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Title

Kinetic Evaluation of the Dissipation of Endosulfan and its Metabolites Endosulfan Sulfate, Endosulfan Diol and Endosulfan Hydroxy Carboxylic Acid in Aerobic Water - Sediment Test Systems

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1 Summary

For the active substance endosulfan (AE F002671) and its aquatic metabolites endosulfan sulfate, endosulfan diol and endosulfan hydroxy carboxylic acid the results of the two aerobic water-sediment systems Krempe and Ohlau [Jonas, 2002] were fitted to a compartment model assuming first order kinetics and using the software package ACSL.

In order to obtain reliable degradation parameters the data of the test-systems had to be pooled and two separate optimisations had to be performed for endosulfan and the metabolites.

For endosulfan, endosulfan sulfate and endosulfan diol the amounts occurring in surface water and in the sediment were analysed separately. For endosulfan hydroxy carboxylic acid the amounts found in surface water were considered only because none was detected in the sediment.

The kinetic analysis demonstrated that for endosulfan, endosulfan sulfate and endosulfan diol identical half life values for surface water and sediment are able to describe the individual measurements sufficiently well and most reliable.

The dissipation of endosulfan from surface water is much faster than the degradation due to significant transfer of endosulfan to the sediment. From the kinetic analysis done here it can be derived that the dissipation half life for endosulfan assumes a value of 0.99 days. This value is practically equivalent to the geometric mean of 1.1 days for the test systems Krempe and Ohlau as reported by Jonas [2002].

The almost instantaneous formation of endosulfan diol by abiotic hydrolysis was considered in the kinetic evaluation by optimising the initial amounts of endosulfan diol in surface water and sediment. Due to the approximation of this pathway as an instantaneous reaction no rate constants were attributed to it during this initial period of incubation, where the abiotic (or even enzymatically accelerated) hydrolysis occurred as a too quick process as being adequately described by the sampling pattern.

The first order half life (DT50) values obtained for the degradation in the total system (water and sediment) by optimisation are shown in **Table 1**. The total system half life values for endosulfan, endosulfan sulfate and endosulfan diol are also valid for degradation in surface water and in sediment.

Table 1: DT50 values for endosulfan and its aquatic metabolites

Compound	DT50 (days)
Endosulfan	38.0
Endosulfan sulfate	53.2
Endosulfan diol	29.5
Endosulfan hydroxy carboxylic acid	106.4

2 Introduction

Aquatic risk assessment requires measures of exposure of an active substance in a typical surface water environment. For this purpose the distribution of active substances between water and sediment phase, and its degradation in each of those phases may be measured in water-sediment systems in the laboratory. These measurements can often be described by simple first order kinetics or to more fully ascribe the possible mechanisms compartment models using first order kinetics for the distribution and degradation processes involved. The kinetic parameters which lead to the best fit between measured and calculated values may be found using a mathematical optimisation algorithm.

The objective of this study was to determine the first order kinetic parameters which describe the behaviour of endosulfan and its metabolites endosulfan sulfate, endosulfan diol and endosulfan hydroxy carboxylic acid in aquatic systems.

3 Substances

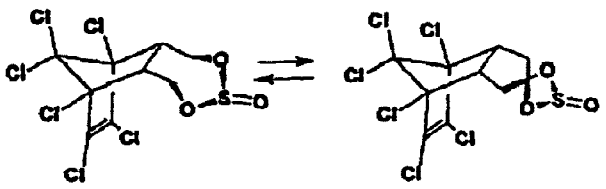
The parent substance endosulfan and its metabolites endosulfan sulfate, endosulfan diol and endosulfan hydroxy carboxylic acid in water-sediment systems may be chemically characterised as follows.

3.1 Endosulfan (AE F002671)

Endosulfan was considered as a mixture of its alpha- and beta-isomers having a molecular mass of 406.9 g mol^{-1}.

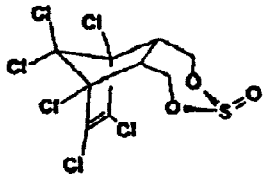
3.1.1 Alpha-endosulfan (AE F052618)

Structure



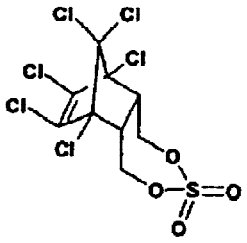
3.1.2 Beta-Endosulfan (AE F052619)

Structure



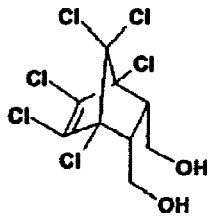
3.2 Endosulfan sulfate (AE F051327)

Structure



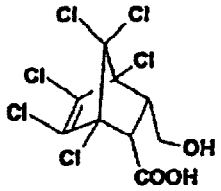
3.3 Endosulfan diol (AE F051329)

Structure



3.4 Endosulfan hydroxy carboxylic acid (AE 0365278)

Structure



4 Experiments and Data

Two studies on the dissipation of [^{14}C]endosulfan, uniformly radio-labelled at all chlorinated carbon atoms of the benzodioxathiepin ring, in aerobic water-sediment systems were used for the determination of the underlying kinetics. These studies are in the following denoted as Krempe and Ohlau [Jonas, 2002]. The samples were incubated at a temperature of 20 °C in the dark for 120 days. Relevant properties of the systems are shown in **Table 2**. The mass recovery for a specific date ranged from 93.8 % to 104.5 % of the total applied dose with the exception of one outlier (114 %).

The metabolites endosulfan sulfate, endosulfan diol and endosulfan hydroxy carboxylic acid were detected at maximum total levels of 25.6 % (Ohlau), 63.5 % (Krempe) and 44.3 % (Ohlau) of the applied radioactivity.

The limit of quantification (LOQ) was given as LOQ = 0.1 % of applied radioactivity. The half of this value, 0.05 %, was attributed to values qualified as below that limit.

Table 2: Physicochemical properties of the water-sediment systems used (CEC is cation exchange capacity). Textural classification follows the German system according to DIN 19682.

Sediment	Texture	pH (water)	Org. carbon	Sand	Silt	Clay	CEC
			--- (g (100 g dry sediment)$^{-1}$) ---				($mmol_c$ (kg dry sediment)$^{-1}$)
Krempe	Silty loam	7.5 – 8.2	3.2	18	60.2	21.8	205
Ohlau	Sand	7.2 – 8.2	0.1	98.8	0	1.2	18

For endosulfan, endosulfan sulfate, endosulfan diol and endosulfan hydroxy carboxylic acid the measured values may be found in section 11.1 in the appendix.

5 Pre-analysis

Preferably each water-sediment test system is evaluated separately, and surface water and sediment compartment are also evaluated separately. Thereby the metabolic pathway as given in the water-sediment study is used and all data are fitted simultaneously.

The first simple first order kinetic analysis of the experimental data (**Table 4** and **Table 5**) showed that this procedure has to be modified in order to achieve reliable kinetic parameter estimates. This was due to large experimental variability and few data remaining which were usable to derive kinetic parameters for the metabolites. These features of the experimental data are briefly illustrated in the following.

Following an expected initial decrease, the total amount of the active endosulfan surprisingly slightly increased in both test-systems (water and sediment) at about 10 - 30 days after application, most pronounced for Krempe.

In this test-system having the higher OC content the hydrolysis product endosulfan diol was formed at a higher level (63.5 % of the applied radioactivity at maximum) than in the system Ohlau (34.6 % of the applied radioactivity at maximum), likely due to an enzymatic increase of the rate of hydrolysis.

The metabolite endosulfan sulfate occurred relatively late in the test-system Ohlau whereas the portion of this metabolite observed in test-system Krempe with the higher OC content was generally marginal, likely due to a higher biodegradation capacity in the system with the higher OC content. The metabolite endosulfan hydroxy carboxylic acid was also formed relatively late, reaching higher levels in the system Ohlau having the lower OC content and therefore obviously a lower biodegradation capacity.

Moreover, at zero time already significant amounts of endosulfan were measured in the sediment and for endosulfan diol in the surface water. Where the first phenomenon may be explained by the extremely high adsorption of endosulfan the

second one suggests a very fast degradation path (hydrolysis) of endosulfan to endosulfan diol possibly related to the fast dissipation of endosulfan from the surface water. The almost instantaneous formation of endosulfan diol was considered in the kinetic evaluation by optimising the initial amounts of endosulfan diol in surface water and sediment. Due to the approximation of this pathway as an instantaneous reaction no rate constants were attributed to it (**Figure 1**).

Trial runs demonstrated that the two data sets had to be pooled to obtain meaningful results.

All attempts to obtain satisfactory results for the parent endosulfan and the metabolites by a simultaneous fit failed. Therefore the kinetic parameters for the metabolites and the parent had to be determined by separate optimisations.

It occurred further that the specific degradation processes in the surface water and in the sediment can be well described by identical half life values. Using different half life parameters has led to unreliable estimates and no superior agreement between measured and calculated values. This was found for all compounds.

Endosulfan hydroxy carboxylic acid occurred in surface water only which was implemented correspondingly in the metabolic scheme used (**Figure 1**).

6 Degradation Pathway

Based on the degradation scheme proposed by [Jonas, 2002], the metabolites considered and the pre-analysis (section 5) the simplified degradation scheme shown in **Figure 1** was developed and used for the kinetic modelling presented in this study.

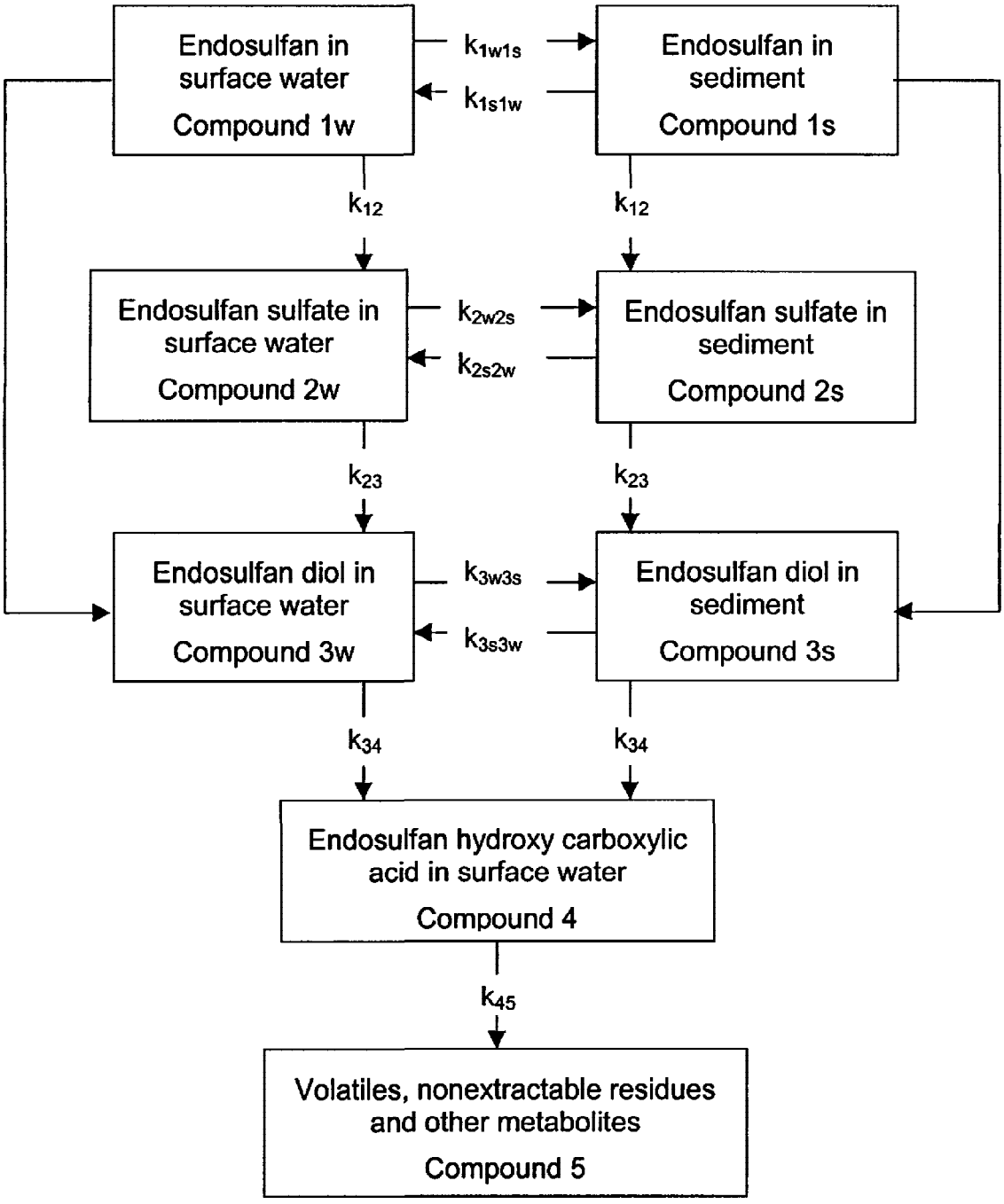


Figure 1: Metabolic pathway for endosulfan in water-sediment systems used for the evaluation of kinetic parameters.

7 Methods

7.1 Hardware / Software

For the kinetic modelling analysis of the aquatic transformation and partitioning data the numerical software package ACSL Optimize 1.2 [ACSL, 1996] was employed. ACSL Optimize uses as object function maximum likelihood which describes the probability to obtain a given set of data assuming that model and model parameter are correct. This includes a submodel of experimental error which can be additionally adjusted by the optimisation procedure. This submodel essentially determines how the assumed experimental error is related to the experimental value. It may be constant, that is independent of the experimental value or proportional to it.

The input differential equations were integrated using a 4^{th} order Runge-Kutta method. The optimisation method was chosen by ACSL Optimize according to the number of parameters. For few parameters the Nelder-Mead algorithm is used for more the Generalized Reduced Gradient method.

Original print outs of relevant ACSL files may be found in section 11.3 in the appendix. Those include the definition of the system of differential equations, a listing of observed and predicted values, the percentage of variation explained by the model, i.e. the coefficient of determination, the optimum parameters, their standard deviation and the parameter correlation matrix.

ACSL Optimize has been frequently used at Bayer CropScience as a flexible tool for comprehensive kinetic analysis.

All calculations were run on an IBM NetVista M41 PC (Intel 845 processor with 4180 MHz, 256 MB RAM) with Microsoft® Windows NT 4.0 as operating system.

7.2 Kinetic Analysis

For the following evaluations it was assumed that all reactions follow first order kinetics. The scheme of transformation of endosulfan in water-sediment systems as shown in **Figure 1** was translated into a corresponding ACSL compartment model (see section 11.2, appendix). As discussed in section 5, separate optimisations had to be performed for endosulfan and its metabolites. Due to the small values of endosulfan sulfate in surface water, the total system values for this compound were used in a first run to obtain a stable fit. In a second run several rate constants were fixed to the previously determined values and it was demonstrated that the degradation rate constant of endosulfan sulfate for the total values is equally valid for the single phases (**Figure 5** and **Figure 6**)

7.2.1 Reaction Equations

The system of reaction equations characterising the compartment model for the total system may be written as

$$dC_{1w}/dt = -(k_{12} + k_{1w1s})\,C_{1w} + k_{1s1w}\,C_{1s} \quad (1)$$

$$dC_{1s}/dt = -(k_{12} + k_{1s1w})\,C_{1s} + k_{1w1s}\,C_{1w} \quad (2)$$

$$dC_{2w}/dt = k_{12}\,C_{1w} - (k_{23} + k_{2w2s})\,C_{2w} + k_{2s2w}\,C_{2s} \quad (3)$$

$$dC_{2s}/dt = k_{12}\,C_{1s} - (k_{23} + k_{2s2w})\,C_{2s} + k_{2w2s}\,C_{2w} \quad (4)$$

$$dC_{3w}/dt = k_{23}\,C_{2} - (k_{34} + k_{3w3s})\,C_{3w} + k_{3s3w}\,C_{3s} \quad (5)$$

$$dC_{3s}/dt = k_{23}\,C_{2} - (k_{34} + k_{3s3w})\,C_{3s} + k_{3w3s}\,C_{3w} \quad (6)$$

$$dC_{4}/dt = -\,k_{45}\,C_{4} + k_{34}(C_{3w} + C_{3s}) \quad (7)$$

where C is the amount of a compound in percentage of applied radioactivity and k_{ij} are rate constants. The subscripts of C and k correspond to the compartment numbers as defined in **Figure 1**.

7.2.2 Initial Condition

Because at zero time already significant amounts of the hydrolysis product endosulfan diol were measured the initial values of endosulfan and endosulfan diol were fitted which is equivalent to the assumption of a very fast reaction from endosulfan to endosulfan diol (see also section 5). The initial values of all other compounds were set to zero.

7.3 Goodness of Fit

Traditionally the goodness of fit is assessed by the coefficient of determination r^2. The basic meaning of this parameter for a non-linear system may be ascribed by the fraction of variance explained by the model. This works satisfactory for datasets having a total variance significantly larger than the measurement uncertainty or error. It fails for cases where the total variance is small compared to the measurement uncertainty. Exactly this is the case for the endosulfan metabolites. Namely endosulfan diol in sediment is a typical example.

As an alternative to assess the goodness of fit the chi-square χ^2 significance test may be used which has not the deficiency of the r^2 mentioned above. A χ^2 value may be calculated as

$$\chi^2 = \sum_{i=1}^{n} (y_i - \hat{y}_i)^2 / \sigma_i^2 \qquad (8)$$

where y_i is a measured and $\hat{y}_i$ is a calculated value, and σ_i is the measurement uncertainty. This χ^2 value can be compared with a tabulated value $\chi^2_{m,\alpha}$ where m is the degrees of freedom which is defined as the number of measurements minus the numbers of model parameter and α is the probability that one may obtain the given χ^2 by chance.

Because σ_i normally is unknown, the procedure most frequently used is to assume a constant σ and to calculate a σ value for the tabulated value $\chi^2_{m,\alpha}$ by inversion of the formula above as

$$\sigma = \sqrt{\sum_{i=1}^{n} (y_i - \hat{y}_i)^2 / \chi^2_{m,\alpha}} \qquad (9)$$

where α = 0.05 is chosen. This σ value represents the minimum measurement uncertainty which has to be presumed in order to pass the χ^2 test. The final assessment is to decide if this σ value corresponds to the experience and expectation of the measurement uncertainty associated with the specific study.

The χ^2 test will be used exclusively in near future in the harmonised evaluation of kinetic data according to FOCUS Kinetics [FOCUS, 2004]. For the calculation of σ presented here the "replicates" of the measured values, i.e., the values for the two systems at identical times, were averaged as recommended by FOCUS [2004].

8 Results

In the following the results of the optimisation are shown. For more statistical details and graphs that show fitted (calculated) and measured data see section 11.3 and **Figure 2** to **Figure 9** in the appendix. Half-life values DT50 are calculated from optimised rate constants k as DT50 = ln(2) / k. Correspondingly, values for DT90 are calculated as DT90 = ln(10) / k. The rate constants k_{ij} are defined in **Figure 1**. The results for endosulfan and its metabolites endosulfan sulfate, endosulfan diol and endosulfan hydroxy carboxylic acid are shown in **Table 3**.

For all compounds reliable parameter estimates were obtained with the exception of endosulfan hydroxy carboxylic acid. Because this is the last metabolite in the considered degradation chain its degradation is overruled by formation during the experimental period. Nevertheless the half life value of 106.4 days obtained appears to be a "worse" case estimation because the calculated line still ascends where the measured values [Jonas, 2002] already stagnate or even decrease (**Figure 9**). Although there is no clear decline of the amounts of endosulfan hydroxy carboxylic acid measured in the experimental period, a degradation rate constant can be identified from the decreasing slope of the experimental curve if the simultaneous formation is numerically subtracted.

Because the variability of the experimental data is necessarily larger when data sets are united and the data of the single test-systems were already relatively variable the coefficient of determination r^2 is smaller as under normal conditions. For the fit of the whole metabolic scheme to the data an overall value of $r^2 = 0.63$ was obtained, where the values measured in surface water were generally better reproduced than those measured in the sediment.

Due to the weakness of r^2 for datasets with small variance, the χ^2 test was applied to assess the goodness of fit as described in section 7.3. For the fit of the whole metabolic scheme to the data an overall measurement error of $\sigma = 3.8$ % of applied radioactivity was obtained to pass the χ^2 test. This is an sufficiently small value compared with the scatter of the data to identify the fits as valid. For details of the χ^2 statistics see section 11.4 in the appendix.

As already mentioned in section 5 the degradation of endosulfan, endosulfan sulfate and endosulfan diol in surface water and in the sediment can be well described by an identical half life value (see **Figure 2** and **Figure 3**, **Figure 7** and **Figure 8**).

For the exchange rate constants for endosulfan in surface water and sediment the values $k_{1w1s} = 0.6822$ days^{-1} to the sediment and $k_{1s1w} = 0.0659$ days^{-1} to surface water were obtained. As expected these values indicate a considerable and fast net flow of endosulfan from the surface water to the sediment (corresponding to an "adsorption" half life value of approximately 1 day). A similar behaviour is found for endosulfan diol with $k_{3w3s} = 0.0391$ days^{-1} to the sediment and $k_{3s3w} = 0$ days^{-1} to the surface water.

From the degradation rate k_{12} and the exchange rate k_{1w1s} a dissipation rate $k_{1\ dissipation}$ for endosulfan in surface water can be calculated as $k_{1\ dissipation} = k_{12} + k_{1w1s} = 0.7005$ days^{-1} where the marginal transfer from the sediment for early times is neglected. This value corresponds to a half life value of $DT50_{\ dissipation} = 0.99$ days for endosulfan which is practically equivalent to the geometric mean of 1.1 days for the test-systems as reported by Jonas [2002].

The optimised values for the initial amounts of endosulfan in surface water were 56 % of applied radioactivity. For endosulfan diol the initial amounts obtained were 27.3 % and 10.5 % of applied radioactivity for surface water and sediment, respectively. As was discussed in section 5 these amounts are attributed to the fast reaction from endosulfan to endosulfan diol (likely abiotic and ezymatically accelerated hydrolysis).

It should be noted again, that the almost instantaneous formation of endosulfan diol was considered in the kinetic evaluation by optimising the initial amounts of endosulfan diol in surface water and sediment. Due to the approximation of this pathway as an instantaneous reaction no rate constants were attributed to it during this initial phase, where the abiotic (or even enzymatically accelerated) hydrolysis was too fast to be adequately described by the sampling pattern.

Table 3: Rate constants k_{ij}, DT50 and DT90 values for the endosulfan and its metabolites.

Compound	Rate constant		DT50	DT90
	Symbol	Value ($days^{-1}$)	(days)	(days)
Endosulfan	k_{12}	0.0183	38.0	126.1
Endosulfan sulfate	k_{23}	0.0130	53.2	176.7
Endosulfan diol	k_{34}	0.0235	29.5	98.1
Endosulfan hydroxy carboxylic acid	k_{45}	0.0065	106.4	353.5

9 Archiving

The detailed simulation results and input data files are archived and can be obtained on diskettes or CD on request.

10 References

ACSL 1996 — ACSL Optimize Version 1.2
MGA Software, Concord, Massachusetts, USA.

FOCUS 2004 — "Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies in EU Registration". Report of the FOCUS Work Group on Degradation Kinetics, Draft of February 2004

Jonas, W. 2002 — Degradation of [^{14}C]Endosulfan in two Aerobic Water/Sediment Systems
Generated by: NATEC Institute
Document No.: C022921
Date: May, 2002

11 Appendix

11.1 Experimental data

Table 4: Measured amounts of endosulfan (alpha- + beta-endosulfan) and the metabolites endosulfan sulfate, endosulfan diol and endosulfan hydroxy carboxylic acid in percentage of applied radioactivity for the test system Krempe [Jonas, 2002]. The abbreviation DAP means days after application, n.d. means not detected.

DAP	Endosulfan		Endosulfan sulfate		Endosulfan diol		Endosulfan hydr. carboxylic acid	
	Water	Sed.	Water	Sed.	Water	Sed.	Water	Sed.
0	66.1	17.6	<0.1	<0.1	16.3	<0.1	<0.1	n.d.
1	27.4	34.3	<0.1	<0.1	31	8.3	<0.1	n.d.
2	6.1	41.2	<0.1	<0.1	35	15	<0.1	n.d.
3	4.3	34.1	0.8	<0.1	24.6	21.3	0.9	n.d.
7	2.6	34.2	<0.1	<0.1	20.1	31	2.3	n.d.
10	1.5	20.4	<0.1	<0.1	22	41.5	3.5	n.d.
14	0.7	45.8	<0.1	<0.1	6.3	32.6	2.5	n.d.
30	0.5	55.6	<0.1	2.1	1	22.7	8.3	n.d.
45	0.3	23.1	<0.1	4.3	1.4	26.6	20.2	n.d.
58	0.2	20.9	<0.1	3.7	1.4	24.5	24.9	n.d.
93	0.2	19.1	0.1	4.5	0.8	14.5	32.9	n.d.
120	0.1	19.4	0.1	7	1.2	15.2	25.3	n.d.

Table 5: Measured amounts of endosulfan (alpha- + beta-endosulfan) and the metabolites endosulfan sulfate, endosulfan diol and endosulfan hydroxy carboxylic acid in percentage of applied radioactivity for the test system Ohlau [Jonas, 2002]. The abbreviation DAP means days after application, n.d. means not detected.

DAP	Endosulfan		Endosulfan sulfate		Endosulfan diol		Endosulfan hydr. carboxylic acid	
	Water	Sed.	Water	Sed.	Water	Sed.	Water	Sed.
0	53.1	20.7	<0.1	<0.1	11.4	<0.1	<0.1	n.d.
1	33.2	46.4	<0.1	<0.1	18.1	<0.1	0.7	n.d.
2	23	45.9	<0.1	<0.1	21.8	4.9	0.9	n.d.
3	17	49	<0.1	<0.1	23.6	5.4	1	n.d.
7	7.8	50.3	<0.1	<0.1	22.9	11.7	1.4	n.d.
10	8	54.4	<0.1	<0.1	17.4	12.4	1.8	n.d.
14	7.5	47.1	0.3	<0.1	16.5	12.2	2.5	n.d.
30	4.3	35.5	2.4	8.5	2.3	4	24.3	n.d.
45	1.7	23.8	3.8	19.9	2.5	2.3	31.7	n.d.
58	0.5	10	5.3	20	3.3	1.7	41.4	n.d.
93	0.5	6.5	3.5	21.2	4.3	1.7	44.3	n.d.
120	0.2	6.2	3.3	22.3	2.8	1.7	44.1	n.d.

11.2 ACSL Program

11.2.1 Endosulfan

```
program acsl
 variable time
 constant k12  = 0.03
 constant k1s1w  = 0.03
 constant k1w1s  = 0.03
 constant c1w0  =0
 constant c1s0  =0

 constant TSTOP = 100.
 initial
  cint = TSTOP/150.
 END
 DYNAMIC
  DERIVATIVE
   dc1w  =  - (k12 + k1w1s) * c1w + k1s1w * c1s
   dc1s  =  - (k12 + k1s1w) * c1s + k1w1s * c1w
  END
  TERMT(time.GE.TSTOP)
 END
 TERMINAL
 END
END
```

11.2.2 Metabolites

```
program acsl
 variable time
 constant k12  = 0.03
 constant k1s1w  = 0.03
 constant k1w1s  = 0.03
 constant k23  = 0.03
 constant k34  = 0.03
 constant k3s3w  = 0.03
 constant k3w3s  = 0.03
 constant k45  = 0.03
 constant c1w0  =0
 constant c1s0  =0
 constant c20  =0
 constant c3w0  =0
 constant c3s0  =0
 constant c40  =0

 constant TSTOP = 100.
 initial
  cint = TSTOP/150.
 END
 DYNAMIC
  DERIVATIVE
   dc1w  =  - (k12 + k1w1s) * c1w + k1s1w * c1s
   dc1s  =  - (k12 + k1s1w) * c1s + k1w1s * c1w
   dc2  =  k12 * c1w + k12 * c1s  - k23  * c2
   dc3w  =  k23 * c2  - k34 * c3w + k3s3w * c3s - k3w3s * c3w
   dc3s  =  k23 * c2  - k34 * c3s - k3s3w * c3s + k3w3s * c3w
   dc4  =  - k45 * c4 + k34 * c3w + k34 * c3s
   c1w    = INTEG(dc1w, C1W0)
   c1s    = INTEG(dc1s, C1S0)
   c2    = INTEG(dc2, C20)
   c3w    = INTEG(dc3w, C3W0)
   c3s    = INTEG(dc3s, C3S0)
   c4    = INTEG(dc4, C40)
  END
  TERMT(time.GE.TSTOP)
 END
 TERMINAL
 END
END
```

11.3 ACSL Optimisation Results

11.3.1 ACSL Output

11.3.1.1 Endosulfan

DESCRIPTION	PARAMETER ESIMATES INITIAL	STANDARD FINAL	DEVIATION
LOG LIKELIHOOD FUNCTION	-18.096	61.587	
K12	0.010159	0.018253	0.0036803
K1W1S	1.05e-008	0.68226	0.089218
K1S1W	1.05e-008	0.065929	0.026564
C1W0	0.56035	0.56036	0.030589
C1S0	0	0.074218	0.043282

TIME	C1W OBSERVED	C1W PREDICTED	PCT ERROR
0	0.661	0.56036	15.2254
0	0.531	0.56036	-5.52919
1	0.274	0.289304	-5.5853
1	0.332	0.289304	12.8603
2	0.061	0.16283	-166.934
2	0.23	0.16283	29.2044
3	0.043	0.103548	-140.809
3	0.17	0.103548	39.0894
7	0.026	0.0515703	-98.3472
7	0.078	0.0515703	33.8843
10	0.015	0.0468254	-212.169
10	0.08	0.0468254	41.4683
14	0.007	0.0433194	-518.848
14	0.075	0.0433194	42.2408
30	0.043	0.0323399	24.791
30	0.005	0.0323399	-546.798
45	0.003	0.0245942	-719.806
45	0.017	0.0245942	-44.6716
58	0.002	0.019399	-869.951
58	0.005	0.019399	-287.981
93	0.002	0.0102408	-412.038
93	0.005	0.0102408	-104.815
120	0.001	0.00625606	-525.606
120	0.002	0.00625606	-212.803

TIME	C1S OBSERVED	C1S PREDICTED	PCT ERROR
0	0.176	0.0742177	57.8309
0	0.207	0.0742177	64.146
1	0.343	0.333796	2.68331
1	0.464	0.333796	28.0612
2	0.412	0.449	-8.98057
2	0.459	0.449	2.17866
3	0.341	0.497215	-45.811
3	0.49	0.497215	-1.47254
7	0.342	0.506894	-48.2146
7	0.503	0.506894	-0.774109
10	0.204	0.48188	-136.216
10	0.544	0.48188	11.4191
14	0.458	0.44816	2.14839
14	0.471	0.44816	4.84918
30	0.355	0.334665	5.72806
30	0.556	0.334665	39.8084
45	0.231	0.25451	-10.1774
45	0.238	0.25451	-6.9369
58	0.209	0.200749	3.94805
58	0.1	0.200749	-100.749
93	0.191	0.105975	44.5156
93	0.065	0.105975	-63.0389
120	0.194	0.0647401	66.6288
120	0.062	0.0647401	-4.41949

STATISTICAL SUMMARY

	MAXIMIZED LOG LIKELIHOOD	WT RESID SUM OF SQUARES	WEIGHTED RESIDUAL SUM	PERCENTAGE VARIATION EXPLAINED	WEIGHTING PARAMETER
C1W	41.61	4.384e-002	-3.317e-002	93.88	0
C1S	19.98	2.659e-001	1.114e-001	51.44	0
OVERALL	61.59	3.098e-001	7.822e-002	75.49	

CORRELATION MATRIX

	K12	K1W1S	K1S1W	C1W0	C1S0
K12	1				
K1W1S	-0.052092	1			
K1S1W	-0.032346	0.63979	1		
C1W0	0.13788	0.40879	0.14253	1	
C1S0	0.37556	-0.32779	-0.063088	-0.53043	1

VARIANCE-COVARIANCE MATRIX

	K12	K1W1S	K1S1W	C1W0	C1S0
K12	1.3544e-005				
K1W1S	-1.7104e-005	0.0079598			
K1S1W	-3.1623e-006	0.0015163	0.00070566		
C1W0	1.5521e-005	0.0011156	0.00011582	0.00093567	
C1S0	5.9821e-005	-0.0012658	-7.2535e-005	-0.00070225	0.0018733

Optimization Method: Generalized Reduced Gradient
INFORM = 0
GRG2 concluded successfully.

Time elapsed: 1.016 seconds
Number of function evaluations: 212

11.3.1.2 Metabolites

DESCRIPTION	PARAMETER ESIMATES INITIAL	PARAMETER ESIMATES FINAL	STANDARD DEVIATION
LOG LIKELIHOOD FUNCTION	79.239	174.24	
K1W1S	0.03	0.69462	0.085008
K1S1W	0.03	0.060862	0.025189
K3W3S	0.03	0.039095	0.01262
K12	0.03	0.0089109	0.0019114
K23	0.03	0.013034	0.0052988
K34	0.03	0.023483	0.004119
K45	0.03	0.0065135	0.0040343
C1W0	0.56	0.55712	0.024425
C3W0	0.37	0.27302	0.022261
C3S0	0.05	0.10516	0.033241
K3S3W	0.03	1e-006	NaN

TIME	C1W OBSERVED	C1W PREDICTED	PCT ERROR
0	0.661	0.557124	15.7149
0	0.531	0.557124	-4.91986
1	0.274	0.282992	-3.28189
1	0.332	0.282992	14.7613
2	0.061	0.155143	-154.332
2	0.23	0.155143	32.5466
3	0.043	0.0954065	-121.876
3	0.17	0.0954065	43.8785
7	0.026	0.0445984	-71.5323
7	0.078	0.0445984	42.8226
10	0.015	0.041301	-175.34
10	0.08	0.041301	48.3737
14	0.007	0.0396296	-466.138
14	0.075	0.0396296	47.1605
30	0.043	0.0343538	20.1075
30	0.005	0.0343538	-587.075
45	0.003	0.0300556	-901.852

45	0.017	0.0300556	-76.7974
58	0.002	0.026768	-1238.4
58	0.005	0.026768	-435.359
93	0.002	0.019596	-879.799
93	0.005	0.019596	-291.92
120	0.001	0.0154056	-1440.56
120	0.002	0.0154056	-670.279
	C1S	C1S	
TIME	OBSERVED	PREDICTED	PCT ERROR
0	0.176	0	100
0	0.207	0	100
1	0.343	0.26919	21.5191
1	0.464	0.26919	41.985
2	0.412	0.392141	4.82022
2	0.459	0.392141	14.5663
3	0.341	0.447022	-31.0914
3	0.49	0.447022	8.77105
7	0.342	0.478836	-40.0106
7	0.503	0.478836	4.80392
10	0.204	0.468326	-129.572
10	0.544	0.468326	13.9106
14	0.458	0.452153	1.27673
14	0.471	0.452153	4.00158
30	0.355	0.392082	-10.4456
30	0.556	0.392082	29.4817
45	0.231	0.343026	-48.4962
45	0.238	0.343026	-44.1287
58	0.209	0.305505	-46.1745
58	0.1	0.305505	-205.505
93	0.191	0.22365	-17.0944
93	0.065	0.22365	-244.077
120	0.194	0.175825	9.3686
120	0.062	0.175825	-183.589
	C2	C2	
TIME	OBSERVED	PREDICTED	PCT ERROR
0	0.001	0	100
0	0.001	0	100
1	0.001	0.00491032	-391.032
1	0.001	0.00491032	-391.032
2	0.001	0.00971349	-871.349
2	0.001	0.00971349	-871.349
3	0.0085	0.0144113	-69.5445
3	0.001	0.0144113	-1341.13
7	0.001	0.0321833	-3118.33
7	0.001	0.0321833	-3118.33
10	0.001	0.0444891	-4348.91
10	0.001	0.0444891	-4348.91
14	0.001	0.0596142	-5861.42
14	0.0035	0.0596142	-1603.26
30	0.109	0.10723	1.62377
30	0.0215	0.10723	-398.745
45	0.0435	0.136545	-213.897
45	0.237	0.136545	42.3861
58	0.0375	0.152741	-307.309
58	0.253	0.152741	39.6281
93	0.046	0.167437	-263.994
93	0.247	0.167437	32.2117
120	0.071	0.161307	-127.193
120	0.256	0.161307	36.9895
	C3W	C3W	
TIME	OBSERVED	PREDICTED	PCT ERROR
0	0.163	0.273024	-67.4994
0	0.114	0.273024	-139.495
1	0.31	0.256494	17.26
1	0.181	0.256494	-41.7094
2	0.35	0.241028	31.1348
2	0.218	0.241028	-10.5634
3	0.246	0.22656	7.9023
3	0.236	0.22656	3.99986
7	0.201	0.177489	11.6971
7	0.229	0.177489	22.4939
10	0.22	0.148486	32.5065
10	0.174	0.148486	14.6634

14	0.063	0.118026	-87.3432
14	0.165	0.118026	28.469
30	0.023	0.05508	-139.478
30	0.01	0.05508	-450.8
45	0.014	0.0374227	-167.305
45	0.025	0.0374227	-49.6907
58	0.014	0.0335591	-139.708
58	0.033	0.0335591	-1.69433
93	0.008	0.0343078	-328.848
93	0.043	0.0343078	20.2144
120	0.012	0.0342475	-185.395
120	0.028	0.0342475	-22.3123

	C3S	C3S	
TIME	OBSERVED	PREDICTED	PCT ERROR
0	0.0005	0.105155	-20931.1
0	0.0005	0.105155	-20931.1
1	0.083	0.112972	-36.1106
1	0.0005	0.112972	-22494.4
2	0.15	0.120052	19.9656
2	0.049	0.120052	-145.003
3	0.213	0.12645	40.6337
3	0.054	0.12645	-134.167
7	0.31	0.146219	52.8327
7	0.117	0.146219	-24.9732
10	0.415	0.156108	62.3837
10	0.124	0.156108	-25.8932
14	0.326	0.164463	49.5513
14	0.122	0.164463	-34.8056
30	0.04	0.169007	-322.519
30	0.227	0.169007	25.5474
45	0.266	0.16094	39.4962
45	0.023	0.16094	-599.74
58	0.245	0.15516	36.6692
58	0.017	0.15516	-812.709
93	0.145	0.150599	-3.86125
93	0.017	0.150599	-785.875
120	0.152	0.149786	1.45681
120	0.017	0.149786	-781.092

	C4	C4	
TIME	OBSERVED	PREDICTED	PCT ERROR
0	0.0005	0	100
0	0.0005	0	100
1	0.0005	0.00874907	-1649.81
1	0.007	0.00874907	-24.9867
2	0.0005	0.0172412	-3348.25
2	0.009	0.0172412	-91.5693
3	0.009	0.0254857	-183.175
3	0.01	0.0254857	-154.857
7	0.023	0.0561607	-144.177
7	0.014	0.0561607	-301.148
10	0.035	0.0769748	-119.928
10	0.018	0.0769748	-327.638
14	0.025	0.102179	-308.716
14	0.025	0.102179	-308.716
30	0.243	0.181025	25.5043
30	0.083	0.181025	-118.102
45	0.202	0.234417	-16.0478
45	0.317	0.234417	26.0515
58	0.249	0.271811	-9.16104
58	0.414	0.271811	34.3452
93	0.329	0.352702	-7.20422
93	0.443	0.352702	20.3833
120	0.253	0.403257	-59.39
120	0.441	0.403257	8.55857

STATISTICAL SUMMARY

	MAXIMIZED LOG LIKELIHOOD	WT RESID SUM OF SQUARES	WEIGHTED RESIDUAL SUM	PERCENTAGE VARIATION EXPLAINED	WEIGHTING PARAMETER
C1W	40.89	4.654e-002	-1.675e-002	93.50	0
C1S	15.9	3.734e-001	-2.805e-001	31.81	0

C2	31.67	1.003e-001	-4.357e-001	48.33	0
C3W	34.62	7.848e-002	-1.914e-001	70.68	0
C3S	18.92	2.905e-001	-3.203e-001	10.38	0
C4	32.24	9.574e-002	-3.090e-001	83.88	0
OVERALL	174.2	9.850e-001	-1.554e+000	62.74	

CORRELATION MATRIX

	K1W1S	K1S1W	K3W3S	K12	K23	K34
K1W1S	1					
K1S1W	0.59942	1				
K3W3S	0.013666	0.0083715	1			
K12	0.092839	0.074298	0.08481	1		
K23	0.1093	0.065061	0.097332	0.63223	1	
K34	0.086139	0.052069	0.07027	0.5176	0.58513	1
K45	0.12187	0.073788	0.067973	0.73523	0.83236	0.69091
C1W0	0.26383	0.10228	0.039114	0.19384	0.32066	0.24943
C3W0	0.0038821	0.0022351	0.50084	0.020616	0.02202	0.04619
C3S0	-0.029401	-0.018092	-0.44327	-0.18444	-0.21239	-0.25665
K3S3W	NaN	NaN	NaN	NaN	NaN	NaN

	K45	C1W0	C3W0	C3S0	K3S3W
K45	1				
C1W0	0.35239	1			
C3W0	0.10303	0.011701	1		
C3S0	-0.041755	-0.083817	-0.40959	1	
K3S3W	NaN	NaN	NaN	NaN	1

VARIANCE-COVARIANCE MATRIX

	K1W1S	K1S1W	K3W3S	K12	K23	K34
K1W1S	0.0072263					
K1S1W	0.0012835	0.00063446				
K3W3S	1.4661e-005	2.6612e-006	0.00015927			
K12	1.5085e-005	3.5771e-006	2.0458e-006	3.6535e-006		
K23	4.9233e-005	8.6836e-006	6.5087e-006	6.4033e-006	2.8077e-005	
K34	3.0161e-005	5.4023e-006	3.6528e-006	4.0751e-006	1.2771e-005	1.6966e-005
K45	4.1794e-005	7.4983e-006	3.4608e-006	5.6696e-006	1.7793e-005	1.1481e-005
C1W0	0.0005478	6.2924e-005	1.2057e-005	9.0496e-006	4.1501e-005	2.5095e-005
C3W0	7.3466e-006	1.2533e-006	0.00014071	8.7722e-007	2.5974e-006	4.2354e-006
C3S0	-8.3081e-005	-1.5149e-005	-0.00018596	-1.1719e-005	-3.741e-005	-3.5141e-005
K3S3W	5.8607e-008	1.0398e-008	-5.448e-007	7.7354e-009	2.3059e-008	2.5778e-008

	K45	C1W0	C3W0	C3S0	K3S3W
K45	1.6276e-005				
C1W0	3.4724e-005	0.00059658			
C3W0	9.2532e-006	6.3625e-006	0.00049557		
C3S0	-5.5997e-006	-6.8053e-005	-0.0003031	0.001105	
K3S3W	1.9734e-008	4.9158e-008	-3.1356e-007	2.5914e-007	-3.4972e-007

Optimization Method: Generalized Reduced Gradient
INFORM = 8
GRG2 stopped because number of function evaluations was exceeded.

Time elapsed: 4.484 seconds
Number of function evaluations: 639

11.3.2 Figures

11.3.2.1 Endosulfan

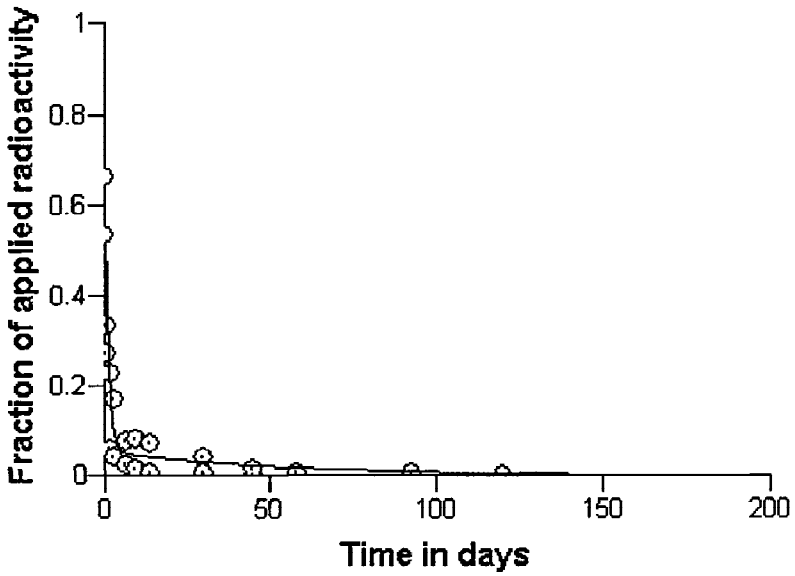


Figure 2: Modelled (solid line) and measured (symbols) amounts of endosulfan in surface water in the test-systems Krempe and Ohlau.

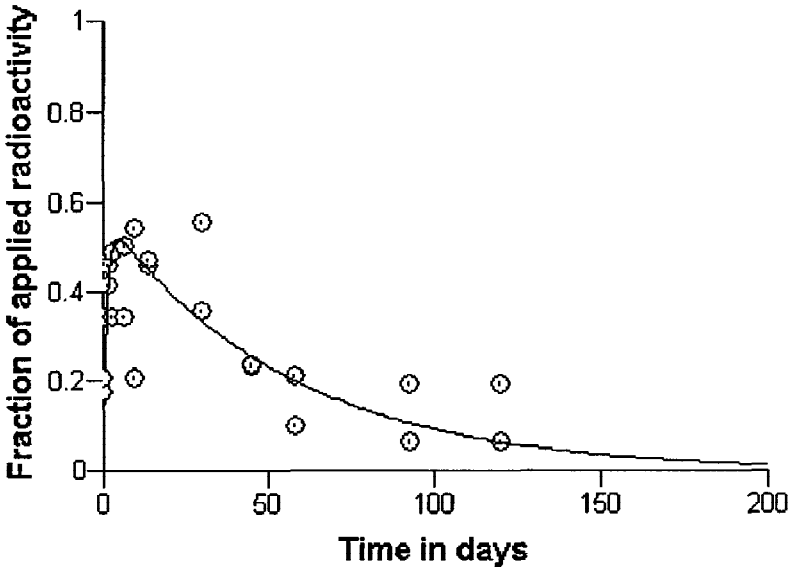


Figure 3: Modelled (solid line) and measured (symbols) amounts of endosulfan in the sediment in the test-systems Krempe and Ohlau.

11.3.2.2 Endosulfan sulfate

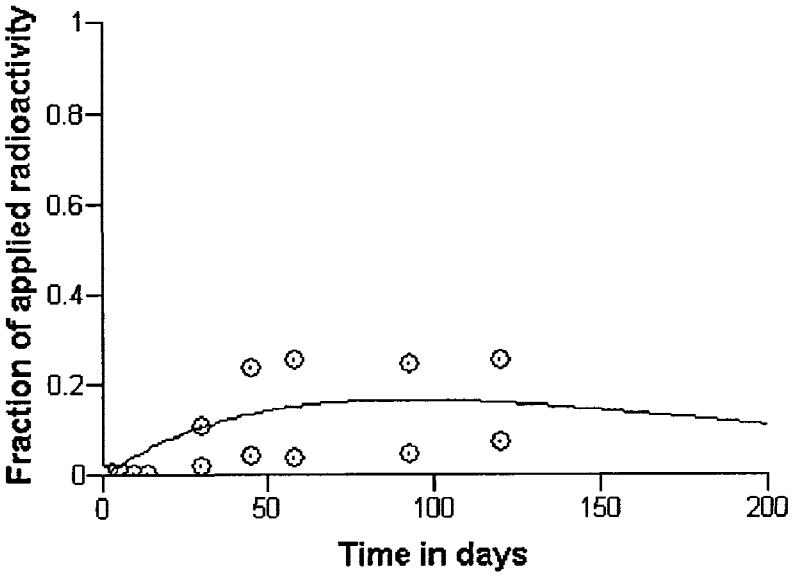


Figure 4: Modelled (solid line) and measured (symbols) total amounts of endosulfan sulfate in the test-systems Krempe and Ohlau.

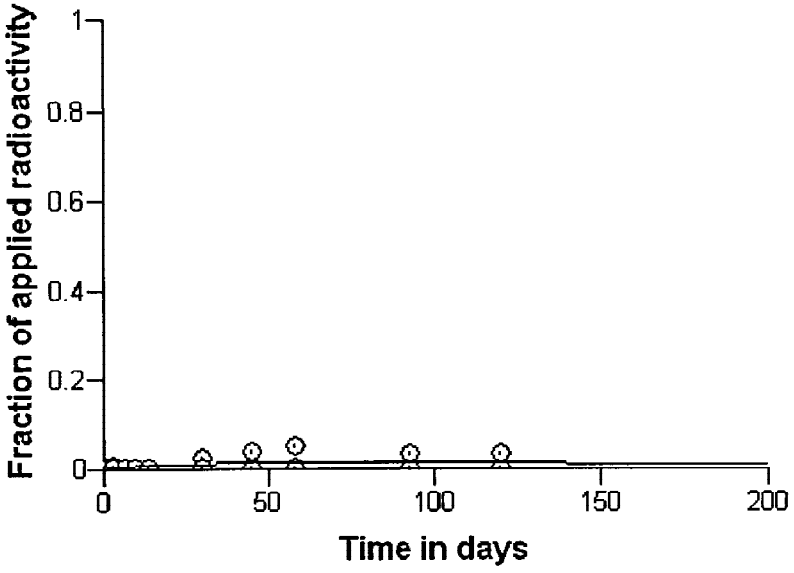


Figure 5: Modelled (solid line) and measured (symbols) amounts of endosulfan sulfate in surface water in the test-systems Krempe and Ohlau.

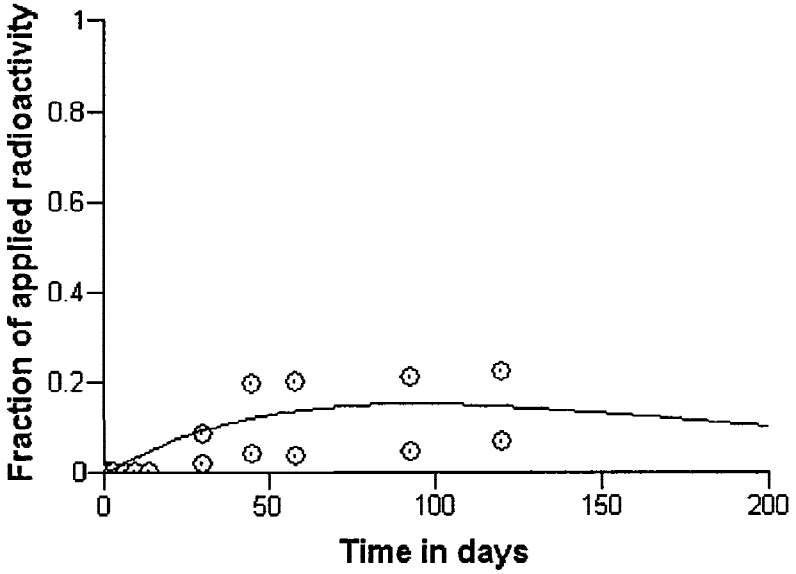


Figure 6: Modelled (solid line) and measured (symbols) amounts of endosulfan sulfate in the sediment in the test-systems Krempe and Ohlau.

11.3.2.3 Endosulfan diol

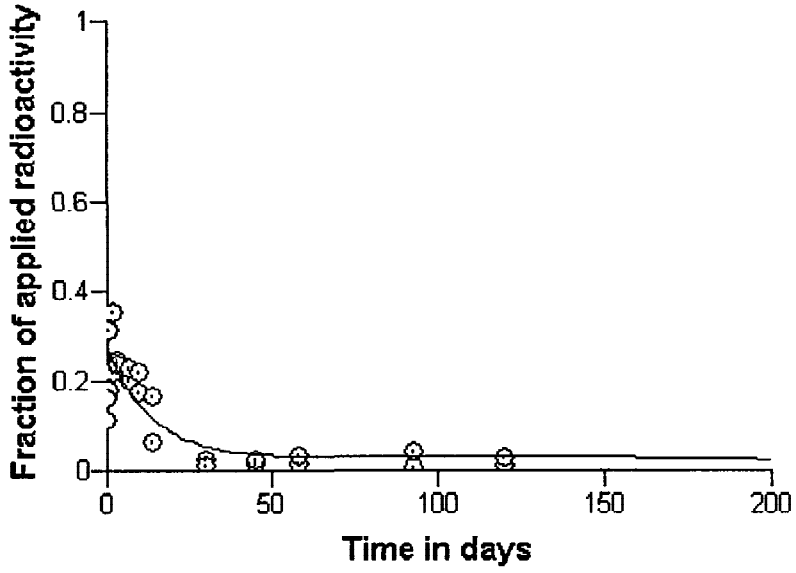


Figure 7: Modelled (solid line) and measured (symbols) amounts of endosulfan diol in surface water for the test-systems Krempe and Ohlau.

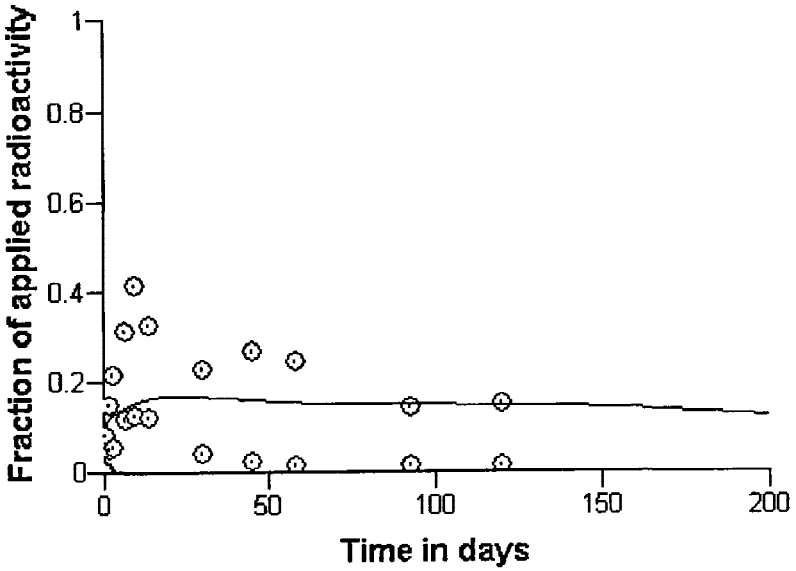


Figure 8: Modelled (solid line) and measured (symbols) amounts of endosulfan diol in the sediment for the test-systems Krempe and Ohlau.

11.3.2.4 Endosulfan hydroxy carboxylic acid

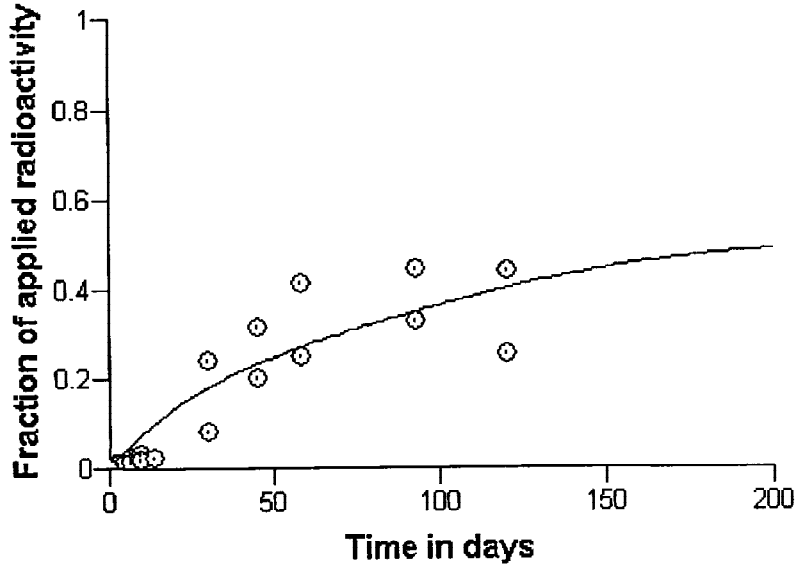


Figure 9: Modelled (solid line) and measured (symbols) amounts of endosulfan hydroxy carboxylic acid in surface water for the test-systems Krempe and Ohlau.

11.4 Chi-square Statistics

Table 6: Chi-square statistics including tabulated values of χ^2 for α = 0.05 and minimum measurement uncertainty σ

Compound	Sum of squares (fraction of a.r.)^2	χ^2	σ (% of a.r.)
Endosulfan water	0.0037	16.92	1.48
Endosulfan sediment	0.1198	16.92	8.42
Endosulfan sulfate water	0.00041	15.51	0.51
Endosulfan sulfate sediment	0.00693	15.51	2.11
Endosulfan diol water	0.02789	15.51	4.24
Endosulfan diol sediment	0.04493	15.51	5.38
Endosulfan hydroxy carboxylic acid water	0.02938	15.51	4.35
Mean			3.79