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ENDOSULFAN

Addendum Volume III

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CHAPTER B-9: Ecotoxicology

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ADDENDUM TO ANNEX B

ENDOSULFAN

B - 9: ECOTOXICOLOGY

B.9 Ecotoxicology

The notifier has presented a lot of information about ecotoxicity of endosulfan. Only the studies that have been considered relevant for the risk assessment have been described in detail by the rapporteur.

B.9.1. Birds

The notifier has presented three new reports about dissipation of endosulfan residues on terrestrial invertebrates under Southern conditions. The results of these studies have been summarised below:

Schäfer and Ebert, 2001

Field trials in cotton in Andalucía (Spain). Two applications of an emulsifiable concentrate of endosulfan at 0.840 kg/ha of the active ingredient per application. 14-day of application interval. Plant dwelling invertebrates were sampled using a D-Vac vaccum machine at day before 2nd application and at days 1, 3, 7 and 14 after 2nd application.

Soil-dwelling invertebrates were sampled by pitfall traps at day before 2nd application and at days 1, 3, 7 after 2nd application.

Plant debris were removed and animals were subdivided into animals > 5mm and < 5 mm. Animals were analysed for the total endosulfan residue (α, β and endosulfan sulfate).

Biotope Cotton field	Category of invertebrates	Total endosulfan residues on invertebrates (ppm)				
		-1 DAA	+1 DAA	+3 DAA	+7 DAA	+14 DAA
Plant zone	Small (<5mm)	1.2	73.1	32.7	3.8	3.4
	Large (>5mm)	0.8	9.5	2.9	1	0.8
	Mix (mass mean)	-	29.2	12.1	1.9	-
Soil zone	Small (<5mm)	-	-	-	-	-
	Large (>5mm)	<0.05	2.1	0.8	0.2	-

With these results the notifier has estimated a dissipation half-life in invertebrates of 1.42 days for all invertebrates.

Klein, 2001

Determination of endosulfan residues and their decline on ground dwelling invertebrates in a cotton field, southern zone. Field trial was in the main cotton growing area of Andalucía, Spain.

One application of formulated product at 840 g ai/ha. Samples were taken before the 1st application and at 1, 3 and 7 days after the 1st application. Samples were analysed for the total endosulfan (α, β and endosulfan sulfate).

Only large invertebrates (> 5mm) were investigated.

Analyte	Crop	Matrix	DAA	Amount of invertebrates g	Residues (mg/kg)
AE F002671	Cotton	invertebrate	-1	31	<0.02
AE F002671	Cotton	invertebrate	1	27	2.13
AE F002671	Cotton	invertebrate	3	62	0.77
AE F002671	Cotton	invertebrate	7	47	0.18

Klein and Martens, 2000

Determination of endosulfan residues and their decline on invertebrate prey of birds in field crops (cotton), southern zone (Andalucía, Spain).

Two applications of formulated product equivalent to 840 g ai/ha. Samples were taken for analysis one day before last application and 1, 3, 7 and 14 days after last application.

Samples of insects were analysed for residues of α, β-endosulfan, (α-AE F052618, β-AE F052619) and endosulfansulfate (AE F051327).

Results on insects from the treated plot are summarised in the table below:

Analyte	Crop	Matrix	DAA	Residues (mg/kg)
AE F051327	Cotton	Insects< 5mm	-1/14*	0.25
AE F051327	Cotton	Insects< 5mm	1	4.20
AE F051327	Cotton	Insects< 5mm	4	9.70
AE F051327	Cotton	Insects< 5mm	7	1.10
AE F051327	Cotton	Plant debris**	7	4.40
AE F051327	Cotton	Insects< 5mm	14	1.40
AE F051327	Cotton	Insects >5mm	-1/14*	0.12
AE F051327	Cotton	Insects >5mm	1	0.98
AE F051327	Cotton	Insects >5mm	4	0.59
AE F051327	Cotton	Insects >5mm	7	0.25
AE F051327	Cotton	Insects >5mm	14	0.33
AE F052618	Cotton	Insects< 5mm	-1/14*	0.37
AE F052618	Cotton	Insects< 5mm	1	33
AE F052618	Cotton	Insects< 5mm	4	9.10
AE F052618	Cotton	Insects< 5mm	7	0.85
AE F052618	Cotton	Plant debris**	7	2
AE F052618	Cotton	Insects< 5mm	14	0.63
AE F052618	Cotton	Insects >5mm	-1/14*	0.24
AE F052618	Cotton	Insects >5mm	1	3.70

Analyte	Crop	Matrix	DAA	Residues (mg/kg)
AE F052618	Cotton	Insects >5mm	4	0.79
AE F052618	Cotton	Insects >5mm	7	0.25
AE F052618	Cotton	Insects >5mm	14	0.13
AE F052619	Cotton	Insects< 5mm	-1/14*	0.66
AE F052619	Cotton	Insects< 5mm	1	51
AE F052619	Cotton	Insects< 5mm	4	22
AE F052619	Cotton	Insects< 5mm	7	1.90
AE F052619	Cotton	Plant debris**	7	6.90
AE F052619	Cotton	Insects< 5mm	14	1.50
AE F052619	Cotton	Insects >5mm	-1/14*	0.41
AE F052619	Cotton	Insects >5mm	1	4.90
AE F052619	Cotton	Insects >5mm	4	1.60
AE F052619	Cotton	Insects >5mm	7	0.51
AE F052619	Cotton	Insects >5mm	14	0.38

*= -1/14 means that the samples were taken 1 day before the 2nd application which is equivalent to 14 days after first application.

** = plant debris: this is the plant material (hairs from the leaves which came of the leaves during sucking the insects out of the crop with an D-VAC suction machine) which was separated from the insects <5mm.

B.9.1.1 Risk assessment on birds

All these values have been used by the rapporteur to refine the risk assessment of insectivorous birds. The initial values have been used to estimated the 90th percentile for the acute risk assessment; this results in a value of 51 ppm. Initial mean values have been used to calculated the short and long-term risk (mean value of initial residues was 18.52 ppm).

Based on these results, the TER values for birds exposed in cotton crops were estimated in accordance with the new Guidance document on risk assessment for birds and mammals under council Directive 91/414/EEC:

Table 9.1.1-1: TER estimations for acute, short-term and long-term studies in cotton.

Endpoint	Toxicity (mg/kgbw/day)	Exposure							TER
		FIR/bw	Init.Concentr	ftwa	PD	PT	Appl. rate	ETE	
Acute	28 mg/kg bw	1.04	51	-	0.5	1	0.84	22.27	1.25
Short-term	161 mg/kg bw/day	1.04	18.52	-	0.5	1	0.84	8.08	19.9
Long-term	30 ppm ≈ 4 mg/kgbw/d	1.04	18.52	0.09	0.5	1	0.84	0.72	5.55

These results showed a potential risk for acute toxicity on insectivorous birds exposed to residues of endosulfan. As the new guidance says " *PT and PD are very specific to both the crop and species*

chosen and therefore any resulting assessment will tend to be Member State specific". The rapporteur has information about the residues of endosulfan in insects of cotton crops; but the notifier has not presented a detailed refinement risk assessment based on the specific fauna of birds in cotton and tomatoes crops in southern European countries, so the final decision will need to be done at the individual Member state level.

B.9.2. Effects on aquatic organisms

B.9.2.1 Acute toxicity to aquatic organisms

B.9.2.1.1 Acute toxicity to fish

Metabolites

Gries and van der Kolk, 2000

^{14}C-endosulfansulfat (metabolite of ^{14}C-endosulfan): Acute toxicity test with rainbow trout under semi-static conditions. LC_{50} value (96 hours) = 0.82 µg ^{14}C-endosulfansulfat/l (0.63-1.2 95% C.I).

B.9.2.2 Chronic toxicity to aquatic organisms

B.9.2.2.1 Chronic toxicity to fish

Dionne, 2002

The chronic toxicity of endosulfan (98.7% active ingredient) to *fathead minnow* was assessed during a full life-cycle exposure. The study was developed in accordance with the standard procedures described in the Pesticide Assessment Guidelines (US.EPA, 1986).*Fathead minnow* were continuously exposed to five concentrations of endosulfan, a dilution water control and a solvent control for a complete life-cycle (260 days). All exposure levels were maintained in duplicate. In addition, their progeny (F_1) were continued in exposure for 30 days post-hatch.

The nominal exposure concentrations of endosulfan selected for the study were 0.038, 0.075, 0.15, 0.30 and 0.60 µg ai/l. Samples of the exposure levels were analysed at least once weekly throughout the exposure period. Based on these analyses, the mean measured exposure concentrations of endosulfan were defined as 0.030, 0.056, 0.11, 0.25 and 0.46 µg ai/l, which ranged from 74 to 82 % of nominal concentrations.

The results of the water quality parameters recorded during the full life-cycle exposure established that dissolved oxygen concentration, temperature, pH, total hardness, total alkalinity and specific conductance varied minimally between exposure aquaria and were unaffected by the concentrations of endosulfan tested. Throughout the 260-day study, the water quality of the test solutions remained within acceptable ranges for the survival, growth and reproduction of the fathead minnow.

Biological endpoints as hatching success, larval survival and larval growth for F_0 and F_1 were recorded. Control and solvent control data were first compared using a t-Test. If there was no statistical difference between control and solvent control data, control data were used for statistical analysis. If a significant difference was demonstrated between control and solvent control data, the solvent control were used for further statistical analysis to determine treatment effects.

F_0 embryos hatched by the fourth day of incubation at all treatment levels and controls. Hatching success ranged from 78 to 82% for embryos exposed to all treatment levels which was statistically similar to the pooled control (79%). Following 30 days post-hatch exposure, F_0 larval survival at the four highest treatment levels ranged from 91 to 98% which was comparable to the pooled control (96%). At the lowest treatment level (0.030 µg ai/l) survival was 88% which was significantly different from the pooled controls. Due to the absence of survival effects at higher concentrations and comparable survival to one replicate of the dilution water control, this survival reduction was not considered toxicant-related. The total length of larval fish exposed to all treatment levels ranged from 30 to 31 mm, which was statistically similar to the pooled control (30 mm).

Following 60 days post-hatch exposure, F_0 survival at the highest treatment level was 83%. Survival in one replicate was 74% and 92% in the other. Based on the treatment mean, survival was statistically reduced compared to the pooled control (96%), however the differences between replicates make it difficult to determine the toxicant response. At the three mid-concentrations, survival ranged from 91 to 97%, which was statistically similar to the pooled controls. The reduced survival at the lowest treatment level observed at 30 days post-hatch remained unchanged. The total length of larval fish exposed to all treatment levels (≤ 0.46 µg ai/l) ranged from 40 to 42 mm, which was statistically similar to the pooled control (41 mm). Wet weight of larval fish exposed to all treatments levels (0.64 to 0.79 g) was statistically similar to the pooled control (0.72 g).

Survival of F_0 adults between test day 64 and 178 was statistically reduced at the highest treatment level (80% versus 97% of the pooled controls). The total length and wet weight of male and female fish was unaffected at all treatment levels.

At test termination (260 days), both total length and wet weight of parental F_0 female fish exposed to treatment levels ≥ 0.11 µgai/l were significantly reduced. F_0 parental males growth was unaffected at all treatment levels. Survival was not evaluated since female mortality during the spawning period of the study is typical due to the aggressive nature of the male fish.

Significant differences were established at the three highest treatment levels for the number of eggs/spawn, spawns/female and number of eggs/female, when compared to the pooled control. Females exposed to 0.11, 0.25 and 0.46 µg ai/l produced 209, 353 and 243 eggs per female, respectively, which was significantly reduced compared to the pooled control (1182). The number of spawns per female for the respective concentrations was 3, 5 and 4, which was significantly reduced compared to the pooled controls (10). The eggs per spawn for the respective concentrations was 69, 68 and 59, which was significantly reduced compared to the pooled controls (114).

A statistically significant difference was indicated for the number of eggs per spawn at the lowest treatment level. Based on the limited amount of reproduction data collected from replicate B, coupled with the good performance of fish at the next higher concentration, the reproductive responses at the 0.030 µg ai/l treatment level were not considered to be related to endosulfan exposure.

Hatching success of F_1 embryos exposed to 0.03, 0.056, 0.11, 0.25 and 0.46 µg ai/l was 89, 85, 88, 84 and 67% which was significantly reduced compared to the solvent control (94%). The hatching success of embryos in the dilution water control was 89% which was also significantly less than the solvent control. Hatching success of embryos at the lowest treatment level (89%), although statistically less than the solvent control, was equal to the dilution water control. Therefore, the responses at the four lowest treatment levels were not considered to be biologically relevant.

Following 30 days of post-hatch exposure, F_1 larval survival at the 0.46 µg ai/l treatment level was 88%, which was significantly lower than the pooled control (95%). Survival at the remaining treatment levels ≤0.25 µg ai/l ranged from 86 to 93%. The survival of the F_1 larval fish exposed to 0.056 µg ai/l was 86% which was statistically different from the pooled control (95%). Since the survival in the next two higher concentrations was higher, this response was not considered toxicant-related.

Total length of larval fish exposed to all treatment levels was similar to the pooled control; however, wet weight of fish exposed to the highest treatment level was significantly reduced compared to the pooled control.

Considering all the information, the rapporteur proposes a NOEC of 0.056 µg ai/l, based on the significant effects of number of eggs/spawn, spawns/female and number of egg/female, when compared to the pooled control. The rapporteur agrees with the conclusion of the notifier that the effects on survival of the F_1 larval fish (30 days of post-hatch exposure) and hatching success of F_1 embryos exposed to 0.056 µg ai/l are not considered toxicant-related.

B.9.2.3 Field studies

Schanne, 2002

[^{14}C]-α,β-Endosulfan formulated as emulsifiable concentrate (352g/l endosulfan): outdoor aquatic microcosm study of the environmental fate and ecological effects.

The objectives of this freshwater field test were the following:

- Fate and relative distribution of 352 g/l EC formulated α,β-Endosulfan and its metabolites in major compartments of outdoor aquatic ecosystems after application as simulated realistic spray drift and run-off.
- Investigation of acute and sublethal effects on bluegill sunfish (*Lepomis macrochirus*) including fish residue analysis.
- Analysis of the community of sediment-dwelling organisms at test end, including residue analysis in these organisms and various compartments of the sediment.

The study was conducted outdoor in order to simulate the conditions in natural systems as closely as possible. For that purpose, sediment, water and other biota were collected from a large shallow water, natural reserve area from the Austrian part of the Lake Costance.

The test design was based on consensus methods proposed by experts at four meetings convened with Europe and North America (SETAC-Europe, 1991; SETAC/RESOLVE, 1991; EWOFFT, 1992; World Wildlife Fund/RESOLVE, 1992; Hill et al., 1994). In addition, the stipulations of the OECD draft guideline document "Freshwater Lentic Field Test" (OECD, 1996) were considered, as well as information provided by European Regulatory Bodies.

The study was conducted as a 7 concentration dose-response study with 4 control systems per application route between August and October 1998: [^{14}C]-α,β-Endosulfan was formulated as emulsifiable concentrate (352 g/l endosulfan THIODAN) and applied up to 3 times to 1 m^3 outdoor microcosm system stocked with 50 juvenile, caged bluegill sunfish. Treatments were performed in increments of two weeks. For spray-drift simulation, the formulation was sprayed homogeneously over the water surface. For run-off simulation, the formulation was applied onto a soil layer, which was aged for one day and applied as soil slurry over the water surface. The identification of the test groups is based on the target concentrations of 0.27, 0.47, 0.84, 1.51, 2.68, 4.64 and 8.38 µg /l for the spray drift application and 0.21, 0.42, 0.84, 2.09, 4.19, 6.29 and 8.39 µg /l for the run-off application.

The following table summarizes the nominal treatment levels, based on the concentrations measured in the stock solutions, given as average per treatment:

Test group	**SD-0.27**[3]	**SD-0.47**[3]	**SD-0.84**[3]	**SD-1.51**[3]	**SD-2.68**[3]	**SD-4.64**[2]	**SD-8.38**[1]
Concentration (µg ai/l)	0.34	0.55	1.16	1.96	3.50	6.40	10.33
Concentration (µg EC/l)	1.03	1.67	3.53	5.96	10.64	19.45	31.4
Drift rate (% of the MRFR)	0.4%	0.7%	1.4%	2.3%	4.2%	7.6%	12.3%
Test group	**RO-0.21**[3]	**RO-0.42**[3]	**RO-0.84**[3]	**RO-2.09**[3]	**RO-4.19**[3]	**RO-6.29**[2]	**RO-8.39**[1]
Concentration (µg SR/l)	0.21	0.42	0.84	2.09	3.99	6.29	8.39
Concentration (µg EC/l)	0.64	1.28	2.55	6.35	12.13	19.12	25.5
Run-off rate (% MRFR)	0.05%	0.1%	0.2%	0.5%	1%	1.5%	2%

[1,2,3] one, two, three treatments at intervals of two weeks; SD; Spray-drift; RO: Run-off; SR: Soil residue after one day ageing (=total endosulfan + metabolites (if any)), EC: Emulsifiable Concentrate (Thiodan 352g/l); MRFR: maximum Recommended Field rate; ai: active ingredient.

Regular observations and sample collection was conducted for 6 weeks. At test end, large samples of water, sediment, macrophytes and tank wall periphyton were collected in order to calculated a mass balance. Furthermore, sediment cores were subdivided into various layers. From these, the residue in the water-sediment interface, pore water, sediment and sediment dwelling organisms were analysed. The populations of sediment-dwelling organisms were taxonomically investigated. All samples taken during the test and at test termination were analysed for their total radioactive residue. Selected samples were characterized by C_{18}-HPLC-UV/RAM and radio-TLC.

During the first approx. 6 hours after each treatment, the total radioactive residue in water (TRR_{water}) showed a gradient from the subsurface water to the deeper water layers. This was mainly seen after spray-drift entry. After run-off entry, a similar gradient was observed, however less prominent. However within 24 hours after each treatment, the TRR was similar at all water levels. Based on the average TRR_{water} the following maximum concentrations were measured:

Test group	**SD-0.27**[3]	**SD-0.47**[3]	**SD-0.84**[3]	**SD-1.51**[3]	**SD-2.68**[3]	**SD-4.64**[2]	**SD-8.38**[1]
Concentration (µg ai/l)	0.34	0.55	1.16	1.96	3.50	6.40	10.33
1st treatment	0.36	0.88	0.98	2.35	4.33	9.17	9.4
2nd treatment	0.56	1.62	1.92	4.81	8.08	20.83	-
3rd treatment	0.49	1.03	1.85	3.85	8.94	-	-
Test group	**RO-0.21**[3]	**RO-0.42**[3]	**RO-0.84**[3]	**RO-2.09**[3]	**RO-4.19**[3]	**RO-6.29**[2]	**RO-8.39**[1]
Concentration (µg SR/l)	0.21	0.42	0.84	2.09	3.99	6.29	8.39
1st treatment	0.19	0.33	0.63	1.52	3.03	4.57	6.07
2nd treatment	0.26	0.74	1.04	2.62	5.37	8.94	-
3rd treatment	0.43	1	1.90	3.89	9.13	-	-

[1,2,3] one, two, three treatments at intervals of two weeks; SD; Spray-drift; RO: Run-off; SR: Soil residue. Test Conc: Average water concentration per treatment based on total radioactivity applied to the enclosures.

The TRR_{water} decreased constantly with time, quite fast during the first days after each treatment and more slowly towards day 42 (test end): about 40% of the maximum TRR_{water} had disappeared from the water. The corresponding DT_{50} values were calculated as 71 days (spray-drift) and 102 days (run-off). A minor part of this residue was associated with the suspended particulate matter (0.8 and 8.9%). Apart from several minor components, the dissolved radioactivity consisted of α- and β-Endosulfan, 4 known and 2 unknown distinct components.

Based on the experimental data the following DT_{50} values were calculated taking the day of maximum concentration as day 0 into account:

Residue	Spray-Drift DT_{50} (days)	Run-off DT_{50} (days)
α,β-Endosulfan	0.2 to 0.7	0.9 to 3
α-endosulfan	0.3 to 0.6	1 to 2
β-Endosulfan	0.4 to 0.6	0.3 to 2
Endosulfan diol	8 to 13	8 to 14
Endosulfan hydroxy ether	13	10

The concentrations of endosulfan lactone, M1 and M4 in water increased constantly during the study, whereas endosulfan sulfate was more or less constant at a low level or slightly decreasing at both entry routes. The total radioactive sediment residue ($TRR_{sediment}$) was increasing during the study to maximum 13.8 µg peq/kg. The same is valid for all components of the residue. The total radioactive residue in macrophytes ($TRR_{macrophytes}$) increased constantly during time to maximum 2236 µg peq/kg

fresh weight. Like for macrophytes, the total radioactive residue in surviving fish (TRR_{fish}) was high at maximum 3960 µg peq/kg fresh weight. The following table summarizes the percent contribution of the metabolites to the corresponding TRR:

Unit	**%**							
Identity	**TRR[1] water**		**TRR[2] sed**		**TRR[3] macrophyte**		**TRR[3] fish**	
Test group	**SD-2.68**	**RO-4.19**	**SD-2.68**	**RO-4.19**	**SD-2.68**	**RO-4.19**	**SD-2.68**	**RO-4.19**
M1	16.7	26.2	0.9	1.1	ND	ND	8-13	12-16
M5	ND	ND	ND	ND	ND	ND	16-25	21-27
Endosulfan diol	26.3	28	38.3	19.7	18.9	13.4	2-3	1-2
Endosulfan hydroxy ether	19.2	17.4	15.3	6	9.7	8.2	1-3	4
Endosulfan lactone	23.4	17.4	8.7	5.1	ND	ND	ND	ND
M4	3.9	3.8	0.7	1.2	ND	ND	ND	ND
Endosulfan sulfate	4	4.8	25.6	23.7	16.7	22.3	41-49	39-47
β-Endosulfan	ND	ND	5.4	20.5	0.9	0.9	8	4-7
α-endosulfan	ND	ND	5.1	20.9	2.9	0.9	5	4
α,β-Endosulfan	ND	ND	10.5	41.3	3.8	1.8	12-13	8-12
M6	ND	ND	ND	ND	1.9	13.5	ND	ND
M7	ND	ND	ND	ND	7.8	6	ND	ND
M8	ND	ND	ND	ND	5	4.2	ND	ND
M9	ND	ND	ND	ND	26.9	19.7	ND	ND

ND not detected; SD: Spray drift; RO: Run-off; [1] test end (days 42/43); [2] day 35/34; [3] at maximum residue level.

At test end almost half of the total residue had disappeared from the ecosystems (spray-drift and run-off, all test levels). The remaining radioactive residue was distributed as follows:

Compartment	**Percent applied**
Water	44.3-61.1 %
Sediment	2.9-16.2 %
Macrophytes	3.8-14.4 %
Tank wall periphyton	< 2.5%
Fish	<1.1%
Difference to 100%	20.9-43.6%

The results obtained for fish mortality showed a steep dose-response. After treatment with 3.99 µg soil residue/l 98% of all fish died within 2 weeks. After spray-drift entry, all fish died latest within few days after the 3rd treatment with average 3.50 µg ai/l per treatment. At higher single dose treatment rates, all fish died within few days after treatment. After triplicate treatment with average 1.96 µg ai/l or 2.09 µg soil residue/l per treatment and below, no test item related mortality was observed. Furthermore, growth

and length of the fish were not affected at these levels. The following table summarized the findings of lethal and sublethal effects (entire system concentrations, average per treatment):

Test system	**NOEC**	**LOEC**
	µg ai/l	**µg ai/l**
Spray-drift entry route	1.96***	3.50*** 3.50** 3.50*
	µg SR/l	µg SR/l
Run-off entry route	2.09***	3.99*** 3.99** 3.99*

SR Soil residue; *** Triplicate treatment at 14 day intervals; ** Duplicate treatment at 14 day intervals; * Single treatment.

The average lethal body load for bluegill sunfish was minimum 2.214 mg peq/kg and maximum 4.410 mg peq/kg. The majority of the residue in fish was represented by α,β-Endosulfan and endosulfan sulfate. The proportion of α-Endosulfan was higher than β-Endosulfan. This is in contrast to the residue in surviving fish, where β-Endosulfan was the major isomer.

The analysis of the sediment residue at test end indicated, that the majority of the residue was found in the top centimetre of the sediments (all test groups and both entry routes). Sediment contamination was higher after run-off due to deposition of treated soil particles. A minor part of the residue was found in the pore water (maximum 4.18 µg peq/l), whereas the majority was associated with the sediment (maximum 64.60 µg peq/kg). A continuous residue gradient was found from the overlaying water to the deeper sediment layer for both pore water and sediment associated residues. The residue found in the sediment-dwelling organisms community indicated that the communities of oligochaetes and detrivorous/predatory chironomids were not affected up to the highest test level. The results are summarized as follows (entire system concentrations, average per treatment):

Test system	**NOEC for sediment organisms**	**LOEC for sediment organisms**
	µg ai/l	**µg ai/l**
Spray-drift entry route	3.50*** 6.40** 10.33*	>3.50*** >6.40** >10.33*
	µg SR/l	**µg SR/l**
Run-off entry route	3.99*** 6.29** 8.39*	>3.99*** >6.29** >8.39*

SR Soil residue; *** Triplicate treatment at 14 day intervals; ** Duplicate treatment at 14 day intervals; * Single treatment.

The biological diversity (taxonomic richness) of sediment-dwelling organisms was slightly lower than in a natural lake environment: 6 to 10 different determination groups (i.e. individual taxa and selected groups of organisms that were analysed together) versus 14 in the lake. A comparison of the physical-chemical parameters in the test systems and at a comparable lake environment indicated similar conditions. Particular the pH were comparable at approximately 8 to 9.

The results lead to the conclusion, that the residue of endosulfan and its metabolites disappears from the water phase with time due to volatilisation after treatment (spray-drift), biodegradation and distribution to other compartments of the ecosystem. This is valid for both entry routes. Endosulfan, endosulfan diol and endosulfan hydroxy ether disappear rather fast from water, whereas other components like endosulfan lactone, M1 and M4 increase with time but stay at low levels throughout the study. Endosulfan sulfate is found at about constant, but low levels in the water. All of the above components are found in sediments and plant materials at different amounts, depending on the matrix and the total residue. The residue of endosulfan in the sediment is higher after run-off, due to deposition of treated particles onto the sediment surface.

The Ecologically Acceptable Concentration (EAC) for toxic effects of endosulfan 352 g/l EC formulation on bluegill sunfish (*Lepomis macrochirus*) is 1.96 µg ai/l after spray-drift entry and 2.09 µg soil residue/l after run-off entry (triplicate treatment at increments of 14 days). The Ecologically Acceptable Concentration (EAC) for toxic effects on sediment-dwelling organisms is 3.50 µg ai/l after spray-drift and 3.99 µg soil residue/l after run-off entry for triplicate treatment scenario at increments of 14 days. The EAC for toxic effects on sediment-dwellers after a single dose treatment is 10.33 µg ai/l (spray-drift) and 8.39 µg soil residue/l (run-off).

The rapporteur deems that this study clearly demonstrates that endosulfan is degraded to metabolites that maintain the chlorinated cyclic structure of endosulfan. These metabolites have the potential of bioaccumulation in fish and macrophytes, and some of them have been demonstrated their potential for persistence in the environment. In addition to this, the study reveals that there are other unknown metabolites with the same potential of bioaccumulation.

The rapporteur has estimated the bioaccumulation factors for spray-drift and run-off routes:
BCF total radioactivity ≈ 1000.
BCF endosulfan-sulphate = 4600 ≈ 5000 (spray-drift)
BCF endosulfan-sulphate = 2900 (run-off)
BCF macrophytes for endosulfan-sulphate = 1000 (spray-drift)
BCF macrophytes for endosulfan-sulphate = 750 (run-off)

Considering the overall information, the rapporteur concludes that this study only allows to estimate the risk of active ingredient, not the metabolites. The rapporteur also concludes that an Ecologically Acceptable Concentration can not be determined based on the results of this study due to the long-term effects of the metabolites can not be established.

Kay, 2001 (See section 8.4.1.2.2 on Fate)

Characterization and analysis of potential vulnerability of aquatic habitats near agriculture. Volume I: Sevilla, Spain.

This study was designed to characterize and assess the relative vulnerability of aquatic habitats associated with agricultural production in the European Union (EU) in order to provide information that can be incorporated into a pesticide risk assessment for the insecticide endosulfan. Information relating to the interaction of various crops and water bodies in the agricultural landscape was generated through analysis of a Land-Use / Land-Cover (LU/LC) classification derived from satellite and aerial imagery, other digital environmental data sets, and hardcopy maps of the study sites. This document discusses methods and results for a study site near Sevilla in Spain. A similar study was conducted at a site in Greece and is reported separately in Volume II.

After examining appropriate markets in the EU for endosulfan, a number of geographically distributed *study areas* associated with target crops of interest were identified. These generalized study areas were reviewed to identify specific *study sites* that maximized the interaction between target crops and surface water and for which suitable imagery could be obtained.

For the Spain study site, in addition to satellite imagery (termed *medium resolutio*n, pixel size = 10m /20m), a statistically significant sample of smaller areas within the study site were identified to further represent areas of significant interaction between crops and water. High-resolution aerial images (pixel size ˜0.5m) were obtained for each of these areas to permit more detailed analysis at what is termed *fine resolution* in this report.

The composition of land-covers surrounding surface water was examined using the medium resolution imagery. These analyses quantified the proportion of target crop(s) in close proximity to water, and the proportion of surface water in close proximity to target crop. Crop composition was characterized in terms of various *margin* distances and was investigated separately for each water body type.

Buffers were characterized in terms of their width and composition as these factors have the potential to significantly mitigate drift and runoff of pesticides to water bodies. The fine resolution imagery was used to characterize the areas between target crops and aquatic habitats (defined as *buffer*s). The proximity of each water body to surrounding crop (within 20m) was examined utilizing the width of the buffers and the amount of direct adjacency and non-cropped perimeter. The perimeter composition and proximity results were refined to include the potential mitigation of certain land-covers in the buffer.

Understanding the potential for spray drift deposition on water bodies is a critical component of most insecticide risk assessments and this study examined drift potential in great detail. A sophisticated analysis to investigate the potential for drift based on the spatial distribution of target crops around water bodies was conducted. Individual water bodies were examined for potential drift from 8 wind directions to better understand the likely deposition arising from any specific wind direction.

Additionally, potential drift was calculated while taking into account the potential mitigation caused by naturally occurring vegetation between cropland and the water bodies. All drift results are expressed in terms of potential maximum concentrations in a water body.

This report documents the results of these spatial analyses for the study site in Spain. The results from this and the Greece study site will be integrated and examined in terms of potential ecological risk in a separate report.

B.9.2.4 Risk assessment for aquatic organisms

The notifier has presented a large number of studies in order to refine the risk assessment for aquatic organisms. This information includes new studies to investigate the fate of endosulfan and its metabolites in water/sediment compartments, a microcosms study, and a set of reports to demonstrate the relevance of different routes of exposure (spray drift versus run-off), and the relevance of different water bodies in relation to the use of endosulfan in cotton and tomatoes crops.

All this information confirms that the identified metabolites still maintain the chlorinated cyclic structure of endosulfan; the toxicity pattern of these metabolites are still of concern for the rapporteur. On the other hand, the fish microcosm study confirms the persistence and potential for bioaccumulation of metabolites maintaining the chlorinated endosulfan structure and the production of additional non identified metabolites also bioaccumulated in fish and other aquatic organisms.

The overall conclusion is that rapporteur can not made a complete risk assessment of aquatic organisms due to a lack of information of endosulfan identified and non-identified metabolites. The microcosms study can not allow the long-term risk assessment due to the existence of the metabolites that have the potential of persistence and bioaccumulation. This study only allows the estimation of the potential risk assessment of the active ingredient, not the metabolites.

Considering the results of microcosms study, the rapporteur has estimated the TER values using the PEC worst-case for cotton (three multiple application) of 11.20 µg ai/l. With this value, the TERs for fish are:

TER (1 m) = 1.96 / 11.20 = 0.17.
TER (30 m) = 1.96 / 0.28 = 7.

These results confirm the potential risk assessment for endosulfan.

B.9.3 Effects on other terrestrial vertebrates

B.9.3.1 Risk assessment

The new guidance document on Risk assessment for birds and mammals established, as indicator species for leafy crops, medium herbivorous mammal. A generic risk assessment for mammals based on this guidance has been made:

Table 9.3.1-1: TER estimations for acute and long-term studies in cotton.

Endpoint	Toxicity (mg/kgbw/day)	Exposure							TER
		FIR/bw	Init.Concentr	ftwa	PD	PT	Appl. rate	ETE	
Acute	10mg/kg bw	0.76	87	1.3	1	1	0.84	72.20	0.13
Long-term	2 mg/kgbw/day	0.76	40	0.53	1	1	0.84	13.53	0.14

A potential risk for mammals has been detected.

B.9.4 Effects on Earthworms

B.9.4.1 Field studies

Forster and Salaün, 2003.

Field study to evaluate the effects of endosulfan 35 EC on earthworms in a grass field in Cornwall, UK.

The study was conducted with three applications of the test item at three treatment rates. This report gives the results of the sampling carried out one occasion before application of treatments, and five subsequent occasions, up to one year (375 days) after the final application.

Application rates: control, (water), 28.6 g ai/ha Endosulfan 35 EC, 490 g ai/ha Endosulfan 35 EC, 840 g ai/ha Endosulfan 35 EC, 4000 g ai/ha carbendazim (reference item) applied on first application occasion only.

Earthworms were sampled using formaline once before treatment and approximately 7 days, 1 month, 4-5 months, 6-7 months and 12 months after the third application of treatments.

The rapporteur concludes that, during the duration of this study, endosulfan 35 EC applied at a rate of 28.6 g ai/ha had no detrimental effect on earthworm populations.

Metabolites

Sowig, 2001

A study on the acute toxicity of endosulfan-sulphate to earthworms of the species *Eisenia fetida* was conducted in an artificial soil test according to OECD guideline 207 and EU guideline 92/69/EWG under GLP. Two different tests were conducted in order to established LC50 and NOEC.

The results of these tests showed that the LC50 14 days was 51.5 mg/kg (32-56 mg/kg 95% confidence limits). Since in the first test no NOEC was achieved the test was repeated with a lower range of dosages. In the second study, the NOEC after 14 days test duration was estimated to be less than 1 mg endosulfan-sulfate/kg dry weight.

B.9.4.2 Risk assessment soil organisms

The fate section of this addendum has established that: " *All identified metabolites contain non-altered hexachlornorborene byclicle. The mineralisation of endosulfan is <5%. Main metabolite is endosulfan*

sulphate that has a mineralisation between 11.01% and 13.08%. These facts suggest a potential high persistence of a soil residue constituted by a number of chlorinated metabolites, which may not account individually for more than 10% of applied dose but that all together may represent high amount of it. Based on their chemical structure it may be expected that the physicochemical properties of these compounds will be similar and generally persistent and bio-accumulable. Therefore, a wider investigation of degradation routes of this compound must be done".

The study of aerobic degradation of endosulfan sulphate (Schnöder, 2002 (C019647)) showed an unknown metabolite that appeared at level above 10% AR, all the attempts made for the identification of this metabolite failed but as a likely structure, a dicarboxilic acid, dihydrodiol metabolite is suspected. The fate section of this addendum concludes that *" The identification of this metabolite is essential for the definition of the degradation route of endosulfan, for the residue definition and for the field dissipation studies required*".

Based on these conclusions, the rapporteur can not developed a complete environmental risk assessment of endosulfan for soil organisms.

B.9.5 Effects on other soil non-target macro-organisms

Klein, 2003.

Effects of endosulfan 352 g/l (nominal) on the decomposition of organic matter enclosed in the litter bags in the field.

The purpose of this study was to investigate possible side-effects of endosulfan 352 g/l on the decomposition of organic matter in the field. A field experiment lasting about seven months was performed. The application rates were:

Drift rate:

1st application: 10.13 g product/ha (according to the long term PEC of the drift rate calculated for a depth of 20 cm)

2nd application: 85.8 g product/ha (according to the seasonal drift rate applied at one application).

Field rate:

1st application: 303.75 g Product/ha (according to the long term PEC of maximum application rate calculated for a depth of 20 cm)

2nd application: 2520 g product/ha (according to the seasonal rate applied at one application).

The conclusion of the study was that endosulfan 352 g/l do not cause an adverse impact on organic matter breakdown under field conditions.

B.9.6 Effects on soil non-target micro-organisms

Metabolites

Karl-Heinz Reis, 2002

Effects of endosulfan-sulfate on the activity of the soil microflora in the laboratory. Two concentrations (1.12 and 11.2 mg/kg soil dry weight) were applied. Based on the results of this study, it can be concluded that it does not have any long term influence on soil microflora when endosulfan-sulfate applied up to 11.2 mg/kg soil dry weight.

B.9.7 Effects on other non-target organisms (flora and fauna) believed to be at risk.

Menne and Pallet, 2001

Evaluation of the efficacy of the insecticide endosulfan on non target plants under greenhouse conditions.

Endosulfan was tested on its herbicidal activity in a standard screening test. The used method included five different crops and their specific weed spectrum of nine monocot and ten dicot plant species.

Conclusion: The insecticide endosulfan showed under greenhouse conditions very slight phytotoxicity symptoms on some plants species investigated. However all plant species recovered. In general with the standard dosage endosulfan was ineffective against the tested plant species.

B.9.8 Effects on biological methods for sewage treatment

Hertl, 2002.

Toxicity of endosulfan substance technical to activated sludge in a respiration inhibition test.

The test measured the respiration rate under defined conditions. The respiration rate (oxygen consumption) of an aerobic activated sludge fed with a standard amount of synthetic sewage was measured in the presence of various concentrations of the test item after an incubation period of 3 hours.

Test concentrations: 10, 32, 100, 320 and 1000 mg endosulfan/l; 3.2, 10, 32 mg 3,5-Dichlorophenol/l and two inoculum controls.

The results of this study showed that 3-hour EC20 and EC50 are clearly higher than 1000 mg/l under the present test conditions (limit of solubility = 0.63 mg/l for mixture of isomers).

Other documents presented by the notifier

The notifier has presented other documents that has been taken into account by the rapporteur; many of these studies has been described in detail in the environmental fate and behaviour section:

Author	Title	No. of Report
Ebert and Romijn	Dissipation of residues on insects. Refined risk assessment for birds and mammals in cotton and tomato (Southern Europe)	C017157
Aventis CropScience	Summary Comments to endosulfan background document on the working group on point and diffuse sources of OSPAR	C018038
Burkle, LW	Agricultural use of endosulfan in cotton: development of a Landscape-based aquatic risk assessment strategy in Spain	C018176

Author	Title	No. of Report
Waltersdorrfer, A	How does the endosulfan NTA fauna study in citrus support cotton use	C015296
Lerche et al.	Selecting chemical substances for the UN-ECE POP Protocol	C025221
Burkle, LW	Endosulfan- assessment against the PBT and vPvB criteria in the context of the European PBT strategy.	C028892
Burkle, LW	Summary of the aquatic ecological risk assessment of endosulfan usage on cotton in Spain.	C021461
Burkle, LW et al.	Aquatic ecological risk assessment for endosulfan uses on cotton in Spain: Part 1: Characterising risks associated with spray drift.	C021321
Mackay N	Estimations of run-off loadings and surface water exposure associated with representative endosulfan usage sites in southern Europe	C021146
Burkle, LW et al.	Aquatic ecological risk assessment for endosulfan uses on cotton in Spain-Part 2: Characterizing risks associated with run-off.	C021344
Mackay N	Site-specific evaluation of realistic usage conditions for endosulfan in Greece, Spain and Portugal	C021352
Mackay, N	Preliminary evaluation of potential study sites for a spatial analysis of agricultural interaction with the environment in southern Europe	C021352
Fischer, R	Endosulfan, risk to sediment dwellers	C022911
Burkle, LW	Aquatic ecological risk assessment of endosulfan usage on tomatoes in Southern Europe.	C022617
Mackay N and Burkle, LW	Aquatic ecological risk assessment for usage of endosulfan on cotton in Greece: Employment of remote sensing data to support candidate safe usage scenario for Annex I of Directive 91/414/EEC.	C023003
Waltersdorfer, A	Relevance of effects on bees after multiple application of AE F002671 00 EC33 C703	C022919
Burkle, LW	NMR investigation of a potential nter-conversion between two metabolites of endosulfan in the context of their acute toxicity: endosulfan lactone and endosulfan hydroxy carboxylic acid.	C023002

B.9.9 Referens on.

Annex IIA, or Annex IIIA point(s)	Year	Author (s) Title Company (insert name) Report No. Source (where different)	GLP GEP Y / N	Published Y / N	Owner	Data Protection

Annex IIA, or Annex IIIA point(s)	Year	Author (s) Title Company (insert name) Report No. Source (where different)	GLP GEP Y / N	Published Y / N	Owner	Data Protection
	2002a	Dionne E. Endosulfan: The Chronic Toxicity to the Fathead Minnow (Pimephales promelas) During Full Life-Cycle Exposure [redacted] Bayer CropScience; Document No: **B004189**	Y	N	Bayer	Y
	2001	Kay Characterization and análisis of potential vulnerability of aquatic habitats near agriculture-Volume I: Sevilla, Spain. Document number: C019473	Y	N	Bayer	Y
	2003a	Forster A., Salaun F. Field Study to evaluate the effects of Endosulfan 35 EC (AE F002671 00 EC33 C702 and AE F002671 00 EC33 C703) on earthworms in a grass field in Cornwall, UK Generated by: Ecotox Limited; Bayer CropScience LP; Ecotoxicology Document No: **B004269**	Y	N	Bayer	Y
	2002b	Hertl J. Toxicity of AE F002671 (endosulfan), substance technical, to activated sludge in a respiration inhibition test Code: AE F002671 00 1D99 0010 Generated by: IBACON GmbH, Rossdorf, DEU; Aventis CropScience GmbH, DEU; Ecotoxicology, Frankfurt Document No: **C018787**	Y	N	Bayer	Y
	2001g	Klein E. H.-J. Determination of endosulfan residues and their decline on ground dwelling invertebrates in a cotton field European Union, southern zone, 1998 Endosulfan emulsifiable concentrate 352 g/L Code: AE F002671 00 EC33 B332 Generated by: Aventis CropScience GmbH, DEU; Residues and Human Exposure, Frankfurt Document No: **C016146**	Y	N	Bayer	Y
	2000	Klein and Martens. Determination of endosulfan residues and their decline on invertebrate prey of birds in field crops (cotton) European Union, Southern zone, 1999. Code: AE F00267100 EC33 B332 Document number: C006600	Y	N	Bayer	Y
	2003a	Klein S. Effects of AE F002671 00 EC33 C704 on the decomposition of organic matter enclosed in litter bags in the field Generated by: IBACON GmbH, Rossdorf, DEU; Bayer CropScience GmbH, DEU; Ecotoxicology, Frankfurt Document No: **C032999**	Y	N	Bayer	Y

Annex IIA, or Annex IIIA point(s)	Year	Author (s) Title Company (insert name) Report No. Source (where different)	GLP GEP Y / N	Published Y / N	Owner	Data Protection
	2000	Gries and van der Kolk 14C endosulfansulfat: acute toxicity test with rainbow trout under semi-static conditions Document number: C016132	Y	N	Bayer	Y
	2002	Karl-Heinz Reis Effects of endosulfan-sulfate on the activity of the soil microflora in the laboratory Code: AE F051327 00 1B98 0006 Document number 022696	Y	N	Bayer	Y
	2001b	Menne H., Pallett K. Evaluation of the efficacy of the insecticide endosulfan (AE F002671) on non target plants under greenhouse conditions Code: AE F002671 Generated by: Aventis CropScience GmbH, DEU; Herbicide Research, Frankfurt Document No: **C017315**	Y	N	Bayer	Y
	2001a	Schaefer D., Ebert E. Dissipation of endosulfan residues on terrestrial invertebrates Code: AE F002671 Generated by: Aventis CropScience GmbH, DEU; Environmental Risk Assessment, Frankfurt Document No: **C016860**	Y	N	Bayer	Y
	2002a	Schanne C. (14C)-alpha, beta-endosulfan (AE F002671) formulated as emulsifiable concentrate (352 g/L endosulfan): Outdoor aquatic microcosm study of the environmental fate and ecological effects Generated by: Springborn Laboratories (Europe) AG, CHE; Aventis CropScience GmbH, DEU; Document No: **C019969**	Y	N	Bayer	Y
	2001d	Sowig P. Acute toxicity to earthworms (Eisenia fetida) Endosulfan-sulfat (AE F051327) substance, pure Code: AE 051327 00 1B99 0004 Generated by: Aventis CropScience GmbH, DEU; Oekotoxikologie, Frankfurt Document No: **C013030**	Y	N	Bayer	Y