		^{14}C was analysed by LSC in one beaker from the 0.1, 0.22, 0.046 and 1.0 µg/l test concentrations, at 0, 7 and 28 days. Sediment and water aliquots were analysed and the flasks were also rinsed with acetone to release radioactivity from the glass walls. In addition, radioactivity in the water compartment was measured daily at these test concentrations.	
5.2	**Results and discussion**	The distribution of radioactivity in whole beakers is given in Table A7.4.3.5.1-8. In vessels where radioactivity was measured daily a bi-phasic disappearance pattern was observed, with an initial rapid decline, followed by a more slow disappearance.	
		In the control, solvent control, and the three lowest test concentrations the **first adults emerged** on day 14. At 0.1 µg/l first emergence occurred at day 15, whereas at higher test concentrations no emergence was observed. Overall mean **emergence rate** was 86% in the control, 79% in the solvent control, and 78%, 63%, 72%, 17% at test concentration of 0.01, 0.022, 0.046 and 0.1 µg/l, respectively (0% at the three highest concentrations). Thus, there was not a clear dose-response relationship. NOEC was set to 0.01 µg/l since this was the only test concentration without significant deviation from control *and* solvent control. LOEC was set to 0.022 µg/l. Sex ratio of emerged midges did not differ significantly from an equal distribution ($p < 0.05$).	
		The **development rate**[1] was not significantly different between the control, solvent control and test concentrations up to 0.046 µg/l. At 0.1 µg/l the development rate increased significantly. NOEC was therefore set to 0.046 µg/l.	
		At the highest test concentration, food consumption was reduced and no obvious larval activity was observed from day 10. No other symptoms of intoxication were observed.	
		The study was well performed and reported. Because of the rapid partitioning of deltamethrin to the sediment phase, as shown in the study report, it can be concluded that the test organisms were exposed to treated sediment for the major part of this chronic study. Therefore this study, although performed as spiked-water study, is deemed suitable for the assessment of the toxicity to sediment dwelling organisms of deltamethrin when used as a biocide.	X
5.3	**Conclusion**		
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

1 The reciprocal of the development time (unit 1/day) represents the daily development of larvae, as the mean time span between day 0 and the emergence of the experimental cohort of midges.

Table A7.4.3.5.1-1 Preparation of TS Solution for Poorly Stable or Volatile Test Substances

Criteria	Details
Dispersion	No
Vehicle	Acetone
Concentration of vehicle	0.48 – 48.52 µl/l
Vehicle control performed	Yes
Other procedures	No

Table A7.4.3.5.1-2 Dilution Water

Criteria	Details
Source	Modified artificial mineral water
Alkalinity (mg/l)	Not reported
Hardness (mg/l)	1.66
pH	6.4 – 7.7
Oxygen content (mg/l)	6.6 – 8.8
Conductance	507 – 546 µS/cm
Holding water different from dilution water	No

Table A7.4.3.5.1-3 Artificial Sediment Composition

10.0%	(1.634 kg) dried and sifted sphagnum peat (residual moisture content of 8.92% taken into calculation)
20.0%	(3.0 kg) kaolin clay (type 1777, Ziegler & Co., Wunsiedel, FRG; kaolinite content 87.5%)
70.0%	(10.5 kg) industrial sand (Quarzsand type 155-0, Gebr. Willersinn GmbH & Co. KG, Ludwigshafen, FRG) Particle analysis: 0.125 mm 40% 0.063 – 0.09 mm 13 – 15%

The values in parentheses refer to the exact amounts as weighed for preparation of the basic substrate. These constituents were blended by a rapid mixer. 90 g Calcium carbonate ($CaCO_3$, pulverised, chemically pure, Riedel-de Haën, Seelze, FRG) were used to adjust the pH of this medium to 5.9.

A small sample was taken from this mixture, and the moisture content was determined by means of a scale with a drying device. The residual moisture content of this substrate was 0.99%. After addition of 5.10 litres deionised water and blending by hand the actual moisture content was determined at 26.35 percent.

Table A7.4.3.5.1-4 Test Conditions

Criteria	Details
Test temperature	19.6 – 20.3°C
Dissolved oxygen (mg/l)	6.6 – 8.8
pH	6.4 – 7.7
Adjustment of pH	No
Aeration of dilution water	Yes
Quality/Intensity of irradiation	Not reported
Photoperiod	16 h light

Table A7.4.3.5.1-5 Test Organisms

Criteria	Details
Species/strain	*Chironomus riparius*
Source	University of Sheffield, UK
Age	First larval stage (L1)
Breeding method	The midges were kept in a cage (60 cm x 60 cm x 50 cm (height)), with a gauze on the inside of the cage. Six crystallising dishes (14 cm diameter, 7 cm height) were set on the bottom of the cage. The crystallising dishes were filled with a 2 – 3 cm layer of quartz sand and a 2 – 3 cm high layer of artificial mineral medium, which was gently aerated. To start the culture in a crystallising dish, 2 – 4 egg masses were placed into the prepared basin. The hatched larvae were fed with an aqueous suspension of fish food. After 2 – 3 weeks the imagines emerged. After mating, female imagines deposited egg masses on the water surface where these were taken to start a new culture or to perform a test. The culture conditions were 20 ± 2°C and a 16:8 hours light-dark-cycle with a 30 minutes dusk and dawn period. The L1 larvae used in the study were obtained by transferring some fresh egg masses in small crystallising dishes with culture medium. After 2 to 3 days the L1-larvae hatched and were transferred to the test beakers.
Kind of food	Tetra-Min
Amount of food	1 mg/larvae/day
Feeding frequency	3 times per week
Pretreatment	No
Feeding of animals during test	Yes (stopped on day 15, as no food was consumed anymore)

Table A7.4.3.5.1-6 Test System

Criteria	Details
Test type	Static water/sediment
Renewal of test solution	No
Volume of test vessels	3000 ml filled with 2.7 l water
Volume/animal	Not reported
Number of animals/vessel	25
Number of vessels/ concentration	4
Test performed in closed vessels due to significant volatility of TS	Covered with plastic caps

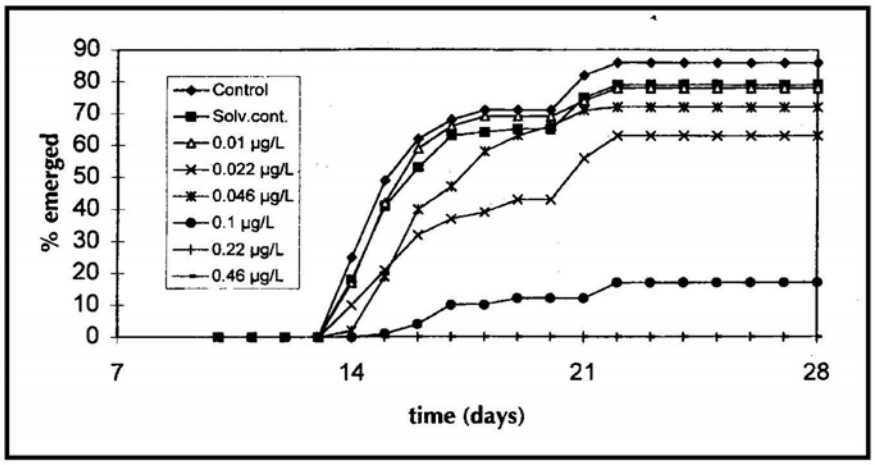
Table A7.4.3.5.1-7 Effects Data: cumulative emergence of *Chironomus riparius*, per treatment

	Treatment	Control	Solvent Control	0.01 µg/l	0.022 µg/l	0.046 µg/l	0.1 µg/l	0.22 µg/l	0.46 µg/l	1.0 µg/l
Males	Mean	10.75	10.25	11.50	9.25	9.75	1.75	0.00	0.00	0.00
	Std. dev	3.40	4.03	2.08	2.63	1.26	1.71	0.00	0.00	0.00
	Min	6	6	9	7	8	0	0	0	0
	max	14	15	14	13	11	4	0	0	0
Females	Mean	10.75	9.50	8.00	6.50	8.25	2.50	0.00	0.00	0.00
	Std dev	2.75	2.89	2.16	1.91	2.06	3.79	0.00	0.00	0.00
	Min	8	6	6	5	6	0	0	0	0
	Max	14	13	11	9	10	8	0	0	0
Both sexes	Mean	21.50	19.75	19.50	15.75	18.00	4.25	0.00	0.00	0.00
	Std dev	1.29	2.50	1.00	3.10	2.83	5.44	0.00	0.00	0.00
	Min	20	16	18	13	14	0	0	0	0
	Max	23	21	20	20	20	12	0	0	0
Emergence rate (%)		86.00	79.00	78.00	63.00	72.00	17.00	0.00	0.00	0.00
Development rate (day^{-1})		0.0651	0.0639	0.0654	0.0597	0.0618	0.0518	-	-	-

Table A7.4.3.5.1-8 Distribution of Radioactivity, as % of Applied

Day	Nominal conc. (µg/l)	Water	Sediment	Glass walls	Total
0	0.1	92	15	5.9	113
7	0.1	35	58	6.4	100
28	0.1	25	72	2.8	99
0	0.22	81	7.7	3.3	92
7	0.22	35	59	3.7	98
28	0.22	27	57	3.1	86
0	0.46	66	6.6	4.2	77
7	0.46	38	55	7.2	100
28	0.46	12	56	2.5	70
0	1.0	67	5.3	3.4	76
7	1.0	32	47	12.3	91
28	1.0	19	55	3.8	78

Figure A7.4.3.5.1-1 Cumulative Emergence of Midges vs Time



EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Not relevant
Materials and methods	Applicant's version is adopted with the following comments: **3.3** RMS considers that some information on the testing procedure was missing, and this is presented below: **Test parameter:** Emergence **Examination:** three times per week for visual assessment of behavioural changes and daily during the period of expected emergence. **Monitoring of TS concentration:** performed daily in water, but only in the 4 highest test concentrations; i.e. 0.1 – 1.0 µg/l. **Statistics:** A comparison of emergence and development rate was performed with a beta-version of EASY ASSAY Toxicity to Chironomids, Version 1.0 to determine the NOEC/LOEC using ANOVA methods (DUNCAN's new multiple range test).
Results	Applicant's version is adopted with the following comments: **4.1.2** The water concentrations of deltamethrin were only analysed in the test concentrations with the highest test concentrations; 0.1 – 1.0 µg/l. There were no analyses of the concentration in the sediment, but after 0, 7, and 28 days, the distribution of the total radioactivity in the compartments water, sediment, and glass walls was determined. It is, however, difficult to convert these results to concentrations of deltamethrin in µg/kg sediment. **4.1.5** There is a tendency of fewer females emerging in the two lowest test concentrations; 0.01 and 0.022 µg/l. In both these test concentrations, 41% of the emerged midges were females, compared to 48% in the solvent control. However, this is only an observation by the RMS and no statistical analysis was performed.
Conclusion	Applicant's version is adopted with the following comments: **5.1** The volume of water in the test vessel was 2.7 l and the amount of sediment was 250 g. The beakers were covered and gently aerated through glass Pasteur pipettes. **5.2** The NOEC based on emergence rate was related to nominal initial water concentration, but the measurements showed that the concentrations decreased quickly, with a mean of about 35% of the initial concentration in the lowest test concentration that was measured; 0.1 µg/l. If it is assumed that the same relationship between initial and actual concentrations is valid also for the NOEC, the NOEC based on measured concentration would be 0.35 * 0.01 = 0.0035 µg/l. It is claimed in the study summary and the Position paper from Grau, that deltamethrin rapidly partitions to the sediment and that the larvae therefore are exposed to deltamethrin through the sediment for the major part of the study. On day 7, 47-59% of the total radioactivity was found in the sediment and by the end of the study (day 28) 55-72% was bound to the sediment. It is therefore remarkable that no attempt was made to determine the concentration of deltamethrin in the upper sediment layer where the larvae dwell, so that the effects could also be related to the sediment concentrations. It is assumed that exposure from the water phase is more rapid and probably determines the toxicity, but the chronic exposure from sediment may also be important.

Section 7 Annex Point IIIA XIII.3.4	**Ecotoxicological Profile Including Environmental Fate and Behaviour** A7.4.3.5.1 Effects on sediment dwelling organisms

Reliability	2
Acceptability	The study is acceptable, but the lack of measurements of the sediment concentrations is considered a deficiency. The NOEC for emergence of Chironomids was 10 ng/l based on nominal water concentrations and 3.5 ng/l based on estimated actual water concentrations.
Remarks	No further remarks

7.4.3.5.2 Aquatic plant toxicity

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [✓]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	Deltamethrin is not an herbicide. Furthermore deltamethrin is used/registered for years for crop protection and is not phytotoxic. Therefore, this study should not be required.	
Undertaking of intended data submission [ ]		

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Not relevant
Evaluation of applicant's justification	The RMS agrees with the applicant's justification.
Conclusion	Applicant's justification is acceptable.
Remarks	No further remarks

7.4.3.5.3 Higher-tier studies

			Official use only
		1. REFERENCE	
1.1	**Reference**	Heimbach, F. *et al* (2005) Biological Effects and Fate of Deltamethrin EW 015 in Outdoor Mesocosm Ponds [redacted] Document MO-05-004459 7.4.3.5.3/01 24 February 2005 Unpublished	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Bayer CropScience AG	
1.2.2	Companies with letter of access	n.a.	
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**	OECD Guidance Document "Simulated Freshwater Lentic Field Tests (Outdoor Microcosms and Mesocosms)", July 2004 (Draft). Guidance Document on Testing Procedures for Pesticides in Freshwater Mesocosms (SETAC-Europe Workshop, Monks Wood, UK, July 1991). Community-Level Aquatic System Studies - Interpretation Criteria (2002) (Proceedings from the CLASSIC Workshop, SETAC 2002).	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**	Deltamethrin formulated as an oil/water emulsion (1.64% w/w deltamethrin)	
3.1.1	Lot/Batch number	AAIM00846	
3.1.2	Specification	As given in Section 2 formulated as an EW	
3.1.3	Purity		
3.1.4	Further relevant properties	-	
3.1.5	Radiolabelling	None	
3.1.6	Method of analysis	Water samples were analysed by HPLC – MS/MS. The method was validated with a LOQ of 0.005 µg/l. Sediments were also analysed by a validated HPLC – MS/MS with a LOQ of 0.1 µg/kg and a LOD of 0.03 µg/kg.	X
3.2	**Reference substance**	None	
3.2.1	Method of analysis for reference substance	Not applicable	

3.3	**Testing procedure**		
3.3.1	Water/sediment	See Tables A7.4.3.5.3-1 and A7.4.3.5.3-2.	
3.3.2	Test organisms	See Effects Data table (Table A7.4.3.5.3-4) for all species found in this study.	
3.3.3	Test system	See Table A7.4.3.5.3-3.	
3.3.4	Duration of the test	105 days	
		4. RESULTS	
4.1	**Experimental data**		
4.1.1	Initial concentrations of test substance	4.8, 10.5, 23, 51, 111 ng as/l.	
4.1.2	Actual concentrations of test substance	An average of 94.1% of the nominal concentration.	X
4.1.3	Effect data	See Table A7.4.3.5.3-4.	
4.1.5	Other effects	-	
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The aim of the study was to determine the ecological effects of a repeated simulated drift contamination with deltamethrin EW 015 on different trophic levels (emergence, zooplankton, macroinvertebrates and phytoplankton) in outdoor mesocosms as an aquatic model ecosystem for lentic aquatic freshwater systems with different trophic levels. The fate of the compound in the individual compartments (water body and sediment) was monitored simultaneously.	
		Twelve test tanks (6 m^3 water, 1 m water depth) which were used in this study are especially designed systems which allow the establishment of almost identical conditions at the start of a study. The bottoms of the artificial tanks were covered with natural sediment (approximately 15 cm in height) seven months prior to the study start.	
		The water was composed of local ground water and water from a nearby uncontaminated pond which was inoculated several times with zooplankton from a natural pond nearby. Natural communities developed spontaneously from seeds and roots of aquatic plants as well as from air borne and naturally transferred stages of planctonic, benthic and filamentous algae organisms during the months before study start. Some weeks before the first application 300 *Asellus aquaticus* were artificially inserted in each pond to establish a stable population of this isopoda. Since the populations could not be maintained in a few ponds during the study, new *Asellus aquaticus* were added to these ponds. In general, the artificial ponds are representative of a small stagnant water body.	X X
		The test substance deltamethrin EW 015 (Batch No.AA1M00846, AZ No.10459) was applied during the early growing season in May 2004 three times at an interval of 7 days onto the water surface of nine test ponds. The treatment levels were 4.8, 10.5, 23, 51 and 111 ng as/l per application (two replicates of 4.8 to 51 ng as/l, one replicate for 111 ng as/l). Three further tanks were used as untreated controls.	X

		The mesocosms were investigated for a period of 14 days before and 105 days after the first treatment (= 91 days after the last treatment). Several times during the study period water and sediment samples were taken and analyzed to investigate the concentration of the test substance in water and sediment. Further parameters studied were the taxonomic composition of zooplankton, phytoplankton, macroinvertebrates and emergence of insects at different days before and after the applications. Since *Asellus aquaticus* was assumed to be one of the most sensitive species in this study, this species was studied intensively in situ on Artificial Substrate Samplers (ASS) and in small cages with leaves which function as traps for these organisms. In addition, bioassays were performed with this species to investigate the potential recovery of a population by immigration of organisms from adjacent water bodies. The physico-chemical water parameters and the content of chlorophyll-a of phytoplankton were also evaluated, as well as the coverage of the sediment with macrophytes and filamentous algae. One diurnal cycle of oxygen concentration, water temperature and pH was recorded during the study.	X X
5.2	**Results and discussion**	The analytical results of water samples taken four hours after each of the three applications show that an average of 94.1% of the nominal concentrations could be found in the mesocosm water confirming nominal concentrations very well. The a.s. disappeared after all applications quickly and steadily with an average half-life in the water column of 22.4 hours. At some sampling dates the percentage of adsorbed a.s. in the water was determined, the results revealed that about two thirds of the total applied amount was bioavailable (solubilised in water) in the pond water, whereas one third was adsorbed to particles as algae or particulate matter.	X
		In the sediment of the two lowest test levels (4.8 and 10.5 ng/l) the test substance could be found only once shortly after the first application (limit of detection = 0.03 µg/kg dry weight). The results of the higher test levels (23 to 111 ng/l) show a slight increase of sediment concentrations during about 7 weeks after application resulting up to 20% of total applied amount in the sediment, and a slow decrease during the later part of the study to less than 6% of total applied amount. The DT_{50} for whole system (water plus sediment) is 31.6 hours.	X
		Direct and indirect effects of the application of deltamethrin to the chemical and physical parameters of the pond water have not been observed at any test concentration. Also no effects on the coverage of the ponds and the biomass of macrophytes and filamentous algae were observed at any treatment level.	
		The biological data showed some minor and major effects on some groups of organisms, as indicated in Table A7.4.3.5.3-4. In this table, the effects were classified according to the following effect categories according to "Guidance Document on Aquatic Ecotoxicology" in the context of the Directive 91/414/EEC (SANCO 3268/2001 rev.4 (final) of 17 October 2002:	

	1	effect could not be demonstrated	- no (statistically significant) effects observed as result of the treatment, and - observed, differences between treatment and controls show no causal relationship	
	2	slight effect	- effects reported in terms of "slight" or "transient" and/or other similar descriptions, and - short-term and/or quantitatively restricted response of sensitive endpoints, and - effects only observed at individual samplings	
	3	pronounced short term effect	- clear response of sensitive endpoints, but total recovery within 8 weeks after the last application, and - effects reported as "temporary effects on less sensitive species/endpoints" and/or other similar descriptions, and - effects observed at some subsequent sampling instances	X
	4	pronounced effect in short-term study(not relevant in this study)	- clear effects (such as strong reductions in densities of sensitive species) observed, but the study is too short to demonstrate complete recovery within 8 weeks after the (last) application	
	5	pronounced long-term effect	- clear response of sensitive endpoints and recovery time of sensitive endpoints is longer than 8 weeks after the last application, and - effects reported as "long-term effects on many sensitive species/endpoints" and/or other similar descriptions, and - effects observed at various subsequent samplings.	

	At the end of the study, no **zooplankton** taxon showed significant differences in abundance compared to controls, demonstrating the recovery of the zooplankton after the third application within 7 weeks. *Chaoborus crystallinus* was identified as the most sensitive zooplankton taxon with consistent effects even at 4.8 ng as/l immediately after application until about two weeks after the last application when a full recovery of the *Chaoborus* population was observed. The crustaceans, especially the copepods and *Daphnia longispina*, proved to be the next most sensitive zooplankton group exhibiting an consistent NOEC of 4.8 ng as/l and 10.5 ng as/l, respectively. The rotifers were either suppressed (especially *Keratella quadrata*, consistent NOEC of 4.8 ng as/l) or promoted (e.g. *Polyarthra spec.*, consistent NOEC of 10.5 ng as/l) obviously by secondary effects. The PRC (Principal Response Curve) and to some degree Similarity and Shannon Diversity Indices reflected these effects on the zooplankton with a community NOEC of 4.8 ng as/l. However, all effected populations recovered shortly after the last application and reached control abundances within some weeks only at all treatment levels including the highest one. Seven weeks after the last application, no taxon showed significant differences in abundance compared to controls, demonstrating the full recovery of the zooplankton community. Due to the missing replication at 111 ng/l the results of this study yield a NOEAEC (no observed ecological adverse effect concentration) of 51 ng/l for the zooplankton.	X
	In **sediment**, significant impacts on the identified species in the ASS (Artificial Substrate Samplers) were only obtained for chironomid larvae at the highest test concentration, resulting in a NOEC of 51 ng as/l for the evenness and 23 ng as/l for the PRC, whereas all other community parameters did not indicate any effect up to the highest test concentration. Observed effects were even short-term only: no long-lasting effects could be detected. In the sediment samples, no effects even up to the highest treatment level could be detected (NOEC 111 ng/l).	
	Direct effects of the test item on the **emergence** of some insects were detected for five taxonomic groups: *Chironomus spec., Orthocladiinee, Psectrotanypus spec., Tanypodinae* (females) and *Chaoborus crystallinus*. Except of *Chaoborus* (same NOEC as in zooplankton), all other groups were affected only at the highest treatment level (*Psectrotanypus spec.* also at 51 ng/l), with a full recovery within eight weeks after last application for the latest for all species. Thus, the community indices yielded a community NOEC of 23 ng/l. Because of the fast and full recovery in emergence (which even included the full aquatic life cycle of the emerged insects) within the first weeks after application on the one hand and the missing replication of the highest treatment level of 111 ng/l on the other hand, the NOEAEC for emergence can also be set as 51 ng/l.	X

	In the mesocosms clear effects on ***Asellus aquaticus*** were demonstrated for the three highest test concentrations both in leaf cages and ASS. At 10.5 ng as/l *Asellus* was only short-term affected after the first application indicating only a decrease in mobility but no mortality. (Both sampling methods indicate a reduction in activity of individuals, which does not necessarily mean mortality). Thus, a consistent NOEC of 10.5 ng as/l can be derived from this study for *Asellus* in the mesocosms. In the three highest test concentrations the abundance of *Asellus* reached mostly the level of controls until study termination. After day 70 the proportion of juveniles in the higher treated ponds reached the level of controls. A full recovery to control level within 8 weeks after last application could not be demonstrated for 23, 51 and 111 ng as/l. However, the differences between control and treatment levels are small and population abundances clearly increased in these ponds, as demonstrated by the increasing number of juvenile organisms and the corresponding reproduction in situ. The bioassay findings confirm that water and food samples from the mesocosms taken at the latest one week after the applications did not have any negative effects on *Asellus aquaticus*. Overall, the NOEAEC for *Asellus* was 51 ng/l due to the missing replication at 111 ng/l.	X
	No direct toxic effects were observed on the **phytoplankton**. During the application period cell densities of some species, as e.g. the dominant *Chroomonas spec.*, were slightly lower at higher treatment levels for a short time than in the controls caused by indirect food web effects, probably by toxic effects of the test item treatments on the copepod populations, which enhanced the rotifer population density by decreased competition. The community NOEC for phytoplankton was 23 ng/l and the NOEAEC 51 ng/l because of the missing replication at the highest treatment level.	
	In conclusion, the fate of deltamethrin demonstrates a steady and fast decline of deltamethrin in the mesocosm water with a mean DT_{50} of 22.4 hours, and a mean DT_{50} of 31.6 hours for the whole test system (water plus sediment). In the sediment of the two lowest test concentrations (4.8 and 10.5 ng/l) the as was only detected once shortly after application. The results of the higher test concentrations (23 to 111 ng/l) show a slight increase of the amount of the test substance in the sediment for about the first seven weeks after application and a slow but constant decrease thereafter.	X X
	Chaoborus crystallinus was identified as the most sensitive taxon with consistent effects even at 4.8 ng as/l immediately after application until about a very few weeks after the last application when a full recovery had been observed even at the highest test level. At 10.5 ng/l also short-term effects for one Rotatoria species (*Keratella quadrata*) and Copopod Nauplii had been observed. *Asellus aquaticus* showed just a reduced activity at this test level for a very few days after application without any sign of mortality or affected reproduction. At 23 and 56 ng/l effects on one to three individual more species had been observed, but also these effects were short-term only with a full recovery within the first weeks after the last application.	X

		The abundance of *Asellus* was clearly reduced after application at this test levels but reached mostly the level of controls until study termination. The differences between control and treatment levels were small and population abundances clearly increased in these ponds during the study, as also demonstrated by the increasing number of juvenile organisms. The bioassay findings confirm that water and food samples from the mesocosms taken at the latest one week after the applications did not have any negative effects on *Asellus aquaticus*. At 111 ng/l the number of affected zooplankton and insect species was distinctly higher, and the effects on *Asellus aquaticus* even more developed as compared to lower treatment levels.	
		Based on these findings and because of the missing replication at the highest test level, 51 ng/l can be concluded as the overall NOEAEC (no observed ecological adverse effect concentration) of this study.	X
5.3	**Conclusion**		
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Table A7.4.3.5.3-1 Test Water

Criteria	Details
Source	Local ground water and uncontaminated pond water
Alkalinity (mg/l)	3 – 7
Hardness (mg/l)	4 – 8
PH	8.0 – 10.3
Oxygen content	6.5 – 26.1
Conductance	384 – 521 µS/cm

Table A7.4.3.5.3-2 Test Sediment

Criteria	Details
Organic carbon (%)	4.2
Nitrogen (%)	0.4
Phosphorus (mg/kg)	820
Cation Exchange Capacity (T-value*), meq/100g sediment	11.8
% Sand	1.5
% Silt	54.5
% Clay	14.0
Classification	Silt loam

* The T-value is the potential maximum quantity of cations which can be retained and released from the sediment.

Table A7.4.3.5.3-3 Test System

Criteria	Details
Test type	Mesocosms (cylindrical tanks of black polyethylene). Each tank was 2.75 m diameter, 1.55m depth, with a surface area of 5.94m^2. Each contained 5.94m^3 water. They were installed in the ground.
Number of treatments levels	5
Number of treatments	3
Method of application	Spraying onto water surface
Number of vessels/ concentration	2 for 4.8 – 51 ng as/l and one for 111 ng as/l

Table A7.4.3.5.3-4 Effects Data: effects classified according to "Guidance Document on Aquatic Ecotoxicology" in the context of the Directive 91/414/EEC (SANCO 3268/2001 rev.4 (final) of 17 October 2002: 1: effect could not be demonstrated – 2: slight effect – 3: pronounced short term effect – 4: pronounced effect in short-term study (not relevant in this study) – 5: pronounced long-term effect

	Test concentration (ng as/l)				
	4.8	**10.5**	**23**	**51**	**111**
Zooplankton					
Phyllopoda					
Daphnia longispina	1	1	3	3	3
Simocephalus vetulus	1	1	1	1	2
Chydorus sphaericus	1	1	1*	2*	2*
Acroperus harpae	1	1	1	1	2*
Eurycercus lamellatus	1	1	1	1	1
Graptoleberis testudinella	1	1	1	1	1
Ostracoda					
Ostracodes (not det.)	1	1	1	1	1
Copepoda					
Cyclopoid Copepods	1	1	2	3	3
Copepod Nauplii	1	2	3	3	3
Rotatoria					
Keratella quadrata	1	3	3	3	3
Lecane lunaris	1	1	1	1	2
Polyarthra spec.	1	1	+	+	+
Lepadella patella	1	1	1	+	+
Asplanchna spec.	1	1	1	+	+
Trichotria pocillum	1	1	1	1	+
Synchaeta spec.	1	1	1	1	+
Testudinella patella	1	1	1	1	1
Cephalodella spec.	1	1	1	1	1
Euchianis deflexa	1	1	1	1	1
Diptera					
Chaoborus crystauinus larvae	3	3	3	3	3
Taxa richness	1	1	1	1	1
Diversity (Shannon Index)	1	1	2	3	3
Evenness	1	1	1	2	2
Similarity (Steinhaus Index)	1	1	3	3	3
Similarity (Stander's Index)	1	1	3	3	3
Principal Response Curves (PRC)	1	2	3	3	3
Community-NOEC	X				
Lowest population-NOEC	< 4.8				
NOEAEC				x*)	

\+ Increase in numbers

* Statistically not significant

*) The NOEAEC was set to 51 ng/l due to the missing replication at 111 ng/l

Table A7.4.3.5.3-4 Effects Data (continued): effects classified according to "Guidance Document on Aquatic Ecotoxicology" in the context of the Directive 91/414/EEC (SANCO 3268/2001 rev.4 (final) of 17 October 2002: 1: effect could not be demonstrated – 2: slight effect – 3: pronounced short term effect – 4: pronounced effect in short-term study (not relevant in this study) – 5: pronounced long-term effect

	Test concentration (ng as/l)				
	4.8	**10.5**	**23**	**51**	**111**
Macroinvertebrates (benthic, ASS)					
Turbellaria					
Dugesias spec.	1	1	1	1	1
Oligochaeta					
Tubificidae	1	1	1	1	1
Stylaria lacustris	1	1	1	1	1
Hirudineae					
Helobdella stagnalis	1	1	1	1	1
Diptera					
Chaoborus crystallinus larvae	see zooplankton evaluation				
Sum of Chironomid larvae	1	1	1	1	2
Ephemeroptera					
Cloeon dipterum	1	1	1	1	1
Odonata					
Ischnura elegans	1	1	1	1	1
Pulmonata					
Gyraulus albus	1	1	1	1	1
Radix ovata	1	1	1	1	1
Taxa richness	1	1	1	1	1
Diversity (Shannon Index)	1	1	1	1	1
Evenness	1	1	1	1	2
Similarity (Steinhaus Index)	1	1	1	1	1
Similarity (Stander's Index)	1	1	1	1	1
Principal Response Curves (PRC)	1	1	1	2	2
Community-NOEC			x		
Lowest population-NOEC **				x	
NOEAEC				x*)	

*) The NOEAEC and NOECs (bentic) were set on 51 ng/l due to the missing replication at 111 ng/l.

Table A7.4.3.5.3-4 Effects Data (continued) : effects classified according to "Guidance Document on Aquatic Ecotoxicology" in the context of the Directive 91/414/EEC (SANCO 3268/2001 rev.4 (final) of 17 October 2002: 1: effect could not be demonstrated – 2: slight effect – 3: pronounced short term effect – 4: pronounced effect in short-term study (not relevant in this study) – 5: pronounced long-term effect

	Test concentration (ng as/l)				
	4.8	**10.5**	**23**	**51**	**111**
Macroinvertebrates (benthic)					
Oligochaeta					
Tubificidae	1	1	1	1	1
Diptera					
Chironomidae larvae	1	1	1	1	1
Ceratopogonidae larvae	1	1	1	1	1
Pulmonata					
Gyraulus albus	1	1	1	1	1
Bivalvia					
Pisidium spec.	1	1	1	1	1
Taxa richness	1	1	1	1	1
Diversity (Shannon Index)	1	1	1	1	1
Evenness	1	1	1	1	1
Similarity (Steinhaus Index)	1	1	1	1	1
Similarity (Stander's Index)	1	1	1	1	1
Principal Response Curves (PRC)	1	1	1	1	1
Community-NOEC				x*)	
Lowest population-NOEC				x*)	
NOEAEC				x*)	

*) The NOEAEC and NOECs (bentic) were set on 51 ng/l due to the missing replication at 111 ng/l.

Table A7.4.3.5.3-4 Effects Data (continued) : effects classified according to "Guidance Document on Aquatic Ecotoxicology" in the context of the Directive 91/414/EEC (SANCO 3268/2001 rev.4 (final) of 17 October 2002: 1: effect could not be demonstrated – 2: slight effect – 3: pronounced short term effect – 4: pronounced effect in short-term study (not relevant in this study) – 5: pronounced long-term effect

	Test concentration (ng as/l)				
	4.8	10.5	23	51	111
Emergence					
Chironomidae					
Sum of Chironominae	1	1	1	1	2
Chironominae (female)	1	1	1	1	2
Chironomus spec. (male)	1	1	1	1	3
Dicrotendipes spec. (male)	1	1	1	1	1
Paratanytarsus spec. (male)	1	1	1	1	1
Einfeldia spec. (male)	1	1	1	1	1
Micropsectra spec. (male)	1	1	1	1	1
Cryptotendipes spec. (male)	1	1	1	1	1
Polypedilum spec. male	1	1	1	1	1
Sum of Orthocladiinae	1	1	1	1	1
Orthocladiinae (female)	1	1	1	1	2
Orthocladiinae (male) cf. *Dratnalia sp.*	1	1	1	1	1
Cricotopus spec. (male)	1	1	1	1	1
Psectrocladius spec. (male)	1	1	1	1	1
Limnophyes spec. (male	1	1	1	1	1
Corynoneura spec. (male)	1	1	1	1	1
Acricotopus spec. (male)	1	1	1	1	1
Sum of Tanypodinae	1	1	1	1	1
Tanypodinae (female)	1	1	1	1	2
Tanypus spec. (male)	1	1	1	1	1
Ablabesmyia spec. (male)	1	1	1	1	1
Holotanypus spec. (male)	1	1	1	1	1
Psectrotanypus spec. (male)	1	1	1	2	2
Monopelopia spec. (male)	1	1	1	1	1
Culicidae					
Anopheles spec.	1	1	1	1	1
Chaoboridae					
Chaoborus crystallinus	2	2	2	2	2
Ephydridae					
Clanoneurum spec.	1	1	1	1	1
Ephemeroptera					
Cloeon spec.	1	1	1	1	1
Taxa richness	1	1	1	1	2
Diversity (Shannon Index)	1	1	1	1	2
Evenness	1	1	1	1	1
Similarity (Steinhaus Index)	1	1	1	1	1
Similarity (Stander's Index)	1	1	1	1	1
Principal Response Curves (PRC)	1	1	1	2	2
Community-NOEC			x		
Lowest population-NOEC	< 4.8				
NOEAEC				x*)	

*) The NOEAEC was set to 51 ng/l due to the missing replication at 111 ng/l.

Table A7.4.3.5.3-4 Effects Data (continued) : effects classified according to "Guidance Document on Aquatic Ecotoxicology" in the context of the Directive 91/414/EEC (SANCO 3268/2001 rev.4 (final) of 17 October 2002: 1: effect could not be demonstrated – 2: slight effect – 3: pronounced short term effect – 4: pronounced effect in short-term study (not relevant in this study) – 5: pronounced long-term effect

	Test concentration (ng as/l)				
	4.8	**10.5**	**23**	**51**	**111**
Asellus aquaticus					
Asellus in mesocosms					
Leaf cages	1	1	3	3	3
ASS	1	1	3	3	3
Leaf cages and ASS	1	1	3	3	3
Asellus bioassay					
Bioassay 1 (Day 2)	1	1	1	2	3
Bioassay 2 (Day 7)	1	1	1	1	2
Bioassay 3(Day 9)	1	1	2	3	3
Bioassay 4 (Day 14)	1	1	1	1	1
Bioassay 5 (Day 16)	1	1	1	2*	2
Bioassay 6 – 13 (Day 21 to Day 70)	1	1	1	1	1
Lowest In situ-NOEC		x			
Lowest bioassay-NOEC		x			
NOEAEC				x*)	
Phytoplankton					
Chlorophyceae	1	1	1	1	1
Scenedesmus spec.	1	1	1	1	+
Schroederia spec.	1	1	1	1	1
Diatomeae	1	1	1	1	2
Nitzschia spec.	1	1	1	1	2
Cryptophyceae	1	1	1	1	2
Chroomonass spec.< 10 µm	1	1	2	2	2
Cryptomonas spec. 10-20 µm	1	1	1	1	2
Cryptomonas spec. 30-40 µm	1	1	1	1	1
Engleonophyta	1	1	1	1	1
Conjugatophyceae	1	1	1	1	1
Cosmarium spec.	1	1	1	1	1
Cyanobacteria (*Merismopedia spec*)	1	1	1	1	+
Sum of filamentous algae	1	1	1	1	1
Taxa richness	1	1	1	1	1
Diversity (Shannon Index)	1	1	1	1	1
Evenness	1	1	1	1	1
Similarity (Steinhaus Index)	1	1	1	1	2
Similarity (Stander's Index)	1	1	1	1	1
Principal Response Curves (PRC)	1	1	1	1	1
Community-NOEC			x		
Lowest population-NOEC		x			
NOEAEC				x*)	

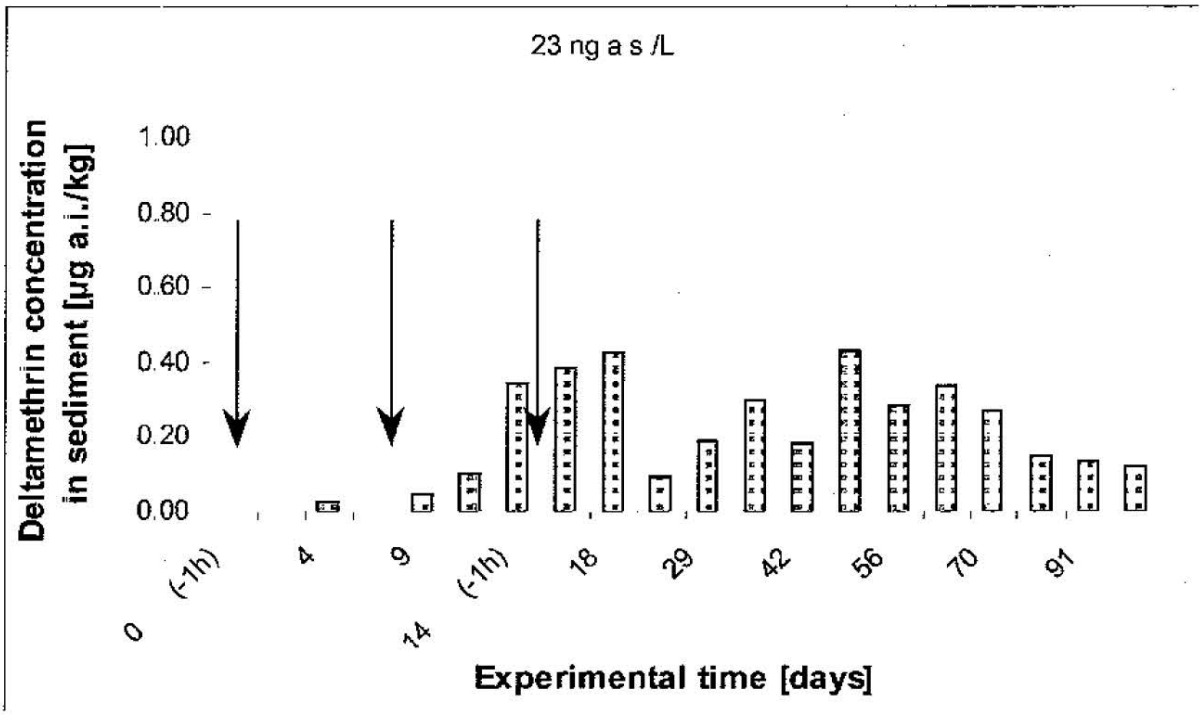
+ Increase in numbers
*) The NOEAEC was set on 51 ng/l due to the missing replication at 111 ng/l.
* Statistically not significant

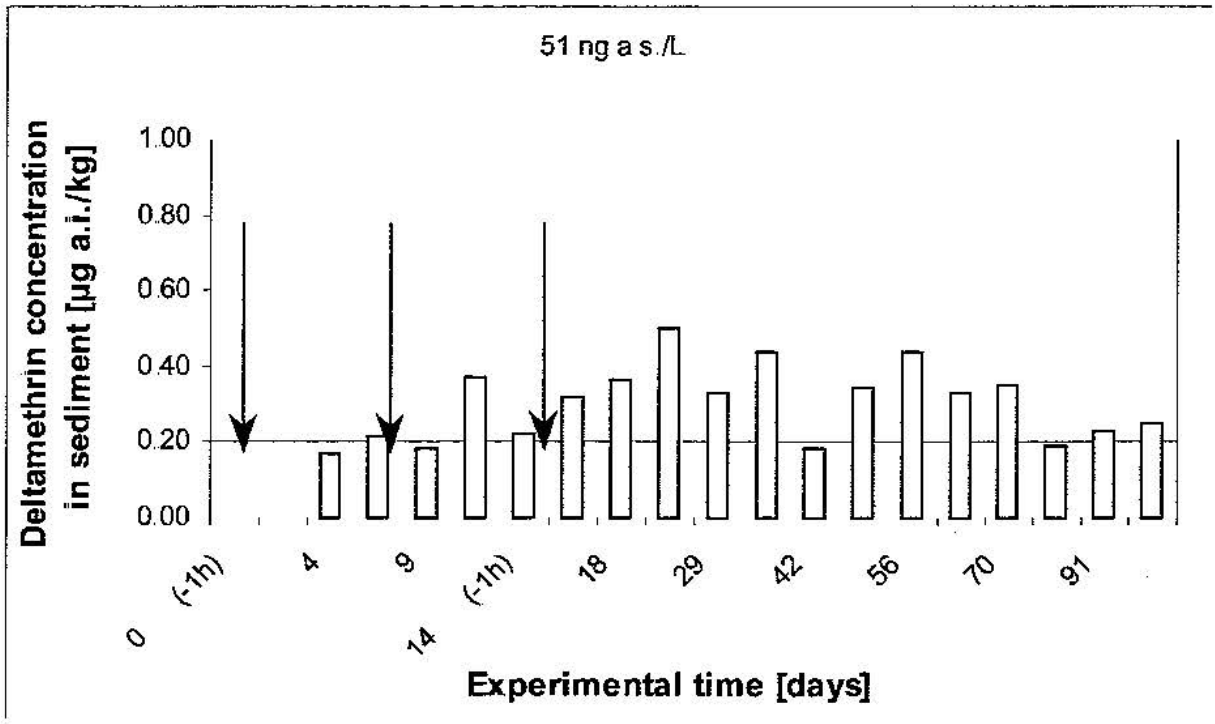
	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Not relevant
Materials and methods	Applicant's version is adopted with the following comments: **3.1.6** The limit of detection (LOD) for water was 0.002 µg/l. **5.1** Until start of the experiment, the water of the 12 mesocosms was exchanged completely 1-2 times per day through the pipe system and the external 13th pond. It is true that the artificial ponds are representative of a small stagnant water body, but the littoral zone is missing in these systems. At applications, the test substance was sprayed onto the water surface of the ponds. Thus, the exposure is not equivalent to the one expected when used as an insecticide in PT18. Emergence traps, placed in the center of each pond, were used to collect emerging insects and these were fixed in 1,2-Ethandiol. Regarding *Asellus aquaticus*, organisms were inserted to the ponds before the start and during experiment to simulate immigration from adjacent water bodies. Prior to start of the experiment, 300 organisms were introduced in each pond at two occasions. Thereafter, 200 individuals were inserted in replicates which only had a few or no individuals after 21 days and further 300 after 53 days. The replicates that received additions of *Asellus* were 23B, 51A and 111 ng/l. In addition, bioassays were conducted with pond water and exposed leaves from the corresponding pond in order to demonstrate the potential of a recovery of the species.
Results	Applicant's version is adopted with the following comments: **4.1.2** The average actual concentration of 94.1% of the nominal concentration was for all test concentrations and applications (see RMS table 1 below). For the first two applications, the average actual concentration for all test concentrations was somewhat higher than the nominal (114–126%) and 4 hours after the last application, the actual concentrations were only 24-70% (mean 43%) of the nominal. **5.2** The active substance may still be bioavailable after ingestion of particles, even if the uptake is slower than through the water phase. The concentration of deltamethrin in the sediment ranged from 0.13 to 0.56 µg/kg dw in the treatment with 23 ng/l, and from 0.15 to 0.71 and 0.79 µg/kg dw, respectively, in the treatments with 51 and 111 ng/l (see figures below). The concentrations were rather variable, but stabilized around 0.20 µg/kg and there was no clear trend of decrease. The effects on the coverage of macrophytes and filamentous algae in the ponds were assessed visually, and the total biomass was determined at the end of the study. The total biomass of macrophytes was somewhat lower in the higher test concentrations; mean 0.74-0.81 mg/kg dw, compared to controls; mean 1.09 mg/kg dw, but no statistical analysis was performed and the data set is probably too small. In the table presenting effect categories according to 91/414/EEC, category 3 is defined as "clear response of sensitive endpoints, but total recovery within 8 weeks after the last application." The question is if recovery is relevant for substances used as insecticides in PT18, where a more or less continuous exposure can be expected? RMS can not agree on the proposed NOEAEC of 51 ng/l for the zooplankton, since recovery may not be possible with insecticide exposure. A community NOEC of 4.8 ng/l is therefore proposed instead.

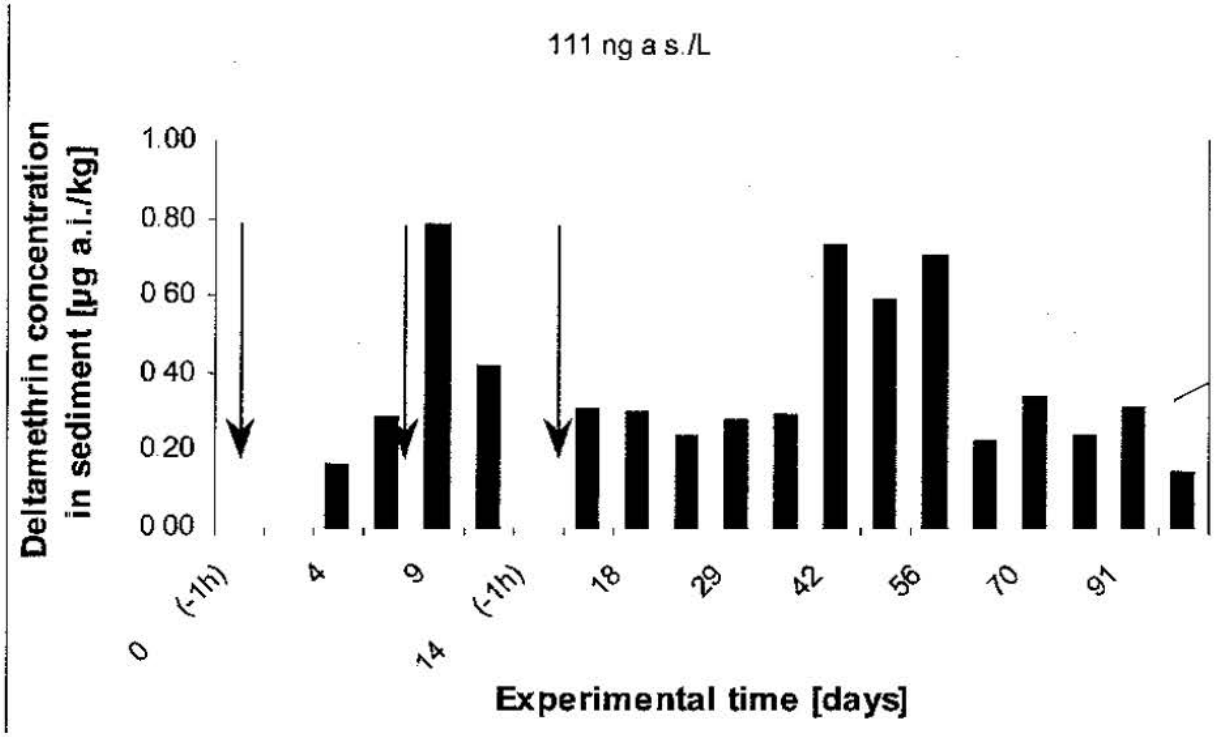
	Sediment and emergence The organic carbon content of the experimental sediment was quite high; 4.2%, which is about twice as much as in artificial sediment used in standard test guidelines. This may have decreased toxicity due to increased sorption. It is true that the emergence reached control levels during the study, but for some species, the emergence was delayed with six weeks at the higher concentrations. This is a strong and ecologically important effect, so even if recovery is observed, the ecosystem may have been affected (i.e. birds feeding on these insects). It is not possible to relate the effects on emergence of insects to sediment concentrations. There does not appear to be a dose-response relationship, since the sediment concentrations are in the same range both when effects are observed, up to five weeks after last application, and when emergence exceeds the control (from day 56). This may partly be due to difficulties when sampling and the fact that a 2 cm layer was sampled and analysed. The active substance was probably sorbed to the surficial sediment layer, and analysing to a depth of 2 cm dilutes the active substance and gives a high variation. The NOEAEC for Asellus of 51 ng/l is not accepted by RMS. A full recovery to control level within 8 weeks after last application could not be demonstrated for the test concentrations 23, 51, and 111 ng as/l in the mesocosms. In the bioassay, NOECs of 10.5 and 23 ng/l were observed two days after the first and second application, repsectively. After the third application, the NOEC was estimated to be 51 ng/l. However, according to the analysis of deltamethrin in water, the mean concentration in the 51 ng/l treatment on day 16 was 18.6 ng/l. If the effects are related to the actual concentrations and not the nominal, NOEC for Asellus is closer to 10.5 than 51 ng/l also in the bioassay. **Fate of deltamethrin** Regarding the estimated half-lives of deltamethrin, the pH values were quite high; 8-9 at the start and 9.0-10.1 at the third application. This probably promoted hydrolyses and degradation of deltamethrin. For further evaluation of fate and DT_{50} in this mesocosm study, see RMS comments on A7.1.2.2.2/03. **Effects on *Chaoborus crystallinus*** Effects were observed up to six weeks after last application, also at 4.8 ng/l, but full recovery was observed after seven weeks.
Conclusion	RMS does not agree on the proposed overall NOEAEC of 51 ng/l, but instead believes that a reasonable NOEC is 4.8 ng/l (n), based on the temporary effects on Chaoborus and the community NOEC for zooplankton.
Reliability	1
Acceptability	The study is regarded as acceptable. However, the mesocosm study and exposure scenario is not considered to be fully representative for the biocidal use of deltamethrin. The purpose of the study was to simulate the exposure conditions in agricultural use. RMS is also of the opinion that recovery can not be regarded to the same extent concerning biocidal use, since the exposure will be more or less continuous and driven by cleaning events that can take place the year around. RMS therefore proposes a NOEC of 4.8 ng/l, even though it is recognised that the most sensitive taxon, *Chaoborus crystallinus*, showed short-term effects also at this concentration.
Remarks	No further remarks

Table 46: Analysed concentrations as % of nominal

	Test Concentration (ng a.s./L)								average
Day	10.5/A	10.5/B	23/A	23/B	51/A	51/B	111	average	4 hours
0 (+4h)	100.5	102.6	116.8	115.7	152.9	131.5	78.7	114.1	after
1	62.4	38.6	64.9	69.7	66.5	32.8	33.0	52.6	the
2	32.4	11.9	45.2	31.7	48.9	8.5	10.8	27.1	3 appl.
4	0.0	0.0	10.9	10.9	20.2	4.9	6.4	7.6	
7	nd	nd	nd	nd	4.9	0.0	2.3	nc	94.1
7 (+4h)	150.7	68.6	150.4	155.9	152.6	86.6	113.6	125.5	+
8	73.3	71.9	39.6	43.6	38.9	26.2	22.2	45.1	48.0
9	69.5	34.8	40.4	41.0	33.6	21.5	16.5	36.8	
11	23.8	0.0	10.9	10.9	15.7	0.0	6.9	9.7	
14	0.0	0.0	0.0	0.0	4.9	0.0	2.3	1.0	
14 (+4h)	25.0	41.0	43.6	66.7	70.2	28.2	23.6	42.6	
15	31.2	17.9	31.5	52.8	57.2	15.5	15.7	31.7	
16	17.9	6.0	13.9	21.6	30.5	6.7	14.2	15.8	
18	nd	nd	nd	0.0	4.9	4.9	0.0	nc	
21	nd	nd	nd	0.0	4.9	0.0	0.0	nc	




51 ng a.s./L
Deltamethrin concentration in sediment [µg a.i./kg]
1.00
0.80
0.60
0.40
0.20
0.00
0
(-1h)
4
9
14
(-1h)
18
29
42
56
70
91
Experimental time [days]


111 ng a.s./L
Deltamethrin concentration in sediment [µg a.i./kg]
1.00
0.80
0 60
0 40
0.20
0 00
0
(-1h)
4
9
14
(-1h)
18
29
42
56
70
91
Experimental time [days]

A7.4.3.5.3/02

			Official use only
		1. REFERENCE	
1.1	**Reference**	Heimbach, F. and Arnold, M. (2005) Bioassay on the Effects of Deltamethrin EW 15 on *Gammarus pulex* in Mesocosm Water [redacted] Document MO-05-004496 **7.4.3.5.3/02** 24 February 2005 Unpublished	
1.2	**Data protection**	Yes	
1.2.1	**Data owner**	Bayer CropScience AG	
1.2.2	Companies with letter of access	n.a.	
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**	OECD Guidance Document "Simulated Freshwater Lentic Field Tests (Outdoor Microcosms and Mesocosms)", July 2004 (Draft). Guidance Document on Testing Procedures for Pesticides in Freshwater Mesocosms (SETAC-Europe Workshop, Monks Wood, UK, July 1991). Community-Level Aquatic System Studies - Interpretation Criteria (2002) (Proceedings from the CLASSIC Workshop).	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**	Deltamethrin EW 15 (oil in water emulsion)	
3.1.1	Lot/Batch number	AAIM00846	
3.1.2	Specification	Not given	
3.1.3	Purity	As given in Section 2, formulated as an EW	
3.1.4	Further relevant properties	-	
3.1.5	Radiolabelling	n.a.	
3.1.6	Method of analysis	Water samples were analysed by HPLC – MS/MS. The method was validated with a LOQ of 0.005 µg/L.	
3.2	**Reference substance**	None	
3.2.1	Method of analysis for reference substance	n.a.	
3.3	**Testing/estimation procedure**		

3.3.1	Test system / performance	Water was taken from nine tanks used in the mesocosm study where deltamethrin had been applied during the early growing season in May 2004, three times at an interval of 7 days onto the water surface of nine test ponds. The treatment levels were 4.8, 10.5, 23, 51 and 111 ng a.s./L per application (two replicates 4.8 to 51 ng a.s./L, one replicate for 111 ng a.s./L). Two and seven days after each application (and 4 hours after the 2nd and 3rd application), and on days 15, 21 and 28 after the last application of the mesocosms, pond water samples were taken from the mesocosms, together with some of the exposed leaves (*Populus spec.*). The bottles were exposed in a climatised room (same climatic conditions as the culture) and slightly aerated. After adaptation of the water samples to room temperature within some hours, ten *Gammarus pulex* of similar size were transferred from the culture into each bottle. The experimental time for each bioassay was three weeks with one to two evaluations weekly. Surviving and dead animals were counted to calculate the survival rate. Water and living animals were refilled into the test bottles each time.	X X X X
3.3.2	Test organism	The test organisms *(Gammarus pulex)* were derived from ditches of the Research institute ALTERRA in Wageningen (The Netherlands). They were cultured in the laboratory at about 12-15 °C, light duration: 16:8 hours in aerated tanks and fed by leaves of *Populus spec.*	
3.3.3	Calculation of NOECs	Univariate analyses	
		4. RESULTS	
4.1	**Experimental data**		
4.1.1	Effects	A reaction of *Gammarus pulex* could be observed at the highest treatment levels of 51 ng a.s./L and 111 ng a.s./L only. A reduction of the numbers of surviving Gammarids was noted at these concentrations in the bioassay water and food samples taken 4 hours to 2 days after application. Nevertheless, no effects were found in bioassays at all test concentrations, even the highest one, which had been established 7 or more days after applications.	
4.1.2	NOEC	A NOEC of 23 ng a.s./L can be calculated from this bioassay study; at higher test concentrations mortality was observed in samples taken in the first two days after application only. Samples taken thereafter did not indicate any toxic effects even at the highest test concentration of 111 ng a.s./L (see Table A7.4.3.5.3-5).	X

		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The aim of the study was to run a bioassay in order to demonstrate the potential recovery of *Gammarus pulex* populations parallel to a mesocosm study on Deltamethrin (see point 7.4.3.5.3/01). This allows a better control and evaluation of the effect of deltamethrin on *Gammarus pulex* as a direct test method for *Gammarus pulex* in the mesocosms, which prefer natural habitats with running water instead of the lentic conditions of the mesocosm study. Water was taken from nine tanks used in the mesocosm study where deltamethrin had been applied during the early growing season in May 2004, three times at an interval of 7 days onto the water surface of nine test ponds. The treatment levels were 4.8, 10.5, 23, 51 and 111 ng a.s./L per application (two replicates 4.8 to 51 ng a.s./L, one replicate for 111 ng a.s./L). Two and seven days after each application (and 4 hours after the 2nd and 3rd application), and on days 15, 21 and 28 after the last application of the mesocosms, pond water samples were taken from the mesocosms, together with some of the exposed leaves (*Populus spec.*). The bottles were exposed in a climatised room (same climatic conditions as the culture) and slightly aerated. After adaptation of the water samples to room temperature within some hours, ten *Gammarus pulex* of similar size were transferred from the culture into each bottle. The experimental time for each bioassay was three weeks with one to two evaluations weekly. Surviving and dead animals were counted to calculate the survival rate. Water and living animals were refilled into the test bottles each time. Univariate analyses were performed to calculate NOECs.	
5.2	**Results and discussion**	A reaction of *Gammarus pulex* could be observed at the highest treatment levels of 51 ng a.s./L and 111 ng a.s./L only. A reduction of the numbers of surviving Gammarids was noted at these concentrations in the bioassay water and food samples taken 4 hours to 2 days after application. Nevertheless, no effects were found in bioassays at all test concentrations, even the highest one, which had been established 7 or more days after applications.	X
		Thus, a NOEC of 23 ng a.s./L can be calculated from this bioassay study; at higher test concentrations mortality was observed in samples taken in the first two days after application only. Samples taken thereafter did not indicate any toxic effects even at the highest test concentration of 111 ng a.s./L.	X
5.3	**Conclusion**		
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Table A7.4.3.5.3-5 Calculated NOECs

	NOEC (ng a.s./L) after		
Time after application	**1st application**	**2nd application**	**3rd application**
4 hours	Not tested	23	23
2 days	23	23	≥111
7 days	≥111	≥111	≥111
15 days	See 2nd application	See 3rd application	≥111
21 days			≥111
28 days			≥111

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Not relevant
Materials and methods	Applicant's version is adopted with the following comments: **3.3.1** The mesocosm study, from which the test water was taken, is summarised in A7.4.3.5.3/01. It has to be pointed out that the aim of the mesocosm study was to simulate spray drift from agricultural use of deltamethrin, and therefore the active substance was sprayed onto the water surface of the test ponds. This procedure is not so relevant for the biocidal exposure and deltamethrin is known to be able to form a film on the water surface and possibly also evaporate before it is dissolved in the water. It is therefore important to measure the actual water concentrations. The bottles mentioned, are the test vessels in which the Gammarus were exposed. For evaluation of the survival of Gammarus, the content in the bottles was poured into a glass ditch and surviving and dead animals were counted. Water and living animals were then filled back into the test bottles.
Results	Applicant's version is adopted with the following comment: **4.1.2** The NOEC of 23 ng as/l is based on the nominal concentrations in the mesocosms. Water samples used in the bioassay were taken 2 and 7 days after each application and then the water concentrations had decreased considerably. The measured concentration in the treatment with 23 ng/l (NOEC) after two days was 7.3-12.2 ng/l, with a mean of about 9 ng/l. In the treatment with a concentration of 51 ng/l, where effects were observed, the measured concentration was 7.3-24.9 ng/l two days after application. A NOEC based on measured water concentrations should therefore be 9 ng/l.
Conclusion	Applicant's version is adopted with the following comments: **5.2** Reaction is a mild word for death. In the two highest concentrations, there was a reduced number of surviving gammarids in the bioassays with water and food samples taken 4 hours to 2 days after application. The NOEC should be related to the measured concentrations of deltamethrin in the water used in the bioassay, and hence the estimated NOEC for Gammarus is 9 ng/l.
Reliability	1
Acceptability	RMS considers that the study is well performed and has a relevant approach. However, the effect concentrations can not be related to the nominal concentrations, which decreased rapidly, and therefore the NOEC for *Gammarus pulex* is 9 ng/l based on measured concentrations and effects on mortality.
Remarks	No further remarks.

			Official use only
		1. REFERENCE	
1.1	**Reference**	Verboom, J. *et al* (2005) A Simulation Model for Spatial Population Dynamics of *Asellus aquaticus* after a Spray Drift Event of Deltamethrin in Aquatic Ecosystems Alterra, Wageningen University and Research Centre, NL Document MO-05-004734 **7.4.3.5.3/03** 24 February 2005 Unpublished	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Bayer CropScience AG	
1.2.2	Companies with letter of access	n.a.	
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**	n.a.	
2.2	**GLP**	n.a.	
2.3	**Deviations**	n.a.	
		3. MATERIALS AND METHODS	
3.1	**Test material**		
3.1.1	Lot/Batch number	n.a.	
3.1.2	Specification	n.a.	
3.1.3	Purity	n.a.	
3.1.4	Further relevant properties	n.a.	
3.1.5	Radiolabelling	n.a.	
3.1.6	Method of analysis	n.a.	
3.2	**Reference substance**	n.a.	
3.2.1	Method of analysis for reference substance	n.a.	
3.3	**Testing/estimation procedure**		

3.3.1	Test system / performance	The population dynamics of *Asellus aquaticus* as the second most sensitive species from the mesocosm study (see point 7.4.3.5.3/01) cannot adequately be investigated *in situ* (including invasion and/or reproduction dynamics of this species) mainly because of the isolation of individual test ponds. Therefore, a metapopulation simulation model on the population dynamics of *Asellus aquaticus* in natural water bodies was performed to evaluate the potential for a population recovery of this species after the contamination of a natural water body through the use of deltamethrin. The metapopulation model describes the effects and recovery of the waterlouse *Asellus aquaticus* population after the exposure to deltamethrin as a result of spray drift using the pond, ditch and stream FOCUS scenario. The exposure modelling was done using the use patterns of deltamethrin, the FOCUS spray drift data and the fate model TOXSWA 1.2. Use patterns resulting in nominal concentrations of 16, 23, 30 and 43 ng/L were evaluated against an untreated control simulation. The linking of exposure and effects was provided using the results of the mesocosm study evaluating the effects of three applications of deltamethrin on *A. aquaticus*.	
3.3.2	Estimation of bioconcentration	n.a.	
		4. RESULTS	
4.1	**Modelling results**	When looking at the total numbers in the pond scenario and the numbers in the treated 100 meter stretch for the ditch and stream scenario, the results show small effects of the 16 ng/L treatment for all three scenarios on the numbers found during the summer peak, which occurred two months after the spraying event. At higher concentrations effects were the largest for the ditch scenario followed by the pond and stream scenario. The differences in peak height between the higher treatment levels were small for the ditch and stream scenario, while the pond scenario resulted in a clear dose-response relation. The ditch scenario proved to be worst-case because the whole 100 meter stretch was treated and no drift of *A. aquaticus* due to wind influence or stream velocity was included. Effects for the pond scenario were smaller because the pond was exposed from one side, so migration from the less contaminated other side was possible. Moreover, in a two-dimensional system such as a pond, recolonisation is easier than in a one-dimensional system such as a ditch. The results of the stream scenario show the importance of the inclusion of drift on the height of the summer peak observed in the treated 100 meter of the stream. It should be noted, however, that the inclusion of drift only had a small influence when the numbers were evaluated for the whole modelled 600 meter stretch.	

		5. APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The population dynamics of *Asellus aquaticus* as the second most sensitive species from the mesocosm study (see point 7.4.3.5.3/01) cannot adequately be investigated *in situ* (including invasion and/or reproduction dynamics of this species) mainly because of the isolation of individual test ponds. Therefore, a metapopulation simulation model on the population dynamics of *Asellus aquaticus* in natural water bodies was performed to evaluate the potential for a population recovery of this species after the contamination of a natural water body through the use of deltamethrin. The metapopulation model describes the effects and recovery of the waterlouse *Asellus aquaticus* population after the exposure to deltamethrin as a result of spray drift using the pond, ditch and stream FOCUS scenario. The exposure modelling was done using the use patterns of deltamethrin, the FOCUS spray drift data and the fate model TOXSWA 1.2. Use patterns resulting in nominal concentrations of 16, 23, 30 and 43 ng/L were evaluated against an untreated control simulation. The linking of exposure and effects was provided using the results of the mesocosm study evaluating the effects of three applications of deltamethrin on *A. aquaticus*.
5.2	**Results and discussion**	When looking at the total numbers in the pond scenario and the numbers in the treated 100 meter stretch for the ditch and stream scenario, the results show small effects of the 16 ng/L treatment for all three scenarios on the numbers found during the summer peak, which occurred two months after the spraying event. At higher concentrations effects were the largest for the ditch scenario followed by the pond and stream scenario. The differences in peak height between the higher treatment levels were small for the ditch and stream scenario, while the pond scenario resulted in a clear dose-response relation. The ditch scenario proved to be worst-case because the whole 100 meter stretch was treated and no drift of *A. aquaticus* due to wind influence or stream velocity was included. Effects for the pond scenario were smaller because the pond was exposed from one side, so migration from the less contaminated other side was possible. Moreover, in a two-dimensional system such as a pond, recolonisation is easier than in a one-dimensional system such as a ditch. The results of the stream scenario show the importance of the inclusion of drift on the height of the summer peak observed in the treated 100 meter of the stream. It should be noted, however, that the inclusion of drift only had a small influence when the numbers were evaluated for the whole modelled 600 meter stretch. In the light of the assumptions and uncertainties discussed in the report, the results of this study seem robust for most factors, but more research is needed to establish the effects of especially density dependence and dispersal (including drift). However, the overall outcome of this study is considered to represent a reasonable worst-case situation for the recovery of a local *A. aquaticus* population when affected by a local use of deltamethrin.
5.3	**Conclusion**	
5.3.1	Reliability	1
5.3.2	Deficiencies	No

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Not relevant
Materials and methods	
Conclusion	
Reliability	4
Acceptability	The study is not considered to be acceptable. This study does not seem to be relevant for the use of deltamethrin in PT18 and it is not clear to RMS how the results can be used in the risk assessment.
Remarks	During the workshop on PT 18 the participating MS agreed that recovery should not be considered for biocidal use of insectides with the major emission route via STP, since the exposure is more or less continuous.

7.5 Effects on terrestrial organisms

7.5.1.1 Inhibition to microbiological activity

			Official use only
		1. REFERENCE	
1.1	**Reference**	Frings, H. and Bock, K.D. (1994a) Deltamethrin: Technical Substance (Hoe 032640 00 ZD99 0001): Investigating the Effect on the Microbial Activity in Soil (short-term effects on aerobic soil respiration in accordance with BBA, VI, 1-1, 2nd edition) [redacted] Document A52240 **7.5.1.1/01** 18 February 1994 Unpublished See Monograph 91/414 from 1998 – Point B.8.8.1	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Bayer CropScience AG	
1.2.2	Companies with letter of access	n.a.	
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; BBA VI, 1-1	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**	Deltamethrin	
3.1.1	Lot/Batch number	2N0398B2	
3.1.2	Specification	As given in Section 2	
3.1.3	Purity	99.6%	
3.1.4	Composition of product	Not applicable	
3.1.5	Further relevant properties	-	
3.1.6	Method of analysis	Not measured	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable	X
3.3	**Reference substance**	Dinoseb-acetate (98%)	

3.3.1	Method of analysis for reference substance	Not measured	
3.4	**Testing procedure**		
3.4.1	Culture medium	Not applicable	
3.4.2	Inoculum / test organisms	Soil microorganisms (not identified)	
3.4.3	Test system	The test system consisted of two soils from an agricultural crop area in Germany. In each case, the soil was taken in the form of a mixed sample from the upper 0 – 20 cm of the top soil (see Table A7.5.1.1-1). Soil samples were taken from a depth of 0 – 20 cm at approximately 10 sites distributed over the area. Rest of plants were removed and the soil was air dried, moving it constantly until sieving was possible. Until the start of the study, the fine soil which had been passed through a 2 mm sieve was stored in aerated containers at +4 -11°C.	X
3.4.4	Test conditions	The test substance and water (45% of the maximum water holding capacity) were added to the test system (2650 g soil) and homogenised. From each of these test series, soil was taken for 4 replicates (650 g each), weighed into aerated stainless steel containers of 2 litre capacity and incubated at 20°C. Evaporation losses were compensated twice weekly by means of weight control, and the soil was homogenised. In order to determine the respiration rates (O_2 consumption), partial quantities (105 g) were removed from each replicate at each measurement date, adding the prescribed quantity of glucose. The O_2 consumption was measured on days 0, 14, and 28 (and on day 56 for the loamy sand).	
3.4.5	Duration of the test	56 days	
3.4.6	Test parameter	Respiration (based on oxygen)	
3.4.7	Analytical parameter	Not applicable	
3.4.8	Sampling	Days 0, 14, 28 and, in one case, 56 (loamy sand).	
3.4.9	Monitoring of TS concentration	No	
3.4.10	Controls	Untreated soil	
3.4.11	Statistics	Ades program used.	
		4. RESULTS	
4.1	**Preliminary test**		
4.1.1	Concentration	None	
4.1.2	Effect data	Not applicable	
4.2	**Results test substance**		

4.2.1	Initial concentrations of test substance	37.5 and 375 g/ha	X
4.2.2	Actual concentrations of test substance	Not measured	
4.2.3	Growth curves	-	
4.2.4	Cell concentration data	-	
4.2.5	Concentration / response curve	-	
4.2.6	Effect data	Only slight deviations of the treated test series from the untreated control were observed in the course of the study. They did, however, not exceed the BBA tolerance value for loamy sand. The largest deviation was observed in the 10 fold dosage on day 28, with + 7.04%.	X
		Although this value was in the range of biological variation, further analysis was carried out on day 56 in sandy loam in order to confirm this interpretation.	
		In silty loam, examination of the test substance yielded no deviations beyond the range of biological variation during the 28-day test period.	
4.2.7	Other observed effects	-	
4.3	**Results of controls**	See Point 4.2.6 above.	
4.4	**Test with reference substance**		
4.4.1	Concentrations	3.0 and 15.0 kg/ha	X
4.4.2	Results	In the reference compound (dinoseb-acetate) deviations of – 25.11% were detected after 28 days, which increased until the end of the study reaching – 39.71% in the loamy sand. Deviations of – 46.65% were detected on day 28 and – 66.67% on day 91 as compared with the control for the silty loam.	
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The short-term effects of deltamethrin (purity 99.6%) on aerobic soil respiration were tested in two soils at application rates of 37.5 g/ha (max. field dose) and 375 g/ha. The respiration rates (O_2-consumption) were determined, after admixture of glucose, on days 0, 14, 28 and 56 (soil 1) during a 12 hour measurement period. The soil characteristics are presented in Table A7.5.1.1-1.	
		Four replicates of 650 g soil were incubated at 20°C in aerated stainless steel containers. 100g of soil were taken at each measure occasion.	

5.2	**Results and discussion**	Only slight deviations of the treated soils from the untreated were observed and they were in the Range 1 according to the BBA-Richtlinie, Teil VI, 1-1. The effects were negligible according to the criteria of Domsch *et al*, 1983.	
5.2.1	NOEC	> 375 g/ha	X
5.3	**Conclusion**		
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Table A7.5.1.1-1 Soil Characteristics for the Two Soils Used in the Soil Respiration Test on Deltamethrin

Texture	pH	Biomass (mg/100g)	Organic carbon capacity (%)	Maximum water holding (%)
Loamy sand	6.1	15.5	0.7	33.5
Silty loam	7.0	66.4	1.4	46.8

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Not relevant
Materials and methods	Applicant's version is adopted with the following comments: **3.2** The active substance (solid) was diluted with quartz powder, before it was mixed into the test soil. **3.4.3** There are some errors in the headings in table A7.5.1.1-1. It should be **Microbial** biomass, Organic carbon **content**, and Maximum water holding **capacity**.
Results	Applicant's version is adopted with the following comments: **4.2.1** The initial concentrations are equivalent to 0.05 and 0.5 mg/kg dw soil. **4.2.6** The largest deviation, +7.04%, was observed in soil 1. **4.4.1** It should be noted that there are much higher test concentrations of the reference substance, compared to the test substance.
Conclusion	Applicant's version is adopted with the following comment: **5.2.1** The NOEC of >375 g/ha is comparable to 0.50 mg/kg dw soil.
Reliability	1
Acceptability	The study was performed according to a recommended guideline and is considered to be acceptable. The NOEC for inhibition to microbiological activity was > 0.50 mg/kg dw soil based on effects on respiration.
Remarks	No further remarks

7.5.1.1/02 Inhibition to microbiological activity

			Official use only
		1. REFERENCE	
1.1	**Reference**	Frings, H. and Bock, K.D. (1994b) Deltamethrin, technical substance (Hoe 032640 00 ZD99 0001): Investigating the Effect on the Nitrogen Cycle in Soil (in accordance with BBA, VI, 1-1 2nd edition) [redacted] Document A52241 **7.5.1.1/02** 21 February 1994 Unpublished See Monograph 91/414 from 1998 – Point B.8.8.1	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Bayer CropScience AG	
1.2.2	Companies with letter of access	n.a.	
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; BBA VI, 1-1	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**	Deltamethrin	
3.1.1	Lot/Batch number	2N0398B2	
3.1.2	Specification	As given in Section 2	
3.1.3	Purity	99.6%	
3.1.4	Composition of product	Not applicable	
3.1.5	Further relevant properties	-	
3.1.6	Method of analysis	None	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**		X
3.3	**Reference substance**	Nitrogen stabiliser (N-serve TG: nitrapyrin 90%)	
3.3.1	Method of analysis for reference substance	Not measured	
3.4	**Testing procedure**		
3.4.1	Culture medium	Not applicable	

3.4.2	Inoculum / test organisms	Not applicable	
3.4.3	Test system	The test system consisted of two soils from an agricultural crop area in Germany. In each case, the soil was taken in the form of a mixed sample from the upper 0 – 20 cm of the top soil (see Table A7.5.1.1-2). Soil samples were taken from a depth of 0 – 20 cm at approximately 10 sites distributed over the area. Rests of plants were removed and the soil was dried in the air, moving it constantly until sieving was possible. Until the start of the study, the fine soil which had been passed through a 2 mm sieve was stored in aerated containers at +4 - 11°C.	X
3.4.4	Test conditions	Lucerne green meal (except for Ts. 1), the test substance and water (45% of the maximum water holding capacity) were added to the test system (1000 g soil in the dry state/Ts.) and homogenised. From each of these test plots, soil was taken for 3 replicates (290 g each in the dry state), weighed into aerated 2 1-stainless steel containers and incubated at 20°C. Evaporation losses were compensated twice weekly by means of weight control, mixing deionised water homogenously into the soil. For the ammonium nitrogen (NH_4-N) and nitrate nitrogen (NO_3-N) determinations, partial quantities (40 g soil in the dry state) were removed from each replicate at each measurement date. Ammonium nitrogen (NH_4-N) and nitrate nitrogen (NO_3-N) were determined on days 0, 7, 14 and 28. On the date of analysis, 40 g soil (dry state) per test sample were extracted with a total of 200 ml extraction solution (1 % potassium aluminium sulphate) over a period of 1 hour. 1 mg NO_3-N/100 g soil was added in the form of 10 ml extraction solution containing KNO_3 in order to ensure detection and to spike the nitrate concentration in the soil which was low in some cases. The added 10 ml and the water volume contained in the moist soil are part of the total extraction solution. Filtration took place via N-deficient plaited filters. The added N (1 mg/100 g soil) was subtracted when recording the measurements. NH_4-N was determined by means of a gas electrode (Orion 95-12) in 50 ml filtrate after addition of 1 ml 10 mol NaOH. In order to ensure more exact measurements of ammonium, 1 mg NH_4-N/100 g soil was added in the form of 10 ml of a solution containing $(NH_4)_2SO_4$ to the above filtrate quantity immediately before measurement. The addition was also subtracted from the measured value. NO_3-N was determined in the remaining filtrate of the samples, which had been kept at 20°C, by means of a sensitive 93-07-01 electrode from Orion after check-up of the pH and correction to 3.2 – 3.5 if necessary.	
3.4.5	Duration of the test	28 days	
3.4.6	Test parameter	Soil respiration based on nitrogen production	
3.4.7	Analytical parameter	Not performed	

3.4.8	Sampling	Days 0, 7, 14 and 28.	
3.4.9	Monitoring of TS concentration	No	
3.4.10	Controls	Untreated soil	
3.4.11	Statistics	-	
		4. RESULTS	
4.1	**Preliminary test**		
4.1.1	Concentration	None	
4.1.2	Effect data	Not applicable	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	37.5 and 375 g/ha	X
4.2.2	Actual concentrations of test substance	Not measured	
4.2.3	Growth curves	-	
4.2.4	Cell concentration data	-	
4.2.5	Concentration / response curve	-	
4.2.6	Effect data	Loamy sand: Determination of the microbial activity in the test system (control without admixture of lucerne green meal (Ts. 1)) yielded an increase of the mineral nitrogen content (NH_4-N and NO_3-N = total N_{min}) from 1.64 to 2.11 mg N/100 g soil during 28 days. In the control with lucerne green meal admixture (Ts. 2), the mineral nitrogen (total N_{min}) increased from 1.65 to 5.05 mg N/100 g soil. The mineralisation rate of the added substrate nitrogen was thus 29.4 % during a period of 28 days after subtraction of the natural mineralisation. In the test series treated with the test substance, no deviations from the control exceeding the BBA tolerance value were observed after the 28-d test period had elapsed. The test series treated at the 1x dose level showed the greatest deviation (-14.4%, day 7) as compared with the control. During the following time, this value declined to -12.5% (day 14) and had decreased to -8.1% by day 28. No appreciable deviations were found in test series 4 (10 fold application rate). They were within the range of biological and analytical variation over the whole of the test period. In the test series treated with the test substance, the development of the oxidation of ammonium nitrogen can be considered as rapid during the entire test period.	

		The determined pH values showed no effects resulting from application of the test substance (Ts. 3 – 4) as compared with the control (Ts. 2). Silty loam: Determination of the activity of the test system without admixture of lucerne green meal (Ts. 1) yielded an increase in the mineral nitrogen content (NH_4-N and NO_3-N = total N_{min}) from 2.53 to 4.29 mg N/100 g soil during 28 days. The mineral nitrogen content (total N_{min}) in the control with admixture of lucerne green meal increased from 2.87 to 6.40 mg N/100 g soil, equivalent to a rate of mineralisation of 21.1% of the added substrate nitrogen after subtraction of the natural N-mineralisation. In the test series treated with the test substance, the deviations from the control (Ts. 2) were within the range of biological and analytical variation during the entire test period. The oxidation from NH_4 to NO_3-N took place rapidly.	X
4.2.7	Other observed effects	-	
4.3	**Results of controls**		
4.4	**Test with reference substance**		
4.4.1	Concentrations	Nitrogen stabiliser (N-serve TG) at 750 and 3750 g/ha.	
4.4.2	Results	The reference substance (N-Serve) showed a maximum deviation from the control of -72.0 % with regard to NO_3-N on day 28 with the silty loam. Nitrification was clearly inhibited.	
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The effects of deltamethrin (purity 99.6%) on the nitrogen cycle were tested in two soils at application rates of 37.5 g/ha (maximum field rate) and 375 g/ha. Lucerne green meal was added at a rate of 10 mg N/100 g soil and the ammonium and nitrate contents were determined on days 0, 7, 14 and 28. The soil characteristics are shown in Table A7.5.1.1-2. Three replicates of 290 g soil for each application and control were incubated at 20°C and 40 g of soil were removed for each measurement date for the ammonium nitrogen and the nitrate nitrogen determinations.	
5.2	**Results and discussion**	No appreciable variations from the treated soils compared to the untreated controls were found. The relative N transformations were within Range I stated in the BBA-Richtlinie, Teil VI, 1-1 and hence the effects of deltamethrin on the nitrogen cycle are negligible according to the criteria of Domsch *et al*, 1983.	
5.3	**Conclusion**		
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Table A7.5.1.1-2 Soil Characteristics for the Two Soils Used in the Soil Respiration Test on Deltamethrin

Texture	pH	Biomass (mg/100g)	Organic carbon capacity (%)	Maximum water holding (%)
Loamy sand	6.1	15.5	0.7	33.5
Silty loam	7.0	66.4	1.4	46.8

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Not relevant
Materials and methods	Applicant's version is adopted with the following comments: **3.2** The active substance (solid) was diluted with quartz powder, before it was mixed into the test soil. It is a bit strange that the test substance was not dissolved or applied using the sand as a carrier, considering the low water solubility. The reference substance, on the other hand, was dissolved in CH_2Cl_2. However, this probably did not affect the outcome of the test. **3.4.3** There are some errors in the headings in table A7.5.1.1-1. It should be **Microbial** biomass, Organic carbon **content**, and Maximum water holding **capacity**. Additionally, the main headline should be "Soil characteristics for the two soils used in the soil **Nitrogen cycle** test"
Results	Applicant's version is adopted with the following comments: **4.2.1** The initial concentrations are equivalent to 0.05 and 0.5 mg/kg dw soil. **4.2.6** In soil 2, the deviation from the control was -2.7% at the most.
Conclusion	Applicant's version is adopted with the following comment: **5.2** The NOEC for inhibition of deltamethrin on the nitrogen cycle can be estimated to >375 g/ha or 0.50 mg/kg dw soil.
Reliability	1
Acceptability	The study was performed according to a recommended guideline and is considered to be acceptable. The NOEC for inhibition to microbiological activity was > 0.50 mg/kg dw soil based on effects on the nitrogen cycle.
Remarks	No further remarks

7.5.1.2 Acute toxicity test to earthworms or other soil non-target organisms

			Official use only
		1. REFERENCE	
1.1	**Reference**	Hoxter, K.A. and Smith, G.J. (1993) Deltamethrin Technical: An Acute Toxicity Study With the Earthworm in an Artificial Soil Substrate [redacted] Document A50956 **7.5.1.2/01** 17 May 1993 Unpublished See Monograph 91/414 from 1998 – Point B.8.6.1	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Bayer CropScience AG	
1.2.2	Companies with letter of access	n.a.	
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**	The pH was higher than recommended and no reference substance was used.	
		3. MATERIALS AND METHODS	
3.1	**Test material**	Deltamethrin	
3.1.1	Lot/Batch number	1N0356B2	
3.1.2	Specification	As given in Section 2	
3.1.3	Purity	98%	
3.1.4	Composition of product	Not applicable	
3.1.5	Further relevant properties	-	
3.1.6	Method of analysis	Soil was extracted with acetone and then acetone:hexane. Combined extracts were filtered and partitioned. Hexane layers were dried and the final extract was then diluted with toluene. Analysis was done by GC/ECD.	
3.2	**Reference substance**	None	
3.2.1	Method of analysis for reference substance	Not applicable	
3.3	**Testing procedure**		

3.3.1	Preparation of the test substance	For each concentration, a calculated amount of acetone was used to dissolve the test substance which was then incorporated into the soil, along with the water.	
3.3.2	Application of the test substance	See Point 3.3.1.	
3.3.3	Test organisms	*Eisenia fetida* (See Table A7.5.1.2-2 for details)	
3.3.4	Test system	See Table A7.5.1.2-3.	
3.3.5	Test conditions	See Table A7.5.1.2-4.	
3.3.6	Test duration	14 days	
3.3.7	Test parameter	Mortalities, body weight, behaviour	
3.3.8	Examination	On Days 7 and 14.	
3.3.9	Monitoring of TS concentration	Yes, at sample preparation and Days 7 and 14.	
3.3.10	Statistics	Pattern of mortality did not allow for calculation of the LC_{50} value. This and the NOEC were determined by visual inspection.	
		4. RESULTS	
4.1	**Filter paper test**		
4.1.1	Concentration	Not applicable	
4.1.2	Number / percentage of animals showing adverse effects	Not applicable	
4.1.3	Nature of adverse effects	Not applicable	
4.2	**Soil test**		
4.2.1	Initial concentrations of test substance	167, 279, 465, 776 and 1290 mg as/kg soil (nominal). Mean measured: 149, 277, 447, 781 and 1290 mg as/kg soil	X
4.2.2	Effect data (mortality)	There were no mortalities at any level.	
4.2.3	Concentration / effect curve	Not applicable	
4.2.4	Other effects	Apparent lethargy or immobility at the two highest doses on Day 7. All worms appeared normal on Day 14.	
4.3	**Results of controls**		
4.3.1	Mortality	There were no mortalities.	
4.3.2	Number / percentage of earthworms showing adverse effects	There were no adverse effects.	

4.3.3	Nature of adverse effects	Not applicable	
4.4	**Test with reference substance**		
4.4.1	Concentrations	None	
4.4.2	Results	Not applicable	
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	In a 14-day acute toxicity study adult earthworms *(Eisenia fetida)* were exposed to deltamethrin (purity 98%) at mean measured concentrations of 149, 277, 447, 781 and 1290 mg/kg. Acetone was used as a solvent. Four replicate test chambers were used for each treatment and the control with 10 worms per chamber. The substrate was an artificial soil with a pH of 7.3 – 8.2 and a moister content of approximately 50 – 55% of WHC. The temperature was 20.0 – 21.4°C and there was continuous light. Observations were made on days 7 and 14.	
5.2	**Results and discussion**	There were no mortalities observed and hence the 14-day LC_{50} was > 1290 mg/kg soil. The NOEC was 447 mg/kg based on signs of toxicity as immobility and lethargy. The mean measured test concentrations were > 89% of nominate.	
5.2.1	NOEC	447 mg/kg soil	X
5.2.2	LC_{50}	> 1290 mg/kg soil	X
5.2.3	LC_{100}	> 1290 mg/kg soil	
5.3	**Conclusion**		
5.3.1	Other conclusions	-	
5.3.2	Reliability	1	
5.3.3	Deficiencies	No	

Table A7.5.1.2-1 Preparation of TS Solution

Criteria	Details
Type and source of dilution water	Not reported
Alkalinity / Salinity	Not reported
Hardness	Not reported
PH	Not reported
Oxygen content	Not reported
Conductance	Not reported
Holding water different from dilution water	Not reported
In case of the use of an organic solvent	
Dispersion	-
Vehicle	Acetone
Concentration of vehicle	-
Vehicle control performed	Yes
Other procedures	-

Table A7.5.1.2-2 Test Organisms

Criteria	Details
Species / strain	*Eisenia foetida*
Source of the initial stock	Laboratory stock
Culturing techniques	OECD guidelines
Age / weight	Adult, 0.5 – 0.6 g
Pretreatment	One day before test initiation, 265 adult earthworms *(Eisenia fetida)* with clitellum, were selected from the Wildlife International Ltd earthworm culture. The culture, originally provided by Vittar and Associates, Inc., Mobile, Alabama, was maintained according to OECD guidelines. The selected worms were placed in a container with approximately 500 g of artificial soil which had a moisture content of approximately 15%, for the acclimation period. On the day of test initiation, worms were rinsed briefly with deionised water, blotted dry with paper towels and placed in labelled beakers to be weighed. Worms were allocated to the tared and labelled beakers in pairs, by indiscriminate draw, until each beaker contained 10 worms.

Table A7.5.1.2-3 Test System

Criteria	Details
Artificial soil test substance	70% sand, 20% kaolin clay, 10% sphagnum peat moss
Test mixture	For each concentration, a calculated amount of acetone was used to dissolve the test substance which was then incorporated into the soil, along with the water.
Size, volume and material of test container	Glass mason jars
Amount of artificial soil (kg)/container	750 g
Nominal levels of test concentrations	167, 279, 465, 776, 1290 mg as/kg soil
Number of replicates/concentration	4
Number of earthworms/test concentration	40
Number of earthworms/container	10
Light source	Continuous light from fluorescent tubes (423 to 680 lux)
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	20.0 – 21.4°C
Moisture content	12.3 – 18.3%
PH	7.28 – 8.18
Adjustment of pH	At study initiation only
Light intensity / photoperiod	Continuous
Relevant degradation products	Not measured

Table A7.5.1.2-5 Validity Criteria for Acute Earthworm Test According to OECD Guideline 207

	Fulfilled	Not fulfilled
Mortality of control animals < 10%	X	

Section 7 **Annex Point IIIA XIII.3.2**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** A7.5.1.2 Acute toxicity test to earthworms or other soil non-target organisms

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Not relevant
Materials and methods	Applicant's version is adopted
Results	Applicant's version is adopted with the following comment: **4.2.1** Both initial and measured concentrations are in mg as/kg **dw** soil.
Conclusion	Applicant's version is adopted with the following comment: **5.2.1** The NOEC is 447 mg/kg dw soil. **5.2.2** The LC_{50} is >1290 mg/kg dw soil.
Reliability	1
Acceptability	The study is acceptable. It mainly follows OECD 207, and was well performed and reported. The NOEC for earthworms was 447 mg/kg dw soil based on signs of immobility and lethargy and the LC_{50} was > 1290 mg/kg dw soil.
Remarks	No further remarks

7.5.1.3 Acute toxicity to plants

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [✓]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	Based on the use pattern of the biocidal products, no exposure to plants is anticipated. Furthermore, deltamethrin is an insecticide used/registered for years for crop protection and is not phytotoxic. Therefore, this study should not be required."	
Undertaking of intended data submission [ ]		

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Not relevant
Evaluation of applicant's justification	The RMS agrees with the applicant's justification.
Conclusion	Applicant's justification is acceptable.
Remarks	No further remarks

Section 7 **Annex Point IIIA XIII.3.2**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** A7.5.2.1 Reproduction study with other soil non-target macro-organisms

7.5.2 Terrestrial tests, long-term tests

7.5.2.1 Reproduction study with other soil non-target macro-organisms

			Official use only
		1. REFERENCE	
1.1	**Reference**	Moser, T. and Scheffczyk, A. (2005) Deltamethrin-Br_2CA: Effects on survival and reproduction of the predaceous mite *Hypoaspis aculeifer* CANESTRINI (Acari: Laelapidae) in standard soil (LUFA 2.1) [redacted] Document M-255441-01-1 7.5.2.1/01 1 August 2005 Unpublished	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Bayer CropScience AG	
1.2.2	Companies with letter of access	n.a.	
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**	SECOFASE, Final Report, improvement and standardisation of test systems for assessing sub-lethal effects of chemicals on fauna in the soil ecosystem (Løkke & van Gestel 1996). Guidance document on regulatory testing procedures for pesticides with non-target arthropods (Barrett *et al.* 1994).	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**	Major metabolite of deltamethrin: Br_2CA	
3.1.1	Lot/Batch number	2N6185C	
3.1.2	Specification	Not given	
3.1.3	Purity	98.8%	
3.1.4	Further relevant properties	-	X
3.1.5	Radiolabelling	n.a.	
3.1.6	Method of analysis	n.a.	
3.2	**Reference substance**	Dimethoate at 5 mg dimethoate/kg	
3.2.1	Method of analysis for reference substance	n.a.	
3.3	**Testing/estimation procedure**		

3.3.1	Test system / performance	Br_2CA was mixed into LUFA 2.1 soil. 20 *Hypoaspis aculeifer* (4-6 days old) were added to 10, 32, 100, 316 and 1000 mg test item/kg soil dry weight (4 replicates), positive control (dimethoate) at 5 mg/kg soil (3 replicates) and water control (5 replicates) at 23.7 - 25.7 °C and permanent dark. Soil pH values were between 6.1 - 6.3 and soil moisture was between 54.4% and 63.1% of the maximum water holding capacity. Mortality/escape rate was determined after 14 days of exposure, reproduction was determined after 34 days.	X
3.3.2	Estimation of bioconcentration	n.a.	
		4. RESULTS	
4.1	**Experimental data**	Three percent of adult mites died in the control and 6.3% mortality was found at a concentration of 10 mg test item/kg soil (dry weight) (corresponding to a corrected mortality according to Abbott (1925) of 3.4%). At all other concentrations of the test item tested 13.3 -22.5% mortality was observed (corresponding to a corrected mortality from 10.7 to 20.1%). Since the mortality observed with the test item was not higher than 22.5%, the LC_{50} value could not be calculated. The ANOVA and the Williams t-test (1-sided, $p \leq 0.05$) showed a significant difference in the mortality after 14 days between the control and the concentrations of 32, 100, 316 and 1000 mg test item/kg soil (dry weight) of the test item tested. Therefore, the $NOEC_{Mortality}$ was determined as 10 mg test item/kg soil (dry weight) and accordingly the $LOEC_{Mortality}$ was determined as 32 mg test item/kg soil (dry weight). However, the observed mortality with all concentrations tested was below the required validity criterion for the control mortality of < 25% and the statistically significant differences are partly due to the low control mortality. Reproduction was examined only for the control and the two highest concentrations of the test item which caused less than 50% mortality (i.e. 316 and 1000 mg test item/kg soil (dry weight). Statistical analysis (Welch t-test; 1-sided, $p \leq 0.05$) showed no significant difference concerning the cumulative number of juveniles per female after 7 days between the control and these two concentrations of the test item tested. Therefore, the $NOEC_{reproduction}$ was determined as $\geq$ 1000 mg test item/kg soil (dry weight). See Table A7.5.2.1-1 for the complete results. After 14 days of exposure, 94.8% corrected mortality according to Abbott (1925) of the adult mites was observed with the positive control which was within the recommended range of 50.0-99.5%.	X

		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	A study in the predaceous mite, *Hypoaspis aculeifer*, was conducted with the major metabolite of deltamethrin in the environment, Br_2CA. The test material was mixed into LUFA 2.1 soil. 20 *Hypoaspis aculeifer* (4-6 days old) were added to 10, 32, 100, 316 and 1000 mg test item/kg soil dry weight (4 replicates), positive control (dimethoate) at 5 mg/kg soil (3 replicates) and water control (5 replicates) at 23.7 - 25.7 °C and permanent dark. Soil pH values were between 6.1 - 6.3 and soil moisture was between 54.4% and 63.1% of the maximum water holding capacity. Mortality/escape rate was determined after 14 days of exposure, reproduction was determined after 34 days.	
5.2	**Results and discussion**	Three percent of adult mites died in the control and 6.3% mortality was found at a concentration of 10 mg test item/kg soil (dry weight) (corresponding to a corrected mortality according to Abbott (1925) of 3.4%). At all other concentrations of the test item tested 13.3 -22.5% mortality was observed (corresponding to a corrected mortality from 10.7 to 20.1%). Since the mortality observed with the test item was not higher than 22.5%, the LC_{50} value could not be calculated. The ANOVA and the Williams t-test (1-sided, $p \leq 0.05$) showed a significant difference in the mortality after 14 days between the control and the concentrations of 32, 100, 316 and 1000 mg test item/kg soil (dry weight) of the test item tested. Therefore, the $NOEC_{Mortality}$ was determined as 10 mg test item/kg soil (dry weight) and accordingly the $LOEC_{Mortality}$ was determined as 32 mg test item/kg soil (dry weight). However, the observed mortality with all concentrations tested was below the required validity criterion for the control mortality of < 25% and the statistically significant differences are partly due to the low control mortality. Reproduction was examined only for the control and the two highest concentrations of the test item which caused less than 50% mortality (i.e. 316 and 1000 mg test item/kg soil (dry weight)). Statistical analysis (Welch t-test; 1-sided, $p \leq 0.05$) showed no significant difference concerning the cumulative number of juveniles per female after 7 days between the control and these two concentrations of the test item tested. Therefore, the $NOEC_{reproduction}$ was determined as ≥ 1000 mg test item/kg soil (dry weight). See Table A7.5.2.1-1 for the complete results. After 14 days of exposure, 94.8% corrected mortality according to Abbott (1925) of the adult mites was observed with the positive control which was within the recommended range of 50.0-99.5%. Conclusions: The LC_{50} value could not be calculated. The $NOEC_{Mortality}$ was determined as 10 mg test item/kg soil (dry weight). The $LOEC_{Mortality}$ was determined as 32 mg test item/kg soil (dry weight). The $NOEC_{Reproduction}$ was > 1000 mg test item/kg soil (dry weight).	X
5.3	**Conclusion**		
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Section 7 **Annex Point IIIA XIII.3.2**	**Ecotoxicological Profile Including Environmental Fate and Behaviour** A7.5.2.1 Reproduction study with other soil non-target macro-organisms

Table A7.5.2.1-1 Effects on Mortality and Reproduction of *Hypoaspis aculeifer*

Concentration [mg test item/kg soil (dry weight)]	Average mortality (%)	Corrected mortality (%)	Mean cumulative number of juveniles / female after 7 days (mean ± sd)	Reduction of juveniles (%)
Control	3.0	0.0	23.4 ± 3.9	0.0
Positive control	95.0	94.8	-	-
10	6.3	3.4	-	-
32	13.3*	10.7	-	-
100	16.3*	13.7	-	-
316	20.0*	17.5	22.9 ± 3.3	2.1
1000	22.5*	20.1	24.8 ± 4.1	-6.2
			Adult mortality	**Reproduction**
LC_{50} [mg test item/ kg soil (dry weight)]			> 1000	-
NOEC [mg test item/ kg soil (dry weight)]			10	≥ 1000
LOEC [mg test item/ kg soil (dry weight)]			32	-

*: significantly different to control (Williams t-test; 1-sided, $p \leq 0.05$)
-: could not be determined

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Not relevant
Materials and methods	Applicant's version is adopted with the following comments: **3.1.4** The test substance is insoluble in water, but soluble in methanol. **3.3.1** The test substance was added in the form of a powder to an amount of LUFA 2.1 soil, which was then mixed with the remainder of the soil to prepare the different test item concentrations. The mites were fed with prey mites or enchytraeids during the test.
Results	Applicant's version is adopted with the following comment: **4.1** The positive control which is referred to is the test with the reference substance dimethoate.
Conclusion	Applicant's version is adopted with the following comment: **5.2** The NOEC and LOEC for mortality are based on nominal concentrations.
Reliability	1
Acceptability	The study is acceptable. It was well performed and reported and the $NOEC_{mortality}$ of Br_2CA for soil non-target macroorganisms was 10 mg/kg dw soil, based on nominal concentrations.
Remarks	No further remarks

7.5.2 Terrestrial tests, long-term tests

7.5.2.1 Reproduction study with other soil non-target macro-organisms

		1. REFERENCE	Official use only
1.1	**Reference**	Luehrs, U. (2004) Deltamethrin EW15: Effects on reproduction and growth of earthworms *Eisenia fetida* in artificial soil with 5% peat. [redacted] Document M-085431-01-1 7.5.2.1/02 19 August 2004 Unpublished	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Bayer CropScience AG	
1.2.2	Companies with letter of access	n.a.	
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; BBA VI, 2-2 (C. Kula, 1994) & ISO 11268-2, 1998	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes, with respect to the properties of the test item (log Pow > 2), 5% instead of 10% peat was used, considering the influence on bioavailability (EPPO 2002).	
		3. MATERIALS AND METHODS	
3.1	**Test material**	Deltamethrin formulated as EW 15	
3.1.1	Lot/Batch number	AAIM00846	
3.1.2	Specification	Deltamethrin formulated as EW 15	
3.1.3	Purity	-	
3.1.4	Composition of product	Deltamethrin 16.42 g/L	
3.1.5	Further relevant properties	-	
3.1.6	Method of analysis	No analysis performed	
3.2	**Reference substance**	Derosal SC 360 g/L (active ingredient carbendazim)	
3.2.1	Method of analysis for reference substance	No information reported	
3.3	**Testing procedure**	Deionised water was used as control	

3.3.1	Preparation of the test substance	The following amounts of deltamethrin EW 15 were weighed using an analytical balance and deionised water was added until a final net weight of 1000 g was reached. Rate 1 (0.8 L/ha): 1.368 g Rate 2 (1.39 L/ha): 2.377 g Rate 3 (2.41 L/ha): 4.121 g Rate 4 (4.18 L/ha): 7.148 g Rate 5 (7.25 L/ha): 12.398 g Rate 6 (12.58 L/ha): 21.510 g To reach a homogeneous emulsion a magnetic stirrer was used.	
3.3.2	Application of the test substance	All dilutions were applied in a singular application according to agricultural practice with a laboratory-spraying equipment (Fa. Schachtner, D-71640 Ludwigsburg) onto the soil of the assigned trays. The inner sides of the test container walls were covered with plastic covers to protect the inside walls. The covers were removed immediately after spraying the trays. Application was done after the introduced test organisms had burrowed into the soil. After application containers were left open for 60 to 110 min following treatment, care was taken to prevent worms from escaping during this time; afterwards the containers were closed with the lids.	
3.3.3	Test organisms	*Eisenia fetida andrei* (Savigny 1826) (See Table A7.5.2.1/02-1 for details)	
3.3.4	Test system	See Table A7.5.2.1/02-2.	X
3.3.5	Test conditions	See Table A7.5.2.1/02-3.	
3.3.6	Test duration	56 days	
3.3.7	Test parameter	Mortality (number of dead adult earthworms at day 28 after application), body weight change (between day 0 and day 28 after application), feeding activity, and reproduction (number of young worms 8 weeks after application)	
3.3.8	Examination	On days 0 and 28	X
3.3.9	Monitoring of TS concentration	No	X
3.3.10	Statistics	Data of mortality were analyzed for significance by using Fisher-exact-test (two-sided, $\alpha = 0.05$). Data of body weight changes and reproduction were tested for normal distribution and homogeneity of variance using Kolmogoroff-Smimov-test and Cochran-test. Because data of body weight changes were normally distributed and homogeneous Dunnett-test was used (multiple comparison, two sided, $\alpha = 0.05$). Because data of reproduction were not homogeneous Bonferroni-U test was used (multiple comparison, one-sided, $\alpha = 0.05$). The software used to perform the statistical analysis was SYSTAT 9 for Windows and ToxRatPro, version 2.07, ToxRat solutions GmbH (2001-2003).	
		4. RESULTS	
4.1	**Filter paper test**		

4.1.1	Concentration	Not applicable	
4.1.2	Number / percentage of animals showing adverse effects	Not applicable	
4.1.3	Nature of adverse effects	Not applicable	
4.2	**Soil test**	See Table A.7.5.2.1/02-4	
4.2.1	Initial concentrations of test substance	Deltamethrin EW 15 was sprayed onto the soil at 0.8; 1.39; 2.41; 4.18; 7.25 & 12.58 L/ha corresponding to 13.1, 22.8, 39.6, 68.6, 119.1, and 206.6 g a.i./ha.	X
4.2.2	Effect data (mortality)	In the rate of 0.8 L/ha a slight mortality (2.5%) was observed, which was not significantly different compared to the control, where also 2.5% of the worms were dead (Fisher exact test, $\alpha = 0.05$). No mortality was observed in other rates.	
4.2.3	Concentration / effect curve	-	
4.2.4	Other effects	The body weight changes were no significantly different compared to the control up to and including the rate 12.58 L test item/ha (Dunnett-test, $\alpha = 0.05$, two sided). The reproduction rates were not significantly different compared to the control in any test item rate (Bonferroni-U test, $\alpha = 0.05$, one-sided smaller). In all treatment group food was consumed. The results show that the turnover of biomass of those earthworms exposed to the six different rates of the test item was comparable to the control. No behavioural abnormalities were observed.	
4.3	**Results of controls**	See Table A.7.5.2.1/02-4	
4.3.1	Mortality	In the control (deionised water), 2.5% of the worms were dead.	
4.3.2	Number / percentage of earthworms showing adverse effects	There were no adverse effects.	
4.3.3	Nature of adverse effects	Not applicable	
4.4	**Test with reference substance**		
4.4.1	Concentrations	See Figure A7.5.2.1/02-1	X
4.4.2	Results	See Figure A7.5.2.1/02-1	

		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Deltamethrin EW 15 was sprayed onto the soil at 0.8; 1.39; 2.41; 4.18; 7.25 & 12.58 L/ha corresponding to 13.1, 22.8, 39.6, 68.6, 119.1, and 206.6 g a.i./ha to which earthworms (*Eisenia fetida*; 40 worms per treatment group) were exposed at 19-21°C, light 440-600 lux, 16 h light/8 h dark, fed weekly with dried cattle manure, initial soil water content 22.4-25.3% (52.1-58.8% of the maximum water holding capacity), water content at experimental termination 24.5-26.7% (57.0-62.1% of the maximum water holding capacity); initial pH 5.5, pH 5.8-6.1 at experimental termination. Endpoints were mortality (number of dead adult earthworms at day 28 after application), body weight change (between day 0 and day 28 after application), feeding activity, and reproduction (number of young worms 8 weeks after application). The toxic standard: Derosal SC 360 (active ingredient carbendazim) is tested at least once a year in a dose response study. The control was sprayed with deionised water.	
5.2	**Results and discussion**	Deltamethrin EW 15 did not show significant lethal effects or effects on body weight changes or reproduction of the earthworm *Eisenia fetida* in artificial soil up to the highest test rate of 12.58 L test item/ha. Therefore, the Lowest Observed Effect Level (LOEL) in this study was determined to be greater than 12.58 L test item/ha. The No-Observed-Effect-Level (NOEL) was determined to be 12.58 L test item/ha, i.e. the highest concentration tested.	
5.2.1	NOEC	12.58 L test item/ha, corresponding to 206.6 g a.s./ha	X
5.2.2	LOEL	> 206.6 g a.s./ha	
5.3	**Conclusion**		
5.3.1	Other conclusions	-	
5.3.2	Reliability	1	X
5.3.3	Deficiencies	No	

Table A7.5.2.1/02-1 Test Organisms

Criteria	Details
Species / strain	*Eisenia fetida Andrei*
Source of the initial stock	[redacted]
Culturing techniques	Bred under standardised conditions (in a breeding medium of cattle manure, peat, sand and straw, fed with cattle manure, stored at room temperature)
Age / weight	Adult (8-9 months) / 300 – 500 mg
Pre-treatment	Earthworms were acclimatized for 2 days, in artificial soil, under test conditions (controlled environment room, in a ventilated area; temperature: 19°C – 20°C; light intensity: 440-600 lux; light regime: 16 h light : 8 h dark; water content checked once a week).

Table A7.5.2.1/02-2 Test System

Criteria	Details
Artificial soil	Based on OECD 207 but with reduced organic matter content: 5.0% Sphagnum-peat, air-dried and finely ground (2 mm); 20% kaolin clay; approx. 0.2% chalk added to adjust pH to 6.0 +/- 0.5; approx. 74.8% fine quartz-sand (F34) containing more than 50% by mass of particle size 0.05 mm to 0.2 mm. With respect to the properties of the test item (log Pow > 2), 5% instead of 10% peat was used considering the influence on bioavailability.
Test mixture	The following amounts of deltamethrin EW 15 were weighed using an analytical balance and deionised water was added until a final net weight of 1000 g was reached. Rate 1 (0.8 L/ha): 1.368 g Rate 2 (1.39 L/ha): 2.377 g Rate 3 (2.41 L/ha): 4.121 g Rate 4 (4.18 L/ha): 7.148 g Rate 5 (7.25 L/ha): 12.398 g Rate 6 (12.58 L/ha): 21.510 g To reach a homogeneous emulsion a magnetic stirrer was used.
Size, volume and material of test container	Plastic boxes (18.3 cm x 13.6 cm x 6 cm with the size *ca.* 16.5 cm x 11.5 cm = 189.75 cm^2 at the level of the soil) with perforated transparent lids to enable exchange of air, to minimise evaporation of the artificial soil, and to prevent the worms from escaping.
Amount of artificial soil (kg)/container	626 g of the prepared soil (approx. 500 g (dw) artificial soil, plus approx. 121 g water, plus approx. 5 g food). The height of the soil layer in the containers was *ca.* 5 cm.
Nominal levels of test concentrations	13.1, 22.8, 39.6, 68.6, 119.1, and 206.6 g a.i./ha
Number of replicates/concentration	4
Number of earthworms/test concentration	40
Number of earthworms/container	10
Light source	Not reported
Test performed in closed vessels due to significant volatility of test substrate	No

Table A7.5.2.1/02-3 Test Conditions

Criteria	Details
Test temperature	19°C – 21°C
Water content	Initial soil water content 22.4-25.3% (52.1-58.8% of the maximum water holding capacity), water content at experimental termination 24.5-26.7% (57.0-62.1% of the maximum water holding capacity)
pH	Initial pH 5.5, pH 5.8-6.1 at experimental termination
Adjustment of pH	No
Light intensity / photoperiod	Light intensity: 440-600 lux Light regime: 16 h light : 8 h dark
Relevant degradation products	Not measured

Table A7.5.2.1/02-4 Effects on Mortality and Reproduction of *Eisenia fetida*

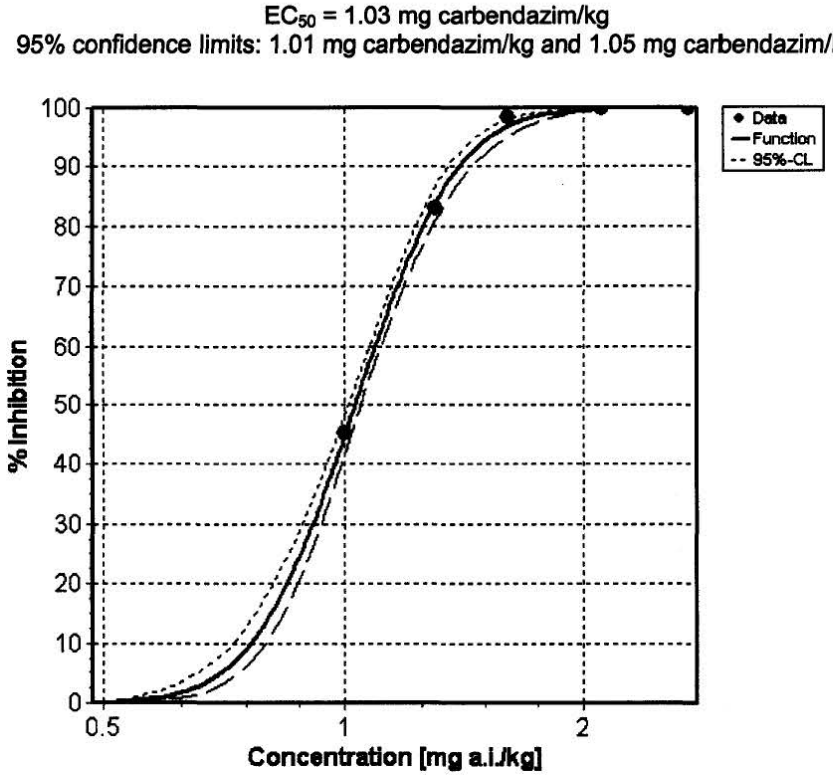
Test species	***Eisenia fetida***						
Exposure	Test item sprayed onto artificial soil						
NOEC	206.6 g a.i./ha						
LOEC	> 206.6 g a.i./ha						
Test item	**control**	**Deltamethrin EW 15**					
Application [g a.i./ha]	deionized water	13.1	22.8	39.6	68.6	119.1	206.6
Mortality [%] ± SD	2.5 ± 5.0	2.5 n.s.[1] ± 5.0	0.0 -	0.0 -	0.0 -	0.0 -	0.0 -
Body weight change [%] ± SD	58.1 ± 4.2	52.3 n.s.[1] ± 13.8	60.9 n.s.[1] ± 9.3	59.5 n.s.[1] ± 9.9	58.0 n.s.[1] ± 4.6	60.1 n.s.[1] ± 5.0	55.3 n.s.1 ± 11.2
Reproduction [# of juveniles] ± SD % of control	272 ± 29 -	231 n.s.[2] ± 87 84.8	278 n.s.[2] ± 28 102.0	288 n.s.[2] ± 25 105.7	250 n.s.[2] ± 25 91.8	299 n.s.[2] ± 26 109.9	285 n.s.[2] ± 28 104.7
Amount of food added [g] ± SD	25.0 ± 0.0	25.0 ± 0.0	25.0 ± 0.0	25.0 ± 0.0	25.0 ± 0.0	25.0 ± 0.0	25.0 ± 0.0

SD = Standard deviation
from control
- Not applicable

1) Dunnett's Test, $\alpha = 0.05$, two-sided
2) Bonferroni-T-Test, $\alpha = 0.05$, one-sided smaller

n.s.: not stat. significantly different

Figure A7.5.2.1/02-1 Reproduction results of the most recent test with the toxic standard reference Derosal SC 360



	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Not relevant
Materials and methods	**3.3.2:** The application of test substance was made according to agricultural practice with laboratory spraying equipment onto the soil. RMS opinion is that this exposure route is neither relevant nor comparable to biocidal exposure routes. *This issue was discussed at TM I 2010 and it was agreed that studies where the test substance is mixed into the substrate would preferred. However, during bilateral discussions after the meeting one MS pointed out that based on the relatively large number of individuals per soil volume it is likely that the soil is effectively mixed by the worms and thereby in practice an even distribution of the test compound would be achieved. The RMS agree with this proposal, and has therefore changed the reliability of this study to 2.* **3.3.4:** RMS considers that the approach to use 5% peat instead of 10% and thus deviate from the guideline was correct. This is also in line with the more recently adopted OECD TG 222 (2004). **3.3.8:** Examination of reproduction (juvenile worms) was performed after 56 days (8 weeks). **3.3.9:** RMS considers that the absence of monitoring of TS in the artificial soil is a big flaw for the relevance of the test although this is not required in the test guidelines. This is because it is not possible to relate a concentration in g as/ha to mg as/kg soil, which is needed to be able to compare the effect concentrations to biocide exposure.
Results	**4.2.1:** The test concentrations should have been reported in mg/kg soil. *Based on RMS calculations, the NOEC value (at the highest test level) corresponds to 0.626 mg/kg ww or 0.784 mg/kg dw.* **4.4.1:** In the test with the reference substance, the test substance must have been applied and mixed with the soil, since the test concentrations are given as mg/kg.
Conclusion	**5.2.1**: To be able to calculate a PNEC from the NOEC, this effect concentration has to be given in mg/kg. *Further, it was proposed at TM I 2010 that the results should be normalised to the standard content of organic matter. The resulting NOEC is then 0.426 mg/kg ww or 0.533 mg/kg dw.*
Reliability	~~3~~**2**
Acceptability	*The RMS originally proposed that despite that the study was performed in accordance with the guidelines it should not be accepted due to the method of application (on the soil surface without mixing of the soil). It was also agreed by the TM I 2010 that studies where the test substance is mixed into the soil is preferred. However, after bilateral communication after the RMS consider it likely that the worms will efficiently mix the soil. Despite this, the NOEC value will not be used for PNEC derivation, since no effects were observed at the highest test concentration, and the next lowest NOEC value was only slightly higher than that from this study.*
Remarks	

7.5.2.1 Reproduction study with other soil non-target macro-organisms

			Official use only
		1. REFERENCE	
1.1	**Reference**	Lechelt-Kunze, C. (2004) Deltamethrin EC 025: Influence on the reproduction of the collembola species *Folsomia candida* tested in artificial soil with 5% peat. [redacted] Document M-233529-01-1 7.5.2.1/03 14 July 2004 Unpublished	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Bayer CropScience AG	
1.2.2	Companies with letter of access	n.a.	
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**	ISO 11267 (1999) Soil quality – Inhibition of reproduction of Collembola (*Folsomia candida*) by soil pollutants	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes, with respect to the properties of the test item (log Pow ≥ 2), 5% instead of 10% peat were used considering the influence on bioavailability (EPPO 2002).	
		3. MATERIALS AND METHODS	
3.1	**Test material**	Deltamethrin formulated as EC 25	
3.1.1	Lot/Batch number	OP240341	
3.1.2	Specification	Deltamethrin formulated as EC 25	
3.1.3	Purity	-	
3.1.4	Composition of the product	Deltamethrin 24.48 g/L	
3.1.5	Further relevant properties	-	
3.1.6	Method of analysis	No analysis performed	
3.2	**Reference substance**	Betosip (active ingredient Phenmedipham). Test concentrations 89, 133, 200, 300 and 450 mg Betosip/kg artificial soil (dw), tested once a year	
3.2.1	Method of analysis for reference substance	No information reported	
3.3	**Testing/estimation procedure**	Deionised water was used as control	

<table>
<tr><td>3.3.1</td><td>Preparation of the test substance</td><td>From the water soluble test item a stock solution was prepared (0.1031 g test item in 200 ml water). The following amounts of the stock solution were taken to prepare 100 ml of the test item solution to realise the demanded concentrations. If less than 100 ml were applied, the missing amount was filled up with deionised water.

<table><tr><th>ml of the stock solution</th><th>+ ml deionised water</th><th>Corresponding nominal test item concentration mg/kg soil (dw)</th></tr><tr><td>6.25
12.5
25
50
100</td><td>93.75
87.5
75
20
-</td><td>6.25
12.50
25.00
50.00
100.00</td></tr></table></td><td>X</td></tr>
<tr><td>3.3.2</td><td>Application of the test substance</td><td>At the start the amount of 100 ml test item solution was mixed homogeneously into 500 mg artificial soil (dw) with a laboratory mixer (Krefft). In a second step 15 ml of deionised water was introduced in the same way. With 115 ml a water content of approximately 50% of the maximum water holding capacity was reached. The control soil contained the corresponding amount of deionised water only. Application rates in this test were 6.25; 12.50; 25.00; 50.00 & 100.00 mg test item/kg artificial soil (dw). Approximately 30 g (wet weight) of the test substrate were filled in each test vessel avoiding compression.</td><td></td></tr>
<tr><td>3.3.3</td><td>Test organisms</td><td>Folsomia candida (See Table A7.5.2.1/03-1 for details)</td><td></td></tr>
<tr><td>3.3.4</td><td>Test system</td><td>See Table A7.5.2.1/03-2.</td><td>X</td></tr>
<tr><td>3.3.5</td><td>Test conditions</td><td>See Table A7.5.2.1/03-3.</td><td></td></tr>
<tr><td>3.3.6</td><td>Test duration</td><td>28 days</td><td></td></tr>
<tr><td>3.3.7</td><td>Test parameter</td><td>Mortality of adult Collembola and the number of alive juveniles 4 weeks after application</td><td></td></tr>
<tr><td>3.3.8</td><td>Examination</td><td>The soil of each replicate was decanted into a Bellaplast vessel (volume: 200 ml, surface: 75 cm²). Each portion was stirred up with 80 ml of deionised water and the Collembola drifted to the surface. The water was coloured with 5 ml black ink (Pelikan) in order to increase the contrast between the water and the white Collembola. From each vessel a digital image was taken. The adult and juvenile Collembola on each digital image were counted automatically. These procedures were carried out with the Lemna Tec Scanalizer. Each image was checked for mistakes by visual inspection.</td><td></td></tr>
<tr><td>3.3.9</td><td>Monitoring of TS concentration</td><td>No</td><td>X</td></tr>
<tr><td>3.3.10</td><td>Statistics</td><td>The software used to perform the statistical analysis was ToxRat Pro 2.09 (Ratte, 2002). Data of reproduction were tested for normal distribution and homogeneity of variance using Kolmogorroff-Smimov Test and Cochran-Test ($\alpha = 0.05$) respectively. Data of reproduction were normally distributed and homogeneity of variances was given, but data were not monotonous. Therefore Dunnett's Test (one-sided-smaller, $\alpha = 0.05$) was used to determine NOEC and LOEC values.</td><td></td></tr>
</table>

		4. RESULTS	
4.1	**Test with test item**	In the control group 4% of the adult Collembola died which is within the tolerated range of ≤ 20% mortality recommended by the guideline (see Table A7.5.2.1/03-4). The highest mortality rate of 10% was found in the test item concentration of 50 mg deltamethrin EC 25/kg soil (dw). Concerning the number of juveniles statistical analysis (Dunett's test, one sided-smaller, $\alpha = 0.05$) reveals significant differences between the control and the highest treatment groups with 100 mg deltamethrin EC 25/kg soil (dw). The NOEC for reproduction: 50 mg deltamethrin EC 25/kg soil (dw). The LOEC for reproduction: 100 mg deltamethrin EC 25/kg soil (dw).	
4.2	**Test with reference substance**	To demonstrate the sensitivity of the test system Betosip as a toxic standard is regularly tested (once a year) at concentrations of 89, 133, 200, 300 and 450 mg test item/kg artificial soil (dw). In the most recent test (Lechelt-Kunze, 2004), the mortality rate of adult Collembola was 10, 18, 66, 100 and 100% at 89, 133, 200, 300 and 450 mg Betosip/kg soil (dw). With 89 mg Betosip/kg soil (dw) the number of juveniles was statistically significantly reduced to 68% (Bonferroni-U-Test, one-sided-smaller, $\alpha = 0.05$) in comparison to the control. The statistically significant effect on the reproduction was in the range of 100 – 200 mg product/kg artificial soil (dw) as recommended by the guideline and showed that the test system was sensitive.	
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Ten Collembola (10-l2 days old) per replicate (5 replicates per treatment group) were exposed to control (water treated), 6.25, l2.50, 25.00, 50.00 and 100.00 mg Deltamethrin EC 25/kg artificial soil (dw) (Deltamethrin EC 025, batch No.: OP24034l; containing deltamethrin 24.48 g/L) at 18 – 22°C, 400 - 800 Lux, l6h light: 8h dark, 5% peat in the artificial soil. During the test they were fed with granulated dry yeast. Mortality and reproduction were determined after 28 days. The toxic standard used was Betosip (active ingredient: Phenmedipham, test concentrations 89, l33, 200, 300 and 450 mg Betosip/kg artificial soil (dw), tested once a year (Lechelt-Kunze, 2004)).	
5.2	**Results and discussion**	In the control group 4% of the adult Collembola died which is within the tolerated range of ≤ 20% mortality recommended by the guideline. The highest mortality rate of 10% was found in the test item concentration of 50 mg deltamethrin EC 25/kg soil (dw). Concerning the number of juveniles statistical analysis (Dunett's test, one sided-smaller, $\alpha = 0.05$) reveals significant differences between the control and the highest treatment groups with 100 mg deltamethrin EC 25/kg soil (dw).	
5.3	**Conclusion**	The NOEC for reproduction: 50 mg deltamethrin EC 25/kg soil (dw). The LOEC for reproduction: 100 mg deltamethrin EC 25/kg soil (dw).	X
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Table A7.5.2.1/03-1 Test Organisms

Criteria	Details
Species / strain	*Folsomia candida* (Collembola, Isotomidae)
Source of the initial stock	The culture of the springtails *Folsomia candida* used in this test has been bred at [redacted] since April 2002. The strain was originally obtained from [redacted]
Culturing techniques	The Collembola were bred in a mixture of Plaster of Paris and activated charcoal and demineralised water (11:1:10 w/w). Bellaplast vessels (9.5 cm ∅) were filled up to a height of 1 cm with this mixture. The vessels, closed with perforated plastic lids, had to be moistened, fed and aerated regularly once a week. The breeding culture was kept under the following conditions: temperature 20-24°C; permanent dark, feeding once a week with bakers dry yeast (Dr. Oetker).
Age	10-12 days
Synchronisation	Twelve days before starting the test, egg clusters from the breeding containers were transferred to fresh breeding substrate to obtain Collembola of a uniform age (10-12 days old at test start). After 47 hours the egg cluster were removed and the remaining Collembola hatched from the eggs were fed with granulated dry yeast.

Table A7.5.2.1/03-2 Test System

<table>
<tr><th>Criteria</th><th>Details</th></tr>
<tr><td>Artificial soil</td><td>The test was conducted in artificial soil according to OECD 207 (1984): 5% sphagnum peat (air dried and finely ground), 20% kaolinite clay (content of kaolinite 56%), 74-75% industrial quartz sand (F36; particle size 0.20 mm – 0.05 mm = 68.2%) and 0.2-1% calcium carbonate (for the adjustment to pH 6.0 +/- 0.5). With respect to the properties of the test item (log Pow ≥ 2), 5% instead of 10% peat were used considering the influence on bioavailability (EPPO 2002).</td></tr>
<tr><td>Test mixture</td><td>From the water soluble test item a stock solution was prepared (0.1031 g test item in 200 ml water). The following amounts of the stock solution were taken to prepare 100 ml of the test item solution to realise the demanded concentrations. If less than 100 ml were applied, the missing amount was filled up with deionised water.
<table>
<tr><th>ml of the stock solution</th><th>+ ml deionised water</th><th>Corresponding nominal test item concentration mg/kg soil (dw)</th></tr>
<tr><td>6.25
12.5
25
50
100</td><td>93.75
87.5
75
20
-</td><td>6.25
12.50
25.00
50.00
100.00</td></tr>
</table></td></tr>
<tr><td>Size, volume and material of test container</td><td>Glass vessels (volume 100 ml; diameter 5 cm) covered with plastic lids</td></tr>
<tr><td>Amount of artificial soil (kg)/container</td><td>30 g (wet weight) of the test substrate were filled in each vessel.</td></tr>
<tr><td>Nominal levels of test concentrations</td><td>6.25; 12.50; 25.00; 50.00 & 100.00 mg test item/kg soil (dw)</td></tr>
<tr><td>Number of replicates/concentration</td><td>5 (+1 without Collembola for measurement of soil moisture during the test and pH and soil moisture at the end of the test)</td></tr>
<tr><td>Number of Collembola/container</td><td>10</td></tr>
<tr><td>Light source</td><td>Artificial light</td></tr>
<tr><td>Test performed in closed vessels due to significant volatility of test substrate</td><td>No</td></tr>
</table>

Table A7.5.2.1/03-3 Test Conditions

Criteria	Details
Test temperature	20 +/- 2 °C
Water content	Start: 17.4 - 18.7% (46.1 - 50.4% of WHCmax) End: 18.1 - 18.8% (48.3 - 50.5% of WHCmax) (guideline requirement: 40 - 60% of WHCmax)
pH	Start: pH 5.53 - 5.57 End: pH 5.52 - 5.54 (guideline requirement: 6.0 +/- 0.5)
Adjustment of pH	No
Light intensity / photoperiod	Light intensity: start: 670 lux; 14 days: 614 lux; end: 703 lux (guideline requirement 400 - 800 lux) Light regime: 16 h light : 8 h dark
Relevant degradation products	Not measured

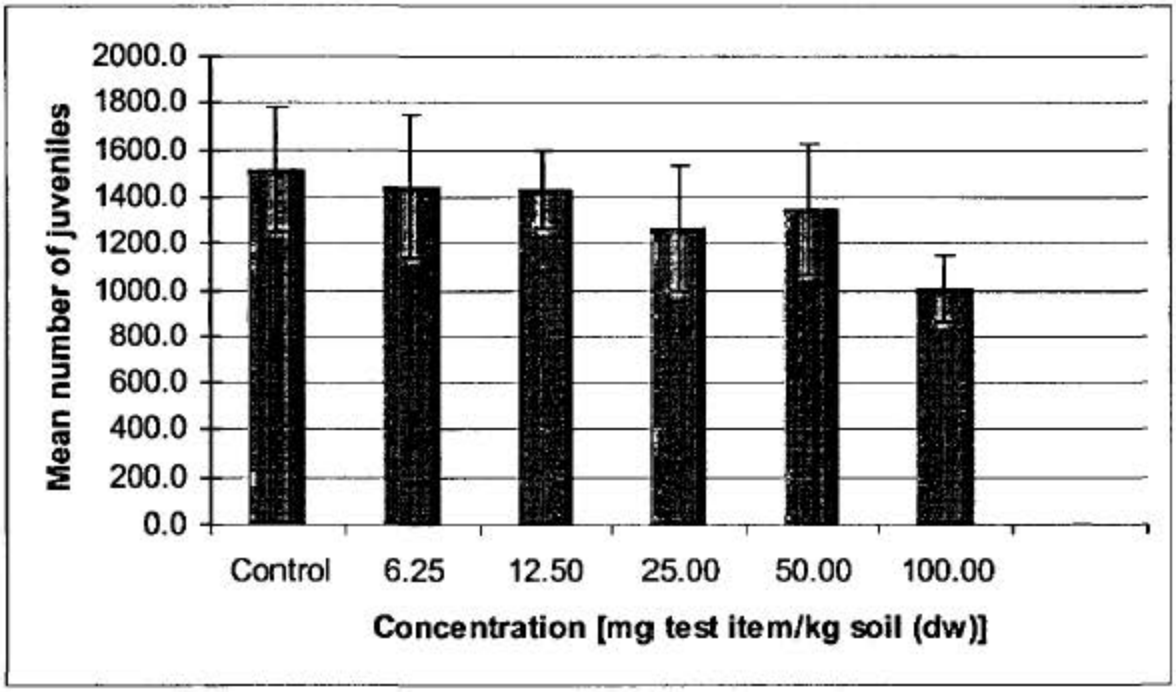
Table A7.5.2.1/03-4 Effects on Mortality and Reproduction of *Folsomia candida*

Test item	Deltamethrin EC25		
Test organism	*Folsomia candida*		
Test substrate	Artificial soil		
mg test item/kg soil (dry weight) nominal concentration	Adult mortality	Mean number of juveniles ± SD	Reproduction (% of control)
Control	4%	1515 ± 263	-
6.25	0%	1443 ± 304	95
12.50	2%	1428 ± 164	94
25.00	6%	1260 ± 269	83
50.00	10%	1348 ± 276	89
100.00	6%	1008 ± 146	67*
			Reproduction
NOEC (mg test item/kg soil (dry weight)) LOEC (mg test item/kg soil (dry weight))			50 100

* Statistically significantly different from deionised water control (Dunnett´s Test, one-sided-smaller, $\alpha = 0.05$).

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Not relevant
Materials and methods	**3.3.1:** It would have been preferred if the test concentrations were given for the active substance, deltamethrin, and not the formulation. **3.3.4:** RMS agrees with the approach to use 5% peat as OM with respect to the hydrophobicity of deltamethrin. This is also in line with OECD TG 232 (2009). **3.3.9:** It would have been preferred if the test concentrations were monitored during and by the end of the test, but RMS realises that this is problematic. This is also not required in the referred test guidelines or in the more recently adopted OECD TG 232 (2009).
Results	RMS has included a graph of the mean reproduction of Collembola as visualisation.
Conclusion	**5.3** The effect concentrations expressed as mg as/kg soil were as follows: The NOEC was 1.25 mg as/kg soil (dw) and the LOEC was 2.50 mg as/kg soil (dw).
Reliability	1
Acceptability	The study is considered to be acceptable. The test seems to have been well performed according to approved test guidelines. The validity criteria of the recently adopted OECD TG 232 (2009) were fulfilled.
Remarks	

Figure 1. Mean Reproduction of Collembola after 4 weeks



7.5.2.1 Reproduction study with other soil non-target macro-organisms

			Official use only
		1. REFERENCE	
1.1	**Reference**	Lechelt-Kunze, C. (2005) Deltamethrin EC25 G: Influence on mortality and reproduction of the soil mite species *Hypoaspis aculeifer* tested in artificial soil with 5% peat. [redacted] Document M-255821-01-1 7.5.2.1/04 11 August 2005 Unpublished	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Bayer CropScience AG	
1.2.2	Companies with letter of access	n.a.	
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**	Recommendations of the Hypoaspis Ring-test group (HASTE) 15th Annual SETAC Europe Meeting, May 22nd – May 27th, 2005 in Lille	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**	Deltamethrin formulated as EC 25	
3.1.1	Lot/Batch number	OP240000	
3.1.2	Specification	Deltamethrin formulated as EC 25	
3.1.3	Purity	-	
3.1.4	Composition of the product	Deltamethrin 26.49 g/L	
3.1.5	Further relevant properties	-	
3.1.6	Method of analysis	No analysis performed	
3.2	**Reference substance**	Dimethoate EC 400, trade name Perfekthion (392,72 g dimethoate/L) was used at 10 mg dimethoate/kg dry weight soil.	
3.2.1	Method of analysis for reference substance	No information reported	
3.3	**Testing/estimation procedure**	Deionised water was used as control	

<table>
<tr><td>3.3.1</td><td>Preparation of the test substance</td><td>All test item solutions were prepared freshly on the day of the application. Prior to the start of the test a stock solution (= solution 1) was prepared with 152.7 mg test item filled up to a volume of 250 ml with deionised water (1.78 mg deltamethrin/kg dry weight soil). The test item solutions were prepared as follows:

<table>
<tr><th>Solutions</th><th>Final volume 250 ml (filled up with deionised water)</th><th>Corresponding nominal test item concentration mg a.s./kg soil (dw)</th></tr>
<tr><td>1</td><td>152.7 mg Deltamethrin EC 25</td><td>1.78</td></tr>
<tr><td>2</td><td>140 ml of solution 1</td><td>1.00</td></tr>
<tr><td>3</td><td>140 ml of solution 2</td><td>0.56</td></tr>
<tr><td>4</td><td>143 ml of solution 3</td><td>0.32</td></tr>
<tr><td>5</td><td>141 ml of solution 4</td><td>0.18</td></tr>
</table></td><td></td></tr>
<tr><td>3.3.2</td><td>Application of the test substance</td><td>A uniform volume of 50 ml was used for all application solutions (starting with the lowest application rate and ending with the highest application rate). The test item was thoroughly mixed into 500 g artificial dry weight soil of each application rate using a laboratory mixer (Krefft). The control group was treated first in the same way as described above but with 50 ml deionised water only. Afterwards the treated soil of each application rate and the control will be portioned out. Each test vessel of the 5 replicates plus the one for measurement purposes will be filled up with 20 g avoiding compression of the soil. The remaining soil will be disposed.</td><td></td></tr>
<tr><td>3.3.3</td><td>Test organisms</td><td>Hypoaspis aculeifer (See Table A7.5.2.1/04-1 for details)</td><td></td></tr>
<tr><td>3.3.4</td><td>Test system</td><td>See Table A7.5.2.1/04-2.</td><td></td></tr>
<tr><td>3.3.5</td><td>Test conditions</td><td>See Table A7.5.2.1/04-3.</td><td></td></tr>
<tr><td>3.3.6</td><td>Test duration</td><td>16 days for exposure to the test item at 20 +/- 2°C plus 2 days of extraction of the mites</td><td></td></tr>
<tr><td>3.3.7</td><td>Test parameter</td><td>Mortality of adult female Hypoaspis in comparison to the initially placed test organisms expressed in % and the number of offspring hatched from the eggs and surviving until the end of the test period per test vessel (reproduction).</td><td></td></tr>
<tr><td>3.3.8</td><td>Examination</td><td>After a period of 16 days, the surviving adults and the living juveniles per test vessel were extracted, applying a temperature gradient. For this purpose the content of each test vessel was carefully transferred to sieve vessels (mesh size approx. 1 mm). Each sieve vessel was put onto another vessel containing a fixing liquid. The vessels were positioned in cooling water in a Berlese-apparatus. The temperature was increased from approximately 25 to 45°C within 2 days. Extracted mites were collected in a fixing solution (20% ethylene glycol, 80% deisonised water; 2 g detergent/L fixing solution were added). All Hypoaspis (adult, females and juveniles) were counted under a binocular.</td><td></td></tr>
<tr><td>3.3.9</td><td>Monitoring of TS concentration</td><td>No</td><td>X</td></tr>
</table>

3.3.10	Statistics	The software used to perform the statistical analysis was ToxRat Pro 2.09 (Ratte, 2002). Data of reproduction were tested for normal distribution and homogeneity of variance using Kolmogorroff-Smimov Test and Cochran-Test ($\alpha = 0.05$) respectively. Data of reproduction were normally distributed and homogeneity of variances was given, but data were not monotonous. Therefore Dunnett's Test (one-sided-smaller, $\alpha = 0.05$) was used to determine NOEC and LOEC values.	
		4. RESULTS	
4.1	**Test with test item**	In the control group 4% of the adult *Hypoaspis* died which is within the recommended range of ≤ 25% mortality. Fisher's Exact Test revealed no significant different mortality rates for all treatment groups. Concerning the number of juveniles statistical analysis (Dunnett's Test, two-sided, $\alpha = 0.05$) revealed no significant differences between the control and all treatment groups. Therefore the No-Observed-Effect-Concentration (NOEC) for mortality and reproduction is considered to be ≥ 1.78 mg deltamethrin/kg dry weight soil, the highest concentration tested. The Lowest-Observed-Effect-Concentration (LOEC) for mortality and reproduction is > 1.78 mg deltamethrin/ kg dry weight soil.	
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Ten adult, fertilized, female *Hypoaspis aculeifer* per replicate (5 replicates per application rate) were exposed to control (water treated), 0.18, 0.32, 0.56, 1.00, 1.78 mg deltamethrin/kg and 10 mg dimethoate/kg dry weight soil. The test item was applied by mixing into the soil. The *Hypoaspis* were of a uniform age not differing more than two days (30 days after start of egg laying). During the test, they were fed with cheese mites bred on brewer's yeast. During the study a temperature of 20 ± 2°C and light regime of 400 – 800 Lux, 16 h light: 8 h dark was applied. The artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis): 75% fine quartz sand, 5% Sphagnum peat, air dried and finely ground, 20% Kaolin clay and approximately 0.2% Calcium carbonate ($CaCO_3$). After a period of 16 days, the surviving adults and the living juveniles were extracted by applying a temperature gradient using a Berlese-apparatus. Extracted mites were collected in a fixing solution (20% ethylene glycol, 80% deionised water; 2 g detergent/L fixing solution were added). All *Hypoaspis* were counted under a Binocular.	