Section A7.4.3.5.1 Effects on sediment dwelling organisms

Annex Point IIIA 13.3.4

Official use only

1 REFERENCE

1.1	**Reference**	**A7.4.3.5.1/02:** Backfisch K and Weltje L (2011): Chronic toxicity of Reg. No. 4078193 (BAS 310 I; alpha-Cypermethrin) to the non-biting midge *Chironomus riparius* – a spiked sediment study. BASF SE, Crop protection, Limburgerhof, Germany, Report no. 383065, September 6, 2011 (unpublished), BASF DocID 2011/1124187 **A7.4.3.5.1/03** Backfisch K and Weltje L (2012): Report amendment No. 1: Chronic toxicity of Reg. No. 4078193 (BAS 310 I; alpha-Cypermethrin) to the non-biting midge *Chironomus riparius* – a spiked sediment study. BASF SE, Crop Protection, Limburgerhof, Germany, Report no. 383065, May 23, 2012 (unpublished), BASF DocID 2012/1156939 Habekost (2013): Additional information: Chronic toxicity of *Chironomus riparius* exposed to alpha-cypermethrin - Re-calculation of endpoints based on wet weight (unpublished), BASF DocID 2013/1289188
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF SE
1.2.2	Companies with letter of access	No
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes OECD 218 (2004): Sediment-Water Chironomid Toxicity Test Using Spiked Sediment
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	TGAI BAS 310 I (alpha-cypermethrin) As given in Section A2.
3.1.1	Lot/Batch number	COD-000595
3.1.2	Specification	As given in Section A2.

Section A7.4.3.5.1 Effects on sediment dwelling organisms

Annex Point IIIA 13.3.4

3.1.3	Purity	99.2%	
3.1.4	Composition of product	Not applicable	
3.1.5	Further relevant properties	Water solubility at 20°C (pH 7): 2.5 µg/L	
3.1.6	Method of analysis	Alpha-cypermethrin in overlaying water and sediment pore water was determined by GC/ECD after liquid/liquid extraction into hexane (LoQ = 5 ng/L). Alpha-cypermethrin in sediment was determined by GC/MS using a modified DFG/Deutsche Forschungsgemeinschaft, German Research Foundation) method S19 (LoQ = 1 µg/kg). The validation of the methods and the analytical reports are included in the study report.	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Please refer to Table A7.4.3.5.1- 1.	X
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance		
3.4	**Testing procedure**		
3.4.1	Dilution water	Elendt M4 medium; details are given in Table A7.4.3.5.1-2	
3.4.2	Test organisms	*Chironomus riparius*, as specified in Table A7.4.3.5.1-3	
3.4.3	Test system	The test system is described in Table A7.4.3.5.1-4	
3.4.4	Test conditions	The exposure to alpha-cypermethrin was achieved via treated (spiked) sediment (thoroughly and evenly distributed, please refer to Table A7.4.3.5.1- 1). The sediment was overlaid with reconstituted water (dilution water). The relevant test conditions are presented in Table A7.4.3.5.1-5.	
3.4.5	Duration of the test	28 d (DAT 5 to DAT 33)	X
3.4.6	Test parameter	Larval development time, emergence rate, behaviour, survival	
3.4.7	Examination/ sampling	Before emergence of the first midge: 3 times per week. Daily inspection for emerged midges. Emerged adults were removed from the vessels daily, their number and gender was recorded.	
3.4.8	Monitoring of TS concentration	Yes, analytical determination of the test substance at test initiation (DAT 5) and at test termination (DAT 33) in the sediment pore water, the overlaying water and the sediment.	

Section A7.4.3.5.1 Effects on sediment dwelling organisms

Annex Point IIIA 13.3.4

3.4.9	Statistics	Statistical determination of the NOEC for emergence rate and development rate was done by analysis of variance (ANOVA, $\alpha = 0.05$) followed by Williams' Multiple Sequential *t*-test Procedure to test for significant differences between the treatments. Statistical determination of the EC_x values for emergence rate and development rate was done by Probit analysis using linear max. likelihood regression. The calculations were done using the commercial software package ToxRatPro, Version 2.10 (ToxRat Solutions GmbH).

Section A7.4.3.5.1 Effects on sediment dwelling organisms

Annex Point IIIA 13.3.4

4 RESULTS

4.1	**Range finding test**	The target concentrations for the study were selected based on the results of a non-GLP range finding test. The detailed results of the range finding study are not given in the study report.
4.1.1	Concentration	
4.1.2	Number/percentage of animals showing adverse effects	
4.1.3	Nature of adverse effects	
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	0, 0, 15, 30, 60, 120 and 240 µg/kg dry weight (nominal)
4.2.2	Actual concentrations of test substance	Sediment: < LoD, < LoD, 10.5, 22.5, 45.0, 84.5 and 148.0 µg/kg dry weight (mean measured, n = 2) Pore water: < LoD, < LoD, 0.029, 0.052, 0.128, 0.066, 0.125 µg/L Overlying water: < LoD, < LoD, < LoD, 0.0185, 0.0355, 0.0185, 0.0370 µg/L The chemical analysis of the sediment, overlaying water and pore water was performed at start and termination of the study period (DAT 5 and DAT 33). An additional vessel for the analysis at test initiation for each treatment group was set up. The analysis on day 33 was conducted in the same test vessels as used for the biological assessment) Please refer to Table A7.4.3.5.1- 6, Table A7.4.3.5.1- 7, and Table A7.4.3.5.1- 8 for the analytical results.

Section A7.4.3.5.1 Effects on sediment dwelling organisms

Annex Point IIIA 13.3.4

4.2.3	Effect data	The first emerged midges were observed on day 14 after insertion of the larvae. Total numbers of emerged midges in the test are presented in Table A7.4.3.5.1- 9. There was no indication for different effects on males and females. To improve statistical power, male and female data were pooled for the calculations (males always emerge earlier than females, a natural phenomenon in *C. riparius*). The effect data are based on mean measured concentrations of alpha-cypermethrin. The emergence rate and the development rate are presented in Table A7.4.3.5.1- 10. Treatments were tested against the solvent control as this was considered to provide the best representation of control conditions, since all treatments (except the water control) received the same amount of acetone. Significant differences in the numbers of emerged midges could be detected at the two highest concentrations (84.5 and 148 µg/kg dry sediment). For development rate, at the three highest concentrations significant differences from control/solvent control were detected (45.0, 84.5 and 148 µg/kg dry sediment). The NOEC for development rate was 22.5 µg/kg dry sediment (0.052 µg/L pore water) and for emergence rate 45.0 µg/kg dry sediment (0.128 µg/L pore water) as determined by ANOVA, Williams' Multiple Sequential t-test Procedure ($p<0.05$). The effects at the NOEC for development rate were 3.2% and for emergence rate 9.72%, respectively. As the observed effects at the NOEC, especially for development rate, are below 10%, EC_x values were calculated, which are considered more suitable for risk assessment. For emergence rate, the calculated EC_{10}, EC_{15} and EC_{50} were 51.4, 58.5 and 101.4 µg/kg dry sediment, respectively. For the development rate the calculated EC_{10} was 74.1 µg/kg dry sediment. The EC_{15} and the EC_{50} for development rate were > 148 µg/kg dry sediment.
4.2.4	Concentration / response curve	Please refer to Figure A7.4.3.5.1- 1, Figure A7.4.3.5.1- 2 and Figure A7.4.3.5.1- 3 for emergence and development rates.
4.2.5	Other effects	
4.3	**Results of controls**	No effects were seen in the solvent or water controls (as given in Table A7.4.3.5.1- 9)
4.4	**Test with reference substance**	Relation to a study with a reference substance, completed in December 2010 at BASF: the chronic toxicity of lindane to the non-biting midge *Chironomus riparius* was tested using spiked water.
4.4.1	Concentrations	Concentrations used are not given.
4.4.2	Results	The NOEC for the development and emergence rate determined in the separate study was 2.0 µg/L (nominal). The results were within the expected range for lindane.

Section A7.4.3.5.1 Effects on sediment dwelling organisms

Annex Point IIIA 13.3.4

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The toxicity of the test substance alpha-cypermethrin to sediment-dwelling organisms was tested using larvae of the midge *Chironomus riparius*, according to OECD 218. According to this guideline, *Chironomus* larvae were exposed to the test substance over 28 days via spiked sediment. Effects on emergence rate, development rate and survival were evaluated.

5.2 Results and discussion

Analytical measurements of BAS 310 I by GC/MS yielded the following mean-measured values:

The overlaying water concentrations measured by GC/ECD ranged from <LoD to 0.037 µg/L.

The pore water concentrations measured by GC/ECD ranged from <LoD to 0.128 µg/L.

In the sediment, <LoD, 10.5, 22.5, 45.0, 84.5 and 148.0 µg/kg dry sediment were measured. These values were used for the statistical evaluation of the biological data.

Conversion from test item concentration in sediment dry weight to sediment wet weight (BASF DocID 2013/1289188):

The conversion from dry weight to wet weight was based on the actual data from the Chironomus study. As documented in the raw data and shown in Table A7.4.3.5.1- 12, the water content (calculated from the weight difference of wet sediment and sediment after drying) in each sediment sample of DAI 0 and DAI 28 ranged from 23.2 to 28.1 % with an average water content in wet sediment of 24.72% (n = 28, coefficient of variation = 5.14 %).

According to the TGD (part II. Chapter 3, table 5, p. 43) the density of sediment solid particles is 2.5 kg/L (=2,500 kg/m^3) and the density of the water phase 1 kg/L (=1,000 kg/m^3). The sediment used in the study consists of 24.72% water and 75.26% solid particles, which results in a 'wet' density of (0.2472 x 1.0 kg/L) + (0.7528 x 2.5 kg/L) = 2.1294 kg/L. The dry weight is consequently (0.7528 x 2.5) = 1.882 kg (per litre wet sediment) and the ratio wet:dry is 1.13:

Calculation of conversion factor:

Density wet sediment: (0.2472 x 1 kg/L) + (0.7528 x 2.5 kg/L) = 2.1294 kg/L

Weight of dry sediment: (0.7528 x 2.5 kg/L) = 1.882 kg per L wet sediment

Conversion factor wwt to dwt: 2.1294 / 1.882 = 1.1315, **rounded 1.13**

The conversion factor of 1.13 is used to calculate the concentrations of the active substance from the dry sediment weight to the wet sediment weight.

Section A7.4.3.5.1 Effects on sediment dwelling organisms

Annex Point IIIA 13.3.4

5.2.1	NOEC	22.5 µg/kg dry sediment and 0.052 µg/L pore water for development rate 19.9 µg/kg wet sediment 45.0 µg/kg dry sediment and 0.128 µg/L pore water for emergence rate 39.8 µg/kg wet sediment
5.2.2	EC_{10}	74.1 µg/kg dry sediment for development rate 65.6 µg/kg wet sediment 51.4 µg/kg dry sediment for emergence rate 45.5 µg/kg wet sediment
5.2.3	EC_{15}	> 148 µg/kg dry sediment for development rate > 130 µg/kg wet sediment 58.5 µg/kg dry sediment for emergence rate 51.8 µg/kg wet sediment
5.2.4	EC_{50}	> 148 µg/kg dry sediment for development rate > 130 µg/kg wet sediment 101.4 µg/kg dry sediment for emergence rate 89.7 µg/kg wet sediment
5.3	**Conclusion**	All validity criteria are fulfilled (please refer to Table A7.4.3.5.1- 11). The study on the young larvae of *C. riparius* is considered to be valid without restriction. At the NOEC for development rate, 22.5 µg/kg dry sediment, there was only 3.2% effect, while the EC10 was 74.1 µg/kg dry sediment. An effect of 3.2% was not considered to be of biological significance as performance at this treatment was even within the validity criteria for the control emphasizing the high quality of the study and low level of (statistically significant) effects. For PNEC derivation it is suggested to use the next higher treatment, i.e. 39.8 µg/kg wet sediment.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	January 2013
Materials and Methods	The Applicant's version is acceptable with the following comments/information: Section 3.2, Table A7.4.3.5.1- 1 : Vehicle: Read: 'After an evaporation time of about 1.5 h under the fume hood, ...' instead of : ' After total evaporation of the acetone'. Section 3.4.5: Duration of the test 28 days (DAT 5 to DAT 33). DAT stands for 'Day after Treatment'.
Results and discussion	BE CA agrees with the applicant's version.
Conclusion	BE CA agrees with the applicant's version.
Reliability	1
Acceptability	Acceptable
Remarks	None
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.4.3.5.1- 1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	No
Vehicle	The exposure in the test was achieved via treated (spiked) sediment. For the spiking procedure, the test substance was dissolved in acetone. Certain amounts of this clear stock solution were mixed with small amounts of quartz sand. After total evaporation of the acetone, the spiked quartz sand was mixed homogenously with the other constituents of the sediment to obtain the different intended test concentrations (µg alpha-cypermethrin/kg sediment, dry weight). The resulting test sediment (artificial substrate, prepared according to OECD test guideline No. 218 and consisting of the following total fractions (dry weight basis, including the spiked quartz sand fraction): ca 5% finely ground sphagnum peat; ca 20% kaolin clay (kaolinite content > 30%); ca. 0.75% $CaCO_3$ (Riedel-de-Haen 12010); ca. 75% quartz sand (ISS-O-VAC, Fa. Gebr. Willersinn), >80% has a particle size > 0.09 and < 0.18 mm. Moisture of the blend: ca. 30% due to addition of water. The pH of the final mixture was determined to be 7.18.
Concentration of vehicle	Example: nominal concentration: 15 µg alpha-cypermethrin/kg dry sediment: 80 g quartz sand spiked with a total of 10.5 µg alpha-cypermethrin, were added to 620 g dry sediment. Likewise the other test concentrations used in this test were prepared (0, 15, 30, 60, 120 and 240 µg/kg dry weight) using respective volumes of the stock solution. For the water control, the quartz sand without solvent was mixed into the sediment.
Vehicle control performed	Yes, solvent control in addition to blank control
Other procedures	In order to include potentially remaining residues from the vessel in which quartz sand and test item solutions were mixed, the vessel was rinsed with deionised water, which afterwards was carefully added to the dry sediment (approx. 100 mL). The resulting moisture content of the sediment was approximately 30%.

Table A7.4.3.5.1-2: Dilution water.

Criteria	Details
Source	Elendt M4 medium, composed on the basis of ultrapure deionised water, at least 24 hours before use to allow stabilisation of the solution.
Hardness	2.53 mmol/L
Oxygen content	8.89 (at 20.9 °C, > 60% air saturation value)
pH	7.94
Conductivity	678 µS/cm
Ca^{2+} / Mg^{2+} ratio	4/1
Na^{+} / K^{+} ratio	10/1
Holding water different from dilution water	No

Table A7.4.3.5.1-3: Test organisms.

Criteria	Details
Strain / Clone	*Chironomus riparius* larvae
Source	In-house laboratory culture, established with egg-ropes obtained from the Zoological Institute of the J.W. Goethe University in Frankfurt M., Germany.
Age	≤ 2 days
Breeding method	Culture conditions and food type are identical to test conditions (Table A7.4.3.5.1-5)
Kind of food	Commercially available fish food, TetraMin, finely ground and suspended in M4 water
Amount of food	Approx. 0.25 – 1.0 mg TetraMin per larva per day (i.e. 5 – 20 mg/vessel/day)
Feeding frequency	Daily up to day 24 of treatment
Pre-treatment	No (not required)
Feeding of animals during test	Yes

Table A7.4.3.5.1-4: Test system.

Criteria	Details
Test type	Static
Renewal of test solution	Not applicable
Volume of test vessels	Volume: 600 mL filled with 100 ± 2 g spiked wet artificial sediment, carefully overlaid with 400 mL reconstituted water (ca 7.5 – 8 cm water layer)
Volume/animal	0.02 L overlaying water, approx. 5 g sediment
Number of animals/vessel	20 first-instar larvae
Number of vessels/concentration	4 (test item concentration and the solvent free or water control) 6 (solvent control, acetone)
Test performed in closed vessels due to significant volatility of TS	No, but the vessels were covered to reduce evaporation and to avoid the escape of emerging midges

Table A7.4.3.5.1-5: Test conditions.

Criteria	Details
Test temperature	20 ± 1 °C (temperature controlled room)
Dissolved oxygen	7.89 – 10.06 mg/L
Ammonium content	Day of insertion of larvae: 0.8 mg/L in the control, solvent control and the 240 µg/kg dry sediment treatment. 28 days after insertion of larvae: 2.0 mg/L in the control, solvent control and in the 240 µg/kg dry sediment treatment.
Total hardness of the M4 water during the test	2.75 to 3.0 mmol/L
pH	7.83 – 8.29
Adjustment of pH	No
Aeration of dilution water	Yes, gentle aeration through a glass pipette with the outlet positioned a few cm above the sediment. The test system was allowed to stabilise for 5 days before addition of the larvae (on DAT 5). During addition of the larvae and for about 24 h afterwards, the aeration was stopped to give the larvae the opportunity to settle into the sediment.
Quality/Intensity of irradiation	536 – 952 Lux
Photoperiod	16:8 h (L:D), 1 h dawn and dusk phase (> 500 lux)

Table A7.4.3.5.1- 6: Concentrations of BAS 310 I in the sediment in µg/kg dry sediment

Nominal target concentration [µg/kg d.s.]	DAT 5	DAT 33	Mean measured
	Measured concentration [µg/kg d.s.]	Measured concentration [µg/kg d.s.]	[µg/kg d.s.]
0 (water control)	<LoD	<LoD	<LoD
0 (solvent control)	<LoD	<LoD	<LoD
15	10	11	10.5
30	22	23	22.5
60	48	42	45.0
120	87	82	84.5
240	146	150	148.0

DAT = day after treatment, DAT 5 = test initiation.
d.s. = dry sediment
Limit of Quantification = 1 µg/kg

Table A7.4.3.5.1- 7: Concentrations of BAS 310 I in overlaying water

Nominal target concentration [µg/kg dry sediment]	DAT 5	DAT 33	Mean measured
	Measured concentration [µg/L]	Measured concentration [µg/L]	[µg/L]
0 (water control)	<LoD	<LoD	<LoD
0 (solvent control)	<LoD	<LoD	<LoD
15	<LoD	<LoD	<LoD
30	0.024	0.013*	0.0185
60	0.035	0.036	0.0355
120	0.018	0.019	0.0185
240	0.049	0.025	0.0370

DAT = day after treatment
Limit of Quantification = 5 ng/L
* = Results from all measurements including the retain samples

Table A7.4.3.5.1- 8: Concentrations of BAS 310 I in pore water

Nominal target concentration [µg/kg dry sediment]	DAT 5	DAT 33	Mean measured
	Measured concentration [µg/L]	Measured concentration [µg/L]	[µg/L]
0 (water control)	<LoD	<LoD	<LoD
0 (solvent control)	<LoD	<LoD	<LoD
15	0.031	0.027	0.029
30	0.080	0.024	0.052
60	0.210	0.046	0.128
120	*	0.066	0.066
240	0.110	0.140	0.125

DAT = day after treatment

Limit of Quantification = 5 ng/L

* = no measurement, sample was lost during centrifugation

Table A7.4.3.5.1- 9: Effect data: Numbers of emerged midges (within 28 days, while the first midges emerged on day 14 after insertion of the larvae)

Nominal target concentration [µg/kg dry sediment]	Vessel 1	Vessel 2	Vessel 3	Vessel 4	Vessel 5*	Vessel 6*	Mean
Control	19	18	17	19	–	–	18.3
Solvent control	18	18	18	17	17	20	18.0
15	18	18	19	17	–	–	18.0
30	17	19	20	15	–	–	17.8
60	17	16	17	15	–	–	16.3
120	17	12	15	14	–	–	14.5
240	2	1	6	1	–	–	2.5

Control and test concentrations: 4 test vessels à 20 larvae; Solvent control: 6 test vessels à 20 larvae

Table A7.4.3.5.1- 10: Emergence rate (ER) and development rate (DR) with respective standard deviations (SD)

Mean measured concentrations [µg/kg dry sediment	**ER (SD)**	**DR (SD)**
Control	0.9125 (0.479)	0.0665 (0.0010)
Solvent control	0.9000 (0.0548)	0.0672 (0.0016)
10.5	0.9000 (0.0408)	0.0652 (0.0020)
22.5	0.8875 (0.1109)	0.0651 (0.0033)
45	0.8125 (0.0479)	0.0618 (0.0020)*
84.5	0.7250 (0.1041)*	0.0594 (0.0013)*
148	0.1250 (0.1190)*	0.0577 (0.0063)*

* statistically significant

Table A7.4.3.5.1- 11: Validity criteria and obtained data for the sediment-water chironomid toxicity test according to OECD Guideline 218.

	Fulfilled
Emergence in the controls ≥ 70%	☑
Emergence in the controls occurred between 12 and 23 days after insertion of the larvae	☑
Oxygen concentration was ≥ 60% of the air saturation value	☑
pH of the water was in the range of 6 – 9	☑
Water temperature did not differ more than ± 1.0 °C	☑

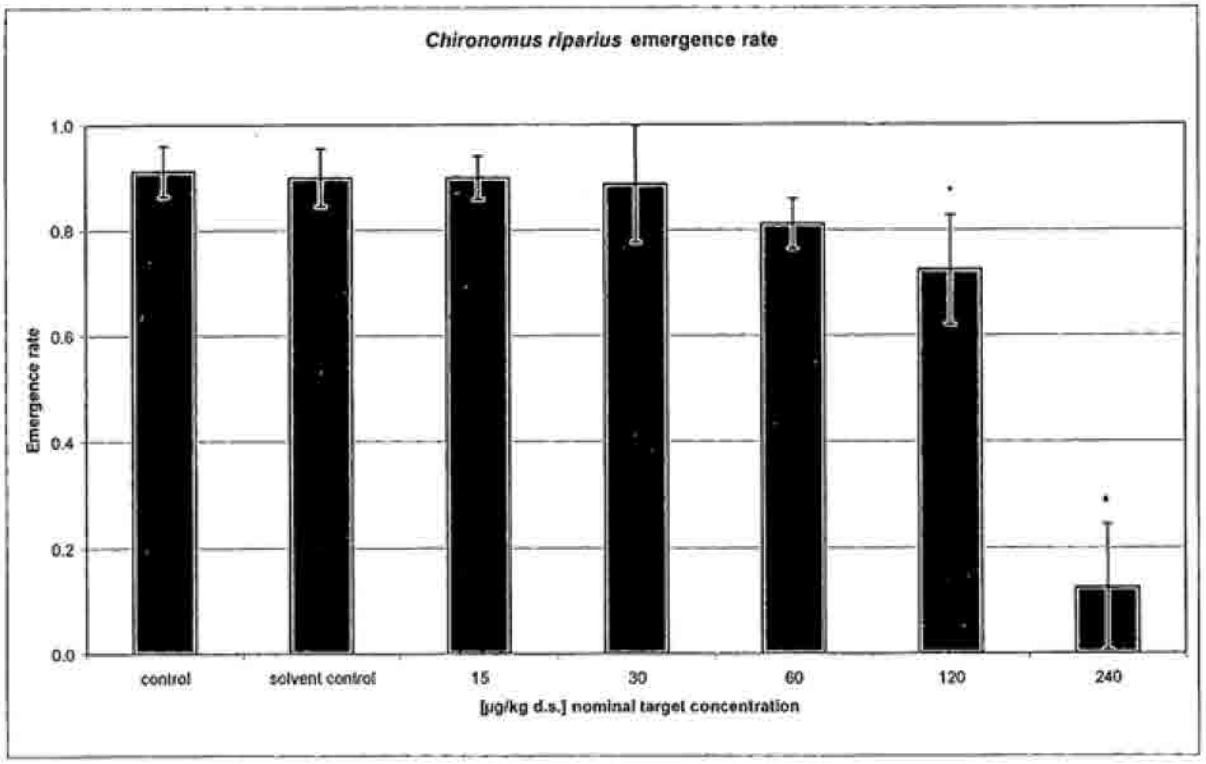

Figure A7.4.3.5.1- 1: Emergence rate of *C. riparius* in the different BAS 310 I treatments and in the controls (d.s. = dry sediment). * = statistically significant

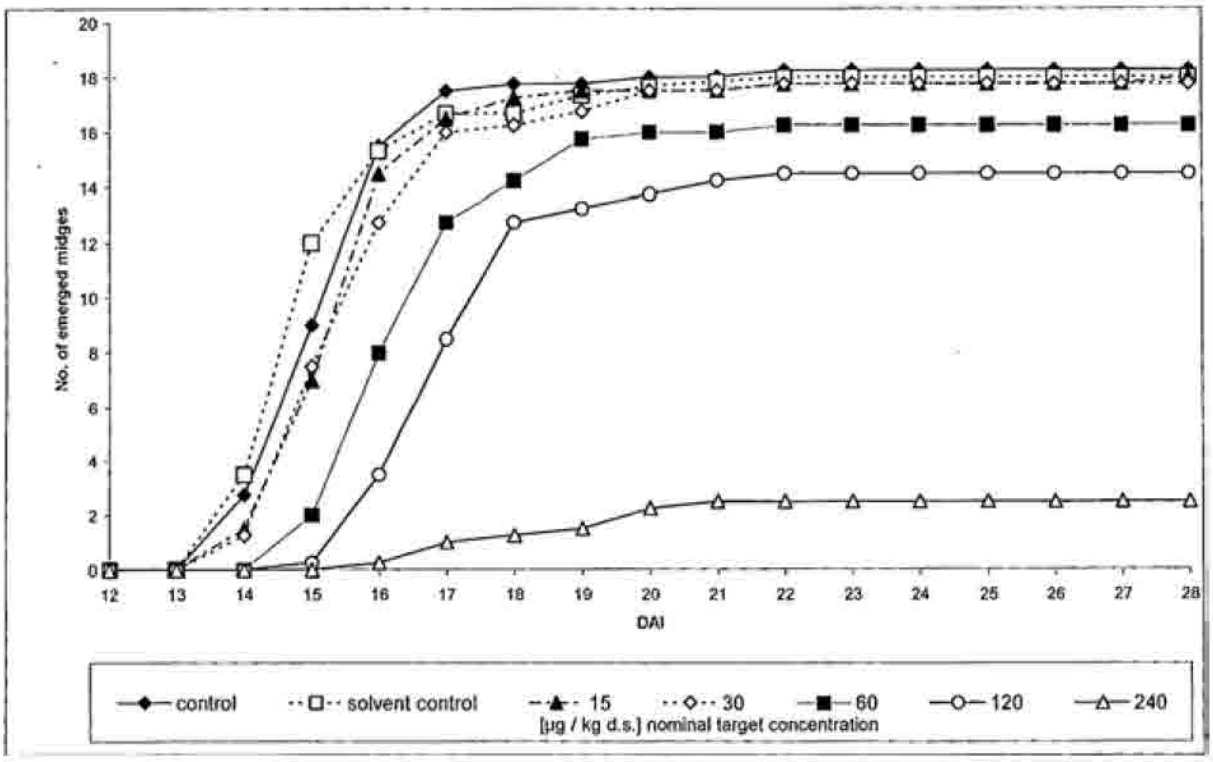

Figure A7.4.3.5.1- 2: Cumulative emergence of *C. riparius* against days after introduction of larvae in the different nominal target concentrations of BAS 310 I and the controls (d.s. = dry sediment).

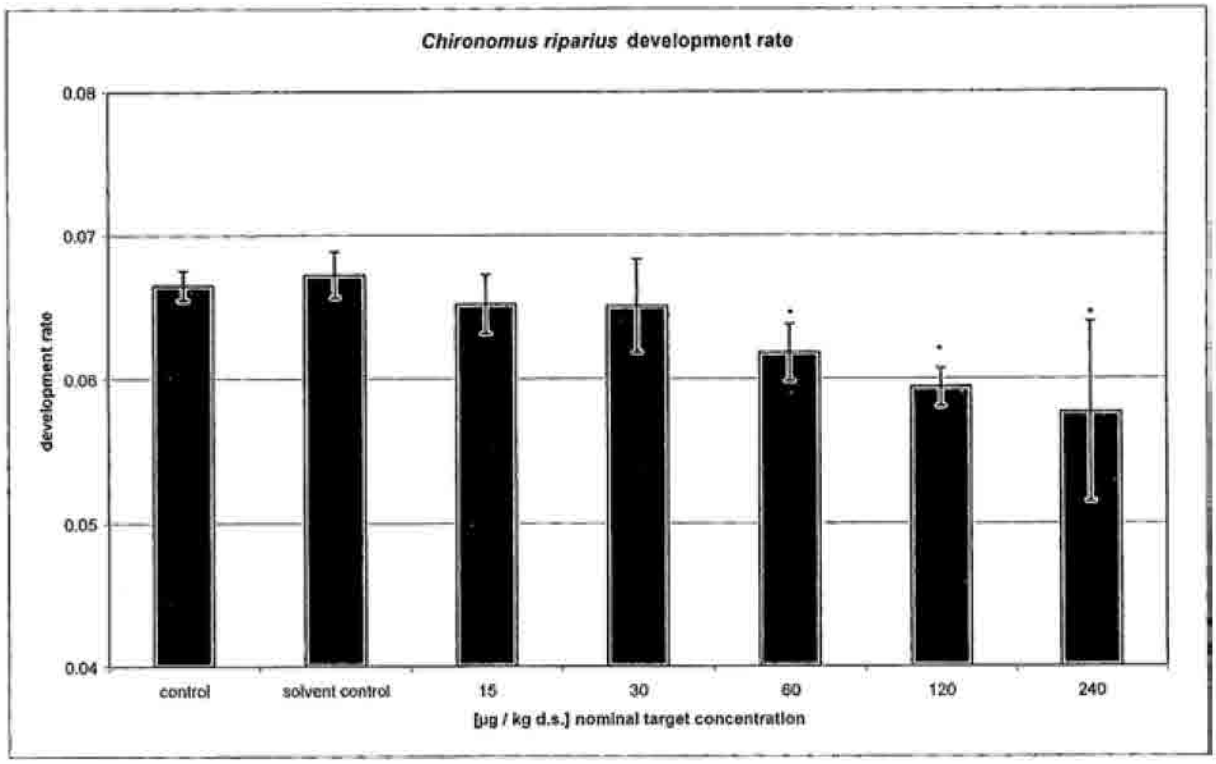

Figure A7.4.3.5.1- 3: Development rate of *C. riparius* in the different nominal target concentrations of BAS 310 I and the controls (d.s. = dry sediment).

Table A7.4.3.5.1- 12: Water content in sediment samples of a spiked sediment *Chironomus* study (retrieved from the raw data, not included in the study report).

DAI [days]	Treatment [µg/kg]	Water content sample 1 [%]	Water content sample 2 [%]	Mean water content [%]
	Water control	24.02	23.57	23.80
	Solvent control	24.36	24.00	24.18
	15	24.11	23.98	24.05
DAI 0	30	24.95	24.35	24.64
	60	24.73	23.67	24.20
	120	24.23	23.86	24.05
	240	24.13	24.11	24.12
	Water control	24.92	24.69	24.81
	Solvent control	23.37	24.46	23.92
	15	25.60	25.33	25.47
DAI 28	30	23.98	26.02	25.09
	60	27.62	28.13	27.88
	120	27.16	26.07	26.62
	240	23.21	23.40	23.31
			Mean	**24.72**
			CV (%)	**5.14**
			n	**28**

DAI = Days after insertion of the larvae

Section A7.4.3.5.1 **Effects on sediment dwelling organisms**
Annex Point IIIA 13.3.4

Official use only

1 REFERENCE

1.1	**Reference**	**A7.4.3.5.1/04:** Gilberg D., Goth M. and Class T.J. (2013): Alpha-cypermethrin (BAS 310 I): A study on the chronic toxicity to the sediment dweller *Lumbriculus variegatus*. ECT Oekotoxikologie GmbH, Flörsheim/Main, Germany (study number: 12CT1LA) and PTRL Labor für Umwelt- und Pestizidchemie GmbH, Ulm, Germany (ID P2712G), July 05, 2013 (unpublished), BASF DocID 2012/1205915 Habekost (2013): Additional information: Chronic toxicity of *Lumbriculus variegatus* exposed to alpha-cypermethrin - Re-calculation of endpoints based on wet weight - (unpublished), BASF DocID 2013/1289189
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF SE
1.2.2	Companies with letter of access	No
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes OECD 225 (2007): Sediment-water *Lumbriculus* toxicity test using spiked sediment
2.2	**GLP**	Yes
2.3	**Deviations**	The variability of the online-measured temperature during the exposure period was ± 1.1 °C instead of ± 1 °C. The Total Organic Carbon (TOC) content was 2.6 % instead of 2 ± 0.5%. An influence on the outcome and the integrity of the study is not expected.

3 MATERIALS AND METHODS

3.1	**Test material**	TGAI BAS 310 I (Alpha-cypermethrin) As given in Section A2.
3.1.1	Lot/Batch number	COD-000595
3.1.2	Specification	As given in Section A2.

Section A7.4.3.5.1 Effects on sediment dwelling organisms

Annex Point IIIA 13.3.4

3.1.3	Purity	99.2 %
3.1.4	Composition of product	Not applicable
3.1.5	Further relevant properties	Water solubility at 20°C (pH 7): 0.004-0.008 mg/L (acc. to study report)
3.1.6	Method of analysis	Alpha-cypermethrin in water was determined by GC/MS after liquid/liquid extraction into dichloromethane (LOQ = 5 ng/L). Alpha-cypermethrin in sediment was determined by GC/MS using a modified DFG (Deutsche Forschungsgemeinschaft, German Research Foundation) method S19 (LOQ = 10 µg/kg dry sediment weight or 17°µg/kg wet sediment weight, respectively). The validation of the methods and the analytical reports are included in the study report.
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Please refer to Table A7.4.3.5.1- 1.
3.3	**Reference substance**	A reference item was not tested.
3.3.1	Method of analysis for reference substance	-
3.4	**Testing procedure**	
3.4.1	Dilution water	Reconstituted water; details are given in Table A7.4.3.5.1-2
3.4.2	Test organisms	*Lumbriculus variegatus*; as specified in Table A7.4.3.5.1-3
3.4.3	Test system	The test system is described in Table A7.4.3.5.1-4
3.4.4	Test conditions	The exposure to alpha-cypermethrin was achieved via treated (spiked) sediment (thoroughly and evenly distributed, please refer to Table A7.4.3.5.1- 1). The sediment was overlaid with reconstituted water (dilution water). The relevant test conditions are presented in Table A7.4.3.5.1-5.
3.4.5	Duration of the test	28 d (day 0 to day 28)
3.4.6	Test parameter	Reproduction (total number of surviving worms) and biomass (dry weight of the surviving organisms) of the test organisms
3.4.7	Examination/ sampling	Aeration was controlled daily (on workdays). Observation in order to assess visually any behavioural differences compared with the control: at least three times a week. Sampling of sediment and overlying water, at start and end of exposure of control, lowest and highest test concentration.
3.4.8	Monitoring of TS concentration	Yes, analytical determination of the test substance at test initiation (day 0) and at test termination (day 28) in the overlaying water and sediment.

Section A7.4.3.5.1 Effects on sediment dwelling organisms

Annex Point IIIA 13.3.4

3.4.9	Statistics	Normal distribution and variance homogeneity of the data were assessed by Shapiro Wilk's test and Levene's test, respectively. Welch t-test and Williams t-test were used for NOEC/LOEC calculation ($p \leq 0.05$). The EC_x-values were calculated using probit analysis. The statistical software package ToxRat Professional 2.10 (ToxRat Solutions GmbH, Naheweg 15, D-52477 Alsdorf, Germany) was used for these calculations. All statistical calculations were done based on the nominal concentrations and then corrected to mean initial mean measured concentrations (total isomers).
		4 RESULTS
4.1	**Range finding test**	In agreement with the applicant the first definitive test (limit test at a concentration of 3.0 mg/kg dry sediment) was cancelled on day 3 of exposure. The detailed results of the first definitive test are not given in the study report.
4.1.1	Concentration	3.0 mg/kg dry sediment
4.1.2	Number/percentage of animals showing adverse effects	Not given
4.1.3	Nature of adverse effects	Not given
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	0 (solvent control), 62.5,125, 250, 500 and 1000 µg/kg dry sediment (nominal concentrations: 0, 2.1, 4.1, 8.3,16.5, 33.0 mg/L)

Section A7.4.3.5.1 Effects on sediment dwelling organisms

Annex Point IIIA 13.3.4

4.2.2	Actual concentrations of test substance	Measured concentration (day 0): in sediment: 74 (lowest) and 1099 (highest) µg total isomers/kg dw recovery sediment: 118 and 110 % of nominal Overlying water: 5.6 and 89 ng total isomers/L Measured concentration (day 28): in sediment 63 (lowest) and 1022 (highest) µg total isomers/kg dw recovery sediment: 101 and 102 % of nominal Overlying water: 31 and 114 total isomers ng/L The chemical analysis of the sediment and overlaying water was performed for the lowest and highest nominal test item concentration (62.5 and 1000 µg/kg dw) at start and termination of the study period (day 0 and day 28). Control and test vessels were sampled in duplicate. One was analysed and the second kept as a reserve. Please refer to Table A7.4.3.5.1- 6 for the analytical results.
4.2.3	Effect data	In the concentration levels of 250 µg/kg sediment dry weight and 100 µg/kg sediment dry weight, one worm was found to be dead after 28 days of exposure. Since no meaningful number of dead worms and no concentration response relation was observed, no statistical evaluation was performed. Total numbers of worms in the test are presented in Table A7.4.3.5.1- 7. The effect data are based on mean measured concentrations of alpha-cypermethrin. The reproduction, total and individual biomass are presented in Table A7.4.3.5.1- 8. Treatments were tested against the solvent control as this was considered to provide the best representation of control conditions, since all treatments received the same amount of acetone. Significant differences in the reproduction could be detected at the concentrations levels 125, 250 and 1000 µg/kg dry sediment, while for 500 µg/kg dry sediment the test could not be performed. For total biomass at the four highest concentrations significant differences from solvent control were detected (125, 250, 500 and 1000 µg/kg dry sediment) and for individual biomass at the three highest concentrations (250, 500 and 1000 µg/kg dry sediment). The NOEC for reproduction and total biomass was 71.3 µg/kg dry sediment and for individual biomass 143.0 µg/kg dry sediment as determined by Shapiro-Wilk's test on normal distribution; significance level $\alpha = 0.05$ and homogeneity of variances (Levene's test; $\alpha = 0.05$). For reproduction the calculated EC_{50} was 278 µg/kg dry weight, for total biomass the calculated EC_{10}, EC_{20} and EC_{50} were 52.0, 75.7 and 155 µg/kg dry sediment, respectively, and for individual biomass an EC_{10}, EC_{20} and EC_{50} of 131, 189 and 383 µg/kg dry sediment were determined.

Section A7.4.3.5.1 Effects on sediment dwelling organisms

Annex Point IIIA 13.3.4

4.2.4	Concentration / response curve	Please refer to Figure A7.4.3.5.1- 1, Figure A7.4.3.5.1- 2 and Figure A7.4.3.5.1- 3 for reproduction, biomass (total biomass) and biomass (individual biomass).	
4.2.5	Other effects	-	
4.3	**Results of controls**	No test item was found above the limit of detection (LOD; based on wet sediment was 1 µg/kg and in the overlying water was 1 ng/L) in the control sample (as given in Table A7.4.3.5.1- 6)	**X**
4.4	**Test with reference substance**	-	
4.4.1	Concentrations	-	
4.4.2	Results	-	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The toxicity of the test substance alpha-cypermethrin to sediment-dwelling organisms was tested using adult worms of the endobenthic oligochaete *Lumbriculus variegatus*, according to OECD 225. According to this guideline, the "synchronised" *Lumbriculus* adults were exposed to the test substance over 28 days via spiked sediment. Effects on reproduction and biomass as well as the survival of the worms were evaluated.	

Section A7.4.3.5.1 Effects on sediment dwelling organisms

Annex Point IIIA 13.3.4

5.2	**Results and discussion**	Analytical measurements of BAS 310 I by GC/MS yielded the following measured values: The overlaying water concentrations measured by GC/MS ranged from 5.6 to 114 ng total isomers/L. For the sediment a concentration range of 63 to 1099 µg total isomers/kg dry sediment were measured. Based on the nominal concentrations, all statistical calculations were done and corrected to initial mean measured concentrations. **Conversion from test item concentration in sediment dry weight to sediment wet weight (BASF DocID 2013/1289189):** The conversion from dry weight to wet weight was based on the actual data from the *Lumbriculus* study. As documented in the raw data and shown in Table A7.4.3.5.1- 10, the water content (calculated from the weight difference of wet sediment and sediment after drying) in each sediment sample of day -11, day 0 and day 28 ranged from 35.4 to 41.3 % with an average water content in wet sediment of 38.16 % (n = 22; coefficient of variation = 4.94 %). According to the TGD (part II, Chapter 3, table 5, p. 43) the density of sediment solid particles is 2.5 kg/L (=2,500 kg/m^3) and the density of the water phase 1 kg/L (=1,000 kg/m^3). The sediment used in the study consists of 38.16 % water and 61.84 % solid particles, which results in a 'wet' density of (0.3816 x 1.0 kg/L) + (0.6184 x 2.5 kg/L) = 1.9276 kg/L. The dry weight is consequently (0.6184 x 2.5) = 1.546 kg (per litre wet sediment) and the ratio wet: dry is 1.25: <u>Calculation of conversion factor:</u> Density wet sediment: (0.3816 x 1.0 kg/L) + (0.6184 x 2.5 kg/L) = 1.9276 kg/L Weight of dry sediment: (0.6184 x 2.5) = 1.546 kg per L wet sediment Conversion factor wwt to dwt: 1.9276 / 1.546 = 1.2468. **rounded 1.25** The conversion factor of 1.25 is used to calculate the concentrations of the active substance from the dry sediment weight to the wet sediment weight.
5.2.1	NOEC	71.3 µg/kg dry sediment (Reproduction) 57.0 µg/kg wet sediment 71.3 µg/kg dry sediment (Biomass (dry weight)) 57.0 µg/kg wet sediment 143 µg/kg dry sediment (Individual biomass (dry weight)) 114 µg/kg wet sediment
5.2.2	LOEC	143 µg/kg dry sediment (Reproduction) 114 µg/kg wet sediment 143 µg/kg dry sediment (Biomass (dry weight)) 114 µg/kg wet sediment 285 µg/kg dry sediment (Individual biomass (dry weight)) 228 µg/kg wet sediment

Section A7.4.3.5.1 Effects on sediment dwelling organisms

Annex Point IIIA 13.3.4

5.2.3	EC_{10}	n.d. µg/kg dry sediment (Reproduction) n.d. µg/kg wet sediment 52.0 µg/kg dry sediment (Biomass (dry weight)) 41.6 µg/kg wet sediment 131 µg/kg dry sediment (Individual biomass (dry weight)) 105 µg/kg wet sediment
5.2.4	EC_{20}	n.d. µg/kg dry sediment (Reproduction) n.d. µg/kg wet sediment 75.7 µg/kg dry sediment (Biomass (dry weight)) 60.6 µg/kg wet sediment 189 µg/kg dry sediment (Individual biomass (dry weight)) 151 µg/kg wet sediment
5.2.5	EC_{50}	278 µg/kg dry sediment (Reproduction) 222 µg/kg wet sediment 155 µg/kg dry sediment (Biomass (dry weight)) 124 µg/kg wet sediment 383 µg/kg dry sediment (Individual biomass (dry weight)) 306 µg/kg wet sediment
5.3	**Conclusion**	All validity criteria are fulfilled (please refer to Table A7.4.3.5.1- 9). The study on the *Lumbriculus variegatus* is considered to be valid without restriction.
5.3.1	Reliability	1
5.3.2	Deficiencies	The variability of the online-measured temperature during the exposure period was ± 1.1 °C instead of ± 1 °C. The Total Organic Carbon (TOC) content was 2.6 % instead of 2 ± 0.5%. An influence on the outcome and the integrity of the study is not expected.

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	

	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	November 2013 The Applicant's version is acceptable with the following comments/information:
Materials and Methods	
Results and discussion	Section 4.3 Table A7.4.3.5.1-10 presents the water content in spiked sediment samples and does not present the analytical results of control solutions as announced by the applicant.
Conclusion	
Reliability	1
Acceptability	Acceptable
Remarks	None

	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.4.3.5.1- 1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	No
Vehicle	The exposure in the test was achieved via treated (spiked) sediment. For the spiking procedure, the test substance was dissolved in acetone and this stock solution was used to prepare a series of application solutions by dissolving the stock solution with acetone. An appropriate volume of these application solutions was mixed with an appropriate amount of quartz sand for each treatment. After total evaporation of the acetone, the coated quartz sand was mixed with the conditioned pre-sediment of each concentration level. The resulting test sediment (artificial substrate, prepared according to OECD test guideline No. 225 and consisting of the following <u>total</u> fractions (dry weight basis, including the spiked quartz sand fraction): **ca. 5 % Peat** (Sphagnum moss peat, air dried, no visible plant remains, finely ground (particle size ≤ 0.5 mm)) **ca. 75 % Quartz sand** (Grain size: < 2 mm; > 50 % of the particles should be in the range of 50 – 200 µm) **ca. 20 % Kaolinite clay** (Kaolinite content ≥ 30 %) **ca. 0.25 Urtica powder** (*Folia urticae*; e. G. Caelo Caesar & Loretz GmbH, Hilden, in addition to dry sediment, finely ground (particle size ≤ 0.5 mm)) **ca. 0.25 Cellulose powder** (e. g., α-Cellulose, in addition to dry sediment) **ca. 2 ± 0.5 % Organic carbon** (Adjusted by addition of peat and sand) **ca. 0.05 – 1 % Calcium carbonate** ($CaCO_3$, pulverised, chemically pure, in addition to dry sediment) **ca. 46 % Deionised water** (Conductivity ≤ 10 µS/cm, in addition to dry sediment) Moisture of the blend: range 30 to 50 % of dry weight of the sediment. The pH of the final mixture was determined to be 6.3 (in expectation of ammonia development).
Concentration of vehicle	<u>Example:</u> For the nominal concentration of 62.5 µg alpha-cypermethrin/kg dry sediment 1.56 ml of the stock solution (33 mg/L) were diluted with 25°mL acetone to reach a nominal concentration in application solution of 2.1 mg/L. From this application solution 17 ml were applied (36 µg alpha-cypermethrin) to 562 g sediment dry weight Likewise the other test concentrations used in this test were prepared (0, 62.5, 125, 250, 500 and 1000 µg/kg dry weight) using respective volumes of the stock solution. For the solvent control, the uncontaminated artificial sediment and reconstituted water (sediment water ration approx. 1:3.5) was mixed.
Vehicle control performed	Yes, solvent control only, since the solvent was evaporated before quartz sand was mixed with the pre-sediment.
Other procedures	To ensure that the test item added to the sediment was evenly distributed within the sediment, the bulk artificial sediment were thoroughly mixed. From these bulk concentration levels, the sediment was distributed to the individual replicates of each concentration level. Prior to application of the test item, the artificial sediment was conditioned for seven days by covering with reconstituted water and under the same conditions which prevailed in the subsequent test.

Table A7.4.3.5.1-2: Dilution water.

Criteria	Details
Source	Reconstituted water with the composition and physical-chemical characteristics are according to OECD guideline No. 203 (OECD 1992).
Hardness ($CaCO_3$)	225.0 mg/L
Oxygen content	8.24 mg/L (≥ 30 % air saturation value)
pH	7.5
Conductivity	614 µS/cm
Ca^{2+} / Mg^{2+} ratio	4/1
Na^{+} / K^{+} ratio	10/1
Holding water different from dilution water	No

Table A7.4.3.5.1-3: Test organisms.

Criteria	Details
Strain / Clone	*Lumbriculus variegates*, freshwater oligochaete
Source	The species of an in-house culture has been cultured at ECT Oekotoxikologie GmbH since January 1998, were originally obtained from Fischfutter Etzbach (D-53894 Mechernich-Bergheim, Germany) and the identity of the cultured organisms was confirmed according to Brinkhurst (1971).
Age	Adult worms
Breeding method	Eleven days before the start of the test, the worms were artificially fragmented ("synchronisation") to avoid "uncontrolled" regeneration and subsequent high variation in test results. Worms were placed onto a glass slide in a drop of culture water and bisected in the mean body region. To regenerate new heads the posterior ends were left in a culture vessel containing a 2 ± 1 cm layer of quartz sand and test medium. They were held at 20 ± 2 °C until start of exposure.
Kind of food	The worms are fed with fish food suspension, TetraMin®.
Amount of food	A suspension of 50 mg/ml finely ground TetraMin®was fed to the regenerated worms once on day seven after dissection.
Feeding frequency	no additional feeding
Pre-treatment	After regenerating, intact complete worms of similar size, which were actively swimming or crawling upon a gentle mechanical stimulus, were used for the test.
Feeding of animals during test	Feed in sediment (*Urtica* powder and cellulose)

Table A7.4.3.5.1-4: Test system.

Criteria	Details
Test type	Static
Renewal of test solution	Not applicable
Volume of test vessels	250 ml glass vessels measuring 6 cm in diameter and 11.5 cm in height. Filled with approximately 80 g spiked wet artificial sediment to a depth of around 1.7 cm sediment layer and about 175 ml overlying water.
Volume/animal	Circa 0.018 L overlaying water, approx. 8 g sediment
Number of animals/vessel	10 at start of exposure
Number of vessels/concentration	Four replicates (test item concentration levels), six replicates (solvent control, acetone) No negative control (sediment without addition of solvent), since the solvent is removed by evaporation. Additional vessels were used for chemical analysis at start and at end of exposure.
Test performed in closed vessels due to significant volatility of TS	No, but the vessels were covered to reduce evaporation.

Table A7.4.3.5.1-5: Test conditions.

Criteria	Details
Test temperature	Manual measurement in the test vessels: 20.3 – 20.7 °C (n = 30; within the required range of 20 ± 2 °C) Online measurement in a separate vessel: 18.2 – 20.4 °C (n = 679)
Dissolved oxygen	8.1 – 8.4 mg/L (n = 30) 90-94 % of air saturation value, ASV
Ammonium content	0.004 - 3.29 (n = 78; values < 1.3 mg/L NH_4^+ were below the limit of quantification (LOQ).
Total hardness of the overlaying water during the test	273.3 – 292.9 mg/L $CaCO_3$ (n = 4)
pH	7.6 – 8.1 (n = 30; within the required range of 6 – 9)
Adjustment of pH	No
Aeration of dilution water	Yes, gentle aeration (up to 4 bubbles per second) during equilibration and exposure. A glass pipette (150 mm) is fixed through a hole of the lid and positioned approximately 2.5 cm above the sediment to minimize perturbation of the sediment (according to OECD test guideline 225).
Quality/Intensity of irradiation	168 – 414 Lux (n = 5; within the required range of 100 – 500 lux)
Photoperiod	16 h light :8 h dark

Table A7.4.3.5.1- 6: Summary of the analytical results.

Nominal test item concentration [µg/kg d.s.]	Test period (days)	Measured concentration in sediment and overlying water and recovery in sediment			
		Sediment [µg total isomers/kg dw]	Sediment [µg total isomers/kg ww]*	Recovery sediment (total isomers) [% of nominal]	Overlying water [ng total isomers/L]
0.0	0	< LOD	< LOD	-	< LOD
0.0	28	< LOD	< LOD	-	< LOD
62.5	0	74	59.2	118	5.6
62.5	28	63	50.4	101	31
1000	0	1099	879.2	110	89
1000	28	1022	817.6	102	114

* calculated with a conversion factor ww/dw of 1.25

Table A7.4.3.5.1- 7: Numbers of worms per replicate sampled after 28 days of exposure.

Replicate	Concentration [µg/kg dw]	Number of worms introduced (day 0)	Number of worms (day 28)
C0LA	0	10	25
C0LB	0	10	22
C0LC	0	10	27
C0LD	0	10	27
C0LE	0	10	22
C0LF	0	10	23
C1A	62.5	10	23
C1B	62.5	10	17
C1C	62.5	10	20
C1D	62.5	10	25
C2A	125	10	10
C2B	125	10	10
C2C	125	10	10
C2D	125	10	12
C3A	250	10	10
C3B	250	10	9
C3C	250	10	10
C3D	250	10	10
C4A	500	10	10
C4B	500	10	10
C4C	500	10	10
C4D	500	10	10
C5A	1000	10	9
C5B	1000	10	10
C5C	1000	10	10
C5D	1000	10	10

Table A7.4.3.5.1- 8: Reproduction, total biomass and individual biomass of worms per replicate (mean value and standard deviation (SD) per treatment).

Concentration [µg/kg dw]	Reproduction after 28 days of exposure		Total biomass in dry weight		Individual biomass in dry weight per worm		Number of replicates
	Mean [n]	SD [n]	Mean [mg]	SD [mg]	Mean [mg]	SD [mg]	
Solvent control	24.3	2.34	30.05	3.87	1.247	0.2156	6
62.5	21.3	3.50	26.63	2.73	1.285	0.2868	4
125	10.5	1.00	14.30	0.98	1.375	0.1915	4
250	9.8	0.50	7.25	0.73	0.748	0.1180	4
500	10.0	0.00	3.35	0.19	0.335	0.0191	4
1000	9.8	0.50	2.28	0.39	0.234	0.0413	4

Table A7.4.3.5.1- 9: Validity criteria and obtained data for the sediment-water *Lumbriculus* toxicity test using spiked sediment according to OECD Guideline 225.

	Fulfilled
The average number of living worms per replicate in the controls should have increased by a factor of at least 1.8 at the end of exposure	☑
Oxygen concentration was ≥ 30% of the air saturation value	☑
pH of the water was in the range of 6 – 9	☑
Water temperature did not differ more than ± 2.0 °C	☑

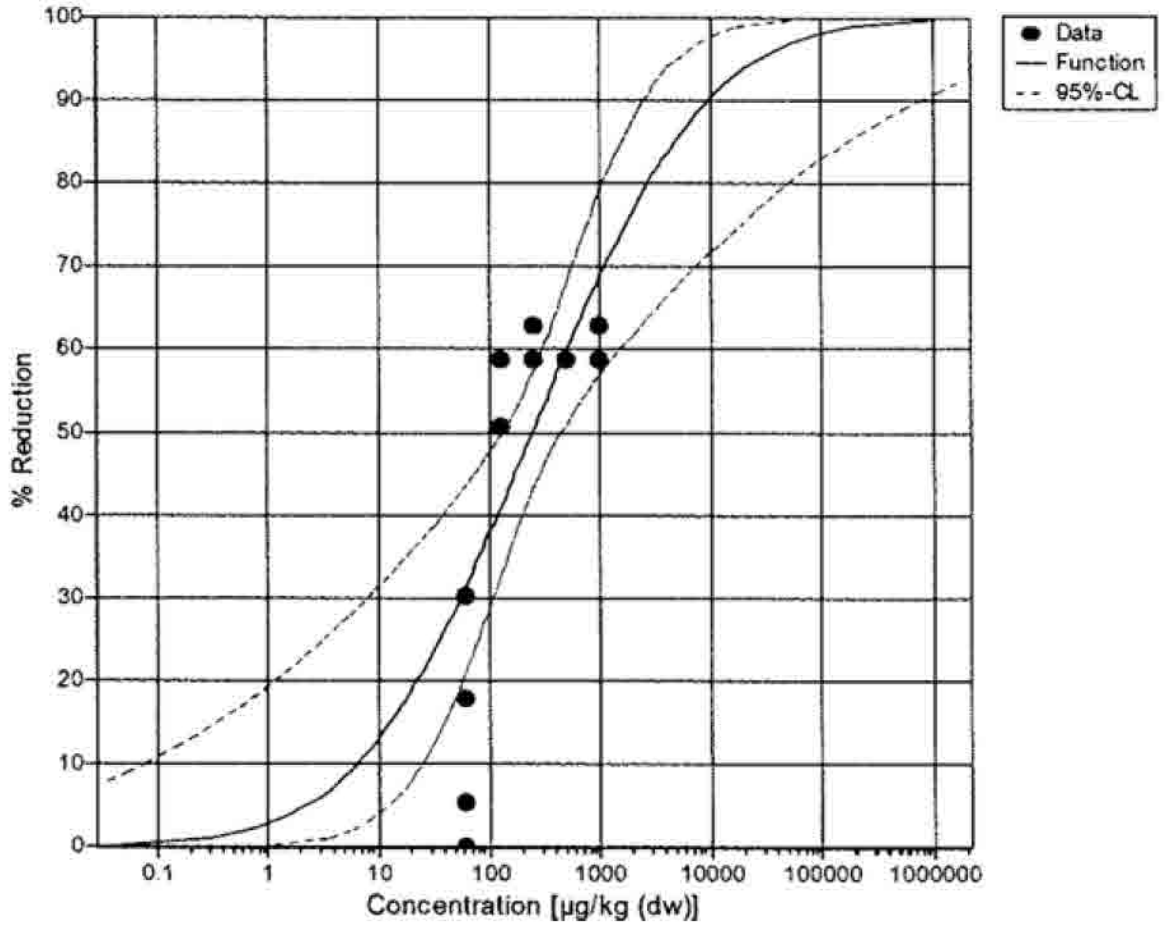

Figure A7.4.3.5.1- 1: Concentration-effect curve showing the influence of the test item on worm number of the introduced *Lumbriculus variegatus* as observed after 28 d (reproduction).

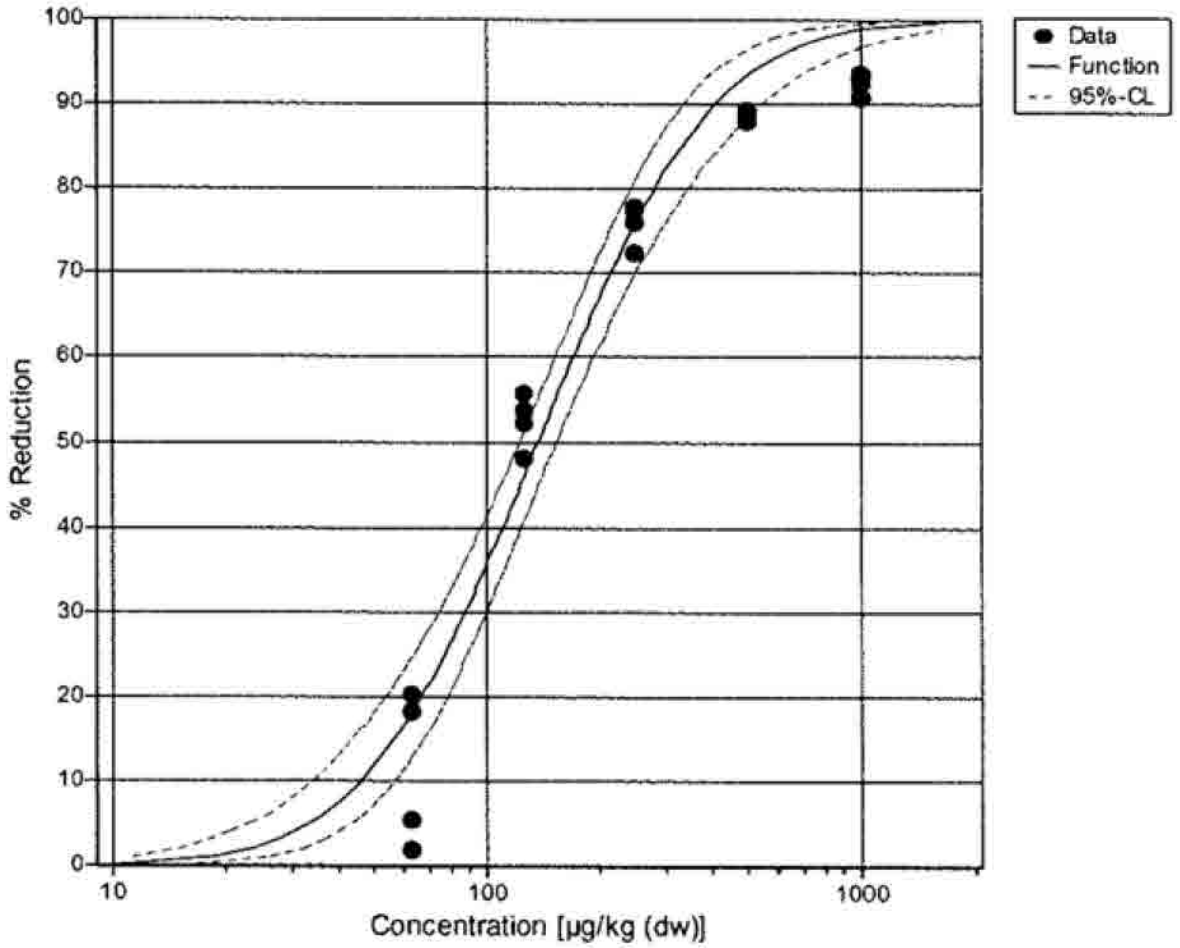

Figure A7.4.3.5.1- 2: Concentration-effect curve showing the influence of the test item on total weight of the introduced *Lumbriculus variegatus* as observed after 28 d (biomass).

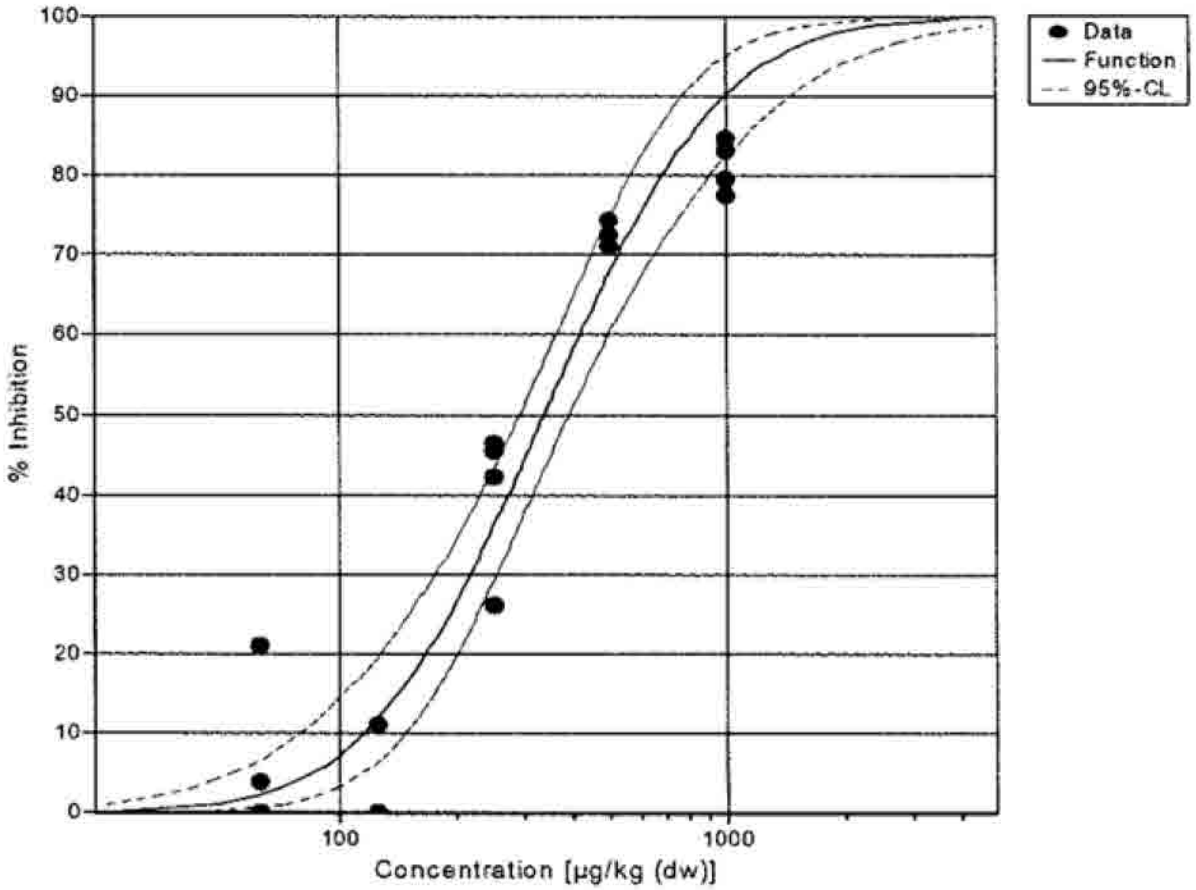

Figure A7.4.3.5.1- 3: Concentration-effect curve showing the influence of the test item on individual weight of the introduced *Lumbriculus variegatus* as observed after 28 d (biomass).

Table A7.4.3.5.1- 10: Water content in sediment samples of a spiked sediment *Lumbriculus* study (values retrieved from the raw data, not included in the study report).

		Weight (wet sample) [g]	Weight (dry sample) [g]	solid particle content [%]	water phase content [%]
day -11	pre-sediment	8.2978	5.3494	64.47	35.53
	pre-sediment	9.5558	6.1762	64.63	35.37
	pre-sediment	9.8442	6.3442	64.45	35.55
	pre-sediment	9.3365	6.0155	64.43	35.57
day 0	C0Li	9.0081	5.5372	61.47	38.53
	CoLj	9.2175	5.6880	61.71	38.29
	C1g	9.4119	5.9367	63.08	36.92
	C1h	8.2470	5.1606	62.58	37.42
	C2f	9.4769	5.9494	62.78	37.22
	C3f	10.6030	6.7077	63.26	36.74
	C4f	10.6938	6.6977	62.63	37.37
	C5g	10.7835	6.7574	62.66	37.34
	C5h	10.9113	6.7906	62.23	37.77
day 28	C0Lg	9.0907	5.3388	58.73	41.27
	C0Lh	9.4505	5.5955	59.21	40.79
	C1e	8.9019	5.4308	61.01	38.99
	C1f	9.8602	5.8350	59.18	40.82
	C2e	8.8988	5.4310	61.03	38.97
	C3e	10.9236	6.5082	59.58	40.42
	C4e	9.9780	5.8681	58.81	41.19
	C5e	9.7465	5.9999	61.56	38.44
	C5f	9.9021	6.0513	61.11	38.89
				overall mean	38.16
				SD	1.887
				% CV	4.94
				n	22

Section A7.4.3.5.1 **Effects on sediment dwelling organisms**
Annex Point IIIA 13.3.4

Official use only

1 REFERENCE

1.1	**Reference**	**A7.4.3.5.1/05:** Höss S. (2013): Chronic toxicity of α-cypermethrin to *Caenorhabditis elegans* exposed via spiked sediment according to ISO guideline 10872 (2010). Ecossa, Starnberg, Germany, BASF Study ID 711682, August 03, 2013 (unpublished), BASF DocID 2013/1250848
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF SE
1.2.2	Companies with letter of access	No
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes ISO guideline 10872 (2010) Water quality – Determination of the toxic effect of sediment and soil samples on growth, fertility and reproduction of *Caenorhabditis elegans* (Nematoda)
2.2	**GLP**	Biological phase non-GLP, however, considering GLP principles. Analytical phase GLP
2.3	**Deviations**	-

3 MATERIALS AND METHODS

3.1	**Test material**	BAS 310 I (Alpha-cypermethrin) As given in Section A2.
3.1.1	Lot/Batch number	COD-000595
3.1.2	Specification	As given in Section A2.
3.1.3	Purity	99.2 %
3.1.4	Composition of product	Not applicable
3.1.5	Further relevant properties	-

Section A7.4.3.5.1 Effects on sediment dwelling organisms

Annex Point IIIA 13.3.4

3.1.6	Method of analysis	Alpha-cypermethrin in sediment was determined by GC/MS using a modified DFG (Deutsche Forschungsgemeinschaft, German Research Foundation) method S19 (LOQ = 0.10 mg/kg dry sediment weight). The validation of the methods and the analytical reports are included in the study report.	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Please refer to Table A7.4.3.5.1-1.	
3.3	**Reference substance**	Benzylcetyldimethylammonium chloride monohydrate (BAC-C16), used in biological phase of the study.	
3.3.1	Method of analysis for reference substance	-	
3.4	**Testing procedure**		
3.4.1	Dilution water	M9-medium according to ISO 10872, composed on the basis of distilled/deionized water. The composition of the M9 medium is given as follows: 6 g/L Na_2HPO_4; 3 g/L KH_2PO_4; 5 g/l NaCl and 0.25 g/L $MgSO_4 \cdot 7\ H_2O$.	
3.4.2	Test organisms	*Caenorhabditis elegans*, juvenile; as specified in Table A7.4.3.5.1-2	
3.4.3	Test system	The test system is described in Table A7.4.3.5.1-3	X
3.4.4	Test conditions	The exposure to alpha-cypermethrin was achieved via treated (spiked) sediment (thoroughly distributed, please refer to Table A7.4.3.5.1-1). The sediment was overlaid with M9-medium. The relevant test conditions are presented in Table A7.4.3.5.1- 4.	
3.4.5	Duration of the test	96 h (0 h to 96 h)	
3.4.6	Test parameter	Toxicity endpoints: growth and reproduction (offspring (second generation)/test organisms) Validity criterion; measured for controls: recovery (mean recovery of exposed test organisms), males (mean percentage of males) and fertility (mean fertility)	
3.4.7	Examination/ sampling	Count the separated exposed test organisms Count the males in the replicates and exclude them from further measurement Gravid exposed test organisms Calculate the replicate growth Count the offspring of each replicate	
3.4.8	Monitoring of TS concentration	Yes, nominal sediment concentrations of the test item are verified by chemical analysis for the start (0 h) and the end of the test (96 h), while sediment concentrations are provided on dry and wet weight basis.	

Section A7.4.3.5.1 Effects on sediment dwelling organisms

Annex Point IIIA 13.3.4

3.4.9	Statistics	Statistical tests were performed to test for significant differences between the solvent control (SC) and the treatments with the test item. After testing for normal distribution (Shapiro Wilks test) and homogeneity of variance (Levene test) an one-way ANOVA was carried out to test for effects (differences to solvent control). The lowest-observed-effect concentration (LOEC) was then defined as the lowest tested concentration that showed a significant effect. The no-observed-effect-concentration (NOEC) was defined as the concentration step below the LOEC.
		4 RESULTS
4.1	**Range finding test**	Final test concentrations were chosen based on a pre-liminary range-finding test. Details are not given in the study report.
4.1.1	Concentration	
4.1.2	Number/percentage of animals showing adverse effects	
4.1.3	Nature of adverse effects	
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	0 (control), 0 (solvent control), 0.3, 1, 3, 10 and 30 mg/kg dry weight
4.2.2	Actual concentrations of test substance	<u>Measured concentration (0 hours):</u> in sediment: 0.233 (lowest), 0.733, 4.39, 7.51 and 28.2 (highest) mg total isomers/kg dw recovery sediment: 73 - 100 %, 1x 146 % (3 mg/kg dry weight) <u>Measured concentration (96 hours):</u> in sediment 0.235 (lowest), 1.04, 3.30, 7.38 and 29.1 (highest) mg total isomers/kg dw recovery sediment: 74 -110 % The chemical analysis of the sediment was performed for all nominal test item concentrations (0.3, 1, 3 10 and 30 mg/kg dw) as well as for the control and solvent control at start and termination of the study period (0 hours and 96 hours). Controls and test vessels were sampled threefold. One was analysed and the rest kept as a reserve. Please refer to Table A7.4.3.5.1- 5 for the analytical results.

Section A7.4.3.5.1 Effects on sediment dwelling organisms

Annex Point IIIA 13.3.4

4.2.3	Effect data	The effect data are based on mean measured concentrations of alpha-cypermethrin. The growth and reproduction are presented in Table A7.4.3.5.1- 6. Treatments were tested against the solvent control as this was considered to provide the best representation of control conditions, since all treatments received the same amount of acetone. No significant inhibitory effect on growth and reproduction of *C. elegans* at any tested concentration (0.3 to 30 mg/kg dry weight; $p > 0.05$, one-way ANOVA, post-hoc test: Dunnett's test) with inhibition values ranging from -6.5 to -1.6 % for growth and -24.4 to 30.5 % for reproduction. In the lowest concentration (Cyp1; 0.3 mg/kg dw) the 30.5 % inhibition did not show to be a significant effect ($p = 0.229$, Dunnet t, on sided < SC; one-way ANOVA: $F = 1.692$) and was not considered to be a treatment related effect. The NOEC for both sublethal toxicity endpoints was determined to be ≥ 28.6 mg/kg dw (≥17.2 mg/kg ww) based on mean measured concentrations.
4.2.4	Concentration / response curve	Please refer to Figure A7.4.3.5.1- 1 and Figure A7.4.3.5.1- 2 for growth and reproduction.
4.2.5	Other effects	-
4.3	**Results of controls**	No test item was found above the limit of detection (LOQ; based on dry sediment was 0.10 mg/kg) in the control sample (as given in Table A7.4.3.5.1- 5 and Table A7.4.3.5.1- 6)
4.4	**Test with reference substance**	Benzylcetyldimethylammonium chloride monohydrate
4.4.1	Concentrations	15 mg/L in aqueous medium
4.4.2	Results	Inhibited the growth of *C. elegans* by 52 %

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The toxicity of the test substance alpha-cypermethrin to sediment-dwelling organisms was tested using juvenile worms of the endobenthic nematodes *Caenorhabditis elegans*, according to ISO guideline 10872 (2010). According to this guideline, the *Caenorhabditis* were exposed to the test substance over 96 hours via spiked sediment. Effects on the growth and reproduction were evaluated.
5.2	**Results and discussion**	Analytical measurements of BAS 310 I by GC/MS yielded the following measured values: For the sediment a concentration range of 0.233 to 29.1 mg/kg dry sediment were measured. Based on the nominal concentrations, all statistical calculations were done and corrected to initial mean measured concentrations.

Section A7.4.3.5.1 Effects on sediment dwelling organisms

Annex Point IIIA 13.3.4

5.2.1	NOEC	Initial measured concentrations: ≥ 28.2 mg/kg dry weight (growth and reproduction) ≥ 16.9 mg/kg wet weight Mean measured concentrations: ≥ 28.6 mg/kg dry weight (growth and reproduction) ≥ 17.2 mg/kg wet weight
5.2.2	LOEC	Could not be determined.
5.3	**Conclusion**	All validity criteria are fulfilled (please refer to Table A7.4.3.5.1- 7 and Table A7.4.3.5.1- 8). The study on effects of *Caenorhabditis elegans* is considered to be valid without restriction.
5.3.1	Reliability	1
5.3.2	Deficiencies	-

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	October the 14th, 2013
Materials and Methods	The Applicant's version is considered to be acceptable with the following amemdment: 3.4.3 Test system Minor editing mistake in Table A7.4.3.5.1-03: Test system. Read: ' Negative control (sediment without addition of solvent), solvent control (addition of acetone without test item) and positive control (addition of 0.5 ml (**30 mg/L**) Benzylcetyldimethylammonium chloride monohydrate (BAC-C16)) were used.' Instead of: 'Negative control (sediment without addition of solvent), solvent control (addition of acetone without test item) and positive control (addition of 0.5 ml (**30°mg/L**) Benzylcetyldimethylammonium chloride monohydrate (BAC-C16)) were used.'
Results and discussion	The Applicant's version is considered to be acceptable.
Conclusion	The Applicant's version is considered to be acceptable.
Reliability	1
Acceptability	Applicant's version is considered to be acceptable
Remarks	The biological phase of the test is not covered by a GLP certificate. However, it does not present any major/minor deficiencies when compared to the reference ISO 10872 Guideline.
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.4.3.5.1-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	No
Vehicle	The exposure in the test was achieved via treated (spiked) sediment. For the spiking procedure, the test substance was dissolved in acetone and this stock solution was used to prepare a series of application solutions by dissolving the stock solution with acetone. The resulting test sediment (artificial substrate, prepared according to ISO guideline 10872: - **40% (w/w) Quartz sand** (0.1-0.4 mm: BayWa; Munich, Germany) - **30% (w/w) Quartz powder** (Millisil W4; Quarzwerke Frechen, Germany) - **20% (w/w) Al_2O_3** (purum, p.a.; Fluka; Batch: 437451/1) - **4.5% (w/w) Fe_2O_3** (puriss. P.a. ≥99%; Fluka; Batch 428807/1) - **0.5% (w/w) Dolomit** (Dolomitwerk Jettenberg; Germany) - **1% (w/w) $CaCo_3$** (Ph Eur; USP; BP, precipitated; Carl-Roth; batch: 14245877) - **4% (w/w) Peat** (untreated, highly decomposed black peat, R.H.P. quality; Klasmann-Deilmann, Geeste, Germany) The dry components are mixed: total amount of 50 g (dry wt) Moisture of the blend: 40% of dry weight of the sediment according to ISO.
Concentration of vehicle	Example: 3 ml of acetone with and without test item were added to 6 g of dry sediment The used concentrations in this test were: 0.3; 1.0; 3.0; 10; 30 mg/kg dry weight) using respective volumes of the stock solution. For the solvent control, the uncontaminated artificial sediment was mixed with acetone without test item.
Vehicle control performed	Yes, control and solvent control were used.
Other procedures	Closed with caps and incubated for 24 h at 20°C

Table A7.4.3.5.1-2: Test organisms.

Criteria	Details
Strain / Clone	Strain: N2 Species: *Caenorhabditis elegans* Genotype: *C. elegans* wild type DR subclone of CB original (Tc1 pattern I)
Source	Caenorhabditis Genetic Center; University of Minnesota; Dept of GCD; 6-160 Jackson Hall; 321 Church Street S.E; Minneapolis; USA
Age	Sent as dauer larvae (July 2012)
Breeding method	Cultivation according to ISO 10872 (2010)
Kind of food	The worms are fed with *Escherichia coli* OP50 from GCD; University of Minnesota.
Amount of food	Bacterial lawn
Feeding frequency	no additional feeding
Pre-treatment	Only J1 juvenile stages were used for the test.
Feeding of animals during test	Feed in sediment (*E. coli*)

Table A7.4.3.5.1-3: Test system.

Criteria	Details
Test type	Static
Renewal of test solution	Not applicable
Volume of test vessels	For the test 5 ml snap-cap glas vessels were used. Filled with 0.5 g spiked wet artificial sediment.
Volume/animal	0.3 g dry sediment moistened with 0.2 ml M9 medium to 0.5 g wet weight sediment (water content 40 %)
Number of animals/vessel	10 juvenile *C. elegans* at start of exposure
Number of vessels/concentration	Following concentrations were used: 0.3, 1, 3, 10, 30 mg/kg dry weight. For each concentration and the controls 12 replicates were set up (6 vessels for toxicity testing, 6 vessels for chemical analysis at start and at end of exposure). Negative control (sediment without addition of solvent), solvent control (addition of acetone without test item) and positive control (addition of 0.5 ml (30°mg/L) Benzylcetyldimethylammonium chloride monohydrate (BAC-C16)) were used.
Test performed in closed vessels due to significant volatility of TS	Yes, test vessels with wet sediment were closed with caps.

Table A7.4.3.5.1- 4: Test conditions.

Criteria	Details
Test temperature	19.5 -20.0 °C
Dissolved oxygen	Not stated
Ammonium content	Not stated
Total hardness of the overlaying water during the test	Not stated However, M9-medium according to ISO 10872 was used to wet the soil 6 g/L Na_2HPO_4 3 g/L KH_2PO_4 5 g/L NaCl 0.25 g/L $MgSO_4 \cdot 7H_2O$
pH	Not stated
Adjustment of pH	Not stated
Aeration of dilution water	No
Quality/Intensity of irradiation	None
Photoperiod	None, cultivation in the dark

Table A7.4.3.5.1- 5: Summary of the analytical results.

Nominal test item concentration [mg/kg d.s.]	Test period (hours)	Measured concentration in sediment and recovery in sediment		
		Sediment [mg/kg dw]	Sediment [mg/kg ww]	Recovery sediment (total isomers) [% of nominal]
n.a.	0		<LOQ	
	96		<LOD	
	0		<LOD	
	96		<LOD	
0.30	0	0.233	0.140	78
0.30	96	0.235	0.141	78
1.0	0	0.733	0.440	73
1.0	96	1.04	0.62	104
3.0	0	4.39	2.63	146
3.0	96	3.30	1.98	110
10	0	7.51	4.51	75
10	96	7.38	4.43	74
30	0	28.2	16.9	100
30	96	29.1	17.5	103

LOQ: 0.10 mg/kg (based on dry sediment weight), LOD: 0.0019 mg/kg

Table A7.4.3.5.1- 6: Toxicity test results: growth and reproduction of C. elegans after 96 h of exposure to α-cypermethrin via spiked sediment in various concentrations (given on a dry weight [dw] and wet weight [ww] basis); C = control; SC = solvent control; SD = standard deviation; Inh = inhibition; initial measured concentration = concentration at 0 h; mean measured concentration = average concentration of 0 h and 96 h values.

	Sediment concentrations (mg/kg)						Toxicity parameters			
	Nominal		Initial measured		Mean measured		Growth (µm)		Reproduction (Offspring / test organism)	
Code	dw	ww	dw	ww	dw	ww	Mean ± SD	% Inh to SC*	Mean ± SD	% Inh to SC*
C	0	0	< LOD		< LOD		1289 ± 59		40.2 ± 11.2	
SC	0	0	< LOD		< LOD		1302 ± 51		37.0 ± 14.0	
Cyp1	0.3	0.18	0.23	0.14	0.23	0.14	1323 ± 45	-1.6	25.7 ± 8.9	30.5
Cyp2	1	0.6	0.73	0.44	0.89	0.53	1387 ± 85	-6.5	40.5 ± 10.6	-9.4
Cyp3	3	1.8	4.39	2.63	3.85	2.31	1340 ± 106	-2.9	39.2 ± 17.6	-5.9
Cyp4	10	6	7.51	4.50	7.45	4.47	1376 ± 42	-5.7	43.0 ± 17.2	-16.0
Cyp5	30	18	28.23	16.94	28.65	17.19	1332 ± 32	-2.3	46.1 ± 8.1	-24.4

* negative inhibition values indicate higher performance than in the solvent control

Table A7.4.3.5.1- 7: Validation criteria in controls; x = fulfilled

Validation criteria	Control	Solvent control	Control (Positive Control)
% Recovery in controls (80 ≤ x ≤ 120 %)	85.0 (x)	80.0 (x)	96.7 (x)
% Males in Controls (≤ 10 %)	0.0 (x)	0.0 (x)	0.0 (x)
% Fertility in controls (≥ 80 %)	98.1 (x)	100.0 (x)	100.0 (x)
Reproduction in controls (≥ 30 offspring/test organisms)	40.2 (x)	37.0 (x)	122.2 (x)

Table A7.4.3.5.1- 8: Validity criteria and obtained data for the sediment-water *Caenorhabditis* toxicity test using spiked sediment according to ISO 10872 (2010).

	Fulfilled
The mean recovery of exposed test organisms from the control is ≥ 80% and ≤ 120%	☑
The mean percentage of males in the control is ≤ 10%; the percentage of males in a single control replicate is ≤ 20%	☑
The mean fertility in the control is ≥ 80%	☑
The mean reproduction in the control is ≥ 30 offspring per exposed test organism	☑

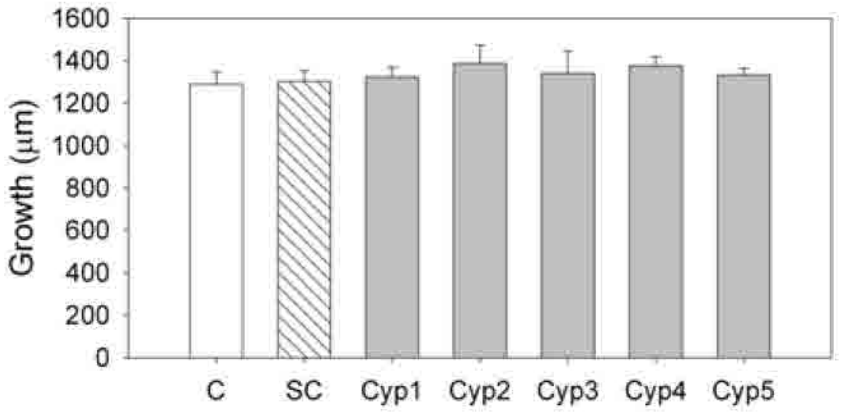

Figure A7.4.3.5.1- 1: Mean growth of *C. elegans* after 96 h of exposure to α-cypermethrin via spiked sediment in various concentrations (for codes see Table 2); error bars = standard deviation.

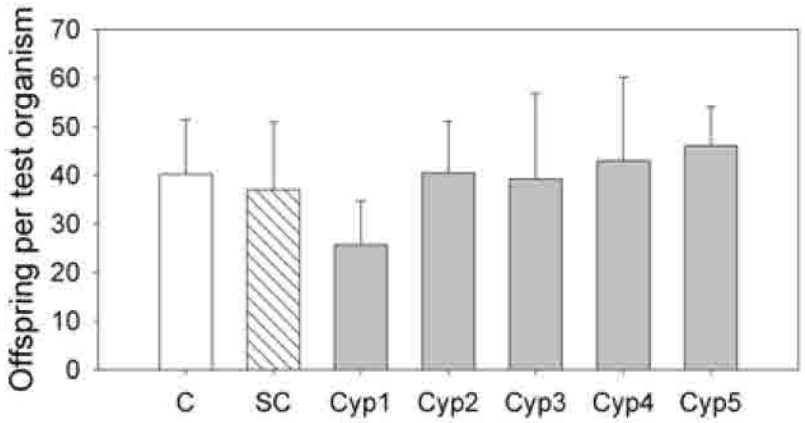

Figure A7.4.3.5.1- 2: Mean reproduction of *C. elegans* after 96 h of exposure to α-cypermethrin via spiked sediment in various concentrations (for codes see Table 2); error bars = standard deviation.

Section A7.4.3.5.2 Aquatic plant toxicity

Annex Point IIIA 13.3.4

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X]	**Technically not feasible** [ ] **Scientifically unjustified** [X]	
Limited exposure [ ]	**Other justification** [ ]	
Detailed justification:	An experimental study on aquatic plant toxicity is not available. Alphacypermethrin is an insecticide. Based on the outcome of the algal growth inhibition test (A7.4.1.3/01), it may be argued that plants are not target organisms for this active substance. This is further supported by higher-tier studies (mesocosm studies, A7.4.3.5/02, 08), where a diverse semi-natural algal community as well as a semi-natural macrophyte community were an integral part of the test system. In these studies, the observed effects on the plant community were considered to be indirect effects (grazing by affected zooplankton). There was no evidence for direct toxic effects of Alphacypermethrin to plants. Thus, it is satisfactorily demonstrated that plants are considerably less susceptible to Alphacypermethrin than freshwater animals. Since a community-based EAC was derived from the mesocosm studies, the conduct of specific plant toxicity studies is not considered to be required.	
Undertaking of intended data submission [ ]		

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)	
Date	March 2009	
Evaluation of applicant's justification	Applicant's justification are considered to be acceptable	
Conclusion	Acceptable	
Remarks		
	COMMENTS FROM ...	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section A7.5.1.1 **Inhibition of microbial activity (terrestrial)**
Annex Point IIA 7.4

Official use only

1 REFERENCE

1.1	**Reference**	**A7.5.1.1/01:** Kölzer U (2006) Effects of BAS 310 I (Reg. No. 4078193) on the activity of the soil microflora – nitrogen transformation test (EC_x). GAB, Niefern-Öschelbronn, Germany, Report no. 20061048/01-ABMF, April 11, 2006 (unpublished), BASF DocID: 2006/1008040.
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF AG
1.2.2	Companies with letter of access	No
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes OECD 216
2.2	**GLP**	Yes
2.3	**Deviations**	Yes The maximum test concentration did not cover the potential EC_{10}, EC_{25} or EC_{50} value (see results section).

3 MATERIALS AND METHODS

3.1	**Test material**	As given in Section A2.
3.1.1	Lot/Batch number	COD-000166
3.1.2	Specification	As given in Section A2.
3.1.3	Purity	99.3% (w/w)
3.1.4	Composition of product	Not applicable
3.1.5	Further relevant properties	Properties potentially negatively affecting the test performance are not known.
3.1.6	Method of analysis	Nitrate and ammonium were quantified using ion-selective electrodes, respectively. LoQ (nitrate-N) = 10.2 mg/kg dry soil LoQ (ammonium-N) = 0.873 mg/kg dry soil Verification of test substance concentrations is not necessary according to OECD 216.

Section A7.5.1.1 Inhibition of microbial activity (terrestrial)

Annex Point IIA 7.4

3.2	**Reference substance**	Yes Dinoterb 97.5%, tested in a separate study at a rate of 13.3 mg/kg
3.2.1	Method of analysis for reference substance	Not required
3.3	**Testing procedure**	
3.3.1	Soil sample/ inoculum/ test organism	As given in Table A7.5.1.1- 1.
3.3.2	Test system	Details are presented in Table A7.5.1.1- 2.
3.3.1	Application of TS	Please refer to Table A7.5.1.1- 3.
3.3.2	Test conditions	Test conditions are described in Table A7.5.1.1- 4.
3.3.3	Test parameter	Inhibition of microbial nitrogen transformation
3.3.4	Analytical parameter	Measurement of nitrate and ammonium concentration
3.3.5	Duration of the test	28 days
3.3.6	Sampling	At day 0, 7, 14 and 28.
3.3.7	Monitoring of TS concentration	No (not required according to OECD 216)
3.3.8	Controls	Yes Control without test substance
3.3.9	Statistics	Shapiro-Wilks test for normality Bartlett's test for homogeneity of variances Dunnett's multiple *t*-test for NOEC determination

4 RESULTS

4.1	**Range finding test**	Not performed
4.1.1	Concentration	
4.1.2	Effect data	
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	1.0, 3.3, 10, 33, and 100 mg/kg dry soil
4.2.2	Actual concentrations of test substance	Nominal
4.2.3	Growth curves	Not appropriate

Section A7.5.1.1 **Inhibition of microbial activity (terrestrial)**
Annex Point IIA 7.4

4.2.4	Cell concentration data	Not appropriate
4.2.5	Concentration/ response curve	Since no inhibition occurred within the tested concentration range, a concentration-response curve cannot be given.
4.2.6	Effect data	For the effect of Alphacypermethrin on nitrate formation see Table A7.5.1.1- 1. Effects on ammonium transformation could not be detected since from day 7 onwards ammonium concentrations were consistently below the LoQ. Nitrate formation was not significantly different from the control at any test concentration. Thus, EC_{50} > 100 mg/kg dry soil NOEC > 100 mg/kg dry soil
4.2.7	Other observed effects	None
4.3	**Results of controls**	See Table A7.5.1.1- 5.
4.4	**Test with reference substance**	Performed
4.4.1	Concentrations	13.3 mg/kg dry soil
4.4.2	Results	+34.7% deviation from the control (i.e., stimulation)

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The impact of Alphacypermethrin on the nitrogen turnover of soil microorganisms was tested according to OECD guideline 216, at active substance concentrations in a range of 1.0 to 100 mg/kg dry soil. This concentration range did not cover the potential EC_{50}. Apart from this, there were no deviations from the guideline.
5.2	**Results and discussion**	Properties of Alphacypermethrin potentially negatively affecting the test performance are not known. Within the tested concentration range, there were no detectable effects of Alphacypermethrin on nitrogen turnover.
5.2.1	NOEC	> 100 mg/kg dry soil
5.2.2	EC_{10}	> 100 mg/kg dry soil
5.2.3	EC_{50}	> 100 mg/kg dry soil
5.3	**Conclusion**	Variation of the test parameter between control replicates was less than 15%. Thus, the test is considered valid. Although none of the effect concentrations in question (NOEC, EC_{10}, EC_{25} or EC_{50}) was covered by the applied concentration range, this does not affect the quality of the study. The potential concentration of Alphacypermethrin to be expected in soil falls far below the tested concentrations. Thus, it is considered fully appropriate to forward to the risk assessment an EC_{50} of > 100 mg/kg dry soil.

Section A7.5.1.1 Inhibition of microbial activity (terrestrial)

Annex Point IIA 7.4

5.3.1	Reliability	1
5.3.2	Deficiencies	No

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	March 2009
Materials and Methods	The Applicant's version is considered to be acceptable
Results and discussion	The Applicant's version is considered to be acceptable
Conclusion	The Applicant's version is considered to be acceptable
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.5.1.1- 1: Microbial sample / inoculum.

Criteria	Details
Nature	Soil sample
Sampling site	D-76877 Offenbach, Germany
Geographical reference on the sampling site	Not stated in the report Upon dossier preparation, the geographical reference was retrieved as: 49° 12' 8" N, 8° 10' 52" E
Data on the history of the site	No plant protection treatment for at least one year prior to sampling
Use pattern	Agricultural field with pumpkin
Depth of sampling [cm]	20
Sand / Silt / Clay content [% dry weight]	65.0 / 33.6 / 1.2
pH	6.75
Organic carbon content [% dry weight]	1.17
Nitrogen content [mg/kg dry weight]	NH_4^+-N: 2.20 NO_3^--N: 27.1
Cation exchange capacity [mval/100 g]	10.1
Initial microbial biomass [% of TOC]	1.0
Reference of methods	Anderson and Domsch (1978), Soil Biol. Biochem. 10: 215–221; ISO 11465; ISO 10390
Collection / storage of samples	Sampling: 18 Nov 2005 Sieving: 02 Dec 2005 Start of storage: 06 Dec 2005 Start of soil conditioning for test: 02 Feb 2006 Storage temperature (mean, min, max): 4, 3.6, 4.5 °C
Preparation of inoculum for exposure	Moisture adjustment to 45% WHC; Thorough mixing with lucerne meal (0.5% of soil dry weight)
Pre-treatment	Sieving (2 mm) prior to storage

Table A7.5.1.1- 2: Test system.

Criteria	Details
Culturing apparatus	2000 mL glass bottles
Number of vessels/concentration	3
Aeration device	–
Measuring equipment	Ion-selective electrodes
Test performed in closed vessels	No

Table A7.5.1.1- 3: Application of test substance.

Criteria	Details
Application procedure	Dissolved in volatile solvent (acetone)
Carrier	Quartz sand
Concentration of liquid carrier [% v/v]	Not applicable, acetone was evaporated after application to sand
Liquid carrier control	–
Other procedures	None

Table A7.5.1.1- 4: Test conditions.

Criteria	Details
Organic substrate	None
Incubation temperature	20 ± 2 °C
Soil moisture	Adjusted to 45% WHC at test start; Every 7 days addition of water as appropriate to compensate weight difference following weighing
Method of soil incubation	Individual sub-samples
Aeration	No

Table A7.5.1.1- 5: Effect data: Nitrate concentrations at the end of the test (28 days).

a.s. concentration [mg/kg dry soil]	Mean nitrogen concentration [mg/kg dry soil]	CV [%]	Deviation from control [%]	Mean NO_3^--N-formation rate [mg/kg dry soil /d]
Nitrate-N				
Control	43.5	1.91	–	1.55
1.0	45.4	3.27	4.42	1.62
3.3	43.8	4.49	0.728	1.57
10	45.7	9.25	4.95	1.63
33	45.1	1.63	3.55	1.61
100	45.5	8.07	4.47	1.62

Remark: All 7, 14 and 28-d ammonium concentrations were below the limit of quantification and are therefore not presented, whereas day 0 ammonium values ranged between 1.56 and 2.20 mg/kg dry soil

Section A7.5.1.1 Inhibition of microbial activity (terrestrial)

Annex Point IIA 7.4

Official use only

1 REFERENCE

1.1	**Reference**	**A7.5.1.1/02:** Kölzer U (2006) Effects of BAS 310 I (Reg. No. 4078193) on the activity of the soil microflora – carbon transformation test (EC_x). GAB, Niefern-Öschelbronn, Germany, Report no. 20061048/02-ABMF, April 11, 2006 (unpublished), BASF DocID: 2006/1008041.
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF AG
1.2.2	Companies with letter of access	No
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes OECD 217 (2000)
2.2	**GLP**	Yes
2.3	**Deviations**	Yes The maximum test concentration did not cover the potential EC_{10}, EC_{25} or EC_{50} value (see results section).

3 MATERIALS AND METHODS

3.1	**Test material**	As given in Section A2.
3.1.1	Lot/Batch number	COD-000166
3.1.2	Specification	As given in Section A2.
3.1.3	Purity	99.3% (w/w)
3.1.4	Composition of product	Not applicable
3.1.5	Further relevant properties	Properties potentially negatively affecting the test performance are not known.
3.1.6	Method of analysis	No Verification of test substance concentrations is not necessary according to OECD 217.

Section A7.5.1.1 Inhibition of microbial activity (terrestrial)

Annex Point IIA 7.4

3.2	**Reference substance**	Yes Dinoterb 97.5%, tested in a separate study at a rate of 13.3 mg/kg
3.2.1	Method of analysis for reference substance	Not required
3.3	**Testing procedure**	
3.3.1	Soil sample/ inoculum/ test organism	As given in Table A7.5.1.1- 6.
3.3.2	Test system	Details are presented in Table A7.5.1.1- 7.
3.3.3	Application of TS	Please refer to Table A7.5.1.1- 8.
3.3.4	Test conditions	Test conditions are described in Table A7.5.1.1- 9.
3.3.5	Test parameter	Organic carbon (glucose) transformation
3.3.6	Analytical parameter	Respiration rate, measured as pressure decrease in the test vessels due to oxygen consumption.
3.3.7	Duration of the test	56 days
3.3.8	Sampling	At day 0, 7, 14, 28, and 56.
3.3.9	Monitoring of TS concentration	No (not required according to OECD 217)
3.3.10	Controls	Yes Control without test substance
3.3.11	Statistics	Shapiro-Wilks test for normality Bartlett's test for homogeneity of variances Dunnett's multiple *t*-test for NOEC determination

4 RESULTS

4.1	**Range finding test**	Not performed
4.1.1	Concentration	
4.1.2	Effect data	
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	1.0, 3.3, 10, 33, and 100 mg/kg dry soil
4.2.2	Actual concentrations of test substance	Nominal
4.2.3	Growth curves	Not appropriate

Section A7.5.1.1 Inhibition of microbial activity (terrestrial)

Annex Point IIA 7.4

4.2.4	Cell concentration data	Not appropriate	
4.2.5	Concentration/ response curve	Since no inhibition occurred within the tested concentration range, a concentration-response curve cannot be given.	
4.2.6	Effect data	For the effect of Alphacypermethrin on the respiration rate see Table A7.5.1.1- 10. Organic carbon transformation was not significantly different from the control at any test concentration. Thus, EC_{50} > 100 mg/kg dry soil NOEC > 100 mg/kg dry soil	
4.2.7	Other observed effects	None	
4.3	**Results of controls**	See Table A7.5.1.1- 10.	
4.4	**Test with reference substance**	Performed	
4.4.1	Concentrations	13.3 mg/kg dry soil	
4.4.2	Results	–34.1% deviation from the control	X

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The impact of Alphacypermethrin on the organic carbon transformation rate of soil micro-organisms (analytical parameter: respiration rate) was tested according to OECD guideline 217, at active substance concentrations in a range of 1.0 to 100 mg/kg dry soil. This concentration range did not cover the potential EC_{50}. Apart from this, there were no deviations from the guideline.	
5.2	**Results and discussion**	Properties of Alphacypermethrin potentially negatively affecting the test performance are not known. Within the tested concentration range, there were no detectable effects of Alphacypermethrin on the respiration rate of the soil samples.	
5.2.1	NOEC	> 100 mg/kg dry soil	
5.2.2	EC_{10}	> 100 mg/kg dry soil	
5.2.3	EC_{50}	> 100 mg/kg dry soil	

Section A7.5.1.1 Inhibition of microbial activity (terrestrial)

Annex Point IIA 7.4

5.3	**Conclusion**	Variation of the test parameter between control replicates was less than 15%, except for the 28-day sampling date. This was the reason for extending the test period to 56 days. Since variation of control replicates returned to below 15% after 56 days, the test is considered valid. Although none of the effect concentrations in question (NOEC, EC_{10}, EC_{25} or EC_{50}) was covered by the applied concentration range, this does not affect the quality of the study. The potential concentrations of Alphacypermethrin to be expected in soil fall far below the tested concentrations. Thus, it is considered fully appropriate to forward to the risk assessment an EC_{50} of > 100 mg/kg dry soil.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	March 2009
Materials and Methods	The Applicant's version is considered to be acceptable
Results and discussion	4.4.2 Reference substance +35,1% deviation from control
Conclusion	The Applicant's version is considered to be acceptable
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.5.1.1- 6: Microbial sample / inoculum.

Criteria	Details
Nature	Soil sample
Sampling site	D-76877 Offenbach, Germany
Geographical reference on the sampling site	Not stated in the report Upon dossier preparation, the geographical reference was retrieved: 49° 12' 8" N, 8° 10' 52" E
Data on the history of the site	No plant protection treatment for at least one year prior to sampling
Use pattern	Agricultural field with pumpkin
Depth of sampling [cm]	20
Sand / Silt / Clay content [% dry weight]	65.0 / 33.6 / 1.2
pH	6.75
Organic carbon content [% dry weight]	1.17
Nitrogen content [mg/kg dry weight]	NH_4^+-N: 2.20 NO_3^--N: 27.1
Cation exchange capacity [mval/100 g]	10.1
Initial microbial biomass [% of TOC]	1.0
Reference of methods	Anderson and Domsch (1978), Soil Biol. Biochem. 10: 215–221; ISO 11465; ISO 10390
Collection / storage of samples	Sampling: 18 Nov 2005 Sieving: 02 Dec 2005 Start of storage: 06 Dec 2005 Start of soil conditioning for test: 02 Feb 2006 Storage temperature (mean, min, max): 4, 3.6, 4.5 °C
Preparation of inoculum for exposure	Moisture adjustment to 45% WHC
Pre-treatment	Sieving (2 mm) prior to storage

Table A7.5.1.1- 7: Test system.

Criteria	Details
Culturing apparatus	2000 mL glass bottles
Number of vessels/concentration	3
Aeration device	–
Measuring equipment	OxiTop Control® pressure measuring system
Test performed in closed vessels	No The soil was stored in the above glass bottles loosely closed by screw caps over the test period; For short-term respiration rate measurements, samples of 100 g were transferred to an OxiTop measurement bottle, incubated for 20–24 h and the pressure decrease recorded over 12 h

Table A7.5.1.1- 8: Application of test substance.

Criteria	Details
Application procedure	Dissolved in volatile solvent (acetone)
Carrier	Quartz sand
Concentration of liquid carrier [% v/v]	Not applicable, acetone was evaporated after application to sand
Liquid carrier control	–
Other procedures	None

Table A7.5.1.1- 9: Test conditions.

Criteria	Details
Organic substrate	Glucose, 300 mg/100 g wet soil (0.3%) added the respective sub-sample prior to each measurement trial
Incubation temperature	20 ± 2 °C
Soil moisture	Adjusted to 45% WHC at test start; Every 7 days addition of water as appropriate to compensate weight difference following weighing
Method of soil incubation	Individual sub-samples
Aeration	No

Table A7.5.1.1- 10: Effect data: Respiration rates of soils treated with Alphacypermethrin.

a.s. concentration [mg/kg dry soil]	Mean O_2 consumption [mg/kg dry soil/h]				Deviation from control [%]			
	7 d	14 d	28 d	56 d	7 d	14 d	28 d	56 d
Control	4.84	4.28	3.77	4.68	–	–	–	–
1.0	4.19	4.56	4.38	4.25	–13.4	+6.40	+16.1	–9.13
3.3	3.70	3.85	3.94	4.43	–23.6	–10.1	+4.38	–5.27
10	4.44	4.72	4.31	4.62	–8.35	+10.3	+14.1	–1.37
33	4.24	4.08	3.30	4.21	–12.5	–4.82	–12.6	–10.1
100	3.38	4.18	3.89	4.61	–30.2	–2.30	+3.15	–1.50

Section A7.5.1.2 Earthworm, acute toxicity test

Annex Point IIIA 13.3.2

Official use only

1 REFERENCE

1.1	**Reference**	**A7.5.1.2/01:** Weyman GS, Canez VM (1998) 14-Day earthworm (*Eisenia foetida*) acute toxicity study with Alphacypermethrin (AC 900049) in a 150 g/kg WG Formulation (RLF 12152). Covance, Harrogate, UK, Report no. 628/110-1018, March 23, 1998 (unpublished), BASF RDI No.: AL-560-019.
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF AG
1.2.2	Companies with letter of access	No
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes OECD 207
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	150 g/kg WG formulation of alphacypermethrin
3.1.1	Lot/Batch number	R1811-088
3.1.2	Specification	White granules: 4.25 mm diameter, 3.8 mm thick.
3.1.3	Purity	151.9 g/kg alphacypermethrin in the formulation (=15.19% w/w)
3.1.4	Composition of product	See above.
3.1.5	Further relevant properties	The formulation selected for this test is appropriate to ensure homogeneous mixing with the test substrate after moistening with water. Expiry date: 7 January 1998 (test performance: 19 October to 12 November 1997).
3.1.6	Method of analysis	GC-ECD Method description SAMS 354-2, as validated in reference A4.2/01.

Section A7.5.1.2 Earthworm, acute toxicity test

Annex Point IIIA 13.3.2

3.2	**Reference substance**	Yes 2-chloroacetamide (CAS-No. 79-07-2)	
3.2.1	Method of analysis for reference substance	The reference substance concentrations were not verified analytically.	
3.3	**Testing procedure**		
3.3.1	Preparation of the test substance	As detailed in Table A7.5.1.2- 1.	
3.3.2	Application of the test substance	Thorough mixing of stock dispersions with the test substrate. Formulated alphacypermethrin was applied in RO (reverse osmosis) water. The amount of water used for treatment was sufficient to bring the moisture content of the test substrate to approximately 35% of dry weight.	
3.3.3	Test organisms	*Eisenia foetida*, as described in Table A7.5.1.2- 2.	
3.3.4	Test system	The test system is described in Table A7.5.1.2- 3.	
3.3.5	Test conditions	Please refer to Table A7.5.1.2- 4.	X
3.3.6	Test duration	14 d	
3.3.7	Test parameter	Mortality, body weight change.	
3.3.8	Examination	7 d, 14 d	
3.3.9	Monitoring of TS concentration	Yes At test initiation.	
3.3.10	Statistics	LC_{50}: probit analysis NOEC of mortality and body weight change: ANOVA, followed by Dunnett's test in case of significant result.	

4 RESULTS

4.1	**Filter paper test**	Not performed However, a *range-finding test* using artificial soil was performed, which is briefly described below.	
4.1.1	Concentration	0.1, 1.0, 10, 100, 1000 mg a.i./kg dry test substrate One replicate per treatment level.	
4.1.2	Number/ percentage of animals showing adverse effects		
4.1.3	Nature of adverse effects	Mortality	

c [mg a.i./kg]	Control	0.1	1.0	10	100	1000
Number	0	0	0	0	1	1
%	0	0	0	0	10	10

Section A7.5.1.2 Earthworm, acute toxicity test

Annex Point IIIA 13.3.2

4.2 Soil test

4.2.1 Initial concentrations of test substance

Mean measured concentrations [mg/kg]:

Nominal	Control	62.5	125	250	500	1000
Measured	< 0.1	78.0	107.4	198.7	509.2	909.3
% of nominal	n.a.	125	86	79	102	91

4.2.2 Effect data (Mortality)

Mortality data are given in Table A7.5.1.2- 5.

For effect concentrations see Table A7.5.1.2- 6.

4.2.3 Concentration / effect curve

Not provided.

4.2.4 Other effects

Treatment related weight loss:

c [mg a.i./kg]	62.5	125	250	500	1000
% weight loss	–7.7	–8.7	–13.5	–14.4	–8.6
Significance	ns	ns	*	*	ns

Furthermore, in all alphacypermethrin treatments a number of worms initially remained on the substrate surface (in the controls, all were submerged after 1 h): Two worms were on the surface in the 62.5, 125 and 250 mg a.i./kg treatments, respectively; at 500 mg a.i./kg there were three worms on the surface; at 1000 mg/kg there were estimated to be 14 worms on the surface. After 24 h, all worms up to and including the 125 mg a.i./kg treatment were submerged. There was one worm on the surface in the 250 mg a.i./kg treatment, none at 500 mg a.i./kg, and four at 1000 mg a.i./kg (1, 2, 1, 0 in replicates 1 to 4, respectively). This indicates a slight repellency of the employed alphacypermethrin formulation to earthworms.

4.3 Results of controls

4.3.1 Mortality

7 d: 2.5%
14 d: 5%

4.3.2 Number/ percentage of earthworms showing adverse effects

7 d: 1
14 d: 2

4.3.3 Nature of adverse effects

Mortality

4.4 Test with reference substance

Performed

4.4.1 Concentrations

10, 19, 34, 61, 110 mg/kg

4.4.2 Results

LC_{50} (14d) = 26.55 mg/kg (95% CI = 229–29.4)

Section A7.5.1.2 **Earthworm, acute toxicity test**
Annex Point IIIA 13.3.2

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The acute toxicity of alphacypermethrin to earthworms was tested in an artificial soil test using *Eisenia foetida*, according to OECD guideline 207 and EC method C.8. Earthworms were exposed to five different test substance concentrations up to 1000 mg/kg soil dry weight and mortality as well as any toxicological symptoms were observed after 7 and 14 days. Deviations from the guidelines were not reported.
5.2	**Results and discussion**	Measured TS concentrations outside the range 80 to 120% of nominal are likely to be largely due to the method of incorporation of the test substance into the test substrate, which may have resulted in some variability of concentration through the substrate, discernible if small sub-samples are analysed. The observed deviations were small, however. In the light of the biological results, this is deemed acceptable and effects are nevertheless assessed based on nominal concentrations. The observed initial repellent effect is not considered to have affected the results since the worms were nevertheless exposed to the test substance for at least 13 days. With respect to body weight loss, a no-effect concentration can be given: NOEC = 125 mg a.i./kg A non-lethal concentration (LC_0) cannot be given due to slight mortality in the lowest concentration. Alternatively, a no-effect concentration with respect to mortality can be given based on statistical analysis as: NOEC =250 mg a.i./kg. Since a median mortality of 50% was not reached at any treatment level, the LC_{50} is estimated to be above 1000 mg a.i./kg dry soil.
5.2.1	LC_0	–
5.2.2	LC_{50}	> 1000 mg a.i./kg dry soil
5.2.3	LC_{100}	> 1000 mg a.i./kg dry soil
5.3	**Conclusion**	Since no deviations from the guidelines or other unusual circumstances were reported, the test was considered valid and reliable.
5.3.1	Reliability	1
5.3.2	Deficiencies	None

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	March 2009
Materials and Methods	The Applicant's version is acceptable with the following amendment: 3.3.5 Continuous lighting, 423 – 501 lux
Results and discussion	The Applicant's version is considered to be acceptable
Conclusion	The Applicant's version is considered to be acceptable
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.5.1.2- 1: Preparation of TS solution.

Criteria	Details
Dispersion	Dispersion in RO water
Vehicle	No
Concentration of vehicle	Not appropriate
Vehicle control performed	Not appropriate
Other procedures	None

Table A7.5.1.2- 2: Test organisms.

Criteria	Details
Species	*Eisenia foetida*
Source of the initial stock	Blades Biological, Cowden, Edenbridge, Kent, UK
Culturing techniques	Holding in damp bulb fibre (various manufacturers)
Age/weight	Age > 2 months, with clitellum Weight 300–600 mg
Pre-treatment	24 h acclimation in basic test substrate in a 10 l glass tank, under same conditions of temperature and lighting as in the test, without food supply

Table A7.5.1.2- 3: Test system.

Criteria	Details
Artificial soil test substrate	10% sphagnum peat 20% kaolinite clay 70% industrial sand pH adjusted to 6.5 ± 0.5 using $CaCO_3$ Moisture adjusted to 35% of dry weight using RO water or TS solution, as appropriate, carried out as an integral part of dosing
Test mixture	
Size, volume and material of test container	Glass crystallising dish of 1 l volume
Amount of artificial soil per container	750 g wet weight
Nominal levels of test concentrations	62.5, 125, 250, 500, 1000 mg a.i./kg artificial soil
Number of replicates/concentration	4
Number of earthworms/test concentration	40
Number of earthworms/container	10
Light source	Artificial light
Test performed in closed vessels due to significant volatility of test substrate	No

Table A7.5.1.2- 4: Test conditions.

Criteria	Details
Test temperature	19.5–21.7°C
Moisture content	24.0–27.5% of wet weight
pH	6.5 ± 0.5 at the start of the test
Adjustment of pH	Yes By addition of $CaCO_3$
Light intensity / photoperiod	Continuous lighting, 430–492 lux
Relevant degradation products	Not determined

Table A7.5.1.2- 5: Mortality data.

Test substance concentration (nominal) [mg a.i./kg artificial soil]	**Mortality**			
	Number		**Percentage**	
	7 d	**14 d**	**7 d**	**14 d**
62.5	3	6	7.5	15
125	4	7	10	17.5
250	4	5	10	12.5
500	12	12	30	30
1000	14	15	35	37.5
Temperature [°C]	19.5–21.7			
pH	6.5 ± 0.5			
Moisture content	24.0–27.5% of wet weight			

Table A7.5.1.2- 6: Effect data.

	14 d [mg/kg dry soil][1]	**95 % CI**
LC_0	–	–
LC_{50}	> 1000	–
LC_{100}	> 1000	–

Table A7.5.1.2- 7: Validity criteria for acute earthworm test according to OECD 207.

	Fulfilled	**Not fulfilled**
Mortality of control animals < 10%	X	

Section A7.5.1.2 Earthworm, acute toxicity test

Annex Point IIIA 13.3.2 – Supportive data –

The following reference is considered to contain additional information concerning acute toxicity to earthworms, addressing the effects of alphacypermethrin at a lower limit dose level and is thus presented in an abbreviated format (adopted from the PPP-dossier) as supportive data:

Reference: **A7.5.1.2/02:**

Inglesfield C, Sherwood C (1983) Toxicity of Cypermethrin and WL85871 to the earthworm *Eisenia foetida* L. (Oligochaeta: Lumbriculidae) in laboratory tests. SRC, Sittingbourne, UK, Report no. SBGR.83.071, April 15, 1983 (unpublished), BASF RDI No.: CY-531-002.

Guidelines: OECD guideline 207

GLP: No, at the time the study was conducted GLP was not compulsory.

Materials and Methods:

Test Substance: Technical grade alphacypermethrin (also known as BAS 310 I and previously known as AC 900049 and WL85871); Batch Number OCD/7; purity: 98.2–99.4%.

Species: Earthworm (*Eisenia fetida*); Origin: S.H. Nash Ltd., Essex, UK.

Test Conditions: The earthworms were exposed to the test substance in an artificial soil consisting of 70% industrial sand, 20% kaolin clay and 10% *Sphagnum* peat. There were 4 replicates per treatment, with ten earthworms per replicate. The test was conducted under the following conditions:

Temperature: 20 ± 1°C

Photoperiod: 24 hours of continuous light

Concentrations tested:

0 (deionized water control), solvent (acetone) control, 0.1, 1.0, 10, and 100 mg a.s./kg of soil.

Treatment/Application or Method:

Stock solutions of the test substance were prepared in acetone. 5 mL of the stock solutions were sprayed onto the surface of the test soils using a Brinkmann TLC hand-held sprayer. The solution was then thoroughly mixed with the soil to ensure even distribution of the test substance. The soil was then left for two hours to allow the acetone to evaporate.

Observation: Earthworm mortality was recorded on days 7 and 14.

Statistics: No statistical analysis was conducted.

Findings:

The number of dead earthworms in each treatment is summarized in the following table:

Table A7.5.1.2- 8: Effect of technical grade Alphacypermethrin on the survival of earthworms in a 14-day acute toxicity test.

Treatment	Day 7 mortality	Day 14 mortality
Control	2.5%	2.5%
Solvent Control	0%	0%
0.1mg/kg	0%	2.5%
1.0 mg/kg	5.0%	5.0%
10 mg/kg	0%	2.5%
100 mg/kg	2.5%	5.0%

Since there was < 50% mortality in all test substance treatment groups, the 14-day LC_{50} is > 100 mg/kg, the highest concentration tested. The NOEC for mortality was 100 mg/kg, since there was not significantly higher mortality in any test substance treatment group in comparison to the controls.

Conclusions:
The 14-day LC_{50} of alphacypermethrin to the earthworm was determined to be > 100 mg a.s./kg of soil. The NOEC was 100 mg/kg of soil.

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	March 2009
Materials and Methods	The Applicant's version is considered to be acceptable
Results and discussion	The Applicant's version is acceptable with the following amendment: Table A7.5.1.2-8: 0.1 and 1.0 mg/kg
Conclusion	The Applicant's version is considered to be acceptable
Reliability	2
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A7.5.1.2 Earthworm, acute toxicity test

Annex Point IIIA 13.3.2 – Supportive data –

The following reference is considered to contain additional information concerning acute toxicity to earthworms, addressing the effects of an identified metabolite and is thus presented in an abbreviated format (adopted from the PPP-dossier) as supportive data:

Reference: **A7.5.1.2/03:**

Staab F (2001) Effect of metabolite CL 206 128 (metabolite of α-Cyper-methrine) on the mortality of the earthworm Eisenia fetida. BASF, Limburgerhof, Germany, Report no. 108413, August 21, 2001 (unpublished), BASF DocID: 2001/1014597.

Guidelines: OECD guideline 207

GLP: Yes (laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Materials and Methods:

Test Substance: CL 206128; Lot Number AC12251-34; purity: 99%.

Species: Earthworm (*Eisenia fetida*); origin: in-house cultures of BASF Aktiengesellschaft, Limburgerhof, Germany.

Test Conditions: The earthworms were exposed to the test substance in an artificial soil consisting of 69% quartz sand, 20% kaolin clay, 10% *Sphagnum* peat, and 1% CaCO3. There were 4 replicates per treatment, with ten earthworms per replicate. The test was conducted in 1.0 L glass jars which were maintained under the following conditions:

Temperature: 20–21°C

Photoperiod: 24 hours of continuous light

Concentrations tested:

0 (control), 10, 18, 32.4, 58.3, 105, 189, and 340.1 mg/kg of soil.

Treatment/Application or Method:

Appropriate amounts of the test substance were weighed in small beakers using an analytical balance. The test substance was suspended in 15 mL of acetone, and poured onto an appropriate amount of artificial soil. The soil was then left for approximately one hour to allow the acetone to evaporate. After one hour, water was added to the test medium, and the medium was thoroughly mixed. Approximately 750 g of prepared substrate was added to each of the four replicate test vessels. Ten earthworms were placed on the soil surface of each test vessel.

Observation: Earthworm mortality was recorded on days 7 and 14. Mean earthworm biomass was determined on days 0 and 14.

Statistics: Probit-, logit-, and log-log analysis (Toxstat ver. 3.5) were performed to estimate the 14-day LC_{50}. The Bonferroni *t*-test (Toxstat ver. 3.5) was used to determine the NOEC based on changes in earthworm biomass.

Findings:

The number of dead earthworms in each treatment is summarized in the following table:

Table A7.5.1.2- 9: Effect of CL 206128 on the survival of earthworms in a 14-day Acute toxicity test.

Treatment	Day 7 Mortality	Day 14 Mortality
Control	0%	0%
10 mg/kg	0%	0%
18 mg/kg	0%	0%
32.4 mg/kg	0%	0%
58.3 mg/kg	0%	0%
105 mg/kg	0%	0%
189 mg/kg	35%	45%
340.1 mg/kg	82.5%	95%

The 14-day LC_{50} was 215 mg/kg. The NOEC for mortality was 105 mg/kg.
The effect of the test substance on earthworm biomass is summarized in the following table:

Table A7.5.1.2- 10: Effect of CL 206128 on earthworm biomass in a 14-day acute toxicity test.

Treatment	**Day 14 Relative Weight Change**
Control	–5.88%
10 mg/kg	–10.50%
18 mg/kg	–10.77%
32.4 mg/kg	–19.70%*
58.3 mg/kg	–23.77%*
105 mg/kg	–33.82%*
189 mg/kg	–46.43%*
340.1 mg/kg	–38.30%*

*Significantly different from controls.

There was a statistically significant in weight loss in all treatments ≥ 32.4 mg/kg. Therefore, the NOEC for effects on biomass was 18 mg/kg.

Conclusions:
The 14-day LC_{50} of CL 206128 to the earthworm was 215 mg/kg of soil. The NOEC was 18 mg/kg of soil.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	March 2009
Materials and Methods	The Applicant's version is considered to be acceptable
Results and discussion	The Applicant's version is acceptable with the following amendment: Table A7.5.1.2-10: -10,70%
Conclusion	The Applicant's version is considered to be acceptable
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A7.5.1.2 Earthworm, acute toxicity test

Annex Point IIIA 13.3.2 – Supportive data –

The following reference is considered to contain additional information concerning acute toxicity to earthworms, addressing the effects of an identified metabolite and is thus presented in an abbreviated format (adopted from the PPP-dossier) as supportive data:

Reference: **A7.5.1.2/04:**

Staab F (2001) Effect of metabolite CL 912554 (metabolite of α-Cypermethrine) on the mortality of the earthworm *Eisenia fetida*. BASF, Limburgerhof, Germany, Report no. 108441, August 27, 2001 (unpublished), BASF DocID: 2001/1014603.

Guidelines: OECD guideline 207

GLP: Yes (laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Materials and Methods:

Test Substance: CL 912554; Lot Number AC12717-65; purity: 99%.

Species: Earthworm (*Eisenia fetida*); origin: in-house cultures of BASF Aktiengesellschaft, Limburgerhof, Germany.

Test Conditions: The earthworms were exposed to the test substance in an artificial soil consisting of 69% quartz sand, 20% kaolin clay, 10% Sphagnum peat, and 1% $CaCO_3$. There were 4 replicates per treatment, with ten earthworms per replicate. The test was conducted in 1.0 L glass jars which were maintained under the following conditions:

Temperature: 20–21°C

Photoperiod: 24 hours of continuous light

Concentrations tested:

0 (control), 10, 19, 36, 69, 130, 248, and 470 mg/kg of soil.

Treatment/application or method:

Appropriate amounts of the test substance were blended with 10 g of quartz sand, and the sand was thoroughly mixed into an appropriate amount of artificial soil. Approximately 750 g of prepared substrate was added to each of the four replicate test vessels. Ten earthworms were placed on the soil surface of each test vessel.

Observation: Earthworm mortality was recorded on days 7 and 14. Mean earthworm biomass was determined on days 0 and 14.

Statistics: Probit analysis was performed to estimate the 14-day LC50. Dunnett's test was used to determine the NOEC based on changes in earthworm biomass. All statistical calculation were performed using Toxstat ver. 3.5.

Findings:

The number of dead earthworms in each treatment is summarized in the following table:

Table A7.5.1.2- 11: Effect of CL 912554 on the survival of earthworms in a 14-day acute toxicity test.

Treatment	Day 7 mortality	Day 14 mortality
Control	0%	0%
10 mg/kg	0%	0%
19 mg/kg	0%	0%
36 mg/kg	0%	0%
69 mg/kg	0%	0%
130 mg/kg	0%	2.5%
248 mg/kg	57.5%	92.5%
470 mg/kg	97.5%	100%

The 14-day LC_{50} was 198 mg/kg. The NOEC for mortality was 69 mg/kg since there was less than 10% mortality in this and all lower treatment concentrations.

The effect of the test substance on earthworm biomass is summarized in the following table:

Table A7.5.1.2- 12: Effect of CL 912554 on earthworm biomass in a 14-day acute toxicity test.

Treatment	Day 14 Relative Weight Change
Control	–4.97%
10 mg/kg	–5.12%
19 mg/kg	–8.83%
36 mg/kg	–14.43%*
69 mg/kg	–15.56%*
130 mg/kg	–24.07%*
248 mg/kg	n.d.**
470 mg/kg	n.d.

*Significantly different from controls.
**n.d. = not determined.

There was a statistically significant in weight loss in all treatments ≥ 36 mg/kg. Therefore, the NOEC for effects on biomass was 19 mg/kg.

Conclusions:

The 14-day LC_{50} of CL 912554 to the earthworm was 198 mg/kg of soil. The NOEC was 19 mg/kg of soil.

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	March 2009
Materials and Methods	The Applicant's version is considered to be acceptable
Results and discussion	The Applicant's version is considered to be acceptable
Conclusion	The Applicant's version is considered to be acceptable
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A7.5.1.3 Acute toxicity to plants

Annex Point IIIA 13.3.4

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible** [ ] **Scientifically unjustified** [X]	
Limited exposure [ ]	**Other justification** [ ]	
Detailed justification:	Alphacypermethrin is an insecticide. As such, plants are not the target organisms of Alphacypermethrin. Instead, the elementary chemical structure of pyrethroids like alphacypermethrin is derived from pyrethrin, a natural insecticide of plant origin. Thus, it is considered reasonable to assume that also Alphacypermethrin does not exhibit significant toxicity to plants. The assumption of low toxicity to plants is additionally supported by the finding that no inhibitory effects to algae were observed (see A7.4.1.3/01), indicating that plants in general may indeed be considered as unsusceptible to Alphacypermethrin. It may therefore be safely assumed that among the organisms from the trophic levels covered by standard terrestrial toxicity tests (decomposers, consumers and primary producers) plants are the least sensitive ones towards Alphacypermethrin. Confirmation of this reasonable assumption by an experimental study is not considered necessary.	
Undertaking of intended data submission [ ]		

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)	
Date	March 2009	
Evaluation of applicant's justification	Applicant's justification are considered to be acceptable	
Conclusion	Acceptable	
Remarks		
	COMMENTS FROM ...	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section A7.5.2.1 **Annex Point IIIA 13.3.2**	**Reproduction study with other soil non-target macro-organisms**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure** [X]	**Technically not feasible** [ ] **Scientifically unjustified** [ ] **Other justification** [ ]	
Detailed justification:	According to chapter 3 of the TNsG on additional data requirements, a test on reproductive effects with soil non-target macro-organisms is only required if the risk assessment for the terrestrial compartment, based on the equilibrium partitioning method indicates a concern for the terrestrial compartment or there is long term exposure. This is not the case with alphacypermethrin for the following reasons: (i) The testing for effects on reproductive effects with soil non-target macro-organisms is not considered to be required for lack of exposure, the justification being as follows: The recommended uses of alphacypermethrin as a domestic insecticide will involve only indoor use and are therefore not expected to include direct discharge to soil. Accordingly, any exposure of soil is only expected to result from application of sewage sludge to agricultural areas which was, however, estimated to be very low in the risk assessment. A significant risk was not identified. Therefore, any quantitatively relevant or long-term exposure of soil non-target macro-organisms is not conceivable. (ii) It is further stated that for some product types, these tests will be required with the core data set. However, for product type 18, (cf. Chapter 2.5), the conduct of these tests is explicitly not required. Thus, the conduct of an earthworm (or other soil organisms) reproduction study is not considered to be necessary.	
Undertaking of intended data submission [ ]		

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)	
Date	March 2009	
Evaluation of applicant's justification	BE CA agree with the Applicant's justifications	
Conclusion	Accepted	
Remarks		
	COMMENTS FROM ...	
Date		
Evaluation of applicant's justification		
Conclusion		

Remarks	

Section A7.5.2.2 Long-term test with terrestrial plants

Annex Point IIIA 13.3.4

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible** [ ] **Scientifically unjustified** [ ]	
Limited exposure [X]	**Other justification** [ ]	
Detailed justification:	According to chapter 3 of the TNsG on additional data requirements, a test for long-term effects on terrestrial plants is only required if the risk assessment for the terrestrial compartment, based on the equilibrium partitioning method indicates a concern for the pedosphere, if there is direct or long term exposure, and if plants are considered as relevant for performing further studies. This is not the case for the following reasons: (i) The testing for long-term effects on terrestrial plants is not considered to be required for lack of exposure. The recommended uses of Alphacypermethrin as a domestic insecticide will not involve direct discharge to soil. Accordingly, any exposure of soil is only expected to result from application of sewage sludge to agricultural areas which was, however, estimated to be very low in the risk assessment. A significant risk was not identified. Therefore, any quantitatively relevant or long-term exposure of plants is not conceivable. (ii) It is further stated that for some product types, these tests will be required with the core data set. However, for product type 18, (cf. Chapter 2.5), the conduct of these tests is explicitly not required. For these reasons, the conduct of a long-term plant toxicity study is not considered to be necessary.	
Undertaking of intended data submission [ ]		

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)	
Date	March 2009	
Evaluation of applicant's justification	Applicant's justification are considered to be acceptable	
Conclusion	Acceptable	
Remarks		
	COMMENTS FROM ...	
Date		
Evaluation of applicant's justification		
Conclusion		

Remarks	

Section A7.5.3.1.1 Acute oral toxicity to birds

Annex Point IIIA 13.1.1

Official use only

1 REFERENCE

1.1	**Reference**	**A7.5.3.1.1/01:** [redacted] (2000) Avian acute oral toxicity test with Alphacypermethrin (AC 900049) technical in Northern bobwhites (*Colinus virginianus*). [redacted] Report no. 105-046-03, September 01, 2000 (unpublished). (BASF RDI No.: AL-505-002)
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF
1.2.2	Companies with letter of access	No
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes SETAC (1995) US-EPA OPPTS 850.2100 (Avian Acute Oral Toxicity Test)
2.2	**GLP**	Yes (self-certified laboratory)
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	Alphacypermethrin (AC 900049) Technical, as given in Section A2.
3.1.1	Lot/Batch number	AC 12395-18
3.1.2	Specification	As given in Section A2.
3.1.3	Purity	96.1%
3.1.4	Further relevant properties	The physical-chemical properties of the test substance, as given in Section A3, are not considered to affect the test performance.
3.1.5	Method of analysis of test concentration	No analysis of test concentration was performed. The amount of test substance administered in gelatine capsules for individual bird was calculated gravimetrically.
3.2	**Administration of the test substance**	Via gelatine capsule, to facilitate uptake and digestion.

Section A7.5.3.1.1 Acute oral toxicity to birds

Annex Point IIIA 13.1.1

3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	Not applicable	
3.4	**Testing procedure**		
3.4.1	Test organisms	Northern bobwhites, as described in Table A7.5.3.1.1-1	
3.4.2	Test system	See Table A7.5.3.1.1- 2.	
3.4.3	Diet	Diet is described in Table A7.5.3.1.1- 2; due to the method of administration, further analysis data are not necessary.	
3.4.4	Test conditions	Test conditions are provided in Table A7.5.3.1.1- 3 and Table A7.5.3.1.1- 4.	
3.4.5	Duration of the test	14 d	
3.4.6	Test parameter	Mortality	X
3.4.7	Examination/ observation	See Table A7.5.3.1.1- 2	
3.4.8	Statistics	Body weight and feed consumption: one-way ANOVA	
		4 RESULTS	
4.1	**Range finding test**	Performed	
4.1.1	Concentration	400, 600, 900, 1350 and 2025 mg/kg bw	
4.1.2	Number/ percentage of animals showing adverse effects	2025 mg/kg bw: two males and two females (100%, respectively), as specified under 4.1.3 below.	
4.1.3	Nature of adverse effects	The following effects were observed: 400 mg/kg: None 600 mg/kg: None 900 mg/kg: None 1350 mg/kg: None 2025 mg/kg: Slight signs of lethargy on one single observation date No other signs of toxicity or mortalities were noted.	
4.2	**Results test substance**		
4.2.1	Applied concentrations	400, 600, 900, 1350 and 2025 mg/kg bw	

Section A7.5.3.1.1 Acute oral toxicity to birds

Annex Point IIIA 13.1.1

4.2.2	Effect data (mortality)	No test substance related mortality, moribundity, or signs of toxicity were noted in the control group or in any of the treatment groups throughout the 14-day test period. The median lethal dose (LD_{50}) of Alphacypermethrin for 23-week-old Northern bobwhite quails was determined to be greater than 2025 mg/kg bw.	
4.2.3	Body weight	Average body weights at each observation point are presented in Table A7.5.3.1.1- 5. Results of ANOVA indicated that there were no dosage related differences in body weight at either observation point.	
4.2.4	Feed consumption	Mean feed consumption at each observation point is presented in Table A7.5.3.1.1- 6. Results of ANOVA indicated that there were no dosage related differences in feed consumption during either observation period.	X
4.2.5	Concentration-response curve	Not applicable.	
4.2.6	Other effects	No gross pathological findings were noted in any of the control or test birds that were subjected to gross pathological examinations at the sacrifice.	
4.3	**Results of controls**		
4.3.1	Number/ percentage of animals showing adverse effects	None of the control animals showed any adverse effects.	
4.3.2	Nature of adverse effects	Not appropriate (see 4.3.1).	
4.4	**Test with reference substance**	Not performed	
4.4.1	Concentrations		
4.4.2	Results		
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Acute oral toxicity of Alphacypermethrin to Northern bobwhite quail was tested according to SETAC (1995) and US-EPA OPPTS 850.2100.	

Section A7.5.3.1.1 Acute oral toxicity to birds

Annex Point IIIA 13.1.1

5.2	**Results and discussion**	No test substance related mortality, moribundity, or signs of intoxication were observed during the definitive test. No significant differences between control and treatment groups in body weight or feed consumption were found. Gross pathological examinations performed on all birds from each group showed no evidence of toxicological effects. Hence, the lowest lethal dose (LLD) and the median lethal dose (LD_{50}) were determined to be greater than 2025 mg/kg bw, the highest tested dose, and the NOEL was determined at 2025 mg/kg bw. Under U.S. EPA classification for inter-chemical comparison for toxic substances, Alphacypermethrin is considered to be "practically non-toxic" to Northern bobwhites quails.
5.2.1	LD_{50}	LD_{50} > 2025 mg/kg
5.3	**Conclusion**	
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	March 2009
Materials and methods	The Applicant's version is considered to be acceptable with the following amendments: 3.4.6 Mortality, moribundity, signs of intoxication 4.2.4 Table A7.5.3.1.1-6
Results and discussion	
Conclusion	The Applicant's version is considered to be acceptable
Reliability	1
Acceptability	Acceptable
Remarks	Birds were medicated before the test.
	COMMENTS FROM ...
Date	
Materials and methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.5.3.1.1- 1: Test organisms.

Criteria	Details
Species/strain	*Colinus virginianus* (Northern Bobwhite)
Source	Stevenson Game Bird Farm, P.O. Box 426, Riverside, TX 77367, USA
Age	16 weeks upon arrival
Sex	47 males, 51 females
Mean body weight at day 0	193.99–196.97 g
Breeding population	Not reported
Amount of food	*Ad libitum*
Age at time of dosing	23 weeks
Health condition/medication	All birds were healthy; Birds were not medicated

Table A7.5.3.1.1- 2: Test system.

Criteria	Details
Test location	Indoor in holding pens
Holding pens	Plastic coated steel wire pens 51 × 25 × 25 cm (l × w × h)
Number of animals	60
Number of animals per pen (cm²/bird)	2 individuals per pen (1 m, 1 f) 637.5 cm²/individual
Number of animals per dose	10 (5 m, 5 f)
Pre-treatment/ acclimation	Acclimation period 19 d Environmental conditions as in the test (see Table A7.5.3.1.1- 3) Feed: Purina® Custom Game Bird Breeder Layena® 28% containing VIP BMD (prophylactic antibacterial) at a rate of 1 lb/100 lbs of food Feed and water available ad libitum
Diet during test	Purina® Custom Game Bird Breeder Layena® 28%; Purina Mills, Inc., 800 Chouteau Avenue, St. Louis, MO 63163 No analysis results reported.
Dosage levels of test substance	Single oral dose administered by gelatine capsule: 0, 400, 600, 900, 1350 and 2025 mg/kg bw.
Replicate/dosage level	Not appropriate
Feed dosing method	Administered of gelatine capsule
Dosing volume per application	Not appropriate
Frequency, duration and method of animal monitoring after dosing	Observation for clinical symptoms: twice daily
Time and intervals of body weight determination	At days 0, 1, 3, 7 and 14
Time and intervals of feed consumption values	At days 3, 7 and 14
Gross pathology	At termination

Table A7.5.3.1.1- 3: Test conditions.

Criteria	Details
Test temperature	Temperatures are listed in a separate table (Table A7.5.3.1.1- 4)
Shielding of the animals	Not stated
Ventilation	Not stated
Relative humidity	Humidity data are listed in a separate table (Table A7.5.3.1.1- 4)
Photoperiod and lighting	8:16 h (L:D) Full spectrum fluorescent lights

Table A7.5.3.1.1- 5: Temperature and humidity data recorded during the test.

Date	Temperature (°C)		Relative humidity (%)
	Min.	Max.	
5/24/00	20	22	72
5/25/00	20	25	48
5/26/00	19	24	62
5/27/00	18	22	61
5/28/00	19	21	66
5/29/00	18	21	70
5/30/00	18	21	71
5/31/00	19	22	67
6/01/00	19	22	72
6/02/00	19	26	71
6/03/00	18	21	71
6/04/00	18	22	71
6/05/00	19	21	62
6/06/00	19	21	62
Mean	**19**	**22**	**66**

Table A7.5.3.1.1- 6: Mean body weights of Northern Bobwhite during the acute oral toxicity definitive test with Alphacypermethrin, including the control group.

Dose level [mg/kg]	Mean body weight [g] at day no.			
	0	3	7	14
0	196.97	203.54	201.62	205.13
400	194.11	197.85	197.79	201.00
600	194.03	196.76	195.84	199.58
900	195.03	199.94	198.85	201.33
1350	195.07	198.74	198.40	200.63
2025	193.99	195.45	198.59	202.74

Table A7.5.3.1.1- 7: Mean daily feed consumption (g × individual^{-1} × d^{-1}) of Northern Bobwhite during the acute oral toxicity definitive test with Alphacypermethrin, including the control group; data are presented as pooled estimates for periods as given in the table.

Dose level [mg/kg]	**Mean feed consumption**		
	Days 0–3	**Days 3–7**	**Days 7–14**
0	14.92	13.98	14.96
400	14.04	13.80	14.36
600	13.68	14.46	14.92
900	13.82	13.86	14.32
13.30/ BE CA correction: 1350	14.52/ BE CA correction: 13.30	14.60/ BE CA correction: 14.52	69.0/BE CA correction: 14.60
2025	14.26	16.62	15.32

Section A7.5.3.1.2 Short-term toxicity on birds

Annex Point IIIA 13.1.2

Official use only

1 REFERENCE

1.1	**Reference**	**A7.5.3.1.2/01:** [redacted] (2001) Alphacypermethrin (BAS 310 I) dietary toxicity (LC50) to the Northern Bobwhite (*Colinus virginianus*). [redacted], Report no. CYD/632, September 24, 2001 (unpublished). (BASF RDI No.: AL-534-003)
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF
1.2.2	Companies with letter of access	No
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes OECD 205 US-EPA OPPTS 850.2200
2.2	**GLP**	Yes
2.3	**Deviations**	Yes Acclimatisation period of 4 days instead of 7 days.

3 MATERIALS AND METHODS

3.1	**Test material**	Alphacypermethrin, as given in Section A2.
3.1.1	Lot/Batch number	AC12395-18
3.1.2	Specification	As given in Section A2.
3.1.3	Purity	96.1% w/w
3.1.4	Further relevant properties	The physical-chemical properties of the test substance, as given in Section A3, are not considered to have affected the test performance.

Section A7.5.3.1.2 Short-term toxicity on birds

Annex Point IIIA 13.1.2

3.1.5	Method of analysis	HPLC A detailed analytical report including description of the method is appended to the original study. The mean concentrations of Alphacypermethrin in test diet formulations prepared for feeding during the study were within ± 2% of nominal concentrations, confirming the accuracy of formulation. The homogeneity of Alphacypermethrin in avian diet formulations was confirmed at nominal concentrations of 156 ppm and 5000 ppm. The stability was confirmed during ambient temperature storage for 8 days, representing the maximum time from preparation to completion of use.	
3.2	**Administration of the test substance**	Dietary administration No vehicle was necessary for incorporation; a pre-mix of suitable strength was prepared by mixing Alphacypermethrin with untreated basal diet. The required concentration was prepared by dilution of the prepared pre-mix.	
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	Not applicable	
3.4	**Testing procedure**		
3.4.1	Test organisms	Northern Bobwhite quail, as described in Table A7.5.3.1.2- 1.	
3.4.2	Test system	See Table A7.5.3.1.2- 2.	
3.4.3	Diet	Basal diet is specified in Table A7.5.3.1.2- 2.	
3.4.4	Test conditions	Test conditions are described in Table A7.5.3.1.2- 4.	
3.4.5	Duration of the test	5 days of exposure, 3 days post-exposure	
3.4.6	Test parameter	Mortality	X
3.4.7	Examination/ observation	Observation of mortalities, behaviour and clinical signs daily. Determination of body weight at days –4, 0, 5 and 8. Group mean food consumption recorded over days –4 to 1, 1 to 5 and 6 to 8. Macroscopic post-mortem examination on all birds from the highest dose group and all birds from one control group (group 1); tissues examined included digestive tract, liver, kidneys, heart, spleen, muscle and subcutaneous fat.	
3.4.8	Statistics	LC_{50} value and 95% confidence interval by probit analysis.	

Section A7.5.3.1.2 **Short-term toxicity on birds**
Annex Point IIIA 13.1.2

4 RESULTS

4.1	**Range finding test**	Not performed
4.1.1	Concentration/dose	
4.1.2	Number/ percentage of animals showing adverse effects	
4.1.3	Nature of adverse effects	
4.2	**Results test substance**	
4.2.1	Applied concentrations	156, 313, 625, 1250, 2500 and 5000 ppm of Alphacypermethrin.
4.2.2	Effect data (mortality)	Cumulative mortalities over the 8-d test period are provided in Table A7.5.3.1.2- 5. As there were no mortalities at the maximum treatment level, the LC_{50} is larger than 5000 ppm.
4.2.3	Body weight	During the treatment period, group mean body weights showed a smaller increase in Group 8 (5000 ppm Alphacypermethrin) compared to the controls. Individual body weights were analysed statistically (Williams' test); on day 5 the mean body weight of group 8 (5000 ppm) was significantly lower than in the pooled control group. There were no statistically significant differences between test and control groups at day 8. Mean body weights and body weight increases for each observation period are presented in Table A7.5.3.1.2- 6.
4.2.4	Feed consumption	Food consumption was variable among groups and there was no evidence to suggest that consumption was affected by treatment. Mean food consumption per bird per day for each observation period is presented in Table A7.5.3.1.2- 7.
4.2.5	Concentration-response curve	Not applicable.

Section A7.5.3.1.2 Short-term toxicity on birds

Annex Point IIIA 13.1.2

4.2.6	Other effects	Clinical signs: All birds treated with 5000 ppm Alphacypermethrin and 5 birds treated with 2500 ppm showed unsteadiness and six of the ten high dosed individuals were subdued. These clinical signs of toxicity were observed for up to four days from the beginning of treatment. Treatment at 1250 ppm caused one bird to develop unsteadiness during day1 only. Two birds in Group 4 (313 ppm Alphacypermethrin) became subdued, and weak or unsteady on day 4 and day 6, respectively. Both birds were considered unlikely to recover and were therefore sacrificed. Another bird in the same group died unexpectedly on day 4. All three birds showed symptoms of pecking by other birds and therefore the clinical signs or death were considered unrelated to treatment. Necropsy: *Post mortem* examination of the two sacrificed birds and the bird of group 4 (313 ppm) that died unexpectedly showed cuts around the beak but no other abnormalities. At termination, no other abnormalities were detected in any of the bird examined (0 ppm, 5000 ppm Alphacypermethrin).
4.3	**Results of controls**	
4.3.1	Number/ percentage of animals showing adverse effects	None of the control animals showed any adverse effects.
4.3.2	Nature of adverse effects	Not appropriate (see 4.3.1).
4.4	**Test with reference substance**	Not performed
4.4.1	Concentrations	
4.4.2	Results	

Section A7.5.3.1.2 Short-term toxicity on birds

Annex Point IIIA 13.1.2

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The short-term dietary toxicity of Alphacypermethrin to Northern Bobwhite quail was determined according to the guidelines OECD 205 and US-EPA OPPTS 850.2200. The study deviated from the guidelines with respect to the acclimatisation period (4 days instead of 7 days). However, this deviation is not considered to have affected the results.
5.2	**Results and discussion**	Clinical signs of toxicity, including subdued behaviour and unsteadiness were noted in groups treated with 1250 ppm Alphacypermethrin and above. There were no treatment-related mortalities. Body weight increase over the treatment period was depressed in the high dosed group. The NOEC was established at 5000 ppm, with respect to mortality as well as clinical and pathological findings. Under US EPA classification Alphacypermethrin is considered to be "practically non-toxic" to young Northern bobwhite quail.
5.2.1	LC_0	LC_0 = 5000 ppm
5.2.2	LC_{50}	LC_{50} > 5000 ppm
5.2.3	LC_{100}	Not applicable
5.3	**Conclusion**	The validity criteria are considered to be fulfilled (Table A7.5.3.1.2- 8). Other circumstances that may have negatively affected the integrity and quality of the results are not reported. Thus, the study was considered to be valid without restrictions.
5.3.1	Reliability	1
5.3.2	Deficiencies	No