Guidance Document SANCO/10181/2013-rev 2.1 (13 May 2013).~~

Report:	CA 5.1.2/1 Birks V., 2016 a 14C-BAS 605 F: Comparative in vitro metabolism studies with rat and human cryopreserved hepatocytes 2014/1000803
Guidelines:	UK Health and Safety Good Laboratory Practice Regulations 1999 (No. 3106), 2004/10/EC of 11 February 2004, OECD Principles of Good Laboratory Practice
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

I. MATERIAL AND METHODS

A. MATERIALS

1.	**Test Material:**	
	Description:	[pyrimidinyl-2-C14]-BAS 605 F (pyrimidinyl label) [pyrimidinyl-1,3-N15]-BAS 605 F (^{15}N label) Unlabeled BAS 605 F
	Lot/Batch #:	1049-1010 (pyrimidinyl label) 1050-0101 (^{15}N label) L83-126 (unlabeled)
	Purity:	99.6% (unlabeled) 99.1% (^{15}N label) Radiochemical purity: 99.7% (pyrimidinyl label) Specific activity: 6.42 MBq/mg (pyrimidinyl label)
	CAS#:	53112-28-0
	Stability of test compound:	
		The test item was stable over the test period. Stability controls without cells showed nearly identical HPLC profiles that contained only peaks corresponding to the unchanged active substance BAS 605 F.
2.	**Vehicle and/or positive control:**	
		Vehicle: hepatocytes Positive control: ^{14}C-testosterone testosterone or ^{14}C-7-ethoxycoumarin instead of the test substances were incubated with hepatocytes from the two different species to indicate the metabolic activity of the different hepatocytes.
3.	**Test animals:**	Mammals
	Species:	Human, rat
	Strain:	-, Sprague Dawley

B. STUDY DESIGN AND METHODS

1. Dates of work: 28-Apr-2015 - 02-Aug-2015

This study was carried out at Quotient Bioresearch (Rushden) Ltd., United Kingdom.

Test procedure

The objective of this study was to compare the *in vitro* metabolism in hepatocytes of rat (the species previously used in toxicological testing of this substance defining the toxicological reference values) to the metabolism in human hepatocyte samples and to determine whether metabolic profiles were similar and whether unique human metabolites occurred.

To address this question, the radiolabeled test item was incubated with hepatocytes from human and rat (all mixed gender) at a final concentration of 10 µM. The concentration was chosen after a cell viability pre-test. ^{14}C-labeled test item (pyrimidinyl-2-^{14}C-BAS 605 F) was used. The viability of the hepatocytes was determined after 180 min of incubation with 1, 5 and 10 µM unlabelled Pyrimethanil (BAS 605 F) using the CellTiter 96® AQ_{ueous} One Solution cell viability assay by Promega.

After incubation for 0, 10, 30, 60 or 180 min, the reaction was terminated by addition of acetonitrile (25% of incubation sample volume) and the resulting supernatant was analyzed by HPLC. Selected samples were additionally investigated by LC-HR-MS.

All the supernatants contained ≥90% of the applied radioactivity (% AR), therefore pellet extraction was not required.

Negative and positive controls were run in parallel to demonstrate the absence of non metabolic degradation and the metabolic activity of the hepatocytes (Phase I and Phase II metabolic reactions), respectively. The control experiments yielded the expected results.

Test design and analytical procedures

Test solutions
Stock solutions of BAS 605 F were prepared in DMSO. The radioactive concentration of the solutions was confirmed by LSC. Radiochemical purity was confirmed on each experimental day by HPLC.
For the unlabeled test item, a 2 mM solution of ^{12}C-BAS 605 F (unlabeled) was prepared by dissolving an aliquot of ^{12}C-BAS 605 F in DMSO.

For the preparation of the application solutions, 2 mM solutions of ^{14}C/^{15}N/^{12}C-BAS 605 F (pyrimidinyl label) were prepared by combining suitable amounts of the prepared solutions to give the final stocks. The final stock solutions were analyzed by LC-HR-MS in order to confirm the identity of BAS 605 F and the initial isotope pattern.

Positive control incubations were performed with ^{14}C-testosterone and ^{14}C-7-ethoxycoumarin, respectively. The final concentration of ^{14}C-testosterone in incubations was 150 µM at a target of 1% (v/v). Therefore, a stock solution of 15 mM ^{14}C-testosterone was prepared. Initially, unlabeled testosterone was weighed and dissolved in methanol to give a 15 mM solution. An aliquot of ^{14}C-testosterone was reduced to dryness under N_2 gas and reconstituted in the unlabeled testosterone. The final concentration of ^{14}C-7-ethoxycoumarin in incubations was 25 µM at a target of 1% (v/v). Therefore, a stock solution of 2.5 mM ^{14}C-7-ethoxycoumarin was prepared. An aliquot of ^{14}C-7-ethoxycoumarin in toluene was reduced to dryness under N_2 gas and reconstituted in methanol.

Negative controls:
In the negative controls no metabolism should occur. For the “stability control”, the application solution was mixed with incubation medium instead of the cell suspension. For the “zero incubation control” (t = 0 min), the reaction was stopped immediately after addition of the cell suspension.

Preparation of hepatocytes
Mixed gender rat hepatocytes were purchased from Bioreclamation IVT. Mixed gender Hepatosure™ Pooled Cryopreserved Human Hepatocytes (100 donors) were sourced from Xenotech. Hepatocytes were stored in liquid nitrogen prior to use. Hepatocytes were resuscitated as per the instruction provided by the supplier.

Viability tests and non-specific binding tests
The viability of human and rat hepatocytes after incubation with 1, 5 and 10 µM BAS 605 F (non-radiolabeled) was tested using the cell proliferation assay AQ$_{ueous}$ One Solution (Promega) in order to select the appropriate concentration of the test item in the *in vitro* assays.
Prior to the species comparison of ^{14}C-BAS 605 F metabolism, an assessment of the non-specific binding to the incubation plate was performed at 10 µM ^{14}C-BAS 605 F.

In vitro assays
On each incubation day, the application solutions in DMSO were diluted with human or rat hepatocyte suspension by a factor of 200 to prepare incubations at a final concentration of 10 µM BAS 605 F. Aliquots of the application media were analysed by LSC to calculate the amounts of applied radioactivity per well (representing 100% AR). The application media were incubated at a final concentration of 10 µM BAS 605 F with human or rat hepatocytes. In the case of testosterone and 7-ethoxycoumarin, the incubations were performed at 150 µM and 25 µM, respectively and were diluted with with human or rat hepatocyte suspension by a factor of 100 to prepare the respective application medium.

Each sample (4.02 mL total incubation volume) comprised 0.02 mL of application medium and 4 mL of hepatocyte cell suspension in one of the wells of a 24-well plate. The final cell concentration was approximately 1 x 10^6 cells/mL. The reactions were performed for 0, 10, 30, 60 or 180 min at 37°C and at 5% CO_2 in an incubator.
Two negative controls (stability and zero incubation control), two positive controls (testosterone and 7-ethoxycoumarin) and a blank control (application medium with DMSO instead of the test item) were performed for each analyzed species.

In each experimental setup, the incubation of BAS 605 F as well as the test compound control assays were performed in triplicates. Incubations with testosterone or 7-ethoxycoumarin were performed in singlicate. In parallel, the viability of the human and rat hepatocytes after 180 minutes' incubation with 10 µM BAS 605 F was tested using the cell proliferation assay.

Sampling and sample storage
The incubation containing BAS 605 F was terminated by pipetting the incubation mixture into a tube containing cold acetonitrile to adjust the sample to an acetonitrile concentration of approximately 20% (v/v). The incubations containing testosterone and 7-ethoxycoumarin were terminated by pipetting the incubation mixture into a tube containing acetonitrile or methanol, respectively. All incubations were sonicated for a few seconds, vortex mixed and centrifuged for 5 min at 16,000 x g at 4°C prior to analysis. The radioactive residues in the supernatants were determined by LSC analysis of aliquots pre and post centrifugation. The remaining pellet was then redissolved in water and the percentage of radioactivity remaining determined by LSC analysis. All samples were stored in a freezer at -20°C.

Work-up of the residual pellet
In the supernatant, the radioactive residues were quantified and ranged from 87.0% to 103%. Therefore, pellet extraction was not required. The remaining pellet was redissolved in water and aliquots were measured via LSC.

Evaluation of the data by HPLC and MS
For the qualitative evaluation of the masses of peaks representing more than 5% AR in human hepatocytes, selected samples of the supernatants were analysed by means of LC-HR-MS and processed using Xcalibur v2.2.
Samples were removed from the freezer and allowed to thaw in the refrigerator. Samples were sonicated for 10 minutes, vortex mixed for ca. 10 seconds and centrifuged at 16,000 x g for 5 minutes at 10°C. An aliquot was transferred to a polypropylene vial for LC-MS analysis. The mass spectra of the ^{14}C peaks were evaluated to obtain the m/z values of prominent ions corresponding to the test item or its conversion products.
The m/z values that were assigned to prominent peaks in the supernatant are listed together with the retention times and the % AR values.

II. RESULTS AND DISCUSSION

The radio-HPLC analyses of human and rat hepatocyte samples were compared in order to determine whether a unique human metabolite occurred or not. Selected human and rat supernatant samples were also analysed by LC-HR-MS to assign m/z values to prominent peaks representing more than 5% AR in human samples.

The HPLC profiles of the negative control samples contained only peaks corresponding to the unchanged active substance pyrimethanil (BAS 605 F). Therefore, no significant metabolism or degradation of pyrimethanil occurred without the influence of hepatocytes.
The positive controls with testosterone showed that the metabolic activity of the hepatocytes with respect to Phase I metabolic reactions was sufficiently high. Testosterone was metabolised (>55%) after incubation with human and rat hepatocytes.
HPLC analyses of the positive controls with 7-ethoxycoumarin revealed mean portions of the metabolised 7-ethoxycoumarin reaching values of 30.7% and 25.5% AR after incubation with human and rat hepatocytes, respectively. Hydroxylated, sulphated and glucuronidated metabolites of 7-ethoxycoumarin were detected in human and rat. In rat, 7HC sulphate was the major metabolite (11% AR). However, in human both 7HC sulphate and 7HC glucuronide were formed at lower levels (4.1-7.2% AR).

Radio HPLC and LC HR-MS analysis of samples after incubation with human hepatocytes with 10 µM BAS 605 F allowed the assignment of one relevant peak, M296.
Apart from the active substance BAS 605 F, the following peak was found in the human hepatocytes samples incubated with 10 µM BAS 605 F:
The peak at 30.9 min (M296; m/z 296) was detected after 30 min of incubation representing 1.6% AR, which increased to a maximum of 14.3% AR after 180 min; no other peaks were detected.

In rat hepatocyte samples, the relevant peak with an m/z 296 at 30.9 min from the incubation with human hepatocytes was detected in addition to the active substance pyrimethanil (BAS 605 F) as well. All the components (pyrimethanil and metabolites thereof) detected in human hepatocytes were also detected in rat hepatocytes.

The results are shown in Table 5.1.2-1.

Table 5.1.2-1: Comparison of the relevant metabolites of BAS 605 F after incubation with human and rat hepatocytes

Incubation time (min)	Species	Range of recovery (% AR) in sample supernatants	Relevant peak (% AR)[1]	
			m/z 296 RT 30.9 min	Pyrimethanil (BAS 605 F)
0 min	Human	97.5 - 103	n.d.	98.53
	Rat	91.6 - 97.2	n.d.	94.40
10 min	Human	99.3 - 102	n.d.	99.77
	Rat	90.3 - 99.7	n.d.	94.28
30 min	Human	97.9 - 101	1.60	95.95
	Rat	89.6 - 101	n.d.	91.93
60 min	Human	90.1 - 101	5.67	88.41
	Rat	94.6 - 97.0	1.19	88.71
180 min	Human	97.9 - 103	14.32	80.10
	Rat	93.7 - 99.7	6.63	75.78

1 % applied radioactivity of supernatant
n.d. not detected

III. CONCLUSION

After the incubation of the active substance pyrimethanil (BAS 605 F) in human hepatocytes, two ^{14}C peaks were detected that represented more than 5% AR at at least one time point. The peaks could be assigned to the unchanged active substance pyrimethanil and a metabolite representing an m/z 296 and an RT of 30.9 min.

All the components (pyrimethanil and metabolites thereof) detected in human hepatocytes were also detected in rat hepatocyte samples. Therefore, it can be concluded that the metabolic degradation in human and rat hepatocytes was highly comparable and there was no unique human metabolite.

Overall conclusion

Pyrimethanil has been extensively studied for absorption, distribution, metabolism and excretion. Taking all studies into consideration, the following general conclusions can be drawn:

- Pyrimethanil is rapidly excreted via urine and feces.
- The majority of the radioactivity was excreted via urine (58-91% of the dose) and smaller amounts via feces (1-12% of the dose). The biliary excretion was more pronounced in female animals (25-27%) compared to male rats (14-19%). The bioavailability is at least 71.6%.
- There is no evidence of any cumulative potential of pyrimethanil. Throughout the time course of the experiments, only very low concentrations, if any, were found in tissues and organs.
- The metabolite patterns in tissues and excreta were largely comparable for both sexes and for all dose groups investigated.
- Metabolic degradation in human and rat hepatocytes was highly comparable and there was no unique human metabolite.
- In total 2 major and 3 minor transformation steps were observed in rats:
    - Hydroxylation at the phenyl- and/or pyrimidinyl-ring and/or methyl group of BAS 605 F, resulting in hydroxyl groups (Phase I) and
    - Subsequent conjugation with glucuronic acid or sulfate of the mono- or di-hydroxylated parent compound (Phase II).
    - Cleavage of two C-N bonds in the pyrimidinyl ring of the parent compound with further hyydroxylation or
    - Cleavage at the secondary amine bond of the parent compound and
    - Glutathione conjugation with subsequent following degradation and cleavage processes to cysteine conjugates.

- The combination of these reactions results in a huge number of metabolites. Based on the toxicological considerations detailed in chapter 5.8, the following grouping was introduced:

- Group 1: Hydroxylation/oxidation products of pyrimethanil and their conjugates
- Group 2: Cleavage products pyrimidin-moiety hydroxylates and conjugates
- Group 3: Cleavage products phenyl-moiety

When the information from previous and new studies is reviewed in total, the following endpoints are proposed (Table 5.1.2-2). In this context, the metabolites being considered for dietary exposure assessment are designated as "significant". After the conduct of the assessments (see M-CA 6.7 and 6.9), none of them was identified as "relevant".

Table 5.1.2-2: Proposed endpoints

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of absorption	Rapid, at least 71.6% within 4 h
Distribution	Widely distributed: highest levels in adrenals, blood, liver, kidney, thyroid, ovaries, renal fat, skin
Potential for accumulation	No potential for accumulation
Rate and extent of excretion	>80-95% (mainly in urine) within 48 h
Metabolism in animals	Extensive. 22 identified metabolites in rat
Toxicologically significant compounds (animals, plants)*	Pyrimethanil and metabolites

*In this context, the metabolites being considered for dietary exposure assessment are designated as "significant". After the conduct of the assessments (see M-CA 6.7 and 6.9), none of them was identified as "relevant".

CA 5.2 Acute Toxicity

Studies evaluated in the draft monograph of Rapporteur Member State Austria of April 15, 2004:

Pyrimethanil has been tested in various species and via different routes of administration. All studies have been evaluated by European authorities and Austria as Rapporteur Member State and were considered to be acceptable. For the convenience of the reviewer, these studies are summarized below as extracted from the monograph and the EFSA conclusion. A tabulated summary is provided in Table 5.2-1.
In order to align the dossier submission and the draft RAR, the information that was present in the dRAR in more detail was included in this dossier update in light green, whereas new information and/or corrections were included in lime green.

Table 5.2-1: Summary of acute toxicity studies with pyrimethanil available in the original monograph

Study	Dosage	Result	Reference (BASF DocID)
Acute toxicity, Oral, Rat, Sprague-Dawley m/f	800, 1600, 3200, 6400 mg/kg bw in 1% aqueous methyl cellulose	LD_{50} 4149 mg/kg bw	A81766
Acute toxicity, Oral Mouse, CD-1, m/f	1250, 2500, 5000 mg/kg bw n 1% aqueous methyl cellulose	LD_{50} 4665 mg/kg bw	A81775
Acute toxicity ,dermal Rat, Sprague-Dawley, m/f	5000 mg/kg bw in 1% aqueous methyl cellulose	LD_{50} > 5000 mg/kg bw	A81769
Acute toxicity, inhalation Rat, Sprague-Dawley, m/f	1.98 mg/L	LC_{50} > 1.98 mg/L	A81820
Skin irritation Rabbit, New Zealand White, f	0.5 g/animal	Non irritant	A81771
Eye irritation Rabbit, New Zealand White, f	0.1 mL/animal	Non irritant	A81773
Skin sensitisation, Buehler Test Guinea pig, Dunkin Hartley, f	Induction: 60% in Alembicol D Challenge: 60% in Alembicol D	Not sensitising	A81778, A55313
Skin sensitisation, Maximisation Test Guinea pig, Dunkin Hartley, m/f	Intrad. induction: 20% in mixture Freund's adjuvant /paraffin oil Epid. induction and challenge: 50% in paraffin oil	Not sensitising	A81848, A89294, A89340

Pyrimethanil is of low acute toxicity in rats by the oral (LD_{50} 4149 mg/kg bw), dermal (LD_{50} > 5000 mg/kg bw) and inhalative route (LC_{50} > 1.98 mg/L; max. attainable concentration). Also in mice, low acute oral toxicity was demonstrated (LD_{50} 4665 mg/kg bw). The following clinical symptoms of acute pyrimethanil intoxication were observed after oral intake: reduced activity, reduced muscle tone, prostration in rat and mice. Rats additionally showed hunched posture and in mice coolness to touch an isolated finding of coma and in male mice urogenital and body soiling was observed. Local effects in the rat after dermal exposure were very slight erythema and very slight sloughing of the superficial skin, from which the animals recovered completely within one week. Exaggerated respiratory movements and matted fur were the only signs observed after inhalation exposure recurring to normal within 3 days latest. Pyrimethanil is non-irritating to the skin and to the eyes of rabbits and is a non-sensitizer in the Buehler test and in the Magnusson and Kligman maximization study as well, conducted in guinea pigs. No classification for acute toxicity is needed.

Studies submitted in this AIR 3 dossier (not yet peer-reviewed):
In accordance with the requirements of Commission Regulation SANCO/11802/2010 an in vitro NRU Phototoxicity Test in Balb/c 3T3 cells has been performed and is described in detail in chapter M-CA 5.2.7. The respective study is listed in Table 5.2-2.

Table 5.2-2: Acute toxicity study with pyrimethanil (not yet peer-reviewed)

Study	Dosage	Result	Reference (BASF DocID)
NRU Phototoxicity Test, in vitro Balb/c 3T3 cells	4.6 – 1000 µg/L in 1% DMSO in PBS	Not phototoxic	2013/1342919

Based on the available data on the acute toxicity, classification of pyrimethanil for the relevant endpoints is not required according to the criteria laid down in Regulation (EU) No. 1272/2008 (CLP). Moreover, the data on acute toxicity of pyrimethanil have already been evaluated in 2006 coming to the conclusion that pyrimethanil does not need to be classified for health effects (ECBI/159/04) as laid down in the 31st ATP to the Council Directive 67/548/EEC (Commission Directive 2009/2/EC of 15 January 2009).
Based on the available studies, the conclusion for relevant endpoints adopted to the new list of endpoint format for the current re-registration remains as follows:

Acute toxicity (Regulation (EU) N° 283/2013, Annex Part A, point 5.2)

Rat LD_{50} oral	4149 mg/kg bw	
Rat LD_{50} dermal	> 5000 mg/kg bw	
Rat LC_{50} inhalation	> 1.98 mg/L; max. attainable concentration	
Skin irritation	Non-irritant	
Eye irritation	Non-irritant	
Skin sensitisation	Not sensitising	
Phototoxicity	Not phototoxic	

CA 5.2.1 Oral

The acute oral toxicity study of pyrimethanil was evaluated and peer reviewed during the previous Annex I inclusion process (for further details please see Table 5.2-1). Pyrimethanil has low acute oral toxicity.

Report:	CA 5.2.1/1 [redacted] 1989 a Technical SN 100 309: Rat acute oral toxicity study A81766
Guidelines:	OECD 401, US EPA (1984) Pesticide Assessment Guidelines. Subdiv. F - Hazard Evaluation: Human and domestic animals (Nov 1984 revised edition)
GLP:	yes (certified by Department of Health and Social Security of the Government of the United Kingdom, United Kingdom)
Deviations:	No (compared to OECD guideline 401 adopted 1987)

Report:	CA 5.2.1/2 [redacted] 1993 a Technical SN 100309: Rat acute oral toxicity A81767
Guidelines:	OECD 401, US EPA (1984) Pesticide Assessment Guidelines. Subdiv. F - Hazard Evaluation: Human and domestic animals (Nov 1984 revised edition)
GLP:	yes

Report:	CA 5.2.1/3 [redacted] 1994 a 1st amendment to report No. TOX/89/223-3: Technical SN 100309: Rat acute oral toxicity study A81768
Guidelines:	OECD 401, US EPA (1984) Pesticide Assessment Guidelines. Subdiv. F - Hazard Evaluation: Human and domestic animals (Nov 1984 revised edition)
GLP:	yes

Material and Methods:
Groups of 5 rats/sex (strain: CD Sprague-Dawley; source: [redacted].) weighing between 142 and 250 g received a single dose of 0 (vehicle control), 800, 1600, 3200 or 6400 mg/kg bw pyrimethanil (batch no. CR 19325/1; purity 98.4 %, suspended in 1 % w/v methyl cellulose in distilled water) by oral gavage. After administration, all animals were kept under observation for 14 days. Clinical observations were made daily. Body weights were recorded on the day of dosing and on days 7 and 14. At termination, all surviving rats were subjected to gross post-mortem examination for external abnormalities and for abnormalities of the thoracic and abdominal viscera. Necropsies were also performed on those animals that had died during the study.

Findings:
Clinical signs and mortality: Death rates in the individual groups were 0/5 (♂) and 0/5 (♀) at 800 mg, 1/5 (♂) and 0/5 (♀) at 1600 mg, 0/5 (♂) and 0/5 (♀) at 3200 mg, and 5/5 (♂) and 3/5 (♀) at 6400 mg, resp. Deaths occurred within 4 – 6 hours after dosing. At the highest dose, the animals exhibited moderate to severe reduced activity, reduced muscle tone, hunched posture and prostration within half an hour after dosing. Rats at 1600 and 3200 mg/kg bw showed slight signs of reduced activity, ataxia and reduced muscle tone with complete recovery within 3 days (1600 mg) and 12 - 14 days (3200 mg). No treatment related effects on body weights were observed.
Pathology: No gross pathological findings were seen in any of the decedents and in rats surviving to day 14.

Conclusion:
Pyrimethanil (suspended in 1 % w/v methyl cellulose in distilled water) is of low acute toxicity in rats after oral administration. The LD_{50} was calculated to be 4149 (3341 – 5153) mg/kg bw in males and 5971 (4252 – 8386) mg/kg bw in females. Based on the results of this study, no classification is required according to the CLP (EC 1272/2009).

Report:	CA 5.2.1/4 [redacted] 1990 a Mouse acute oral study A81775
Guidelines:	OECD 401, US EPA (1984) Pesticide Assessment Guidelines. Subdiv. F - Hazard Evaluation: Human and domestic animals (Nov 1984 revised edition)
GLP:	yes (certified by Department of Health and Social Security of the Government of the United Kingdom, United Kingdom)
Deviations:	No (compared to OECD guideline 401 adopted 1987)

Material and Methods:
Groups of 5 mice/sex (strain: CRL:CD-1 [ICR]; source: [redacted].) weighing between 26 and 34 g were given a single dose of 0 (vehicle control), 1250, 2500 or 5000 mg/kg bw pyrimethanil (batch no. CR 19325/1; purity 98.4 %, suspended in 1 % w/v methyl cellulose in distilled water) by oral gavage. After administration, all animals were kept under observation for further 14 days. Body weights were recorded on the day of dosing and on days 7 and 14. Clinical observations were made daily. At termination, all surviving mice were subjected to gross post-mortem examination for external abnormalities and for abnormalities of the thoracic and abdominal viscera. Necropsies were also performed on those mice that had died during the study period.

Findings:
Clinical signs and mortality: At the highest dose, signs of systemic toxicity like reduced activity, reduced muscle tone and prostration were seen in animals with three males and two females being killed in extremis within 5 hours after administration. No deaths were seen at any other dose level. Mice of the two lower dose levels showed also signs of reduced activity (approx. 2 hours after dosing) with complete recovery within 1 day. Body weights were not adversely affected by treatment.
Pathology: Macroscopic post-mortem examination of the decedents and of mice surviving to day 14 showed no treatment-related effects.

Conclusion:
Pyrimethanil (suspended in 1 % w/v methyl cellulose in distilled water) is of low acute toxicity in mice after oral administration. The LD_{50} was calculated to be 4665 (3322 – 6552) mg/kg bw in males and 5359 (3816 – 7526) mg/kg bw in females. Based on the results of this study, no classification for acute oral toxicity is required according to the CLP (EC 1272/2009).

CA 5.2.2 Dermal

The acute dermal toxicity study of pyrimethanil was evaluated and peer reviewed during the previous Annex I inclusion process (for further details please see Table 5.2-1). Pyrimethanil has low acute dermal toxicity.

Report:	CA 5.2.2/1 [redacted] 1989 b Technical SN 100 309: Rat acute dermal toxicity study A81769
Guidelines:	OECD 402, US EPA (1984) Pesticide Assessment Guidelines. Subdiv. F - Hazard Evaluation: Human and domestic animals (Nov 1984 revised edition)
GLP:	yes (certified by Department of Health and Social Security of the Government of the United Kingdom, United Kingdom)
Deviations:	No (compared to OECD guideline 402 adopted 1987)

Report:	CA 5.2.2/2 [redacted] 1994 b 1st amendment to report No. TOX/89/223-4 - Technical SN 100309: Rat acute dermal toxicity study A81770
Guidelines:	OECD 402, US EPA (1984) Pesticide Assessment Guidelines. Subdiv. F - Hazard Evaluation: Human and domestic animals (Nov 1984 revised edition)
GLP:	yes

Material and Methods:
Groups of five male and five female Sprague-Dawley rats (source: [redacted].) weighing between 207 and 318 g received a topical application of pyrimethanil technical (batch no. CR 19325/1; purity 98.4 %; suspended in 1 % w/v methyl cellulose in distilled water) at a dose level of 0 and 5000 mg/kg bw at a dose volume of 10 mL/kg bw. Aluminium foil was applied to the shaved area of the dorsal region of the animals (6 x 10 cm) and held in place with a waterproof adhesive bandage. The test suspension was injected between the aluminium foil and the skin. After the 24-hour exposure period, bandage and foil were removed and the treated skin was washed with water and soap. Within a 14 days observation period, clinical signs were recorded once per day, body weight of the animals were recorded on days 0, 7 and 14. All animals were subjected to a macroscopic post mortem examination.

Findings:
There were no mortalities or signs of systemic toxicity observed during the 14-day study period. 2 males of the 5000 mg dose group showed very slight focal erythema at the test sites 1 day after treatment with complete recovery within 2 days. All animals gained normal weight during the study. There were no macroscopic pathological changes observed at necropsy.

Conclusions:
Pyrimethanil (suspended in 1 % w/v methyl cellulose in distilled water) is of low acute dermal toxicity in rats. The LD_{50} was greater than 5000 mg/kg bw for males and females. Based on the results of this study, no classification for acute dermal toxicity is required according to the CLP (EC 1272/2008).

CA 5.2.3 Inhalation

The acute inhalation toxicity study of pyrimethanil was evaluated and peer reviewed during the previous Annex I inclusion process (for further details please see Table 5.2-1). Pyrimethanil has low acute inhalation toxicity.

Report:	CA 5.2.3/1 [redacted] 1992 a Technical SN 100309: Rat acute (4-hour exposure) inhalation toxicity study A81820
Guidelines:	EPA 81-3, OECD 403, EEC Method B2
GLP:	yes (certified by Department of Health and Social Security of the Government of the United Kingdom, United Kingdom)
Deviations:	No (compared to OECD guideline 403 adopted 2009)

Report:	CA 5.2.3/2 [redacted] 1994 a 1st amendment to report No. TOX/92/223-48: Technical SN 100309: Rat acute (4-hour exposure) inhalation toxicity study A89514
Guidelines:	EPA 81-3, OECD 403, EEC Method B2
GLP:	yes

Material and Methods:
Five male and five female Sprague-Dawley rats (strain CRL:CD, source: [redacted]) weighing between 199 and 224 g were exposed for 4 hours via nose-only inhalation to a dust aerosol (dynamic conditions) of pyrimethanil technical (batch no. CR 19325/C1/900304; purity: 96.4 %) at an analytical concentration of 1.98 mg/L air (nominal concentration 12.7 mg/L), the highest attainable concentration. The mass median aerodynamic diameter (MMAD) was 8.2 µm with a geometric standard deviation of 3 µm. Analysis showed that 38 % of the particles were 6 µm or less and 71.2 % of the particles were 9.8 µm or less. An additional group of 5 males and 5 females were exposed to clean air for 4 hours. After exposure, the rats were kept under observation for 14 days. Body weights were estimated daily. At termination, all animals were subjected to a detailed macroscopic examination including estimation of lung weight.

Findings:
Clinical signs and mortality: There were no mortalities observed during the 14-day study period. During exposure soiling of the fur was observed in test and control animals. Post exposure, abnormal respiration was noted in animals treated with pyrimethanil during the first two days following treatment; recovery was complete in all animals by day 3 of the observation period. Slight reductions in body weight gain were recorded on the day following exposure procedure in all groups (control and test animals).
Pathology: At termination of the study, no macroscopic findings were noted at necropsy. In addition, lung to body weight ratios were not adversely affected by treatment.

Conclusion:
Based on the mortality results, the LC_{50} value (4 h exposure) for male and female rats is in excess of 1.98 mg/~~l~~L of air, the highest attainable concentration. No classification required.

CA 5.2.4 Skin irritation

The acute skin irritation study of pyrimethanil was evaluated and peer reviewed during the previous Annex I inclusion process (for further details please see Table 5.2-1). Pyrimethanil is not irritant to the skin.

Report:	CA 5.2.4/1 [redacted] 1989 c Technical SN 100 309: Rabbit skin irritancy study A81771
Guidelines:	OECD 404
GLP:	yes (certified by Department of Health and Social Security of the Government of the United Kingdom, United Kingdom)
Deviations:	Yes, animals were exposed simultaneously instead of a stepwise approach (compared to OECD guideline 404, adopted 2015)

Report:	CA 5.2.4/2 [redacted] 1994 c 1st amendment to report No. TOX/89/223-5 - Technical SN 100309: Rabbit skin irritancy study A81772
Guidelines:	OECD 404
GLP:	yes

Material and Methods:
Pyrimethanil (batch no. CR 19325/1; purity 98.4 %; 0.5 g a.i. moistened with 0.5 mL of distilled water) was applied to a 2.5 cm^2 gauze pad, and applied to a 10 cm x 10 cm area of the clipped dorso-lumbar region of 3 female New Zealand White rabbits (source: [redacted]) under a semi-occluded wrap (Elastoplast) for a 4-hour exposure period. Following the exposure period, the test sites were washed with water and soap to remove any remaining test material and dried. The skin sites were examined 30 to 60 minutes after treatment and then daily for 3 days.

Findings:
Clinical signs: Throughout the study period, the treated sites of all animals appeared normal; no oedema or erythema were noted. In addition, no signs of toxicity were observed. Pyrimethanil showed a primary irritation score of 0.00 when applied for 4 hours (semi-occluded) to intact rabbit skin.

Conclusion:
According to the results of the study, pyrimethanil is not irritant to the intact, shaved rabbit skin. No classification for dermal irritation is required according to the CLP (EC 1272/2008).

CA 5.2.5 Eye irritation

The acute eye irritation study of pyrimethanil was evaluated and peer reviewed during the previous Annex I inclusion process (for further details please see Table 5.2-1). Pyrimethanil is not irritant to the eye.

Report:	CA 5.2.5/1 [redacted] 1989 d Technical SN 100 309: Rabbit eye irritancy study A81773
Guidelines:	OECD 405
GLP:	yes (certified by Department of Health and Social Security of the Government of the United Kingdom, United Kingdom)
Deviations:	No (compared to OECD guideline 405 adopted 2012)

Report:	CA 5.2.5/2 [redacted] 1994 d 1st amendment to report No. TOX/89/223-6 - Technical SN 100309: Rabbit eye irritancy study A81774
Guidelines:	OECD 405
GLP:	yes

Material and Methods:
Approximately 100 mg of pyrimethanil (batch no. CR 19325/1; purity 98.4 %) were placed into the lower conjunctival sac of the left eye of 3 New Zealand White rabbits (source: [redacted]). The eyelids were held together for one second following instillation. The collateral eyes remained untreated and served as a control. The eyes were examined 1 hour after the instillation, and then 1, 2, 3 and 4 days thereafter. Grading and scoring of the ocular lesions were performed using the numerical scoring system given in the mentioned OECD guideline.

Findings:
Only one animal showed very slight conjunctival redness, one hour after instillation. The finding was completely reversible within 24 hours. The other 2 rabbits showed no signs of irritation. The overall mean scores from the 24-, 48-, and 72-hour observations for redness, chemosis, corneal opacity and iritis were 0.00, each.

Conclusion:
According to the results of the study, pyrimethanil technical is non-irritating to the rabbit eye. No classification for eye irritation is required according to the CLP (EC 1272/2008).

CA 5.2.6 Skin sensitisation

The skin sensitisation studies (Buehler Test and Maximisation Test) of pyrimethanil were evaluated and peer-reviewed during the previous Annex I inclusion process (for further details please see Table 5.2-1). Pyrimethanil is not sensitising.

Report:	CA 5.2.6/1 [redacted]., 1990 a SN 100 309: Skin sensitisation in guinea pig (BUEHLER test) A81778
Guidelines:	OECD 406, EEC 79/831 A V B 6
GLP:	yes (certified by Department of Health and Social Security of the Government of the United Kingdom, United Kingdom)
Deviations:	Yes, only 10 test animals have been used, positive control data are presented in a separate report (compared to OECD guideline 405 adopted 2012).

Report:	CA 5.2.6/2 [redacted] 1990 b T10, addendum #1: SN 100309: Skin sensitization in guinea pig - Positive control results A55313
Guidelines:	OECD 406, EPA 81-6, EEC 79/831 A V B 6
GLP:	yes (certified by Department of Health and Social Security of the Government of the United Kingdom, United Kingdom)

Material and Methods:
For induction, 10 female Dunkin/Hartley guinea pigs (source: [redacted].) received topical applications of 0.5 mL pyrimethanil (batch no. CR 19325/1, purity 99.7 %; 60% w/v suspensions in Alembicol „D“ [fractionated coconut oil]) on the clipped skin of the left shoulder region for 6 hours under occlusive dressing. Control animals were treated similarly with Alembicol „D“ alone. This concentration used for treatment in the main study was chosen on the basis of a preliminary study with concentrations of 10, 20, 40 and 60 % of the test material in Alembicol “D“ to determine the maximum non-irritant concentration. In the main study, induction treatments were made on the same day each week for further two weeks. Two weeks after the final induction application, topical challenge application with 0.5 mL pyrimethanil (60% w/v in Alembicol „D“) on the right (untreated) flank of the animals was carried out for 6 hours under occlusive dressing. The control animals were treated with vehicle alone. Readings for dermal changes were performed 24, 48 and 72 h after patch removal. Sensitivity of the strain has been assessed by the use of positive controls with 1-chloro-2,4-dinitrobenzene (induction 1 % in 70 % ethanol; challenge 0.025 and 0.05 %) (Addendum #1).

Findings:
In the preliminary test, no dermal reactions were seen in any of the animals, at any concentration. During induction phase of the main study, very slight dermal reactions (erythemas) were seen in 3 of the test animals mainly after the first application, which were not persistent and considered to be due to slight irritancy. After challenge application with pyrimethanil, there were no dermal reactions seen in any of the test and control animals.

Conclusion:
This study is considered as supplementary information only because of the low numbers of animals used. However, based on unequivocal results obtained, it can be concluded that pyrimethanil technical has shown no sensitizing properties in the guinea pig.

Report:	CA 5.2.6/3 [redacted], 1994 a Pyrimethanil (Code: SN 100309) - Guinea-pig skin sensitisation study A81848
Guidelines:	OECD 406, EEC 92/69 B 6, EPA Subdiv. F Ser. 81
GLP:	yes (certified by Groupe Interministeriel des Produits Chimiques, France)
Deviations:	No (compared to OECD guideline 405 adopted 2012).

Report:	CA 5.2.6/4 [redacted] 1996 a 1st amendment to report TOX/94/223-82: Technical SN 100309 - Guinea-pig skin sensitisation study (Magnusson and Kligman Maximisation assay) A89294
Guidelines:	OECD 406, EEC 92/69 B 6, EPA Subdiv. F Ser. 81
GLP:	yes (certified by Groupe Interministeriel des Produits Chimiques, France)

Report:	CA 5.2.6/5 [redacted] 1996 b 2nd amendment to report TOX/94/223-82: Technical SN 100309 - Guinea-pig skin sensitisation study (Magnusson and Kligman Maximisation assay) A89340
Guidelines:	OECD 406, EEC 92/69 B 6, EPA Subdiv. F Ser. 81
GLP:	yes (certified by Groupe Interministeriel des Produits Chimiques, France)

Material and Methods:
20 guinea pigs (10 males, 10 females; strain: Dunkin Hartley; source: [redacted]) were given pyrimethanil (batch no. CR 19325/1; purity 96.5 %) intradermally and topically. Additionally, 5 male and 5 female guinea pigs were used as control group. The concentrations used for the treatment in this study were based on the results of a preliminary study. In the main study, for intradermal induction (day 1) pyrimethanil was used as a 20 % (w/w) concentration in paraffin oil and as a 1:1 preparation of Freund's complete Adjuvant plus pyrimethanil 20 % in paraffin oil. These intradermal injections (0,1 mL) were applied at the dorsal scapular areas (both sides), which were clipped free of hair before application. Topical induction was carried out on day 8 using a concentration of 50 % pyrimethanil in paraffin oil (0.5 mL/animal). As the test substance has shown no irritating properties after occlusive cutaneous treatment in the preliminary test, the guinea pigs were pre-treated on day 7 with 0.5 mL of sodium lauryl sulfate (10%) in Vaseline. For cutaneous treatment, a patch was saturated with the test solution, placed over the injection sites of the test animals and held in place for 48 hours by an occlusive dressing. The control animals were treated according to the same scheme using only paraffin oil. For the challenge phase (two weeks after topical induction), pyrimethanil was administered topically in a concentration of 50 % in paraffin oil (0.5 mL) to the test animals and the control animals and applied on their right flanks by an occlusive dressing. On the left flanks 0.5 mL of the vehicle only was applied. The dressings were removed 24 hours later and skin reactions were quantified 24 and 48 hours after removal. The sensitivity of the animals was checked in an additional study with dinitro 2,4-chlorobenzene (1st amendment).

Findings:
In the preliminary study, signs of skin irritation were observed 24 und 48 hours after intradermal application of 20 % pyrimethanil in paraffin oil. After topical treatment with 50 % pyrimethanil in paraffin oil for 24 hours, no cutaneous reactions were observed. However, no further details of this preliminary study are given in the report.
In the main study, no mortalities or treatment-related clinical signs were observed. All animals gained weight during the study. On day 10 (one hour after removal of the dressing for topical induction), no necrosis or ulcera in control and test animals were observed at the treated sites. After challenge application, no dermal reactions were observed in either control or test animals (scores of zero).

Conclusion:
The test substance showed no skin sensitizing properties (contact allergenic potential) in guinea pigs under the test conditions used. No classification for skin sensitisation is required according to the CLP (EC 1272/2008)

CA 5.2.7 Phototoxicity

Report:	CA 5.2.7/1 Cetto V.,Landsiedel R., 2014a BAS 605 F (Pyrimethanil) - In vitro 3T3 NRU phototoxicity test 2013/1342919
Guidelines:	OECD 432 (2004) In vitro 3T3 NRU Phototoxicity test, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.41 No. L 142
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Deviations:	No (compared to OECD guideline 432 adopted 2004).

Executive Summary

Pyrimethanil (batch No. 0004268152; purity 99.7%) was tested for its ability to induce phototoxic effects in Balb/c 3T3 cells in vitro. The photo-cytotoxicity was estimated by the means of the Neutral Red Uptake (NRU) method. A pretest and two main experiments were carried out with and without irradiation with an UVA source. Vehicle and positive controls were included into the experiment.

Based on an initial range-finding phototoxicity test for the determination of the experimental concentrations, the following concentrations were tested in the first experiment with and without UVA irradiation: 4.6, 10.0, 21.5, 46.4, 100.0, 215.4, 464.2 and 1000 µg/mL. As result of the experiment a slightly increased cytotoxicity was observed in the presence of UVA irradiation. A Photo-Irritancy-Factor (PIF) of 2.1 was calculated. Therefore, a second experiment was conducted to confirm the probable phototoxic potency from the first experiment. In the second experiment the following concentrations were tested with and without UVA irradiation: 150, 200, 250, 300, 350, 400, 450 and 500 µg/mL. The results from the first experiment were not confirmed in the second experiment (PIF 1.0). Additionally, the calculated Mean Photo Effect (MPE) values of 0.096 and 0.003 in the first and second experiment, respectively, did not show phototoxic potential.

Furthermore, precipitation was observed in the absence and presence of UVA irradiation at 464.2 µg/mL and above in the first experiment and at 350 µg/mL and above in the second experiment. In addition, pyrimethanil was clearly cytotoxic with and without UVA irradiation at the highest concentrations.

The positive control chlorpromazine led to the expected increased cytotoxicity with UVA irradiation as indicated by PIF values of 60.1 and 22.8.

Based on the results of the present study and taking into account the classification rules pyrimethanil was predicted to have no phototoxic potential in the in vitro 3T3 NRU Phototoxicity Test using Balb/c 3T3 cells.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material Pyrimethanil (BAS 605 F)

Description:	Solid, white
Lot/Batch #:	0004268152
Purity:	99.7% (tolerance ± 1.0%)
Stability of test compound:	Expiry date: 31.12.2014
Solvent used:	Dimethylsulfoxide (DMSO)

2. Control Materials:

Vehicle control:	1% (v/v) DMSO in PBS
Positive control compounds:	Chlorpromazine (CPU) was dissolved in DMSO; 8 concentrations tested - 1.9 to 180 µg/mL without radiation, 0.03 to 3.2 µg/mL with radiation

3. Test organisms: BaIb/c 3T3, clone A31: fibroblast cell line isolated from the muscle tissue of a mouse embryo. The Balb/c 3T3 cell line which was used in this experiment was obtained from the "European Collection of Cell Cultures" Salisbury, Wiltshire SP4 OJG, UK on 09 Aug 2006 and is stored at -196°C (liquid nitrogen).

4. Culture media and reagents:

Culture medium:	Dulbecco's Modified Eagle's Medium (DMEM) supplemented with - 10% (v/v) newborn calf serum (NCBS) - 4 mM L-glutamine - 100 IU penicillin - 100 µg/mL streptomycin
Neutral Red solution:	- 0.4 g Neutral Red powder (NR; Sigma N4638) - 100 mL deionized water
Neutral Red medium:	- 1 mL Neutral Red solution - 79 mL culture medium (DMEM incl. supplements) (incubated overnight at 37° C with 5% CO_2 and filtered with a 0.22 µm filter prior to use)

Other solutions and reagents:
- phosphate buffered saline (PBS) without Ca/Mg
- trypsin/EDTA solution (0.05%; 0.02%)
- Neutral Red desorb solution
(1 mL acetic acid, 50 mL ethanol, 49 mL deionized water)

5. Irradiation source:

The Sol 500 solar simulator (Dr. Hönle AG, 82166 Gräfelfing, Germany) used with filter H1 produced wavelength > 320 nm. The exposure rates were determined with UV-meter RM-12 (Dr. Gröbel GmbH, 76275 Ettlingen, Germany).

6. Test concentrations:

Pretest:

Concentrations of 4.6 to 1000 µg/mL with and without irradiation were tested. The EC_{50} values determined were 331.7 µg/mL without and 160.6 µg/mL with UVA irradiation.

Main NRU test:

Based on the results of the pretest the following concentrations were used in the main study:

Experiment 1:

With and without UVA: 4.6, 10.0, 21.5, 46.4, 100.0, 215.4, 464.2, 1000 µg/mL

Experiment 2:

With and without UVA: 150, 200, 250, 300, 350, 400, 450, 500 µg/mL

B. TEST PERFORMANCE

1. Dates of experimental work: 05-Nov-2013 to 10-Feb-2014

2. Treatment and NRU Phototoxicity Test:

Two 96 well-plates per substance (test substance or positive control) were used for cultivation of cells (1.5 x 10^5 cells/well). After an attachment period of about 24 hours the cells were washed once with 100 µL PBS and subsequently treated with the respective substance (8 concentrations each with 6 replicates of the test substance or the positive control) and the vehicle control. After pre-incubation for 1 hour in the dark (5% (v/v) CO_2, ≥ 90% humidity; 37°C), one 96 well-plate per substance was irradiated for 50 minutes with UVA (UV intensity underneath the lid 1.5 - 2.1 mW/cm^2 = 5 J/cm^2) whereas the respective reference plate was kept in the dark for the same period. Thereafter the test-respectively control-substance was removed and the cells washed at least once with 100 µL PBS. After replenishing the wells with culture medium, the cells were incubated overnight under the conditions indicated above. The medium was removed 24 hours after the start of treatment and after washing with 100 µL PBS the wells were filled with 100 µL medium containing 50 µg/mL Neutral Red. Subsequently the plates were incubated for another 3 hours. Each step was performed under light protected conditions in the lab to prevent uncontrolled photo activation. Finally, the cells were washed again with 100 µL and the dye was extracted by 100 µL Neutral Red desorb solution. Cytotoxicity was determined by measuring the Neutral Red Uptake by means of a microplate reader (Perkin Elmer, Waltham, Massachusetts, US; Wallac 1420 multilabel counter) equipped with a 550 nm filter to read the absorption of the extracted dye. The absorption shows a linear relationship with the number of surviving cells.

3. Evaluation/Assessment

3.1 Cytotoxicity

The mean absorbance values obtained for each test group of every plate were used to calculate the percentage of cell viability relative to the respective vehicle control, which is arbitrarily set at 100 %.

$$Viability^{\S}\ [\%] = \frac{\text{Absorbance}_{\text{mean}}\ \text{of the test group}}{\text{Absorbance}_{\text{mean}}\ \text{of the vehicle control}} \times 100$$

§ The authors of the study denominate the above quotient as 'cytotoxicity', which is strictly speaking not correct. Thus, in this summary the appropriate term 'viability' is used. This applies also to Table 5.2.7-1 to Table 5.2.7-4.

In case of cytotoxicity, an EC_{50} value (concentration at which the viability is reduced by 50% relative to the respective vehicle control) was calculated by a linear interpolation method (linear dose-response curve). Therefore two viability values were needed: one between 100% and 50% and one between 50% and 0%. From these two points the concentration that inhibits the Neutral Red uptake by 50% of the respective control was calculated.

For the assessment of the phototoxic potential of a compound two prediction models are currently available:

- The Photo-Irritancy-Factor (PIF) prediction model for substances which allow the comparison of two equi-effective concentrations (EC_{50}) in the concurrently performed experiments in the presence and absence of light. This model includes two special cases: Case 1 accounts for situations in which an EC_{50} can only be calculated in the presence of UVA irradiation. Case 2 accounts for situations where an EC_{50} cannot be calculated in absence and presence of irradiation. These special cases do not apply to this study. Even though described in the report these cases are not described in this summary.
- The Mean Photo Effect prediction model which is used if no EC_{50} was obtained in the absence and presence of UV light.

3.2 Photo-Irritancy-Factor (PIF)

For substances which induce a 50 % cytotoxicity (EC_{50}) in the presence and absence of light the Photo-Irritancy-Factor (PIF) is calculated based on comparison of the EC_{50} values in the absence (-UVA) and presence (+UVA) of UVA irradiation.

$$PIF = \frac{EC_{50}\ (-UVA)}{EC_{50}\ (+UVA)}$$ resulting in the following classification rules:

PIF ≥ 5	phototoxic potential predicted
2 < PIF < 5:	probable phototoxic potential predicted
PIF ≤ 2:	no phototoxic potential predicted

3.3 Mean Photo Effect (MPE)

A major limitation of the Photo-Irritancy-Factor prediction model is the fact that the PIF is based on the comparison of two equi-effective concentrations (EC_{50}) in the concurrently performed experiments in the absence and presence of UV light, which cannot be determined in every case. To overcome this limitation, a novel measure for the phototoxic potential of chemicals, the Mean Photo Effect (MPE), has recently been proposed. It is based on comparison of the +UVA and -UVA concentration response curves on a grid of concentrations c_i (i = 1,…,N) chosen from the common concentration range of the (-UVA) and (+UVA) experiments. The photo effect (PE_i) at concentration c_i is calculated as the product of the concentration effect (CE_i) and the response effect (RE_i). The MPE is obtained by averaging across all PE_i values. Analogous to PIF, the MPE can be used in a prediction model for the phototoxic potential of chemicals by comparing it with a critical cut-off value, MPE_c. The cut-off value MPE_c = 0.1 was derived from a first application of the MPE-based prediction model to data obtained in phase II of the EU/COLIPA study in a test carried out by the FRAME/University of Nottingham laboratory according to the same test design, but with primary human keratinocytes instead of 3T3 cells:

The resulting classification rules are:

MPE ≥ 0.1:	phototoxic potential predicted
MPE < 0.1:	no phototoxic potential predicted

3.4 Other parameters

pH:
The pH was measured at least for the two top doses and for the vehicle controls with and without irradiation.

Osmolarity:
Osmolarity was measured at least for the two top doses and for the vehicle controls with and without irradiation.

Solubility:
Test substance precipitation was checked immediately after treatment and at the end of treatment.

Cell morphology:
Test cultures of all test groups were examined microscopically before staining with NRU, which allows conclusions to be drawn about attachment of the cells.

4. Statistics:

No special statistical tests were performed.
Mean absorbance values and standard deviations were calculated from the single values using calculation software (e.g. MS Excel). The calculations were made using the unedited values. For the report the values were rounded, therefore there may be deviations in the given relative values. If technical errors occurred in single wells (outlier) at least 4 single values per test group were sufficient for calculating reliable mean values. Outliers are defined as values that have half or double the value of the respective mean.

5. Acceptance criteria:

The assay has to be considered valid if the following criteria are met:

- The vehicle control needs to fulfill the following criteria:
  - The mean OD550 value (with and without UVA irradiation) should be > 0.3.
  - Cell viability after irradiation should be at least 80% of the concurrent non-irradiated vehicle control.
  - The standard deviation of the mean values of both vehicle control rows should not exceed ± 15%.
- The positive control chlorpromazine needs to fulfill the following criteria:
  - the EC_{50} value should be in the ranges:
    With irradiation (+UVA): 0.1 - 2.0 µg/mL
    Without irradiation (-UVA): 7.0 - 90.0 µg/mL
  - and the PIF ≥ 6.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Osmolarity and pH values were not influenced by test substance treatment. In this study, in the absence and the presence of UVA irradiation test substance precipitation in culture medium was observed at 464.2 µg/mL and above in the first experiment and at 350 µg/mL in the second experiment.
In addition, changes in cell morphology were observed at the end of exposure period at 464.2 µg/mL and above without irradiation and from 215.4 µg/mL onward with irradiation in the first experiment. There were changes in the morphology from 400 µg/mL onward with and without irradiation in the second experiment.

B. CYTOTOXICITY OF THE TEST SUBSTANCE

After treatment with pyrimethanil clear cytotoxic effects indicated by Neutral Red absorbance values below 50% of the control were observed in the first experiment and second experiment in the absence and presence of UVA irradiation at least in the highest applied concentrations.

In detail, without UVA irradiation, there was a decrease in the cell number from 464.2 µg/mL (EC_{50}: 343.5 µg/mL) onward in the first experiment and from 400.0 µg/mL (EC_{50}: 365.4 µg/mL) onward in the second experiment [see Table 5.2.7-1 and Table 5.2.7-2].

In addition, with UVA irradiation, there was a decrease in the cell number at 215.4 µg/mL (EC_{50}: 166.7 µg/mL) and above in the first experiment and at 400.0 µg/mL (EC_{50}: 367.3 µg/mL) and above in the second experiment [see Table 5.2.7-1 and Table 5.2.7-2].

Table 5.2.7-1: Mean relative viability of pyrimethanil with (+) and without (-) UVA irradiation in Balb 3T3 cells (Experiment 1)

Test group	UVA irradiation[2]	Precipitation[3]	Mean $OD_{corr.}$[4]	Relative viability [% of control]	
				Mean	SD
Vehicle control 1	-	-	0.357	-	3.8
Vehicle control 2	-	-	0.418	-	5.5
Vehicle mean[1]	-	-	0.387	100.0	9.4
Pyrimethanil					
4.6 µg/mL	-	-	0.405	104.5	3.8
10.0 µg/mL	-	-	0.410	106.0	1.1
21.5 µg/mL	-	-	0.410	105.8	3.4
46.4 µg/mL	-	-	0.421	108.6	3.3
100.0 µg/mL	-	-	0.420	108.4	3.2
215.4 µg/mL	-	-	0.398	102.8	3.0
464.2 µg/mL	-	+	0.001	0.3	0.2
1000.0 µg/mL	-	+	0.001	0.2	0.2
Vehicle control 1	+	-	0.333	-	3.7
Vehicle control 2	+	-	0.383	-	2.3
Vehicle mean[1]	+	-	0.358	100	7.8
Pyrimethanil					
4.6 µg/mL	+	-	0.347	97.0	1.7
10.0 µg/mL	+	-	0.366	102.3	2.9
21.5 µg/mL	+	-	0.363	101.4	4.9
46.4 µg/mL	+	-	0.377	105.4	4.2
100.0 µg/mL	+	-	0.356	99.4	6.3
215.4 µg/mL	+	-	0.050	13.9	8.6
464.2 µg/mL	+	+	0.002	0.5	0.2
1000.0 µg/mL	+	+	0.004	1.1	1.2

[1] 1% (v/v) DMSO
[2] Irradiation with Sol 500 solar simulator for 50 minutes (approx. 5 J/cm²)
[3] Precipitation in PBS at the end of exposure period
[4] Mean OD corrected: mean absorbance (test group) minus mean absorbance (blank)

Table 5.2.7-2: Mean relative viability of pyrimethanil with (+) and without (-) UVA irradiation in Balb 3T3 cells (Experiment 2)

Test group	UVA irradiation[2]	Precipitation[3]	Mean $OD_{corr.}$[4]	Relative viability [% of control]	
				Mean	SD
Vehicle control 1	-	-	0.437	-	5.8
Vehicle control 2	-	-	0.412	-	3.8
Vehicle mean[1]	-	-	0.425	100	5.6
Pyrimethanil					
150 µg/mL	-	-	0.374	88.0	10.6
200 µg/mL	-	-	0.386	90.8	7.0
250 µg/mL	-	-	0.347	81.6	7.7
300 µg/mL	-	-	0.318	4.8	12.4
350 µg/mL	-	+	0.285	67.0	11.3
400 µg/mL	-	+	0.049	11.6	13.1
450 µg/mL	-	+	0.005	1.1	0.8
500 µg/mL	-	+	0.034	8.0	4.0
Vehicle control 1	+	-	0.430	-	2.7
Vehicle control 2	+	-	0.429	-	2.9
Vehicle mean[1]	+	-	0.429	100	2.6
Pyrimethanil					
150 µg/mL	+	-	0.386	90.0	3.6
200 µg/mL	+	-	0.359	83.7	3.6
250 µg/mL	+	-	0.268	62.4	15.3
300 µg/mL	+	-	0.339	78.9	10.1
350 µg/mL	+	+	0.306	71.4	5.0
400 µg/mL	+	+	0.042	9.8	5.4
450 µg/mL	+	+	0.009	2.1	2.6
500 µg/mL	+	+	0.041	9.6	4.4

[1] 1% (v/v) DMSO
[2] Irradiation with Sol 500 solar simulator for 50 minutes (approx. 5 J/cm²)
[3] Precipitation in PBS at the end of exposure period
[4] Mean OD corrected: mean absorbance (test group) minus mean absorbance (blank)

Based on the EC_{50} values a PIF of 2.1 (probable phototoxic potential) was obtained in the first experiment and a PIF of 1 (no phototoxic potential) was obtained in the second experiment.

In addition, the Mean Photo Effect (MPE) for all experiments was detected. In the first experiment a MPE=0.096 (no phototoxic potential) and in the second experiment MPE=0.003 (no phototoxic potential) was obtained.

C. CYTOTOXICITY OF THE POSITIVE CONTROL

After treatment with the positive control chlorpromazine, clear cytotoxic effects indicated by Neutral Red absorbance values below 50% of the control were observed in the first experiment and second experiment in the absence and the presence of UVA irradiation at least in the highest applied concentrations.

In detail, without UVA irradiation, there was a decrease in the cell number from 60 µg/mL (EC_{50}: 35.4 µg/mL) onward in the first experiment and from 30 µg/mL (EC_{50}: 22.6 µg/mL) onward in the second experiment [see Table 5.2.7-3 and Table 5.2.7-4].

In addition, with UVA irradiation, there was a decrease in the cell number at 0.8 µg/mL (EC_{50}: 0.6 µg/mL) and above in the first experiment and from 1.6 µg/mL (EC_{50}: 1.0 µg/mL) onward in the second experiment [see Table 5.2.7-3 and Table 5.2.7-4].

Table 5.2.7-3: Mean relative viability of chlorpromazine with (+) and without (-) UVA irradiation in Balb/c 3T3 cells (Experiment 1)

Test group	UVA irradiation	Mean OD [2]	Mean $OD_{corr.}$ [3]	Relative viability [% of control]	
				Mean	SD
Vehicle control 1	-	0.419	0.384	-	4.1
Vehicle control 2	-	0.424	0.389	-	1.6
Vehicle mean[1]	-	0.422	0.386	100	3.0
Chlorpromazine					
1.9 µg/mL	-	0.433	0.397	102.8	3.7
3.8 µg/mL	-	0.456	0.421	108.9	3.3
7.5 µg/mL	-	0.455	0.419	108.6	3.0
15.0 µg/mL	-	0.436	0.400	103.6	5.6
30.0 µg/mL	-	0.270	0.234	60.7	35.8
60.0 µg/mL	-	0.040	0.004	1.1	2.8
90.0 µg/mL	-	0.037	0.001	0.2	0.6
180.0 µg/mL	-	0.037	0.001	0.2	0.2
Vehicle control 1	+	0.376	0.341	-	1.4
Vehicle control 2	+	0.384	0.348	-	1.7
Vehicle mean[1]	+	0.380	0.345	100	1.9
Chlorpromazine					
0.03 µg/mL	+	0.365	0.329	95.6	2.3
0.05 µg/mL	+	0.380	0.345	100.0	2.7
0.10 µg/mL	+	0.372	0.337	97.8	2.7
0.20 µg/mL	+	0.366	0.331	96.0	3.6
0.40 µg/mL	+	0.311	0.276	80.1	5.9
0.80 µg/mL	+	0.092	0.056	16.4	8.6
1.60 µg/mL	+	0.035	0.000	0.1	0.1
3.20 µg/mL	+	0.039	0.004	1.1	0.2

[1] 1% (v/v) DMSO
[2] Mean absorbance at 550 nm of 6 wells, in general
[3] Mean absorbance (test group) minus mean absorbance (blank)

Table 5.2.7-4: Mean relative viability of chlorpromazine with (+) and without (-) UVA irradiation in Balb/c 3T3 cells (Experiment 2)

Test group	UVA irradiation	Mean OD [2]	Mean $OD_{corr.}$ [3]	Relative viability [% of control]	
				Mean	SD
Vehicle control 1	-	0.481	0.046	-	7.1
Vehicle control 2	-	0.491	0.456	-	6.0
Vehicle mean[1]	-	0.486	0.451	100.0	6.4
Chlorpromazine					
1.9 µg/mL	-	0.480	0.444	98.6	3.3
3.8 µg/mL	-	0.491	0.455	101.0	3.8
7.5 µg/mL	-	0.484	0.448	99.5	8.5
15.0 µg/mL	-	0.402	0.366	81.3	4.0
30.0 µg/mL	-	0.122	0.087	19.2	3.8
60.0 µg/mL	-	0.036	0.000	0.1	0.1
90.0 µg/mL	-	0.036	0.001	0.1	0.1
180.0 µg/mL	-	0.037	0.002	0.4	0.1
Vehicle control 1	+	0.509	0.470	-	6.8
Vehicle control 2	+	0.503	0.464	-	5.2
Vehicle mean[1]	+	0.506	0.467	100.0	5.9
Chlorpromazine					
0.03 µg/mL	+	0.509	0.470	100.6	8.5
0.05 µg/mL	+	0.534	0.495	106.0	7.5
0.10 µg/mL	+	0.515	0.476	102.0	5.9
0.20 µg/mL	+	0.498	0.459	98.2	2.8
0.40 µg/mL	+	0.491	0.452	96.7	8.0
0.80 µg/mL	+	0.345	0.306	65.4	12.1
1.60 µg/mL	+	0.041	0.002	0.5	0.6
3.20 µg/mL	+	0.043	0.004	0.8	0.9

[1] 1% (v/v) DMSO
[2] Mean absorbance at 550 nm of 6 wells, in general
[3] Mean absorbance (test group) minus mean absorbance (blank)

Based on the EC_{50} values, a PIF of 60.1 and 22.8 was obtained in the first and second experiment, respectively. Thus, the phototoxic potential of the positive control groups were demonstrated.

III. CONCLUSIONS

According to the results of the present study, pyrimethanil is not considered to be a phototoxic substance in the in vitro 3T3 NRU Phototoxicity Test.

CA 5.3 Short-Term Toxicity

Studies evaluated in the draft monograph of Rapporteur Member State Austria of April 15, 2004:

Short-term toxicity studies (up to 90 days) with oral administration in three different species (rats, mice, dogs) and a 1-year dog study have been evaluated by European authorities and Austria as Rapporteur Member State and were considered to be acceptable. For the convenience of the reviewer, these studies are summarized below as extracted from the monograph and the EFSA conclusion. A tabulated summary is provided in Table 5.3-1.
In order to align the dossier submission and the draft RAR, the information that was present in the dRAR in more detail was included in this dossier update in light green, whereas new information and/or corrections were included in lime green.

Table 5.3-1: Summary of short-term toxicity studies conducted with pyrimethanil available in the original mongraph

Study	Dosages (mg/kg bw/ day)	NOAEL (mg/kg bw/day)	Main adverse effect	Reference (BASF DocID)
10-day gavage Sprague-Dawley rat	M&F: 0, 10, 100 and 1000 mg/kg bw/day suspended in 1% aqueous methyl cellulose	Not established due to limited investigations performed	Transient body weight gain reduction in males, increased plasma cholesterol (both sexes), colloid depletion and follicular epithelial hyperplasia in thyroid (both sexes)	A81776, A81777
4-week feeding Sprague-Dawley rat	M: 844, 1135 and 2434 F: 844, 1187 and 2967 [0, 10000, 15000, 30000 ppm]	Cannot be determined	Effects on body weight, food consumption, haematology, clinical chemistry, organ weights, gross pathology and histopathology.	A81779
13-week feeding Sprague-Dawley rat	M: 5.4, 54.5 and 529.1 F: 6.8, 66.7 and 625.9 [0, 80, 800, 8000 ppm]	~~5.4~~ 54 (M) ~~6.8~~ 68 (F) [800 ppm]	Reduced body weight (gain) and food consumption, haematological changes, liver hypertrophy and changes in thyroid pathology.	A81783, C023548, A81784, A81785, 2003/1023036
4-week feeding CD-1 mouse	M: 167, 567 and 1960 F: 236, 667 and 2357 [0, 1000, 3000, 10000 ppm]	167 (M) 236 (F) [1000 ppm]	Reduced body weight (gain), increased liver and adrenal weights, clinical-chemical changes and histopathological findings in thyroid, kidneys and urinary bladder.	A81781
13-week feeding CD-1 mouse	M: 12, 139 and 1864 F: 18, 203 and 2545 [0, 80, 900, 10000 ppm]	139 (M) 203 (F) [900 ppm]	Reduced body weight (gain), increased liver weights and histopathological changes in thyroid, kidneys and urinary bladder.	A81792
13 days, gavage Beagle dog	Phase 1: M&F: Increasing dose levels from 10 to 2000 mg/kg bw Phase 2: M: 1200 (800) F: 1200	Cannot be determined	Reduced body weight and vomitus.	A81763

Table 5.3-1: Summary of short-term toxicity studies conducted with pyrimethanil available in the original mongraph

Study	Dosages (mg/kg bw/ day)	NOAEL (mg/kg bw/day)	Main adverse effect	Reference (BASF DocID)
4 weeks, gavage Beagle dog	M&F: 100, 500 and 1000/800	100 (M&F)	Reduced food consumption in females and vomitus in both sexes.	A81764
13 weeks, gavage Beagle dog	M&F: 6, 80 and 1000/800	6 (M&F)	Reduced body weight (gain) and food consumption, vomitus and other clinical signs, clinical-chemical changes and decreased water intake.	A81790
1-year, gavage Beagle dog	M&F: 2, 30 and 400/250	30 (M&F)	Reduced body weight (gain), vomitus and diarrhoea, haematological changes and decreased water intake.	A81809

Short-term toxicity of pyrimethanil was studied in dietary up to 90-days (13 weeks) studies in rats and mice, and up to 90-days (13 weeks) and 1-year studies in dogs.

In the rat study, the main target organs were liver (increase of weight, hypertrophy) and thyroid (follicular epithelial hypertrophy and colloid depletion). In mice, relevant findings concerned the liver (clinical chemistry findings, increased organ weight), thyroid (necrosis of follicular epithelial cells), kidneys (tubular dilatation) and urinary bladder (hyperplasia of the epithelium, uroliths). In dogs, relevant findings comprised clinical signs, retardation of body weight gain and some minor effects in haematological and biochemical parameters. In addition, in both dog studies a dose-related marked decrease in water intake was observed, which was considered as an adverse effect.

The relevant NOAEL is ~~5.4~~ 30 mg/kg bw/day from the ~~90-days rat~~ 1-year dog study.

Studies submitted in this AIR 3 dossier (not yet peer-reviewed):
The only new study to consider whether it could affect the short-term relevant NOAEL/NOEL would be the 90-day neurotoxicity study in rats as presented in section CA 5.7. However, the determined study NOAEL of 38.7 mg/kg bw/day in male rats was clearly above the derived ~~lowest~~ relevant NOAEL of the ~~90-day rat~~ 1-year dog study of 30 mg/kg bw/day and no additional targets/critical effects were determined.

Based on the available data on the short-term toxicity, classification of pyrimethanil for this endpoint is not required according to the criteria laid down in Regulation (EU) No. 1272/2008 (CLP). Moreover, the data on short-term toxicity of pyrimethanil have already been evaluated in 2006, coming to the conclusion that pyrimethanil does not need to be classified for health effects (ECBI/159/04) as laid down in the 31st ATP to the Council Directive 67/548/EEC (Commission Directive 2009/2/EC of 15 January 2009). The not yet peer-reviewed 90-day neurotoxicity study as presented in section CA 5.7 has not been submitted for evaluation of classification. The results of this studies will however not change the classification and labelling of pyrimethanil.

Thus, the conclusion for relevant endpoints adopted to the new list of endpoint format for the current re-registration is as follows:

Short-term toxicity (Regulation (EU) N° 283/2013, Annex Part A, point 5.3)

Target organ / critical effect	Rat: Reduced body weight and retarded body weight gain, proteinuria, histological findings in liver and thyroid. Mouse: Reduced body weight and retarded weight gain, liver (hypertrophy), histological findings in thyroid, kidneys and urinary bladder. Dog: Clinical signs (vomiting), retarded body weight gain, decreased water consumption (both sexes) and hematological changes	
Relevant oral NOAEL	90-day, rat: ~~5.4~~ 54 mg/kg bw/day 90-day, mouse: 139 mg/kg bw/day 90-day dog: ~~30~~ 6 mg/kg bw/day 1-year dog: ~~6~~ 30 mg/kg bw/day Overall NOAEL dog: 30 mg/kg bw per day	
Relevant dermal NOAEL	No data - not required	
Relevant inhalation NOAEL	No data - not required	

CA 5.3.1 Oral 28-day study

Pyrimethanil, rat 10-day gavage repeated dose study [redacted] 1992)
(BASF DocID A81776 and BASF DocID A81777)

Guidelines:	According to OECD guideline 408 (1981) and US EPA Pesticide Assessment Guidelines, Subdivision F
Deviations:	Major deviations: small number animals per dose (3 per sex); organ weights not recorded, limited histopathology (liver and thyroid only)
GLP:	Yes
Acceptance:	Limited validity, considered only as supplementary information

Pyrimethanil (batch no. CR 19325/1; purity 99.7 %; suspended in 1 % w/v methyl cellulose in distilled water) was administered to groups of 3 male and 3 female Sprague Dawley rats (CR1:COBS CD BR; source: [redacted].) for 10 consecutive days at dose levels of 0 (vehicle control), 10, 100 or 1000 mg/kg bw/d by gavage.
No mortalities and no signs of toxicity were observed at any dose level. Transient 10% reduction in body weight gain was recorded in males of the highest dose group. All other animals were unaffected in body weight. Clinical chemistry analyses revealed a slight and moderate increase of the plasma cholesterol level in males and females of the highest dose group, respectively. Histopathological examination of the thyroids revealed a test item induced colloid depletion and follicular cell hypertrophy in animals of both sexes of the highest dose group. Other organs were found unaffected by the treatment.
Taking into account the limitations of this study, a scientifically derived NOAEL cannot be determined. However, it was concluded in the report, that 1000 mg pyrimethanil/kg bw by gavage was tolerated by the rat when administered daily for 10 consecutive days.

~~**Pyrimethanil, rat 28-day dietary and gavage repeated dose study ([redacted] 1991) (BASF DocID A81779)**~~

~~**Guidelines:** According to OECD guideline 408 (1981) and US EPA Pesticide Assessment Guidelines, Subdivision F~~
~~**Deviations:**~~
~~**GLP:** Yes~~
~~**Acceptance:** Considering the dose levels selected, the study is regarded only as supplementary information.~~

Report:	CA 5.3.1/1 [redacted] 1991 a Technical SN 100 309: 28 day dietary and gavage repeated dose study in rats A81779
Guidelines:	OECD 408, JMAFF, US EPA (1984) Pesticide Assessment Guidelines. Subdiv. F - Hazard Evaluation: Human and domestic animals (Nov 1984 revised edition)
GLP:	yes (certified by Department of Health and Social Security of the Government of the United Kingdom, United Kingdom)
Deviations:	Yes, detailed clinical observations once a week, sensory reactivity to stimuli of different types and functional observation battery were not conducted. Weight determination of prostate and seminal vesicles with coagulating glands were not performed. Histopathology was not conducted for different tissues, such as urinary bladder, lymph nodes, peripheral nerve, skeletal muscle, bone and bone marrow (compared to OECD guideline 407 adopted 3 October 2008).

Executive summary

Pyrimethanil (batch no. CR 19325/1; purity 99.2%) was administered to groups of 5 male and 5 female Sprague Dawley rats (source [redacted]) either via the diet at concentrations of 0, 10000, 15000 and 30000 ppm (equivalent to 844, 1135 and 2434 mg/kg bw/day in males and 844, 1187 and 2967 mg/kg bw/day in females) for 28 days or by gavage at a dose level of 1500 mg/kg bw/day for 11 days followed by 1000 mg/kg bw/day for the remaining 17 days of the study.

Concerning clinical signs, most animals treated via gavage and the majority of animals given 15000 and 30000 ppm pyrimethanil via diet showed clinical signs. Treatment related effect on body weight development and food consumption was noticed after dietary as well as gavage administration. Reduced water intake was noted after dietary administration. Clinical chemistry analyses revealed a spectrum of significant treatment-related disturbances in several parameters and hematology gave indication of anemia both after dietary as well as gavage administration. Kidney, liver and thyroid were assessed as being target organs. After gavage administration, inflammation of the upper gastrointestinal tract was noticed.
Taking into account the dosing regime and the findings of this study, a NOAEL cannot be determined. However, it was concluded in the report, that 10000 ppm pyrimethanil in the diet exceeded the maximum tolerated dose and was considered unsuitable for subchronic administration.

Material and Methods:
Pyrimethanil (batch no. CR 19325/1; purity 99.2 %) was administered to groups of 5 male and 5 female Sprague Dawley rats (source [redacted]) either via the diet at concentrations of 0, 10000 (equiv. to 844 mg/kg bw/d [♂,♀]), 15000 (equiv. to 1135 [♂] and 1187 [♀] mg/kg bw/d) and 30000 ppm (equiv. to 2434 [♂] and 2967 [♀] mg/kg bw/d) for 28 days or by gavage at a dose level of 1500 mg/kg bw/d for 11 days followed by 1000 mg/kg bw/d for the remaining 17 days of the study.
Animals were observed daily for clinical signs and mortalities. Individual body weights were recorded twice weekly during treatment, food consumption was measured weekly and water intake for a four-days period during week 3 of treatment. Ophthalmological examinations were performed prior to treatment and on day 28 of treatment.
Blood samples for clinical chemistry (total protein, albumin, globulin, calcium, phosphate, sodium, potassium, urea, creatinin, glucose, cholesterol, bilirubin, chloride, carbon dioxide, anion gap, AST, ALT, AP, GGT, CPK) and haematology (haematocrit, haemoglobin, RBC, reticulocyte count, MCHC, MCV, MCH, WBC total, WBC differential, platelets count) were taken on day 29 of treatment. At necropsy, all animals were examined macroscopically and the weights of selected organs (adrenals, brain, heart, kidneys, liver, pituitary, spleen, ovaries, testes) were recorded. Following organs/tissues from all animals were examined for histopathological changes: adrenals, brain, caecum, colon, duodenum, epididymites, heart, ileum, jejunum, kidneys, liver, lungs, oesophagus, ovaries, pancreas, pituitary, spleen, stomach, testes, thyroid with parathyroid, any other tissue showing macroscopic abnormalities.

Findings:
General observation: Two females treated with 1500/1000 mg/kg bw by gavage died or were killed in extremis on days 7 and 9, resp. and one male and one female were killed in extremis on day 15. (Necropsy of these animals showed evidence of dosing accidents). No mortalities occurred in animals treated via the diet. Concerning clinical signs, most animals treated via gavage and the majority of animals given 15000 and 30000 ppm pyrimethanil via diet showed reduced activity, hunched posture, urogenital soiling and emaciation. The onset of the majority of clinical signs was between days 7 and 17 of dosing and persisted until the end of treatment. No treatment-related signs were observed in animals treated with 10000 ppm.
There was a dose-related loss of body weight in all groups and both sexes treated via the diet during the first four days of treatment, which persisted until study termination in rats treated with 30000 ppm only. There was also a marked dose-related reduction of body weight gain in all dietary treatment groups (reported to be in a range of 49 – 121 % in males and 15 – 64 % in females) compared to controls. Rats treated with 1500/1000 mg/kg by gavage showed also a marked reduction in body weight gain (25 % in males and 10 % in females).
Food consumption was reduced in all dietary treatment groups (ranging from 25 – 56 % in males and 19 – 33 % in females) and also in the group dosed by gavage (14 % in males and 10 % in females) compared to control values.
Concerning water intake, there was a marked reduction in males seen at 30000 ppm (group mean value 12 mL/animal/day [52 %]) and 10000 ppm (group mean value 19.7 mL/animal/day [21 %]) and to a lesser extent in females at 30000 ppm (group mean value 17.8 mL/animal/day [15 %]) compared to control animals (24.8 mL/animal/day in males and 21.0 mL/animal/day in females). There were no effects in water intake in both sexes at 15000 ppm or 1500/1000 mg/kg bw.

Clinical chemistry analyses revealed a spectrum of significant treatment-related disturbances in several parameters: Following treatment with 30000 ppm, significant increases in urea, bilirubin, ALT (males only) and GGT, and significant decreases in total protein, albumin, globulin, AP and glucose (females only) were recorded. At 15000 ppm, there were significant increases in GGT, cholesterol (males only), alkaline phosphatase (males only), phosphate (females only), bilirubin (females only) and decreased glucose and creatine kinase (females only). Following treatment with 10000 ppm, there was a significant increase in cholesterol in both sexes compared to controls. Rats treated with 1500/1000 mg/kg by gavage showed increased levels of potassium, ALT (males only) and cholesterol, and a decrease in glucose in females only. Haematological examinations revealed significant reductions in MCV and MCH in all dietary treatment groups compared to controls. Following treatment with 1500/1000 mg/kg by gavage, there were significant decreases in haemoglobin, MCV and MCH.
Organ weight analysis showed a significant treatment-related reduction in absolute kidney weight at 15000 and 30000 ppm, and a significant increase in relative liver weight (evident in all treated groups).
Pathological/histopathological findings comprised treatment-related effects in the kidneys (increased luminal diameter, degenerative changes to basophilic tubules, tubular necrosis, karyomegaly in tubular epithelium and inflammatory infiltration; seen in diet-dosed and gavage-dosed animals), liver (accentuated lobular pattern; in diet-dosed and gavage-dosed animals), oesophagus (acute inflammation of the muscularis; in rats treated by gavage), stomach (epithelial hyperplasia and mucosal ulceration in animals treated by gavage) and thyroid (colloid depletion follicular epithelial hypertrophy and hyperplasia in animals treated by gavage). In rats treated via diet, the effects described were predominantly recorded at 30000 ppm though some findings were also evident at 10000 and 15000 ppm.

Conclusion:
Taking into account the dosing regime and the findings of this study, a NOAEL cannot be determined. However, it was concluded in the report, that 10000 ppm pyrimethanil in the diet exceeded the maximum tolerated dose and was considered unsuitable for subchronic administration.

~~**Pyrimethanil, mouse 28-day dietary repeated dose study ([redacted], 1991)**~~
~~**(BASF DocID A81781)**~~

~~**Guidelines:** According to OECD guideline 451 and US EPA Pesticide Assessment Guidelines, Subdivision F~~
~~**Deviations:** Deviations (small number of animals per dose group and limited histopathology) are noted.~~
~~**GLP:** Yes~~
~~**Acceptance:** Due to deviations, the study is regarded as supplementary information only.~~

Report:	CA 5.3.1/2 [redacted] 1991 b Technical SN 100 309: Mouse 28-day dietary repeat dose study A81781
Guidelines:	OECD 408, US EPA (1984) Pesticide Assessment Guidelines. Subdiv. F - Hazard Evaluation: Human and domestic animals (Nov 1984 revised edition), JMAFF
GLP:	yes (certified by Department of Health and Social Security of the Government of the United Kingdom, United Kingdom)
Deviations:	Yes, small number of animals per dose group, limited histopathology, no detailed clinical observations as well as sensory reactivity to stimuli of different types should be performed and functional observation battery (compared to OECD guideline 407 (adopted 2008).

Executive summary

Groups of 5 male and 5 female mice (strain: CD-1; source: [redacted].) were fed diet containing 0, 1000, 3000, 10000 or 30000 ppm pyrimethanil (batch no. CR 19325/2; purity 95.3%), equivalent to 0, 167, 567 and 1960 mg/kg bw/day in males and 0, 236, 667 and 2357 mg/kg bw/day in females, respectively (mg/kg-equivalents of mice treated at 30000 ppm not given) for 28 days.

In the report, the NOAEL for dietary administration of pyrimethanil to CD-1 mice for 28 days was set at 1000 ppm (equivalent to 167 and 236 mg/kg bw/day for males and females, respectively) based on retarded body weight gain, increased liver weight and pathological/histopathological findings in thyroid, kidneys and urinary bladder. However, taking into account the limited histopathological examinations, the results of the study should be considered as supplementary information only.

Material and Methods:
Groups of 5 male and 5 female mice (strain: CD-1; source: [redacted] were fed diet containing 0, 1000, 3000, 10000 or 30000 ppm pyrimethanil (batch no. CR 19325/2; purity 95.3 %), equivalent to 0, 167, 567 and 1960 mg/kg bw/d in males and 0, 236, 667 and 2357 mg/kg bw/d in females, resp. (mg/kg-equivalents of mice treated at 30000 ppm not given) for 28 days. Diets were prepared weekly; stability and homogeneity was confirmed by analysis.
Animals were observed daily for clinical signs. Body weight and food consumption were measured weekly. Haematology (haematocrit, haemoglobin, RBC, MCHC, MCV, MCH, WBC total, WBC differential, platelets count) and clinical chemistry analysis (total protein, albumin, globulin, calcium, phosphate, sodium, potassium, urea, creatinin, glucose, cholesterol, bilirubin, chloride, AST, ALT, AP, GGT, CPK) were carried out in blood samples collected at the end of treatment.
At necropsy, all animals were examined macroscopically, the weights of selected organs (liver, heart, brain, adrenals, spleen, kidneys, testes and ovaries) were recorded and subsequently histopathological examinations were performed on liver, kidneys, thyroid and urinary bladder.

Findings:
General observations: All animals treated with 30000 ppm died within the first week of treatment. Before death, clinical signs like reduced activity, ataxia, hypothermia and prostration were associated with severe emaciation.
At the next lower dose level (10000 ppm), slight emaciation was observed in females together with a slight actual body weight loss, and a reduced body weight gain in males during the first two weeks of treatment. Overall, there was a reduction of the body weight gain in both sexes (12 % in males, 11 % in females) compared to control animals. At 3000 ppm, there was also a slight reduction in body weight gain in both sexes (3 % in males, 4 % in females) during the first week of treatment (Table 5.3.1-1).

Table 5.3.1-1: 28-day dietary repeat dose study in mice; group mean body weight and body weight change

	0 ppm ♂	0 ppm ♀	1000 ppm ♂	1000 ppm ♀	3000 ppm ♂	3000 ppm ♀	10000 ppm ♂	10000 ppm ♀
Group mean body weigh (g)								
day 1	26.8	21.8	28.2	21.7	25.9	21.8	26.5	21.7
day 15	31.1	24.3	30.8	23.0	30.0	24.0	27.5	19.7*
day 29	33.8	26.2	32.6	25.8	32.4	25.4	30.3	23.7
Group mean body weight change (g/animal/day)								
day 1 – 8	+ 0.35	+ 0.25	+ 0.24	+ 0.21	+ 0.24	+ 0.14	+ 0.11*	- 0.05*
day 8 – 15	+ 0.36	+ 0.10	+ 0.13	- 0.01	+ 0.35	+ 0.17	+ 0.03*	- 0.23*
day 15 – 22	+ 0.18	+ 0.10	+ 0.12	+ 0.26	+ 0.10	0.05	+ 0.13	+ 0.46
day 22 – 29	+ 0.20	+ 0.17	+ 0.13	+ 0.14	+ 0.24	+ 0.17	+ 0.28	+ 0.11

* (p = 0.05) significantly different from controls (Student's t-test)

In addition, food consumption was reduced in females treated with 3000 (15 – 19 %) and 10000 ppm (17 – 28 %) during the first two weeks of treatment. However, there were no consistent differences in food conversion ratios at any dose level.
Clinical chemistry, haematology: There was a statistically significant increase in plasma cholesterol in females at 10000 ppm (3.26 mmol/l) at study termination, compared to controls (2.49 mmol/l). For all other parameters investigated, no toxicologically significant treatment-related effects were observed at any dose level.
Pathology: Organ weight analysis revealed statistically significant increased liver weights relative to body weight in females only at 10000 ppm and moderate increase in absolute and relative adrenal weights in males only at 3000 and 10000 ppm (Table 5.3.1-2).

Table 5.3.1-2: 28-day dietary repeat dose study in mice; organ weights (mean values)

	0 ppm		1000 ppm		3000 ppm		10000 ppm	
	♂	♀	♂	♀	♂	♀	♂	♀
Liver (absolute; g)	1.762	1.274	1.717	1.251	1.828	1.338	1.696	1.366
Liver (relative; % of bw)	5.422	5.087	5.389	5.089	5.832	5.416	5.864	6.043**
Adrenals (absolute; g)	0.005	0.010	0.005	0.011	0.007	0.010	0.006	0.008
Adrenals (relative; % of bw)	0.015	0.040	0.015	0.042	0.021*	0.042	0.021	0.038

* (p = 0.05); ** (p = 0.01) significantly different from controls (Student's t-test)

Histopathological changes were seen in the thyroid gland (pigmentation of the follicular cells) of both males and females at 10000 ppm, in the kidneys (slight tubular degeneration) of females at 10000 ppm, and in the urinary bladder (minimal to moderate urothelial hyperplasia; urolithiasis) of males at 10000 ppm. No treatment-related changes were seen in mice treated at 1000 and 3000 ppm (Table 5.3.1-3).

Table 5.3.1-3: 28-day dietary repeat dose study in mice; Incidences of histopathological findings

	0 ppm		1000 ppm		3000 ppm		10000 ppm	
	♂	♀	♂	♀	♂	♀	♂	♀
Thyroid (pigmentation of follicular cells)	0/5	0/5	0/5	0/5	0/5	0/5	5/5	2/5
Kidneys tubular degeneration)	0/5	0/5	0/5	0/5	0/5	0/5	0/5	2/5
urinary bladder (urothelial hyperplasia)	0/5	0/5	0/5	0/5	0/5	0/5	3/5	0/5

Conclusion:
In the report, the NOAEL for dietary administration of pyrimethanil to CD-1 mice for 28 days was set at 1000 ppm (equivalent to 167 and 236 mg/kg bw/d for males and females, resp.) based on retarded body weight gain, increased liver weight and pathological/histopathological findings in thyroid, kidneys and urinary bladder. However, taking into account the limited histopathological examinations, the results of the study should be considered as supplementary information only.

~~**Pyrimethanil, dog 28-day repeated dose study ([redacted], 1990) (BASF DocID A81764)**~~

~~**Guidelines:** It was stated, that the study was conducted according to the prevailing published guidelines of US EPA Pesticide Assessment Guidelines, Subdivision F, and OECD guidelines (no. not specified).~~
~~**GLP:** Yes~~
~~**Acceptance:** Because of some limitations in this study (e.g. low number of animals/dose), the study is regarded only as supplementary information.~~

Report: CA 5.3.1/3
[redacted] 1990 a
Technical SN 100 309: 28-day repeat dose study in dogs
A81764
Guidelines: JMAFF, US EPA (1984) Pesticide Assessment Guidelines. Subdiv. F - Hazard Evaluation: Human and domestic animals (Nov 1984 revised edition), OECD Guidelines for Testing of Chemicals (Paris 1981)
GLP: yes
(certified by Department of Health and Social Security of the Government of the United Kingdom, United Kingdom)

Executive summary

Groups of 2 male and 2 female beagle dogs (source [redacted]) were given pyrimethanil (batch no. CR 19325/1; purity 99.0%; suspended in 1% methyl cellulose in distilled water) by gavage at dose levels of 0 (solvent control), 100, 500 and 1000 mg/kg bw/day. The highest dose level was reduced to 800 mg/kg bw/day on Day 7 of treatment.

It was concluded that 800 mg/kg bw/day produced vomiting and other signs indicating a dose level approximating to the MTD. The NOAEL in this subacute study was set at 100 mg/kg bw/day.

Material and Methods:
Groups of 2 male and 2 female beagle dogs (source [redacted]) were given pyrimethanil (batch no. CR 19325/1; purity 99.0 %; suspended in 1 % methyl cellulose in distilled water) by gavage at dose levels of 0 (solvent control), 100, 500 and 1000 mg/kg bw/d. The highest dose level was reduced to 800 mg/kg bw/d on day 7 of treatment.
The dogs were observed daily for clinical signs with body weight and food intake measured weekly. Ophthalmoscopy and electrocardiography was performed in all animals prior to and towards the end of treatment.
Clinical chemistry (total protein, albumin, globulin, calcium, phosphate, sodium, potassium, urea, creatinine, glucose, cholesterol, bilirubin, chloride, carbon dioxide, anion gap, AST, ALT, AP, GGT, CPK) and haematology analysis (haematocrit, haemoglobin, RBC, reticulocyte count, MCHC, MCV, MCH, WBC total, WBC differential, platelets count, Thrombotest, Kaolin cephalin time) according to relevant guidelines were conducted prior to and after 15 and 28 days of treatment.
Urinalysis (colour, pH, protein, glucose, ketones, bilirubin, urobilinogen, blood) was done on urine samples taken directly from the bladder at necropsy.
At necropsy the organ weights of adrenals, brain, gonads, heart, kidneys, liver and thyroid (with parathyroid) were recorded and subsequently histopathological examination was performed on following organs/tissues: adrenals, aorta, brain [3 levels], caecum, colon, duodenum, epididymites, eyes, femur, gall bladder, heart, ileum, jejunum, kidneys, lacrimal gland, liver, lungs, mandibular lymph node, mammary gland, mesenteric lymph node, oesophagus, optic nerve, ovaries, pancreas, pituitary, prostate, rectum, salivary gland, sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord [3 levels], spleen, sternum, submandibular lymph node, stomach, testes, thymus, thyroid with parathyroid, tongue, trachea, urinary bladder, uterus, vagina.

Findings:
General observations: There were no deaths during the study. Frequent vomiting was observed in all dogs given 1000 mg/kg bw. After reducing this dose level to 800 mg/kg bw (at day 7), vomiting occurred only occasionally. At 500 mg/kg bw, one male and one female vomited on days 1, 2, 3, 17 and 24. In all cases, vomiting occurred 2 – 4 hours after dosing.
Body weight: At 1000 mg/kg, slight weight loss was seen in both sexes (2 - 3 %) during the first week of treatment when compared with controls. Following dose reduction to 800 mg/kg, the overall body weight gain throughout the study period remained slightly reduced in females. In addition, food consumption was reduced significantly (25 %) in females during the first week of treatment. At 500 mg/kg bw, food consumption in females was also slightly reduced (6 %) during the first 3 weeks of the study.
Ophthalmoscopic and electrocardiographic examinations as well as haematology and urinalysis revealed no treatment-related findings at any dose level.
Examination of clinical chemistry parameters revealed a slight but not statistically significant increase in plasma cholesterol levels after 15 and 28 days of treatment which could be observed in both sexes treated with 1000/800 mg/kg bw/d compared with control values.
Pathology: At necropsy, there was an apparent small dose-related increase in absolute and relative liver weights in females of all dose groups compared with controls. However, it was stated in the report that this finding was considered due to a single control female with a low relative liver weight. One male dog at 1000/800 mg/kg bw had reduced absolute and relative testes weight (approximately 50 %), but this finding was not considered to be related to treatment. Histopathological examinations showed no treatment-related findings at any dose level.

Conclusion:
It was concluded that 800 mg/kg bw/d produced vomiting and other signs indicating a dose level approximating to the MTD. The NOAEL in this subacute study was set at 100 mg/kg bw/d.

CA 5.3.2 Oral 90-day study

~~**Pyrimethanil, rat 13-week oral (dietary) toxicity study followed by a 4-week regression period ([redacted] 1990)**~~
~~**(BASF DocID A81783, BASF DocID C023548, BASF DocID A81784, BASF DocID A81785 and BASF DocID 2003/1023036)**~~

~~**Guidelines:** According to OECD guideline 408 (1981) and US EPA Pesticide Assessment Guidelines, Subdivision F~~
~~**Deviations:** Small deviations are noted but do not limit the scientific validity of the study.~~
~~**GLP:** Yes~~
~~**Acceptance:** The study is considered acceptable in the EU registration process 2004-2006~~

Report:	CA 5.3.2/1 [redacted] 1990 a SN 100 309: 13-week oral (dietary) toxicity study in the rat followed by a 4-week regression period A81783
Guidelines:	FIFRA 40CFR part 160, JMAFF 59 NohSan No 4200, OECD
GLP:	yes (certified by Department of Health and Social Security of the Government of the United Kingdom, United Kingdom)
Deviations:	Yes, small deviations (results of dietary analyses for homogeneity are reported separately. Technical error on feeding control diet to high dose animals during the first three days of week 6, and also minor problem with stability; analyses indicated that low concentration of 80 ppm was not stable for more than 4 days, so from week 6 diets at 80 ppm were frozen after preparation) are noted but do not limit the scientific validity of the study.

[see KCA 5.3.2/2 A81784]
[see KCA 5.3.2/3 A81785]
[see KCA 5.3.2/4 A81787]

Executive summary

Groups of 10 male and 10 female Sprague Dawley rats/dose (source: [redacted].) received pyrimethanil (batch no. CR 19325/2; ~~purity not stated in the report~~ 95.3%) with the diet at dose levels of 0, 80, 800 and 8000 ppm (equivalent to 0, 5.4, 54.5 and 529.1 mg/kg bw/day in males and 0, 6.8, 66.7 and 625.9 mg/kg bw/day in females) for 13 weeks. In addition, a recovery study was conducted with further groups of 10 rats/sex/dose, which received pyrimethanil at 0 and 8000 ppm for 13 weeks, and thereafter control diet for 4 weeks.

Following a 13-week dietary administration of pyrimethanil to Sprague-Dawley rats, adverse effects were observed at the high dose group (8000 ppm). Treatment-related findings included reductions in body weight gain and food consumption, proteinuria, organ weight effects and histopathological findings in liver and thyroid, which have been identified as the main target organs. Histological alterations comprised centrilobular hypertrophy in the liver and follicular epithelial hypertrophy and colloid depletion in the thyroid. Slight hypertrophy of centrilobular hepatocytes was also evident at the next lower dose level (800 ppm) in this study, however, was reversible within 4-week recovery period.
Based on the results of the 90-day dietary study, the short-term NOAEL in the rat can be established at 800 ppm (equivalent to ~~5.4~~ 54 mg/kg bw/day in males and ~~6.8~~ 68 mg/kg bw/day in females).

Material and Methods:
Groups of 10 male and 10 female Sprague Dawley rats/dose (source: [redacted].) received pyrimethanil (batch no. CR 19325/2; purity: ~~not stated in the report~~ 95.3% (the same batch as used in Mouse 28-day dietary repeat dose study with report No. TOX/89/223-12)) with the diet at dose levels of 0, 80, 800 and 8000 ppm for 13 weeks (equivalent to 0, 5.4, 54.5 and 529.1 mg/kg bw/d for males and 0, 6.8, 66.7 and 625.9 mg/kg bw/d for females). In addition, a recovery study was conducted with further groups of 10 rats/sex/dose which received pyrimethanil at 0 and 8000 ppm for 13 weeks, and thereafter control diet for 4 weeks. Test diets were prepared freshly each week and stability of the test material was confirmed by analysis.
All examinations including clinical and ophthalmological examinations, estimations of body weight gain, food and water consumption, haematology (haematocrit, haemoglobin, RBC, MCHC, MCV, MCH, WBC total, WBC differential, platelets count), biochemistry (total protein, albumin, globulin, calcium, phosphate, sodium, potassium, urea, creatinine, glucose, bilirubin, chloride, AST, ALT, AP), urinalysis (colour, volume, pH, specific gravity, protein, glucose, ketones, bilirubin, urobilinogen, blood cells), gross necropsy, organ weight analysis (adrenals, brain, heart, kidneys, liver, pituitary, spleen, ovaries, testes, thymus) and histopathology (adrenals, aorta, brain [4 levels], caecum, colon, duodenum, epididymites, eyes, femur, heart, ileum, jejunum, kidneys, lacrimal gland, liver, lungs, mammary gland, mesenteric lymph node, oesophagus, ovaries, pancreas, pituitary, prostate, rectum, salivary gland, sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord [3 levels], spleen, sternum, submandibular lymph node, stomach, testes, thymus, thyroid with parathyroid, trachea, urinary bladder, uterus, vagina) were performed according to the requirements of the relevant OECD directive mentioned.

Findings:
General observations: There were no treatment-related deaths during the study. Additionally, no treatment-related clinical symptoms or signs of systemic toxicity were observed at any dose level.
Body weight: At 8000 ppm, mean body weights of both sexes were lower than controls resulting in a marked decrease of body weight gain (28 % in males and 33 % in females) throughout the study period. During the reversibility phase, some recovery in weight gain was apparent, but at termination of the reversibility phase, moderate decreases in mean weight gain (18 % in males and 21 % in females) over the whole study period were still evident, compared to controls (Table 5.3.2-1).
Mean body weights and weight gain of animals treated with 80 or 800 ppm were similar to that of the controls over the treatment period.

Table 5.3.2-1: 13 week oral toxicity study in rats; body weights (mean values in g)

Sampling time	0 ppm ♂	0 ppm ♀	80 ppm ♂	80 ppm ♀	800 ppm ♂	800 ppm ♀	8000 ppm ♂	8000 ppm ♀
week 1	175	122	164	120	172	118	169	116
week 7	385	227	367	222	378	221	313	185
week 14	476	263	445	248	455	244	387	211
after recovery phase	511	276	-	-	-	-	432	241

Food consumption was also reduced by 33 % in both sexes given 8000 ppm during the first week and by approximately 18 % for the remainder of the study compared to control values. Food intake of rats given 80 and 800 ppm were comparable to controls throughout the study.
Water intake of all treated groups was similar to that of controls.
Ophthalmoscopic examination revealed no evidence of treatment-related changes.
Haematology, clinical chemistry, urinalysis: Evaluation of haematological parameters (performed prior to treatment, at week 4, week 13 and after the recovery period) revealed significant increases in the values of mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) at week 4 and 13 and after recovery phase for both sexes given 8000 ppm. However, these findings were not considered to be of toxicological significance in the report.
Blood chemistry parameters (performed during week 4, week 13/14 and after the recovery phase) showed no evidence of treatment-related effects. Although various parameters (e.g. AST, urea, total protein) occasionally attained intergroup differences with statistical significance at some dose levels, none were considered to be of toxicological significance. It was explained in the report that this is because the apparent differences were not of the magnitude expected for diagnostic significance, or were not consistently changed between the two occasions during the treatment period.

Table 5.3.2-2: 13 week oral toxicity study in rats; Clinical chemistry parameters related to the liver function; Group mean values in males and females

Parameters \ Dose [ppm]	Males		Females	
	0	8000	0	8000
Week 4 of treatment				
ALP [U/l]	236	264	195	170
ALT [U/l]	27	22	19	16
AST [U/l]	55	43**	46	45
Bilirubin [mg %]	0.4	0.4	0.2	0.3
Week 13/14 of treatment				
ALP [U/l]	109	91	64	76
ALT [U/l]	26	19	22	17**
AST [U/l]	60	47	56	46
Bilirubin [mg %]	0.6	0.3***	0.3	0.3
Week 4 of the reversibility period				
ALP [U/l]	77	101	47	58
ALT [U/l]	27	42	21	25
AST [U/l]	52	58	46	50
Bilirubin [mg %]	0.3	0.3	0.3	0.3

significantly different from controls ** p<0.01, *** p<0.001

Urinalysis exhibited higher incidences of positive scores for proteinuria in males given 8000 ppm, compared to controls. In addition, changes in the colour of the urine of males given 800 ppm and both sexes given 8000 ppm were apparent. It was assumed in the report that this altered colouration of the urine might be attributed to a compound-metabolite of pyrimethanil. After the recovery period, the urine specimens of both sexes given 8000 ppm were similar to those of the controls.
Organ weight: The analysis of absolute and relative organ weights revealed statistically significant changes in several organs in animals treated with 8000 ppm compared to controls (mean values are given in Table 5.3.2-3) which were considered to be related with the marked growth retardation seen at this dose level. Changes in organ weights recorded at the dose levels of 80 ppm and 800 ppm were reported to be within historical control ranges. After the recovery period, no changes in organ weights were observed in animals previously given 8000 ppm when compared with controls.

Table 5.3.2-3: 13 week oral toxicity study in rats; substantial findings in absolute (g) and relative organ weights (%, mean values)

	0 ppm ♂	♀	80 ppm ♂	♀	800 ppm ♂	♀	8000 ppm ♂	♀
Adrenals (g)	0.049	0.056	0.050	0.056	0.052	0.058	0.039**	0.040***
Heart (g)	1.34	0.86	1.33	0.86	1.34	0.88	1.17***	0.74***
Kidney (g)	2.96	1.67	2.77	1.76	2.75	1.77	2.68	1.52*
Spleen (g)	0.65	0.45	0.69	0.46	0.70	0.45	0.59	0.37*
Thymus (g)	0.312	0.266	0.350	0.211	0.288	0.221	0.266	0.190**
Brain (% of bw)	0.45	0.75	0.47	0.79	0.46	0.77	0.55***	0.93***
Gonads ♂ (% of bw)	0.72	-	0.76	-	0.78	-	0.90**	-
Kidney (% of bw)	0.65	0.68	0.64	0.74	0.63	0.76**	0.74*	0.79***
Liver (% of bw)	3.59	3.19	3.49	3.61*	3.50	3.58*	4.69**	4.20***

* ($p \leq 0.05$); ** ($p \leq 0.01$); *** ($p \leq 0.001$) significantly different from controls (Analysis of variance)

Pathology: Macroscopic pathology exhibited no treatment-related findings at any dose level. Histopathologically, treatment with pyrimethanil was associated with minimal hypertrophy of centrilobular hepatocytes in animals treated with 800 ppm (♂) and 8000 ppm (♂/♀). In addition, there was also a decrease in incidence of margination of hepatocyte cytoplasm in males treated at 800 and 8000 ppm which was considered secondary to the hypertrophy. In the thyroid, a treatment-related increase in incidence and severity of thyroid follicular epithelial hypertrophy and an increased incidence of follicular epithelial brown pigment (which stained positively with Schmorl's method for lipofuscin) was observed in both sexes at the highest dose level. Incidences of these histological changes observed are given in Table 5.3.2-4 and Table 5.3.2-5.

Table 5.3.2-4: 13 week oral toxicity study in rats; incidences of microscopic findings

	0 ppm		80 ppm		800 ppm		8000 ppm	
	♂	♀	♂	♀	♂	♀	♂	♀
Centrilobular hypertrophy of hepatocytes	0/10	0/10	0/10	0/10	2/10	0/10	9/10	3/10
Thyroid follicular epithelial hypertrophy	3/10	0/10	2/10	1/10	2/10	1/10	9/10	6/10
Brown pigment in follicular epithelium	0/10	0/10	0/10	0/10	0/10	0/10	8/10	7/10

Table 5.3.2-5: 13 week oral toxicity study in rats; Incidence of histopathological findings in the liver of males and females

Parameters \ Dose [ppm]	Males (10)				Females (10)			
	0	80	800	8000	0	80	800	8000
Necrosis	1/10	1/10	1/10	0/10	0/10	0/10	0/10	0/10
Fibrosis	1/10	1/10	1/10	0/10	0/10	0/10	0/10	0/10
Congestion	1/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
Siderosis	0/10	1/10	1/10	0/10	0/10	0/10	0/10	0/10
Marg. of cytoplasm	6/10	5/10	3/10	2/10	1/10	0/10	0/10	0/10
Chronic inflammation	0/10	0/10	0/10	0/10	1/10	0/10	0/10	1/10

Following the 28 day recovery period, hypertrophic changes in the livers and the thyroids as well were not longer apparent in animals previously given 8000 ppm.

Conclusion:
Following a 13 week dietary administration of pyrimethanil to Sprague-Dawley rats, adverse effects were observed at 8000 ppm (reduced body weights and retarded body weight gain; proteinuria; histopathological findings in liver and thyroid). Hypertrophy of centrilobular hepatocytes and of the thyroid follicular epithelium were reversible following the 4 week withdrawal period. The NOAEL for this study can be established at 800 ppm (equivalent to 54 mg/kg bw/d in males and 68 mg/kg bw/d in females) based on the adverse effects observed at top dose.

~~**Pyrimethanil, mouse 90-day dietary repeated dose study ([redacted] 1991) (BASF DocID A81792)**~~

~~**Guidelines:** According to OECD guideline 408 (1981)~~
~~**Deviations:** Deviations (no ophthalmoscopic examination was performed; only 10 animals/sex/dose of 20 were subjected to necropsy, the others were used for haematology and clinical chemistry) were noted, but do not limit the scientific validity of the study.~~
~~**GLP:** Yes~~
~~**Acceptance:** The study is considered acceptable in the EU registration process 2004-2006~~

Report: CA 5.3.2/5
[redacted] 1991 a
SN 100309 (CR 19325/3): Mouse 90-day dietary repeat dose study
A81792
Guidelines: OECD 408, US EPA (1984) Pesticide Assessment Guidelines. Subdiv. F - Hazard Evaluation: Human and domestic animals (Nov 1984 revised edition), JMAFF
GLP: yes
(certified by Department of Health and Social Security of the Government of the United Kingdom, United Kingdom)

Deviations: Yes, no ophthalmoscopic examination was performed; only 10 animals/sex/dose of 20 were subjected to necropsy, the others were used for hematology and clinical chemistry. Additionally, no detailed clinical observations as well as no sensory reactivity to stimuli of different types and functional observation battery were performed (compared to OECD guideline 408 (adopted 21 September 1998). The deviations are considered not to limit the scientific validity of the study.

Executive summary

Groups of 20 male and 20 female CD-1 BR mice (source: [redacted].) were fed diet containing 0, 80, 900 and 10000 ppm pyrimethanil (batch no. CR 19325/3; purity 97.7 – 97.9%) for 13 weeks (equivalent to 0, 12, 139 and 1864 mg/kg bw/day in males and 0, 18, 203 and 2545 mg/kg bw/day in females).

In the 90-day dietary study in mice, there was clear evidence of toxicity at the high dose level (10000 ppm) including retarded body weight gain, clinical chemistry findings, organ weight effects and pathological/histopathological findings. Relevant treatment-related pathological findings in this species concerned the thyroid (necrosis of follicular epithelial cells), kidneys (tubular dilatation) and urinary bladder (hyperplasia of the epithelium, uroliths). Increased liver weights were evident at 10000 ppm, but no distinct treatment-related microscopic changes were observed. However, levels of cholesterol and total bilirubin in plasma were elevated at this dose level.
The NOAEL could be established at 900 ppm (equivalent to 139 mg/kg bw/day in males and 203 mg/kg bw/day in females).

Material and Methods:
Groups of 20 male and 20 female CD-1 BR mice (source: [redacted].) were fed diet containing 0, 80, 900 and 10000 ppm pyrimethanil (batch no. CR 19325/3; purity 97.7 – 97.9%) for 13 weeks (equivalent to 0, 12, 139 and 1864 mg/kg bw/d for males and 0, 18, 203, and 2545 mg/kg bw/d for females). Diets were prepared twice weekly; stability and homogeneity was confirmed by analysis.
Animals were observed daily for clinical signs. Body weight and food consumption were measured weekly. Water intake was recorded over a 4-days period during weeks 4/5, 8/9 and 11/12. Haematology (haematocrit, haemoglobin, RBC, MCHC, MCV, MCH, WBC total, WBC differential, platelets count) and clinical chemistry (total protein, albumin, globulin, calcium, phosphate, sodium, potassium, urea, creatinine, glucose, cholesterol, bilirubin, chloride, AST, ALT, AP, GGT, CPK) investigations were carried out after 12/13 weeks of treatment. At necropsy, the weights of selected organs (liver, heart, brain, adrenals, spleen, kidneys, testes and ovaries) were recorded and subsequently all major organs and tissues (adrenals, bone marrow, bone sternum, caecum, colon, duodenum, heart, ileum, jejunum, kidneys, liver, lungs, mesenteric lymph node, oesophagus, ovaries, pancreas, spleen, stomach, testes, thymus, thyroid with parathyroid, urinary bladder, uterus) from control and high dosed mice were examined. In addition, liver, kidneys, lungs, uterus, thyroid, urinary bladder and macroscopic abnormalities from the low and mid dose groups were also examined.

Findings:
General observations: No treatment-related mortality or clinical signs of toxicity were noted during the 13-week study period at any dose level.
At 10000 ppm, there was a reduction in body weight gain over the whole treatment period (12.4 % in males; 7.2 % in females) compared to controls. In contrast, food consumption of males and females at this dose level was significantly increased (by 14.1 % in males and 9.8 % in females) compared to untreated mice. Food conversion ratios were reduced in males and females at this dose level, mainly during the first two weeks of treatment. There were no treatment-related effects in food conversion ratios following lower dose levels.
Measurement of water intake showed no consistent effects of treatment at any dose level.
Haematology: Investigations of haematological parameters performed at study termination revealed no toxicological significant effects of treatment at any dose level.
Concerning clinical chemistry parameters, there was a statistically significant increase in cholesterol and total bilirubin in females when compared to controls. Additionally, statistically significant increases in phosphate levels were observed in males following treatment with 80 and 10000 ppm, but not in males dosed with 900 ppm and in females at any dose level (Table 5.3.2-6).

Table 5.3.2-6: 90 day dietary repeat dose study in mice; relevant clinical chemistry findings (mean values) at termination

	0 ppm ♂	♀	80 ppm ♂	♀	900 ppm ♂	♀	10000 ppm ♂	♀
Phosphate (mmol/l)	1.40	1.99	1.79***	1.70	1.65	1.87	1.78***	1.88
Total bilirubin (mmol/l)	3.6	5.4	4.4	4.0	4.0	4.4	4.3	7.7***
Cholesterol (mmol/l)	4.05	2.53	3.44	2.40	3.82	2.28	3.60	2.98**

** ($p \leq 0.01$); *** ($p \leq 0.001$) significantly different from controls (Student's t-test)

Organ weights: Statistically significant increases in relative liver weights were noted for males and females at 10000 ppm, as compared to controls.
Pathology: At necropsy, a dark discolouration of the thyroid gland in 8/10 males of the top dose was observed. Females at 10000 ppm showed fluid filled ovarian bursa in 5/10 mice compared with 4/10 animals at 900 ppm and 1/10 animals at 80 ppm and in the control group, resp. However, in the absence of histopathological changes, this finding was considered indicative of normal oestrous cyclicity.
At 10000 ppm, histopathological treatment-related findings were detected in the kidneys (slight tubular dilatation in kidneys), urinary bladder (uroliths, slight to moderate hyperplasia of the bladder epithelium) and thyroid gland (slight to severe exfoliative necrosis of follicular cells; slight to severe lipofuscin pigmentation of the follicular cells). Incidences of these findings are given in Table 5.3.2-7.

Table 5.3.2-7: 90 day dietary repeat dose study in mice; relevant necropsy findings (organ weight changes; incidences of histological changes)

	0 ppm ♂	♀	80 ppm ♂	♀	900 ppm ♂	♀	10000 ppm ♂	♀
Liver weight absolute (g)	1.98	1.39	1.95	1.51	2.00	1.40	2.17	1.59*
relative (% of bw)	4.95	4.65	4.99	5.04	5.02	4.83	5.78**	5.62**
Kidney – slight tubular dilatation	0/10	0/10	0/10	0/10	0/10	0/10	3/10	0/10
Thyroid – exfoliative necrosis of follicular cells	0/10	0/10	0/10	0/10	0/10	0/10	8/10	1/10
Thyroid – pigmentation of follicular cells	0/10	0/10	0/10	0/10	0/10	0/10	10/10	9/10
Urinary bladder – urothelial hyperplasia	0/10	0/10	0/10	0/10	0/10	0/10	0/10	3/10
Urinary bladder – uroliths	0/10	0/10	0/10	0/10	0/10	0/10	1/10	4/10

* ($p \leq 0.05$); ** ($p \leq 0.01$) significantly different from controls (Dunnet's test)

In addition, staining intensity of liver sections from male mice with PAS ("periodic acid Schiff") method showed marked depletion of glycogen at 10000 ppm. At 900 ppm, also some slight glycogen depletion in male mice was indicated by reduced staining intensity, but not considered to represent a toxicological adverse effect.

Conclusion:
The NOAEL for dietary administration of pyrimethanil to CD-1 mice for 13 weeks can be set at 900 ppm (equivalent to 139 and 203 mg/kg bw/d for males and females) based on retarded body weight gain, increased liver weight and histopathological findings in thyroid, kidneys and urinary bladder, seen at 10000 ppm.

~~**Pyrimethanil, dog 90-day oral (gavage) repeated dose study ([redacted], 1991) (BASF DocID A81763 and BAS DocID A81790)**~~

~~**Guidelines:** According to OECD guideline 409 (1981)~~
~~**GLP:** Yes~~
~~**Acceptance:** The study is scientific valid and acceptable in the EU registration process 2004-2006.~~

Report:	CA 5.3.2/6 [redacted] 1991 c Technical SN 100309: Dog 90-day oral (gavage) repeat dose study A81790
Guidelines:	US EPA (1984) Pesticide Assessment Guidelines. Subdiv. F - Hazard Evaluation: Human and domestic animals (Nov 1984 revised edition), OECD 408, JMAFF
GLP:	yes (certified by Department of Health and Social Security of the Government of the United Kingdom, United Kingdom)
Deviations:	Yes, minor limitations (organ weight of uterus, gall bladder and thymus were not recorded) were noted (compared to OECD guideline 409 (adopted 21 Sep 1998), but do not limit the scientific validity of the study.

Executive summary

Pyrimethanil (batch no. CR 19325/3; purity 97.7%, suspended in 0.5% w/v methyl cellulose in water) was given to groups of 4 male and 4 female beagle dogs (source [redacted]) at dose levels of 0 (vehicle control), 6, 80 and 1000 mg/kg bw/day (reduced to 800 mg/kg bw/day by Day 7 of treatment) by gastric intubation (constant dosing volume 5 mL/kg bw) for at least 90 consecutive days.

Continuous treatment of dogs with pyrimethanil by gavage for 13 weeks caused a slight reduction in body weight and food consumption at 1000/800 mg/kg bw/day and induced vomiting at 80 and 1000/800 mg/kg bw/day. In addition, a marked decrease of water consumption at these both dose levels was observed. Since the toxicological significance of this reduced water intake is not clarified, this finding should be considered as an adverse effect. There were also some findings on clinical chemistry parameters, already seen at 80 mg/kg bw/day, which were considered of limited toxicological relevance. Therefore, the NOAEL in this study is to be set at 6 mg/kg bw/day.

Material and Methods:
Pyrimethanil (batch no. CR 19325/3; purity 97.7%, suspended in 0.5 % w/v methyl cellulose in water) was given to groups of 4 male and 4 female beagle dogs (source [redacted]) at dose levels of 0 (vehicle control), 6, 80 and 1000 mg/kg bw/d (reduced to 800 mg/kg bw/d by day 7 of treatment) by gastric intubation (constant dosing volume 5 mL/kg bw) for at least 90 consecutive days. Dosing suspensions were prepared the day before use; stability and homogeneity was confirmed by analysis.
Clinical observations and food consumptions were recorded daily, body weights were determined weekly. Water intake was measured over a 4-days period during weeks 3 - 4, 7 - 8 and 11 - 12 of treatment. Ophthalmoscopic examinations and electrocardiograms were made prior to the start of treatment and during the final week of treatment.
Blood samples for biochemical (total protein, albumin, globulin, calcium, phosphate, sodium, potassium, urea, creatinine, glucose, cholesterol, bilirubin, chloride, carbon dioxide, anion gap, AST, ALT, AP, GGT, CPK) and haematological investigations (haematocrit, haemoglobin, RBC, MCHC, MCV, MCH, WBC total, WBC differential, platelets count, erythrocyte sedimentation rate, Thrombotest, Kaolin cephalin time) were collected prior to treatment and after 4 and 13 weeks.
Urine samples (colour, volume, pH, specific gravity, protein, glucose, ketones, bilirubin, urobilinogen, blood cells, bacteria, sediment) were taken at necropsy from the urinary bladder.
At termination of the study, all animals were subjected to gross pathological and histopathological examinations (adrenals, aorta, bone marrow smear, brain, caecum, colon, duodenum, epididymites, eyes, femur, gall bladder, heart, ileum, jejunum, kidneys, lacrimal gland, liver, lungs, mammary gland, mandibular and mesenteric lymph node, oesophagus, optic nerve, ovaries, pancreas, pituitary, prostate, rectum, salivary glands, sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord [3 sections], spleen, sternum, submandibular lymph node, stomach, testes, thymus, thyroid with parathyroid, tongue, trachea, urinary bladder, uterus, vagina) and the following organs were weighed: adrenals, brain, heart, kidneys, liver, lungs, pituitary, spleen, testes, thyroid, ovaries.

Findings:
General observations: There were no treatment-related mortalities during the study. At 1000 mg/kg , vomiting was observed in all dogs during the first 6 days of treatment (1 – 4 hours after dosing). The dose level was therefore reduced to 800 mg/kg to alleviate vomiting. However, occasional to frequent vomiting was observed further on in the majority of animals, the incidence of vomiting was subsequent to approx. 9% of all administrations at this dose level. Other signs observed included salivation and reduced activity. Also at 80 mg/kg, infrequent vomiting was evident, the overall incidence of vomiting was less than 2 % of all administrations in this dose group.
During the first week of treatment, very slight body weight loss (- 3%) was seen in both sexes treated with 1000 mg/kg compared to controls. In addition, food consumption was reduced during this week among top dose animals (- 23 % in males, -16 % in females). After reduction of the dose to 800 mg/kg, the overall reduction in body weight gain remained slight in both sexes (less than 3 %) throughout the study compared to controls. No effects on body weights and food consumption were seen in animals given 6 and 80 mg/kg bw.
At 1000/800 mg/kg, water consumption was decreased in both sexes and also in females treated with 80 mg/kg bw during the entire treatment period. Concerning this finding, no further explanation on the toxicological significance is given in the report. No effect on water consumption was seen at 6 mg/kg bw in either sex.
The overall mean water intakes are presented in Table 5.3.2-8 and Table 5.3.2-9.

Table 5.3.2-8: 90 day repeat dose study in dogs; group mean water consumption (mL/two dogs/day)

	0 mg/kg ♂	♀	**6 mg/kg** ♂	♀	**80 mg/kg** ♂	♀	**1000/800 mg/kg** ♂	♀
days 19 – 22 (% of controls)	2990	2726	3280 (109.6)	2726 (100)	2618 (87.5)	2369 (86.9)	2269 (75.8)	2335 (85.6)
days 47 – 50 (% of controls)	2870	2679	3301 (115.0)	2670 (99.6)	2563 (89.3)	2380 (88.8)	1963 (68.4)	2389 (89.1)
days 75 – 78 (% of controls)	2809	3106	3300 (117.4)	2700 (86.9)	2748 (97.8)	2338 (75.2)	1806 (64.3)	2188 (70.4)
overall mean (% of controls)	2890	2838	3294 (116.0)	2699 (95.1)	2643 (91.4)	2362 (83.2)	2013 (69.6)	2304 (81.1)

Table 5.3.2-9: Dog 90 days oral (gavage) repeat dose study; Group mean body weight [kg]

Dose [mg/kg bw] / Day	Males				Females			
	0	6	80	1000/800	0	6	80	1000/800
1	11.5	11.4	11.5	11.6	10.3	10.2	10.2	10.3
15	11.7	12.0	11.9	11.6	10.5	10.6	10.4	10.4
29	12.3	12.6	12.5	12.2	11.1	11.0	10.9	10.9
43	12.5	12.9	12.2	12.5	11.4	11.4	10.9	11.4
57	12.7	13.1	12.8	12.9	11.8	11.6	11.3	11.5
71	13.0	13.3	13.0	13.2	12.1	11.8	11.4	11.5
92	13.4	13.7	13.4	13.5	12.3	12.0	11.6	12.0

Ophthalmoscopic and electrocardiographic investigations revealed no treatment-related changes in any dose group. Concerning haematological parameters and urinalysis, no toxicologically significant treatment-related effects were observed in any dose group. It was mentioned in the report that all existing intergroup differences with statistical significance at some dose levels showed no consistency, and were therefore considered to be of biological variability with no toxicological relevance.
Clinical chemistry: In week 4, there were statistically significant reductions in phosphate in male dogs treated with 800 mg/kg and 80 mg/kg compared with control values. Slight, but not statistically significant decreases were also evident in males at both dose levels in week 13 (Table 5.3.2-10)

Table 5.3.2-10: 90 day repeat dose study in dogs; Phosphate values (mmol/l) in the plasma of male dogs (mean values)

Sampling time	0 ppm	6 mg/kg bw	80 mg/kg bw	1000/800 mg/kg bw
Phosphate, week 0	2.59	2.46	2.32	2.24
week 4	2.40	2.33	2.13*	1.97*
week 13	2.00	2.04	1.84	1.74

* ($p \leq 0.05$) significantly different from controls

In addition, there were also slight reductions in sodium, anion gap and total protein in females treated with 1000/800 mg/kg in week 4. These differences in clinical chemistry parameters were considered in the report to be of limited toxicological significance. Other intergroup differences occasionally attaining statistical significance at some dose levels were considered as biological variability with no toxicological relevance because they were not dose-related or were within the normal range of controls.
Organ weight analysis revealed no significant treatment-related effects at any dose level.

Table 5.3.2-11: Dog 90 days oral (gavage) repeat dose study; Group mean organ weight [g]

Organs \ Dose [mg/kg bw]	Males				Females			
	0	6	80	1000/800	0	6	80	1000/800
Testes	21.2	22.4	23.5	20.9				
Ovaries					1.00	1.08	1.13	1.08
Thyroids	1.02	0.82	0.87	1.28	0.73	0.80	0.83	0.89
Kidneys	55.9	58.8	57.2	60.2	49.8	50.8	48.1	48.2
Pituitary	0.0725	0.0750	0.0700	0.0750	0.0725	0.0650	0.0600	0.0650
Liver	402.0	403.5	387.9	370.5	347.6	352.6	349.1	350.0

Pathology: The results of the pathological and histopathological examination showed no treatment-related morphological changes at any dose group compared to controls. However, there was some depletion of hepatic glycogen in dogs treated with 800 mg/kg compared to control animals. The levels of glycogen in the liver of animals given 6 and 80 mg/kg were not significantly different from controls.

Table 5.3.2-12: Dog 90 days oral (gavage) repeat dose study; Incidence of histopathological findings in the liver

Parameters \ Dose [mg/kg bw]	Males (4)				Females (4)			
	0	6	80	1000/800	0	6	80	1000/800
Periductular Mo infiltration (minimal)	2	0	0	3	1	2	1	3
Microgranuloma	2	1	2	4	4	2	3	3
Glycogen content (total)	4	4	4	3	4	4	4	2
Focal pericholangitis (slight)	0	0	1	0	0	0	0	-
Subcapsular fibrosis (slight)	0	0	1	1	1	0	0	2

Mo = mononuclear cell

Conclusion:
Continuous treatment of dogs with pyrimethanil by gavage for 13 weeks caused a slight reduction in body weight and food consumption at 1000/800 mg/kg and induced vomiting at 80 and 1000/800 mg/kg bw/d. In addition, a marked decrease of water consumption at these both dose levels was observed. Since the toxicological significance of this reduced water intake is not clarified, this finding should be considered as an adverse effect. There were also some findings on clinical chemistry parameters, already seen at 80 mg/kg, which were considered of limited toxicological relevance. Therefore, the NOAEL in this study is to be set at 6 mg/kg bw/d.

~~**Pyrimethanil, dog 1-year oral (gavage) repeated dose study (1992) (BASF DocID A81809)**~~

~~**Guidelines:** According to OECD guideline 452 (1981)~~
~~**GLP:** Yes~~
~~**Acceptance:** The study is scientific valid and acceptable in the EU registration process 2004-2006.~~

Report:	CA 5.3.2/7 1992 a Technical SN 100309: Dog 12 month oral (gavage) repeat dose study A81809
Guidelines:	US EPA (1984) Pesticide Assessment Guidelines. Subdiv. F - Hazard Evaluation: Human and domestic animals (Nov 1984 revised edition), OECD 452, JMAFF, EEC 79/831 A V B 1
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)
Deviations:	The animal room temperature was 19-23 ºC, but it should be within a range of 15-21 ºC. At the beginning of the study, the body weight variation for each sex of animals should not exceed ± 20% of the mean weight, however, this requirement was not met in males. The addition of the fourth test group is recommended if large intervals (6-10 fold) between dosages are used. Wet weights of epididymes and uterus of animals were not determined.

Executive summary

Groups of 4 male and 4 female beagle dogs (source:) were treated daily for 12 months with pyrimethanil (batch no. 19325/4; purity 96.3 – 96.9%; suspended in 0.5% w/v methyl cellulose in water) at dose levels of 0 (vehicle control), 2, 30 and 400/250 mg/kg bw/day, administered by gastric intubation (constant dosing volume 5 mL/kg bw). The top dose was reduced on study Day 8 from 400 to 250 mg/kg bw/day.

The treatment-related effects observed included vomiting, retardation of body weight gain, increase in white blood cells (mainly neutrophils) in males only and a dose-related distinct decrease in water intake. Based on the results, the NOAEL of this study can be established at 30 mg/kg bw/day.

Material and Methods:
Groups of 4 male and 4 female beagle dogs (source: [redacted]) were treated daily for 12 months with pyrimethanil (batch no. 19325/4; purity 96.3 – 96.9 %; suspended in 0.5 % w/v methyl cellulose in water) at dose levels of 0 (vehicle control), 2, 30 and 400/250 mg/kg bw/d, administered by gastric intubation (constant dosing volume 5 mL/kg bw). The top dose was reduced on study day 8 from 400 to 250 mg/kg bw/d. Dosing suspensions were prepared the day before use; stability and homogeneity was confirmed by analysis.
Animals were observed daily for physical conditions and clinical signs, whilst body weight was recorded weekly. Food consumption was measured daily. Water intake was recorded during weeks 13, 26 and 52. Ophthalmoscopy and electrocardiogram were made prior to and towards the end of treatment.
Clinical chemistry (total protein, albumin, globulin, calcium, phosphate, sodium, potassium, urea, creatinine, glucose, cholesterol, bilirubin, chloride, carbon dioxide, anion gap, AST, ALT, AP, GGT, CPK), urinalysis (colour, volume, pH, specific gravity, protein, glucose, ketones, bilirubin, urobilinogen, blood cells, bacteria, sediment) and haematology (haematocrit, haemoglobin, RBC, MCHC, MCV, MCH, WBC total, WBC differential, platelets count, erythrocyte sedimentation rate, Thrombotest, Kaolin cephalin time, reticulocyte count) were performed before treatment and during weeks 13, 26 and at termination.
At termination of the study, all animals were subjected to gross pathological examinations and the following organs were weighed: adrenals, brain, heart, kidneys, liver, lungs, pituitary, spleen, testes, thyroid, ovaries. Following fixation, histopathological examinations (adrenals, aorta, bone marrow smear, brain, caecum, colon, duodenum, epididymites, eyes, femur, gall bladder, heart, ileum, jejunum, kidneys, lacrimal gland, liver, lungs, mammary gland, mandibular and mesenteric lymph node, oesophagus, optic nerve, ovaries, pancreas, pituitary, prostate, rectum, salivary glands, sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord [3 sections], spleen, sternum, stomach, testes, thymus, thyroid with parathyroid, tongue, trachea, urinary bladder, uterus, vagina) were performed.

Findings:
General observations: There were no mortalities during the study. Following treatment with 400 mg/kg bw, frequent vomiting was recorded in males and females 0.5 to 6 hours after dosing. The incidence of vomiting was approx. 36 % after all doses administered for both sexes. Therefore, the dose level was reduced to 250 mg/kg bw on study day 8, after which vomiting was only sporadically seen (approx. 1 % of all doses administered in both sexes during the remainder of the study period). Colouration of faeces and diarrhoea was also observed following 250 mg/kg bw. At 30 mg/kg the overall incidence of vomiting was 0.4 % of doses administered in males only.
Measurements of rectal temperature at the end of treatment revealed no abnormalities at any dose level.
Body weight: At 400 mg/kg slight body weight loss occurred during the first week of treatment at both sexes (0.8 % in males and 2.5 % in females). Subsequent to the reduction of the dose level to 250 mg/kg bw/d, the overall group mean body weight gain was reduced for the remainder of the study by approx. 6 % in males and 17 % in females compared with control values. In addition, lower group mean food consumption (-3 % in males, - 16 % in females) and reduced food efficiency in males and females were seen in dogs treated with 250 mg/kg bw compared with controls.

Table 5.3.2-13: 12 months oral (gavage) repeat dose study in dogs; cumulative group mean body weight change (g/animal/study period)

	0 mg/kg		2 mg/kg		30 mg/kg		400/250 mg/kg	
	♂	♀	♂	♀	♂	♀	♂	♀
weeks 1 – 52	1600	2600	1400	2800	2000	2000	800	600

Water consumption was distinctly reduced at weeks 13, 26 and 52 for high dose males and at weeks 26 and 52 for high dose females. The overall water consumption was reduced by 35 % in males and 26 % in females, resp., compared with control values (Table 5.3.2-14). Water consumption was not effected in dogs at the two lower dose levels.

Table 5.3.2-14: 12 months oral (gavage) repeat dose study in dogs; group mean water consumption (mL/two dogs/day)

Sampling period	0 mg/kg		2 mg/kg		30 mg/kg		400/250 mg/kg	
	♂	♀	♂	♀	♂	♀	♂	♀
days 89 – 92 (% of controls)	3300	2512	3312 (100.3)	2596 (103.3)	3448 (104.4)	2936 (116.8)	1956 (59.2)	2414 (96.0)
days 180 – 183 (% of controls)	2782	2992	2778 (99.8)	2398 (80.1)	2994 (107.6)	2628 (87.8)	1956 (70.3)	1882 (62.9)
days 362 – 365 (% of controls)	2890	2400	2688 (93.0)	2238 (93.2)	2982 (103.1)	2030 (84.6)	1948 (67.4)	1588 (66.1)
overall mean (% of controls)	2990	2634	2926 (97.8)	2410 (91.5)	3140 (105.0)	2530 (96.1)	1956 (65.4)	1960 (74.4)

Haematology, clinical chemistry, urinalysis: At the high dose level a statistically significant increase in white blood cells (mainly neutrophils) were seen in male dogs after 3, 6 and 12 months of treatment (Table 5.3.2-15).

Table 5.3.2-15: 12 months oral (gavage) repeat dose study in dogs; Haematological value white blood cells (x 10^9/ŁL)

	0 mg/kg ♂	♀	2 mg/kg ♂	♀	30 mg/kg ♂	♀	400/250 mg/kg ♂	♀
before treatment	13.01	12.89	12.45	11.66	12.17	10.71	12.92	11.52
after 3 months	10.87	11.04	11.14	10.91	12.08	10.65	14.18*	11.11
after 6 months	9.28	9.91	9.64	10.16	10.18	9.78	12.92**	10.42
after 12 months	9.88	10.22	11.08	10.20	9.91	11.71	13.76**	11.46

* ($p \leq 0.05$); ** ($p \leq 0.01$) significantly different from controls

Table 5.3.2-16: 12 months oral (gavage) repeat dose study in dogs; Group mean body weight [kg] in males

Dose [mg/kg bw]	Day					
	1	64	127	183	246	365
0	12.7	13.3	13.3	13.7	14.0	14.3
2	12.1	12.5	12.7	13.0	13.2	13.5
30	12.0	12.8	13.2	13.5	13.6	14.0
400/250	12.5	13.0	13.2	13.3	13.3	13.3

Table 5.3.2-17: 12 months oral (gavage) repeat dose study in dogs; Group mean body weight [kg] in females

Dose [mg/kg bw]	Day					
	1	64	127	183	246	365
0	11.7	12.2	12.6	13.4	13.4	14.3
2	11.7	12.4	13.0	13.1	13.6	14.5
30	11.6	12.1	12.7	13.0	12.9	13.6
400/250	11.8	11.9	11.9	12.0	12.1	12.4

Table 5.3.2-18: 12 months oral (gavage) repeat dose study in dogs; Group mean organ weight [g]

Organs \ Dose [mg/kg bw]	Males				Females			
	0	2	30	400/250	0	2	30	400/250
Testes	31.9	34.1	27.7	30.9				
Ovaries					1.63	1.57	2.03	1.10
Thyroids	1.14	0.99	0.91	1.20	0.92	0.97	0.87	0.96
Kidneys	58.0	61.9	61.5	59.9	53.8	50.8	49.1	49.5
Pituitary	0.0695	0.0738	0.0690	0.0765	0.0803	0.0803	0.0708	0.0755
Liver	386.7	365.8	399.1	404.9	352.1	350.0	361.2	353.6

In addition, a mild but statistically significant reduction in clotting time (Thrombotest) was observed in both sexes after 12 months of treatment at this dose level (15.6 [♂] sec. and 15.3 [♀] sec. compared to 16.5 [♂] sec. and 16.1 [♀] sec. in controls).
All other differences of haematological or also biochemical parameters investigated with statistical significance attaining at some dose levels were considered to be of no toxicological significance because they were either not dose-related or were n ot consistently changed or were within the normal range of controls.
Ophthalmoscopic examinations and electrocardiographic investigations revealed no treatment-related changes.
Organ weight analysis revealed no significant treatment-related effects at any dose level.
Pathology: The results of the pathological and histopathological examination showed no treatment-related changes at any dose group.

Conclusion:
The NOAEL for this study can be set at 30 mg/kg bw/d. This NOAEL is supported by clinical signs (vomiting), distinct decrease in water consumption and also haematological findings, seen at the highest concentration tested.

Relevant NOAEL for short-term toxicity in dogs

The NOAEL in the 90-day dog study is 6 mg/kg bw/d and in the 12-month dog study is 30 mg/kg bw/d. However, considering the dose levels selected, the comparable study design, the end-points addressed, and strain of animal, it is appropriate to establish an overall subchronic NOAEL of 30 mg/kg bw/d in dogs, based on the collective results from the 90-day and the one-year dog studies.

CA 5.3.3 Other routes

Neither subacute/subchronic dermal nor inhalation studies with pyrimethanil have been submitted.

CA 5.4 Genotoxicity Testing

Studies evaluated in the draft monograph of Rapporteur Member State Austria of April 15, 2004:

A sufficient data-package of in vitro genotoxicity studies in bacterial and mammalian cell systems and of in vivo genotoxicity has been evaluated by European authorities and Austria as Rapporteur Member State and was considered to be acceptable. For the convenience of the reviewer, these studies are summarized below as extracted from the monograph and the EFSA conclusion. Tabulated summaries are provided in Table 5.4-1.
In order to align the dossier submission and the draft RAR, the information that was present in the dRAR in more detail was included in this dossier update in light green, whereas new information and/or corrections were included in lime green.

Table 5.4-1: Mutagenicity studies conducted with pyrimethanil available in the original monograph

Study type	Test System	With S-9 mix	Result	Reference (BASF DocID)
In vitro Mutagenicity in bacterial cells (Ames test)	*Salmonella typhimurium* (TA 1535, 100, 1537, 98); *Escherichia coli* (CM 881 trp uv resistant pKM 101; CM 891 WP2 trp uvrA pKM 101); Concentration up to 1500 µg/plate	No Yes	Negative Negative	A81788, A81805
In vitro Mutagenicity in mammalian cells	CHO/HPRT; Concentrations up to 400 µg/mL	No Yes	Negative Negative	A81827; A89515
In vitro Cytogenicity	Chromosome aberration in human lymphocytes; Wide range of concentrations up to 500 µg/mL	No Yes	Negative Negative	A81789
In vitro DNA damage (Bacterial (REC) mutation assay)	*Bacillus subtilis* (H17 (rec^+), M45 (rec^-); Dose range: 0 – 5000 µg/disk	No Yes	Negative Negative	A81859
In vivo Cytogenicity	Mouse Micronucleus test (0, 225, 450 and 900 mg/kg bw); oral gavage	Not applicable	Negative	A81802
In vivo DNA damage and repair	USD, rat primary hepatocytes (0, 100, 300 and 1000 mg/kg bw); oral gavage	Not applicable	Negative	A81803

Pyrimethanil was tested in a battery of in vitro and in vivo mutagenicity assays with purity values ranging from 96.4% to 99.4%.
Results from these studies showed that pyrimethanil does not induce base-pair or frame-shift mutation in any of the bacterial tester strains (*S. typhimurium*, *E.coli*), or gene mutation in mammalian cells in culture (CHO-HRPT assay). No potential for clastogenicity was observed in the in vitro metaphase chromosome analysis assay in human lymphocytes or in the in vivo mouse micronucleus assay and in the UDS assay in rat hepatocytes with in vivo treatment, as well.
In conclusion, there is no evidence from the available studies of a mutagenic or clastogenic potential of pyrimethanil.

Studies submitted in this AIR 3 dossier (not yet peer-reviewed):
An in vivo study characterising the genotoxicity in germ cells was performed with pyrimethanil, which were not submitted during the previous Annex I inclusion process. Furthermore, the literature evaluation identified further in vitro studies in mammalian cells which was added as supplemental information.

Table 5.4-2: Genotoxicity study with pyrimethanil (not yet peer-reviewed)

Study type	Test System	With S-9 mix	Result	Reference (BASF DocID)
In vivo Cytogenicity Chromosome aberration in mouse spermatogonial cells	NMRI mice; 6 males / group Single oral administration by gavage of 0, 500, 1000 and 2000 mg/kg bw in 1% aqueous methyl cellulose	Not applicable	Negative	C032060
High Throughput Screen genotoxicity assays (GreenScreen, CellCiphr, CellSensor)	Mammalian cells	-	Negative	2009/1130462

No reduction of the mitotic index was observed, indicating that pyrimethanil was not cytotoxic for spermatogonial cells at the indicated doses and application route. The analysis of the chromosome aberration rates showed that pyrimethanil did not induce a biologically relevant and statistically significant increase of the aberration rates in the mouse spermatogonial cells at any of the tested doses or sampling times. Thus, it can be stated that under the experimental conditions pyrimethanil did not induce genotoxicity in germ cells. The published literature supported the lacking evidence for genotoxicity of pyrimethanil.

Pyrimethanil was not phototoxic in the 3T3 NRU Phototoxicity Test and thus did not trigger the need for further investigations on photo-effects. The data-requirement on photomutagenicity testing is waived as no agreed/validated test method is in place. No guidance on which test is to be performed under what conditions is available. This is of particular relevance, as although test systems have been developed, efforts of their validation have proven to be futile up to now. Indeed, a statement on photogenotoxicity testing by the committee on mutagenicity of chemicals in food consumer products and the environment (COM/13/S1) does not recommend photomutagenicity testing due to lack of validated test systems. This is in line with the recommendation given in the Report of the International Workshop on Genotoxicity Testing Working Group [see KCA 5.4/1 2010/1233312]. Accordingly the Guidance for applicants on preparing dossiers" (SANCO/10181/2013-rev. 2.1) states that when no agreed test methods or guidance documents are available waiving of these particular data requirements is considered acceptable.

Based on the available data on the genotoxicity, classification of pyrimethanil for this endpoint is not required according to the criteria laid down in Regulation (EU) No. 1272/2008 (CLP). Moreover, the data on genotoxicity of pyrimethanil have already been evaluated in 2006, coming to the conclusion that pyrimethanil does not need to be classified for health effects (ECBI/159/04) as laid down in the 31st ATP to the Council Directive 67/548/EEC (Commission Directive 2009/2/EC of 15 January 2009). The not yet peer-reviewed genotoxicity studies, in vivo chromosome aberration in mouse spermatogonial cells and the supplemental mammalian cell screening assays described in public literature have not been submitted for evaluation of classification. The results of these studies will however not change the classification and labelling of pyrimethanil.

Based on the available studies, the conclusion for relevant endpoints adopted to the new list of endpoint format for the current re-registration is as follows:

Genotoxicity (Regulation (EU) N° 283/2013, Annex Part A, point 5.4)

In vitro studies	Ames test: Negative CHO-HRPT assay: Negative Chromosome analysis assay in human lymphocytes: Negative Bacterial (REC) mutation assay: Negative High Throughput Screen genotoxicity assays (GreenScreen, CellCiphr, CellSensor) in mammalian cells: Negative	
In vivo studies	Mouse micronucleus assay: Negative UDS assay: Negative Chromosome analysis assay in mouse spermatogonial cells: Negative	
Photomutagenicity	Not required	
Potential for genotoxicity	No evidence for genotoxic potential	

CA 5.4.1 In vitro studies

The in vitro genotoxicity studies of pyrimethanil were evaluated and peer reviewed during the previous Annex I inclusion process (for further details please see Table 5.4-1). All of these tests were clearly negative.
Additional literature data, not yet peer reviewed during the previous Annex I inclusion process, are provided below.

Report:	CA 5.4.1/1 Jones E.,Gant R.A., 1990 a Technical SN 100309: Bacterial mutation assay A81788
Guidelines:	EPA 84-2, OECD 471
GLP:	yes (certified by Department of Health and Social Security of the Government of the United Kingdom, United Kingdom)
Deviations:	The recommended bacterial concentration is 109 cells/ml, however, bacterial cultures used in the assay contained 2 x 109 cells/mL. 2-aminoanthracene should not be used as the only indicator of S9 mix efficacy. For assays performed with TA1535 or TA100 strains without metabolic activation, sodium azide is recommended as a positive control. No historical control data provided.

Material and Methods:
Pyrimethanil (batch no. CR 19325/1; purity 98.7 %) was tested in the Ames test using histidine dependent auxotrophic mutants of *Salmonella typhimurium* (strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100). Pyrimethanil (dissolved in DMSO) was added at concentrations of 0 (solvent control), 15, 50, 150, 500 and 1500 µg/plate in the presence and absence of S-9 mix (liver preparations from Aroclor 1254-induced male Sprague-Dawley rats). Dose levels were established on the basis of a preliminary range finding test. (Pyrimethanil at a concentration of 5000 µg/plate induced cytotoxicity and precipitation on the plates was seen.) In two separate occasions three replicates per concentration were incubated at 37°C for 3 days.
As positive controls 9-aminoacridine (80 µg/plate administered to TA 1537), 2-nitrofluorene (1 and 2 µg/plate administered to TA 98 and TA 1538, resp.), N-ethyl-N'-nitro-N-nitroso guanidine (3 and 5 µg/plate administered to TA 100 and 1535) and 2-aminoanthracene (0,5 – 2 µg/plate administered to all strains with metabolic activation) were used.
Evaluation criteria: The test was considered positive if there is an increase in revertant colonies of at least twice the solvent control, with some evidence of a positive dose-relationship, with any bacterial strain either with or without S-9 mix. If the treatment does not produce reproducible increases of at least 1.5 times the solvent controls, at any dose level with any bacterial strain, it is considered to show no evidence of mutagenic activity. No statistical analysis is performed.

Table 5.4.1-1: **Bacterial mutation assay; Revertant colony counts per plate; IL = incomplete bacterial lawn, NL = no bacterial lawn, + = presence, - = absence**

Strain	Dose level [µg/plate]	Mean revertant colony counts	
		Test 1 -S9 mix/+S9 mix	Test 2 -S9 mix/+S9 mix
TA 1535	**1500**	IL/IL	IL/IL
	500	10/12	9/9
	150	10/12	9/11
	50	12/11	8/8
	15	12/14	6/10
	Solvent	14/15	8/10
TA 1537	**1500**	IL/IL	IL/IL
	500	14/9	7/11
	150	12/11	12/10
	50	9/11	13/12
	15	11/10	5/6
	Solvent	13/11	7/11
TA 1538	**1500**	IL/IL	IL/IL
	500	7/9	7/15
	150	8/10	11/11
	50	7/10	7/10
	15	8/10	8/11
	Solvent	6/8	8/10
TA 98	**1500**	IL/IL	NL/IL
	500	21/19	IL/7
	150	20/23	17/20
	50	21/19	16/16
	15	19/21	17/15
	Solvent	22/25	15/14
TA 100	**1500**	IL/IL	IL/IL
	500	59/82	IL/IL
	150	113/123	53/64
	50	119/110	77/76
	15	111/125	79/81
	Solvent	132/136	84/93

Table 5.4.1-2: **Bacterial mutation assay; Revertant colony counts per plate: positive control; + = presence, - = absence, ENNG = N-ethyl-N-nitro-N-nitroso guanidine, 9AC = 9-aminoacridine, NF = 2-nitrofluorene, AA = 2-aminoanthracene**

Strain	Compound	Dose level [µg/plate]	S9 mix	Mean revertant colony counts	
				Test 1	Test 2
TA 1535	**ENNG**	**5**	-	409	634
TA 1537	**9AC**	**80**	-	1233	2205
TA 1538	**NF**	**2**	-	69	57
TA 98	**NF**	**1**	-	126	53
TA 100	**ENNG**	**3**	-	539	341
TA 1535	**AA**	**2**	+	87	41
TA 1537	**AA**	**2**	+	79	64
TA 1538	**AA**	**0.5**	+	76	61
TA 98	**AA**	**0.5**	+	81	54
TA 100	**AA**	**1**	+	273	158

Findings:
In both trials cytotoxicity was observed at 1500 µg/plate with and without S-9 mix. However, no substantial increases in revertant colony numbers of any of the strains tested were observed following treatment with pyrimethanil at any dose level, either in the presence or absence of metabolic activation. The positive control materials elicited the expected positive responses.

Conclusion:
It can be concluded that pyrimethanil was not mutagenic when tested at dose levels up to 1500 µg/plate in this test system.

Report:	CA 5.4.1/2 Jones E.,Gant R.A., 1991 a Technical SN 100309: Bacterial mutation assay with Escherichia coli A81805
Guidelines:	EPA 84-2, OECD 472
GLP:	yes (certified by Department of Health and Social Security of the Government of the United Kingdom, United Kingdom)
Deviations:	The recommended bacterial concentration is 109 cells/ml, however, bacterial cultures used in the assay contained 2 x 109 cells/mL. 2-aminoanthracene should not be used as the only indicator of S9 mix efficacy. No historical control data provided.

Material and Methods:
Pyrimethanil (batch no. CR 19325/01/900304; purity 96.4 %) was tested in the Ames test using tryptophan dependent auxotrophic mutants of *Escherichia coli* (strains: CM 881 WP2 trp uv resistant pKM 101 and CM 891 WP2 trp uvrA pKM 101). The test substance (dissolved in DMSO) was added at concentrations of 0 (solvent control), 15, 50, 150, 500 and 1500 µg/plate in the presence and absence of S-9 mix (liver preparations from Aroclor 1254-induced male Sprague-Dawley rats). Dose levels were established on the basis of the preliminary range finding test mentioned before. In two separate trials three replicates per concentration were incubated at 37°C for 3 days.
N-ethyl-N'-nitro-N-nitroso guanidine (2 µg/plate) was used as positive control in the absence of the activating system, and 2-aminoanthracene (20 µg/plate) in its presence.
Evaluation criteria: The same evaluation criteria were used as described in the previous test.

Findings:
In both trials, no substantial increases in revertant colony numbers in both strains tested were observed following treatment with pyrimethanil at any dose level, either in the presence or absence of metabolic activation. The positive control materials elicited the expected positive responses.

Table 5.4.1-3: Bacterial mutation assay with Escherichia coli; Revertant colony counts per plate; + = presence, - = absence

Strain	Dose level [µg/plate]	Mean revertant colony counts	
		Test 1 -S9 mix/+S9 mix	Test 2 -S9 mix/+S9 mix
CM 881	**1500**	29/29	21/27
	500	63/59	59/64
	150	50/50	72/83
	50	55/55	71/79
	15	59/53	75/78
	Solvent	51/62	80/82
CM 891	**1500**	100/98	194/192
	500	194/206	212/193
	150	232/211	241/247
	50	279/228	213/216
	15	208/225	219/211
	Solvent	253/211	206/172

Table 5.4.1-4: **Bacterial mutation assay with Escherichia coli; Revertant colony counts per plate: positive control; + = presence, - = absence, ENNG = N-ethyl-N-nitro-N-nitroso guanidine, AA = 2-aminoanthracene**

Strain	Compound	Dose level [µg/plate]	S9 mix	Mean revertant colony counts	
				Test 1	Test 2
CM 881	**ENNG**	**2**	-	1097	1323
CM 891	**ENNG**	**2**	-	1221	1444
CM 881	**AA**	**20**	+	114	232
CM 891	**AA**	**20**	+	873	961

Conclusion:
It can be concluded that pyrimethanil was not mutagenic when tested at dose levels up to 1500 µg/plate in this bacterial test system.

Report:	CA 5.4.1/3 Gant R.A.,Jones E., 1994 a Pyrimethanil: Bacterial (REC) assay for DNA damage A81859
Guidelines:	EPA 84-2, JMAFF 59 NohSan No 4200
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)
Deviations:	The stability of the test compound in the solvent was not determined.

Material and Methods:
Pyrimethanil (batch no. CR19325/01/900304; purity: 96.2%; dissolved in DMSO) was added on 6 mm ∅ filter paper disks at concentrations of 0 (solvent control, 5, 150, 500, 1500 and 5000 µg/disk and these disks were placed on petri dishes containing cultures of *Bacillus subtilis* (strain H17 [repair proficient rec^+] and M45 [repair deficient rec^-]) in agar in the absence and presence of rat liver S-9 mix (liver preparations from Aroclor 1254-induced male Sprague-Dawley rats). All plates were incubated at 37°C for 18 – 20 hours. After this period the diameter of any zone of toxicity and/or inhibition surrounding the disks were measured. The test was conducted on two separate occasions.
AF-2 (0.0005 - 0.016 µg/disk) was used as positive control in the absence of the activating system, and 2-aminofluorene (25 – 400 µg/disk) in its presence. As negative controls (in order to demonstrate the integrity of both tester strains), kanamycin (without S-9mix) and streptomycin (with S-9 mix) were used.
Evaluation criteria: The growth inhibition ratio of strain H17 : strain M45 of less than 0.75 indicates preferential killing of the rec^- strain. If this is observed in two separate experiments, with some evidence of dose-relation, the test substance will be considered to cause damage to the bacterial DNA. If no preferential killing of the rec^- strain is observed the test substance will be considered not to cause damage to the bacterial DNA.

Findings:
Following treatment with pyrimethanil, in both tests, zones of toxicity and/or inhibition were observed at dose levels between 500 – 5000 µg/disk in the absence of S-9 mix, and between 150 – 5000 µg/disk in the presence of S-9 mix. No substantial differences in toxicity were observed between the two strains in either the first or the second spot test. The growth inhibition ratio was in the range of 0.83 – 1.10 (without S-9 mix) and 0.82 – 1.00 (with S-9 mix).
The positive control AF-2 produced little or no toxicity towards strain H17 but showed clear toxicity towards strain M45, thereby demonstrating sensitivity of the assay. Results with 2-aminofluorene were less marked although slight differential cytotoxicity was seen in the first assay. The negative controls induced little or no consistent preferential killing in either bacterial strain.

Conclusion:
It is concluded that, when tested at dose levels up to 5000 µg/disk in DMSO, pyrimethanil showed no potential to damage bacterial DNA.

Report:	CA 5.4.1/4 Adams K. et al., 1992 a Technical SN 100 309: In vitro Chinese hamster ovary/HPRT locus gene mutation assay A81827
Guidelines:	OECD 476, EPA 84-2
GLP:	yes (certified by Department of Health and Social Security of the Government of the United Kingdom, United Kingdom)

Report:	CA 5.4.1/5 Jackson C.M.,Everett D.J., 1994 a 1st amendment to report No. TOX/92/223-57 - Technical SN 100309: In vitro Chinese hamster ovary/HPRT locus gene mutation assay A89515
Guidelines:	OECD 476, EPA 84-2
GLP:	yes (certified by Department of Health and Social Security of the Government of the United Kingdom, United Kingdom)
Deviations	Absence of mycoplasma contamination of CHO cells should be mentioned. Sufficient number of cells (but never less than 2 million) should be cultured during the expression period and plated for mutant selection. This was not met in the study. The cytotoxicity was expressed as cloning efficiency instead of relative survival (RS); the recommended formula for RS calculation was not used. No appropriate historical control data are provided.

Material and Methods:

Pyrimethanil (batch no. CR 19325/01/900304; purity 97.2 %; dissolved in DMSO) was tested for its ability to induce point mutations at the hypoxanthine-guanine-phosphoribosyl-transferase(HPRT) locus of Chinese hamster ovary (CHO-K1-BH_4) cells both with and without metabolic activation (liver S-9 homogenate fraction from Aroclor 1254 induced male Sprague Dawley rats). The CHO cells were exposed to pyrimethanil for a period of 4 hours at concentrations of 0, 10, 50, 100, 150, 200, 220, 230, 240, 250, 300 and 400 µg/mL culture without activation and at concentrations of 0, 10, 50, 100, 150, 200, 250, 265, 280, 300 and 400 µg/mL culture with S-9 mix. These dose levels were established on the basis of preliminary toxicity test with dose levels of 0, 5, 15, 30, 70, 125, 250, 500, 1000 and 1775 µg/mL. DMSO was used as negative control and ethylmethan sulfonate (250 µg/mL) and 20-methylcholanthrene (5 µg/mL) were used as positive controls with and without S-9 activation resp. After a mutation expression period of 7 days at 37°C 6-thioguanine was added as selective agent and 5 plates for each treatment group were seeded with 2 x 10^5 cells. In addition, cell samples for the estimation of plating efficiency (3 replicate plates) were seeded with 200 cells each without addition of the selective agent. After a subsequent incubation for further 7 days, the cells were fixed, stained and counted. Two independent tests in the absence as well as in the presence of metabolic activation were carried out.

Evaluation criteria: To be an adverse effect, the mutant frequency must exhibit a reproducible, significant increase compared both to the concurrent solvent control and the historical control of the laboratory with evidence of a dose relationship to the response observed.

Findings:
In the preliminary toxicity test, concentrations of 250 µg pyrimethanil/mL (without S-9 mix) and 500 µg/mL (with S-9 mix) induced marked cytotoxicity.
In the main study (without S-9 mix), treatment of cells with 10 – 400 µg/mL (1st test) and 10 – 240 µg/mL (2nd test) resulted in mean cell survivals of 95 – 0 % and 93 – 54 %, resp. Cultures treated with 10, 50, 100, 150 and 200 µg/mL and 150, 200, 220, 230 and 240 µg/mL were therefore assessed for viability and induced mutations. No significant increases in mutant frequency were observed in both tests. The positive control EMS induced highly signifcant increases in mutant frequency.
In the presence of S-9 mix, treatment of cells with 10 – 400 µg/mL (1st test) and 10 – 280 µg/mL (2nd test) resulted in mean cell survival rates of 106 – 0% and 145 – 42 %, resp. Cultures treated with 50, 100, 150, 200 and 250 µg/mL and 150, 200, 250, 265 and 280 µg/mL were assessed for viability and induced mutations. Statistically significant increases in mutant frequency were observed at the top two dose levels in test 1 but only at one intermediate dose level in test 2 with no significant increases at higher dose levels. Thus, the findings were neither reproducible nor dose-related. Moreover, these increases were less than the historical control level of 15 and therefore, not considered to be toxicologically significant. 20-methylcholanthrene, the positive control, induced highly significant increases in mutant frequency.
The results of cytotoxicity, plating efficiency and mutant frequency in CHO cells are presented in Table 5.4.1-5.

Table 5.4.1-5: Cytotoxicity and mutant frequency in CHO cells (mean values)

Compound	conc. (µg/mL) 1st test	conc. (µg/mL) 2nd test	mean cell survival (% of control) 1st test	mean cell survival (% of control) 2nd test	plating efficiency [1] (%) 1st test	plating efficiency [1] (%) 2nd test	Mean mutant frequency per 10^6 survivors [2] 1st test	Mean mutant frequency per 10^6 survivors [2] 2nd test
without S-9 mix								
DMSO	-	-	100	100	61.7	54.5	5	5
Pyrimethanil	10		95		63		3	
	50		77		59.5		5	
	100		89		56.5		3	
Pyrimethanil	150	150	78	81	61.5	51	3	3
	200	200	61	63	63,5	53.5	4	9
		220		86		58		2
		230		54		51.5		4
		240		59		52.5		10
EMS	250	250	76	54	45	39.5	247***	320***
with S-9 mix								
DMSO	-	-	100	100	72.5	64.2	5	2
Pyrimethanil	50		106		67.5		6	
	100		99		66		5	
	150	150	93	85	68.5	62	3	4
	200	200	97	113	81.5	66	9*	9*
	250	250	58	100	62.5	58.5	14***	5
		265		67		58		2
		280		42		56		3
Methyl-cholanthrene	5	5	103	98	62.5	45	302***	426***

* ($p \leq 0.05$) significantly different from control; *** ($p \leq 0.001$) significantly different from control;

1) % plating efficiency = total number of colonies on non-selective plates x 100/number of cells seeded (600);

2) total number of colonies on selective plates x 600/number of colonies on non-selective plates

Conclusion:
It was concluded that pyrimethanil did not demonstrate mutagenic potential in this in vitro test system.

Report:	CA 5.4.1/6 Brooker P.C. et al., 1990 a Technical SN 100309: Metaphase chromosome analysis of human lymphocytes cultured in vitro A81789
Guidelines:	EPA 84-2, OECD 473
GLP:	yes (certified by Department of Health and Social Security of the Government of the United Kingdom, United Kingdom)
Deviations:	The report should include information on the source of blood lymphocytes, i.e. the state of health, number, age, and sex of donors. The following experimental conditions should be conducted: (i) a short term treatment with test substance in the absence of S9 mix; (ii) a short term treatment with test substance in the presence of S9 mix; (iii) a long term treatment with test substance in the absence of S9 mix; The first one was not performed. In addition, during the short term treatment, the cells should be exposed to the test substance for 3-6 hours instead of 2 hours. Ethylmethanesulphonate has been already excluded from the list of reference substances recommended as a positive control not requiring the metabolic activation. At least 300 well-spread metaphases should be scored per each concentration of test substance and control, which was not met in the study. Appropriate historical control data are not provided.

Material and Methods:

Cultures of human lymphocytes (stimulated to divide by treatment with phytohaem-agglutinin) were exposed to pyrimethanil (batch no. CR 19325/3; purity: 99.4 %; dissolved in DMSO) with and without metabolic activation (liver preparations from Aroclor 1254-induced male Sprague-Dawley rats) at 37°C at concentrations of 0 (solvent control), 1, 2, 3.9, 7.8, 15.6, 31.3, 62.5, 125, 250 and 500 µg/mL. Cells which did not receive S-9 metabolic activation were harvested at 24 and 42 hours after continuous exposure. Cells which received S-9 metabolic activation were treated with the test material for 2 hours and then placed in fresh media and incubated for further 22 and 40 hours. About 2 h prior to harvesting the cells, colchicine (0,25 µg/mL) was added. After hypotonic treatment, fixation of the cells and staining of 5 slides per culture, metaphase analysis was carried at a dose level causing a decrease in mitotic index of 50 – 80 % of the solvent control value or, if there was no decrease, at the maximum achievable concentration. The highest dose level alone was analysed at the 42 hour harvest. However, at the 24 hours harvest, intermediate and low dose levels were also analysed which were 50 % and 12.5 % of the highest concentration investigated. All samples were run in duplicate for each concentration tested. As positive controls, ethylmethane sulfonate (1000 µg/mL) and cyclophosphamide (20 µg/mL) were used for non-activation series and for metabolic activation series, resp.

Evaluation criteria: 100 metaphase figures from each duplicate were examined with normally a maximum of 25 from each slide. The cells were evaluated for the presence of structural chromosomal aberrations and gaps; statistical analysis (Fisher' test) was performed.

Findings:
Cytotoxicity: In the absence of metabolic activation 24 hours after initiation of the treatment pyrimethanil caused a dose-dependent decrease in mitotic index at the four highest dose levels compared to solvent control with the highest dose (500 µg/mL) yielding no live cells. For the dose levels 125 and 62.5 µg/mL, mitotic indices of 34.2 % and 64.6 % of the solvent control value (mitotic index of 7.9 %) were reported. Cultures harvested after 42 hours again showed dose-dependent decreases in mitotic indices with the concentration of 125 µg/mL, yielding a mitotic index of 49 % of the solvent control value (mitotic index of 5.1 %).
In the presence of S-9 mix, pyrimethanil caused a dose-dependent decrease in mitotic index at the two highest dose levels for both the 24 and 42 hour harvest. The second highest dose level caused a decrease in mitotic index to 56.4 and 46.6 % of the solvent control value (mitotic indices of 9.4 and 10,3 %), resp.
Based in these results following concentrations were selected for metaphase analysis: 24 hours harvest: 7.8, 31.3 and 62.5 µg/mL without S-9 mix; 31.3, 125 and 250 µg/mL with S-9 mix; 42 hours harvest: 125 µg/mL without S-9 mix and 250 µg/mL with S-9 mix.
Metaphase analysis: In both the presence and absence of metabolic activation pyrimethanil caused no statistical significant increase in the proportion of metaphase figures containing structural chromosomal aberrations at either 24 or 42 hours after initiation of treatment when compared with the solvent control. The mean % of aberrant cells (no. aberrant cells/total no. cells x 100) are given in Table 5.4.1-6. Both positive control compounds caused statistically significant increases in chromosomal damage.

Table 5.4.1-6: Mean % of aberrant cells (including and excluding gaps)

	Without metabolic activation				With metabolic activation			
Dose (µg/mL)	**24 hours**		**42 hours**		**24 hours**		**42 hours**	
	Excl. Gaps	**Incl. gaps**	**Excl. gaps**	**Incl. gaps**	**Excl. gaps**	**Incl. gaps**	**Excl. Gaps**	**Incl. gaps**
DMSO (solvent control)	0	0	0	0	0	0	0.25	0.25
7.8	0	0	-	-	-	-	-	-
31.3	0	0.5	-	-	0	0	-	-
62.5	0.5	0.5	-	-	-	-	-	-
125	-	-	0.5	0.5	0.5	0.5	-	-
250	-	-	-	-	0.5	0.5	0.5	0.5
Ethylmethansulphonate	14.5*	14.5*	-	-	-	-	-	-
Cyclophosphamide	-	-	-	-	15*	15*	-	-

* significantly different from DMSO at $p \leq 0.001$

Conclusion:
Pyrimethanil technical has shown no evidence of clastogenic activity under the conditions of this in vitro assay.

Literature data

Report:	CA 5.4.1/7 Knight A.W. et al., 2009 a Evaluation of high-throughput genotoxicity assays used in profiling the US EPA ToxCast chemicals 2009/1130462
Guidelines:	none
GLP:	no
Deviations:	not appropriate

Executive Summary of the Literature

Three high-throughput screening (HTS) genotoxicity assays-GreenScreen HC GADD45a-GFP (Gentronix Ltd.), CellCiphr p53 (Cellumen Inc.) and CellSensor p53RE-bla (Invitrogen Corp.)-were used to analyse the collection of 320 predominantly pesticide active compounds being tested in Phase I of US. Environmental Protection Agency's ToxCast research project. Between 9% and 12% of compounds were positive for genotoxicity in the assays. However, results of the varied tests only partially overlapped, suggesting a strategy of combining data from a battery of assays. The HTS results were compared to mutagenicity (Ames) and animal tumorigenicity data. Overall, the HTS assays demonstrated low sensitivity for rodent tumorigens, likely due to: screening at a low concentration, coverage of selected genotoxic mechanisms, lack of metabolic activation and difficulty detecting non-genotoxic carcinogens. Conversely, HTS results demonstrated high specificity, >88%. Overall concordance of the HTS assays with tumorigenicity data was low, around 50% for all tumorigens, but increased to 74-78% (vs. 60% for Ames) for those compounds producing tumours in rodents at multiple sites and, thus, more likely genotoxic carcinogens. The aim of the present study was to evaluate the utility of HTS assays to identify potential genotoxicity hazard in the larger context of the ToxCast program, to aid prioritization of environmentally relevant chemicals for further testing and assessment of carcinogenicity risk to humans.

For pyrimethanil negative results were observed in all three screening assays, indicating that pyrimethanil has no genotoxic properties supporting available in vitro and in vivo data.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Pyrimethanil
Description: not specified
Batch/purity #: not specified
Stability of test compound: not specified

2. Control Materials:
Vehicle control: GreenScreen HC GADD45a-GFP: DMSO
CellCiphr p53: DMSO
CellSensor p53 RE-bla: DMSO
Positive control: GreenScreen HC GADD45a-GFP: not specified
CellCiphr p53: mentioned, but not specified
CellSensor p53 RE-bla: Nultilin-3 (12 µM)

3. Test Organisms: GreenScreen HC GADD45a-GFP: human lymphoblastoid TK6 cell line (GADD45a-GFP reporter strain and out-of-frame EGFP gen control strain)
CellCiphr p53: human HepG2 cell line
CellSensor p53 RE-bla: HCT-116 cell line

4. Test Concentrations: GreenScreen HC GADD45a-GFP: 50, 100 and 200 µM
CellCiphr p53: 0.39 – 200 µM
CellSensor p53 RE-bla: 1.2 nM – 92 µM

B. TEST PERFORMANCE:

1. Cytotoxicity assay:

GreenScreen HC GADD45a-GFP:
Inhibition of cell proliferation was detected by reduction of optical absorbance.

CellCiphr p53:
Cell loss was recorded by measuring the cell counts after Hoechst 3342 staining with Arrayscan HCS Reader, and IC_{50} values were calculated.

CellSensor p53 RE-bla:
not specified

2. Genotoxicity assay:

GreenScreen HC GADD45a-GFP:
Human *GADD45a* mediated growth arrest and DNA damage was recorded by p53 regulated induction of GFP protein via fluorescence. A control strain containing an out-of-frame EGFP gene with non-functional GFT protein was used for corrections of auto-fluorescence or non-specific induced cellular fluorescence.

CellCiphr p53:
DNA damage was recorded by measurement of p53 activation via a fluorescent anti-p53 antibody (Alexa Fluor 488). Half-maximal activity (AC_{50}) values were determined by fitting the data to the Hill equation using the Condoseo module of Genedata Screener (Genedata AG, Basel, Switzerland).

CellSensor p53 RE-bla:
Activation of the p53 controlled beta-lactamase were recorded via proprietary "GeneBLAzer" technology based on fluorescence resonance energy transfer (FRET). Data were expressed as the ratio of emissions at 460 nm/530 nm (excitation at 405 nm). For primary data analysis, readings for each titration point were first normalized relative to the Nultilin-3 control (12 µM, 100%) and wells containing the vehicle only (basal, 0%), and then corrected by applying a pattern correction algorithm using control plates containing the DMSO diluent alone. Concentration–response titration points for each compound were fitted to the Hill equation and concentrations of half-maximal activity (AC_{50}) and maximal response (efficacy) values were calculated.

3. Evaluation criteria:

GreenScreen HC GADD45a-GFP:

If the cell density relative to a vehicle-treated control fell below 80% at 1 test concentration the compound was deemed cytotoxic and if extended over 2 or 3 concentrations, strongly cytotoxic. Otherwise the compound was considered negative for cytotoxicity.

If induction of GFP fluorescence relative to a vehicle-treated control exceeded 50% at 1 test concentration the compound was deemed genotoxic and if extended over 2 or 3 concentrations, strongly genotoxic. Otherwise the compound was considered negative for genotoxicity.

CellCiphr p53:

A positive result was concluded if the p53 AC_{50} was calculated to be below 200 µM, provided the AC_{50} was lower than the IC_{50} for cell loss/cytotoxicity at for that time point.

CellSensor p53 RE-bla:

A positive result was concluded if the p53 AC_{50} was calculated to be below 92 µM.

II. RESULTS AND DISCUSSION

Negative result were obtained with pyrimethanil in the GreenScreen HC GADD45a-GFP, CellCiphr p53 and CellSensor p53RE-bla assays tested up to 200 µM.

III. CONCLUSION

According to the results of the present study, the test substance pyrimethanil is not mutagenic in the GreenScreen HC GADD45a-GFP, CellCiphr p53 and CellSensor p53RE-bla assays under the experimental conditions chosen here.

Classification of study: Supplementary information

CA 5.4.2 In vivo studies in somatic cells

The in vivo genotoxicity studies of pyrimethanil were evaluated and peer reviewed during the previous Annex I inclusion process (for further details please see Table 5.4-1). All of these tests were clearly negative.

Report:	CA 5.4.2/1 [redacted] 1991 a Technical SN 100309: Mouse micronucleus test A81802
Guidelines:	EPA 84-2, OECD 474, EPA 560/6-83-001
GLP:	yes (certified by Department of Health and Social Security of the Government of the United Kingdom, United Kingdom)
Deviations:	The mice were not acclimated to the laboratory conditions for at least five days. Individual weight of animals at the beginning and end of the study were not included in the report. At least 4000 immature erythrocytes per animal should be examined for evaluation of micronucleated immature erythrocyte incidence, which was not met in the study. The report did not include analysis of food and water quality.

[see KCA 5.4.2/2 C002477]

Material and Methods:
Pyrimethanil (batch no. CR 19325/4; purity 97.6 %; suspended in 1 % w/v methyl cellulose in distilled water) was administered to 15 male and 15 female CD mice (source: [redacted]) per dose group by gastric intubation: Single treatments have been performed at doses of 0 (vehicle control), 225, 450 and 900 mg/kg bw. Dose levels were based on a preliminary study with 900 mg/kg bw being the maximum tolerated dose estimated. For each dose group, bone marrow smears from the femurs were obtained from 5 males and 5 females at 24, 48 and 72 hours after application. The positive control group (5 males and 5 females) received a single oral dose of mitomycin C (12 mg/kg bw); bone marrow smears were taken 24 hours after dosing. One smear from each animal in the high dose group, the positive and vehicle groups were examined for (i) the presence of micronucleated cell per 1000 polychromatic erythrocytes per animal, (ii) the ratio of polychromatic to normochromatic erythrocytes of at least 1000 erythrocytes, and (iii) the number of micronucleated normochromatic erythrocytes. In addition (1st addendum), slides from the low dose and mid dose groups (5 males and 5 females per dose level) at the 24 and 48 hour sampling time were analysed by examining 2000 polychromatic erythrocytes per animal.
Evaluation criteria: A positive response is indicated by a significant increase in the incidence of micronucleated polychromatic erythrocytes compared to the incidence in vehicle control for at least one of the sampling time.

Findings:
Clinical findings: Signs of systemic toxicity (decreased respiratory rate, hunched posture, lethargy) were seen in all groups treated with pyrimethanil. No signs were seen in the vehicle control and positive control groups.
Histology: Pyrimethanil at any dose level tested did not cause any statistically significant increase in the number of micronucleated polychromatic and normochromatic erythrocytes in any of the three time points investigated. All group mean values and individual values for animals treated with pyrimethanil fell within the range of the laboratory historical control data. In addition, there was no significant decrease in the ratio of polychromatic to normochromatic erythrocytes. Mitomycin C caused statistically significant increases in the frequency of micronucleated polychromatic erythrocytes and also significant decreases in the ratio of polychromatic to normochromatic erythrocytes. Detailed results are given in Table 5.4.2-1.

Table 5.4.2-1: Incidence of micronucleated erythrocytes and the ratio of polychromatic to normochromatic erythrocytes

Sampling time	Treatment	Dose (mg/kg)	Ratio p/n (mean)	Incidence mnp (mean ‰)	Incidence mnn (mean ‰)
24 hours	Vehicle control	-	1.159	0.4	0.6
	Pyrimethanil	225	1.150	0.6	0.4
		450	0.984	1.0	0.37
		900	1.394	0.5	0.6
	Mitomycin C	12	0.663*	41.3*	1.2
48 hours	Vehicle control	-	1.015	0.4	0.2
	Pyrimethanil	225	1.254	0.95	0.84
		450	1.322	0.85	0.21
		900	0.983	1.2	0.6
72 hours	Vehicle control	-	1.216	0.4	0.0
	Pyrimethanil	900	1.175	0.7	0.4

* significantly different vom vehicle control at $p \leq 0.001$ (Wilcoxon's sum of ranks test)
p/n - ratio of polychromatic to normochromatic erythrocytes
mnp - number of micronucleated cells per 1000 polychromatic erythrocytes examined
mnn - number of micronucleated cells per 1000 normochromatic erythrocytes examined

Conclusion:
Based on these findings, pyrimethanil technical is considered negative in the *in vivo* mouse micronucleus assay. Therefore, it is concluded that pyrimethanil technical did not cause chromosomal damage *in vivo*.

Report:	CA 5.4.2/3 [redacted] 1991 a Technical SN 100 309: Unscheduled DNA synthesis assay in rat hepatocytes treated in vivo A81803
Guidelines:	EPA 84-2, OECD 482, EPA 560/6-83-001
GLP:	yes (certified by Department of Health and Social Security of the Government of the United Kingdom, United Kingdom)
Deviations:	no

[see KCA 5.4.2/4 A89513]

Material and Methods:
Groups of 6 male rats (strain: Sprague Dawley CD; source: [redacted] were given single oral doses (gastric intubation) of 0 (vehicle control), 100, 300 and 1000 mg/kg bw pyrimethanil (batch no. CR 19325/4; purity 97.6 %; suspended in 1 % w/v methyl cellulose in distilled water); these dose levels were based on the results of a preliminary toxicity test.
2 and 14 hours after dosing of the rats hepatocytes were isolated from 3 animals per dose by enzymatic dissociation. Cells were cultured (3 replicate cultures per animal) in vitro with [methyl–^{3}H] thymidine with a final activity of 10 µCi/mL for 4 hours followed by a further 24 hours incubation with unlabelled thymidine. After fixing and staining of the cells, incorporation of thymidine into nuclear DNA was quantified autoradiographically in three slides per animal. Usually 50 nuclei were examined per culture together with 50 equivalent cytoplasmic areas. As positive control 3 male rats were treated with 4 mg dimethylnitrosamine/kg bw by the oral route; examination of the cells was performed for the 2 hours expression time only.
Evaluation criteria: Unscheduled DNA synthesis (DNA repair) was assessed by comparing both gross and net nuclear grain counts from treated animals with concurrent control levels (one-way analysis of variance). A positive response is indicated by a significant increase in the net nuclear grain count over concurrent control values and an associated increase in the corresponding gross nuclear grain count.

Findings:
Clinical signs: Animals treated with pyrimethanil at 1000 mg/kg only showed lethargy and piloerection 2 hours after dosing. No mortalities occurred.
Autoradiography: No statistical significant increases in the net nuclear grain count were obtained in any dose level after either the 2 or the 14 hours exposure period in animals treated with pyrimethanil. A small, statistically significant increase in the gross nuclear grain count was seen at the 14 hour expression time with 1000 mg/kg pyrimethanil. This finding was considered to have arisen by chance since the concurrent control count was very low. In addition, there was no significant increase in the net nuclear grain count at this concentration point.
The positive control substance gave the expected significant increase in net nuclear grain count accompanied by a large increase in the gross nuclear grain count. Mean values of nuclear grain counts are given in Table 5.4.2-2.

Table 5.4.2-2: Nuclear grain count (mean values of 3 cultures and of 3 animals, resp.)

Dose	After 2 hours		After 14 hours	
(mg/kg bw)	Gross nuclear grain count	Net nuclear grain count	Gross nuclear grain count	Net nuclear grain count
0 (vehicle control)	12.9	-1.9	9.7	-2.3
100	14.2	-1.7	11.3	-2.1
300	12.2	-2.4	13.1	-2.1
1000	13.2	-2.1	13.6*	-1.7
Dimethyl-nitrosamine	36.3*	24.7**	-	-

significantly different from vehicle control at (*) $p \leq 0{,}01$; (**) $p \leq 0{,}001$ (analysis of variance)

Conclusion:
It can be concluded that pyrimethanil showed no potential to induce unscheduled DNA synthesis in rat hepatocytes treated in vivo.

CA 5.4.3 In vivo studies in germ cells

Report:	CA 5.4.3/1 [redacted] 2003b In vivo chromosome aberration assay in mouse spermatogonial cells with Pyrimethanil C032060
Guidelines:	OECD 483
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Landwirtschaft und Forsten, Wiesbaden)
Deviations:	no

Executive Summary

Pyrimethanil (batch No. OP2150064; purity 99.6%) was tested in vivo for the ability to induce chromosome and numerical aberrations in spermatogonial cells of male NMRI mice. Six animals per dose group and sampling point were treated with 500, 1000, 2000 mg/kg bw (24 h sacrifice) and 2000 mg/kg bw (48 h sacrifice) pyrimethanil by gavage with an administration volume of 10 mL/kg bw. Directly after sacrifice, spermatogonial cells were prepared from testicles, fixed with methanol/glacial acetic acid fixative (3+1), and stained with Giemsa. Vehicle (aqueous methyl cellulose, 1%) and positive control (Adriblastin, 5 mg/kg bw) were included to demonstrate the sensitivity of the test system.
No reduction of the mitotic index was observed, indicating that the test item was not cytotoxic for spermatogonial cells at the indicated doses and application route. The analysis of the chromosome aberration rates showed that pyrimethanil did not induce a biologically relevant and statistically significant increase of the aberration rates in the mouse spermatogonial cells at any of the tested doses or sampling times. The obtained values were within the range of the historical control data. The treatment of the animals with Adriblastin, however, induced significantly higher aberration rates.
Based on the results of this study, it can be stated that under the experimental conditions reported, pyrimethanil failed to induce chromosome aberration in the mouse spermatogonial cells.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Pyrimethanil (BAS 605 F)

Description:	Solid, light yellow
Lot/Batch #:	OP2150064
Purity:	99.6%
Stability of test compound:	The stability of the test substance under storage conditions was guaranteed until 31 July 2003 by the sponsor. The stability of the test substance at room temperature dissolved in the vehicle aqueous methyl cellulose (1%) was verified analytically.
Solvent used:	1% aqueous methyl cellulose

2. Control Materials:

Negative control:	A negative control was not employed in this study.
Solvent control:	1% aqueous methyl cellulose
Positive control:	Adriblastin (Doxorubicinhydrochlorid) 5 mg/kg bw

3. Test animals:

Species:	Mouse
Strain:	NMRI
Sex:	male
Age:	approximately 8-10 weeks
Weight at dosing (mean):	33.8 ± 3.1 g
Source:	[redacted]
Acclimation period:	at least 5 days
Diet:	pelleted standard diet (Altromin, Lage/Lippe, Germany), ad libitum
Water:	tap water, ad libitum
Housing:	single housing in Makrolon Type I cages with wire mesh tops (Ehret GmbH, Emmendingen, Germany)
Environmental conditions:	
Temperature:	19 - 25°C
Humidity:	30 - 85%
Air changes:	no details
Photo period:	alternating 12-hour light and dark cycles, artificial light from 6 am to 6 pm

6. Test concentrations:

a) Preliminary experiment: 2000 mg/kg bw

b) Main experiment: 500, 1000, and 2000 mg/kg bw

B. STUDY DESIGNS AND METHODS:

1. Dates of experimental work: 04-Nov-2002 to 17-Jan-2003

2. Preliminary experiment:
In a preliminary experiment 2 animals received a single dose of 2000 mg/kg bw (20 mL/kg bw) of the test item by gavage. Animals were observed for mortality and clinical signs at 1, 2-4, 6, 24, 30, and 48 h after treatment.

3. Main experiment:

Treatment
Based on the results from the preliminary study three dose groups were used in the main study (500, 1000, and 2000 mg/kg bw). Six animals were assigned to each test group per sacrifice time point. 24 h after the single treatment with 500, 1000, and 2000 mg/kg bw of the test item the spermatogonial cells were collected for chromosome analysis. In addition, in the 2000 mg/kg bw dose group cells were collected from six further animals 48 h after treatment. Five animals received a single intraperitoneal injection of 5 mg/kg bw Adriblastin and were sacrificed 24 h after treatment. Approximately 18 h before treatment the animals received no food but water ad libitum. The animals were weighed at the beginning of the treatment. Animals were observed for mortality and clinical signs at 1, 2-4, 6, and 24 h after treatment.

Preparation of the animals
The animals were sacrificed by cervical dislocation. Both testicles were prepared and the tunica removed. The tubuli were mechanically separated in physiological salt solution and treated with 1.5% collagenase H for 30 min at 37°C to obtain single cell suspension. After centrifugation, the cells were treated with a hypotonic sodium citrate solution for 20 min at 37°C. The cells were sedimented by centrifugation and the pellet was fixed in methanol/glacial acetic acid (3+1) for 20 min. The fixative cell suspension was spread by flame, dried and stained with Giemsa. Cover slips were mounted with EUKITT. One or more slides were made from each sample.

Analysis of metaphase cells
Per animal 100 well spread metaphases were scored for gaps, breaks, fragments, deletions, multiple aberrations, exchanges, and chromosomal disintegrations. Slides were coded prior to analysis. The number of chromosome aberrations per metaphase was determined. Only metaphases with the characteristic chromosome number of 40 ± 2 were included in the analysis. A mitotic index based on 1000 cells per culture was determined for all evaluated test groups as a cytotoxicity measure.

4. Statistics:

As an aid in evaluating the results the non-parametric Mann Whitney U test is used.

5. Acceptance and evaluation criteria:

Acceptance criteria

The study was considered valid, if the following criteria are met:

- The negative controls are in the range of the historical control data (up to 2% aberrant cells exclusive gaps)
- The frequency of aberrant cells of the positive controls is significantly higher than the mean value of the negative control
- At least 80% of animals are evaluable

Evaluation criteria

The test chemical is classified mutagenic, if it induces either a dose-related increase in the number of aberrant cells, which clearly exceeds the negative control range or a relevant positive response for at least one of the test points.

A test item producing neither a dose-related increase in the number of aberrant cells nor a positive response at any of the test points is considered non-mutagenic in this system.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS:

Chemical analysis of the test item formulation demonstrated that the nominal dose levels had been achieved with the analysed concentration, and homogeneity values (deviations from the nominal values ranged between -0.3 to 17.5%) being within the accepted range of nominal concentration (100 ± 20%).

B. PRELIMINARY EXPERIMENT:

A preliminary toxicity test was performed to determine appropriate test substance doses for the main test. The animals treated with 2000 mg/kg bw showed reduction of spontaneous activity, ruffled fur, abdominal position, and apathy (see Table 5.4.3-1). No mortality was observed.

Table 5.4.3-1: Overview of clinical signs observed in the 2 animals of the preliminary experiment

	Hours post-treatment					
Clinical signs	**1**	**2-4**	**6**	**24**	**30**	**48**
Reduction of spontaneous activity	2	2	2	0	0	0
Ruffled fur	2	2	2	2	2	2
Abdominal position	2	1	0	0	0	0
Apathy	1	0	0	0	0	0

On the basis of these data 2000 mg/kg bw (the maximum level recommended in the guideline) was selected as the top dose for the main experiment. Two lower doses of 500 and 1000 mg/kg bw were also selected.

C. MAIN EXPERIMENT:

Table 5.4.3-2: Overview of clinical signs observed in the animals of the main experiment

	Hours post-treatment			
Clinical signs	**1**	**2-4**	**6**	**24**
Reduction of spontaneous activity				
500 mg/kg bw (24 h)	6	5	5	4
1000 mg/kg bw (24 h)	6	6	6	1
2000 mg/kg bw (24 and 48 h)	12	9	8	8
Ruffled fur				
500 mg/kg bw (24 h)	5	6	5	4
1000 mg/kg bw (24 h)	6	6	6	6
2000 mg/kg bw (24 and 48 h)	12	12	8	9
Abdominal position				
500 mg/kg bw (24 h)	1	0	0	0
1000 mg/kg bw (24 h)	3	2	0	0
2000 mg/kg bw (24 and 48 h)	7	3	3	2
Eyelid closure				
500 mg/kg bw (24 h)	-	-	-	-
1000 mg/kg bw (24 h)	1	0	0	0
2000 mg/kg bw (24 and 48 h)	3	3	3	1
Apathy				
500 mg/kg bw (24 h)	-	-	-	-
1000 mg/kg bw (24 h)	1	0	0	0
2000 mg/kg bw (24 and 48 h)	2	3	3	2

As a measure of cytotoxicity for each animal only the mitotic index was calculated by scoring the number of mitotic cells in 1000 spermatogonial cells. The data below show that the mitotic indices (percentage of cells in mitosis) of the treated animals (7.88 – 11.42%) were not decreased as compared to the vehicle controls (7.56%).

Table 5.4.3-3: Mitotic index observed in the spermatogonial cells of the animals of the main experiment

Experimental group	Dose [mg/kg bw]	Sampling time	Total No. of cells scored	No. of mitotic cells in 5000 analysed cells	Mean mitotic index (%)
1. Vehicle	0	24	5000	378	7.56
2. Pyrimethanil	500	24	5000	571	11.42
3. Pyrimethanil	1000	24	5000	530	10.60
4. Pyrimethanil	2000	24	5000	394	7.88
5. Adriblastin	5	24	5000	309	6.18
6. Pyrimethanil	2000	48	5000	487	9.74

The analysis of the chromosome aberration rates showed that pyrimethanil did not induce a biologically relevant and statistically significant increase of the aberration rates (0.4 – 1.4% excluding gaps) in the mouse spermatogonial cells at any of the tested doses or sampling times, as compared to the control (0.4% excluding gaps). The obtained values were within the historical range of the negative control data (2%). The treatment of the animals with the positive control Adriblastin, however, induced significantly higher aberration rates (7% excluding gaps).

Table 5.4.3-4: Chromosome aberration rates in spermatogonial cells of mice treated with pyrimethanil

Experimental group	Dose [mg/kg bw]	Sampling time	Total No. of cells scored	% aberrant cells	
				incl. gaps	excl. gaps
1. Vehicle	0	24	500	0.4	0.4
2. Pyrimethanil	500	24	500	0.8	0.6
3. Pyrimethanil	1000	24	500	0.4	0.4
4. Pyrimethanil	2000	24	500	1.8	1.4
5. Adriblastin	5	24	500	8.2	7.0*
6. Pyrimethanil	2000	48	500	1.0	1.0

* ($p \leq 0.05$) significantly different from controls (Mann Whitney U test)

No increased incidence of aberration types was observed in animals receiving pyrimethanil. No iso-gaps, iso-breaks, deletions and no chromosome disintegrations were observed in any dose group including solvent and positive control group.

Table 5.4.3-5: Chromosome aberration types in spermatogonial cells of mice treated with pyrimethanil

Experimental group	Dose [mg/kg bw]	Sampling time	Gap	Break	Fragment	Iso-fragment	Multiple aberrations (>5 per cell, excluding gaps)	Exchange
1. Vehicle	0	24	0	1	1	0	0	0
2. Pyrimethanil	500	24	1	3	0	0	0	0
3. Pyrimethanil	1000	24	0	1	1	0	0	0
4. Pyrimethanil	2000	24	2	3	2	2	0	0
5. Adriblastin	5	24	6	14	6	3	1	11
6. Pyrimethanil	2000	48	0	3	1	0	0	1

III. CONCLUSION

In conclusion, it can be stated that under the experimental conditions reported, pyrimethanil failed to induce chromosome aberration in the mouse spermatogonial cells.

CA 5.5 Long-Term Toxicity and Carcinogenicity

Studies evaluated in the draft monograph of Rapporteur Member State Austria of April 15, 2004:

A combined chronic toxicity and carcinogenicity study in rats and a carcinogenicity study in mice have been evaluated by European authorities and Austria as Rapporteur Member State and were considered to be acceptable. For the convenience of the reviewer, these studies are summarised below as extracted from the monograph and the EFSA conclusion. A tabulated summary is provided in Table 5.5-1.

Table 5.5-1: Summary of long-term toxicity/carcinogenicity studies conducted with pyrimethanil

Study	Dosages (mg/kg bw/day)	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Main adverse effect	Reference (BASF DocID)
24-month combined chronic toxicity and carcinogenicity Sprague-Dawley rat	M: 1.3, 17 and 221 F: 1.8, 22 and 291 [0, 32, 400, 5000 ppm]	17 (M) 22 (F) [400 ppm]	221 (M) 291 (F) [5000 ppm]	Systemic toxicity: Reduced body weight gain, clinical-chemical changes and pathological/histo-pathological findings in liver and thyroid. Carcinogenicity: Increased incidences of benign follicular cell tumours in the thyroid gland (not statistically significant and evaluated not human relevant): Weight of evidence not carcinogenic	A81806, 2003/1023033¥, A54965, A81808 2003/1023036¥ 2003/1023032¥
18-month carcinogenicity CD-1 mouse	M: 1.7 - 2.7, 17.3 - 26.7 and 177.8 - 281.1 F: 2.2 - 3.3, 22.3 - 32.0 and 221.9 - 327.5 [0, 16, 160, 1600 ppm]	17.3 (M) 22.3 (F) [160 ppm]	178 (M) 222 (F) [1600 ppm]	Systemic toxicity: Necropsy findings in the urogenital tract. Carcinogenicity: No evidence of carcinogenicity.	A81811, A81814, A89479, A89480, A89481, 2003/1023034¥, A81813 2003/1023032¥

¥ Note: These supplemental documents were submitted in course of the evaluation of the initial dossier and have already been peer-reviewed during Annex I inclusion of pyrimethanil as reported in the Draft Monograph of April 2004 prepared by the former RMS Austria.

A combined 2-year chronic toxicity/carcinogenicity study was conducted in rats and an 18-months carcinogenicity study was performed with mice.
In rats, liver and thyroid have been identified as the target organs. Liver pathology comprised changes in biochemical parameters, increased organ weight and histological alterations at 5000 ppm. In the thyroid, microscopic examination revealed higher incidences of colloid depletion, hypertrophy of the follicular epithelium, deposition of intracytoplasmic brown pigment and focal hyperplasia of the follicular epithelium also at 5000 ppm. In addition, increased incidences of benign follicular cell tumours of the thyroid gland were evident in males and females at this high dose level. However, statistical significance was not reached. In mechanistic studies effects of pyrimethanil on hepatic enzymes in rats and mice and, in particular, on the thyroid in rats have been investigated. It was demonstrated that enhanced hepatic metabolism induced by treatment with pyrimethanil produced thyroid hormone imbalance due to increased thyroid hormone clearance resulting in a chronic stimulation of the thyroid. The NOAEL for chronic toxicity /carcinogenicity in the rat was set at 400 ppm (equivalent to 17 [♂] and 22 [♀] mg/kg bw/day).
In mice, there were no treatment-related increases in the incidence of tumours following long-term treatment with pyrimethanil up to 1600 ppm suggestive of a carcinogenic effect. Additionally, there were no treatment-related differences in mortality, clinical signs, body weight or haematological parameters at any dose level. There was an increased incidence in morbidity and mortality in males of all groups, particularly during the first 52 weeks of the study, which was associated with lesions in the urogenital tract. These findings (with no evidence of a clear dose-response relationship) were mostly considered to be caused by male aggression. However, the slightly increased incidence of urinary bladder distension evident in decedent males at 1600 ppm was suggested to be a possible effect of treatment. The NOAEL for this study was set at 160 ppm (equivalent to 17.3 – 26.7 [♂] and 22.3 – 32.0 [♀] mg/kg bw/day).
The relevant NOAEL for chronic toxicity/carcinogenicity was set at 17 mg/kg bw/day, from the 2-year study in rat.

Studies submitted in this AIR 3 dossier (not yet peer-reviewed):
No additional data on long-term toxicity and carcinogenicity of pyrimethanil were generated by the applicant. The only new study to consider whether it could affect the long-term relevant NOAEL/NOEL would be the 90-day neurotoxicity study in rats as presented in section CA 5.7. However, the determined study NOAEL of 38.7 mg/kg bw/day in male rats was clearly above the derived lowest relevant NOAEL of the chronic toxicity/carcinogenicity study in rat and no additional targets/critical effects were determined.

Based on the available data on the long-term toxicity and carcinogenicity, classification of pyrimethanil for this endpoint is not required according to the criteria laid down in Regulation (EU) No. 1272/2008 (CLP). Moreover, the data on long-term toxicity and carcinogenicity of pyrimethanil have already been evaluated in 2006, coming to the conclusion that pyrimethanil does not need to be classified for health effects (ECBI/159/04) as laid down in the 31st ATP to the Council Directive 67/548/EEC (Commission Directive 2009/2/EC of 15 January 2009). The not yet peer-reviewed 90-day neurotoxicity study, as presented in section CA 5.7, has not been submitted for evaluation of classification. The results of this study will however not change the classification and labelling of pyrimethanil.

Thus, the conclusion for relevant endpoints adopted to the new list of endpoint format for the current re-registration is presented as follows:

Long-term toxicity and carcinogenicity (Regulation (EU) N°283/2013, Annex Part A, point 5.5)

Long-term effects (target organ/critical effect)	Rat: Reduced body weight gain, increased plasma cholesterol, increased liver weights, pathological and histopathological findings in liver and thyroid in both sexes. Mouse: Slightly increased incidence of urinary bladder distension in males.	
Relevant long-term NOAEL	2-year combined chronic and carcinogenicity rat: 17 mg/kg bw/day 18-month carcinogenicity mouse: 17.3 mg/kg bw/day	
Carcinogenicity (target organ, tumour type)	Rat: Benign follicular cell tumours of the thyroid gland in both sexes (not statistically significant and evaluated to be not of human relevance). Weight of evidence not carcinogenic. Mouse: No evidence of carcinogenicity. Pyrimethanil is unlikely to pose a carcinogenic hazard to humans.	
Relevant NOAEL for carcinogenicity	2-year combined chronic and carcinogenicity rat: 17 mg/kg bw/day 18-month carcinogenicity mouse: 178 mg/kg bw/day	

Pyrimethanil, rat 104-week dietary combined chronic toxicity and carcinogenicity study [redacted] 1993)
(BASF doc ID A81806 and related documents 2003/1023033, A54965, A81808, 2003/1023036, 2003/1023032)

Guidelines: According to OECD guideline 453 and US EPA Pesticide Assessment Guidelines, Subdivision F
Deviations: The survival rate at 24 months is 38 – 56% for males and 26 – 42% for females. Therefore this study does not meet the OECD guidelines with regard to animal survival rate (50% survival of 50 rats/sex/dose at 24 months is required), but it is acceptable within the range of US EPA guidelines (50% survival rate at 18 months and at least 25% survival rate at 24 months in rats), except the female control group. However, for this group survival after 100 weeks was 36% (18/50). Therefore, it can be concluded that the rats survived long enough to allow a valid interpretation of the potential oncogenicity of the tested compound. In addition, historical control data from 21 studies (1993 – 2000) on survival of Sprague Dawley rats from [redacted]) have been submitted demonstrating that the test groups have very similar figures of percentage survival at week 104 to the historical control data (males 50.6 %; females 37.6 %).
GLP: Yes
Acceptance: The study is considered acceptable in the EU registration process 2004-2006.

Report:	CA 5.5/1 [redacted] 1993 a Technical SN 100309: 104 week rat combined chronic toxicity and oncogenicity study A81806
Guidelines:	US EPA (1984) Pesticide Assessment Guidelines. Subdiv. F - Hazard Evaluation: Human and domestic animals (Nov 1984 revised edition), OECD 453, JMAFF, EEC 79/831 A V B 1
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)
Deviations:	The survival rate at 24 months is 38 – 56% for males and 26 – 42% for females. Therefore this study does not meet the OECD guidelines with regard to animal survival rate (50% survival of 50 rats/sex/dose at 24 months is required), but it is acceptable within the range of US EPA guidelines (50% survival rate at 18 months and at least 25% survival rate at 24 months in rats), except the female control group. However, for this group survival after 100 weeks was 36% (18/50). Therefore, it can be concluded that the rats survived long enough to allow a valid interpretation of the potential oncogenicity of the tested compound. In addition, historical control data from 21 studies (1993 – 2000) on survival of Sprague Dawley rats from [redacted]) have been submitted demonstrating that the test groups have very similar figures of percentage survival at week 104 to the historical control data (males 50.6 %; females 37.6 %). The addition of the fourth test group is recommended if large intervals (6-10fold) between dosages are used. As for haematological parameters, prothrombin time and activated partial thromboplastin time should be investigated. Epididymes, uterus, and thyroid should be weighted at necropsy after the chronic toxicity phase.

Report:	CA 5.5/2 [redacted] 1993 a Technical SN 100309: 104 week rat combined chronic toxicity and oncogenicity study A54965
Guidelines:	US EPA (1984) Pesticide Assessment Guidelines. Subdiv. F - Hazard Evaluation: Human and domestic animals (Nov 1984 revised edition), OECD 453, JMAFF, EEC 79/831 A V B 1
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Report:	CA 5.5/3 Healing G., 1994 e 1st addendum to report No. TOX/92/223-62: Technical SN 100309: 104 week rat combined chronic toxicity and oncogenicity study - Report of individual pathology findings A81808
Guidelines:	US EPA (1984) Pesticide Assessment Guidelines. Subdiv. F - Hazard Evaluation: Human and domestic animals (Nov 1984 revised edition), OECD 453, JMAFF, EEC 79/831 A V B 1
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

Groups of 70 male and 70 female Sprague Dawley CRL:CD(SD)BR rats (source: [redacted] at start of treatment approx. 6 weeks old) received pyrimethanil (batch no. CR 19325/01/900304; purity 97.2%) with the diet at dose levels of 0, 32, 400 and 5000 ppm (equivalent to mean achieved doses of 0, 1.3, 17 and 221 mg/kg bw/day [males] and 0, 1.8, 22 and 291 mg/kg bw/day [females]) for two years. 20 males and 20 females of each group were sacrificed for interim examination, after 52 weeks, the remaining were scheduled for sacrifice after 104 weeks of treatment.

At 5000 ppm, there was an overall reduction in body weight gain in animals of both sexes throughout the study period. Food consumption was also reduced in females at this dose level. Liver and thyroid have been identified as the target organs. Liver pathology comprised increased organ weight, centrilobular hypertrophy and increased incidences of eosinophilic foci at 5000 ppm. Some changes in biochemical parameters (increased levels of cholesterol and GGT in plasma) can be considered as the result of liver toxicity at the high dose level. In the thyroid, microscopic examination revealed higher incidences of colloid depletion, hypertrophy of the follicular epithelium, deposition of intracytoplasmic brown pigment and focal hyperplasia of the follicular epithelium in males and females after 52 and 104 weeks at 5000 ppm. In addition, increased incidences of benign follicular cell tumours of the thyroid gland were evident in males and females at this high dose level. However, statistical significance was not reached. The NOAEL for chronic toxicity/carcinogenicity in the rat was set at 400 ppm (equivalent to 17 [males] and 22 [females] mg/kg bw/day).

In mechanistic studies [see section CA 5.8.2; DocID A81625, C001378, A81829 and A81828], effects of pyrimethanil on hepatic enzymes in rats and mice and, in particular, on the thyroid in the rat have been investigated. It was demonstrated that pyrimethanil administered at 5000 ppm in the diet to rats for 7 days did not show a direct thyroid blocking activity [see section CA 5.8.2; DocID A81829]. There were no indications of inhibition of iodine uptake into the thyroid (i.e: tyrosine iodination) or of a significant discharge of [^{125}I] after perchlorate challenge. The results after pyrimethanil treatment were similar to those obtained with phenobarbital, which modifies also thyroid homeostasis by an indirect mechanism. In a second study [see section CA 5.8.2; DocID A81828], dietary administration of 5000 ppm pyrimethanil to rats induced significant increases in mean levels of thyroid-stimulating hormone (TSH) and significant reductions in mean levels of T_3 and T_4 after 3 days of treatment. After 7 and 14 days of treatment, mean TSH levels in treated animals were higher than controls but plasma T_3 and T_4 levels had returned to untreated levels (via the normal homeostatic mechanism). In addition, marked and significant increase of the hepatic uridine diphosphate-glucuronosyltransferase (UDP-GT) activity was observed. This was considered to lead to an increased thyroid hormone clearance via enhanced hepatic metabolism. Reversibility of all these changes was demonstrated. These data indicate that pyrimethanil elicits its effects on the thyroid via a reversible and extrathyroidal, indirect mechanism. It can be concluded that the chronic reduction of circulating thyroid hormone levels was produced by increased thyroid hormone clearance via enhanced hepatic metabolism resulting in increased TSH levels and a chronic stimulation of the thyroid gland with development of increased cell division, increased size and number of thyroid cells. Such effects may lead to changes in thyroid functionality and morphology. Rodents are known to be particularly sensitive to this type of effect. However, it is considered that this mechanism has little relevance to man and such thyroidal effects seen in rodents may not be considered to be relevant to humans.

Therefore, pyrimethanil was considered as not carcinogenic. The no observed adverse effect level (NOAEL) for chronic toxicity/carcinogenicity in both sexes was 400 ppm (equivalent to 17 mg/kg bw/day in males and 22 mg/kg bw/day in females).

Material and Methods:
Groups of 70 male and 70 female Sprague Dawley CRL:CD(SD)BR rats (source: [redacted]; at start of treatment approx. 6 weeks old) received pyrimethanil (batch no. CR 19325/01/900304; purity 97.2 %) with the diet at dose levels of 0, 32, 400 and 5000 ppm (equivalent to mean achieved doses of 0, 1.3, 17 and 221 mg/kg bw/d [males] and 0, 1.8, 22 and 291 mg/kg bw/d [females]) for two years. Test diets were prepared twice weekly; stability and homogeneity of the test diets was confirmed by analysis.
All animals were observed daily for clinical signs. Body weights were recorded weekly until week 13 and once every two weeks thereafter. Food consumption was recorded twice weekly. Water intake was recorded over a 4-day-period during weeks 9, 16, 32 and 48. Biochemistry (total protein, albumin, globulin, calcium, phosphate, sodium, potassium, urea, creatinine, glucose, cholesterol, bilirubin, chloride, AST, ALT, AP, GGT, CPK), haematology (haematocrit, haemoglobin, RBC, MCHC, MCV, MCH, WBC total, WBC differential, platelets count, reticulocyte count) and urinalysis (colour, volume, pH, specific gravity, protein, glucose, ketones, bilirubin, urobilinogen, blood cells, bacteria, sediments) were carried out after 13, 26, 52, 78 and 102 weeks. An additional blood sample was also taken during week 72 for measurement of thyroid hormones. Ophthalmoscopy was performed prior to start the treatment and during weeks 50 and 102. After 52 weeks of treatment, an interim kill of approx. 20 animals of each group of each sex was undertaken. At interim and terminal necropsy, the weights of selected organs (adrenals, brain, heart, kidneys, liver, spleen, ovaries, testes) were recorded and histopathology was performed on adrenals, aorta, bone marrow smear, brain, caecum, cervical lymph node, colon, duodenum, epididymites, eyes, femur, harderian gland, heart, ileum, jejunum, kidneys, lacrimal gland, liver, lungs, mammary gland, mesenteric lymph node, oesophagus, optiv nerve, ovaries, pancreas, pituitary, prostate, rectum, salivary glands, sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord, spleen, sternum, stomach, testes, thymus, thyroid with parathyroid, tongue, trachea, urinary bladder, uterus and vagina.

Findings:
General observations: There were no treatment-related clinical signs of toxicity at any dose level. There was also no evidence of significant adverse intergroup differences in mortalities and survival, respectively. The incidences of total mortality, which occurred mainly during the last months of the study, were 32/70, 27/70, 27/70 and 22/70 for males and 39/70, 37/70, 32/70 and 29/70 for females at 0, 32, 400 and 5000 ppm, resp. The survival of animals per dose group are given in **Table 5.5-2**.

Table 5.5-2: Survival data: number of survived rats (% survival)

	0 ppm ♂	♀	32 ppm ♂	♀	400 ppm ♂	♀	5000 ppm ♂	♀
total number	70	70	70	70	70	70	70	70
week 52	67	68	69	68	65	67	69	69
rats after 1st kill	48	49	50	48	45	48	49	49
week 80	38	34	46	36	40	38	44	46
week 92	28	23	35	26	36	29	39	38
week 104	19/50 (38 %)	13/50 (26 %)	24/50 (48 %)	14/50 (28 %)	23/50 (46 %)	19/50 (40 %)	28/50 (56 %)	21/50 (42 %)

Overall the data suggest that survival was not affected by the presence of pyrimethanil in the diet. In addition, historical control data from 21 studies (1993 – 2000) on survival of Sprague Dawley rats from [redacted]) have been submitted demonstrating that the test groups have very similar figures of percentage survival at week 104 to the historical control data (males 50.6 %; females 37.6 %).

At 5000 ppm, there was an overall reduction in body weight gain of approx. 5 % and 42 % in males and females resp. throughout the study period. Also, food consumption was reduced by approx. 5 % in males and 11 % in females at this dose level, compared with controls.
No treatment-related effects on water consumption were noted at any dose level. Ophthalmoscopic findings were consistent with the strain and age of the animals.
Haematology: In animals of the high dose, there were a number of minor differences compared with controls, some of which achieved variable statistical significance. There was a slightly higher platelet count in males after 13 and 26 weeks and in females after 13, 26, 52 and 78 weeks. In males, slightly lower mean cell haemoglobin after 52 and 78 weeks, and in females lower haemoglobin concentrations after 13, 26, 52 and 78 weeks were observed. However, there were no notable differences evident in any parameters after 102 weeks at this dose level. None of the haematological findings were considered to be of toxicological significance because of factors such as lack of dose relationship, inconsistency of effects with time or the changes falling within the normal range of controls, There were no effects at 400 or 32 ppm.
Clinical chemistry: At 5000 ppm, slightly higher GGT levels were observed after 78 and 102 weeks in males indicating liver effects. In addition, there were slight to moderate higher plasma cholesterol levels in males after 13 and 26 weeks and in females at all sampling time points compared to concurrent controls (Table 5.5-3).

Table 5.5-3: Clinical chemistry finding – group mean values of plasma cholesterol

Cholesterol (mmol/l)	0 ppm ♂	♀	32 ppm ♂	♀	400 ppm ♂	♀	5000 ppm ♂	♀
13 weeks	2.18	2.12	1.74	2.39	1.94	2.11	3.13**	2.91**
26 weeks	2.47	2.05	2.05	2.34	2.27	2.36	3.13*	3.35***
52 weeks	3.14	3.03	2.41	2.89	2.48	3.31	3.64	4.39***
78 weeks	3.10	3.55	3.59	3.96	3.35	4.55	3.81	5.84*
102 weeks	3.77	3.76	13.84	3.92	4.34	4.48	4.88	4.94*

* ($p \leq 0.05$) significantly different from controls

** ($p \leq 0.01$) significantly different from controls

*** ($p \leq 0.001$) significantly different from controls

There were occasional incidences of findings on other parameters at the different dose levels achieving statistically significant differences ($p<0.001$) from control (e.g. slightly higher total bilirubin levels in females after 7, 18 and 24 months at 5000 ppm; slightly higher plasma urea levels in males after 3, 7 and 12 months at 400 and 5000 ppm; slightly lower phosphate levels in treated males after 3, 7, 12 and 18 months), but it was concluded that non were considered of toxicological significance because they were neither dose-related, nor consistent with time or were within the normal age-dependent range of historical controls (historical control data from [redacted] for the clinical chemistry parameters urea and phosphate in Sprague Dawley rats have been submitted).

Thyroid hormone levels were normal at any dose level compared with control levels when sampled in week 72.

Urinalysis: A brownish-black urine colouration was observed in animals of both sexes at 5000 ppm throughout the treatment period and also occasionally in some individuals in the other dose groups. This finding was considered to be due to the presence of urinary metabolites of pyrimethanil.

Pathology: After 52 weeks following treatment at 5000 ppm, absolute liver weights were 13 % higher in males and 5 % in females. After 104 weeks at 5000 ppm, absolute liver weights were higher in males only (24 %). At termination, significantly higher relative liver weights were recorded in males (26 %) and females (21%) compared with control animals (Table 5.5-4). There were no treatment-related effects at 400 or 32 ppm.

Table 5.5-4: Absolute (g) and relative (% of bw) liver weights

		0 ppm ♂	0 ppm ♀	32 ppm ♂	32 ppm ♀	400 ppm ♂	400 ppm ♀	5000 ppm ♂	5000 ppm ♀
Liver weight absolute (g)	52 weeks	21.27	11.82	21.76	11.71	21.48	12.46	23.93	12.38
	104 weeks	19.28	16.44	21.24	17.22	23.26*	15.97	23.85**	14.41
Liver weight relative (% of bw)	52 weeks	2.94	3.04	2.90	2.92	3.01	2.93	3.59**	3.47**
	104 weeks	2.59	3.25	2.67	3.40	2.96	3.30	3.26**	3.92**

* ($p \leq 0.05$) significantly different from controls

** ($p \leq 0.01$) significantly different from controls

Macroscopic examination: Following treatment with 5000 ppm, dark thyroids were observed in one male and four females (out of 20) after 52 weeks, and in 6 males and 17 females (out of 50) after 104 weeks. There were no other treatment-related effects.

At 5000 ppm, histopathological changes comprised treatment-related effects in the liver (minimal to moderate centrilobular hepatocyte hypertrophy, minimal to severe eosinophilic foci) and the thyroid. In the thyroid gland, there was a higher incidence of minimal to slight colloid depletion and hypertrophy of the follicular epithelium than in controls. A higher incidence of minimal to moderate deposition of intracytoplasmic brown pigment (which stained positively by Schmorl's method for lipofuscin) was seen in the thyroid follicular epithelium. Focal hyperplasia of the follicular epithelium was also seen in males and females both after 52 and 104 weeks. Incidences of relevant findings are given in Table 5.5-6:.

No treatment-related effects were recorded following treatment with 32 or 400 ppm after 52 or 104 weeks.

Concerning neoplastic findings, the only tissue showing a higher incidence of tumours than in controls was the thyroid gland in animals of both sexes at the high dose level with benign follicular cell tumours in 9/70 males (an additional 1/70 males had a malignant tumour) and 7/70 females compared with 3/70 in control males and 0/70 in control females. These incidences were not statistically significantly different from controls on a pair wise comparison but it was stated in the report that a positive trend for both sexes was evident.

Table 5.5-5: 104 week oral toxicity study in rats; substantial necropsy findings

		0 ppm ♂	0 ppm ♀	32 ppm ♂	32 ppm ♀	400 ppm ♂	400 ppm ♀	5000 ppm ♂	5000 ppm ♀
Liver									
Centrilobular hypertrophy	52 weeks	0/19	0/19	0/19	0/19	0/20	0/19	20/20	1/20
	104 weeks	0/51	0/51	0/51	0/51	0/50	0/51	32/50	6/50
Eosinophilic foci	52 weeks	0/19	0/19	0/19	0/19	0/20	0/19	1/20	0/20
	104 weeks	2/21	0/51	3/21	0/51	3/50	0/51	19/50	0/50
Thyroid									
Colloid depletion	52 weeks	9/19	6/19	8/19	5/19	12/20	6/19	18/20	13/20
	104 weeks	25/51	15/51	21/51	19/51	24/50	13/51	36/50	38/50
Follicular epithe-lium hypertrophy	52 weeks	9/19	6/19	8/19	5/19	12/20	5/19	18/20	13/20
	104 weeks	25/51	15/51	21/51	19/51	24/50	13/51	36/50	38/50
Focal follicular epithelium hyperplasia	52 weeks	0/19	0/19	0/19	0/19	0/20	0/19	1/20	0/20
	104 weeks	2/51	1/51	2/51	0/51	4/50	1/51	9/50	7/50
Intracytoplasmic brown pigment in follicular epithelium	52 weeks	1/19	1/19	0/19	0/19	0/20	0/19	18/20	19/20
	104 weeks	0/51	0/51	0/51	0/51	0/50	0/51	38/50	47/50
benign follicular tumour	52 weeks	0/19	0/19	0/19	0/19	0/20	0/19	3/20	1/20
	104 weeks	3/51	0/51	4/51	3/51	2/50	3/51	7/50	6/50

52 week interim excludes decedents; 104 week terminal includes decedents

Table 5.5-6: 104 week oral toxicity study in rats; Overall tumour incidence in males; I = interim kill, D = decedent, T = terminal kill

Dose [ppm]	0			32			400			5000		
Number of animals	I	D	T	I	D	T	I	D	T	I	D	T
Primary tumours	19	32	19	19	27	24	20	27	23	20	22	28
Absent	16	3	3	18	4	2	16	4	1	13	4	2
Benign	3	17	11	1	11	16	3	17	9	6	7	20
Malignant	0	12	5	0	12	6	1	6	13	1	11	6
Multiple primary tumours												
Absent	19	15	6	19	13	13	20	18	7	20	15	14
Benign	0	14	13	0	13	11	0	9	16	0	6	13
Malignant	0	3	0	0	1	0	0	0	0	0	1	1

Table 5.5-7: 104 week oral toxicity study in rats; Overall tumour incidence in females; I = interim kill, D = decedent, T = terminal kill

Dose [ppm]	0			32			400			5000		
Number of animals	I 19	D 39	T 12	I 19	D 37	T 14	I 19	D 32	T 19	I 20	D 29	T 21
Primary tumours												
Absent	11	2	1	14	0	3	14	1	1	16	4	3
Benign	8	24	7	4	24	6	4	19	14	4	20	12
Malignant	0	13	4	1	13	5	1	12	4	0	5	6
Multiple primary tumours												
Absent	18	15	5	16	13	4	18	13	5	20	12	11
Benign	1	22	7	3	22	9	1	18	13	0	17	10
Malignant	0	2	0	0	2	1	0	1	1	0	0	0

Conclusion:
The NOAEL for chronic toxicity/carcinogenicity from this study was 400 ppm (equivalent to 17 [♂] and 22 [♀] mg/kg bw/d). This NOAEL was supported by evidence of retarded body weight gain, increased plasma cholesterol levels, increased liver weights and pathological / histopathological findings in the liver and thyroid in males and females at 5000 ppm, the highest concentration tested.

~~Pyrimethanil, mouse 80-week dietary carcinogenicity study ([redacted] 1993)~~
~~(BASF doc ID A81811 and related documents A81814, A89479, A89480, A89481, 2003/1023034, A81813, 2003/1023032)~~

~~**Guidelines:** According to OECD guideline 451 and US EPA Pesticide Assessment Guidelines 83-2~~
~~**Deviations:** Minor deviations from the protocol are noted but do not limit the scientific validity of the study.~~
~~**GLP:** Yes~~
~~**Acceptance:** The study is scientific valid and acceptable in the EU registration process 2004-2006~~

Report:	CA 5.5/4 [redacted] 1993 a Technical SN 100 309: 80 week oral (dietary administration) carcinogenicity study in the mouse A81811
Guidelines:	EPA 83-2, OECD 451
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)
Deviations:	Shorter duration of the study than 24 months should be justified. The animal room temperature was not strictly held within a range of 22 ± 3 ºC. The addition of the fourth test group is recommended if large intervals (6-10fold) between dosages are used.

Report:	CA 5.5/5 [redacted] 1995 a 1st addendum to report SN 100309/T27/2 - Pyrimethanil - 80 week oral (dietary administration) carcinogenicity study in the mouse A81813
Guidelines:	EPA 83-2, OECD 451
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Report:	CA 5.5/6 [redacted] 1994 a 1st amendment to report No. TOX/92/223-68 - Technical SN 100309: 80 week oral (dietary administration) carcinogenicity study in the mouse A81814
Guidelines:	EPA 83-2, OECD 451
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Report:	CA 5.5/7 [redacted]., 1995 a 2nd amendment to report No. TOX/92/223-68: Technical SN 100309: 80 week oral (dietary administration) carcinogenicity study in the mouse A89479
Guidelines:	EPA 83-2, OECD 451
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Report:	CA 5.5/8 [redacted] 1995 a 3rd amendment to report No. TOX/92/223-68: Technical SN 100309: 80 week oral (dietary administration) carcinogenicity study in the mouse A89480
Guidelines:	EPA 83-2, OECD 451
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Report:	CA 5.5/9 [redacted] 1996 a 4th amendment to report No. TOX/92/223-68: Technical SN 100309: 80 week oral (dietary administration) carcinogenicity study in the mouse A89481
Guidelines:	EPA 83-2, OECD 451
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive summary

Groups of 51 male and 51 female mice (strain: Crl:CD-1(ICR)BR, source: [redacted].) were treated with pyrimethanil (batch no. CR19325/4; purity 96.0%) via diet at dose levels of 0, 16, 160 and 1600 ppm (equivalent to 0, 1.7 – 2.7, 17.3 – 26.7 and 177.8 – 281.1 mg/kg bw/day in males and 0, 2.2 – 3.3, 22.3 – 32.0 and 221.9 – 327.5 mg/kg bw/day in females) for 80 weeks.

In CD-1 mice, there were no treatment-related increases in the incidence of tumours following long-term treatment with pyrimethanil up to 1600 ppm suggestive of a carcinogenic effect. Additionally, there were no treatment-related differences in mortality, clinical signs, body weight or haematological parameters at any dose level. There was an increased incidence in morbidity and mortality in males of all groups, particularly during the first 52 weeks of the study, which was associated with lesions in the urogenital tract. The findings showed no evidence of a clear dose-response relationship and were considered to be caused by male aggression, which is often manifested as lesions in the urogenital region. However, the slightly increased incidence of urinary bladder distension evident in decedent males at 1600 ppm was suggested to be a possible effect of treatment. The no observed adverse effect level (NOAEL) for this study was set at 160 ppm (equivalent to 17.3 – 26.7 mg/kg bw/day in males and 22.3 – 32.0 mg/kg bw/day in females).

Material and Methods:
Groups of 51 male and 51 female mice (strain: Crl:CD-1(ICR)BR, source: [redacted].) were treated with pyrimethanil (batch no. CR19325/4; purity 96.0 %) via diet at dose levels of 0, 16, 160 and 1600 ppm (equivalent to 0, 1.7 – 2.7, 17.3 – 26.7 and 177.8 – 281.1 mg/kg bw/d in males and 0, 2.2 – 3.3, 22.3 – 32.0 and 221.9 – 327.5 mg/kg bw/d in females) for 80 weeks. Test diets were prepared twice weekly; stability and homogeneity of the test diets was confirmed by analysis.
Animals were observed daily for mortalities and clinical signs. All animals were also given a detailed clinical examination weekly. Body weights were recorded before treatment, weekly until week 16 and once every four weeks thereafter and at necropsy. Food consumption and water intake by each cage was determined at weekly intervals to week 16 and for one week in every four thereafter. Blood samples for haematological investigations (differential white blood cells) were obtained from 10 animals/sex/dose in weeks 26, 52 and 80. At terminal necropsy, the weights of selected organs (adrenals, brain, kidneys, liver, testes) were recorded. Histopathology was performed in all tissues to be evaluated (adrenals, aorta, brain, caecum, colon, duodenum, epididymites, eyes, femur including marrow, heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes, mammary gland, oesophagus, ovaries, pancreas, pituitary, prostate, rectum, salivary glands, sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord, spleen, sternum, stomach, testes, thymus, thyroid with parathyroid, trachea, urinary bladder, uterus) according to the requirements of the directive from all animals in control and high dose groups, from animals that died or were killed in extremis, and gross lesions and tissue masses from all animals, and on lungs, liver and kidneys from animals in the low and intermediate dose groups.

Findings:
General observations: There was no evidence of treatment-related significant adverse intergroup differences in mortalities and survival, respectively. At the end of the study (week 80) the survival of males was 67 %, 43 %, 71 % and 59 %, and of females 76 %, 80 %, 76 % and 78 % in the control, low, intermediate and high dose groups, resp. There was also no difference in the nature or incidence of clinical signs to indicate an effect of treatment at any dose level. However, it was reported that several males from all groups had injuries, such as sores in the genital region, which resulted from fighting/self mutilation.
During the first 52 weeks of treatment, there was an increased incidence in morbidity and mortality in males of all groups (4/51, 9/51, 6/51 and 11/51 at 0, 16, 160 and 1600 ppm, resp.) associated with lesions in the urogenital region. These lesions consisted of balanoposthitis, preputial gland adenitis/abscesses, seminal vesiculitits/distension, prostatitis, urinary bladder distension and cystitis. Incidences of the necropsy findings in the urogenital tract of males which died during the first 52 weeks of the study are given in Table 5.5-8.

Table 5.5-8: Incidences of necropsy findings at the urogenital tract in male mice which died during the first 52 weeks

	Dose level (ppm)			
	0	16	160	1600
	number of animals affected/number of animals examined			
Urinary bladder distension	0/4	4/9	2/6	8/11
Urinary bladder cystitis	1/4	1/9	1/5	3/11
Balanoposthitis	0/1	5/5	1/1	7/7
Adenitis/abscess of preputial gland	0/0	1/2	1/1	4/6
Distension of seminal vesicle	1/4	4/9	1/5	5/11
Vesiculitis	1/4	0/9	0/5	3/11
coagulating gland distension	0/3	5/9	3/5	0/11
coagulating gland adenitis	0/3	0/9	0/5	3/11
Prostatitis	0/3	2/9	1/5	5/11

Concerning these findings, it was explained that in this study the mice were housed three to a cage and the clinical observation clearly indicated male aggression and related injuries in the genital area from fighting/self-mutilation, which can lead to secondary changes in the urogenital tract e.g. obstruction or inflammation. These findings were prominent during the first 52 weeks of the study when many of the unscheduled deaths occurred and were associated with urinary bladder distension. In this context, it was further stated that according to *Glaister J.R. (Principles of Toxicological Pathology; Pub. Taylor and Frances, Chapter 4, pp 182 –203; 1986),* lesions in the urogenital region (of males) led to a large proportion of the morbidity and mortality. The incidence (%) of morbidity and mortality in male CD-1 mice due to the urogenital tract lesions at different study intervals is reported by *Glaister* to be up to 31 % during the first 52 weeks (no further details given).

Body weight: There were no adverse effects of treatment on body weight or body weight gain at any dose level. Concerning food consumption, high dose males tended to eat more compared with control animals. In weeks 56 to 80 the difference in food consumption was statistically significant (39.2 and 44.4 g/animal/week in control and high dose males, resp.). Also treated females tended to eat more food than controls but the difference was small (within 7 %) and there was no consistent relationship with dose level. There were no statistically significant treatment-related effects on water intake.

Haematology: In week 26, high dose males had a greater proportion of neutrophils than controls ($p<0.05$) and a lower proportion of lymphocytes ($p<0.01$). The proportion of monocytes was also slightly increased. However, this shift in proportion of white blood cells was not maintained at the other samplings and was therefore considered of no toxicological significance.
Pathology: Organ weight analysis showed no treatment-related findings.
Macroscopic as well as histopathological evaluation of non-neoplastic changes revealed a variety of findings in controls and treated groups, the majority of which was consistent with the known background data of the ageing CD-1 mouse used. However, an increased incidence of urogenital tract lesions characterized by preputial gland adenitis/abscess, distension/vesiculitis of the seminal vesicles, coagulating gland distension and prostatitis was observed in males, mainly during the first 52 weeks of the study with the highest incidence occurring in the high dose group. In addition, the incidence of urinary bladder distension was increased in the low and high dose group male decedents, primarily during the first 52 weeks. The incidence of these findings in all decedent and terminal-kill male mice is given in Table 5.5-9.

Table 5.5-9: Incidences of relevant histopathological findings at the urogenital tract in male mice (decedents and terminal kill)

	Dose level (ppm)			
	0	**16**	**160**	**1600**
Decedents: number of animals affected/number of animals examined				
seminal vesicle distension	8/17	9/28	5/15	9/21
seminal vesicle vesiculitis	2/17	1/28	2/15	4/21
coagulating gland distension	6/16	13/28	6/15	3/21
Prostatitis	0/16	3/28	4/15	6/21
preputial gland adenitis/abscess	1/3	4/10	4/5	7/10
urinary bladder distension	3/17	7/27	4/15	9/21
urinary bladder cystitis	1/17	6/27	3/15	5/21
inflammatory cell foci in bladder	4/17	5/27	4/15	2/21
Terminal kill: number of animals affected/number of animals examined				
seminal vesicle distension	10/34	3/3	4/5	16/30
seminal vesicle vesiculitis	1/34	0/3	3/5	1/30
preputial gland adenitis/abscess	3/9	4/5	5/8	7/8
coagulating gland distension	15/34	3/3	4/4	17/30
Prostatitis	2/34	0/3	0/4	0/30
urinary bladder distension	1/34	0/0	0/2	0/30
inflammatory cell foci in bladder	15/34	0/0	1/2	14/30

Table 5.5-10: 80 week oral (dietary administration) carcinogenicity study in mice; Overall tumour incidence (% tumour-bearing animals).

Primary tumours \ Dose [pm]	Males				Females			
	0	16	160	1600	0	16	160	1600
Benign	22/51 (43 %)	16/51 (31 %)	20/51 (39 %)	17/51 (33 %)	7/51 (14 %)	9/51 (18 %)	9/51 (18 %)	13/51 (25 %)
Malignant	6/51 (12 %)	3/51 (6 %)	6/51 (12 %)	5/51 (10 %)	10/51 (20 %)	7/51 (14 %)	6/51 (12 %)	6/51 (12 %)

In a position paper concerning these findings (*Reader S.; Pyrimethanil: Statements on the 80-week carcinogenicity study in the mouse; 2003*) it was explained that the overall pattern of the urogenital tract findings in male mice in this study concurs with the type of effects as highlighted by *Glaister (1986).* In addition, the findings showed no evidence of a clear dose-response relationship and were mostly considered to be related to male aggression. Moreover, the pattern of findings suggests that there was an underlying inflammatory response (particularly of the urinary bladder) in male mice throughout the study. This was corroborated at terminal necropsy, where more than 40 % of the control males had inflammatory cell foci in the urinary bladder.
However, concerning urinary bladder distension, the incidence from historical control data for male CD-1 mice decedents to 52 weeks was reported to be 40 % and for all male animals up to 80 weeks was 21 %. These values are higher than recorded in the concrete study with the exception of males at the highest dose where approx. 43 % of the decedent males had this finding. It was concluded that for this slightly higher incidence of bladder distension for the decedent males at 1600 ppm, a possible effect of treatment at this dose level could not be excluded.
With respect to neoplastic findings, the overall incidence of tumour-bearing animals was 53.1 %, 37.5 %, 52.2 % and 46.8 % in males, and 31.4 %, 30 %, 28 % and 33.3 % in females in the control, low, intermediate and high dose groups, resp. Tumours of the liver, lung and haem/lymph/ reticular system accounted for the majority of tumours. Overall summary of tumour-bearing animals is given in the table 6.5-9. However, it was stated that the spectrum of tumours seen in control animals and treated animals was consistent with that expected in mice of this strain and age. There was no evidence of any increase in the incidence of tumour-bearing animals nor any specific tumour type suggestive of a carcinogenic effect attributable to treatment with pyrimethanil in CD-1 mice.

Conclusion:
It can be concluded that dietary administration of pyrimethanil to mice up to and including 1600 ppm for 80 weeks showed no evidence on any treatment-related increase in the type or incidence of neoplastic findings in this study suggestive of a carcinogenic effect. There was an increased incidence in morbidity and mortality in males of all groups, particularly during the first 52 weeks of the study, which was associated with lesions in the urogenital tract. However, the findings showed no evidence of a clear dose-response relationship and were mostly considered to be caused by male aggression, which is often manifested as lesions in the urogenital region. Based on the increased incidence of urinary bladder distension at 1600 ppm, slightly exceeding the value from historical control data, the mid dose level of 160 ppm (equivalent to 17.3 – 26.7 [♂] and 22.3 – 32.0 [♀] mg/kg bw/d) was considered a NOAEL in the study.

CA 5.6 Reproductive Toxicity

Studies evaluated in the draft monograph of Rapporteur Member State Austria of April 15, 2004:

A two-generation study in rats and developmental toxicity studies in rats and rabbits have been evaluated by European authorities and Austria as Rapporteur Member State and were considered to be acceptable. For the convenience of the reviewer, these studies are summarized below as extracted from the monograph and the EFSA conclusion. A tabulated summary is provided in Table 5.6-1.

In order to align the dossier submission and the draft RAR, the information that was present in the dRAR in more detail was included in this dossier update in light green, whereas new information and/or corrections were included in lime green.

Table 5.6-1: Summary of reproduction toxicity studies conducted with pyrimethanil

Study	Dosages (mg/kg bw/day)	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Main adverse effect	Reference (BASF DocID)
2-Generation, oral, feed, Sprague-Dawley rat	M: 1.5, 18.4 and 238.9 F: 1.9, 23.4 and 294.4 [0, 32, 400, 5000 ppm]	Parental toxicity: 18.4 (M) 23.4 (F) [400 ppm] Developmental toxicity: 18.4 (M) 23.4 (F) [400 ppm]	Parental toxicity: 239 (M) 294 (F) [5000 ppm] Developmental toxicity: 239 (M) 294 (F) [5000 ppm]	Parental toxicity: Reduced body weight gain in F0 parental animals and reduced body weight in F1 parental animals. Developmental toxicity: Reduced body weights and retarded weight gain in F1 and F2 pups and slight retardation in somatic development of F2 pups	A81822, A89219, A89218
Developmental toxicity, gavage (days 6-15), Sprague-Dawley rat	F: 7, 85 and 1000	Maternal toxicity: 85 Developmental toxicity: 85	Maternal toxicity: 1000 Developmental toxicity: 1000	Maternal toxicity: Reduced body weight gain and food intake and other clinical signs. Developmental toxicity: Reduced litter and fetal body weight.	A81800, 2003/1023035¥
Developmental toxicity, gavage (days 7-19), New Zealand White rabbit	F: 7, 45 and 300	Maternal toxicity: 45 Developmental toxicity: 45	Maternal toxicity: 300 Developmental toxicity: 300	Maternal toxicity: Reduced body weight gain and food consumption, and other clinical signs. Developmental toxicity: Reduced fetal body weight, retardation on fetal development, increased incidences of skeletal variations (13th vertebrae and ribs) and increased number of runts.	A81798

¥ Note: These supplemental document was submitted in course of the evaluation of the initial dossier and has already been peer-reviewed during Annex I inclusion of pyrimethanil as reported in the Draft Monograph of April 2004 prepared by the former RMS Austria.

In the 2-generation reproduction study conducted with Sprague-Dawley rats, pyrimethanil showed no adverse effects on mating performance, fertility, gestation, parturition, pup numbers, sex ratio, survival rates and pup viability at concentrations up to and including 5000 ppm. However, at 5000 ppm, there was a significant reduction in mean body weight of F1 pups from day 1 post-partum to weaning. Also for the F2 offspring, there was a reduction in mean body weight from day 1 post-partum and a moderate reduction in body weight gain during lactation phase. Parental systemic toxicity was evident in terms of reduction in mean body weight gain of males and females at the top dose in both generations. The results from this study support a NOAEL of 400 ppm (18.4 – 23.4 mg/kg bw/day) for both parental toxicity as well as reproductive effects.
Pyrimethanil was not teratogenic in rats and rabbits after oral administration. In the developmental toxicity study in rats, the NOAEL for maternal toxicity was 85 mg/kg bw/day based on reduced maternal body weight gain and food consumption but also on clinical signs that were evident in the next higher dose group (1000 mg/kg bw/day). In the pups decreased mean litter weights and mean fetal body weights at 1000 mg/kg bw/day were noticed. Therefore, the NOAEL for fetal effects was also set at 85 mg/kg bw/day.
In the developmental study conducted with rabbits, maternal toxicity was evident by reduction in food consumption and initial body weight loss followed by deficits in weight gain, and also by clinical signs observed at 300 mg/kg bw/day. The maternal NOAEL was 45 mg/kg bw/day. Fetotoxicity was evident at 300 mg/kg bw/day by decreased mean fetal body weights, retardations on fetal development (runted fetuses) and increased incidences of skeletal variations (additional 13th vertebra and extra ribs). This finding was considered to be not a direct adverse effect on embryonic development but be related to maternal toxicity. The developmental NOAEL was 45 mg/kg bw/day.

Studies submitted in this AIR 3 dossier (not yet peer-reviewed):

No additional data on reproductive toxicity of pyrimethanil were generated by the applicant. There are however, screening-study results available with pyrimethanil in the open literature as part of the ToxCast Program that could principally affect the overall evaluation for reproductive toxicity.

Table 5.6-2: Summary of reproduction toxicity studies conducted with pyrimethanil after peer-review for Annex I inclusion

Study	Dosages	Endpoint	Result	Reference (BASF DocID)
Zebrafish Developmental Screening Assay	1 nM – 80 µM	Death, hatching performance and overt structural defects in Zebrafish larvae day 6 post fertilization	Equivocal finding: Malformation at 26.6 and 80 µM, without dose-response, No death or effect on hatching	2012/1368722

In the publication of Padilla et al. 2011 [see KCA ~~5.6.2/1~~ 5.6.2/3, DocID 2012/1368722] there is limited and inconclusive evidence for reproduction toxicity effects for pyrimethanil in the Zebrafish developmental screening assay at high concentrations. No dose-response relationship was obtained and thus no AC_{50} was derived. These high dose effects may not be indicative for a specific reproduction toxicity effect. The limited findings of a screening assay were not confirmed by the apical study results in rats and rabbits. Thus, these findings are not affecting the general conclusions and endpoints drawn for reproduction toxicity based on the apical studies already evaluated in the peer review for Annex I inclusion of pyrimethanil.

Based on the available data on the reproductive toxicity, classification of pyrimethanil for this endpoint is not required according to the criteria laid down in Regulation (EU) No. 1272/2008 (CLP). Moreover, the data on reproductive toxicity of pyrimethanil have already been evaluated in 2006 coming to the conclusion that pyrimethanil does not need to be classified for health effects (ECBI/159/04) as laid down in the 31st ATP to the Council Directive 67/548/EEC (Commission Directive 2009/2/EC of 15 January 2009). The limited newly available screening data do not affect the evaluation of classification of pyrimethanil.

Thus, the conclusion for relevant endpoints adopted to the new list of endpoint format for the current re-registration remains as follows:

Reproductive toxicity (Regulation (EU) N° 283/2013, Annex Part A, point 5.6)

Reproduction toxicity

Reproduction target / critical effect	Parental toxicity: Reduced body weight gain in F0 parental animals and reduced body weight in F1 parental animals. Reproductive toxicity: No adverse effect observed in rat 2-generation study. Offspring's toxicity: Pup body weight gain reduced in the presence of parental toxicity.	
Relevant parental NOAEL	18.4 mg/kg bw/day	
Relevant reproductive NOAEL	239 mg/kg bw/day (highest dose tested)	
Relevant offspring NOAEL	18.4 mg/kg bw/day	

Developmental toxicity

Developmental target / critical effect	Rat: Maternal toxicity: Reduced body weight gain and food consumption, and clinical signs. Developmental toxicity: Reduced litter and fetal body weight. Rabbit: Maternal toxicity: Reduced body weight gain and food consumption, and clinical signs. Developmental toxicity: Reduced fetal body weight, retardation on fetal development and increased incidences of skeletal variations related to maternal toxicity.	
Relevant maternal NOAEL	Rat: 85 mg/kg bw/day Rabbit: 45 mg/kg bw/day	
Relevant developmental NOAEL	Rat: 85 mg/kg bw/day Rabbit: 45 mg/kg bw/day	

CA 5.6.1 Generational studies

~~**Pyrimethanil, rat 2-generation dietary reproduction toxicity study ([redacted], 1993) (BASF DocID A81822, BASF DocID A89219 and BASF DocID A89218)**~~

~~**Guidelines:** According to OECD guideline 416 and US EPA Guideline 83-4~~
~~**GLP:** Yes~~
~~**Acceptance:** The study is scientific valid and acceptable in the EU registration process 2004-2006~~

Report:	CA 5.6.1/1 [redacted] 1993 a Technical SN 100309: Two generation oral (dietary administration) reproduction toxicity study in the rat A81822
Guidelines:	EPA 83-4, OECD 416
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)
Deviations:	In general, the addition of the fourth test group is recommended if large intervals (6-10fold) between dosages are used. In dietary studies the dose interval should not exceed 3fold. In F1 weanlings selected for mating, the age at vaginal opening or preputial separation was not determined in the study. Sperm parameters, i.e. total cauda epididymal sperm number, percent progressively motile sperm, percent morphologically normal sperm, and percent of sperm with each identified abnormality were not evaluated in the study. The following organs: uterus, ovaries, testes, prostate, seminal vesicles with coagulating gland, brain, liver, kidneys, spleen, pituitary, thyroid and adrenal gland of all P and F1 parental animals should be weighed at the time of termination. Brain, spleen, and thymus of randomly selected F1 and F2 pups of each sex and litter were not weighed in the study. Quantitative evaluation of primordial follicles of F1 females was not performed in the study.

Report:	CA 5.6.1/2 [redacted] 1993 b 1st amendment to report No. TOX/91/223-49: Technical SN 100309: Rat two generation dietary reproduction toxicity study A89218
Guidelines:	EPA 83-4, OECD 416
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Report:	CA 5.6.1/3 [redacted] 1995 a 2nd amendment to report No. TOX/91/223-49: Technical SN 100309: Rat two generation dietary reproduction toxicity study A89219
Guidelines:	EPA 83-4, OECD 416
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive summary

Groups of 30 rats/sex (strain: Sprague Dawley CRL-CD(SD)BR; source: [redacted].) of the F0 generation received pyrimethanil (batch no. CR19325/4; purity 96.2 – 97.3%) via diet at dose levels of 0, 32, 400 and 5000 ppm (equivalent to 1.5, 18.4 and 238.9 mg/kg bw/day in males and 1.9, 23.4 and 294.4 mg/kg bw/day in females) for 14 weeks before mating, during the mating, gestation and lactation period until terminal sacrifice. The rats were paired on a basis of 1 male : 1 female. Offspring were culled on day 4 post-partum to give four male and four female offspring per litter where possible. At weaning (day 21 post-partum), 25 male and 25 female F1 pups were selected to form the F1 generation. These animals were maintained for 14 weeks and were then paired. F1 females were allowed to litter and rear their F2 offspring to weaning.

In the 2-generation reproduction study conducted with Sprague-Dawley rats, pyrimethanil showed no adverse effects on mating performance, fertility, gestation, parturition, pup numbers, sex ratio, survival rates and pup viability at concentrations up to and including 5000 ppm. However, at 5000 ppm, there was a significant reduction in mean body weight of F1 pups from day 1 post-partum to weaning. Also for the F2 offspring, there was a reduction in mean body weight from day 1 post-partum and a moderate reduction in body weight gain during lactation phase. In addition, performance in the air righting test at 5000 ppm was significantly lower than in controls, but it was stated that this finding might be attributable to the slight retardation in somatic development which is likely to have occurred in association with the reduced mean body weight. Parental systemic toxicity was evident in terms of reduction in mean body weight gain of males and females at the top dose in both generations. The results from this study support a NOAEL of 400 ppm (equivalent to 18.4 – 23.4 mg/kg bw/day) for parental/developmental toxicity and a NOAEL of 5000 ppm (highest dose tested; equivalent to 239 and 294 mg/kg bw/day) for reproduction toxicity.

Material and Methods:
Groups of 30 rats/sex (strain: Sprague Dawley CRL-CD(SD)BR; source: [redacted].) of the F_0 generation received pyrimethanil (batch no. CR19325/4; purity 96.2 – 97.3 %) via diet at dose levels of 0, 32, 400 and 5000 ppm for 14 weeks before mating, during the mating, gestation and lactation period until terminal sacrifice. The rats were paired on a basis of 1 male : 1 female. Offspring were culled on day 4 post partum to give four male and four female offspring per litter where possible. At weaning (day 21 post partum), 25 male and 25 female F_1 pups were selected to form the F_1 generation. These animals were maintained for 14 weeks and were then paired. F_1 females were allowed to litter and rear their F_2 offspring to weaning. The F_1 animals and the F_2 pups were then necropsied.
Observations included clinical signs and mortalities once per day. Body weights of males were recorded weekly and body weights of females were recorded weekly during maturation period, on days 0, 6, 12, 15 and 20 of pregnancy, and on days 1, 4, 7, 14 and 21 post-partum. Food consumption was measured at the same time. At birth, the number and sex of live and dead fetuses were recorded. Number, sex and weight of pups were recorded on days 1, 4, 7, 14 and 21 post partum. Surviving pups were assessed for the age at which pinna unfolding, incisor eruption and eye opening took place, and on day 1 for surface righting reflex, day 17 for air righting reflex, and day 21 for grip reflex, pupillary reflex and auditory startle response. All adult F_0 and F_1 animals were necropsied and examined macroscopically. A comprehensive range of tissues (ovaries, uterus, cervix, vagina, pituitary gland, testes, epididymites, seminal vesicles, prostate, coagulating gland, any lesion) were examined histopathologically in F_0 and F_1 control animals and those treated with 5000 ppm. F_1 pups culled on day 4 post partum and postweaning offsprings not selected for continuation of the study and F_2 pups were examined macroscopically

Findings:
General observation: No treatment-related mortalities or significant clinical signs of toxicity were observed in either the F0 or F1 parental animals throughout the study period.
Body weight: At the high dose level, there was a 5.5 % deficit in absolute group mean weight in F0 males by week 14 (pairing) and a similar difference from controls was maintained until termination. In high dose F0 females, absolute mean weight was 3.4 % lower than the control value during the pre-mating period increasing to 6.4 % difference at day 20 of gestation. During lactation, this difference was lowered to 3.7 %. The overall deficit in body weight gain from initiation of treatment to termination was 10 % and 6 % in F0 males and F0 females, resp., compared to controls. At the start of the F1 generation, the mean body weights of high dose F1 males and F1 females were approximately 16 % lower than their respective controls. During the study, this difference was reduced to a 13 % difference in F1 males at week 24, a 14.4 % difference from controls in F1 females at day 20 of gestation, and, following higher mean weight gain than in controls during lactation, an 8.7 % difference at day 21 post-partum. In intermediate and low dose males and females, body weights and weight gain were comparable with control values.
Food intake: There were no marked adverse effects of treatment on mean food intake of parental F0 at any dose level. However, the mean food intake in high dose F0 and F1 females was slightly but statistically significant higher than in controls during gestation and lactation with some evidence that results were influenced by a tendency to scatter the food.
Test article intake: During the pre-mating period, pyrimethanil intake showed a general decline in animals of both sexes. During the reproductive phase (gestation and lactation), female intake increased. The calculated pyrimethanil intakes for males and females (ranges at the different segments of the study) are given in Table 5.6.1-1.

Table 5.6.1-1: Group mean intakes of pyrimethanil (mg/kg bw/d); ranges at different segments of the study

Dose level (ppm)	F_0	F_1	F_0	F_1	F_0	F_1
	♂ (premating weeks) ♀ (premating weeks)		♀ (gestation)		♀ (lactation)	
32	1.5 – 2.8 1.9 – 2.8	1.5 – 4.3 2.0 – 4.3	2 – 3	3	3 – 6	3 – 7
400	18.4 – 34.7 23.4 – 33.7	19.1 – 45.5 25.2 – 52.4	28 – 31	32 – 36	43 – 85	45 – 87
5000	238.9 – 418.3 294.4 – 411.4	252.6 – 722.7 342.3 – 706.9	365 - 416	557 – 628	874 – 1173	930 – 1333

Effects on fertility: The pregnancy rate was marginally lower in high dose F0 animals (96.7, 93.3, 90,0 and 83.3 % at dose levels of 0, 32, 400 and 5000 ppm, resp.) but as the pregnancy rate was 100 % in F1 parental animals at this dose level and was within historical control range, the difference was considered unrelated to treatment.
At the high dose, mean F1 pup weights from day 1 post-partum (- 4.7 %) to weaning were significantly lower than in controls. A weight deficit of 17.9 % was present at weaning. Also in F2 pups, there was a reduction in mean pup weight at 5000 ppm on day 1 post-partum followed by a lower percentage mean weight increase from day 1 to 21 at this dose level, resulting in a weight deficit of 15.9 % compared to controls. At the intermediate dose, mean F2 pup weights were also slightly lower than in concurrent controls. However, it was stated that the mean values (of both sexes combined) were almost identical to the corresponding control value for the F0 generation (Table 5.6.1-2).

Table 5.6.1-2: Mean pup weights (g)

Dose levels	day 1		day 4		day 7		day 14		day 21	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
F_1 0 ppm	6.7	6.3	9.7	9.2	16.8	15.8	35.2	33.8	58.7	56.1
32 ppm	6.6	6.0	10.0	9.1	16.9	15.5	35.8	33.6	60.1	56.2
400 ppm	6.7	6.4	9.9	9.4	16.7	15.8	35.3	34.0	60.0	57.3
5000 ppm	6.3	5.9	8.6**	8.1**	13.9***	13.0***	29.3***	28.2***	48.0***	46.0***
F_2 0 ppm	6.7	6.3	10.0	9.4	17.2	16.6	36.7	35.4	59.3	56.6
32 ppm	6.6	6.2	9.7	9.1	16.5	15.9	35.8	35.1	58.0	56.1
400 ppm	6.3	5.9	9.2	8.7	15.9	15.0	34.9	33.2	57.2	54.5
5000 ppm	6.4	6.0	9.0	8.6**	15.2**	14.3***	31.5***	30.1***	49.7***	47.7***

** ($p \leq 0.01$) significantly different from controls
*** ($p \leq 0.001$) significantly different from controls

Table 5.6.1-3: **Two generation reproduction toxicity study in rat; Group mating data and group mean litter data in P and F1 generation, * ($p \leq 0.05$) significantly different from controls**

Parameter \ Dose [ppm]	P generation				F1 generation			
	0	32	400	5000	0	32	400	5000
Fertility index in males [%]	90.0	86.7	86.7	83.3	76.0	83.3	83.3	100.0
Fertility index in females [%]	96.7	93.3	90.0	83.3*	88.0	91.7	80.0	100.0
Fecundity index in males [%]	100.0	92.9	89.7	89.3	95.0	90.9	90.9	100.0
Fecundity index in females [%]	100.0	93.3	90.0	86.2*	91.7	91.7	87.0	100.0
No. of implantation sites (Mean no. / female)	442 (15.8)	379 (14.6)	375 (15.6)	373 (15.5)	338 (15.4)	321 (15.3)	308 (17.1)	345 (14.4)
Post-implantation survival index [%]	89.8	91.0	88.3	90.6	91.7	92.2	91.6	96.8
No. of pups born (Mean no. / female)	414 (14.3)	345 (13.3)	331 (13.8)	338 (14.1)	310 (14.1)	312 (14.2)	282 (15.7)	334 (13.9)
Live birth index [%]	95.7	94.2	96.7	97.9	97.7	98.1	96.8	98.8

In addition, performance in the air righting test at 5000 ppm was significantly lower than in controls, but it was stated that this finding might be attributable to the slight retardation in somatic development which is likely to have occurred in association with the reduced mean body weight. For all other parameters investigated concerning mating, fertility or gestation indices, the parturition data or the litter data, no treatment-related effects were observed at both generations at dietary concentrations up to and including 5000 ppm.

Pathology: Necropsy findings in F0 parental animals, F1 offspring (culled on day 4 or on day 21), F1 parental animals and F2 pups (culled on day 4 or on day 21) showed no treatment-related changes at any dose level. No treatment-related microscopic findings were noted in reproductive organs from F0 and F1 parental animals at 5000 ppm compared with controls.

Conclusion:
The NOAEL for reproductive/developmental effects in offsprings and also for parental systemic effects was 400 ppm (equivalent to 18.4 [♂] and 23.4 [♀] mg/kg bw/d, the lowest value of the range indicated in table 6.6.1-1). 5000 ppm pyrimethanil in the diet led to reductions in body weight gain of parents and a reduction in body weight of F_1 and F_2 pups. In F_2 pups, this weight reduction was associated with a poorer performance of the air-righting reflex. However, there were no adverse effects on reproductive performance or offspring viability at concentrations up to and including 5000 ppm.

CA 5.6.2 Developmental toxicity studies

~~**Pyrimethanil, rat developmental toxicity (teratogenicity) study ([redacted], 1991) (BASF DocID A81800)**~~

~~**Guidelines:** Although no specific test guideline is cited in the report, the study complies to a great extend with the requirements of the OECD guideline 414.~~
~~**GLP:** Yes~~
~~**Acceptance:** The study is scientific valid and acceptable in the EU registration process 2004-2006~~

Report:	CA 5.6.2/1 [redacted] 1991 a Technical SN 100309: Rat oral developmental toxicity (teratogenicity) study A81800
Guidelines:	none
GLP:	yes (certified by Department of Health and Social Security of the Government of the United Kingdom, United Kingdom)
Deviations:	The relative humidity in the animal room was 40 – 80%, however, it should not exceed 70%. The report did not include analysis of food and water quality. The age of females enrolled in the study was not mentioned. The addition of the fourth test group is recommended if large intervals (6-10fold) between dosages are used. Gravid uteri including the cervix were not weighed in the study.

Executive summary

Groups of 30 female rats (strain: Sprague Dawley CR1:COBS CD; source: [redacted]) received pyrimethanil (batch no. CR 19325/4; purity 96.3%; suspended in 1% w/v methyl cellulose in distilled water) from day 6 to 15 of pregnancy by oral gavage at a constant dosing volume of 10 mL/kg bw. Dose levels were 0 (vehicle control), 7, 85 and 1000 mg/kg bw/day.

In the rat study, maternal toxicity was evident by reductions in food consumption and body weight gains, as well as by occurrence of clinical signs at 1000 mg/kg bw/day. There were no treatment-related effects in the number of corpora lutea, pre- and post-implantation loss, litter size or sex ratio. Fetotoxicity was demonstrated by decreased mean litter weights and mean fetal body weights at 1000 mg/kg bw/day. The described external malformations - isolated findings of unilateral/bilateral forelimb flexure and unilateral missing testis -were not corroborated by more extensive skeletal or morphological examinations, were not determined in F1 males from the multi-generation study (missing testis) and/or were within historical control ranges. Concerning visceral and skeletal malformations, the low incidences and group distribution of all these findings showed no consistency, and did not indicate any treatment-related effect. The NOAEL for maternal toxicity as well as for developmental toxicity is 85 mg/kg bw/day.

Material and Methods:
Groups of 30 female rats (strain: Sprague Dawley CR1: COBS CD; source: [redacted]) received pyrimethanil (batch no. CR 19325/4; purity 96.3 %; suspended in 1 % w/v methyl cellulose in distilled water) from day 6 to 15 of pregnancy by oral gavage at a constant dosing volume of 10 mL/kg bw. Dose levels were 0 (vehicle control), 7, 85 and 1000 mg/kg bw/d. These dose levels were chosen based on results from a dose-ranging study, which showed maternal toxicity like reductions in body weight gain during treatment at a dose level of 1000 mg/kg bw/d. Suspensions were prepared fresh every two days. Stability of the test material was confirmed by analysis during the study. Observations for deaths, behaviour and clinical signs of animals were made most daily. Body weights were recorded on days 1, 3, 6 to 16, 18 and 20 p.c., food consumption was measured between days 3 - 5, 6 - 8, 9 – 11, 12 – 15, 16 – 17 and 18 – 19 p.c. On gestation day 20, females were subjected to a post-mortem macroscopic examination. The content of each uterine horn was examined for the number of corpora lutea, resorption sites, number of live or dead fetuses, weight and sex of each live fetus and external malformations. Approximately one-half of the fetuses from each litter was examined for soft tissue alterations; the remaining fetuses from each litter were processed for skeletal evaluations.

Findings:
Maternal effects: One control female and one low-dose female were found dead on days 13 and 18 p.c., resp., possibly resulting from intubation error. At the highest dose level, maternal toxicity was reflected in significant reduced body weight gain during treatment (between days 12 – 20 p.c.) compared to controls and also in clinical signs (hairloss, slight to moderate emaciation, hunched posture). In addition, there was a significant reduction in food intake during days 6 – 15 p.c. at this dose level. There were no effects on parameters investigated at the two lower dose levels.
Litter data/fetal parameters: There were no treatment-related effects in the number of corpora lutea, pre- and post-implantation loss, litter size or sex ratio. Fetotoxicity, evident at 1000 mg/kg bw/d, was reflected in significantly reduced mean litter weight and mean fetal body weight compared to controls, but values were within the historical control range from the laboratory.
Concerning external malformations, three fetuses (from 272 fetuses examined) at 1000 mg/kg showed unilateral/bilateral forelimb flexure, however this finding was not corroborated by the more extensive skeletal examinations. In addition, it was reported that the incidence was within historical control range and therefore not considered treatment-related. Also the low incidence of one missing testis in 0, 2, 2 and 3 fetuses each of the dose groups 0, 7, 85 and 1000 mg/kg resp., recorded at necropsy, was not corroborated by the more extensive morphological examinations carried out at free-hand serial sectioning nor in the F_1 males from the rat multigeneration study and was not considered treatment-related, therefore.
Concerning visceral and skeletal malformations, following isolated findings were reported: 2 fetuses with an interventricular septal defect, 1 fetus with unilateral microphthalmia and 1 fetus with unilateral macrophthalmia of the low dose (173 fetuses examined); 1 fetus with unilateral anophthalmia of the mid dose (138 fetuses examined); 1 fetus with agnathia/microphthalmia and 1 fetus with an interventricular septal defect of the high dose (139 fetuses examined). In addition, some variations in the degree of ossification of the cranium, ribs, limbs and metacarpals/metatarsals were observed in fetuses of each dose group including controls. However, as also stated in the report, the low incidences and group distribution of all these findings showed no consistency, and did not indicate any treatment-related effect.

Table 5.6.2-1: Rat oral developmental toxicity (teratogenicity) study; summary of substantial study findings (mean values)

Parameter	0 mg/kg	7 mg/kg	85 mg/kg	1000 mg/kg
Maternal body weight (g)				
day 12 p.c.	292.2	294.4	297.9	271.5***
day 20 p.c.	388.4	392.3	396.1	353.3***
Food consumption (g/animal/d))				
days 9 – 11 p.c.	23.9	24.3	24.0	18.8*
days 12 – 15 p.c.	25.3	26.4	25.5	20.7*
Pregnancy rate (%) at day 20	87	90	70	80
Pre-/postimplantation loss (%)	9.6/6.3	7.5/4.0	7.0/7.1	9.7/8.6
Mean no. of live fetuses/litter	12.2	12.2	12.5	11.3
Mean litter weight (g)	47.53	48.21	48.99	40.73**
Mean fetal body weight (g)	3.88	3.95	3.92	3.62**

* ($p \leq 0.05$) significantly different from controls (Dunnett's test)

** ($p \leq 0.01$) significantly different from controls (Mann Whitney U test)

*** ($p \leq 0.001$) significantly different from controls (Mann Whitney U test)

Conclusion:
The NOAEL for maternal toxicity was 85 mg/kg bw/d based on clinical signs and retarded body weight gain. Fetal toxicity was demonstrated by decreased mean litter weights and mean fetal body weights at 1000 mg/kg bw/d. There was no evidence of treatment-related teratogenic effects at any dose level in this study. Therefore, the dose level of 85 mg/kg bw/d can be considered as the NOAEL for developmental toxicity.

Pyrimethanil, rabbit developmental toxicity (teratogenicity) study ([redacted] 1991) (BASF DocID A81798)

Guidelines:	Although no specific test guideline is cited in the report, the study complies to a great extend with the requirements of the OECD guideline 414.
GLP:	Yes
Acceptance:	The study is scientific valid and acceptable in the EU registration process 2004-2006

Report:	CA 5.6.2/2 [redacted], 1991 a Technical SN 100 309: Oral (gavage) development toxicity (teratogenicity) study in the New Zealand white rabbit A81798
Guidelines:	EPA 83-3
GLP:	yes (certified by Department of Health and Social Security of the Government of the United Kingdom, United Kingdom)
Deviations:	Each experimental group should consist at least of 16 pregnant females at the time of necropsy, however, at the end of the study (day 28), only 14 females were alive in the highest dose group (3 of dams were terminated on study day 15, 22 and 24). The animal room temperature was not strictly held within a range of 18 ± 3 °C recommended for rabbit studies.

Executive summary

Groups of 18 or 19 pregnant animals/dose (strain: New Zealand White; source: [redacted].) were treated with pyrimethanil (batch no.CR 19325/3; purity 97.1%; suspended in 1% w/v methyl cellulose in distilled water) from day 7 to 19 of pregnancy by oral gavage. The dose levels of 0 (vehicle control), 7, 45 and 300 mg/kg bw/day were selected following examination of results in a preliminary range-finding study.

In the rabbit study, maternal toxicity was evident by reduction in food consumption and initial body weight loss followed by deficits in weight gain, and also by clinical signs observed at 300 mg/kg bw/day. Three females of this dose group were killed on days 15, 22 and 25, respectively, because of treatment-related emaciation. Mean numbers of corpora lutea, implantations and live fetuses and mean pre- and post-implantation losses were within expected ranges in all groups. The sex ratio of the live fetuses was comparable in all groups with near equal proportions of males and females. Concerning malformations, the number of fetuses with these findings were considered to be spontaneous and not related to the treatment. Fetotoxicity was demonstrated at the same dose level (300 mg/kg bw/day) by decreased mean fetal body weights, retardations on fetal development (runted fetuses) and increased incidences of skeletal variations (additional 13th vertebra and extra ribs). Since this finding was a well-known variation, it was considered to be related to maternal toxicity and not as a direct adverse effect on embryonic development. Fetuses in this dose group showed higher, but not statistically significant incidences of minor skeletal abnormalities (non-ossification of the pubic bone and astragalus, bilobed, bipartite, mis-shapen or misaligned sternebrae) and also an increased frequency (not significant) of variants of skeletal ossification (retarded or non-ossification of fore and hind limb epiphysis). A NOAEL of 45 mg/kg bw/day was determined for both maternal and foetal toxicity.

Material and Methods:
Groups of 18 or 19 pregnant does/dose (strain: New Zealand White; source: [redacted].) were treated with pyrimethanil (batch no.CR 19325/3; purity 97.1 %; suspended in 1 % w/v methyl cellulose in distilled water) from day 7 to 19 of pregnancy by oral gavage. The dose levels of 0 (vehicle control), 7, 45 and 300 mg/kg bw/d were selected following examination of results in a preliminary range-finding study. Stability and homogeneity of the test formulation was confirmed by analysis. Clinical signs and mortality were checked daily. Body weight of the does was determined on day 3, daily from day 7 to 19 and on days 22, 25 and 28 of pregnancy. Food consumptions was recorded every two days. On day 28 of pregnancy, all females were examined for the presence of corpora lutea, implantation sites, resorption sites (early or late) and of live or dead fetuses. Live fetuses were weighed, sexed and examined for external, soft tissue and skeletal malformations and variations, resp.

Findings:
Maternal effects: Treatment at 300 mg/kg resulted in maternal toxicity comprising initial body weight loss with deficits in weight gain (mainly between days 7 and 9), reduced production and size of faecal pellets, and reduced food consumption, compared to controls. Three females of this dose group were killed on days 15, 22 and 25, resp., because of treatment-related emaciation. At the mid dose, there was only a slightly higher incidence of females with reduced production and size of faecal pellets compared to controls. However, there were no effects of treatment on body weight and on food consumption in the groups dosed at 7 and 45 mg/kg.
Litter data/fetal parameters: Mean numbers of corpora lutea, implantations and live fetuses and mean pre- and post-implantation losses were within expected ranges in all groups. The sex ratio of the live fetuses was similar in all groups with near equal proportions of males and females. Mean fetal weight was significantly lower in the high dose group, compared to controls which was considered to be due to embryonic growth retardation.
Concerning malformations the number of fetuses with these findings were 3/144, 6/142, 4/144 and 1/140 in the control group and group treated at 7, 45 and 300 mg/kg , resp. The observed abnormalities included microphthalmia, gastroschisis, scoliosis, heart abnormalities and craniofacial abnormalities and were considered to be spontaneous and not related to treatment. However, in the high dose group there was a significantly higher number of runted fetuses (< 20 g bodyweight) compared to current controls and also to reported historical control range (0 – 1.4%). In addition, fetuses at this dose group showed higher, but not statistically significant incidences of minor skeletal abnormalities (non-ossification of the pubic bone and astragalus; bilobed, bipartite, mis-shapen or misaligned sternebrae) and also an increased frequency (not significant) of variants of skeletal ossification (retarded or non-ossification of fore and hind limb epiphysis). There was also a significant increase in the incidence of fetuses with 13 thoracic vertebrae and uni- or bilateral extra 13th ribs in comparison with controls. This finding was considered as a well-known variation to be related to maternal toxicity but not to be a direct adverse effect on embryonic development.

Table 5.6.2-2: Rabbit oral developmental toxicity (teratogenicity) study; Summary of substantial study findings (mean values)

Parameter	0 mg/kg	7 mg/kg	45 mg/kg	300 mg/kg
Maternal body weight (kg) day 7 p.c. day 9 p.c. day 15 p.c.	 3.80 3.83 4.05	 3.76 3.79 4.01	 3.76 3.78 4.00	 3.58** 3.52*** 3.71***
Food consumption (g/rabbit/d) days 7 – 11 p.c. days 11 – 15 p.c.	 168 133	 164 158	 159 157	 97*** 97***
Pregnancy rate (%) on day 28	89	89	94	78
Mean no. of live fetuses/litter	9.0	8.9	8.5	10.0
Pre-/postimplantation loss (%)	15.7/13.8	18.5/12.1	19.3/14.6	7.3/11.7
Mean fetal body weight (g)	36.4	35.5	36.0	32.6**
No. of runted fetuses	1/144 (0.4 %)	1/142 (0.6 %)	1/144 (0.6 %)	6/140 (3.7 %)*
No. of fetuses with bilobed, bipartite, mis-shapen or misaligned sternebrae	0/144 (0.0 %)	2/142 (1.3 %)	1/144 (0.5 %)	4/140 (2.8 %)
No. of fetuses with 13^{th} thoracic vertebra 13^{th} ribs	 43/144 (28.4 %) 28/144 (19.7 %)	 46/142 (33.8 %) 35/142 (26.0 %)	 57/144 (38.4 %) 43/144 (26.4 %)	 94/140 (65.9 %)*** 82/140 (56.8%)***

* ($p \leq 0.05$) significantly different from controls (Dunn's multiple comparison test)

** ($p \leq 0.01$) significantly different from controls (Student's test)

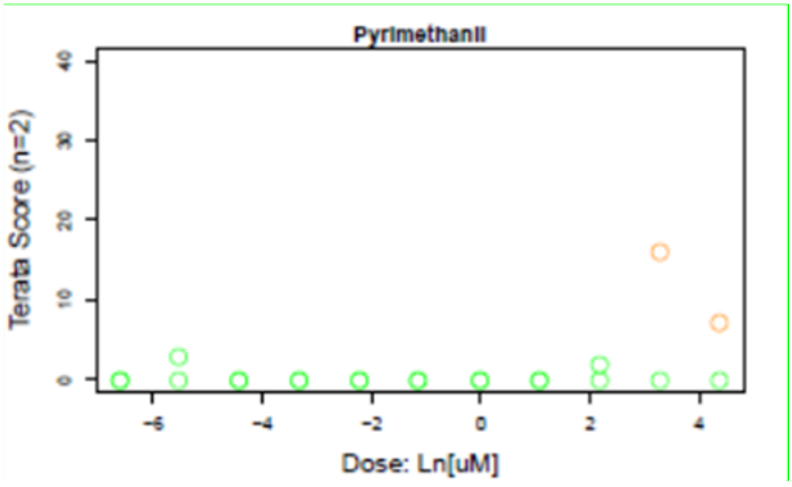
*** ($p \leq 0.001$) significantly different from controls (Student's test; Dunn's multiple comparison test)

Conclusion:
The NOAEL for maternal toxicity can be set at 45 mg/kg bw/d based on decreased body weight gain during treatment and also on clinical signs evident at 300 mg/kg bw/d. Fetotoxicity was demonstrated at 300 mg/kg bw/d by decreased fetal body weights, retardations on fetal development (runted fetuses) and increased incidences of skeletal variations (additional 13^{th} vertebra and extra ribs). There was no evidence of teratogenicity in this study. The developmental NOAEL can be set at 45 mg/kg bw/d.

Pyrimethanil, Zebrafish developmental screening (Padilla, 2012)

Report: CA 5.6.2/3
Padilla S. et al., 2011 a
Zebrafish developmental screening of the ToxCast Phase I chemical library
2012/1368722
Guidelines: none
GLP: no

Zebrafish (Danio rerio) is an emerging toxicity screening model for both human health and ecology. As part of the Computational Toxicology Research Program of the U.S. EPA, the toxicity of the 309 ToxCastTM Phase I chemicals was assessed using a zebrafish screen for potential developmental toxicity in man. All exposures were by immersion from 6–8 h post fertilization (hpf) to 5 days post fertilization (dpf); nominal concentration range of 1 nM–80µM. 2 larvae per concentration were assessed. On 6 dpf larvae were assessed for death and overt structural defects. Results revealed that the majority (62%) of chemicals were toxic to the developing zebrafish; both toxicity incidence and potency was correlated with chemical class and hydrophobicity (logP); and inter-and intra-plate replicates showed good agreement. The numerical score groups into lethality (40), non-hatching (20) and malformation index (<20).
This publication aims to develop and validate a screening method within the ToxCast Screening program and Pyrimethanil was one of the about 300 substances evaluated. A toxicity score of 30 was calculated for pyrimethanil based on the single concentration study, indicating reproduction toxicity at 80 µM (highest dose tested). No AC50 was derived for pyrimethanil in the dose-response experiment as no concentration-related effect could be observed. No death was noticed and hatching was not affected. At high concentrations 26.6 and 80 µM) either a normal larvae or a larvae with malformations were observed. At lower concentrations (8.8 µM and below) no indications of malformations after pyrimethanil treatment were observed. These high dose effects may not be indicative for a specific reproduction toxicity effect. In conclusion, given the lacking dose response equivocal effects were observed at high (toxic) concentrations.
The study is considered to be supplemental



CA 5.7 Neurotoxicity Studies

Studies evaluated in the draft monograph of Rapporteur Member State Austria of April 15, 2004:

No specific neurotoxicity studies with pyrimethanil have been submitted. In addition, there are no indications from available mammalian toxicity studies that pyrimethanil causes acute, chronic or developmental neurotoxic effects. Therefore, the European authorities and Austria as Rapporteur Member State concluded that it is not necessary to perform specific neurotoxicity studies with pyrimethanil.

Thus, the following conclusion was given in the list of endpoints for Annex I listing of pyrimethanil [see EFSA Scientific Report (2006) 61, 1-70, Conclusion on the peer review of pyrimethanil].

Neurotoxicity / Delayed neurotoxicity (Annex IIA, point 5.7)

No data - no concern from other studies

Studies submitted in this AIR 3 dossier (not yet peer-reviewed):

The following acute and subchronic neurotoxicity studies are not required for registration in the EU. However, the studies were conducted due to US data requirements and are provided for the sake of completeness. This data have not been evaluated previously on EU level.
The results of these studies are listed in Table 5.7-1; comprehensive summaries are provided further below (see CA 5.7.1/1, CA 5.7.1/2 and CA 5.7.1/3).

Table 5.7-1: Neurotoxicity studies with pyrimethanil (not yet peer-reviewed)

Study	Dosages (mg/kg bw/day)	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Main adverse effect	Reference (BASF DocID)
Acute toxicity behavioral peak effect determination Sprague-Dawley rat	M&F 500, 1000 and 2000 mg/kg bw in 0.5% aqueous methyl cellulose	Not applicable	Not applicable	Slight effects on locomotor activity gait and arousal, reduced body temperature, peak effects from 1.5 to 4 hours post dosing	B003425
Acute neurotoxicity, gavage Sprague-Dawley rat	M&F: 30, 100 and 1000 mg/kg bw In 0.5% aqueous methyl cellulose	Systemic toxicity: 30 (M) 100 (F) Neurotoxicity: 1000 (M&F)	Systemic toxicity: 100 (M) 1000 (F) Neurotoxicity: >1000 (M&F)	Transient signs of systemic toxicity. No adverse signs of neurotoxicity observed.	B003483
Subchronic neurotoxicity (3-month feeding) Sprague-Dawley rat	M: 4.0, 38.7 and 391.9 F: 4.6, 44.3 and 429.9 [0, 60, 600, 6000 ppm]	Systemic toxicity: 38.7 (M) 44.3 (F) Neurotoxicity 391.9 (M) 429.9 (F)	Systemic toxicity: 391.9 (M) 429.9 (F) Neurotoxicity: >391.9 (M) >429.9 (F)	Reduced body weight and food intake. No signs of neurotoxicity observed.	B003519

In the acute neurotoxicity study, the neurotoxic potential of pyrimethanil in rats after a single administration of 30, 100 or 1000 mg/kg bw via gavage followed by a 14-day observation period was investigated. Based on the observed peak effect of 1.5 to 4 hours in the pre-study conducted, the initial functional observation battery and motor activity assessment was conducted 1.5 to 2.5 hours after dosing. No overt clinical signs of neurotoxicity, no mortality, and no significant differences in body weight or food consumption between control and treated groups were observed. However, administration of pyrimethanil to rats resulted in transient changes such as gait abnormalities, lower motor activity and reduced body temperature in both sexes at 1000 mg/kg bw. Furthermore, decreased hind-limb grip strength was observed in males at 1000 mg/kg bw in the functional observational on days 1. Several females of this high dose group had dilated pupils. At 100 mg/kg bw, the effect was limited to a transient reduced body temperature for males on day 1. No other differences clearly attributed to treatment were noted on day 1 and all animals showing findings on day 1 appeared normal at the day 8 and day 15 assessment. No significant differences in brain measurements were determined and no gross or neuropathological findings occurred that were attributed to treatment.
The transient effects observed on the day of bolus administration are therefore not considered to be indicative for neurotoxicity but of general transient systemic toxicity with clear recovery. The NOAEL for neurotoxicity was thus 1000 mg/kg bw/day (highest dose tested) in both sexes.

In the subchronic neurotoxicity study, the effects of pyrimethanil on neurotoxicity in rats after dietary exposure of 60, 600, and 6000 ppm (equivalent to 4.0, 38.7, and 391.9 mg/kg bw/day in males and 4.6, 44.3, and 429.9 mg/kg bw/day in females) over three months were investigated. No toxicologically significant effects on behavioural assessments attributed to pyrimethanil were observed. No significant differences in brain measurements were determined and no gross or neuropathological findings occurred that were attributed to treatment. Furthermore, no overt clinical signs of toxicity and no mortality were observed. However, the test substance caused signs of general toxicity at 6000 ppm in line with findings observed in the 13-week rat toxicity study. The treatment resulted in slightly lower body weights and a transient reduction in food intake for males and significant reductions in body weight and food intake for females at the highest dose level.
The NOAEL for neurotoxicity was 6000 ppm (equivalent to 391.9 mg/kg bw/day in males and 429.9 mg/kg bw/day in females, respectively).

Since no indications for neurotoxic potential of pyrimethanil were observed in the studies conducted, it can be assumed that pyrimethanil do not exhibit neurotoxicity. Furthermore, clinical observations and histopathological examinations in short-term (28 - 90 days) and long-term toxicity studies (18 - 24 months) revealed no treatment-related behavioural changes and structural alterations in the brain compared to the controls. Nevertheless, a significantly lower performance in the air-righting reflex was reported for F2 pups in the two-generation reproduction toxicity study. The poorer performance was considered to be associated with a slight retardation in somatic development which was likely to have occurred in combination with the reduced mean body weight. In conclusion, pyrimethanil is not considered to cause neurotoxic effects.

The data on neurotoxicity have not been evaluated in 2006 for evaluation whether pyrimethanil is to be classified for health effects (ECBI/159/04) as laid down in the 31st ATP to the Council Directive 67/548/EEC (Commission Directive 2009/2/EC of 15 January 2009). Based on the available data on neurotoxicity, classification of pyrimethanil for this endpoint is not required according to the criteria laid down in Regulation (EU) No. 1272/2008 (CLP). The results of these studies would not change the classification and labelling of pyrimethanil.

Thus, the conclusion for relevant endpoints adopted to the new list of endpoint format for the current re-registration remains as follows:

Neurotoxicity (Regulation (EU) N° 283/2013, Annex Part A, point 5.7)

Acute neurotoxicity	No findings indicative of neurotoxic potential reported $NOAEL_{neurotoxicity}$: 1000 mg/kg bw/day (highest dose tested) $NOAEL_{systemic}$: 30 mg/kg bw/day	
Repeated neurotoxicity	No findings indicative of neurotoxic potential reported $NOAEL_{neurotoxicity}$: 391.9 mg/kg bw/day (highest dose tested) $NOAEL_{systemic}$: 38.7 mg/kg bw/day	
Additional studies (e.g. delayed neurotoxicity, developmental neurotoxicity)	No data submitted for delayed neurotoxicity or developmental neurotoxicity - not required	

CA 5.7.1 Neurotoxicity studies in rodents

Report: CA 5.7.1/1
[redacted] 2001a
A time of peak behavioral effects study of Pyrimethanil technical in the rat
B003425
Guidelines: EPA 870.6200
GLP: yes
(certified by United States Environmental Protection Agency)
Deviations: no

Executive Summary

The objective of this study was to establish the time of peak effects of pyrimethanil on behavior following a single oral dose to rats, performed as a pre-study for a subsequent acute neurotoxicity study in the same species. The test substance (in 0.5% aqueous MC) was administered to groups of 3 male and 3 female Sprague Dawley (CD) rats at doses of 500, 1000, and 2000 mg/kg bw at a dose volume of 5 mL/kg bw. Treatment caused dose-related behavioral changes (such as gait, locomotor activity, arousal) and reduced body temperature, and the severity of the changes generally peaked at around 1.5 to 4 hours post-dosing. No mortality, no effect on body weight and no further clinical signs of toxicity were observed.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Pyrimethanil (BAS 605 F)

Description:	solid / powder, beige light yellow
Lot/Batch #:	14/00
Purity:	99.8%
Stability of test compound:	Expiry date of the test substance: Nov 2002. The test substance preparation was stable for a period of 7 days.

2. Vehicle and/or positive control: aqueous methylcellulose (MC) solution (0.5%)

3. Test animals:

Species:	Rats
Strain:	CD (crl:CD®[SD]IGS BR)
Sex:	Male and female
Age:	50-53 days (start of administration)
Weight at dosing:	males: 244 - 277 g females: 174 - 190 g
Source:	[redacted]
Acclimation period:	11-12 days
Diet:	pelleted laboratory diet (PMI certified rodent chow 5002), ad libitum
Water:	water, ad libitum
Housing:	single housing in stainless-steel mesh-bottomed cages

Environmental conditions:

Temperature:	19 - 25°C
Humidity:	30 - 70%
Air changes per hour:	not specified
Photo period:	12 h light / 12 h dark

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 8-Jan-2001 (start of administration) to 16-Jan-2001 (euthanasia)

2. Animal assignment and treatment:
Pyrimethanil was administered once to groups of 3 male and 3 female rats at doses of 500, 1000, and 2000 mg/kg bw by gavage with a dosing volume of 5 mL/kg bw.

3. Test substance preparation and analysis:
The test substance was applied as a suspension. To prepare the suspension, the appropriate amount of test substance was weighed out depending on the desired concentration. Then the vehicle (water containing 0.5% methylcellulose) was filled up to the desired volume, and subsequently mixed using a magnetic stirrer. The test substance preparations were made once before the first administration.
The stability of the test substance formulation at refrigeration over a period of a maximum of 7 days was proven by analysis. Homogeneity analyses of the test substance preparations were performed in samples of all concentrations administered at the beginning. These samples also served for concentration control analysis.

4. Statistics:
Means and standard deviations (SD) of each test group were calculated for several parameters.

C. METHODS

1. Observations:

A check for moribund and dead animals was made twice daily. In addition, a complete detailed examination was performed once prior to treatment, then daily thereafter. Any observed clinical sign was recorded individually.

2. Body weight:

The body weight was determined at least twice during the acclimation period, as well as on days 1 and 8.

3. Clinical assessment of neurotoxicity:

Functional observational battery

All animals were tested prior to treatment (pre-study) and at approximately 0.5, 1, 1.5, 2, 4, 6, 8, and 24 h post-dosing. Testing was performed by the same trained technicians, who were unaware of the animals' treatment group.

The FOB was performed with equipment built for this purpose. The arena was a square of Plexiglas (approximately 60 cm x 60 cm) placed on a raised platform. The tests were conducted in the room housing the animals, where temperature, humidity and photoperiod were monitored. Odors in the room were minimized by maintaining adequate air changes and cleaning of equipment, as necessary.

Open field/handling observations:

The animals were transferred to a standard arena and the following parameters were examined:

1. overall gait incapacity	7. lacrimation
2. locomotor activity level	8. tremors, twitches, convulsions
3. arousal	9. bizarre/stereotypic behaviour
4. salivation	10. body temperature
5. diarrhea	11. urinary staining
6. respiration	

4. Terminal procedures:

All animals were euthanized on completion of the study (day 8) and discarded without further examination. The method of euthanasia was CO_2 asphyxiation.

II. RESULTS AND DISCUSSION

A. TEST SUBSTANCE ANALYSES

Test substance preparations were found to be homogenous and of correct concentrations (85-115% of nominal concentrations), or slightly lower after 1 day storage and within the acceptance range or slightly above after 7 days storage. The differences were attributed to analytical and/or sampling variability as no downward trend was observed. However, in order to eliminate any concern about potential instability of the test article in the vehicle, the dose formulations used for administration to the animals were prepared fresh on each day of use.

B. OBSERVATIONS

1. Clinical signs of toxicity

No treatment-related overt clinical signs of toxicity were observed, except as stated in the results of the FOB.

2. Mortality

No mortality was observed and no animals were euthanized prior to scheduled termination.

C. BODY WEIGHT AND FOOD CONSUMPTION

No test substance-related findings on body weight data were found from day 1 to 8 between the control and treated rats.

Table 5.7.1-1: Mean body weights and body weight changes after single administration

	Dose level (mg/kg bw)							
	0		500		1000		2000	
	male	female	male	female	male	female	male	female
Body weight (g)								
Day 1	257	183	263	186	261	179	267	180
Day 8	296	203	304	203	295	200	305	196
Body weight change (g)								
Days 1 - 8	40	20	42	17	33	21	38	16

D. CLINICAL ASSESSMENT OF NEUROTOXICITY

Functional observational battery

For the 2000 mg/kg bw group, one male showed very slight locomotor activity at 1 hour post-dosing and at 1.5 and 2 hours post-dosing all males showed very slight locomotor activity, while one female showed very slight activity at 2 hours post-dosing and all females showed very slight activity at 4 hours post-dosing. The largest difference from control values in average score for locomotor was at 2 hours post-dosing for males and females. One male had a severely decreased arousal score at 1.5 hours post-dosing and another male showed a similar score at 2 hours post-dosing, while one female showed severely decreased arousal at 4 and 6 hours post-dosing. The largest differences from control values in average arousal score occurred at 1.5 hours post-dosing for males and 2 hours post-dosing for females. Two males and one female showed an overall gait incapacity characterized by ataxia as early as 0.5 hour post-dosing and at 1.5 and 2 hours post-dosing these same animals plus an additional female showed this gait alteration. The respiratory rate was decreased for one male and one female at 1 hour post-dosing. One male showed a decreased respiration as late as 8 hours post-dosing. One male showed red muzzle staining at 1.5 and 2 hours post-dosing. Diarrhea was noted for one male at 24 hours post-dosing. The lowest average body temperature was at 4 hours post-dosing for males and females.

For the 1000 mg/kg bw group, there were no obvious differences in locomotor activity or arousal. One male and one female showed an overall gait incapacity (characterized primarily by ataxia) as early as 0.5 hour post-dosing, and for this male the gait alteration was seen up to 6 hours post-dosing. Diarrhea was noted for one male between 0.5 and 1.5 hours and again at 24 hours post-dosing, but may not have been treatment-related because it also occurred pre-dosing for this animal, while one female had diarrhea at 1 and 6 hours post-dosing. The lowest average body temperature was at 4 and 6 hours post-dosing for males and 2 hours post-dosing for females.

For the 500 mg/kg bw group, there were no clear effects on locomotor activity or arousal. One female showed an overall gait incapacity (characterized by ataxia) at 0.5 hour post-dosing. One male showed a reduced respiration at 4 hours post-dosing while one female had a decreased respiration at 1 hour post-dosing, but the significance of this finding is unclear. The lowest average body temperature was at 4 hours post-dosing for males, and 2 hours post-dosing for females.

III. CONCLUSIONS

A single oral administration of 500, 1000, or 2000 mg/kg bw pyrimethanil to rats by gavage caused dose-related behavioral changes (such as gait, locomotor activity, arousal) and reduced body temperature, and the severity of the changes generally peaked at around 1.5 to 4 hours post-dosing.

Report: CA 5.7.1/2
[redacted] 2001b
An acute oral neurotoxicity study of Pyrimethanil technical in rats
B003483
Guidelines: EPA 870.6200
GLP: yes
(certified by United States Environmental Protection Agency)
Deviations: no

Executive Summary

The aim of this study was to determine the effect of pyrimethanil on neurotoxicity in rats after a single administration via gavage and an observation period of 14 days. The test substance (in 0.5% aqueous MC) was administered to groups of 12 male and female Sprague Dawley (CD) rats at doses of 30, 100 and 1000 mg/kg bw at a dose volume of 10 mL/kg bw. Administration of pyrimethanil to rats resulted in transient changes such as gait abnormalities, reduced body temperature, decreased grip strength for males, and lower motor activity at 1000 mg/kg bw. At 100 mg/kg bw, the effects were limited to a reduced body temperature for males. No significant differences in brain measurements were determined and no gross or neuropathological findings occurred that were attributed to treatment. No overt clinical signs of toxicity, no mortality, and no significant differences in body weight or food consumption between control and treated groups were observed. . The transient effects observed on the day of bolus administration are therefore not considered to be indicative for neurotoxicity but of general transient systemic toxicity with clear recovery. The NOAEL for neurotoxicity was thus 1000 mg/kg bw/day (highest dose tested) in both sexes. The NOAEL for systemic toxicity was 30 mg/kg bw/day in males and 100 mg/kg bw/day in females

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Pyrimethanil (BAS 605 F)

Description:	solid / powder, beige light yellow
Lot/Batch #:	14/00
Purity:	99.8%
Stability of test compound:	Expiry date of the test substance: Nov 2002. The test substance preparation was stable for a period of 7 days.

2. Vehicle and/or positive control: aqueous methylcellulose (MC) solution (0.5%)

3. Test animals:

Species:	Rats
Strain:	CD (crl:CD®[SD]IGS BR)
Sex:	Male and female
Age:	51 ± 2 days (start of administration)
Weight at dosing:	males: 250 - 311 g females: 170 - 205 g
Source:	[redacted]
Acclimation period:	approx. 2 weeks
Diet:	pelleted laboratory diet (PMI certified rodent chow), ad libitum
Water:	water, ad libitum
Housing:	single housing in stainless-steel mesh-bottomed cages

Environmental conditions:

Temperature:	19 - 25°C
Humidity:	30 - 70%
Air changes per hour:	not specified
Photo period:	12 h light / 12 h dark

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 9-Apr-2001 (start of administration) to 27-Apr-2001 (necropsy)

2. Animal assignment and treatment:
Pyrimethanil was administered once to groups of 12 male and 12 female rats at doses of 30, 100, and 1000 mg/kg bw by gavage with a dosing volume of 5 mL/kg bw.

3. Test substance preparation and analysis:
The test substance was applied as a suspension. To prepare the suspension, the appropriate amount of test substance was weighed out depending on the desired concentration. Then the vehicle (water containing 0.5% methylcellulose) was filled up to the desired volume, and subsequently mixed using a magnetic stirrer. During administration of the test substance, preparations were kept homogeneous using a magnetic stirrer. The test substance preparations were made once before the first administration.
The stability of the test substance formulation at 4°C over a period of a maximum of 7 days was proven by analysis. Homogeneity analyses of the test substance preparations were performed in samples of all concentrations administered at the beginning. These samples also served for concentration control analysis.

4. Statistics:
Means and standard deviations (SD) of each test group were calculated for several parameters. Further parameters and statistical tests are listed below.

Parameters	Statistical test
Body weight, food consumption, quantitative FOB, brain measurements	Bartlett´s test was used for group variances. When the differences were not significant ($p > 0.001$), a one-way analysis of variance (ANOVA) was performed. If significant differences ($p < 0.05$) were indicated by the ANOVA, Dunnett´s test was used to compare the control and treated groups. If $p < 0.001$ by Bartlett´s test, then the Kruskal-Wallis test was performed. If then significant ($p < 0.05$), Dunnett´s test (or Wilcoxon/Mann Whitney U test) were applied.
Qualitative FOB	Fisher´s exact probability test.
Motor activity	Bartlett´s test was used for group variances. When the differences were not significant ($p > 0.001$), a one-way analysis of variance (ANOVA) for the pre-study period and an analysis of covariance (ANCOVA) for the days 1, 8 and 15 (with pre-study data as covariate) was performed using the GLM procedure of the SAS statistical package. If significant differences ($p < 0.05$) were indicated by the ANOVA, a t-test was used to compare control and treated groups.

C. METHODS

1. Observations:

A check for moribund and dead animals was made twice daily. In addition, a complete detailed examination was performed once prior to the treatment and daily thereafter. Any observed clinical sign was recorded individually.

2. Body weight:

The body weight was determined at least twice during the acclimation period, as well as on days 1, 8, and 15, and prior to terminal euthanasia.

3. Food and water consumption:

Individual food consumption was determined weekly, commencing on the last week of the acclimation period.

4. Clinical assessment of neurotoxicity:

Functional observational battery

All animals were tested prior to treatment (pre-study) and on days 1 (approximately 1.5 to 2 hours post-dosing), 8 and 15. Testing was performed by the same trained technicians, who were unaware of the animals' treatment group. The FOB evaluations preceded motor activity testing.

The FOB was performed with equipment built for this purpose. The arena was a square of Plexiglas (approximately 60 cm x 60 cm) placed on a raised platform. The tests were conducted in the room housing the animals, where temperature, humidity and photoperiod were monitored. Odors in the room were minimized by maintaining adequate air changes and cleaning of equipment, as necessary.

For home cage observations attention was paid to: body position, tremor, twitches, convulsions, bizarre/stereotypic behaviour. At removal of the home cage easiness of removal and vocalization were checked.

Open field observations:

The animals were transferred to a standard arena and the following parameters were examined:

1. overall gait incapacity	8. respiration
2. locomotor activity level	9. piloerection
3. arousal	10. tremors, twitches, convulsions
4. grooming	11. bizarre/stereotypic behaviour
5. olfactory response	12. defecation
6. palpebral closure	13. urination
7. rearing	14. ataxic, hypotonic and impaired gait

Handling observations:

The animals were removed from the open field and subjected to following sensorimotor and reflex tests:

1. salivation	9. extensor thrust
2. urinary staining	10. pain perception ("toe and tail pinch")
3. diarrhea	11. visual placing
4. pupillary reflex	12. lacrimation
5. pinna reflex	13. auricular startle
6. cornea reflex	14. air righting reflex
7. salivation	15. positional passivity
8. body tone	

Furthermore, grip strength, hind-limb splay and body temperature were determined.

Motor activity assessment

Motor activity (MA) measurements were carried out in all animals prior to treatment, on study days 0, 8 and 15. The sessions were of 1-hour duration and the activity counts were recorded in 6 successive 10-minute intervals by a computer. Temperature and humidity were monitored and a background sound level of approximately 70 dBA and illumination of 600-1000 Lux were maintained throughout testing.

5. Neuropathology:

On completion of the study (day 16), the animals were euthanized by exsanguination from the abdominal aorta following isoflurane anesthesia and were subjected to a gross pathological examination. Samples from abnormal tissues were retained in neutral buffered 10% formalin or other appropriate preservative for possible future examination. All animals were fasted overnight prior to scheduled euthanasia.

Tissue preservation

Five surviving animals per test group and sex were subjected to deep anesthesia with sodium pentobarbital and sacrificed by perfusion fixation. Heparinized sodium chloride solution was used as a rinsing solution and a glutaraldehyde-paraformaldehyde solution was used as a fixative. The animals fixed by perfusion were necropsied with regard to the question of neuropathology, and the visible organs or organ sections were assessed by gross pathology as accurately as is possible after a perfusion fixation.

Organ/tissue preservation list

Paraffin embedding, sectioning, staining and preservation:

The tissues listed below were prepared for examination of the high dose and control animals by embedding in paraffin wax and sectioning at 6 microns. The sections were stained with hematoxylin and eosin and then examined by light microscopy.

- Brain (7 levels): olfactory bulbs, forebrain (through the septum), center of the cerebrum (through the hypothalamus), midbrain, cerebellum and pons, midcerebellum and medulla oblongata, and medulla oblongata
- Spinal cord: cervical, thoracic, and lumbar (longitudinal and cross sections)
- Skeletal muscle: gastrocnemius (longitudinal and cross sections)
- Grossly abnormal central nervous system tissues

Brain weight (excluding olfatory bulbs), length and maximum coronal width were recorded prior to trimming.

Epoxy embedding, sectioning, staining and preservation:

For the control and high dose group animals, the tissues were rinsed in sodium cacodylate buffer (0.1 M) and placed in 2% osmium tetroxide for 2 hours. The tissues were rinsed and stained in an aqueous solution of uranyl acetate (1%) for 2 hours. The tissues were then rinsed in distilled water, dehydrated in ascending concentrations of ethyl alcohol and embedded in a mixture of Jembed and Araldite. Epoxy sections (0.5 µm) were obtained with a glass knife, stained with borate-buffered 1% toluidine blue, cover slipped and examined by light microscopy.

Peripheral nervous system (PNS)

Sciatic nerve (mid-thigh region) (longitudinal and cross-sections)

Sciatic nerve (at sciatic notch) (longitudinal and cross-sections)

Sural nerve (at knee) (longitudinal and cross-sections)

Tibial nerve (at knee) (longitudinal and cross-sections)

Central nervous system (CNS)

Gasserian ganglion – left (cross-section)

Lumbar dorsal root ganglion (L4) (cross-section)

Lumbar dorsal root (L4) (cross-section)

Lumbar ventral root (L4) (longitudinal and cross-section)

Cervical dorsal root ganglion (C5) (cross-section)

Cervical dorsal root (C5) (cross-section)

Cervical ventral root (C5) (longitudinal and cross-section)

Grossly abnormal central or peripheral nervous system tissues.

II. RESULTS AND DISCUSSION

A. TEST SUBSTANCE ANALYSES

Test substance preparations were found to be homogenous and of correct concentrations (85-115% of nominal concentrations). The stability was confirmed for 7 days at 4°C.

B. OBSERVATIONS

1. Clinical signs of toxicity

No treatment-related overt clinical signs of toxicity were observed, except as stated in the results of the FOB.

2. Mortality

No mortality was observed and no animals were euthanized prior to scheduled termination.

C. BODY WEIGHT AND FOOD CONSUMPTION

No test substance-related findings on body weight were found. No significant differences in food consumption were detected between the control and treated groups.

Table 5.7.1-2: Mean body weights and body weight changes after single administration

	Dose level (mg/kg bw)							
	0		30		100		1000	
	male	female	male	female	male	female	male	female
Body weight (g)								
Day 1	228	169	227	166	227	166	226	166
Day 6	281	188	281	185	283	187	278	186
Day 8	331	208	336	204	338	205	325	203
Day 15	379	228	383	221	387	226	372	221
Body weight change (g)								
Days 0 - 14	151	59	156	55	160	59	146	55

D. CLINICAL ASSESSMENT OF NEUROTOXICITY

Functional observational battery

The FOB performed on day 1 indicated the following findings for the 1000 mg/kg bw group: A significant number of animals (male/female) displayed a slight to moderate overall gait capacity characterized by ataxic gait while in the arena. Several females had dilated pupils and one male showed an abnormal response to the air-righting reflex. In addition, hind-limb grip strength was significantly decreased for males, and both sexes had significantly lower body temperature.

At 100 mg/kg bw, males showed a significantly lower body temperature on day 1.

No other differences clearly attributed to the treatment were noted on day 1 and all animals showing findings on day 1 appeared normal at the day 8 assessment.

Motor activity assessment

On day 1, the total activity counts of males and females in the 1000 mg/kg bw group were significantly reduced when compared to the control group. The linear constructed variable, or rate of linear change, was also significantly altered for both sexes in the 1000 mg/kg bw group on this day.
No other significant differences were detected between the control and treated groups.

E. NEUROPATHOLOGY

There were no gross pathological or histopathological findings attributed to treatment. There were no significant differences in brain weight, length or width measurements between the control and treated groups.

III. CONCLUSIONS

A single oral administration of pyrimethanil to rats by gavage resulted in transient changes such as gait abnormalities, reduced body temperature, decreased grip strength for males and lower motor activity for both sexes at 1000 mg/kg bw. At 100 mg/kg bw the effects were limited to a reduced body temperature for males. No significant differences in brain measurements were determined and no gross or neuropathological findings occurred that were attributed to treatment. The transient effects observed on the day of bolus administration are therefore not considered to be indicative for neurotoxicity but of general transient systemic toxicity with clear recovery. The NOAEL for neurotoxicity was thus 1000 mg/kg bw/day (highest dose tested) in both sexes. The NOAEL for systemic toxicity was 30 mg/kg bw/day in males and 100 mg/kg bw/day in females

Report:	CA 5.7.1/3 [redacted] 2001c A 13-week dietary neurotoxicity study of Pyrimethanil technical in rats B003519
Guidelines:	EPA 870.6200
GLP:	yes (certified by United States Environmental Protection Agency)
Deviations:	no

Executive Summary

The aim of this study was to determine the effect of pyrimethanil on neurotoxicity in rats after a dietary exposure for 13 weeks. The test substance was administered via the diet to groups of 12 male and female Sprague Dawley (CD) rats at concentrations of 60, 600, and 6000 ppm. The overall study mean achieved intake of pyrimethanil was 4.0, 38.7, and 391.9 mg/kg bw/day for males, and 4.6, 44.3, and 429.9 mg/kg bw/day for females, in the 60, 600, and 6000 ppm groups, respectively. Treatment resulted in slightly lower body weights and a transient reduction in food intake for males and significant reductions in body weight and food intake for females at the 6000 ppm dose level. There were no toxicologically significant effects on behavioral assessments or structural changes of nervous system tissue attributed to the test article. No overt clinical signs of toxicity and no mortality were observed. The NOAEL for neurotoxicity was 6000 ppm, equivalent to 391.9 mg/kg bw/day in males and 429.9 mg/kg bw/day in females, respectively. The NOAEL for systemic toxicity was 600 ppm, equivalent to 38.7 mg/kg bw/day in males and 44.3 mg/kg bw/day in females.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Pyrimethanil (BAS 605 F)

Description:	solid / powder, beige light yellow
Lot/Batch #:	14/00
Purity:	99.8%
Stability of test compound:	Expiry date of the test substance: Nov 2002. The test substance preparation was stable for a period of 14 days.

2. Vehicle and/or positive control: N.A.

3. Test animals:

Species:	Rats
Strain:	CD (crl:CD®[SD]IGS BR)
Sex:	Male and female
Age:	48-51 days (start of administration)
Weight at dosing:	males: 227-282 g females: 164-196 g
Source:	[redacted]
Acclimation period:	approx. 2 weeks
Diet:	pelleted laboratory diet (PMI certified rodent chow 5002), ad libitum
Water:	water, ad libitum
Housing:	single housing in stainless-steel mesh-bottomed cages

Environmental conditions:

Temperature:	19 - 25°C
Humidity:	30 - 70%
Air changes per hour:	not specified
Photo period:	12 h light / 12 h dark

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 6-Feb-2001 (start of administration) to 11-May-2001 (necropsy)

2. Animal assignment and treatment:
Pyrimethanil was administered to groups of 12 male and 12 female rats at doses of 60, 600, and 6000 ppm via the diet over a period of 13 weeks. Rats were distributed into groups based on the body weight data by means of a computer-based selection procedure.

3. Test substance preparation and analysis:
Dose formulations were prepared once weekly, divided into aliquots and stored frozen prior to use. On the day of use, the appropriate aliquots were removed from the freezer, equilibrated to room temperature, and used for feeding the animals for 3 - 4 days.
The 6000 and 600 ppm dose formulations were prepared by by mixing appropriate amounts of the test item with basal diet using a hand whisk and Hobart mixer (with whisk attachment). No correction was made for purity of the test article. The 60 ppm dose formulation was prepared by serial dilution by mixing appropriate amounts of the 600 ppm dose formulation with basal diet using a hand whisk and Hobart mixer (with whisk attachment). Control group animals received only powdered basal diet.
The stability of the test substance formulation at room temperature or at -20°C over a period of a maximum of 14 days was proven by analysis. Homogeneity analyses of the test substance preparations were performed in samples of all concentrations administered at the beginning. These samples also served for concentration control analysis.

4. Statistics:
Means and standard deviations (SD) of each test group were calculated for several parameters. Further parameters and statistical tests are listed below.

Parameters	Statistical test
Body weight, food consumption, quantitative FOB, brain measurements	Bartlett´s test was used for group variances. When the differences were not significant ($p > 0.001$), a one-way analysis of variance (ANOVA) was performed. If significant differences ($p < 0.05$) were indicated by the ANOVA, Dunnett´s test was used to compare the control and treated groups. If $p < 0.001$ by Bartlett´s test, then the Kruskal-Wallis test was performed. If then significant ($p < 0.05$), Dunnett´s test (or Wilcoxon/Mann Whitney U test) were applied.
Qualitative FOB	Fisher´s exact probability test.
Motor activity	Bartlett´s test was used for group variances. When the differences were not significant ($p > 0.001$), a one-way analysis of variance (ANOVA) for the pre-study period and an analysis of covariance (ANCOVA) for the Days 1, 8 and 15 (with pre-study data as covariate) was performed using the GLM procedure of the SAS statistical package. If significant differences ($p < 0.05$) were indicated by the ANOVA, a t-test was used to compare control and treated groups.

C. METHODS

1. Observations:

A check for moribund and dead animals was made twice daily. In addition, a complete detailed examination was performed once weekly. Any observed clinical sign was recorded individually.

2. Body weight:

The body weight was determined at least twice during the acclimation period, as well as weekly during the study including days of behavioral testing and prior to terminal euthanasia.

3. Food and water consumption:

Individual food consumption was determined in the last week of the acclimation period and twice weekly thereafter. Group mean achieved intake of the test item was calculated on a weekly basis.

4. Clinical assessment of neurotoxicity:

Functional observational battery

All animals were tested prior to treatment (pre-study) and during the 4th, 8th and 13th week of the treatment. Testing was performed by the same trained technicians, who were unaware of the animals' treatment group. The FOB evaluations preceded motor activity testing.

The FOB was performed with equipment built for this purpose. The arena was a square of Plexiglas (approximately 60 cm x 60 cm) placed on a raised platform. The tests were conducted in the room housing the animals, where temperature, humidity and photoperiod were monitored. Odors in the room were minimized by maintaining adequate air changes and cleaning of equipment, as necessary.

For home cage observations, attention was paid to body position, tremor, twitches, convulsions and bizarre/stereotypic behaviour. At removal of the home cage easiness of removal and vocalization were checked.

Open field observations:

The animals were transferred to a standard arena and the following parameters were examined:

1. overall gait incapacity	8. respiration
2. locomotor activity level	9. piloerection
3. arousal	10. tremors, twitches, convulsions
4. grooming	11. bizarre/stereotypic behaviour
5. olfactory response	12. defecation
6. palpebral closure	13. urination
7. rearing	14. ataxic, hypotonic and impaired gait

Handling observations:

The animals were removed from the open field and subjected to the following sensorimotor and reflex tests:

1. salivation	9. extensor thrust
2. urinary staining	10. pain perception ("toe and tail pinch")
3. diarrhea	11. visual placing
4. pupillary reflex	12. lacrimation
5. pinna reflex	13. auricular startle
6. cornea reflex	14. air righting reflex
7. salivation	15. positional passivity
8. body tone	

Furthermore, grip strength, hind-limb splay and body temperature were determined.

Motor activity assessment

Motor activity (MA) measurements were carried out in all animals prior to treatment and during the 4th, 8th and 13th week of treatment. The sessions were of 1-hour duration and activity counts were recorded in 6 successive 10-minute intervals by a computer. Temperature and humidity were monitored, and a background sound level of approximately 70 dBA and illumination of 600-1000 Lux were maintained throughout testing.

4. Terminal procedures:

Animals not selected for perfusion

On completion of the study (day 93), the animals were euthanized by exsanguination from the abdominal aorta following isoflurane anesthesia and were subjected to a gross pathological examination. Samples from abnormal tissues were retained in neutral buffered 10% formalin or other appropriate preservative for possible future examination. All animals were fasted overnight prior to scheduled euthanasia.

Animals selected for perfusion - Tissue preservation

Five surviving animals per test group and sex were subjected to deep anesthesia with sodium pentobarbital and sacrificed by perfusion fixation. Heparinized sodium chloride solution was used as a rinsing solution and a glutaraldehyde/paraformaldehyde solution was used as a fixative. The animals fixed by perfusion were necropsied with regard to the question of neuropathology, and the visible organs or organ sections were assessed by gross pathology as accurately as is possible after a perfusion fixation.

Animals selected for perfusion - Neuropathology

On completion of the perfusion, the calvarum and the dorsal vertebral column were exposed by removing the skin and underlying muscle. The skin on the lateral surface of the hind-limbs was removed. The skin and tissues on the ventral surface of the abdomen and thorax were removed and discarded. The thoracic and abdominal organs were removed as two groups of tissues and placed in a separate tissue bag containing neutral buffered 10% formalin (not evaluated). The remaining carcass including the brain, spinal cord and limbs was placed in another tissue bag containing neutral buffered 10% formalin.

Tissues from the high dose and control animals were processed for neuropathological evaluation. The trimmed tissues from the low and mid dose groups were kept in neutral buffered 10% formalin (not evaluated). The remaining carcasses from all animals were retained. The nervous system tissues of animals in all groups were grossly examined at the time of sampling and any pathology observed was recorded and reported.

Organ/tissue preservation list

Paraffin embedding, sectioning, staining and preservation:

The tissues listed below for the high dose and control animals were prepared for examination by embedding in paraffin wax and sectioning at 6 microns. The sections were stained with hematoxylin and eosin and then examined by light microscopy.

- Brain (7 levels): olfactory bulbs, forebrain (through the septum), center of the cerebrum (through the hypothalamus), midbrain, cerebellum and pons, midcerebellum and medulla oblongata, and medulla oblongata
- Spinal cord: cervical, thoracic, and lumbar (longitudinal and cross sections)
- Skeletal muscle: gastrocnemius (longitudinal and cross sections)
- Grossly abnormal central nervous system tissues

Brain weight (excluding olfatory bulbs), length and maximum coronal width were recorded prior to trimming.

Epoxy embedding, sectioning, staining and preservation:

For the control and high dose group animals, the tissues were rinsed in sodium cacodylate buffer (0.1 M) and placed in 2% osmium tetroxide for 2 hours. The tissues were rinsed and stained in an aqueous solution of uranyl acetate (1%) for 2 hours. The tissues were then rinsed in distilled water, dehydrated in ascending concentrations of ethyl alcohol and embedded in a mixture of Jembed and Araldite. Epoxy sections (0.5 µm) were obtained with a glass knife, stained with borate-buffered 1% toluidine blue, cover slipped and examined by light microscopy.

Peripheral nervous system (PNS)

Sciatic nerve (mid-thigh region) (longitudinal and cross-sections)
Sciatic nerve (at sciatic notch) (longitudinal and cross-sections)
Sural nerve (at knee) (longitudinal and cross-sections)
Tibial nerve (at knee) (longitudinal and cross-sections)

Central nervous system (CNS)

Gasserian ganglion – left (cross-section)
Lumbar dorsal root ganglion (L4) (cross-section)
Lumbar dorsal root (L4) (cross-section)
Lumbar ventral root (L4) (longitudinal and cross-section)
Cervical dorsal root ganglion (C5) (cross-section)
Cervical dorsal root (C5) (cross-section)
Cervical ventral root (C5) (longitudinal and cross-section)

Grossly abnormal central or peripheral nervous system tissues.

II. RESULTS AND DISCUSSION

A. TEST SUBSTANCE ANALYSES

Test substance preparations were found to be homogenous and of correct concentrations (85-115% of nominal concentrations), although there was slightly more variability in the results for the 60 ppm level. The stability was confirmed for 14 days at room temperature or in the refrigerator. However, because of some variability in the data from the 60 ppm level and results from previous studies by the sponsor indicating possible instability of the test article in the diet, it was decided to prepare the diets for each group once weekly, then store frozen, and change the aliquots twice weekly.

B. OBSERVATIONS

1. Clinical signs of toxicity

No treatment-related overt clinical signs of toxicity were observed.

2. Mortality

No mortality was observed and no animals were euthanized prior to scheduled termination.

C. BODY WEIGHT AND FOOD CONSUMPTION

For males, the body weight gain of the 6000 ppm group during the first week of treatment was significantly lower than in the control group. No statistically significant difference was observed in terms of body weight, although the 6000 ppm group did tend to show slightly lower body weights than the control group throughout the treatment period.

For females, there was no significant difference in body weight gain between treated and control groups. However, 6000 ppm females showed lower body weights than the control group feemales generally throughout the treatment period with significantly lower values occurring on days 71, 85 and 92.

The food intake of the 6000 ppm males was significantly lower than in the control group from days 1 to 5. No other significant differences between treated and control groups were noted for males.

Females in the 6000 ppm group had lower food consumption throughout the treatment period with significantly lower values during several weeks. No other significant differences were observed between treated and control group females. A significant lower value for the 600 ppm females from days 61 to 64 was considered incidental.

Table 5.7.1-3: Relevant mean body weights and body weight changes

	Dose level (mg/kg bw)							
	0		60		600		6000	
	male	female	male	female	male	female	male	female
Body weight (g)								
Day 1	204	161	203	161	205	161	204	162
Day 6	252	178	250	178	252	180	250	179
Day 71	544	286	547	279	535	283	524	263*
Day 85	570	295	575	285	560	297	552	272*
Day 92	576	291	583	282	567	291	559	268*
Body weight change (g)								
Days 1 - 8	50.4	19.3	51.6	19.2	51.6	18.1	39.9**	16.9

* ($p \leq 0.05$); ** ($p \leq 0.01$) significantly different from controls (Dunnett's test)

D. Achieved test substance intake

The group mean weekly intake of pyrimethanil ranged from 3.0 to 6.2, 30.1 to 61.8, and 307.4 to 571.3 mg/kg bw/day for males and from 3.9 to 6.0, 37.0 to 57.2, and 370.8 to 518.6 mg/kg bw/day for females, in the 60, 600, and 6000 ppm groups, respectively.
The overall study mean achieved intake of Pyrimethanil was 4.0, 38.7, and 391.9 mg/kg bw/day for males, and 4.6, 44.3, and 429.9 mg/kg bw/day for females, in the 60, 600, and 6000 ppm groups, respectively.

E. CLINICAL ASSESSMENT OF NEUROTOXICITY

Functional observational battery

~~B~~No differences were observed between the treated and control groups at any of the assessment intervals that were considered to be treatment-related.

Motor activity assessment

There were no significant differences in total counts or the linear constructed variable between the treated and control groups that were related to treatment. A statistically significantly lower value for total counts at the week 4 assessment for the 60 ppm males was attributed to intergroup variability.

F. NEUROPATHOLOGY

There were no gross pathological or histopathological findings attributed to treatment. There were no significant differences in brain weight, length or width measurements between the control and treated groups.

III. CONCLUSIONS

Dietary treatment of male and female rats with pyrimethanil for 13 weeks at concentrations of 60, 600 or 6000 ppm resulted in slightly lower body weights and a transient reduction in food intake for males and significant reductions in body weight and food intake for females at the 6000 ppm level. There were no toxicologically significant effects on behavioral assessments or structural changes to nervous system tissue attributed to the test article. The NOAEL for neurotoxicity was 6000 ppm, equivalent to 391.9 mg/kg bw/day in males and 429.9 mg/kg bw/day in females, respectively. The NOAEL for systemic toxicity was 600 ppm, equivalent to 38.7 mg/kg bw/day in males and 44.3 mg/kg bw/day in females.

CA 5.7.2 Delayed polyneuropathy studies

As there was no indication for neurotoxicity and/or neuropathy from any of the studies conducted and as pyrimethanil does not belong to a chemical class suspected to induce delayed neuropathies, no study is considered to be necessarily, and thus no further study was conducted.

CA 5.8 Other Toxicological Studies

In order to align the dossier submission and the draft RAR, the information that was present in the dRAR in more detail was included in this dossier update in light green, whereas new information and/or corrections were included in lime green.

CA 5.8.1 Toxicity studies of metabolites

Studies evaluated in the draft monograph of Rapporteur Member State Austria of April 15, 2004:
Acute oral toxicity and an Ames test studies on rat metabolite M605F007 (AN7, group 2) have already been evaluated for Annex I inclusion of pyrimethanil. For the convenience of the reviewer, these are summarized below as extracted from the monograph including addenda together with the new studies conducted meanwhile for the identified metabolites.
Since the time of the dossier submission, additional QSAR tools, that are more reliable and predictive, are available to the applicant. Therefore, the metabolite section of pyrimethanil was updated taking into account all valuable information.

Based on the available data the following assessment was provided in the Annex I listing of pyrimethanil:

Toxicity studies of rat and soil metabolite M605F007 (AN7)	acute oral rat LD50 735 mg/kg bw no evidence of genotoxic activity (Ames test)

With regard to toxicological relevance the following metabolites have to be taken into consideration and are addressed in this dossier section. A summary of the conducted studies / evaluations is provided in Table 5.8.1-1.

Table 5.8.1-1: Pyrimethanil metabolites considered for potential toxicological relevance

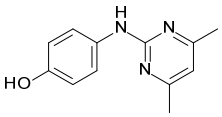
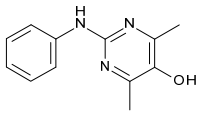
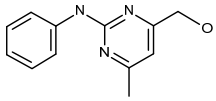
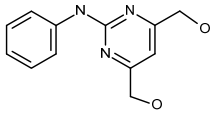
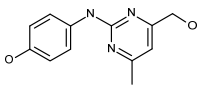
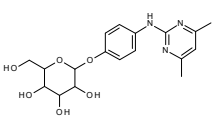
Metabolite	Structure	Reason for relevance assessment	Study / Test system	Findings	Reference (DocID)
Group 1: Hydroxylation/oxidation products and their conjugates					
M605F002 (AN2) ~~p. 162~~		Livestock, plant and rotational crop metabolite	QSAR: CaseUltra, OECD toolbox, OASIS Times, Vega	By weight of evidence not genotoxic	2017/1100634 2017/1100635 2017/1100632 2017/1100633 2014/1326432 2015/1198645 2015/1186244 2015/1198463
			Covered by rat metabolism and toxicological test of pyrimethanil see chapter 5 of AIR 3 dossier. ADI: 0.17 mg/kg bw/day (pyrimethanil)		
M605F003 (AN3) ~~p.165~~		Livestock, plant and rotational crop metabolite	QSAR: CaseUltra, OECD toolbox, OASIS Times, Vega	By weight of evidence not genotoxic	2017/1100634 2017/1100635 2017/1100632 2017/1100633 2014/1326432 2015/1198645 2015/1186244 2015/1198463
			Covered by rat metabolism and toxicological test of pyrimethanil see chapter 5 of AIR 3 dossier. ADI: 0.17 mg/kg bw/day (pyrimethanil)		
M605F004 (AN4) ~~p. 168~~		Livestock, plant and rotational crop metabolite	QSAR: CaseUltra, OECD toolbox, OASIS Times, Vega	By weight of evidence not genotoxic	2017/1100634 2017/1100635 2017/1100632 2017/1100633 2014/1326432 2015/1198645 2015/1186244 2015/1198463
			Covered by rat metabolism and toxicological test of pyrimethanil see chapter 5 of AIR 3 dossier. ADI: 0.17 mg/kg bw/day (pyrimethanil)		
M605F005 (AN5) ~~p. 171~~		Rotational crop metabolite	QSAR: CaseUltra, OECD toolbox, OASIS Times, Vega	By weight of evidence not genotoxic	2017/1100634 2017/1100635 2017/1100632 2017/1100633 2014/1326432 2015/1198645 2015/1186244 2015/1198463
			Covered by rat metabolism and toxicological test of pyrimethanil see chapter 5 of AIR 3 dossier. ADI: 0.17 mg/kg bw/day (pyrimethanil)		
M605F006 (AN6) ~~p. 174~~		Livestock and rotational crop metabolite	QSAR: CaseUltra, OECD toolbox, OASIS Times, Vega	By weight of evidence not genotoxic	2017/1100634 2017/1100635 2017/1100632 2017/1100633 2014/1326432 2015/1198645 2015/1186244 2015/1198463
			Covered by rat metabolism and toxicological test of pyrimethanil see chapter 5 of AIR 3 dossier. ADI: 0.17 mg/kg bw/day (pyrimethanil)		
M605F001 (AN1) β-O glucoside of M605F002 ~~p. 179~~		Plant metabolite	Covered by evaluation of M605F002 By weight of evidence not genotoxic Covered by rat metabolism and toxicological test of pyrimethanil see chapter 5 of AIR 3 dossier. ADI: 0.17 mg/kg bw/day (pyrimethanil)		

Table 5.8.1-1: Pyrimethanil metabolites considered for potential toxicological relevance

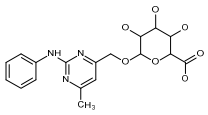
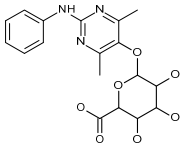
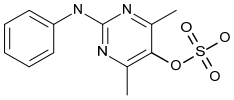
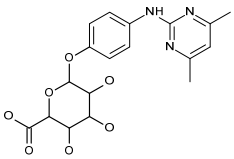
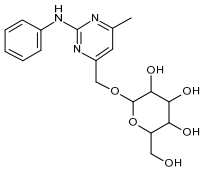
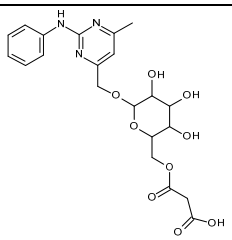
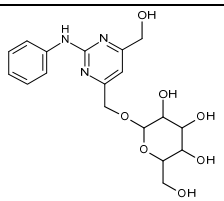
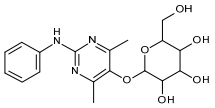
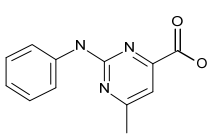
Metabolite	Structure	Reason for relevance assessment	Study / Test system	Findings	Reference (DocID)
M605F014 Glucuronide conjugate of M605F004 p. 179		Livestock metabolite	Covered by evaluation of M605F004 By weight of evidence not genotoxic Covered by rat metabolism and toxicological test of pyrimethanil see chapter 5 of AIR 3 dossier. ADI: 0.17 mg/kg bw/day (pyrimethanil)		
M605F020 Glucuronide conjugate of M605F003 p. 180		Livestock metabolite	Covered by evaluation of M605F003 By weight of evidence not genotoxic Covered by rat metabolism and toxicological test of pyrimethanil see chapter 5 of AIR 3 dossier. ADI: 0.17 mg/kg bw/day (pyrimethanil)		
M605F021 Sulfate of M605F003 p. 180		Livestock metabolite	QSAR: OECD Toolbox, CaseUltra	Not genotoxic (QSAR predictions "in domain")	2017/1100634 2017/1100635 2017/1100632 2017/1100633
			Covered by evaluation of M605F003 By weight of evidence not genotoxic Covered by rat metabolism and toxicological test of pyrimethanil see chapter 5 of AIR 3 dossier. ADI: 0.17 mg/kg bw/day (pyrimethanil)		
M605F023 Glucuronide of M605F002 p. 180		Livestock metabolite	Covered by evaluation of M605F002 and QSAR prediction using CaseUltra By weight of evidence not genotoxic Covered by rat metabolism and toxicological test of pyrimethanil see chapter 5 of AIR 3 dossier. ADI: 0.17 mg/kg bw/day (pyrimethanil)		
M605F027 ß-O-glucoside of M605F004 p. 181		Plant and rotational crop metabolite	Covered by evaluation of M605F004 By weight of evidence not genotoxic Covered by rat metabolism and toxicological test of pyrimethanil see chapter 5 of AIR 3 dossier. ADI: 0.17 mg/kg bw/day (pyrimethanil)		
M605F028 Malonyl-β-O glucoside of M605F004 p. 181		Plant and rotational crop metabolite	Covered by evaluation of M605F004 By weight of evidence not genotoxic Covered by rat metabolism and toxicological test of pyrimethanil see chapter 5 of AIR 3 dossier. ADI: 0.17 mg/kg bw/day (pyrimethanil)		
M605F029 β-O-glucoside of M605F005 p. 182		Plant metabolite	Covered by evaluation of M605F005 By weight of evidence not genotoxic Covered by rat metabolism and toxicological test of pyrimethanil see chapter 5 of AIR 3 dossier. ADI: 0.17 mg/kg bw/day (pyrimethanil)		
M605F030 β-O-glucoside of M605F003 p. 182		Plant and rotational crop metabolite	Covered by evaluation of M605F003 By weight of evidence not genotoxic Covered by rat metabolism and toxicological test of pyrimethanil see chapter 5 of AIR 3 dossier. ADI: 0.17 mg/kg bw/day (pyrimethanil)		
M605F034 (Pyrimethanil -COOH) p. 177		Livestock metabolite	QSAR: CaseUltra, OECD toolbox, OASIS Times, Vega	By weight of evidence not genotoxic	2017/1100634 2017/1100635 2017/1100632 2017/1100633 2015/1186253 2015/1186247 2015/1186245 2015/1198463
			Covered by evaluation of M605F004 By weight of evidence not genotoxic Covered by rat metabolism and toxicological test of pyrimethanil see chapter 5 of AIR 3 dossier.		

Table 5.8.1-1: Pyrimethanil metabolites considered for potential toxicological relevance

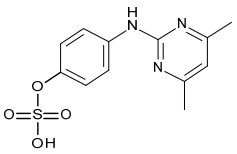
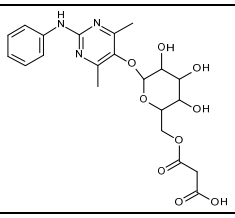
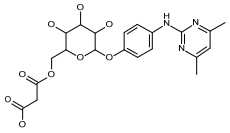
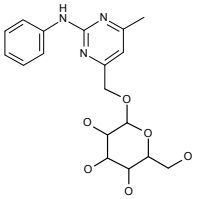
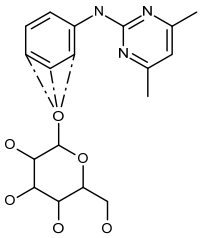
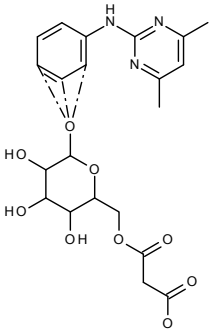
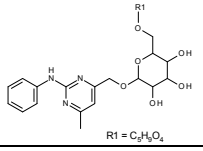
Metabolite	Structure	Reason for relevance assessment	Study / Test system	Findings	Reference (DocID)
			ADI: 0.17 mg/kg bw/day (pyrimethanil)		
M605F035 Sulfate of M605F002 ~~p. 182~~		Livestock metabolite	QSAR: OECD toolbox, CaseUltra	Not genotoxic (QSAR predictions "in domain")	2017/1100634 2017/1100635 2017/1100632 2017/1100633
			Covered by evaluation of M605F002 By weight of evidence not genotoxic Covered by rat metabolism and toxicological test of pyrimethanil see chapter 5 of AIR 3 dossier. ADI: 0.17 mg/kg bw/day (pyrimethanil)		
M605F036 Malonyl-β-O-glucoside of M605F003 ~~p. 183~~		Plant and rotational crop metabolite	Covered by evaluation of M605F003 By weight of evidence not genotoxic Covered by rat metabolism and toxicological test of pyrimethanil see chapter 5 of AIR 3 dossier. ADI: 0.17 mg/kg bw/day (pyrimethanil)		
M605F037 Malonyl-β-O glucoside of M605F002 ~~p. 184~~		Plant metabolite	Covered by evaluation of M605F002 By weight of evidence not genotoxic Covered by rat metabolism and toxicological test of pyrimethanil see chapter 5 of AIR 3 dossier. ADI: 0.17 mg/kg bw/day (pyrimethanil)		
M605F038 C-6 sugar conjugate of M605F004 ~~p. 184~~		Plant metabolite	Covered by evaluation of M605F004 By weight of evidence not genotoxic Covered by rat metabolism and toxicological test of pyrimethanil see chapter 5 of AIR 3 dossier. ADI: 0.17 mg/kg bw/day (pyrimethanil)		
M605F039 "Glucose conjugate…" ~~p. 185~~		Plant and rotational crop metabolite	Covered by evaluation of M605F002 By weight of evidence not genotoxic Covered by rat metabolism and toxicological test of pyrimethanil see chapter 5 of AIR 3 dossier. ADI: 0.17 mg/kg bw/day (pyrimethanil)		
M605F040 "Malonyl glucose conj. …" ~~p. 185~~		Plant and rotational crop metabolite	Covered by evaluation of M605F002 By weight of evidence not genotoxic Covered by rat metabolism and toxicological test of pyrimethanil see chapter 5 of AIR 3 dossier. ADI: 0.17 mg/kg bw/day (pyrimethanil)		
M605F041 $C_5H_9O_4$ glucose conjugate of M605F004 ~~p. 186~~		Plant metabolite	Covered by evaluation of M605F004 By weight of evidence not genotoxic Covered by rat metabolism and toxicological test of pyrimethanil see chapter 5 of AIR 3 dossier. ADI: 0.17 mg/kg bw/day (pyrimethanil)		

Table 5.8.1-1: Pyrimethanil metabolites considered for potential toxicological relevance

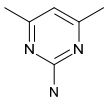
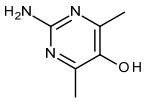
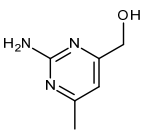
Metabolite	Structure	Reason for relevance assessment	Study / Test system	Findings	Reference (DocID)
Group 2: Pyrimidin-moiety cleavage products and their derivatives and conjugates					
M605F007 (AN7) ~~p. 188~~		Rotational crop metabolite	QSAR: CaseUltra, OECD toolbox, OASIS TIMES, VEGA	~~Inconclusive alerts~~ By weight of evidence not genotoxic	2017/1100634 2017/1100635 2017/1100632 2017/1100633 2015/1186251 2015/1186250 2015/1186244 2015/1198463
			Acute toxicity, Oral, Rat, Sprague-Dawley 100, 200, 400, 800, 1600 mg/kg bw in 1% aqueous methyl cellulose	LD50 735 mg/kg bw	C001117
			Mutagenicity in bacterial cells (Ames test) Salmonella typhimurium (TA 1535, TA 1537, TA98, TA 100); Escherichia coli (CM 891); Concentration up to 5000 µg/plate Without and with S-9 mix	Not mutagenic	C000864
			In vitro micronucleus assay (Mammalian cells) Human lymphocytes, 4h –S9 and + S9 up to 1250 µg/mL; 20 h – S9 up to 750 µg/mL	Not genotoxic	2015/1112086
			Application of the TTC-threshold for non-genotoxic Cramer Class III molecules for risk assessment purposes: 1.5 µg/kg bw/day		
M605F008 (AN8) ~~p. 203~~		Rotational crop metabolite	QSAR: CaseUltra, OECD toolbox, OASIS TIMES, VEGA	Not genotoxic	2017/1100634 2017/1100635 2017/1100632 2017/1100633 2014/1326432 2015/1198645 2015/1186244 2015/1198463
			Covered by toxicological testing of M605F007 (grouping approach) Not genotoxic. Application of the TTC-threshold for non-genotoxic Cramer Class III molecules for risk assessment purposes: 1.5 µg/kg bw/day		
M605F016 (Py-Hydroxy of M605F007) ~~p. 205~~		Rotational crop metabolite	QSAR: CaseUltra, OECD toolbox, OASIS TIMES, VEGA	~~Inconclusive alerts~~ By weight of evidence not genotoxic	2017/1100634 2017/1100635 2017/1100632 2017/1100633 2015/1198713 2015/1186248 2015/1186245 2015/1198463
			Covered by toxicological testing of M605F007 (grouping approach). By weight of evidence not genotoxic. Application of the TTC-threshold for non-genotoxic Cramer Class III molecules for risk assessment purposes: 1.5 µg/kg bw/day		
M605F032 (Me-Hydroxy of M605F007) ~~p. 207~~		Plant and rotational crop metabolite	QSAR: CaseUltra, OECD toolbox, OASIS TIMES, VEGA	~~Inconclusive alerts~~ By weight of evidence not genotoxic	2017/1100634 2017/1100635 2017/1100632 2017/1100633 2015/1198714 2015/1186249 2015/1186245 2015/1198463
			Covered by toxicological testing of M605F007 (grouping approach) By weight of evidence not genotoxic. Application of the TTC-threshold for non-genotoxic Cramer Class III molecules for risk assessment purposes: 1.5 µg/kg bw/day		

Table 5.8.1-1: Pyrimethanil metabolites considered for potential toxicological relevance

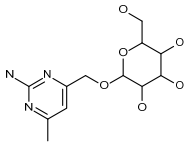
Metabolite	Structure	Reason for relevance assessment	Study / Test system	Findings	Reference (DocID)
M605F033 (Glucose conjugate of M605F032) ~~P 209~~		Plant and rotational crop metabolite	Covered by evaluation of M605F032 By weight of evidence not genotoxic. Covered by toxicological testing of M605F007 (grouping approach) Application of the TTC-threshold for non-genotoxic Cramer Class III molecules for risk assessment purposes: 1.5 µg/kg bw/day		

Table 5.8.1-1: Pyrimethanil metabolites considered for potential toxicological relevance

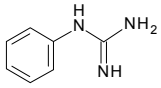
Metabolite	Structure	Reason for relevance assessment	Study / Test system	Findings	Reference (DocID)
Group 3: Phenyl-moiety cleavage product					
M605F025 (Phenyl-guanidine) ~~P 210~~		Livestock, plant and rotational crop metabolite	QSAR: CaseUltra, OECD toolbox, OASIS TIMES, VEGA	Not genotoxic	2017/1100634 2017/1100635 2014/1326434 2014/1326433 2015/1192118 2015/1198463
			Mutagenicity in bacterial cells (Ames test) Salmonella typhimurium (TA 1535, TA 1537, TA98, TA 100,); Escherichia coli (WP2 uvrA); Concentration 20.6 to 5000 µg/plate Without and with S-9 mix	Not mutagenic	1992/1005537
			Mutagenicity in mammalian cells HPRT assay in V79 cells 4 h (± S9): 650 – 2600 µg/mL	Not mutagenic	2017/1124121
			In vitro choromosome aberration (Mammalian cells) CHO CCL 61 18h –S9: 103.13, 206.25, 412.5 µg/ml 3 h (18 h sampling) + S9: 412.5, 825 and 1650 µg/mL; 42 h – S9: 412.4, 825, 1650 µg/mL 3 h (42 h sampling) + S9: 825, 1650, 3300 µg/mL	Equivocal	1992/1005538
			In vivo chromosome aberration Micronucleus test Wistar rat 24 h 125, 250, 500 mg/kg bw 48 h 500 mg/kg bw	Not genotoxic	2015/1186908 2017/1005941 2015/1225521 2015/1223832 2016/1321106
			90-day rat study Wistar 0, 50, 300, 2000 and 8000 ppm 3.06/3.52, 17.8/22.1, 131/147, 677/639 mg/kg bw/day	clinical symptoms (8000 ppm) significant impairment of body weight development (≥ 2000 ppm) severity indicates that 8000 ppm exceeded MTD; reduced food consumption, potential target organs hematopoetic system, liver, kidney, several organ weight alterations secondary to malnutrition of the animals. NOAEL 300 ppm (17.8 mg/kg bw/day)	2002/1027718

Table 5.8.1-1: Pyrimethanil metabolites considered for potential toxicological relevance

Metabolite	Structure	Reason for relevance assessment	Study / Test system	Findings	Reference (DocID)
			Developmental toxicity, rat Wistar 0, 20, 200, 400, 600 mg/kg bw/day	- prenatal toxicity study in rat: clinical symptoms, impairment of body weight development and reduced food consumption indicate maternal toxicity at ≥200 mg/kg bw/day; severity indicates that 600 mg/kg bw/day exceeded MTD. Reduced foetus body weights and delayed ossification (secondary to retarded body weight development) was observed at ≥200 mg/kg bw/day. NOAEL (systemic and developmental): 20 mg/kg bw/day	2002/1027717
			ADI based on the 90-day rat study: 0.089 mg/kg bw/day		

Thus, the conclusion for relevant endpoints for the current renewal was based on the evaluations conducted amended as follows:

Other toxicological studies (SANCO/11802 data point 5.8)

Studies performed on metabolites or impurities	**Group 1: Hydroxylation/oxidation products and their conjugates:** *Group 1 hydroxylates and carboxylate* **M605F002 (AN2) (**metabolite in plants, rotational crops, livestock and rat) - metabolite group covered by toxicological testing of pyrimethanil **Conclusion:** **ADI: 0.17 mg/kg bw/day (pyrimethanil)** **M605F003 (AN3) (**metabolite in plants, rotational crops, livestock and rat) metabolite group covered by toxicological testing of pyrimethanil **Conclusion: ADI: 0.17 mg/kg bw/day (pyrimethanil)** **M605F004 (AN4) (**metabolite in plants, rotational crops, livestock and rat) metabolite group covered by toxicological testing of pyrimethanil **Conclusion: ADI: 0.17 mg/kg bw/day (pyrimethanil)** **M605F005 (AN5) (**metabolite in soil and rotational crops) metabolite group covered by toxicological testing of pyrimethanil **Conclusion: ADI: 0.17 mg/kg bw/day (pyrimethanil)** **M605F006 (AN6) (**metabolite in rotational crops, livestock and rat) metabolite group covered by toxicological testing of pyrimethanil **Conclusion: ADI: 0.17 mg/kg bw/day (pyrimethanil)** **M605F034 (Pyrimethanil-COOH)** (livestock metabolite) metabolite group covered by toxicological testing of pyrimethanil **Conclusion: ADI: 0.17 mg/kg bw/day (pyrimethanil)** *Group 1 conjugates* **M605F001** (plant metabolite) - metabolite group covered by toxicological testing of pyrimethanil **Conclusion:** **ADI: 0.17 mg/kg bw/day (pyrimethanil)** **M605F014** (livestock metabolite) - metabolite group covered by toxicological testing of pyrimethanil **Conclusion:** **ADI: 0.17 mg/kg bw/day (pyrimethanil)**

M605F020 (livestock and rat metabolite)
- metabolite group covered by toxicological testing of pyrimethanil
Conclusion:
ADI: 0.17 mg/kg bw/day (pyrimethanil)

M605F021 (livestock and rat metabolite)
- metabolite group covered by toxicological testing of pyrimethanil
Conclusion:
ADI: 0.17 mg/kg bw/day (pyrimethanil)

M605F023 (livestock and rat metabolite)
- metabolite group covered by toxicological testing of pyrimethanil
Conclusion:
ADI: 0.17 mg/kg bw/day (pyrimethanil)

M605F027 (metabolite in plant and rotational crops)
- metabolite group covered by toxicological testing of pyrimethanil
Conclusion:
ADI: 0.17 mg/kg bw/day (pyrimethanil)

M605F028 (metabolite in plant and rotational crops)
- metabolite group covered by toxicological testing of pyrimethanil
Conclusion:
ADI: 0.17 mg/kg bw/day (pyrimethanil)

M605F029 (metabolite in plants)
- metabolite group covered by toxicological testing of pyrimethanil
Conclusion:
ADI: 0.17 mg/kg bw/day (pyrimethanil)

M605F030 (metabolite in plant and rotational crops)
- metabolite group covered by toxicological testing of pyrimethanil
Conclusion:
ADI: 0.17 mg/kg bw/day (pyrimethanil)

M605F035 (metabolite in livestock and rat)
- metabolite group covered by toxicological testing of pyrimethanil
Conclusion:
ADI: 0.17 mg/kg bw/day (pyrimethanil)

M605F036 (metabolite in plant and rotational crops)
- metabolite group covered by toxicological testing of pyrimethanil
Conclusion:
ADI: 0.17 mg/kg bw/day (pyrimethanil)

M605F037 (metabolite in plants)
- metabolite group covered by toxicological testing of pyrimethanil

Conclusion:
ADI: 0.17 mg/kg bw/day (pyrimethanil)

M605F038 (metabolite in plants)
- metabolite group covered by toxicological testing of pyrimethanil
Conclusion:
ADI: 0.17 mg/kg bw/day (pyrimethanil)

M605F039 (metabolite in plant and rotational crops)
- metabolite group covered by toxicological testing of pyrimethanil
Conclusion:
ADI: 0.17 mg/kg bw/day (pyrimethanil)

M605F040 (metabolite in plant and rotational crop)
- metabolite group covered by toxicological testing of pyrimethanil
Conclusion:
ADI: 0.17 mg/kg bw/day (pyrimethanil)

M605F041 (metabolite in plants)
- metabolite group covered by toxicological testing of pyrimethanil
Conclusion:
ADI: 0.17 mg/kg bw/day (pyrimethanil)

Group 2: Pyrimidin-moiety cleavage products and their derivatives and conjugates:

M605F007 (AN7) (metabolite in soil, water (sediment), rotational crops and rat)
- Bacterial mutagenicity negative, chromosome aberration in vitro test: negative

Conclusion: risk assessment based on TTC-threshold of 1.5 µg/kg bw/day

M605F008 (AN8) (metabolite in soil and rotational crops)
- not genotoxic in vitro based on grouping approach

Conclusion: risk assessment based on TTC-threshold of 1.5 µg/kg bw/day

M605F016 (Py-HO of AN7) (metabolite in rotational crops)
- not genotoxic in vitro based on grouping approach

Conclusion: risk assessment based on TTC-threshold of 1.5 µg/kg bw/day

M605F032 (Me-HO of AN7) (metabolite in plant and rotational crops)
- not genotoxic in vitro based on grouping approach

Conclusion risk assessment based on TTC-threshold of 1.5 µg/kg bw/day

Group 2 conjugates

M605F033 (Glucoside of AN7) (metabolite in plant and rotational crops)
- not genotoxic in vitro based on grouping approach

Conclusion risk assessment based on TTC-threshold of 1.5 µg/kg bw/day

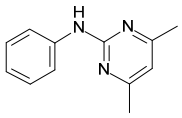
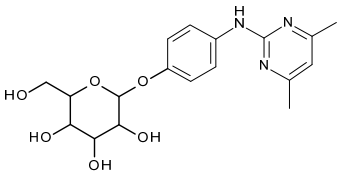
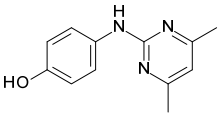
Group 3: Phenyl-moiety cleavage products:

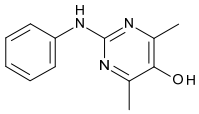
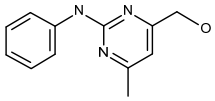
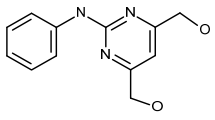
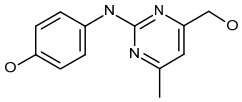
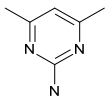
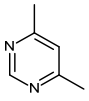
M605F025 (Phenylguanidine)
(metabolite in plant, rotational crops, livestock and rat)
- By weight of evidence not genotoxic: Bacterial mutagenicity (Ames test): negative, mammalian-cell mutagenicity (HPRT test): negative, chromosome aberration in vitro test: equivocal; rat micronucleus test: negative
- 90-day rat study: clinical symptoms (8000 ppm) significant impairment of body weight development (≥ 2000 ppm) severity indicates that 8000 ppm exceeded MTD; reduced food consumption, potential target organs hematopoetic system, liver, kidney, several organ weight alterations secondary to malnutrition of the animals.
  NOAEL 300 ppm (17.8 mg/kg bw/day)
- prenatal toxicity study in rat: clinical symptoms, impairment of body weight development and reduced food consumption indicate maternal toxicity at ≥200 mg/kg bw/day; severity indicates that 600 mg/kg bw/day exceeded MTD. Reduced foetus body weights and delayed ossification (secondary to retarded body weight development) was observed at ≥200 mg/kg bw/day.
  NOAEL (systemic and developmental): 20 mg/kg bw/day

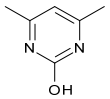
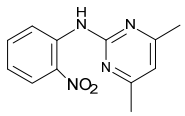
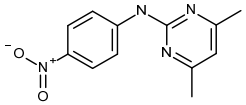
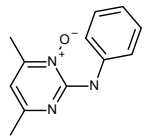
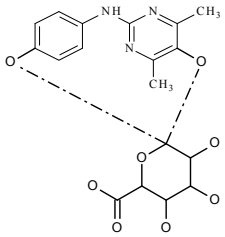
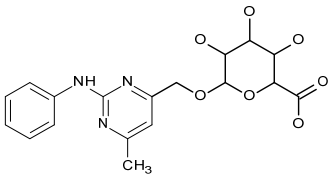
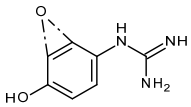
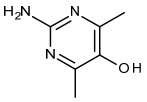
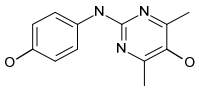
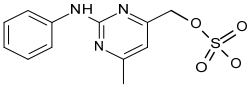
Conclusion: ADI based on the 90-day rat study: 0.089 mg/kg bw/day

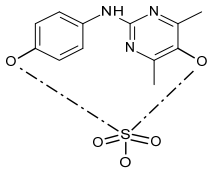
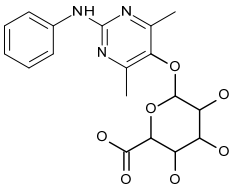
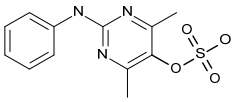
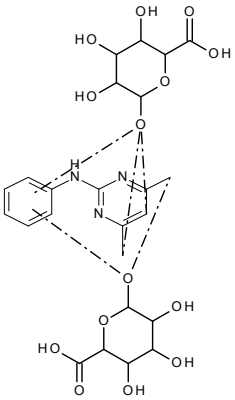
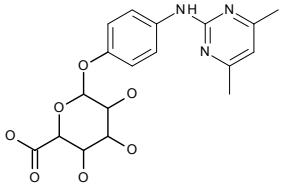
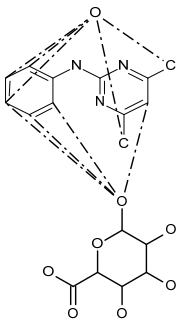
Pyrimethanil is extensively metabolised in all matrices (mammalian, plant and soil) resulting in a number of metabolites identified as described in more detail in M-CA 5.1. Of note, no significant groundwater metabolites have been identified. All significant metabolites, which have to be toxicologically evaluated, are listed in the following table.

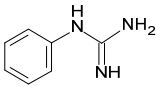
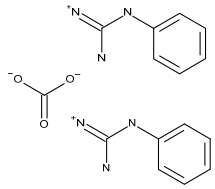
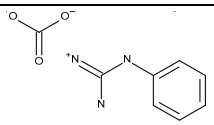
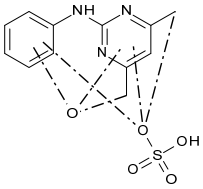
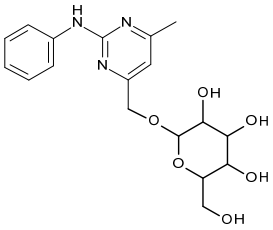
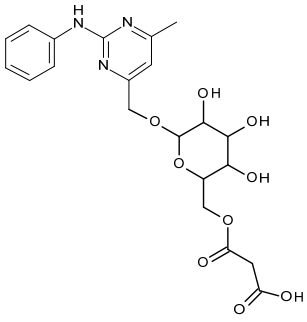
Table 5.8.1-2: Notations of parent and metabolites of pyrimethanil

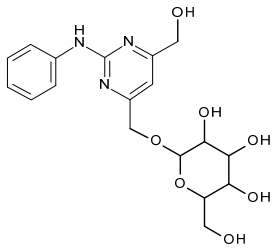
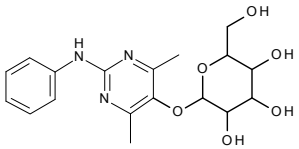
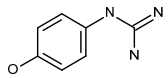
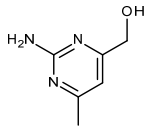
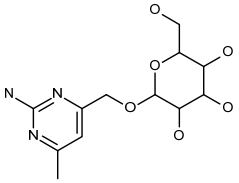
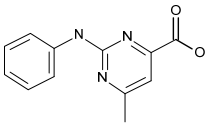
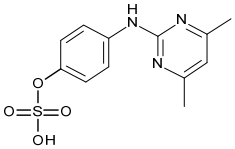
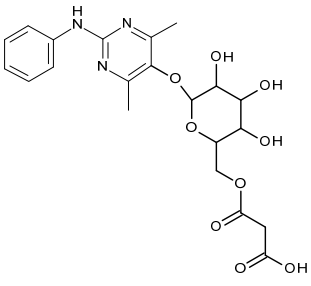
Metabolite designation			Compound found in	Molecular structure
Code number	Reg. No	Synonyms		
Pyrimethanil **BAS 605 F** **M605F000**	236999	AE B100309 SN 100309 AN1	Soil, Water, Sediment Plants: Leafy crops (Lettuce) Fruit (Apple (peel, pulp, leaves)) Fruit (Vine (grapes, leaves)) Fruit (Tomato (fruit, leaves)) Root crops (Carrot (foliage, roots) (foliar or soil treatment)) *Leafy crops (Lettuce)* Rotational crops: Lettuce, radish (tops, roots), wheat (forage, grain, straw) *Wheat (forage, hay, straw, grain), spinach (immature, mature), radish (tuber, foliage)* Animals: Rat (feces) Rat (plasma) *Rat (feces, liver, kidney, fat, plasma)*	
M605F001	-	β-O glucoside of SN 614 276/ M605F002 Unk B T1-T3 M1	Plants: Fruit ((probably) Tomato (fruit, leaves) (might be other C-6 / disaccharide conjugate in addition to glc), Vine (grapes, leaves)) Root crops (Carrot foliage (foliar or soil treatment), roots (soil treatment))	
M605F002	4739173	SN 614 276 AE C614276 AN2	Plants: Leafy crops (Lettuce (released after hydrolysis)) Fruit & root crops (intermediate in vine and apple leaves, carrot foliage) Rotational crops: Lettuce, radish (tops, roots), wheat (forage, grain, straw) Animals (main metabolite): Cow (kidney, urine) *Hen (egg, liver, muscle, fat), goat (liver, kidney)* Rat (urine, feces; major met.) Rat (plasma) *Rat (urine, feces)*	

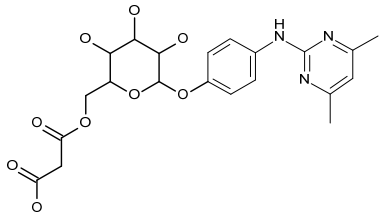
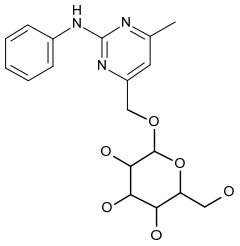
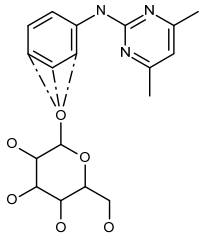
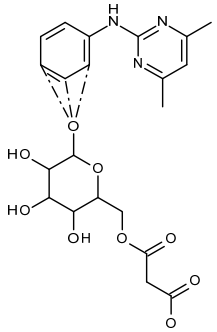
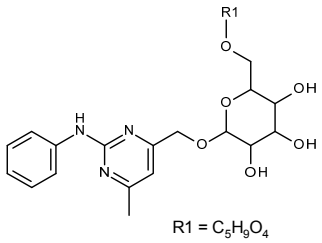
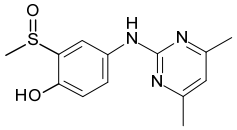
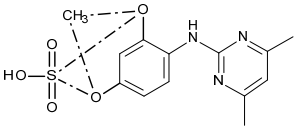
Metabolite designation			Compound found in	Molecular structure
Code number	Reg. No	Synonyms		
M605F003	5079484	SN 614 277 AE C614277 AN3	Plants: Leafy crops (Lettuce (released after hydrolysis)) Rotational crops: Lettuce, radish (tops), wheat (forage, grain, straw) Animals: Cow (milk, kidney, urine, rat excreta (liver feeding)) Rat (urine, feces) Rat (plasma) *Rat (feces)*	
M605F004	5079485	SN 614278 SX 614278 AE 614278 AN4	Plants: Fruit & root crops (Intermediate in vine and apple leaves, carrot foliage) *Leafy crops & cereal/grass crops (lettuce, wheat (forage; ST))* Rotational crops: Lettuce, radish (tops, roots), wheat (forage, grain, straw) *Wheat (forage, hay, straw), spinach (mature), radish (tuber)* Animals: Rat excreta (liver feeding) Rat (urine, feces) Rat (plasma) *Goat (liver)* *Rat (feces, liver, kidney, plasma)*	
M605F005	5079487	C 621 312 AE C621312 AN5	Soil Plants: Fruit (Intermediate in vine and apple leaves) Rotational crops: Lettuce, radish (tops, roots), wheat (forage, grain, straw)	
M605F006	5079486	SN 614800 AE C614 800 AN6	Rotational crops: Radish (tops, roots), wheat (forage, grain, straw) Animals: Cow (kidney, urine, rat excreta (liver feeding)) *Hen (liver, egg), goat (liver, kidney)* Rat (urine, feces)	
M605F007	40603	AE F132593 SN 512 723 ZK 512723 AE C512723 NC 12723 S151 CL 2869 AN7	Soil, Water/Sediment Rotational crops: Lettuce, radish (tops), wheat (forage, grain, straw) *Wheat (forage, hay, straw, grain), spinach (immature, mature), radish (foliage)* Animals: *Rat (urine, feces, bile)*	
M605F008	4245969	AE 0025462 AN8	Soil Rotational crops: Lettuce, radish (tops), wheat (forage, grain, straw)	

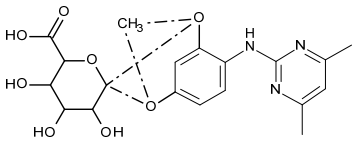
Metabolite designation			Compound found in	Molecular structure
Code number	Reg. No	Synonyms		
M605F009	51589	SN 469 626 AE F132512 CL 17345 Std. 155 AN9	Soil, Water, Sediment	
M605F010		AZ 196 920	Soil	
M605F011	4180488	SN 617 916	Soil, Water/Sediment	
M605F012	5079483	SN 603 193	Soil, Water/Sediment	
M605F013			Animals: *Rat (urine, bile)*	
M605F014		Glucuronide conjugate of SN 614278 / 5079485 / M605F004	Animal: *Goat (liver, kidney)*	
M605F015			Animals: *Rat (feces)*	
M605F016			Rotational crops: *Wheat (forage, hay, straw, grain), spinach (immature, mature), radish (foliage, tubers)*	
M605F017		SN 615 224 SN 615 244	Animals: Rat excreta (liver feeding) Rat (urine, feces) *Rat (urine, feces)*	
M605F018		Sulfate conjugate of SN 614278 / M605F004	Animals: *Rat (urine, bile, liver, kidney, fat, plasma)*	

Metabolite designation			Compound found in	Molecular structure
Code number	Reg. No	Synonyms		
M605F019		Sulfate conjugate of M605F017	Animals: *Rat (urine, bile, kidney, plasma)*	
M605F020		Glucuronide conjugate of 5079484 / M605F003	Animal: *Goat (kidney)* *Rat (urine, bile)*	
M605F021		Sulfate conjugate of 5079484 / M605F003	Animal: *Goat (liver, kidney, milk)* *Rat (urine, bile, liver, kidney, plasma)*	
M605F022		Glucuronide/ glucuronide conjugate of BAS 605 F	Animals: *Rat (was detected by structure elucidation, but no radiopeak could be assigned)*	
M605F023		Glucuronide of 4739173 / M605F002	Animals: *Hen (egg, liver, muscle, fat), goat (liver, kidney, milk)* *Rat (urine, bile, plasma)*	
M605F024		Glucuronide conjugate of hydroxylated BAS 605 F	Animals: *Rat (urine, bile)*	

Metabolite designation			Compound found in	Molecular structure
Code number	Reg. No	Synonyms		
M605F025	4182909	Phenyl guanidine CGA 263208 Metab I11 N-(4-hydroxyphenyl-4-cyclopropyl-6-methyl-2-pyrimidamine N-phenylguanidine	Plants: *Leafy crops (lettuce)* *Cereal/grass crops (wheat (hay, straw, forage; ST))* Rotational crops: *Wheat (forage, hay, straw, grain), spinach (immature, mature), radish (foliage, tuber)* Animals: *Goat (liver, kidney, milk)* *Hen (liver)* *Rat (urine, feces)*	
-		Phenylguanidine carbonate CA 1139A CGA 263208 tech.	(Not applicable)	
-	102020	Phenylguanidine hydrogen carbonate 1-phenylguanidinium hydrogen carbonate AE F132306 CL 47054	(Not applicable)	
M605F026		Sulfate conjugate of hydroxylated BAS 605 F	Animals: *Rat (urine, bile)*	
M605F027		β-O-glucoside of SN 614278 M605F004 U2 M5 Unk F T1-T3	Plants: Fruit (Apple (peel, pulp, leaves)) Fruit (Vine (leaves)) Fruit ((probably) Tomato (fruit, leaves)) Root crops (Carrot (foliage, roots) (foliar or soil treatment)) *Leafy crops & cereal/grass crops (Lettuce, wheat (hay, straw, forage; ST))* Rotational crops: *Wheat (forage, hay, straw), radish (foliage, tuber)*	
M605F028		Malonyl-β-O glucoside of SN 614 278 / M605F004 Unk A T1-T3	Plants: Root crops (Carrot (foliage, roots) (foliar or soil treatment)) *Leafy crops & cereal/grass crops (Lettuce, wheat (hay, straw, forage; ST))* Rotational crops: *Wheat (forage, hay, straw), spinach (mature), radish (tuber, foliage)*	

Metabolite designation			Compound found in	Molecular structure
Code number	Reg. No	Synonyms		
M605F029		β-O-glucoside of C621 312/ M605F005 U1	Plants: Fruit (Apple (peel, pulp, leaves))	
M605F030		Glucose conjugate of 5079484 / M605F003	Plants: *Leafy crops (Lettuce)* Rotational crops: *Wheat (forage, hay, straw), radish (foliage)*	
M605F031			Animals: *Rat (feces, bile)*	
M605F032		Me-Hydroxy of AN7, 40603 / M605F007 4-Pyrimidinemethanol, 2-amino-6-methyl-	Plants: *Cereal/grass crops (wheat (hay, straw, forage; ST))* Rotational crops: *Wheat (forage, hay, straw), spinach (immature, mature), radish (foliage, tubers)*	
M605F033		Glucose conjugate of M605F032	Plants: *Leafy crops & cereal/grass crops (Lettuce, wheat (hay, straw, forage; ST))* Rotational crops: *Wheat (forage, hay, straw, grain), spinach (immature, mature), radish (foliage, tubers)*	
M605F034		Pyrimethanil Carboxylic Acid	Animals: *Goat (liver, kidney)*	
M605F035		Sulfate of SN 614 276 / M605F002	Animals: *Goat (liver, milk)* *Hen (egg, liver, muscle, fat)* Rat (urine, feces; major met.)	
M605F036		Malonyl glucose conjugate of 5079484 / M605F003	Plants: *Leafy crops & cereal/grass crops (Lettuce, wheat (forage, hay; ST))* Rotational crops: *Wheat (forage, hay, straw), spinach (mature, immature), radish (foliage)*	

Metabolite designation			Compound found in	Molecular structure
Code number	Reg. No	Synonyms		
M605F037		Malonyl-β-O glucoside of SN 614 276 / M605F002 Unk C T1-T3	Plants: Root crops (Carrot foliage (foliar or soil treatment), roots (soil treatment))	
M605F038		C-6 sugar conjugate of SN 614278 M605F004 U3 M4 T1-T3	Plants: Fruit (Apple (peel, pulp, leaves) (**not** cleaved by β-glucosidase)) Fruit (Vine (grapes, leaves) (**not** cleaved by β-glucosidase)) Fruit ((probably) Tomato (fruit, leaves))	
M605F039			Plants: *Leafy crops (Lettuce)* Rotational crops: *Spinach (mature, immature)*	
M605F040			Plants: *Leafy crops (Lettuce)* Rotational crops: *Spinach (mature, immature)*	
M605F041			Plants: *Leafy crops (Lettuce)*	 R1 = $C_5H_9O_4$
M605F042			Animals: *Rat (urine, feces)*	
M605F043			Animals: *Rat (urine)*	

Metabolite designation			Compound found in	Molecular structure
Code number	Reg. No	Synonyms		
M605F044			Animals: *Rat (urine, bile)*	

Grouping approach for toxicological relevance assessment

~~For the toxicological relevance assessment of the metabolites, a grouping approach was applied and if applicable, key structures were selected for toxicological relevance assessment.~~
In order to avoid unnecessary animal studies, a grouping approach was applied in which the above metabolites (except *per se* non-relevant metabolites like rat-only metabolites) were assigned to three different groups and representative key structures were selected. To select the metabolites for toxicological relevance assessment, different criteria (**A – C**) were considered, as described below.

The grouping proposal takes into account:

A) Chemical similarity,
B) Coverage by mammalian toxicity studies conducted with parent pyrimethanil.
C) Information on chemical reactivity (structural alerts)

A Chemical Similarity

With regard to evaluation of chemical similarity the general proposals given by e.g. by the EFSA Scientific Opinion on Evaluation of the Toxicological Relevance of Pesticide Metabolites for Dietary Risk Assessment [EFSA Journal 2012;10(07):2799] were followed. The following general molecular modifications were considered to probably not cause higher toxicity of the metabolites:

- Simple demethylation of the ring or side chain
- Simple hydroxylation of the ring system without any cleavage of the ring
- Hydroxylation of another position (i.e. side chain)
- Conjugation of metabolites with amino acids, sulfates or sugar residues

Comparison was made to parent as well as to the grouped metabolites in order to select key metabolites for testing. In addition consideration of increased hydrophilicity and thus considered faster excretion of the grouped metabolites as compared to the tested key metabolites and/or parent was taken into account.

B Coverage of metabolites of concern by mammalian toxicity studies

Although not comparable some overlap exists in the metabolism of pyrimethanil in animals, plant and soil. Thus general conclusions drawn from the animal metabolism of pyrimethanil as well as overlaps of metabolite structures were taken into consideration when grouping the metabolites with regard to human exposure from the source livestock, plant or rotational crop. It was considered whether either the metabolites under consideration or similar structures were formed in the metabolisms studies conducted in mammals.
Moreover, it is taken into consideration that metabolites after uptake in to the body could be transformed by known metabolic pathways into structures that have been identified in the mammalian metabolism studies conducted.
Phase II conjugation of hydroxylates (o-bound sugar conjugation, glucuronidation or sulfatation were considered to be intrinsic metabolic pathway to increase the excretability of the metabolite of concern. As presented in section 5.1 above the hydroxylates and the glucuronidation and sulfatation conjugates thereof represent the excretion pathway in the rat and are thus covered by the rat metabolism of the parent molecule. With regard to o-bound sugar conjugates representing the conjugation pathway in plants these o-bound are expected to be cleaved in the mammalian GIT back-transforming the conjugated metabolite to the respective hydroxylates, which are assessed accordingly. For this reason, the corresponding aglycones of the sugar conjugates are considered for toxicity relevance assessment.

C Presence of Structural alerts

For all metabolites identified as potentially relevant except the conjugates, the presence for potential structural alerts was evaluated with different SAR/QSAR models. The conjugates were considered to be covered by the structural alerts of the predecessor hydroxylates (i.e. their aglycones) as the conjugate structures are not expected to contribute to the toxicity.

Models used, were CASE Ultra, OASIS TIMES, the OECD toolbox (identification of alerts as well as read-across, where applicable) and VEGA. The QSAR predictions for metabolites of pyrimethnail made with CASE Ultra and the QSAR Toolbox were all “in domain” and thus reliable for Ames mutagenicity and the potential to induce structural and/or numerical aberrations (micronuclei). ~~However,~~ In contrast, the QSAR predictions obtained from OASIS TIMES and Vega are to some extend limited by the reliability as most of the structures evaluated were not in the prediction domain. ~~Thus, given the structural relationship of the metabolites evaluated inter alia and in relation to the parent molecule pyrimethanil, the predicted alerts were compared to those for the parent and those metabolites were toxicological data were available in order to overcome the limitations of the predictions made.~~ Moreover, the systems used do not distinguish chiral structures.
For this reason, the predictions made by CASE Ultra and the QSAR Toolbox were given a higher weight compared to the other tools. Alerts fired for the metabolites were also compared to those alerts being fired for pyrimethanil. Since negative experimental data on the parent compound were available, common alerts were identified not to be predictive for a positive genotoxic effect and some limitations of the predictions made by OASIS TIMES and Vega could be addressed accordingly.

Grouping proposal for pyrimethanil metabolites

Group 1: Hydroxylation/oxidation products of pyrimethanil and their conjugates

This group comprises of the single or double hydroxylation products of pyrimethanil namely: M605F002, M605F003, M605F004, M605F005, M605F006.
In addition, the further oxidized pyrimethanil-carboxylic acid (M605F034) is also considered a member of this group.

Moreover, the phase II conjugates of these hydroxylates are assigned to this group i.e.
the O-bound sugar conjugates M605F001 (conjugate of M605F002),
M605F027 (conjugate of M605F004),
M605F029 (conjugate of M605F005),
M605F030 (conjugate of M605F003),
M605F038 (conjugate of M605F004)
M605F039,
M605F041 (conjugate of M605F004),
the O-bound sugar-malonyl conjugates M605F028 (conjugate of M605F004),
M605F036 (conjugate of M605F003),
M605F037 (conjugate of M605F002),
M605F040,
the glucuronide conjugates M605F014 (conjugate of M605F004),
M605F020 (conjugate of M605F003),
M605F023 (conjugate of M605F002)
as well as the sulfates M605F021 (conjugate of M605F003) and M605F035 (conjugate of M605F002).
The O-bound sugar and sugar-malonyl conjugate plant metabolites are expected to be cleaved in the mammalian gastrointestinal tract to the corresponding predecessor hydroxylates (i.e. aglycones), which are assessed as primary relevant structures. The two sulfate conjugates are assessed as they are, although cleavage to their corresponding aglycone may also occur.

Coverage of certain metabolites by toxicity testing with the parent compound

With regard to the rat metabolism investigations [see M-CA 5.1] in urine, the respective studies identified the metabolites M605F002 and its further downstream metabolites (conjugation, further hydroxylation and subsequent conjugation) in sum with up to 39.2% in the peer-reviewed phenyl label study and up to 43.9% in the new pyrimidinyl label study. M605F003 and its conjugates were found in sum up to 7.9% in the phenyl label study and up to 14.2% in the pyrimidinyl label study. M605F004 and conjugation metabolites are in sum up to 2.3% in the phenyl label study and up to 11.0% in the pyrimidinyl label study. M605F017 the double hydroxylated derivative of M605F002 or M605F003 respectively and conjugation metabolites are in sum up to 5.0% in the phenyl label study and up to 17.8% in the pyrimidinyl label study. Phase I metabolite M605F006 was found in the peer-reviewed study only with up to 5.7%. Phase II metabolite M605F026 was found in the new study only with up to 9.6%.

Overall the metabolites of this major transformation steps (hydroxylation and subsequent conjugation - group 1) were found in rat urine in a range of 17.4% to 45.7% in the phenyl label study and in range of 53.7% to 75.2% in the pyrimidinyl label study and thus considered to be well covered by the toxicological profile of the parent molecule pyrimethanil.

Group 2: Cleavage products pyrimidine-moiety hydroxylates and conjugates

Group 2 comprises cleavage products of pyrimethanil which contain the dimethyl pyrimidine moiety in their molecular structure, all occurring in plants.

M605F007 is defined to be the marker metabolite of this group. A deamination of this metabolite leads to M605F008, while a ring or side-chain hydroxylation leads to metabolites M605F016 or M605F032, respectively.

M605F033 as last representative of group 2 represents the sugar conjugate of M605F032. The O-bound sugar conjugate is expected to be cleaved in the mammalian gastrointestinal tract to the corresponding predecessor hydroxylates (i.e. M605F032), which is assessed as primary relevant structure.

Group 3: Cleavage product with phenyl-moiety

Only M605F025 was assigned to group 3, which is the only cleavage product containing the phenyl moiety.

Overview of the grouping approach

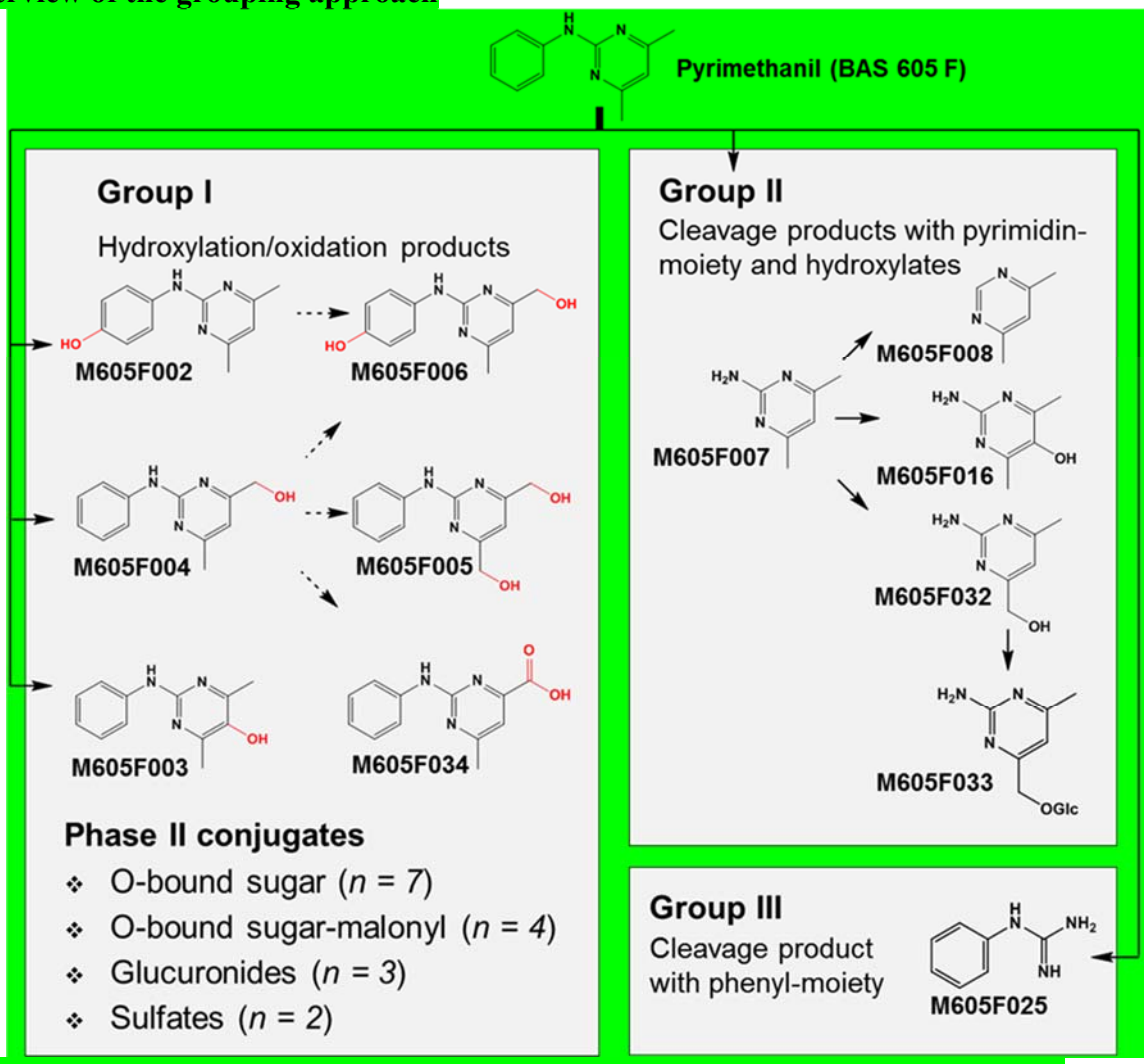


Figure 5.8.1-1: Basic structure of grouping proposal for pyrimethanil metabolites.

Note: the corresponding conjugates are not shown but are discussed in detail below.

Genotoxicity hazard assessment

For all metabolites identified with potential relevance or as corresponding group members (see below), presence for potential structural alerts associated with genotoxicity was evaluated using different QSAR models. Models used were CASE Ultra, the OECD Toolbox - modified by inclusion of read across where applicable -, OASIS TIMES, and VEGA. In addition to simply identify the molecular structures of the metabolites, also overall predictions of their potential to induce genotoxicity (i.e. Ames and MNT/CA) were obtained with a considerable reliability (i.e. 'in domain' predictions using CASE Ultra and the OECD Toolbox)).
The QSAR models being used for this approach are described in more detail in the following section.

Overview of the QSAR tools being used to evaluate genotoxicity

CASE Ultra

CASE Ultra (Version 1.6.0.0) is a combination of a number of statistical QSAR models to predict Ames mutagenicity or the potential to form micronuclei (in vivo MNT).
QSAR models being used were:

- GT1_A7B for Bacterial mutagenicity in diverse Salmonella sub-strains (TA97, TA 98, TA100, TA1535, TA1536, TA1537, TA1538)
- GT1_AT_ECOLI (featuring data from Salmonella TA102 and E.coli)
- GT3_MNT_Mouse

The QSAR model reporting formats (QMRFs) for the models included in the commercial package are supplied with this update [see KCA 5.8.1/46 2015/1282049, KCA 5.8.1/47 2015/1282048, KCA 5.8.1/48 2015/1282047, KCA 5.8.1/49 2015/1282367 and KCA 5.8.1/50 2017/1066625].
Each statistical model works as multilinear regression QSAR. A set of multilinear regressions are derived with a stepwise increment method, one set per structural alert. Structural alerts are derived by fragmentation of the training dataset with an initial pool of ~1000 descriptors. Fragments are counted and compared to the number of substances in the training database containing this fragment and their respective activity in the test organism. Additional structural alerts as well as positive and negative modulators are taken into consideration.
In addition, CASE Ultra also features a konsolidator which combines test results and compares the test chemical and alerts with the test results of 11461 chemicals with experimental data. The consolidator also allows to add experimental data to the evaluation. Thus, experimental data available for parent and tested metabolites/impurities are taken into consideration. The konsolidator is only available for Ames mutagenicity.

BASF has further trained the above-mentioned models using genotoxicity information for plant protection chemistry as derived from EFSA conclusions over a time-period spanning 2006 to 2016. Thereby, the respective training dataset for the statistical models have been expanded form

Prediction model, number of substances underlying standard version (respective left number) and number of substances in trained version (respective right number):

- GT1_A7B for Bacterial mutagenicity 3979 4624
- GT1_AT_ECOLI 1199 1861
- GT3_MNT_Mouse 610 1106

o Adding additional proprietary BASF plant protection data
610 1168

Statistical features of the original models were retained. Models were validated using three different methods, as available under default settings for CASE Ultra:

- Leave N% Out Cross Validation
- Bootstrap
- Y Scrambling

For Ames mutagenicity, CASE Ultra also features an expert, rule-based model, which is not trainable. The model is described in *Alexander Sedykh, Suman Chakravarti, and Roustem Saiakhov. MultiCASE rule-based expert system for mutagenicity prediction: creating and validating Genetic Toxicity Association Annual Meeting, 2015, Delaware. Poster presentation.*

The model is based on structural fragments probability for each structural alert and trained on 10.777 compounds, for which experimental results derived in the Ames test are available. The model includes 175 structural alerts, of which 40 represent basic alerts associated with general genotoxicity mechanisms such as DNA binding, while the remainder is implemented as refining factors, e.g. having an activating or deactivating effect on a basic alert. Expert alerts were collected from the public literature, refined and benchmarked on a reference set of over 11.000 chemicals with known mutagenicity outcomes.

It should be noted that this reference dataset comprises a wide range of molecular structures being used in the sectors of chemicals, pharmaceuticals and partially plant protection chemistry. However, the data set does generally not contain data on metabolites or conjugates. Glutathione conjugates or glucuronides are generally missing. Sulfuric acid is present as a structural moiety or functional group, but not as part of phase II conjugates. Sugar conjugates as typically seen in plant metabolism are also clearly under represented.

OASIS TIMES

OASIS TIMES is a hybrid statistical and knowledge-based model for toxicity prediction. The Tissue Metabolism Simulator (TIMES), developed by LMC (Bourgas University, Bulgaria; http://oasis-lmc.org/) integrates on the same platform a metabolic simulator and QSAR models for predicting toxicity of selected metabolites. The metabolic simulator generates plausible metabolic maps from a comprehensive library of biotransformations and abiotic reactions. It allows prioritization of chemicals according to toxicity of their metabolites. Of OASIS TIMES the prediction models for Ames test and in vitro chromosome aberration were considered and therefore predictivity is limited to these test systems only.
The reactivity model describing interactions of chemicals with DNA is based on an alerting group approach. Only those toxicophores extracted from the training set having clear interpretation for the molecular mechanism causing the ultimate effect included in the model. The mechanistic interrelation between alerts and related parametric ranges generalizing the effect of the rest of the molecules on the alert is also considered. The structural component of the model is based on the structural similarity between chemicals in the training set which were correctly predicted by the model. The structural neighborhood of atom-centered fragments is used to determine this similarity. The training set consists of 1514 chemicals for Ames and 808 chemicals for chromosomal aberration.
The derived model is combined with metabolic simulator TIMES used for predicting metabolic activation of chemicals with the S9 mix. The metabolic simulator is trained to reproduce documented maps for mammalian liver metabolism for 261 chemicals. Parent chemicals and each of the generated metabolites are submitted to a battery of models to screen for a general effect and mutagenicity mechanisms. Thus, chemicals are predicted to be mutagenic as parents only, parents and metabolites, and metabolites only. Mutagenicity could be due to the parent chemical only or as a result of its metabolic activation (i.e., the parent is inactive but it is transformed to a mutagenic metabolite), or both parent structure and metabolites could be mutagenic.
This OASIS QSAR system is also included in the OECD Toolbox (but not in combination with TIMES), in order to make use of (Q)SAR approaches also in the assessment of chemicals under REACH (OECD, 2008). The BASF-internal version has the advantage that it is capable to consider metabolic transformation.

Q(SAR) Model Reporting Formats (QMRF) for both endpoints Ames and Chromosomal Aberration are provided in JCA DocIDs 2013/1414242 and 2013/1414460.

VEGA

Using the VEGA platform, access to a series of QSAR (quantitative structure-activity relationship) models for regulatory purposes was obtained. Of the models offered by VEGA [http://www.VEGA-qsar.eu/] only the three independent statistical prediction models for mutagenicity (Ames) were selected. The first one is an implementation of CAESAR, which makes predictions based on the comparison of the structure of interest to the CAESAR database of mutagenicity data of substances in the structure database. A score is provided for the match of the structures, and the mutagenicity data of the closest related substances compared to the structure of interest. Consequently, if a structure is not adequately presented in the database, the prediction is only of very limited validity. The QMRF report is available in JCA DocID 2015/1186934.

The second algorithm **SarPy** searches for isolated structural alerts of substructures in the molecule. Again this is based on the mutagenicity data provided in the structure database.

The third one is a part of the Toxtree module. Toxtree is a open-source application that places chemicals into categories and predicts various kinds of toxic effect by applying decision tree approaches. The version included into the VEGA platform is the version 1.0.0 just relying on the Benigni-Bossa rulebase. The underlying Benigni-Bossa rulebase and its applicability are reported in a JRC Report [JCA DocID 2008/1102916]. This Benigni-Bossa rulebase QSAR model included in Vega considers:

1. the mutagenic activity of aromatic amines in the *Salmonella typhimurium* TA100 strain (Ames test);
2. the mutagenic activity of αβ -unsaturated aldehydes in the *Salmonella typhimurium* TA100 strain (Ames test).

The Toxtree model is also covered in the OECD toolbox above.

OECD Toolbox (Profiling module)

The OECD toolbox version 3.3 was used for the initial evaluation, while version 3.4 was used in the update. Both versions are publicly available and as downloadable via link of the ECHA webpage [http://www.qsartoolbox.org/download.html] was used for the evaluation. The outcome of the OECD toolbox profiling conducted for the metabolites was exported and collected [see KCA 5.8.1/1 2015/1198463] as the report function of the current OECD toolbox version did not work properly. The profiling module provided structural alerts for different endpoints. Of particular interest were the modules dealing with protein- or DNA-binding capacity as well as genotoxicity and/or carcinogenicity predictions. It should be noticed that the profiles just provide structural alerts without consideration on probability that these alerts may become active or inactive due to chemical reactivity and/or sterical hindrance. The current version of the OECD toolbox does not allow to generate reports out of the conducted evaluations, instead the toxicological profiles obtained with the different modules were exported to EXCEL. These exported profiles however, do not include the explanation that are available for the different alerts identified, these can be obtained when running the evaluation with the OECD toolbox as available and have been included for evaluation of the individual compounds below.

OECD Toolbox (Read-across module)

In addition to profiling, a modified OECD Toolbox module including read across was created [see KCA 5.8.1/53 2017/1100632 (AMES) and KCA 5.8.1/54 2017/1100633 (in vivo MNT)] and used to get QSAR predictions with domain estimations within the OECD Toolbox version 3.4.

Therefore, all six modules of the workflow were stepwise approached (i.e. i. Input, ii. Profiling, iii. Endpoint, iv. Category Definition, v. Data Gap Filling, vi. Report). To get a higher reliability of the predictions, available experimental data on pyrimethanil metabolites were imported into the QSAR Toolbox using the Import wizard. In accordance with the Residue Guidance for dietary risk assessment, all proposed profilers related to genotoxity were used to characterize each metabolite (i.e. DNA binding by OASIS v1.4, DNA binding by OECD, DNA alerts for Ames by OASIS v1.4, DNA alerts for CA and MNT by OASIS v1.1, in vitro mutagenicity (Ames test) alerts by ISS, in vivo mutagenicity (Micronucleus) alerts by ISS and Organic Functional Groups). If no experimental data on genotoxicity were stored in the data bases of the QSAR Toolbox (module "Endpoint"), suitable analogues for read-across were searched in the module "Category Definition". In this module, the above profilers on DNA binding and genotoxicity were used to identify analogues as well as the empiric profiler for structural similarity. If suitable analogues were available, a read-across approach was performed (module "Data Gap Filling") to predict Ames mutagenicity as well as the potential to form structural or numerical chromosomal aberrations (MNT in vivo). For all predictions, QPRFs were created in the module "Report" and are exportable as pdf files.

CAVEAT on reliability of QSAR modules implied

With regard to the QSAR evaluations as implied in OASIS TIMES and VEGA it should be noted that for nearly all analysis the algorithm reported are out of structural domain error. As a consequence the predictivity is solely based on the proposed DNA-interaction via the structural alert, not (VEGA) or not appropriately (OASIS TIMES) taking into account possible functional group interaction and stereochemical hindrance. It is well acknowledged that these structural activity predictions are therefore of limited validity. To overcome these limitations the evaluation was conducted mainly in comparison to the parent compound pyrimethanil or metabolites with available toxicological data, in order to assess whether same or other predictions than for the compared compound were made. ~~For the convenience of the reviewer and for better comparison, the results of the QSAR analysis for the parent substance pyrimethanil are summarized as follows.~~

The trained modules of the OECD toolbox and CASE Ultra were of better reliability showing a higher rate of molecules to be assessed within the prediction domain.

For overview in the following tables the QSAR-predictions for all metabolites assessed is summarized. Table 5.8.1-3 provides the information for Ames mutagenicity and Table 5.8.1-4 the information related to chromosomal aberration in vitro/in vivo.

Table 5.8.1-3: Summary of QSAR-predictions for Ames mutagenicity for Pyrimethanil metabolites

Metabolite	Aglycon	Test system	CASE Ultra	OECD Toolbox incl. Read Across	OASIS TIMES	Vega Caesar	Vega SarPy
		Ames	Statistical Model Konsolidated prediction; trained model	Mechanistic model	Mixed model	Statistical Model	
Pyrimethanil		**Negative (tested)**	**Negative (known)**	**Negative (known)**	Positive	Positive	Negative
Group 1							
M605F002		**Negative** (>10% ADME rat)	**Negative (known)**	**Negative (known)**	Positive[1]	Negative	Negative
M605F003			**Negative**	**Negative**	Positive[1]	Negative	Negative
M605F004			**Negative**	**Negative**	Positive[1]	Negative	Negative
M605F005			**Negative**	**Negative**	Positive[1]	Negative	Negative
M605F006			**Negative**	**Negative**	Positive[1]	Negative	Negative
M605F034			**Negative**	**Negative**	Positive[1]	Negative	Negative
M605F035			**Negative**	**Negative**	not tested	not tested	not tested
M605F021		**Negative** (>10% ADME rat)	**Negative (known)**	**Negative (known)**	not tested	not tested	not tested
M605F019		**Negative** (>10% ADME rat)	no need to check (rat-only metabolite)				
M605F023		**Negative** (>10% ADME rat)	**Negative (known)**	**Negative (known)**	not tested	not tested	not tested

Metabolite	Aglycon	Test system	CASE Ultra	OECD Toolbox incl. Read Across	OASIS TIMES	Vega Caesar	Vega SarPy
		Ames	Statistical Model Konsolidated prediction; trained model	Mechanistic model	Mixed model	Statistical Model	
M605F001	M605F002		no need to check (covered by aglycon)				
M605F037	M605F002		no need to check (covered by aglycon)				
M605F039	M605F002		no need to check (covered by aglycon)				
M605F040	M605F002		no need to check (covered by aglycon)				
M605F020	M605F003		no need to check (covered by aglycon)				
M605F030	M605F003		no need to check (covered by aglycon)				
M605F036	M605F003		no need to check (covered by aglycon)				
M605F014	M605F004		no need to check (covered by aglycon)				
M605F027	M605F004		no need to check (covered by aglycon)				
M605F028	M605F004		no need to check (covered by aglycon)				
M605F038	M605F004		no need to check (covered by aglycon)				
M605F041	M605F004		no need to check (covered by aglycon)				
M605F029	M605F005		no need to check (covered by aglycon)				
Group 2							
M605F007		**Negative (tested)**	**Negative (known)**	**Negative (known)**	Negative	Negative	Negative
M605F008			**Negative**	**Negative**	Negative	Negative	Negative
M605F016			**Negative**	**Negative**	Negative	Negative	Negative
M605F032			**Negative**	**Negative**	Negative	Negative	Negative
M605F033	M605F032		no need to check (covered by aglycone)				
Group 3							
M605F025		**Negative (tested)**	**Negative**	**Negative (known)**	not tested	not tested	not tested

In domain predictions are depicted in bold; Out of domain predictions are not highlighted; [1] Out of domain and/or same alerts as parent that was negative in experimental genotoxicity studies

Table 5.8.1-4: Summary of QSAR-predictions for chromosomal aberration in vitro/in vivo for Pyrimethanil metabolites

Metabolite	Aglycon	Chromosomal aberration tested		CASE Ultra	OECD Toolbox incl. Read Across	OASIS TIMES
		CA / MNT in vitro	MNT in vivo	Statistical Model Konsolidated prediction; trained model	Mechanistic model	Mixed model
Pyrimethanil		**Negative (tested)**	**Negative (tested)**	**Negative (known)**	**Negative (known)**	Positive
Group 1						
M605F002		**Negative** (>10% ADME rat)		**Negative (known)**	**Negative (known)**	Positive
M605F003				**Negative**	**Negative**	Positive[1]
M605F004				**Negative**	**Negative**	Positive[1]
M605F005				**Negative**	**Negative**	Positive[1]
M605F006				**Negative**	**Negative**	Positive[1]
M605F034				**Negative**	**Negative**	Positive[1]
M605F035				**Negative**	**Negative**	not tested
M605F021		**Negative** (>10% ADME rat)		**Negative (known)**	**Negative (known)**	not tested
M605F019		**Negative** (>10% ADME rat)		no need to check (rat-only metabolite)		
M605F023		**Negative** (>10% ADME rat)		**Negative (known)**	**Negative (known)**	not tested
M605F001	M605F002			no need to check (covered by aglycon)		
M605F037	M605F002			no need to check (covered by aglycon)		
M605F039	M605F002			no need to check (covered by aglycon)		
M605F039	M605F002			no need to check (covered by aglycon)		
M605F040	M605F002			no need to check (covered by aglycon)		
M605F020	M605F003			no need to check (covered by aglycon)		
M605F030	M605F003			no need to check (covered by aglycon)		
M605F036	M605F003			no need to check (covered by aglycon)		
M605F014	M605F004			no need to check (covered by aglycon)		
M605F027	M605F004			no need to check (covered by aglycon)		
M605F028	M605F004			no need to check (covered by aglycon)		
M605F038	M605F004			no need to check (covered by aglycon)		
M605F041	M605F004			no need to check (covered by aglycon)		
M605F029	M605F005			no need to check (covered by aglycon)		

Metabolite	Aglycon	Chromosomal aberration tested		CASE Ultra	OECD Toolbox incl. Read Across	OASIS TIMES
		CA / MNT in vitro	MNT in vivo	Statistical Model Konsolidated prediction; trained model	Mechanistic model	Mixed model
Group 2						
M605F007		**Negative (tested)**		**Negative (known)**	**Negative (known)**	Negative
M605F008				**Negative**	**Negative**	Negative
M605F016				**Negative**	**Negative**	Negative
M605F032				**Negative**	**Negative**	Negative
M605F033	M605F032			no need to check (covered by aglycone)		
Group 3						
M605F025		Equivocal (tested)	**Negative (tested)**	**Negative**	**Negative (known)**	not tested

In domain predictions are depicted in bold; Out of domain predictions are not highlighted; [1] Out of domain and/or same alerts as parent that was negative in experimental genotoxicity studies

Systemic toxicity assessment

Group 1: Hydroxylation/oxidation products of pyrimethanil and their conjugates

Group 1 comprises hydroxylation products (phase I metabolites) of pyrimethanil as well as further oxidized conjugates (phase II metabolites), which are of high structural similarity to the parent since they all contain the intact core-structure.

For the hazard assessment of group 1, the toxicological properties of the parent molecule are taken into consideration as a pragmatic approach. Pyrimethanil is not classified to be toxic either by acute or by sub-chronic to chronic exposure. Moreover, pyrimethanil is neither classified for genotoxicity, carcinogenicity nor reproduction or developmental toxicity. Thus, metabolites would not require a toxicological testing for these endpoints. The metabolites assigned to group 1 do all contain the pyrimethanil core structure just with additional hydroxylations and oxidations as modifications by phase II enzymes. Since these metabolites were *i.* structurally similar to the parent with respect to the molecule and *ii.* phase I and II reactions are usually associated with a gain in polarity and hydrophilicity leading to faster excretion, all metabolites of group 1 are covered by toxicological testing of pyrimethanil.

Thus, the **ADI of 0.17 mg/kg b.w.** calculated for parent is also valid for all metabolites of this group and no further testing is required.

Group 2: Cleavage products pyrimidine-moiety hydroxylates and conjugates

Group 2 comprises cleavage products of pyrimethanil which contain the dimethyl pyrimidine moiety in their molecular structure, all occurring in plants.

An acute oral toxicity study on rat metabolite M605F007 (AN7) with an LD_{50} of 735 mg/kg has already been evaluated for Annex I inclusion of pyrimethanil. Further modifications like deamination, ring hydroxylation or sidechain hydroxylation with subsequent sugar conjugation result in die other metabolites assigned to group 2 (i.e. M605F008, M605F016, M605F032, M605F033, respectively).

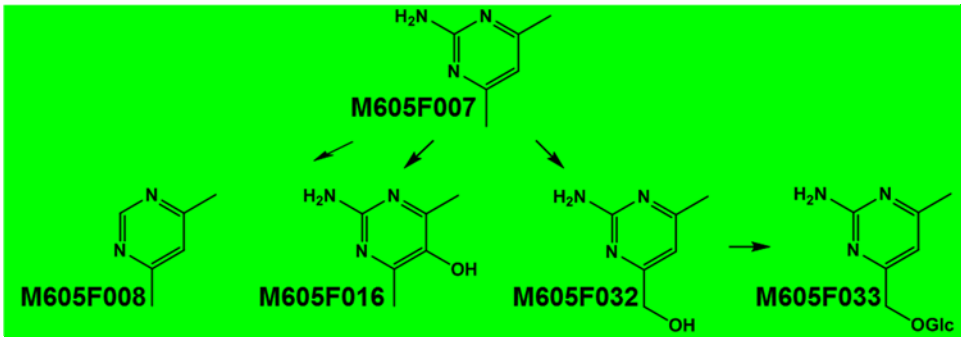


Figure 5.8.1-2: Metabolites of pyrimethanil assigned to group 2.

Threshold of toxicological concern (TTC) concept for toxicological relevance assessment

The TTC concept comprises an approach to assess whether chemical structures are of concern, for which no or only limited information on the toxicological profile is available. A TTC value is a human exposure threshold value for a chemical of unknown toxicity below which there is no appreciable risk to health following oral exposure for a lifetime. The use of the TTC principle eliminates the necessity of extensive toxicity testing (in particular animal testing) and safety evaluations when human intakes of a chemical are below a certain level of concern.
The TTC concept as initially developed [Cramer et al 1978a, see KCA 5.8.1/5 1978/1001324]; [Munro et al 1996a, see KCA 5.8.1/6 1996/1005180]; [Kroes et al. 2004a, see KCA 5.8.1/7 2004/1036074] concluded on structurally derived threshold values based on the 5th percentile of the underlying database. For genotoxic compounds of the structural Cramer class III (the class relevant for almost all organic structures to be considered in plant protection safety evaluation is 0.0025 µg/kg bw/day for presumed genotoxic compounds and 1.5 µg/kg bw/day for non-genotoxic compounds.
Since the development the TTC-concept has been further extended by validation with additional toxicological databases as summarized in Table 5.8.1-5 below. Thus, the robustness of the derived values and the coverage of further toxicological endpoints like reprotoxicity, neurotoxicity and endocrine modes of action (except for steroids) could be demonstrated.

Table 5.8.1-5: Extension of endpoints and robustness of TTC-concept by additional dataset evaluations

Database	Cramer class III 5th percentile NOAEL	Cramer class III TTC for non-genotoxic compounds	Reference
Original TTC database	0.0005 mmol/kg bw/day or 0.15 mg/kg bw/day	1.5 µg/kg bw/day	Munro et al., 1996 [see KCA 5.8.1/6 1996/1005180]
RepDose Database about 600 substances (repeated dose studies)	0.0016 mmol/kg bw/day		Escher and Mangelsdorf, 2009 [see KCA 5.8.1/8 2009/1132142]
861 industrial chemicals (28- and 90-day studies)	0.8 mg/kg bw/day	8 mg/kg bw/day	Kalkhof et al. 2012 [see KCA 5.8.1/9 2012/1365802]
328 pesticides incl. 43 carbamates and organophosphates	0.20 mg/kg bw/day	2.0 µg/kg bw/day	Feigenbaum et al 2015 [see KCA 5.8.1/10 2015/1182409]
91 chemicals and 507 pharmaceuticals Endpoints fertility and developmental toxicity **Safety factor of 1000**	Developmental toxicity: 1 mg/kg bw/day Fertility: 1.5 mg/kg bw/day	Developmental toxicity: 1 µg/kg bw/day Fertility: 1.5 µg/kg bw/day	Bernauer et al 2008 [see KCA 5.8.1/11 2008/1102896]
111 chemicals Endpoint Developmental toxicity in rats **Safety factor of 500**	4 mg/kg bw/day	8 µg/kg bw/day	Van Ravenzwaay et al 2011 [see KCA 5.8.1/12 2011/1295931]
104 chemicals Endpoint Developmental toxicity in rabbits **Safety factor of 500**	2 mg/kg bw/day	4 µg/kg bw/day	Van Ravenzwaay et al. 2012 [see KCA 5.8.1/13 2012/1365804]
300 substances Endpoint Developmental and reproduction toxicity	0.31 mg/kg bw/day	3 µg/kg bw/day	Laufersweiler et al. 2012 [see KCA 5.8.1/14 2012/1365803]

The TTC-concept has meanwhile been considered in the EU e.g. for the evaluation of chemicals under the REACH regulation [ECHA (2012) Guidance on information requirements and chemical safety assessment Chapter R.8: Characterization of dose [concentration]-response for human health] and has been employed or considered for the evaluation of food flavorings [EFSA, 2010e. Guidance on the data required for the risk assessment of flavorings to be used in or on foods. European Food Safety Authority. The EFSA Journal 8(6): 1623. http://www.efsa.europa.eu/en/efsajournal/doc/1623.pdf] or pesticide degradation products by EFSA [EFSA, 2012b. Scientific Opinion: Exploring options for providing preliminary advice about possible human health risks based on the concept of Threshold of Toxicological Concern (TTC). European Food Safety Authority (EFSA) Scientific Committee, Parma, Italy; EFSA Journal, 10(7), 2750 and EFSA, 2012a. Scientific opinion on evaluation of the toxicological relevance of pesticide metabolites for dietary risk assessment. EFSA Panel on Plant Protection Products and their Residues (PPR), European Food Safety Authority (EFSA), Parma, Italy; EFSA Journal, 10(07), 2799].

The proposed threshold levels for pesticide metabolites are 0.0025 µg/kg bw/day for potentially genotoxic compounds and 1.5 µg/kg bw/day for non-genotoxic Cramer Class III compounds. Thus, where applicable for the grouping approach and the subsequent consumer exposure assessment the TTC concept was taken into account.

The exposure thresholds provided in the SANCO guidance document of 2003 (*SANCO/221/2000 –rev.10- final 25 February 2003; ASB2012-3097*) are based on the threshold of toxicological concern (TTC) concept (*Cramer et al., 1978, CHE2006-1120; Kroes et al., 2004, TOX2004-1275; Munro et al., 1996, TOX2004-1274*), which has been further developed since then and steadily updated. Consequently, in the group evaluations below, these developments have been taken into consideration for the overall relevance assessment. The European Food Safety Authority (EFSA) has in its recent evaluation on the TTC concept concluded that the TTC values based on Cramer classes are protective against systemic endpoints such as reproduction and developmental toxicity (*EFSA, 2012b. Scientific Opinion: Exploring options for providing preliminary advice about possible human health risks based on the concept of Threshold of Toxicological Concern (TTC). European Food Safety Authority (EFSA) Scientific Committee, Parma, Italy; EFSA Journal, 10(7), 2750, (ASB2015-2796) and its applicability on the assessment of plant metabolites (EFSA, 2012a, Scientific opinion on evaluation of the toxicological relevance of pesticide metabolites for dietary risk assessment, EFSA Panel on Plant Protection Products and their Residues (PPR), European Food Safety Authority (EFSA), Parma, Italy; EFSA Journal, 10(07), 2799 (ASB2012-10281)*).

For systemic toxicity assessment of group 2 metabolites, the threshold of toxicological concern concept (TTC) was applied. A TTC value is a human exposure threshold value for a chemical of unknown toxicity, below which there is no appreciable risk to health following oral exposure for a lifetime. The use of the TTC principle eliminates the necessity of extensive toxicity testing (in particular animal testing) and safety evaluations when human intakes of a chemical are below a certain level of concern.

To further substantiate that the metabolites of pyrimethanil bear no concern for systemic toxicity, an exposure estimate summing up all non-tested metabolites were evaluated in relation to the threshold of toxicological concern for chronic systemic exposure. It is assumed that the non-relevant rat and plant metabolites of group 2 would be assigned to Cramer class III (most toxic group), for which the TTC trigger for systemic exposure is 90 µg/person.

Table 5.8.1-6: Summary table of all group 2 metabolites and the contribution to chronic dietary risk.

Metabolites	**Contribution**	**Exposure** [µg/person per day]
M605F007	Rotational crop	5.49 (metabolism data)
		8.46 (field data)
M605F008	Rotational crop	2.97
M605F016	Rotational crop	3.24
M605F032	Rotational crop	1.89
M605F033	Plant	50.67
	Rotational crop	8.1
	Overall	53.73
Sum [µg/person per day]		67.32 (metabolism data)
		70.29 (field data)
Sum TTC systemic exposure Cramer Class III		**74.8%** (metabolism data)
		78.1% (field data)

The calculated exposure of the sum of the group 2 metabolites (worst-case assumption) is 70.3 µg/person. Thus, the sum of metabolites covers 74.8% (worst-case assumption) of the TTC value so that the estimates for the plant metabolites of group 2 are well below the TTC trigger for systemic toxicity of Cramer class 3 molecules. More details on exposure values are listed in section M-CA 6.9.

The metabolites of group 2 are in sum well below the TTC trigger for systemic toxicity of 90 µg/person and are thus without further concern.

Group 3: Cleavage product with phenyl-moiety

Only M605F025 was assigned to group 3, which is the only cleavage product containing the phenyl moiety. M605F025 was tested in a 90-day oral toxicity study in rats with M605F025, which is described in more detail in the following sections. Based on the NOAEL 300 ppm (17.8 mg/kg bw/day) of this study, an **ADI of 0.089 mg/kg b.w.** was derived for M605F025 using a safety factor of 100 and an additional extrapolation factor of 2 (subchronic to chronic).

Parent substance: pyrimethanil

Structural alerts for pyrimethanil

Metabolite	QSAR tools applied	Structural alerts for genotoxicity (Pyrimethanil)	Structural alerts for genotoxicity (in silico generated metabolites)	Reliability	Evaluation
Pyrimethanil	OE	No alert	Protein binding alert for CA Alert: "Michael addition to the quinoid type structures N-Substituted Aromatic Amines"	Low	Alerts not confirmed by genotoxicity testing conducted
	OA	Ames Not mutagenic	Ames Limited structural alert for elastogenicity of one derivative "Quinoneimines"	Low	
		CA in vitro Not genotoxic	CA Limited structural alert for elastogenicity of one derivative "Quinoneimines"	Low	
	VE	Ames mutagenic in CAESAR module	-	Reasonable	
		Ames not mutagenic in SarPy and Toxtree modules	-	Low	

OE = OECD-toolbox
OA = OASIS-times
DE = DEREK
VE = Vega
CA = Chromosomal aberration

Parent compound	QSAR tools applied	Structural alerts for genotoxicity	Prediction on genotoxicity	Reliability	Evaluation
Pyrimethanil	CU	Ames Limited structural alert "Dimethylpyrimidine"	Ames Not mutagenic / Inconclusive / Known negative	High (In Domain)	WoE: non-genotoxic
		in vivo MNT Limited structural alert "Phenyl"	in vivo MNT Not genotoxic / Known negative	High (In Domain)	
	OE incl. read across	-	Ames: experimental data available	High (In Domain)	non-genotoxic
		-	CA/MNT: experimental data available	High (In Domain)	
	OE	Protein binding alert for CA Alert: "Michael addition to the quinoid type structures N-Substituted Aromatic Amines"	No alert	- (No Domain definition)	alerts overruled by experimental data
	OA	Ames Limited structural alert for clastogenicity of one derivative "Quinoneimines"	Ames Mutagenic	Low (Out of Domain)	prediction overruled by experimental data
		CA Limited structural alert for clastogenicity of one derivative "Quinoneimines"	CA in vitro Genotoxic	Low (Out of Domain)	
	VE	-	Ames mutagenic in CAESAR module	Reasonable by QSAR, low by WoE	prediction overruled by experimental data
		-	Ames not mutagenic in SarPy and Toxtree modules	Low (Out of Domain)	non-genotoxic

CU = CASE Ultra; OE = OECD Toolbox; OA = OASIS-times; VE = Vega; CA = Chromosomal aberration; MNT = Micronucleus Test; WoE = weight of evidence

The CASE Ultra analysis revealed a negative prediction for the bacterial mutagenicity by several modules: the Ames Konsolidator, Expert model, E. coli trained and S. thyphimurium trained and untrained models, but an inconclusive prediction by E. coli untrained module [see KCA 5.8.1/51 2017/1100634]. The probability of the positive prediction in the later module is 46.6% with a model's probability threshold of 50% and a grey zone in the range of 40% to 60%. However, the structural alert "Dimethylpyrimidine" underlying the positive prediction was rejected due to existing experimental data with negative outcome.
A negative prediction for in vivo chromosomal aberration was obtained in the MNT mice model. Available experimental data on the test item were used to train the model, and thus the trained model versions revealed a "known negative" outcome, albeit a limited structural alert "Phenyl" with a positive prediction probability of 21.9% (model's probability threshold: 50%) was obtained by the in vivo MNT mice model trained on EFSA and BASF data sets [see KCA 5.8.1/52 2017/1100635]. However, this structural alert "Phenyl" was rejected due to existing experimental data with negative outcome. All CASE Ultra predictions were of high reliability (i.e. *in domain*).

OASIS Times [see molecule 7 of report KCA 5.8.1/2 2014/1326432] predicted pyrimethanil to be not mutagenic in the Ames test without metabolic activation with the limitation that the molecule was out of the prediction domain. An in silico generated metabolite was predicted positive yielding "Quinoneimines" as structural alert; however again the prediction was out of the total domain for this model.
In the module for chromosomal aberration [see KCA 5.8.1/3 2015/1186246] the prediction was negative for the parent substance pyrimethanil. The overall prediction was positive as the structural alert "Quinoneimines" was received for an in silico generated metabolite; however, the prediction was out of the total domain for this model.

The VEGA prediction [see KCA 5.8.1/4 2015/1186245] in two out of three modules (TOXTREE, SarPy) was not mutagenic. The reliability of these predictions was low in both modules. The CAESAR module resulted in a positive prediction for mutagenicity (Ames). The prediction was reliable and the compound was in the model applicability domain, however the structural similarity was limited. This result although considered reliable is however not reflected by the toxicological testing of pyrimethanil.

~~Information from OECD Toolbox [see KCA 5.8.1/1 2015/1198463] revealed no alerts for DNA binding or genotoxicity as evaluated in various modules.~~
For the modified OECD Toolbox including read across, available experimental data on pyrimethanil were used to train the model for increasing predictivity and reliability.
Information from the profiling approach as performed with the previous version of the OECD Toolbox [see DocID 2015/1198463] revealed no alerts for DNA binding or genotoxicity as evaluated in various modules.

In conclusion, regarding Ames mutagenicity there was an alert in one of the set of employed tools while the other tools predicted a negative result. Regarding chromosomal aberration structure activity evaluation tools employed there was no alert for pyrimethanil itself but a limited alert for a degradate of pyrimethanil in vitro.

These predictions are considered of no relevance, as a full set of in vitro and in vivo genotoxicity tests is available for pyrimethanil, which indicate that pyrimethanil has no genotoxic properties [see CA 5.4].

Grouping proposal for pyrimethanil metabolites

Based on the above given considerations the following grouping was introduced:

Group 1: Hydroxylation/oxidation products of pyrimethanil and their conjugates

The following metabolites were assigned to that group:

- M605F002 (former AN2)
- M605F003 (former AN3)
- M605F004 (former AN4)
- M605F005 (former AN5)
- M605F006 (former AN6)
- M605F034 (Pyrimethanil-Carboxylic-Acid)
- O-bound sugar conjugates:
  - M605F001 (conjugate of M605F002)
  - M605F027 (conjugate of M605F004)
  - M605F029 (conjugate of M605F005)
  - M605F030 (conjugate of M605F003)
  - M605F038 (conjugate of M605F004)
  - M605F039
  - M605F041 (conjugate of M605F004)
- O-bound sugar-malonyl conjugates :
  - M605F028 (conjugate of M605F004)
  - M605F036 (conjugate of M605F003)
  - M605F037 (conjugate of M605F002)
  - M605F040
- Glucuronide conjugates:
  - M605F014 (conjugate of M605F004)
  - M605F020 (conjugate of M605F003)
  - M605F023 (conjugate of M605F002)
- Sulfates:
  - M605F021 (conjugate of M605F003)
  - M605F035 (conjugate of M605F002)

Group 2: Cleavage products pyrimidin-moiety hydroxylates and conjugates

The following metabolites were assigned to that group:

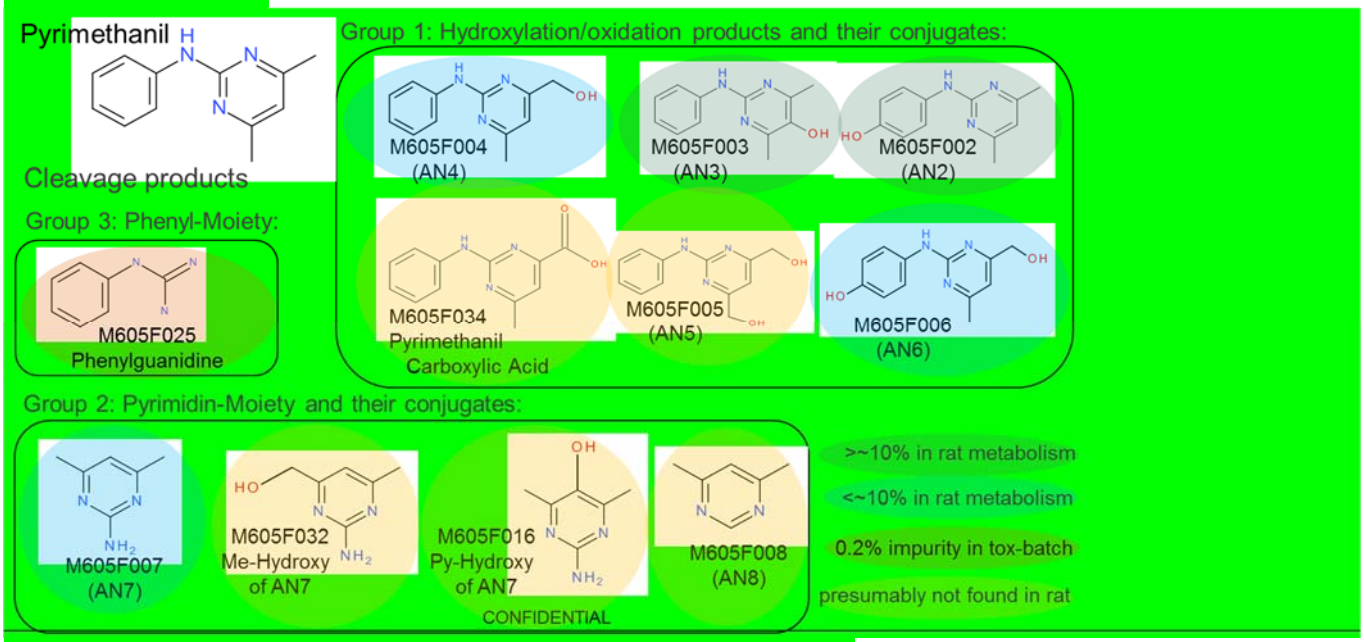
- M605F007 (former AN7)
- M605F008 (former AN8)
- M605F016
- M605F032
- O-bound sugar conjugate:
  - M605F033 (conjugate of M605F032)

Group 3: Cleavage products phenyl-moiety

The following metabolite was assigned to that group:

- M605F025

Figure 5.8.1-1: Basic structures of grouping proposal for pyrimethanil metabolites



Note: the concurrent conjugates are not shown but are discussed in detail below

TOXICOLOGICAL ASSESSMENT OF METABOLITES

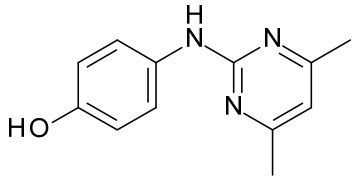
Group 1: Hydroxylation/oxidation products of pyrimethanil and their conjugates

This group comprises of the single or double hydroxylation products of pyrimethanil namely M605F002, M605F003, M605F004, M605F005, M605F006. Also the conjugates of these hydroylates are subsummarized in this group i.e. M605F001, M605F014, M605F020, M605F021, M605F023, M605F027, M605F028, M605F029, M605F030, M605F035, M605F036, M605F037, M605F038, M605F039, M605F040 and M605F041. The O-bound sugar and sugar-malonyl conjugate plant metabolites are expected to be cleaved in mammalian gastrointestinal tract to the corresponding predecessor hydroxylates, which are assessed as primary relevant structures. In addition the further oxidized pyrimethanil-carboxylic acid (M605F034) is also considered a member of this group.

With regard to the rat metabolism investigations [see MCA 5.1] in urine, the respective studies identified the metabolites M605F002 and its further downstream metabolites (conjugation, further hydroxylation and subsequent conjugation) in sum with up to 39.2% in the peer-reviewed phenyl label study and up to 43.9% in the new pyrimidinyl label study. M605F003 and its conjugates were found in sum up to 7.9% in the phenyl label study and up to 14.2% in the pyrimidinyl label study. M605F004 and conjugation metabolites are in sum up to 2.3% in the phenyl label study and up to 11.0% in the pyrimidinyl label study. M605F017 the double hydroxylated derivative of M605F002 or M605F003 respectively and conjugation metabolites are in sum up to 5.0% in the phenyl label study and up to 17.8% in the pyrimidinyl label study. Phase I metabolite M605F006 was found in the peer-reviewed study only with up to 5.7%. Phase II metabolite M605F026 was found in the new study only with up to 9.6%.

Overall the metabolites of this major transformation steps (hydroxylation and subsequent conjugation - group 1, see CA5.1.2 and 5.8) were found in rat urine in a range of 17.4% to 45.7% in the phenyl label study and in range of 53.7% to 75.2% in the pyrimidinyl label study and thus considered to be well covered by the toxicological profile of the parent molecule pyrimethanil.

Metabolite M605F002 (former assigned AN2)



M605F002 is a metabolite of pyrimethanil that was determined in rats, livestock, plants and rotational crops. Furthermore, three conjugates of M605F002 were identified, occurring either in rat and livestock or plant. In the following the toxicological relevance of M605F002 and its conjugates M605F001, M605F023 and M605F037 are evaluated.

A Structural alerts for M605F002

Metabolite	QSAR tools applied	Structural alerts for genotoxicity	Reliability	Evaluation
M605F002	OE	No alert	Low	By weight of evidence not genotoxic
	OA	Ames No other alert than parent	Low	
		CA No other alert than parent	Low	
	VE	Not mutagenic	Low	

OE = OECD-toolbox; OA = OASIS-times; VE = Vega
CA = Chromosomal aberration

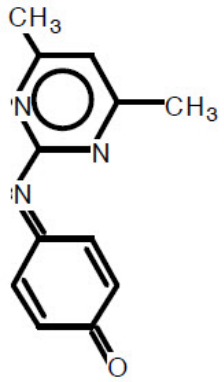
Metabolite	QSAR tools applied	Structural alerts for genotoxicity	Prediction on genotoxicity	Reliability	Evaluation
M605F002	CU	Ames No other alert than parent	Ames Not mutagenic / Inconclusive / Known negative	High (In Domain)	WoE: non-genotoxic
		in vivo MNT Limited structural alert "Hydroxyphenyl"	in vivo MNT Not genotoxic / Known negative	High (In Domain)	
	OE incl. read across	-	Ames: Known negative - covered by pyrimethanil	High (In Domain)	non-genotoxic
		-	CA/MNT: Known negative - covered by pyrimethanil	High (In Domain)	
	OE	No alert	No alert	- (No Domain definition)	non-genotoxic
	OA	Ames No other alert than parent	Ames Mutagenic	Low (Out of Domain)	WoE: non-genotoxic
		CA No other alert than parent	CA in vitro Genotoxic	Low (Out of Domain)	
	VE	-	Ames Mot mutagenic	Low (Out of Domain)	non-genotoxic

CU = Case Ultra; OE = OECD-toolbox; OA = OASIS-times; VE = Vega
CA = Chromosomal aberration; MNT = Micronucleus Test; WoE = weight of evidence

The CASE Ultra analysis revealed a negative prediction for the bacterial mutagenicity by several modules: the Ames Konsolidator, Expert model, E. coli trained and S. thyphimurium trained and untrained models, but an inconclusive prediction by E. coli untrained module based on the same structural alert "Dimethylpyrimidine" that was already obtained for the parent compound [see KCA 5.8.1/51 2017/1100634] and rejected based on available experimental data.
Negative prediction for the chromosomal aberration was obtained for in vivo endpoint by MNT mice models, albeit a limited structural alert "Hydroxyphenyl" with a positive prediction probability of 28.5% (model's probability threshold: 50%) was obtained by the MNT mice model trained on EFSA data set. Due to coverage of the genotoxicity potential of the rat metabolite M605F002 (> 10% in ADME studies) by genotoxicity studies on parent compound, the MNT mice model trained on EFSA and BASF data set revealed a "known negative" outcome. [see KCA 5.8.1/52 2017/1100635]. Therefore, the structural alert "Hydroxyphenyl" was considered as not relevant and rejected. All Case Ultra predictions were of high reliability.

OASIS Times [see molecule 1 of report KCA 5.8.1/2 2014/1326432] predicted M605F002 to be not mutagenic in the Ames test without metabolic activation with the limitation that the molecule was out of the prediction domain. An in silico generated metabolite was predicted positive yielding "Quinoneimines" as structural alert. This prediction is not considered reliable as i) the molecule was out of the prediction domain, and ii) this structural alert was also identified for the parent molecule pyrimethanil [see Figure 5.8.1-2 below] which gave negative test results in all available in vitro and in vivo genotoxicity tests, and was thus not considered of relevance.
In the CA tool [see molecule 1 of KCA 5.8.1/15 2015/1198645] again the structure itself was predicted negative while an in silico generated metabolite yielding "Quinoneimines" as structural alerts were predicted positive. As the same structural alert was identified for pyrimethanil without being confirmed in the in vitro and in vivo genotoxicity testing conducted the alert was not considered to be of relevance.

Figure 5.8.1-2: Strucutral similarity of QSAR Quinoneimine alert for parent and exemplary metabolites

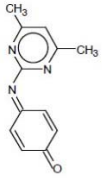


Predicted value: in vitro Ames positive

Predicted value: in vitro CA positive

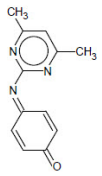
Pyrimethanil-Quinoneimine-Derivative

1.2
Metabolite



Predicted Ames Mutagenicity	in vitro Ames positive
Alert info	Quinoneimines
ModelReliability	High, >= 60% (n>=10)
Total Domain	Out of Domain

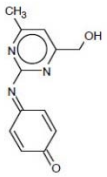
1.2
Metabolite



Predicted CA with S9	in vitro CA positive
Predicted Mechanism	Interaction with DNA &Interactions with topoisomerases / proteins
Alert info	Quinoneimines,alpha,beta-Unsaturated Carbonyls and Related Compounds
ModelReliability	High, >= 60% (n>=10)
Total Domain	N/A

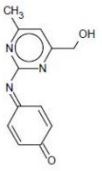
M605F002-Quinonemine-Derivative

3.7
Metabolite



Predicted Ames Mutagenicity	in vitro Ames positive
Alert info	Quinoneimines
ModelReliability	High, >= 60% (n>=10)
Total Domain	Out of Domain

3.7
Metabolite



Predicted CA with S9	in vitro CA positive
Predicted Mechanism	Interaction with DNA &Interactions with topoisomerases / proteins
Alert info	Quinoneimines,alpha,beta-Unsaturated Carbonyls and Related Compounds
ModelReliability	High, >= 60% (n>=10)
Total Domain	N/A

M605F004-Quinonemine-Derivative

The VEGA prediction in all three modules (CAESAR, SarPy and TOXTREE) was not mutagenic for Ames, however the reliability of this prediction was low [see molecule 1 of report KCA 5.8.1/16 2015/1186244].

For the modified OECD Toolbox including read across, M605F002 was used to train the model for increasing predictivity and reliability, due to coverage of the genotoxicity potential of this rat metabolite (> 10% in ADME studies) by genotoxicity studies on parent compound, that were clearly negative.
Information from OECD Toolbox (previous version v3.3) revealed no alerts for DNA binding or genotoxicity (in vitro and in vivo) as evaluated in various modules [see KCA 5.8.1/1 2015/1198463].

In conclusion, in the structure activity evaluation tools employed there was a limited alert for genotoxicity, which is considered of no relevance as the same structural alert was identified for pyrimethanil without being confirmed in the in vitro and in vivo genotoxicity testing conducted.

B Toxicity studies for M605F002

Studies evaluated in the draft monograph of rapporteur member state Austria of April, 2004: No toxicity studies were evaluated during Annex I inclusion of pyrimethanil.

New toxicity studies not already evaluated in the draft monograph of rapporteur member state Austria of April, 2004: No new studies are available.

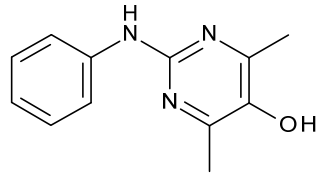
C Toxicological evaluation of metabolite M605F002 and conjugates M605F001, M605F023 and M605F037

The hydroxylated metabolites M605F002, M605F003, M605F004 and M605F006 and further hydroxylates and conjugates thereof add up in the rat metabolism of pyrimethanil in urine in a range of 17.4% to 45.7% in the phenyl label study and in range of 53.7% to 75.2% in the pyrimidinyl label study and are thus considered to be well covered by the toxicological profile of the parent molecule pyrimethanil.

The structural alert for mutagenicity in silico identified for M605F002 was the same structural alert as for the parent substance pyrimethanil. However, in various in vitro and in vivo genotoxicity tests pyrimethanil proved to be non-genotoxic [for details refer to section CA 5.4]. Moreover, toxicity evaluation of M605F002 in vivo is covered by studies performed with pyrimethanil based on the fact that the metabolite and further hydroxylates and conjugates thereof were found in the rat metabolism to a significant extend. As excretion products, the conjugated M605F002 metabolites are considered to be not more toxic than the parent M605F002 when comparing the chemical structure.

Thus, M605F002 and the conjugates thereof (M605F001, M605F023 and M605F037) are considered to be of no toxicological relevance and the ADI derived for pyrimethanil can be applied to this metabolite as well.

Metabolite M605F003 (former assigned AN3)



M605F003 is a metabolite of pyrimethanil that was determined in plants, rotational crops, livestock and rats. Furthermore, three conjugates of M605F003 were identified, occurring either in livestock, plant and/or rotational crop. In the following the toxicological relevance of M605F003 and its conjugates M605F020, M605F030, and M605F036 are evaluated.

A Structural alerts for M605F003

Metabolite	QSAR tools applied	Structural alerts for genotoxicity	Reliability	Evaluation
M605F003	OE	Alert for MN in vivo in ISS module No alert for MNT, Ames, CA in OASIS module	Low	By weight of evidence not genotoxic
	OA	Ames No other alert than parent	Low	
		CA No other alert than parent	Low	
	VE	Not mutagenic	Low	

OE = OECD toolbox; OA = OASIS times; VE = Vega
CA = Chromosomal aberration; MN = Micronuclei

Metabolite	QSAR tools applied	Structural alerts for genotoxicity	Prediction on genotoxicity	Reliability	Evaluation
M605F003	CU	Ames No alerts	Ames Not mutagenic	High (In Domain)	non-genotoxic
		in vivo MNT No other alert than parent	in vivo MNT Not genotoxic	High (In Domain)	
	OE incl. read across	Ames No alerts	Ames Not mutagenic	High (In Domain)	non-genotoxic
		MNT/CA No alert	MNT/CA Not genotoxic	High (In Domain)	
	OE	Alert for MN in vivo in ISS module No alert for MNT, Ames, CA in OASIS module	Ames Not mutagenic MNT Inconclusive	- (No Domain definition)	WoE: non-genotoxic
	OA	Ames No other alert than parent	Ames Mutagenic	Low (Out of Domain)	WoE: non-genotoxic
		CA No other alert than parent	CA in vitro Genotoxic	Low (Out of Domain)	
	VE	-	Ames Not mutagenic i	Low (Out of Domain)	non-genotoxic

CU = Case Ultra; OE = OECD-toolbox; OA = OASIS-times; VE = Vega
CA = Chromosomal aberration; MNT = Micronucleus Test; WoE = weight of evidence

The CASE Ultra analysis revealed a negative prediction for the bacterial mutagenicity by all modules: the Ames Konsolidator, Expert model, untrained and trained E. coli and S. thyphimurium models [see KCA 5.8.1/51 2017/1100634].
Negative prediction for the chromosomal aberration was obtained for in vivo endpoint by MNT mice models, albeit a limited structural alert “Phenyl” with a positive prediction probability of 21.9% (model’s probability threshold: 50%) was obtained by the MNT mice model trained on EFSA and BASF data sets [see KCA 5.8.1/52 2017/1100635]. Furthermore, the same alert was obtained for the parent compound – pyrimethanil, and rejected based on available experimental data.All Case Ultra predictions were of high reliability.

OASIS Times [see molecule 2 of report KCA 5.8.1/2 2014/1326432] predicted M605F003 to be not mutagenic in the Ames test without metabolic activation with the limitation that the molecule was out of the prediction domain. One in silico generated metabolite was predicted positive yielding "Quinoneimines" as structural alert. This prediction is not considered reliable as i) the molecule was out of the prediction domain, and ii) this structural alert was also identified for the parent molecule pyrimethanil which gave negative test results in all available in vitro and in vivo genotoxicity tests, and was thus not considered of relevance.
In the CA tool [see molecule 2 of KCA 5.8.1/15 2015/1198645] again the structure itself was predicted negative while an in silico generated metabolite yielding "Quinoneimines" as structural alerts was predicted positive. As the same structural alert was identified for pyrimethanil without being confirmed in the in vitro and in vivo genotoxicity testing conducted the alert was not considered to be of relevance.

The VEGA prediction in all three modules (CAESAR, SarPy and TOXTREE) was not mutagenic; however the reliability of this prediction was low [see molecule 2 of report KCA 5.8.1/16 2015/1186244].

Negative prediction was obtained for the Ames [see KCA 5.8.1/53 2017/1100632] and in vivo Micronucleus [see KCA 5.8.1/54 DocID 2017/1100633] endpoints by the modified OECD-toolbox based on read across with parent compound pyrimethanil and/or its metabolites M605F007, M605F025 M605F002; M605F019, M605F021 and M605F023. Due to this modification, both predicted endpoints were "in the domain".
Information from OECD Toolbox (previous version v3.3) revealed no alerts for DNA binding or genotoxicity as evaluated in various modules, including OASIS DNA alerts for Ames, MN, and CA. [see KCA 5.8.1/1 2015/1198463]. The alert "H-acceptor-path3-H-acceptor" for in vivo mutagenicity (Micronucleus) was received from the ISS module. However positive predictivity varies within the ISS module and is rather low for the structural alert SA_34 ("H-acceptor-path3-H-acceptor") [Benigni et al., 2012, KCA 5.8.1/17 2012/1368942]. This alert explores the possibility that a chemical interacts with DNA and/or proteins via non-covalent binding, such as DNA intercalation or groove-binding [Snyder et al., 2006, KCA 5.8.1/18 2006/1051853]. In the publication from Snyder at al. several molecules were discussed having chromosome aberration properties. The structures of the example molecules are considered **not relevant/relevant with low reliability** for the metabolite of pyrimethanil receiving this alert based on the following points:

- Positive predictivity is rather low for the structural alert SA_34.
- The structures described in Snyder et al. (2006) possess a N-dialkyl moiety in contrast to the metabolite of pyrimethanil.
- Classical intercalating substances have rather high molecular weights (e.g. ethidium bromide).

In conclusion, in the structure activity evaluation tools employed there was a limited alert for mutagenicity, which is considered of no relevance for the in vivo situation.

B Toxicity studies for M605F003

Studies evaluated in the draft monograph of rapporteur member state Austria of April, 2004: No toxicity studies were evaluated during Annex I inclusion of pyrimethanil.

New toxicity studies not already evaluated in the draft monograph of rapporteur member state Austria of April, 2004: No new studies are available.

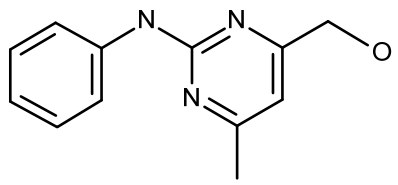
C Toxicological evaluation of metabolite M605F003 and conjugates M605F020, M605F030 and M605F036

The hydroxylated metabolites M605F002, M605F003, M605F004 and M605F006 and further hydroxylates and conjugates thereof add up in the rat metabolism of pyrimethanil in urine in a range of 17.4% to 45.7% in the phenyl label study and in range of 53.7% to 75.2% in the pyrimidinyl label study and are thus considered to be well covered by the toxicological profile of the parent molecule pyrimethanil.

The structural alert for mutagenicity in silico identified for M605F003 was the same structural alert as for the parent substance pyrimethanil or considered of no relevance given the lacking structural similarity. However, in various in vitro and in vivo genotoxicity tests pyrimethanil proved to be non-genotoxic (for details refer to chapter 05.04). For M605F003, which is a hydroxylated metabolite of the parent substance, higher toxicity as for the parent is not assumed. Moreover, toxicity evaluation of M605F003 in vivo is covered by studies performed with pyrimethanil based on the fact that the metabolite and further hydroxylates and conjugates thereof were found in the rat metabolism to a significant extend. As excretion products, the conjugated M605F003 metabolites are considered to be not more toxic than the parent M605F002 when comparing the chemical structure.

Thus, M605F003 and the conjugates thereof (M605F020, M605F030 and M605F036) are considered to be of no toxicological relevance and the ADI derived for pyrimethanil can be applied to this metabolite as well.

Metabolite M605F004 (former assigned AN4)



M605F004 is a metabolite of pyrimethanil that was determined in rats, plants, rotational crop and livestock. Furthermore, four conjugates of M605F004 were identified, occurring either in livestock, plant and/or rotational crop. In the following the toxicological relevance of M605F004 and its conjugates M605F014, M605F027, M605F028 and M605F038 are evaluated.

A Structural alerts for M605F004

Metabolite	QSAR tools applied	Structural alerts for genotoxicity	Reliability	Evaluation
M605F004	OE	Alert for MN in vivo in ISS module No alert for MNT, Ames, CA in OASIS module	Low	By weight of evidence not genotoxic
	OA	Ames No other alert than parent	Low	
		CA No other alert than parent	Low	
	VE	Not mutagenic	Low	

OE = OECD toolbox; OA = OASIS times; VE = Vega
CA = Chromosomal aberration; MN = Micronuclei

Metabolite	QSAR tools applied	Structural alerts for genotoxicity	Prediction on genotoxicity	Reliability	Evaluation
M605F004	CU	Ames No other alert than parent	Ames Not mutagenic / Inconclusive	High (In Domain)	WoE: non-genotoxic
		in vivo MNT No other alert than parent	in vivo MNT Not genotoxic	High (In Domain)	
	OE incl. read across	-	Ames: Not mutagenic	High (In Domain)	non-genotoxic
		-	CA/MNT: Not genotoxic	High (In Domain)	
	OE	Alert for MN in vivo in ISS module No alert for MNT, Ames, CA in OASIS module	Ames Not mutagenic MNT Inconclusive	- (No Domain definition)	WoE: non-genotoxic
	OA	Ames No other alert than parent	Ames Mutagenic	Low (Out of Domain)	WoE: non-genotoxic
		CA No other alert than parent	CA in vitro Genotoxic	Low (Out of Domain)	
	VE	-	Ames Not mutagenic	Low (Out of Domain)	non-genotoxic

CU = Case Ultra; OE = OECD-toolbox; OA = OASIS-times; VE = Vega
CA = Chromosomal aberration; MNT = Micronucleus Test; WoE = weight of evidence

The CASE Ultra analysis revealed a negative prediction for the bacterial mutagenicity by several modules: the Ames Konsolidator, Expert model, E. coli trained and S. thyphimurium trained and untrained models, but an inconclusive prediction by E. coli untrained module based on the same structural alert “Dimethylpyrimidine” that was already obtained for the parent compound [see KCA 5.8.1/51 2017/1100634] and rejected based on available experimental data.
Negative prediction for the chromosomal aberration was obtained for in vivo endpoint by MNT mice models, albeit a limited structural alert “Phenyl” with a positive prediction probability of 21.9% (model’s probability threshold: 50%) was obtained by the MNT mice model trained on EFSA and BASF data sets [see KCA 5.8.1/52 2017/1100635]. Furthermore, the same alert was obtained for the parent compound – pyrimethanil, and rejected based on available experimental data. All Case Ultra predictions were of high reliability.

OASIS Times [see molecule 3 of report KCA 5.8.1/2 2014/1326432] predicted M605F004 to be not mutagenic in the Ames test without metabolic activation with the limitation that the molecule was out of the prediction domain. Some in silico generated metabolites were predicted positive yielding "Quinoneimines" as structural alert. This prediction is not considered reliable as i) the molecule was out of the prediction domain, and ii) this structural alert was also identified for the parent molecule Pyrimethanil which gave negative test results in all available in vitro and in vivo genotoxicity tests, and was thus not considered of relevance.
In the CA tool [see molecule 3 of KCA 5.8.1/15 2015/1198645] again the structure itself was predicted negative while an in silico generated metabolite yielding "Quinoneimines" as structural alerts was predicted positive. As the same structural alert was identified for pyrimethanil without being confirmed in the in vitro and in vivo genotoxicity testing conducted the alert was not considered to be of relevance.

The VEGA prediction in all three modules (CAESAR, SarPy and TOXTREE) was not mutagenic; however the reliability of this prediction was low [see molecule 3 of report KCA 5.8.1/16 2015/1186244].

Negative prediction was obtained for the Ames [see KCA 5.8.1/53 2017/1100632] and in vivo Micronucleus [see KCA 5.8.1/54 DocID 2017/1100633] endpoints by the modified OECD-toolbox based on read across with parent compound pyrimethanil and/or its metabolites M605F007, M605F025 M605F002; M605F019, M605F021 and M605F023. Due to this modification, both predicted endpoints were "in the domain".
Information from OECD Toolbox (previous version v3.3) revealed no alerts for DNA binding or genotoxicity as evaluated in various modules, including OASIS DNA alerts for Ames, MN, and CA [see KCA 5.8.1/1 2015/1198463]. The alert "H-acceptor-path3-H-acceptor" for in vivo mutagenicity (Micronucleus) was received from the ISS module. However positive predictivity varies within the ISS module and is rather low for the structural alert SA_34 ("H-acceptor-path3-H-acceptor") [Benigni et al., 2012, KCA 5.8.1/17 2012/1368942]. This alert explores the possibility that a chemical interacts with DNA and/or proteins via non-covalent binding, such as DNA intercalation or groove-binding [Snyder et al., 2006, KCA 5.8.1/18 2006/1051853]. In the publication from Snyder at al. several molecules were discussed having chromosome aberration properties. The structures of the example molecules are considered **not relevant/relevant with low reliability** for the metabolite of pyrimethanil receiving this alert based on the following points:

- Positive predictivity is rather low for the structural alert SA_34.
- The structures described in Snyder et al. (2006) possess a N-dialkyl moiety in contrast to the metabolite of pyrimethanil.
- Classical intercalating substances have rather high molecular weights (e.g. ethidium bromide).

In conclusion, in the structure activity evaluation tools employed there was a limited alert for mutagenicity, which is considered of no relevance for the in vivo situation.

B Toxicity studies for M605F004

Studies evaluated in the draft monograph of rapporteur member state Austria of April, 2004: No toxicity studies were evaluated during Annex I inclusion of pyrimethanil.

New toxicity studies not already evaluated in the draft monograph of rapporteur member state Austria of April, 2004: No new studies are available.

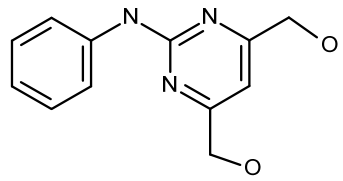
C Toxicological evaluation of metabolite M605F004 and conjugates M605F014, M605F027, M605F028 and M605F038

The hydroxylated metabolites M605F002, M605F003, M605F004 and M605F006 and further hydroxylates and conjugates thereof add up in the rat metabolism of pyrimethanil in urine in a range of 17.4% to 45.7% in the phenyl label study and in range of 53.7% to 75.2% in the pyrimidinyl label study and are thus considered to be well covered by the toxicological profile of the parent molecule pyrimethanil.

The toxicological alert for mutagenicity in silico identified for M605F004 was the same structural alert as for the parent substance pyrimethanil. However, in various in vitro and in vivo genotoxicity tests pyrimethanil proved to be non-genotoxic (for details refer to chapter 05.04). Moreover, toxicity evaluation of M605F004 in vivo is covered by studies performed with pyrimethanil based on the fact that the metabolite and further hydroxylates and conjugates thereof were found in the rat metabolism to a significant extend. As excretion products, the conjugated M605F004 metabolites are considered to be not more toxic than the parent M605F004 when comparing the chemical structure.

Thus, M605F003 and the conjugates thereof (M605F014, M605F027, M605F028 and M605F038) are considered to be of <u>no</u> toxicological relevance and the ADI derived for pyrimethanil can be applied to this metabolite as well.

Metabolite M605F005 (former assigned AN5)



M605F005 is a double hydroxylated metabolite of pyrimethanil occurring in soil and rotational crop. Furthermore, one conjugate of M605F005 was identified, occurring in plants. In the following the toxicological relevance of M605F005 and its conjugate M605F029 are evaluated.

A Structural alerts for M605F005

Metabolite	QSAR tools applied	Structural alerts for genotoxicity	Reliability	Evaluation
M605F005	OE	Alert for MN in vivo in ISS module No alert for MNT, Ames, CA in OASIS module	Low	By weight of evidence not genotoxic
	OA	Ames No other alert than parent	Low	
		CA No other alert than parent	Low	
	VE	Not mutagenic	Low	

OE = OECD-toolbox; OA = OASIS-times; VE = Vega
CA = Chromosomal aberration; MN = Micronuclei

Metabolite	QSAR tools applied	Structural alerts for genotoxicity	Prediction on genotoxicity	Reliability	Evaluation
M605F005	CU	Ames No alerts	Ames Not mutagenic	High (In Domain)	WoE: non-genotoxic
		in vivo MNT No other alert than parent	in vivo MNT Not genotoxic	High (In Domain)	
	OE incl. read across	Ames No alerts	Ames Not mutagenic	High (In Domain)	non-genotoxic
		MNT/CA No alert	MNT/CA Not genotoxic	High (In Domain)	
	OE	Alert for MN in vivo in ISS module No alert for MNT, Ames, CA in OASIS module	Ames Not mutagenic MNT Inconclusive	- (No Domain definition)	WoE: non-genotoxic
	OA	Ames No other alert than parent	Ames Mutagenic	Low (Out of Domain)	WoE: non-genotoxic
		CA No other alert than parent	CA in vitro Genotoxic	Low (Out of Domain)	
	VE	-	Ames Not mutagenic	Low (Out of Domain)	non-genotoxic

CU = Case Ultra; OE = OECD-toolbox; OA = OASIS-times; VE = Vega
CA = Chromosomal aberration; MNT = Micronucleus Test; WoE = weight of evidence

The CASE Ultra analysis revealed a negative prediction for the bacterial mutagenicity by all modules: the Ames Konsolidator, Expert model, untrained and trained E. coli and S. thyphimurium models [see KCA 5.8.1/51 2017/1100634].
Negative prediction for the chromosomal aberration was obtained for in vivo endpoint by MNT mice models, albeit a limited structural alert “Phenyl” with a positive prediction probability of 21.9% (model’s probability threshold: 50%) was obtained by the MNT mice model trained on EFSA and BASF data sets [see KCA 5.8.1/52 2017/1100635]. Furthermore, the same alert was obtained for the parent compound – pyrimethanil, and rejected based on available experimental data.All Case Ultra predictions were of high reliability.

OASIS Times [see molecule 4 of report KCA 5.8.1/2 2014/1326432] predicted M605F005 to be not mutagenic in the Ames test without metabolic activation but with the limitation that the molecule was out of the prediction domain. Some in silico generated metabolites were predicted positive yielding "Quinoneimines" as structural alert. This prediction is not considered reliable as i) the molecule was out of the prediction domain, and ii) this structural alert was also identified for the parent molecule Pyrimethanil which gave negative test results in all available in vitro and in vivo genotoxicity tests, and was thus not considered of relevance.
In the CA tool [see molecule 4 of KCA 5.8.1/15 2015/1198645] again the structure itself was predicted negative while an in silico generated metabolite yielding "Quinoneimines" as structural alerts was predicted positive. As the same structural alert was identified for pyrimethanil without being confirmed in the in vitro and in vivo genotoxicity testing conducted the alert was not considered to be of relevance.

The VEGA prediction in all three modules (CAESAR, SarPy and TOXTREE) was not mutagenic; however the reliability of this prediction was low [see molecule 4 of report KCA 5.8.1/16 2015/1186244].

Negative prediction was obtained for the Ames [see KCA 5.8.1/53 2017/1100632] and in vivo Micronucleus [see KCA 5.8.1/54 DocID 2017/1100633] endpoints by the modified OECD-toolbox based on read across with parent compound pyrimethanil and/or its metabolites M605F007, M605F025 M605F002; M605F019, M605F021 and M605F023. Due to this modification, both predicted endpoints were "in the domain".
Information from OECD Toolbox (previous version v3.3) revealed no alerts for DNA binding or genotoxicity as evaluated in various modules, including OASIS DNA alerts for Ames, MN, and CA. [see KCA 5.8.1/1 2015/1198463]. The alert "H-acceptor-path3-H-acceptor" for in vivo mutagenicity (Micronucleus) was received from the ISS module. However positive predictivity varies within the ISS module and is rather low for the structural alert SA_34 ("H-acceptor-path3-H-acceptor") [Benigni et al., 2012, KCA 5.8.1/17 2012/1368942]. This alert explores the possibility that a chemical interacts with DNA and/or proteins via non-covalent binding, such as DNA intercalation or groove-binding [Snyder et al., 2006, KCA 5.8.1/18 2006/1051853]. In the publication from Snyder at al. several molecules were discussed having chromosome aberration properties. The structures of the example molecules are considered **not relevant/relevant with low reliability** for the metabolite of pyrimethanil receiving this alert based on the following points:

- Positive predictivity is rather low for the structural alert SA_34.
- The structures described in Snyder et al. (2006) possess a N-dialkyl moiety in contrast to the metabolite of pyrimethanil.
- Classical intercalating substances have rather high molecular weights (e.g. ethidium bromide).

In conclusion, in the structure activity evaluation tools employed there was a limited alert for mutagenicity, which is considered of no relevance for the in vivo situation.

B Toxicity studies for M605F005

Studies evaluated in the draft monograph of rapporteur member state Austria of April, 2004: No toxicity studies were evaluated during Annex I inclusion of pyrimethanil.

New toxicity studies not already evaluated in the draft monograph of rapporteur member state Austria of April, 2004: No new studies are available.

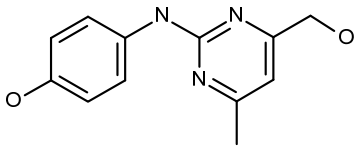
C Toxicological evaluation of metabolite M605F005 and conjugate M605F029

The hydroxylated metabolites M605F002, M605F003, M605F004 and M605F006 and further hydroxylates and conjugates thereof add up in the rat metabolism of pyrimethanil in urine in a range of 17.4% to 45.7% in the phenyl label study and in range of 53.7% to 75.2% in the pyrimidinyl label study and are thus considered to be well covered by the toxicological profile of the parent molecule pyrimethanil.

The structural alert for mutagenicity in silico identified for M605F005 was the same structural alert as for the parent substance pyrimethanil or considered of no relevance given the lacking structural similarity. However, in various in vitro and in vivo genotoxicity tests pyrimethanil proved to be non-genotoxic (for details refer to chapter 05.04). Moreover, toxicity evaluation of M605F005 in vivo is covered by studies performed with pyrimethanil based on the fact that the metabolite and further hydroxylates and conjugates thereof were found in the rat metabolism to a significant extend. As an excretion product, the conjugated M605F005 metabolite is considered to be not more toxic than the parent M605F005 when comparing the chemical structure.

Thus, M605F005 and the conjugate M605F029 are considered to be of <u>no</u> toxicological relevance and the ADI derived for pyrimethanil can be applied to this metabolite as well.

Metabolite M605F006 (AN6)



M605F006 is a double hydroxylated metabolite of pyrimethanil, occurring in rat, livestock, and rotational crops.

A Structural alerts for M605F006

Metabolite	QSAR tools applied	Structural alerts for genotoxicity	Reliability	Evaluation
M605F006	OE	Alert for MN in vivo in ISS module No alert for MNT, Ames, CA in OASIS module	Low	By weight of evidence not genotoxic
	OA	Ames No other alert than parent	Low	
		CA No other alert than parent	Low	
	VE	Not mutagenic	Low	

OE = OECD toolbox; OA = OASIS times; VE = Vega
CA = Chromosomal aberration; MN = Micronuclei

Metabolite	QSAR tools applied	Structural alerts for genotoxicity	Prediction on genotoxicity	Reliability	Evaluation
M605F006	CU	Ames No alerts	Ames Not mutagenic	High (In Domain)	WoE: non-genotoxic
		in vivo MNT Limited structural alert "Hydroxyphenyl"	in vivo MNT Not genotoxic	High (In Domain)	
	OE incl. read across	Ames No alerts	Ames Not mutagenic	High (In Domain)	non-genotoxic
		MNT/CA No alert	MNT/CA Not genotoxic	High (In Domain)	
	OE	Alert for MN in vivo in ISS module No alert for MNT, Ames, CA in OASIS module	Ames Not mutagenic MNT Inconclusive	- (No Domain definition)	WoE: non-genotoxic
	OA	Ames No other alert than parent	Ames Mutagenic	Low (Out of Domain)	WoE: non-genotoxic
		CA No other alert than parent	CA in vitro Genotoxic	Low (Out of Domain)	
	VE	-	Ames Not mutagenic i	Low (Out of Domain)	non-genotoxic

CU = Case Ultra; OE = OECD-toolbox; OA = OASIS-times; VE = Vega
CA = Chromosomal aberration; MNT = Micronucleus Test; WoE = weight of evidence

The CASE Ultra analysis revealed a negative prediction for the bacterial mutagenicity by several modules: the Ames Konsolidator, Expert model, E. coli trained and S. thyphimurium trained and untrained models, but an inconclusive prediction by E. coli untrained module based on the same structural alert "Dimethylpyrimidine" that was already obtained for the parent compound [see KCA 5.8.1/51 2017/1100634] and rejected based on available experimental data.
Negative prediction for the chromosomal aberration was obtained for in vivo endpoint by MNT mice models, albeit a limited structural alert "Hydroxyphenyl" with a positive prediction probability of 28.5% (model's probability threshold: 50%) was obtained by the MNT mice model trained on EFSA data set [see KCA 5.8.1/52 2017/1100635]. However, the same the structural alert "Hydroxyphenyl" was obtained for the known negative metabolite M605F002 but was considered as not relevant and rejected. All Case Ultra predictions were of high reliability.

OASIS Times [see molecule 5 of report KCA 5.8.1/2 2014/1326432] predicted M605F006 to be not mutagenic in the Ames test without metabolic activation but with the limitation that the molecule was out of the prediction domain. Some in silico generated metabolites were predicted positive yielding "Quinoneimines" as structural alert.
This prediction is not considered reliable as i) the molecule was out of the prediction domain, and ii) this structural alert was also identified for the parent molecule Pyrimethanil which gave negative test results in all available in vitro and in vivo genotoxicity tests, and was thus not considered of relevance.
In the CA tool [see molecule 5 of KCA 5.8.1/15 2015/1198645] again the structure itself was predicted negative while an in silico generated metabolite yielding "Quinoneimines" as structural alerts was predicted positive. As the same structural alert was identified for pyrimethanil without being confirmed in the in vitro and in vivo genotoxicity testing conducted the alert was not considered to be of relevance.

The VEGA prediction in all three modules (CAESAR, SarPy and TOXTREE) was not mutagenic; however the reliability of this prediction was low [see molecule 5 of report KCA 5.8.1/16 2015/1186244].

Negative prediction was obtained for the Ames [see KCA 5.8.1/53 2017/1100632] and in vivo Micronucleus [see KCA 5.8.1/54 DocID 2017/1100633] endpoints by the modified OECD-toolbox based on read across with parent compound pyrimethanil and/or its metabolites M605F007, M605F025 M605F002; M605F019, M605F021 and M605F023. Due to this modification, both predicted endpoints were "in the domain".
Information from OECD Toolbox (previous version v3.3) revealed no alerts for DNA binding or genotoxicity as evaluated in various modules, including OASIS DNA alerts for Ames, MN, and CA. [see KCA 5.8.1/1 2015/1198463]. The alert "H-acceptor-path3-H-acceptor" for in vivo mutagenicity (Micronucleus) was received from the ISS module. However positive predictivity varies within the ISS module and is rather low for the structural alert SA_34 ("H-acceptor-path3-H-acceptor") [Benigni et al., 2012, KCA 5.8.1/17 2012/1368942]. This alert explores the possibility that a chemical interacts with DNA and/or proteins via non-covalent binding, such as DNA intercalation or groove-binding [Snyder et al., 2006, KCA 5.8.1/18 2006/1051853]. In the publication from Snyder at al. several molecules were discussed having chromosome aberration properties. The structures of the example molecules are considered **not relevant/relevant with low reliability** for the metabolite of pyrimethanil receiving this alert based on the following points:

- Positive predictivity is rather low for the structural alert SA_34.
- The structures described in Snyder et al. (2006) possess a N-dialkyl moiety in contrast to the metabolite of pyrimethanil.
- Classical intercalating substances have rather high molecular weights (e.g. ethidium bromide).

In conclusion, in the structure activity evaluation tools employed there was a limited alert for mutagenicity, which is considered of no relevance for the in vivo situation.

B Toxicity studies for M605F006

Studies evaluated in the draft monograph of rapporteur member state Austria of April, 2004: No toxicity studies were evaluated during Annex I inclusion of pyrimethanil.

New toxicity studies not already evaluated in the draft monograph of rapporteur member state Austria of April, 2004: No new studies are available.

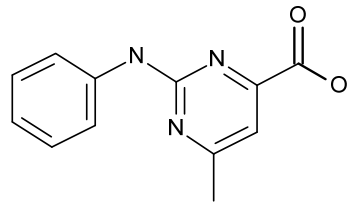
C Toxicological evaluation of metabolite M605F006

The hydroxylated metabolites M605F002, M605F003, M605F004 and M605F006 and further hydroxylates and conjugates thereof add up in the rat metabolism of pyrimethanil in urine in a range of 17.4% to 45.7% in the phenyl label study and in range of 53.7% to 75.2% in the pyrimidinyl label study and are thus considered to be well covered by the toxicological profile of the parent molecule pyrimethanil.

The structural alert for mutagenicity in silico identified for M605F006 was the same structural alert as for the parent substance pyrimethanil or considered of no relevance given the lacking structural similarity. However, in various in vitro and in vivo genotoxicity tests Pyrimethanil proved to be non-genotoxic (for details refer to chapter 05.04).
Moreover, toxicity evaluation of M605F002 in vivo is covered by studies performed with pyrimethanil based on the fact that the metabolite and further hydroxylates and conjugates thereof were found in the rat metabolism to a significant extend. For M605F006, which is a double hydroxylated metabolite of the parent substance, higher toxicity as for the parent is not assumed.

Thus, M605F006 is considered to be of no toxicological relevance and the ADI derived for pyrimethanil can be applied to this metabolite as well.

Metabolite M605F034 (Pyrimethanil Carboxylic Acid)



M605F034 is a livestock metabolite of Pyrimethanil.

A Structural alerts for M605F034

Metabolite	QSAR tools applied	Structural alerts for genotoxicity	Reliability	Evaluation
M605F034	OE	Alert for MN in vivo in ISS module No alert for MNT, Ames, CA in OASIS module	Low	By weight of evidence not genotoxic
	OA	No other alert than parent	Low	
		No other alert than parent	Low	
	VE	Not mutagenic	Low	

OE = OECD toolbox; OA = OASIS-times; VE = Vega
CA = Chromosomal aberration; MN = Micronuclei

Metabolite	QSAR tools applied	Structural alerts for genotoxicity	Prediction on genotoxicity	Reliability	Evaluation
M605F034	CU	Ames No other alert than parent	Ames Not mutagenic	High (In Domain)	WoE: non-genotoxic
		in vivo MNT No other alert than parent	in vivo MNT Not genotoxic	High (In Domain)	
	OE incl. read across	Ames No alerts	Ames Not mutagenic	High (In Domain)	non-genotoxic
		MNT/CA No alert	MNT/CA Not genotoxic	High (In Domain)	
	OE	Alert for MN in vivo in ISS module No alert for MNT, Ames, CA in OASIS module	Ames Not mutagenic MNT Inconclusive	- (No Domain definition)	WoE: non-genotoxic
	OA	Ames No other alert than parent	Ames Mutagenic	Low (Out of Domain)	WoE: non-genotoxic
		CA No other alert than parent	CA in vitro Genotoxic	Low (Out of Domain)	
	VE	-	Ames Not mutagenic	Low (Out of Domain)	non-genotoxic

CU = Case Ultra; OE = OECD-toolbox; OA = OASIS-times; VE = Vega
CA = Chromosomal aberration; MNT = Micronucleus Test; WoE = weight of evidence

The CASE Ultra analysis revealed a negative prediction for the bacterial mutagenicity by several modules: the Ames Konsolidator, Expert model, E. coli trained and S. thyphimurium trained and untrained models, but an inconclusive prediction by E. coli untrained module based on the same structural alert “Dimethylpyrimidine” that was already obtained for the parent compound [see KCA 5.8.1/51 2017/1100634] and rejected based on available experimental data.
Negative prediction for the chromosomal aberration was obtained for in vivo endpoint by MNT mice models, albeit a limited structural alert “Phenyl” with a positive prediction probability of 21.9% (model’s probability threshold: 50%) was obtained by the MNT mice model trained on EFSA and BASF data sets [see KCA 5.8.1/52 2017/1100635]. Furthermore, the same alert was obtained for the parent compound – pyrimethanil, and rejected based on available experimental data. All Case Ultra predictions were of high reliability.

OASIS Times [see KCA 5.8.1/19 2015/1186253] predicted M605F034 to be not mutagenic in the Ames test without metabolic activation with the limitation that the molecule was out of the prediction domain. One in silico generated metabolite was predicted positive yielding "Quinoneimines" as structural alert. This prediction is not considered reliable as i) the molecule was out of the prediction domain, and ii) this structural alert was also identified for the parent molecule pyrimethanil which gave negative test results in all available in vitro and in vivo genotoxicity tests, and was thus not considered of relevance.
In the CA tool [see KCA 5.8.1/20 2015/1186247] again the structure itself was predicted negative while an in silico generated metabolite yielding "Quinoneimines as structural alerts was predicted positive. As the same structural alert was identified for pyrimethanil without being confirmed in the in vitro and in vivo genotoxicity testing conducted the alert was not considered to be of relevance.

The VEGA prediction in all three modules (CAESAR, SarPy, Toxtree) was not mutagenic, with a low to moderate reliability [see molecule 2 of report KCA 5.8.1/4 2015/1186245].

Negative prediction was obtained for the Ames [see KCA 5.8.1/53 2017/1100632] and in vivo Micronucleus [see KCA 5.8.1/54 DocID 2017/1100633] endpoints by the modified OECD-toolbox based on read across with parent compound pyrimethanil and/or its metabolites M605F007, M605F025 M605F002; M605F019, M605F021 and M605F023. Due to this modification, both predicted endpoints were "in the domain".
Information from OECD Toolbox (previous version v3.3) revealed no alerts for DNA binding or genotoxicity as evaluated in various modules, including OASIS DNA alerts for Ames, MN, and CA. [see KCA 5.8.1/1 2015/1198463]. The alert "H-acceptor-path3-H-acceptor" for in vivo mutagenicity (Micronucleus) was received from the ISS module. However positive predictivity varies within the ISS module and is rather low for the structural alert SA_34 ("H-acceptor-path3-H-acceptor") [Benigni et al., 2012, KCA 5.8.1/17 2012/1368942]. This alert explores the possibility that a chemical interacts with DNA and/or proteins via non-covalent binding, such as DNA intercalation or groove-binding [Snyder et al., 2006, KCA 5.8.1/18 2006/1051853]. In the publication from Snyder at al. several molecules were discussed having chromosome aberration properties. The structures of the example molecules are considered **not relevant/relevant with low reliability** for the metabolite of pyrimethanil receiving this alert based on the following points:

- Positive predictivity is rather low for the structural alert SA_34.
- The structures described in Snyder et al. (2006) possess a N-dialkyl moiety in contrast to the metabolite of pyrimethanil.
- Classical intercalating substances have rather high molecular weights (e.g. ethidium bromide).

In conclusion, in the structure activity evaluation tools employed there was a limited alert for mutagenicity, which is considered of no relevance for the in vivo situation.

B Toxicity studies for M605F034

Studies evaluated in the draft monograph of rapporteur member state Austria of April, 2004: No toxicity studies were evaluated during Annex I inclusion of pyrimethanil.

New toxicity studies not already evaluated in the draft monograph of rapporteur member state Austria of April, 2004: No new studies are available.

C Toxicological evaluation of metabolite M605F034

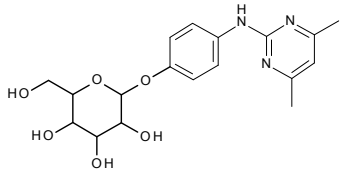
The structural alert for mutagenicity in silico identified for M605F034 was either the same structural alert as for the parent substance pyrimethanil or considered of no relevance given the lacking structural similarity. However, in various in vitro and in vivo genotoxicity tests Pyrimethanil proved to be non-genotoxic (for details refer to chapter 05.04). Moreover, toxicity evaluation of M605F002 in vivo is covered by studies performed with pyrimethanil based on the fact that the metabolite and further hydroxylates and conjugates thereof were found in the rat metabolism to a significant extend.
For M605F034, which is a carboxylated metabolite of the parent substance, higher toxicity as for the parent is not assumed.

Thus, M605F034 is considered to be of no toxicological relevance and the ADI derived for pyrimethanil can be applied to this metabolite as well.

Conjugates of hydroxylation/oxidation products

Except for M605F021, M605F023 and M605F035, ~~N~~no additional QSAR evaluations were performed with the conjugated metabolites. The conjugated metabolites listed below are formed either with endogenous phase II substrates (glucuronic acid, sulfate) or are expected to be cleaved in the mammalian gastrointestinal tract to form endogenous molecules (sugars) that have no toxicologically relevant properties in addition to the predecessor hydroxylates. Therefore, the conjugated molecules are supposed to have the same or less toxicologically relevant properties as the parent molecule. The possibility of hydrolysis is covered by the respective QSAR evaluations for the parent molecules that are discussed above.

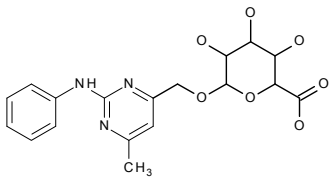
Metabolite M605F001 (former assigned AN1)



Metabolite M605F001 is the β-O-glucoside of M605F002, occurring in plants. The excretion product M605F001 is considered to be not more toxic than the predecessor hydroxylate M605F002, which in turn is considered to be covered by the toxicological testing of pyrimethanil [see "Toxicological evaluation of metabolite M605F002" above].

Thus, M605F001 is considered to be of no toxicological relevance and the ADI derived for pyrimethanil can be applied to this metabolite as well.

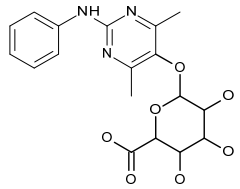
Metabolite M605F014



Livestock metabolite M605F014 is the glucuronide conjugate of M605F004. The excretion product M605F014 is considered to be not more toxic than the predecessor hydroxylate M605F004, which in turn is considered to be covered by the toxicological testing of pyrimethanil [see "Toxicological evaluation of metabolite M605F004" above].

Thus, M605F014 is considered to be of no toxicological relevance and the ADI derived for pyrimethanil can be applied to this metabolite as well.

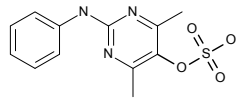
Metabolite M605F020



Livestock and rat metabolite M605F020 is the glucuronide conjugate of M605F003. The excretion product M605F020 is considered to be not more toxic than the predecessor hydroxylate M605F003, which in turn is considered to be covered by the toxicological testing of pyrimethanil [see "Toxicological evaluation of metabolite M605F003" above].

Thus, M605F020 is considered to be of no toxicological relevance and the ADI derived for pyrimethanil can be applied to this metabolite as well.

Metabolite M605F021



Livestock and rat metabolite M605F021 is the sulfate conjugate of M605F003. The excretion product M605F021 is considered to be not more toxic than the predecessor hydroxylate M605F003, which in turn is considered to be covered by the toxicological testing of pyrimethanil [see "Toxicological evaluation of metabolite M605F003" above].

A Structural alerts for M605F021

Metabolite	QSAR tools applied	Structural alerts for genotoxicity	Prediction on genotoxicity	Reliability	Evaluation
M605F021	CU	Ames Alert "Sulfate"	Ames Not mutagenic / Inconclusive	High (In Domain)	WoE: non-genotoxic
		in vivo MNT Alerts "Sulfate" and "Phenyl"	in vivo MNT Mutagenic / Not mutagenic / Known negative	High (In Domain)	
	OE incl. read across	Ames No alert	Ames Known negative - covered by pyrimethanil	High (In Domain)	non-genotoxic
		MNT/CA No alert	MNT/CA Known negative - covered by pyrimethanil	High (In Domain)	

CU = Case Ultra; OE = OECD-toolbox
CA = Chromosomal aberration; MNT = Micronucleus Test; WoE = weight of evidence

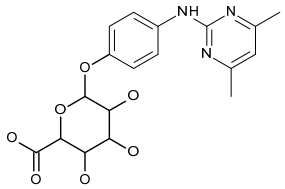
The CASE Ultra analysis revealed a negative prediction for the bacterial mutagenicity by several modules: the Ames Konsolidator as well as trained E. coli and S. thyphimurium moduls, but an inconclusive prediction by Expert as well as untrained E. coli and S. thyphimurium modules based on the structural alert "Sulfate" [see KCA 5.8.1/51 2017/1100634]. The probability of the positive prediction in the untrained S. thyphimurium module is 42.9% with a model's probability threshold of 45% and a grey zone in the range of 40% to 60%. The probability of the positive prediction in the untrained E. coli module is 52.8% with a model's probability threshold of 50% and a grey zone in the range of 40% to 60%. The probability of the positive prediction in the Expert module is 42.8% with a model's probability threshold of 50% and a grey zone in the range of 40% to 60%. However, since the rat metabolite M605F021 (> 10% in ADME studies) is considered to be covered by genotoxicity studies on parent compound that were clearly negative, the structural alert "Sulfate" is considered to be not relevant and is rejected.
Positive prediction for the chromosomal aberration was obtained for in vivo endpoint by untrained MNT mice model with the structural alert "Sulfate", revealing a positive prediction probability of 64.3% (model's probability threshold: 45%, grey zone: 35% to 55%). However, the trained modules revealed a negative prediction, albeit a limited structural alert "Phenyl" that was already demonstrated but rejected for the parent compound - pyrimethanil, was obtained by the MNT mice model trained on EFSA and BASF data set. Additionally, due to coverage of the genotoxicity potential of the rat metabolite M605F021 (> 10% in ADME studies) by genotoxicity studies on parent compound, the MNT mice model trained on EFSA and BASF data set revealed a "known negative" outcome [see KCA 5.8.1/52 2017/1100635]. Therefore, the structural alerts "Sulfate" and "Phenyl" were considered as not relevant and rejected.
All Case Ultra predictions were of high reliability.

For the modified OECD Toolbox including read across, M605F021 was used to train the model for increasing predictivity and reliability, due to coverage of the genotoxicity potential of this rat metabolite (> 10% in ADME studies) by genotoxicity studies on parent compound, that were clearly negative.

In conclusion, in the structure activity evaluation tools employed there was a limited alert for mutagenicity or genotoxicity, which are considered of no relevance for the in vivo situation.

Thus, M605F021 is considered to be of no toxicological relevance and the ADI derived for pyrimethanil can be applied to this metabolite as well.

Metabolite M605F023



Livestock and rat metabolite M605F023 is the glucuronide conjugate of M605F002. The excretion product M605F023 is considered to be not more toxic than the predecessor hydroxylate M605F002, which in turn is considered to be covered by the toxicological testing of pyrimethanil [see "Toxicological evaluation of metabolite M605F002" above].

A Structural alerts for M605F023

Metabolite	QSAR tools applied	Structural alerts for genotoxicity	Prediction on genotoxicity	Reliability	Evaluation
M605F023	CU	Ames No other alert than parent	Ames Not mutagenic / Inconclusive	High (In Domain)	WoE: non-genotoxic
		in vivo MNT Alert "Alkoxy - ether; sugar conjugate" and "Alkoxy - hydroxy; sugar conjugate"	in vivo MNT Mutagenic / Not mutagenic / Known negative	High (In Domain)	
	OE incl. read across	Ames No alert	Ames Known negative - covered by pyrimethanil	High (In Domain)	non-genotoxic
		MNT/CA No alert	MNT/CA Known negative - covered by pyrimethanil	High (In Domain)	

CU = Case Ultra; OE = OECD Toolbox;
CA = Chromosomal aberration; MNT = Micronucleus Test; WoE = weight of evidence

All Case Ultra predictions were of high reliability.
The CASE Ultra analysis revealed a negative prediction for the bacterial mutagenicity by several modules: the Ames Konsolidator, Expert model, E. coli trained and S. thyphimurium trained and untrained models, but an inconclusive prediction by E. coli untrained module based on the same structural alert "Dimethylpyrimidine" that was already obtained for the parent compound [see KCA 5.8.1/51 2017/1100634] and rejected based on available experimental data.

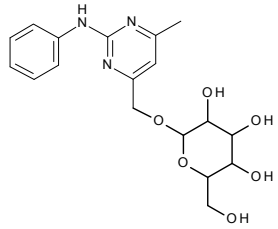
Negative prediction for the chromosomal aberration was obtained for in vivo endpoint by the trained MNT mice models, albeit two limited structural alerts "Sugar conjugates" with a positive prediction probability of 53.1% (model's probability threshold: 45%; grey zone: 35% - 55%) was obtained by the untrained MNT mice model [see KCA 5.8.1/52 2017/1100635]. The two structural alerts originate from a substructure of the glycoside substructure. This substructure is common in all kinds of sugars. Unfortunately, sugars are not yet included into the training data set. It can be assumed that the alerts originating from sugars do not represent a real structural alert. The substances that carry the same structural alert are not sugars and thus can be regarded as wrong positives for this substructure. Consequently, the prediction by the untrained MNT mice model would shift from inconclusive to out of domain.

For the modified OECD Toolbox including read across, M605F023 was used to train the model for increasing predictivity and reliability, due to coverage of the genotoxicity potential of this rat metabolite (> 10% in ADME studies) by genotoxicity studies on parent compound, that were clearly negative.

In conclusion, in the structure activity evaluation tools employed there was a limited alert for mutagenicity or genotoxicity, which are considered of no relevance for the in vivo situation.

Thus, M605F023 is considered to be of no toxicological relevance and the ADI derived for pyrimethanil can be applied to this metabolite as well.

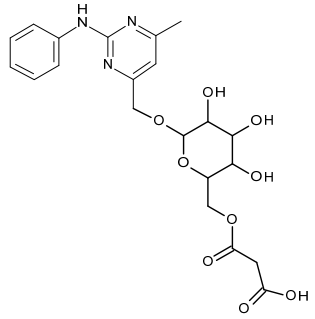
Metabolite M605F027



Metabolite M605F027, the β-O-glucoside of M605F004, is a plant and rotational crop metabolite. The excretion product M605F027 is considered to be not more toxic than the predecessor hydroxylate M605F004, which in turn is considered to be covered by the toxicological testing of pyrimethanil [see "Toxicological evaluation of metabolite M605F004" above].

Thus, M605F027 is considered to be of no toxicological relevance and the ADI derived for pyrimethanil can be applied to this metabolite as well.

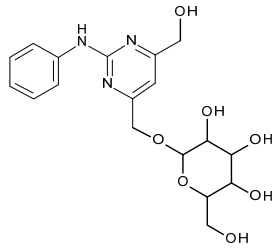
Metabolite M605F028



Metabolite M605F028, the malonyl-β-O-glucoside of M605F004, is a plant and rotational crop metabolite. The excretion product M605F028 is considered to be not more toxic than the predecessor hydroxylate M605F004, which in turn is considered to be covered by the toxicological testing of pyrimethanil [see "Toxicological evaluation of metabolite M605F004" above].

Thus, M605F028 is considered to be of no toxicological relevance and the ADI derived for pyrimethanil can be applied to this metabolite as well.

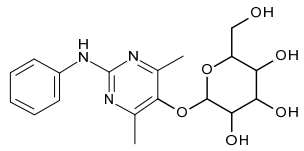
Metabolite M605F029



Metabolite M605F029, the β-O-glucoside of M605F005, is a plant metabolite. The excretion product M605F029 is considered to be not more toxic than the predecessor hydroxylate M605F005, which in turn is considered to be covered by the toxicological testing of pyrimethanil [see "Toxicological evaluation of metabolite M605F005" above].

Thus, M605F029 is considered to be of no toxicological relevance and the ADI derived for pyrimethanil can be applied to this metabolite as well.

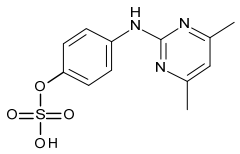
Metabolite M605F030



Metabolite M605F030, the glucose conjugate of M605F003, is a plant and rotational crop metabolite. The excretion product M605F030 is considered to be not more toxic than the predecessor hydroxylate M605F003, which in turn is considered to be covered by the toxicological testing of pyrimethanil [see "Toxicological evaluation of metabolite M605F003" above].

Thus, M605F030 is considered to be of no toxicological relevance and the ADI derived for pyrimethanil can be applied to this metabolite as well.

Metabolite M605F035



Metabolite M605F035, the sulfate conjugate of M605F002, is a rat and life stock metabolite. The excretion product M605F035 is considered to be not more toxic than the predecessor hydroxylate M605F002, which in turn is considered to be covered by the toxicological testing of pyrimethanil [see "Toxicological evaluation of metabolite M605F002" above].

A Structural alerts for M605F035

Metabolite	QSAR tools applied	Structural alerts for genotoxicity	Prediction on genotoxicity	Reliability	Evaluation
M605F035	CU	Ames Alerts "Dimethylpyrimidine" and "Sulfate"	Ames Mutagenic / Not mutagenic / Inconclusive	High (In Domain)	WoE: non-genotoxic
		in vivo MNT Alert "Sulfate"	in vivo MNT Mutagenic / Not mutagenic	High (In Domain)	
	OE incl. read across	Ames No alert	Ames Not mutagenic	High (In Domain)	non-genotoxic
		MNT/CA No alert	MNT/CA Not genotoxic	High (In Domain)	

CU = Case Ultra; OE = OECD-toolbox;
CA = Chromosomal aberration; MNT = Micronucleus Test; WoE = weight of evidence

The CASE Ultra analysis revealed a negative prediction for the bacterial mutagenicity by several modules: the Ames Konsolidator, Expert model, E. coli trained and S. thyphimurium trained and untrained models, but an inconclusive prediction due to a limited alert "Sulfate" by Expert and untrained S. thyphimurium modules with a positive probability of 42.9% (S. thyphimurium module probability threshold: 45%; grey zone: 35% - 55%; Expert module probability threshold: 50%; grey zone: 40% - 60%). Furthermore, a positive prediction was obtained by the untrained E. coli module based on the same structural alert "Dimethylpyrimidine" that was already obtained for the parent compound and rejected based on available experimental data and the alert "Sulfate", resulting in a positive probability of 76.3% with the probability threshold of 50% and a grey zone ranging from 40% to 60%. [see KCA 5.8.1/51 2017/1100634]. However, since the same alert "Sulfate" was obtained for other sulfate conjugated metabolite M605F021, that is a rat metabolite (> 10% in ADME studies) considered to be negative due to coverage by the genotoxicity profile of the parent – pyrimethanil, the present alert "Sulfate" was considered not to be of relevance and rejected.

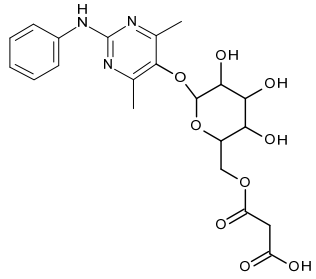
Negative prediction for the chromosomal aberration was obtained for in vivo endpoint by the trained MNT mice models, albeit the structural alert "Sulfate" with a positive prediction probability of 64.3% (model's probability threshold: 45%; grey zone: 35% - 55%) was obtained by the untrained MNT mice model [see KCA 5.8.1/52 2017/1100635]. However, since the same alert "Sulfate" was obtained for other sulfate conjugated metabolite M605F021, that is a rat metabolite (> 10% in ADME studies) considered to be negative due to coverage by the genotoxicity profile of the parent – pyrimethanil, the present alert "Sulfate" was considered not to be of relevance and rejected.
All Case Ultra predictions were of high reliability.

Negative prediction was obtained for the Ames [see KCA 5.8.1/53 2017/1100632] and in vivo Micronucleus [see KCA 5.8.1/54 DocID 2017/1100633] endpoints by the modified OECD-toolbox based on read across with parent compound pyrimethanil and/or its metabolites M605F007, M605F025 M605F002; M605F019, M605F021 and M605F023. Due to this modification, both predicted endpoints were "in the domain".

In conclusion, in the structure activity evaluation tools employed there was a limited alert for mutagenicity or genotoxicity, which are considered of no relevance for the in vivo situation.

Thus, M605F035 is considered to be of no toxicological relevance and the ADI derived for pyrimethanil can be applied to this metabolite as well.

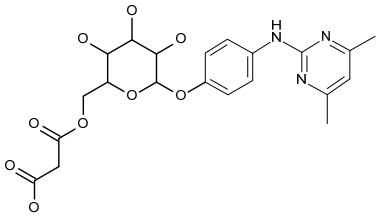
Metabolite M605F036



Metabolite M605F036, the malonyl glucose conjugate of M605F003, is a plant and rotational crop metabolite. The excretion product M605F036 is considered to be not more toxic than the predecessor hydroxylate M605F003, which in turn is considered to be covered by the toxicological testing of pyrimethanil [see "Toxicological evaluation of metabolite M605F003" above].

Thus, M605F036 is considered to be of no toxicological relevance and the ADI derived for pyrimethanil can be applied to this metabolite as well.

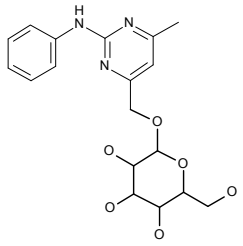
Metabolite M605F037



Metabolite M605F037, the malonyl-β-O-glucoside of M605F002, is a plant metabolite. The excretion product M605F037 is considered to be not more toxic than the predecessor hydroxylate M605F002, which in turn is considered to be covered by the toxicological testing of pyrimethanil [see "Toxicological evaluation of metabolite M605F002" above].

Thus, M605F037 is considered to be of no toxicological relevance and the ADI derived for pyrimethanil can be applied to this metabolite as well.

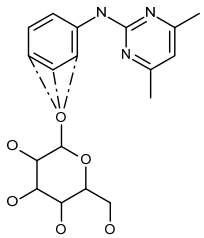
Metabolite M605F038



Metabolite M605F038, the C-6 sugar conjugate of M605F004, is a plant metabolite. The excretion product M605F038 is considered to be not more toxic than the predecessor hydroxylate M605F004, which in turn is considered to be covered by the toxicological testing of pyrimethanil [see "Toxicological evaluation of metabolite M605F004" above].

Thus, M605F038 is considered to be of no toxicological relevance and the ADI derived for pyrimethanil can be applied to this metabolite as well.

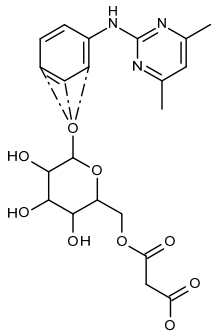
Metabolite M605F039



Metabolite M605F039, a undefined glucose conjugate of a phenylring-hydroxylate, is a plant and rotational crop metabolite. The excretion product M605F039 is considered to be not more toxic than the predecessor hydroxylates like M605F002, which in turn is considered to be covered by the toxicological testing of pyrimethanil [see "Toxicological evaluation of metabolite M605F002" above].

Thus, M605F039 is considered to be of no toxicological relevance and the ADI derived for pyrimethanil can be applied to this metabolite as well.

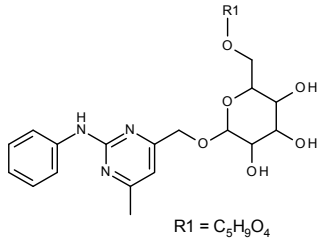
Metabolite M605F040



Metabolite M605F040, a undefined malonyl glucose conjugate of a phenylring-hydroxylate, is a plant and rotational crop metabolite. The excretion product M605F040 is considered to be not more toxic than the predecessor hydroxylates like M605F002, which in turn is considered to be covered by the toxicological testing of pyrimethanil [see "Toxicological evaluation of metabolite M605F002" above].

Thus, M605F040 is considered to be of no toxicological relevance and the ADI derived for pyrimethanil can be applied to this metabolite as well.

Metabolite M605F041



Metabolite M605F041, the $C_5H_9O_4$ glucose conjugate of M605F004, is a plant metabolite. The excretion product M605F041 is considered to be not more toxic than the predecessor hydroxylate M605F004, which in turn is considered to be covered by the toxicological testing of pyrimethanil [see "Toxicological evaluation of metabolite M605F004" above].

Thus, M605F041 is considered to be of no toxicological relevance and the ADI derived for pyrimethanil can be applied to this metabolite as well.

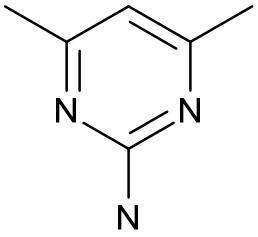
Conclusion for group 1 metabolites:

Based on the metabolic transformation (e.g. hydroxylation) metabolites of group 1 can generally be considered equally or less toxic than the parent substance pyrimethanil. QSAR evaluation of group 1 metabolites results in a common structural alert “Quinoneimines” which is considered not relevant as the same structural alert is identified for pyrimethanil, which is clearly not genotoxic based on several in vitro and in vivo studies. The structural alert identified in the ISS module of the OECD toolbox is not considered relevant given the lacking structural similarity. Furthermore, as the hydroxylate metabolites of this group and conjugates thereof were detected in a rat metabolism study with pyrimethanil as the major metabolic pathway, no further testing is required and the risk assessment can be based on the parent ADI.

Group 2: Pyrimidin-moiety and their conjugates

This group comprises of the metabolites M605F007, M605F008, M605F016, M605F032 and M605F033 which represent a pyrimidine structure as basis. M605F007 the basic derivative is either desaminated leading to M605F008 or hydroxylated leading to M605F016 or M605F032 and the O-bound sugar-conjugate thereof (M605F033). As both degradation steps (desamination or hydroxylation are expected to result in generally equally or less toxic derivative M605F007 is considered as relevant group representative. This assessment is well supported by the QSAR genotoxicity prediction as presented below and summarized in Table 5.8.1-1. Consequently, M605F007 was chosen as group representative for toxicity evaluation.

Metabolite M605F007 (AN7)



M605F007 is a metabolite of pyrimethanil that was determined in rat, soil and water (sediment), and rotational crops.

A Structural alerts for M605F007

Metabolite	QSAR tools applied	Structural alerts for genotoxicity	Reliability	Evaluation
M605F003	OE	Alert for Ames, MN in ISS module No alert for MN, Ames, CA in OASIS module	Low	Inconclusive alerts not confirmed by genotoxicity testing
	OA	Ames Not mutagenic	Low	
		CA Not clastogenic	Low	
	VE	Inconclusive mutagenic in TOXTREE module not mutagenic in CAESAR and SarPy module	Low to moderate	

OE = OECD-toolbox; OA = OASIS-times; VE = Vega
CA = Chromosomal aberration; MN = Micronuclei

Metabolite	QSAR tools applied	Structural alerts for genotoxicity	Prediction on genotoxicity	Reliability	Evaluation
M605F007	CU	Ames Alerts “Dimethylpyrimidine”, “Aminopyrimidine” and “Primary aromatic amines”	Ames Mutagenic / Not mutagenic / Inconclusive	High (In Domain)	Alerts overruled by experimental data
		in vivo MNT No alerts	in vivo MNT Not genotoxic / Known negative	High (In Domain)	
	OE incl. read across	Ames “Primary aromatic amines”	Ames Known negative	High (In Domain)	Alerts overruled by experimental data
		MNT/CA “Primary aromatic amines”	MNT/CA Known negative	High (In Domain)	
	OE	Alert for Ames, MNT in vivo in ISS module No alert for MNT, Ames, CA in OASIS module	Ames Inconclusive MNT Inconclusive	- (No Domain definition)	Alerts overruled by experimental data
	OA	Ames No alert	Ames Not mutagenic	Low (Out of Domain)	Non-genotoxic
		CA No alert	CA in vitro Not genotoxic	Low (Out of Domain)	
	VE	-	Ames Inconclusive CEASAR and SarPy – not mutagenic Toxtree - mutagenic	Reasonable by QSAR, low by WoE	Prediction overruled by experimental data

CU = Case Ultra; OE = OECD-toolbox; OA = OASIS-times; VE = Vega
CA = Chromosomal aberration; MNT = Micronucleus Test; WoE = weight of evidence

The CASE Ultra analysis revealed a negative prediction for the bacterial mutagenicity by several modules: the Ames Konsolidator, E. coli trained and S. thyphimurium trained and untrained models, but a positive prediction by the Expert and E. coli untrained module [see KCA 5.8.1/51 2017/1100634]. The untrained E. coli module revealed the structural alerts “Dimethylpyrimidine” and “Aminopyrimidine” which were, however not verified by available experimental Ames test data, and thus was rejected. The same is true for the structural alert “primary aromatic amines” obtained by the Expert model.
Negative prediction for the chromosomal aberration was obtained for in vivo endpoint by all applied MNT mice models [see KCA 5.8.1/52 2017/1100635].
All Case Ultra predictions were of high reliability.

OASIS Times [see KCA 5.8.1/21 2015/1186251] predicted M605F007 and in silico generated metabolites to be not mutagenic in the Ames test with or without metabolic activation but with the limitation that the molecule was out of the prediction domain. For chromosomal aberration the prediction was as well negative for M605F007 and in silico generated metabolites thereof [see KCA 5.8.1/22 2015/1186250].

The VEGA prediction in two out of three modules (CAESAR, SarPy) was not mutagenic. The TOXTREE module resulted in a positive prediction for mutagenicity (Ames), yielding the alert "Primary aromatic amine, hydroxyl amine and its derived esters (with restrictions)". The reliability of these predictions was low to moderate [see molecule 6 of report KCA 5.8.1/16 2015/1186244].

For the modified OECD Toolbox including read across, the available experimental data on M605F007 were used to train the model for increasing predictivity and reliability. Due to negative results that were experimentally obtained in an Ames test and an in vitro MNT test in human lymphocytes (see below), the obtained structural alert "Primary aromatic amines" by ISS, was considered not relevant and rejected.
Information from OECD Toolbox (previous version v3.3) [see KCA 5.8.1/1 2015/1198463] revealed no alerts for DNA binding or genotoxicity as evaluated in various modules, including OASIS DNA alerts for Ames, MN, and CA. The alert "Primary aromatic amine, hydroxyl amine and its derived esters" for mutagenicity (Ames, Micronucleus) was received from the ISS module.

In conclusion, in the structure activity evaluation tools employed there was a limited inconclusive alerts for mutagenicity, which is considered of no relevance for the in vivo situation, as negative in vitro Ames and micronucleus assays are available with M605F007.

B Toxicity studies for M605F007

Studies evaluated in the draft monograph of rapporteur member state Austria of April, 2004: An acute oral toxicity study in rats and an Ames test were evaluated during Annex I inclusion of pyrimethanil. For the convenience of the reviewer short summaries of these are provided below.

Table 5.8.1-7: Summary of toxicity studies with M605F007 available in the original monograph

Study	Dosage / Test system	Result	Reference (BASF DocID)
Acute toxicity, Oral, Rat, Sprague-Dawley m/f	100, 200, 400, 800, 1600 mg/kg bw in 1% aqueous methyl cellulose	LD_{50} 735 mg/kg bw	C001117
Mutagenicity in bacterial cells (Ames test)	*Salmonella typhimurium* (TA 1535, TA 1537, TA98, TA 100,); *Escherichia coli* (CM 891); Concentration up to 5000 µg/plate Without and with S-9 mix	Not mutagenic	C000864

New toxicity studies not already evaluated in the draft monograph of rapporteur member state Austria of April, 2004: A micronucleus assay was performed with M605F007 as summarized below yielding negative results with and without metabolic evaluation [see KCA 5.8.1/25 2015/1112086].

Table 5.8.1-8: Summary of toxicity studies with M605F007 not yet peer-reviewed

Study	Dosage / Test system	Result	Reference (BASF DocID)
In vitro micronucleus assay (Mammalian cells)	Human lymphocytes, 4h –S9 and + S9 up to 1250 µg/mL; 20 h – S9 up to 750 µg/mL	Not genotoxic	2015/1112086

Rat acute oral toxicity with M605F007:

~~Reference: Weir L., [redacted] 1998; Report No. TOX/98/223-98~~
~~DocID C001117~~
~~The study was conducted according to OECD guideline 401 and in compliance with GLP principles. The study is scientific valid and therefore acceptable.~~

Report:	CA 5.8.1/23 [redacted] 1998 a AE F132593 (soil photolysis metabolite of Pyrimethanil) - Code: AE F132593 00 1D99 0001 - Rat acute oral toxicity C001117
Guidelines:	OECD 401, EPA 81-1, EEC 92/69 B 1, JMAFF 59 NohSan No 4200
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)
Previous evaluation:	DAR (2004)
Acceptability:	The study is scientific valid and therefore acceptable

Material and method:
Groups of 5 rats/sex (strain: CD Sprague-Dawley; source: [redacted].) weighing between 182 and 210 g received a single dose of 0 (vehicle control), 100, 200, 400, 800 and 1600 mg/kg bw M605F007 called AE F132593 in the study report (batch no. C1D990001; purity 98.7%, suspended in 1% w/v methyl cellulose in distilled water) by oral gavage. After administration all animals were kept under observation for 14 days. Clinical observations were made daily. Body weights were recorded on the day of dosing and on days 7 and 14. At termination all surviving rats were necropsied. Necropsies were also performed on those animals that had died during the study.

Findings:
Clinical signs and mortality: At 1600 mg/kg, all males and females were culled due to a moribund condition between approx. 30 minutes to 4 hours post-dosing. At 800 mg/kg, 3 males and 2 females and one female at 400 mg/kg were culled between 1 – 6 hours post dosing. There were no mortalities at 100 and 200 mg/kg. Clinical signs observed in animals treated at 1600, 800, 400 and in females only at 200 mg/kg were included salivation, prostration, altered respiration, unsteady gait, reduced activity, reduced muscle tone and occasional findings in the eyes (red discharge). These findings were observed in a dose related manner with respect to onset and severity with complete recovery by day 7 post dosing. No treatment related effects on body weights were observed.
Pathology: Necropsy of decedents revealed mottled kidneys, accentuated lobular pattern and all lobes mottled in the liver and dark thoracic fluid and multiple areas in the lungs. No gross pathological findings were seen in any of the rats surviving to day 14.

Conclusion:
The oral LD_{50} for the soil metabolite M605F007 was calculated to be 735 (575 – 939) mg/kg bw in male and female rats.

Bacterial reverse mutation assay (Ames test) with M605F007:

~~Reference: Kitching J., 1998; Report No. TOX/98/223-99~~
~~DocID C000864~~
~~The study was performed according to OECD guideline 471 and US EPA guideline 84-2 and in compliance with GLP principles. Deviation: The stability of the test compound in the solvent was not determined. The study is of scientific validity and considered acceptable.~~

Report:	CA 5.8.1/24 Kitching J., 1998 a AE F132593 (soil metabolite of Pyrimethanil) - Code: AE F132593 00 ID99 0001 - Bacterial reverse mutation assay C000864
Guidelines:	OECD 471, EEC 92/69 B 13, EEC 92/69 B 14, JMAFF 59 NohSan No 4200, EPA 40 CFR Part 799
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)
Previous evaluation:	DAR (2004)
Acceptability:	The study is scientific valid and therefore acceptable

Material and method:
M605F007 (batch no. 59474, purity 98.7%) was tested in the Ames test using histidine dependent auxotrophic mutants of Salmonella typhimurium (strains TA 1535, TA 1537, TA 98 and TA 100) and a tryptophan dependent mutant of Escherichia coli (strain CM891). The test substance (dissolved in DMSO) was added at concentrations of 0 (solvent control), 5, 15, 50, 150, 500, 1500 and 5000 µg/plate in the presence and absence of S-9 mix (liver preparations from Aroclor 1254-induced male Sprague-Dawley rats). Two independent mutation tests were performed. The first was a standard plate incorporation assay with three replicates per concentration incubated at 37°C for 3 days. The second involved a pre-incubation stage in which the bottles were incubated at 37°C for 30 minutes with shaking before the addition of the agar overlay.
As positive controls 9-aminoacridine (30 µg/plate administered to TA 1537), 2-nitrofluorene (1 µg/plate administered to TA 98), N-ethyl-N'-nitro-N-nitrosoguanidine (2, 3 and 5 µg/plate administered to CM891, TA 100 and 1535, resp.), 2-aminoanthracene (2 and 10 µg/plate administered to TA 1535 and CM891, resp.) and Benzo[a]pyrene (5 µg/plate for TA 1537, TA 98 and TA 100) were used.

Evaluation criteria:
The test is considered positive if there is an increase in revertant colonies of at least twice the solvent control, with some evidence of a positive dose-relationship, with any bacterial strain either with or without S-9 mix. If the treatment does not produce reproducible increases of at least 1.5 times the solvent controls, at any dose level with any bacterial strain, it is considered to show no evidence of mutagenic activity. No statistical analysis is performed.

Findings:
In both tests cytotoxicity was observed at 5000 µg/plate with and without S-9 mix. However, no substantial increases in revertant colony numbers of any of the strains tested were observed following treatment with M605F007 at any dose level, either in the presence or absence of metabolic activation. The positive control materials elicited the expected positive responses.

Table 5.8.1-9: Bacterial mutation assay; Revertant colony counts per plate; IL = incomplete bacterial lawn, + = presence, - = absence

Strain	Dose level [µg/plate]	Mean revertant colony counts	
		Test 1 -S9 mix/+S9 mix	Test 2 -S9 mix/+S9 mix
TA 1535	5000	IL/IL	IL/IL
	1500	13/14	18/18
	500	12/10	20/22
	150	15/18	15/20
	50	13/15	15/19
	15	11/18	17/18
	5	15/18	-
	Solvent	12/16	17/20
TA 1537	5000	IL/IL	IL/IL
	1500	6/7	15/14
	500	9/15	14/13
	150	11/12	11/13
	50	12/10	15/14
	15	12/10	14/16
	5	12/13	-
	Solvent	8/11	14/12
TA 98	5000	IL/IL	IL/IL
	1500	22/27	28/31
	500	23/23	25/27
	150	20/27	20/31
	50	23/22	26/27
	15	22/29	19/27
	5	18/30	-
	Solvent	20/25	24/29
TA 100	5000	IL/IL	IL/IL
	1500	77/91	120/114
	500	118/119	117/112
	150	115/122	114/124
	50	118/112	118/120
	15	125/113	109/112
	5	126/124	-
	Solvent	104/110	123/121
CM 891	5000	IL/IL	IL/IL
	1500	127/105	183/140
	500	172/147	201/165
	150	194/152	218/183
	50	213/174	215/219
	15	227/181	233/201
	5	203/165	-
	Solvent	175/142	252/198

Table 5.8.1-10: **Bacterial mutation assay; Revertant colony counts per plate: positive control; + = presence, - = absence, ENNG = N-ethyl-N-nitro-N-nitrosoguanidine, 9AC = 9-aminoacridine, NF = 2-nitrofluorene, AA = 2-aminoanthracene, B[a]P = benzo[a]pyrene**

Strain	Compound	Dose level [µg/plate]	S9 mix	Mean revertant colony counts	
				Test 1	Test 2
TA 1535	ENNG	5	-	135	462
TA 1537	9AC	30	-	211	759
TA 98	NF	1	-	286	117
TA 100	ENNG	3	-	427	451
CM 891	ENNG	2	-	824	1003
TA 1535	AA	2	+	84	175
TA 1537	B[a]P	5	+	97	165
TA 98	B[a]P	5	+	161	300
TA 100	B[a]P	5	+	312	515
CM 891	AA	10	+	701	875

Conclusion:
It can be concluded that the metabolite M605F007 was not mutagenic when tested at dose levels up to 5000 µg/plate in this test system.

Mammalian Cell Chromosomal aberation (MNT) in vitro with M605F007

Report:	CA 5.8.1/25 Sokolowski A., 2015a Reg.No. 40603 (metabolite of BAS 605 F, Pyrimethanil): Micronucleus test in human lymphocytes in vitro 2015/1112086
Guidelines:	OECD 487 (2014), Commission Regulation EU No. 640/2012 of 06 July 2012 - B.49: In vitro Mammalian Cell Micronucleus Test
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)
Deviations:	no

Report:	CA 5.8.1/26 Sokolowski A., 2015 b Report Amendment No. 1: Reg.No. 40603 (metabolite of BAS 605 F, Pyrimethanil): Micronucleus test in human lymphocytes in vitro 2015/1182723
Guidelines:	OECD 487 (2014), Commission Regulation EU No. 640/2012 of 06 July 2012 - B.49: In vitro Mammalian Cell Micronucleus Test
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

M605F007 called Reg.No. 40603 (Metabolite of BAS 605 F) in the study report (Batch: L72-129; Purity: 98.6%) was tested for its potential to induce micronuclei in human lymphocytes *in vitro* in the absence and presence of metabolic activation by S9 mix. Three independent experiments were performed where the cells were incubated for 4 (±S9 mix) or 20 hours (-S9 mix) with the test substance at concentrations in the range of 8.1 to 1250 µg/mL. Appropriate mutagens were used as positive controls. Treatments started after a 48 hour stimulation period with phytohaemagglutinin. Cytokinesis-block proliferation index and cytostasis were determined in 500 binucleated cells/culture as cytotoxicity parameters and number of micronucleated cells were determined in 1000 binucleated cells/culture for evaluation of mutagenicity.

No precipitation of the test item in the culture medium was observed. No relevant influence on osmolarity or pH was observed. In Experiment IA and II in the absence of S9 mix concentrations showing clear cytotoxic effects were not evaluable for cytogenetic damage. In Experiment 1A clear cytotoxic effects were observed from 233.2 µg/mL onwards. In Experiment IB in the absence of S9 mix and Experiment IA and II in the presence of S9 mix, no cytotoxicity was observed up to the highest applied concentration.

In all experiments, no relevant increase in the number of micronucleated cells was observed after treatment with the test item. The micronucleus rates of the cells after treatment with the test item did not exceed the range of the solvent control and were within the range of the laboratory historical control data. In Experiment IA in the presence of S9 mix one single statistically significant increase was observed after treatment with 408.2 µg/mL (0.60 % micronucleated cells). Since the value is clearly within the range of the laboratory historical solvent control data (0.15 – 1.70 % micronucleated cells), the finding has to be regarded as biologically irrelevant. The positive controls induced statistically significant increases in cells with micronuclei.

In conclusion, Reg.No. 40603 (Metabolite of BAS 605 F) is considered to be non-genotoxic in this *in vitro* micronucleus test when tested up to cytotoxic concentrations.

(BASF DocID 2015/1112086)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Reg. No. 40603 (Metabolite of BAS 605 F, Pyrimethanil)

Description: solid / white

Lot/Batch #: L72-129

Purity/content: 98.6%

Stability of test compound: The stability of the test item under storage conditions over the study period was guaranteed by the sponsor (expiry date Feb, 2020). Stable in deionized water (60°C) over 4 h.

Vehicle used: Deionised water

2. Control Materials:

Negative: No negative control was employed in this study.

Vehicle control: Culture medium with 10% deionized water

Positive control: Without metabolic activation:
Mitomycin C (MMC, 2 and 3 µg/mL; pulse treatment) dissolved in deionized water;
Demecolcin (50 ng/mL; continuous treatment) dissolved in deionized water
With metabolic activation:
Cyclophosphamide (CCP, 15 and 17.5 µg/mL) dissolved in saline (0.9% NaCl)

3. Activation: S9 was produced from the livers of rats pretreated with β-naphthoflavone/phenobarbital. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction is thawed at room temperature mixed with an appropriate volume of S9-supplement (cofactors). This preparation, the so-called S9-mix, was kept on ice until used.
The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	100 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM

An appropriate quantity of S9 mix per mL culture medium were added yielding a final protein concentration of 0.75 mg/mL in the cultures. Each batch of S9 was routinely tested for its capability to activate the known mutagens benzo[a]pyrene and 2-aminoanthracene in the Ames test.

4. Test organism: Human peripheral blood lymphocytes

5. Culture media:

Culture medium: Dulbecco´s Modified Eagles medium/Ham´s F12 (1:1) with GlutaMAX™ (200 mM) supplemented with 10% (v/v) fetal bovine serum (FBS), Pen/Strep (100 U/mL/100 µg/mL), HEPES (10 mM), heparin (125 U.S.P.-U/mL), phytohaemeagglutinin (PHA, 3 µg/mL).

6. Test concentrations:

Micronucleus assay

Experiment IA

(4-h exposure, -S9): 8.1, 14.2, 24.9, **43.5, 76.2, 133.3**, 233.2, 408.2, 714.3, 1250 µg/mL

(4-h exposure, +S9): 8.1, 14.2, 24.9, 43.5, 76.2, 133.3, 233.2, **408.2, 714.3, 1250** µg/mL

Experiment IB

(4-h exposure, -S9):

First trial: 25, 50, 100, 125, 150, 175, 200, 225, 250, 300, 400 µg/mL

Second trial: 25, 50, 100, 150, 200, 250, 300, 400, 500, 600, 700, 800 µg/mL

Third trial: 14.2, 24.9, 43.5, 76.2, 133.3, 233.2, 408.2, **714.3, 1000, 1250** µg/mL

Experiment II

(4-h exposure, +S9): 62.5, 125, 250, 500, **750, 1000, 1250** µg/mL

(20-h exposure, -S9): 25, 50, 100, 200, 300, **400, 500, 750**, 1000, 1250 µg/mL

Experiment IB was repeated twice due to lack of cytotoxicity. Numbers shown in bold indicate experimental points that were evaluated for micronucleus formation.

B. TEST PERFORMANCE

1. Dates of experimental work: 07-Jan-2015 to 23-Apr-2015

2. Dose selection:

Dose selection was performed according to the current OECD Guideline for the *in vitro* micronucleus test. The highest test item concentration should be 10 mM, 2 mg/mL, or 2 µL/mL, whichever is the lowest. At least three test item concentrations should be evaluated for cytogenetic damage.

With regard to the molecular weight and the purity (98.6 %) of the test item, 1250.0 µg/mL (approx. 10 mM) were applied as top concentration for treatment of the cultures in the pre-test. Test item concentrations ranging from 8.1 to 1250.0 µg/mL (with and without S9 mix) were chosen for the evaluation of cytotoxicity. In the pre-test for toxicity, no precipitation of the test item was observed at the end of treatment. Since the cultures fulfilled the requirements for cytogenetic evaluation in the presence of S9 mix, this preliminary test was designated Experiment IA. The experimental part without S9 mix was repeated three times with top doses of 400.0, 800.0 and 1250.0 µg/mL to obtain evaluable concentrations in a cytotoxic range (Exp. IB).

In Experiment IA (with S9 mix) and IB (without S9 mix) after 4 hours exposure no cytotoxicity was observed. Therefore, 1250.0 µg/mL were chosen as top treatment concentration for Experiment II.

The cytogenetic evaluation of concentrations in Experiment IA and II (without S9 mix) was limited due to strong test item-induced toxic effects (low cell numbers, partially paralleled by poor cell quality).

3. Pre-experiment:

A preliminary cytotoxicity test was performed to determine the concentrations to be used in the main experiment. Cytotoxicity is characterized by the percentages of reduction in the CBPI in comparison with the controls (% cytostasis) by counting 500 cells per culture in duplicate. The experimental conditions in this pre-experimental phase were identical to those required and described below for the mutagenicity assay.
The pre-test was performed with 10 concentrations of the test item separated by no more than a factor of √10 and a solvent and positive control. All cell cultures were set up in duplicate. Exposure time was 4 hrs (with and without S9 mix). The preparation interval was 40 hrs after start of the exposure.

4. Micronucleus test:

Pulse exposure:

About 48 h after seeding 2 blood cultures (10 mL each) were set up in parallel in 25 cm² cell culture flasks for each test item concentration. The culture medium was replaced with serum-free medium containing the test item. For the treatment with metabolic activation 50 µL S9 mix per mL culture medium was added. After 4 h the cells were spun down by gentle centrifugation for 5 minutes. The supernatant was discarded and the cells were resuspended in and washed with "saline G" (pH 7.2, containing 8000 mg/L NaCl, 400 mg/L KCl, 1100 mg/L glucose • H_2O, 192 mg/L Na_2HPO_4 • 2 H_2O and 150 mg/L KH_2PO_4). The washing procedure was repeated once as described. The cells were resuspended in complete culture medium with 10 % FBS (v/v) and cultured for a 16-hour recovery period. After this period Cytochalasin B (4 µg/mL) was added and the cells were cultured another approximately 20 hours until preparation.

Continuous exposure

About 48 h after seeding 2 blood cultures (10 mL each) were set up in parallel in 25 cm² cell culture flasks for each test item concentration. The culture medium was replaced with complete medium (with 10 % FBS) containing the test item. After 20 hours the cells were spun down by gentle centrifugation for 5 minutes. The supernatant was discarded and the cells were re-suspended in and washed with "saline G". The washing procedure was repeated once as described. After washing the cells were re-suspended in complete culture medium containing 10 % FBS (v/v). Cytochalasin B (4 µg/mL) was added and the cells were cultured another approximately 20 hours until preparation.

Preparation of cells

The cultures were harvested by centrifugation 40 h after beginning of treatment. The cells were spun down by gentle centrifugation for 5 minutes. The supernatant was discarded and the cells were re-suspended in approximately 5 mL saline G and spun down once again by centrifugation for 5 minutes. Then the cells were resuspended in 5 mL KCl solution (0.0375 M) and incubated at 37°C for 20 minutes. 1 mL of ice-cold fixative mixture of methanol and glacial acetic acid (19 parts plus 1 part, respectively) was added to the hypotonic solution and the cells were resuspended carefully. After removal of the solution by centrifugation the cells were resuspended for 2 x 20 minutes in fixative and kept cold. The slides were prepared by dropping the cell suspension in fresh fixative onto a clean microscope slide. The cells were stained with Giemsa.

5. Statistics:

The number of polychromatic erythrocytes with micronuclei was analyzed by comparing the dose groups with the vehicle control using the Chi square test.

6. Cytotoxicity evaluation:

Evaluation of cytotoxicity and cytogenetic damage

Evaluation of the slides was performed using NIKON microscopes with 40 x objectives. The micronuclei were counted in cells showing a clearly visible cytoplasm area. The criteria for the evaluation of micronuclei are described in the publication of Countryman and Heddle (1976). The micronuclei have to be stained in the same way as the main nucleus. The area of the micronucleus should not extend the third part of the area of the main nucleus. At least 1000 binucleate cells per culture were scored for cytogenetic damage on coded slides. The frequency of micronucleated cells was reported as % micronucleated cells. To describe a cytotoxic effect the CBPI was determined in 500 cells per culture and cytotoxicity is expressed as % cytostasis. A CBPI of 1 (all cells are mononucleate) is equivalent to 100 % cytostasis.

$$CBPI = \frac{(MONC \times 1) + (BINC \times 2) + (MUNC \times 3)}{n}$$

CBPI	Cytokinesis-block proliferation index
n	Total number of cells
MONC	Mononucleate cells
BINC	Binucleate cells
MUNC	Multinucleate cells

$$\text{Cytostasis \%} = 100 - 100\,[(CBPI_T - 1) / (CBPI_C - 1)]$$

T	Test item
C	Solvent control

7. Evaluation criteria:

Acceptability criteria:

- The rate of micronuclei in the solvent controls falls within the historical laboratory control data range.
- The rate of micronuclei in the positive controls is statistically significant increased.
- The quality of the slides must allow the evaluation of a sufficient number of analyzable cells.

Evaluation criteria:

A test item can be classified as non-clastogenic and non-aneugenic if:

- the number of micronucleated cells in all evaluated dose groups is in the range of the historical laboratory control data and
- no statistically significant or concentration-related increase of the number of micronucleated cells is observed in comparison to the respective solvent control.

A test item can be classified as clastogenic and aneugenic if:

- the number of micronucleated cells is not in the range of the historical laboratory control data and
- either a concentration-related increase in three test groups or a statistically significant increase in the number of micronucleated cells is observed.

If the above mentioned criteria for the test item are not clearly met, the test item will be classified as equivocal or a confirmatory experiment may be performed. However, results may remain questionable regardless of the number of times the experiment is repeated.

An increase in the number of micronucleated mononucleate cells may indicate that the test item has aneugenic potential.

II. RESULTS AND DISCUSSION

A. CYTOTOXICITY AND TREATMENT CONDITIONS

No precipitation of the test item in the culture medium was observed. No relevant influence on osmolarity or pH was observed.

In each experimental group two parallel cultures were analysed. 1000 binucleate cells per culture were evaluated for cytogenetic damage on coded slides. To determine a cytotoxic effect the CBPI was determined in 500 cells per culture and cytotoxicity is described as % cytostasis.

In Experiment IA and II in the absence of S9 mix, concentrations showing clear cytotoxic effects were not evaluable for cytogenetic damage. In Experiment 1A clear cytotoxic effects were observed from 233.2 µg/mL onwards. In Experiment IB in the absence of S9 mix and Experiment IA and II in the presence of S9 mix, no cytotoxicity was observed up to the highest applied concentration. In experiment II, in the absence of S9 mix, strong cytotoxicity was observed at 1000 µg/mL indicated by low cell numbers partially paralleled by poor cell quality.

B. MICRONUCLEUS ASSAY

In all experiments, no relevant increase in the number of micronucleated cells was observed after treatment with the test item. The micronucleus rates of the cells after treatment with the test item (0.05 – 0.90 % micronucleated cells) did not exceed the range of the solvent control values (0.20 – 0.90 % micronucleated cells) and were within the range of the laboratory historical control data, which was 0.1-1.3 and 0.3-1.45 for pulse and continuous treatment experiments, respectively, in aqueous solution.
In Experiment IA in the presence of S9 mix one single statistically significant increase was observed after treatment with 408.2 µg/mL (0.60 % micronucleated cells). Since the value is clearly within the range of the laboratory historical solvent control data (0.15 – 1.70 % micronucleated cells), the finding has to be regarded as biologically irrelevant.
Either Demecolcin (50.0 ng/mL), MMC (2.0 or 3.0 µg/mL) or CPA (15.0 or 17.5 µg/mL) were used as positive controls and showed distinct increases in cells with micronuclei.

Table 5.8.1-11: Summary of results of the in vitro micronucleus test in human lymphocytes with Reg.No. 40603 (Metabolite of BAS 605 F) in the absence of S9 mix

Exp.	Preparation interval	Test item concentration in µg/mL	Proliferation index CBPI	Cytostasis in %[a]	Micronucleated cells in %[b]
Exposure period 4 hrs without S9 mix					
IA	40 hrs	Solvent control[1]	1.88	-	0.35
		Positive control[2]	1.15	83.1	**7.85***
		43.5	1.70	20.3	0.35
		76.2	1.73	16.7	0.05
		133.3	1.58	34.3	0.20
IB	40 hrs	Solvent control[1]	1.67	-	0.55
		Positive control[3]	1.12	82.0	**13.30***
		714.3	1.65	2.4	0.65
		1000	1.66	0.6	0.55
		1250	1.62	7.2	0.75
Exposure period 20 hrs without S9 mix					
II	40 hrs	Solvent control[1]	1.85	-	0.45
		Positive control[4]	1.77	9.1	**3.90***
		400	1.74	12.4	0.45
		500	1.63	25.7	0.90
		750	1.56	34.0	0.90

a: For the positive control groups and the test item treatment groups the values are related to the solvent controls
b: The number of micronucleated cells was determined in a sample of 2000 binucleated cells

*: The number of micronucleated cells is statistically significantly higher than corresponding control values ($p \leq 0.05$)

1 Deionised water 10% (v/v)

2 MMC 3.0 µg/mL

3 MMC 2.0 µg/mL

4 Demecolcin 50.0 ng/mL

Table 5.8.1-12: Summary of results of the in vitro micronucleus test in human lymphocytes with Reg.No. 40603 (Metabolite of BAS 605 F) in the presence of S9 mix

Exp.	Preparation interval	Test item concentration in µg/mL	Proliferation index CBPI	Cytostasis in %[a]	Micronucleated cells in %[b]
Exposure period 4 hrs with S9 mix					
IA	40 hrs	Solvent control[1]	1.93	-	0.20
		Positive control[2]	1.71	23.9	**3.00***
		408.2	1.86	8.2	**0.60***
		714.3	1.87	6.7	0.05
		1250	1.85	8.7	0.50
II	40 hrs	Solvent control[1]	1.84	-	0.90
		Positive control[3]	1.77	8.8	**2.70***
		750	1.87	n.c.	0.65
		1000	1.82	1.8	0.55
		1250	1.85	n.c.	0.55

a: For the positive control groups and the test item treatment groups the values are related to the solvent controls
b: The number of micronucleated cells was determined in a sample of 2000 binucleated cells

*: The number of micronucleated cells is statistically significantly higher than corresponding control values ($p \leq 0.05$)

1 Deionised water 10% (v/v)

4 CPA 15 µg/mL

4 CPA 17.5 µg/mL

III. CONCLUSION

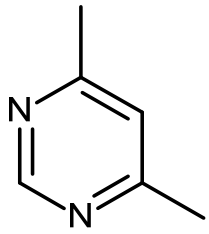
In conclusion, it can be stated that under the experimental conditions reported, the test item did not induce micronuclei as determined by the *in vitro* micronucleus test in human lymphocytes. Therefore, **Reg.No. 40603 (Metabolite of BAS 605 F, Pyrimethanil)** is considered to be **non-mutagenic** in this *in vitro* micronucleus test, when tested up to the highest required or evaluable concentrations.

C Toxicological evaluation of metabolite M605F007

The limited structural alert for mutagenicity in silico identified for M605F007 was clearly rejected by the available toxicological tests yielding clear negative results in an Ames test and an in vitro micronucleus test. Furthermore, M605F007 has shown to be moderately acutely toxic in rats after oral administration.

Thus, M605F007 is considered to be not genotoxic and the TTC-threshold of 1.5 µg/kg bw/day can be applied for risk assessment purposes.

Metabolite M605F008 (former assigned AN8)



M605F008 is a metabolite of Pyrimethanil that was determined in soil and rotational crops. M605F008 is the desaminated successor of M605F007. The desaminated M605F008 is considered to be less toxic than the predecessor M605F007.

A Structural alerts for M605F008

Metabolite	QSAR tools applied	Structural alerts for genotoxicity	Reliability	Evaluation
M605F008	OE	Not genotoxic	Low	Not genotoxic
	OA	Ames Not mutagenic	Low	
		CA Not clastogenic	Low	
	VE	Not mutagenic	Low to moderate	

OE = OECD-toolbox; OA = OASIS-times; VE = Vega
CA = Chromosomal aberration; MN = Micronuclei

Metabolite	QSAR tools applied	Structural alerts for genotoxicity	Prediction on genotoxicity	Reliability	Evaluation
M605F008	CU	Ames No other alert than parent	Ames Not mutagenic / Inconclusive	High (In Domain)	WoE: non-genotoxic
		in vivo MNT No alert	in vivo MNT Not genotoxic	High (In Domain)	
	OE incl. read across	Ames No alerts	Ames Not mutagenic	High (In Domain)	non-genotoxic
		MNT/CA No suitable read across candidate	MNT/CA -	-	
	OE	No alert	Ames Not mutagenic MNT Not mutagenic	- (No Domain definition)	non-genotoxic
	OA	Ames No alert	Ames Not mutagenic	Low (Out of Domain)	non-genotoxic
		CA No alert	CA in vitro Not genotoxic	Low (Out of Domain)	
	VE	-	Ames Not mutagenic	Low (Out of Domain)	non-genotoxic

CU = Case Ultra; OE = OECD-toolbox; OA = OASIS-times; VE = Vega
CA = Chromosomal aberration; MNT = Micronucleus Test; WoE = weight of evidence

The CASE Ultra analysis revealed a negative prediction for the bacterial mutagenicity by several modules: the Ames Konsolidator, Expert model, E. coli trained and S. thyphimurium trained and untrained models, but an inconclusive prediction by E. coli untrained module based on the same structural alert “Dimethylpyrimidine” that was already obtained for the parent compound [see KCA 5.8.1/51 2017/1100634] and rejected based on available experimental data.
Negative prediction for the chromosomal aberration was obtained for in vivo endpoint by all MNT mice models applied [see KCA 5.8.1/52 2017/1100635].
All Case Ultra predictions were of high reliability.

OASIS Times [see molecule 6 of report KCA 5.8.1/2 2014/1326432] predicted M605F008 and in silico generated metabolites to be not mutagenic in the Ames test with or without metabolic activation with the limitation that the molecule was out of the prediction domain. In the module for chromosomal aberration [see KCA 5.8.1/15 2015/1198645] the prediction was negative for the relevant metabolite itself and further in silico generated metabolites; however the prediction was out of the total domain for this model.

The VEGA prediction in all three modules (CAESAR, SarPy, TOXTREE) was not mutagenic, however the reliability of these predictions was rather low as the structures could be out of the domain [see molecule 7 of report KCA 5.8.1/16 2015/1186244].

Negative prediction was obtained for the Ames endpoint [see KCA 5.8.1/53 2017/1100632 by the modified OECD-toolbox based on read across with parent compound pyrimethanil and/or its metabolites M605F007, M605F025 M605F002; M605F019, M605F021 and M605F023. Due to this modification, the predicted mutagenicity endpoint was "in the domain". For the chromosomal aberration endpoint, no suitable read across candidate could be found, and thus no prediction on that endpoint was possible.
Information from OECD Toolbox (previous version v3.3) [see KCA 5.8.1/1 2015/1198463] revealed no alerts for DNA binding or genotoxicity as evaluated in various modules.

When compared to M605F007 as presented above both metabolites do not raise any relevant alert in any of the QSAR models applied. Moreover, the TIMES metabolic pathway prediction in the OASIS model are comparable for both metabolites. Thus, the QSAR data support the consideration to group both metabolites together.

In conclusion, in the structure activity evaluation tools employed there was no alert for mutagenicity.

B Toxicity studies for M605F008

Studies evaluated in the draft monograph of rapporteur member state Austria of April, 2004: No toxicity studies were evaluated during Annex I inclusion of pyrimethanil.

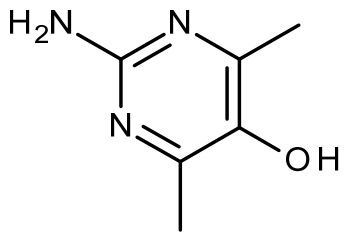
New toxicity studies not already evaluated in the draft monograph of rapporteur member state Austria of April, 2004: No new studies are available.

C Toxicological evaluation of metabolite M605F008

No structural alert for mutagenicity was identified in the applied QSAR models. M605F008 differs from M605F007 by the desaminated side chain. This transition is considered not to increase toxicity. In particular for the considerations on genotoxicity none of the QSAR approaches applied raised any concern for M605F008. For the parental metabolite M605F007 the limited and inconclusive concern raised in silico was not confirmed by genotoxicity testing. Thus it was considered reasonable to group both metabolites together and to conclude based on the QSAR predictions in combination with the genotoxicity data for M605F007 yielding clear negative results in an Ames test and an in vitro micronucleus test that no genotoxic concern is given for M605F008.

Thus, M605F008 is by applying the grouping approach considered to be not genotoxic and the TTC-threshold of 1.5 µg/kg bw/day can be applied for risk assessment purposes.

Metabolite M605F016 (Py-Hydroxy of AN7)



M605F016 the pyrazole ring hydroxylate of M605F007 is a metabolite of Pyrimethanil that was determined in rotational crops.

A Structural alerts for M605F016

Metabolite	QSAR tools applied	Structural alerts for genotoxicity	Reliability	Evaluation
M605F016	OE	Alert for Ames, MN in ISS module No alert for MN, Ames, CA in OASIS module	Low	Inconclusive alerts covered by toxicological testing of M605F007
	OA	Ames Not mutagenic	Low	
		CA Not clastogenic	Low	
	VE	Inconclusive mutagenic in TOXTREE module not mutagenic in CAESAR and SarPy module	Low to moderate	

OE = OECD-toolbox; OA = OASIS-times; VE = Vega
CA = Chromosomal aberration; MN = Micronuclei

Metabolite	QSAR tools applied	Structural alerts for genotoxicity	Prediction on genotoxicity	Reliability	Evaluation
M605F016	CU	Ames Alerts “Aminopyrimidine” and “Primary aromatic amines”	Ames Mutagenic / Not mutagenic / Inconclusive	High (In Domain)	Alerts overruled by experimental data on M605F007
		in vivo MNT No alerts	in vivo MNT Not genotoxic	High (In Domain)	
	OE incl. read across	Ames “Primary aromatic amines”	Ames Not mutagenic	High (In Domain)	Alerts overruled by experimental data on M605F007
		MNT/CA No suitable read across candidate	MNT/CA -	-	
	OE	Alert for Ames, MNT in vivo in ISS module No alert for MNT, Ames, CA in OASIS module	Ames Inconclusive MNT Inconclusive	- (No Domain definition)	Alerts overruled by experimental data on M605F007
	OA	Ames No alert	Ames Not mutagenic	Low (Out of Domain)	Non-gentoxic
		CA No alert	CA in vitro Not genotoxic	Low (Out of Domain)	
	VE	-	Ames Inconclusive CEASAR and SarPy – not mutagenic Toxtree - mutagenic	Reasonable by QSAR, low by WoE	Prediction overruled by experimental data on M605F007

CU = Case Ultra; OE = OECD-toolbox; OA = OASIS-times; VE = Vega
CA = Chromosomal aberration; MNT = Micronucleus Test; WoE = weight of evidence

The CASE Ultra analysis revealed a negative prediction for the bacterial mutagenicity by several modules: the Ames Konsolidator, E. coli trained and S. thyphimurium trained and untrained models, but a positive prediction by the Expert based on the alert “primary aromatic amines” with a positive prediction probability of 77.7% (model’s probability threshold: 50%). The untrained E.coli module revealed an inconclusive outcome due to the structural alerts “Aminopyrimidine” with a positive prediction probability of 47.5% (model’s probability threshold: 50%; grey zone: 40% – 60%) [see KCA 5.8.1/51 2017/1100634]. However, since a simple hydroxylation of the ring system without any cleavage of the ring is not considered to induce higher toxicity, the negative results obtained experimentally for M605F007 are considered to be applicable also for M605F016. Therefore, the alerts obtained in silico are overruled by the existing experimental data that were negative.
Negative prediction for the chromosomal aberration was obtained for in vivo endpoint by all applied MNT mice models [see KCA 5.8.1/52 2017/1100635].
All Case Ultra predictions were of high reliability.

OASIS Times [see KCA 5.8.1/27 2015/1198713] predicted M605F016 and in silico generated metabolites to be not mutagenic in the Ames test with or without metabolic activation with the limitation that the molecule was out of the prediction domain. In the module for chromosomal aberration [see KCA 5.8.1/28 2015/1186248] the prediction was negative for the relevant metabolite itself and further in silico generated metabolites; however the prediction was out of the total domain for this model.

The VEGA prediction in two out of three modules (CAESAR, SarPy) was not mutagenic, however the reliability of these predictions was rather low as the structures were out of the domain. A positive prediction and the structural alert SA_28 "Primary aromatic amine, hydroxyl amine and its derived esters (with restrictions)" was received in the Toxtree module, being of moderate reliability [see molecule 8 of report KCA 5.8.1/4 2015/1186245].

Negative prediction was obtained for the Ames endpoint [see KCA 5.8.1/53 2017/1100632 by the modified OECD-toolbox based on read across with parent compound pyrimethanil and/or its metabolites M605F007, M605F025 M605F002; M605F019, M605F021 and M605F023. Due to this modification, the predicted mutagenicity endpoint was "in the domain". For the chromosomal aberration endpoint, no suitable read across candidate could be found, and thus no prediction on that endpoint was possible.
Information from OECD Toolbox (previous version v3.3) [see KCA 5.8.1/1 2015/1198463] revealed no alerts for DNA binding or genotoxicity as evaluated in various modules, including OASIS DNA alerts for Ames, MN, and CA. The alerts "H-acceptor-path 3-H-acceptor" and "Primary aromatic amine, hydroxyl amine and its derived esters" for mutagenicity were received from the ISS module. However, positive predictivity varies within the ISS module and is rather low for the structural alert SA_34 ("H-acceptor-path3-H-acceptor") [Benigni et al., 2012, DocID 2012/1368942]. The alert SA_28 "Primary aromatic amine, hydroxyl amine and its derived esters (with restrictions)" is also available for M605F007 and was clearly rejected by toxicological testing. Thus, taken together the similar chemical structure and the comparable TIMES metabolic pathway prediction in the OASIS model, this alert is considered to be not relevant.

In conclusion, in the structure activity evaluation tools employed there was a limited alert for mutagenicity, which is considered of no relevance for the in vivo situation, as negative in vitro Ames and micronucleus assays are available with the structural analogue M605F007.

B Toxicity studies for M605F016

Studies evaluated in the draft monograph of rapporteur member state Austria of April, 2004: No toxicity studies were evaluated during Annex I inclusion of pyrimethanil.

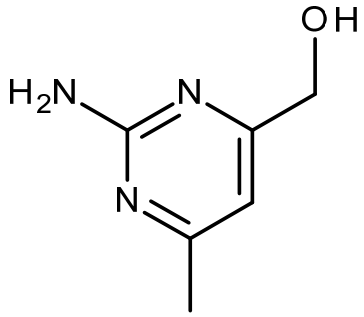
New toxicity studies not already evaluated in the draft monograph of rapporteur member state Austria of April, 2004: No new studies are available.

C Toxicological evaluation of metabolite M605F016

The structural alert for mutagenicity in silico identified for M605F016 was clearly rejected by the available toxicological tests yielding clear negative results in an Ames test and an in vitro micronucleus test with the analogue substance M605F007.
Furthermore, exposure to the metabolite M605F016 is below the Cramer Class III-threshold of 1.5 µg/kg bw/day).

Thus, M605F016 is by applying the grouping approach considered to be not genotoxic and the TTC-threshold of 1.5 µg/kg bw/day can be applied for risk assessment purposes.

Metabolite M605F032 (Me-Hydroxy of AN7)



M605F032 the methyl hydroxylated derivative of M605F007 is a metabolite of Pyrimethanil that was determined in plants and rotational crops.

A Structural alerts for M605F032

Metabolite	QSAR tools applied	Structural alerts for genotoxicity	Reliability	Evaluation
M605F032	OE	Alert for Ames, MN in ISS module No alert for MN, Ames, CA in OASIS module	Low	Inconclusive alerts covered by toxicological testing of M605F007
	OA	Ames Not mutagenic	Low	
		CA Not clastogenic	Low	
	VE	Inconclusive mutagenic in TOXTREE module not mutagenic in CAESAR and SarPy module	Moderate	

OE = OECD-toolbox; OA = OASIS-times; VE = Vega
CA = Chromosomal aberration; MN = Micronuclei

Metabolite	QSAR tools applied	Structural alerts for genotoxicity	Prediction on genotoxicity	Reliability	Evaluation
M605F032	CU	Ames Alerts “Dimethylpyrimidine”, “Aminopyrimidine” and “Primary aromatic amines”	Ames Inconclusive	High (In Domain)	Alerts overruled by experimental data on M605F007
		in vivo MNT No alerts	in vivo MNT Not genotoxic / Known negative	High (In Domain)	
	OE incl. read across	covered by experimental data on M605F007			non-genotoxic
	OE	Alert for Ames, MNT in vivo in ISS module No alert for MNT, Ames, CA in OASIS module	Ames Inconclusive MNT Inconclusive	- (No Domain definition)	Alerts overruled by experimental data on M605F007
	OA	Ames No alert	Ames Not mutagenic	Low (Out of Domain)	Non-gentoxic
		CA No alert	CA in vitro Not genotoxic	Low (Out of Domain)	
	VE	-	Ames Inconclusive CEASAR and SarPy – not mutagenic Toxtree - mutagenic	Reasonable by QSAR, low by WoE	Prediction overruled by experimental data on M605F007

CU = Case Ultra; OE = OECD-toolbox; OA = OASIS-times; VE = Vega
CA = Chromosomal aberration; MNT = Micronucleus Test; WoE = weight of evidence

The CASE Ultra analysis revealed a negative prediction for the bacterial mutagenicity by several modules: the Ames Konsolidator, E. coli trained and S. thyphimurium trained and untrained models, but a positive prediction by the Expert and E. coli untrained module [see KCA 5.8.1/51 2017/1100634]. The untrained E. coli module revealed a positive outcome due to the structural alerts “Dimethylpyrimidine” and “Aminopyrimidine” with a positive prediction probability of 72.3% (model’s probability threshold: 50%; grey zone: 40% – 60%). The Expert module revealed an inconclusive outcome due to the structural alerts “Aminopyrimidine” with a positive prediction probability of 77.7% (model’s probability threshold: 50%; grey zone: 40% – 60%). However, since a simple hydroxylation of the ring system without any cleavage of the ring is not considered to induce higher toxicity, the negative results obtained experimentally for M605F007 are considered to be applicable also for M605F016. Therefore, the alerts obtained in silico are overruled by the existing experimental data that were negative.
Negative prediction for the chromosomal aberration was obtained for in vivo endpoint by all applied MNT mice models [see KCA 5.8.1/52 2017/1100635].
All Case Ultra predictions were of high reliability.

OASIS Times [see KCA 5.8.1/29 2015/1198714] predicted M605F032 and in silico generated metabolites to be not mutagenic in the Ames test with or without metabolic activation with the limitation that the molecule was out of the prediction domain. In the module for chromosomal aberration [see KCA 5.8.1/30 2015/1186249] the prediction was negative for the relevant metabolite itself and further in silico generated metabolites; however the prediction was out of the total domain for this model.

The VEGA prediction in two out of three modules (CAESAR, SarPy) was not mutagenic, however the reliability of these predictions was rather low as the structures were out of the domain. A positive prediction and the structural alert SA28 “Primary aromatic amine, hydroxyl amine and its derived esters (with restrictions)” was received in the Toxtree module, being reasonably reliable [see molecule 7 of report KCA 5.8.1/4 2015/1186245].

For the modified OECD Toolbox including read across, the available experimental data on M605F007 were considered to be applicable for its hydroxylated metabolite, and thus M605F032 was assessed to be a “known negative”.
Information from OECD Toolbox (previous version v3.3) [see KCA 5.8.1/1 2015/1198463] revealed no alerts for DNA binding or genotoxicity as evaluated in various modules, including OASIS DNA alerts for Ames, MN, and CA. The alerts “H-acceptor-path 3-H-acceptor” and “Primary aromatic amine, hydroxyl amine and its derived esters” for mutagenicity were received from the ISS module. However, positive predictivity varies within the ISS module and is rather low for the structural alert SA_34 (“H-acceptor-path3-H-acceptor”) [Benigni et al., 2012, KCA 5.8.1/17 2012/1368942]. The alert SA_28 “Primary aromatic amine, hydroxyl amine and its derived esters (with restrictions)” is also available for M605F007 and was clearly rejected by toxicological testing. Thus, taken together the similar chemical structure and the comparable TIMES metabolic pathway prediction in the OASIS model, this alert is considered to be not relevant.

In conclusion, in the structure activity evaluation tools employed there was a limited alert for mutagenicity, which is considered of no relevance for the in vivo situation, as negative in vitro Ames and micronucleus assays are available with the structural analogue M605F007.

B Toxicity studies for M605F032

Studies evaluated in the draft monograph of rapporteur member state Austria of April, 2004: No toxicity studies were evaluated during Annex I inclusion of pyrimethanil.

New toxicity studies not already evaluated in the draft monograph of rapporteur member state Austria of April, 2004: No new studies are available.

C Toxicological evaluation of metabolite M605F032

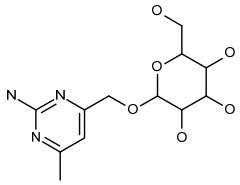
The structural alert for mutagenicity in silico identified for M605F032 was clearly rejected by the available toxicological tests yielding negative results in an Ames test and an in vitro micronucleus test with the analogue substance M605F007.
Furthermore, exposure to the metabolite M605F032 is below the Cramer Class III-threshold of 1.5 µg/kg bw/day).

Thus, M605F032 is by applying the grouping approach considered to be not genotoxic and the TTC-threshold of 1.5 µg/kg bw/day can be applied for risk assessment purposes.

Conjugates of hydroxylation products

No additional QSAR evaluations were performed with the conjugated metabolites. The conjugated metabolite M605F033 is the Glucose-conjugate of M605F033. It is expected that this sugar conjugate will be cleaved in the mammalian gastrointestinal tract to form endogenous glucose without toxicologically relevant properties in addition to the predecessor hydroxylate. The conjugated molecule is supposed to have the same or less toxicologically relevant properties as the parent molecule. The possibility of hydrolysis is covered by the respective QSAR evaluation for the parent molecule that id discussed above.

Metabolite M605F033



Metabolite M605F033 is the glucose conjugate of M605F032, occurring in plants and rotational crops. The excretion product M605F033 is considered to be not more toxic than the predecessor hydroxylate M605F032, which in turn is considered to be covered by the toxicological testing of the unhydroxylated predecessor M605F007 [see "Toxicological evaluation of metabolite M605F007" above].

Thus, M605F033 is by applying the grouping approach considered to be not genotoxic and the TTC-threshold of 1.5 µg/kg bw/day can be applied for risk assessment purposes.

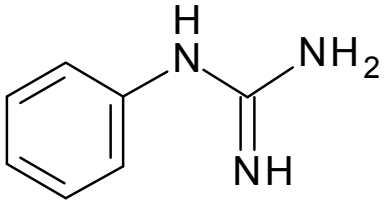
Conclusion for group 2 metabolites:

QSAR evaluation of group 2 metabolites was inconconclusive, with a low reliability. A positive prediction was received for M605F007 and hydroxylates thereof (M605F016 and M605F032) in one of the three VEGA modules (Toxtree) as well as in the ISS modules of the OECD toolbox which are both based on the same prediction model (Benigni Bossa rules) again with a low reliability. In contrast the OASIS predictions for Ames and chromosomal aberration for the structures itself as well as for in silico derivatives thereof was negative in all cases. The desaminated derivative M605F008 revealed no alert for genotoxicity in any of the models applied. The positive genotoxicity predictions based on the Benigni-Bossa rules are considered not relevant as an Ames test and an in vitro micronucleus test performed with M605F007 showed clearly negative results. Thus, group 2 metabolites are considered to be not genotoxic.
Although the results of the acute oral toxicity of M605F007 indicate a slightly higher toxicity compared to the parent substance pyrimethanil, this is considered not relevant as all group 2 metabolites are found below the TTC-trigger of 1.5 mg/kg bw/day for Cramer-class III substances.

Group 3: Cleavage products phenyl-moiety

The only cleavage product attributed to the phenyl moiety of pyrimethanil is M605F025 (Phenylguanidin).

Metabolite M605F025 (Phenylguanidine)



M605F025 (Phenylguanidine) is a rat, livestock, plant and rotational crop metabolite of pyrimethanil.

A Structural alerts for M605F025 (Phenylguanidine)

Metabolite	QSAR tools applied	Structural alerts for genotoxicity	Reliability	Evaluation
M605F025	OE	Not genotoxic	Low	Not genotoxic
	OA	Ames Not genotoxic	Low	
		CA Not clastogenic	Low	
	VE	Not mutagenic	Reasonable	

OE = OECD toolbox; OA = OASIS-times; VE = Vega
CA = Chromosomal aberration; MN = Micronuclei

Metabolite	QSAR tools applied	Structural alerts for genotoxicity	Prediction on genotoxicity	Reliability	Evaluation
M605F025	CU	Ames no alert	Ames Not mutagenic	High (In Domain)	Alerts overruled by experimental data on M605F025
		in vivo MNT Alert “Guanidine” and “Phenyl”	in vivo MNT Gentoxic / Not genotoxic	High (In Domain)	
	OE incl. read across	known negative due to available experimental data			non-genotoxic
	OE	No alert	Ames Not mutagenic MNT Not genotoxic	- (No Domain definition)	non-genotoxic
	OA	Ames No alert	Ames Not mutagenic	Low (Out of Domain)	non-gentoxic
		CA No alert	CA in vitro Not genotoxic	Low (Out of Domain)	
	VE	-	Ames Not mutagenic	Moderate (Possible out of Domain)	non-gentoxic

CU = Case Ultra; OE = OECD-toolbox; OA = OASIS-times; VE = Vega
CA = Chromosomal aberration; MNT = Micronucleus Test; WoE = weight of evidence

The CASE Ultra analysis revealed a negative prediction for the bacterial mutagenicity by all modules applied: the Ames Konsolidator, trained and untrained E. coli and S. thyphimurium module, and Expert module, without identification of any alert [see KCA 5.8.1/51 2017/1100634]. Positive prediction for the chromosomal aberration was obtained for in vivo endpoint by untrained MNT mice model with the structural alert “Guanidine”, revealing a positive prediction probability of 61.1% (model’s probability threshold: 45%, grey zone: 35% to 55%). However, the trained modules revealed a negative prediction, albeit a limited structural alert “Phenyl” that was already demonstrated but rejected for the parent compound - pyrimethanil, was obtained by the MNT mice model trained on EFSA and BASF data set [see KCA 5.8.1/52 2017/1100635]. Additionally, both alerts were not verified by the existing experimental data on M605F025 that were negative. Therefore, the structural alerts “Guanidine” and “Phenyl” were considered as not relevant and rejected.
All Case Ultra predictions were of high reliability.

OASIS Times [see KCA 5.8.1/31 2014/1326434] predicted M605F025 and in silico generated metabolites to be not mutagenic in the Ames test with or without metabolic activation with the limitation that the molecule was out of the prediction domain. In the module for chromosomal aberration [see KCA 5.8.1/32 2014/1326433] the prediction was negative for the relevant metabolite itself and further in silico generated metabolites; however the prediction was out of the total domain for this model.

The VEGA prediction [see KCA 5.8.1/33 2015/1192118] in all three modules (CAESAR, SarPy, TOXTREE) was "**non-mutagen"**, however the reliability of these predictions was varying. While the CAESAR prediction had a moderate reliability the reliability of the SarPy prediction was low but the TOXTREE prediction reliability was high.

Based on the available experimental data on M605F025 that were negative, this metabolite was considered to be "known negative" by OECD toolbox including read across models.
In the OECD toolbox (previous version v3.3) [see KCA 5.8.1/1 2015/1198463] there was no structural alert identified related to protein or DNA-binding and genotoxicity (Ames, MN, and CA) by OASIS.

In conclusion, no alerts for mutagenicity were received in any module evaluated.

B Toxicity studies for M605F025

Studies evaluated in the draft monograph of rapporteur member state Austria of April, 2004: No toxicity studies were evaluated during Annex I inclusion of pyrimethanil.

New toxicity studies not already evaluated in the draft monograph of rapporteur member state Austria of April, 2004: Two valid Ames tests, and a supplemental chromosome aberration in vitro study with phenylguanidine-carbonate are available and are discussed below. A rat micronucleus test ~~is currently~~ was conducted subsequently to clarify the equivocal results obtained in the in vitro chromosome aberration study which has several methodological flaws. Final Study report ~~results are expected by end of October 2015 and will be~~ was provided to the RMS in December 2015 ~~as will be the final report of as soon as available~~. Furthermore, a 90-day and a developmental toxicity study in Wistar rats are provided below. The material applied for testing also called CGA 263208 tech. or CA 1139A was the carbonate salt of phenylguanidine for stabilization of the test-compound. CA 1139A tested as an intermediate was a compound presumably analysed for phenylguanidine content of 76.2%. It remains unclear whether a carbonate or hydrocarbonate was the testing material. CGA 263208 tech. tested as a metabolite had a stated purity of about 95% and is assumed to represent the phenylguandine carbonate salt content. As no information on CAS-No is assigned in the reports and naming varies to be either phenylguanidine-carbonate or phenylguanidine-hydrocarbonate, the hydration status of the salt remains again unclear. This is however not considered to impair the toxicological evaluation of phenylguanidine and thus the provided studies are considered applicable to evaluate the toxicity of the metabolite M605F025.

Report:	CA 5.8.1/34 Hertner T., 1994 a Salmonella and Escherichia/mammalian-microsome mutagenicity test 1994/1005589
Guidelines:	OECD 471, EPA 798.5265, Official Journal of the European Communities L383A (1992)
GLP:	yes (certified by Swiss Federal Office of Public Health, Berne, Switzerland)
Deviations:	no

Executive Summary

S. typhimurium strains TA 98, TA 100, TA 102, TA 1535 and TA 1537, and a strain of *E. coli* WP2 uvrA were exposed to phenylguanidine-carbonate (Batch: EA168376, Purity: ca. 95.2%) using DMSO as a solvent in the presence and absence of metabolic activation for 48 hours. Vehicle and positive controls were included in each experiment.

In the Ames standard plate test (SPT) the test item was tested in five concentrations in a range of 61.73 to 5000 µg/plate with and without S9 mix (Aroclor-induced rat liver S9 mix).

No precipitation of the test substance was found up to the highest concentration. Background growth was reduced only with strain TA100 treated with the highest concentration (5000 µg/plate) with metabolic activation, but number of revertants was unaffected, thus no cytotoxicity was apparent at any concentration with or without metabolic activation in the main experiments.

An increase in the number of his^+ and trp^+ revertants was not observed in standard plate tests either without S9 mix or after the addition of a metabolizing system. The positive controls induced the appropriate response in the corresponding strains, thus demonstrating the sensitivity of the test system.

According to the results of the study, the test substance phenylguanidine-carbonate is not mutagenic in the Ames standard plate test under the experimental conditions of the study.

(BASF DocID 1994/1005589)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Description:	CGA 263208 tech. (phenylguanidine-carbonate) solid
Lot/Batch #:	EA168376
Purity:	95.2%
Stability of test compound:	No data concerning stability of the test compound was presented in the study report, except values from HPLC analysis, where analysed concentrations were found to be 86.3% and 88.5% of the nominal concentrations.
Solvent used:	DMSO

2. Control Materials:

Negative control:	None
Vehicle control:	The vehicle control with and without S9 mix only contained the vehicle used for the test substance at the same concentration and volume for all tester strains.
Solvent/final concentration:	100 µL/plate

Positive control compounds tested without addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	Sodium azide	Water (bidest.)	5 µg/plate
TA 102	Mitocycin C	Water (bidest.)	2 µg/plate
TA 1535	Sodium azide	Water (bidest.)	5 µg/plate
TA 1537	9-Aminoacridine (AAC)	DMSO	150 µg/plate
TA 98	2-Nitrofluorene	DMSO	20 µg/plate
WP2 uvrA	4-Nitroquinoline-N-oxide (4-NQO)	DMSO	2 µg/plate

Positive control compounds tested with addition of rat metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	2-Aminoanthracene	DMSO	10 µg/plate
TA 102	2-Aminoanthracene	DMSO	20 µg/plate
TA 1535	Cyclophosphamide	Water (bidest.)	400 µg/plate
TA 1537	2-Aminoanthracene	DMSO	10 µg/plate
TA 98	2-Aminoanthracene	DMSO	10 µg/plate
WP2 uvrA	2-Aminoanthracene	DMSO	50 µg/plate

3. Activation:

S9 was produced from the livers of 7-week old male RAI rats (Tif:RAIf[SPF]), weighting 177 – 197 g, that were treated with Aroclor 1254 (500 mg/kg bw/day) i. p. for 5 days.

The rat liver S9-mix had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	100 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10%

4. Test organisms:

S. typhimurium strains: TA 98, TA 100, TA 102, TA 1535, TA 1537
E. coli strains: WP2 uvrA
The bacterial strains are checked for the following characteristics at regular intervals: deep rough character (rfa), ampicillin resistance (R factor plasmid), and UV-light sensitivity (absence of uvrB and uvrA genes in Salmonella and E. coli strains, respectively).

Histidine and tryptophan auxotrophy was automatically proven in each experiment via the spontaneous rate.

5. Test concentrations:

Preliminary range-finding test: TA 102 and WP2 uvrA were exposed to dose levels of 20.58, 61.73, 185.19, 555.56, 1666.67 and 5000 µg/plate with and without metabolic activation.

Standard plate test: TA 98, TA 100, TA 102, TA 1535, TA 1537 and WP2 uvrA were exposed to dose levels of 61.73, 185.19, 555.56, 1666.67 and 5000 µg/plate with and without metabolic activation in two independent experiments.

B. TEST PERFORMANCE:

1. Dates of experimental work: 15-June – 01-July-1994, finalization date: 18-July-1994

2. Preliminary range-finding assay:

A mixture of 2-mL portions of warm top agar (0.6% agar and 0.6% NaCl, supplemented with 10% of 0.5 mM L-histidine + 0.5 mM (+)-biotin or 0.5 mM L-tryptophan), 0.1 mL test solution or vehicle, 0.1 mL fresh bacterial culture, 0.5 mL S9-mix (in tests with metabolic activation) or 0.5 mL phosphate buffer (in tests without metabolic activation) was poured onto minimal agar in Petri dishes. Each Petri dish contained about 20 mL minimal agar (1.5% agar supplemented with 2.0% salts of Vogel-Bonner Medium E and 2.0% glucose). After horizontal shaking (130 - 140 rounds/minute) incubation at 37°C for 48 hours in the dark, reduction in background lawn was determined.

3. Standard plate test:

A mixture of 2-mL portions of warm top agar (0.6% agar and 0.6% NaCl, supplemented with 10% of 0.5 mM L-histidine + 0.5 mM (+)-biotin or 0.5 mM L-tryptophan), 0.1 mL test solution or vehicle, 0.1 mL fresh bacterial culture, 0.5 mL S9-mix (in tests with metabolic activation) or 0.5 mL phosphate buffer (in tests without metabolic activation) was poured onto minimal agar in Petri dishes. Each Petri dish contained about 20 mL minimal agar (1.5% agar supplemented with 2.0% salts of Vogel-Bonner Medium E and 2.0% glucose). After horizontal shaking (130 – 140 rounds/minute) incubation at 37°C for 48 hours in the dark, the bacterial colonies (his^+ or try^+ revertants) were counted.

4. Statistics:

No special statistical tests were performed.

5. Evaluation criteria:

The test chemical is considered positive in this assay if the following criteria are met:

- At least a reproducible doubling of the mean number of revertants per plate above that of the negative control at any concentration for one or more of the following strains: TA 98, TA 1535, TA 1537, E.coli WP2 uvrA.
- A reproducible increase of the mean number of revertants per plate for any concentration above that of the negative control by at least a factor of 1.5 for strains TA 100 or TA 102.

Generally a concentration-related effect should be demonstrated.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Values from HPLC analyses (86.3% and 88.5%) were stated to be in agreement with the intended concentrations, thus said to demonstrate the stability of the test substance in vehicle, but no formal data were presented to support this claim.

B. TOXICITY

A preliminary cytotoxicity assay was performed prior to the main experiments. Background growth was reduced with TA 100 at 5000 µg/plate with metabolic activation. Thus, 5000 µg/plate was selected as the highest concentration ± S9.

No cytotoxicity was apparent at any concentration tested in the SPT experiments with or without metabolic activation. Strain TA 100 showed reduced background growth at 5000 µg/plate with metabolic activation, but the numbers of revertant colonies were not reduced.

C. MUTATION ASSAYS

In the SPT experiments with and without metabolic activation no biologically relevant increase in number of revertants was observed in any strain tested [see Table 5.8.1-13]. The positive controls yielded revertant numbers in a range expected for the respective strains and thus demonstrated the sensitivity of the test system.

No test item precipitation was observed with and without S9 mix.

Table 5.8.1-13: Standard plate test with phenylguanidine-carbonate - Mean number of revertants

Experiment 1: Standard plate test												
Strain	**TA 98**		**TA 100**		**TA 1535**		**TA 1537**		**TA 102**		**WP2 uvrA**	
Metabol. activation	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**
Vehicle control (DMSO)	21.67	32.00	105.00	130.67	16.00	11.33	8.67	9.67	245.00	236.67	21.67	22.00
Test item												
61.73 µg/plate	20.00	29.67	90.00	103.00	13.67	13.67	10.00	9.33	237.00	230.67	21.67	24.33
185.19 µg/plate	18.33	32.00	97.33	101.33	15.00	16.33	10.00	13.33	219.33	261.67	18.33	27.33
555.56 µg/plate	22.00	37.67	103.00	91.67	15.67	13.67	8.33	8.00	235.67	243.00	27.67	22.33
1666.67 µg/plate	19.00	37.67	103.67	92.33	14.67	10.33	8.33	9.00	245.00	261.00	22.33	27.67
5000 µg/plate	21.00	31.67	107.00	99.00	12.33	12.33	10.33	12.33	213.33	255.33	29.67	26.00
Pos. control§	2003	2166	1539	1559	1161	442	2120	229	1729	1586	912	861
Experiment 2: Standard plate test												
Strain	**TA 98**		**TA 100**		**TA 1535**		**TA 1537**		**TA 102**		**WP2 uvrA**	
Metabol. activation	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**
Vehicle control (DMSO)	22.00	37.33	119.33	105.33	13.33	11.00	13.00	7.33	296.00	303.33	19.00	19.33
Test item												
61.73 µg/plate	25.00	40.67	108.67	111.33	13.67	15.00	10.00	9.67	283.67	304.67	25.33	21.33
185.19 µg/plate	22.67	46.33	93.67	109.67	12.00	13.00	9.67	7.67	293.67	320.00	20.33	22.67
555.56 µg/plate	26.33	40.00	105.33	100.33	15.33	13.33	14.00	11.00	299.00	305.67	23.33	27.67
1666.67 µg/plate	25.67	38.00	123.00	105.00	12.00	12.67	10.67	4.67	282.33	268.33	21.67	20.67
5000 µg/plate	19.67	30.67	102.67	108.00	12.00	12.67	12.67	10.33	230.33	265.33	27.00	23.67
Pos. control§	1911	1962	1540	1491	1290	359	2159	269	1482	2113	926	833

§ = Compound and concentrations see Material and Methods (I.A.2.) above

III. CONCLUSION

According to the results of the present study, the test substance phenylguanidine-carbonate is not mutagenic in the Ames standard plate test under the experimental conditions chosen here.

Report:	CA 5.8.1/35 Hertner T., 1992 a CA 1139 A - Salmonella and Escherichia/liver-microsome test 1992/1005537
Guidelines:	OECD 471, EPA 798.5265
GLP:	yes (certified by Swiss Federal Office of Public Health, Berne, Switzerland)
Deviations:	no

Executive Summary

S. typhimurium strains TA 98, TA 100, TA 1535 and TA 1537, and a strain of *E. coli* WP2 uvrA were exposed to CA1139A (phenylguanidine-carbonate) (Batch: P.201025, Purity: 76.2%) using DMSO as a solvent in the presence and absence of metabolic activation for 48 hours. Vehicle and positive controls were included in each experiment.

In the Ames standard plate test (SPT) the test item was tested in five concentrations in a range of 312.5 to 5000 µg/plate with and without S9 mix (Aroclor-induced rat liver S9 mix).

No precipitation of the test substance was found up to the highest concentration. Background growth and the number of revertants was not affected by the test item with and without metabolic activation.

An increase in the number of his^+ and trp^+ revertants was not observed in standard plate tests either without S9 mix or after the addition of a metabolizing system. The positive controls induced the appropriate response in the corresponding strains, thus demonstrating the sensitivity of the test system.

According to the results of the study, the test substance phenylguanidine-carbonate is not mutagenic in the Ames standard plate test under the experimental conditions of the study.

(BASF DocID 1992/1005537)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material CA1139A (phenylguanidine-carbonate)

Description: solid (crystalline powder) / beige

Lot/Batch #: P.201025

Purity: 76.2%

Stability of test compound: Stability and concentration (lowest concentration used) determination of the test compound was presented in the study report via HPLC-UV data, where analysed concentrations were found to be in a range of 102.2 - 106.3% and 105.7 - 108.0% of nominal concentrations in the main and confirmatory experiments, respectively.

Solvent used: DMSO

2. Control Materials:

Negative control: None

Vehicle control: The vehicle control with and without S9 mix only contained the vehicle used for the test substance at the same concentration and volume for all tester strains.

Solvent/final concentration: 100 µL/plate

Positive control compounds tested without addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	Sodium azide	Water (bidest.)	5 µg/plate
TA 1535	Sodium azide	Water (bidest.)	5 µg/plate
TA 1537	9-Aminoacridine (AAC)	DMSO	150 µg/plate
TA 98	2-Nitrofluorene	DMSO	20 µg/plate
WP2 uvrA	4-Nitroquinoline-N-oxide (4-NQO)	DMSO	2 µg/plate

Positive control compounds tested with addition of rat metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	2-Aminoanthracene	DMSO	2.5 µg/plate
TA 1535	Cyclophosphamide	Water (bidest.)	400 µg/plate
TA 1537	2-Aminoanthracene	DMSO	2.5 µg/plate
TA 98	2-Aminoanthracene	DMSO	2.5 µg/plate
WP2 uvrA	2-Aminoanthracene	DMSO	50 µg/plate

3. Activation:

S9 was produced from the livers of approximately 7-week old male RAI rats (Tif:RAIF[SPF]), weighting 143 - 159 g and 198 - 221 g, that were treated with Aroclor 1254 (500 mg/kg bw/day in sesame oil) i. p. for 5 days.

The rat liver S9-mix had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	100 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10%

4. Test organisms:

S. typhimurium strains: TA 98, TA 100, TA 1535, TA 1537
E. coli strains: WP2 uvrA
The bacterial strains are checked monthly for the following characteristics at regular intervals: deep rough character (rfa), ampicillin resistance (R factor plasmid), and UV-light sensitivity (absence of uvrB and uvrA genes in Salmonella and E. coli strains, respectively).

Histidine and tryptophan auxotrophy was automatically proven in each experiment via the spontaneous rate.

5. Test concentrations:

Preliminary range-finding test: TA 100 and WP2 uvrA were exposed to doses ranging from 20.6 to 5000 µg/plate with and without metabolic activation.

Standard plate test: TA 98, TA 100, TA 1535, TA 1537 and WP2 uvrA were exposed to doses of 312.5, 625, 1250, 2500 and 5000 µg/plate with and without metabolic activation in two independent experiments.

B. TEST PERFORMANCE:

1. Dates of experimental work: 26-June – 17-July-1992, finalisation date: 20-July-1992

2. Preliminary range-finding assay:

A mixture of 2-mL portions of warm top agar (0.6% agar and 0.6% NaCl, supplemented with 10% of 0.5 mM L-histidine + 0.5 mM (+)-biotin or 0.5 mM L-tryptophan), 0.1 mL test solution or vehicle, 0.1 mL fresh bacterial culture, 0.5 mL S9-mix (in tests with metabolic activation) or 0.5 mL of 100 mM sodium phosphate buffer (in tests without metabolic activation) was poured onto minimal agar in Petri dishes. Each Petri dish contained about 20 mL minimal agar (1.5% agar supplemented with 2.0% salts of Vogel-Bonner Medium E and 2.0% glucose). After horizontal shaking (130 - 140 rounds/minute) incubation at 37°C for 48 hours in the dark, reduction in background lawn was determined.

3. Standard plate test:

A mixture of 2-mL portions of warm top agar (0.6% agar and 0.6% NaCl, supplemented with 10% of 0.5 mM L-histidine + 0.5 mM (+)-biotin or 0.5 mM L-tryptophan), 0.1 mL test solution or vehicle, 0.1 mL fresh bacterial culture, 0.5 mL S9-mix (in tests with metabolic activation) or 0.5 mL of 100 mM sodium phosphate buffer (in tests without metabolic activation) was poured onto minimal agar in Petri dishes. Each Petri dish contained about 20 mL minimal agar (1.5% agar supplemented with 2.0% salts of Vogel-Bonner Medium E and 2.0% glucose). After horizontal shaking (130 - 140 rounds/minute) incubation at 37°C for 48 hours in the dark, the bacterial colonies (his^+ or try^+ revertants) were counted.

4. Statistics:

No special statistical tests were performed.

5. Evaluation criteria:

The test chemical is considered positive in this assay if the following criteria are met:

- At least a reproducible doubling of the mean number of revertants per plate above that of the negative control at any concentration for one or more of the following strains: TA 98, TA 1535, TA 1537, E.coli WP2 uvrA.
- A reproducible increase of the mean number of revertants per plate for any concentration above that of the negative control by at least a factor of 1.5 for strains TA 100.

Generally a concentration-related effect should be demonstrated.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Values from HPLC-UV analyses were in agreement with the intended concentrations (102.2 - 106.3% and 105.7 - 108.0% of the nominal concentrations in the main and confirmatory experiments, respectively), thus the stability of the test substance in vehicle is confirmed.

B. TOXICITY

A preliminary cytotoxicity assay was performed with and without metabolic activation prior to the main experiments to define the highest concentration to be used. Background growth and number of revertants were not affected by the test item with TA 100 and WP2 uvrA with and without metabolic activation. Thus, 5000 µg/plate was selected as the highest concentration for testing with and without metabolic activation.

No cytotoxicity was apparent at any concentration tested in the SPT experiments (main and confirmatory) with or without metabolic activation.

C. MUTATION ASSAYS

In the SPT experiments with and without metabolic activation no biologically relevant increase in number of revertants was observed in any strain tested [see Table 5.8.1-14]. The positive controls yielded revertant numbers in a range expected for the respective strains and thus demonstrated the sensitivity of the test system.

No test item precipitation was observed with and without S9 mix.

Table 5.8.1-14: Standard plate test with phenylguanidine-carbonate - Mean number of revertants

Experiment 1: Standard plate test										
Strain	**TA 100**		**TA 1535**		**TA 1537**		**TA 98**		**WP2 uvrA**	
Metabol. activation	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**
Vehicle.control (DMSO)	109.3 ± 12.9	114.7 ± 14.0	7.0 ± 1.0	11.7 ± 0.6	5.0 ± 2.0	25.0 ± 9.6	13.0 ± 3.6	5.3 ± 1.2	21.7 ± 6.4	19.3 ± 5.1
Test item										
3125 µg/plate	104.0 ± 7.9	106.7 ± 4.9	7.3 ± 1.5	12.3 ± 2.5	4.0 ± 1.0	25.3 ± 3.8	13.3 ± 1.5	5.0 ± 2.0	16.0 ± 6.2	16.7 ± 4.2
625 µg/plate	99.7 ± 5.9	117.0 ± 4.9	8.3 ± 4.0	7.7 ± 0.6	7.0 ± 1.7	30.0 ± 6.6	9.0 ± 3.0	5.3 ± 2.9	15.3 ± 0.6	13.7 ± 1.5
1250 µg/plate	99.7 ± 6.7	99.0 ± 4.4	7.3 ± 0.6	12.7 ± 0.6	3.7 ± 1.5	25.0 ± 4.0	12.0 ± 7.9	4.7 ± 2.1	17.0 ± 3.0	21.7 ± 3.1
2500 µg/plate	107.7 ± 10.2	98.7 ± 18.9	8.3 ± 3.5	12.7 ± 4.5	6.3 ± 2.1	33.7 ± 6.7	13.3 ± 5.1	7.0 ± 0.0	21.0 ± 5.2	13.7 ± 1.5
5000 µg/plate	93.0 ± 8.5	101.0 ± 9.0	6.0 ± 1.0	7.3 ± 2.1	4.3 ± 1.2	30.7 ± 2.5	9.7 ± 4.0	6.0 ± 1.0	17.0 ± 2.6	16.7 ± 3.1
Pos. control[§]	888.3 ± 90.2	1386.0 ± 102.7	745.7 ± 44.5	378.0 ± 136.4	1005.3 ± 43.1	1205.7 ± 64.6	986.0 ± 16.1	99.7 ± 19.7	817.0 ± 145.7	948.3 ± 223.8
Experiment 2: Standard plate test										
Strain	**TA 100**		**TA 1535**		**TA 1537**		**TA 98**		**WP2 uvrA**	
Metabol. activation	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**
Vehicle.control (DMSO)	119.3 ± 3.8	134.0 ± 6.6	13.7 ± 1.5	10.0 ± 4.4	8.3 ± 3.2	8.0 ± 3.6	24.7 ± 6.4	44.7 ± 11.0	21.0 ± 6.0	14.3 ± 2.1
Test item										
3125 µg/plate	124.7 ± 10.4	121.0 ± 24.3	13.7 ± 1.2	12.0 ± 4.6	8.7 ± 6.7	6.0 ± 1.0	18.3 ± 2.9	34.3 ± 9.5	16.3 ± 1.5	16.7 ± 3.5
625 µg/plate	111.7 ± 6.8	117.0 ± 16.6	9.0 ± 5.3	9.3 ± 2.5	8.7 ± 1.5	8.7 ± 0.6	20.0 ± 5.0	31.7 ± 9.5	17.7 ± 2.5	17.3 ± 1.5
1250 µg/plate	113.3 ± 9.0	120.3 ± 26.5	11.3 ± 6.4	12.7 ± 4.0	9.0 ± 2.6	8.3 ± 3.2	15.0 ± 2.0	33.3 ± 2.5	14.7 ± 1.5	18.7 ± 3.2
2500 µg/plate	125.0 ± 2.0	123.0 ± 7.0	9.0 ± 2.0	14.7 ± 1.5	8.3 ± 2.1	9.7 ± 2.9	20.0 ± 5.6	40.7 ± 9.3	15.3 ± 4.9	17.0 ± 4.4
5000 µg/plate	97.0 ± 8.9	107.7 ± 34.1	9.0 ± 2.6	13.0 ± 2.6	10.3 ± 2.9	5.7 ± 2.1	15.7 ± 2.5	38.3 ± 6.4	19.3 ± 4.2	19.3 ± 5.5
Pos. control[§]	1274.0 ± 140.6	1998.0 ± 262.2	955.0 ± 110.6	449.0 ± 134.3	1758.7 ± 606.4	158.3 ± 34.5	1699.0 ± 199.1	2375.7 ± 270.5	711.3 ± 110.7	1278.0 ± 85.9

[§] = Compound and concentrations see Material and Methods (I.A.2.) above

III. CONCLUSION

According to the results of the present study, the test substance phenylguanidine-carbonate is not mutagenic in the Ames standard plate test under the experimental conditions chosen here.

Report:	CA 5.8.1/36 Chang S., 2017 a CA1139 - Gene mutation assay in chinese hamster - V79 cells in vitro (V79/HPRT) 2017/1124121
Guidelines:	OECD 476
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Klimaschutz, Landwirtschaft und Verbraucherschutz, Wiesbaden)

covered by LoA [see KCA 5.8.1/55 2017/1190281]

Executive Summary

CA1139 (Phenylguanidine carbonate salt, Metabolite M605F025 of pyrimethanil, Batch: WRS 1233/1, Purity: 93.8% as carbonate or 76.6% as phenylguanidine) was tested in vitro for its ability to induce forward mutations in mammalian cells by assessing the mutation of the HPRT locus in V79 cells. Three independent experiments were conducted in the presence and/or absence of metabolic activation. Based on the results of a preliminary cytotoxicity assay and a correction factor for purity of 1.3, concentrations of up to 2600 µg/mL were used in the main experiments. The treatment intervals for both experiments in the presence and absence of metabolic activation were 4 hours. Ethylmethanesulfonate (EMS) and 7,12-Dimethylbenöz(a)anthracene (DMBA) served as positive controls in the experiments without and with metabolic activation, respectively. After the incubation period treatment media were replaced by culture medium in both experiments and the cells were incubated for one week for expression of mutant cells. This was followed by incubation of cells in selection medium containing 6-thioguanine for about 1 week.

The recommended toxic range of approximately 10-20% relative survival was covered in nearly all experiments with and without metabolic activation.

No relevant increase in the number of mutant colonies was observed either with or without S9 mix. Albeit some inconsistent increases of the mutant frequencies above the 95% control limit were observed, statistical significant increase was lacking and all values were within the range of the historical control data.

Treatment with the positive controls EMS and DMBA resulted in a marked increase in the number of mutant colonies as well as of mutant frequencies in all experiments, thus demonstrating the sensitivity of the test.

Based on the results of the study it is concluded that under the conditions of this test CA1139 (Phenylguanidine carbonate salt, Metabolite M605F025 of pyrimethanil) does not induce forward mutations in mammalian cells in vitro.

(BASF DocID 2017/1124121)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material	CA1139 (Phenylguanidine carbonate salt, Metabolite M605F025 of pyrimethanil,)
Description:	powder (crystalline), light beige
Lot/Batch #:	WRS 1233/1
Purity:	93.8% (as carbonate salt) 76.6% (as phenylguanidine)
Stability of test compound:	The stability of the test substance under storage conditions was guaranteed by the sponsor. The expiry date was 30 Sept 2022.
Solvent used:	Dimethylsulfoxide (DMSO)
2. Control Materials:	
Negative control:	A negative control was not employed in this study
Vehicle control:	1% (v/v) DMSO in culture medium
Positive control -S9:	Ethylmethanesulfonate (EMS) 300 µg/mL (2.4 mM)
Positive control +S9:	7,12-Dimethylbenöz(a)anthracene (DMBA) 2.3 µg/mL (8.9 µM)
3. Activation:	S9 was produced from the livers of induced 8 – 12 week old male Wistar rats (RjHan:WI, weight approx. 220 – 320 g), supplier: Janvier Labs, Saint-Berthevin Cedex, France). The rats received a peroral application of 80 mg/kg bw phenobarbital and ß-naphthoflavone on 3 consecutive days. 24-hours after the last application, the livers were isolated, S9 fraction prepared by centrifugation at 9000 g and stored at -80°C in small batches. Each batch was routinely tested for metabolic capacity with known pro-mutagens in the Ames Test. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction is thawed at room temperature and S9-fraction is mixed with S9-supplement (cofactors). This preparation, the so-called S9-mix, was kept on ice until used.
	The protein content of the S9 preparations used was 28.1 mg/mL for the 1st experiment and 30.1 mg/L for the 2nd experiment.

Component	Concentration
Phosphate buffer (pH 7.4)	100 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM

0.75 mg/mL of the S9 mix was used for the experiments with metabolic activation leading to a final S9 concentration of 5% in the medium.

4. Test organism: V79 cells.

V79 cells have a high proliferation rate (doubling time about 12-16 h), sufficient high cloning efficiency (more than 50%) and karyotype with a modal number of 22 chromosomes. Stocks of the V79 cell line were maintained at -196°C in liquid nitrogen. Each batch used for mutagenicity testing was checked for mycoplasma contamination, karyotype stability and spontaneous mutant frequency. HPRT-deficient mutants were eliminated from the stock cultures by growing the cells in the HAT-medium, blocking de-novo synthesis of nucleotides.

5. Culture media:

Culture medium: MEM (minimal essential medium) containing Hank's salts, neomycin (5 µg/mL) and amphotericin B (1%).

Selection medium: ("TG" medium): Culture medium supplemented with 6-thioguanine at a final concentration of 11 µg/mL

6. Locus examined: hypoxanthine-guanine-phosphoribosyl transferase (H(G)PRT)

7. Test concentrations:

a) Preliminary toxicity assay: Eight concentrations ranging from 20.3 to 2600 µg/mL

b) Mutation assay:

± S9 162.5, 325, **650, 1300, 1733.3, 2166.6**, and **2600** µg/mL (evaluated concentrations are indicated in bold)

B. TEST PERFORMANCE:

1. Dates of experimental work: 27-Oct-2016 to 18-Apr-2016
Finalisation date: 31-May-2017

2. Preliminary cytotoxicity assay and concentration selection:

Cytotoxicity was assessed by determination of the cloning efficiency. About 500 cells (duplicate cultures per concentration level) were incubated as described for mutation assay – determination of cytotoxicity. After treatment with the test item, the colony forming ability was observed and compared to the controls. In addition to the cloning efficiency the following parameters were measured: pH, osmolarity and the determination of precipitates (solubility) at the beginning and at the end of treatment.

According to the current OECD Guideline 476 (2016) for cell gene mutation tests at least four analysable concentrations should be used. For freely-soluble and non-cytotoxic test items the maximum concentration should be 2 mg/mL, 2 µL/mL or 10 mM, whichever is the lowest. For cytotoxic test items the maximum concentration should result in approximately 10% to 20% relative adjusted cloning efficiency I and the analysed concentrations should cover a range from the maximum to little or no cytotoxicity. Relatively insoluble test items should be tested up to the highest concentration that can be formulated in an appropriate solvent as solution or homogenous suspension. These test items should be tested up to or beyond their limit of solubility.

3. Mutation Assay:

Seeding of Cells:	Two to three days after subcultivation stock cultures were trypsinized at 37 °C for 5 minutes. Then the enzymatic digestion was stopped by adding complete culture medium with 10% FBS and a single cell suspension was prepared. The trypsin concentration for all subculturing steps was 0.2% in phosphate buffered saline (PBS).
	Prior to the trypsin treatment the cells were treated with PBS containing 200 mg/L EDTA. Approximately 0.7 to 1.2×10^7 cells were seeded in plastic flasks. The cells were grown for 24 hours prior to treatment. With the cell doubling time of approximately 12 h this ensures a population of more than 20×10^6 cells treated with the test substance.

Cell treatment:	24 hours after seeding the cells, the medium was replaced with serum-free medium containing the test item, either without S9 mix or with 50 µL/mL S9 mix. Concurrent solvent and positive controls were treated in parallel. After 4 hours, this medium was replaced with complete medium following two washing steps with "saline G". Immediately after the end of treatment the cells were trypsinised as described above and sub-cultivated. At least 2.0×10^6 cells per experimental point (concentration series plus controls) were subcultured in 175 cm² flasks containing 30 mL medium.
Expression:	Three days after first sub-cultivation approximately 2.0×10^6 cells per experimental point were sub-cultivated in 175 cm² flasks containing 30 mL medium.
Selection:	Following the expression time of approximately 7 days five 80 cm² cell culture flasks were seeded with about 3 – $5x10^5$ cells each in medium containing 6-TG (11 µg/mL). After 7 – 10 days the colonies were stained with 10% methylene blue in 0.01% KOH solution. Colonies with more than 50 cells were counted. If in doubt, the colony size was checked with a preparation microscope.
Determination of Cytotoxicity:	Cloning efficiency 1 (survival): The survival (cloning efficiency 1; CE_1) was determined in parallel to the mutagenicity test. Approximately 500 cells per dose group were seeded into duplicate 25 cm^2 flasks and treated as described above. The colonies were fixed and stained 6 to 8 days after treatment as described below. Cloning efficiency 2 (viability): The viability (cloning efficiency 2; CE_2) was determined after the expression period, parallel to the mutant selection. Two additional 25 cm² flasks were seeded with approx. 500 cells each in non-selective medium to determine the viability.

Calculations:

Mutant frequency:

$$MF = \frac{\text{mean number of mutant colonies after selection period}}{\text{number of cells survived } (CE_{absolute})} \times 10^6$$

Cloning efficiency (CE,%) absolute:

$$CE_{absolute} = \frac{\text{mean number of colonies per flask}}{\text{number of cells seeded}} \times 100$$

relative, in comparison to control:

$$CE_{relative} = \frac{CE_{absolute} \text{ of the test group}}{CE_{absolute} \text{ of the vehicle control}} \times 100$$

Relative cell density (% of control):

$$CD_{relative} = \frac{\text{CD at 1st subculativation}}{\text{CD at 1st subculativation of the vehicle control}} \times 100$$

Relative survival (RS):

$$RS = \frac{\text{CE}_{relative}}{\text{CD}_{relative}} \times 100$$

4. Statistics:

The statistical analysis was performed in the mean values of culture I and II for all experiments.
A linear regression analysis (least squares, calculated using a validated excel spreadsheet) was performed to assess a possible dose dependency of mutant frequencies. The numbers of mutant colonies generated with the test item were compared to the solvent control groups. A trend is judged as significant whenever the p-value (probability value) is below 0.05.
A t-test was performed using a validated test script of "R", a language and environment for statistical computing and graphics, to evaluate an isolated increase of the mutation frequency at a test point exceeding the 95% confidence interval and other if necessary. Again, a t-test is judged as significant if the p-value (probability value) is below 0.05.
However, both, biological and statistical significance was considered together.

5. Acceptability criteria:

The gene mutation assay is considered acceptable if it meets the following criteria:

- The mean values of the numbers of mutant colonies per 106 cells found in the solvent controls of both parallel cultures remain within the 95% control limit of the laboratory historical control data range and are acceptable for addition to the laboratory historical control database.
- The positive control substances should produce a significant increase in mutant colony frequencies and remain within the historical control range of positive controls
- The cloning efficiency II (absolute value) of the solvent controls must exceed 50%.

6. Evaluation criteria:

A test chemical is considered to be clearly positive if, in any of the experimental conditions examined all of the following criteria are met:

- at least one of the test concentrations exhibits a statistically significant increase compared with the concurrent solvent control in both parallel cultures,
- the increase is concentration-related when evaluated with an appropriate trend test,
- any of the results are outside the distribution of the historical solvent control data.

When all of these criteria are met, the test chemical is then considered able to induce gene mutations in cultured mammalian cells in this test system.

Providing that all acceptability criteria are fulfilled, a test chemical is considered clearly negative if, in all experimental conditions examined:

- none of the test concentrations exhibits a statistically significant increase compared with the concurrent negative control,
- there is no concentration-related increase when evaluated with an appropriate trend test,
- all results are inside the distribution of the historical negative control data.

The test chemical is then considered unable to induce gene mutations in cultured mammalian cells in this test system.

There is no requirement for verification of a clearly positive or negative response.
In cases when the response is neither clearly negative nor clearly positive as described above, or in order to assist in establishing the biological relevance of a result, the data should be evaluated by expert judgement and/or further investigations. Performing a repeat experiment possibly using modified experimental conditions (e.g. concentration spacing) could be useful. In rare cases, even after further investigations, the data set will preclude making a conclusion of positive or negative results. Therefore, the test chemical response should be concluded to be equivocal (interpreted as equally likely to be positive or negative).

II. RESULTS AND DISCUSSION

A. PRE-TEST

The pre-experiment was performed in the presence and absence of metabolic activation. Test item concentrations between 20.3 µg/mL and 2600 µg/mL were used. The highest concentration was chosen with respect to the current OECD Guideline 476 (2016) and the purity of the test item.

A relevant toxic effect was observed in the experimental part without metabolic activation at the maximum concentration of 2600 µg/mL.

The test medium was checked for precipitation or phase separation at the end of each treatment period (4 hours) before the test item was removed. No precipitation or phase separation occurred up to the highest concentration with and without metabolic activation.

There was no relevant shift of the osmolarity and pH value even at the maximum concentration of the test substance in the pre-experiment (solvent control: 469 mOsm and pH 7.36 versus 452 mOsm and pH 7.76 at 2600 µg/mL).

The concentrations used in the main experiment were selected based on the data of the pre-experiment. The individual concentrations were generally spaced by a factor of 2. Narrower spacing was used at the highest concentrations to cover the toxic range more closely. Top dose was 2600 µg/mL for all three main experiments.

B. MAIN EXPERIMENT - MUTANT FREQUENCY

No relevant increase in the number of mutant colonies was observed either with or without S9 mix. Albeit some inconsistent increases of the mutant frequencies above the 95% control limit were observed, statistical significant increase was lacking and all values were within the range of the historical control data.
Treatment with the positive controls EMS and DMBA resulted in a marked increase in the number of mutant colonies as well as of mutant frequencies in all experiments, thus demonstrating the sensitivity of the test.

C. MAIN EXPERIMENT - CYTOTOXICITY

Except for repeat Experiment III, the recommended toxic range of approximately 10-20% RS was covered in all other experiments with and without metabolic activation.

Table 5.8.1-15: Gene mutation in mammalian cells - 1st experiment

Test group	Rel. survival -RS (%)	Mutant frequency (per 10^6 cells)	Cloning efficiency CE (%) relative	Relative cell density (%)
Without metabolic activation; 4-hour exposure period				
Vehicle (DMSO, 1%)	100	22.9	100	100
HCD 95% control limit		1.7 – 30.2		
range		3.4 – 41.0		
Test item				
650.0 µg/mL	93.6	22.0	120.0	77.0
1300.0 µg/mL	58.3	19.8	106.0	55.2
1733.3 µg/mL	54.8	25.5	89.7	61.2
2166.6 µg/mL	20.1	12.7	66.1	30.3
2600.0 µg/mL	7.5	12.3	40.9	18.9
Positive control EMS				
300.0 µg/mL	76.5	293.2	88.8	86.0
With metabolic activation; 4-hour exposure period				
Vehicle (DMSO)	100	29.7	100	100
HCD 95% control limit		2.0 – 29.4		
range		2.4 – 39.2		
Test item				
650.0 µg/mL	85.9	23.1	124.1	68.8
1300.0 µg/mL	95.4	15.6	106.2	89.9
1733.3 µg/mL	103.3	17.8	102.7	100.6
2166.6 µg/mL	69.5	24.5	80.4	85.6
2600.0 µg/mL	17.9	30.9	30.7	45.5
Positive control DMBA				
2.3 µg/mL	107.7	60.6	104.6	103.8

HCD = historical control data (including data of 105 – 111 HPRT studies performed between 2014 – 2016)

Table 5.8.1-16: Gene mutation in mammalian cells – 2nd and 3rd experiment

Test group	Rel. survival -RS (%)	Mutant frequency (per 10^6 cells)	Cloning efficiency CE (%) relative	Rel. cell density (%)
Without metabolic activation; 4-hour exposure period – 3rd experiment				
Vehicle (DMSO, 1%)	100	29.3	100	100
HCD 95% control limit		1.7 – 30.2		
range		3.4 – 41.0		
Test item				
650.0 µg/mL	82.6	30.5	85.9	96.0
1300.0 µg/mL	79.0	24.7	77.7	99.6
1733.3 µg/mL	60.9	21.6	62.5	97.6
2166.6 µg/mL	36.6	19.8	41.4	89.7
2600.0 µg/mL	12.9	21.7	17.6	77.5
Positive control EMS				
300.0 µg/mL	90.1	202.8	79.0	112.9
With metabolic activation; 4-hour exposure period – 2nd experiment				
Vehicle (DMSO)	100	23.2	100	100
HCD 95% control limit		2.0 – 29.4		
range		2.4 – 39.2		
Test item				
650.0 µg/mL	86.8	23.0	90.4	96.5
1300.0 µg/mL	84.7	15.6	86.6	98.2
1733.3 µg/mL	79.0	19.6	89.1	88.7
2166.6 µg/mL	60.5	19.9	66.9	90.5
2600.0 µg/mL	45.8	10.8	66.4	69.1
Positive control DMBA				
2.3 µg/mL	87.3	167.4	93.2	93.7

HCD = historical control data (including data of 105 – 111 HPRT studies performed between 2014 – 2016)

III. CONCLUSION

Based on the results of the study it is concluded that under the conditions of this test CA1139 (Phenylguanidine carbonate salt, Metabolite M605F025 of pyrimethanil) does not induce forward mutations in mammalian cells in vitro.

Report: CA 5.8.1/37
Hertner T., 1992 b
Cytogenetic test on chinese hamster cells in vitro (EC-conform)
1992/1005538

Guidelines: OECD 473, MAFF Testing Guidelines for Toxicology Studies: Metabolism Animals (Japan), EPA 798.5375, EEC B.10 (19 September 1984) rev. June 1989

GLP: yes
(certified by Ministry of Agriculture, Forestry and Fisheries of Japan, Japan)

Ex

Deviations: to the current OECD test guideline (OECD 473, 2014):

- In this study, the exposure time in experiments without metabolic activation was 18 h and 42 h, while one of the demanded test conditions should include also an exposure time of 3-6 hours without metabolic activation.
- In this study, 200 metaphases were evaluated in the test substance and vehicle control groups, and 50 metaphases were evaluated in the positive control group, while 300 metaphases per treatment are required in the OECD 473, 2014.
- In this study, the cytotoxicity is evaluated by the suppression of the mitotic index to 50 - 80% related to vehicle control that is equivalent to a mitotic index of 50 – 20% related to the vehicle control. The current OECD test guideline recommends the usage of mitotic index for cytotoxicity purpose only for human lymphocytes, and the maximum concentration tested for mutagenicity should be ≥ 45 ± 5% of the negative control. For cell lines, relative population doubling (RPD) or relative increase in cell count (RICC) are recommended by the current OECD test guideline.
- For the confirmatory study first and second experiment - 18 hours without metabolic activation and 3 hours exposure (18 hour sampling time) with metabolic activation - no cytoxicity determination (mitotic index) was reported. Thus, no judgement on the determined chromosomal aberration effects in relation to unspecific cytotoxicity is possible.
- No positive control data are reported for the 42 h exposure period
- No individual culture data are reported
- The evaluation criteria are not fully in line with the current guideline

Acceptance: ~~Due to the limitations in design and reporting the study is considered supplemental.~~
Despite the specified deviations, the study is valid and assigned reliability factor of 2 (reliable with restrictions)

Executive Summary

Phenylguanidine-carbonate (metabolite of BAS 605 F; Batch: P.201025, Purity: 76.2%) was tested in vitro for its ability to induce structural chromosome aberrations in Chinese Hamster Ovary (CHO CCL 61) cells in the presence and absence of metabolic activation. Based on the solubility limit (3300 µg/mL) the of the test item in the vehicle (culture medium (-S9) or nutrient mixture F-12 (+S9)) or induced cytotoxicity (suppression of mitotic index), phenylguanidine-carbonate was tested at concentrations of 103.13, 206.25, and 412.5 µg/mL (18 h exposure/18 h sampling time) and 51.56, 103.13 and 206.25 µg/mL (42 h exposure/ 42 h sampling time) in the absence of metabolic activation, and 412.5, 825, and 1650 µg/mL (3 h exposure/ 18 h sampling time) and 825, 1650 and 3300 µg/mL (3 h exposure / 42 h sampling time) in the presence of metabolic activation, respectively. For each independent experiment, vehicle and positive controls (cyclophosphamide (CPA) and mitomycin C (MMC) for the experiment with and without metabolic activation, respectively) were included to demonstrate the sensitivity of the test system. Metaphase arrest was induced by colcemid and cells were stained with orcein. At least 200 metaphases per treatment group and vehicle control, and 50 metaphases per positive control group were analysed for chromosomal aberrations.

Additionally, the DNA distribution of cell cultures was determined by flow cytometry after cell fixation and staining with DAPI.

The positive controls MMC and CPA induced substantial chromosome damage in any experiment. In the experiments without metabolic activation the study result is considered equivocal as the statistic significances obtained after 18 h exposure are not consistent when comparing the initial and the confirmatory repeat experiments or are not dose dependent in the 42 h exposure experiment. In the experiments with metabolic activation the study result is considered equivocal as well as the statistic significances obtained at high dose after 3 h exposure (18 h sampling) are not consistent when comparing the initial and the confirmatory repeat experiments. The statistical increase at the 42 h sampling after 3 h exposure with metabolic activation is attributed to indirect cytotoxic and/or osmotic effects at this high dose level. It is acknowledged that the statistically significant increases in chromosome aberration for the 18 h sampling time point was as for the experiment without metabolic action seen in the confirmatory experiment only for which no cytotoxicity is reported. Thus a shift in cytotoxicity cannot be ruled out. As stated above the study has several limitations in study design and reporting.
Thus, under the experimental conditions chosen here, the conclusion is drawn that phenyl-guanidine-carbonate (Metabolite of BAS 605 F) is equivocal for chromosome-damaging (clastogenic) properties under in vitro conditions using CHO cells.

Cell cycle analysis data indicated a shift in the DNA distribution in the absence of metabolic activation at the two highest concentrations tested (206.25 and 412.5 µg/mL). In the presence of metabolic activation, a shift in the DNA distribution profile could be detected at the highest concentration of 1650 µg/mL. Therefore, evidence was obtained for cell cycle disturbing effects in the CHO cells caused by the test substance or its metabolite at the higher concentrations tested.

(BASF DocID 1992/1005538)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: CA 1139A (Phenylguanidine-carbonate)
Description: Solid (crystalline powder) / beige
Lot/Batch #: P. 201025
Purity: 76.2%
Stability of test compound: The stability of the test substance in appropriate vehicle was confirmed analytically. The analytical report is not a part of the study report, but the raw data are stored at the testing facility.
Solvent used: Culture medium (without metabolic activation)
Nutrient mixture F-12 (with metabolic activation)

2. Control Materials:
Negative control: An absolute control without any treatment was performed only for cell cycle analysis
Vehicle control: Culture medium (without metabolic activation)
Nutrient mixture F-12 (with metabolic activation)

Positive control, -S9: Mitomycin C (MMC) 0.2 µg/mL (culture medium)
Positive control, +S9: Cyclophosphamide (CPA) 20.0 µg/mL (nutrient mixture F-12)
Historical controls: Vehicle control data (culture medium and DMSO) from studies performed in 1991 are present in the study report.

3. Activation: S9 was produced from the livers of approximately 7-week old male RAI rats (Tif:RAIF[SPF]), weighting 150 - 250 g, that were treated with Aroclor 1254 (500 mg/kg bw/day) i. p. for 5 days.

The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Isocitric acid (trisodium salt)	15.3 mM
NADP	3.14 mM
S9	1.5 %

4. Test organisms:

Cell line:	Chinese hamster ovary cells (CHO CCL 61)
Source:	ATCC (American Type Culture Collection)
Passages used:	38/26, 39/6, 39/10, 43/3 and 43/5
Doubling time:	12 - 13 h
Genetic stability:	The stability of the genome of the cell line is assessed on the basis of the regular cytogenetic analysis of control cultures in the course of the cytogenetic studies and is judged to be adequate for the particular purpose.
Absence of mycoplasma:	Is verified by periodically examinations (no data shown in the report).

5. Culture medium: Medium consisting of Nutrient Mixture F-12 supplemented with 10% (v/v) fetal calf serum (FCS) and Penicillin/ Streptomycin (1000 U/mL and 100 µg/mL).

5. Test concentrations:

a) Cytotoxicity assay:

Experiment with S9:

Main Experiment I:	25.78, 51.56, 103.12, 206.25, 412.5, 825, 1650 and 3300 µg/mL (3 hours exposure and 18 h sampling time)
Confirmatory Experiment I.B:	25.78, 51.56, 103.13, 206.25, 412.5, 825, 1650 and 3300 µg/mL (3 hours exposure and 42 h sampling time)

Experiment without S9:

Main Experiment II:	25.78, 51.56, 103.12, 206.25, 412.5, 825, 1650 and 3300 µg/mL (18 h exposure and 18 h sampling time)
Confirmatory Experiment II.B:	6.45, 12.89, 25.78, 51.56, 103.13, 206.25, 412.5, 825, 1650 and 3300 µg/mL (42 h exposure and 42 h sampling time)

b) Mutation assay:

Experiment with S9

Main Experiment I:	412.5, 825 and 1650 µg/mL (3 hours exposure and 18 h sampling time)
Confirmatory Experiment I.A:	412.5, 825 and 1650 µg/mL (3 hours exposure and 18 h sampling time)
Confirmatory Experiment I.B:	825, 1650 and 3300 µg/mL (3 hours exposure and 42 h sampling time)

Experiment without S9:

Main Experiment II:	103.12, 206.25 and 412.5 µg/mL (18 h exposure and 18 h sampling time)
Confirmatory Experiment II.A:	103.12, 206.25 and 412.5 µg/mL (18 h exposure and 18 h sampling time)
Confirmatory Experiment II.B:	51.56, 103.12 and 206.25 µg/mL (42 h exposure and 42 h sampling time)

c) Cell cycle analysis:
Experiment with S9
Main Experiment III: 103.12, 206.25, 412.5, 825, 1650 and 3300 µg/mL (3 h exposure and 18 h sampling time)

Experiment without S9
Main Experiment IV: 25.78, 51.56, 103.12, 206.25 and 412.5 µg/mL (18 h exposure and 18 h sampling time)

B. TEST PERFORMANCE:

1. Dates of experimental work: 18-May-1992 to 14-Sep-1992, finalisation date: 7-Oct-1992

2. Cytotoxicity assay:
The cytotoxicity tests were performed as an integral part of the mutagenicity tests with and without metabolic activation. The highest concentration tested (3300 µg/mL) was based on solubility limit of the test item in the solvent (culture medium or nutrient mixture F-12). Cytotoxicity was determined based on mitotic index of 2000 cells/treatment and the highest concentration for analysis was based on suppression of mitotic activity of 50-80% compared to the control group.

3. Cytogenicity Assay:

Cell treatment: Cells (at least 1 x 10^4 cells/mL) were pre-incubated on a series of glass slides in quadruple culture dishes at 37°C and 5% CO_2 for 29 hours. Exposure to the test substance, vehicle or positive control was performed for 3 hours in the presence of metabolic activation or 18 h and 42 h in the absence of metabolic activation. Sampling was performed 15 and 39 h after treatment for 3 h (+S9) and at the end of the 18 and 42 h treatment (-S9). Quadruplicate cultures were prepared for each group in each assay.

Spindle inhibition: Colcemide (0.4 µg/mL final concentration) was added to the cultures 2 hours prior to harvesting.

Cell harvest: At the end of the sampling time the culture medium was completely removed. For hypotonic treatment 0.075 M KCl solution was added and the cells were fixed by addition of methanol/ acetic acid (3:1 v/v).

Slide preparation: The cells on the slides were stained with orcein.

Metaphase analysis: Slides were coded prior to analysis. Whenever possible 200 well-spread metaphase figures with 19 to 21 centromeres from two cultures (100 metaphases/ replicate culture) were scored in the vehicle control and in the treated groups. At least 50 metaphases were scored in the positive controls (25 metaphases/ replicate culture). Following aberrations were recorded:

- Specific aberrations: breaks, exchanges, deletions and fragments
- Unspecific aberrations: gaps and chromosome decay
- Numerical alterations (metaphases with > 21 centromeres

Using the Vernier scale on the microscope stage, the coordinates of all metaphases with specific aberrations were recorded.

4. Cell cycle analysis:

Cell treatment: Cells were maintained in tissue culture flasks at 37°C and 5% CO_2. Exposure to the test substance and vehicle was performed for 3 hours in the presence of metabolic activation or 18 h in the absence of metabolic activation. Sampling was performed 15 h after treatment for 3 h (+S9) and at the end of the 18 h treatment (-S9). Additionally an absolute control was run in both experiments without any treatment in order to check the condition of the cultures.

Cell harvest: At the end of the sampling time, cells were detached with trypsin-EDTA. The monodispersed cells were fixed with ice-cold water/methanol/ethanol/isopropanol (30/56/7/7 (v/v)) for 2 h.

Sample preparation: DNA was stained with DAPI-solution (5 µM 2,4-diamidino-2-phenylindole in Tris-buffer, pH 7.4), supplemented with FITC-labeled microbeads (as an internal standard) for 1 h at 4°C. The proportion of microbeads and cells in the total particle count indicates the growth of the cultured cells.

Cell cycle analysis: Cell cycle distribution was analysed in a flow cytometer.

5. Statistics:

The data were analysed using a chi-square test.

6. Evaluation criteria:

The test chemical is considered positive in this assay if the following criteria are met:

- The percentage of metaphases containing specific aberrations in a treatment group is higher than 6.0 and differs statistically significant from the respective value of the negative control
- A concentration-related response should be demonstrated

The test chemical is considered negative in this assay if the following criteria are met:

- The percentage of metaphases containing specific aberrations in a treatment group is less than or equal to 6.0 and does not differ statistically significant from the respective value of the negative control

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS:

The test substance in vehicle was analysed to confirm the intended concentrations to be used in the mutagenicity tests and the stability of the test substance in the vehicle used. The concentration values determined were 104.3% and 111.6% of the nominal concentration, thus indicating a sufficient stability of the test substance in the vehicle.

B. CYTOTOXICITY ASSAYS:

Without S9-mix

Main Experiment II:

The highest concentration selected for mutagenicity testing was 412.5 µg/mL, based on a suppression of mitotic index of 77.4% [see Table 5.8.1-17].

Confirmatory Experiment II.B:

The highest concentration selected for mutagenicity testing was 206.25 µg/mL that caused a suppression of mitotic index of 47.4% [see Table 5.8.1-18]. At the next higher concentration of 412.5 µg/mL the mitotic index was suppressed by 92.3%.

With S9-mix

Main Experiment I:

The highest concentration selected for mutagenicity testing was 1650 µg/mL that caused a suppression of mitotic index of 38.5%. At the next higher concentration of 3300 µg/mL only few cells remained on the slides due to toxicity [see Table 5.8.1-19].

Confirmatory Experiment I.B:

The highest concentration selected for mutagenicity testing was 3300 µg/mL that caused a suppression of mitotic index of 5.9%. Higher concentrations were not tested due to solubility limitations [see Table 5.8.1-20].

C. CYTOGENICITY ASSAYS:

Exposure without metabolic activation

Main Experiment II, 18 h exposure:
There was no statistically difference in cells with chromosomal aberration excluding or including gaps. All treatment group values are within the historical control range [see Table 5.8.1-17].

Confirmatory Experiment II.A, 18 h exposure:
The value obtained at the highest concentration (412.5 µg/mL) shows a statistically significant difference (excluding and including gaps) compared with the respective vehicle control, but remains below the trigger-value of 6.0% and is within the laboratory historical control range [see Table 5.8.1-17].

Confirmatory Experiment II.B, 42 h exposure
After 42 h exposure the value obtained at 103.13 µg/mL concentration shows a statistically significant difference compared with the respective vehicle control, but remains below the trigger-value of 6%, is within the historical control range and is not a part of dose-dependency [see Table 5.8.1-18].

Overall in the experiments without metabolic activation the study result is considered equivocal as the statistic significances obtained are not consistent when comparing the initial and the confirmatory repeat experiments or are not dose dependent. As no cytotoxicity is reported for the confirmatory experiment with 18 h exposure, a shift in cytotoxicity cannot be ruled out. The study was evaluated by the study director not to be indicative for genotoxicity as the laboratory evaluation criteria were not met.

Table 5.8.1-17: Chromosome aberration test with phenylguanidine-carbonate without metabolic activation (18 hours treatment, 18 hours sampling time)

Compound	Concentration [µg/mL]	Mitotic index (% of control)	No. of cells examined	% of cells with aberrations	
				excluding gaps	including gaps
Main Experiment II					
Vehicle control	0	100	200	1	4
Test substance	103.13	84.68	200	0.5	1.5
	206.25	59.68	200	0	1.5
	412.5	22.58	200	2.5	5
HC Range (95th percentile)				**0-4 (3.35)**	**1-9.5 (7.4)**
MMC	0.2	-	50	**44*** **	**60[a]**
Confirmatory Experiment II.A					
Vehicle control	0	-	200	0	3
Test substance	103.13	-	200	2	6
	206.25	-	200	1.5	3.5
	412.5	-	200	**3.5****	**8[a]**
HC Range (95th percentile)				**0-4 (3.35)**	**1-9.5 (7.4)**
MMC	0.2	-	50	**28*** **	**36[a]**

[a] = not statistically evaluated
* = 0.05 ≥ p > 0.01; ** = 0.01 ≥ p > 0.001; *** = p ≤ 0.001
HC = Historical control

Table 5.8.1-18: Chromosome aberration test with phenylguanidine-carbonate without metabolic activation (42 hours treatment, 42 hours sampling time)

Compound	Concentration [µg/mL]	Mitotic index (% of control)	No. of cells examined	% of cells with aberrations	
				excluding gaps	including gaps
Confirmatory Experiment II.B					
Vehicle control	0	100	200	1.5	5.5
Test substance	51.56	126.02	200	1	5
	103.13	108.16	200	**4***	**8.5[a]**
	206.25	52.55	200	1	7
HC Range (95th percentile)				0-5 (3.5)	0.5-9.5 (7.75)

[a] = not statistically evaluated
* = 0.05 ≥ p > 0.01; ** = 0.01 ≥ p > 0.001; *** = p ≤ 0.001
HC = Historical control

Exposure with S9-mix
Main Experiment I, 3 h exposure, 18 h sampling:
There is no statistically difference in cells with chromosomal aberration excluding or including gaps. All treatment group values are within the historical control range [see Table 5.8.1-11].

Confirmatory Experiment I.A, 3 h exposure, 18 h sampling:
The value obtained at the highest concentration (1650 µg/mL) shows a statistically significant difference (excluding and including gaps) compared with the respective vehicle control, but remains below the trigger-value of 6.0%. The values excluding or including gaps are however outside of the laboratory historical control range [see Table 5.8.1-11].

Confirmatory Experiment I.B 3 h exposure, 42 h sampling:
In the experiment with 42 h sampling period the value obtained at the highest concentration shows a statistically significant difference, compared with the respective vehicle control and matches the trigger-value of 6% and is outside the historical range. However, although the mitotic index obtained 42 h after treatment was normal [see Table 5.8.1-12], the 18 hours experiments indicate serious cytotoxicity at concentrations of 1650 µg/mL and higher. Furthermore, it is well known, that increased osmotic strength of the culture media may exert genetic damage through indirect mechanisms. Such effect may appear when compounds are added to cell cultures at concentrations higher than about 10 mM. The concentration of 3300 µg/mL corresponds to 18.2 mM. The genetic damage observed 42 h after treatment with 3300 µg/mL of the test item is therefore not regarded to be caused by a direct interaction of the test substance or its metabolites with DNA, but is rather induced indirectly through cytotoxicity and/or osmotic effect.

Overall in the experiments with metabolic activation the study result is considered equivocal as the statistic significances obtained are not consistent when comparing the initial and the confirmatory repeat experiments. It is acknowledged that the statistically significant increases in chromosome aberration for the 18 h sampling time point was as for the experiment without metabolic action seen in the confirmatory experiment only for which no cytotoxicity is reported. Thus a shift in cytotoxicity cannot be ruled out. The statistical increase at the 42 h sampling is attributed to indirect cytotoxic and/or osmotic effects at this high dose level. The study was evaluated by the study director not to be indicative for clastogenicity of the compound.

Table 5.8.1-19: Chromosome aberration test with phenylguanidine-carbonate with metabolic activation (3 hours treatment, 18 hour sampling time)

Compound	Concentration [µg/mL]	Mitotic index (% of control)	No. of cells examined	% of cells with aberrations	
				excluding gaps	including gaps
Main experiment I					
Vehicle control	0	100	200	1	2.5
Test substance	412.5	100.56	200	1	4.5
	825	101.68	200	1	3.5
	1650	61.45	200	0.5	1.5
HC Range (95th percentile)				**0-3.5 (2.5)**	**1-6.5 (5.7)**
MMC	20	-	50	32***	42[a]
Confirmatory experiment I.A					
Vehicle control	0	-	200	1	3
Test substance	412.5	-	200	2	4.5
	825	-	200	2	4
	1650	-	200	**5****	**8.5**[a]
HC Range (95th percentile)				**0-3.5 (2.5)**	**1-6.5 (5.7)**
MMC	20	-	50	24***	24[a]

[a] = not statistically evaluated
* = 0.05 ≥ p > 0.01; ** = 0.01 ≥ p > 0.001; *** = p ≤ 0.001
HC = Historical control

Table 5.8.1-20: Chromosome aberration test with phenylguanidine-carbonate with metabolic activation (3 hours treatment, 42 hours sampling time)

Compound	Concentration [µg/mL]	Mitotic index (% of control)	No. of cells examined	% of cells with aberrations	
				excluding gaps	Including gaps
Confirmatory Experiment I.B					
Vehicle control	0	100	200	2.5	7
Test substance	825	132.35	200	0.5	2.5
	1650	111.76	200	0.5	2.5
	3300	94.12	200	**6.5****	**14.5**[a]
HC Range (95th percentile)				0-4 (2.175)	0-8.5 (7.1)

* = 0.05 ≥ p > 0.01; ** = 0.01 ≥ p > 0.001; *** = p ≤ 0.001
HC = Historical control

Unspecific chromosomal aberrations in the form of chromatid gaps found in all experiments were within the frequency generally observed**.**

Positive controls

The treatment of the cultures with mitomycin C (0.2 µg/mL) and cyclophosphamide (20.0 µg/mL) without and with metabolic activation system, respectively, induce a high incidence (p ≤ 0.001) of specific chromosomal aberrations in the Main Experiment II and Confirmatory Experiment II.A without metabolic activation (44% and 28%), and in the Main Experiment I and Confirmatory Experiment I.A with metabolic activation (32% and 24%).

D. CELL CYCLE ANALYSIS:

Exposure without S9

At all concentrations tested, test item induced a weak concentration-dependent increase in the proportion of cells in the $G_{1/0}$-stage. Simultaneously, the proportion of cells in the S-stage was slightly reduced [see Table 5.8.1-21].

These effects are interpreted as evidence for cell cycle arresting effects in the $G_{1/0}$- or in the early S-stage, respectively.

Table 5.8.1-21: Cell cycle analysis with phenylguanidine-carbonate without metabolic activation (18 hours treatment, 18 hours sampling time)

Compound	Concentration [µg/mL]	Total particle count#	Particles, identified as cells	Cell cycle Phase (Distribution %)		
				$G_{1/0}$	S	G_2 + M
Negative control	0	20 000	17 182	43.7	41.9	14.4
Vehicle control	0	20 000	16 573	40.1	42.8	17.1
Test substance	25.78	20 000	16 181	43.5	42.0	14.5
	51.56	20 000	16 551	44.1	40.9	15.0
	103.13	20 000	16 400	46.5	40.1	13.4
	206.25	20 000	15 728	51.9	35.2	12.9
	412.50	20 000	14 893	59.0	26.8	14.2

= including internal standard beads

With S9

At the concentration of 1650 µg/mL a weak increase in the proportion of cells in the $G_{1/0}$-stage was observed. Simultaneously, the proportion of cells in the S- and G_2+M-stages was reduced. In addition, the amount of particles, identified as cells, was reduced by about 73% in comparison with the solvent control [see Table 5.8.1-22].

These effects are interpreted as evidence for a cell cycle arresting activity of the metabolites of the test substance in the $G_{1/0}$- and S-stage at the highest concentration tested. Furthermore, the metabolites of the test substance exerts marked cytotoxic effects resulting in the reduced cell number at this concentration.

Table 5.8.1-22: Cell cycle analysis with phenylguanidine-carbonate with metabolic activation (3 hours treatment, 18 hours sampling time)

Compound	Concentration [µg/mL]	Total particle count#	Particles, identified as cells	Cell cycle Phase (Distribution %)		
				$G_{1/0}$	S	G_2 + M
Negative control	0	20 000	17 603	46.6	39.2	14.2
Vehicle control	0	20 000	17 174	41.6	45.3	13.1
Test substance	103.13	20 000	17 168	42.0	46.9	11.1
	206.25	20 000	16 932	42.6	45.1	12.3
	412.50	20 000	17 334	40.6	45.5	13.9
	825.00	20 000	16 455	35.4	47.8	16.8
	1650.00	10 000	2 700[§]	57.6[§]	37.2[§]	5.2[§]

= including internal standard beads
[§] = estimated

III. CONCLUSION

The results from this study with phenylguanidine-carbonate were equivocal with regard to induction of chromosome damage in cultured CHO cells either in the presence or in the absence of S9 mix under the experimental conditions applied. At higher concentrations, the test item induce cell cycle disturbing effects in the CHO cells.

Report:	CA 5.8.1/38 [redacted] 2015 a Reg.No. 4182909 (metabolite of BAS 605 F, Pyrimethanil) tested as carbonate salt: Micronucleus Assay in bone marrow cells of the rat 2015/1186908
Guidelines:	OECD 474 (2014), (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.12, EPA 870.5395
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Klimaschutz, Landwirtschaft und Verbraucherschutz, Wiesbaden)
Deviations:	no

Report:	CA 5.8.1/39 [redacted] 2017 a Report Amendment 1: Reg.No. 4182909 (metabolite of BAS 605 F, Pyrimethanil) tested as carbonate salt: Micronucleus assay in bone marrow cells of the rat 2017/1005941
Guidelines:	OECD 474 (2014), (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.12, EPA 870.5395
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Klimaschutz, Landwirtschaft und Verbraucherschutz, Wiesbaden)
Previous evaluation:	New information

Report:	CA 5.8.1/40 Grauert E.,Kamp H., 2015 a Reg.No. 4182909 (metabolite of BAS 605 F, Pyrimethanil) tested as carbonate salt - Concentration control analyses in dimethyl sulfoxide + polyehtylene glycol (3+7; v/v) 2015/1225521
Guidelines:	none
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Report:	CA 5.8.1/41 Grauert M.E.,Kamp H., 2015 b Reg.No. 4182909 (metabolite of BAS 605 F, Pyrimethanil) tested as carbonate salt - Plasma analysis for external studies 2015/1223832
Guidelines:	none
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Report:	CA 5.8.1/42 Seibold A., 2016 a Determination of Reg. 4182909 (metabolite of BAS 605 F, Pyrimethanil) in rodent plasma samples originating from sponsor study ID 06M0319/15X114 2016/1321106
Guidelines:	OECD Principles of Good Laboratory Practice
GLP:	yes (certified by Landesamt fuer Umwelt, Messungen und Naturschutz Baden Wuerttemberg, Karlsruhe, Germany)

Executive Summary

M605F025 (Phenylguanidine, Reg.No. 4182909; Batch: L85-142; Purity: 73.7% phenylguanidine) was tested as the stable carbonate salt for the ability to induce micronuclei in polychromatic erythrocytes (PCE) in the bone marrow of the rat. For this purpose, the test substance dissolved in DMSO / PEG 400 (3/7) was administered once orally to groups of 7 male rat at dose levels of 125, 250 and 500 mg/kg body weight in a volume of 10 mL/kg body weight. The vehicle served as negative and cyclophosphamide as positive control (5 male animals/control). The animals were sacrificed 24 hours (all dose groups) or 48 hours (additional high dose and vehicle groups) after the administration and the bone marrow of the femora was prepared. After staining of the preparations, 4000 PCE were evaluated per animal and investigated for micronuclei. The normocytes occurring per 4000 PCE were also recorded.

The highest dose selected was based on two pre-experiments indicating exceeding toxicity (mortality at 1000 mg/kg bw). Clinical symptoms in the main experiment included abdominal posture, partially closed eyes, slightly reduced spontaneous activity, salivation, as well as sleepiness. The animals of all dose levels were affected. The animals treated with the vehicle control did not exhibit any clinical symptoms.
The observed systemic toxicity at the tested doses is indicative for a systemic distribution of the test item. Thus, bioavailability of the test item under the tested conditions is demonstrated. This was additionally confirmed by analytical detection of the test item in plasma qualitatively [DocID 2015/1223832] and quantitatively [see BASF DocID 2016/1321106].
After treatment with the test item the number of PCEs was not substantially decreased as compared to the mean value of PCEs of the vehicle control thus indicating that M605F025 tested as carbonate salt did not exert any cytotoxic effects in the bone marrow. There was no biologically relevant or statistically significant enhancement in the frequency of the detected micronuclei at any preparation interval after administration of the test item and with any dose level used.
Cyclophosphamide used as positive control showed a substantial increase of induced micronucleus frequency.
In conclusion, M605F025 tested as carbonate salt did not induce micronuclei as determined by the micronucleus test with bone marrow cells of the rat. Therefore, M605F025 tested as carbonate salt is considered to be non-mutagenic in this micronucleus assay.

(BASF DocID 2015/1186908,
2015/1225521, 2015/1223832 and 2016/1321106)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Reg.No. 4182909 (Metabolite of BAS 605 F, Pyrimethanil) tested as carbonate salt

Description:	Solid, white
Lot/Batch #:	L85-142
Purity:	73.7%
Stability of test compound:	
Solvent used:	DMSO / PEG 400 (3/7) 30% DMSO, 70% Polyethyleneglycol

2. Control Materials:

Negative:	No negative control was employed in this study.
Solvent control:	DMSO / PEG 400 (3/7)
Positive control:	Cyclophosphamide (CPA) 40 mg/kg dissolved in sterile water

3. Test animals:

Species:	Rat
Strain:	Wistar
Sex:	Male for the main study; male and female for the range finding study
Age:	6 - 10 weeks
Weight at dosing:	Males mean value: 185.1 g (range: 162.8 – 200.8)
Source:	[redacted]
Number of animals per dose:	
Range finding study:	2 males and 2 females for each pre-test
Micronucleus assay:	7 males/dose; 5 males/control
Acclimation period:	At least 5 days
Diet:	Pelleted standard diet (certified), ad libitum
Water:	Tap water, ad libitum
Housing:	Single housing in Makrolon Type III/VI cages, with wire mesh top

4. Environmental conditions:

Temperature:	20 - 24 °C
Humidity:	45% - 65%
Air changes:	frequency not indicated
Photo period:	12-hour light-dark cycle (06:00 - 18:00, 18:00 - 06:00)

5. Test compound doses:

Range finding test:	1000 and 500 mg/kg (administered once orally)
Micronucleus assay:	125, 250 and 500 mg/kg The test substance was administered once by oral gavage using an application volume of 20 mL/kg.

6. Analytics:

Sampling:	A sample of each dose group and the vehicle control group of the micrnonucleus test was collected by the performing laboratory and sended to the analytical laboratory.
Sampling date:	25-Sep-2015
Date of analysis:	02-Oct-2015
Analytical method:	HPLC – MS LC – MS/MS

B. TEST PERFORMANCE

1. Dates of experimental work: 03-Sep-2015 to 02-Oct-2015

2. Preliminary range finding test:

2 Male and 2 female Wistar rat were treated once by oral gavage with a test substance dose of 1000 (1st pre-test) and 500 mg/kg bw (2nd pre-test).

3. Micronucleus test:

Treatment and sampling:	Groups of male mice were treated once with either the vehicle or 125, 250 and 500 mg M605F025 / kg bw (tested as carbonate salt) by oral gavage. Additional test groups treated with the vehicle control and the high dose animals were treated for the second sampling period. The application volume was 10 mL/kg bw and the volume to be applied was calculated based on actual weight on the day of administration. The positive control substance CPA was administered once by oral gavage. The animals were surveyed for evident clinical signs of toxicity throughout the study.

	Twenty-four hours after the administration the mice were killed and the femora were prepared free of all soft tissue. After cutting the epiphyses the bone marrow was flushed out in a centrifugation tube with fetal calf serum and subsequently centrifuged at 390 x g for 10 minutes. The supernatant was discharged and the pellet resuspended. The sampling of the femora from the additional high dose and vehicle control group was performed as described above 48 h after the treatment.
Slide preparation:	A small drop of the suspension was spread on a clean microscopic slide and smears were prepared. After air drying the smears were stained with May-Grünwald/Giemsa. Cover slips were mounted with EUKITT. At least one slide was made from each bone marrow sample.
Slide evaluation:	In general, 2000 polychromatic erythrocytes (PCEs) from each male animal of every test group were evaluated and investigated for micronuclei (MN). The normochromatic erythrocytes (NCEs) that occurred were also scored. To describe a cytotoxic effect the ratio between polychromatic and normochromatic erythrocytes was determined in the same sample and expressed in polychromatic erythrocytes per 2000 erythrocytes.

4. Statistics:

The number of polychromatic erythrocytes with micronuclei was analyzed by comparing the dose groups with the vehicle control using the Mann-Whitney U-test.

5. Evaluation criteria:

A test item was considered mutagenic if it induces either a dose-related increase or a clear increase in the number of micronucleated polychromatic erythrocytes in a single dose group above the laboratory's historical solvent control data range. Statistical methods were used as an aid in evaluating the results, if necessary. However, the primary point of consideration was the biological relevance of the results.
A test item that fails to produce a biological relevant increase in the number of micronucleated polychromatic erythrocytes was considered non-mutagenic in this system.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATION

The measured values of M605F025 tested as carbonate salt in 30% DMSO, 70% Polyethyleneglycol were found to be in the range of 99.4% and 101.4% of the nominal concentration. These results demonstrate the correctness of the concentrations applied.

B. PRELIMINARY RANGE FINDING TEST

At 1000 mg/kg bw/day one male was sacrificed in moribund state on the day of application. 2 females were found dead on the next day. No mortality was observed at 500 mg/kg bw/day. However the animals of both sex revealed clear signs of toxicity (reduced activity, apathy at high dose only, abdominal position, salivation, piloerection) on the day of administration. As no sex specific difference in toxicity was observed, only male animals were used for the main experiment.

C. MICRONUCLEUS ASSAY

1. Clinical symptoms

The clinical symptoms of the main study are summarized in the table below clearly indicating systemic exposure of the rats towards M605F025 tested as its stable carbonate salt. No clinical symptoms were observed in animals treated with the vehicle solely. No clinical observations were conducted for positive control animals.

Table 5.8.1-23: M605F025 administered as stable carbonate salt by oral gavage: Clinical observations in main study

	500 mg/kg bw			250 mg/kg bw		125 mg/kg bw	
Preparation interval	**24 h (7 males) and 48 h (7 males)**		**48 h (7 males)**	**24 h (7 males)**		**24 h (7 males)**	
Observation	**0 – 6 h**	**24 h**	**48 h**	**0 – 6 h**	**24 h**	**0 – 6 h**	**24 h**
Symptoms							
Abdominal posture	14	0	0	7	0	5	0
Partially closed eyes	5	0	0	5	0	3	0
Slightly reduced spontaneous activity	14	14	7	7	2	7	2
Salivation	14	0	0	7	0	7	0
Burrows itself in the bedding	14	0	0	7	0	7	0
Sleepy	14	0	0	7	0	7	0

2. Analytical detection of M605F025 tested as stable carbonate salt in blood plasma

The free base of the M605F025 carbonate salt was qualitatively detected in 5 out of 6 samples of the high dose group of 500 mg/kg bw and wasn't detected in any of the 3 vehicle control group samples.

Quantitative plasma analysis revealed M605F025 concentrations in the range of 7.6 to 8.4 mg/L, 1 hour post-treatment. Four-hour samples revealed M605F025 concentrations in the range of 6.95 to 19.15 mg/L. No test substance could be detected in the control rat plasma samples both, qualitatively and quantitatively (LOQ = 0.05 mg/L).

This result confirms that M605F025 reaches the plasma and thus can become effective in the target tissue of the rat micronucleus test.

3. Cell analysis in polychromatic erythrocytes of rat bone marrow

The results obtained in the cell analysis in the main study are summarized in the table below.
The mean number of polychromatic erythrocytes (PCE) was not substantially decreased after treatment with M605F025 as compared to the vehicle control indicating that M605F025 tested as its stable carbonate salt did not have any cytotoxic properties in the bone marrow. When compared to the concurrent vehicle control there was no statistical significant or biological relevant increase in the frequency of micronuclei in any dose level and of any preparation intervals. The values of the treated groups were below or equal to the value of the respective vehicle control. The positive control cyclophosphamide showed a statistically significant increase in induced micronuclei at both preparation intervals

Table 5.8.1-24: M605F025 administered as stable carbonate salt by oral gavage: Cell analysis in polychromatic erythrocytes in rat bone marrow

Treatment	Sampling time	MN / 4000 PCE [mean ± S.D.	Range	PCE per total Erythrocytes (% ratio vehicle)
24 h sampling				
Vehicle	24	11.4 ±5.8	6 - 20	0.573 (100%)
M605F025				
125 mg/kg bw	24	8.3 ± 2.6	5 - 13	0.562 (98.08)
250 mg/kg bw	24	9 ± 3.6	4 - 12	0.584 (101.92)
500 mg/kg bw	24	8.6 ± 2.9	6 - 13	0.575 (100.35)
Positive control				
Cyclophosphamide	24	102.6 ± 39.7	49 - 154	0.527 (91.97)
48 h sampling				
Vehicle	48	15.2 ± 5.8	10 - 25	0.575 (100)
M605F0251				
500 mg/kg bw	48	14.0 ± 3.5	8 - 18	0.545 (94.78)

MN = micronuclei
PCE = polychromatic erythrocytes

III. CONCLUSION

In conclusion, it can be stated that during the study described and under the experimental conditions reported, M605F025 administered as its carbonate salt did not induce micronuclei as determined by the micronucleus test in the bone marrow cells of the rat.

Report:	CA 5.8.1/43 Altmann B., 2002 a 90-day oral toxicity study in rats (administration in food) - CGA 263208 tech. [redacted]
Guidelines:	OECD 408, EPA 870.3100, EEC 87/302, JMAFF No 12 Nosan No 8147
GLP:	yes (certified by Swiss Federal Office of Public Health, Berne, Switzerland)
Deviations:	Yes, sensory reactivity to stimuli was not determined

Executive Summary

In a 90-day oral toxicity study phenylguanidine-carbonate, technical (Batch: 014941D0; Purity: 94.4%) was administered to 10 albino HanBrl:WIST (SPF) rats/sex/dose in diet at 0, 50, 300, 2000 and 8000 ppm (equivalent to mean daily intake of 0, 3.06, 17.8, 131 and 677 mg/kg bw for males and to 0, 3.52, 22.1, 147 and 638 mg/kg bw for females; correcting for food spillage gives 140 mg/kg bw for 2000 ppm females and 536 mg/kg bw and 616 mg/kg bw for 8000 ppm males and females, respectively). The study included a further group treated with 8000 ppm of cyprodinil as the concurrent active ingredient to this metabolite testing. However, these data are not reported in this study summary.

Signs of mortality were recorded twice daily, and sings of toxicity were observed daily. Individual body weights were recorded weekly. Clinical examinations were conducted on a once-a-week basis. Functional observational battery (FOB) and measurements of motor activity, fore-paw and hind-paw grip strength and landing foot splay were made towards the end of the treatment period.

Treatment related findings were noticed in the mid and high dose group of 2000 and 8000 ppm.
Body weight and body weight gain were significantly affected at the 2000 ppm group and above. The severely reduced body weight development in the high dose group animals leading to about 23% body weight gain as compared to control in males and about 32% in females indicates that the maximum tolerable dose has been exceeded at this dose level.
Observed clinical signs were restricted to the high dose and included mainly transient occurrence of reduced muscle tone and piloerection. In the FOB no specific neurotoxic effect were noticed but the observed reduced grip strength and decrease in landing food splay at 2000 ppm and above were attributed to the notably reduced body weight gain.
At the beginning of the study a dose-related reduction in food and water consumption was observed which returned to normal the weeks thereafter. Food spillage was noticed mainly in the high dose group and in one mid dose group animal. Taken together these findings indicate an impaired palatability of the compound.

Various parameters of the hematopoietic system were altered in the high dose group, including changes of the hematological profile and microscopic findings in the bone marrow and the spleen. The increase in gamma-glutamyl-transpeptidase in conjunction with increase in alkaline phosphatase suggests compromising effects on hepatobiliary function in line with the changes observed for bilirubin and globulin to albumin ratio. The noticed reduced absolute liver weights in correlation with reduced glycogen deposition in the hepatocytes and the cytoplasmic vacuolation of the pancreas in some high dose males in turn indicates physiological adoption to the decrease in body weight of the affected group.
Increased plasma urea levels, higher plasma creatinine levels and proteinurea were observed at the high dose group suggesting disturbance in kidney function. However, in the absence of histopathological correlate these findings may as well be related to the nutritional deficiency.
Reduction in weights of male reproduction organs (testes and epididymidis) were observed after treatment with 8000 ppm in correlation with macroscopic findings of diminished size and histopathological occurrence of precipitate, and reduced spermatozoa in the epididymidis and reduced secretion in prostate and seminal vesicle and reduced spermatogenic activity and increased incidence and severity of tubular atrophy in the testis. However, as these finding were associated with the pronounced reduction in body weight gain they may be unspecific representing the poor general state of the animals. The sexual organ weight reduction in females (uterus and ovaries) as well as several other affected organ weights without histopathological correlate like heart and brain in both sexes, adrenals (females only) and thyroid (males only) are considered to be related to the reduced body weight gain.

There were no conclusive indications of an effect at dose levels of 50 and 300 ppm. A significantly lower relative spleen weight in 300 ppm females, although a part of a dose-related trend, is not considered biologically relevant as the mean absolute spleen weight was not significantly different and no histopathological correlate was determined.

Based on these findings, it was concluded that the LOAEL for phenylguanidine-carbonate is 2000 ppm (=131 mg/kg bw/day for males and 140 mg/kg bw/day for females), and the NOAEL is 300 ppm (17.8 mg/kg bw/day for male and 22.1 mg/kg bw/day for female).

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: CGA 263208 tech. (phenylguanidine-carbonate)

Description:	solid (powder) / white
Lot/Batch #:	014941D0
Purity:	94.4%
Stability of test compound:	Analytical investigations (HPLC) indicated stability of the test item in pelleted food for 7 weeks when stored at room temperature.

The concurrently tested active ingredient CGA 219471 (cyprodinil) is not reported in this study summary as not being relevant for the evaluation of pyrimethanil and its metabolite.

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rats
Strain:	Albino, HanBrl:WIST (SPF)
Sex:	Male and female
Age:	7 – 8 weeks (start of administration)
Weight at dosing (mean):	males: 186 - 243 g females: 136 - 186 g
Source:	[redacted]
Acclimation period:	25 days
Diet:	pelleted standard diet (Nafag No. 8900), ad libitum
Water:	tap water, ad libitum
Housing:	individual housing in macrolon cages (900 cm²)

Environmental conditions:

Temperature:	22 ± 2°C
Humidity:	55 ± 10%
Air changes:	16 – 20 / h
Photo period:	12 h light / 12 h dark

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 16-Jul-2001 – 8-Feb-2002
(In life dates: 16-Jul-2001 (start of administration) to 15/18-Oct-2001 (sacrifice))

2. Animal assignment and treatment:
The test item was administered to groups of 10 male and 10 female ~~mice~~ rats at dietary concentrations of 0, 50, 300, 2000 and 8000 ppm for at least 90 days. Animals were assigned to the test groups by computer randomization.

3. Test substance preparation and analysis:
The test substance was applied via the oral route in the diet. Diet was prepared at three week intervals by mixing appropriate amounts of test substance with Nafag No. 8900 FOR GLP and was stored in stainless steel containers at room temperature. Homogeneity and stability were tested prior to study initiation. During the study, samples of treated food were analyzed for every preparation (weeks 1-2, 3-6, 7-10, and 11 to treatment end) for concentration. Stability investigation was performed at week 8 with samples of the diet used for dosing on weeks 1-2.

4. Statistics:
Means and standard deviations (S.D.) of each test group were calculated for several parameters. Statistical analyses on in-life and organ weight data were carried out using the statistical routines in the NOVATOX System. Quantitative data such as body and organ weights were analyzed either using two-sided parametric or non-parametric statistical test following a pre-test for uniformity (Bartlett's test of homogeneity of variances). Tests used included analysis of variance (ANOVA), Dunnett's multiple comparison, and Kruskal-Wallis non-parametric test. Statistical significance was reported at $p \leq 0.05$ and $p \leq 0.01$.

C. METHODS

1. Observations:
Animals were inspected daily for signs of toxicity and twice daily for mortality. Clinical examinations were conducted once a week.

2. Body weight:
Body weight was determined before the start of the administration period and thereafter in weekly intervals.

3. Food consumption and compound intake:
Individual food consumption was determined in weekly intervals and calculated for periods of one week as g food/animal/day. Food consumption for each animal was determined and mean daily diet intake was calculated as g food/kg bw/day. Food utilization efficiency (body weight gain in g/food consumption in g per unit time x 100) and compound intake (mg/kg bw/day) values were calculated in a time-weighted averages from the consumption and body weight gain data.

4. Clinical assessment of neurotoxicity:
All animals were evaluated in a Functional Observation battery (FOB) that included observations in the home cage, observations and gait evaluation in a standard arena, and observations during handling, followed by a neurological examination (CNS activity, CNS over-excitation, sensorimotor, autonomic, and physiological functions) towards the end of the treatment period. Motor activity was measured shortly after completion of the FOB.

5. Hematology and clinical chemistry:
Blood was collected on all surviving rats of each dose group early in the morning on study days 92 and 93. The following hematological and clinical chemistry parameters were determined for all animals:

Hematology:					
	Red blood cells		*White blood cells*		*Clotting Potential*
✓	Erythrocyte count (RBC)	✓	Total leukocyte count (WBC)	✓	Thrombocyte count
✓	Hemoglobin (HGB)	✓	Leukocyte differential count	✓	Prothrombin time (PT)
✓	Hemoglobin concentration distribution width (HDW)	✓	Platelet distribution width		
✓	Hematocrit (HCT)				
✓	Mean corp. volume (MCV)				
✓	Mean corp. hemoglobin (MCH)				
✓	Mean corp. Hb. conc. (MCHC)				
	Reticulocytes (RET)				
✓	Red cell distribution width				

Clinical chemistry:					
	Electrolytes		*Metabolites and Proteins*		*Enzymes:*
✓	Calcium	✓	Albumin	✓	Alanine aminotransferase (ALAT)
✓	Chloride	✓	Albumin/globulin ratio	✓	Aspartate aminotransferase (ASAT)
✓	Phosphorus (inorganic)		Bile acids	✓	Alkaline phosphatase (AP)
✓	Potassium	✓	Bilirubin (total)	✓	γ-glutamyl transpeptidase (GGT)
✓	Sodium	✓	Cholesterol (total)	✓	Glutamate dehydrogenase (GLDH)
		✓	Creatinine		
		✓	Glucose		
		✓	Protein (total)		
		✓	Triglycerides		
		✓	Urea nitrogen		
		✓	Globulins		

6. Urinalysis:
Urine was collected overnight from individual fasted animals while they were housed in metabolism cages at the end of the treatment period.

The following quantitative or semi-quantitative parameters were determined for all animals:

Urinalysis					
	Quantitative parameters:		*Semi quantitative parameters*		
✓	Urine volume	✓	Bilirubin	✓	Protein
✓	Specific gravity/osmolality	✓	Blood/blood cells	✓	pH-value
		✓	Colour	✓	Urobilinogen
		✓	Glucose		Sediment (microscopical exam.)
		✓	Ketones		

7. Ophthalmoscopy:
Eyes were examined in all animals before the start of treatment and those of all control and high dose animals were examined again in week 13 (day 86).

8. Sacrifice and pathology:
All animals that died and those sacrificed on schedule were subjected to gross pathological examinations.

The following organs were sampled, weighed and examined histopathologically after immersion fixation:

Pathology:											
The following organs were collected (column C), weighed (W) and examined histopathologically (H).											
C	W	H		C	W	H		C	W	H	
✓	✓	✓	adrenal glands	✓			lacrimal glands	✓	✓	✓	spleen
✓		✓	aorta	✓	✓	✓	liver	✓		✓	stomach
✓		✓	bone[§]	✓		✓	lung	✓	✓	✓	testes
✓			bone marrow*	✓		✓	lymph nodes[$]	✓	✓	✓	thymus
✓	✓	✓	brain	✓		✓	mammary gland	✓		✓	tongue
✓		✓	caecum	✓			nose#	✓	✓	✓	thyroid/parathyroid
✓		✓	colon	✓	✓	✓	ovaries	✓		✓	trachea
✓		✓	duodenum	✓			oviduct	✓		✓	urinary bladder
✓	✓	✓	epididymides	✓		✓	pancreas	✓	✓	✓	uterus
✓			esophagus	✓		✓	Peripheral nerve[α]	✓		✓	vagina
✓		✓	eyes (with optic nerve)	✓		✓	Peyer's patches	✓		✓	Zymbal's gland
✓		✓	gross lesions	✓		✓	pituitary gland				
✓			Harderian glands	✓		✓	prostate				
✓	✓	✓	heart	✓		✓	rectum				
✓	✓	✓	heart	✓			salivary gland				
✓		✓	ileum	✓			seminal vesicles				
✓		✓	jejunum	✓			skeletal muscle				
✓		✓	joint (femurotibial)	✓		✓	skin				
✓	✓	✓	kidneys	✓		✓	spinal cord[ß]				
[§] sternum and/or femur; * 2nd femur and sternum, [$] axillary & mesenteric, [#]muzzle, [α] sciatic, [ß] 3 levels											

II. RESULTS AND DISCUSSION

A. TEST SUBSTANCE ANALYSES

Diets were found to be homogenous and of correct concentrations. The individual concentrations varied in the range from ± 3% of the mean concentrations, and the overall mean concentrations of the diet samples varied ± 2% of the nominal concentrations.
The stability of the test item in food was confirmed by analysis of 7 weeks old diet-preparations (prepared on or before July 12, 2001 and analysed on August 23, 2001) which showed acceptable test item concentrations (± 8% of the nominal concentrations). However, the control diet which was originally assayed 0 ppm test item on July 12, 2001 showed 40.02 ppm test item on August 23, 2001 and the control diet from August 07, 2001 that was assayed on August 23, 2001 showed 46.20 ppm test item (see p. 538 and p. 539 of MRID 45614706), but no clarification or explanation of that issue is provided in the study report.
The analytical data indicate that the mixing procedure was adequate and that the variance between nominal and actual dosage to the rats was acceptable.

B. OBSERVATIONS

1. Clinical signs of toxicity

Treatment-related clinical signs were observed mainly in 8000 ppm dose group animals, and consisted of piloerection, and reduced muscle tone. Hunched posture was seen in females at treatment end, and discharge (genital region) was observed in females during the second half of the study.

Table 5.8.1-25: Summary of general clinical observations of rats administered phenylguanidine-carbonate for 90 days

Dose level	Males					Females				
[ppm]	0	50	300	2000	8000	0	50	300	2000	8000
[mg/kg bw/d]	-	3.06	17.8	131	536	-	3.52	22.1	140	616
No. of animals	10	10	10	10	10	10	10	10	10	10
Total affected	1	0	2	0	10	1	0	0	1	10
Neuromuscular										
Muscle tone ↓	0	0	0	0	7	0	0	0	0	8
Hunched posture	0	0	0	0	0	0	0	0	0	1
Discharges										
discharge	0	0	0	0	0	0	0	0	1	4
Behavior										
stereotypies	0	0	0	0	0	1	0	0	0	0
Skin/Fur										
Lesion	1	0	1	0	1	0	0	0	0	1
Piloerection	0	0	0	0	4	0	0	0	0	8
Hair loss	0	0	1	0	0	0	0	0	0	0

↓ = decrease

Findings from other dose groups consisted primarily of hair loss and lesions that were considered to be sporadic and incidental.

2. Mortality

1/10 (control group) and 3/10 (8000 ppm dose group) males died at week 14 after blood sampling, and these deaths were considered to be unrelated to treatment.

C. BODY WEIGHT AND BODY WEIGHT GAIN

In males of the 8000 ppm dose group mean body weights were decreased from week 2 through 14, resulting in a significantly reduced mean body weight gain of 22.7% compared with the control group. In females of the 8000 ppm dose group mean body weights were significantly decreased from week 2 through 14, resulting in a significantly reduced mean body weight gain of 33.8% compared with the control group.

In males of the 2000 ppm dose group mean body weights were significantly depressed at week 4, and during week 5 through 14, resulting in a significantly reduced mean body weight gain of 66.4% compared with the control group. For 2000 ppm females the mean body weights were lower (but not significantly) than those of control and lower-dose groups from week 2 through week 14, but resulting in a significantly reduced mean body weight gain of 79.2% compared with the control group.

The weekly body weights and the body weight gain was not adversely affected in animals of either sex treated at 50 and 300 ppm dose groups [see Table 5.8.1-26].

Table 5.8.1-26: Mean body weight and body weight gain of rats administered phenylguanidine-carbonate for 90 days

Dose level	Males					Females				
[ppm]	0	50	300	2000	8000	0	50	300	2000	8000
[mg/kg bw/d]	-	3.06	17.8	131	536	-	3.52	22.1	140	616
Body weight [g]										
Week -1	212.9 ± 11.2	213.7 ± 12.9	210.4 ± 13.4	213.5 ± 18.9	212.7 ± 14.6	161.1 ± 16.4	158.1 ± 14.1	160.7 ± 12.4	159.2 ± 12.9	154.5 ± 12.8
Week 1	253.4 ± 13.1	255.5 ± 18.3	251.7 ± 16.2	256.4 ± 20.9	254.3 ± 15.3	177.3 ± 18.0	174.3 ± 14.2	180.0 ± 12.7	177.7 ± 15.2	170.4 ± 13.3
Week 2	285.5 ± 14.6	287.6 ± 19.3	283.7 ± 19.9	273.4 ± 21.9	**235.8**** ± 19.8	191.3 ± 19.4	189.7 ± 16.6	195.9 ± 14.3	186.1 ± 11.9	**153.3**** ± 13.3
Week 8	394.0 ± 25.2	389.9 ± 29.1	385.3 ± 27.6	**340.1**** ± 34.9	**280.4**** ± 18.6	231.1 ± 20.2	228.6 ± 17.5	237.1 ± 19.9	223.7 ± 12.7	**184.1**** ± 12.2
Week 14	440.7 ± 31.7	438.1 ± 36.1	432.4 ± 31.5	**380.4**** ± 44.6	**296.7**** ± 21.9	248.1 ± 19.8	247.4 ± 19.1	254.3 ± 20.9	233.8 ± 18.3	**194.3**** ± 14.4
Mean body weight gain [g]										
Week 1-14	187.3 ± 19.9	182.6 ± 22.4	180.6 ± 18.6	**124.3**** ± 28.6	**42.5**** ± 14.2	70.8 ± 9.6	73.1 ± 11.2	74.3 ± 10.7	**56.1*** ± 14.8	**23.9**** ± 5.4
% of control	100	97.5	96.4	66.4	22.7	100	103.2	104.9	79.2	33.8

Values presented as group mean ± S.D.
* = p≤0.05; ** = p≤0.01

D. FOOD CONSUMPTION, WATER CONSUMPTION AND COMPOUND INTAKE

At 8000 ppm a significant reduction of food intake during the first week was observed in both sexes [see Table 5.8.1-27], but was comparable with corresponding control groups in the following weeks, except in males at the end of the study (week 14) where a significant reduced food intake was observed. At 2000 ppm a significant reduction of food intake during the first week was observed in males and females, respectively, but was comparable with corresponding control groups in the following weeks. The reduced food consumption at week 1 in the higher dose groups (2000 and 8000 ppm) was considered consistent with the introduction of an unpalatable diet. The lacking palatability was also reflected by the observed food spillage in one 2000 ppm group female and in several high dose animals. Consequently, the food consumption values measured were too high at various time points and in turn might be the reason for the dose-related reduction in overall body weight gain. Thus, the mean test item intake may also be overestimated in particular in high dose group males.

No differences of food intake were observed in the animals of 50 and 300 ppm dose groups.

Table 5.8.1-27: Mean food and water consumption of rats administered phenyl-guanidine-carbonate for 90 days

Dose level	Males					Females				
[ppm]	0	50	300	2000	8000	0	50	300	2000	8000
[mg/kg bw/d]	-	3.06	17.8	131	536	-	3.52	22.1	140	616
Mean food consumption/rat/day [g]										
Week -1	22.54 ± 1.35	23.70 ± 1.88	22.16 ± 1.81	24.05 ± 2.34	23.65 ± 1.45	16.77 ± 1.12	16.66 ±1.56	17.25 ± 1.24	17.73 ± 1.40	16.06 ± 1.49
Week 1	22.20 ± 1.41	23.12 ± 1.89	21.26 ± 1.62	**17.79*** ± 1.63	**10.12**** ± 2.64	16.12 ± 1.60	15.38 ± 1.31	15.86 ± 1.51	**12.23**** ± 1.57	**5.67**** ± 1.22
Week 2	23.49 ± 1.09	24.11 ± 2.05	22.67 ± 1.35	21.54 ± 2.24	21.38 ± 2.44	17.38 ± 1.56	16.54 ± 1.40	17.00 ± 1.67	15.92 ± 1.55	14.89 ± 2.09
Week 8	20.54 ± 1.89	20.97 ± 2.06	20.42 ± 1.95	21.52 ± 2.61	29.51 ± 11.01	14.22 ± 2.02	13.93 ± 0.78	16.08 ± 1.74	16.39 ± 2.15	15.91 ± 2.88
Week 14	21.62 ± 1.22	22.42 ± 2.44	21.14 ± 1.38	19.99 ± 3.70	**20.09*** ± 8.12	15.67 ± 1.90	15.27 ± 1.22	17.16 ± 2.41	15.79 ± 4.89	14.52 ± 1.71
Mean water consumption/rat/day [g]										
Week -1	22.81 ± 2.14	23.15 ± 2.14	22.73 ± 2.03	24.02 ± 3.40	24.71 ± 3.43	18.16 ± 3.71	19.83 ± 1.33	19.31 ± 3.61	20.25 ± 2.82	18.41 ± 2.76
Week 1	26.85 ± 1.28	27.27 ± 2.51	26.89 ± 2.35	30.95 ± 4.80	21.29 ± 6.44	22.71 ± 3.50	21.77 ± 2.87	25.92 ± 4.86	25.31 ± 5.97	**15.25**** ± 4.86
Week 2	26.66 ± 2.84	25.11 ± 2.06	26.18 ± 3.50	31.35 ± 5.48	28.68 ± 5.54	20.47 ± 4.63	21.41 ± 3.22	24.01 ± 4.95	**25.81*** ± 4.58	22.29 ± 3.84
Week 8	24.86 ± 3.50	23.36 ± 2.25	24.76 ± 2.97	26.43 ± 4.16	26.13 ± 8.22	20.50 ± 4.07	22.41 ± 4.15	**27.90*** ± 5.37	27.26 ± 8.70	21.36 ± 3.17
Week 12	23.72 ± 3.11	21.79 ± 2.80	27.96 ± 3.12	26.61 ± 4.43	24.54 ± 6.62	20.90 ± 4.39	22.33 ± 4-36	**27.11*** ± 5.31	24.46 ± 5.15	21.11 ± 4.64
Week 13	24.50 ± 2.92	23.21 ± 4.34	24.51 ± 4.07	27.32 ± 5.56	25.62 ± 7.12	19.30 ± 3.83	22.82 ± 4.08	26.68 ± 7.05	26.31 ± 8.72	21.10 ± 3.99

Values presented as group mean ± S.D.
* = p≤0.05; ** = p≤0.01

The statistically significantly reduced water consumption in the high dose animals at week one might be correlated with reduced food intake during this period.
In the absence of a dose-relationship, the statistically significant increase in water consumption in 2000 ppm females at week 2, and in 300 ppm females at weeks 8 and 12 was considered incidental and not treatment-related. However, it might be also a consequence of unpalatable diet consumption.

The calculated average test article intake is presented in Table 5.8.1-28 below.

Table 5.8.1-28: Mean daily substance intake of rats administered phenylguanidine-carbonate for 90 days

Dose level [ppm]	0	50	300	2000	8000
Substance intake [mg/kg bw/day]					
Males	-	3.06	17.8	131	677 (**536*)**
Females	-	3.52	22.1	147 (140)	638 (616)

(*): Calculation of test item intake based on animals without food spillage

E. Clinical assessment of neurotoxicity

No abnormal FOB findings were observed in animals of both sexes in 50 and 300 ppm dose groups and in females at 2000 and 8000 ppm.
FOB findings in 2000 ppm males included noticeably, but not significantly reduced grip strengths for fore-paws and hind-paws, as well as decrease in landing foot splay. These findings were considered to be secondary to body weight reductions and not indicative of a specific neurotoxic effect [see Table 5.8.1-29].

FOB findings in 8000 ppm males included reduced landing foot splay and statistically significant decreases in fore-paw and hind-paw strengths. However, relative grip strength (grip strength/g body weight) was higher compared with control animals. These effects were thus considered to be secondary to lower body weight and not a direct effect of the test item. Similar to grip strength, the reduction in landing food splay was considered to be incidental and not related to exposure to the test item because in case of peripheral neuropathy an increase in landing foot splay would be expected.

Table 5.8.1-29: FOB findings in males at week 13

	FOB findings				
	Males				
Dose level [ppm]	**0**	**50**	**300**	**2000**	**8000**
Dose level [mg/kg bw/d]	**-**	**3.06**	**17.8**	**131**	**536**
Mean body weight [g]	437.8 ± 33.5	432.0 ± 34.5	428.8 ± 33.7	**376.0**** ± 41.1	**297.2**** ± 20.3
Grip strength (fore-paw) [g]	1843 ± 174	1870 ± 289	1810 ± 215	1615 ± 265	**1523*** ± 275
Relative grip strength (fp)	4.21	4.33	4.22	4.30	5.12
Grip strength (hind-paw) [g]	1465 ± 194	1453 ± 79	1413 ± 170	1278 ± 168	**1193**** ± 181
Relative grip strength (hp)	3.35	3.36	3.30	3.40	4.01
Landing foot splay [cm]	12.31 ±1.09	11.35 ± 0.88	11.40 ± 1.66	11.05 ± 1.82	9.90 ± 2.77

Values presented as group mean ± S.D.

No treatment-related effects on the various motor activity parameters tested were observed in treated groups.

F. BLOOD ANALYSIS

1. Hematology

There were no significant hematological variations in males in 50 ppm and females in 50, 300 and 2000 ppm dose groups [see Table 5.8.1-30].

Regarding the red blood cell system males at 8000 ppm had a significant lower mean values for hemoglobin concentration (HGB) and hematocrit (HCT), which were associated with slight (statistically not significant) reduced erythrocyte count (RBC). Females at the high dose of 8000 ppm had a significantly higher hemoglobin distribution width (HDW). The reduction of red blood cell parameters was considered to be secondary to reduction of body weight and food consumption and the consequential alteration of bone marrow cellularity, which is further discussed in the histopathology part. The red blood cell parameters in males were generally at the lower boundary of the historical control data in the control and dose groups. RBC value of the concurrent control 8.39 were even slightly below the historical control (HC) mean range from 8.46 - 9.40. This may be due to the fact that already in the control animals the incidence of bone marrow changes (fatty atrophy) was present, and thus the hematopoiesis was already reduced in the controls. Two factors for the change of bone marrow cellularity may have acted together in this study. First the background level of bone marrow change, which is age- and species-dependent, and second the dietary influence on bone marrow cellularity, where it was shown that diet deprivation leads to the bone marrow fatty atrophy.

In the high and mid dose males a significant lower prothrombin activity (PT) was noted. PT was also decreased in females at the high dose of 8000 ppm. Lower PT parameters in males were still within the historical control data (mean values ranging from 0.74 - 0.93).

A significant decrease in leukocyte count (WBC), was noticed in males at 300 ppm, to a lesser extent at 2000 ppm which was not noted at the highest dose tested (8000 ppm). Females treated with 8000 ppm test item had an increased WBC associated with lymphocytosis and higher values for eosinophils, basophils and monocytes. WBC parameters in males were within the mean historical control range. The WBC decrease in males was not considered to be treatment related but incidental in nature due to the lacking dose response and being within the HC range. The WBC parameters in the 8000 ppm dose group in females was outside the HC range [see Table 5.8.1-30]. This may partly be due to a wide range of WBC parameters from 3.87 to a very high value in a single animal of 11.18. Furthermore, fatty atrophy in bone marrow as depicted in Table 5.8.1-35 is associated with an increase in WBC count, especially of myeloid cells. This is also reflected by the observation that number of monocytes, eosinophils and basophiles were statistically increased in females of the top dose.

In conclusion, no direct substance-related effect on hematology parameters is supposed.

Table 5.8.1-30: Selected haematology data at Days 92-93

		Males					Females				
Dose level	**[ppm]**	**0**	**50**	**300**	**2000**	**8000**	**0**	**50**	**300**	**2000**	**8000**
	[mg/kg bw/day]	**-**	**3.06**	**17.8**	**131**	**536**	**-**	**3.52**	**22.1**	**140**	**616**
RBC [10^{12}/L]		8.39 ± 0.54	8.45 ± 0.35	8.52 ± 0.36	8.28 ± 0.50	7.95 ± 0.41	7.62 ± 0.35	7.68 ± 0.28	7.72 ± 0.24	7.55 ± 0.37	7.93 ± 0.29
		HC: 8.46 - 9.40					HC: 7.55 - 8.39				
HGB [mmol/L]		9.44 ± 0.25	9.24 ± 0.29	9.26 ± 0.23	9.12 ± 0.23	**8.83**** ± 0.31	8.82 ± 0.31	9.11 ± 0.26	8.86 ± 0.31	8.59 ± 0.44	8.84 ± 0.18
		HC: 9.3 - 10.2					HC: 8.8 - 9.7				
HCT [L/L]		0.46 ± 0.001	0.45 ± 0.02	0.45 ± 0.02	0.45 ± 0.02	**0.42**** ± 0.02	0.43 ± 0.02	0.44 ± 0.01	0.44 ± 0.02	0.42 ± 0.02	0.44 ± 0.01
		HC: 0.46 - 0.50					HC: 0.44 - 0.47				
HDW ¥ [mmol/L]		1.58 ± 0.08	1.70 ± 0.16	1.57 ± 0.11	1.66 ± 0.10	1.73 ± 0.17	1.23 ± 0.07	1.31 ± 0.12	1.27 ± 0.09	1.33 ± 0.14	**1.44**** ± 0.15
		HC: 1.37 - 1.87					HC: 1.18 - 1.55				
WBC [10^9/L]		7.86 ± 1.49	6.87 ± 1.26	**5.71**** ± 1.18	**6.09*** ± 0.86	7.52 ± 1.61	3.81 ± 0.56	4.06 ± 0.95	4.84 ± 0.93	4.19 ± 0.77	**7.75**** ± 2.01
		HC: 5.27 - 8.91					HC: 2.41-5.44				
Mono [10^9/L]		0.18 ± 0.06	0.16 ± 0.05	**0.12*** ± 0.05	**0.10**** ± 0.04	0.17 ± 0.08	0.09 ± 0.03	0.09 ± 0.02	0.11 ± 0.03	0.08 ± 0.03	**0.13*** ± 0.03
		HC:0.16 - 0.32					HC: 0.06 - 0.24				
Eos [10^9/L]		0.12 ± 0.04	0,10 ± 0.04	0.12 ± 0.03	0.10 ± 0.02	0.12 ± 0.05	0.07 ± 0.02	0.08 ± 0.02	0.08 ± 0.02	0.07 ± 0.03	**0.14*** 0.07
		HC: 0.08 - 0.17					HC: 0 05 - 0.10				
Baso [10^9/L]		0.08 ± 0.03	0.06 ± 0.02	0.05 ± 0.02	0.05 ± 0.01	0.08 ± 0.05	0.03 ± 0.01	0.05 ± 0.02	0.04 ± 0.02	0.03 ± 0.02	**0.07**** 0.03
		HC: 0.01 - 0.04					HC: 0.00 - 0.02				
PT [rel. 1]		0.93 ± 0.10	0.93 ± 0.13	0.89 ± 0.10	**0.74**** ± 0.12	**0.76**** ± 0.19	1.07 ± 0.11	0.97 ± 0.03	1.17 ± 0.07	1.01 ± 0.10	0.95 ± 0.12
		HC: 0.74 - 0.93					HC: 0.82 - 1.08				

Values presented as group mean ± S.D.
* = p≤0.05; ** = p≤0.01
¥ representing the standard deviation of the haemoglobin concentration histogram.
HC = historical control range of study means (N = 19)

2. Clinical chemistry findings

Males had a significant reduction of total protein and globulin at 2000 ppm and above, as well as increased albumin-to-globulin (A/G) ratio [see Table 5.8.1-31]. Females were only affected for these parameters in the high dose group of 8000 ppm. Furthermore, the albumin concentration was significantly reduced in females of the high dose group. A significant increase of total bilirubin was noticed in males at the highest dose only.

Significantly increased urea was seen in both sexes at 8000 ppm. Significant elevations in creatinine was noticed in females only at both 2000 and 8000 ppm but without a clear dose-dependency. Furthermore, significant elevations in potassium in high dose females was determined.

Thisese findings are consistent with effects on renal and hepatic functions in conjunction with malnutrition and/or malabsorption.

In high dose groups of both sexes increased alkaline phosphatase (AP) activities were detected that were considered treatment related. Females treated with 8000 ppm test item showed a significantly increased gamma-glutamyl-transpeptidase (GGT) activity in five out of 10 animals, while in males only one animal was affected not leading to statistical significance. The increase in GGT in conjunction with increase in AP suggests compromising effects on hepatobiliary function in line with the changes observed for bilirubin and A/G ratio which might also be attributed to liver toxicity

Calcium levels were significantly decreased in high dose males and in females of the 50, the 2000 and the 8000 ppm group. There was however no clear dose-dependency in females and the decrease was attributed to an unusual high control group value. All values were within the historical control range [see Table 5.8.1-31]. In males the slight decrease was also considered to be incidental as being clearly within the historical control range.

The decreased glutamate dehydrogenase (GLDH) activities in high dose females as well as the decreased alaninamino transferase levels in mid dose females were not considered to be adverse as decreases of the parameters are not considered to be indicative of toxicity.

With an exception of a reduced mean blood calcium level in females in the 50 ppm treatment group, considered to be an incidental finding, there were no treatment related alterations in blood chemistry at the 50 and 300 ppm dietary levels.

Table 5.8.1-31: Selected clinical chemistry data on Days 92 - 93

	Males					Females				
Dose level [ppm]	**0**	**50**	**300**	**2000**	**8000**	**0**	**50**	**300**	**2000**	**8000**
Dose level [mg/kg bw/day]	**-**	**3.06**	**17.8**	**131**	**536**	**-**	**3.52**	**22.1**	**140**	**616**
Urea [mmol/L]	5.69 ± 0.66	5.94 ± 0.47	6.48 ± 1.20	6.39 ± 0.99	**8.13**** ± 1.35	7.25 ± 0.88	7.13 ± 0.71	7.12 ± 0.62	8.02 ± 0.66	**9.98**** ± 1.20
	HC: 5.49 - 7.02					HC: 6.51 - 8.60				
Creatinine [µmol/L]	28.02 ± 2.47	27.99 ± 1.80	30.84 ± 3.08	28.23 ± 3.19	30.50 ± 2.47	29.82 ± 2.69	30.03 ± 3.28	30.27 ± 2.98	**34.83**** ± 2.40	**34.05*** ± 4.65
	HC: 23.9 - 39.5					HC: 25.3 - 43.0				
Total Bilirubin [µmol/L]	1.57 ± 0.34	1.47 ± 0.24	1.43 ± 0.31	1.71 ± 0.43	**2.05*** ± 0.43	1.73 ± 0.59	1.37 ± 0.28	1.53 ± 0.28	1.68 ± 0.96	1.93 ± 0.76
	HC: 1.25 - 1.77					HC: 1.53 - 2.29				
Total protein [g/L]	64.97 ± 1.95	64.52 ± 2.26	63.24 ± 1.86	**62.45*** ± 2.31	**56.78**** ± 1.72	68.87 ± 2.39	66.25 ± 2.87	67.29 ± 1.99	65.56 ± 3.94	**57.47**** ± 2.01
	HC: 62.45 - 71.13					HC: 65.36 - 74.01				
Albumin [g/L]	40.31 ± 1.65	41.19 ± 1.59	40.47 ± 1.93	41.49 ± 1.67	39.47 ± 1.60	46.24 ± 2.16	44.43 ± 2.62	45.82 ± 2.30	43.82 ± 3.32	**40.97**** ± 1.50
	HC: 40.47 - 41.35					HC: 46.02 - 47.47				
Globulin [g/L]	24.66 ± 1.75	23.33 ± 2.03	22.77 ± 2.32	**20.96*** ± 0.97	**17.49**** ± 0.84	22.63 ± 0.98	21.82 ± 0.94	21.47 ± 0.70	21.74 ± 2.07	**16.50**** ± 1.82
	HC: 21.10 - 26.10					HC: 20.03 - 21.59				
A/G ratio	1.64 ± 0.15	1.78 ± 0.18	1.80 ± 0.24	**1.98**** ± 0.09	**2.26**** ± 0.15	2.05 ± 0.13	2.04 ± 0.14	2.14 ± 0.16	2.03 ± 0.26	**2.51**** ± 0.33
	HC: 1.58 - 1.97					HC: 2.14 - 2.37				
K^+ [mmol/L]	4.23 ± 0.39	4.12 ± 0.16	4.53 ± 1.00	4.04 ± 0.27	4.95 ± 0.86	3.54 ± 0.27	3.73 ± 0.36	3.45 ± 0.24	3.30 ± 0.28	**4.12**** ± 0.38
	HC: 3.16 - 4.01					HC: 2.67 - 3.45				
Ca^{2+} [µmol/L]	2.78 ± 0.09	2.71 ± 0.05	2.75 ± 0.04	2.71 ± 0.03	**2.67*** ± 0.07	2.83 ± 0.06	**2.74*** ± 0.09	2.78 ± 0.06	**2.74*** ± 0.05	**2.65*** ± 0.06
	HC: 2.45 - 2.75					HC: 2.47 - 2.74				
ALAT [U/L]	32.27 ± 6.94	38.10 ± 20.17	28.64 ± 4.73	32.73 ± 11.86	51.21 ± 47.96	33.70 ± 13.86	23.44 ± 4.53	28.06 ± 3.87	**21.70**** **± 2.69**	34.57 ± 8.20
	HC: 25.7 - 43.3					HC: 20.4 - 42.0				
AP [U/L]	63.04 ± 9.87	63.54 ± 7.13	63.56 ± 15.44	66.25 ± 11.31	**92.30**** ± 11.65	22.93 ± 2.25	28.46 ± 7.78	31.02 ± 15.14	29.58 ± 10.42	**80.55**** ± 24.07
	HC: 63.9 - 81.5					HC: 23.7 - 35.7				
GGT [U/L]	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.26 ± 0.82	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	**1.27**** ± 1.36
	HC: 0.0 - 0.0					HC: 0.0 - 0.3				
GLDH [U/L]	10.14 ± 6.29	10.03 ± 4.29	8.31 ± 3.15	6.63 ± 3.20	9.30 ± 4.29	28.95 ± 31.34	8.11 ± 5.79	15.22 ± 11.06	5.73 ± 0.72	**5.74*** ± 2.40
	HC: n. a.					HC: n. a.				

Values presented as group mean ± S.D.
HC = historical control range of study means (N = 3 - 19)
n. a. = not available
* = p≤0.05; ** = p≤0.01

G. URINALYSIS

At 8000 ppm significant elevated protein levels (in both sexes), increased bilirubin level in both sexes (significant only in females), and a significant increase in pH in females (no indications of a pH effect in males) [see Table 5.8.1-32] were observed.

The observed proteinuria coinciding with the observed effects on in blood chemistry of higher plasma urea levels and higher plasma creatinine levels suggest a disturbance of kidney function. The major mechanisms producing proteinuria include increased tubular cell secretion, decreased tubular resorption in normal filtered proteins, and an increase of filtered proteins caused by altered glomerular capillary permeability. However, no treatment-related histopathological correlate was determined for the kidney and the reported absolute weight decreases [see Table 5.8.1-33] without corroborating relative organ weight changes are attributed to the body weight impairment instead of an organ specific effect. Thus, the toxicological relevance of these blood chemistry and urinalysis changes is considered equivocal.

The increased levels in urinary bilirubin are consistent with the increased serum values in both sexes, and suggest some compromise of hepatobiliary function (also indicated by elevated GGT and AP levels).

No treatment related alterations in urine cellularity or composition in animals of both sexes at the 50, 300 and 2000 ppm dietary levels were observed.

Table 5.8.1-32: Selected urinalysis parameters for rats treated with phenylguanidine-carbonate after 90 Days

	Males					Females				
Dose level [ppm]	**0**	**50**	**300**	**2000**	**8000**	**0**	**50**	**300**	**2000**	**8000**
Dose level [mg/kg bw/d]	**-**	**3.06**	**17.8**	**131**	**536**	**-**	**3.52**	**22.1**	**140**	**616**
Protein [g/L]	0.7 ± 0.2	0.7 ± 0.2	0.7 ± 0.2	0.8 ± 0.2	**1.0*** ± 0.4	0.5 ± 0.3	0.3 ± 0.2	0.4 ± 0.2	0.5 ± 0.3	**1.3*** ± 1.3
Bilirubin [µmol/L]	10.2 ± 8.8	15.3 ± 5.4	15.3 ± 5.4	15.3 ± 5.4	17.0 ± 0.0	5.1 ± 8.2	3.4 ± 7.2	3.4 ± 7.2	13.6 ± 7.2	**15.3*** ± 5.4
pH	6.6 ± 0.3	6.3 ± 0.5	6.5 ± 0.2	6.4 ± 0.6	7.0 ± 0.6	5.2 ± 0.4	5.5 ± 0.6	5.6 ± 0.8	5.8 ± 0.4	**6.7*** ± 0.4

Values presented as group mean ± S.D.
* = p≤0.05; ** = p≤0.01

H. Ophthalmoscopy

There were no ocular changes or abnormalities that could be attributed to treatment.

I. NECROPSY

1. Organ weight

Carcass weight in both sexes of the 2000 and 8000 ppm group was reduced and reflected the changes of the body weight development reported in Table 5.8.1-33.

Absolute spleen weights were decreased in both sexes at the 2000 and the 8000 ppm group. The relative weights were significantly decreased at the 8000 ppm group in males and at the 300, 2000 and 8000 ppm group in females. No histopathological correlate was determined in the 2000 ppm group [see Table 5.8.1-35]. For the high dose a decreased splenic hematopoietic activity was determined. The weight effects in the 2000 and 8000 ppm groups were considered secondary to the malnutrition status of the animals of these dose groups. The effect on relative (but not absolute) spleen weight in the 300 ppm dose group was at least partly affected by the higher body weight in this dose group. The relative weight reduction without affection of food consumption and body weight development or histopathological correlate was thus considered to be incidental ant not treatment related.

Absolute liver weight decreases were noticed at 8000 ppm in both sexes. However, as these weight changes did not coincide by relative weight changes, but were only supported by a noticed decreased glycogen storage at this dose level [see Table 5.8.1-35 below] these finding is considered secondary to the reduced food consumption and impairment of bodyweight development reported above. The statistically significant increase in relative weight of the 2000 ppm males without concurrent effect on absolute weight and without dose-response relationship was considered to be incidental.

The determined absolute kidney and heart weight reductions in the 2000 and 8000 ppm males and the 8000 ppm females without affected relative weights and without histopathological correlate are considered secondary to the malnutrition status of the animals of those dose groups.

Absolute organ weights of the reproductive system (testis, epididymidis, uterus and ovaries) were reduced in the high dose group (uterus weights without statistical significance) [see Table 5.8.1-33]. Relative weights were not affected for any of these organs. In females the weight reductions were without histopathological correlate.

Relative brain weight increases in high dose animals were determined for both sexes. The absolute weights were, however, not affected in males or even slightly reduced in females indicating that these relative changes reflect the reduced carcass weights instead of organ weights alterations.

Absolute adrenal weights were decreased in females of the high dose group and relative weights were increased in males. The effect on relative adrenal weight in high dose males may be based on the calculation of the ratio organ weight/body weight instead of organ/brain weight, which is considered more appropriate for evaluation of relative adrenal weights. In the thyroid relative weights were significantly increased in high dose males but the absolute weights were in the normal range. In the absence of any histopathological correlate these alterations are considered to reflect a physiological response to the effects on the body weight development and thus not a primary adverse effect.

No treatment related alterations of organ weights in animals of both sexes at the 50 ppm and in males at the 300 ppm dietary intake levels were observed.

Table 5.8.1-33: Absolute and relative organ weight data for males and females

	Weights									
	Males					Females				
Dose level [ppm]	**0**	**50**	**300**	**2000**	**8000**	**0**	**50**	**300**	**2000**	**8000**
Dose level [mg/kg bw/day]	**-**	**3.06**	**17.8**	**131**	**536**	**-**	**3.52**	**22.1**	**140**	**616**
Carcass weight	410.30 ± 28.30	416.60 ± 33.40	406.80 ± 31.40	**358.10**** ± 43.40	**282.50**** ± 23.10	234.40 ± 20.60	234.60 ± 21.00	241.50 ± 18.90	221.80 ± 18.50	**182.70**** ± 14.30
	HC: 361.30 - 401.60					HC: 204.50 - 227.40				
Spleen, abs. [g]	0.77 ± 0.14	0.76 ± 0.11	0.70 ± 0.06	**0.62*** ± 0.05	**0.44*** ± 0.10	0.63 ± 0.09	0.60 ± 0.09	0.57 ± 0.06	**0.48**** ± 0.07	**0.39**** ± 0.06
	HC: 0.71 - 0.77					HC: 0.52 - 0.62				
Spleen, rel.	1.91 ± 0.22	1.83 ± 0.21	1.74 ± 0.18	1.74 ± 0.14	**1.55* ± 0.26**	2.70 ± 0.29	2.56 ± 0.27	**2.36* ± 0.28**	**2.18**** ± 0.27**	**2.14**** ± 0.22**
	HC mean range: 1.87 - 2.12					HC mean range: 2.46 - 2.95				
Liver, abs. [g]	13.87 ± 1.06	14.82 ± 1.62	14.43 ± 1.71	13.18 ± 1.97	**10.04 ± 1.09****	8.74 ± 0.79	8.65 ± 1.01	9.15 ± 1.08	8.30 ± 0.91	**6.27**** ± 0.57
	HC: 12.54 - 14.22					HC: 7.06 - 8.37				
Liver, rel.	33.80 ± 1.80	35.50 ± 1.90	35.40 ± 2.60	**36.70 ± 2.10***	35.50 ± 1.50	37.30 ± 1.80	36.90 ± 3.70	37.90 ± 3.90	37.50 ± 3.20	34.40 ± 2.90
	HC: 34.30 - 36.20					HC: 33.78 - 37.98				
Kidney, abs [g].	2.67 ± 0.16	2.63 ± 0.23	2.50 ± 0.22	**2.37*** ± 0.29	**1.95**** ± 0.20	1.69 ± 0.14	1.72 ± 0.24	1.78 ± 0.22	1.63 ± 0.09	**1.34**** ± 0.13
	HC: 2.14 - 2.43					HC: 1.43 - 1.66				
Kidney, rel.	6.53 ± 0.41	6.32 ± 0.27	6.15 ± 0.29	6.65 ± 0.70	6.90 ± 0.28	7.22 ± 0.52	7.31 ± 0.68	7.36 ± 0.75	7.38 ± 0.48	7.36 ± 0.78
	HC: 5.92 - 6.25					HC: 6.86 - 7.68				
Heart, abs. [g]	1.11 ± 0.09	1.12 ±0.08	1.09 ± 0.09	**0.98*** ± 0.11	**0.80**** ± 0.09	0.79 ± 0.07	0.79 ± 0.09	0.83 ± 0.11	0.72 ± 0.08	**0.59**** ± 0.05
	HC: 1.02 - 1.20					HC: 0.70 - 0.83				
Heart, rel.	2.72 ± 0.17	2.70 ± 0.07	2.68 ± 0.16	2.75 ± 0.17	2.81 ± 0.18	3.38 ± 0.21	3.39 ± 0.39	3.41 ± 0.36	3.27 ± 0.27	3.22 ± 0.16
	HC: 2.77 - 3.20					HC: 3.38 - 3.83				
Thyroid & Parathyroid, abs. [mg]	24.60 ± 4.10	25.30 ± 6.00	22.40 ± 6.10	22.50 ± 4.20	24.90 ± 5.30	22.60 ± 6.80	21.90 ± 4.00	24.00 ± 4.90	22.20 ± 3.70	19.90 ± 5.20
	HC: 27.22 - 46.09					HC: 24.90 - 35.26				
Thyroid & Parathyroid, rel.	0.06 ± 0.01	0.06 ±0.01	0.06 ± 0.01	0.06 ± 0.01	**0.09**** ± 0.02**	0.10 ± 0.03	0.09 ± 0.02	0.10 ± 0.02	0.10 ± 0.02	0.11 ± 0.03
	HC: 0.07 - 0.13					HC: 0.11 - 0.16				

Table 5.8.1-33: Absolute and relative organ weight data for males and females

	Weights									
	Males					Females				
Dose level [ppm]	**0**	**50**	**300**	**2000**	**8000**	**0**	**50**	**300**	**2000**	**8000**
Dose level [mg/kg bw/day]	**-**	**3.06**	**17.8**	**131**	**536**	**-**	**3.52**	**22.1**	**140**	**616**
Adrenals, abs. [mg]	60.10 ± 5.80	60.80 ± 8.10	59.60 ± 8.00	53.00 ± 6.30	51.60 ± 8.30	~~0.07~~ 74.00 ± ~~0.01~~ 13.90	~~0.07~~ 69.40 ± ~~0.01~~ 13.30	~~0.08~~ 74.60 ± ~~0.01~~ 12.20	~~0.07~~ 72.70 ± ~~0.01~~ 11.80	~~**0.05****~~ **52.30**** ± ~~0.01~~ 8.50
	HC: 64.47 - 81.63					HC: 78.66 - 97.20				
Adrenals, rel.	0.15 ± 0.01	0.15 ± 0.02	0.15 ± 0.02	0.15 ± 0.02	**0.18**** ± 0.03	0.32 ± 0.06	0.30 ± 0.04	0.31 ± 0.04	0.33 ± 0.05	0.29 ± 0.04
	HC: 0.17 - 0.22					HC: 0.38 - 0.47				
Brain, abs. [g]	2.16 ± 0.07	2.14 ± 0.08	2.13 ± 0.07	2.08 ± 0.11	2.05 ± 0.15	2.00 ± 0.07	2.00 ± 0.10	2.05 ± 0.09	1.94 ± 0.06	**1.88**** ± 0.04
	HC: 2.18 - 2.30					HC: 2.00 - 2.11				
Brain, rel.	5.28 ± 0.33	5.15 ± 0.41	5.27 ± 0.46	5.86 ± 0.59	**7.27**** ± 0.61	8.55 ± 0.50	8.54 ± 0.49	8.51 ± 0.69	8.78 ± 0.69	**10.4**** ± 0.8
	HC: 5.72 - 6.30					HC: 8.99 - 10.18				
Testes, abs. [g]	4.04 ± 0.36	3.91 ± 0.37	3.81 ± 0.33	3.70 ± 0.18	**2.95**** ± 0.80	-	-	-	-	-
	HC: 3.53 - 3.84					-				
Testes, rel.	9.84 ± 0.42	9.39 ± 0.51	9.36 ± 0.86	10.40 ± 1.10	10.30 ± 2.40	-	-	-	-	-
	HC: 9.37 - 10.44					-				
Epididymides, abs. [g]	1.40 ± 0.12	1.42 ± 0.16	1.41 ± 0.1495	1.24 ± 0.10	**0.93**** ± 0.26	-	-	-	-	-
	HC: 1.33 - 1.41					-				
Epididymides, rel.	3.41 ± 0.29	3.41 ± 0.40	3.48 ± 0.47	3.49 ± 0.42	3.25 ± 0.82	-	-	-	-	-
	HC: 3.55 - 3.92					-				
Ovaries, abs. [g]	-	-	-	-	-	0.15 ± 0.02	0.16 ± 0.04	0.17 ± 0.03	0.16 ± 0.03	**0.10**** ± 0.03
	-					HC: 0.16 - 0.20				
Ovaries, rel.	-	-	-	-	-	0.63 ± 0.07	0.68 ± 0.14	0.69 ± 0.11	0.71 ± 0.10	0.57 ± 0.14
	-					HC: 0.76 - 0.97				
Uterus, abs. [g]	-	-	-	-	-	0.84 ± 0.29	0.86 ± 0.15	0.92 ± 0.30	0.75 ± 0.22	0.60 ± 0.35
	-					HC: n. a.				
Uterus, rel.	-	-	-	-	-	3.59 ± 1.16	3.72 ± 0.89	3.78 ± 1.15	3.36 ±0.96	3.34 ± 2.10
	-					HC: n. a.				

Values presented as group mean ± S.D.
HC = historical control range of study means (N = 3 - 7)
n. a. = not available
* = p≤0.05; ** = p≤0.01

2. Gross lesions

At 8000 ppm males had smaller-sized epididymides, prostate glands, testes and seminal vesicles. These findings were associated with microscopic observations such as reduced secretion, reduced spermatozoa, reduced spermatogenic activity and testicular tubular atrophy.

At 2000 ppm one male had a smaller-sized prostate gland and the same animal showed diminished seminal vesicle. A smaller sized seminal vesicle was reported for one male of the low dose group of 50 ppm. However, for the single occurrence in the 2000 and the 50 ppm group animal no microscopic correlate was determined. Thus these isolated findings are not considered treatment related.

No findings were observed in female animals.

Table 5.8.1-34: Incidences of macroscopic findings in males

	Incidences of macroscopic findings				
	Males				
Dose level [ppm]	**0**	**50**	**300**	**2000**	**8000**
Dose level [mg/kg bw/day]	**-**	**3.06**	**17.8**	**131**	**536**
Epididymides • Diminished in size	-	-	-	-	3
Prostate gland • Diminished in size	-	-	-	1	6
Testes • Diminished in size	-	-	-	-	3
Seminal vesicle • Diminished in size	-	1	-	1	6

3. Histopathology

At 8000 ppm both sexes showed increased severity of fatty atrophy of the bone marrow and decreased splenic hematopoietic activity. These histopathological findings are considered in relation with the hematological alterations as treatment related findings. Spleen weights were decreased in the 2000 and 8000 ppm groups associated with decreased hematopoietic activity in the high dose group animals [see Table 5.8.1-30]. The background incidence of bone marrow fatty atrophy was high in the control group [Table 5.8.1-35], probably contributing to the rather low hematology parameters related to red blood cells (at the lower boundary of the historical control data) and the extramedullary hematopoiesis in the spleen. It is known that bone marrow fatty atrophy is related to age and species/strain, and thus may occur independent of treatment. Furthermore, fatty atrophy in bone marrow is also known to be induced by food deprivation. Thus, the effects on RBC (and WBC) and bone marrow parameters may be secondary to reduced food consumption and impaired body weight development in the dose groups.

The decreased incidence and severity of hepatocellular glycogen deposition and the increased incidence of cytoplasmic vacuolation of pancreatic acinar cells are considered physiological changes which probably reflect the decrease in body weight development of the affected groups.

In males the occurrence of precipitate and reduced numbers of sperm in the epididymides, reduced secretion in the prostate and seminal vesicle, and reduced spermatogenic activity in the testes were observed in high dose group. Furthermore, there was an increased incidence of tubular atrophy in testes. These findings correlated with the observed weight reductions and macroscopic observations in this dose group. The findings in the male reproductive system were however associated with a pronounced reduction in body weight gain (23% of control) indicating that the maximum tolerable dose was exceeded. Therefore these findings may be unspecific representing a poor general condition of the animals also in line with the clinical observations. The weight reductions in the female reproductive organs were without any histopathological correlate and might be related to the lower body weight gain as well.

No microscopic changes associated with the test substance were observed in animals of both sexes treated with 50, 300 and 2000 ppm test item.

Table 5.8.1-35: Incidences of microscopic findings after treatment with phenylguanidine-carbonate

		Incidences of microscopic findings									
		Males									
Dose level [ppm]		**0**	**50**	**300**	**2000**	**8000**	**0**	**50**	**300**	**2000**	**8000**
Dose level [mg/kg bw/day]		**-**	**3.06**	**17.8**	**131**	**536**	**-**	**3.52**	**22.1**	**140**	**616**
Bone marrow	# affected	10	9	10	10	**10**	8	10	8	8	**10**
• Fatty atrophy	Grade 1	2	2	-	-	**-**	2	1	1	1	**-**
	Grade 2	6	6	7	7	**-**	4	3	5	4	**-**
	Grade 3	2	1	3	3	**3**	2	6	2	3	**4**
	Grade 4	-	-	-	-	**6**	-	-	-	-	**6**
	Grade 5	-	-	-	-	**1**	-	-	-	-	**-**
Spleen	# affected	10	10	10	10	**10**	10	10	10	10	**10**
• Extramedullary hematopoiesis	Grade 1	1	1	-	-	**3**	-	-	-	-	**-**
	Grade 2	6	4	9	6	**6**	1	1	1	2	**8**
	Grade 3	3	4	1	3	**1**	2	4	2	2	**5**
	Grade 4	-	1	-	1	**-**	7	5	7	6	**4**
Liver • Deposition of glycogen	# affected	6	7	7	8	-	5	6	8	8	2
Pancreas • Cytoplasmic vacuolation	# affected	-	-	-	-	3	-	-	-	-	-
Thyroid gland	# affected	9	9	10	10	9	2		1	2	3
• Follicular hypertrophy	Grade 1	7	6	7	4	6	2	-	1	2	2
	Grade 2	2	2	2	5	2	-		-	-	1
	Grade 3	-	1	1	1	1	-		-	-	-
Epididymides • Precipitate	# affected	-	-	-	-	3					
Epididymides • Reduced spermatozoa	# affected	-	-	-	-	5					
Prostate gland • Reduced secretion	# affected	-	-	-	-	7					
Seminal vesicle • Reduced secretion	# affected	-	-	-	-	6					
Testes	# affected	1	2	3	1	**5**					
• Tubular atrophy	Grade 1	1	2	3	1	**4**					
	Grade 2	-	-	-	-	**1**					
Testes • Reduced spermatogen.	# affected	-	-	-	-	3					

III. CONCLUSIONS

The administration of phenylguanidine-carbonate induced treatment-related changes in animals in ≥ 2000 ppm dose groups. At these dose levels clinical signs were noted and depression in food consumption, water consumption and body weight development were observed. Laboratory investigations and histopathological examinations point to the hematopoietic and lympho-reticular system, reproductive system, liver and pancreas as potential target organs. In addition, changes in laboratory parameters indicate disturbance in kidney function. However, some of this alterations may be related to nutritional deficiency and not indicative of a target organ specific toxic effect.

The lowest observed effect level (LOAEL) for phenylguanidine-carbonate is 2000 ppm (equivalent to 131 mg/kg bw/day for males and 140 mg/kg bw/day for females), based on significantly lower mean body weight gains for both sexes, and a number of significant elevations and depressions in blood chemistry parameters. In addition, males had significantly reduced absolute and relative mean spleen weights.

The no observed effect level (NOAEL) for phenylguanidine-carbonate is 300 ppm (equivalent to 17.8 mg/kg bw/day for males and 22.1 mg/kg bw/day for females).

Report: CA 5.8.1/44
Khalil S., 2002 a
Prenatal developmental toxicity study in the rat - CGA 263208 tech.
[redacted]

Guidelines: OECD 414, EEC 87/302, EPA 870.3700, JMAFF No 12 Nosan No 8147

GLP: yes
(certified by Swiss Federal Office of Public Health, Berne, Switzerland)

Deviations: A new dose group (400 mg/kg bw/day) was introduced in the course of the study. 8 untreated animals (No. 121 to 128), starting of treatment one week later and the remaining 16 animals from the 1000 mg/kg bw/day group (No. 105 to 120; already treated with 1000 mg/kg bw/day for 3 days) were used in the new 400 mg/kg bw/day group. Although the dosing regime was somewhat inconsistent the obtained results did fit well into the other groups. Thus this deviation is not considered to compromise the overall validity of the study.

Executive Summary

Groups of 24 mated female Hanlbm:WIST (SPF) rats received phenylguanidine as the carbonate salt (Batch: 014941D0, Purity: 94.4%) in 0.5% w/w aqueous solution of sodium carboxy-methylcellulose (CMC), supplemented with 0.1% w/w aqueous polysorbate 80/ Tween, administered by gavage at 0, 20, 200, 600 and 1000 mg/kg bw/day on days 6 to 20 of gestation. Control animals received the vehicle alone. Based on the high maternal toxicity of the test item at 1000 mg/kg bw/day (8/24 females were found dead or were sacrificed moribund after 3 days of treatment), treatment at this dose level was discontinued. A new dose group (400 mg/kg bw/day) was introduced. Obviously, 8 untreated animals (No. 121 to 128) and the remaining 16 animals from the 1000 mg/kg bw/day group (No. 105 to 120; already treated with 1000 mg/kg bw/day for 3 days) were used in the new 400 mg/kg bw/day group. Although the dosing regime was somewhat inconsistent for this group the obtained results did fit well into the other groups. Thus this deviation is not considered to compromise the overall validity of the study. Animals were observed for mortality twice daily. Clinical signs and individual body weights were recorded daily. Food consumption data were recorded on gestation days 3, 6, 9, 11, 13, 16, 19 and 21. Termination and necropsy on day 21 of presumed gestation was followed by processing and detailed visceral and skeletal examination of foetuses.

At 600 mg/kg bw/day 2/24 females died around day 20, indicating that this dose exceeded the maximum tolerable dose. Animals of this dose group showed significantly reduced mean body weight and body weight change (-56% of control) or even body weight loss (-7.9% day 6 to necropsy) when considering corrected body weights (minus gravid uterus). These findings together indicated that the maximum tolerable dose was exceeded. Further contributing signs of maternal toxicity were their significant lower food consumption from day 6 to day 21, the occurrence of piloerection and salivation, and the bloody and clear vaginal discharges seen in almost all animals. Furthermore, abnormal gait, respiratory sounds and chromodacryorrhea were observed.
At 400 mg/kg bw/day animals showed significantly reduced mean body weight and body weight gain (-25% of control, respectively -57% of control when corrected for gravid uterus) throughout the treatment period, accompanied by significant lower food consumption from day 6 to day 21, the occurrence of piloerection and salivation, and the bloody and clear vaginal discharges seen in almost all animals.
At 200 mg/kg bw/day dams showed salivation and piloerection. Significantly lower mean food consumption was observed at the start of the treatment, but not subsequently. At necropsy, reddish and/or mottled thymus was observed, that was not the case in the animals of control and 20 mg/kg bw/day dose groups. This finding (bleeding) is however a secondary effect of the sacrifice of the animals and not considered related to treatment.
There were no indications of maternal toxicity at 20 mg/kg bw/day.

At 600 mg/kg bw/day foetuses showed significant reduced mean foetal body weight (75.5% of control) and significant delays in ossification. Micrognathia was observed in one foetus and cleft palate in another. As these findings were absent in the historical control data from 2426 foetuses, it is concluded that these malformations were due to exposure to the test material but clearly seen in relation to the exceeded toxicity of the dams.
At 400 mg/kg bw/day foetuses showed significant reduced mean foetal body weight (89.8% of control) and delays in ossification (not significant).
At 200 mg/kg bw/day foetuses showed reduced body weight (not significant) that was below the historical control data minimum. A doubling of incidence of the variation of unossified metatarsal 1 (not significant) that was outside the historical control range and a part of a dose-related trend was observed. This delayed ossification is attributed to the retardation in body weight development and thus considered a secondary effect.
There were no indications of fetal toxicity at 20 mg/kg bw/day.

Based on that findings, it was concluded that the maternal LOAEL for phenylguanidine-carbonate is 200 mg/kg bw/day and maternal NOAEL is 20 mg/kg bw/day.

Based on that findings, it was concluded that the developmental LOAEL for phenylguanidine-carbonate is 200 mg/kg bw/day and developmental NOAEL is 20 mg/kg bw/day.

(BASF DocID 2002/1027717)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:	phenylguanidine-carbonate salt, technical
Description:	solid (powder) / white
Lot/Batch #:	014941D0
Purity:	94.4%
Stability of test compound:	Analytical investigations (HPLC) indicated stability of the test item in suspension for 2 hours when stored at room temperature.
2. Vehicle and/or positive control:	5% w/w aqueous solution of sodium carboxymethyl-cellulose (CMC, pharmacopoeia quality, high viscosity (Hercules Powder Co., Product No. 7HF) supplemented with 0.1% w/w aqueous polysorbate 80 / Tween (Novartis Pharma AG) to improve suspendability.
3. Test animals:	
Species:	Rat
Strain:	Albino, Hanlbm:WIST (SPF)
Sex:	Female
Age (at mating):	Approximately 10 weeks
Weight (day 0 of gestation):	189.3 – 191.7 g
Source:	[redacted]
Acclimation period:	Approximately 1-2 weeks
Time of dosing:	Days 6 to 20 of gestation
Diet:	Pelleted certified standard diet (Nafag No. 890 FOR GLP), ad libitum
Water:	Tap water, ad libitum
Housing:	During acclimatisation: in groups of up to four females in suspended stainless steel cages with grid floors and lids. During mating (one pair per cage) and gestation (one female): in high density polypropylene cages with stainless steel grid floors and lids.
Environmental conditions:	
Temperature:	19 - 25°C
Humidity:	30 - 70%
Air changes:	16 - 20 per hour
Photo period:	12 h light / 12 h dark

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 11-Jun-2001 - 04-Feb-2002

2. Animal assignment and treatment:
Animals were assigned to dose groups each consistent of 24 mated females using a method of randomization by weight stratification. The dose levels were selected based on the results from a developmental toxicity study in Tif:RAIf (SPE) albino rats with the metabolic precursor cyprodinil, and were 0, 20, 200, 600 and 1000 mg/kg bw day. Based on the high maternal toxicity of the test item at 1000 mg/kg bw/day (8/24 females were found dead or were sacrificed moribund after 3 days of treatment), treatment at this dose level was discontinued. A new dose group (400 mg/kg bw/day) was introduced. Obviously, 8 untreated animals (No. 121 to 128) and the remaining 16 animals from the 1000 mg/kg bw/day group (No. 105 to 120; already treated with 1000 mg/kg bw/day for 3 days) were used in the new 400 mg/kg bw/day group. Although the dosing regime was somewhat inconsistent in this dose group - two thirds of the animals receiving 1000 ppm up to 3 days and subsequently 400 ppm from day 4 to day 20, while 8 animals were treated with 400 ppm throughout the whole dosing period, the obtained results did fit well into the other groups. Thus this deviation is not considered to compromise the overall validity of the study. All doses were administered once daily by gavage on gestation days 6 through 20, in a volume of 10 mL/kg bw/day, based on the body weight on the most recent body weight determination (made daily).

3. Test substance preparation and analysis:
Test material-vehicle mixtures were prepared daily (within 2 hours of administration) by mixing appropriate amounts of test substance with 5% w/w aqueous solution of sodium carboxymethyl-cellulose (CMC, pharmacopoeia quality, high viscosity (Hercules Powder Co., Product No. 7HF) supplemented with 0.1% w/w aqueous polysorbate 80 / Tween (Novartis Pharma AG) to improve the solubility. Test item-vehicle mixtures were prepared using a high-speed homogenizer (Polytron PT6000). A magnetic stirrer was used to maintain homogeneity of the mixtures during administration. The nominal concentrations of the test item in the resulting mixtures were 0, 2, 20, 40 and 60 mg/mL.
Analyses to verify test item concentration, stability and homogeneity in prepared suspensions were conducted on three dates during the administration period. On each date, samples of each dose concentration were collected in duplicate before and after dosing, with samples from before dosing taken from the top, middle and bottom of the container and samples from after dosing being taken from the middle of the container. The samples were stored frozen (-20°C) until analytical investigations.

4. Statistics:
Statistical analyses were conducted using the routines in the NOVATOX system. Quantitative data such as body and organ weights were analyzed using either parametric or non-parametric statistical tests after checking for uniformity of within group variances using Bartlett's test of homogeneity of variances. If differences were not significant, a one-way analysis of variance (ANOVA) was used, and if this was significant at $p \leq 0.05$, comparisons were made between the control group and each of the treatment groups by Dunnett's multiple comparison test. If Bartlett's test was significant, the Kruskal-Wallis non-parametric test of differences between groups was used, and if this in turn was significant, comparison was made between controls and each of the treatment groups using Dunn's multiple comparison test.
Data, such as percentage of lethal malformations in a litter were analyzed using the non-parametric Kruskal-Wallis test. If this test indicated $p \leq 0.05$, comparison were made between the control group and each of the treatment groups using Dunn's multiple comparison test.
Binomial data, such as presence or absence of fetal malformations in a litter, were evaluated by a chi-square test of association. If this gave $p \leq 0.05$, comparison were made between the controls and each treatment group using Fisher's exact test with Bonferroni correction.
All statistical tests were two-sided, and significant differences between groups at $p \leq 0.05$ and $p \leq 0.01$ were flagged (* and **, respectively) in the report tables.

C. METHODS

1. Observations:
Animals were observed for mortality twice daily. Signs of toxicity were recorded daily.

2. Body weight:
Individual body weights were measured daily.

3. Food consumption and compound intake:
Food consumption data were recorded on gestation days 3, 6, 9, 11, 13, 16, 19 and 21.

4. Sacrifice and pathology:
Dams were sacrificed on day 21 of gestation. Examinations at sacrifice consisted of:

- Macroscopic examination of the main organs of the thoracic and abdominal cavities, particularly the genitals
- Gravid uterine weight
- Number of corpora lutea in each ovary
- Uterine content:
    - Number and location of live and dead fetuses
    - Number and location of early and late [embryonic/fetal] losses
    - Examination of non-gravid uteri with ammonium sulfide to confirm the non-pregnant status.

Fetuses were sexed (using anogenital distance), weighed and examined for external and orifice abnormalities. Fetuses were then killed by subcutaneous injection of 5% w/v Pentothal in the neck. Fetuses were assigned to either visceral or skeletal evaluation at an approximate 1:1 ratio (starting with skeletal) for each litter independent of sex. For those fetuses with a gross external anomaly or malformation, allocation to examination type was based on the type and incidence of the finding. In the external examination of fetuses, there was particular attention to the following body regions and possible finding:

- Body surface (including generalized or localized edema, hemorrhage)
- Head (including cranioschisis, encephalocele, cleft palate)
- Trunk (including rachischisis, atresia of a body orifice, omphalocele)
- Extremities (including deformations, limb position anomaly, kinked tail)

For the soft tissue (visceral examinations), approximately one-half of the fetuses per litter were fixed in Bouin's solution for at least two weeks, and were then micro-dissected (with removal of the limbs, tail, and skin of the trunk), the head was sectioned, and the trunk was opened and the body walls and ribs peeled back to access the abdominal and thoracic organs. Examination included the morphology and position of the following organs and organ system:

- Skin
- Central nervous system: Brain (olfactory bulbs, cerebrum, lateral and medial ventricles), and spinal cord
- Eyes: lens, vitreous, retina
- Body cavities: thorax and abdomen, including diaphragm
- Respiratory system: nasal cavity (nasal septum, turbinates, choanae), trachea, bronchi, lungs, pleura
- Digestive system: oral cavity, palate, tongue, esophagus, stomach, intestine, rectum, liver, peritoneum
- Endocrine system: thyroid, pancreas, adrenals, thymus, pituitary
- Circulatory system: spleen, pericardium, heart (atria, ventricles, septae), major vessels
- Excretory system: kidneys (renal papillae, renal pelvis), ureters, urinary bladder
- Genital system: testes, epididymides, vas deferens, seminal vesicles; ovaries, oviducts, uterus

For skeletal examination, approximately half the fetuses for each litter were assessed using a modification of the staining technique of Dawson, involving staining with alizarin red S and alcian blue. Specimen were stored in glycerol. The following skeletal elements were included in the examination:

- Facial bones: nasal, premaxillary, maxillary and zygomatic bones, mandible
- Cranial bones: frontal, parietal, interparietal, occipital and exoccipital bones, fontanel
- Sternum: sternebrae 1 to 6
- Shoulder girdle: scapula and clavicle
- Forelimbs: humerus, ulna, radius, metacarpals and proximal phalanges 2 to 5, distal phalanges of anterior digits 1 to 5
- Pelvic girdle: ilium, ischium, pubis
- Hindlimbs: femur, tibia, fibula, calcaneus, metatarsals and distal phalanges 1 to 5, proximal phalanges 2 to 5 of posterior digits
- Ribs: anteroposterior 1 to 13
- Spinal column: cervical vertebral centers and arches 1 to 7, thoracic vertebral centers and arches 1 to 13, lumbar vertebral centers and arches 1 to 6, sacral vertebral centers and arches 1 to 4
- Cartilage: ribs, spinal column

Indices

The following indices were calculated from cesarean section records of animals in the study: total number of corpora lutea, pre-implantation loss %, post-implantation loss %; and mean fetal weight per litter (as the sum of weights of live fetuses divided by the number of live fetuses).

II. RESULTS AND DISCUSSION

A. TEST SUBSTANCE ANALYSES

Homogeneity values were within 8%, except for one nominal 60 mg/mL (for 600 ppm dose group) suspension (prepared on July 17, 2001) where the variation (values ranged from 59.22 to 74.60 mg/mL) was 26% of the nominal value.
The stability analysis revealed the effective concentrations of the test item in the 12 samples ranged from 100.4 to 111.7% of the nominal value. Thus, the stability of the suspension at room temperature for 2 h was confirmed.
The concentration analysis revealed the mean concentration of the test item ranged from 95.8 to 108.1% of the nominal value.
The analytical data indicated that the mixing procedure was adequate and that the variance between the nominal and actual dosage to the study animals was acceptable.

B. OBSERVATIONS

1. Clinical signs of toxicity
In most, if not all females at 400 and 600 mg/kg bw/day bloody and clear vaginal discharge as well as piloerection and salivation were observed. Symptoms seen only in females at 600 mg/kg bw/day included abnormal gait, respiratory sounds and chromodacryorrhea [see Table 5.8.1-36].
At 200 mg/kg bw/day, only in 2 animals clinical symptoms (piloerection and salivation) were observed.
No clinical signs were seen in control group and treatment group animals dosed with 20 mg/kg bw/day and in 22/24 females of the 200 mg/kg bw/day group.

2. Mortality
2 females (2/22 pregnant females) of the 600 mg/kg bw/day dose group were found dead on day 20 of gestation.
All other females in all other groups survived to termination [see Table 5.8.1-36].

C. BODY WEIGHT AND BODY WEIGHT GAIN

A significant reduction of the body weight and body weight gain was observed during the treatment period (days 6 - 20) in females exposed to 400 and 600 mg/kg bw/day [see Table 5.8.1-36]. The corrected body weight (minus gravid uterus) even showed body weight loss at 600 ppm, supporting the evaluation that the maximum tolerable dose was exceeded. Even at 400 ppm the corrected body weight gain was -57% of the control, indicating a severe toxic effect on the dams.
No alterations of the body weight gain in females at 20 and 200 mg/kg bw/day were observed during the whole study period.

D. FOOD CONSUMPTION

A dose-dependency was noticed in dose groups from 200 to 600 mg/kg bw/day starting on the day of the test item application (day 6) until the end of the study on day 21 for every observed time period [see Table 5.8.1-36]. In rats dosed at 400 and 600 mg/kg bw/day food consumption was significantly lower compared to control from day 6 to 21. In rats dosed at 200 mg/kg bw/day significantly reduced food consumption in the periods from days 6-13 were observed, but did not reach statistical significance thereafter, although food consumption values were consistently below those of control animals.
There was no significant effect in rats dosed at 20 mg/kg bw/day.

Table 5.8.1-36: Summary of maternal data in the teratology study with phenylguanidine-carbonate

Parameter	Dose [mg/kg bw/day]				
	0	**20**	**200**	**400**	**600**
Disposition of females					
Number mated	24	24	24	24	24
Number pregnant	23	22	20	23	20
Pregnancy rate (%)	95.8	91.7	83.3	95.8	83.3
Died pregnant	0	0	0	0	2
Died non-pregnant	0	0	0	0	0
Number with live young at termination	23	22	20	23	18
Number of litters examined	23	22	20	23	18
Clinical signs					
Piloerection	0/24	0/24	2/24	24/24	24/24
Salivation	0/24	0/24	2/24	22/24	24/24
Bloody vaginal discharge	0/24	0/24	0/24	7/24	19/24
Clear vaginal discharge	0/24	0/24	0/24	8/24	9/24
Abnormal gait	0/24	0/24	0/24	0/24	5/24
Respiratory system	0/24	0/24	0/24	0/24	2/24
Chromodacryorrhea	0/24	0/24	0/24	0/24	2/24
Bodyweight [g]					
Day 0	191.5 ± 9.3	189.3 ± 9.6	191.7 ± 10.0	189.5 ± 9.1	190.0 ± 9.9
Day 6	214.0 ± 11.4	211.7 ± 12.3	216.6 ± 12.9	212.7 ± 9.8	210.7 ± 11.6
Day 21	319.0 ± 26.2	306.7 ± 24.4	316.1 ± 20.6	**291.6**** ±17.6	**256.7**** ± 21.2
At necropsy, minus gravid uterus	244.5 ± 17.1	239.1 ± 18.9	246.2 ± 17.0	**225.9**** ± 11.6	**202.7**** ± 16.4
Net weight change from Day 6	30.2 ± 8.7	27.4 ± 10.3	29.6 ± 6.9	**13.1**** ± 8.3	**-7.9**** ± 14.7
Gravid uterine weight					
Gravid uterine weight [g]	75.2 ± 13.4	67.7 ± 19.1	70.0 ± 11.9	**65.7*** **± 11.9**	**53.9**** **± 9.2**
Gravid uterine weight [% difference from control]	0	-9.97	-6.91	-12.63[a]	-28.32[a]
Bodyweight [% difference from control]					
Day 21	0	-3.86	-0.91	**-8.59[a]**	**-19.53[a]**
At necropsy, minus gravid uterus	0	-2.21	0.70	**-7.61[a]**	**-17.10[a]**

Table 5.8.1-36: Summary of maternal data in the teratology study with phenylguanidine-carbonate

Parameter	Dose [mg/kg bw/day]				
	0	**20**	**200**	**400**	**600**
Bodyweight change [g]					
Days 0-6	22.8 ± 4.3	22.3 ± 4.1	24.9 ± 5.1	23.2 ± 4.2	20.6 ± 4.6
Days 6-21	105.4 ± 17.3	95.1 ± 15.8	99.5 ± 12.3	**78.9**** ± 13.8	**46.0**** ± 19.6
Day 6 to necropsy, minus gravid uterus	30.2 ± 8.7	27.4 ± 10.3	29.6 ± 6.9	**13.1**[a] ± 8.3	**-7.9**[a] ± 14.7
Bodyweight change [% difference from control]					
Days 0-6	0	-2.19	9.21	1.75	-9.65
Days 6-21	0	-9.77	-5.60	**-25.14****	**-56.36****
Day 6 to necropsy, minus gravid uterus	0	-9.27	-1.99	**-56.62**[a]	**-126.16**[a]
Food consumption [g/rat/day]					
Days 0-3	19.7 ± 1.5	19.7 ± 2.0	20.0 ± 2.0	19.9 ± 2.0	19.0 ± 2.0
Days 3-6	21.3 ± 1.8	20.8 ± 2.3	21.8 ± 2.1	21.4 ± 2.3	20.4 ± 1.9
Days 6-9	21.9 ± 2.4	21.1 ± 2.2	**19.3**** **± 2.5**	**15.8**** **± 2.2**	**13.3**** **± 2.5**
Days 9-11	22.9 ± 2.7	21.5 ± 2.8	**20.3**** **± 2.1**	**16.7**** **± 2.4**	**13.4**** **± 2.5**
Days 11-13	25.1 ± 2.5	23.6 ± 2.7	**22.4**** **± 2.4**	**19.0**** **± 2.8**	**15.0**** **± 3.3**
Days 13-16	25.2 ± 2.4	24.4 ± 2.6	24.3 ± 2.2	**20.3**** **± 2.2**	**15.9**** **± 2.7**
Days 16-19	26.8 ± 2.7	25.3 ± 3.0	24.6 ± 2.2	**21.6**** **± 2.7**	**16.2**** **± 4.0**
Days 19-21	26.0 ± 3.4	24.3 ± 2.9	23.3 ± 2.4	**19.6**** **± 3.1**	**13.2**** **± 4.7**

Data represent group mean ± S.D.
* = p≤0.05; ** = p≤0.01
[a] = statistical significance not reported

E. NECROPSY

1. Macroscopic findings

12/24 and 6/24 females dosed at 400 and 600 mg/kg bw/day, respectively, had solid contents (bedding material) in the stomach.
2/20, 6/23 and 4/20 pregnant females dosed at 200, 400 and 600 mg/kg bw/day, respectively, showed reddish and/or mottled thymus.
All females surviving until scheduled termination had live young on day 20 of gestation [seeTable 5.8.1-36].

2. Litter data

No statistically significant variations were observed in number of corpora lutea, number of implantations and number of live fetuses per dam in all groups.
Sex ratio was unaffected by treatment and no statistically significant increase of pre- and post-implantation losses were observed, even though the 600 mg/kg bw/day treatment group had a post-implantation loss double as high as the control group (3.3% vs. 8.6%) based on increased number of early resorptions (17 vs. 8) [see Table 5.8.1-37]. The increased being outside the historical control range is considered treatment related but related to the excessive dosing of the dams resulting even in mortality.

3. Litter and fetal weights

At 400 and 600 mg/kg bw/day statistically significant ($p \leq 0.01$) reduction of all male, female and total fetal body weights were observed [see Table 5.8.1-37]. Although no statistically significant impact in the fetal weight of the 200 mg/kg bw treatment group was determined, the mean fetal weight (4.8 ± 0.3 g) were below the historical range (4.9 - 5.0 g).
At 20 mg/kg bw/day fetal weight was unaffected by treatment.

4. Fetal (litter) examinations

At 600 mg/kg bw/day treatment, one foetus (#87/1) had micrognathia (small lower jaw) and another (#79/14) had cleft palate. The historical data do not include this findings among controls.
Significant increases in incomplete ossification of metacarpal 5 and in variations such as unossified sternebra, unossified metatarsal-1 and unosssified cervical vertebral centers were observed [see Table 5.8.1-39].
At 400 mg/kg bw/day treatment, foetuses showed increased incidences in variations such as unossified metatarsal-1, unossified cervical vertebral centers, unossified proximal phalanges of anterior digits, unossified proximal phalanges of posterior digits and incomplete ossification of distal phalanx of posterior digits.
At 200 mg/kg bw/day treatment, foetuses showed doubling of the incidences of the variation of unossified metatarsal 1 (24 vs. 11 in controls), and this was a part of a dose-related trend [see Table 5.8.1-39] and was outside the historical control ranges. Thus this finding is considered treatment related. Delayed ossifications are correlated with retardations in body weight developments thus the trend in impairment of body weight development is well in line with the trend in these delays in ossification.
At 20 mg/kg bw/day treatment, two foetuses (from two different litters) showed a renal pelvic dilatation that was not seen in other groups. Due to lacking dose-response relationship, this finding was considered incidental. Thus, at 20 mg/kg bw/day no treatment related effect on foetal development was noticed.

Table 5.8.1-37: Summary of litter data in the teratology study with phenylguanidine-carbonate

Parameter					
Dose [mg/kg bw/day]	**0**	**20**	**200**	**400**	**600**
Number of litters examined	**23**	**22**	**20**	**23**	**18**
Litter values:					
Total # Corpora lutea	283	254	242	276	216
Corpora lutea/Dam	12.3 ± 1.4	11.5 ± 2.3	12.1 ± 1.4 (HC: 10.9 – 12.8)	12.0 ± 1.6	12.0 ± 1.4
Total # Implantations	263	231	218	258	203
Implantations/Dam	11.4 ± 2.1	15.5 ± 3.3	10.9 ± 2.2	11.2 ± 1.9	11.3 ± 1.4
Pre-implantation losses (%)	6.8 ± 14.5	10.6 ± 18.0	9.7 ± 15.8 (HC: 3.9 – 11.1)	5.9 ± 15.3	5.6 ± 9.0
Post-implantation losses (%)	3.3 ± 6.1	4.8 ± 6.7	3.5 ± 6.3 (HC: 2.8 – 4.2)	4.4 ± 6.3	8.6 ± 14.9
Total # resorptions	9	13	8	12	18
Total # early resorptions	8	13	8	12	17
Total # late resorptions	1	0	0	0	1
Total # live fetuses	254	218	210	246	185
Live fetuses/Dam	11.0 ± 2.1	9.9 ± 3.1	10.5 ± 2.2 (HC: 9.6 – 10.9)	10.7 ± 1.8	10.3 ± 1.9
Sex ratio [male fetuses/live fetuses %]	49.4	45.0	48.4	45.5	46.5
Fetal weight [g]					
Males	5.0 ± 0.3	5.0 ± 0.3	4.9 ± 0.3 (HC: 5.0 – 5.1)	**4.5**** ± 0.3	**3.8**** ± 0.3
Females	4.8 ± 0.3	4.8 ± 0.3	4.7 ± 0.3 (HC: 4.7 – 4.8)	**4.3**** ± 0.2	**3.6**** ± 0.3
Combined sexes	4.9 ± 0.3	4.9 ± 0.3	4.8 ± 0.3 (HC: 4.9 – 5.0)	**4.4**** ± 0.2	**3.7**** ± 0.3
% difference from control					
Number of live fetuses	0	-14.17	-17.32	-3.15	-27.16
Fetal weight – combined sexes	0	0	-2.04	-10.20	-24.49

Litter incidences and weights are given as group mean ± S.D.
HC = historical control range of group means
* = p≤0.05; ** = p≤0.01

Table 5.8.1-38: Summary of fetal external and visceral examinations

Fetal (litters) observations	Dose [mg/kg bw/day]				
	0	**20**	**200**	**400**	**600**
External examination:					
Number examined:	254 (23)	218 (22)	210 (20)	246 (23)	185 (18)
Number affected:	0 (0)	0 (0)	0 (0)	0 (0)	2 (2)
Anomalies and variants:					
Micrognathia:	0 (0)	0 (0)	0 (0)	0 (0)	1 (1)
Cleft Palate:	0 (0)	0 (0)	0 (0)	0 (0)	1 (1)
Visceral examination:					
Number examined:	120 (23)	101 (22)	104 (20)	117 (23)	90 (18)
Number affected:	0 (0)	0 (0)	0 (0)	0 (0)	1 (1)
Malformations:					
Cleft Palate	0 (0)	0 (0)	0 (0)	0 (0)	1 (1)
Variations:					
Thymic remnant in neck [no.]	3 (2)	1 (1)	2 (2)	2 (2)	2 (2)
Thymic remnant in neck [%]	2.17 (8.7)	0.91 (4.5)	2.25 (10.0)	1.74 (8.7)	2.22 (11.1)
	(HC: 0.0-2.2 foetal incidence; 0.0-10.9 litter incidence)				
Liver accessory lobulet [no.]	1 (1)	2 (2)	2 (2)	3 (3)	2 (2)
Liver accessory lobulet [%]	0.87 (4.3)	1.89 (9.1)	2.08 (10.0)	2.61 (13.0)	2.04 (11.1)
	(HC: 0.0-2.2 foetal incidence; 0.0-10.6 litter incidence)				
Renal pelvic dilatation	0 (0)	2 (2)	0 (0)	0 (0)	0 (0)

Data given as fetal incidences (litter incidences in brackets)
HC = historical control mean range %

Table 5.8.1-39: Summary of fetal skeletal examinations

Fetal (litters) observations	Dose [mg/kg bw/day]				
	0	**20**	**200**	**400**	**600**
Skeletal examination:					
Number examined:	134 (23)	117 (22)	106 (20)	129 (20)	95 (18)
Number affected:	0 (0)	0 (0)	0 (0)	0 (0)	1 (1)
Malformations:					
Reduced mandibula	0 (0)	0 (0)	0 (0)	0 (0)	1 (1)
Anomalies:					
Total Foetal Skeletal/Cartilage Anomalies [no.]	6 (5)	5 (5)	2 (2)	6 (6)	9 (8)
Total Foetal Skeletal/Cartilage Anomalies [%]	4.14 (21.7)	3.98 (22.7)	2.25 (10.0)	5.36 (26.1)	9.63 (44.4)
	(HC: 6.7 fetal incidence; 29.9 litter incidence)				
Incomplete Ossification Metacarpal 5 [no.]	0 (0)	1 (1)	0 (0)	2 (2)	**8** **(7**)**
Incomplete Ossification Metacarpal 5 [%]	0 (0)	0.91 (4.5)	0 (0)	1.59 (8.7)	**8.52**** **(38.9)**
	(HC: 0.0-1.7 fetal incidence; 0.0-6.4 litter incidence)				
Variants:					
Unossified Sternebra 6 [no.]	0 (0)	0 (0)	1 (1)	1 (1)	**5** **(5**)**
Unossified Sternebra 6 [%]	0 (0)	0 (0)	1.0 (5.0)	1.45 (4.3)	**5.37**** **(27.8)**
	(HC: 0.0-0.4 fetal incidence; 0.0-2.2 litter incidence)				
Incomplete Ossification Frontal Bone [no.]	2 (2)	2 (2)	2 (2)	3 (3)	7 (5)
Incomplete Ossification Frontal Bone [%]	1.59 (8.7)	1.56 (9.1)	1.83 (10.0)	3.04 (13.0)	7.41 (27.8)
	(HC: 0.0-0.8 fetal incidence; 0.0-4.8 litter incidence)				
Unossiefied Metatarsal-1 [no.]	11 (7)	13 (9)	24 (10)	42 (15)	**63** **(17**)**
Unossiefied Metatarsal-1 [%]	8.53 (30.4)	11.49 (40.9)	22.72 (50.0)	**34.18*** **(65.2)**	**64.15**** **(94.4)**
	(HC: 3.3-10.3 fetal incidence; 14.3-32.6 litter incidence)				
Unossified Cervical Vert. Centers [no.]	34 (13)	20 (10)	24 (12)	58 (18)	**66** **(18**)**
Unossified Cervical Vert. Centers [%]	24.01 (56.5)	19.0 (4.5)	22.58 (60.0)	43.44 (78.3)	**70.74**** **(100)**
	(HC: 14.7-24.2 fetal incidence; 44.7-77.8 litter incidence)				
Ant. Digit 2 – Unossified Proximal Phalanx [no.]	38 (15)	29 (15)	49 (17)	76 (22)	64 (16)
Ant. Digit 2 – Unossified Proximal Phalanx [%]	29.93 (65.2)	25.54 (68.2)	46.36 (85.0)	**60.59*** **(96.7)**	**65.48**** **(88.9)**
	(HC: 20.8-35.0 fetal incidence; 47.8-81.0 litter incidence)				
Post. Digit 2 - Unossified Proximal Phalanx [no.]	72 (21)	65 (22)	73 (20)	111 (23)	90 (17)
Post. Digit 2 - Unossified Proximal Phalanx [%]	56.05 (91.3)	56.49 (100)	69.54 (100)	**86.33**** **(100)**	**91.30*** **(94.4)**
	(HC: 41.6-57.1 fetal incidence; 73.9-95.2 litter incidence)				

Table 5.8.1-39: Summary of fetal skeletal examinations

Fetal (litters) observations	Dose [mg/kg bw/day]				
	0	**20**	**200**	**400**	**600**
Post. Digit 5 – Incomplete Ossific. Distal Phalanx [no.]	13 (8)	10 (9)	15 (6)	28 (12)	**30 (15*)**
Post. Digit 5 – Incomplete Ossific. Distal Phalanx [%]	10.33 (34.8)	9.51 (40.9)	12.81 (30.0)	21.59 (52.2)	**30.79** ** **(83.3)**
	(HC: 12.5-53.7 fetal incidence; 57.1-88.9 litter incidence)				

Data given as fetal incidences (litter incidences in brackets)
HC = historical control mean range %
* = p≤0.05; ** = p≤0.01

III. CONCLUSIONS

Maternal toxicity at the high dose of 600 mg/kg bw/day clearly exceeded the maximum tolerable dose as indicated by mortality, clinical symptoms and body weight loss (corrected body weight) in conjunction with significant reduced food consumption throughout the treatment period. Significant maternal toxicity was also evident at the next lower dose level of 400 ppm and to a lesser extend at 200 mg/kg bw/day based on impaired body weight development, lower food consumption and clinical symptoms.

The increased incidence of post-implantation loss (early resorptions) at the highest dose level as well as the single incidences of malformations (1 fetus with mandibular micrognathia and 1 fetus with cleft palate) at this dose were clearly attributed to the exceedance of the maternal tolerable dose and thus not considered indicative for specific embryotoxic effects.

Foetotoxicity was evident at the two higher dose levels (400 and 600 mg/kg bw/day) in correlation to the maternal toxicity, in form of significantly reduced mean foetal body weights and delays in ossification. The delayed ossification was considered secondary to the retarded body weight development. As for the dams the effects in the pups at 200 mg/kg bw/day were less evident (mostly without statistical significance) but reflected the overall trend.

In conclusion the No Observed Adverse Effect Level (NOAEL) for phenylguanidine-carbonate in the rat was 20 mg/kg/day for maternal as well as for developmental toxicity, and the Lowest Observed Adverse Effect Level (LOAEL) was 200 mg/kg/day for both the pregnant female and the developing foetus.

Literature data

Literature search and search in chemical evaluation internet-databases did provide some additional information on the pharmacological properties of phenylguanidine. Phenylguanidine was used as a model compound in studies on 5-HT3 serotonine receptor, on human organic cation transporters (hOCT1 and hOCT2), on inhibition of dimethylarginine dimethylaminohydrolase activity or binding to trypsin. These studies intending to understand receptor ligand interaction are not considered of relevance for toxicological evaluation of the metabolite and were thus not taken into account.

C Toxicological evaluation of metabolite M605F025

No structural alert for mutagenicity was identified in any of the applied QSAR models. This was supported by the in genotoxicity conducted with phenylguanidine-carbonate as the stable form of M605F025, yielding negative results in two Ames tests and equivocal results in a supplemental in vitro chromosomal aberration test that has methodological flaws. A rat micronucleus test was clearly negative without any indication for chromosomal aberration in vivo. Systemic toxicity and potential developmental toxicity of phenylguanidine-carbonate was investigated in a 90-day rat and in a prenatal toxicity study in rats. In both studies the highest dose tested 8000 ppm (equivalent to about 536 mg/kg bw/day in males and 616 mg/kg bw/day in females) in the 90-day study and 600 mg/kg bw/day in the developmental toxicity study clearly exceeded the maximum tolerable dose. The LOAEL of the 90-day study was 2000 ppm (about 130 mg/kg bw/day in males and 140 mg/kg bw/day in females based on significantly lower body weight gains, alterations in clinical chemistry parameters and significantly reduced spleen weights. The NOAEL was 17.8 mg/kg bw/day in males and 22.1 mg/kg bw/day in females. In the developmental toxicity study the maternal LOAEL was 200 mg/kg bw/day based on based on impaired body weight development, lower food consumption and clinical symptoms. The maternal NOAEL was 20 mg/kg bw/day. Regarding developmental effects the LOAEL was 200 mg/kg bw/day based on retarded foetal weight and delayed ossification considered secondary to the impairment of body weight. The developmental NOAEL was thus also 20 mg/kg bw/day.
The NOAELs derived for the carbonate salt of phenylguanidine are considered applicable for phenylguanidine, since the salt is expected to be dissociated in the mammalian gastrointestinal tract and the dissociated phenylguanidine is considered the contributing toxicant for the effects observed.

Based on the NOAEL of 17.8 mg/kg bw/day in males from the 90-day study an **ADI of 0.089 mg/kg bw/day** is proposed for phenylguanidine. The ADI was derived by applying an additional uncertainty factor of 2 to derive from subchronic to chronic exposure [see EFSA Scientific Opinion: Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data, EFSA Journal 2012;10(3):2579 and ECHA Guidance on information requirements and chemical safety assessment, Chapter R.8: Characterisation of dose [concentration]-response for human health, Version 2.1, November 2012] in addition to the standard factor of 100. Although this ADI is lower than the respective ADI of pyrimethanil, this is not considered to reflect a significant higher toxicity but attributed to the wide dose spacing between LOAEL and NOAEL and the application of the additional safety factor. Note the NOAEL for the 90-day rat study of pyrimethanil is 5.4 mg/kg bw/day and the LOAEL is 54 mg/kg bw/day and thus below the respective endpoints for the metabolite.

Thus, M605F025 is considered to be of no toxicological relevance and an ADI of 0.089 mg/kg bw/day can be derived from the available toxicological database for risk-assessment purposes.

CA 5.8.2 Supplementary studies on the active substance

Studies evaluated in the draft monograph of Rapporteur Member State Austria of April 15, 2004:

In supplementary studies, effects of pyrimethanil on hepatic enzymes in rats and mice and on the thyroid in the rat have been investigated. These studies have been evaluated by European authorities and Austria as Rapporteur Member State and were considered to be acceptable. For the convenience of the reviewer, these studies are summarized below as extracted from the monograph and the EFSA conclusion (January 13, 2006). A tabulated summary is provided in **Table 5.8.2-1**.

Table 5.8.2-1: Summary of supplementary studies conducted with pyrimethanil

Study	Dosages (mg/kg bw/day)	Main adverse effect	Reference (BASF DocID)
Liver enzyme induction 4 days, gavage, twice daily Sprague-Dawley rat	M: 100 and 200	Weak induction of ethoxyresorufin-*O*-deethylase and pentoxyresorufin-*O*-dealkylase and increased concentration of cytochrome B5.	[see KCA 5.8.2/1 A81625]
Liver enzyme induction 4-day feeding CD-1 mouse	F: 162 [0, 900 ppm]	Low potency inducer of cytochrome P-450 and 7-pentoxyresorufin-*O*-dependentylase	[see KCA 5.8.2/2 C001378]
Perchlorate discharge assay 7-day feeding Sprague-Dawley rat	M: 1009 [0, 5000 ppm]	No direct thyroid blocking activity (no iodine discharge) of pyrimethanil. Increase thyroidal iodine uptake as indicator for an increased thyroid hormone turnover by an indirect extrathyroidal mechanism	[see KCA 5.8.2/3 A81829]
14-day feeding Sprague-Dawley rat	M: 379 [0, 5000 ppm]	Reduced body weight gain and marked induction of hepatic uridine diphosphate-glucuronosyltransferase (UDP-GT).	[see KCA 5.8.2/4 A81828]

In mechanistic studies, effects of pyrimethanil on hepatic enzymes (rats and mice) and, in particular, on the thyroid in rats have been investigated. It was demonstrated that treatment with pyrimethanil enhanced hepatic metabolism, inducing increased thyroid hormone clearance. Thus, the resulting thyroid hormone imbalance induced a chronic stimulation of the thyroid.

For the convenience of the reviewer short summaries of the already peer reviewed supplementary studies are provided below:

Studies submitted in this AIR 3 dossier (not yet peer-reviewed):

No additional data were generated by the applicant but information from a comprehensive literature search is available. Literature data developed in the ToxCast program established further information on potential toxicological cellular mode of action. Pyrimethanil was tested positive for the following nuclear receptor pathways in transactivation: ATG_PPARg_TRANS, ATG_PXRE_CIS, ATG_PXRE_CIS, Tox21_AhR and gene expression assays linked to AhR, CAR, and PXR. Pyrimethanil was not associated with the activation in other assays targeting estrogen receptor and/or androgen receptor. In a safety pharmacology testing battery, pyrimethanil induced transient apathy an increased hexobarbital sleeping time at a high dose of 1000 mg/kg bw. These findings are in line with the observed transient effects in the acute toxicity and acute neurotoxicity testing [see MCA 5.2.1 and MCA 5.7.1]. Moreover, serotonine induced gut contraction was dose-dependently inhibited. The other tests on cardiovascular and respiratory system, on gut functionality, neuro-musuculare interaction and/or blood haemolysis/coagulation showed no treatment related effects. The respective studies are listed in Table 5.8.2-2.

Table 5.8.2-2: Summary of supplementary studies conducted with pyrimethanil (not yet peer-reviewed)

Type of study	Test substance	Result Classification	Reference (BASF DocID)
Nuclear receptor transactivation assays	Pyrimethanil	Activation of AhR, CAR and PXR related receptor pathways.	[redacted]. 2011 2011/1295091
Xenobiotic-metabolizing enzyme and transporter gene expression in primary cultures of human hepatocytes	Pyrimethanil	Expression of genes that were induced by pyrimethanil were linked to AhR, CAR, and PXR.	[redacted]. 2010a 2010/1233112
Safety Pharmacology Testing: Irwin Screen, rat	Pyrimethanil	1000 mg/kg bw: Transient slight apathy (30 min after dosing); NOEL 141 mg/kg bw	[redacted] 1994 A81858
Safety Pharmacology Testing: Hexobarbital sleeping time, rat	Pyrimethanil	1000 mg/kg bw: Significant prolongation of sleeping time; NOEL 141 mg/kg bw	[redacted] 1994 A81858
Safety Pharmacology Testing: Isolated Guinea pig ileum	Pyrimethanil	Inhbition of serotonine induced contraction in a dose dependent manner. No effects on acetylcholine, histamine or BaCl induced contractions. NOEL 1 µg/ml:	[redacted] 1994 A81858
Safety Pharmacology Testing: Cardiovascular system and respiration in the dog	Pyrimethanil	No effects on blood pressure, heart rate, ECG, cardiac function, blood flow or respiration in anesthetized dog. NOEL 1000 mg/kg bw.	[redacted] 1994 A81858
Safety Pharmacology Testing: Charcoal propulsion test, mice	Pyrimethanil	No effects on charcoal propulsion (gut travelling distance). NOEL 1000 mg/kg bw.	[redacted] 1994 A81858
Safety Pharmacology Testing: Isolated phrenic nerve-diaphragm	Pyrimethanil	No effect on nerve contraction after electric stimulation. NOEL 100 µg/ml:	[redacted] 1994 A81858
Safety Pharmacology Testing: In vitro haemolytic effect, rabbit erythrocytes	Pyrimethanil	No haemolytic activity. NOEL 100 µg/ml:	[redacted] 1994 A81858
Safety Pharmacology Testing: Blood coagulation, rat	Pyrimethanil	No effects on prothrombin time, activated partial thromboplastin time or whole blood clotting time. NOEL 1000 mg/kg bw.	[redacted] 1994 A81858

Thus, the conclusion for relevant endpoints for the current renewal was amended as follows:

Other toxicological studies (SANCO/11802 data point 5.8)

Supplementary studies on the active substance	Pyrimethanil induces AhR, CAR and PXR related enzymes. Thyroid hormone imbalance due to increased clearance as secondary effect to enhanced hepatic metabolism. No direct thyroid blocking activity. Transient apathy and induced hexobarbital sleeping time in safety pharmacology testing: Inhibition of serotonine induced ileum contraction.

Short summaries of the already peer reviewed supplementary studies:

The effect of SN 100 309 on the hepatic mixed function oxidase system of male rats following oral administration

~~Reference: [redacted] 1991; Report No. TOX/90/223-20, BASF DocID A81625~~

~~The study is conducted in compliance with GLP. However, no specific guideline is mentioned. It is scientific valid and acceptable.~~

Report:	CA 5.8.2/1 [redacted] 1991 a The effect of SN 100 309 on the hepatic mixed function oxidase system of male rats following oral administration at 100 or 200 mg/kg bodyweight A81625
Guidelines:	OECD 417
GLP:	yes (certified by Department of Health and Social Security of the Government of the United Kingdom, United Kingdom)
Previous evaluation	DAR (2004)
Acceptability:	The study is scientifically valid and acceptable

Material and method:

Pyrimethanil (batch no. CR 19298/1; purity 99.4%, suspended in 0.5% gum tragacanth in distilled water) was administered orally by gavage to groups of six male rats/dose (strain Sprague Dawley CD-1) at dose levels of 0 (vehicle control), 100 and 200 mg/kg bw twice daily for 4 days. Additional "positive" groups were given 0.1% phenobarbitone in drinking water for at least 14 days, an intraperitoneal dose of phenobarbitone (in water) at 80 mg/kg bw for 4 days, an intraperitoneal dose of ß-naphthaflavone (in cornoil) at 80 mg/kg for 4 days and an intraperitoneal dose of clofibrate (in corn oil) at 400 mg/kg bw for 4 days. 17 hours and 24 hours (positive groups), respectively, after the last dosing, animals were necropsied, microsomal suspensions prepared from their livers and levels of liver enzymes (cytochrome b_5, cytochrome P-450, lauric acid hydrolase, ethoxyresorufin-O-deethylase, pentoxyresorufin-O-dealkylase) were determined.

Findings:
Results showed that dosing with pyrimethanil had only a minor effect on the liver enzyme showing extremly low levels of induction of ethoxyresorufin-O-deethylase and pentoxyresorufin-O-dealkylase. However, there was a statistically significant increase in liver weight at 100 mg/kg only and concentration of cytochrome b_5 at 200 mg/kg only. It was stated in the report that the pattern of induction was similar to the phenobarbitone-type. The magnitude of increase of ethoxyresorufin-O-deethylase is below that seen with phenobarbitone and very much less than that seen with ß-naphthoflavone. On the other hand, the increase of pentoxyresorufin-O-dealkylase, whilst being much less that caused by phenobarbitone, was significantly greater than that caused by ß-naphthoflavone. Lauric acid hydrolase activity was not increased significantly; therefore pyrimethanil was not considered to be a clofibrate-type inducer of cytochrome P-450.

Table 5.8.2-3: Effects of oral dosing with pyrimethanil on liver and liver enzymes of the male rat (mean values)

	Pyrimethanil			**positive control groups**		
	0 mg/kg	100 mg/kg	200 mg/kg	clofibrate	ß-naphtha-flavone	phenobar-bitone
Liver weight (% of bw)	2.92	3.20*	3.11	4.41**	3.70**	4.72**
microsomal protein (mg/g liver)	33.83	33.26	33.27	38.39**	36.63	46.85**
Enzyme concentration (nmoles/mg protein)						
Cytochrome P-450	0.63	0.78	0.82	1.06**	1.0*	1.76**
Cytochrome b_5	0.18	0.23	0.26*	0.19	0.32**	0.46**
Enzyme activity (nmoles/min/mg protein						
Lauric acid hydrolase	0.09	0.143*	0.182**	0.130	1.49**	0.337**
Ethoxyresorufin-O-deethylase	6.41	7.88	6.72	21.5**	4.63	5.7
Pentoxyresorufin-O-dealkylase	0.02	0.038**	0.029*	0.057*	0.023	3.72**

* ($p = 0.05$) significantly different from control
** ($p = 0.01$) significantly different from control

Conclusion:
Pyrimethanil administered at 100 and 200 mg/kg bw to the rat was a very weak inducer of the hepatic mixed-function oxidase system, predominantly of the phenobarbitone-type.

Pyrimethanil: Investigation of liver enzyme induction following dietary administration to female CD-1 mice for 4 days

~~**Reference: [redacted] 1998; Report No. TOX/98/223-100, BASF DocID C001378**~~

Report:	CA 5.8.2/2 [redacted]., 1998 a Pyrimethanil - Investigation of liver enzyme induction following dietary administration to female CD-1 mice for 4 days C001378
Guidelines:	JMAFF 59 NohSan No 4200, EEC Annex II A Section 5.1.3
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)
Previous evaluation	DAR (2004)
Acceptability:	The study is scientifically valid and acceptable

Aim of the study was to investigate the effect on the hepatic enzyme activities and also on the oestrus cycle of female CD-1 mice following dietary administration of pyrimethanil. ~~The study is conducted in compliance with GLP. However, no specific guideline is mentioned. It is scientific valid and acceptable.~~

Material and method:

Groups of 15 female CD-1 mice (source [redacted].) were fed diet containing 0 or 900 ppm (equivalent to 162 mg/kg bw/d) pyrimethanil (batch no. 400204E; purity 97.5%) for 4 days. Animals were observed daily for clinical signs. Bodyweight and food consumption were recorded. In addition, vaginal smears were prepared before treatment and again on day 4 and the cell types were examined to identify the stage of the cycle. Following 4 days administration, livers were removed and weighed, and microsomal suspensions were prepared for determination of protein concentration, cytochrome P450 and 7-pentoxyresorufin-O-deethylase activity.

Findings:
General observations: There were no mortalities or clinical signs observed during the study period that were considered to be related to treatment. In addition, body weight gain and food consumption for the treated mice was essentially similar to that of untreated controls. Investigations on vaginal smears exhibited no overt differences between treated and control groups. However, these investigations were limited and only over a very short treatment period. Therefore, no conclusion can be drawn on this investigation.
Liver enzymes: The concentration of microsomal protein/g liver was statistically significant increased by 8% ($p<0.05$) in females receiving pyrimethanil compared to controls. In addition, there were small but statistically significant increases in mean cytochrome P450 concentration of 5% (results per mg protein) and 13% (results per g liver) above control. Also 7-pentoxyresorufin-O-dependentylase activity was increased by 38% (expressed per mg protein) or 50% (expressed per g liver), when compared to controls.

Conclusion:
It was concluded that pyrimethanil was acting only as a low potency inducer of cytochrome P450, including 7-pentoxyresorufin-O-dependentylase (murine Cyp2b subfamily).The effects were very small and therefore thought to be of little biological consequence.

Technical SN 100 309: Rat 7-day dietary thyroid function test using perchlorate discharge as a diagnostic test

~~**Reference:** [redacted] **1992(a); Report No. TOX/92/223-55, BASF DocID A81829**~~

Report:	CA 5.8.2/3 [redacted] 1992 a Technical SN 100 309: Rat 7-day dietary thyroid function test using perchlorate discharge as a diagnostic test A81829
Guidelines:	none
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)
Previous evaluation	DAR (2004)
Acceptability:	The study is scientifically valid and acceptable

Report:	CA 5.8.2/4 [redacted] 1993 c Addendum 1: Technical SN 100 309: Rat 7-day dietary thyroid function test using perchlorate discharge as a diagnostic test A81830
Guidelines:	none
GLP:	yes

The purpose of this study was to investigate whether the thyroid effects seen following repeated dose of pyrimethanil to rats occurs via a direct effect on the thyroid or an indirect mechanism mediated via the liver. The study used the "perchlorate discharge test" to investigate defects in the iodine trapping mechanism. ~~The study is conducted in compliance with GLP principles. However, no specific guidelines mentioned. It is scientific valid and acceptable.~~

Material and method:
Groups of 12 male rats (strain: Sprague Dawley CRL CD(SD)BR, source [redacted].) were fed for seven days diet containing either pyrimethanil (batch no. CR 19325/01/900304; purity 96.2%) at dose levels of 0 and 5000 ppm (equivalent to 1009 mg/kg bw/d), propylthiouracil (2000 ppm – equivalent to 353 mg/kg bw/d) or phenobarbital (1000 ppm – equivalent to 217 mg/kg bw/d). On day 8, all rats were given a 100 µl i.p. dose of radiolabelled [^{125}I]. Six hours later, six rats per dose group were given 10 mg/kg potassium perchlorate i.p. dissolved in physiological saline, whilst the other six rats per dose group received 10 mL/kg i.p. 0.9% w/v saline. Exactly 2.5 minutes later, the rats were killed, blood samples were taken, the thyroids were weighed and radioactivity in the thyroid and in blood was recorded.

Findings:
General observations: Treatment with pyrimethanil produced no adverse clinical effects. However, body weight gain of the animals was reduced by 42% and food consumption was reduced by 21% when compared to controls. Propylthiouracil treatment resulted in reduced activity and piloerection between days 3 and 4. Body weight gain was reduced by 65% and food consumption by 36%. After phenobarbital treatment, the rats showed reduced activity, abnormal gait, reduced muscle tone, piloerection and wasted body conditions between days 3 and 8. No effects on body weight gain and food consumption were evident.
Thyroid effects: Pyrimethanil treatment induced a statistically significantly higher iodine uptake in the thyroid of animals compared to untreated controls. No significant discharge of iodine occurred after perchlorate treatment.
Also after administration of phenobarbital, iodine uptake into the thyroids was statistically significantly higher than that of the concurrent controls, indicating fully organification. As after pyrimethanil administration, there was no significant discharge of [^{125}I] in the phenobarbital treated rats after perchlorate administration.
In contrast, iodine uptake in the thyroid of animals treated with 2000 ppm propylthiouracil, which is known a direct acting goitrogenic substance by directly blocking thyroid function via inhibition of the thyroid peroxidase (essential for the iodination of tyrosine in the thyroid follicles and thereby for the elaboration of thyroxin) was statistically significantly lower than in untreated animals (70%), and a significant discharge of iodine occurred after perchlorate treatment. In addition, thyroid weights and relative thyroid weights were significantly increased [see Table 5.8.2-4].

Table 5.8.2-4: Relevant thyroid findings

	control		**pyrimethanil (5000 ppm)**		**propylthiouracil (2000 ppm)**		**phenobarbital (1000 ppm)**	
	+ saline	**+ perchlorate**	**+ saline**	**+ perchlorate**	**+ saline**	**+ perchlorate**	**+ saline**	**+ perchlorate**
Thyroid weight (g)	0.017	0.016	0.017	0.015	0.03**	0.029**	0.022	0.019
Thyroid weight (% bw)	0.008	0.008	0.009	0.009	0.017**	0.017**	0.01	0.09
Thyroid count (dpm*1000)	86	99	129*	159**	56*	22***	190**	188**
Blood count (dpm*1000)	6	9	7	7	9	10	8	7
Thyroid:blood ^{125}I (*1000)	1.0	0.9	1.3	1.4*	0.3***	0.1***	1.4	1.4

dpm = disintigrations per minute;
significantly different from untreated; * p = 0.05, ** p = 0.01, *** p = 0.001

Conclusion:
It was concluded that pyrimethanil administered at 5000 ppm in the diet to rats does not have a direct thyroid blocking activity. Results are similar to those obtained with phenobarbital on the basis of which modifies thyroid homeostasis by reducing plasma hormone levels by an extrathyroidal indirect mechanism, due to increased hepatic enzyme activity and hormone clearance. This modification of thyroid hormone homeostasis leads to histopathological changes in the thyroid consistent with stimulation.

Technical SN 100 309: Rat 14-day dietary study to investigate the mechanism of thyroid response

~~**Reference: [redacted] 1992(b); Report No. TOX/92/223-56, BASF DocID A81828**~~

Report:	CA 5.8.2/5 [redacted] 1992 b Technical SN 100 309: Rat 14-day dietary study to investigate the mechanism of thyroid response A81828
Guidelines:	OECD 407
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)
Previous evaluation	DAR (2004)
Acceptability:	The study is scientifically valid and acceptable

The purpose of this study was to characterise the mechanism of thyroid effects using repeated dietary administration for 14 days followed by a withdrawal period of 14 days to monitor reversibility. ~~The study is conducted in compliance with GLP, but no specific guideline is mentioned. However, it is scientific valid and acceptable.~~

Material and method:

Pyrimethanil (batch no. CR19325/01/900304; purity 96.2%) was administered in the diet to groups of 10 male rats (strain: Sprague Dawley CRL CD(SD)BR, source [redacted].) for 14 days at dose levels of 0 and 5000 ppm (equivalent to 379 mg/kg bw/d). After that all rats received untreated diet for a further 14 days. Animals were observed daily for clinical signs, bodyweights and food consumption were recorded twice weekly. Blood samples for clinical chemistry (comprising plasma pituitary thyroid hormone estimations [thyroxin T4, triiodothyronine T3, reverse triiodothyronine RT3, thyroid stimulating hormone TSH] were taken before treatment, after 1 (6 hours after commencing dosing), 2, 4, 8 and 15 days of treatment, and after the withdrawal period. An interim necropsy was undertaken after 14 days on 5 rats from each dose group.

At necropsy (interim and terminal), the weights of the liver, pituitary and thyroid were recorded and the organs were examined histologically. In addition, microsomal preparations in order to estimate the activity of uridine diphosphoglucuronyl transferase [UDPGT] were performed. Results were expressed per milligram of microsomal protein measured.

Findings:
General observations: There were neither mortalities nor treatment-related clinical signs. A significant decrease in body weight gain (by 23%) occurred in treated rats during the first week, compared to controls.
Clinical chemistry: Following treatment with pyrimethanil for 6 hours, one animal had a reduced level of T4 and elevated TSH. After 24 hours, a statistically significant increase in mean group TSH and RT3 levels was observed. By day 4, a moderate statistically significant reduction in T4 and T3 and a concomitant increase in TSH was seen. On days 8 and 15, group mean TSH levels were still higher than in controls, although T3 and T4 levels had returned to normal levels. After the recovery period, no significant effects on hormone levels were observed.
Investigations on hepatic uridine diphosphoglucuronyl transferase (UDPGT) revealed a marked statistically significant increase after 14 days of treatment. Levels returned to normal following the 14 day recovery period.

Table 5.8.2-5: Substantial clinical chemistry findings

Parameter	6 hours		24 hours		day 4		day 8		day 15		day 29	
	0 ppm	5000 ppm	0 ppm	5000 ppm	0 ppm	5000 ppm	0 ppm	5000 ppm	0 ppm	5000 ppm	0 ppm	5000 ppm
Total T_3 (nmol/l)	1.7	1.4	1.3	1.1	1.1	0.9*	1.3	1.2	1.0	1.0	1.1	1.2
Total T_4 (nmol/l)	107	91	88	85	84	64**	76	75	69	78	65	80
Reverse T_3 (pmol/l)	20.3	21.7	17.3	20.9*	16.3	20.0	14.8	17.8	15.6	17.8	13.8	15.2
TSH (ng/mL)	6.6	10.2	6.0	9.7**	8.3	12.9*	10.2	14.0	7.2	15.5**	9.5	8.8
UDPGT (U/l/mg)	-	-	-	-	-	-	-	-	71	317***	41	67**

significantly different from untreated; * p = 0.05, ** p = 0.01, *** p = 0.001 (student's t-test)

Pathology: Following treatment with pyrimethanil, there was a statistically significant decrease in absolute and relative thyroid weight and a statistically significant increase in absolute and relative liver weight compared to controls. Histopathology revealed minimal to slight centrilobular hepatocyte enlargement in all treated animals; complete recovery of this finding was recorded after the withdrawal period. Thyroid effects included moderate to severe colloid depletion and slight to moderate follicular cell hypertrophy in 5/5 rats and minimal to moderate follicular epithelial hyperplasia in 4/5 animals.
After the withdrawal period, there was a marked recovery of colloid depletion, follicular cell hypertrophy and follicular cell hyperplasia in treated rats, indicating reversibility of the effects on the thyroid gland.

Conclusion:
Results demonstrate that pyrimethanil increased the levels of liver enzyme UDPGT. This was considered to lead to increased thyroid hormone clearance via enhanced hepatic metabolism. This in turn resulted in an extrathyroidal, indirect effect on thyroid hormone homeostasis, which via normal pituitary feedback led to increased TSH levels and histopathological changes in the thyroid consistent with thyroid gland stimulation.

Literature data

The EPA ToxCast Database as accessible via the iCSSDashboard ~~http://actor.epa.gov/actor/faces/CSSDashboardLaunch.jsp~~ https://actor.epa.gov/dashboard/ provides a large and ongoing growing database on high-throughput-screening assays of molecular interaction with chemicals. A set of publications providing summary information on parts of these tests and the results obtained with 1814 chemicals including pyrimethanil has meanwhile been published. Summary information on all individual test outcomes are accessible via the internet. The ToxCast data on pyrimethanil related to the toxicological modes of action under discussion are summarized below together with other data available from public literature.

Report: CA 5.8.2/6
Shah I. et al., 2011a
Using nuclear receptor activity to stratify hepatocarcinogens
2011/1295091
Guidelines: none
GLP: no

Executive Summary

This study is part of the EPA ToxCast program. The authors investigated the effects of 309 environmental pesticides on nuclear receptors using primary human hepatocytes, HepG2 cells transfected with a multiple reporter transcription unit (MRTU) library consisting of 48 transcription factor binding sites, cis reporter gene assays and cell free and cell based cytochrome P450 assays. The resulting data was used to calculate an aggregate scaled activity score for different nuclear receptors (CAR, PXR, PPAR, AhR, SR, RXR), which was then correlated to lesion progression data extracted from EPA's pesticide database.
Pyrimethanil, together with boscalid and propanil, was grouped into category VI E; group E indicated, that the compound can activate the nuclear receptors AhR, CAR and PXR. The rationale for this grouping is largely based on the activation of the reporter genes.

Pyrimethanil was tested positive in the following activation assays: ATG_PPARg_TRANS, ATG_PXRE_CIS, ATG_PXRE_CIS, Tox21_AhR. Pyrimethanil was not associated with the activation in other assays targeting estrogen receptor and/or androgen receptor.

Classification of study: Supplementary information

Report: CA 5.8.2/7
Rotroff D.M. et al., 2010a
Xenobiotic-metabolizing enzyme and transporter gene expression in primary cultures of human hepatocytes modulated by toxcast chemicals
2010/1233112
Guidelines: none
GLP: no

Executive Summary

This study is part of the EPA ToxCast program. Primary human hepatocyte cultures as model system were used to characterize the concentration- and time-response of the 320 ToxCast chemicals for changes in expression of genes regulated by nuclear receptors. Fourteen gene targets were monitored in quantitative nuclease protection assays: six representative cytochromes P-450, four hepatic transporters, three Phase II conjugating enzymes, and one endogenous metabolism gene involved in cholesterol synthesis. These gene targets are sentinels of five major signalling pathways: AhR, CAR, PXR, FXR, and PPARalpha. Besides gene expression, the relative potency and efficacy of these chemicals to modulate cellular health and enzymatic activity were assessed. Results demonstrated that the culture system was an effective model of chemical-induced responses by prototypical inducers such as phenobarbital and rifampicin. Gene expression results identified various ToxCast chemicals that were potent or efficacious inducers of one or more of the 14 genes, and potent to interfere the 5 nuclear receptor signalling pathways. Significant relative risk associations with rodent in vivo chronic toxicity effects are reported for the five major receptor pathways. These gene expression data are being incorporated into the larger ToxCast predictive modelling effort.
Pyrimethanil induced gene expression of following genes: CYP1A1, CYP1A2, CYP2B6, CYP3A4, and UGT1A1. No effect was observed on CYP2C9 and SULT2A1.
Expression of genes that were induced by pyrimethanil were linked to AhR, CAR, and PXR. Expression of UGT and CYP2C are also linked to AhR or PXR, respectively, but were not affected by pyrimethanil incubation. In conclusion, these results together with the results of the transactivation studies (Shah et al., 2011a) should be used as supporting screening information only.

Classification of study: Supplementary information

Report: CA 5.8.2/8
[redacted] 1994a
Pyrimethanil technical - Safety pharmacology studies
A81858
Guidelines: none
GLP: yes

Executive Summary

Pyrimethanil tech. (Batch: CR 19325/01/900304; Purity: 96.2%) was studied in a series of safety pharmacology studies. Pyrimethanil was used at dose levels of 0, 20, 141, 500, and 1000 mg/kg bw for the *in vivo* assays and at concentrations of 0, 1, 10, and 100 µg/mL for the *in vitro* tests.

Pyrimethanil showed no anticoagulant, haemolytic or neuromuscular blocking activity. It had no effect on gastrointestinal activity in mice and produced no cardiovascular or respiratory effects in the anaesthetized dog. Thus, the NOEL corresponds to the highest dose/concentration applied for the aforementioned tests.
However, pyrimethanil produced transient apathy and induced a prolonged hexobarbital sleeping time in rats at 1000 mg/kg bw (NOEL 141 mg/kg bw). Furthermore, inhibitory effects against 5-HT-induced contractions of the isolated guinea pig ileum were observed from 10 µg/mL onward (NOEL 1 µg/mL) after incubation with pyrimethanil.

(BASF DocID A81858)

A. MATERIAL

Test Material:	Technical pyrimethanil
Lot/Batch #:	CR 19325/01/900304
Purity:	96.2%
Stability of test compound:	The test substance was stable over the study period (Expiry date July 12, 1996).

B. METHODS

Pyrimethanil was used at dose levels of 0, 20, 141 and 1000 mg/kg bw for the *in vivo* assays in rodents, at 500 and 1000 mg/kg bw in dogs and at concentrations of 0, 1, 10, and 100 µg/mL for the *in vitro* tests. All animals were killed at the end of the assessment.

Rat clinical and behavioural observations (Irwin Screen)
Groups of 4 male Sprague-Dawley rats were given a single dose of pyrimethanil (in 0.5% aqueous methylcellulose). Animals were assessed at 30, 90, 150 and 300 minutes after dosing, and were kept for a 7-day period to assess delayed mortalities.

Hexobarbital sleeping time
Groups of 10 (5 male and 5 female) Sprague-Dawley rats were given a single dose of pyrimethanil (in 0.5% aqueous methylcellulose). Two similar groups were given either the vehicle alone or 15 mg chlorpromazine/kg bw. Sleeping time was measured for each animal following hexobarbital treatment.

Isolated guinea-pig ileum
A sub-maximal contraction of the ileum to each spasmogen (acetylcholine, histamine, 5-HT or BaCl) was obtained. The sub-maximal dose of spasmogen was re-applied to the bathing fluid in the presence of pyrimethanil. In addition, the direct effects of technical pyrimethanil on the isolated ileum were also assessed.

Cardiovascular system and respiration in the dog
Dogs were anaesthetised and various cardiovascular and respiratory parameters measured for an equibrilation period of 30 minutes. The effect of vehicle administration was then examined on the parameters for a 60-minute period. The effects of an intraduodenal dose of 500 or 1000 mg/kg pyrimethanil was then examined on the recorded parameters for a period of 180 min.

Charcoal propulsion test
Groups of 10 mice were given 20, 141 or 1000 mg/kg bw pyrimethanil (in 0.5% aqueous methylcellulose). Two similar groups were given either the vehicle alone or 10 mg/kg bw morphine sulphate. Forty-five minutes after dosing the mice received additional charcoal by gavage. After a further 30-min period, the mice were killed and the distance the charcoal had travelled along the gastrointestinal tract was measured.

Isolated phrenic nerve-diaphragm
The phrenic nerve and associated portion of the diaphragm were suspended in an organ bath. The nerve was electrically stimulated and the contraction of the diaphragm recorded. The effects of the addition of pyrimethanil to the bathing fluid, on the contraction of the diaphragm, were assessed.

In vitro haemolytic effect
The erythrocytes from 3 rabbits were prepared as a 3% suspension in saline. One mL of the suspension was mixed with 3 mL of the pyrimethanil solutions. After 4 h the degree of haemolysis was assessed.

Blood coagulation
Groups of 10 male Sprague-Dawley rats were given a single dose of pyrimethanil (in 0.5% aqueous methylcellulose). Whole blood clotting was measured in vivo. In addition, a blood sample was taken and prothrombin time and activated partial thromboplastin time were measured.

II. RESULTS AND DISCUSSION

Rat clinical and behavioural observations (Irwin Screen)
Transient slight apathy was observed in 3 animals 30 min after dosing with 1000 mg/kg bw. All animals appeared normal from 150 minutes post-dose onwards. The NOEL was 141 mg/kg bw.

Hexobarbital sleeping time
A statistically significant prolongation of the hexobarbital sleeping time was observed in male and female rats dosed with 1000 mg/kg bw. No relevant effects were observed at the lower dose levels 20 and 141 mg/kg bw. The NOEL was 141 mg/kg bw.

Isolated Guinea-pig ileum
Contractions of the guinea-pig ileum induced by serotonine were inhibited by 10 and 100 µg/mL of pyrimethanil in a concentration-dependent manner, with marked responses at 100 µg/mL. No significant effects on acetylcholine, histamine or BaCl-induced contractions were recorded. Pyrimethanil alone produced no spasmogenic activity. The NOEL was 1 µg/mL.

Cardiovascular system and respiration in the dog
No treatment-related effects on blood pressure, heart rate, ECG, cardiac function, blood flow or respiration in the anaesthetized dog was observed at any dose level. The NOEL was 1000 mg/kg bw.

Charcoal propulsion test
No significant effects on charcoal propulsion in any of the groups treated with pyrimethanil was observed, whereas a marked inhibition of the travelling distance was observed for the positive control. The NOEL was 1000 mg/kg bw.

Isolated phrenic nerve-diaphragm
No effect on contractions of the diaphragm in the presence of pyrimethanil at the concentration tested when the phrenic nerve was electrically stimulated. The NOEL was 100 µg/mL.

In vitro haemolytic effect
Pyrimethanil caused no haemolytic activity. The NOEL was 100 µg/mL.

Blood coagulation
No effect of pyrimethanil on prothrombin time, activated partial thromboplastin time or whole blood clotting time was observed. The NOEL was 1000 mg/kg bw.

III. CONCLUSIONS

Pyrimethanil was mostly inactive in the safety pharmacology studies resulting in NOEL values corresponding to the highest dose/concentration tested. However, pyrimethanil produced transient apathy and induced a prolonged hexobarbital sleeping time in rats at 1000 mg/kg bw. Furthermore, inhibitory effects against 5-HT-induced contractions of the isolated guinea pig ileum were observed from 10 µg/mL onward after incubation with pyrimethanil.

Classification: Supplementary information

CA 5.8.3 Endocrine disrupting properties

Pyrimethanil is neither classifiable for carcinogenicity nor for reproduction toxicity and it thus does not fall under the interim criteria for endocrine disruption, as depicted from Regulation 1107/2009. Up to now, there is no other scientific criteria available on the regulatory definition of an endocrine disrupting compound.

One target organ in rats and mice after dietary administration of pyrimethanil is the thyroid. In the following a short overview of the relevant studies and NOAELs/LOAELs identified for thyroid toxicity is given and an assessment on different susceptibilities towards expected relevance and exposure to humans is provided. Furthermore, for a comprehensive evaluation of endocrine disrupting properties of pyrimethanil, the available data addressing estrogen- and androgen-related effects were also evaluated.

Studies evaluated in the draft monograph of Rapporteur Member State Austria of April 15, 2004:

The evaluation of potential endocrine disruption was not a data-requirement at the time of Annex I inclusion of pyrimethanil. However, all relevant pivotal in vivo studies as well as mechanistic studies already evaluated for Annex I conclusion were used for evaluation of possible endocrine disrupting effects and did not provide any indication of such [see studies listed in tables 5.8.3-2 to 5.8.3-4] The noticed effect of pyrimethanil on the thyroid was assessed as induced via a reversible and extra-thyroidal, indirect mechanism. No evidence for disruption of the sexual endocrine system was given from the pivotal repeated dose and reproductive toxicity studies conducted.

In conclusion, there was no evidence for endocrine disrupting properties for pyrimethanil from the data-package evaluated for Annex I inclusion.

Studies submitted in this AIR 3 dossier (not yet peer-reviewed):

Literature data and an inhouse test revealed limited and inconclusive evidence for anti-androgenic effects and even less conclusive androgenic activity in vitro caused by pyrimethanil in transactivation assays at high dose levels. There was no indication for receptor binding in a competitive ligand binding assay and no androgen sensitive cell-proliferation was induced by pyrimethanil in vitro. These data are judged to represent supplemental information only and do not provide relevant endpoints. By weight of evidence there is no conclusive indication for androgenic or anti-androgenic activity of pyrimethanil.

With regard to estrogenic or anti-estrogenic effects the evidence for pyrimethanil was again inconclusive. While there was a limited increase of estrone production in a steroidogenesis assay, no receptor binding or dimerization, no induction of receptor regulated genes, and no receptor transactivation, neither agonism nor antagonism was reported. Regarding cell proliferation, the slight effect observed in an E-screen assay at high dose was not confirmed in an independent second assay in the same cell line nor in another cell line. These data are judged to represent supplemental information only and do not provide relevant endpoints. By weight of evidence there is no conclusive indication for estrogenic or anti-estrogenic activity of pyrimethanil.

There was some indication that pyrimethanil activated PXRE and AhR.

With regard to the thyroid hormone system, there is an alert from the ToxCast data publication that pyrimethanil might interact with the thyroid hormone pathway. However, the in vitro studies on receptor mediated activation are negative. Instead as discussed above the thyroid toxicity observed in the pivotal toxicity studies is related to a reversible and extra-thyroidal, indirect mechanism of increased thyroid hormone clearance by liver enzyme induction.

The respective studies related to potential endocrine disrupting properties are listed in Table 5.8.3-1.

Table 5.8.3-1: Summary of endocrine disrupting properties with pyrimethanil (not yet peer-reviewed)

Type of study	Test substance	Result Classification	Reference (BASF DocID)
QSAR Profiling Tool (based on results from Shah et al, 2011)	Pyrimethanil	Medium activity in LogP and KEGG path Weak activity for Ingenuity path, Other NR, other XME/ADME High activity in the TR pathways No activity in the AR- or ER-pathways	Reif et al., 2010 DocID 2010/1231552
QSAR (AR-binding)	Pyrimethanil	No AR-binding	Orton et al., 2011 2011/1291251
Steroidogenesis assay	Pyrimethanil	Increase of estrone by 47 ± 20%	Prutner et al., 2013 2013/1419984
Gene expression analysis of nuclear receptor regulated genes	Pyrimethanil	Increase: CYP1A1, CYP1A2, CYP2B6, CYP3A4, and UGT1A1 No effect: CYP2C9, SULT2A1 Expression of genes was linked to AhR and PXR receptor	Rotroff et al., 2010 2010/1233112
ER binding assays Human (NVS-hER), bovine (NVS_bER) and murine (NVS_mERa)	Pyrimethanil	No receptor binding	Rotroff et al., 2014 2014/1323273
Competitive ligand binding assay (LBA) Androgen receptor	Pyrimethanil	No competitive inhibition.	Medjakovic et al., 2013 2013/1419983
Protein-fragment complementation assays on estrogen receptor alpha (ERα) and beta (ERβ) homo- and heterodimerization (ERα-ERα; OT_ERaERa_1440, ERα-ERβ; OT_ERaERb_1440, ERβ-ERβ; OT_ERbERb_1440)	Pyrimethanil	No dimerization activity	Rotroff et al., 2014 2014/1323273
Pathway specific protein stabilization OT_AR_ARSRC1_0480 OT_AR_ARSRC1_0960	Pyrimethanil	No protein stabilization	ToxCast Data accessible via iCSSDashboard ~~http://actor.epa.gov/actor/faces/CSSDashboardLaunch.jsp~~ https://actor.epa.gov/dashboard/

Table 5.8.3-1: Summary of endocrine disrupting properties with pyrimethanil (not yet peer-reviewed)

Type of study	Test substance	Result Classification	Reference (BASF DocID)
Transactivation assays (with nuclear receptors)	Pyrimethanil	Activation of PPAR, PXRE and AhR No activation of ER or AR	Shah et al., 2011 2011/1295091
Yeast AhR transactivation assay	Pyrimethanil	Slight binding potential	Medjakovic et al., 2013 2013/1419983
Yeast Androgen Screening (YAS) transactivation assay with the hAR yeast strain	Pyrimethanil	Anti-androgenic effects were observed at the highest dose level of 100 µM.	Woitkowiak, 2011 2011/1140604
YAS transactivation assay	Pyrimethanil	Anti-androgenic activity but no androgenic activity	Orton et al., 2011 2011/1291251
yAS transactivation assay	Pyrimethanil	Not androgenic no AR-transactivation	Medjakovic et al., 2013 2013/1419983
yESα	Pyrimethanil	Not estrogenic No ERα-transactivation	Medjakovic et al., 2013 2013/1419983
MDA-kb2 Transactivation assay	Pyrimethanil	Slight androgenic and anti-androgenic activity	Orton et al., 2011 2011/1291251
MDA-kb2 Transactivation assay	Pesticide mixtures including Pyrimethanil	Addition of effects of pure AR antagonists	Orton et al., 2012 2012/1368704
Reporter gene assay: mRNA transcription factor ATG_ERE_CIS and ATG_ERa_TRANS ATG_ERRa_TRANS_up ATG_ERRg_TRANS_up	Pyrimethanil	No mRNA transcription	Rotroff et al., 2014 2014/1323273 ToxCast Data accessible via iCSSDashboard ~~http://actor.epa.gov/actor/faces/CSSDashboardLaunch.jsp~~ https://actor.epa.gov/dashboard/
Reporter gene assay: mRNA transcription factor ATG_AR_trans_up	Pyrimethanil	No mRNA transcription	ToxCast Data accessible via iCSSDashboard ~~http://actor.epa.gov/actor/faces/CSSDashboardLaunch.jsp~~ https://actor.epa.gov/dashboard/
Reporter gene assay: mRNA transcription factor ATG_THRa1_ trans_up	Pyrimethanil	TR transcription factor mRNA transcription	ToxCast Data accessible via iCSSDashboard ~~http://actor.epa.gov/actor/faces/CSSDashboardLaunch.jsp~~ https://actor.epa.gov/dashboard/
Receptor mediated pathway activation OT_AR_ARELUC_ AG_1440	Pyrimethanil	No pathway activation	ToxCast Data accessible via iCSSDashboard ~~http://actor.epa.gov/actor/faces/CSSDashboardLaunch.jsp~~ https://actor.epa.gov/dashboard/

Table 5.8.3-1: Summary of endocrine disrupting properties with pyrimethanil (not yet peer-reviewed)

Type of study	Test substance	Result Classification	Reference (BASF DocID)
ERa Transactivation assay Tox21_ERa_BLA_agonist, Tox21_ERa_BLA_antagonist,	Pyrimethanil	No receptor agonism, no receptor antagonism	Rotroff et al., 2014 2014/1323273 ToxCast Data accessible via iCSSDashboard ~~http://actor.epa.gov/actor/faces/CSSDashboardLaunch.jsp~~ https://actor.epa.gov/dashboard/
ERa Transactivation assay Tox21_ERa_LUC_BG1_agonist Tox21_ERa_LUC_BG1_antagonist	Pyrimethanil	No receptor agonism, no receptor antagonism	Rotroff et al., 2014 2014/1323273 ToxCast Data accessible via iCSSDashboard ~~http://actor.epa.gov/actor/faces/CSSDashboardLaunch.jsp~~ https://actor.epa.gov/dashboard/
AR Transactivation assay Tox21_AR_BLA_agonist Tox21_AR_BLA_ antagonist	Pyrimethanil	No receptor agonism, no receptor antagonism	ToxCast Data accessible via iCSSDashboard ~~http://actor.epa.gov/actor/faces/CSSDashboardLaunch.jsp~~ https://actor.epa.gov/dashboard/
AR Transactivation assay Tox21_AR_LUC_ MDAKB2_agonist Tox21_AR_LUC_ MDAKB2_antagonist	Pyrimethanil	No receptor agonism, no receptor antagonism	ToxCast Data accessible via iCSSDashboard ~~http://actor.epa.gov/actor/faces/CSSDashboardLaunch.jsp~~ https://actor.epa.gov/dashboard/
NR Transactivation assay TOX21_TR_LUC_GHR_ Agonist TOX21_TR_LUC_GHR_ Antagonist	Pyrimethanil	No receptor agonism, no receptor antagonism	ToxCast Data accessible via iCSSDashboard ~~http://actor.epa.gov/actor/faces/CSSDashboardLaunch.jsp~~ https://actor.epa.gov/dashboard/

Table 5.8.3-1: Summary of endocrine disrupting properties with pyrimethanil (not yet peer-reviewed)

Type of study	Test substance	Result Classification	Reference (BASF DocID)
Cell proliferation T47D cell growth assay (ACEA_T47D_80hr_Positive)	Pyrimethanil	No estrogen sensitive cell proliferation	Rotroff et al., 2014 2014/1323273 ToxCast Data accessible via iCSSDashboard ~~http://actor.epa.gov/actor/faces/CSSDashboardLaunch.jsp~~ https://actor.epa.gov/dashboard/
E-screen (MCF-7 cells)	Pyrimethanil	Slight proliferative effect	Bitsch et al., 2002 2011/1281908
Estrogen sensitive cell proliferation assay (MCF-7)	Pyrimethanil	No proliferative or proliferation inhibition effect in estrogen sensitive (ERα-positive) cell line MCF-7	Medjakovic et al., 2013 2013/1419983
Androgen sensitive Proliferation assay (LN CAP)	Pyrimethanil	No proliferative nor proliferation inhibition effect in androgen sensitive cell line LNCaP	Medjakovic et al., 2013 2013/1419983
Reporter gene assays	Pyrimethanil	No indication for estrogen receptor pathway	Judson et al. 2015 2015/1279970
Reporter gene assays and cytotoxicity assays	Pyrimethanil	Cytotoxicity information as additional information for assessment of receptor pathway involvement of pyrimethanil	Judson et al. 2016 2016/1227708

Discussion

Pyrimethanil showed some borderline effects in some in vitro screening assays for androgenicity or estrogenicity, although in the majority of screening tests pyrimethanil had no effect. Effects noted were only seen at high test-concentrations. Furthermore, no indications of androgenic/estrogenic effects were observed in several in vivo studies. There were no alerts for endocrine disruption from the evaluation of reproductive organs, mating performance and fertility or from pre- or post-natal development of the offspring. Pathology and histopathology of subchronic, chronic or carcinogenicity studies in mice, rats, and dogs revealed no abnormalities related to androgenicity or estrogenicity.
Indeed, effects of pyrimethanil on the thyroid pathology/histopathology were observed in several in vivo studies. However, further mechanistic studies and the results of additional literature evaluation clearly indicated that pyrimethanil elicits its effects on the thyroid via a reversible and extra-thyroidal, indirect mechanism. These data are supported by screening information from transactivation assays where no thyroid receptor-dependent transactivation was observed with pyrimethanil. In comparison to laboratory animals humans are expected to be less sensitive to thyroid inhibitors due to several reasons:

- Humans possess the thyroxine-binding globulin, a serum protein that is missing in laboratory rodents, and which prevents the glucuronidation and thus excretion of thyroid hormones.
- The half-lives of thyroid hormones are much longer in humans than in rats.
- Humans do develop goiter rather than neoplasms in case of thyroid insufficiency, which is also an important factor for human risk assessment.

The available screening, mechanistic, and in vivo studies indicate that pyrimethanil has thyroidal relevance in rats and mice, but following a mechanism that is not relevant for humans. This lack of relevance for humans is strongly supported by the mechanistic studies performed with pyrimethanil and already discussed in the conclusion on the peer review of Pyrimethanil (EFSA, 2006).

In conclusion, pyrimethanil is considered to have no thyroidal, endocrine related effects that are relevant for humans.

Thus, the conclusion for relevant endpoints for the current renewal was amended as follows:

Other toxicological studies (SANCO/11802 data point 5.8)

Endocrine disrupting properties	No relevant endocrine effects on the oestrogen, androgen hormone system is observed. Effects observed on the thyroid hormone system are based on an indirect mechanism not related to endocrine disruption and not considered of relevance for humans. Not classifiable for endocrine disruption according to interim criteria

Estrogenic/anti-estrogenic effects

In the following all available data are evaluated regarding estrogenic or anti-estrogenic effects. Available information includes a 2-generation study in rats, developmental toxicity studies in rats and rabbits, and ~~three~~ screening studies from public literature (see Table 5.8.3-2)
The screening information is extracted from the ToxCast database. The relevant background information related to test details and evaluation parameters of the individual tests are summarised in the publication from Judson et al. (DocID 2015/1279970). In addition, Judson et al. (DocID 2016/1227708) describe the influence of cytotoxicity on the assay outcome.

Table 5.8.3-2: Overview on available information from studies/literature regarding estrogenicity.

Study type	Endpoint (Conclusion)	Main effects/target organ	Reference
In vivo studies in rat and rabbit			
2-Generation, oral, feed, Sprague-Dawley rat (see CA 5.6)	Toxicity to reproduction (parental/developmental NOAEL 18.4 (M)/23.4 (F) mg/kg bw/d)	Parental toxicity: Reduced body weight gain Developmental toxicity: Reduced body weights and retarded weight gain	A81822¥, A89219¥, A89218¥
Developmental toxicity, gavage (days 6-15), Sprague-Dawley rat (see CA 5.6)	Developmental toxicity (Maternal NOAEL 85 mg/kg bw/d Fetal NOAEL 85 mg/kg bw/d)	Maternal toxicity: Reduced body weight gain and food intake and other clinical signs Developmental toxicity: Reduced litter and fetal body weight	A81800¥, 2003/1023035¥
Developmental toxicity, gavage (days 7-19), New Zealand White rabbit (see CA 5.6)	Developmental toxicity Maternal NOAEL 45 mg/kg bw/d Fetal NOAEL 45 mg/kg bw/d	Maternal toxicity: Reduced body weight gain and food consumption, and other clinical signs (disturbed fecal excretion). Developmental toxicity: Reduced fetal body weight, retardation on fetal development, increased incidences of skeletal variations (13th vertebrae and ribs) and increased number of runts.	A81798¥

Table 5.8.3-2: Overview on available information from studies/literature regarding estrogenicity.

Study type	Endpoint (Conclusion)	Main effects/target organ	Reference
Level 1 screening studies (according to OECD guidance on EDC, GD150)			
QSAR Profiling Tool (based on results from Shah et al, 2011)	Activity on ER pathway (no effect)	No ER pathway activation	Reif et al., 2010 DocID 2010/1231552
Level 2 screening studies (according to OECD guidance on EDC, GD150)			
High-throughput screening assays for the estrogen receptor	ER binding, dimerization, chromatin binding, transcriptional avtivation, and ER-dependent cell proliferation	No indication for estrogen receptor pathway	Judson et al., 2015 2015/1279970 Judson et al., 2016 2016/1227708
Steroidogenesis (H295 R human adrenocortical carcinoma cells)	Estrone concentration in cell culture medium (Slight effects on estrone production)	Increase of estrone of 47±20% (at 100 µM)	Prutner et al., 2013 2013/1419984
Gene expression analysis of nuclear receptor regulated genes	CYP1A1, CYP1A2, CYP2B6, CYP3A4, UGT1A1, CYP2C9 SULT2A1 (Expression of genes linked to AhR and PXR receptor)	Increase: CYP1A1, CYP1A2, CYP2B6, CYP3A4, and UGT1A1	Rotroff et al., 2010 2010/1233112
ER binding assays Human (NVS-hER), bovine (NVS_bER) and murine (NVS_mERa)	ER Receptor binding (no effect)	Inactive: No receptor binding	Rotroff et al., 2014 2014/1323273 ToxCast Data accessible via iCSSDashboard ~~http://actor.epa.gov/actor/faces/CSSDashboardLaunch.jsp~~ https://actor.epa.gov/dashboard/
Protein-fragment complementation assays on estrogen receptor alpha (ERα) and beta (ERβ) homo- and heterodimerization (ERα-ERα; OT_ERaERa_1440, ERα-ERβ; OT_ERaERb_1440, ERβ-ERβ; OT_ERbERb_1440)	ER Receptor dimerization (no effect)	Inactive: No dimerization activity	Rotroff et al., 2014 2014/1323273 ToxCast Data accessible via iCSSDashboard ~~http://actor.epa.gov/actor/faces/CSSDashboardLaunch.jsp~~ https://actor.epa.gov/dashboard/
yESα	ERα-transactivation	not estrogenic (10 nM-100 µM)	Medjakovic et al., 2013

Table 5.8.3-2: Overview on available information from studies/literature regarding estrogenicity.

Study type	Endpoint (Conclusion)	Main effects/target organ	Reference
	(no effect)		2013/1419983
mRNA transcription ATG_ERE_CIS and ATG_ERa_TRANS ATG_ERRa_TRANS_up ATG_ERRg_TRANS_up	ER transcription factor mRNA transcription (no effect)	Inactive: No mRNA transcription	Rotroff et al., 2014 2014/1323273 ToxCast Data accessible via iCSSDashboard ~~http://actor.epa.gov/actor/faces/CSSDashboardLaunch.jsp~~ https://actor.epa.gov/dashboard/
Transactivation assay Tox21_ERa_BLA_agonist, Tox21_ERa_BLA_antagonist,	ERa-transactivation (no effect)	Inactive: No receptor agonist or antagonist	Rotroff et al., 2014 2014/1323273 ToxCast Data accessible via iCSSDashboard ~~http://actor.epa.gov/actor/faces/CSSDashboardLaunch.jsp~~ https://actor.epa.gov/dashboard/
Transactivation assay Tox21_ERa_LUC_BG1_agonist Tox21_ERa_LUC_BG1_antagonist	ERa-transactivation (no effect)	Inactive: No receptor agonist or antagonist	Rotroff et al., 2014 2014/1323273 ToxCast Data accessible via iCSSDashboard ~~http://actor.epa.gov/actor/faces/CSSDashboardLaunch.jsp~~ https://actor.epa.gov/dashboard/
Cell proliferation T47D cell growth assay (ACEA_T47D_80hr_Positive)	Cell prolifation (no effect)	Inactive: No estrogen sensitive cell proliferation	Rotroff et al., 2014 2014/1323273 ToxCast Data accessible via iCSSDashboard ~~http://actor.epa.gov/actor/faces/CSSDashboardLaunch.jsp~~ https://actor.epa.gov/dashboard/
E-screen (MCF-7 cells)	ERα-dependent cell proliferation (borderline increase in cell proliferation)	Increase at 100 µM (RPE of 15% of 17β-estradiol)	Bitsch et al., 2002 2011/1281908
Estrogen sensitive cell proliferation assay (MCF-7)	Hormone dependent cell proliferation (no effect)	Not estrogenic (10 µM and 100 µM)	Medjakovic et al., 2013 2013/1419983

¥ studies already peer-reviewed.

The available screening studies indicate that pyrimethanil is not a potent estrogen or anti-estrogen. The yeast-assay and ERα-transactivation assays performed within the EPA ToxCast program were clearly negative. The E-screen showed borderline results but had several limitations preventing conclusive evaluation [see CA 5.8.3/6]. Furthermore, the same assay type was reported to be negative in the recent publication of Medjakovic et a. 2013 [see CA 5.8.3/7] In the available in vivo studies no indication of an estrogenic or anti-estrogenic effect was observed.
No effects on gestation, gestation length, implantation loss, malformations or histopathological abnormalities have been observed in the 2-generation study in rats, and the developmental toxicity studies in rats and rabbits.

In conclusion, pyrimethanil is considered to have no estrogenic or anti-estrogenic properties based on the available information.

Androgenic effects

In the following all available data are evaluated regarding androgenic or anti-androgenic effects. Available information includes several screening assays as well as subchronic, chronic, and carcinogenicity studies in mice, rats, or dogs (see table Table 5.8.3-3).

Table 5.8.3-3: Overview on available information from studies/literature regarding androgenicity.

Study type	Endpoint (Conclusion)	Results	Reference
In vivo studies in rat, mouse and dog			
4-week feeding Sprague-Dawley rat (see CA 5.3)	Clinical signs, Pathology, Histology (no NOAEL determined)	No effects observed related to androgen-linked parameters or tissues	A81779¥
13-week feeding Sprague-Dawley rat (see CA 5.3)	Clinical signs, Pathology, Histology (NOAEL 5.4 (m) and 6.8 (f) mg/kg bw/day)	No effects observed related to androgen-linked parameters or tissues	A81783¥, C023548¥, A81784¥, A81785¥, 2003/1023036¥
4-week feeding CD-1 mouse (see CA 5.3)	Clinical signs, Pathology, Histology (NOAEL 167 (m) and 236 (f) mg/kg bw/day)	No effects observed related to androgen-linked parameters or tissues	A81781¥
13-week feeding CD-1 mouse (see CA 5.3)	Clinical signs, Pathology, Histology (NOAEL 139 (m) and 203 (f) mg/kg bw/day)	No effects observed related to androgen-linked parameters or tissues	A81792¥
13 days, gavage Beagle dog (see CA 5.3)	Clinical signs, Pathology, Histology (no NOAEL determined)	No effects observed related to androgen-linked parameters or tissues	A81763¥
4 weeks, gavage Beagle dog (see CA 5.3)	Clinical signs, Pathology, Histology (NOAEL 100 (m+f) mg/kg bw/day)	No effects observed related to androgen-linked parameters or tissues	A81764¥
13 weeks, gavage Beagle dog (see CA 5.3)	Clinical signs, Pathology, Histology (NOAEL 6 (m+f) mg/kg bw/day)	No effects observed related to androgen-linked parameters or tissues	A81790¥
1-year, gavage Beagle dog (see CA 5.3)	Clinical signs, Pathology, Histology (NOAEL 30 (m+f) mg/kg bw/day)	No effects observed related to androgen-linked parameters or tissues	A81809¥
24-month combined chronic toxicity and carcinogenicity Sprague-Dawley rat (see CA 5.5)	Clinical signs, Pathology, Histology (NOAEL 17 (m) and 22 (f) mg/kg bw/day)	No effects observed related to androgen-linked parameters or tissues	A81806¥, 2003/1023033¥, A54965¥, A81808¥
18-month carcinogenicity CD-1 mouse (see CA 5.5)	Clinical signs, Pathology, Histology (NOAEL 17.3 (m) and 22.3 (f) mg/kg bw/day)	No effects observed related to androgen-linked parameters or tissues	A81811¥, A81814¥, A89479¥, A89480¥, A89481¥, 2003/1023034¥, A81813¥

Table 5.8.3-3: Overview on available information from studies/literature regarding androgenicity.

Study type	Endpoint (Conclusion)	Results	Reference
Level 1 screening studies (according to OECD guidance on EDC, GD150)			
QSAR	Interaction with AR (no effect)	inactive (no interaction predicted)	Orton et al., 2011 2011/1291251
Level 2 screening studies (according to OECD guidance on EDC, GD150)			
Gene expression analysis of nuclear receptor regulated genes	CYP1A1, CYP1A2, CYP2B6, CYP3A4, UGT1A1, CYP2C9 SULT2A1 (Expression of genes linked to AhR and PXR receptor)	Increase: CYP1A1, CYP1A2, CYP2B6, CYP3A4, and UGT1A1	Rotroff et al., 2010 2010/1233112
Competitive ligand binding assay (LBA) Androgen receptor	Ligand binding inhibition (no effect)	No competitive inhibition	Medjakovic et al., 2013 2013/1419983
Receptor mediated pathway activation OT_AR_ARELUC_ AG_1440	Gene expression regulation (no effect)	Inactive: No activation of AR pathway	ToxCast Data accessible via iCSSDashboard ~~http://actor.epa.gov/actor/faces/CSSDashboardLaunch.jsp~~ https://actor.epa.gov/dashboard/
Pathway specific protein stabilization OT_AR_ARSRC1_0480 OT_AR_ARSRC1_0960	Gene expression regulation (no effect)	Inactive: No protein stabilization	ToxCast Data accessible via iCSSDashboard ~~http://actor.epa.gov/actor/faces/CSSDashboardLaunch.jsp~~ https://actor.epa.gov/dashboard/
mRNA transcription ATG_AR_trans_up	AR transcription factor mRNA transcription (no effect)	Inactive: no mRNA transcription	ToxCast Data accessible via iCSSDashboard ~~http://actor.epa.gov/actor/faces/CSSDashboardLaunch.jsp~~ https://actor.epa.gov/dashboard/
Transactivation assay Tox21_AR_BLA_agonist Tox21_AR_BLA_ antagonist	AR-transactivation (no effect)	Inactive: Not a receptor agonist or antagonist	Shah et al., 2011 2011/1295091 ToxCast Data accessible via iCSSDashboard ~~http://actor.epa.gov/actor/faces/CSSDashboardLaunch.jsp~~ https://actor.epa.gov/dashboard/
Transactivation assay Tox21_AR_LUC_ MDAKB2_agonist	AR-transactivation (no effect)	Inactive: Not a receptor agonist or antagonist	Shah et al., 2011 2011/1295091

Table 5.8.3-3: Overview on available information from studies/literature regarding androgenicity.

Study type	Endpoint (Conclusion)	Results	Reference
Tox21_AR_LUC_ MDAKB2_antagonist			ToxCast Data accessible via iCSSDashboard ~~http://actor.epa.gov/actor/faces/CSSDashboardLaunch.jsp~~ https://actor.epa.gov/dashboard/
Testing for androgenic and anti-androgenic potential in yeast (YAS)	Induction or AR-transactivation (no androgenic but anti-androgenic activity)	not androgenic (100 pM-100 µM) anti-androgenic 100 µM (decrease to 25%) not cytotoxic (100 pM-100 µM)	Woitkowiak, 2011 2011/1140604
Testing for androgenic and anti-androgenic potential in yeast (YAS)	AR- transactivation (anti-androgenic activity)	Anti-androgenic (EC_{20} 9.15 µM) Cytotoxicity: (EC_{20} 167 µM)	Orton et al., 2011 2011/1291251
Testing for androgenic and anti-androgenic potential in yeast (YAS)	AR- transactivation (no effect)	not androgenic (10 nM-100 µM)	Medjakovic et al., 2013 2013/1419983
Testing for androgenic and anti-androgenic potential in mammalian cells (MDA-kb2)	AR- transactivation (slight effects observed)	androgenic (EC_{20}: 27.8 µM) anti-androgenic (IC_{20}: 27.2 µM) cytotoxicity (EC_{20} >125 µM)	Orton et al., 2011 2011/1291251
Testing for androgenic effect of mixture in mammalian cells (MDA-kb2)	AR- transactivation (addition of effects of pure AR agonists)		Orton et al., 2011 2011/1291251
Androgen sensitive Proliferation assay (LN CAP)	Hormone dependent cell proliferation (no effect)	No proliferative nor proliferation inhibition effect in androgen sensitive cell line LNCaP	Medjakovic et al., 2013 2013/1419983

¥ studies already peer-reviewed.

The available screening studies indicate that pyrimethanil is not a potent androgen or anti-androgen. Slight AR-binding and induced gene expression was observed after incubation with pyrimethanil in some assays not confirmed in others. In two YAS-assays and a cell proliferation assay no androgenic effects were observed. This is also true for the various in vivo studies were no effects related to androgens were observed.

Slight anti-androgenic effects were observed in two transactivation assays, not confirmed in two other assay conducted as part of the ToxCast testing battery. No indication of anti-androgenic activity of pyrimethanil in vivo was observed.

No effects on male sexual organs were noticed in any of the repeated dose toxicity studies conducted. No effects on gestation, gestation length, implantation loss, malformations or histopathological abnormalities have been observed in the 2-generation study in rats, and the developmental toxicity studies in rats and rabbits.

In conclusion, pyrimethanil is considered to have no androgenic or anti-androgenic properties based on the available information.

Thyroid effects

In the following all available data are evaluated regarding effects on the thyroid. Available information includes several screening assays, mechanistic studies as well as subchronic, chronic, and carcinogenicity studies in mice, rats, or dogs (see table Table 5.8.3-4).

Table 5.8.3-4: Overview on available information from studies/literature regarding effects on the thyroid.

Study type	Endpoint	Results	Reference
In vivo studies in rat, mouse and dog			
4-week feeding Sprague-Dawley rat (see CA 5.3)	Clinical signs, Pathology, Histology	Effects on body weight, food consumption, haematology, clinical chemistry, organ weights, gross pathology and histopathology.	A81779¥
13-week feeding Sprague-Dawley rat (see CA 5.3)	Clinical signs, Pathology, Histology	Changes in thyroid pathology	A81783¥, C023548¥, A81784¥, A81785¥, 2003/1023036¥
4-week feeding CD-1 mouse (see CA 5.3)	Clinical signs, Pathology, Histology	Histopathological findings in thyroid	A81781¥
13-week feeding CD-1 mouse (see CA 5.3)	Clinical signs, Pathology, Histology	Histopathological changes in thyroid	A81792¥
13 days, gavage Beagle dog (see CA 5.3)	Clinical signs, Pathology, Histology	No effects observed related to the thyroid	A81763¥
4 weeks, gavage Beagle dog (see CA 5.3)	Clinical signs, Pathology, Histology	No effects observed related to the thyroid.	A81764¥
13 weeks, gavage Beagle dog (see CA 5.3)	Clinical signs, Pathology, Histology	No effects observed related to the thyroid	A81790¥
1-year, gavage Beagle dog (see CA 5.3)	Clinical signs, Pathology, Histology	No effects observed related to the thyroid	A81809¥
24-month combined chronic toxicity and carcinogenicity Sprague-Dawley rat (see CA 5.5)	Clinical signs, Pathology, Histology	Systemic toxicity: pathological/histo-pathological findings in the thyroid. Carcinogenicity: Increased incidences of benign follicular cell tumours in the thyroid gland (not statistically significant and evaluated not human relevant)	A81806¥, 2003/1023033¥, A54965¥, A81808¥
18-month carcinogenicity CD-1 mouse (see CA 5.5)	Clinical signs, Pathology, Histology	No effects observed related to the thyroid	A81811¥, A81814¥, A89479¥, A89480¥, A89481¥, 2003/1023034¥, A81813¥

Table 5.8.3-4: Overview on available information from studies/literature regarding effects on the thyroid.

Study type	Endpoint	Results	Reference
Mechanistic studies			
4 days, gavage, twice daily Sprague-Dawley rat	Enzyme activity (EROD, PEROD, CYP b_5)	Weak induction of ethoxyresorufin-*O*-deethylase and pentoxyresorufin-*O*-dealkylase and increased concentration of cytochrome b_5.	A81625¥
7-day feeding Sprague-Dawley rat	Iodine uptake into the thyroid	Reduced body weight gain and food consumption, and significant higher iodine uptake	A81829¥
14-day feeding Sprague-Dawley rat	Clinical chemistry, enzyme activity	Increased TSH levels, normal T_3/T_4 levels, marked induction of hepatic uridine diphosphate-glucuronosyltransferase	A81828¥
4-day feeding CD-1 mouse	Enzyme activity	Low potency inducer of cytochrome P-450 and 7-pentoxyresorufin-*O*-depentylase	C001378¥
Level 1 screening studies (according to OECD guidance on EDC, GD150)			
QSAR Profiling Tool (based on results from Shah et al, 2011)	Activity on TR pathway	TR pathway activation	Reif et al., 2010 DocID 2010/1231552
Level 2 screening studies (according to OECD guidance on EDC, GD150)			
mRNA transcription ATG_THRa1_ trans_up	TR mRNA transcription factor transcription	Active: TR transcription factor mRNA transcription	ToxCast Data accessible via iCSSDashboard ~~http://actor.epa.gov/actor/faces/CSSDashboardLaunch.jsp~~ https://actor.epa.gov/dashboard/
Transactivation assay TOX21_TR_LUC_GHR_Agonist TOX21_TR_LUC_GHR_Antagonist	TR transactivation	Inactive. No receptor agonist or antagonist	ToxCast Data accessible via iCSSDashboard ~~http://actor.epa.gov/actor/faces/CSSDashboardLaunch.jsp~~ https://actor.epa.gov/dashboard/

¥ studies already peer-reviewed.

In supplementary mechanistic studies, effects of pyrimethanil on hepatic enzymes in rats and mice and, in particular, on the thyroid in the rat have been investigated. It was demonstrated that pyrimethanil administered at 5000 ppm in the diet to rats for 7 days did not show a thyroid blocking activity. There were no indications of inhibition of iodine uptake into the thyroid (i.e. tyrosine iodination) or of a significant discharge of [^{125}I] after perchlorate challenge. Results after treatment with pyrimethanil were similar to those obtained with phenobarbital which modifies also thyroid homeostasis by an indirect mechanism. In a second study, dietary administration of 5000 ppm pyrimethanil to rats induced significant increases in mean levels of TSH and significant reductions in mean levels of T3 and T4 after 3 days of treatment. After 7 and 14 days of treatment, mean TSH levels in treated animals were higher than controls but plasma T3 and T4 levels had returned to untreated levels (via the normal homeostatic mechanism). In addition, marked and significant increases of the hepatic uridine diphosphoglucuronyl transferase activity were observed. Reversibility of all these changes was demonstrated.
These data indicate that pyrimethanil elicits its effects on the thyroid via a reversible and extra-thyroidal, indirect mechanism. It can be concluded that the chronic reduction of circulating thyroid hormone levels was produced by increased thyroid hormone clearance via enhanced hepatic metabolism resulting in increased TSH levels and a chronic stimulation of the thyroid gland with development of increased cell division, increased size and number of thyroid cells. [Hurley et al. 1998; KCA 5.8.3/13 1998/1009835]
These data are supported by screening information from transactivation assays were no thyroid receptor-dependent transactivation was observed with pyrimethanil.

In comparison to laboratory animals, humans are expected to be less sensitive to thyroid inhibitors. Reasons are the existence of the thyroxine-binding globulin, a serum protein that is missing in laboratory rodents. Furthermore, the half-lives of thyroid hormones are much longer in humans than in rats. Another important point for risk assessment is the fact that development of thyroid cancer in humans is rather rare compared with rats. Humans rather develop goiter than neoplasm in case of thyroid insufficiency [Choksi et al., 2003; KCA 5.8.3/14 2003/1034659].

The available screening, mechanistic, and in vivo studies indicate that pyrimethanil has thyroidal relevance in rats and mice, but following a mechanism that is not relevant for humans. This lack of relevance for humans is strongly supported by the mechanistic studies performed with pyrimethanil and already discussed in the conclusion on the peer review of Pyrimethanil [EFSA, 2006].

In conclusion, pyrimethanil is considered to have no thyroidal effects that are relevant for humans.

Report:	CA 5.8.3/1 Woitkowiak C., 2011a BAS 605 F (Pyrimethanil) - Testing for potential androgenic and antiandrogenic activity using the YAS-assay (AR) (yeast androgen screening) 2011/1140604
Guidelines:	none
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Deviations:	none

Executive Summary

Pyrimethanil (Batch No. COD-000078; purity 99.6%) was tested to assess an androgenic and/or antiandrogenic activity by using the Yeast Androgen Screening (YAS) assay with the hAR yeast strain.

Two independent experiments were carried out. Vehicle (DMSO) and positive controls for androgenic effects (5α-dihydrotestosterone: 10 pM-1 µM) and antiandrogenic effects (5α-dihydrotestosterone, 5 nM; hydroxyflutamide, 10 µM) were included into the experiment. Pyrimethanil was tested at concentrations from 100 pM up to 100 µM. No precipitation and no cytotoxicity were observed up to the highest concentration tested. An increase in the androgen receptor dependent enzyme expression (colour development) was not observed. A reproducible inhibition of the androgen effect compared to 5 nM 5α-dihydrotestosterone was observed at a concentration of 100 µM only.
Under the experimental conditions of the study, pyrimethanil did not exert androgenic effects while antiandrogenic effects were observed in the Yeast Androgen Screening (YAS) assay using the hAR yeast strain.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material	Pyrimethanil (BAS 605 F)
Description:	Solid, white
Lot/Batch #:	COD-000078
Purity:	99.6% (tolerance ± 1.0%)
Stability of test compound:	Expiry date: 01.06.2012
Solvent used:	Dimethylsulfoxide (DMSO)
2. Control Materials:	
Vehicle control:	DMSO 1% (v/v)
Positive control compounds:	Androgenic control: 5α-dihydrotestosterone Antiandrogenic control: 5α-dihydrotestosterone combined with hydroxyflutamide
3. Test organisms:	Yeast cells (*Saccharomyces cerevisiae*) have been stably transformed with a gene encoding the human androgen receptor (hAR), which is constitutively expressed. Additionally, these cells are stably transformed with a reporter gene plasmid, containing an androgen response element and the *LacZ* gene, which encodes the reporter enzyme β-galactosidase. The hAR yeast strain was obtained from "Technische Universität Dresden", Prof. Dr. G. Vollmer on 11 Feb 2010.

B. TEST PERFORMANCE:

1. Dates of experimental work: 23-May-2011 to 27-May-2011

2. Test substance preparation:
The test substance was weighed and topped up with the chosen vehicle to achieve the required concentration of the stock solution. The test substance was dissolved in DMSO. To achieve a clear solution of the test substance in the vehicle, the test substance preparation was shaken thoroughly. The further concentrations were diluted according to the planned concentrations. All test substance formulations were prepared immediately before administration. The stability of the test substance in the vehicle DMSO and in water was not determined analytically.
Final test substance concentrations: 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} mol/L

2. Test method:
A deep-frozen (-80°C) yeast stock culture was thawed at room temperature, inoculated in growth medium and incubated for preculture (24-72 h) and growth medium was exchanged after 72 h before use. Of the preculture, optical density (OD) was determined at 690 nm. For preparation of the test culture, 0.50 mL of the preculture with an OD of 1.0 was transferred into 50 mL fresh culture medium including 0.5 mL chromogenic substrate CPRG (chlorophenol red-β-D-galacto-pyranoside).
The study was carried out in 96-well microtiter plates in which 2 µL of different test substance solutions had been pipetted. 200 µL of the test culture was added to each well. The plates were sealed with breathable tape and incubated until measurement of the OD.

3. Controls:
Each experiment includes a negative control (vehicle control) and positive controls for the verification of the detection of androgenic and antiandrogenic activity in the yeast cells.

<u>Negative controls / Vehicle controls</u>
The vehicle control contains 2 µL of the vehicle used for the test substance. The final concentration of the vehicle in the culture medium will be 1% (v/v).

<u>Positive controls</u>
Androgenic control:
5α-dihydrotestosterone (dissolved in ethanol)
Final concentrations: 10^{-11}, 10^{-10}, 10^{-9}, $5x10^{-9}$; 10^{-8}, 10^{-7}, and 10^{-6} mol/L

Antiandrogenic Control:
5α-dihydrotestosterone combined with hydroxyflutamide (dissolved in DMSO)
Final concentrations:
$5x10^{-9}$ mol/L (5α-dihydrotestosterone)/ 10^{-5} mol/L (hydroxyflutamide)

The stability of the selected positive controls is well-defined under the chosen culture conditions since they are well established reference endocrine disruptors.

4. Evaluation/Assessment

4.1 Endocrine activity

After 48 h (±4 h) incubation, absorbance of the plates is measured at 570 nm (colour development, androgen receptor dependent enzyme expression) and 690 nm (turbidity due to growth of the yeast). Evaluation is performed by calculating the difference of the measured ODs at the two wavelengths (absorption at 570 nm - absorption at 690 nm). For the 4 replicates per concentration the median, minimum and maximum value are presented in a diagram. The findings of the first experiment were confirmed in an additional assay. The concentrations and test conditions were the same as in the first investigation.

4.2 Cytotoxicity

Cytotoxicity of the test substance is indicated by decrease of the yeast growth (measurement of the turbidity at 690 nm). For the evaluation of antiandrogenic activity only nontoxic test substance concentrations are taken into consideration.

4.2 Acceptance criteria

Generally, the experiment is considered valid, if the following criteria are met:

- The positive controls induced an agonistic / antagonistic effect within the range of the historical control data.
- The concentration $5x10^{-9}$ mol/L 5α-dihydrotestosterone achieved at least 40 percent of the maximum effected androgen receptor dependent enzyme expression of the positive control (colour development) based on the experiment.
- The vehicle control did not show colour development at 570 nm.

4.3 Assessment criteria

Generally, cytotoxicity (decrease of the yeast growth) is considered for data interpretation especially in the case of antiandrogenic activity.

A test substance is generally considered non-androgenic in this assay, if:

- Androgen receptor dependent enzyme expression was within the historical negative control range under all experimental conditions in two experiments carried out independently.

The test substance is considered as androgenic in this assay, if:

- A concentration-dependent and reproducible increase of the androgen receptor dependent enzyme expression (colour development) by at least 20% compared to the vehicle control was observed.
- If a concentration-dependent and reproducible increase of the androgen receptor dependent enzyme expression (colour development) by at least 10% and less than 20% compared to the vehicle control was observed, the test substance is considered to be slightly androgenic.

The test substance is considered as antiandrogenic in this assay, if:

- A concentration-dependent and reproducible inhibition of the androgenic effect compared to $5x10^{-9}$ mol/L 5α-dihydrotestosterone (partly or total suppression of expected colour development, without signs for cytotoxicity) by at least 20% was observed compared to $5x10^{-9}$ mol/L 5α-dihydrotestosterone alone.
- If a concentration-dependent and reproducible inhibition of the androgenic effect compared to $5x10^{-9}$ mol/L 5α-dihydrotestosterone (partly or total suppression of expected colour development, without signs for cytotoxicity) by at least 10% but less than 20% compared to $5x10^{-9}$ mol/L 5α-dihydrotestosterone alone was observed, the test substance is considered to be slightly antiandrogenic.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

No analytical determination of the test substance solutions was performed. No test substance precipitation was found.

B. ENDOCRINE ACTIVITY

Androgenicity:

An increase in the androgen receptor dependent enzyme expression (colour development) was not observed.

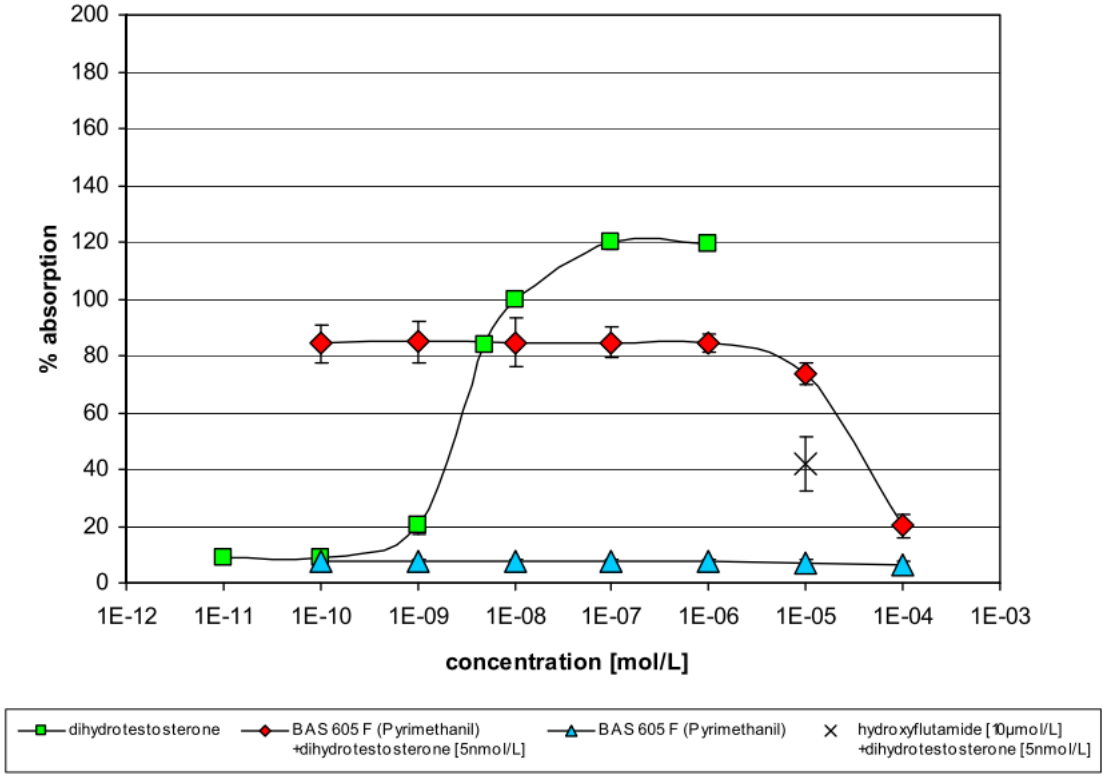
Antiandrogenicity:

A reproducible inhibition of the androgen effect compared to $5x10^{-9}$ mol/L 5α-dihydro-testosterone (partly or total suppression of expected colour development) was observed at a concentration of 10^{-4} mol/L onward.

C. CYTOTOXICITY OF THE TEST SUBSTANCE

No cytotoxic effect (decrease of the yeast growth) was observed.

Figure 5.8.3-1: Androgen receptor-dependent enzyme expression; Normalized median (% absorption calculated relative to the absorbance values obtained for 10^{-8} M dihydrotestosterone)



III. CONCLUSIONS

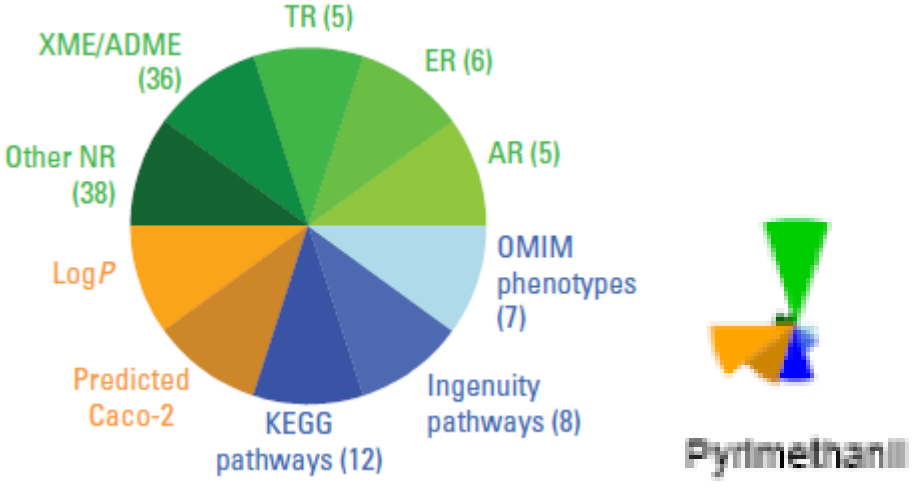
Under the experimental conditions of the study, pyrimethanil did not exert androgenic effects while antiandrogenic effects were observed in the Yeast Androgen Screening (YAS) assay using the hAR yeast strain.

Literature data
As many other pesticides and chemicals, pyrimethanil is part of the US ToxCast program. Several assays react to varying doses of pyrimethanil, however, no conclusive picture has emerged. The following publications related to endocrine effects are discussed.

Report:	CA 5.8.3/2 Reif D.M. et al., 2010a Endocrine profiling and prioritization of environmental chemicals using ToxCast data 2010/1231552
Guidelines:	none
GLP:	no

Executive Summary of the Literature
This publication describes a profiling tool developed on the ToxCast database to prioritize chemicals with regard to endocrine disruption evaluation/testing as a decision support tool. Thus this priorization tool was applied also to pyrimethanil being part of the ToxCast program. The priorization tool focused on estrogen, androgen and thyroid pathways and thus incorporated those screening assays of the ToxCast program considered relevant for putative endocrine profiles. In addition it incorporated external molecular pathway databases i.e. Kyoto Encyclopedia of Genes and Genomes (KEGG), Ingenuity software and the Online Mendelian Inheritance in Men repository. The tests in which pyrimethanil showed an activity are the same as described in the publication of Shah et al. 2011 [see M-CA 5.8.2/7]. The current evaluation of the results can be seen on EPA's dashboard [http://actor.epa.gov/dashboard/]. The so-called ToxPi profile for pyrimethanil (supplementary information) indicates medium activity in LogP and KEGG path, and weak activity for Ingenuity path, other NR, other XME/ADME and high activity in the TR pathways. The publication does not provide an absolute ranking but in visual comparison to activity alerts for other compound the ranking of activities for pyrimethanil is moderate. No linkage between pyrimethanil and other endpoints is provided.

The summary information (ToxPi) for pyrimethanil is provided below:



It is important to note that both Ingenuity Pathways and KEGG rely on literature data often generated in completely different cellular setting. In particular Ingenuity pathways are highly curated and quality controlled. However, genes/proteins have linkage to multiple pathways. An estimation from Lockhart et al. (Nature 405, 827-836, (15 June 2000)) indicated that a typical protein is associated to 7 other proteins. This is much more profound in key cellular regulators or chaperones (e.g. heat shock proteins). Those are implicated in multiple pathways and often regulation on gene expression level is not associated to any functional change in the pathway, as cells have large pools for these proteins and can compensate for changes. A regulation of such an accessory protein thus will indicate a positive association to multiple pathways, without giving any information on a functional change. Current approaches in bioinformatics therefore apply a weight of evidence approach, which means that multiple parameters within a pathway need to be regulated, before this is considered to be affected. Please also note that neither KEGG nor Ingenuity pathway analysis takes the direction of the regulation into account. This is an important limitation, as an up regulation can have fundamentally different consequences than a down regulation. A really meaningful prediction using either KEGG or Ingenuity would require a detailed evaluation of the affected network and prior knowledge of the regulation of this network in the used cell system. On their own, KEGG or Ingenuity pathways can only be seen as indicator or roadsigns pointing a researcher into a direction.

Another factor which needs to be taken into account is the level of complexity for each pathway. A grouping according to nuclear receptor regulation does not provide any information, which receptor is regulated. The same is true for a group like KEGG path: Which of the more than 500 KEGG pathways is regulated? What does it mean?

Also this analysis does not take into account that multiple assays exist for the same pathway or endpoint within the ToxCast program. In principle, a chemical acting on any given pathway should score at multiple assays associated with that endpoint. Individual hits should be disregarded in the context of a weight of evidence. Another factor not taken into account is that assay results achieved at cytotoxic concentrations have low reliability (please see discussion in M-CA 5.8.2). Using the EPA dashboard it is apparent, that positive activity calls were largely seen at cytotoxic concentration. Furthermore, different assays for the indicated outcome did not show concordance. Therefore, we consider this information to be supplementary only.

Classification of study: Supplementary information

Report: CA 5.8.3/3
Orton F. et al., 2011a
Widely used pesticides with previously unknown endocrine activity revealed as in vitro antiandrogens
2011/1291251
Guidelines: none
GLP: no

Executive Summary of the Literature
This publication investigated the androgenic and anti-androgenic activity of pesticides based on the heights of exposure and known or QSAR predicted interaction with the androgen receptor. A total of 37 pesticides were tested in the MDA-kb2 assay using MDA-kb2 human breast cancer cells transfected with a fire-fly luciferase reporter gene that is driven by an androgen-response element-containing promoter. Selected pesticides were also tested in the YAS-assay.

The QSAR analysis of pyrimethanil was 'inactive'. The MDA-kb2 assay was positive in the presence or absence of DHT co-exposure (0.25 nM), indicating a slight androgenic and antiandrogenic activity of pyrimethanil. An IC_{20} of 27.2 µM was determined regarding anti-androgenicity. An EC_{20} of 27.8 µM was determined regarding androgenicity. The EC_{20} for anti-androgenicity in the YAS assay was 9.15 µM but androgenicity was not reported. The cytotoxicity in the MDA.kb2 assay was relatively low with an EC_{20} >125 µM and was comparable with the EC_{20} in the YAS assay yielding 167 µM.

Classification of study: Supplementary information

Report: CA 5.8.3/4
Orton F. et al., 2012a
Competitive androgen receptor antagonism as a factor determining the predictability of cumulative antiandrogenic effects of widely used pesticides 2012/1368704
Guidelines: none
GLP: no

Executive Summary of the Literature
In this publication the authors investigated the antiandrogenic activity of pesticides in combination with other pesticides to gain more information regarding the combined effects and the competitive AR antagonism of pesticide mixtures. Furthermore comparison with two mixture prediction models (CA "dose addition" and IA "response addition" models) was applied. Three mixtures containing either 8 pure antagonists ("8 mix"), a combination of five AR antagonists showing agonist activity ("5 mix"), and a combination of all pesticides ("13 mix") were tested in the MDA-kb2 assay using MDA-kb2 human breast cancer cells transfected with a fire-fly luciferase reporter gene that is driven by an androgen-response element-containing promoter.

A good agreement between the effects of the mixture of eight "pure" AR antagonists and the responses predicted by CA was observed. Schild plot analysis revealed that the 8 mix acted by competitive AR antagonism. However, the observed responses of the 5 mix and the 13 mix fell within the "prediction window" boundaries defined by the predicted regression curves of CA and IA. Schild plot analysis with these mixtures yielded anomalous responses incompatible with competitive receptor antagonism.

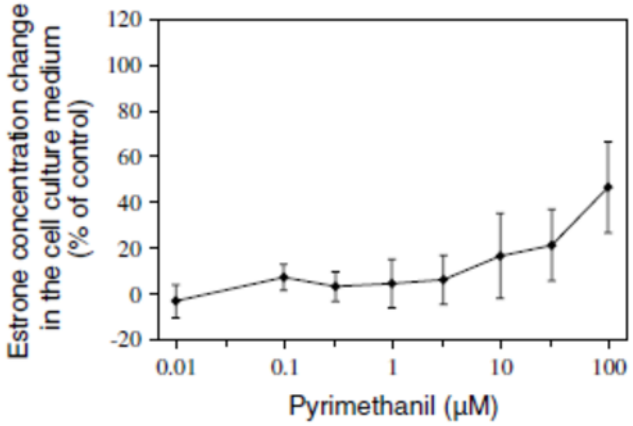
Classification of study: Supplementary information

Report: CA 5.8.3/5
Prutner W. et al., 2013a
Effects of single pesticides and binary pesticide mixtures on estrone production in H295R cells
2013/1419984
Guidelines: none
GLP: no

Executive Summary of the Literature

The aim of the present study was to determine whether the human adrenocortical carcinoma cell line H295R can be used as an in vitro test system to investigate the effects of binary pesticide combinations on estrone production as biological endpoint. Therefore, H295R cells were incubated with 0.01, 0.1, 0.3, 1, 3, 10, 30, and 100 µM pyrimethanil (DMSO as solvent; 0.1% v/v) for 24 h. 70 µL of the supernatant were used for determination of estrone concentration by means of an ELISA kit. Cytotoxicity was determined after incubation by means of the CytoTox-One™ Homogeneous Membrane Integrity Assay. Pyrimethanil increased the total amount of estrone in the supernatant by 47 ± 20% (compared to the solvent control) as determined in 3 independent experiments. The maximum induction was observed at 100 µM (highest concentration tested). Cytotoxicity was not observed up to 100 µM for pyrimethanil. Cyprodinil and pyrimethanil, which belong to the same chemical group increase estrone, showed a partial additive effect on estrone concentration, when tested as a mixture at specific concentrations.

Figure 5.8.3-2: Pyrimethanil estrone concentration change in supernatant



Comments from reviewer:
The study has some limitations questioning the effect of pyrimethanil. No statistical evaluation was performed, thus limiting the scientific value of the results. Furthermore, no clear dose-response effect can be observed. A possible effect was only seen at the highest dose tested (100 µM) but no detailed cytotoxicity data were provided.

Classification of study: Supplementary information

Report: CA 5.8.3/6
Bitsch N. et al., 2002a
In vitro screening of the estrogenic activity of active components in pesticides
2011/1281908
Guidelines: none
GLP: no

Executive Summary of the Literature
The aim of the present study was to determine the possible estrogenic activity of 57 active ingredients of pesticides and growth regulators certificated in Germany, using in vitro tests (MCF-7 E-Screen).
Therefore, MCF-7 cells were incubated in 24-well plates (10000 cells per well) with 10 nM to 100 µM pyrimethanil (DMSO as solvent) for 6 days. Furthermore, a co-incubation with the antiestrogen tamoxifen (1 µM) was performed under the same conditions, only using pyrimethanil concentrations yielding a positive result in the E-Screen. Proliferative effect was determined by means of sulforhodamine-B-assay.
Pyrimethanil resulted in an induction of proliferation, which was statistically significant ($p<0.01$). The maximum effect was observed at 100 µM pyrimethanil with a relative proliferative effect of 15% of 17β-estradiol.

Comments from reviewer:
The study has some limitations questioning the effect of pyrimethanil and the final conclusion in the publication. No raw data were available for further evaluation, only logarithmised values were available. No dose-response information was provided. A possible effect was only seen at the highest dose tested (100 µM) but no detailed cytotoxicity data were provided. Furthermore a RPE of 15% is mentioned as threshold accepted in the general literature. As pyrimethanil showed an RPE of 15% at the highest dose tested, the reviewer concludes that the results do not indicate a clear estrogenic effect of pyrimethanil.

Classification of study: Supplementary information

Report: CA 5.8.3/7
Medjakovic S. et al., 2013a
Effect of nonpersistent pesticides on estrogen receptor, androgen receptor, and aryl hydrocarbon receptor
2013/1419983
Guidelines: none
GLP: no

Executive Summary

Executive Summary of the Literature

The aim of the present study was to determine the transactivation potential of pyrimethanil and other pesticidal active ingredients when tested on human estrogen receptor a (ERα), androgen receptor (AR) and arylhydrocarbon receptor (AhR) in vitro. Moreover, pesticidal products containing the considered active ingredients were tested for transactivation potential, one of which was Scala. Relative binding affinities of the pure pesticide constituents for AR and their proliferative effect on human breast cancer and prostate cancer cell lines were evaluated.

Pyrimethanil showed a binding and transactivation potential to the AhR yielding an EC_{50} value of 4.6 µM. No activity regarding EC_{50} for AR or ERα or IC_{50} regarding AR was detectable. No ERα transactivating potential of pyrimethanil was observed when tested in the range of 10 nM to 100 µM in the yeast estrogen assay α (yESα). Furthermore, no AR transactivating potential of pyrimethanil was observed when tested in the range of 10 nM to 100 µM in the yeast androgen assay (yAS). Pyrimethanil had no effect on the cell growth of LNCaP (androgen sensitive), DU-145 (androgen insensitive), MCF-7 (ERα positive), and MDA-MB-231 cells (ERα negative). In conclusion, pyrimethanil showed a slight AhR binding potential, but no effects on AR or ERα regulated transactivation or cell growth.

Classification of study: Supplementary information

Report: CA 5.8.3/8
Shah I. et al., 2011a
Using nuclear receptor activity to stratify hepatocarcinogens
2011/1295091
Guidelines: none
GLP: no

Executive Summary of the Literature

This publication has already been presented in section MCA 5.8.2 above.

Pyrimethanil, together with boscalid and propanil, was grouped into category VI E; group E indicated, that the compound can activate the nuclear receptors AhR, CAR and PXR. The rationale for this grouping is largely based on the activation of the reporter genes. There was however no linkage to sexual hormone pathway related receptors.

Pyrimethanil was tested positive in the following activation assays: ATG_PPARg_TRANS, ATG_PXRE_CIS, ATG_PXRE_CIS, Tox21_AhR. Pyrimethanil was not associated with the activation in other assays targeting estrogen receptor and/or androgen receptor.

Classification of study: Supplementary information

Report: CA 5.8.3/9
Rotroff D.M. et al., 2010a
Xenobiotic-metabolizing enzyme and transporter gene expression in primary cultures of human hepatocytes modulated by toxcast chemicals
2010/1233112
Guidelines: none
GLP: no

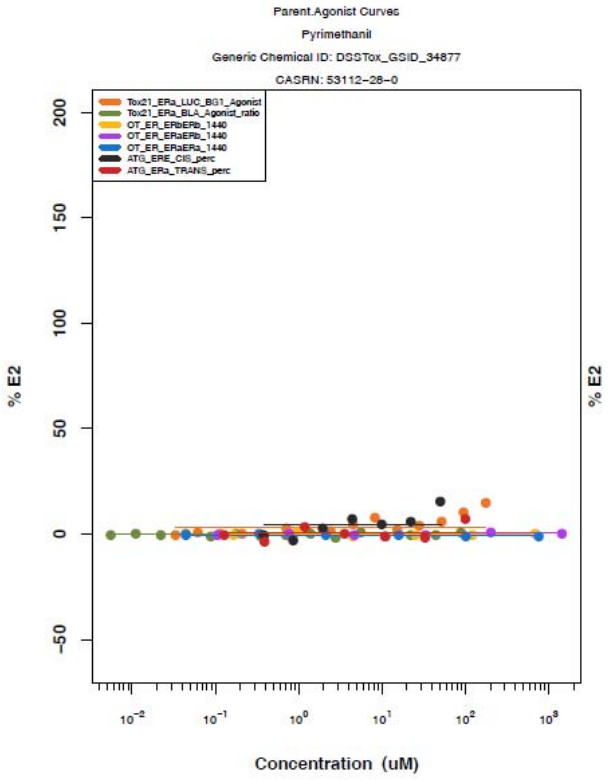
This publication has already been presented in section MCA 5.8.2 above.

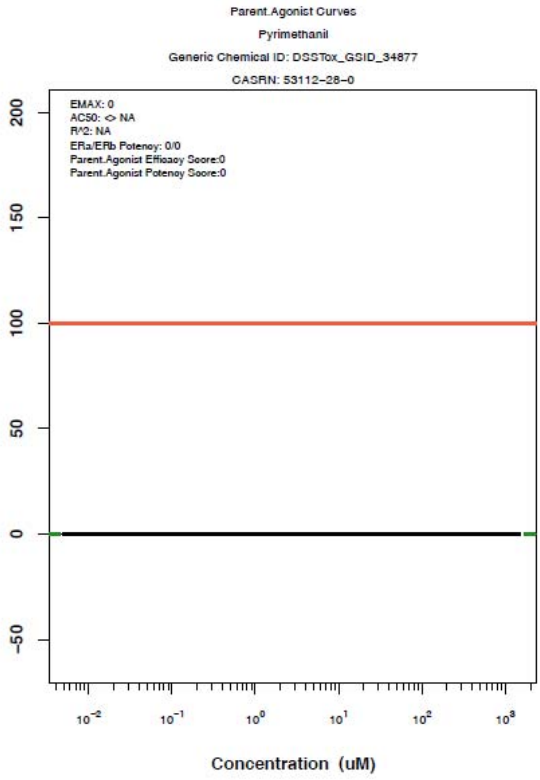
Pyrimethanil induced gene expression of following genes: CYP1A1, CYP1A2, CYP2B6, CYP3A4, and UGT1A1. No effect was observed on CYP2C9 and SULT2A1.
Expression of genes that were induced by pyrimethanil were linked to AhR, CAR, and PXR. Expression of UGT and CYP2C are also linked to AhR or PXR, respectively, but were not affected by pyrimethanil incubation. There was no link to ER or AR mediated pathway.

Classification of the study: Supplementary information

Report: CA 5.8.3/10
Rotroff D.M. et al., 2014a
Predictive endocrine testing in the 21st century using in vitro assays of estrogen receptor signaling responses
2014/1323273
Guidelines: none
GLP: no

Executive Summary: Thousands of environmental chemicals are subject to regulatory review for their potential to be endocrine disruptors (ED). In vitro high-throughput screening (HTS) assays have emerged as a potential tool for prioritizing chemicals for ED-related whole-animal tests. In this study, 1814 chemicals including pesticide active and inert ingredients, industrial chemicals, food additives, and pharmaceuticals were evaluated in a panel of 13 in vitro HTS assays. The panel of in vitro assays interrogated multiple end points related to estrogen receptor (ER) signalling, namely binding, agonist, antagonist, and cell growth responses. Competitive binding assays on human (extracts of MCF-7 human breast cancer cell; NVS_hER), bovine (extracts of bovine uterus membrane; NVS_bER) and murine (recombinant mouse ERα of ligand binding domain; NVS_mERa) estrogen receptor were conducted. Protein-fragment complementation assays on estrogen receptor alpha (ERα) and beta (ERβ) homo- and heterodimerization (ERα-ERα; OT_ERaERa_1440, ERα-ERβ; OT_ERaERb_1440, ERβ-ERβ; OT_ERbERb_1440) were employed. Included were a cis- and a trans-activation reporter gene assay (ATG_ERE_CIS and ATG_ERa_TRANS). Moreover two further reporter gene assays were run in agonist (Tox21_ERa_BLA_agonist, Tox21_ERa_LUC_BG1_agonist) and antagonist (Tox21_ERa_BLA_antagonist, Tox21_ERa_LUC_BG1_antagonist) mode. Cell proliferation was investigated in the T47D cell growth assay (ACEA_T47D_80hr_Positive). The results from the in vitro assay battery were used to create an ER Interaction Score. For the ∼1,800 chemicals evaluated in this study, 82% did not display indications of interacting with the ER signalling pathway and would be low priorities for additional ER testing. If maximum sensitivity is desired, the model can be run with narrower confidence intervals around the composite curves. This would result in an increased false positive rate and a decreased false negative rate.
For this dossier the data for pyrimethanil is relevant and thus the results are described in the following figures. In summary, it is seen that pyrimethanil is for estrogen receptor (ER) signalling endpoints, namely binding, agonist, antagonist and cell growth responses negative. For Pyrimethanil the ER Interaction Score was found to be 0. Taking together, Pyrimethanil is one of the 82% chemicals which did not display indications of interacting with the ER signalling pathway.


Parent.Agonist Curves
Pyrimethanil
Generic Chemical ID: DSSTox_GSID_34877
CASRN: 53112-28-0
Tox21_ERa_LUC_BG1_Agonist
Tox21_ERa_BLA_Agonist_ratio
OT_ER_ERbERb_1440
OT_ER_ERaERb_1440
OT_ER_ERaERa_1440
ATG_ERE_CIS_perc
ATG_ERa_TRANS_perc
% E2
Concentration (uM)


Parent.Agonist Curves
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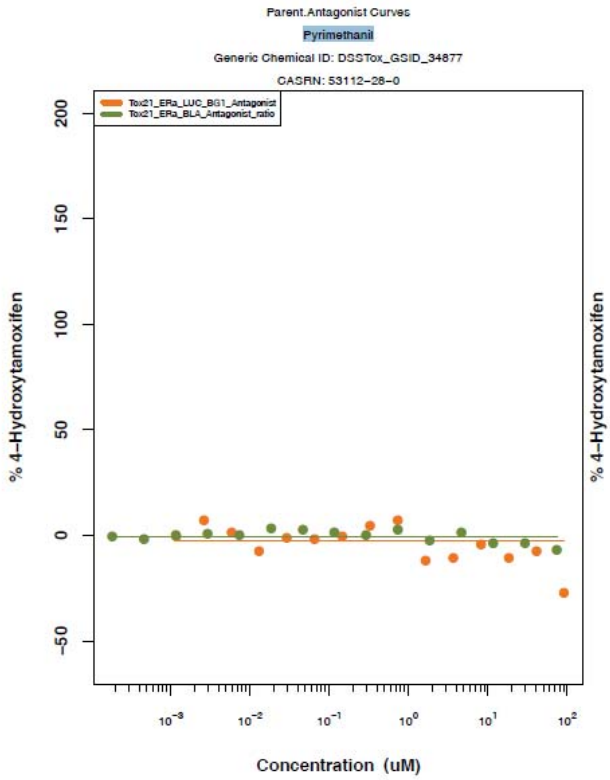
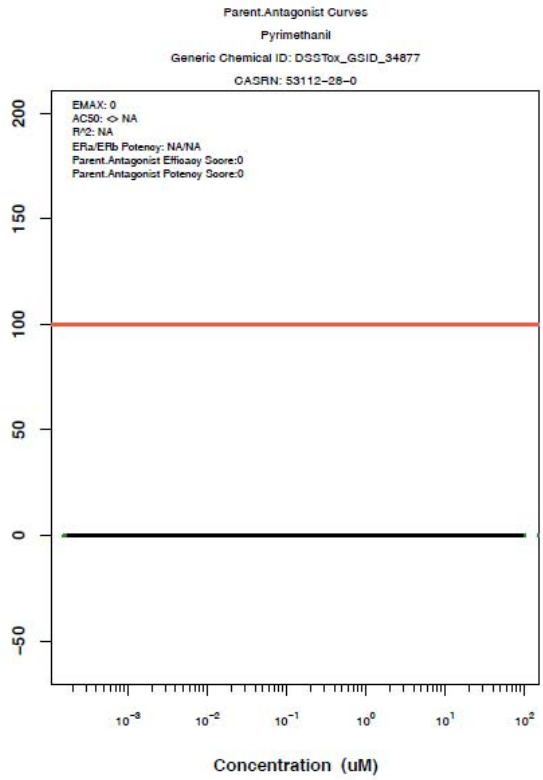
Generic Chemical ID: DSSTox_GSID_34877
CASRN: 53112-28-0
EMAX: 0
AC50: <> NA
R^2: NA
ERa/ERb Potency: 0/0
Parent.Agonist Efficacy Score:0
Parent.Agonist Potency Score:0
% E2
Concentration (uM)


Parent.Antagonist Curves
Pyrimethanil
Generic Chemical ID: DSSTox_GSID_34877
CASRN: 53112-28-0
Tox21_ERa_LUC_BG1_Antagonist
Tox21_ERa_BLA_Antagonist_ratio
% 4-Hydroxytamoxifen
Concentration (uM)


Parent.Antagonist Curves
Pyrimethanil
Generic Chemical ID: DSSTox_GSID_34877
CASRN: 53112-28-0
EMAX: 0
AC50: <> NA
R^2: NA
ERa/ERb Potency: NA/NA
Parent.Antagonist Efficacy Score:0
Parent.Antagonist Potency Score:0
% 4-Hydroxytamoxifen
Concentration (uM)

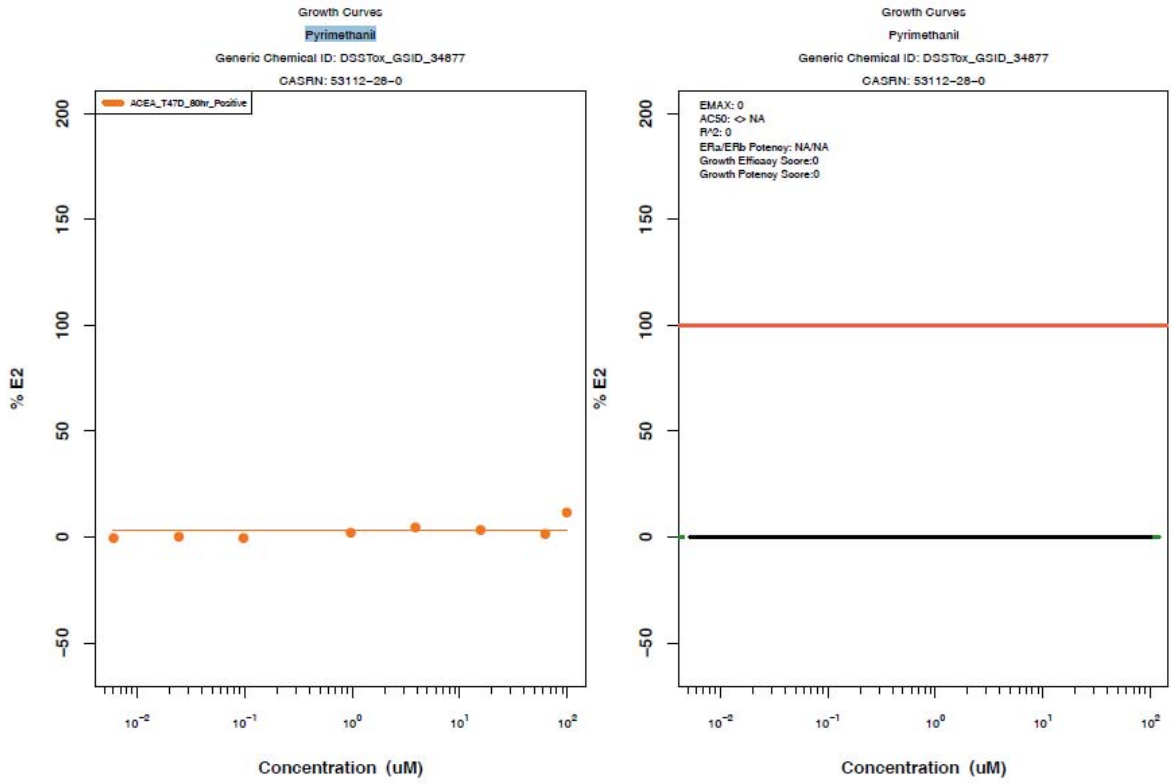


Figure 5.8.3-3: Pyrimethanil estrogen receptor signaling endpoints

Conclusion of the author: An ER Interaction Score was developed by aggregating data from 13 different in vitro ER assays based on the known cellular ER signalling pathways. This model produced scores for an overall likelihood of a chemical being estrogenic, and these scores were highly correlated with in vivo data and ER reference chemical classifications, indicating that the model is capable of predicting estrogenic likelihood with a high degree of accuracy.

Conclusion of the applicant: This study analyses around 1800 substances concerning their potential to be an endocrine disruptor. Pyrimethanil was one of them and found to be zero for the binding group, agonist group, antagonist group, growth group and the ER Interaction Score and would be therefore of low priority for additional ER testing. This study is considered to be relevant for human risk assessment.

Classification of the study: Supplementary information

Report:	CA 5.8.3/11 Judson R.S. et al., 2015 a Integrated model of chemical perturbations of a biological pathway using 18 in vitro high-throughput screening assays for the estrogen receptor 2015/1279970
Guidelines:	none
GLP:	no
Previous evaluation:	New information

Executive Summary of the Literature

This publication illustrates a computational network model that integrates 18 in vitro, high-throughput screening assays measuring estrogen receptor (ER) binding, dimerization, chromatin binding, transcriptional avtivation, and ER-dependent cell proliferation. The data used were generated by the EPA ToxCast program. The network model uses activity patterns across the in vitro assays to predict whether a chemical is an ER agonist or antagonist, or is otherwise influencing the assays through a manner dependent on the physics and chemistry of the technology platform ("assay interference"). The method is applied to a library of 1812 commercial and environmental chemicals, including 45 ER positive and negative reference chemicals. The results for Pyrimethanil are presented in the following figure:

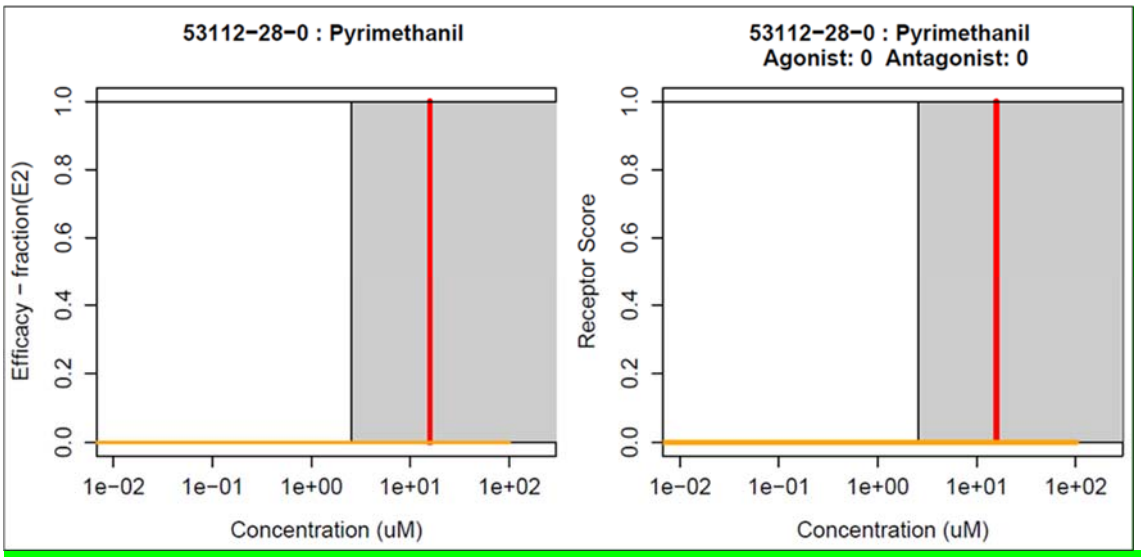


Figure 5.8.3-4: Summarized information retrieved for Pyrimethanil in the HTS-assays as evaluated using the modelling approach from Judson et al.

The left-hand panel shows in principle the synthetic concentration-response data for the available assays (orange line: Tox21 Era beta-lactamase antagonist reporter gene). The right-hand panel shows the corresponding magnitude of the modelled receptor responses.

For chemicals with cell-stress/cytotoxicity activity, the cell-stress/cytotoxicity center is indicated by a vertical red line, and the cell-stress/cytotoxicity region (starting 3 cell-stress/cytotoxicity MAD below the cell-stress/cytotoxicity center) is indicated by the gray shaded region. In case of Pyrimethanil, no activity was observed at all.

Among the reference chemicals, the network model correctly identified the agonists and antagonists with the exception of very weak compounds whose activity was outside the concentration range tested. The model agonist score also correlated with the expected potency class of the active reference chemicals. Of the 1812 chemicals evaluated, 111 (6.1%) were predicted to be strongly ER active in agonist or antagonist mode. This dataset and model were also used to begin a systematic investigation of assay interference. The most prominent cause of false-positive activity (activity in an assay that is likely not due to interaction of the chemical with ER) is cytotoxicity. Cytotoxicity was measured using a collection of 35 assays in the ToxCast battery that detect cytotoxicity or other forms of cell loss across several cell lines and primary cell types. The single experiments performed with Pyrimethanil indicated no activity. Based on the single results, no in vivo activity of Pyrimethanil is expected.

Classification of study: Supplementary information

Report:	CA 5.8.3/12 Judson R., 2016 a Analysis of the effects of cell stress and cytotoxicity on in vitro asssay activity across a diverse chemical and assay space 2016/1227708
Guidelines:	none
GLP:	no
Previous evaluation:	New information

Executive Summary of the Literature

In this publication data that were generated by the EPA ToxCast program and which were discussed in the publication from Judson et al. (2015) were assessed in detail against the background of cytotoxicity. Chemical toxicity can arise from disruption of specific biomolecular functions or through more generalized cell stress and cytotoxicity-mediated processes. In this publication responses of 1060 chemicals including pharmaceuticals, natural products, pesticides, consumer, and industrial chemicals across a battery of 815 in vitro assay endpoints from 7 high-throughput assay technology platforms were analyzed in order to distinguish between these types of activities. Both cell-based and cell-free assays showed a rapid increase in the frequency of responses at concentrations where cell stress/cytotoxicity responses were observed in cell-based assays. Chemicals that were positive on at least 2 viability/cytotoxicity assays within the concentration range tested (typically up to 100 µM) activated a median of 12% of assay endpoints whereas those that were not cytotoxic in this concentration range activated 1.3% of the assays endpoints. The results suggest that activity can be broadly divided into: (1) specific biomolecular interactions against one or more targets (e.g. receptors or enzymes) at concentrations below which overt cytotoxicity-associated activity is observed; and (2) activity associated with cell stress or cytotoxicity, which may result from triggering specific cell stress pathways, chemical reactivity, physico-chemical disruption of proteins or membranes, or broad low-affinity non-covalent interactions. The analyses presented here provide context for use of these data in ongoing studies to predict in vivo toxicity from chemicals lacking extensive hazard assessment.

The summarized cytotoxicity data for Pyrimethanil are provided in the following figure:

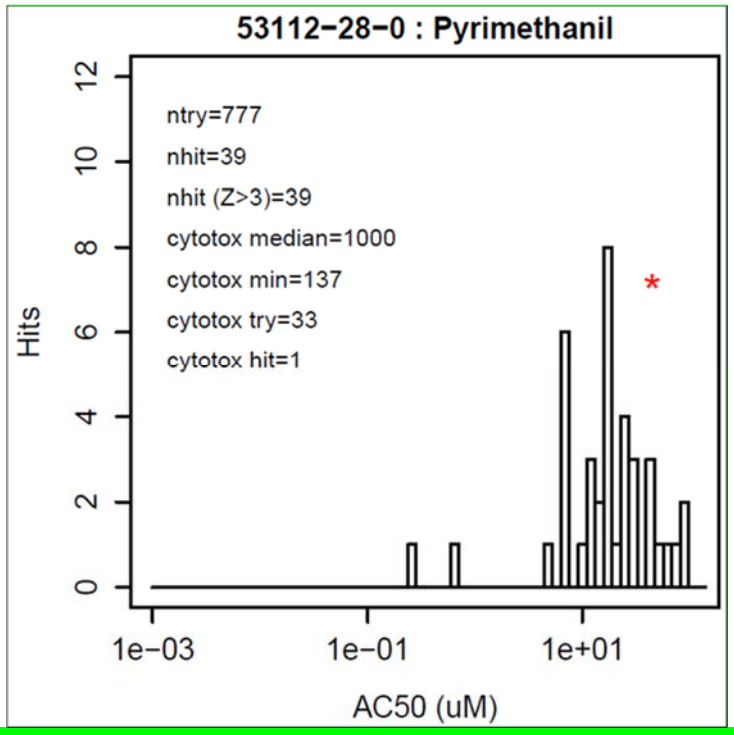


Figure 5.8.3-5: Summarized information retrieved for Pyrimethanil showing assay activities almost outside the region of cytotoxicity.
The histogram shows the distribution of AC50 values across the full assay set for each chemical. The red star indicates the AC50 value for the cytotoxicity assays activated by these chemicals (8 or more cytotoxity-related assays were active in each case). In the legend, ntry is the number of assay endpoints tested, excluding cytotoxicity endpoints, nhit is the number active, and nhit (Z>3) is the number active with Z>3. The cytotoxicity median and min are in units of µM.

The cytotoxicity was assessed according to the following scheme. For chemicals with 2 or more hits in cytotoxicity assays, the mean median $logAC_{50}$(cytotox) and the MAD of the $logAC_{50}$(cytotox) was calculated for the hits. Next, the median of the MAD of the $logAC_{50}$(cytotox) distributions across all chemicals was calculated to define the global cytotoxicity MAD. A new value (the Z-score) was then assigned to each chemical assay hit combination:

$$Z\ (chemical, assay) = \frac{-logAC50(chemical,assay) - median[-LogAC50(chemical,cytotox)]}{global\ cytotoxicity\ MAD}$$

The single experiments performed with Pyrimethanil indicated that most of the hits were observed outside the cytotoxic region.

Classification of study: Supplementary information

CA 5.9 Medical Data

Information evaluated in the draft monograph of Rapporteur Member State Austria of April 15, 2004:
No documentation was submitted other than a statement by the notifier concerning the possible occupational exposure of manufacturing plant personnel. It is stated that "*all personnel concerned are examined routinely... and the examinations and questionnaires yielded no indications of product-specific health disturbances...*". In addition, it was stated that "*no clinical cases or incidences of poisoning in humans have been reported from possible exposure to pyrimethanil"* and "*no adverse, work-related health effects have been recorded to date"*.

Thus, the following conclusion was given in the list of endpoints for Annex I listing of Pyrimethanil:

Medical data (Annex IIA, point 5.9)

	Available data indicate no detrimental effects on health of plant personnel in manufacturing of pyrimethanil; no clinical cases or poisoning incidents have been reported

Information on medical data obtained since then has been collected and evaluated and a literature search has been conducted to extend the evaluation basis. Thus, the conclusion for relevant endpoints for the current re-registration was adopted as follows:

A search in the literature databases listed below - restricted to "pps=human" and "ct d human" - has been performed on Augusr 3rd, 2015 via DIMDI-host for the following terms:
Pyrimethanil*
CAS 53112-28-0

Medline 66 (NLM)
Medline alert (NLM)
Embase 74 (Elsevier)
Embase alert (Elsevier)
Biosys (Thomson Reuters)
IPA International Pharmaceutical Abstracts (Thomson Reuters)

Cross check via Internet available databases:
CHEMID (http://chem2.sis.nlm.nih.gov/chemidplus/chemidlite.jsp)
PubMed (http://www.ncbi.nlm.nih.gov/pubmed)
Toxnet (http://toxnet.nlm.nih.gov/)

and FAUST (GUA internal literature database)

Thus, the conclusion for relevant endpoints for the current re-registration was adopted as follows:

Medical data (Regulation (EU) N° 283/2013, Annex Part A, point 5.9)

No adverse health effects reported during research, production and use of pyrimethanil and its formulations.

CA 5.9.1 Medical surveillance on manufacturing plant personnel and monitoring studies

All persons handling crop protection products are surveyed by regular medical examinations. There are no specific parameters available for effect monitoring of Pyrimethanil. Thus, the medical monitoring program is designed as a general health check-up, with special interest in the primary target organs presumed to be relevant by analogy from animal experiments.
The surveillance program includes a general physical examination including neurological status, red and white blood cell counts, liver enzymes. Adverse health effects suspected to be related to Pyrimethanil exposure have not been observed.

CA 5.9.2 Data collected on humans

Medical data on Pyrimethanil is limited, but no reports of adverse effects were identified during routine monitoring of production plant workers and among personnel involved in the experimental biological testing or field trials. There is no evidence or data available to support any findings in relation to poisoning with Pyrimethanil.
No associations of adverse health effects due to Pyrimethanil have been documented in the BASF-internal medical files.

No data on exposure have been registered in the BASF-internal clinical incident log in persons exposed to Pyrimethanil.

CA 5.9.3 Direct observations

Few cases of exposure to Pyrimethanil are reported to BASF. None did result in any health disorder.

CA 5.9.4 Epidemiological studies

No epidemiological studies with Pyrimethanil have been conducted.

Neither data on exposure of the general public nor epidemiologic studies are available for BASF SE, nor is BASF SE aware on any epidemiologic studies performed by third parties.

No monitoring of the general population nor epidemiological studies are available for Pyrimethanil. As such, no observations regarding health effects after exposure of the general public are known to us.

CA 5.9.5 Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical tests

Pyrimethanil can be detected in urine [Faniband et al. (2015); see KCA 4.2/17 2015/1186269], in blood [Teng et al. (2015) see KCA 4.2/15 2015/1181153 and Dulaurent et al. (2010); [see KCA 4.2/14 2010/1233172] and in hair samples [Schummer et al. (2010); [see KCA 4.2/16 2010/1233254].
The analytical methods are summarized in section MCA 4.2 of this dossier.

No specific symptoms of poisoning are seen.

CA 5.9.6 Proposed treatment: first aid measures, antidotes, medical treatment

See safety data sheet / precautions; symptomatic and supportive treatment, no specific antidote known.

CA 5.9.7 Expected effects of poisoning

Expected effects were derived from acute and subacute studies in animals. No effects were reported for humans.

Pyrimethanil

DOCUMENT M-CA, Section 6

RESIDUES IN OR ON TREATED PRODUCTS, FOOD AND FEED AND PLANT METABOLISM

Compiled by:

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Fax.No.:
E-mail:

Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
Oct 2015	Initial AIR Dossier	BASF DocID 2015/1004012
April, 2016	Inclusion of final rotational crop study report in CA 6.6.2/1	BASF DocID 2016/1118408
Sept 2016	Second interim report of Freezing storage stability of M605F005, M605F007 and Phenylguanidine (CA 6.1/2)	BASF DocID 2016/1287831
Sept 2017	CA 6.1/2 Final Study report was added BASF Doc ID 2017/1136008. CA 6.3 - Representative Uses, Typo in the BBCH was corrected to 53-77 for the proposed uses in pome fruit and EPPO Codes for crops were added. CA 6.4 and 6.7.2 Animal model 2016 added CA 6.6.1 Pyrimidyl changed to pyrimidinyl. CA 6.6.2/2 Rotational crop trial in wheat lost due to crop failure in the Netherlands – new trial was added BASF DocID 2016/1067813. CA 6.9 proposed MRL values more specified CA 6.9 Table 6.9-7 Typo for ADI group 2 and 3 corrected.	BASF DocID 2017/1134390

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CA 6 RESIDUES IN OR ON TREATED PRODUCTS, FOOD AND FEED AND PLANT METABOLISM

Pyrimethanil (BAS 605 F), a fungicide for use in various fruits and vegetables as well as several other crops, is registered in Europe since many years. It was fully reviewed under Directive 91/414/EEC and included in Annex I by Commission Directive No 2006/74/EC. Inclusion entered into force on 01 June 2007. The approval was transferred to the new Regulation (EC) No 1107/2009 in Commission Implementing Regulation (EU) No 540/2011. Approval extension was granted until 30 April 2018 by Regulation (EU) No 678/2014.

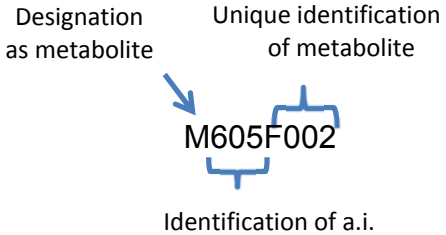
All relevant information on the first Annex I review and the endpoints used in consumer dietary assessments can be found in the monograph of pyrimethanil and in the SANCO/10019/2006 final (EU Review Report).

For the current renewal of approval under Regulation 1107/2009, a data gap analysis according to new guidelines, new guidance documents and new policies in exposure assessments was performed. New studies / evaluations were initiated where considered necessary. Furthermore, a literature search was performed and scientific publications were evaluated for their endpoint relevance and quality.

Although titles and abstracts of several publications indicated a potential connection to respective consumer safety chapters of this dossier, the detailed evaluation of the majority of these publications showed no endpoint of sufficient reliability which could be used for the required risk assessments. For consumer safety only public literature data on processing of pyrimethanil was regarded as relevant and is summarized in chapter MCA 6.5.3. Further information on the literature assessment and respective justifications can be found in MCA 9.

An overview of metabolites identified during consumer safety studies is given below. The information in the table allows a comparison between the pathways in different test systems.

The metabolite overview in the table below is including the different code numbers that are available for each metabolite. Due to historic reasons (e.g. several changes of ownership of pyrimethanil and different companies have different coding systems, different sources of metabolites: soil, water, plant, live-stock and thus different metabolite codes in different dossier sections), it is unfortunately not possible to use always only one and the same metabolite code for a certain metabolite. Therefore BASF has introduced a harmonized coding system to be used.



In the following chapters and study summaries synonym metabolite codes are given in addition where deemed to be helpful.

The following studies in this section are covered by LoA [see KCA 6.5.3/11 2007/1028269] :

[see KCA 6.5.3/1 B002853],
[see KCA 6.5.3/3 A81725],
[see KCA 6.5.3/5 A89698].

Table 6-1: Notations of parent and metabolites of pyrimethanil

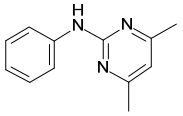
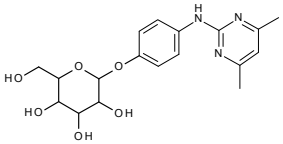
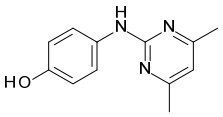
Code number	Reg. No	Synonyms	Description (IUPAC name / CAS-No)	Compound found in	Structure
Pyrimethanil **BAS 605 F** **M605F000**	236999	AE B100309 SN 100309 AN1	N-(4,6-dimethylpyrimidine-2-yl)aniline / 53112-28-0	Soil, Water, Sediment Plants: Leafy crops (Lettuce) Fruit (Apple (peel, pulp, leaves)) Fruit (Vine (grapes, leaves)) Fruit (Tomato (fruit, leaves)) Root crops (Carrot (foliage, roots) (foliar or soil treatment)) *Leafy crops (Lettuce)* Rotational crops: Lettuce, radish (tops, roots), wheat (forage, grain, straw) *Wheat (forage, hay, straw, grain), spinach (immature, mature), radish (tuber, foliage)* Animals: Rat (feces) Rat (plasma) *Rat (feces, liver, kidney, fat, plasma)*	
M605F001	-	β-O glucoside of SN 614 276 / M605F002 Unk B T1-T3 M1	β-O-glucoside of 2-(4-hydroxyanilino)-4,6-dimethylpyrimidine	Plants: Fruit ((probably) Tomato (fruit, leaves) (might be other C-6 / disaccharide conjugate in addition to glc), Vine (grapes, leaves)) Root crops (Carrot foliage (foliar or soil treatment), roots (soil treatment))	
M605F002	4739173	SN 614 276 AE C614276 AN2	2-(4-hydroxyanilino)-4,6-dimethylpyrimidine / 81261-84-9	Plants: Leafy crops (lettuce (released after hydrolysis)) Fruit & root crops (intermediate in vine and apple leaves, carrot foliage) Rotational crops: Lettuce, radish (tops, roots), wheat (forage, grain, straw) Animals (main metabolite): Cow (kidney, urine) *Hen (egg, liver, muscle, fat), goat (liver, kidney)* Rat (urine, feces; major met.) Rat (plasma) *Rat (urine, feces)*	

Table 6-1: Notations of parent and metabolites of pyrimethanil

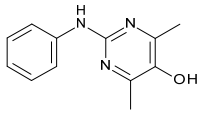
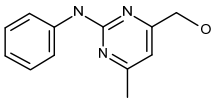
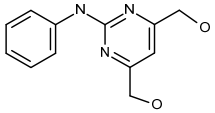
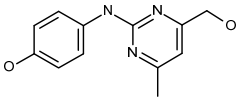
Code number	Reg. No	Synonyms	Description (IUPAC name / CAS-No)	Compound found in	Structure
M605F003	5079484	SN 614 277 AE C614277 AN3	2-anilino-4,6-dimethylpyrimidine-5-ol	Plants: Leafy crops (Lettuce (released after hydrolysis)) Rotational crops: Lettuce, radish (tops), wheat (forage, grain, straw) Animals: Cow (milk, kidney, urine, rat excreta (liver feeding)) Rat (urine, feces) Rat (plasma) *Rat (feces)*	
M605F004	5079485	SN 614278 SX 614278 AE 614278 AN4	2-anilino-6-methylpyrimidine-4-methanol / 116389-9	Plants: Fruit & root crops (Intermediate in vine and apple leaves, carrot foliage) *Leafy crops & cereal/grass crops (lettuce, wheat (straw, forage; ST))* Rotational crops: Lettuce, radish (tops, roots), wheat (forage, grain, straw) *Wheat (forage, hay, straw), spinach (mature), radish (tuber)* Animals: Rat excreta (liver feeding) Rat (urine, feces) Rat (plasma) *Goat (liver)* *Rat (feces, liver, kidney, plasma)*	
M605F005	5079487	C 621 312 AE C621312 AN5	2-anilino-4,6-dihydroxymethyl-pyrimidine	Soil Plants: Fruit (Intermediate in vine and apple leaves) Rotational crops: Lettuce, radish (tops, roots), wheat (forage, grain, straw)	
M605F006	5079486	SN 614800 AE C614 800 AN6	2-(4-hydroxyanilino)-4-hydroxymethyl-6-methylpyrimidine	Rotational crops: Radish (tops, roots), wheat (forage, grain, straw) Animals: Cow (kidney, urine, rat excreta (liver feeding)) *Hen (liver, egg), goat (liver, kidney)* Rat (urine, feces)	

Table 6-1: Notations of parent and metabolites of pyrimethanil

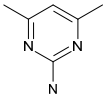
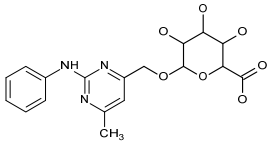
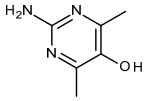
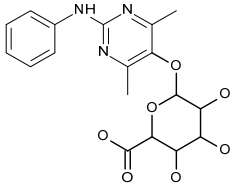
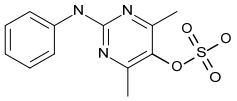
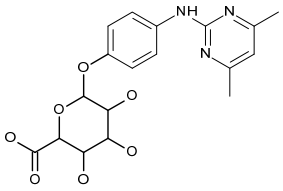
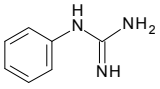
Code number	Reg. No	Synonyms	Description (IUPAC name / CAS-No)	Compound found in	Structure
M605F007	40603	AE F132593 SN 512 723 ZK 512723 AE C512723 NC 12723 S151 CL 2869 AN7	2-amino-4,6-dimethylpyrimidine / 767-15-7	Soil, Water/Sediment Rotational crops: Lettuce, radish (tops), wheat (forage, grain, straw) *Wheat (forage, hay, straw, grain), spinach (immature, mature), radish (foliage)* Animals: *Rat (urine, feces, bile)*	
M605F008	4245969	AE 0025462 AN8	4,6-dimethylpyrimidine / 1558-17-4	Soil Rotational crops: Lettuce, radish (tops), wheat (forage, grain, straw)	
M605F014		Glucuronide conjugate of 5079485 / M605F004		Animal: *Goat (liver, kidney)*	
M605F016			2-Amino-4,6-dimethylpyrimidin-5-ol / 685897-68-1	Rotational crops: *Wheat (forage, hay, straw, grain), spinach (immature, mature), radish (foliage, tubers)*	
M605F020		Glucuronide conjugate of 5079484 / M605F003		Animal: *Goat (kidney)* *Rat (urine, bile)*	
M605F021		Sulfate conjugate of 5079484 / M605F003		Animal: *Goat (liver, kidney, milk)* *Rat (urine, bile, liver, kidney, plasma)*	
M605F023		Glucuronide of 4739173 / M605F002		Animals: *Hen (egg, liver, muscle, fat), goat (liver, kidney, milk)* *Rat (urine, bile, plasma)*	
M605F025	418209	Phenylguanidine CGA 263208 Metab I11 N-(4-hydroxyphenyl-4-cyclopropyl-6-methyl-2-pyrimidamine N-phenyl-guanidin	phenyl-1-guanidine / 2002-16-6	Plants: *Leafy crops (lettuce)* *Cereal/grass crops (wheat (hay, straw, forage; ST))* Rotational crops: *Wheat (forage, hay, straw, grain), spinach (immature, mature), radish (foliage, tuber)* Animals: *Goat (liver, kidney, milk)* *Hen (liver)* *Rat (urine, feces)*	

Table 6-1: Notations of parent and metabolites of pyrimethanil

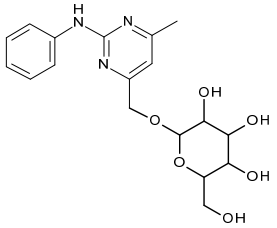
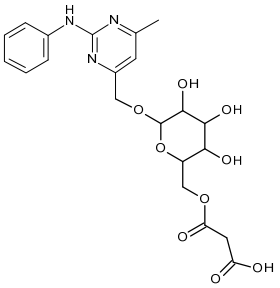
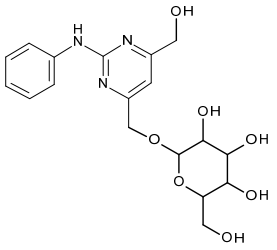
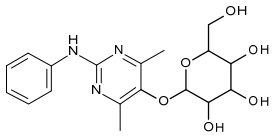
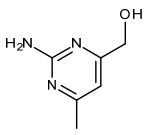
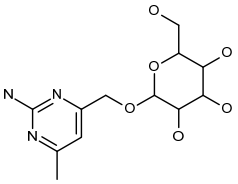
Code number	Reg. No	Synonyms	Description (IUPAC name / CAS-No)	Compound found in	Structure
M605F027		β-O-glucoside of M605F004 U2 M5 Unk F T1-T3	β-O-glucoside of 2-anilino-4-hydroxymethyl-6-methylpyrimidine	Plants: Fruit (Apple (peel, pulp, leaves)) Fruit (Vine (leaves)) Fruit ((probably) Tomato (fruit, leaves)) Root crops (Carrot (foliage, roots) (foliar or soil treatment)) *Leafy crops & cereal/grass crops (Lettuce, wheat (hay, straw, forage; ST))* Rotational crops: *Wheat (forage, hay, straw), radish (foliage, tuber)*	
M605F028		Malonyl-β-O glucoside of SN 614 278 / M605F004 Unk A T1-T3	Malonyl-β-O glucoside of 2-anilino-4-hydroxymethyl-6-methylpyrimidine	Plants: Root crops (Carrot (foliage, roots) (foliar or soil treatment)) *Leafy crops & cereal/grass crops (Lettuce, wheat (hay, straw, forage; ST))* Rotational crops: *Wheat (forage, hay, straw), spinach (mature), radish (tuber, foliage)*	
M605F029		β-O-glucoside of C621 312/ M605F005 U1	β-O-glucoside of 2-anilino-4,6-dihydroxymethyl-pyrimidine	Plants: Fruit (Apple (peel, pulp, leaves))	
M605F030		Glucose conjugate of 5079484 / M605F003		Plants: *Leafy crops (Lettuce)* Rotational crops: *Wheat (forage, hay, straw), radish (foliage)*	
M605F032		Me-Hydroxy of AN7 4-Pyrimidine-methanol, 2-amino-6-methyl-	110429-43-1	Plants: *Cereal/grass crops (wheat (hay, straw, forage; ST))* Rotational crops: *Wheat (forage, hay, straw), spinach (immature, mature), radish (foliage, tubers)*	
M605F033		Glucose conjugate of 40603 / M605F032		Plants: *Leafy crops & cereal/grass crops (Lettuce, wheat (hay, straw, forage; ST))* Rotational crops: *Wheat (forage, hay, straw, grain), spinach (immature, mature), radish (foliage, tubers)*	

Table 6-1: Notations of parent and metabolites of pyrimethanil

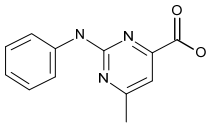
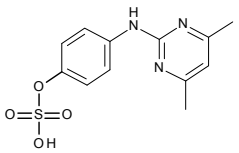
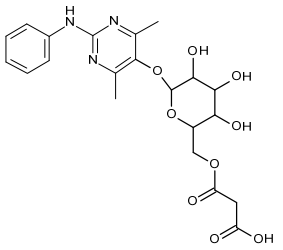
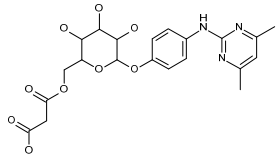
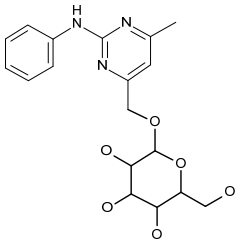
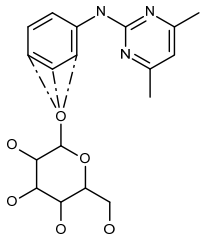
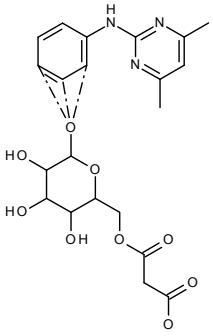
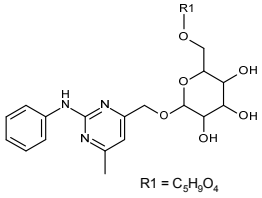
Code number	Reg. No	Synonyms	Description (IUPAC name / CAS-No)	Compound found in	Structure
M605F034		Pyrimethanil Carboxylic Acid	4-Pyrimidinecarboxylic acid, 6-methyl-2-(phenylamino)- / 1354513-00-0	Animals: *Goat (liver, kidney)*	
M605F035		Sulfate of SN 614 276 / M605F002		Animals: *Goat (liver, milk)* *Hen (egg, liver, muscle, fat)* Rat (urine, feces; major met.)	
M605F036		Malonyl glucose conjugate of 5079484 / M605F003		Plants: *Leafy crops & cereal/grass crops (Lettuce, wheat (forage, hay; ST))* Rotational crops: *Wheat (forage, hay, straw), spinach (mature, immature), radish (foliage)*	
M605F037		Malonyl-β-O glucoside of SN 614 276 / M605F002 Unk C T1-T3	Malonyl-β-O-glucoside of 2-(4-hydroxyanilino)-4,6-dimethylpyrimidine	Plants: Root crops (Carrot foliage (foliar or soil treatment), roots (soil treatment))	
M605F038		C-6 sugar conjugate of M605F004 U3 M4 T1-T3	C-6 sugar conjugate of 2-anilino-6-methylpyrimidine-4-methanol	Plants: Fruit (Apple (peel, pulp, leaves) (**not** cleaved by β-glucosidase)) Fruit (Vine (grapes, leaves) (**not** cleaved by β-glucosidase)) Fruit ((probably) Tomato (fruit, leaves))	
M605F039			"glucose conjugate …"	Plants: *Leafy crops (Lettuce)* Rotational crops: *Spinach (mature, immature)*	
M605F040			"Malonyl glucose conj.	Plants: *Leafy crops (Lettuce)* Rotational crops: *Spinach (mature, immature)*	

Table 6-1: Notations of parent and metabolites of pyrimethanil

Code number	Reg. No	Synonyms	Description (IUPAC name / CAS-No)	Compound found in	Structure
M605F041			$C_5H_9O_4$ glucose conjugate of Reg. No. 5079485 resp M605F004 resp. 2-anilino-6-methylpyrimidine-4-methanol $C_{23}H_{31}N_3O_{10}$ M = 509 u	Plants: *Leafy crops (Lettuce)*	

Text in *italics* indicates new studies
ST Seed treatment

CA 6.1 Storage stability of residues

The stability of residues was reviewed during the previous Annex I inclusion process (Annex II, section 4, point 6.1) and no further data was requested.

The following information is copied from the EFSA Conclusion (EFSA Scientific Report (2006) 61, 1-70, Conclusion on the peer review of pyrimethanil):

Stability of residues (Annex IIA, point 6 introduction, Annex IIIA, point 8 introduction)

Stable for 9-12 months (carrots, lettuce, apples, tomatoes, grapes, wine, dried peas)

EFSA Reasoned Opinion on MRLs (European Food Safety Authority; Review of the existing maximum residue levels (MRLs) for pyrimethanil according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2011;9(11):2454. [65 pp.] doi:10.2903/j.efsa.2011.2454. Available online: www.efsa.europa.eu/efsajournal) referred to the EFSA Conclusion: "Storage stability of pyrimethanil was demonstrated for a period of 12 months at -18°C in commodities with high water (lettuce, tomatoes, carrots, apples) and high acid (grapes) content as well as dry commodities (dried peas)".

For pyrimethanil in **plant** matrices, a new storage stability study has been conducted in order to cover a storage period of about 24 months for matrices of all five categories (high water, oil, protein, starch and acid content) defined in the OECD Guideline 506. In addition, a storage stability study on metabolites M605F005, M605F007 and M605F025 (phenylguanidine) to cover a storage period of about 24 months is ~~in progress; interim results for up to 150 days of storage are~~ presented in this document.

In case of **animal** matrices, no storage stability study was performed. This was considered acceptable by EFSA (EFSA Reasoned Opinion on MRLs) on the basis that samples were stored less than 1 month under freezer conditions before analysis.

Report: CA 6.1/1
Oppinger M., 2015a
Investigation of the storage stability of BAS 605 F (RegNo. 236999) in plant matrices
2014/1000681
Guidelines: EEC 7032/VI/95 rev. 5, OECD 506 (Oct. 2007), EPA 860.1380
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: Pyrimethanil (BAS 605 F)
Lot/Batch #: 486213
Purity: 99.8%
CAS#: 53112-28-0
Spiking levels: 0.1 mg/kg

2. Test Commodity:

Crop: Apple (fruit), oilseed rape (seed), dry pea (seed), wheat (grain) and grape (fruit)
Sample size: 5 g (stored sample)

B. STUDY DESIGN

1. Test procedure

Plant matrices (apple fruit, oilseed rape seed, dried pea seed, wheat grain and grape fruit) were fortified with pyrimethanil at a concentration level of 0.1 mg/kg (10 x LOQ). The spiked samples were stored under the usual storage conditions for field samples (about -20°C in the dark) and analyzed after about 0, 30 (±2), 90 (±7), 180 (±7), 365 (±7), 565 (±7) and 755 (±7) days. The exact sampling days are given in the table below.

2. Description of analytical procedures

The residues of pyrimethanil were quantitated using BASF method No L0066/02 (542/2). Pyrimethanil was extracted from plant material with a mixture of methanol and water. An aliquot of the extract was centrifuged and diluted. Final determination was performed by HPLC-MS/MS. The limit of quantitation of the method was 0.01 mg/kg.
Freshly fortified specimens were analyzed concurrently with specimens dosed and stored frozen. Acceptable procedural recovery data were obtained except for oilseed rape seed at day 567 (50.1%).

II. RESULTS AND DISCUSSION

The table below shows a summary of the stability data. The results are expressed as average percentage of the nominal fortification. In order to account possible variation over the time investigated, the mean corrected recovery results are given in addition in parentheses.

After a storage time of 753-756 days the mean recovery results of the nominal in all stored samples were above 70% and therefore show stability in all matrices for pyrimethanil.
Two earlier sampling points were just below 70%: for rape seed at day 180 the uncorrected recovery of the nominal was 69% (corrected 93.9%) and for wheat grain at day 96 the uncorrected recovery of the nominal was 67.3% (corrected 85.4%). Hence, this minor lower recovery is deemed irrelevant.
One sampling point at day 567 for oilseed rape seed is not further considered for calculation of mean recovery of stored samples as the procedural recoveries were as low as 50%. At all other sampling points procedural recoveries were acceptable >70% for each matrix.

Table 6.1-1: Storage stability of pyrimethanil in plant matrices

Mean Recovery (%)										
Storage time (days)	**Apple fruit**		**Oilseed rape seed**		**Dried pea seed**		**Wheat grain**		**Grape fruit**	
	A: in stored samples, % of nominal					B: procedural, in freshly spiked sample				
	A[1)]	B[2)]	**A[1)]**	B[2)]	**A[1)]**	B[2)]	**A[1)]**	B[2)]	**A[1)]**	B[2)]
0	**96.8 (100)**	96.8	**101 (100)**	101.0	**98.3 (100)**	98.3	**98.1 (100)**	98.0	**97.5 (100)**	97.4
29	**89.1 (85.3)**	104.5	**87.9 (101)**	87.4	**85.5 (83.0)**	103.0	**98.8 (91.9)**	107.5	**99.2 (86.6)**	114.5
90-96	**82.2 (74.4)**	110.5	**90.8 (92.9)**	97.7	**96.2 (87.1)**	110.5	**67.3 (85.4)**	78.8	**85.5 (77.0)**	111.0
177-180	**93.9 (89.0)**	105.5	**69.0 (93.9)**	73.5	**80.1 (89.3)**	89.7	**86.7 (92.0)**	94.2	**95.5 (85.0)**	112.3
368-371	**70.9 (80.5)**	88.1	**77.2 (93.2)**	82.8	**90.1 (90.9)**	99.1	**73.8 (87.4)**	84.4	**77.9 (81.1)**	96.0
567-568	**96.7 (89.1)**	108.5	**-***	-*	**76.3 (97.1)**	78.6	**88.9 (89.9)**	98.9	**94.8 (89.0)**	106.5
753-756	**88.1 (90.8)**	97.1	**92.6 (90.8)**	102.0	**90.8 (102)**	89.4	**93.1 (82.3)**	113.1	**88.2 (90.0)**	98.0

1 A = mean % recovery in stored samples
2 B = mean % procedural recovery for freshly spiked samples
() Mean corrected recovery results are given in parentheses
* Sampling point at day 567 is not further considered for calculation of mean recovery of stored samples as the procedural recoveries were as low as 50%

III. CONCLUSION

The results obtained in this storage stability study demonstrate that pyrimethanil is stable in plant matrices with high water, high oil, high protein, high starch and high acid content when stored frozen for up to 24 months. Recoveries slightly below 70% in intermediate samples are not considered to have an impact on this conclusion.

Report:	CA 6.1/2 Morgenthal K., 2015a Interim report: Freezing storage stability of M605F005, M605F007 and Phenylguanidine in five plant matrices over 2 years at -18 °C 2015/1050228
Guidelines:	OECD 506 (Oct. 2007), EEC 7032/VI/97 rev. 5, EPA 860.1380
GLP:	yes (certified by Swiss Federal Office of Public Health, Berne, Switzerland)

Report:	CA 6.1/2 Homazava N., 2016 a Second Interim Report - Freezing storage stability of M605F005, M605F007 and Phenylguanidine in five plant matrices over 2 years at -18 °C 2015/1279709
Guidelines:	OECD 506, EEC 7032/VI/95 rev. 5, EPA 860.1380
GLP:	yes (certified by Swiss Federal Office of Public Health)

Report:	CA 6.1/2 Homazava N., 2016 b Freezing storage stability of M605F005, M605F007 and Phenylguanidine in five plant matrices over 2 years at -18 °C 2017/1136008
Guidelines:	OECD 506, EEC 7032/VI/95 rev. 5, EPA 860.1380, EPA 712-C-95-177
GLP:	yes (certified by Swiss Federal Office of Public Health)

The storage stability study on pyrimethanil metabolites over 2 years was initiated in March 2015 and is still in progress. The results of the second interim report covering storage periods of up to 512 months are summarized below.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	M605F005; M605F007; M605F025 (phenylguanidine)
Lot/Batch #:	DJS3880/4CR; L72-129; 9210956
Purity:	99.7%; 98.6%; 89.7%
CAS#:	Not available; 767-15-7; 6685-76-3
Spiking levels:	0.1 mg/kg

2. Test Commodity:

Crop:	Lettuce, strawberry, oilseed rape (seeds), dried beans, wheat (grain)
Sample size:	5 g (stored sample)

B. STUDY DESIGN

1. Test procedure

Plant matrices (lettuce, strawberry, oilseed rape seeds, dried beans and wheat grain) were fortified with pyrimethanil metabolites M605F005, M605F007 and M605F025 at a concentration level of 0.1 mg/kg (10 x LOQ). The spiked samples were stored under the usual storage conditions for field samples (≤-18°C in the dark) and analyzed after about 0, 3, 5, 8 (only M605F005 in wheat grain and M625F007 in oilseed rape seeds) 12, 18 and 24 months~~. (0, 95, and 149 days). This interim report shows the results for up to 512 months.~~

2. Description of analytical procedures

Pyrimethanil metabolites M605F005, M605F007 and M605F025 were quantified using BASF method No L0274/01. The compounds were extracted from plant material with a mixture of methanol and water. For the extraction of M605F025 from oilseed rape seeds, dried beans and wheat grains, the extraction mixture was acidified with formic acid. An aliquot of the extract was centrifuged and diluted with water. Final determination was performed by LC-MS/MS using mass transitions m/z 232 → 144, 124 → 67 and 136 → 77 for M605F005, M605F007 and M605F025, respectively. The limit of quantitation of the method was 0.01 mg/kg per analyte.
Freshly fortified specimens were analyzed concurrently with specimens dosed and stored frozen. Acceptable procedural recovery data were obtained except for M605F007 in lettuce at day 0 (133%). However, results for the confirmatory transition of M605F007 in lettuce showed acceptable procedural recovery of 97.7% . The discrepancy is caused by the inpresition in peak integration on the primary transition.

II. RESULTS AND DISCUSSION

The table below shows a summary of the stability data. The results are expressed as average percentage of the nominal fortification. In order to account for possible variation over the time investigated, the mean corrected recovery results are given in addition in parentheses.

The recoveries obtained for M605F005, M605F007 and M605F025 in stored homogenized samples of lettuce, strawberry, ~~oilseed rape seeds~~, dried beans and wheat grains provide sufficient evidence for ~~5 12~~ 24 months' stability.

~~Although the residue levels observed for M605F025 in 5 months stored oilseed rape (69.4%) and for M605F005 in 5 months stored dried beans samples (62.1%) show a decline, the interpretation and consideration of stability remains speculative, as the informative value is limited due to the restricted number of sampling intervals.~~

~~The residue level observed for M605F025 in oilseed rape (60.3%) for 12 months' storage is considered acceptable as the corrected value was >70% (73.9%). The residue levels observed for M605F005 in wheat grains (max. 37.2%) and for M605F007 in oilseed rape (max. 52.4%) for 12 months' storage period show a decline, the interpretation and consideration of stability remains open, as the informative value is limited due to the restricted number of sampling intervals. To clarify the question of M605F005 stability in wheat grains and M605F007 stability in oilseed rape an additional storage interval of 8 months will be evaluated.~~

~~For the final assessment of stability, further testing intervals have to be evaluated.~~

In oilseed rape commodity, the storage stability was found to be stable for 12 months for phenylguanidine M605F025, 5 months for M605F007 and 18 months for M605F005.

For wheat grain for M605F005 the recoveries showed some inconclusive variations. However, the storage stability is confirmed to be 8 months, with all recoveries > 70%. The data from the further storage at 12 months intervals are inconclusive with recoveries at 34.8% or 37.2%, they are followed by 70.2% recovery at 18 months and 66.6% recovery at 24 months. However, a new set of two fortified samples aged for 12 months resulted in a recovery of 94.1%. Therefore, that low recoveries of the two initial measurement (34.8% and 37.2%, respectively) at 12 months are possibly caused by unknown technical mistake and M605F005 can be considered stable for 24 months.

Table 6.1-2: Storage stability of pyrimethanil metabolites in plant matrices

Mean Recovery (%)										
Storage time (days / month)	Lettuce		Strawberry		Oilseed rape seed		Dried beans		Wheat grain	
	A: in stored samples, % of nominal					B: procedural, in freshly spiked sample				
	A[1)]	B[2)]	A[1)]	B[2)]	A[1)]	B[2)]	A[1)]	B[2)]	A[1)]	B[2)]
M605F005										
0	**86.0 (108)**	79.4	**87.3 (107)**	81.3	**81.5 (104)**	78.4	**85.9 (96.6)**	88.9	**95.2 (111)**	85.7
95 / 3	**77.4 (86.1)**	89.9	**75.1 (89.2)**	84.2	**77.4 (95.6)**	81.0	**94.2 (92.4)**	102	**74.6 (74.1)**	101
149 / 5	**89.4 (110)**	81.6	**87.9 (106)**	83.1	**84.3 (117)**	72.2	**62.1 (65.1)**	95.5	**96.9 (117)**	83.1
~~240~~245 / 8	-	-	-	-	-	-	-	-	~~Due Feb 2017~~ **74.5 (94.3)**	79.0
392 / 12	**73.8 (84.2)**	87.7	**77.9 (89.2)**	87.3	**75.5 (86.4)**	87.4	**70.6 (84.8)**	83.3	**34.8 (36.7)**	94.7
400 (repeat)	-	-	-	-	-	-	-	-	**37.2 (44.7)**	83.2
365 (new spike) / 12	-	-	-	-	-	-	-	-	**94.1 (95.3)**	98.7
550 / 18	**71.1 (77.6)**	91.7	**66.7 (75.4)**	88.5	**56.4 (79.9) [69.2]**	70.6	**65.5 (70.0)**	93.6	**70.2 (88.6)**	79.3
732 / 24	**69.9 (86.4)**	81.0	**62.4 (79.6)**	78.4	**40.0 (56.1)**	71.3	**87.2 (119)**	73.0	**66.6 (82.9) [81.3]**	80.3
M605F007										
0	**132 (99.4) 95.5* (97.8)***	133 97.7*	**95.7 (103)**	93.3	**92.5 (99.6)**	92.9	**91.9 (101)**	91.0	**82.4 (96.7)**	85.3
95 / 3	**77.8 (86.3)**	90.2	**89.2 (99.7)**	89.5	**74.9 (97.7)**	76.7	**90.4 (99.2)**	91.1	**85.2 (88.1)**	96.6
149 / 5	**98.2 (105)**	93.9	**87.9 (97.0)**	90.7	**73.3 (88.9)**	82.4	**92.7 (89.2)**	104	**91.8 (96.7)**	95.0
~~240~~245 / 8	-	-	-	-	~~Due Feb 2017~~ **19.4 (26.3)**	**73.9**	-	-	-	-
392 / 12	**89.0 (84.2)**	106	**104 (96.9)**	107	**52.4 (50.9)**	103	**89.8 (83.3)**	108	**80.2 (76.0)**	106
400 (repeat)	-	-	-	-	**37.1 (49.8)**	74.5	-	-	-	-
550 / 18	**88.9 (93.4)**	95.2	**77.6 (79.3)**	97.9	**11.6 (15.1)**	77.0	**81.6 (90.6)**	90.0	**83.0 (98.4)**	84.3
732 / 24	**89.2 (86.1)**	104	**101 (113)**	89.3	**22.0 (24.8)**	88.7	**78.1 (89.9)**	86.9	**89.1 (93.8)**	95.0

Mean Recovery (%)										
Storage time (days / month)	**Lettuce**		**Strawberry**		**Oilseed rape seed**		**Dried beans**		**Wheat grain**	
	A: in stored samples, % of nominal					B: procedural, in freshly spiked sample				
	A[1)]	B[2)]	**A[1)]**	B[2)]	**A[1)]**	B[2)]	**A[1)]**	B[2)]	**A[1)]**	B[2)]
M605F025										
0	**105 (99.2)**	106	**107 (97.2)**	110	**85.3 (100)**	85.2	**88.9 (95.8)**	95.8	**952.6 (106)**	87.5
95 and 111 / 3	**95.5 (108)**	88.0	**97.0 (110)**	88.1	~~n.r.~~[3] **68.2 (62.4)**	109	~~n.r.~~[3] **82.7 (92.5)**	89.4	~~n.r.~~[3] **79.5 (76.9)**	103
149 / 5	**86.6 (100)**	86.6	**89.7 (95.7)**	93.7	**69.4 (82.3)**	84.3	**78.6 (89.2)**	88.1	**77.5 (87.7)**	88.4
392 / 12	**91.2 (98.1)**	93.0	**112 (106)**	105	-	-	**75.9 (74.8)**	101	**93.1 (84.5)**	110
400 (repeat)	-	-	-	-	**60.3 (73.9) [70.7]**	81.6	-	-	-	-
550 and555 / 18	**106 (103)**	103	**108 (111)**	97.8	**32.1 (31.7)**	101	**91.1 (93.3)**	97.6	**77.9 (81.3)**	95.8
732 / 24	**88.9 (86.0)**	103	**93.7 (87.8)**	107	**29.6 (30.4)**	97.3	**89.2 (87.2)**	102	**89.9 (91.1)**	98.7

1 A = mean % recovery in stored samples
2 B = mean % procedural recovery for freshly spiked samples
3 Not reported; initiation of storage: 26.08.2015; due 25.11.2015
() Mean corrected recovery results are given in parentheses
* normalized to “day0”. The normalisation to the “day 0” helps interpreting the data. The latter is also stated in Reg. 283/2013: “Results shall be presented as absolute values in mg/kg and not adjusted by recovery, as well as percentage of nominal spike value”.
*: results for the confirmatory transition of M605F007. The discrepancy is caused by the imprecision in peak integration on the primary transition.

III. CONCLUSION

~~metabolites M605F005, M605F007 and M605F025 are stable in plant matrices with high water, high oil, high protein, high starch and high acid content when stored frozen for up to 5 months. Although the residue levels observed for M605F025 and for M605F005 in 5 months stored oilseed rape and dried beans samples show a decline, the interpretation and consideration of stability remains speculative, as the informative value is limited due to the restricted number of sampling intervals.~~

Overall, the recoveries obtained for pyrimethanil metabolites M605F005, M605F007 and phenylguanidine M605F025 in stored homogenized samples of lettuce, strawberry, wheat grain and dried beans provide sufficient evidence for ~~12~~ 24 months' stability. ~~The residue levels observed for M605F005 in wheat grains and for M605F007 in oilseed rape for 12 months' storage period show a decline, the interpretation and consideration of stability remains open, as the informative value is limited due to the restricted number of sampling intervals. To clarify the question of M605F005 stability in wheat grains and M605F007 stability in oilseed rape an additional storage interval of 8 months will be evaluated.~~ In oilseed rape commodity the storage stability was found to be 12 months for phenylguanidine M605F025, 5 months for M605F007 and 18 months for M605F005.

Stability of residues in sample extracts

For extract of plant and animal matrices, please refer to chapters 4.1.2 and 4.2; data on stability in sample extracts are given in the respective analytical method summaries.

CA 6.2 Metabolism, distribution and expression of residues

CA 6.2.1 Metabolism, distribution and expression of residues in plants

In context of the previous submission for Annex I inclusion, plant metabolism studies have been submitted in tomatoes, apples, grapes, lettuce and carrots, covering the crop categories fruits and fruiting vegetable, leafy and root crops (all foliar and for carrots in addition soil application), see Annex II chapter 6.1.

According to the results of these studies, pyrimethanil is the major compound; small but significant portions of conjugated metabolites were found in crop leaves. Based on the compounds detected, the metabolism of pyrimethanil takes place via

- Hydroxylation and
- Conjugation of the parent compound

The metabolic spectrum found was independent to the radiolabel (anilino- or pyrimidinyl-label) used, indicating that both moieties of the molecule were still present in all detectable conversion products. The studies are considered as suitable and valid for describing the degradation behavior of pyrimethanil in plants.

During the re-evaluation of the established MRLs according to Art.12 of Regulation 396/2005 (EFSA Journal 2011;9(11):2454), EFSA noted that that "during the peer review process the significance of metabolites was evaluated on the basis of the percentage of TRR alone. During a previous reconsideration of the data it was observed that many of the metabolites are present at levels exceeding 0.05 mg/kg (after accounting for the N rate). This is considered to be due to the very high residue levels (mg/kg) found in the metabolism studies. The high levels observed are found principally in fruit studies; when residue levels in the apple and grape metabolism studies are compared to apple and grape residues found at time zero (i.e. when parent will be at a maximum) in decline trials the metabolism studies are shown to be a considerable overestimate".

The following conclusion is taken from the Reasoned Opinion (see: EFSA Journal 2011;9(11):2454), the new Metabolite Codes were added for the readers convenience:
From the carrots studies, it appears that the route of degradation is the same with the two kinds of applications. The parent compound predominated in both roots and foliage. In carrot roots, parent represented 70-93% of TRR; however, in foliage there was evidence of further metabolism with parent representing as little as 45.7% of the TRR. The identified metabolites indicated that the metabolism proceeded via hydroxylation of either the aniline or pyrimidinyl ring followed by conjugation to glucose or malonic acid. The main conjugate identified was malonyl-β-O-glucoside of SN 614 278 (M605F028) representing a maximum of 16.8% of TRR in the carrot foliage and 1.3% of TRR in the roots. All other metabolites were present at less than 0.3% TRR in roots and less than 7.6% TRR in foliage.
In tomatoes parent pyrimethanil predominated representing 95-97% of the TRR in fruit and leaves. Five metabolites were resolved, three of which were shown to be hydroxylated conjugates of parent and all of which were present at levels <1.13% TRR.

In apples parent predominated in both fruit and leaves. In fruits parent represented 69-77% of TRR; in leaves parent represented 54-63 % of TRR. Metabolism proceeded via hydroxylation of either the aniline or pyrimidinyl ring followed by conjugation to glucose or other 6 carbon sugars. The principle conjugate determined was β-O-glucoside of C621 312 (M605F029) representing a maximum of 16.5% of TRR in leaves and 1.5% of TRR in fruit. All other metabolites were present at less than 3.4% TRR in fruit and less than 7.5% TRR in leaves.
In grapes parent predominated in both fruit and leaves. In fruit parent represented 91% of TRR however in foliage there was evidence of further metabolism with parent representing as little as 31% of TRR. The principle metabolite identified was a C6 sugar of SN 614 276 (M605F001) present at 0.6% of TRR in fruit and 16.8% of TRR in leaves. Three other metabolites were resolved, all of which were present at less than 2.8% of TRR.
In lettuce parent compound represents 92% of the TRR with a zero day PHI and falls to 80% and 44% with a 7 and 21 day PHI, respectively. Only two metabolites were resolved, conjugated of SN 614 276 (aglycon M605F002) and conjugated of SN 614 277 (aglycon M605F003) which were present at <4.5% and 7.9 % of TRR, respectively.

It appears that the metabolic pathway is similar in the three crop groups evaluated. In all these available metabolism studies, the conjugated and hydroxylated metabolites of pyrimethanil were present at levels significantly below those of the parent. All other metabolites were present at levels below 10% TRR. Consequently, the residue definition for foliar and post-harvest treatment in all crop groups is defined as pyrimethanil only for both enforcement and risk assessment. These conclusions reached by EFSA reflect the views of RMS and are also in line with the findings of the JMPR (FAO, 1995).

Summary of available metabolism studies in plants (EFSA Reasoned Opinion 2011, Art. 12 review)

Group	Crop *(Study code)*	Label position	Application and sampling details				
			Method, F or G[1]	Rate (kg a.s./ha)	No	Sampling (DAT)	Remarks
Fruits and fruiting vegetable	Tomatoes *(A91817)*	2-[U-^{14}C]-anilino and 2-[^{14}C]-pyrimidinyl labelled	Foliar treatment, G	0.8	4	Immediately after each treatment. Final sampling: 8	-
	Apples *(A81633)*	2-[U-^{14}C]-anilino and 2-[^{14}C]-pyrimidinyl labelled	Foliar treatment,	0.45	4	42	-
	Grapes *(A81628)*	2-[U-^{14}C]-anilino labelled	Foliar treatment (automatic pipette)	0.8	2	21	-
Leafy vegetables	Lettuce *(A91255)*	2-[^{14}C]-pyrimidinyl-labelled	Foliar treatment, F	0.8	2	Immediately after the first treatment, 7 and 21	-
Root and tuber vegetables	Carrots *(C010122)*	n.r.	Foliar and soil treatment	0.8	2	1 and 21 days after the 1st and the 2nd treatment	-
			Foliar treatment	2.4	2		

n.r. Not reported
1 Outdoor/field application (F) or glasshouse/protected/indoor application (G)

Since pyrimethanil is authorized for seed treatment on cereals, for which no representative metabolism study is available, EFSA considered that in order to extend the proposed residue definition to this kind of treatment, a representative metabolism study on cereals with seed treatment has to be submitted. Thus, a new seed treatment metabolism study on wheat was conducted and is summarized below (2014/1000808). Furthermore, a new metabolism study on lettuce (2014/1000805) was conducted to provide a second label.

In brief, in the **new lettuce metabolism study** (foliar application), parent pyrimethanil was the predominant residue, confirming the findings of the peer-reviewed studies. Several conjugates were identified in minor amounts all <10 %TRR, the aglycons of which were detected in peer-reviewed study on lettuce after hydrolysis as well as other hydroxylates and the conjugates. Two new minor metabolites were identified in the polar region: M605F025 (phenylguanidine) and M605F033. M605F033 is a conjugated derivative of soil metabolite M605F007 hydroxylate (intermediate M605F032 - not found in the lettuce study).
In the **new wheat metabolism study** (seed treatment), no parent pyrimethanil was found. No residues >0.01 mg/kg in wheat grain occurred. Thus, wheat grain was not subjected to metabolite identification since total radioactive residues were too low. In wheat straw, hay and forage in addition to hydroxylated metabolite M605F004 and three conjugated compounds, two new metabolites were identified in the polar region: M605F032 and M605F033. They are hydroxylated (M605F032) and further conjugated (M605F033) derivatives of soil metabolite M605F007. Another polar compound (M605F025) was found as minor metabolite.

The new studies in lettuce and wheat showed that the metabolism of pyrimethanil in plants includes the following reactions in addition to the two concluded from the peer-reviewed studies:

- Cleavage of amine bond and
- Ring opening of the pyrimidine ring

The new studies summarized below were performed using the phenyl-^{14}C- and pyrimidinyl-^{14}C-labeled pyrimethanil.

Report:	CA 6.2.1/1 Gordon L.,Johnson J., 2015a The metabolism of 14C-Pyrimethanil (BAS 605 F, Reg. No. 236999) in lettuce 2014/1000805
Guidelines:	OECD-ENV/JM/MONO/(2009)31 (OECD No. 64), Test No. 501: Metabolism in crops, EPA 860.1000, EPA 860.1300, PMRA Residue Chemistry Guidelines Section 97.2 Nature of the Residue - Plants - Livestock (Canada), JMAFF No 12 Nosan No 8147, Appendix A of Guideline 1607/VI/97
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	[Pyrimidinyl-2-^{14}C]-BAS 605 F (pyrimidinyl label) [Pyrimidinyl-1,3-^{15}N]-BAS 605 F Unlabeled BAS 605 F [Phenyl-U-^{14}C]-BAS 605 F (phenyl label) [Phenyl-1,2,3,4,5,6-^{13}C]-BAS 605 F
Lot/Batch #:	1049-1010 (^{14}C-pyrimidinyl label) 1050-1003 (^{15}N-pyrimidinyl label) L83-126 (unlabeled) 1036-1011 (^{14}C-phenyl label) 1052-1003 (^{13}C-phenyl label)
Purity:	Radiochemical purity: 99.7% (^{14}C-pyrimidinyl label) 99.2% (^{14}C-phenyl label) Specific activity: 6.42 MBq/mg (^{14}C-pyrimidinyl label) 3.98 MBq/mg (pyrimidinyl mixture) 8.53 MBq/mg (^{14}C-phenyl label) 4.46 MBq/mg (phenyl mixture)
CAS#:	53112-28-0
Stability of test compound:	The test item was stable over the test period.

2. Test Commodity:

Crop:	Lettuce
Type:	Leafy crops
Variety:	Salad Bowl
Botanical name:	*Lactuca sativa* L.
Crop parts(s) or processed commodity:	Immature and mature lettuce (foliage)
Sample size:	Not relevant

3. Soil: A sandy loam was used. The soil physicochemical properties are described below (see Table 6.2.1-1).

Table 6.2.1-1: Soil physicochemical properties

Soil series	Soil type	pH	OM[3] %	Sand %	Silt %	Clay %	MWHC[4] %	CEC[5] meq/100 g
N/A	Sandy loam[1]	7.3[2]	2.4	65*	20*	15*	n.r.	10.0

N/A = not applied; 1 USDA scheme; 2 H_2O; 3 Organic matter; 4 Maximum Water Holding Capacity; n.r. = not reported; 5 Cation exchange capacity

B. STUDY DESIGN

The metabolism study was conducted with [^{14}C]-pyrimethanil (pyrimidinyl label and phenyl label) during 2013-2015. The study was carried out at Charles River, Tranent, Edinburgh, EH33 2NE, UK.

1. Test procedure

The active substance was foliar applied in form of the formulated product BAS 605 04 F to lettuce at an application rate of nominally 2x 800 g a.s./ha (actually 834+801 g a.s./ha and 795+803 g a.s./ha for the pyrimidinyl label and phenyl label, respectively) with a 14 day retreatment interval. Immature lettuce was harvested 14 days after the first application (immediately prior to the second application) and mature lettuce (BBCH 49) was harvested 14 DALA to provide a 14 day PHI.

2. Description of analytical procedures

Radioanalysis: For the determination of the TRR, and the measurement of solid residues following solvent extraction or solubilization procedures, homogenized subsamples were combusted using a sample oxidizer. The resultant $^{14}CO_2$ was absorbed, mixed with a scintillation fluid and the radioactivity determined by liquid scintillation counting (LSC). ^{14}C standards were combusted at the beginning and at regular intervals throughout each batch of analyses. Measurements of radioactivity were not corrected for oxidizer efficiency; however combustion efficiencies were generally in excess of 97%.

Extraction: Homogenized subsamples of milled tissue were extracted three times with methanol, each followed by centrifugation. The residue was re-extracted twice with water in the same manner. The volume of each extract was measured prior to analysis by LSC. The combined results of methanol extractions and water extractions are referred to as extractable radioactive residues (ERR).
The debris remaining after solvent extraction of each sample was dried under a gentle stream of nitrogen gas. Aliquots were combusted for the determination of the residual radioactive residue (RRR).
The immature lettuce foliage samples were extracted twice. The debris remaining following the initial solvent extraction was extracted a further two times with methanol/water (7:3 v/v) using an ultrasonic bath. The ultrasonic bath was set at *ca.* 25°C for the first extract and *ca.* 40°C for the second extract. Each extract was separated from the residue by centrifugation. The volume of each extract was measured prior to analysis by LSC. The two extracts were finally combined before again analyzing by LSC. For the purposes of this report results of the aqueous solvent extractions are included in the definition of extractable radioactive residues (ERR).

The debris remaining after solvent extraction of immature and mature lettuce foliage was subjected to several enzyme treatments, i.e driselase, cellulase and macerozyme, β-glucosidase, tyrosinase and laccase, protease, artificial gastric juice and artificial intestinal fluid, and analyzed by LSC.
The debris after bound residue extractions of each sample was dried and homogenized. Aliquots were combusted for the determination of the remaining debris (terminal unextracted residue).

3. Identification of metabolites

Selected extracts containing significant radioactive residues (≥0.010 mg/kg) were analyzed using reversed phase HPLC and LC-MS methods for metabolite characterization and identification. Thin layer chromatography (TLC) was used for additional investigation into reference item M605F025.

II. RESULTS AND DISCUSSION

A. TOTAL RADIOACTIVE RESIDUES (TRRs)

The calculated TRR of immature lettuce foliage was 1.325 and 0.401 mg/kg for the phenyl and pyrimidinyl treated samples, respectively. The calculated TRR of mature lettuce foliage was 2.501 and 1.880 mg/kg for the phenyl and pyrimidinyl treated samples, respectively. The calculated TRR was set to 100% TRR for all matrices.
Additionally, the TRR was measured by direct combustion followed by LSC analyses. The measured TRR of all lettuce matrices was in the same order of magnitude as the calculated TRR values.

Table 6.2.1-2: Total radioactive residues (TRR) of [^{14}C]-pyrimethanil in lettuce following foliar treatment

TRRs in treated lettuce samples					
Matrix	DAT	TRR combusted [mg/kg]		TRR calculated* [mg/kg]	
		Pyrimidinyl label	Phenyl label	Pyrimidinyl label	Phenyl label
Immature lettuce	14 DAT1	0.327	1.130	0.401 (122.6%)	1.325 (117.3%)
Mature lettuce	14 DAT2	1.728	2.042	1.880 (108.8%)	2.501 (122.5%)

DAT = Days after treatment; either treatment 1 (DAT1) or treatment 2 (DAT2; 14 days after treatment 1)
* TRR was calculated as the sum of ERR + RRR

B. EXTRACTION, CHARACTERISATION AND IDENTIFICATION OF RESIDUES

1. Extractability of residues in lettuce

The extractability of the lettuce matrices with methanol and water is summarized in the table below. The extractability was 85.4-87.8% TRR (0.352-1.131 mg/kg) for immature and 80.0-82.4% TRR (1.550-2.002 mg/kg) for mature foliage. For both matrices the majority of the radioactivity was extracted with methanol (immature: 82.3-85.1% TRR and mature: 73.9-77.7% TRR) and only minor amounts were subsequently released with water (immature: 2.5-3.0% TRR and mature: 4.3-5.8% TRR) and aqueous methanol (immature: 0.1-0.2% TRR and mature: 0.3-0.4% TRR).

Table 6.2.1-3: Extractability of the total radioactivity of [^{14}C]-pyrimethanil in lettuce following foliar treatment

Matrix	DAT	TRR calc. *	Distribution of radioactive residues						ERR		RRR	
			Methanol		Water		Methanol/ water 7:3					
		[mg/kg]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]
Pyrimidinyl label												
Immature lettuce	14 DAT1	0.401	0.341	85.1	0.010	2.5	0.001	0.2	0.352	87.8	0.049	12.2
Mature lettuce	14 DAT2	1.880	1.461	77.7	0.081	4.3	0.008	0.4	1.550	82.4	0.329	17.5
Phenyl label												
Immature lettuce	14 DAT1	1.325	1.090	82.3	0.040	3.0	0.001	0.1	1.131	85.4	0.192	14.5
Mature lettuce	14 DAT2	2.501	1.849	73.9	0.145	5.8	0.008	0.3	2.002	80.0	0.500	20.0

DAT = Days after treatment
* TRR was calculated as the sum of ERR + RRR

2. Identification and characterization of extractable residues in lettuce

Structural elucidation of metabolites was based on mass spectrometric analysis of the methanol extract of [Py-^{14}C] treated mature lettuce. HPLC-MS analysis of the fractions led to the identification of the parent compound BAS 605 F and its metabolites M605F004, M605F025, M605F027, M605F028, M605F030, M605F033, M605F036, M605F039, M605F040 and two isomers of M605F041.

In the ERR (methanol and water extracts) of immature lettuce, unchanged parent compound BAS 605 F was the major residue and accounted for 0.614 mg/kg or 46.3% TRR and 0.181 mg/kg or 45.2% TRR in [Ph-^{14}C] and [Py-^{14}C] treated samples, respectively. Carbohydrate conjugates M605F027, M605F028, M605F036, M605F041 and M605F039 were all present at similar levels (0.059-0.018 mg/kg; 7.4-4.3% TRR) in both samples. Metabolite M605F030 and the two isomers of M605F040 were below or equal to 0.056 mg/kg or 4.2% TRR. The remaining identified component, M605F033 (0.013 mg/kg and 3.2% TRR) was seen in the [Py-^{14}C] treated sample only. In the ERR of immature lettuce 1.125 mg/kg or 84.9% TRR and 0.312 mg/kg or 77.4% TRR were identified and additional 0.017 mg/kg or 1.4% TRR and 0.005 mg/kg or 1.8% TRR were characterized by HPLC in [Ph-^{14}C] and [Py-^{14}C] treated samples, respectively. An additional 1.3-1.0% of the TRR (0.019-0.003 mg/kg) was characterized from the RRR by sequential solubilization with enzymes. Low levels (1.0-0.9% TRR, 0.013-0.003 mg/kg) of radioactive residues were released by artificial gastric juice and artificial intestinal fluid extractions.

In the ERR (methanol and water extracts) of mature lettuce, unchanged parent compound BAS 605 F was the major residue and accounted for 0.551 mg/kg or 22.0% TRR and 0.382 mg/kg or 20.3% of the TRR in [Ph-^{14}C] and [Py-^{14}C] treated samples, respectively. Carbohydrate conjugates, M605F027, M605F028, M605F03, M605F036, M605F039, and M605F041 were all present at similar levels (0.216-0.060 mg/kg; 8.6-3.2% TRR) in both samples. Metabolites M605F040 (isomer 1) and M605F040 isomer 2) were below or equal to 0.142 mg/kg or 5.7% TRR. Metabolite M605F004 was also identified in low levels accounting for ≤3.0% TRR. M605F025 and M605F033 were present in the methanol extract of [Py-^{14}C] treated mature foliage only and accounted for 0.033 mg/kg or 1.7% TRR and 0.129 mg/kg or 6.9% TRR respectively. In the ERR of mature lettuce 1.881 mg/kg or 75.0% TRR and 1.340 mg/kg or 71.2% TRR were identified and an additional 0.048 mg/kg or 2.1% TRR and 0.076 mg/kg or 3.9% TRR were characterized by HPLC in [Ph-^{14}C] and [Py-^{14}C] treated samples, respectively. An additional 2.2-2.1% TRR (0.054-0.042 mg/kg) was characterized from the RRR by sequential solubilization with enzymes. Low levels (1.6-1.4% TRR, 0.035-0.031 mg/kg) of radioactive residues were released by artificial gastric juice and artificial intestinal fluid extractions.

This resulted in final unextracted residues of 0.039-0.183 mg/kg or 9.8-13.8% TRR in immature foliage and 0.212-0.465 mg/kg or 11.3-18.6% TRR in mature foliage. The low levels of radioactive residues released by the enzyme solubilization indicated that minor residues are incorporated into natural plant matrices such as cell walls. The artificial gastric juice and artificial intestinal fluid treatment indicated that the remaining bound residues are not readily bioavailable.

Table 6.2.1-4: Residues of [pyrimidinyl-2-^{14}C]-pyrimethanil in lettuce following 2 foliar applications at a rate of 800 g a.s./ha

Test substance	Pyrimidinyl label			
Sample/metabolite	Immature foliage		Mature foliage	
	mg/kg	% TRR	mg/kg	% TRR
BAS 605F (pyrimethanil)	0.181	45.2	0.382	20.3
M605F004	<0.001	<0.1	0.030	1.6
M605F025	-	-	0.033	1.7
M605F027	0.022	5.4	0.107	5.7
M605F028	0.018	4.4	0.135	7.1
M605F030	0.003	0.7	0.060	3.2
M605F033	0.013	3.2	0.099	5.3
M605F036	0.018	4.3	0.129	6.8
M605F039	0.018	4.6	0.129	6.9
M605F040 (isomer 1)	0.010	2.5	0.094	5.0
M605F040 (isomer 2)	0.011	2.7	0.062	3.3
M605F041	0.018	4.4	0.080	4.3
Total identified	**0.312**	**77.4**	**1.340**	**71.2**
Others[1]	0.005	1.8	0.076	3.9
Total characterized by HPLC	0.005	1.8	0.076	3.9
Characterized by further aqueous methanol extracts	0.001	0.2	0.008	0.4
Total identified and/or characterized from ERR[2]	**0.318**	**79.5**	**1.424**	**75.5**
Unextractable (RRR)[3]	0.049	12.2	0.329	17.5
Driselase solubilizate	<0.001	0.1	0.008	0.4
Macerozyme/Cellulase solubilizate	0.001	0.3	0.008	0.4
β-glucosidase solubilizate	<0.001	0.1	0.004	0.2
Laccase/Tyrosinase solubilizate	0.001	0.2	0.009	0.5
Protease solubilizate	0.001	0.3	0.013	0.7
Sum of solubilized radioactive residues	0.003	1.0	0.042	2.2
Pepsin in artificial gastric juice solubilizate	0.001	0.3	0.008	0.4
Pancreatin in artificial intestinal fluid solubilizate	0.002	0.6	0.023	1.2
Sum of bioavailable residues	**0.003**	**0.9**	**0.031**	**1.6**
Final residue	0.039	9.8	0.212	11.3
Grand total	**0.367**	**91.6**	**1.753**	**93.0**

1 Up to 9 peaks characterized (≤0.005 mg/kg or 1.2% TRR) (immature) / up to 10 peaks characterized (≤0.018 mg/kg or 1.0% TRR) (mature)
2 ERR = extractable radioactive residue
3 RRR = residual radioactive residue

Table 6.2.1-5: Residues of [phenyl-U-^{14}C]-pyrimethanil in lettuce following 2 foliar applications at a rate of 800 g a.s./ha

Test substance	Phenyl label			
Sample/metabolite	Immature foliage		Mature foliage	
	mg/kg	% TRR	mg/kg	% TRR
BAS 605F (pyrimethanil)	0.614	46.3	0.551	22.0
M605F004	0.015	1.2	0.076	3.0
M605F027	0.077	5.8	0.183	7.3
M605F028	0.084	6.4	0.194	7.7
M605F030	0.027	2.1	0.128	5.1
M605F036	0.065	4.9	0.216	8.6
M605F039	0.099	7.4	0.195	7.8
M605F040 (isomer 1)	0.056	4.2	0.142	5.7
M605F040 (isomer 2)	0.029	2.1	0.078	3.1
M605F041	0.059	4.5	0.118	4.7
Total identified	**1.125**	**84.9**	**1.881**	**75.0**
Others[1]	0.017	1.4	0.048	2.1
Total characterized by HPLC	0.017	1.4	0.048	2.1
Characterized by further aqueous methanol extracts	0.001	0.1	0.008	0.3
Total identified and/or characterized from ERR[2]	**1.143**	**86.4**	**1.937**	**77.4**
Residual radioactive residue (RRR)[3]	0.192	14.5	0.500	20.0
Driselase solubilizate	0.003	0.2	0.008	0.3
Macerozyme/Cellulase solubilizate	0.003	0.2	0.010	0.4
β-glucosidase solubilizate	0.003	0.2	0.008	0.3
Laccase/Tyrosinase solubilizate	0.003	0.2	0.015	0.6
Protease solubilizate	0.007	0.5	0.013	0.5
Sum of solubilized radioactive residues	0.019	1.3	0.054	2.1
Pepsin in artificial gastric juice solubilizate	0.004	0.3	0.010	0.4
Pancreatin in artificial intestinal fluid solubilizate	0.009	0.7	0.025	1.0
Sum of bioavailable residues	**0.013**	**1.0**	**0.035**	**1.4**
Final residue	0.183	13.8	0.465	18.6
Grand total	**1.335**	**100.9**	**2.437**	**97.4**

1 Up to 13 peaks characterized (≤0.002 mg/kg (immature) / ≤0.006 mg/kg (mature) or 0.2% TRR)
2 ERR = extractable radioactive residue
3 RRR = residual radioactive residue

3. Proposed metabolic pathway

Pyrimethanil (BAS 605 F) is metabolized in lettuce foliage and degradation products are eventually incorporated into natural plant products. Unchanged parent BAS 605 F remained the major component in immature foliage (harvested 14 days after application 1) and mature foliage (harvested 14 days after application 2).
The metabolism of BAS 605 F includes the following reactions:

- Hydroxylation of the parent compound
- Formation of glucose and malonyl glucose conjugates of metabolites via hydroxylation of the parent compound
- Cleavage of amine bond
- Ring opening of the pyrimidine ring

The proposed metabolic pathway of BAS 605 F in lettuce is shown in Figure 6.2.1-1.

4. Storage stability

The solvent extracts (methanol and water) of immature and mature lettuce foliage were extracted and analyzed by HPLC with a maximum of 66 days between sampling and analysis. Quantitation analysis was performed using HPLC with a maximum of 316 days between sampling and analysis.
Repeat HPLC analysis of the combined methanol extracts of [Ph-^{14}C] mature foliage and [Py-^{14}C] mature foliage were very similar to the metabolite pattern of the initial analysis. The storage stability analysis was conducted 663 days after the initial analysis. It was concluded, therefore, that the composition of the extracts was stable throughout the period of the study.

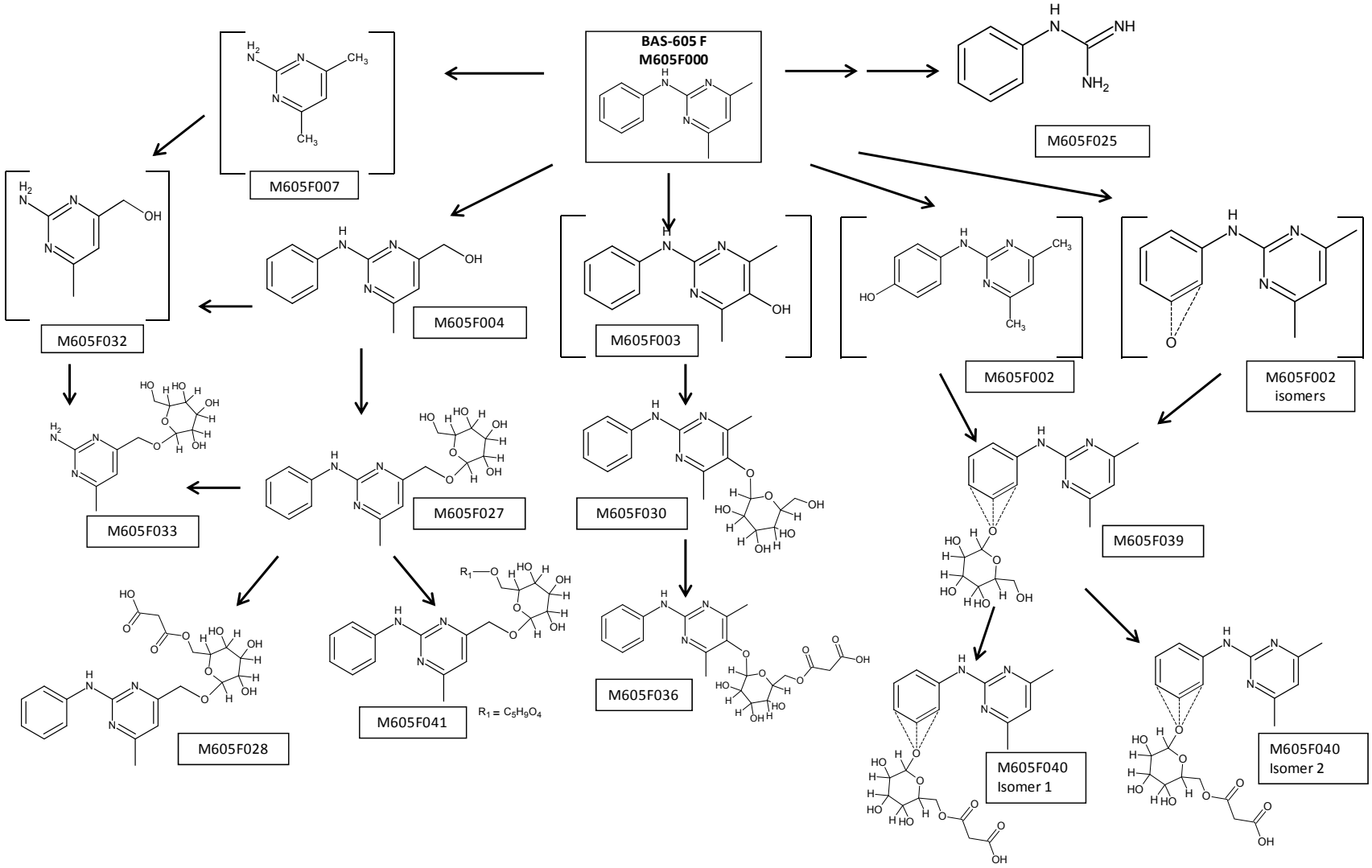
III. CONCLUSION

The majority of the soluble radioactive residues were extracted with methanol (immature foliage: 82.3-85.1% TRR and mature foliage: 73.9-77.7% TRR), while only minor amounts were subsequently released with water (≤5.8% TRR) and aqueous methanol (≤0.4% TRR).
Sequential solubilization of the residues after extraction with enzymes released an additional 1.3% TRR from [Ph-^{14}C] treated immature lettuce, 1.0% TRR from [Py-^{14}C] treated immature lettuce, 2.1% TRR from [Ph-^{14}C] treated mature lettuce and 2.2% TRR from [Py-^{14}C] treated mature lettuce.
Treatment with artificial gastric juice and artificial intestinal fluid released an additional 1.0-0.9% TRR from immature foliage and 1.6-1.4% TRR from mature foliage. This resulted in a final residue level of 13.8% TRR in [Ph-^{14}C] treated immature lettuce, 9.8%TRR in [Py-^{14}C] treated immature foliage, 18.6% TRR in [Ph-^{14}C] treated mature lettuce and 11.3% TRR in [Py-^{14}C] treated mature lettuce. The low levels of radioactive residues released by the artificial gastric juice and artificial intestinal fluid extractions indicated that the remaining bound residues are not readily bioavailable.
In all matrices, the unchanged parent compound BAS 605 F was the major component identified (20.3-46.3% TRR). The remaining identified components M605F004, M605F025, M605F027, M605F028, M605F030, M605F033, M605F036, M605F039, M605F041 and two isomers of M605F040 ranged from <0.1-8.6% of the TRR.
The metabolism of BAS 605 F includes the following reactions:

- Hydroxylation of the parent compound
- Formation of glucose and malonyl glucose conjugates of metabolites via hydroxylation of the parent compound
- Cleavage of amine bond
- Ring opening of the pyrimidine ring

The degradation products of BAS 605 F are eventually incorporated into natural products.
A radiovalidation of the crop residue method BASF No 542/2 was conducted. This showed comparable extractability; the amounts of BAS 605 F detected were greater than the amounts detected in the metabolism extraction analysis. The extraction efficiency of the residue method for parent BAS 605 F was 139%, therefore the residue method BASF No 542/2 is able to extract and quantify pyrimethanil.

Figure 6.2.1-1: Proposed metabolic pathway of pyrimethanil in lettuce



Report:	CA 6.2.1/2 Begley K.,Gordon L., 2015a The metabolism of 14C-Pyrimethanil (BAS 605 F, Reg. No. 236999) in wheat following a seed dressing application 2014/1000808
Guidelines:	OECD-ENV/JM/MONO/(2009)31 (OECD No. 64), OECD 501, EPA 860.1000, EPA 860.1300, PMRA Residue Chemistry Guidelines Section 97.2 Nature of the Residue - Plants - Livestock (Canada), Appendix A of Guideline 1607/VI/97, JMAFF No 12 Nosan No 8147
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Report:	CA 6.2.1/3 Thiaener J. et al., 2015a In life phase and storage stability belonging to 14C-Pyrimethanil (14C-BAS 605 F) metabolism study in wheat after seed treatment - Study protocol 812012 (Charles River, Edinburgh, UK) 2015/1093635
Guidelines:	EPA 860.1300: Nature of the Residue in Plants Livestock, EPA 860.1000: Background - PMRA Section 97.2 (Canada): Residue Chemistry Guidelines: Plants and Lifestock (June 1997), JMAFF 59 NohSan No 4200, OECD 501, EEC 7028/VI/95 rev. 3 Appendix A (EU): Metabolism and distribution in plants
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

The study consists of two parts: storage stability investigations (with in-life phase) and metabolism investigations. These parts are separately indicated in the summary below.

I. MATERIAL AND METHODS

A. MATERIALS

2. Test Material:

Description:	[Pyrimidinyl-2-^{14}C]-BAS 605 F (pyrimidinyl label) [Phenyl-U-^{14}C]-BAS 605 F (pyrimethanil)
Lot/Batch #:	1049-1010 (pyrimidinyl label) 1036-1011 (phenyl label)
Purity:	Radiochemical purity: 99.7% (pyrimidinyl label) 99.2% (phenyl label) Specific activity: 6.42 MBq/mg (pyrimidinyl label) 8.53 MBq/mg (phenyl label)
CAS#:	53112-28-0
Stability of test compound:	The test item was stable over the test period.

2. Test Commodity:

Crop:	Wheat
Type:	Cereal / grass crops
Variety:	WS Thasos
Botanical name:	*Triticum aestivum* L.
Crop parts(s) or processed commodity:	Forage, hay, grain, straw
Sample size:	Not relevant

3. Soil: A sandy loam was used. The soil physicochemical properties are described below (see Table 6.2.1-6).

Table 6.2.1-6: Soil physicochemical properties

Soil series	Soil type	pH	OM %	Sand %	Silt %	Clay %	MWHC*** %	CEC cmol/kg
Bruch West	Sandy loam*	7.9**	1.40	70.1*	20.6*	9.3*	27.7	11.8

* USDA scheme **H_2O ***Maximum Water Holding Capacity

B. STUDY DESIGN

The metabolism study was conducted with [^{14}C]-pyrimethanil (pyrimidinyl label and phenyl label) during 2012-2015. The field phase of the study was performed at the Agricultural Research Centre of BASF in Limburgerhof, Germany.

1. Test procedure

The active substance was applied in form of the formulated product BAS 604 01 F to wheat seeds at an application rate of nominally 18.9 g a.s./ha (actually 19.272 g a.s./ha and 18.681 g a.s./ha for the pyrimidinyl label and phenyl label, respectively). After seed treatment with the test item; seeds were sown and cultivated in climatic chambers, simulating the natural climatic conditions of a typical spring wheat growing area. Samples of wheat matrices were collected at growth stage 49 (forage and hay) and 89 (grain and straw) for both the pyrimidinyl and phenyl label.

Storage stability investigations

The samples were homogenized and the total radioactivity was measured. Aliquots of the samples were extracted with methanol and water and the storage stability investigations of methanol extracts of wheat matrices were conducted within 6 months after treatment, corresponding to the final analyses of samples in the metabolism study of approximately 29 months.

Metabolism investigations

Corresponding samples were shipped for the metabolism investigations study to Charles River, Edinburgh (UK).

After completion of the metabolism investigations stored samples of wheat matrices were extracted with methanol to investigate the stability of stored material. The HPLC chromatograms of the stored methanol extracts from the beginning of this study and the new extracts were compared to the HPLC chromatograms conducted at the study start to evaluate the sample and extract stability.

2. Description of analytical procedures

Storage stability and metabolism investigations
Radioanalysis: Homogenized solid plant samples and due to high chemo luminescence, aliquots of separate and pooled methanol extracts of the initial extraction were added to a combustion cone containing paper tissue and combusted by means of an automatic sample oxidizer. The $^{14}CO_2$ was trapped by an absorption and scintillation liquid, and the collected radioactivity was measured by liquid scintillation counting (LSC). ^{14}C standards were combusted to determine the recovered radioactivity and the measurements were corrected accordingly (instrument factor 1.021-1.045).
Extraction: Aliquots of homogenized wheat samples were extracted with methanol for three times, each followed by centrifugation and filtration. The residues were subsequently extracted with water. The remaining methanol extracts were combined and fractionated by SPE. No further sample preparation steps were carried out for the residues after solvent extraction. The extractable residual residues were subjected to HPLC analyses. The residual radioactive residues (RRR) were determined by combustion and LSC measurement.

Metabolism investigations
Radioanalysis: For the determination of the TRR combusted, and the measurement of solid residues following solvent extraction or solubilization procedures, subsamples were combusted using a sample oxidizer. The resultant $^{14}CO_2$ was absorbed, mixed with scintillation fluid and the radioactivity determined by liquid scintillation counting. ^{14}C standards were combusted at the beginning and at regular intervals throughout each batch of analyses. Measurements of radioactivity were not corrected for oxidizer efficiency, however combustion efficiencies were between 97-100%.
Extraction: Subsamples of milled tissue were extracted three times with methanol. Each extract was separated from the residue by centrifugation and decanted, while the residue was subjected to the next extraction step. The methanol extracts were analyzed by LSC. The residue was re-extracted twice with milli-Q water in the same manner which were also analyzed by LSC. The combined results of methanol extractions and water extractions are referred to as extractable radioactive residues (ERR).
The debris remaining after solvent extraction of each sample was combusted for the determination of the residual radioactive residue (RRR).
The RRR of [Ph-^{14}C] treated wheat hay and straw and [Py-^{14}C] treated forage, hay and straw was sequentially solubilized with driselase, macerozyme/cellulase, β-glucosidase, laccase/tyrosinase and protease.
In [phenyl-U-^{14}C] treated wheat hay and straw and [pyrimidinyl-2-^{14}C] treated forage, hay and straw methanol extracts were concentrated and analyzed by HPLC. Water extracts of [pyrimidinyl-2-^{14}C] treated hay were also concentrated and analyzed by HPLC.
Two dimensional thin layer chromatography (TLC) analyses were carried out on selected extracts.

3. Identification of metabolites

Metabolism investigations

The peak assignments were generally based on comparison of the retention times of reference items with the ^{14}C-signals of the quantitative and confirmatory HPLC analyses.

The assignments of the radio-peaks were mainly based on co-chromatography with [pyrimidinyl-2-^{14}C] treated mature lettuce (see BASF DocID 2014/1000805 summarized above), where metabolites of interest had been confirmed by mass spectrometry analysis. Another liquid chromatography system was used to confirm the identity of M605F025 and for co-chromatography analysis. It was also used to demonstrate that the polar region in [phenyl-U-^{14}C] treated straw was made up of more than one component (including M605F032 and M605F033).

II. RESULTS AND DISCUSSION

A. TOTAL RADIOACTIVE RESIDUES (TRRs)

Storage stability investigations

For storage stability investigations, the total radioactive residue (TRR) was calculated by summarizing the extractable radioactive residue (ERR) and the residual radioactive residue (RRR) after solvent extraction. The calculated TRR values were set to 100% TRR for all matrices except for wheat grain, where the TRR was obtained only from direct combustion. As detected values were very low (0.002 mg/kg for the pyrimidinyl label, <0.001 mg/kg for the phenyl label), the matrix wheat grain was not further investigated.

For wheat forage, the calculated TRR accounted for 0.023 mg/kg (59 DAT, pyrimidinyl label) and 0.005 mg/kg (58 DAT, phenyl label). The highest TRR amounts were detected for wheat hay with 0.125 mg/kg for the pyrimidinyl label (59 DAT) and 0.035 mg/kg for the phenyl label (58 DAT).

The TRR of straw was 0.037 mg/kg for the pyrimidinyl label (114 DAT) and 0.028 mg/kg for the phenyl label (114 DAT).

Additionally, the TRR was measured by direct combustion followed by LSC analyses. The measured TRR of all wheat matrices showed no major differences to the calculated TRR values.

Table 6.2.1-7: Total radioactive residues (TRR) of [^{14}C]-pyrimethanil in wheat matrices following seed treatment

TRRs in treated wheat samples					
Matrix	DAT	TRR combusted [mg/kg]		TRR calculated [mg/kg]	
		Pyrimidinyl label	Phenyl label	Pyrimidinyl label	Phenyl label
Forage	58-59	0.024	0.005	0.023	0.005
Hay	58-59	0.126	0.033	0.125	0.035
Straw	114	0.036	0.027	0.037	0.028
Grain	114	0.002	<0.001	-	-

DAT = Days after treatment

Metabolism investigations
In the metabolism study, the total radioactive residue (TRR) was calculated by summing the extractable radioactive residues (ERR) and the residual radioactive residue (RRR) after solvent extraction. The calculated TRR in wheat forage was 0.008 and 0.033 mg/kg; wheat hay was 0.035 and 0.152 mg/kg; wheat straw was 0.025 and 0.037 mg/kg and in wheat grain was 0.001 and 0.002 mg/kg in the phenyl and pyrimidinyl treated samples, respectively. The calculated TRR was set to 100% TRR for all matrices.
Additionally, the TRR was measured by direct combustion analysis of the homogenized harvest tissue followed by LSC. The measured TRR of all matrices/labels showed no major differences to the calculated TRR values.

Table 6.2.1-8: Total radioactive residues (TRR) of [^{14}C]-pyrimethanil in wheat matrices following seed treatment

TRRs in treated wheat samples					
Matrix	DAT	TRR combusted [mg/kg]		TRR calculated [mg/kg]	
		Pyrimidinyl label	Phenyl label	Pyrimidinyl label	Phenyl label
Forage	58-59	0.027	0.006	0.033	0.008
Hay	58-59	0.115	0.037	0.152	0.035
Straw	114	0.031	0.017	0.037	0.025
Grain	114	0.001	0.001	0.002	0.001

DAT = Days after treatment

B. EXTRACTION, CHARACTERISATION AND IDENTIFICATION OF RESIDUES

1. Extractability of residues in wheat

Storage stability investigations
During the storage stability investigations, the extractability of wheat matrices accounted for 42.4% to 63.9% TRR. Wheat matrices were extracted with methanol and water.
For wheat forage, the extractability with methanol and water accounted for 55.6% (pyrimidinyl) and 51.5 % TRR (phenyl), with the main part being extracted with methanol (49.0% and 45.0% TRR for the pyrimidinyl and phenyl label, respectively). The extractability of wheat hay with methanol and water accounted for 57.3 % TRR for the pyrimidinyl label and 63.9 % TRR for the phenyl label. For wheat hay treated with the pyrimidinyl labelled test item, the major part of the residues was extracted with methanol (43.2% TRR), whereas for the phenyl label similar amounts were extracted with methanol and water (methanol: 30.9% TRR, water: 33.1% TRR). For the straw, 44.5% (pyrimidinyl label) and 42.4% TRR (phenyl label) were extracted with methanol and water. The residues extracted with methanol were approximately 2-fold higher than with water (pyrimidinyl: 30.1% TRR methanol and 14.4% TRR water; phenyl: 30.3% TRR methanol and 12.2% TRR water).

Table 6.2.1-9: Extractability of the total radioactivity of [^{14}C]-pyrimethanil in wheat matrices following seed treatment

Matrix	DAT	TRR calc. *	Distribution of radioactive residues				ERR		RRR	
			Methanol		Water					
		[mg/kg]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]
Pyrimidinyl label										
Forage	59	0.023	0.012	49.0	0.002	6.6	0.013	55.6	0.010	44.4
Hay	59	0.125	0.054	43.2	0.018	14.1	0.072	57.3	0.054	42.7
Straw	114	0.037	0.011	30.1	0.005	14.4	0.017	44.5	0.021	55.5
Phenyl label										
Forage	58	0.005	0.002	45.0	0.000	6.5	0.003	51.5	0.003	48.5
Hay	58	0.035	0.011	30.9	0.012	33.1	0.022	63.9	0.013	36.1
Straw	114	0.028	0.008	30.3	0.003	12.2	0.012	42.4	0.016	57.6

DAT = Days after treatment
* TRR was calculated as the sum of ERR + RRR

Metabolism investigations

In the metabolism study, the extractability was 42.4-69.1% TRR (0.006-0.067 mg/kg) for all matrices in both labels except wheat grain, where extracts contained less than the limit of quantitation (<LOQ) in the [phenyl-U-^{14}C] label and 23.9% of TRR (<0.001 mg/kg) was extracted in [pyrimidinyl-2-^{14}C] label. For both labels and all matrices, soluble radioactive residues were predominantly extracted with methanol except grain samples, where all quantifiable residues were extracted in water extracts.

Sequential enzyme incubations released between 19.7% and 24.6% of the TRR (0.006-0.009 mg/kg) in [phenyl-U-^{14}C] treated samples and between 7.9% and 19.4% of the TRR (0.002-0.026 mg/kg) in [pyrimidinyl-2-^{14}C] treated samples.

Table 6.2.1-10: Extractability of the total radioactivity of [^{14}C]-pyrimethanil in wheat matrices following seed treatment

Matrix	TRR calc. *	Distribution of radioactive residues				ERR		RRR	
		Methanol		Water					
	[mg/kg]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]
Pyrimidinyl label									
Forage	0.033	0.013	38.0	0.001	4.4	0.014	42.4	0.019	57.6
Hay	0.152	0.045	29.7	0.022	14.2	0.067	43.9	0.085	56.0
Straw	0.037	0.011	30.8	0.007	19.0	0.018	49.8	0.019	50.2
Grain	0.002	<LOQ	<LOQ	<0.001	23.9	<0.001	23.9	0.002	76.1
Phenyl label									
Forage	0.008	0.005	62.3	0.001	6.8	0.006	69.1	0.002	30.9
Hay	0.035	0.010	29.6	0.006	16.0	0.016	45.6	0.019	54.3
Straw	0.025	0.008	32.9	0.005	19.6	0.013	52.5	0.012	47.5
Grain	0.001	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.001	100.0

DAT = Days after treatment
* TRR was calculated as the sum of ERR + RRR

2. Identification and characterization of extractable residues in wheat

Metabolism investigations

[Phenyl-U-^{14}C] treated wheat hay and straw and [pyrimidinyl-2-^{14}C] treated forage, hay and straw methanol extracts contained residues of ≥0.008 mg/kg and so were concentrated and analyzed by HPLC. Water extracts of [pyrimidinyl-2-^{14}C] treated hay were also concentrated and analyzed by HPLC.

In [phenyl-U-^{14}C] treated wheat, M605F025 was the most abundant component (6.2-8.8% TRR; ≤0.003 mg/kg) identified by co-chromatography. M605F027, M605F008 and M605F036 were assigned by comparison of retention times with reference item on one HPLC system; these metabolites accounted for 0.6% to 2.6% of the TRR (≤0.001 mg/kg). The unidentified polar regions at retention times 2.70 and 3.10 minutes accounted for 6.8% and 9.5% of the TRR in straw; this was shown to be multiple components in analysis using another HPLC system. The remaining unidentified components in hay and straw were below or equal to 2.6% of the TRR.

In [pyrimidinyl-2-^{14}C] treated wheat samples, significant attempts were made at identifying the polar region at retention time 3.30-3.50 min. This was initially identified as the metabolite M605F033 by co-chromatography in one HPLC system with a reference item ([pyrimidinyl-2-^{14}C] treated mature lettuce from study BASF DocID 2014/1000805 summarized above). This was the most abundant component in all analyzed [pyrimidinyl-2-^{14}C] treated wheat matrices, accounting for 10.5% to 27.2% of the TRR (0.004-0.041 mg/kg). To investigate this region further, [pyrimidinyl-2-^{14}C] treated hay methanol extract was run on another HPLC system using reference items generated under study BASF DocID 2015/1000181 (see chapter 6.6) for co-chromatography. Results from this analysis showed the presence of both M605F032 and M605F033, indicating that this polar region consists of multiple components, including M605F032 and M605F033.

The presence of M605F032 and M605F033 was confirmed in [pyrimidinyl-2-^{14}C] treated forage, hay and straw concentrated methanol SPE elutes using a contrasting TLC system.

M605F025 was second highest component in [pyrimidinyl-2-^{14}C] treated wheat accounting for 3.3% to 6.9% of the TRR (≤0.005 mg/kg) which was identified by co-chromatography. M605F027, M605F028, M605F004 and M605F036 were assigned by comparison of retention times with reference items; metabolites accounted for 0.5% to 3.6% of the TRR. An unidentified region at retention time 2.90 min accounted for 0.6% to 7.3% of the TRR. Analysis with another HPLC system showed this to be multiple components. The remaining unidentified components were below or equal to 2.0% (0.003 mg/kg) of the TRR.

Table 6.2.1-11: Residues of [pyrimidinyl-2-^{14}C]-pyrimethanil in wheat following seed treatment at a rate of 18.9 g a.s./ha

Test substance	Pyrimidinyl label					
Sample/metabolite	Forage		Hay		Straw	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
M605F004	<0.001	0.5	-	-	-	-
M605F025	0.002	4.6	0.005	3.3	0.003	6.9
M605F027	<0.001	1.0	0.001	0.8	0.001	1.4
M605F028	0.001	3.6	0.002	1.3	<0.001	0.9
M605F032/M605F033	0.006	16.5	0.041	27.3	0.004	10.5
M605F036	<0.001	1.4	-	-	-	-
Total identified	**0.009**	**27.6**	**0.049**	**32.7**	**0.008**	**19.7**
Characterized by HPLC	0.003	8.3	0.020	13.3	0.003	10.5
Water extraction	0.001	4.4	-	-	0.007	19.0
Diselase solubilizate	0.001	3.8	0.016	10.7	0.005	14.2
Macerozyme/cellulase solubilizate	0.001	1.7	0.004	2.7	0.001	1.9
B-glucosidease solubilizate	<0.001	1.2	0.003	2.0	0.001	1.4
Tyrosine/laccase solubilizate	<0.001	0.8	0.002	1.0	0.001	1.9
Protease solubilizate	<0.001	0.4	0.001	0.4	<0.001	<0.1
Pepsin in artificial gastric juice solubilizate	<0.001	0.3	<0.001	0.3	<0.001	<0.1
Pancreatin in artificial intestinal fluid solubilizate	<0.001	0.4	<0.001	0.3	<0.001	<0.1
Total characterized	**0.006**	**21.3**	**0.046**	**30.7**	**0.018**	**48.9**
Total identified and/or characterized	**0.015**	**48.9**	**0.095**	**63.4**	**0.026**	**68.6**
Final residue	0.006	19.4	0.024	16.1	0.010	27.4
Grand total	**0.021**	**68.3**	**0.119**	**79.5**	**0.036**	**96.0**

Table 6.2.1-12: Residues of [phenyl-2-^{14}C]-pyrimethanil in wheat following seed treatment at a rate of 18.9 g a.s./ha

Test substance	Phenyl label			
Sample/metabolite	Hay		Straw	
	mg/kg	% TRR	mg/kg	% TRR
M605F025	0.003	8.8	0.001	6.2
M605F027	0.001	1.9	<0.001	0.9
M605F028	0.001	2.6	<0.001	0.6
M605F036	<0.001	0.7	-	-
Total identified	**0.005**	**14.0**	**0.001**	**7.7**
Characterized by HPLC	0.005	14.4	0.006	22.9
Water extraction	0.006	16.0	0.005	19.6
Diselase solubilizate	0.006	16.9	0.004	13.3
Macerozyme/cellulase solubilizate	0.001	4.1	0.001	3.1
B-glucosidease solubilizate	0.001	2.0	<0.001	1.3
Tyrosine/laccase solubilizate	0.001	1.6	0.001	2.0
Protease solubilizate	<0.001	<0.1	<0.001	<0.1
Pepsin in artificial gastric juice solubilizate	<0.001	<0.1	<0.001	<0.1
Pancreatin in artificial intestinal fluid solubilizate	<0.001	<0.1	<0.001	<0.1
Total characterized	**0.020**	**55.0**	**0.017**	**62.2**
Total identified and/or characterized	**0.025**	**69.0**	**0.018**	**69.9**
Final residue	0.007	20.5	0.008	32.7
Grand total	**0.032**	**89.5**	**0.026**	**102.6**

3. Proposed metabolic pathway

Metabolism investigations

Pyrimethanil (BAS 605 F) is extensively metabolized and degradation products are incorporated in to forage, hay and straw fractions of the sample tissue. The metabolism of BAS 605 F includes the following reactions:

- Hydroxylation of the parent compound;
- Formation of glucose and malonyl glucose conjugates of metabolites via hydroxylation of the parent compound;
- Cleavage of the amine bond prior to hydroxylation and conjugation;
- Cleavage of the pyrimidinyl ring

The major routes of metabolism in wheat grown from seed treated with BAS 605 F were the ring opening of the pyrimidinyl ring of parent compound BAS 605 F to form M605F025 and the cleavage of the amine bond of M605F004 to form M605F032 prior to conjugation to form the glucoside M605F033 in [pyrimidinyl-2-^{14}C] treated samples.

Minor metabolism of BAS 605 F occurs via hydroxylation on the pyrimidinyl ring of BAS 605 F to form M605F004 and M605F003. The hydrolyzed metabolites are then subject to conjugation at the hydroxyl group by a malonyl glucoside to form M605F028 and M605F036, respectively, or conjugation of a glucoside to form M605F027.

The proposed metabolic pathway of BAS 605 F in wheat is shown in Figure 6.2.1-2.

4. Storage stability

All samples were generally stored at approximately -18°C during the course of this study.

Storage stability investigations

Storage stability investigations were carried out in wheat forage for the pyrimidinyl label and wheat hay and straw for both labels. A comparison of the extractabilities and of the metabolite patterns (HPLC analyses) obtained at the beginning and at the end of the investigation period showed that there was no relevant change in the nature of the radioactive residues during storage of the samples in methanol extracts and plant materials for up to approximately 29 months.

Metabolism investigations

Wheat samples were extracted and analyzed by HPLC within a maximum of 54 days of receipt of sample, except [phenyl-U-^{14}C] treated wheat straw where sample was not initially analyzed as residues in methanol extracts were <0.010 mg/kg. The [phenyl-U-^{14}C] treated wheat straw sample was extracted after 14 days and analyzed by HPLC 104 days after sample receipt.

III. CONCLUSION

Storage stability investigations

During storage stability investigations, the highest TRR amounts were detected for wheat hay with 0.125 mg/kg for the pyrimidinyl label (59 DAT) and 0.035 mg/kg for the phenyl label (58 DAT). For wheat forage, the TRR accounted for 0.023 mg/kg (59 DAT, pyrimidinyl label) and 0.005 mg/kg (58 DAT, phenyl label). The TRR of straw was 0.037 mg/kg for the pyrimidinyl label (114 DAT) and 0.028 mg/kg for the phenyl label (114 DAT). For wheat grain, the detected TRR were very low (0.002 mg/kg for the pyrimidinyl label, 0.001 mg/kg for the phenyl label). Therefore this matrix was not further investigated.

A comparison of the extractabilities and of the metabolite patterns (HPLC analyses) obtained at the beginning and at the end of the investigation period, which corresponded to the investigation period of samples for the BAS 605 F metabolism study, showed that there was no relevant change in the nature of the radioactive residues during storage of the samples in methanol extracts and plant materials over a period of approximately 29 months.

Metabolism investigations

In the metabolism study, the calculated TRR of [phenyl-U-^{14}C] wheat was between 0.008 and 0.035 mg/kg and the calculated TRR of [pyrimidinyl-2-^{14}C] wheat was between 0.033 and 0.152 mg/kg for forage, hay and straw. Low levels of TRR were detected in grain samples of both labels between 0.001 and 0.002 mg/kg.

Solubilization of the residues after solvent extraction was conducted with enzymes on the post extraction solids where remaining radioactive residues (RRR) were greater than 0.010 mg/kg. The enzymes released between 19.7% and 24.6% of the TRR in [phenyl-U-^{14}C] treated wheat and between 7.9% and 19.4% of the TRR in [pyrimidinyl-2-^{14}C] treated samples. To investigate bioavailability of the RRR, the remaining tissue was treated with pepsin in artificial gastric juice followed by pancreatin in artificial intestinal fluid. This released very small levels of radioactive residues showing that the RRR was not readily bioavailable. This resulted in a final unextracted non-bioavailable residue level of 20.5-32.7%TRR in [phenyl-U-^{14}C] treated samples and 16.1-27.4% TRR in [pyrimidinyl-2-^{14}C] treated samples.

In [phenyl-U-^{14}C] treated wheat, M605F025 was the most abundant component (6.2-8.8% TRR). M605F027, M605F028 and M605F036 accounted for 0.6% to 2.6% of the TRR. The unidentified polar regions at retention times 2.90 and 3.50 minutes accounted for 6.8% and 9.5% of the TRR in straw contained multiple components. The remaining unidentified components in hay and straw were below or equal to 2.6% of the TRR.

In [pyrimidinyl-2-^{14}C] treated wheat samples, the polar region at retention time 3.30-3.50 min was initially identified as the metabolite M605F033 by co-chromatography in one HPLC system. This was the most abundant component in all analyzed [pyrimidinyl-2-^{14}C] treated wheat matrices, accounting for 10.5% to 27.2% of the TRR. To investigate this region further, [pyrimidinyl-2-^{14}C] treated hay methanol extract was run on another HPLC system. Results from this analysis showed the presence of both M605F032 and M605F033, confirmed using a contrasting TLC method, indicating that this polar region consists of multiple components, including M605F032 and M605F033.

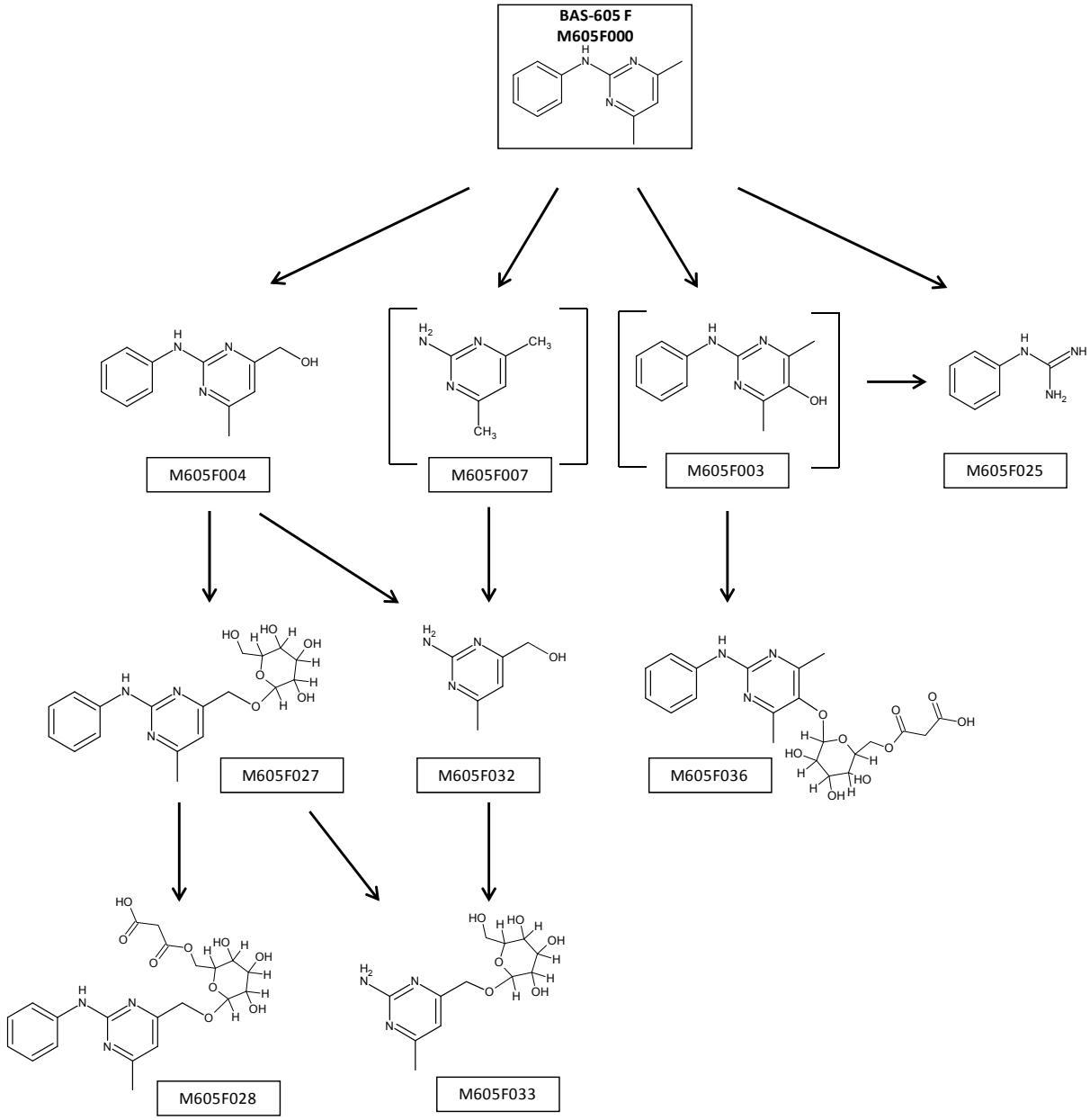
M605F025 was the second largest component in [pyrimidinyl-2-^{14}C] treated wheat accounting for 3.3% to 6.9% of the TRR. M605F027, M605F028, M605F004 and M605F036 accounted for 0.5% to 3.6% of the TRR. An unidentified region at retention time 2.90 accounted for 0.6% to 7.3% of the TRR. This was shown to be multiple components. The remaining unidentified components were below or equal to 2.0% of the TRR.

The metabolism of BAS 605 F includes the following reactions:

- Hydroxylation of the parent compound
- Formation of glucose and malonyl glucose conjugates of metabolites via hydroxylation of the parent compound.
- Cleavage of amine bond
- Ring opening of the pyrimidine ring

The degradation products of BAS 605 F are eventually incorporated into natural products.

Figure 6.2.1-2: Proposed metabolic pathway of pyrimethanil in wheat



CA 6.2.2 Poultry

No poultry metabolism study was conducted for the previous Annex I inclusion process. According to Reg. (EU) No 283/2013, metabolism studies on poultry shall be provided where the plant protection product is to be used in crops whose parts or products, also after processing, are fed to poultry and where the intake is expected to exceed 0.004 mg/kg bw/day. For the feed burden calculation see chapter 6.7. To fulfill the current data requirements, a new study has been performed.

Report:	CA 6.2.2/1 [redacted] 2015a The metabolism of 14C-Reg. No. 236999 (BAS 605 F) in laying hens 2014/1000807
Guidelines:	EPA 860.1300: Nature of the Residue in Plants Livestock, EEC 91/414 (7030(VI/95 Rev. 3), OECD Test Guideline 503 - Metabolism in livestock
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Description: [Pyrimidinyl-2-^{14}C]-BAS 605 F (pyrimidinyl label)
[Pyrimidinyl-1,3-^{15}N]-BAS 605 F
Unlabeled BAS 605 F

Lot/Batch #: 1049-1010 (^{14}C-pyrimidinyl label)
1050-0101 (^{15}N-pyrimidinyl label)
486213 (unlabeled)

Purity: Radiochemical purity: 99.7% (^{14}C-pyrimidinyl label)
Specific activity: 6.42 MBq/mg (^{14}C-pyrimidinyl label)
2.27 MBq/mg (pyrimidinyl mixture)

CAS#: 53112-28-0

Development code: 236999 (Reg. No)

Stability of test compound: The test item was stable for the test period

2. Test Animals

Species:	Hen
Variety:	Isa Warren
Gender:	Female
Age:	About 26 weeks
Weight at dosing:	1.657-2.065 kg
Number of animals:	10
Acclimation period:	8 days
Diet:	Non-medicated feed ("Golden Yolk Layer pellets"), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	In metabolism cages
Environmental conditions	
Temperature:	21-25°C during dosing
Humidity:	16-60% during dosing (The low humidity results recorded are of no welfare concern to the birds as they had ad libitum access to water)
Photoperiod:	16 h light / 8 h dark

B. STUDY DESIGN AND METHODS

1. Dosing regime

Oral:	**Amount of dose:**	12 mg/kg feed/day (nominal)
	Food consumption:	151-174 g food (dry weight)/animal/day (excludes day 14 since no fill 24 h period)
	Vehicle:	Gelatin capsule by oral gavage
	Timing:	Once daily
	Duration:	14 days

2. Sample collection

Egg collection:	Twice daily
Excreta collection:	Daily
Interval from last dose to sacrifice:	Approximately 6 h
Tissues harvested & analyzed:	Liver, peritoneal fat, subcutaneous fat (with skin attached), combined leg and thigh muscle, breast muscle, GI tract and contents, bile and carcass, blood

3. Test system

The metabolism and distribution of pyrimethanil was investigated in ten laying hens following a repeated oral administration of [pyrimidinyl-2-^{14}C]-BAS 605 F at a dose level of nominally 12 mg/kg feed for 14 consecutive days. The test item was prepared in gelatin capsules and administered orally by gavage. The mean daily dose administered was 11.26 mg/kg food consumed (dry weight equivalent). Details of the study outline are summarized in Table 6.2.2-1.

Table 6.2.2-1: Dosing of laying hens with ^{14}C-pyrimethanil

Animal	Treatment days	Nominal daily dose	Actual daily dose (mean)	
		mg/kg feed intake	mg/kg feed intake[1]	mg/kg bw[2]
1-10	14	12	9.348-15.843	0.851-1.042

1 Mean excludes day 14 since no full 24 h period
2 Based on mean body weight on study days 1 and 14 for animals 1-10 as well as on mean total test item in capsule (1.761 mg)

4. Sampling and Storage

Excreta were collected prior to dose administration and at 24 h intervals thereafter until the time of sacrifice. The daily excreta samples were pooled. Following each excreta collection cages were rinsed with the minimum amount of methanol/water (1:1 v/v). The daily cagewash samples were pooled.
Eggs were collected pre-dose and then twice daily until sacrifice (am and pm) during the dosing period. Eggs were separated into yolk and white. A composite egg yolk sample was prepared by combining the yolk samples from each hen. Similarly a composite egg white sample was prepared by combining the individual egg white samples. Egg shells were retained but were not analyzed.
The hens were sacrificed 6 h following administration of the 14th and final dose. The following samples were retained *post mortem*: blood (collected into appropriate anti-coagulant blood tubes), liver, peritoneal fat, subcutaneous fat, leg and thigh muscle, breast muscle, GI tract and contents, bile, partially formed eggs, carcass.

Samples were analyzed initially using HPLC. Egg yolk and liver extracts contained significant radiolabeled residues and were analyzed using HPLC after all other experimental work was complete to investigate the stability of radiolabeled components during storage.

5. Description of analytical methods

For the determination of the measured TRR, homogenized material (excreta with addition of methanol; tissues with the aid of dry ice; eggs; blood) was combusted by means of a sample oxidizer before LSC measurement. For samples not requiring combustion (bile and cage wash), radioactivity was directly determined by mixing with a suitable scintillation fluid and LSC analysis.
Fat was dissolved in dichloromethane. The dichloromethane soluble fraction was dried under nitrogen, then reconstituted in hexane and partitioned with methanol/water (4:1 v/v). The dichloromethane insoluble fraction was extracted using methanol and methanol/water (4:1 v/v and 3:7 v/v). Liver, egg yolk, muscle and egg white were also extracted using methanol and methanol/water (4:1 v/v and 3:7 v/v).
Analysis of solvent extracts of yolk and tissue extracts and quantitation of metabolites in egg yolk, egg white, liver, muscle and fat was performed by HPLC.
Polar metabolites were analyzed by radio-TLC which allowed identification of M605F025 by co-chromatography with the reference standard. Reference standards were visualized by quenching under UV light (254 nm).
Egg yolk, egg white, liver, muscle and fat extracts were analyzed by HPLC-MS to confirm assignments made by co-chromatography. Where metabolites were detected in several samples the most representative data which were obtained from analysis of egg yolk and egg white was reported.

II. RESULTS AND DISCUSSION

A. TOTAL RADIOACTIVE RESIDUES (TRRs)

In the analytical phase of the study, the TRR was calculated by summarizing the extractable radioactive residue (ERR) and the residual radioactive residue after solvent extraction (RRR). The calculated TRR was then set to 100%. The amounts of radioactivity which were detected in matrices relevant for the metabolism investigations are summarized in Table 6.2.2-2.
The majority of the radioactivity dosed was recovered in excreta. Approximately 5-7% of the total dose administered was recovered in excreta each day with 94.99% total dose recovered in excreta collected throughout the dosing period. The concentration of radioactivity in excreta ranged from 3.366-11.691 mg/kg/day.
Radioactive residues in egg yolks increased steadily to a plateau of ca 0.050 mg/kg after 7 days. Residues in egg whites were lower and reached a plateau of ca 0.006 mg/kg after 2 days. Recovery of radioactivity in egg yolks and egg whites accounted for <0.01% of the dose. Egg yolks and egg whites collected from the plateau phase (day 8-13) were combined to produce a single egg yolk sample and a single egg white sample.
Residues in liver were 0.336 mg/kg. Residues in fat were 0.031 and 0.040 mg/kg in peritoneal fat and subcutaneous fat and skin, respectively. Residues in muscle were 0.010 mg/kg and 0.011 mg/kg in breast muscle and leg and thigh muscle, respectively. The residues were comparable in both fat types and in both muscle types and for that reason these tissues were pooled to produce a single fat sample and a single muscle sample for investigation of the nature of the residue.

The TRR of all matrices was also measured by combustion analysis. For all matrices, the TRR measured was similar to the TRR calculated.

Table 6.2.2-2: Total radioactive residues in edible matrices after dosing of laying hens with [pyrimidinyl-2-^{14}C]-pyrimethanil

Matrix	TRR [mg/kg]	
	Measured	Calculated
Liver	0.300	0.336
Breast muscle	0.010	0.011[2]
Leg and thigh muscle	0.011	
Skin with fat	0.040	0.034[2]
Peritoneal fat	0.031	
Egg yolk	0.050[1]	0.052
Egg white	0.006[1]	0.006

TRR: Total radioactive residue
Calculated: Sum of ERR + RRR
1 Theoretical radioactivity in pooled sample
2 The TRR in the individual fat and muscle types was similar and for that reason pooled fat and pooled muscle samples were prepared. TRR was determined by sum of extractable and non-extractable residues and by direct measurement. By direct measurement the TRR was 0.012 mg/kg for the pooled muscle and 0.037 mg/kg for the pooled fat.

B. EXTRACTION OF RESIDUES

Egg yolk, liver, muscle and fat were extracted with methanol and methanol/water mixtures. Sub-samples of extracts were concentrated to a smaller volume and the recovery of radioactivity monitored by LSC analysis throughout sample processing.
Soluble radioactive residues extracted with methanol and methanol/water mixtures in egg and tissues ranged from 50.9% TRR (0.171 mg/kg) in liver to 95.3% TRR (0.033 mg/kg) in fat. Radioactive residues remaining after solvent extraction accounted for 14.5% TRR (0.001 mg/kg) and 11.9% TRR (0.006 mg/kg) for egg white and yolk, respectively, 49% TRR (0.165 mg/kg) for liver, 4.7% TRR (0.002 mg/kg) for fat and 14.9% TRR (0.002 mg/kg) for muscle. The nature of non-extractable residues in egg yolk, liver and muscle was investigated by digestion with protease enzyme. This released an additional 7.7% TRR (0.004 mg/kg) from egg yolk, 12.3% TRR (0.041 mg/kg) for liver and 7.5% TRR (0.001 mg/kg) for muscle. It was assumed that the remaining radioactivity was bound to the liver structure and was not bioavailable.

Table 6.2.2-3: Extractability of edible matrices with solvents (methanol, methanol/water) after dosing of laying hens with [pyrimidinyl-2-^{14}C]-pyrimethanil

Matrix	TRR (calculated) mg/kg	Solvent extract (ERR)		RRR	
		mg/kg	% TRR	mg/kg	% TRR
Egg White	0.006	0.005	85.5	0.001	14.5
Egg Yolk	0.052	0.046	88.0	0.006	11.9
Liver	0.336	0.171	50.9	0.165	49
Fat	0.034	0.033	95.3	0.002	4.7
Muscle	0.011	0.009	85.1	0.002	14.9

TRR: Total radioactive residue (sum of ERR + RRR), set as 100% TRR
ERR Extractable radioactive residue (solvents: methanol, methanol/water)
RRR: Residual radioactive residue after solvent extraction (solvents: methanol, water/methanol)

C. IDENTIFICATION AND CHARACTERIZATION OF RESIDUES

A summary of all identified metabolites and their distribution in egg, muscle, fat and liver is given in Table 6.2.2-4.

Unchanged BAS 605 F was not detected in any of the samples analyzed. The main residues identified in egg and edible tissues were M605F002 (0.001-0.026 mg/kg, 5.1-44.8% TRR), M605F023 (<0.001-0.060 mg/kg, 5.0-22.6% TRR) and M605F035 (0.001-0.024 mg/kg, 7.1-21.9% TRR). M605F006 was a minor metabolite identified in egg white (<0.001 mg/kg, 5.0% TRR) and in liver (0.018 mg/kg, 5.3% TRR).

Investigation of the minor unknown components with RT *ca* 12.9 min and 14.4 min indicated the presence of mass spectra for *m/z* 214 and 230, respectively. The structure of these metabolites could not be assigned from the mass spectra although it was clear from the isotope pattern and the accurate mass that these ions were related to BAS 605 F. These metabolites were minor (≤0.003 mg/kg, ≤12.7% TRR) and the characterization achieved by mass spectrometry was sufficient. Metabolites with retention time <5 min by radio-HPLC were characterized as polar in nature. These were not adequately retained to allow identification by HPLC. Polar metabolites were isolated and analyzed by radio-TLC which identified the metabolite M605F025 by co-chromatography with the reference standard Reg. No 102020 (M605F025). M605F025 accounted for 0.004 mg/kg (1.3% TRR).

Table 6.2.2-4: Summary of identified and characterized residues in edible matrices of laying hens after dosing with [pyrimidinyl-2-^{14}C]-BAS 605 F

Components	Egg yolk		Egg white		Whole egg[1]		Liver		Muscle		Fat	
	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]
M605F002	44.8	0.023	23.9	0.001	40.3	0.008	7.7	0.026	5.1	0.001	25.8	0.009
M605F023	22.6	0.012	5.0[3]	<0.001[3]	18.8[4]	0.004[4]	17.9	0.060	10.8	0.001	8.0	0.003
M605F006	-	-					5.3	0.018	-	-	-	-
M605F035	11.8	0.006	12.6	0.001	12.0	0.002	7.1	0.024	19.5	0.002	21.9	0.007
Total identified	**79.2**	**0.041**	**41.5**	**0.002**	**71.1**	**0.014**	**38.0**	**0.128**	**35.4**	**0.004**	**55.7**	**0.019**
Polar region (RT <5 min)	-	-	14.9[2]	0.001[2]	3.2[2]	<0.001[2]	1.5[5]	0.005[5]	29.8[6]	0.003[6]	2.4	0.001
Characterized unknown, *m/z* 214	-	-	12.7	0.001	2.7	0.001	1.0	0.003	-	-	-	-
Characterized unknown, *m/z* 230	-	-	4.6	<0.001	1.0	<0.001	0.5	0.002	-	-	1.6	0.001
Unknown at RT ca 9.38 min	-	-	2.4	<0.001	0.5	<0.001	-	-	-	-	-	-
Unknown at RT ca 17.13 min	-	-	-	-	-	-	2.0	0.007	-	-	-	-
Unknown at RT ca 17.63 min	-	-	-	-	-	-	2.3	0.008	-	-	-	-
Unknown at RT ca 21.38 min	-	-	-	-	-	-	-	-	-	-	3.1	0.001
Unknown at RT ca 22.63 min	-	-	0.8	<0.001	0.2	<0.001	-	-	-	-	-	-
Unknown at RT ca 23.38 min	-	-	-	-	-	-	1.3	0.004	-	-	-	-
Unknown at RT ca 26.88 min	-	-	-	-	-	-	-	-	-	-	1.2	<0.001
Unknown at RT ca 28.88 min	-	-	-	-	-	-	1.6	0.005	-	-	-	-
Unknown at RT ca 31.38 min	-	-	1.2	<0.001	0.3	<0.001	-	-	-	-	-	-
Unknown at RT ca 31.63 min	-	-	-	-	-	-	-	-	8.5	0.001	-	-
Unknown at RT ca 33.38 min	-	-	0.5	<0.001	0.1	<0.001	-	-	-	-	-	-
Unknown at RT ca 34.13 min	-	-	-	-	-	-	-	-	-	-	1.5	<0.001
Unknown at RT ca 35.88 min	-	-	-	-	-	-	-	-	5.1	0.001	-	-
Unknown at RT ca 36.63 min	-	-	-	-	-	-	-	-	7.7	0.001	-	-
Unknown at RT ca 39.88 min	-	-	0.6	<0.001	0.1	<0.001	-	-	-	-	-	-
DCM insoluble extract	-	-	-	-	-	-	-	-	-	-	5.6	0.002

Table 6.2.2-4: Summary of identified and characterized residues in edible matrices of laying hens after dosing with [pyrimidinyl-2-^{14}C]-BAS 605 F

Components	Egg yolk		Egg white		Whole egg[1]		Liver		Muscle		Fat	
	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]
Hexane fraction	-	-	4.0	<0.001	n.r.	n.r.	-	-		-	18.3	0.006
Total characterized	-	-	41.7	0.001	8.1[7]	0.002	10.2	0.034	51.1	0.006	33.7	0.011
Total identified and/or characterized	**79.2**	**0.041**	**83.2**	**0.003**	**79.2**	**0.016**	**48.2**	**0.162**	**86.5**	**0.010**	**89.4**	**0.030**
Released by protease	7.7	0.004	-	-	n.r.	n.r.	12.3	0.041	7.5	0.001	-	-
Final residue	4.2	0.002	14.5	0.001	n.r.	n.r.	36.7[8]	0.123[8]	7.4	0.001	4.7	0.002
Grand total	**91.1**	**0.047**	**97.7**	**0.004**	n.r.	n.r.	**97.2**	**0.327**	**101.4**	**0.012**	**94.1**	**0.032**

1 Residues in whole egg were calculated from the total residues in yolk and white.
2 Comprised of at least 4 components, none of which individually exceed 6.9% TRR, <0.001 mg/kg
3 Spectra for both M605F023 and M605F006 were observed in this region which indicated that both metabolites were present but were not resolved. In total, these unresolved metabolites accounted for 5.0% TRR (<0.001 mg/kg) and for that reason it was not necessary to attempt to resolve and quantify the individual metabolites.
4 M605F006 was unresolved from M605F023 in egg white but was not detected in egg yolk. Based on the low TRR for these metabolites in egg white and the detection of only M605F023 in egg yolk, M605F006 was a minor metabolite in egg.
5 Comprised of M605F025 which accounted for 0.004 mg/kg (1.3% TRR).
6 Comprised of at least 3 components, none of which individually exceed 12.0% TRR, 0.001 mg/kg
7 Characterized from ERR
8 It is assumed that the remaining radioactivity was bound to the liver structure and was not bioavailable.
n.r. Not reported
Note: Investigation of components with RT ca 12.88 min and 14.38 min indicated the presence of mass spectra for m/z 214 and m/z 230, respectively. The structure of these metabolites could not be assigned from the mass spectra although it was clear from the isotope pattern and the accurate mass that these ions were related to BAS 605 F.

1. Metabolic pathway

The proposed metabolic pathway of BAS 605 F in hen is shown in Figure 6.2.2-1:
BAS 605 F was extensively metabolized in the laying hen. Unchanged parent BAS 605 F was not detected in egg yolk or white or in edible tissues.

The metabolism of BAS 605 F includes the following reactions:

- Hydroxylation of the parent compound
- Formation of glucuronide and sulphate conjugates of metabolites via hydroxylation of the parent compound
- Cleavage of the pyrimidine ring

2. Storage stability

Profiles for egg yolk and liver at initial analysis and after all other experimental work was complete were comparable. Some minor differences in retention time and resolution were observed and these were deemed to be due to inter-assay variation through use of different instrumentation used for the initial profiling and the stability investigation. The main metabolites identified in the hen were the glucuronide conjugate M605F023 and the sulphate conjugate M605F035 which would not be formed by degradation. The presence of these metabolites and the similarity between the initial analysis and the stability analysis confirmed that stability during storage was acceptable.

III. CONCLUSION

Approximately, 99.3% of the total dose was recovered, the majority of which was present in the excreta (95.0%).

Radioactive residues in egg yolks increased steadily to a plateau of *ca* 0.050 mg/kg after 7 days. Residues in egg whites were low and increased slowly to a plateau of *ca* 0.020 mg/kg after 7 days. Recovery of radioactivity in egg yolks and egg whites accounted for <0.01% of the dose.

Measured residues in liver were 0.300 mg/kg. Residues in fat were 0.031 and 0.040 mg/kg in peritoneal fat and subcutaneous fat and skin, respectively. Residues in muscle were 0.010 mg/kg and 0.011 mg/kg in breast muscle and leg and thigh muscle, respectively. The residues were comparable in both fat types and in both muscle types and for that reason these tissues were pooled to produce a single fat sample and a single muscle sample for investigation of the nature of the residue.

Soluble radioactive residues extracted with methanol and methanol/water mixtures in egg and tissues ranged from 50.9% TRR in liver to 95.3% TRR in fat. Radioactive residues remaining after solvent extraction accounted for 14.5% TRR and 11.9% TRR for egg white and yolk, respectively, 49% TRR for liver, 4.7% TRR for fat and 14.9% TRR for muscle. The nature of non-extractable residues in egg yolk, liver and muscle was investigated by digestion with protease enzyme. This released an additional 7.7% TRR from egg yolk, 12.3% TRR for liver and 7.5% TRR for muscle. It was assumed that the remaining radioactivity was bound to the liver structure and was not bioavailable.

Unchanged BAS 605 F was not detected in any of the samples analyzed. The main residues identified in egg and edible tissues were M605F002 (5.1-44.8% TRR), M605F023 (5.0-22.6% TRR) and M605F035 (7.1-21.9% TRR). M605F006 was a minor metabolite identified in egg white (5% TRR) and in liver (5.3% TRR).

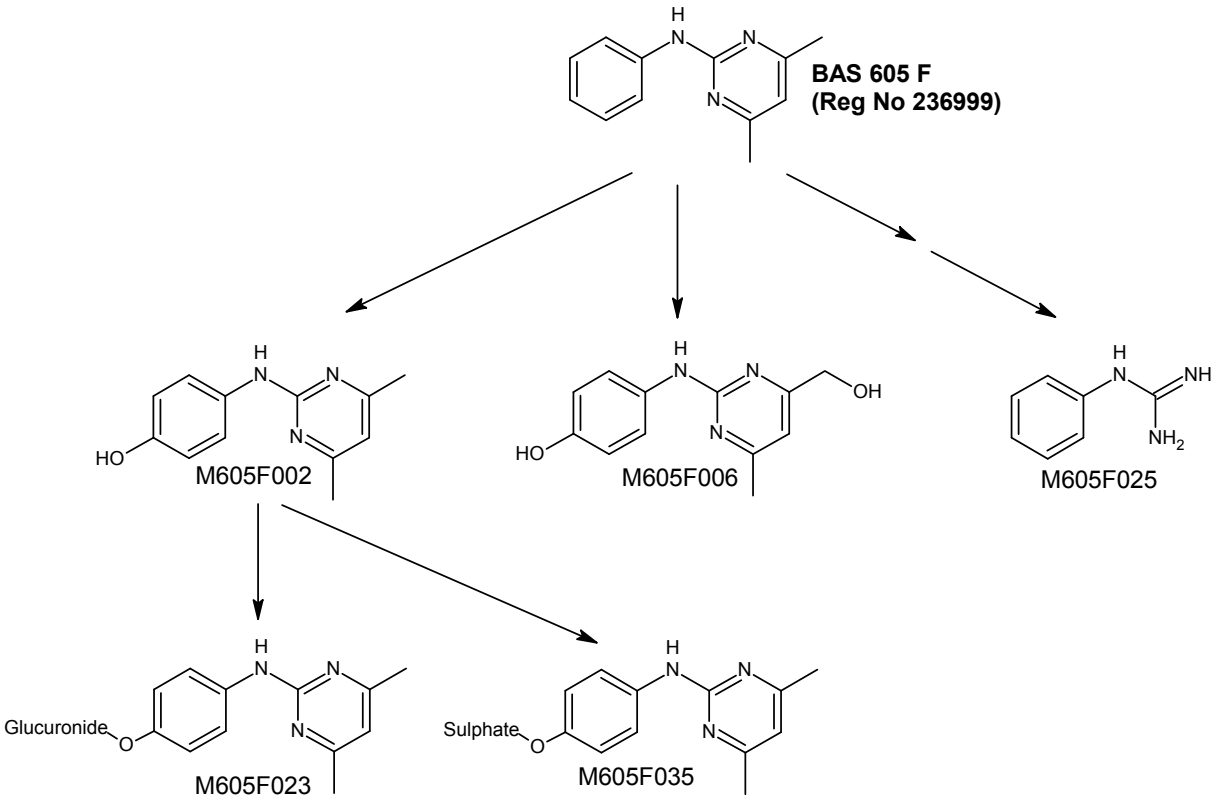
Investigation of the minor unknown components with RT *ca* 12.9 min and 14.4 min indicated the presence of mass spectra for *m/z* 214 and 230, respectively. The structure of these metabolites could not be assigned from the mass spectra although it was clear from the isotope pattern and the accurate mass that these ions were related to BAS 605 F. These metabolites were minor (≤0.003 mg/kg, ≤12.7% TRR) and the characterization achieved by mass spectrometry was sufficient. Metabolites with retention time <5 min by radio-HPLC were characterized as polar in nature. These were not adequately retained to allow identification by HPLC. Polar metabolites were isolated and analyzed by radio-TLC which identified the metabolite M605F025 by co-chromatography.

BAS 605 F was extensively metabolized in the laying hen. Unchanged parent BAS 605 F was not detected in egg yolk or white or in edible tissues.

The metabolism of BAS 605 F includes the following reactions:

- Hydroxylation of the parent compound
- Formation of glucuronide and sulphate conjugates of metabolites via hydroxylation of the parent compound
- Cleavage of the pyrimidine ring

Figure 6.2.2-1: Proposed metabolic pathway of BAS 605 F in hen



CA 6.2.3 Lactating ruminants

The nature of pyrimethanil residues in commodities of animal origin was investigated in the framework of Directive 91/414/EEC.

A cow metabolism study conducted with labeled pyrimethanil was evaluated during the original Annex I inclusion process (Study A81627, see Annex II chapter 6.2.1.2).

The following conclusion is copied from the EFSA Reasoned Opinion on the re-evaluation of established MRLs according to Art.12 of Regulation 396/2005 (EFSA Journal 2011;9(11):2454), the new Metabolite Codes were added for the readers convenience:
"One lactating cow was dosed with 0.4 mg/kg bw/d of labelled pyrimethanil. In the study, no sign of accumulation was observed. Pyrimethanil itself could not be identified in any of the tissues investigated. The major metabolite present in milk was identified as being SN 614 277 *M605F003* (64% TRR). In kidney, 46% of the TRR was identified as SN 614 276 *M605F002*. In both tissues, others metabolites were characterized as highly polar. Residues in muscle and fat were too low for isolation and identification. In liver the extractability of residues was low and the extracted material was mainly incorporated in low molecular weight proteins and peptides as well as in RNA and DNA.
As uses considered during the peer review did not result in exposure of livestock and consequently no residues were expected in foods of animal origin, EFSA did not take position on the similarity between rat and ruminant metabolism and on the fat solubility of pyrimethanil in its conclusions. However, in 2007, the JMPR concluded that the metabolism in ruminant and rat is similar and that the residue is not fat-soluble; EFSA agrees with this proposal. "

The study is only partly meeting today's requirements; an insufficient identification rate was achieved in liver. Therefore a new goat metabolism study (2014/1000806) with two labels has been performed.

In general, the metabolism in both studies is comparable. In the **new goat study** (pyrimidinyl and phenyl label), structure elucidation was performed using state-of-the-art technology in unaltered samples, which enable the identification of phase II metabolites and minor transformation steps. In the peer-reviewed cow study (phenyl label), samples were hydrolyzed and treated before structure elucidation, which results in identification of the phase I metabolites (aglycons) rather than phase II metabolites.
In neither study parent pyrimethanil was identified, and no indication of accumulation was observed. Radioactivity in muscle and fat was too low (<0.01 mg/kg) for further investigation.
The deficiency of poor identification rate in liver was solved in the new study. Several hydroxylated and conjugated metabolites were identified in liver. In the polar region, M605F025 (phenylguanidine) was identified with up to 10.4% TRR (0.059 mg/kg). This metabolite was also found in kidney and milk. Another new metabolite, M605F034 (pyrimethanil carboxylic acid) was detected in kidney (up to 19.8% TRR; 0.125 mg/kg) and liver (2.7% TRR; 0.014 mg/kg).
In the peer-reviewed metabolism study on cows, the main metabolites were M605F002 in tissues and M605F003 in milk. In the new study on goats, M605F023, the glucuronide conjugate of metabolite M605F002, was the predominant component in ruminant liver, kidney and milk, in combination with M605F034 for kidney and M605F021, the sulfate conjugate of M605F003, for milk.

In brief, the metabolic pathway derived from the new study is comparable the peer-reviewed one. Further conjugated compounds were detected in addition to ring cleavage and oxidation to carboxylic acid:

- Mono- and di-hydroxylation of the parent compound
- Formation of glucuronide and sulphate conjugates of metabolites via hydroxylation of the parent compound
- Cleavage of the pyrimidine ring
- Oxidation of the hydroxylated parent to produce a carboxylic acid metabolite

Report: CA 6.2.3/1
[redacted] 2015b
The metabolism of [14C]-Reg.No. 236999 (BAS 605 F) in lactating goats
2014/1000806
Guidelines: EPA 860.1300, OECD Test Guideline 503 - Metabolism in livestock, EEC 91/414 (7030/VI/95)
GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Description: [Pyrimidinyl-2-^{14}C]-BAS 605 F (pyrimidinyl label)
[Pyrimidinyl-1,3-^{15}N]-BAS 605 F
[Phenyl-U-^{14}C]-BAS 605 F (phenyl label)
[Phenyl-1,2,3,4,5,6-^{13}C]-BAS 605 F
Unlabeled BAS 605 F

Lot/Batch #: 1049-1010 (^{14}C-pyrimidinyl label)
1050-0101 (^{15}N-pyrimidinyl label)
1036-1011 (^{14}C-phenyl label)
1052-1003 (^{13}C-phenyl label)
486213 (unlabeled)

Purity: Radiochemical purity: 99.7% (^{14}C-pyrimidinyl label)
99.2% (^{14}C-phenyl label)
Specific activity: 6.42 MBq/mg (^{14}C-pyrimidinyl label)
1.91 MBq/mg (pyrimidinyl mixture)
8.53 MBq/mg (^{14}C-phenyl label)
1.59 MBq/mg (phenyl mixture)

CAS#: 53112-28-0
Development code: 236999
Stability of test compound: The test item was stable for the test period

2. Test Animals

Species:	Goat
Variety:	"Saanen cross Toggenburg"
Gender:	Female
Age:	Not reported
Weight at dosing:	71.0-78.5 kg
Number of animals:	4 (2 per label)
Acclimation period:	9-11 days
Diet:	2 x 0.5 kg non-medicated concentrate + hay *ad libitum*
Water:	Drinking water, *ad libitum*
Housing:	Individually in pens during acclimation; in metabolism cages during dosing
Environmental conditions	
Temperature:	15-20°C
Humidity:	19-59% (The low humidity results recorded are of no welfare concern to the animals as they had ad libitum access to water)
Photoperiod:	Not reported

B. STUDY DESIGN AND METHODS

1. Dosing regime

Oral:	**Amount of dose:**	12 mg/kg feed/day (nominal)
	Food consumption:	0.871-2.545 kg/animal/day (during dosing)
	Vehicle:	Gelatin capsule by oral gavage
	Timing:	Once daily
	Duration:	8 days

2. Sample collection

Milk collection:	Twice daily
Urine and feces collection:	Daily
Interval from last dose to sacrifice:	3 h
Tissues harvested & analyzed:	Liver, kidneys, omental fat, renal fat, subcutaneous fat, flank muscle, loin muscle, GI tract and contents, bile, blood, carcass

3. Test system

The metabolism and distribution of pyrimethanil was investigated in four lactating goats following a repeated oral administration of [pyrimidinyl-2-^{14}C]- or [phenyl-2-^{14}C]-BAS 605 F at a dose level of nominally 12 mg/kg feed for 8 consecutive days. The test item was prepared in gelatin capsules and administered orally by gavage to two goats per label. Based on days 1 to 7, the mean achieved daily dose administered was 11.7-12.7 mg/kg food consumed (dry weight equivalent). Details of the study outline are summarized in Table 6.2.3-1.

Table 6.2.3-1: Dosing of lactating goats with ^{14}C-pyrimethanil

Animal	Treatment days	Nominal daily dose	Actual daily dose		Sacrifice time after last dose (hours)
		mg/kg feed intake	mg/kg feed intake[1]	mg/kg bw[2]	
Pyrimidinyl-2-^{14}C label					
3+4	8	12	12.668-12.605	0.172-0.280	3
Phenyl-U-^{14}C label					
1+2	8	12	11.672-12.706	0.277-0.322	3

1 Mean excludes day 8 since no full 24 h period
2 Based on mean body weight on study days -1 and 8 for animals 1-4 (68.0-69.5 kg for animals 3+4, 73.8-77.8 kg for animals 1-2) as well as on mean total test item in capsule (11.719-19.456 mg for animals 3+4, 20.425-25.013 mg for animals 1+2)

4. Sampling and storage

Blood samples were taken prior to first dose and at 1, 2, 3, 4, 6, 8, 10, 12 and 24 h post first dose. Urine and feces samples were collected for the 24 h period prior to first dose and for each 24 h period until sacrifice. The goat was milked twice daily and immediately prior to sacrifice. Considering the time of the maximum residue concentration in plasma (t_{max}) at approximately 3 h post final dose the goat was sacrificed and edible tissues (liver, kidney, muscle and fat), bile, blood and the GI tract collected.

Liver, kidney and milk extracts were analysed by HPLC within 6 months of sample collection. Liver, kidney and milk extracts were analyzed using HPLC after all other experimental work was complete to investigate the stability of radiolabeled components during storage.

5. Description of analytical methods

For measurement of radioactivity, solid samples were combusted by means of a sample oxidizer. The resultant $^{14}CO_2$ generated was absorbed and mixed with scintillation fluid. Samples were then subject to LSC analysis. Liquid and fat samples were directly analyzed by LSC.
A sample of each milk pool was partitioned with dichloromethane. Separation of the aqueous miscible and organic fractions for the phenyl label milk sample was assisted by addition of water. The organic fraction was decanted and partitioned with dichloromethane/water. The aqueous fraction was extracted using methanol and methanol/water (4:1 v/v and 3:7 v/v). More concentrated milk extracts were prepared to aid metabolite identification. Bulk milk samples were partitioned several times with dichloromethane; then the aqueous fraction was extracted using methanol. The methanol extract was concentrated, the concentrated extract partitioned with hexane and the resulting aqueous fraction filtered such that the final sample was suitable for radio-LC-MS analysis.
Liver, kidney and feces were extracted using methanol and methanol/water (4:1 v/v and 3:7 v/v).
Analysis of solvent extracts of milk and tissue extracts and quantitation of metabolites in urine, feces, milk and tissues was performed by HPLC.
Polar metabolites were analyzed by radio-TLC which afforded better resolution of metabolites and allowed identification of M605F025 by co-chromatography with the reference standard. Reference standards were visualized by quenching under UV light (254 nm).
Milk, liver and kidney were analyzed by HPLC-MS to confirm assignments made by co-chromatography. Where metabolites were detected in several samples the most representative data were reported.

II. RESULTS AND DISCUSSION

A. TOTAL RADIOACTIVE RESIDUES (TRRs)

For goats 1 and 2 given phenyl labeled [^{14}C]-BAS 605 F approximately 93%-99% of the total dose was recovered. The majority of the radioactivity was present in the urine (60-71% dose) and feces (16%-20% dose). There was also a large proportion remaining in the GI tract contents (6.3-9.4%). Radioactivity in the cagewash accounted for 2.4%-6.0% dose. Radioactivity associated with edible portions (milk and tissues) accounted for ≤0.72% of the total dose.
For goats 3 and 4 given pyrimidinyl labeled [^{14}C]-BAS 605 F approximately 91-92% of the total dose was recovered. The majority of the radioactivity was present in the urine (66%-69% dose) and feces (12%-14% dose). There was also a large proportion remaining in the GI tract contents (5.1%-6.4%). Radioactivity in the cagewash accounted for 1.7%-6.9% dose. Radioactivity associated with edible portions (milk and tissues) accounted for ≤0.75% of the total dose.

For each single sample an initial measurement of radioactivity was performed. For milk, liver and kidney the residues were >0.01 mg/kg and were thus further investigated. Residues in fat and muscle were low (≤0.012 mg/kg in muscle and ≤0.006 mg/kg in fat) for both goats (001F and 002F, phenyl label). Residues in fat and muscle were low (≤0.007 mg/kg in muscle and ≤0.005 mg/kg in fat) for both goats (003F and 004F, pyrimidinyl label). Due to the low residues in muscle and fat no further investigations were performed.

The total radioactive residues (TRR) in all matrices are summarized in Table 6.2.3-2.

Table 6.2.3-2: Total radioactive residues in edible matrices and excreta after dosing of lactating goats with [pyrimidinyl-2-^{14}C]- or [phenyl-U-^{14}C]-pyrimethanil

Matrix	TRR [mg/kg]	
	measured	calculated
Pyrimidinyl-2-^{14}C label		
Milk (day 6-7)	0.092	0.091
Liver	0.529	0.572
Kidney	0.366	0.383
Urine (day 6-7)	8.804	10.673
Feces (day 6-7)	1.892	2.466
Phenyl-U-^{14}C label		
Milk (day 6-7)	0.104	0.085
Liver	0.496	0.502
Kidney	0.612	0.631
Urine (day 6-7)	9.217	9.226
Feces (day 6-7)	1.414	1.492

TRR: Total radioactive residue (sum of ERR + RRR)

B. EXTRACTION OF RESIDUES

The extractability of radioactive residues in feces, milk, liver and kidney ranged from 58.2% (pyrimidinyl label liver) to 98.4% (phenyl label milk) of the TRR. Radioactive residues remaining in the debris after solvent extraction accounted for 32% TRR and 41.7% TRR for phenyl and pyrimidinyl label liver, respectively and 9.6% TRR and 22.7% TRR for the phenyl and pyrimidinyl label kidney, respectively. The nature of non-extractable residues was investigated by digestion with protease enzyme. This released an additional 14.3% and 7.8% TRR from liver (phenyl and pyrimidinyl labels, respectively and 4.4% and 15.5% TRR for kidney (phenyl and pryimidinyl labels, respectively).

Table 6.2.3-3: Extractability of edible matrices with solvents (methanol, methanol/water) after dosing of lactating goats with [pyrimidinyl-2-^{14}C]- or [phenyl-U-^{14}C]-pyrimethanil

Matrix	TRR (calculated) mg/kg	Solvent extract (ERR)		RRR	
		mg/kg	% TRR	mg/kg	% TRR
Pyrimidinyl-2-^{14}C label					
Milk	0.091	0.085	94.1	0.005	5.5
Liver	0.572	0.333	58.2	0.239	41.7
Kidney	0.383	0.296	77.3	0.087	22.7
Phenyl-U-^{14}C label					
Milk	0.085	0.084	98.4	0.001	1.5
Liver	0.502	0.341	68.0	0.161	32.0
Kidney	0.631	0.570	90.4	0.061	9.6

TRR: Total radioactive residue (sum of ERR + RRR), set as 100% TRR
ERR: Extractable radioactive residue (solvents: methanol, methanol/water)
RRR: Residual radioactive residue after solvent extraction

Soluble radioactive residues extracted with methanol and methanol/water mixtures in milk and tissues ranged from 68% TRR (0.341 mg/kg) in liver to 98.4% TRR (0.084 mg/kg) in milk for the phenyl label and 58.2% TRR (0.333 mg/kg) in liver to 94.1% TRR (0.085 mg/kg) in milk for the pyrimidinyl label. Radioactive residues remaining after solvent extraction accounted for 1.5% TRR (0.001 mg/kg) in phenyl label milk and 5.5% TRR (0.005 mg/kg) in the pyrimidinyl milk. These residues were not significant and were not investigated further. Radioactive residues remaining after solvent extraction of liver and kidney accounted for 32% TRR (0.161 mg/kg) and 41.7% TRR (0.239 mg/kg) in liver from the phenyl and pyrimidinyl labels, respectively and 9.6% TRR (0.061 mg/kg) and 22.7% TRR (0.087 mg/kg) in kidney for the phenyl and pyrimidinyl labels, respectively. The nature of the non-extractable residues in liver and kidney was investigated by digestion with protease enzyme. This released an additional 14.3% TRR (0.072 mg/kg) and 7.8% TRR (0.045 mg/kg) from liver, and an additional 4.4% TRR (0.028 mg/kg) and 15.5% TRR (0.059 mg/kg) from kidney for the phenyl and pyrimidinyl labels, respectively. It was assumed that the remaining radioactivity was bound to the liver and kidney structure and was not bioavailable.

C. IDENTIFICATION AND CHARACTERIZATION OF RESIDUES

A summary of all identified metabolites and their distribution in liver, kidney and milk is given in Table 6.2.3-4 to Table 6.2.3-6.

Unchanged BAS 605 F was not detected in any of the samples analyzed. The residues identified in milk and edible tissues were M605F023 (0.007-0.153 mg/kg, 7.8-29.3% TRR), M605F034 (0.014-0.125 mg/kg, 2.7-19.8% TRR), M605F021 (0.006-0.042 mg/kg, 1.3-27.1% TRR), M605F002 (0.011-045 mg/kg, 2-7.1% TRR), M605F014 (0.006-0.040 mg/kg, 1.5-6.4% TRR), M605F020 (0.018-0.020 mg/kg, 3.1-4.8% TRR), M605F035 (0.003-0.009 mg/kg, 0.9-6.5% TRR), M605F006 (0.022 mg/kg, 4.3% TRR) and M605F004 (0.007 mg/kg, 1.3% TRR).

Unidentified components with RT *ca* 14.4 min and 19.6 min which accounted for 0.003-0.025 mg/kg (1.6-22.8% TRR) were characterized as sulphate conjugates of BAS 605 F metabolites with *m/z* 310 by mass spectrometry using both positive and negative ionization modes and fragmentation analysis. It was clear from the isotope pattern that these metabolites were related to BAS 605 F and accurate mass measurement indicated that these were sulphate conjugates. Despite thorough investigation by mass spectrometry an exact structure of these metabolites could not be assigned.

Metabolites with retention time <8 min by radio-HPLC were characterized as polar in nature. These were not adequately retained to allow identification by HPLC. Polar metabolites in liver (both labels), kidney and milk (pyrimidinyl label only) were isolated and analyzed by radio-TLC which identified the metabolite M605F025 by co-chromatography with the reference standard Reg. No 102020 (M605F025). M605F025 accounted for 0.001-0.059 mg/kg (0.8-10.4% TRR).

Table 6.2.3-4: Summary of identified and characterized residues in edible matrices of lactating goats after dosing with [pyrimidinyl-2-^{14}C]-BAS 605 F

Components	Liver		Kidney		Milk	
	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]
M605F002	2.0	0.011	4.4	0.017	-	-
M605F014	-	-	1.5	0.006	-	-
M605F020	-	-	4.8	0.018	-	-
M605F021	2.7	0.015	6.6	0.025	22.6	0.021
M605F023	9.2	0.053	22.2	0.085	7.8[7]	0.007[7]
M605F034	-	-	7.3	0.028	-	-
M605F035	0.9	0.005	-	-	3.1	0.003
Total identified	**14.8**	**0.084**	**46.8**	**0.179**	**33.5**	**0.031**
Polar region (RT <8 min), including M605F025 (see Table 6.2.3-6)	21.3[4]	0.122[4]	20.0[5]	0.076[5]	21.8[6]	0.020[6]
Characterized unknown, *m/z* 310, RT ca 15.88 min	4.3[2]	0.025[2]	-	-	22.8[2,7]	0.021[2,7]
Characterized unknown, *m/z* 310, RT ca 20.13 min	1.6	0.009	-	-	6.5[2]	0.006[2]
Unknown at RT ca 8.88 min	-	-	1.5	0.006	-	-
Unknown at RT ca 18.38 min	1.5	0.009	-	-	-	-
Unknown at RT ca 22.63 min	1.0	0.006	-	-	-	-
Unknown at RT ca 23.63 min	1.1	0.006	-	-	-	-
Unknown at RT ca 26.13 min	1.3	0.007	-	-	-	-
DCM extract	-	-	-	-	3.5	0.003
Hexane fraction	3.6	0.020	1.2	0.005	0.7	0.001
Total characterized	35.7	0.204	22.7	0.087	55.3	0.051
Total identified and/or characterized	**50.5**	**0.288**	**69.5**	**0.266**	**88.8**	**0.082**
Final residue	41.7	0.239	22.7	0.087	5.5	0.005
Grand total	**92.2**	**0.527**	**92.2**	**0.353**	**94.3**	**0.087**

2 These regions were characterized in milk and components with similar retention times in tissues were assigned by comparison with the milk. Ions with *m/z* 310 (molecular ion) and 230 (in source fragment ion) were detected and were related to BAS 605 F, indicating the metabolites were sulphate conjugates. However the exact structure could not be assigned. Corresponding deprotonated ions were detected in negative ionization mode.

4 The polar region was comprised of at least 2 discrete components which were resolved and quantified by TLC. M605F025 was identified by TLC and accounted for 10.4% TRR (0.059 mg/kg). The unidentified component accounted for 7.2% TRR (0.041 mg/kg). Material that remained at the origin accounted for 3.1% TRR (0.018 mg/kg).

5 The polar region was comprised of at least 6 discrete components which were resolved and quantified by TLC. M605F025 was identified by TLC and accounted for 1.6% TRR (0.006 mg/kg). The 5 unidentified components individually accounted for 1.4-5.9% TRR (0.005-0.023 mg/kg).

6 The polar region was comprised of at least 4 discrete components which were resolved and quantified by TLC. M605F025 was identified by TLC and accounted for 0.8% TRR (0.001 mg/kg). The 3 unidentified components individually accounted for 2.2-7.4% TRR (0.002-0.007 mg/kg).

7 These components were not fully resolved therefore could not be accurately quantified individually. These peaks were resolved in the bulk milk extract which indicated that component characterized as a sulphate conjugate was more abundant than the M605F023.

Table 6.2.3-5: Summary of identified and characterized residues in edible matrices of lactating goats after dosing with [phenyl-2-^{14}C]-BAS 605 F

Components	Liver		Kidney		Milk	
	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]
M605F002	4.0	0.020	7.1	0.045	-	-
M605F004	1.3	0.007	-	-	-	-
M605F006	4.3	0.022	-	-	-	-
M605F014	2.1	0.011	6.4	0.040	-	-
M605F020	-	-	3.1	0.020	-	-
M605F021	1.3	0.006	6.7	0.042	27.1	0.023
M605F023	15.2	0.076	24.3	0.153	29.3	0.025
M605F034	2.7	0.014	19.8	0.125	-	-
M605F035	1.8	0.009	-	-	6.5	0.006
Total identified	**32.7**	**0.165**	**67.4**	**0.425**	**62.9**	**0.054**
Polar region (RT <8 min), including M605F025 (see Table 6.2.3-6)	14.1[1]	0.071[1]	5.5	0.035	10.5	0.009
Characterized unknown, *m/z* 310, RT ca 12.38-14.38 min	3.5[2]	0.018[2]	3.6[2]	0.023[2]	22.5[2]	0.019[2]
Characterized unknown, RT ca 16.63 min, unresolved M605F023 and M605F006[3]	-	-	7.8[3]	0.049[3]	-	-
Characterized unknown, *m/z* 310, RT ca 19.63 min	1.7[2]	0.009[2]	-	-	4.1[2]	0.003[2]
Unknown at RT ca 16.13 min	4.0	0.020	-	-	-	-
Unknown at RT ca 22.63 min	1.2	0.006	-	-	-	-
Unknown at RT ca 23.38 min	1.1	0.006	-	-	-	-
Unknown at RT ca 25.13 min	0.9	0.005	-	-	-	-
Unknown at RT ca 35.38 min	0.8	0.004	-	-	-	-
DCM extract	-	-	-	-	0.8	0.001
Hexane fraction	3.5	0.018	-	-	-	-
Total characterized	30.8	0.157	16.9	0.107	37.9	0.032
Total identified and/or characterized	**63.5**	**0.322**	**84.3**	**0.532**	**100.8**	**0.086**
Final residue	32.0	0.161	9.6	0.061	1.5	0.001
Grand total	**95.5**	**0.483**	**93.9**	**0.593**	**102.3**	**0.087**

1 The polar region was comprised of at least 3 discrete components which were resolved and quantified by TLC. M605F025 was identified by TLC and accounted for 7.3% TRR (0.037 mg/kg). Neither of the 2 unidentified components individually exceeded 1.5% TRR (0.008 mg/kg). Material that remained at the origin accounted for 4.5% TRR (0.023 mg/kg).

2 These regions were characterized in milk and components with similar retention times in tissues were assigned by comparison with the milk. Ions with *m/z* 310 (molecular ion) and 230 (in source fragment ion) were detected and were related to BAS 605 F, indicating the metabolites were sulphate conjugates. However the exact structure could not be assigned. Corresponding deprotonated ions were detected in negative ionization mode.

3 This region was characterized as being comprised of the M605F023 and M605F006 as mass spectra for both components were detected. Following enzyme deconjugation no peak was detected in this region for the glucuronide and a small peak was detected that had comparable retention time with the 5069486 reference standard. This indicated that, while present, M605F006 was a minor component (1.4% TRR, 0.009 mg/kg). For these reasons this region has been characterized as unresolved M605F023 and M605F006.

Table 6.2.3-6: Summary of polar residues in edible matrices of lactating goats after dosing with [^{14}C]-BAS 605 F (TLC measurement)

Components	Phenyl label		Pyrimidinyl label					
	Liver		Liver		Kidney		Milk	
	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]
M605F025	7.3	0.037	10.4	0.059	1.6	0.006	0.8	0.001
Unidentified (RRf[1]=0.9)	1.2	0.006	-	-	1.4	0.005	-	-
Unidentified (RRf[1]=0.8)	1.5	0.008	7.2	0.041	-	-	2.2	0.002
Unidentified (RRf[1]=0.7)	-	-	-	-	5.0	0.019	6.9	0.006
Unidentified (RRf[1]=0.5)	-	-	-	-	1.4	0.005	-	-
Unidentified (RRf[1]=0.3)	-	-	-	-	5.9	0.023	-	-
Unidentified (RRf[1]=0.1)	-	-	-	-	2.8	0.011	7.4	0.007
Origin	4.5	0.023	3.1	0.018	1.8	0.007	-	-
Diffuse unassigned material (total)	3.0	0.015	2.1	0.012	-	-	4.4	0.004

1 RRf relative to M605F025

1. Metabolic pathway

The proposed metabolic pathway of BAS 605 F in goat is shown in Figure 6.2.3-1. BAS 605 F was extensively metabolized in the lactating goat. Unchanged parent pyrimethanil was not detected in milk, edible tissues, urine or feces.

The metabolism of BAS 605 F includes the following reactions:

- Mono- and di-hydroxylation of the parent compound
- Formation of glucuronide and sulphate conjugates of metabolites via hydroxylation of the parent compound
- Cleavage of the pyrimidine ring
- Oxidation of the hydroxylated parent to produce a carboxylic acid metabolite

2. Storage stability

Liver, kidney and milk extracts were analyzed by HPLC within 6 months of sample collection. The liver, kidney and milk extracts were analyzed again using HPLC after all other experimental work was complete to investigate the stability of radiolabeled components during storage.
For liver and kidney extracts comparison of the initial profiles with the stability profiles showed no significant differences between the profiles. This demonstrated that the sample extracts had remained stable during storage.
Differences were observed between the initial profile and the stability profile for milk, which had no influence on the outcome of the study. The stability of residues in milk during storage was accepted on the basis that the sulphate and glucuronic acid conjugates identified in milk would not be formed by degradation and thus the milk analysis provided a true representation of the residues in milk.

III. CONCLUSION

Radioactivity in plasma increased to a maximum of 0.061 mg/kg for goat 1 3 h after dosing and 0.065 mg/kg for goat 2 2 h after dosing. Radioactivity in plasma from goats 3 and 4 reached a maximum of 0.07% mg/kg (goat 3) and 0.04§ m/kg (goat 4) 2 h after dosing.

Approximately 91-100% of the total dose was recovered with the majority in the urine (60-71% dose) and feces (12-20% dose). A large proportion remained in the GI tract contents (5.1-9.6% dose). Radioactivity associated with edible portions (milk and tissues) accounted for ≤0.75% of the total dose.

Residues in the milk from goats 1 and 2 given the phenyl label reached a plateau maximum of 0.114 mg/kg after 6 days for goat 1 and 0.117 mg/kg after 5 days for goat 2. Residues in milk from goat 3 given the pyrimidinyl label reached a plateau maximum of 0.108 mg/kg after 6 days for goat 3. For goat 4 given the pyrimidinyl label, residues remained relatively constant throughout the dose period.
The ratio of residues in the cream to skimmed milk from goats given the phenyl label was 1:1.5 and 1:1 for goats 001F and 002F, respectively. For goats given the pyrimidinyl label the ratio of residues in the cream to skimmed milk was 1:1.1 and 1:1 for goats 003F and 004F, respectively.
Residues in liver were 0.428-0.563 mg/kg and residues in kidney were 0.305-0.701 mg/kg. Residues in muscle and fat were low (≤0.012 mg/kg in muscle, ≤0.006 mg/kg in fat).

Soluble radioactive residues extracted with methanol and methanol/water mixtures in milk and tissues ranged from 68% TRR in liver to 98.4% TRR in milk for the phenyl label and 58.2% TRR in liver to 94.1% TRR in milk for the pyrimidinyl label. Radioactive residues remaining after solvent extraction accounted for 1.5% TRR in phenyl label milk and 5.5% TRR in the pyrimidinyl milk. These residues were not significant and were not investigated further. Radioactive residues remaining after solvent extraction of liver and kidney accounted for 32% TRR and 41.7% TRR in liver from the phenyl and pyrimidinyl labels, respectively and 9.6% TRR and 22.7% TRR in kidney for the phenyl and pyrimidinyl labels, respectively. The nature of the non-extractable residues in liver and kidney was investigated by digestion with protease enzyme. This released an additional 14.3% TRR and 7.8% TRR from liver, and an additional 4.4% TRR and 15.5% TRR from kidney for the phenyl and pyrimidinyl labels, respectively. It was assumed that the remaining radioactivity was bound to the liver and kidney structure and was not bioavailable.

Unchanged BAS 605 F was not detected in any of the samples analyzed. The residues identified in milk and edible tissues were M605F023 (7.8-29.3% TRR), M605F034 (2.7-19.8% TRR), M605F021 (1.3-27.1% TRR), M605F002 (2-7.1% TRR), M605F014 (1.5-6.4% TRR), M605F020 (3.1-4.8% TRR), M605F035 (0.9-6.5% TRR), M605F006 (4.3% TRR) and M605F004 (1.3% TRR).

Unidentified components with RT *ca* 14.4 min and 19.6 min which accounted for 1.6-22.8% TRR were characterized as sulphate conjugates of BAS 605 F metabolites with *m/z* 310 by mass spectrometry using both positive and negative ionization modes and fragmentation analysis. It was clear from the isotope pattern that these metabolites were related to BAS 605 F and accurate mass measurement indicated that these were sulphate conjugates. Despite thorough investigation by mass spectrometry an exact structure of these metabolites could not be assigned.

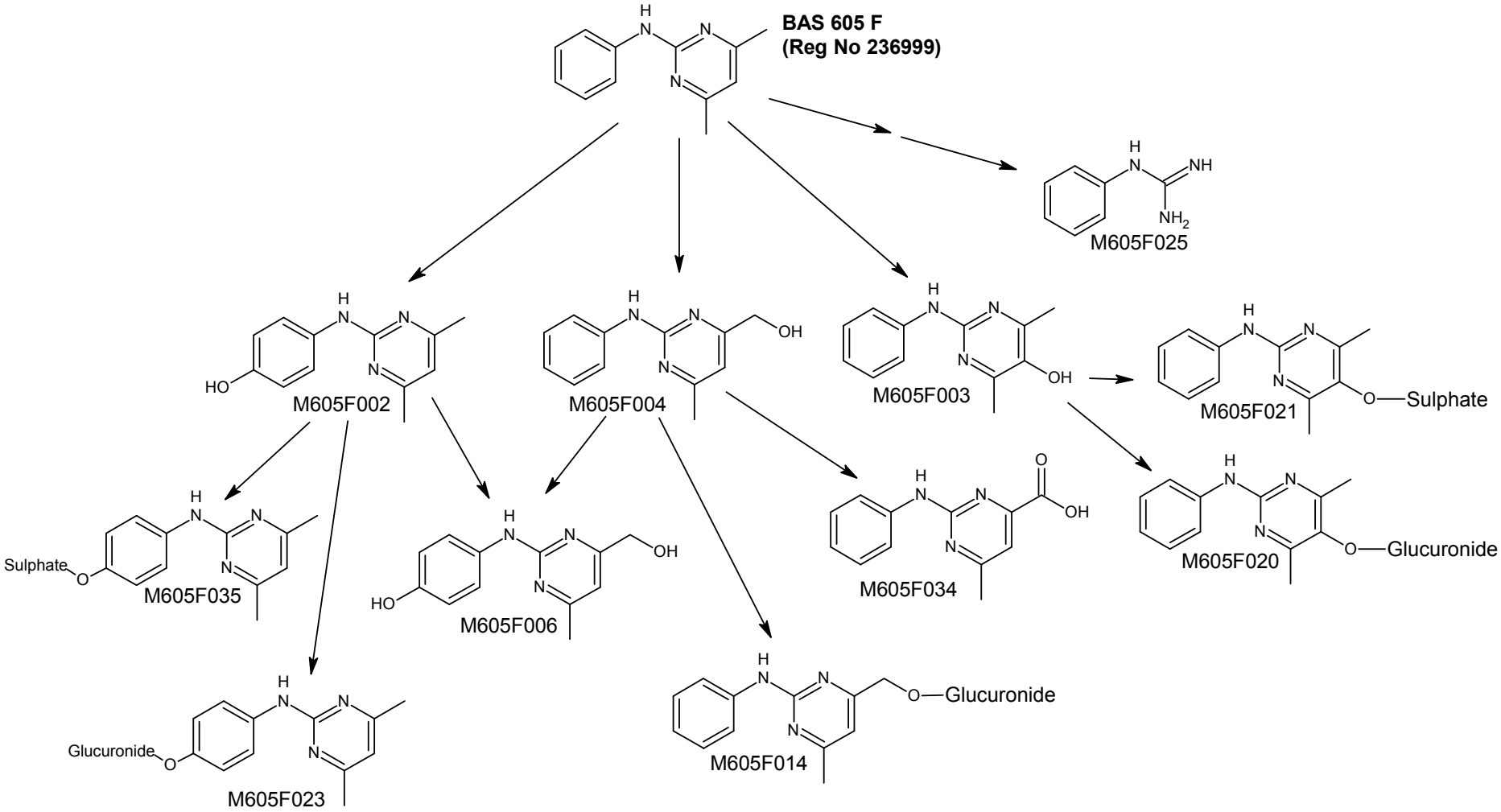
Metabolites with retention time <5 min by radio-HPLC were characterized as polar in nature. These were not adequately retained to allow identification by HPLC. Polar metabolites in liver (both labels), kidney and milk (pyrimidinyl label only) were isolated and analyzed by radio-TLC which identified the metabolite M605F025 by co-chromatography with the reference standard Reg. No 102020 (M605F025). M605F025 accounted for 0.8-10.4% TRR.

BAS 605 F was extensively metabolized in the lactating goat. Unchanged parent pyrimethanil was not detected in milk, edible tissues, urine or feces.

The metabolism of BAS 605 F includes the following reactions:

- Mono- and di-hydroxylation of the parent compound
- Formation of glucuronide and sulphate conjugates of metabolites via hydroxylation of the parent compound
- Cleavage of the pyrimidine ring
- Oxidation of the hydroxylated parent to produce a carboxylic acid metabolite

Figure 6.2.3-1: Proposed metabolic pathway of BAS 605 F in goat



CA 6.2.4 Pigs

According to Reg. (EU) No 283/2013, pig metabolism studies shall be provided where the plant protection product is used in crops whose parts or products, also after processing, are fed to pigs and where it becomes apparent that metabolic pathways differ significantly in the rat as compared to ruminants and where the intake is expected to exceed 0.004 mg/kg bw/day.

For pyrimethanil, no metabolism study was performed in pigs, since the metabolite patterns in rodents (rats) and ruminants (cows and goats) did not differ significantly.

The following conclusion is copied from the EFSA Reasoned Opinion on the re-evaluation of established MRLs according to Art.12 of Regulation 396/2005 (EFSA Journal 2011;9(11):2454): "As uses considered during the peer review did not result in exposure of livestock and consequently no residues were expected in foods of animal origin, EFSA did not take position on the similarity between rat and ruminant metabolism and on the fat solubility of pyrimethanil in its conclusions. However, in 2007, the JMPR concluded that the metabolism in ruminant and rat is similar and that the residue is not fat-soluble; EFSA agrees with this proposal."

CA 6.2.5 Fish

No bioaccumulation study has been performed in context of the previous Annex I inclusion process.

In the Conclusion 2006, EFSA considered the risk for bioconcentration in fish to be low as the log Pow is <3 for pyrimethanil. This is confirmed in this dossier (see chapter MCA 8.2.2): A bioconcentration study is not required since the log Pow of pyrimethanil and its metabolite M605F007 (= AE F132593; Reg. No. 40603) is <3.
As uses considered during the peer review did not result in exposure of livestock and consequently no residues were expected in foods of animal origin, EFSA did not take position on the fat solubility of pyrimethanil in its Conclusion. In the Reasoned Opinion on the re-evaluation of established MRLs (EFSA Journal 2011;9(11):2454), EFSA agreed with the JMPR conclusion that the residue is not fat-soluble.

As a new data requirement according to Regulation 283/2013, metabolism studies on fish may be required where the plant protection product is used in crops whose parts or products, also after processing, are fed to fish and where residues in feed may occur from the intended applications. Results from studies on bioconcentration in fish may be used if it can be demonstrated with scientific evidence that the results of these studies may be assumed to be equivalent. The bioconcentration of the substance shall be assessed where:
- the log Pow is >3 (20 or 25°C, effect of pH (4 to 10) to be reported if pKa 2-12) or there are other indications of bioconcentration, and
- the substance is considered stable, that is to say there is less than 90% loss of the original substance over 24 hours via hydrolysis (see point 7.2.1.1).

Pyrimethanil is registered mainly in fruit and vegetable crops which are not regarded as fish feed items. Only the use on cereals and legume vegetables would contribute to a small amount. Furthermore, the representative crops (apple, grape, strawberry and lettuce) are no fish feed items.
It is also hydrolytically stable in sterile buffer solutions at environmental relevant pH and temperature. However, no bioconcentration study in fish needs to be conducted since the log Pow is ≤3, independent of the pH.

In conclusion, BASF considers a fish metabolism study not required.

CA 6.3 Magnitude of residues trials in plants

Pyrimethanil is registered in several crops belonging to different EU crop groups. Within this dossier residue data are only provided for the representative uses supporting the renewal of approval. The solo SC formulation BAS 605 04 F has been selected as representative formulation. Several other closely related formulations have also been used to support these uses. All formulations are described in Document J.

Consequently, in this dossier section the relevant data for the following crops are summarized:

- Apples
- Grapes
- Strawberries
- Lettuce

The data for apples and grapes have been evaluated before in the context of the previous active substance inclusion process and have been evaluated within the MRL re-evaluation process according to Reg. 396/2005, Art. 12. Consequently they are considered as peer-reviewed.

Strawberries and lettuce were not part of representative uses of original Annex I inclusion process. However some studies were provided to EFSA in the context of the MRL evaluation process according to Reg. 396/2005, Art. 12. Those studies are summarized below and are indicated accordingly by a remark above the study header so that a differentiation between “old” and new data should be possible.

In M-CA 6.7 the residue levels found in samples destined for human food or for animal feed are compared with the data that have already been evaluated by EFSA. The relevant residue levels for MRL derivation are underlined.

CA 6.3.1 Pome fruit

The use in pome fruit was part of the previous active substance inclusion process. Sufficient data supporting the representative GAP were submitted to Austria as the designated Rapporteur Member State and were evaluated on EU level. Meanwhile, the GAP has changed regarding the application rate to 0.48 kg a.s./ha, which is within 25% tolerance of the evaluated 0.6 kg a.s./ha. Therefore, no further residue studies have been conducted. Both the current GAP and the one used in the initial evaluation are presented in Table 6.3.1-1. The corresponding individual trial results are presented in **Table 6.3.1-2** for the reviewer's convenience.

Table 6.3.1-1: Representative GAP for the use of pyrimethanil (BAS 605 F) in/on pome fruit

Crop	Maximum applied dose (kg a.s./ha)	Water volume (L/ha)	PHI (days)	Application method	Application timing
Pome fruit (apple, pear) (current GAP) MABSD, PYUCO	5 x 0.48	200-1500	56	Foliar spray	BBCH 53-77
Apples (peer-reviewed GAP) MABSD	5# x 0.6	500-2000	56	Foliar spray	Before BBCH 59 and after BBCH 71

PHI Pre-harvest interval
3 applications at 0.6 kg a.s./ha before end of flowering and 2 applications at 0.4 kg a.s./ha after fruit formation

Table 6.3.1-2: Trial results peer reviewed during the initial active substance approval procedure and supporting the current representative GAP

Commodity	Region	F, G or I	Individual trial results (mg/kg)	STMR (mg/kg)	HR (mg/kg)	MRL[1] (mg/kg)	Comments
Apples	NEU	F	0.12; 2 x 0.18; 0.20; 0.22; 0.30; 0.33; 0.45; 0.6	0.22	0.6	1 (OECD 0.9)	EFSA Conclusion 2006 (studies with BASF DocIDs C017379 and C028773)
Apples	SEU	F	<0.05; 0.11; 0.15; 2 x 0.16; 0.18; 0.22; 0.30; 0.37	0.16	0.37	0.5 (OECD 0.6)	EFSA Conclusion 2006 (studies with BASF DocIDs C017380 and C028774)

1 MRLs given in the respective official documents (EFSA, etc.) using the EU procedure for MRL calculation; the MRL calculated with the current version of the OECD MRL calculator is given in parentheses.

During Article 12 review (EFSA Reasoned Opinion 2011), the same and multiple additional pyrimethanil residue data in apples and pears were evaluated, supporting more critical GAPs and in addition post-harvest uses, which are non-BASF uses. They are not shown here. The current EU MRL for pome fruit was derived from the existing Codex MRL (CXL) of 15 mg/kg. The data basis for this CXL/MRL is given in the JMPR Report 2013 and is presented in Table 6.3.1-3. The apple and pear data evaluated for this CXL match a GAP for uses of pyrimethanil as post-harvest thermofog treatment (1 x 8 g a.s./ton fruits) and are presented in Table 6.3.1-3. The CXL was adopted to EU legislation in June 2015 (see Commission Regulation (EU) 2015/845).

Table 6.3.1-3: Trial results evaluated during CXL setting (CXL = current EU MRL)

Commodity	Region	F, G or I	Individual trial results (mg/kg)	STMR (mg/kg)	HR (mg/kg)	MRL (mg/kg)	Comments
Apples, pears		I (post-harvest)	Apples: 1.1; 2 x 1.4; 1.5; 1.6; 4.9; 6.4; 7.1 Pears: 1.0; 1.6; 1.8; 3.5	1.6	7.1	15	JMPR Report 2013; current EU MRL (adopted CXL) Non-BASF data

The current EU MRL covers the representative GAP. Furthermore, all uses evaluated during Article 12 review are also covered.

CA 6.3.2 Grape

The use in grapes was part of the previous active substance inclusion process. Sufficient data supporting the representative GAP were submitted to Austria as the designated Rapporteur Member State and were evaluated on EU level. The GAP has changed only slightly since. Therefore, no further residue studies have been conducted. Both the current GAP and the one used in the initial evaluation are presented in Table 6.3.2-1. The corresponding individual trial results are presented in **Table 6.3.2-2** for the reviewer's convenience.

Table 6.3.2-1: Representative GAP for the use of pyrimethanil (BAS 605 F) in/on grapes

Crop	Maximum applied dose (kg a.s./ha)	Water volume (L/ha)	PHI (days)	Application method	Application timing
Grapes (wine) (current GAP) VITVI	1 x 1.0	200-2000	21	Foliar spray	BBCH 68-85
Grapes (wine) (peer-reviewed GAP) VITVI	1 x up to 1	150-2000	21	Foliar spray	BBCH 62-81

PHI Pre-harvest interval

Table 6.3.2-2: Trial results peer-reviewed during the initial active substance approval procedure and supporting the recent representative GAP

Commodity	Region	F, G or I	Individual trial results (mg/kg)	STMR (mg/kg)	HR (mg/kg)	MRL[1] (mg/kg)	Comments
Grapes (wine)	NEU	F	0.37; 0.38; 0.44; 0.59; 0.83; 0.84; 0.97; 2 x 1.1	0.83	1.1	2 (OECD 3)	EFSA Conclusion 2006 (studies with BASF DocIDs C017382 and C028776)
Grapes (wine)	SEU	F	0.28; 0.38; 0.41; *0.42*; 0.48; 0.58; 0.83; 2 x *1.0*; 1.5; 2 x *1.6*; 1.98[2]	0.83	1.98	3 (OECD 4)	EFSA Conclusion 2006 (studies with BASF DocIDs A81716, A81696, A81702, A81740 and C017384); Values in *italics* are actually table grapes

1 MRLs given in the respective official documents (EFSA, etc.) using the EU procedure for MRL calculation; the MRL calculated with the current version of the OECD MRL calculator is given in parentheses.

2 This residue was found at 56 DALA; no samples were taken at PHI 21 (sampling 50-127 DALA). Excluding this value from the calculation leads to an MRL proposal of 3 mg/kg (OECD calculator).

During Article 12 review (EFSA Reasoned Opinion 2011), additional pyrimethanil residue data in grapes (table and wine) were evaluated, supporting more critical GAPs (3 x 0.75 (NEU) or 3 x 0.96 kg a.s./ha (SEU), PHI 21 days) and being the data basis for the current EU MRL of 5 mg/kg (see **Table 6.3.2-3**).

Table 6.3.2-3: Trial results evaluated during Article 12 review (current EU MRL)

Commodity	Region	F, G or I	Individual trial results (mg/kg)	STMR (mg/kg)	HR (mg/kg)	MRL[1] (mg/kg)	Comments
Grapes (wine, table)	NEU	F	<0.05; 0.19; 0.37; 0.38; 2 x 0.44; 0.47; 0.59; 0.67; 0.83; 0.84; 0.97; 2 x 1.1; 1.2; 1.7; 2.22; 2.27	0.75	2.27	3 (OECD 4)	More critical GAP (3x 0.75 kg a.s./ha); EFSA RO 2011 (Art 12 review) (studies with BASF DocIDs C017382, C028776 and additional trials)
Grapes (wine, table)	SEU	F	0.35; *0.39*; *1.0*; 1; 1.1, *1.2*; 2 x 1.33; 1.44; 1.48; *1.5*; 1.67; 2 x 1.78; 1.82; *1.9*; 1.91; 1.97; 2 x 2.1; 2.26; 2.64; 2 x *2.7*; 2 x 2.8; *3.2*; 3.55; *3.9*; 4.59	1.86	4.59	5 (OECD 6)	More critical GAP (3x 0.96 kg a.s./ha); EFSA RO 2011 (Art 12 review); (studies with BASF DocIDs A81747, A81746 and C017385 and additional trials); Values in *italics* are actually table grapes

1 MRLs given in the respective official documents (EFSA, etc.) using the EU procedure for MRL calculation; the MRL calculated with the current version of the OECD MRL calculator is given in parentheses.

The current EU MRL covers the representative GAP and the uses evaluated during Article 12 review.

CA 6.3.3 Strawberry

The use in strawberries was not part of the previous active substance inclusion process. Sufficient data supporting the corresponding GAP were evaluated during Article 12 review. Both the current GAPs and the ones used during Article 12 review are presented in Table 6.3.3-1. The corresponding individual trial results are presented in **Table 6.3.3-2** for the reviewer's convenience.

Table 6.3.3-1: Representative GAP for the use of pyrimethanil (BAS 605 F) in/on strawberries

Crop	Maximum applied dose (kg a.s./ha)	Water volume (L/ha)	PHI (days)	Application method	Application timing
Strawberries (current GAP) FRASS	3 x 0.8	200-2000	3	Foliar spray (field)	BBCH 55-89 (7-14 day interval)
Strawberries (current GAP) FRASS	2 x 0.8	200-2000	3	Foliar spray (greenhouse)	BBCH 55-89 (7-14 day interval)
Strawberries (evaluated GAP) FRASS	2 x 0.8	n.r.	1 (NEU) 3 (SEU)	Foliar spray (field)	BBCH 55-67
Strawberries (evaluated GAP) FRASS	2 x 0.8	n.r.	3	Foliar spray (indoor)	From BBCH 60 (21 day interval)

n.r. Not reported

Table 6.3.3-2: Trial results evaluated during Article 12 review (current EU MRL) and partly supporting the recent representative GAP

Commodity	Region	F, G or I	Individual trial results (mg/kg)	STMR (mg/kg)	HR (mg/kg)	MRL (mg/kg)	Comments
Strawberries	NEU	F	0.61; 0.88; 0.91; 1.0; 1.1; 1.2; 1.6; 2.4*; 4.5; 4.6	1.15	4.6	5 (OECD 8)	EFSA RO 2011 (Art 12 review); values at 0-1 DALA (studies with BASF DocIDs C021414 and C028245)
Strawberries	SEU	F	0.37; 0.49; 0.53; 0.67; 0.68; 1.1; 1.3; 1.7; 1.8	0.68	1.8	3 (OECD 3 with 1.1 mg/kg)	EFSA RO 2011 (Art 12 review); values at PHI 3; 1.8 mg/kg (1 DALA) should read 1.1 mg/kg (PHI 3) (studies with BASF DocIDs C021415 and C028171)
Strawberries	EU	I	0.38; 0.46; 2 x 0.52; 0.54*; 0.73; 0.79*; 0.82	0.53	0.82	2 (OECD 2)	EFSA RO 2011 (Art 12 review) (studies 2013/1399899 and 2013/1399900)

1 MRLs given in the respective official documents (EFSA, etc.) using the EU procedure for MRL calculation; the MRL calculated with the current version of the OECD MRL calculator is given in parentheses.
* Non-BASF data

The Northern European residue data on strawberry evaluated during Article 12 review were the data basis for the current EU MRL of 5 mg/kg (see Table 6.3.3-2). The current EU MRL also covers the representative GAPs (see chapter MCA 6.7 and the following study summaries).

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Report: CA 6.3.3/1
Oxspring S., 2010b
Study on the residue behaviour of Pyrimethanil in strawberry (protected) after treatment with BAS 605 04 F in Northern Europe during 2010
2010/1156071
Guidelines: none
GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: BAS 605 04 F (SC)
Lot/Batch #: 0003007090 (400 g/L pyrimethanil, nominal)
Purity: Not reported
CAS#: 53112-28-0
Development code: Not reported
Spiking levels: 0.01-5.0 mg/kg

2. Test Commodity:

Crop: Strawberry
Type: Berries and other small fruits
Variety: Sonata, Elsanta, Zumba, Cordelia, Albion
Botanical name: *Fragaria x ananassa*
Crop parts(s) or processed commodity: Fruits
Sample size: ≥1 kg / 12 units

B. STUDY DESIGN

1. Test procedure

During the 2010 growing season 8 decline trials in protected strawberry were conducted in the United Kingdom to determine the residue level of pyrimethanil in or on raw agricultural commodities (RAC). BAS 605 04 F (SC) containing 400 g/L pyrimethanil was applied twice at individual rates equivalent to 0.800 kg pyrimethanil/ha in a spray volume of 200 L/ha. One untreated plot of each trial served as control. The applications were performed 9-11 and 2-4 days before the expected harvest (PHI 3). Specimens of fruits were collected immediately and 1, 2-4 and 6-8 days after the application. Samples were stored deep-frozen for a maximum of 67 days until analysis.

Table 6.3.3-3: Target application rates and timings for strawberry

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/ Timing
2011	8	2	P	BAS 605 04 F (SC)	BAS 605 F	0.800	200	10±1 DBH 3±1 DBH

DBH Days before harvest

2. Description of analytical procedures

The specimens were analyzed for pyrimethanil with BASF method No 542/2 (L0066/02) quantifying the analyte with a limit of quantitation (LOQ) of 0.01 mg/kg. The analyte was extracted with a mixture of methanol and water. An aliquot of the extract was centrifuged and diluted. The final determination was performed using HPLC-MS/MS.

Table 6.3.3-4: Summary of procedural recovery data for pyrimethanil

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
BASF method No 542/2; LOQ = 0.01 mg/kg		**Pyrimethanil (BAS 605 F)**		
Strawberry fruit	0.01, 1.0, 5.0	6	78	7.1

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.3-5, detailed residue levels are shown in Table 6.3.3-6.
Residues of pyrimethanil in treated fruit specimens collected on the day of the last application (0 DALA) ranged from 0.57 to 2.31 mg/kg. At the PHI (2-4 DALA) residues declined in six trials and increased in two trials. Residues found at the PHI (2-4 DALA) ranged from 0.51 to 2.11 mg/kg. At 6-8 DALA a further decline was observed in five trials and residues ranged from 0.36 to 1.77 mg/kg.
No residues of pyrimethanil above the limit of quantitation (0.01 mg/kg) were found in any of the analyzed untreated specimens.

Table 6.3.3-5: Summary of residues in strawberries treated with BAS 605 04 F

Region	Year	DALA[1]	Growth stage[2] (BBCH)	Range of residues (mg/kg)	
				Matrix	Pyrimethanil
Protected	2010	0	81-87	Fruits	0.57-2.31
		1	81-87	Fruits	0.51-2.11
		2-4	85-89	Fruits	0.24-1.13
		6-8	85-89	Fruits	0.36-1.77

1 Days after last application
2 At sampling

III. CONCLUSION

Residues of pyrimethanil in treated fruit specimens collected at the PHI (2-4 DALA) ranged from 0.24 to 1.13 mg/kg. At 6-8 DALA residues ranged from 0.36 to 1.77 mg/kg.

Table 6.3.3-6: Residues of pyrimethanil in protected strawberry after two applications of BAS 605 04 F

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)	
							Matrix	Pyrimethanil
Study code:	392246	Strawberry	United Kingdom	BAS 605 04 F 2 x 0.800	81-85	0	Fruits	0.66
Doc ID:	2010/1156071					1	Fruits	1.03
Trial No:	S10-02446-01					3	Fruits	0.24
GLP:	yes					7	Fruits	<u>0.76</u>
Year:	2010							
Study code:	392246	Strawberry	United Kingdom	BAS 605 04 F 2 x 0.800	81-87	0	Fruits	1.78
Doc ID:	2010/1156071					1	Fruits	1.53
Trial No:	S10-02446-02					3	Fruits	1.09
GLP:	yes					6	Fruits	<u>1.16</u>
Year:	2010							
Study code:	392246	Strawberry	United Kingdom	BAS 605 04 F 2 x 0.800	85-87	0	Fruits	0.96
Doc ID:	2010/1156071					1	Fruits	0.63
Trial No:	S10-02446-03					4	Fruits	<u>0.98</u>
GLP:	yes					6	Fruits	0.68
Year:	2010							
Study code:	392246	Strawberry	United Kingdom	BAS 605 04 F 2 x 0.800	85-87	0	Fruits	1.23
Doc ID:	2010/1156071					1	Fruits	1.20
Trial No:	S10-02446-04					4	Fruits	<u>0.68</u>
GLP:	yes					6	Fruits	0.66
Year:	2010							
Study code:	392246	Strawberry	United Kingdom	BAS 605 04 F 2 x 0.800	85	0	Fruits	2.31
Doc ID:	2010/1156071					1	Fruits	2.11
Trial No:	S10-02446-05					3	Fruits	1.13
GLP:	yes					7	Fruits	<u>1.77</u>
Year:	2010							
Study code:	392246	Strawberry	United Kingdom	BAS 605 04 F 2 x 0.800	81-85	0	Fruits	1.01
Doc ID:	2010/1156071					1	Fruits	0.51
Trial No:	S10-02446-06					3	Fruits	<u>0.60</u>
GLP:	yes					8	Fruits	0.48
Year:	2010							
Study code:	392246	Strawberry	United Kingdom	BAS 605 04 F 2 x 0.800	81-85	0	Fruits	1.65
Doc ID:	2010/1156071					1	Fruits	0.90
Trial No:	S10-02446-07					4	Fruits	<u>0.52</u>
GLP:	yes					8	Fruits	0.36
Year:	2010							
Study code:	392246	Strawberry	United Kingdom	BAS 605 04 F 2 x 0.800	85-87	0	Fruits	0.57
Doc ID:	2010/1156071					1	Fruits	0.86
Trial No:	S10-02446-08					2	Fruits	<u>0.79</u>
GLP:	yes					6	Fruits	0.50
Year:	2010							

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
_ Underlined values were used for MRL calculation

This study has been already evaluated (greenhouse application only) in the context of the Article 12 review (European Food Safety Authority; Review of the existing maximum residue levels (MRLs) for pyrimethanil according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2011;9(11):2454. [65 pp.] doi:10.2903/j.efsa.2011.2454EFSA Journal 2012; 10(3):2606. [36 pp.] doi:10.2903/j.efsa.2012.2606). Four field trials are also part of the studies but not according to supportive GAP.

Report:	CA 6.3.3/2 Pigeon O., 2007a Determination of Pyrimethanil residues in strawberries after treatment with Pyrimethanil 400 SC - 2006 2013/1399899
Guidelines:	FAO, EEC 96/46, EEC 96/68, EEC 91/414, SANCO/3029/99, SANCO/825/00, EEC 396/2005, SANCO/10232/2006
GLP:	yes (certified by Groupe Interministeriel des Produits Chimiques, France)

[see LoA under KCA 6.3.3/8 2013/1420180]

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	PYRIMETHANIL 400 SC (SC)
Lot/Batch #:	96450/1-3 (400 g/L pyrimethanil, nominal)
Purity:	Not reported
CAS#:	53112-28-0
Development code:	Not reported
Spiking levels:	0.05-5.0 mg/kg

2. Test Commodity:

Crop:	Strawberry
Type:	Berries and other small fruits
Variety:	Garriguette, Cléry, Cirafine, Charlotte, Darselect, Ciloé
Botanical name:	*Fragaria x ananassa*
Crop parts(s) or processed commodity:	Fruits
Sample size:	≥1 kg from min. 12 plants

B. STUDY DESIGN

1. Test procedure

During the 2006 growing season 2 greenhouse and 4 field trials in strawberry were conducted in Northern and Southern France to determine the residue level of pyrimethanil in or on raw agricultural commodities (RAC). PYRIMETHANIL 400 SC containing 400 g/L pyrimethanil was applied twice at individual rates equivalent to 0.800 kg pyrimethanil/ha in a spray volume of 600 L/ha. One untreated plot of each trial served as control. The applications were performed 23-24 and 3 days before the expected harvest (PHI 3). Specimens of fruits were collected 3 and 7 days after the application. Samples were stored deep-frozen until analysis.

Table 6.3.3-7: Target application rates and timings for strawberry

Year	No of trials	No of Appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/ Timing
2006	2	2	G	PYRIMETHANIL 400 SC (SC)	BAS 605 F	0.800	600	24±1 DBH 3±1 DBH
2006	4	2	F	PYRIMETHANIL 400 SC (SC)	BAS 605 F	0.800	600	24±1 DBH 3±1 DBH

DBH Days before harvest

2. Description of analytical procedures

The specimens were analyzed for pyrimethanil using Walloon Agricultural Research Centre method No MEREPYRIME rev 3 quantifying the analyte with a limit of quantitation (LOQ) of 0.05 mg/kg. The analyte was extracted with acetone, acidified and liquid/liquid partitioned into n-hexane. After basification and liquid/liquid partitioning into n-hexane/ethyl acetate the samples was cleaned up with solid phase extraction (SPE) on silica gel. The final determination was performed using GC-NPD.

Table 6.3.3-8: Summary of procedural recovery data for pyrimethanil

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
GC-NPD; LOQ = 0.05 mg/kg		**Pyrimethanil (BAS 605 F)**		
Strawberry fruit	0.05, 0.5, 5.0	16	84	9.5

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.3-9; detailed residue levels are shown in Table 6.3.3-10 to Table 6.3.3-12.
Residues of pyrimethanil in treated fruit specimens collected at the PHI (3 DALA) residues ranged from 0.518 to 0.725 mg/kg in the greenhouse samples and from 0.189 to 0.691 mg/kg in the outdoor samples. At 7 DALA residues ranged from 0.199 to 0.336 mg/kg in the greenhouse samples and from 0.167 to 0.375 mg/kg in the field samples.
No residues of pyrimethanil above the limit of quantitation (0.05 mg/kg) were found in any of the analyzed untreated specimens.

Table 6.3.3-9: Summary of residues in strawberries treated with PYRIMETHANIL 400 SC

Region	Year	DALA[1]	Growth stage[2] (BBCH)	Range of residues (mg/kg)	
				Matrix	Pyrimethanil
Greenhouse	2006	3	87	Fruits	0.518-0.725
		7	87	Fruits	0.199-0.336
Northern EU	2006	3	89	Fruits	0.415-0.691
		7	89	Fruits	0.246-0.375
Southern EU	2006	3	87	Fruits	0.189-0.597
		7	89	Fruits	0.167-0.343

1 Days after last application
2 At sampling

III. CONCLUSION

Residues of pyrimethanil in treated fruit specimens collected at the PHI (3 DALA) ranged from 0.518 to 0.725 mg/kg in the greenhouse samples and from 0.189 to 0.691 mg/kg in the outdoor samples. At 7 DALA residues ranged from 0.199 to 0.336 mg/kg in the greenhouse samples and from 0.167 to 0.375 mg/kg in the field samples.

Table 6.3.3-10: Residues of pyrimethanil in strawberry after two greenhouse applications of PYRIMETHANIL 400 SC

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)	
							Matrix	Pyrimethanil
Study code:	21196	Strawberry	France	PYRIMETHANIL	87	3	Fruits	0.725
Doc ID:	2013/1399899			400 SC		7	Fruits	0.336
Trial No:	06 F CL CH P12			2 x 0.800				
GLP:	yes							
Year:	2006							
Study code:	21196	Strawberry	France	PYRIMETHANIL	87	3	Fruits	0.518
Doc ID:	2013/1399899			400 SC		7	Fruits	0.199
Trial No:	06 F CL CH P13			2 x 0.800				
GLP:	yes							
Year:	2006							

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
_ Underlined values were used for MRL calculation

Table 6.3.3-11: Residues of pyrimethanil in strawberry after two applications of PYRIMETHANIL 400 SC in Northern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)	
							Matrix	Pyrimethanil
Study code:	21196	Strawberry	France	PYRIMETHANIL	89	3	Fruits	0.691
Doc ID:	2013/1399899			400 SC		7	Fruits	0.375
Trial No:	06 F CL CH P14			2 x 0.800				
GLP:	yes							
Year:	2006							
Study code:	21196	Strawberry	France	PYRIMETHANIL	89	3	Fruits	0.415
Doc ID:	2013/1399899			400 SC		7	Fruits	0.246
Trial No:	06 F CL CH P15			2 x 0.800				
GLP:	yes							
Year:	2006							

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application

Table 6.3.3-12: Residues of pyrimethanil in strawberry after two applications of PYRIMETHANIL 400 SC in Southern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)	
							Matrix	Pyrimethanil
Study code:	21196	Strawberry	France	PYRIMETHANIL	85	3	Fruits	0.189
Doc ID:	2013/1399899			400 SC		7	Fruits	0.167
Trial No:	06 F CL CH P16			2 x 0.800				
GLP:	yes							
Year:	2006							
Study code:	21196	Strawberry	France	PYRIMETHANIL	87	3	Fruits	0.597
Doc ID:	2013/1399899			400 SC		7	Fruits	0.343
Trial No:	06 F CL CH P17			2 x 0.800				
GLP:	yes							
Year:	2006							

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application

This study has been already evaluated (greenhouse application only) in the context of the Article 12 review (European Food Safety Authority; Review of the existing maximum residue levels (MRLs) for pyrimethanil according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2011;9(11):2454. [65 pp.] doi:10.2903/j.efsa.2011.2454EFSA Journal 2012; 10(3):2606. [36 pp.] doi:10.2903/j.efsa.2012.2606). Seven field trials are part of the studies but not to supportive GAP.

Report:	CA 6.3.3/3 Pigeon O., 2008a Determination of Pyrimethanil residues in strawberries after treatment with Pyrimethanil 400 SC in France 2007 2013/1399900
Guidelines:	EEC 96/46, EEC 96/68, EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex II (Part A Section 8), SANCO/3029/99, SANCO/825/00 rev. 7 (17 March 2004), EEC 396/2005, FAO
GLP:	yes (certified by Groupe Interministeriel des Produits Chimiques, France)

[see LoA under KCA 6.3.3/8 2013/1420180]

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	PYRIMETHANIL 400 SC (SC)
Lot/Batch #:	96450/1-3 (400 g/L pyrimethanil, nominal)
Purity:	Not reported
CAS#:	53112-28-0
Development code:	Not reported
Spiking levels:	0.05-5.0 mg/kg

2. Test Commodity:

Crop:	Strawberry
Type:	Berries and other small fruits
Variety:	Marat, Pajaro, Charlotte, Cléry, Cirafine, Elsanta, Daisy, Florence, Darselect
Botanical name:	*Fragaria x ananassa*
Crop parts(s) or processed commodity:	Fruits
Sample size:	≥0.4 kg from min. 12 plants

B. STUDY DESIGN

1. Test procedure

During the 2007 growing season 4 greenhouse and 7 field trials in strawberry were conducted in Northern and Southern France to determine the residue level of pyrimethanil in or on raw agricultural commodities (RAC). PYRIMETHANIL 400 SC containing 400 g/L pyrimethanil was applied twice at individual rates equivalent to 0.800 kg pyrimethanil/ha in a spray volume of 600 L/ha. Only in one trial (07 F CL CH P01) 0.916 kg a.s./ha were applied in 693 L/ha of water in the second application. One untreated plot of each trial served as control. The applications were performed 23-24 and 3 days before the expected harvest (PHI 3). Specimens of fruits were collected 3 and 7 days after the application and additionally at DALA 0 and 1 in 2 greenhouse and 4 outdoor trials. Samples were stored deep-frozen until analysis.

Table 6.3.3-13: Target application rates and timings for strawberry

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/ Timing
2007	4	2	G	PYRIMETHANIL 400 SC (SC)	BAS 605 F	0.800	600	24±1 DBH 3±1 DBH
2007	7	2	F	PYRIMETHANIL 400 SC (SC)	BAS 605 F	0.800	600	24±1 DBH 3±1 DBH

DBH Days before harvest

2. Description of analytical procedures

The specimens were analyzed for pyrimethanil using Walloon Agricultural Research Centre method No RESSM007 rev 1 (previously named MEREPYRIME method) quantifying the analyte with a limit of quantitation (LOQ) of 0.05 mg/kg. The analyte was extracted with acetone, acidified and liquid/liquid partitioned into n-hexane. After basification and liquid/liquid partitioning into n-hexane/ethyl acetate the samples was cleaned up with solid phase extraction (SPE) on silica gel. The final determination was performed using GC-NPD.

Table 6.3.3-14: Summary of procedural recovery data for pyrimethanil

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
GC-NPD; LOQ = 0.05 mg/kg		**Pyrimethanil (BAS 605 F)**		
Strawberry fruit	0.05, 0.5, 5.0	26	94	11

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.3-15; detailed residue levels are shown in Table 6.3.3-16 to Table 6.3.3-18.
Residues of pyrimethanil in treated fruit specimens collected at the PHI (3 DALA) residues ranged from 0.383 to 0.818 mg/kg in the greenhouse samples and from 0.170 to 0.684 mg/kg in the outdoor samples. At 7 DALA residues ranged from 0.115 to 0.508 mg/kg in the greenhouse samples and from 0.117 to 0.643 mg/kg in the field samples.
No residues of pyrimethanil above the limit of quantitation (0.05 mg/kg) were found in any of the analyzed untreated specimens except in the ones from one Southern European trial (07 F CL CH P07) where residues in control samples were 0.138-0.219 mg/kg at 3 DALA and 0.090-0.179 mg/kg at 7 DALA. These residues are probably due to an error of the producer who did a generalized treatment with pyrimethanil on the field, but this information cannot be confirmed by field data.

Table 6.3.3-15: Summary of residues in strawberries treated with PYRIMETHANIL 400 SC

Region	Year	DALA[1]	Growth stage[2] (BBCH)	Range of residues (mg/kg)	
				Matrix	Pyrimethanil
Greenhouse	2007	0	87-89	Fruits	0.696-1.02
		1	87-89	Fruits	0.816-0.906
		3	87-89	Fruits	0.383-0.818
		7	87-89	Fruits	0.115-0.508
Northern EU	2007	0	85	Fruits	0.616-0.744
		1	85	Fruits	0.529-0.966
		3	85-87	Fruits	0.487-0.684
		7	87-89	Fruits	0.441-0.643
Southern EU	2007	0	87	Fruits	0.105-0.306
		1	87	Fruits	0.178-0.424
		3	87-89	Fruits	0.170-0.391
		7	87-89	Fruits	0.117-0.305

1 Days after last application
2 At sampling

III. CONCLUSION

Residues of pyrimethanil in treated fruit specimens collected at the PHI (3 DALA) ranged from 0.383 to 0.818 mg/kg in the greenhouse samples and from 0.170 to 0.684 mg/kg in the outdoor samples. At 7 DALA residues ranged from 0.115 to 0.508 mg/kg in the greenhouse samples and from 0.117 to 0.643 mg/kg in the field samples.

Table 6.3.3-16: Residues of pyrimethanil in strawberry after two greenhouse applications of PYRIMETHANIL 400 SC

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)	
							Matrix	Pyrimethanil
Study code:	21495	Strawberry	France	PYRIMETHANIL	87	3	Fruits	0.818
Doc ID:	2013/1399900			400 SC		7	Fruits	0.290
Trial No:	07 F CL CH P01			2 x 0.800[3]				
GLP:	yes							
Year:	2007							
Study code:	21495	Strawberry	France	PYRIMETHANIL	89	3	Fruits	0.383
Doc ID:	2013/1399900			400 SC		7	Fruits	0.115
Trial No:	07 F CL CH P02			2 x 0.800				
GLP:	yes							
Year:	2007							
Study code:	21495	Strawberry	France	PYRIMETHANIL	87	0	Fruits	0.696
Doc ID:	2013/1399900			400 SC		1	Fruits	0.816
Trial No:	07 F CL CH P03			2 x 0.800		3	Fruits	0.460
GLP:	yes					7	Fruits	0.135
Year:	2007							
Study code:	21495	Strawberry	France	PYRIMETHANIL	89	0	Fruits	1.02
Doc ID:	2013/1399900			400 SC		1	Fruits	0.906
Trial No:	07 F CL CH P04			2 x 0.800		3	Fruits	0.521
GLP:	yes					7	Fruits	0.508
Year:	2007							

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
3 In the second application, 916 kg a.s./ha were applied
_ Underlined values were used for MRL calculation

Table 6.3.3-17: Residues of pyrimethanil in strawberry after two applications of PYRIMETHANIL 400 SC in Northern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)	
							Matrix	Pyrimethanil
Study code:	21495	Strawberry	France	PYRIMETHANIL	85	3	Fruits	0.684
Doc ID:	2013/1399900			400 SC		7	Fruits	0.441
Trial No:	07 F CL CH P05			2 x 0.800				
GLP:	yes							
Year:	2007							
Study code:	21495	Strawberry	France	PYRIMETHANIL	85	0	Fruits	0.616
Doc ID:	2013/1399900			400 SC		1	Fruits	0.966
Trial No:	07 F CL CH P09			2 x 0.800		3	Fruits	0.487
GLP:	yes					7	Fruits	0.637
Year:	2007							
Study code:	21495	Strawberry	France	PYRIMETHANIL	85	0	Fruits	0.744
Doc ID:	2013/1399900			400 SC		1	Fruits	0.529
Trial No:	07 F CL CH P10			2 x 0.800		3	Fruits	0.622
GLP:	yes					7	Fruits	0.643
Year:	2007							

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application

Table 6.3.3-18: Residues of pyrimethanil in strawberry after two applications of PYRIMETHANIL 400 SC in Southern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)	
							Matrix	Pyrimethanil
Study code:	21495	Strawberry	France	PYRIMETHANIL	89	3	Fruits	0.328
Doc ID:	2013/1399900			400 SC		7	Fruits	0.264
Trial No:	07 F CL CH P07			2 x 0.800				
GLP:	yes							
Year:	2007							
Study code:	21495	Strawberry	France	PYRIMETHANIL	87	3	Fruits	0.170
Doc ID:	2013/1399900			400 SC		7	Fruits	0.117
Trial No:	07 F CL CH P08			2 x 0.800				
GLP:	yes							
Year:	2007							
Study code:	21495	Strawberry	France	PYRIMETHANIL	85	0	Fruits	0.306
Doc ID:	2013/1399900			400 SC		1	Fruits	0.424
Trial No:	07 F CL CH P11			2 x 0.800		3	Fruits	0.391
GLP:	yes					7	Fruits	0.281
Year:	2007							
Study code:	21495	Strawberry	France	PYRIMETHANIL	87	0	Fruits	0.105
Doc ID:	2013/1399900			400 SC		1	Fruits	0.178
Trial No:	07 F CL CH P12			2 x 0.800		3	Fruits	0.371
GLP:	yes					7	Fruits	0.305
Year:	2007							

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application

This study has been already evaluated in the context of the Article 12 review (European Food Safety Authority; Review of the existing maximum residue levels (MRLs) for pyrimethanil according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2011;9(11):2454. [65 pp.] doi:10.2903/j.efsa.2011.2454EFSA Journal 2012; 10(3):2606. [36 pp.] doi:10.2903/j.efsa.2012.2606).

Report:	CA 6.3.3/4 Sonder K.-H., 2002a Residues behaviour in strawberries; European Union (Northern zone) 2001 - Pyrimethanil, AE B100309 - Water miscible suspension concentrate (SC) 37.38% w/w (= 400 g/L) C021414
Guidelines:	EEC 7029/VI/95 rev. 5
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Landwirtschaft und Forsten, Wiesbaden)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	AE B100309 00 SC37 A407 (SC)
Lot/Batch #:	OP210191 (400 g/L pyrimethanil, nominal)
Purity:	Not reported
CAS#:	53112-28-0
Development code:	Not reported
Spiking levels:	0.05-5.0 mg/kg

2. Test Commodity:

Crop:	Strawberry
Type:	Berries and other small fruits
Variety:	Elsanta, Corona, Siloe, Chandler
Botanical name:	*Fragaria ananassa*
Crop parts(s) or processed commodity:	Fruits
Sample size:	≥1 kg (exception: trial 01R690-4, 200-500 g)

B. STUDY DESIGN

1. Test procedure

During the 2001 growing season 5 field trials in strawberry were conducted in Northern Europe to determine the residue level of pyrimethanil in or on raw agricultural commodities (RAC). AE B100309 00 SC37 A407 containing 400 g/L pyrimethanil was applied three times at individual rates equivalent to 1.0 kg pyrimethanil/ha in a spray volume of 250-300 L/ha. One untreated plot of each trial served as control. The applications were performed at BBCH 65-81, 73-85 and 81-89 at a 6-8 day interval. Specimens of fruits were collected immediately after the last application (0 DALA) and 1 and 3 days thereafter. Samples were stored deep-frozen until analysis for a maximum of 223 days.

Table 6.3.3-19: Target application rates and timings for strawberry

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/ Timing
2001	5	3	F	AE B100309 00 SC37 A407 (SC)	BAS 605 F	1.0	250-300	17 DBH 10 DBH 3 DBH

DBH Days before harvest

2. Description of analytical procedures

The specimens were analyzed for pyrimethanil using Covance method No DGM C05/98-0 quantifying the analyte with a limit of quantitation (LOQ) of 0.05 mg/kg. The analyte was extracted by maceration with acetone. The acidified extract was cleaned up by washing with hexane followed by partition of the pyrimethanil into solvent from basified extract. Final clean-up of the extract was achieved by silica SPE prior to determination by gas chromatography with mass spectrometric detection (GC-MS).

Table 6.3.3-20: Summary of procedural recovery data for pyrimethanil

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
GC-MS; LOQ = 0.05 mg/kg		**Pyrimethanil (BAS 605 F)**		
Strawberry fruit	0.05, 0.5, 5.0	6	89	12

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.3-21; detailed residue levels are shown in Table 6.3.3-22.
Residues of pyrimethanil in treated fruit specimens collected at the PHI (3 DALA) residues ranged from 0.33 to 2.7 mg/kg. Residues declined from 0 to 3 DALA.
No residues of pyrimethanil above the limit of quantitation (0.05 mg/kg) were found in any of the analyzed untreated specimens.

Table 6.3.3-21: Summary of residues in strawberries treated with AE B100309 00 SC37 A407

Region	Year	DALA[1]	Growth stage[2] (BBCH)	Range of residues (mg/kg)	
				Matrix	Pyrimethanil
Northern EU	2001	0	81-89	Fruits	0.61-4.6
		1	81-89	Fruits	0.48-4.5
		3	85-89	Fruits	0.33-2.7

1 Days after last application
2 At sampling

III. CONCLUSION

Residues of pyrimethanil in treated fruit specimens collected at the PHI (3 DALA) ranged from 0.33 to 2.7 mg/kg.

Table 6.3.3-22: Residues of pyrimethanil in strawberry after three applications of AE B100309 00 SC37 A407 in Northern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)	
							Matrix	Pyrimethanil
Study code:	01 R 690	Strawberry	Germany	AE B100309 00	81	0	Fruits	3.6
Doc ID:	C021414			SC37 A407		1	Fruits	4.5
Trial No:	01R690-1			3 x 1.0		3	Fruits	2.2
GLP:	yes							
Year:	2001							
Study code:	01 R 690	Strawberry	Germany	AE B100309 00	87	0	Fruits	1.0
Doc ID:	C021414			SC37 A407		1	Fruits	0.64
Trial No:	01R690-2			3 x 1.0		3	Fruits	0.56
GLP:	yes							
Year:	2001							
Study code:	01 R 690	Strawberry	France	AE B100309 00	87	0	Fruits	4.6
Doc ID:	C021414			SC37 A407		1	Fruits	4.5
Trial No:	01R690-3			3 x 1.0		3	Fruits	2.7
GLP:	yes							
Year:	2001							
Study code:	01 R 690	Strawberry	France	AE B100309 00	89	0	Fruits	0.61
Doc ID:	C021414			SC37 A407		1	Fruits	0.48
Trial No:	01R690-4			3 x 1.0		3	Fruits	0.33
GLP:	yes							
Year:	2001							
Study code:	01 R 690	Strawberry	United Kingdom	AE B100309 00	87	0	Fruits	1.3
Doc ID:	C021414			SC37 A407		1	Fruits	1.6
Trial No:	01R690-5			3 x 1.0		3	Fruits	1.0
GLP:	yes							
Year:	2001							

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
_ Underlined values were used for MRL calculation

This study has been already evaluated in the context of the Article 12 review (European Food Safety Authority; Review of the existing maximum residue levels (MRLs) for pyrimethanil according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2011;9(11):2454. [65 pp.] doi:10.2903/j.efsa.2011.2454EFSA Journal 2012; 10(3):2606. [36 pp.] doi:10.2903/j.efsa.2012.2606).

Report:	CA 6.3.3/5 Sonder K.-H., 2003a Residues behaviour in strawberries - European Union (Northern zone) 2002 - Pyrimethanil, AE B100309 water miscible suspension concentrate (SC) 37.38% w/w (= 400 g/L) C028245
Guidelines:	EEC 7029/VI/95 rev. 5
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Landwirtschaft und Forsten, Wiesbaden)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	AE B100309 00 SC37 A408 (SC)
Lot/Batch #:	OP211001 (400 g/L pyrimethanil, nominal)
Purity:	Not reported
CAS#:	53112-28-0
Development code:	Not reported
Spiking levels:	0.05-0.5 mg/kg

2. Test Commodity:

Crop:	Strawberry
Type:	Berries and other small fruits
Variety:	Elsanta, Elsanta A+, Florence
Botanical name:	*Fragaria ananassa*
Crop parts(s) or processed commodity:	Fruits
Sample size:	≥1 kg

B. STUDY DESIGN

1. Test procedure

During the 2002 growing season 4 field trials in strawberry were conducted in Northern Europe to determine the residue level of pyrimethanil in or on raw agricultural commodities (RAC). AE B100309 00 SC37 A408 containing 400 g/L pyrimethanil was applied three times at individual rates equivalent to 1.0 kg pyrimethanil/ha in a spray volume of 200-300 L/ha. One untreated plot of each trial served as control. The applications were performed at BBCH 65-73, 73-85 and 81-87 at a 6-7 day interval. Specimens of fruits were collected immediately after the last application (0 DALA) and 3 days thereafter. Samples were stored deep-frozen until analysis for a maximum of 160 days.

Table 6.3.3-23: Target application rates and timings for strawberry

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/ Timing
2002	4	3	F	AE B100309 00 SC37 A408 (SC)	BAS 605 F	1.0	200-300	17 DBH 10 DBH 3 DBH

DBH Days before harvest

2. Description of analytical procedures

The specimens were analyzed for pyrimethanil using Covance method No DGM C05/98-0 quantifying the analyte with a limit of quantitation (LOQ) of 0.05 mg/kg. The analyte was extracted by maceration with acetone. The acidified extract was cleaned up by washing with hexane followed by partition of the pyrimethanil into solvent from basified extract. Final determination was performed by gas chromatography with mass spectrometric detection (GC-MS).

Table 6.3.3-24: Summary of procedural recovery data for pyrimethanil

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
GC-MS; LOQ = 0.05 mg/kg		**Pyrimethanil (BAS 605 F)**		
Strawberry fruit	0.05, 0.5	4	97	6.7

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.3-25; detailed residue levels are shown in Table 6.3.3-26.
Residues of pyrimethanil in treated fruit specimens collected at the PHI (3 DALA) residues ranged from 0.67 to 0.84 mg/kg. Residues declined from 0 to 3 DALA.
No residues of pyrimethanil above the limit of quantitation (0.05 mg/kg) were found in any of the analyzed untreated specimens.

Table 6.3.3-25: Summary of residues in strawberries treated with AE B100309 00 SC37 A408

Region	Year	DALA[1]	Growth stage[2] (BBCH)	Range of residues (mg/kg)	
				Matrix	Pyrimethanil
Northern EU	2002	0	81-87	Fruits	0.88-1.2
		3	85-89	Fruits	0.67-0.84

1 Days after last application
2 At sampling

III. CONCLUSION

Residues of pyrimethanil in treated fruit specimens collected at the PHI (3 DALA) ranged from 0.67 to 0.84 mg/kg.

Table 6.3.3-26: Residues of pyrimethanil in strawberry after three applications of AE B100309 00 SC37 A408 in Northern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)	
							Matrix	Pyrimethanil
Study code:	02 R 690	Strawberry	Germany	AE B100309 00	87	0	Fruits	0.91
Doc ID:	C028245			SC37 A408		3	Fruits	0.72
Trial No:	02R690-1			3 x 1.0				
GLP:	yes							
Year:	2002							
Study code:	02 R 690	Strawberry	Germany	AE B100309 00	85	0	Fruits	0.88
Doc ID:	C028245			SC37 A408		3	Fruits	0.84
Trial No:	02R690-2			3 x 1.0				
GLP:	yes							
Year:	2002							
Study code:	02 R 690	Strawberry	France	AE B100309 00	81	0	Fruits	1.2
Doc ID:	C028245			SC37 A408		3	Fruits	0.67
Trial No:	02R690-3			3 x 1.0				
GLP:	yes							
Year:	2002							
Study code:	02 R 690	Strawberry	United Kingdom	AE B100309 00	87	0	Fruits	1.1
Doc ID:	C028245			SC37 A408		3	Fruits	0.73
Trial No:	02R690-4			3 x 1.0				
GLP:	yes							
Year:	2002							

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
_ Underlined values were used for MRL calculation

This study has been already evaluated in the context of the Article 12 review (European Food Safety Authority; Review of the existing maximum residue levels (MRLs) for pyrimethanil according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2011;9(11):2454. [65 pp.] doi:10.2903/j.efsa.2011.2454EFSA Journal 2012; 10(3):2606. [36 pp.] doi:10.2903/j.efsa.2012.2606).

Report:	CA 6.3.3/6 Sonder K.-H., 2002b Residues behaviour in strawberries - European Union (Southern zone) 2002 - Pyrimethanil, AE B100309 - Water miscible suspension concentrate (SC) 37.38% w/w (= 400 g/L) C021415
Guidelines:	EEC 7029/VI/95 rev. 5
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Landwirtschaft und Forsten, Wiesbaden)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	AE B100309 00 SC37 A407 (SC)
Lot/Batch #:	OP210191 (400 g/L pyrimethanil, nominal)
Purity:	Not reported
CAS#:	53112-28-0
Development code:	Not reported
Spiking levels:	0.05-5.0 mg/kg

2. Test Commodity:

Crop:	Strawberry
Type:	Berries and other small fruits
Variety:	Camarosa, Darselat, Marmolada
Botanical name:	*Fragaria ananassa*
Crop parts(s) or processed commodity:	Fruits
Sample size:	Not reported

B. STUDY DESIGN

1. Test procedure

During the 2001 growing season 5 field trials in strawberry were conducted in Southern Europe to determine the residue level of pyrimethanil in or on raw agricultural commodities (RAC). AE B100309 00 SC37 A407 containing 400 g/L pyrimethanil was applied three times at individual rates equivalent to 1.0 kg pyrimethanil/ha in a spray volume of 300-1500 L/ha. One untreated plot of each trial served as control. The applications were performed at BBCH 65-81, 81-85 and 85-87 at a 6-8 day interval. Specimens of fruits were collected immediately after the last application (0 DALA) and 1 and 3 days thereafter. Samples were stored deep-frozen until analysis for a maximum of 244 days.

Table 6.3.3-27: Target application rates and timings for strawberry

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/ Timing
2001	5	3	F	AE B100309 00 SC37 A407 (SC)	BAS 605 F	1.0	300-1500	17 DBH 10 DBH 3 DBH

DBH Days before harvest

2. Description of analytical procedures

The specimens were analyzed for pyrimethanil using Covance method No DGM C05/98-0 quantifying the analyte with a limit of quantitation (LOQ) of 0.05 mg/kg. The analyte was extracted by maceration with acetone. The acidified extract was cleaned up by washing with hexane followed by partition of the pyrimethanil into solvent from basified extract. Final clean-up of the extract was achieved by silica SPE prior to determination by gas chromatography with mass spectrometric detection (GC-MS).

Table 6.3.3-28: Summary of procedural recovery data for pyrimethanil

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
GC-MS; LOQ = 0.05 mg/kg		**Pyrimethanil (BAS 605 F)**		
Strawberry fruit	0.05, 0.5, 5.0	8	77	6.2

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.3-29; detailed residue levels are shown in Table 6.3.3-30.
Residues of pyrimethanil in treated fruit specimens collected at the PHI (3 DALA) residues ranged from 0.49 to 1.3 mg/kg. Residues declined from 0 to 3 DALA in all trials but one.
No residues of pyrimethanil above the limit of quantitation (0.05 mg/kg) were found in any of the analyzed untreated specimens.

Table 6.3.3-29: Summary of residues in strawberries treated with AE B100309 00 SC37 A407

Region	Year	DALA[1]	Growth stage[2] (BBCH)	Range of residues (mg/kg)	
				Matrix	Pyrimethanil
Southern EU	2001	0	85-87	Fruits	0.80-4.0
		1	85-89	Fruits	0.66-2.8
		3	87-89	Fruits	0.49-1.3

1 Days after last application
2 At sampling

III. CONCLUSION

Residues of pyrimethanil in treated fruit specimens collected at the PHI (3 DALA) ranged from 0.49 to 1.3 mg/kg.

Table 6.3.3-30: Residues of pyrimethanil in strawberry after three applications of AE B100309 00 SC37 A407 in Southern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)	
							Matrix	Pyrimethanil
Study code:	01 R 691	Strawberry	Spain	AE B100309 00	87	0	Fruits	1.4
Doc ID:	C021415			SC37 A407		1	Fruits	1.8
Trial No:	01R691-1			3 x 1.0		3	Fruits	1.1
GLP:	yes							
Year:	2001							
Study code:	01 R 691	Strawberry	Spain	AE B100309 00	85	0	Fruits	0.80
Doc ID:	C021415			SC37 A407		1	Fruits	0.69
Trial No:	01R691-2			3 x 1.0		3	Fruits	0.49
GLP:	yes							
Year:	2001							
Study code:	01 R 691	Strawberry	France	AE B100309 00	87	0	Fruits	4.0
Doc ID:	C021415			SC37 A407		1	Fruits	2.8
Trial No:	01R691-3			3 x 1.0		3	Fruits	1.1
GLP:	yes							
Year:	2001							
Study code:	01 R 691	Strawberry	Italy	AE B100309 00	85	0	Fruits	0.87
Doc ID:	C021415			SC37 A407		1	Fruits	0.66
Trial No:	01R691-4			3 x 1.0		3	Fruits	0.68
GLP:	yes							
Year:	2001							
Study code:	01 R 691	Strawberry	Italy	AE B100309 00	87	0	Fruits	1.1
Doc ID:	C021415			SC37 A407		1	Fruits	1.1
Trial No:	01R691-5			3 x 1.0		3	Fruits	1.3
GLP:	yes							
Year:	2001							

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
_ Underlined values were used for MRL calculation

This study has been already evaluated in the context of the Article 12 review (European Food Safety Authority; Review of the existing maximum residue levels (MRLs) for pyrimethanil according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2011;9(11):2454. [65 pp.] doi:10.2903/j.efsa.2011.2454EFSA Journal 2012; 10(3):2606. [36 pp.] doi:10.2903/j.efsa.2012.2606).

Report:	CA 6.3.3/7 Sonder K.-H., 2003b Residues behaviour in strawberries - European Union (Southern zone) 2002 - Pyrimethanil, AE B100309 water miscible suspension concentrate (SC) 37.38% w/w (= 400 g/L) C028171
Guidelines:	EEC 7029/VI/95 rev. 5
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Landwirtschaft und Forsten, Wiesbaden)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	AE B100309 00 SC37 A408 (SC)
Lot/Batch #:	OP211001 (400 g/L pyrimethanil, nominal)
Purity:	Not reported
CAS#:	53112-28-0
Development code:	Not reported
Spiking levels:	0.05-2.0 mg/kg

2. Test Commodity:

Crop:	Strawberry
Type:	Berries and other small fruits
Variety:	Camarosa, Darselect, Pajaro, Marmolada
Botanical name:	*Fragaria ananassa*
Crop parts(s) or processed commodity:	Fruits
Sample size:	≥1 kg

B. STUDY DESIGN

1. Test procedure

During the 2002 growing season 4 field trials in strawberry were conducted in Southern Europe to determine the residue level of pyrimethanil in or on raw agricultural commodities (RAC). AE B100309 00 SC37 A408 containing 400 g/L pyrimethanil was applied three times at individual rates equivalent to 0.87-1.0 kg pyrimethanil/ha in a spray volume of 250-350 L/ha. One untreated plot of each trial served as control. The applications were performed at BBCH 73-87, 81-87 and 85-87 at a 7 day interval. Specimens of fruits were collected immediately after the last application (0 DALA) and 3 days thereafter. Samples were stored deep-frozen until analysis for a maximum of 187 days.

Table 6.3.3-31: Target application rates and timings for strawberry

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/ Timing
2002	4	3	F	AE B100309 00 SC37 A408 (SC)	BAS 605 F	1.0	250-350	17 DBH 10 DBH 3 DBH

DBH Days before harvest

2. Description of analytical procedures

The specimens were analyzed for pyrimethanil using Covance method No DGM C05/98-0 quantifying the analyte with a limit of quantitation (LOQ) of 0.05 mg/kg. The analyte was extracted by maceration with acetone. The acidified extract was cleaned up by washing with hexane followed by partition of the pyrimethanil into solvent from basified extract. Final clean-up of the extract was achieved by silica SPE prior to determination by gas chromatography with mass spectrometric detection (GC-MS).

Table 6.3.3-32: Summary of procedural recovery data for pyrimethanil

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
GC-MS; LOQ = 0.05 mg/kg		**Pyrimethanil (BAS 605 F)**		
Strawberry fruit	0.05, 2.0	4	83	4.5

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.3-33; detailed residue levels are shown in Table 6.3.3-34.
Residues of pyrimethanil in treated fruit specimens collected at the PHI (3 DALA) residues ranged from 0.37 to 1.7 mg/kg. Residues declined from 0 to 3 DALA.
No residues of pyrimethanil above the limit of quantitation (0.05 mg/kg) were found in any of the analyzed untreated specimens.

Table 6.3.3-33: Summary of residues in strawberries treated with AE B100309 00 SC37 A408

Region	Year	DALA[1]	Growth stage[2] (BBCH)	Range of residues (mg/kg)	
				Matrix	Pyrimethanil
Southern EU	2002	0	85-87	Fruits	0.39-2.2
		3	87	Fruits	0.37-1.7

1 Days after last application
2 At sampling

III. CONCLUSION

Residues of pyrimethanil in treated fruit specimens collected at the PHI (3 DALA) ranged from 0.37 to 1.7 mg/kg.

Table 6.3.3-34: Residues of pyrimethanil in strawberry after three applications of AE B100309 00 SC37 A408 in Southern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)	
							Matrix	Pyrimethanil
Study code:	02 R 691	Strawberry	Spain	AE B100309 00	87	0	Fruits	1.5
Doc ID:	C028171			SC37 A408		3	Fruits	0.67
Trial No:	02R691-1			3 x 1.0				
GLP:	yes							
Year:	2002							
Study code:	02 R 691	Strawberry	France	AE B100309 00	87	0	Fruits	0.39
Doc ID:	C028171			SC37 A408		3	Fruits	0.37
Trial No:	02R691-2			3 x 1.0				
GLP:	yes							
Year:	2002							
Study code:	02 R 691	Strawberry	France	AE B100309 00	87	0	Fruits	2.2
Doc ID:	C028171			SC37 A408		3	Fruits	1.7
Trial No:	02R691-3			3 x 0.87				
GLP:	yes							
Year:	2002							
Study code:	02 R 691	Strawberry	Italy	AE B100309 00	85	0	Fruits	0.78
Doc ID:	C028171			SC37 A408		3	Fruits	0.53
Trial No:	02R691-4			3 x 1.0				
GLP:	yes							
Year:	2002							

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
_ Underlined values were used for MRL calculation

CA 6.3.4 Lettuce

The use in lettuce was not part of the previous active substance inclusion process. Sufficient data supporting the corresponding GAP were evaluated during Article 12 review. The representative GAP is only slightly different regarding the application timing. Both the current GAPs and the ones used during Article 12 review are presented in Table 6.3.4-1. The corresponding individual trial results are presented in Table 6.3.4-2 for the reviewer's convenience.

Table 6.3.4-1: Representative GAP for the use of pyrimethanil (BAS 605 F) in/on lettuce

Crop	Maximum applied dose (kg a.s./ha)	Water volume (L/ha)	PHI (days)	Application method	Application timing
Lettuce (current GAP) LACSA	2 x 0.8	200-2000	14	Foliar spray (field and greenhouse)	BBCH 10-47 (10-14 day interval)
Lettuce (evaluated GAP) LACSA	2 x 0.8	n.r.	14	Foliar spray (field)	Until BBCH 19 (NEU) n.r. (SEU)
Lettuce (evaluated GAP) LACSA	2 x 0.8	n.r.	14	Foliar spray (indoor)	BBCH 37-45 (7 day interval)

n.r. Not reported

Table 6.3.4-2: Trial results evaluated during Article 12 review (current EU MRL) and partly supporting the recent representative GAP

Commodity	Region	F, G or I	Individual trial results (mg/kg)	STMR (mg/kg)	HR (mg/kg)	MRL[1] (mg/kg)	Comments
Lettuce → scarole	NEU	F	2 x <0.05; 0.06; 0.11; 0.3; 0.34; 0.42; 0.43; 0.55	0.30	0.55	1 (OECD 1)	EFSA RO 2011 (Art 12 review) (studies with BASF DocID A91280 and C003112)
Lettuce	SEU	F	0.05; 0.14; 0.31; 0.62; 0.68; 0.78; 1.2	0.62	1.2	2 (OECD 3)	EFSA RO 2011 (Art 12 review) (studies with BASF DocID A91282 and C003103)
Lettuce → scarole, herbs, leaves and sprouts of brassica	EU	I	1.48; 2.06; 3.07; 3.14[2]; 4.18[3]; 5.73[2]; 6.18; 12.45	3.66	12.45	20 (OECD 20; 4.18 → 4.81: 20)	EFSA RO 2011 (Art 12 review) and EFSA RO 2011 (lettuce and scarole); (study with BASF DocID 2013/1399903)

1 MRLs given in the respective official documents (EFSA, etc.) using the EU procedure for MRL calculation; the MRL calculated with the current version of the OECD MRL calculator is given in parentheses.
2 Head lettuce varieties; remaining values from open leaf varieties
3 4.18 should read 4.81 (typo)

The indoor residue data on lettuce evaluated during Article 12 review and in EFSA Reasoned Opinion 2011 (European Food Safety Authority; Modification of the existing MRLs for pyrimethanil in lettuce and scarole. EFSA Journal 2011;9(4):2148) were the data basis for the current EU MRL of 20 mg/kg (see Table 6.3.4-2); they are summarized here again. Residues produced by the representative GAPs are covered by the current EU MRL.
In addition, the field trials evaluated during Article 12 review are summarized below for the reviewer's convenience.

This study has been already evaluated (greenhouse application only) by Austria in the context of a previous submission. It was used as basis for the corresponding EFSA Reasoned Opinion (European Food Safety Authority; Modification of the existing MRLs for pyrimethanil in lettuce and scarole. EFSA Journal 2011;9(4):2148) and in the context of the Article 12 review (European Food Safety Authority; Review of the existing maximum residue levels (MRLs) for pyrimethanil according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2011;9(11):2454. [65 pp.] doi:10.2903/j.efsa.2011.2454EFSA Journal 2012; 10(3):2606. [36 pp.] doi:10.2903/j.efsa.2012.2606).

Report:	CA 6.3.4/1 Lagrasse S., 2010a Determination of Pyrimethanil residues in lettuces conducted under protected conditions following two applications of Pyrimethanil 400 SC - Decline curve and harvest residue trials conducted in France, Spain, Italy, Greece and Germany, 2008 2013/1399903
Guidelines:	EEC 96/46, EEC 96/68, EEC 1607/VI/97 rev. 2 10.06.1999, EEC 7029/VI/95 rev. 5 (July 22 1997), EEC 7525/VI/95 rev. 7, SANCO/825/00 rev. 7 (17 March 2004), SANCO/3029/99 rev. 4
GLP:	yes (certified by Groupe Interministeriel des Produits Chimiques, France)

Report:	CA 6.3.4/2 Witte A., 2009 a Pyrimethanil, analytical report to #2013/1399903 2009/1132162
Guidelines:	EEC 91/414, SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes (certified by Umweltministerium Baden-Wuerttemberg, Stuttgart)

[see LoA under KCA 6.3.4/8 2013/1420180]

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	PYRIMETHANIL 400 SC (SC)
Lot/Batch #:	7027710/4-8 (400 g/L pyrimethanil, nominal)
Purity:	Not reported
CAS#:	53112-28-0
Development code:	Not reported
Spiking levels:	0.01-25.0 mg/kg

2. Test Commodity:

Crop:	Lettuce
Type:	Leaf vegetables
Variety:	Leaf lettuce: Kinshasa, Carolo, Gentilina, Simpson, Grünetta, Candela Head lettuce: Iceberg, Panisse, Gisela, Armonica, Alexandria, Soleillan, Kamikaze
Botanical name:	*Lactuca sativa*
Crop parts(s) or processed commodity:	Lettuce
Sample size:	≥1.0 kg

B. STUDY DESIGN

1. Test procedure

During the 2008 growing season 8 greenhouse and 10 field trials in head and leaf lettuce were conducted in Northern and Southern Europe to determine the residue level of pyrimethanil in or on raw agricultural commodities (RAC). PYRIMETHANIL 400 SC containing 400 g/L pyrimethanil was applied twice at individual rates equivalent to 0.800 kg pyrimethanil/ha in a spray volume of 1000 L/ha. One untreated plot of each trial served as control. The applications were performed targeting 21 and 14 days before the expected harvest (PHI 13-15). Specimens of leaves / heads were collected 13-15 days after the application and additionally at DALA 0, 1, 3 and 7-8 in 4 greenhouse and 4 outdoor trials on two head and leaf lettuce crops each. Samples were stored deep-frozen until analysis.

Table 6.3.4-3: Target application rates and timings for lettuce

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/ Timing
2008	8	2	G	PYRIMETHANIL 400 SC (SC)	BAS 605 F	0.800	1000	21±1 DBH 14±1 DBH
2008	10	2	F	PYRIMETHANIL 400 SC (SC)	BAS 605 F	0.800	1000	21±1 DBH 14±1 DBH

DBH Days before harvest

2. Description of analytical procedures

The specimens were analyzed for pyrimethanil using Chemisches Institut Pforzheim method No 08A01038-01-RALE quantifying the analyte with a limit of quantitation (LOQ) of 0.01 mg/kg. The analyte was extracted by maceration with acetone. The extracts were subject to two liquid/liquid partition phases (acidic and basic), then cleaned by solid phase extraction (SPE). The final determination was performed using HPLC-MS/MS.

Table 6.3.4-4: Summary of procedural recovery data for pyrimethanil

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
HPLC-MS/MS; LOQ = 0.01 mg/kg		**Pyrimethanil (BAS 605 F)**		
Head lettuce	0.01, 1.0, 25.0	14	76	8.2
Leaf lettuce	0.01, 1.0, 25.0	14	83	6.0

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.4-5, detailed residue levels are shown in Table 6.3.4-6 to Table 6.3.4-8.
Residues of pyrimethanil in treated lettuce specimens collected at the PHI (13-15 DALA) ranged from 3.14 to 5.73 mg/kg in head lettuce and from 1.48 to 12.5 mg/kg in leaf lettuce in the greenhouse samples. In the outdoor samples, residues ranged from 0.026 to 1.25 mg/kg in head lettuce and from 0.17 to 0.99 mg/kg in leaf lettuce. In the decline trials, a general residue decline could be observed from 0 to 13-15 DALA.
No residues of pyrimethanil above the limit of quantitation (0.01 mg/kg) were found in any of the analyzed untreated specimens except in four trials (08 F CL CH P03, -P19, -P20, -P33) where residues in control samples were 0.010-0.027 mg/kg.

Table 6.3.4-5: Summary of residues in lettuce treated with PYRIMETHANIL 400 SC

Region	Year	DALA[1]	Growth stage[2] (BBCH)	Range of residues (mg/kg)	
				Matrix	Pyrimethanil
Greenhouse	2008	0	45	Head lettuce	17.7-20.9
		1	45	Head lettuce	15.0-21.5
		3	45	Head lettuce	12.6-16.6
		7-8	47	Head lettuce	7.24-10.7
		15	49	Head lettuce	3.14-5.73
Greenhouse	2008	0	37-41	Leaf lettuce	13.8-17.0
		1	38-42	Leaf lettuce	11.9-13.9
		3	39-43	Leaf lettuce	11.0-12.5
		7-8	44-46	Leaf lettuce	7.63-8.65
		13-15	49	Leaf lettuce	1.48-12.5
Northern EU	2008	14	49	Head lettuce	0.026
Northern EU	2008	15	49	Leaf lettuce	0.46
Southern EU	2008	0	41-43	Head lettuce	14.1-21.5
		1	41-43	Head lettuce	9.30-15.7
		3	42-45	Head lettuce	7.45-7.50
		7-8	45-47	Head lettuce	2.16-3.45
		13-14	49	Head lettuce	0.16-1.25
Southern EU	2008	0	38	Leaf lettuce	9.48-15.4
		1	38	Leaf lettuce	4.32-12.5
		3	43	Leaf lettuce	3.43-5.79
		7	47	Leaf lettuce	1.18-2.27
		14	49	Leaf lettuce	0.17-0.99

1 Days after last application
2 At sampling

III. CONCLUSION

Residues of pyrimethanil in treated lettuce specimens collected at the PHI (13-15 DALA) ranged from 3.14 to 5.73 mg/kg in head lettuce and from 1.48 to 12.5 mg/kg in leaf lettuce in the greenhouse samples. In the outdoor samples, residues ranged from 0.026 to 1.25 mg/kg in head lettuce and from 0.17 to 0.99 mg/kg in leaf lettuce.

Table 6.3.4-6: Residues of pyrimethanil in lettuce after two greenhouse applications of PYRIMETHANIL 400 SC

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)	
							Matrix	Pyrimethanil
Study code:	08 F CL CH P/B	Leaf lettuce	France	PYRIMETHANIL 400 SC 2 x 0.800	44	14	Lettuce	4.81
Doc ID:	2013/1399903							
Trial No:	08 F CL CH P05							
GLP:	yes							
Year:	2008							
Study code:	08 F CL CH P/B	Leaf lettuce	Spain	PYRIMETHANIL 400 SC 2 x 0.800	41	14	Lettuce	6.18
Doc ID:	2013/1399903							
Trial No:	08 F CL CH P17							
GLP:	yes							
Year:	2008							
Study code:	08 F CL CH P/B	Leaf lettuce	Spain	PYRIMETHANIL 400 SC 2 x 0.800	42	14	Lettuce	2.06
Doc ID:	2013/1399903							
Trial No:	08 F CL CH P18							
GLP:	yes							
Year:	2008							
Study code:	08 F CL CH P/B	Leaf lettuce	Italy	PYRIMETHANIL 400 SC 2 x 0.800	45	13	Lettuce	12.5[3]
Doc ID:	2013/1399903							
Trial No:	08 F CL CH P33							
GLP:	yes							
Year:	2008							
Study code:	08 F CL CH P/B	Head lettuce	France	PYRIMETHANIL 400 SC 2 x 0.800	45	0	Lettuce (head)	20.9
Doc ID:	2013/1399903					1	Lettuce (head)	21.5
Trial No:	08 F CL CH P03					3	Lettuce (head)	16.6
GLP:	yes					8	Lettuce (head)	10.7
Year:	2008					15	Lettuce (head)	5.73[4]
Study code:	08 F CL CH P/B	Head lettuce	France	PYRIMETHANIL 400 SC 2 x 0.800	45	0	Lettuce (head)	17.7
Doc ID:	2013/1399903					1	Lettuce (head)	15.0
Trial No:	08 F CL CH P04					3	Lettuce (head)	12.6
GLP:	yes					7	Lettuce (head)	7.24
Year:	2008					15	Lettuce (head)	3.14
Study code:	08 F CL CH P/B	Leaf lettuce	Spain	PYRIMETHANIL 400 SC 2 x 0.800	41	0	Lettuce	13.8
Doc ID:	2013/1399903					1	Lettuce	11.9
Trial No:	08 F CL CH P19					3	Lettuce	11.0[5]
GLP:	yes					7	Lettuce	7.63[5]
Year:	2008					14	Lettuce	3.07
Study code:	08 F CL CH P/B	Leaf lettuce	Spain	PYRIMETHANIL 400 SC 2 x 0.800	37	0	Lettuce	17.0
Doc ID:	2013/1399903					1	Lettuce	13.9
Trial No:	08 F CL CH P20					3	Lettuce	12.5
GLP:	yes					7	Lettuce	8.65[6]
Year:	2008					14	Lettuce	1.48

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
3 Mean of duplicate analyses; residue in control sample 0.027 mg/kg
4 Residue in control sample 0.012 mg/kg
5 Residue in control sample 0.010 mg/kg at 3 DALA and 0.021 mg/kg at 7 DALA
6 Residue in control sample 0.011 mg/kg
_ Underlined values were used for MRL calculation

Table 6.3.4-7: Residues of pyrimethanil in lettuce after two applications of PYRIMETHANIL 400 SC in Northern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)	
							Matrix	Pyrimethanil
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	08 F CL CH P/B 2013/1399903 08 F CL CH P41 yes 2008	Head lettuce	Germany	PYRIMETHANIL 400 SC 2 x 0.800	19-41	14	Lettuce	0.026
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	08 F CL CH P/B 2013/1399903 08 F CL CH P42 yes 2008	Leaf lettuce	Germany	PYRIMETHANIL 400 SC 2 x 0.800	18-41	15	Lettuce	0.46

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
_ Underlined values were used for MRL calculation

Table 6.3.4-8: Residues of pyrimethanil in lettuce after two applications of PYRIMETHANIL 400 SC in Southern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)	
							Matrix	Pyrimethanil
Study code:	08 F CL CH P/B	Head lettuce	Spain	PYRIMETHANIL 400 SC 2 x 0.800	37	14	Lettuce	0.25
Doc ID:	2013/1399903							
Trial No:	08 F CL CH P21							
GLP:	yes							
Year:	2008							
Study code:	08 F CL CH P/B	Head lettuce	Spain	PYRIMETHANIL 400 SC 2 x 0.800	38	14	Lettuce	0.16
Doc ID:	2013/1399903							
Trial No:	08 F CL CH P22							
GLP:	yes							
Year:	2008							
Study code:	08 F CL CH P/B	Leaf lettuce	France	PYRIMETHANIL 400 SC 2 x 0.800	37	14	Lettuce	0.99
Doc ID:	2013/1399903							
Trial No:	08 F CL CH P07							
GLP:	yes							
Year:	2008							
Study code:	08 F CL CH P/B	Leaf lettuce	Greece	PYRIMETHANIL 400 SC 2 x 0.800	16	14	Lettuce	0.17
Doc ID:	2013/1399903							
Trial No:	08 F CL CH P38							
GLP:	yes							
Year:	2008							
Study code:	08 F CL CH P/B	Head lettuce	France	PYRIMETHANIL 400 SC 2 x 0.800	43	0	Lettuce (head)	14.1
Doc ID:	2013/1399903					1	Lettuce (head)	9.30
Trial No:	08 F CL CH P06					3	Lettuce (head)	7.50
GLP:	yes					7	Lettuce (head)	3.45
Year:	2008					14	Lettuce (head)	1.25
Study code:	08 F CL CH P/B	Leaf lettuce	Spain	PYRIMETHANIL 400 SC 2 x 0.800	38	0	Lettuce	9.48
Doc ID:	2013/1399903					1	Lettuce	4.32
Trial No:	08 F CL CH P23					3	Lettuce	3.43
GLP:	yes					7	Lettuce	1.18
Year:	2008					14	Lettuce	0.37
Study code:	08 F CL CH P/B	Leaf lettuce	Spain	PYRIMETHANIL 400 SC 2 x 0.800	38	0	Lettuce	15.4
Doc ID:	2013/1399903					1	Lettuce	12.5
Trial No:	08 F CL CH P24					3	Lettuce	5.79
GLP:	yes					7	Lettuce	2.27
Year:	2008					14	Lettuce	0.19
Study code:	08 F CL CH P/B	Head lettuce	Italy	PYRIMETHANIL 400 SC 2 x 0.800	41	0	Lettuce	21.5
Doc ID:	2013/1399903					1	Lettuce	15.7
Trial No:	08 F CL CH P34					3	Lettuce	7.45
GLP:	yes					8	Lettuce	2.16
Year:	2008					13	Lettuce	1.01

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
_ Underlined values were used for MRL calculation

This study has been already evaluated in the context of the Article 12 review (European Food Safety Authority; Review of the existing maximum residue levels (MRLs) for pyrimethanil according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2011;9(11):2454. [65 pp.] doi:10.2903/j.efsa.2011.2454EFSA Journal 2012; 10(3):2606. [36 pp.] doi:10.2903/j.efsa.2012.2606).

Report:	CA 6.3.4/3 Old J.,Smith A., 1998a Pyrimethanil suspension concentrate 400 g/L - Code: AE B100309 00 SC A304 - Residue trials in field lettuce for establishment of an MRL, following two applications in Northern Europe 1997 A91280
Guidelines:	none
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	AE B100309 00 SC37 A304 (SC)
Lot/Batch #:	08450342 (400 g/L pyrimethanil, nominal)
Purity:	Not reported
CAS#:	53112-28-0
Development code:	Not reported
Spiking levels:	0.05-1.0 mg/kg

2. Test Commodity:

Crop:	Lettuce
Type:	Leaf vegetables
Variety:	Enrica, Nadine, Saladin
Botanical name:	*Lactuca sativa*
Crop parts(s) or processed commodity:	Lettuce
Sample size:	Not reported

B. STUDY DESIGN

1. Test procedure

During the 1997 growing season 5 field trials in lettuce were conducted in Northern Europe to determine the residue level of pyrimethanil in or on raw agricultural commodities (RAC). AE B100309 00 SC37 A304 containing 400 g/L pyrimethanil was applied twice at individual rates equivalent to 0.8 kg pyrimethanil/ha in a spray volume of 400 L/ha. One untreated plot of each trial served as control. The applications were performed at BBCH 17-33 and 41-45 with a 12-16 days interval. Specimens of lettuce were collected immediately after the last application as well as 3, 7, 14 and 21 days thereafter. Samples were stored deep-frozen until analysis.

Table 6.3.4-9: Target application rates and timings for lettuce

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/ Timing
1997	5	2	F	AE B100309 00 SC37 A304 (SC)	BAS 605 F	0.8	400	28±1 DBH 14±1 DBH

DBH Days before harvest

2. Description of analytical procedures

The specimens were analyzed for pyrimethanil using AgrEvo method RESID/97/47 (see EU-reviewed method with DocID C000292 in chapter 4.1.2) quantifying the analyte with a limit of quantitation (LOQ) of 0.05 mg/kg. Residues of pyrimethanil were extracted by homogenization with acetone. Dilution with water under acid conditions was followed by washing with hexane. Neutralization with sodium hydrogen carbonate allowed extraction into hexane + ethyl acetate (3 + 1). Final clean-up was by silica solid phase extraction (SPE) which enabled the pyrimethanil residue to be determined by gas chromatography with mass selective detection (GC-MS).

Table 6.3.4-10: Summary of procedural recovery data for pyrimethanil

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
GC-MS; LOQ = 0.05 mg/kg		**Pyrimethanil (BAS 605 F)**		
Lettuce	0.05, 0.10, 0.25, 1.0	9	93	18

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.4-11, detailed residue levels are shown in Table 6.3.4-12.
Residues of pyrimethanil in treated lettuce specimens collected at the PHI (14 DALA) ranged from 0.06 to 0.55 mg/kg. A general residue decline could be observed from 0 to 21 DALA.
No residues of pyrimethanil above the limit of quantitation (0.05 mg/kg) were found in any of the analyzed untreated specimens.

Table 6.3.4-11: Summary of residues in lettuce treated with AE B100309 00 SC37 A304

Region	Year	DALA[1]	Growth stage[2] (BBCH)	Range of residues (mg/kg)	
				Matrix	Pyrimethanil
Northern EU	1997	0	41-45	Lettuce	4.1-35
		3	41-45	Lettuce	1.5-4.6
		7	43-47	Lettuce	0.11-2.1
		14	47-49	Lettuce	0.06-0.55
		21	49-53	Lettuce	<0.05-0.28

1 Days after last application
2 At sampling

III. CONCLUSION

Residues of pyrimethanil in treated lettuce specimens collected at the PHI (14 DALA) ranged from 0.06 to 0.55 mg/kg.

Table 6.3.4-12: Residues of pyrimethanil in lettuce after two applications of AE B100309 00 SC37 A304 in Northern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)	
							Matrix	Pyrimethanil
Study code:	390063	Head lettuce	Germany	AE B100309 00 SC37 A304 2 x 0.8	41	0	Lettuce	35
Doc ID:	A91280					3	Lettuce	4.5
Trial No:	5					7	Lettuce	1.5
GLP:	yes					14	Lettuce	0.30
Year:	1997					21	Lettuce	<0.05
Study code:	390063	Head lettuce	France	AE B100309 00 SC37 A304 2 x 0.8	45	0	Lettuce	9.3
Doc ID:	A91280					3	Lettuce	2.9
Trial No:	3					7	Lettuce	1.1
GLP:	yes					14	Lettuce	0.34
Year:	1997					21	Lettuce	0.13
Study code:	390063	Head lettuce	France	AE B100309 00 SC37 A304 2 x 0.8	43	0	Lettuce	26
Doc ID:	A91280					3	Lettuce	2.2
Trial No:	4					7	Lettuce	2.1
GLP:	yes					14	Lettuce	0.42
Year:	1997					21	Lettuce	0.28
Study code:	390063	Head lettuce	United Kingdom	AE B100309 00 SC37 A304 2 x 0.8	41	0	Lettuce	13
Doc ID:	A91280					3	Lettuce	4.6
Trial No:	1					7	Lettuce	0.47
GLP:	yes					14	Lettuce	0.55
Year:	1997					21	Lettuce	<0.05
Study code:	390063	Head lettuce	United Kingdom	AE B100309 00 SC37 A304 2 x 0.8	41	0	Lettuce	4.1
Doc ID:	A91280					3	Lettuce	1.5
Trial No:	2					7	Lettuce	0.11
GLP:	yes					14	Lettuce	0.06
Year:	1997					21	Lettuce	<0.05

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
_ Underlined values were used for MRL calculation

This study has been already evaluated in the context of the Article 12 review (European Food Safety Authority; Review of the existing maximum residue levels (MRLs) for pyrimethanil according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2011;9(11):2454. [65 pp.] doi:10.2903/j.efsa.2011.2454EFSA Journal 2012; 10(3):2606. [36 pp.] doi:10.2903/j.efsa.2012.2606).

Report:	CA 6.3.4/4 Old J.,Anderson I., 1999a Field lettuce: Residues of Pyrimethanil: EU (Northern zone): 1998 - Report of field and analytical phase - Pyrimethanil SC suspension concentrate 400 g/L C003112
Guidelines:	none
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Report:	CA 6.3.4/5 Old J.,Anderson I., 1999 b 1st Amendment to report No. 17108: Pyrimethanil suspension concentrate 400 g/L - Field lettuce: Residues of Pyrimethanil - EU (Northern zone): 1998 - Report of field and analytical phase C004097
Guidelines:	<none>
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	AE B100309 00 SC37 A402 (SC)
Lot/Batch #:	AE B100309 00 SC37 A402 (400 g/L pyrimethanil, nominal)
Purity:	Not reported
CAS#:	53112-28-0
Development code:	Not reported
Spiking levels:	0.05-0.50 mg/kg

2. Test Commodity:

Crop:	Lettuce
Type:	Leaf vegetables
Variety:	Brandon, Enrica, Lincoln, Nadine
Botanical name:	*Lactuca sativa*
Crop parts(s) or processed commodity:	Lettuce
Sample size:	Not reported

B. STUDY DESIGN

1. Test procedure

During the 1998 growing season 4 field trials in lettuce were conducted in Northern Europe to determine the residue level of pyrimethanil in or on raw agricultural commodities (RAC). AE B100309 00 SC37 A402 containing 400 g/L pyrimethanil was applied twice at individual rates equivalent to 0.8 kg pyrimethanil/ha in a spray volume of 400 L/ha. One untreated plot of each trial served as control. The applications were performed at BBCH 18-41 and 41-48 with a 13-14 days interval. Specimens of lettuce were collected immediately after the last application as well as 7 and 14 days thereafter. Samples were stored deep-frozen until analysis.

Table 6.3.4-13: Target application rates and timings for lettuce

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/ Timing
1998	4	2	F	AE B100309 00 SC37 A402 (SC)	BAS 605 F	0.8	400	28±1 DBH 14±1 DBH

DBH Days before harvest

2. Description of analytical procedures

The specimens were analyzed for pyrimethanil using AgrEvo method RESID/99/6 (see EU-reviewed method with DocID C000292 in chapter 4.1.2) quantifying the analyte with a limit of quantitation (LOQ) of 0.05 mg/kg. Residues of pyrimethanil were extracted by homogenization with acetone. Dilution with water under acid conditions was followed by washing with hexane. Neutralization with sodium hydrogen carbonate allowed extraction into hexane + ethyl acetate (3 + 1). Final clean-up was by silica solid phase extraction (SPE) which enabled the pyrimethanil residue to be determined by gas chromatography with mass selective detection (GC-MS).

Table 6.3.4-14: Summary of procedural recovery data for pyrimethanil

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
GC-MS; LOQ = 0.05 mg/kg		**Pyrimethanil (BAS 605 F)**		
Lettuce	0.05, 0.50	4	98	15

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.4-15, detailed residue levels are shown in Table 6.3.4-16.
Residues of pyrimethanil in treated lettuce specimens collected at the PHI (14 DALA) ranged from <0.05 to 0.43 mg/kg. A general residue decline could be observed from 0 to 14 DALA.
No residues of pyrimethanil above the limit of quantitation (0.05 mg/kg) were found in any of the analyzed untreated specimens.

Table 6.3.4-15: Summary of residues in lettuce treated with AE B100309 00 SC37 A402

Region	Year	DALA[1]	Growth stage[2] (BBCH)	Range of residues (mg/kg)	
				Matrix	Pyrimethanil
Northern EU	1998	0	12-47	Lettuce	2.4-22
		7	43-49	Lettuce	0.15-1.3
		14	47-49	Lettuce	<0.05-0.43

1 Days after last application
2 At sampling

III. CONCLUSION

Residues of pyrimethanil in treated lettuce specimens collected at the PHI (14 DALA) ranged from <0.05 to 0.43 mg/kg.

Table 6.3.4-16: Residues of pyrimethanil in lettuce after two applications of AE B100309 00 SC37 A402 in Northern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)	
							Matrix	Pyrimethanil
Study code:	391926	Head lettuce	United Kingdom	AE B100309 00 SC37 A402 2 x 0.8	41	0	Lettuce	12
Doc ID:	C003112					7	Lettuce	0.80
Trial No:	/CUPAR					14	Lettuce	<u><0.05</u>
GLP:	yes							
Year:	1998							
Study code:	391926	Head lettuce	The Netherlands	AE B100309 00 SC37 A402 2 x 0.8	43-45	0	Lettuce	18
Doc ID:	C003112					7	Lettuce	1.2
Trial No:	SIEBENGEWALD					14	Lettuce	<u>0.43</u>
GLP:	yes							
Year:	1998							
Study code:	391926	Head lettuce	United Kingdom	AE B100309 00 SC37 A402 2 x 0.8	48	0	Lettuce	2.4
Doc ID:	C003112					7	Lettuce	0.15
Trial No:	SOUTHERY					14	Lettuce	<u><0.05</u>
GLP:	yes							
Year:	1998							
Study code:	391926	Head lettuce	Germany	AE B100309 00 SC37 A402 2 x 0.8	43	0	Lettuce	22
Doc ID:	C003112					7	Lettuce	1.3
Trial No:	STRAELEN					14	Lettuce	<u>0.11</u>
GLP:	yes							
Year:	1998							

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
_ Underlined values were used for MRL calculation

This study has been already evaluated in the context of the Article 12 review (European Food Safety Authority; Review of the existing maximum residue levels (MRLs) for pyrimethanil according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2011;9(11):2454. [65 pp.] doi:10.2903/j.efsa.2011.2454EFSA Journal 2012; 10(3):2606. [36 pp.] doi:10.2903/j.efsa.2012.2606).

Report:	CA 6.3.4/6 Old J.,Smith A., 1998b Pyrimethanil suspension concentrate 400 g/L - Code: AE B100309 00 SC37 A304 - Residue trials in field lettuce for establishment of an MRL, following two applications in Southern Europe 1997 A91282
Guidelines:	none
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	AE B100309 00 SC37 A304 (SC)
Lot/Batch #:	08450342 (400 g/L pyrimethanil, nominal)
Purity:	Not reported
CAS#:	53112-28-0
Development code:	Not reported
Spiking levels:	0.05-1.0 mg/kg

2. Test Commodity:

Crop:	Lettuce
Type:	Leaf vegetables
Variety:	Veraniega, Justin, Rougette de Montpellier, Romana, Parris Island Cos
Botanical name:	*Lactuca sativa*
Crop parts(s) or processed commodity:	Lettuce
Sample size:	Not reported

B. STUDY DESIGN

1. Test procedure

During the 1997 growing season 5 field trials in lettuce were conducted in Southern Europe to determine the residue level of pyrimethanil in or on raw agricultural commodities (RAC). AE B100309 00 SC37 A304 containing 400 g/L pyrimethanil was applied twice at individual rates equivalent to 0.8 kg pyrimethanil/ha in a spray volume of 400 L/ha. One untreated plot of each trial served as control. The applications were performed at BBCH 15-44 and 42-46 with a 12-16 days interval. Specimens of lettuce were collected immediately after the last application as well as 3, 7-8, 14 and 21-22 days thereafter. Samples were stored deep-frozen until analysis.

Table 6.3.4-17: Target application rates and timings for lettuce

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/ Timing
1997	5	2	F	AE B100309 00 SC37 A304 (SC)	BAS 605 F	0.8	400	28±1 DBH 14±1 DBH

DBH Days before harvest

2. Description of analytical procedures

The specimens were analyzed for pyrimethanil using AgrEvo method RESID/98/7 (see EU-reviewed method with DocID C000292 in chapter 4.1.2) quantifying the analyte with a limit of quantitation (LOQ) of 0.05 mg/kg. Residues of pyrimethanil were extracted by homogenization with acetone. Dilution with water under acid conditions was followed by washing with hexane. Neutralization with sodium hydrogen carbonate allowed extraction into hexane + ethyl acetate (3 + 1). Final clean-up was by silica solid phase extraction (SPE) which enabled the pyrimethanil residue to be determined by gas chromatography with mass selective detection (GC-MS).

Table 6.3.4-18: Summary of procedural recovery data for pyrimethanil

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
GC-MS; LOQ = 0.05 mg/kg		**Pyrimethanil (BAS 605 F)**		
Lettuce	0.05, 0.10, 0.25, 1.0	10	85	13

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.4-19, detailed residue levels are shown in Table 6.3.4-20.
Residues of pyrimethanil in treated lettuce specimens collected at the PHI (14 DALA) ranged from 0.05 to 1.2 mg/kg. A general residue decline could be observed from 0 to 21 DALA.
No residues of pyrimethanil above the limit of quantitation (0.05 mg/kg) were found in any of the analyzed untreated specimens, except for the Greek trial where a residue of 0.182 mg/kg was found at 3 DALA; no explanation was given for this.

Table 6.3.4-19: Summary of residues in lettuce treated with AE B100309 00 SC37 A304

Region	Year	DALA[1]	Growth stage[2] (BBCH)	Range of residues (mg/kg)	
				Matrix	Pyrimethanil
Southern EU	1997	0	41-46	Lettuce	4.6-22
		3	43-47	Lettuce	1.2-7.8
		7-8	47-50	Lettuce	0.32-4.5
		14	49-61	Lettuce	0.05-1.2
		21-22	49-69	Lettuce	<0.05-0.77

1 Days after last application
2 At sampling

III. CONCLUSION

Residues of pyrimethanil in treated lettuce specimens collected at the PHI (14 DALA) ranged from 0.05 to 1.2 mg/kg.

Table 6.3.4-20: Residues of pyrimethanil in lettuce after two applications of AE B100309 00 SC37 A304 in Southern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)	
							Matrix	Pyrimethanil
Study code:	390105	Head lettuce	Spain	AE B100309 00 SC37 A304 2 x 0.8	42	0	Lettuce	11
Doc ID:	A91282					3	Lettuce	1.7
Trial No:	R153/ALBORAYA					7	Lettuce	0.45
GLP:	yes					14	Lettuce	0.05
Year:	1997					21	Lettuce	<0.05
Study code:	390105	Head lettuce	Italy	AE B100309 00 SC37 A304 2 x 0.8	43	0	Lettuce	13
Doc ID:	A91282					3	Lettuce	1.2
Trial No:	R153/GUIDIZZOLO					7	Lettuce	0.40
GLP:	yes					14	Lettuce	0.31
Year:	1997					21	Lettuce	<0.05
Study code:	390105	Head lettuce	France	AE B100309 00 SC37 A304 2 x 0.8	43	0	Lettuce	22
Doc ID:	A91282					3	Lettuce	7.8
Trial No:	R153/MONTFRIN					7	Lettuce	4.5[3]
GLP:	yes					14	Lettuce	0.78
Year:	1997					21	Lettuce	0.77
Study code:	390105	Head lettuce	Italy	AE B100309 00 SC37 A304 2 x 0.8	45	0	Lettuce	17
Doc ID:	A91282					3	Lettuce	4.6
Trial No:	R153/ROSETO					7	Lettuce	0.32
GLP:	yes					14	Lettuce	0.14
Year:	1997					21	Lettuce	0.11
Study code:	390105	Head lettuce	Greece	AE B100309 00 SC37 A304 2 x 0.8	46	0	Lettuce	4.6[3]
Doc ID:	A91282					3	Lettuce	1.9
Trial No:	R153/SOUTH PECITSA					8	Lettuce	1.9
GLP:	yes					14	Lettuce	1.2
Year:	1997					22	Lettuce	0.62

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
3 Mean of two analyses
_ Underlined values were used for MRL calculation

This study has been already evaluated in the context of the Article 12 review (European Food Safety Authority; Review of the existing maximum residue levels (MRLs) for pyrimethanil according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2011;9(11):2454. [65 pp.] doi:10.2903/j.efsa.2011.2454EFSA Journal 2012; 10(3):2606. [36 pp.] doi:10.2903/j.efsa.2012.2606).

Report: CA 6.3.4/7
Sonder K.-H., 1999a
Decline of residues in leaf lettuce - European Union, Southern zone, 1998 - Pyrimethanil (proposed ISO) - Water miscible suspension concentrate (SC) 400 g/L
C003103
Guidelines: none
GLP: yes
(certified by Hessisches Ministerium fuer Umwelt, Energie, Jugend, Familie und Gesundheit, Wiesbaden)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	AE B100309 00 SC37 A402 (SC)
Lot/Batch #:	11471189 (400 g/L pyrimethanil, nominal)
Purity:	Not reported
CAS#:	53112-28-0
Development code:	Not reported
Spiking levels:	0.05-1.0 mg/kg

2. Test Commodity:

Crop:	Lettuce
Type:	Leaf vegetables
Variety:	Nevada, Atraxion, Winter haven, Vanity
Botanical name:	*Lactuca sativa*
Crop parts(s) or processed commodity:	Lettuce
Sample size:	Not reported

B. STUDY DESIGN

1. Test procedure

During the 1998 growing season 4 field trials in lettuce were conducted in Southern Europe to determine the residue level of pyrimethanil in or on raw agricultural commodities (RAC). AE B100309 00 SC37 A402 containing 400 g/L pyrimethanil was applied twice at individual rates equivalent to 0.8 kg pyrimethanil/ha in a spray volume of 250-500 L/ha. One untreated plot of each trial served as control. The applications were performed at BBCH 17-43 and 39-48 with a 14-19 days interval. Specimens of lettuce were collected immediately after the last application as well as 7 and 14 days thereafter, except for the Portuguese trial where no 14 DALA samples was taken. Samples were stored deep-frozen until analysis for a maximum of 272 days.

Table 6.3.4-21: Target application rates and timings for lettuce

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/ Timing
1998	4	2	F	AE B100309 00 SC37 A402 (SC)	BAS 605 F	0.8	250-500	28±1 DBH 14±1 DBH

DBH Days before harvest

2. Description of analytical procedures

The specimens were analyzed for pyrimethanil using AgrEvo method RESID/98/36 (see EU-reviewed method with DocID C000292 in chapter 4.1.2) quantifying the analyte with a limit of quantitation (LOQ) of 0.05 mg/kg. Residues of pyrimethanil were extracted by homogenization with acetone. Dilution with water under acid conditions was followed by washing with hexane. Neutralization with sodium hydrogen carbonate allowed extraction into hexane + ethyl acetate (3 + 1). Final clean-up was by silica solid phase extraction (SPE) which enabled the pyrimethanil residue to be determined by gas chromatography with mass selective detection (GC-MS).

Table 6.3.4-22: Summary of procedural recovery data for pyrimethanil

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
GC-MS; LOQ = 0.05 mg/kg		**Pyrimethanil (BAS 605 F)**		
Lettuce	0.05, 0.10, 1.0	5	98	11

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.4-23, detailed residue levels are shown in Table 6.3.4-24.
Residues of pyrimethanil in treated lettuce specimens collected at the PHI (14 DALA) ranged from 0.62 to 7.5 mg/kg. A general residue decline could be observed from 0 to 14 DALA.
No residues of pyrimethanil above the limit of quantitation (0.05 mg/kg) were found in any of the analyzed untreated specimens.

Table 6.3.4-23: Summary of residues in lettuce treated with AE B100309 00 SC37 A402

Region	Year	DALA[1]	Growth stage[2] (BBCH)	Range of residues (mg/kg)	
				Matrix	Pyrimethanil
Southern EU	1998	0	39-48	Lettuce	13-33
		7	41-49	Lettuce	1.7-17
		14	44-49	Lettuce	0.62-7.5

1 Days after last application
2 At sampling

III. CONCLUSION

Residues of pyrimethanil in treated lettuce specimens collected at the PHI (14 DALA) ranged from 0.62 to 7.5 mg/kg.

Table 6.3.4-24: Residues of pyrimethanil in lettuce after two applications of AE B100309 00 SC37 A402 in Southern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1]	Residues found (mg/kg)	
							Matrix	Pyrimethanil
Study code:	ER 98 ECS 260	Leaf lettuce	France	AE B100309 00 SC37 A402 2 x 0.8	41	0	Lettuce	14
Doc ID:	C003103					7	Lettuce	2.7
Trial No:	FRA0001					14	Lettuce	<u>0.62</u>
GLP:	yes							
Year:	1998							
Study code:	ER 98 ECS 260	Leaf lettuce	Greece	AE B100309 00 SC37 A402 2 x 0.8	39	0	Lettuce	33
Doc ID:	C003103					7	Lettuce	17[3]
Trial No:	GRC0001					14	Lettuce	7.5[3,4]
GLP:	yes							
Year:	1998							
Study code:	ER 98 ECS 260	Leaf lettuce	Italy	AE B100309 00 SC37 A402 2 x 0.8	48	0	Lettuce	20
Doc ID:	C003103					7	Lettuce	2.4
Trial No:	ITA0001					14	Lettuce	<u>0.68</u>
GLP:	yes							
Year:	1998							
Study code:	ER 98 ECS 260	Leaf lettuce	Portugal	AE B100309 00 SC37 A402 2 x 0.8	47	0	Lettuce	13
Doc ID:	C003103					7	Lettuce	1.7
Trial No:	PRT0001							
GLP:	yes							
Year:	1998							

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
3 Mean of two analyses
4 Sample not considered for MRL calculation since the crop (BBCH 44) had not yet reached commercial maturity (BBCH 49)
_ Underlined values were used for MRL calculation

CA 6.4 Feeding studies

The requirements for feeding studies are set out according to Commission Regulation (EU) No 283/2013 with data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market as well as the Appendix G (Lundehn document 7031/VI/95 rev.4, July 1996) and OECD guidelines. Feeding studies are required:

(1) if significant residues (≥ 0.1 mg/kg of the total diet as received, except special cases, such as active substances which accumulate) occur in crops or part of the crops fed to livestock, and

(2) if metabolism studies indicate that significant residues (above 0.01 mg/kg for each analyte) may occur in any edible animal tissue, taking into account the residue levels in potential feeding stuff obtained at the 1x dose rate.

(3) However, feeding studies shall not be required where intake is below 0.004 mg/kg bw/d, except in cases where the residue, that is to say the active substance, its metabolites or breakdown products, as defined in the residue definition for risk assessment, tends to accumulate.

Data/information on poultry and lactating ruminant feeding studies (BASF doc ID B003807, Annex II chapter 6.4.1.2) for pyrimethanil were reviewed during the Annex I inclusion process and were considered to be acceptable. The following section was copied from the Draft Assessment Report, which was prepared by RMS Austria.

Table 6.4-1: Residues from livestock feeding studies (Annex IIA, point 6.4, Annex IIIA, point 8.3)

Intakes by livestock ≥ 0.1 mg/kg diet/day:	Ruminant: no	Poultry: no	Pig: no
Muscle	-	-	-
Liver	-	-	-
Kidney	-	-	-
Fat	-	-	-
Milk	-	-	-
Eggs	-	-	-

For the Annex I inclusion process data were submitted covering the safe uses in grapes (wine), apples and protein peas.

During Article 12 review of all EU MRLs (see EFSA Reasoned Opinion, EFSA Journal 2011;9(11):2454), the calculated dietary burdens for all groups of livestock were found to exceed the trigger value of 0.1 mg/kg DM/day; further investigation of residues would therefore be required in all commodities of animal origin. "Considering however that there is only a slight exceedance of the livestock dietary burden for poultry and that the real median residue for cereal grains is expected to be at least twice as low as the limit of quantitation (LOQ) used in the calculation, the actual livestock dietary burden is not expected to exceed the trigger value in practice. Further investigation of residues in poultry is therefore currently not required but will be required if the livestock dietary burden is further increased in the future." (EFSA Reasoned Opinion 2011)
The results of the dietary burden calculation are reported in the table below.

Table 6.4-2: Results of the dietary burden calculation (EFSA Reasoned Opinion, 2011)

	Maximum dietary burden (mg/kg bw/d)	Median dietary burden (mg/kg bw/d)	Highest contributing commodity	Max dietary burden (mg/kg DM)	Trigger exceeded (Y/N)
Risk assessment residue definition: pyrimethanil					
Dairy ruminants	0.13	0.13	Orange pomace	3.59	Y
Meat ruminants	0.45	0.45	Orange pomace	10.41	Y
Poultry	0.01	0.01	Potatoes	0.12	Y
Pigs	0.01	0.01	Potatoes	0.23	Y

In the context of this document, new feed burden calculations were performed (see chapter 6.7) using the OECD calculator and the Animal Model 2016 as well as EFSA PROFile considering all pyrimethanil uses, including non-BASF uses, i.e. a worst case scenario. The representative uses in strawberry and lettuce are no feed items in either model; apple is a feedstuff in ~~both~~ all scenarios, while grape is only considered in the OECD model.

In addition to the worst case calculations in chapter 6.7, a **feed burden calculation with BASF uses only** was performed using the OECD calculator and the Animal Model 2016 to show that no new feeding study is required, i.e. due to the newly found metabolites M605F025 (phenylguanidine) and M605F034.
Metabolites M605F004, M605F006 and M605F014 are not further evaluated here since they occur only in insignificant amounts.
The input values presented in chapter 6.7 were modified accordingly by deleting citrus fruits and replacing the input values for pome fruit by BASF data presented in this dossier, i.e. STMR of 0.22 mg/kg and a median processing factor of 1.6 for press cake (wet pomace).

The doses to be used (achieved with both the OECD calculator and the Animal Model 2016) when estimating the maximum residues in products of animal origin are for

Dairy cattle	0.04 mg/kg bw/d (1.16 mg/kg feed DM)
Beef cattle	0.03 mg/kg bw/d (1.16 mg/kg feed DM)
Poultry	0.04 mg/kg bw/d (0.61 mg/kg feed DM, layer)
Pig	0.04 mg/kg bw/d (1.17 mg/kg feed DM, finishing)

Thus requirement 1 and 3 are fulfilled with BASF uses. Following requirement 2, if metabolism studies indicate that significant residues (above 0.01 mg/kg for each analyte) may occur, is evaluated for the major and the new metabolites found in the new goat metabolism studies (see chapter MCA 6.2). In addition for all metabolites found in livestock their relevance for the consumer risk assessment was evaluated (see chapters MCA 6.7 and 6.9).

Overdosing factors

Beef cattle:
FB beef (1.16 mg/kg DM) / feeding level metabolism study goat (12.4 mg/kg DM) = 0.09
Dairy cattle:
FB dairy (1.16 mg/kg DM) / feeding level metabolism study goat (12.4 mg/kg DM) = 0.09
Poultry:
FB poultry (0.61 mg/kg DM) / feeding level metabolism study hen (11.3 mg/kg DM) = 0.05
Pig:
FB pig (1.17 mg/kg DM) / feeding level metabolism study goat (12.4 mg/kg DM) = 0.09

Extrapolation to TRR (mg/kg M605F034 in parent equiv.) in metabolism studies
Liver: 0.014 mg/kg TRR*0.09 = 0.001 mg/kg
Kidney: 0.125 mg/kg TRR*0.09 = 0.01 mg/kg
Milk: Not found

Since all values for M605F034 are ≤0.01 mg/kg, no new feeding study is required. A summary is also given in Table 6.4-3.

Extrapolation to TRR (mg/kg sum M605F002 + M605F023 + M605F035 in parent equiv.) in metabolism studies
Liver: 0.105 mg/kg TRR*0.09 = 0.009 mg/kg
Kidney: 0.198 mg/kg TRR*0.09 = 0.02 mg/kg
Milk: 0.031 mg/kg TRR*0.09 = 0.003 mg/kg

Only in kidney there was a finding exceeding the trigger value of 0.01 mg. However, since the peer-reviewed cow feeding study includes an enzymatic cleavage step in the method used (B003807) and thus analyzed for free M605F002, the respective conjugates are covered as well. Therefore, M605F002 and its conjugates are covered by the peer-reviewed feeding study.

Extrapolation to TRR (mg/kg sum M605F003 + M605F020 + M605F021 in parent equiv.) in metabolism studies
Liver: 0.015 mg/kg TRR*0.09 = 0.001 mg/kg
Kidney: 0.062 mg/kg TRR*0.09 = 0.006 mg/kg
Milk: 0.023 mg/kg TRR*0.09 = 0.002

Since all values are ≤0.01 mg/kg, no feeding study is required. A summary is also given in Table 6.4-3.

Extrapolation to TRR (mg/kg M605F025 in parent equiv.) in metabolism studies
Liver: 0.059 mg/kg TRR*0.09*MCF (0.68) = 0.0036 mg/kg
Kidney: 0.006 mg/kg TRR*0.09*MCF (0.68) = 0.0004 mg/kg
Milk: 0.001 mg/kg TRR*0.09*MCF (0.68) = 0.0001 mg/kg

Since all values for M605F025 are ≤0.01 mg/kg, no new feeding study is required. A summary is also given in Table 6.4-3.

Table 6.4-3: Summary of expected residues in ruminant matrices

Matrix	Max. mg/kg metabolite in goat metabolism study				Expected residues expressed as pyrimethanil (mg/kg)			
	Sum of M605F002 and its conjugates	Sum of M605F003 and its conjugates	M605F034	M605F025	Sum of M605F002 and its conjugates (mg/kg TRR x OF[1])	Sum of M605F003 and its conjugates (mg/kg TRR x OF[1])	M605F034 (mg/kg TRR x OF[1])	M605F025 (mg/kg TRR x OF[1] x MCF[2])
Bovine liver	0.105	0.015	0.014	0.059	0.009	0.001	0.001	0.0036
Bovine kidney	0.198	0.062	0.125	0.006	**0.018**	0.006	0.01	0.0004
Milk	0.031	0.023	-	0.001	0.003	0.002	-	0.0001

1 Beef cattle: FB beef (1.16 mg/kg DM OECD) / feeding level metabolism study goat (12.4 mg/kg DM) OF 0.09
Dairy cattle: FB dairy (1.16 mg/kg DM) / feeding level metabolism study goat (12.4 mg/kg DM) OF 0.09
2 Molecular weight correction factor ($MW_{M605F025}$ (135.1691 g/mol) / MW_{parent} (199.2557 g/mol)) MCF 0.68

Poultry is considered in chapter MCA 6.7 for MRL derivation; therefore the calculations are not repeated here. It was shown that all values are ≤0.01 mg/kg using the worst-case feed burden calculation; no feeding study in poultry is necessary.

CA 6.4.1 Poultry

For poultry, the calculated dietary burden performed during Article 12 review was only slightly exceeding the trigger value of 0.1 mg/kg DM. Taking into consideration that this calculation includes values at LOQ, for cereal grain in particular, EFSA was of the opinion that the real exposure will be lower than 0.1 mg/kg DM and that MRLs in poultry are not required for the time being (see EFSA Reasoned Opinion 2011).
Since the feed burden using EFSA PROFile calculation did not change for this dossier, no feeding study with poultry was considered necessary for active substance approval renewal. Furthermore, the residues in poultry matrices extrapolated from the hen metabolism study are <0.01 mg/kg, supporting that no poultry feeding study is needed.

CA 6.4.2 Ruminants

During the peer review under Directive 91/414/EEC the magnitude of pyrimethanil residues in livestock was investigated in a feeding study (B003807) with lactating cows. Four groups of lactating cows, each consisting of three animals, except the highest dose group consisting of 4 animals, were dosed for 28 days with pyrimethanil at levels of 0.03, 0.1, 0.3 and 1.5 mg/kg bw/day. Results of this livestock feeding study are summarized in the table below; no residues at or above the LOQ were found in any matrix of the lowest dose group.

In the Article 12 review, EFSA based MRLs and risk assessment values for ruminant and pig products on this peer-reviewed livestock feeding study in compliance with the latest international recommendations on this matter (FAO, 2009). EFSA was of the opinion that maximum residue values in tissues were not reported. Individual feeding study results for each animal at each feeding level (ruminants) were therefore considered desirable but not essential.
To meet EFSA's wish, individual results are added to the table below in italic font for kidney and milk, since these two matrices were the only ones which showed any residues; it should be noted that parent pyrimethanil residues were below the limit of detection (LOD = 1/3 LOQ; 0.017 mg/kg for kidney and 0.0033 mg/kg for milk) if analyzed. In contrast to the Reasoned Opinion, metabolite values have been converted to parent equivalent for the individual cows. EFSA noted that for ruminant kidney a slight underestimation of the MRL cannot be excluded considering that the maximum residue values in the livestock feeding study were not reported. It can be clarified here that this is not the case since in the dose level 0.3 mg/kg bw/d the mean and maximum residue are similar.

Table 6.4.2-1: Overview of the values derived from the livestock feeding study

Commodity	Dose level (mg/kg bw/d)	No of animals *Name of individual animal*	Result for enforcement[1]		Result for risk assessment[1]	
			Mean (mg/kg)	Max (mg/kg)	Mean (mg/kg)	Max (mg/kg)
Residue definition for enforcement and risk assessment: sum of pyrimethanil and SN 614 276 (M605F002), expressed as pyrimethanil						
Ruminant meat	0.10	3	<0.02[3]	n.r.	<0.02[3]	n.r.
	0.30	3	<0.02[3]	n.r.	<0.02[3]	n.r.
	1.50	4	<0.02[3]	n.r.	<0.02[3]	n.r.
Ruminant fat	0.10	3	<0.02[3]	n.r.	<0.02[3]	n.r.
	0.30	3	<0.02[3]	n.r.	<0.02[3]	n.r.
	1.50	4	<0.02[3]	n.r.	<0.02[3]	n.r.
Ruminant liver	0.10	3	<0.02[3]	n.r.	<0.02[3]	n.r.
	0.30	3	<0.02[3]	n.r.	<0.02[3]	n.r.
	1.50	4	<0.02[3]	n.r.	<0.02[3]	n.r.
Ruminant kidney	0.10	3	0.08 *(0.06)*	n.r.	0.08 *(0.06)*	n.r.
		3688	*0.08 (0.06)*	*0.09 (0.07)*	*0.08 (0.06)*	*0.09 (0.07)*
		3703	*0.09 (0.07)*		*0.09 (0.07)*	
		3692	*0.08 (0.06)*		*0.08 (0.06)*	
	0.30	3	0.13 *(0.11)*	n.r.	0.13 *(0.11)*	n.r.
		3694	*0.13 (0.12)*	*0.13 (0.12)*	*0.13 (0.12)*	*0.13 (0.12)*
		3695	*0.13 (0.12)*		*0.13 (0.12)*	
		3698	*0.12 (0.10)*		*0.12 (0.10)*	
	1.50	4	0.49 *(0.45)*	n.r.	0.49 *(0.45)*	n.r.
		3691	*0.83 (0.81)*	*0.83 (0.81)*	*0.83 (0.81)*	*0.83 (0.81)*
		3693	*0.23 (0.21)*		*0.23 (0.21)*	
		3701	*0.73 (0.71)*		*0.73 (0.71)*	
		3702	*<0.07 (<0.05)*		*<0.07 (<0.05)*	
Pig meat[2]	0.10	3	<0.02[3]	n.r.	<0.02[3]	n.r.
	0.30	3	<0.02[3]	n.r.	<0.02[3]	n.r.
	1.50	4	<0.02[3]	n.r.	<0.02[3]	n.r.
Pig fat[2]	0.10	3	<0.02[3]	n.r.	<0.02[3]	n.r.
	0.30	3	<0.02[3]	n.r.	<0.02[3]	n.r.
	1.50	4	<0.02[3]	n.r.	<0.02[3]	n.r.
Pig liver[2]	0.10	3	<0.02[3]	n.r.	<0.02[3]	n.r.
	0.30	3	<0.02[3]	n.r.	<0.02[3]	n.r.
	1.50	4	<0.02[3]	n.r.	<0.02[3]	n.r.
Pig kidney[2]	0.10	3	0.08	n.r.	0.08	n.r.
	0.30	3	0.13	n.r.	0.13	n.r.
	1.50	4	0.49	n.r.	0.49	n.r.

Table 6.4.2-1: Overview of the values derived from the livestock feeding study

Commodity	Dose level (mg/kg bw/d)	No of animals *Name of individual animal*	Result for enforcement[1]		Result for risk assessment[1]	
			Mean (mg/kg)	Max (mg/kg)	Mean (mg/kg)	Max (mg/kg)
Residue definition for enforcement and risk assessment: sum of pyrimethanil and SN 614 277 (M605F003), expressed as pyrimethanil						
Milk	0.10	3	<0.020 *(<0.01)*	n.a.	<0.020 *(<0.01)*	n.a.
		3688	*<0.02 (<0.01)*	*n.a.*	*<0.02 (<0.01)*	*n.a.*
		3703	*<0.02 (<0.01)*		*<0.02 (<0.01)*	
		3692	*<0.02 (<0.01)*		*<0.02 (<0.01)*	
	0.30	3	0.025 *(0.016)*	n.a.	0.025 *(0.016)*	n.a.
		3694 (Day 22)	*0.016 (0.015)*	*n.a.*	*0.016 (0.015)*	*n.a.*
		3695 (Day 22)	*0.025 (0.023)*		*0.025 (0.023)*	
		3698 (Day 22)	*<0.02 (<0.01)*		*<0.02 (<0.01)*	
	1.50	4	0.070 *(0.063)*	n.a.	0.070 *(0.063)*	n.a.
		3691 (Day 27)	*0.059 (0.056)*	*n.a.*	*0.059 (0.056)*	*n.a.*
		3693 (Day 27)	*0.047 (0.044)*		*0.047 (0.044)*	
		3701 (Day 27)	*0.084 (0.081)*		*0.084 (0.081)*	
		3702 (Day 27)	*0.075 (0.072)*		*0.075 (0.072)*	

n.a. Not applicable - only the mean values are considered for calculating MRLs in milk
n.r. Not reported - MRLs were calculated based on the mean result instead of the maximum result
Information in *italics* was added to EFSA's table
1 Values in parentheses show the values of the respective individual metabolite expressed as parent equivalents.
2 The feeding studies were carried out with ruminants; according to the metabolism pathway, an extrapolation between ruminant and pig is acceptable
3 This should read <0.1 since the LOQ of the analytical method used in the feeding study was 0.05 mg/kg per analyte for tissues.

As the available ruminant feeding study was considered acceptable by EFSA during Article 12 review, no new feeding study was conducted, also with regard to animal welfare rules.

The new feed burden calculations performed (see chapter 6.7) using the OECD calculator as well as EFSA PROFile result in no residues above the current EU MRL of 0.1 mg/kg for ruminant muscle, fat, liver and edible offal; 0.2 mg/kg for ruminant kidney and 0.05 mg/kg for milk are anticipated, regardless of the method for feed burden calculation. No new MRLs are proposed.

Furthermore, none of the metabolites identified in the new goat metabolism study which were not covered by the analytical method used in the peer-reviewed cow feeding study would exceed 0.01 mg/kg in milk or animal tissues, taking the BASF uses in consideration. This is an additional strong argument for the conclusion that a new cow feeding study is not necessary.

CA 6.4.3 Pigs

In case of pyrimethanil, the metabolism in rats and ruminants are similar. Therefore no pig feeding study is required.

CA 6.4.4 Fish

According to the Commission Regulations (EU) No 283/2013 (active substances) and 284/2013 (plant protection products) as of 1 March 2013, fish feeding studies might be required active substances whose approval expires on 1 January 2016 or later. A fish feeding study may be required where residues at levels above 0.01 mg/kg may be reasonably expected in edible tissues, based on the findings of the fish metabolism study and the estimated maximum residues which might occur in fish feed. Particular attention should be laid on lipophilic substances with an intrinsic tendency for accumulation.
For pyrimethanil, no fish metabolism study was regarded necessary since EFSA considered the risk for bioconcentration in fish to be low as the log Pow is ≤3 for pyrimethanil (see chapter 6.2.5). EFSA agreed with the JMPR conclusion that the residue is not fat-soluble. Thus, it can be reasonably assumed that residue levels in edible fish tissues will not accumulate.

Further a feeding on fish is only required

(1) where significant residues (≥ 0.1 mg/kg of the total diet as received, except special cases, such as active substances which accumulate) occur in crops or part of the crops fed to fish,

and

(2) the log Pow is >3.

According to the working document on the nature of pesticide residues in fish, fish diet for trout and carp mainly consists of cereals, pulses, oilseeds and processed fractions thereof. Thus the representative uses of this dossier apples, grapes, strawberry and lettuce are not considered as fish diet. Furthermore, MRLs for fish matrices will not be set in close future. Due to these facts, but also driven by the lack of any suitable EU guideline / guidance document for the conduct of fish feeding studies, a respective study was not performed.

CA 6.5 Effects of Processing

CA 6.5.1 Nature of the residue

Data/information on processing studies were reviewed during the Annex I inclusion process and were considered acceptable.

The effect of processing on the nature of the pyrimethanil residue was investigated in a standard hydrolysis study (C004545, Annex II chapter 6.5.1) performed at three test conditions (20 minutes at 90°C, pH 4; 60 minutes at 100°C, pH 5; 20 minutes at 120°C, pH 6). It was concluded that pyrimethanil is stable under all conditions tested and that no formation of toxicologically relevant metabolites occurs. Thus, for processed commodities the same residue definition as for raw agricultural commodities (RAC) is applicable (see EFSA Reasoned Opinion, EFSA Journal 2011;9(11):2454).

A separate high temperature hydrolysis study for M605F002 and M605F003 (included in proposed residue definition in animal matrices for monitoring and risk assessment) is not considered necessary for the following reason: M605F002's and M605F003's chemical structures differ from their parent pyrimethanil only by hydroxyl groups (-OH) on the phenyl- or pyrimidinyl-ring, respectively. As no cleavage or ring opening occurred under all tested conditions in the high temperature hydrolysis study it is highly unlikely that M605F002 and M605F003 will react differently. Therefore the metabolites and their respective conjugates can be considered as covered by the previous high temperature hydrolysis studies for parent.

CA 6.5.2 Distribution of the residue in inedible peel and pulp

All representative uses to be evaluated in this dossier (apples, grapes, strawberries and lettuce) are crops with edible peel only. Therefore, studies on the distribution between peel and pulp are not required.

CA 6.5.3 Magnitude of residues in processed commodities

In the framework of the peer review, processing studies on apples (processed products: puree and juice; by-products: peel and press cake) and on grapes (processing in wine) have been submitted. In apple by-products such as peel and press cake, residues of pyrimethanil increase with calculated processing factors of 1.91 (peel; mean value) and 1.27 (press cake; mean value).
No concentration of pyrimethanil could be observed during the processing of grapes in wine. Two results showed a concentration of pyrimethanil residues after processing of grapes in wine but they were considered negligible compared to the large dataset available for wine. Processing of apples in juice did not lead to a concentration of pyrimethanil. (see EFSA Reasoned Opinion, EFSA Journal 2011;9(11):2454; see also Table 6.5.3-1).

In the framework of an MRL application, processing studies in peas and beans were also evaluated. The pea study was conducted using incurred residues (1.39 to 2.18 mg/kg in peas without pods) derived from an application of 1.8 kg a.s./ha with a 6 day PHI. Residues were of sufficient magnitude both before and after processing to derive processing factors for washing, blanching and canning. No significant concentration of residues was seen in any of the processed products. The green bean study was conducting using incurred residues (0.12 to 0.37 mg/kg in green beans) derived from two applications of 0.856 kg a.s./ha. Residues were of sufficient magnitude both before and after processing to derive processing factors for canned, blanched and frozen green beans. No concentration of residues was seen in any of the processed bean products (see EFSA Reasoned Opinion, EFSA Journal 2011;9(11):2454); see also Table 6.5.3-1).

An overview of all present processing studies is available in the tables below. Robust processing factors for enforcement purposes could be derived for all crops, except for blanched beans with pods because a minimum of 3 studies is normally required. Further processing studies are not required as they are not expected to affect the outcome of the risk assessment required (see EFSA Reasoned Opinion, EFSA Journal 2011;9(11):2454).

Table 6.5.3-1: Overview of the available processing studies (EFSA Reasoned Opinion 2011; EFSA Conclusion 2006; DAR 2004)

Processed commodity	Number of studies	Median PF[1]	Median CF[2]	Comments
Enforcement residue definition: Pyrimethanil				
Processing factors recommended for enforcement and risk assessment (sufficiently supported by data)				
Apples, dry pomace	4	0.67[3]	1.00	*Studies peer-reviewed: A81719, A81749* *BASF comment: pomace is referred to as such in study report A81719 but actually puree was produced (see also JMPR Evaluation Report 2007 and overview apples PF Table 6.5.3-2)*
Apples, wet pomace (study: A81719, A81749)	4	0.67[3]	1.00	*Studies peer-reviewed: A81719, A81749* *BASF comment: pomace is referred to as such in study report A81719 but actually puree was produced (see also JMPR Evaluation Report 2007 and overview apples PF Table 6.5.3-2)*
Apple sauce (puree)	4	0.69[3]	1.00	*Studies peer-reviewed: A81719, A81749*
Apple juice	4	0.62[3]	1.00	*Studies peer-reviewed: A81719, A81749*
Apple, peel	4	1.91[3]	1.00	*Studies peer-reviewed: A81719, A81749*
Apple, press cake	4	1.28[3]	1.00	*Studies peer-reviewed: A81719, A81749* *BASF comment: press cake is referred to as such in study reports A81719 and A81749 but actually pomace was produced (see also JMPR Evaluation Report 2007 and overview apples PF Table 6.5.3-2)*
Wine grapes, wine	52	0.43[3]	1.00	*Studies peer-reviewed: A81696, A81697, A81702, A81731* Based on the available data (EFSA, 2006), no distinction can be made between different types of wine. Further clarification might be required later on. *BASF comment: The red wine making includes a heating process and therefore the PF could differ from white wine. However, for pyrimethanil the hydrolysis study showed no influence of heating on the nature of the residue. Therefore there should be no significant difference between red and white wine making. See grape PF Table 6.5.3-12 and Table 6.5.3-13*

Table 6.5.3-1: Overview of the available processing studies (EFSA Reasoned Opinion 2011; EFSA Conclusion 2006; DAR 2004)

Processed commodity	Number of studies	Median PF[1]	Median CF[2]	Comments
Peas, washed	4	0.93	1.0	*MRL review*
Peas, blanched	4	1.02	1.0	*MRL review*
Peas, canned	4	0.86	1.0	*MRL review (available BASF studies: DocID 2008/1077929)*
Beans with pods, canned	4	0.40	1.0	*MRL review (available BASF studies: DocID C012166)*
Beans with pods, frozen	4	0.45	1.0	*MRL review*
Indicative processing factors (limited data sets)				
Beans with pods, blanched	1	0.67	1.0	*MRL review*

1 The median processing factor is obtained by calculating the median of the individual processing factors of each processing study.
2 The median conversion factor for enforcement to risk assessment is obtained by calculating the median of the individual conversion factors of each processing study.
3 The value reported corresponds to a mean processed factor and not a median processing factor.
Italic BASF additional information and comments

For the representative uses to be evaluated in this dossier (apples, grapes, strawberries and lettuce), the following processing procedures are essential (category 1) according to OECD guideline 508 and OECD guidance document 96: production of juice and wet pomace for apple, production of must, wine, juice and wet pomace for grape and production of juice for strawberry; for lettuce, no processing data are required. A brief summary of the processing factors and studies available is given below, followed by more detailed study summaries.

Apple:

The studies reviewed on EU level cover the processing of apple into juice and wet pomace as well as the optional product apple sauce (puree) and the additional products peel and press cake. Two additional studies are summarized further below in this chapter which were either evaluated by JMPR or submitted for product registration on Member State level, therefore not yet peer-reviewed. All transfer factors are summarized in Table 6.5.3-2 and again in Table 6.5.3-7.

Table 6.5.3-2: Overall mean processing factors for pyrimethanil residues in essential apple processed fractions

Source: DocID (trial location or reference)	Juice	Press cake (wet pomace)	Status of evaluation	Comment
A81719 + A81721 (Horneburg-Neuenkirchen)	0.43	2.0	Peer-reviewed; see Draft Assessment Report 2004, Volume 3, Annex B.7 Residue data Evaluated in JMPR Evaluation Report 2007	Pomace and press cake are referred to as such in study report A81719 but actually puree and pomace were produced, respectively (see also JMPR Evaluation Report 2007); thus, the study was carefully reconsidered in this document
A81719 + A81721 (Ersdorf)	0.61	0.63	Peer-reviewed; see Draft Assessment Report 2004, Volume 3, Annex B.7 Residue data JMPR Evaluation Report 2007	
A81749 (Tönisvorst)	0.45	0.88	Peer-reviewed; see Draft Assessment Report 2004, Volume 3, Annex B.7 Residue data JMPR Evaluation Report 2007	Press cake is referred to as such in study report A81749 but actually pomace was produced (see also JMPR Evaluation Report 2007); thus, the study was carefully reconsidered in this document
A81749 (Orsingen-Nenzingen)	1.0	1.3	Peer-reviewed; see Draft Assessment Report 2004, Volume 3, Annex B.7 Residue data JMPR Evaluation Report 2007	
B002853 (R11-01)	0.35	4.1	JMPR Evaluation Report 2007	Full summary is provided
C029321 (AG61632/2)	0.58*	1.9	Submitted for product registration on Member State level	Full summary is provided
Mean	**0.57**	**1.8**		
Median	**0.52**	**1.6**		

* Cloudy juice; value for clear juice is 0.51

Grapes:

The studies reviewed on EU level cover the processing of grapes into wine. Processing into wine, juice and wet pomace is covered within this dossier, as well as the optional product raisins. Processing into must as well as juice, pomace and wine is described in public literature, which is summarized further below.
According to OECD guidance document 96, the theoretical processing factor for raisins is 5. The available processing study (see BASF DocID A81725 + A89698, summaries further below) confirmed residue concentration but to a lesser extent.
The results on processing into wine described in public literature support the findings of the peer-reviewed studies (see Table 6.5.3-1). Residues do not concentrate in either white or red wine, supporting the BASF statement made in Table 6.5.3-1. In the following tables a brief summary of the studies and public literature submitted in this dossier is given.

Table 6.5.3-3: Overall mean processing factors for pyrimethanil residues in essential and optional white grape processed fractions

Source: DocID (trial location or reference)	Juice	Wet pomace (cake)	Raisins	Must[1]	Bottled wine	Status of evaluation	Comment
A81725+A89698 (Fresno, CA, USA)	0.69	2.41	1.63	-	-	JMPR Evaluation Report 2007	
2014/1326452 (Casablanca Valley, Chile)	0.36	0.98	-	0.25	0.10	Not yet evaluated	Public literature
2010/1233252 (Slovenia)	-	3.9	-	0.36	-	Not yet evaluated	Public literature
2005/1044080 (Spain, D)	-	1.03	-	0.58[2]	0.50	Not yet evaluated	Public literature
Mean	**0.53**	**2.08**	**1.63**	**0.40**	**0.30**		

1 Fermented juice
2 Worst case at 5 days
3 Worst case

Table 6.5.3-4: Overall mean processing factors for pyrimethanil residues in essential red grape processed fractions

Source: DocID (trial location or reference)	Fermented juice (must; drop wine)	Fermented juice (must; press wine)	Fermented pomace / wet pomace (cake)	Bottled wine	Status of evaluation	Comment
2014/1326452 (Casablanca Valley, Chile)	0.26	0.39	1.5	0.09	Not yet evaluated	Public literature
2010/1233252 (Slovenia)	-	-	3.5	-	Not yet evaluated	Public literature
2005/1044080 (Spain, A)	-	0.31	3.04	0.12	Not yet evaluated	Public literature
2005/1044080 (Spain, B)	-	0.39	1.69	0.28	Not yet evaluated	Public literature
2005/1044080 (Spain, C)	-	0.13	2.25	0.08	Not yet evaluated	Public literature
Mean	**0.26**	**0.31**	**2.4**	**0.14**		
2005/1044080 (Spain, D[1])	-	0.88[2]	0.92	0.48	Not yet evaluated	Public literature

1 Not included in mean since this refers to rosé wine making
2 Worst case at 5 days
3 Worst case

Strawberry:

For strawberry, two processing studies (BASF DocIDs 2008/1022961 and A89298) are available and summarized further below in which strawberries were processed into washed berries, jam and canned strawberries. None of these processing products are essential. No juice was produced in the available processing studies. However, extrapolation is possible from grape juice. It was shown that pyrimethanil residues do not concentrate in grape juice (see BASF DocID A81725 + A89698 and 2014/1326452, Alister *et al.*, 2014).

Apple

As stated above, the studies reviewed on EU level cover the processing of apple into juice and wet pomace as well as the optional product apple sauce (puree) and the additional products peel and press cake. Two additional studies (B002853, C029321) are summarized below which were either evaluated by JMPR or submitted for product registration on Member State level, therefore not yet peer-reviewed. All transfer factors are summarized in Table 6.5.3-7.

Report:	CA 6.5.3/1 Brady S.S., 2000a Magnitude of Pyrimethanil residues in or on apples and processed apple commodities resulting from four applications of Scala, USA, 1999 B002853
Guidelines:	EPA 860.1520
GLP:	yes (certified by United States Environmental Protection Agency)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	Scala (SC)
Lot/Batch #:	AABA00049 (400 g/L pyrimethanil, nominal)
CAS#:	53112-28-0

2. Test Commodity:

Crop:	Apple
Type:	Pome fruit
Variety:	Red Delicious
Botanical name:	*Malus domestica*
Crop parts(s) or processed Commodity:	Fruits, wet pomace, juice

B. STUDY DESIGN

1. Test procedure

During the 1999 growing season, one field trial was conducted at a representative apple growing area in the USA to determine the residue level of pyrimethanil (BAS 605 F) in apple processed fractions.
Scala was foliar applied four times at an exaggerated target rate of 2.25 kg a.s./ha to apple trees with a retreatment interval of 7 days. The applications were made 52, 59, 66 and 73 days prior to harvest. The spray volume used was 879-917 L/ha.
Fruit RAC samples were harvested in duplicate 73 days after the last application. Apple fruit samples were processed according to simulated commercial procedures into wet pomace and juice. Samples were stored deep-frozen until analysis for a maximum of 200 days.

2. Description of analytical procedures

Residues of pyrimethanil were determined using AgrEvo method No DGM C05/98-0. Residues of pyrimethanil were extracted with acetone. After acidification and partitioning with hexane, the hexane layer was made basic and extracted with ethyl acetate/hexane. Depending on the matrix, the extract was further cleaned up by silica gel solid phase extraction. The final determination was performed by GC-MS. The validated limit of quantitation was 0.05 mg/kg.
Average concurrent recoveries of pyrimethanil were 100% with fortification levels of 0.05 and 1.0 mg/kg.

II. RESULTS AND DISCUSSION

Residues of pyrimethanil were in the range of 0.11 to 0.23 mg/kg for apple fruits at 73 DALA.
The mean transfer factor was below 1 in juice. Therefore it can be concluded that pyrimethanil does not concentrate in juice. The mean transfer factor was above 1 (4.1) in wet pomace, which is not destined for human consumption.

III. CONCLUSION

It was shown that pyrimethanil concentrated in wet pomace but not in juice, which is destined for human consumption.

Table 6.5.3-5: Residues of pyrimethanil in apple processed fractions

Trial location / Trial number	Processed commodity	Application rate (kg a.s./ha)	DALA[1]	Residues found[3] (mg/kg)	Processing factor[2]
				Pyrimethanil	
Yakima, WA, USA (R11-01) 1999	**Fruit (RAC)**	4 x 2.25	73	**0.17** (0.11, 0.23)	-
	Wet pomace			0.70 (0.75, 0.64)	4.1
	Juice			0.06 (0.06, 0.06)	0.35

1 Days after last application
2 The processing factor is calculated by dividing the residue in the processed fraction by the residue in the RAC sample
3 Mean of two field samples; individual values are given in parentheses

Report:	CA 6.5.3/2 Chambers J.G.,Taylor N.W., 2003a AE B100309 00 SC37 A4 (EXP10588A), AE C597265 00 1K10 A1 (EXP10979A) - Determination of residues in juice, puree and processing fractions following the application of a tank-mix to apples trees C029321
Guidelines:	none
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	AE B100309 00 SC37 A4 / EXP10588A (SC)
Lot/Batch #:	OP210191 (400 g/L pyrimethanil, nominal)
CAS#:	53112-28-0

2. Test Commodity:

Crop:	Apple
Type:	Pome fruit
Variety:	Cox Orange Pippin
Botanical name:	*Malus domestica*
Crop parts(s) or processed Commodity:	Fruits, wash water, wet pomace, cloudy juice, clear juice, pasteurized juice, peel, cores, puree, pasteurized puree

B. STUDY DESIGN

1. Test procedure

During the 2001 growing season, one field trial was conducted at a representative apple growing area in United Kingdom to determine the residue level of pyrimethanil (BAS 605 F) in apple processed fractions.

AE B100309 00 SC37 A4 (in a tank mix with a fluquinconazole-containing formulation) was foliar applied twice at a target rate of 0.800 kg a.s./ha to apple trees with a retreatment interval of 16 days. The applications were made 30 and 14 days prior to harvest. The spray volume used was about 1000 L/ha.

Fruit RAC samples were harvested 14 days after the last application. Apple fruit samples were processed according to simulated commercial procedures into juice and puree. Samples were stored deep-frozen until analysis.

2. Description of analytical procedures

Residues of pyrimethanil were determined using AgrEvo method No DGM C05/98-0. Residues of pyrimethanil were extracted with acetone. After concentration, clean-up was done by solvent partition. The final determination was performed by GC-MS. The validated limit of quantitation was 0.01 mg/kg.
Average concurrent recoveries of pyrimethanil were 85% with fortification levels of 0.01-4.0 mg/kg.

II. RESULTS AND DISCUSSION

Residues of pyrimethanil were 0.85 mg/kg for apple fruits at 14 DALA.
The mean transfer factor was below 1 in wash water, cloudy juice, clear juice, pasteurized juice, cores, puree and pasteurized puree. Therefore it can be concluded that pyrimethanil does not concentrate in these matrices. The mean transfer factor was above 1 in wet pomace and peel, which are not destined for human consumption.

III. CONCLUSION

It was shown that pyrimethanil did not concentrate in any matrix but wet pomace and peel, which are not destined for human consumption.

Table 6.5.3-6: Residues of pyrimethanil in apple processed fractions

Trial location / Trial number	Processed commodity	Application rate (kg a.s./ha)	DALA[1]	Residues found (mg/kg)	Processing factor[2]
				Pyrimethanil	
Longstanton, United Kingdom (AG61632/2) 2001	**Fruit (RAC)**	2 x 0.800	14	**0.85**	-
	Wash water			<0.01	0.01
	Wet pomace			1.6	1.9
	Cloudy juice			0.49	0.58
	Clear juice			0.43	0.51
	Pasteurized juice			0.45	0.53
	Peel			1.5	1.8
	Cores			0.45	0.53
	Puree			0.35	0.41
	Pasteurized puree			0.35	0.41

1 Days after last application
2 The processing factor is calculated by dividing the residue in the processed fraction by the residue in the RAC sample; residues <LOQ were set LOQ

Table 6.5.3-7: Overall mean processing factors for pyrimethanil residues in apple processed fractions

Source: DocID (trial location or reference)	Peel	Cores	Puree	Puree (heat-treated)	Juice	Juice (heat-treated)	Press cake (wet pomace)	Press cake (pomace) (heat-treated)
A81719 + A81721 (Horneburg-Neuenkirchen)[1,2,4]	2.9	-	0.57	0.67	0.43	0.39	2.0	2.3
A81719 + A81721 (Ersdorf)[1,2,4]	1.4	-	0.74	0.67	0.61	0.66	0.63	0.52
A81749 (Tönisvorst)[1,2,4]	1.2	-	0.36	0.38	0.45	0.50	0.88	-
A81749 (Orsingen-Nenzingen)[1,2,4]	2.1	-	1.0	0.98	1.0	1.3	1.3	-
B002853 (R11-01)[2,5]	-	-	-	-	0.35	-	4.1	-
C029321 (AG61632/2)[3,5]	1.8	0.53	0.41	0.41	0.58*	0.53	1.9	
Mean	**1.9**	**0.53**	**0.62**	**0.62**	**0.57**	**0.68**	**1.8**	**1.4**
Median	**1.8**	**0.53**	**0.57**	**0.67**	**0.52**	**0.53**	**1.6**	**1.4**

1 Peer-reviewed; see Draft Assessment Report 2004, Volume 3, Annex B.7 Residue data
2 Evaluated in JMPR Evaluation Report 2007
3 Submitted for product registration on Member State level
4 Studies already peer-reviewed
5 Studies not yet peer-reviewed, full summary is provided
* Cloudy juice; value for clear juice is 0.51

Grape

For grapes, processing into wine, juice and wet pomace is covered, as well as the optional product raisins. Processing into must as well as juice, pomace and wine is described in public literature (see BASF DocID 2014/1326452, Alister et al., 2014; BASF DocID 2014/1326452, Čuš et al., 2010; BASF DocID 2005/1044080, Fernandez M.J. et al., 2005). No further studies need to be submitted.
According to OECD guidance document 96, the theoretical processing factor for raisins is 5. The available processing study (see BASF DocID A81725 + A89698) confirmed residue concentration but to a lesser extent.

Report:	CA 6.5.3/3 Brady S.S., 1994a Pyrimethanil SC - SN 100309-derived residues in or on processed grape commodities following treatment with SN 100309 SC - USA 1992 A81725
Guidelines:	none
GLP:	yes (certified by United States Environmental Protection Agency)

Report:	CA 6.5.3/4 Brady S.S., 1995 a SN 100309-derived residues in or on processed grape commodities following treatment with SN 100309 SC, USA, 1992 A87662
Guidelines:	none
GLP:	yes

Report:	CA 6.5.3/5 Brady S.S., 1996a Amendment No.2 - SN 100309-derived residues in or on processed grape commodities following treatment with SN 100309 SC, USA, 1992 A89698
Guidelines:	none
GLP:	no

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	SN 100309 40 SC (CQ 1294) (SC)
Lot/Batch #:	920101 (400 g/L pyrimethanil, nominal)
CAS#:	53112-28-0

2. Test Commodity:

Crop:	Grape
Type:	Berries and other small fruits
Variety:	Thompson Seedless
Botanical name:	*Vitis vinifera*
Crop parts(s) or processed Commodity:	Fruits, juice, wet pomace, dry pomace, raisins, raisin waste

B. STUDY DESIGN

1. Test procedure

During the 1992 growing season, a field trial was conducted in California, USA, to determine the residue level of pyrimethanil (BAS 605 F) in grape processed fractions.
SN 100309 40 SC was ground applied four times at an exaggerated target rate of 1.0 kg a.s./ha to grapes. The applications were made at flowering, grape closure, color change and approximately 21 days prior to harvest of mature fruits. The spray volume used was 852-1305 L/ha.
Fruit RAC samples were harvested 21 days after the last application. Grape fruit samples were processed according to simulated commercial procedures into the following samples: juice, wet pomace, dry pomace, raisins and raisin waste. Samples were stored at ambient temperature until start of processing and frozen until analysis. The longest interval between sampling and analysis in this study was 335 days.

2. Description of analytical procedures

The samples were analyzed for pyrimethanil by a method (see DocID A83777 in chapter 4.1.2: Bayer (Schering) method UPSR 60/90 - PA 100 309.5/16) which extracts the residue with methanol, partitions it into ethyl acetate, cleans up the residues on silica gel, and analyzes the extracts by high performance liquid chromatography (HPLC) using UV detection at 268 nm for fruit, juice, wet pomace and dry pomace and by gas chromatography (GC) with nitrogen specific detection (NPD) for juice, dry pomace, raisins and raisin waste. The validated limit of quantitation was 0.02 mg/kg for fruit, juice and pomace and 0.05 mg/kg for raisins and raisin waste.
The mean procedural recoveries were the following: for juice 88.9% with HPLC and 96.3% with GC; for fruit 96.9% with HPLC; for wet pomace 94.9% with HPLC; for dry pomace 74.2% with HPLC and 99.6% with GC; for raisins 104.3% with GC; and for raisin waste 93.1% with GC.

II. RESULTS AND DISCUSSION

Residues of pyrimethanil were 0.49 mg/kg in grape fruits at 21 DALA.
The mean transfer factors representing the different processing steps were below 1 in juice. Therefore it can be concluded that pyrimethanil does not concentrate in juice. For the other matrices the study indicated concentration of residues with processing factors of 2.41 for wet pomace, 6.80 for dry pomace, 1.63 for raisins and 18.94 for raisin waste.

III. CONCLUSION

It was shown that pyrimethanil did not concentrate in juice but in raisins, wet and dry pomace and raisin waste.

Table 6.5.3-8: Residues of pyrimethanil in grape processed fractions

Trial location / Trial number	Processed commodity	Application rate (kg a.s./ha)	DALA[1]	Residues found (mg/kg)	Processing factor[2]
				Pyrimethanil	
Fresno, CA, USA 1992	**Fruit (RAC)**	4 x 1.0	21	**0.49 (HPLC)**	-
	Juice			0.34[3] (0.29 (HPLC) 0.39 (GC))	0.69
	Wet pomace			1.18 (HPLC)	2.41
	Dry pomace			3.33[3] (2.88 (HPLC) 3.77 (GC))	6.80
	Raisins			0.80 (GC)	1.63
	Raisin waste			9.28 (GC)	18.94

1 Days after last application
2 The processing factor is calculated by dividing the residue in the processed fraction by the residue in the RAC sample
3 Mean of two analysis methods; individual results are given in parentheses

Report: CA 6.5.3/6
Alister C. et al., 2014a
Effects of wine grape cultivar, application conditions and the winemaking process on the dissipation of six pesticides
2014/1326452
Guidelines: none
GLP: no

During the 2012 growing season, wine grape cultivars Sauvignon Blanc (white) and Pinot Noir (red) in Casablanca Valley, Valparaiso region, Chile, received an individual application of 2.6862 (white) or 2.0066 L/ha (red) of pyrimethanil SC formulation. In addition, five other pesticides were applied. The spray volume used was 1792.8 (white) or 1337.7 L/ha (red). Fruit RAC samples were harvested in triplicate immediately after the application has dried and at 2, 9, 20, 30, 40 and 50 days thereafter. Samples were stored at 4±1°C until transportation and deep-frozen at -18°C until analysis.
Three days after application, red grape fruit samples were processed according to simulated commercial procedures into de-stemmed fruits, stems, fermented juice (drop wine and press wine), fermented pomace, malolactic fermented wine and wine; white grape fruits were processed into juice, pomace, fermented juice (must), unfiltered wine and wine. Both red and white wine were stored in bottles for ten months at 20°C and 80% relative humidity in the dark.
Pyrimethanil residues were extracted from homogenized samples with acetonitrile/methanol. After shaking with magnesium sulfate and sodium chloride, the samples were concentrated to dryness and re-dissolved in ethyl acetate. Final analysis was performed by GC-MS using ion mass (m/z) 198 for quantitation. The limit of detection was 0.0114 mg/kg; the recovery was 88.0%.

Table 6.5.3-9: Residues of pyrimethanil in grape processed fractions

Trial location	Processed commodity	Application rate (L product/ha)	DALA[1]	Residues found (mg/kg)	Processing factor[2]
				Pyrimethanil	
Casablanca Valley, Chile 2012	**Red grapes (RAC)**	2.0066	3	**5.084**	-
	De-stemmed fruits			1.543	0.30
	Stems			9.663	1.9
	Fermented juice (must; drop wine)			1.313	0.26
	Fermented juice (must; press wine)			1.986	0.39
	Fermented pomace			7.373	1.5
	Malolactic fermented wine			0.421	0.08
	Bottled wine			0.437	0.09
	Stored bottled wine			0.198	0.04
	White grapes (RAC)	2.6862		**4.688**	-
	Juice			1.684	0.36
	Pomace			4.583	0.98
	Fermented juice (must)			1.152	0.25
	Cleared fermented juice (must)			1.095	0.23
	Unfiltered wine			0.624	0.13
	Bottled wine			0.480	0.10
	Stored bottled wine			0.291	0.06

1 Days after last application
2 The processing factor is calculated by dividing the residue in the processed fraction by the residue in the RAC sample

The transfer factor was below 1 in all processed fractions from red and white wine making except stems (1.9) and fermented pomace (1.5) from processing into red wine. Therefore it can be concluded that pyrimethanil only concentrates in these two fractions, which are not destined for human consumption.

Report: CA 6.5.3/7
Cus. F. et al., 2010a
Pesticide residues in grapes and during vinification process
2010/1233252
Guidelines: none
GLP: no

During the 2007 growing season, wine grape cultivars Pinot Gris (white) and Blaufränkisch (red) in Slovenia received an application of pyrimethanil SC formulation (Mythos; Pyrus 400 SC). In addition, several other pesticides were applied. Fruit RAC samples were harvested 40 (white) or 45 (red) days after the application.
Red grape fruit samples were processed according to simulated commercial procedures into crushed grapes, cake, must, lees and wine; white grape fruits were processed into cake, must, clarified must, lees and wine. Both red and white wine were stored in bottles for six months.
Pyrimethanil residues were extracted from homogenized samples with acetone/petroleum ether/dichloromethane. The organic phase was evaporated to dryness and the residue was re-dissolved in cyclohexane/ethyl acetate. After filtration and gel-permeation clean-up, final analysis was performed by GC-MS. The limit of detection was 0.01 mg/kg. Calibration was performed using matrix-matched standards.
Only cake from red wine making and cake, must, clarified must and lees form white wine making were analyzed for pyrimethanil.

Table 6.5.3-10: Residues of pyrimethanil in grape processed fractions

Trial location	Processed commodity	Application rate (L product/ha)	DALA[1]	Residues found[3] (mg/kg)	Processing factor[2]
				Pyrimethanil	
Slovenia 2007	**Red grapes (RAC)**	n.r.	35[3]	**0.04**	-
	Cake (pomace)		76	0.14	3.5
	White grapes (RAC)		71	**0.50**	-
	Cake (pomace)			1.96	3.9
	Must			0.18	0.36
	Clarified must			0.23	0.46
	Lees			0.90	1.8

1 Days after last application
2 The processing factor is calculated by dividing the residue in the processed fraction by the residue in the RAC sample
3 As no residues of pyrimethanil were detected in red grapes at the 3rd sampling (71 DALA) and thus no processing factor could be derived, the lowest residues of 1st (35 DALA) and 2nd sampling (53 DALA) were used for calculation of a worst-case processing factor
n.r. Not reported

The transfer factor was below 1 for must and above 1 in cake (pomace) and lees. Therefore it can be concluded that pyrimethanil only concentrates in these two fractions, which are not destined for human consumption.

Report: CA 6.5.3/8
Fernandez M.J. et al., 2005a
Fungicide dissipation curves in winemaking processes with and without maceration step
2005/1044080
Guidelines: none
GLP: no

Wine grape cultivars Airén (white) and Monstrell (red) collected from fields in Spain received a post-harvest spray treatment of Scala 40% SC, containing the active substance pyrimethanil, at a rate of 200 cm^3/hL. Two hours later, grape fruits were processed according to simulated commercial procedures into pomace, must, clarified must, lees, racked wine, unclarified wine and clarified wine using four different procedures:
- traditional red wine making
- carbonic maceration red wine making
- red wine of long maceration and pre-fermentation
- wine making without maceration (white and rosé)

Pyrimethanil residues were extracted from samples with acetone/dichloromethane (1:1, v/v), followed by filtration and concentration of the obtained extract. The dry extract was re-dissolved in isooctane/toluene (1:1, v/v). Final analysis was performed by GC-NPD. The limit of quantitation was 0.01 mg/kg; the recovery was 89-103% with variability coefficients of 1.0-14%.

Table 6.5.3-11: Residues of pyrimethanil in grape processed fractions

Trial location	Processed commodity	Application rate (cm^3 product/hL)	DALA[1]	Residues found[3] (mg/kg) Pyrimethanil				Processing factor[2]			
				A	B	C	D	A	B	C	D
Spain	**Red grapes (RAC)**	200	0	N/A	**4.47**	N/A	N/A	N/A	-	N/A	N/A
	Half maceration			N/A	4.48	N/A	N/A	N/A	1.00	N/A	N/A
	Grape bunch			N/A	4.15	N/A	N/A	N/A	0.93	N/A	N/A
	Crushed red grapes (RAC)			**5.40**	N/A	**5.35**	**3.52**	-	N/A	-	-
	Freeze maceration			N/A	N/A	3.72	N/A	N/A	N/A	0.70	N/A
	Maceration			5.22	N/A	3.61	N/A	0.97	N/A	0.67	N/A
	Pomace			16.41	7.57	12.02	3.25	3.04	1.69	2.25	0.92
	Must			1.67	1.76	0.712	6.69	0.31	0.39	0.13	1.90
	Lees			N/A	N/A	N/A	11.10	N/A	N/A	N/A	3.15
	Clarified must			N/A	N/A	N/A	4.77	N/A	N/A	N/A	1.36
	Must, 5 days			N/A	N/A	N/A	3.09	N/A	N/A	N/A	0.88
	Must, 8 days			1.39	N/A	N/A	2.77	0.26	N/A	N/A	0.79
	Must, 12 days			N/A	1.76	N/A	N/A	N/A	0.39	N/A	N/A
	Lees			6.72	1.49	3.56	14.60	1.24	0.33	0.67	4.15
	Racked wine			0.91	1.79	0.46	1.95	0.17	0.40	0.09	0.55
	Unclarified wine			0.73	1.39	0.46	1.80	0.14	0.31	0.09	0.51
	Clarified wine			0.66	1.26	0.43	1.70	0.12	0.28	0.08	0.48
	Crushed white grapes (RAC)			**N/A**	**N/A**	**N/A**	**5.18**	**N/A**	**N/A**	**N/A**	-
	Pomace			N/A	N/A	N/A	5.32	N/A	N/A	N/A	1.03
	Must			N/A	N/A	N/A	7.01	N/A	N/A	N/A	1.35
	Lees			N/A	N/A	N/A	13.60	N/A	N/A	N/A	2.63
	Clarified must			N/A	N/A	N/A	3.30	N/A	N/A	N/A	0.64
	Must, 5 days			N/A	N/A	N/A	2.99	N/A	N/A	N/A	0.58
	Must, 8 days			N/A	N/A	N/A	2.86	N/A	N/A	N/A	0.55
	Lees			N/A	N/A	N/A	8.93	N/A	N/A	N/A	1.72
	Racked wine			N/A	N/A	N/A	3.02	N/A	N/A	N/A	0.58
	Unclarified wine			N/A	N/A	N/A	2.59	N/A	N/A	N/A	0.50
	Clarified wine			N/A	N/A	N/A	2.58	N/A	N/A	N/A	0.50

1 Days after last application
2 The processing factor is calculated by dividing the residue in the processed fraction by the residue in the RAC sample
3 Mean of three values
A Traditional red wine making
B Carbonic maceration red wine making
C Red wine of long maceration and pre-fermentation
D Wine making without maceration (white and rosé)
N/A Not applicable

The transfer factor (TF or PF) was above 1 in pomace and lees from traditional red wine making, in pomace from carbonic maceration and long maceration/pre-fermentation red wine making and in must (before fermentation), clarified must and lees from wine making without maceration (white and rosé). Therefore it can be concluded that pyrimethanil only concentrates in these fractions, which are not destined for human consumption except must. In macerated crushed grapes from traditional red wine making, in half macerated grapes and grape bunches from carbonic maceration and in pomace from wine making without maceration (white and rosé), residues remained at about the same level with PFs 0.92-1.03. In all other processed fractions, residue decline was shown.

Table 6.5.3-12: Overall mean processing factors for pyrimethanil residues in white grape processed fractions

Trial number	Juice	Wet pomace (cake)	Dry pomace	Raisins	Raisin waste	Must[1]	Clarified must	Unfiltered wine	Bottled wine	Stored bottled wine	Lees
Fresno, CA, USA 1992	0.69	2.41	6.80	1.63	18.94	-	-	-	-	-	-
Casablanca Valley, Chile 2012	0.36	0.98	-	-	-	0.25	0.23	0.13	0.10	0.06	-
Slovenia 2007	-	3.9	-	-	-	0.36	0.46	-	-	-	1.8
Spain, D	-	1.03	-	-	-	0.58[2]	-	0.50	0.50	-	2.63[3]
Mean	**0.53**	**2.08**	**6.80**	**1.63**	**18.94**	**0.40**	**0.35**	**0.32**	**0.30**	**0.06**	**2.22**

1 Fermented juice
2 Worst case at 5 days
3 Worst case

Table 6.5.3-13: Overall mean processing factors for pyrimethanil residues in red grape processed fractions

Trial number	De stemmed fruits	Stems	Fermented juice (must; drop wine)	Fermented juice (must; press wine)	Fermented pomace / wet pomace (cake)	Lees	(Malolactic) Fermented wine	Bottled wine	Stored bottled wine
Casablanca Valley, Chile 2012	0.30	1.9	0.26	0.39	1.5	-	0.08	0.09	0.04
Slovenia 2007	-	-	-	-	3.5	-	-	-	-
Spain, A	-	-	-	0.31	3.04	1.24	0.17	0.12	-
Spain, B	-	-	-	0.39	1.69	0.33	0.40	0.28	-
Spain, C	-	-	-	0.13	2.25	0.67	0.09	0.08	-
Mean	**0.30**	**1.9**	**0.26**	**0.31**	**2.4**	**0.74**	**0.18**	**0.14**	**0.04**
Spain, D[1]	-	-	-	0.88[2]	0.92	4.15[3]	0.55	0.48	-

1 Not included in mean since this refers to rosé wine making
2 Worst case at 5 days
3 Worst case

The results on processing into wine described in public literature support the findings of the peer-reviewed studies (see Table 6.5.3-1). Residues do not concentrate in either white or red wine, supporting the BASF statement made in Table 6.5.3-1.

Strawberry

For strawberry, two processing studies (BASF DocIDs 2008/1022961 and A89298) are available and summarized below in which strawberries were processed into washed berries, jam and canned strawberries. No juice was produced in the available processing studies. However, extrapolation is possible from grape juice. It was shown that pyrimethanil residues do not concentrate in grape juice (see BASF DocID A81725 + A89698 and 2014/1326452 Alister *et al.*, 2014).

Report:	CA 6.5.3/9 Harant H., 2008a Determination of residues of BAS 605 F in strawberries and its processed products after three applications of BAS 605 01 F in Germany 2008/1022961
Guidelines:	EEC 7029/VI/95 rev. 5, IVA Guideline I-III (1992), BBA IV 3-3, BBA IV 3-4, EEC 7035/VI/95 rev. 5
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 605 01 F (SC)
Lot/Batch #:	1001 (400 g/L pyrimethanil, nominal)
CAS#:	53112-28-0

2. Test Commodity:

Crop:	Strawberry
Type:	Berries and other small fruits
Variety:	Elsanta
Botanical name:	*Fragaria x ananassa*
Crop parts(s) or processed Commodity:	Fruits, washed fruits, wash water, jam before cooking, jam after cooking, vegetable stock, canned fruit

B. STUDY DESIGN

1. Test procedure

During the 2006 growing season, four field trials were conducted at representative strawberry growing areas in Germany to determine the residue level of pyrimethanil (BAS 605 F) in strawberry processed fractions.
BAS 605 01 F was foliar applied three times at an exaggerated target rate of 1.0 kg a.s./ha to strawberries with a retreatment interval of 7 days. The applications were made 17, 10 and 3 days prior to harvest of mature fruits. The spray volume used was about 400 L/ha.
Fruit RAC samples were harvested on the day of the last application and 3 days thereafter (BBCH 89). Strawberry fruit samples were processed according to simulated commercial procedures into the following samples: washed fruits, wash water, jam before cooking, jam after cooking, vegetable stock and canned fruits. Samples were stored at 5-8°C until start of processing and deep-frozen until analysis.

2. Description of analytical procedures

Residues of pyrimethanil were determined using BASF method No 542/2 (L0066). Residues of pyrimethanil were extracted with a mixture of methanol and water. After centrifugation and dilution, the final determination was performed by HPLC-MS/MS. The validated limit of quantitation was 0.01 mg/kg.
Average concurrent recoveries of pyrimethanil were 99% with fortification levels of 0.01 to 1.0 mg/kg.

II. RESULTS AND DISCUSSION

Residues of pyrimethanil were in the range of 0.86 to 24.52 mg/kg for strawberry fruits at the day of the last application and of 0.50 to 1.76 mg/kg at 3 DALA.
The mean transfer factors representing the different processing steps were below 1 in all processed fractions except canned fruit where residues averagely remained at the same level. Therefore it can be concluded that pyrimethanil does not concentrate in washed fruits, wash water, jam before cooking, jam after cooking, vegetable stock and canned fruit.

III. CONCLUSION

It was shown that pyrimethanil did not concentrate in any processed fraction.

Table 6.5.3-14: Residues of pyrimethanil in strawberry processed fractions

Trial location / Trial number	Processed commodity	Application rate (kg a.s./ha)	DALA[1]	Residues found (mg/kg)	Processing factor[2]
				Pyrimethanil	
96181 Untersteinbach, Germany (FR 15/06/50) 2006	Fruit	3 x 1.0	0	24.52	-
	Fruit (RAC)		3	**1.76**	-
	Washed fruit			1.16	0.66
	Wash water			0.11	0.06
	Jam before cooking			0.81	0.46
	Jam after cooking			0.54	0.31
	Vegetable stock			0.51	0.30
	Canned fruits			1.30	0.74
04685 Nerchau, Germany (FR 15/06/70) 2006	Fruit	3 x 1.0	0	2.32	-
	Fruit (RAC)		3	**1.73**	-
	Washed fruit			1.14	0.66
	Wash water			0.13	0.08
	Jam before cooking			0.50	0.29
	Jam after cooking			0.49	0.28
	Vegetable stock			0.44	0.25
	Canned fruits			1.42	0.82
04808 Wurzen, Germany (FR 15/06/30) 2006	Fruit	3 x 1.0	0	0.86	-
	Fruit (RAC)		3	**0.50**	-
	Washed fruit			0.59	1.18
	Wash water			0.06	0.12
	Jam before cooking			0.26	0.52
	Jam after cooking			0.26	0.52
	Vegetable stock			0.29	0.58
	Canned fruits			0.71	1.42
04703 Minkwitz, Germany (FR 15/06/75) 2006	Fruit	3 x 1.0	0	1.91	-
	Fruit (RAC)		3	**1.16**	-
	Washed fruit			1.16	1.00
	Wash water			0.09	0.08
	Jam before cooking			0.53	0.46
	Jam after cooking			0.51	0.44
	Vegetable stock			0.44	0.38
	Canned fruits			1.26	1.09

1 Days after last application
2 The processing factor is calculated by dividing the residue in the processed fraction by the residue in the RAC sample

Table 6.5.3-15: Mean processing factors for pyrimethanil residues in strawberry processed fractions

Trial number	Washed fruit	Wash water	Jam before cooking	Jam after cooking	Vegetable stock	Canned fruits
FR 15/06/50	0.66	0.06	0.46	0.31	0.30	0.74
FR 15/06/70	0.66	0.08	0.29	0.28	0.25	0.82
FR 15/06/30	1.18	0.12	0.52	0.52	0.58	1.42
FR 15/06/75	1.00	0.08	0.46	0.44	0.38	1.09
Mean	**0.88**	**0.09**	**0.43**	**0.39**	**0.38**	**1.02**

Report: CA 6.5.3/10
Wrede A., 1996a
Pyrimethanil suspension concentrate 400 g/L (Code: CQ 1294) - Scala: Residues in strawberries and strawberry marmalade Germany 1993
A89298
Guidelines: none
GLP: yes
(certified by Senatsverwaltung fuer Gesundheit und Soziales, Berlin, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	CQ 1294 / Scala (SC)
Lot/Batch #:	Not reported (400 g/L pyrimethanil, nominal)
CAS#:	53112-28-0

2. Test Commodity:

Crop:	Strawberry
Type:	Berries and other small fruits
Variety:	Elvira, Bogota
Botanical name:	*Fragaria x ananassa*
Crop parts(s) or processed Commodity:	Fruits, jam

B. STUDY DESIGN

1. Test procedure

During the 1993 growing season, three field trials were conducted at representative strawberry growing areas in Germany to determine the residue level of pyrimethanil (BAS 605 F) in strawberry processed fractions.
CQ 1294 (Scala) was foliar applied to strawberries three times at an exaggerated target rate of 1.0 kg a.s./ha. One trial accidentally received another treatment at a rate of 0.5 kg a.s./ha one day before the first sampling, resulting in a total application rate of 3.5 kg a.s./ha, with a retreatment interval of 4-8 days. The applications were made at BBCH 63, 65 and 67. The spray volume used was 2000 L/ha.
Mature fruit RAC samples were taken between 1 and 29 DALA. Strawberry fruit samples from two trial sites were processed according to domestic procedures into jam. Samples were stored deep-frozen until analysis.

2. Description of analytical procedures

Residues of pyrimethanil were determined using Bayer (Schering) method No R/V 2/94 - PA 100 309.5/16. Residues of pyrimethanil were extracted with organic solvents. After clean-up using liquid/liquid partition and a silica gel column, the final determination was performed by HPLC-UV. The validated limit of quantitation was 0.05 mg/kg.
Average concurrent recoveries of pyrimethanil were 88% with fortification levels of 0.05 and 0.5 mg/kg.

II. RESULTS AND DISCUSSION

Residues of pyrimethanil in fruit generally declined over time. In RAC samples at 27-28 DALA, residues were in the range of 0.13 to 0.36 mg/kg.
The mean transfer factor for jam was below 1. Therefore it can be concluded that pyrimethanil does not concentrate in jam.

III. CONCLUSION

It was shown that pyrimethanil did not concentrate in jam.

Table 6.5.3-16: Residues of pyrimethanil in strawberry processed fractions

Trial location / Trial number	Processed commodity	Application rate (kg a.s./ha)	DALA[1]	Residues found (mg/kg)	Processing factor[2]
				Pyrimethanil	
42489 Ratingen-Wulfrath, Germany (C-15-93) 1993	Fruit	3 x 1.0	3	3.4	-
	Fruit		10	0.96	-
	Fruit		13	0.92	-
	Fruit		16	1.1	-
	Fruit		20	0.69	-
78333 Stockach-Hindelwangen, Germany (I-12-93) 1993	Fruit	3 x 1.0 1 x 0.5	1	2.4	-
	Fruit		6	0.83	-
	Fruit		12	0.61	-
	Fruit		20	0.22	-
	Fruit (RAC)		28	**0.13**	-
	Jam			0.097	0.75
53501 Eckendorf, Germany (Q-08-93) 1993	Fruit	3 x 1.0	18	0.45	-
	Fruit		22	0.51	-
	Fruit		25	0.24	-
	Fruit (RAC)		27	**0.36**	-
	Fruit		29	0.33	-
	Jam		27	0.21	0.58

1 Days after last application
2 The processing factor is calculated by dividing the residue in the processed fraction by the residue in the RAC sample

Table 6.5.3-17: Mean processing factors for pyrimethanil residues in strawberry processed fractions

	Jam
I-12-93	0.75
Q-08-93	0.58
Mean	**0.67**

Table 6.5.3-18: Overall mean processing factors for pyrimethanil residues in strawberry processed fractions

Trial number	Washed fruit	Wash water	Jam before cooking	Jam after cooking	Vegetable stock	Canned fruits
FR 15/06/50	0.66	0.06	0.46	0.31	0.30	0.74
FR 15/06/70	0.66	0.08	0.29	0.28	0.25	0.82
FR 15/06/30	1.18	0.12	0.52	0.52	0.58	1.42
FR 15/06/75	1.00	0.08	0.46	0.44	0.38	1.09
I-12-93	-	-	-	0.75	-	-
Q-08-93	-	-	-	0.58	-	-
Mean	**0.88**	**0.09**	**0.43**	**0.48**	**0.38**	**1.02**

CA 6.6 Residues in Rotational Crops

A **confined rotational crop study** (**B003517**, Annex II chapter 6.6.2) was submitted and evaluated during the previous Annex I inclusion process. The residue levels and the nature of residues were investigated in three different succeeding crops (lettuce, radish, wheat) at an application rate of 2.4 kg a.s./ha. In the study ^{14}C-pyrimidinyl labeled pyrimethanil was applied to bare soil. The study is considered to be still scientifically valid and it is meeting the requirements included in OECD guideline 502, with the exception that only one label was used and an insufficient identification rate of the polar region was achieved.
In addition, a **field rotational crop study** (**C031659**, Annex II chapter6.6.3) was submitted and evaluated during the previous Annex I inclusion process. The residue levels were investigated in different succeeding crops (lettuce, cauliflower, curly kale, winter wheat) at different plant-back intervals after two applications of 0.8 kg a.s./ha to lettuce at BBCH 16-19 and 43-47 with an interval of 10-11 days and a PHI of 14 days. The study does not fully meet the requirements included in OECD guideline 504.

For pyrimethanil, the following conclusion was made by the EFSA in the course of the Annex I inclusion process. The relevant endpoint was copied from the EFSA Conclusion 2006 (EFSA Scientific Report (2006) 61, 1-70, Conclusion on the peer review of pyrimethanil).

Metabolism in plants (Annex IIA, point 6.1 and 6.7, Annex IIIA, point 8.1 and 8.6)

Rotational crops	Lettuce, radish, wheat (confined study with radioactive substance) Lettuce, cauliflower, curly kale, wheat (field study)

Residues in succeeding crops (Annex IIA, point 6.6, Annex IIIA, point 8.5)

No residues in succeeding crops to be expected above 0.05 mg/kg (based on field studies)

New confined and field rotational crop studies (one each) were conducted to fully meet the requirements of OECD guideline 502 and 504, respectively. Full summaries are provided below (see chapter 6.6.1 and 6.6.2). Both old and new studies were considered in relevance of metabolite investigation for residue definition and risk assessment (see chapter MCA 6.7, 6.9 and relevance of metabolites document 2015/1188589).
Please note that the new rotational crop studies were conducted according to the critical GAP for strawberry at the time of study initiation (3x 1 kg a.s./ha, total 3 kg a.s./ha). In the meantime, such cGAP had to be reconsidered in light of the outcome of ecotoxicological risk assessments according to latest guidelines on Birds&Mammals. The application rate of the cGAP was finally decreased to 3 x 0.8 kg a.s./ha (total 2.4 kg a.s./ha), resulting in an overdosing factor of 0.8 (2.4 kg a.s./ha / 3 kg a.s./ha). This does not affect the validity of the study.

Preliminary considerations

During the peer review under Directive 91/414/EEC, it was demonstrated in several degradation studies that pyrimethanil is moderate to medium persistent in soil under dark aerobic conditions. DT_{90} values exceed the trigger value of 100 days ($DT_{90\ lab}$ = 93-238 days; $DT_{90\ field}$ = 82-180 days). The major transformation product was AE F132593 (2-amino-4,6-dimethyl-pyrimidine, M605F007). This metabolite is moderate to high persistent in soil. Dissipation in field trials in Germany confirms that pyrimethanil is a moderate persistent substance in soil ($DT_{50\ field}$ = 23-54 days). (EFSA Conclusion 2006)

The new DT_{50} and DT_{90} values calculated in this dossier are the following:

Parent pyrimethanil: DT_{50lab} = 26.8-71.8 days
DT_{90lab} = 89.1-377.1 days
$DT_{50field}$ = 17.5-43.6 days
$DT_{90field}$ = 83.7-264.7 days

M605F007: DT_{50lab} = 15.6-207.3 days
DT_{90lab} = 51.8-688.5 days
$DT_{50field}$ = 28.1-142.9 days
$DT_{90field}$ = 93.2-474.7 days

Based on field soil dissipation studies pyrimethanil and its metabolite are not expected to accumulate in soil (see MCA chapter 7).

In context of the EU MRL review according to Article 12 (EFSA Journal 2011;9(11):2454) the assessment was confirmed by EFSA: The following paragraphs were taken from the relevant Reasoned Opinion.

Nature of residues

One **confined rotational crop study** (B003517, Annex II chapter 6.6.2) was considered in the framework of the **peer review**. The study was conducted with an application of 2.4 kg a.s./ha ^{14}C-pyrimidinyl labelled pyrimethanil to bare soil. Wheat, lettuce and radish were planted/sowed 30, 130 and 300 days after treatment. Wheat was harvested at an immature stage and at maturity; lettuce and radish were harvested at maturity.

In contrast to the metabolism in primary crops, metabolites were identified at levels equivalent or higher to that of the parent compound. Identification of metabolites was mainly carried out on 30 day plots, but it is noted that the total radioactive residues in grains were shown to be higher after 300 days (0.03 mg eq/kg) that after 130 days (0.015 mg eq/kg). The same increase in TRR was identified in wheat straw. The metabolite generally found at the highest levels was C 621 312 (2-anilino-4,6-dihydroxymethyl-pyrimidine; M605F005). The amounts of pyrimethanil and its metabolites found in edible parts of plants sowed or planted 30 days after ageing period were such that residue levels in the range of common analytical limits of quantitation could be expected under practical conditions in case of early installation of rotational crops. Therefore, a field study was required.

Table 6.6-1: Summary of confined metabolism study in rotational crops (peer-reviewed)

Crop group	Crop	Label position	Application and sampling details				
			Method, F or G[1]	Rate (kg a.s./ha)	Sowing intervals (DAT)	Harvest intervals (DAP)	Remarks
Leafy vegetables	Lettuce	2-[^{14}C]-pyrimidinyl-labeled pyrimethanil	Bare soil application, G	2.4	30	46-79	At maturity
					130	46-79	
					300	46-79	
Root and tuber vegetables	Radish	2-[^{14}C]-pyrimidinyl-labeled pyrimethanil	Bare soil application, G	2.4	30	46-79	At maturity
					130	46-79	
					300	46-79	
Cereals	Wheat	2-[^{14}C]-pyrimidinyl-labeled pyrimethanil	Bare soil application, G	2.4	30	35-148 and 73-190	Immature stage and at maturity
					130	35-148 and 73-190	
					300	35-148 and 73-190	

1 Outdoor/field application (F) or glasshouse/protected application (G)

In brief, in the **new confined rotational crop study**, minor new metabolites (<10% TRR and <0.05 mg/kg) were identified in the polar region: M605F016, M605F032 (exception: major in wheat forage plant back interval: 120 day after treatment) and M605F033. They are hydroxylated (M605F016, M605F032) or further conjugated (M605F033) derivatives of soil metabolite M605F007, which had already been identified in the peer-reviewed confined rotational crop study. Another new polar compound (M605F025) was found as major metabolite (>10% TRR and >0.05 mg/kg) in wheat forage (plant back interval: 30 DAT), hay and straw (plant back interval: 30, 120, 365 DAT), in radish foliage (plant back interval: 30 and 120 DAT), and in radish tubers (plant back interval: 30, 120, 365 DAT). Metabolite M605F005 (C 621 312), which was the major metabolite in the peer-reviewed confined rotational crop study, was not found in any sample of the new confined rotational crop study. This is in accordance to the peer-reviewed field rotational crop data. From the hydroxylation and further conjugation pathway the only major metabolite is M605F028 (a Malonyl-O glucoside of SN 614278/M605F004) in wheat forage (plant back interval: 30 DAT) and hay (plant back interval. 30, 120 DAT).

Magnitude of residues

One **rotational crop study in field** conditions (C031659, Annex II chapter6.6.3) was considered in the framework of the **peer review** (EFSA, 2006). The study was conducted with 2 applications of 0.8 kg a.s./ha to lettuce at growth stages of BBCH 16-19 and BBCH 43-47 with an interval of 10-11 days and a PHI of 14 days. The following crops were sampled at harvest (lettuce: PHI = 70-119 days, brassica: 109-249 days and winter wheat – straw and grain: PHI = 321-330 days). In those succeeding crops, no residues of pyrimethanil or metabolite C 621 312 (M605F005) were present above the limit of quantitation (0.05 mg/kg). This gives indication that the possible presence of residual compounds resulting from the use of pyrimethanil in rotational crops is limited to low amounts and that the resulting toxicological burden can be considered as minor, taking into account the ADI value established for pyrimethanil. Thus, EFSA concludes that there is no need for a plant-back restriction.

In brief, in the **new field rotational study** pyrimethanil was applied to bare soil with the maximal seasonal application rate (overdosing factor 0.8 as explained above) and the following crops were replanted after 30, 120 and 365 days: wheat, carrot/radish, cauliflower/broccoli and lettuce/spinach. The samples taken were analyzed for parent pyrimethanil (BAS 605 F), M605F005, M605F007 and M605F025 as marker substances for each metabolite group (see relevance of metabolites document 2015/1188589 and MCA 6.9 and 6.7 for further explanation). ~~An interim~~ Reports including the results of the 30, 120 and 365 day replant interval ~~is~~ are summarized below. ~~It is considered to cover the relevant replant intervals, since~~ In food items (grain, carrot/radish roots and mature lettuce/spinach) at replant interval 120 days residues were below the LOQ of 0.01 mg/kg, with the exception of one spinach sample with M605F007 residues at 0.035 mg/kg. In accordance to the decline of residues seen in the confined rotational crop study no residues are found ~~expected to be even lower~~ at the 365 day replant interval.
After 30 day replant interval parent pyrimethanil was found >0.01 mg/kg in food items of root and leafy vegetables; M605F007 was found >0.01 mg/kg in leafy crop food items.
As parent residues cannot be excluded, **an MRL in root & tuber and leafy vegetables is applied for** (see chapter MCA 6.7). Risk assessments for both parent pyrimethanil and metabolite M605F007 show that there will be no risk for the consumer. Thus, there is no need to include M605F007 in any residue definition. See also chapter MCA 6.7, 6.9 and relevance of metabolites document (BASF doc ID 2015/1188589) for further reasoning.
No M605F025 was found in any food item at 30 and 120 day replant interval. Furthermore, in compliance with the new confined rotational crop study, residues of M605F005 were below 0.01 mg/kg in all treated plant specimens at 30 and 120 day replant interval. Thus, M605F005 and M605F025 are regarded as not relevant. For further justification see the evaluation of the relevance of metabolites in chapters MCA 6.7 and 6.9.

CA 6.6.1 Metabolism in rotational crops

The new conducted confined rotational crop study is summarized below:

Report: CA 6.6.1/1
MacDonald R.,Henderson S., 2015a
The uptake and metabolism of 14C-Pyrimethanil (BAS 605 F, Reg. No. 236999) in confined rotational crops
2015/1000181

Guidelines: OECD-ENV/JM/MONO/(2009)31 (OECD No. 64), OECD 502 Metabolism in Rotational Crops (January 2007), EPA 860.1000: EPA Residue Chemistry Test Guidelines, EPA 860.1850: EPA Residue Chemistry Test Guidelines, PMRA Residue Chemistry Guidelines Section 97.13 Confined Accumulation in Rotational Crops (Canada)

GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: Phenyl-U-^{14}C -labeled pyrimethanil
Phenyl-1,2,3,4,5,6-^{13}C
Pyrimidinyl-2-^{14}C-labeled pyrimethanil
Pyrimidinyl-1,3-^{15}N-labeled pyrimethanil
Unlabeled pyrimethanil

Lot/Batch #: 1036-1011 (phenyl label)
1052-1003 (^{13}C-label)
1049-1010 (pyrimidinyl label)
1050-1003 (^{15}N-label)
L83-126 (unlabeled)

Purity: Radiochemical purity: 99.2% (phenyl label)
99.7% (pyrimidinyl label)
Chemical purity a.s.: 96.6% (^{13}C-label)
98.7% (^{15}N-label)
99.6% (unlabeled)
Specific activity: 258154 dpm/µg (application solution, phenyl label)
244671 dpm/µg (application solution, pyrimidinyl label)

CAS#: 53112-28-0

Stability of test compound: Test compound was stable over investigation time.

2. Test Commodity:

Crop:	Spring wheat	spinach	radish
Type:	Cereals	leafy vegetables	root & tuber vegetables
Variety:	Paragon	Renegade F1	French Breakfast 3
Botanical name:	*Triticum aestivum*	*Spinacia oleracea*	*Raphanus sativus*
Crop part/processed commodity:	Wheat forage, hay, straw, grain	Immature spinach, mature spinach	Radish foliage, tubers
Sample size:	0.063-1.607 kg		

3. Soil: A sandy loam soil was used. The soil physicochemical properties are described below (see Table 6.6.1-1).

Table 6.6.1-1: Soil physicochemical properties

Soil series	Soil type	pH	OM[2] %	Sand %	Silt %	Clay %	Max. water holding capacity	CEC[1] meq/100 g
Not specified	Sandy loam*	6.5**	2.4	65*	20*	15*	n.r.	10.0

1 Cation exchange capacity
2 Organic matter; organic carbon 1.4%
* USDA scheme
** $CaCl_2$; pH in water 7.3
n.r. Not reported

B. STUDY DESIGN

The study was conducted during the period of April 2013 to September 2015 at Charles River, Tranent, Edinburgh, United Kingdom.

1. Test procedure

The active substance in the SC formulation BAS 605 04 F was applied to bare outdoor soil in plastic containers at a nominal application rate of 3000 g a.s./ha using a hand held sprayer system. In two separate setups differently labeled test items were applied (either ^{14}C-labeled on the phenyl ring or ^{14}C-labeled on the pyrimidine ring (pyrimidinyl label). The actual application rates achieved were 2965 g a.s./ha for the [phenyl-U-^{14}C] label and 2960 g/ha [pyrimidinyl-^{14}C] label. After application, the soil was aged for approximately 30 days (simulating an emergency plant back), 120 days (simulating a fall plant back) and 365 days (one year after treatment). At 28 days after application (2 days prior to the first sowing), the containers were moved indoors. For each plant back interval, the crops spinach, radish and spring wheat were sowed.

2. Sampling

Spring wheat was sampled just above the soil level at time points representative of forage (BBCH 30), hay (BBCH 61-69) and maturity (BBCH 89). At the maturity harvest, the wheat was separated into straw (including chaff) and grain. Radish was sampled at maturity and separated into foliage and tubers (BBCH 49). Spinach foliage was sampled at an immature stage (BBCH 36) and at maturity (BBCH 49).
Soil samples were taken after ploughing for each plant back interval.
Samples not analysed immediately were stored in a freezer set to maintain -20°C until taken for analysis and following analysis returned to the freezer.

3. Description of analytical procedures

Radioanalysis: For the determination of the TRR combusted, and the measurement of solid residues following solvent extraction or solubilization procedures, plant subsamples were combusted using a sample oxidizer. The resultant $^{14}CO_2$ was absorbed, mixed with scintillation fluid and the radioactivity determined by liquid scintillation counting (LSC). ^{14}C standards were combusted at the beginning and at regular intervals throughout each batch of analyses. Measurements of radioactivity were not corrected for oxidizer efficiency; however combustion efficiencies were in excess of 97%.
Extraction: Homogenized subsamples of milled tissue were extracted three times with methanol, each followed by centrifugation. The residue was re-extracted twice with water in the same manner. The volume of each extract was measured prior to analysis by LSC. The combined results of methanol extractions and water extractions are referred to as ERR.
The residue remaining after solvent extraction of each sample was dried under a gentle stream of nitrogen gas. Aliquots were combusted for the determination of the RRR.
Characterization: Investigation of RRR after solvent extraction was performed on samples where residues were >10% TRR and >0.050 mg/kg. A combination of sequential solubilization steps with 1% ammonia solution, macerozyme / cellulase, β-glucosidase, tyrosinase / laccase, α-amylase and protease was used. Following enzyme treatment the remaining residues were incubated with pepsin and pancreatin. Samples with a sufficient level of radioactivity were subjected to HPLC analysis.
Identification of metabolites: The peak assignments for BAS 605 F and its metabolites were based on mass spectrometry. Mass spectrometry was conducted on 30 DAT wheat hay (phenyl label), 30 DAT wheat straw (pyrimidinyl label), 120 DAT wheat forage (both labels), 120 DAT wheat hay (phenyl label), 120 DAT wheat straw (pyrimidinyl label), 120 DAT mature spinach (phenyl label) and 120 DAT radish foliage (both labels). Assignments of the metabolites in other samples was made by co-chromatography experiments, with characterised samples or reference standards, or by comparison with retention times.
HPLC analysis was used for purity check, analysis of the application solution, analysis of extracts, quantification of metabolites, confirmatory analysis and analysis of α-amylase extracts.

II. RESULTS AND DISCUSSION

A. TOTAL RADIOACTIVE RESIDUES (TRRs)

Plant matrices

For spring wheat, the matrices with the highest residue levels were hay and straw. The residue levels in straw ranged from 0.457 mg/kg (365 DAA, phenyl label) to 5.451 mg/kg (30 DAA, phenyl label), and from 0.323 mg/kg (365 DAA, pyrimidinyl label) to 5.244 mg/kg (30 DAA, pyrimidinyl label). In hay the levels ranged from 0.095 mg/kg (365 DAA, phenyl label) to 2.170 mg/kg (30 DAA, phenyl label), and from 0.100 mg/kg (365 DAA, pyrimidinyl label) to 1.342 mg/kg (30 DAA, pyrimidinyl label). The TRR in grain accounted for 0.026 mg/kg (365 DAA, phenyl label) to 0.142 mg/kg (30 DAA, phenyl label), and from 0.017 mg/kg (365 DAA, pyrimidinyl label) to 0.174 mg/kg (30 DAA, pyrimidinyl label). Lower levels of radioactive residues were found in wheat forage, ranging from 0.057 mg/kg (365 DAA, both labels) to 1.606 mg/kg (30 DAA, both labels). The residue levels in immature spinach ranged from 0.007 mg/kg (365 DAA, phenyl label) to 0.140 mg/kg (30 DAA, phenyl label), and from 0.011 mg/kg (365 DAA, pyrimidinyl label) to 0.125 mg/kg (120 DAA, pyrimidinyl label). In mature spinach the levels ranged from 0.009 mg/kg (365 DAA, phenyl label) to 0.140 mg/kg (120 DAA, phenyl label), and from 0.010 mg/kg (365 DAA, pyrimidinyl label) to 0.179 mg/kg (120 DAA, pyrimidinyl label). The residue levels in radish foliage ranged from 0.104 mg/kg (365 DAA, phenyl label) to 2.156 mg/kg (30 DAA, phenyl label), and from 0.036 mg/kg (365 DAA, pyrimidinyl label) to 1.232 mg/kg (30 DAA, pyrimidinyl label). The TRR in radish tubers accounted for 0.051 mg/kg (365 DAA, phenyl label) to 0.393 mg/kg (120 DAA, phenyl label), and from 0.015 mg/kg (365 DAA, pyrimidinyl label) to 0.206 mg/kg (30 DAA, pyrimidinyl label).

Table 6.6.1-2: Total radioactive residues in crops after treatment with ^{14}C-pyrimethanil

Matrix	TRR combusted [mg/kg]		TRR calculated[1] [mg/kg]	
	Phenyl label	Pyrimidinyl label	Phenyl label	Pyrimidinyl label
Plant back interval: 30 DAT				
Wheat forage	1.443	1.679	1.527	1.606
Wheat hay	2.007	1.346	2.170	1.342
Wheat straw[3]	5.228	5.628	5.451	5.244
Wheat grain	0.137	0.193	0.142	0.174
Immature spinach	0.116	0.071	0.140	0.071
Mature spinach	0.112	0.108	0.128	0.104
Radish foliage	2.054	1.149	2.156	1.232
Radish tubers	0.235	0.186	0.273	0.206
Plant back interval: 120 DAT				
Wheat forage	1.070	0.933	1.093	0.922
Wheat hay	1.515	0.841	1.461	0.873
Wheat straw[3]	4.412	1.680	4.146	1.625
Wheat grain	0.170	0.497	0.185	0.500
Immature spinach	0.145	0.141	0.140	0.125
Mature spinach	0.140	0.181	0.145	0.179
Radish foliage	1.437	0.750	1.512	0.793
Radish tubers	0.327	0.184	0.393	0.199
Plant back interval: 365 DAT				
Wheat forage	0.057	0.058	0.058	0.057
Wheat hay	0.091	0.091	0.095	0.100
Wheat straw[3]	0.461	0.343	0.457	0.323
Wheat grain	0.026	0.018	0.026	0.017
Immature spinach	0.007	0.012	0.007[2]	0.011
Mature spinach	0.009	0.011	0.009[2]	0.012
Radish foliage	0.088	0.043	0.104	0.036
Radish tubers	0.046	0.013	0.051	0.015

1 TRR was calculated as the sum of ERR (extraction with methanol and water) + RRR
2 TRR combusted. TRR calculated was not determined because of low amount of radioactivity
3 Spring wheat chaff was added to straw
DAT: Days after treatment

Soil

The values for the TRR in soil samples are summarized in the table below.

Table 6.6.1-3: Total radioactive residues in soil after treatment with ^{14}C-pyrimethanil

Soil samples (Days After Treatment DAT)	TRRs [mg/kg] determined by direct combustion	
	Phenyl label	Pyrimidinyl label
Plant back interval: 30 DAT		
At sowing		
Wheat (crate 1)	0.701	0.639
Wheat (crate 2)	1.085	0.869
Spinach (crate 4)	0.887	0.582
Radish (crate 6)	1.743	1.387
Plant back interval: 120 DAT		
At sowing		
Wheat (crate 3)	0.933	0.461
Spinach (crate 5)	0.702	0.664
Radish (crate 7)	1.411	0.701
Plant back interval: 365 DAT		
At sowing		
Wheat (crate 1)	0.815	0.689
Wheat (crate 2)	0.616	0.456
Spinach (crate 4)	0.530	0.472
Radish (crate 6)	0.605	0.608

B. EXTRACTION AND CHARACTERIZATION OF RESIDUES

1. Extraction and characterization of residues in rotational crops

The extractability of the radioactive residues with methanol and water ranged for rotational crop matrices from 88.3 to 23.7% TRR in the phenyl label and from 90.3 to 15.1% TRR for the pyrimidinyl label. For wheat grain, a tendency for low extractability with both solvents was found for both labels (less than 61.5% TRR). With the exception of wheat grain, the major portion of the radioactive residues of both labels were extracted with methanol (from 86.1 to 40.3% TRR) compared to water (from 20.2 to 2.1% TRR). For wheat grain (both labels) the portion extracted with methanol (from 44.7 to 5.9% TRR) compared to water (from 20.0 to 9.2% TRR) was higher for 30 DAA and lower for 365 DAA plant pack intervals.

Table 6.6.1-4: Extractability of radioactive residues in rotational crop samples (phenyl label)

Days after treatment	TRR calculated[1]	Methanol extract		Water extract		ERR[2]		RRR	
DAT	[mg/kg]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]
Wheat forage									
30	1.527	1.289	84.5	0.058	3.8	1.347	88.3	0.179	11.7
120	1.093	0.673	61.6	0.052	4.7	0.725	66.3	0.368	33.7
365	0.058	0.032	56.3	0.005	9.6	0.037	65.9	0.020	34.1
Wheat hay									
30	2.170	1.511	69.6	0.223	10.3	1.734	79.9	0.438	20.2
120	1.461	0.945	64.7	0.140	9.6	1.085	74.3	0.375	25.7
365	0.095	0.052	54.4	0.013	12.7	0.065	67.1	0.031	32.9
Wheat straw									
30	5.451	3.150	57.8	0.577	10.6	3.727	68.4	1.717	31.5
120	4.146	2.106	50.8	0.638	15.4	2.744	66.2	1.401	33.8
365	0.457	0.184	40.3	0.092	20.2	0.276	60.5	0.181	39.5
Wheat grain									
30	0.142	0.063	44.7	0.017	12.1	0.080	56.8	0.061	43.1
120	0.185	0.047	24.9	0.037	20.0	0.084	44.9	0.102	55.1
365	0.026	0.002	8.2	0.004	15.5	0.006	23.7	0.020	76.3
Immature spinach									
30	0.140	0.111	79.6	0.003	2.1	0.114	81.7	0.026	18.3
120	0.140	0.101	72.4	0.004	2.7	0.105	75.1	0.035	24.9
Mature spinach									
30	0.128	0.093	72.7	0.004	3.1	0.097	75.8	0.031	24.2
120	0.145	0.096	66.3	0.007	4.6	0.103	70.9	0.042	29.1
Radish foliage									
30	2.156	1.686	78.2	0.121	5.6	1.807	83.8	0.351	16.3
120	1.512	1.266	83.7	0.042	2.8	1.308	86.5	0.204	13.5
365	0.104	0.073	69.9	0.011	10.2	0.084	80.1	0.021	19.9
Radish tubers									
30	0.273	0.176	64.4	0.019	7.1	0.195	71.5	0.078	28.4
120	0.393	0.310	78.8	0.021	5.4	0.331	84.2	0.062	15.8
365	0.051	0.030	59.7	0.003	5.8	0.033	65.5	0.018	34.6

1 TRR was calculated as the sum of ERR + RRR and set 100%
2 ERR calculated as sum of methanol and water extract

Table 6.6.1-5: Extractability of radioactive residues in rotational crop samples (pyrimidinyl label)

Days after treatment	TRR calculated[1]	Methanol extract		Water extract		ERR[2]		RRR	
DAT	[mg/kg]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]
Wheat forage									
30	1.606	1.383	86.1	0.068	4.2	1.451	90.3	0.156	9.7
120	0.922	0.635	68.8	0.049	5.3	0.684	74.1	0.239	25.9
365	0.057	0.033	58.9	0.005	8.8	0.038	67.7	0.018	32.3
Wheat hay									
30	1.342	0.994	74.1	0.086	6.4	1.080	80.5	0.260	19.4
120	0.873	0.451	51.8	0.071	8.1	0.522	59.9	0.350	40.1
365	0.100	0.053	53.7	0.013	12.3	0.066	66.0	0.034	33.9
Wheat straw									
30	5.244	2.999	57.2	0.724	13.8	3.723	71.0	1.526	29.1
120	1.625	0.617	38.0	0.254	15.6	0.871	53.6	0.752	46.3
365	0.323	0.140	43.3	0.061	18.8	0.201	62.1	0.122	37.9
Wheat grain									
30	0.174	0.075	43.1	0.032	18.4	0.107	61.5	0.067	38.5
120	0.500	0.030	5.9	0.047	9.2	0.077	15.1	0.425	85.0
365	0.017	<LOD	<LOD	0.003	17.6	0.003	17.6	0.014	82.4
Immature spinach									
30	0.071	0.057	79.7	0.001	2.3	0.058	82.0	0.013	18.1
120	0.125	0.094	74.9	0.003	2.7	0.097	77.6	0.028	22.3
365	0.011	0.008	69.7	<LOD	<LOD	0.008	69.7	0.003	30.3
Mature spinach									
30	0.104	0.072	69.3	0.003	2.7	0.075	72.0	0.029	28.1
120	0.179	0.147	81.8	0.005	2.7	0.152	84.5	0.028	15.4
365	0.010	0.007	70.9	<LOD	<LOD	0.007	70.9	0.003	29.1
Radish foliage									
30	1.232	0.973	79.0	0.080	6.5	1.053	85.5	0.177	14.4
120	0.793	0.664	83.7	0.028	3.5	0.692	87.2	0.102	12.8
365	0.036	0.017	46.3	0.005	13.3	0.022	59.6	0.015	40.5
Radish tubers									
30	0.206	0.124	60.3	0.012	5.6	0.136	65.9	0.070	34.1
120	0.199	0.156	77.9	0.013	6.5	0.169	84.4	0.031	15.5
365	0.015	0.007	46.5	0.001	9.1	0.008	55.6	0.007	44.4

1 TRR was calculated as the sum of ERR + RRR and set 100%
2 ERR calculated as sum of methanol and water extract

Considerable amounts of the radioactive residues were not extractable with methanol and water. The RRRs after solvent extraction, >10% TRR and >0.050 mg/kg (wheat forage, hay, straw, grain and radish foliage and tubers), were further characterized using a combination of sequential solubilization steps with 1% ammonia solution, macerozyme / cellulase, β-glucosidase, tyrosinase / laccase, α-amylase and protease. These steps investigated the residues which had been embedded / incorporated in insoluble plant material (e. g. proteins, cell wall polymers and starch). Following enzyme treatment the remaining residues were incubated with pepsin and pancreatin to investigate the bioavailability of the RRR.

The most effective solubilization step for wheat forage, hay and straw RRR was treatment with 1% ammonia solution (13.2-2.5% TRR; 0.060-0.040 mg/kg). Subsequent enzymatic incubation steps and treatments with pepsin and pancreatin released only minor portions of radioactive components (1.4-0.2% TRR; 0.015-0.002 mg/kg). This resulted in a final unextracted, not bioavailable residue level ranging from 23.4-2.7% TRR (0.380-0.041 mg/kg).
For radish foliage and tubers RRR only minor portions of radioactivity were released from ammonia, enzymatic, pepsin and pancreatin incubations (2.8-0.1% TRR; 0.006-0.002 mg/kg). This resulted in a final unextracted, not bioavailable residue level ranging from 26.5-4.2% TRR (0.055-0.064 mg/kg).
The most effective solubilization step overall and for wheat grain was treatment with α-amylase (49.0 (pyrimidinyl label, 120 DAT) to 13.0% TRR (phenyl label, 30 DAT); 0.245-0.018 mg/kg) indicating natural incorporation into starch. For the pyrimidinyl label (120 DAT), analysis of the extracts after α-amylase incubation using HPLC yielded a chromatogram with a peak at 10.10 min which was identified as naturally incorporated ^{14}C-glucose. Subsequent treatments with pepsin and pancreatin released only minor portions of radioactive components (6.9-0.2% TRR; 0.035-0.001 mg/kg). This resulted in a final unextracted, not bioavailable residue level ranging from 17.7-9.8% TRR (0.031-0.049 mg/kg).

2. Identification and quantification of extractable residues in rotational crops

In spring wheat matrices, M605F025 was the major component (27.0 to 0.1% TRR; 1.044 to 0.001 mg/kg) formed by ring opening of the pyrimidine ring. The remaining identified components BAS 605 F, M605F004, M605F007, M605F016, M605F027, M605F028, M605F030, M605F032, M605F033 and M605F036 ranged from 13.0 to 0.3% TRR (0.515 to <0.001 mg/kg, phenyl label) or from 16.3 to 0.1% TRR (0.359 to <0.001 mg/kg, pyrimidinyl label).
In spinach, the unchanged parent compound BAS 605 F (48.3 to 2.4% TRR; 0.068 to 0.003 mg/kg) and the malonic ester M605F040 (18.1 to 7.9% TRR; 0.026 to 0.006 mg/kg) were the major components. The remaining identified components M605F004, M605F007, M605F016, M605F025, M605F028, M605F032, M605F033, M605F036 and M605F039 ranged from 7.2 to 1.3% TRR (0.010 to 0.002 mg/kg, phenyl label) or from 17.5 to 0.6% TRR (0.031 to 0.001 mg/kg, pyrimidinyl label).
In radish, M605F025 was the major component (49.4 to 7.6% TRR; 0.899 to 0.002 mg/kg) formed by ring opening of the pyrimidine ring. The remaining identified components BAS 605 F, M605F004, M605F007, M605F016 M605F027, M605F028, M605F030, M605F032, M605F033 and M605F036 ranged from 7.8 to 0.8% TRR (0.035 to 0.001 mg/kg, phenyl label) or from 5.2 to 0.5% TRR (0.022 to <0.001 mg/kg, pyrimidinyl label).

Table 6.6.1-6: Summary of identified and characterized components - phenyl label

Metabolite	Crop parts							
	Wheat forage		Wheat hay		Wheat straw		Wheat grain	
	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]
Plant back interval: 30 DAT								
BAS 605 F	0.115	7.5	0.044	2.0	0.072	1.3	-	-
M605F004	0.062	4.0	0.047	2.2	0.115	2.1	-	-
M605F025	0.256	16.8	0.472	21.8	1.044	19.2	0.012	8.2
M605F027	0.106	6.9	0.034	1.6	0.193	3.5	-	-
M605F028	0.167	10.9	0.264	12.2	0.515	9.5	-	-
M605F030	0.021	1.4	0.011	0.5	0.081	1.5	-	-
M605F036	0.054	3.5	0.087	4.0	0.142	2.6	-	-
Sum of identified components	**0.781**	**51.0**	**0.959**	**44.3**	**2.162**	**39.7**	**0.012**	**8.2**
Total characterized by HPLC	**0.392**	**25.5**	**0.713**	**33.0**	**1.366**	**25.0**	**0.059**	**44.3**
Total characterized	**0.450**	**29.3**	**-**	**-**	**-**	**-**	**-**	**-**
Total identified and characterized	**1.231**	**80.3**	**1.672**	**77.3**	**3.528**	**64.7**	**0.071**	**52.5**
Residual Radioactive Residue (RRR)	0.179	11.7	0.438	20.2	1.717	31.5	0.061	43.1
Total characterized from RRR	**0.128**	**8.4**	**0.234**	**10.7**	**0.692**	**12.7**	**0.035**	**25.1**
Total identified and characterized from ERR+RRR	**1.359**	**88.7**	**1.906**	**88.0**	**4.220**	**77.4**	**0.106**	**77.6**
Total identified and characterized + RRR	**1.400**	**91.4**	**2.060**	**95.1**	**5.038**	**92.4**	**0.127**	**92.3**
Plant back interval: 120 DAT								
BAS 605 F	0.073	6.7	0.190	13.0	0.020	0.5	0.007	3.9
M605F004	0.049	4.5	0.022	1.5	0.040	1.0	-	-
M605F025	0.109	10.0	0.335	23.0	0.671	16.2	0.006	3.4
M605F027	0.033	3.0	-	-	0.046	1.1	-	-
M605F028	0.067	6.1	0.156	10.7	0.239	5.8	-	-
M605F030	-	-	-	-	0.015	0.4	-	-
M605F036	0.019	1.8	0.042	2.9	0.065	1.6	-	-
Sum of identified components	**0.350**	**32.1**	**0.745**	**51.1**	**1.096**	**26.6**	**0.013**	**7.3**
Total characterized by HPLC	**0.326**	**30.1**	**0.388**	**26.5**	**1.359**	**32.7**	**0.063**	**34.1**
Total characterized	**0.378**	**34.8**	**-**	**-**	**-**	**-**	**-**	**-**
Total identified and characterized	**0.728**	**66.9**	**1.133**	**77.6**	**2.455**	**59.3**	**0.076**	**41.4**
Residual Radioactive Residue (RRR)	0.368	33.7	0.375	25.7	1.401	33.8	0.102	55.1
Total characterized from RRR	**0.191**	**17.5**	**0.199**	**13.6**	**0.738**	**17.8**	**0.072**	**38.6**
Total identified and characterized from ERR+RRR	**0.919**	**84.4**	**1.332**	**91.2**	**3.193**	**77.1**	**0.148**	**80.0**
Total identified and characterized + RRR	**1.004**	**92.2**	**1.392**	**95.3**	**3.612**	**87.2**	**0.176**	**94.9**

Table 6.6.1-6: Summary of identified and characterized components - phenyl label

Metabolite	Crop parts							
	Wheat forage		Wheat hay		Wheat straw		Wheat grain	
	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]
Plant back interval: 365 DAT								
BAS 605 F	<0.001	0.7	-	-	0.002	0.3	-	-
M605F004	0.001	1.2	<0.001	0.5	0.002	0.5	-	-
M605F025	0.005	9.2	0.016	16.4	0.061	13.4	-	-
M605F027	0.001	1.7	-	-	-	-	-	-
M605F028	0.003	5.6	0.006	6.7	0.017	3.7	-	-
M605F030	<0.001	0.4	-	-	-	-	-	-
M605F036	0.001	1.2	0.002	1.7	0.005	1.1	-	-
Sum of identified components	**0.011**	**20.0**	**0.024**	**25.3**	**0.087**	**19.0**	**-**	**-**
Total characterized by HPLC	**0.017**	**29.4**	**0.031**	**32.2**	**0.152**	**33.3**	**0.003**	**12.8**
Total characterized	**0.022**	**39.0**	**-**	**-**	**-**	**-**	**0.005**	**21.0**
Total identified and characterized	**0.033**	**59.0**	**0.055**	**57.5**	**0.239**	**52.3**	**-**	**-**
Residual Radioactive Residue (RRR)	**0.020**	**34.1**	**0.031**	**32.9**	**0.181**	**39.5**	**0.020**	**76.3**
Total characterized from RRR	**-**	**-**	**-**	**-**	**0.093**	**20.2**	**-**	**-**
Total identified and characterized from ERR+RRR	**-**	**-**	**-**	**-**	**0.332**	**72.5**	**-**	**-**
Total identified and characterized + RRR	**0.053**	**93.1**	**0.086**	**90.4**	**0.386**	**84.4***	**0.025**	**97.3**

* Small procedural losses occured during preparation of samples for HPLC and further extraction of the RRR. Individually these losses were less than or equal to 5.4 % TRR or 0.025 mg/kg.

Table 6.6.1-7: Summary of identified and characterized components - phenyl label

Metabolite	Crop parts							
	Immature spinach		Mature spinach		Radish foliage		Radish tubers	
	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]
Plant back interval: 30 DAT								
BAS 605 F	0.068	48.3	0.007	5.2	-	-	0.021	7.8
M605F004	-	-	0.002	1.3	-	-	-	-
M605F025	0.003	2.1	0.002	1.7	0.899	41.7	0.095	34.7
M605F027	-	-	-	-	0.035	1.6	-	-
M605F028	-	-	0.005	4.3	0.033	1.5	-	-
M605F030	-	-	-	-	0.035	1.6	-	-
M605F036	-	-	0.002	1.4	0.022	1.0	-	-
M605F039	0.005	3.7	0.006	4.9	-	-	-	-
M605F040	0.013	9.1	0.014	11.2	-	-	-	-
Sum of identified components	**0.089**	**63.2**	**0.038**	**30.0**	**1.024**	**47.4**	**0.116**	**42.5**
Total characterized by HPLC	**0.028**	**19.7**	**0.041**	**32.5**	**0.675**	**31.4**	**0.063**	**22.9**
Total characterized	**0.031**	**21.8**	**0.045**	**35.6**	**0.796**	**37.0**	**0.082**	**30.0**
Total identified and characterized	**0.120**	**85.0**	**0.083**	**65.6**	**1.820**	**84.4**	**0.198**	**72.5**
Residual Radioactive Residue (RRR)	0.026	18.3	0.031	24.2	0.351	16.3	0.078	28.4
Total characterized from RRR	**-**	**-**	**-**	**-**	**0.108**	**5.0**	**0.033**	**11.5**
Total identified and characterized from ERR+RRR	**-**	**-**	**-**	**-**	**1.928**	**89.4**	**0.231**	**84.0**
Total identified and characterized + RRR	**0.146**	**103.3**	**0.114**	**89.8**	**2.081**	**96.5**	**0.283**	**103.0**
Plant back interval: 120 DAT								
BAS 605 F	0.009	6.3	0.022	15.2	0.017	1.1	0.006	1.5
M605F004	-	-	0.007	5.2	-	-	-	-
M605F025	0.002	1.6	0.002	1.5	0.685	45.3	0.194	49.4
M605F027					0.019	1.2	0.003	0.8
M605F028	-	-	-	-	0.025	1.7	-	-
M605F030					0.016	1.1	-	-
M605F036	0.010	7.0	0.008	5.8	0.014	0.9	-	-
M605F039	0.010	7.2	0.006	4.3	-	-	-	-
M605F040	0.024	17.4	0.026	18.1	-	-	-	-
Sum of identified components	**0.055**	**39.5**	**0.071**	**50.1**	**0.776**	**51.3**	**0.203**	**51.7**
Total characterized by HPLC	**0.038**	**27.7**	**0.026**	**18.2**	**0.535**	**35.4**	**0.093**	**23.3**
Total characterized	**0.042**	**30.4**	**0.033**	**22.8**	**0.577**	**38.2**	**0.114**	**28.7**
Total identified and characterized	**0.097**	**69.9**	**0.104**	**72.9**	**1.353**	**89.5**	**0.317**	**80.4**
Residual Radioactive Residue (RRR)	0.035	24.9	0.042	29.1	0.204	13.5	0.062	15.8
Total characterized from RRR	**-**	**-**	**-**	**-**	**0.094**	**6.2**	**0.019**	**4.7**
Total identified and characterized from ERR+RRR	**-**	**-**		**--**	**1.447**	**95.7**	**0.336**	**85.1**
Total identified and characterized + RRR	**0.132**	**94.8**	**0.146**	**102.0**	**1.511**	**99.9**	**0.381**	**96.6**

Table 6.6.1-7: Summary of identified and characterized components - phenyl label

Metabolite	Crop parts							
	Immature spinach		Mature spinach		Radish foliage		Radish tubers	
	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]
Plant back interval: 365 DAT								
BAS 605 F	-	-	-	-	-	-	-	-
M605F004	-	-	-	-	-	-	-	-
M605F025	-	-	-	-	0.027	26.0	0.012	23.6
M605F027	-	-	-	-	0.004	3.8	0.001	2.7
M605F028	-	-	-	-	-	-	-	-
M605F030	-	-	-	-	-	-	-	-
M605F036	-	-	-	-	-	-	-	-
M605F039	-	-	-	-	-	-	-	-
M605F040	-	-	-	-	-	-	-	-
Sum of identified components	**-**	**-**	**-**	**-**	**0.031**	**29.8**	**0.013**	**26.3**
Total characterized by HPLC	**-**	**-**	**-**	**-**	**0.052**	**48.4**	**0.012**	**25.3**
Total characterized	**-**	**-**	**-**	**-**	**-**	**-**	**0.015**	**31.1**
Total identified and characterized	**-**	**-**	**-**	**-**	**0.083**	**78.2**	**0.028**	**57.4**
Residual Radioactive Residue (RRR)	-	-	-	-	0.021	19.9	0.018	34.6
Total characterized from RRR	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
Total identified and characterized from ERR+RRR	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
Total identified and characterized + RRR	**-**	**-**	**-**	**-**	**0.104**	**98.1**	**0.046**	**92.0**

Table 6.6.1-8: Summary of identified and characterized components - pyrimidinyl label

Metabolite	Crop parts							
	Wheat forage		Wheat hay		Wheat straw		Wheat grain	
	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]
Plant back interval: 30 DAT								
BAS 605 F	0.183	11.4	0.034	2.5	-	-	-	-
M605F004	0.066	4.1	0.037	2.8	0.085	1.6		
M605F025	0.216	13.4	0.362	27.0	1.020	19.4	0.015	8.4
M605F027	0.117	7.3	0.036	2.7	0.162	3.1		
M605F028	0.182	11.3	0.219	16.3	0.359	6.8		
M605F030	0.021	1.3			0.049	0.9		
M605F036	0.057	3.5	0.051	3.8	0.103	2.0		
Sum of identified components	**0.842**	**52.3**	**0.739**	**55.1**	**1.778**	**33.8**	**0.015**	**8.4**
Total characterized by HPLC	**0.476**	**29.6**	**0.418**	**31.2**	**1.521**	**29.3**	**0.082**	**48.6**
Total characterized	**0.544**	**33.8**	-	-	-	-	-	-
Total identified and characterized	**1.386**	**86.1**	**1.157**	**86.3**	**3.299**	**63.1**	**0.097**	**57.0**
Residual Radioactive Residue (RRR)	0.156	9.7	0.260	19.4	1.526	29.1	0.067	38.5
Total characterized from RRR	**0.107**	**6.7**	**0.125**	**9.3**	**0.733**	**14.0**	**0.046**	**25.9**
Total identified and characterized from ERR+RRR	**1.493**	**92.8**	**1.282**	**95.6**	**4.032**	**77.1**	**0.143**	**82.9**
Total identified and characterized + RRR	**1.575**	**97.9**	**1.400**	**104.4**	**4.903**	**93.7**	**0.174**	**100.6**
Plant back interval: 120 DAT								
M605F007	0.078	8.4	0.043	5.0	0.062	3.8	<0.001	0.1
M605F016	0.053	5.8	0.041	4.7	0.036	2.2	0.001	0.1
M605F025	0.064	6.9	0.050	5.8	0.107	6.6	0.001	0.1
M605F032	0.127	13.8	0.024	2.8	0.032	2.0		
M605F033	0.039	4.3	0.058	6.6	0.065	4.0	0.004	0.8
Sum of identified components	**0.361**	**39.2**	**0.216**	**24.9**	**0.302**	**18.6**	**0.006**	**1.1**
Total characterized by HPLC	**0.250**	**27.0**	**0.301**	**34.6**	**0.565**	**34.7**	**0.046**	**9.4**
Total characterized	**0.299**	**32.3**	-	-	-	-	-	-
Total identified and characterized	**0.660**	**71.5**	**0.517**	**59.5**	**0.867**	**53.3**	**0.052**	**10.5**
Residual Radioactive Residue (RRR)	0.239	25.9	0.350	40.1	0.752	46.3	0.425	85.0
Total characterized from RRR	**0.174**	**18.7**	**0.134**	**15.2**	**0.311**	**19.1**	**0.369**	**73.6**
Total identified and characterized from ERR+RRR	**0.834**	**90.2**	**0.651**	**74.7**	**1.178**	**72.4**	**0.421**	**84.1**
Total identified and characterized + RRR	**0.862**	**93.2**	**0.792**	**90.9**	**1.558**	**95.8**	**0.470**	**93.9**

Table 6.6.1-8: Summary of identified and characterized components - pyrimidinyl label

Metabolite	Crop parts							
	Wheat forage		Wheat hay		Wheat straw		Wheat grain	
	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]
Plant back interval: 365 DAT								
M605F007	0.001	2.4	0.004	3.7	0.005	1.5	-	-
M605F016	0.001	1.1	0.003	3.0	0.005	1.6	-	-
M605F025	0.004	6.7	0.010	10.1	0.031	9.5	-	-
M605F032	0.002	4.1	0.002	1.6	0.010	3.0	-	-
M605F033	0.004	6.6	0.004	4.3	0.001	0.4	-	-
Sum of identified components	**0.012**	**20.9**	**0.023**	**22.7**	**0.052**	**16.0**	**-**	**-**
Total characterized by HPLC	**0.016**	**29.7**	**0.043**	**40.8**	**0.130**	**40.2**	**0.002**	**14.5**
Total characterized	**0.021**	**38.5**	**-**	**-**	**-**	**-**	**0.002**	**14.5**
Total identified and characterized	**0.033**	**59.4**	**0.066**	**63.5**	**0.182**	**56.2**	**-**	**-**
Residual Radioactive Residue (RRR)	0.018	32.3	0.034	33.9	0.122	37.9	0.014	82.4
Total characterized from RRR	**-**	**-**	**-**	**-**	**0.068**	**21.0**	**-**	**-**
Total identified and characterized from ERR+RRR	**-**	**-**	**-**	**-**	**0.250**	**77.2**	**-**	**-**
Total identified and characterized + RRR	**0.051**	**91.7**	**0.100**	**97.4**	**0.298**	**92.0**	**0.016**	**96.9**

Table 6.6.1-9: Summary of identified and characterized components - pyrimidinyl label

Metabolite	Crop parts							
	Immature spinach		Mature spinach		Radish foliage		Radish tubers	
	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]
Plant back interval: 30 DAT								
BAS 605 F	0.021	28.7	0.005	4.5	-	-	0.008	3.8
M605F004	-	-	0.001	0.6	-	-	0.003	1.2
M605F007	0.005	6.7	0.007	6.3	-	-	-	-
M605F016	0.001	1.8	0.002	1.8	-	-	-	-
M605F025	0.001	1.8	0.002	1.7	0.446	36.2	0.071	34.2
M605F027	-	-	-	-	0.018	1.5	-	-
M605F028	-	-	0.004	3.4	0.014	1.2	0.001	0.5
M605F030	-	-	-	-	0.013	1.0	-	-
M605F033	0.005	7.5	0.008	8.0	-	-	-	-
M605F036	-	-	0.002	1.5	-	-	-	-
M605F039	0.002	3.0	0.003	2.8	-	-	-	-
M605F040	0.006	7.9	0.010	9.7	-	-	-	-
Sum of identified components	**0.043**	**59.0**	**0.044**	**40.3**	**0.491**	**39.9**	**0.083**	**39.7**
Total characterized by HPLC	**0.013**	**18.3**	**0.027**	**26.5**	**0.436**	**35.5**	**0.047**	**22.8**
Total characterized	**0.014**	**20.6**	**0.030**	**29.2**	**0.516**	**42.0**	**0.059**	**28.4**
Total identified and characterized	**0.057**	**79.6**	**0.074**	**69.5**	**1.007**	**81.9**	**0.142**	**68.1**
Residual Radioactive Residue (RRR)	0.013	18.1	0.029	28.1	0.177	14.4	0.070	34.1
Total characterized from RRR	**-**	**-**	**-**	**-**	**0.066**	**5.3**	**0.027**	**13.6**
Total identified and characterized from ERR+RRR	**-**	**-**	**-**	**-**	**1.073**	**87.2**	**0.169**	**81.7**
Total identified and characterized + RRR	**0.070**	**97.7**	**0.103**	**97.6**	**1.193**	**96.9**	**0.224**	**108.2**
Plant back interval: 120 DAT								
BAS 605 F	0.003	2.4	-	-	-	-	0.003	1.5
M605F007	0.009	7.5	0.031	17.5	0.022	2.8	-	-
M605F016	0.009	7.4	0.025	14.1	0.007	0.9	-	-
M605F025	-	-	-	-	0.304	38.3	0.090	45.1
M605F027	-	-	-	-	-	-	0.001	0.6
M605F032	0.006	4.7	0.012	6.7	0.007	0.9	-	-
M605F033	0.011	8.8	0.030	16.5	0.021	2.7	-	-
M605F036	0.006	4.7	-	-	-	-	-	-
M605F039	0.006	4.9	-	-	-	-	-	-
M605F040	0.017	13.2	-	-	-	-	-	-
Sum of identified components	**0.067**	**53.6**	**0.098**	**54.8**	**0.361**	**45.6**	**0.094**	**47.2**
Total characterized by HPLC	**0.035**	**27.3**	**0.053**	**29.3**	**0.325**	**41.1**	**0.049**	**23.5**
Total characterized	**0.038**	**30.0**	**0.058**	**32.0**	**0.353**	**44.6**	**0.062**	**30.0**
Total identified and characterized	**0.105**	**83.6**	**0.156**	**86.8**	**0.714**	**90.2**	**0.156**	**77.2**
Residual Radioactive Residue (RRR)	0.028	22.3	0.028	15.4	0.102	12.8	0.031	15.5
Total characterized from RRR	**-**	**-**	**-**	**-**	**0.048**	**6.0**	**-**	**-**
Total identified and characterized from ERR+RRR	**-**	**-**	**-**	**-**	**0.762**	**96.2**	**-**	**-**
Total identified and characterized + RRR	**0.133**	**105.9**	**0.184**	**102.2**	**0.813**	**102.6**	**0.187**	**92.7**

Table 6.6.1-9: Summary of identified and characterized components - pyrimidinyl label

Metabolite	Crop parts							
	Immature spinach		Mature spinach		Radish foliage		Radish tubers	
	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]
Plant back interval: 365 DAT								
M605F007	-	-	-	-	0.001	4.0	-	-
M605F016	-	-	-	-	0.001	3.5	<0.001	2.5
M605F025	-	-	-	-	0.003	7.6	0.002	13.1
M605F032	-	-	-	-	<0.001	1.2	<0.001	1.7
M605F033	-	-	-	-	0.002	5.2	0.001	4.8
Sum of identified components	**-**	**-**	**-**	**-**	**0.007**	**21.5**	**0.003**	**22.1**
Total characterized by HPLC	**0.006**	**50.5**	**0.005**	**64.5**	**0.007**	**21.2**	**0.004**	**31.2**
Total characterized	**0.006**	**50.5**	**0.005**	**64.5**	**0.012**	**34.5**	**0.005**	**40.3**
Total identified and characterized	**-**	**-**	**-**	**-**	**0.019**	**56.0**	**0.008**	**62.4**
Residual Radioactive Residue (RRR)	0.003	30.3	0.003	29.1	0.015	40.5	0.007	44.4
Total characterized from RRR	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
Total identified and characterized from ERR+RRR	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
Total identified and characterized + RRR	**0.009**	**80.8**	**0.008**	**93.6**	**0.034**	**96.5**	**0.015**	**106.8**

3. Proposed metabolic pathway

The proposed metabolic pathway of BAS 605 F in rotational crops after application to soil is shown in Figure 6.6.1-1.

BAS 605 F is extensively metabolized; degradation products have been shown to be incorporated in to specific fractions of sample tissue. The metabolism of BAS 605 F includes the following reactions:

- Hydroxylation of a methyl group on the pyrimidinyl ring of BAS 605 F to form M605F004.
- Hydroxylation of the phenyl ring and pyrimidinyl ring of BAS 605 F to form intermediates M605F002 and/or isomers thereof and M605F003 respectively.
- Conjugation of the hydroxyl group of M605F004 to form glucoside M605F027 and subsequent esterification to furnish the malonic ester derivative M605F028.
- Conjugation of the hydroxyl groups of M605F002, and/or isomers thereof, and M605F003 to form glucosides M605F039 and M605F030 respectively, and subsequent esterification to furnish the malonic ester derivatives M605F040 and M605F036 respectively.
- Cleavage of the secondary amine linkage in BAS 605 F to form M605F007 or the hydroxylated derivatives M605F032 and M605F016.
- Conjugation of the hydroxyl group of M605F032 to form glucoside M605F033
- Cleavage/ring opening of the pyrimidinyl ring of BAS 605 F to form M605F025.

The major route of metabolism of BAS 605 F in wheat (forage, hay and straw fractions) and radish matrices was ring-opening of the pyrimidinyl moiety to furnish M605F025. A secondary pathway via hydroxylation of BAS 605 F (M605F004), followed by glucose conjugation (M605F027) and subsequent esterification to form the malonic ester derivative (M605F028) represented another significant route of metabolism in wheat (forage, hay and straw fractions) matrices.

The unchanged parent BAS 605 F remained as the primary component in immature spinach after the 30 DAA plant back interval. After the 120 DAA plant back interval, the major route of metabolism of BAS 605 F in spinach matrices was initial hydroxylation of the phenyl ring of BAS 605 F (M605F002 and/or isomers), followed by glucose conjugation (M605F039) and subsequent esterification to form the malonic ester derivative M605F040.
Significant levels of secondary amine cleavage products, pyrimidine M605F007 and its hydroxylated derivatives M605F016 and M605F032 were present in the majority of matrices. Furthermore, the glucoside M605F033, which could be formed via a number of possible intermediates was also present as a major component.

4. Storage stability

The solvent extracts (methanol and water) of representative samples were initially extracted and HPLC analyzed within a maximum of 31 days.

Subsequently, samples were reconstituted in solvents which were identical to the starting conditions of the mobile phase used for HPLC analysis. For this reason the sampling to analysis interval for quantification was up to 468 days for some of the representative samples.

The metabolite pattern of an early extraction (10 days after sampling), the concentrated methanol extracts of radish foliage (120 DAA, phenyl label), was obtained 485 days after extraction with HPLC and was virtually identical to the metabolite pattern of the same sample used for initial analysis. This was also true of the methanol extracts of wheat straw (120 DAA, pyrimidinyl label), wheat grain (120 DAA, pyrimidinyl label), mature spinach (30 DAA phenyl label) and all other representative samples. Chromatography experiments were conducted for the concentrated methanol extracts of wheat straw (120 DAA, both labels), wheat grain (30 DAA, both labels), mature spinach (30 DAA, both labels) and radish foliage (120 DAA, both labels). Hence, the corresponding metabolites were stable throughout the period of investigation (minimum of 567 days).

III. CONCLUSION

The homogenized plant samples were extracted with methanol and water. The total radioactive residues (TRR) were determined by addition of the extractable radioactive residues (ERR) and the residual radioactive residues (RRR) after solvent extraction. At all plant back intervals, the highest TRR levels were found in spring wheat straw and radish foliage but the concentration of residues decreased after a plant back interval of 120 and 365 days. For the other plant matrices lower TRR values were found.

The extractability of the radioactive residues with methanol and water ranged for rotational crop matrices from 88.3 to 23.7% TRR in the phenyl label and from 90.3 to 15.1% TRR for the pyrimidinyl label. For wheat grain, a tendency for low extractability with both solvents was found for both labels (less than 61.5% TRR). With the exception of wheat grain, the major portion of the radioactive residues of both labels were extracted with methanol (from 86.1 to 40.3% TRR) compared to water (from 20.2 to 2.1% TRR). For wheat grain (both labels) the portion extracted with methanol (from 44.7 to 5.9% TRR) compared to water (from 20.0 to 9.2% TRR) was higher for 30 DAA and lower for 365 DAA plant pack intervals.

Considerable amounts of the radioactive residues were not extractable with methanol and water. The RRR after solvent extraction, >10% TRR and >0.050 mg/kg, were further characterized using a combination of sequential solubilization steps with ammonia solution and several enzymes. These steps investigated the residues which had been embedded / incorporated in insoluble plant material. Following enzyme treatment the remaining residues were incubated with pepsin and pancreatin to investigate the bioavailability of the RRR. The most effective solubilization step was the incubation of wheat grain with α-amylase indicating natural incorporation of residues into starch. This was supported by chromatography experiments which confirmed the presence of naturally incorporated ^{14}C-glucose in wheat grain (120 DAA, pyrimidinyl label). In general residues from each solubilization step were low indicating that the RRR was not bioavailable.

The major component in spring wheat matrices, M605F025 (27.0 to 0.1% TRR), was formed by ring opening of the pyrimidine ring of BAS 605 F. The remaining identified components BAS 605 F, M605F004, M605F007, M605F016, M605F027, M605F028, M605F030, M605F032, M605F033 and M605F036 ranged from 13.0 to 0.3% TRR (phenyl label) or from 16.3 to 0.1% TRR (pyrimidinyl label).

In spinach, the unchanged parent compound BAS 605 F (48.3 to 2.4% TRR) and the malonic ester M605F040 (18.1 to 7.9% TRR) were the major components. The remaining identified components M605F004, M605F007, M605F016, M605F025, M605F028, M605F032, M605F033, M605F036 and M605F039 ranged from 7.2 to 1.3% TRR (phenyl label) or from 17.5 to 0.6% of the TRR (pyrimidinyl label).

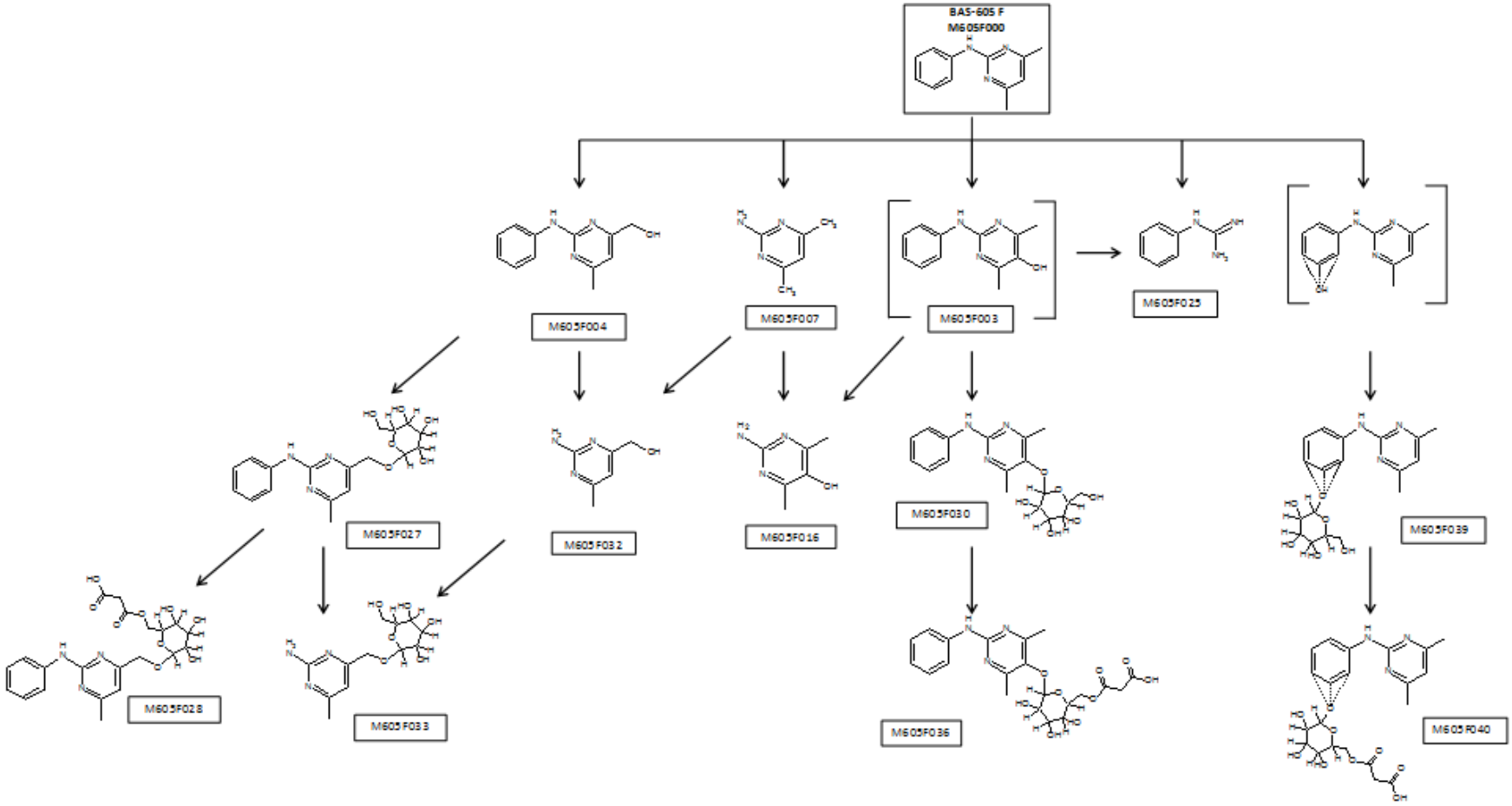
In radish, M605F025 was the major component (49.4 to 7.6% TRR) formed by ring opening of the pyrimidine ring. The remaining identified components BAS 605 F, M605F004, M605F007, M605F016 M605F027, M605F028, M605F030, M605F032, M605F033 and M605F036 ranged from 7.8 to 0.8% TRR (phenyl label) or from 5.2 to 0.5% TRR (pyrimidinyl label).

The metabolism of BAS 605 F includes the following reactions:

- Hydroxylation of the parent compound
- Formation of glucose and malonyl glucose conjugates of metabolites via hydroxylation of the parent compound
- Cleavage of secondary amine bond
- Ring opening of the pyrimidine ring

The degradation products of BAS 605 F are eventually incorporated into natural plant products.

Figure 6.6.1-1: Proposed metabolic pathway of pyrimethanil (BAS 605 F) in rotational crops



CA 6.6.2 Magnitude of residues in rotational crops

According to Reg. 283/2013, studies on the magnitude of residues in rotational crops are required under the following circumstances:

If the metabolism studies indicate that residues of the active substance or of relevant metabolites or breakdown products either from plant or soil metabolism may occur (> 0.01 mg/kg), limited field studies and, if necessary, field trials shall be carried out.

The new conducted field rotational crop study is summarized below. ~~Please note that at this point in time, interim data covering 30 and 120 DAA replant intervals are available only. However, since residues were generally much lower after the 120 DAA replant interval, and in accordance to the decline of residues seen in the confined rotational crop study, residues are expected to be even lower at the 365 day replant interval (plots 5 and 6). Therefore suitable information should be available for a sound evaluation.~~

~~**Report:**~~	~~CA 6.6.2/1~~ ~~Erdmann H.-P., 2015a~~ ~~Interim report: Rotational crops wheat, carrots/radish, cauliflower/broccoli, lettuce/spinach after one application of BAS 605 04 F to bare soil 30, 120, 365 days prior planting field conditions in Germany Netherlands Italy Spain 2014-2015~~ ~~2015/1112007~~
~~**Guidelines:**~~	~~EEC 7029/VI/95 rev. 5 (July 22 1997), EEC 7525/VI/95 rev. 9 (March 2011), EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, OECD 504~~
~~**GLP:**~~	~~yes~~ ~~(certified by Land Brandenburg Ministerium fuer Umwelt, Gesundheit und Verbraucherschutz, Potsdam, Germany)~~

Report:	CA 6.6.2/1 Erdmann H.-P., 2016 a Study on the residue behavior of BAS 605 F on rotational crops after one application of BAS 605 04 F to bare soil 30, 120 and 365 days prior planting under field conditions in DE, NL, Italy and Spain, 2014 - 2015 2016/1026893
Guidelines:	EEC 7029/VI/95 rev. 5 (July 22 1997), EEC 7525/VI/95 rev. 9 (March 2011), EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, OECD 504
GLP:	yes (certified by Land Brandenburg Ministerium fuer Umwelt, Gesundheit und Verbraucherschutz, Potsdam, Germany)

Report:	CA 6.6.2/2 Erdmann H.-P., 2017 a Study on the residue behavior of BAS 605 F (Pyrimethanil) on the rotational crop: Wheat after one application of BAS 605 04 F to bare soil 30, 120 and 365 days prior seeding under field conditions in The Netherlands, 2015 - 2016 2016/1067813
Guidelines:	EEC 7029/VI/95 rev. 5 (July 22 1997), EEC 7525/VI/95 rev. 9 (March 2011), EC 1107/2009 of the European Parliament, EEC 79/117, EEC 91/414, OECD 504, OECD 509 Crop Field Trial (2009)
GLP:	yes (certified by Land Brandenburg Ministerium der Justiz und fuer Europa und fuer Verbraucherschutz, Potsdam, Germany)

Executive Summary

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Pyrimethanil (BAS 605 F)
Description: BAS 605 04 F
Lot/Batch #: FRE-001002, FRE-001053
Purity: Not relevant
CAS#: 53112-28-0
Development code: 236999 (Reg. No pyrimethanil)
Spiking levels: 0.01-0.5 mg/kg

2. Test Commodity:

Crop:	1) Wheat, 2) carrot/radish, 3) cauliflower/broccoli, 4) lettuce/spinach
Type:	1) Cereals, 2) root and tuber vegetables, 3) brassica vegetables, 4) leafy vegetables
Variety:	1) Kadrilj, Valbona, Artur Nick, Apertus, Tabasco 2) Merida, Mokum, Amsterdam 2, Candela di Fuoco, 3) Chambord, Marathon, Snow Crown F1, Sirente, 4) Whale, Corvair, Spargo F1, Viroflay
Botanical name:	1) *Triticum aestivum*, 2) *Daucus carota*/*Raphanus sativus*, 3) *Brassica oleracea*, 4) *Lactuca sativa/Spinacia oleracea*
Crop parts(s) or processed commodity:	1) Whole plant without roots, grain, straw, 2) whole plant with roots, top, root, 3) whole plant without roots, inflorescence, 4) leaves
Sample size:	At least 0.5 kg plant material

B. STUDY DESIGN AND METHODS

1. Test procedure

During the growing seasons of 2014 to ~~and 2015~~ 2016, ~~5~~ 6 trials were conducted to determine the magnitude of residues of pyrimethanil and its metabolites M605F005, M605F007 and M605F025 (phenylguanidine) in four field rotational crops (wheat, carrot/radish, cauliflower/broccoli and spinach/lettuce) after one application of BAS 605 04 F (400 g BAS 605 F/L, SC) at a rate of 7.5 L/ha, equivalent to 3.0 kg a.s./ha, to bare soil 30±1, 120±3 and 365±5 days before sowing/planting. The water volume used was 200 L/ha.
Four trials (2 EU-North and 2 EU-South) consisted of three untreated (plot 1, 3, 5) and three treated plots (plot 2, 4, 6) which were planted after different time intervals (plot 2: 30±1 Days after application plot 4: 120±3 DAA, plot 6: 365±5 DAA). Plots 1 to 6 were divided into four subplots planted with different crops: wheat, carrots/radish, cauliflower/broccoli and spinach/lettuce, exception in trial L140122: plot 1 and 2 were divided into three subplots planted with different crops: carrots, broccoli and spinach, plot 3 and 4 were also divided into two subplots planted with different crops: carrots and broccoli.
One trial (L140746) consisted of one untreated (plot 3) and one treated plot (plot 4) which were planted after 120±3 DAA. Plot 3 and 4 were divided into one subplot planted with spinach, to repeat parts of trial L140122.
As a crop failure occurred in wheat of trial L0140122, wheat as a rotational crop for all 3 plant back intervals (plots 2, 4 and 6) was repeated as trial L150373 in a separate study (2016/1067813).
In all trials the applications were done 29-31 days before planting on plot 2, 119-123 days before planting on plot 4 and 361-365 days before planting on plot 6 (exception: in trial L140122 the replant interval on plot 6 was 389 days before planting). The plots 1, 3 and 5 remained untreated.

Specimens collected from the crops are summarized in the following list:

- Wheat: Whole plants without roots were collected at BBCH 30-33 and 65. Grain and straw were collected at BBCH 89.
- Carrots/radish (small): Whole plants with roots were collected at BBCH 41, roots and tops at BBCH 49.
- Cauliflower/broccoli: Whole plants without roots were collected at BBCH 41, inflorescences at BBCH 49.
- Spinach/lettuce (seeded): Leaves were collected at BBCH 41 and 49.

Samples were generally stored at or below -18°C from sampling to analysis for a maximum of 380 days for pyrimethanil and 448 days for metabolites M605F005, M605F007 and M605F025.

2. Description of analytical procedures

The plant specimens were analyzed for residues of pyrimethanil according to the BASF method No 542/2 (L0066/02) and for M605F005, M605F007 and phenylguanidine (M605F025) according to BASF method No L0274/01.
Furthermore, soil specimens (application verification samples) were analyzed for pyrimethanil according to BASF method No L0249/01.
The limit of quantitation (LOQ) for each analyte (pyrimethanil, M605F005, M605F007 and M605F025) is 0.01 mg/kg.
Method No 542/2 (L0066/02): The homogenized specimens were extracted using a mixture of methanol and water (70:30, v/v). An aliquot of the extract was centrifuged and a portion of the clear supernatant was taken and diluted with methanol/water (50:50, v/v) to measuring concentration. Measurement was performed by LC-MS/MS. The limit of detection (LOD) is 0.001 mg/kg only for L150373 (2016/1067813) 0.002 mg/kg.
Method No L0274/01: The homogenized specimens were extracted using a mixture of methanol and water (40:60, v/v). An aliquot of the extract was centrifuged and a portion of the clear supernatant was taken and diluted with water to measuring concentration. After filtering, measuring was performed by LC-MS/MS. The limit of detection (LOD) is 0.002 mg/kg.
Method No L0274/01 for straw: The homogenized specimens were extracted using a mixture of methanol/water/formic acid (40:60:0.1, v/v/v). An aliquot of the extract was centrifuged and a portion of the clear supernatant was taken and diluted with water and methanol to measuring concentration. Measurement was performed by LC-MS/MS. The limit of detection (LOD) is 0.002 mg/kg.

Table 6.6.2-1: Summary of recoveries of pyrimethanil and metabolites in plant commodities

Matrix	Fortification level (mg/kg)	n	Summary recoveries							
			Mean (%)	RSD (%)	Mean (%	RSD (%)	Mean (%)	RSD (%)	Mean (%)	RSD (%)
Method No 542/2 (L0066/02)[1] Method No L0274/01[2]			BAS 605 F (pyrimethanil)		M605F005		M605F007		M605F025 (phenylguanidine)	
Wheat whole plant[3]	0.01/0.1	6	87	3.7	79.3	9.7	89.2	10.7	84.4	6.0
Wheat grain	0.01/0.1	4	98	7.6	82.4	7.5	92.9	7.6	73.5	8.6
Wheat straw	0.01/0.1	4	85	5.2	85.5	2.2	90.7	4.2	91.4	4.2
Carrot whole plant[4]	0.01/0.1/0.5	4[1]/2[2]	85	3.6	82.5	N/A	91.1	N/A	80.5	N/A
Carrot roots	0.01/0.1	2	86	N/A	81.9	N/A	94.7	N/A	80.5	N/A
Carrot tops	0.01/0.1	4[1]/2[2]	88	0.7	80.1	N/A	91.5	N/A	83.6	N/A
Radish whole plant with roots	0.01/0.1	2[1]/4[2]	85	N/A	79.5	7.0	91.9	3.2	91.0	5.7
Radish tops (leaves + stem)	0.01/0.1	2	85	N/A	82.8	N/A	89.1	N/A	86.8	N/A
Radish roots	0.01/0.1	2	86	N/A	79.6	N/A	88.2	N/A	85.6	N/A
Cauliflower whole plant[3]	0.01/0.1	2	89	N/A	76.1	N/A	85.5	N/A	81.5	N/A
Cauliflower inflorescences	0.01/0.1	2	85	N/A	82.2	N/A	88.4	N/A	86.9	N/A
Broccoli whole plant[3]	0.01/0.1	2	87	N/A	70.1	N/A	77.8	N/A	74.5	N/A
Broccoli inflorescences	0.01/0.1	2	87	N/A	72.2	N/A	81.5	N/A	85.4	N/A
Spinach leaves	0.01/0.1	8[1]/12[2]	94	9.6	85.9	7.6	92.9	5.9	86.3	7.6
Lettuce	0.01/0.1/0.5	3/2	93	1.3	78.5	N/A	85.1	N/A	84.5	N/A
Wheat whole plant[3]	0.01/0.1/0.5[1]	9[1]/8[2]	92	6.6	79	8.4	90	9.4	85	8.0
Wheat grain	0.01/0.1	6	98	5.9	79	8.9	91	7.1	73	11
Wheat straw	0.01/0.1	6	94	15	88	4.0	93	5.0	92	3.7
Wheat whole plant[3]	0.01/0.1/0.3[1]/1[2]	7	87	3.3	79	6.5	92	7.6	85	6.4
Wheat grain	0.01/0.1/1[2]	6[1]/7[2]	88	5.3	88	2.6	92	5.3	73	5.2
Wheat straw	0.01/0.1/1[2]	6[1]/7[2]	91	7.8	90	6.0	88	5.6	96	3.6
Carrot whole plant[4]	0.01/0.1/0.5[1]	6[1]/4[2]	87	5.2	79	6.1	94	6.5	85	6.4
Carrot roots	0.01/0.1	4	88	3.6	83	2.9	94	2.4	81	1.7
Carrot tops	0.01/0.1	6[1]/4[2]	88	0.9	81	4.9	94	2.7	86	7.3

Table 6.6.2-1: Summary of recoveries of pyrimethanil and metabolites in plant commodities

Matrix	Fortification level (mg/kg)	n	Summary recoveries							
			Mean (%)	RSD (%)	Mean (%	RSD (%)	Mean (%)	RSD (%)	Mean (%)	RSD (%)
Method No 542/2 (L0066/02)[1] Method No L0274/01[2]			**BAS 605 F (pyrimethanil)**		**M605F005**		**M605F007**		**M605F025 (phenylguanidine)**	
Radish whole plant with roots	0.01/0.1	2[1]/4[2]	85	N/A	80	7.0	92	3.2	91	5.7
Radish tops (leaves + stem)	0.01/0.1	2	85	N/A	83	N/A	89	N/A	87	N/A
Radish roots	0.01/0.1	2	86	N/A	80	N/A	88	N/A	86	N/A
Cauliflower whole plant[3]	0.01/0.1	4	92	6.9	79	5.2	86	2.2	85	5.5
Cauliflower inflorescences	0.01/0.1	4	87	4.0	80	5.6	94	9.8	88	4.2
Broccoli whole plant[3]	0.01/0.1	2	87	N/A	70	N/A	78	N/A	75	N/A
Broccoli inflorescences	0.01/0.1	4	84	8.3	77	7.7	86	6.4	90	8.7
Spinach leaves	0.01/0.1	8[1]/12[2]	94	9.6	86	7.6	93	5.9	86	7.6
Lettuce	0.01/0.1/0.5[1]	5[1]/4[2]	94	1.5	79	0.9	89	5.6	84	6.1

N/A Not applicable
1 Pyrimethanil
2 Metabolites; the method was modified for analysis of wheat straw
3 Without roots
4 With roots

Furthermore, the matrix wheat straw was validated following the BASF method No L0274/01 with changes in chromatography and extraction for the analytes M605F005, M605F007 and M605F025.

A detailed summary of the method validation is given below.

Principle of the method: Homogenized wheat specimens were extracted using a mixture of methanol/water/formic acid (40:60:0.1, v/v/v). An aliquot of the extract was centrifuged and a portion of the clear supernatant was taken and diluted with water and methanol to measuring concentration. Measurement was performed by LC-MS/MS. Analysis was accomplished on a Waters Acquity UPLC HSS T3 column applying an acetonitrile-pure water gradient using 5 mmol ammonium acetate/formic acid as modifier. Detection of M605F005, M605F007 and M605F025 was accomplished in ESI+ mode at mass transitions m/z 232 → 196, 124 → 67 and 136 → 76.6 for quantitation and 232 → 144, 124 → 82 and 136 → 93.9 for confirmation, respectively.

Recovery findings: ~~In all matrices~~ For all analytes tested in matrix straw, the mean recovery values were between 70% and 110%. The detailed results are given in the table below.

Table 6.6.2-2: Validation results of method L0274/01: pyrimethanil metabolites M605F005, M605F007 and M605F025 in wheat straw

Test substance	Crop	Fortification level (mg/kg)	No of tests	Average recovery (%)		Relative standard deviation (%)	
Transition				**232 → 196**	**232 → 144**	**232 → 196**	**232 → 144**
M605F005	Wheat straw	0.01	5	74	77	5.5	7.0
		0.1	5	78	78	2.3	2.6
				124 → 67	**124 → 82**	**124 → 67**	**124 → 82**
M605F007	Wheat straw	0.01	5	83	87	1.3	2.5
		0.1	5	85	84	2.9	1.5
				136 → 76.6	**136 → 93.9**	**136 → 76.6**	**136 → 93.9**
M605F025	Wheat straw	0.01	5	93	87	4.8	2.3
		0.1	5	86	85	1.3	1.8

Linearity: Good linearity was observed over the concentration range tested. Linear correlations with coefficients >0.99 were obtained for M605F005, M605F007 and M605F025. At least 6 calibration points distributed over a concentration range of 0.025-5.0 ng/mL were used.

Specificity: LC-MS/MS is a highly specific detection technique and therefore no confirmatory technique is required. Analysis is possible at two different mass transitions per analyte, hence no additional confirmatory analytical technique is required.

Matrix Effects:	Matrix matched standards were used if a significant matrix effect (>10%) was determined in the course of the method implementation (non-GLP system suitability test). The chromatographic systems for the determination of M605F005, M605F007 and M605F025 was calibrated using matrix matched standards. One additional dilution step with methanol/water (2:8, v/v) to decrease matrix effects was carried out.
Interference:	The detector signals of M605F005, M605F007 and M605F025 in all of the control specimens were below 30% of the detector signals of the specimens fortified at the LOQ. The specificity of the analytical method is acceptable, since no significant interferences from the specimen matrices were detected at the retention time of interest.
Limit of Quantitation:	The limit of quantitation of the analytical method is 0.01 mg/kg in wheat straw for all analytes tested.
Repeatability:	The relative standard deviations (RSD, %) for all analytes were <10%. The detailed values are shown in Table 6.6.2-2.
Stability of Solutions:	Working solutions of each reference item were tested for stability during the time period of the laboratory work. The stability was tested with satisfactory results (recovery 88-106%) for a storage period of 123 days. The stability of each analyte in the specimen extract of wheat straw was tested by re-analyzing specimen extract fortified at 10-fold after a period of 6 days using fresh standard solutions. The extract was stored at 2-8°C. Mean recoveries of 91-102% were obtained, proving stability of extracts for at least 6 days.
Conclusion:	**It could be demonstrated that the method L0274/01 fulfils the requirements with regard to linearity, specificity, repeatability, limit of quantitation, and recoveries and is therefore applicable and suitable to correctly determine residues of pyrimethanil metabolites M605F005, M605F007 and M605F025 in wheat straw.**

II. RESULTS AND DISCUSSION

~~The Interim results of plots 2 and 4 (30 and 120 days replanting interval)~~ are summarized below ~~(except of wheat specimens from plot 3 and 4 at BBCH 65 and BBCH 89 for trials L140121 and L140123). The results for the plots 5 to 6 and for the application verifications specimens will be given in the final report.~~

Table 6.6.2-3: Range of results after ~~30 and 120~~ 30, 120 and 365 DAA replanting interval

Sub Plot No.	Crop	Portion analyzed	Growth Stage [BBCH]	n	Range of residues			
					BAS 605 F [mg/kg]	M605F005 [mg/kg]	M605F007 [mg/kg]	M605F025 [mg/kg]
PLOT 2: 30 ± 1 DAA replanting interval								
1	Wheat	Whole plant[1]	31-32	~~3~~ 4	<0.01-0.26	<0.01	<0.01	<0.01-0.058
		Whole plant[1]	65	~~3~~ 4	<0.01-0.037	<0.01	<0.01	<0.01-0.041
		Grain	89	~~3~~ 4	<0.01	<0.01	<0.01	<0.01
		Straw	89	~~3~~ 4	<0.01-0.018	<0.01	<0.01-0.052	<0.01-0.055
2	Carrots/ Radish	Whole plant[2]	41	4	<0.01-0.26	<0.01	<0.01-0.011	<0.01
		Roots	49	4	<0.01-0.025	<0.01	<0.01	<0.01
		Tops	49	4	<0.01-0.097	<0.01	<0.01	<0.01-0.018
3	Cauliflower/ Broccoli	Whole plant[1]	41	4	<0.01-0.079	<0.01	<0.01-0.028	<0.01
		Inflorescences	49	4	<0.01	<0.01	<0.01	<0.01
4	Spinach/ Lettuce (seeded)	Leaves	41	4	<0.01-0.42	<0.01	<0.01-0.11	<0.01
			49	4	<0.01-0.044	<0.01	<0.01-0.042	<0.01
PLOT 4: 120 ± 3 DAA replanting interval								
1	Wheat	Whole plant[1]	30-32	~~3~~ 4	<0.01	<0.01	<0.01	<0.01
		Whole plant[1]	65	~~3~~ 4	<0.01	<0.01	<0.01	<0.01
		Grain	89	~~3~~ 4	<0.01	<0.01	<0.01	<0.01
		Straw	89	~~3~~ 4	<0.01	<0.01	<0.01	<0.01
2	Carrots/ Radish	Whole plant[2]	41	4	<0.01-0.016	<0.01	<0.01	<0.01
		Roots	49	4	<0.01	<0.01	<0.01	<0.01
		Tops	49	4	<0.01	<0.01	<0.01	<0.01
3	Cauliflower/ Broccoli	Whole plant[1]	41	4	<0.01	<0.01	<0.01	<0.01
		Inflorescences	49	4	<0.01	<0.01	<0.01	<0.01
4	Spinach/ Lettuce (seeded)	Leaves	41	4	<0.01-0.075	<0.01	<0.01-0.054	<0.01
			49	4	<0.01	<0.01	<0.01-0.035	<0.01
PLOT 6: 365 ± 5 DAA and 389 (Netherlands) replanting interval								
1	Wheat	Whole plant[1]	31-32	~~3~~ 4	<0.01	<0.01	<0.01	<0.01
		Whole plant[1]	65	~~3~~ 4	<0.01	<0.01	<0.01	<0.01
		Grain	89	~~3~~ 4	<0.01	<0.01	<0.01	<0.01
		Straw	89	~~3~~ 4	<0.01	<0.01	<0.01	<0.01
2	Carrots/ Radish	Whole plant[2]	41	4	<0.01	<0.01	<0.01	<0.01
		Roots	49	4	<0.01	<0.01	<0.01	<0.01
		Tops	49	4	<0.01	<0.01	<0.01	<0.01
3	Cauliflower/ Broccoli	Whole plant[1]	41	4	<0.01	<0.01	<0.01	<0.01
		Inflorescences	49	4	<0.01	<0.01	<0.01	<0.01
4	Spinach/ Lettuce (seeded)	Leaves	41	4	<0.01	<0.01	<0.01	<0.01
			49	4	<0.01	<0.01	<0.01	<0.01

1 Without roots
2 With roots

30 days replanting interval (Plot 2)

Pyrimethanil (BAS 605 F)

In plot 2 (30 days replanting interval) residues of pyrimethanil were found in the specimens of wheat (whole plant with roots) taken at BBCH 31-32 in the range of <0.01-0.26 mg/kg and at BBCH 65 in the range of <0.01-0.037 mg/kg. At BBCH 89 no residues were found in wheat (grain) (<0.01 mg/kg) and residues were found in wheat (straw) in the range of <0.01-0.018 mg/kg.

Residues of pyrimethanil were found in the specimens of carrots/radish (whole plant with roots) taken at BBCH 41 in the range of <0.01-0.26 mg/kg. At BBCH 49 residues were analyzed in carrots/radish (roots) in the range of <0.01-0.025 mg/kg and in carrots/radish (tops) in the range of <0.01-0.097 mg/kg.

In cauliflower/broccoli (whole plant without roots) specimens taken at BBCH 41 residues of pyrimethanil were found in the range of <0.01-0.079 mg/kg. At harvest time (BBCH 49) no residues above the LOQ were found in the inflorescences specimens of cauliflower/broccoli.

In lettuce/spinach (leaves) specimens taken at BBCH 41 residues of pyrimethanil were found in the range of <0.01-0.42 mg/kg. At BBCH 49 residues were found in lettuce/spinach (leaves) specimens in the range of <0.01-0.044 mg/kg.

M605F005

No residues of M605F005 above the limit of quantitation (LOQ) were found in any of the treated plant specimens of wheat, carrots/radish, cauliflower/broccoli and lettuce/spinach.

M605F007

In plot 2 no residues above the LOQ (0.01 mg/kg) of M605F007 were found in the specimen of wheat (whole plant without roots) at BBCH 31-32 and BBCH 65. Also, no residues above the LOQ were found in the specimens of wheat (grain) at BBCH 89. In the specimens of wheat (straw) at BBCH 89 residues were found in the range of <0.01-0.052 mg/kg.

In carrots/radish (whole plant with roots) specimens taken at BBCH 41 residues of metabolite M605F007 were found in the range of <0.01-0.011 mg/kg. At BBCH 49 (harvest time) no residues above the LOQ were found in any other carrot / radish tops or roots specimens.

In cauliflower/broccoli (whole plant without roots) specimens taken at BBCH 41 residues of metabolite M605F007 were found between <0.01 and 0.028 mg/kg. At BBCH 49 (harvest time) no residues above the LOQ were found in any other cauliflower/broccoli (inflorescences) specimens.

In spinach/lettuce (leaves) specimens taken at BBCH 41 residues of metabolite M605F007 were found in the range of <0.01-0.11 mg/kg and at BBCH 49 (harvest time) in the range of <0.01-0.042 mg/kg.

M605F025 (phenylguanidine)

In plot 2 residues of M605F025 were found in the specimen of wheat (whole plant without roots) at BBCH 31-32 in the range of <0.01-0.058 mg/kg and at BBCH 65 in the range of <0.01-0.041 mg/kg. No residues above the LOQ were found in the specimens of wheat (grain) at BBCH 89. In the specimens of wheat (straw) at BBCH 89 residues were found in the range of <0.01-0.055 mg/kg.

Furthermore, residues of M605F025 were found in carrots/radish tops specimen taken at BBCH 49 up to 0.018 mg/kg. In all other treated plant specimen of carrots/radish, cauliflower/broccoli and lettuce/spinach no residues of M605F025 were analyzed above the limit of quantitation (LOQ).

120 days replanting interval (Plot 4)

Pyrimethanil (BAS 605 F)

In plot 4 (120 days replanting interval) no residues of pyrimethanil above the LOQ were found in any of the treated wheat specimens.

Residues of pyrimethanil were found in the specimens of carrots/radish (whole plant with roots) taken at BBCH 41 in the range of <0.01-0.016 mg/kg. No residues of pyrimethanil above the LOQ were found in any of the other treated carrots/radish specimens.

No residues of pyrimethanil above the LOQ were found in any of the treated cauliflower/broccoli specimens.

Residues of pyrimethanil were found in the specimens of spinach/lettuce (leaves) taken at BBCH 41 in the range of <0.01-0.075 mg/kg. No residues of pyrimethanil above the LOQ were found in the treated spinach/lettuce (leaves) specimens taken at BBCH 49 (harvest time).

M605F005

No residues of M605F005 above the limit of quantitation (LOQ) were found in any of the treated plant specimens of wheat, carrots/radish, cauliflower/broccoli and lettuce/spinach.

M605F007

No residues of M605F007 above the limit of quantitation (LOQ) were found in any of the treated plant specimens of wheat, carrots/radish and cauliflower/broccoli.

Residues of M605F007 were found in the spinach/lettuce specimens taken at BBCH 41 in the range of <0.01-0.054 mg/kg and in the range of <0.01-0.035 mg/kg for specimens taken at BBCH 49.

M605F025 (phenylguanidine)

No residues of M605F025 above the limit of quantitation (LOQ) were found in any of the treated plant specimens of wheat, carrots/radish, cauliflower/broccoli and lettuce/spinach.

365 days replanting interval (Plot 6)

In plot 6 (365 days replanting interval) no residues of pyrimethanil, M605F005, M605F007 or M605F025 (phenylguanidine) above the LOQ were found in any of the treated wheat, carrots/radish, cauliflower/broccoli and spinach/lettuce specimens.

III. CONCLUSION

Pyrimethanil residues of up to 0.26 mg/kg and 0.018 mg/kg were found in wheat whole plant without roots and straw, respectively, at the 30 day replanting interval (PBI). No residues above the LOQ were found in wheat grain or in any of the wheat specimens at 120 and 365 day PBI. Residues of up to 0.26 mg/kg and 0.097 mg/kg were found in carrots/radish whole plant with roots and tops, respectively, at the 30 day PBI. At 120 day PBI, residues were lower with up to 0.016 mg/kg in whole plant with roots and <LOQ in the other carrots/radish specimens. At 365 day PBI, residues were <LOQ. In cauliflower/broccoli (whole plant without roots) specimens residues up to 0.079 mg/kg were found at 30 day PBI. No residues above the LOQ were found in inflorescences or in any of the cauliflower/broccoli specimens at 120 and 365 day PBI. In 30 day PBI lettuce/spinach leaves taken at BBCH 41 residues of up to 0.42 mg/kg were found. At BBCH 49 residues were up to 0.044 mg/kg. At 120 day PBI, residues were lower with up to 0.075 mg/kg at BBCH 41 and <LOQ at BBCH 49. At 365 day PBI, residues were <LOQ.
No residues of M605F005 above the limit of quantitation (LOQ) were found in any of the treated plant specimens of wheat, carrots/radish, cauliflower/broccoli and lettuce/spinach at the 30 day or 120 day or 365 day replant interval.

No residues above the LOQ (0.01 mg/kg) of M605F007 were found in wheat whole plant without roots and grain. In straw residues of up to 0.052 mg/kg were found at 30 days PBI. In carrots/radish whole plant with roots residues were up to 0.011 mg/kg at 30 days PBI. No residues above the LOQ were found in any other carrot/radish specimen. In cauliflower/broccoli whole plant without roots residues of up to 0.028 mg/kg were found at 30 days PBI. No residues above the LOQ were found in any other cauliflower/broccoli specimen. In spinach/lettuce leaves taken at BBCH 41 residues of up to 0.11 mg/kg were found and at BBCH 49 residues were up to 0.042 mg/kg. At 120 day PBI, residues were lower with up to 0.054 mg/kg at BBCH 41 and 0.035 mg/kg at BBCH 49. At 365 day PBI, residues were <LOQ.

M605F025 residues of up to 0.058 mg/kg, 0.041 mg/kg and 0.055 mg/kg were found in wheat whole plant without roots at BBCH 31-32 and at BBCH 65 and in straw, respectively, at the 30 day PBI. Furthermore, residues of M605F025 were found in carrots/radish tops up to 0.018 mg/kg. In all other plant specimens of wheat, carrots/radish, cauliflower/broccoli and lettuce/spinach no residues of M605F025 were analyzed above the limit of quantitation (LOQ).

In the following table, values are reported distinguishing between LOD (0.001 mg/kg and 0.002 mg/kg for pyrimethanil and 0.002 mg/kg for metabolites), LOQ (0.01 mg/kg), values between LOD and LOQ (given in parentheses) and values above LOQ.

Table 6.6.2-4: Residues in succeeding crops at 30, 120 and ~~120~~ 365 DAA replanting interval

Study details	Formulation, Appl. rate (kg a.s./ha)	Crop	Country	GS[1]	Residues found (mg/kg)				
					Matrix	BAS 605 F	M605F005	M605F007	M605F025
PLOT 2: 30 ± 1 DAA replanting interval									
Study code: 413468 **Doc ID:** ~~2015/1112007~~ 2016/1026893 2016/1067813 **GLP:** Yes **Year:** 2014/15	BAS 605 04 F 1 x 3.0 to bare soil	Wheat	Germany	31-32	Whole plant without roots	0.26	<0.01 (0.002)	<0.01 (0.004)	0.058
			Italy	31-32	Whole plant without roots	0.022	<0.01 (0.004)	<0.01 (0.004)	0.031
			Spain	31-32	Whole plant without roots	<0.001	~~<0.001~~ <0.002	<0.01 (0.006)	~~0.001~~<0.002
			Netherlands	31-32	Whole plant without roots	<0.002	<0.002	<0.010 (0.004)	<0.002
			Germany	65	Whole plant without roots	0.037	~~0.001~~<0.002	~~0.001~~ <0.002	0.041
			Italy	65	Whole plant without roots	<0.01 (0.004)	~~0.001~~<0.002	<0.01 (0.003)	<0.01 (0.007)
			Spain	65	Whole plant without roots	<0.001	~~0.001~~<0.002	<0.01 (0.008)	~~0.001~~<0.002
			Netherlands	65	Whole plant without roots	<0.002	<0.002	<0.002	<0.002
			Germany	89	Grain	<0.01 (0.007)	~~0.001~~<0.002	~~0.001~~ <0.002	<0.01 (0.002)
			Italy	89	Grain	<0.01 (0.001)	~~0.001~~<0.002	~~0.001~~ <0.002	~~0.001~~<0.002
			Spain	89	Grain	<0.001	~~0.001~~<0.002	~~0.001~~ <0.002	~~0.001~~<0.002
			Netherlands	89	Grain	<0.002	<0.002	<0.002	<0.002
			Germany	89	Straw	0.018	<0.01 (0.004)	<0.01 (0.005)	0.055
			Italy	89	Straw	<0.01 (0.006)	<0.01 (0.005)	0.024	0.028
			Spain	89	Straw	<0.001	~~0.001~~<0.002	0.052	<0.01 (0.003)
			Netherlands	89	Straw	<0.002	<0.002	<0.002	<0.002
		Carrots	Germany	41	Whole plant with roots	0.26	~~0.001~~<0.002	<0.01 (0.003)	<0.01 (0.008)
			Netherlands	41	Whole plant with roots	0.13	~~0.001~~<0.002	0.011	<0.01 (0.005)
			Italy	41	Whole plant with roots	0.087	~~0.001~~<0.002	0.011	<0.01 (0.003)
			Germany	49	Roots	0.025	~~0.001~~<0.002	~~0.001~~ <0.002	~~0.001~~<0.002
			Netherlands	49	Roots	0.022	~~0.001~~<0.002	<0.01 (0.002)	~~0.001~~<0.002
			Italy	49	Roots	0.015	~~0.001~~<0.002	~~0.001~~ <0.002	~~0.001~~<0.002
			Germany	49	Tops	0.088	~~0.001~~<0.002	~~0.001~~ <0.002	<0.01 (0.01)
			Netherlands	49	Tops	0.097	~~0.001~~<0.002	<0.01 (0.005)	<0.01 (0.004)
			Italy	49	Tops	0.065	~~0.001~~<0.002	<0.01 (0.004)	~~0.001~~<0.002
		Radish (small)	Spain	41	Whole plant with roots	<0.001	~~0.001~~<0.002	~~0.001~~ <0.002	~~0.001~~<0.002
			Spain	49	Tops (leaves + stem)	<0.01 (0.009)	~~0.001~~<0.002	<0.01 (0.003)	0.018
			Spain	49	Roots	<0.001	~~0.001~~<0.002	<0.01 (0.006)	~~0.001~~<0.002

Table 6.6.2-4: Residues in succeeding crops at 30, 120 and ~~120~~ 365 DAA replanting interval

Study details	Formulation, Appl. rate (kg a.s./ha)	Crop	Country	GS [1]	Residues found (mg/kg)				
					Matrix	BAS 605 F	M605F005	M605F007	M605F025
		Cauli-flower	Germany	41	Whole plant without roots	0.079	~~0.001~~<0.002	<0.01 (0.004)	<0.01 (0.007)
			Italy			<0.001	~~0.001~~<0.002	<0.01 (0.004)	~~0.001~~<0.002
			Spain			<0.001	~~0.001~~<0.002	<0.01 (0.003)	~~0.001~~<0.002
			Germany	49	Inflorescence	<0.01 (0.003)	~~0.001~~<0.002	~~0.001~~ <0.002	~~0.001~~<0.002
			Italy			<0.001	~~0.001~~<0.002	~~0.001~~ <0.002	~~0.001~~<0.002
			Spain			<0.001	~~0.001~~<0.002	~~0.001~~ <0.002	~~0.001~~<0.002
		Broccoli	Netherlands	41	Whole plant without roots	0.027	~~0.001~~<0.002	0.028	<0.01 (0.005)
			Netherlands	49	Inflorescence	<0.01 (0.001)	~~0.001~~<0.002	<0.01 (0.003)	~~0.001~~<0.002
		Spinach	Netherlands	41	Leaves	<0.01 (0.007)	~~0.001~~<0.002	<0.01 (0.006)	~~0.001~~<0.002
			Italy			0.039	~~0.001~~<0.002[2]	0.11[2]	~~0.001~~<0.002[2)]
			Spain			<0.001	~~0.001~~<0.002	<0.01 (0.003)	~~0.001~~<0.002
			Netherlands	49	Leaves	<0.01 (0.003)	~~0.001~~<0.002	0.011	~~0.001~~<0.002
			Italy			<0.01 (0.007)	~~0.001~~<0.002[3]	0.042[3]	~~0.001~~<0.002[3)]
			Spain			<0.001	~~0.001~~<0.002	~~0.001~~ <0.002	~~0.001~~<0.002
		Lettuce (seeded)	Germany	41	Leaves	0.42	~~0.001~~<0.002	<0.01 (0.003)	~~0.001~~<0.002
			Germany	49	Leaves	0.044	~~0.001~~<0.002	~~0.001~~ <0.002	~~0.001~~<0.002

Table 6.6.2-4: Residues in succeeding crops at 30, 120 and ~~120~~ 365 DAA replanting interval

Study details	Formulation, Appl. rate (kg a.s./ha)	Crop	Country	GS [1]	Residues found (mg/kg)				
					Matrix	BAS 605 F	M605F005	M605F007	M605F025
PLOT 4: 120 ± 3 DAA replanting interval									
Study code: 413468 **Doc ID:** ~~2015/1112007~~ 2016/1026893 2016/1067813 **GLP:** Yes **Year:** 2015	BAS 605 04 F 1 x 3.0 to bare soil	Wheat	Germany	30-32	Whole plant without roots	<0.001	~~0.001~~<0.002	~~0.001~~ <0.002	~~0.001~~<0.002
			Italy			<0.001	~~0.001~~<0.002	~~0.001~~ <0.002	~~0.001~~<0.002
			Spain			<0.001	~~0.001~~<0.002	~~0.001~~ <0.002	~~0.001~~<0.002
			Netherlands			<0.002	<0.002	<0.002	<0.002
			Germany	65		<0.001	<0.002	<0.002	<0.002
			Italy			<0.001	~~0.001~~<0.002	~~0.001~~ <0.002	~~0.001~~<0.002
			Spain			<0.001	~~0.001~~<0.002	~~0.001~~ <0.002	~~0.001~~<0.002
			Netherlands			<0.002	<0.002	<0.002	<0.002
			Germany	89	Grain	<0.001	<0.002	<0.002	<0.002
			Italy			<0.001	<0.002	<0.002	<0.002
			Spain			<0.001	~~0.001~~<0.002	~~0.001~~ <0.002	~~0.001~~<0.002
			Netherlands			<0.002	<0.002	<0.002	<0.002
			Germany	89	Straw	<0.001	<0.002	<0.002	<0.002
			Italy			<0.001	<0.002	<0.002	<0.002
			Spain			<0.001	~~0.001~~<0.002	~~0.001~~ <0.002	~~0.001~~<0.002
			Netherlands			<0.002	<0.002	<0.002	<0.002
		Carrots	Germany	41	Whole plant with roots	0.016	~~0.001~~<0.002	~~0.001~~ <0.002	~~0.001~~<0.002
			Netherlands			<0.01 (0.002)	~~0.001~~<0.002	<0.01 (0.004)	~~0.001~~<0.002
			Italy			<0.001	~~0.001~~<0.002	~~0.001~~ <0.002	~~0.001~~<0.002
			Germany	49	Roots	<0.01 (0.002)	~~0.001~~<0.002	~~0.001~~ <0.002	~~0.001~~<0.002
			Netherlands			<0.001	~~0.001~~<0.002	~~0.001~~ <0.002	~~0.001~~<0.002
			Italy			<0.001	~~0.001~~<0.002	~~0.001~~ <0.002	~~0.001~~<0.002
			Germany	49	Tops	<0.01 (0.007)	~~0.001~~<0.002	~~0.001~~ <0.002	~~0.001~~<0.002
			Netherlands			<0.01 (0.002)	~~0.001~~<0.002	~~0.001~~ <0.002	~~0.001~~<0.002
			Italy			<0.01 (0.002)	~~0.001~~<0.002	~~0.001~~ <0.002	~~0.001~~<0.002
		Radish (small)	Spain	41	Whole plant with roots	<0.001	~~0.001~~<0.002	~~0.001~~ <0.002	<0.01 (0.002)
			Spain	49	Tops (leaves + stem)	<0.001	~~0.001~~<0.002	~~0.001~~ <0.002	~~0.001~~<0.002
			Spain	49	Roots	<0.001	~~0.001~~<0.002	~~0.001~~ <0.002	~~0.001~~<0.002

Table 6.6.2-4: Residues in succeeding crops at 30, 120 and ~~120~~ 365 DAA replanting interval

Study details	Formulation, Appl. rate (kg a.s./ha)	Crop	Country	GS [1]	Residues found (mg/kg)				
					Matrix	BAS 605 F	M605F005	M605F007	M605F025
		Cauli-flower	Germany	41	Whole plant without roots	<0.001	~~0.001~~<0.002	<0.01 (0.002)	~~0.001~~<0.002
			Italy			<0.001	~~0.001~~<0.002	~~0.001~~ <0.002	~~0.001~~<0.002
			Spain			<0.001	~~0.001~~<0.002	<0.01 (0.002)	~~0.001~~<0.002
			Germany	49	Inflorescence	<0.001	~~0.001~~<0.002	~~0.001~~ <0.002	~~0.001~~<0.002
			Italy			<0.001	~~0.001~~<0.002	~~0.001~~ <0.002	~~0.001~~<0.002
			Spain			<0.001	~~0.001~~<0.002	~~0.001~~ <0.002	~~0.001~~<0.002
		Broccoli	Netherlands	41	Whole plant without roots	<0.001	~~0.001~~<0.002	~~0.001~~ <0.002	~~0.001~~<0.002
			Netherlands	49	Inflorescence	<0.001	~~0.001~~<0.002	~~0.001~~ <0.002	~~0.001~~<0.002
		Spinach	Italy	41	Leaves	<0.001	~~0.001~~<0.002	~~0.001~~ <0.002	~~0.001~~<0.002
			Spain			<0.001	~~0.001~~<0.002	~~0.001~~ <0.002	~~0.001~~<0.002
			Netherlands			0.041[3]	~~0.001~~<0.002	0.054	~~0.001~~<0.002
			Italy	49	Leaves	<0.001	~~0.001~~<0.002	~~0.001~~ <0.002	~~0.001~~<0.002
			Spain			<0.001	~~0.001~~<0.002	~~0.001~~ <0.002	~~0.001~~<0.002
			Netherlands			<0.01 (0.003)[3]	~~0.001~~<0.002	0.035	~~0.001~~<0.002
		Lettuce (seeded)	Germany	41	Leaves	0.075	~~0.001~~<0.002	<0.01 (0.003)	~~0.001~~<0.002
			Germany	49	Leaves	<0.01 (0.007)	~~0.001~~<0.002	~~0.001~~ <0.002	~~0.001~~<0.002

Table 6.6.2-4: Residues in succeeding crops at 30, 120 and ~~120~~ 365 DAA replanting interval

Study details	Formulation, Appl. rate (kg a.s./ha)	Crop	Country	GS [1]	Residues found (mg/kg)				
					Matrix	BAS 605 F	M605F005	M605F007	M605F025
PLOT 6: 365 ± 5 DAA or 389 (Netherlands) replanting interval									
Study code: 413468 **Doc ID:** 2016/1026893 2016/1067813 **GLP:** Yes **Year:** 2015	BAS 605 04 F 1 x 3.0 to bare soil	Wheat	Germany	31-33	Whole plant without roots	<0.001	<0.002	<0.002	<0.002
			Italy			<0.001	<0.002	<0.002	<0.002
			Spain			<0.001	<0.002	<0.002	<0.002
			Netherlands			<0.002	<0.002	<0.002	<0.002
			Germany	65		<0.001	<0.002	<0.002	<0.002
			Italy			<0.001	<0.002	<0.002	<0.002
			Spain			<0.001	<0.002	<0.002	<0.002
			Netherlands			<0.002	<0.002	<0.002	<0.002
			Germany	89	Grain	<0.001	<0.002	<0.002	<0.002
			Italy			<0.001	<0.002	<0.002	<0.002
			Spain			<0.001	<0.002	<0.002	<0.002
			Netherlands			<0.002	<0.002	<0.002	<0.002
			Germany	89	Straw	<0.001	<0.002	<0.002	<0.002
			Italy			<0.001	<0.002	<0.002	<0.002
			Spain			<0.001	<0.002	<0.002	<0.002
			Netherlands			<0.002	<0.002	<0.002	<0.002
		Carrots	Germany	41	Whole plant with roots	<0.01 (0.002)	<0.002	<0.002	<0.002
			Netherlands			<0.01 (0.003)	<0.002	<0.002	<0.002
			Italy			<0.001	<0.002	<0.002	<0.002
			Germany	49	Roots	<0.001	<0.002	<0.002	<0.002
			Netherlands			<0.001	<0.002	<0.002	<0.002
			Italy			<0.001	<0.002	<0.002	<0.002
			Germany	49	Tops	<0.01 (0.002)	<0.002	<0.002	<0.002
			Netherlands			<0.01 (0.002)	<0.002	<0.002	<0.002
			Italy			<0.001	<0.002	<0.002	<0.002
		Radish (small)	Spain	41	Whole plant with roots	<0.001	<0.002	<0.002	<0.002
			Spain	49	Tops	<0.001	<0.002	<0.002	<0.002
			Spain	49	Roots	<0.001	<0.002	<0.002	<0.002
		Cauli-flower	Germany	41	Whole plant without roots	<0.01 (0.002)	<0.002	<0.002	<0.002
			Italy			<0.001	<0.002	<0.002	<0.002
			Spain			<0.001	<0.002	<0.002	<0.002
			Germany	49	Inflorescence	<0.001	<0.002	<0.002	<0.002
			Italy			<0.001	<0.002	<0.002	<0.002
			Spain			<0.001	<0.002	<0.002	<0.002
		Broccoli	Netherlands	41	Whole plant without roots	<0.01 (0.001)	<0.002	<0.002	<0.002
			Netherlands	49	Inflorescence	<0.001	<0.002	<0.002	<0.002
		Spinach	Italy	41	Leaves	<0.001	<0.002	<0.002	<0.002
			Spain			<0.001	<0.002	<0.002	<0.002
			Netherlands			<0.01 (0.002)	<0.002	<0.002	<0.002
			Italy	49	Leaves	<0.001	<0.002	<0.002	<0.002
			Spain			<0.001	<0.002	<0.002	<0.002
			Netherlands			<0.001	<0.002	<0.002	<0.002

Table 6.6.2-4: Residues in succeeding crops at 30, 120 and ~~120~~ 365 DAA replanting interval

Study details	Formulation, Appl. rate (kg a.s./ha)	Crop	Country	GS [1]	Residues found (mg/kg)				
					Matrix	BAS 605 F	M605F005	M605F007	M605F025
		Lettuce (seeded)	Germany	41	Leaves	<0.01 (0.006)	<0.002	<0.002	<0.002
			Germany	49	Leaves	<0.001	<0.002	<0.002	<0.002

1 BBCH
2 Mean value of five determinations
3 Mean value of three determinations

CA 6.7 Proposed residue definitions and maximum residue levels

CA 6.7.1 Proposed residue definitions

The residue definitions currently established in the EU and supported in future are compiled in Table 6.7.1-1. In the subsequent section, detailed justifications for BASF's proposal are provided. The proposal is based on a careful evaluation of all studies being available at the time point of submission. Consequently it includes considerations for all those crops for which an EU MRL is established. It is not limited to the representative uses in apples, grapes, strawberries and lettuce.

Table 6.7.1-1: Residue definition - pyrimethanil

End-Point	**Active substance: Pyrimethanil**	
	EU agreed endpoints (EFSA Scientific Report (2006) 61, 1-70, Conclusion on the peer review of pyrimethanil; EFSA Journal 2011;9(11):2454)	Residue definitions proposed in the context of this dossier
Residue definition in plant matrices for risk assessment	Parent compound (Pyrimethanil)	Parent compound (Pyrimethanil)
Residue definition in plant matrices for monitoring	Parent compound (Pyrimethanil)	Parent compound (Pyrimethanil)
Residue definition in animal matrices for risk assessment	Milk: sum of pyrimethanil and SN 614 277[1], expressed as pyrimethanil Livestock tissues (except poultry): sum of pyrimethanil and SN 614 276[2], expressed as pyrimethanil	Milk: sum of pyrimethanil and SN 614 277[1], expressed as pyrimethanil Livestock tissues (except poultry): sum of pyrimethanil and SN 614 276[2], expressed as pyrimethanil
Residue definition in animal matrices for monitoring	Milk: sum of pyrimethanil and SN 614 277[1], expressed as pyrimethanil Livestock tissues (except poultry): sum of pyrimethanil and SN 614 276[2], expressed as pyrimethanil	Milk: sum of pyrimethanil and SN 614 277[1], expressed as pyrimethanil Livestock tissues (except poultry): sum of pyrimethanil and SN 614 276[2], expressed as pyrimethanil
Conversion factors between residue definitions (animal)	-	-

1 2-anilino-4,6-dimethylpyrimidine-5-ol, M605F003
2 2-(4-hydroxyanilino)-4,6-dimethylpyrimidine, M605F002

For deriving appropriate residue definitions for monitoring and risk assessment purposes the principles described in the following document were considered:

- OECD GUIDANCE DOCUMENT ON THE DEFINITION OF RESIDUE (as revised in 2009), SERIES ON TESTING AND ASSESSMENT No. 63 and SERIES ON PESTICIDES No. 31 (ENV/JM/MONO(2009)30)
- EFSA Scientific Opinion on Evaluation of the Toxicological Relevance of Pesticide Metabolites for Dietary Risk Assessment, EFSA Journal 2012;10(07): 2799

The first document covers both aspects whereas the purpose of the PPR Scientific Opinion is limited to the residue definition for risk assessment purposes. The corresponding EU guidance document is in preparation and will be available earliest by end of 2015.

Plant Matrices

For proposing a suitable residue definition in plant matrices, multiple investigations were performed. As presented in sections 6.2, 6.3, 6.5 and 6.6 plant studies were performed in which pyrimethanil was applied according to the intended use patterns.

For deriving a suitable **residue definition for food of plant origin**, five peer-reviewed crop metabolism studies in three different crop categories were considered covering the categories of root vegetables, fruits and leafy crops. In addition, a foliar study on lettuce and a seed treatment study on wheat were conducted using ^{14}C-phenyl- and/or ^{14}C-pyrimidinyl-labeled pyrimethanil, covering the categories of leafy vegetables and cereals. The effect of processing on the nature of the residue was investigated in the framework of the original inclusion into Annex I according to Directive 91/414/EEC using test conditions simulating pasteurization, baking, brewing, boiling and sterilization. Information on the residue situation in succeeding or rotational crops is available from each a peer-reviewed and a new confined ([^{14}C]-radiolabeled) and non-radiolabeled crop rotational study conducted with pyrimethanil, six peer-reviewed aerobic soil metabolism (degradation) studies, a peer-reviewed anaerobic soil metabolism (degradation) study and a new soil photolysis study.

In general, metabolism of pyrimethanil in plants comprises

- Hydroxylation of the parent compound
- Conjugation of the parent compound
- Cleavage of amine bond
- Ring opening of the pyrimidine ring

The plant metabolism studies indicate that parent pyrimethanil is the predominant residue in edible plant parts after foliar treatment. For the seed treatment use in wheat it was shown that no residues >0.01 mg/kg in grain occur. Pyrimethanil is a major component in several rotational crops as well. In the new conducted confined rotational crop studies and the field study for pyrimethanil a significant uptake (>0.01 mg/kg) in edible parts of leafy and root vegetables was shown after a short re-plant interval of approximately one month. In the field rot crop study, in addition to parent pyrimethanil, metabolites M605F005, M605F007 and M605F025 were analyzed as marker substances; for assessment of their relevance see below and chapter MCA 6.9 (document 2015/1188589 gives even more detailed information).
Pyrimethanil was found to be stable to hydrolysis at simulated processing conditions for pasteurization, baking/brewing/boiling and sterilization. Thus, for processed commodities the same residue definition as for raw agricultural commodities (RAC) is applicable.

Residue definition for monitoring purposes

According to the OECD Guidance Document, the residue definition for tolerance/MRL enforcement purposes should focus on those analytes which would indicate a possible misuse of the pesticide and which can be easily detected/measured by a broad base of national laboratories (use of a multi-residue method). The analyte(s) to be selected for monitoring purposes should occur in large quantities, and should be common to all commodities in which residues are expected. Ideally, a monitoring method should be based on one single analyte ('marker or indicator compound').

In case of pyrimethanil and its metabolites in food of plant origin, there is only one component which meets all criteria listed in the OECD guidance document. Based on the plant studies available, in which parent BAS 605 F was the most prominent residue, the following residue definition is proposed for monitoring purposes in plant commodities (including process fractions thereof):

For monitoring / enforcement: Pyrimethanil

This is compliant with the current EU residue definition and is in line with the one in force for JMPR (CODEX).

Residue definition for data generation / risk assessment purposes

The derivation of a suitable residue definition for risk assessment purposes is much more complex; according to the OECD guidance document the contribution of each metabolite/degradate to a potential dietary risk needs to be considered.

In general, two factors must be addressed:

- **Potential for exposure** to the metabolite/degradate in the human diet
- **Relative toxicity** of the metabolite/degradate to the parent

Metabolites/degradates with higher potential exposures and toxicities are more likely to be included in the dietary assessment. The OECD guidance document provides a first hint on how an indicative risk assessment can be performed if metabolites are not readily available as reference substances. For such cases, the document recommends to calculate parent/metabolite ratios from the metabolism studies and to apply these ratios in a second step to the residue level being measured during e.g. supervised field trials. The approach is described much more in detail in the EFSA Scientific Opinion 2799 (2012) which also includes the concept of the threshold of toxicological concern (TTC) as screening tool for pesticide metabolites. Main "purpose" of the TTC concept is to check whether there is negligible exposure.

In order to propose a suitable residue definition for risk assessment purposes, the pyrimethanil metabolites found in plant metabolism studies and during processing were grouped in total into 3 different groups.

- Group 1 - Hydroxylation/oxidation products and their conjugates:
  Hydroxylation/oxidation products of parent: M605F002, M605F003, M605F004, M605F005, M605F006 and their conjugates (Glucose and Malonylglucose conjugates): M605F001, M605F027, M605F028, M605F029, M605F030, M605F036, M605F037, M605F038, M605F039, M605F040, M605F041
- Group 2 - Pyrimidin-moiety:
  M605F007, M605F008, M605F016, M605F032 and its conjugates M605F033
- Group 3 - Phenyl-moiety:
  M605F025

The dietary exposure for each group was assessed separately for identifying the contributions of the plant metabolites to the total dietary risk. The assessments were limited to those target crops from which a contribution to the dietary risk could be expected.

Table 6.7.1-2: Summary table of all metabolites and the contribution to chronic dietary risk

Metabolite group	Metabolites	ADI [mg/kg bw/day]	Contribution	ADI utilization [%]
1	M605F001	0.17	Plant	0.2
	M605F002		Plant Rot crop	0.1 0.1
	M605F003		Plant Rot crop	0.2 0.1
	M605F004		Plant Rot crop	0.2 0.1
	M605F005		Rot crop	0.2
	M605F006		Rot crop	0.04
	M605F027		Plant Rot crop Overall	0.8 0.03 0.8
	M605F028		Plant Rot crop Overall	0.4 0.02 0.4
	M605F029		Plant	0.5
	M605F030		Plant	0.3
	M605F036		Plant Rot crop Overall	0.5 0.01 0.5
	M605F037		Plant	0.01
	M605F038		Plant	0.5
	M605F039		Plant Rot crop Overall	0.4 0.01 0.5
	M605F040		Plant Rot crop Overall	0.5 0.04 0.5
	M605F041		Plant	0.3
2	M605F007	0.0015 TTC Cramer class III	Rot crop	6.1 (metabolism data) 9.4 (field data)
	M605F008		Rot crop	3.3
	M605F016		Rot crop	3.6
	M605F032		Rot crop	2.1
	M605F033		Plant Rot crop Overall	56.3 9.0 59.7
3	M605F025	0.089	Plant Rot crop Overall	0.1 3.3 (metabolism data) 0.03 (field data) 3.3

Group 1
The parent ADI of 0.17 mg/kg bw/d was used (for explanation see chapter MCA 6.9 and 2015/1188589). With the current EFSA model the maximum chronic risk assessment per metabolite ranges from 0.01 to 0.8% of the ADI. The diet with the highest TMDI is "DE child" with 0.8% of the ADI due to intake of M605F027. For this diet, the highest contributor is pome fruit with 0.3% of the ADI.

Group 2
The TTC Cramer class III reference value of 0.0015 mg/kg bw/d was used (for explanation see chapter MCA 6.9 and 2015/1188589). With the current EFSA model the maximum chronic risk assessment per metabolite ranges from 2.1 to 59.7% of the ADI. The diet with the highest TMDI is "ES adult" with 59.7% of the ADI due to intake of M605F033. For this diet, the highest contributor is lettuce with 57.3% of the ADI.
Looking at the contribution to the dietary burden of M605F033, it was shown that residues in rotational crops have a comparably low influence; the majority of the ADI utilization arises from residues after foliar application. Either way, the ADI utilization was far below 100%.
Comparing the risk assessment of M605F007 using rotational crop data from the metabolism study on the one hand and ~~interim~~ field rotational crop data on the other, it was shown that there is no chronic risk in either scenario; the field data are still safe. As metabolite M605F007 was analyzed as marker compound for group 2, it can be reasonably assumed that this statement is accurate for the whole group.

Group 3
A metabolite-specific ADI of 0.089 mg/kg bw/d was used (for explanation see chapter MCA 6.9 and 2015/1188589). With the current EFSA model the chronic risk assessment ranges from 0.2 to 3.3% of the ADI considering both plant and rotational crop data. The diet with the highest TMDI is "UK Toddler" with 3.3% of the ADI. For this diet, the highest contributor is sugar beet (root) with 2.7% of the ADI.

The data show that the contributions of the metabolites to the dietary risk are quite small - the majority showing ADI utilizations <10% - even under unrealistic worst case assumptions. None of the groups should be included in the residue definition for dietary risk assessment. Due to the favorable outcome, no further refinement than indicated above was performed (e.g. by inclusion of processing factors for vegetable crops which are always cooked prior to consumption).

Based on the findings summarized above the following residue definition for pyrimethanil in plant matrices is proposed:

For dietary risk assessment: Pyrimethanil

This is compliant with the current EU residue definition and is in line with the one in force for JMPR (CODEX).

Animal matrices

For proposing a suitable residue definition in animal matrices, multiple investigations were performed considering livestock metabolism and feeding studies (see chapters 6.2 and 6.4).

For deriving a suitable **residue definition for food of animal origin**, a peer-reviewed cow metabolism and cow feeding studies were considered. In addition, a metabolism study on goat and one on hen were conducted using ^{14}C-phenyl- and/or ^{14}C-pyrimidinyl-labeled pyrimethanil.

In general, metabolism of pyrimethanil in livestock comprises
- Hydroxylation of the parent compound
- Formation of glucuronide and sulfate conjugates of metabolites via hydroxylation of the parent compound
- Cleavage of the pyrimidine ring

In goat, oxidation of the hydroxylated parent to produce a carboxylic acid metabolite was observed in addition.

The main biotransformation reactions (hydroxylation and conjugation) were observed in rats, goats and laying hens, thus there is a consistent picture of the metabolism of ^{14}C-pyrimethanil in all animal species investigated.
In the peer-reviewed metabolism study on cows, no parent pyrimethanil could be identified. The main metabolites were M605F002 in tissues and M605F003 in milk. In the course of the new metabolism studies (goat and hen), parent pyrimethanil could not be identified in any matrix. M605F023, the glucuronide conjugate of metabolite M605F002, is the predominant component in ruminant liver, kidney and milk, in combination with M605F034 (pyrimethanil carboxylic acid) for kidney and M605F021, the sulfate conjugate of M605F003, for milk. In addition, hydroxylation and conjugation products M605F002 and M605F004 (monohydroxy), M605F006 (dihydroxy), M605F014 (glucuronide conjugate of M605F004), M605F020 (glucuronide conjugate of M605F003), M605F035 (sulfate conjugate of M605F002) and group 3 metabolite M605F025 (phenylguanidine) were identified in minor amounts in goat matrices.
In hen, the main metabolites were M605F002 and its glucuronide (M605F023) and sulfate conjugates (M605F035). In addition, M605F006 (dihydroxy) was identified in minor amounts. All studies, including excretion studies in rat, indicate that pyrimethanil does not accumulate in animal matrices.
M605F003 is part of the current residue definition for milk; since the peer-reviewed method used in the cow feeding study (B003870) included an enzymatic cleavage step, it was analyzed with its conjugates M605F020 (glucuronide) and M605F021 (sulfate) as free M605F003. Likewise, M605F002 is part of the residue definition for tissues; it was analyzed with its conjugates M605F023 (glucuronide) and M605F035 (sulfate) as free M605F002.

A suitable enforcement method (L0210/01) is available and summarized in chapter 4.2.

Residue definition for monitoring purposes

In case of pyrimethanil and its metabolites in food of animal origin, the situation is more complex than in plant matrices. In the livestock studies available, in tissues hydroxy metabolite M605F002 as well as its conjugates M605F023 and M605F035 and in milk hydroxy metabolite M605F003 as well as its conjugates M605F020 and M605F021 were the most prominent residue. Since the methods used in the cow feeding study included an enzymatic cleavage step, M605F002 and M605F003 were analyzed with their conjugates as free M605F002 or M605F003, respectively. Thus, the following residue definition is proposed for monitoring purposes in animal commodities:

For monitoring / enforcement:

Milk:	**Sum of pyrimethanil and M605F003, expressed as pyrimethanil**
Tissues (except poultry):	**Sum of pyrimethanil and M605F002, expressed as pyrimethanil**

Due to low residues, no enforcement residue definition is proposed for poultry matrices. This is compliant with the current EU residue definitions and the current JMPR (CODEX) residue definitions, where also none was set for poultry. However, if considered necessary, the residue definition for ruminant and pig tissues could also be applied to poultry matrices.

Residue definition for data generation / risk assessment purposes

In order to propose a suitable residue definition for risk assessment purposes, the pyrimethanil metabolites found in animal metabolism studies were grouped in total into 2 different groups (group 2 metabolites were not identified in edible livestock matrices).

- Group 1- Hydroxylation/oxidation products and their conjugates:
  Hydroxylation/oxidation products of parent: M605F002, M605F003, M605F004, M605F006, M605F034 and their conjugates (Glucuronides and Sulfates): M605F014, M605F020, M605F021, M605F023, M605F035
- Group 3 - Phenyl-moiety:
  M605F025

The dietary exposure for each group was assessed separately for identifying the contributions of the animal metabolites to the total dietary risk (see Table 6.7.1-2**:**). To derive input values, two routes were considered: livestock metabolism studies were carefully evaluated for the presence of relevant metabolites (route 1) and a feed burden was calculated for M605F025 to estimate residues due to direct consumption of M605F025 residues in feed (route 2).

Table 6.7.1-3: Summary table of all metabolites and the contribution to chronic dietary risk

Metabolite group	Metabolites	ADI [mg/kg bw/day]	Contribution	ADI utilization [%]
1	M605F002	0.17	Animal	0.01
	M605F003		Animal	0.3
	M605F004		Animal	0.001
	M605F006		Animal	0.003
	M605F014		Animal	0.002
	M605F020		Animal	0.001
	M605F021		Animal	0.2
	M605F023		Animal	0.2
	M605F034		Animal	0.01
	M605F035		Animal	0.05
3	M605F025	0.089	Animal	0.03

Group 1
The parent ADI of 0.17 mg/kg bw/d was used (for explanation see chapter MCA 6.9 and 2015/1188589). With the current EFSA model the maximum chronic risk assessment per metabolite ranges from 0.001 to 0.3% of the ADI considering route 1. The diet with the highest TMDI is "FR toddler" with 0.3% of the ADI due to intake of M605F003. For this diet, the highest contributors are milk and cream with 0.3% of the ADI.

Group 3
A metabolite-specific ADI of 0.089 mg/kg bw/d was used (for explanation see chapter MCA 6.9 and 2015/1188589). With the current EFSA model the chronic risk assessment ranges from 0.002 to 0.03% of the ADI considering both routes. The diet with the highest TMDI is "UK Infant" with 0.03% of the ADI. For this diet, the highest contributors are milk and cream with 0.03% of the ADI.

The data show that the contributions of the metabolites to the dietary risk are very small - showing ADI utilizations <1% - even under unrealistic worst case assumptions. No additional metabolite should be included in the residue definition for dietary risk assessment; M605F002 and M605F003 (as well as their glucuronide and sulfate conjugates due to the enzymatic cleavage step included in the methods used in the cow feeding study) are already part of the current residue definitions.

Based on the findings summarized above the following residue definitions for pyrimethanil in animal matrices are proposed:

For dietary risk assessment:

Milk: **Sum of pyrimethanil and M605F003, expressed as pyrimethanil**

Tissues (except poultry): **Sum of pyrimethanil and M605F002, expressed as pyrimethanil**

This is compliant with the current EU residue definitions and is in line with the one in force for JMPR (CODEX). Due to low residues, no residue definition is proposed for poultry matrices. However, if considered necessary, the residue definition for ruminant and pig tissues could also be applied to poultry matrices.

CA 6.7.2 Proposed maximum residue levels (MRLs) and justification of the acceptability of the levels proposed

The following table shows the current EU MRLs for pyrimethanil (mg/kg) as of June 04, 2015 (Commission Regulation (EU) 2015/845) (source: European Commission website http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=pesticide.residue.CurrentMRL&language=EN&pestResidueId=199).

Table 6.7.2-1: EU MRLs set for the uses of pyrimethanil

Code number	Groups and examples of individual products to which the MRLs apply	Pyrimethanil (R)
100000	. FRUITS, FRESH or FROZEN; TREE NUTS	
110000	. Citrus fruits	8
120000	. Tree nuts	
120010	. Almonds	0.2
120020	. Brazil nuts	0.02*
120030	. Cashew nuts	0.02*
120040	. Chestnuts	0.02*
120050	. Coconuts	0.02*
120060	. Hazelnuts/cobnuts	0.02*
120070	. Macadamias	0.02*
120080	. Pecans	0.02*
120090	. Pine nut kernels	0.02*
120100	. Pistachios	0.2
120110	. Walnuts	0.02*
120990	. Others (2)	0.02*
130000	. Pome fruits	15
140000	. Stone fruits	
140010	. Apricots	10
140020	. Cherries (sweet)	4
140030	. Peaches	10
140040	. Plums	2
140990	. Others (2)	0.01*
150000	. Berries and small fruits	
151000	. (a) Grapes	5
152000	. (b) Strawberries	5
153000	. (c) Cane fruits	
153010	. Blackberries	10
153020	. Dewberries	0.01*
153030	. Raspberries (red and yellow)	10
153990	. Others (2)	0.01*
154000	. (d) other small fruits and berries	
154010	. Blueberries	5
154020	. Cranberries	5
154030	. Currants (black, red and white)	5
154040	. Gooseberries (green, red and yellow)	5
154050	. Rose hips	5
154060	. Mulberries (black and white)	5
154070	. Azaroles/Mediterranean medlars	15
154080	. Elderberries	5

Table 6.7.2-1: EU MRLs set for the uses of pyrimethanil

154990	. Others (2)	5
160000	. Miscellaneous fruits with	
161000	. (a) edible peel	
161010	. Dates	0.01*
161020	. Figs	0.01*
161030	. Table olives	0.02*
161040	. Kumquats	0.01*
161050	. Carambolas	0.01*
161060	. Kaki/Japanese persimmons	15
161070	. Jambuls/jambolans	0.01*
161990	. Others (2)	0.01*
162000	. (b) inedible peel, small	0.01*
163000	. (c) inedible peel, large	
163010	. Avocados	0.01*
163020	. Bananas	0.1
163030	. Mangoes	0.01*
163040	. Papayas	0.01*
163050	. Granate apples/pomegranates	0.01*
163060	. Cherimoyas	0.01*
163070	. Guavas	0.01*
163080	. Pineapples	0.01*
163090	. Breadfruits	0.01*
163100	. Durians	0.01*
163110	. Soursops/guanabanas	0.01*
163990	. Others (2)	0.01*
200000	. VEGETABLES, FRESH or FROZEN	
210000	. Root and tuber vegetables	
211000	. (a) potatoes	0.05*
212000	. (b) tropical root and tuber vegetables	0.01*
213000	. (c) other root and tuber vegetables except sugar beets	
213010	. Beetroots	0.01*
213020	. Carrots	1
213030	. Celeriacs/turnip rooted celeries	0.01*
213040	. Horseradishes	0.01*
213050	. Jerusalem artichokes	0.01*
213060	. Parsnips	0.01*
213070	. Parsley roots/Hamburg roots parsley	0.01*
213080	. Radishes	0.01*
213090	. Salsifies	0.01*
213100	. Swedes/rutabagas	0.01*
213110	. Turnips	0.01*
213990	. Others (2)	0.01*
220000	. Bulb vegetables	
220010	. Garlic	0.01*
220020	. Onions	0.2
220030	. Shallots	0.01*
220040	. Spring onions/green onions and Welsh onions	3
220990	. Others (2)	0.01*

Table 6.7.2-1: EU MRLs set for the uses of pyrimethanil

230000	. Fruiting vegetables	
231000	. (a) solanacea	
231010	. Tomatoes	1
231020	. Sweet peppers/bell peppers	2
231030	. Aubergines/eggplants	1
231040	. Okra/lady's fingers	0.01*
231990	. Others (2)	0.01*
232000	. (b) cucurbits with edible peel	0.7
233000	. (c) cucurbits with inedible peel	0.01*
234000	. (d) sweet corn	0.01*
239000	. (e) other fruiting vegetables	0.01*
240000	. Brassica vegetables (excluding brassica roots and brassica baby leaf crops)	0.01*
250000	. Leaf vegetables, herbs and edible flowers	
251000	. (a) lettuces and salad plants	
251010	. Lamb's lettuces/corn salads	0.01*
251020	. Lettuces	20
251030	. Escaroles/broad-leaved endives	20
251040	. Cresses and other sprouts and shoots	0.01*
251050	. Land cresses	0.01*
251060	. Roman rocket/rucola	0.01*
251070	. Red mustards	0.01*
251080	. Baby leaf crops (including brassica species)	20
251990	. Others (2)	0.01*
252000	. (b) spinaches and similar leaves	0.01*
253000	. (c) grape leaves and similar species	0.01*
254000	. (d) watercresses	0.01*
255000	. (e) witloofs/Belgian endives	0.01*
256000	. (f) herbs and edible flowers	20
260000	. Legume vegetables	
260010	. Beans (with pods)	3
260020	. Beans (without pods)	0.01*
260030	. Peas (with pods)	3
260040	. Peas (without pods)	0.2
260050	. Lentils	0.01*
260990	. Others (2)	0.01*
270000	. Stem vegetables	
270010	. Asparagus	0.01*
270020	. Cardoons	0.01*
270030	. Celeries	0.01*
270040	. Florence fennels	0.01*
270050	. Globe artichokes	0.01*
270060	. Leeks	1
270070	. Rhubarbs	0.01*
270080	. Bamboo shoots	0.01*
270090	. Palm hearts	0.01*
270990	. Others (2)	0.01*
280000	. Fungi, mosses and lichens	0.01*
290000	. Algae and prokaryotes organisms	0.01*

Table 6.7.2-1: EU MRLs set for the uses of pyrimethanil

300000	. PULSES	
300010	. Beans	0.5
300020	. Lentils	0.5
300030	. Peas	0.5
300040	. Lupins/lupini beans	0.5
300990	. Others (2)	0.01*
400000	. OILSEEDS AND OIL FRUITS	0.02*
500000	. CEREALS	
500010	. Barley	0.05*[1]
500020	. Buckwheat and other pseudo-cereals	0.01*
500030	. Maize/corn	0.01*
500040	. Common millet/proso millet	0.01*
500050	. Oat	0.05*[1]
500060	. Rice	0.05*
500070	. Rye	0.05*[1]
500080	. Sorghum	0.05*
500090	. Wheat	0.05*[1]
500990	. Others (2)	0.01*
600000	. TEAS, COFFEE, HERBAL INFUSIONS, COCOA AND CAROBS	
610000	. Teas	0.05*
620000	. Coffee beans	0.05*
630000	. Herbal infusions from	
631000	. (a) flowers	0.05*
632000	. (b) leaves and herbs	0.05*
633000	. (c) roots	
633010	. Valerian	0.05*
633020	. Ginseng	1.5
633990	. Others (2)	0.05*
639000	. (d) any other parts of the plant	0.05*
640000	. Cocoa beans	0.05*
650000	. Carobs/Saint John's breads	0.05*
700000	. HOPS	0.05*
800000	. SPICES	
810000	. Seed spices	0.05*
820000	. Fruit spices	0.05*
830000	. Bark spices	0.05*
840000	. Root and rhizome spices	
840010	. Liquorice	0.05*
840020	. Ginger	0.05*
840030	. Turmeric/curcuma	0.05*
840040	. Horseradish	[2]
840990	. Others (2)	0.05*
850000	. Bud spices	0.05*
860000	. Flower pistil spices	0.05*
870000	. Aril spices	0.05*
900000	. SUGAR PLANTS	0.01*

Table 6.7.2-1: EU MRLs set for the uses of pyrimethanil

1000000	. PRODUCTS OF ANIMAL ORIGIN -TERRESTRIAL ANIMALS	[3]
1010000	. Tissues from	[3]
1011000	. (a) swine	0.1*[3]
1012000	. (b) bovine	[3]
1012010	. Muscle	0.1*[3]
1012020	. Fat tissue	0.1*[3]
1012030	. Liver	0.1*[3]
1012040	. Kidney	0.2[3]
1012050	. Edible offals (other than liver and kidney)	0.1*[3]
1012990	. Others (2)	0.1*[3]
1013000	. (c) sheep	[3]
1013010	. Muscle	0.1*[3]
1013020	. Fat tissue	0.1*[3]
1013030	. Liver	0.1*[3]
1013040	. Kidney	0.2[3]
1013050	. Edible offals (other than liver and kidney)	0.1*[3]
1013990	. Others (2)	0.1*[3]
1014000	. d) goat	[3]
1014010	. Muscle	0.1*[3]
1014020	. Fat tissue	0.1*[3]
1014030	. Liver	0.1*[3]
1014040	. Kidney	0.2[3]
1014050	. Edible offals (other than liver and kidney)	0.1*[3]
1014990	. Others (2)	0.1*[3]
1015000	. (e) equine	[3]
1015010	. Muscle	0.1*[3]
1015020	. Fat tissue	0.1*[3]
1015030	. Liver	0.1*[3]
1015040	. Kidney	0.2[3]
1015050	. Edible offals (other than liver and kidney)	0.1*[3]
1015990	. Others (2)	0.1*[3]
1016000	. (f) poultry	0.05*[3]
1017000	. (g) other farmed terrestrial animals	[3]
1017010	. Muscle	0.1*[3]
1017020	. Fat tissue	0.1*[3]
1017030	. Liver	0.1*[3]
1017040	. Kidney	0.2[3]
1017050	. Edible offals (other than liver and kidney)	0.1*[3]
1017990	. Others (2)	0.1*[3]
1020000	. Milk	0.05[3]
1030000	. Birds eggs	0.01*[3]

Table 6.7.2-1: EU MRLs set for the uses of pyrimethanil

1040000	. Honey and other apiculture products	0.05*[3]
1050000	. Amphibians and Reptiles	0.05*[3]
1060000	. Terrestrial invertebrate animals	0.05*[3]
1070000	. Wild terrestrial vertebrate animals	0.05*[3]

(R) The residue definition differs for the following combinations pesticide-code number:
Pyrimethanil - code 1020000: Sum of pyrimethanil and 2-anilino-4.6-dimethylpyrimidine-5-ol, expressed as pyrimethanil
Pyrimethanil - codes 1011000/1012000/1013000/1014000/1015000/1017000: Sum of pyrimethanil and 2-(4-hydroxyanilino)-4.6-dimethylpyrimidine, expressed as pyrimethanil

* Indicates lower limit of analytical determination

1 The European Food Safety Authority identified some information on crop metabolism with seed treatment as unavailable. When re-viewing the MRL, the Commission will take into account the information referred to in the first sentence, if it is submitted by 5 February 2016, or, if that information is not submitted by that date, the lack of it.

2 The applicable maximum residue level for horseradish (*Armoracia rusticana*) in the spice group (code 0840040) is the one set for horseradish (*Armoracia rusticana*) in the vegetables category, root and tuber vegetables group (code 0213040) taking into account changes in the levels by processing (drying) according to Art. 20 (1) of Regulation (EC) No 396/2005.

3 The European Food Safety Authority identified some information on analytical methods as unavailable. When re-viewing the MRL, the Commission will take into account the information referred to in the first sentence, if it is submitted by 5 February 2016, or, if that information is not submitted by that date, the lack of it.

Plant Matrices

For pyrimethanil MRLs are established in several crops. In order to support the renewal of approval for pyrimethanil, additional residue trials are presented for some of the intended uses (strawberries, lettuce). In the chapters below, the new data are evaluated using statistical means and compared with the data being included in the most recent EFSA Reasoned Opinions. The established EU MRLs cover the representative uses (apple, grape, strawberry and lettuce).

Apples

The use in pome fruit was part of the previous active substance inclusion process. Sufficient data supporting the representative GAP were submitted to Austria as the designated Rapporteur Member State and were evaluated on EU level (EFSA Conclusion 2006). Meanwhile, the GAP has changed regarding the application rate to 0.48 kg a.s./ha, which is within 25% tolerance of the evaluated 0.6 kg a.s./ha. Therefore, no further residue studies have been conducted. The residues at PHI 56 are repeated below for the reviewer's convenience.

Northern Europe (n=9), field: 0.12, 0.18 (2x), 0.20, 0.22, 0.30, 0.33, 0.45, 0.6 mg/kg

Southern Europe (n=9), field: <0.05, 0.11, 0.15, 0.16 (2x), 0.18, 0.22, 0.30, 0.37 mg/kg

In contrast to the EFSA Conclusion, where the EU procedure was used for MRL calculation, in this document the current version of the OECD MRL calculator was used. In order to derive the most robust MRL, all trials were considered.

Table 6.7.2-2: MRL calculation for pome fruit in the EU for pyrimethanil based on parent residues - representative GAP, already peer-reviewed

	Pyrimethanil [mg/kg]	
	North (n=9)	**South (n=9)**
STMR	0.22	0.16
HR	0.6	0.37
OECD	**0.9**	**0.6**

During Article 12 review (EFSA Reasoned Opinion 2011), several additional pyrimethanil residue data in apples and pears were evaluated, supporting more critical GAPs. However, the current EU MRL for pome fruit was derived from the existing Codex MRL (CXL) of 15 mg/kg. The data basis for this CXL/MRL is given in the JMPR Report 2013. The apple and pear data evaluated match a GAP for uses of pyrimethanil as post-harvest thermofog treatment (1 x 8 g a.s./ton fruits).

JMPR 2013 (n=12), indoor (post-harvest): Apples: 1.1, 1.4 (2x), 1.5, 1.6, 4.9, 6.4, 7.1 mg/kg
Pears: 1.0, 1.6, 1.8, 3.5 mg/kg

The current **EU MRL** of **15 mg/kg** (STMR: 1.6 mg/kg; HR: 7.1 mg/kg) covers the representative GAP. Furthermore, all uses evaluated during Article 12 review are also covered. The corresponding dataset is used in this document for feed burden calculation and risk assessment.

Grapes

The use in grapes was part of the previous active substance inclusion process. Sufficient data supporting the representative GAP were submitted to Austria as the designated Rapporteur Member State and were evaluated on EU level (EFSA Conclusion 2006). The GAP has changed only slightly since. Therefore, no further residue studies have been conducted. The residues at PHI 21 are repeated below for the reviewer's convenience.

Northern Europe (n=9), field: 0.37; 0.38; 0.44; 0.59; 0.83; 0.84; 0.97; 2 x 1.1 mg/kg

Southern Europe (n=9), field: 0.28; 0.38; 0.41; 0.42; 0.48; 0.58; 0.83; 2 x 1.0; 1.5; 2 x 1.6; 1.98 mg/kg

In contrast to the EFSA Conclusion, where the EU procedure was used for MRL calculation, in this document the current version of the OECD MRL calculator was used. In order to derive the most robust MRL, all trials were considered.

Table 6.7.2-3: MRL calculation for grapes in the EU for pyrimethanil based on parent residues - representative GAP, already peer-reviewed

	Pyrimethanil [mg/kg]	
	North (n=9)	**South (n=13)**
STMR	0.83	0.83
HR	1.1	1.98
OECD	**3**	**4**

During Article 12 review (EFSA Reasoned Opinion 2011), additional pyrimethanil residue data in grapes were evaluated, supporting more critical GAPs (3 x 0.75 (NEU) or 3 x 0.96 kg a.s./ha (SEU), PHI 21 days) and being the data basis for the current EU MRL of 5 mg/kg.

EFSA 2011 (n=18), NEU: <0.05, 0.19, 0.37, 0.38, 2x 0.44, 0.47, 0.59, 0.67, 0.83, 0.84, 0.97, 2x 1.1, 1.2, 1.7, 2.22, 2.27 mg/kg

EFSA 2011 (n=30), SEU: 0.35, 0.39, 1.0, 1, 1.1, 1.2, 2x 1.33, 1.44, 1.48, 1.5, 1.67, 2 x 1.78, 1.82, 1.9, 1.91, 1.97, 2x 2.1, 2.26, 2.64, 2x 2.7, 2x 2.8, 3.2, 3.55, 3.9, 4.59 mg/kg

The current **EU MRL** of **5 mg/kg** covers the representative GAP. No new MRL is proposed even though an EU MRL of 6 mg/kg would be derived using the same dataset and the OECD calculator:

Table 6.7.2-4: MRL calculation for grapes in the EU for pyrimethanil based on parent residues - critical GAP, Article 12 review

	Pyrimethanil [mg/kg]	
	North (n=18)	**South (n=30)**
STMR	0.75	1.86
HR	2.27	4.59
OECD	**4**	**6**

Strawberries

In the growing season 2010, in total eight protected trials were performed in Northern Europe (United Kingdom). The trials were conducted with formulation BAS 605 04 F according to the critical GAP of this formulation (± 25%; 2 x 0.800 kg a.s./ha).

In the growing seasons 2006 and 2007, in total six greenhouse trials were performed in Northern and Southern Europe (France). The trials were conducted with formulation Pyrimethanil 400 SC according to the critical GAP of this formulation (± 25%; 2 x 0.800 kg a.s./ha).

The following parent compound residues were found in strawberry fruit samples at a PHI of 3 days or thereafter (if higher residues occurred):

Europe (n=14), protected/greenhouse: *0.383, 0.460, 0.518*, 0.52, *0.521*, 0.60, 0.68, *0.725*, 0.76, 0.79, *0.818*, 0.98, 1.16, 1.77 mg/kg

Some of these data (indicated in italics) have already been evaluated by EFSA (EFSA Reasoned Opinion 2011 (Article 12 review)).

For MRL calculation the current version of the OECD MRL calculator was used. In order to derive the most robust MRL, all trials were considered.

Table 6.7.2-5: MRL calculation for strawberry in the EU for pyrimethanil based on parent residues (mg/kg) - evaluated and new data

	Pyrimethanil [mg/kg]
	Greenhouse/protected (n=14)
STMR	0.703
HR	1.77
OECD	**3**

The field use in strawberries was evaluated during Article 12 review. No additional data supporting the representative GAP are presented. For Northern Europe, values at the intended PHI of 3 days (supporting the current GAP) instead of 0-1 days (supporting the evaluated GAP) were chosen here. The residues at PHI 3 are shown below.

Northern Europe (n=9), field: 0.33; 0.56; 0.67; 0.72; 0.73; 0.84; 1.0; 2.2; 2.7 mg/kg

Southern Europe (n=9), field: 0.37; 0.49; 0.53; 0.67; 0.68; 2 x 1.1; 1.3; 1.7 mg/kg

In contrast to the EFSA Conclusion, where the EU procedure was used for MRL calculation, in this document the current version of the OECD MRL calculator was used. In order to derive the most robust MRL, all trials were considered.

Table 6.7.2-6: MRL calculation for strawberries in the EU for pyrimethanil based on parent residues (mg/kg) - representative GAP

	Pyrimethanil [mg/kg]	
	North (n=9)	**South (n=9)**
STMR	0.73	0.68
HR	2.7	1.7
OECD	**5**	**3**

The data show that the calculated MRL for pyrimethanil in strawberry is covered by the current MRL of 5 mg/kg. Therefore BASF proposes to keep the current EU MRL for pyrimethanil at 5 mg/kg for strawberry.

Code number 152000 (strawberries): 5 mg/kg

Lettuce

In the growing season 2008, in total eight greenhouse (6 leaf lettuce, *2 head lettuce*) and ten field trials (5 leaf lettuce, *5 head lettuce*) were performed in Northern and Southern Europe (France, Germany, Greece, Italy, Spain). The trials were conducted with formulation Pyrimethanil 400 SC according to the critical GAP of this formulation (± 25%; 2 x 0.800 kg a.s./ha).

The following parent compound residues were found in lettuce head samples at a PHI of 14 days or thereafter (if higher residues occurred); values in italics indicate head lettuce:

Europe (n=8), greenhouse: 1.48, 2.06, 3.07, *3.14*, 4.81, *5.73*, 6.18, 12.45 mg/kg

These greenhouse data have already been evaluated by EFSA (EFSA Reasoned Opinion 2011 (lettuce + scarole); EFSA Reasoned Opinion 2011 (Article 12 review)). In this document, typos were corrected and MRL calculation performed with the OECD calculator.

Northern Europe (n=2), field: *0.026*, 0.46 mg/kg

Southern Europe (n=8), field: *0.16*, 0.17, 0.19, *0.25*, 0.37, 0.99, *1.01*, *1.25* mg/kg

These field data have not been evaluated on EU level.

In addition, several field trials on *head lettuce* and leaf lettuce performed with the same GAP were evaluated by EFSA during Article 12 review. In the growing seasons 1997 and 1998, in total 16 field trials (2 leaf lettuce, *14 head lettuce*) were performed in Northern and Southern Europe (France, Germany, Greece, Italy, The Netherlands, Portugal, Spain, United Kingdom). The relevant residues are shown below.

Northern Europe (n=9), field: *<0.05(2x), 0.06, 0.11, 0.3, 0.34, 0.42, 0.43, 0.55* mg/kg

Southern Europe (n=7), field: *0.05, 0.14, 0.31*, 0.62, 0.68*; 0.78, 1.2* mg/kg

For MRL calculation the current version of the OECD MRL calculator was used. In order to derive the most robust MRL, all trials were considered.

Table 6.7.2-7: MRL calculation for lettuce in the EU for pyrimethanil based on parent residues (mg/kg) - evaluated and new data

	Pyrimethanil [mg/kg]		
	Greenhouse (n=8)	**North (n=11)**	**South (n=15)**
STMR	3.975	0.30	0.37
HR	12.5	0.55	1.25
OECD	**20**	**1**	**3**

The data show that the calculated MRL for pyrimethanil in lettuce is covered by the current MRL of 20 mg/kg. Therefore BASF proposes to keep the current EU MRL for pyrimethanil at 20 mg/kg for lettuce.

Code number 251020 (lettuce): 20 mg/kg

Rotational crops: root and tuber vegetables

In the growing season 2014/2015, in total four rotational crop trials on carrot (3) and radish (1) were performed in Northern and Southern Europe (Germany, The Netherlands, Italy and Spain). The trials were conducted with solo formulation BAS 605 04 F applied to soil once at a rate of 3.0 kg a.s./ha).
The following parent compound residues were found in carrot root samples at BBCH 49 or radish roots at BBCH 89 after a 30 day re-plant interval:

Northern Europe (n=2): 0.022, 0.025 mg/kg

Southern Europe (n=2): <0.01, 0.015 mg/kg

For MRL calculation the current version of the OECD MRL calculator was used. In order to derive the most robust MRL, all trials were considered.

Table 6.7.2-8: MRL calculation for root and tuber vegetables in the EU for pyrimethanil based on parent residues (mg/kg)

	Pyrimethanil [mg/kg]
	Rotational crops - carrot and radish
STMR	0.019
HR	0.025
OECD	**0.05**

The data show that the calculated MRL for pyrimethanil in root and tuber vegetables is not covered by the current MRL of 0.01-0.05 mg/kg (at the respective LOQ), except for carrot (1 mg/kg). Therefore BASF proposes to raise the current EU MRLs for pyrimethanil for the following commodities to avoid MRL exceedances from rotational crops:

Code number 210000 (root and tuber vegetables), except carrots (code 213020):
0.05 mg/kg

As bulb vegetables belong to the same rotational crop category (root crops) according to OECD guidance 501, Annex 1, it is proposed to set the MRL of 0.05 mg/kg also for the following commodities:

Code number 220010 (garlic), 220030 (shallots) and 220990 (other bulb vegetables)
0.05 mg/kg

For onions and spring onions, the calculated MRL is covered by the existing ones of 0.2 and 3 mg/kg, respectively.

Rotational crops: leafy and Brassica vegetables

In the growing season 2014/2015, in total twelve rotational crop trials on spinach and lettuce (4), cauliflower and broccoli (4) and carrot and radish (4) were performed in Northern and Southern Europe (Germany, The Netherlands, Italy and Spain). The trials were conducted with solo formulation BAS 605 04 F applied to soil once at a rate of 3.0 kg a.s./ha).
The following parent compound residues were found in spinach and lettuce leaves, cauliflower and broccoli inflorescences and carrot top samples at BBCH 49 or radish tops at BBCH 89 after a 30 day re-plant interval:

Northern Europe (n=2,): leafy: <0.01, 0.044 mg/kg
Brassica: <0.01 (2x) mg/kg
root crop leaves: 0.088, 0.097 mg/kg

Southern Europe (n=2): leafy: <0.01 (2x) mg/kg
Brassica: <0.01 (2x) mg/kg
root crop leaves: <0.01, 0.065 mg/kg

For MRL calculation the current version of the OECD MRL calculator was used. In order to derive the most robust MRL, all trials and leafy matrices were considered.

Table 6.7.2-9: MRL calculation for leafy and Brassica vegetables in the EU for pyrimethanil based on parent residues (mg/kg)

	Pyrimethanil [mg/kg]
	Rotational crops - spinach and lettuce, cauliflower and broccoli, carrot and radish tops
STMR	0.010
HR	0.097
OECD	**0.2**

The data show that the calculated MRL for pyrimethanil in leaf and Brassica vegetables is not covered by the current MRL of 0.01 mg/kg, except for lettuce, escarole, leaves and sprouts of Brassica and herbs (20 mg/kg). Therefore BASF proposes to raise the current EU MRLs for pyrimethanil for the following commodities to avoid MRL exceedances from rotational crops:

Code number 240000 (Brassica vegetables) and 250000 (leaf vegetables and fresh herbs), except lettuce (code 251020), escarole (code 251030), leaves and sprouts of Brassica (code 251080) and herbs (code 256000):

0.2 mg/kg

As stem vegetables and hops belong to the same rotational crop category (leafy crops) according to OECD guidance 501, Annex 1, it is proposed to set the MRL of 0.2 mg/kg also for the following commodities:

Code number 270000 (stem vegetables), except leek (code 270060), and 700000 (hops)

0.2 mg/kg

For **leek**, the calculated MRL is covered by the existing one of 1 mg/kg. A new MRL application for leek was submitted in France in January 2015 in course of the product re-registration (evaluation on going) and applies for a raise of the current MRL to **3 mg/kg**.

Animal matrices

In 2011, the most recent calculation of the overall feed burden has been performed by EFSA in context of the MRL re-evaluation according to EEC 396/2005, Article 12. The output is shown in **Table 6.7.2-10**.

Table 6.7.2-10: Results of the dietary burden calculation by EFSA (EFSA Reasoned Opinion 2011; Article 12 review)*

	Maximum dietary burden (mg/kg bw/d)	Highest contributing commodity	Max dietary burden (mg/kg DM)	Trigger exceeded (Y/N)
Risk assessment residue definition: pyrimethanil				
Dairy ruminants	0.13	Orange pomace	3.59	Y
Meat ruminants	0.45	Orange pomace	10.41	Y
Poultry	0.01	Potatoes	0.12	Y
Pigs	0.01	Potatoes	0.23	Y

* EFSA Journal 2011;9(11):2454

In this document, the current version of the OECD feed burden calculator (using the OECD methodology) was applied in the first place. In addition, the feed burden was calculated using EFSA PROFile and the results are presented further below. Furthermore, the recent Animal Model 2016 was applied for comparison reasons. All pyrimethanil uses, including non-BASF uses (i.e. citrus fruits and post-harvest use on pome fruits), were considered as a worst-case scenario.

The following input values were used for calculation of the Results for Reasonable Worst Case Feeding Levels (RWCFL). According to the Animal Model 2016, almond hulls and grape pomace are not present in EU animal diets.

Table 6.7.2-11: Input values for the revised dietary burden calculation (OECD methodology)

Commodity	RWCFL EU	
	Input value (mg/kg)	Comment
Forages		
Barley, straw[1]	0.05	HR = STMR
Cabbage heads, leaves	0.2	MRL (rotational crop)[10]
Kale, leaves	0.2	MRL (rotational crop)[10]
Oat, straw[1]	0.05	HR = STMR
Pea, hay[5]	1.0/0.20	HR/STMR
Rye, straw[1]	0.05	HR = STMR
Wheat, straw[1]	0.05	HR = STMR
Roots & Tubers		
Carrot, culls[19,6]	0.54/0.14[7]	HR/STMR
Cassava/tapioca, roots	0.05	MRL (rotational crop)[10]
Potatoes, culls[2]	0.05	MRL (rotational crop)[10]
Swede	0.05	MRL (rotational crop)[10]
Turnip	0.05	MRL (rotational crop)[10]
Cereal Grain/Crops Seeds		
Barley, grain[1]	0.05	STMR
Bean, seed[1]	0.07	STMR
Corn, field, grain[1]	0.05	STMR
Corn, pop, grain[1]	0.05	STMR
Lupin, seed[1]	0.07	STMR
Oat, grain[1]	0.05	STMR
Pea, seed[5]	0.09	STMR
Rye, grain[1]	0.05	STMR
Wheat, grain[1]	0.05	STMR
By-Products		
Almond, hulls[5]	2.6	STMR
Apple, pomace, wet[5,87]	6.6	STMR (1.6 mg/kg[8]) x PF (4.1[5,11])
Citrus, dried pulp[1,3]	7.85	STMR (3.14 mg/kg) x 2.5
Grape, pomace, wet[5,9]	1.7	STMR (0.71 mg/kg) x 2.4
Potato, process waste[2]	0.05	MRL (rotational crop)[10]
Wheat, milled byproducts[1,4]	0.05	STMR

1 See EFSA Journal 2011;9(11):2454
2 Potato tuber
3 Citrus pomace (no BASF-use)
4 Wheat bran
5 See JMPR Evaluation Report 2007
6 Carrots
~~7 HR/STMR in context of a JMPR submission leading to CODEX MRL, which was later adopted by EFSA~~
87 See JMPR Report 2013, BASF-use: see chapter MCA 6.3 and 6.5 STMR (0.22 mg/kg) x PF (1.6)
98 BASF-use grape: see chapter MCA 6.3 and 6.5 STMR (0.83 mg/kg) x PF (2.08) leads to same input value of 1.7 mg/kg
109 As MRLs were derived from a field rotational crop study, these values were favored over the respective HR and STMR values to ensure to cover a worst-case scenario since no residue trials were conducted on these crops as primary crops.
110 This is also the highest processing factor of this dossier (see chapter MCA 6.5) and was used as worst case.

The results of the total maximum dietary burdens are presented below (see **Table 6.7.2-12**).

Table 6.7.2-12: Summary of the results for RWCFL (EU)

	Cattle Beef	Cattle Dairy	Sheep Ram/Ewe	Sheep Lamb	Swine Breeding	Swine Finishing	Poultry Broiler	Poultry Layer	Poultry Turkey
Body weight (kg)	500	650	75	40	260	100	1.7	1.9	7
Daily intake (kg DM)	12	25	2.5	1.7	6	3	0.12	0.13	0.5
Dietary burden (mg/kg bw)	**0.103**	**0.106**	**0.093**	**0.119**	**0.062**	**0.035**	**0.035**	**0.042**	**0.035**
Feed burden (mg/kg DM)	**4.282**	**2.761**	**2.800**	**2.806**	**2.669**	**1.168**	**0.501**	**0.609**	**0.495**

The tables below show the detailed results of the feed burden calculation. It should be noted that the doses assume that the diet completely consists of plant material which had been treated with pyrimethanil.

Table 6.7.2-13: Detailed results for RWCFL (EU): cattle (beef)

Category	Crop	Feedstuff	% of diet	Dietary contribution (mg/kg bw)	Cumulative % diet	Adjusted % Diet	Adjusted dietary contribution (mg/kg bw)	Feed burden (mg/kg DM)
By-products	Apple	pomace, wet	20	0.079	20	20	0.079	3.300
Roots/tubers	Carrot	culls	15	0.016	35	15	0.016	0.675
Forages	Pea	hay	25	0.007	60	25	0.007	0.284
Cereal grains	Corn, field	grain	80	0.001	100	40	0.001	0.023
Total			**140**	**0.103**	**-**	**100**	**0.103**	**4.282**

Table 6.7.2-14: Detailed results for RWCFL (EU): cattle (dairy)

Category	Crop	Feedstuff	% of diet	Dietary contribution (mg/kg bw)	Cumulative % diet	Adjusted % Diet	Adjusted dietary contribution (mg/kg bw)	Feed burden (mg/kg DM)
By-products	Citrus	dried pulp	20	0.066	20	20	0.066	1.725
Roots/tubers	Carrot	culls	15	0.026	35	15	0.026	0.675
Forages	Pea	hay	30	0.013	65	30	0.013	0.341
Cereal grains	Rye	grain	40	0.001	100	35	0.001	0.020
Total			**105**	**0.106**	**-**	**100**	**0.106**	**2.761**

Table 6.7.2-15: Detailed results for RWCFL (EU): sheep (ram/ewe)

Category	Crop	Feedstuff	% of diet	Dietary contribution (mg/kg bw)	Cumulative % diet	Adjusted % Diet	Adjusted dietary contribution (mg/kg bw)	Feed burden (mg/kg DM)
By-products	Apple	pomace, wet	10	0.055	10	10	0.055	1.650
Roots/tubers	Carrot	culls	20	0.030	30	20	0.030	0.900
Forages	Pea	hay	20	0.008	50	20	0.008	0.227
Cereal grains	Rye	grain	40	0.001	90	40	0.001	0.023
Total			**90**	**0.093**	**-**	**90**	**0.093**	**2.800**

Table 6.7.2-16: Detailed results for RWCFL (EU): sheep (lamb)

Category	Crop	Feedstuff	% of diet	Dietary contribution (mg/kg bw)	Cumulative % diet	Adjusted % Diet	Adjusted dietary contribution (mg/kg bw)	Feed burden (mg/kg DM)
By-products	Apple	pomace, wet	10	0.070	10	10	0.070	1.650
Roots/tubers	Carrot	culls	20	0.038	30	20	0.038	0.900
Forages	Pea	hay	20	0.010	50	20	0.010	0.227
Cereal grains	Barley	grain	60	0.001	100	50	0.001	0.028
Total			**110**	**0.119**	**-**	**100**	**0.119**	**2.806**

Table 6.7.2-17: Detailed results for RWCFL (EU): swine (breeding)

Category	Crop	Feedstuff	% of diet	Dietary contribution (mg/kg bw)	Cumulative % diet	Adjusted % Diet	Adjusted dietary contribution (mg/kg bw)	Feed burden (mg/kg DM)
By-products	Citrus	dried pulp	15	0.030	15	15	0.030	1.294
Roots/tubers	Carrot	culls	25	0.026	40	25	0.026	1.125
Forages	Pea	hay	20	0.005	60	20	0.005	0.227
Cereal grains	Barley	grain	80	0.001	100	40	0.001	0.023
Total			**140**	**0.062**	**-**	**100**	**0.062**	**2.669**

Table 6.7.2-18: Detailed results for RWCFL (EU): swine (finishing)

Category	Crop	Feedstuff	% of diet	Dietary contribution (mg/kg bw)	Cumulative % diet	Adjusted % Diet	Adjusted dietary contribution (mg/kg bw)	Feed burden (mg/kg DM)
Roots/tubers	Carrot	culls	25	0.034	25	25	0.034	1.125
Cereal grains	Barley	grain	80	0.001	100	75	0.001	0.043
By-products	Wheat	milled bypdts	50	0.001	100	0	0.000	0.000
Forages	Wheat	silage	0	0.000	100	0	0.000	0.000
Total			**155**	**0.036**	**-**	**100**	**0.035**	**1.168**

Table 6.7.2-19: Detailed results for RWCFL (EU): poultry (broiler)

Category	Crop	Feedstuff	% of diet	Dietary contribution (mg/kg bw)	Cumulative % diet	Adjusted % Diet	Adjusted dietary contribution (mg/kg bw)	Feed burden (mg/kg DM)
Roots/tubers	Carrot	culls	10	0.032	10	10	0.032	0.450
Cereal grains	Rye	grain	70	0.003	80	70	0.003	0.040
By-products	Wheat	milled bypdts	20	0.001	100	20	0.001	0.011
Forages	Wheat	silage	0	0.000	100	0	0.000	0.000
Total			**100**	**0.035**	**-**	**100**	**0.035**	**0.501**

Table 6.7.2-20: Detailed results for RWCFL (EU): poultry (layer)

Category	Crop	Feedstuff	% of diet	Dietary contribution (mg/kg bw)	Cumulative % diet	Adjusted % Diet	Adjusted dietary contribution (mg/kg bw)	Feed burden (mg/kg DM)
Roots/tubers	Carrot	culls	10	0.031	10	10	0.031	0.450
Forages	Pea	hay	10	0.008	20	10	0.008	0.114
Cereal grains	Barley	grain	100	0.004	100	80	0.003	0.045
By-products	Wheat	milled bypdts	20	0.001	100	0	0.000	0.000
Total			**140**	**0.043**	**-**	**100**	**0.042**	**0.609**

Table 6.7.2-21: Detailed results for RWCFL (EU): poultry (turkey)

Category	Crop	Feedstuff	% of diet	Dietary contribution (mg/kg bw)	Cumulative % diet	Adjusted % Diet	Adjusted dietary contribution (mg/kg bw)	Feed burden (mg/kg DM)
Roots/tubers	Carrot	culls	10	0.032	10	10	0.032	0.450
Cereal grains	Rye	grain	60	0.002	70	60	0.002	0.034
By-products	Wheat	milled bypdts	20	0.001	90	20	0.001	0.011
Forages	Wheat	silage	0	0.000	90	0	0.000	0.000
Total			**90**	**0.035**	**-**	**90**	**0.035**	**0.495**

Using the OECD calculator, the doses to be used when estimating the maximum residues in products of animal origin are for

dairy cattle 0.11 mg/kg bw/d (2.76 mg/kg feed DM)
beef cattle 0.10 mg/kg bw/d (4.28 mg/kg feed DM)
lamb 0.12 mg/kg bw/d (2.81 mg/kg feed DM)
poultry 0.04 mg/kg bw/d (0.61 mg/kg feed DM, layer)
pig 0.06 mg/kg bw/d (2.67 mg/kg feed DM, breeding)

The recent Animal Model 2016 was applied for comparison reasons. The input values shown in Table 6.7.2-11 were used, except almond hulls and grape pomace are not present in EU animal diets.

Table 6.7.2-22: Results of the dietary burden calculation using Animal Model 2016

Animal species	Median dietary burden (mg/kg bw/d)	Maximum dietary burden (mg/kg bw/d)	Highest contributing commodity	Max dietary burden (mg/kg DM)	Trigger exceeded (Y/N)
Risk assessment residue definition: pyrimethanil					
Beef cattle*	0.090	0.102	Apple wet pomace	4.26	Y
Dairy cattle*	0.084	0.106	Citrus dried pulp	2.76	Y
Ram / ewe	0.068	0.093	Apple wet pomace	2.79	Y
Lamb	0.087	0.119	Apple wet pomace	2.80	Y
Breeding swine	0.040	0.062	Citrus dried pulp	2.67	Y
Finishing swine*	0.010	0.035	Carrot culls	1.17	Y
Broiler poultry	0.012	0.035	Carrot culls	0.50	Y
Layer poultry*	0.016	0.042	Carrot culls	0.61	Y
Turkey	0.012	0.035	Carrot culls	0.50	Y

* These categories correspond to those (formerly) assessed at EU level.

Using the Animal Model 2016, the doses to be used when estimating the maximum residues in products of animal origin are for

dairy cattle 0.11 mg/kg bw/d (2.76 mg/kg feed DM)
beef cattle 0.10 mg/kg bw/d (4.26 mg/kg feed DM)
lamb 0.12 mg/kg bw/d (2.80 mg/kg feed DM)
poultry 0.04 mg/kg bw/d (0.61 mg/kg feed DM, layer)
pig 0.06 mg/kg bw/d (2.67 mg/kg feed DM, breeding)

The results are almost the same compared to the OECD calculator.

In addition to the OECD method, the procedure of EFSA (PROFile) has been applied for calculation of the feed burden.

The following input values were used for calculation of maximum dietary burden:

Table 6.7.2-23: Input values for the revised dietary burden calculation (EU methodology)

Commodity	Maximum dietary burden	
	Input value (mg/kg)	Comment
Cabbage	0.2	MRL (rotational crop)[6]
Kale	0.2	MRL (rotational crop)[6]
Orange pomace[1,3]	7.85	STMR (3.14 mg/kg) x 2.5
Grapefruit pomace[1,3]	7.85	STMR (3.14 mg/kg) x 2.5
Lemon pomace[1,3]	7.85	STMR (3.14 mg/kg) x 2.5
Lime pomace[1,3]	7.85	STMR (3.14 mg/kg) x 2.5
Mandarin pomace[1,3]	7.85	STMR (3.14 mg/kg) x 2.5
Apple pomace[2,5]	6.6	STMR (1.6 mg/kg[5]) x PF (4.1[2,7])
Wheat grain[1]	0.05	STMR
Barley grain[1]	0.05	STMR
Rye grain[1]	0.05	STMR
Oat grain[1]	0.05	STMR
Maize grain[1]	0.05	STMR
Wheat bran[1]	0.05	STMR
Rye bran[1]	0.05	STMR
Wheat straw[1]	0.05	HR
Barley straw[1]	0.05	HR
Rye straw[1]	0.05	HR
Oat straw[1]	0.05	HR
Peas (dry)[2]	0.09	STMR
Beans (dry)[1]	0.07	STMR
Lupines (dry)[1]	0.07	STMR
Potatoes[1,2]	0.05	MRL (rotational crop)[6]
Turnips	0.05	MRL (rotational crop)[6]
Swedes	0.05	MRL (rotational crop)[6]

1 See EFSA Journal 2011;9(11):2454
2 See JMPR Evaluation Report, 2007
3 No BASF-use
4 STMR in context of a JMPR submission leading to CODEX MRL, which was later adopted by EFSA
5 See JMPR Report 2013, BASF-use: see chapter MCA 6.3 and 6.5 STMR (0.22 mg/kg) x PF (1.6)
6 As MRLs were derived from a field rotational crop study, these values were favored over the respective HR and STMR values to ensure to cover a worst-case scenario since no residue trials were conducted on these crops as primary crops.
7 This is also the highest processing factor of this dossier (see chapter MCA 6.5) and was used as worst case.

The results are summarized in Table 6.7.2-24.

Table 6.7.2-24: Results of the dietary burden calculation

	Maximum dietary burden (mg/kg bw/d)	Highest contributing commodity	Max dietary burden (mg/kg DM)	Trigger exceeded (Y/N)
Risk assessment residue definition: pyrimethanil				
Dairy ruminants	0.13	Orange pomace	3.61	Y
Meat ruminants	0.45	Orange pomace	10.55	Y
Poultry	0.01	Turnips	0.23	Y
Pigs	0.02	Turnips	0.54	Y

The following tables show the calculation of the maximum dietary burden for each relevant livestock species, which are based on the highest and the median residue levels of pyrimethanil. It should be noted that the doses assume that the diet completely consists of plant material which had been treated with pyrimethanil.

Table 6.7.2-25: Detailed results of the livestock dietary burden calculations for dairy ruminants

Commodity	DM intake (%)	Residue intake over DM intake	Actual contribution to total DM intake (%)	Actual contribution to total residue intake (mg/kg bw/d)	Total DM intake (%)	Total residue intake (mg/kg bw/d)
Orange pomace	10	0.012411	10	0.124111	10	0.124111
Turnips	30	0.000182	30	0.005455	40	0.129565
Peas (dry)	20	0.000038	20	0.000761	60	0.130326
Wheat grain	40	0.000021	40	0.000846	100	0.131172

Maximum dietary burden: 0.131172 mg/kg bw/d

Table 6.7.2-26: Detailed results of the livestock dietary burden calculations for meat ruminants

Commodity	DM intake (%)	Residue intake over DM intake	Actual contribution to total DM intake (%)	Actual contribution to total residue intake (mg/kg bw/d)	Total DM intake (%)	Total residue intake (mg/kg bw/d)
Orange pomace	30	0.014627	30	0.438820	30	0.438820
Turnips	60	0.000214	60	0.012857	90	0.451677
Peas (dry)	20	0.000045	10	0.000449	100	0.452126

Maximum dietary burden: 0.452126 mg/kg bw/d

Table 6.7.2-27: Detailed results of the livestock dietary burden calculations for poultry

Commodity	DM intake (%)	Residue intake over DM intake	Actual contribution to total DM intake (%)	Actual contribution to total residue intake (mg/kg bw/d)	Total DM intake (%)	Total residue intake (mg/kg bw/d)
Cabbage	5	0.000902	5	0.004511	5	0.004511
Turnips	20	0.000316	20	0.006316	25	0.010827
Peas (dry)	30	0.000066	30	0.001983	55	0.012810
Wheat grain	70	0.000037	45	0.001652	100	0.014462

Maximum dietary burden: 0.014462 mg/kg bw/d

Table 6.7.2-28: Detailed results of the livestock dietary burden calculations for pigs

Commodity	DM intake (%)	Residue intake over DM intake	Actual contribution to total DM intake (%)	Actual contribution to total residue intake (mg/kg bw/d)	Total DM intake (%)	Total residue intake (mg/kg bw/d)
Cabbage	15	0.000571	15	0.008571	15	0.008571
Turnips	60	0.000200	60	0.012000	75	0.020571
Peas (dry)	40	0.000042	25	0.001047	100	0.021618

Maximum dietary burden: 0.021618 mg/kg bw/d

The doses to be used when estimating the maximum residues in products of animal origin are for

dairy cattle 0.13 mg/kg bw/d (3.61 mg/kg feed DM)
beef cattle 0.45 mg/kg bw/d (10.55 mg/kg feed DM)
chicken 0.01 mg/kg bw/d (0.23 mg/kg feed DM
pig 0.02 mg/kg bw/d (0.54 mg/kg feed DM)

Proposed animal MRLs

The doses to be used when estimating the maximum residues in products of animal origin are summarized in the table below. Highlighted cells indicate worst case of PROFile and OECD.

Table 6.7.2-29: Results of the dietary burden calculation - comparison of OECD and PROFile calculation

	Maximum dietary burden (mg/kg bw/d)		Highest contributing commodity		Max dietary burden (mg/kg DM)		Trigger exceeded (Y/N)
	PROFile (EFSA, 2011)[1]	OECD = Model 2016	PROFile (EFSA, 2011)[1]	OECD = Model 2016	PROFile (EFSA, 2011)	OECD = Model 2016	
Risk assessment residue definition: pyrimethanil							
Dairy ruminants	0.13	0.11	Orange pomace	Citrus dried pulp (citrus pomace)	3.61 (3.59)	2.76	Y
Meat ruminants	0.45	0.10[2]	Orange pomace	Apple pomace, wet	10.55 (10.41)	4.28 4.26	Y
Poultry	0.01	0.04[3]	Turnips	Carrot culls (carrots)	0.23 (0.12)	0.61[3]	Y
Pigs	0.02 (0.01)	0.06[4]	Turnips	Citrus dried pulp (citrus pomace)	0.54 (0.23)	2.67[4]	Y

1 Values given in EFSA Reasoned Opinion 2011 only shown if different from PROFile calculation performed for AIR 3
2 Worst case of cattle and sheep is lamb: 0.12 mg/kg bw/d; highest contributor: apple pomace, wet
3 Layer (worst case)
4 Breeding (worst case); finishing: 0.035 mg/kg bw/d or 1.17 mg/kg DM; highest contributor: carrot culls (carrots)
Highlighted cells indicate worst case of PROFile and OECD

Cattle (sheep, goat) products

A residue transfer study (BASF DocID B003807, Annex II chapter 6.4.1.2) with pyrimethanil was conducted in cows and was evaluated during Article 12 review (EFSA Reasoned Opinion 2011). The animals were dosed with 1, 3, 10 and 50 mg/kg feed (dry matter) equal to 0.03, 0.1, 0.3 and 1.5 mg/kg bw/d for a period of 28 days. In muscle, liver, fat and milk, no residues (<0.05 mg/kg for tissues and <0.01 mg/kg for milk) of parent pyrimethanil or metabolite M605F002 were found in any dose group. Maximum group mean residues of M605F003 of 0.016 and 0.063 mg/kg (expressed as parent equivalents) were found in milk of the two highest dose groups, respectively. In kidney, maximum residues of M605F002 of <0.05, 0.07, 0.12 and 0.81 mg/kg (expressed as parent equivalents) were found, respectively. No pyrimethanil residues were detected.
The calculated feed burdens are within the range of the feeding study. Therefore, no residues are to be expected in ruminant muscle, liver and fat. Estimated residues in milk and kidney are described below.

OECD approach
The feed burden calculation presented above, considering lamb as worst case of the meat ruminant species and dairy ruminants, indicates that the 3x dose level (0.1 mg/kg bw/d) for tissues and for milk is the most appropriate for covering the uses of pyrimethanil.
Thus, a factor was calculated (calculated feed burden / dose level in feeding study, in mg/kg bw/d). The factor derived for tissues is 1.2 (0.12 mg/kg bw / 0.1 mg/kg bw). Applying this factor to the M605F002 residue of 0.07 mg/kg found in kidney at the 3x dose level results in a calculated residue of 0.08 mg/kg. Addition of parent residue (<0.017 mg/kg (LOD)) gives a total pyrimethanil residue of 0.10 mg/kg for kidney.
In case of milk, a factor of 1.1 (0.11 mg/kg bw / 0.1 mg/kg bw) was calculated. Residues of both parent and M605F003 were below the LOQ of 0.01 mg/kg in the 3x dose group. As worst case, the total residue was set to 0.02 mg/kg, which leads to a calculated residue of 0.02 mg/kg.

Animal Model 2016 approach
Inserting the results of the cow feeding study into the Animal Model 2016 the lowest feeding level is identified as the closest level. For muscle, fat and liver, an MRL of 0.1 mg/kg is derived by both interpolation and linear regression as all residues were below the LOQ for this level; a transfer factor could not be applied. In case of kidney, the transfer factor gives the highest residue compared to interpolation and linear regression, resulting in an MRL proposal of 0.1 mg/kg. For milk, an MRL of 0.02 mg/kg is derived by interpolation.

PROFile approach (worst case)
The feed burden calculation presented above indicates that the 10x dose level (0.3 mg/kg bw/d) for tissues and the 3x dose level (0.1 mg/kg bw/d) for milk are the most appropriate for covering the uses of pyrimethanil. The feed burden calculation (PROFile) performed in the context of this document differs only marginally from the one performed by EFSA.
Thus, a factor was calculated (calculated feed burden / dose level in feeding study, in mg/kg bw/d). The factor derived for tissues is 1.5 (0.45 mg/kg bw / 0.3 mg/kg bw). Applying this factor to the M605F002 residue of 0.12 mg/kg found in kidney at the 10x dose level results in a calculated residue of 0.18 mg/kg. Addition of parent residue (<0.017 mg/kg (LOD) gives a total pyrimethanil residue of 0.20 mg/kg for kidney.
In case of milk, a factor of 1.3 (0.13 mg/kg bw / 0.1 mg/kg bw) was calculated. Residues of both parent and M605F003 were below the LOQ of 0.01 mg/kg in the 3x dose group. As worst case, the total residue was set to 0.02 mg/kg, which leads to a calculated residue of 0.03 mg/kg.

Therefore, no residues above the current EU MRL of 0.1 mg/kg for ruminant muscle, fat, liver and edible offals, 0.2 mg/kg for ruminant kidney and 0.05 mg/kg for milk are anticipated, regardless of the method for feed burden calculation.

No new MRLs are proposed.

Pig products

No separate feeding study with pigs has been performed since common metabolic pathways have been observed in rats and goats and therefore significant differences in the metabolic pathways from pigs as compared to ruminants are very unlikely. The proposals for maximum residue levels for pig products can therefore be derived from the cattle feeding study.

OECD approach (worst case)
The calculated feed burden is between the 1x (0.03 mg/kg bw/d) and the 3x dose level (0.1 mg/kg bw/d) of the cow feeding study. The residue levels of parent pyrimethanil and M605F002 found at the 1x dose level were below the LOQ of 0.05 mg/kg (sum: 0.10 mg/kg) in all matrices. Thus, a factor of 0.6 (0.06 mg/kg bw / 0.1 mg/kg bw) is derived considering the 3x dose level. At the 3x dose level, only residues of M605F002 in kidney were found at 0.07 mg/kg. Applying the factor of 0.6 to the M605F002 residue results in a calculated residue of 0.04 mg/kg. Addition of parent residue (<0.017 mg/kg) gives a total pyrimethanil residue of 0.06 mg/kg for kidney.

Animal Model 2016 approach
Inserting the results of the cow feeding study into the Animal Model 2016 the lowest feeding level is identified as the closest level. For muscle, fat and liver, an MRL at LOQ is derived by both interpolation and linear regression as all residues were below the LOQ for this level; a transfer factor could not be applied. In case of kidney, the transfer factor gives the highest residue compared to interpolation and linear regression, resulting in an MRL proposal of 0.06 mg/kg.

PROFile approach
The 1x dose level (0.03 mg/kg bw/d) of the cow feeding study was considered, which exceeds the calculated feed burden by a factor of about 1.5. The residue levels of parent pyrimethanil and M605F002 found at the 1x dose level were below the LOQ of 0.05 mg/kg (sum: 0.10 mg/kg) in all matrices. Therefore, no residues are to be expected in pig matrices.

Therefore, no residues above the current EU MRL of 0.1 mg/kg for swine tissues are anticipated, regardless of the method for feed burden calculation.

No new MRLs are proposed.

Poultry products

For poultry, the calculated dietary burden performed during Article 12 review was only slightly exceeding the trigger value of 0.1 mg/kg DM. Taking into consideration that this calculation includes values at LOQ, for cereal grain in particular, EFSA was of the opinion that the real exposure will be lower than 0.1 mg/kg DM and that MRLs in poultry are not required for the time being (see EFSA Reasoned Opinion 2011).
No feeding study was considered necessary. However, a metabolism study on hen was conducted which can serve as a feeding study in this case.

OECD approach = Animal Model 2016 (worst case)
A feed burden of 0.61 mg/kg feed (DM) was calculated for poultry. In the metabolism study, hens were dosed with an average of 11.3 mg/kg DM. Thus, an overdosing factor of 0.61 mg/kg DM / 11.3 mg/kg DM = 0.054 is derived. This factor is applied to the sum of metabolites of pyrimethanil found in eggs and tissues, as a worst case scenario; parent residues were not detected. An overview is given in the table below.

Table 6.7.2-30: Residues[1] from hen metabolism study and estimated residues in poultry matrices

	M605F002	M605F006	M605F023	M605F035	M605F025	Sum	Sum x overdosing factor (0.054)
Egg yolk	0.023	Not detected	0.012	0.006	Not detected	0.041	**0.002**
Egg white	0.001	<0.001		0.001	Not detected	<0.003	<0.0002
Liver	0.026	0.018	0.060	0.024	0.004	0.132	**0.007**
Muscle	0.001	Not detected	0.001	0.002	Not detected	0.004	**0.0002**
Fat	0.009	Not detected	0.003	0.007	Not detected	0.019	**0.001**

1 Residues expressed as parent equivalents

PROFile approach
A feed burden of 0.23 mg/kg feed (DM) was calculated for poultry. In the metabolism study, hens were dosed with an average of 11.3 mg/kg DM. Thus, an overdosing factor of 0.23 mg/kg DM / 11.3 mg/kg DM = 0.020 is derived. This factor is applied to the sum of metabolites of pyrimethanil found in eggs and tissues, as a worst case scenario; parent residues were not detected. An overview is given in the table below.

Table 6.7.2-31: Residues[1] from hen metabolism study and estimated residues in poultry matrices

	M605F002	M605F006	M605F023	M605F035	M605F035	Sum	Sum x overdosing factor (0.020)
Egg yolk	0.023	Not detected	0.012	0.006	Not detected	0.041	**0.0008**
Egg white	0.001	0.001		0.001	Not detected	<0.003	0.00006
Liver	0.026	0.018	0.060	0.024	0.04	0.132	**0.003**
Muscle	0.001	Not detected	0.001	0.002	Not detected	0.004	**0.00008**
Fat	0.009	Not detected	0.003	0.007	Not detected	0.019	**0.0004**

1 Residues expressed as parent equivalents

The calculated residues in eggs and poultry tissues are below the LOQ of 0.01 mg/kg and thus do not exceed the default MRLs of 0.05 mg/kg for tissues and 0.01 mg/kg for eggs.

No MRLs are proposed.

CA 6.7.3 Proposed maximum residue levels (MRLs) and justification of the acceptability of the levels proposed for imported products (import tolerance)

The MRLs listed in M-CA 6.7.2 include domestic uses, but also values for imported crops. Prior to final approval, the import tolerances have been carefully evaluated by EFSA. Parts of them are resulting from the adoption of CODEX MRLs (CXLs). Pyrimethanil and its crops have been assessed by JMPR (2007, 2013).

CA 6.8 Proposed safety intervals

Pre-harvest interval
For apples, the application is intended to meet a pre-harvest interval of 56 days.
For grapes (wine), the application is intended to meet a pre-harvest interval of 21 days.
For strawberry, application is intended to meet a pre-harvest interval of 3 days.
For lettuce, application is intended to meet a pre-harvest interval of 14 days.

Re-entry period for livestock to areas to be grazed
Because pyrimethanil is not intended to be used in areas to be grazed, no re-entry period for livestock has to be defined.

Re-entry period for man to treated crops
Re-entry assessments are given for the representative uses in the supplemental product dossiers (MCP chapter 7.2). Re-entry is possible on the day after application given the worker is wearing adequate work clothing.

Withholding period for animal feed stuffs
Grape, strawberry and lettuce are no feed items in Europe.
Treated apple feed items (apple, pomace wet) may be used as fodder for livestock. Pyrimethanil derived residues in this feed item are assessed in MCA chapter 6.7 by providing updated calculations of livestock dietary burdens and deriving suitable MRLs for animal products covering the intended uses. There is no additional withholding period needed for animal feeds with regard to pyrimethanil derived residues.

Waiting period between application and crop sowing or planting the crop to be protected
No waiting period is necessary since pyrimethanil is not intended in a pre-emergence use.

Waiting period between application and handling treated produce
This is not relevant here since a post-harvest treatment is not intended for apples, grapes, strawberries and lettuce in the context of this dossier.

Waiting period between last application and sowing or planting succeeding crops

In the confined rotational crop studies conducted (see MCA chapter 6.6), uptake of BAS 605 F residues from soil into plants was observed at all three replant intervals tested (30, 120 and 365 DAT) and for all representative crops examined. In order to investigate the uptake under more realistic conditions and to show if residues >0.01 mg/kg would occur, field rotational crop studies were conducted.
The peer-reviewed study was conducted with 2 applications of 0.8 kg a.s./ha to lettuce at growth stages of BBCH 16-19 and BBCH 43-47 with an interval of 10-11 days and a PHI of 14 days. The following crops were sampled at harvest (lettuce: PHI = 70-119 days, brassica: 109-249 days and winter wheat – straw and grain: PHI = 321-330 days). In those succeeding crops, no residues of pyrimethanil or metabolite C 621 312 (M605F005) were present above the limit of quantitation (0.05 mg/kg).
A new field rotational crop study was performed (four locations across Europe) were pyrimethanil was applied to bare soil with the maximal seasonal application rate of 3 kg a.s./ha (overdosing factor 0.8) and the following crops were replanted after 30, 120 and 365 days: wheat, carrot/radish, cauliflower/broccoli and lettuce/spinach. Pyrimethanil residues of >0.01 mg/kg but <0.05 mg/kg were found in food items (carrot roots and mature lettuce). Based on those results MRLs were proposed for leafy and root vegetables as succeeding crops.
The uptake of BAS 605 F residues from soil into the other crop categories was not relevant or covered by the current MRL. In particular, this applies to cereal grains where no detectable residues were found in the field rotational crop study and a MRL is set to 0.05 mg/kg after seed treatment use. Based on the results for wheat grain, it is expected that the residue levels of all other grains and seeds (e.g. oilseed rape, maize) being planted in rotation will also be below the LOQ of 0.01 mg/kg.
Further, for all metabolites occurring in succeeding crops it could be shown that they are of no relevance (see chapter MCA 6.7 and 6.9).
No replant restriction is needed since either the MRL proposals included in MCA chapter 6.7 are covering potential residues taken up from soil, or any residue above LOQ can be excluded in crops investigated following current guidelines.

CA 6.9 Estimation of the potential and actual exposure through diet and other sources

Assessments of the potential chronic and acute dietary consumer risk due to exposure to residues of pyrimethanil were performed using the EFSA model for chronic and acute risk assessment - rev. 2_0 (Model PRIMo). The EFSA model was used since it considers all the different diets in the EU and all consumer groups.

The ADI and ARfD for the active substance pyrimethanil are summarized in the table below. Due to their substructures, the same toxicological endpoints are also applying to the hydroxylated/oxidized and conjugated metabolites M605F001-006, M605F014, M605F020-021, M605F023, M605F027-030 and M605F034-041 (group 1).

Table 6.9-1: Toxicological endpoints - pyrimethanil and metabolites of group 1

Endpoint	Value	Study	Safety factor	Reference
Acceptable Daily Intake (ADI)	0.17 mg/kg bw/d	90 day and 2 year rat study, multigeneration study in rats	100	EFSA Conclusion (EFSA Scientific Report (2006) 61, 1-70, Conclusion on the peer review of pyrimethanil)
Acute Reference Dose (ARfD)	Not allocated - not necessary			

The ADI and ARfD for the metabolite M605F025 (phenylguanidine, group 3) are summarized in the table below. For the metabolite, genotoxicity tests have been performed as well as a 90-day toxicity study and a prenatal toxicity study in rat. They are summarized in MCA chapter 5.8.1. By weight of evidence M605F025 is not considered to be genotoxic. Based on the lowest NOAEL (17.8 mg/kg bw/d) from 90-day toxicity study in rat, an ADI was derived for metabolite M605F025. The toxicological profile of M605F025 gives no indication for derivation of an Acute Reference Dose (ARfD).

Table 6.9-2: Toxicological endpoints – M605F025 (group 3)

Endpoint	Value	Study	Safety factor	Reference
Acceptable Daily Intake (ADI)	0.089 mg/kg bw/d	90 day study in rats	200	M-CA 5.8.1
Acute Reference Dose (ARfD)	Not allocated - not necessary			

The ADI used for the metabolites M605F007 and M605F008, the hydroxylates M605F016 and M605F032 of M605F007 and the glucose conjugate M605F033 of M605F032 (group 2) is summarized in the table below. For group 2 metabolites, genotoxicity tests have been performed. As none of them showed any genotoxic potential, TTC values (Cramer class III) can be used for an indicative dietary exposure assessment. A detailed evaluation is given in chapter MCA 5.8.1.

Table 6.9-3: Toxicological endpoints – TTC approach (used for metabolites of group 2)

Endpoint	Value	Study	Safety factor	Reference
Acceptable Daily Intake (ADI)	0.0015 mg/kg bw/d	None	Not relevant	TTC

Since the data for parent do not lead to the need for an Acute Reference Dose (ARfD), no ARfD is proposed for the metabolites, either. Pyrimethanil is not neurotoxic and does not cause developmental toxicity.

Acceptable Daily Intake (ADI) and Dietary Exposure Calculation

Pyrimethanil

TMDI calculation

A revised chronic exposure assessment was performed using all crops and maximum residue levels (established and proposed EU MRL values based on rotational crop data from root and tuber vegetables and leafy and Brassica vegetables) summarized in Table 6.9-4.
The summary of the calculation using the EFSA model rev 2.0 is presented in Table 6.9-5. For the assessment, an ADI of 0.17 mg/kg bw/day was used. According to the EFSA model the TMDI has been simultaneously calculated for adults, children, toddlers and infants (different age groups), vegetarian and elderly in different EU countries.

Table 6.9-4: Pyrimethanil - MRL values used for risk assessment

Code number	Groups and examples of individual products to which the MRLs apply	Pyrimethanil (R)
100000	. FRUITS, FRESH or FROZEN; TREE NUTS	
110000	. Citrus fruits	8
120000	. Tree nuts	
120010	. Almonds	0.2
120020	. Brazil nuts	0.02*
120030	. Cashew nuts	0.02*
120040	. Chestnuts	0.02*
120050	. Coconuts	0.02*
120060	. Hazelnuts/cobnuts	0.02*
120070	. Macadamias	0.02*
120080	. Pecans	0.02*
120090	. Pine nut kernels	0.02*
120100	. Pistachios	0.2
120110	. Walnuts	0.02*
120990	. Others (2)	0.02*
130000	. Pome fruits	15
140000	. Stone fruits	
140010	. Apricots	10
140020	. Cherries (sweet)	4
140030	. Peaches	10
140040	. Plums	2
140990	. Others (2)	0.01*

Table 6.9-4: Pyrimethanil - MRL values used for risk assessment

150000	. Berries and small fruits	
151000	. (a) Grapes	5
152000	. (b) Strawberries	5
153000	. (c) Cane fruits	
153010	. Blackberries	10
153020	. Dewberries	0.01*
153030	. Raspberries (red and yellow)	10
153990	. Others (2)	0.01*
154000	. (d) other small fruits and berries	
154010	. Blueberries	5
154020	. Cranberries	5
154030	. Currants (black, red and white)	5
154040	. Gooseberries (green, red and yellow)	5
154050	. Rose hips	5
154060	. Mulberries (black and white)	5
154070	. Azaroles/Mediterranean medlars	15
154080	. Elderberries	5
154990	. Others (2)	5
160000	. Miscellaneous fruits with	
161000	. (a) edible peel	
161010	. Dates	0.01*
161020	. Figs	0.01*
161030	. Table olives	0.02*
161040	. Kumquats	0.01*
161050	. Carambolas	0.01*
161060	. Kaki/Japanese persimmons	15
161070	. Jambuls/jambolans	0.01*
161990	. Others (2)	0.01*
162000	. (b) inedible peel, small	0.01*
163000	. (c) inedible peel, large	
163010	. Avocados	0.01*
163020	. Bananas	0.1
163030	. Mangoes	0.01*
163040	. Papayas	0.01*
163050	. Granate apples/pomegranates	0.01*
163060	. Cherimoyas	0.01*
163070	. Guavas	0.01*
163080	. Pineapples	0.01*
163090	. Breadfruits	0.01*
163100	. Durians	0.01*
163110	. Soursops/guanabanas	0.01*
163990	. Others (2)	0.01*

Table 6.9-4: Pyrimethanil - MRL values used for risk assessment

200000	. VEGETABLES, FRESH or FROZEN	
210000	. Root and tuber vegetables	
211000	. (a) potatoes	0.05* / 0.05[4]
212000	. (b) tropical root and tuber vegetables	0.01* / 0.05[4]
213000	. (c) other root and tuber vegetables except sugar beets	
213010	. Beetroots	0.01* / 0.05[4]
213020	. Carrots	1
213030	. Celeriacs/turnip rooted celeries	0.01* / 0.05[4]
213040	. Horseradishes	0.01* / 0.05[4]
213050	. Jerusalem artichokes	0.01* / 0.05[4]
213060	. Parsnips	0.01* / 0.05[4]
213070	. Parsley roots/Hamburg roots parsley	0.01* / 0.05[4]
213080	. Radishes	0.01* / 0.05[4]
213090	. Salsifies	0.01* / 0.05[4]
213100	. Swedes/rutabagas	0.01* / 0.05[4]
213110	. Turnips	0.01* / 0.05[4]
213990	. Others (2)	0.01* / 0.05[4]
220000	. Bulb vegetables	
220010	. Garlic	0.01* / 0.05[4]
220020	. Onions	0.2
220030	. Shallots	0.01* / 0.05[4]
220040	. Spring onions/green onions and Welsh onions	3
220990	. Others (2)	0.01* / 0.05[4]
230000	. Fruiting vegetables	
231000	. (a) solanacea	
231010	. Tomatoes	1
231020	. Sweet peppers/bell peppers	2
231030	. Aubergines/eggplants	1
231040	. Okra/lady's fingers	0.01*
231990	. Others (2)	0.01*
232000	. (b) cucurbits with edible peel	0.7
233000	. (c) cucurbits with inedible peel	0.01*
234000	. (d) sweet corn	0.01*
239000	. (e) other fruiting vegetables	0.01*
240000	. Brassica vegetables (excluding brassica roots and brassica baby leaf crops)	0.01* / 0.2[4]
250000	. Leaf vegetables, herbs and edible flowers	
251000	. (a) lettuces and salad plants	
251010	. Lamb's lettuces/corn salads	0.01* / 0.2[4]
251020	. Lettuces	20
251030	. Escaroles/broad-leaved endives	20
251040	. Cresses and other sprouts and shoots	0.01* / 0.2[4]
251050	. Land cresses	0.01* / 0.2[4]
251060	. Roman rocket/rucola	0.01* / 0.2[4]
251070	. Red mustards	0.01* / 0.2[4]
251080	. Baby leaf crops (including brassica species)	20
251990	. Others (2)	0.01* / 0.2[4]
252000	. (b) spinaches and similar leaves	0.01* / 0.2[4]
253000	. (c) grape leaves and similar species	0.01* / 0.2[4]
254000	. (d) watercresses	0.01* / 0.2[4]
255000	. (e) witloofs/Belgian endives	0.01* / 0.2[4]
256000	. (f) herbs and edible flowers	20

Table 6.9-4: Pyrimethanil - MRL values used for risk assessment

260000	. Legume vegetables	
260010	. Beans (with pods)	3
260020	. Beans (without pods)	0.01*
260030	. Peas (with pods)	3
260040	. Peas (without pods)	0.2
260050	. Lentils	0.01*
260990	. Others (2)	0.01*
270000	. Stem vegetables	
270010	. Asparagus	0.01* / 0.2[4]
270020	. Cardoons	0.01* / 0.2[4]
270030	. Celeries	0.01* / 0.2[4]
270040	. Florence fennels	0.01* / 0.2[4]
270050	. Globe artichokes	0.01* / 0.2[4]
270060	. Leeks	3[5]
270070	. Rhubarbs	0.01* / 0.2[4]
270080	. Bamboo shoots	0.01* / 0.2[4]
270090	. Palm hearts	0.01* / 0.2[4]
270990	. Others (2)	0.01* / 0.2[4]
280000	. Fungi, mosses and lichens	0.01*
290000	. Algae and prokaryotes organisms	0.01*
300000	. PULSES	
300010	. Beans	0.5
300020	. Lentils	0.5
300030	. Peas	0.5
300040	. Lupins/lupini beans	0.5
300990	. Others (2)	0.01*
400000	. OILSEEDS AND OIL FRUITS	0.02*
500000	. CEREALS	
500010	. Barley	0.05*[1]
500020	. Buckwheat and other pseudo-cereals	0.01*
500030	. Maize/corn	0.01*
500040	. Common millet/proso millet	0.01*
500050	. Oat	0.05*[1]
500060	. Rice	0.05*
500070	. Rye	0.05*[1]
500080	. Sorghum	0.05*
500090	. Wheat	0.05*[1]
500990	. Others (2)	0.01*
600000	. TEAS, COFFEE, HERBAL INFUSIONS, COCOA AND CAROBS	
610000	. Teas	0.05*
620000	. Coffee beans	0.05*
630000	. Herbal infusions from	
631000	. (a) flowers	0.05*
632000	. (b) leaves and herbs	0.05*
633000	. (c) roots	
633010	. Valerian	0.05*
633020	. Ginseng	1.5
633990	. Others (2)	0.05*
639000	. (d) any other parts of the plant	0.05*
640000	. Cocoa beans	0.05*
650000	. Carobs/Saint John's breads	0.05*

Table 6.9-4: Pyrimethanil - MRL values used for risk assessment

700000	. HOPS	0.05* / 0.2[4]
800000	. SPICES	
810000	. Seed spices	0.05*
820000	. Fruit spices	0.05*
830000	. Bark spices	0.05*
840000	. Root and rhizome spices	
840010	. Liquorice	0.05*
840020	. Ginger	0.05*
840030	. Turmeric/curcuma	0.05*
840040	. Horseradish	[2]
840990	. Others (2)	0.05*
850000	. Bud spices	0.05*
860000	. Flower pistil spices	0.05*
870000	. Aril spices	0.05*
900000	. SUGAR PLANTS	0.01*
1000000	. PRODUCTS OF ANIMAL ORIGIN -TERRESTRIAL ANIMALS	[3]
1010000	. Tissues from	[3]
1011000	. (a) swine	0.1*[3]
1012000	. (b) bovine	[3]
1012010	. Muscle	0.1*[3]
1012020	. Fat tissue	0.1*[3]
1012030	. Liver	0.1*[3]
1012040	. Kidney	0.2[3]
1012050	. Edible offals (other than liver and kidney)	0.1*[3]
1012990	. Others (2)	0.1*[3]
1013000	. (c) sheep	[3]
1013010	. Muscle	0.1*[3]
1013020	. Fat tissue	0.1*[3]
1013030	. Liver	0.1*[3]
1013040	. Kidney	0.2[3]
1013050	. Edible offals (other than liver and kidney)	0.1*[3]
1013990	. Others (2)	0.1*[3]
1014000	. d) goat	[3]
1014010	. Muscle	0.1*[3]
1014020	. Fat tissue	0.1*[3]
1014030	. Liver	0.1*[3]
1014040	. Kidney	0.2[3]
1014050	. Edible offals (other than liver and kidney)	0.1*[3]
1014990	. Others (2)	0.1*[3]
1015000	. (e) equine	[3]
1015010	. Muscle	0.1*[3]
1015020	. Fat tissue	0.1*[3]
1015030	. Liver	0.1*[3]
1015040	. Kidney	0.2[3]
1015050	. Edible offals (other than liver and kidney)	0.1*[3]
1015990	. Others (2)	0.1*[3]
1016000	. (f) poultry	0.05*[3]

Table 6.9-4: Pyrimethanil - MRL values used for risk assessment

1017000	. (g) other farmed terrestrial animals	[3]
1017010	. Muscle	0.1*[3]
1017020	. Fat tissue	0.1*[3]
1017030	. Liver	0.1*[3]
1017040	. Kidney	0.2[3]
1017050	. Edible offals (other than liver and kidney)	0.1*[3]
1017990	. Others (2)	0.1*[3]
1020000	. Milk	0.05[3]
1030000	. Birds eggs	0.01*[3]
1040000	. Honey and other apiculture products	0.05*[3]
1050000	. Amphibians and Reptiles	0.05*[3]
1060000	. Terrestrial invertebrate animals	0.05*[3]
1070000	. Wild terrestrial vertebrate animals	0.05*[3]

(R) The residue definition differs for the following combinations pesticide-code number:
Pyrimethanil - code 1020000: Sum of pyrimethanil and 2-anilino-4.6-dimethylpyrimidine-5-ol, expressed as pyrimethanil
Pyrimethanil - codes 1011000/1012000/1013000/1014000/1015000/1017000: Sum of pyrimethanil and 2-(4-hydroxyanilino)-4.6-dimethylpyrimidine, expressed as pyrimethanil

* Indicates lower limit of analytical determination

1 The European Food Safety Authority identified some information on crop metabolism with seed treatment as unavailable. When re-viewing the MRL, the Commission will take into account the information referred to in the first sentence, if it is submitted by 5 February 2016, or, if that information is not submitted by that date, the lack of it.

2 The applicable maximum residue level for horseradish (*Armoracia rusticana*) in the spice group (code 0840040) is the one set for horseradish (*Armoracia rusticana*) in the vegetables category, root and tuber vegetables group (code 0213040) taking into account changes in the levels by processing (drying) according to Art. 20 (1) of Regulation (EC) No 396/2005.

3 The European Food Safety Authority identified some information on analytical methods as unavailable. When re-viewing the MRL, the Commission will take into account the information referred to in the first sentence, if it is submitted by 5 February 2016, or, if that information is not submitted by that date, the lack of it.

4 Proposed in this document based on field rotational crop data

5 New MRL in leek currently applied for

With the current EFSA model the chronic risk assessment ranges from 13 to 149% of the ADI (see Table 6.9-5). The diet with the highest TMDI is "DE child" with 149.1% of the ADI. For this diet, the highest contributors are pome fruit with 112.2% of the ADI. The diet with the second highest TMDI is “NL child” with 93.2% of the ADI, in which also pome fruit are the major contributors with 59.6% of the ADI.

The ADI utilization exceeds the ADI for one diet only when using MRL values. Thus, a refinement was performed (see NEDI calculation below).

Table 6.9-5: Pyrimethanil (BAS 605 F): TMDI calculation based on input values listed in Table 6.9-4

Pyrimethanil			
Status of the active substance:	**Approved**	Code no.	**BAS 605 F**
LOQ (mg/kg bw):		proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	**0.17**	ARfD (mg/kg bw):	**Not applicable**
Source of ADI:	**Dir 2006/74**	Source of ARfD:	**Dir 2006/74**
Year of evaluation:	**2006**	Year of evaluation:	**2006**

Chronic risk assessment

TMDI (range) in % of ADI minimum - maximum	
13	149
No of diets exceeding ADI:	1

Highest calculated TMDI values in % of ADI	MS Diet	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities	pTMRLs at LOQ (in % of ADI)
149.1	DE child	112.2	Pome fruit	21.6	Citrus fruit	3.7	Table and wine grapes	
93.2	NL child	59.6	Pome fruit	19.1	Citrus fruit	2.4	Scarole (broad-leaf endive)	
47.2	FR toddler	25.4	Pome fruit	11.0	Citrus fruit	1.9	Beans (with pods)	
40.7	WHO Cluster diet B	12.4	Pome fruit	7.3	Citrus fruit	6.3	Table and wine grapes	
40.5	IE adult	13.2	Pome fruit	12.6	Citrus fruit	3.4	Table and wine grapes	
37.2	FR infant	24.9	Pome fruit	5.0	Citrus fruit	1.6	Carrots	
34.7	ES child	14.4	Pome fruit	11.0	Citrus fruit	4.9	Lettuce	
34.3	DK child	26.5	Pome fruit	1.7	Lettuce	1.4	Citrus fruit	
33.0	UK Toddler	16.6	Pome fruit	10.9	Citrus fruit	0.8	Table and wine grapes	
29.2	NL general	12.0	Pome fruit	8.7	Citrus fruit	2.5	Table and wine grapes	
28.6	PT General population	12.5	Pome fruit	8.1	Table and wine grapes	3.6	Citrus fruit	
28.0	ES adult	9.8	Pome fruit	6.8	Citrus fruit	6.3	Lettuce	
27.5	UK Infant	16.0	Pome fruit	6.3	Citrus fruit	1.1	Milk and cream,	
25.0	FR all population	12.1	Table and wine grapes	5.3	Pome fruit	3.1	Citrus fruit	
25.0	WHO cluster diet E	9.1	Pome fruit	5.3	Table and wine grapes	3.9	Citrus fruit	
24.6	SE general population 90th percentile	12.4	Pome fruit	6.5	Citrus fruit	0.9	Peaches	
24.5	PL general population	20.5	Pome fruit	0.9	Table and wine grapes	0.6	Citrus fruit	
23.5	IT kids/toddler	10.9	Pome fruit	3.5	Citrus fruit	3.4	Lettuce	
22.8	WHO regional European diet	7.5	Pome fruit	4.4	Lettuce	3.8	Citrus fruit	
22.0	IT adult	9.1	Pome fruit	4.4	Lettuce	2.7	Citrus fruit	
20.6	WHO Cluster diet F	6.8	Pome fruit	5.3	Citrus fruit	3.5	Lettuce	
20.6	LT adult	17.9	Pome fruit	0.7	Lettuce	0.5	Citrus fruit	
17.2	UK vegetarian	5.9	Pome fruit	4.9	Citrus fruit	2.6	Table and wine grapes	
16.4	DK adult	8.7	Pome fruit	4.3	Table and wine grapes	1.2	Citrus fruit	
14.9	WHO cluster diet D	6.7	Pome fruit	2.2	Citrus fruit	1.6	Table and wine grapes	
13.7	UK Adult	4.1	Pome fruit	3.3	Table and wine grapes	3.3	Citrus fruit	
12.8	FI adult	5.3	Citrus fruit	3.8	Pome fruit	1.0	Table and wine grapes	

IEDI calculation

Since the ADI utilization exceeds the ADI for one diet when using MRL values, a refinement was performed using the STMR value of 1.6 mg/kg for pome fruit (see chapter 6.7.2).

The refined chronic risk assessment ranges from 5 to 49% of the ADI (see Table 6.9-6). The diet with the highest TMDI is "DE child" with 48.9% of the ADI. For this diet, the highest contributors are citrus fruit with 21.6% of the ADI. The diet with the second highest TMDI is “NL child” with 40.0% of the ADI, in which also citrus fruit are the major contributors with 19.1% of the ADI.

According to the presented IEDI calculation a chronic intake of pyrimethanil residues is unlikely to present a public health concern.

Table 6.9-6: Pyrimethanil (BAS 605 F): IEDI calculation based on input values listed in Table 6.9-4 and STMR for pome fruit

Pyrimethanil			
Status of the active substance:	**Approved**	Code no.	**BAS 605 F**
LOQ (mg/kg bw):		proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	**0.17**	ARfD (mg/kg bw):	**Not applicable**
Source of ADI:	**Dir 2006/74**	Source of ARfD:	**Dir 2006/74**
Year of evaluation:	**2006**	Year of evaluation:	**2006**

Chronic risk assessment

TMDI (range) in % of ADI minimum - maximum	
5	49
No of diets exceeding ADI:	---

Highest calculated TMDI values in % of ADI	MS Diet	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities	pTMRLs at LOQ (in % of ADI)
48.9	DE child	21.6	Citrus fruit	12.0	Pome fruit	3.7	Table and wine grapes	
40.0	NL child	19.1	Citrus fruit	6.4	Pome fruit	2.4	Scarole (broad-leaf endive)	
29.7	WHO Cluster diet B	7.3	Citrus fruit	6.3	Table and wine grapes	4.2	Lettuce	
28.7	IE adult	12.6	Citrus fruit	3.4	Table and wine grapes	3.3	Peaches	
24.5	FR toddler	11.0	Citrus fruit	2.7	Pome fruit	1.9	Beans (with pods)	
21.9	ES child	11.0	Citrus fruit	4.9	Lettuce	1.5	Pome fruit	
20.3	FR all population	12.1	Table and wine grapes	3.1	Citrus fruit	1.1	Lettuce	
19.2	ES adult	6.8	Citrus fruit	6.3	Lettuce	1.4	Table and wine grapes	
18.5	NL general	8.7	Citrus fruit	2.5	Table and wine grapes	1.4	Lettuce	
18.2	UK Toddler	10.9	Citrus fruit	1.8	Pome fruit	0.8	Table and wine grapes	
17.5	PT General population	8.1	Table and wine grapes	3.6	Citrus fruit	2.1	Peaches	
16.9	WHO cluster diet E	5.3	Table and wine grapes	3.9	Citrus fruit	1.1	Lettuce	
16.1	WHO regional European diet	4.4	Lettuce	3.8	Citrus fruit	1.2	Peaches	
15.0	FR infant	5.0	Citrus fruit	2.7	Pome fruit	1.6	Carrots	
14.5	WHO Cluster diet F	5.3	Citrus fruit	3.5	Lettuce	2.2	Table and wine grapes	
13.9	IT adult	4.4	Lettuce	2.7	Citrus fruit	2.2	Peaches	
13.8	IT kids/toddler	3.5	Citrus fruit	3.4	Lettuce	2.0	Peaches	
13.5	SE general population 90th percentile	6.5	Citrus fruit	1.3	Pome fruit	0.9	Peaches	
13.2	UK Infant	6.3	Citrus fruit	1.7	Pome fruit	1.1	Milk and cream,	
12.0	UK vegetarian	4.9	Citrus fruit	2.6	Table and wine grapes	1.7	Lettuce	
10.7	DK child	2.8	Pome fruit	1.7	Lettuce	1.4	Citrus fruit	
10.0	UK Adult	3.3	Table and wine grapes	3.3	Citrus fruit	1.4	Lettuce	
9.4	FI adult	5.3	Citrus fruit	1.0	Table and wine grapes	0.9	Lettuce	
9.0	WHO cluster diet D	2.2	Citrus fruit	1.6	Table and wine grapes	0.8	Herbs	
8.5	DK adult	4.3	Table and wine grapes	1.2	Citrus fruit	0.9	Pome fruit	
6.2	PL general population	2.2	Pome fruit	0.9	Table and wine grapes	0.6	Citrus fruit	
4.6	LT adult	1.9	Pome fruit	0.7	Lettuce	0.5	Citrus fruit	

Metabolites

The main purpose of the information presented below is to support the establishment of a robust residue definition for risk assessment purposes. Therefore, the data being summarized are going far beyond the scope of this dossier which supports the uses in apples, grapes, strawberry and lettuce. In the assessment the contributions to the chronic dietary risk of all crops that are currently registered in the EU (including import tolerances) were taken into account.

For facilitating the evaluation of the exposure assessment performed, the relevant exposure data for the metabolites are derived in a separate report.

Report:	CA 6.9/1 Benz-Birck A.,Arndt I., 2015a Pyrimethanil (BAS 605 F): Evaluation of relevance of Pyrimethanil metabolites in dietary risk assessment 2015/1188589
Guidelines:	none
GLP:	no

B. STUDY DESIGN AND METHODS

Grouping

In order to assess the contribution of the metabolites to the chronic dietary risk, the metabolites being present in the plant and livestock metabolism studies were grouped into three groups. Grouping of metabolites was done based on toxicological assessment. This means that the same endpoints (Acceptable Daily Intake - ADI) are applicable for all metabolites in one group. Grouping does not concern the consumer risk assessment in any other way, i.e. risk assessments were performed for each single metabolite with the respective group endpoints. A combined risk assessment for all metabolites in one group is not necessary.

The assignment to groups is based on substructures. For details on grouping see chapter MCA 5.8.1.

- Group 1: Hydroxylation/oxidation products and their conjugates (Hydroxylation/oxidation products of parent: M605F002, M605F003, M605F004, M605F005, M605F006, M605F034 and their conjugates (Glucose conjugates, Malonylglucose conjugates, Glucuronides and Sulfates): M605F001, M605F014, M605F020, M605F021, M605F023, M605F027, M605F028, M605F029, M605F030, M605F035, M605F036, M605F037, M605F038, M605F039, M605F040, M605F041)
- Group 2: Pyrimidin-moiety (M605F007, M605F008, M605F016, M605F032) and conjugate (M605F033)
- Group 3: Phenyl-moiety (M605F025)

Occurrence
The metabolite occurrence and residue levels are described in detail for each group in document 2015/1188589. A general overview is given below.

Group 1
In the plant metabolism studies, several metabolites with the relevant substructure have been identified. None of these plant metabolites exceeds 10% TRR and 0.01 mg/kg in food items, except M605F001 in vine leaves with 15.8-16.8% TRR.
In the rotational crop metabolism study the same metabolites where found and in addition a di-hydroxylate (M605F006). Only M605F005 and M605F040 exceed 10% TRR and 0.01 mg/kg in food items. M605F005 was only found in the "old" rotational crop study and not in the newly conducted one; it was never found in the field rotational crop study >0.01 mg/kg and is therefore not considered relevant.
All metabolites found in livestock belong to group 1 with the exception of M605F025 (phenylguanidine, group 3).

Group 2
Pyrimidin-moiety metabolites are cleavage products of pyrimethanil and were found in the newly conducted metabolism studies on lettuce (foliar application) and wheat (seed treatment) as well as the old and new rotational crop studies.
In the plant metabolism studies, the metabolites M605F032 and M605F033 with the relevant substructure have been identified. None of these plant metabolites exceeds 10% TRR and 0.01 mg/kg in food items.
In the rotational crop metabolism study all metabolites of this group (M605F007, M605F008, M605F016, M605F032 and M605F033) have been identified. M605F007, M605F016 and M605F033 exceed 10% TRR and 0.01 mg/kg in food items.
Pyrimidin-moiety metabolites (group 2) were not identified in edible livestock matrices.

Group 3
Phenyl-moiety metabolite M605F025 is a cleavage product of pyrimethanil and was exclusively found in the newly conducted metabolism studies on lettuce (foliar application) and wheat (seed treatment) as well as the new rotational crop study and the livestock metabolism studies in goat and hen.
In the plant metabolism studies, metabolite M605F025 does not exceed 10% TRR and 0.01 mg/kg in food items.
In the rotational crop metabolism study M605F025 exceeds 10% TRR and 0.01 mg/kg in food items. In the field rotational crop study, M605F025 was not found in food items at or above the LOQ of 0.01 mg/kg. Nevertheless, values <LOQ and >LOD were used in risk assessment to show non-relevance.
In livestock metabolism studies, M605F025 exceeds 10% TRR and 0.01 mg/kg in goat liver.

II. RESULTS AND DISCUSSION

Risk assessment

An assessment of the potential chronic dietary consumer risk due to exposure to residues of pyrimethanil metabolites was performed using the EFSA model (PRIMo rev. 2_0). The EFSA model was used since it considers all the different diets in the EU and all consumer groups.
Chronic assessment calculations use a number of worst-case scenarios with respect to dietary burden. It is assumed that the market penetration is 100%, i.e. all crops have been treated with the respective active substance. Effects of processing are not considered. Thus, the actual consumer exposure will be significantly lower in reality.

The input values used for the assessment are presented in document 2015/1188589. The results of the risk assessments are summarized below.

Table 6.9-7: Summary table of all metabolites and the contribution to chronic dietary risk

Metabolite group	Metabolites	ADI [mg/kg bw/day]	Contribution	ADI utilization [%]
1	M605F001	0.17	Plant	0.2
	M605F002		Plant Rot crop Animal Overall	0.1 0.1 0.01 0.2
	M605F003		Plant Rot crop Animal Overall	0.2 0.1 0.3 0.4
	M605F004		Plant Rot crop Animal Overall	0.2 0.1 0.001 0.2
	M605F005		Rot crop	0.2
	M605F006		Rot crop Animal Overall	0.04 0.003 0.04
	M605F014		Animal	0.002
	M605F020		Animal	0.001
	M605F021		Animal	0.2
	M605F023		Animal	0.2
	M605F027		Plant Rot crop Overall	0.8 0.03 0.8
	M605F028		Plant Rot crop Overall	0.4 0.02 0.4
	M605F029		Plant	0.5
	M605F030		Plant	0.3
	M605F034		Animal	0.01
	M605F035		Animal	0.05
	M605F036		Plant Rot crop Overall	0.5 0.01 0.5
	M605F037		Plant	0.01
	M605F038		Plant	0.5
	M605F039		Plant Rot crop Overall	0.4 0.01 0.5
	M605F040		Plant Rot crop Overall	0.5 0.04 0.5
	M605F041		Plant	0.3

Table 6.9-7: Summary table of all metabolites and the contribution to chronic dietary risk

Metabolite group	Metabolites	ADI [mg/kg bw/day]	Contribution	ADI utilization [%]
2	M605F007	~~0.17~~ 0.0015	Rot crop	6.1 (metabolism data) 9.4 (field data)
	M605F008		Rot crop	3.3
	M605F016		Rot crop	3.6
	M605F032		Rot crop	2.1
	M605F033		Plant Rot crop Overall	56.3 9.0 59.7
3	M605F025	~~0.17~~ 0.089	Plant Rot crop Animal Overall	0.1 3.3 (metabolism data) 0.03 (field data) 0.03 3.3 (metabolism data) 0.2 (field data)

Group 1
With the current EFSA model the maximum chronic risk assessment per metabolite ranges from 0.001 to 0.8% of the ADI. The diet with the highest TMDI is "DE child" with 0.8% of the ADI due to intake of M605F027. For this diet, the highest contributor is pome fruit with 0.3% of the ADI. The diet with the second highest TMDI is “NL child” with 0.7% of the ADI due to intake of M605F027, in which citrus fruit is the major contributor with 0.2% of the ADI.

Group 2
With the current EFSA model the maximum chronic risk assessment per metabolite ranges from 2.1 to 59.7% of the ADI. The diet with the highest TMDI is "ES adult" with 59.7% of the ADI due to intake of M605F033. For this diet, the highest contributor is lettuce with 57.3% of the ADI. The diet with the second highest TMDI is “WHO Cluster diet B” with 55.1% of the ADI, again due to intake of M605F027, in which also lettuce is the major contributor with 38.4% of the ADI.
Looking at the contribution to the dietary burden of M605F033, it was shown that residues in rotational crops have a comparably low influence; the majority of the ADI utilization arises from residues after foliar application.
Comparing the risk assessment of M605F007 using rotational crop data from the metabolism study on the one hand and ~~interim~~ field rotational crop data on the other, it was shown that there is no chronic risk in either scenario; the field data are still safe. As metabolite M605F007 was analyzed as marker compound for group 2, it can be reasonably assumed that this statement is accurate for the whole group.

Group 3
With the current EFSA model the chronic risk assessment ranges from 0.2 to 3.3% of the ADI. The diet with the highest TMDI is "UK Toddler" with 3.3% of the ADI. For this diet, the highest contributor is sugar beet (root) with 2.7% of the ADI. The diet with the second highest TMDI is “UK Infant” with 1.9% of the ADI, in which also sugar beet (root) is the major contributor with 1.2% of the ADI.

III. CONCLUSION

Pyrimethanil follows a common pathway in crops and livestock. In general, the following key transformation steps were found:

- Hydroxylation / oxidation of the parent compound
- Conjugation of the hydroxylated / oxidized products
- Ring opening of the pyrimidine ring
- Cleavage of the amine bound (not in livestock)

These steps are common in all metabolism studies (except for cleavage of the amine bond which does not occur in livestock); the differences observed are more on the quantitative level.

For performing an indicative assessment, the metabolites were first grouped according to the resulting substructures. In a second step, the exposure was estimated based on all available data (mainly of metabolism information and data from succeeding crop residue field trials). Subsequently, chronic dietary exposure assessments was performed for identifying the contributions of the metabolites to the total dietary risk.

The exposure estimates applying worst case assumptions did not indicate any dietary concern. The calculation of the % ADI resulted in values far below 100%. No significant contribution to the chronic dietary risk can be expected from any plant or livestock metabolite.

Acute Reference Dose (ARfD) and Dietary Exposure Calculation

Pyrimethanil

No acute reference dose has been set or is considered necessary. The following statement was copied from the EFSA Conclusion 2006:

"Acute oral toxicity studies demonstrate the low acute toxicity of pyrimethanil. No adverse effects were observed early in repeated dose studies at dose levels that were relevant for human exposure. No developmental toxicity was induced by pyrimethanil at dose levels below maternal toxicity. From the evaluation of the available toxicological data base of pyrimethanil it can be concluded that there is no need to establish an ARfD."

The 2007 JMPR also decided that an ARfD is unnecessary. The Meeting therefore concluded that the short-term intake of pyrimethanil residues is unlikely to present a public health concern.

The statements of EFSA and JMPR are still valid - no ARfD is necessary for pyrimethanil.

Metabolites

Since the data for parent do not lead to the need for an Acute Reference Dose (ARfD), no ARfD is proposed for the metabolites, either pyrimethanil is not neurotoxic and does not cause developmental toxicity.

CA 6.10 Other studies

No other/special studies are deemed necessary. The studies and information provided in the previous sections are considered adequate and sufficient.

CA 6.10.1 Effect on the residue level in pollen and bee products

The objective of these studies shall be to determine the residue in pollen and bee products for human consumption resulting from residues taken up by honeybees from crops at blossom.

In the absence of validated test method or guidance documents, and in agreement with the RMS this data requirement is waived in accordance to SANCO Guidance Document SANCO/10181/2013-rev 2.1 (13 May 2013).

Pyrimethanil

DOCUMENT M-CA, Section 7

FATE AND BEHAVIOUR IN THE ENVIRONMENT

Compiled by:

[redacted]

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E-mail:

Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
Oct. 2015	Initial AIR3 dossier	BASF DocID 2015/1004013
Sept 2017	CA 7.1.2.1.2 Aerobic degradation of metabolite, breakdown and reaction products: additional kinetic evaluation added in order to address concerns of the RMS (BASF DocID 2017/1066535)	BASF DocID 2017/1134391
Sept 2017	CA 7.2.3: Additional information regarding the effect of water treatment procedures on the nature of residues when surface water or groundwater are abstracted for drinking water (BASF DocID 2017/1121801).	BASF DocID 2017/1134391

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CA 7 FATE AND BEHAVIOUR IN THE ENVIRONMENT

Pyrimethanil (BAS 605 F), a fungicide for use in pome fruits, grapevine, strawberries, lettuce and various other crops, is registered in Europe since many years. It was fully reviewed under Directive 91/414/EEC and included in Annex I by Commission Directive 2006/74/EC of 21 August 2006. Inclusion entered into force on 1 June 2007. The approval was transferred to the new Regulation (EC) No 1107/2009 in Commission Implementing Regulation (EU) No 540/2011. Approval extension was granted until 30 April 2018 by Regulation No 678/2014/EU.

All relevant information on the first Annex I review and the endpoints used in environmental risk assessments can be found in the DAR of pyrimethanil and in the EFSA scientific report (13 January 2006) 61, 1 - 70.

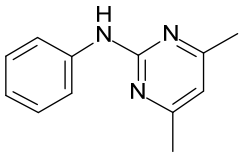
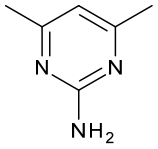
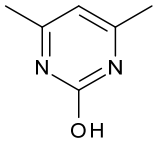
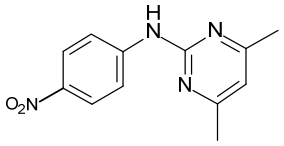
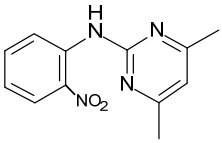
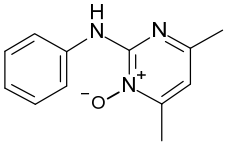
For the current registration renewal under Regulation 1107/2009, a data gap analysis according to new guidelines, new guidance documents and new procedures in kinetic evaluations and exposure assessments was performed and new studies or kinetic evaluations were initiated where considered necessary. All new data are provided in this section.

Furthermore, a literature search was performed and scientific publications were evaluated for their endpoint relevance and quality. Although title and abstract of several publications indicated a potential connection to respective environmental fate chapters of this dossier, the detailed evaluation of most publications showed no endpoint of sufficient reliability which could be used for the required exposure or risk assessments. In cases where publications were summarized and included into this dossier, the information is considered only as supplemental or as confirmation of the existing GLP data.

Studies on the route of degradation were performed using the phenyl-^{14}C or pyrimidine-^{14}C labeled pyrimethanil.

An overview of metabolites and substance names and designations discussed in this section is given below. The table is including the different code numbers that are available for each metabolite. In the following chapters and study summaries, synonym metabolite codes are given in brackets where deemed to be helpful.

Table 7-1: Substance codes and chemical structures of pyrimethanil and metabolites used in environmental fate dossier chapters

Substance/ Metabolite Code	Reference code (Reg.No.)	Synonyms	Structure	Compound found in:
Pyrimethanil BAS 605 F	236999	AE B100309 SN 100309 HOE 123521 CL 189865 M605F000		soil water sediment
M605F007 2-amino-4,6-dimethyl-pyrimidine (ADMP)	40603	AE F132593 SN 512723 ZK 512723 AE C512723 NC 12723 S151 CL 2869		soil water sediment
M605F009 2-hydroxy-4,6-dimethyl-pyrimidine	51589	AE F132512 SN 469626 CL 17345 Std. 155		soil water/sediment
M605F011*	4180488	SN 617916*		soil water/sediment
M605F012*	5079483	SN 603193*		soil water/sediment
M605F010*	n.a.	AZ 196920*		soil

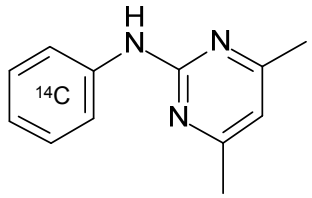
* only observed at extremely high pyrimethanil soil appl. rates of ≥ 100 mg/kg (occurrence < 1% of applied radioactivity)
n.a. = not assigned

Note: The order of the study summaries shown below might be differing compared to the information given in the application submitted for renewal of approval. In case references are summarized that were not contained in the application or in case references listed in the application are not contained in this chapter, comments are made where appropriate.

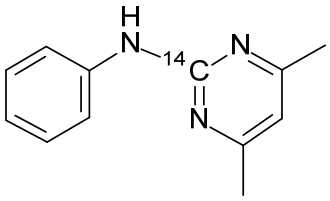
CA 7.1 Fate and behaviour in soil

CA 7.1.1 Route of degradation in soil

The route of degradation in soil of pyrimethanil was overall investigated with two different radio-labels (phenyl- and pyrimidinyl-label).



U-^{14}C-phenyl-label



2-^{14}C-pyrimidinyl-label

Most information on the route of degradation in soil is derived from studies already peer-reviewed during the previous Annex I inclusion process. Only a new soil photolysis study was initiated since the old study showed considerable material balance deficiencies.

CA 7.1.1.1 Aerobic degradation

No new aerobic soil metabolism study with pyrimethanil was performed. The studies in the chapters on route and rate of degradation in soil of the previous dossier and which were already peer-reviewed during the previous Annex I inclusion process are considered still valid.

Already peer-reviewed studies, on which the conclusions on the route of degradation of pyrimethanil in aerobic soil are based upon:

Feyerabend M. 1992, BASF DocID A81889, IIA 7.1.1.1.1/1;
Feyerabend M. 1993, BASF DocID A81890, IIA 7.1.1.1.1/2;
Feyerabend M. 1991, BASF DocID A81878,IIA 7.1.1.2.1/1;
Feyerabend M. 1991, BASF DocID A81879,IIA 7.1.1.2.1/2;
Feyerabend M. 1993, BASF DocID A81904,IIA 7.1.1.2.1/3;
Forster V. 1992, BASF DocID A81888,IIA 7.1.1.2.1/4

The route of degradation of pyrimethanil in aerobic soil studies was characterized by a rather low mineralization rate (4 - 7% of applied radioactivity (AR) within approx. 100 days) and a formation of high amounts of bound residues (about 42 - 62% AR within approx. 100 days).

Only one transformation product reached > 5% AR. It was formed by cleavage of the amino bridge to yield the major metabolite 2-amino-4,6-dimethyl-pyrimidine, now designated as M605F007. The metabolite code used in the EFSA *Scientific Report* 2006 was AE F132593. Its maximum observed occurrence during all aerobic soil studies was ~ 8 % AR.

One minor metabolite which could also be observed when using pyrimidinyl-labelled test item was the 2-hydroxy-4,6-dimethyl-pyrimidine (SN 469626, M605F009), but it never reached more than 2.7% AR in soil. Some additional minor metabolites (all < 1% AR) resulting from nitration of the phenyl ring or oxidation of one of the pyrimidinyl nitrogens were identified when using artificially high application rates for incubation (≥ 100 mg pyrimethanil/kg soil).

The proposed route of degradation of pyrimethanil in soil is shown in Figure 7.1.1.3-1.

CA 7.1.1.2 Anaerobic degradation

No new anaerobic soil metabolism study was performed. The already peer-reviewed study of *Tarara, G. (1996) (BASF DocID A89445)* is considered still valid.

Already peer-reviewed study, on which the conclusions on the route of degradation of pyrimethanil in anaerobic soil are based upon:

Tarara, G. 1996, BASF DocID A89445, IIA 7.1.1.1.2/1

Pyrimethanil decreased during the 30 day aerobic preincubation from 99.4 to 28.3% AR. After initiating anaerobic conditions by water-logging and nitrogen-flushing of test vessels, no significant further degradation of pyrimethanil took place. After 120 days, it amounted still to 25.8% AR.

Metabolite 2-amino-4,6-methylpyrimidine was the major degradation product formed during the aerobic pre-incubation phase (max. 13.6% AR at day 30). During the anaerobic phase it declined very slowly to 10.0% AR. Also traces of metabolite 2-hydroxy-4,6-methylpyrimidine were detected (max. 2.2% AR at day 37). All other unidentified metabolites never exceeded 3.8% AR.

Mineralization was overall negligible (1.6% AR), whereas the non-extractable residues increased during aerobic incubation to 50.6% at day 37 and then remained stable during the anaerobic phase (51.3% AR at day 120).

Analysis of the non-extractable residues of a 120 day sample resulted in a distribution of residues in the fulvic acids, ß-humic acids and humic acids of about 9, 5, and 9% AR, respectively. The rest could not be extracted by 0.1 M NaOH.

CA 7.1.1.3 Soil photolysis

A new soil photolysis study with pyrimethanil was initiated, since the old soil photolysis study as submitted in the previous dossier (*Tschampel M., 1993; BASF DocID A81918; IIA 7.1.1.1.2/2*) was performed only with the phenyl-label and showed considerable material balance deficiencies. It is therefore considered no longer valid and is replaced by the new study described below.

New, not yet peer-reviewed study

Report:	CA 7.1.1.3/1 Hassink J.,Kretschmar G., 2015a Soil photolysis of Pyrimethanil 2015/1039821
Guidelines:	EPA 161-3, EPA 835.2410, SETAC Procedures for assessing the environmental fate and ecotoxicity for pesticides (March 1995), OECD Draft Guideline Phototransformation of Chemicals on Soil Surfaces (January 2002), EEC 91/414 Annex II
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

A soil photolysis was conducted with pyrimidinyl-^{14}C- and phenyl-^{14}C-labeled pyrimethanil (BAS 605 F) to investigate the behavior in soil under the influence of light.

The soil was passed through a 2 mm sieve before use and was treated with either with pyrimidinyl-^{14}C- or phenyl -^{14}C-labeled test item at a nominal rate of 6.7 mg per kg dry soil which corresponds to a field application rate of 1000 g pyrimethanil per hectare. Soil aliquots of 30 g (dry weight basis) were weighed into test dishes and placed into a Suntest apparatus for continuous irradiation (light intensity: 3 mW cm^{-2}) and aeration. The incubation temperature was kept at 22°± 1°C and the soil moisture was daily adjusted to pF 2.0-2.5, i.e. corresponding to 50% of the maximum water holding capacity. A closed incubation system with continuous aeration was used with an attached trapping system for the determination of volatile compounds. The samples for the dark control were kept in an incubation cabinet and treated analogously but without irradiation.

Samples were taken at 0, 2, 5, 7, 12 and 15 days after treatment (DAT). All soil samples were worked up in duplicate. The soil samples were extracted three times with methanol and three times with methanol/water (1/1, v/v). The combined methanol extracts as well as the combined methanol/water extracts were analyzed by liquid scintillation counting (LSC) and HPLC. The non-extractable residues were determined by combustion and LSC. The soil residues after solvent extraction of the 5, 7, 12 and 15 day samples (both labels, irradiated and dark samples) were further extracted with NaOH to determine the amount of alkali-soluble components. The dried soil was then combusted, in order to determine the amount of non-extractable soil bound residues. A full material balance was provided for each sampling interval.

The overall mean values for the material balance in the photolysis and the dark control for the pyrimidinyl-label were in the range of 98.7 to 105.2% TAR and for the phenyl-label 97.9 to 107.2% TAR. Carbon dioxide was the only volatile degradation product trapped, however, only reaching 0.07/0.8% TAR (pyrimidinyl/phenyl) after 15 days in the photolysis test and 0.12/0.46% TAR (pyrimidinyl/phenyl) in the dark control.

The extraction behaviour between irradiated and non-irradiated samples did not significantly differ. At the end of the study, approximately 10.6/14.9% TAR (pyrimidinyl/phenyl) were non-extractable from the irradiated soil samples. About 11.1/13.2% TAR (pyrimidinyl/phenyl) were non-extractable at the end of incubation of the dark control samples. The alkali-soluble radioactivity amounted in the photolysis test to 5.1-6.2 and 5.5-9.0% TAR for pyrimidinyl- and phenyl-label, repectively. The dark control resulted in similar values (approx. 4.3-5.2 and 5.2-8.7% TAR for pyrimidinyl- and phenyl-label, respectively).

The concentration of the test item pyrimethanil declined to 89.2/81.3% TAR (pyrimidinyl-/phenyl-label) within 15 days under continuous irradiation. The corresponding amounts in the dark control samples were 93.4 and 93.2% TAR for the pyrimidinyl- and phenyl-label, respectively.

Aliquots of all samples were analyzed by radio-HPLC (RHPLC). No degradation product exceeded 1% TAR at any sampling time.

Overall it can be concluded that the degradation rate of pyrimethanil as well as metabolite formation was not significantly influenced by irradiation. The calculated half-lives were all by far longer than the experiment duration. The DT_{50} values (SFO) were 84.1 and 50.9 days for the (continuously) irradiated experiment and 178 and 99.2 days for the dark control.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

The test item pyrimethanil (BAS 605 F) was used in two ^{14}C-labeled forms.

Internal code:	BAS 605 F
Reg. No.:	236999
CAS No.:	53112-28-0
Chemical name (IUPAC):	N-(4,6-dimethylpyrimidin-2-yl)aniline
Molecular mass:	199.26 g mol^{-1}
Molecular formula:	$C_{12}H_{13}N_3$

1. Pyrimidinyl-2-^{14}C-label

Batch No.:	1049-1010
Specific radioactivity of a.s.:	6.42 MBq mg^{-1}
Radiochemical purity:	99.7%
Purity:	99.8%

2. Phenyl-U-^{14}C-label

Batch No.:	1036-1011
Specific radioactivity of a.s.:	8.53 MBq mg^{-1}
Radiochemical purity:	99.2%
Purity:	99.4%

Unlabeled

Batch No.:	486213
Purity:	99.8% (± 1.0%)

2. Soil

The German agricultural soil LUFA 2.2 (BASF soil No. 736) from LUFA (Landwirtschaftliche Untersuchungs- und Forschungsanstalt, Speyer, Germany) was used in this study. Since the experiments with the two labels were performed consecutively, two different soil batches were used. After collecting the soil from 0-20 cm depth from the field, the soil was passed through a 2 mm sieve, remoistened to approximately 8-12% soil moisture and stored at about 4°C in the dark (no longer than 3 months before use). An overview of soil parameters is listed in Table 7.1.1.3-1.

Table 7.1.1.3-1: Characteristics of soils used for pyrimethanil soil photolysis

Soil designation	**LUFA 2.2** **BASF soil No. 12/736/05** Germany (Origin LUFA Speyer)	**LUFA 2.2** **BASF soil No. 12/736/04** Germany (Origin LUFA Speyer)
DIN 4220 Particle size distribution *[%]* sand 0.063 – 2 mm silt 0.002 – 0.063 mm clay < 0.002 mm textural class	 68.5 20.0 11.5 loamy sand	 72.0 18.7 9.3 loamy sand
USDA Particle size distribution *[%]* sand 0.050 – 2 mm silt 0.002 – 0.050 mm clay < 0.002 mm textural class	 69.9 18.6 11.5 sandy loam	 73.7 17.0 9.3 sandy loam
Organic C *[%]*	1.60	1.47
pH [H_2O] pH [$CaCl_2$]	6.4 5.4	5.9 5.4
Cation exchange capacity *[$cmol^+ kg^{-1}$]*	8.6	8.2
Max. water holding capacity *[g per 100 g dry weight]*	31.0	31.9
Microbial biomass (start of study) *[mg C per 100 g dry soil]*	42.1	32.8

B. STUDY DESIGN

1. Experimental conditions

For each label, twenty small stainless steel dishes (88 mm x 44 mm x 10 mm) were filled with 30 g soil (dry weight equivalents), respectively. Ten dishes were used for photolysis testing and ten for dark control. After treatment of the soil surface of each dish with appropriate amounts of application solution, the dishes for photolysis were arranged in a rectangular bowl with a connected thermostat. The temperature was adjusted to 22 ± 1°C and controlled by an external tempering unit, while the dishes for dark control were put into an incubator at 22 ± 1°C. The bowl was closed airtight with a quartz glass covering and the whole incubation device was continuously aerated with CO_2-depleted (0.5 M NaOH) and remoistened air via an air inlet and an air outlet.

In order to trap potentially evolving volatiles (including $^{14}CO_2$), the emergent air was bubbled through three different trapping solutions located between dish and pump: 1. NaOH (0.5 M); 2. ethylene glycol; 3. H_2SO_4 (0.5 M).

The incubation bowl for photolysis was placed under a SUNTEST CPS plus (Atlas) equipped with a Xenon lamp emitting light with a spectrum similar to sunlight and with an intensity of about 3 mW cm^{-2} (UVA range). This corresponds to a clear summer day in Southern Germany (about 49° N). Wavelengths < 290 nm were filtered off to simulate natural sunlight.

To maintain the temperature especially on the quartz glass surface in order to avoid a rapid drying of the soil surface, the air space between lamp and quartz glass within the SUNTEST device was cooled by an external apparatus (Yeti, Seveso). To maintain the initial water content as constant as possible, dishes were weighed at each incubation day and the evaporated water was replaced.

The amount of test item to be applied on the soil surface was calculated based on a recommended field application rate of 1000 g ha^{-1}. If a soil layer of 1 cm and bulk density of 1.5 kg L^{-1} is assumed the application rate corresponds to about 6.66 mg test item per kg dry soil (and about 200 µg per dish).

2. Sampling

The sampling dates were 0, 2, 5, 7, 12 and 15 days after treatment (DAT) for both label treatments. Two vessels were taken at each sampling time from each photolysis test system and the dark control (with exception of DAT 0, where no dark control samples were taken). At each sampling time, the respective volatile trapping solutions were removed.

3. Description of analytical procedures

Each soil sample was consecutively extracted once with 80 mL of methanol, two times with 60 mL of methanol, once with 80 mL of methanol/water (1:1, v/v) followed by two times with 60 mL of methanol/water (1:1, v/v). For each extraction step, the suspension was shaken for 15 min, and then solids and extract separated by centrifugation and filtration. The three corresponding methanol and three methanol/water extracts were combined, respectively, and measured by liquid scintillation counting (LSC) as well as by radio-HPLC.

After the last extraction, the soil residues were air-dried and stored at room temperature. For determination of the amount of non-extractable residues by combustion, the residues were homogenized by milling. Aliquots of each sample were combusted in a sample oxidizer. The trapped $^{14}CO_2$ was analyzed by LSC.

Since amounts of 5-15% of the total applied radioactivity (TAR) were found non-extractable in the 5, 7, 12 and 15 day samples of the photolysis and the dark control, the soil residues were further extracted with NaOH for humic substance fractionation.

Samples were extracted three times with 0.5 M NaOH on a rotary shaker (minimum extraction time 6 hours) and twice washed with water. Aliquots of the NaOH and the water extracts were analyzed by LSC. NaOH extracts and water extracts were pooled, representing together the fulvic and humic acid fraction. Since the amount of radioactivity exceeded 5% TAR in several cases, acidic precipitation of the fulvic acids was performed by adjusting the pH of the combined NaOH extracts to pH 1 with conc. HCl. After precipitation, samples were centrifuged and the supernatant was removed. Aliquots of the supernatants representing the fulvic acids were measured by LSC. The precipitate, the humic acid fraction, was dissolved in 0.5 M NaOH and also analyzed by LSC. The fulvic acid fraction of two samples (15 DAT samples of both labels) was further characterized by partitioning into water-soluble and ethyl acetate soluble phase. Both phases were analyzed by LSC.

The soil residue after the last washing was air-dried, homogenized and the weight was determined. Three aliquots were combusted and analyzed by LSC in order to determine the amount of radioactivity in the non-soluble humins.

4. Calculation of the degradation rate

Kinetic evaluation was performed in order to derive degradation parameters as triggers for additional work (trigger endpoints). Kinetic analysis and calculation of $DegT_{50}$ and $DegT_{90}$ values was performed following the recommendations of the FOCUS Kinetics workgroup [*FOCUS (2006) "Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration" Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005 version 1.0 (November 2011), 436pp.*]. The software package KinGUII (version 2.2014.224.1704) was used for parameter fitting [*Meyer et al. (2014) KinGUII v2.1. Manual for the Graphical User Interface. Bayer CropScience AG*]. The error tolerance and the number of iterations of the optimization tool were set to 0.000001 and 100, respectively.

For each data set, the kinetic models proposed by the FOCUS Kinetics guidance document were tested to identify the best-fit model. The recommended kinetic models, i.e. the single first order kinetics (SFO), the Gustafson-Holden model (FOMC) and bi-exponential (DFOP) kinetics, are already implemented in KinGUI. The Goodness-of-fit was evaluated by visual assessment, χ^2 minimum error, and type-I-error rate (t-test) [for details see Chapter 6.3 in *FOCUS (2006)*].

Replicate measurements were considered for the parameter estimation. The initial concentration of the applied test item was set to the material balance recovered at day 0. The χ^2 value was calculated for the kinetic model as recommended by FOCUS, considering the average of replicate measurements. For the individual parameters, the t-test statistics were based on number of individual measurements.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

Total recoveries of radioactivity extracted from soil are summarized in Table 7.1.1.3-2 to Table 7.1.1.3-5. The overall mean values for the material balance in the photolysis and in the dark control were in the range of 97.9 to 107.2% TAR.

Table 7.1.1.3-2: Recovery and distribution of radioactivity in soil LUFA 2.2 after treatment with pyrimidinyl-2-^{14}C-labeled pyrimethanil and incubation under irradiated conditions [% TAR]

Days after treatment	MeOH	MeOH/H_2O	total extractable	non-extractable (NER)	volatiles [a]	material balance
0/I	98.0	1.20	99.2	0.28	n.a.	99.5
0/II	99.1	1.16	100.3	0.27	n.a.	100.5
0 mean	**98.5**	**1.18**	**99.7**	**0.27**	**n.a.**	**100.0**
2/I	89.9	4.83	94.7	3.57	0.01	98.3
2/II	97.6	5.44	103.0	3.40	0.01	106.4
2 mean	**93.7**	**5.14**	**98.9**	**3.49**	**0.01**	**102.4**
5/I	90.4	2.76	93.2	5.63	0.01	98.8
5/II	92.5	2.93	95.4	6.11	0.01	101.5
5 mean	**91.4**	**2.84**	**94.3**	**5.87**	**0.01**	**100.2**
7/I	90.6	3.37	94.0	6.59	0.02	100.6
7/II	94.8	3.76	98.5	8.01	0.02	106.6
7 mean	**92.7**	**3.57**	**96.3**	**7.30**	**0.02**	**103.6**
12/I	85.5	4.16	89.6	10.66	0.05	100.3
12/II	85.5	4.18	89.7	10.38	0.05	100.1
12 mean	**85.5**	**4.17**	**89.7**	**10.52**	**0.05**	**100.2**
15/I	88.0	5.43	93.4	10.88	0.07	104.3
15/II	81.4	5.70	87.1	10.36	0.07	97.5
15 mean	**84.7**	**5.57**	**90.2**	**10.62**	**0.07**	**100.9**

TAR = total applied radioactivity
n.a. = not analyzed
[a] no other volatiles than CO_2 were found

Table 7.1.1.3-3: Recovery and distribution of radioactivity in soil LUFA 2.2 after treatment with pyrimidinyl-2-^{14}C-labeled pyrimethanil and incubation under dark conditions [% TAR]

Days after treatment	MeOH	MeOH/H_2O	total extractable	non-extractable (NER)	volatiles [a]	material balance
2/I	95.6	4.56	100.2	2.83	0.00	103.0
2/II	94.6	3.48	98.0	3.11	0.00	101.2
2 mean	**95.1**	**4.02**	**99.1**	**2.97**	**0.00**	**102.1**
5/I	92.8	1.95	94.8	5.53	0.02	100.3
5/II	89.8	1.78	91.6	5.40	0.02	97.0
5 mean	**91.3**	**1.87**	**93.2**	**5.46**	**0.02**	**98.7**
7/I	93.7	2.45	96.1	7.79	0.03	103.9
7/II	91.8	2.36	94.2	6.79	0.03	101.0
7 mean	**92.7**	**2.41**	**95.2**	**7.29**	**0.03**	**102.5**
12/I	94.6	2.71	97.3	9.08	0.08	106.5
12/II	91.7	2.51	94.3	9.56	0.08	103.9
12 mean	**93.2**	**2.61**	**95.8**	**9.32**	**0.08**	**105.2**
15/I	90.5	3.92	94.4	11.41	0.12	106.0
15/II	89.4	3.51	92.9	10.74	0.12	103.8
15 mean	**90.0**	**3.71**	**93.7**	**11.08**	**0.12**	**104.9**

TAR = total applied radioactivity, n.a. = not analyzed
[a] no other volatiles than CO_2 were found

Table 7.1.1.3-4: Recovery and distribution of radioactivity in soil LUFA 2.2 after treatment with phenyl-U-^{14}C-labeled pyrimethanil and incubation under irradiated conditions [% TAR]

Days after treatment	MeOH	MeOH/H_2O	total extractable	non-extractable (NER)	volatiles [a]	material balance
0/I	98.7	1.11	99.8	0.27	n.a.	100.1
0/II	98.6	1.03	99.6	0.28	n.a.	99.9
0 mean	**98.7**	**1.07**	**99.7**	**0.28**	**n.a.**	**100.0**
2/I	95.8	2.49	98.3	3.21	0.09	101.6
2/II	93.0	2.54	95.5	3.53	0.09	99.2
2 mean	**94.4**	**2.52**	**96.9**	**3.37**	**0.09**	**100.4**
5/I	93.2	3.25	96.5	6.39	0.3	103.2
5/II	93.6	3.38	96.9	6.42	0.3	103.7
5 mean	**93.4**	**3.32**	**96.7**	**6.41**	**0.3**	**103.4**
7/I	86.3	3.78	90.1	9.80	0.5	100.4
7/II	89.9	4.08	94.0	9.64	0.5	104.1
7 mean	**88.1**	**3.93**	**92.0**	**9.72**	**0.5**	**102.3**
12/I	82.8	4.60	87.4	12.43	0.7	100.6
12/II	80.1	4.79	84.9	14.91	0.7	100.6
12 mean	**81.5**	**4.70**	**86.2**	**13.67**	**0.7**	**100.6**
15/I	81.4	4.18	85.6	15.44	0.8	101.9
15/II	74.4	4.29	78.7	14.46	0.8	94.0
15 mean	**77.9**	**4.23**	**82.2**	**14.95**	**0.8**	**97.9**

TAR = total applied radioactivity, n.a. = not analyzed
[a] no other volatiles than CO_2 were found

Table 7.1.1.3-5: Recovery and distribution of radioactivity in soil LUFA 2.2 after treatment with phenyl-U-^{14}C-labeled pyrimethanil and incubation under dark conditions [% TAR]

Days after treatment	MeOH	MeOH/H_2O	total extractable	non-extractable (NER)	volatiles [a]	material balance
2/I	99.6	1.80	101.4	3.26	0.02	104.7
2/II	97.5	2.04	99.6	3.23	0.02	102.8
2 mean	**98.6**	**1.92**	**100.5**	**3.24**	**0.02**	**103.7**
5/I	94.5	2.42	96.9	6.45	0.07	103.4
5/II	94.5	2.33	96.9	6.71	0.07	103.6
5 mean	**94.5**	**2.38**	**96.9**	**6.58**	**0.07**	**103.5**
7/I	93.8	2.43	96.2	9.04	0.13	105.4
7/II	92.8	2.43	95.3	8.56	0.13	103.9
7 mean	**93.3**	**2.43**	**95.7**	**8.80**	**0.13**	**104.7**
12/I	86.1	3.04	89.2	13.47	0.33	103.0
12/II	85.4	3.86	89.2	12.22	0.33	101.8
12 mean	**85.7**	**3.45**	**89.2**	**12.84**	**0.33**	**102.4**
15/I	89.8	3.83	93.6	13.89	0.46	107.9
15/II	90.4	3.18	93.6	12.49	0.46	106.5
15 mean	**90.1**	**3.50**	**93.6**	**13.19**	**0.46**	**107.2**

TAR = total applied radioactivity, n.a. = not analyzed
[a] no other volatiles than CO_2 were found

B. EXTRACTABLE AND BOUND RESIDUES

The amount of extractable radioactive residues in the study with the pyrimidinyl-label decreased from 99.7% TAR on day 0 to 90.2% TAR on day 15 in the photolysis test, and from 99.1% TAR on day 2 to 93.7% TAR on day 15 in the dark control.

In the study with the phenyl-label the amount of extractable radioactive residues decreased from 99.7% TAR on day 0 to 82.2% TAR on day 15 in the photolysis test, and from 100.5% TAR to 93.6% TAR in the dark control.

In the study with the pyrimidinyl-label, the amount of non-extractable residues (NER) increased from 0.3% TAR on day 0 to 10.6% TAR in photolysis compared to 11.1% TAR in dark control after 15 days. The amount of NER in the study with the phenyl-label increased from 0.3% TAR on day 0 to 15.0% TAR in photolysis compared to 13.2% TAR in dark control after 15 days.

C. VOLATILIZATION

Carbon dioxide was the only trapped volatile degradation product found in the trapping solutions. After 15 days of treatment, 0.07% TAR (pyrimidinyl-label) and 0.80% TAR (phenyl-label) were mineralized in the photolysis test and 0.12% and 0.46% TAR in the dark control.

D. TRANSFORMATION OF PARENT COMPOUND

Results of radio-HPLC analyses are presented in Table 7.1.1.3-6 and Table 7.1.1.3-7.

After 15 days, the amount of pyrimidinyl-labeled pyrimethanil decreased to 89.2% TAR in the photolysis experiment and to 93.4% TAR in the dark control samples. The amount of phenyl-labeled pyrimethanil decreased in the same time (15 days) to 81.3% TAR in the photolysis experiment and to 93.2% TAR in the dark control samples.

For both labels, several degradation products were detected in the extracts, as a sum not exceeding 1.0% TAR at any sampling date.

Table 7.1.1.3-6: Radio-HPLC analysis of soil extracts after treatment of soil LUFA 2.2 with pyrimidinyl-2-^{14}C-labeled pyrimethanil (sum of MeOH and MeOH/Water extracts) [% TAR]

days after treatment	Photolysis test			Dark control		
	Total	pyrimethanil t_R~41.5'	Sum others*	Total	pyrimethanil t_R~41.5'	Sum others [a]
0 I	99.2	99.2	n.d.	n.a.	n.a.	n.a.
0 II	100.3	100.3	n.d.	n.a.	n.a.	n.a.
0 mean	**99.7**	**99.7**	**n.d.**	**n.a.**	**n.a.**	**n.a.**
2 I	94.7	94.5	0.3	100.2	100.2	n.d.
2 II	103.0	102.9	0.1	98.0	97.9	0.1
2 mean	**98.9**	**98.7**	**0.2**	**99.1**	**99.1**	**0.1**
5 I	93.2	92.9	0.3	94.8	94.5	0.3
5 II	95.4	95.1	0.3	91.6	91.3	0.2
5 mean	**94.3**	**94.0**	**0.3**	**93.2**	**92.9**	**0.2**
7 I	94.0	93.5	0.5	96.1	95.8	0.3
7 II	98.5	98.2	0.3	94.2	94.1	0.2
7 mean	**96.3**	**95.8**	**0.4**	**95.2**	**94.9**	**0.3**
12 I	89.6	88.9	0.7	97.3	96.8	0.5
12 II	89.7	89.0	0.7	94.3	93.9	0.4
12 mean	**89.7**	**89.0**	**0.7**	**95.8**	**95.3**	**0.5**
15 I	93.4	92.5	0.9	94.4	93.8	0.6
15 II	87.1	85.9	1.1	92.9	92.9	0.03
15 mean	**90.2**	**89.2**	**1.0**	**93.7**	**93.4**	**0.3**

TAR = total applied radioactivity
t_R = retention time [min]
n.a. = not applicable
n.d. = not detected
[a] sum of unknown peaks, each individual peak ≤ 1% TAR

Table 7.1.1.3-7: Radio-HPLC analysis of soil extracts after treatment of soil LUFA 2.2 with phenyl-U-^{14}C-labeled pyrimethanil (sum of MeOH and MeOH/Water extracts) [% TAR]

days after treatment	Photolysis test			Dark control		
	Total	pyrimethanil t_R~41.5'	Sum others*	Total	pyrimethanil t_R~41.5'	Sum others [a]
0 I	99.8	99.8	n.d.	n.a.	n.a.	n.a.
0 II	99.6	99.6	n.d.	n.a.	n.a.	n.a.
0 mean	**99.7**	**99.7**	**n.d.**	**n.a.**	**n.a.**	**n.a.**
2 I	98.3	98.0	0.3	101.4	101.4	0.03
2 II	95.5	95.2	0.3	99.6	99.5	0.08
2 mean	**96.9**	**96.6**	**0.3**	**100.5**	**110.4**	**0.05**
5 I	96.5	96.2	0.3	96.9	96.8	0.1
5 II	96.9	96.8	0.2	96.9	96.7	0.2
5 mean	**96.7**	**96.5**	**0.2**	**96.9**	**96.8**	**0.1**
7 I	90.1	89.3	0.8	96.2	96.0	0.2
7 II	94.0	93.4	0.5	95.3	95.1	0.1
7 mean	**92.0**	**91.4**	**0.7**	**95.7**	**95.6**	**0.1**
12 I	87.4	86.7	0.7	89.2	88.8	0.3
12 II	84.9	83.7	1.3	89.2	88.8	0.4
12 mean	**86.2**	**85.2**	**1.0**	**89.2**	**88.8**	**0.4**
15 I	85.6	84.7	0.9	93.6	93.2	0.4
15 II	78.7	77.9	0.8	93.6	93.1	0.5
15 mean	**82.2**	**81.3**	**0.8**	**93.6**	**93.2**	**0.4**

TAR = total applied radioactivity
t_R = retention time [min]
n.a. = not applicable
n.d. = not detected
[a] sum of unknown peaks, each individual peak ≤ 1% TAR

E. CHARACTERIZATION OF NON-EXTRACTABLE RESIDUES (NER)

Results of the non-extractable residue characterization performed by humic substance fractionation are given in Table 7.1.1.3-8 to Table 7.1.1.3-10.

The alkali-soluble radioactivity amounted to 4.5% and 4.6% TAR at day 15 in the photolysis and dark control with the pyrimidinyl-label and to 8.3% and 8.1% TAR at day 15 in the photolysis and dark control with the phenyl-label, respectively. Further separation into humic and fulvic acid fraction showed that the NaOH extracted radioactivity was more related to the fulvic (two-third) than to the humic acid fraction (one-third) in the photolysis test. For the dark control samples, the NaOH extracted radioactivity was almost equally distributed between these two fractions. Further separation into ethyl acetate and water soluble phases was performed with two irradiated samples of 15 DAT (both labels), exhibiting an almost equal distribution.

Table 7.1.1.3-8: Characterization of non-extractable residues (NER) in soil LUFA 2.2 after treatment with ^{14}C-pyrimethanil [% TAR]

days after treatment	Position of radiolabel	Incubation	NER initial	NaOH extraction	Water extraction	Sum of NaOH and water extracts	Soil residues after extraction (Humins)	Sum
5d II	pyrimidinyl-^{14}C	irradiated	6.11	2.20	0.33	2.53	1.99	4.52
7d II			8.01	2.82	0.44	3.26	2.34	5.59
12d I			10.66	5.68	0.56	6.24	3.17	9.41
15d I			10.88	4.48	0.66	5.13	3.36	8.49
5d I		dark	5.53	1.99	0.33	2.31	1.89	4.20
7d I			7.79	2.93	0.43	3.35	2.37	5.72
12d II			9.56	3.74	0.55	4.29	2.94	7.23
15d I			11.41	4.55	0.68	5.22	3.21	8.43
5d II	phenyl-^{14}C	irradiated	6.42	3.41	0.29	3.70	2.10	5.80
7d I			9.80	5.41	0.48	5.89	2.67	8.56
12d I			12.43	4.83	0.66	5.49	4.33	9.82
15d I			15.44	8.29	0.71	9.00	4.99	14.0
5d II		dark	6.71	3.45	0.29	3.74	2.04	5.79
7d I			9.04	4.77	0.39	5.16	2.48	7.63
12d I			13.47	7.11	0.66	7.77	3.76	11.53
15d I			13.89	8.11	0.59	8.70	3.79	12.49

TAR = total applied radioactivity
NER = non-extractable radioactive residues

Table 7.1.1.3-9: Separation of fulvic acid and humic acid fraction in soil LUFA 2.2 after treatment with ^{14}C-pyrimethanil [% TAR]

days after treatment	Position of radiolabel	Incubation	Sum of NaOH and water extracts	Fulvic acids	Humic acids	Sum
12d I	pyrimidinyl-^{14}C	irradiated	6.24	4.32	1.39	5.71
15d I			5.14	4.44	1.82	6.26
15d I		dark	5.23	3.89	3.29	7.18
7d I	phenyl-^{14}C	irradiated	5.89	3.34	2.13	5.47
12d I			5.50	4.51	2.28	6.79
15d I			9.00	5.55	2.69	8.24
7d I		dark	5.16	2.47	2.29	4.76
12d I			7.77	3.91	3.13	7.05
15d I			8.70	4.17	3.65	7.82

TAR = total applied radioactivity

Table 7.1.1.3-10: Liquid/liquid extraction of fulvic acid fractions with ethyl acetate in soil samples after treatment with ^{14}C-pyrimethanil [% TAR]

days after treatment	Position of radiolabel	Incubation	Fulvic acids	Aqueous residue	Ethyl acetate extract	Sum of aqueous residue + ethyl ac.
15d I	pyrimidinyl-^{14}C	irradiated	4.44	2.22	2.30	4.52
15d I	phenyl-^{14}C	irradiated	5.55	2.56	2.75	5.31

TAR = total applied radioactivity

F. KINETIC MODELING RESULTS

Degradation rates of BAS 750 F were estimated using the software package KinGUI 2 following the recommendations of the FOCUS Kinetics workgroup. The soil residues for the irradiated and the dark control experiment could both be best described by the SFO kinetic fit approach. The $DegT_{50}$/$DegT_{90}$ values obtained with the selected best-fit model are presented in Table 7.1.1.3-11: .

Table 7.1.1.3-11: Trigger endpoints for pyrimethanil in soil photolysis and dark control

Test system	$DegT_{50}$ *[d]*	$DegT_{90}$ *[d]*	Kinetic	χ^2 error
Photolysis (pyrimidinyl)	84.1*	279	SFO	1.05
Photolysis (phenyl)	50.9*	169	SFO	1.05
Dark control (pyrimidinyl)	178	590	SFO	1.53
Dark control (phenyl)	99.2	329	SFO	1.60

* continuous irradiation

III. CONCLUSION

Irradiation experiments with pyrimidinyl-2-^{14}C- and with phenyl-U-^{14}C-labeled pyrimethanil did not show a significant influence of light on the degradation rate and metabolite formation in soil. There was no relevant degradation during the study period, indicating that the substance pyrimethanil is more or less stable on an irradiated soil surface.

Summary: Route of degradation of pyrimethanil in soil

The overall understanding of soil degradation of pyrimethanil did not change since the evaluation during the previous Annex I inclusion process (according to EU Directive 91/414/EEC).

The behavior of pyrimethanil in soil is characterized by a rather low mineralization rate and the formation of considerable amounts of bound residues (both labels). Most of the non-extractable residues are associated with NaOH-insoluble humins and with high-molecular humic acids.

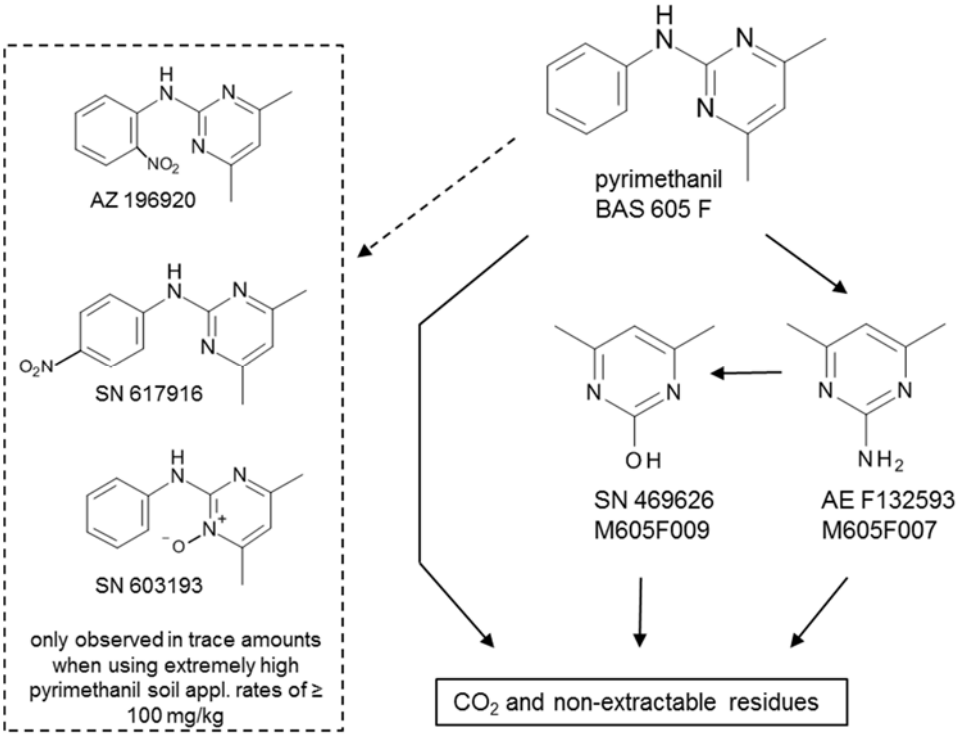
Major route of aerobic degradation consists of elimination of the phenyl-ring to yield the major metabolite 2-amino-4,6-dimethyl-pyrimidine (max. ~8% AR in aerobic soil studies, max. 13.6% after aerobic pre-incubation phase in anaerobic soil study), which can be further metabolized to the 2-hydroxy-4,6-dimethyl-pyrimidine (max. 2.7% AR). With the phenyl-labelled pyrimethanil, no distinct metabolite could be observed.

Soil photolysis does not have a significant influence on the degradation behaviour. Under anaerobic conditions, the degradation is slowed down considerably. No photolytical breakdown products or anaerobic metabolites were observed.

A few minor side reactions at the parent molecule (nitro substitutions or hydroxylations, all < 1% AR) were observed at very exaggerated dose rates in laboratory studies. However, none of them was further considered for exposure or risk assessments.

A scheme on the proposed route of degradation is given in Figure 7.1.1.3-1.

Figure 7.1.1.3-1: Proposed route of degradation of pyrimethanil in soil



CA 7.1.2 Rate of degradation in soil

CA 7.1.2.1 Laboratory studies

CA 7.1.2.1.1 Aerobic degradation of the active substance

No new degradation rate studies with pyrimethanil were performed. The already peer-reviewed studies as presented in the previous dossier are considered still valid.

Already peer-reviewed studies, on which the rate of degradation of pyrimethanil and its metabolite in aerobic soil are based upon:

Feyerabend M. 1991, BASF DocID A81878,IIA 7.1.1.2.1/1;
Feyerabend M. 1991, BASF DocID A81879,IIA 7.1.1.2.1/2;
Feyerabend M. 1993, BASF DocID A81904,IIA 7.1.1.2.1/3;
Forster V. 1992, BASF DocID A81888,IIA 7.1.1.2.1/4;
Koehn D. 2001, BASF DocID C011694; IIA 7.1.1.2.1/8

The degradation rates were re-calculated by using the old experimental data and analysing the kinetic parameters according to the current FOCUS guidance. A summary table of all obtained laboratory soil degradation values for pyrimethanil can be found at the end of this chapter (best-fit and normalized to 20°C, pF 2 and 10°C, pF 2).

New, not yet peer-reviewed kinetic evaluations:

Report:	CA 7.1.2.1.1/1 Sopena-Vazquez F.,Budde E., 2013a Kinetic evaluation of laboratory soil degradation studies with Pyrimethanil and its metabolite (AE F132593) according to FOCUS degradation kinetics 2012/1321627
Guidelines:	FOCUS Kinetics (2006) SANCO/10058/2005 version 2.0
GLP:	no

Executive Summary

The degradation behavior of pyrimethanil (BAS 605 F) and its soil metabolite 2-amino-4,6-dimethyl-pyrimidine (M605F007, previously referred to as AE F132593) in aerobic soil has been investigated in five laboratory degradation studies. In four of these studies with one soil each, pyrimethanil was applied as test item, while in the fifth study with five soils, the metabolite was used as test item.

The purpose of this evaluation is to analyze the degradation kinetics observed in the studies, taking into account the current guidance of the FOCUS workgroup on degradation kinetics.

Kinetic evaluation was performed in order to derive degradation parameters as triggers for additional work (trigger endpoints) and degradation parameters for environmental fate models (modeling endpoints)

The appropriate kinetic model to derive trigger and modeling endpoints was identified considering the procedures and kinetic models proposed by the FOCUS workgroup on degradation kinetics. The best-fit model to derive trigger endpoints was selected based on visual and statistical assessment.

Trigger endpoints for pyrimethanil based on best-fit models were derived from bi-phasic (three soils) as well as from SFO (one soil) kinetics. The endpoints ranged from 26.8 to 71.8 days (DT_{50}) and from 89.1 to 377.1 days (DT_{90}).

Trigger endpoints for the pyrimethanil metabolite M605F007 were derived from SFO (six soils) as well as from DFOP (one soil) kinetics and ranged from15.6 to 207.3 days (DT_{50}) and from 51.8 to 688.5 days (DT_{90}).

Modeling endpoints for pyrimethanil could be derived from SFO kinetics. The normalized (20°C, pF2) modeling DT_{50} values ranged between 22.6 and 81.9 days.

For the metabolite M605F007, normalized modeling-DT_{50} values were derived from SFO (six soils) and DFOP (one soil) kinetics and ranged from 15.6 to 208.5 days.

I. MATERIAL AND METHODS

The degradation of pyrimethanil and its soil metabolite 2-amino-4,6-dimethyl-pyrimidine (M605F007, previously referred to as AE F132593) was analyzed in four different studies under laboratory conditions using different soils from Germany [*BASF DocID A81904; BASF DocID A81878; BASF DocID A81879; BASF DocID A81888*].

In addition, the degradation of metabolite M605F007 has been investigated in one study with five soils from Europe [*BASF DocID C011694*].

During the evaluation, the current guidance of the FOCUS workgroup on degradation kinetics [*FOCUS (2006): "Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration" Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005 version 2.0, 436 pp*] was taken into account.

Kinetic modeling strategy

Kinetic evaluation was performed in order to derive trigger and modeling endpoints. The appropriate kinetic model was identified considering the procedures and kinetic models proposed by the FOCUS workgroup on degradation kinetics [*FOCUS (2006)*]. The appropriate model was selected based on visual and statistical assessment. DT_{50} values suitable for use in environmental fate models were derived depending on the kinetic model.

Kinetic models included in the evaluations

For each data set, the kinetic models proposed by FOCUS Kinetics [*FOCUS (2006)*] were tested in order to identify the best-fit model, i.e. single first order (SFO) kinetics, the Gustafson-Holden model (FOMC), the Hockey-stick (HS) kinetics, and the bi-exponential (DFOP) kinetics. The respective model descriptions and corresponding equations for calculating endpoints (DT_{50}, DT_{90}) are shown in the FOCUS Kinetics guidance [*FOCUS (2006)*], Box 5-1, Box 5-2, Box 5-3, and Box 5-4.

The goodness-of-fit of the kinetic models was assessed by visual inspection and statistical measures, as recommended by the FOCUS Kinetics guidance [*FOCUS (2006)*].

Data handling and software for kinetic evaluation

When available, replicate measurements were used in the parameter estimation. The experimental data were derived from the study reports and adjusted according to FOCUS [*FOCUS (2006)*].

The software package KinGUI version 2.2012.320.1629 was used for parameter fitting. The error tolerance and the number of iterations of the optimization tool (IRLS) were set to $1x10^{-5}$ and 100, respectively.

Normalization of degradation rates to reference conditions

According to FOCUS [*FOCUS (2006)*] the DT_{50} values obtained from laboratory studies were normalized for modeling purposes to reference conditions at a temperature of 20°C and soil moisture at pF2 to account for the different soil temperature and moisture conditions during incubation.

Experimental data

The kinetic evaluation was conducted for different soils from Europe deriving from five aerobic laboratory soil degradation studies, thereof four for the parent compound [*BASF DocID A81904; BASF DocID A81878; BASF DocID A81879; BASF DocID A81888*] and one for metabolite M605F007 (AE F132593) [*BASF DocID C011694*].

The test substance was applied at a nominal rate of 1.33 mg kg^{-1} dry soil [*BASF DocID A81904*], 1.40 mg kg^{-1} dry soil [BASF *DocID A81878];* 1.41 mg kg^{-1} dry soil [*BASF DocID A81879*], or 2.0 mg kg^{-1} dry soil [*BASF DocID A81888*]. Metabolite M605F007 was applied at a nominal rate of 0.066 mg kg^{-1} soil [*BASF DocID C011694*].

The soil characteristics are summarized in Table 7.1.2.1.1-1 to Table 7.1.2.1.1-5.

Table 7.1.2.1.1-1: Soil characteristics of study A81879

Parameter	Schering Soil No. 178
Soil type	Loam (USDA)
Particle size distribution [%]	
Sand (50 - 2000 µm)	41.1
Silt (2 - 50 µm)	37.7
Clay (< 2µm)	21.2
Organic carbon [%]	3.5
Microbial biomass [mg C/kg dry soil] before start of study / at end of study	506 / 534
CEC [mVal/100g]	18
pH [$CaCl_2$]	7.5
Max. water holding capacity [g/100g soil]	30.2

Table 7.1.2.1.1-2: Soil characteristics of study A81878

Parameter	German Standard Soil 2.2
Soil type	Loamy sand (USDA)
Particle size distribution [%]	
Sand (50 - 2000 µm)	75.0
Silt (2 - 50 µm)	23.1
Clay (<2 µm)	1.9
Organic carbon [%]	2.4
Microbial biomass [mg C/kg dry soil] before start of study / at end of study	459 / 116
CEC [mVal/100g]	10.0
pH [$CaCl_2$]	5.8
Max. water holding capacity [g/100g soil]	38.3

Table 7.1.2.1.1-3: Soil characteristics of study A81904

Parameter	German Standard Soil 2.3 SP 389
Soil type	Sandy loam (USDA)
Particle size distribution [%]	
Sand (50 - 2000 µm)	60.7
Silt (2 - 50 µm)	32.6
Clay (<2 µm)	6.7
Organic carbon [%]	0.95
Microbial biomass [mg C/100g dry soil] before start of study / at day 154 / at end of study	329 / 214 / 122
CEC [mVal/100g]	8
pH [$CaCl_2$]	5.9
Max. water holding capacity [g/100g soil]	37.2

Table 7.1.2.1.1-4: Soil characteristics of study A81888

Parameter	German Standard Soil 2.1
Soil type	Sand (USDA)
Particle size distribution [%]	
Sand (50 - 2000 µm)	93.3
Silt (2 - 50 µm)	4.7
Clay (<2 µm)	2
Organic carbon [%]	0.74
Microbial biomass [mg C/100g dry soil]	not measured
CEC [mVal/100g]	5
pH [$CaCl_2$]	5.4
Max. water holding capacity [g/100g soil]	25.4

Table 7.1.2.1.1-5: Soil characteristics of study C011694

Parameter	LS 2.2	SL 2.3	SLS	Bologna	Ferrara
Soil type	Loamy sand (USDA)	Sandy loam (USDA)	Silt loam (USDA)	Sandy clay loam (USDA)	Silty clay (USDA)
Particle size distribution [%]					
Sand (50 - 2000 µm)	79.3	62.5	17.8	54.9	3.9
Silt (2 - 50 µm)	11.2	26	62.4	21	46.6
Clay (<2 µm)	9.4	11.6	19.8	24.1	49.5
Organic carbon [%]	1.95	1.51	2.08	2.1	2.4
Microbial biomass [mg C/100g dry soil] before start of study / at day 120	38.5 / 21.0	18.6 / 15.1	29.4 / 37.5	17.9 / 45.9	116.2 / 96.3
CEC [mVal/100g]	7.9	10.3	14.2	18.9	25.1 / 95.6
pH [$CaCl_2$]	6	6.9	7	7.6	7.6
Max. water holding capacity [g/100g soil]	46.4	42.7	55	48.1	56.9

An overview of the experimental conditions of the studies is given in Table 7.1.2.1.1-6.

Table 7.1.2.1.1-6: Overview on experimental conditions

Study (DocID)	Test item (label)	Soil	Soil type (USDA)	Moisture [% MWHC]	Temp. [°C]	Duration [d]	Residues at end of study [% TAR]
A81879	Pyrimethanil (phe)	Schering Soil No. 178	Loam	40	20	364	7.4
A81878		German Standard Soil 2.2	Loamy sand	40	20	364	10.9
A81904	Pyrimethanil (pyr)	German Standard Soil 2.3 SP 389	Sandy loam	40	20	364	4.5
A81888		German Standard Soil 2.1	Sand	40	21	155	17.1
C011694	M605F007 (pyr)	LS 2.2	Loamy sand	50	20	230	33.1
		SL 2.3	Sandy loam	50	20	230	31.6
		SLS	Silt loam	50	20	153	4.7
		Bologna	Sandy clay loam	50	20	230	2.0
		Ferrara	Silty clay	50	20	153	7.0

(phe) UL-^{14}C-phenyl-^{14}C-labeled pyrimethanil
(pyr) 2-^{14}C-pyrimidinyl-^{14}C-labeled pyrimethanil or M605F007

The measured data as well as resulting datasets submitted to kinetic analysis are given in Table 7.1.2.1.1-7 to Table 7.1.2.1.1-11.

Table 7.1.2.1.1-7: Data for pyrimethanil in Schering Soil No. 178 used for kinetic evaluation (study A81879)

DAT	Experimental data [% TAR]	Input data according to FOCUS [% TAR]
0	98.4	98.4
0	98.6	98.6
7	87.6	87.6
7	88.5	88.5
14	79.2	79.2
14	81.6	81.6
30	65.3	65.3
30	71.3	71.3
58	51.9	51.9
58	54.7	54.7
91	46.1	46.1
91	39.4	39.4
154	28.1	28.1
154	24.7	24.7
245	13.8	13.8
245	13.9	13.9
364	7.7	7.7
364	7.1	7.1

DAT = Days after Treatment
TAR = Total applied radioactivity

Table 7.1.2.1.1-8: Data for pyrimethanil in German Standard Soil 2.2 used for kinetic evaluation (study A81878)

DAT	Experimental data [% TAR]	Input data according to FOCUS [% TAR]
0	97.2	97.2
0	97.9	97.9
1	93.3	93.3
1	95.0	95.0
3	91.6	91.6
3	92.2	92.2
7	84.3	84.3
7	87.3	87.3
14	82.1	82.1
14	83.5	83.5
30	71.5	71.5
30	71.8	71.8
50	60.6	60.6
50	55.6	55.6
91	39.4	39.4
91	40.6	40.6
154	24.6	24.6
154	26.2	26.2
245	17.2	17.2
245	17.5	17.5
364	10.6	10.6
364	11.2	11.2

DAT = Days after Treatment
TAR = Total applied radioactivity

Table 7.1.2.1.1-9: Data for pyrimethanil and its metabolite M605F007 (AE F132593) in German Standard Soil 2.3 SP 389 used for kinetic evaluation (study A81904)

DAT	Experimental data [% TAR]		Input data according to FOCUS [% TAR]	
	Pyrimethanil	M605F007	Pyrimethanil	M605F007
0	92.4	n.d.	92.4	0.0
0	94.1	n.d.	94.1	0.0
7	84.8	1.3	84.8	1.3
7	84.4	0.7	84.4	0.7
14	76.5	1.0	76.5	1.0
14	78.8	1.2	78.8	1.2
28	45	5.4	45	5.4
28	51.1	4.1	51.1	4.1
62	6.9	9.8	6.9	9.8
62	10.8	6.8	10.8	6.8
90	11.8	5.1	11.8	5.1
90	14.2	5.3	14.2	5.3
153	5.3	3.0	5.3	3.0
153	6.3	4.5	6.3	4.5
244	5.9	2.2	5.9	2.2
244	5.6	2	5.6	2.0
364	4.3	1.2	4.3	1.2
364	4.7	1.0	4.7	1.0

DAT = Days after Treatment
TAR = Total applied radioactivity
n.d. = Not detected

Table 7.1.2.1.1-10: Data for pyrimethanil and its metabolite M605F007 (AE F132593) in German Standard Soil 2.1 used for kinetic evaluation (study A81888)

DAT	Experimental data [% TAR]		Input data according to FOCUS [% TAR]	
	pyrimethanil	M605F007	Pyrimethanil	M605F007
0	97.3	n. d.	97.3	0.0
0.1	93.3	n. d.	93.3	0.025 [a]
7	80.2	1.2	80.2	1.2
14	72.9	2.4	72.9	2.4
30	50.4	5.9	50.4	5.9
70	28.2	9.7	28.2	9.7
105	23.5	11.5	23.5	11.5
155	17.1	10.6	17.1	10.6

DAT = Days after Treatment
TAR = Total applied radioactivity
n.d. = Not detected
[a] Metabolite not detected, according to FOCUS, the value for the metabolite was set to ½ LOD

Table 7.1.2.1.1-11: Data for kinetic evaluation of the metabolite M605F007 (AE F132593) (study C011694)

days after treatment	Experimental data [% TAR]	Input data according to FOCUS [% TAR]	days after treatment	Experimental data [% TAR]	Input data according to FOCUS [% TAR]
SLS			**Ferrara**		
0	97.1	97.1	0	94.4	94.4
7	69.7	69.7	7	86.6	86.6
14	51.6	51.6	14	75	75.0
28	26.2	26.2	28	53.5	53.5
62	8.3	8.3	62	35.4	35.4
90	4.7	4.7	90	12.1	12.1
120	4.5	4.5	120	10.0	10.0
153	4.7	4.7	153	7.0	7.0
Bologna			**LS 2.2**		
0	89.6	89.6	0	98.9	98.9
7	77.5	77.5	7	82.5	82.5
14	64.7	64.7	14	73.4	73.4
28	32.2	32.2	28	64.7	64.7
62	11.6	11.6	62	58.2	58.2
90	3.7	3.7	90	48.9	48.9
120	3.7	3.7	120	40.3	40.3
153	2.3	2.3	153	42.6	42.6
230	2.0	2.0	230	33.1	33.1
SL 2.3					
0	100.0	100.0			
7	85.7	85.7			
14	82.3	82.3			
28	68.9	68.9			
62	65.1	65.1			
90	52.5	52.5			
120	45.7	45.7			
153	31.9	31.9			
230	31.6	31.6			

TAR = Total applied radioactivity

II. RESULTS AND DISCUSSION

Degradation kinetics of pyrimethanil and its metabolite M605F007 (AE F132593) were evaluated according to FOCUS [*FOCUS (2006)*] from the results of laboratory studies on aerobic soil degradation of pyrimethanil and M605F007 in order to derive trigger as well as modeling endpoints. For the experiments under standard incubation conditions (study with pyrimethanil: 20 - 21°C, 40% of MWHC; study with M605F007: 20°C, 50% of MWHC) the resulting DT_{50} values were further normalized to reference conditions (20°C, pF 2) according to the recommendations of the FOCUS groundwater workgroup [*FOCUS (2014) Generic guidance for FOCUS groundwater scenarios, v 2.2*].

The DT_{50} values derived as trigger endpoints for additional work for pyrimethanil based on best-fit models are summarized in Table 7.1.2.1.1-12. The respective DT_{50} values for the metabolite M605F007 are reported in chapter CA 7.1.2.1.2, Table 7.1.2.1.2-2:

The DT_{50} values at study conditions, the parameters included in the normalization procedure, and the resulting DT_{50} values normalized to reference conditions (modeling endpoints) for pyrimethanil in Table 7.1.2.1.1-13 and Table 7.1.2.1.1-14.
The respective normalization parameters and normalized DT_{50} values for the metabolite M605F007 are provided in chapter CA 7.1.2.1.2, Table 7.1.2.1.2-3 and Table 7.1.2.1.2-4.

Table 7.1.2.1.1-12: Trigger endpoints for additional work for pyrimethanil based on best-fit models

Study (DocID)	Soil	Test item (label)	Best-fit model	χ^2 error	Trigger endpoints	
					DT_{50} [d]	DT_{90} [d]
Aerobic soil degradation of pyrimethanil						
A81879	Schering Soil No. 178 (Loam)	parent (phe)	DFOP	0.96	69.8	298.3
A81878	German Standard Soil 2.2 (Loamy sand)		FOMC	1.9	71.8	377.1
A81904	German Standard Soil 2.3 SP 389 (Sandy loam)	parent (pyr)	SFO	12.4	26.8	89.1
A81888	German Standard Soil 2.1 (Sand)		FOMC	3.0	35.3	252.0 *
				Geo mean	**46.7**	**224.2**

* Since 10% TAR was not reached within the experimental period, DT_{90} values should be interpreted carefully
(phe), (pyr) position of 14C-label (phenyl, pyrimidinyl)

Table 7.1.2.1.1-13: Calculated factors for normalization of degradation rates to standard conditions

Study (DocID)	Soil	T_{act} [°C]	T_{ref} [°C]	θ_{act} [g/100g]	θ_{ref} [g/100g]	f_{temp}	f_{moist}
Aerobic soil degradation of pyrimethanil							
A81879	Schering Soil No. 178 (Loam)	20	20	12.1	25	1.0	0.601
A81878	German Standard Soil 2.2 (Loamy sand)	20	20	15.3	14	1.0	1.000
A81904	German Standard Soil 2.3 SP 389 (Sandy loam)	20	20	14.9	19	1.0	0.843
A81888	German Standard Soil 2.1 (Sand)	21	20	10.4	12	1.1	0.890

T_{act}	actual temperature during incubation	[°C]
T_{ref}	reference temperature (20°C)	[°C]
θ_{act}	actual soil moisture	[g / 100 g dry soil]
θ_{ref}	reference soil moisture at field capacity (pF 2, 10 kPa) according to FOCUS	[g / 100 g dry soil]
f_{temp}	temperature correction factor	[-]
f_{moist}	moisture correction factor	[-]

Table 7.1.2.1.1-14: Modeling endpoints for pyrimethanil

Study (DocID)	Soil	Test item (label)	Best-fit model	χ^2 error	Modeling endpoints	
					DT_{50} [d]	Normalized DT_{50} [d]
Aerobic soil degradation of pyrimethanil						
A81879	Schering Soil No. 178 (Loam)	parent (phe)	SFO	4.4	80.1	48.1
A81878	German Standard Soil 2.2 (Loamy sand)		SFO	4.4	81.9	81.9
A81904	German Standard Soil 2.3 SP 389 (Sandy loam)	parent (pyr)	SFO	12.4	26.8	22.6
A81888	German Standard Soil 2.1 (Sand)		SFO	7.0	44.7	43.7
					Geo mean	**44.4**

* Modeling endpoint derived from the slow phase of bi-phasic fit
(phe), (pyr) position of ^{14}C-label (phenyl, pyrimidinyl)

III. CONCLUSION

Trigger endpoints for additional work and modeling endpoints were derived for pyrimethanil and its metabolite M605F007 in five laboratory degradation studies.

Trigger endpoints for pyrimethanil based on best-fit models were derived from biphasic (three soils) as well as from SFO (one soil) kinetics. The trigger endpoints ranged from 26.8 to 71.8 days (DT_{50}) and from 89.1 to 377.1 days (DT_{90}).
Modeling endpoints for pyrimethanil could be derived from SFO kinetics. The normalized modeling DT_{50} values ranged between 43.7 and 81.9 days..

The following publication contains information on the degradation rate of pyrimethanil in soil which can be used as supplemental or supporting data.

Report: CA 7.1.2.1.1/2
Marin-Benito J.M. et al., 2012a
Dissipation of fungicides in a vineyard soil amended with different spent mushroom substrates
2012/1368542
Guidelines: none
GLP: no

Executive Summary

In this publication, the degradation kinetics and formation of metabolites for fungicides of different chemical classes (iprovalicarb, metalaxyl, penconazole, and pyrimethanil) and determination of bound residues for metalaxyl and penconazole were studied in both an unamended vineyard soil and in the same soil amended with two spent mushroom substrates (composted (C-SMS1) and fresh (F-SMS2)). The degradation kinetics was fitted to single first-order or first-order multicompartment patterns.

In this summary, only the investigations and results of the unamended soil experiment performed with pyrimethanil are reported, since all other information is not considered relevant in the context of pyrimethanil re-registration in the EU.

Unlabelled pyrimethanil was incubated at a concentration of 2 mg/kg (dry soil) under aerobic conditions in a sandy clay from a vineyard in Aldeanueva (La Rioja, Spain) at 20°C in the dark. The soil moisture was adjusted to 40% of the max.WHC. Samples were taken in duplicates at 10 different time points up to 58 days. Soil samples were extracted with methanol, the extracts separated from soil by centrifugation, then filtered and concentrated and finally analysed by GC/MS. Pyrimethanil was quantified via ion m/z 198.0. The LOQ was listed as 1.782 µg/L. Kinetic evaluation was done according to recommendations of the FOCUS work group (2006).

The results showed a fast degradation of pyrimethanil in soil with a DT_{50} (SFO) of 15.3 days.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Internal code:	BAS 605 F (pyrimethanil)
Reg. No.:	304428
CAS-No.:	175013-18-0
Chemical name (IUPAC):	methyl N-(2-{[1-(4-chlorophenyl)-1H-pyrazol-3 yl]oxymethyl}phenyl)-(N-methoxy)carbamate
Molar mass:	387.8 g mol^{-1} (unlabeled)

Substance was purchased by Dr. Ehrenstorfer GmbH (Augsburg, Germany), purity > 97.5%.

2. Soil

Soil was collected from the surface layer (0−15 cm) of a vineyard in Aldeanueva de Ebro (La Rioja, Spain). The sandy clay loam soil was sieved (<2 mm) and stored at 4 °C until further use.

The soil characteristics were determined by the usual analytical methods (MAPA Madrid, Spain). The pH was determined in a soil/water suspension (1:2 w/v ratio), and particle size distribution was determined using the pipet method. The OC content and DOC content in soil extracts (1:2 w/v ratio) in Milli-Q ultrapure water were determined as and ash percentage was determined by weight difference after ignition at 540 °C for 24 h. Organic carbon (OC) content was determined by oxidation (Walkley−Black method). Dissolved organic carbon (DOC) was determined in a suspension of residue in Milli-Q ultrapure water (1:100 w/v ratio) after 24 h of shaking at 20 °C, centrifuging (20 min at 12857g), and filtering (Minisart NY25 filter 0.45 µm, Sartorius Stedim Biotech, Germany) using a Shimadzu 5050 organic carbon analyzer (Shimadzu, Columbia, MD, USA).

Table 7.1.2.1.1-15: Characteristics of soil used for determination of the degradation rate of pyrimethanil

origin	soil type	pH (H_2O)	Org. C [%]	DOC [%]	sand [%]	silt [%]	clay [%]
Aldeanueva de Ebro (vineyard), La Rioja, Spain	sandy clay loam	7.87	0.59	0.005	64.4	14.2	21.4

B. STUDY DESIGN

Initially, the investigated fungicides were individually dissolved in methanol to give a concentration of 1000 mg L^{-1}. Solutions of each fungicide were then prepared in Milli-Q ultrapure water, and a volume of 10 mL of appropriate concentration was added to 300 g of fresh weight of unamended or amended soils to give a concentration of 2 mg kg^{-1} dry soil.

Soil samples were incubated at 20 °C in the dark. The moisture content of soil samples was previously adjusted to 40% of the maximum soil water-holding capacity, and it was maintained by adding sterile Milli-Q ultrapure water when necessary. Each soil treatment was prepared in duplicate.

A sterilized soil sample was also prepared by autoclaving soil at 120 °C for 1 h on three consecutive days. Sterilized unamended soil was treated with each fungicide and incubated as indicated above, and these samples were used as controls to check the chemical degradation of fungicides. Finally, soils for microbiological control were prepared by adding only sterile Milli-Q ultrapure water. All soils were thoroughly stirred with a sterilized spatula, and all of the steps were performed in a sterile cabinet.

Soil samples were taken at day 0 for fungicide analysis and thereafter repeatedly at different time intervals (up to 258 days) depending on the degradation rate of each fungicide. For pyrimethanil, in total 10 samplings from 0 to 58 days are indicated in the respective graph showing the pyrimethanil degradation curve.

At each sampling time, 2 × 5 g of each duplicate treatment (300 g sample treated with different fungicides) was taken and shaken at 20 °C for 24 h with methanol (10 mL) in glass tubes. The samples were then centrifuged at 5045g for 15 min, and the fungicide extracts were filtered in a GHP Acrodisc filter (Waters Corp.) to remove particles >0.45 µm. For the determination of the fungicides and their metabolites, a volume of the extract (6 mL) was transferred to a clean glass tube and evaporated at 25 °C under a nitrogen stream using an EVA-EC2-L evaporator (VLM GmbH, Bielefeld, Germany) until dryness. The residue was dissolved in 0.75 mL of methanol and transferred to a glass vial for analysis. The recoveries of the extraction method were determined by spiking three unamended soil samples with analytical grade fungicide to a final concentration of 2 mg kg^{-1} and performing the extraction procedure as described above. The mean recovery values varied between 87 and 111% for all of the fungicides studied.

Pyrimethanil was quantified using a 7890A Agilent gas chromatograph coupled to a 5975C Agilent mass spectrometer (Agilent Technologies, Avondale, AZ, USA) using a 30 m × 0.25 mm i.d., 0.25 µm film thickness, HP-5MS capillary column (J&W, Folsom, CA, USA). A volume of 1.0 µL was injected in splitless mode at 250 °C. The carrier gas was ultrapure helium at a flow of 1.5 mL min^{-1}. Measurements were performed in SIM mode. The more abundant ion was chosen for pyrimethanil quantification (m/z 198.0).

The LOQ for determination of pyrimethanil is stated with 1.782 µg L^{-1} (LOD 0.540 µg L^{-1}).

The degradation kinetics for the fungicide was fitted to a single first-order (SFO) kinetic model ($C = C0\ e^{-kt}$) or first-order multicompartment (FOMC) model ($C = C0/((t/\beta) + 1)^{\alpha}$), known also as the Gustafson and Holden model. C is the fungicide concentration at time t, C0 is the initial fungicide concentration, k (day^{-1}) is the degradation rate, α is a shape parameter determined by the coefficient of variation of k values, and β is a location parameter. For the selection of the kinetic model that best describes the degradation results, FOCUS work group guidance recommendations were followed. The coefficient of determination (r^2) and the chi-square (χ^2) test were calculated as indicators of the goodness of fit.

II. RESULTS AND DISCUSSION

The plotted graph of the results obtained from the pyrimethanil experiment showed a well- fitted SFO decline curve. After 58 days, only 2 - 7% of the initial applied pyrimethanil was left in soil.

The kinetic parameters for the degradation of pyrimethanil are shown in Table 7.1.2.1.1-16.

Table 7.1.2.1.1-16: Kinetic parameters for the degradation of pyrimethanil in a Spanish soil

Kinetic		DT_{50} ± SD [d]	r^2	χ^2
SFO	k (day^{-1}) = 0.045	15.3 ± 0.3	0.972	8.7
FOMC	α = 4.82 x 10^4 β = 1.07 x 10^6	15.3 ± 0.4	0.972	9.1

III. CONCLUSION

This publication is considered only as supplemental. Although the study description gives the impression of an overall reliable experiment, detailed information on soil parameters (e.g. max. water holding capacity), measured results (only graphs, no tables) and kinetic evaluation (residual plots) is missing . The half-life of pyrimethanil in a Spanish soil was determined to be 15.3 days.

Summary of degradation endpoints for pyrimethanil in different soils under aerobic conditions

Table 7.1.2.1.1-17: Summary table on best-fit degradation endpoints of pyrimethanil as obtained in aerobic soil (laboratory, 20-21°C, 40%MWHC)s

BASF DocID	Soil (Soil type)	pH ($CaCl_2$)	Org. C [%]	Temp. [°C]	Moisture [% MWHC]	Best-fit $DegT_{50}$ / $DegT_{90}$ [d]	Kinetic model	χ^2 error level
A81879, 2012/1321627	Schering Soil No. 178 (Loam)	7.5	3.5	20	40	69.8 / 298.3	DFOP	0.96
A81878, 2012/1321627	German Standard Soil 2.2 (Loamy sand)	5.8	2.4	20	40	71.8 / 377.1	FOMC	1.9
A81904, 2012/1321627	German Standard Soil 2.3 SP 389 (Sandy loam)	5.9	0.95	20	40	26.8 / 89.1	SFO	12.4
A81888, 2012/1321627	German Standard Soil 2.1 (Sand)	5.4	0.74	21	40	35.3 / 252.0	FOMC	3.0
2012/ 1368542 [a]	Aldeanueva de Ebro (sandy clay loam)	7.87 [b]	0.59	20	40	15.3 / 50.8 [c]	SFO	8.7

MWHC maximum water holding capacity

[a] reported as supplementary information only

[b] pH measured in H_2O

[c] DT_{90} calculated as DT_{50} * 3.32 (constant ratio between DT_{50} and DT_{90} for SFO kinetics)

Table 7.1.2.1.1-18: Summary table on degradation endpoints for modeling of pyrimethanil as obtained in laboratory soil studies (normalized to 20°C, pF2)

BASF DocID	Soil (Soil type)	pH ($CaCl_2$)	Org. C [%]	Temp. [°C]	Moisture [% MWHC]	$DegT_{50}$ at study conditions [d]	$DegT_{50}$ normalized to 20°C, pF2 [d]	Kinetic model	χ^2 error level
A81879, 2012/1321627	Schering Soil No. 178 (Loam)	7.5	3.5	20	40	80.1	48.1	SFO	4.4
A81878, 2012/1321627	German Standard Soil 2.2 (Loamy sand)	5.8	2.4	20	40	81.9	81.9	SFO	4.4
A81904, 2012/1321627	German Standard Soil 2.3 SP 389 (Sandy loam)	5.9	0.95	20	40	26.8	22.6	SFO	12.4
A81888, 2012/1321627	German Standard Soil 2.1 (Sand)	5.4	0.74	21	40	44.7	43.7	SFO	7.0
2012/ 1368542 [a]	Aldeanueva de Ebro (sandy clay loam)	7.87 [b]	0.59	20	40	15.3 [c]	- [c]	SFO	8.7
						Geo mean	**44.4**		

MWHC maximum water holding capacity

[a] reported as supplementary information only

[b] pH measured in H_2O

[c] supplementary information only, no normalized $DegT_{50}$ calculated

Calculation of degradation rates at 10°C

$DegT_{50}$ values at a temperature of 10°C were calculated by multiplying the normalized $DegT_{50}$ values at 20°C and pF 2 with a default Q_{10} value of 2.58 [*EFSA (2007): "Opinion on a request from EFSA related to the default Q_{10} value used to describe the temperature effect on transformation rates of pesticides in soil. Scientific Opinion of the Panel on Plant Protection Products and their Residues (PPR-Panel). Question No EFSA-Q-2007-048. The EFSA Journal (2007) 622, 1-32*]. A summary of the calculated half-lives of pyrimethanil at 10°C is given in the table below.

Table 7.1.2.1.1-19: Summary table on degradation endpoints of pyrimethanil obtained in laboratory soil studies, re-calculated to 10°C and pF 2

BASF DocID	Soil (Soil type)	pH ($CaCl_2$)	Org. C [%]	Temp. [°C]	Moisture [% MWHC]	$DegT_{50}$ at 20°C, pF2 [d]	$DegT_{50}$ at 10°C, pF2 [d]	Method of calculation
A81879, 2012/1321627	Schering Soil No. 178 (Loam)	7.5	3.5	20	40	48.1	124.1	Q_{10} = 2.58
A81878, 2012/1321627	German Standard Soil 2.2 (Loamy sand)	5.8	2.4	20	40	81.9	211.3	Q_{10} = 2.58
A81904, 2012/1321627	German Standard Soil 2.3 SP 389 (Sandy loam)	5.9	0.95	20	40	22.6	58.3	Q_{10} = 2.58
A81888, 2012/1321627	German Standard Soil 2.1 (Sand)	5.4	0.74	21	40	43.7	112.7	Q_{10} = 2.58

MWHC maximum water holding capacity

CA 7.1.2.1.2 Aerobic degradation of metabolites, breakdown and reaction products

The metabolite 2-amino-4,6-dimethyl-pyrimidine (M605F007, synonym: AE F132593) was the only metabolite of pyrimethanil occurring at amounts of > 5% AR in aerobic soil (Table 7.1.2.1.2-1). For this metabolite, the rate of degradation was studied in different soils.

Table 7.1.2.1.2-1: Maximum occurrence of pyrimethanil metabolite M605F007 in laboratory aerobic soil studies (20°C, 40% MWHC)

Soil study	BASF DocID	Parent Label	Soil	Maximum % AR (at day x)
Aerobic soil metabolism	A81904 [a]	pyrimidinyl	German Standard Soil 2.3 (Sandy loam)	8.3 (62 d)
Aerobic soil metabolism	A81888 [a]	pyrimidinyl	German Standard Soil 2.1 (Sand)	11.5 (105 d)
Anaerobic soil metabolism (30 d aerobic pre-incubation phase)	A89445 [a]	pyrimidinyl	German Standard Soil 2.3 (Sandy loam)	13.6 (30 d)

MWHC maximum water holding capacity
[a] already peer-reviewed during former Annex I listing

New, not yet peer-reviewed kinetic evaluations:

The report on kinetic re-evaluation of all the aerobic soil studies is already included in full detail in chapter CA 7.1.2.1.1 for the parent degradation rates (reference CA 7.1.2.1.1/1). Since this report provides also the respective degradation parameters (trigger and modeling endpoints) for the metabolite, it is included here again. However, the summary below reports briefly only the resulting degradation endpoints for M605F007. For data handling and more details about the kinetic evaluation please see CA 7.1.2.1.1/1.

Report: CA 7.1.2.1.2/1
Sopena-Vazquez F.,Budde E., 2013a
Kinetic evaluation of laboratory soil degradation studies with Pyrimethanil and its metabolite (AE F132593) according to FOCUS degradation kinetics 2012/1321627
Guidelines: FOCUS Kinetics (2006) SANCO/10058/2005 version 2.0
GLP: no

Executive Summary

The degradation behavior of pyrimethanil (BAS 605 F) and its soil metabolite 2-amino-4,6-dimethyl-pyrimidine (M605F007, previously referred to as AE F132593) in aerobic soil has been investigated in five laboratory degradation studies. In four of these studies with one soil each, pyrimethanil was applied as test item, while in the fifth study with five soils, the metabolite was used as test item.

The purpose of this re-evaluation is to analyze the degradation kinetics observed in the studies, taking into account the current guidance of the FOCUS workgroup on degradation kinetics. Kinetic evaluation was performed in order to derive degradation parameters as triggers for additional work (trigger endpoints) and degradation parameters for environmental fate models (modeling endpoints).

The appropriate kinetic model to derive trigger and modeling endpoints was identified considering the procedures and kinetic models proposed by the FOCUS workgroup on degradation kinetics. The best-fit model to derive trigger endpoints was selected based on visual and statistical assessment.

Trigger endpoints for the pyrimethanil metabolite M605F007 were derived from SFO (six soils) as well as from DFOP (one soil) kinetics. The endpoints ranged from15.6 to 207.3 days (DT_{50}) and from 51.8 to 688.5 days (DT_{90}).

Modeling endpoints for the metabolite M605F007were derived from SFO (six soils) and DFOP (one soil) kinetics. Normalized modeling DT_{50} values ranged from 15.6 to 208.5 days.

I. MATERIAL AND METHODS

The material and methods of the kinetic evaluation and normalization procedure are presented in detail in reference CA 7.1.2.1.1/1.

II. RESULTS AND DISCUSSION

For the metabolite M605F007, valid degradation endpoints could derived from two parent studies (one soil each) as well as from an additional study (five soils) where the metabolite was applied as test item.

The resulting best-fit DT_{50} and DT_{90} values for the metabolite are summarized in Table 7.1.2.1.2-2.
The DT_{50} values at study conditions, the correction factors for temperature and moisture correction, and the resulting DT_{50} values normalized to reference conditions (modeling endpoints) for metabolite M605F007 are summarized in Table 7.1.2.1.2-3 and Table 7.1.2.1.2-4.

Table 7.1.2.1.2-2: Trigger endpoints for additional work for pyrimethanil metabolite M605F007 based on best-fit models

Study (DocID)	Soil	Test item (label)	Best-fit model	χ^2 error	Trigger endpoints	
					DT_{50} [d]	DT_{90} [d]
Aerobic soil degradation of the metabolite M605F007						
A81904	German Standard Soil 2.3 SP 389 (Sandy loam)	parent (pyr)	SFO	26.7	87.2	289.5
A81888	German Standard Soil 2.1 (Sand)		SFO	5.7	207.3	688.5
C011694	SLS (Silt loam)	metabolite M605F007 (pyr)	SFO	6.2	15.6	51.8
	Bologna (Sandy clay loam)		SFO	8.1	21.3	70.8
	Ferrara (Silty clay)		SFO	5.5	36.8	122.3 *
	LS 2.2 (Loamy sand)		DFOP	3.3	95.0	579.2 *
	SL 2.3 (Sandy loam)		SFO	6.7	117.9	391.7 *
				Geo mean	**59.0**	**213.6**

* Since 10% TAR was not reached within the experimental period, DT_{90} values should be interpreted carefully
(pyr) = position of 14C-label (pyrimidinyl)

Table 7.1.2.1.2-3: Calculated factors for normalization of degradation rates to standard conditions

Study (DocID)	Soil	T_{act} [°C]	T_{ref} [°C]	θ_{act} [g/100g]	θ_{ref} [g/100g]	f_{temp}	f_{moist}
Aerobic soil degradation of the metabolite M605F007							
A81904 [a]	German Standard Soil 2.3 SP 389 (Sandy loam)	20	20	14.9	19	1.0	0.843
A81888 [a]	German Standard Soil 2.1 (Sand)	21	20	10.4	12	1.1	0.890
C011694 [b]	SLS (Silt loam)	20	20	27.5	26	1.0	1.0
	Bologna (Sandy clay loam)	20	20	24.1	22	1.0	1.0
	Ferrara (Silty clay)	20	20	28.5	40	1.0	0.788
	LS 2.2 (Loamy sand)	20	20	23.2	14	1.0	1.0
	SL 2.3 (Sandy loam)	20	20	21.4	19	1.0	1.0

T_{act}	actual temperature during incubation	[°C]
T_{ref}	reference temperature (20°C)	[°C]
θ_{act}	actual soil moisture	[g / 100 g dry soil]
θ_{ref}	reference soil moisture at field capacity (pF 2, 10 kPa) according to FOCUS	[g / 100 g dry soil]
f_{temp}	temperature correction factor	[-]
f_{moist}	moisture correction factor	[-]

[a] pyrimethanil was the applied test item
[b] the metabolite was the applied test item

Table 7.1.2.1.2-4: Modeling endpoints for pyrimethanil metabolite M605F007

Study (DocID)	Soil	Test item (label)	Best-fit model	χ^2 error	Modeling endpoints		
					DT_{50} [d]	Normalized DT_{50} [d]	FF [-]
Aerobic soil degradation of the metabolite M605F007 (AE F132593)							
A81879	German Standard Soil 2.3 SP 389 (Sandy loam)	parent (pyr)	SFO	26.7	87.2	73.5	0.106 ±0.03
A81878	German Standard Soil 2.1 (Sand)		SFO	5.7	207.3	202.8	0.188 ±0.03
C011694	SLS (Silt loam)	metabolite M605F007 (pyr)	SFO	6.2	15.6	15.6	-
	Bologna (Sandy clay loam)		SFO	8.1	21.3	21.3	-
	Ferrara (Silty clay)		SFO	5.5	36.8	29.0	-
	LS 2.2 (Loamy sand)		DFOP [d]	3.3	208.5 [c]	208.5 *	-
	SL 2.3 (Sandy loam)		SFO	6.7	117.9	117.9	-
					Geo mean	**62.0**	
					Arithm mean		**0.147**

* Modeling endpoint derived from the slow phase of bi-phasic fit

III. CONCLUSION

Trigger endpoints for additional work and modeling endpoints were derived for pyrimethanil and its metabolite M605F007 in five laboratory degradation studies.

Trigger endpoints for the pyrimethanil metabolite M605F007 derived from SFO (six soils) as well as from DFOP (one soil) kinetics ranged from15.6 to 207.3 days (DT_{50}) and from 51.8 to 688.5 days (DT_{90}).

Modeling endpoints for M605F007, normalized modeling-DT_{50} values were derived from SFO (six soils) and DFOP (one soil) kinetics and ranged from 15.6 to 208.5 days.

The following report was provided in order to address concerns raised by the Rapporteur Member State. The aim was to provide additional information regarding the kinetics and rate of degradation in soil for the metabolite M605F007, specifically addressing concerns related to

a. Temperature normalization of the study C011694, investigating the potential effect of temperature deviation during the incubation
b. Re-fitting of metabolite kinetics in study A81904 using the latest version of the fitting tool KinGUI

Report:	CA 7.1.2.1.2/2 Erzgraeber B., 2017 a BAS 605 F - Pyrimethanil: Additional information related to the degradation kinetics of Pyrimethanil and its metabolite in soil 2017/1066535
Guidelines:	none
GLP:	no

a. Temperature normalization of study C011694

I. MATERIAL AND METHODS

In the aerobic soil degradation study with pyrimethanil metabolite M605F007 (2-amino-2,4-dimethylpyrimidine) [Koehn, D.M. (2001), DocID C011694], the incubation temperature had increased for a short time from 20°C to 27°C due to technical issues. The deviation from the default incubation temperature of 20°C was described in the study report and occurred between 20th Dec. 1997 and 3rd Jan. 1998, i.e. for a time span of 14 days and between sampling day 120 and sampling day 153. Thus, only samplings at 153 DAT (days after treatment) and 240 DAT were effected.

The kinetic analysis presented in Sopena-Vazquez, F., Budde E. (2013a) [DocID 2012/1321627] had not specifically considered this temperature increase, assuming that it had only negligible impact on the overall degradation rates due to the short time interval and late occurrence of the deviation. This hypothesis is investigated in the current work.

In order to assess the impact of the short deviation from the default temperature on the degradation kinetics and DT_{50} / DT_{90} values, a time-step normalization approach was applied. This normalization procedure is similar to the normalization usually done for field studies [FOCUS (2006, 2014)], where fluctuating temperature and moisture are corrected for during the kinetic evaluation. In this approach a normalized “day length” is calculated based on daily variation in soil temperature and moisture. The daily values are calculated and the cumulative time between sampling time points determined and used as input into a standard kinetic evaluation.

In the present case, only temperature normalization is considered. Equation 7.1.2-1 was applied to derive normalized time-steps corrected to 20°C and the data were then re-fitted with the kinetic analysis tool KinGUI (version 2.2014.224.1704), following the FOCUS Kinetics guidance. The actual and temperature-corrected sampling time points are given in Table **7.1.2.1.2-5:** .

Equation 7.1.2-1 Calculation of temperature correction factor and normalized time-steps

a) $D_{norm} = D \cdot f_{temp}$

b) $t_i = \sum_{t=1}^{i-1} D_{norm}$

with

t_i	time from application till sampling at day i	[d]
D_{norm}	normalized day length (20° C, pF 2)	[d]
i	time span between application and sampling	[d]

c) $f_{temp} = Q_{10}^{\frac{Tact-Tref}{10}}$

with

D_{norm}	normalized day length (temperature and moisture)	[d]
f_{temp}	temperature correction factor	[-]
D	1 d	
T_{act}	actual temperature [°C]	
T_{ref}	reference temperature (20°C)	[°C]
Q_{10}	factor of increase of degradation rate with an increase in temperature of 10°C (Q10=2.58)	[-]

Table 7.1.2.1.2-5: Actual and temperature-corrected sampling time points, valid for all soils in study C011694

actual sampling time point [DAT]	temperature corrected sampling time point [d]
0	0
7	7
14	14
28	28
62	62
90	90
120	120
153	167.1
230	244.1

The temperature corrected sampling time points were used in combination with the modelling data provided above (Table 7.1.2.1.2-5).

II. RESULTS AND DISCUSSION

The statistical and visual assessment of the different kinetic models tested for metabolite M605F007 are presented in the following tables for the soils SLS, Bologna, Ferrara, LS 2.2, and SL 2.3, respectively.

Table 7.1.2.1.2-6: Statistical and visual assessment of different degradation kinetic models for the metabolite M605F007 in soil SLS

Kinetic model	χ^2 error	parameters	95% CI		p (t-test)	DT_{50} [d]	DT_{90} [d]	Visual assessment
			lower	upper				
SFO	**6.3**	**k: 0.0445**	**-**	**-**	**k: <0.001**	**15.6**	**51.7**	**good**
FOMC	4.7	α: 3.6 β: 68.4	0.89 6.68	6.32 130.2	-	14.5	61.3	good

⇨ SFO provides a good fit to the experimental data, the χ^2 error is small and the degradation rate is statistically significant.
⇨ FOMC improves the χ^2 error slightly, but the visual fit is not improved compared to the SFO kinetics. DT_{50} and DT_{90} values are very similar to the SFO endpoints.
⇨ **Conclusion: SFO is used for trigger endpoint and for modeling endpoint.**

Table 7.1.2.1.2-7: Statistical and visual assessment of different degradation kinetic models for the metabolite M605F007 in soil Bologna

Kinetic model	χ^2 error	parameters	95% CI		p (t-test)	DT_{50} [d]	DT_{90} [d]	Visual assessment
			lower	upper				
SFO	**8.2**	**k: 0.0326**	**-**	**-**	**k: <0.001**	**21.3**	**70.7**	**good**
FOMC	8.6	α: 9141 β: 280700	7548 280700	10734 280777	-	21.3	70.7	good

⇨ SFO provides a good fit to the experimental data, the χ^2 error is small and the degradation rate is statistically significant.
⇨ FOMC does not improve the fit compared to the SFO kinetics.
⇨ **Conclusion: SFO is used for trigger endpoint and for modeling endpoint.**

Table 7.1.2.1.2-8: Statistical and visual assessment of different degradation kinetic models for the metabolite M605F007 in soil Ferrara

Kinetic model	χ^2 error	parameters	95% CI		p (t-test)	DT_{50} [d]	DT_{90} [d]	Visual assessment
			lower	upper				
SFO	**5.7**	**k: 0.0188**	**-**	**-**	**k: <0.001**	**37.0**	**122.8**	**good**
FOMC	6.1	α: 1121 β: 59750	-499000 -26610000	501300 26730000	-	36.9	122.8	good

⇨ SFO provides a good fit to the experimental data, the χ^2 error is small and the degradation rate is statistically significant.
⇨ FOMC does not improve the fit compared to the SFO kinetics.
⇨ **Conclusion: SFO is used for trigger endpoint and for modeling endpoint.**

Table 7.1.2.1.2-9: Statistical and visual assessment of different degradation kinetic models for the metabolite M605F007 in soil LS 2.2

Kinetic model	χ^2 error	parameters	95% CI lower	95% CI upper	p (t-test)	DT_{50} [d]	DT_{90} [d]	Visual assessment
SFO	9.8	k: 0.0051	-	-	k: <0.001	137.2	455.7	moderate
FOMC	3.6	α: 0.316 β: 10.42	0.2265 2.191	0.405 18.64	-	83.0	>1000	good
DFOP	**3.7**	**k1: 0.0797** **k2: 0.0029** **g: 0.335**	**0.234**	**0.436**	**k1: <0.05** **k2: <0.01**	**96.9**	**643.9**	**good**

⇨ SFO provides a moderate fit to the experimental data, the χ^2 error <10% and the degradation rate is statistically significant.
⇨ FOMC improves the visual fit. Since DT_{90} is not achieved, DFOP kinetics is tested.
⇨ DFOP provides a good fit with low χ^2 error and statistically significant parameters.
⇨ **Conclusion: DFOP is selected to derive trigger endpoints and for modeling endpoints. For modeling, use the slow phase DT_{50} value (DT_{50} = 235.6d).**

Table 7.1.2.1.2-10: Statistical and visual assessment of different degradation kinetic models for the metabolite M605F007 in soil SL 2.3

Kinetic model	χ^2 error	parameters	95% CI lower	95% CI upper	p (t-test)	DT_{50} [d]	DT_{90} [d]	Visual assessment
SFO	**6.8**	**k: 0.0056**	**-**	**-**	**k: <0.001**	**123.2**	**409.3**	**good**
FOMC	5.2	α: 0.713 β: 61.45	0.131 -25.33	1.295 148.2	-	101.0	>1000	good
DFOP	**4.7**	**k1: 0.1068** **k2: 0.0045** **g: 0.195**	**0.056**	**0.334**	**k1: 0.12** **k2: < 0.001**	**105.0**	**460.1**	**good**

⇨ SFO provides a good fit to the experimental data, the χ^2 error is low and the degradation rate is statistically significant.
⇨ FOMC improves the visual fit slightly. Since DT_{90} is not achieved, DFOP kinetics is tested.
⇨ DFOP provides a good fit with low χ^2 error and statistically significant slow-phase degradation rate.
⇨ **Conclusion: SFO is acceptable to derive modeling endpoints. DFOP is selected to derive trigger endpoints.**

A comparison of degradation endpoints derived by Sopena-Vazquez F., Budde E. (2013a) and the time-step normalized endpoints is given in the following table.

Table 7.1.2.1.2-11: Comparison of degradation endpoints provided by Sopena-Vazquez F., Budde E. (2013a) and time-step normalized endpoints

Soil	Sopena-Vazquez F., Budde E. (2013a)			timestep normalization (Tref = 20°C)		
Trigger endpoints						
	Kinetic model	DT_{50} [d]	DT_{90} [d]	Kinetic model	DT_{50} [d]	DT_{90} [d]
SLS	SFO	15.6	51.8	SFO	15.6	51.7
Bologna	SFO	21.3	70.8	SFO	21.3	70.7
Ferrara	SFO	36.8	122.3	SFO	37.0	122.8
LS 2.2	DFOP	95.0	579.2	DFOP	96.9	643.9
SL 2.3	SFO	117.9	391.7	DFOP	105.0	460.1
Modeling endpoints (at incubation moisture)						
	Kinetic model	DT_{50} [d]		Kinetic model	DT_{50} [d]	
SLS	SFO	15.6		SFO	15.6	
Bologna	SFO	21.3		SFO	21.3	
Ferrara	SFO	36.8		SFO	37.0	
LS 2.2	DFOP	208.5 [a]		DFOP	235.6 [a]	
SL 2.3	SFO	117.9		SFO	123.2	
Geo mean		**49.6**			**51.3**	

[a] modeling endpoint derived from the slow phase of the DFOP model

III. CONCLUSION

Despite a short-term deviation of the incubation temperature and increase to 27°C in study C011694, it is possible to derive normalized degradation endpoints as trigger and for modeling by applying a time-step normalization approach similar to the evaluation usually done for field studies. The temperature deviation can be considered by adjusting the sampling time points accordingly.

Kinetic analysis of the time-step normalized data resulted in DT_{50} and DT_{90} endpoints very similar to those reported by Sopena-Vazquez F., Budde E. (2013a). In soils SLS, Bologna and Ferrara, the temperature deviation had no impact on the overall degradation kinetics. Only in soils LS 2.2 and SL 2.3 a slight increase of the DT_{50} / DT_{90} is noted. The geometric mean DT_{50} across all soils is marginally affected.

The time-step normalized endpoints therefore confirm the previously reported DT_{50} and DT_{90} values for the metabolite M605F007.

b. Metabolite kinetics in study A81904

I. MATERIAL AND METHODS

The kinetic analysis reported in Sopena-Vazquez F., Budde E. (2013a) included the study A81904 [Feyerabend M. (1993)], from which parent and metabolite degradation endpoints had been derived from a combined pathway fit. The parameter estimation at that time was done with KinGUI Version 2.2012.320.1629.
In the meantime, KinGUI has been improved and updated, particularly with regards to the optimization algorithms. Therefore, the kinetic evaluation is repeated with the latest version of the fitting tool KinGUI. The modelling data used are the same as reported in Sopena-Vazquez F., Budde E. (2013a) above (Table 7.1.2.1.1-9).

In addition to the pathway fit, a metabolite decline fit (from the maximum onwards) is tested. Conservative estimates of metabolite DT_{50} and DT_{90} values can often be derived by fitting a decline curve from the time of maximum onwards. This can be considered as a more conservative estimate for the true degradation rate of the metabolite. Since in study the study, a clear decline phase for the metabolite M605F007 was established, a decline fit from the time of maximum was conducted. The modelling data for this decline fit are provided in the following table.

Table 7.1.2.1.2-12: Data for metabolite M605F007 in German Standard Soil 2.3 SP 389 used for metabolite decline fit from maximum (study A81904)

Time after maximum [d]	Experimental data [%TAR]	Input data according to FOCUS [%TAR]
0	9.8	9.8
0	6.8	6.8
28	5.1	5.1
28	5.3	5.3
91	3.0	3.0
91	4.5	4.5
182	2.2	2.2
182	2	2.0
302	1.2	1.2
302	1.0	1.0

II. RESULTS AND DISCUSSION

The statistical assessment of the pathway fit for pyrimethanil and its metabolite M605F007 in German Standard Soil 2.3 SP 389 is shown in the following table.

Table 7.1.2.1.2-13: Statistical and visual assessment of degradation kinetics for pyrimethanil and its metabolite M605F007 (AE F132593) in German Standard Soil 2.3 SP 389 in a combined pathway fit

Kinetic model	χ^2 error	parameter	95% CI		p (t-test)	visual assessment	DT_{50} [d]	DT_{90} [d]	formation fraction
			lower	upper					
par: SFO	12.5	k: 0.0255	0.021	0.030	k: <0.001	moderate	27.2	90.4	-
met: SFO	25.1	k: 0.0080	0.004	0.012	k: <0.001	good	87.2	289.5	0.107
⇨ SFO visual fit is visually acceptable, the derived parameters are statistically significant. ⇨ **Conclusion: SFO can be used to derive metabolite endpoints for trigger and for modeling**.									

In the combined pathway fit, an acceptable visual fit was obtained and the estimated degradation rates are statistically significant (p-values <0.001). In one of two replicate measurements at DAT 62, the maximum occurrence of the metabolite was higher than the predicted curve, however, the deviation between the measured amount (mean of two replicates) and the predicted amount at this sampling time point is only 2.1%. This is considered an acceptable deviation.

In addition to the pathway fit, a fit from maximum was tested for the metabolite. The results of the metabolite decline fit are given below.

Table 7.1.2.1.2-14: Statistical and visual assessment of degradation kinetics for metabolite M605F007 in German Standard Soil 2.3 SP 389 in a metabolite decline fit

Kinetic model	χ^2 error	parameter	95% CI		p (t-test)	visual assessment	DT_{50} [d]	DT_{90} [d]
			lower	upper				
SFO	10.8	0.0079	0.005	0.011	k: <0.001	good	89.1	295.9
⇨ SFO visual fit is visually acceptable, the derived parameters are statistically significant. ⇨ **Conclusion: the SFO decline fit provides a good description of the decline phase of M605F007, it can be used to derive metabolite endpoints for trigger and for modeling. The decline fit confirms the degradation rate obtained in the pathway fit**.								

III. CONCLUSION

It was shown, that a combined fit for pyrimethanil and its metabolite M605F007 for study A81904 does result in an acceptable overall fit and statistically reliable parameters.
In one of two replicate measurements at DAT 62, the maximum occurrence of the metabolite was higher than the predicted curve, however, the deviation between the measured amount (mean of two replicates) and the predicted amount at this sampling time point is only 2.1%. This is considered an acceptable deviation.

The metabolite DT_{50} was confirmed by a decline fit starting at the time of maximum occurrence, which can be considered to provide a conservative estimate for the metabolite degradation. SFO kinetics provided a good description of the decline phase and the resulting DT_{50} value of 89 days compares very well with the DT_{50} value estimated from the pathway fit (87 days).

Therefore, the kinetic parameters derived for this soil for the metabolite M605F007 are acceptable for deriving trigger and modelling endpoints.

Summary of degradation endpoints for pyrimethanil metabolite M605F007 in different soils under aerobic conditions

Table 7.1.2.1.2-15: Summary table on best-fit degradation endpoints of pyrimethanil metabolite M605F007 (AE F132593) as obtained in aerobic soil (laboratory, 20-21°C, 40-50%MWHC)

BASF DocID	Test Item (label)	Soil (Soil type)	pH ($CaCl_2$)	Org. C [%]	Temp. [°C]	Moisture [%MWHC]	Best-fit $DegT_{50}$/ $DegT_{90}$ [d]	Kinetic model	χ^2 error level
A81904, 2012/ 1321627	parent (pyr)	German Standard Soil 2.3 SP 389 (Sandy loam)	5.9	0.95	20	40	87.2 / 289.5	SFO	26.7
A81888, 2012/ 1321627	parent (pyr)	German Standard Soil 2.1 (Sand)	5.4	0.74	21	40	207.3 / 688.5	SFO	5.7
C011694, 2012/ 1321627	M605F007 (pyr)	SLS (Silt loam)	7.0	2.08	20	50	15.6 / 51.8	SFO	6.2
		Bologna (Sandy clay loam)	7.6	2.1	20	50	21.3 / 70.8	SFO	8.1
		Ferrara (Silty clay)	7.6	2.4	20	50	36.8 / 122.0	SFO	5.5
		LS 2.2 (Loamy sand)	6.0	1.95	20	50	95.0 / 579.2	DFOP	3.3
		SL 2.3 (Sandy loam)	6.9	1.51	20	50	117.9 / 391.7	SFO	6.7

MWHC maximum water holding capacity
(pyr) = position of 14C-label (pyrimidinyl)

Table 7.1.2.1.2-16: Summary table on degradation endpoints for modeling of pyrimethanil metabolite M605F007 as obtained in laboratory soil studies (normalized to 20°C, pF2)

BASF DocID	Test Item (label)	Soil (Soil type)	pH ($CaCl_2$)	Org. C [%]	$DegT_{50}$ at study conditions [d]	$DegT_{50}$ normalized to 20°C, pF2 [d]	Kinetic model	χ^2 error level	FF [-]
A81904, 2012/ 1321627	parent (pyr)	German Standard Soil 2.3 SP 389 (Sandy loam)	5.9	0.95	87.2	73.5	SFO	26.7	0.106 ±0.03
A81888, 2012/ 1321627	parent (pyr)	German Standard Soil 2.1 (Sand)	5.4	0.74	207.3	202.8	SFO	5.7	0.188 ±0.03
C011694, 2012/ 1321627	M605F007 (pyr)	SLS (Silt loam)	7.0	2.08	15.6	15.6	SFO	6.2	-
		Bologna (Sandy clay loam)	7.6	2.1	21.3	21.3	SFO	8.1	-
		Ferrara (Silty clay)	7.6	2.4	36.8	29.0	SFO	5.5	-
		LS 2.2 (Loamy sand)	6.0	1.95	208.5 *	208.5 *	DFOP *	3.3	-
		SL 2.3 (Sandy loam)	6.9	1.51	117.9	117.9	SFO	6.7	-
					Geo mean	**62.0**			
					Arithm mean				**0.147**

* Modeling endpoint derived from the slow phase of bi-phasic fit
MWHC maximum water holding capacity
(pyr) = position of 14C-label (pyrimidinyl)

CA 7.1.2.1.3 Anaerobic degradation of the active substance

No new experimental data were generated. The old, already peer-reviewed study (*Tarara G. (1996), BASF DocID A89445, IIA 7.1.1.2.1/9*) is considered still valid. A brief summary of the results of this study can be found above in chapter CA 7.1.1.2.

Since the kinetic evaluations provided in the old reports are outdated, new kinetic evaluations for pyrimethanil were performed according to current FOCUS guidelines.

New, not yet peer-reviewed kinetic evaluation:

Report: CA 7.1.2.1.3/1
Studenroth S.,Budde E., 2014a
Kinetic evaluation of anaerobic laboratory soil metabolism study with Pyrimethanil according to FOCUS Degradation Kinetics
2014/1000842
Guidelines: FOCUS Kinetics (2006) SANCO/10058/2005 version 2.0
GLP: no

Executive Summary

The degradation behavior of pyrimethanil (BAS 605 F) in soil under anaerobic conditions has been investigated in a laboratory metabolism study with one soil using 2-^{14}C-pyrimidinyl-labeled pyrimethanil. The purpose of this evaluation was to analyze the degradation kinetics observed in the study, taking into account the current guidance of the FOCUS workgroup on degradation kinetics.

The calculated kinetic endpoints of pyrimethanil and its metabolite 2-amino-4,6-dimethylpyrimidine (M605F007, previously referred to as AE F132593) are summarized in the following table.

Table 7.1.2.1.3-1: Endpoints for anaerobic degradation of pyrimethanil and its metabolite M605F007 (AE F132593)

Compound	Kinetic model	χ^2 error	DT_{50} [d]	DT_{90} [d]	Model parameters
Pyrimethanil	HS	7.5	18.7 (aerobic) [a] > 1000 (anaerobic) [a]	62.0 (aerobic) [b] > 1000 (anaerobic) [b]	k1 [d^{-1}]: 0.03715 k2 [d^{-1}]: 2.3 x 10^{-14} tb [d]: 38.44
M605F007 (AE F132593)	SFO	2.2	216.2 [c]	718.4	k: 0.003205

[a] DT_{50} calculated for aerobic and anaerobic phase, with $DT_{50} = ln2/k$ ($k_{aerobic}$ = 0.03715, $k_{anaerobic}$ = 2.3 x 10^{-14})
[b] DT_{90} calculated for aerobic and anaerobic phase, with $DT_{90} = ln10/k$ ($k_{aerobic}$ = 0.03715, $k_{anaerobic}$ = 2.3 x 10^{-14})
[c] Fit starting with maximum observed concentration on day 30

For pyrimethanil, degradation was observed during the aerobic phase, while no significant degradation was observed under the applied anaerobic conditions. The estimated DT_{50} values for pyrimethanil were derived with the HS kinetic model with 18.7 days for the aerobic phase and > 1000 days for the anaerobic phase. For the metabolite M605F007, degradation during the anaerobic phase was observed and the SFO model was considered appropriate to derive a dissipation endpoint with $DisT_{50}$ of 216.2 days.

I. MATERIAL AND METHODS

Anaerobic degradation of pyrimethanil (2-^{14}C-pyrimidinyl-labeled) and its metabolite 2-amino-4,6-dimethylpyrimidine (M605F007, previously referred to as AE F132593) was investigated in one soil in the laboratory at 20°C in the dark [*Tarara, G. (1996) BASF DocID A89445*]. The purpose of this evaluation was to analyze the degradation kinetics observed in the study according to current guidance of the FOCUS workgroup on degradation kinetics [*FOCUS (2006): "Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration" Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005 version 1.0, 436 pp*].

Kinetic modeling strategy

The kinetic evaluation was performed in order to derive degradation endpoints for the parent and dissipation endpoints for the metabolite (i.e., from the maximum observed concentration). The appropriate kinetic model was identified based on visual and statistical assessment considering the procedures and kinetic models proposed by the FOCUS workgroup on degradation kinetics [*FOCUS (2006)*]. Appropriate DT_{50} values were derived depending on the kinetic model.

Kinetic models included in the evaluations

For the pyrimethanil data set, the kinetic models proposed by FOCUS Kinetics [*FOCUS (2006)*] was tested in order to identify the best-fit model, i.e. single first order (SFO) kinetics and the bi-exponential (FOMC, DFOP, HS) kinetics. For the assessment of the formation and degradation parameters of the metabolite, the SFO and the FOMC model were used.

The goodness-of-fit of the kinetic models was assessed by visual inspection and statistical measures, as recommended by the FOCUS Kinetics guidance [*FOCUS (2006)*].

Data handling and software for kinetic evaluation

The experimental data were derived from the study reports and adjusted according to FOCUS [*FOCUS (2006)*].

The software package KinGUI version 2.2014.224.1704 was used for parameter fitting. The error tolerance and the number of iterations of the optimization tool (IRLS) were set to $1x10^{-6}$ and 100, respectively.

Experimental data

The kinetic evaluation was based on the findings of a laboratory anaerobic soil metabolism study with one soil from Germany [*BASF DocID A89445*]. The soil characteristics are summarized in Table 7.1.2.1.3-2.

Table 7.1.2.1.3-2: Soil characteristics

Parameter	**German Standard Soil 2.3**
Soil type (USDA)	Sandy loam
Particle size distribution [%]	
Sand (50 - 2000 µm)	64.99
Silt (2 - 50 µm)	23.25
Clay (< 2 µm)	11.76
Organic carbon [%]	1.31
Microbial biomass [mg C kg^{-1} dry soil weight]	37.8
CEC [mVal $100g^{-1}$ dry weight]	8.83
pH ($CaCl_2$)	6.2
Max. water holding capacity [g $100g^{-1}$ soil]	26.85

The test soil was treated with 2-^{14}C-pyrimidinyl-labeled pyrimethanil at a nominal application rate of 1.33 mg kg^{-1} dry soil (corresponding to a field application rate of 1 kg a.s. ha^{-1}), incubated aerobically for 30 days (pre-incubation) and anaerobically for 90 days in the dark at 20°C. Soil samples were taken at 0, 7, 14, 30 days after treatment (DAT; aerobic phase) and at 37 (7), 44 (14), 64 (34), 90 (60), and 120 (90) DAT (anaerobic phase).

The measured data as well as resulting data sets submitted to kinetic analysis are given in Table 7.1.2.1.3-3.

Table 7.1.2.1.3-3: Data for kinetic evaluation of anaerobic metabolism of 2-14C-pyrimidinyl-pyrimethanil and its metabolite M605F007 (AE F132593) in German Standard Soil 2.3

Day	Experimental data [%TAR]		Input data according to FOCUS [%TAR]	
	Pyrimethanil	M605F007 (AE F132593)	Pyrimethanil	M605F007 (AE F132593)
0	99.8		101.0 [b]	
0	99.0		100.2 [b]	
7	75.85		75.85	
7	82.30		82.30	
14	67.95		67.95	
14	72.38		72.38	
30 (0)[a]	27.83	15.21	27.83	15.21
30 (0)[a]	28.86	12.00	28.86	12.00
37 (7)[a]	22.42	13.56	22.42	13.56
37 (7)[a]	26.31	11.01	26.31	11.01
44 (14)[a]	22.92	10.32	22.92	10.32
44 (14)[a]	22.52	12.04	22.52	12.04
64 (34)[a]	25.67	10.45	25.67	10.45
64 (34)[a]	24.51	12.06	24.51	12.06
90 (60)[a]	28.15	8.77	28.15	8.77
90 (60)[a]	22.59	11.17	22.59	11.17
120 (90)[a]	30.22	9.91	30.22	9.91
120 (90)[a]	21.45	10.15	21.45	10.15

TAR = Total Applied Radioactivity
[a] Anaerobic phase after water-logging
[b] Set to material balance

II. RESULTS AND DISCUSSION

Pyrimethanil degraded during aerobic pre-incubation with a DT_{50} of 18.7 days, but was stable under anaerobic conditions; therefore, no statistically significant degradation rate could be derived for the anaerobic phase of the study.
For the metabolite M605F007, the SFO model was considered appropriate to derive a $DisT_{50}$ value from the anaerobic degradation study. The results are summarized in Table 7.1.2.1.3-4.

Table 7.1.2.1.3-4: Endpoints for anaerobic degradation of pyrimethanil and its metabolite M605F007 (AE F132593)

Compound	Kinetic model	χ^2 error	DT_{50} [d]	DT_{90} [d]	Model parameters
Pyrimethanil	HS	7.5	18.7 (aerobic) [a] > 1000 (anaerobic) [a]	62.0 (aerobic) [b] > 1000 (anaerobic) [b]	k1 [d^{-1}]: 0.03715 k2 [d^{-1}]: 2.3 x 10^{-14} tb [d]: 38.44
M605F007 (AE F132593)	SFO	2.2	216.2 [c]	718.4	k: 0.003205

[a] DT_{50} calculated for aerobic and anaerobic phase, with $DT_{50} = ln2/k$ ($k_{aerobic} = 0.03715$, $k_{anaerobic} = 2.3 \times 10^{-14}$)
[b] DT_{90} calculated for aerobic and anaerobic phase, with $DT_{90} = ln10/k$ ($k_{aerobic} = 0.03715$, $k_{anaerobic} = 2.3 \times 10^{-14}$)
[c] Fit starting with maximum observed concentration on day 30

III. CONCLUSION

According to the guidance of the FOCUS workgroup on degradation kinetics, degradation kinetics for pyrimethanil and its metabolite M605F007 were performed. For pyrimethanil, degradation was observed during the aerobic phase, while no significant degradation was observed under the applied anaerobic conditions. The estimated DT_{50} values for pyrimethanil were derived with the HS kinetic model, with 18.7 days for the aerobic phase and > 1000 days for the anaerobic phase. For the metabolite M605F007, degradation during the anaerobic phase was observed and the SFO model was considered appropriate to derive a dissipation endpoint with $DisT_{50}$ of 216.2 days.

CA 7.1.2.1.4 Anaerobic degradation of metabolites, breakdown and reaction products

No extra study on the anaerobic degradation of soil metabolite M605F007 (AE F132593) was performed. Information on the behaviour in anaerobic soil could be deduced from the parent study, where the metabolite was formed in significant amounts during the 30 day aerobic pre-incubation phase. The metabolite was included in the report for the kinetic evaluation of pyrimethanil degradation in anaerobic soil, and the results are presented above in chapter CA 7.1.2.1.3.

CA 7.1.2.2 Field studies

CA 7.1.2.2.1 Soil dissipation studies

The already peer-reviewed field soil dissipation studies running from 1991/1992 with results from eight locations in Germany are still considered valid.

Already peer-reviewed studies :

former Annex point / Reference Number:	*Report:*	*BASF DocID:*	*Trial sites:*
A II M 7.1.1.2.2/1	*Wrede-Ruecker A. 1993*	*A81908*	*Goch-Nierswalde* *Weeze-Wemb* *Emmerich-Elten* *Schwichteler*
A II M 7.1.1.2.2/2	*Seiler P. 1993*	*A81909*	*field and weather data for A81908*
A II M 7.1.1.2.2/3	*Wrede A. 1994*	*A81928*	*Goch* *Meissner-Vockerrode* *Schwichteler* *Eisenfeld-Rueck*
A II M 7.1.1.2.2/4	*Schulz J. 1994*	*A81929*	*field and weather data for A81928*

All trials were performed under test conditions where surface processes were not excluded. These studies are considered suitable for derivation of persistence endpoints.

A new field dissipation study with pyrimethanil was initiated in 2012, in order to cover the requirements for calculating degradation rates in the soil matrix for modelling following the new EFSA guidance [*EFSA (2010)*], i.e. excluding soil surface loss processes. This study was accompanied by a respective storage stability study which is also summarized below.

Kinetic evaluations of the old as well as new field soil dissipation studies were performed according to current FOCUS and EFSA guidelines.

A summary on the calculation of trigger and modelling endpoints for pyrimethanil obtained from old and new field studies are presented at the end of this chapter.

New, not yet peer-reviewed studies:

Report:	CA 7.1.2.2.1/1 Richter T., Kuhnke G., 2015a Field soil dissipation study of BAS 605 F (Pyrimethanil) in the formulation BAS 605 04 F on bare soil at five sites in Europe, 2012 - 2013 2014/1000661
Guidelines:	NAFTA Guidance Document for Conducting Terrestrial Field Dissipation Studies Regulatory Directive DIR2006-01 (March 2006), EPA 835.6100, SANCO/3029/99 rev. 4 (11 July 2000), SETAC Procedures for assessing the environmental fate and ecotoxicity for pesticides (March 1995), EFSA Panel on Plant Prptection Products and their Residues (PPR)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Report:	CA 7.1.2.2.1/2 Richter T., 2017 a Amendment No. 1 - Field soil dissipation study of BAS 605 F (Pyrimethanil) in the formulation BAS 605 04 F on bare soil at five sites in Europe, 2012 - 2013 2016/1344943
Guidelines:	NAFTA Guidance Document for Conducting Terrestrial Field Dissipation Studies Regulatory Directive DIR2006-01 (March 2006), EPA 835.6100, SANCO/3029/99 rev. 4 (11 July 2000), SETAC Procedures for assessing the environmental fate and ecotoxicity for pesticides (March 1995), EFSA Panel on Plant Prptection Products and their Residues (PPR)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Remark: All samples from this terrestrial field dissipation study as described in chapter M-CA 7.1.2.2.1/1 were analysed with the residue analytical method L0249/01 as described in detail in chapter M-CA 4.1.2/2 (2013/1374394). The originally developed residue method developed (refer to M-CA 4.1.2/1; 2005/1027614) was originally developed but then updated to the most recent, and currently valid guidelines. The updated method L0249/01 (M-CA 4.1.2/2 (2013/1374394).) is the data generation method applied.

Executive Summary

The degradation of pyrimethanil (BAS 605 F) under field conditions was investigated at five sites in Europe (Germany, Northern France, Southern France, Italy, and Spain). All sites represent typical regions of agricultural practice representative for growing crops including apples. All trial sites consisted of an untreated and a treated plot, the latter being subdivided into 3 subplots that were assigned for replicates.

The product BAS 605 04 F, formulated as a suspension concentrate (SC), was broadcast applied to bare soil in a single application at a nominal rate of 1000 g a.s. ha^{-1} using a target water volume of 400 L ha^{-1}. Applications were conducted between mid of June and end of July 2012 using a calibrated boom sprayer. The actual application rates for each trial were determined by quantifying the amount of spray discharged and ranged from 1001 to 1031 g a.s. ha^{-1}, with an average of 1013 g a.s. ha^{-1}. Results from spray broth analysis for the individual trial sites revealed concentrations between 81 and 114% of the nominal value with an average of 98% across all sites. Dose verification conducted via application verification samples yielded recovery values for the individual sites ranging from 68 to 129% of the target rate and an average recovery of 103% across all sites.

Immediately after application of the test item, the plots were covered with a layer of sand of at least 3 – 5 mm depth to protect the applied product from surface loss processes like photolysis or volatilization, and to exclude any potential impact on the degradation of the test item caused by any of these processes. The layer of sand was controlled up to at least 30 days after application and was renewed when needed. Within this time period of 30 days, the individual fields received a total precipitation (rain and irrigation) of 72.5 mm (France North), 95 mm (Germany), 55.8 mm (France South), 47.2 mm (Italy) and 29.4 mm (Spain), respectively.

No tillage or fertilization was performed during the course of the study and no crops were grown throughout any of the trials. The plots were kept generally free of vegetation via the application of glyphosate, quinoclamin, and glufosinate ammonium.

Rainfall was supplemented with irrigation at sites in Northern France (169.7 mm), Germany (230 mm), Southern France (135 mm), Italy (543 mm) and Spain (363.2 mm) and the total water input was at least 108.1% of the historical average rainfall during the study period at the test sites.

Soil core samples were taken at several intervals up to 489 days after application and down to a maximum soil depth of 90 cm. Soil cores were cut into 10 cm sections. Soil segments of the same depth and subplot from a defined sampling event were pooled and homogenized and a representative sub-sample of each depth was taken for residue analysis. Soil specimens were stored at about -18°C within a maximum of 6 hours (in some exceptional cases 7 h 22 min) after beginning of sampling. Soil specimens remained frozen until analysis.

In order to demonstrate stability of the residues in soil during storage and shipment, shipment verification specimens were prepared at selected sampling occasions by fortifying untreated soil from the field sites with known amounts of pyrimethanil. These specimens were stored and shipped under the same conditions as the actual residue specimens. Analysis of the shipment verification specimens on pyrimethanil yielded average recovery values, corrected for procedural recovery, of 73 to 107% across all sites confirming residue stability during all storage and shipment procedures.

Soil specimens were analyzed for pyrimethanil and its metabolite 2-amino-4,6-dimethyl-pyrimidine (M605F007, synonym AE F132593, sometimes also referred to as ADMP) according to BASF method L0249/01. The analytical method involved extraction of the soil with methanol, dilution of an aliquot with water, clean- up of this dilution by solid phase extraction (SPE), concentration by evaporation, and final determination of the analytes by HPLC-MS/MS. The limit of quantification (LOQ) was 0.01 mg kg^{-1} for each individual analyte. The limit of detection (LOD) was set at 0.002 mg kg^{-1} (20% of LOQ).

Field samples from the treated plots were analyzed down to a depth until at least two consecutive soil segments were free of quantifiable residues (< LOQ). Analysis was performed until a maximum of 489 days after treatment (DAT). Application monitors (Petri dish samples) and shipment verification specimens were analyzed for parent only using the same analytical method L0249/01 with minor adaptations.

No residues above 30% of the LOQ of any analyte were detected in any of the untreated control samples proving that there were no interferences of the untreated soil material with the analytical procedure used. Procedural recovery experiments performed with untreated soils spiked with the two analytes at concentrations of 0.01, 0.05, 0.1, 0.5, and 5.0 mg kg^{-1} yielded overall mean recovery rates between 80 and 95% for the individual analytes, confirming the validity of the analytical method used in this study.

Residue values of pyrimethanil and metabolite M605F007 in mg kg^{-1} dry soil were converted to residue rates in g ha^{-1} taking into account the actual dry soil density of the individual field samples, and were summed up for all depths between 0 and 40 cm analyzed. Residue values were not corrected for procedural recoveries except for results obtained from petri dish and shipment verification analysis.

Pyrimethanil degraded relatively fast under field conditions in soil at all five European field sites. The total amount of pyrimethanil residues detected in the soil profiles decreased from an average of 930 g ha^{-1}at day 0 to an average of 84 g ha^{-1} (range 0-183 g ha^{-1}) after 140 days. No residues above the LOQ (0.01 mg kg^{-1}) were detectable after 1 year in any of the trials (at the latest). Degradation endpoints were not provided in this report, but were calculated in a separate modeling report summarized below [see KCA 7.1.2.2.1/4 2014/1000843].

Residues of pyrimethanil in the soil profiles were exclusively found in the top 0-10 cm layer of the soils, except for one detect at a depth of 10-20 cm ($\leq$ 0.026 mg kg^{-1}, equivalent to < 37 g ha^{-1}) in the trial conducted in Germany (at 8 DAT). No residues above the LOQ were detected below 20 cm in any sample at any site. Altogether, pyrimethanil did not show any tendency to move into deeper soil layers.

Metabolite M605F007 was detected in small amounts at all sites reaching maximum amounts of values of 21 g ha^{-1} (Northern France), 131 g ha^{-1} (Germany), 27 g ha^{-1} (Southern France), 40 g ha^{-1} (Italy), and 15 g ha^{-1} (Spain) soil layer related. Thereafter, residues declined again and were no longer detected after 308 days at the latest.

M605F007 was exclusively found in the top 0-10 cm soil layer, except for a single detect in the 10-20 cm layer at the site in Italy. No residues of M605F007 above the LOQ were observed in deeper soil layers in any sample at any site.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Test item (formulation):	BAS 605 04 F
Active substance (a.s.):	Pyrimethanil (BAS 605 F, Reg.No. 236999)
Type of formulation:	SC
Chemical name (IUPAC):	N-(4,6-dimethylpyrimidin-2-yl)aniline
Batch No.:	0004695275 (certificate of analysis 179677_6)
Content of a.s.:	416.9 g L^{-1} (nominal 400.0 g L^{-1})
Expiration date:	November 30, 2012

2. Test sites

The dissipation of pyrimethanil under field conditions was investigated a fives sites in Europe representative of Central and Southern EU conditions. One trial was performed in Germany, Northern France, Southern France, Italy and Spain, respectively. The site characteristics are presented Table 7.1.2.2.1-1**:** to Table 7.1.2.2.1-3. Soil parameters were determined from soil samples taken before application from boundaries of the treated plot following segmentation according to the soil horizons.

Table 7.1.2.2.1-1: Soil characteristics of the trial sites L120562 and L120563 used to investigate the field dissipation of pyrimethanil (BAS 605 F)

Trial	**L120562**				**L120563**		
Location	**Schaeffersheim, France North**				**Goch-Nierswalde, Germany**		
Soil properties	0 – 30 cm	30 – 50 cm	50 – 70 cm	70 – 90 cm	0 – 30 cm	30 – 60 cm	60 – 90 cm
Soil class (DIN 4220)	Clay silt (Ut4)	Silty clay (Tu4)	Clay silt (Ut4)	Clay silt (Ut3)	Clay silt (Ut2)	Clay silt (Ut2)	Loamy sand (Sl2)
sand [%]	9.4	6.1	5.9	7.5	19.0	20.7	80.9
silt [%]	67.6	67.5	72.9	79.1	72.3	70.8	13.5
clay [%]	23.0	26.4	21.3	13.4	8.7	8.6	5.6
Soil class (USDA)	Silt loam	Silt loam	Silt loam	Silt loam	Silt loam	Silt loam	Loamy sand
sand [%]	9.9	7.0	6.7	8.1	24.1	24.7	81.9
silt [%]	67.0	66.5	72.0	78.4	67.1	66.8	12.5
clay [%]	23.0	26.4	21.3	13.4	8.7	8.6	5.6
Total organic C [%]	2.16	0.83	0.55	0.25	1.74	0.34	0.09
Organic matter [%] [a]	3.72	1.43	0.95	0.43	3.00	0.59	0.16
pH [$CaCl_2$]	7.2	7.5	7.7	7.7	6.6	6.3	4.5
pH [H_2O]	8.1	8.3	8.4	8.5	7.2	7.1	5.9
CEC [$cmol^+ kg^{-1}$]	21.3	18.1	14.4	11.9	6.2	0.9	0.7
MWHC [g $100g^{-1}$ dry weight]	33.7	35.7	35.7	31.8	34.8	28.5	19.3
pF 2.0 [g $100g^{-1}$ dry weight] [b]	27.4	27.5	27.4	26.6	33.4	24.9	12.0
pF 2.5 [g $100g^{-1}$ dry weight] [b]	23.5	22.3	24.4	19.6	19.5	13.6	7.6
Dry bulk density [g cm^{-3}] [c]	1.091 (10-20 cm)	-	-	-	1.521 (10-20 cm)	-	-
Soil taxonomy	Haplic Calcisol (according to WRB classification)				Pseudogley-Cambisol / Pseudogley-Paracambisol (according to FAO classification)		

CEC = Cation exchange capacity
MWHC = Maximum water holding capacity
[a] Organic matter = Organic carbon x 1.724 (organic carbon = C_{org})
[b] Water retention characteristics, soil moisture at 0.1 or 0.33 bar
[c] Mean of three replicates

Table 7.1.2.2.1-2: Soil characteristics of the trial sites L120564 and L120565 used to investigate the field dissipation of pyrimethanil (BAS 605 F)

Trial	**L120564**			**L120565**		
Location	**Barry-d'Islemade, France (South)**			**Dugliolo, Italy**		
Soil properties	0 – 30 cm	30 – 60 cm	60 – 90 cm	0 – 30 cm	30 – 60 cm	60 – 90 cm
Soil class (DIN 4220)	Loamy sand (Sl3)	Sandy loam (Ls3)	Sandy loam (Ls4)	Loamy sand (Sl4)	Loamy sand (Sl3)	Sand (Ss)
sand [%]	61.4	50.7	56.7	60.9	65.6	89.8
silt [%]	28.0	30.0	20.2	26.4	23.1	6.8
clay [%]	10.7	19.3	23.1	12.7	11.3	3.4
Soil class (USDA)	Sandy loam	Sandy loam	Sandy clay loam	Sandy loam	Sandy loam	Sand
sand [%]	64.3	52.3	58.0	66.7	72.3	91.7
silt [%]	25.1	28.4	18.9	20.6	16.4	5.0
clay [%]	10.7	19.3	23.1	12.7	11.3	3.4
Total organic C [%]	0.61	0.37	0.25	2.94	2.94	2.92
Organic matter [%] [a]	1.05	0.64	0.43	5.07	5.07	5.03
pH [$CaCl_2$]	5.2	5.7	6.1	7.5	7.7	7.8
pH [H_2O]	6.0	6.5	6.9	8.4	8.5	8.7
CEC [$cmol^+ kg^{-1}$]	4.9	9.2	12.9	8.0	7.0	7.4
MWHC [g $100g^{-1}$ dry weight]	30.4	28.8	31.2	27.2	25.5	27.1
pF 2.0 [g $100g^{-1}$ dry weight] [b]	16.6	19.3	22.7	19.6	15.8	10.5
pF 2.5 [g $100g^{-1}$ dry weight] [b]	13.3	15.1	17.2	13.3	11.0	6.7
Dry bulk density [g cm^{-3}] [c]	1.577 (0-20 cm)	-	-	1.324 (10-20 cm)	-	-
Soil taxonomy	Endoeutric Albeluvisol (according to WRB classification)			Calcareous land of river dikes (according to FAO classification)		

CEC = Cation exchange capacity
MWHC = Maximum water holding capacity
[a] Organic matter = Organic carbon x 1.724 (organic carbon = C_{org})
[b] Water retention characteristics, soil moisture at 0.1 or 0.33 bar
[c] Mean of three replicates

Table 7.1.2.2.1-3: Soil characteristics characteristics of the trial sites L120566 used to investigate the field dissipation of pyrimethanil (BAS 605 F)

Trial	**L120566**			
Location	**Utrera, Spain**			
Soil properties	0 – 20 cm	20 – 60 cm	60 – 80 cm	80 – 90 cm
Soil class (DIN 4220)	Sand (Ss)	Sand (Ss)	Sand (Ss)	Clay sand (St3)
sand [%]	93.6	91.9	89.7	70.8
silt [%]	3.5	4.6	5.5	6.1
clay [%]	2.9	3.4	4.8	23.0
Soil class (USDA)	Sand	Sand	Sand	Sandy clay loam
sand [%]	95.3	94.2	91.3	73.2
silt [%]	1.8	2.4	4.0	3.7
clay [%]	2.9	3.4	4.8	23.0
Total organic C [%]	0.39	0.15	0.09	0.22
Organic matter [%] [a]	0.67	0.26	0.16	0.38
pH [$CaCl_2$]	7.0	7.2	7.1	6.9
pH [H_2O]	7.7	7.5	7.5	7.6
CEC [$cmol^+ kg^{-1}$]	2.6	0.7	0.9	10.7
MWHC [g $100g^{-1}$ dry weight]	25.2	23.6	23.6	31.5
pF 2.0 [g $100g^{-1}$ dry weight] [b]	10.9	12.2	14.4	25.1
pF 2.5 [g $100g^{-1}$ dry weight] [b]	5.6	6.1	7.0	18.5
Dry bulk density [g cm^{-3}] [c]	1.540 (12–15 cm)	-	-	-
Soil taxonomy	Eutric planosol (according to FAO classification)			

CEC = Cation exchange capacity
MWHC = Maximum water holding capacity
[a] Organic matter = Organic carbon x 1.724 (organic carbon = C_{org})
[b] Water retention characteristics, soil moisture at 0.1 or 0.33 bar
[c] Mean of three replicates

The selected fields represented typical regions with agricultural practice and soils suitable for growing apples that had been under cultivation for many years. The sites were flat without any significant slopes. Before commencement of the first treatment, the soil at each trial site was prepared as for sowing and was rolled if considered necessary, but then was left fallow. No product containing the active substance of the test item had been used on the test plots during the last three years.

B. STUDY DESIGN

1. Experimental treatments

The trial area at each site was divided into two plots, one untreated control plot (size: 30 - 90 m^2) and one treated plot (size: 306 - 441 m^2). The treated plot consisted of three equal sized subplots A, B, and C that were assigned for replicates.

The product, formulated as suspension concentrate (SC), was broadcast applied to bare soil in a single application at a nominal rate of 1000 g a.s. ha^{-1} using a target water volume of 400 L ha^{-1}. Applications were conducted between mid of June and end of July 2012 using calibrated boom sprayers. Treated subplots were three-fold replicated with subplot size ranging from 102 to 147 m^2. For each treated replicate, a separate spray mixture was prepared and the test item was applied to each subplot individually. Each spray mixture was visually checked for homogeneity and small aliquots of the spray mixture were taken before and after application of each individual subplot for later analysis.

The actual application rates determined by quantifying the amount of spray discharged ranged from 1001 to 1031 g a.s. ha^{-1} averaged over the three replicates of each treated plot. In addition, the dose was verified by sampling Petri dishes filled with untreated soil from the trial site (approximately 50 g per dish, sieved to 2 mm). The Petri dishes with an inner diameter of about 11.0 cm were placed on the treated plot (ten in each subplot) before application. After completion of the application, the Petri dishes were collected from the field, closed with a lid, sealed with adhesive tape and stored chilled after collection. They were either placed in a freezer within 30 min after application or stored on blue or dry ice after collection and placed in a freezer within less than 4 h. Further details on the application are presented in Table 7.1.2.2.1-4 below.

Table 7.1.2.2.1-4: Application rates of field trial sites treated with BAS 605 04 F (SC)

Trial Country	Application Method	No. of applications	Subplot (m^2)	Application rate per treatment				Application date
				nominal [g a.s. ha^{-1}]	actual [a] [g a.s. ha^{-1}]	dose verification [b]		
						[g a.s ha^{-1}]	% of nominal	
L120562 France (North)	broadcast spray to bare soil	1	A (141)	1000	1030	984	98	24-July-2012
			B (141)	1000	1030	1125	113	
			C (141)	1000	1032	1180	118	
			Average	**1000**	**1031**	**1096**	**110**	
L120563 Germany	broadcast spray to bare soil	1	A (102)	1000	1050	719	72	27-June-2012
			B (102)	1000	1001	669	67	
			C (102)	1000	999	654	65	
			Average	**1000**	**1017**	**681**	**68**	
L120564 France (South)	broadcast spray to bare soil	1	A (147)	1000	1037	1110	111	22-June-2012
			B (147)	1000	997	1080	108	
			C (147)	1000	992	1030	103	
			Average	**1000**	**1009**	**1073**	**107**	
L120565 Italy	broadcast spray to bare soil	1	A (102)	1000	1014	1184 [c]	118	29-June-2012
			B (102)	1000	1021	1499 [d]	150	
			C (102)	1000	992	1181	118	
			Average	**1000**	**1009**	**1288**	**129**	
L120566 Spain [e]	broadcast spray to bare soil	1	A (102)	1000	1015	1053	105	12-June-2012
			B (102)	1000	993	960	96	
			C (102)	1000	995	934	93	
			Average	**1000**	**1001**	**983**	**98**	

[a] Determined by calculation of spray liquid applied
[b] Determined by means of petri dishes filled with soil (recovery corrected)
[c] Corrected for outlier according to Dixon test
[d] Remark: due to two exceptional high values, however no outliers
[e] Dose verification values were calculated for 10.8 cm diameter since the type of dishes appeared to be equal to dishes from other trials (difference therefore due to measuring error)

Immediately after application of the test item and before subsequent soil core sampling, the control plot and the treated replicates were covered with a thin layer of sand to protect the applied product from surface loss processes like photolysis or volatilization, and to exclude any potential impact on the degradation of the test item caused by any of these processes. The application of sand was conducted manually or using a box- or rotary spreader until complete coverage of the soil surface. The thickness of the sand layer necessary for complete coverage of the soil was between 3 and 10 mm.

The layer of sand was controlled for 30±2 days after application and renewed if necessary. If the field had received a total of at least 10 mm of precipitation (rainfall or/and irrigation) within the first month, further check and renewal of the sand cover was no longer required. Within this time period of 30 days, the individual fields received a total precipitation (rain and irrigation) of 72.5 mm (France North), 95 mm (Germany), 55.8 mm (France South), 47.2 mm (Italy), and 29.4 mm (Spain), respectively.

No tillage or fertilization was performed during the course of the study from first to last sampling and no crops were grown throughout any of the trials. The plots were kept free of vegetation via the application of glyphosate, quinoclamin, and glufosinate ammonium.

Rainfall was supplemented with irrigation at sites in Northern France (169.7 mm), Germany (230 mm), Southern France (135 mm), Italy (543 mm), and Spain (363.2 mm) in order to adjust precipitation to historic values (Germany, Northern and Southern France), or to compensate for evaporation at bare soil conditions (Italy and Spain)..

Actual weather data are based on records of appropriate weather stations located on-site (maximum 500 m distance). Monthly summary results on temperature, precipitation, and irrigation are presented in Table 7.1.2.2.1-5 and Table 7.1.2.2.1-6.

Table 7.1.2.2.1-5: Summary of climatic conditions at field trial sites used to investigate the dissipation of pyrimethanil (trial sites L120562, L120563, and L120564)

Trial	L120562			L120563			L120564		
Location	Schaeffersheim			Goch-Nierswalde			Barry d'Islemade		
	France (North)			Germany			France (South)		
Climatic conditions	T_{mean} Air [°C]	Prec. [mm]	Irrigation [mm]	T_{mean} Air [°C]	Prec. [mm]	Irrigation [mm]	T_{mean} Air [°C]	Prec. [mm]	Irrigation [mm]
Month		∑	∑		∑	∑		∑	∑
Jun 12	-	-	-	19.3 [a]	1.6 [a]	-	22.1 [a]	3.2 [a]	0.0
Jul 12	20.3 [a]	30.8 [a]	0.0	16.9	148.0	10	20.4	53.2	13.9
Aug 12	19.5	44.4	18.9	18.4	45.0	25	22.7	86.2	0.0
Sep 12	14.2	52.6	31.7	13.5	48.8	25	18.2	19.6	17.3
Oct 12	9.2	95.0	0.0	10.0	84.6	10	14.5	35.2	18.9
Nov 12	6.0	109.6	0.0	6.5	34.6	15	8.9	65.0	0.0
Dec 12	3.3	54.6	0.0	4.3	121.0	0	6.5	104.0	0.0
Jan 13	1.6	15.8	0.0	1.8	46.2	0	4.8	152.2	0.0
Feb 13	0.8	33.6	0.0	1.2	37.6	0	4.5	69.0	0.0
Mar 13	2.9	18.4	13.2	2.8	39.8	0	8.9	169.6	0.0
Apr 13	10.0	84.0	21.5	8.4	45.2	15	11.6	77.4	0.0
May 13	11.9	154.4	0.0	11.8	60.8	15	12.9	142.0	0.0
Jun 13	17.4	93.0	0.0	15.8	64.8	25	17.4	80.4	16.0
Jul 13	21.0	21.4	51.4	19.3	66.2	30	22.8	37.0	34.4
Aug 13	18.3	75.0	33.1	18.1	18.6	50	21.8	90.6	0.0
Sept 13	14.7	94.8	0.0	14.2	59.4	0	17.8	56.0	18.3
Oct 13	11.5	140.2	0.0	12.0 [a]	34.4 [a]	10	16.4 [a]	52.0 [a]	16.2
Nov 13	7.0 [a]	57.6 [a]	0.0	-	-	-	-	-	-

[a] Actual weather data refer to time period from start of trial (day of application) until end of trial (day of last sampling)

Table 7.1.2.2.1-6: Summary of climatic conditions at field trial sites used to investigate the dissipation of pyrimethanil (trial sites L120565 and L120566)

Trial	L120565			L120566		
Location	Dugliolo			Utrera		
	Italy			Spain		
Climatic conditions	T_{mean} Air [°C]	Prec. [mm]	Irrigation [mm]	T_{mean} Air [°C]	Prec. [mm]	Irrigation [b] [mm]
Month		∑	∑		∑	∑
Jun 12	28.0 [a]	0 [a]	0	27.4 [a]	0 [a]	14.9
Jul 12	25.89	0.2	77	27.4	0	45.7
Aug 12	26.30	1.0	130	28.1	0	44.2
Sep 12	19.88	134.8	20	24.6	78.0	26.9
Oct 12	14.71	79.8	0	20.0	127.0	0.0
Nov 12	9.96	80.6	0	15.8	135.5	0.0
Dec 12	1.51	24.2	0	12.4	37.0	0.0
Jan 13	2.72	110	0	11.9	49.5	0.0
Feb 13	2.47	93.4	0	11.1	55.0	0.0
Mar 13	7.21	110.0	0	14.6	165.5	0.0
Apr 13	13.30	57.0	21	18.1	47.0	0.0
May 13	16.62	68.2	5	20.1	8.0	38.6
Jun 13	21.37	45.4	55	25.0	0	43.7
Jul 13	24.88	8.0	92	28.2	0	56.8
Aug 13	23.97	45.0	76	28.7	0	72.9
Sept 13	20.16	31.0	67	25.5	21.5	19.5
Oct 13	14.3 [a]	73.4 [a]	0	23.6 [a]	30.0 [a]	0.0

[a] Actual weather data refer to time period from start of trial (day of application) until end of trial (day of last sampling)
[b] Treated plot, irrigation of the untreated plot is specified in the field phase report

Due to additional irrigation, the total water input at the test sites during the study was at least 108.1% of the historical average rainfall, which is considered sufficient to allow the cultivation of crops like apples. Details on the historical (long-term) weather data on precipitation and average air temperature are given in the study report.

2. Sampling

Replicate soil specimens (8 per treated subplot and 10 or 15 per control plot) were taken at intervals up to 489 days after treatment (DAT) and down to a maximum soil depth of 90 cm. At day 0, immediately after application, the treated plots were sampled down to 10 cm only. The detailed sampling intervals are presented in Table 7.1.2.2.1-7.

Table 7.1.2.2.1-7: Summary of sampling intervals of residue soil samples at each field trial site

Trial	Country	Sampling intervals [days after treatment]
L120562	France (North)	-12, 0, 3, 7, 15, 30, 63, 92, 134, 184, 238, 304, 358, 483
L120563	Germany	-1, 0, 3, 8, 14, 33, 61, 85, 132, 175, 253, 294, 356, 485
L120564	France (South)	-1, 0, 3, 6, 12, 27, 60, 95, 140, 180, 245, 293, 368, 489
L120565	Italy	-3, 0, 3, 6, 13, 31, 60, 90, 137, 173, 308, 370, 480
L120566	Spain	-1, 0, 3, 8, 15, 30, 55, 90, 139, 183, 246, 309, 351, 483

Untreated specimens were collected from the control plot on two or three occasions, between one and twelve days before application down to a depth of 90 cm, after about nominally 240 DAT (all trials with exception of L120565), and nominally 480 DAT to a depth of 10 cm. The specimens were taken randomly from the untreated plot each time and pooled according to soil depth.

The 15 cores collected at the first sampling interval were taken using a common soil probe equipped with a plastic liner of 3.9 to 5.0 cm diameter. In trial L120564, the 0-10 cm sample at the first sampling were collected using a metal tube of 8.3 cm diameter. The 10 cores (0-10 cm) taken after about 250 and 480 DAT were also collected with this metal tube. In trial L120562, the 480 DAT sample was taken using a HGL corer with an inner diameter of 8.0 cm.

Treated soil specimens were taken randomly from eight points of each of the three treated subplots A – C and pooled according to subplot and depth. All soil specimens from 0-10 cm depth collected from the treated plots were taken separately from the deeper layers using a metal tube of 7.2 to 11 cm diameter in the first step. Soil samples from the top layer were taken by pressing the metal tube described above into the ground and collecting the soil with a spoon or similar device. Soil specimens deeper than 10 cm were collected through the center of the excavation hole contained by the guard collar, using a common soil corer fitted with a plastic liner of diameter 3.9 to 5.0 cm. Sampling of these cores was conducted in one run or in up to four consecutive steps.

Immediately after sampling and before freezing, all soil cores collected with the soil probe were sectioned into 10 cm segments and pooled by depth. In addition to the main sampling described above, a second complete sampling (double sampling) was carried out. The reserve samples were not sectioned into 10 cm segments but directly put into freezers at the field test sites.

All soil specimens intended for residue analysis were stored at about -18°C latest 7.3 h after sampling and remained frozen through storage, shipment, and processing until final analysis. Sample processing was conducted in frozen state in a mill together with dry ice.

Shipment verification specimens were prepared to demonstrate stability of the residues in soil during storage and through any shipment processes. The samples were prepared at three occasions by fortification of soil with 0.15 mg kg^{-1} pyrimethanil and were subsequently handled in the same manner as the actual residue samples. The analytical results demonstrated no significant losses from the shipment verification samples. The recovery of pyrimethanil was in the range of 73 - 107% (mean of trials, corrected for the mean recovery of the respective analytical set).

3. Analytical procedure

Field soil specimens, were analyzed for pyrimethanil and its metabolite 2-amino-4,6-dimethyl-pyrimidine (M605F007, AE F132953) according to the analytical method L0249/01 [see KCA 4.1.2/1 2005/1027614] provided by BASF. Petri dish and shipment verification specimens were analyzed for pyrimethanil according to the same method with minor adaptations to account for the larger quantity of soil to be extracted.

The analytical method involved extraction of the soil with methanol, dilution of an aliquot with water, clean-up of this dilution by solid phase extraction (SPE), concentration by evaporation, and final determination of the analytes by HPLC-MS/MS.

Spray broth specimens were diluted with acetone/water (10/90, v/v) to the appropriate concentration and analyzed for pyrimethanil using HPLC-MS/MS.

The limit of quantification (LOQ; related to wet soil) was 0.01 mg kg^{-1} for each individual analyte. The limit of detection (LOD) was set at 0.002 mg kg^{-1} (20% of LOQ).

Analysis of field soil specimens originating from the treated plots was conducted down to a depth until at least two consecutive soil segments were free of quantifiable residues (< LOQ of 0.01 mg kg^{-1}). Analysis was performed up to a maximum of 489 DAT.

The validity of the analytical method was proven within the present study by analysis of untreated control and fortified samples within each analytical sample set.

4. Storage stability experiments

Storage stability of Pyrimethanil and its metabolite M605F007 in frozen soil was investigated in a separate study [see KCA 7.1.2.2.1/2 2013/1352188].

5. Calculation of degradation times

No calculation of degradation rates is provided in the study report. A detailed kinetic evaluation of the degradation behavior of pyrimethanil and its metabolite M605F007 in the five European field soils is presented in a separate modeling report [see KCA 7.1.2.2.1/4 2014/1000843].

II. RESULTS AND DISCUSSION

1. Spray broth concentration and application verification

Analyzed concentrations of pyrimethanil averaged for the individual trial sites were in the range of 2.03 to 2.86 g L^{-1} corresponding to 81 - 114% of the target concentration of 2.5 g L^{-1}. The analytical results were not corrected for procedural recoveries and confirm the integrity of the test item used in the trials.

Application verification was conducted by means of petri dishes filled with fine untreated soil from the trial site. As a result, the obtained application rates for the individual trials ranged from 681 to 1288 g a.s. ha^{-1} representing 68 - 129% of the target application rate. The applied amount determined via the application monitors in these trials is in agreement with the nominal value of 1000 g ha^{-1}, and the results from spray broth analysis.

2. Residues in field soil samples

Untreated soil specimens (control samples) of the respective soil depths from each trial were analyzed for residues of pyrimethanil and metabolite M605F007. No residues above 30% LOQ of any analyte were detected in any of the control samples, showing that there were no interferences of the untreated soil material with the analytical procedures used.

Procedural recovery experiments performed with untreated field soil specimens spiked with a mix of the two analytes at concentration levels of 0.01, 0.05, 0.1, 0.5, and 5.0 mg kg^{-1} yielded overall mean recovery rates for the individual analytes between 80 and 95%, confirming the validity of the analytical method used in this study. Detailed results are summarized in Table 7.1.2.2.1-8.

Table 7.1.2.2.1-8: Procedural recoveries of soil residue method

Analyte	Fortification level [mg kg^{-1}]	n	Mean recovery ± RSD* [%]
Pyrimethanil	0.01	54	89 ± 10
	0.05	4	89 ± 5
	0.1	38	95 ± 8
	0.5	12	95 ± 13
	5.0	1	92
	All fortification levels	**109**	**92 ± 10**
M605F007 (ADMP)	0.01	53	80 ± 12
	0.05	3	82 ± 9
	0.1	37	87 ± 9
	0.5	12	91 ± 14
	5.0	1	95
	All fortification levels	**106**	**84 ± 12**

*RSD = Relative standard deviation

These data prove that the analytical method applied was able to determine residues of pyrimethanil and M605F007 in soil samples accurately down to a concentration of 0.01 mg kg^{-1} for each analyte.

Field soil specimens from the treated plots were analyzed down to a depth until at least two consecutive soil segments were free of quantifiable residues (< LOQ of 0.01 mg kg^{-1}, maximum depth of 40 cm). If samples were analyzed in triplicate, the individual numbers were averaged to produce a mean for the respective soil sample.

Residue values for pyrimethanil and M605F007 are presented in Table 7.1.2.2.1-9 to Table 7.1.2.2.1-12 in µg kg^{-1} and g ha^{-1}. All residue values presented in these tables are related to the dry weight of the soil and are not corrected for procedural recoveries. Residue levels of the two analytes in µg kg^{-1} dry soil were converted to residue rates in g ha^{-1} taking into account the actual dry soil density of the field samples, and were summed up for all depths between 0 and 40 cm analyzed.

Table 7.1.2.2.1-9: Total residues of pyrimethanil under field conditions in soil calculated to µg kg^{-1} and summed up for all depths analyzed

Trial Country	L120562 Schaeffersheim, France (North)			L120563 Goch-Nierswalde, Germany			
days after treatment	Subplot 1 [µg kg^{-1}]	Subplot 2 [µg kg^{-1}]	Subplot 3 [µg kg^{-1}]	days after treatment	Subplot 1 [µg kg^{-1}]	Subplot 2 [µg kg^{-1}]	Subplot 3 [µg kg^{-1}]
0	1420	1060	1190	**0**	520	500	680
3	730	820	860	**3**	580	480	650
7	560	730	980	**8**	556	684	711
15	680	790	600	**14**	460	620	580
30	330	290	270	**33**	61	160	130
63	130	170	190	**61**	10	12	16
92	170	110	120	**85**	<10	<10	14
134	69	25	86	**132**	<10	17	12
184	63	56	48	**175**	11	12	19
238	47	24	48	**253**	<10	10	12
304	21	16	19	**294**	11	14	13
358	--	--	--	**356**	<10	<10	10
483	--	--	--	**485**	<10	<10	<10
Trial Country	**L120564 Barry d' Islemade, France (South)**			**L120565 Dugliolo, Italy**			
days after treatment	**Subplot 1 [µg kg^{-1}]**	**Subplot 2 [µg kg^{-1}]**	**Subplot 3 [µg kg^{-1}]**	**days after treatment**	**Subplot 1 [µg kg^{-1}]**	**Subplot 2 [µg kg^{-1}]**	**Subplot 3 [µg kg^{-1}]**
0	610	670	650	**0**	670	580	600
3	680	670	630	**3**	580	500	720
6	480	520	690	**6**	490	950	620
12	690	480	590	**13**	430	420	720
27	360	390	390	**31**	210	460	560
60	200	240	170	**60**	110	220	240
95	100	130	130	**90**	220	190	330
140	77	76	69	**137**	120	88	140
180	83	53	74	**173**	180	190	55
245	48	21	62	**308**	72	33	43
293	17	21	14	**370**	33	23	23
368	<10	<10	<10	**480**	21	14	12
489	<10	<10	<10				

Table 7.1.2.2.1-9: Total residues of pyrimethanil under field conditions in soil calculated to µg kg^{-1} and summed up for all depths analyzed

Trial Country	L120566 Utrera, Spain		
days after treatment	Subplot 1 [µg kg^{-1}]	Subplot 2 [µg kg^{-1}]	Subplot 3 [µg kg^{-1}]
0	640	680	660
3	410	540	490
8	400	430	460
15	420	320	260
30	250	270	340
55	200	260	230
90	170	160	150
139	55	46	49
183	28	21	35
246	18	16	17
309	--	--	--
351	--	--	--
483	--	--	--

LOQ (limit of quantification): 10 µg kg^{-1}
-- samples not analyzed

Table 7.1.2.2.1-10: Total residues of M605F007 under field conditions in soil calculated to µg kg^{-1} and summed up for all depths analyzed

Trial Country	L120562 Schaeffersheim, France (North)			L120563 Goch-Nierswalde, Germany			
days after treatment	Subplot 1 [µg kg^{-1}]	Subplot 2 [µg kg^{-1}]	Subplot 3 [µg kg^{-1}]	days after treatment	Subplot 1 [µg kg^{-1}]	Subplot 2 [µg kg^{-1}]	Subplot 3 [µg kg^{-1}]
0	<10	<10	<10	**0**	<10	<10	<10
3	<10	<10	<10	**3**	<10	<10	<10
7	<10	<10	<10	**8**	<10	<10	<10
15	14	12	10	**14**	<10	<10	<10
30	20	16	16	**33**	91	36	57
63	19	21	20	**61**	69	70	88
92	21	11	18	**85**	23	52	19
134	10	15	11	**132**	16	21	19
184	<10	18	<10	**175**	<10	13	<10
238	<10	<10	<10	**253**	<10	13	<10
304	<10	<10	<10	**294**	<10	<10	<10
358	--	--	--	**356**	<10	<10	<10
483	--	--	--	**485**	<10	<10	<10
Trial Country	**L120564 Barry d' Islemade, France (South)**			**L120565 Dugliolo, Italy**			
days after treatment	**Subplot 1 [µg kg^{-1}]**	**Subplot 2 [µg kg^{-1}]**	**Subplot 3 [µg kg^{-1}]**	**days after treatment**	**Subplot 1 [µg kg^{-1}]**	**Subplot 2 [µg kg^{-1}]**	**Subplot 3 [µg kg^{-1}]**
0	<10	<10	<10	**0**	<10	<10	<10
3	<10	<10	<10	**3**	<10	<10	<10
6	<10	<10	<10	**6**	15	<10	<10
12	<10	<10	<10	**13**	23	<10	<10
27	<10	<10	<10	**31**	21	23	13
60	<10	14	<10	**60**	11	12	<10
95	10	10	12	**90**	<10	11	20
140	18	14	<10	**137**	<10	<10	30
180	19	12	13	**173**	<10	18	48
245	17	12	14	**308**	31	10	14
293	12	<10	14	**370**	<10	<10	<10
368	<10	<10	<10	**480**	<10	<10	<10
489	<10	<10	<10				

Table 7.1.2.2.1-10: Total residues of M605F007 under field conditions in soil calculated to µg kg^{-1} and summed up for all depths analyzed

Trial Country	L120566 Utrera, Spain		
days after treatment	Subplot 1 [µg kg^{-1}]	Subplot 2 [µg kg^{-1}]	Subplot 3 [µg kg^{-1}]
0	<10	<10	<10
3	<10	<10	<10
8	<10	<10	<10
15	<10	<10	<10
30	<10	<10	<10
55	10	11	10
90	<10	<10	<10
139	<10	<10	<10
183	<10	<10	<10
246	<10	<10	<10
309	--	--	--
351	--	--	--
483	--	--	--

LOQ (limit of quantification): 10 µg kg^{-1}
-- samples not analyzed

Table 7.1.2.2.1-11: Total residues of pyrimethanil under field conditions in soil calculated to g ha^{-1} and summed up for all depths analyzed

Trial Country	L120562 Schaeffersheim, France (North)			L120563 Goch-Nierswalde, Germany			
days after treatment	Subplot 1 [g ha^{-1}]	Subplot 2 [g ha^{-1}]	Subplot 3 [g ha^{-1}]	days after treatment	Subplot 1 [g ha^{-1}]	Subplot 2 [g ha^{-1}]	Subplot 3 [g ha^{-1}]
0	1301	980	1163	**0**	761	777	1113
3	672	797	844	**3**	666	602	724
7	513	715	909	**8**	645	772	842
15	620	793	543	**14**	496	679	610
30	312	287	260	**33**	88	221	182
63	130	166	183	**61**	16	18	22
92	147	101	115	**85**	0	0	22
134	71	28	90	**132**	0	21	13
184	52	44	46	**175**	14	14	19
238	49	25	52	**253**	0	12	15
304	21	17	18	**294**	11	12	12
358	--	--	--	**356**	0	0	11
483	--	--	--	**485**	0	0	0
Trial Country	**L120564 Barry d' Islemade, France (South)**			**L120565 Dugliolo, Italy**			
days after treatment	**Subplot 1 [g ha^{-1}]**	**Subplot 2 [g ha^{-1}]**	**Subplot 3 [g ha^{-1}]**	**days after treatment**	**Subplot 1 [g ha^{-1}]**	**Subplot 2 [g ha^{-1}]**	**Subplot 3 [g ha^{-1}]**
0	721	799	795	**0**	957	843	842
3	764	769	701	**3**	775	666	916
6	617	599	818	**6**	629	1224	850
12	711	533	661	**13**	520	553	969
27	393	460	442	**31**	261	576	757
60	232	270	204	**60**	145	310	361
95	115	144	146	**90**	328	254	466
140	114	118	108	**137**	170	131	183
180	115	78	99	**173**	203	218	68
245	55	27	73	**308**	92	45	51
293	24	29	23	**370**	41	31	32
368	0	0	0	**480**	31	20	18
489	0	0	0				

Table 7.1.2.2.1-11: Total residues of pyrimethanil under field conditions in soil calculated to g ha^{-1} and summed up for all depths analyzed

Trial Country	L120566 Utrera, Spain		
days after treatment	Subplot 1 [g ha^{-1}]	Subplot 2 [g ha^{-1}]	Subplot 3 [g ha^{-1}]
0	970	1025	899
3	614	769	743
8	562	585	638
15	578	422	379
30	359	369	476
55	269	350	337
90	237	222	206
139	81	64	71
183	42	31	52
246	27	23	25
309	--	--	--
351	--	--	--
483	--	--	--

LOQ (limit of quantification): Residue values <10 µg kg^{-1}
(<LOQ) were reported and treated as zero
-- samples not analyzed

Table 7.1.2.2.1-12: Total residues of M605F007 under field conditions in soil calculated to g ha^{-1} and summed up for all depths analyzed

Trial Country	L120562 Schaeffersheim, France (North)			L120563 Goch-Nierswalde, Germany			
days after treatment	Subplot 1 [g ha^{-1}]	Subplot 2 [g ha^{-1}]	Subplot 3 [g ha^{-1}]	days after treatment	Subplot 1 [g ha^{-1}]	Subplot 2 [g ha^{-1}]	Subplot 3 [g ha^{-1}]
0	0	0	0	**0**	0	0	0
3	0	0	0	**3**	0	0	0
7	0	0	0	**8**	0	0	0
15	13	12	9	**14**	0	0	0
30	19	16	15	**33**	131	50	80
63	19	21	19	**61**	108	104	121
92	18	10	17	**85**	33	78	29
134	10	17	12	**132**	19	25	20
184	0	14	0	**175**	0	16	0
238	0	0	0	**253**	0	16	0
304	0	0	0	**294**	0	0	0
358	--	--	--	**356**	0	0	0
483	--	--	--	**485**	0	0	0
Trial Country	**L120564 Barry d' Islemade, France (South)**			**L120565 Dugliolo, Italy**			
days after treatment	**Subplot 1 [g ha^{-1}]**	**Subplot 2 [g ha^{-1}]**	**Subplot 3 [g ha^{-1}]**	**days after treatment**	**Subplot 1 [g ha^{-1}]**	**Subplot 2 [g ha^{-1}]**	**Subplot 3 [g ha^{-1}]**
0	0	0	0	**0**	0	0	0
3	0	0	0	**3**	0	0	0
6	0	0	0	**6**	19	0	0
12	0	0	0	**13**	28	0	0
27	0	0	0	**31**	26	29	18
60	0	16	0	**60**	14	17	0
95	11	11	13	**90**	0	15	28
140	27	22	0	**137**	0	0	39
180	26	18	17	**173**	0	21	66
245	19	15	16	**308**	40	14	17
293	17	0	23	**370**	0	0	0
368	0	0	0	**480**	0	0	0
489	0	0	0				

Table 7.1.2.2.1-12: Total residues of M605F007 under field conditions in soil calculated to g ha^{-1} and summed up for all depths analyzed

Trial Country	L120566 Utrera, Spain		
days after treatment	Subplot 1 [g ha^{-1}]	Subplot 2 [g ha^{-1}]	Subplot 3 [g ha^{-1}]
0	0	0	0
3	0	0	0
8	0	0	0
15	0	0	0
30	0	0	0
55	13	15	15
90	0	0	0
139	0	0	0
183	0	0	0
246	0	0	0
309	--	--	--
351	--	--	--
483	--	--	--

LOQ (limit of quantification): Residue values <10 µg kg^{-1}
(<LOQ) were reported and treated as zero
-- samples not analyzed

As is evident from the analytical data, pyrimethanil degraded relatively fast at all five European field sites. The total amount of pyrimethanil residues detected in the soil profiles decreased from an average of 930 mg kg^{-1} at day 0 to an average of 84 g ha^{-1} (range 0-183 g ha^{-1}) after 140 days. No residues above the LOQ (0.01 mg kg^{-1}) were detectable after one year in trials L120562 (Northern France), L120563 (Germany), and L120564 (Southern France) and after 309 days in trial 120566 (Spain). In trial L120565 (Italy), 23 g ha^{-1} (average of three replicates) were measured after 480 days.

Considering the distribution of pyrimethanil residues in the soil profiles, residues were exclusively found in the top 0-10 cm layer of the soils, except for one detect at a depth of 10-20 cm (≤ 0.026 mg kg^{-1}, equivalent to ≤ 37 g ha^{-1}) at trial site Germany (8 DAT). No residues above the LOQ were detected below 20 cm in any sample at any site. Altogether, it can be concluded that pyrimethanil does not show any tendency to move into deeper soil layers.

Metabolite M605F007 was detected in small amounts at all sites. It was detected earliest from 6 DAT on, reaching maximum concentrations of 21 g ha^{-1} (Northern France), 131 g ha^{-1} (Germany), 27 g ha^{-1} (Southern France), 40 g ha^{-1} (Italy), and 15 g ha^{-1} (Spain), which were related to single 10 cm layers. Thereafter, the residues declined and were no longer detectable after 308 days at the latest.

M605F007 was exclusively found in the top 0-10 cm soil layer, except for a single detect in the 10-20 cm layer at the trial site in Italy. No residues of M605F007 above the LOQ were observed in deeper soil layers in any sample at any site.

3. Shipment verification specimens

Shipment verification specimens were prepared to demonstrate stability of the residues in soil during storage and through any shipment process. The samples were prepared at nominal 0, 30, and 90 DAT by fortification of soil aliquots with 0.15 mg kg^{-1} pyrimethanil and were subsequently handled in the same manner as the actual residue samples. Concentrations of pyrimethanil analyzed were corrected for the mean recovery of the respective analytical set ranging from 73 to 107%.

The analytical results demonstrated no significant losses from the shipment verification samples. The average amount of pyrimethanil from the spiked field samples was 93% across all trials. It was concluded that pyrimethanil was stable in all soils under the storage and shipment conditions used.

4. Time of storage

The predominant part of the samples was analyzed within one year. Very few individual samples were stored for a longer time period prior to analysis. The maximum period any soil sample from the present field soil dissipation study was stored from the time of sampling to extraction was 499 days. Petri dish specimens were stored for up to 374 days after application. The maximum storage period of the spray broth samples was 384 days. Shipment verification specimens were stored for a maximum of 353 days between spiking and analysis. To confirm residue stability over the maximal storage period, a storage stability study was set up [see KCA 7.1.2.2.1/2 2013/1352188].

III. CONCLUSION

Pyrimethanil degraded rather fast under field conditions in soil at all five European field sites. The half-lives in soil are supposed to be short and are subject of a separate modeling report [see KCA 7.1.2.2.1/4 2014/1000843].

Pyrimethanil residues were mainly detected in the upper 10 cm of the soils. No residues above the LOQ were detected below 20 cm in any sample at any site, and it can be concluded that pyrimethanil shows no tendency to move into deeper soil layers. Metabolite M605F007 was detected only in small amounts. It declined again in all trials and was no longer detectable latest after 308 days. It was almost exclusively found in the top 0-10 cm soil layer and showed also no tendency to leach.

Overall, the study showed that pyrimethanil (and its metabolite) does not persist in soil under field conditions and pose no risk for accumulation or leaching.

Report: CA 7.1.2.2.1/3
Gasso-Brown D., 2015a
Determination of the storage stability of Pyrimethanil and its metabolite 2-amino-4,6-dimethylpyrimidine in soil following frozen storage for approximately 18 months
2013/1352188

Guidelines: EPA 860.1380, EEC 7032/VI/95 rev. 5, OECD 506 (Oct. 2007), OECD-ENV/JM/MONO/(2007)17, SANCO/3029/99 rev. 4 (11 July 2000)

GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

In the present study, the storage stability of pyrimethanil and its metabolite 2-amino-4,6-dimethylpyrimidine (M605F007, AE F132593) was investigated by fortifying control soil samples taken from five separate field test sites conducted as part of the new terrestrial field dissipation study [see KCA 7.1.2.2.1/1 2014/1000661]. The control samples were fortified at a level of 0.1 mg kg^{-1} with the test items. The samples were analyzed after different storage periods (nominal 0, 30, 60, 120, 240, 360 and 540 days) at ≤ -18°C.

The validated BASF analytical method L0249/01 was used in this study. It was re-validated to be in accordance to the newest guidelines by EAS Chem UK as part of a validation study presented in section CA 4.1.2/2 [see KCA 4.1.2/2 2013/1374394].

Residues of pyrimethanil and its metabolite M605F007 were extracted with methanol. After dilution and clean up by solid phase extraction (SPE), the retained analytes were eluted, concentrated, and analyzed by LC-MS/MS. The limit of quantification (LOQ) was defined as the lowest fortification level with mean recoveries ranging between 70% and 110% with a relative standard deviation not exceeding 20%. For the analytical method (L0249/01) used during this study, this was set at 0.01 mg kg^{-1}.

After 540 days of storage, 80% (L120562), 83% (L120563), 121% (L120564), 79% (L120565), and 79% (L120566) of the initial concentration of pyrimethanil (at 0 days) was found in spiked soil samples.

For metabolite M605F007, 78% (L120562), 63% (L120563), 83% (L120564), 89% (L120565), and 81% (L120566) of the initial concentration (at 0 days) was found after 540 days of storage in spiked soil samples.

According to EC guideline 7032/VI/95 and U.S. EPA guideline OPPTS 860.1380, residues are regarded as stable if the mean recovery at a given storage period does not fall below 70% of the initial value. For soil L120563, the procedural recoveries were low at certain time points. The stability samples were therefore corrected for the procedural recoveries. It was confirmed that M605F007 is stable under deep-frozen conditions (≤ -18°C) for 540 days of storage.

Therefore, results confirmed that pyrimethanil and M605F007 are stable under deep-frozen conditions (≤ -18°C) in soil for at least 540 days of storage.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Materials

1. Pyrimethanil (BAS 605 F)

Internal code:	BAS 605 F
Reg. No.:	236999
CAS No.:	53112-28-0
Chemical name (IUPAC):	N-(4,6-dimethylpyrimidin-2-yl)aniline
Molecular mass:	199.3 g mol^{-1}
Molecular formula:	$C_{12}H_{13}N_3$
Batch No.:	486213 / L83-126
Purity:	99.8 / 99.6%

2. Pyrimethanil metabolite M605F007 (also referred to as ADMP)

Internal code:	M605F007
Reg. No.:	40603
CAS No.:	767-15-7
Chemical name (IUPAC):	2-amino-4,6-dimethylpyrimidine
Molecular mass:	123.2 g mol^{-1}
Molecular formula:	$C_6H_9N_3$
Batch No.:	L72-129
Purity:	98.6%

2. Soil

Untreated soil samples from five trial sites of a field dissipation study [see KCA 7.1.2.2.1/1 2014/1000661] were used for testing.

B. STUDY DESIGN

1. Experimental Conditions

Untreated soil samples were transferred into brown glass jars and fortified individually with either pyrimethanil or M605F007 at a level of 0.1 mg kg^{-1}. Individual fortification solutions of pyrimethanil and M605F007 in methanol were distributed drop wise onto the soil, and the glass jar was closed and placed into the deep freezer (typically ≤ -18°C). For each storage interval and soil type, a set of eight samples, consisting of two controls, two controls for recovery determination, two stored pyrimethanil samples and two stored M605F007 samples were prepared. In addition, three spare sets per soil type were prepared in case re-analysis or additional time storage intervals were required. Furthermore, procedural recovery samples were prepared by fortification of soil at a level of 0.1 mg kg^{-1} with pyrimethanil or M605F007.

2. Sampling

The following samples were analyzed:

- Pyrimethanil
  After fortification and storage for 0, 31, 62, 124, 242, 363 and 542 days for soils from L120562 and L120563, 0, 31, 63, 123, 246, 361 and 545 days for soil from L120564 and 0, 29, 62, 120, 238, 364 and 540 days for soils from L120565 and L120566 (all samples in duplicate).
- M605F007
  After fortification and storage for 0, 31, 62, 124, 242, 363 and 542 days for soils from L120562 and L120563, 0, 31, 63, 123, 246, 361 and 545 days for soil from L120564 and 0, 29, 62, 120, 238, 364 and 540 days for soils from L120565 and L120566 (all samples in duplicate).
- In addition, untreated control samples were analyzed at the date of analysis in duplicate as well as two freshly fortified recovery samples at 0.1 mg kg^{-1} for method validation purpose.

3. Description of analytical procedures

Analytical method L0249/01 [see KCA 4.1.2/1 2005/1027614] was provided by the sponsor with a LOQ of 0.01 mg kg^{-1}. It was also re-validated to be in accordance to the newest guidelines by EAS Chem UK as part of a validation study presented in CA 4.1.2/2 [see KCA 4.1.2/2 2013/1374394].

Residues of pyrimethanil and its metabolite 2-amino-4,6-dimethylpyrimidine (M605F007, AE F132593) were extracted with methanol. An aliquot of the extract was diluted with water and cleaned up by SPE (0.2 g Lichrolut EN). The retained analytes were eluted from the SPE material with acetone, concentrated to near dryness, and reconstituted with water to have a final solvent ratio of acetone/water 1:9 (v/v). Analysis was performed by LC-MS/MS monitoring two parent - daughter ion transitions per analyte.

The limit of quantification (LOQ) was defined as the lowest fortification level with mean recoveries ranging between 70% and 110% with a relative standard deviation not exceeding 20%. For the analytical method used during this study, this was set at 0.01 mg kg^{-1} for pyrimethanil and M605F007. The limit of detection (LOD) was defined as 20% of LOQ, i.e. 0.002 mg kg^{-1}.

II. RESULTS AND DISCUSSION

A summary of the recovery results after storage is presented in Table 7.1.2.2.1-13 and Table 7.1.2.2.1-14. The procedural recovery were in the range of 70 to 110%. Data reported in the summary tables were not corrected for the procedural recovery. Where appropriate, residues were corrected for the mean untreated residue. The percent of initial was calculated from mean percentage recovery obtained for the storage stability samples. The mean recovery at 0 days was set to 100% of initial.

After 540 days of storage, 80% (L120562), 83% (L120563), 121% (L120564), 79% (L120565), and 79% (L120566) of the initial concentration of pyrimethanil (at 0 days) was found in spiked soil samples fortified with pyrimethanil at 0.1 mg kg^{-1}.

After 540 days of storage, 78% (L120562), 63% (L120563), 83% (L120564), 89% (L120565) and 81% (L120566) of the initial concentration of M605F007 (at 0 days) was found in spiked soil samples fortified with the M605F007 at 0.1 mg kg^{-1}.

According to EC guideline 7032/VI/95 and U.S. EPA guideline OPPTS 860.1380, residues can be regarded as stable if the mean recovery at a given storage period does not fall below 70% of the initial value. For soil L120563, the stored recoveries were low (in % of initial) at some time points, indicating an extraction issue. The stability samples were therefore corrected for the procedural recovery revealing that M605F007 is stable under deep-frozen conditions (≤ -18°C) for 540 days of storage.

Table 7.1.2.2.1-13: Storage stability of pyrimethanil in frozen soil

Soil	Storage period [days]	Recovery [%]		Mean Recovery [%]	% of Initial [a]
L120562	0	108	109	109	100
	31	89	93	91	84
	62	97	110	104	95
	124	98	120	109	100
	242	93	93	93	86
	363	97	96	96	89
	542	83	91	87	80
L120563	0	110	105	107	100
	31	84	85	85	79
	62	91	79	85	80
	124	99	96 [b]	98	91
	242	103	94	98	92
	363	88	91	89	83
	542	89	90	89	83
L120564	0	88	91	90	100
	31	95	104	100	111
	63	96	100	98	109
	123	112	112	112	125
	246	97	93	95	106
	361	90	96	93	104
	545	111	107	109	121
L120565	0	103	101	102	100
	29	86	82	84	82
	62	91	88	89	87
	120	89	96	92	91
	238	95	94	95	93
	364	99	93	96	94
	540	76	87	81	79
L120566	0	100	104	102	100
	29	77	77	77	76
	62	90	96	93	91
	120	95	94	95	93
	238	89	91	90	88
	364	94	92	93	91
	540	84	78	81	79

RSD = relative standard deviation
[a] mean recovery at 0 days was set to 100% of initial
[b] mean of two injections of the same sample extract

Table 7.1.2.2.1-14: Storage stability of pyrimethanil metabolite M605F007 in frozen soil

Soil	Storage period [days]	Recovery [%]		Mean Recovery [%]	% of Initial [a]
L120562	0	96	100	98	100
	31	74	81	77	79
	62	89	87	88	90
	124	93	87	90	92
	242	88	90	89	91
	363	81	82	82	83
	542	73	80	76	78
L120563	0	97	98	98	100
	31	75	70	72	74
	62	81	78	80	82
	124	84	84	84	86
	242	70	69	69	71
	363	59	65	62	64
	542	61	62	62	63
L120564	0	65	67	66	100
	31	55	57	56	85
	63	64	62	63	95
	123	57	57	57	86
	246	55	55	55	83
	361	54	54	54	82
	545	54	56	55	83
L120565	0	89	84	86	100
	29	73	76	75	86
	62	78	76	77	89
	120	83	77	80	93
	238	80	82	81	94
	364	82	85	83	97
	540	76	78	77	89
L120566	0	89	90	89	100
	29	72	75	74	82
	62	78	77	78	87
	120	81	81	81	90
	238	66	66	66	74
	364	84	82	83	92
	540	74	71	72	81

RSD = relative standard deviation
[a] mean recovery at 0 days was set to 100% of initial
[b] mean of two injections of the same sample extract

III. CONCLUSION

This study demonstrates that pyrimethanil (BAS 605 F) and its metabolite M605F007 (AE F132593) are stable under deep-frozen conditions (≤ -18°C) in soil for at least 540 days of storage.

Report: CA 7.1.2.2.1/4
Budde E., 2014a
Kinetic evaluation of eight field dissipation trials with BAS 605 F - Pyrimethanil conducted in 1991 and 1992: Determination of trigger and modeling endpoints according to Focus degradation kinetics and EFSA Guidance
2013/1278722

Guidelines: FOCUS Kinetics (2006) SANCO/10058/2005 version 2.0, EFSA Guidance to obtain DegT50 values in soil (2010)

GLP: no

Executive Summary

The dissipation behaviour of pyrimethanil (BAS 605 F) in soil has been investigated in two field dissipation studies (1991 and 1992) including a total of eight field trials at six different locations in Germany. The purpose of this evaluation was to analyse the degradation kinetics of pyrimethanil under different climatic conditions and to derive trigger endpoints for additional work and modeling endpoints according to the current guidance of the FOCUS workgroup on degradation kinetics and the EFSA guidance on estimating DT_{50} values in soil for modeling purposes.

Kinetic evaluation of pyrimethanil was performed in order to derive field dissipation parameters that are adequate to be used as trigger endpoints for higher-tier experiments. The best-fit kinetic model was selected based on a visual and statistical assessment. Kinetic evaluation showed that the dissipation behavior of pyrimethanil was best described using single first-order (SFO) kinetics in four out of eight trials, whereas for one trial FOMC kinetics was selected as best-fit model. For three trials, no adequate model was found for the available data. Field half-live values ($DissT_{50}$) ranged from 25.2 to 53.6 days with $DissT_{90}$ values ranging from 83.7 to 264.7 days.

Prior to kinetic evaluation for modeling endpoints, the sampling intervals of the field studies were normalized to reference conditions regarding soil moisture and temperature according to the time-step normalization technique. Kinetic evaluation was performed on the normalized sampling interval dataset in order to derive degradation parameters that can be used as modeling endpoints.

Four field trials had to be excluded from kinetic analysis according to EFSA, due to a low number of remaining data points (< 4) after > 10 mm cumulative precipitation.

For two of the remaining four field trials, kinetic evaluation of the time-step normalized dataset (20°C, pF2) resulted in normalized field half-lives ($DegT_{50}$) for pyrimethanil between 16.3 and 22.3 days, when all sampling days were included, and between 19.9 and 21.8 days, when only data with > 10 mm rainfall were considered. For the other two field trials, no adequate model fit could be derived.

I. MATERIAL AND METHODS

Soils

The degradation behavior of pyrimethanil was investigated in two field dissipation studies [*Wrede-Rücker, A. (1993) Pyrimethanil: SC (Scala); Residues in soil, Germany 1991, BASF DocID A81908; Wrede, A. (1993) Pyrimethanil suspension concentrate 400 g/l; Residues in soil, Germany 1992, BASF DocID A81928*] with eight field trials in total. The soil characteristics of the eight field trials are summarized in Table 7.1.2.2.1-15.

Table 7.1.2.2.1-15: Soil characteristics

Trial	**Goch-Nierswalde (1991)**	**Weeze-Wemb**	**Emmerich-Elten**	**Schwichteler (1991)**
Location	**Germany**	**Germany**	**Germany**	**Germany**
Soil layer for soil analysis	0 - 30 cm	0 - 30 cm	0 - 30 cm	0 - 30 cm
Soil texture (DIN 19682) fine silt [%] middle silt [%] clay [%]	Humus sand 3.5 11.5 8.3	Humus sand 1.3 1.6 2.8	Humus sand 11.8 19.2 22.6	Sandy loam 1.5 4.1 6.0
Organic matter [%]	2.5	1.8	1.5	2.8
pH (KCl) [-]	6.2	5.49	6.9	5.8
Trial	**Goch (1992)**	**Meissner-Vockerode**	**Schwichteler (1992)**	**Elsenfeld-Rück**
Location	**Germany**	**Germany**	**Germany**	**Germany**
Soil layer for soil analysis	0 - 30 cm	0 - 30 cm	0 - 30 cm	0 - 30 cm
Soil texture (DIN 19682) fine silt [%] middle silt [%] clay [%]	Silty loam 4.9 13.4 14.0	loam 17.4 4.8 14.7	Humus sandy loam 2.5 5.8 10.1	Silty loam 5.8 16.5 15.7
Organic matter [%]	3.5	2.1	6.3	2.1
pH (KCl) [-]	5.9	6.4	6.3	7.0

The trials were situated in typical agricultural regions in Germany, considering a range of different soils and climatic conditions.

Kinetic modeling

The software package KinGUI version 2.2012.320.1629 was used for parameter fitting [*SCHÄFER et al. (2007)*]. The error tolerance and the number of iterations of the optimization tool (IRLS) were set to the default values of 1×10^{-6} and 100, respectively.

Datasets were prepared for kinetic evaluation as follows:

- Values below the quantification or detection limit were treated as recommended by the FOCUS workgroup [*FOCUS (2006)*]. The limit of quantification (LOQ) for pyrimethanil reported in the two studies was 0.05 mg kg^{-1}. The limit of detection (LOD) was 0.01 mg kg^{-1} [*BASF DocID A81908; BASF DocID A81928*]. According to FOCUS, values below LOD were set to 0.5 x LOD = 0.005 mg kg^{-1}.
- For each sampling point, the concentration of a compound in the single soil layer given in mg kg^{-1} was transformed to its residue given in kg ha^{-1} considering the thickness of the respective segment and a default soil bulk density of 1.5 g cm^{-3}. The total residues in the sampled soil core were calculated as the sum of residues of the single soil core segments.

Experimental data

The nominal application rate was 1500 g a.s. ha^{-1} at all trial sites. Applications (using the SC formulation 'SCALA') were made by spraying to bare soil and were conducted in June 1991 [*BASF DocID A81908*] and May 1992 [*BASF DocID A81928*]. Soil samples were taken at up to 369 days after treatment (DAT).

The measured data for pyrimethanil as well as resulting datasets submitted to kinetic analysis are given in Table 7.1.2.2.1-16 to Table 7.1.2.2.1-23.

Table 7.1.2.2.1-16: Experimental data from field trial Goch (1991) used for kinetic evaluation of pyrimethanil

days after treatment	Concentration in individual soil layer [mg kg^{-1}]			Total residue
	0-10 cm	10-20 cm	20-30 cm	[kg ha^{-1}]
0	0.09	n.d.		
1	0.07	n.d.		
3	0.08	n.d.		
7	0.14	n.d.		
14	n.d.	n.d.		
28	n.d.	n.d.		
61	n.d.	n.d.		
92	n.d.	n.d.		
184	n.d.	n.d.		
280	n.d.	n.d.		
369	n.d.	n.d.		

Data used as input for kinetic modeling of trigger / modeling endpoints according to FOCUS [*FOCUS (2006)*]

days after treatment	D_{norm} [a] [d]	Concentration in individual soil layer [mg kg^{-1}]			Total residue
		0-10 cm	10-20 cm	20-30 cm	[kg ha^{-1}]
0		0.090	0.005		0.143
1		0.070	0.005		0.113
3		0.080	0.005		0.128
7		0.140	0.005		0.218
14		**0.005**			0.008

n.d. Not detected

[a] Normalization not performed

bold numbers: set to ½ LOD according to FOCUS (2006), based on time course

underlined numbers: set to ½ LOD according to FOCUS (2006), based on soil layer sequence

Table 7.1.2.2.1-17: Experimental data from field trial Weeze-Wemb used for kinetic evaluation of pyrimethanil

days after treatment	Concentration in individual soil layer [mg kg^{-1}]			Total residue
	0-10 cm	10-20 cm	20-30 cm	[kg ha^{-1}]
0	0.54	n.d.		
1	0.15	n.d.		
3	n.d.	n.d.		
7	0.07	n.d.		
14	0.23	n.d.		
28	0.10	n.d.		
61	n.d.	n.d.		
92	0.10	n.d.		
183	n.d.	n.d.		
278	n.d.	n.d.		
369	n.d.	n.d.		

Data used as input for kinetic modeling of trigger / modeling endpoints according to FOCUS [*FOCUS (2006)*]

days after treatment	D_{norm} [a] [d]	Concentration in individual soil layer [mg kg^{-1}]			Total residue
		0-10 cm	10-20 cm		[kg ha^{-1}]
0	**0.0**	0.540	0.005		0.818
1	**0.4**	0.150	0.005		0.233
3	**1.4**	**0.005**			0.008
7	**4.4**	0.070	0.005		0.113
14	**9.1**	0.230	0.005		0.353
28	**23.8**	0.100	0.005		0.158
61	**52.3**	**0.005**			0.008
92	**74.4**	0.100	0.005		0.158
183	**104.2**	**0.005**			0.008

n.d. Not detected

[a] Normalized (20 °C, pF2) day lengths

bold numbers: set to ½ LOD according to FOCUS (2006), based on time course

underlined numbers: set to ½ LOD according to FOCUS (2006), based on soil layer sequence

Table 7.1.2.2.1-18: Experimental data from field trial Emmerich-Elten used for kinetic evaluation of pyrimethanil

days after treatment	Concentration in individual soil layer [mg kg^{-1}]			Total residue
	0-10 cm	10-20 cm	20-30 cm	[kg ha^{-1}]
0	0.06	n.d.		
1	0.42	n.d.		
3	0.12	n.d.		
7	0.14	n.d.		
14	n.d.	n.d.		
30	n.d.	n.d.		
61	n.d.	n.d.		
92	n.d.	n.d.		
183	n.d.	n.d.		
277	n.d.	n.d.		
368	n.d.	n.d.		

Data used as input for kinetic modeling of trigger / modeling endpoints according to FOCUS [*FOCUS (2006)*]

days after treatment	D_{norm} [a] [d]	Concentration in individual soil layer [mg kg^{-1}]			Total residue
		0-10 cm	10-20 cm	20-30 cm	[kg ha^{-1}]
0		0.060	0.005		0.098
1		0.420	0.005		0.638
3		0.120	0.005		0.188
7		0.140	0.005		0.218
14		**0.005**			0.008

n.d. Not detected

[a] Normalization not performed

bold numbers: set to ½ LOD according to FOCUS (2006), based on time course

underlined numbers: set to ½ LOD according to FOCUS (2006), based on soil layer sequence

Table 7.1.2.2.1-19: Experimental data from field trial Schwichteler (1991) used for kinetic evaluation of pyrimethanil

days after treatment	Concentration in individual soil layer [mg kg^{-1}]			Total residue
	0-10 cm	10-20 cm	20-30 cm	[kg ha^{-1}]
0	0.48	n.d.		
1	1.4	n.d.		
3	0.99	n.d.		
7	0.57	n.d.		
13	0.43	n.d.		
28	0.34	n.d.		
61	0.18	n.d.		
92	0.67	n.d.		
184	n.d.	n.d.		
276	0.18	n.d.		
359	0.12	n.d.		

Data used as input for kinetic modeling of trigger / modeling endpoints according to FOCUS [*FOCUS (2006)*]

days after treatment	D_{norm} [a] [d]	Concentration in individual soil layer [mg kg^{-1}]			Total residue
			10-20 cm		[kg ha^{-1}]
0	0.0	0.480	0.005		0.728
1	0.7	1.400	0.005		2.108
3	2.0	0.990	0.005		1.493
7	4.4	0.570	0.005		0.863
13	10.3	0.430	0.005		0.653
28	22.1	0.340	0.005		0.518
61	47.9	0.180	0.005		0.278
92	66.5	0.670	0.005		1.013
184	94.2	**0.005**			0.008
276	111.6	0.180	0.005		0.278
359	157.6	0.120	0.005		0.188

n.d. Not detected

[a] Normalized (20 °C, pF2) day lengths

bold numbers: set to ½ LOD according to FOCUS (2006), based on time course

underlined numbers: set to ½ LOD according to FOCUS (2006), based on soil layer sequence

Table 7.1.2.2.1-20: Experimental data from field trial Goch (1992) used for kinetic evaluation of pyrimethanil

days after treatment	Concentration in individual soil layer [mg kg^{-1}]			Total residue
	0-10 cm	10-20 cm	20-30 cm	[kg ha^{-1}]
0	0.60	n.d.		
1	0.47	n.d.		
3	0.95	n.d.		
7	n.d.	n.d.		
13	0.59	n.d.		
29	0.62	n.d.		
61	0.32	n.d.		
91	0.13	n.d.		
184	n.d.	n.d.		
274	n.d.	n.d.		
363	n.d.	n.d.		

Data used as input for kinetic modeling of trigger / modeling endpoints according to FOCUS [*FOCUS (2006)*]

days after treatment	D_{norm} [a] [d]	Concentration in individual soil layer [mg kg^{-1}]			Total residue
			10-20 cm		[kg ha^{-1}]
0		0.600	0.005		0.908
1		0.470	0.005		0.713
3		0.950	0.005		1.433
7		**0.005**			0.008
13		0.590	0.005		0.893
29		0.620	0.005		0.938
61		0.320	0.005		0.488
91		0.130	0.005		0.203
184		**0.005**			0.008

n.d. Not detected

[a] Normalization not performed

bold numbers: set to ½ LOD according to FOCUS (2006), based on time course

underlined numbers: set to ½ LOD according to FOCUS (2006), based on soil layer sequence

Table 7.1.2.2.1-21: Experimental data from field trial Meissner-Vockerode used for kinetic evaluation of pyrimethanil

days after treatment	Concentration in individual soil layer [mg kg^{-1}]			Total residue
	0-10 cm	10-20 cm	20-30 cm	[kg ha^{-1}]
0	0.53	n.d.		
1	0.75	n.d.		
3	0.50	n.d.		
6	0.63	n.d.		
13	0.51	n.d.		
30	0.23	n.d.		
60	0.14	n.d.		
94	0.22	n.d.		
187	n.d.	n.d.		
277	n.d.	n.d.		
369	n.d.	n.d.		

Data used as input for kinetic modeling of trigger / modeling endpoints according to FOCUS [*FOCUS (2006)*]

days after treatment	D_{norm} [a] [d]	Concentration in individual soil layer [mg kg^{-1}]			Total residue
			10-20 cm		[kg ha^{-1}]
0	**0.0**	0.530	0.005		0.803
1	**0.4**	0.750	0.005		1.133
3	**1.0**	0.500	0.005		0.758
6	**2.3**	0.630	0.005		0.953
13	**6.5**	0.510	0.005		0.773
30	**19.5**	0.230	0.005		0.353
60	**41.3**	0.140	0.005		0.218
94	**73.2**	0.220	0.005		0.338
187	**117.5**	**0.005**			0.008

n.d. Not detected

[a] Normalized (20 °C, pF2) day lengths

bold numbers: set to ½ LOD according to FOCUS (2006), based on time course

underlined numbers: set to ½ LOD according to FOCUS (2006), based on soil layer sequence

Table 7.1.2.2.1-22: Experimental data from field trial Schwichteler (1992) used for kinetic evaluation of pyrimethanil

days after treatment	Concentration in individual soil layer [mg kg^{-1}]			Total residue
	0-10 cm	10-20 cm	20-30 cm	[kg ha^{-1}]
0	0.76	n.d.		
1	2.1	n.d.		
3	0.83	n.d.		
8	1.1	n.d.		
15	1.1	n.d.		
32	0.19	n.d.		
62	0.38	n.d.		
92	0.13	n.d.		
187	0.15	n.d.		
278	n.d.	n.d.		
362	n.d.	n.d.		
Data used as input for kinetic modeling of trigger / modeling endpoints according to FOCUS [*FOCUS (2006)*]				
days after treatment	**D_{norm} [a] [d]**	**Concentration in individual soil layer [mg kg^{-1}]**		**Total residue**
		10-20 cm		**[kg ha^{-1}]**
0	**0.0**	0.760	0.005	1.148
1	**0.4**	2.100	0.005	3.158
3	**1.3**	0.830	0.005	1.253
8	**3.3**	1.100	0.005	1.658
15	**7.8**	1.100	0.005	1.658
32	**22.6**	0.190	0.005	0.293
62	**45.9**	0.380	0.005	0.578
92	**71.8**	0.130	0.005	0.203
187	**121.9**	0.150	0.005	0.233
278	**140.6**	**0.005**		0.008

n.d. Not detected

[a] Normalized (20 °C, pF2) day lengths

bold numbers: set to ½ LOD according to FOCUS (2006), based on time course

underlined numbers: set to ½ LOD according to FOCUS (2006), based on soil layer sequence

Table 7.1.2.2.1-23: Experimental data from field trial Elsenfeld-Rück used for kinetic evaluation of pyrimethanil

days after treatment	Concentration in individual soil layer [mg kg^{-1}]			Total residue
	0-10 cm	10-20 cm	20-30 cm	[kg ha^{-1}]
0	1.5	n.d.		
1	1.5	n.d.		
3	0.94	n.d.		
7	0.9	n.d.		
14	1.0	n.d.		
28	0.66	n.d.		
61	0.27	n.d.		
91	0.12	n.d.		
180	n.d.	n.d.		
280	n.d.	n.d.		
359	n.d.	n.d.		

Data used as input for kinetic modeling of trigger / modeling endpoints according to FOCUS [*FOCUS (2006)*]

days after treatment	D_{norm} [a] [d]	Concentration in individual soil layer [mg kg^{-1}]			Total residue
			10-20 cm		[kg ha^{-1}]
0		1.500	0.005		2.258
1		1.500	0.005		2.258
3		0.940	0.005		1.418
7		0.900	0.005		1.358
14		1.000	0.005		1.508
28		0.660	0.005		0.998
61		0.270	0.005		0.413
91		0.120	0.005		0.188
180		**0.005**			0.008

n.d. Not detected

[a] Normalization not performed

bold numbers: set to ½ LOD according to FOCUS (2006), based on time course

underlined numbers: set to ½ LOD according to FOCUS (2006), based on soil layer sequence

Normalization procedure

Evaluation of the suitability of field dissipation data for normalization was performed according to the evaluation criteria for normalization compiled by the Dutch regulatory authority (CTB criteria) [*CTB (1999)*].

Temperature correction factors (f_{temp}) were determined to account for differences between actual daily soil temperatures as calculated by FOCUS-PEARL 4.4.4 and a reference temperature of 20 °C using the Q_{10} approach as described in the report of the FOCUS soil modeling working group [see *FOCUS (1997)*]. The Q_{10} response function was applied for temperatures above 0 °C (see Equation 7.1.2.2.1-1 c). Below field temperatures of 0 °C it was assumed that no degradation occurs (Equation 7.1.2.2.1-1 c). For the evaluation, the EFSA opinion on the default Q_{10} value [see *EFSA (2007)*] was followed and a Q_{10} value of 2.58 was included in the assessment.

Moisture correction factors (f_{moist}) were determined to account for differences between actual daily soil moisture as calculated by FOCUS-PEARL 4.4.4 and the reference soil moisture at field capacity (pF 2) (Equation 7.1.2.2.1-1 d).

The normalized day lengths were derived according to Equation 7.1.2.2.1-1 a. For DAT 0, no normalization was considered and application was assumed to occur at the time point zero. Normalized sampling days (DAT_{norm}) after application were calculated by cumulatively summing up normalized day lengths according to Equation 7.1.2.2.1-1 b.

Equation 7.1.2-2: Calculation of normalized day length based on combination of soil moisture and soil temperature correction factors

a) $$D_{norm} = D * f_{temp} * f_{moisture}$$

b) $$t_i = \sum_{t=1}^{i-1} D_{norm}$$

with:

t_i =	Time from application till sampling at day i	[d]
D_{norm} =	Normalized day length (20°C, pF2)	[d]
i =	Time span between application and sampling	[d]

c) $$f_{temp} = \begin{cases} Q_{10}^{\frac{Tact-Tref}{10}} & \text{for } T_{act} > 0°C \\ 0 & \text{for } T_{act} \leq 0°C \end{cases}$$

d) $$f_{moist} = \begin{cases} (\frac{\theta_{act}}{\theta_{ref}})^B & \text{for } \theta_{ref} > \theta_{act} \\ 1 & \text{for } \theta_{ref} \leq \theta_{act} \end{cases}$$

with:

D_{norm} =	normalized day length (temperature and moisture)	
D =	actual day length (1 d)	[days]
f_{temp} =	temperature correction factor	[-]
f_{moist} =	moisture correction factor	[-]
T_{act} =	actual soil temperature (°C)	[C°]
T_{ref} =	reference temperature (20 °C)	[C°]
Q_{10}=	factor of increase of degradation rate with an increase in temperature of 10 °C (Q_{10} = 2.58 [EFSA 2007])	[-]
θ_{act} =	actual soil moisture (vol. water content)	[m^3 m^{-3}]
θ_{ref} =	reference soil moisture at pF2	[m^3 m^{-3}]
B =	exponent of the moisture response function, B = 0.7	[-]

Time-step normalization was not performed for trials Goch-Nierswalde (1991), Emmerich-Elten, Goch (1992) and Elsenfeld-Rück, because of too few data points. Field sampling dates for the remaining four trial locations and the normalized (20 °C, pF2) day lengths based on soil moisture and soil temperature data as simulated by FOCUS-PEARL 4.4.4 are given in Table 7.1.2.2.1-16 to Table 7.1.2.2.1-23.

II. RESULTS AND DISCUSSION

The best-fit kinetic model was selected to derive trigger endpoints for additional work for pyrimethanil, based on a visual and statistical assessment. Kinetic evaluation showed that the dissipation behavior of pyrimethanil in four of the eight trials was best described using single first-order (SFO) kinetics, whereas for one trial FOMC was chosen as best-fit kinetics. For the other three trials, no adequate model was found for the available data.

The best-fit kinetics and derived non-normalized trigger endpoints for pyrimethanil are summarized in Table 7.1.2.2.1-24

Table 7.1.2.2.1-24: Summary of endpoints for use as triggers for additional work of pyrimethanil

Study (DocID)	Field trial	Soil type	Best-fit kinetic model	χ^2 error	Rate k [d^{-1}]	DT_{50} [d]	DT_{90} [d]
A81908	Goch-Nierswalde (1991)	Sandy loam	No adequate kinetic model derived				
	Weeze-Wemb	Loam	No adequate kinetic model derived				
	Emmerich-Elten	Humus sand	No adequate kinetic model derived				
	Schwichteler (1991)	Sandy silt	FOMC	34.5	-	29.6	264.7
A81928	Goch (1992)	Silty loam	SFO	24.6	0.0129	53.6	178.1
	Meißner-Vockerode	Loam	SFO	18.3	0.0205	33.9	112.5
	Schwichteler (1992)	Humus sandy loam	SFO	32.2	0.0177	39.3	130.5
	Elsenfeld-Rück	Silty loam	SFO	15.6	0.0275	25.2	83.7

Prior to kinetic evaluation for deriving modeling endpoints of pyrimethanil, the data sets of the eight trials were evaluated for the criterion of 10 mm cumulative rainfall. The field studies were assessed with regard to an appropriate number of data points for a kinetic assessment if samplings before 10 mm of cumulative precipitation are excluded. The evaluation showed that it was not possible to perform kinetic analysis for four out of eight trial locations, because of too few data points.

The dissipation behavior of pyrimethanil in the four remaining field trials was analyzed in a step-wise approach to derive modeling endpoints. For two of the four field trials, no adequate model fit could be derived. A summary of the results of the kinetic evaluation are presented in Table 7.1.2.2.1-25.

Table 7.1.2.2.1-25: Summary of endpoints for use in modeling of pyrimethanil

Study (DocID)	Field trial	Soil type	Kinetic model	χ^2 error (all data/ data >10 mm)	All data included		Data > 10 mm rainfall	
					Rate k [d^{-1}]	DT_{50} [d]	Rate k [d^{-1}]	DT_{50} [d]
A81908	Goch-Nierswalde (1991)	Sandy loam	-	Less than five data points after 10 mm rain				
	Weeze-Wemb	Loam	-	No adequate kinetic model derived				
	Emmerich-Elten	Humus sand	-	Less than five data points after 10 mm rain				
	Schwichteler (1991)	Sandy silt	-	No adequate kinetic model derived				
A81928	Goch (1992)	Silty loam	-	Less than five data points after 10 mm rain				
	Meißner-Vockerode	Loam	SFO	18.8 / 19.6	0.0311	22.3	0.0348	19.9
	Schwichteler (1992)	Humus sandy loam	SFO	42.9 / 30.0	0.0425	16.3	0.0318	21.8
	Elsenfeld-Rück	Silty loam	-	Less than five data points after 10 mm rain				
							Geo mean	**20.8**

III. CONCLUSION

Kinetic evaluation of eight field trials with pyrimethanil, originating from two field dissipation studies, was conducted in order to derive reliable trigger endpoints for additional work and normalized modeling endpoints according to the current guidance of the FOCUS workgroup on degradation kinetics.

Kinetic evaluation for the derivation of trigger endpoints showed that the dissipation behavior of pyrimethanil was best described using single first-order (SFO) kinetics for five out of eight trials. For three trials, no adequate model was found for the available data. Field half-live values ($DegT_{50}$) used as trigger endpoints ranged from 25.2 to 53.6 days with $DegT_{90}$ values ranging from 83.7 to 264.7 days.

Kinetic evaluation of the time-step normalized dataset (20°C, pF2) was possible for four out of eight field trials. Normalized field half-lives ($DegT_{50}$) for pyrimethanil used as modeling endpoints were in the range between 16.3 and 22.3 days, when all sampling days were included, and between 19.9 and 21.8 days, when only data with > 10 mm rainfall were considered.

The latter one (i.e. data with >10mm rainfall included) was considered relevant for finally selecting the degradation endpoints for modeling purposes.

Report:	CA 7.1.2.2.1/5 Dalkmann P., 2015a Kinetic evaluation of field dissipation study with BAS 605 F - Pyrimethanil conducted between 2012 and 2013 in Europe: Determination of best-fit and modeling endpoints according to FOCUS 2014/1000843
Guidelines:	FOCUS Kinetics (2006) SANCO/10058/2005 version 2.0, EFSA Guidance to obtain DegT50 values in soil (2014)
GLP:	no

Executive Summary

The degradation behavior of BAS 605 F – pyrimethanil in soil has been investigated in a field dissipation study tailored for determining the degradation in soil matrix ($DegT_{50matrix}$ field study) including five field trials [see KCA 7.1.2.2.1/1 2014/1000661].

The purpose of the present kinetic evaluation was to analyze the degradation kinetics of pyrimethanil observed in the five soils according to the current guidance of the FOCUS workgroup on degradation kinetics, under consideration of the recommendations provided in the EFSA guidance to obtain $DegT_{50}$ values in soil for modeling purposes.

In a first step, a non-normalized kinetic evaluation was performed in order to derive best-fit field degradation parameters for pyrimethanil. The best-fit kinetic model was selected based on a visual and statistical assessment under consideration of the FOCUS guidance for deriving non-normalized best-fit endpoints.

The best-fit endpoints ($DegT_{50}$) for pyrimethanil and its metabolite 2-amino-4,6-dimethyl-pyrimidine (M605F007; also referred to as ADMP or AE F132593) ranged from 17.5 to 44.0 days and from 28.1 to 142.9 days, respectively. The corresponding $DegT_{90}$ values ranged from 59.4 to 262.3 days and from 93.2 to 474.7 days.

In a second step, prior to kinetic evaluation, the sampling intervals of the field studies were normalized to reference conditions (20°C, pF2) regarding soil moisture and temperature according to the time-step normalization technique. Kinetic evaluation was performed on the normalized sampling interval dataset, and degradation parameters that can be used as modeling endpoints were derived. The respective degradation parameters were derived based on a visual and statistical assessment under the consideration of the EFSA Guidance as well as the recommendations of the FOCUS kinetics working group.

Normalized modeling $DegT_{50}$ values for pyrimethanil ranged from 14.5 to 77.5 days. For the metabolite M605F007, normalized modeling $DegT_{50}$ values between 15.6 to 48.2 days were derived.

I. MATERIAL AND METHODS

Soils

The degradation behavior of pyrimethanil was investigated in a field dissipation study with five field trials. The field study and corresponding data are reported in CA 7.1.2.2.1/1 [see KCA 7.1.2.2.1/1 2014/1000661]. The trials were situated in typical agricultural regions in Germany, Italy, Spain, and France (two trials), considering a range of different soils and climatic conditions. Soil characteristics of the different sites are provided above in Table 7.1.2.2.1-1 to Table 7.1.2.2.1-3, a summary of the climatic conditions can be found in Table 7.1.2.2.1-5 and Table 7.1.2.2.1-6.

Kinetic modeling strategy

The appropriate kinetic model was identified considering the procedures and kinetic models proposed by the FOCUS workgroup on degradation kinetics [*FOCUS (2006)*]. In a first step, a non-normalized kinetic evaluation was performed in order to derive best-fit field degradation parameters for pyrimethanil (trigger endpoints). In a second step, a kinetic evaluation was performed to derive degradation parameters that can be used as modeling endpoints. Prior to kinetic evaluation, the sampling intervals of the field studies were normalized to reference conditions (20°C, pF2) regarding soil moisture and temperature according to the time-step normalization technique. Kinetic evaluation was performed on the normalized sampling interval dataset.

Data handling and software for kinetic evaluation

Replicate measurements were used in the parameter estimation. The experimental data were derived from the study report and adjusted according to FOCUS [*FOCUS (2006)*].

As surface processes had been excluded by covering the soil with sand in the field study, all data points were considered in this evaluation regardless of the 10 mm rain criterion described in an EFSA guidance document [*EFSA (2014) EFSA Guidance Document for evaluating laboratory and field dissipation studies to obtain DegT50 values of active substances of plant protection products and transformation products of these active substances in soil. EFSA Journal 2014;12(5):3662,37 pp*].

The software package KinGUI version 2.2012.320.1629 was used for parameter fitting [*SCHÄFER et al. (2007)*]. The error tolerance and the number of iterations of the optimization tool (IRLS) were set to the default values of 1×10^{-6} and 100, respectively.

Datasets were prepared for kinetic evaluation as follows:

- Values below the quantification or detection limit were treated as recommended by the FOCUS workgroup [*FOCUS (2006)*]. The LOQ for pyrimethanil reported in the study was 0.01 mg kg^{-1} [see KCA 7.1.2.2.1/1 2014/1000661]. Values below LOQ were set to 0.5 × LOQ = 0.005 mg kg^{-1}.
- For each sampling point, the concentration of a compound in the single soil layer given in mg kg^{-1} was transformed to its residue given in g ha^{-1} considering the thickness of the respective segment and undisturbed soil bulk densities for each soil layer. The total residues in the sampled soil core were calculated as the sum of residues of the single soil core segments.

The measured data as well as resulting datasets submitted to kinetic analysis are provided in the original evaluation report.

Normalization procedure

Evaluation of the suitability of field dissipation data for normalization was performed according to the evaluation criteria for normalization compiled by the Dutch regulatory authority (CTB criteria) [*CTB (1999)*].

Temperature correction factors (f_{temp}) were determined to account for differences between actual daily soil temperatures as calculated by FOCUS-PEARL 4.4.4 and a reference temperature of 20°C using the Q_{10} approach as described in the report of the FOCUS soil modeling working group [see *FOCUS (1997)*]. The Q_{10} response function was applied for temperatures above 0°C (see Equation 7.1.2.2.1-1 c above). Below field temperatures of 0°C it was assumed that no degradation occurs (Equation 7.1.2.2.1-1 c). For the evaluation, the EFSA opinion on the default Q_{10} value [see *EFSA (2007)*] was followed and a Q_{10} value of 2.58 was included in the assessment.

Moisture correction factors (f_{moist}) were determined to account for differences between actual daily soil moisture as calculated by FOCUS-PEARL 4.4.4 and the reference soil moisture at field capacity (pF2) (see Equation 7.1.2.2.1-1 d).

The normalized day lengths were derived according to Equation 7.1.2.2.1-1 a. For DAT 0, no normalization was considered and application was assumed to occur at the time point zero. Normalized sampling days (DAT_{norm}) after application were calculated by cumulatively summing up normalized day lengths as described above in Equation 7.1.2.2.1-1 b.

Table 7.1.2.2.1-26 shows the field sampling dates for the trial locations and the normalized (20°C, pF2) day lengths based on soil moisture and soil temperature data as simulated by FOCUS-PEARL 4.4.4.

Table 7.1.2.2.1-26: Time-step normalized (temperature and moisture) sampling days

L120562 (France North)		L120563 (Germany)		L120564 (France South)	
days after treatment	D_{norm} [d]	days after treatment	D_{norm} [d]	days after treatment	D_{norm} [d]
0	0	0	0	0	0
3	3.1	3	2.7	3	2.8
7	7.0	8	6.6	6	6.8
15	14.5	14	11.7	12	13.9
30	29.7	33	26.1	27	28.2
63	52.0	61	51.5	60	71.1
92	65.0	85	67.3	95	108.9
134	75.9	132	86.9	140	136.7
184	83.9	175	97.1	180	149.5
238	91.8	253	111.0	245	167.1
304	117.9	294	119.5	293	183.1
358	158.6	356	150.9	368	229.8
		485	247.1	489	359.5
L120565 (Italy)		**L120566 (Spain)**			
days after treatment	D_{norm} [d]	days after treatment	D_{norm} [d]		
0	0	0	0		
3	6.4	3	2.8		
6	12.4	8	8.6		
13	24.8	15	20.3		
31	53.9	30	45.6		
60	107.3	55	99.8		
90	140.2	90	182.8		
137	170.3	139	252.7		
173	180.0	183	283.2		
308	222.1	246	316.4		
370	282.2	309	357.7		
480	417.6	351	403.2		
		483	670.9		

II. RESULTS AND DISCUSSION

The derived non-normalized degradation endpoints (best-fit) of pyrimethanil and its metabolite 2-amino-4,6-dimethyl-pyrimidine (M605F007) are summarized in Table 7.1.2.2.1-27 and Table 7.1.2.2.1-28.

Table 7.1.2.2.1-27: Summary of best-fit endpoints of pyrimethanil derived from European field dissipation trials (2012-2013)

Field trial	Soil type (USDA)	Best-fit kinetic model	χ^2 error [%]	Estimated parameters	$DegT_{50}$ [d]	$DegT_{90}$ [d]
L120562	Silt loam	FOMC	15.3	α: 2.074 β: 45.20 d^{-1}	18.0	92.4
L120563	Silt loam	SFO	19.4	k: 0.0388 d^{-1}	17.9	59.4
L120564	Sandy loam	DFOP	4.6	k1: 0.02876 d^{-1} k2: 0.00556 d^{-1} g: 0.7358	33.5	181.9
L120565	Sandy loam	FOMC	10.6	α: 1.5890 β: 80.4778 d^{-1}	44.0	262.3
L120566	Sand	DFOP	5.0	k1: 0.2859 d^{-1} k2: 0.0121 d^{-1} g: 0.3858	17.5	150.4

Table 7.1.2.2.1-28: Summary of best-fit endpoints of metabolite M605F007 derived from European field dissipation trials (2012-2013)

Field trial	Soil type (USDA)	Best-fit kinetic model	χ^2 error [%]	Estimated parameters	Formation fraction	$DegT_{50}$ [d]	$DegT_{90}$ [d]
L120562	Silt loam	SFO [a]	13.4	k: 0.0077 d^{-1}	0.064	90.2	299.5
L120563	Silt loam	SFO [b]	20.0	k: 0.0247 d^{-1}	-	28.1	93.2
L120564	Sandy loam	SFO [b]	15.9	k: 0.0049 d^{-1}	-	142.9	474.7
L120565	Sandy loam	No reliable endpoints					
L120566	Sand	M605F007 detected at one sampling date only					

[a] Parent FOMC
[b] Decline fit

The degradation behavior of pyrimethanil and its metabolite M605F007 in the five field trials was analyzed in a step-wise approach to derive modeling endpoints. The summarized results of the kinetic evaluation are presented in Table 7.1.2.2.1-29 and Table 7.1.2.2.1-30.

Table 7.1.2.2.1-29: Summary of modeling endpoints of pyrimethanil derived from European field dissipation trials (2012-2013)

Field trial	Soil type (USDA)	Kinetic model	χ^2 error [%]	Estimated parameters	DegT$_{50}$ [d] [a]
L120562	Silt loam	SFO	14.8	k: 0.0368 d^{-1}	18.8
L120563	Silt loam	SFO	19.3	k: 0.0477 d^{-1}	14.5
L120564	Sandy loam	SFO	5.5	k: 0.0158 d^{-1}	44.0
L120565	Sandy loam	SFO	10.4	k: 0.0093 d^{-1}	74.9
L120566	Sand	DFOP	8.4	k1:0.3070 d^{-1} k2:0.0070 d^{-1} g: 0.3983	77.5 [b]
				Geometric mean	**37.0**

[a] Reference conditions 20°C, pF2 (time-step normalized)
[b] Calculated as DT$_{50}$ = DT$_{90}$ / 3.32 (DT$_{90}$ is reached within the study period)

Table 7.1.2.2.1-30: Summary of modeling endpoints of metabolite M605F007 derived from European field dissipation trials (2012-2013)

Field trial	Soil type (USDA)	Kinetic model	χ^2 error [%]	Estimated parameters	Formation fraction	DegT$_{50}$ [d] [a]
L120562	Silt loam	SFO	21.9	k: 0.0200 d^{-1}	0.079	34.6
L120563	Silt loam	SFO [b]	13.3	k: 0.0445 d^{-1}	-	15.6
L120564	Sandy loam	SFO [b]	9.6	k: 0.0144 d^{-1}	-	48.2
L120565	Sandy loam	No reliable endpoint				
L120566	Sand	M605F007 detected at one sampling date only				
					Geometric mean	**29.6**

[a] Reference conditions 20°C, pF2 (time-step normalized)
[b] Derived from SFO decline fit for the metabolite

III. CONCLUSION

Kinetic evaluation of five field trials with pyrimethanil, originating from one field dissipation study, was conducted in order to derive reliable non-normalized best-fit and normalized modeling endpoints according to the current guidance of the FOCUS workgroup on degradation kinetics.

Non-normalized degradation endpoints for pyrimethanil based on best-fit models were derived from biphasic (four field trials) as well as from SFO (one field trial) kinetics and ranged from 17.5 to 44.0 days ($DegT_{50}$) and from 59.4 to 262.3 days ($DegT_{90}$).

Non-normalized endpoints for the metabolite M605F007 derived from SFO (three field trials) ranged from 28.1 to 142.9 days ($DegT_{50}$) and from 93.2 to 474.7 days ($DegT_{90}$). For two field trials, no best-fit endpoints for M605F007 could be derived.

Modeling endpoints for pyrimethanil could be derived from SFO (four field trials) as well as from DFOP (one field trial) kinetics. The normalized modeling $DegT_{50}$ values ranged from 14.5 to 77.5 days.

For the metabolite 2-amino-4,6-dimethyl-pyrimidine (M605F007), normalized modeling $DegT_{50}$ values were derived from SFO (three field trials) kinetics and ranged from 15.6 to 48.2 days. For two field trials, no modeling endpoints for M605F007 could be derived.

Summary of degradation endpoints for pyrimethanil and its metabolite M605F007 in terrestrial field dissipation studies

Table 7.1.2.2.1-31: Summary of best-fit field half-lives of pyrimethanil obtained in terrestrial field dissipation studies

BASF DocID	Trial / Location	Soil type	pH	Org. C [%]	Best-fit DT_{50} / DT_{90} [d]	Kinetic model	χ^2 error
A81908 [e], 2013/1278722	Goch-Nierswalde, Germany (1991)	Sandy loam	6.2 [a]	1.45 [c]	- [d]	-	-
	Weeze-Wemb, Germany (1991)	Loam	5.5 [a]	1.04 [c]	- [d]	-	-
	Emmerich-Elten, Germany (1991)	Humus sand	6.9 [a]	0.87 [c]	- [d]	-	-
	Schwichteler, Germany (1991)	Sandy silt	5.8 [a]	1.62 [c]	29.6 / 264.7 [e]	FOMC	34.5
A81928 [e], 2013/1278722	Goch, Germany (1992)	Silty loam	5.9 [a]	2.03 [c]	53.6 / 178.1 [e]	SFO	24.6
	Meißner-Vockerode, Germany (1992)	Loam	6.4 [a]	1.22 [c]	33.9 / 112.5 [e]	SFO	18.3
	Schwichteler, Germany (1992)	Humus sandy loam	6.3 [a]	3.65 [c]	39.3 / 130.5 [e]	SFO	32.2
	Elsenfeld-Rück, Germany (1992)	Silty loam	7.0 [a]	1.22 [c]	25.2 / 83.7 [e]	SFO	15.6
2014/1000661 [f], 2014/1000843	Schaeffersheim, France (2012)	Silt loam	7.2 [b]	2.16	18.0 / 92.4 [f]	FOMC	15.3
	Goch-Nierswalde, Germany (2012)	Silt loam	6.6 [b]	1.74	17.9 / 59.4 [f]	SFO	19.4
	Barry-d'Islemade, France (2012)	Sandy loam	5.2 [b]	0.61	33.5 / 181.9 [f]	DFOP	4.6
	Dugliolo, Italy (2012)	Sandy loam	7.5 [b]	2.94	44.0 / 262.3 [f]	FOMC	10.6
	Utrera, Spain (2012)	Sand	7.0 [b]	0.39	17.5 / 150.4 [f]	DFOP	5.0

[a] measured in KCl
[b] measured in CaCl2
[c] calculated from organic matter (OM) content by OC=OM/1.724
[d] no adequate kinetic model derived
[e] field dissipation study including surface loss processes; best-fit endpoints serve as trigger endpoints for additional work
[f] covered field dissipation study; only to derive modeling endpoints in the soil matrix, excluding surface loss processes [EFSA, 2010]; best-fit endpoints should not be used as triggers for additional work

Table 7.1.2.2.1-32: Summary of normalized field half-lives of pyrimethanil suitable for modeling

BASF DocID	Trial / Location	Soil type	pH	Org. C [%]	$DegT_{50}$ (20°C, pF2) [d]	Kinetic model	χ^2 error
A81908, 2013/1278722	Goch-Nierswalde, Germany (1991)	Sandy loam	6.2 [a]	1.45 [c]	- [d]	-	-
	Weeze-Wemb, Germany	Loam	5.5 [a]	1.04 [c]	- [e]	-	-
	Emmerich-Elten, Germany	Humus sand	6.9 [a]	0.87 [c]	- [d]	-	-
	Schwichteler, Germany (1991)	Sandy silt	5.8 [a]	1.62 [c]	- [e]	-	-
A81928, 2013/1278722	Goch, Germany (1992)	Silty loam	5.9 [a]	2.03 [c]	- [d]	-	-
	Meißner-Vockerode, Germany	Loam	6.4 [a]	1.22 [c]	19.9	SFO	19.6
	Schwichteler, Germany (1992)	Humus sandy loam	6.3 [a]	3.65 [c]	21.8	SFO	30.0
	Elsenfeld-Rück, Germany	Silty loam	7.0 [a]	1.22 [c]	- [d]	-	-
2014/1000661 [g], 2014/1000843	Schaeffersheim, France	Silt loam	7.2 [b]	2.16	18.8	SFO	14.8
	Goch-Nierswalde, Germany	Silt loam	6.6 [b]	1.74	14.5	SFO	19.3
	Barry-d'Islemade, France	Sandy loam	5.2 [b]	0.61	44.0	SFO	5.5
	Dugliolo, Italy	Sandy loam	7.5 [b]	2.94	74.9	SFO	10.4
	Utrera, Spain	Sand	7.0 [b]	0.39	77.5 [f]	DFOP	8.4
Geometric mean					**31.4**		

[a] measured in KCl
[b] measured in CaCl2
[c] calculated from organic matter (OM) content by OC=OM/1.724
[d] less than five data points after 10 mm rain
[e] no adequate kinetic model derived
[f] calculated as $DT_{50} = DT_{90}/3.32$ since DT_{90} was reached with the study period
[g] covered field dissipation study, excluding surface loss processes [EFSA, 2010]

Table 7.1.2.2.1-33: Summary of best-fit field half-lives of pyrimethanil metabolite M605F007 obtained in terrestrial field dissipation studies

BASF DocID	Trial / Location	Soil type	pH	Org. C [%]	Best-fit DT_{50} / DT_{90} [d]	FF [-]	Kinetic model	χ^2 error
2014/1000661 [h], 2014/1000843	Schaeffersheim, France	Silt loam	7.2 [b]	2.16	90.2 / 299.5 [d]	0.064	SFO	13.4
	Goch-Nierswalde, Germany	Silt loam	6.6 [b]	1.74	28.1 / 93.2 [e]	- [e]	SFO	20.0
	Barry-d'Islemade, France	Sandy loam	5.2 [b]	0.61	142.9 / 474.7 [e]	- [e]	SFO	15.9
	Dugliolo, Italy	Sandy loam	7.5 [b]	2.94	- [f]	-	-	-
	Utrera, Spain	Sand	7.0 [b]	0.39	- [g]	-	-	-

[a] measured in KCl
[b] measured in CaCl2
[c] calculated from organic matter (OM) content by OC=OM/1.724
[d] Fit in a linked model, parent kinetic FOMC
[e] Fit of decline from maximum
[f] no adequate kinetic model derived
[g] metabolite detected at one sampling date only
[h] covered field dissipation study, excluding surface loss processes [EFSA, 2010]

Table 7.1.2.2.1-34: Summary of normalized field half-lives of pyrimethanil metabolite M605F007 suitable for modeling

BASF DocID	Trial / Location	Soil type	pH	Org. C [%]	$DegT_{50}$ (20°C, pF2) [d]	FF [-]	Kinetic model	χ^2 error
2014/1000661 [h], 2014/1000843	Schaeffersheim, France	Silt loam	7.2 [b]	2.16	34.6 [d]	0.079	SFO	21.9
	Goch-Nierswalde, Germany	Silt loam	6.6 [b]	1.74	15.6 [e]	-	SFO	13.3
	Barry-d'Islemade, France	Sandy loam	5.2 [b]	0.61	48.2 [e]	-	SFO	9.6
	Dugliolo, Italy	Sandy loam	7.5 [b]	2.94	- [f]	-	-	-
	Utrera, Spain	Sand	7.0 [b]	0.39	- [g]	-	-	-
Geometric mean					**29.6**			
Arithmethic mean						**0.079**		

[a] measured in KCl
[b] measured in CaCl2
[c] calculated from organic matter (OM) content by OC=OM/1.724
[d] Fit in a linked model, parent kinetic SFO
[e] Fit of decline from maximum
[f] no adequate kinetic model derived
[g] metabolite detected at one sampling date only
[h] covered field dissipation study, excluding surface loss processes [EFSA, 2010]

CA 7.1.2.2.2 Soil accumulation studies

No experimental data available. Since all best-fit pyrimethanil DT_{90} values from the field soil dissipation study used for derivation of trigger endpoints are < 1 year (see Table 7.1.2.2.1-31), accumulation data are not needed for the active substance.

CA 7.1.3 Adsorption and desorption in soil

CA 7.1.3.1 Adsorption and desorption

CA 7.1.3.1.1 Adsorption and desorption of the active substance

No new studies on adsorption/desorption behavior of the active substance pyrimethanil were performed. The two studies as peer-reviewed during the previous Annex I listing process are considered to be still valid. The adsorption values as listed in the DAR (April 2004) and the EFSA Scientific Report (2006) are shown in Table 7.1.3.1.1-1.

Already peer-reviewed studies on the adsorption and desorption of pyrimethanil:

Forster V. 1992, BASF DocID A81886, IIA 7.1.2/1;
Sadowsky-Dunkmann I. 1994, BASF DocID A81931, IIA 7.1.2/2

Table 7.1.3.1.1-1: Adsorption of pyrimethanil on different soils (DAR April 2004)

Soil	Soil type	org. C [%]	pH [-]	K_f [mL g^{-1}]	K_{foc} [mL g^{-1}]	1/n [-]
Lufa 2.1	sand	0.75	6.1	3.75	500	0.90
Lufa 2.2	loamy sand	2.45	7.1	7.83	320	0.87
Lufa 2.3	sandy loam	0.95	5.9	2.51	265	0.86
Schering soil 178	sandy loam	3.5	7.5	2.69	75	0.85
Schering soil 181	sandy clay loam	0.9	7.6	3.08	345	0.86
Schering soil 183	loamy sand	1.32	7.8	3.95	299	0.857
Schering soil 184	sand	3.12	4.8	23.44	751	0.833
Schering soil 185	loamy sand	3.23	4.4	17.53	543	0.841
Schering soil 192	sandy loam	1.56	4.6	10.65	682	0.907

CA 7.1.3.1.2 Adsorption and desorption of metabolites, breakdown and reaction products

The adsorption/desorption behavior of the pyrimethanil metabolite M605F007 (AE F132593, 2-amino-4,6-dimethylpyrimidine) was already evaluated during the previous Annex I listing process. This study is considered still valid, and the adsorption values as given in the DAR (April 2004) and the EFSA Scientific Report (2006) are presented in Table 7.1.3.1.2-1.

Already peer-reviewed studies on the adsorption and desorption of pyrimethanil metabolite M605F007 (AE F132593):

Sadowsky-Dunkmann I., Eyrich U. 1994, BASF DocID A81935, IIA 7.1.2/3

Table 7.1.3.1.2-1: Adsorption of metabolite M605F007 (AE F132593) on different soils as listed in the DAR (April 2004)

Soil	Soil type	org. C [%]	pH [-]	K_f [mL g^{-1}]	K_{foc} [mL g^{-1}]	1/n [-]
Schering soil 178	loam	3.5	7.6	1.93	56	0.784
Schering soil 181	clay loam	0.9	7.5	2.16	240	0.819
Schering soil 183	loamy sand	1.32	7.8	1.24	94	0.806
Schering soil 184	sand	3.12	4.8	5.20	167	0.772
Schering soil 185	loamy sand	3.23	4.4	3.46	107	0.795
Schering soil 192	sandy loam	1.56	4.6	3.12	197	0.696

CA 7.1.3.2 Aged sorption

No experimental data are available. They are not considered necessary for leaching assessment of pyrimethanil. PEC_{gw} calculations were performed with the regular Freundlich sorption coefficients as presented in chapter CA 7.1.3.1.

CA 7.1.4 Mobility in soil

CA 7.1.4.1 Column leaching studies

CA 7.1.4.1.1 Column leaching of the active substance

No new experimental data are available. The column leaching studies already peer-reviewed during the previous Annex I inclusion process are considered still valid (*Förtsch A. (1991), BASF DocID A81875, Zumdick A. (1993), BASF DocID A81902*).

Pyrimethanil (phenyl ring labelled) showed no tendency of leaching through soil columns (4 soils tested). The total amounts of radioactivity found in leachates were always ≤ 1.5% AR.

In a second study, where pyrimethanil was applied formulated as SCALA (3 soils tested), the parent compound in the leachates amounted only to 0.1 - 0.2% of applied substance.

CA 7.1.4.1.2 Column leaching of metabolites, breakdown and reaction products

No new experimental data are available. The aged soil column leaching study already peer-reviewed during the previous Annex I inclusion process is considered still valid (*Sadowsky-Dunkmann I., Eyrich U. (1994), BASF DocID A81935*). The total amount of radioactivity in the leachate was ~3.6% AR after 5 days of irrigation with 235 - 270 mL per day. Only a small part of the radioactivity in the leachate was identified as pyrimethanil (0.27% AR) and metabolite AE F132593 (0.19% AR).

CA 7.1.4.2 Lysimeter studies

No new experimental data are available. The soil mobility of pyrimethanil and its metabolites was evaluated during the previous Annex I inclusion process in a lysimeter study performed in Neustadt, RP, Germany with two soil cores (*Kubiak R. (1995), BASF DocID A84664*). Each lysimeter was planted with one vine plant.

Three applications were made to each lysimeter between July 11 and Aug. 20, 1991, and these applications were repeated on one lysimeter core in the following year (1992). The actual amounts applied were between 1.4 and 1.7 kg active substance/ha/year.

Pyrimethanil or metabolite AE F132593 were never detected in any of the leachate samples. The yearly average concentrations for the lysimeter treated only in the first year were 0.26 - 0.50 µg/L ai equivalents, and for the lysimeter treated in two consecutive years 0.52 - 0.85 µg/L ai equivalents. No individual compounds were found in the leachates at any time. The radioactivity in the leachates consisted of CO_2, non-extractables (from the water phase) and unknown polars.

CA 7.1.4.3 Field leaching studies

No field leaching study was performed. The leaching assessment in the current renewal dossier is based on PEC_{gw} calculations performed with updated endpoints according to the newest guidelines and guidance documents.

CA 7.2 Fate and behaviour in water and sediment

CA 7.2.1 Route and rate of degradation in aquatic systems (chemical and photochemical degradation)

CA 7.2.1.1 Hydrolytic degradation

No new data on hydrolytic degradation of pyrimethanil were produced. The already peer-reviewed study of Tschampel, M. 1989 (BASF DocID A81864) is considered still valid.

Pyrimethanil was hydrolytically stable at 50°C at pH 4, 5, 7, and 9 over 120 hours (5 days) and at 22°C at pH 5, 7, and 9 over 31 (36) days.

CA 7.2.1.2 Direct photochemical degradation

No new data on direct photochemical degradation of pyrimethanil are submitted. The already peer-reviewed studies of the previous dossier are still considered sufficient to address this Annex point.

Already peer-reviewed studies on the direct photochemical degradation of pyrimethanil:

Tschampel M. 1992,	BASF DocID A81887,	IIA 7.2.1.1/2
Tschampel M. 2000,	BASF DocID C006829,	IIA 7.2.1.1/3
Mamouni A. 2001,	BASF DocID C014860,	IIA 7.2.1.1/5

In the study of Tschampel, M. 1992 (BASF DocID A81887) and its addendum Tschampel, M. 2000 (BASF DocID C006829), non-labelled pyrimethanil was used for incubation under photolytic conditions at 30°C, and metabolite quantification was done with HPLC/UV assuming the same absorption values for metabolites as for parent.

In this study, some slow photolysis was reported to take place at pH 7 (DT50 of ~77 days), whereas at pH 4 a rather fast photolysis was postulated. However, the observed photolysis at pH 4 had to be attributed to the use of citrate buffer where citrate acted as a photosensitizer. Overall, the HPLC chromatograms showed several peaks, but none of them exceeded 10% based on UV detection. Since material balance could not be given due to the use of non-labelled material, already for the previous EU registration a new aqueous photolysis with radio-labelled test item was initiated.

In the more recent study of Mamouni, A. 2001 (BASF DocID C014860), radiolabelled pyrimethanil was used for incubation under a Suntest apparatus in buffer solutions pH 4, 5, 7, and 9 at a temperature of 22°C. Pyrimethanil proved to be stable to direct photolysis in sterile aqueous buffer solutions at pH 5, 7, and 9. In pH 4 solution (citrate buffer), a moderately fast degradation was noted (DT50 ~6 - 7 days). However, this effect, as also observed in the earlier study, had to be attributed to citrate acting as photosensitizer.

Overall, it can be concluded that direct photolysis does not contribute to the environmental degradation of pyrimethanil in water.

CA 7.2.1.3 Indirect photochemical degradation

The results of the already peer-reviewed study of Tschampel, M. 1994 (BASF DocID A81930) showed that indirect photolysis of pyrimethanil may occur via photosensitizers present in the water (e.g. humic acids). Since non-labelled test item was used in this old study, no reliable information could be obtained on photodegradates, although the parent had declined to ~25% of the initial concentration after 4 days of continuous irradiation.

Therefore, a new study on indirect photolysis of pyrimethanil was initiated using 14C-labelled test item.

New, not yet peer-reviewed study:

Report:	CA 7.2.1.3/1 Hassink J.,Kretschmar G., 2015b Photolysis of Pyrimethanil in sterile natural water 2015/1106125
Guidelines:	FAO Revised Guidelines on Environmental Criteria for the Registration of Pesticides Revision 3 (28 August 1993), JMAFF No 12 Nosan No 8147
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Report:	CA 7.2.1.3/2 Hassink J., 2016 a Report Amendment No. 1: Photolysis of Pyrimethanil in sterile natural water 2015/1253633
Guidelines:	FAO Revised Guidelines on Environmental Criteria for the Registration of Pesticides Revision 3 (28 August 1993), JMAFF No 12 Nosan No 8147
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

Photolysis of pyrimethanil (BAS 605 F) in sterile natural water was investigated with [phenyl-U-14C]-labeled and [pyrimidinyl-2-14C]-labeled pyrimethanil over a testing period of 15 days with continuous irradiation. For both labels, the photolysis experiments showed recoveries in the range of 91.7 to 100.6% TAR. For the dark control experiments, the recoveries ranged from 97.0 to 101.1% TAR.

In the photolysis experiment with phenyl-labeled pyrimethanil, the concentration of the test item declined from 100.0% at day 0 to 39.3% TAR at the end of the study after 15 days. During the study, numerous small HPLC peaks were detected ranging over the entire measuring time and were for each individual unknown degradation product < 4.1% TAR. Mineralization to CO2 reached about 7% TAR.

In the photolysis experiment with pyrimidinyl-labeled pyrimethanil, the measured values for the test item declined from 100.0% at day 0 to 49.4% TAR at the end of the study after 15 days. As with the phenyl-label, numerous small HPLC peaks were detected ranging over the entire measuring time, however, none of the individual unknown degradation product ever exceeded 3.0% TAR. Mineralization to CO2 was rather low reaching only about 1% TAR.

In dark control samples no degradation of the test item occurred.
Under irradiated conditions the calculated DT50 and the DT90 values were 11.5 and 38.3 days for phenyl-labeled pyrimethanil, respectively. For the pyrimidinyl-label, a DT50 of 16.3 days and a DT90 54.0 days were derived.

It can be concluded that indirect irradiation via photosensitizers present in natural water has a significant effect on the degradation of pyrimethanil in aqueous systems, however, no distinct degradation product occurred in amounts higher than 4.1% of the applied radioactivity.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

The test item pyrimethanil (BAS 605 F) was used in two 14C-labeled forms.

Internal code: BAS 605 F
Reg. No.: 236999
CAS No.: 53112-28-0
Chemical name (IUPAC): N-(4,6-dimethylpyrimidin-2-yl)aniline
Molecular mass: 199.26 g mol-1
Molecular formula: C12H13N3

1. Phenyl-U-14C-label
Batch No.: 1036-1011
Specific radioactivity of a.s.: 8.53 MBq mg-1
Radiochemical purity: 99.2%, see certificate of analysis
Purity: 99.4%

2. Pyrimidinyl-2-14C-label
Batch No.: 1049-1010
Specific radioactivity of a.s.: 6.42 MBq mg-1
Radiochemical purity: 99.7%, see certificate of analysis
Purity: 99.8%

Unlabeled
Batch No.: 486213
Purity: 99.8% (± 1.0%)

2. Test system

The test system was taken from a small pond named "Kleiner Waldsee" (Kastenbergheide) west of the town Schifferstadt, Rhineland Palatinate, Germany. Sterility of the water samples was achieved by sterile filtration (0.2 µm). Characteristics of the pond water are given in Table 7.2.1.3-1.

Table 7.2.1.3-1: Characteristics of the pond water used for water photolysis of pyrimethanil

Origin	Kleiner Waldsee, Kastenbergheide near Schifferstadt, RP, Germany
Sampling date	07.10.2013
pH*	7.89 a
O2 content (22°C) [mg L-1]	8.7 b
Total organic carbon [mg L-1]	18
Nitrate [mg L-1]	< 1
Conductivity at 25°C [µS cm-1]	309
Suspended particles [mg L-1]	11
Evaporation residues (105°C) [mg L-1]	210

a Mean values of two measurements at the beginning of the study
b pH measurements of the samples were proceeded on regular basis throughout the study period, values were in the range of 7.89 to 8.91

B. STUDY DESIGN

1. Experimental conditions

Ten glass vessels were filled with 18 mL test solution (containing 0.05 mg mL-1 test item), respectively, and were closed with a quartz glass covering. The vessels were placed into a temperature controlled stainless steel incubation block (22 ± 1°C) under a SUNTEST CPS exposure unit (Heraeus, Germany) fitted with a xenon arc light source emitting light with a spectrum similar to that of sunlight at an intensity of about 3 mW cm-2 (UVA range). This corresponds to a clear summer day in Southern Germany (about 49°N). Wavelengths < 290 nm were filtered off to simulate natural sunlight.

Each vessel was equipped with a magnetic stirrer constantly mixing gently the test water. The test vessels were equipped with an air inlet and an air outlet. The incoming air was led through a 0.5 M NaOH solution to remove CO2 and then through water to remoisten the air. By using a sterile filter, contamination through air borne germs should be prevented. Potential volatiles from the test solution (including 14CO2) were trapped in three different trapping solutions located after the air outlet: 1. NaOH (0.5 M); 2. H2SO4 (0.5 M); 3. Ethylene glycol.

The dark control samples were stored in appropriate flasks in a climatic chamber at 22 ± 1°C without irradiation. Since pyrimethanil was expected to be stable under the incubation conditions, no mineralization was measured.

2. Sampling

Samples were taken 0, 2, 5, 8, 12, and 15 days after treatment (DAT). At each sampling date, the water was analyzed by liquid scintillation counting (LSC) and radio-HPLC.

Additionally, the three trapping solutions (mineralization) were analyzed for total radioactive material by LSC.

3. Description of analytical procedures

All samples were measured for radioactivity (LSC) and were analyzed by radio-HPLC to determine a metabolite pattern. No extraction was performed, samples were analyzed directly.

II. RESULTS AND DISCUSSION

A. MATERIAL BALANCE

Total recoveries of radioactivity are summarized in Table 7.2.1.3-2 and Table 7.2.1.3-3.

For both labels, the photolysis experiments showed recoveries in the range of 91.5 to 100.6% of the total applied radioactivity (TAR). For the dark control experiments, the recoveries ranged from 97.0 to 101.1% TAR. During photolysis with phenyl-labelled pyrimethanil, radioactive volatiles were found in the NaOH-traps up to 7% after 15 days. Precipitation by BaCl2 proved that this radioactivity consisted of CO2. With the pyrimidinyl-label, mineralization was lower reaching only about 1% TAR.

Table 7.2.1.3-2: Recovery and distribution of radioactivity in natural water after treatment with phenyl-U-14C-labeled pyrimethanil (mean values) [% TAR]

Days after treatment	Irradiated					Dark control
	Water	Volatiles			Material balance	Water
		NaOH	Ethylene glycol	H2SO4		
0	100.0	n.a.	n.a.	n.a.	100.0	n.a.
2	100.4	0.22	0.0	0.02	100.6	99.5
5	99.4	0.88	0.01	0.08	100.3	101.1
8	95.2	2.82	0.02	0.66	98.0	100.2
12	89.1	6.88	0.02	0.36	96.0	100.1
15	84.6	6.89	0.02	0.25	91.5	101.0

TAR = total applied radioactivity
n.a. = not applicable

Table 7.2.1.3-3: Recovery and distribution of radioactivity in natural water after treatment with pyrimidinyl-2-14C-labeled pyrimethanil (mean values) [% TAR]

Days after treatment	Irradiated					Dark control
	Water	Volatiles			Material balance	Water
		NaOH	Ethylene glycol	H2SO4		
0	100.0	n.a.	n.a.	n.a.	100.0	n.a.
2	98.9	0.01	0.00	0.00	98.9	99.7
5	98.2	0.12	0.00	0.07	98.3	99.6
8	97.5	0.37	0.00	0.07	97.9	98.0
12	97.4	0.84	0.00	0.04	98.3	97.0
15	98.7	1.23	0.00	0.10	99.9	97.0

TAR = total applied radioactivity
n.a. = not applicable

B. TRANSFORMATION OF PARENT COMPOUND

Results of radio-HPLC analyses are presented in Table 7.2.1.3-4 to Table 7.2.1.3-5.

The amounts of pyrimethanil in irradiated solutions decreased from 100% TAR to 39.3% TAR (phenyl label) and 49.4% TAR (pyrimidinyl label) at the end of the study after 15 days of incubation. Pyrimethanil degraded to numerous degradates, however, due to their occurrence in only very low amounts (< 4.1% TAR).

The dark control solutions revealed no significant degradation of pyrimethanil. The amount of pyrimethanil remaining in solution after 15 days was in the range 97.0 – 101.0% TAR for both labels. No degradates were detected.

Table 7.2.1.3-4: Radio-HPLC analysis of natural water samples after treatment with phenyl-U-14C-labeled pyrimethanil and incubation under irradiated and dark conditions (mean values) [% TAR]

Days after treatment	Irradiated			Dark control		
	sum of unknown a	pyrimethanil Rt = 108.0	Sum	sum of unknown	pyrimethanil Rt = 108.0	Sum
0	n.d.	100.0	100.0	n.d.	100.0	100.0
2	3.8	96.6	100.4	n.d.	99.5	99.5
5	12.0	87.4	99.4	n.d.	101.1	101.1
8	27.7	67.6	95.2	n.d.	100.2	100.2
12	41.2	47.9	89.1	n.d.	100.1	100.1
15	45.3	39.3	84.6	n.d.	101.0	101.0

TAR = total applied radioactivity
Rt = retention time [min]
n.d. = not detected
a each unknown compound < 4.1% TAR (total applied radioactivity)

Table 7.2.1.3-5: Radio-HPLC analysis of natural water samples after treatment with pyrimidinyl-2-14C-labeled pyrimethanil and incubation under irradiated and dark conditions (mean values) [% TAR]

Days after treatment	Irradiated			Dark control		
	sum of unknown a	pyrimethanil Rt = 108.0	Sum	sum of unknown	pyrimethanil Rt = 108.0	Sum
0	n.d.	100.0	100.0	n.d.	100.0	100.0
2	n.d.	98.9	98.9	n.d.	99.7	99.7
5	13.9	84.3	98.2	n.d.	99.6	99.6
8	21.9	75.7	97.5	n.d.	98.0	98.0
12	31.6	65.8	97.4	n.d.	97.0	97.0
15	49.2	49.4	98.7	n.d.	97.0	97.0

TAR = total applied radioactivity
Rt = retention time [min]
n.d. = not detected
a each unknown compound < 3.0% TAR (total applied radioactivity)

C. KINETIC MODELING RESULTS

The SFO fit was selected as the best-fit model for derivation of pyrimethanil DT50 and DT90 values for the natural water photolysis (Table 7.2.1.3-6). In the dark control, no degradation of the test item was observed.

Table 7.2.1.3-6: DT50 and DT90 values for pyrimethanil in aqueous photolysis in sterile natural water

Treatment	Label	DT50 [d]	DT90 [d]	Kinetic model	χ2 error
irradiated	pyrimidinyl	16.3	54.0	SFO	3.482
irradiated	phenyl	11.5	38.3	SFO	5.511

III. CONCLUSION

Photolysis of pyrimethanil (BAS 605 F) in sterile natural pond water showed a rather rapid degradation of the test item within 15 days. With both tested radiolabels numerous photolysis products were formed, however, none of them ever exceeded 4.1 % TAR at any sampling time. Mineralization with the phenyl label of about 7% TAR within 15 days indicates that the phenyl ring can be opened and further degraded to CO2. The calculated half-lives under continuous irradiation were 11.5 days and 16.3 days for the phenyl- and pyrimidinyl-label, respectively.

This study shows that although pyrimethanil is not susceptible to direct photolysis, indirect photolysis in natural water can lead to a quick breakdown so that persistence in natural surface waters is very unlikely.

CA 7.2.2 Route and rate of biological degradation in aquatic systems

CA 7.2.2.1 "Ready biodegradability"

The already peer-reviewed study of Jenkins, W.R. 1992 (BASF DocID A81871) is still considered valid. Pyrimethanil proved to be not readily biodegradable in a Modified Sturm Test according to OECD 301D. The degradation of pyrimethanil was only 1% ThCO2 after 28 days.

CA 7.2.2.2 Aerobic mineralisation in surface water

New, not yet peer-reviewed study:

Report:	CA 7.2.2.2/1 Yeomans P., 2015a (14C)-Pyrimethanil (BAS 605 F): Aerobic mineralisation in surface water 2014/1000623
Guidelines:	OECD 309 (April 2004)
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

The purpose of this study was to determine the mineralization and degradation rates of pyrimethanil in an aquatic system under dark conditions. The pelagic test system was chosen for this study.

The test was performed at two different pyrimethanil concentrations (10 µg L-1 and 100 µg L-1) using two differently 14C-labeled test items (pyrimidinyl and phenyl label), respectively. Sterile samples were also incubated for each label of the higher concentration. The test vessels were attached to a flow-through system for continuous aeration and incubated at a temperature of 20 ± 2°C in the dark. Samples for the experiment were taken at 0, 3, 7, 14, 21, 35 and 59 days after treatment.

The amount and nature of radioactivity in the water samples was determined by liquid scintillation counting (LSC) and radio-HPLC. Volatiles were trapped in 2 M sodium hydroxide and also analyzed by LSC. Parent substance identification was done by co-chromatography with the corresponding reference items on HPLC and TLC. TLC also confirmed the quantification of parent substance.

From the obtained results it can be concluded that pyrimethanil is not significantly degraded in the natural water environment provided in this test. For the pyrimidinyl label, 93.4 to 95.9% of the total applied radioactivity (TAR) was recovered from the biotic samples as the unchanged active substance after 59 days. With the phenyl label, 95.3 to 96.6% TAR was recovered as the unchanged active substance after 35 days, however, at day 59 various additional peaks were observed in the chromatograms of the high concentration and one replicate of the low concentration. After further characterization of those peaks, the results suggested that the observed components are no real metabolites but rather experimental artefacts, equivalents of the parent compound or the sum of various minor components.

The known environmental metabolites (M605F007, also referred to AE F132593, Reg. No. 40603) and M605F009 (also referred to as AE F132512, Reg. No. 51589) were not detected at any time.

Radioactivity in the volatile traps did not exceed 0.4 or 2.4% TAR for the pyrimidinyl and phenyl label, respectively, indicating a low rate of mineralization.

Also in the sterilized samples degradation was low with still more than 95.8% TAR recovered as unchanged parent at the end of the study for both labels.

Overall, the compound was considered to be stable in the test systems. Degradation kinetics were not reported as no significant degradation was observed.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material

The test item pyrimethanil was used in two 14C-labeled forms.

Internal code: BAS 605 F
Reg. No.: 236999
Common name: Pyrimethanil
CAS No.: 53112-28-0
Chemical name (IUPAC): N-(4,6-dimethylpyrimidin-2-yl)aniline
Molecular mass: 199.3 g mol-1
Molecular formula: C12H13N3

1. Pyrimidinyl-2-14C-label
Batch No.: 1049-1010
Specific radioactivity of a.s.: 6.42 MBq mg-1
Radiochemical purity: 99.7%, see certificate of analysis (measurement prior to application: 99.9 – 100%)
Purity: 99.8%

2. Phenyl-U-14C-label
Batch No.: 1036-1011
Specific radioactivity of a.s.: 8.53 MBq mg-1
Radiochemical purity: 99.2%, see certificate of analysis (measurement prior to application: 99.3 – 99.9%)
Purity: 99.4%

Unlabeled
Batch No.: L83-126
Purity: 99.6%

2. Test system

Surface water and sediment were collected from The Lake at Studley Royal (Ripon, United Kingdom). The physico-chemical properties of the system are summarized in Table 7.2.2.2-1.

Prior to use the sediment and water were stored together in the dark at 4 ± 2°C with free access to air. Water was filtered through a 0.1 mm sieve and sediment was passed through a 2 mm sieve.

Table 7.2.2.2-1: Characterization of the water/sediment system

Designation Origin		Fountains Abbey The Lake, Studley Royal, Ripon, UK	
Water			
Temperature a	[°C]	6.4	
pH water a	[-]	8.73	
Redox potential (Eh) a	[mV]	189.2	
Oxygen concentration a	[mg L-1]	11.83	
Total organic carbon	[mg L-1]	4.55	
Total N	[%]	0.00938	
Total P	[mg L-1]	0.19	
Hardness (CaCO3)	[mg L-1]	146	
Sediment			
Textural class	[-]	UK Particle Size Distribution	USDA
Sand	[%]	78	79
Silt	[%]	17	16
Clay	[%]	5	5
Soil type	[-]	Sandy loam	Sandy loam
pH (H2O)	[-]	7.9	
pH (CaCl2)	[-]	7.4	
Redox potential (Eh) a	[mV]	-79.5	
Organic carbon	[%]	1.4	

a measured directly at sampling site

B. STUDY DESIGN

1. Experimental conditions

A total of 94 test vessels was prepared: 18 test vessels for each radiolabel (pyrimidinyl and phenyl) and each nominal concentration (10 and 100 µg L-1), 9 vessels for the sterile incubation (both labels; 100 µg L-1), 2 vessels as system control with radiolabeled benzoic acid and 2 vessels with benzoic acid plus treatment solvent.

The vessels were filled with about 100 mL water. Trace amounts of suspended sediment was added (10 mg/L) in order to provide a minimum source of minerals and nutrients for microbes. The test systems were still considered pelagic. Appropriate amounts of the respective application solutions prepared in acetonitrile were pipetted to the water surface to achieve a nominal test concentration of 10 µg pyrimethanil L-1 or 100 µg pyrimethanil L-1, respectively.

The systems were incubated at 20 ± 2°C in a metabolism apparatus (incubator) with a gas flow system providing a continuous flow of fresh air. Each test vessel was connected to a volatile trapping system of three gas washing bottles (one empty bottle followed by two 2 M NaOH bottles). Test vessels containing sterile water were also aerated, however, the air stream was led through sterile filters to avoid contamination of the test system by airborne germs.

Control vessels treated with 14C-benzoic acid were incubated under the same conditions as the pyrimethanil treated vessels in order to prove that the test water contained an active microbial community.

2. Sampling

Samples, including the sterile groups, were taken at 0, 3, 7, 14, 21, 35 and 59 days after treatment (DAT). For sampling, the test vessels were removed from the rigs and temperature, O2 content, pH, and redox potential of the water was measured in test vessels of one replicate.

3. Description of analytical procedures

Water
The water in the test vessels was transferred into glass jars and weighed. The test vessels were then washed (with sonication) with dichloromethane (DCM). Weighed aliquots of the water and DCM were measured directly by liquid scintillation counting (LSC).

For the high concentration samples (100 µg L-1), radio-HPLC analysis was carried out without further workup. For the low concentration samples (10 µg L-1), sub-samples of the water were partitioned with DCM (three times). The DCM was then concentrated to dryness and the samples reconstituted in acetonitrile or acetonitrile : water (1:1, v/v) prior to chromatography. Procedural recoveries were checked by LSC and were found to be 90% or higher. Where the radioactivity in water after partitioning was ≥ 5% TAR, HPLC fraction collection of unextracted water was used. The tabulated values do therefore not show any activity remaining after partitioning for these samples. Parent substance identification was done by co-chromatography with the corresponding reference items on HPLC and TLC.

Volatiles
Throughout the test, volatile traps were collected from sampled test vessels. From further incubated test vessels, the trapping solutions were also collected, but replaced with fresh solutions at 3, 7, 14, 21, 29, 35, 43 and 51 DAT. Control vessels had traps collected at the same intervals. Aliquots of the trapping solutions were measured by LSC.

The smallest identified peak was deemed to be the limit of detection/quantification (LOD/LOQ) and accounted for 0.2% of the total applied radioactivity (TAR).

4. Calculation of the degradation/dissipation rates

Degradation kinetics were not reported in a non-GLP annex as stated in the protocol because insufficient degradation was observed to allow calculation of meaningful results.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

The material balances and the distribution of radioactivity between the different workup fractions of the water samples treated with 14C-pyrimethanil (pyrimidinyl and phenyl label) is presented in Table 7.2.2.2-2 to Table 7.2.2.2-5.

The actual applied amount of test item per test vessel containing 100 mL of water was 10.3 µg (high concentration) and 1.0 µg (low concentration) for the pyrimidinyl label, and 10.1 µg (high concentration) and 1.0 µg (low concentration) for the phenyl label.

In general, no significant differences were found in pyrimethanil behavior between the high and the low test concentration. Therefore, the results expressed as % TAR in the text include those of both concentrations and both labels, if not stated otherwise.

The material balances ranged from 97.2 to 101.2% TAR in the viable test vessels, and from 97.7 to 101.9% TAR in the sterilized vessels.

At the end of the study (59 DAT) the radioactivity in the water accounted for 95.2 to 97.4% TAR for the viable test vessels and for 97.2 to 98.1% TAR for the sterilized vessels. Adsorption to the test vessel surface was negligible with only 0 – 1.4% TAR found in the rinsing solution of single test vessels.

Radioactivity in the volatile traps did not exceed 0.4 or 2.4% TAR for the pyrimidinyl and phenyl label, respectively, indicating a low rate of mineralization.

Table 7.2.2.2-2: Material balance and distribution of radioactivity after application of [pyrimidinyl-2-14C] pyrimethanil to lake water (non-sterile) [% TAR]

Sample	Days after treatment	Water	Vessel wash a	Total in NaOH Traps	Material balance
Low concentration (10 µg L-1)					
A1		97.2	0.6	n.a.	97.8
A2	0	99.5	0.9	n.a.	100.4
Mean		98.4	0.8	n.a.	99.1
A3		97.9	1.4	n.d.	99.3
A4	3	96.0	1.2	n.d.	97.2
Mean		97.0	1.3	n.d.	98.3
A5		98.3	n.d.	0.1	98.4
A6	7	98.3	n.d.	n.d.	98.3
Mean		98.3	n.d.	0.1	98.4
A7		97.0	0.8	n.d.	97.8
A8	14	99.5	0.9	n.d.	100.4
Mean		98.3	0.9	n.d.	99.1
A9		97.7	1.1	n.d.	98.8
A10	21	96.5	1.3	n.d.	97.8
Mean		97.1	1.2	n.d.	98.3
A11		93.3	1.0	0.2	94.5
A12	35	99.2	1.4	n.d.	100.6
Mean		96.3	1.2	0.1	97.6
A13		97.9	n.d.	0.5	98.4
A14	59	96.5	n.d.	0.2	96.7
Mean		97.2	n.d.	0.4	97.6
High concentration (100 µg L-1)					
B1		97.7	0.9	n.a.	98.6
B2	0	98.7	0.9	n.a.	99.6
Mean		98.2	0.9	n.a.	99.1
B3		97.2	1.1	n.d.	98.3
B4	3	96.3	1.1	n.d.	97.4
Mean		96.8	1.1	n.d.	97.9
B5		97.3	0.4	n.d.	97.7
B6	7	99.4	0.5	n.d.	99.9
Mean		98.4	0.5	n.d.	98.8
B7		96.6	0.5	n.d.	97.1
B8	14	96.8	0.5	n.d.	97.3
Mean		96.7	0.5	n.d.	97.2
B9		97.6	0.8	n.d.	98.4
B10	21	97.6	0.9	n.d.	98.5
Mean		97.6	0.9	n.d.	98.5
B11		96.8	1.2	n.d.	98.0
B15	35	96.1	1.2	n.d.	97.3
Mean		96.5	1.2	n.d.	97.7
B13		95.7	0.6	n.d.	96.3
B14	59	97.9	0.6	n.d.	98.5
Mean		96.8	0.6	n.d.	97.4

TAR = total applied radioactivity
n.a. = not applicable
n.d. = not detected (or < 0.1%)
a dichloromethane wash of the incubation vessel

Table 7.2.2.2-3: Material balance and distribution of radioactivity after application of [phenyl-U-14C] pyrimethanil to lake water (non-sterile) [% TAR]

Sample	Days after treatment	Water	Vessel wash a	Total in NaOH Traps	Material balance
Low concentration (10 µg L-1)					
D1		99.5	1.1	n.a.	100.6
D2	0	98.6	0.8	n.a.	99.4
Mean		99.1	1.0	n.a.	100.0
D3		97.9	1.0	n.d.	98.9
D4	3	99.4	1.4	0.1	100.9
Mean		98.7	1.2	0.1	99.9
D5		99.2	0.8	n.d.	100.0
D6	7	98.4	0.5	n.d.	98.9
Mean		98.8	0.7	n.d.	99.5
D7		99.7	0.7	n.d.	100.4
D8	14	97.3	1.1	n.d.	98.4
Mean		98.5	0.9	n.d.	99.4
D9		98.4	1.2	0.1	99.7
D10	21	98.4	0.8	n.d.	99.2
Mean		98.4	1.0	0.1	99.5
D11		98.8	1.5	0.2	100.5
D12	35	97.2	1.2	0.1	98.5
Mean		98.0	1.4	0.2	99.5
D13		96.0	1.2	0.9	98.1
D14	59	94.4	0.6	3.0	98.0
Mean		95.2	0.9	2.0	98.1
High concentration (100 µg L-1)					
E1		99.4	0.7	n.a.	100.1
E2	0	99.9	0.7	n.a.	100.6
Mean		99.7	0.7	n.a.	100.4
E3		100.5	1.1	0.1	101.7
E4	3	99.6	1.0	0.0	100.6
Mean		100.1	1.1	0.1	101.2
E5		99.2	0.3	0.3	99.8
E6	7	99.0	0.4	0.1	99.5
Mean		99.1	0.4	0.2	99.7
E7		100.0	0.4	0.2	100.6
E8	14	98.9	0.3	0.2	99.4
Mean		99.5	0.4	0.2	100.0
E9		98.2	0.9	0.2	99.3
E10	21	97.5	0.8	0.3	98.6
Mean		97.9	0.9	0.3	99.0
E11		98.4	1.3	0.4	100.1
E12	35	99.4	0.8	0.3	100.5
Mean		98.9	1.1	0.4	100.3
E13		97.5	0.5	3.3	101.3
E14	59	97.2	0.8	1.5	99.5
Mean		97.4	0.7	2.4	100.4

TAR = total applied radioactivity
n.a. = not applicable
n.d. = not detected (or < 0.1%)
a dichloromethane wash of the incubation vessel

Table 7.2.2.2-4: Material balance and distribution of radioactivity after application of [pyrimidinyl-2-14C] pyrimethanil to lake water (sterile) [% TAR]

Sample	Days after treatment	Water	Vessel wash a	Total in NaOH Traps	Material balance
Sterilized water (100 µg L-1)					
C1	0	98.9	0.6	n.a.	99.5
C2	3	97.4	0.7	n.d.	98.1
C3	7	98.0	0.2	n.d.	98.2
C4	14	98.4	0.3	n.d.	98.7
C5	21	99.3	0.5	n.d.	99.8
C6	35	97.3	0.7	n.d.	98.0
C7	59	97.2	0.5	n.d.	97.7

TAR = total applied radioactivity
n.a. = not applicable
n.d. = not detected (or < 0.1%)
a dichloromethane wash of the incubation vessel

Table 7.2.2.2-5: Material balance and distribution of radioactivity after application of [phenyl-U-14C] pyrimethanil to lake water (sterile) [% TAR]

Sample	Days after treatment	Water	Vessel wash a	Total in NaOH Traps	Material balance
Sterilized water (100 µg L-1)					
F1	0	100.3	0.9	n.a.	101.2
F2	3	98.3	1.1	n.d.	99.4
F3	7	98.5	0.3	n.d.	98.8
F4	14	101.6	0.3	n.d.	101.9
F5	21	99.5	0.6	n.d.	100.1
F6	35	97.9	0.7	n.d.	98.6
F7	59	98.1	0.4	n.d.	98.5

TAR = total applied radioactivity
n.a. = not applicable
n.d. = not detected (or < 0.1%)
a dichloromethane wash of the incubation vessel

B. TRANSFORMATION OF PARENT COMPOUND

The summary of radio-HPLC analysis of the water phases is presented in Table 7.2.2.2-6 to Table 7.2.2.2-9.

The HPLC chromatograms of water samples treated with the pyrimidinyl-labeled pyrimethanil predominantly showed only the unchanged parent. No metabolite above 2.6% TAR was detected in any sample.

For the test vessels treated with the phenyl-label at the low concentration, also no significant degradation of pyrimethanil was observed. However, one replicate of the 59 day sampling (sample D14) contained two components which accounted for 7.9 and 17.2% TAR, respectively. Sample D14 was frozen for 53 days prior to analysis, and since the same components were also observed in a high concentration sample (E14) that had been stored for 97 days prior to an additional analysis, the peaks have to be considered as artefacts caused by sample storage.

For the test vessels treated with the phenyl-label at the high concentration, the chromatograms of the two replicates at day 59 (E13, E14) showed also some inconsistent peak pattern. For sample E13, numerous minor components were observed, including one accounting for 8.8% TAR. However, when zooming into this peak, its shape indicates that it consists of several minor compounds. For sample E14, a single additional component accounting for 31.1% TAR was quantified and were proven as a parent equivalent by MS analysis.

Results obtained for the sterilized samples revealed similar results to the non-sterilized samples /except for the 59 DAT phenyl labeled samples).

Table 7.2.2.2-6: Metabolite overview for the water phase after application of [pyrimidinyl-2-14C] pyrimethanil to lake water (non-sterile) [% TAR]

Sample	Days After Treatment	pyrimethanil	Sum of unknowns	Largest Unknown	Unresolved Background	Aqueous phase	Total
Low concentration (10 µg L-1)							
A1		96.2	n.d.	n.d.	0.7	0.3	97.2
A2	0	99.0	n.d.	n.d.	0.1	0.4	99.5
Mean		97.6	0.0	0.0	0.4	0.3	98.4
A3		97.7	n.d.	n.d.	0.2	ND	97.9
A4	3	93.8	n.d.	n.d.	0.7	1.6	96.1
Mean		95.7	0.0	0.0	0.4	0.8	97.0
A5		97.7	n.d.	n.d.	0.6	ND	98.3
A6	7	95.9	2.1	2.1	0.3	ND	98.3
Mean		96.8	1.0	1.0	0.4	0.0	98.3
A7		94.6	n.d.	n.d.	0.5	1.9	97.1
A8	14	97.9	n.d.	n.d.	0.4	1.2	99.5
Mean		96.3	0.0	0.0	0.4	1.6	98.3
A9		94.6	n.d.	n.d.	1.4	1.7	97.7
A10	21	92.0	n.d.	n.d.	1.4	3.3	96.6
Mean		93.3	0.0	0.0	1.4	2.5	97.2
A11 a		82.5	9.6	2.6	1.3	n.a.	93.3
A12	35	92.0	2.8	1.6	0.6	3.9	99.2
Mean		87.2	6.2	2.1	0.9	1.9	96.3
A13		95.0	n.d.	n.d.	0.1	2.8	98.0
A14	59	91.9	n.d.	n.d.	1.0	3.8	96.6
Mean		93.4	0.0	0.0	0.6	3.3	97.3
High concentration (100 µg L-1)							
B1		97.3	n.d.	n.d.	0.4	-	97.7
B2	0	98.2	n.d.	n.d.	0.5	-	98.7
Mean		97.7	n.d.	n.d.	0.5	-	98.2
B3		96.9	n.d.	n.d.	0.3	-	97.2
B4	3	96.1	n.d.	n.d.	0.2	-	96.3
Mean		96.5	n.d.	n.d.	0.2	-	96.8
B5		97.1	n.d.	n.d.	0.2	-	97.3
B6	7	99.3	n.d.	n.d.	0.1	-	99.4
Mean		98.2	n.d.	n.d.	0.2	-	98.4
B7		95.5	n.d.	n.d.	1.1	-	96.6
B8	14	96.5	n.d.	n.d.	0.3	-	96.8
Mean		96.0	n.d.	n.d.	0.7	-	96.7
B9		96.0	n.d.	n.d.	1.6	-	97.6
B10	21	96.8	n.d.	n.d.	0.8	-	97.6
Mean		96.4	n.d.	n.d.	1.2	-	97.6
B11		96.7	n.d.	n.d.	0.1	-	96.8
B12	35	91.7	4.2	2.1	0.2	-	96.1
Mean		94.2	2.1	1.0	0.2	-	96.5
B13		94.9	n.d.	n.d.	0.8	-	95.7
B14	59	96.9	n.d.	n.d.	1.0	-	97.9
Mean		95.9	n.d.	n.d.	0.9	-	96.8

TAR = total applied radioactivity
n.d. = not detected (or < 0.1%)
a Analyzed using fraction collection of unextracted water. % TAR in aqueous phase therefore shown as n.a.

Table 7.2.2.2-7: Metabolite overview for the water phase after application of [pyrimidinyl-2-14C] pyrimethanil to lake water (non-sterile) [% TAR]

Sample	Days After Treatment	pyrimethanil	Sum of unknowns	Largest Unknown	Unresolved Background	Aqueous phase	Total
Low concentration (10 µg L-1)							
D1		98.6	n.d.	n.d.	0.5	0.4	99.5
D2	0	97.9	n.d.	n.d.	0.3	0.4	98.6
Mean		98.3	0.0	0.0	0.4	0.4	99.1
D3		97.5	n.d.	n.d.	0.4	n.d.	97.9
D4	3	99.3	n.d.	n.d.	0.1	n.d.	99.4
Mean		98.4	0.0	0.0	0.3	0.0	98.7
D5		97.7	n.d.	n.d.	0.5	1.0	99.2
D6	7	97.9	n.d.	n.d.	0.5	n.d.	98.4
Mean		97.8	0.0	0.0	0.5	0.5	98.8
D7		97.9	n.d.	n.d.	0.5	1.3	99.7
D8	14	93.7	n.d.	n.d.	1.8	1.8	97.3
Mean		95.8	0.0	0.0	1.2	1.5	98.5
D9		95.8	n.d.	n.d.	1.4	1.3	98.4
D10	21	96.9	n.d.	n.d.	0.3	1.2	98.4
Mean		96.3	0.0	0.0	0.9	1.3	98.4
D11		96.0	n.d.	n.d.	0.6	2.3	98.8
D12	35	94.6	n.d.	n.d.	0.3	2.3	97.3
Mean		95.3	0.0	0.0	0.4	2.3	98.0
D13 a		90.1	4.6	1.1	1.3	NA	96.0
D14 a	59	58.0	35.0 b	17.2	1.5	NA	94.4
Mean		74.0	19.8	9.1	1.4	0.0	95.2
High concentration (100 µg L-1)							
E1		98.9	n.d.	n.d.	0.5	-	99.4
E2	0	98.8	n.d.	n.d.	1.1	-	99.9
Mean		98.9	0.0	0.0	0.8	-	99.7
E3		100.2	n.d.	n.d.	0.3	-	100.5
E4	3	99.3	n.d.	n.d.	0.3	-	99.6
Mean		99.7	0.0	0.0	0.3	-	100.1
E5		98.6	n.d.	n.d.	0.6	-	99.2
E6	7	98.6	n.d.	n.d.	0.4	-	99.0
Mean		98.6	0.0	0.0	0.5	-	99.1
E7		99.8	n.d.	n.d.	0.2	-	100.0
E8	14	98.9	n.d.	n.d.	0.0	-	98.9
Mean		99.3	0.0	0.0	0.1	-	99.5
E9		97.7	n.d.	n.d.	0.5	-	98.2
E10	21	97.0	n.d.	n.d.	0.5	-	97.5
Mean		97.4	0.0	0.0	0.5	-	97.9
E11		97.8	n.d.	n.d.	0.6	-	98.4
E12	35	95.4	3.7	2.0	0.3	-	99.4
Mean		96.6	1.9	1.0	0.4	-	98.9
E13		55.5	40.6	8.8 c	1.3	-	97.5
E14	59	64.2	31.1	31.1 d	1.9	-	97.2
Mean		59.8	35.9	20.0	1.6	-	97.3

TAR = total applied radioactivity
n.d. = not detected (or < 0.1%)
a Analyzed using fraction collection of unextracted water. % TAR in aqueous phase therefore shown as n.a.
b The two largest peaks have to be considered as artefacts caused by sample storage.
c The peak shape indicates that it probably consists of several compounds.
d Compound was proven as a parent equivalent by MS analysis.

Table 7.2.2.2-8: Metabolite overview for the water phase after application of [pyrimidinyl-2-14C] pyrimethanil to lake water (sterile) [% TAR]

Sample	Days After Treatment	Pyrimethanil	Sum of unknowns	Largest Unknown	Unresolved Background	Total
Sterilized water (100 µg L-1)						
C1	0	98.5	n.d.	0.4	98.9	98.9
C2	3	97.2	n.d.	0.2	97.4	97.4
C3	7	97.3	n.d.	0.7	98.0	98.0
C4	14	97.9	n.d.	0.5	98.4	98.4
C5	21	99.0	n.d.	0.3	99.3	99.3
C6	35	97.1	n.d.	0.2	97.3	97.3
C7	59	95.8	n.d.	1.4	97.2	97.2

TAR = total applied radioactivity
n.d. = not detected (or < 0.1%)

Table 7.2.2.2-9: Metabolite overview for the water phase after application of [phenyl-U-14C] pyrimethanil to lake water (sterile) [% TAR]

Sample	Days After Treatment	Pyrimethanil	Sum of unknowns	Largest Unknown	Unresolved Background	Total
Sterilized water (100 µg L-1)						
F1	0	100.0	n.d.	n.d.	0.3	100.3
F2	3	97.7	n.d.	n.d.	0.6	98.3
F3	7	97.7	n.d.	n.d.	0.8	98.5
F4	14	101.1	n.d.	n.d.	0.5	101.6
F5	21	99.4	n.d.	n.d.	0.1	99.5
F6	35	94.4	3.1	3.1	0.4	97.9
F7	59	96.9	n.d.	n.d.	1.2	98.1

TAR = total applied radioactivity
n.d. = not detected (or < 0.1%)

Control samples
The control vessels treated with [14C]-sodium benzoate showed that the test system was microbially active both without and with the addition of acetonitrile. The total recoveries of trapped volatile radioactivity after 59 days were 87.2 and 86.1% TAR and the material balances were 92.6 and 93.7% TAR for the samples without and with acetonitrile, respectively.

A material balance was also established for intermediate sampling times, where water samples were radioassayed (after 0, 14 and 29 days). The material balance ranged from 73.0 and 74.2% TAR at 0 DAT to 90.8 and 91.9% TAR at 59 DAT for the samples without and with acetonitrile, respectively. It was assumed that the low recoveries at 0 DAT were due to poor initial homogeneity at sampling. Later, radioactivity present as 14CO2 dissolved in the water may have escaped during sampling and LSC measurement.

III. CONCLUSION

From the obtained results, it can be concluded that pyrimethanil (BAS 605 F) is not significantly degraded in the natural water environment provided in this test. Radioactivity in the volatile traps did not exceed 2.4% TAR in any sample, indicating a low rate of mineralization.

Overall, the compound was considered to be stable in the test systems. Degradation kinetics were not reported as no significant degradation was observed.

CA 7.2.2.3 Water/sediment studies

No new water/sediment study was performed. The already peer-reviewed study of Bieber, W.D. 1994 (BASF DocID A81917) is still considered valid. The study was performed only with the 14C-pyrimidyl-label, since the only phenyl-labelled metabolites which could be formed by cleavage would be simple phenyl-rings or (more likely) low molecular, aliphatic breakdown products after ring-opening.

The behaviour of pyrimethanil in the two water/sediment systems was characterized by a moderately fast movement from the water phase into sediment. After 100 days, only 3.4 - 8.6% of the total applied radioactivity (TAR) was left in the water as unchanged parent. In the sediment, it increased until day 14 or 30 to max. values of 46 - 68% TAR and declined afterwards to 12.5 - 42.8% TAR at day 100.

Low amounts of the known metabolite M605F007 (AE F132593) were observed in water (max. 2.4 - 6.0% TAR) and sediment (max. 3.7 - 4.4% TAR). All other metabolites never exceeded 3% TAR in any sample.

Mineralization was overall rather low reaching 2.4 - 9.1% TAR after 100 days. Non-extractable residues increased up to 31.4 - 58.1% TAR at day 100. Further analysis of the NERs showed the majority of radioactivity associated with humic acids and fulvic acids.

The half-lives of pyrimethanil in water / sediment systems were re-calculated according to the newest guidelines and guidance documents. The kinetic analysis is presented in the following report.

New, not yet peer-reviewed kinetic evaluation:

Report:	CA 7.2.2.3/1 Erzgraeber B., 2008a BAS 605 F - Pyrimethanil: Kinetic evaluation of a water / sediment study according to FOCUS 2008/1065209
Guidelines:	FOCUS (2006): Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration Sanco/10058/2005 version 2.0 434 pp.
GLP:	no

Report:	CA 7.2.2.3/2 Erzgraeber B., 2010a Addendum No. 1 to report - BAS 605 F - Pyrimethanil: Kinetic evaluation of a water / sediment study according to FOCUS 2010/1057334
Guidelines:	FOCUS (2006): Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration Sanco/10058/2005 version 2.0 434 pp.
GLP:	no

Executive Summary

The fate and behavior of pyrimethanil (BAS 605 F) in aquatic systems has been studied in an aerobic water/sediment study with two different test systems, one with a sandy loam sediment rich in organic matter content, the other one with a sandy sediment low in organic matter. The degradation of 14C-pyrimethanil (pyrimidine labeled) and the formation and degradation of its metabolite 2-amino-4,6-dimethyl-pyrimidine (M605F007, previously referred to as AE F132593), as well as the partitioning of both substances between water and sediment have been studied over a period of 100 days under laboratory conditions.

The present modeling study aimed at the evaluation of dissipation and degradation kinetics of pyrimethanil in these water/sediment systems and to derive modeling endpoints appropriate to be used in FOCUS surface water modeling. The kinetic analysis was done following the recommendations of the FOCUS work group on degradation kinetics.

Kinetic evaluation was performed for the parent substance pyrimethanil, considering the different levels proposed by the FOCUS kinetics guidance. The analysis at Level P-I (one compartment approach) was assessed for degradation in the whole system as well as dissipation from the water phase and dissipation in the sediment phase of the test systems. At Level P-II, the kinetic analysis considered the degradation in water and sediment and the partitioning between both phases. For metabolite M605F007, a pronounced decline phase in the water/sediment systems was not visible, therefore, an analysis at Level M-I (decline of metabolite in whole system, water, or sediment) was not performed.

Level P-I kinetic analysis of the whole system resulted in DegT50 values of 132.5 and 49.5 days for the Krempe and Ohlau test system, respectively. Dissipation half-lives for the water phase ranged from 3.8 to 11.8 days in the Krempe and Ohlau system, respectively. DT90 values for the water phase were calculated to be 77.9 days in the Krempe system and 77.1 days in the Ohlau system. The decline fit of pyrimethanil in sediment resulted in DT50 values of 109.4 and 48.3 days in systems Krempe and Ohlau.

The Level P-II kinetic analysis revealed DegT50 values in sediment of 122.2 and 25.2 days in the Krempe and Ohlau test system, respectively. However, degradation rates in the water phase could not be estimated reliably. Consequently, no modeling endpoints were derived from the Level P-II evaluation.

Additional information regarding the kinetic analysis of pyrimethanil in the water/sediment systems is provided in the addendum 1 of the study report. The data include the estimated parameter values and their reliability, as well as table and plots of observed and calculated values and the residual for all kinetic models tested at the P-l level analysis of pyrimethanil in the whole system, the water phase and the sediment phase.

I. MATERIAL AND METHODS

Kinetic evaluation of the dissipation and degradation behavior of pyrimethanil and its metabolite 2-amino-4,6-dimethyl-pyrimidine (M605F007) was conducted for an aerobic water/sediment study with two test systems, a sandy loam sediment rich in organic matter (system "Krempe") and a sandy sediment low in organic matter (system "Ohlau") [BASF DocID A81917]. Major characteristics of the water/sediment systems are summarized in Table 7.2.2.3-1. Kinetic evaluation was performed in order to derive best-fit degradation and dissipation parameters as well as degradation parameters suitable for modeling purposes (modeling endpoints) according to the recommendation of the FOCUS workgroup on degradation kinetics [FOCUS (2006)].

Table 7.2.2.3-1: Characteristics of the water/sediment systems used for incubation with 14C-pyrimethanil

Parameter			System Krempe		System Ohlau	
			Water	Sediment	Water	Sediment
Temperature a	[°C]		17	-	11	-
pH a	(CaCl2)		8.1	6.8	8.5	6.5
O2 content a	[mg L-1]	surface 5 cm above sediment	9.2 8.5	- -	13.8 13.7	- -
Total N	[mg L-1] [%]	beginning end	4.2 0.7 -	- - 0.21	4.2 8.5 -	- - 0.03
Total P	[mg L-1] [%]	beginning end	1.7 1.5	 0.14	0.5 1.4 -	- - 0.04
DOC	[%]		22	-	6	-
TOC / org. C	[%]		-	2.4	-	0.22
Water hardness	[mmol L-1]		2.0	-	1.3	-
Dry matter	[%]		-	45	-	78
CEC	[cmol+ kg-1]		-	18.8	-	2.5

a Measured directly at sampling site
DOC = Dissolved organic carbon
TOC = Total organic carbon
CEC = Cation exchange capacity

The test vessels were treated with pyrimidine-14C-labeled pyrimethanil at a concentration of 0.34 mg kg-1. The test system was incubated for up to 100 days at 20°C in the dark.

Kinetic modeling

The appropriate kinetic model was identified considering the procedures and kinetic models proposed by the FOCUS workgroup on degradation kinetics [FOCUS (2006)]. According to FOCUS, degradation and dissipation endpoints were derived for use as modeling inputs.

Kinetic models included in the assessment

Kinetic evaluation at Level P-I (one-compartment approach) was performed for pyrimethanil degradation in the total system as well as for dissipation from the water and sediment phase of the test systems.

At Level P-II (two-compartment approach: water and sediment), the kinetic analysis considered the degradation in water and sediment and the partitioning between both phases.

For metabolite M605F007 (AE F132593), a pronounced decline phase in the water/sediment systems was not visible, therefore, an analysis at level M-I (decline of metabolite in whole system, water, or sediment) was not performed.

As the purpose of the study was to derive modeling endpoints, all four kinetic models proposed by FOCUS were used during the evaluation (SFO, FOMC, DFOP, and HS). Details on the models are given in the FOCUS Kinetics guidance [FOCUS (2006)].

The appropriateness of a distinct kinetic model to describe degradation can be tested with the following checks recommended by FOCUS [FOCUS (2006)]:

Visual assessment of goodness-of-fit
Estimation of the error percentage at which the $\chi 2$ test is passed [Equation 6-2 in FOCUS (2006)]
t-test to evaluate whether estimated degradation parameters differ from zero.

A kinetic model is considered appropriate if the residuals are randomly distributed, the $\chi 2$ - error value is low (ideally below 15% but larger values may be acceptable if the visual fit is acceptable) and the t-test for the degradation parameters is passed at 10% error level.

Data handling

At Level P-I and P-II of the analysis, the kinetic evaluation started on the day of treatment (i.e. 0 DAT). The initial concentration of the parent substance in the total system or in water was set to the material balance recovered at 0 DAT as recommended by FOCUS [FOCUS (2006)]. Accordingly, the initial concentration in the sediment phase was assumed to be zero at Level P-II.

The assessment of dissipation in sediment at Level P-I only requires kinetics to be fitted to the corresponding decline data, starting from the maximum observed concentration in the compartment. The dissipation of the respective compound was thus evaluated starting at the day of maximum occurrence that was defined as 0 days after maximum concentration (0 DAMC). All later time points were adjusted accordingly as days after maximum concentrations (DAMC).

Software for kinetic evaluation

The software package ModelMaker (version 3.0.4) was used for parameter fitting [Anonymus (1997) Model Maker User Manual, Version 3, Cherwell Scientific Publishing Limited].

Experimental data

The experimental data of pyrimethanil and its metabolite M605F007 used as model input values for the kinetic evaluations are given in Table 7.2.2.3-2.

Table 7.2.2.3-2: Experimental data of pyrimethanil used for kinetic evaluation of dissipation/degradation in water/sediment

days after treatment	Pyrimethanil residues -system "Krempe" [% TAR]			Pyrimethanil residues -system "Ohlau" [% TAR]		
	Total system	Water	Sediment	Total system	Water	Sediment
0	98.4 a	98.4 a	0 b	100.8 a	100.8 a	0 b
0	98.9 a	98.9 a	0 b	99.7 a	99.7 a	0 b
0.25	93.8	80.3	13.5	96.0	89.8	6.2
0.25	93.5	82.1	11.4	97.3	89.7	7.6
1	95.5	72.3	23.2	95.5	74.7	20.8
1	94.7	72.5	22.2	96.2	78.1	18.1
2	96.2	57.9	38.3	97.7	71.8	25.9
2	97.0	60.1	36.9	97.9	67.8	30.1
7	96.7	43.9	52.8	95.3	57.8	37.5
7	91.4	42.1	49.3	93.9	60.0	33.9
14	91.0	32.0	59.0	91.1	50.4	40.7
14	94.6	32.9	61.7	86.3	33.7	52.6
30	88.4	21.0	67.4	70.4	30.3	40.1
30	87.8	19.3	68.5	68.2	27.5	40.7
58	78.8	16.4	62.4	41.6	18.8	22.8
59	77.2	15.5	61.7	53.5	24.6	28.9
100	47.9	7.2	40.7	24.8	6.6	18.2
100	54.9	10.0	44.9	7.0	0.3	6.7

DAT = Days after treatment
TAR = Total applied radioactivity
Bold numbers: peak concentration considered for single-compartment evaluation; previous values were omitted; sampling dates were adjusted accordingly.
a Set to material balance.
b Set to zero for kinetic evaluation at Level P-II.

II. RESULTS AND DISCUSSION

The datasets for each water/sediment system were analyzed considering the procedures and kinetic models proposed by the FOCUS workgroup on degradation kinetics [FOCUS (2006)]. The complete fitting procedure is given in the original study report.

The evaluation of both test systems at Level P-I resulted in reliable endpoints for pyrimethanil for degradation in the total system as well as for dissipation in the water and sediment phase.
The kinetic evaluation revealed no reliable fit for any of the evaluated water/sediment systems at Level P-II.

An overview of the estimated modeling endpoints for pyrimethanil at Level P-I and Level P-II in both water/sediment systems is given in Table 7.2.2.3-3 and Table 7.2.2.3-4.

Table 7.2.2.3-3: DT50 and DT90 values for pyrimethanil in water/sediments at Level P-I

System	Kinetic model	$\chi 2$ error	DegT50 [d]	DegT90 [d]
Total System				
System "Krempe"	SFO	3.4	132.5	440.0
System "Ohlau"	SFO	4.4	49.5	164.3
Geo mean			81.0	268.9
Water				
System "Krempe"	DFOP	6.3	3.8	77.9
System "Ohlau"	DFOP	5.3	11.8	77.1
Geo mean			6.7	77.5
Sediment				
System "Krempe"	SFO	4.1	109.4	363.6
System "Ohlau"	SFO	4.0	48.3	160.4
Geo mean			72.7	241.5

Table 7.2.2.3-4: DT50 and DT90 values for pyrimethanil in water/sediments at Level P-II

System	Kinetic model	$\chi 2$ error	DegT50 [d]	DegT90 [d]
Water				
System "Krempe"	SFO	11.1	No reliable endpoint could be derived	
System "Ohlau"	SFO	8.2	No reliable endpoint could be derived	
Geo mean			-	-
Sediment				
System "Krempe"	SFO	11.1	122.2	406.1
System "Ohlau"	SFO	12.8	25.2	83.9
Geo mean			55.5	184.6

III. CONCLUSION

The dissipation and degradation of pyrimethanil in two water/sediment systems was evaluated according to the FOCUS kinetics recommendations.

Level P-I kinetic analysis of the whole system resulted in DegT50 values (SFO kinetics) of 132.5 and 49.5 days for the Krempe and Ohlau test system, respectively. The geometric mean DegT50 value was 81.0 days.

The dissipation of pyrimethanil from the water phase (Level P-I) was best described using the DFOP kinetic model, with corresponding DT50 values of 3.8 and 11.8 days in the Krempe and Ohlau system, respectively. DT90 values for the water phase were calculated to be 77.9 days in the Krempe system and 77.1 days in the Ohlau system. Decline of pyrimethanil in sediment (Level P-I) could be described by SFO kinetics, with corresponding DT50 values of 109.4 and 48.3 days in systems Krempe and Ohlau.

The Level P-II kinetic analysis revealed DegT50 values in sediment of 122.2 and 25.2 days in the Krempe and Ohlau test system, respectively, however, no reliable degradation rates could be estimated for the water phase. Consequently, no modeling endpoints were derived from the Level P-II evaluation.

Based on the results of these kinetic evaluations and considering the latest FOCUS guidance documents [FOCUS (2015): Generic guidance for FOCUS surface water Scenarios, version 1.4. 367 pp.], it is proposed to select endpoints for FOCUS surface water modeling as follows:

Table 7.2.2.3-5: Decision scheme for estimation and use of modelling endpoints

FOCUS surface water Step	Selected endpoint	Justification
Step 1	81.0 days	Geometric mean system DegT50 (n = 2)
Step 2	81.0 days for water and sediment	Geometric mean system DegT50 (n = 2)
Step 3 a	1000 days for water 81.0 days for sediment	Default approach due to unreliable degradation rate in water at Level P-II. geometric mean system DegT50 for sediment

a Based on newest guidance documents [FOCUS (2015): Generic guidance for FOCUS surface water Scenarios, version 1.4. 367 pp.] the recommendation is to ascribe the whole system DT50 to the water phase for compounds with a Koc< 100 mL/g or to the sediment phase for compounds with a Koc > 2000mL/g and use a default of 1000 days for the other compartment. For compounds with Koc between 100 and 2000mL/g , the FOCUS kinetics advice regarding running simulations with both combinations.

CA 7.2.2.4 Irradiated water/sediment study

An irradiated water/sediment study was not performed. Since no photo-degradates were detected at direct or at indirect photolysis of pyrimethanil, an irradiated water/sediment study is not considered necessary.

CA 7.2.3 Degradation in the saturated zone

Due to low leaching potential, pyrimethanil or its metabolite is not expected to reach deeper soil layers or saturated zones. Therefore, investigations on the degradation in the saturated zone are considered to be not necessary.

Effect of water treatment procedures

The following report was submitted in order to address the effect of water treatment procedures on the nature of residues when surface water or groundwater are abstracted for drinking water.

Report:	CA 7.2.3/1 Erzgraeber B., 2017 b BAS 605 F – Pyrimethanil - Estimation of concentrations in water at drinking water abstraction points 2017/1121801
Guidelines:	none
GLP:	no

Executive Summary

This document aims to provide additional information regarding the estimation of predicted concentrations of BAS 605 F – pyrimethanil and its metabolite M605F007 in water at drinking water abstraction points. The exposure concentrations at drinking water abstraction points were estimated following a peer-reviewed methodology developed for the Netherlands considering surface water and groundwater contribution.

Based on the calculations performed for surface water and groundwater, concentrations of pyrimethanil and its metabolite M605F007 at drinking water abstraction points are considered to be negligible and hence, no adverse effect of water treatment procedure are to be expected.

I. MATERIAL AND METHODS

There is currently no agreed guidance on the EU level on how to address the effect of water treatment procedures, which is an approval condition mentioned in Article 4.3(b) of Regulation (EC) No 1107/2009, neither experimentally nor with regard to the exposure assessment. However, the exposure concentrations at drinking water abstraction points may be estimated following a peer-reviewed methodology developed for the Netherlands considering surface water and groundwater contribution.

Exposure of pesticides to groundwater and surface water is conservatively estimated on the field scale for a one hectare agricultural field. Abstraction of drinking water is performed at large intake areas covering whole catchments of agricultural fields. The concentration of pesticides in water from the local field towards the abstraction area are therefore subject to dissipation and dilution processes. Based on these processes a Tier 1 calculation method was developed in the Netherlands to estimate the concentration (PEC) in water at abstraction points from surface water exposure considering all crops of relevance [*Adriaanse et al.(2008). Development of an assessment methodology to evaluate agricultural use of plant protection products for drinking water production from surface waters. Alterra report 1635.*].

In the absence of other guidance, the assessment approach from the Netherlands is used to provide an estimate of the concentrations of pyrimethanil and its metabolite M605F007 at drinking water abstraction points, considering Dutch specific input data. In the Netherlands, a total of 10 drinking water scenarios are relevant. For each scenario, a PEC in surface water for the abstraction of drinking water is derived following subsequent methodology.

Entry pathway surface water

Equation 7.2.3-1 Calculation of Tier1 PEC in surface water at point of drinking water abstraction

$$PEC_{Tier\ 1} = \sum_{all}^{crops} ((PEC_{FOCUS\_D3} \cdot f_{corrFOCUSscen}) f_{use\_intensity}) \cdot f_{timing} \cdot f_{dissipation} \cdot f_{add\_dilution}$$

PEC_{Tier1} = PEC in surface water at location where it is abstracted for drinking water preparation (µg/l)

PEC_{FOCUS_D3} = global maximum PEC edge-of-field for the FOCUS D3 scenario (µg/l)

$f_{corrFOCUSscen}$ = correction factor for implicit choices concerning contributing areas made in FOCUS D3 scenario (-) *[entry pathway drainage, $f_{corrFOCUSscen}$ = 3; entry pathway drift, $f_{corrFOCUSscen}$ = 1]*

$f_{use\_intensity}$ = factor considering the use of the pesticide (-)

$= RCA \cdot f_{market} \cdot f_{relevant\_contributing\_area}$

RCA = relative cropped area for a specific crop (-)

$= {area_{crop}}/{area_{dw\_abstraction}}$

$Area_{crop}$ = crop area on which the active substance is potentially used within the drinking water intake area (ha)

$Area_{dw\_abstraction}$ = total intake area of abstraction point (ha)

f_{market} = market share of active substance [*default value 0.4*]

$f_{relevant\_contributing\_Area}$ = fraction of the area contributing to the main entry route (-) [*0.5 for drift entries and 1.0 for drainage entries*]

f_{timing} = factor considering the difference in timing of application within the area of use (-) *[default value 0.5]*

$f_{dissipation}$ = factor considering the dissipation from the edge-of-field watercourse to the abstraction point (-)

$= e^{-k_{diss}t}$, where

t = time of pesticide in water since last application [*average value 6 days*]

k_{diss} = dissipation rate constant of pesticide in surface water (d^{-1})

$f_{add\_dilution}$ = factor considering additional dilution, e.g. by considerable water flows entering the intake area, or by lakes via which water travels to abstraction points [*default = 1; scenario Andijk = 1/6*]

The representative uses of pyrimethanil include application to pome/stone fruits, grapevine, lettuce and strawberries. For these crops, Step 3 and Step 4 FOCUS PEC_{sw} were calculated for the active substance pyrimethanil [please refer to M-CP 9.2.5, Sacher S. (2015b), DocID 2015/1169411].
For the metabolite M605F007 only Step 2 PEC_{sw} values are required for the exposure assessment to aquatic organisms. As a worst-case estimate these can be used for the estimation of metabolite concentrations at the drinking water abstraction points.

For the representative uses in pome/stone fruits, lettuce and strawberries, the corresponding crop groups selected in the Dutch model were "*tall fruit cultures*", "*leafy vegetables*" and "*strawberries*". For grapevines, no corresponding crop group was available.

Entry pathway groundwater

For groundwater, similar dissipation and dilution factors as with surface water could be applied, however there is currently no guidance available for the estimation of groundwater concentrations reaching abstraction points for drinking water. In the absence of such guidance, the edge-of-field PEC_{gw} values [reported in M-CP 9.2.4, Sachers S. (2015a), DocID 2015/1169410] may provide worst-case estimates.

II. RESULTS AND DISCUSSION

Entry pathway surface water

The respective PEC_{sw} values and estimated concentrations at drinking water abstraction points according to the methodology of *Adriaanse et al.(2008)* are provided in Table 7.2.3-1 and Table 7.2.3-2.

Table 7.2.3-1: Edge-of-field PEC_{sw} values for pyrimethanil and its metabolite M605F007 (based on Sacher S. 2015b)

Compound	PECsw (µg/l) (edge-of-field)	Entry pathway	Scenario
	Pome/stone fruit		
Pyrimethanil	4.115	Drift	D3 – ditch Step 4: 20m buffer zone
M605F007	19.355	n.a.	Step 2 South Europe
	Lettuce		
Pyrimethanil	5.076	Drift	D3 – ditch Step 3
M605F007	15.913	n.a.	Step 2 South Europe
	Strawberries		
Pyrimethanil	5.072	Drift	D3 – ditch Step 3
M605F007	9.640	n.a.	Step 2 South Europe

n.a. = not applicable

Based on the PEC_{sw} values, potential occurrence at point of drinking water abstraction are estimated following the methodology described by *Adriaanse et al.(2008)*. These are shown in Table 7.2.3-2.

Table 7.2.3-2: PEC_{sw} at abstraction points for drinking water for pyrimethanil and its metabolite M605F007

	PECsw at abstraction point [µg/L]	
Scenario:	**Pyrimethanil**	**M605F007***
De Punt	0.000	0.001
Andijk	0.000	0.001
N'gein	0.013	0.061
Heel	0.007	0.031
A'dam	0.010	0.048
Brakel	0.004	0.014
Petrus	0.004	0.014
Twente	0.00	0.00
ScheelH	0.006	0.024
BommelerW	0.012	0.055
Overall maximum PEC	**0.013**	**0.061***

*based on FOCUS Step2 PECsw values and not including mitigation measures as needed for the parent compound

The overall maximum PEC_{sw} at abstraction points for pyrimethanil and its metabolite M605F007 are estimated to be <0.1 µg/l. Therefore, it can be concluded that the risk of quantifiable concentrations that reach drinking water abstraction points is low.

Entry pathway groundwater

For groundwater, similar dissipation and dilution factors as with surface water could be applied, however there is no guidance available for the estimation of groundwater concentrations reaching abstraction points for drinking water.

The assessment of PEC_{gw} for pyrimenthanil and its metabolite M605F007 according to FOCUS resulted in PEC_{gw} values <0.1 µg/L for all representative uses and all scenarios [see M-CP 9.2.4, Sachers, S. (2015a), DocID 2015/1169410]. Consequently, it is highly unlikely, that pyrimethanil or its metabolite occur at the point of drinking water abstraction at relevant concentrations.

III. CONCLUSION

Based on the calculations performed for entry pathways surface water and groundwater, concentrations of pyrimethanil and its metabolite M605F007 at drinking water abstraction points are considered to be negligible. and hence, no adverse effect of water treatment procedure can be expected.

Concentrations of the active substance pyrimethanil and its metabolite M605F007 at raw water abstraction points will be very low. It is therefore highly unlikely that water treatment processes such as ozonation or chlorination will result in the formation of significant levels of by-products. Consequently, further information on the effect of water treatment processes on the nature of residues present in surface water and groundwater is not considered necessary.

CA 7.3 Fate and behaviour in air

CA 7.3.1 Route and rate of degradation in air

No new experimental data are available, but a new calculation of the photochemical oxidative degradation in air (Atkinson) according to the newest model is provided below.

Report: CA 7.3.1/1
Hassink J., 2013a
Photochemical oxidative degradation of BAS 605 F (QSAR estimates)
2013/1375100
Guidelines: EC 1107/2009 of the European Parliament
GLP: no

Executive Summary

The degradation rates for reactions of pyrimethanil with OH radicals and ozone in the atmosphere were calculated using the AOPWIN program based on ATKINSON's increment method. Based on the resulting degradation rate of k_{OH} = 200.2720 10^{-12} cm^3 $molecule^{-1}s^{-1}$, the atmospheric degradation half-life of the substance via this reaction route is $t_{1/2}$ = 0.05 d (12 h day). Although O_3 is likely to react with pyrimethanil, the degradation rate resulting from ozone attack could not be estimated by the OECD method due to lack of increments.

I. MATERIAL AND METHODS

Internal Code: BAS 605 F (pyrimethanil)
Reg. No.: 236999
CAS No.: 53112-28-0
Chem. Name: N-(4,6-dimethylpyrimidin-2-yl)aniline
Molar Mass: 199.26 g mol^{-1}
Emprirical Formula: $C_{12}H_{13}N_3$

OH-radical attack
Using the computer program AOPWIN (AOPWIN Program (Atmospheric Oxidation Program for Microsoft Windows 3.1) Version 1.88, Syracuse Research Corp.) which is based on the increment system published by Atkinson, the degradation rate for reactions of pyrimethanil with hydroxyl radicals is calculated based on the structural formula. Assuming a pseudo-first order reaction, the degradation half-life via this reaction route is calculated by taking into account the diurnally and seasonally averaged concentration of hydroxyl radicals in the troposphere.

The degradation of a compound A by OH-radicals can then be calculated by

$-d[A]/dt = k' \cdot [A]$ (1)
with $k' = k \cdot [OH\text{-radicals}]$

The half-life of this process can be calculated by equation (2):

$t_{1/2} = \ln 2/k' = \ln 2 / (k \cdot [OH\text{-radicals}])$ (2)

Ozone attack
The degradation rate resulting from ozone attack can be determined with an increment method. The half-life for this process can then be derived as described in equation 3 by taking into account the concentration of ozone molecules in the air:

$t_{1/2} = \ln 2/k' = \ln 2/k \cdot [ozone\ molecules]$ (3)

II. RESULTS AND DISCUSSION

The AOPWIN program uses the SMILES notation as basis for the calculation. The SMILES code for pyrimethanil is:

c1ccccc1Nc2nc(C)cc(C)n2

For some chemical groups increment data for the Atkinson method are missing. The missing data were estimated using "assumed values" by AOPWIN. An "assumed value" is a value of a structure fragment that has not been assigned a numeric value by Atkinson or derived explicitly from experimental values.

The total rate constant is $k = 200.2720\ 10^{-12}\ cm^3\ molecule^{-1}s^{-1}$.

The weighted global average tropospheric hydroxyl radical concentration is $1.5\ 10^6$ mol cm^{-3}. Conclusively, the half-life for the degradation of pyrimethanil by OH-radicals as calculated with equation (2) is:

$t_{1/2} = \ln 2 / (200.2720\ 10^{-12}\ 1.5\ 10^6)$ s
$= 0.641$ h
$= 0.05$ d (12 h day)

Although pyrimethanil contains reactive sites for an ozone attack no increments are available and a reasonable approximation by AOPWIN is not possible. Therefore, although O_3 is likely to react with pyrimethanil no degradation estimation can be given.

CA 7.3.2 Transport via air

Pyrimethanil is characterized by a medium high vapor pressure (1.1 x 10^{-3} Pa at 20°C) and shows some volatilization from soil (~10%) and plant surfaces (~27%) within 24h. However, its calculated half-life in air according to the method of Atkinson is only 0.6 hours. Therefore, no significant transport to non-target areas is expected.

CA 7.3.3 Local and global effects

Due to the calculated very short half-life in air, no local or global effects after use of pyrimethanil are expected.

CA 7.4 Definition of the residue

CA 7.4.1 Definition of the residue for risk assessment

According to the results presented in M-CA 7.1 – 7.3 the following compounds have to be considered for the environmental risk assessment:

Soil:
Pyrimethanil and its aerobic soil metabolite 2-amino-4,6-dimethylpyrimidine (AE F132593, Reg.No. 40603, M605F007)

Metabolite M605F007 was tested in acute and chronic earthworm studies and on nitrogen transformation in soil. Based on the results and the soil risk assessment, it can be concluded that the risk of metabolite for soil organisms and nitrogen transformation is negligible.

Groundwater:
Pyrimethanil and its aerobic soil metabolite 2-amino-4,6-dimethylpyrimidine (AE F132593, Reg.No. 40603, M605F007)

Based on the high adsorption and/or the fast degradation rates in soil, neither the parent molecule nor its metabolite poses any risk of leaching to groundwater. The predicted annual leachate concentrations of pyrimethanil and its soil metabolite were below 0.001 µg L^{-1}.

Surface Water:
Pyrimethanil and metabolite 2-amino-4,6-dimethylpyrimidine (AE F132593, Reg.No. 40603, M605F007)

Metabolite M605F007 was tested for its acute toxicity to fish, daphnia and algae. The metabolite showed low toxicity to aquatic organisms and sufficient margins of safety were reached already after FOCUS surface water step 1-2 calculations (worst case). Hence, it is concluded that the risk of the metabolite for aquatic organisms is very low.

Sediment:
Pyrimethanil

No pyrimethanil metabolites were found in sediment in amounts > 5% of the applied radioactivity.

Air:
Pyrimethanil

No volatile metabolite was detected.

CA 7.4.2 Definition of the residue for monitoring

According to the results of the risk assessment the following compounds should be considered for environmental monitoring:

Soil: Pyrimethanil (parent only)

Ground Water: Pyrimethanil (parent only)

Surface Water: Pyrimethanil (parent only)

Sediment: Pyrimethanil (parent only)

Air: Pyrimethanil (parent only)

CA 7.5 Monitoring data

According to the knowledge of the applicant, there are currently no published environmental monitoring data available for pyrimethanil or its metabolite, which would provide knowledge on the environmental behaviour not covered by this dossier.

Pyrimethanil

DOCUMENT M-CA, Section 8

ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE

Compiled by:

[redacted]

[redacted]

Tel.No.: [redacted]
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E-mail:

Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
2015-10-30	Initial AIR Dossier	BASF DocID 2015/1004014
2016-07-22	Inclusion of study CA 8.2.2.1/2 (study on zebrafish developmental screening). The study was found in scientific peer-reviewed literature, it was seen as not relevant for the ecotoxicological evaluation of pyrimethanil and originally only included in the toxicological section (CA 5.6.2). Due to the request of the RMS, the study was shifted to the ecotoxicological section.	BASF DocID 2016/1220151
Sept 2017	CA 8.2 – Due to a request of the RMS, further calculations of additional EC10 and EC20 endpoints for chronic ecotoxicological laboratory studies have been performed (reference is made to BASF DocID 2017/1074959). Furthermore, studies summaries for the relevant already peer reviewed studies were included (Mattock S.D., 1998, A91867 - Barber I.,Lattimore A.E., 1992, A81880 - Schupner J.K.,Christ M.T., 1993, A81898). The recalculated EC10 values for the relevant aquatic studies were included in the LoEPs.	BASF DocID 2017/1134392
Sept 2017	CA 8.2 – The pyrimethanil green algae study by Jenkins C., 1992 (A81883, DocID 1992/1001357) was statistically re-evaluated using mean measured test concentrations and a test duration of only 72 hours (reference is made to BASF DocID 2017/1073965).	BASF DocID 2017/1134392

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CA 8 ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE

The following studies in this section are covered by LoA [see KCA 8.1/5 2007/1028269] :

[see KCA 8.1.1.3/1 B003540],
[see KCA 8.2.1/1 B003476],
[see KCA 8.2.1/2 B003330],
[see KCA 8.2.2.1/1 B003492],
[see KCA 8.2.4.2/1 B003321],
[see KCA 8.2.4.2/2 B003335],
[see KCA 8.2.5.2/1 B003354],
[see KCA 8.2.6.2/1 B003422],
[see KCA 8.2.6.2/2 B003496],
[see KCA 8.2.6.2/3 B003421],
[see KCA 8.2.7/1 B003716].

CA 8.1 Effects on birds and other terrestrial vertebrates

Studies conducted for use in the risk assessments for birds and mammals

In addition to studies on effects on birds or terrestrial vertebrates, new studies on the residue behaviour of pyrimethanil on plants relevant for the higher tier risk assessment of bird and terrestrial vertebrates are summarized under this chapter from M-CA 8.1/1 to 8.1/4. The studies have not been peer-reviewed on EU level, which is indicated prior to the respective summary. For completeness some older studies are submitted additionally, which have not been submitted during the previous Annex I inclusion process (*e.g.* because there is no respective data requirement in the EU).

A study on the residue behaviour of pyrimethanil after application of BAS 605 04 F on young pea plants was conducted and has not been evaluated previously on EU level.

Report:	CA 8.1/1 Moreno S.,Galvez O., 2015a Study on the residue behaviour of Pyrimethanil (BAS 605 F) on pea after treatment with BAS 605 04 F under field conditions in North and South Europe, season 2013 - 2014 2014/1000928
Guidelines:	EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EEC 7029/VI/95 rev. 5 Appendix B, OECD 509 Crop Field Trial (2009), EEC 7525/VI/95 rev. 9 (March 2011)
GLP:	yes (certified by ENAC, Entidad Nacional de Acreditación, Madrid, Spain)

Executive Summary

The objective of the study was to determine the magnitude of residues of pyrimethanil (BAS 605 F) on pea (young plants) after one application of BAS 605 04 F. Samplings were carried out directly after and at subsequent time intervals after the application. The selected application rate corresponds to the maximum single rate of the Good Agricultural Practice (critical GAP) of BAS 605 04 F.

I. MATERIAL AND METHODS

A. MATERIALS

Test Material:	BAS 605 04 F
Description:	BAS 605 04 F (pyrimethanil)
Lot/Batch #:	360033; BAS 605 F: 400 g/kg (BAS 605 04 F, SC)
Purity:	not relevant
CAS#:	pyrimethanil (BAS 605 F): 53112-28-0
Crop parts(s) or processed commodity:	pea (young plants without roots; BBCH 12-30)
Sample size:	50.2-157.4 g

B. STUDY DESIGN

Study site

During the 2013/2014 growing season a total of eight trials were conducted in representative pea growing areas in Germany, the Netherlands, Spain and Italy.

Test item and application

Each trial consisted of a control plot (untreated) and one treated plot (plot 2) without replication. No product containing the test item was used on the test plots during the season.

The test item BAS 605 04 F was foliar applied on plot 2 at a nominal application rate of 2.5 L product per ha with a spray volume of 200 L/ha at three leaves unfolded growth stage (BBCH 12/13).

Sampling information

For this study treated specimens were collected as wheat whole plants without roots 1 hour after the last application (HALA) as well as 1, 2, 3, 4, 5, 7, 10, 12 and 14 days thereafter. Untreated specimens were collected also as pea whole plants without roots at 0 DBLA (days before last application) as well as 5 and 14 days thereafter. Each specimen was collected in duplicate ("ship" and "retain"), so that reserve specimens were available in case of potential problems during sample shipment, storage or analysis.

Damaged plants were not harvested. Each specimen was collected randomly from a minimum of 12 different places within each plot.

Untreated specimens were obtained prior to treated specimens when coincided at sampling timings. Each specimen was placed into an individual plastic bag and was subsequently double bagged with a second plastic bag. Specimen labels were fixed to the inner plastic bags detailing the specimen type, specimen number, trial and study number. The retain specimens were harvested at the same time of the original ones and were destroyed in the facilities of each test site or the Test Facility by agreement of the Study Director and Sponsor.

All specimens were stored at ≤ -18ºC and were sent to Specimen Management in BASF SE Agricultural Center Limburgerhof.

Description of analytical methods

All specimens were analysed for pyrimethanil (BAS 605 F) according to BASF method No. 542/2 (L0066/02). The method has a limit of quantitation of 0.01 mg/kg.

For further details on the analytical methods, please consult the consumer safety part (M-CA, section 4, chapter CA 4.1.2 and M-CA, section 6, chapter CA 6.3).

The results of procedural recovery experiments averaged at about 97.6% for BAS 605 F at fortification levels between 0.01 and 43 mg/kg.

II RESULTS AND DISCUSSION

Pyrimethanil

The pyrimethanil residues in the pea specimens taken 0 DALA (1 HALA) ranged from 10 – 73 mg/kg. They decreased to 5.8 – 63 mg/kg in the specimens taken 1 DALA and further to 3.4 – 51 mg/kg at 2 DALA. In the specimens taken 3 DALA 2.9 – 34 mg/kg were determined. The residue level in the specimens taken 4 DALA was 2.3 – 17 mg/kg), whereas in those taken 5 DALA < 0.01 – 20 mg/kg were found. Afterwards a steady decline was observed in the specimens taken 7 DALA (0.86 – 11 mg/kg), 10 DALA (0.30 – 6.4 mg/kg) and 12 DALA (0.12 – 4.7 mg/kg). At the last sampling (14 DALA they remained at this level (0.091 – 3.0 mg/kg).

No residues of pyrimethanil above the limit of quantitation were found in any of the analysed untreated specimens with the exception of one sample (L1307570002) which showed residues of 0.012 mg/kg.

Table 8.1-1: Summary of residues of pyrimethanil in pea (whole plant without roots)

Trial details	Sampling timing	Sampling no.	Date	Crop growth stage (BBCH)	Pyrimethanil residues [mg/kg]
Trial no. L130757 Study site: North Rhine-Westphalia, Germany	1 HALA	1	25.04.2014	13	22
	1 DALA	2	26.04.2014	13	14
	2 DALA	3	27.04.2014	14	4.9
	3 DALA	4	28.04.2014	14	3.6
	4 DALA	5	29.04.2014	15	3.2
	5 DALA	6	30.04.2014	15	2.1
	7 DALA	7	02.05.2014	16	0.96
	10 DALA	8	05.05.2014	17	0.6
	12 DALA	9	07.05.2014	19	0.28
	14 DALA	10	09.05.2014	30	0.29
Trial no. L130758 Study site: North Rhine-Westphalia, Germany	1 HALA	1	25.04.2014	13	13
	1 DALA	2	26.04.2014	13	14
	2 DALA	3	27.04.2014	14	5.0
	3 DALA	4	28.04.2014	14	3.8
	4 DALA	5	29.04.2014	15	3.0
	5 DALA	6	30.04.2014	15	1.8
	7 DALA	7	02.05.2014	16	1.1
	10 DALA	8	05.05.2014	17	0.79
	12 DALA	9	07.05.2014	19	0.28
	14 DALA	10	09.05.2014	30	0.38
Trial no. L130759 Study site: Limburg, Netherlands	1 HALA	1	05.05.2014	13	10
	1 DALA	2	06.05.2014	13	11
	2 DALA	3	07.05.2014	14	7.5
	3 DALA	4	08.05.2014	14	3.5
	4 DALA	5	09.05.2014	15	3.2
	5 DALA	6	10.05.2014	15	1.7
	7 DALA	7	12.05.2011	16	1.2
	10 DALA	8	15.05.2014	17	0.74
	12 DALA	9	17.05.2014	18	0.39
	14 DALA	10	19.05.2014	19	0.26
Trial no. L130760 Study site: Gelderland, Netherlands	1 HALA	1	05.05.2014	13	14
	1 DALA	2	06.05.2014	13	10
	2 DALA	3	07.05.2014	14	5.2
	3 DALA	4	08.05.2014	14	2.9
	4 DALA	5	09.05.2014	15	2.9
	5 DALA	6	10.05.2014	15	<0.01
	7 DALA	7	12.05.2014	16	0.98
	10 DALA	8	15.05.2014	17	0.72
	12 DALA	9	17.05.2014	18	0.46
	14 DALA	10	19.05.2014	19	0.18
Trial no. L130761 Study site: Huelva, Spain	1 HALA	1	23.01.2014	12	73
	1 DALA	2	24.01.2014	12	63
	2 DALA	3	25.01.2014	12	51
	3 DALA	4	26.01.2014	12	34
	4 DALA	5	27.01.2014	12	17
	5 DALA	6	28.01.2014	13	20
	7 DALA	7	30.01.2014	13	11
	10 DALA	8	02.02.2014	14	6.4
	12 DALA	9	04.02.2014	14	4.7
	14 DALA	10	07.02.2014	14-15	3.0

Trial no. L130762 Study site: Seville, Spain	1 HALA	1	23.04.2014	12	38
	1 DALA	2	24.04.2014	12	31
	2 DALA	3	25.04.2014	12-13	3.4
	3 DALA	4	26.04.2014	12-13	5.5
	4 DALA	5	27.04.204	12-13	3.6
	5 DALA	6	28.04.2014	13-14	2.4
	7 DALA	7	30.04.2014	14	0.86
	10 DALA	8	03.05.2014	14-15	0.30
	12 DALA	9	05.05.2014	15	0.12
	14 DALA	10	07.05.2014	16	0.091
Trial no. L130763 Study site: Caserta,, Italy	1 HALA	1	18.11.2013	12-13	14
	1 DALA	2	19.11.2013	12-13	7.3
	2 DALA	3	20.11.2013	13-14	6.5
	3 DALA	4	21.11.2013	14	5.0
	4 DALA	5	22.11.2013	14-15	2.3
	5 DALA	6	23.11.2013	14-15	2.5
	7 DALA	7	25.11.2013	15	1.2
	10 DALA	8	28.11.2013	15-16	0.5
	12 DALA	9	30.11.2013	15-16	0.37
	14 DALA	10	02.12.2013	15-16	0.13
Trial no. L130764 Study site: Piacenza, Italy	1 HALA	1	14.05.2014	12-13	11
	1 DALA	2	15.05.2014	12-13	5.8
	2 DALA	3	16.05.2014	13-14	6.5
	3 DALA	4	17.05.2014	14	4.2
	4 DALA	5	18.05.2014	14-15	2.6
	5 DALA	6	19.05.2014	14-15	2.8
	7 DALA	7	21.05.2014	16-17	1.5
	10 DALA	8	24.05.2014	17-18	0.60
	12 DALA	9	26.05.2014	18-19	0.30
	14 DALA	10	28.05.2014	19	0.14

HALA: hours after last application; DALA: days after last application

III. CONCLUSION

The pyrimethanil residues in the pea specimens taken 0 DALA (1 HALA) ranged from 10 – 73 mg/kg. They continuously decreased until the last sampling (14 DALA) to a range of 0.091 – 3.0 mg/kg.

A study on the residue behaviour of pyrimethanil after application of BAS 605 04 F on young wheat plants was conducted and has not been evaluated previously on EU level.

Report:	CA 8.1/2 Martin T., 2014a Study on the residue behavior ob BAS 605 F (Pyrimethanil) on wheat (young plants) after the application of BAS 605 04 F under field conditions in Germany, Netherlands, Italy and Spain, 2013/2014 2014/1000926
Guidelines:	EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EEC 7029/VI/95 rev. 5 Appendix B (July 22 1997), EEC 7525/VI/95 rev. 7
GLP:	yes (certified by ENAC, Entidad Nacional de Acreditación, Madrid Spain)

Executive Summary

The objective of the study was to determine the magnitude of residues of pyrimethanil (BAS 605 F) on wheat (young plants) after one application of BAS 605 04 F. Samplings were carried out directly after and at subsequent time intervals after the application. The selected application rate corresponds to the maximum single rate of the Good Agricultural Practice (critical GAP) of BAS 605 04 F.

I. MATERIAL AND METHODS

A. MATERIALS

Test Material:	BAS 605 04 F
Description:	BAS 605 04 F (pyrimethanil)
Lot/Batch #:	360033; BAS 605 F: 400 g/kg (BAS 605 04 F, SC)
Purity:	not relevant
CAS#:	pyrimethanil (BAS 605 F): 53112-28-0
Crop parts(s) or processed commodity:	wheat (young plants without roots; BBCH 11-22)
Sample size:	23.4-110.13 g

B. STUDY DESIGN

Study site

During the 2013/2014 growing season a total of eight trials were conducted in representative wheat growing areas in Germany, the Netherlands, Spain and Italy.

Test item and application

Each trial consisted of a control plot (untreated) and one treated plot (plot 2) without replication. No product containing the test item was used on the test plots during the season.

The test item BAS 605 04 F was foliar applied on plot 2 at a nominal application rate of 2.5 L product per ha with a spray volume of 200 L/ha at three leaves unfolded growth stage (BBCH 11-13 with priority at BBCH 13).

Sampling information

For this study treated specimens were collected as wheat whole plants without roots 1 hour after the last application (HALA) as well as 1, 2, 3, 4, 5, 7, 10, 12 and 14 days thereafter. Untreated specimens were collected also as wheat whole plants without roots 1 hour after application as well as 5 and 14 days thereafter. Each specimen was collected in duplicate ("ship" and "retain") except for the trials from BASF SE L130746, Mistakenly the double specimen L1307460011B was shipped together with the ship specimen to the Specimen Management Limburgerhof. The retain sample was included in the shipment of main specimens by error.

Untreated specimens were obtained prior to treated specimens when coincided at sampling timings.

All specimens were stored at ≤ -18ºC and were sent to Specimen Management in BASF SE Agricultural Center Limburgerhof.

Description of analytical methods

All specimens were analysed for pyrimethanil (BAS 605 F) according to BASF method No. 542/2 (L0066/02). The method has a limit of quantitation of 0.01 mg/kg.

For further details on the analytical methods, please consult the consumer safety part (M-CA, section 4, chapter CA 4.1.2 and M-CA, section 6, chapter CA 6.3).

The results of procedural recovery experiments averaged at about 98.8% for BAS 605 F at fortification levels between 0.01 and 150 mg/kg.

II RESULTS AND DISCUSSION

The pyrimethanil residues in the wheat specimens taken 0 DALA (1 HALA) ranged from 23 – 124 mg/kg. They decreased to 18 – 67 mg/kg in the specimens taken 1 DALA and further to 12 – 66 mg/kg at 2 DALA. In the specimens taken 3 DALA 5.3 – 61 mg/kg were determined. The residue level in the specimens taken 4 DALA was 5.6 – 49 mg/kg), whereas in those taken 5 DALA 3.8 – 31 mg/kg were found. Afterwards a steady decline was observed in the specimens taken 7 DALA (1.3 – 17 mg/kg), 10 DALA (0.38 – 9.7 mg/kg) and 12 DALA (0.29 – 4.1 mg/kg). At the last sampling (14 DALA they remained at this level (0.18 – 2.6 mg/kg).

No residues of pyrimethanil above the limit of quantitation were found in any of the analysed untreated specimens with the exception of three samples (L1307470002, L1307480001 and L1307480002) which showed residues of 0.011, 0.010 and 0.012 mg/kg respectively. These results in the control specimens can be explained by slight contamination that could have happened in the field, in the specimen management or in the laboratory. Nevertheless, the small residue amounts found in the untreated specimens do not affect the residue results of the study.

Table 8.1-2: Summary of residues of pyrimethanil in wheat (whole plant without roots)

Trial details	Sampling timing	Sampling no.	Date	Crop growth stage (BBCH)	Pyrimethanil residues [mg/kg]
Trial no. L130745 Study site: Brandenburg, Germany	1 HALA	1	21.10.2013	11	42
	1 DALA	2	22.10.2013	11	35
	2 DALA	3	23.10.2013	11-12	20
	3 DALA	4	24.10.2014	11-12	17
	4 DALA	5	25.10.2014	11-12	8.8
	5 DALA	6	26.10.2014	12-13	4.9
	7 DALA	7	28.10.2014	12-13	2.4
	10 DALA	8	31.10.2014	13	1.2
	12 DALA	9	02.11.2013	13-21	0.86
	14 DALA	10	04.11.2013	13-21	0.59
Trial no. L130746 Study site: Brandenburg, Germany	1 HALA	1	06.03.2014	13	57
	1 DALA	2	07.03.2014	13	41
	2 DALA	3	08.03.2014	13	34
	3 DALA	4	09.03.2014	13-14	38
	4 DALA	5	10.03.2014	13-21	24
	5 DALA	6	11.03.2014	13-21	23
	7 DALA	7	13.03.2014	13-22	14
	10 DALA	8	16.03.2014	21-22	3.4
	12 DALA	9	18.03.2014	21-22	1.9
	14 DALA	10	20.02.2014	21-22	1.4
Trial no. L130747 Study site: Ottersum, Netherlands	1 HALA	1	10.03.2014	13	54
	1 DALA	2	11.03.2014	13	55
	2 DALA	3	12.30.2014	13	45
	3 DALA	4	13.03.2014	14	51
	4 DALA	5	14.03.2014	14	21
	5 DALA	6	15.03.2014	14	20
	7 DALA	7	17.03.2014	15	8.6
	10 DALA	8	20.03.2014	18	2.7
	12 DALA	9	22.03.2014	21	1.4
	14 DALA	10	24.03.2014	21	0.94
Trial no. L130748 Study site: Horst aan de Maas, Netherlands	1 HALA	1	10.03.2014	13	62
	1 DALA	2	11.03.2014	13	54
	2 DALA	3	12.03.2014	13	55
	3 DALA	4	13.03.2014	14	26
	4 DALA	5	14.03.2014	14	12
	5 DALA	6	15.03.2014	14	17
	7 DALA	7	17.03.2014	15	8.4
	10 DALA	8	20.03.2014	18	2.2
	12 DALA	9	22.03.2014	21	1.3
	14 DALA	10	24.03.2014	21	0.86
Trial no. L130749 Study site: Seville, Spain	1 HALA	1	31.10.2013	12	50
	1 DALA	2	01.11.2013	12	36
	2 DALA	3	02.11.2013	12	18
	3 DALA	4	03.11.2013	12-13	15
	4 DALA	5	04.11.2013	13	6.7
	5 DALA	6	05.11.2013	13	3.8
	7 DALA	7	07.11.2013	13	1.3
	10 DALA	8	10.11.2013	13	0.38
	12 DALA	9	12.11.2013	14	0.29
	14 DALA	10	14.11.2013	14	0.18

Trial no. L130750 Study site: Seville, Spain	1 HALA	1	18.02.2014	12	45
	1 DALA	2	19.02.2014	12	39
	2 DALA	3	20.02.2014	12-13	25
	3 DALA	4	21.02.2014	13	13
	4 DALA	5	22.022014	13	11
	5 DALA	6	23.02.2014	13	7.3
	7 DALA	7	25.02.2014	13	2.6
	10 DALA	8	28.02.2014	14	0.89
	12 DALA	9	02.03.2014	14	1.1
	14 DALA	10	04.03.2014	14	0.36
Trial no. L130751 Study site: Foggia, Italy	1 HALA	1	06.12.2013	13	124
	1 DALA	2	07.12.2013	13	67
	2 DALA	3	08.12.2013	13	66
	3 DALA	4	09.12.2013	13	61
	4 DALA	5	10.12.2013	13	49
	5 DALA	6	11.12.2013	13	31
	7 DALA	7	13.12.2013	13	17
	10 DALA	8	16.12.2013	14	9.7
	12 DALA	9	18.12.2013	14	4.1
	14 DALA	10	20.12.2013	14	2.6
Trial no. L130752 Study site: Bologna, Italy	1 HALA	1	11.11.2013	12-13	23
	1 DALA	2	12.11.2013	12-13	18
	2 DALA	3	13.11.2013	13	12
	3 DALA	4	14.11.2013	13	5.3
	4 DALA	5	15.11.2013	13	5.6
	5 DALA	6	16.11.2013	13	8.9
	7 DALA	7	18.11.2013	13-14	6
	10 DALA	8	21.11.2013	13-14	1.4
	12 DALA	9	23.11.2013	14	0.97
	14 DALA	10	25.11.2013	14-15	0.72

HALA: hours after last application; DALA: days after last application

III. CONCLUSION

The pyrimethanil residues in the wheat specimens taken 0 DALA (1 HALA) ranged from 23 – 124 mg/kg. They continuously decreased until the last sampling (14 DALA) to a range of 0.18 – 2.6 mg/kg.

A study on the dissipation of pyrimethanil after application of BAS 605 04 F on young wheat and pea plants in the Northern Zone of Europe was conducted and has not been evaluated previously on EU level.

Report:	CA 8.1/3 Delgado Cartay M.D., 2015a Dissipation of BAS 605 F (Pyrimethanil) on young plants (wheat and peas) from field trials conducted in the Northern Zone of Europe - Calculation of DT50/DT90 dissipation times 2015/1029552
Guidelines:	FOCUS (2006): Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration Sanco/10058/2005 version 2.0 434 pp.
GLP:	no

This modelling report provides kinetic analysis and estimation of dissipation times (DT_{50} values) for pyrimethanil on young plants.

MATERIAL AND METHODS

Calculation of DT_{50}

The concentration time curves were described by a single first order (SFO) kinetic model, and this was fitted against the results of the individual trials.

The goodness-of-fit of the kinetic models was assessed by visual inspection and statistical measures, as recommended by the FOCUS guidance. For visual inspection, the recommended graphical representations of observed and modeled decline curves versus time and the residuals versus time are presented. As goodness-of-fit measures, the $\chi 2$ minimum error level is provided.

RESULTS

Table 8.1-3: DT_{50} values of pyrimethanil in young wheat and pea plants

Plant	Trial	Country	DT50 [d]	Kinetic model	$\chi 2$ error
Wheat	L130745	Germany	1.9	SFO	11.86
Wheat	L130746	Germany	3.4	SFO	12.04
Wheat	L130747	Netherlands	3.3	SFO	22.63
Wheat	L130748	Netherlands	2.6	SFO	21.41
Peas	L130757	Germany	1.2	SFO	14.98
Peas	L130758	Germany	1.8	SFO	28.41
Peas	L130759	Netherlands	2.3	SFO	22.35
Peas	L130760	Netherlands	1.5	SFO	14.91

CONCLUSION

The decline of pyrimethanil residues on young plants was well described by this first order

A study on the dissipation of pyrimethanil after application of BAS 605 04 F on young wheat and pea plants in the Southern Zone of Europe was conducted and has not been evaluated previously on EU level.

Report:	CA 8.1/4 Delgado Cartay M.D., 2015b Dissipation of BAS 605 F (Pyrimethanil) on young plants (wheat and peas) from field trials conducted in the Southern Zone of Europe - Calculation of DT50/DT90 dissipation times 2015/1029553
Guidelines:	FOCUS (2006): Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration Sanco/10058/2005 version 2.0 434 pp.
GLP:	no

This modelling report provides kinetic analysis and estimation of dissipation times (DT_{50} values) for pyrimethanil on young plants.

MATERIAL AND METHODS

Calculation of DT_{50}

The concentration time curves were described by a single first order (SFO) kinetic model, and this was fitted against the results of the individual trials.

The goodness-of-fit of the kinetic models was assessed by visual inspection and statistical measures, as recommended by the FOCUS guidance. For visual inspection, the recommended graphical representations of observed and modeled decline curves versus time and the residuals versus time are presented. As goodness-of-fit measures, the $\chi 2$ minimum error level is provided.

RESULTS

Table 8.1-4: DT_{50} values of pyrimethanil in young wheat and pea plants

Plant	Trial	Country	DT50 [d]	Kinetic model	$\chi 2$ error
Wheat	L130749	Spain	1.5	SFO	10.49
Wheat	L130750	Spain	1.9	SFO	13.02
Wheat	L130751	Italy	2.7	SFO	15.49
Wheat	L130752	Italy	2.4	SFO	20.49
Peas	L130761	Spain	2.6	SFO	11.39
Peas	L130762	Spain	1.1	SFO	37.85
Peas	L130763	Italy	1.8	SFO	13.94
Peas	L130764	Italy	2.3	SFO	16.20

CONCLUSION

The decline of pyrimethanil residues on young plants was well described by this first order kinetics.

CA 8.1.1 Effect on birds

CA 8.1.1.1 Acute oral toxicity to birds

No new study available.

CA 8.1.1.2 Short-term dietary toxicity to birds

No new study available.

CA 8.1.1.3 Sub-chronic and reproductive toxicity to birds

The following study on reproductive toxicity of pyrimethanil to the mallard has not been evaluated on EU level before (originally performed for submission outside EU) and is thus now submitted.

Report:	CA 8.1.1.3/1 [redacted]., 2001a Pyrimethanil: A reproduction study with the mallard B003540
Guidelines:	EPA 71-4, OECD 206
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

The objective of this study was to evaluate the effects upon the adult mallard (*Anas platyrhynchos*) of dietary exposure to BAS 605 F (pyrimethanil), over a period of approximately 21 weeks. Effects on adult health, body weight, and feed consumption were evaluated. In addition, the effects of adult exposure to pyrimethanil on the number of eggs laid, fertility, embryo viability, hatchability, offspring survival, and egg shell thickness were evaluated.

Mallard ducks (64 males and 64 females) were exposed in pairs, 1 male and 1 female per pen, to the pyrimethanil concentrations; 160, 320 or 640 ppm in diet. An equivalent control group was fed diet comparable to the pyrimethanil treatment group, but without the addition of the test substance. The birds were exposed to pyrimethanil for approximately 21 weeks, 10 weeks prior to egg production and throughout approximately 11 weeks of egg production.

Each treatment and control group contained a total of 16 pairs of birds. All adult birds were observed daily throughout the test for signs of toxicity or abnormal behaviour, and effects on adult health, body weight, feed consumption and reproductive parameters were evaluated. Reproductive parameters included egg production, egg shell thickness, egg fertility, embryo viability, egg hatchability, and offspring survival and growth to day 14.

Samples of the birds' diet from the control and test substance treatments were taken at intervals throughout the study and pyrimethanil concentrations measured.

Concentrations of the test substance in the diet were not adjusted to 100% active ingredient. Therefore, dietary concentrations are expressed as parts per million (ppm) in the diet.

Results

There were no treatment-related mortalities, overt signs of toxicity or treatment-related effects upon body weight or feed consumption of the birds at any of the pyrimethanil concentrations tested. Additionally, there were no treatment-related effects upon any of the reproductive parameters measured at the 160, 320 or 640 ppm test concentrations. The no observed effect concentration (NOEC) for mallard ducks exposed to pyrimethanil in the diet during the study was therefore 640 ppm, the highest concentration tested. Analysis of the test diet provided to the birds during the study showed that the mean measured concentrations of pyrimethanil in the treatments were 152, 311 and 641 ppm (i.e. 95 to 100% of nominal concentrations). Pyrimethanil was not detected in any of the control samples.

CONCLUSION

There were no treatment-related mortalities, overt signs of toxicity or treatment-related effects upon body weight or feed consumption at any of the concentrations tested. Additionally, there were no treatment-related effects upon any of the reproductive parameters measured at the 160, 320 or 640 ppm test concentrations. The no-observed-effect concentration for mallard exposed to pyrimethanil in the diet during the study was 640 ppm, the highest concentration tested.

MATERIAL AND METHODS

Test item:	Pyrimethanil techn. AE B100309 (BAS 605 F, CAS No. 53112-28-0), Batch No. AACA01168, purity: 99.4 %
Test species:	Mallard duck (*Anas platyrhynchos*), phenotypically indistinguishable from wild type; adults, age: 29 weeks (before beginning of first egg-laying period); supplier: [redacted]
Test design:	Mallard ducks approaching their first breeding season were kept in a group of 1 male and 1 female in a pen per replicate. 16 pens were allocated to the control and each treatment group. The final evaluation of substance mediated effects and the statistical analysis is based on 15 pens in the control and 16 in each of the treatment groups. All adult birds and their offspring were given feed and water *ad libitum* during acclimation and testing. The study period was divided into eight phases: 1. Study Initiation; 2. Acclimation – Approximately 9 weeks; 3. Pre-photostimulation – 3 weeks; 4. Photostimulation; 5. Egg laying – Approximately 11 weeks; 6. First Eggs Set; 7. post-adult termination – 6 weeks; 8.Experimental Termination. All eggs were collected, washed, artificially incubated and hatched; the young birds were maintained for 14 days. Adult birds were sacrificed after the egg-laying period, young birds after 14 days.
Endpoints:	Adult birds: mortalities, clinical observations, gross necropsy, adult body weight, adult feed consumption Reproductive parameters: Eggs laid, eggs cracked, eggs set, viable embryos, live 3-week embryos, hatchlings, body weight hatchlings, 14-day old survivors, body weight 14-day old survivors, egg shell thickness Establishing of NOEC
Test concentrations:	0 (Control), 160, 320 and 640 ppm pyrimethanil in the diet (nominal).

Test conditions:	Adult mallard study room: temperature 22.2 ± 0.8°C (SD); relative humidity: 63 ± 17% (SD); ventilation: 15 times the room air /h; photoperiod: ≤ 8 hours light (week 1 – 10), lengthened photoperiod to 17 hours light (week 11 – to the end of study); approximately 193 lux Egg collection and storage: collected daily, washed before storing in cold room until incubation: temperature: 13 ± 0.4°C (SD), relative humidity: 70 ± 6% (SD). Eggs set for incubation: temperature: 37.5 ± 0.0°C (SD), average wet bulb temperature: 30.6 ± 0.1°C (SD), relative humidity approximately 60%; after 24 days transferred to the hatcher: temperature: 37.2 ± 0.0°C (SD), average wet bulb temperature: 33.6 ± 0.3°C (SD) relative humidity approximately 77%. Hatchlings: temperature approximately 38°C from hatching 5-7th day, afterwards approximately 29°C; average ambient room temperature 25.5 ± 0.9°C (SD), relative humidity: 81 ± 6 % (SD); photoperiod: 16 hours light per day
Analytics:	The test substance concentrations were analysed using HPLC
Statistics:	Analysis of variance (ANOVA) were used for differences between groups, Dunnett´s multiple comparison procedure were used for both datasets of "life 3-week embryos of viable embryos" and "hatchlings of 3-weeks embryos", respectively, and Dunnett´s method following arcsine square root transformation for percentage data

RESULTS AND DISCUSSION

Analytical measurements:
The result of the analytical verification of the test substance (Batch No. AACA01168) was 99.4 %. Concentrations of the test substance in the diet were not adjusted to 100% active ingredient. Therefore, dietary concentrations are expressed as parts per million (ppm) in the diet.

Biological results

Parental generation
No substance-related effects in the parent generation on mortality, clinical observations, in the gross necropsy, adult body weight, adult feed consumption could be detected in any of the concentration groups. Avoidance of feed was not observed.

Reproductive results
There were no apparent treatment-related effects upon reproductive performance at any of the concentrations tested. When compared to the control group, there were no statistically significant differences in any of the reproductive parameters measured in the 160 or 640 ppm treatment groups. However, at the 320 ppm test concentration there was a statistically significant ($p < 0.05$) reduction in the number of eggs laid as a percentage of maximum eggs laid. The reduction was further reflected as statistically significant ($p <0.05$) reductions in the numbers of both hatchlings and 14-day old survivors as percentages of the maximum number of eggs set. Due to lesions of egg yolk peritonitis or other lesions that impacted reproduction possibilities of the two females, which resulted in the reduced egg number (only one per female), the differences were not considered treatment related. There were no other statistically significant differences in the reproductive parameters measured between the control group and the 320 ppm treatment group. There were no apparent treatment related effects upon egg shell thickness and upon offspring body weight at any of the concentrations tested.

The results of the study are summarized in Table 8.1.1.3-1 to Table 8.1.1.3-3.

Table 8.1.1.3-1: Effects of pyrimethanil on the parental generation of mallard ducks (*Anas platyrhynchos*)

Parameter	Treatment group [ppm in the diet]			
	Control	160 ppm	320 ppm	640 ppm
No. of replicates (1 male and 1 female per replicate/pen)	15	16	16	16
No. of substance-related mortalities of adult birds [1)]	2	0	0	0
Mean food consumption per treatment group [g feed/bird/day] [2)]	163.6	153.4	157.1	183.9
Adult body weight [g] at the end of study (male / female)	1171 / 1146	1122 / 1139	1129 / 1124	1178 / 1128
Gain of adult body weight [g] at the end of study (male/female)	34 / 111	-37 / 98	-20 / 94	22 / 95

1) Note that mortalities were not treatment related
2) Mean value calculated for each group over 21 weeks (values from appendix A). Calculated from total no. per group (15 pens in the control group and 16 pens in each dose group). These values were not given in the study but were calculated additionally.

Generally, birds were in good health throughout the experimental period. Clinical signs that could have been attributed to the test substance were not observed.

Table 8.1.1.3-2 Effects of pyrimethanil on the reproduction of mallard ducks (*Anas platyrhynchos*)

Parameter	Treatment group [ppm in the diet]			
	Control	160 ppm	320 ppm	640 ppm
Total eggs laid	828	846	598	750
Eggs laid/hen	55	53	37	47
Eggs laid/hen/day	0.76	0.72	0.51	0.64
Eggs cracked	25	27	11	21
Mean egg shell thickness (mm)	0.385 ± 0.025	0.392 ± 0.025	0.381 ± 0.029	0.376 ± 0.017
Eggs set	727	740	523	642
Viable Embryos	676	662	506	605
Mean body weight (g) of hatchlings per group	37 ± 4	37± 2	38± 3	37 ± 3
Live 3-week embryos	670	658	499	603
Mean bodyweight (g) of 14-day old survivors	297 ± 27	289 ± 28	286 ± 14	287 ± 23
Ducklings hatched[1)]	578	533	400	559
14-day old survivors	567	528	391	553
14-day old survivors/hen	38	33	24	35

Table 8.1.1.3-3 Effects of pyrimethanil on the reproduction of mallard ducks (*Anas platyrhynchos*) expressed as percentages

Parameter	Treatment group [ppm in the diet]			
	Control	160 ppm	320 ppm	640 ppm
% viable embryos of eggs set	93	90	96	93
% eggs laid/maximum laid	77	73	52*	65
% live 3-week embryos/viable embryos	99	99	98	100
%hatchlings/live 3-week old embryos	85	81	74	93
% hatchlings/eggs set	79	73	70	86
% 14-day old survivors/eggs set	77	73	68	85
% 14-day survivors of ducklings hatched	98	99	98	99
% cracked eggs of eggs laid	3	3	2	3
% hatchlings/maximum set	59	51	39*	54
% 14-day old survivors/maximum set	58	51	38*	53

* Significantly different form the control at $p<0.05$

CONCLUSION

There were no treatment-related mortalities, overt signs of toxicity or treatment-related effects upon body weight or feed consumption at any of the concentrations tested. Additionally, there were no treatment-related effects upon any of the reproductive parameters measured at the 160, 320 or 640 ppm test concentrations. The no-observed-effect concentration for mallard exposed to pyrimethanil in the diet during the study was 640 ppm, the highest concentration tested.

CA 8.1.2 Effects on terrestrial vertebrates other than birds

CA 8.1.2.1 Acute oral toxicity to mammals

No new study available.

CA 8.1.2.2 Long-term and reproductive toxicity to mammals

No new study available.

CA 8.1.3 Effects of active substance bioconcentration in prey of birds and mammals

No new study available.

CA 8.1.4 Effects on terrestrial vertebrate wildlife (birds, mammals, reptiles and amphibians)

According to the revised data requirements under regulation 1107/2009 (Commission Regulations (EU) 283/2013 and 284/2013 for the active ingredient and the plant protection products, respectively), the risk to amphibians and reptiles shall be addressed. Nevertheless, unlike birds and mammals, toxicity tests for amphibian and reptile species are not requested. In the EU there is no guidance or validated regulatory protocols yet available neither on the type of regulatory testing necessary or on how to conduct a risk assessment for amphibian and reptiles. In the case of pyrimethanil, there are no studies in the literature on the toxicity of this substance on amphibians and reptiles.

According to the new aquatic guidance document (EFSA, 2013) amphibian should be included in the aquatic and terrestrial risk assessment. In absence of GLP studies the assessment should be based on any existing relevant information (testing of amphibian is not recommended at first instance due to animal welfare reasons and the absence of standard guidelines for amphibian testing). With regard to the aquatic risk assessment several data analyses indicate that the risk assessment for aquatic organisms (and fish in particular) covers the risk assessment for aquatic phases of amphibians (Fryday and Thompson, 2012 available on http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/343e.pdf, and see KCA 8.1.4/3 2013/1416700, Weltje et al., 2013]).
This is supported by the results of two peer-reviewed scientific studies by Araujo et al. [see KCA 8.1.4/1 2014/1327212 and see KCA 8.1.4/2 2014/1327213] (2014a, 2014b). In these studies, avoidance responses by amphibian tadpoles (*i.e.* tadpoles of two amphibian species, *Leptodactylus latrans* and *Lithobates catesbeianus*) and juvenile fish (*Danio rerio*) after exposure to pyrimethanil have been investigated (with high, environmentally unrealistic concentrations). Overall, the outcome of the studies indicates, that fish are more sensitive to pyrimethanil exposure than amphibian tadpoles (*i.e.* avoidance responses for fish were more pronounced, occurred early in the test and at lower concentrations compared to the study on amphibian tadpoles).

The literature studies were both considered as relevant but not reliable (RI 3; for justification and further details please see the literature search and evaluation files also provided within the submission for Annex I Renewal). Thus, reference is made to these studies here but no study summaries are provided since studies are not suitable for an ecotoxicological risk assessment.

Compared to aquatic studies, regulatory ecotoxicological information on amphibians based on dosing studies (LD50) is rather scarce. However, in the few cases where terrestrial stages of amphibians were tested in this kind of study as birds and mammals, the general pattern is that amphibians are less sensitive than the latter two taxa (see Table 12 and 13 in Fryday and Thompson, 2012).

In the case of reptiles there is even less information available than for amphibians (see the revision by Fryday and Thompson, 2009 : http://www.efsa.europa.eu/en/supporting/pub/13e).
The risk from dietary exposure can be assumed to be lower for reptiles than for birds and mammals (Fryday and Thompson 2009). This is because reptiles are poikilotherm (i.e., do not maintain a constant body temperature) and as a result feeding activity will peak at warm days and will be zero during hibernation or at cold days. In contrast, birds and mammals will have to maintain a constant body temperature and, hence, will need to feed every day (Fryday & Thompson 2009). Uncertainties remain on the contribution of dermal exposure to the overall exposure to reptiles. However, in contrast to amphibians the skin of reptiles is much less permeable; its functions is in general protection and barrier and not an organ used for respiration or water/mineral exchange with the environment. Accordingly, reptiles are considered less vulnerable to dermal exposure as compared to amphibians. Nevertheless, some uncertainty with respect to the risk to reptiles, i.e. whether they are sufficiently covered by other (more standard) ecotoxicological data will remain and further research is needed.

However, pyrimethanil has been used for many years in many countries worldwide. So far, there are no publications indicating a potential risk of this compound to amphibians / reptiles and despite the long term use worldwide, we are not aware of a single findings (or incidence) reports that amphibians / reptiles were harmfully affected by applications of this substance.

References:

Commission Regulation (EU) No 283/2013 setting out data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. Official Journal of the European Union: 1st March 2013.

Commission Regulation (EU) No 284/2013: setting out the data requirements for plant protection products, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. Official Journal of the European Union: 1st March 2013.

Fryday S and Thompson H (2009): Literature reviews on ecotoxicology of chemicals with a special focus on plant protection products. Lot 1. Exposure of reptiles to plant protection products. EFSA (CFT/EFSA/PPR/2008/01).
http://www.efsa.europa.eu/en/supporting/pub/13e

Fryday S and Thompson, H (2012): Toxicity of pesticides to aquatic and terrestrial life stages of amphibians and occurrence, habitat use and exposure of amphibian species in agricultural; Food and Environment research agency, UK.
http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/343e.pdf,

CA 8.1.5 Endocrine disrupting properties

Based on the physical, chemical and structural characteristics of the active substance pyrimethanil as well as based on results of available long-term bird (see M-CP 10.1.1) and mammal studies (see M-CA 5.8.3), there is no indication of endocrine effects on the oestrogen, androgen hormone system for this active substance and effects observed on the thyroid hormone system are not toxicologically relevant. This is supported by a comprehensive overall assessment (see M-CA 5.8.3). Thus, no further studies are required.

CA 8.2 Effects on aquatic organisms

Since Annex I inclusion of pyrimethanil (BAS 605 F), new toxicity studies on the active substance have been performed and as a result there are new endpoints which are now used in the aquatic risk assessment. For completeness this includes some older studies, which have not been submitted during the previous Annex I inclusion process (*e.g.* because there is no respective data requirement in the EU). In addition, summaries are provided for peer-reviewed scientific literature that was considered to be of relevance for the aquatic risk assessment of pyrimethanil.

Details on the EU agreed studies which have been already evaluated within the Annex I inclusion of pyrimethanil are provided in the EU Review documents of the active substance (*i.e.* EFSA Conclusion of pyrimethanil (Draft Assessment Report (Vol. 3, Annex B.9, January 2005); Addendum to the DAR, September 2005 and EFSA Scientific Report (2006) 61, 1-70).

For better transparency and traceability of the active substance history, the results of all studies are summarized in Table 8.2-1. Full references used within the following chapters are given at the end of MCA 8.2. Document N3 contains structures and synonyms for the relevant metabolite of pyrimethanil.

Table 8.2-1: Summary of the toxicity values for aquatic organisms obtained in studies with the active substance pyrimethanil (BAS 605 F) and its major metabolite

Organism	Endpoint	Value [mg/L]	Reference (BASF Name / DocID)	EU agreed (Justification for submission of new data)
active substance: pyrimethanil (BAS 605 F)				
Fish				
Oncorhynchus mykiss	96 h LC_{50}	**10.56**	A81872 / 1992/1001351	yes
Cyprinus carpio	96 h LC_{50}	35.36	A81881 / 1992/1001355	yes
Lepomis macrochirus [1)]	96 h LC_{50}	29.0	B003476 / 2001/1025878	no (conducted for registrations outside EU)
Cyprinodon variegatus [1), 2)]	96 h LC_{50}	**2.8**	B003330 / 2001/1025870	no (conducted for registrations outside EU)
Oncorhynchus mykiss	21 d NOEC 21 d EC_{10}	1.6 1.692	A81898 / 1993/1001479 + 2017/1074959 (ECx recal.)	yes
Oncorhynchus mykiss	91 d NOEC (ELS study)	0.07	A91863 / 1998/1003069	no (submitted during Annex I inclusion process; however, study was not accepted by EFSA) *
Oncorhynchus mykiss [1)]	89 d NOEC (ELS study)	**0.077**	B003492 / 2001/1025880	no (new study; replaces older ELS study)

Organism	Endpoint	Value [mg/L]	Reference (BASF Name / DocID)	EU agreed (Justification for submission of new data)
Aquatic invertebrates				
Daphnia magna	48 h EC_{50}	**2.9**	A81876 / 1992/1001352	yes
Americamysis bahia [1), 2)]	48 h LC_{50}	**4.1** [4)]	B003321 / 2001/1025869	no (conducted for registrations outside EU)
Crassostrea virginica [1), 2)]	96 h EC_{50}	3.9	B003335 / 2001/1025871	no (conducted for registrations outside EU)
Daphnia magna	21 d NOEC 21 d EC_{10}	**0.97** **1.4**	A81880 / 1992/1001354 + 2017/1074959 (ECx recal.)	yes
Americamysis bahia [1), 2)]	28 d NOEC	**0.50**	B003354 / 2000/1023232	no (conducted for registrations outside EU)
Sediment dwelling aquatic invertebrates				
Chironomus riparius (spiked water study)	28 d NOEC 28 d EC_{10}	4.0 (8.152) [5)] 3.531	A91867 / 1998/1003070 + 2017/1074959 (ECx recal.)	yes
Algae [6)]				
Selenastrum capricornutum (Syn. Pseudokirchneriella subcapitata)	72 h E_rC_{50} ~~96 h E_rC_{50}~~ 72 h E_bC_{50} ~~96 h E_bC_{50}~~	**5.521** ~~**5.84**~~ 0.916 ~~1.20~~	A81883 / 1992/1001357 + 2017/1073965 (statistical recal.)	yes
Navicula pelliculosa [1)]	72 h & 96 h E_rC_{50} / E_bC_{50}	> 3.8	B003421 / 2001/1025873	no (conducted for registrations outside EU)
Anabaena flos-aquae [1)]	72 h & 96 h E_rC_{50} / E_bC_{50}	> 3.9	B003422 / 2001/1025874	no (conducted for registrations outside EU)
Skeletonema costatum [1), 2)]	72 h & 96 h E_rC_{50} / E_bC_{50}	> 6.6	B003496 / 2001/1025881	no (conducted for registrations outside EU)
Aquatic macrophytes [6)]				
Lemna gibba [1)]	7 d E_rC_{50} 7 d E_bC_{50}	**> 30.0** [7)] 15.3 [7)] / 8.7 [8)]	B003716 / 2002/1019556	no (conducted for registrations outside EU)

Organism	Endpoint	Value [mg/L]	Reference (BASF Name / DocID)	EU agreed (Justification for submission of new data)
metabolite: M605F007 (= AE F132593; Reg. No. 40603)				
Fish				
Oncorhynchus mykiss	96 h LC_{50}	> 100	C012529 / 2001/1023291	yes
Aquatic invertebrates				
Daphnia magna	48 h EC_{50}	> 100	C012530 / 2001/1023292	yes
Algae [6)]				
Pseudokirchneriella subcapitata	72 h & 96 h E_rC_{50} / E_bC_{50}	> 100	C012804 / 2001/1023293	yes

Bold figures: Where several endpoints are available for the same group or where several endpoints are available for one study based on different effect parameters (*e.g.* for algae and macrophytes), the relevant endpoint is used in the TER calculations presented in chapter 10.2 of the MCP dossier part for Annex I renewal.

ELS = early life stage

* The 91 d ELS study on *O. mykiss* study was not accepted by EFSA (EFSA Scientific Report (2006)), therefore a second ELS study (DocID 2001/1025880 or B003492) was conducted.

1) Study has not been submitted during the Annex I inclusion process of pyrimethanil; a study summary is provided below.

2) Marine / saltwater species

4) In accordance with the new regulation 283/2013 the 48 h endpoint obtained in the 96 h study is considered as relevant endpoint and is presented here.

5) Value in mg/kg dry sediment; based on sediment concentration in spiked water study after 7 days at NOEC level.

6) In accordance to the EFSA Aquatic Guidance Document (EFSA 2013) and OECD guidelines 201 (OECD, 2011) and 221 (OECD, 2006), only the EC_{50} values for the more relevant endpoint 'growth rate' (E_rC_{50}) are considered for the risk assessment for aquatic primary producers.

7) based on frond no.

8) based on dry weight

CA 8.2.1 Acute toxicity to fish

The following acute study with *Lepomis macrochirus* performed with the active substance pyrimethanil was conducted for registrations outside the EU. The study is provided for completeness and has not been evaluated previously on EU level.

Report:	CA 8.2.1/1 [redacted] 2001a 96 hour acute toxicity to the bluegill sunfish, Lepomis macrochirus, in a static system - Pyrimethanil technical 99.4% w/w B003476
Guidelines:	EPA 72-1, OECD 203
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

In a 96-hour static acute toxicity laboratory study, juvenile bluegill sunfish (*Lepomis macrochirus*) were exposed to nominal concentrations of 3.125, 6.25, 12.5, 25 and 50 mg pyrimethanil/L in groups of 10 animals per test vessel containing 15 L water. Fish were observed for survival and symptoms of toxicity within 3 hour after start of exposure and 6, 24, 48, 72 and 96 hours after start of exposure.

The biological results are based on the nominal concentrations. After 96 hours of exposure no mortality was observed in the control and at concentrations of up to and including 12.5 mg pyrimethanil/L, whereas 30% and 100% mortality was observed at the two highest test item concentrations of 25 mg a.s./L and 50 mg a.s./L, respectively. Sub-lethal effects (*i.e.* loss of equilibrium, swimming ceased and dark pigmentation) were found at 12.5 mg a.s./L after 96 hours.

In a static acute toxicity study with bluegill sunfish (*Lepomis macrochirus*) the LC_{50} (96 h) of pyrimethanil was 29 mg a.s./L based on nominal concentrations. The NOEC (96 h) was determined to be 12.5 mg a.s./L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Pyrimethanil (BAS 605 F; Reg. no.: 236 999), batch no. AACA00707, purity: 99.4%.

B. STUDY DESIGN

Test species: Bluegill sunfish (*Lepomis macrochirus*), juveniles; mean body length 2.2 cm (1.9 - 2.6 cm); mean body weight 0.251 g (0.149 - 0.387 g); supplied by "Osage Catfisheries", Osage Beach, Missouri, USA.

Test design: Static system (96 hours); 5 test item concentrations plus control, 10 fish per test vessel and per concentration (loading 0.167 g fish/L); assessment of mortality and symptoms of toxicity within 3 hour after start of exposure and 6, 24, 48, 72 and 96 hours after start of exposure.

Endpoints: LC_{50}, NOEC, mortality and sub-lethal effects.

Test concentrations: Control, 3.125, 6.25, 12.5, 25 and 50 mg pyrimethanil/L (nominal).

Test conditions: Glass tanks (39.4 x 24.5 x 20.2 cm), test volume: 15 L, blended, filtered well water, temperature: 21.6 - 22.5 °C; pH 8.2 - 8.6; oxygen content: 6.0 mg/L - 9.0 mg/L; hardness: 136 mg $CaCO_3$/L; conductivity: approx. 900 - 1000 µmhos/cm; photoperiod 16 h light : 8 h dark; light intensity: approx. 1800 lux; no aeration; no feeding.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with UV detection.

Statistics: Descriptive statistics; Spearman-Karber-method for calculation of LC_{50}.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentration was conducted in each concentration at the beginning and at the end of the test. The analyzed contents of pyrimethanil over the course of the study ranged from 80% to 106%. As the mean measured concentrations of pyrimethanil did not vary by more than 20% of nominal concentrations, the following biological results are based on nominal concentrations.

Biological results: After 96 hours of exposure no mortality was observed in the control and at concentrations of up to and including 12.5 mg pyrimethanil/L, whereas 30% and 100% mortality was observed at the two highest test item concentrations of 25 mg a.s./L and 50 mg a.s./L, respectively. Sub-lethal effects (*i.e.* loss of equilibrium, swimming ceased and dark pigmentation) were observed at 12.5 mg a.s./L after 96 hours. The results are summarized in Table 8.2.1-1.

Table 8.2.1-1: Acute toxicity (96 h) of pyrimethanil on bluegill sunfish (*Lepomis macrochirus*)

Concentration [mg/L] (nominal)	**Control**	**3.125**	**6.25**	**12.5**	**25**	**50**
Mortality [%]	0	0	0	0	30	100
Symptoms*	none	none	none	none	E, S, D	n.d.
Endpoints [mg pyrimethanil/L] (nominal)						
LC_{50} (96 h)	29 (95% confidence limits: 23 - 35)					
NOEC (96 h)	12.5					

* Symptoms after 96 h: E = loss of equilibrium, S = swimming ceased, D = dark pigmentation
n.d. = not determined; all animals dead

III. CONCLUSION

In a static acute toxicity study with bluegill sunfish (*Lepomis macrochirus*) the LC_{50} (96 h) of pyrimethanil was 29 mg a.s./L based on nominal concentrations. The NOEC (96 h) was determined to be 12.5 mg a.s./L (nominal).

The following acute flow-through toxicity study with *Cyprinodon variegatus* performed with the active substance pyrimethanil was conducted for registrations outside the EU. The study is provided for completeness and has not been evaluated previously on EU level.

Report:	CA 8.2.1/2 [redacted] 2001a Pyrimethanil: A 96-hour flow-through acute toxicity test with the sheepshead minnow (Cyprinodon variegatus) B003330
Guidelines:	EPA 850.1075
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

In a 96-hour flow-through acute toxicity laboratory study, juvenile sheepshead minnows (*Cyprinodon variegatus*) were exposed to a dilution water control, a solvent control and to nominal concentrations of 0 (control), 0.63, 1.3, 2.5, 5.0 and 10.0 mg pyrimethanil/L (corresponding to mean measured concentrations of 0.61, 1.2, 2.4, 5.2 and 7.4 mg a.s./L) in groups of 10 animals per test vessel containing 15 L water. Fish were observed for survival and symptoms of toxicity 6, 24, 48, 72 and 96 hours after start of exposure.

The biological results are based on mean measured concentrations. After 96 hours of exposure no mortality was observed in the control and at concentrations of up to and including 1.2 mg pyrimethanil/L, whereas 15% and 95% mortality occurred at 2.4 mg a.s./L and 5.2 mg a.s./L, respectively. At the highest test item concentration of 7.4 mg a.s./L, 100% mortality was observed. Sub-lethal effects (*i.e.*, surfacing and lying on bottom) were found at 2.4 mg a.s./L and at 5.2 mg a.s./L after 96 hours.

In a flow-through acute toxicity study with sheepshead minnows (*Cyprinodon variegatus*) the LC_{50} (96 h) of pyrimethanil was 2.8 mg/L based on mean measured concentrations. The NOEC (96 h) was determined to be 1.2 mg a.s./L (mean measured concentrations).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Pyrimethanil (BAS 605 F; Reg. no.: 236 999), batch no. AACA01168, purity: 99.4%.

B. STUDY DESIGN

Test species: Sheepshead minnow (*Cyprinodon variegatus*), juveniles; mean body length 32 mm (26 - 37 mm); mean wet weight 1.1 g (0.71 - 1.6 g); supplied by "Aquatic Bio Systems", Fort Collins, Colorado, USA.

Test design: Flow-through system (96 hours); 5 test item concentrations plus a dilution water control and a solvent control; 10 fish per replicate (loading 0.12 g fish/L); 2 replicates per concentration; assessment of mortality and symptoms of toxicity 6, 24, 48, 72 and 96 hours after start of exposure.

Endpoints: LC_{50}, NOEC, mortality and sub-lethal effects.

Test concentrations: Control (dilution water), solvent control (0.1 mL dimethylformamide/L), 0.63, 1.3, 2.5, 5.0 and 10.0 mg pyrimethanil/L (nominal), corresponding to mean measured concentrations of 0.61, 1.2, 2.4, 5.2 and 7.4 mg pyrimethanil/L.

Test conditions: Stainless steel aquaria (25 L), test volume: approx. 15 L, 2 replicate test chambers; filtered natural seawater diluted with freshwater and aerated, salinity: 20‰; flow-rate: 6 volume additions per 24 hours per test chamber; temperature: 21.7 - 22.0 °C; pH 8.1 - 8.3; oxygen content: 6.2 mg/L - 8.1 mg/L; photoperiod 16 h light : 8 h dark; light intensity: approx. 184 lux; no feeding.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with UV detection.

Statistics: Descriptive statistics; moving average-method for calculation of LC_{50}.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentration was conducted in each concentration at the beginning and at the end of the test. The analyzed contents of pyrimethanil ranged from 86.6% - 100% of nominal at test initiation and from 62.4% to 107% of nominal at test termination. The following biological results are based on mean measured concentrations.

Biological results: After 96 hours of exposure no mortality was observed in the control and at concentrations of up to and including 1.2 mg pyrimethanil/L, whereas 15% and 95% mortality occurred at 2.4 mg a.s./L and 5.2 mg a.s./L, respectively. At the highest test item concentration of 7.4 mg a.s./L, 100% mortality was observed. Sub-lethal effects (*i.e.*, surfacing and lying on bottom) were found at 2.4 mg a.s./L and at 5.2 mg a.s./L after 96 hours. The results are summarized in Table 8.2.1-2.

Table 8.2.1-2: Acute toxicity (96 h) of pyrimethanil on sheepshead minnows (*Cyprinodon variegatus*)

Concentration [mg/L] (nominal)	**Control**	**Solvent control**	**0.63**	**1.3**	**2.5**	**5.0**	**10.0**
Concentration [mg/L] (mean measured)	**--**	**--**	**0.61**	**1.2**	**2.4**	**5.2**	**7.4**
Mortality [%]	0	0	0	0	15	95	100
Symptoms*	none	none	none	none	A	R	n.d.
Endpoints [mg pyrimethanil/L] (mean measured)							
LC_{50} (96 h)	2.8 (95% confidence limits: 2.4 - 3.3)						
NOEC (96 h)	1.2						

* Symptoms after 96 h: A = surfacing, R = lying on bottom
n.d. = not determined; all animals dead

III. CONCLUSION

In a flow-through acute toxicity study with sheepshead minnows (*Cyprinodon variegatus*) the LC_{50} (96 h) of pyrimethanil was 2.8 mg/L based on mean measured concentrations. The NOEC (96 h) was determined to be 1.2 mg a.s./L (mean measured concentrations).

CA 8.2.2 Long-term and chronic toxicity to fish

A 21 d chronic fish study performed with pyrimethanil was already evaluated during the previous Annex I inclusion process. The summary of the study and new statistical (ECx) recalculations is provided below.

Report:	CA 8.2.2/1 [redacted] 1993 a The prolonged toxicity of SN 100 309 technical to rainbow trout, Oncorhynchus mykiss, in a flow through system A81898
Guidelines:	OECD 203, EPA 540/9-86-138
GLP:	yes (certified by United States Environmental Protection Agency)

Material and methods:
Test material: SN 100309 (pyrimethanil)
Lot/Batch No: 390902 E 00000
Purity: 98.3% w/w

The toxicity of pyrimethanil to rainbow trout was assessed under flow through conditions over a 21 day exposure period. The 74 days old fishes were exposed to six nominal concentrations (0.94, 1.6, 2.6, 4.3 and 7.2 mg/L), one dilution water control and one solvent control (DMF). The trouts (ten per treatment and control) were incubated at 12.9 ± 0.31°C, under a 16/8-hour light/dark photoperiod and were fed twice a day during the study. Daily the mortality, behaviour and appearance of fish in each test vessel were checked. The length and weight of the fish were measured after 21 d.
Samples of the test solutions from each treatment replicate were analysed for pyrimethanil ption to test initiation, weekly thereafter, and 24 hours before termination (day 20).

Table 8.2.2-1: Measured concentrations of pyrimethanil in test samples

Nominal Concentration (mg/L)	Mean Measured Concentrations (mg/L)		
	Rep. 1 [a] n = 4	Rep. 2 [a] n = 4	Rep. 1 & 2 n = 8
Control	ND	ND	ND
Solvent Control	ND	ND	ND
0.94	1.1	1.1	1.1
1.6	1.5	1.6	1.6
2.6	2.7	2.8	2.7
4.3	4.4	4.4	4.4
7.2	7.3	7.3	7.3

[a] Mean measured concentrations from: 7/29/93, 8/5/93, 8/12/93, and 8/19/93.
ND = Not Detected

Results:
The mean measured concentrations were determined as 1.1, 1.6, 2.7, 4.4 and 7.3 mg/L (100 – 122 % of nominal). The endpoints are based on mean measured concentrations.

No mortalities were observed at concentrations up to 2.7 mg/L, at 4.4 and 7.3 mg/L the mortality was 30 and 40 %. Therefore no EC_{50} calculation was possible. Significant reduction in weight and length was noted in treatment levels at 2.7, 4.4 and 7.3 mg/L and. Based on the most sensitive endpoint (weight) the NOEC was 1.6 mg/L and the LOEC was 2.7 mg/L.

Table 8.2.2-2: Mean survival, wet weight and length of rainbow trout at the end of the exposure period to pyrimethanil

Nominal Concentration (mg a.S./l)	Mean Fish Weight and Length		
	No. fish surviving	Mean Weight (g)	Mean Length (cm)
Untreated Control	100	1.680 (0.4593)	4.54 (0.342)
Solvent Control	95	1.710 (0.5060)	4.59 (0.418)
1.1	100	1.583 (0.3889)	4.56 (0.344)
1.6	100	1.688 (0.4083)	4.64 (0.359)
2.7	100	1.393 (0.3188)	4.43 (0.279)
4.4	70	0.620 (0.1971)	3.69 (0.318)
7.3	61	0.696 (0.2313)	3.80 (0.290)

Calculation of ECx values (DocID: 2017/1074959)

In accordance with current requirements, additional calculations of ECx values have been performed:

Data for the recalculation were taken from the report. Only data on the most sensitive parameter fish weight was recalculated for EC_x, since survival and length measurements showed less distinct effects. The effects are compared to mean measured concentrations. Statistical recalculation was performed with ToxRatProfessional Version 2.10.

	Test concentrations [mg a.s./L]						
	Control	Solvent Control	1.1	1.6	2.7	4.4	7.3
Fish mean weight (g)	1.680	1.710	1.583	1.688	1.393	0.620	0.696
% effect on weight after 21d	--	--	6.6	0.4	17.8	63.4	58.9

No statistical significant difference exists between control and solvent control. Comparison of treatment effects was therefore done to the pooled control.

Parameter of the probit analysis:

Parameter	Value
Computation runs:	5
Slope b:	2,79091
Intercept a:	-1,91908
Variance of b:	9,13388
Goodness of Fit	
Chi^2:	0,31671
Degrees of freedom:	3
$p(Chi^2)$:	0,95686
Log EC50:	0,68762
SE Log EC50:	0,23364
g-Criterion:	1,25345
Residual Variance (Chi^2/df):	0,10557
r^2:	0,729
F:	8,078
p(F) (df: 1;3):	0,066

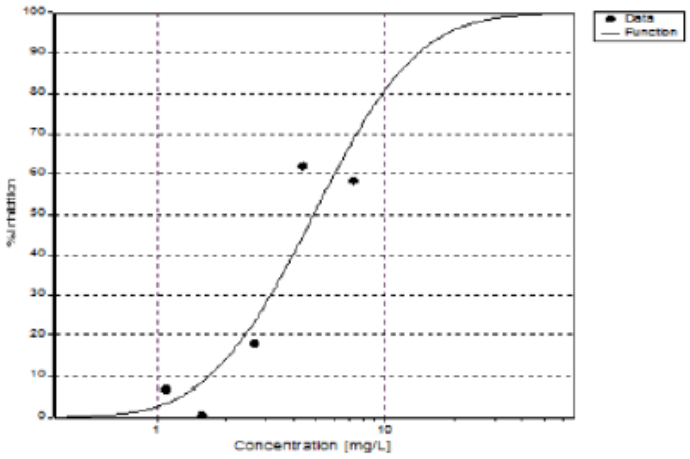


Figure: Concentration-effect curve showing the influence of the test item on weight of the introduced Oncorhynchus mykiss as observed after 21 d.

Since no confidence interval could be determined for the EC_x calculations, EC_{10} and EC_{20} endpoints are of low reliability. This is caused due to the not steady increase of effects in the dose-response. The NOEC should be retained as the principal endpoint of the study.

Results: The results based on mean measured concentrations are:

Endpoint	EC_{10} (mg/L)	EC_{20} (mg/L)	NOEC (mg/L)
Weight after 21 days	1.692 (95%-CL: n.d.)	2.433 (95%-CL: n.d.)	1.6

n.d. = not determined due to mathematical reasons or inappropriate data

Conclusion:
The 21-day LC_{50} is >7.3 mg pyrimethanil /L, the 21-day no-observed-effect concentration (NOEC) is 1.6 mg pyrimethanil /L and EC_{10} 1.692 mg pyrimethanil /L, based on mean measured concentrations.

CA 8.2.2.1 Fish early life stage toxicity test

In the EFSA Conclusion on the peer review of pyrimethanil (EFSA Scientific Report (2006), it was stated that the 91 d early life stage study on *O. mykiss* which was conducted before Annex I inclusion of pyrimethanil (BASF DocID A91863) is not accepted by EFSA since the NOEC of 0.07 mg/L was extrapolated (lower than the lowest test concentration). Thus, EFSA requested a new early life stage study on fish. Therefore, the following fish early life stage toxicity study performed with pyrimethanil on rainbow trout was conducted. It has not been evaluated previously on EU level. It confirms the toxicity result from the first ELS study.

Report:	CA 8.2.2.1/1 [redacted] 2001a Pyrimethanil: An early life-stage toxicity test with the rainbow trout (Oncorhynchus mykiss) B003492
Guidelines:	EPA 850.1400, OECD 210
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

The chronic toxicity of pyrimethanil to rainbow trout (*Oncorhynchus mykiss*) embryos and fry was investigated in an 89-day early life-stage test under flow-through conditions. Embryos were exposed to a dilution water control and a solvent control and to pyrimethanil at nominal concentrations of 0.020, 0.040, 0.080, 0.160 and 0.320 mg a.s./L (corresponding to mean measured concentrations of 0.020, 0.039, 0.077, 0.153 and 0.312 mg a.s./L). Four replicates, each with 30 embryos, were tested. Hatchability, survival rate, time to hatch and swim up and signs of toxicity or abnormal behavior of rainbow trout embryos and larvae were assessed throughout the study. On day 30 post-hatch, the total length of fish was determined photographically. Individual fish lengths and weights were measured at test termination on day 62 post-hatch.

The biological results are based on mean measured concentrations of the test item. No statistically significant treatment-related effects on hatching success, time to swim-up, survival and total length of rainbow trout embryos and larvae were observed at up to and including the highest tested concentration of 0.312 mg a.s./L. No abnormal behavior was observed in any of the test groups. At test termination, fish exposed to 0.153 and 0.312 mg a.s./L had significantly reduced wet weights in comparison to the pooled controls. Fish dry weight was statistically significantly reduced compared to the pooled controls at mean measured concentrations of 0.039, 0.153 and 0.312 mg a.s./L. Since there was no significant difference in the 0.077 mg a.s./L group, the effect in the 0.039 mg a.s./L group was considered to be not treatment related.

In an early life stage study with rainbow trout (*Oncorhynchus mykiss*), the overall NOEC (89 d) for pyrimethanil was determined to be 0.077 mg a.s./L, based on mean measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Pyrimethanil (BAS 605 F, Reg. no. 236 999; product code: AE B100309 00 1D99 0010), batch no. AACA01168; purity: 99.4% (w/w).

B. STUDY DESIGN

Test species: Rainbow trout (*Oncorhynchus mykiss*), newly-fertilized embryos (< 24 hours old); unfertilized eggs and sperm were obtained from "Troutlodge, Inc.", Sumner, Washington, USA; gametes from 4 females and 15 males were used; eggs were fertilized in-house and the test started within four hours of fertilization.

Test design:	Flow-through system; total exposure period: 89 days (including 27 day hatching period and 62-day post-hatch period); 5 test item concentrations plus a dilution water control and a solvent control; 4 replicate test chambers per treatment and control group, with each test chamber containing two incubation cup; 15 newly-fertilized eggs per incubation cub, resulting in a total of 30 embryos per replicate and 120 embryos per treatment. The test was initiated with the distribution of newly-fertilized eggs to the incubation cups. An additional 30 embryos were held in each of four incubation cups in dilution water and were sacrificed on day 12 to evaluate the fertilization success. After hatching, the larvae from all test concentrations were counted and released into the test chambers. When > 90% of the negative control group reached the swim-up stage (*i.e.* on day 21 post-hatch), the number of larvae was reduced to 15 per replicate in all treatments (60 fish per treatment). Embryo survival (hatching success), time to hatch, time to swim-up of the larvae, and the post-hatch growth and survival were measured for the rainbow trout in each treatment and control group. Daily observations were made during the embryo incubation and post-hatch periods. Fish lengths were measured at 30 days post-hatch and at test termination. The wet weight and dry weight of each surviving fish were measured at test termination.
Endpoints:	NOEC values based on hatchability, survival, toxic signs and growth.
Test concentrations:	Water control, solvent control (0.1 mL dimethyl formamide/L), 0.020, 0.040, 0.080, 0.160 and 0.320 mg a.s./L (nominal); corresponding to mean measured concentrations of 0.020, 0.039, 0.077, 0.153 and 0.312 mg a.s./L.
Test conditions:	Test chambers: 9 L glass aquaria filled with a test volume of 7 L; two glass incubation cups per test chamber (used during embryo stage) with approx. 50 mm diameter and 425 µm nylon screen mesh attached to the bottom; test medium: filtered and sterilized freshwater obtained from a well (moderately-hard water); water temperature: 11.0 °C - 13.5 °C; photoperiod: embryos/larvae were kept in darkness except during observations until one week after hatching; thereafter, 16 hours light : 8 hours dark; light intensity: approx. 47 lux; conductivity: 265 - 330 µmhos/cm; pH 7.8 - 8.3; oxygen content: 5.0- 10 mg/L; total hardness: 128 - 140 mg $CaCO_3$/L; alkalinity: 178 - 186 mg $CaCO_3$/L; flow rate: 6.4 volume additions per test chamber per 24 hours; feeding: larval fish were fed with commercial salmon-starter mash beginning on day 48 (end of swim-up stage) until approx. 52 hours before study termination, larvae were fed three times per day on weekdays and at least two times daily on weekends/holidays.

Analytics: Analytical verification of pyrimethanil concentrations was conducted using an HPLC-method with UV-detection.

Statistics: Descriptive statistics; 2X2 contingency tables or Student`s t-test for comparison of negative control and solvent control data ($\alpha = 0.05$); 2X2 contingency tables and chi square test for hatching success, time to swim-up and survival data ($\alpha = 0.05$); Bonferroni t-test or Wilcoxon's rank sum test for length and weight data ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical measurements of pyrimethanil concentrations were conducted in samples collected from each treatment at test initiation, once per week during the test and at test termination. The mean measured concentrations of pyrimethanil in the test-substance treatments during the 89-day exposure ranged from 96% to 100% of the nominal concentrations. The following biological results are based on mean measured concentrations.

Biological results: There were no statistically significant differences between the effects in the negative and solvent control groups (2X2 contingency tables or Student`s t-test; $p > 0.05$) for all measured parameters and thus, the controls were pooled for comparisons among the treatment groups.
No statistically significant treatment-related effects on hatching success, time to swim-up, survival and total length of rainbow trout embryos and larvae were observed at up to and including the highest tested concentration of 0.312 mg a.s./L ($p > 0.05$).
No abnormal behavior was observed in any of the test groups.
Mean control survival prior to thinning and after thinning was 95% and 98%, respectively. Survival in the 0.153 mg a.s./L treatment prior to thinning was 87% and was significantly different from the pooled controls (2X2 contingency tables, $p \leq 0.05$), however, this was not considered to be treatment-related due to the lack of effect at 0.312 mg a.s./L.
Rainbow trout embryos began hatching on day 25 and all surviving embryos in the control and treatment groups had hatched by day 29. Hatching success in the negative control and solvent control groups averaged 78% and 69%, respectively.
Rainbow trout larvae began swimming up from the bottom of the test chambers on day 13 post-hatch. By day 21 post-hatch, 94% of the negative control fish had attained swim-up.
At test termination, fish exposed to 0.153 and 0.312 mg a.s./L had significantly reduced wet weights in comparison to the pooled controls (Bonferroni t-test, $p \leq 0.05$). Fish dry weight was statistically significantly reduced compared to the pooled controls at mean measured concentrations of 0.039, 0.153 and 0.312 mg a.s./L (Bonferroni t-test, $p \leq 0.05$). Since there was no significant difference in the 0.077 mg a.s./L group, the effect in the 0.039 mg a.s./L group was considered to be not treatment related.

The results are summarized in Table 8.2.2.1-1.

Table 8.2.2.1-1: Effects of pyrimethanil on early life stages of rainbow trout (*Oncorhynchus mykiss*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.020**	**0.040**	**0.080**	**0.160**	**0.320**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**0.020**	**0.039**	**0.077**	**0.153**	**0.312**
Hatching success [%]	77.5	69.2	75.8	80.0	80.8	77.5	85.8
Start of hatch [d]	25	26	25	26	26	25	25
End of hatch [d]	27	28	28	28	27	28	27
Post-hatch survival [%] (day 1 to day 21 post-hatch; before thinning)	93.5	96.4	89.0	93.8	93.8	87.1 *	89.3
Post-hatch survival [%] (day 22 to day 62 post-hatch; after thinning to test end)	96.6	100	100	100	100	98.3	98.3
Behavioral abnormalities	none	none	none	none	none	none	none
Mean total length [mm] (day 62 post-hatch)	53.3	54.5	55.5	54.0	54.3	52.5	52.7
Mean wet weight (day 62 post-hatch)	1.652	1.558	1.612	1.557	1.576	1.379 *	1.405 *
Mean dry weight (day 62 post-hatch)	0.358	0.335	0.343	0.322 *	0.331	0.290 *	0.283 *
Endpoints [mg pyrimethanil/L] (mean measured)							
$NOEC_{hatching\ success,\ time\ to\ swim\ up,\ survival\ \&\ total\ length}$ (89 d)	≥ 0.312						
$NOEC_{wet\ weight\ \&\ dry\ weight}$ (89 d)	0.077						
$NOEC_{overall}$ (89 d)	**0.077**						

* Statistically significantly different from the pooled controls using 2X2 contingency tables for survival data and Bonferroni t-test for growth data (both with $p \leq 0.05$).

III. CONCLUSION

In an early life stage study with rainbow trout (*Oncorhynchus mykiss*), the overall NOEC (89 d) for pyrimethanil was determined to be 0.077 mg a.s./L, based on mean measured concentrations.

Calculation of ECx values (DocID: 2017/1074959)
In accordance with current requirements, additional calculations of ECx values have been performed.

Fish ELS; *Oncorhynchus mykiss*	2001/1025880 B003492	0.077	--	--	--	No reliable ECx can be calculated, since the study does not provide a clear dose-response effect. At the highest test concentrations (≥ 160 µg a.s/L), growth effects of 10-20% were seen which do not allow a reliable curve fitting.

Conclusion:
The lowest-observed-effect concentration (LOEC) is 0.0153 mg pyrimethanil/L and the no-observed-effect concentration (NOEC) is 0.077 mg pyrimethanil /L, based on mean measured concentrations. No reliable ECx can be calculated.

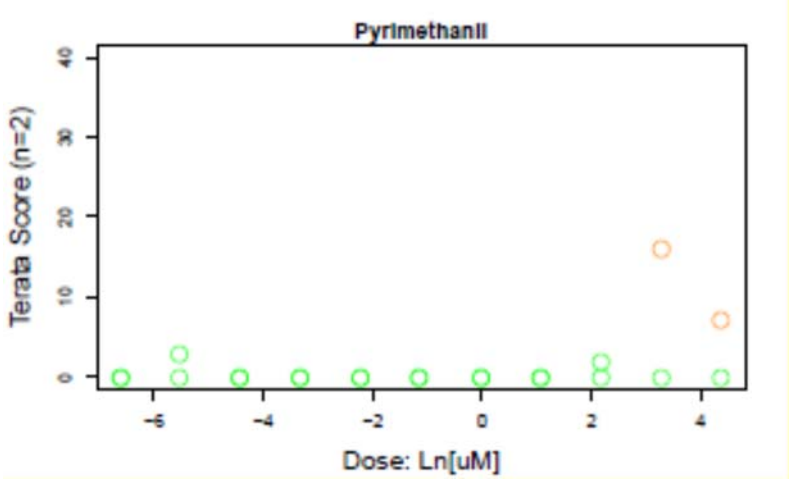
The following study on zebrafish developmental screening, found in scientific peer-reviewed literature, was seen as not relevant for the ecotoxicological evaluation of pyrimethanil and originally only included in the toxicological section (CA 5.6.2). Due to the request of the RMS, the study and its summary were shifted to the ecotoxicological section. Further evaluation of this study can be found in the toxicological part (CA 5.6)

Pyrimethanil, Zebrafish developmental screening (Padilla, 2012)

Report:	CA 8.2.2.1/2 Padilla S. et al., 2011 a Zebrafish developmental screening of the ToxCast Phase I chemical library 2012/1368722
Guidelines:	none
GLP:	no

Zebrafish (*Danio rerio*) is an emerging toxicity screening model for both human health and ecology. As part of the Computational Toxicology Research Program of the U.S. EPA, the toxicity of the 309 ToxCastTM Phase I chemicals was assessed using a zebrafish screen for developmental toxicity. All exposures were by immersion from 6–8 h post fertilization (hpf) to 5 days post fertilization (dpf); nominal concentration range of 1 nM–80µM. 2 larvae per concentration were assessed. On 6 dpf larvae were assessed for death and overt structural defects. Results revealed that the majority (62%) of chemicals were toxic to the developing zebrafish; both toxicity incidence and potency was correlated with chemical class and hydrophobicity (logP); and inter-and intra-plate replicates showed good agreement. The numerical score groups into lethality (40), non-hatching (20) and malformation index (<20).

This publication aims to develop and validate a screening method within the ToxCast Screening program and Pyrimethanil was one of the about 300 substances evaluated. A toxicity score of 30 was calculated for pyrimethanil based on the single concentration study, indicating reproduction toxicity at 80 µM (highest dose tested). No AC_{50} was derived for pyrimethanil in the dose-response experiment as no concentration-related effect could be observed. No death was noticed and hatching was not affected. At high concentrations (26.6 and 80 µM) either a normal larvae or a larvae with malformations were observed. At lower concentrations (8.8 µM and below) no indications of malformations after pyrimethanil treatment were observed. These high dose effects may not be indicative for a specific reproduction toxicity effect. In conclusion, given the lacking dose response, equivocal effects were observed at high (toxic) concentrations. The study is considered to be supplemental.


Pyrimethanil
Terata Score (n=2)
0
10
20
30
40
-6
-4
-2
0
2
4
Dose: Ln[uM]

CA 8.2.2.2 Fish full life cycle test

The chronic toxicity to fish is fully addressed by a 21 d chronic fish study and two early life stage studies (one of these early life stage studies was already evaluated during the previous Annex I inclusion process, but was not accepted by EFSA due to extrapolation of the endpoint; a summary for the second study is provided above). No additional fish full life cycle study is required and no (new) study has been conducted.

CA 8.2.2.3 Bioconcentration in fish

A bioconcentration study is not required since the log Pow of pyrimethanil and its metabolite M605F007 (= AE F132593; Reg. No. 40603) is < 3.

CA 8.2.3 Endocrine disrupting properties

Based on the physical, chemical and structural characteristics of the active substance pyrimethanil as well as based on results of available long-term fish studies (and studies on terrestrial vertebrates; see chapter MCA-8.1.5) there is no indication of endocrine disrupting properties of this active substance. This is supported by several impact assessments of different organizations (see MCA 5.8.3). Thus, no further studies are required.

CA 8.2.4 Acute toxicity to aquatic invertebrates

CA 8.2.4.1 Acute toxicity to *Daphnia magna*

This point is not addressed via new toxicity studies.

CA 8.2.4.2 Acute toxicity to an additional aquatic invertebrate species

The following acute flow-through toxicity study with the saltwater mysid *Americamysis bahia* performed with the active substance pyrimethanil was conducted for registrations outside the EU. The study is provided for completeness and has not been evaluated previously on EU level.
The 48 h LC_{50} obtained in the 96 h study on *A. bahia* is used as relevant endpoint for the risk assessment in accordance with the EU Regulation 283/2013 (European Commission, 2013) which describes the data requirements for active substances. Also the recent EFSA Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA, 2013) advises to use the 48-h endpoint, which allows an easy comparison to the other standard acute invertebrate tests. Therefore the 48 h results are presented below additionally to the 96 h results.

Report:	CA 8.2.4.2/1 Drottar K.R. et al., 2001b Pyrimethanil: A 96-hour flow-through acute toxicity test with the saltwater mysid (Mysidopsis bahia) B003321
Guidelines:	EPA 850.1035, ASTM E 729-88a
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

In a flow-through acute toxicity laboratory study, saltwater mysids were exposed to a dilution water control, a solvent control and to nominal concentrations of 0.38, 0.75, 1.5, 3.0 and 6.0 mg pyrimethanil/L (corresponding to mean measured concentrations of 0.37, 0.71, 1.5, 2.9 and 5.8 mg a.s./L) in groups of 10 animals per test chamber containing 6.5 L water with 2 replicates per concentration. The mysids were observed for survival and symptoms of toxicity 5, 24, 48, 72 and 96 hours after start of exposure.

The biological results are based on mean measured concentrations. After 96 hours of exposure no mortality and no other toxic effects occurred in the control, the solvent control and the lowest test item concentration of 0.37 mg a.s./L, whereas 10%, 5% and 30% mortality were observed at the test item concentrations of 0.71, 1.5 and 2.9 mg a.s./L, respectively. At the highest test item concentration of 5.8 mg a.s./L, 100% mortality was determined. Sub-lethal effects (*i.e.*, lethargy and loss of equilibrium) were found at 2.9 mg a.s./L after 96 hours.

In a flow-through acute toxicity study with saltwater mysids (*Americamysis bahia*) the LC_{50} (96 h) for pyrimethanil was determined to be 3.4 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was 0.37 mg a.s./L (mean measured). The LC_{50} (48 h) for pyrimethanil was determined to be 4.1 mg a.s./L (mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Pyrimethanil (BAS 605 F, Reg. No. 236 999); product code: AE B100309 00 1D99 0010, batch no. AACA01168, purity: 99.4%.

B. STUDY DESIGN

Test species: Saltwater mysid (*Mysidopsis bahia;* nowadays called: *Americamysis bahia*), juveniles, age: less than 24 hours old; source: in-house cultures.

Test design: Flow-through system (96 hours); 5 test item concentrations plus a dilution water control and a solvent control; 10 mysids per test chamber, two replicate test chambers per concentration; assessment of mortality and symptoms of toxicity 5, 24, 48, 72 and 96 hours after start of exposure.

Endpoints: LC_{50} (96 h), NOEC, mortality and sub-lethal effects.

Test concentrations: Control (dilution water), solvent control (0.1 mL dimethyl formamide/L), 0.38, 0.75, 1.5, 3.0 and 6.0 mg pyrimethanil/L (nominal), corresponding to mean measured concentrations of 0.37, 0.71, 1.5, 2.9 and 5.8 mg a.s./L.

Test conditions: Stainless steel aquaria (8 L); test volume: 6.5 L; test compartments: 500 mL glass beakers with nylon screen covering two holes on opposite sides of the beaker; filtered natural seawater, diluted with freshwater and aerated, flow-rate: 14 volume additions per 24 hours per test chamber; salinity: 20‰; temperature: 24.8 °C - 24.9 °C; pH 8.2 - 8.4; oxygen content: 5.2 mg/L - 6.8 mg/L; photoperiod 16 h light : 8 h dark; light intensity: 118 lux; juvenile mysids were fed daily with brine shrimps nauplii (*Artemia* sp.).

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with UV detection.

Statistics: Descriptive statistics; binominal method for calculation of LC_{50}.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentration was conducted in each concentration at test initiation and at test termination. Measured concentrations of pyrimethanil ranged from 96.0% to 99.4% of nominal concentrations at test initiation and from 89.6% to 98.9% of nominal at test termination. The following biological results are based on mean measured concentrations.

Biological results: After 96 hours of exposure no mortality and no other toxic effects occurred in the control, the solvent control and the lowest test item concentration of 0.37 mg a.s./L, whereas 10%, 5% and 30% mortality were observed at the test item concentrations of 0.71, 1.5 and 2.9 mg a.s./L, respectively. At the highest test item concentration of 5.8 mg a.s./L, 100% mortality was determined. Sub-lethal effects (*i.e.*, lethargy and loss of equilibrium) were found at 2.9 mg a.s./L after 96 hours. The results are summarized in Table 8.2.4.2-1.

Table 8.2.4.2-1: Acute toxicity (96 h) of pyrimethanil to saltwater mysids (*Americamysis bahia*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.38**	**0.75**	**1.5**	**3.0**	**6.0**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**0.37**	**0.71**	**1.5**	**2.9**	**5.8**
Mortality [%] (96 h)	0	0	0	10	5	30	100
Symptoms * (96 h)	none	none	none	none	none	C, N	n.d.
Endpoints [mg pyrimethanil/L] (mean measured)							
LC_{50} (48 h)	4.1 (95% confidence limits: 2.9 - 5.8)						
LC_{50} (96 h)	3.4 (95% confidence limits: 1.5 - 5.8)						
NOEC (96 h)	0.37						

* Symptoms after 96 hours: C = lethargy; N = loss of equilibrium
n.d. = not determined; all mysids dead

III. CONCLUSION

In a flow-through acute toxicity study with saltwater mysids (*Americamysis bahia*) the LC_{50} (96 h) for pyrimethanil was determined to be 3.4 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was 0.37 mg a.s./L (mean measured). The LC_{50} (48 h) for pyrimethanil was determined to be 4.1 mg a.s./L (mean measured).

The following toxicity study with *Crassostrea virginica* performed with the active substance pyrimethanil was conducted for registrations outside the EU. The study is provided for completeness and has not been evaluated previously on EU level.

Report:	CA 8.2.4.2/2 Drottar K.R. et al., 2001c Pyrimethanil: A 96-hour shell deposition test with the eastern oyster (Crassostrea virginica) B003335
Guidelines:	EPA 850.1025, ASTM E 729
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

A study was conducted to determine the effects of pyrimethanil on the shell deposition of eastern oysters during a 96-hour exposure period under flow-through test conditions. The eastern oysters were exposed to a dilution water control, a solvent control and to nominal test item concentrations of 0.63, 1.3, 2.5, 5.0 and 10.0 mg pyrimethanil/L (corresponding to mean measured concentrations of 0.65, 1.3, 2.4, 4.8 and 9.6 mg a.s./L) in groups of 20 oysters in stainless steel aquaria each containing approximately 13 L seawater with one replicate per treatment. Eastern oysters were observed for survival and symptoms of toxicity within 1.5 h after test initiation and 24, 48, 72 and 96 h after start of exposure. Measurements of shell deposition for each oyster were made after 96 hours.

The biological results are based on mean measured concentrations. After 96 hours of exposure, no mortality and no sublethal effects were observed in any of the test item concentrations tested. Shell growth was statistically significantly reduced at the three highest test item concentrations of 2.4, 4.8 and 9.6 mg pyrimethanil/L in comparison to the pooled control.

In a flow-through acute toxicity study with eastern oysters (*Crassostrea virginica*), the EC_{50} (96 h) for pyrimethanil was 3.9 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be 1.3 mg a.s./L (mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Pyrimethanil (BAS 605 F; Reg. no.: 236 999); product code: AE B100309 00 1D99, batch no. AACA01168; purity: 99.4%.

B. STUDY DESIGN

Test species: Eastern oysters (*Crassostrea virginica*), average length 33.8 mm (range: 26.2 - 40.4 mm); source: "Middle Peninsula Aquaculture", North, Virginia, USA.

Test design: Flow-through system (96 hours); 5 test item concentrations plus a dilution water control and a solvent control, 1 replicate for each test item concentration and the controls with 20 oysters per replicate; assessment of mortality and symptoms of toxicity within 1.5 h after test initiation and 24, 48, 72 and 96 h after start of exposure; measurements of shell deposition 96 hours after start of exposure.

Endpoints: EC_{50} (96 h) and NOEC for shell growth inhibition; mortality and symptoms of toxicity.

Test concentrations: Control (dilution water), solvent control (0.1 mL dimethylformamide/L), 0.63, 1.3, 2.5, 5.0 and 10.0 mg pyrimethanil/L (nominal), corresponding to mean measured concentrations of 0.65, 1.3, 2.4, 4.8 and 9.6 mg a.s./L.

Test conditions: 52 L stainless steel aquaria, test volume approximately 13 L, natural seawater diluted with well water and aerated; flow rate: 39 volume additions/test chamber/24 h; salinity: 21‰ - 22‰; temperature: 21.4 °C - 22.1 °C; pH 8.1 - 8.2; oxygen content: 5.6 mg/L - 7.8 mg/L; photoperiod 16 h light : 8 h dark; light intensity: 247 lux; supplementary diet for oysters: algal suspension at 2.9 x 10^9 cells/oyster/day.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with UV detection.

Statistics: Descriptive statistics; Student's t-test for comparison of the control data ($\alpha = 0.05$); linear interpolation for calculation of EC_{50} (96 h), ANOVA followed by Bonferroni t-test ($\alpha = 0.05$) for shell deposition data

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at test initiation, 24 h, 48 h, 72 h and 96 h after start of exposure, as well as at test termination. Mean measured concentrations for pyrimethanil ranged from 94.0% to 103% of nominal concentrations at test initiation and from 81.9% to 94.1% of nominal at test termination. The following biological results are based on mean measured concentrations.

Biological results: No statistically significant differences were determined between the control and the solvent control data (Student's t-test; $\alpha = 0.05$). Thus, the data from both control groups were pooled and the pooled data set was used for statistical evaluation of treatment related effects. After 96 hours of exposure, no mortality and no sublethal effects were observed in any of the test item concentrations tested. Shell growth was statistically significantly reduced at the three highest test item concentrations of 2.4, 4.8 and 9.6 mg a.s./L in comparison to the pooled control (ANOVA followed by Bonferroni t-test, $\alpha = 0.05$). The results are summarized in Table 8.2.4.2-2.

Table 8.2.4.2-2: Acute toxicity (96 h) of pyrimethanil to eastern oysters (*Crassostrea virginica*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.63**	**1.3**	**2.5**	**5.0**	**10**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**0.65**	**1.3**	**2.4**	**4.8**	**9.6**
Shell growth inhibition after 96 h [%] [1)]	--	--	17	9	36 *	59 *	93 *
Endpoints [mg pyrimethanil/L] (mean measured)							
EC_{50} (96 h)	3.9 (95% confidence limits: 2.4 - 5.4)						
NOEC (96 h)	1.3						

* Statistically significant difference compared to pooled control (Bonferroni t-test, $\alpha = 0.05$).
1) Percent inhibition compared to the pooled control.

III. CONCLUSION

In a flow-through acute toxicity study with eastern oysters (*Crassostrea virginica*), the EC_{50} (96 h) for pyrimethanil was 3.9 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be 1.3 mg a.s./L (mean measured).

CA 8.2.5 Long-term and chronic toxicity to aquatic invertebrates

CA 8.2.5.1 Reproductive and development toxicity to *Daphnia magna*

This point is not addressed via new toxicity studies.

A 21 d chronic study on *Daphnia* performed with pyrimethanil was already evaluated during the previous Annex I inclusion process. The summary of the study and new statistical (ECx) recalculations is provided below.

Report:	CA 8.2.5.1/1 Barber I.,Lattimore A.E., 1992 a Determination of the effects of (14C)-SN 100309 on the life-cycle of Daphnia magna A81880
Guidelines:	EPA 540/9-86-141, OECD 202 Part II (1984)
GLP:	yes (certified by Department of Health and Social Security of the Government of the United Kingdom, United Kingdom)

Material and methods:
Test material: SN100309 (pyrimethanil)
Lot/Batch No: CR 19325/4
Purity: 96.1% w/w

The chronic effects of [^{14}C]-pyrimethanil on the survival, growth and reproduction on *Daphnia magna* were determined. Four replicates of 10 daphnids (< 24 hours old) were incubated under semi static conditions for 21 days with daily feeding and observation. The nominal exposure concentrations were 0.2, 0.44, 0.97, 2.13 and 4.67 mg/L, further one water and one solvent (DMT) control were prepared. Test solutions were renewed every 2 – 3 days and samples of the freshly prepared and used test solutions were analysed for pyrimethanil.
The temperature of the test media was 20 ± 1°C and the mean measured pH-values ranged from 7.63 – 7.74. The water hardness was 252 ± 2 mg/L (as $CaCO_2$) and the dissolved oxygen content was between 8.0 to 8.4 mg O_2/L.
At the end of the test survival, growth and reproduction of treated groups were compared to the control groups and results were analysed with different statistical test methods.

Results:
The analytical data indicated that test concentrations were maintained within ± 20 % of nominal concentration during the study. Therefore the results are based on nominal concentrations.

Table 8.2.5.1-1: Measured concentrations of pyrimethanil as a percentage of nominal in freshly prepared and used test solutions

	Fresh Solutions						Used Solutions				
Time of Preparation	0.2	0.44	0.97	2.13	4.67	5.00[a]	0.2	0.44	0.97	2.13	4.67
0	100.7	98.4	99.9	101.0	104.0	-	101.9	101.4	101.2	102.9	103.7
2	102.3	108.3	105.7	107.2	104.9	106.5	102.2	108.3	105.2	106.3	108.4
4	106.8	101.5	104.4	108.1	110.1	107.8	101.6	102.4	104.7	106.8	-
6	102.1	102.6	103.7	101.9	-	103.4	101.0	101.9	100.9	103.2	-
9	95.4	95.1	101.8	104.0	-	103.1	103.1	99.4	101.1	102.0	-
11	98.2	100.1	101.8	101.4	-	101.4	100.7	98.8	101.4	99.2	-
13	103.3	103.0	105.0	104.1	-	102.4	101.1	101.0	101.7	104.0	-
16	104.6	104.4	105.1	103.8	-	104.1	102.0	105.8	103.1	103.0	-
18	101.0	105.9	105.3	105.9	-	105.6	106.4	109.0	108.8	108.8	-

(a) stock solution
(-) not measured

Survival and reproduction of daphnids were significantly reduced at 2.13 mg/L, but the growth of survived daphnids at this treatment level was not significantly influenced. At the highest concentrations 4.67 mg/L all daphnids died. The 21 day EC_{50} was calculated as 1.87 mg/L (95 % CL 1.63 – 2.14). Based on the most sensitive endpoints (reproduction and mortality) the NOEC was determined to be 0.94 mg/L and the LOEC was 2.13 mg/L.
The results of the study are summarized in the tables below.

Table 8.2.5.1-2: Total parental *Daphnia magna* survival on Day 7, 14 and 21

	Percent mortality (a) Nominal [^{14}C]-SN 100309 concentration (mgl $^{-1}$)						
	Control	Solvent Control	0.2	0.44	0.97	2.13	4.67
7 days	0 (0/30)	3 (1/30)	0 (0/30)	0 (0/30)	0 (0/30)	0 (0/30)	100 (30/30)
14 days	3 (1/30)	7 (2/30)	0 (0/30)	0 (0/30)	0 (0/30)	17 (5/30)	100 (30/30)
21 days	3 (1/30)	10 (3/30)	0 (0/30)	0 (0/30)	0 (0/30)	67 (20/30)	100 (30/30)

(a) - percent mortality (number of daphnids dead/number of daphnids exposed)

Table 8.2.5.1-3: Cumulative live offspring production per adult over the duration of the study

Daphnia	Nominal [^{14}C]-SN 100309 concentration (mgl $^{-1}$) Control	Solvent Control	0.2	0.44	0.97	2.13
1	124	113	157	161	136	0
2	149	115	131	144	122	2
3	131	132	135	149	132	-
4	132	116	121	131	140	0
5	126	118	138	144	126	0
6	130	122	136	138	142	2
7	118	-	109	137	134	0
8	119	126	120	131	138*	-
9	132	132	128	135	139	-
10	126	121	136	125	137	-
Mean	128.7	121.7	131.1	139.5	134.6	0.7
St. error	2.8	2.4	4.1	3.3	2.0	0.4

(*) Animal died on day 20 after releasing 5 broods, so value included in subsequent analyses.

(-) Animal died before end of study, so value not included in subsequent analyses.

Table 8.2.5.1-4: Body lengths (mm) of surviving adult daphnids at the end of the study

	Nominal [^{14}C]-SN 100309 concentration (mg l^{-1})					
Daphnia	Control	Solvent Control	0.2	0.44	0.97	2.13
1	4.307	4.400	4.585	4.307	4.446	4.029
2	4.261	4.307	4.307	4.354	4.400	4.400
3	4.493	4.400	4.354	4.400	4.307	-
4	4.400	4.261	4.307	4.168	4.400	4.400
5	4.354	4.354	4.354	4.354	4.400	4.354
6	4.354	4.539	4.354	4.354	4.400	4.307
7	4.307	-	4.307	4.261	4.400	4.400
8	4.354	4.493	4.354	4.446	-	-
9	4.446	4.493	4.354	4.354	4.400	-
10	4.400	4.400	4.400	4.400	4.400	-
Mean	4.368	4.405	4.368	4.340	4.395	4.315
st. error	0.022	0.030	0.026	0.025	0.012	0.059

(-) Animal died before end of study.

Table 8.2.5.1-5: Summary of toxicity values

Parameter	NOEC (mg l^{-1})	LOEC (mg l^{-1})
Survival	0.97	2.13
Growth	2.13	4.67
Reproduction	0.97	2.13

Calculation of ECx values (DocID: 2017/1074959)

In accordance with current requirements, additional calculations of ECx values have been performed:

Data for the recalculation were taken from the report. The effects are compared to nominal concentrations since concentration control analytics proved that test concentrations were in the range of ±20% of nominal. Statistical recalculation was performed with ToxRatProfessional Version 2.10.

	Test concentrations [mg a.s./L]					
	Control	Solvent Control	0.2	0.44	0.97	2.13
Mean number of living young after 21d	128.7	121.7	131.1	139.5	134.6	0.67
% effect on reproduction after 21d*	--	--	-4.6	-11.3	-7.4	99.5

*negative values indicate a better reproduction than in the control

Reproduction (mean cumulative offspring): Parameter of the probit analysis; results of the regression analysis:

Parameter	Value
Computation runs:	13.0000
Slope b:	21.0543
Intercept a:	-4.35934
Variance of b:	1944246912
Goodness of Fit	
Chi²:	0.0000
Degrees of freedom:	2.0000
p(Chi²):	1.0000
Log EC50:	0.2071
SE Log EC50:	254.0946
g-Criterion:	0.0271
Residual Variance	
(Chi²/df):	0.0000
r²:	0.9970
F:	684.0270
p(F) (df: 1;2):	0.0010

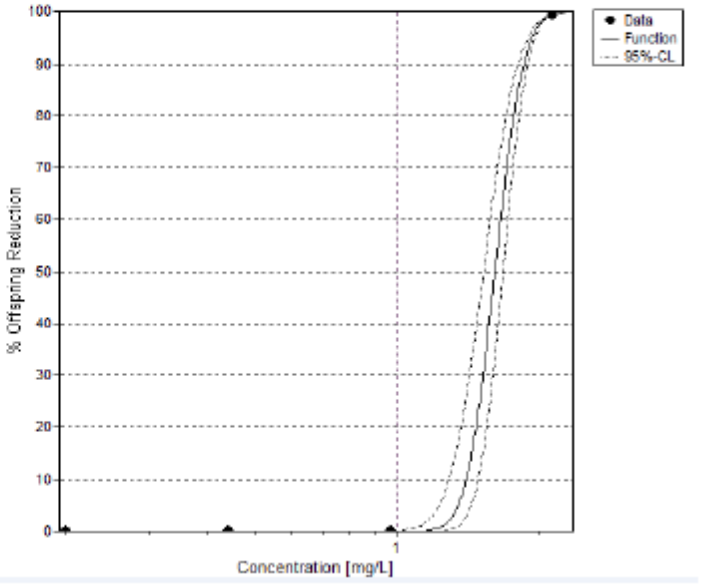


Figure: Concentration-effect curve showing the influence of the test item on mean cumulative offspring of the introduced Daphnia magna as observed after 21d.

Results:

Endpoint	EC_{10} (mg/L)	EC_{20} (mg/L)	NOEC
Reproduction	1.400 (95%-CL 1.289 – 1.486)	1.469 (95%-CL 1.366 – 1.548)	0.97

Conclusion:
The 21-day no-observed-effect concentration (NOEC) is 0.97 mg pyrimethanil /L (reproduction and mortality) and EC_{10} is 1.4 mg pyrimethanil /L, based on nominal concentrations.

CA 8.2.5.2 Reproductive and development toxicity to an additional aquatic invertebrate species

The following flow-through life-cycle study with the saltwater mysid *Americamysis bahia* was conducted for registrations outside the EU. The study is provided for completeness and has not been evaluated previously on EU level.

Report:	CA 8.2.5.2/1 Drottar K.R. et al., 2000a Pyrimethanil: A flow-through life-cycle toxicity test with the saltwater mysid (Mysidopsis bahia) B003354
Guidelines:	EPA 850.1350
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

The chronic toxicity of pyrimethanil to saltwater mysids (*Americamysis bahia*) was evaluated in a 28-day full-life cycle test under flow-through conditions. Mysids were exposed to a dilution water control, a solvent control and to nominal test item concentrations of 0.13, 0.25, 0.50, 1.0 and 2.0 mg pyrimethanil/L (corresponding to mean measured concentrations of 0.12, 0.25, 0.50, 1.0 and 2.0 mg a.s./L). Survival, reproductive success and symptoms of toxicity were assessed throughout the study. Length and dry weight of saltwater mysids was determined at test termination.

The biological results are based on mean measured concentrations. Survival of juvenile mysids on day 14 was statistically significantly affected compared to the pooled control at the highest test item concentrations of 2.0 mg pyrimethanil/L. Because all mysids in the highest treatment were dead on day 14, the 2.0 mg a.s./L treatment group was excluded from the statistical analyses of survival on day 28, reproduction, length and dry weight. Length of adult mysids showed statistically significant differences to the pooled control at the second highest test item concentrations of 1.0 mg a.s./L. Comparison of reproduction rates and dry weight data for males and females did not establish statistically significant effects, compared to the pooled control data.

In a flow-through chronic toxicity study with saltwater mysids (*Americamysis bahia*), the overall NOEC (28 d) for pyrimethanil was determined to be 0.50 mg a.s./L based on mean measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Pyrimethanil (BAS 605 F; Reg. no.: 236 999); product code: AE B100309 00 1D99 0010, batch no. AACA01168, purity: 99.4%.

B. STUDY DESIGN

Test species: Saltwater mysid (*Mysidopsis bahia;* nowadays called: *Americamysis bahia*), juveniles, age: less than 24 hours old; source: in-house cultures.

Test design: Flow-through system (28 d); 5 test item concentrations plus a dilution water control and a solvent control; 4 replicate test chambers for each test item concentration, the control and the solvent control, prior to sexual maturity, 15 mysids were held in one compartment placed in each replicate test chamber (giving a total of 60 mysids per concentration); when mysids attained sexual maturity (day 14), reproductive pairs were placed in reproductive compartments (1 pair per compartment, 5 pairs per test chamber); immature mysids and extra females were discarded, left over sexually mature males were maintained in separate compartments within the test chambers; daily assessment of survival and symptoms of toxicity, assessment of reproduction (number of offspring produced by each female); determination of length and dry weight of surviving first-generation mysids at test termination.

Endpoints: NOEC based on survival, reproductive success, length and dry weight.

Test concentrations: Control (dilution water), solvent control (0.1 mL dimethylformamide/L), 0.13, 0.25, 0.50, 1.0 and 2.0 mg pyrimethanil/L (nominal), corresponding to mean measured concentrations of 0.12, 0.25, 0.50, 1.0 and 2.0 mg a.s./L.

Test conditions: Test chambers: 9 L glass aquaria, test volume: 5 L; compartments prior to pairing of mysids: glass culture dishes with nylon mesh screen attached to two holes on opposite sides; reproductive compartments: glass petri dishes with sides of nylon mesh screen; dilution water: natural seawater diluted with well water, filtered, aerated and sterilized; flow rate: approximately 9 volume additions/test chamber/24 h; salinity: 20‰; temperature: 23.6 - 26.1 °C; pH 8.2 - 8.3; oxygen content: 4.7 mg/L - 6.8 mg/L; photoperiod 16 h light : 8 h dark; light intensity: 60 lux; mysids were fed with live brine shrimps nauplii two to four times per day (except for the last day of the test).

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with UV detection.

Statistics: Descriptive statistics; NOEC values were determined using 2 x 2 contingency tables for survival data ($\alpha = 0.05$); Bonferroni t-test for growth data ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at test initiation and after 7, 14, 21 and 28 days, except for the highest test item concentrations where analytical measurements were only conducted at test initiation and after 7 and 14 days. The mean measured concentrations for pyrimethanil in the test substance treatments during the 28-day exposure ranged from 92% to 100% of the nominal concentrations. The following biological results are based on mean measured concentrations.

Biological results: No statistically significant differences were determined between the control and the solvent control data. Thus, the data from both control groups were pooled and the pooled data set was used for statistical evaluation of treatment related effects. Survival of juvenile mysids on day 14 was statistically significantly affected compared to the pooled control at the highest test item concentrations of 2.0 mg pyrimethanil/L (2 x 2 contingency table, $\alpha = 0.05$). Because all mysids in the highest treatment were dead on day 14, the 2.0 mg a.s./L treatment group was excluded from the statistical analyses of survival on day 28, reproduction, length and dry weight. Length of adult mysids was statistically significantly different to the pooled control at the second highest test item concentrations of 1.0 mg a.s./L (Bonferroni t-test, $\alpha = 0.05$). Comparison of reproduction rates and dry weight data for males and females did not establish statistically significant effects, compared to the pooled control data. The results are summarized in Table 8.2.5.2-1.

Table 8.2.5.2-1: Chronic toxicity (28 d) of pyrimethanil to saltwater mysids (*Americamysis bahia*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.13**	**0.25**	**0.50**	**1.0**	**2.0**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**0.12**	**0.25**	**0.50**	**1.0**	**2.0**
Survival (juvenile mysids) on day 14 [%]	92	98	98	92	98	95	0 *
Survival (adult mysids) on day 28 [%]	71	82	66	80	78	82	n.d.
Reproductive success [mean number of young per reproductive day]	0.462 ± 0.0596	0.434 ± 0.147	0.350 ± 0.104	0.359 ± 0.0948	0.383 ± 0.185	0.261 ± 0.123	n.d.
Mean length of adult mysids on day 28 [mm]	7.70 ± 0.102	7.69 ± 0.0938	7.60 ± 0.202	7.66 ± 0.122	7.55 ± 0.0909	7.29 ± 0.120 #	n.d.
Mean dry weight of adult mysids on day 28 [mg]	0.992 ± 0.0756	0.978 ± 0.0691	0.904 ± 0.0492	1.05 ± 0.0796	0.971 ± 0.0445	0.902 ± 0.0651	n.d.
Endpoints [mg pyrimethanil/L] (mean measured)							
NOEC $_{overall}$ (28 d)	0.50						

* Statistically significant differences compared to the pooled control (2 x 2 contingency table, $\alpha = 0.05$).
\# Statistically significant differences compared to the pooled control (Bonferroni t-test, $\alpha = 0.05$).
n.d. = not determined, all individuals dead

III. CONCLUSION

In a flow-through chronic toxicity study with saltwater mysids (*Americamysis bahia*), the overall NOEC (28 d) for pyrimethanil was determined to be 0.50 mg a.s./L based on mean measured concentrations.

Calculation of ECx values (DocID: 2017/1074959)

In accordance with current requirements, additional calculations of ECx values have been performed.

No meaningful ECx could be calculated for this study due to unclear dose-effect response in the different test concentrations. As the table below (inhibition values in mean offspring) shows, the first three test concentrations show a similar quantitative response, which means that the slope of the statistically determined relationship is not significant different from zero. Due to lacking concentrations-response in the lower test concentrations, reliable EC_x values cannot be calculated.

	Test concentrations [mg a.s./L]						
	Control	Solvent control	0.12	0.25	0.5	1.0	2.0
Mean number of living young per reproductive day after 21d	0.46	0.43	0.35	0.36	0.38	0.26	--*
% effect on reproduction	--	--	21.8	19.9	14.5	41.7	100

*all animals dead at test end

Results:

Endpoint	EC_{10} (mg/L)	EC_{20} (mg/L)	NOEC
Reproduction	n.d. (95%-C: n.d.)	n.d. (95%-CL: n.d.)	0.5

n.d. = not determined due to mathematical reasons or inappropriate data

Conclusion:

The 28-day no-observed-effect concentration (NOEC) is 0.5 mg pyrimethanil /L (growth), based on mean measured concentrations. No reliable ECx can be calculated.

CA 8.2.5.3 Development and emergence in Chironomus riparius

A spiked water toxicity study on *Chironomus riparius* performed with pyrimethanil was already evaluated during the previous Annex I inclusion process. The summary of the study and new statistical (ECx) recalculations is provided below.
No additional studies are required and no (new) study has been conducted.

Report:	CA 8.2.5.3/1 Mattock S.D., 1998 a Pyrimethanil - Substance technical 99% w/w - Code: AE B100309 00 1D99 00 - Pyrimethanil: Effects on sediment dwelling Chironomus riparius in a water sediment system A91867
Guidelines:	OECD 207, BBA proposal: Effects of plant protection products on the development of sediment dwelling larvae of Chironomus riparius in water-sediment system 1995
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Material and methods:
Test material: Pyrimethanil technical
Lot/Batch No: CR 19325/01/900304
Purity: 97.5% w/w

The toxicity of ^{14}C-pyrimethanil to sediment dwelling larvae of *Chironomus riparius* was investigated in a 28 day static sediment toxicity test. For each tested treatment (0.5, 1.0, 2.0, 4.0 and 8.0 mg/L), five test vessels (3 biological replicates and 2 analytical samples) were prepared containing ca. 300 g (2 cm depth) of an artificial sediment and 2.5 l overlying water. 25 first instar larvae were applied to each biological test vessel after a standing period of 7 days. 24 h after the addition of the test organisms pyrimethanil was applied to the water sediment test system. For the test period the following mean water quality parameters were reported: Temperature 20.2°C (19.6 – 22.1°C), pH 7.0 (6.1 – 7.5), oxygen saturation 98 % (90 – 101 %) and conductivity 251 µS (227 – 294µS).
Samples for sediment analysis were taken from additional analytical test vessels on days 0 and 7, samples taken on day 28 were taken from one of the replicate biological test vessels. Pore water samples were taken on day 0, 7 and 28. Samples for analysis of overlying water were taken from analytical test vessels at concentrations 0.5, 2.0 and 8.0 mg/L on day 0, 7, 14, 21 and 28.

Results:
Results of chemical analysis of the pyrimethanil concentrations in the overlying water within one hour of treatment indicated that the nominal concentrations of 0.5, 1.0, 2.0, 4.0 and 8.0 mg/L were achieved. Therefore, the toxicity of pyrimethanil to *Chironomus riparius* is expressed based on the nominal initial concentrations of pyrimethanil in overlying water.

Table 8.2.5.3-1: Results of the chemical analysis: measured ^{14}C-pyrimethanil concentrations in overlaying water, pore water and sediment

Test sample [mg/L]	0.5	1.0	2.0	4.0	8.0
Overlaying water [mg/L]					
Day 0	0.535	1.060	2.146	4.324	8.269
Day 7	0.280	0.601	1.257	2.778	5.389
Day 14	0.224	0.438	0.915	2.138	4.317
Day 21	0.196	0.402	0.796	1.865	3.915
Day 28	0.150	0.353	0.727	1.513	3.419
Pore water [mg/L]					
Day 0	0.008	0.017	0.035	0.081	0.102
Day 7	0.20	0.106	0.267	0.634	1.079
Day 28	0.016	0.065	0.095	0.237	0.495
Sediment [mg/kg]					
Day 0	0.130	0.100	0.505	0.204	1.925
Day 7	1.101	2.484	4.058	8.152	14.716
Day 28	1.443	3.397	5.651	18.779	26.622

The emergence of *Chironomus riparius* started in all treatments (except 8.0 mg/L) on day 13. In the highest treatment the emergence started on day 14, 15 and 16 respectively. No significant difference of development between male and female midges was noticed.

Table 8.2.5.3-2: Emergence and development rate of *Chironomus riparius* exposed to ^{14}C-pyrimethanil

test sample [mg/L]	control	solvent	0.5	1.0	2.0	4.0	8.0
emergence [%]	87	91	91	97	96	72	27***
development rate [%/day]	7.1	7.3	7.1	7.0	7.3	6.9	6.2*

* P<0.05, *** P<0.001 (2-sides t-test; 1-sided Dunnetts test)

The 28 day EC_{50} (emergence) based on nominal concentrations was calculated with 6.13 mg parent equivalents/L (CL95% 5.61 – 6.76). The NOEC was determined to be 4 mg/L.

Calculation of ECx values (DocID: 2017/1074959)
In accordance with current requirements, additional calculations of ECx values have been performed:

Data for the recalculation was taken from the report. The effects are compared to nominal test concentrations. Statistical recalculation was performed with ToxRatProfessional Version 2.10.

Nominal conc. (mg/L)	Day 28 emergence and survival [%]	% Effect compared to the pooled control*	Development rate	% Inhibition*
Control	87	-	0.072	-
Solvent control	91	-	0.073	-
0.5	91	-2.3	0.071	1.9
1.0	97	-9.0	0.070	2.7
2.0	96	-7.9	0.073	-1.6
4.0	72	19.9	0.069	5.1
8.0	25	71.9	0.062	13.9

*negative values indicate a better performance than in the control group.

No statistical significant difference exists between control and solvent control. Comparison of treatment effects was therefore done to the pooled control. No reliable EC_X calculation was possible for developmental rate due to the low occurrence of effects.

Emergence rate: Parameter of the probit analysis; results of the regression analysis:

Parameter	Value
Computation runs:	9.0000
Slope b:	5.1209
Intercept a:	-4.08726
Variance of b:	0.4099
Goodness of Fit	
Chi^2:	0.6401
Degrees of freedom:	3.0000
p(Chi^2):	0.8872
Log EC50:	0.7982
SE Log EC50:	0.0227
g-Criterion:	0.0601
F:	299.8410
p(F) (df: 1;3):	0.0000

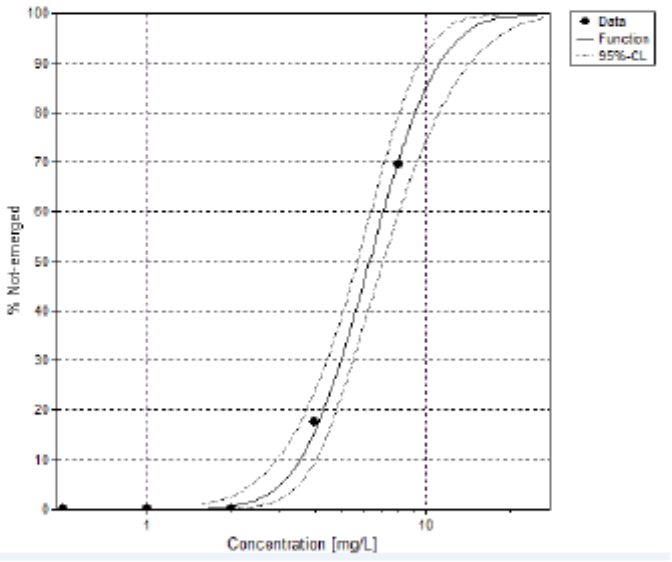


Figure: Concentration-effect curve showing the influence of the test item on emergence rate of males and females of the introduced Chironomus riparius as observed after 28 d.

Results: The results based on measured concentrations are:

Endpoint	EC_{10} (mg/L)	EC_{20} (mg/L)
Emergence rate of male/ females	3.531 (95%-CL 2.92 – 4.02)	4.30 (95%-CL 3.74 – 4.79)
Developmental rate males/ females	n.d.	n.d.

n.d. = not determined due to mathematical reasons

Conclusion:
The 28-day statistically significant no-observed-effect concentration (NOEC) is 4 mg pyrimethanil /L and EC10 is 3.531 mg pyrimethanil /L, based on initial nominal concentrations.

CA 8.2.5.4 Sediment dwelling organisms

This point is not triggered and not addressed via (new) toxicity studies.

CA 8.2.6 Effects on algal growth

CA 8.2.6.1 Effects on growth of green algae

A study on *Selenastrum capricornutum* performed with pyrimethanil was already evaluated during the previous Annex I inclusion process. The summary of the study and new statistical recalculations is provided below. No additional studies are required and no (new) study has been conducted.

Report:	CA 8.2.6.1/1 Jenkins C.A., 1991 a SN 100 309: Determination of its EC50 to (Selenastrum capricornutum) A81883
Guidelines:	OECD 201
GLP:	yes (certified by Department of Health and Social Security of the Government of the United Kingdom, United Kingdom)

Material and methods:
Test material: SN100309 (pyrimethanil)
Lot/Batch No: CR 19325/4
Purity: 95.5% w/w

The objective of the study was to assess the effects of pyrimethanil on the growth of the unicellular green alga *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*). The algal cultures (1 x 10^4 cells/mL) were exposed to five nominal concentrations: 0.32, 3.2, 10, 32 and 100 mg/L, as well as to one dilution and one solvent control. The test samples were incubated up to 96 hours under static conditions, at temperatures from 21.5 – 22.4 °C and continuously illumination. The cell density was determined by cell counts with a haemocytometer. For the determination of algistatic or algicidal effects at the end of the test, samples of the two highest concentrations (32 and 100 mg/L) were transferred to fresh test medium without test substance, and incubated up to ten days.

Results:
The initial mean measured test concentrations at concentration levels up to 3.2 mg/L were within 20 % of nominal concentrations, whilst the higher concentrations were significantly lower than nominal. After 96 h the measured test concentrations were between 94 and 125 % of initial. Since all effect concentrations were observed within the initial measured concentration range, thus reported results are based on nominal test concentrations.

Table 8.2.6.1-1: Measured concentrations of pyrimethanil in test samples

Nominal conc. (mg/l)	Measured SN 100309 concentrations (ug/l) 0 hours	mean values as % of nominal	96 hours	mean values as % of nominal	% of t0
0	nd,nd	-	nd,nd	-	-
0.32	0.33,0.32	102	0.33,0.33	103	100
1.0	1.01,1.01	101	1.08,1.03	106	104
3.2	2.65,2.68	83	2.80,2.85	88	106
10	4.28,4.68	45	5.55,5.67	56	125
32	13.5,15.9	46	16.6,16.7	52	113
100	23.6,27.8	26	23.0,25.4	24	94

nd none detected.

% of t0 mean measured concentrations after 96 hours, expressed as a percentage of the mean starting concentrations.

The algal biomass (area under the curve) was significantly inhibited at all test concentrations compared to the solvent control, whereas growth rate (slope) was reduced at concentrations greater than 1 mg/L. Thus, the NOEC for biomass and for growth rate were < 0.32 mg/L and 1 mg/L. The 96 h E_bC_{50} and E_rC_{50} were estimated to be 1.2 mg/L (95% CL 0.67 – 1.91 mg/L) and 5.84 mg/L (95% CL 4.84 - 7.04 mg/L).
The subsequent determination of algistatic or algicidal effects at the end of the test indicated that pyrimethanil was algicidal at 32 and 100 mg/L.

Table 8.2.6.1-2: Average specific growth and biomass (0-96 hours) in test and control cultures

Nominal test material conc. (mg/l)	Flask number	Average specific growth rate x 10^{-2}	Mean x 10^{-2}	Biomass	Mean
0 (control)	1	5.102		4215	
	2	4.547	4.779	2822	3329
	3	4.689		2951	
0 (acetone)	5	5.013		3666	
	6	4.682	4.830	3144	3360
	7	4.797		3270	
0.32	9	4.805		2937	
	10	4.513	4.537	2201	2072
	11	4.293		1077	
1.0	13	4.582		2600	
	14	4.153	4.368	1697	2080
	15	4.368		1944	
3.2	17	3.261		899	
	18	3.414	3.495	921	1087
	19	3.810		1440	
10	21	2.880		575	
	22	3.878	3.316	1151	843
	23	3.190		803	
32	25	0.232		0	
	26	-0.139	-0.132	-14	-3
	27	-0.490		5	
100	29	-0.139		-14	
	30	-1.022	-0.728	-44	-31
	31	-1.022		-35	

Recalculations (BASF DocID 2017/1073965):
In accordance with current requirements, additional recalculations based on mean measured concentrations after 72 h and 96 h have been performed. In addition, the EC_{10} and EC_{20} values have been calculated.

Data for the recalculation were taken from the report. The effects are compared to mean measured concentrations. Statistical recalculation was performed with ToxRatProfessional Version 2.10.

Calculation of mean measured concentrations:

Nominal conc. (mg/L)	0.32	1	3.2	10	32	100
Mean found over 96h (%)	102.5	103.5	85.5	50.5	49	25
Mean conc. over 96h (mg/L)	0.328	1.035	2.736	5.05	15.68	25

Inhibition of growth rate and yield after 72h and 96h test duration:

Mean measured concentration (mg/L)	Inhibition %			
	Growth rate (72h)	Growth rate (96h)	Yield (72h)	Yield (96h)
Control	--	--	--	--
0.328	12.1	5.6	38.5	22.7
1.04	14.3	9.1	44.6	34.8
2.74	28.1	27.2	69.3	72.0
5.05	33.2	31.0	76.0	75.1
15.68	100	100	100	100
25	100	100	100	100

No statistical significant difference exists between control and solvent control. Comparison of treatment effects was therefore done to the pooled control.

Yield (72h): the following parameters were used for the probit analysis:

Parameter	Value
Computation runs:	7
Slope b:	1.12708
Intercept a:	0.04298
Variance of b:	0.41016
Goodness of Fit	
Chi^2:	1.52336
Degrees of freedom:	16
$p(Chi^2)$:	1.00000
Log EC50:	-0.03814
SE Log EC50:	0.31107
g-Criterion:	0.13826
Residual Variance (Chi^2/df):	0.09521
r^2:	0.670
F:	32.529
p(F) (df: 1;16):	0.000

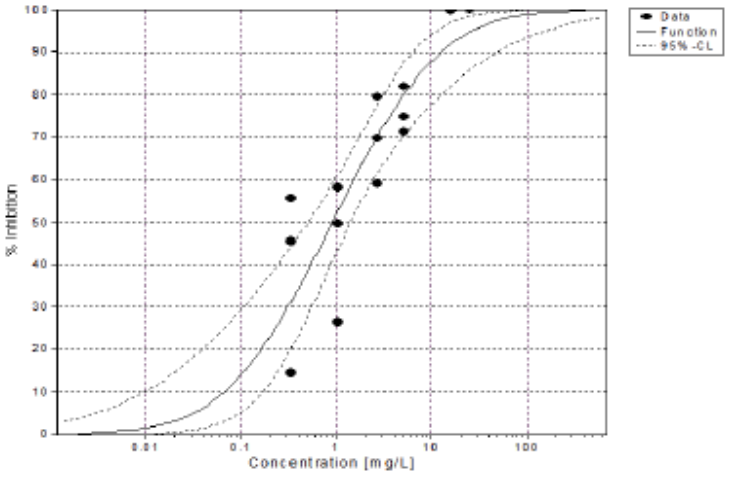


Figure: Concentration-effect curve showing the influence of the test item on yield of the introduced P. subcapitata as observed after 72 h.

Growth rate (72h): the following parameters were used for the probit analysis:

Parameter	Value
Computation runs:	13
Slope b:	2.76127
Intercept a:	-2.04890
Variance of b:	2.97946
Goodness of Fit	
Chi^2:	1.42254
Degrees of freedom:	16
$p(Chi^2)$:	1.00000
Log EC50:	0.74201
SE Log EC50:	0.16099
g-Criterion:	0.15626
Residual Variance (Chi^2/df):	0.08891
r^2:	0.643
F:	28.783
p(F) (df: 1;16):	0.000

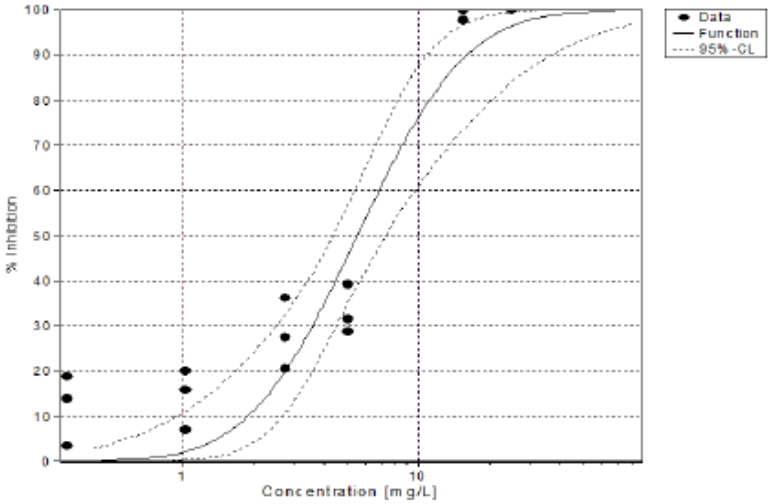


Figure: Concentration-effect curve showing the influence of the test item on growth rate of the introduced P. subcapitata as observed after 72 h.

Results for 72h and 96h with mean measured test concentrations:

Endpoint	NOEC (mg/L)	EC_{10} (mg/L)	EC_{20} (mg/L)	EC_{50} (mg/L)
Growth rate (72h)	< 0.328	1.896 (95%-CL 0.95 – 2.7)	2.737 (95%-CL 1.69 – 3.6)	5.521 (95%-CL 4.36 – 7.3)
Yield (72h)	< 0.328	n.d.	0.164 (95%-CL 0.04 - 0.33)	0.916 (95%-CL 0.51 - 1.4)
Growth rate (96h)	0.328	2.138 (95%-CL 1.27 – 2.8)	3.016 (95%-CL 2.09 – 3.8)	5.825 (95%-CL 4.79 – 7.3)
Yield (96h)	< 0.328	0.206 (95%-CL 0.07 - 0.37)	0.399 (95%-CL 0.18 - 0.63)	1.413 (95%-CL 0.97 –1.95)

n.d. = not determined due to mathematical reasons or inappropriate data

For the purpose of the renewal of pyrimethanil, the coefficients of variation of average specific growth rates were calculated for both 96- and 72-hour period and are presented in the tables below:

Table 8.2.6.1-3: Average coefficient of variance at 0-96 hours and section-by-section in the control cultures

Negative control							
Replicates	**0-96 h**			**Section by section (day 0-1, 1-2, 2-3, 3-4)**			
	Average growth rate (day^{-1})	**St Dev**	**CV (%)**	**Average growth rate (day^{-1})**	**St Dev**	**CV (%)**	**Mean CV (%)**
A				1.22	0.55	45.02	
B	1.15	0.069	6.04	1.09	0.59	53.97	49.77
C				1.13	0.57	50.30	
Solvent control							
Replicates	**0-96 h**			**Section by section (day 0-1, 1-2, 2-3, 3-4)**			
	Average growth rate (day^{-1})	**St Dev**	**CV (%)**	**Average growth rate (day^{-1})**	**St Dev**	**CV (%)**	**Mean CV (%)**
A				1.20	0.50	41.36	
B	1.16	0.40	3.50	1.12	0.60	53.38	48.84
C				1.15	0.60	51.78	

Table 8.2.6.1-4: Average coefficient of variance at 0-72 hours and section-by-section in the control cultures

Negative control							
Replicates	**0-72 h**			**Section by section (day 0-1, 1-2, 2-3)**			
	Average growth rate (day^{-1})	**St Dev**	**CV (%)**	**Average growth rate (day^{-1})**	**St Dev**	**CV (%)**	**Mean CV (%)**
A				1.46	0.35	23.83	
B	1.40	0.052	3.75	1.37	0.23	17.01	22.11
C				1.37	0.35	25.48	
Solvent control							
Replicates	**0-72 h**			**Section by section (day 0-1, 1-2, 2-3)**			
	Average growth rate (day^{-1})	**St Dev**	**CV (%)**	**Average growth rate (day^{-1})**	**St Dev**	**CV (%)**	**Mean CV (%)**
A				1.42	0.30	20.87	
B	1.41	0.007	0.51	1.41	0.22	15.76	21.10
C				1.41	0.37	26.66	

According to current OECD 201 guideline (2006) the validity criteria were met for 72 hours test period (the specific growth rate in control was 1.40 and 1.41 per day within the 72-hour period in negative and solvent control, respectively (should be greater than 0.92 per day); the mean coefficient of variation for section-by section specific growth rates in the control cultures was 22.11 and 21.10% in negative and solvent control, respectively (should be less than 35%); the coefficient of variation of average specific growth rates during the whole test period in replicate control cultures was 3.75 and 0.51% in negative and solvent control, respectively (should be less than 7%)). For the 96-hour test period the validity criteria were also met, except for the mean coefficient of variation for section-by section specific growth rates in the control cultures: it was 49.77 and 48.84% in negative and solvent control, respectively (should be less than 35%).
Thus, for the 72-hour test period, the study is considered valid and acceptable for regulatory use.

Conclusion:
The 72-hour E_rC_{50} is 5.521 mg pyrimethanil/L , the 72-hour E_yC_{50} is 0.916 mg pyrimethanil /L, the 72-hour no-observed-effect concentration (NOEC) is <0.328 mg pyrimethanil /L, based on mean measured concentrations.

CA 8.2.6.2 Effects on growth of an additional algal species

The following algae studies performed with the active substance pyrimethanil were conducted for registrations outside the EU. The studies are provided for completeness and have not been evaluated previously on EU level. In accordance to the EFSA Aquatic Guidance Document (EFSA, 2013) and OECD guideline 201 (2011) the 72 h endpoints obtained in the 96 h studies are considered as relevant endpoints for the aquatic risk assessment, therefore both the 72 h and 96 h endpoints are reported in the study summaries below if appropriate.

Report:	CA 8.2.6.2/1 Desjardins D. et al., 2001d Pyrimethanil: A 96-hour toxicity test with the freshwater alga (Anabaena flos-aquae) B003422
Guidelines:	EPA 850.5400, OECD 201, EEC 92/69 C 3, ASTM E 1218-90
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

In a 96-hour static toxicity laboratory study, the effect of pyrimethanil on growth of the blue-green alga *Anabaena flos-aquae* was investigated. The following nominal concentrations were applied: 0.25, 0.50, 1.0, 2.0 and 4.0 mg pyrimethanil/L (corresponding to mean measured concentrations of 0.22, 0.47, 0.94, 1.9 and 3.9 mg a.s./L). Additionally, a solvent control (dimethylformamide) and a dilution water control were set up. Assessment of growth was conducted 24 h, 48 h, 72 h and 96 h after test initiation.

The biological results are based on mean measured concentrations. After 96 hours of exposure, no morphological effects on algae were observed in the control groups and at any of the test item concentrations tested. Furthermore, no statistically significant differences in algal growth compared to the pooled control were observed at any test item concentration.

In a 96-hour algae toxicity test with *Anabaena flos-aquae* both the E_bC_{50} and the E_rC_{50} of pyrimethanil were determined to be > 3.9 mg a.s./L (72h and 96h) based on mean measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Pyrimethanil (BAS 605 F, Reg. No. 236 999); product code: AE B100309 00 1D99 0010, batch no. AACA01168, purity: 99.4% (w/w).

B. STUDY DESIGN

Test species: Freshwater blue-green alga, *Anabaena flos-aquae*; in-house culture; stock originally obtained from "UTCC - The University of Toronto Culture Collection of Algae and Cyanobacteria".

Test design: Static system (96 hours); 5 test concentrations with 3 replicates for each test item concentration, the dilution water control and the solvent control; daily assessment of growth.

Endpoints: EC_{50} with respect to biomass development and growth rate after exposure over 96 hours.

Test concentrations: Control (dilution water), solvent control (0.1 mL dimethylformamide/L), 0.25, 0.50, 1.0, 2.0 and 4.0 mg pyrimethanil/L (nominal), corresponding to mean measured concentrations of 0.22, 0.47, 0.94, 1.9 and 3.9 mg a.s./L.

Test conditions: 250 mL flasks; test volume: 100 mL; sterilized freshwater algal medium; pH 7.1 - 7.4 at test initiation and pH 8.1 - 8.2 at test termination; temperature: 22.5 °C - 23.9 °C; initial cell densities: 1 x 10^4 cells/mL; continuous light at 1950 - 2340 lux, continuous shaking at 100 rpm.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with UV detection.

Statistics: Descriptive statistics; non-linear regression analysis for determination of EC_{50} values for cell density, area under the growth curve and growth rate; Student's t-test ($\alpha = 0.05$) for comparison of control and solvent control data; Dunnett's test for determination of the NOEC values ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each test concentration at the beginning and at the end of the test. Measured concentrations of pyrimethanil ranged from 91.0% to 99.4% of nominal concentrations at test initiation and from 88% to 96.7% of nominal at test termination. The following biological results are based on mean measured concentrations.

Biological results: No statistically significant differences were determined between the control and the solvent control data (Student's t-test, $\alpha = 0.05$). Thus, the data from both control groups were pooled and the pooled data set was used for statistical evaluation of treatment related effects. After 96 hours of exposure, no morphological effects on algae were observed in the control groups and at any of the test item concentrations tested. Furthermore, no statistically significant differences in algal growth compared to the pooled control were observed at any test item concentration (Dunnett's test, $\alpha = 0.05$). The effects on algal growth are summarized in Table 8.2.6.2-1.

Table 8.2.6.2-1: Effect of pyrimethanil on the growth of the blue-green alga *Anabaena flos-aquae*

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.25**	**0.50**	**1.0**	**2.0**	**4.0**
Concentration [mg a.s./L] (mean measured)	--	--	**0.22**	**0.47**	**0.94**	**1.9**	**3.9**
Inhibition in 96 h (mean cell density) [%] *[1)]	--	--	-39	3.7	17	5.7	-32
Inhibition in 96 h (mean area under growth curve) [%] *[1)]	--	--	-18	6.9	13	12	-17
Inhibition in 96 h (growth rate) [%] *[1)]	--	--	-8.9	-0.90	2.5	-0.13	-5.9
	Endpoints [mg pyrimethanil/L] (mean measured)						
EC_{50} (72h & 96 h) E_bC_{50} (72h & 96 h) E_rC_{50} (72h & 96 h) [2)]	> 3.9						

* Negative values indicate stimulated growth compared to the pooled control.

1) Percent inhibition compared to the pooled control.

2) No significant difference between control and solvent control cultures (Student's t-test, $\alpha = 0.05$), therefore data from the control and solvent control was pooled for EC_{50} calculation.

III. CONCLUSION

In a 96-hour algae toxicity test with *Anabaena flos-aquae* both the E_bC_{50} and the E_rC_{50} of pyrimethanil were determined to be > 3.9 mg a.s./L (72h and 96h) based on mean measured concentrations.

Report:	CA 8.2.6.2/2 Abedi J.,Young B.M., 2001a Effect to Skeletonema costatum (marine diatom) in a growth inhibition test - Pyrimethanil technical 99.4 % w/w B003496
Guidelines:	OECD 201, EPA 123-2
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

In a 96-hour static toxicity laboratory study, the effect of pyrimethanil on the growth of the marine diatom *Skeletonema costatum* was investigated. The following nominal concentrations were applied: 0.5, 1.0, 2.0, 4.0 and 8.0 mg pyrimethanil /L (corresponding to mean measured concentrations of 0.46, 0.96, 2.0, 3.9 and 6.6 mg a.s./L). Additionally, a solvent control (*N*,*N*-dimethylformamide) and a dilution water control were set up. Assessment of growth was conducted daily.

The biological results are based on mean measured concentrations. After 96 hours of exposure, statistically significant effects compared to the pooled control based on biomass and growth rate data were observed at the highest tested concentration.

In a 96-hour algae test with *Skeletonema costatum*, the E_bC_{50} (72 h and 96 h) and the E_rC_{50} (72 h and 96 h) of pyrimethanil were both determined to be > 6.6 mg a.s./L based on mean measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Pyrimethanil (BAS 605 F; Reg. no.: 236 999); product code: AE B100309 00 1D99 0099, batch no. AACA00707, purity: 99.4% (w/w).

B. STUDY DESIGN

Test species: Marine diatom, *Skeletonema costatum*, strain CCMP 1332, in-house culture, obtained from Provasoli-Guillard National Center for Culture of Marine Phytoplankton, Bigelow Laboratories, West Boothbay Harbor, Maine, USA.

Test design: Static system (96 hours); 5 test concentrations plus a dilution water control and a solvent control with 3 replicates for each; daily assessment of growth.

Endpoints: EC_{50} with respect to biomass and growth rate, NOEC.

Test concentrations: Control (dilution water), solvent control (0.1 mL *N*,*N*-dimethylformamide/L), 0.5, 1.0, 2.0, 4.0 and 8.0 mg pyrimethanil/L (nominal), corresponding to mean measured concentrations of 0.46, 0.96, 2.0, 3.9 and 6.6 mg a.s./L.

Test conditions: 250 mL glass flasks; test volume: 100 mL; sterilized MAA growth medium; pH 8.1 at test initiation and pH 8.9 - 9.0 at test termination; oxygen content: 6.2 mg/L - 6.4 mg/L at test initiation and 9.7 mg/L - 10.7 mg/L at test termination; temperature: 20.9 °C - 21.0 °C at test initiation and 20.2 °C - 20.6 °C at test termination; salinity: 30‰ at test initiation and 28‰ - 30‰ at test termination; initial cell densities: 1.0 x 10^4 cells/mL; photoperiod of 14 h light: 10 h darkness, light intensity: 4300 ± 645 lux.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with UV detection.

Statistics: Descriptive statistics, t-test for comparison of the control data ($\alpha = 0.05$); Williams Test for determination of 96 h NOEC value ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each test concentration at the beginning and at the end of the test. Measured values for pyrimethanil ranged from 97.7% to 113.0% of nominal concentrations at test initiation and from 66.8% to 88.9% of nominal at test termination. The following biological results are based on mean measured concentrations.

Biological results: No statistically significant differences were observed between the controls (t-test, $\alpha = 0.05$). Hence, the controls were pooled and used as the reference in all evaluations. After 96 hours of exposure, statistically significant effects compared to the pooled control based on biomass and growth rate data were observed at the highest tested concentration (William`s Test, $\alpha = 0.05$). The effects on biomass development and algal growth rate are summarized in Table 8.2.6.2-2.

Table 8.2.6.2-2: Effect of pyrimethanil on the growth of the marine diatom *Skeletonema costatum*

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.5**	**1.0**	**2.0**	**4.0**	**8.0**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**0.46**	**0.96**	**2.0**	**3.9**	**6.6**
Inhibition 96 h (biomass) [%] #	--	--	5	-6	12	10	27 *
Inhibition in 96 h (growth rate) [%] #	--	--	2	-1	5	2	8 *
Endpoints [mg a.s./L] (mean measured)							
E_bC_{50} (72 h & 96 h), E_rC_{50} (72 h & 96 h)	> 6.6						
NOEC (96 h)	3.9						

\# Negative values indicate stimulated growth compared to the pooled control.
* Statistically significant differences compared to the pooled control (William`s Test, $\alpha = 0.05$).

III. CONCLUSION

In a 96-hour algae test with *Skeletonema costatum*, the E_bC_{50} (72 h and 96 h) and the E_rC_{50} (72 h and 96 h) of pyrimethanil were both determined to be > 6.6 mg a.s./L based on mean measured concentrations.

Report:	CA 8.2.6.2/3 Desjardins D. et al., 2001b Pyrimethanil: A 96-hour toxicity test with the freshwater diatom (Navicula pelliculosa) B003421
Guidelines:	EPA 850.5400, OECD 201, EEC 92/69 C 3
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

In a 96-hour static toxicity laboratory study, the effect of pyrimethanil on of the growth of the freshwater diatom *Navicula pelliculosa* was investigated. The following nominal concentrations were applied: 0.25, 0.50, 1.0, 2.0 and 4.0 mg pyrimethanil/L (corresponding to mean measured concentrations of 0.24, 0.49, 0.94, 1.8 and 3.8 mg a.s./L). Additionally, a solvent control (dimethylformamide) and a dilution water control were set up. Assessment of growth was conducted 24 h, 48 h, 72 h and 96 h after test initiation.

The biological results are based on mean measured concentrations. After 96 hours of exposure, there were no noticeable changes in cell shape, size or color in any of the test item treatments. There was evidence of cell aggregation in all test item treatments and the control groups, however, because aggregation was observed in all test item treatment levels it was not considered to be treatment-related. No statistically significant differences compared to the pooled control were observed in any of the test item concentrations tested after 96 hours of exposure.

In a 96 hour algae toxicity test with *Navicula pelliculosa* both the E_bC_{50} (72 h and 96 h) and the E_rC_{50} (72 h and 96 h) for pyrimethanil were determined to be > 3.8 mg a.s./L based on mean measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Pyrimethanil (BAS 605 F; Reg. no.: 236 999), batch no. AACA01168, purity: 99.4%.

B. STUDY DESIGN

Test species: Freshwater diatom, *Navicula pelliculosa*; in-house culture; stock originally obtained from "UTEX - the Culture Collection of Algae, University of Texas, Austin, USA.

Test design: Static system; test duration 96 hours; 5 test concentrations with 3 replicates for each test item concentration, the dilution water control and the solvent control; daily assessment of growth.

Endpoints: EC_{50} with respect to cell density, biomass and growth rate after exposure over 96 hours.

Test concentrations: Control (dilution water), solvent control (0.1 mL dimethylformamide/L), 0.25, 0.50, 1.0, 2.0 and 4.0 mg pyrimethanil/L (nominal), corresponding to mean measured concentrations of 0.24, 0.49, 0.94, 1.8 and 3.8 mg a.s./L.

Test conditions: 250 mL flasks; test volume 100 mL; freshwater algal medium; pH 7.3 - 7.4 at test initiation, pH 7.8 - 8.2 at test termination; temperature: 23.5 °C - 24.0 °C; initial cell densities 1 x 10^4 cells/mL; continuous light at 3890 - 4140 lux; constant shaking at approximately 100 rpm.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with UV detection.

Statistics: Descriptive statistics; Student's t-test ($\alpha = 0.05$) for comparison of the control data; non- linear regression analysis for determination of EC_{50} values, Dunnett's test for determination of the NOEC value ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

Biological results: After 96 hours of exposure, there were no noticeable changes in cell shape, size or color in any of the test item treatments. There was evidence of cell aggregation in all test item treatments and the control groups, however, because aggregation was observed in all test item treatment levels it was not considered to be treatment-related. No statistically significant differences compared to the pooled control were observed in any of the test item concentrations tested (Dunnett's test, $\alpha = 0.05$) after 96 hours of exposure. The effects on algal growth are summarized in Table 8.2.6.2-3.

Table 8.2.6.2-3: Effect of pyrimethanil on growth of the freshwater diatom *Navicula pelliculosa*

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.25**	**0.50**	**1.0**	**2.0**	**4.0**
Concentration [mg a.s./L] (mean measured)	--	--	**0.24**	**0.49**	**0.94**	**1.8**	**3.8**
Inhibition in 96 h (cell density) [%] [1), 2)]	--	--	0.15	-1.2	2.3	-1.1	3.7
Inhibition in 96 h (area under the growth curve) [%] [1), 2)]	--	--	-9.2	-1.6	-6.7	-2.7	-7.9
Inhibition in 96 h (growth rate) [%] [1), 2)]	--	--	-0.072	-0.37	0.40	-0.35	0.72
	Endpoints [mg pyrimethanil/L] (mean measured)						
EC_{50} (72 h and 96 h) E_bC_{50} (72 h and 96 h) E_rC_{50} (72 h and 96 h) [3)]	> 3.8						
NOEC (72 h and 96 h) [3)]	≥ 3.8						

1) Percent inhibition was calculated relative to the pooled control.
2) Negative values indicate stimulated growth compared to the pooled control.
3) No significant difference between control and solvent control cultures (Student's t-test, $\alpha = 0.05$), therefore data from the control and solvent control was pooled for EC_{50} and NOEC calculation.

III. CONCLUSION

In a 96 hour algae toxicity test with *Navicula pelliculosa* both the E_bC_{50} (72 h and 96 h) and the E_rC_{50} (72 h and 96 h) for pyrimethanil were determined to be > 3.8 mg a.s./L based on mean measured concentrations.

CA 8.2.7 Effects on aquatic macrophytes

The following aquatic plant toxicity study performed with the active substance pyrimethanil was conducted for registrations outside the EU. The study is provided for completeness and has not been evaluated previously on EU level.

Report:	CA 8.2.7/1 Christ M.T.,Abedi J., 2002a Effect to Lemna gibba (duckweed) in a growth inhibition test - Pyrimethanil technical 99.4% w/w B003716
Guidelines:	EPA 123-2, OECD 221
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

In a 7-day static toxicity laboratory study, the effect of pyrimethanil on the growth of the duckweed *Lemna gibba* was investigated. The following nominal concentrations were applied: 0 (control), 0 (solvent control), 1.9, 3.8, 7.5, 15 and 30 mg pyrimethanil/L. Assessment of growth and other effects was conducted 2, 4 and 7 days after test initiation. Average specific growth rate (rate of change in frond number with time) and biomass (the productivity of the culture determined as area under the growth curves) were both calculated. Effects on final biomass were determined based on the dry weight of fronds at study termination.

The biological results are based on nominal concentrations. The duckweed population in the control vessels showed sufficient growth, increasing from 15 fronds in the pooled control vessels at test initiation to an average of 314 fronds per vessel at test termination, corresponding to a 21 x multiplication. Morphological changes like colony separation, chlorotic and dwarf fronds were observed at the two highest tested concentrations of 15 and 30 mg a.s./L.

In a 7-day aquatic plant test with *Lemna gibba* the E_rC_{50} and the E_bC_{50} of pyrimethanil based on frond no. were determined to be > 30 mg a.s./L and 15.3 mg a.s./L (nominal). The E_bC_{50} based on dry weight was 8.7 mg a.s./L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Pyrimethanil (BAS 605 F, Reg. no. 236 999); batch no. AACA00704, purity: 99.4% (w/w).

Test species: Duckweed (*Lemna gibba* G3), inocula 7 - 10 days old cultures; cultures maintained in-house; stock obtained from the University of Minnesota, Horticultural Science, USA.

B. STUDY DESIGN

Test design: Static-renewal system (7 days); 7 treatment groups (5 test item concentrations, control, solvent control) with 3 replicates for each treatment; 5 plants with 3 fronds, total number of fronds at test initiation: 15 per replicate; assessment of growth and other effects on days 2, 4 and 7.

Endpoints: EC_{50} with respect to growth rate and biomass after exposure over 7 days.

Test concentrations: Control, solvent control (0.5 mL DMF/L), 1.9, 3.8, 7.5, 15 and 30 mg pyrimethanil/L (nominal).

Test conditions: 1.2 L glass beakers, test volume 500 mL, 20x-AAP nutrient medium, pH 7.5 - 8.8; water temperature: 24.4 °C - 25.6 °C; oxygen content: 6.3 mg/L - 10.0 mg/L; continuous light, light intensity: 4200 lux - 5200 lux.

Analytics: Analytical verification of the test item was conducted using a HPLC-method with UV detection.

Statistics: Descriptive statistics; non-linear regression analysis for determination of the EC_{50} values.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each test concentration at test initiation, at test solution renewal on day 4 and at test termination. Mean measured values for pyrimethanil ranged from 90% to 105% of nominal in freshly prepared samples and from 95% to 106% of nominal in old study samples. As analytical data confirmed correct application of the test item, the following biological results are based on nominal concentrations.

Biological results: The duckweed population in the control vessels showed sufficient growth, increasing from 15 fronds in the pooled control vessels at test initiation to an average of 314 fronds per vessel at test termination, corresponding to a 21 x multiplication. Morphological changes like colony separation, chlorotic and dwarf fronds were observed at the two highest tested concentrations of 15 and 30 mg a.s./L. Effects on growth rate and yield are summarized in Table 8.2.7-1.

Table 8.2.7-1: Effect of pyrimethanil on the growth of duckweed *Lemna gibba*

Concentration [mg a.s./L] (nominal)	**1.9**	**3.8**	**7.5**	**15**	**30**
Inhibition after 7 d [%] * (growth rate based on frond no.)	7	13	15	25	47
Inhibition after 7 d [%] * (biomass based on frond no)	16	27	31	44	70
Inhibition after 7 d [%] * (biomass based on dry weight)	26	38	37	61	87
Endpoints [mg pyrimethanil/L] (nominal)					
E_rC_{50} (7 d) based on frond no.	> 30				
E_bC_{50} (7 d) based on frond no.	15.3				
E_bC_{50} (7 d) based on dry weight	8.7				

* Inhibition of frond growth was calculated relative to the pooled control group.

III. CONCLUSION

In a 7-day aquatic plant test with *Lemna gibba* the E_rC_{50} and the E_bC_{50} of pyrimethanil based on frond no. were determined to be > 30 mg a.s./L and 15.3 mg a.s./L (nominal). The E_bC_{50} based on dry weight was 8.7 mg a.s./L (nominal).

Calculation of ECx values (DocID: 2017/1074959)

In accordance with current requirements, additional calculations of ECx values have been performed.

Data for the recalculation were taken from the report. The effects are compared to nominal concentrations. Statistical recalculation was performed with ToxRatProfessional Version 2.10.

Nominal Test concentration (mg/L)	Inhibition (0 - 7 days) %			
	Frond number		Dry weight	
	Growth rate	Yield	Growth rate	Yield
1.9	4.84	19.57	8.72	26.25
3.8	9.32	32.72	13.98	37.74
7.5	10.73	36.71	13.60	36.92
15	18.15	54.39	26.89	60.71
30	34.60	77.57	54.65	86.14

No statistical significant difference exists between control and solvent control. Comparison of treatment effects was therefore done to the pooled control.

Yield and growth rate calculations for the parameter "frond number":

Yield (frond no.): the following parameters were used for the probit analysis:

Parameter	Value
Computation runs:	5.0000
Slope b:	1.2691
Intercept a:	-1.29262
Variance of b:	1.8536
Goodness of Fit	
Chi²:	0.0629
Degrees of freedom:	3.0000
p(Chi²):	0.9959
Log EC50:	1.0186
SE Log EC50:	0.4130
g-Criterion:	0.2444
Residual Variance	
(Chi²/df):	0.0210
r²:	0.9320
F:	41.4350
p(F) (df: 1;3):	0.0080

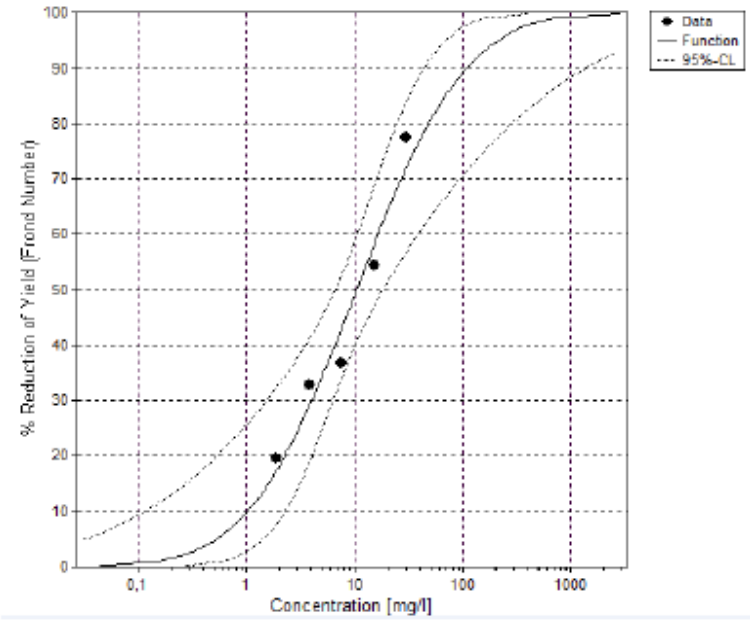


Figure: Concentration-effect curve showing the influence of the test item on yield (frond no.) of Lemna gibba as observed after 7d.

Growth rate (frond no.): the following parameters were used for the probit analysis:

Parameter	Value
Computation runs:	8.0000
Slope b:	1.1746
Intercept a:	-2.18519
Variance of b:	6.3726
Goodness of Fit	
Chi²:	0.0158
Degrees of freedom:	3.0000
p(Chi²):	0.9995
Log EC50:	1.8604
SE Log EC50:	1.5201
g-Criterion:	0.2466
Residual Variance	
(Chi²/df):	0.0053
r²:	0.9320
F:	41.0520
p(F) (df: 1;3):	0.0080

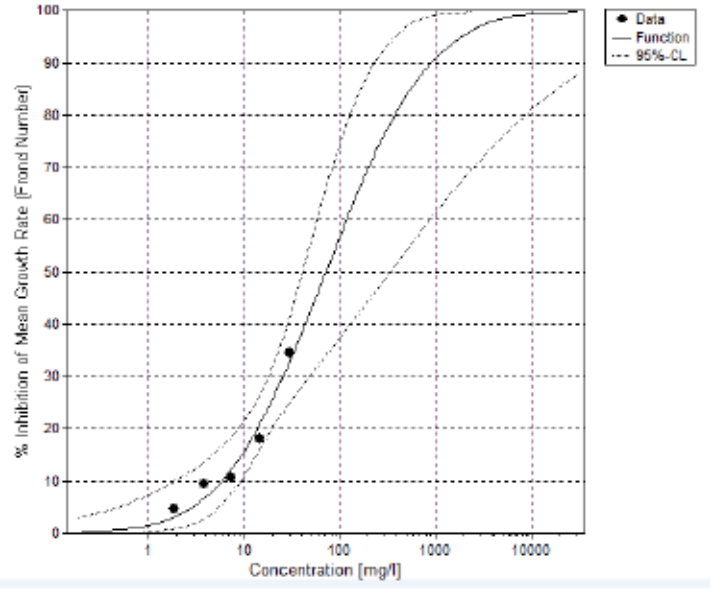


Figure: Concentration-effect curve showing the influence of the test item on growth rate (frond no.) of Lemna gibba as observed after 7d.

Yield and growth rate calculations for the parameter "dry weight":

Yield (dry weight): the following parameters were used for the probit analysis:

Parameter	Value
Computation runs:	4.0000
Slope b:	1.2899
Intercept a:	-1.16647
Variance of b:	1.8244
Goodness of Fit	
Chi²:	0.1696
Degrees of freedom:	3.0000
p(Chi²):	0.9823
Log EC50:	0.9043
SE Log EC50:	0.3968
g-Criterion:	0.6277
Residual Variance	
(Chi²/df):	0.0565
r²:	0.8430
F:	16.1300
p(F) (df: 1;3):	0.0280

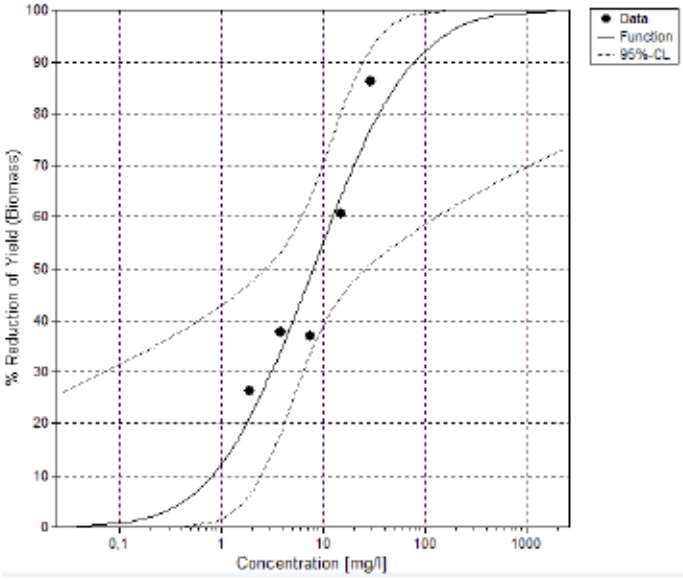


Figure: Concentration-effect curve showing the influence of the test item on yield (dry weight) of Lemna gibba as observed after 7d.

Growth rate (dry weight): the following parameters were used for the probit analysis:

Parameter	Value
Computation runs:	10.0000
Slope b:	1.5392
Intercept a:	-2.26921
Variance of b:	4.7880
Goodness of Fit	
Chi²:	0.0801
Degrees of freedom:	3.0000
p(Chi²):	0.9941
Log EC50:	1.4743
SE Log EC50:	0.5735
g-Criterion:	0.5465
Residual Variance	
(Chi²/df):	0.0267
r²:	0.8610
F:	18.5280
p(F) (df: 1;3):	0.0230

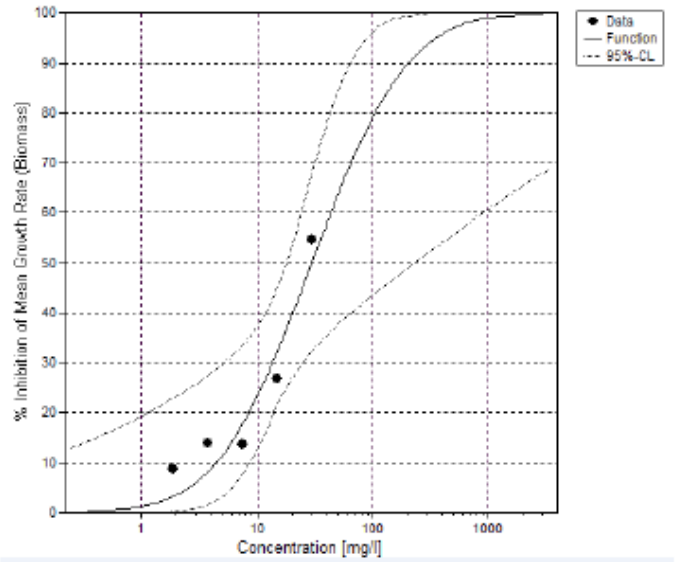


Figure: Concentration-effect curve showing the influence of the test item on growth rate (dry weight) of Lemna gibba as observed after 7d.

ECx Results for Lemna gibba, 7 day study duration:

Endpoint	EC_{10} (mg/L)	EC_{20} (mg/L)
Growth rate (frond no.)	5.9 (95%-CL 1.9 – 9.2)	13.9 (95%-CL 8.7 – 19.9)
Yield (frond no.)	1.0 (95%-CL 0.1 – 2.3)	2.3 (95%-CL 0.5 – 4.0)
Growth rate (dry weight)	4.4 (95%-CL 0.1 – 8.6)	8.5 (95%-CL 1.1 – 14.1)
Yield (dry weight)	n.d.	1.8 (95%-CL 0.0 – 4.2)

Data on growth rates and doubling times in the control replicates throughout the test are presented in the table below.

Growth rates and doubling times in control replicates

Negative control		
Time (days)	Mean growth rate (day^{-1})	T_d (days)
0-7	0.42	1.65
0-2	0.46	1.50
2-4	0.28	2.52
4-7	0.60	1.16
Solvent control		
Time (days)	Mean growth rate (day^{-1})	T_d (days)
0-7	0.44	1.56
0-2	0.42	1.64
2-4	0.30	2.30
4-7	0.68	1.02

The validity criteria stated in the current OECD 221 (2006) guideline were fulfilled althouth the doubling time for negative control section 2-4 days is 2.52 days , however, this is still considered acceptable. The study is considered valid and acceptable for regulatory use.

Conclusion:
The 7-day E_rC_{50} based on frond number is >30 mg pyrimethanil/L, the 7-day E_bC_{50} based on frond number is 15.3 mg pyrimethanil /L, 7-day E_bC_{50} based on dry weight is 8.7 mg pyrimethanil /L. The overall 7-day no-observed-effect concentration (NOEC) is 1.9 mg pyrimethanil /L, based on nominal concentrations.

CA 8.2.8 Further testing on aquatic organisms

From the literature search the following peer-reviewed scientific study was considered relevant and reliable although with restrictions (RI 2; as this is a non-GLP study). For details please see the literature search and evaluation files also provided within the submission for Annex I Renewal.
In this study, literature was analyzed and 6 standard toxicity studies with standard organisms were conducted to determine the effect profile and toxicity of pyrimethanil on different trophic levels and systematic groups. Only the endpoints obtained in the standard toxicity studies were considered in the summary below, whereas the endpoints obtained from secondary literature were not further evaluated. As only limited information on the conduct of the toxicity studies is given in this peer-reviewed scientific study, it is not possible to provide an OECD-format summary, however a condensed version is presented below providing all details available on the 6 standard toxicity studies. The endpoints obtained in the 6 standard toxicity studies are in a similar range compared to the endpoints from respective GLP studies conducted with pyrimethanil (see Table 8.2-1 above). Therefore, the results from the peer-reviewed scientific study are presented as additional information, but are not considered for the aquatic risk assessment.

Report:	CA 8.2.8/1 Seeland A., 2012a Aquatic ecotoxicity of the fungicide Pyrimethanil: Effect profile under optimal and thermal stress conditions 2012/1368903
Guidelines:	none
GLP:	no

Executive Summary

Aquatic laboratory standard toxicity studies on the green algae *Desmodesmus subspicatus* (72 h), the aquatic plant species *Lemna minor (*chronic, 7 d), the blackworm *Lumbriculus variegatus* (chronic, 28 d), the sediment-dwelling aquatic insect *Chironomus riparius* (acute, 48 h) and the aquatic crustacean species *Daphnia magna* (acute, 48 h and chronic, 21 d) were conducted with pyrimethanil. All toxicity studies were carried out according to the respective OECD Guidelines (with minor deviations from the guidelines for tests on *L. variegatus and C. riparius*).

In 6 aquatic laboratory toxicity studies on *Desmodesmus subspicatus* (72 h), *Lemna minor* (chronic, 7 d), *Lumbriculus variegatus* (chronic, 28 d), *Chironomus riparius* (acute, 48 h) and *Daphnia magna* (acute, 48 h and chronic, 21 d) the LC_{50} / EC_{50} values of pyrimethanil were 13.7, 23.4, 12.7, 2.92, 3.61 and 1.18 mg a.s./L, respectively. The NOEC values were 5.0, 2.5, 4.0, 0.5, 2.0 and 0.5 mg a.s./L, respectively.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Pyrimethanil (BAS 605 F; Reg. no.: 236 999; CAS No: 53112-28-0), analytical standard (99.9% purity)) purchased from Sigma-Aldrich (Steinheim, Germany).

B. STUDY DESIGN

All organisms tested in the bioassays originated from in-house cultures of the Goethe University Frankfurt am Main.
Chronic pyrimethanil effects on algal/plant growth and on the mortality and reproduction of two invertebrates were studied.
L. variegatus was exposed in a sediment-water-system spiked with pyrimethanil via water phase (OECD 225, 2007). The sediment consisted of quartz sand and kaolin but contrary to the guideline no peat was added.
In addition, the acute effects of pyrimethanil on the survival of *C. riparius* was tested according to OECD 235 (2011) with minor deviations. Briefly, 24 first instar larvae of *C. riparius* were introduced to 24-multiwell plates (2 mL spiked reconstituted water / cavity, pH 7.9 - 8.4, conductivity 540 µS/cm). One cethyl alcohol pellet was added to each well to reduce the surface tension. The larvae were exposed to two control (with/without cetyl alcohol) and seven pyrimethanil treatments at a light : dark cycle of 16:8 h under dim light without feeding at 20 ± 1 °C. After 48 h the larvae were checked for their mobility.

A summary of the relevant information on the different bioassays (organisms, endpoints, OECD guideline number, test duration and used concentrations) is given in Table 8.2.8-1.

Table 8.2.8-1: Information on bioassays with several aquatic test organisms conducted to assess the aquatic ecotoxicity of pyrimethanil

Test organism	Test system	Endpoint	OECD guideline	Test duration	Test concentration range [mg a.s./L]
Desmodesmus subspicatus	Growth inhibition test	Number of cells	201 (OECD, 2002)	72 h	0.6 - 50
Lemna minor	Growth inhibition test	Number of fronds	221 (OECD, 2006)	7 d	1.25 - 80
Lumbriculus variegatus	Sediment-water toxicity test	Number of worms	225 (OECD, 2007)	28 d	0.25 - 8.0
Chironomus riparius	Acute immobilization test	Number of immobile individuals	235 * (OECD, 2011)	48 h	0.5 - 32
Daphnia magna	Acute immobilization test	Number of immobile individuals	202 (OECD, 2004)	48 h	0.25 - 16
D. magna	Reproduction test	Number of offspring	211 (OECD, 2008)	21 d	0.03 -2.0

* modified as described above.

Data are reported as mean [± standard deviation]. The 10% and 50% effect concentrations ($EC1_0$, EC_{50}) for the acute and chronic toxicity tests were derived using a non-linear regression curve fit model (x = log(x)). To calculate the no-observed-effect-concentration (NOEC) and the lowest observed effect-concentration (LOEC), a one-way ANOVA ($p \leq 0.05$) and subsequently a Dunnett's post hoc test were applied. If the assumption for an ANOVA was not given, the Kruskall-Wallis-test followed by Dunn's post hoc test was used ($p \leq 0.05$). For the comparison of two means (e.g. acute immobilization test with *D. magna*), the unpaired t-test for data sets with normal distribution or the Mann-Whitney-test was applied. In addition, the produced neonates of the pyrimethanil treatment and the control of a dedicated generation were compared using a two-way ANOVA followed by Bonferroni posttest ($p \leq 0.05$).

II. RESULTS AND DISCUSSION

Table 8.2.8-2 gives an overview of the observed and calculated effect concentrations derived from the conducted bioassays.

Table 8.2.8-2: Ecotoxicity of pyrimethanil [mg a.s./L] (95% confidence interval) on aquatic model organisms and the respective endpoint

Species	Test parameter	NOEC	LOEC	LC_{10} / EC_{10} (CI)	LC_{50}/EC_{50} (CI)
Desmodesmus subspicatus (chronic, 72 h)	growth	5	10	6.83 (5.60 - 8.32)	13.7 (12.2 - 15.3)
Lemna minor (chronic, 7 d)	growth	2.5	5	3.07 (2.31 - 5.93)	23.4 (20.7 - 26.4)
Lumbriculus variegatus (chronic, 28 d)	reproduction	4	8	1.52 (0.68 - 3.40)	12.7 (6.42 - 25.0)
Chironomus riparius (acute, 48 h)	immobility	0.5	1	0.91 (0.09 - 9.16)	2.92 (1.11 - 7.69)
Daphnia magna (acute, 48 h)	immobility	2	4	1.02 (0.48 - 2.19)	3.61 (2.62 - 4.97)
D. magna (chronic, 21 d)	reproduction	0.5	1	0.95 (0.67 - 1.35)	1.18 (0.42 - 3.32)

NOEC = no observed effect concentration
LOEC = lowest observed effect concentration
LC_{10} / EC_{10} = concentration causing 10% mortality / effect
LC_{50}/EC_{50} = concentration causing 50% mortality/effect
CI = confidence interval

III. CONCLUSION

In 6 aquatic laboratory toxicity studies on *Desmodesmus subspicatus* (chronic, 72 h), *Lemna minor* (chronic, 7 d), *Lumbriculus variegatus* (chronic, 28 d), *Chironomus riparius* (acute, 48 h) and *Daphnia magna* (acute, 48 h / chronic, 21 d) the LC_{50} / EC_{50} values of pyrimethanil were 13.7, 23.4, 12.7, 2.92, 3.61 and 1.18 mg a.s./L, respectively. The NOEC values were 5.0, 2.5, 4.0, 0.5, 2.0 and 0.5 mg a.s./L, respectively.

From the literature search the following peer-reviewed scientific study was considered relevant and reliable although with restrictions (RI 2; as this is a non-GLP study). For details please see the literature search and evaluation files also provided within the submission for Annex I Renewal.
The following study investigates life-stage specific temperature-dependent ecotoxicity of pyrimethanil on the water snail *Physella acuta* at 15, 20 and 25°C. However, the thermal optimum of *Physella acuta* was reported to be between 20 and 30°C (see literature cited in this study). Endpoints and findings resulting from tests conducted at 15°C are therefore not further evaluated. Furthermore, the reliability of results generated in tests conducted at 25°C is questionable as F0 adult snails in the control treatments revealed a considerably increased mortality at 25°C, although no mortality could be observed in the F1 adults at all temperatures. The increased mortality of adults in the F0 may owe to the fact that the adult snails were taken from the breeding stock cultured at 20°C for several generations and acclimated to the test temperatures of 25°C for a too short adaption time. Furthermore, the variability of the data derived from the tests conducted at 25°C is high (percentage variances of the confidence intervals up to 498%). Thus, in the following summary only the experimental data and results from tests conducted at 20°C are presented. The resulting endpoints are in a similar range compared to the endpoints from respective GLP studies conducted with pyrimethanil (see Table 8.2-1 above). Therefore, the results from the peer-reviewed scientific study are presented as additional information but are not considered for the aquatic risk assessment.

Report:	CA 8.2.8/2 Seeland A. et al., 2012a Life stage-specific effects of the fungicide Pyrimethanil and temperature on the snail Physella acuta (Draparnaud, 1805) disclose the pitfalls for the aquatic risk assessment under global climate change 2012/1368902
Guidelines:	none
GLP:	no

Executive Summary

In embryo, juvenile, half- and full-life-cycle toxicity laboratory tests the snail *Physella acuta* was exposed under semi-static conditions. Animals in all tests were exposed to a dilution water control and to concentrations of 0.06, 0.12, 0.25 and 0.5 and/or 1.0 mg pyrimethanil/L at a test temperature of 20°C.

The biological results are based on nominal concentrations of the test item.

Embryo Test:
No statistically significant difference to the control for mortality or embryonic deformations could be detected in the three lowest pyrimethanil treatment of 0.06, 0.12 and 0.25 mg a.s./L. At higher treatment levels (0.5 and 1 mg a.s./L), the number of deformed or deceased embryos increased.

Juvenile growth test:
Compared to the control treatment, the size of the juveniles increased after exposure to 0.06 and 0.12 mg pyrimethanil/L by 47.2% and 43.8%, respectively, which is not seen as an adverse substance related effect. No effect was observed at 0.25 mg a.s/L, whereas an actual adverse effect to juvenile growth was overserved at the highest test item concentration of 0.5 mg a.s./L.

Half life cycle test:
Significant effects on mortality in the F0 generation occurred at 0.5 and 1 mg a.s./L (19.8% and 46.4% respectively), no significant mortality occurred in the F0 generation for the control and test concentrations of 0.06 to 0.25 mg a.s./L. Egg mass production of F0 decreased in dependence on pyrimethanil concentration, likely due to the general toxicity effect indicated by mortality in the two highest test concentrations. At the two highest test item concentrations of 0.5 and 1.0 mg a.s./L the amount of egg masses/snail was significantly reduced. No statistically different effect on egg mass/snail could be detected for test concentrations up to and including 0.25 mg a.s./L.

Full life cycle test:
No mortality in snails of the F1 generation was observed (test concentrations up to 0.25 mg a.s./L). A significant decrease in egg mass/snail was observed at 0.25 mg a.s./L, while no effect on egg mass was observed for 0.06 and 0.12 mg a.s./L. Fertility of eggs mirrored the effects observed for the endpoint egg mass/snail, i.e. fertility was slightly decreased at 0.25 mg a.s/L (67.8% fertile eggs compared to 87.3% fertile eggs in the control).

In embryo, juvenile, half- and full-life-cycle toxicity laboratory tests the snail *Physella acuta* was exposed to pyrimethanil at a test temperature of 20°C. The overall NOEC was determined to be 0.12 mg a.s./L.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Pyrimethanil (BAS 605 F; Reg. no.: 236 999; CAS No.: 53112-28-0), analytical standard (99.9% purity) purchased from Sigma-Aldrich (Steinheim, Germany).

B. STUDY DESIGN

Test species: Water snail (*Physella acuta*), from in-house culture.
Embryo test: eggs (24 h old).
Juvenile growth test: freshly hatched juveniles.
Half life cycle test: fertile adult snails (F0 generation).
Full life cycle test: embryos (F1 generation).

Test design: Embryo test: Semi-static (2 - 4 weeks); 5 test item concentration plus dilution water control, 12 replicates per treatment with one egg each, daily observation of development.
Juvenile growth test: Semi-static (6 weeks), 4 test item concentration plus dilution water control, 12 replicates per treatment, weekly measurement of shell length.
Half life cycle test: Semi-static (28 d); 5 test item concentration plus dilution water control, snails were acclimated to the test temperature for 14 d; 5 replicates with three snails per replicate, removal of egg masses and counting of dead snails twice a week.
Full life cycle test: Semi-static, combination of all tree tests; 5 test item concentration plus dilution water control.

Endpoints: Embryo test: hatching, deformation and mortality, heartbeat.
Juvenile growth test: final size.
Half life cycle test: egg masses per F0 snail.
Full life cycle test: egg masses per F1 snail.

Test concentrations: Embryo test: Control (dilution water), 0.06, 0.12, 0.25, 0.5 and 1.0 mg a.s./L.
Juvenile growth test: Control (dilution water), 0.06, 0.12, 0.25 and 0.5 mg a.s./L.
Life cycle tests: Control (dilution water), 0.06, 0.12, 0.25, 0.5 and 1.0 mg a.s./L (full-life cycle including F1 generation only up to 0.25 mg a.s./L).

Test conditions:	ISO medium (according to OECD guideline 202, 2004); test temperature: 20°C for all test. Embryo test: 24-well plates (2 mL); weekly water renewal. Juvenile growth test: 12-well-plates (5 mL) for the first 3 weeks, afterwards 100 mL glass beakers containing 50 mL test solution (covered with glass lids shell length at test beginning: 0.808 ± 0.004 mm; weekly water renewal, food: Tetra Min® once a week. Half life cycle test: 250 mL glass beakers (covered with glass lids), aeration via Pasteur pipettes, weekly water renewal, food: 5 mg Tetra Min®/snail/day twice a week; removal of egg masses twice a week and transfer to 1 L containers. Full life cycle test: 10 L aquaria, weekly water renewal, food: Tetra Min® ad libitum once a week, egg masses produced by sexually mature F1 individuals were transferred to 24 well plates (see embryo test).
Analytics:	The test item concentrations were analyzed using a HPLC method.
Statistics:	Descriptive statistics, non-linear regression curve fit model for calculation of ECx values; two-factorial ANOVA followed by Tukey posttest for determination of the NOEC values ($\alpha = 0.05$).

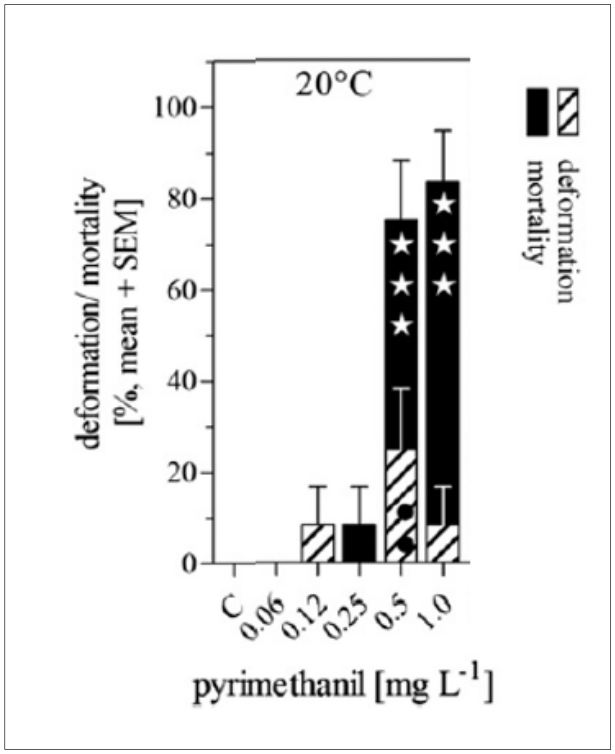
II. RESULTS AND DISCUSSION

Analytical measurements: The chemical behavior of pyrimethanil was exemplarily measured in the concentration range of 0.15 - 2.5 mg a.s./L during six days. Pyrimethanil in the test media degraded slightly (14.8 ± 15.1%) within six days. The following biological results are based on nominal concentrations.

Biological results:

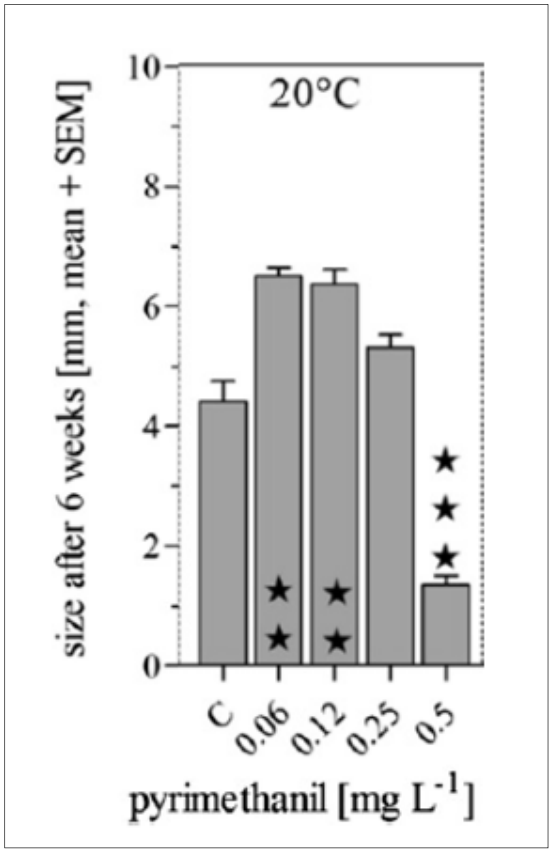
Embryo Test:
The experiment lasted for two weeks in minimum and for four weeks in maximum, depending on the temperature-dependent full hatching success in the controls. No statistically significant difference to the control for mortality or embryonic deformations could be detected in the three lowest pyrimethanil treatment of 0.06, 0.12 and 0.25 mg a.s./L. At higher treatment levels (0.5 and 1 mg a.s./L), the number of deformed or deceased embryos increased (see Figure 8.2.8-1). In the highest pyrimethanil test concentration of 1 mg a.s./L the embryo mortality reached 83.3%.

Figure 8.2.8-1: **Embryo toxicity test with *Physella acuta*.** Deformation [%, mean ± SEM] and mortality [%, mean ± SEM] of embryonic snails after exposure to 0.06 - 1.0 mg a.s./L of pyrimethanil and a control treatment at 20°C. Significant differences to control (n = 12): ●● = $p < 0.01$ for deformation; ★★★ = $p < 0.001$ for mortality. C = control.



Juvenile growth test:
The control snails grew to a size of 4.40 ±1.13 mm. Compared to the control treatment, the size of the juveniles increased significantly after exposure to 0.06 and 0.12 mg pyrimethanil/L by 47.2% and 43.8%, respectively (see Figure 8.2.8-2), which is not seen as an adverse substance related effect. No effect was observed at 0.25 mg a.s/L. An actual adverse effect to juveniles was overserved at the highest test item concentration only. At 0.5 mg a.s./L the growth was significantly reduced compared to the control (reduction by 69.4%).

Figure 8.2.8-2: **Juvenile growth test with *Physella acuta*.** Size of juvenile snails [mm, mean ± SD] after six weeks of exposure to 0.06 - 0.5 mg a.s./L of pyrimethanil and a control treatment at 20°C. Significant differences to control (n = 12): ★★ = $p < 0.01$; ★★★ = $p < 0.001$.



Half life cycle test:
Significant effects on mortality in the F0 generation occurred at 0.5 and 1 mg a.s./L (19.8% and 46.4% respectively, see Table 8.2.8-3), no significant mortality occurred in the F0 generation for the control and test concentrations of 0.06 to 0.25 mg a.s./L. Egg mass production of F0 decreased in dependence on pyrimethanil concentration, likely due to the general toxicity effect indicated by mortality in the two highest test concentrations. At the two highest test item concentrations of 0.5 and 1.0 mg a.s./L the amount of egg masses/snail was significantly reduced. No statistically different effect on egg mass/snail could be detected for test concentrations up to and including 0.25 mg a.s./L (see Figure 8.2.8-3 A).

Full life cycle test:
First it should be noted that as a result of the poor reproduction of F0 adults at 0.5 and 1.0 mg a.s./L, a F1 could not be established for these pyrimethanil treatments. However, the reproductive power of the controls (F0 and F1) did not differ among the two life-cycle-tests for 20°C (F0: 10.1 ± 1.79 egg masses/snail, F1: 9.66 ± 1.13 egg masses/snail).

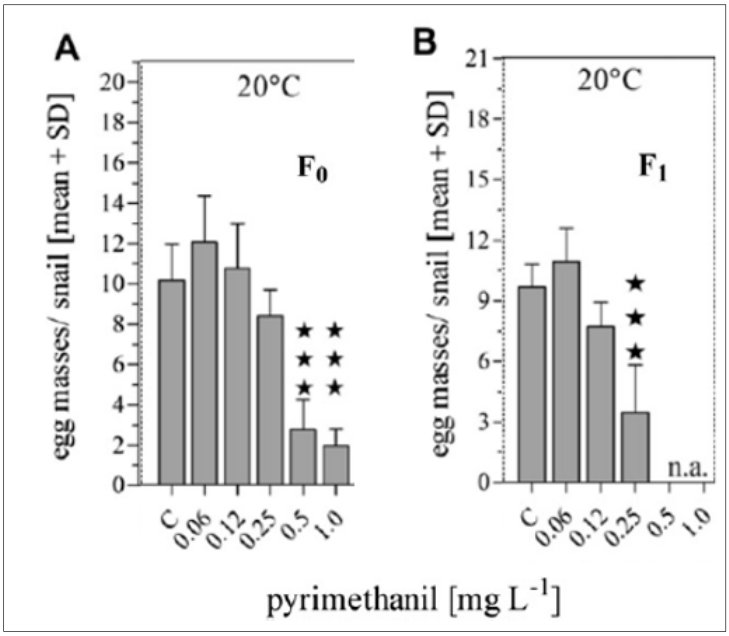
No mortality in snails of the F1 generation was observed (test concentrations up to 0.25 mg a.s./L, see Table 8.2.8-3). A significant decrease in egg mass/snail was observed at 0.25 mg a.s./L, while no effect on egg mass was observed for 0.06 and 0.12 mg a.s./L (see Figure 8.2.8-3 B). Fertility of eggs mirrored the effects observed for the endpoint egg mass/snail, i.e. fertility was slightly decreased at 0.25 mg a.s/L (67.8% fertile eggs compared to 87.3% fertile eggs in the control).

Table 8.2.8-3: Effects of the active substance pyrimethanil to *Physella acuta* in half- and full life cycle tests at 20°C

Concentration (nominal) [mg a.s./L]	**Control**	**0.06**	**0.12**	**0.25**	**0.5**	**1.0**
Mortality (adult organisms) [%] – F0 generation	6.6	13.2	6.6	6.6	19.8	46.4
Mortality (adult organisms) [%] – F1 generation	0	0	0	0	x	**x**
Fertility [%] - F0 generation	*	*	*	*	+	+
Fertility [%] – F1 generation	87.3	85.8	78.4	67.8	**x**	**x**

\+ Poor reproduction (too few egg masses available to asses a second generation)
x No data available
* Continuation to F1 (therefore no calculation of fertility)

Figure 8.2.8-3: **Reproduction test with *Physella acuta*.** Produced egg masses of each adult [mean ± SD] after 28 days of exposure to 0.06 - 1.0 mg a.s./L(F0) and 0.06 - 0.25 mg a.s./L (F1) of pyrimethanil and a control treatment at 20°C. A: reproduction of the F0 generation, where only adults incurred fungicide exposure; B: reproduction of the F1 generation, which parents were already exposed to the fungicide. Significant differences to control (n = 5): ★★★ = $p < 0.001$.



Relevant endpoint values for all tests are summarized in Table 8.2.8-4.

Table 8.2.8-4: **Endpoints obtained for *Physella acuta* at 20°C in an embryo toxicity test, a juvenile growth test, a half life cycle test and a full life cycle test with the active substance pyrimethanil**

Test (endpoint)	NOEC [mg a.s./L]	EC_{50} [CI] [mg a.s./L]
Embryo toxicity test (mortality)	0.25	0.402 ± [0.339 - 0.475]
Juvenile growth test (final size)	0.25 *	n.d.
Half life cycle test (egg masses per F0 snail)	0.25	0.395 ± [0.327 - 0.477]
Full life cycle test (egg masses per F1 snail)	0.12	0.204 ± [0.167 - 0.248]
Overall	**0.12**	--

* No adverse effects were observed at 0.25 mg a.s./L (effect: increase in growth) - therefore a NOEC of 0.25 mg a.s./L was considered reliable (contrary to the reported NOEC of 0.06 mg/L in the study report).
n.d. not determined

III. CONCLUSION

In embryo, juvenile, half- and full-life-cycle toxicity laboratory tests the snail *Physella acuta* was exposed to pyrimethanil at a test temperature of 20°C. The overall NOEC was determined to be 0.12 mg a.s./L.

From the literature search the following peer-reviewed scientific study was considered relevant and reliable although with restrictions (RI 2; as this is a non-GLP study). For details please see the literature search and evaluation files also provided within the submission for Annex I Renewal.
A 28 d life-cycle toxicity test with *Chironomus riparius* was conducted according to OECD guideline 219 (OECD 2004). Additionally, a bifactorial multigeneration study was conducted in order to test if Global Climate Change conditions alter the effects of low pesticide concentrations on life history and genetic diversity of the aquatic model organism *C. riparius*. In the multigeneration study 2 mg pyrimethanil/L were applied, which is below the NOEC level determined in the 28 d life-cycle toxicity test (*i.e.* half of the NOEC). The results from the multigeneration study are not further evaluated as no significant effects are expected after treatment below the NOEC level and the study was designed to evaluate temperature mediated effects. Therefore, in the following summary only the experimental data and results from the 28 d life-cycle toxicity test are presented. The resulting endpoints are in a similar range compared to the endpoints from the respective GLP study conducted with pyrimethanil (see Table Error**!** Use the Home tab to apply Überschrift 2 to the text that you want to appear here.**-1** above). Therefore, the results from the peer-reviewed scientific study are presented as additional information but are not considered for the aquatic risk assessment.

Report:	CA 8.2.8/3 Mueller R. et al., 2011a Simulated climate change conditions unveil the toxic potential of the fungicide Pyrimethanil on the midge Chironomus riparius: a multigeneration experiment 2011/1297771
Guidelines:	none
GLP:	no

Executive Summary

In a 28-day static (spiked water) study, non-biting midge larvae (*Chironomus riparius*) were exposed to pyrimethanil in the presence of sediment. Nominal test concentrations were 2, 4, 8, 16, 24, and 32 mg a.s./L, corresponding to time-weighted mean measured concentrations of 1.36, 2.83, 5.32, 9.07, 19.6 and 21.7 mg a.s./L. Additionally, a solvent (ethyl acetate) control and a water control were set up. All test item concentrations and the controls had 6 replicates. 60 larvae were added to each test vessel.

The biological results are based on nominal water concentrations and additionally on time-weighted mean measured concentrations. Survival of controls was ≥ 72% and emergence took place between day 13 and 24 with 0.06 midges per day. Mean emergence time (E_mT_{50}) of untreated females averaged to 18.4 ± 0.99 days in controls and 18.2 ± 0.85 days in solvent controls. Proportion of emerged females (compared to males) amounted to 53 ± 14% in controls and 47 ± 7% in solvent controls. Females produced 8981 ± 3447 and 7551 ± 1824 eggs, 45 ± 16% and 56 ± 27% thereof were fertile. The calculated population growth rate (PGR) was 1.21 ± 0.05 and 1.22 ± 0.05 (control/solvent control), respectively.
At the lower range of tested pyrimethanil concentrations, development and reproduction were less affected than survival. The estimation of effective concentrations for the endpoint emergence time was not possible as regressions were too steep.
E_mT_{50} was slightly reduced until 24 mg a.s./L (16.2 ± 3.11 days at 24 mg a.s./L; 15.0 days at 32 mg a.s./L) albeit E_mT_{50} did not differ in variance at all.
The development rate of 0.06 midges per day was unaffected up to 8 mg a.s./L. Both the total and fertile egg production per female were significantly enhanced by 2 mg a.s./L compared to controls.

In a 28-day spiked water test (including sediment) with *Chironomus riparius* the NOEC value for mortality was determined to be 4 mg pyrimethanil/L (nominal; corresponding to time weighted mean measured concentration of 2.83 mg a.s./L). The NOEC value for reproduction and population growth rate is 8 mg pyrimethanil/L (nominal; corresponding to time weighted mean measured concentration of 5.32 mg a.s./L).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Pyrimethanil (BAS 605 F; Reg. no.: 236 999), analytical standard (Fluka) purchased from Sigma-Aldrich (Taufkirchen, Germany).

B. STUDY DESIGN

Test species: Non-biting midge (*Chironomus riparius*), first instar larvae; culture maintained at Goethe University, Frankfurt am Main, Germany.

Test design: Static system including sediment (28 days); 6 test concentrations plus a solvent (ethyl acetate) control and a water control, 5 replicates per treatment; 60 larvae per vessel (0.1 larvae /cm^2); daily monitoring of number and sex of emerged adults and dead pupae.

Endpoints: NOEC and EC_{50} (regarding mortality, reproduction and population growth rate).

Test concentrations: Solvent control (200 µL ethyl acetate/L), water control, 2, 4, 8, 16, 24, and 32 mg a.s./L (nominal), corresponding to time-weighted mean measured concentrations (average actual concentrations (ACC)) of 1.36, 2.83, 5.32, 9.07, 19.6 and 21.7 mg a.s./L.

Test conditions: 2 L quartz glass test vessels filled with aged sediment (5 days) and 1 L reconstituted water (reconstituted water (conductivity 537 µS/cm^2, pH 8.4); sediment consisted of washed and sterilized (24 h at 220 °C) quartz sand (QuickMix®, quick-mix Gruppe, Osnabrück, Germany) with following granulometry: 0.1% > 500 µm, 34.3% > 250 µm, 50.0% > 150 µm, 10.6% > 125 µm, 5.0% > 63 µm, and 0.03% > 20 µm. In addition, sediment contained 0.4% leaves of *Fagus sylvatica* (particle size < 500 µm; wetted with 111 mL reconstituted water per gram); one day after application, vessels were slightly aerated and covered with gauze (mesh size 1.5 mm); during the test pH ranged between 7.1 - 8.2; oxygen saturation was ≥ 70%; temperature: 20 ± 1.5 °C, humidity 60%; light intensity: 1800 lux; photoperiod: 16 h light : 8 h dark; feeding every other day, larvae were fed with finely grounded fish food (TetraMin®, Tetra GmbH, Melle, Germany) with 0.25 mg/larvae/day (day 1 - 5), 0.5 mg/larvae–1/day (day 6 - 11) and 1 mg/larvae/day from day 12 onwards.

Analytics: Analytical verification of test item concentrations in test medium was conducted using a HPLC-method with UV detection.

Statistics: Descriptive statistics, EC_{50}/LC_{50} values were calculated from concentration–response relationships by means of nonlinear regression analysis ($x = \log(x)$). To detect the NOEC Tukey's multiple comparison test was performed, subsequent homogeneity of variances (Bartlett's test, $p < 0.05$) was proven and unifactorial Model I analysis of variance (ANOVA) ($p < 0.05$) accomplished. Data were arcsine transformed in the case of percentage data and square root transformed if heterogeneous. Still nonparametric data were tested with Kruskal–Wallis test followed by Dunn's test ($p < 0.05$).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations in test medium was sampled from all test vessels approximately 1 h after application and at the end of the experiment. The biological results are based on nominal water concentrations and additionally on time-weighted mean measured concentrations.

Biological results: Survival of controls was ≥ 72% and emergence took place between day 13 and 24 with 0.06 midges per day. Mean emergence time (E_mT_{50}) of untreated females averaged to 18.4 ± 0.99 days in controls and 18.2 ± 0.85 days in solvent controls. Proportion of emerged females (compared to males) amounted to 53 ± 14% in controls and 47 ± 7% in solvent controls. Females produced 8981 ± 3447 and 7551 ± 1824 eggs, 45 ± 16% and 56 ± 27% thereof were fertile. The calculated population growth rate (PGR) was 1.21 ± 0.05 and 1.22 ± 0.05 (control/solvent control), respectively.
At the lower range of tested pyrimethanil concentrations, development and reproduction were less affected than survival. The estimation of effective concentrations for the endpoint emergence time was not possible as regressions were too steep.
E_mT_{50} was slightly reduced until 24 mg a.s./L (16.2 ± 3.11 days at 24 mg a.s./L; 15.0 days at 32 mg a.s./L) albeit E_mT_{50} did not differ in variance at all.
The development rate of 0.06 midges per day was unaffected up to 8 mg a.s./L. Both the total and fertile egg production per female were significantly enhanced by 2 mg a.s./L compared to controls ($p < 0.05$).

The endpoints are summarized in Table 8.2.8-5.

Table 8.2.8-5: Summary of endpoints for pyrimethanil

	Endpoints [mg pyrimethanil/L] (nominal)	
	nominal	time-weighted mean measured
LC_{50}	9.27	5.95
$EC_{50\ PGR}$	--*	13.1
$NOEC_{mortality}$	4	2.83
$NOEC_{reproduction}$ & $NOEC_{PGR}$	8	5.32

* EC_{50} only reported based on time-weighted mean measured concentrations (AAC-corrected)

III. CONCLUSION

In a 28-day spiked water test (including sediment) with *Chironomus riparius* the NOEC value for mortality was determined to be 4 mg pyrimethanil/L (nominal; corresponding to time weighted mean measured concentration of 2.83 mg a.s./L). The NOEC value for reproduction and population growth rate is 8 mg pyrimethanil/L (nominal; corresponding to time weighted mean measured concentration of 5.32 mg a.s./L).

From the literature search the following peer-reviewed scientific study was considered relevant and reliable although with restrictions (RI 2; as this is a non-GLP study). For details please see the literature search and evaluation files also provided within the submission for Annex I Renewal. In this peer-reviewed scientific study, survival of *Tubifex tubifex* was investigated after exposure to pyrimethanil at various concentrations over 7 days. Additionally, effects of pyrimethanil on protein contents, and signs of oxidative stress in *T. tubifex* have been investigated. However, these measurements are not relevant for the aquatic risk assessment and are thus not described in the following summary.
The LC_{50} values derived from this study indicates a lower toxicity of pyrimethanil to *T. tubifex* than to other aquatic invertebrate species (including the sediment dweller *C. riparius*) tested in GLP studies (see Table 8.2-1 above). Therefore, the results from the peer-reviewed scientific study are presented as additional information but are not considered for the aquatic risk assessment.

Report:	CA 8.2.8/4 Mosleh Y.Y. et al., 2013a Biological effects of Pyrimethanil on aquatic worms (Tubifex tubifex) under laboratory conditions 2013/1420260
Guidelines:	none
GLP:	no

Executive Summary

In a 7 day static acute toxicity study, sludge worms (*Tubifex tubifex*) were exposed to pyrimethanil at nominal test concentrations of 0 (control), 5, 10, 15, 20 and 25 mg a.s./L (corresponding to mean measured concentrations of 0, 2.9, 7.5, 11.3, 14.7 and 19.0 mg a.s./L) in 3 replicates per concentration, containing 40 worms each. Worms were observed for mortality 1, 2, 4 and 7 days after start of exposure.

The biological results are based on nominal concentrations of the test item. For the control group no mortality was observed. Mortality increased with pyrimethanil concentration.

In a 7 day static acute toxicity study with *Tubifex tubifex* the LC_{50} (7 d) of pyrimethanil was determined to be 39.5 mg/L.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Pyrimethanil (BAS 605 F, Reg. No. 236 999)

B. STUDY DESIGN

Test species: Sludge worms (*Tubifex tubifex*); source: in-house culture, originally obtained from a retention basin near Cormicy sur Marne (Marne, France).

Test design: Static system, 5 test concentrations plus control, 3 replicates per concentration and control; 40 worms (approximately 1.5 g fresh weight) per replicate; assessment of mortality on day 1, 2, 4 and 7 (end of the test).

Endpoints: LC_{50}, mortality.

Test concentrations: Control (spring water), 5, 10, 15, 20 and 25 mg/L pyrimethanil (nominal); corresponding to mean measured concentrations of 0, 2.9, 7.5, 11.3, 14.7 and 19.0 mg a.s./L

Test conditions: Crystallizing dishes (Ø = 8 cm), test volume100 mL; pH of the control: 7 ± 0.1; hardness of the control: 300 ± 10 mg L^{-1} $CaCO_3$; water temperature: 21 ± 1°C; photoperiod: 12 h light: 12 h dark.

Analytics: The test item concentrations were analyzed using a HPLC method.

Statistics: Descriptive (statistics), graphical determination of LC_{50} values.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentration was conducted in each concentration at the beginning, at day 4 and at the end of the test. Mean measured values for pyrimethanil ranged from 76 to 91% of nominal concentrations at test initiation, from 50 to 79% at day 4 and from 45 to 63% at test termination. The following biological results are based on nominal concentrations.

Biological results: For the control group no mortality was observed. Mortality increased with pyrimethanil concentration. For results see Table 8.2.8-6.

Table 8.2.8-6: Lethal concentrations [mg a.s./L] of pyrimethanil in *Tubifex tubifex* after 1, 2, 4, and 7 days of exposure

Toxicity	Time (days)			
	1	2	4	7
LC_{50} (7 days)	10.8 ± 0.09	9.5 ± 0.08	8.3 ± 0.08	8.05 ± 0.04
LC_{50} (7 days)	25.6 ± 0.15	22.3 ± 0.08	20.2 ± 0.09	19.3 ± 0.03
LC_{50} (7 days)	49.2 ± 0.58	45.6 ± 0.98	42.3 ± 0.08	39.5 ± 0.95

III. CONCLUSION

In a 7 day static acute toxicity study with *Tubifex tubifex* the LC_{50} (7 d) of pyrimethanil was determined to be 39.5 mg a.s/L.

From the literature search the following peer-reviewed scientific study was considered relevant and reliable although with restrictions (RI 2; as this is a non-GLP study). For details please see the literature search and evaluation files also provided within the submission for Annex I Renewal. In this peer-reviewed scientific study two green algae species were exposed to pyrimethanil at various concentrations and effects were determined by measuring growth rate and algal chlorophyll fluorescence emission. According to the current OECD guideline 201 for alga testing (OECD, 2011) the relevant endpoint for toxicity tests with algae is inhibition of growth. Therefore, in the summary provided below, only the results based on the effect parameter growth rate are presented. The results derived from this study are in a similar range compared to the results from respective GLP studies on several algae species with pyrimethanil (see Table 8.2-1 above). Therefore, the results from the peer-reviewed scientific study are presented as additional information but are not considered for the aquatic risk assessment.

Report: CA 8.2.8/5
Dosnon-Olette R. et al., 2010a
Fungicide and herbicide removal in Scenedesmus cell suspensions
2010/1232972
Guidelines: none
GLP: no

Executive Summary

In a 96 h static acute toxicity laboratory study, the effect of pyrimethanil on the growth of the green algae *Scenedesmus obliquus* and *Scenedesmus quadricauda* were investigated. Nominal concentrations of 0.200, 0.400, 0.600 and 0.800 mg pyrimethanil were applied. Additionally different control treatments were set up. Assessment of growth was conducted once at the beginning and every 24 h thereafter.

The biological results are based on nominal test item concentrations. After 96 hours of exposure, growth rate of *S. obliquus* was statistically significantly reduced at all tested concentrations compared to the control, whereas statistically significantly differences in growth rate of *S. quadricauda* compared to the control occurred only at the highest tested concentration of 0.800 mg a.s./L. After 96 h of exposure at the highest concentration tested, the growth rate inhibition reached a maximum of 24%.

In a 96 h algae test with the green algae *Scenedesmus obliquus* and *Scenedesmus quadricauda*, the EC_{50} for pyrimethanil was determined to be > 0.800 mg a.s./L for both algae species

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Pyrimethanil (BAS 605 F; Reg. no.: 236 999); tested as formulated product Scala (BASF Agro, France) containing 400 g a.s./L (nominal).

B. STUDY DESIGN

Test species: Green algae *Scenedesmus obliquus* and *Scenedesmus quadricauda* (SAG 276-3a and 276-4b; Göttingen, Germany).

Test design: Static system; test duration 4 d (96 hours); 4 test item concentrations plus control, all experiments were repeated three times and each sample was run in triplicate; three different controls were carried out in parallel: # 1 algae in a pesticide free medium, # 2 dead-frozen algae in a medium containing pesticides and # 3 medium containing pesticides but free of algae; daily assessment of growth by measurement of optical density.

Endpoint: Inhibition of growth rate after exposure over 4 days (96 h).

Test concentrations: Control; 0.200, 0.400, 0.600 and 0.800 mg a.s./L. Pesticide concentrations given in this study refer to the actual, and not to the nominal concentrations of pyrimethanil.

Test conditions: 250 mL Erlenmeyer flasks containing 100 mL culture medium; exponentially growing cell cultures with an initial cell concentration of 1.4×10^6 cells/mL; data on physical/chemical test conditions (pH, conductivity, light intensity, temperature and hardness not reported.

Analytics: Analytical measurements were conducted using an HPLC-method with UV detection.

Statistics: Descriptive statistics, significant differences between controls and test item treatments were determined by One Way ANOVA tests ($p < 0.05$).

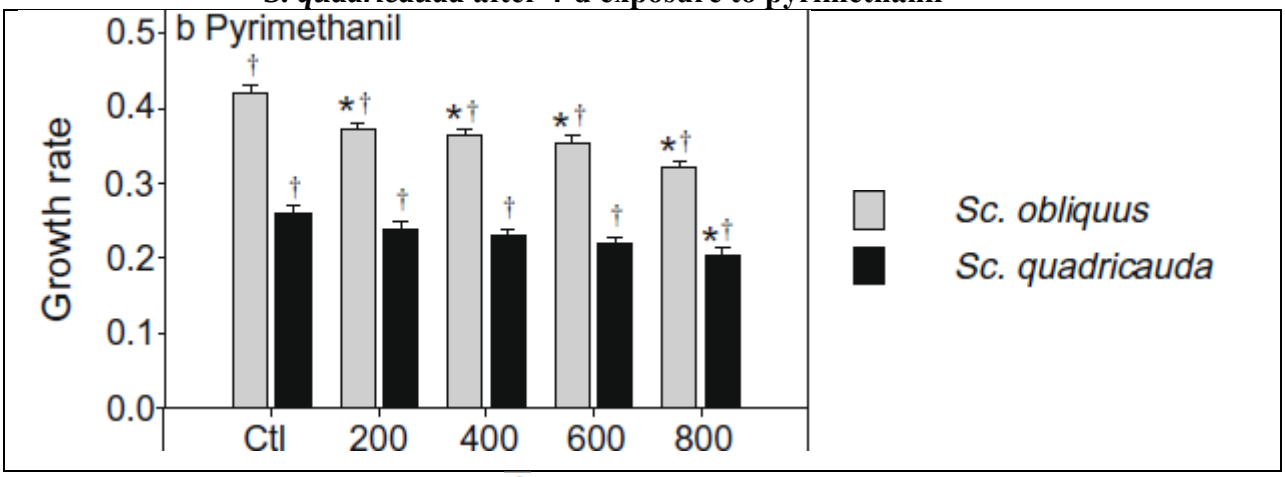
II. RESULTS AND DISCUSSION

Analytical measurements: Analytical measurements were conducted daily in the controls. No pesticide was detected in control # 1. The following biological results are based on nominal test item concentrations.

Biological results: After 4 days of exposure, growth rate of *Sc. obliquus* was statistically significantly reduced at all tested concentrations compared to the control, whereas statistically significantly differences in growth rate of *Sc. quadricauda* compared to the control occurred only at the highest tested concentration of 0.800 mg a.s./L (One Way ANOVA ($p < 0.05$). After 4 days of exposure at the highest concentration tested the growth rate inhibition reached a maximum of 24%.

The results are shown in Figure 8.2.8-4.

Figure 8.2.8-4: Effect of pyrimethanil on the growth rate of *S. obliquus* and *S. quadricauda* after 4-d exposure to pyrimethanil



* Data are significantly different from control and † for data significantly different between the two algae ($P < 0.05$).

III. CONCLUSION

In a 96 h algae test with the green algae *Scenedesmus obliquus* and *Scenedesmus quadricauda*, the EC_{50} for pyrimethanil was determined to be > 0.800 mg a.s./L for both algae species

From the literature search the following peer-reviewed scientific study was considered relevant and reliable although with restrictions (RI 2; as this is a non-GLP study). For details please see the literature search and evaluation files also provided within the submission for Annex I Renewal. Pyrimethanil-contaminated samples were taken from outdoor mesocosms 1 and 10 days after pyrimethanil application. Different dilutions were prepared using both nutrient-rich culture medium (LC Oligo) and non-contaminated mesocosm samples to assess cell growth inhibition. In the summary below, only the results from the treated mesocosm water samples compared to the reference (*i.e.* non-contaminated mesocosm water) are presented. A comparison of pyrimethanil containing mesocosm water samples to nutrient rich culture medium is not reasonable as nutrient enrichment itself can induce effects on algae (see also discussion in the scientific paper itself). The EC_{50} based on the area under the growth curve (*i.e.* biomass) obtained by comparison of pyrimethanil treatments to non-contaminated mesocosm water (as control) is in the same range as the endpoints from the GLP studies on several algae species conducted with pyrimethanil (see Table 8.2-1 above). Therefore, the results from the peer-reviewed scientific study are presented as additional information but are not considered for the aquatic risk assessment.

Report:	CA 8.2.8/6 Shinn C. et al., 2014a Immediate and mid-term effects of Pyrimethanil toxicity on microalgae by simulating an episodic contamination 2014/1326517
Guidelines:	none
GLP:	no

Executive Summary

In a 72-hour static acute toxicity laboratory study, the effect of pyrimethanil on the growth of the green alga *Selenastrum capricornutum* was investigated using dilutions of 25%, 50%, 75% and 100% of pyrimethanil treated mesocosm water. Additionally pyrimethanil treated mesocosm water samples taken at day 10 post-application were used as a test item concentration. Non-contaminated mesocosm water served as test medium and was used to set up the control replicates. Assessment of growth was conducted 24 h, 48 h, 72 h after test initiation.

The biological results are based on the nominal test item concentrations. In the reference samples (non-contaminated mesocosm water), cells multiplied 56 times after 72 h of exposure, while when exposed to pyrimethanil at 100% they multiplied only 7 times. Growth in all pyrimethanil samples diluted with reference sample were statistically different to growth in the reference sample after 72 h of exposure. Growth inhibition of cells exposed to pyrimethanil and diluted with reference sample was ca. 20% for the three lowest dilutions (pyrimethanil at 25, 50 and 75% + reference) when compared with undiluted reference sample, while growth inhibition of the cells exposed to the pyrimethanil-contaminated mesocosm sample was significantly reduced by 80% (±6.5). After 72 h of exposure, the EC_{50} based on the area under the growth curve (*i.e.* biomass) was 85 ± 2% (*i.e.* 1.2 ± 0.03 mg a.s./L) when compared with the reference sample.
The toxicity of samples taken from contaminated mesocosms at day 10 was attenuated to 34% (±15) when compared with reference sample.

In a 72-hour algae test with *Selenastrum capricornutum* using pyrimethanil treated mesocosm water, the 72 h E_bC_{50} of pyrimethanil was determined to be 1.2 mg a.s./L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Pyrimethanil (BAS 605 F; Reg. no.: 236 999), applied as commercial formulation Mythos® (containing 300 g pyrimethanil/L) to mesocosms.

B. STUDY DESIGN

Test species: Unicellular fresh water green alga, *Selenastrum capricornutum* (syn. *Pseudokirchneriella subcapitata*), algal cells used in the assay were three days old (exponential growth phase).

Test design: Static system; test duration 72 hours; samples collected at day 1 post-application from mesocosms treated with pyrimethanil were diluted with samples from non-contaminated mesocosms (reference) to prepare different pyrimethanil dilutions (P25 + R, P50 + R, P75 + R, P100 - day 1), additionally pyrimethanil treated mesocosm water samples taken at day 10 post-application were used as test concentration (P100 – day 10); 3 replicates per treatment; daily assessment of cell growth; growth inhibition percentages were calculated by comparison of the area under the curve of the control with the pyrimethanil-treated samples

Endpoint: EC_{50} with respect to biomass after exposure over 72 hours.

Test concentrations: Reference (non-contaminated mesocosm water); 25%, 50%, 75% and 100% dilutions of pyrimethanil treated mesocosm water (day 1 post-application - pyrimethanil concentration of 1.40 ± 0.06 mg a.s./L) and day 10 post-application pyrimethanil treated mesocosm water (pyrimethanil at 0.78 ± 0.06 mg a.s./L).

Test conditions: 125 mL glass Erlenmeyer flasks; test volume 50 mL; dilution water: non-contaminated mesocosm water; pH of non-contaminated mesocosm water 6.92 at day 1 and 5.92 at day 10; pH of treated mesocosm water 6.82 at day 1 and 5.10 at day 10; dissolved oxygen of non-contaminated mesocosm water 7.1 mg/L at day 1 and 7.7 at day 10; Dissolved oxygen of treated mesocosm water 6.7 mg/L at day 1 and 7.7 at day 10; initial cell density of 10^4 cells/mL.

Analytics: Analytical verification was conducted in the treated mesocosm water at day 1 and day 10 post-application using a HPLC-method.

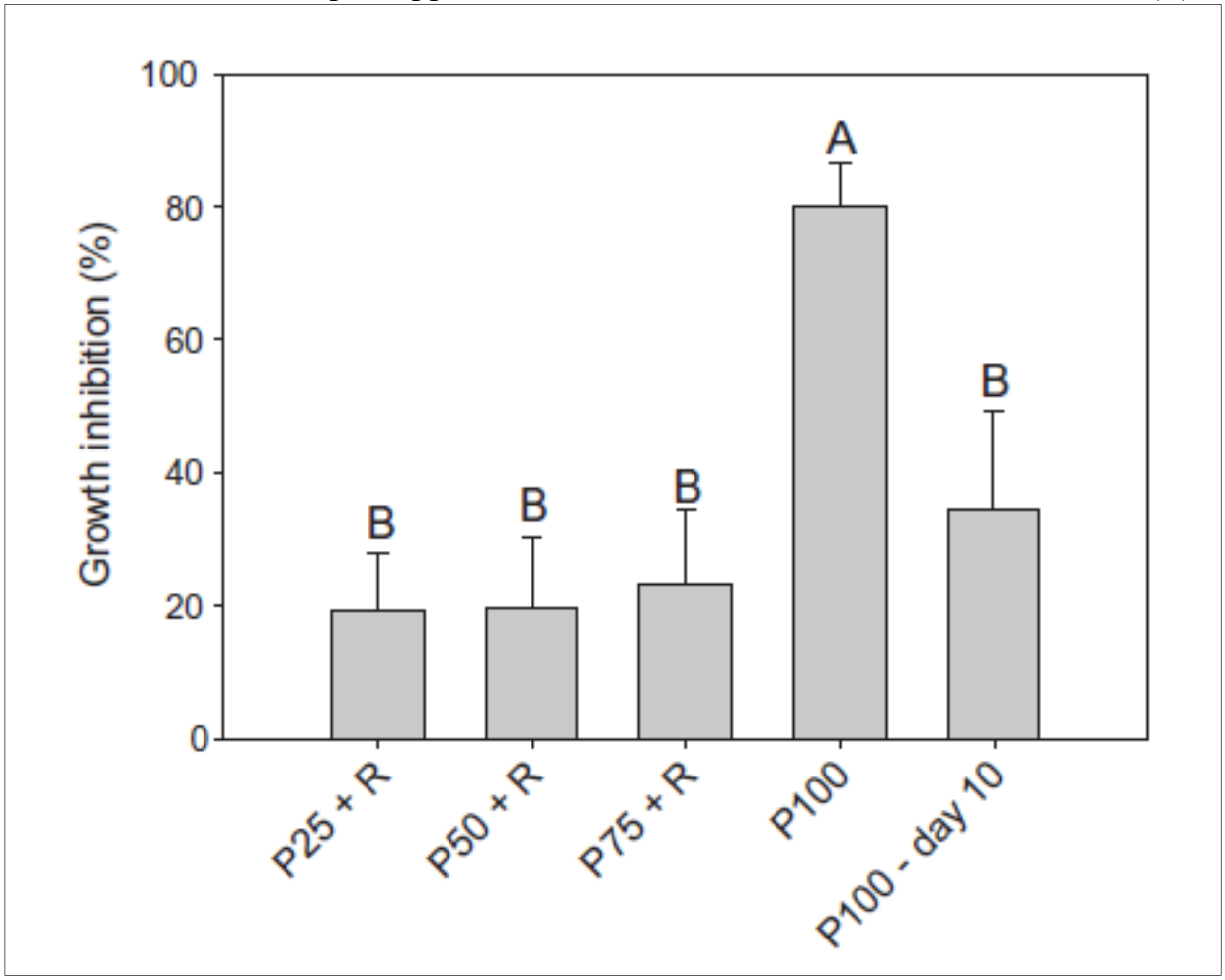
Statistics: Descriptive statistics, trimmed Spearman Karber method to determine E_bC_{50} values; ANOVA followed by Dunnet multiple comparison test to compare all treatments with growth recorded in the reference sample, ANOVA followed by Bonferroni multiple comparison test to compare all treatments simultaneously, one way ANOVA followed by Bonferroni post hoc test to compare between cell growth in samples taken at day 1 and 10.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentration was conducted at day 1 and day 10 post-application in the treated mesocosm water. Pyrimethanil concentrations in treated mesocosm water were 1.40 ± 0.06 mg a.s./L and 0.78 ± 0.06 mg a.s./L day 1 and day 10 post-application, respectively. As no analytical measurements were conducted for the pyrimethanil dilutions prepared for the growth inhibition test, the biological results are based on the nominal test item concentrations.

Biological results: In the reference samples, cells multiplied 56 times after 72 h of exposure, while when exposed to pyrimethanil at 100% they multiplied only 7 times. Growth in all pyrimethanil samples diluted with reference sample were statistically different to growth in the reference sample after 72 h of exposure ($p < 0.05$; Dunnet Multiple Comparisons Test).
Growth inhibition of cells exposed to pyrimethanil and diluted with reference sample was ca. 20% for the three lowest dilutions (pyrimethanil at 25, 50 and 75% + reference) when compared with undiluted reference sample (see Figure 8.2.8-5), while growth inhibition of the cells exposed to the pyrimethanil-contaminated mesocosm sample was significantly reduced by 80% (±6.5) ($p < 0.05$; Bonferroni Multiple Comparisons Test).
After 72 h of exposure, the EC_{50} based on the area under the growth curve (*i.e.* biomass) was 85 ± 2% (*i.e.* 1.2 ± 0.03 mg a.s./L) when compared with the reference sample.
The toxicity of samples taken from contaminated mesocosms at day 10 was attenuated to 34% (±15) when compared with reference sample.

Figure 8.2.8-5: Percentages of growth inhibition of cells of the microalgae *S. capricornutum* exposed for 72 h to pyrimethanil-treated (P) mesocosm water taken at days 1 and 10 post-application and diluted with reference mesocosm water (R).



Different letters indicate statistically significant differences among treatments at the end of the assay ($p < 0.05$; Bonferroni Multiple Comparisons Test).

P25 + R	25% pyrimethanil dilution with mesocosm water taken at day 1 post application
P50 + R	50% pyrimethanil dilution with mesocosm water taken at day 1 post application
P75 + R	75% pyrimethanil dilution with mesocosm water taken at day 1 post application
P100 - Day 1	100% mesocosm water taken at day 1 post application
P100 - Day 10	100% mesocosm water taken at day 10 post application

III. CONCLUSION

In a 72-hour algae test with *Selenastrum capricornutum* using pyrimethanil treated mesocosm water, the 72 h E_bC_{50} of pyrimethanil was determined to be 1.2 mg a.s./L (nominal).

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CA 8.3 Effects on arthropods

CA 8.3.1 Effects on bees

Since Annex I inclusion of pyrimethanil (BAS 605 F), new studies on bees have been performed with the active substance (tested with the solo-formulation BAS 605 04 F due to technical reasons). As a result there are new endpoints which are now considered in the honeybee risk assessment. Summaries of these new studies are provided in CP 10.3.1.1, 10.3.1.2, and 10.3.1.3.

Details on the EU agreed studies which have been already evaluated within the Annex I inclusion of pyrimethanil are provided in the EU Review documents of pyrimethanil (Draft Assessment Report, vol. 1, 2005; EFSA Scientific Report, Jan. 2006).

The results of all studies are summarized in Table 8.3.1-1.

Table 8.3.1-1: List of studies and endpoints with honeybees and the active substance pyrimethanil (BAS 605 F)

Substance	Test species	Endpoint	Value	Reference (BASF DocID)	EU agreed
pyrimethanil	honeybee	48 h acute oral LD_{50}	> 100 µg a.s./bee	Barrett and Barber, A81877	yes
		48 h acute contact LD_{50}	> 100 µg a.s./bee		
pyrimethanil [1)]	bumblebee	96 h acute oral LD_{50}	> 579 µg a.s./bee	Amsel, 2014/1000863	no, new study
		96 h acute contact LD_{50}	> 400 µg a.s./bee		
pyrimethanil [1)]	honeybee	10 d chronic LD_{50}	55.6 µg a.s./bee/day	Kleebaum, 2013/1376402	no, new study
		10 d chronic NOED	18.9 µg a.s./bee/day		
		10 d chronic LC_{50}	2.84 g a.s./kg food		
		10 d chronic NOEC	0.803 g a.s./kg food		
pyrimethanil [1)]	honeybee	72 h acute oral larvae LD_{50}	> 99.2 µg a.s./larva	Kleebaum, 2013/1376403	no, new study
		72 h acute oral larvae LC_{50}	> 2.926 g a.s./kg food		

[1)] Studies were conducted with the solo-formulation BAS 605 04 F (containing 40% pyrimethanil, nominally) due to technical reasons.

CA 8.3.1.1 Acute toxicity to bees

CA 8.3.1.1.1 Acute oral toxicity

The tests for bumblebees were performed with the active substance as contained in BAS 605 04 F and reference is made to chapter CP 10.3.1.1.1 (see DocID 2014/1000863).

CA 8.3.1.1.2 Acute contact toxicity

The tests for bumblebees were performed with the active substance as contained in BAS 605 04 F and reference is made to chapter CP 10.3.1.1.2 (see DocID 2014/1000863).

CA 8.3.1.2 Chronic toxicity to bees

The test was performed with the active substance as contained in BAS 605 04 F and reference is made to chapter CP 10.3.1.2 (see DocID 2013/1376402).

CA 8.3.1.3 Effects on honeybee development and other honeybee life stages

The tests were performed with the active substance as contained in BAS 605 04 F and reference is made to chapter CP 10.3.1.3 (see DocID 2013/1376403).

CA 8.3.1.4 Sub-lethal effects

No new studies are available. However, two semi-field tunnel tests (DocID 2012/1159662 and DocID 2014/1000867) with the formulation BAS 605 04 F are available, which sufficiently address effects of the active substance to honeybees. Please refer to CP 10.3.1.5 for details. Furthermore, a field test with BAS 605 04 F (DocID 2014/1000868) was conducted, which sufficiently addresses effects of the active substance to honeybees. Please refer to CP 10.3.1.6 for details.

CA 8.3.2 Effects on non-target arthropods other than bees

No new studies are available.

CA 8.3.2.1 Effects on Aphidius rhopalosiphi

No new studies are available.

CA 8.3.2.2 Effects on Typhlodromus pyri

No new studies are available. Several studies were conducted with the formulated product (reference is made to 10.3.2).

CA 8.4 Effects on non-target soil meso- and macrofauna

Since Annex I inclusion of the active substance pyrimethanil (BAS 605 F), one new study on soil macro organisms has been performed with a soil metabolite of pyrimethanil, M605F007 (= AE F132593, Reg.No. 40 603). As a result, there is one new endpoint which is considered in the respective risk assessment. A summary of this new study is provided below.

Details on the EU agreed studies which have been already evaluated within the Annex I inclusion of pyrimethanil are provided in the EU Review documents of pyrimethanil (Draft Assessment Report, vol. 1, 2005; EFSA Scientific Report, Jan. 2006).

An overview on studies and endpoints is given in Table 8.4-1.

Table 8.4-1: Toxicity to non-target soil meso- and macrofauna of pyrimethanil and relevant metabolites

Substance	Species	Endpoint	Value [mg/kg dry soil]	Reference (BASF Doc ID)	EU agreed
pyrimethanil	*Eisenia fetida*	$LC_{50, CORR}$	313 *	Barrett, A81882	yes
M605F007 (=AE F132593)	*Eisenia fetida*	LC_{50}	> 1000	Sowig, C012176	yes
M605F007 (=AE F132593)	*Eisenia fetida*	NOEC	≥ 8.0	Luehrs, 2005/1026415	no, new study

* Toxicity endpoint is adjusted using a soil factor of 2 to address the organic content of the soil (peat 10%), and the log P_{ow} of the substance is > 2.

CA 8.4.1 Earthworms – sub-lethal effects

Report:	CA 8.4.1/1 Luehrs U., 2005a Effects of AE F132 593 (Metabolite of BAS 605 F, Pyrimethanil) on reproduction and growth of earthworms Eisenia fetida in artificial soil with 5% peat 2005/1026415
Guidelines:	OECD 222, ISO 11268-2 (1998)
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Landwirtschaft und Forsten, Wiesbaden)

Executive Summary

In a chronic toxicity study, adults of *Eisenia fetida* (Annelida: Oligochaeta), were exposed to M605F007 (= AE F132593, Reg. no. 40 603), a metabolite of pyrimethanil. The test item was mixed into artificial soil (5% peat only) at concentrations of 0.5, 1.0, 2.0, 4.0 and 8.0 mg M605F007/kg dry soil. For the control treatment, the soil was left untreated. Assessment of worm mortality, body weight and feeding activity was carried out after 28 days and reproduction (number of juveniles) was assessed after 56 days.

After 28 days of exposure no mortality was observed in the control compared to a slight, but statistically not significantly different mortality of 2.5% in the test item treatment groups. No statistically significant differences on body weight were observed up to and including the highest test item concentration of 8.0 mg M605F007/kg dry soil. No statistically significant differences on reproduction were observed up to and including the highest test item concentration of 8.0 mg M605F007/kg dry soil. No behavioral abnormalities were observed in any of the treatment groups. The feeding activity in all test item treated groups was comparable to the control.

In a 56-day reproduction study with M605F007 on earthworms (*Eisenia fetida*), the NOEC was determined to be ≥ 8.0 mg/kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: M605F007 (= AE F132593, metabolite of BAS 605 F, pyrimethanil), Reg. no.: 40 603, batch no. L72-129, purity: 98.6%.

Test species: *Eisenia fetida* (earthworm); adult worms with clitellum and weight of 350 mg - 550 mg, approx. 11 months old; source: in-house culture.

B. STUDY DESIGN

Test design: 56-day test in treated artificial soil according to OECD 222 (5% peat), different concentrations of the test item are mixed homogeneously into the soil; 6 treatment groups were set up (5 concentrations of the test item, untreated control) with 4 replicates for the test item treatment groups and 8 replicates for the control; each with 10 worms. Assessment of worm mortality, behavioral effects and biomass development was done after 28 days of exposure; after an additional 28 days (56 days after application) reproduction (number of juveniles) was assessed.

Endpoints: NOEC.

Reference item: Brabant Carbendazim Flowable (carbendazim, 500 g/L nominal). The effects of the reference item were investigated in a separate study.

Test concentrations: Control, 0.5, 1.0, 2.0, 4.0 and 8.0 mg M605F007/kg dry soil.

Test conditions: Artificial soil according to OECD 222 with a reduced content of peat (5%); pH 5.7 - 6.0 at test initiation, 5.9 - 6.1 at test termination; water content 52.8% - 54.3% of its max. water holding capacity (WHC) at test initiation and 41.8% - 68.5% WHC at test termination; temperature: 18 °C - 21 °C; photoperiod: 16 hours light: 8 hours dark, light intensity: 420 lux - 800 lux; food: cattle manure.

Statistics: Descriptive statistics; Fisher's Exact test for mortality data and Dunnett's test for weight change and reproduction data ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

After 28 days of exposure no mortality was observed in the control compared to a slight, but statistically not significantly different mortality of 2.5% in the test item treatment groups (Fisher's Exact test, $\alpha = 0.05$). No statistically significant differences on body weight were observed up to and including the highest test item concentration of 8.0 mg M605F007/kg dry soil (Dunnett's test, $\alpha = 0.05$). No statistically significant differences on reproduction were observed up to and including the highest test item concentration of 8.0 mg M605F007/kg dry soil (Dunnett's test, $\alpha = 0.05$).
No behavioral abnormalities were observed in any of the treatment groups. The feeding activity in all test item treated groups was comparable to the control. The results are summarized in Table 8.4.1-1.

Table 8.4.1-1: Effects of M605F007 on *Eisenia fetida* in a 56-day reproduction study

M605F007 [mg/kg dry soil]	**Control**	**0.5**	**1.0**	**2.0**	**4.0**	**8.0**
Mortality (28 d) [%]	0.0	2.5	2.5	2.5	2.5	2.5
Weight change (28 d) [%]	34.4	30.7	32.6	37.8	35.8	38.2
Number of juveniles (56 d)	367	322	256	343	302	315
Reproduction [% of control] (56 d)	--	87.7	69.6	93.4	82.4	85.7
Endpoints [mg M605F007/kg dry soil]						
$NOEC_{mortality,\ weight\ change}$ (28 d)	≥ 8.0					
$NOEC_{reperoduction}$ (56 d)	≥ 8.0					

III. CONCLUSION

In a 56-day reproduction study with M605F007 on earthworms (*Eisenia fetida*), the NOEC was determined to be ≥ 8.0 mg/kg dry soil.

Further studies were conducted with the formulated product (reference is made to chapter CP 10.4).

CA 8.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

No new studies are available. Studies were conducted with the formulated product (reference is made to chapter CP 10.4).

CA 8.4.2.1 Species level testing

No new studies are available.

CA 8.5 Effects on nitrogen transformation

Since Annex I inclusion of the active substance pyrimethanil (BAS 605 F), a new study on nitrogen transformation has been performed with the metabolite M605F007 (= AE F132593, Reg. no. 40 603). As a result, there is a new endpoint which is considered in the risk assessment. A summary of this new study is provided below.

Details on the EU agreed studies which have been already evaluated within the Annex I inclusion of pyrimethanil are provided in the EU Review documents of pyrimethanil (Draft Assessment Report, vol. 1, 2005; EFSA Scientific Report, Jan. 2006).

An overview on studies and endpoints is given in Table 8.5-1 below.

Table 8.5-1: Toxicity to nitrogen transformation of pyrimethanil and its metabolites

Substance	Endpoint	Endpoint (< 25% effect) [mg/kg dry soil]	Reference (BASF DocID)	EU agreed
pyrimethanil [1)]	Effects on nitrogen transformation	1.08	Vonk, A81885	yes
M605F007 (=AE F132593)	Effects on nitrogen transformation	0.6	Schulz, 2005/1027568	no, new study

1) Test carried out with a pyrimethanil solo-formulation (40% w/w pyrimethanil suspension concentrate).

Report:	CA 8.5/1 Schulz L., 2005a Effects of AE F132593 (metabolite of BAS 605 F, Pyrimethanil) on the activity of soil microflora (nitrogen transformation test) 2005/1027568
Guidelines:	OECD 216 (2000)
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

In a soil microbial activity study, the effect of M605F007 (= AE F132593, Reg. no. 40 603), a metabolite of pyrimethanil, on nitrogen transformation was tested in a loamy sand soil. M605F0073 was applied to samples of the soil, at nominal concentrations of 0.06 and 0.60 mg/kg dry soil. Triplicate samples of each treatment were removed for analysis of NH_4-nitrogen and NO_3-nitrogen 0, 7, 14, and 28 days after application.

No unacceptable effects of M605F007 on nitrogen transformation in soil were observed in the test item concentrations of 0.06 and 0.60 mg/kg dry soil after 28 days. Only negligible deviations from the control of +0.4 and +0.1% were observed in the treatment groups after 28 days.

Based on the results of this study, M605F007 caused no unacceptable short-term and no unacceptable long-term effects on the soil nitrogen transformation (measured as NO_3-N production) in a loamy sand soil tested up to a concentration of 0.60 mg/kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: M605F007 (= AE F132593, metabolite of BAS 605 F, pyrimethanil), Reg. no.: 40 603, batch no. L72-129, purity: 98.6%.

Test soil: Biologically active agricultural soil: loamy sand soil, pH 6.8, C_{org}: 1.44%, WHC: 42.32%.

B. STUDY DESIGN

Test design: Determination of N-transformation (NO_3-nitrogen production) in soil enriched with lucerne meal (concentration in soil 0.5%). Three treatment groups were set up (one untreated control group and two concentrations of the test item) with three replicates per treatment. Comparison of test item treated soil with a non-treated soil. NH_4-nitrogen formed from organically bound nitrogen and NO_3-nitrogen formed from the nitrification process was determined by using the Autoanalyzer II (Bran + Luebbe). Sampling scheme: 0, 7, 14, and 28 days after treatment, sub-samples were withdrawn from each of the 3 replicates and subjected to extraction and nitrogen measurement.

Endpoints: Effects on NO_3-nitrogen production 28 days after exposure.

Test concentrations: Control, 0.06 and 0.60 mg M605F007/kg dry soil.

Reference item: Dinoterb. The reference item was tested in a separate study at 6.8 and 16 mg/kg dry soil.

Test conditions: Water content: approx. 45% of its maximum water holding capacity; pH 6.8. Soil samples were incubated at 20 °C ± 2 °C while stored in glass flasks in the dark.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

No unacceptable effects of M605F007 on nitrogen transformation in soil were observed in the test item concentrations of 0.06 and 0.60 mg/kg dry soil after 28 days. Only negligible deviations from the control of +0.4 and +0.1% were observed in the treatment groups after 28 days. The results are summarized below in Table 8.5-2.

Table 8.5-2: Effects of M605F007 on soil micro-organisms (nitrogen transformation) on days 7, 14, and 28 of incubation

Soil (days)	Control	0.06 mg M605F007/kg dry soil		0.60 mg M605F007/kg dry soil	
	NO_3-N [mg/kg dry soil]	NO_3-N [mg/kg dry soil]	% Deviation from control [1)]	NO_3-N [mg/kg dry soil]	% Deviation from control [1)]
Loamy sand (7 d)	31.3	33.1	+5.9	32.0	+2.2
Loamy sand (14 d)	33.9	34.6	+2.1	34.3	+1.2
Loamy sand (28 d)	45.8	46.0	+0.4	45.8	+0.1

[1)] Based on NO_3-nitrogen production; - = inhibition, + = stimulation

In a separate study the reference item dinoterb produced the expected level of effect (+41.6% on day 28).

III. CONCLUSION

Based on the results of this study, M605F007 caused no unacceptable short-term and no unacceptable long-term effects on the soil nitrogen transformation (measured as NO_3-N production) in a loamy sand soil tested up to a concentration of 0.60 mg/kg dry soil.

CA 8.6 Effects on terrestrial non-target higher plants

No new studies are available.

CA 8.6.1 Summary of screening data

No new studies are available.

CA 8.6.2 Testing on non-target plants

No new studies are available with the active substance. Studies were performed with the formulated product and reference is made to chapter CP 10.6.

CA 8.7 Effects on other terrestrial organisms (flora and fauna)

The following study is no longer a data requirement for risk assessments; therefore, it is presented as additional information.

Report: CA 8.7/1
Schulz L., 2005b
Effects of AE F132593 (metabolite of BAS 605 F, Pyrimethanil) on the activity of soil microflora (carbon transformation test)
2005/1027569
Guidelines: OECD 217 (2000)
GLP: yes
(certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

In a soil microbial activity study, the effect of M605F007 on carbon transformation was investigated in a loamy sand soil. M605F007 was applied to samples of the soil, in a laboratory, at nominal concentrations of 0.06 and 0.60 mg/kg dry soil. Triplicate samples of each treatment were removed for analysis of carbon transformation (oxygen consumption) 0, 7, 14 and 28 days after application.

No unacceptable effects of M605F007 on carbon transformation in soil were observed in the test item concentrations of 0.06 and 0.60 mg/kg dry soil after 28 days. Only negligible deviations from the control of +0.6 and +0.2% were observed in the treatment groups after 28 days.

Based on the results of this study, M605F007 caused no unacceptable short-term and no unacceptable long-term effects on carbon transformation (measured as oxygen consumption) in a loamy sand soil tested up to a concentration of 0.60 mg/kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: M605F007 (= AE F132593, metabolite of BAS 605 F, pyrimethanil), Reg. no.: 40 603, batch no. L72-129, purity: 98.6%.

B. STUDY DESIGN

Test soil: Biologically active agricultural soil: loamy sand soil, pH 6.8, C_{org}: 1.44%, WHC: 42.32%.

Test design: Determination of carbon-transformation in soil after addition of glucose (concentration in soil: 0.6%). Three treatment groups were set up (one untreated control group and two concentrations of the test item) with three replicates per treatment. Comparison of test item treated soil with a non-treated soil. A respirometer system was used to measure the oxygen consumption over a period of maximum 24 hours at different sampling intervals. Sampling scheme: 0, 7, 14 and 28 days after treatment, sub-samples were withdrawn from the bulk batches and subjected to the measurement.

Test concentrations: Control, 0.06 and 0.60 mg M605F007/kg dry soil.

Endpoints: Effects on O_2 consumption 28 days after exposure.

Reference item: Dinoterb. The reference item was tested in a separate study at 6.8 and 16 mg/kg dry soil.

Test conditions: Water content: approx. 45% of its maximum water holding capacity; pH 6.8. Soil samples were incubated at 20 °C ± 2 °C while stored in steel vessels in the dark.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

No unacceptable effects of M605F007 on carbon transformation in soil were observed in the test item concentrations of 0.06 and 0.60 mg/kg dry soil after 28 days. Only negligible deviations from the control of +0.6 and +0.2% were observed in the treatment groups after 28 days. The results are summarized below in Table 8.7-1.

Table 8.7-1: Effects of M605F007 on soil micro-organisms (carbon transformation) on days 7, 15 and 28 of incubation

Soil (days)	Control	0.06 mg M605F007/kg dry soil		0.60 mg M605F007/kg dry soil	
	O_2 consumption [mg/kg dry soil/h]	O_2 consumption [mg/kg dry soil/h]	% Deviation from control [1)]	O_2 consumption [mg/kg dry soil/h]	% Deviation from control [1)]
Loamy sand (7 d)	7.86	7.62	-3.0	7.78	-1.0
Loamy sand (14 d)	7.45	7.34	-1.6	7.45	-0.1
Loamy sand (28 d)	7.31	7.35	+0.6	7.32	+0.2

[1)] Based on O_2 consumption; - = inhibition, + = stimulation

In a separate study the reference item dinoterb produced the expected level of effect (38.5% inhibition on day 28).

III. CONCLUSION

Based on the results of this study, M605F007 caused no unacceptable short-term and no unacceptable long-term effects on carbon transformation (measured as oxygen consumption) in a loamy sand soil tested up to a concentration of 0.60 mg/kg dry soil.

CA 8.8 Effects on biological methods for sewage treatment

The results of the already peer-reviewed and accepted study are still valid and they are summarized in Table 8.8-1. No new study has been performed.

Table 8.8-1: Effects on biological methods for sewage treatment

Test item	Study type	Endpoint [mg a.s./L]	Reference (BASF DocID)	EU agreed
BAS 605 F (pyrimethanil)	Respiration inhibition test (inhibition of oxygen consumption activated sludge from wastewater plant)	The 3 hour EC_{50} for inhibition of respiration of sewage sludge micro-organisms is 360 mg/L. Thus, the risk to biological methods of sewage treatment is considered to be low.	C003915	yes

CA 8.9 Monitoring data

According to the knowledge of the applicant, there are currently no published ecotoxicological monitoring data available for pyrimethanil or its metabolites, which would provide additional knowledge on the ecotoxicological assessment not covered by this dossier.

Pyrimethanil

Document M-CA, Section 9

LITERATURE DATA

Compiled by:

[redacted]

Tel.No.: [redacted]
Fax.No.:
E-mail:

Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CA 9 LITERATURE DATA

A literature search on Pyrimethanil and the common product trade names was performed by the BASF Group Information Center. The Literature Search Report on Pyrimethanil describes the general search and evaluation process as well as details on search profiles, search histories and summary tables.

The complete search report is provided in K-CA 9 [see KCA 9/1 2015/1223349].

The first step of the search result processing based on summary records was done by the Information Center and involved the separation into "hits" and "ballast" (obviously irrelevant records).
The “ballast” was not further processed; whereas the “hits” were further evaluated by the scientific experts and categorized into “relevant” (yes/no), “reliable” (yes/no), and “used for dossier”.

To facilitate a comprehensible listing of the "Hits" in the different regulatory areas: Mammalian Toxicology (including Human Toxicology and Operator Exposure), Metabolism (incl. Animal and Plant), Analytics, E-Fate and Ecotoxicology (incl. Wildlife, Aquatic, Terrestric and General), an Excel file was generated for each section with 4 typical sheets, namely:

- "Check for ballast"
- "Check for relevance"
- "Check for reliability"
- “Used for dossier"

The nine relevant Excel files are attached to the search report in K-CA 9 with the file names and results as listed in table below:

Pyrimethanil Literature analytics	10 hits were identified to be discussed in the dossier
Pyrimethanil Literature Ecotoxicology aquatic	8 hits were identified to be discussed in the dossier
Pyrimethanil Literature Ecotoxicology general	No literature was identified to be of interest for use in the dossier
Pyrimethanil Literature Ecotoxicology terrestric	No literature was identified to be of interest for use in the dossier
Pyrimethanil Literature Ecotoxicology wildlife	No literature was identified to be of interest for use in the dossier
Pyrimethanil Literature E-fate	1 hit was identified to be discussed in the dossier
Pyrimethanil Literature Toxicology	17 hits were identified to be discussed in the dossier
Pyrimethanil Literature_metabolism animal	No literature was identified to be of interest for use in the dossier
Pyrimethanil Literature_metabolism residue plant	5 hits were identified to be discussed in the dossier

Besides, a specific literature search was conducted by medical centre – see MCA 5.9

In addition, a search was conducted (i) on EU and (ii) on non-EU institutional websites.

(i) From EU websites, following documentation was retrieved:

- Pyrimethanil DAR (2005), Pyrimethanil peer-Review report, EFSA conclusion (2006) and Pyrimethanil EU review report (2006 & 2010 update) (not provided in K-CA 9 of the present dossier but as separate supportive document)

- Pyrimethanil EFSA opinion on MRLs (2010-2011) in K-CA 9 :
  (BASF DocID 2011/1297731)
  (BASF DocID 2010/1233234)
  (BASF DocID 2011/1297732)

- Pyrimethanil related regulation (Annex I inclusion 2006/74/EC and extension of approval period 540/2011) in K-CA 9 :
  (BASF DocID 2006/1051814)
  (BASF DocID 2014/1326414)

- Records from data bases (Agritox, University of Hertfordshire) in K-CA 9 :
  (BASF DocID 2012/1368842)
  (BASF DocID 2014/1326415)

- Pyrimethanil containing products : National evaluation from ANSES, Ctgb, BVL, PSD in K-CA 9 :
  (BASF DocID 2012/1368843)
  (BASF DocID 2012/1368844)
  (BASF DocID 2007/1071025)
  (BASF DocID 2013/1420162)
  (BASF DocID 2011/1299733)
  (BASF DocID 2009/1132182)
  (BASF DocID 1995/1008594)

(ii) Following documentation from JMPR, Authority from Colombia, Australian APVMA, US EPA, Californian EPAn and Canadian PMRA have been retrieved and are provided in K-CA 9:

- Pyrimethanil JMPR Evaluation (2007) :

(BASF DocID 2007/7017697)
(BASF DocID 2007/7017698)

- Pyrimethanil JMPR Report (2007) :

(BASF DocID 2007/7017699)

- Pyrimethanil Colombian Registration Document :

(BASF DocID 2009/3001791)
(BAS DocID 2011/3008943)
(BASF DocID 2010/3002702)
(BASF DocID 2009/3001732)
(BASF DocID 2005/3001741)
(BASF DocID 2011/3008944)
(BASF DocID 2010/3002703)

- Pyrimethanil Australian APVMA (NRA) Evaluation :

(BASF DocID 2011/8000241)

- Pyrimethanil US EPA Human Health :

(BASF DocID 2008/7023600)
(BASF DocID 2009/7010896)
(BASF DocID 2010/7018806)
(BASF DocID 2012/7007790)
(BASF DocID 2015/7003583)

- Pyrimethanil US EPA Dietary Exposure :

(BASF DocID 2012/7007789)

- Pyrimethanil US EPA Ecotox Report :

(BASF DocID 2014/7004863)
(BASF DocID 2014/7004864)

- Pyrimethanil US EPA EFED Ecological Risk Assessment :

(BASF DocID 2010/7018805)

- Pyrimethanil US EPA Pesticide Tolerances :

(BASF DocID 2004/7014525)
(BASF DocID 2012/7007793)

- Pyrimethanil California EPA :
(BASF DocID 2005/7009154)

- Pyrimethanil Canadian PMRA :
(BASF DocID 2013/7006023)
(BASF DocID 2013/7006024)
(BASF DocID 2006/7017252)

- Pyrimethanil Toxnet HSDB Full Record (2010) :
(BASF DocID 2012/7007791)

Pyrimethanil

Document M-CA, Section 10

CLASSIFICATION AND LABELLING OF THE ACTIVE SUBSTANCE

Compiled by:

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Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CA 10 CLASSIFICATION AND LABELLING OF THE ACTIVE SUBSTANCE

The following harmonized classification and labelling was adopted for pyrimethanil:

Table 10-1: Harmonized classification of Pyrimethanil according to (EC) No 1272/2008

Legislation	Classification	Labelling	Concentration limits
Directive 67/548/EEC (31st ATP)	N R51-53	N R: 51/53 S: 61	
Regulation (EC) No 1272/2008	Hazard class and category code: Aquatic Chronic 2 Hazard statement code: H411	 Hazard statement code: H411	

Regarding toxicology the evaluation conducted within this supplemental dossier submission has not changed the classification. In particular the new studies/data submitted do not affect the health effect classification and labelling. Thus, the harmonized classification as laid down in Regulation (EU) No. 1272/2008 (CLP) based on the evaluation laid down in the 31st ATP to the Council Directive 67/548/EEC (Commission Directive 2009/2/EC of 15 January 2009) is still applicable to pyrimethanil.

Regarding ecotoxicology, new evaluation leads to a change of classification for aquatic chronic hazard, since the lowest chronic endpoint for pyrimethanil is below 100 µg/L (see Table 10-3, 89d ELS fish NOEC). Therefore, BASF proposed the following classification and labelling for pyrimethanil:

Table 10-2: Proposed Classification and Labelling for Pyrimethanil according to (EC) No 1272/2008

Legislation	Classification	Labelling	Concentration limits
Regulation (EC) No 1272/2008	Aquatic Chronic 1 Hazard statement code: H410	 Warning Hazard statement code: H410	M-factor = 1

Table 10-3: Ecotoxicology/Environment data relevant for Classification of pyrimethanil[1]

Study Type (duration)	Results	Reference (BASF DocID)
Cyprinodon variegatus **(96 h)** [$]	**96 h LC_{50} = 2.8 mg/L**	**B003330**
Oncorhynchus mykiss **(89 d)** [$]	**89 d NOEC = 0.077 mg/L**	**B003492**
Daphnia magna (48 h)	48 h EC_{50} = 2.9 mg/L	A81876
Daphnia magna (21 d)	21 d NOEC = 0.97 mg/L	A81880
Selenastrum capricornutum (72 h)	72 h E_rC_{50} = 5.84 mg/L	A81883
	72 h NOEC = 1 mg/L	
Lemna gibba (7 d) [$]	7 d E_rC_{50} > 30 mg/L	B003716
	7 d NOEC = 1.9 mg/L	
Biodegradation	Pyrimethanil is not readily biodegradable	A81871 / A81921

[$] Study was not submitted during Annex I inclusion process of the active substance (for details see chapter CA 8.2).

[1] The lowest acute and chronic endpoint (basis for classification) is marked in **bold**.