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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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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The study "[^{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 " has been performed in compliance with the Swiss Ordinance relating to Good Laboratory Practice, adopted February 2nd, 2000 [RS 813.016.5]. This Ordinance is based on the OECD Principles of Good Laboratory Practice, as revised in 1997 and adopted November 26th, 1997, by decision of the OECD Council [C(97)186/Final] (OECD 1998; Eidg. Dept. des Innern; Switzerland, 2000).

The following were not performed and reported under GLP.

1) Annex III: Installation and Equipment of the Testing Basins until July 31, 1998 (date of study plan signature)

2) Annex IV: Macrophyte Community and Biomass Assessment until July 31, 1998 (date of study plan signature)

3) Annex V: Pre-study Physical-chemical Monitoring of the Testing Basin until July 31, 1998 (date of study plan signature)

4) Annex VI: Collection and Supply of Zooplankton from Lake Constance (Food for Bluegill Sunfish)

5) Annex XII: Weather Record

Stability, characterisation, and verification of the test substance and analytical standards identity as well as maintenance of records are the responsibility of the Sponsor. Maintenance of a sample of the test substance and the analytical standards is the responsibility of the Sponsor. The raw data, the study plan and the final report will be kept in the archives of Springborn Laboratories (Europe) AG for at least 10 years. Samples and specimens will be retained at Springborn Laboratories (Europe) AG only as long as the quality of preparation permits evaluation.

SPRINGBORN LABORATORIES (EUROPE) AG

C. Schanné 15/Feb/2002

Dr. Clemens Schanné Date
Study Director

QUALITY ASSURANCE UNIT STATEMENT

The raw data and report for "[^{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" were inspected by the Quality Assurance Unit (QAU) at Springborn Laboratories (Europe) AG to determine adherence with the study plan and laboratory standard operating procedures. In addition, inspections of certain phases of the in-life portion of the study were performed. Dates and types of study inspections, dates reported to the Study Director and to Management are listed below.

The GLP Certificate of Springborn Laboratories (Europe) AG is given under Annex I.

Based on these inspections, it was determined that this report accurately reflects the raw data collected during this study.

Date of Inspection	Type of Inspection	Reported to Study Director	Reported to Management
24.07.98	Study Plan Review	24.07.98	31.07.98
31.07.98	Study Plan Review	31.07.98	31.07.98
26.08.98	In-Life Inspections	26.08.98	16.09.98
27.08.98	In-Life Inspection	27.08.98	16.09.98
10.09.98	In-Life Inspections	10.09.98	16.09.98
13.07.99	Data Books Audits	13.07.99	30.07.99
26.01.01	In-Life Inspection	26.01.01	28.01.01
05.02. -11.04.01	Data Books Audits	05.02. -11.04.01	30.04.01
28.11. -18.12.01	Data Books Audits	28.11. -18.12.01	03.01.02
21.01. -11.02.02	Data Book / Draft Report Audits	21.01. -11.02.02	13.02.02
12.02.02	Draft Report Audit	12.02.02	13.02.02
13.02.02	Final Report	13.02.02	13.02.02

SPRINGBORN LABORATORIES (EUROPE) AG

A. Schenk Feb 13, 2002

Ms. Astrid Schenk Date

Quality Assurance

SIGNATURES AND APPROVAL

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Feb. 13, 2002

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13. Feb., 2002

Dr. Héctor Galicia Date
Test Facility Management

TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES ANNEX III TO ANNEX X

LIST OF FIGURES

Page

LIST OF FIGURES ANNEX III TO ANNEX XIII

Page

LIST OF DOCUMENTS ANNEX III TO ANNEX XIII

Page

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.

2. MATERIALS AND METHODS

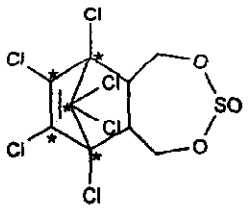
2.1 Test Item (Thiodan, i.e. 352 g α,β-endosulfan per litre; 329 g α,β-endosulfan per kg)

The study was conducted with radioisotopically diluted α,β-endosulfan formulated as Thiodan emulsifiable concentrate (352 g/L α,β-endosulfan). The following components were used to prepare the formulation: Radiolabelled α,β-endosulfan (2.1.1), the non-labelled analytical standard of α,β-endosulfan (2.1.2) and the blank formulation of Thiodan® (2.1.3).

The certifcates of analysis of all component were supplied by the sponsor and are given in Annex II.

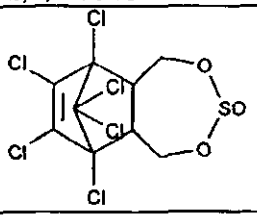
2.1.1 Radiolabeled active ingredient (α,β-endosulfan)

The radiolabelled test item was delivered by the sponsor and has the following characteristics:

Name of the test item	*[^{14}C]-endosulfan*
Structural formula / position of labelling (*) [U-6,7,8,9,10-^{14}C]-labelled	
Chemical name	6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9,-methano-2,4,3-benzo-dioxathiepin-3-oxide, alpha- and beta-isomer
Molar mass	406.92 g/ml (unlabelled)
Aventis code	AE F002671
Batch	Z28070-0
Specific radioactivity	3765 MBq/g (101.8 mCi/g)
Ratio of α and β endosulfan	69:31
Radiopurity as given by the supplier	99 % (radio-HPLC, radio-TLC)
Storage	at - 20 °C

Upon arrival at Springborn Laboratories (Europe) AG, the external packaging of the material was inspected for damage. The packaging was removed and the primary storage container was also inspected for leakage or damage. The sample identity was recorded and the material was stored under the conditions specified. A continuous documentation of the test material removed from the container was recorded in the raw data and the substance log book. The radiopurity was determined by C_{18}-HPLC-UV/RAM prior to use with the analytical method supplied by the sponsor.

2.1.2 Unlabelled analytical standard (α,β-endosulfan)

Common name	Endosulfan
Aventis/Hoechst Nos.	AE F002671, Hoe 002671
Aventis-Code	AE F002671 00 1B99 0007
Batch number	27946-30
Aventis certificate number	07479
Activity	Insecticide
Mode of action	Insecticide
CAS registry No.	115-29-7
Chemical name	6,7,8,9,10,10,hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzo-dioxathiepin-3-oxide
Chemical Structure	
Empirical formula	$C_9H_6Cl_6O_3S$
Purity (%)	99.3%
Ratio of α and β endosulfan	69:31
Expiration date (at -20 °C)	28 July 2002
Storage conditions	-20 °C

2.1.3 Blank formulation

Common name	Thiodan Blank Formulation
Batch	AE F 002671 00 EC 00 A 219
Storage conditions	Room Temperature

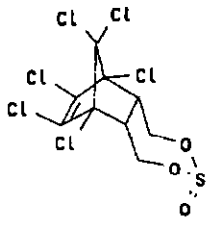
Upon arrival at Springborn Laboratories (Europe) AG, the external packaging of the unlabelled test item and the blank formulation was inspected for damage. The packaging was removed and the primary storage container was also inspected for leakage or damage. The sample identity was recorded and the material was stored under the conditions specified and used before the expiration date. A continuous documentation of the test material removed from the container was recorded in the raw data and the substance log book

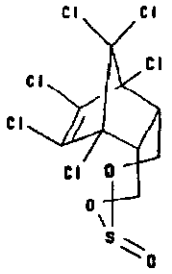
2.2 Analytical Standards (unlabelled)

The following analytical standards were used as reference compounds to facilitate identification of residual parent and its degradates formed during the course of the study. They were furthermore used to conduct method validation experiments. All compounds and data as delivered by the sponsor (Annex II).

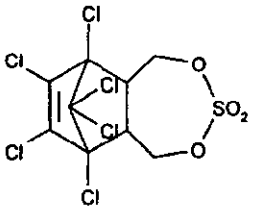
2.2.1 α,β-endosulfan

The unlabelled analytical standard as described in paragraph 2.1.2 was used.
General information concerning the two isomers is presented below.

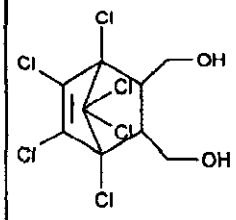
Common name	α-endosulfan
Aventis/Hoechst Nos.	AE F052618, Hoe 052618
Chemical Structure	
Empirical formula	$C_9H_6Cl_6O_3S$
CAS registry No.	959-98-8

Common name	β-endosulfan
Aventis/Hoechst Nos.	AE F052619, Hoe 052619
Chemical Structure	
Empirical formula	$C_9H_6Cl_6O_3S$
CAS registry No.	33213-65-9

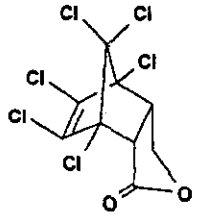
2.2.2 Endosulfan sulfate

Common name	Endosulfan sulfate
Aventis/Hoechst Nos.	AE F051327, Hoe 051327
Aventis-Code	AE F051327 00 1B98 0005
Batch numbers	42340 and CIW999
Aventis certificate number	07133 and 07693
CAS registry No.	1031-07-8
Chemical name	6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3,3-dioxide
Chemical structure	
Empirical formula	$C_9H_6Cl_6O_4S$
Purity	98.2% and 99.4%
Expiration date (at -20 °C)	26 November 2001 and 11 January 2001
Storage conditions	-20 °C

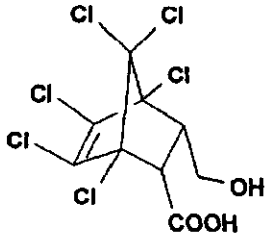
2.2.3 Endosulfan diol

Common name	Endosulfan diol
Aventis/Hoechst Nos.	AE F051329, Hoe 051329
Aventis-Code	AE F051329 00 1B99 005
Batch number	C02352073
Aventis certificate number	06446
CAS registry No.	2157-19-9
Chemical name	1,4,5,6,7,7-hexachloro-bicyclo-(2,2,1)-hept-5-ene-2,3-dimethanol
Chemical structure	
Empirical formula	$C_9H_8Cl_6O_2$
Purity	99.6% (w/w)
Expiration date (stored under storage conditions)	26 April 2000
Storage conditions	-20 °C

2.2.4 Endosulfan lactone

Common name	Endosulfan lactone
Aventis/Hoechst Nos.	AE F051328, Hoe 051328
Aventis-Code	AE F051328 00 1B99 0004
Batch number	8273X
Aventis certificate number	07642
CAS registry No.	3868-61-9
Chemical Structure	
Purity	99.6 % (w/w)
Expiration date (stored under storage conditions)	11 December 2002
Storage conditions	-20 °C

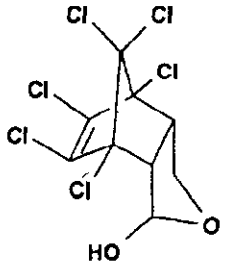
2.2.5 Endosulfan hydroxy carboxylic acid

Common name	Endosulfan hydroxy carboxylic acid
Aventis No.	AE 0365278
Aventis-Code	AE 0365278 00 1C59 0001
Chemical Structure	
Batch number	RSS1807

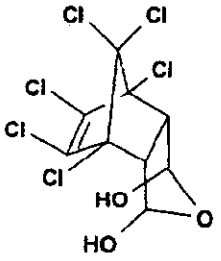
Aventis certificate number	08090
Purity	58.6 % (w/w)
Expiration date (stored under storage conditions)	17 September 2001
Storage conditions	-20 °C

* Contains AE F051328 (endosulfan lactone) as impurity : 39.8% (w/w)

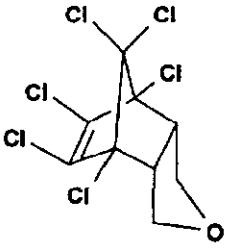
2.2.6 Endosulfan hydroxy ether

Common name	Endosulfan hydroxy ether
Aventis/Hoechst Nos.	AE F051326, Hoe 051326
Aventis-Code	AE F051326 00 1B96 0002
Chemical Structure	
Batch number	REW 1008-7
Aventis certificate number	07684
Purity	96.2 % (w/w)
Expiration date (stored under storage conditions)	8 January 2002
Storage conditions	-20°C

2.2.7 Endosulfan dihydroxy ether

Common name	Endosulfan dihydroxy ether
Aventis No.	AE 0035655
Aventis-Code	AE 0035655 00 1B98 0001
Chemical Structure	
Batch number	GH9826
Aventis certificate number	07483
Purity	97.9 % (w/w)
Expiration date (stored under storage conditions)	14 July 2000
Storage conditions	-20 °C

2.2.8 Endosulfan ether

Common name	Endosulfan ether
Aventis/Hoechst Nos.	AE F051330, Hoe 051330
Aventis-Code	AE F051330 00 1B99 0004
Chemical structure	
Batch number	23510
Aventis certificate number	06895

CAS registry No.	3369-52-6
Purity	99.2 % (w/w)
Expiration date (stored under storage conditions)	7 May 2001
Storage conditions	-20°C

Upon arrival at Springborn Laboratories (Europe) AG, the external packaging of the standards was inspected and stored as described above. The standards were dissolved in acetone, typically at concentrations of 1 mg/mL. The purity of the standards was checked during the study at regular intervals by comparison with the first chromatograms produced for the study.

2.3 Analytical Standards (radiolabelled)

The following radiolabeled analytical standards were supplied by the sponsor and used as reference compounds to conduct method validation work. All data as supplied by the sponsor.

2.3.1 [^{14}C]-α-endosulfan

Code (including the position of labeling)	AE F 052618-[6,7,8,9,10-^{14}C]
Lot	Z 25029-2
Specific radioactivity	2001 MBq/g
Storage conditions	- 20°C protected from light

2.3.2 [^{14}C]-β-endosulfan

Code (including the position of labeling)	AE F 052619-[6,7,8,9,10-^{14}C]
Lot	Z 27041-0
Specific radioactivity	3184 MBq/g
Storage conditions	- 20°C protected from light

2.3.3 [^{14}C]-endosulfan sulfate

Code (including the position of labeling)	AE F 051327-[6,7,8,9,10-^{14}C]
Lot	Z 25034-1
Specific radioactivity	2401 MBq/g
Storage conditions	Store under inert atmosphere at - 20°C protected from light

2.3.4 [^{14}C]-endosulfan diol

Code (including the position of labeling)	AE F 051329-[1,4,5,6,7-^{14}C]
Lot	Z 28025-0
Specific radioactivity	4147 MBq/g
Storage conditions	- 20°C protected from light

Upon arrival at Springborn Laboratories (Europe) AG, the external packaging of the radiolabelled materials was inspected and stored as described above. The radiopurity was determined by C_{18}-HPLC-RAM prior to use with the analytical method supplied by the sponsor. The certificates of analysis are provided under Annex II.

2.4 Test System: Outdoor Aquatic Microcosms

Enclosures made of stainless steel (diameter: 1.10 m, height: approximately 1.5 m) and equipped with equilibrated natural water-sediment, including biota taken from Lake Constance, were used as test systems. The sediment layer in the enclosures was about 20 cm and the water layer 1 m at the 1st treatment. The volume of the water column was approximately 1190 L. This lake is a representative Central-European freshwater ecosystem and a major drinking water reservoir, 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 site of the Lake of Constance nearby Springborn's Testing Facility in Horn, Switzerland.

The collection of water, sediment and biota as well as the establishment and equilibration of the systems until signature of the study plan are described in detail under Annex III. Furthermore, the characterisation data of sediment and water are provided under this Annex.

In addition to the macrophytes from the Lake of Constance, macrophytes as obtained from a commercial breeder were planted as integral part of the ecosystems. A detailed description of the planting and assessment of the macrophyte community and biomass prior to the signature of the study plan is given under Annex IV. All macrophyte related work conducted after the signature of the study plan is described under sections 3.2, 3.4 and 3.7 of this report.

2.5 Test Species: Bluegill Sunfish

Bluegill sunfish, *Lepomis macrochirus*, was selected by the study sponsor as a representative warm water fish species, to be used for the LC_{50} and NOEC determinations during the microcosm study. The laboratory LC_{50} for the active substance is 3.3 µg α,β-endosulfan per liter (Pickering and Henderson, 1966). With the batch of fish used in the experiment, acute toxicity studies following OECD requirements were conducted in parallel under separate study plans (SL projects 1049.008.140 and 1049.010.140). A detailed description of fish origin, maintenance, acclimation and feeding until randomisation and introduction into the test systems is given under sections 3.1 and 3.7.

2.6 Test Conditions

The study was conducted outdoor under field conditions in order to simulate the conditions in natural systems as closely as possible. Monitoring of the testing basin prior to signature of the study plan is provided under Annex V. All other determinations are provided under Annex X.

3. STUDY CONDUCT

An overall time schedule is provided under Table 1. Radioisotopically diluted endosulfan was formulated as 352 g/L (329 g/kg) Thiodan and applied 3 times to 1 m^3 outdoor microcosm systems stocked with 50 juvenile, caged bluegill sunfish. Treatments were performed in increments of two weeks. The first treatment took place on August 26, 1998. Two entry routes were simulated: spray drift and run-off. Each entry route was tested with 7 concentrations and 4 controls. For the spray drift entry, the formulation was sprayed homogeneously over the water surface. For the run-off entry, the formulation was sprayed homogeneously onto soil. The treated soil was aged for one day and suspended thereafter with water. The soil slurry was homogeneously distributed over the water surface of the microcosms.

After the applications, the following samples were taken at defined intervals (Table 2 to 5): water, sediment cores, macrophytes and fish. Fish populations were observed daily for mortality. Dead fish were removed from the cages. Weight and length of the fish, removed for residue analysis, were determined during the study. 6 weeks after the first application, the outdoor phase of the test was terminated: All remaining fish and macrophytes were removed as well as large samples of water, sediment and tank wall periphyton in order to calculate the mass balance and to analyse the communities of sediment organisms. Length and weight of all fish was determined.

All samples taken during the test and at test termination were analysed for their total radioactive residue. Selected samples were characterized by chromatographic methods, i.e. to determine the concentration of endosulfan and its degradates in the various compartments of the ecosystem.

At test termination, sediment cores were subdivided into various layers. From these, the residue in the water-sediment interphase, pore water, sediment and sediment-dwelling organisms were analysed. The populations of sediment-dwelling organisms were taxonomically investigated.

Some of the methods, which were used to conduct this study, e.g. the spraying procedure, certain sampling techniques and analytical methods were validated prior to the 1st treatment.

The detailed results of these experiments are provided under Annex VII of this report. All methods used to conduct this project are described below in detail.

3.1 Delivery, Acclimation and Maintenance of the Bluegill Sunfish until Application

The bluegill sunfish used for the test were obtained from Osage Catfisheries, Inc., Osage Beach, Missouri 65065, USA. The test organisms (approximately 1500 fish) were shipped to Springborn Laboratories (Europe) AG, Horn, Switzerland, where they arrived on July 31, 1998 in good conditions. The fish were transferred into a large cage (2 mm nylon net) prepared with a cover of the same mesh size and exposed to one of Springborn's outdoor basins. The majority of the acclimatized fish were transferred from the transportation vessels into this cage (Figure 9). The fish and the cage were transferred into the testing basin mid of August 1998. About 50 fish were transferred into Springborn's Testing Facility laboratory building with the purpose to conduct an acute LC_{50} study. This work was carried out under SL project number 1049.008.140.

During the pre-study acclimation phase, the caged fish were fed 2 times a day with a mixture of live brine shrimp (*Artemia nauplii*, in-house culture), concentrated zooplankton from the Lake of Constance (cf. Annex VI), commercial flake food and commercial trout starter. Apart from this addition of food, the natural zooplankton populations of Springborn's outdoor basins served as food during the pre-study period. Further details about the feeding regime after transfer of the test fish into the enclosures are provided under section 3.6.

3.2 Determination of *Myriophyllum spicatum* biomass

The biomass of the established population of *Myriophyllum spicatum* (c.f. Annex IV) was estimated as follows: Each *Myriophyllum* macrophyte was given a unique number. Furthermore a drawing was established providing the location of each *Myriophyllum* macrophyte with respect to a grid of the testing basin 1 (Figure 1). Thereafter, the length and diameter of each macrophyte was estimated and the volume was calculated from these data. Representative macrophytes, which occupied volumes of the low, medium and high categories, were removed from the basins and dried until a constant weight was reached and weighed again. Based on these data, a linear regression curve was calculated. This

curve was used to calculate the dry weight of each individual macrophyte based on its estimated volume. This work was conducted on August 5/6, 1998, i.e. just before placement of the enclosures. The data were used to select, from the existing population, those macrophytes with the most similar volume in order to provide similar biomass to each of the enclosures for the test. Representative pictures of *Myriophyllum* are provided under Figure 10. The estimated amounts and volumes of introduced *Myriophyllum* biomass are summarized under Annex VIII.

3.3 Placement of Enclosures into the Testing Basins

The enclosures, which were used to conduct this study, were stainless steel cylinders with a diameter of 1.10 m and a total height of 1.68 m. The upper edge of each enclosure exceeded the basins water spill over by about 10 cm in order to prevent water overflow from the inside out and vice versa. Each enclosure was equipped with 8 holes near the upper edge to enable e.g. fixing of transportation devices and sample collection equipment. The bottom edge was covered by rubber to prevent any exchange from the inside of the enclosure to the outer basin and vice versa.

The placement procedure was fully documented by video documentation and took place on August 7, 1998 using a large crane. Representative pictures are provided under Figures 11 and 12. A total of 22 enclosures was placed into the testing basin with a minimum distance of about 15 cm between each other. Each enclosure were thoroughly cleaned before placement into the testing basin. The enclosure was positioned at the required location within the testing basin: It was agreed with the sponsor to position the enclosures in a way, that the macrophyte was located towards West. After positioning with respect to the macrophytes, the enclosures were lowered into the water and finally the sediment. The enclosures were carefully pressed into the sediment until settling, in order to make sure, that the enclosure was placed on the bottom of the basin so that the sealing became effective.

3.4 Sampling, Preparation and Stocking of the Enclosures with Lake Born *Elodea canadensis*.

Elodea canadensis was collected from the littoral zone of Lake Constance on August 11, 1998. The collection site was located in the area of Horn harbour. Following collection from

the lake, the macrophytes were kept in a mixture of tap and lake water for 3 days. Thereafter, the macrophytes were bundled using stainless steel wires and transferred into the testing basins.

On August 20, 1998, the fresh weight of each bundle was determined and noted on a label. In order to provide quantitative data about the *Elodea* biomass introduced into each enclosure, an experiment was conducted using 14 *Elodea* bundles of different weight with the purpose to correlate the fresh weight and the dry weight after drying at 105°C for 24 hours.

Immediately prior to the first treatment, i.e. on August 24 1998, bundles of two size classes were selected: size class A (32 to 68 g fresh weight) and size class B (70 to 100 g fresh weight). Bundles of size class A received one Telfon ring and bundles of size class B received two Teflon rings. Thereafter, each enclosure was equipped with 3 bundles of size class A and 3 bundles of size class B with a similar total weight in order to equip each test system with a similar amount of Elodea biomass. The bundles were distributed evenly around the inside of each enclosure except in the area occupied by *Myriophyllum*. The estimated amounts and volumes of introduced *Elodea* biomass are summarized under Annex VIII.

3.5 Equipment of the Enclosures with Sample Collection Devices and a Fish Cage

A schematic drawing of a fully equipped enclosure is provided under Figure 2.

On August 13, 1998, each enclosure was equipped with a fish cage. This cage consisted of a 3 mm stainless steel cylinder with a closed stainless steel bottom. It's height was 1.25 m, its diameter 0.42 m. The cage was positioned into each enclosure as such that the bottom was located at a few centimeters above the sediment. For that purpose, that fish cage was hooked onto a stainless steel outer cage which had been carefully pushed into the sediment, down to the bottom of the testing basins. The fish cage equipment was ideally positioned as far as possible into the centre of each enclosure taking into account the location of the *Myriophyllum* macrophyte.

On August 20, 1998, each enclosure was equipped with the collection devices for water samples and wall-adsorbed radioactivity of which representative pictures are shown under Figures 13 to 15:

- One stainless steel device, equipped with 3 teflon tubes. One tube was located at about 10 cm above the sediment, one at about the middle water column and one at about 10 cm below the water surface. This collection device enabled the collection of water samples from the different water depths without disturbing the water surface. Each tube was individually labelled with the corresponding water level.

- 8 stainless steel strips (1 m long and 10 cm broad). These strips were regularly distributed around the walls of each enclosure. They were used to analyse the residue on the tank walls at the end of the study.

3.6 Introduction of the Test Fish into the Enclosures and Feeding until the First Application

On August 21, 1998, i.e. five and six days prior to treatment of the spray drift and run-off enclosures, respectively, the fish were counted and added to the enclosures. Most of the fish (approximately 95%) were netted out of the cage and placed into a 300 litres fibreglass tank which contained the same water as the impoundment. Any fish not deemed appropriate to be used in the test were removed from the tank (i.e., any fish which were obviously stressed or exhibited adverse behaviour such as darkened pigmentation or lethargy). In general fish were in excellent condition and only a few fish (<20, i.e. <1.5%) were removed. Twenty-three plastic containers, each containing approximately 20 L of water were prepared. Approximately 100 to 150 fish were netted out of the tank and into a bucket containing 10 L water. Fish were then netted from the bucket into the 20 L containers. Fish were added 5 at a time to each 20 L container until all 20 L containers contained 50 fish. One of the 23 containers was used to determine the mean weight and length of the fish. After length and weight had been determined, the fish were returned to the culture cage. The fish in the remaining twenty-two 20 L containers were assigned to the test enclosures. Most of the water was decanted from the container after which the fish were carefully poured into the enclosure. Representative pictures are given under Figure 16 .

The representative sample (N = 50) of fish, taken from the test population during randomisation, had a mean wet weight of 0.87 g (range 0.30 to 1.69 g) and a mean total length of 36 mm (range 30 to 47 mm). Based on the mean wet weight of the fish the loading of each enclosure was calculated to be 43.5 grams of biomass per enclosure (0.037 g/L). The fish were fed Artemia nauplii and concentrated zooplankton from the Lake Constance once or twice a day, except for Sundays. The amount of food, based on dry weight, corresponded to a total of approximately 3% of their total biomass. After the first application of the test item, about one quarter of test fish developed symptoms of a virus infection. Further details are described under Annex XIII.

3.7 Randomization of the Enclosures based on *Myriophyllum spicatum* Biomass and Assessment and the Location of the Fish Cage

As described above, 22 enclosures had been positioned in the testing basins 11 enclosures were assigned for each entry route, run-off and spray drift, respectively. Of each set of 11 enclosures, 7 enclosures were assigned for treatment and 4 as controls. As described above, the fish cages had been positioned in the centre of each enclosure. However, this was not always possible due to the location of the *Myriophyllum* macrophyte. It was decided upon prior consent with the sponsor, that those enclosure should be used for spray drift applications, in which the fish caging equipment was more or less in the centre. Hence two groups of enclosures were defined, one group for spray drift and one for run-off entry route testing. In order to reduce the risk, that the difference in macrophyte biomass (although small) would bias the results of the test, randomised numbers were associated to the enclosures of each group and the various treatment levels and controls planned for this group. This was done on August 25, 1998. Based of the randomisation, each enclosure was individually labelled with a permanently installed label (cf. Figure 3).

Figure 17 provides an overview of the testing basin, fully equipped with macrophytes, enclosures and sampling devices ready for the first treatment.

3.8 Determination of System Quality Parameters

System quality parameters and the water level were determined in all enclosures one day before the first treatment and during the conduct of the study, according to the schedule

provided in Tables 4 and 5. Furthermore a representative sample of water and sediment was removed from the testing basins immediately prior to treatment in order to characterize water and sediment according to the respective guidelines for water-sediment studies.

The measurements of pH, Dissolved Oxygen (DO), temperature, conductivity of the water were conducted at about half of the water depth if not particularly specified. The samples for the determination of N/P, alkalinity, hardness and TOC/DOC were collected as depth integrated samples.

The pH of the water and the sediment was measured using a pH meter (WTW Multiline P4) and a combined glass electrode. The dissolved oxygen concentration was measured with a dissolved oxygen meter and probe (WTW Multiline P4). Total hardness was measured by the EDTA titrimetric method and total alkalinity was determined by potentiometric titration to an endpoint of pH 4.5 (pH meter Metrohm 619). Specific conductivity was measured with a Metrohm Model E587 conductivity meter and probe. Further details about sample processing for the determination of alkalinity, hardness, TOC/DOC and Suspended Particulate Matter (SPM) are provided in section 3.11.2.2.

3.9 Shading and Aeration of the Enclosures prior to the 1st Treatment

During the pre-treatment acclimatisation period, an increase of the pH value was observed, most likely due to the proton depletion caused by photosynthesis of the established macrophyte flora and the phytoplankton. Due to the susceptibility of the test item and some of its metabolites to hydrolysis under alkaline conditions, it was decided in agreement with the sponsor, to take all natural means to achieve a decrease of the pH value below 9, particularly prior to the first treatment. For that purpose, the enclosures were shaded during the day and aerated during the nights. Shading was performed by large green shading covers equipped with a fine mesh, which allowed air exchange (Figure 18). During the nights, each enclosure was individually aerated. For that purpose, air was bubbled into the water at about half of the water column (Figure 19). Care was taken to deliver the approximate same air-flow into each enclosure. Further details are given under Annex V.

The physical-chemical characterization data one day prior the 1st treatment are provided under Annex VIII.

3.10 Applications Procedures

The application techniques described below were based on procedures as developed and validated prior to the first treatment. The results of this work are provided under Annex VII.

3.10.1 Spray drift

The test item, i.e. α,β-endosulfan formulated as 352 g/L EC formulation (Thiodan) was sprayed 3 times at 14-day intervals on the microcosm water surface by using an agricultural low-pressure sprayer. The following were the target concentrations in the water body of the microcosms per treatment: 0.27, 0.47, 0.84, 1.51, 2.68, 4.69 and 8.38 µg α,β-endosulfan per litre water. In order to reach these target concentrations an excess amount of about 40% of the target value was applied based on the pre-study validation experiments (Annex VII). All test groups were treated accordingly. The spray drift control enclosures were not treated at all.

The following table overviews, which treatment were performed:

Test group	Target a.i. concentration (a.i.)	Target product concentration (Thiodan)	1st Treatment	2nd Treatment	3rd Treatment
SD-0.27	0.27 µg ai/L	0.82 µg/L	X	X	X
SD-0.47	0.47 µg ai/L	1.43 µg/L	X	X	X
SD-0.84	0.84 µg ai/L	2.55 µg/L	X	X	X
SD-1.51	1.51 µg ai/L	4.59 µg/L	X	X	X
SD-2.68	2.68 µg ai/L	8.15 µg/L	X	X	X
SD-4.69	4.69 µg ai/L	14.26 µg/L	X	X	NP
SD-8.38	8.38 µg ai/L	25.47 µg/L	X	NP	NP

a.i.: active ingredient (α-endosulfan : ß-endosulfan = 69 : 31)

NP: not performed, in agreement with the sponsor

SD: Spray drift entry route

Basically, the spray solutions were prepared as follows: A stock solution of the radiolabelled active ingredient was prepared in acetone. A stock solution of the blank formulation was prepared indeionized (MilliQ) water. Appropriate aliquots of these stock solutions were

combined and made up with MilliQ water to the required spray volume. Each spray solution was prepared and quantified separately. The quality of these solutions was analysed prior to and after the applications (selected cases).

3.10.1.1 Preparation of stock and spray solutions

3.10.1.1.1 First application

A stock was prepared by weighing 41.6 mg of the radiolabelled test item (^{14}C-endosulfan, Aventis lot Z28070-0) into a 5 mL volumetric flask and dissolving this into 5 mL acetone (Scharlau, HPLC grade, 27156). Three times 20 µL of this stock solution was taken and diluted to 10 mL using acetone. Three times 50 µL of each dilution was added to Scintillation cocktail (Ready SafeTM, Beckman) to determine the concentration of total radioactivity present. Based on these LSC counts, the measured concentration of radioactivity in the stock solution, the actual amount of active ingredient in the stock solution was calculated to be 39.63 mg a.i. in 5 mL. The radiopurity was determined by analysing 10 µL of one of the dilutions by HPLC-RAM.

To prepare the Thiodan blank formulation, 99.8 mg of the blank formulation (SL-SRC code 7-006, Aventis code AE F002671 00 EC00 A219) was weighed into a 10 mL volumetric flask and diluted with Milli-Q water to 10 mL.

Since the spray solution for the highest test concentration was radioisotopically diluted with the unlabelled test item analytical standard, an additional stock solution was performed dissolving 32.9 mg unlabelled α,β-endosulfan (Aventis lot 27946-30) in 2 mL acetone.

Seven 20 mL volumetric flasks were prepared for the spray solutions. An appropriate aliquot of the 14C stock solution was introduced into each flask. Thereafter, a corresponding volume of the blank formulation emulsion was added. For the highest concentration (SD-8.38), an aliquot of the unlabelled stock was added. All spray solution was made up with water to the final volume of 20 mL resulting in the ready-to-use spray liquid. Each solution was quantified by LSC counting 5 aliquots of 50 µL each.

The new specific radioactivity of the spray solutions for the highest test concentration was determined as follows: The amount of radiolabelled test item was calculated based on the

LSC measurement of the spray solution, the specific radioactivity of the 14C test item active ingredient and the weight of analytical standard, added to the spray solution. The calculated new specific radioactivity for the highest test concentration was 49.9 µCi/mg.

The following table overviews the composition of each individual treatment solution:

Test group	SD-0.27	SD-0.47	SD-0.84	SD-1.51	SD-2.68	SD-4.69	SD-8.38
Target concentration	0.27µg ai/L	0.47µg ai/L	0.84µg ai/L	1.51µg ai/L	2.68µg ai/L	4.69µg ai/L	8.38µg ai/L
Volume [mL]	20	20	20	20	20	20	20
Radiolabelled active ingredient (α,β = 69 : 31), [mg]	0.45	0.80	1.51	2.45	4.30	8.79	7.76
Unlabelled active ingredient, [mg]	NA	NA	NA	NA	NA	NA	8.08
Total active ingredient, [mg]	0.45	0.80	1.51	2.45	4.3	8.79	15.84
Blank Formulation (Thiodan), [mg]	1.08	1.90	3.38	6.1	10.85	18.98	33.9
Composition of the Formulation [g a.i./kg Thiodan]	294	296	309	287	284	317	318

The radiopurity of the spray solutions was determined by C_{18}-HPLC-RAM prior to each application in all treatment solutions. Since the solutions for the spray drift entry were kept under more drastic environmental conditions in the field, representative aliquots of these treatment solutions were kept at the same environmental conditions as the spray solutions. These aliquots were analysed by HPLC after the treatments. The results are given under section 5.1.

3.10.1.1.2 Second application

A stock was prepared by weighing about 24.2 mg of the radiolabelled test item into a 5 mL volumetric flask and solving this in 5 mL acetone (Scharlau, HPLC grade, 27156). Three times 10 µL of this stock solution was taken and diluted to 10 mL using acetone. Three times 100 µL were taken of each dilution and put into scintillation cocktail (Ready SafeTM, Beckman) to determine the concentration of total radioactivity present. Based on these LSC

counts, the measured concentration of radioactivity in the stock solution, the actual amount of active ingredient in the stock solution was calculated to be 22.54 mg a.i. in 5 mL. The radiopurity was determined by analysing 10 µL of one of the dilutions by HPLC-RAM.

To prepare the Thiodan blank formulation, 99.9 mg of the blank formulation (SL-SRC code 7-006, Aventis code AE F002671 00 EC00 A219) was weighed into a 10 mL volumetric flask and diluted to 10 mL with Milli-Q water.

Six 20 mL volumetric flasks were prepared for the spray solutions. An appropriate aliquot of the 14C stock solution was introduced into each flask. Thereafter, a corresponding volume of the blank formulation emulsion was added. All spray solution was made up with Milli-Q water to the final volume of 20 mL resulting in the ready-to-use spray liquid. Each solution was quantified by LSC counting 3 aliquots of 25 µL each.

The following table overviews the composition of each individual treatment solution:

Test group	**SD-0.27**	**SD-0.47**	**SD-0.84**	**SD-1.51**	**SD-2.68**	**SD-4.69**
Target concentration	0.27 µg ai/L	0.47 µg ai/L	0.84 µg ai/L	1.51 µg ai/L	2.68 µg ai/L	4.69 µg ai/L
Volume [mL]	20	20	20	20	20	20
Radiolabelled active ingredient (α:ß = 69 : 31), [mg]	0.58	0.97	1.73	3.05	5.45	9.62
Blank Formulation (Thiodan), [mg]	1.08	1.90	3.38	6.10	10.85	18.98
Composition of the Formulation [g a.i./kg Thiodan]	349	338	339	333	334	336

The radiopurity of the spray solutions was determined by C18-HPLC-RAM prior to each application in all treatment solutions. Since the solutions for the spray drift entry were kept under more drastic environmental conditions in the field, representative aliquots of these treatment solutions were kept at the same environmental conditions as the spray solutions. These aliquots were analysed by HPLC after the treatments. The results are given under section 5.1.

3.10.1.1.3 Third application

A stock was prepared by weighing 12.8 mg of the radiolabelled test item (SL-SRC code RAD-023, Aventis lot 228 028Z) into a 5 mL volumetric flask and solving this into 5 mL acetone (Scharlau, HPLC grade, 27465/1405). Three times 10 µL of this stock solution was taken and diluted to 10 mL using acetone. Three times 100 µL were taken of each dilution and put into scintillation cocktail (Ready SafeTM, Beckman) to determine the concentration of total radioactivity present. Based on these LSC counts, the measured concentration of radioactivity in the stock solution, the actual amount of active ingredient in the stock solution was calculated to be 12.09 mg a.i. in 5 mL. The radiopurity was determined by analysing 10 µL of one of the dilutions by HPLC-RAM.

The Thiodan blank formulation was prepared as follows: 104.4 mg of the blank formulation (SL-SRC code 7-006, Aventis code AE F002671 00 EC00 A219) was weighed into a 10 mL volumetric flask.

Five 20 mL volumetric flasks were prepared for the spray solutions. An appropriate aliquot of the 14C stock solution was introduced into each flask. Thereafter, a corresponding volume of the blank formulation emulsion was added. All spray solution was made up with water to the final volume of 20 mL resulting in the ready to use spray liquid. Each solution was quantified by LSC counting 3 aliquots of 50 µL each.

The following table overviews the composition of each individual treatment solution:

Test group	SD-0.27	SD-0.47	SD-0.84	SD-1.51	SD-2.68
Target water concentration	0.27µg ai/L	0.47µg ai/L	0.84µg ai/L	1.51µg ai/L	2.68µg ai/L
Volume [mL]	20	20	20	20	20
Radiolabelled active ingredient (α:ß = 69 : 31), [mg]	0.53	1.01	1.82	3.04	5.38
Blank Formulation (Thiodan), [mg]	1.08	1.90	3.38	6.10	10.85
Composition of the Formulation [g a.i./kg Thiodan]	329	347	350	333	331

The radiopurity of the spray solutions was determined by C_{18}-HPLC-RAM prior to each application in all treatment solutions. Since the solutions for the spray drift entry were kept under more drastic environmental conditions in the field, representative aliquots of these treatment solutions were kept at the same environmental conditions as the spray solutions. These aliquots were analysed by HPLC after the treatments. The results are given under section 5.1.

3.10.1.2 Treatment of the enclosures

The spray solutions were sprayed onto the surface of the corresponding enclosure by means of a special spraying device. This spraying device consisted of a stainless steel frame holding the device with the mounted spray nozzle at a height of about 100 cm above the water surface. Furthermore, the frame held a removable polyethylene cover. This plastic cover not only minimised any external influence such as wind disturbance, but also trapped any droplets of the spray fog of the pesticide not reaching the target area during the spraying procedure. The following commercial nozzle for agricultural purpose was used at a spraying pressure of 6 bar: Spraying Systems Co., TEEJET TG SS 0.3.

At each application, the spray solutions were transferred into the spraying device. The volumetric flasks were rinsed 2 times with 5 mL of Milli-Q water. After addition of the rinse to the spraying device the solution was sprayed. An additional rinse of the volumetric flasks was performed with 10 mL Milli-Q water, which was also sprayed.

After each spraying procedure, the spraying device was rinsed with acetone to determine the residual radioactivity. For that purpose, 3 aliquots of 5 mL of the rinse were counted by LSC.

The protective polyethylene sheet was removed after spraying of each test group. Ten stripes, each 30 cm long and 10 cm broad, were cut out of the lower end of the protective sheet at regular distance from each other. Each piece was cut into smaller pieces which were counted by LSC after addition of 15 mL Ready Safe™. Strips of about 1 x 5 cm were placed into one liquid scintillation vial. Ready Safe™ (15 mL) was added to the vials which were then analysed by LSC.

Based on the data obtained for the spraying device rinse and the protective sheet LSC counts, the amount of radioactivity which reached the surface was calculated for each application and dose group. The results are provided under section 5.1.2.

3.10.2 Run-off

Run-off applications were conducted 3 times at 14-day intervals with a pre-defined quantity of soil which had been treated with the test item at a target concentration of 30 - 32 mg a.i./kg dry soil and aged for 1 day prior to treatment of the enclosures. This relatively high soil concentration (approximately 30-times higher than achieved according to agricultural use practice) was selected in order to reduce the influence of the soil slurry to fish. Based on the residue determination of the soil after ageing, different aliquots of soil were introduced into the enclosures in order to meet the following target concentrations: 0.21, 0.42, 0.84, 2.09, 4.19, 6.29, 8.39 µg soil-adsorbed residue per litre. The 4 control groups obtained amounts of untreated soil equivalent to the highest 4 test group soil loads.

3.10.2.1 Test soil and treatment regime

The soil for test item treatments was a non-contaminated agricultural soil, originating from Spain (cf. document A-VII-2). After collection from the field site, the soil was transported to the laboratory where it arrived air-dried end of July 1998. This soil was characterised as a sandy loam with clay, silt and sand contents of about 44%, 44% and 12%, respectively, a pH of about 7.7 and an organic carbon content of about 1.20 g per 100 g dry soil.

The soil was sieved through a 0.5 mm screen. Thereafter the soil was adjusted to about 5% of its Maximum Water Holding capacity. This moisture was found to be optimal during pre-tests since the addition of the required volume of the spray solutions did not damage the surface structure of the soil. (e.g. by clumping due to inappropriate high moisture).

The following table overviews, which treatments were performed:

Test group	Target a.i. concentration	Target product concentration (Thiodan)	1st Treatment	2nd Treatment	3rd Treatment
RO-0.21	0.21 µg ai/L	0.64 µg/L	X	X	X
RO-0.42	0.42 µg ai/L	1.28 µg/L	X	X	X
RO-0.84	0.84 µg ai/L	2.55 µg/L	X	X	X
RO-2.09	2.09 µg ai/L	6.35 µg/L	X	X	X
RO-4.19	4.19 µg ai/L	12.74 µg/L	X	X	X
RO-6.29	6.29 µg ai/L	19.12 µg/L	X	X	NP
RO-8.39	8.39 µg ai/L	25.50 µg/L	X	NP	NP

a.i.: active ingredient (α-endosulfan : ß-endosulfan = 69 : 31)

NP: not performed, on in agreement with the sponsor

RO: Run-off entry route

3.10.2.2 Preparation of stock and spray solutions

3.10.2.2.1 First application

A stock was prepared by weighing 48.9 mg of the radiolabelled test item and solving this into 5 mL acetone (Scharlau, HPLC grade, 27156). Three times 20 µL of this stock solution were taken and diluted to 10 mL using acetone. Three times 200 µL were taken of each dilution and put into Scintilation Cocktail (Ready Safe™, Beckman) to determine the concentration of total radioactivity present. The radiopurity was determined by analysing 10 µL of one of the dilutions by HPLC-RAM. The radiopurity was 97.91% (Figure 20). Based on the radiopurity and the measured concentration of radioactivity in the stock solution, the actual amount of active ingredient in the stock solution was calculated to be 43.4 mg a.i. in 5 mL.

To prepare the Thiodan formulation 88.6 mg of the blank formulation (AE F002671 00 EC00 A219) was weighed into a 20 mL volumetric flask. All of the stock solution with the radiolabelled test item was added. The 5 mL flask of the radiolabelled stock was rinsed with 4 to 5 drops of acetone and a few times with Milli-Q water. The volume of the formulation

stock (application solution) was brought to 20 mL with Milli-Q water. Five times 10 µL of the application stock was counted by LSC. The total amount of a.i. in the application solution was 43.3 mg a.i in 20 mL stock.

3.10.2.2.2 Second application

Of the stock solution prepared for the first spray drift application 730 µl was left which corresponded to 5.85 mg active ingredient. The radiopurity of this stock solution as determined by HPLC-RAM was 96.3%. Another stock was prepared by weighing 25.6 mg of the radiolabelled test item and addition of the remaining of the stock solution of the first spray drift application. The total volume was brought to 5 mL with acetone (Scharlau, HPLC grade, 27156). Three times 10 µL of this stock solution were taken and diluted to 10 mL using acetone. Three times 100 µL were taken of each dilution and put into Scintilation Cocktail (Ready SafeTM, Beckman) to determine the concentration of radioactivity present. The radiopurity was determined by analysing 10 µL of one of the dilutions by HPLC. The radiopurity was 98.62%. Based on the radiopurity and the measured concentration of radioactivity in the stock solution, the actual amount of active ingredient in the stock solution was calculated to be 30.23 mg a.i. in 5 mL.

To prepare the Thiodan formulation 61.6 mg of the blank formulation (AE F002671 00 EC00 A219) was weighed into a 20 mL volumetric flask. All of the stock solution with the radiolabelled test item was added. The 5 mL flask of the radiolabelled stock was rinsed with acetone and the rinse also added to the volumetric flask. The volume of the formulation stock (application solution) was brought to volume of 20 mL with Milli-Q water. Five times 10 µL of the application stock was counted by LSC. The amount of a.i. in the application solution was 30.0 mg a.i in 20 mL.

3.10.2.2.3 Third application

No stock solution was prepared for the third application, since the remainders of the soil dosed with the test item used for the first and second application were used. The stability of the samples during freezer storage was verified (cf. section 5.1.3 and Figure 26).

3.10.2.3 Application of soil

3.10.2.3.1 First application

The moisture content of the soil (described in Annex VII) was determined by weighing of four aliquots before and after drying at 104 °C. The moisture content was 4.89% based on dry weight. One kilogram of soil (based on dry weight, 1048.90 g wet soil) was put into a flat stainless steel tub (length 62 cm, width 50 cm, height 20 cm). The soil was evenly spread as a thin layer with approximately 0.5 cm. The 20 mL application solution containing 43.3 mg a.i. was put into a TLC-sprayer (CAMAG, Muttenz, Switzerland). The container was partly covered with a glass plate to avoid drift of the spray vapour. A small opening was left to allow the spraying. The application solution was sprayed close to the soil surface to avoid drift outside the soil area. The spray procedure was interrupted for approximately 8.5 hours to recharge the battery of the sprayer. During the interruption, Argon was put on the soil to avoid loss of the test item due to volatilisation and the container was closed with the glass plate and aluminium foil. After the interruption the rest of the application solution was sprayed. The volumetric flask which had contained the application solution was rinsed with 5 mL Milli-Q water. The rinsate was transferred to the sprayer and sprayed on the surface of the soil. The container with the applied soil was filled with Argon, after which the container was closed with aluminium foil. The applied soil was left to age overnight. The glass plate, the volumetric flask and the sprayer were rinsed and the total radioactivity in the rinsates was determined. Since no significant residue was found affixed to the spray equipment after the first treatment, this step was omitted during the 2nd and 3rd treatment.

3.10.2.3.2 Second application

The same procedures as described for the first application were used, apart from:

- moisture content of the soil was 3.55% based on dry weight.
- seven hundred grams of dry soil equivalent were used (724.86 g wet weight).
- no interruption in the application occurred.
- the volumetric flask which had contained the application solution was rinsed with 10 mL MilliQ-water. The rinse was sprayed onto the soil.

3.10.2.3.3 Third application

For the third application the remainders of the applied soils of the first and second application, which had been stored at approximately -20°C were used. To verify that the quality of the stored soils were comparable with the freshly applied soils, approximately one gram of the soil of each application was taken and extracted with acetone by accelarated solvent extraction (ASE). The volume of the extract was determined to be 12.5 mL for both samples. The total radioactivity of three times 100 µL of each extract was measured by LSC. The quality of the radioactivity in the extract was measured by HPLC-RAM and was compared with the quality of the extracts of the soils directly after the first and second application. Based on the results obtained it was concluded that the stored soils could be used for the third application. The weight of the soil dosed for the first application was 138.52 g, the weight of the soil for the second application 150.42 g. The two batches of soil were combined and the mixture was thoroughly shaken. The moisture content of the mixed soil was determined to be 2.92% based on dry weight.

3.10.2.4 Determination of the amount of radioactivity in the aged soil

3.10.2.4.1 First application

The moisture content of the soil used to dose the control enclosures was 4.95%, whereas the treated soil had a moisture content of 3.03% based on dry weight. The treated soil was mixed within the stainless steel tub with a large spatula following 1-day ageing. Then the soil was transferred to a bowl and was mixed for 5 minutes with a Hobart mixer. During the whole procedure the soil was covered with Argon. After mixing of the soil, 12 aliquots were taken of which 10 samples of 0.1 g were taken to determine the total radioactivity after combustion in a Packard Model 306 sample oxidizer. The resulting $^{14}CO_2$ was trapped as a carbonate salt in Carbosorb® (a basic amine, Packard), dispensed into a glass scintillation vial, and Permafluor V® (a toluene based cocktail, Packard) was then added. The efficiency of the oxidizer to combust radiolabelled material on soil was verified by adding a standard reference material (Spec Check [^{14}C]-Standard) to 0.1 g soil and comparing the measured value to that of a concurrently fortified standard. An efficiency of 97.0% was found, which showed that the oxidizer was working properly. The concentration of total radiolabelled test item in the soil was determined to be 32.0 mg endosulfan/kg moist soil.

3.10.2.4.2 Second application

The same procedures were used as for the first application. The moisture contents of the control and treated soil were 3.30 and 6.04% based on dry weight, respectively. The combustion efficiency of the oxidizer was found to be 95.3%. The concentration of total radiolabelled test item in the soil was 30.0 mg endosulfan/kg moist soil.

3.10.2.4.3 Third application

For the third application a mixture of the remainders of the treated soil for the first and second application were used. The total amount of radioactivity in the soil was determined with the Packard oxidizer following intensive homogenisation. The combustion efficiency with soil was determined to be 98.2%. The concentration of total radiolabelled test item in the soil was 30.3 mg endosulfan/kg moist soil.

3.10.2.5 Preparation and addition of the slurry to the enclosures

The soils were applied to the enclosures as a slurry with a dry soil:water ratio of 1:16.67. The amounts of soil and water applied to the enclosures at the three applications are presented in the table below. The average load of soil bound residue introduced into each enclosure is given in Table 8.

	Application 1		Application 2		Application 3	
Target concentration (dose level)	weight of moist soil [g]	volume of water [mL]	weight of moist soil [g]	volume of water [mL]	weight of moist soil [g]	volume of water [mL]
0 µg ai/L (RO-CTRL-1)	79.25	1255	80.79	1301	82.31	1329
0 µg ai/L (RO-CTRL-2)	158.81	2514	161.90	2608	141.44	2284
0 µg ai/L (RO-CTRL-3)	238.07	3768	242.71	3909	n.p.[a]	n.p.[a]
0 µg ai/L (RO-CTRL-4)	317.63	5030	n.p.[a]	n.p.[a]	n.p.[a]	n.p.[a]
0.21 µg ai/L (RO-0.21)	7.81	126	8.33	130	8.25	133
0.42 µg ai/L (RO-0.42)	15.62	252	16.65	261	16.50	267
0.84 µg ai/L (RO-0.84)	31.24	504	33.31	522	32.96	534
2.09 µg ai/L (RO-2.09)	77.77	1256	82.93	1299	82.15	1329
4.19 µg ai/L (RO-4.19)	155.87	2518	166.20	2602	141.31[b]	2284
6.29 µg ai/L (RO-6.29)	233.65	3774	249.13	3903	n.p.[a]	n.p.[a]
8.39 µg ai/L (RO-8.39)	311.70	5035	n.p.[a]	n.p.[a]	n.p.[a]	n.p.[a]

[a] n.p. not performed. These enclosures were not dosed, due to 100% mortality in the highest test concentrations in agreement with the sponsor.

[b] Due to a shortage of applied soil this enclosure was dosed with less than used for the other two applications. The dosage level was 86% of the amount used at the other two applications.

The slurries were prepared in 500 or 2000 mL separation funnels. The larger amounts were added in several portions. The slurry was homogenised by thorough shaking and was applied on the surface of the water within the enclosure in a circle shaped movement. The suspension formed a "cloud" in the water which spread evenly over the enclosure. The volumetric flasks were rinsed with 100 to 1000 mL water after each addition of slurry to the enclosure. The rinsates were also added to the enclosures during which care was taken that any slurry which stuck on the top of the fish cages was rinsed off.

3.10.2.6 Desorption of endosulfan from aged soil, dosed one day prior to application

After the first and second application, 6 aliquots of approximately 1.5 g dry weight of the aged soil were weighed into Teflon centrifuge tubes. Twenty-five milliliter of deionized water (Milli-Q, Springborn Laboratories In-house) was added. To save radiolabelled test item, as little as possible soil was used for the desorption test, so that only 3 aliquots of approximately 1 g dry weight of the aged soil were taken at the last application. To this 16.7 mL of deionized water was added. The ratio between the amount of soil and the deionized water was the same as used for the preparation of the run-off slurries, i.e. 1:16.7.

The samples were shaken vigorously for approximately 1 minute after which they were centrifuged for 10 to 15 minutes at 2000 rpm (centrifuge used: Mistral 2000). The supernatants were decanted and the volume was determined. To determine the total radioactivity in the supernatant 3 times 100 µl were taken and put into 15 ml of scintillation cocktail (Ready Safe™, Beckman), after which the samples were counted by LSC (counter used: Beckman LS 6000TA). To avoid degradation of endosulfan during further processing of the samples, the supernatants were acidified with a drop of 85% H_3PO_4 (Fluka).

The acidified supernatants were filtered through a 0.7 µm glass filter (Whatman GF/F), to determine the fraction of dissolved radioactivity. The containers which contained the supernatants were rinsed with 1 mL deionized water. The rinses were also filtered through the 0.7 µm filter, after which the filter was rinsed with 1 mL deionized water. The total volume of the filtrates was determined. Three 100 µl samples were taken from each filtrate and counted by LSC. The filters were cut into pieces, put into 15 mL of Ready Safe™ and the radioactivity was counted by LSC.

3.11 Sample Collection

Various samples were collected from all or selected enclosures according to the time schedule for the project (Tables 2 and 3).

3.11.1 Surface Film

Samples of the surface endosulfan film on the water surface following spraying were taken according to the procedure provided under Annex VII. These samples were taken after each

treatment at regular intervals post each spray drift treatment. The target times were 1, 3, 6 and 24 hours post treatment.

The following table overviews the collection times post treatment:

Test group	Target water concentration	First Treatment	Second Treatment	Third Treatment
		Time after treatment [hrs:min]	Time after treatment [hrs:min]	Time after treatment [hrs:min]
SD-0.27	0.27 µg ai/L	1:00/2:15/7:45/23:20	0:57/4:27/6:12/18:57	0:58/3:15/5:58/nn
SD-0.47	0.47 µg ai/L	1:00/3:05/6:25/19:35	1:16/3:59/6:16/19:11	1:02/3:07/5:50/nn
SD-0.84	0.84 µg ai/L	1:00/4:10/5:58/19:08	1:00/3:35/6:02/18:14	0:54/2:44/5:59/nn
SD-1.51	1.51 µg ai/L	0:55/3:23/5:43/18:43	1:40/3:50/6:00/16:43	1:09/3:12/7:12/nn
SD-2.68	2.68 µg ai/L	1:00/3:55/5:20/17:45	1:13/3:17/5:09/15:50	0:53/3:02 /6:10/nn
SD-4.69	4.69 µg ai/L	1:18/4:03/6:00/NP	2:00/3:20/5:30/15:52	NP
SD-8.38	8.38 µg ai/L	0:55/2:42/NP/NP	NP	NP

NP: not performed

SD: Spray Drift

nn: the 24 hours samples were taken, but the exact time was not not noted.

Stainless steel nets were prepared as described under Annex VII. Each net was weighed and the weight was recorded in a daily log table. At each sampling, 3 nets were carefully attached onto the surface of the water and removed after about 5 seconds equilibration. One net was exposed at about the centre of each enclosure, one at half of the radius and one at about 10 cm from the enclosures border. After exposure, each net was weighted again and the weight was recorded in the daily log table. The three nets per enclosure were placed into a glass vessel and sealed. Acidified acetone were added and the nets were shaken vigorously in order to wash the residue from the net. The volume of the net rinse was quantified followed by LSC counting of the acetone.

3.11.2 Water

Water was collected in order to receive samples for the determination of physical-chemical parameters, Suspended Particulate Material (SPM) analysis, total radioactive residue

determination and qualitative analysis of the residual radioactivity. The collection scheme is given in Tables 2 and 3. The methodology is summarized under Figure 4.

3.11.2.1 Collection during the first 24 hours post each treatment

In order to monitor the vertical distribution of the radioactivity in the enclosures, after each treatment, water was collected at the times of surface film collection (i.e. after target 1, 3, 6, 24 hours) in the Spray drift entry route scenario (the timing of samples collection after the applications see section 3.11.1). For the Run-off entry route scenario the following table overviews the timing of samples collection after the applications:

Run-off (RO):

Test group	Target concentration	First Treatment	Second Treatment	Third Treatment
		Time after treatment [hrs:min]	Time after treatment [hrs:min]	Time after treatment [hrs:min]
RO-0.21	0.21 µg SR ai/L	0:52/2:45 /6:10	1:15/3:22/6:09	1:03/3:10/6:09
RO-0.42	0.42 µg SR ai/L	1:05/2:49/6:25	1:13/3:20/6:10	1:06/3:08/6:20
RO-0.84	0.84 µg SR ai/L	1:00/3:00/6:40	1:10/3:16/6:15	1:02/3:05/6:22
RO-2.09	2.09 µg SR ai/L	0:55/3:00/6:50	1:06/3:12/6:29	1:00/3:02/6:27
RO-4.19	4.19 µg SR ai/L	0:57/3:25/6:30	1:11/3:09/7:03	0:58/3:01/6:28
RO-6.29	6.29 µg SR ai/L	0:55/2:52/6:05	0:45/2:40/7:00	NP
RO-8.39	8.39 µg SR ai/L	1:00/2:55/5:50	NP	NP

NP: Not performed

SR: Residue originally sorbed to soil

a.i.: active ingredient

The following methods were applied:

Aliquot water samples were taken from treated and control enclosures using teflon tubes installed at the 3 water levels. For that purpose, an aliquot of about 1000 mL was removed from the low, medium and high level by means of a vacuum pump. About 100 mL of each level (low (~5 cm subsurface), medium (~65 cm subsurface) and high (~10 cm above sediment, i.e. ~120 cm subsurface)) were stored separately in flasks. After acidification with

concentrated phosphoric acid of the samples, they were stored in the freezer until further analysis (LSC). Equal aliquots of each level were pooled in a 1 L measuring cylinder and thoroughly mixed to prepare depth-integrated water samples. 1 L aliquots were filled into appropriate flasks, which were deep frozen after acidification with concentrated phosphoric acid.

3.11.2.2 Collection during the study

During the study, the samples were collected as described above. However, further aliquots of the pooled sample were collected to conduct the following determinations:

- Determination of alkalinity and hardness of the fresh samples.
- TOC/DOC measurements of the fresh samples by means of a Dohrmann DC-80 TOC analyser or after storage of an aliquot in appropriately labelled glass vials in the freezer until analysis.
- Determination of the suspended particulate matter (SPM) by filtration of 1.5 L through pre-conditioned, dried and weighed Whatman GF/F filters (0.7 µm exclusion size). The filters were weighed to determine the SPM of each sample. 0.5 L of the resulting percolate were stored in the freezer for HPLC analysis of the dissolved residue of the test item and its degradates.

3.11.2.3 Collection at the end of the study

At the end of the study, aliquots of 10 L each from the 3 depth levels were collected acidified and individually deep-frozen for further analytical purpose. Furthermore 330 mL from each depth level was combined and used for water quality measurements.

3.11.3 Sediment

Sediment was collected at regular intervals (cf. Tables 2 and 3) in order to receive samples for the analysis of the sediment adsorbed residue determination and qualitative analysis of the residual radioactivity. Details are described under Figure 5.

3.11.3.1 Collection during the study

Sediment samples for chemical analysis were removed by means of a special stainless steel corer. Three cores of 9 cm^2 each were taken at each sampling. Care was taken, that the cores were removed from different locations within each enclosure. The core material was removed from the corer and combined in glass containments. A few mL of phosphoric acid were added to the sediment and thoroughly mixed in order to stabilize the quality of the residue. Thereafter, the containments were sealed and deep frozen until further analytical work.

3.11.3.2 Collection at the end of the study

At the end of the study, basically the same collection procedure was applied. However, fifteen replicate cores were collected of which ten replicate cores were taken for biological analysis and five for residue analysis. The procedure after removal of the cores was as follows (Figure 5): The supernatant water was removed from the top of each core by means of a vacuum pump. Care was taken, that no organic material debris was removed. Thereafter, the water-sediment interphase was removed from the collection device by the vacuum pump. Thereafter, the top sediment layer (about 1 cm) was removed and combined with the water-sediment interphase. Thereafter the remaining sediment core was cut into different layers: 0 to 1 cm, 1 to 5 cm, > 5 cm.

The corresponding samples of the replicate cores were combined to one sample for further analysis. All samples due for biological analysis were either processed directly or after storage for maximum one week in the refrigerator. The samples for chemical analysis were acidified as described above and deep frozen.

3.11.3.3 Samples for biological evaluation

The following samples were processed based on a validated method (cf. Annex XI for details).

- Combined water-sediment interphase and top 1 cm of the sediment core
- 1 to 5 cm layer

The samples of the different sediment layers were wet sieved through a 500 µm screen following conventional procedures in order to collect the sediment-dwelling organisms. Taxonomic determination of these organisms was conducted based on appropriate literature.

After taxonomic determination, the total radioactive residue in sediment dwelling organisms was determined by LSC.

3.11.3.4 Samples for chemical evaluation

The following samples were analysed

- Supernatant water
- Combined water-sediment interphase and top 1 cm of the sediment core
- 1 to 5 cm layer
- > 5 cm layer

The supernatant water was submitted to LSC counting and HPLC analysis. The water of the combined sediment-water interphase and top 1 cm sediment layer was decanted and measured by LSC. The sediments of replicate cores were combined for further analysis. The sediment samples were centrifuged. Supernatants and sediment were submitted to radiochemical analysis.

3.11.4 Macrophytes

Macrophytes were collected in order to receive samples for residue determination. The general collection scheme is given in Tables 2 and 3. The methodology is summarized under Figure 6.

Aliquot samples of the *Elodea canadensis* macrophyte type were removed from the enclosures. The water was removed as far as possible by shaking. Thereafter, they were introduced in glass vessels and sealed. Their fresh weight was determined followed by addition of about 50 mL acidified water. Thereafter, the samples were sealed and deep frozen. At selected intervals, *Myriophyllum* was collected and processed as described above.

At test termination all macrophytes, *Elodea canadensis* and *Myriophyllum spicatum* were completely removed. Filamentous algae that had developed on the macrophyte surfaces, were removed with the macrophytes. All samples were processed as described above.

3.11.5 Tank Wall Residue

The tank wall periphyton was collected in order to receive samples for residue determination. As described in section 3.5, removable stainless steel stripes were used to determine the tank wall residues. At the end of the test, all stripes were removed from the enclosures followed by scraping off the periphyton. This material was transferred into glass vials, acidified acetone and deep frozen for further analyses, (cf. Figure 6).

3.11.6 Fish

Fish were collected for residue determination and information about length and weight. The general collection scheme is given in Tables 4 and 5. The methodology is summarized in Figure 7. Two fish per enclosure were randomly collected at each sampling interval (spray drift: days 3, 7, 13, 16, 17, 21, 27, 30, 31, 35 and 41, run-off: days 4, 7, 12, 14, 16, 18, 21, 26, 28, 30, 32, 35 and 42). These fish were placed into glass liquid scintillation vials followed by addition of acidified water. The vials were sealed and deep frozen until further work-up.

3.12 Feeding of Test Fish during the Study

As already stated in section 3.6, the fish were fed *Artemia nauplii* and concentrated zooplankton from the Lake of Constance (cf. Annex VI) once or twice a day, except for Sundays. The amount of food, based on dry weight, corresponded to a total of approximately 3% of the total fish biomass. In case mortality occurred in an enclosure the feeding quantity was reduced proportional to the mortality. The biomass calculations were updated every 14 days based on the most recent weight and length of the sampled fish. The dry weight of the Artemia and concentrated zooplankton was determined once a week.

3.13 Biological Observations of Test Fish

The fish were monitored daily for mortality and behavioural changes. For that purpose, the fish cages were slowly hooked up on the outer holding cage. The dead fish were removed by means of a net. A detailed schedule is given in Tables 4 and 5.

3.14 Analytical methods

The samples collected from the enclosures at the various time intervals were processed and analysed by instrumental analysis as will be described below. The samples were either analysed directly or after clean-up, extraction or solubilization of the tissues.

The total radioactive residue was determined in all samples. The radioactivity of selected samples from selected concentrations was characterized by C_{18} HPLC-UV-RAM.

3.14.1 Sample processing and clean up

3.14.1.1 Extraction of soil used for run-off applications

Two times 10 g of the treated soil used to dose the enclosures at the first and second application were extracted with acetone in an Accelerated Solvent Extractor (ASE) on the same day as the application. The quality of the soil for the third application (remainders of the first and second application) was checked before use. The following ASE method was used:

Heat:	5 minutes
Static:	10 minutes
Flush:	20 % vol.
Purge:	120 sec.
Cycles:	3
Pressure:	1500 psi
Temperature:	RT

3.14.1.2 Water samples

3.14.1.2.1 Analysis of the Suspended Particulate Matter (SPM) by filtration

To determine the concentration and quality of dissolved radio-labelled test item in the water samples the samples were filtered through a 0.7 µm glass filter (Whatman GF/F, Ø 47 mm). Although in general 0.45 µm is used as the division between dissolved and non dissolved material, a 0.7 µm filter was used since it was the smallest glass filter available. Membrane

filters with pores of 0.45 µm of other materials than glass were tested during preliminary tests (Annex VII). Filtration of solutions of radio-labelled parent resulted in recoveries between 46.1 and 82.8% showing that the loss of radio-labelled test item through binding to the filter material was too large. The recoveries found for filtration through the 0.7 µm glass filter were larger than 90%.

The suspended particulate matter (defined as particles larger than 0.7 µm) was also determined. The 0.7 µm glass filters (Whatman GF/F, Ø 47 mm) were dried for 2 hours at 105°C. The filters were allowed to come to room temperature in an excicator, before the weight was taken. An appropriate amount of the water sample of which the total radioactivity had been determined by LSC was filtered through a 0.7 µm glass filter. The glass filters with the suspended particle matter were dried again for 2 hours at 105°C. After cooling down to room temperature in an exsiccator, the weight was taken. The total radioactivity in the suspended particulate matter was determined by LSC by putting the dried filter plus the suspended particulate matter in 15 mL scintillation cocktail (Ready Safe™).

The total radioactivity in the filtrate (dissolved fraction with particles <0.7 µm) was determined by LSC by putting 1-5 mL samples into 15 mL scintillation cocktail, after which the filtrate was used for further processing by Solid Phase Extraction (SPE).

3.14.1.2.2 Solid Phase Extraction (SPE) of the supernatant water

A glass reaction tube with a teflon frit (Supelco/Fluka) was filled with 1.0 g Chromabond C18 ec (Macherey & Nagel). The Chromabond C18 ec was conditioned with 10 ml acetonitrile (Scharlau, HPLC grade) and 10 mL Milli-Q water (Springborn Laboratories (Europe) AG in-house production, batch #004). An appropriate amount of the filtered sample of pore water sample was applied to the SPE column. The total volume of the eluate was determined and a 1 mL sample was taken for LSC analysis. The column was extracted with 3 mL acetonitrile. The total volume of the acetonitrile was taken and 50 µL were taken for LSC analysis. The sample was spiked with 50 µl of the mixture of non-labelled standards. The sample was analysed directly by HPLC or was stored at -20°C before HPLC analysis.

3.14.1.3 Sediment samples

Due to the fact, that the residue in the sediment samples was too low for direct LSC combustion, larger sediment samples were extracted to obtained higher amounts of radioactivity for qualitative and quantitative analysis.

The following describes the procedures used to determine the concentration of the total radioactivity in the sediment samples taken during the study from SD-2.68 and RO-4.19 and the samples taken at test termination.

The following devices and materials were used:
- Accelerated Solvent Extractor (ASE 200), Dionex
- Hydromatrix (CHEM ELUT) sample preparation products, Harbor City, CA90710, USA
- Balance, Mettler
- Liquid Scintillation Counter, LS 6000 TA, Beckman
- Liquid Scintillation Cocktail, Ultima™ Flow AF, Packard
- Mistral 2000 Centrifuge
- Acetone, Scharlau, HPLC grade

3.14.1.3.1 Procedures of the samples taken during the study

The total weight of the sediment was taken and the sediment was mixed thoroughly. Eighty grams of the homogenized sediment was taken and mixed with 20 grams of Hydromatrix. This was divided over four ASE extraction cartridges and the samples were extracted with acetone on the ASE, according to the program described in the next paragraph:

3.14.1.3.2 Procedure for the samples taken at test termination

The three segments were analysed separately. The pore water was isolated from the sediment samples by centrifuging an aliquot of the sediment at 3000 rpm for 16 minutes. The supernatant was decanted and three times 5 mL were counted by LSC. The pellet was put back to the rest of the sample. The extraction of the three segments of the sediment (0-1, 1-5, > 5 cm) was performed as described for the samples taken during the study.

- Heat: 5 minutes
- Static: 10 minutes
- Flush: 20%
- Purge: 120 seconds
- Cycles: 2
- Pressure: 1500 psi
- Temperature: 80 °C

The extracts of each individual segment were combined and the total volume was determined. Three 500 µL samples were taken and the total radioactivity in the samples was determined by LSC. Appropriate aliquots were analyzed by radio-TLC and C_{18} HPLC-UV/RAM.

3.14.1.4 Macrophytes

The deep frozen plant material as well as the tank wall residue were handled as follows: The acidified water was decanted and analysed by LSC. The remaining macrophyte material was homogenized under dry ice. The total weight of the macrophytes was taken and the material was mixed thoroughly. Aliquots were combusted in order to determine the total radioactive residue. For qualitative analysis, eighty grams of the homogenized macrophyte material was taken and mixed with 20 grams of Hydromatrix. This was divided over four ASE extraction cartridges and the samples were extracted with acetone on the ASE, according to the following program:

- Heat: 5 minutes
- Static: 10 minutes
- Flush: 50%
- Purge: 120 seconds
- Cycles: 2
- Pressure: 1500 psi
- Temperature: 80 °C

Three 100 µL samples were taken and the total radioactivity in the samples was determined by LSC Appropriate aliquot were used to conduct HPLC-RAM analysis.

3.14.1.5 Fish tissues

3.14.1.5.1 Determination of total radioactivity

The fish that had been stored at -20°C were thawed. In general, two fish per test concentration and sampling interval had been taken. The thawed fish were blotted dry with a paper tissue and the weight was taken. The fish were cut in approximately 4 pieces and put into liquid N_2. After the pieces were frozen, they were transferred to a mortar, which had been pre-cooled in dry-ice. The tissue pieces were homogenised using a pestle. The homogenate was filled into a pre-tarred LSC vial and the weight was taken. Approximately 150 mg of the homogenate was weighed into a pre-tarred vial. Two mL BTE-450 (Beckman) was added. The fish tissue was completely digested overnight at room temperature or for approximately 4 hours at 55 °C in a waterbath. Ten milliliter liquid scintillation cocktail, Ready Organic (Beckman) acidified with acetic acid was added. The mixture was shaken well. The mixture was left for 1 hour, after which the LSC counts were determined using the Liquid Scintillation Counter (LS 6000 TA, Beckman).

The fish taken at test termination were stored at -20°C. Acidified water had been added to the fish taken during the study before storage. The total volume of the acidified water was taken and three times 1 mL was analysed by LSC to determine the amount of radio-labelled test item which had moved to the acidified water during storage. The scintillation cocktail used for this analysis was Ultima flow (Packard).

The total radioactivity determined in the fish homogenate and the acidified water were summed up and the concentration of endosulfan equivalent per kilogram fresh fish weight was calculated.

3.14.1.5.2 Extraction of fish tissues

Aliquots of the homogenized fish was taken and mixed with 20 grams of Hydromatrix. This was divided over four ASE extraction cartridges and the samples were extracted with acetone on the ASE, according to the following program:

- Heat: 5 minutes
- Static: 10 minutes
- Flush: 50%
- Purge: 120 seconds
- Cycles: 2
- Pressure: 1500 psi
- Temperature: 80 °C

The four extracts were combined and the total volume was determined. Three 100 µL samples were taken and the total radioactivity in the samples was determined by LSC.

3.14.1.6 Sediment dwelling organisms

The counts for Oligochaeta and Chiromidae collected from the various sediment layers were taken in-vivo. The level of TRR in the sediment-dwelling organisms were determined following the methodology used for fish homogenates.

Due to the rather low biomass of the sediment-dwelling organisms per sediment layer, all collected organisms were placed into a glass liquid scintillation vial. Two milliliter BTE-450 (Beckman) was added. The organisms were completely digested overnight at room temperature or for about 4 hours at 55 °C in a waterbath. Ten milliliter liquid scintillation cocktail, Ready Organic (Beckman) acidified with acetic acid was added. The mixture was shaken well. The mixture was left for 1 hour, after which the LSC counts were determined using the Liquid Scintillation Counter (LS 6000 TA, Beckman).

3.14.2 Measurement of radioactivity

Radioactivity was quantified with a Beckman LS 6000TA liquid scintillation counter equipped with DPM and luminescence options. All measurements were performed at least in duplicate, corrected automatically for background and counted for a time interval allowing a counting error below 5% or for a maximum of 5 minutes.

Aqueous samples: Aqueous samples were mixed carefully before the aliquots for counting were taken. At least triplicate aliquots of maximum 5 mL were removed from each individual sample. Ten milliliter Ultima Flow AF® was used for LSC.

Organic samples (acetone and aqueous acetone): These samples were also mixed carefully before the aliquots for counting were taken. At least 3 aliquots were taken and measured. Ultima Flow AF® or Ready Safe™ (15 mL) were used for LSC.

Samples obtained by combustion: To determine the residual radioactivity in soil, 3 sub-samples were combusted in a stream of oxygen on a Packard Oxidizer (Model 306) using a platinum heating coil as a catalyst. Liberated $^{14}CO_2$ was absorbed in 9 mL Carbosorb® E and 9 mL Permafluor® E+ and the radioactivity was quantified by LSC.

3.14.3 Characterisation of radioactivity

Samples and extracts due for the characterization of the total radioactive residue were analysed using co-chromatography C_{18}-HPLC-UV/RAM. For that purpose, the analytical standard, as supplied by the sponsor, was used. Other techniques like radio-TLC on silica gel matrix were also conducted to confirm the identity of the test item and major degradates which exceeded 10% of the applied radioactivity.

3.14.3.1 High Performance Liquid Chromatography (HPLC)

The following HPLC system equipped with a radioactivity monitor (HPLC-RAM) was used:

Device	Hewlett-Packard 1050	pump
	Hewlett-Packard 1050	autosampler
	Hewlett-Packard	Chemstation
Detectors	**Radiometric-detector**	
	Canberra Packard Flow Scintillation Analyzer 500 TR series	
	Nuclide:	^{14}C
	Cell Type:	liquid
	Cell Volume:	500 µL
	Scintillator:	Packard Flow Scint A
	Flow Rate Scintillator operated at:	3.0 mL/min

UV Detector
Hewlett-Packard 1050 detector

The temperature during the analyses was kept constant at 20°C by using a column thermostat (Bischoff).

Typically, stock solutions of analytical standard of α,β-endosulfan and the main metabolites were added to the HPLC samples to verify the quality of the radioactivity in the samples. They were used as external standards in case of high UV signals (sample matrix).

The following columns and solvent systems were used to generate the HPLC chromatograms shown in this report:

Method 1:

Column: Hibar RT LiChrosorb RP-18 (5 µm) , 4 mm * 250 mm (Merck)

Mobile Phase: A: Acetonitrile

B: Milli-Q water/0.1 mol/L ammoniumacetate

Flow rate: 1.5 mL

Time (min)	% A	% B
0	50	50
20	100	0
25	100	0
30	50	50
40	50	50

This method was supplied by the sponsor and was used to determine the radiopurity of the test substance in stock and spray solutions. The percent distribution of the peaks in the HPLC-RAM chromatograms was calculated using the corresponding Packard software tools. The retention times varied depending on the batch of the column used. However, co-elution was conducted for almost all the samples. In case the sample matrix UV signals were too high, external standard mixtures were used.

Method 2

Column: Hibar RT LiChrosorb RP-18 (5 µm) , 4 mm * 250 mm (Merck)

Mobile Phase: A: Acetonitrile

B: Milli-Q water

Flow rate: 1.0 mL/min

Time (min)	% A	% B
0	50	50
20	100	0
25	100	0
30	50	50
40	50	50

This method was adapted by Springborn Laboratories and used to characterize the radioactivity in water, sediment, macrophytes and fish material. The percent distribution of the peaks in the HPLC-RAM chromatograms was calculated using the corresponding Packard software tools.

3.14.3.2 Thin Layer Chromatography (TLC)

In addition to reversed phase HPLC, normal phase co-chromatography radio-TLC was performed in order to characterize the TRR as described above. For that purpose, an aliquot of the solutions to be applied to the plate was sprayed using nitrogen as a 2-cm band onto pre-coated 5 cm * 20 cm chromatographic plates by means of an automated spraying device (Linomat). Other plates were prepared by hand spotting the same sample with a capillary, i.e., without spraying.

The radiolabelled reference standards were used as TLC reference standards. The following chromatographic plates were used: Silica gel 60 F 254, 0.25 mm, Merck (# 1.05714), 5 cm * 20 cm. The plates were developed with the following solvent systems:

Toluol: Petrolether: Ethylacetate = 10:1:2

Other methods were used during the method development. A detailed record is given in the raw data.

The TLC plates were developed in glass tanks under saturated conditions. The length of each run was recorded (typically 15 cm). After the chromatography, the plates were air-dried prior to radio-scanning. Radio-detection was performed on a TLC linear analyser (Tracemaster 20/LB 2821).

4. CALCULATIONS

4.1 Radioactivity Levels

The following equations were used to quantity the residues in water, sediment and macrophytes. The specific radioactivities are given under section 3.10.

4.1.1 Total Radioactive Residue (TRR) in water

The total radioactivity residue (TRR) found in the water samples of each enclosure were expressed as µg Parent Equivalent (peq)/L as determined from the dpm found in the samples. The following equation was used:

$$\mu g\ peq/L = \frac{dpm/L}{specific\ activity\ (\mu Ci/mg)*2.22*10^6\ (dpm/\mu Ci)*1000\ \mu g/mg}$$

For balance calculations, the following equation was used to calculate the total amount (µg) in the water of the enclosure (total volume 1190 litres):

$$\mu g\ peq\ in\ water\ phase\ of\ enclosure = \mu g\ peq/L*1190L$$

4.1.2 TRR in Sediment

A subsample of approximately 80 g from the homogenized sediment sample was taken, mixed with 20 g hydromatrix after which the sample was extracted with ASE. The amount of radioactivity found in the extract was determined by taking 3 times 500 µL and determinating the activity by LSC. The total dpm/g was calculated with:

$$dpm/g = \frac{dpm\ measured*total\ volume\ extract}{0.5\ mL*weight\ of\ sample(g)}$$

The concentration of µg peq/kg sediment was calculated using the following equation:

$$\mu g\ peq/kg = \frac{dpm/g * 1000 g/kg}{specific\ activity\ (\mu Ci/mg) * 2.22 * 10^6\ (dpm/\mu Ci) * 1000\ \mu g/mg}$$

To determine the total amount of radioactivity in the sediment at test termination (mass balance) 5 cores of the sediment were taken and mixed. The total weight of this mixed sample was determined. The weight of the total sediment in the enclosure was calculated using the following equation:

$$total\ weight\ sediment = weight\ of\ 5\ cores * \frac{area\ of\ enclosure}{area\ of\ 5\ cores}$$

where: area of enclosure = 9499 cm^2

area of 5 cores = 45 cm^2

4.1.3 TRR in Macrophytes including Tank Wall Periphyton

The weight of the plants was determined at sampling. All of the plant material was homogenised using Hydromatrix® and the weight of the homogenised sample was determined. The radioactivity in the acid water added to the plants before storage was determined by taking 3 times 5 mL and determining the radioactivity by LSC.

The amount of radioactivity found in the homogenised sample was determined by combustion of 5 sub-samples of approximately 0.1 - 0.8 g. The total dpm/g was calculated with:

$$dpm/g = \frac{dpm\ measured}{weight\ of\ sub\text{-}sample\ (g)}$$

The total dpm in the homogenised plant material was calculated using the following equation:

$$total\ dpm\ in\ homogenate = dpm/g * total\ weight\ homogenate\ (g)$$

Further:

Total dpm in plant material = total dpm in homogenate + total dpm in acid water

The µg peq/kg fresh plant was determined using the following equation:

$$\mu g\ peq/kg = \frac{total\ dpm/weight\ plant\ material\ (kg)}{specific\ activity\ (\mu Ci/mg)*2.22*10^{6}\ (dpm/\mu Ci)*1000\ \mu g/mg}$$

At test termination all plant material was taken out of the enclosure and the total fresh weight was determined.

4.1.4 TRR in Fish

The fish had been stored in acidified water at approximately –20°C for 8 to 9 months. The total activity in the acidified water was determined by:

Total dpm in acidified water = dpm determined *total volume (mL)

The fish were homogenized and subsamples were taken and processed as described above. The dpm/g homogenate were calculated by:

$$dpm/g = \frac{dpm\ determined}{weight\ of\ subsamples}$$

The total dpm in the fish samples were determined by:

*dpm total = dpm/g * total weight of homogenate + total dpm in acidified water.*

The dpm/g fish at sampling was calculated by:

$$dpm/g\ fresh\ fish = \frac{dpm\ total}{weight\ of\ fish\ at\ sampling}$$

The mg peq/kg was calculated by:

$$\text{mg peq/kg fresh fish} = \frac{\text{dpm/g fresh fish} * 1000\ \text{g/kg}}{\text{specific activity } (\mu\text{Ci/mg}) * 2.22*10^6\ (\text{dpm/}\mu\text{Ci})}$$

4.2 Quantitative Contribution of Individual Radioactive residues

The percent values of each individual radioactive residue as quantified by HPLC-RAM and/or radio TLC in terms of percent HPLC run or percent TLC run, were expressed in terms of total applied radioactivity according to the following equation:

$$\text{(\%) individual radioactive residue} = \frac{\text{(\%) of dose in sample} \times \text{(\%) individual radioactive residue}_{HPLC/TLC}}{100\%}$$

More details about the evaluation of the HPLC-RAM and radio-TLC chromatograms are provided under section 3.14.2. The corresponding concentrations for individual radioactive fractions and metabolites in mg/L or mg/kg were calculated as given under 4.1.

4.3 Mass Balance

The percentage of TTR in each compartment (water, sediment or plants) at test termination was calculated by:

$$\text{\% in compartment} = \frac{\text{total dpm found}}{\text{total dpm applied}} * 100\%$$

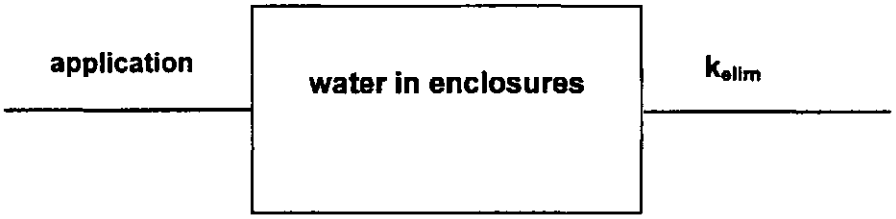
4.4 DT_{50} Calculations

The following commercially available software was used: PKAnalyst® (MicroMath® scientific software, Microsoft Windows versions 2.0 and 1.0, respectively). This software was used to best fit mathematical models to the experimental data.

The following models were used:

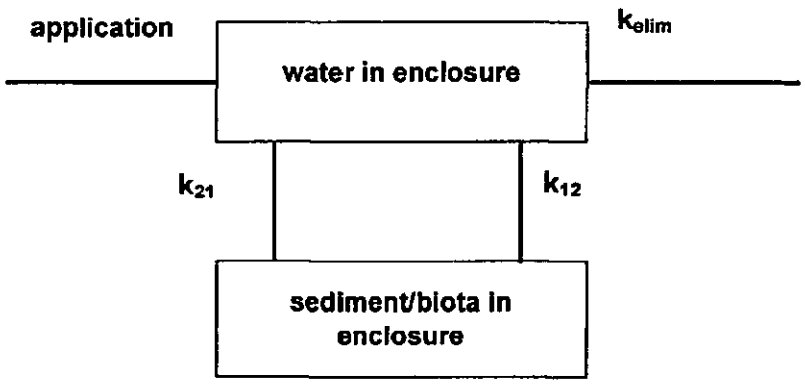
1. A first order, one compartment model, with a single dose input and a first order output:

$$C_t = C_0 * e^{-k_{elim}t}$$



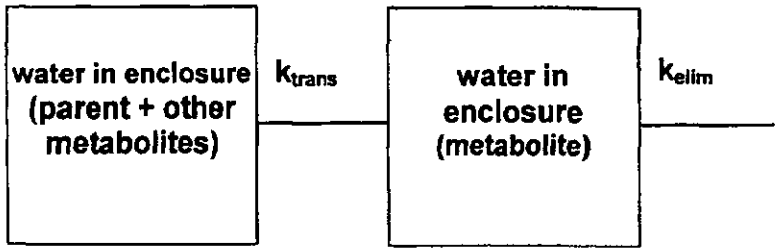
2. A two-compartment (bi-exponential) model with a single dose input and first order output

$$C_t = A * e^{(-\alpha * t)} + B * e^{(-\beta * t)}$$



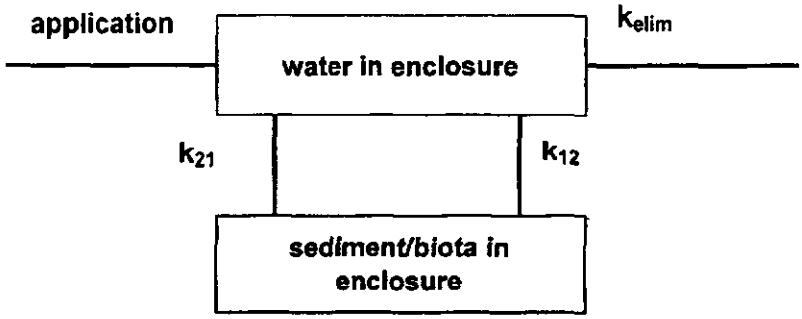
3. A one compartment model with first order input and a first order output

$$C_t = \frac{Dose * k_{trans}}{Volume(k_{trans} - k_{elim})}\{e^{k_{elim} * t} - e^{k_{trans} * t}\}$$



4. A two compartment model with a constant input and a first order output

$$C_t = A * e^{(-\alpha * t)} + B * e^{(-\beta * t)} + C * e^{(k_{trans} * t)}$$



4.5 LC_{50} Calculations

Based on the observations on fish mortality, a computer program, modified from the program of C. Stephan (Peltier and Weber, 1985) was used to calculate the LC_{50} values and 95% confidence intervals. Three statistical methods are available in the computer

programme: moving average angle analysis, probit analysis, and non-linear interpolation with 95% confidence intervals calculated by binomial probability. The selection of reported LC_{50} values and 95% confidence intervals were based upon an examination of the database and the results of the computer analysis. Selection criteria included the establishment of a concentration-effect relationship, the number of concentrations causing partial responses and the span of responses bracketing the LC_{50} value. If two or more statistical methods produced acceptable results, then the method which yielded the smallest 95% confidence interval was selected. The methods used are presented in the tables.

4.6 Statistical Methods (fish weight and length)

The data were tested for normality using the Shapiro-Wilk test. The data for the spray drift and run-off weights were normal distributed after transformation to log 10. The data for the run-off length were normally distributed without transformation. The data for the spray drift length were not normally distributed.

For the normal distributed data, an analysis of variance was performed to determine whether statistical differences between groups existed. If the analysis of variance showed statistical significant differences ($p<0.05$), the Duncan test was performed to determine between which groups the differences were statistical significant.

For non-normal distributed data a Kruskal-Wallis ANOVA, median test was performed to determine whether statistical differences between groups existed. If the Kruskal-Wallis ANOVA, median test showed statistical significant differences ($p<0.05$), the Mann Whitney U-test was performed to determine between which groups the differences were statistical significant.

5. RESULTS

5.1 Treatments

The results are summarized in Table 6 to 9 and Figures 20 to 26.

5.1.1 Radiochemical purity of α,β-endosulfan in stock solutions

The radiochemical purity of [^{14}C]-α,β-endosulfan as supplied by the sponsor was determined by C_{18}-HPLC-UV/RAM in all stock solutions prior to each application (spray drift and run-off). The results show that the radiopurity prior to and after the treatments was > 98% in all solutions. Representative chromatograms are given in Figures 20 to 22. Due to the fact, that deep frozen stock solutions from the first treatment were used to prepare application solutions for the second treatment, the stability of the active ingredient in acetone was proven after 2 weeks storage in a freezer. Figure 20B shows the corresponding HPLC chromatogram.

5.1.2 Spray drift: Quantification of the test item applied to the enclosures

The following results were obtained: Amounts of 9 to 23%, 2 to 11% and 2 to 23% of the amounts present in the stock solutions were rinsed from the spraying device after the 1st, 2nd and 3rd treatment, respectively. An average of 1.0 % was lost to the plastic cover. This value results from determinations after the 1st treatment, which was also applied to the 2nd and 3rd treatment. Based on the pre-study method validation experiment, the average loss of radioactivity on the fish cage above the water surface was 10%. The amounts of radioactivity lost per treatment were subtracted from the total amount in the corresponding spray solutions: Table 6 presents the expected average water concentrations per treatment. These concentrations are used as test concentrations in this report. The values may be compared with the measured residue concentrations in water 3 to 6 hours after the corresponding treatments (cf. section 5.2.1).

The spray solutions were analyzed by C_{18}-HPLC-UV/RAM prior to and post each application. The stability during application was maintained as shown in representative chromatograms of Figures 23 and 24.

5.1.3 Run-off: Amount of soil residue introduced into the enclosures

The following total radioactive soil residue (SR) was determined in the soil for run-off preparation following treatment with α and β-endosulfan and ageing for one day at room temperature: 33.1 mg/kg dry equivalent soil (1st treatment), 31.9 mg/kg dry soil equivalent (2nd treatment) and 31.9 mg/kg dry soil equivalent (3rd treatment).

HPLC-RAM/UV analysis of post ageing soil extracts showed that parent α and β-endosulfan were responsible for 96.2 to 97.3 % of the total radioactivity. Representative chromatograms are given in Figures 25 and 26.

The total amounts of aged soil, which were introduced as slurry into the enclosures are provided in Table 7. Based on the different amounts introduced into each test system, the total residue load per enclosure and the expected average water concentrations per treatment were calculated. Table 8 summarizes the results. As for the spray drift entry, the expected average water concentrations per treatment [µg SR/L] are used as test concentrations in this report. These values may be compared with the measured residue concentrations in water 3 to 6 hours after the corresponding treatments (cf. section 5.2.1).

It should be stressed, that the expected average concentrations represent soil bound and desorbed residue. A desorption experiment was conducted in order to quantify the proportion of soluble and insoluble residue introduced into the enclosures. An overview of the percentage of radioactivity desorbed from the soil is presented in Table 9. The mean amount of radioactivity desorbed from the soil was 11.38, 7.93 and 7.65 mg per kg dry soil expressed as endosulfan, corresponding to mean percentages of 34, 25 and 24% for the 1st, 2nd and 3rd application, respectively. Hence, most of the residue was still bound to the soil particles after the desorption experiment.

5.2 Residue in Water

The results are summarized in Tables 10 to 36 and Figures 27 to 77.

The following sections describe the concentration and nature of the residue in water after the 1st treatment until the end of the study. Due to the very low water solubility of the test item, which for spray drift simulation was applied as a fine fog onto the surface of the water body, the concentration of the test item in the surface film during 1 day post treatment will be

described below. Furthermore, the vertical distribution of the test item in the water phase during time, i.e. from the upper water layers (including surface film after spray drift) into the deeper water layers will be illustrated. The method validation experiments for surface film collection are provided in Annex VII.

5.2.1 Quantification of the total radioactive residue (TRR) in water

5.2.1.1 Spray Drift

The results are summarized in Tables 10 to 16 as well as in Figures 27 to 34.

The distribution of the residue in water was comparable for all concentrations and treatment regimes. This is valid for the surface film residue as well as for the depth integrated raw water samples and the raw water samples collected from the 3 water depth levels. A sharp and continuous concentration gradient was found within the first 24 hours after each treatment in the various samples (Tables 10 to 12 and Figures 27 to 33): The highest residue levels were found in the surface film, followed by the residue concentrations in the subsurface, mid water and near sediment water samples. After about 1 day post each treatment, the TRR was equally distributed in the water column and the concentration in the surface film had remarkably dropped to almost the concentration levels of water.

It is obvious from the results, that the residue concentrations in the surface film during about 6 hours post treatment were remarkably higher than the concentrations in the deeper water layers and the depth integrated water concentrations (Table 13).

The maximum exposure concentrations in surface film, water at about 10 cm below surface and depth integrated water samples are summarized in Table 14. The results clearly indicate, that the exposure concentrations during the first 6 hours post treatment were remarkably higher than the expected concentrations in water. The maximum exposure concentrations in depth integrated water were either slightly higher or similar to the expected average concentrations in water.

As already mentioned above, the residue concentration in water from the various depth levels was comparable after 1 day post treatment. The average concentrations for all test groups during total incubation are summarized in Table 16 and Figure 34. The results clearly indicate, that the average residue in water decreased rather quickly within 24 hours after the

treatments, followed by a much slower dissipation. The results show, that up to 40% of the initial water residue had disappeared from the water within 24 hours. It is assumed, that the rather fast mitigation of the residue is most likely due to volatilisation from the surface film and/or binding of the residue to other compartments of the ecosystem. The dissipation kinetic of the TRR water will be described below under section 5.2.3.

5.2.1.2 Run-Off

The results are summarized in Tables 17 to 22 as well as in Figures 35 to 42.

The TRR in the raw water at the various depth levels was more or less comparable during the study, however with one exception: During the first 6 hours after the second and third treatment (if any), the residue in the subsurface water layers was typically higher than in the mid level and above sediment surface layers. The latter were comparable during the study at all concentration levels. The relatively high concentrations in the subsurface layers might have been caused by the presence of contaminated clay/silt particles during the hours after the treatment. After settlement of these particles and/or partly release of the aged materials from the soil followed by degradation, the residue in subsurface water was comparable with the residue in the deeper water layers.

There is no explanation, why this was not observed during the first treatment. One reason might be the sampling regime: Samples were taken later after the 1st treatment than samples taken after the 2nd and 3rd treatment (cf. section 3.11.1).

Typically, the maximum exposure concentrations were found in the subsurface water layers within the first 24 hours post each treatment. These concentrations as well as the maximum exposure concentrations in depth integrated water samples as summarized in Table 20.

The residue concentration in water from the various depth levels was comparable latest one day post treatment. The depth integrated water concentrations for all test groups during incubation are summarized in Table 22 and Figure 42. The results clearly indicate, that the average residue in the water decreased rather quickly within 24 hours after the treatments, followed by a much slower dissipation, as described for spray drift simulation, however at a lower rate. The results show, that up to 40% of the initial water residue had disappeared from the water. It is assumed, that the mitigation of the residue is most likely due to

sedimentation of the contaminated soil particles and/or binding of the desorbed residue to other compartments of the ecosystem. The dissipation kinetic of the TRR water will be described below.

5.2.1.3 DT_{50} of the TRR in water

The results provided in Tables 15 and 22 indicate, that the TRR in water more rapidly decreased during the initial 24 hours after treatment followed by a phase with a slower decrease. In the second phase, the TRR decreased constantly until the end of the incubation period, but this period did not cover a full half-life interval in all test groups. This is valid for both entry routes (Figure 43). The data obtained after single treatment with 10.33 µg ai/L (SD-8.38) and 8.39 µg SR/L (RO-8.39, SR: residue originally sorbed to soil) were suitable to calculate DT_{50} values using kinetics models which well fitted the available experimental data. After spray drift, the DT_{50} for the TRR_{water} was calculated to be 71 days. The corresponding DT_{50} for the TRR_{water} after run-off treatment was calculated as 102 days after the treatment.

5.2.2 Characterization of the total radioactive residue (TRR) in water

The total radioactive residue in raw water was analyzed by filtration through 0.7 µm sieves in order to detect the residue associated with the suspended particulate matter (SPM). In addition, HPLC-UV/RAM was used to characterize the dissolved active ingredient and its metabolites in water. The elution pattern was compared with that of a 1st series of analytical standards: α,β-endosulfan, endosulfan sulfate and endosulfan diol as supplied by the sponsor at test initiation (Figure 44). Chromatograms of freezer stored, acidified raw water samples are provided under Annex IX to show the storage stability to dissolved residues. The storage period covers a time interval until the last series of water analyses. Selected water and solid-phase-extracted (SPE) samples were re-analysed in order to enrich and identify unknown major components in water using a 2nd series of analytical standards containing endosulfan lactone, endosulfan hydroxy carboxylic acid, endosulfan hydroxy ether, endosulfan dihydroxy ether and endosulfan ether, supplied by the sponsor (Figure 45). The corresponding storage stability chromatograms are provided under Annex IX.

5.2.2.1 Suspended Particulate Matter (S.P.M)

The results are summarized in Tables 23 and 24 as well as in Figures 46 to 51.

Analysis of the amount of residue, associated with the suspended particulate matter > 0.7 µm was conducted throughout the study using raw water samples from test groups SD-2.68 and RO-4.19.

The results obtained from spray drift showed, that the majority of the residue was found in the percolate (Table 23): Between 90.9 and 109.5 % of the raw water radioactivity was available as dissolved radioactivity. Small portions of only 0.8 to 8.9 % of the raw water radioactivity were associated with suspended particulate matter. A particular time of increased or decreased portions of SPM associated residue was not found during the study.

Very similar results were obtained for the run-off entry route (Table 24). Between 93.9 and 100.7% of the raw water residue were dissolved radioactivity. Amounts of 0.7 to 2.4 % were associated to the suspended particulate matter.

Samples obtained from test groups SD-2.68, SD-8.38, RO-4.19 and RO-8.39 were used to conduct HPLC analysis of the raw water and filtered water samples. The results are shown under Figures 46 to 51. As expected, the pattern of radioactive components was quite similar before and after filtration. This is valid for both run-off and spray drift entry routes.

5.2.2.2 Chromatographic analysis of residue dissolved in water

The results are summarized in Tables 25 to 36 and Figures 52 to 72.

The following test groups were selected to conduct detailed HPLC analysis of the metabolites with the purpose to monitor the concentrations of the test item active ingredient and its major degradates during time: Test groups SD-2.68 and RO-4.19 (3 treatments), test groups SD-4.69 and RO-6.29 (2 treatments) and test groups SD-8.38 and RO-8.39 (single treatment).

Furthermore, the pattern of metabolites from different test groups was compared at the same selected intervals in order to show a potential influence of the test concentration. As expected, the test concentration did not change the pattern as demonstrated in Figure 52.

In the course of the study, up to 16 radioactive components were detected. Of these, 7 were identified as α,β-endosulfan or its known metabolites by means of HPLC co-elution with the corresponding analytical standards. Representative HPLC chromatograms are provided under Figures 53 to 56 (spray drift) and 57 to 59 (run-off). HPLC chromatograms presented under Figures 60 to 63 illustrate representative re-analysis traces to identify unknown components using a flatter elution gradient.

The composition of the dissolved residues is listed in Tables 25 to 29 for spray drift and in Tables 30 to 34 for run-off entry.

5.2.2.2.1 α,β-endosulfan

The active ingredient of the test item showed a rather fast degradation after the treatments. This is valid for both isomers. The results show, that the 90% disappearance of the active ingredient was faster after the 2nd and 3rd treatment. This is valid for both entry routes of the test item.

After spray drift entry of the test item, the concentration of α/β endosulfan dropped below 10% of the total residue in water within 7 days, 2 days and 6 hours (1st, 2nd and 3rd treatment of test group SD-2.68, respectively), 12 hours (2nd treatment of test group SD-4.69) and 7 days after the single treatment of test group SD-8.38. Quite similar disappearance rates were obtained after run-off entry: the concentration of α/β endosulfan dropped below 10% of the total residue in water within 12 days, 2 days and 3 hours (1st, 2nd and 3rd treatment of test group RO-4.19, respectively), 4 days (2nd treatment of test group RO-6.29) and 7 days after treatment of test group RO-8.39. Further details about the DT_{50} of endosulfan and its isomers are provided under section 5.2.3.1.

5.2.2.2.2 Endosulfan diol

The major metabolite besides the parent compound was endosulfan diol. The concentration of this component sharply increased after each treatment to a peak. Thereafter, the component dissipated rather quickly. This is valid for all test levels and both entry routes. Further details concerning the dissipation kinetics can be obtained from section 5.2.3.2.

After spray drift entry, the maximum portions of the diol were observed on the following days: 2, 2 and 1 after the 1st, 2nd and 3rd treatment of test group SD-2.68, respectively; 2 after the

2nd treatment of test group SD-4.69 and 2 to 7 days after treatment of test group SD-8.38. The corresponding values for the run-off entry are: 2, 2 and 1 days after the 1st, 2nd and 3rd treatment of test group RO-4.19, respectively; 2 days after the 2nd treatment of test group RO-6.29 and 4 days after treatment of test group RO-8.39. Hence, very similar results were obtained for both entry-routes.

The following maximum residues of endosulfan diol were found after spray drift entry of the test item: 4.29 µg peq/L (test group SD-2.68, triplicate treatment), 7.01 µg peq/L (test group SD-4.69, duplicate treatment) and 4.33 µg peq/L (test group SD-8.38, single treatment). After 42 days, the residue of endosulfan diol after single treatment at test group SD-8.39 had dropped to 9.3% (0.49 µg peq/L) of the total residue.

The corresponding values for the run-off entry were: 4.22 µg peq/L (test group RO-4.19, triplicate treatment), 4.66 µg peq/L (test group RO-6.29, duplicate treatment) and 3.03 µg peq/L (test group RO-8.39, single treatment). As for the spray drift entry, the residue of endosulfan diol after single treatment at tets group RO-8.39 had dropped to 9.5% (0.41 µg peq/L) of the total residue after 43 days.

The results obtained indicate, that endosulfan diol represents the major residue, which disappears fairly quickly from the water compartment of the ecosystem.

5.2.2.2.3 Endosulfan hydroxy ether

Endosulfan hydroxy ether was detected regularly in the water after spray drift and run-off entry.

The concentration of this metabolite increased constantly between each application and remained at about constant levels throughout the study.

The following maximum residue of endosulfan hydroxy ether were found after spray drift entry of the test item: 1.48 µg peq/L (test group SD-2.68, triplicate treatment), 2.01 µg peq/L (test group SD-4.69, duplicate treatment) and 2.47 µg peq/L (test group SD-8.38, single treatment). The corresponding values for the run-off entry are: 1.57 µg peq/L (test group RO-4.19, triplicate treatment), 1.82 µg peq/L (test group RO-6.29, duplicate treatment) and 1.65 µg peq/L (test group RO-8.39, single treatment).

5.2.2.2.4 Endosulfan lactone

Like endosulfan hydroxy ether, endosulfan lactone was detected regularly in the water samples of both entry routes. The following maximum residue levels were reached at the end of the spray drift part of the study: 1.48 µg peq/L (test group SD-2.68, triplicate treatment), 1.85 µg peq/L (test group SD-4.69, duplicate treatment) and 1.96 µg peq/L (test group SD-8.38, single treatment). The corresponding values for the run-off entry are: 1.63 µg peq/L (test group RO-4.19, triplicate treatment), 1.84 µg peq/L (test group RO-6.29, duplicate treatment) and 1.65 µg peq/L (test group RO-8.39, single treatment).

5.2.2.2.5 Endosulfan sulfate

Endosulfan sulfate occurred regularly in all water samples throughout the study at more or less constant concentrations at a fairly low level, when compared to the above metabolites. This is valid for both entry routes. However, there is indication, that the concentrations towards the end of the study rather decreased. The concentrations of endosulfan sulfate ranged from 0.01 to 0.39 µg peq/L (test group SD-2.68, triplicate treatment), from 0.29 to 0.62 µg peq/L (test group SD-4.69, duplicate treatment) and from 0.19 to 0.54 µg peq/L (test group SD-8.38, single treatment). The corresponding ranges for the run-off entry route are: <0.01 to 0.77 µg peq/L (test group RO-4.19, triplicate treatment), 0.05 to 0.95 µg peq/L (test group RO-6.29, duplicate treatment) and 0.09 to 0.75 µg peq/L (test group RO-8.39, single treatment).

5.2.2.2.6 M1

M1 represented the most polar fraction, which eluted from the HPLC column only a short time after the dead time. The peak shape indicated, that this fraction consists of one component. Generally speaking, the concentrations of M1 in water varied over time. However, the maximum residues of M1 were rather found towards the end of the outdoor phase of the study. The following maximum residues in water after spray drift entry were found: 1.26 µg peq/L (test group SD-2.68, triplicate treatment), 1.14 µg peq/L (test group SD-4.69, duplicate treatment) and 1.96 µg peq/L (test group SD-8.38, single treatment). The corresponding value for the run-off entry are: 2.01 µg peq/L (test group RO-4.19, triplicate treatment), 1.64 µg peq/L (test group RO-6.29, duplicate treatment) and 1.20 µg peq/L (test

group RO-8.39, single treatment). These values correspond to 8.9 to 28.7% of the total amount of test item applied per enclosure. No further identification was performed.

5.2.2.2.7 M4

This metabolite was found mainly towards the end of the outdoor incubation phase and only after duplicate and triplicate treatment with the test item. Until day 21, M4 was found in traces, only. This is valid for all treatment levels and both entry routes. The residue increased constantly towards test termination, where the following maximum residue levels were found: 0.25 µg peq/L (test group SD-2.68, triplicate treatment) and 0.42 µg peq/L (test group SD-4.69, duplicate treatment). After single treatment at test group SD-8.38 M4 was detected up to 0.47 µg peq/L. The corresponding values for the run-off entry are: 0.29 µg peq/L (test group RO-4.19, triplicate treatment) and 0.63 µg peq/L (test group RO-6.29, duplicate treatment). As for the spray drift entry, M4 was detected up to 0.39 µg peq/L after single treatment with at test group RO-8.39. These values correspond to 2.4 to 5.0% of the total amount of test item applied per enclosure. No further identification was performed

5.2.2.2.8 Minor unknown degradates of endosulfan

Apart from the above known and major (>10%) unknown metabolites of endosulfan, several minor (≤10%) components were detected. They amounted to maximum 0.42 µg peq/L including at least 5 individual components after triplicate treatment at test group SD-2.68, 0.25 µg peq/L for 4 components after duplicate treatment at test group SD-4.69 and to 0.36 µg peq/L including at least 6 components after single treatment at test group SD-8.38. Very similar maximum concentration of unidentified components were found after run-off entry of the test item: 0.33 µg peq/L including at least 3 individual components after triplicate treatment at test group RO-4.19, 0.63 µg peq/L including at least 3 components after duplicate treatment at test group RO-6.29 and to 0.24 µg peq/L including at least 5 components after single treatment at test group RO-8.39.

5.2.3 Degradation Kinetics of TRR, parent and metabolites in water

The dissipation dated for (α+β)-endosulfan, α-endosulfan, β-endosulfan, and it's metabolites endosulfan diol and endosulfan hydroxy ether in water were plotted vs. time and the DT_{50}

were calculated. An overview of the corresponding DT_{50} values is presented in Tables 35 and 36 for spray drift and run-off, respectively. The plots of the concentrations found in the water phase against time for test groups SD-8.38 and RO-8.39 are presented in Figures 73 to 77.

5.2.3.1 DT_{50} of Endosulfan, (α- and β-endosulfan)

A fast dissipation of both α- and β-endosulfan was found for test groups SD-2.68 and RO-4.19 (triplicate treatment) and SD-8.38 and RO-8.39 (single treatment) with DT_{50} values of <1 day for the spray drift application and ≤2 days for the run-off application (Figure 73). The slower dissipation after run-off applications was most likely caused by continuous desorption of the soil bound residue into the water. The dissipation of α-endosulfan (Figure 74) and β-endosulfan (Figure 75) can be described by a two compartment model. First a rapid distribution of the test item to the various compartments of the ecosystem took place after which other factors became effective in the ecosystem, like e.g. biotic degradation of the test item. No α- or β-endosulfan was found in the water after 12 days of exposure in test groups SD-8.38 and RO 8.39.

5.2.3.2 DT_{50} of Endosulfan diol

Figure 76 shows that the dissipation of endosulfan diol follows a first order kinetic. The DT_{50} values of endosulfan diol at test groups SD-2.68 and RO-4.19 (triplicate treatment) and SD-8.38 and RO-8.39 (single treatment) are between 8 and 14 days for both spray drift and run-off entry routes.

5.2.3.3 DT_{50} of endosulfan hydroxy ether

The DT_{50} values for endosulfan hydroxy ether were calculated as 13 and 10 days for test groups SD-8.38 and RO-8.39 (single treatment) (Figure 77). For test groups SD-2.68 and RO-4.19 (triplicate treatment), the time after the last application was too short to allow for a definite pattern, but an increase in the concentration was found up to day 35, after which a decrease was seen until day 43.

5.3 Residue in other Compartments of the Ecosystem

The results are summarized in Tables 37 to 43 as well as in Figures 78 to 86.

Investigations of the TRR in macrophyte materials were conducted using spray drift and run-off test groups SD-2.68 and RO-4.19, i.e. those enclosures, which had been treated 3 times at 14-day intervals.

The comparative Figures 59 and 60 obtained from both entry routes clearly demonstrate, that by far, the highest residue concentration was found in or on the macrophyte material, whereas the residue concentrations in the sediments were minor.

5.3.1 Residue (TRR) in macrophytes

5.3.1.1 Quantification of the total radioactive residue (TRR) in macrophytes

The residue in *Elodea canadensis* increased constantly during the study (Table 37 and Figure 78 and 79). A decrease of the residue was not observed until the end of the outdoor phase. The maximum residue after spray drift entry was 2.2 mg peq/kg (wet weight) macrophyte material. After run-off entry of the test item, maximum 2.1 mg peq/kg were found. Taking into account, that the maximum concentration in water was between 11 and 15 µg peq/L, it becomes obvious, that there is significant adsorption and /or accumulation of the residue by the macrophytes.

5.3.1.2 Characterization of the total radioactive residue (TRR) in macrophytes at the maximum residue level

The pattern of radioactive components was analyzed at the maximum residue level, i.e. on day 31 (test group SD-2.68) and on day 26 (test group RO-4.19). The results are summarized in Table 38. The corresponding chromatograms are shown in Figures 80 and 81.

Numerous components were detected in the extracts obtained from *Elodea canadensis* of which two were identified as α- and β-endosulfan. The concentrations of α-endosulfan were at 47 µg peq/kg (spray drift) and 19 µg peq/kg (run-off). The corresponding concentrations of β-endosulfan were at 15 and 19 µg peq/kg, respectively. Three of the other components

were identified as known metabolites of endosulfan, i.e. endosulfan sulfate, endosulfan diol and endosulfan hydroxy ether. The concentrations of these metabolites were between 156 and 305 µg peq/kg after spray drift entry and between 170 and 460 µg peq/kg after run-off entry.

Four distinct unknown components (M6 to M9) were found: at the following maximum levels (both entry routes): 434 µg peq/kg (M9), 279 µg peq/kg (M6), 126 µg peq/kg (M7) and 87 µg peq/kg (M8). Apart from these components, several unknown radioactive components were detected. Up to thirteen of these were minor: Their total concentration did not exceed 173 µg peq/kg macrophyte material.

5.3.2 Residue (TRR) in Sediment

5.3.2.1 Quantification of the total radioactive residue (TRR) in sediment

The residue in the total sediment cores was significantly lower than in the macrophyte material (Table 39 and Figures 78 and 79). After spray drift entry the residue constantly increased during time. A maximum concentration of 6.2 µg peq/kg sediment was found. After run-off treatment, the residue peaked on the days of treatment. This is most likely due to deposition of contaminated soil residue on the sediment surface. After the peak phase and a short term drop of the concentration, the residue increased again. A maximum concentration of 13.8 µg peq/kg was found.

As will be shown later (cf. section 5.6) the majority of the residue was found in the top centimeter sediment layer which included the water/sediment interphase material.

5.3.2.2 Characterization of the total radioactive residue (TRR) in sediment

The results are summarized in Tables 40 to 43. Representative chromatograms are shown in Figures 82 to 85. The extracted sediment samples were shown to be stable in the freezer, cf. figure 85a. This sediment sample had been extracted about half a year after sampling and the extracts were measured by HPLC 2 years later.

The results indicate that the radioactivity, which had been extracted from the sediments at the various time intervals, consisted of several components. The majority of the residue was characterized using available standards of the parent component and its metabolites.

5.3.2.2.1 α- and β-endosulfan

The parent components (α/β endosulfan) were found at about constant concentrations after spray drift entry of the test item: Between 0.31 and 0.69 µg/kg were found in the sediment after triplicate treatment at test group SD-2.68. The contribution of α/β endosulfan to the TRR of the sediment was rather low: It decreased from maximum 68.7% after the 1st treatment to 10.5% at test end (Table 41).

After run-off entry, the residue of the parent component peaked at the treatment times, most likely caused by deposition of contaminated soil particles. For the same reason, the residue was remarkably higher after run-off entry than after spray drift entry. After the treatments, the concentration of α/β endosulfan dropped to a lower level due to desorption/degradation of the parent. In contrast to the spray drift entry route, the majority of the residue was represented by the parent compound: 100% of the residue consisted of endosulfan after the 1st treatment. At the end of the test, still 41% of the TRR were represented by the parent component (Table 43).

Maximum residue concentrations at 8.5 µg/kg were found after the 3rd treatment of test group RO-4.19. At the end of the study 0.65 and 3.76 µg/kg sediment were detected in the sediments of test groups SD-2.68 and RO-4.19, respectively.

5.3.2.2.2 Endosulfan sulfate

Endosulfan sulfate was detected in the sediment after both entry routes at similar concentrations. The concentration of this component increased constantly with time: At the end of the study, the following residues were found: 1.60 µg peq/kg (test group SD-2.68) as well as 2.16 µg peq/kg (test group RO-4.19). Endosulfan sulfate contributed maximum 34 and 24% to the TRR of the sediment, SD-2.68 and RO-4.19, respectively.

5.3.2.2.3 Endosulfan diol

The concentrations of endosulfan diol were similar to the concentrations of endosulfan sulfate. They furthermore showed a very similar development during the study: The concentrations of endosulfan diol constantly increased towards the end of the test, where 2.39 µg peq/kg (test group SD-2.68) and 1.80 µg peq/kg (test group RO-4.19) were

detected. Endosulfan diol contributed maximum 38 and 21% to the TRR of the sediment at test groups SD-2.68 and RO-4.19, respectively.

5.3.2.2.4 Endosulfan hydroxy ether and endosulfan lactone

Both components showed a –more ore less- constant increase during the study at similar concentration levels. The following residues were found at the end of the study: endosulfan hydroxy ether contributed 0.96 and 0.54 µg peq/kg after SD-2.68 and RO-4.19 entry of the test item. The corresponding values for endosulfan lactone are 0.54 and 0.47 µg peq/kg sediment. Each of these components did not contribute more than 28% and 8.0% to the TRR of the sediment with spray drift and run-off entry.

5.3.2.2.5 Unkown components

Apart from the above metabolites of endosulfan, several minor unknown components were detected in the sediment extracts. None of them exceeded 0.16 µg peq/kg sediment. This corresponds to a contribution of maximum 3.2% of the TRR for each individual component.

A comparative HPLC pattern of compounds found in water, sediment and macrophytes is provided in Figure 86.

5.4 Effects on Fish

The following effects on fish were monitored during the study: Mortality and sublethal effects, the latter by analysis of growth and weight during the study and at the end of the test. Furthermore, fish were removed at regular intervals in order to monitor the TRR in fish and the number and nature of metabolites. The results are summarized in Tables 44 to 54 as well as in Figures 87 to 122.

5.4.1 Fish Mortality

According to Tables 4 and 5, the fish populations per enclosure were checked after each treatment for dead fish which were removed from the enclosures. It should be mentioned here, that after the treatments, the fish frequently moved to the water surface. This was particularly observed for the 2 highest test groups (SD-8.38 and RO-8.39).

The cumulative mortality data are presented in Tables 44 (spray drift) and 45 (run-off) and summarized in Figure 87. The results indicate a very steep dose response.

At test termination a cumulative mortality of 0, 3, 10, 13, 100, 100 and 100 % was found in test groups SD-0.27 to SD-8.38 of the spray drift application, respectively. For the run-off entry a cumulative mortality of 3, 3, 13, 7, 100, 100 and 100% was found for test groups RO-0.21 to RO-8.39, respectively.

LC_{50} values were calculated using the mortality data during and at the end of the study. For that purpose, the average concentration of α,β-endosulfan in water per treatment (cf. Table 6 and 8) were used to conduct these calculations in agreement with the sponsor. The results are summarized in Table 46 and 47 as well as in Figure 88. The LC_{50} values found for the spray drift application sharply decreased after the first and second treatment and stayed at more or less constant level thereafter, i.e. at 2.39 (day 29) and 1.86 µg/L (day 41). The corresponding LC_{50} values for the run-off application decreased constantly after the first treatment and remained thereafter at a similar level i.e. at 2.96 µg/L after 13 days, 2.86 µg/L after 27 days and 2.79 µg/L after 42 days.

At lower treatments, the mortality of fish was either comparable to the controls or slightly enhanced: After spray drift of the lowest test groups SD-0.27 and SD-0.47, respectively, 0 and 3% of the fish had died at the end whereas 0 to 8% had died in the controls for spray drift and run-off, respectively. The mortality at test groups SD-0.84 and SD-1.51 was slightly enhanced: 10 and 13% of the fish had died, respectively. After run-off application, 3 to 7% of the fish had died in test groups RO-0.21, RO-0.42 and RO-2.09. After treatment at test group RO-0.84, the mortality was slightly enhanced, since 13% of the fish had died at the end.

It is assumed, that the slightly enhanced mortality at test groups SD-0.84, SD-1.51 and RO-0.84 is rather related to factors like the virus infection of fish as shown under Annex XIII than to toxicity of the test item: Figures 89 and 90 clearly demonstrate, that the mortality events at the 4 lowest test concentrations are not related to the treatments as demonstrated for the higher test groups (cf. Figure 89 top, spray drift). Furthermore, a consistent dose-response for mortality was not detected at test groups SD-0.27 to SD-1.51 and RO-0.21 to RO-2.09.

5.4.2 *Sublethal Effects*

All surviving/remaining fish were removed at the end of the test. Before the fish were deep frozen for analytical purpose, their weight and length were recorded. During the study, 2 fish had been randomly removed from each enclosure at each sampling time for residue determination. Their weight and length were determined as well before the fish were deep frozen for residue analysis.

5.4.2.1 Fish Length and Weight at test termination

The average weight and length at test termination and the number of fish per enclosure are presented in Table 48. The corresponding box and whisker plots and provided in Figures 91 to 94.

5.4.2.2 Length and Weight after spray drift application

The mean weights of the control fish after spray drift application were 1.44 ± 0.28, 1.57 ± 0.50, 1.67 ± 0.48 and 1.47 ± 0.44 g in the four replicate enclosures, respectively. As no significant difference was found between the four controls (ANOVA, $p>0.05$), the results were combined for further statistical analysis. The mean weights in the combined controls and test groups SD-0.27 to SD-1.51 were 1.57 ± 0.37, 1.32 ± 0.32, 1.29 ± 0.34, and 1.21 ± 0.29 g, respectively. No significant difference between the combined controls and the groups was found (ANOVA, $p>0.05$).

The mean lengths of the control fish after spray drift application were 49 ± 4, 49 ± 5, 50 ± 6 and 47 ± 5 mm in the four replicate enclosures, respectively. Significant differences were found between control group 2 and 4 and between control groups 3 and 4. Therefore, control group 4 was not used for further statistical evaluation. No significant difference was found between the three other control groups (ANOVA, $p>0.05$). Therefore, these results were combined for further statistical analysis. The mean lengths in the combined control and test groups SD-0.27 to SD-1.51 were 48 ± 4, 46 ± 5, 46 ± 4, 46 ± 4 and 46 ± 4 mm, respectively. No significant difference between the combined controls and the test groups was found (ANOVA, $p>0.05$).

Based on the above results, the NOEC for fish growth in the spray drift application was determined to be at test group SD-1.51 (triplicate spray drift application at 14 day intervals), corresponding to an average water concentration of 1.96 µg a.i./L per treatment.

5.4.2.3 Length and Weight after run-off application

The mean weights of the control fish after run-off application were 1.46 ± 0.43, 1.47 ± 0.40, 1.36 ± 0.36 and 1.48 ± 0.53 g in the four replicate enclosures, respectively. As no significant difference was found between the four controls (ANOVA, p> 0.05), the results were combined for further statistical analysis. The mean weights in the combined control and the RO-0.21 to RO-2.09 test groups were 1.38 ± 0.43, 1.25 ± 0.30, 1.24 ± 0.29, and 1.26 ± 0.26 g, respectively. No significant difference between the combined controls and any of test concentrations was found (ANOVA, p>0.05).

The mean lengths of the control fish after run-off application were 48 ± 5, 48 ± 4, 47 ± 6 and 47 ± 6 mm in the four replicate enclosures, respectively. As no significant difference was found between the four controls (ANOVA, p> 0.05), the results were combined for further statistical analysis. The mean lengths in the combined control and the RO-0.21 to RO-2.09 test groups were 47 ± 5, 46 ± 5, 46 ± 4, and 47 ± 2 mm, respectively. No significant difference between the combined controls and any of the test concentrations was found (ANOVA, p>0.05).

Based on the above results, the NOEC for fish growth was determined to be at test group RO-2.09 (triplicate run-off application at 14 day intervals), corresponding to an average water concentration of 2.09 µg SR/L per treatment.

5.4.2.4 Fish Length and Weight during the test

The results are summarized in Figures 95 to 102.

The results clearly indicate, that the slopes of the regression curves after treatment with the test item are very similar to the slopes of the corresponding controls. This is valid for both parameters at all sublethal test levels and both entry routes.

In conclusion, it can be stated, that the results for sublethal effects, obtained during the study, support the results obtained at the end of test, i.e. no sublethal effects were found at the four lowest test groups after spray drift and run-off treatment.

5.4.3 NOEC Values for Mortality and Sublethal Effects

The NOEC values are summarized in Table 49. The NOEC values for both endpoints are at test groups SD-1.51 and RO-2.09, i.e. triplicate spray drift entry of average 1.96 µg ai/L per treatment (spray drift) and average 2.09 µg SR/L per treatment (run-off) at 14 day intervals.

5.4.4 Residues in Fish

The results are summarized in Tables 50 to 55 as well as in Figures 103 to 123.

5.4.4.1 Determination of the TRR in surviving fish

In Figures 103 to 112, the total radioactive residue in fish is related to the TRR in water and the mortality at the various concentration levels. Tables 50 and 51 as well as Figures 94 and 95 summarize the fish residue. In Figures 96 and 97, the TRR and parent residue in water is related to mortality and the residue in fish.

The concentration of total radioactivity in the fish increased directly after each application. In general a slower increase in concentration was seen in the run-off application when compared with the spray drift application. After the increase in concentration, a relatively rapid decrease of the concentration to 50% of the highest level was seen within approximately 4 days. The highest concentration measured in the fish was by a factor of 1218 (spray drift entry) and 1562 (run-off entry) higher than the nominal concentrations in the water phase of the microcosm study, indicating bioconcentration of the residue in fish followed by depuration within rather short time. The following maximum TRR's were found: 0.414 mg peq/kg (test groups SD-0.27, triplicate treatment), 0.619 mg peq/kg (test group SD-0.47, triplicate treatment), 0.943 mg peq/kg (test group SD-0.84, triplicate treatment), 2.228 mg peq/kg (test group SD-1.51, triplicate treatment) and 3.960 mg peq/L (test group SD-2.68, triplicate treatment). The corresponding values after run-off are: 0.328 mg peq/kg (test group RO-0.21, triplicate treatment), 0.441 mg peq/kg (test group RO-0.42, triplicate treatment), 0.833 mg peq/kg (test group RO-0.84, triplicate treatment), 1.535 mg peq/kg

(test group RO-2.09, triplicate treatment) and 3.463 mg peq/kg (tets group RO-4.19, triplicate treatment).

5.4.4.2 Characterisation of the residue in surviving fish

The residue of fish obtained from test groups SD-1.51 and RO-2.09 were analysed in 2 samples at the maximum residue level in order to characterize the nature of the TRR. The results are shown in Table 52. Representative chromatograms are shown in Figures 117 and 118.

The pattern of metabolites obtained from the 2 samples was very similar (run-off and spray drift): The majority of the residue, i.e. 38.8% to 49.0% of the TRR was identified as endosulfan sulfate at day 30 of incubation including three treatments. These values correspond to 0.395 to 0.518 mg peq/kg fish. M5 represented the second major metabolite in fish: up to 0.552 mg peq/kg (24.8 % of the TRR) were found. This metabolite had not been found in water and macrophytes, but in sediment. α,β-endosulfan represented a major portion of radioactivity: 7.9 % (0.080 mg/kg) to 13.3 % (0.295 mg/kg) were detected. Typically, β-endosulfan represented more than 50% of the endosulfan residue. Furthermore the very polar fraction M1 was major, representing up to 15.5 % of the sample TRR (0.158 mg peq/kg fish). Endosulfan diol and endosulfan hydroxy ether were detected at minor amounts. Maximum 2.7 % (0.029 mg/kg) and 3.9 % (0.060 mg/kg) were found, respectively. Apart from the above components an unspecific residue was detected at maximum 0.214 mg peq/kg including at least 15 distinct components.

5.4.4.3 Quantification and Characterization of the residue in dead fish

The residue of dead fish was analysed in order to determine the lethal dose for bluegill sunfish as well as the nature of the accumulated residue. The results are summarized in Tables 53 to 54 as well as in Figures 119 to 121.

The average residue after spray drift treatment at test group SD-4.69 was 2.214 mg peq/kg and 3.499 mg peq/kg on day 1 and 2 after treatment (fish which have been dead for a maximum of 1 day between observations). After duplicate run-off entry at test group RO-6.29, the average residue amounted to 3.294 mg peq/kg on day 2 after the treatment.

After dosing of the enclosures at test group SD-8.38, the residue amounted to 4.220 mg peq/kg after one day and 4.410 mg peq/kg after two days. The corresponding values at test group RO-8.39 are 4.020 mg peq/kg and 3.900 mg peq/kg.

The results indicate, that the lethal body load for fish is minimum 2.214 mg peq/kg and increased in this study to 4.410 mg peq/kg.

The characterization of the residue demonstrated, that the pattern of radioactivity in dead fish was different from the pattern obtained from the surviving fish of test groups SD-1.51 and RO-2.09. In dead fish the majority of the residue was represented by α,β-endosulfan (59.3 to 68.3 % of the TRR or 1.296 and 2.845 mg/kg, respectively). The α-isomer of endosulfan represented more than half of the amount. In surviving fish of test groups SD-1.51 and RO-2.09 α,β-endosulfan represented only 11.6 to 13.3% of the total residue and β-endosulfan represented the major isomer.

Endosulfan sulfate was the second major component of the TRR in dead fish ranging from 24.0 to 30.3 % of the TRR (1.001 to 0.742 mg peq/kg). M1, M5 and endosulfan hydroxy ether represented minor fractions of the TRR at maximum 4.3 % (0.106 mg peq/kg) for each individual component.

A HPLC comparison of the pattern of metabolites in water and fish is provided in Figure 122.

The overall pattern of metabolites in the various compartments of the ecosystem is summarized in Table 55.

5.5 Balance of Radioactivity at test termination

At the end of the outdoor phase of the study, a mass balance was calculated for each individual treated enclosure. For that purpose, the total dpm found in the various compartments of the systems, i.e. water, macrophytes, tank wall and fish, were added up and given in percent of the applied radioactivity at test initiation. The difference between the total radioactivity recovered and the total radioactivity applied is supposed to be radioactivity which disappeared from the systems due to biodegradation and evaporation of the test item. The values obtained from water and sediment were extrapolated from the aliquots taken to the total amount of water and sediment per enclosures.

The results are shown in Figures 123 and 124. The TRR in water decreased to values between 44.3 and 61.1% from the initial values in all enclosures. A dose-dependence was not observed. The residue in macrophytes showed no explicate dose-response at the two entry routes except for the 2 highest test concentrations: The residue of test groups SD-0.27 to SD-2.68 and RO-0.21 to RO-4.19 ranged from 4.2 to 8.1%, whereas residues between 9.2 and 14.1% were found in test-groups SD/RO 6 and 7. Very similar findings were made for the sediment: between 2.9% and 16.9% of the total applied radioactivity were found at the end at all test groups.

The tank wall periphyton contained less than 2.5% of the total applied radioactivity.

The residue in fish amounted to less than 1.1% after spray drift and run-off entry of the test item.

The results indicate that between 20.9 and 43.6% of the residue had disappeared from the test system after a total of 6 weeks.

5.6 Detailed Chemical and Biological Analysis of the sediments at test termination

At test termination, the sediment samples were collected in a different way as it was done during the study: The sediment cores were sectioned into layers and analyzed chemically and biologically in order to obtain the following information:

1. Vertical distribution of the residue
2. Distribution of the residue between sediment solids and the pore water
3. Determination of the residue in sediment-dwelling organisms
4. Effects on the abundance of sediment-dwelling organisms in the various sediment layers
5. Effects on eco-functional groups of sediment-dwellers (detritivorous and predatory organisms)

Parallel investigations in the testing basin and the Lake site of removal of water-sediments were conducted for comparison purpose.

All results are summarized in Tables 56 to 61 as well as in Figures 125 to 137.

5.6.1 Quantification of the residue in the sediment compartment

The results are summarized in Tables 56 and 57 as well as in Figures 125 to 129.

The results obtained for both entry routes and all concentrations indicate, that the residue in the aqueous compartments, i.e. water overlaying the sediment, including sediment-water interphase water as well as pore water of the top 1 cm sediment layer decreased remarkably when compared to the residue in the free water of the enclosures. This gradient indicates that the sediment is a sink for the residue. The concentrations found in the pore water depended on the total applied concentration: After treatments with 10.5 (3 times average 3.5 µg/L per treatment (test group SD-3.5)) to 12.8 µg a.i./L (2 times average 6.4 µg/L per treatment (test group SD-6.4)) applied as single dose or in 2 or 3 portions at intervals of 14 days the residue in the top layer pore water was between 0.11 and 4.18 µg peq/L. This is valid for both entry routes. As for the highest treatment levels, pore water concentrations were similar after spray drift and run-off at the lower concentration levels.

In the sediment, a gradient was observed from the top centimeter to the deeper layers of the sediment. This is valid for the pore water and the remaining residue in the sediments solid (Figures 125 to 129, respectively), i.e. the concentrations are lower in the deeper sediment layers. As for the pore water, a dose-dependency is observed for both entry-routes. It is obvious from the tables, that the residue in the top sediment layer is remarkably higher after run-off treatment when compared to the corresponding dose levels after spray drift treatment. This is most likely due to settled soil particles, which had been introduced into the systems during the treatment(s). Maximum residues were found at 30 µg peq/kg sediment after spray drift entry of 3 * average 3.5 µg a.i./L per treatment (test group SD-2.68) and 65 µg peq/kg sediment after run-off entry of 3 * average 3.99 µg SR/L per treatment (test group RO-4.19).

A very similar picture is seen for the residue of the sediment-dwelling organisms (Figure 128): The higher the dose, the higher the residue. However, only organisms found in the top 5 centimeters of the sediment showed a residue above the detection limit. Depending on the concentrations, the residue ranged between 0.02 to 1.58 µg peq/kg for the lowest spray drift concentration (3 * average 0.34 µg ai/L per treatment, test group SD-0.27) and the highest run-off concentration (single treatment with 8.39 µg SR/L, test group RO-8.39), respectively.

A comparable view of the residue in the pore water, the sediment and the sediment dwelling organisms is provided in Figure 129.

5.6.2 Characterization of the residue of the top one cm sediment layer

The results are summarized in Table 58 and Figures 130 to 131.

The residue in the top centimeter layer of the sediment, which included the solid materials of the sediment-water interphase, was characterized by HPLC and TLC. The samples obtained from test groups SD-2.68 and RO-4.19 were selected to conduct this work.

As can be seen from the corresponding table, the residue after spray drift consisted of at least 13 components, of which none exceeded 8.9 µg peq/kg sediment. 6 of the 13 components were identified as parent compound and known metabolites of the test item.

The known components accounted for total 23.7 µg peq/kg of 30.4 µg peq/kg total residue. None of the unknown components exceeded 2.8 µg peq/kg.

After run-off treatment, at least 15 components were detected. Of these, only 2 exceeded 10 µg peq/kg: alpha and beta endosulfan. Their total concentration was 35 µg/kg of 64.6 µg peq/kg total residue. The known components below an individual concentration of 10 µg peq/L contributed 22.3 µg peq/kg sediment. None of the at least seven unknown components exceeded 7.1 µg peq/kg.

5.6.3 Analysis of Sediment Dwelling Organisms

The results are summarized in Tables 59 to 61 and Figures 132 to 137.

The organisms were collected from the following sediment layers and evaluated in vivo at test termination:

1. Top centimeter of the sediment combined with the water-sediment interphase material
2. 1 to 5 cm layer

Based on pre-study investigations, no significant counts of sediment-dwelling organisms were found in the deeper sediment layers, i.e. > 5 cm. This corresponds to respective findings in the Lake (cf. Annex XI).

The results obtained from the top 5 cm of the sediment cores at test termination were compared with the finding from sediment cores in the testing basin and from the Bay of Fussach, i.e. the origin of the water-sediment systems to conduct this study. These investigations were conducted parallel to the activities at test termination, i.e. at about the same season.

Due to the fact, that the water-sediment interphase was collected and pooled with the top centimetres of the sediment, various types of non-sediment dwelling macroinvertebrate larvae and other organisms were found: Ostracoda, Ephemeroptera, Trichoptera, Bryozoa, Hydrozoa, Gastropoda, Bivalvia, Acari, Chaoboridae and Ceratopogonidae. Typically, their abundances were too low for quantitative evaluation and hence, they are not considered here.

The counts for the following groups of organisms (determination groups) were taken into account to assess the effects of the test item on sediment-dwelling organisms: Oligochaeta and Chironomidae.

Several lower taxa were combined to form these groups due to low abundance of the individual lower taxa. The following lower taxa contribute to the groups:

Oligochaeta **(detritivorous)**:

Branchiura soverbyi

Tubificidae

Naididae

Chironomidae **(detritivorous)**:

Chironomus plumosus

Chrionomus thummi

Chironomini

Tanytarsinae (detritivorous)

Chironomidae **(predatory)**:

Tanypodinae

5.6.3.1 Results after Spray drift and Run-Off Application

The abundance of sediment dwelling organisms in the top centimeter of the control spray drift and run-off sediments was remarkable higher than in the deeper sediment layer (1- 5 cm). This is mainly valid for the detritivorous oligochaetes and the predatory midge larvae. The counts of detritivorous chironomids were generally very low in the control sediments. The total counts of detritivorous and predatory sediment dwellers were comparable in the control enclosures for spray drift and run-off entry of the test item. Hence, the treatment of

the enclosures with control soil did not have an influence on the abundance of sediment dwellers.

The results shown under Tables 59 and 60 as well as in Figures 133 to 135 indicate, that the abundance of all groups of organisms after treatment with the test item is comparable to the abundance in the controls. A particular dose-response was not found. Due to the fact, that no replicates were available for the treatment test groups, the results of all groups (i.e. including the controls) were analyzed using Box and Whisker plots (non-parametric test to e.g. identify outlyers of a data base). Outlying values occured very rarely, i.e. values, which were 1.5 times above or below the interquartile range from the 3rd quartile (75th percentile). After spray drift treatment, the detirivorous sediment-dweller of only one of 4 controls showed higher abundances than all other groups. This is valid for all sediment layers and the whole cores and was due to the increased abundance of the chironomids. After run-off treatment, no systematic response was observed. However, outlying high abundances with respect to all other test groups were found for one of the contol test groups and the treatments test groups RO-021 (lowest concentration) and RO-8.39 (highest concentration). In conclusion, the Box and Whisker plots support the finding, that the NOEC values are given at the highest test concentrations for both entry routes (Table 61)

5.6.3.2 Sediment Dwelling Organisms in enclosures, testing basin and the Bay of Fussach

The counts obtained for the sediment-dwelling oligochaeta were typically lower in the control enclosures for spray drift and run-off than in the testing basin and the Bay of Fussach, where comparable counts were made. However, the predatory chironomids occurred with similar abundance in the Bay and the control enclosures. Predatory chironomids were found at about equal abundance in the testing basin and the control enclosures of both entry routes. However, none of them were found in the Bay of Fussach. Apart from the quantitative differences in the population of sediment-dwellers it can be stated, that the compositions of sediment dwelling organisms is similar in the enclosures, the testing basin and a comparable environment of the Lake.

5.6.3.3 Comparison of the biological variability of organisms, found in and on the sediment (sediment-water interphase, benthos).

The above statement, i.e. the comparability of the test system with the Lake is supported by the fact, that the biological diversity in the enclosures, the testing basin and a corresponding Lake environment is quite similar (Figure 137). Between 8 and 10 distinct taxa and determination groups were found in the spray drift controls, between 6 and 9 in the run-off controls. The corresponding values for the Testing Basin is 10 and the Bay of Fussach is 14.

6. CONCLUSIONS

The results lead to the conclusion, that the residue of endosulfan and its metabolites dissapears 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 dissapear rather fast from water, whereas others like endosulfan lactone, M1 and M4 increase with time. 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 SR/L after run-off entry (triplicate treatment at increments of 14 days). The Ecologically Acceptable Concentration for toxic effects on sediment-dwelling organisms is 3.50 µg ai/L after spray-drift entry and 3.99 µg SR/L after run-off entry (triplicate treatment 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).

7. REFERENCES

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8. TABLES

Table 1. Overall time schedule.

Day Spray Drift	Day Run-Off	Week 1998	Date (1998)	Event
		20	15 May	Equipment of the testing basin with sediment
		21	19 May	Equipment with Macrophytes
			23 May	Addition of Lake water to the testing basin
		22	26 May	Addition of lake borne zooplankton
		23		-
		24	9 June	Addition of lake borne macro-zoobenthic organisms
		25		-
		26		-
		27	2 July	Initiation of regular physical-chemical monitoring
		28		-
		29		-
		30		Assessment of macrophyte biomass
-26	-27	31	31 July	Study Plan signed
			31 July	Arrival of Test Fish at the SRC of Springborn in one of Springborn's basin Distribution of fish (majority into a large cage basin, remaining fish into laboratory culture acclimation to conduct SL project 1049.008.310)
-21	-22	32	5/6 August	Assessment of macrophyte biomass
-19	-20		7 August	Establishment of the Enclosures
-13	-14	33	12-14 August	Establishment of Fish Cages
			mid of August	Transfer of the test fish holding cage into the definitive testing basin.
-6	-7	34	20 August	Establishment of the water collection device and stainless steel stripes for the collection of the tank wall residue
-5	-6		21 August	Randomization of fish and introduction into the enclosures
-1	-1	35	25 August	Day –1 characterization (physical-chemical parameters)
0		35	26 August	Spray drift: 1st Treatment Concentrations 1,2,3,4,5,6,7
	0		27 August	Run-Off: 1st Treatment Concentrations 1,2,3,4,5,6,7
		36		-
14		37	9 September	Spray drift: 2nd Treatment Concentrations 1,2,3,4,5,6
	14		10 September	Run-Off: 2nd Treatment Concentrations 1,2,3,4,5,6
		38		-
28		39	23 September	Spray drift: 3rd Treatment Concentrations 1,2,3,4,5
	28		24 September	Run-Off: 3rd Treatment Concentrations 1,2,3,4,5
		40		-
41		41	06 October	Spray drift: Test Termination
	42		08 October	Run-Off: Test Termination

- No study related activities

Table 2. Spray drift entry: Collection of surface film (SF), water (W), sediment (S) and macrophytes *Myriophyllum* (Myr) and Elodea (El).

Day Spray Drift	Week 1998	Date (1998)	Event	SF	W	S	Myr	El
0	35	26. Aug.	1st Treatment (SD-0.27 to 8.38)	x	x			
1		27. Aug.		x	x			
2		28. Aug.			x	x	x	
3		29. Aug.			x	x	x	x
4		30. Aug.						
5	36	31. Aug.						
6		1. Sep.						
7		2. Sep.			x	x		x
8		3. Sep.						
9		4. Sep.						
10		5. Sep.						
11		6. Sep.						
12	37	7. Sep.						
13		8. Sep.			x	x	x	x
14		9. Sep.	2nd Treatment (SD-0.27 to 4.69)	x	x			
15		10. Sep.		x	x			
16		11. Sep.			x	x		x
17		12. Sep.			x	x		
18		13. Sep.						
19	38	14. Sep.						
20		15. Sep.			x	x		x
21		16. Sep.						
22		17. Sep.						
23		18. Sep.						
24		19. Sep.						
25		20. Sep.						
26	39	21. Sep.						
27		22. Sep.			x	x		
28		23. Sep.	3rd Treatment (SD-0.27 to 2.68)	x	x			
29		24. Sep.		x	x			
30		25. Sep.			x	x		x
31		26. Sep.			x	x		x
32		27. Sep.						
33	40	28. Sep.						
34		29. Sep.						
35		30. Sep.			x	x		x
36		1. Oct.						
37		2. Oct.						
38		3. Oct.						
39		4. Oct.						
40	41	5. Oct.						
41		6. Oct.			x			
42		7. Oct.	Test End (last observation fish)			x	x	

Myr *Myriophyllum spicatum*
El *Elodea canadensis*

Table 3. Run-off entry: Collection of surface film (SF), water (W), sediment (S) and macrophytes *Myriophyllum* (Myr) and Elodea (El).

Day Run-Off	Week1998	Date (1998)	Event	SF	W	S	Myr	El
0		27. Aug.	1st Treatment (RO-0.21 to 8.39)		x	x		
1		28. Aug.			x	x		
2		29. Aug.			x	x	x	x
3		30. Aug.						
4	36	31. Aug.			x	x		x
5		1. Sep.						
6		2. Sep.						
7		3. Sep.			x	x		x
8		4. Sep.						
9		5. Sep.						
10		6. Sep.						
11	37	7. Sep.						
12		8. Sep.			x		x	x
13		9. Sep.				x		
14		10. Sep.	2nd Treatment (SD-0.27 to 6.29)		x	x		
15		11. Sep.			x	x		
16		12. Sep.			x	x		x
17		13. Sep.						
18	38	14. Sep.			x	x		x
19		15. Sep.						
20		16. Sep.						
21		17. Sep.			x	x		x
22		18. Sep.						
23		19. Sep.						
24		20. Sep.						
25	39	21. Sep.						
26		22. Sep.			x	x		x
27		23. Sep.						
28		24. Sep.	3rd Treatment (SD-0.27 to 4.19)		x	x		
29		25. Sep.			x	x		
30		26. Sep.			x	x		x
31		27. Sep.						
32	40	28. Sep.			x	x		x
33		29. Sep.						
34		30. Sep.				x		x
35		1. Oct.						
36		2. Oct.						
37		3. Oct.						
38		4. Oct.						
39	41	5. Oct.						
40		6. Oct.						
41		7. Oct.						
42		8. Oct.	Test End (last observation fish)			x	x	x
43		9. Oct.			x			

Myr *Myriophyllum spicatum*
El *Elodea canadensis*

Table 4. Spray drift entry: Water quality, fish observations and collection of fish for residue analysis.

Day Spray Drift	Week 1998	Date (1998)	Event	Water Quality	Observations	Collection
0	35	26. Aug.	1st Treatment (SD-0.27 to 8.38)		X	
1		27. Aug.		X	X	
2		28. Aug.		X	X	
3		29. Aug.		X	X	X
4		30. Aug.			X	
5	36	31. Aug.		X	X	
6		1. Sep.			X	
7		2. Sep.		X	X	X
8		3. Sep.		X	X	
9		4. Sep.		X	X	
10		5. Sep.			X	
11		6. Sep.			X	
12	37	7. Sep.		X	X	
13		8. Sep.			X	X
14		9. Sep.	2nd Treatment (SD-0.27 to 4.69)	X	X	
15		10. Sep.		X	X	
16		11. Sep.			X	X
17		12. Sep.			X	X
18		13. Sep.			X	
19	38	14. Sep.			X	
20		15. Sep.		X	X	
21		16. Sep.			X	X
22		17. Sep.			X	
23		18. Sep.			X	
24		19. Sep.			X	
25		20. Sep.			X	
26	39	21. Sep.		X	X	
27		22. Sep.			X	X
28		23. Sep.	3rd Treatment (SD-0.27 to 2.68)		X	
29		24. Sep.		X	X	
30		25. Sep.			X	X
31		26. Sep.			X	X
32		27. Sep.			X	
33	40	28. Sep.			X	
34		29. Sep.			X	
35		30. Sep.			X	X
36		1. Oct.		X	X	
37		2. Oct.			X	
38		3. Oct.			X	
39		4. Oct.			X	
40	41	5. Oct.			X	
41		6. Oct.			X	X
42		7. Oct.			X	

Table 5. Run-off: Water quality, fish observations and collection of fish for residue analysis.

Day Run-Off	Week 1998	Date (1998)	Event	Water Quality	Observations	Collections
0		27. Aug.	1st Treatment (RO-0.21 to 8.39)	X	X	
1		28. Aug.		X	X	
2		29. Aug.		X	X	
3		30. Aug.			X	
4	36	31. Aug.		X	X	X
5		1. Sep.			X	
6		2. Sep.		X	X	
7		3. Sep.		X	X	X
8		4. Sep.		X	X	
9		5. Sep.			X	
10		6. Sep.			X	
11	37	7. Sep.			X	
12		8. Sep.		X	X	X
13		9. Sep.			X	
14		10. Sep.	2nd Treatment (RO-0.21 to 6.29)	X	X	
15		11. Sep.		X	X	
16		12. Sep.			X	X
17		13. Sep.			X	
18	38	14. Sep.			X	X
19		15. Sep.		X	X	
20		16. Sep.			X	
21		17. Sep.			X	X
22		18. Sep.			X	
23		19. Sep.			X	
24		20. Sep.			X	
25	39	21. Sep.		X	X	
26		22. Sep.			X	X
27		23. Sep.			X	
28		24. Sep.	3rd Treatment (RO-0.21 to 4.19)	X	X	
29		25. Sep.			X	
30		26. Sep.			X	X
31		27. Sep.			X	
32	40	28. Sep.			X	X
33		29. Sep.			X	
34		30. Sep.			X	
35		1. Oct.		X	X	X
36		2. Oct.			X	
37		3. Oct.			X	
38		4. Oct.			X	
39	41	5. Oct.			X	
40		6. Oct.			X	
41		7. Oct.			X	
42		8. Oct.			-	X

Table 6. Spray drift entry route: Target treatment rates and expected water concentrations, based on the total amount of radioactivity introduced into each enclosure.

Test Group	SD-0.27	SD-0.47	SD-0.84	SD-1.51	SD-2.68	SD-4.69	SD-8.38
TARGET [% of the MRFR] per treatment	**0.32%**	**0.56%**	**1.0%**	**1.8%**	**3.2%**	**5.6%**	**10%**
TARGET Concentration in Water [µg ai/L*] per treatment	**0.27**	**0.47**	**0.84**	**1.51**	**2.68**	**4.69**	**8.38**
TARGET Concentration in Water [µg EC/L*] per treatment	**0.82**	**1.43**	**2.55**	**4.59**	**8.15**	**14.26**	**25.47**
Number of treatments at intervals of 2 weeks	**3**	**3**	**3**	**3**	**3**	**2**	**1**
1st treatment Total amount per microcosm [mg ai]	0.29	0.53	1.122	1.872	3.208	6.935	12.292
1st: Concentration in water [µg ai/L*]	0.24	0.45	0.94	1.57	2.70	5.83	10.33
1st: Concentration in water [% target]	90	95	112	104	101	124	123
2nd: Total amount per microcosm [mg ai]	0.451	0.794	1.471	2.532	4.644	8.297	NP
2nd: Concentration in water [µg ai/L*]	0.38	0.67	1.24	2.13	3.90	6.97	NP
2nd: Concentration in water [% target]	140	142	147	141	146	149	NP
3rd: Total amount per microcosm [mg ai]	0.458	0.655	1.558	2.591	4.643	NP	NP
3rd: Concentration in water [µg ai/L*]	0.39	0.55	1.31	2.18	3.9	NP	NP
3rd: Concentration in water [% target]	143	117	156	144	146	NP	NP
Sum: Total amount per microcosm [mg ai]	1.199	1.979	4.151	6.995	12.495	15.232	12.292
Sum: Concentration in water [µg ai/L*]	1.01	1.66	3.49	5.88	10.50	12.80	10.33
Sum: Concentration in water [% target]	124	118	138	130	131	136	123
Average concentration in water per treatment [µg ai/L*]	**0.34**	**0.55**	**1.16**	**1.96**	**3.50**	**6.40**	**10.33**
Average concentration in water per treatment [µg EC/L*]	**1.03**	**1.67**	**3.53**	**5.96**	**10.64**	**19.45**	**31.40**
Average drift rate [% of the MRFR]	**0.4%**	**0.7%**	**1.4%**	**2.3%**	**4.2%**	**7.6%**	**12.3%**

SD: Spray drift
MRFR: Maximum Recommended Field Rate: 1.05 kg ai/ha
*: Concentrations are calculated based on the total volume of 1190 L per enclosure
EC: Emulsifiable Concentrate (Thiodan 352 g/L)
NP: Application not performed

Table 7. Run-off applications: Amounts of untreated and treated soil introduced into the respective enclosures during 3 applications.

Test Group	1st treatment	2nd treatment	3rd treatment	Sum of 3 treatments	Mean of 3 treatments	st. dev. of 3 treatments
	moist soil[1] [g]	moist soil[1] [g]	moist soil[1] [g]	moist soil[2] [g]	moist soil[2] [g]	moist soil[2] [g]
TSR [mg/kg]	33.1	31.9	31.9	-	-	-
RO-0.21	7.8	8.3	8.3	24.4	8.1	0.3
RO-0.42	15.6	16.6	16.5	48.8	16.3	0.6
RO-0.84	31.2	33.3	33.0	97.5	32.5	1.1
RO-CTRL-1	79.3	80.8	82.3	242.4	80.8	1.5
RO-2.09	77.8	82.9	82.1	242.8	80.9	2.7
RO-CTRL-2	158.8	161.9	141.4	462.1	154.0	11.1
RO-4.19	155.9	166.2	141.3	463.4	154.5	12.5
RO-CTRL-3	238.1	242.7	NP	480.8	240.4	3.3
RO-6.29	233.6	249.1	NP	482.7	241.4	11.0
RO-CTRL-4	317.6	NP	NP	317.6	317.6	NA
RO-8.39	311.7	NP	NP	311.7	311.7	NA

RO: Run-off
st. dev.: Standard Deviation
[1] per application
[2] per 3 applications
CTRL Control
NP: Not Performed
NA: Not Applicable (one treatment only)
TSR: Total soil residue

Table 8. Run-off entry route: Target treatment rates and theoretical water concentrations, based on the amount of aged soil introduced into each enclosure.

Test Group	RO-0.21	RO-0.42	RO-0.84	RO-2.09	RO-4.19	RO-6.29	RO-8.39
Target [% of the MRFR] per treatment	**0.05%**	**0.1%**	**0.2%**	**0.5%**	**1.0%**	**1.5%**	**2.0%**
Target load [mg SR] per microcosm and treatment	**0.25**	**0.5**	**1.0**	**2.49**	**4.99**	**7.48**	**9.98**
Target water conc. [µg SR/L*] per treatment	**0.21**	**0.42**	**0.84**	**2.09**	**4.19**	**6.29**	**8.39**
Target water con. [µg EC/L*] per treatment	**0.64**	**1.28**	**2.55**	**6.35**	**12.74**	**19.12**	**25.50**
Number of treatments at intervals of 2 weeks	**3**	**3**	**3**	**3**	**3**	**2**	**1**
1st: Total amount applied per microcosm [mg SR/L]	0.25	0.5	1.0	2.49	4.99	7.48	9.98
1st: Concentration in water [µg SR/L*]	0.21	0.42	0.84	2.09	4.19	6.29	8.39
1st: Concentration in water [% target]	100	100	100	100	100	100	100
2nd: Total amount applied per microcosm [mg SR/L]	0.25	0.5	1.0	2.49	4.99	7.48	NP
2nd :Concentration in water [µg SR/L*]	0.21	0.42	0.84	2.09	4.19	6.29	NP
2nd :Concentration in water [% target]	100	100	100	100	100	100	NP
3rd :Total amount applied per microcosm [mg SR/L]	0.25	0.5	1.0	2.49	4.279[a]	NP	NP
3rd :Concentration in water [µg SR/L*]	0.21	0.42	0.84	2.09	3.60[a]	NP	NP
3rd :Concentration in water [% target]	100	100	100	100	86[a]	NP	NP
Sum: Total amount applied per microcosm [mg SR/L]	0.75	1.5	3.0	7.47	14.259	14.960	9.98
Sum: Concentration in water [µg SR/L*]	0.63	1.26	2.52	6.28	11.98	12.57	8.39
Sum: Concentration in water [% target]	100	100	100	100	95	100	100
Average load [mg SR] per microcosm and treatment	**0.25**	**0.5**	**1.0**	**2.49**	**4.75**	**7.48**	**9.98**
Average concentration in water per treatment [µg SR/L*]	**0.21**	**0.42**	**0.84**	**2.09**	**3.99**	**6.29**	**8.39**
Average concentration in water per treatment [µg EC/L*]	**0.64**	**1.28**	**2.55**	**6.35**	**12.13**	**19.12**	**25.5**
Average Run-Off Contamination rate [% of the MRFR]	**0.05%**	**0.1%**	**0.2%**	**0.5%**	**1.0%**	**1.5%**	**2.0%**

RO: Run-off
MRFR: Maximum Recommended Field Rate: 1.05 kg a.i./ha.
SR: Soil Residue (after a one day ageing under aerobic conditions).
*: concentrations are calculated based on the total volume of 1190 L per enclosure and total desorption.
EC: Emulsifiable Concentrate (Thiodan 352 g/L).
NP: Application not performed.
a. Lower dosing due to limited amount of radiolableled ai, in agreement with the sponsor

Table 9. Concentration of total radioactivity in the soil, aerobically aged for one day, as well as amount and percentage of radioactivity desorped per kg dry soil.

	Concentration of endosulfan in aged soil [mg/kg dry soil]	Amount of endosulfan desorbed per kg dry soil [mg]	Mean amount of endosulfan desorbed per kg dry soil [mg]	Percentage of desorbed endosulfan [%]	Mean percentage of desorbed endosulfan [%]
Application 1	33.1	9.85[a]	**11.38**	-	**34**
		11.06		33	
		11.20		34	
		11.46		35	
		11.75		36	
		11.43		35	
Application 2	31.9	6.86	**7.93**	21	**25**
		8.02		25	
		8.14		25	
		8.68		27	
		8.30		26	
		7.62		24	
Application 3	31.9	5.77[a]	**7.65**	-	**24**
		7.60		24	
		7.70		24	

[a] Outlier according to Dixon test for outliers at α=0.05, not used for further calculations.

Table 10. Spray drift: Total Radioactive Residue (TRR) in the surface film, at 3 subsurface water levels and in depth integrated water: Test period after the 1st application.

Test Day		1 hr	3 hrs	6 hrs	1 day	2 days	3 days	7 days	13 days
Test Conc. (Test Group)	Sample	[µg peq/L]							
0.34 µg ai/L*** (SD-0.27)	SF	46	13	7	2	NP	NP	NP	NP
	TRR_{SS}	0.67	1.04	0.74	0.17	0.18	0.17	0.16	0.13
	TRR_{M}	0.03	0.02	0.03	0.18	0.17	0.16	0.15	0.13
	TRR_{SSe}	0.02	0.02	0.02	0.16	0.17	0.16	0.15	0.13
	[a]AVG_{TRR}	0.24	0.36	0.26	0.17	0.17	0.16	0.15	0.13
0.55 µg ai/L*** (SD-0.47)	SF	204	124	59	88	NP	NP	NP	NP
	TRR_{SS}	2.58	2.52	1.15	0.36	0.36	0.34	0.32	0.28
	TRR_{M}	0.03	0.03	0.03	0.36	0.35	0.34	0.32	0.28
	TRR_{SSe}	0.03	0.02	0.03	0.29	0.36	0.33	0.31	0.28
	[a]AVG_{TRR}	0.88	0.86	0.40	0.34	0.36	0.34	0.32	0.28
1.16 µg ai/L*** (SD-0.84)	SF	104	61	21	9	NP	NP	NP	NP
	TRR_{SS}	1.70	2.86	2.28	0.75	0.66	0.65	0.59	0.53
	TRR_{M}	0.04	0.05	0.05	0.72	0.68	0.66	0.60	0.52
	TRR_{SSe}	0.02	0.03	0.03	0.49	0.68	0.67	0.58	0.52
	[a]AVG_{TRR}	0.59	0.98	0.79	0.65	0.68	0.66	0.59	0.52
1.96 µg ai/L*** (SD-1.51)	SF	1175	830	586	151	NP	NP	NP	NP
	TRR_{SS}	6.93	6.16	3.54	1.25	1.15	1.11	1.04	0.93
	TRR_{M}	0.09	0.11	0.07	1.21	1.16	1.14	1.05	0.93
	TRR_{SSe}	0.02	0.04	0.04	0.68	1.14	1.11	1.03	0.93
	[a]AVG_{TRR}	2.35	2.10	1.22	1.05	1.15	1.12	1.04	0.93
3.50 µg ai/L** (SD-2.68)	SF	128	51	45	12	NP	NP	NP	NP
	TRR_{SS}	12.75	7.43	6.68	2.29	2.08	2.00	1.91	1.70
	TRR_{M}	0.18	0.11	0.22	2.25	2.08	2.05	1.92	1.74
	TRR_{SSe}	0.05	0.05	0.05	1.94	2.16	2.06	1.94	1.74
	[a]AVG_{TRR}	4.33	2.53	2.32	2.16	2.11	2.04	1.92	1.73
	[b]AVG_{TRR}	3.91	2.62	NP	NP	NP	1.81	NP	1.76
6.40 µg ai/L** (SD-4.69)	SF	943	421	NP	NP	NP	NP	NP	NP
	TRR_{SS}	27.21	15.03	NP	4.24	3.75	3.71	3.50	3.19
	TRR_{M}	0.23	0.91	NP	4.21	3.80	3.77	3.61	3.23
	TRR_{SSe}	0.07	0.09	NP	3.53	3.77	3.76	3.43	3.19
	[a]AVG_{TRR}	9.17	5.34	NP	3.99	3.77	3.75	3.52	3.20
10.33 µg ai/L* (SD-8.38)	SF	1670	250	NP	NP	NP	NP	NP	NP
	TRR_{SS}	25.53	27.81	NP	6.96	7.74	7.61	7.09	6.59
	TRR_{M}	0.37	0.23	NP	8.75	7.70	7.65	7.21	6.63
	TRR_{SSe}	0.14	0.17	NP	8.90	8.06	7.67	7.06	6.58
	[a]AVG_{TRR}	8.68	9.40	NP	8.20	7.83	7.64	7.12	6.60

Test Conc.: Average water concentration per treatment based on total radioactivity applied to the enclosures
SD: Spray drift
SF: Surface Film
TRR: Total Radioactive Residue
AVG: Average
SS: about 10 cm sub-surface
M: Mid water level
SSe: about 10 cm above sediment surface
[a]: average of SS, M and SSe values
[b]: measurement of depth integrated samples
*, **, ***: one, two or three treatments
NP: not performed
peq: parent equivalents

Table 11. Spray drift: Total Radioactive Residue (TRR) in the surface film, at 3 subsurface water levels and average: Test Period after the 2nd application.

Test Day		14 days 1 hrs	14 days 3 hrs	14 days 6 hrs	15 days	16 days	17 days	21 days	27 days
Test Conc. (Test Group)	Sample	[µg peq/L]							
0.34 µg ai/L*** (SD-0.27)	SF	335	112	58	13	NP	NP	NP	NP
	TRR_{SS}	0.96	1.39	1.07	0.53	0.37	0.35	0.30	0.29
	TRR_{M}	0.13	0.14	0.15	0.48	0.36	0.34	0.31	0.28
	TRR_{SSe}	0.13	0.15	0.14	0.14	0.31	0.33	0.32	0.30
	[a]AVG_{TRR}	0.41	0.56	0.46	0.38	0.35	0.34	0.31	0.29
0.55 µg ai/L*** (SD-0.47)	SF	241	55	27	6	NP	NP	NP	NP
	TRR_{SS}	1.71	4.28	3.05	1.03	0.78	0.75	0.64	0.61
	TRR_{M}	0.28	0.29	0.30	1.00	0.74	0.74	0.65	0.60
	TRR_{SSe}	0.27	0.29	0.29	0.30	0.71	0.73	0.65	0.60
	[a]AVG_{TRR}	0.76	1.62	1.21	0.78	0.74	0.74	0.65	0.60
1.16 µg ai/L*** (SD-0.84)	SF	361	37	32	13	NP	NP	NP	NP
	TRR_{SS}	0.62	2.08	4.68	1.95	1.36	1.32	1.15	1.12
	TRR_{M}	0.53	0.55	0.53	1.63	1.34	1.33	1.20	1.11
	TRR_{SSe}	0.51	0.56	0.54	0.56	1.06	1.25	1.19	0.88
	[a]AVG_{TRR}	0.55	1.06	1.92	1.38	1.25	1.30	1.18	1.04
1.96 µg ai/L*** (SD-1.51)	SF	491	108	51	21	NP	NP	NP	NP
	TRR_{SS}	8.74	12.49	8.96	3.41	2.46	2.42	2.21	2.04
	TRR_{M}	0.98	1.00	0.98	3.10	2.55	2.44	2.22	2.05
	TRR_{SSe}	0.91	0.95	0.92	0.93	2.23	2.30	2.19	1.93
	[a]AVG_{TRR}	3.54	4.81	3.62	2.48	2.41	2.38	2.21	2.01
3.50 µg ai/L** (SD-2.68)	SF	878	139	152	79	NP	NP	NP	NP
	TRR_{SS}	16.75	20.59	16.24	5.45	4.72	4.59	4.14	3.84
	TRR_{M}	1.81	1.86	1.84	4.44	4.68	4.54	4.19	3.91
	TRR_{SSe}	1.79	1.80	1.76	3.55	4.35	4.52	4.16	3.88
	[a]AVG_{TRR}	6.79	8.08	6.61	4.48	4.58	4.55	4.16	3.88
	[b]AVG_{TRR}	6.91	7.46	6.31	4.60	NP	4.59	4.12	3.66
6.40 µg ai/L** (SD-4.69)	SF	265	101	89	16	NP	NP	NP	NP
	TRR_{SS}	43.79	55.80	37.76	14.35	9.21	8.98	7.88	7.41
	TRR_{M}	3.38	3.47	3.32	13.89	9.23	8.95	7.83	7.47
	TRR_{SSe}	3.23	3.24	3.24	3.23	9.32	7.75	8.01	7.48
	[a]AVG_{TRR}	16.80	20.83	14.77	10.49	9.25	8.56	7.91	7.45
10.33 µg ai/L* (SD-8.38)	SF	NP	NP	NP	NP	NP	NP	NP	NP
	TRR_{SS}	NP	NP	NP	NP	NP	NP	5.97	6.03
	TRR_{M}	NP	NP	NP	NP	NP	NP	6.12	6.18
	TRR_{SSe}	NP	NP	NP	NP	NP	NP	6.07	6.55
	[a]AVG_{TRR}	NP	NP	NP	NP	NP	NP	6.06	6.26

Test Conc.: Average water concentration per treatment based on total radioactivity applied to the enclosures
SD: Spray drift
SF: Surface Film
TRR: Total Radioactive Residue
AVG: Average
SS: about 10 cm sub-surface
M: Mid water level
SSe: about 10 cm above sediment surface
a: average of SS, M and SSe values
b: measurement of depth integrated samples
*, **, ***: one, two or three treatments
NP: not performed peq: parent equivalents

Table 12. Spray drift: Total Radioactive Residue (TRR) in the surface film, at 3 subsurface water levels and in depth integrated water: Test period after the 3rd application.

Test Day		28 days 1 hrs	28 days 3 hrs	28 days 6 hrs	29 days	30 days	31 days	35 days	42 days
Test Conc. (Test Group)	Sample	[µg peq/L]							
0.34 µg ai/L*** (SD-0.27)	SF	400	170	202	NP	NP	NP	NP	NP
	TRR_{SS}	0.89	0.49	0.57	0.50	0.47	0.47	0.47	0.42
	TRR_{M}	0.28	0.30	0.30	0.48	0.47	0.48	0.40	0.47
	TRR_{SSe}	0.28	0.30	0.31	0.49	0.49	0.49	0.57	0.45
	[a]AVG_{TRR}	0.49	0.36	0.39	0.49	0.48	0.48	0.48	0.45
0.55 µg ai/L*** (SD-0.47)	SF	618	135	164	NP	NP	NP	NP	NP
	TRR_{SS}	1.32	1.06	1.20	1.03	1.00	0.98	1.01	0.87
	TRR_{M}	0.59	1.10	0.62	1.02	1.00	1.02	0.99	0.92
	TRR_{SSe}	0.61	0.60	0.60	1.00	1.04	1.01	1.10	0.83
	[a]AVG_{TRR}	0.84	0.92	0.80	1.02	1.01	1.00	1.03	0.87
1.16 µg ai/L*** (SD-0.84)	SF	3508	621	214	NP	NP	NP	NP	NP
	TRR_{SS}	2.82	2.90	2.97	1.86	1.82	1.83	1.77	1.43
	TRR_{M}	1.04	1.10	1.12	1.89	1.82	1.86	1.76	1.66
	TRR_{SSe}	1.08	1.06	1.10	1.81	1.74	1.78	1.90	1.63
	[a]AVG_{TRR}	1.65	1.68	1.73	1.85	1.79	1.83	1.81	1.57
1.96 µg ai/L*** (SD-1.51)	SF	3403	1440	770	NP	NP	NP	NP	NP
	TRR_{SS}	6.53	6.66	3.54	3.56	3.62	3.53	3.42	3.24
	TRR_{M}	2.02	2.43	3.76	3.62	3.64	3.57	3.42	3.32
	TRR_{SSe}	2.01	2.45	3.20	3.44	3.71	3.59	4.34	3.23
	[a]AVG_{TRR}	3.52	3.85	3.50	3.54	3.66	3.56	3.72	3.26
3.50 µg ai/L** (SD-2.68)	SF	407	163	173	NP	NP	NP	NP	NP
	TRR_{SS}	5.08	19.10	8.35	7.24	7.09	6.99	6.71	NP
	TRR_{M}	3.80	3.88	4.66	7.26	7.15	7.11	6.34	6.39
	TRR_{SSe}	3.79	3.83	3.86	7.04	6.59	6.91	6.98	6.43
	[a]AVG_{TRR}	4.22	8.94	5.62	7.18	6.94	7.00	6.68	6.41
	[b]AVG_{TRR}	4.25	11.08	5.89	7.42	6.17	7.24	7.01	NP
6.40 µg ai/L** (SD-4.69)	SF	NP	NP	NP	NP	NP	NP	NP	NP
	TRR_{SS}	NP	NP	NP	NP	NP	NP	7.12	6.85
	TRR_{M}	NP	NP	NP	NP	NP	NP	7.35	7.05
	TRR_{SSe}	NP	NP	NP	NP	NP	NP	7.31	6.86
	[a]AVG_{TRR}	NP	NP	NP	NP	NP	NP	7.26	6.92
10.33 µg ai/L* (SD-8.38)	SF	NP	NP	NP	NP	NP	NP	NP	NP
	TRR_{SS}	NP	NP	NP	NP	NP	NP	5.64	5.56
	TRR_{M}	NP	NP	NP	NP	NP	NP	5.67	5.62
	TRR_{SSe}	NP	NP	NP	NP	NP	NP	5.86	4.77
	[a]AVG_{TRR}	NP	NP	NP	NP	NP	NP	5.72	5.32

Test Conc.: Average water concentration per treatment based on total radioactivity applied to the enclosures
SD: Spray drift
SF: Surface Film
TRR: Total Radioactive Residue
AVG: Average
SS: about 10 cm sub-surface
M: Mid water level
SSe: about 10 cm above sediment surface
[a]: average of SS, M and SSe values
[b]: measurement of depth integrated samples
*, **, ***: one, two or three treatments
NP: not performed
peq: parent equivalents

Table 13. Ratio of TRR in the surface film and in the water.

Test Group	Test Conc µg ai/L]	Sample	1st treatment				2nd treatment				3rd treatment			
Hours after application			1:00	2:15	7:45	23:20	0:57	4:27	6:12	18:57	0:58	3:15	5:58	NP
SD-0.27	0.34	SF_{SS}	69	12	10	13	350	80	54	25	447	347	355	NP
		SF_{M}	1481	563	252	13	2537	792	391	27	1427	573	673	NP
		SF_{SSe}	2160	593	423	14	2537	745	403	93	1414	573	660	NP
		SF_{AVG}	191	36	29	14	823	199	128	34	823	471	515	NP
Hours after application			1:00	3:05	6:25	19:35	1:16	3:59	6:16	19:11	1:02	3:02	5:50	NP
SD-0.47	0.55	SF_{SS}	79	49	52	243	140	13	9	5	466	126	137	NP
		SF_{M}	7345	4961	1779	246	846	187	91	6	1050	122	267	NP
		SF_{SSe}	7846	5939	2365	309	887	191	94	19	1005	223	271	NP
		SF_{AVG}	232	145	148	263	318	34	23	7	733	146	204	NP
Hours after application			1:00	4:10	5:58	19:08	1:00	3:35	6:02	18:14	0:54	2:44	5:59	NP
SD-0.84	1.16	SF_{SS}	61	21	9	11	582	18	7	7	1244	214	72	NP
		SF_{M}	2490	1290	392	12	684	67	61	8	3377	566	192	NP
		SF_{SSe}	4261	2361	784	18	707	66	60	23	3238	586	195	NP
		SF_{AVG}	177	63	27	13	653	35	17	9	2129	369	124	NP
Hours after application			0:55	3:23	5:43	18:43	1:40	3:50	6:00	16:43	1:09	3:12	7:12	NP
SD-1.51	1.96	SF_{SS}	170	135	165	122	56	9	6	6	522	216	218	NP
		SF_{M}	12762	7818	8866	125	503	108	52	7	1687	591	205	NP
		SF_{SSe}	59428	22162	16278	222	538	114	56	22	1692	589	241	NP
		SF_{AVG}	501	395	482	145	139	23	14	8	967	374	220	NP
Hours after application			1:00	3:55	5:20	17:45	1:13	3:17	5:09	15:50	0:53	3:02	6:10	NP
SD-2.68	3.50	SF_{SS}	10	7	7	5	52	7	9	15	80	9	21	NP
		SF_{M}	699	459	202	5	485	75	82	18	107	42	37	NP
		SF_{SSe}	2628	964	874	6	490	77	86	22	107	43	45	NP
		SF_{AVG}	30	20	19	6	129	17	23	18	96	18	31	NP
Hours after application			1:18	4:03	NP	NP	2:00	3:20	5:30	15:52	NP	NP	NP	NP
SD-4.69	6.40	SF_{SS}	35	28	NP	NP	44	56	38	14	NP	NP	NP	NP
		SF_{M}	4063	465	NP	NP	3	3	3	14	NP	NP	NP	NP
		SF_{SSe}	13437	4565	NP	NP	3	3	3	3	NP	NP	NP	NP
		SF_{AVG}	103	79	NP	NP	17	21	15	10	NP	NP	NP	NP
Hours after application			0:55	2:42	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
SD-8.38	10.33	SF_{SS}	65	9	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
		SF_{M}	4476	1109	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
		SF_{SSe}	11758	1480	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
		SF_{AVG}	192	27	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP

Test Conc.: Average water concentration per treatment based on total radioactivity applied to the enclosures
SD: Spray drift
SF: Surface Film
TRR: Total Radioactive Residue
AVG: Average
SS: about 10 cm sub-surface
M: Mid water level
SSe: about 10 cm above sediment surface
NP: not performed
Note: Calculations performed with unrounded numbers

Table 14. Spray drift: Maximum exposure concentrations

A) Surface film

Test Group	Test Conc.	1st treatment		2nd treatment		3rd treatment	
		[µg peq/L]	[hrs]	[µg peq/L]	[hrs]	[µg peq/L]	[hrs]
SD-0.27	0.34 µg ai/L***	46	1	335	1	400	1
SD-0.47	0.55 µg ai/L***	204	1	241	1	618	1
SD-0.84	1.16 µg ai/L***	104	1	361	1	3508	1
SD-1.51	1.96 µg ai/L***	1175	1	491	1	3403	1
SD-2.68	3.50 µg ai/L***	128	1	878	1	407	1
SD-4.69	6.40 µg ai/L**	943	1	265	1	NP	NP
SD-8.38	10.33 µg ai/L*	1669	1	NP	NP	NP	NP

B) Subsurface water layers

Test Group	Test Conc.	1st treatment		2nd treatment		3rd treatment	
		[µg peq/L]	[hrs]	[µg peq/L]	[hrs]	[µg peq/L]	[hrs]
SD-0.27	0.34 µg ai/L***	1.04	3	1.39	3	0.89	1
SD-0.47	0.55 µg ai/L***	2.58	1	4.28	3	1.32	1
SD-0.84	1.16 µg ai/L***	2.86	3	4.68	6	2.97	6
SD-1.51	1.96 µg ai/L***	6.93	1	12.49	3	6.66	3
SD-2.68	3.50 µg ai/L***	12.75	1	20.59	3	19.1	3
SD-4.69	6.40 µg ai/L**	27.21	1	55.80	3	NP	NP
SD-8.38	10.33 µg ai/L*	27.81	3	NP	NP	NP	NP

Test Conc.: Average water concentration per treatment based on total radioactivity applied to the enclosures
SD: Spray drift
NP: Not performed
*, **, ***: one, two or three treatments
peq: parent equivalents

Table 14. Spray drift: Maximum exposure concentrations (continued).

C) Depth integrated water

Test Group	Test Conc.	1st treatment		2nd treatment		3rd treatment	
		[µg peq/L]	[hrs]	[µg peq/L]	[hrs]	[µg peq/L]	[hrs]
SD-0.27	0.34 µg ai/L***	0.36	3	0.56	3	0.49	1, 24
SD-0.47	0.55 µg ai/L***	0.88	1	1.62	3	1.03	7 days
SD-0.84	1.16 µg ai/L***	0.98	3	1.92	6	1.85	24
SD-1.51	1.96 µg ai/L***	2.35	1	4.81	3	3.85	3
SD-2.68	3.50 µg ai/L***	4.33	1	8.08	3	8.94	3
SD-4.69	6.40 µg ai/L**	9.17	1	20.83	3	NP	NP
SD-8.38	10.33 µg ai/L*	9.40	3	NP	NP	NP	NP

Test Conc.: Average water concentration per treatment based on total radioactivity applied to the enclosures
SD: Spray drift
NP: Not performed
*, **, ***: one, two or three treatments
peq: parent equivalents

Table 15. Spray drift: Mean TRR of raw water samples. The values represent either an average of 3 different water layers or depth-integrated values, given in µg of parent equivalents (peq) per L (µg peq/L).

A: Test Period after the 1st application

	Test Day	1 hrs	3 hrs	6 hrs	1 day	2 days	3 days	7 days	13 days
Test Group	Test Conc.	[µg peq/L]							
SD-0.27	0.34 µg ai/L***	0.24	0.36	0.26	0.17	0.17	0.16	0.15	0.13
SD-0.47	0.55 µg ai/L***	0.88	0.86	0.40	0.34	0.36	0.34	0.32	0.28
SD-0.84	1.16 µg ai/L***	0.59	0.98	0.79	0.65	0.68	0.66	0.59	0.52
SD-1.51	1.96 µg ai/L***	2.35	2.10	1.22	1.05	1.15	1.15	1.05	0.93
SD-2.68	3.50 µg ai/L***	3.91	2.62	2.32	2.16	2.11	1.81	1.92	1.76
SD-4.69	6.40 µg ai/L**	9.17	5.34	NP	3.99	3.77	3.75	3.52	3.20
SD-8.38	10.33 µg ai/L*	8.68	9.40	NP	8.2	7.83	7.64	7.12	6.60

B: Test period after the 2nd application

	Test Day	14 days 1 hrs	14 days 3 hrs	14 days 6 hrs	15 days	16 days	17 days	21 days	27 days
Test Group	Test Conc.	[µg peq/L]							
SD-0.27	0.34 µg ai/L***	0.41	0.56	0.46	0.38	0.35	0.34	0.31	0.29
SD-0.47	0.55 µg ai/L***	0.76	1.62	1.21	0.78	0.74	0.74	0.65	0.6
SD-0.84	1.16 µg ai/L***	0.55	1.06	1.92	1.38	1.25	1.30	1.18	1.04
SD-1.51	1.96 µg ai/L***	3.54	4.81	3.62	2.48	2.41	2.38	2.21	2.01
SD-2.68	3.50 µg ai/L***	6.91	7.46	6.31	4.60	4.58	4.59	4.12	3.66
SD-4.69	6.40 µg ai/L**	16.8	20.83	14.77	10.49	9.25	8.56	7.91	7.45
SD-8.38	10.33 µg ai/L*	NP	NP	NP	NP	NP	NP	6.06	6.26

C: Test period after the 3rd application

	Test Day	28 days 1 hrs	28 days 3 hrs	28 days 6 hrs	29 days	30 days	31 days	35 days	42 days
Test Group	Test Conc.	[µg peq/L]							
SD-0.27	0.34 µg ai/L***	0.49	0.36	0.39	0.49	0.48	0.48	0.48	0.45
SD-0.47	0.55 µg ai/L***	0.84	0.92	0.8	1.02	1.01	1.0	1.03	0.87
SD-0.84	1.16 µg ai/L***	1.65	1.68	1.73	1.85	1.79	1.83	1.81	1.57
SD-1.51	1.96 µg ai/L***	3.52	3.85	3.5	3.54	3.66	3.56	3.72	3.26
SD-2.68	3.50 µg ai/L***	4.25	11.08	5.89	7.42	6.17	7.24	7.01	6.41
SD-4.69	6.40 µg ai/L**	NP	NP	NP	NP	NP	NP	7.26	6.92
SD-8.38	10.33 µg ai/L*	NP	NP	NP	NP	NP	NP	5.72	5.32

Test Conc.: Average water concentration per treatment based on total radioactivity applied to the enclosures
SD: Spray drift
NP: Not performed
*, **, ***: one, two or three treatments
peq: parent equivalents

Table 16. Spray drift: Mitigation of the Total Radioactive Residue (TRR).

		1st Treatment			2nd Treatment			3rd Treatment		
Test Group	Test Conc.	A	B	C	A	B	C	A	B	C
	[µg ai/L]	[µg ai/L]	[$µg_{peq}$/L]	[%]	[$µg_{peq}$/L]	[$µg_{peq}$/L]	[%]	[$µg_{peq}$/L]	[$µg_{peq}$/L]	[%]
SD-0.27	0.34***	0.24	0.17	71	0.38	0.25	66	0.39	0.2	51
SD-0.47	0.55***	0.45	0.34	76	0.67	0.50	75	0.55	0.42	76
SD-0.84	1.16***	0.94	0.65	69	1.24	0.86	69	1.31	0.81	53
SD-1.51	1.96***	1.57	1.05	67	2.13	1.55	73	2.18	1.53	70
SD-2.68	3.50***	2.70	2.16	80	3.90	2.75	71	3.9	3.76	96
SD-4.69	6.40**	5.83	3.99	68	6.97	7.29	105	NP	NP	NP
SD-8.38	10.33*	10.33	8.20	79	NP	NP	NP	NP	NP	NP
Average	-	-	-	73	-	-	73	-	-	71

Test Conc.: Average water concentration per treatment based on total radioactivity applied to the enclosures
SD: Spray drift
A: Average concentration in water after treatment (cf. Tables 6)
B: Relative TRR on day 1 after treatment (i.e. TRR of day 1 after treatment minus TRR on day –1 prior to each treatment) (Tables 10 -12)
C: B in percent of A
*, **, ***: one, two or three treatments
NP: Not performed
peq: parent equivalents

Table 17. Run-off: Total Radioactive Residue (TRR) at 3 subsurface water levels and in depth integrated samples: Test period after the 1st application.

Test Day		1 hrs	3 hrs	6 hrs	1 day	2 days	4 days	7 days	12 days
Test Conc. (Test Group)	Sample	[µg peq/L]							
0.21 µg SR/L*** (RO-0.21)	TRR_{SS}	0.20	0.20	0.20	0.16	0.15	0.16	0.17	0.14
	TRR_{M}	0.17	0.19	0.19	0.16	0.15	0.16	0.16	0.14
	TRR_{SSe}	0.18	0.17	0.17	0.17	0.15	0.15	0.16	0.14
	[a]AVG_{TRR}	0.18	0.19	0.19	0.16	0.15	0.16	0.16	0.14
0.42 µg SR/L*** (RO-0.42)	TRR_{SS}	0.31	0.32	0.33	0.29	0.30	0.30	0.29	0.26
	TRR_{M}	0.31	0.31	0.34	0.31	0.30	0.32	0.30	0.26
	TRR_{SSe}	0.31	0.30	0.32	0.31	0.32	0.31	0.30	0.28
	[a]AVG_{TRR}	0.31	0.31	0.33	0.30	0.31	0.31	0.30	0.27
0.84 µg SR/L*** (RO-0.84)	TRR_{SS}	0.61	0.61	0.63	0.60	0.59	0.61	0.57	0.52
	TRR_{M}	0.60	0.62	0.63	0.61	0.60	0.71	0.57	0.52
	TRR_{SSe}	0.62	0.62	0.62	0.66	0.58	0.59	0.60	0.54
	[a]AVG_{TRR}	0.61	0.61	0.63	0.62	0.59	0.64	0.58	0.53
2.09 µg SR/L*** (RO-2.09)	TRR_{SS}	1.47	1.46	1.52	1.46	1.44	1.49	1.41	1.32
	TRR_{M}	1.43	1.45	1.50	1.49	1.48	1.54	1.42	1.35
	TRR_{SSe}	NP	1.43	1.49	1.60	1.54	1.46	1.52	1.39
	[a]AVG_{TRR}	NP	1.44	1.50	1.52	1.49	1.50	1.45	1.36
3.99 µg SR/L*** (RO-4.19)	TRR_{SS}	2.94	2.89	2.99	2.87	2.78	2.79	2.75	2.56
	TRR_{M}	2.90	2.85	3.02	2.88	2.80	2.94	2.81	2.58
	TRR_{SSe}	2.88	2.77	2.97	3.35	3.01	3.07	2.81	2.63
	[a]AVG_{TRR}	2.91	2.84	2.99	3.03	2.86	2.93	2.79	2.59
	[b]AVG_{TRR}	4.12	2.86	2.87	2.64	2.52	NP	NP	2.49
6.29 µg SR/L** (RO-6.29)	TRR_{SS}	4.52	4.33	4.52	4.17	4.11	4.12	4.06	3.68
	TRR_{M}	4.58	4.36	4.53	4.37	4.19	4.33	4.16	3.79
	TRR_{SSe}	4.60	4.32	4.41	5.13	4.36	4.31	4.21	3.88
	[a]AVG_{TRR}	4.57	4.34	4.49	4.56	4.22	4.26	4.14	3.78
8.39 µg SR/L* (RO-8.39)	TRR_{SS}	6.18	5.77	5.95	5.46	5.57	5.80	5.74	5.34
	TRR_{M}	6.11	5.75	5.82	5.74	5.64	6.17	5.74	5.46
	TRR_{SSe}	5.91	5.79	5.99	6.00	5.69	5.93	5.90	5.49
	[a]AVG_{TRR}	6.07	5.77	5.92	5.73	5.63	5.97	5.79	5.43

Test Conc.: Average water concentration per treatment based on total radioactivity applied to the enclosures
RO: Run-off
TRR: Total Radioactive Residue
AVG: Average
SS: about 10 cm sub-surface
M: Mid water level
SSe: about 10 cm above sediment surface
SR: Soil residue
a: average of SS, M and SSe values
b: measurement of depth integrated samples
NP: not performed
*, **, ***: one, two or three treatments
peq: parent equivalents

Table 18. Run-Off: Total Radioactive Residue (TRR) at 3 subsurface water levels and in depth integrated samples: Test period after the 2nd application.

Test Day		14 days 1 hrs	14 days 3 hrs	14 days 6 hrs	15 days	16 days	18 days	21 days	26 days
Test Conc. (Test Group)	Sample	[µg peq/L]							
0.21 µg SR/L*** (RO-0.21)	TRR_{SS}	0.22	0.45	0.38	0.26	NP	0.26	0.24	NP
	TRR_{M}	0.15	0.16	0.21	0.26	NP	0.26	0.25	NP
	TRR_{SSe}	0.15	0.17	0.19	0.24	NP	0.27	0.24	NP
	[a]AVG_{TRR}	0.17	0.26	0.26	0.25	NP	0.26	0.24	NP
0.42 µg SR/L*** (RO-0.42)	TRR_{SS}	1.47	0.71	0.64	0.49	0.48	0.47	0.45	NP
	TRR_{M}	0.35	0.42	0.40	0.49	0.45	0.48	0.45	NP
	TRR_{Sse}	0.39	0.42	0.47	0.52	0.51	0.47	0.45	NP
	[a]AVG_{TRR}	0.74	0.52	0.50	0.50	0.48	0.47	0.45	NP
0.84 µg SR/L*** (RO-0.84)	TRR_{SS}	0.98	1.48	0.75	0.99	NP	0.98	0.95	NP
	TRR_{M}	0.61	0.83	1.37	0.99	NP	1.01	0.93	NP
	TRR_{Sse}	0.66	0.80	0.94	1.06	NP	0.99	0.94	NP
	[a]AVG_{TRR}	0.75	1.04	1.02	1.01	NP	0.99	0.94	NP
2.09 µg SR/L*** (RO-2.09)	TRR_{SS}	4.24	4.43	3.53	2.54	NP	2.64	2.43	NP
	TRR_{M}	1.62	1.66	2.09	2.47	NP	2.49	2.49	NP
	TRR_{Sse}	1.59	1.59	1.96	2.41	NP	2.73	2.40	NP
	[a]AVG_{TRR}	2.48	2.56	2.52	2.47	NP	2.62	2.44	NP
3.99 µg SR/L*** (RO-4.19)	TRR_{SS}	10.33	8.63	7.11	5.01	NP	5.27	5.08	NP
	TRR_{M}	3.21	3.34	3.79	4.96	NP	5.30	5.09	NP
	TRR_{Sse}	NP	2.12	4.38	4.93	NP	5.54	5.04	NP
	[a]AVG_{TRR}	NP	4.70	5.10	4.97	NP	5.37	5.07	NP
	[b]AVG_{TRR}	5.68	5.18	4.79	4.95	5.42	NP	5.06	4.91
6.29 µg SR/L** (RO-6.29)	TRR_{SS}	16.58	14.20	13.68	7.31	NP	7.66	7.26	NP
	TRR_{M}	4.91	5.92	5.07	7.30	NP	7.74	7.21	NP
	TRR_{SSe}	5.33	4.65	7.13	7.86	NP	7.87	7.53	NP
	[a]AVG_{TRR}	8.94	8.26	8.63	7.49	NP	7.76	7.34	NP
8.39 µg SR/L* (RO-8.39)	TRR_{SS}	NP	NP	NP	NP	NP	NP	4.93	NP
	TRR_{M}	NP	NP	NP	NP	NP	NP	4.90	NP
	TRR_{SSe}	NP	NP	NP	NP	NP	NP	4.91	NP
	[a]AVG_{TRR}	NP	NP	NP	NP	NP	NP	4.91	5.10

Test Conc.: Average water concentration per treatment based on total radioactivity applied to the enclosures
RO: Run-off
TRR: Total Radioactive Residue
AVG: Average
SS: about 10 cm sub-surface
M: Mid water level
SSe: about 10 cm above sediment surface
SR: Soil residue
[a]: average of SS, M and SSe values
[b]: measurement of depth integrated samples
*, **, ***: one, two or three treatments
NP: not performed peq: parent equivalents

Table 19. Run-off: Total Radioactive Residue (TRR) at 3 subsurface water levels and in depth integrated samples: Test period after the 3rd application.

Test Day		28 days 1 hrs	28 days 3 hrs	28 days 6 hrs	29 days	30 days	32 days	35 days	43 days
Test Conc. (Test Group)	Sample	[µg peq/L]							
0.21 µg SR/L*** (RO-0.21)	TRR_{SS}	0.53	0.61	0.50	0.35	NP	NP	0.38	0.33
	TRR_{M}	0.27	0.38	0.36	0.36	NP	NP	0.37	0.32
	TRR_{SSe}	0.30	0.31	0.34	0.36	NP	NP	0.38	0.33
	[a]AVG_{TRR}	0.37	0.43	0.40	0.36	NP	NP	0.38	0.33
0.42 µg SR/L*** (RO-0.42)	TRR_{SS}	1.89	0.94	0.94	0.66	NP	NP	0.66	0.56
	TRR_{M}	0.56	0.64	0.62	0.67	NP	NP	0.66	0.61
	TRR_{SSe}	0.56	0.67	0.70	0.64	NP	NP	NP	0.59
	[a]AVG_{TRR}	1.00	0.75	0.75	0.66	NP	NP	NP	0.59
0.84 µg SR/L*** (RO-0.84)	TRR_{SS}	3.45	2.16	2.07	1.44	NP	NP	1.45	0.90
	TRR_{M}	1.09	1.30	1.24	1.41	NP	NP	1.45	1.24
	TRR_{SSe}	1.16	1.17	1.25	1.41	NP	NP	1.91	1.31
	[a]AVG_{TRR}	1.90	1.54	1.52	1.42	NP	NP	1.60	1.15
2.09 µg SR/L*** (RO-2.09)	TRR_{SS}	5.73	5.73	4.69	4.72	3.84	NP	3.83	NA
	TRR_{M}	2.95	2.95	3.30	3.11	3.78	NP	3.81	NA
	TRR_{SSe}	2.99	2.99	3.25	3.33	3.76	NP	4.13	NA
	[a]AVG_{TRR}	3.89	3.89	3.75	3.72	3.79	NP	3.92	3.30
3.99 µg SR/L*** (RO-4.19)	TRR_{SS}	14.71	14.71	12.61	10.94	7.50	NP	NP	7.71
	TRR_{M}	6.42	6.42	6.59	6.35	7.56	NP	NP	7.53
	TRR_{SSe}	6.25	6.25	6.37	6.69	7.67	NP	NP	7.80
	[a]AVG_{TRR}	9.13	9.13	8.52	7.99	7.58	NP	NP	7.68
	[b]AVG_{TRR}	8.33	7.89	7.68	7.82	7.78	7.55	7.49	NP
6.29 µg SR/L** (RO-6.29)	TRR_{SS}	NP	NP	NP	NP	NP	NP	7.29	6.56
	TRR_{M}	NP	NP	NP	NP	NP	NP	7.10	7.04
	TRR_{SSe}	NP	NP	NP	NP	NP	NP	7.45	6.95
	[a]AVG_{TRR}	NP	NP	NP	NP	NP	NP	7.28	6.85
8.39 µg SR/L* (RO-8.39)	TRR_{SS}	NP	NP	NP	NP	NP	NP	4.70	4.34
	TRR_{M}	NP	NP	NP	NP	NP	NP	4.71	4.25
	TRR_{SSe}	NP	NP	NP	NP	NP	NP	NP	4.52
	[a]AVG_{TRR}	NP	NP	NP	NP	NP	NP	4.71	4.37

Test Conc.: Average water concentration per treatment based on total radioactivity applied to the enclosures
RO: Run-off
TRR: Total Radioactive Residue
AVG: Average
SS: about 10 cm sub-surface
M: Mid water level
SSe: about 10 cm above sediment surface
SR: Soil residue
[a]: average of SS, M and SSe values
[b]: measurement of depth integrated samples
*, **, ***: one, two or three treatments
NP: not performed
peq: parent equivalents

Table 20. Run-off: Maximum exposure concentration in depth integrated water samples.

A: Depth-integrated water

Test Group	Test Conc [µg SR/L]	1st Treatment		2nd Treatment		3rd Treatment	
		[µg peq/L]	[hrs]]	[µg peq/L]	[hrs]]	[µg peq/L]	[hrs]]
RO-0.21	0.21***	0.19	3	0.26	6	0.43	3
RO-0.42	0.42***	0.33	6	0.74	1	1.0	1
RO-0.84	0.84***	0.63	6	1.04	3	1.90	1
RO-2.09	2.09***	1.52	1 day	2.62	4 days	3.89	1, 3
RO-4.19	3.99***	3.03	1 day	5.37	4 days	9.13	1
RO-6.29	6.29**	4.57	1	8.94	1	NP	NP
RO-8.39	8.39*	6.07	1	NP	NP	NP	NP

B: Subsurface water

Test Group	Test Conc [µg SR/L]	1st Treatment		2nd Treatment		3rd Treatment	
		[µg peq/L]	[hrs]]	[µg peq/L]	[hrs]]	[µg peq/L]	[hrs]]
RO-0.21	0.21***	0.20	1, 3, 6	0.45	3	0.61	3
RO-0.42	0.42***	0.33	6	1.47	1	1.89	1
RO-0.84	0.84***	0.63	6	1.48	3	3.45	1
RO-2.09	2.09***	1.52	6	4.43	3	5.73	1, 3
RO-4.19	3.99***	2.99	6	10.33	1	14.71	1
RO-6.29	6.29**	4.52	1	16.58	1	NP	NP
RO-8.39	8.39*	6.18	1	NP	NP	NP	NP

Test Conc.: Average water concentration per treatment based on total radioactivity applied to the enclosures
RO: Run-off
SR: Soil residue
*, **, ***: one, two or three treatments
NP: not performed
peq: parent equivalents

Table 21. Run-off: Mitigation of the Total Radioactive Residue (TRR).

		1st Treatment			2nd Treatment			3rd Treatment		
Test Group	Test Conc.	A	B	C	A	B	C	A	B	C
	[µg SR/L]	[$\mu g_{peq}/L$]	[$\mu g_{peq}/L$]	[%]	[$\mu g_{peq}/L$]	[$\mu g_{peq}/L$]	[%]	[$\mu g_{peq}/L$]	[$\mu g_{peq}/L$]	[%]
RO-0.21	0.21***	0.21	0.16	76	0.21	0.11	52	0.21	0.12	57
RO-0.42	0.42***	0.42	0.30	71	0.42	0.23	55	0.42	0.21	50
RO-0.84	0.84***	0.84	0.62	74	0.84	0.48	57	0.84	0.48	57
RO-2.09	2.09***	2.09	1.52	73	2.09	1.11	53	2.09	1.28	61
RO-4.19	3.99***	4.19	3.03	72	4.19	2.38	57	3.60	2.76	81
RO-6.29	6.29**	6.29	4.56	72	6.29	3.71	59	NP	NP	NP
RO-8.39	8.39*	8.39	5.73	68	NP	NP	NP	NP	NP	NP
Average		-	-	71	-	-	56	-	-	61

Test Conc.: Average water concentration per treatment based on total radioactivity applied to the enclosures
RO: Run-off
A: Average concentration in water after treatment (cf. Table 8)
B: Relative TRR on day 1 after treatment (i.e. TRR of day 1 after treatment minus TRR on day –1 prior to each treatment) (Tables 17 - 19)
C: B in percent of A
NP: Not performed
*, **, ***: one, two or three treatments
SR: Soil residue
peq: parent equivalents

Table 22. Run-off: Mean TRR of raw water samples. The values represent either an average of 3 different water layers or depth-integrated values, given in µg of parent equivalents (peq) per L (µg peq/L).

A: Test Period after the 1st application

	Test Day	1 hrs	3 hrs	6 hrs	1 day	2 days	4 days	7 days	13 days
Test Group	Test Conc. [µg SR/L]	[µg peq/L]							
RO-0.21	0.21***	0.18	0.19	0.19	0.16	0.15	0.16	0.16	0.14
RO-0.42	0.42***	0.31	0.31	0.33	0.30	0.31	0.31	0.30	0.27
RO-0.84	0.84***	0.61	0.61	0.63	0.62	0.59	0.64	0.58	0.53
RO-2.09	2.09***	1.45	1.44	1.50	1.52	1.49	1.50	1.45	1.36
RO-4.19	3.99***	4.12	2.86	2.87	2.64	2.52	2.93	2.79	2.49
RO-6.29	6.29**	4.57	4.34	4.49	4.56	4.22	4.26	4.14	3.78
RO-8.39	8.39*	6.07	5.77	5.92	5.73	5.63	5.97	5.79	5.43

B: Test period after the 2nd application

	Test Day	14 days 1 hrs	14 days 3 hrs	14 days 6 hrs	15 days	16 days	18 days	21 days	26 days
Test Group	Test Conc. [µg SR/L]	[µg peq/L]							
RO-0.21	0.21***	0.17	0.26	0.26	0.25	NP	0.26	0.24	NP
RO-0.42	0.42***	0.74	0.52	0.5	0.5	0.48	0.47	0.45	NP
RO-0.84	0.84***	0.75	1.04	1.02	1.01	NP	0.99	0.94	NP
RO-2.09	2.09***	2. 48	2.56	2.52	2.47	NP	2.62	2.44	NP
RO-4.19	3.99***	5.68	5.18	4.79	4.95	5.42	5.37	5.06	4.91
RO-6.29	6.29**	8.94	8.26	8.63	7.49	NP	7.76	7.34	NP
RO-8.39	8.39*	NP	NP	NP	NP	NP	NP	4.91	5.1

Test Conc.: Average water concentration per treatment based on total radioactivity applied to the enclosures
*, **, ***: one, two or three treatments
NP: Not performed
RO: Run-off
SR: *Soil residue* peq: *parent equivalents*

Table 22. Run-off: Mean TRR of raw water samples. Representing either an average of 3 different water layers or depth-integrated values, given in µg of parent equivalents (peq) per L (µg peq/L) (continued).

C: Test period after the 3rd application

	Test Day	28 days 1 hrs	28 days 3 hrs	28 days 6 hrs	29 days	30 days	32 days	35 days	43 days
Test Group	Test Conc. [µg SR/L]	[µg peq/L]							
RO-0.21	0.21***	0.37	0.43	0.4	0.36	NP	NP	0.38	0.33
RO-0.42	0.42***	1.00	0.75	0.75	0.66	NP	NP	NP	0.59
RO-0.84	0.84***	1.90	1.54	1.52	1.42	NP	NP	1.6	1.15
RO-2.09	2.09***	3.89	3.89	3.75	3.72	3.79	NP	3.92	3.30
RO-4.19	3.99***	8.33	7.89	7.68	7.82	7.78	7.55	7.49	7.68
RO-6.29	6.29**	NP	NP	NP	NP	NP	NP	7.28	6.85
RO-8.39	8.39*	NP	NP	NP	NP	NP	NP	4.71	4.37

Test Conc.: Average water concentration per treatment based on total radioactivity applied to the enclosures
*, **, ***: one, two or three treatments
NP: Not performed
RO: Run-off
SR: Soil residue
peq: parent equivalents

Table 23. Spray drift test group SD-2.68: Determination of the Suspended Particulate Matter (SPM).

A: Test Period after the 1st application (Spray drift)

Test Day	1 hrs	3 hrs	6 hrs	1 day	2 days	3 days	7 days	13 days
	[%]							
TRR in percolate	95.2	95.1	100.2	97.1	NP	98.5	97.7	98.0
TRR in 0.7 µm filter	1.3	2.2	2.0	2.0	NP	0.8	2.0	2.5
Recovery	96.5	97.3	102.2	99.1	NP	99.3	99.7	100.5

B: Test period after the 2nd application (Spray drift)

Test Day	14 days 1 hrs	14 days 3 hrs	14 days 6 hrs	15 days	16 days	17 days	21 days	27 days
	[%]							
TRR in percolate	90.9	95.1	94.6	98.5	NP	98.1	98.1	99.4
TRR in 0.7 µm filter	2.3	2.4	2.4	2.2	NP	1.6	2.0	8.1
Recovery	93.3	97.4	97.0	100.7	NP	99.6	100.1	107.4

C: Test period after the 3rd application (Spray drift)

Test Day	28 days 1 hrs	28 days 3 hrs	28 days 6 hrs	29 days	30 days	31 days	35 days	42 days
	[%]							
TRR in percolate	97.4	91.7	97.2	97.2	109.5	97.3	95.2	NP
TRR in 0.7 µm filter	2.3	2.9	6.9	2.6	8.9	3.2	3.5	NP
Recovery	99.6	96.6	104.1	99.8	118.4	100.5	98.6	NP

TRR: Total radioactive residue
NP: Not performed

Table 24. Run-off test group RO-4.19: Determination of the Suspended Particulate Matter (SPM).

Test Period after the 1st application (Run-Off)

Test Day	1 hrs	3 hrs	6 hrs	1 day	2 days	3 days	7 days	13 days
	[%]							
TRR in percolate	99.4	94.7	94.1	93.9	100.7	NP	NP	100.7
TRR in 0.7 µm filter	2.0	0.7	1.1	0.9	1.0	NP	NP	1.9
Recovery	101.4	95.4	95.2	94.8	101.7	NP	NP	102.6

Test period after the 2nd application (Run-off)

Test Day	14 days 1 hrs	14 days 3 hrs	14 days 6 hrs	15 days	16 days	17 days	21 days	27 days
	[%]							
TRR in percolate	95.9	95.8	95.5	98.6	NP	NP	98.2	100.0
TRR in 0.7 µm filter	1.6	1.5	1.5	1.6	NP	NP	1.6	2.4
Recovery	97.5	97.3	97.0	100.2	NP	NP	99.8	102.3

Test period after the 3rd application (Run-Off)

Test Day	28 days 1 hrs	28 days 3 hrs	28 days 6 hrs	29 days	30 days	31 days	35 days	42 days
	[%]							
TRR in percolate	99.2	100.5	97.6	96.1	97.1	100.8	98.4	NP
TRR in 0.7 µm filter	2.0	2.0	1.8	2.1	2.3	2.4	2.0	NP
Recovery	101.2	102.6	99.3	98.2	99.4	103.2	100.4	NP

TRR: Total radioactive residue
NP: Not Performed

Table 25. Spray drift: Characterisation of the TRR in water of test group SD-2.68 after triplicate treatment. The metabolite concentrations are given in µg peq/L.

TRR water [µg peq/L]	3.91	2.62	2.32	2.16	2.11	1.81	1.92	1.76
Test Day	**0.05**	**0.16**	**0.22**	**0.74**	**2**	**3**	**7**	**13**
Identity	[µg peq/L]							
M1	0.04	ND	0.01	0.02	0.03	0.06	0.07	0.06
Endosulfan diol	0.77	0.98	0.96	1.14	1.16	0.89	0.69	0.35
Endosulfan hydroxy ether	0.04	0.02	0.02	0.11	0.21	0.28	0.60	0.66
Endosulfan lactone	0.03	ND	ND	0.01	0.04	0.06	0.18	0.36
M4	ND	ND	ND	ND	ND	ND	ND	ND
Endosulfan sulfate	0.03	0.02	0.01	0.06	0.14	0.16	0.26	0.23
β-endosulfan	*0.91*	*0.40*	*0.37*	*0.19*	*0.14*	*0.09*	*0.02*	*0.00*
α-endosulfan	*2.07*	*1.18*	*0.96*	*0.63*	*0.38*	*0.25*	*0.07*	*0.04*
Endosulfan (α+β)	2.98	1.59	1.33	0.81	0.52	0.34	0.09	0.04
Minor components (#)	0.03 (2)	0.01 (1)	0.01 (1)	0.01 (2)	0.01 (1)	0.03 (3)	0.03 (4)	0.07 (4)

TRR water [µg peq/L]	6.91	7.46	6.31	4.6	4.58	4.59	4.12	3.66
Test Day	**14.05**	**14.14**	**14.21**	**14.66**	**16**	**17**	**21**	**27**
Identity	[µg peq/L]							
M1	0.13	0.18	0.27	0.16	0.18	NP	0.32	0.57
Endosulfan diol	*1.45*	*3.01*	*2.67*	*2.17*	*2.24*	*NP*	*1.37*	*0.90*
Endosulfan hydroxy ether	0.84	0.77	0.70	0.66	0.87	NP	1.13	0.94
Endosulfan lactone	0.42	0.39	0.43	0.39	0.37	NP	0.69	0.76
M4	ND	ND	ND	ND	ND	NP	ND	ND
Endosulfan sulfate	0.24	0.22	0.23	0.29	0.39	NP	0.38	0.31
β-endosulfan	*1.22*	*0.88*	*0.53*	*0.21*	*0.06*	*NP*	*ND*	*0.01*
α-endosulfan	*2.50*	*1.86*	*1.36*	*0.59*	*0.20*	*NP*	*ND*	*0.01*
Endosulfan (α+β)	3.72	2.73	1.90	0.80	0.26	NP	ND	0.03
Minor components (#)	0.12 (4)	0.17 (5)	0.10 (4)	0.13 (5)	0.28 (4)	NP	0.23 (2)	0.15 (6)

TRR water [µg peq/L]	4.25	11.08	5.89	7.42	6.17	7.24	7.01	6.41
Test Day	**28.04**	**28.13**	**28.26**	**29**	**30**	**31**	**35**	**42**
Identity	[µg peq/L]							
M1	0.53	1.26	0.36	0.48	0.47	0.84	0.98	1.07
Endosulfan diol	1.54	3.72	3.37	4.29	3.24	3.49	2.77	1.68
Endosulfan hydroxy ether	0.70	0.96	0.84	0.91	1.20	1.30	1.48	1.23
Endosulfan lactone	0.77	0.93	0.88	1.06	0.85	1.10	1.27	1.50
M4	ND	ND	ND	ND	ND	ND	ND	0.25
Endosulfan sulfate	0.23	0.27	0.23	0.29	0.25	0.28	0.22	0.26
β-endosulfan	*0.08*	*0.13*	*0.05*	*0.05*	*ND*	*ND*	*ND*	*ND*
α-endosulfan	*0.27*	*0.38*	*0.10*	*0.17*	*ND*	*ND*	*ND*	*ND*
Endosulfan (α+β)	0.34	3.52	0.15	0.22	0.03	ND	ND	ND
Minor components (#)	0.13 (5)	0.42 (5)	0.06 (1)	0.16 (4)	0.14 (4)	0.23 (3)	0.29 (6)	0.42 (4)

ND: Not detected (limit of detection: 0.01 µg peq/L)
NP: Not performed
peq: parent equivalents
M1, M4: Unknown components
TRR: Total radioactive residue

Table 26. Spray drift: Characterisation of the Total Radioactive Residue (TRR) in water of test group SD-2.68 after triplicate treatment. The values are given in percent of the TRR per sample.

TRR water [µg peq/L]	3.91	2.62	2.32	2.16	2.11	1.81	1.92	1.76
Test Day	**0.05**	**0.16**	**0.22**	**0.74**	**2**	**3**	**7**	**13**
Identity	[%]							
M1	1.1	ND	0.3	1.0	1.4	3.5	3.8	3.5
Endosulfan diol	19.6	37.6	41.2	52.9	54.9	49.3	36.0	20.1
Endosulfan hydroxy ether	0.9	0.8	0.7	5.1	10.0	15.3	31.1	37.3
Endosulfan lactone	0.8	ND	ND	0.5	2.1	3.1	9.3	20.2
M4	ND	ND	ND	ND	ND	ND	ND	ND
Endosulfan sulfate	0.7	0.8	0.4	2.6	6.8	8.6	13.3	12.9
β-endosulfan	*23.2*	*15.3*	*15.8*	*8.6*	*6.5*	*5.0*	*1.1*	*0.2*
α-endosulfan	*53.0*	*45.2*	*41.5*	*29.0*	*18.0*	*13.7*	*3.8*	*2.0*
Endosulfan (α+β)	76.2	60.5	57.4	37.6	24.5	18.7	4.9	2.2
Minor components (#)	0.8 (2)	0.4 (1)	0.2 (1)	0.5 (2)	0.3 (1)	1.5 (3)	1.5 (4)	3.8 (4)

TRR water [µg peq/L]	6.91	7.46	6.31	4.6	4.58	4.59	4.12	3.66
Test Day	**14.05**	**14.14**	**14.21**	**14.66**	**16**	**17**	**21**	**27**
Identity	[%]							
M1	1.9	2.4	4.2	3.6	3.9	NP	7.9	15.6
Endosulfan diol	20.9	40.4	42.3	47.2	48.9	NP	33.2	24.6
Endosulfan hydroxy ether	12.1	10.3	11.2	14.3	19.1	NP	27.4	25.7
Endosulfan lactone	6.1	5.2	6.9	8.4	8.0	NP	16.8	20.9
M4	ND	ND	ND	ND	ND	NP	ND	ND
Endosulfan sulfate	3.4	3.0	3.7	6.4	8.5	NP	9.1	8.5
β-endosulfan	*17.6*	*11.8*	*8.5*	*4.6*	*1.3*	*NP*	*ND*	*0.4*
α-endosulfan	*36.2*	*24.9*	*21.6*	*12.8*	*4.3*	*NP*	*ND*	*0.3*
Endosulfan (α+β)	53.8	36.6	30.1	17.4	5.6	*NP*	ND	0.7
Minor components (#)	1.7 (4)	2.2 (5)	1.7 (4)	2.8 (5)	6.1 (4)	NP	5.6 (2)	4.1 (6)

TRR water [µg peq/L]	4.25	11.08	5.89	7.42	6.17	7.24	7.01	6.41
Test Day	**28.04**	**28.13**	**28.26**	**29**	**30**	**31**	**35**	**42**
Identity	[%]							
M1	12.5	11.4	6.2	6.5	7.6	11.6	13.9	16.7
Endosulfan diol	36.3	33.6	57.3	57.9	52.6	48.3	39.6	26.3
Endosulfan hydroxy ether	16.4	8.7	14.3	12.3	19.5	17.9	21.1	19.2
Endosulfan lactone	18.1	8.4	14.9	14.3	13.7	15.2	18.2	23.4
M4	ND	ND	ND	ND	ND	ND	ND	3.9
Endosulfan sulfate	5.5	2.5	3.9	3.9	4.0	3.9	3.1	4.0
β-endosulfan	*1.8*	*1.2*	*0.9*	*0.7*	*ND*	*ND*	*ND*	*ND*
α-endosulfan	*6.3*	*3.5*	*1.6*	*2.3*	*0.4*	*ND*	*ND*	*ND*
Endosulfan (α+β)	8.1	31.7	2.5	3.0	0.4	0.0	0.0	0.0
Minor components (#)	3.1 (5)	3.8 (5)	1.1 (1)	2.2 (4)	2.2 (4)	3.1 (3)	4.2 (6)	6.5 (4)

ND: Not detected (limit of detection: 0.3%)
NP: Not performed
peq: parent equivalents
M1, M4: Unknown components
TRR: Total radioactive residue

Table 27. Spray drift: Characterisation of the Total Radioactive Residue (TRR) in water of test group SD-4.69 after duplicate treatment. The values are given in µg peq/L (top) and in percent of the TRR per sample (bottom).

TRR water [µg peq/L]	10.49	9.25	8.56	7.91	7.45	7.26	6.92
Test Day	**14.66**	**16**	**17**	**21**	**27**	**35**	**42**
Identity	[μg_{peq}/L]						
M1	0.31	0.29	0.10	0.49	0.68	0.81	1.14
Endosulfan diol	7.01	6.68	5.95	4.64	3.03	2.20	1.71
Endosulfan hydroxy ether	1.38	1.09	1.24	1.70	2.01	1.75	1.31
Endosulfan lactone	0.43	0.36	0.42	0.66	1.03	1.56	1.85
M4	0.07	0.05	0.09	ND	0.14	0.38	0.42
Endosulfan sulfate	0.43	0.51	0.62	0.43	0.44	0.29	0.44
β-endosulfan	*0.14*	*ND*	*ND*	*ND*	*0.01*	*0.08*	*ND*
α-endosulfan	*0.47*	*0.05*	*0.04*	*<0.01*	*<0.01*	*0.07*	*ND*
Endosulfan (α+β)	0.61	0.05	0.04	<0.01	0.01	0.15	ND
Minor components (#)	0.25 (4)	0.22 (5)	0.11 (2)	ND	0.12 (2)	0.12 (1)	0.06 (1)

TRR water [µg peq/L]	10.49	9.25	8.56	7.91	7.45	7.26	6.92
Test Day	**14.66**	**16**	**17**	**21**	**27**	**35**	**42**
Identity	[%]						
M1	3.0	3.1	1.1	6.2	9.1	11.1	16.4
Endosulfan diol	66.8	72.2	69.5	58.7	40.7	30.3	24.7
Endosulfan hydroxy ether	13.2	11.8	14.5	21.4	26.9	24.2	19.0
Endosulfan lactone	4.1	3.9	5.0	8.3	13.8	21.5	26.7
M4	0.7	0.5	1.1	ND	1.8	5.2	6.0
Endosulfan sulfate	4.1	5.5	7.2	5.4	5.9	4.0	6.4
β-endosulfan	*1.3*	*ND*	*ND*	*ND*	*0.2*	*1.1*	*ND*
α-endosulfan	*4.5*	*0.5*	*0.5*	*ND*	*<0.1*	*1.0*	*ND*
Endosulfan (α+β)	5.8	0.5	0.5	ND	0.2	2.1	ND
Minor components (#)	2.4 (4)	2.41 (5)	1.2 (2)	ND	1.6 (2)	1.7 (1)	0.8 (1)

ND: Not detected (limit of detection: 0.01 µg peq/L or 0.2%)
peq: Parent equivalents
M1, M4: Unknown components
TRR: Total radioactive residue

Table 28. Spray drift: Characterisation of the Total Radioactive Residue (TRR) in water of test group SD-8.38 after single treatment. The metabolite concentrations are given in µg peq/L.

TRR water [µg peq/L]	8.68	9.40	7.83	7.64	7.12
Test Day	**0.04**	**0.13**	**2**	**3**	**7**
Identity	[μg_{peq}/L]				
M1	0.05	ND	0.08	0.16	0.06
Endosulfan diol	0.89	4.33	4.19	3.54	3.81
Endosulfan hydroxy ether	ND	0.27	0.99	ND	1.85
Endosulfan lactone	ND	ND	0.04	ND	0.52
M4	ND	ND	ND	ND	ND
Endosulfan sulfate	ND	ND	0.19	0.31	0.54
β-*endosulfan*	*2.42*	*1.38*	*0.44*	*0.25*	*0.04*
α-*endosulfan*	*5.32*	*3.25*	*1.69*	*1.05*	*0.16*
Endosulfan (α+β)	7.74	4.64	2.14	1.29	0.20
Minor components (#)	ND	0.17 (2)	0.2 (6)	0.36 (6)	0.14 (3)

TRR water [µg peq/L]	6.6	8.0	6.26	5.72	5.32
Test Day	**12**	**15**	**27**	**35**	**42**
Identity	[μg_{peq}/L]				
M1	1.65	1.28	0.95	1.96	1.36
Endosulfan diol	2.47	2.13	1.21	0.76	0.49
Endosulfan hydroxy ether	ND	2.47	1.51	0.84	0.51
Endosulfan lactone	ND	1.80	1.73	1.46	1.96
M4	ND	ND	0.27	0.35	0.47
Endosulfan sulfate	0.47	0.26	0.42	0.19	0.31
β-endosulfan	*ND*	*ND*	*ND*	*ND*	*ND*
α-endosulfan	*ND*	*ND*	*ND*	*ND*	*ND*
Endosulfan (α+β)	ND	ND	ND	ND	ND
Minor components (#)	0.18 (1)	0.06 (1)	0.17 (3)	0.17 (3)	0.22 (3)

ND: Not detected (limit of detection 0.01 µg peq/L)
peq: parent equivalents
M1, M4: *Unknown components*
TRR: Total radioactive residue

Table 29. Spray drift: Characterisation of the Total Radioactive Residue (TRR) in water of test group SD-8.38 after single treatment. The values are given in percent of the TRR per sample.

TRR water [µg peq/L]	8.68	9.4	7.83	7.64	7.12
Test Day	**0.04**	**0.13**	**2**	**4**	**7**
Identity	[%]				
M1	0.6	ND	1.0	2.1	0.8
Endosulfan diol	10.3	46.0	53.5	46.4	53.5
Endosulfan hydroxy ether	ND	2.9	12.7	ND	26.0
Endosulfan lactone	ND	ND	0.6	ND	7.3
M4	ND	ND	ND	ND	ND
Endosulfan sulfate	ND	ND	2.4	4.0	7.5
β-*endosulfan*	*27.9*	*14.7*	*5.7*	*3.2*	*0.6*
α-*endosulfan*	*61.3*	*34.6*	*21.6*	*13.7*	*2.2*
Endosulfan (α+β)	89.1	49.3	27.3	16.9	2.8
Minor components (#)	ND	1.8 (2)	2.6 (6)	4.7 (6)	2.0 (3)

TRR water [µg peq/L]	6.6	8.0	6.26	5.72	5.32
Test Day	**12**	**15**	**27**	**35**	**42**
Identity	[%]				
M1	25.0	16.0	15.2	34.2	25.5
Endosulfan diol	37.5	26.6	19.3	13.2	9.3
Endosulfan hydroxy ether	ND	30.8	24.2	14.6	9.6
Endosulfan lactone	ND	22.6	27.6	25.5	36.8
M4	ND	ND	4.3	6.1	8.8
Endosulfan sulfate	7.2	3.3	6.8	3.4	5.9
β-*endosulfan*	*ND*	*ND*	*ND*	*ND*	*ND*
α-*endosulfan*	*ND*	*ND*	*ND*	*ND*	*ND*
Endosulfan (α+β)	ND	ND	ND	ND	ND
Minor components (#)	2.8 (1)	0.8 (1)	2.7 (3)	3.0 (3)	4.2 (3)

ND: Not detected (limit of detection 0.1%)
peq: parent equivalents
M1, M4: Unknown components
TRR: *Total radioactive residue*

Table 30. Run-off: Characterisation of the Total Radioactive Residue (TRR) in water of test group RO-4.19 after triplicate treatment. The metabolite *concentrations are given in µg peq/L.*

TRR water [µg peq/L]	4.12	2.86	2.87	2.64	2.52	2.93	2.79	2.49
Test Day	**0.05**	**0.13**	**0.25**	**1**	**2**	**4**	**7**	**12**
Identity	[µg peq/L]							
M1	ND	NP	ND	ND	ND	ND	ND	ND
Endosulfan diol	0.30	NP	0.36	0.99	1.22	1.27	ND	0.65
Endosulfan hydroxy ether	0.05	NP	0.04	0.10	0.17	0.47	ND	0.91
Endosulfan lactone	ND	NP	ND	0.03	0.04	0.09	ND	0.33
M4	ND	NP	ND	ND	ND	ND	ND	ND
Endosulfan sulfate	ND	NP	0.04	0.17	0.24	0.39	ND	0.39
β-endosulfan	*1.25*	*NP*	*0.81*	*0.39*	*0.20*	*0.11*	*ND*	*0.02*
α-endosulfan	*2.52*	*NP*	*1.62*	*0.96*	*0.65*	*0.37*	*ND*	*0.07*
Endosulfan (α+β)	3.77	NP	2.43	1.35	0.85	0.49	ND	0.09
Minor components (#)	ND	NP	ND	ND	0.01 (1)	0.23 (9)	ND	0.11 (4)

TRR water [µg peq/L]	5.68	5.18	4.79	4.95	5.42	5.37	5.06	4.91
Test Day	**14.04**	**14.13**	**14.25**	**15**	**16**	**18**	**21**	**26**
Identity	[µg peq/L]							
M1	0.06	ND	0.01	0.05	0.04	0.22	0.04	0.60
Endosulfan diol	1.13	1.60	1.68	2.16	2.49	2.23	2.13	1.31
Endosulfan hydroxy ether	1.07	1.25	0.91	1.01	0.92	1.16	1.40	1.22
Endosulfan lactone	0.55	0.44	0.48	0.48	0.57	0.67	0.76	1.08
M4	ND	ND	ND	ND	ND	ND	ND	0.09
Endosulfan sulfate	0.39	0.38	0.44	0.54	0.76	0.77	0.62	0.61
β-endosulfan	*0.62*	*0.32*	*0.46*	*0.15*	*0.08*	*0.06*	*ND*	*ND*
α-endosulfan	*1.83*	*0.88*	*0.85*	*0.51*	*0.40*	*0.16*	*0.04*	*ND*
Endosulfan (α+β)	2.45	1.20	1.30	0.67	0.49	0.22	0.04	ND
Minor components (#)	0.03 (1)	0.31 (4)	0.12 (5)	0.05 (1)	0.15 (6)	0.11 (3)	0.07 (2)	ND

TRR water [µg peq/L]	8.33	7.89	7.68	7.82	7.78	7.55	7.49	7.68
Test Day	**28.04**	**28.13**	**28.25**	**29**	**30**	**32**	**35**	**43**
Identity	[µg peq/L]							
M1	0.27	0.27	0.22	0.13	0.43	0.41	0.42	2.01
Endosulfan diol	3.36	3.57	4.03	4.22	4.05	3.83	2.88	2.15
Endosulfan hydroxy ether	1.33	1.51	1.16	1.28	1.23	1.37	1.57	1.33
Endosulfan lactone	1.06	1.24	1.14	1.21	1.26	1.27	1.63	1.33
M4	ND	ND	ND	ND	ND	ND	ND	0.29
Endosulfan sulfate	0.54	0.50	0.57	0.54	0.43	0.47	0.57	0.37
β-endosulfan	*0.41*	*0.09*	*0.11*	*0.07*	*0.08*	*ND*	*ND*	*ND*
α-endosulfan	*1.31*	*0.56*	*0.34*	*0.13*	*0.08*	*ND*	*0.09*	*ND*
Endosulfan (α+β)	1.72	0.65	0.45	0.20	0.16	ND	0.09	ND
Minor components (#)	0.06 (1)	0.14 (2)	0.10 (2)	0.25 (2)	0.23 (2)	0.19 (1)	0.33 (3)	ND

ND: Not detected (limit of detection 0.01 µg peq/L)
NP: Not performed
peq: parent equivalents
M1, M4: Unknown components
TRR: Total radioactive residue

Table 31. Run off: Characterisation of the Total Radioactive Residue (TRR) in water of test group RO-4.19 after triplicate treatment. The values are given in percent of the TRR per sample.

TRR water [µg peq/L]	4.12	2.86	2.87	2.64	2.52	2.93	2.79	2.49
Test Day	**0.05**	**0.13**	**0.25**	**1**	**2**	**4**	**7**	**12**
Identity	[%]							
M1	ND	NP	ND	ND	ND	ND	ND	ND
Endosulfan diol	7.2	NP	12.5	37.4	48.3	43.4	ND	26.3
Endosulfan hydroxy ether	1.3	NP	1.4	3.7	6.7	15.9	ND	36.5
Endosulfan lactone	ND	NP	ND	1.2	1.4	3.2	ND	13.2
M4	ND	NP	ND	ND	ND	ND	ND	ND
Endosulfan sulfate	ND	NP	1.5	6.6	9.4	13.2	ND	15.9
β-endosulfan	*30.3*	*NP*	*28.1*	*14.8*	*8.0*	*3.9*	*ND*	*1.0*
α-endosulfan	*61.2*	*NP*	*56.5*	*36.3*	*25.9*	*12.7*	*ND*	*2.8*
Endosulfan (α+β)	91.5	NP	84.6	51.1	33.9	16.6	ND	3.8
Minor components (#)	ND	NP	ND	ND	0.4 (1)	7.7 (9)	ND	4.5 (4)

TRR water [µg peq/L]	5.68	5.18	4.79	4.95	5.42	5.37	5.06	4.91
Test Day	**14.04**	**14.13**	**14.25**	**15**	**16**	**18**	**21**	**26**
Identity	[%]							
M1	1.0	ND	0.2	0.9	0.8	4.0	0.8	12.2
Endosulfan diol	19.9	30.9	35.1	43.6	45.9	41.6	42.0	26.6
Endosulfan hydroxy ether	18.9	24.2	19.0	20.5	16.9	21.6	27.6	24.9
Endosulfan lactone	9.7	8.6	10.0	9.6	10.6	12.5	15.0	22.1
M4	ND	ND	ND	ND	ND	ND	ND	1.9
Endosulfan sulfate	6.8	7.2	9.1	11.0	14.1	14.3	12.3	12.5
β-endosulfan	*10.9*	*6.3*	*9.5*	*3.1*	*1.6*	*1.1*	*ND*	*ND*
α-endosulfan	*32.2*	*16.9*	*17.7*	*10.4*	*7.4*	*3.0*	*0.8*	*ND*
Endosulfan (α+β)	43.1	23.2	27.2	13.5	9.0	4.0	0.8	ND
Minor components (#)	0.6 (1)	5.9 (4)	2.4 (5)	0.9 (1)	2.7 (6)	2.0 (3)	1.4 (2)	ND

TRR water [µg peq/L]	8.33	7.89	7.68	7.82	7.78	7.55	7.49	7.68
Test Day	**28.04**	**28.13**	**28.25**	**29**	**30**	**32**	**35**	**43**
Identity	[%]							
M1	3.2	3.4	2.8	1.7	5.5	5.4	5.6	26.2
Endosulfan diol	40.3	45.2	52.5	53.9	52.0	50.8	38.5	28.0
Endosulfan hydroxy ether	15.9	19.2	15.1	16.4	15.8	18.2	21.0	17.4
Endosulfan lactone	12.7	15.8	14.9	15.4	16.3	16.9	21.8	17.4
M4	ND	ND	ND	ND	ND	ND	ND	3.8
Endosulfan sulfate	6.5	6.4	7.5	6.9	5.5	6.3	7.6	4.8
β-endosulfan	*5.0*	*1.1*	*1.5*	*0.9*	*1.0*	*ND*	*ND*	*ND*
α-endosulfan	*15.7*	*7.1*	*4.4*	*1.7*	*1.0*	*ND*	*1.2*	*ND*
Endosulfan (α+β)	20.7	8.2	5.9	2.6	2.0	ND	1.2	ND
Minor components (#)	0.8 (1)	1.8 (2)	1.4 (2)	3.1 (2)	3.0 (2)	2.5 (1)	4.5 (3)	ND

ND: Not detected (limit of detection 0.1%)
NP: Not performed
peq: parent equivalents
M1, M4: Unknown components
TRR: Total radioactive residue

Table 32. Run off: Characterisation of the Total Radioactive Residue (TRR) in water of test group RO-6.29 after duplicate treatment. The values are given in µg peq/L (top) and in percent of the TRR per sample (bottom).

TRR water [µg peq/L]	8.94	8.63	7.49	7.90	7.76	7.60	7.28	6.85
Test Day	**14.04**	**14.25**	**15**	**16**	**18**	**26**	**35**	**43**
Identity	[$\mu g_{peq}/L$]							
M1	0.01	0.25	0.39	0.45	0.30	1.06	1.64	1.42
Endosulfan diol	1.19	4.66	3.72	4.50	4.21	2.54	1.59	1.01
Endosulfan hydroxy ether	0.09	1.04	1.04	1.49	1.25	1.82	1.30	0.87
Endosulfan lactone	0.05	0.58	0.57	0.58	0.49	1.23	1.84	1.82
M4	ND	ND	0.06	ND	ND	ND	0.30	0.63
Endosulfan sulfate	0.05	0.56	0.58	0.87	0.73	0.95	0.50	0.46
β-endosulfan	*2.70*	*0.36*	*0.22*	*ND*	*0.09*	*ND*	*ND*	*ND*
α-endosulfan	*5.91*	*1.06*	*0.68*	*ND*	*0.42*	*ND*	*ND*	*ND*
Endosulfan (α+β)	8.61	1.42	0.91	ND	0.51	ND	ND	ND
Minor components (#)	0.02 (1)	0.11 (5)	0.23 (5)	ND	0.26 (2)	ND	ND	0.63 (3)

TRR water [µg peq/L]	8.94	8.63	7.49	7.90	7.76	7.60	7.28	6.85
Test Day	**14.04**	**14.25**	**15**	**16**	**18**	**26**	**35**	**43**
Identity	[%]							
M1	0.2	2.9	5.2	5.7	3.9	13.9	22.6	20.7
Endosulfan diol	13.3	54.0	49.6	57.0	54.3	33.5	21.8	14.8
Endosulfan hydroxy ether	1.0	12.1	13.9	18.9	16.1	23.9	17.8	12.7
Endosulfan lactone	0.5	6.7	7.6	7.4	6.3	16.2	25.3	26.6
M4	ND	ND	0.8	ND	ND	ND	4.2	9.2
Endosulfan sulfate	0.6	6.5	7.7	11.1	9.4	12.5	6.8	6.8
β-endosulfan	*30.3*	*4.2*	*3.0*	ND	*1.1*	*ND*	*ND*	*ND*
α-endosulfan	*66.1*	*12.3*	*9.1*	ND	*5.4*	*ND*	*ND*	*ND*
Endosulfan (α+β)	96.3	16.5	12.1	ND	6.6	ND	ND	ND
Minor components (#)	0.2 (1)	1.2 (5)	3.1 (5)	ND	3.4 (2)	ND	ND	9.3 (3)

ND: Not detected (limit of detection: 0.01 µg peq/L or 0.1%)
peq: parent equivalents
M1, M4: Unknown components
TRR: Total radioactive residue

Table 33. Run-off: Characterisation of the Total Radioactive Residue (TRR) in water of test group RO-8.39 after single treatment. The metabolite concentrations are given in µg peq/L.

TRR water [µg peq/L]	6.07	5.77	5.92	5.73	5.63	5.97
Test Day	**0.04**	**0.13**	**0.25**	**1**	**2**	**4**
Identity	[$\mu g_{peq}/L$]					
M1	0.04	0.03	ND	ND	0.10	0.02
Endosulfan diol	0.57	*0.48*	*0.61*	*1.36*	*2.38*	*3.03*
Endosulfan hydroxy ether	0.16	0.17	0.09	0.16	0.67	0.65
Endosulfan lactone	0.11	0.10	ND	ND	0.13	0.11
M4	ND	ND	ND	ND	ND	ND
Endosulfan sulfate	0.12	0.19	0.09	0.19	0.43	0.62
β-endosulfan	*1.82*	*1.57*	*1.57*	*1.28*	*0.52*	*0.38*
α-endosulfan	*3.24*	*3.15*	*3.50*	*2.74*	*1.31*	*1.08*
Endosulfan (α+β)	5.07	4.72	5.06	4.02	1.83	1.47
Minor components (#)	ND	0.08 (3)	0.06 (3)	ND	0.1 (3)	0.07 (2)

TRR water [µg peq/L]	5.79	5.43	4.91	5.10	4.71	4.37
Test Day	**7**	**12**	**21**	**26**	**35**	**43**
Identity	[$\mu g_{peq}/L$]					
M1	0.04	0.20	0.55	0.93	1.20	1.00
Endosulfan diol	2.84	1.80	0.90	0.69	0.49	0.41
Endosulfan hydroxy ether	1.23	1.65	1.46	1.38	0.78	0.50
Endosulfan lactone	0.38	0.60	1.08	1.34	1.55	1.65
M4	ND	ND	0.21	0.26	0.30	0.39
Endosulfan sulfate	0.75	0.61	0.70	0.51	0.38	0.42
β-endosulfan	*0.17*	*0.08*	*ND*	*ND*	*ND*	*ND*
α-endosulfan	*0.28*	*0.25*	*ND*	*ND*	*ND*	*ND*
Endosulfan (α+β)	0.44	0.34	ND	ND	ND	ND
Minor components (#)	0.11 (5)	0.24 (5)	ND	ND	ND	ND

ND: Not detected (limit of detection: 0.01 µg peq/L)
peq: parent equivalents
M1, M4: Unknown components
TRR: Total radioactive residue

Table 34. Run-off: Characterisation of the Total Radioactive Residue (TRR) in water of test group RO-8.39 after single treatment. The values are given in percent of the TRR per sample.

TRR water [µg peq/L]	6.07	5.77	5.92	5.73	5.63	5.97
Test Day	**0.04**	**0.13**	**0.25**	**1**	**2**	**4**
Identity	[%]					
M1	0.7	0.5	ND	ND	1.8	0.3
Endosulfan diol	9.4	8.3	10.3	23.8	42.3	50.7
endosulfan hydroxy ether	2.6	2.9	1.6	2.8	11.9	11.0
Endosulfan lactone	1.8	1.8	ND	ND	2.2	1.8
M4	ND	ND	ND	ND	ND	ND
Endosulfan sulfate	2.0	3.3	1.6	3.2	7.6	10.4
β-endosulfan	30.0	27.2	26.5	22.4	9.2	6.4
α-endosulfan	53.4	54.6	59.1	47.8	23.2	18.2
Endosulfan (α+β)	83.5	81.9	85.5	70.2	32.4	24.6
Minor components (#)	ND	1.4 (3)	1.1 (3)	ND	1.7 (3)	1.3 (2)

TRR water [µg peq/L]	5.79	5.43	4.91	5.10	4.71	4.37
Test Day	**7**	**12**	**21**	**26**	**35**	**43**
Identity	[%]					
M1	0.7	3.6	11.3	18.2	25.5	23.0
Endosulfan diol	49.0	33.1	18.3	13.5	10.5	9.5
endosulfan hydroxy ether	21.2	30.4	29.8	27.0	16.5	11.5
Endosulfan lactone	6.6	11.0	22.1	26.2	33.0	37.7
M4	ND	ND	4.3	5.2	6.4	8.8
Endosulfan sulfate	12.9	11.3	14.3	9.9	8.1	9.6
β-endosulfan	2.9	1.5	ND	ND	ND	ND
α-endosulfan	4.8	4.7	ND	ND	ND	ND
Endosulfan (α+β)	7.7	6.2	ND	ND	ND	ND
Minor components (#)	1.9 (5)	4.4 (5)	ND	ND	ND	ND

ND: Not detected (limit of detection 0.2%)
peq: parent equivalents
M1, M4: Unknown components
TRR: Total radioactive residue

Table 35. Spray drift: DT_{50} values of endosulfan and its major degradation products in water.

DT_{50} values (calculation model used)			
Test Group (Test Conc.)	***first application***	***second application***	***third application***
(α+β)-Endosulfan			
SD-2.68 (3.50 µg ai/L***)	0.5 (2)	0.2 (2)	NC
SD-8.38 (10.33 µg ai/L*)	0.7 (2)	NA	NA
α-endosulfan			
SD-2.68 (3.50 µg ai/L***)	0.6 (2)	0.3 (1)	NC
SD-8.38 (10.33 µg ai/L*)	0.4 (2)	NA	NA
β-endosulfan			
SD-2.68 (3.50 µg ai/L***)	0.4 (2)	NC	NC
SD-8.38 (10.33 µg ai/L*)	0.6 (2)	NA	NA
Endosulfan diol			
SD-2.68 (3.50 µg ai/L***)	9 (3)	8 (1)	11 (1)
SD-8.38 (10.33 µg ai/L*)	13 (3)	NA	NA
Endosulfan sulfate			
SD-2.68 (3.50 µg ai/L***)	NC	NC	NC
SD-8.38 (10.33 µg ai/L*)	NC	NA	NA
M1			
SD-2.68 (3.50 µg ai/L***)	NC	NC	NC
SD-8.38 (10.33 µg ai/L*)	NC	NA	NA
Endosulfan hydroxy ether			
SD-2.68 (3.50 µg ai/L***)	NC	NC	NC
SD-8.38 (10.33 µg ai/L*)	13 (1)	NA	NA
Endosulfan lactone			
SD-2.68 (3.50 µg ai/L***)	NC	NC	NC
SD-8.38 (10.33 µg ai/L*)	NC	NA	NA

NA: Not applicable, since concentration SD-8.38 was only applied once.
NC: Not calculated, since no model fitted the data.

Test Conc.: *Average water concentration per treatment based on total radioactivity applied to the enclosures*
*, ***: one or three treatments

Models:
1. A first order, one compartment model, with a bolus input and a first order output.
2. A two-compartment (bi-exponential) model with a bolus input and first order output
3. A one compartment model with first order input and a first order output

Table 36. Run-off: DT_{50} values of endosulfan and its major degradation products in water.

DT_{50} values (calculation model used)			
Dose Group (Test Conc.)	**first application**	**second application**	**third application**
(α+β)-endosulfan			
RO-4.19 (4.19 µg ai/L***)	0.9 (2)	1 (1)	NC
RO-8.39 (8.39 µg ai/L*)	2 (2)	NA	NA
α-endosulfan			
RO-4.19 (4.19 µg ai/L***)	1 (2)	2 (1)	NC
RO-8.39 (8.39 µg ai/L*)	2 (2)	NA	NA
β-endosulfan			
RO-4.19 (4.19 µg ai/L***)	0.7 (2)	0.3 (7)	NC
RO-8.39 (8.39 µg ai/L*)	2 (2)	NA	NA
Endosulfan diol			
RO-4.19 (4.19 µg ai/L***)	9 (3)	NC	14 (1)
RO-8.39 (8.39 µg ai/L*)	8 (4)	NA	NA
endosulfan sulfate			
RO-4.19 (4.19 µg ai/L***)	NC	NC	NC
RO-8.39 (8.39 µg ai/L*)	NC	NA	NA
M1			
RO-4.19 (4.19 µg ai/L***)	NC	NC	NC
RO-8.39 (8.39 µg ai/L*)	NC	NA	NA
Endosulfan hydroxy ether			
RO-4.19 (4.19 µg ai/L***)	NC	NC	NC
RO-8.39 (8.39 µg ai/L*)	10 (1)	NA	NA
Endosulfan lactone			
RO-4.19 (4.19 µg ai/L***)	NC	NC	NC
RO-8.39 (8.39 µg ai/L*)	NC	NA	NA

NA: Not applicable, since concentration RO-8.39 was only applied once.
NC: Not calculated, since no model fitted the data.

Test Conc.: Average water concentration per treatment based on total radioactivity applied to the enclosures
*, ***: one or three treatments

Models:
1. A first order, one compartment model, with a bolus input and a first order output.
2. A two-compartment (bi-exponential) model with a bolus input and first order output
1. A one compartment model with first order input and a first order output

Table 37. Total Radioactive Residue in the macrophyte *Elodea canadensis* after triplicate treatment (spray drift test group SD-2.68 and run-off test group RO-4.19).

A: Spray drift test period after the 1st application

Test Day	3 hrs	6 hrs	12 hrs	1	2	3	7	13
Test Group SD-2.68***	[µg peq/kg]							
3.50 µg ai/L[a]	NP	NP	NP	NP	243	420	370	875

B: Spray drift test period after the 2nd application

Test Day	14 days 3 hrs	14 days 6 hrs	14 days 12 hrs	15	16	17	20	27
Test Group SD-2.68***	[µg peq/kg]							
3.50 µg ai/L[a]	NP	NP	NP	NP	NP	1112	1827	1191

C: Spray drift test period after the 3rd application

Test Day	28 days 3 hrs	28 days 6 hrs	28 days 12 hrs	29	30	31	35	42
Test Group SD-2.68***	[µg peq/kg]							
3.50 µg ai/L[a]	NP	NP	NP	NP	1'859	1'616	1'884	2'236

D: Run-off test period after the 1st application

Test Day	3 hrs	6 hrs	12 hrs	1	2	4	7	12
Test Group RO-4.19***	[µg peq/kg]							
3.99 µg SR/L[a]	NP	NP	NP	NP	450	565	609	881

E: Run-off test period after the 2nd application

Test Day	14 days 3 hrs	14 days 6 hrs	14 days 12 hrs	15	16	18	21	26
Test Group RO-4.19***	[µg peq/kg]							
3.99 µg SR/L[a]	NP	NP	NP	NP	1'318	1'188	1'537	2'065

F: Run-off test period after the 3rd application

Test Day	28 days 3 hrs	28 days 6 hrs	28 days 12 hrs	29	30	32	34	42
Test Group RO-4.19***	[µg peq/kg]							
3.99 µg SR/L[a]	NP	NP	NP	NP	1'565	1'449	1'862	NP

[a] *Test Conc.: Average water concentration per treatment based on total radioactivity applied to the enclosures*

NP: Not Performed
***: three treatments
SR: Soil residue
peq: parent equivalents

Table 38. Characterization of residual radioactivity in *Elodea canadensis* after triplicate treatment (spray drift test group SD-2.68 and run-off test group RO-4.19).

A: Spray Drift Entry Route:

Total Radioactive Residue (TRR)	1.616 mg peq/kg fresh weight (Day 31)	100 %
Identity	[mg peq/kg fresh weight]	[%]
Endosulfan diol	0.305	18.9
Endosulfan hydroxy ether	0.156	9.7
Endosulfan sulfate	0.270	16.7
β-Endosulfan	*0.015*	*0.9*
α-Endosulfan	*0.047*	*2.9*
α/β-Endosulfan	0.062	3.8
M6	0.031	1.9
M7	0.126	7.8
M8	0.081	5.0
M9	0.434	26.9
Minor components (#)	0.117 (13)	7.2 (13)

B: Run-Off Entry Route:

Total Radioactive Residue (TRR)	2.065 mg peq/kg fresh weight (Day 26)	100 %
Identity	[mg peq/kg fresh weight]	[%]
Endosulfan diol	0.277	13.4
Endosulfan hydroxy ether	0.170	8.2
Endosulfan sulfate	0.460	22.3
β-Endosulfan	*0.019*	*0.9*
α-Endosulfan	*0.019*	*0.9*
α/β-Endosulfan	0.038	1.8
M6	0.279	13.5
M7	0.123	6
M8	0.087	4.2
M9	0.407	19.7
Minor components (#)	0.173 (13)	8.4 (13)

peq: parent equivalents
M6-M9 Unknown components
TRR: total radioactive residue

Table 39. Total Radioactive Residue in sediment cores after triplicate treatment (spray drift test group SD-2.68 and run-off test group RO-4.19).

A: Spray drift Test Period after the 1st application

Test Day	1 hrs	3 hrs	6 hrs	1	2	3	7	13
Test Group SD-2.68***	[µg peq/L]							
3.50 µg ai/L[a]	NP	NP	NP	NP	1.0	1.0	1.2	1.2

B: Spray drift Test period after the 2nd application

Test Day	14 days 1 hrs	14 days 3 hrs	14 days 6 hrs	15	16	17	20	27
Test Group SD-2.68***	[µg peq/L]							
3.50 µg ai/L[a]	NP	NP	NP	NP	NP	2.1	2.5	2.8

C: Spray drift Test period after the 3rd application

Test Day	28 days 1 hrs	28 days 3 hrs	28 days 6 hrs	29	30	31	35	42
Test Group SD-2.68***	[µg peq/L]							
3.50 µg ai/L[a]	NP	NP	NP	NP	4.9	4.7	6.2	NP

D: Run-off Test Period after the 1st application

Test Day	1 hrs	3 hrs	6 hrs	1	2	4	7	13
Test Group RO-4.19***	[µg peq/L]							
3.99 µg SR/L[a]	NP	NP	3.5	3.8	3.4	3.8	3.4	3.7

E: Run-off Test period after the 2nd application

Test Day	14 days 1 hrs	14 days 3 hrs	14 days 6 hrs	15	16	18	21	26
Test Group RO-4.19***	[µg peq/L]							
3.99 µg SR/L[a]	NP	NP	9.2	4.1	3.5	8.1	8.4	9.3

F: Run-off Test period after the 3rd application

Test Day	28 days 1 hrs	28 days 3 hrs	28 days 6 hrs	29	30	32	34	42
Test Group RO-4.19***	[µg peq/L]							
3.99 µg SR/L[a]	NP	NP	13.8	NP	11.3	8.2	9.1	NP

[a] Test Conc.: Average water concentration per treatment based on total radioactivity applied to the enclosures

NP: Not Performed

SR: Soil residue

***: three treatments

peq: parent equivalents

Table 40. Spray drift: Characterisation of the TRR of sediment cores after triplicate treatment (spray drift test group SD-2.68). The metabolite concentrations are given in µg peq/kg.

TRR water [µg peq/kg]	1.00	0.97	1.17	1.20	2.14
Test Day	2	3	7	13	17
Identity	[µg peq/kg]				
M1	ND	ND	ND	0.04	ND
Endosulfan diol	0.12	0.15	0.23	0.04	0.54
Endosulfan hydroxy ether	0.03	0.11	0.24	0.24	0.19
Endosulfan lactone	ND	ND	0.05	0.08	0.11
M4	ND	ND	ND	ND	ND
Endosulfan sulfate	0.16	0.09	0.25	0.40	0.62
β-endosulfan	0.18	0.26	0.06	0.13	0.13
α-endosulfan	0.51	0.29	0.34	0.28	0.56
Endosulfan (α+β)	0.69	0.55	0.40	0.41	0.69
Minor components (#)	ND	0.07 (1)	ND	ND	ND

TRR water [µg peq/kg]	2.54	2.84	4.88	4.76	6.24
Test Day	20	27	30	31	35
Identity	[µg peq/kg]				
M1	0.05	ND	0.15	ND	0.06
Endosulfan diol	0.56	0.57	1.59	1.39	2.39
Endosulfan hydroxy ether	0.70	0.53	0.81	0.91	0.96
Endosulfan lactone	0.30	0.36	0.44	0.51	0.54
M4	ND	ND	ND	ND	0.04
Endosulfan sulfate	0.62	0.97	1.15	1.60	1.60
β-endosulfan	0.10	0.10	0.32	0.15	0.34
α-endosulfan	0.21	0.30	0.37	0.19	0.32
Endosulfan (α+β)	0.31	0.40	0.68	0.34	0.65
Minor components (#)	ND	ND	0.05 (1)	ND	ND

ND: Not detected
peq : parent equivalents
M1, M4: Unknown component

Table 41. Spray drift: Characterisation of the Total Radioactive Residue (TRR) in sediment cores after triplicate treatment (spray drift test group SD-2.68). The values are given in percent of the TRR per sample.

TRR water [µg peq/kg]	1.00	0.97	1.17	1.20	2.14
Test Day	2	3	7	13	17
Identity	[%]				
M1	ND	ND	ND	3.0	ND
Endosulfan diol	11.9	15.1	19.4	3.0	25.0
Endosulfan hydroxy ether	3.0	11.3	20.4	20.0	9.0
Endosulfan lactone	ND	ND	4.3	7.0	5.2
M4	ND	ND	ND	ND	ND
Endosulfan sulfate	16.4	9.4	21.5	33.0	28.8
β-endosulfan	17.9	26.4	5.4	11.0	6.13
α-endosulfan	50.8	30.2	29.0	23.0	25.9
Endosulfan (α+β)	68.7	56.6	34.4	34.0	32.1
Minor components (#)	ND	7.6 (1)	ND	ND	ND

TRR water [µg peq/kg]	2.54	2.84	4.88	4.76	6.24
Test Day	20	27	30	31	35
Identity	[%]				
M1	1.9	ND	3.1	ND	0.9
Endosulfan diol	21.9	20.1	32.6	29.2	38.3
Endosulfan hydroxy ether	27.5	18.8	16.6	19.2	15.3
Endosulfan lactone	11.9	12.8	9.1	10.8	8.7
M4	ND	ND	ND	ND	0.7
Endosulfan sulfate	24.5	34.2	23.6	33.7	25.6
β-endosulfan	4.1	3.4	6.5	3.2	5.4
α-endosulfan	8.2	10.7	7.5	4.0	5.1
Endosulfan (α+β)	12.3	14.1	14.0	7.1	10.5
Minor components (#)	ND	ND	1.0 (1)	ND	ND

ND: Not detected
peq: parent equivalents
M1, M4: Unknown component

Table 42. Run-off: Characterisation of the TRR sediment cores after triplicate treatment (run-off test group RO-4.19). The metabolite concentrations are given in µg peq/kg.

TRR water [µg peq/kg]	3.45	3.81	3.43	9.18	4.15	3.49
Test Day	0	1	7	14	15	16
Identity	[µg peq/kg]					
M1	ND	ND	0.07	0.15	ND	ND
Endosulfan diol	ND	0.12	0.40	1.29	0.45	0.63
Endosulfan hydroxy ether	ND	ND	0.22	0.18	0.23	0.28
Endosulfan lactone	ND	ND	ND	ND	ND	ND
M4	ND	ND	ND	ND	ND	ND
Endosulfan sulfate	ND	0.11	0.46	0.61	0.50	0.47
β-endosulfan	1.38	1.22	0.91	2.86	0.99	0.78
α-endosulfan	2.07	2.32	1.36	4.08	1.98	1.32
Endosulfan (α+β)	3.45	3.54	2.27	6.94	2.98	2.11
Minor components (#)	ND	0.04 (1)	ND	ND	ND	ND

TRR water [%]	8.12	9.32	13.82	11.26	8.21	9.11
Test Day	18	26	28	30	32	34
Identity	[µg peq/kg					
M1	0.12	0.12	0.05	0.05	0.16	0.10
Endosulfan diol	1.15	1.62	1.58	1.81	1.69	1.80
Endosulfan hydroxy ether	0.40	0.68	0.42	0.44	0.38	0.54
Endosulfan lactone	0.28	0.23	0.42	0.42	0.39	0.47
M4	ND	0.03	0.08	ND	ND	0.11
Endosulfan sulfate	1.11	1.32	2.70	1.81	1.86	2.16
β-endosulfan	2.21	2.38	3.80	2.58	1.80	1.86
α-endosulfan	2.58	2.92	4.70	4.05	1.73	1.90
Endosulfan (α+β)	4.80	5.31	8.50	6.62	3.53	3.76
Minor components (#)	0.26 (3)	ND	0.06 (1)	0.11 (1)	0.20 (3)	0.16 (3)

ND: Not detected
peq: parent equivalents
M1, M4: Unknown components

Table 43. Run-off: Characterisation of the Total Radioactive Residue (TRR) in sediment cores after triplicate treatment (Run-off test group RO-4.19). The values are given in percent of TRR per sample.

TRR water [µg peq/kg]	3.45	3.81	3.43	9.18	4.15	3.49
Test Day	**0**	**1**	**7**	**14**	**15**	**16**
Identity	[%]					
M1	ND	ND	2.1	1.6	ND	ND
Endosulfan diol	ND	3.2	11.8	14.1	10.9	18.0
Endosulfan hydroxy ether	ND	ND	6.5	2.0	5.4	8.0
Endosulfan lactone	ND	ND	ND	ND	ND	ND
M4	ND	ND	ND	ND	ND	ND
Endosulfan sulfate	ND	2.9	13.5	6.7	12.0	13.6
β-endosulfan	39.9	32.0	26.5	31.2	23.9	22.4
α-endosulfan	60.1	60.9	39.7	44.4	47.8	38.0
Endosulfan (α+β)	100	93.0	66.2	75.7	71.7	60.4
Minor components (#)	ND	0.96 (1)	ND	ND	ND	ND

TRR water [µg peq/kg]	8.12	9.32	13.82	11.26	8.21	9.11
Test Day	**18**	**26**	**28**	**30**	**32**	**34**
Identity	[%]					
M1	1.5	1.3	0.4	0.4	2.0	1.1
Endosulfan diol	14.2	17.4	11.4	16.1	20.6	19.7
Endosulfan hydroxy ether	4.9	7.3	3.0	3.9	4.6	6.0
Endosulfan lactone	3.4	2.5	3.0	3.7	4.8	5.1
M4	ND	0.4	0.6	ND	ND	1.2
Endosulfan sulfate	13.6	14.2	19.6	16.1	22.6	23.7
β-endosulfan	27.3	25.6	27.5	22.9	21.9	20.5
α-endosulfan	31.8	31.3	34.0	36.0	21.1	20.9
Endosulfan (α+β)	59.1	56.9	61.5	58.8	43.0	41.3
Minor components (#)	3.2 (3)	ND	0.5 (1)	1.0 (1)	2.4 (3)	1.7 (3)

ND Not detected
peq parent equivalents
M1, 4 Unknown component.

Table 44. Cumulative percent fish mortality found after spray drift application.

Test Group	Test Conc.	Cumulative Percent Mortality (%)[1] First Application Day 0				
	(µg ai/L)	Day 1	Day 2	Day 4	Day 7	Day 13
SD-CTRL-1	0	0	0	0	0	0
SD-CTRL-2	0	0	0	2	2	2
SD-CTRL-3	0	0	0	0	0	2
SD-CTRL-4	0	0	0	0	2	2
SD-0.27***	0.34	0	0	0	0	0
SD-0.47***	0.55	0	0	0	0	0
SD-0.84***	1.16	2	2	2	2	2
SD-1.51***	1.96	0	0	2	2	2
SD-2.68***	3.50	2	20	35	35	38
SD-4.69**	6.40	68	100	100	100	100
SD-8.38*	10.33	74	84	100	100	100

Test Group	Test Conc.	Cumulative Percent Mortality (%)[1] Second Application Day 14				
	(µg ai/L)	Day 15	Day 16	Day 18	Day 21	Day 27
SD-CTRL-1	0	0	0	2	2	2
SD-CTRL-2	0	2	2	2	2	2
SD-CTRL-3	0	2	2	2	2	2
SD-CTRL-4	0	2	2	2	2	2
SD-0.27***	0.34	0	0	0	0	0
SD-0.47***	0.55	0	0	0	0	0
SD-0.84***	1.16	2	5	5	5	5
SD-1.51***	1.96	5	5	8	8	11
SD-2.68***	3.50	67	67	77	79	84
SD-4.69**	6.40	100	100	100	100	100
SD-8.38*	10.33	100	100	100	100	100

Test Group	Test Conc.	Cumulative Percent Mortality (%)[1] Third Application Day 28				
	(µg ai/L)	Day 29	Day 30	Day 32	Day 35	Day 41
SD-CTRL-1	0	2	2	3	3	3
SD-CTRL-2	0	2	2	3	3	3
SD-CTRL-3	0	2	2	3	3	3
SD-CTRL-4	0	2	2	3	5	5
SD-0.27***	0.34	0	0	0	0	0
SD-0.47***	0.55	0	0	0	3	3
SD-0.84***	1.16	6	6	6	6	10
SD-1.51***	1.96	11	11	13	13	13
SD-2.68***	3.50	89	89	100	100	100
SD-4.69**	6.40	100	100	100	100	100
SD-8.38*	10.33	100	100	100	100	100

Test Conc.: Average water concentration per treatment based on total radioactivity applied to the enclosures
[1] The numbers were corrected for the fish taken for residue analysis.
CTRL: Control
SD: Spray drift *, **, ***: one, two or three treatments

Table 45. Cumulative percent fish mortality found after run-off application.

Test Group	Test Conc.	Cumulative Percent Mortality (%)[1] First Application Day 0				
	(µg SR/L)	Day 1	Day 2	Day 4	Day 7	Day 13
RO-CTRL-1	0	0	0	0	0	0
RO-CTRL-2	0	0	0	0	0	0
RO-CTRL-3	0	0	0	0	0	0
RO-CTRL-4	0	0	0	0	0	2
RO-0.21***	0.21	0	0	0	0	0
RO-0.42***	0.42	0	0	0	0	0
RO-0.84***	0.84	2	4	6	6	7
RO-2.09***	2.09	0	0	2	2	2
RO-4.19***	3.99	4	54	76	88	98
RO-6.29**	6.29	0	100	100	100	100
RO-8.39*	8.39	10	100	100	100	100

Test Group	Test Conc.	Cumulative Percent Mortality (%)[1] Second Application Day 14				
	(µg SR/L)	Day 15	Day 16	Day 18	Day 21	Day 27
RO-CTRL-1	0	0	0	0	2	2
RO-CTRL-2	0	0	0	0	0	0
RO-CTRL-3	0	0	0	0	0	0
RO-CTRL-4	0	2	2	2	5	5
RO-0.21***	0.21	0	0	0	0	0
RO-0.42***	0.42	0	0	0	0	0
RO-0.84***	0.84	7	7	7	8	8
RO-2.09***	2.09	2	2	2	3	3
RO-4.19***	3.99	98	98	100	100	100
RO-6.29**	6.29	100	100	100	100	100
RO-8.39*	8.39	100	100	100	100	100

Test Group	Test Conc.	Cumulative Percent Mortality (%)[1] Third Application Day 28				
	(µg SR/L)	Day 29	Day 30	Day 32	Day 35	Day 42
RO-CTRL-1	0	2	2	3	5	8
RO-CTRL-2	0	0	0	0	0	0
RO-CTRL-3	0	0	0	0	0	0
RO-CTRL-4	0	5	5	5	5	5
RO-0.21***	0.21	3	3	3	3	3
RO-0.42***	0.42	0	0	0	0	3
RO-0.84***	0.84	8	8	9	9	13
RO-2.09***	2.09	3	3	3	3	7
RO-4.19***	3.99	100	100	100	100	100
RO-6.29**	6.29	100	100	100	100	100
RO-8.39*	8.39	100	100	100	100	100

Test Conc.: Average water concentration per treatment based on total radioactivity applied to the enclosures
[1] The numbers were corrected for the fish taken for residue analysis.
CTRL: Control
RO: Run-off
SR: Soil Residue *, **, ***: one, two or three treatments

Table 46. LC_{50} values and 95% confidence intervals for the spray drift entry route based on average water concentrations per treatment based on total radioactivity applied to the enclosures.

First Application

Day	1	2	3	4	7	13
LC_{50} (µg/L)	**5.19**	**4.09**	**3.48**	**3.40**	**3.40**	**3.34**
95% conf. interval low	3.50	3.50	3.10	3.02	3.02	2.96
95% conf. interval high	5.83	5.83	3.90	3.82	3.82	3.76
test used	B.T.	B.T.	M.A.	M.A.	M.A.	M.A.

Second Application

Day	15	16	17	18	21	27
LC_{50} (µg/L)	**2.96**	**2.94**	**2.68**	**2.60**	**2.57**	**2.47**
95% conf. interval low	2.70	2.67	2.40	2.32	2.30	2.20
95% conf. interval high	3.26	3.25	3.04	2.94	2.91	2.79
test used	M.A.	M.A.	M.A.	M.A.	M.A.	M.A.

Third Application

Day	29	30	31	32	35	41
LC_{50} (µg/L)	**2.39**	**2.39**	**2.30**	**2.02**	**1.93**	**1.86**
95% conf. interval low	2.13	2.13	2.04	1.76	1.68	1.61
95% conf. interval high	2.71	2.71	2.62	2.33	2.22	2.15
test used	M.A.	M.A.	M.A.	M.A.	M.A.	M.A.

B.T. Binomial Test
M.A. Moving Average

Table 47. LC_{50} values and 95% confidence intervals for the run-off entry route based on average water concentrations per treatment based on total radioactivity applied to the enclosures.

First Application

Days	1	2	3	4	7	13
LC_{50} (µg/L)	**>8.39**	**4.04**	**3.69**	**3.39**	**3.17**	**2.96**
95% conf. interval low		2.09	2.09	2.09	2.09	2.09
95% conf. interval high		6.29	4.19	4.19	4.19	4.19
test used		B.T.	B.T.	B.T.	B.T.	B.T.

Second Application

Days	15	16	17	18	21	27
LC_{50} (µg/L)	**2.96**	**2.96**	**2.88**	**2.88**	**2.87**	**2.86**
95% conf. interval low	2.09	2.09	2.09	2.09	2.09	2.09
95% conf. interval high	4.19	4.19	4.19	4.19	4.19	4.19
test used	B.T.	B.T.	B.T.	B.T.	B.T.	B.T.

Third Application

Days	29	30	31	32	35	42
LC_{50} (µg/L)	**2.86**	**2.86**	**2.86**	**2.86**	**2.85**	**2.79**
95% conf. interval low	2.09	2.09	2.09	2.09	2.09	2.09
95% conf. interval high	4.19	4.19	4.19	4.19	4.19	4.19
test used	B.T.	B.T.	B.T.	B.T.	B.T.	BT

B.T. Binomial Test

Table 48. Mean weight and length of fish at test termination. The average weight at study initation was 0.87 g, the average length was 36 mm.

A. Spray drift

Test Group	Test Conc.	Number of Fish	Mean Weight ± S.D.	Number of Fish	Mean Length ± S.D.
	[µg ai/L]	-	[g]	-	[mm]
SD-CTRL-1	0	33	1.44 ± 0.28	37	49 ± 4
SD-CTRL-2	0	36	1.57 ± 0.50	37	49 ± 5
SD-CTRL-3	0	33	1.67 ± 0.48	35	50 ± 6
SD-CTRL-4	0	35	1.47 ± 0.44	37	47 ± 5
SD-0.27***	0.34	31	1.57 ± 0.37	31	48 ± 4
SD-0.47***	0.55	28	1.32 ± 0.40	28	46 ± 5
SD-0.84***	1.16	27	1.29 ± 0.34	27	46 ± 4
SD-1.51***	1.96	21	1.21 ± 0.21	23	46 ± 4

Note: No significant differences were found between the control and treated test groups for weight and length (ANOVA, p>0.05)

B. Run-off

Test Group	Test Conc.	Number of Fish	Mean Weight ± S.D.[a]	Number of Fish	Mean Length ± S.D.
	[µg SR/L]	-	[g]	-	[mm]
RO-CTRL-1	0	34	1.46 ± 0.43	35	48 ± 5
RO-CTRL-2	0	38	1.47 ± 0.40	38	48 ± 4
RO-CTRL-3	0	35	1.36 ± 0.36	38	47 ± 6
RO-CTRL-4	0	36	1.48 ± 0.53	36	47 ± 6
RO-0.21***	0.21	30	1.38 ± 0.43	30	47 ± 5
RO-0.42***	0.42	25	1.25 ± 0.30	29	46 ± 5
RO-0.84***	0.84	25	1.24 ± 0.29	26	46 ± 4
RO-2.09***	2.09	27	1.26 ± 0.26	29	47 ± 4

Note: No significant differences were found between the control and treated test groups for weight and length (ANOVA, p>0.05)

Test Conc.: Average water concentration per treatment based on total radioactivity applied to the enclosures
SR: Soil residue
SD: Spray drift
RO: Run-off
CTRL: Control
***: three treatments

Table 49. Summary of effects of endosulfan on fish. The values are given as average water concentrations per treatment based on total radioactivity applied to the enclosures.

A. Mortality

Test System	NOEC (Day 7-42)	LOEC (Day 7-42)	LC_{50} (Day 7-42)
	[µg ai/L]	[µg ai/L]	[µg ai/L]
Spray drift Entry Route	1.96***	3.50*** 3.50** 3.50*	1.86-3.40
	[µg SR/L]	[µg SR/L]	[µg SR/L]
Run-off Entry route	2.09***	3.99*** 3.99** 3.99*	2.79-3.17

SR: Soil Residue

Note: Due to the data distribution and the very steep dose response curve, the calculated LC_{50} is below the experimentally determined NOEC.

B. Sublethal effects

Test System	NOEC (Day 7-42)
	[µg ai/L]
Spray drift Entry Route	1.96***
	[µg SR/L]
Run-off Entry route	2.09***

SR Soil Residue

*** Triplicate treatment at 14 day intervals

** Duplicate treatment at 14 day intervals

* Single treatment at test inititation

Table 50. Spray drift total radioactivity in fish, expressed as endosulfan equivalents. The values are given in mg peq/kg fresh fish.

	Concentration of Endosulfan equivalent in Fish (mg peq/kg)				
Test Day	Test Conc. 0.34 µg/L***	Test Conc. 0.55 µg/L***	Test Conc. 1.16 µg/L***	Test Conc. 1.96 µg/L***	Test Conc. 3.50 µg/L***
	SD-0.27	SD-0.47	SD-0.84	SD-1.51	SD-2.68
Application 1 (day 0)					
3	0.109	0.244	0.455	0.931	3.053
7	0.052	0.125	0.225	0.605	1.806
13	0.040	0.086	0.169	0.347	0.849
Application 2 (day 14)					
16	-[a]	0.619	0.789	1.808	3.960
17	0.212	0.580	0.636	2.228	1.883
21	0.109	0.190	0.356	0.837	1.579
27	-[b]	0.116	0.204	0.535	0.926
Application 3 (day 28)					
30	0.414	0.557	0.943	1.056	1.329
31	0.358	0.534	0.450	1.071	1.063
35	0.237	0.296	0.603	0.895	-[b]

[a] A concentration of 2.510 mg/kg was found for these samples, which most likely not reflects a fish of the 0.34 µg/L test concentration. Therefore it was omitted from the table.

[b] No value is available for this test concentration at this sampling interval.

Test Conc.: Average water concentration per treatment based on total radioactivity applied to the enclosures

SD Spray drift

***: three treatments

Table 51. Run-off total radioactivity in fish, expressed as endosulfan equivalents. The values are given in mg peq/kg fresh fish.

	Concentration of Endosulfan equivalent in Fish (mg peq/kg)				
Test Day	Test Conc. 0.21 µg/L***	Test Conc. 0.42 µg/L***	Test Conc. 0.84 µg/L***	Test Conc. 2.09 µg/L***	Test Conc. 3.99 µg/L***
	RO-0.21	RO-0.42	RO-0.84	RO-2.09	RO-4.19
Application 1 (day 0)					
4	0.192	0.330	0.676	1.535	3.307
7	0.111	0.146	0.331	0.756	2.236
12	0.067	0.083	0.239	0.470	0.999
Application 2 (day 14)					
16	0.212	0.290	0.833	1.284	2.857
18	0.328	0.335	0.818	1.534	3.463
21	0.158	0.257	0.650	0.629	-[b]
26	-[a]	0.149	0.318	0.692	-[b]
Application 3 (day 28)					
30	0.149	0.325	0.518	1.017	-[b]
32	0.197	0.441	0.480	1.022	-[b]
35	-[a]	0.308	0.409	0.973	-[b]

[a] No value is available for this test concentration at this sampling interval.

[b] No value available since all fish had died by day 18 of the test in this test concentration.

Test Conc.: Average water concentration per treatment based on total radioactivity applied to the enclosures

RO Run-off

***: three treatments

Table 52. Characterization of residual radioactivity in *Lepomis macrochirus* (Bluegill sunfish) at maximum residue levels after triplicate treatment (spray drift test group SD-1.51 and run-off test group RO-2.09).

A. Spray drift.

Total Radioactive Residue (TRR)	2.228 mg peq/kg fresh weight	100 %	1.056 mg peq/kg fresh weight	100 %
Test Day	17		30	
Identity	[mg peq/kg]	[%]	[mg peq/kg]	[%]
M1	0.187	8.4	0.136	12.9
M5	0.552	24.8	0.173	16.3
Endosulfandiol	0.039	1.8	0.029	2.7
endosulfan hydroxy ether	0.028	1.3	0.028	2.6
Endosulfansulfat	0.913	41.0	0.518	49.0
β-Endosulfan	*0.188*	*8.4*	*0.083*	*7.9*
α-Endosulfan	*0.107*	*4.8*	*0.047*	*4.5*
α/β-Endosulfan	0.295	13.3	0.130	12.3
Minor components (#)	0.214 (15)	9.6 (15)	0.043 (7)	4.1 (7)

B. Run-off.

Total Radioactive Residue (TRR)	1.534 mg peq/kg fresh weight	100 %	1.017 mg peq/kg fresh weight	100 %
Test Day	18		30	
Identity	[mg peq/kg]	[%]	[mg peq/kg]	[%]
M1	0.188	12.3	0.158	15.5
M5	0.325	21.2	0.273	26.8
Endosulfandiol	0.016	1.0	0.022	2.2
endosulfan hydroxy ether	0.060	3.9	0.039	3.9
Endosulfansulfat	0.727	47.4	0.395	38.8
β-Endosulfan	*0.112*	*7.3*	*0.036*	*3.5*
α-Endosulfan	*0.065*	*4.3*	*0.044*	*4.3*
α/β-Endosulfan	0.177	11.6	0.080	7.9
Minor components (#)	0.040 (5)	2.6 (5)	0.050 (9)	4.9 (9)

Test Conc.: Average water concentration per treatment based on total radioactivity applied to the enclosures
peq parent equivalents
M1, M5 Unknown components

Table 53. Quantification of residual radioactivity in *Lepomis macrochirus* (Bluegill sunfish) which died within one and 2 days after the 1st treatment.

A: Spray drift

Test Group	SD-4.69	SD-8.38
Test Conc.	6.4 µg ai/L**	10.33 µg ai/L*
Day 1 post 1st Treatment	[mg peq/kg]	[mg peq/kg]
Sample 1	2.185 RC	4.370
Sample 2	2.175	4.064 RC
Sample 3	2.452 RC	4.566
Sample 4	2.042	4.180 RC
Sample 5	-	3.923
Average	**2.214**	**4.220**
Day 2 post 1st Treatment	[mg peq/kg]	[mg peq/kg]
Sample 1	3.583	4.082
Sample 2	3.335	4.110
Sample 3	3.474	4.956
Sample 4	3.605	4.091
Sample 5	-	4.810
Average	**3.499**	**4.410**

B: Run-off

Test Group	RO-6.29	RO-8.39
Test Conc.	6.29 µg SR/L**	8.39 µg SR/L*
Day 1 post 1st Treatment	[mg peq/kg]	[mg peq/kg]
Sample 1	NP	3.994
Sample 2	NP	4.119
Sample 3	NP	3.946
Sample 4	NP	-
Sample 5	NP	-
Average	**NP**	**4.020**
Day 2 post 1st Treatment	[mg peq/kg]	[mg peq/kg]
Sample 1	2.669	3.700
Sample 2	3.177	3.950
Sample 3	3.328	3.692
Sample 4	3.930	4.135
Sample 5	3.368	4.025
Average	**3.294**	**3.900**

Test Conc.: Average water concentration per treatment based on total radioactivity applied to the enclosures
RC; Residue characterization was performed (cf. Table 54)
SR: Soil Residue
NP: Not performed
*, **: one or two treatments

Table 54. Spray drift: Characterization of residual radioactivity in *Lepomis macrochirus* (bluegill Sunfish) which died within one day post 1st treatment.

A. Test group SD-4.69

Total Radioactive Residue (TRR)	2.185 mg peq/kg fresh weight	100 %	2.452 mg peq/kg fresh weight	100 %
Test Day	1		1	
Identity	[mg/kg]	[%]	[mg/kg]	[%]
M1	0.094	4.3	0.106	4.3
M5	0.083	3.8	0.082	3.3
Endosulfan diol	0.033	1.5	0.028	1.2
Endosulfan hydroxy ether	0.007	0.3	ND	ND
Endosulfan sulfate	0.646	29.6	0.742	30.3
β-Endosulfan	*0.550*	*25.2*	*0.646*	*26.3*
α-Endosulfan	*0.746*	*34.2*	*0.837*	*34.1*
α/β-Endosulfan	1.296	59.3	1.482	60.5
Minor components (#)	0.026 (4)	1.2 (4)	0.011 (1)	0.5 (1)

B. Test group SD-8.38

Total Radioactive Residue (TRR)	4.064 mg peq/kg fresh weight	100 %	4.180 mg peq/kg fresh weight	100 %
Test Day	1		1	
Identity	[mg/kg]	[%]	[mg/kg]	[%]
M1	0.135	3.3	0.078	1.9
M5	0.104	2.6	0.071	1.7
Endosulfan diol	0.084	2.1	0.109	2.6
Endosulfan hydroxy ether	0.020	0.5	0.023	0.5
Endosulfan sulfate	1.189	29.3	1.001	24.0
β-Endosulfan	*1.062*	*26.1*	*1.159*	*27.7*
α-Endosulfan	*1.410*	*34.7*	*1.695*	*40.6*
α/β-Endosulfan	2.472	60.8	2.854	68.3
Minor components (#)	0.060 (7)	1.5 (7)	0.044 (3)	1.1 (3)

ND: Not detected
peq: Parent equivalents
M1, M5: Unknown components

Table 55. Pattern of radioactive components, which were detected in the various compartments of the ecosystem.

Identity	Water	Fish	Macrophyte	Sediment
M1	X	X	ND	X
M5	ND	X	ND	ND
Endosulfan diol	**X**	**X**	**X**	**X**
Endosulfan hydroxy ether	X	X	X	X
Endosulfan lactone	X	ND	ND	X
M4	X	ND	ND	X
Endosulfan sulfate	**X**	**X**	**X**	**X**
β-Endosulfan	X	X	X	X
α-Endosulfan	X	X	X	X
α/β-Endosulfan	**X**	**X**	**X**	**X**
M6	ND	ND	X	ND
M7	ND	ND	X	ND
M8	ND	ND	X	ND
M9	ND	ND	X	ND
Minor components (#)	X	X	X	X

ND: Not detected
M1, M4, M5 and M6 to M9 are unknown degradates of the test item.

Table 56. **Spray drift test termination: TRR of endosulfan in water, overlaying water and the sediment including sediment-water interphase material.**

A. Test groupsSD-0.27 to SD-2.68, which had been treated 3 times at intervals of 14 days

Test group Test Conc.	SD-0.27*** 0.34 µg ai/L	SD-0.47*** 0.55 µg ai/L	SD-0.84*** 1.16 µg ai/L	SD-1.51*** 1.96 µg ai/L	SD-2.68*** 3.50 µg ai/L
Depth integrated water [µg peq/L]	0.45	0.87	1.57	3.26	6.41
Overlaying Water Sediment/Water Interphase water (µg peq/L)	0.25	0.59	1.01	1.81	3.21
Pore Water 0-1 cm [µg peq/L]	NA	0.40	0.18	0.82	2.61
Pore Water 1-5 cm [µg peq/L]	0.03	0.08	0.14	0.16	0.61
Pore Water > 5 cm [µg peq/L]	< 0.01	0.01	0.03	0.04	0.10
Sediment Layer 0-1 cm [µg peq/kg]	1.98	4.46	5.81	17.24	30.42
Sediment Layer 1-5 cm [µg peq/kg]	0.57	0.39	0.53	0.98	0.48
Sediment Layer > 5 cm [µg peq/kg]	0.01	0.12	0.30	0.59	0.43
SDO [µg peq/kg sed], 0-1 cm	0.12	0.07	0.11	0.25	1.20
SDO [µg peq/kg sed], 1-5 cm	0.02	0.08	0.04	0.11	0.05
SDO [µg peq/kg sed], > 5 cm	ND	ND	ND	ND	ND

B. Test groups SD-4.69 and SD-8.38, which had been treated twice and once, respectively.

Test group Test Conc.	*SD-4.69*** 6.40 µg ai/L	*SD-8.38** 10.33 µg ai/L
Depth integrated water [µg peq/L]	6.92	5.32
Overlaying Water Sediment/Water Interphase water (µg peq/L)	3.09	2.63
Pore Water 0-1 cm [µg peq/L]	4.04	4.09
Pore Water 1-5 cm [µg peq/L]	1.17	1.48
Pore Water > 5 cm [µg peq/L]	0.14	0.19
Sediment Layer 0-1 cm [µg peq/kg]	10.63	23.97
Sediment Layer 1-5 cm [µg peq/kg]	2.98	7.68
Sediment Layer > 5 cm [µg peq/kg]	0.25	0.61
SDO [µg peq/kg sed], 0-1 cm	0.38	0.61
SDO [µg peq/kg sed], 1-5 cm	0.21	0.30
SDO [µg peq/kg sed], > 5 cm	ND	ND

Test Conc.: Average water concentration per treatment based on total radioactivity applied to the enclosures
*, **, ***: one, two or three treatments
ND: Not determined
peq: Parent equivalents
SDO: Sediment Dwelling Organisms
SD: Spray drift

Table 57. Run-off test termination: TRR of endosulfan in water, overlaying water and the sediment including sediment-water interphase material.

A. Test Groups RO-0.21 to RO-4.19, which had been treated 3 times at intervals of 14 days

Test group Test Conc.	RO-0.21*** 0.21 µg SR/L	RO-0.42*** 0.42 µg SR/L	RO-0.84*** 0.84 µg SR/L	RO-2.09*** 2.09 µg SR/L	RO-4.19*** 3.99 µg SR/L
Depth integrated water [µg peq/L]	0.33	0.59	1.15	3.92	7.68
Overlaying Water Sediment/Water Interphase water (µg peq/L)	0.21	0.42	0.79	2.36	4.16
Pore Water 0-1 cm [µg peq/L]	0.13	0.22	0.11	0.68	1.53
Pore Water 1-5 cm [µg peq/L]	0.11	0.05	0.11	0.15	0.34
Pore Water > 5 cm [µg peq/L]	0.00	0.01	0.01	0.02	0.10
Sediment Layer 0-1 cm [µg peq/kg]	2.31	5.32	7.40	30.43	64.60
Sediment Layer 1-5 cm [µg peq/kg]	0.11	0.11	0.46	2.25	2.15
Sediment Layer > 5 cm [µg peq/kg]	0.05	0.11	0.31	0.48	0.68
SDO [µg peq/kg sed], 0-1 cm	0.13	0.24	0.16	0.13	0.76
SDO [µg peq/kg sed], 1-5 cm	0.02	0.07	0.03	0.03	0.20
SDO [µg peq/kg sed], > 5 cm	ND	ND	ND	ND	ND

B. Test groups RO-6.29 and RO-8.39, which had been treated twice and once, respectively.

Test group Test Conc.	RO-6.29** 6.29 µg SR/L	RO-8.39* 8.39 µg SR/L
Depth integrated water [µg peq/L]	6.85	8.92
Overlaying Water Sediment/Water Interphase water (µg/L)	4.14	3.38
Pore Water 0-1 cm [µg/L]	3.13	4.18
Pore Water 1-5 cm [µg/L]	1.04	1.38
Pore Water > 5 cm [µg/L]	0.17	0.28
Sediment Layer 0-1 cm [µg/kg]	27.26	46.88
Sediment Layer 1-5 cm [µg/kg]	2.25	4.17
Sediment Layer > 5 cm [µg/kg]	2.21	4.29
SDO [µg/kg sed], 0-1 cm	0.34	1.58
SDO [µg/kg sed], 1-5 cm	0.05	0.31
SDO [µg/kg sed], >5 cm	ND	ND

Test Conc.: Average water concentration per treatment based on total radioactivity applied to the enclosures
*, **, ***: one, two or three treatments
SR: Soil residue (after 1 day ageing)
ND: *Not determined*
peq: Parent equivalents
SDO: Sediment Dwelling Organisms RO: Run-off

Table 58. Test groups SD-2.68 and RO-4.19: Characterization of residual radioactivity in the top centimeter of the sediment.

A. Spray drift.

Total Radioactive Residue (TRR)	30.4 µg peq/kg sediment* (Day 42, Test End)	100 %
Identity	[µg peq/kg]	[%]
M1	1.0	3.4
M5	1.0	3.4
Endosulfan diol	8.9	29.3
Endosulfan hydroxy ether	3.1	10.3
Endosulfan lactone	1.3	4.3
M4	1.6	5.2
Endosulfan sulfate	6.3	20.7
β-Endosulfan	*1.0*	*3.4*
α-Endosulfan	*3.1*	*10.3*
Endosulfan (α+β)	4.1	13.7
Minor components (#)	2.8 (3)	9.4 (3)

*after centrifugation to remove the pore water

B: Run-off:

Total Radioactive Residue (TRR)	64.6 µg peq/kg sediment* (Day 43, Test End)	100 %
Identity	[µg peq/kg]	[%]
M1	1.0	1.6
M5	ND	ND
Endosulfan diol	*7.0*	*10.8*
Endosulfan hydroxy ether	4.1	6.3
Endosulfan lactone	ND	ND
M4	1.6	2.5
Endosulfan sulfate	8.6	13.3
β-Endosulfan	*14.3*	*22.2*
α-Endosulfan	*20.7*	*32.0*
Endosulfan I + II	35.0	54.2
Minor components (#)	7.1 (7)	11.3 (7)

*after centrifugation to remove the pore water

Test Conc.: Average water concentration per treatment based on total radioactivity applied to the enclosures
peq: Parent equivalents
M1, M4, M5: Unknown components
SR: Soil residue
ND: Not determined

Table 59. Spray drift: Abundance of Sediment-Dwelling Organisms at test end.

A. 0-1 cm

Test Conc. [µg ai/L]	Test Group	Oligochaeta (Detritivorous)	Chironomidae (Detritivorous)	Total DV	Chironomidae (Predatory)	Total SDO
0	Lake	52	0	52	0	52
0	Basin	18	17	35	25	60
0	SD-CTRL-1	38	15	53	7	60
0	SD-CTRL-2	11	0	11	6	17
0	SD-CTRL-3	5	4	9	22	31
0	SD-CTRL-4	10	2	12	26	38
0.34	SD-0.27***	37	1	38	18	56
0.55	SD-0.47***	15	2	17	14	31
1.16	SD-0.84***	4	3	7	18	25
1.96	SD-1.51***	9	1	10	5	15
3.50	SD-2.68***	0	0	0	27	27
6.40	SD-4.69**	24	0	24	30	54
10.33	SD-8.38*	22	1	23	28	51

B: 1-5 cm

Test Conc. [µg ai/L]	Test Group	Oligochaeta (Detritivorous)	Chironomidae (Detritivorous)	Total DV	Chironomidae (Predatory)	Total SDO
0	Basin	26	3	29	0	29
0	Lake	16	17	33	23	56
0	SD-CTRL-1	7	4	11	2	13
0	SD-CTRL-2	1	0	1	0	1
0	SD-CTRL-3	3	1	4	1	5
0	SD-CTRL-4	3	1	4	1	5
0.34	SD-0.27***	3	0	3	0	3
0.55	SD-0.47***	4	0	4	1	5
1.16	SD-0.84***	6	1	7	0	7
1.96	SD-1.51***	7	0	7	0	7
3.50	SD-2.68***	2	0	2	2	4
6.40	SD-4.69**	4	0	4	1	5
10.33	SD-8.38*	1	0	1	2	3

C: Total 0 to 5 cm

Test Conc. [µg ai/L]	Test Group	Oligochaeta (Detritivorous)	Chironomidae (Detritivorous)	Total DV	Chironomidae (Predatory)	Total SDO
0	Basin	78	3	81	0	81
0	Lake	34	34	68	48	116
0	SD-CTRL-1	45	19	64	9	73
0	SD-CTRL-2	12	0	12	6	18
0	SD-CTRL-3	8	5	13	23	36
0	SD-CTRL-4	13	3	16	27	43
0.34	SD-0.27***	40	1	41	18	59
0.55	SD-0.47***	19	2	21	15	36
1.16	SD-0.84***	10	4	14	18	32
1.96	SD-1.51***	16	1	17	5	22
3.50	SD-2.68***	2	0	2	29	31
6.40	SD-4.69**	28	0	28	31	59
10.33	SD-8.38*	23	1	24	30	54

Test Conc.: Average water concentration per treatment based on total radioactivity applied to the enclosures

*, **, ***: one, two or three treatments SD: Spray drift

SDO: Sediment dwelling organisms DV: Detritivorous

Table 60. Run-off: Abundance of Sediment-Dwelling Organisms at test end.

A. 0-1 cm

Test Conc. [µg SR/L]	Test Group	Oligochaeta (Detritivorous)	Chironomidae (Detritivorous)	Total DV	Chironomidae (Predatory)	Total SDO
0	Lake	52	0	52	0	52
0	Basin	18	17	35	25	60
0	Control 1	17	3	20	12	31
0	Control 2	8	0	8	12	20
0	Control 3	7	2	9	9	18
0	Control 4	10	0	10	14	24
0.21	RO-0.21***	31	10	41	0	41
0.42	RO-0.42***	23	0	23	3	26
0.84	RO-0.84***	22	0	22	9	31
2.09	RO-2.09***	9	0	9	12	21
3.99	RO-4.19***	26	0	26	3	29
6.29	RO-6.29**	20	1	21	6	27
8.39	RO-8.39*	41	0	41	6	47

B: 1-5 cm

Test Conc. [µg SR/L]	Test Group	Oligochaeta (Detritivorous)	Chironomidae (Detritivorous)	Total DV	Chironomidae (Predatory)	Total SDO
0	Lake	26	3	29	0	29
0	Basin	16	17	33	23	56
0	Control 1	3	0	3	2	5
0	Control 2	8	3	11	1	12
0	Control 3	3	0	3	0	3
0	Control 4	3	0	3	0	3
0.21	RO-0.21***	2	1	3	0	3
0.42	RO-0.42***	14	0	14	0	14
0.84	RO-0.84***	8	0	8	0	8
2.09	RO-2.09***	2	0	2	0	2
3.99	RO-4.19***	12	0	12	0	12
6.29	RO-6.29**	2	0	2	0	2
8.39	RO-8.39*	7	0	7	0	7

C: Total 0 to 5 cm

Test Conc. [µg SR/L]	*Test Group*	*Oligochaeta* (Detritivorous)	*Chironomidae* (Detritivorous)	*Total DV*	*Chironomidae* (Predatory)	*Total SDO*
0	Lake	78	3	81	0	81
0	Basin	34	34	68	48	116
0	Control 1	20	3	23	14	37
0	Control 2	16	3	19	13	32
0	Control 3	10	2	12	9	21
0	Control 4	13	0	13	14	27
0.21	RO-0.21***	33	11	44	0	44
0.42	RO-0.42***	37	0	37	3	40
0.84	RO-0.84***	30	0	30	9	39
2.09	RO-2.09***	11	0	11	12	23
3.99	RO-4.19***	38	0	38	3	41
6.29	RO-6.29**	22	1	23	6	29
8.39	RO-8.39*	48	0	48	6	54

Test Conc.: Average water concentration per treatment based on total radioactivity applied to the enclosures
*, **, ***: one, two or three treatments
SR: Soil residue (after 1 day ageing) RO: Run-off
SDO: Sediment dwelling organisms DV: Detritivorous

Table 61. Toxicity to Sediment Dwelling Organisms at test termination (6 Weeks of Exposure). The values are given as average water concentrations per treatment based on total radioactivity applied to the enclosures.

A. Triplicate treatment at 14 day intervals

Test System	NOEC (Day 42)	LOEC (Day 42)	EC_5 (Day 42)	EC_{10} (Day 42)	EC_{50} (Day 42)
	[µg ai/L]	[µg ai/L]	[µg ai/L]	[µg ai/L]	[µg ai/L]
Spray drift entry route	3.50[1]	> 3.50	> 3.50	> 3.50	> 3.50
	[µg SR/L]	[µg SR/L]	[µg SR/L]	[µg SR/L]	[µg SR/L]
Run-off entry route	3.99[1]	> 3.99	> 3.99	> 3.99	> 3.99

SR: Soil Residue
[1] Highest individual treatment rate for this series of test groups

B. Duplicate treatment at 14 day intervals.

Test System	NOEC (Day 42)	LOEC (Day 42)	EC_5 (Day 42)	EC_{10} (Day 42)	EC_{50} (Day 42)
	[µg ai/L]	[µg ai/L]	[µg ai/L]	[µg ai/L]	[µg ai/L]
Spray drift entry route	6.40[1]	> 6.40	> 6.40	> 6.40	> 6.40
	[µg SR/L]	[µg SR/L]	[µg SR/L]	[µg SR/L]	[µg SR/L]
Run-off entry route	6.29[1]	> 6.29	> 6.29	> 6.29	> 6.29

SR: Soil Residue
[1] Highest individual treatment rate

C. Single treatment at test initiation.

Test System	NOEC (Day 42)	LOEC (Day 42)	EC_5 (Day 42)	EC_{10} (Day 42)	EC_{50} (Day 42)
	[µg ai/L]	[µg ai/L]	[µg ai/L]	[µg ai/L]	[µg ai/L]
Spray drift entry route	10.33[1]	> 10.33	> 10.33	> 10.33	> 10.33
	[µg SR/L]	[µg SR/L]	[µg SR/L]	[µg SR/L]	[µg SR/L]
Run-off entry route	8.39[1]	> 8.39	> 8.39	> 8.39	> 8.39

SR: Soil Residue
[1] Highest individual treatment rate

9. FIGURES

Figure 1. **Schematic drawing of the distribution and volume classification of *Myriophyllum spicatum* prior to application. The percentage represents the volume occupied by *Myriophyllum* with respect to a total enclosure volume of 1 m^3.**

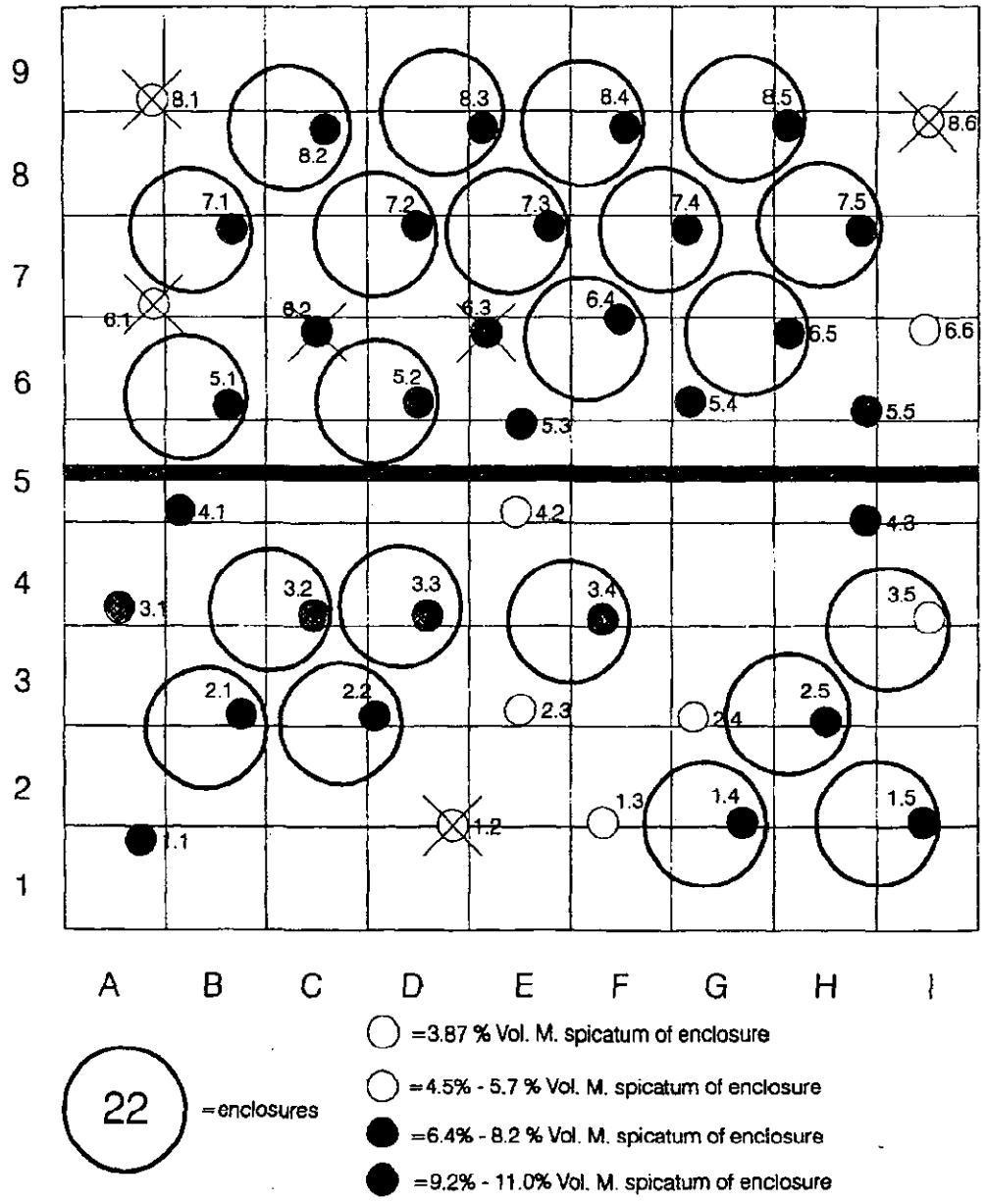


Figure 2. **Schematic drawing of an enclosure exposed to the testing basin of Springborn's outdoor microcosm testing facility.**

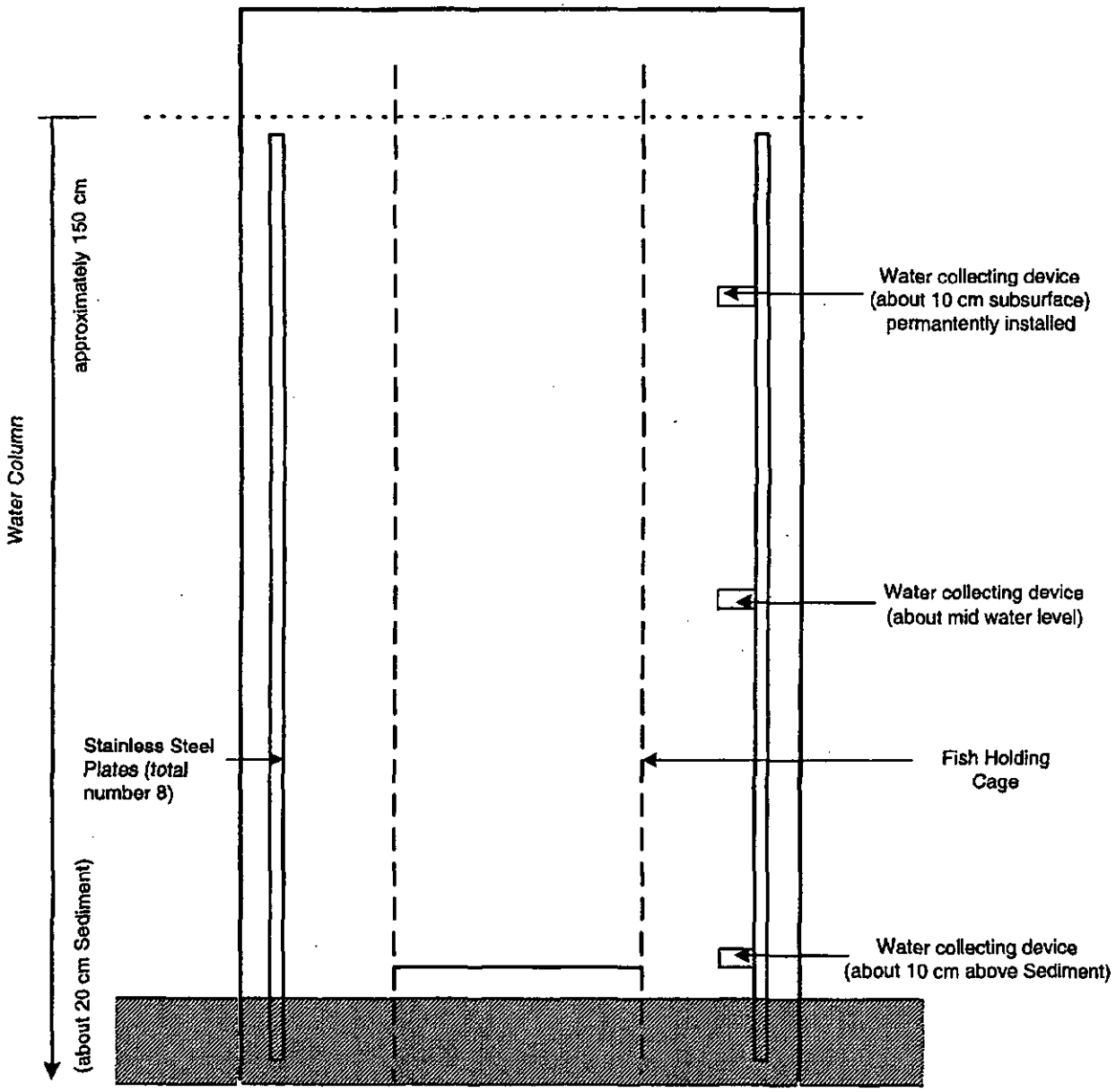


Figure 3. **Numbering, designation and labelling of each individual enclosure after randomization. SD: Spray drift entry route, RO: Run-off entry route. CONC: Concentration, CONT: Control. The number in brackets *and the colour code corresponds to Figure 1.***

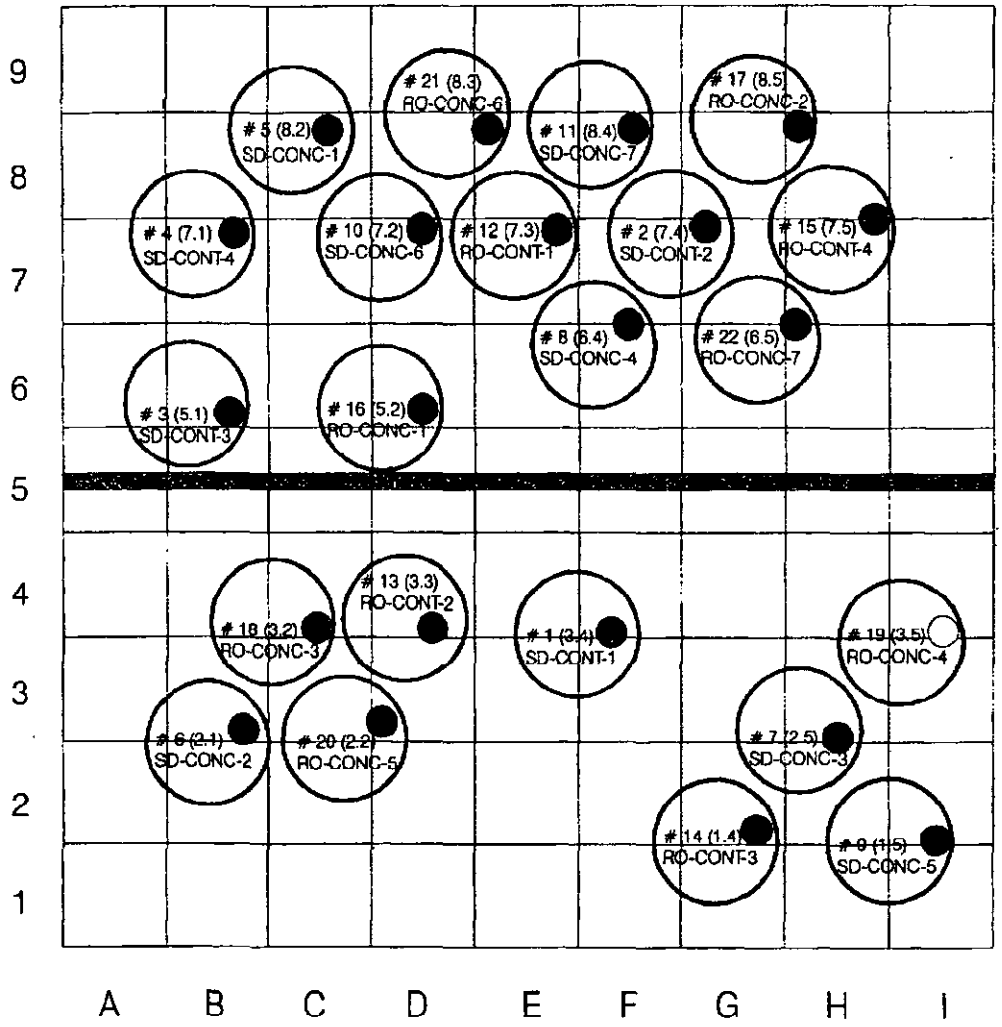


Figure 4. Analytical methods for water.

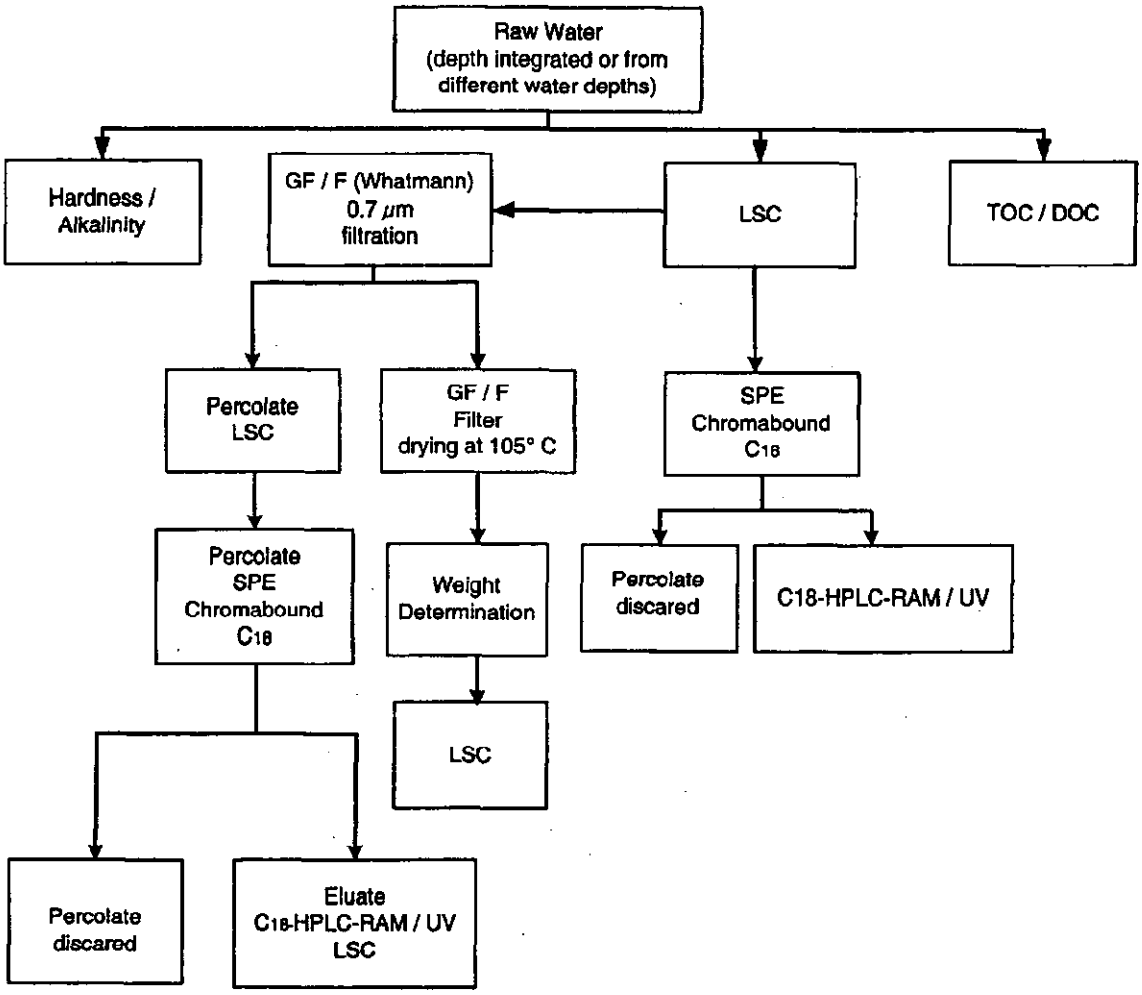


Figure 5. Analytical methods for sediment.

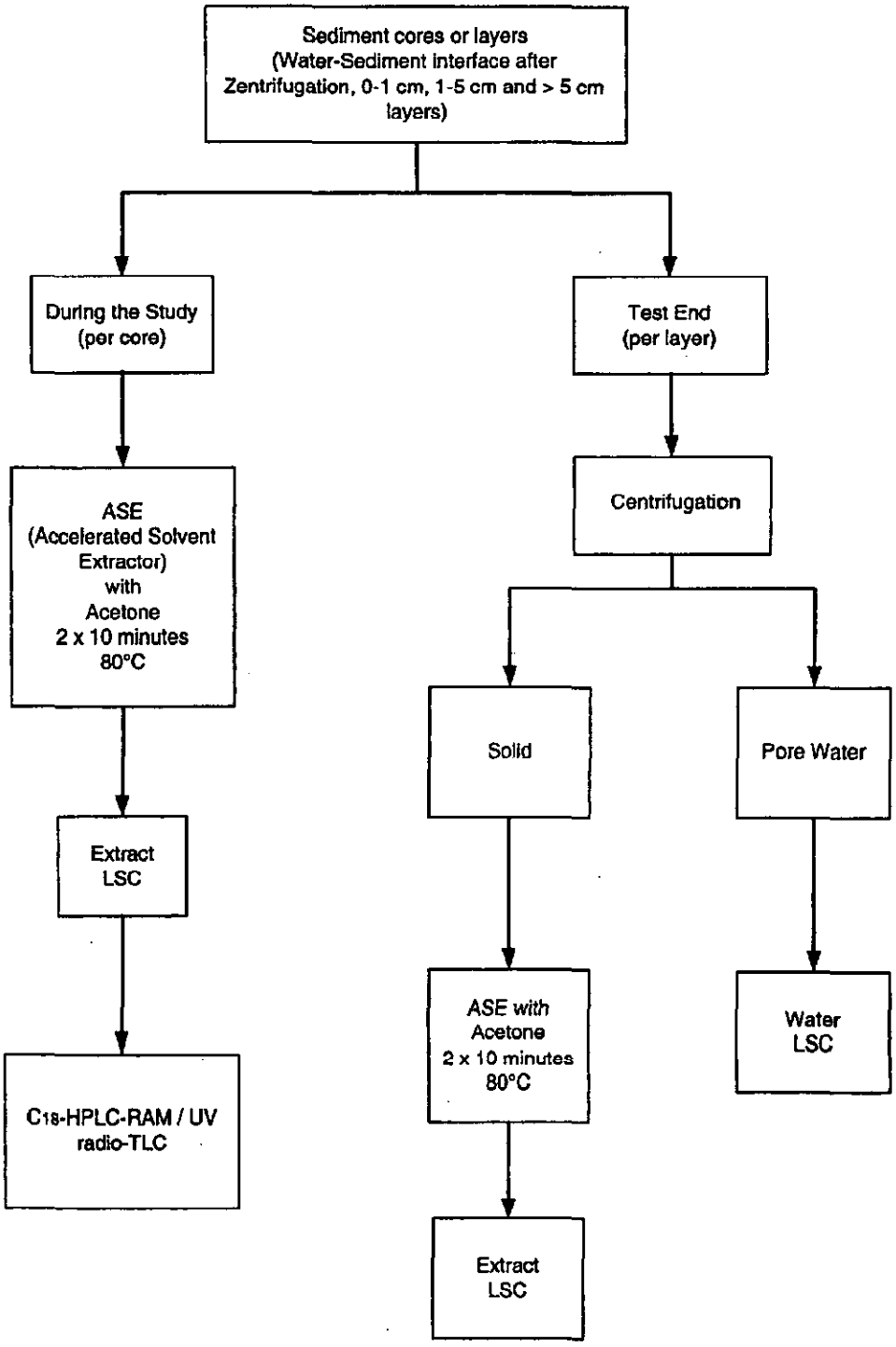


Figure 6. Analytical methods for macrophytes and periphyton (tank wall residue).

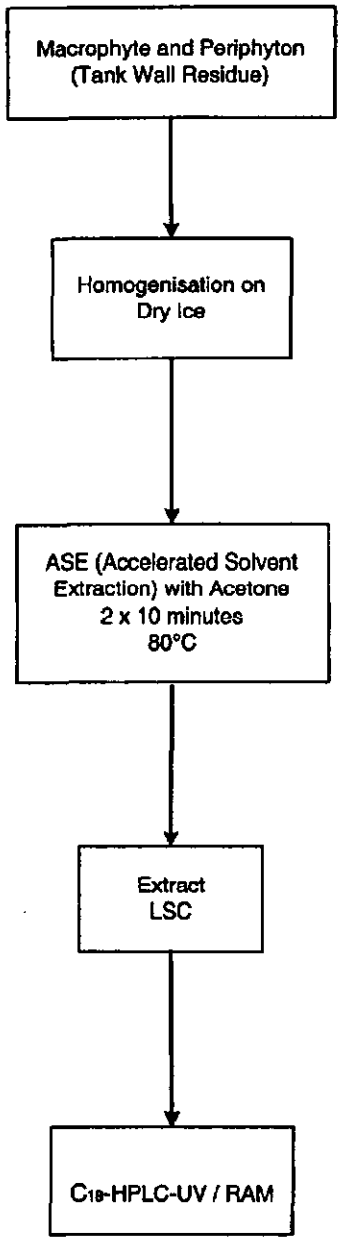


Figure 7. Analytical methods for fish and sediment-dwelling organisms.

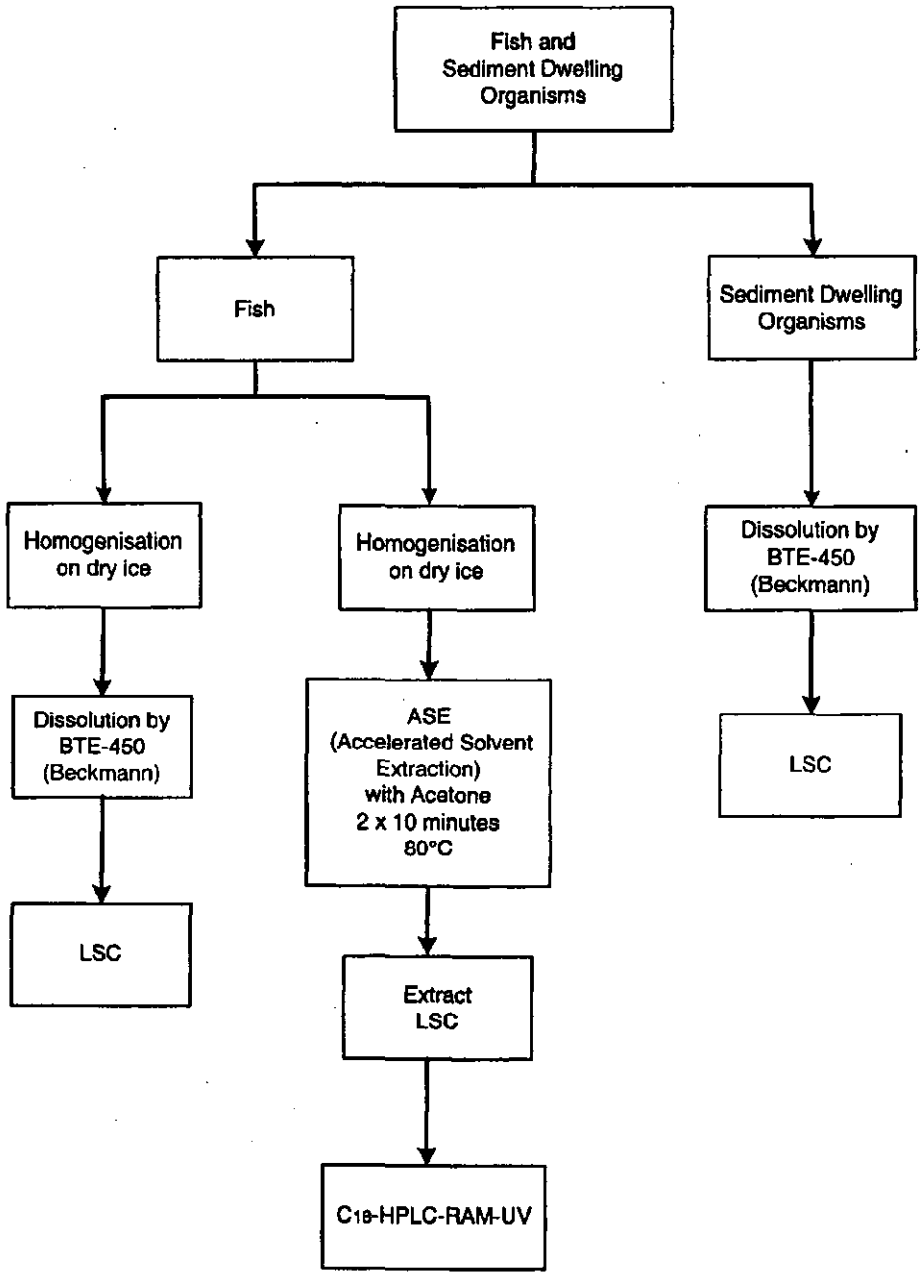


Figure 8. Test basin during equilibration.

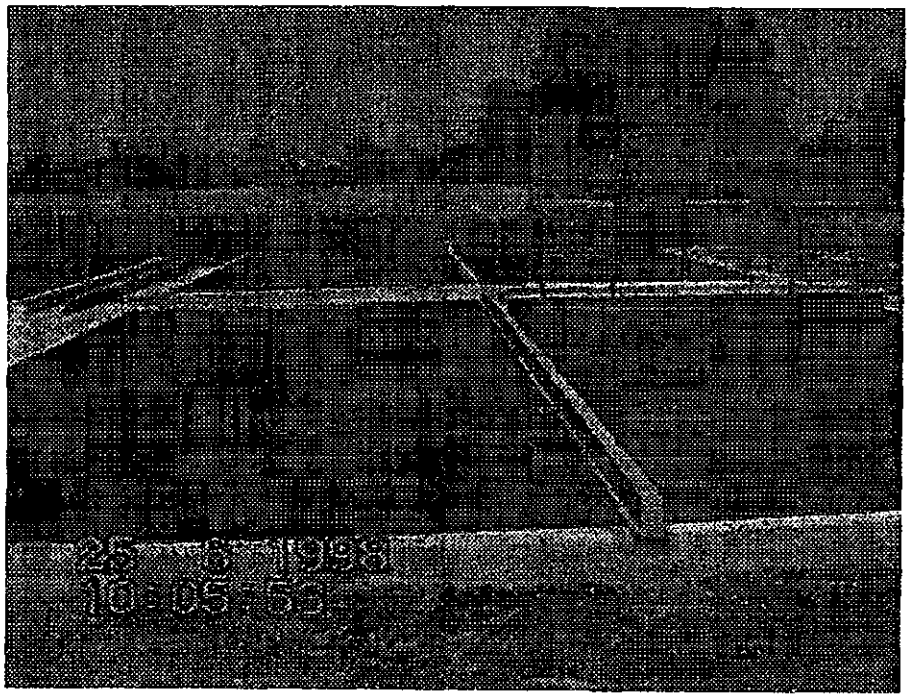


Figure 9. Fish cage exposed to one of the Springborn's outdoor basins.

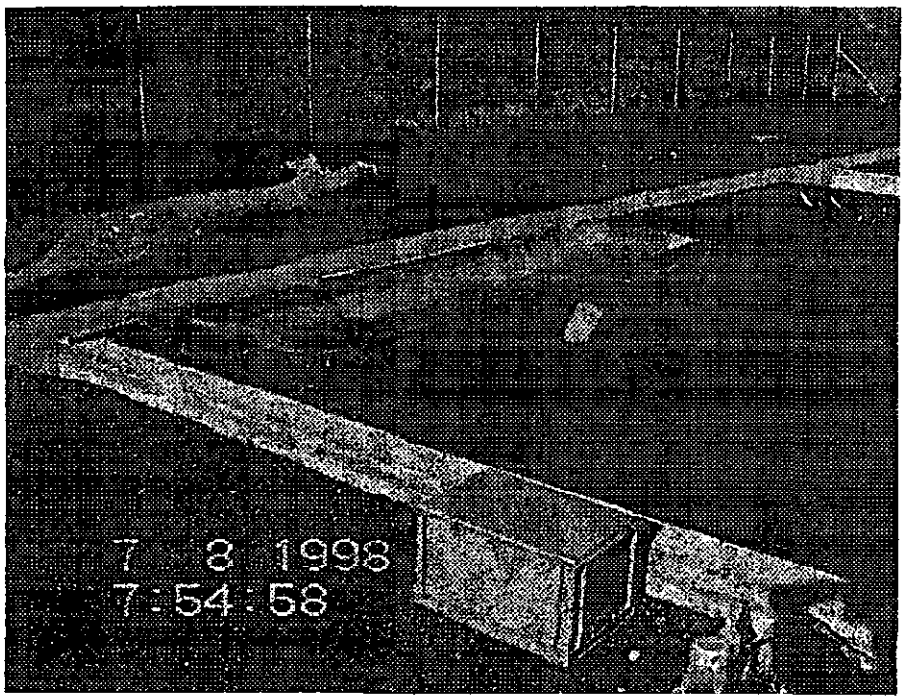


Figure 10. ***Myriophyllum spicatum*** **distributed evenly over the testing basin.**

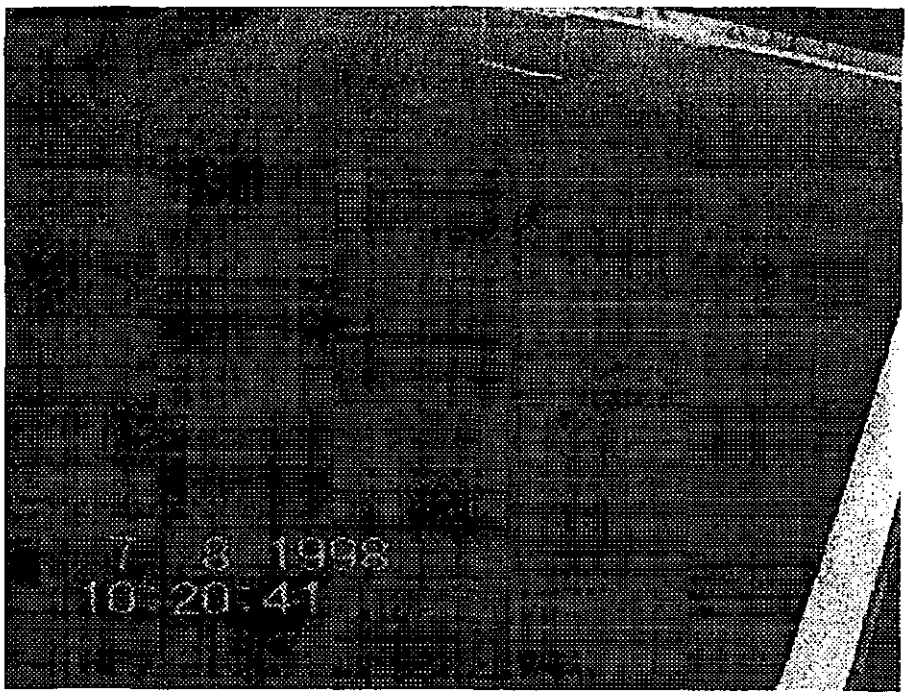


Figure 11. Placement of the enclosures into the testing basin.

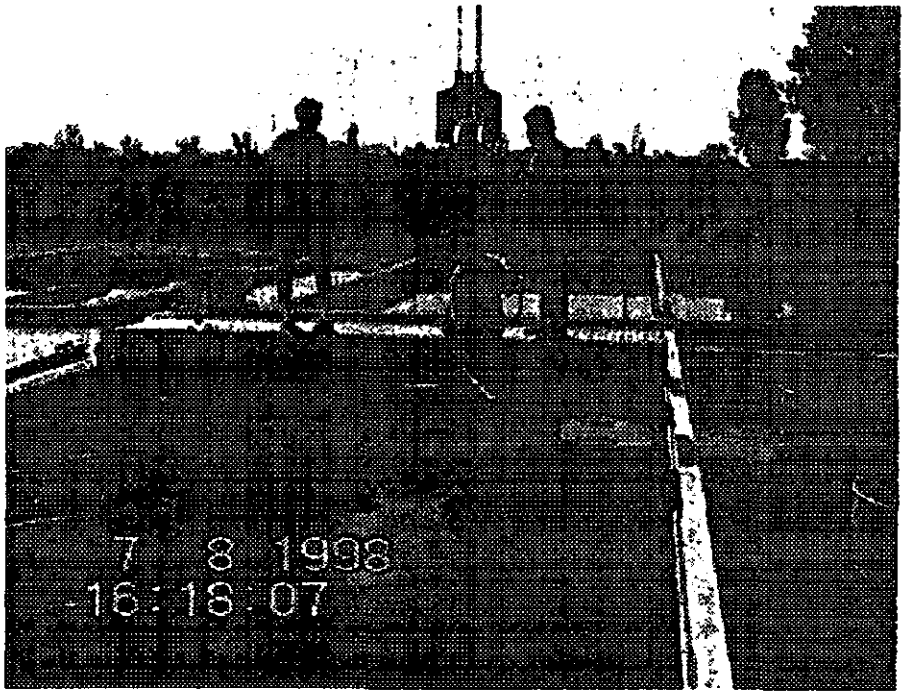


Figure 12. Some enclosures placed into the testing basin.

Figure 13. **Equipment of the enclosures with fish cage and sampling devices (1): Fish cage.**

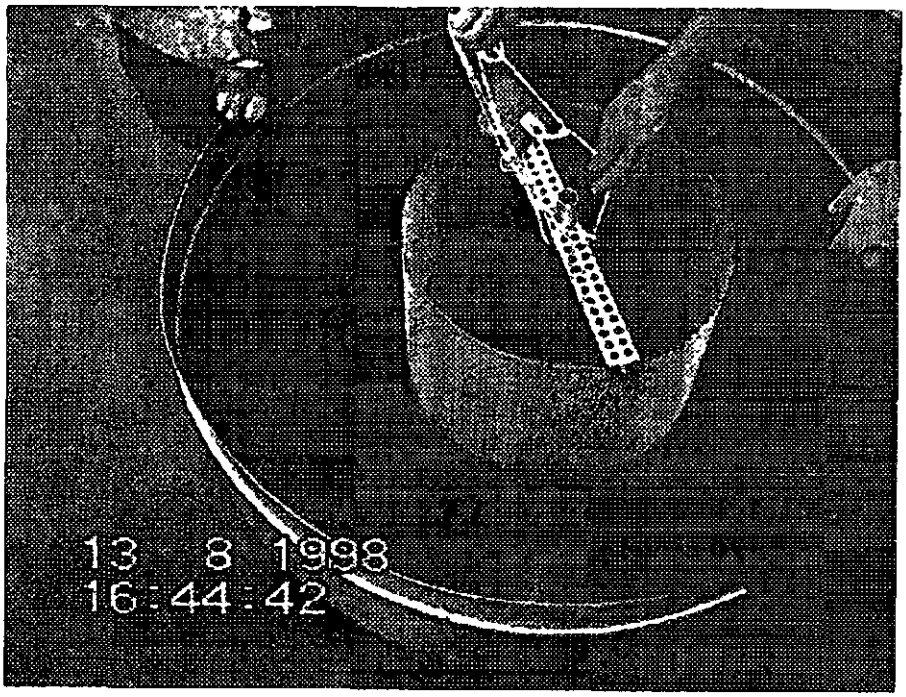


Figure 14. **Equipment of the enclosures with fish cage and sampling devices (2): Fish cage plus macrophyte.**

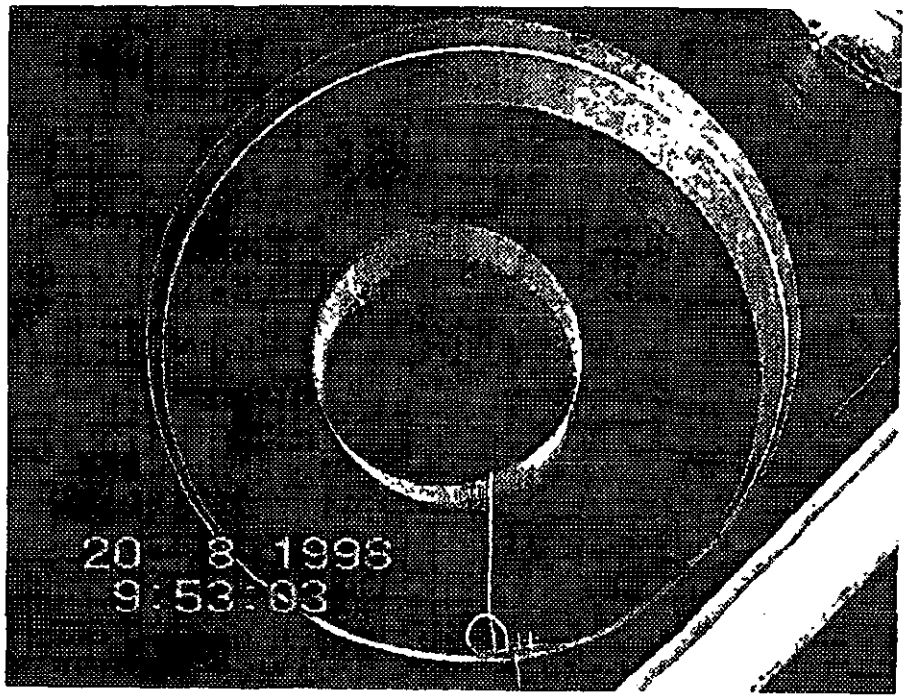


Figure 15. Equipment of the enclosures with fish cage and sampling devices (3): Teflon tubes for water collection.

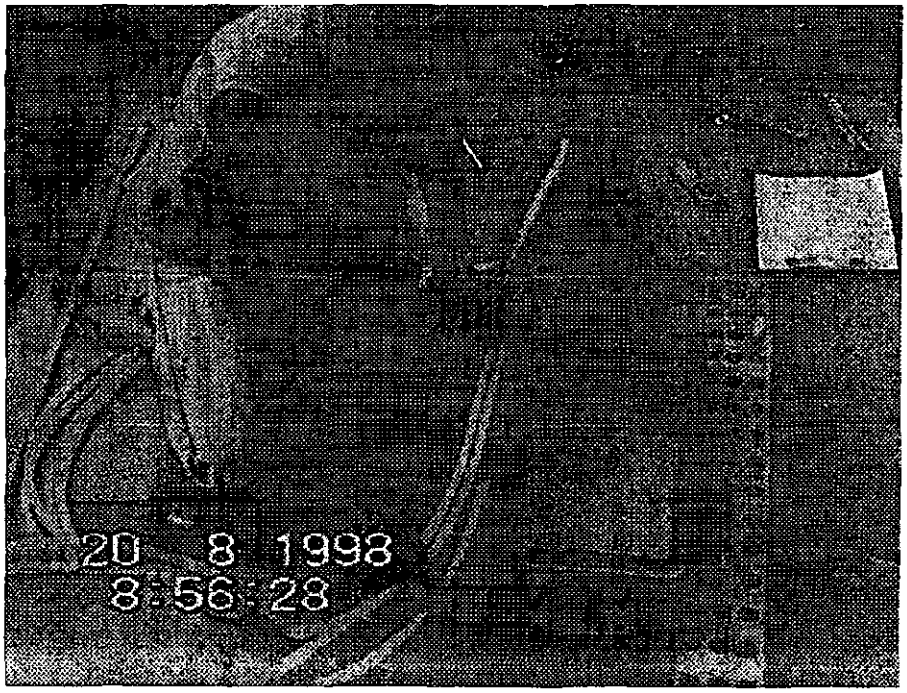


Figure 16. Transfer of fish into each individual enclosure.

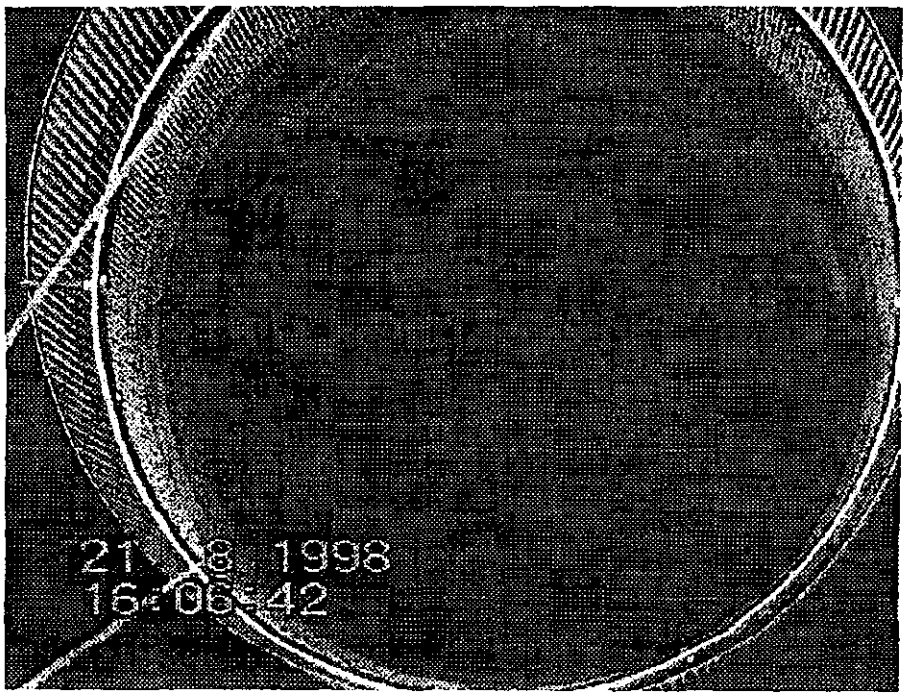


Figure 17 **Testing basin and a fully equipped enclosure immediately prior to the first treatment.**

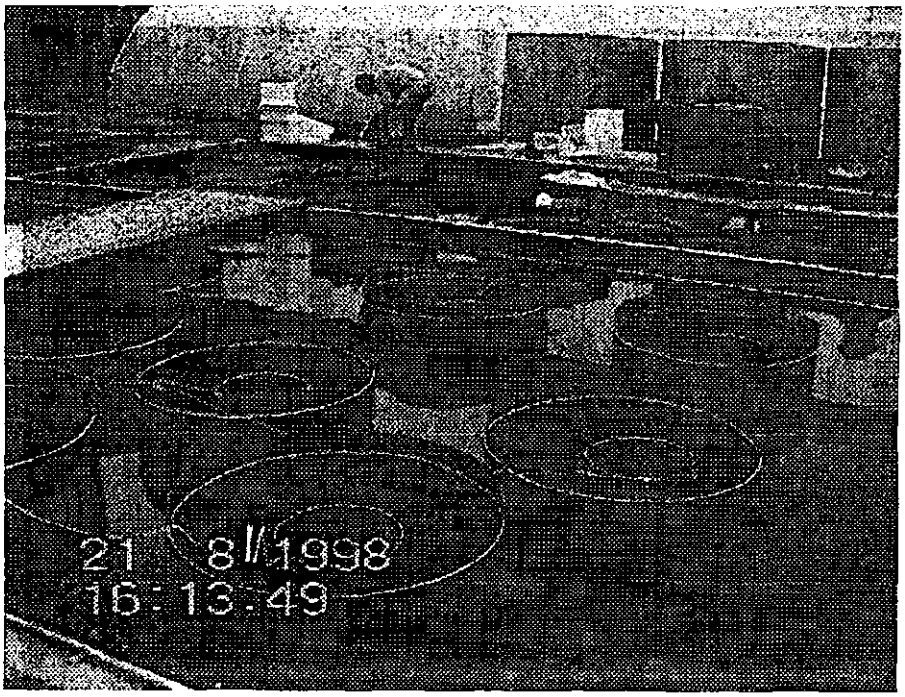


Figure 18. **Shading equipment used to decease the pH value of the systems prior to the first treatment.**

Figure 19. **Overnights aeration of each individual enclosure in order to decrease the pH value prior to the first treatment.**

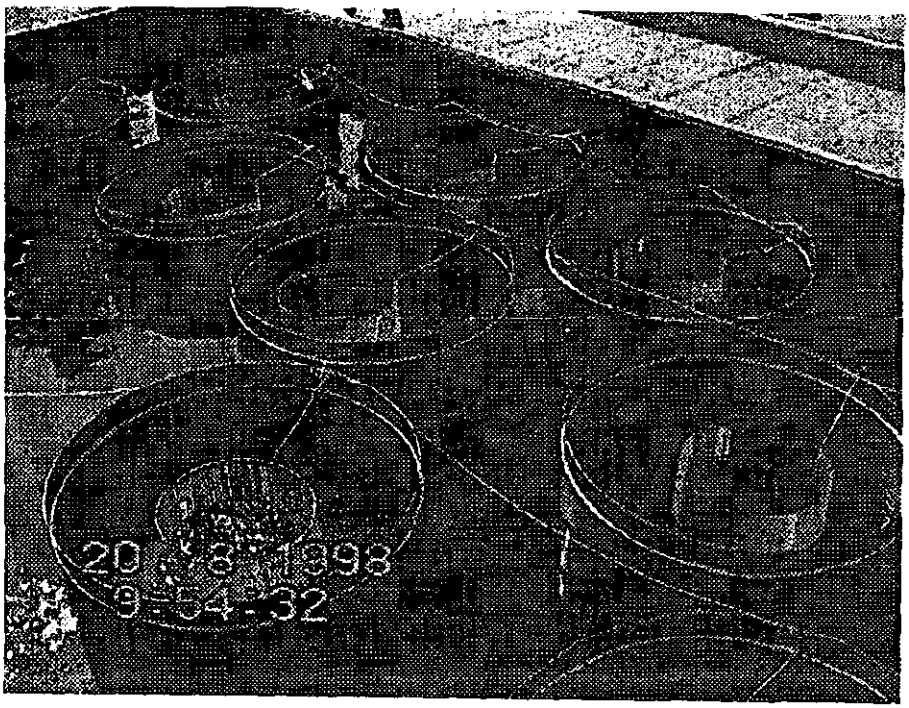


Figure 20. **Radiopurity of α,β-endosulfan (batch Z28070-0) in acetonic stock solution used for the 1st application (A) and after 2 weeks storage in the freezer (B). HPLC method 1 was applied. β-endosulfan: 11.00 min; α-endosulfan: 11.80/11.90 min.**

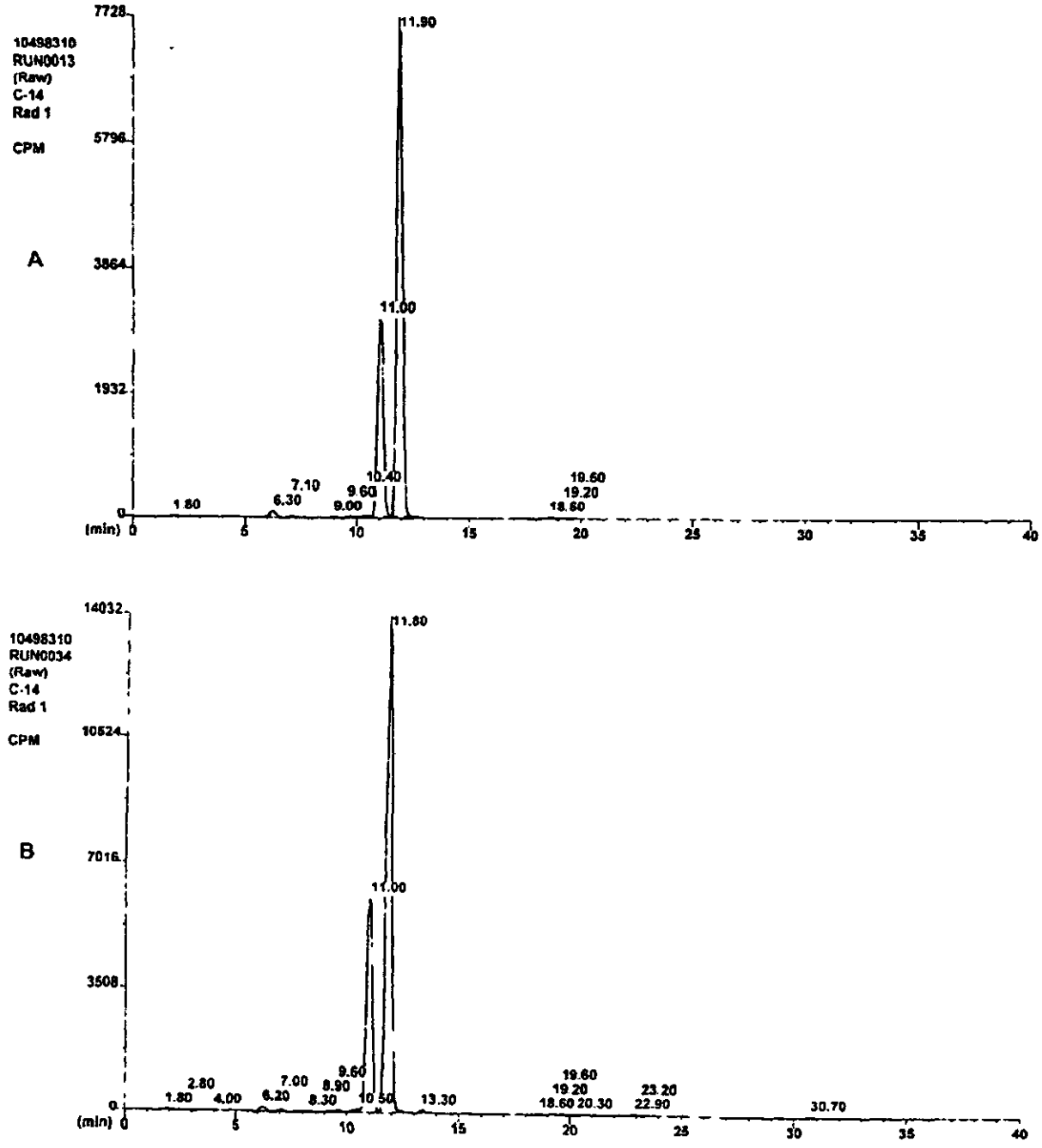


Figure 21. **Radiopurity of α,β-endosulfan (batch Z28070-0) as used for the 2nd application. A: Spray drift, B: Run-off. HPLC method 1 was applied. β-endosulfan: 11.00 min; α-endosulfan: 11.80 min.**

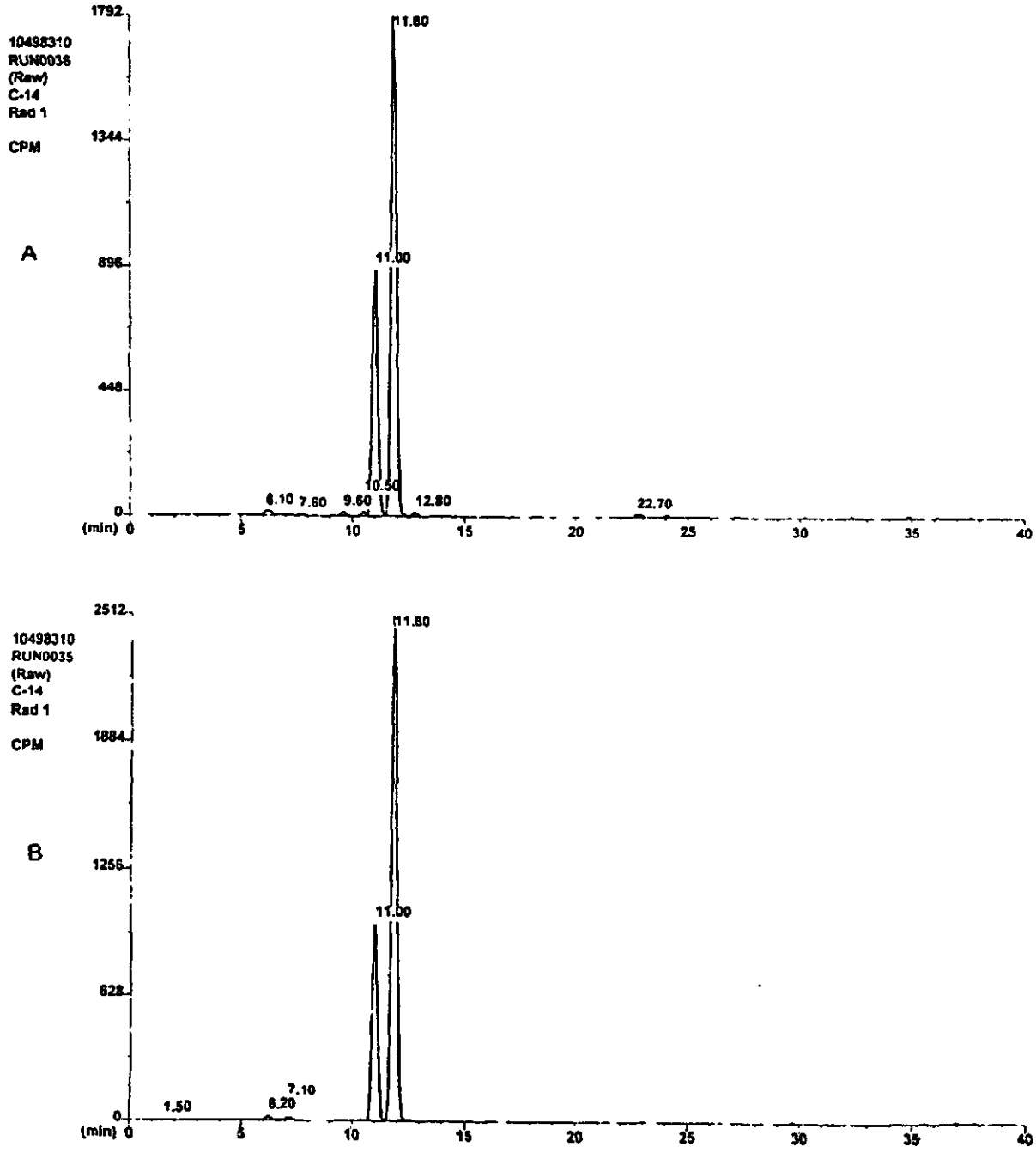


Figure 22. **Radiopurity of α,β-endosulfan (batch Z28070-0) as used for the 3rd application. A: Spray drift, B: Run-off. HPLC method 1 was applied. β-endosulfan: 10.90 min; α-endosulfan: 11.80 min.**

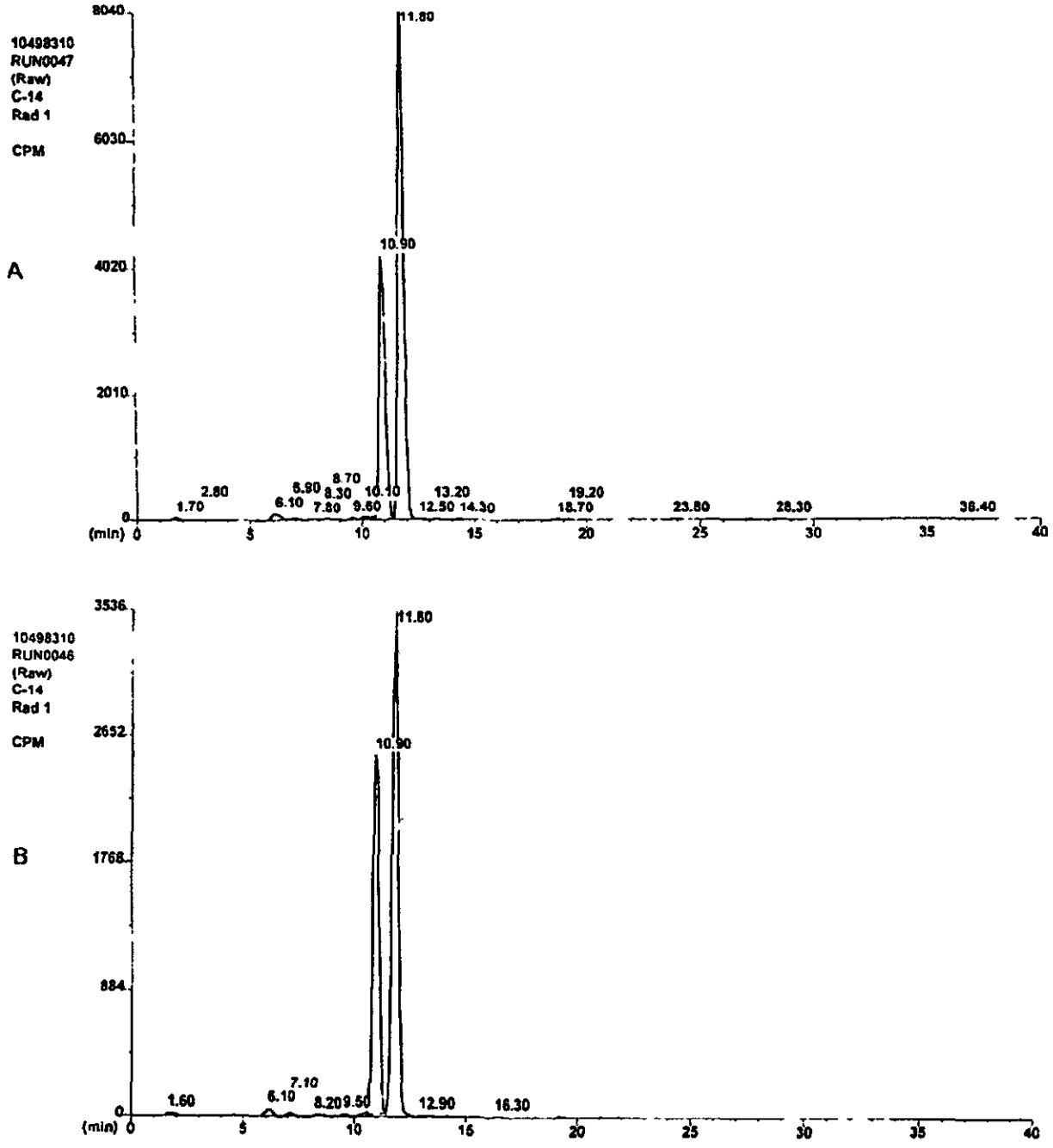
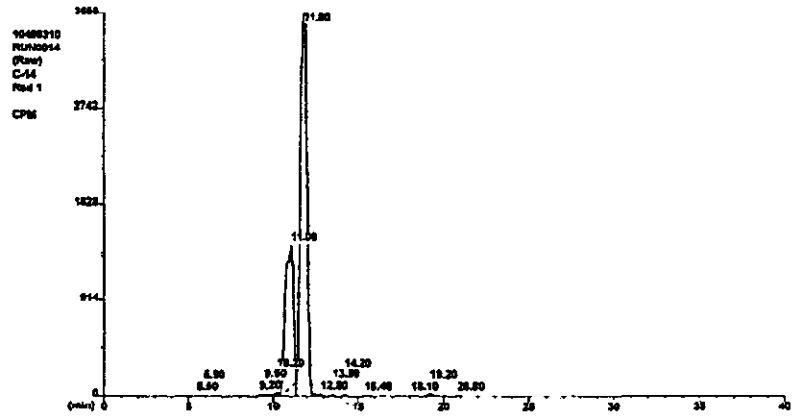
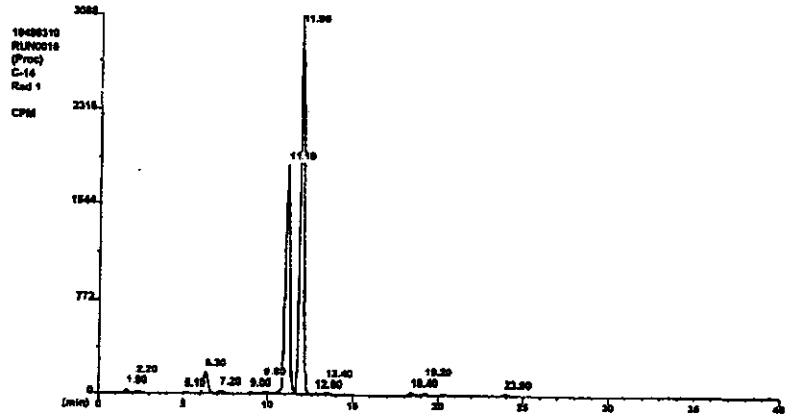


Figure 23. **Spray drift: Representative HPLC-RAM chromatograms of the rinse obtained from the spraying device after the 1st treatment. A: Test group SD-0.27 (lowest concentration), B: Test Group SD-1.51 (medium concentration), C: Test Group SD-8.38 (highest concentration). HPLC method 1 was applied. β-endosulfan: 11.00/11.10 min; α-endosulfan: 11.80/11.90 min.**

A



B



C

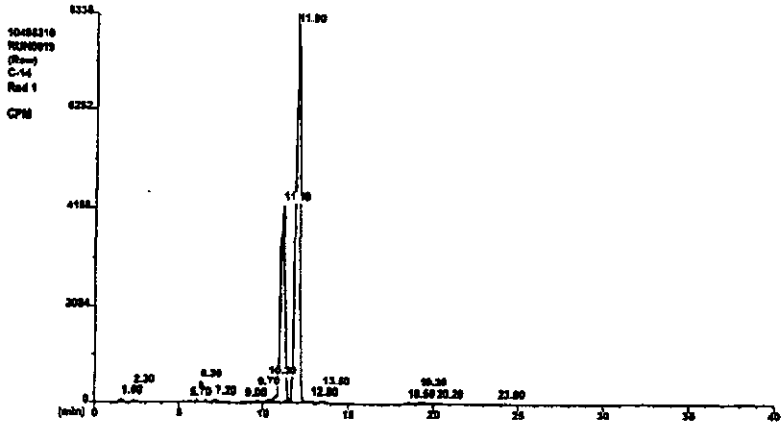
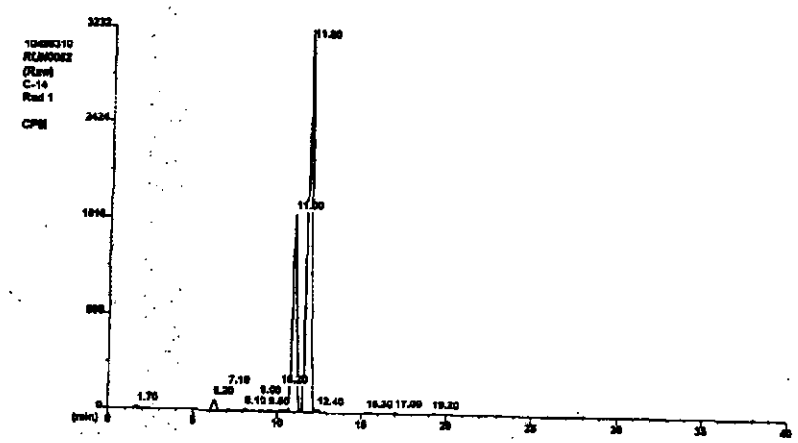
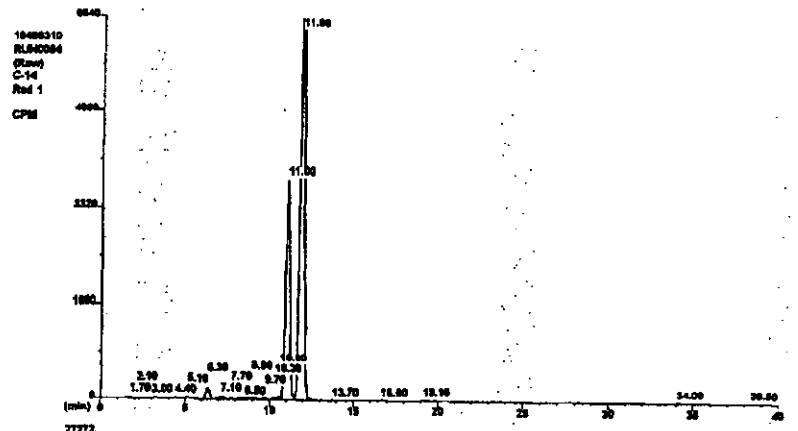


Figure 24. **Spray drift: Representative HPLC-RAM chromatograms of treatment solutions after the 3rd treatment. A: Test group SD-0.27 (lowest concentration), B: Test Group SD-0.84 (medium concentration), C: Test Group SD-8.38 (highest concentration). HPLC method 1 was applied. β-endosulfan: 11.00 min; α-endosulfan: 11.80 min.**

A



B



C

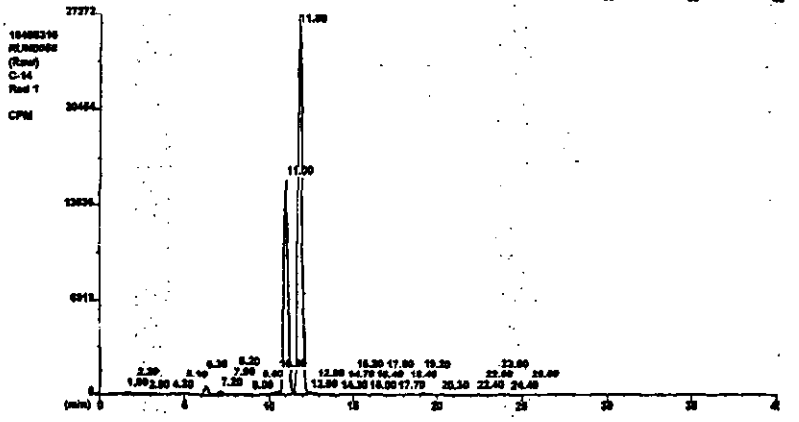


Figure 25. **Run-off: Representative HPLC-UV/RAM chromatogram of treated soil after one day ageing and ASE extraction with acetone/water (1st treatment). A: Radiochromatogram, B: UV-trace of the analytical standards. HPLC method 1 was applied.**

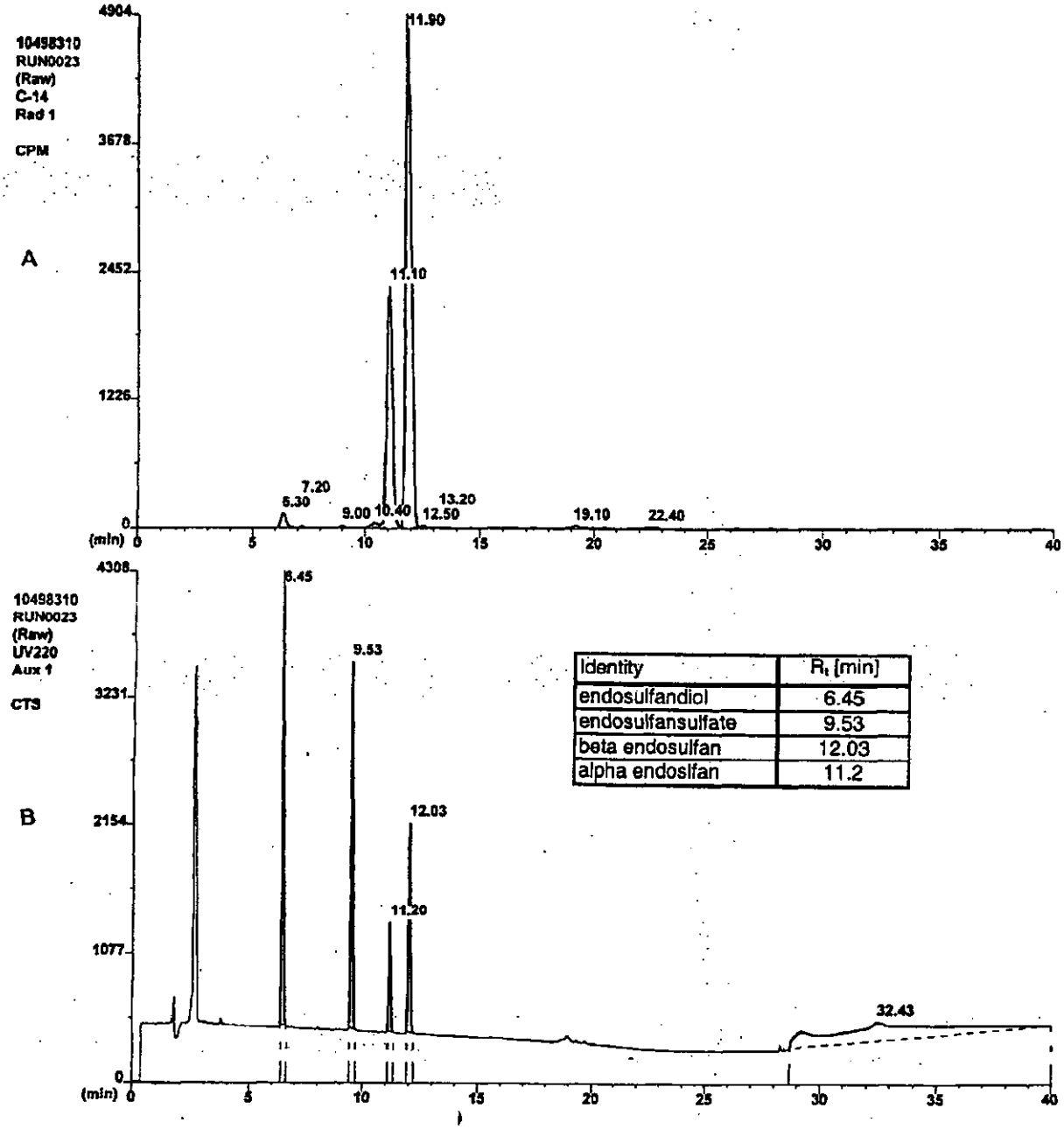
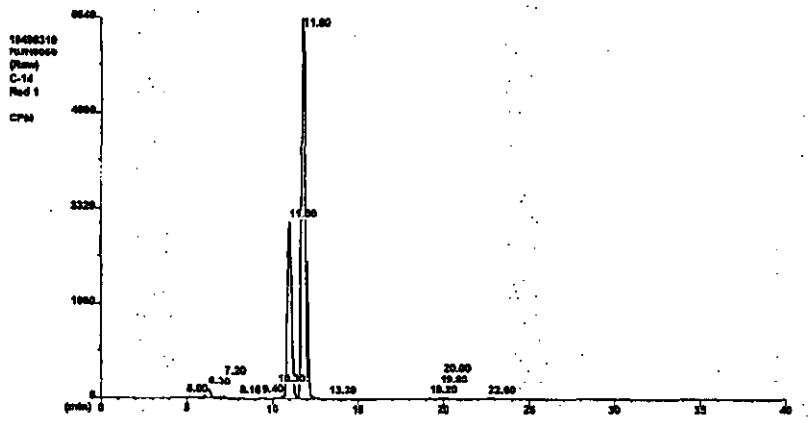
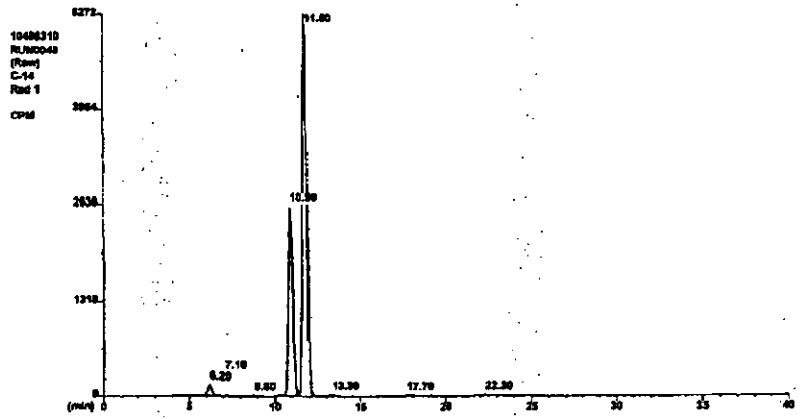


Figure 26. **Run-off: Representative HPLC-RAM chromatograms of treated soil after one day ageing and ASE extraction with acetone/water. A: 2nd treatment, B: 3rd Treatment, remainder of the first treatment after about 4 weeks storage in the freezer, C: Remainder of the 2nd treatment after about 2 weeks storage in the freezer. HPLC method 1 was applied. β-endosulfan: 10.90/11.00 min; α-endosulfan: 11.80 min.**

A



B



C

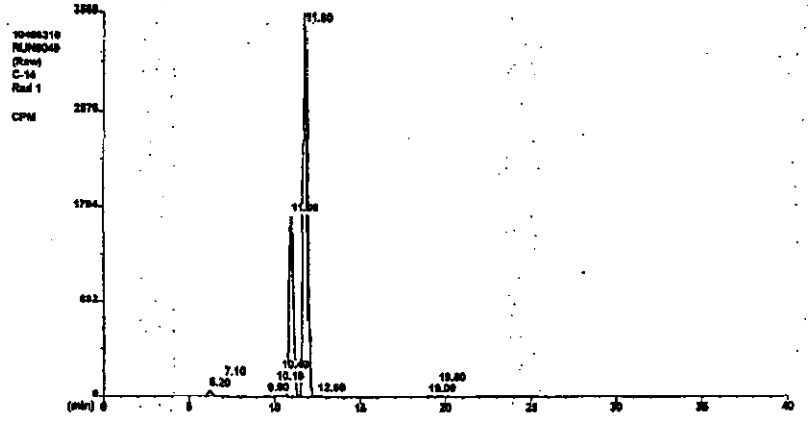
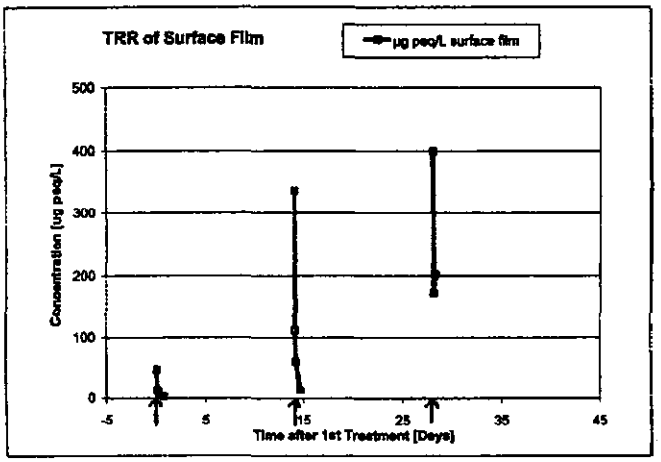
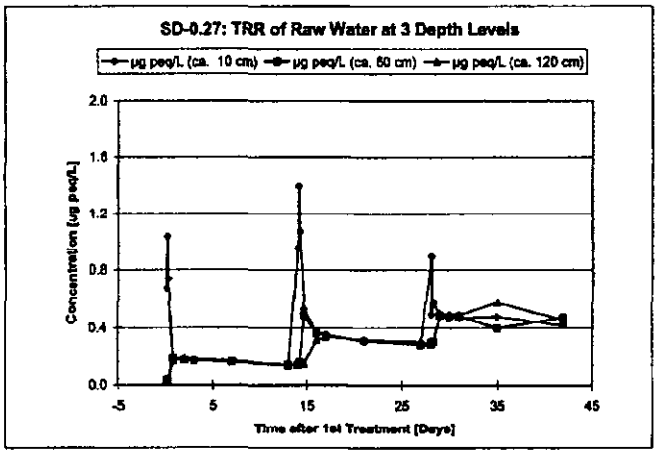


Figure 27. **Spray drift test group SD-0.27: Total Radioactive Residue (TRR) in raw water. A: surface film, B: 3 depth levels, C: mean of 3 depth levels. The arrows point to the days of treatment.**

A



B



C

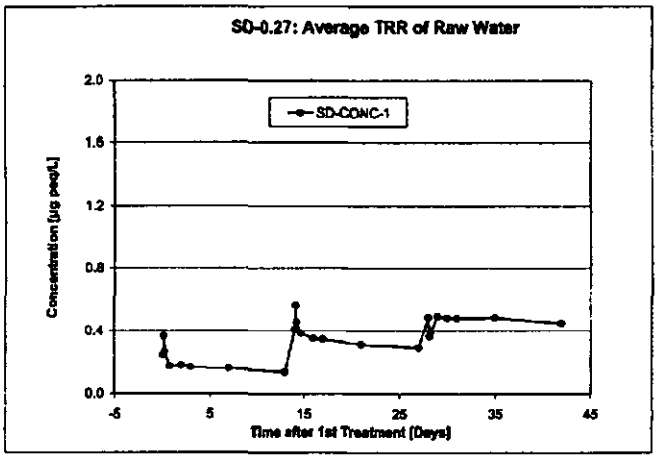
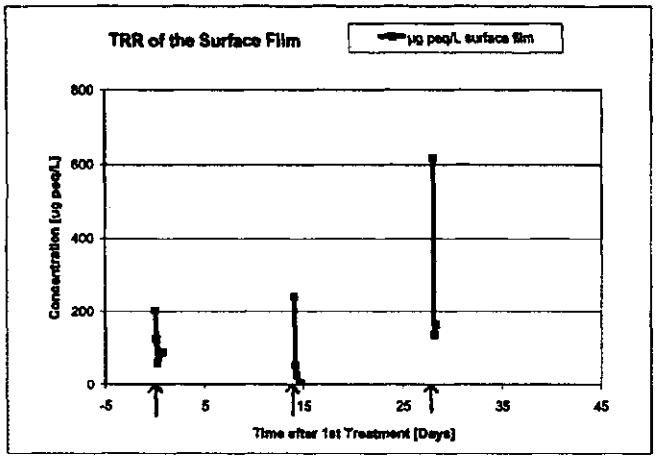
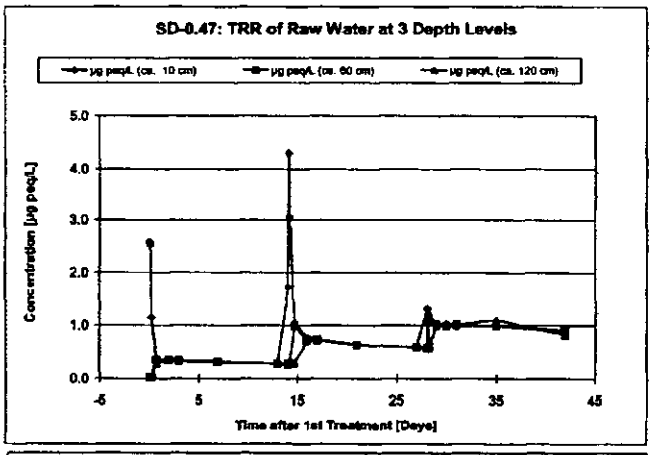


Figure 28. **Spray drift test group SD-0.47: Total Radioactive Residue (TRR) in raw water. A: surface film, B: 3 depth levels, C: mean of 3 depth levels. The arrows point to the days of treatment.**

A



B



C

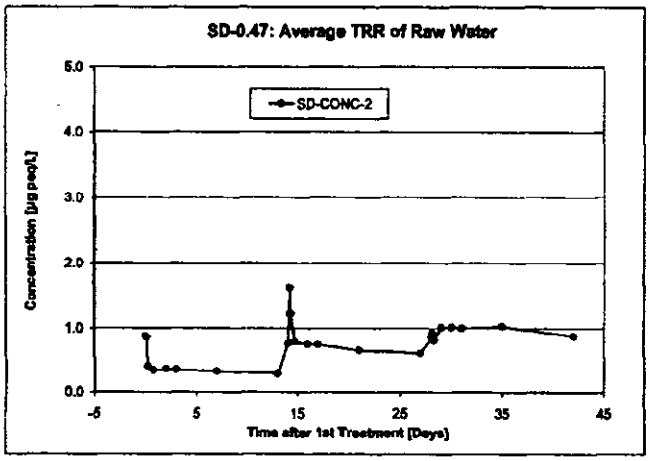
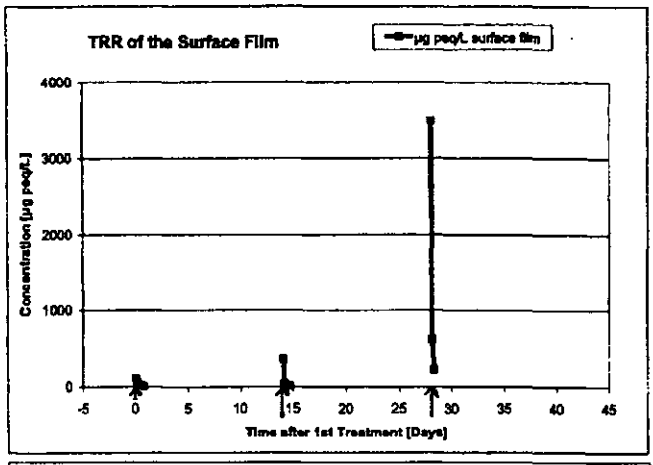
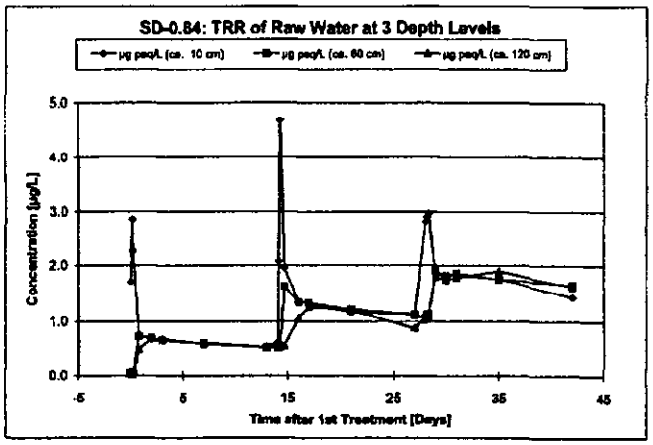


Figure 29. **Spray drift test group SD-0.84: Total Radioactive Residue (TRR) in raw water. A: surface film, B: 3 depth levels , C: mean of 3 depth levels. The arrows point to the days of treatment.**

A



B



C

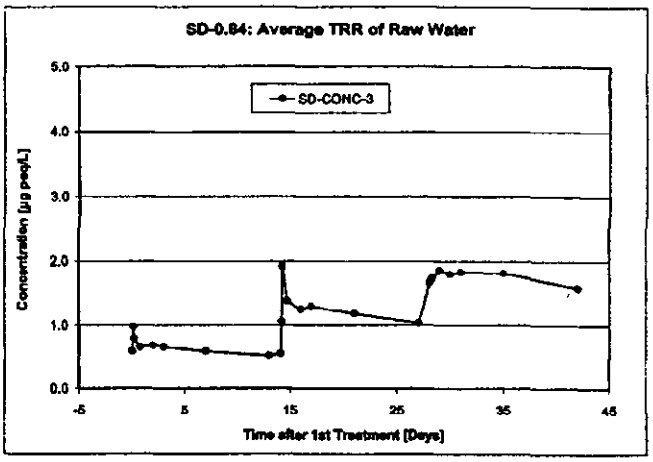
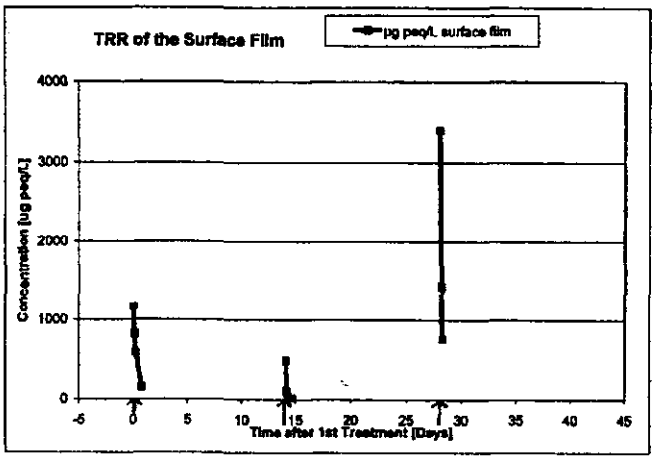
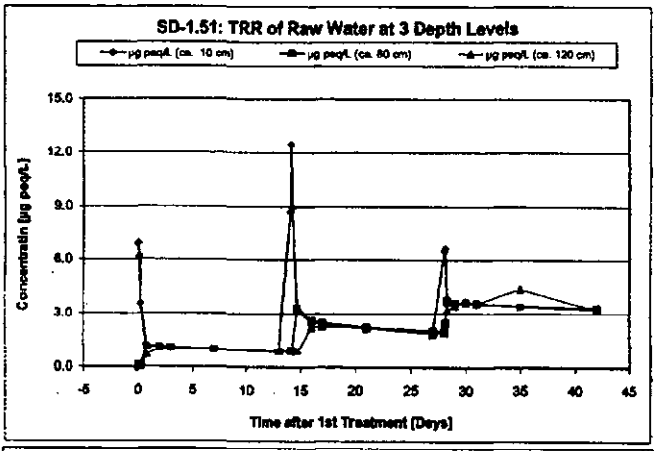


Figure 30. **Spray drift test group SD-1.51: Total Radioactive Residue (TRR) in raw water. A: surface film, B: 3 depth levels , C: mean of 3 depth levels. The arrows point to the days of treatment.**

A



B



C

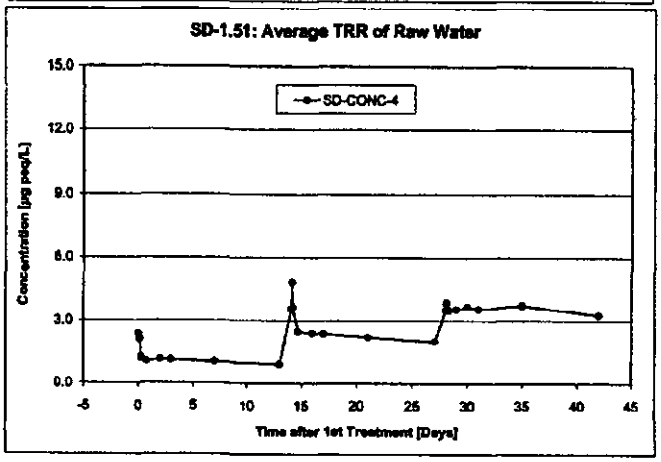
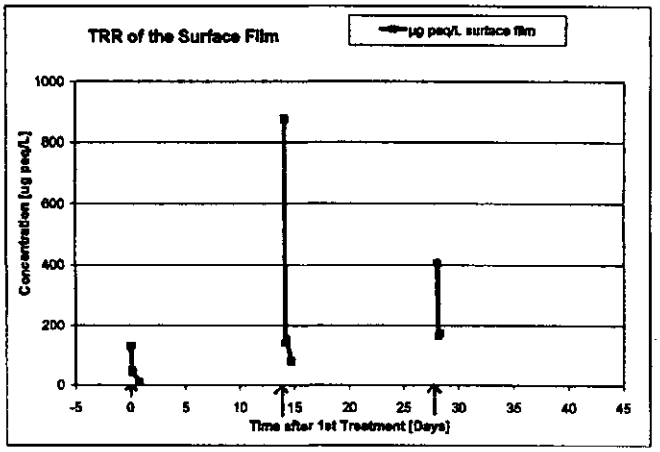
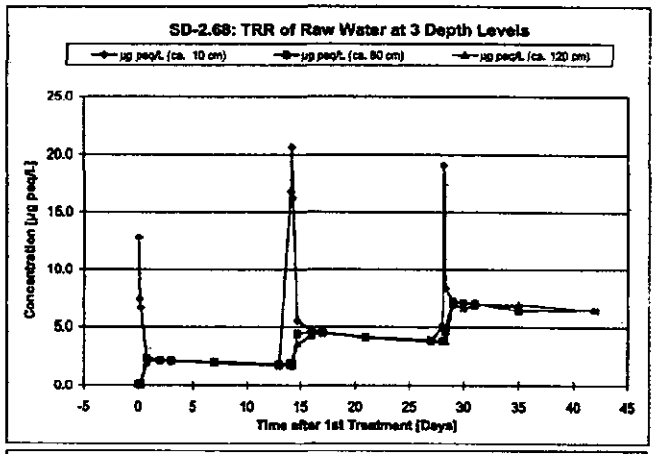


Figure 31. **Spray drift test group SD-2.68: Total Radioactive Residue (TRR) in raw water. A: surface film, B: 3 depth levels , C: mean of 3 depth levels. The arrows point to the days of treatment.**

A



B



C

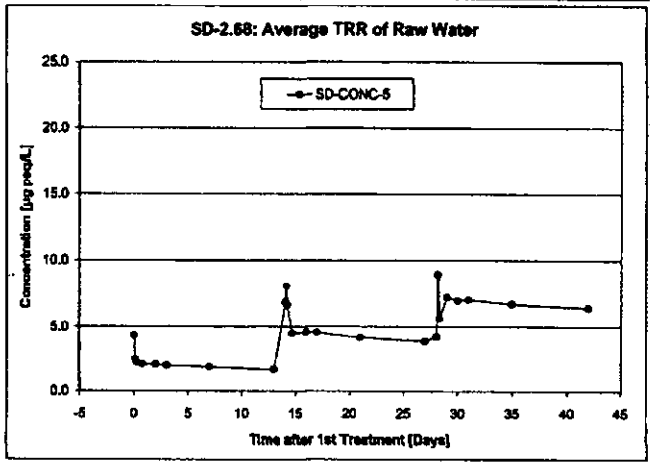
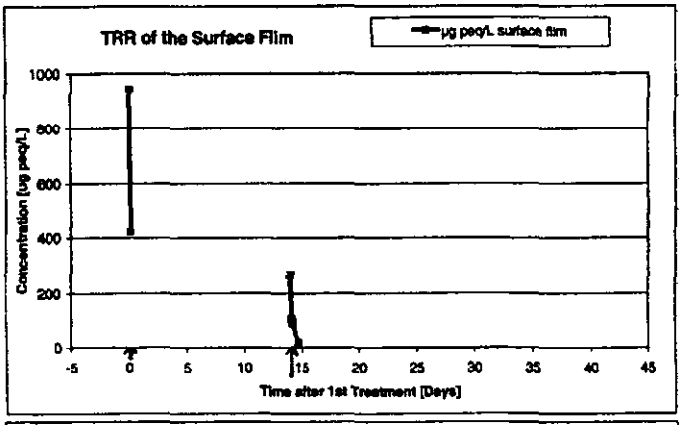
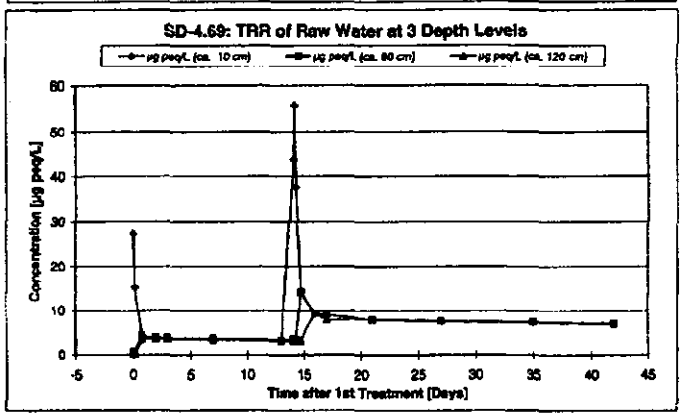


Figure 32. **Spray drift test group SD-4.69: Total Radioactive Residue (TRR) in raw water. A: surface film, B: 3 depth levels , C: mean of 3 depth levels. The arrows point to the days of treatment.**

A



B



C

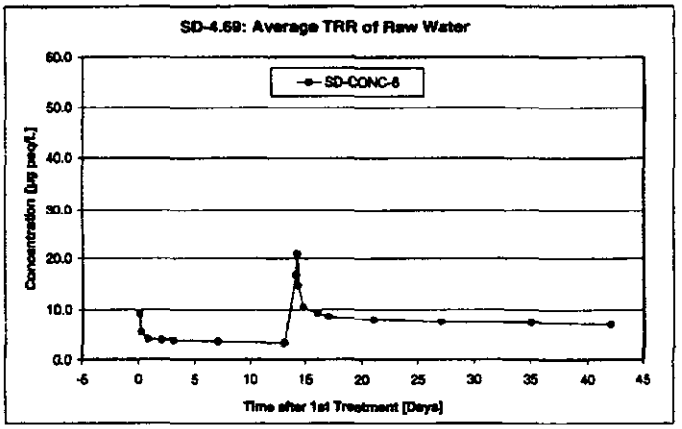
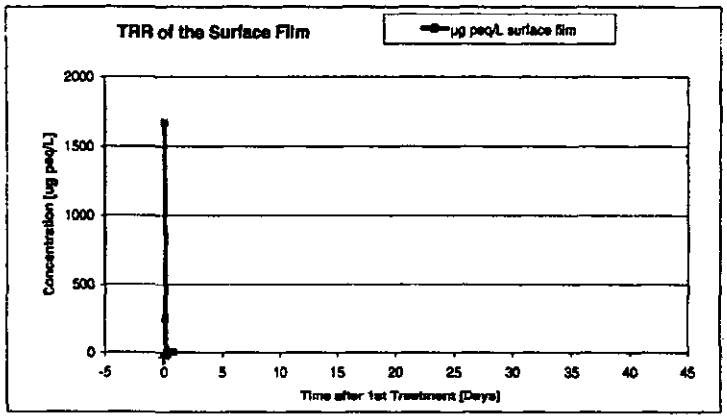
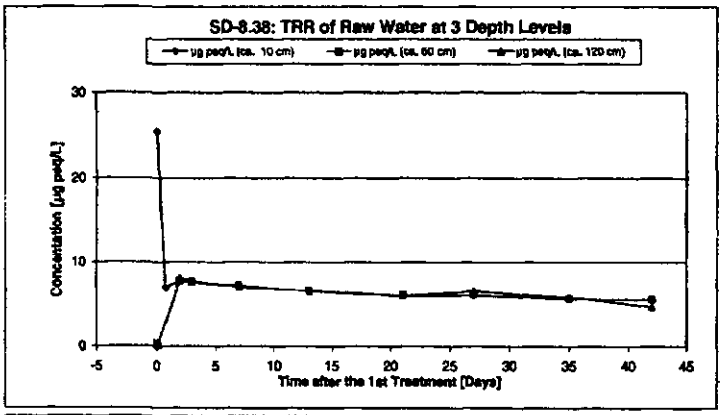


Figure 33. **Spray drift test group SD-8.38: Total Radioactive Residue (TRR) in raw water. A: surface film, B: 3 depth levels , C: mean of 3 depth levels. The arrow points to the day of treatment.**

A



B



C

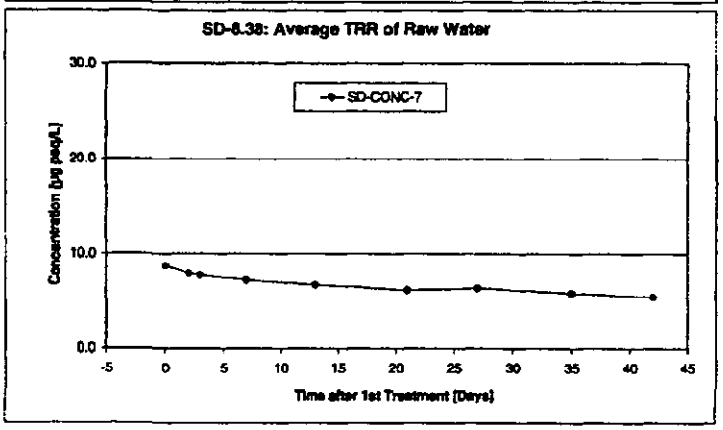


Figure 34. Spray drift test groups SD-0.27 to SD-2.68: Total Radioactive Residue (TRR) of raw water (mean of 3 depth levels).

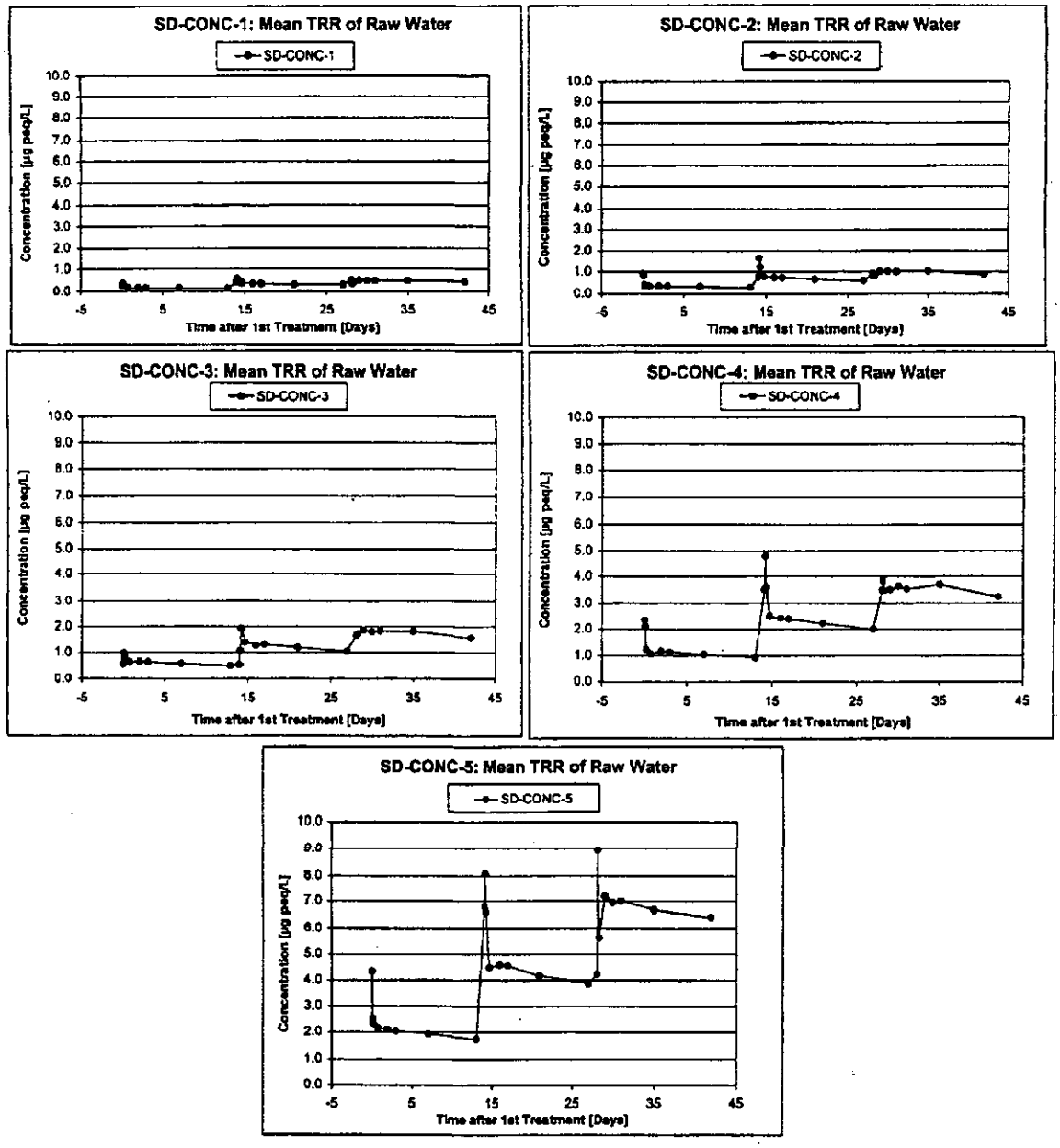
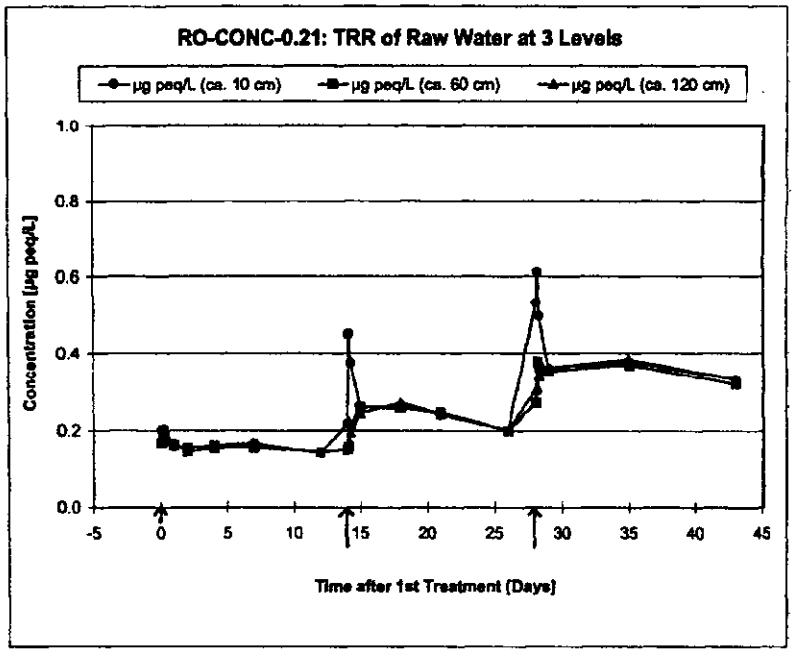


Figure 35. **Run-off test group RO-0.21: Total Radioactive Residue (TRR) in raw water. A: 3 depth levels, B: mean of 3 depth levels. The arrows point to the days of treatment.**

A



B

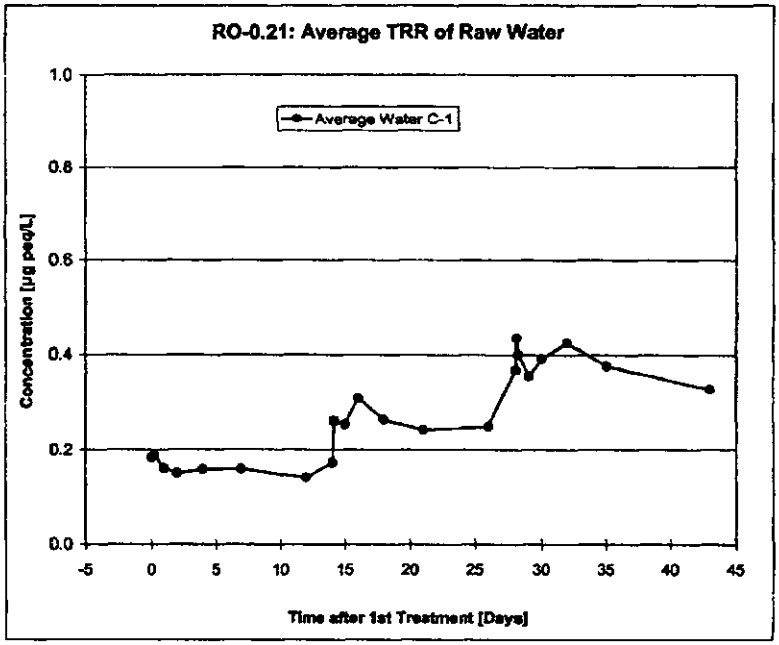
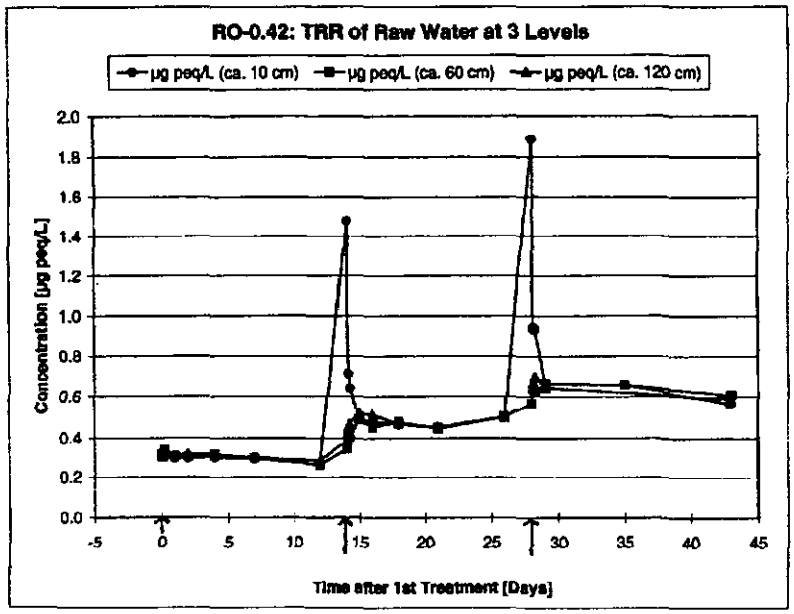


Figure 36. **Run-off test group RO-0.42: Total Radioactive Residue (TRR) *in raw* water. A: 3 depth levels, B: mean of 3 depth levels. The arrows point to the days of treatment.**

A



B

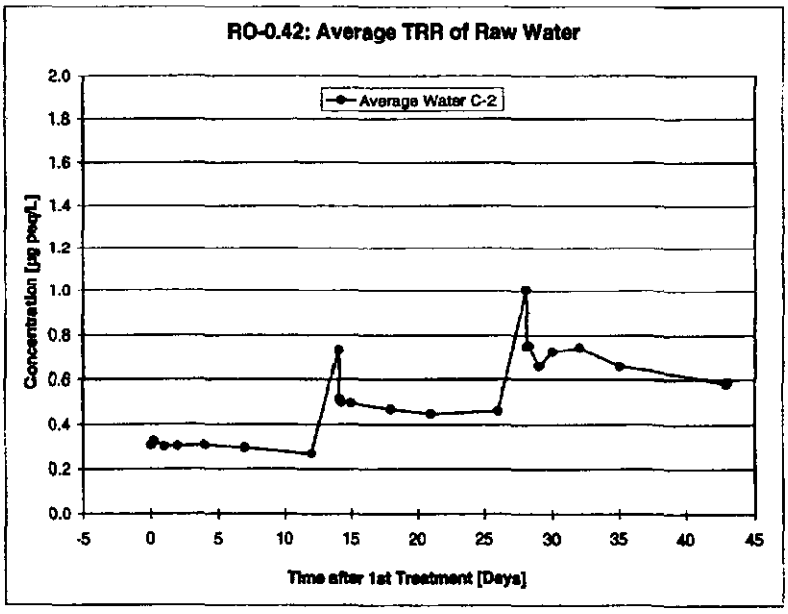
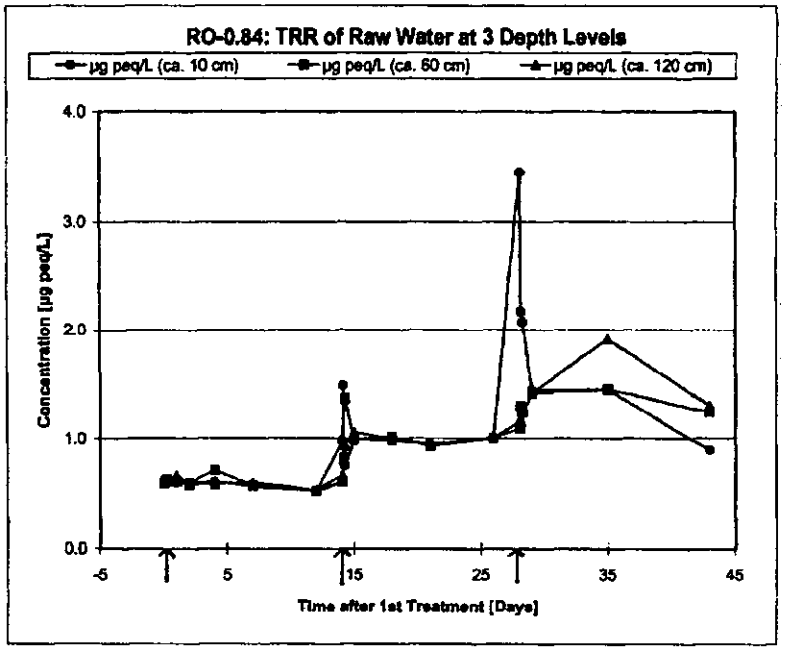


Figure 37. **Run-off test group RO-0.84: Total Radioactive Residue (TRR) in raw water. A: 3 depth levels, B: mean of 3 depth levels. The arrows point to the days of treatment.**

A



B

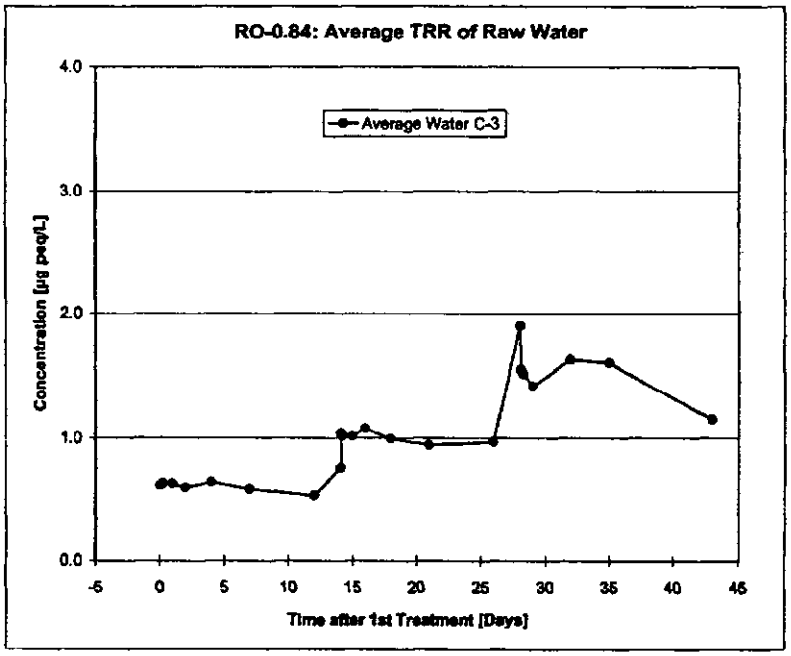
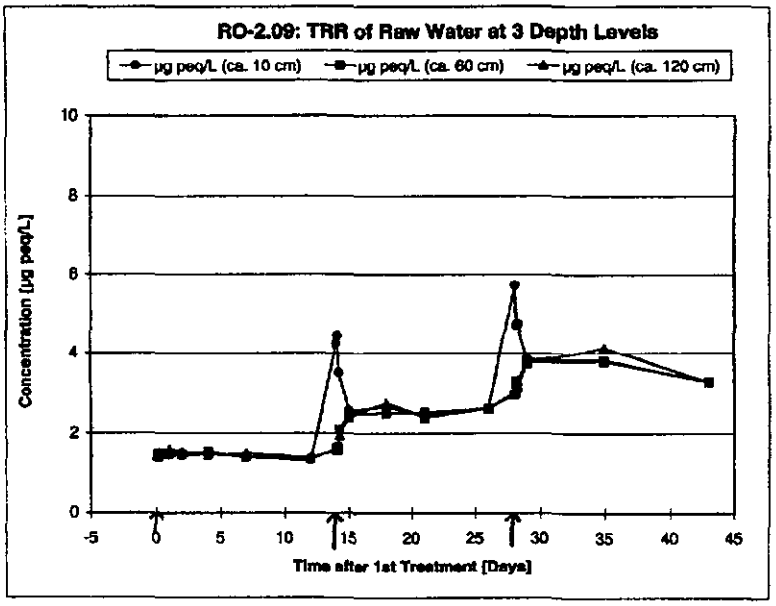


Figure 38. **Run-off test group RO-2.09: Total Radioactive Residue (TRR) in raw water. A: 3 depth levels , B: mean of 3 depth levels. The arrows point to the days of treatment.**

A



B

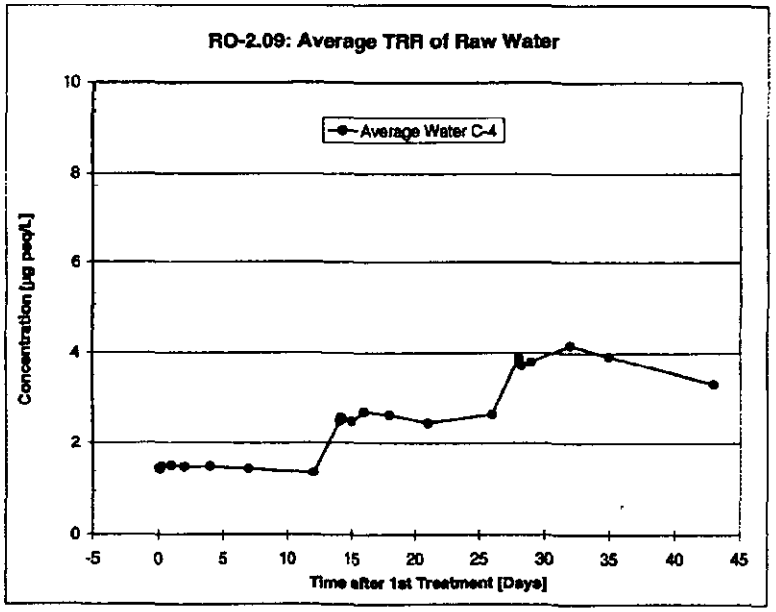
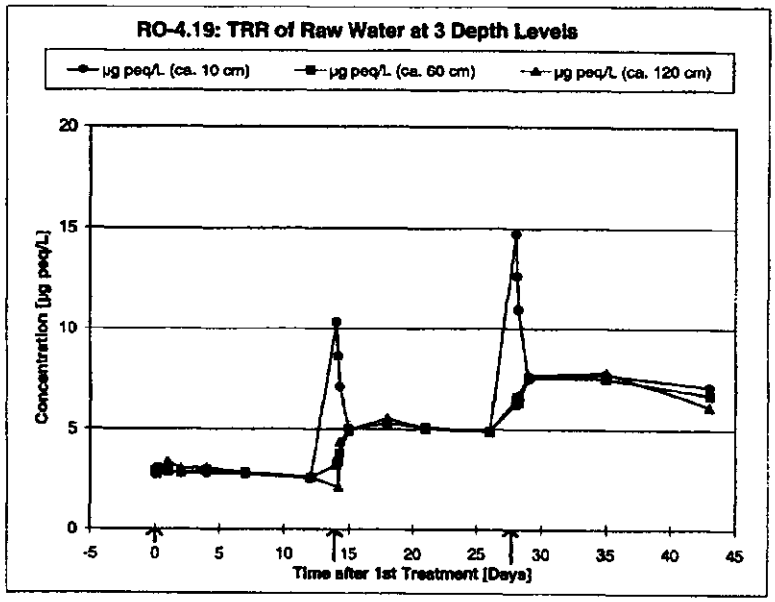


Figure 39. **Run-off test group RO-4.19: Total Radioactive Residue (TRR) in raw water. A: 3 depth levels , B: mean of 3 depth levels. The arrows point to the days of treatment.**

A



B

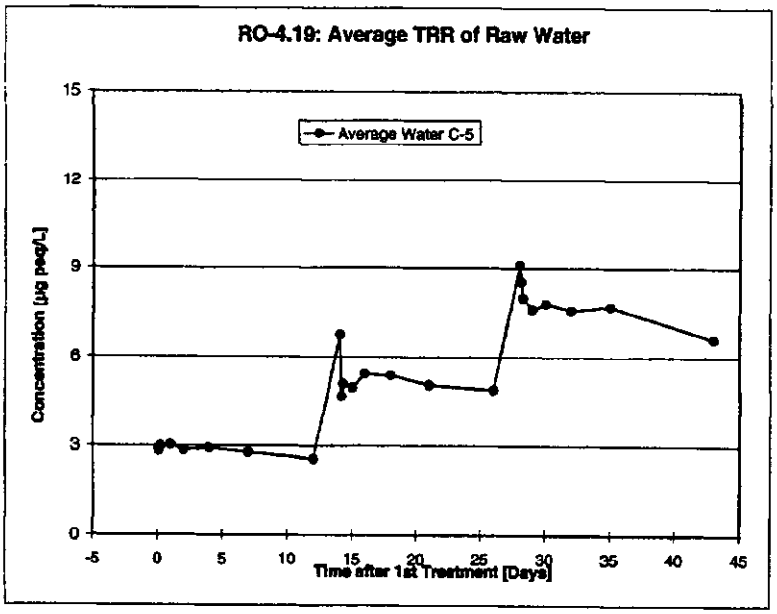
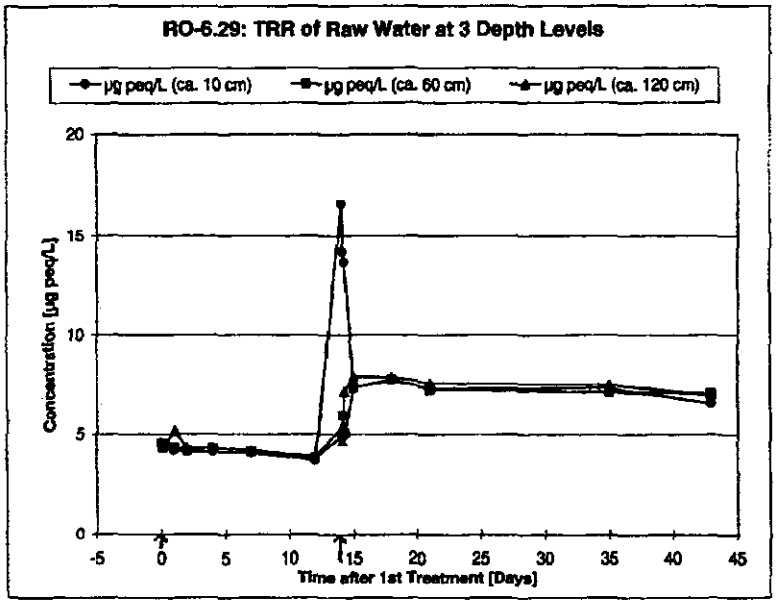


Figure 40. **Run-off test group RO-6.29: Total Radioactive Residue (TRR) in raw water. A: 3 depth levels , B: mean of 3 depth levels. The arrows point to the days of treatment.**

A



B

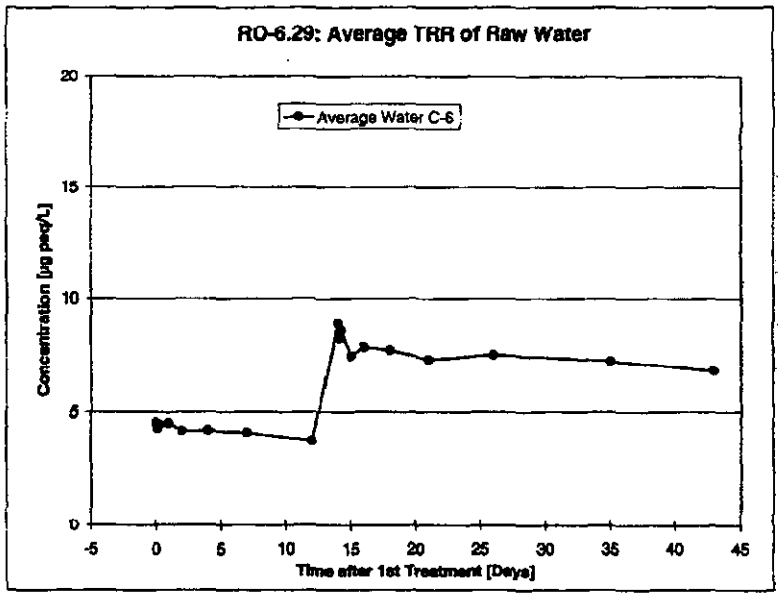
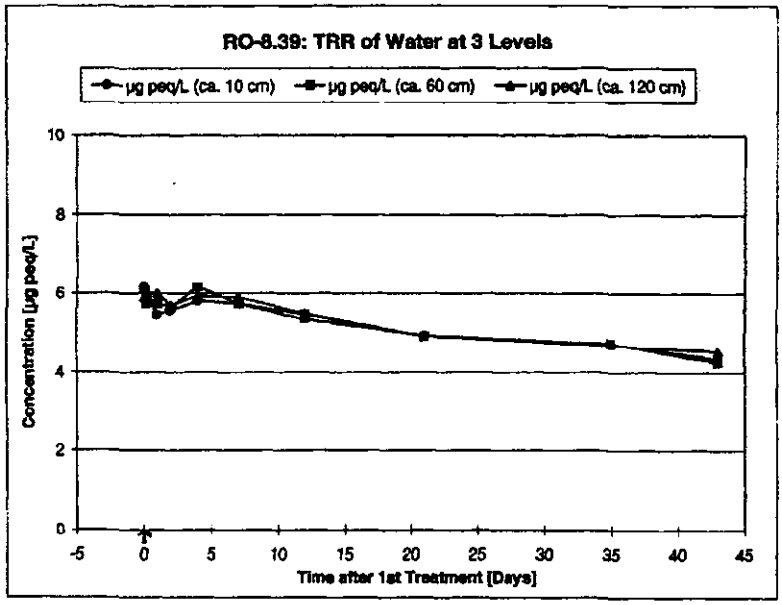


Figure 41. **Run-off test group RO-8.39: Total Radioactive Residue (TRR) in raw water. A: 3 depth levels , B: mean of 3 depth levels. The arrow points to the day of treatment.**

A



B

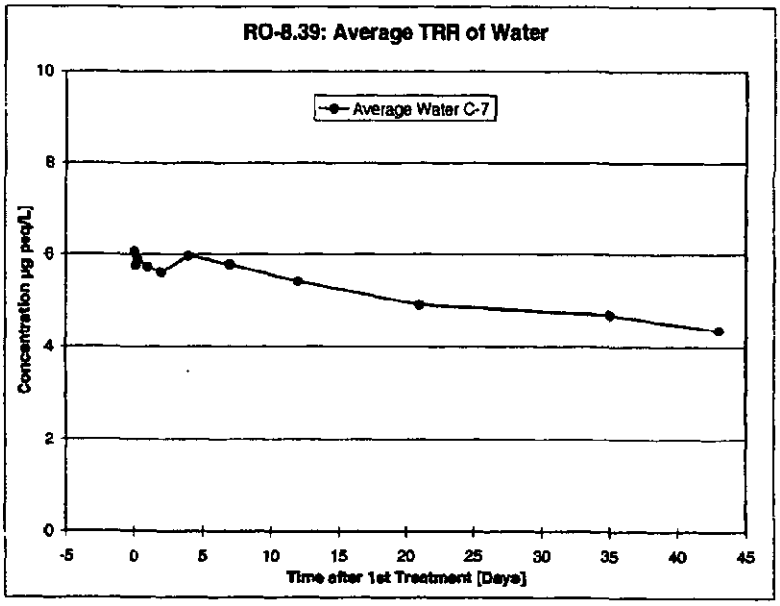


Figure 42. Run-off test groups RO-0.21 to RO-4.19: Total Radioactive Residue (TRR) of raw water (mean of 3 depth levels).

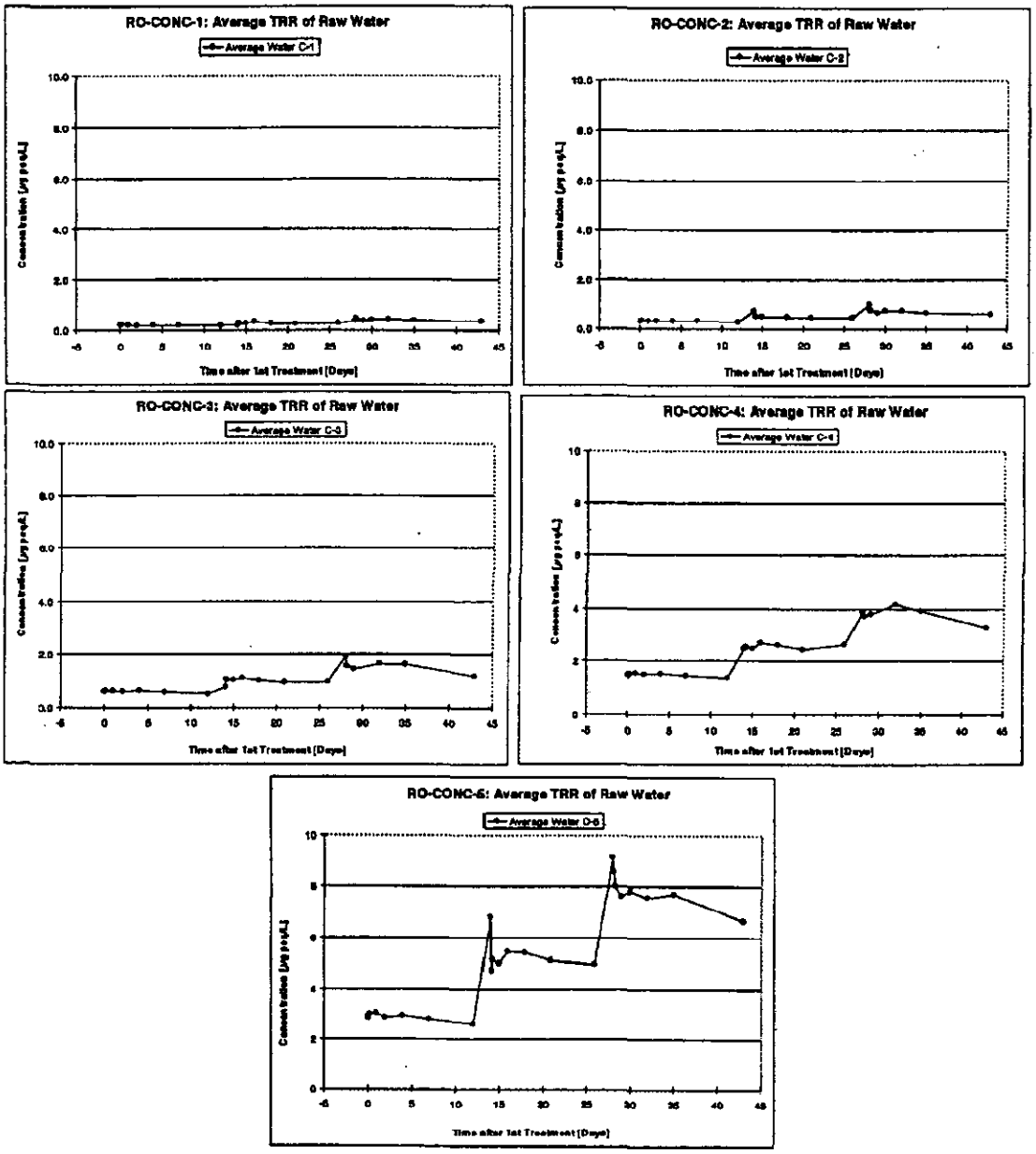
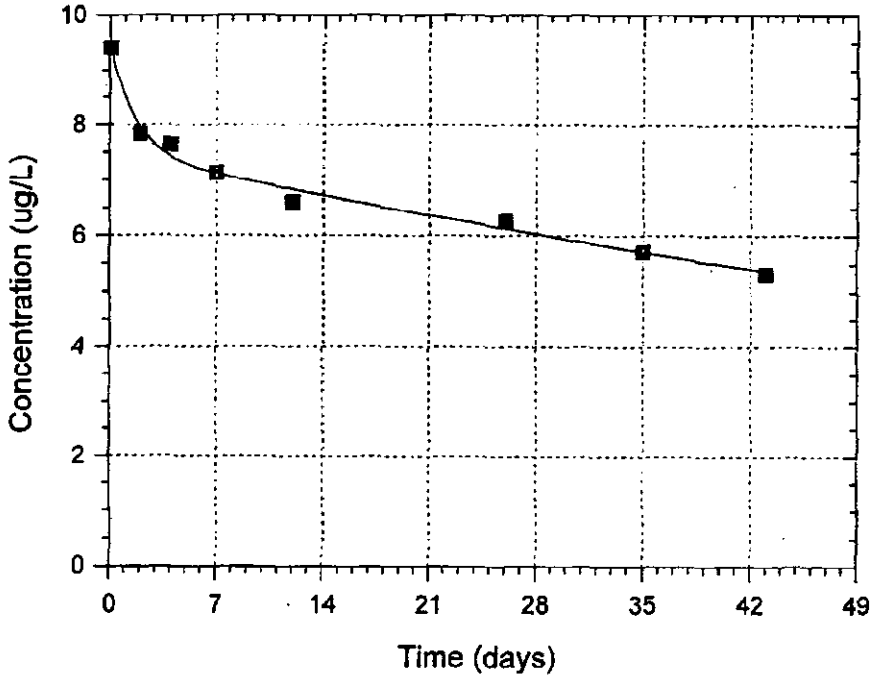


Figure 43. ***Concentration of the TRR in water vs. time after treatment with the highest test concentrations (SD-8.38:top; RO-8.39:bottom).***



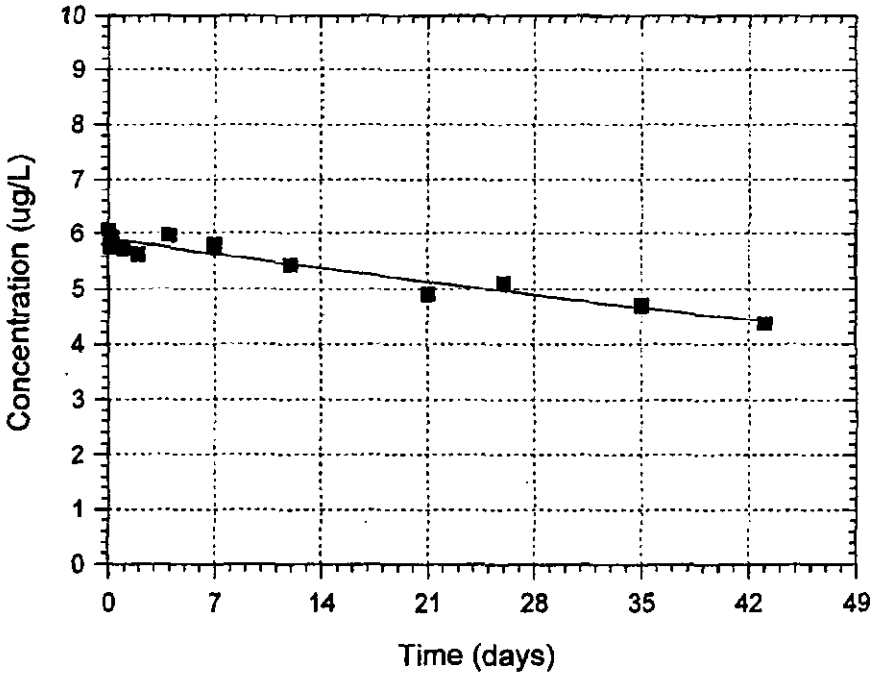
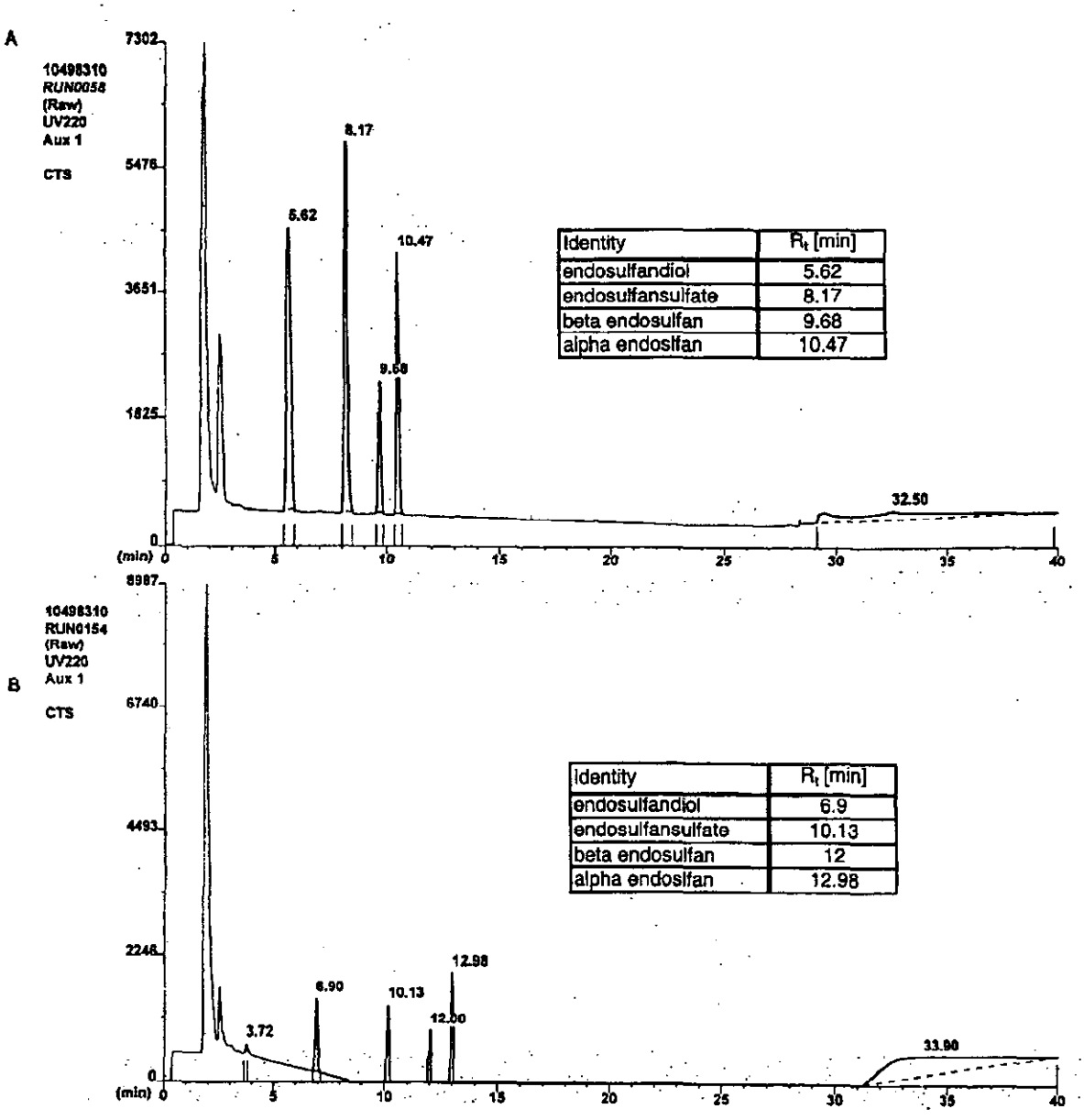


Figure 44. **Representative HPLC chromatograms of analytical standards using HPLC method 1. A: *radiolabelled analytical standards, B: unlabelled* analytical standards (1st series as delivered by the sponsor).**



Identity	R_t [min]
endosulfandiol	5.62
endosulfansulfate	8.17
beta endosulfan	9.68
alpha endosulfan	10.47

Identity	R_t [min]
endosulfandiol	6.9
endosulfansulfate	10.13
beta endosulfan	12
alpha endosulfan	12.98

Figure 45. **Representative HPLC-UV chromatograms of analytical standards: Top: 1st series (Method 1), Bottom: combined 1st and 2nd series delivered by the sponsor (Method 2).**

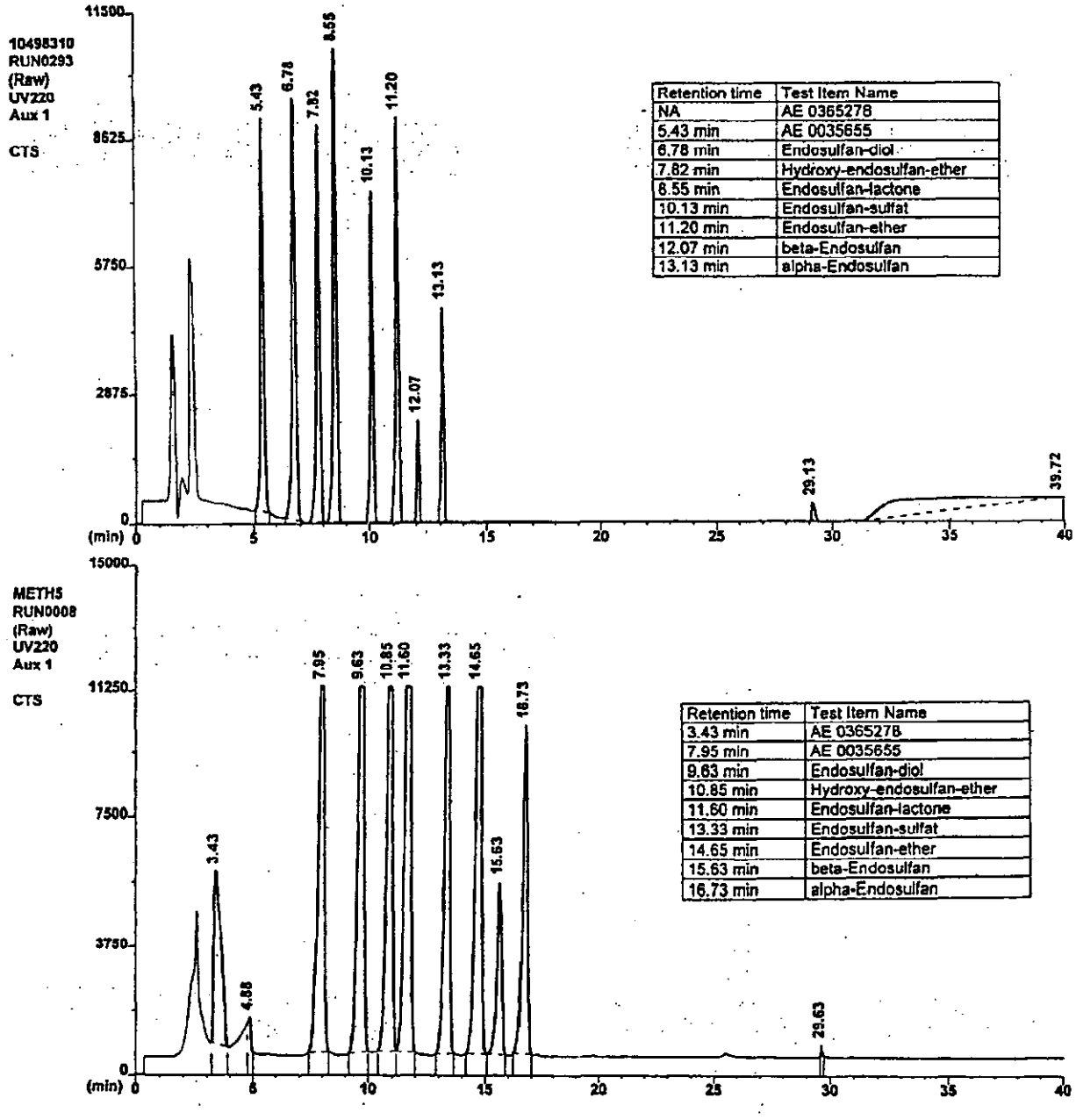
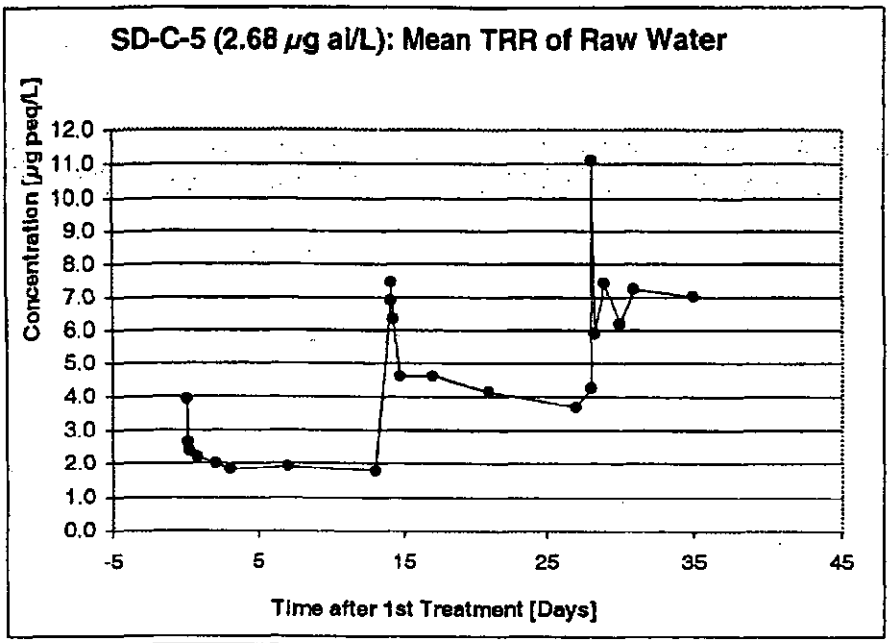


Figure 46. **Spray drift test group SD-2.68: Total Radioactive Residue (TRR) of water: A: Depth integrated raw water, B: Depth integrated water after 0.7 µm filtration.**

A



B

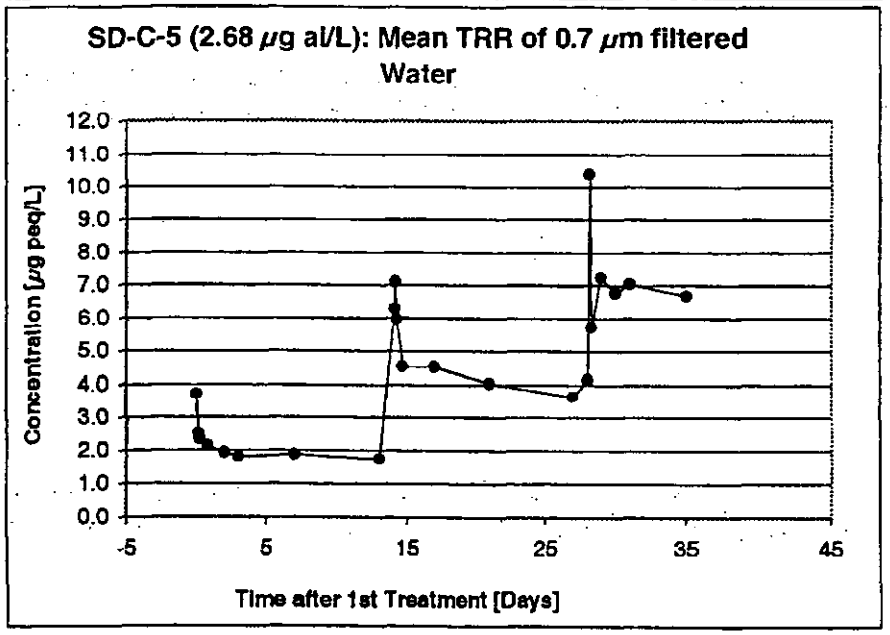
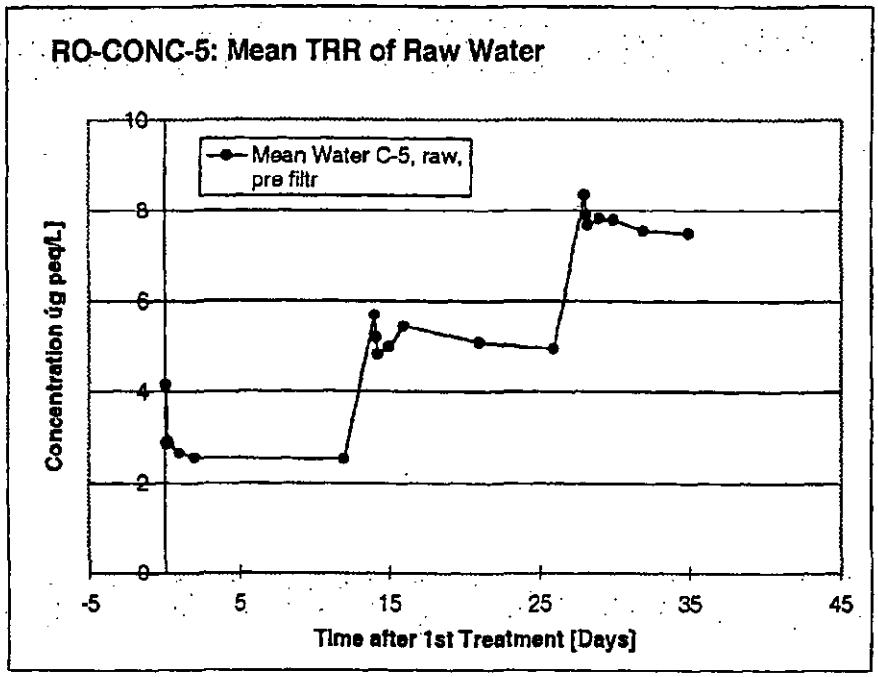


Figure 47. **Run-off test group RO-4.19: Total Radioactive Residue (TRR) of water: A: Depth integrated raw water, B: Depth integrated water after 0.7 µm filtration.**

A



B

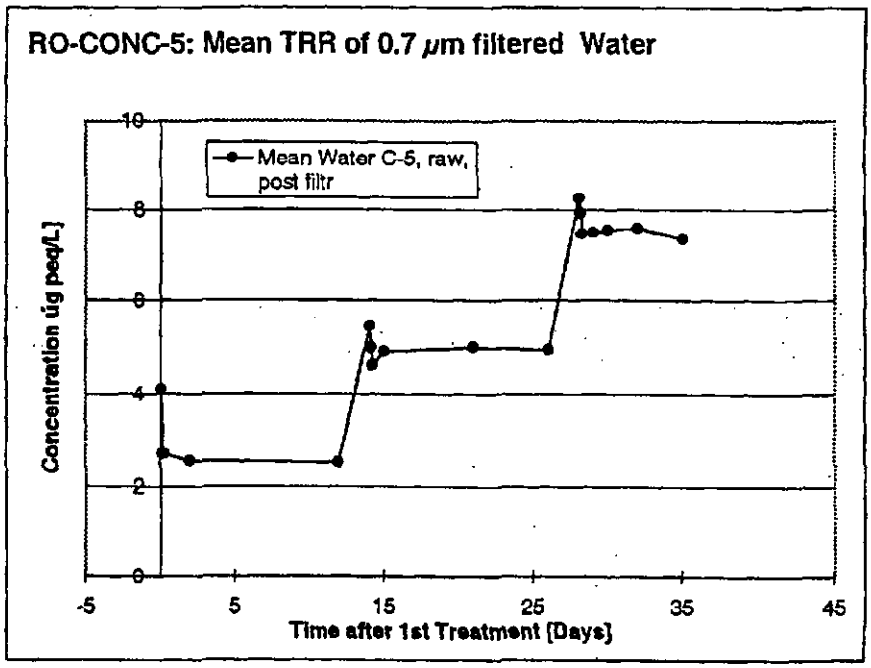


Figure 48. **Run-off test group RO-8.39, one hour after treatment: HPLC-RAM of raw (A) and 0.7 µm filtered (B) raw water. HPLC method 1 was applied.**

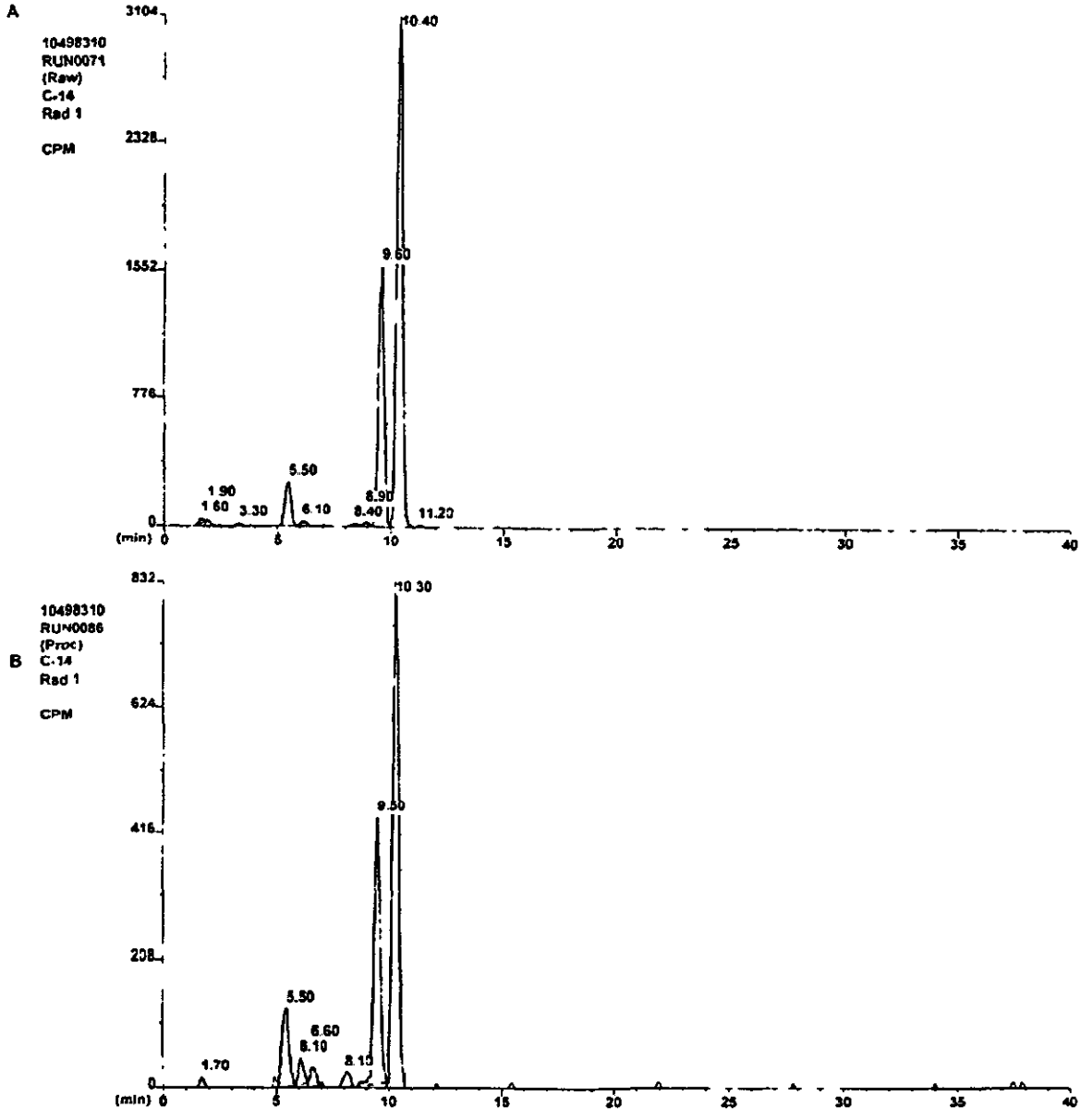


Figure 49. **Run-off test group RO-8.39, one day after 1st treatment: HPLC-RAM of raw (A) and 0.7 µm filtered (B) raw water. HPLC method 1 was applied.**

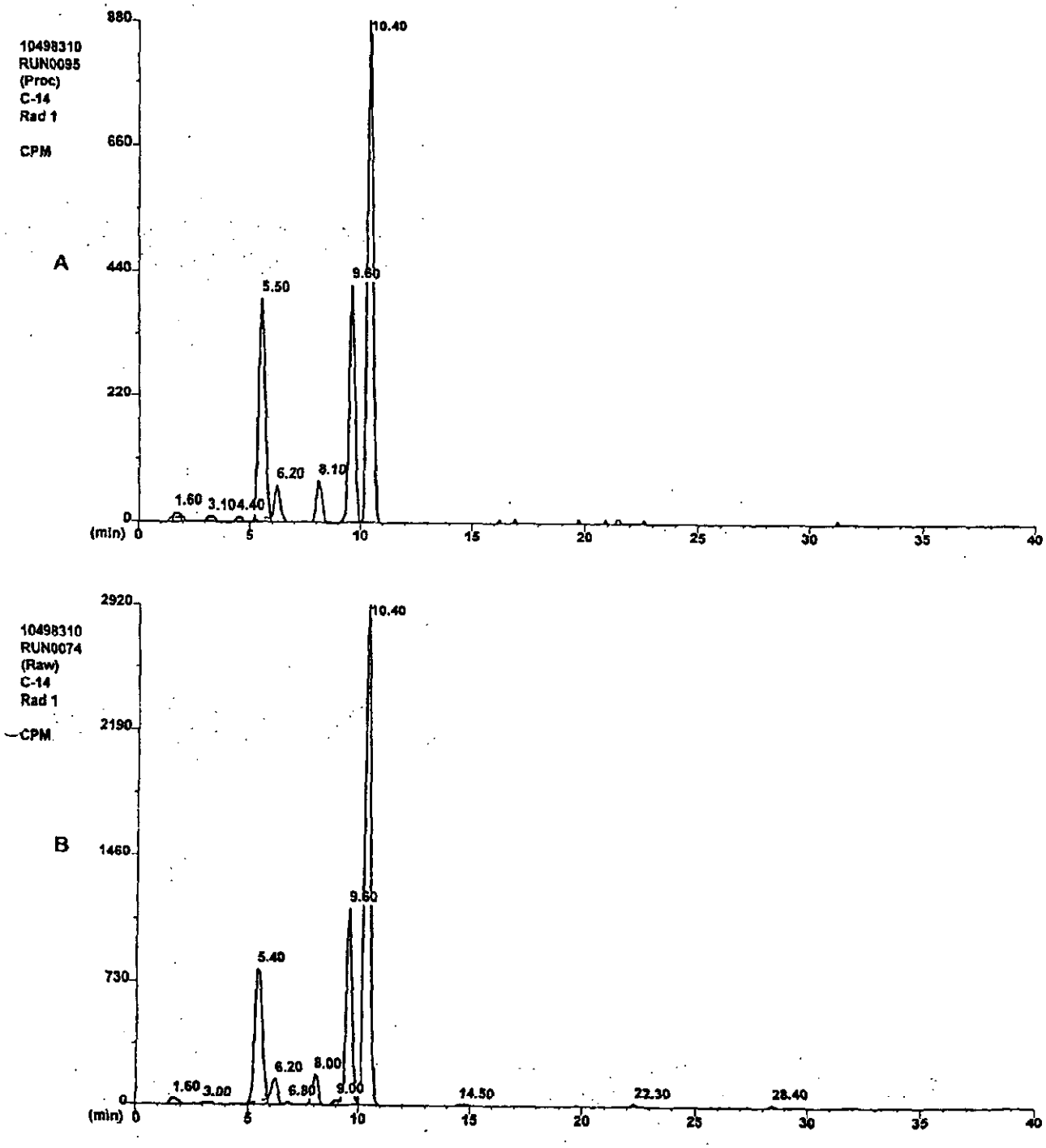


Figure 50. **Run-off test group RO-8.39, seven days after 1st treatment: HPLC-RAM of raw (A) and 0.7 µm filtered (B) raw water. HPLC method 1 was applied.**

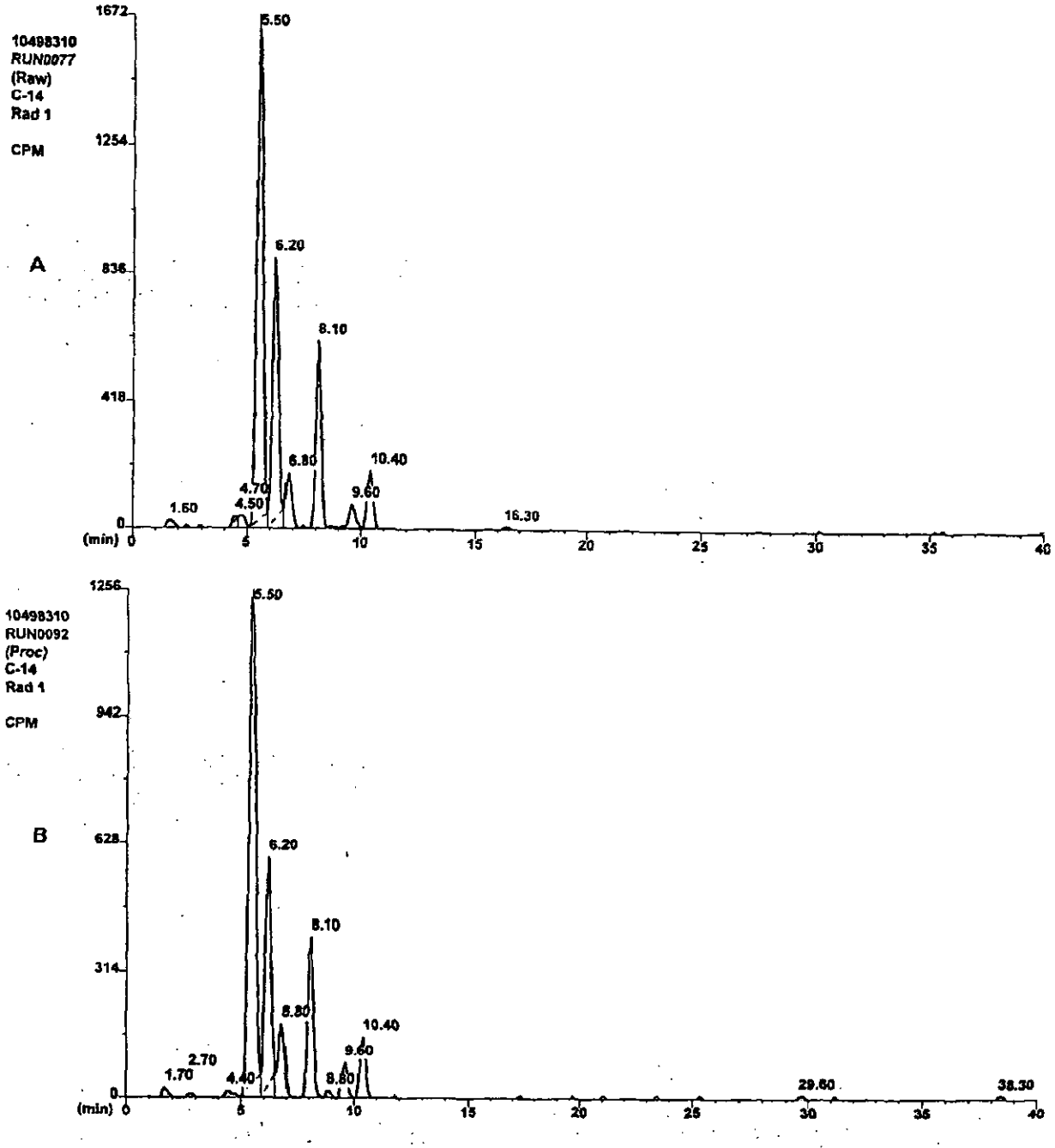


Figure 51. **Spray drift test group SD-2.68, one hour after 1st treatment: HPLC-RAM of raw (A) and 0.7 µm filtered (B) raw water. HPLC method 1 was applied.**

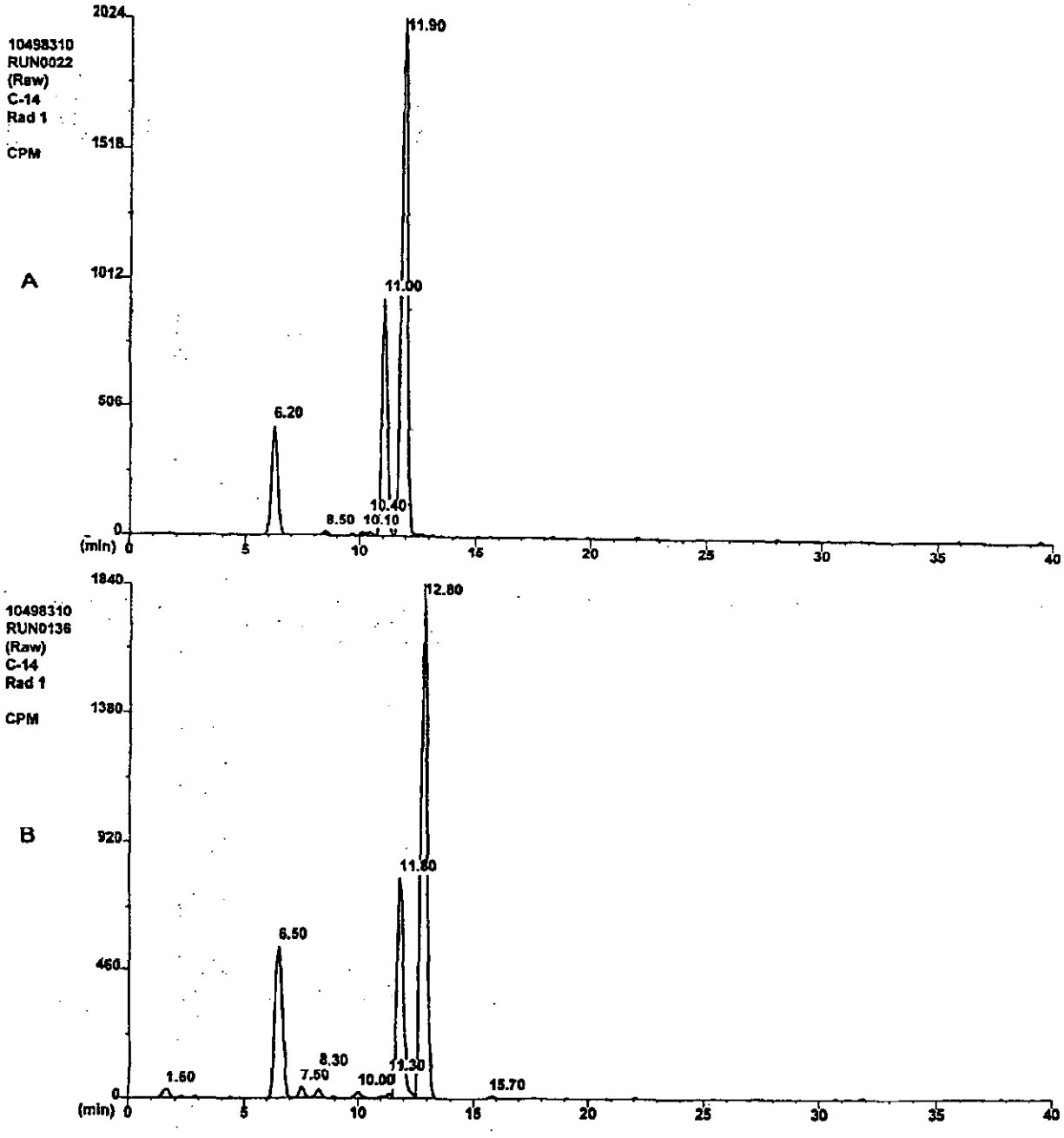


Figure 52. **Representative HPLC-RAM chromatograms of water from test groups SD-0.47 (top), SD-0.84 (middle), and SD-8.38 (bottom), collected on the same sampling day.**

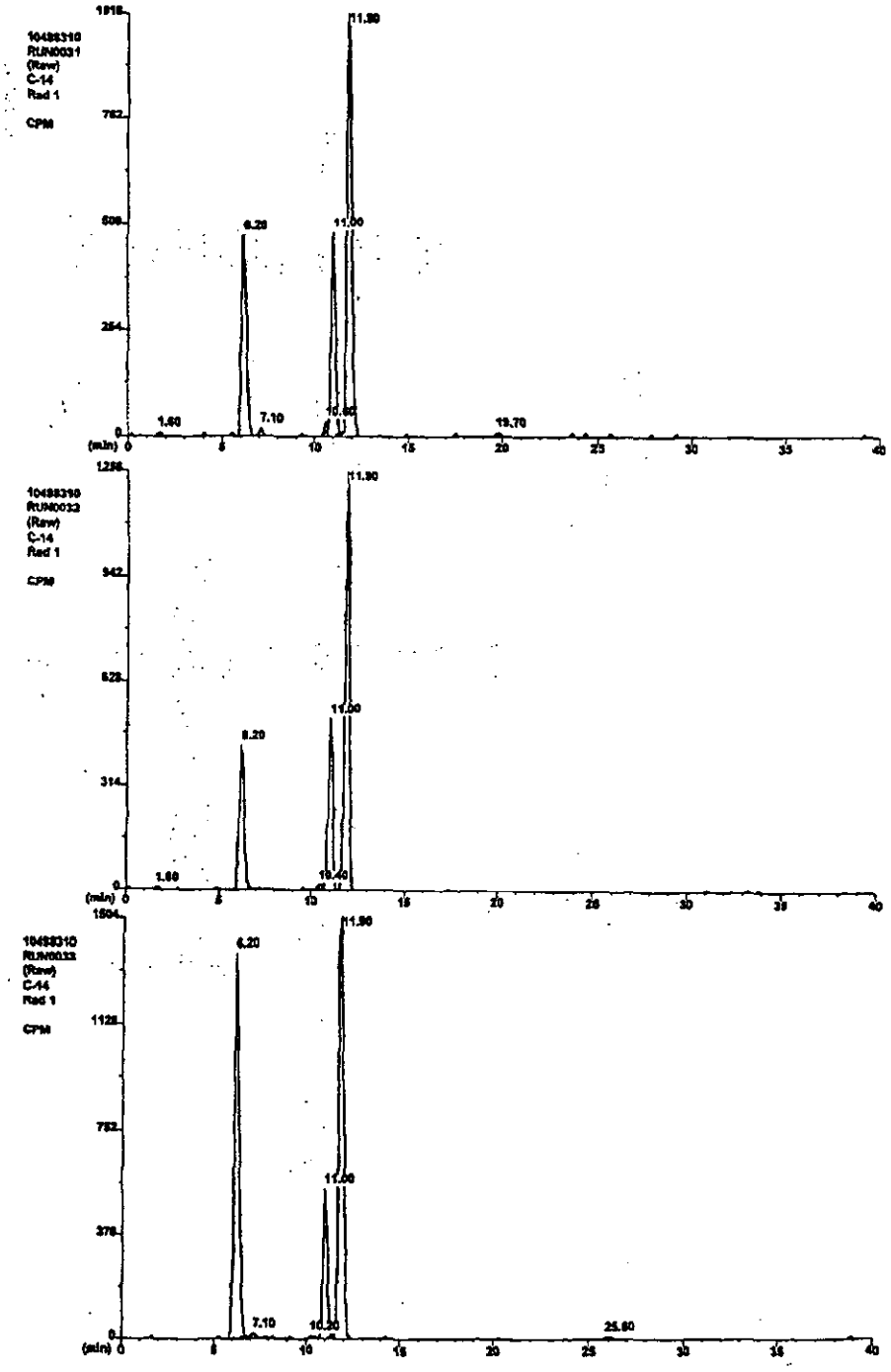
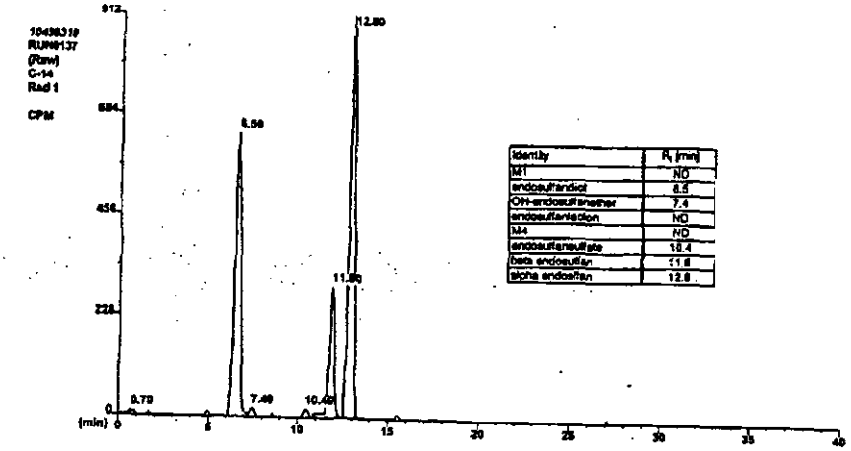
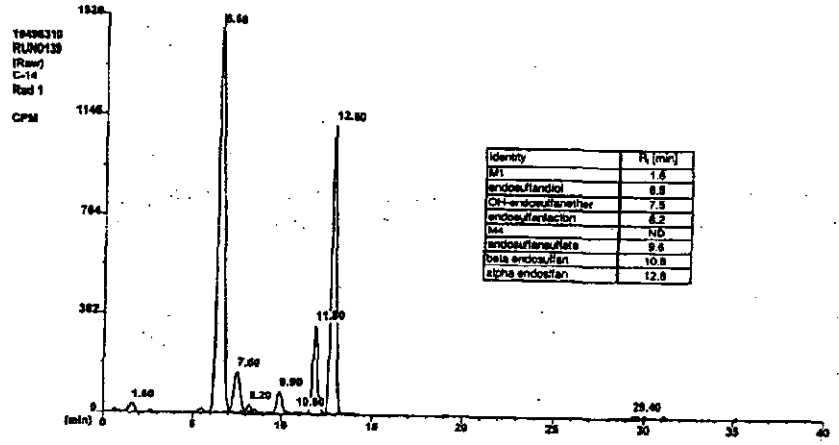


Figure 53. **Spray drift test group SD-2.68: Representative HPLC-RAM chromatograms of water (depth integrated sample after filtration through 0.7 µm filters). A: day 0, 3 hours, B: day 1, C: day 3 after 1st treatment. HPLC method 2 was applied.**

A



B



C