FLUVARIUM CHANNEL EXPERIMENTS

Fluvarium channels were used as described by Zhmud *et al.*, (1997).

Channels without sediment

Experiments were performed in the absence of sediment under a range of conditions as shown in Table 1. At the beginning of each experiment the channels were filled with 24 litres of either 10 mmol/litre $KHCO_3$ to simulate the ionic strength of river water or water that had been in contact with sediment (taken from the end of the experiment with the channel containing sediment as described below). Each channel was then spiked with a solution containing both isomers of permethrin in acetone and either exposed to natural light or covered and kept dark. Samples of water were collected at different times and analysed for permethrin. The volume of water in channels was maintained constant. The solution pH, dissolved oxygen concentration and temperature were recorded at intervals through the experiment.

Table 1. Summary of the experiments with the channels containing only water. Mean pH and temperature are given for both channels. RW: River Water.

Exp. No.	Spiking level (µg)		Characteristics				No samples
	cis	*trans*	Mean pH	Mean temp (°C)	Channel 1	Channel 2	
1	480	480	8.9	13.0	light	light	6
2	480	480	8.6	11.6	light	light	8
3	480	1200	8.8	11.6	light	dark	25
4	480	480	9.0	14.2	light / RW	dark / RW	17

Channel containing water and sediment

Surface bed-sediment (< 5 cm depth) was collected from the River Calder in Yorkshire (NGR SE409258) in May 2000 and sieved (2 mm) onsite. One of the channels was filled to 50 mm depth with the sediment and 20 litres of 10 mmol/litre $KHCO_3$. The channel was spiked with 400 µg of *cis* and *trans* permethrin and left for 6 weeks. Experiments with other solutes have shown a mixing time for the channels of *ca* 10 min. Water samples were taken at intervals for permethrin analysis and the solution pH, dissolved oxygen concentration and temperature recorded throughout the experiment. Within a week, a layer of diatom biofilm developed at the interface that subsequently diversified to a filamentous community. After four weeks, the channel was divided widthways into two equal sections: Section A which naturally contained few native *oligochaete* worms (as judged by their activity at the surface) with Section B supplemented by the equivalent of 1000 worms/m^2. The worms were also collected in May 2000, from surface bed sediments from the River Aire in Yorkshire (NGR: SE534255) and were identified as *Limnodrilus* spp. and *Potamothrix* spp. with some *Tubifex* spp. (probably *Tubifex tubifex*). After 6 weeks, the water was removed and sediment horizontally sectioned every millimetre down to 5 mm, then single sections between 5-10 mm, 10-30 mm and finally every 10-mm down to the bottom using a slicing tool. Sediment sections were sub-sampled for permethrin analysis, porosity determination, and centrifuged to collect porewaters for dissolved silicon analysis. The latter results provide information on bioturbation effects on

porewater movement (Zhmud *et al.*, 1997). A sample of biofilm was also collected at the end of the experiment and analysed for permethrin.

RESULTS AND DISCUSSION

Recoveries for both SPE and SFE were similar to those given by Long *et al.*, (2000).

Adsorption-degradation reactions

The mean temperature and pH for both channels are given in Table 1. The dissolved oxygen concentrations were not measured in Experiments 1 and 2 but were between 95 and 100 % saturation in Experiments 3 and 4. In all of the four experiments in the absence of sediment (as listed in Table 1), the variation in total mass of permethrin present in solution over time followed a similar trend. Experiment 3 was typical (Figure 1).

The data from each experiment were divided into a set where sorption was the main process occurring, and a second one where degradation was the main pathway for loss of permethrin from the overlying water. The separation into two sets of data was done from the intercept of the two linear portions of the first-order rate plots. First-and second-order reaction kinetics were applied to these data to obtain net sorption and degradation rates for both isomers, i.e. isomer interconversion was not taken into account. This analysis indicated that the rapid loss (at < 1 d) was caused by adsorption on the walls of the channels, whereas the much slower rate after one day was caused by degradation. Permethrin is highly hydrophobic (pK_{ow}=6.1 at 20 °C) and adsorbs to glass and PTFE (Sharom *et al.*, 1981; House & Ou, 1992).

Rate constants of sorption and degradation (k_{ads} and k_{deg} respectively) were deduced from first-order kinetic plots. The results are summarised in Tables 2 and 3 and show an intercept close to zero and good correlations, i.e. $r^2 > 0.6$. The correlation coefficients for the first-order plots were consistently higher than the second-order plots. The first-order degradation rate constant for the *cis* isomer is in good agreement with the value of 8.75 x 10^{-6} min^{-1} determined in sandy- loam soils by Jordan *et al.*,(1982) but the value for the *trans* isomer is much smaller in comparison i.e. 34.0 x 10^{-6} min^{-1}.

Table 2. First-order rate constants and standard deviations for the degradation and sorption for *cis* and *trans* permethrin determined from changes in overlying water concentration.

	Permethrin	Type of reaction	k_{ads} / $10^{-3}min^{-1}$
Water	*cis*	sorption	1.57 ± 0.21
Water	*trans*	sorption	1.71 ± 0.22
Sediment	*cis*	sorption	2.72
Sediment	*trans*	sorption	3.01
			k_{deg}/ $10^{-6}min^{-1}$
Water	*cis*	degradation	8.04 ± 5.85
Water	*trans*	degradation	8.78 ± 2.44
Sediment	*cis*	degradation	10.44
Sediment	*trans*	degradation	7.43

A statistical t-test (95 % confidence limit) applied to the set of data presented in Table 3 did not show any significant differences between the results from the experiments, *viz*: light/dark, *cis* / *trans* isomers and river water/synthetic solution, with the exception of Experiment 3. This

showed higher k_{deg} for the *trans* isomer compared with the *cis*. The t-test also showed no differences in the rate constants over the temperature range measured (Table 1).

Table 3. Degradation rates and half-lives in the different experimental conditions.

Compound	Exp. No.	Conditions	Range $k_{deg}/10^{-6}min^{-1}$	Half-life/days
cis permethrin	3	Light	6.33	76.0
	3	Dark	5.15	93.5
	4	Light, river water	16.7	28.8
	4	Dark, river water	7.07	68.1
trans permethrin	3	Light	10.08	47.8
	3	Dark	9.78	49.2
	4	Light, river water	10.14	47.5
	4	Dark, river water	5.12	94.0

Uptake of permethrin by the bed-sediment

The mean temperature, pH and dissolved oxygen saturation of the overlying solution was 19.8 °C, 8.8 and 90 % respectively. In the presence of sediment the concentration of permethrin in the solution overlying the sediment decreased rapidly during the first day as shown in Figure 1 with final concentrations after 43 d of 0.03 and 0.01 µg/litre for the *cis* and *trans* isomers respectively. Generally the *trans* isomer was found at a slightly lower concentration than the *cis* isomer throughout the experiment. As expected, the sorption rates in the experiment containing sediment are higher than with water only (Table 2). However, the degradation rates are in the range of values obtained with the channels containing only water (Table 2). After 43 days, permethrin was observed to have penetrated to a maximum depth of 20 mm with a concentration of the *trans* isomer generally higher than the *cis* (Table 4). The profile of total permethrin in Section A was steeper than in Section B which contained an enhanced density of tubificids (Figure 2). The concentrations in the deeper sediment, i.e. > 5 mm, were consistently slightly higher in Section B compared with Section A. The concentration in the biofilm was found to be approximately 470 ng/g (dry weight) for both isomers. Overall the results show that the sediment was a sink for permethrin with 97 % of the total permethrin in the sediment bed.

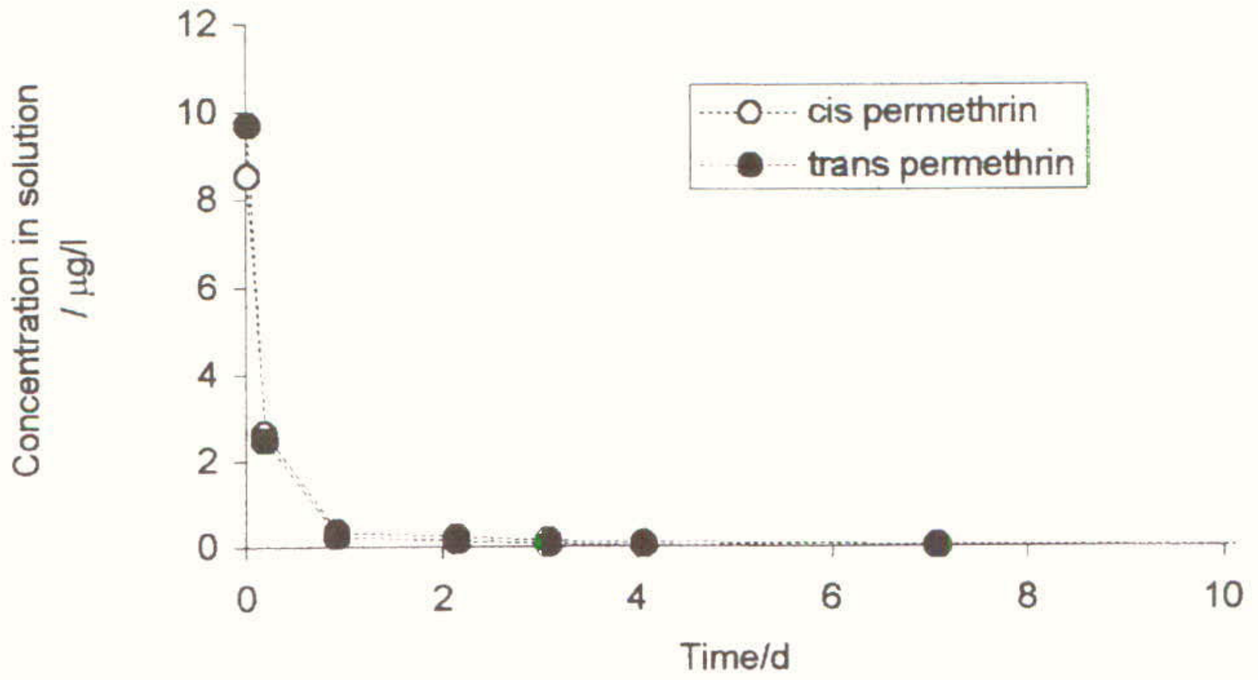

Figure 1. Changes in the solution concentration of the permethrin isomers overlying the bed-sediment in the channel in Experiment 3.

Table 4. Profiles of the isomers and porosity in horizontally sliced sediments. All concentrations expressed as μg/kg (dry weight of sediment). ND: Not Detected.

Depth (mm)	Section A		Section B		Porosity
	cis	*trans*	*cis*	*trans*	
0.5	407	630	108	476	0.74
1.5	272	306	125	350	0.73
2.5	110	164	110	239	0.62
3.5	92	109	80	167	0.86
4.5	65	43	66	103	0.68
7.5	ND	70	57	64	0.54
20	ND	ND	25	ND	0.59
35	ND	ND	ND	ND	0.65
45	ND	ND	ND	ND	0.49

However, the mass balance at the end of the experiment was relatively poor with only 40 % of the initially spike (i.e. 800 μg of total permethrin) accounted for in the overlying solution and sediment. This difference may be a result of several factors including the adsorption of permethrin to the channel sides, degradation in the water and sediment and losses to the biofilm not quantified in the experiment. In particular the concentration found at the first sampling time at t= 15 min was much lower than the expected value of 20 μg/litre for each isomer. This decrease was not caused by adsorption to the channel as a prediction using the rate constant in Table 2 gave a decrease of *ca* 1 μg/litre in 15 min and was too fast for degradation reactions (see above). Hence the reason for this decrease is as yet unknown and further experiments to investigate this are underway.

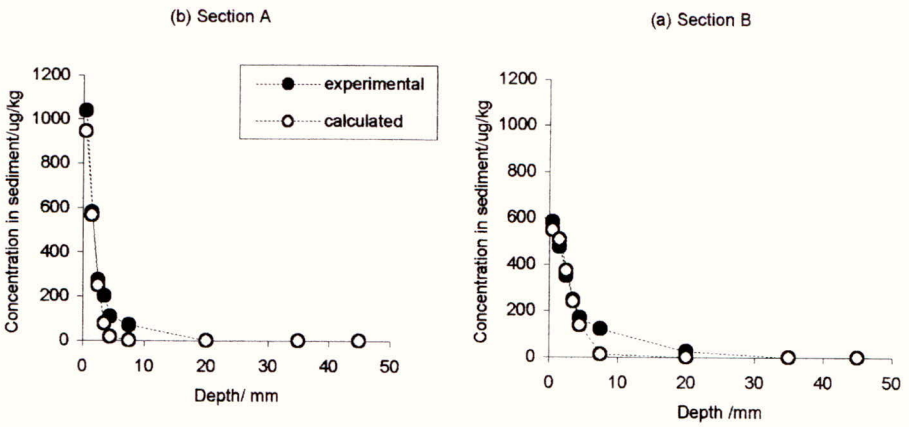

Figure 2. Comparison of the sediment profiles for total permethrin generated by MATHCAD for the effective diffusion coefficient, $D=0.85 \times 10^{-10}$ m^2/s for Section A and $D=2.2 \times 10^{-10}$ m^2/s for Section B. The sediment partition coefficient K_d was taken as 652 litres/kg for both isomers.

The movement of total permethrin into the sediment was analysed for the two channel sections: (a) Section A, with no enhancement of worms and (b) Section B, with enhancement of worms. A MATHCAD model was used to optimise agreement in a conservative system between the measured and calculated concentrations in the overlying water and concentrations found in the sediment profile by adjusting an effective diffusion coefficient for a chosen value of the sediment distribution coefficient, K_d (Daniels *et al.*, 1998).

The results from the MATHCAD model are shown in Figure 2 for the two sediment sections, A and B. The sediment partition coefficient was fixed at 652 litre/kg, the average value measured for the isomers in adsorption batch experiments at 10°C after 5 days shaking. The best predictions for the concentrations in the overlying solution were 17.2 and 3.6 µg/litre for Section A and 16.8 and 2.4 µg/litre for Section B at t=15 min and at the end of the experiment respectively. The higher initial concentration (*cf* experimental value of 18.2 µg/litre for the total isomer concentration) and lower final concentration in the solution are consistent with losses through adsorption and degradation not included in this model. The optimised values of the effective diffusion coefficient (Figure 2) are of the expected magnitude and close to the range of 0.5 to 1.6 x 10^{-10} m^2/s found for simazine and lindane in sediment core experiments (Daniels *et al.*, 1998). The higher value found for Section B might reflect bioturbation effects although the dissolved silicon profiles in the porewaters from the two sections were in close agreement.

ACKNOWLEDGEMENT

We thank Dr Patrick Armitage for help with the experiments involving bioturbation. I, Allan thanks NERC for the award of a Ph.D. studentship.

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Assessment of the environmental properties and effects of pesticide degradation products

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ABSTRACT

When released into the environment, pesticides may be degraded by plants, micro-organisms and chemical processes. Under EU Directive 91/414/EEC the environmental impact of relevant transformation products needs to be assessed. Currently the only approach for assessing the potential risk of a transformation product is to perform a series of experimental investigations. This is a drain on resources. This study is therefore being performed to 1) explore relationships between parent and metabolite toxicity that can be used to identify potentially relevant metabolites in the future; and 2) assess the use of quantitative structure-activity relationships for predicting metabolite toxicity. A large dataset has been compiled containing information on the ecotoxicity of a range of pesticide metabolites and their parent compounds. The dataset has been used to explore relationships between parent and metabolite ecotoxicity and to test quantitative structure-activity relationship (QSAR) techniques. Results obtained to date indicate that, in general, metabolites are of similar or lower toxicity than the parent compounds. A small proportion of metabolites were more toxic. These differences in toxicity could be explained by an enhancement in the uptake of the metabolite compared to the parent (due to changes in hydrophobicity or dissociation constant) or the presence of pesticidal activity in a metabolite. For a large proportion of substances, predictions of ecotoxicity using a QSAR for daphnids were similar to experimentally-derived data. There were however a large number of substances where toxicity predictions were unreliable.

INTRODUCTION

When released into the environment, plant protection products may be degraded by micro-organisms and/or chemical processes. Under EU Directive 91/414/EEC, the environmental impact of selected pesticide transformation products needs to be assessed. Draft guidelines on the assessment of metabolites have been recently developed (CTB, 1999).

Currently, the only approach for assessing the fate and effects of degradation products is to perform experimental studies (e.g. LC50 fish studies). This is a drain on resources in terms of both cost and time. A more pragmatic approach would be extremely useful, particularly one that could be used in a lower tier of the risk assessment process. This could aid in the identification of relevant metabolites, the results acting as a trigger for relevant experimental work.

One possible alternative is to use information on the properties, biodegradability, ecotoxicity and mode of action of the parent compound along with modelling approaches to predict the

environmental fate and effects of degradation products. By using these approaches it may be possible to assess the environmental fate and effects of a metabolite based primarily on its structure. However, before such approaches can be incorporated into the risk assessment process, their suitability for metabolite assessment needs to be demonstrated.

This study is therefore being performed to assess the suitability of these approaches and to develop a framework that integrates predictive approaches and experimental testing to assess the environmental risk of metabolites. This paper presents initial results from the project and includes an assessment of:

1) the relative toxicity of metabolites compared to parent compounds;

2) the suitability of structure-activity relationships for predicting the ecotoxicity of metabolites to non-target organisms.

MATERIALS AND METHODS

An extensive search of the scientific literature, environmental databases and PSD disclosure documents was performed to obtain data on the ecotoxicity of pesticides and their metabolites. The resulting data were input into an Accord for Excel spreadsheet. For those substances where multiple assay values were available, the median value was calculated and these values were used in the analyses described below.

Data on the toxicity of parent compounds and associated metabolites were compared to determine the proportion of metabolites that were more or less toxic than parent compounds. To account for the inherent variability in ecotoxicity test results, a metabolite that was more than an order of magnitude more or less toxic than the parent compound was considered to have either enhanced or reduced toxicity.

The acute toxicity of the metabolites to *Daphnia magna* was predicted using Topkat Version 6.0 (Accelrys, 2001). Topkat is an *in silico* method for predicting the fate, toxicity and ecotoxicity of organic chemicals. The relationships used by the programme are based on high quality data. All predictions obtained using Topkat that satisifed the validation criteria of the programme (i.e. those that were classed by the programme as reliable and within the optimum prediction space) were compared with experimental data to assess the suitability of the programme for predicting metabolite ecotoxicity.

RESULTS

Data were obtained on the ecotoxicity of thirty-five active compounds and forty-seven associated environmental degradation products. The data covered a range of organisms and endpoints including aquatic and terrestrial test species. The compounds covered a wide range of pesticide classes (Table 1).

Table 1. Pesticidal and target class identification of active compounds used in the data analysis. Numbers of parent compounds represented are shown in parentheses

Insecticides	Fungicides	Herbicides
Carbamate (2)	N-trihalomethyl thio (1)	Sulfonylurea (3)
Pyrethroid (2)	Strobilurin analogue (1)	Aryloxyalkanoic acid (2)
Organophosphate (4)	Azole (1)	Quinolinecarboxylic acid (1)
Benzoylurea (1)		Benzonitrile (1)
Oxime carbamate (2)		Chloroacetanilide (1)
Cyclodiene organochloroine (1)		Urea (1)
		1,3,5-triazine (2)
		Aryloxyohenoxypropionate (1)
		Alkanamide (1)
		Anilide (1)
		Bis-carbamate (1)

(* pesticidal classes from Tomlin, 2000) Compounds without class (4)

Comparison of metabolite toxicity with parent toxicity for fish, daphnids and algae (Figure 1, 2 and 3 respectively) indicated that the majority of the metabolites have a toxicity equal to or less than the parent compound.

For fish, only one out of 30 of the metabolites tested was more toxic than the parent, whereas for daphnids, 3 out of 30 metabolites were more toxic than their parent. For algae, all of the metabolites had similar or lower toxicity values than their parent.

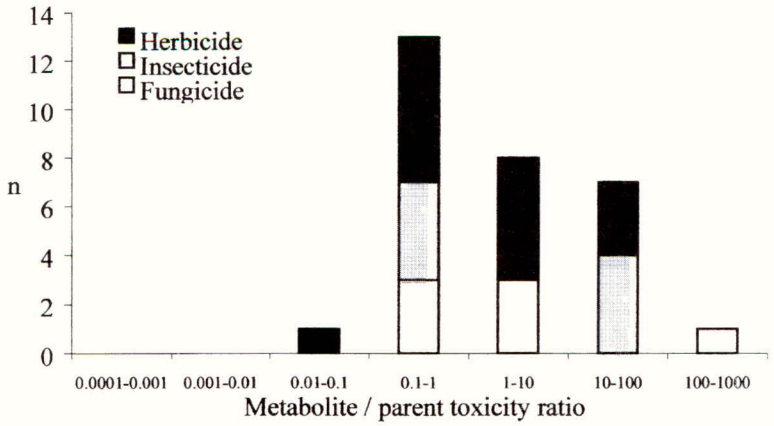

Figure 1. Comparison of metabolite toxicity to parent toxicity to fish (OECD recommended species), 96h LC50.

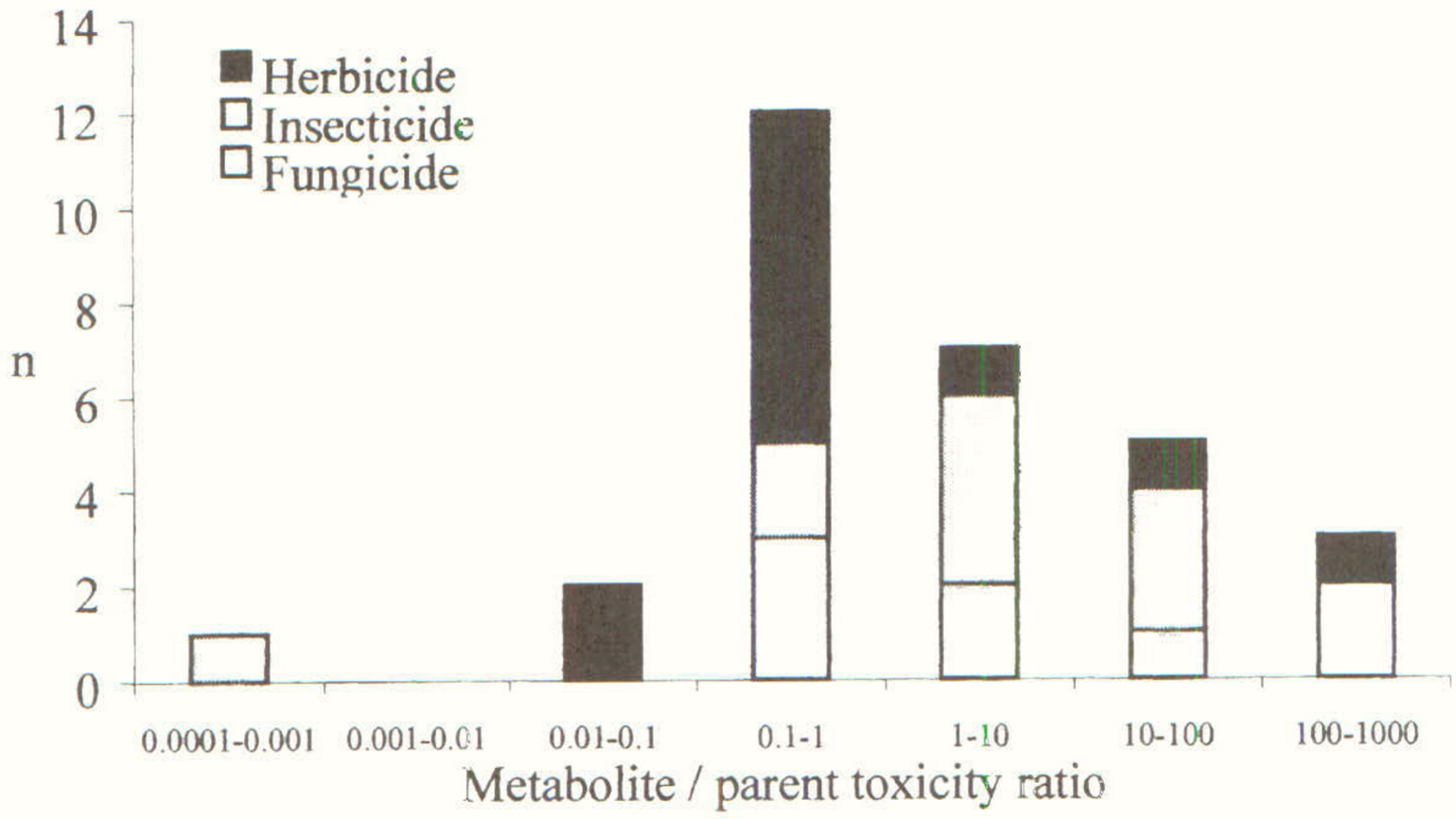

Figure 2. Comparison of metabolite toxicity to parent toxicity to Daphnia (*D. magna* and *D. pulex*), 48h LC50 (mortality) / EC_{50} (intoxiciation).

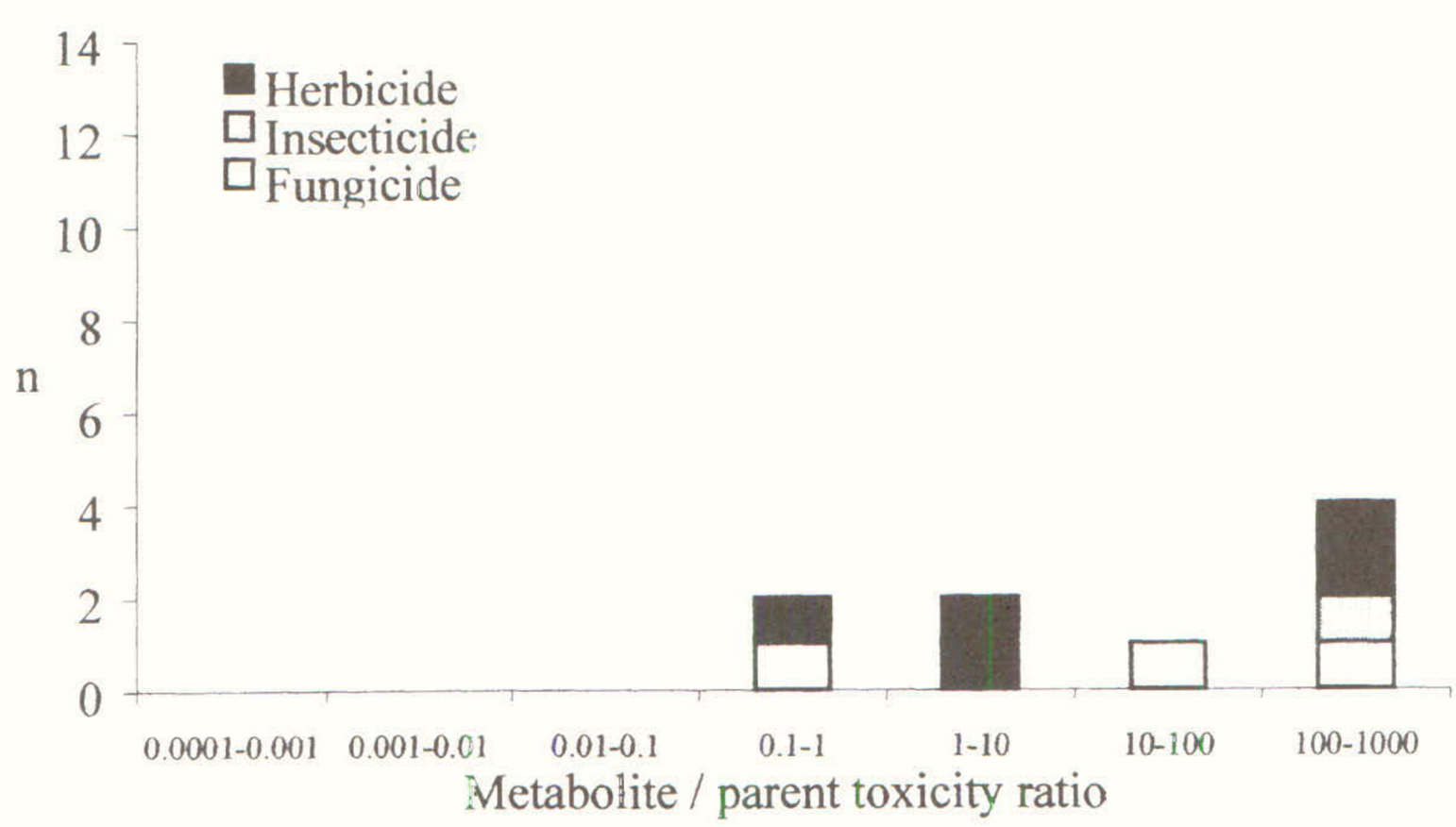

Figure 3. Comparison of metabolite toxicity to parent toxicity to green algae, 72h EC50.

Predictions for a large proportion of the metabolites studied (i.e. 56%) were either outside the Optimum Prediction Space or classified by the Topkat programme as unreliable. These were therefore not considered in the comparison of predicted and experimental data.

Predictions that satisfied all validation criteria were compared to experimentally-derived data (Figure 4). For a large proportion of the substances (61%), predictions were similar to experimental values (i.e. within an order of magnitude). There were however, a number of

substances where the predictions differed from experimental values by more than an order of magnitude.

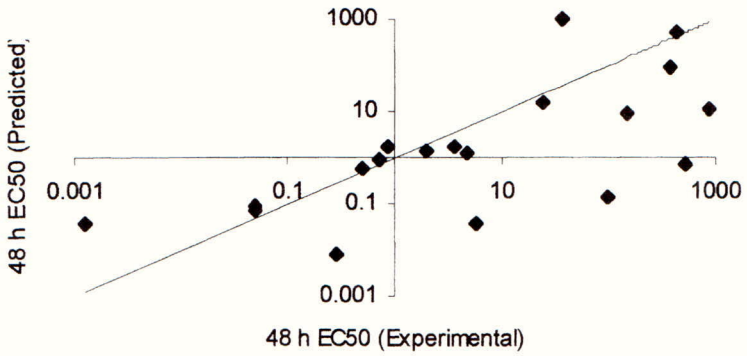

Figure 4. Relationship between experimental and predicted 48 h EC50 values for *Daphnia magna*. The line x=y is shown.

DISCUSSION

When released to the environment pesticides can be subjected to degradation by microorganisms and/or chemicals processes resulting in the formation of metabolites. It is generally thought that these degradation products have less pesticidal activity and lower toxicity than their active parent compounds. The results of this study support this assumption with the majority (94%) of metabolites examined having either similar or lower toxicity than their parent compound.

However, for four metabolites, the toxicity of the metabolite was greater than the toxicity of the parent compound. For two of the metabolites, 3,5,6-trichloro-2-pyridinol (a degradation product of triclopyr) and formaldehyde (a degradation product of glyphosate), the metabolites were more hydrophobic than the parent compounds (based on data from Hansch *et al.*, 1995 and Tomlin, 1997). For these compounds, it is possible therefore that the change in hydrophobicity results in an increased bioconcentration potential and hence increased toxicity.

2,4-dichlorophenol, was more toxic to daphnids than its parent 2,4-D. 2,4-dichlorophenol has a substantially higher pKa value than 2,4-D (i.e. 7.89 compared to 2.73) (Serjeant & Dempsey, 1979; Tomlin, 2000). Therefore under environmental pH conditions, the parent is likely to be more dissociated than the metabolite. The dissociated parent would therefore have a lower bioconcentration factor than the metabolite, explaining the increase in toxicity.

For the organophosphorus compound acephate, the degradation product methamidophos was more toxic than the parent compound. This metabolite is a commercial active ingredient so would be expected to have a specific mode of action.

The suitability of the Topkat programme for predicting the toxicity of metabolites was assessed. Predictions for a large proportion of metabolites obtained were similar to experimentally-derived data. However, there were a number of instances where Topkat either under-predicted or over-predicted metabolite toxicity. The initial work is therefore promising but further work is required before QSARs can be usefully applied in the assessment of metabolite toxicity.

In summary, the work to date has identified a number of possible rules that can be used in the identification of relevant metabolites. Initial assessments of the use of QSARs indicate that the models are suitable for predicting the ecotoxicity of selected compounds. Future work will involve the further expansion and analysis of the database.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the support of the Pesticides Safety Directorate (DEFRA). Opinions expressed within this paper are those of the authors and do not necessarily reflect the opinion of the sponsoring organisations.

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Estimation of standardized transformation rates of a pesticide and its four soil metabolites from field dissipation studies for use in environmental fate modelling

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ABSTRACT

In this study, first-order transformation rates of a pesticide and its four main soil metabolites were estimated from results of seven field dissipation studies using the ModelMaker 4.0 software. A standardization procedure was incorporated in the models to calculate degradation rates at reference conditions defined in FOCUS, i.e. soil temperature of 20°C and soil moisture at pF2. The corrections were imple-mented using the same equations as in the FOCUS groundwater models, i.e. the Arrhenius and Walker equations for temperature and moisture, respectively, and the actual daily temperature and soil moisture values measured in the field studies. The resulting transformation rates were evaluated statistically. Estimates of the average field dissipation behavior of the parent compound and its metabolites were provided. The average standardized half-life of the parent was used to successfully predict disappearence in three independent field trials. The main advantages of the standardization method are 1/ it accounts for seasonal climatic variability, and therefore provides an accurate description of the field data, and 2/ the standardized parameters are applicable in a broad range of climate conditions.

INTRODUCTION

Pesticide degradation parameters derived from field studies may be used in the FOCUS tier 1 risk assessment of leaching to groundwater (FOCUS, 2000). The automatic correction of the transformation rates to actual soil temperature and moisture conditions then needs to be disabled in the simulation models "unless the modeller attempts to standardise the results accounting for differences between field and reference soil temperature/moisture". Without those corrections, the estimated transformation rates can only be used for modeling scenarios with climatic conditions similar to those of the field experiments.

Moreover, the description of field dissipation data with lumped transformation rates is often difficult due to the seasonal variability of the climatic conditions over the study duration. A refined approach standardising the field transformation rates to the FOCUS reference soil moisture and temperature conditions is presented here, that can be used for parent and metabolites of a pesticide substance.

METHODS AND MATERIALS

Description of the field dissipation studies

The field dissipation of a pesticide a.i., referred to as parent has been studied on ten bare-soil trial sites, covering a wide spectrum of soil characteristics (texture, organic carbon content, pH), and climatic conditions (temperature, precipitation) representative of arable agriculture in Europe and the USA (Table 1).

At seven sites, soil concentrations of the parent and its four metabolites were available, while at the three other sites only three metabolites were analyzed.

Table 1. Characteristics of the field dissipation trials. Trials 1 to 7 were used for parameter estimation, trials 8 to 10 were used for validation.

Trial	Texture of topsoil, C_{org} [%]/ pH	Trial duration [d]	Number of data points	Soil/air temp. source	Average air temp.	Precipitation measurement, sum	Soil moisture determination (depth used)
1	Loam 2.3 / 7.9 (H_2O)	635	17	nearby	5.7	on site 1146	TDR*, on site (0-0.3 m)
2	Sandy loam, 0.5 / 7.8 (H_2O)	537	18	nearby	17.9	on site 2915	TDR*, on-site (0-0.3 m)
3	Loam, 2.1 / 6.3 (H_2O)	529	18	on site	13.3	on site 2041	TDR*, on site (0-0.3 m)
4	Sandy loam, 0.7 / 5.1 (H_2O)	540	16	nearby	22.0	on site 3067	Calculated (0-0.3 m)
5	Sandy loam, 0.6 / 7.7 ($CaCl_2$)	356	7	nearby	19.0	nearby 896	not measured
6	Sand, 0.5 / 7.7 ($CaCl_2$)	362	7	nearby	17.5	nearby 648	not measured
7	Loamy sand, 1.8 / 5.6 ($CaCl_2$)	368	7	nearby	9.5	nearby 644	not measured
8	Sandy loam, 1.1 / 6.2 ($CaCl_2$)	352	7	nearby	9.5	nearby 672	not measured
9	Loam, 1.2 / 5.0 ($CaCl_2$)	354	8	nearby	9.6	nearby 903	not measured
10	Sandy loam, 0.6 / 5.6 ($CaCl_2$)	357	7	nearby	10.1	nearby 632	not measured

TDR = Time Domain Reflectometry

Model for calculation of standardized dissipation parameters

Based on the proposed route of dissipation of the pesticide in soil, a mathematical compartment model was developed using the parameter estimation and simulation software ModelMaker V.4.0 (Cherwell ScientificPublishing Ltd., Oxford, UK) to describe the field data (Fig. 1). The model was adapted from this general model for the trials where a metabolite was not detected. The model consists of a system of differential equations with specific pa-

rameters. Transformation processes (flows) between the compartments were described using first-order reactions. The standardization of transformation parameters to the reference soil temperature (20°C) and moisture (pF2) is shown in the model.

For each standardized degradation rate constant k, a variable $k_{act}\,(t,k,T,\theta)$ was created, representing the actual daily transformation rate, function of time t, the daily temperature T and the daily soil moisture θ listed in the look-up table T1, and calculated using the correction equations of Arrhenius and Walker with the default parameters from FOCUS (2000).

Optimization method and statistics

The transformation rates were optimized using the Marquardt algorithm (ordinary least-squares). The overall coefficient of determination r^2, the standard deviations and type-1 error rates of the estimated parameters, as well as the correlation matrix were determined for each data set. In addition, all distibutions of residuals were evaluated.

The standard deviation $\sigma_{k\ Parent}$ of the lumped degradation rate of the parent molecule, which is the sum of the degradation rates from parent to the metabolite and sink compartments, was calculated from the variances σ^2 of the individual rate constants calculated in ModelMaker:

$$\sigma_{k\ Parent} = \sqrt{\sum \sigma^2_{k\ individual}}$$

The type-1 error rates α for the estimated parameters were calculated using a two-sided t-distribution on the ratio $t=\hat{a}_i/\sigma_i$ of the estimated parameter $\hat{a}_i$ and its standard deviation σ_i, with df degrees of freedom: $\alpha = \text{Prob}\,(T \geq |t|; df)$.

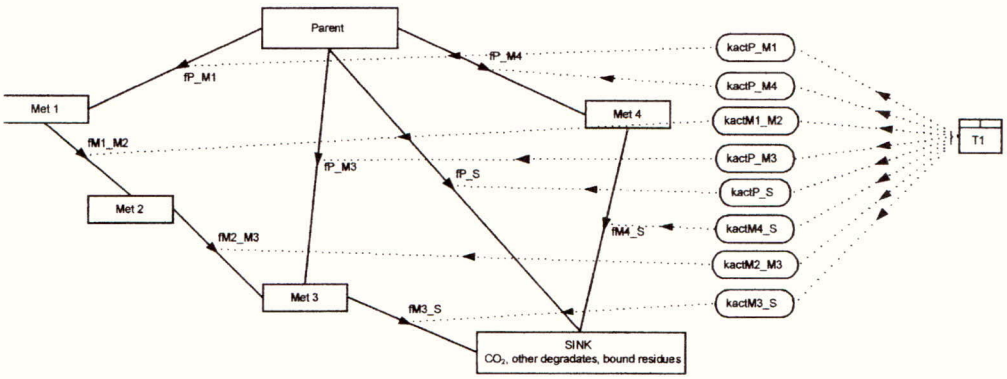

Figure 1. ModelMaker 4.0 compartment model showing the soil dissipation pathway (solid arrows are mass fluxes) and the implementation of the influence of temperature and soil moisture on dissipation (dotted arrows), via a lookup table T1 containing the daily values.

Calculation of average formation fractions and half-lives

For each individual field trial the molar formation fractions for the metabolites were calculated from the formation rates using the following equation: The mean formation fraction for each metabolite had to be normalized so they sum up to 1 (mass conservation).

$$ff_{met\_i} = \frac{k_{parent \rightarrow met\_i}}{\sum_{j=1,3,4,Sink} k_{parent \rightarrow met\_j}}$$

with ff_{met_i} = molar formation fraction
i = 1, 3, 4, Sink

Calculation of average half-lives

From the standardized transformation rates k_{Parent} and k_{M1_M2}, k_{M2_M3}, K_{M3_Sink}, and k_{M4_Sink} obtained from the parameter optimization procedure, the half-live values $t_{1/2}$ for each molecule at each trial site were calculated using the equation: $t_{1/2}=\ln 2/k$. Only the half-lives with a type-1 error rate of less than 5 % were considered significant and averaged arithmetically.

RESULTS

Description of soil dissipation by first-order kinetics

Examples of the results of the optimizations performed with the compartment model (with standardization) are listed in Table 2 for site 4. The optimized kinetic parameters obtained for parent and metabolites are given with their standard deviation, type-1-error rate, the significance decision, and the half-life corresponding to the reported rate, and with the overall coefficients of determination r^2 of the optimization.

The dissipation of parent, equivalent to the sum of the transformation to the metabolites 1, 3, and 4, and to the sink, was well described in all sites, as shown in Figure 2 for site 4. Here, for all substances except Met 2 the model described the data very well, which was also reflected by the statistical evaluation: The dissipation rates of all substances but Met 2 could be shown to be significant in site 4.

Overall, the measured data for all sites were well described by the first-order compartment models with corrections for temperature and soil moisture (r^2 = 0.94 to 0.99). This was confirmed by the significance levels of the lumped rate constants of the dissipation of the parent compound of about 95% for site 1, and above 99.9% for all other locations, indicating that the parameters contribute significantly to the fits, and therefore can be estimated by this procedure. The standard deviations of the lumped parameters are low, indicating that the estimates are reliable. In addition, the residuals were evenly distributed.

cance ranging from about 92% for one site, to above 95% for two other sites, to more than 99.9% for the three remaining sites. Overall, the residuals were evenly distributed. The description of the disappearance of metabolites 1, 2, and 3 was more difficult and gave variable results.

Still, the type-1-error rates of the individual parameters showed a level of significance about or above 95% for three, two, and five of the seven sites. The distribution of the residuals was therefore not equally good for all sites.

Calculation of field degradation half-lives

The half-life values of the field degradation of the parent and metabolites standardized to reference conditions, calculated from the estimated rate constants are listed in

Table 3. The standardized half-life for the parent compound varied from less than 3 d at one site to about 33 d at two sites. The average half-life in the seven field dissipation trials was 14.1 d.

Using this half-life for prediction, the field dissipation of the parent in the three independent trials was described accurately. The standardized half-lives varied from 57 to 90 d for Met 1, 10 to 126 d for Met 2, 19 to 116 d for Met 3, and 3 to 13 d for Met 4. The average field half-lives of Met 1, Met 2, Met 3 and Met 4 are 70, 68, 70 and 10 d, respectively.

Calculation of the formation of the metabolites

The fractions of the metabolites formed from the degradation of the parent compound are listed in

Table 4. Met 2 is formed from Met 1 and therefore not listed. The formation fractions of the metabolites 1, 3, and 4 range from 5 to 37 %, 7 to 34 %, and 9 to 59 % and reflect natural variability of the degradation process.

The transformation fractions to the sink compartment range from 2 to 86 % and indicate a natural variability in the formation of bound residues, other de gradates or CO_2. The normalized arithmetic means of the formation fractions of Met 1, Met 3, and Met 4 were 17.1, 16.5, and 32.9 %, whereas the average transformation to the sink compartment amounted to 33.5 %.

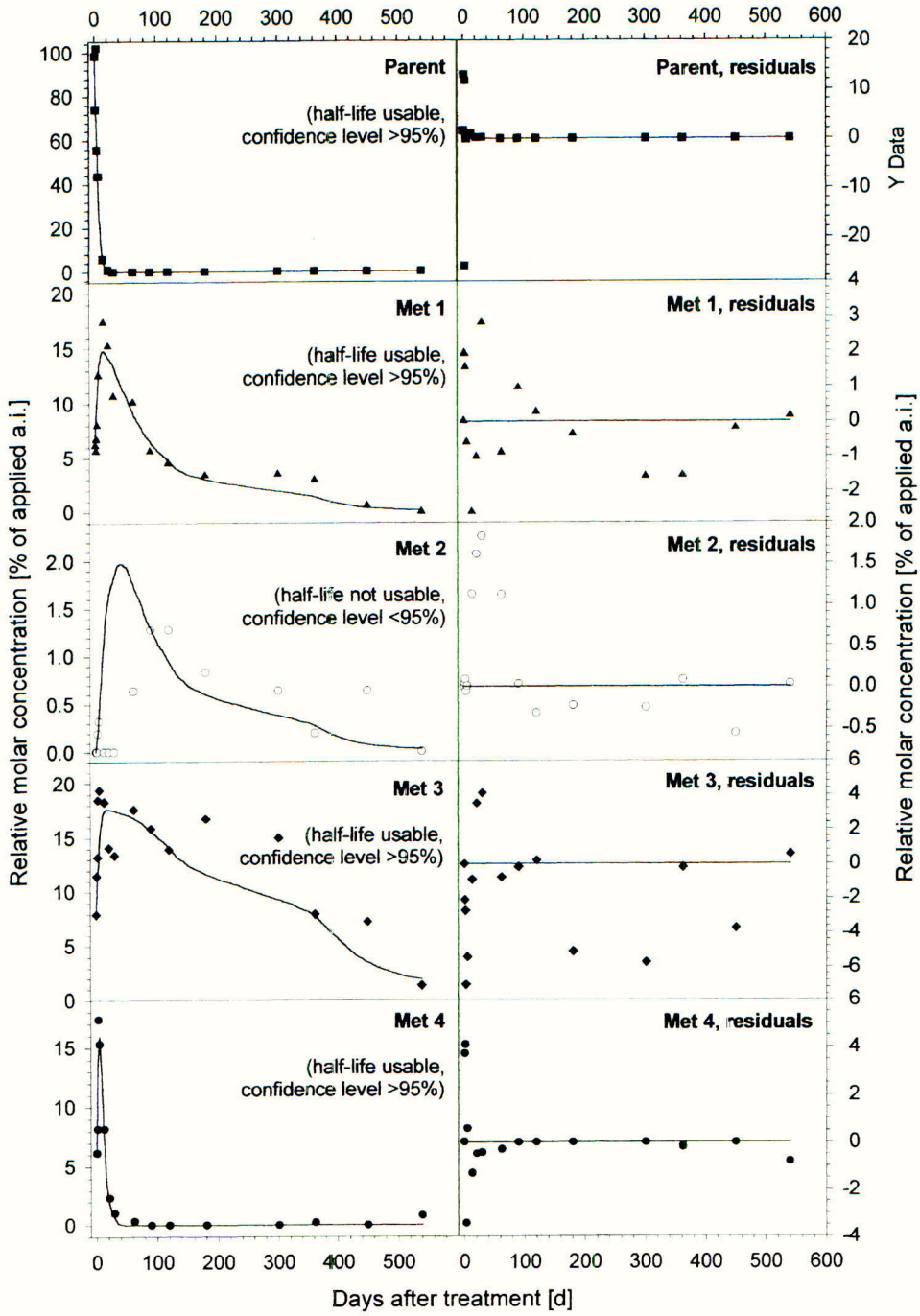

Figure 2. Left: Measured and fitted residues of parent and the four metabolites at site 4. Right: Corresponding distribution of residuals.

Table 2: Site 4: optimization results (79 degrees of freedom, r^2=0.9556).

Parameter	Estimated rate [d^{-1}]	Standard deviation [d^{-1}]	Type-1 error rate	Significance (95% confidence level)	Corresponding half-life [d]
k_{P_M1}	0.0289	0.0025	1.16E-18	+	
k_{P_M3}	0.0299	0.0006	1.88E-61	+	
k_{P_M4}	0.0985	0.0025	1.05E-53	+	
k_{P_Sink}	0.1093	0.0003	3.46E-129	+	
k_{Parent}	**0.2666**	**0.0036**	**9.2E-75**	+	**2.6**
k_{M1_M2}	0.0121	0.0028	4.47E-05	+	**57.3**
k_{M2_M3}	0.0723	0.0532	0.1780	-	**9.6**
k_{M3_Sink}	0.0093	0.0004	4.46E-37	+	**74.5**
k_{M04_Sink}	0.2761	0.0133	9.91E-34	+	**2.5**

Table 3. Half life values of Parent and Metabolites in 7 field trials corresponding to the lumped, standardized degradation rates *k* (Table 2, Figure 2).

Site	1	2	3	4	5	6	7	Arithmetic mean
Parent	32.5	32.7	5.4	2.6	11.0	5.9	8.4	**14.1**
Met 1	n.s.	62.4	90.0	57.3	n.s.	n.s.	n.s.	**69.9**
Met 2	n.s.	126.0	9.5	n.s.	n.s.	n.a.	n.a.	**67.7**
Met 3	n.s.	115.5	47.2	74.5	66.0	n.s.	18.6	**64.4**
Met 4	4.1	5.6	23.1	2.5	n.s.	n.a.	13.0	**9.7**

n.s.: non-significant, confidence level of the estimated degradation rate < 95%
n.a.: non-applicable, no significant levels of the compound detected in the field trial

Table 4. Molar formation fractions of the metabolites directly formed by the degradation of BAS 635 H.

From parent	1 [%]	2 [%]	3 [%]	4 [%]	5 [%]	6 [%]	7 [%]	Mean [%]	Normalized mean [%]
Met 1	10.8	10.3	23.5	31.1	5.1	7.2	37.4	17.9	17.1
Met 3	11.2	17.8	25.6	7.1	19.2	6.6	33.7	17.3	16.5
Met 4	36.9	54.0	21.0	59.4	9.4		26.5	34.5	32.9
Sink	41.0	17.8	29.8	2.4	66.4	86.2	2.4	35.1	33.5
Sum	100.0	100.0	100.0	100.0	100.0	100.0	100.0	104.9	100.0

CONCLUSION

Average standardized halflives and formation fractions were estimated from field studies for a parent and its metabolites after exclusion of non-acceptable datasets by statistical criteria. The resulting standardized parameters were used successfully to predict the transformation

of the parent in three independent trial sites. The standardization is doubly advantageous as it allows a more accurate description of the field data over the seasons, and the standardized rates are valid for the prediction of environmental concentrations under a variety of climatic conditions.

REFERENCES

FOCUS (2000). *FOCUS groundwater scenarios in the EU review of active substances,* report of the FOCUS groundwater scenarios workgroup, EC Doc. Ref. SANCO/321/2000 rev.2.