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FINAL REPORT 1(500)

[^{14}C]-α,β-Endosulfan (AE F002671) Formulated as Emulsifiable Concentrate (352 g/l endosulfan): Outdoor Aquatic Microcosm Study of the Environmental Fate and Ecological Effects

Guidance Documents:
European Workshop on Freshwater Field Tests (EWOFFT), 1992

Hill, J. et al.: Fresh Water Field Tests for Hazard Assessment of Chemicals, 1994

OECD: Draft Proposal for a Guidance Document "Freshwater Lentic Field Tests", 1996

SETAC Guidance Document on Testing Procedures for Pesticides in Freshwater Static Mesocosms, 1991

SETAC/RESOLVE: Workshop on Aquatic Microcosms for Ecological Assessment of Pesticides, 1991

World Wildlife Fund/RESOLVE: Improving Aquatic Risk Assessment under FIFRA. Report of the Aquatic Effects Dialogue Group, 1992

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SUMMARY

The objectives of this freshwater field test were the following:

1. 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 surface run-off.
2. Investigation of acute and sublethal effects on bluegill sunfish (Lepomis macrochirus) including fish residue analysis.
3. 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 outdoors 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 Constance.

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 Tests" (OECD, July 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 (AE F002671) was formulated as Emulsifiable Concentrate (352 g/L endosulfan, THIODAN®) and applied up to 3 times to 1 m^3 outdoor microcosm systems 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 simlulation, 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%	1.0%	1.5%	2.0%

[1], [2], [3]: one, two or three treatments at intervals of 2 weeks; SD: Spray-Drift; RO: Run-off; SR: Soil Residue after one day ageing (= total endosulfan + metabolites (if any)); EC: Emulsifiable Concentrate (Thiodan 352 g/L); MRFR: Maximum Recommended Field Rate; a.i.: 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 calculate 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 was 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]
Test Conc. [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]
Test Conc. [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	1.0	1.90	3.89	9.13	-	-

[1], [2], [3]: one, two or three treatments at intervals of 2 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 ($TRR_{Macrophyte}$) increased constantly during time to maximum 2236 µg peq/kg fresh weight. Like for the 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-1.51**	**RO-2.09**
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	28.0	38.3	19.7	18.9	13.4	2-3	1-2
endosulfan hydroxy ether	19.2	17.4	15.3	6.0	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	4.0	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	6.0	ND	ND
M8	ND	ND	ND	ND	5.0	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 lastest 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 centimeter 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 were negligible at < 1.58 µg/kg. Analysis of the sediment-dwelling organism community indicated, that the communities of oligochaetes and detritivorous/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""	>3.50""
	6.40"	>6.40"
	10.33'	>10.33'
	[µg SR/L]	[µg SR/L]
Run-off Entry route	3.99""	>3.99""
	6.29"	>6.29"
	8.39'	>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 values 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 volatilization 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 disapear 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 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 entry and 3.99 µg soil residue/L after run-off entry for a 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).

1. OBJECTIVES

The objectives of this freshwater field test were the following:

1. *Determination of the relative distribution of endosulfan, applied both as simulated* realistic spray drift and as realistic surface run-off, in five major ecosystem components: water, suspended particular matter, sediment, fish and aquatic plants (macrophytes).
2. Measurement of acute effects of endosulfan on fish survival under natural exposure conditions.
3. Analysis of sublethal effects on fish.
4. Analysis of the community of sediment – dwelling organisms at test termination, including analysis of the residue in these organisms and various sediment compartments.

Spray drift and run-off are assumed to be the major pathways for potential contamination of surface waters with residues of a pesticide when used according to Good Agricultural Practice.

From the scientific data currently available, there are strong indications, that in real aquatic ecosystems, i.e. under natural conditions, and the influence of natural processes, the exposure of aquatic organisms may be considerably reduced when compared with the generic assumptions used in initial steps of the risk assessment.

Therefore, the study was conducted outdoors in order to simulate the conditions in natural systems as closely as possible. For the same reason, abiotic components (sediment, water) and biota were used from a natural lake, i.e. from Lake Constance. This lake is a representative Central-European freshwater ecosystem, with borders to Germany, Switzerland and Austria. The sediment and its associated waters were taken from the "Bay of Fussach" located on the Austrian shore of the lake, which is acknowledged to be typical for a large littoral area. The bay is part of the natural reserve area around the delta of the alpine river Rhine. About ¼ of the water used to establish the microcosms was taken from

the „Bay of Fussach". The remaining ¾ were obtained from a non-polluted lake site nearby Springborn's Testing Facility in Horn, Switzerland.

The test set-up and the endpoints were selected based on the available physico-chemical properties of the test item as well as on data on its environmental fate and ecotoxicological behaviour. The test design was defined based on information given by the Sponsor.

The study was conducted as a seven-concentration dose-response study with four control systems per application route (spray drift or run-off). Radiolabelled material was used in order to be able to monitor the fate and behaviour of the test item and its degradates in the compartments of the artificial ecosystem and fish, even at very low concentrations. For that purpose, [^{14}C]-α,β-Endosulfan (AE F002671) was formulated as Emulsifiable Concentrate (352 g/L endosulfan) and sprayed on the water surface (drift-simulation) or applied as 1-day aged soil slurry (run-off simulation) followed by regular chemical and biological sampling and monitoring.

Due to the lack of definitive written guidance under the European notification process and under national regulations, 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). The SETAC (1991) and EWOFFT (1992) documents are recommended by directive 96/12/EC amending European Commission directive 91/414/EEC. In addition, the stipulations of the OECD draft guideline document "Freshwater Lentic Field Tests" (OECD, July 1996) were considered, as well as information provided by European Regulatory Bodies.

The study was initiated on 31 July 1998, the date the study director signed the study plan and completed the study director signed the final report. The experimental phase of the study started on 31 July 1998 and the last analytical measurements were performed on 7 February 2001. The study was performed in the outdoor microcosm facility and the laboratories of Springborn Laboratories (Europe) AG, Horn, Switzerland.

Study plan and study plan amendment(s) are given under Annex XIV.