Pesticide biotransformation and fate in heterogeneous environments

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Pesticide biotransformation and fate in heterogeneous environments

Jos P.M. Vink

Proefschrift
ter verkrijging van de graad van doctor
op gezag van de rector magnificus,
van de landbouwuniversiteit Wageningen
Dr. C.M. Karssen,
in het openbaar te verdedigen
op vrijdag 19 september 1997
des namiddags te vier uur in de Aula
van de Landbouwuniversiteit te Wageningen

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Voor mijn ouders

Deze dissertatie is tevens verschenen
in de serie Van Zee tot Land 63

Ministerie van Verkeer en Waterstaat
Directoraat-Generaal Rijkswaterstaat
Directie IJsselmeergebied

Lelystad 1997

ISBN 90-5485-710-2

Stellingen

N[illegible]2317

1. In dit land is geen toekomst voor promovendi.
   - *Jo Ritzen, minister van onderwijs*

2. De algemene consensus om systeemkarakteristieken te verdisconteren in de terrestrische risicobeoordeling van bestrijdingsmiddelen dient ook aangewend en uitgewerkt te worden voor het aquatische milieu.

3. Het via batchexperimenten bestuderen van stofgedrag in een aquatisch medium is uiterst risiscovol, indien niet een veelheid aan basale mediumkarakteristieken wordt meebestudeerd. Veranderende beschikbaarheid van voor het microbieel metabolisme essentiële elementen kan leiden tot ernstige misinterpretaties.
   - *Feakin, S.J. et al. 1994. Wat. Res. 11:2289-2296*
   - *Dit proefschrift*

4. Indien overeenstemming bestaat over de hypothese dat de aerobe transformatieprodukten van aldicarb in toxische zin synergistisch of minimaal complementair zijn, dan moet de doseringsaanbeveling van de stof verlaagd worden.
   - *Canna, S. and P. Piera. 1994. Proc. 8th IUPAC congress, Washington DC.*
   - *Dit proefschrift*

5. Vanwege het persistente gedrag onder specifieke omgevingsomstandigheden is het gebruik van een aantal tot nu toe risicoloos veronderstelde chloorfenoxycarbonzuur-herbiciden toe aan herevaluatie.
   - *Dit proefschrift*

6. Indien toxicologische risico's van contaminanten worden beoordeeld aan de hand van blootstelling van vooraf geselecteerde organismen in het leefmilieu, kan van alarmerende concentraties in diep grondwater geen sprake zijn.

7. Een toelatings- of risicocriterium voor pesticiden dat stelt dat een bepaalde halfwaardetijd onder veldsituaties niet overschreden mag worden herbergt hetzelfde kanselement als de toto.

8. Het is waarschijnlijk dat Gilles de la Tourette de voorloper was van het onderzoek naar het transformatiegedrag van pesticiden in heterogene milieus.

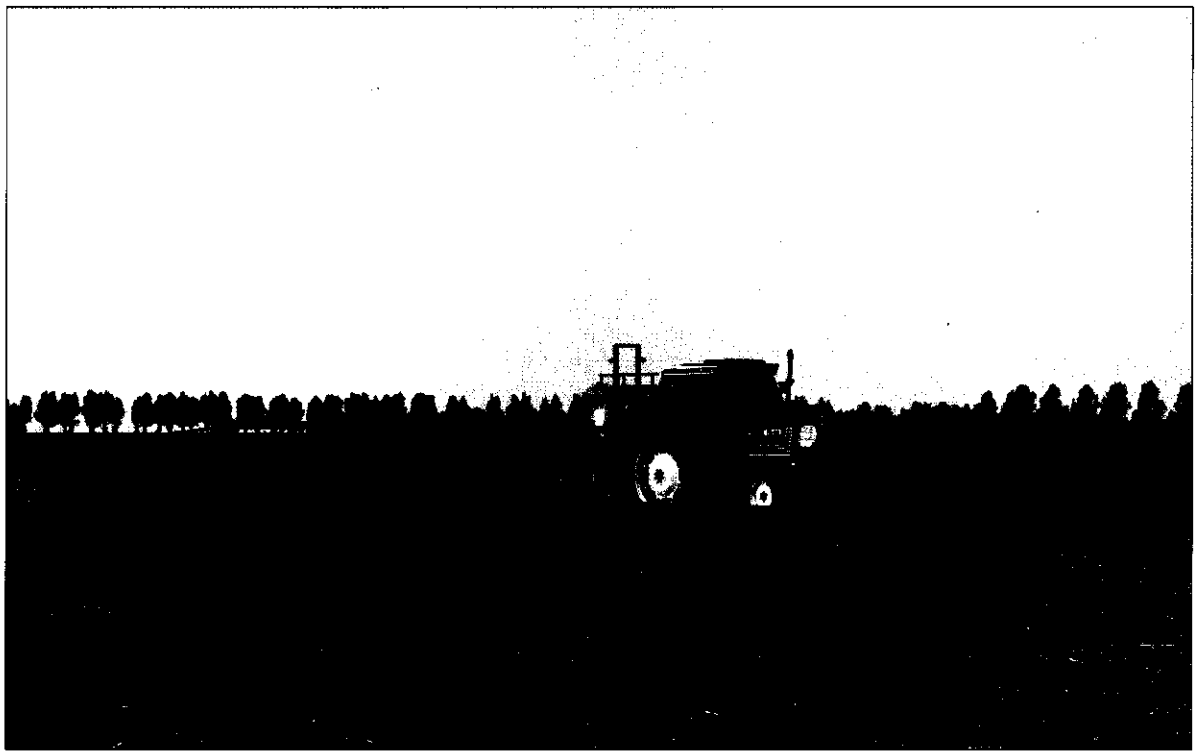
9. Milieubewustzijn heeft eerder een emotionele dan een feitelijke basis.

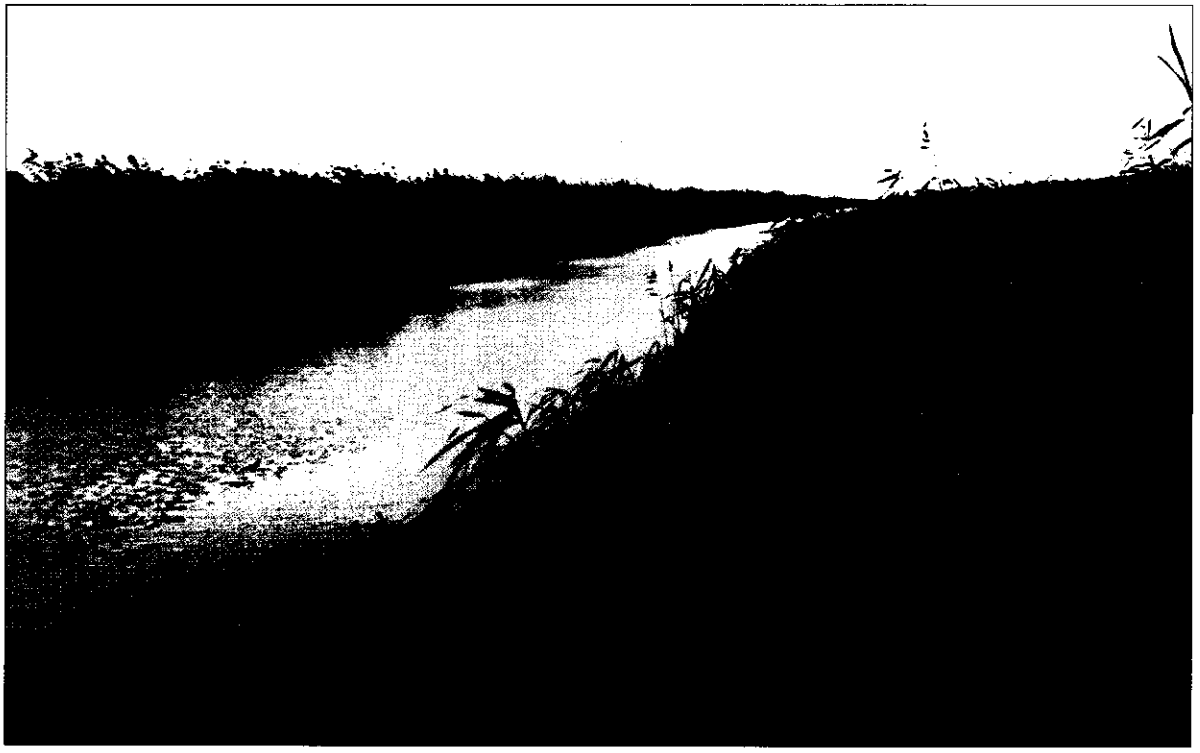
10. Risk assessment: 'Pest side story'.

11. Onduidelijkheid over normen bestaat niet uitsluitend in de bodemverontreiniging. Het is met name een urgent maatschappelijk probleem.

12. Wie de wetenschappelijke waarheid zoekt kan evengoed manden vlechten.

Stellingen behorend bij het proefschrift *Pesticide biotransformation and fate in heterogeneous environments* door Jos P.M. Vink. Wageningen, 19 september 1997.





figuren: Evers Design
omslag: Fiel v.d. Veen
omslagfoto: Willem Kolvoort
foto's binnenwerk: Bert Boekhoven, Jos Vink
DTP en drukwerk: Evers Litho & Druk
coördinatie productie: Henk Bos

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Chapter 1

Introduction

Introduction

Pesticide, or crop protection product, is the collective noun for a large variety of chemicals which are designed to have toxic or inhibitory effects on specific or non-specific organisms. Their effect is stipulated by the molecular composition of the active compound and directly determines its mode of action. At present, over a thousand organic and inorganic chemicals with pesticidal activity are registered, and are applied on a global scale.

The evaluation of risks that pesticides impose on the environment is a fairly young discipline. Although serious incidents of pesticide pollution are infrequent and levels of pesticides in the environment are mostly relatively low, the long-term effects may be substantial due to their ability to concentrate as they move up in the food chain. Once in the aquatic environment, pesticides can cause the closure of drinking water abstraction intakes or the death of any aquatic life. In 1965, Edwards reported as one of the first on 'some side effects of the use of insecticides' in soils. It was not until the early 1970's that some normalised procedures on 'plant protection products' were proposed by the Council of Europe and the Bundesanstalt für Land und Forstwirtschaft. These procedures concerned studies on leaching and degradation of pesticides. One of the earliest guidelines for pesticide risk evaluations came from the Food and Agricultural Organisation (FAO) in 1989, which was followed by the recommendations of the International Union of Pure and Applied Chemistry (IUPAC) in 1980. It took the Organisation for Economic Cooperation and Development (OECD) until 1992 to include pesticides in the Chemical programme, after it was recognised that differences remained in national approaches to pesticide registration. The Environmental Risk Assessment Scheme was only recently launched by the European Plant Protection Organisation (EPPO), in 1993. This programme aims to provide information on the interpretation of test methods, and to produce a reliable assessment of environmental risks. The growing awareness of pesticide-associated problems in the environment has increased the activities of many organisations on international collaboration (e.g., British Crop Protection Council (BCPC), Society of Environmental Toxicology and Chemistry (SETAC) and the Environmental Protection Agency (EPA)). A fair amount of ecological and environmental risk evaluation methods for pesticides have been proposed (e.g., Dushof *et al.*, 1994; Goss, 1992; Grieg-Smith, 1992; Hawkins and Nordquist, 1991; Kovach *et al.*, 1992; Norton *et al.*, 1992; Tiebout and Brugger, 1995) and reviewed (Levitan *et al.*,1995). The presented approaches range from holistic guidelines and frameworks to computer models, each of which has its advantages and draw-backs. For accurate risk managements however, semi-quantitative risk approximations such as the GUS (Groundwater Ubiquity Score; Gustafson, 1988), the PLI (Pesticide Leaching Index; Goss and Maizel, 1992) and the SLC (Soil Leaching Class; Brown, 1996) are no longer sufficient. To evaluate environmental risks of pesticides, a better understanding is needed on the specific dynamics of chemical transformation processes and microbial metabolism in various environmental compartments. Risk evaluation now covers a wide variety of disciplines and extends far beyond chemistry.

Environmental problems

Pesticides should be used in such a way that pests such as insects, weeds, and nematodes are controlled without harming non-target organisms in the environment. Ideally, these compounds persist long enough to control target organisms, and then degrade into inert products. Leaching and runoff losses, however, lead to pollution and accumulation in surface and ground water (Edwards, 1973; Klingman and Ashton, 1982;

Nicholson, 1968; Plimmer, 1977; Schnoor, 1992).

There is only partial understanding about the actual mechanisms how pesticides move between the aerobic, terrestrial soil and aquatic environments, and only little progress has been made on predicting biotransformation in the environment. Transformation of the parent pesticide molecule plays a crucial role in processes that determine transport behaviour in soil layers and subsequent emission to the aquatic environment. The impact of conventional and new pesticides on the environment is generally tested with leaching models, using parameters that are derived from and apply to terrestrial conditions. However, when leached into environments with lower oxygen concentrations, e.g., subsoil, surface waters and saturated sediments, both transformation rates and pathways may change drastically as a result of altered, mostly unfavourable conditions for aerobic microorganisms. Many publications report on compounds that are highly stable in aqueous systems (Anderson, 1995; Ashley and Leigh, 1963; Boesten *et al.,* 1991; Bromilow *et al.,* 1986; Edwards, 1973; Gerstl *et al.,* 1977; Reese *et al.,* 1972). The occurrence of generally unstable organochlorine pesticides in fresh water sediments and the accumulation in aquatic organisms have been reported by many authors (Donald and Syrgiannis, 1995; Goutner *et al.,* 1997; Kenaga, 1980; Stickel, 1968; Tan and Vijayaletchumy, 1994; Zaranyika, 1994).

The general agreement to include soil properties and soil type characterization in pesticide behaviour assessments has not yet been implemented in surface water risk assessments. Only few authors (e.g., Bull, 1985; Cook and Hutter, 1981; Feakin *et al.,* 1994; Kuhlman *et al.,* 1995; Lewis *et al.,* 1986; Tett *et al.,* 1994; Wolfe *et al.,* 1987) report on surface water characteristics and their effect on biotransformation of individual pesticides. Although it may be concluded that the composition of surface waters dictates the overall transformation rate of a specific compound, very little is known about the quantitative contribution of individual characteristics and the possible synergistic or antagonistic effects in combinations of characteristics. Models correlating chemical structure with biotransformation potential have been proposed to address the fate of chemicals in the environment (Alexander and Aleem, 1961; Larson, 1984; Paris and Wolfe, 1980; Wolfe *et al.,* 1987). A major weakness in these models is that they do not account for the diversity of environmental factors affecting biotransformation (Davis and Madsen, 1996). Top layers of sediments in water courses and lakes can become anaerobic during the summer months, allowing the overall transformation to proceed along different pathways (Lehman *et al.,* 1993; Wolfe *et al.,* 1986). Under reduced conditions, some pesticides may undergo partial transformation. Still, only limited information is available on pesticide transformation rates or pathways in low-oxygen environments. Therefore, the influence of transformation in the low-oxygen environments on the overall fate of pesticides has not sufficiently been evaluated.

Mechanisms and uncertainties

Next to the potential hazards of ineffective agricultural use, the fate of pesticides in the various environmental compartments is determined by *in-situ* conditions. The significance and relative contribution of individual environmental properties in the overall transformation process has, if studied in any general context, much been debated. It is, for example, generally believed that the dissolved fraction of a compound, as opposed to the sorbed fraction, is much better available to microorganisms and is therefore metabolised and degraded rapidly. For surface waters however, it has been suggested that sorption may in fact enhance biotransformation by concentrating the target compound (Olmstead and Weber, 1991; Voice *et al.,* 1992), by concentrating nutrients (Tranvik and Jørgensen, 1995), and by providing a large surface area for the attachment of bacteria which are then protected from shear forces by water movement (Shimp and Pfaender, 1987). In contrast, Meakins *et al.* (1994) found in their study to increase transformation rates of simazine in municipal waste waters, that varying the total suspended solid concentration had no effect on the degree of removal. Possibly, organic carbon and/or mineral nutrients that can be assimilated and are required for the biodegradation process, became limiting (Feakin *et al.,* 1994). Biotransformation rates of organic pesticides rely largely on the occurrence and activity of microorganisms that are able to utilise a specific compound as an energy source to perform their primary functions. In most cases, organic pesticides act as a carbon source, but additional N, P or S which is incorporated in the molecule may be beneficial for the devel-

opment of a microbial population. If a compound is present in very small concentrations, it may be insufficient to act as a substrate and hence as an energy source, and decomposition may stop. If it is not completely metabolised as a primary substrate, it may be transformed as a secondary substrate if a chemically simpler or better available carbon source is present. This phenomenon is known as co-metabolism: the compound undergoes microbial transformation without supplying the microorganisms with sufficient carbon or other nutrients (Alexander, 1981).

The rate in which a compound is transformed in the environment is primarily dictated by the population size of microbial communities *in-situ*. The actual size of any microbial population is maintained or is stimulated by favourable conditions in the surrounding environment. Figure 1 illustrates the prevailing activity of aerobic bacteria and fungi in soil layers measured in the month of June. Relevant activity is restricted to a small soil layer increment due to less optimal conditions in deeper layers. In this soil, the transition zone over active and non-active soil is abrupt. Metabolic transformation of pesticides is directly affected by the route and the velocity in which the compounds move through the soil matrix. Numerous authors (Birk and Roadhouse, 1964; Harris, 1969; Huggenberger *et al.*, 1972; Letey and Farmer, 1974; Walker *et al.*, 1995; Weber, 1972) have reported on sorption of pesticides on various soil components. In many soils however, leaching along preferential routes is common. Once a pesticide has leached through the active 'intercept-layers', or by-passed these layers due to preferential flow through macropores or cracks, it may be expected that transformation rates decline, and that transformation routes possibly change. However, individual fields often contain several distinctly different soil types or coherent soil series. This heterogeneity in geochemical, physical and biochemical properties seriously complicates accurate risk evaluations (Hornsby, 1992).

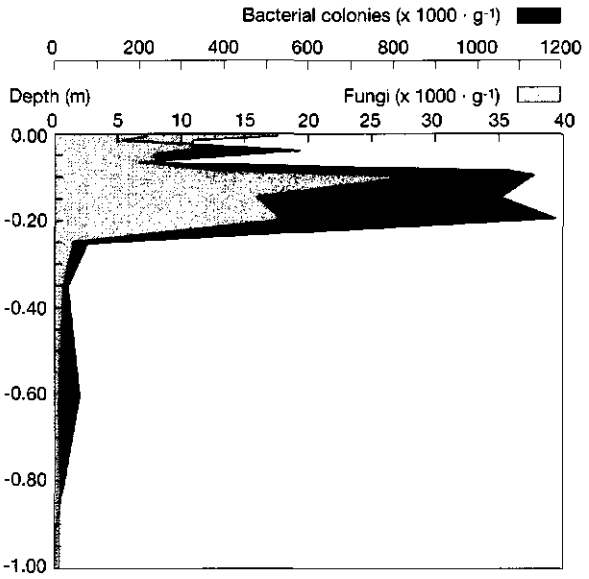


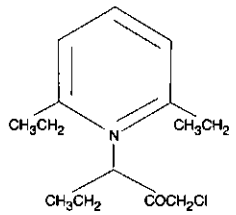
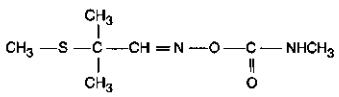
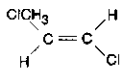
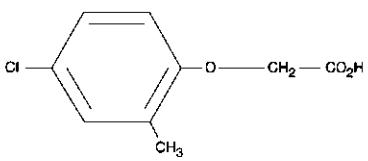
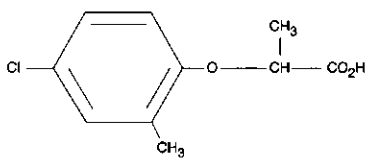
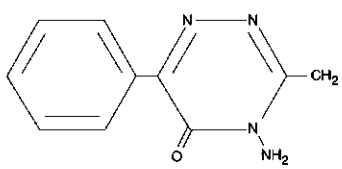
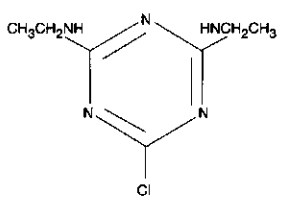
Fig. 1. Colony forming units of oxygen utilising microorganisms in a clay soil in the month of June (average temperature 17 °C). Relevant activity of both bacteria and fungi is restricted to the top 0.25 m of the soil. Soil properties are listed in Chapter 3, Table 1.

Remaining issues

Chemical transformation and microbial metabolism results in alterations of the original molecular structure, subsequently altering its chemical property. These altered structures are referred to as metabolites or transformation products. Organophosphate and organosulfur insecticides commonly have initial transformation products with well-established insecticidal activity, often of greater potency than the parent compound (Barrett, 1996). Of much concern is the fact that these toxicologically active transformation products tend to be more mobile than the respective parent compound (Bottoni *et al.*, 1996; Donati *et al.*, 1994; Donati *et al.*, 1996; Liu *et al.*, 1996; Meyer, 1994; Miles, 1991). The formation of a variety of intermediate products of simazine, which includes deaminisation and ring cleavage, may occur in aqueous systems (Cook, 1987; Erickson and Lee, 1989). Also, little is known about the actual mechanisms over which commonly used pesticides, such as the widely applied herbicide mecoprop, is transformed. In this area, many contradictory findings have been reported (Agertved *et al.*, 1992; Amrein, 1982; Kilpy, 1980; Lappin *et al.*, 1985; Lindholm *et al.*, 1982; Mackay *et al.*, 1985; McCarty *et al.*, 1981; Smith, 1985; Tett *et al.*, 1994). More knowledge is needed on the stability of such compounds under various environmental conditions and the fate of relevant transformation products.

Another issue is proclaimed by the combined action

Table 1. Selected pesticides

Molecular structure	Nomenclature [1])	Common name
	2-chloro-2',6'-diethyl-*N*-methoxymethylacetanilide	Alachlor
	2-methyl-2-(methylthio)propionaldehyde *O*-methylcarbamoyloxime	Aldicarb
	1,3-dichloropropene	1,3-D
	(4-chloro-2-methylphenoxy)acetic acid	MCPA
	(*RS*)-2-(4-chloro-*o*-2-tolyloxy)propionic acid	Mecoprop
	4-amino-4,5-dihydro-3-methyl-6-phenyl-1,2,4-triazin-5-one	Metamitron
	6-chloro-N^2,N^4-diethyl-1,3,5-triazine-2,4-diamine	Simazine

[1]) Systematic chemical name according to the rules of the International Union of Pure and Applied Chemistry.

of pesticides. According to the published literature, the toxicity of many pesticide combinations is at least additive. In some cases, pesticide mixtures - particularly those involving insecticides - have been shown to be synergistic, with increases in toxicity of up to 100-fold (Bernhard and Philogene, 1993; Cohen, 1984; Colin and Belzunces, 1992; Macek, 1975; Thompson, 1996). The environmental implications caused by the combined action of pesticides has been evaluated by some authors (Faust *et al.*, 1993; Faust *et al.*, 1994; Koneman, 1981). Canna and Piera (1994) concluded that aldicarb and its aerobic metabolites, when present in mixtures, may lead to a 50% higher toxicity to some bacteria than when exposed to the individual compounds. The increase in toxicity is attributed to synergistic toxic effects. This is an important conclusion, considering the probable co-existence of aldicarb and its aerobic metabolites in the environment.

In the following chapters, knowledge of geochemical, microbial and physico-chemical processes is generated in order to better understand the behaviour of pesticides in specific environmental compartments that occur along the pesticide's emission route. These include aerobic terrestrial soils, low-oxygeneous subsoils, surface waters and anaerobic sediments. Compounds that were used in these studies (excluding transformation products) are listed in Table 1. Selection of these compounds was based on i) their ability to represent their chemical group; ii) their common use and wide application in agricultural managements; iii) their likelihood to be detected in ground water and surface waters (Bailey *et al.*, 1995).

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Chapter 2

Sorption and Leaching Behaviour of the ^{14}C-Labelled Herbicide Alachlor in a Soil Specific Management; Effect of Landscape Geochemistry

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Published in *Soil Use and Management* **8** (1992) 26-30.

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Sorption and leaching behaviour of the ^{14}C-labelled herbicide alachlor in a soil specific management; effect of landscape geochemistry

Abstract - Uniform application rates of fertilizers and herbicides may result in over-treating some soils and under-treating others; costs may be unnecessarily large and soil, ground water and surface waters may be contaminated. An alternative is site specific treatment, tailored to individual soil types which are present in agricultural fields of any size. To study the pollution hazards of the herbicide alachlor, leaching and sorption experiments were set up, using disturbed samples and undisturbed soil columns. Adjoining Ves, Normania and Webster soil series (Udic Haplustoll; Aquic Haplustoll; Typic Haplaquoll) were sampled and accurately analysed on various soil characteristics. Ring uniformly ^{14}C-radiolabelled alachlor was used to study sorption and leaching characteristics within these soils. Results show distinctly different alachlor behaviour in topsoil and subsoil layers within this soil series sequence.

1 Introduction

Herbicides should be used in such a way that weeds are controlled without harming non-target plants or the environment. Leaching and runoff losses lead to accumulation and pollution of surface and ground water (Edwards, 1965; Nicholson, 1968; Plimmer, 1977; Klingman and Ashton, 1982). Ideally, herbicides persist long enough to control weeds, and then degrade into inert products. Ineffective agricultural use of chemicals is a potential hazard to the environment.

Numerous authors (Birk and Roadhouse, 1964; Harris, 1966; Huggenberger, 1972; Letey and Farmer, 1974) report on sorption of pesticides on various soil components. Individual fields often contain several distinctly different soil types or coherent soil series. Robert and Anderson (1986) have promoted soil specific management, rather than the conventional uniform application rates; they report financial and environmental benefits.

Alachlor, 2-chloro-2',6'-diethyl-*N*-(methoxymethyl)-acetanilide (tradename Lasso™), is a widely used herbicide, applied as a preplanting, pre-emergent or early post-emergent treatment to control annual grasses, certain annual broadleaf weeds and yellow nutsedge in maize (all types), peanuts, soybean, patatoes, dry beans, peas, cotton, and woody ornamentals. It is often used with other herbicides to increase the spectrum of weeds controlled.

In this study, we investigated the environmental impact of alachlor within a series of related and adjacent soil types. Leached and adsorbed amounts of alachlor were measured in disturbed soil samples and in successive horizons of undisturbed soil cores of the Ves-Normania-Webster complex (Udic Haplustoll; Aquic Haplustoll; Typic Haplaquoll). ^{14}C-Labelled alachlor was used, so that leached and adsorbed fractions of the doses applied to undisturbed soil columns could be determined accurately.

2 Materials and methods

Ves, Normania, and Webster soils were sampled in 20 cm increments to a depth of 120 cm, and analysed for pH, organic carbon content, particle size distribution and dry bulk density. Nine undisturbed cores (15 cm diameter) were excavated by driving PVC pipes, 25 cm long and sharpened at one end into a cutting edge, into the soil. Conical base caps, with a centered opening 1 cm across, were used to receive leachates. A Teflon™ tube was mounted in the opening and covered with

glass wool. The bottom of each cap was then covered with 1 kg coarse sand (> 200 μm). The sand was acid treated and washed to remove oxides and organic compounds, which could interfere with the leachate. The surface was levelled, covered with two layers of glass wool and moisturised. The cores were placed on the caps and sealed with silicone so that a vacuum could be applied to the columns. A 10.0 ml aliquot of 100 μmole · L^{-1} ring uniformly labelled ^{14}C-alachlor (27 mCi · $mmole^{-1}$) was applied to the columns. This amount corresponds to conventional application rates in agricultural systems. To apply the solution uniformly to the soil surface, a 10 x 10 cm grid with 100 openings was used, pipetting 0.1 ml aliquots. Bromide was used as a tracer, using 10.0 ml 1000 mg · L^{-1} Br^-, and the grid described before.

Leaching was performed by using a ceramic, funnel-shaped rain simulator, 10 cm diameter and 1.8 openings per cm^2. When placed onto the columns, water would drip from a 5 cm height. A buffer zone of 2.5 cm from the edge of the column was allowed, thus preventing by-pass flow along the column edge. Some authors (Clay *et al.*, 1988a) use different methods as concerned to the leaching experiment itself. A peristaltic pump is being used, thus providing the column with a constant amount of water. Draw backs of this method is the hazard (or negligence) of by-pass flow along the column edges. Moreover, an anaerobic situation is being created, which may affect sorption or decomposition processes. The previously described method is probably closer to natural conditions and is therefore to be prefered in herbicide leaching studies.

Columns were periodically leached with 200 ml aliquots, corresponding to 11.3 mm rainfall, over a period of 22 days. The total feed volume amounted 2200 ml. Leachates were sampled in duplicate, taking 1.00 ml aliquots, and analysed in a Liquid Scintillation Counter (Packard 1500 Tri-carb LSC). Bromide concentrations were measured with an ORION EA920 ion electrode. After leaching, soil samples were taken from the columns, using a cylinder (diameter 2 cm) taking samples of 3 cm increments. Samples were taken in triplicate and mixed. Concentrations of remaining alachlor were measured.

To determine specific sorption isotherms, alachlor solutions in 0.01 M $CaCl_2$ containing 5.0, 10.0, 25.0, 50.0, and 100.0 μmole · L^{-1} alachlor respectively (15,000 decays per minute) were added in 10.0 ml aliquots to 10.0 g soil, using a 25 ml glass centrifuge tube, sealed with a Teflon™ lined cap. Tubes were shaken for 24 hours to equilibrate the soil solution with the adsorption complex. Suspensions were centrifuged at 8000 rpm (± 8000 *g*) for 30 minutes, 1.00 ml aliquots of supernatant were removed and 15 ml OPTI-FLUOR was added. Alachlor concentrations were measured by the Liquid Scintillation Counter. All sorption studies were carried out at a constant temperature of 18 °C. Sorption isotherms were described using the Freundlich sorption equation, which is defined as:

$$q = K_f \cdot C_e^n \qquad (1)$$

in which:

- q = The quantity of compound adsorbed per unit weight of adsorber;
- C_e = The equilibrium concentration in the solution of the compound once sorption has been established;
- K_f and n = Empirical constants, which may be derived from experimental sorption data.

Although the average half-life time of alachlor varies between 40-70 days (WSSA, 1974) or 3-12 months at the usual rate of application (Klingman and Ashton, 1982), it should be noted that during the time period of the leaching experiment, metabolism of alachlor is likely to occur to some extent.

3 Results and discussion

Based on soil composition, the following nine soil layers were selected and used for sorption experiments: Ves 0-20 cm and 60-80 cm, Normania 0-20 cm and 60-80 cm, and Webster 0-20 cm, 20-40 cm, 40-60 cm, 60-80 cm, and 80-100 cm. Particle size distribution of sand fractions (> 50 μm) indicated a change of parent material in the Webster soil below 100 cm.

Figure 1 shows sorption isotherms of the various soil layers. All are logarithmic linear functions with a correlation of around 0.99, indicating close adherence to the Freundlich model. In table 1, some relevant soil properties and the Freundlich parameters are represented. Data are based on initial alachlor concentra-

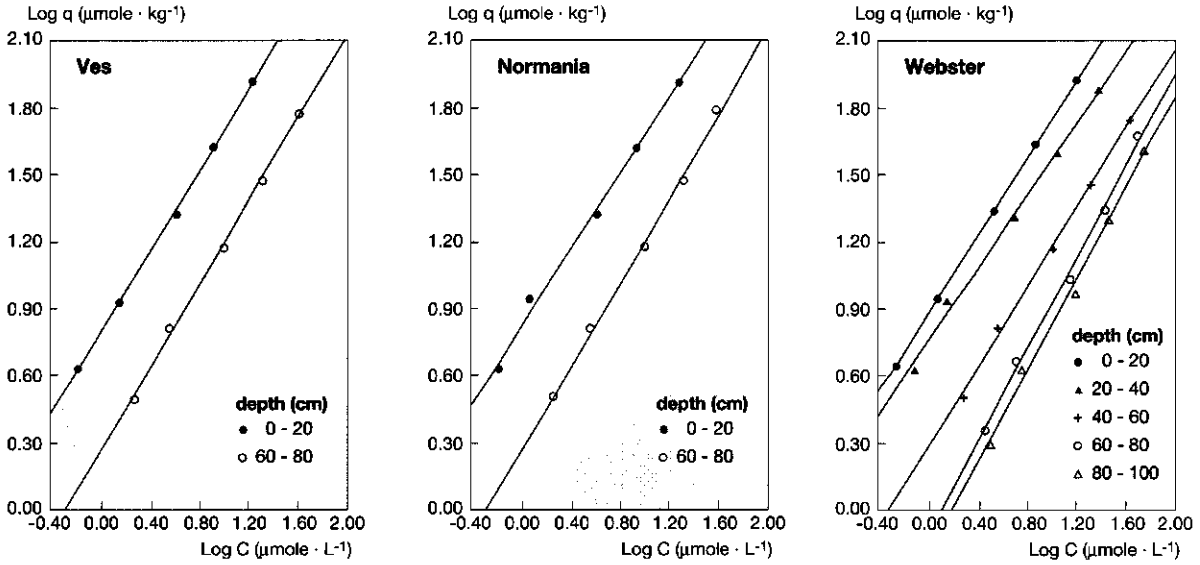

Fig. 1. Sorption isotherms for the Ves, Normania and Webster soils at various depths.

Table 1. Soil properties and experimental Freundlich parameters of various soil layers (K_f in mmole $^{n-1}$ · kg^{-1} · L^n).

Soil series	layer depth (cm)	pH $CaCl_2$	clay < 2 µm	org.C (%)	bulk density (g.cm^{-3})	Freundlich parameters		
						n	K_f	K_{oc}
Ves	0-20	5.4	28.2	3.5	1.36	0.90	6.17	176
Ves	60-80	6.1	29.7	0.7	1.51	0.92	1.85	264
Normania	0-20	5.4	27.9	3.3	1.16	0.85	6.80	206
Normania	60-80	6.4	37.1	0.7	1.38	0.92	1.97	281
Webster	0-20	6.1	30.3	4.2	1.06	0.83	8.24	196
Webster	20-40	6.2	29.4	2.9	1.30	0.77	6.22	214
Webster	40-60	6.4	29.6	1.5	1.35	0.86	2.08	139
Webster	60-80	6.7	24.9	0.6	1.54	0.95	1.12	187
Webster	80-100	6.9	16.7	0.3	1.64	0.98	0.71	237

tions ranging from $5.0.10^{-6}$ to $1.0.10^{-4}$ M.

The capacity for adsorbing alachlor decreases with depth in all studied soils. The top 20 cm of the Webster soil shows a greater adsorbing capacity than the Ves and Normania topsoils, and a sharper decrease at 60-80 cm. The Webster soil shows an exponential decrease of adsorbing capacity with depth.

Figures 2 and 3 show leached amounts of ^{14}C-labelled alachlor in time. Different proportions of the applied doses were leached from the various soil series and soil layers, the amounts corresponding with trends indicated by the sorption isotherms.

Results from the core samples show that after leaching a total amount of 124 mm, approximately

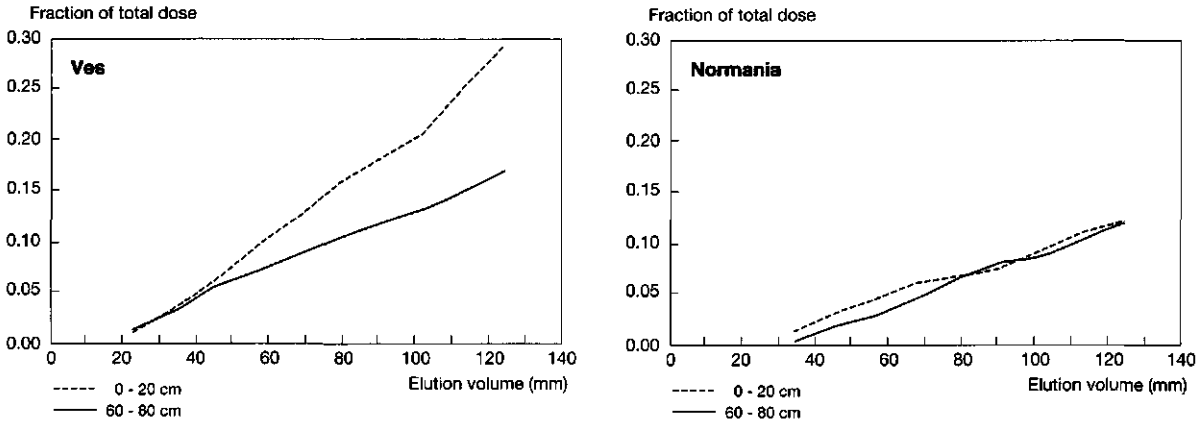

Fig. 2. Cumulative ^{14}C-labelled alachlor leaching from Ves and Normania topsoils and subsoils.

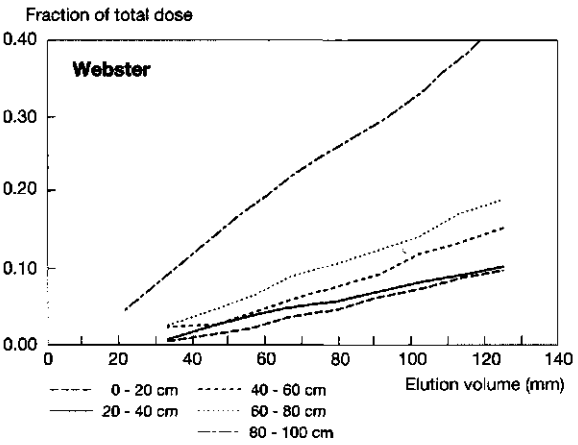

Fig. 3. Cumulative ^{14}C-labelled alachlor leaching from the Webster soil.

74% of the remaining alachlor in the Webster topsoil (0-20 cm) is still present in the top 6 cm. For Ves and Normania, the equivalent amounts are approximately 55% and 61% respectively. Disregarding the precise shape of the concentration curve of alachlor in the core, one may distinguish the soils in terms of mean depth of penetration of the ion fed into the column. The total amount of ions fed into the column, $V.C_e$, is defined by the relation:

$$V \cdot C_e = X_p \cdot (q + \theta C_e) \qquad (2)$$

or, after simple transformation:

$$X_p = \frac{V}{\theta} \cdot \frac{1}{(1 + R_d)} \qquad (3)$$

in which:

X_p = Mean depth of penetration (m)
V = Feed volume ($m^3 \cdot m^{-2}$)
θ = Volume fraction of water (%)
R_d = Distribution ratio = K_f / W, in which W represents the mass moisture content in cm^3 per 100 g soil.

Table 2 shows calculated and measured penetration depths of alachlor at estimated values of 0.3 for θ and 0.4 for W. Corrections of θ and W for different soil layers based on dry bulk density measurements appeared to have little effect (< 10%) on the value of X_p. Measured and calculated values of mean penetration depths can be compared in cases where relatively small amounts have leached through the cores, as in Ves (0-20 cm), Normania (0-20 cm), and Webster (0-20, 20-40, 40-60 cm). The mean penetration depths are 4, 3, 3, 4 and 7 cm respectively. Since the mean depth of penetration of the non-adsorbed bromide ion, and

Table 2. Mean alachlor penetration depth (cm) after 22 days at a total feed volume of 2200 ml.

Soil series	layer depth	mean penetration depth	
	(cm)	calculated	measured
Ves	0-20	2.3	4
Ves	60-80	8.5	-
Normania	0-20	2.7	3
Normania	60-80	8.0	-
Webster	0-20	2.3	3
Webster	20-40	3.3	4
Webster	40-60	8.5	7
Webster	60-80	13.3	-
Webster	80-100	15.5	-

hence the water front, is expressed by V/θ, measurements of Br^- concentration peaks indicated breakthrough at a feed volume of 800 ml; This means that the approximate leaching velocity of the water front is 0.3 cm · mm^{-1} rainfall. This is valid for all studied soils. Mean alachlor penetration velocities ranged from 0.02 cm · mm^{-1} rainfall (Webster 0-20 cm) to 0.1 cm · mm^{-1} rainfall (Webster 80-100 cm).

There is a linear relationship between organic carbon content ($\underline{C}$) and the distribution coefficient K_f of all studied soils. A simple relationship is given by $K_{oc} = K_f / \underline{C}$, in which K_{oc} is a distribution constant accounting for organic carbon. The experimental data for all studied soil series and soil layers resulted in K_{oc} = 139 till 264 $cm^3 \cdot g^{-1}$. With a correlation between K_f and $\underline{C}$ of 0.931, these results seem reliable, and seem to correspond with findings of Gustafson (1988), who investigated sorption behaviour of various pesticides related to organic matter. For alachlor, he found K_{oc} = 161 $cm^3 \cdot g^{-1}$. Of all soil properties measured, organic matter usually gives the best correlation with sorption of herbicides. Mobility is inversely correlated with organic carbon content (Helling, 1971; Gerber and Guth, 1973).

The Webster soil is characterized by a high retention of the herbicide in the topsoil, whereas the Ves and Normania topsoils show low retention. These soils could cause ground water pollution under certain conditions (precipitation exceeding evapotranspiration). The Webster subsoil, however, retains very little amounts. Alachlor, present in these soil layers, has to be considered very mobile and potentially hazardous to ground water. Leached amounts from the Webster subsoil layers were approximately 5 times as high as the Ves and Normania subsoils, whereas calculated values were up to 7 times as high. In an agricultural system where alachlor is being used periodically, decreased application rates for the Webster soils series may be recommended under certain circumstances. Field studies in areas that display morphological and hydrological heterogeneity seem to confirm these findings. Verstraeten (1994) found that concentrations of alachlor and degrades differed across landscape position at selected depths. In general, it may be stated that soil screening (survey, sampling and evaluation), matched with soil specific management, seems to be a practice that may contribute to decrease herbicidal pollution to surface and ground water in soils that are susceptible to leaching, and are potentially hazardous to the environment.

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Chapter 3

Simulation and Model Comparison of Unsaturated Movement of Pesticides from a Large Clay Lysimeter

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Simulation and model comparison of unsaturated movement of pesticides from a large clay lysimeter

Abstract - A long-term (> 10 months) leaching experiment was conducted with a large clay soil column and a rain simulator to study unsaturated transport of the nematicide aldicarb and the herbicide simazine in a cracked clay soil. Water retention and soil conductivity were derived from experimental outflow data and deterministic parameter estimation techniques. Under conventional application rates and realistic rain events, aldicarb's aerobic metabolites were found in very high concentrations, and did not meet the EC-norm level for water during the entire measuring period. A mass balance for aldicarb showed that 0.35% of the initial dose was leached. However, when the two isosteric metabolites aldicarb-sulfoxide and aldicarb-sulfone were included in the mass balance, this percentage increased dramatically to 19.7%. Simazine was found in relatively low concentrations of 0.05-0.6 $\mu g \cdot L^{-1}$, and only 0.11% of the initial dose was leached over 280 days. The absence of a 'breakthrough behaviour' (peak exposure) implies long term delivery (chronic exposure) of the compound from the soil. The predictive performances of the widely used pesticide leaching models VARLEACH, LEACHP, MACRO, PELMO, PESTLA and SIMULAT, which differ in their basic concepts for calculating water and solute transport and pesticide behaviour, were compared. This ring test revealed that none of the models were able to describe both water percolation and pesticide leaching to a complete satisfying degree. Moreover, there is little agreement on maximum pesticide concentrations and the time period in which these occur. This conclusion seriously limits the possibilities of model application and conducting reliable risk assessments for pesticides which are applied on the studied or similar type of clay soils.

1 Introduction

Lysimeters are useful tools to study the behaviour of pesticides in soils, as fluxes can be easily monitored, and the data can well be used to calibrate and validate simulation models. In practice however, conditions may occur in which water and solutes are transported by macropores and by-pass the reactive soil matrix as was shown by Brown *et al.* (1995) and Beck *et al.* (1995) for clay soils. Hence, the pesticide reaction times with sorption sites, as well as the overall exposure of pesticides to the sorbing surface decrease significantly. It is commonly believed that this is a main reason that various pesticides, including those that are assumed to leach only marginally, are found in ground water and surface waters (Edwards, 1973; Beven and Germann, 1982; Armstrong *et al.*, 1995; Johnson *et al.*, 1996).

Leaching models are used as both supporting and decision tools in pesticide management as well as for risk assessments for existing and newly developed pesticides. In many countries, it is recommended or may even be mandatory that the model used for this purpose has been validated. As a number of models have been developed that are currently used for 'screening' purposes, it is of interest to assess how these models perform under various circumstances, in particular for those cases that deviate from homogenous advective water and solute flow as found for clay soils. Diekkrüger *et al.* (1995b) concluded from a study with 19 different models and users, using identical data sets, that model validation is mainly determined by the availability of measurements. They recommended to develop methods for deriving model parameters from other information, like hydraulic conductivity from

soil texture. Combining those transfer functions with models (as was done in this study) may lead to tools which may improve the evaluation of environmental risks from a scientific as well as from a management point of view.

The objectives of this study were: 1) To monitor leaching behaviour of both aldicarb, including its aerobic metabolites, and simazine from a cracked clay soil; 2) To compare the predictive performance of six widely applied simulation models; 3) To evaluate implications for pesticide hazard assessments for ground and surface waters in terms of concentrations and exposure time.

For the leaching experiments, a rain simulator was designed to simulate precipitation amounts and intensities that occurred in the field. The nematicide aldicarb and the herbicide simazine were selected on basis of their wide use and application, their variation in properties, and the representativeness of their chemical group. The selected soil is abundant in the central part of the Netherlands. In a pesticide management, these soils are potentially hazardous to ground and surface waters because of their ability to rapidly by-pass water and solutes. Nevertheless, these clay soils are rarely studied because of the complex hydraulic properties.

2 Methods

2.1 Soil core sampling and lysimeter preparation

To derive representative and useful conclusions from lysimeter experiments, it is of the utmost importance to characterize the stratification and morphological properties of the soil *in-situ* prior to sampling. This will not only contribute to an optimal experimental design, but may also enable the experimenter to transfer the necessary implications for a field scale with larger certainty. To do so, we studied the vertical and horizontal soil heterogeneity in a large field pit on an experimental field station in the central part of The Netherlands. Characterization focused on the occurrence and size classes of macropores. The soil (Calcic Fluvisol (FAO); cracked Hydric Fluvaquent (USDA)) consisted of a relative homogeneous top layer of 15 cm, overlying a layer in which small cracks occurred that increased in diameter with depth. At 45 cm and downward, the soil consisted of prismatic structures with a diameter of approximately 5 cm. The diameter of cracks reached up to 2 cm. The horizontal distance over which cracks and pores repeated in a relative predictable pattern was determined at 30 cm. Diameter and length of the lysimeter were derived from these findings.

To obtain a large undisturbed soil column, a stainless steel casing (0.8 m length, 0.3 m inner diameter) was made, which was rounded at the top to withstand pressure and had a cutting edge at the bottom. From an experimental field station in a polder in the central part of The Netherlands, an undisturbed soil column was taken by simultaneously excavating the soil and gently edging the lysimeter casing downward. The soil density was determined by weighing the lysimeter before and after sampling. At the time of sampling, the moisture content ranged from 0.35 (5-15 cm) to 0.40 (40-50 cm) $cm^3 \cdot cm^{-3}$. (A stainless steel, funnel-shaped leachate collector, 0.3 m diameter, was filled with coarse sand (> 2 mm) and tightly attached to the bottom of the lysimeter casing with a three-way locking system. The edges were luted with polyethylene to allow the establishment of hydraulic suction during leaching. A rain simulator was developed to apply water to the lysimeter surface. The rain simulator consisted of a water reservoir (5 L) with a discharge reading in mm, an adjustable faucet, a cone shaped water distributor, and a drip-tray with nine (0.5 mm) openings in a concentric pattern. Faucet positions were calibrated to deliver a range of outlet intensities. A 5 cm buffer zone between the drip-tray outer area and the lysimeter outer area (surface area ratio 4:5) was allowed to minimise preferential flow along the column sides. The experimental set-up is shown in Fig. 1. Soil and lysimeter properties are listed in Table 1.

2.2 Determining water retention and unsaturated conductivity by transient flow experiments

To predict water flow and pesticide movement through the unsaturated zone, the hydraulic conductivity (K), water content (θ) and pressure head (h) need to be known. Traditional methods to determine $\theta(h)$ relations (i.e. water retention characteristic; pF) for undisturbed columns involve the stepwise equilibrium drainage or laboratory experiments on core samples. For the direct determination of $K(\theta)$ relations, steady

Table 1. Soil and lysimeter properties

	Soil properties		
	Layer 1 5-15 cm	Layer 2 25-35 cm	Layer 3 40-50 cm
Fraction < 2 mm	87.4	87.5	86.6
Fraction < 63 μm	84.3	81.3	84.7
Fraction < 16 μm	55.8	54.7	59.8
Fraction < 2 μm	35.2	34.5	37.7
Dry matter (%)	74.3	75.4	69.7
ρ-moist ($g \cdot cm^{-3}$)	1.53	1.67	1.55
ρ-dry ($g \cdot cm^{-3}$)	1.20	1.17	1.13
θ_F ($cm^3 \cdot cm^{-3}$)	0.374	0.416	0.413
Φ ($cm^3 \cdot cm^{-3}$)	0.576	0.629	0.585
Org. carbon (%)	2.8	2.9	3.2
$CaCO_3$ (%)	8.0	8.0	8.0
pH (H_2O)	8.1	8.2	8.2
Total N (%)	0.24	0.28	0.28
P_2O_5 ($mg \cdot 100\ g^{-1}$)	36.7	37.6	47.8
Bromide ($mg \cdot kg^{-1}$)	0.3	0.2	0.4

	Lysimeter properties	
Height	0.51	m
Diameter	0.295	m
Surface area	0.068	m^2
Volume	0.0349	m^3
ρ-moist	1.55	$g \cdot cm^{-3}$
K_s	32.4	$m \cdot day^{-1}$
θ_s	0.522	-
θ_r	0.300	-
α	$1 \cdot 10^{-4}$	cm^{-1}
L	1.0	-
n	1.11	-

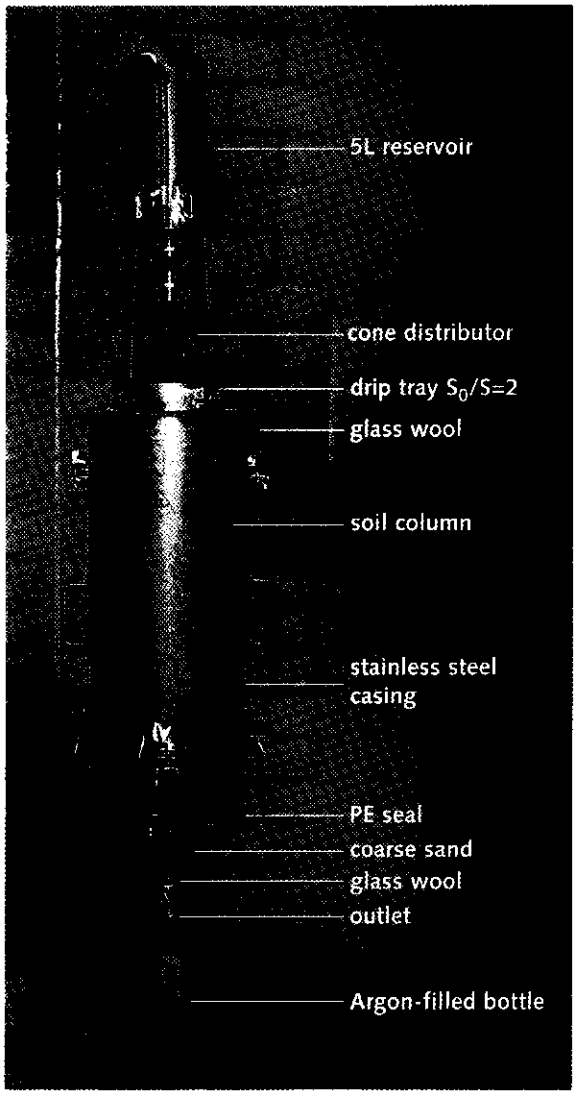

Fig. 1. Experimental set-up.

state flow experiments on soil cores at a number of flow rates are performed. Methods of determining K(θ) from transient flow experiments commonly require prior knowledge of θ(h) and are constrained with regard to initial and boundary conditions. Common techniques for simultaneous determination of K(θ) and θ(h) in situ involve the measurement of θ and h at selected times and depths during transient drainage experiments and are generally tedious. Furthermore, these measurements may be near to impossible to conduct in a soil with macropores, such as cracked clay soils. Except for the top 20 cm, cracks in our clay soil reached throughout the column. Therefore, we chose an alternative approach in which K(θ) is estimated from more easily measured θ(h) data and from the saturated hydraulic conductivity. Unknown parameters can be estimated by minimising deviations between observed and predicted flow variables such as water content and cumulative infiltration (Hornung and Messing, 1982; Zachmann *et al.*, 1982). We determined these parameters from transient outflow experiments which were performed with the lysimeter.

A constant inundation of 2 cm was enforced by connecting a large water reservoir with the lysimeter surface using a siphon. Infiltration and discharge were measured continuously. After reaching steady state, the infiltration was stopped, and gravitational drainage was monitored during seven hours. To optimise the various flow parameters, we used the computer program SFIT (Kool and Parker, 1987a,b). Parameter estimation is based on the simultaneous fit of the water retention characteristic (Equation 1) and the $K(h)$ relationship (Equation 2, Van Genuchten, 1980):

$$\theta = \theta_r + \frac{\theta_s - \theta_r}{(1 + (\alpha h)^n)^m} \quad (1)$$

$$K(h) = K_s \frac{((1 + (\alpha h)^n)^m - (\alpha h)^{n-1})^2}{(1 + (\alpha h)^n)^{m(L+2)}} \quad (2)$$

The Van Genuchten model has been found to describe with reasonable accuracy the hydraulic properties of a variety of soils, in particular when θ is in the vicinity of θ_s (Kool *et al.*, 1985; Parker *et al.*, 1985). Initial input values of the empirical parameters θ_s, θ_r, α and n were approximated according to the methods proposed by Vereecken *et al.* (1989, 1990), allowing the estimation of hydraulic properties from easily measurable soil characteristics such as texture, bulk density and organic carbon content. Data analyses revealed that bulk density is the most important predicting variable in estimating θ_s (Vereecken *et al.*, 1989) and is therefore directly affected by porosity and, consequently, the occurrence of macropores. The estimation is based on a modified structure of the Van Genuchten model:

$$S_e = (1 + \alpha h^n)^{-1} \quad (3)$$

with

$$S_e = (\theta - \theta_r) / (\theta_s - \theta_r) \quad (4)$$

and the hydraulic conductivity equation proposed by Gardner (1958):

$$K = K_s / (1 + (\alpha h)^n) \quad (5)$$

The parameters θ_s and L were fixed as boundary conditions in SFIT, and α, n, θ_r and K_s were optimised within certain boundaries.

2.3 Leaching of bromide, aldicarb (-sulfoxide, -sulfone) and simazine

At day 1, 0.2 g (≈ 29.4 kg · ha^{-1} active ingredient) aldicarb (2-methyl-2-(methylthio)propionaldehyde *O*-methylcarbamoyloxime, Temik™10) and 50.0 mg (≈ 7.4 kg · ha^{-1}) simazine (6-chloro-N^2,N^4-diethyl-1,3,5-triazine-2,4-diamine, Luxan™500) were applied to the column surface. Aldicarb was applied in a granular form directly onto the soil, and simazine was applied as a solute, equivalent to 7.8 mm water. This application was considered as the 'first rain event', and was applied with a 3 mm · h^{-1} intensity. Bromide (5.0 mg, ≈ 0.74 kg · ha^{-1} Br$^-$) was applied to act as a tracer. Pesticide properties are listed in Table 2. Properties of aldicarb-sulfoxide and aldicarb-sulfone which could not be determined experimentally were estimated. These variables were allowed to vary in the simulation exercises.

The leaching water in the reservoir was slightly acidified with 0.01 M HCl till pH = 5.7, which corresponds to pH-values measured in local rain water. During 280 days, water was applied with the rain simulator corresponding to amounts and intensities recorded in 1 hour intervals at a nearby meteorological station. Besides pesticides, metabolites and bromide, concentrations of dissolved oxygen, total-N and pH were measured periodically. Leachates were collected in dark, argon-gas filled bottles attached to the collector. The argon gas was used to expel oxygen from the bot-

Table 2. Pesticide properties (Bol *et al.*, 1992†; Vink and Van der Zee, 1997‡.)

		Aldicarb	Sulfoxide	Sulfone	Simazine
S	†	6160	6160^e	6160^e	3.5
P_v	†	$1.3 \cdot 10^{-2}$	$1.3 \cdot 10^{-2e}$	$1.3 \cdot 10^{-2e}$	$8.1 \cdot 10^{-7}$
H	†	$2.2 \cdot 10^{-7}$	$2.2 \cdot 10^{-7e}$	$2.2 \cdot 10^{-7e}$	$1.6 \cdot 10^{-8}$
Log K_{OW}	†	1.13	>1.13^e	>1.13^e	2.18
K_{OC}	‡	27	< 27^e	< 27^e	171
n	‡	1.3	1^e	1^e	0.89
μ layer 1	‡	0.116	0.058	0.058	0.035
μ layer 3	‡	0.063	0.017	0.017	0.028
Q10	‡	2	1^e	1^e	2
γ	‡	0.05	0.05	0.05	0.05
E_{ACT}	‡	25^e	25^e	25^e	57.4

e = estimated value

tles, thus limiting microbial transformation and/or oxidation of the compounds between collecting periods. Since its density is higher than air, argon gas does not leave the collection bottle until it is expelled by the leachate. The use of dark glass-bottles avoided photolyses. The bottles were weighed after each collection to determine the quantity of leachate. During the experiment the ambient air temperature varied between 17-18 °C.

In the period 1992-1994, drainage water of two field plots at and nearby the experimental station was collected periodically, and concentrations of nutrients and pesticides were monitored (Brongers and Groen, 1995; Brongers *et al.*, 1996). Results of these monitoring programmes are compared to concentrations from the lysimeter experiment in order to relate these laboratory findings to the actual field situation and to assess with some confidence the representivity of the lysimeter.

2.4 Models

The following simulation models were used: VARLEACH 2.0 (Walker, 1987), LEACHP 3.1 (Hutson and Wagenet, 1992), MACRO 3.1 (Jarvis, 1994), PELMO 1.5 (Klein, 1994), PESTLA 2.3 (Boesten, 1993) and SIMULAT 2.4 (Diekkrüger *et al.*, 1995a). These models differ substantially in their basic concepts for calculating water flow (capacity concept vs. Richards' equation), solute transport (displacement vs. convection/dispersion) and the transformation and sorption behaviour of pesticides (linear distribution coefficients vs. Freundlich isotherm; constant K_d vs. variable K_d).

VARLEACH, a derivate of CALF (Nicholls *et al.*, 1982), is one of the very few widely used models that accounts for a slow increase of sorption in time ('aging'). Water flow is based on the two-region tipping bucket principle. Dispersion is fixed by mobile-immobile water separation and layer depth. The model does not account for volatilisation of the pesticides. The largest sensitivity is shown by the application layer thickness and the distribution coefficient (Del Re and Trevisan, 1993).

The LEACHP model was initially developed as a deterministic, mechanistic research model. It is based on a time-dependent local water flux between two positions z1 and z2, and therefore is sensitive to $\theta(z,t)$ functions. The model assumes that a pesticide partitions between the sorbed, liquid, and gas phase. The diffusion-dispersion in the liquid phase is composed of i) mechanical dispersion due to different flow velocities between pores, and ii) chemical diffusion according to Fick's law. Dispersion is required as input. Pesticide volatilisation is included in the model.

An important feature of PELMO is that is developed primarily for regulatory purposes. A large part of the scenario parameters are hard-coded into the model, which reduces the input choices to only a few specific properties. Consequently, retrospective 'tuning' of the predicted values to measured data is minimised.

PESTLA is based on the convection-dispersion equation and assumes equilibrium sorption, described by a Freundlich-type equation modified for a reference concentration, and first-order transformation kinetics. Sensitivity analysis has shown that leaching is very sensitive to transformation and sorption parameters (Boesten, 1991).

Whereas the other four models assume water flow through a homogeneous matrix, both MACRO and SIMULAT account for macropore/bypass flow of water and solutes. Both models describe water fluxes in macropores as gravitational fluxes, but MACRO uses the Brooks and Cores definition of the retention curve. Therefore, water flow at low hydraulic pressures is assumed to be pure matrix flow. In SIMULAT, a Van Genuchten type retention curve is included, and water infiltration from the saturated upper layer is computed from the numerical solution of Richards' equation. Both models assume that infiltration of water into macropores is in equilibrium with moisture in the upper layer, and that dispersion of solutes in macropores may be neglected. Soil shrinkage is supported only by MACRO. SIMULAT provides an optional module for the transformation process, which offers metabolic/cometabolic degradation, substrate inhibition, Michaelis-Menten kinetics, Arrhenius and O'Neill equations to account for temperature, and three approaches for soil moisture dependency. In this exercise, we used Freundlich sorption.

Formation of metabolites is supported only by LEACHP and SIMULAT. An overview of the conceptual model characteristics is given in Table 3.

Table 3. Model characteristics.

	VARLEACH 2.0	LEACHP 3.1	MACRO 3.1	PELMO 1.5	PESTLA 2.3	SIMULAT 2.4
Water and solute transport	2 regions model Daily equilibration	Richards' equation; convection/dispersion	Richards' equation; convection/dispersion + two domain	Capacity model; convection/dispersion	Richards' equation; convection/dispersion	Richards' equation; convection/dispersion + two domain
Macropore flow	no	no	yes	no	no	yes
Sorption	Variable in time K_{dt}	Freundlich isotherm (constant)	Freundlich isotherm (constant)	Freundlich isotherm (variable)	Modified Freundlich isotherm (constant)	Equilibrium/kinetic Freundlich/Langmuir
Transformation	$\mu(T,\theta) = e^{\frac{-E_a}{RT}} A\,\theta^b$	$\mu(T,\theta) = r(T_{ref},\theta_{ref}) \cdot \frac{\max(\theta,\theta_{wp})-\theta_{wp}}{\theta_{min}-\theta_{wp}} Q_{10}^{\frac{T-T_{ref}}{10}}$	$\mu(T,\theta) = r(T_{ref},\theta_{ref}) \cdot \min\left[1,\left(\frac{\theta}{\theta_{ref}}\right)^b\right] f_z$	$\mu(T,\theta) = r(T_{ref},\theta_{ref}) \cdot \left(\frac{\theta}{\theta_{ref}}\right)^{-0.847} Q_{10}^{\frac{T-T_{ref}}{10}}$	$\mu(T,\theta) = e^{\lvert\tau(T-T_{ref})\rvert} \cdot \min\left[1,\left(\frac{\theta}{\theta_{ref}}\right)^b\right] f_z$	Optional
Metabolite formation	no	yes	no	no	no	yes
Time interval	5 days	0.1 day	1 day	1 day	1 day	1 h - 1 day

3 Results

3.1 Leaching behaviour and concentrations

Bromide, which acted as a tracer, leached almost instantaneously from the soil column. A simulated rain-event of 7.8 mm was sufficient to reach maximum concentrations after only 5 days in the leachate. This is a strong indication that preferential flow of water and solute occurred in the soil column.

High aldicarb concentrations of approximately 900 $\mu g \cdot L^{-1}$ were measured in the leachate within the first 10 days (15 mm percolate). After 100 days (210 mm percolate), concentrations reached below 0.1 $\mu g \cdot L^{-1}$, which is the EU-standard for water. Aldicarb's aerobic metabolite, aldicarb-sulfoxide, was found in concentrations of 2000-3000 $\mu g \cdot L^{-1}$ during day 20 till 55, and did not decrease below 0.1 $\mu g \cdot L^{-1}$ until day 205 (500 mm). Maximum aldicarb-sulfone concentrations were approximately 600 $\mu g \cdot L^{-1}$, measured during days 30 till 125 and did not decrease below 0.1 $\mu g \cdot L^{-1}$ until approximately day 245 (540 mm) [1]). A mass balance for aldicarb was made by multiplying measured concentrations with leached volumes. It appeared that 0.35% of the initial dose was leached through the column over 245 days. However, when the two metabolites aldicarb-sulfoxide and aldicarb-sulfone were included in the mass balance, this percentage increased to 19.7%.

With the exception of a high concentration in the first leachate that was retrieved (260 $\mu g \cdot L^{-1}$ after 5 days), simazine was found in relatively small concentrations of 0.05-0.6 $\mu g \cdot L^{-1}$ throughout the entire leaching period of 280 days ($\approx$ 587 mm percolate) [2]). Only 0.11% of the initial dose was leached after this period. We observed conservative behaviour of simazine immediately after application, and assume non-sorptive by-pass to be responsible for this temporal high concentration in the leachate. Kookana *et al.* (1993) showed that during transport of simazine in soils the sorption processes are not in equilibrium, due to intra-organic diffusion. Sorption appeared to be time-dependent.

The difference in leaching behaviour of aldicarb and simazine may be partly explained by their sorption affinity to this clay soil. In a sorption study (Vink and Van der Zee, 1997), conducted with undisturbed micro columns, we found that sorption affinity for simazine was up to 6 times larger than for aldicarb (Table 2). Half lives for simazine were approximately three times longer and were soil-layer dependent. Therefore, an increased residence time of simazine in the lysimeter was to be expected.

Leachate pH-values were measured in 19 samples and ranged from 7.6 till 8.7, despite the use of slightly acidified leaching water (pH $\approx$ 5.7). The solution is therefore buffered by soil constituents such as $CaCO_3$. Dissolved O_2-concentrations were measured 11 times and ranged from 8.3 till 9.6 mg $\cdot L^{-1}$ which is close to saturation. Total-N concentrations, measured in 9 samples, ranged from 1.1 to 2.1 mg $\cdot L^{-1}$ [3]). It is likely that environmental variables like oxygen, nutrient availability and pH do not restrict biotransformation rates of these compounds in the lysimeter.

3.2 Estimation of water retention and conductivity

Using the gravitational drainage data over time, SFIT used 5 iterations to optimise the Van Genuchten parameters (Equations 1 and 2). Figure 2 shows the measured and calculated drainage from the soil column starting at water saturated conditions. The correlation coefficient (r^2) is 0.958. The resulting $\theta(h)$ and $K(\theta)$ relations, estimated from the methods proposed by Vereecken *et al.* (1989, 1990), appear familiar to the soil type.

3.3 Model performances

Figure 3 shows the actual percolation of water from the lysimeter, and the results of model calculations. PESTLA and VARLEACH tend to underestimate percolation, whereas LEACHP, MACRO and PELMO overestimate percolation as a function of time for this clay soil. Calculations with SIMULAT were carried

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[1]) Concentrations of aldicarb sulfoxide and aldicarb sulfone that were measured in drainage water from a field plot were much lower (4$\mu g \cdot L^{-1}$) than those observed in the lysimeter leachates. As opposed to the site were the lysimeter was taken, the field plot had been treated with aldicarb in previous years. Hence, microbial adaptation to the compound and subsequent accelerated transformation of aldicarb has occurred in the field but not in the lysimeter soil.

[2]) Field measurements were 0.1-0.8 $\mu g \cdot L^{-1}$ with maximum concentrations of 6.9 $\mu g \cdot L^{-1}$ at 43 days after field application (Brongers and Groen, 1995).

[3]) Field measurements: 0.9-2.5 μg N.L^{-1} (Brongers *et al.*, 1996).

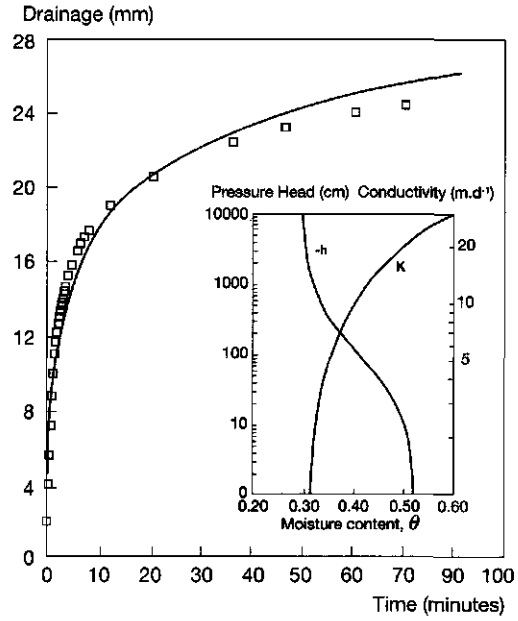

Fig. 2. Measured [□] and calculated [——] percolation, and resulting θ(h) and K(θ) relations of the soil column.

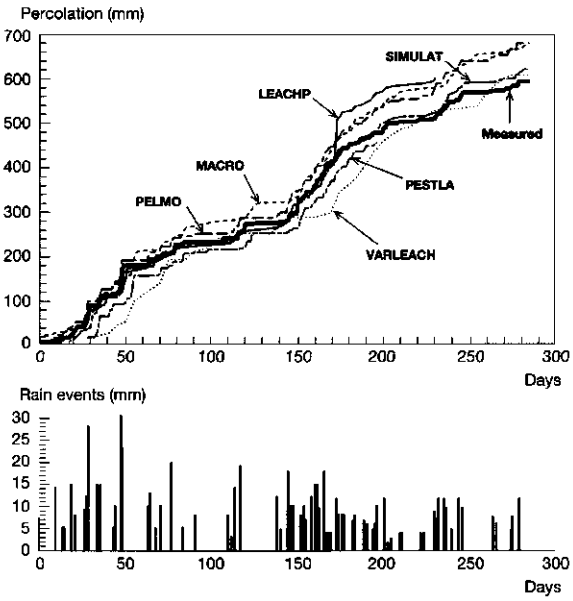

Fig. 3. Measured and calculated percolation from the soil column (top) and simulated rain events (below). Total amount applied was 715 mm, total percolation was 587 mm. Storage in and evaporation from the lysimeter was calculated at each sampling event.

out twice: the first time with the assumption that macropore flow occurs (presented in Fig. 3), and the second time with the same settings but without macropore flow. In Table 4, predicted percolation of the models are compared statistically to the actual measurements. The mean error (ME) is defined as the sum of variance of predicted and observed values at each time step. A positive value indicates a general overestimation, a negative value represents a general underestimation of the time-dependent water flow. The Ratio of Total Percolation (RTP) is defined as the fraction of predicted total amount of water that has percolated the soil column, compared to the actual measurement. Ideally, ME is close to 0, and RTP should be close to 1. The calculations that were closest to the measured data were done with SIMULAT, run in the macropore flow mode. In spite of the macro pore flow option, the MACRO computations of water percolation were not flawless. This may be attributed to its specific and caretaking handling: In the MACRO model, the soil and its

Table 4. Statistical comparison of measured and simulated water flow. ME = mean error; RTP = Ratio of Total Percolation; O = observed value; P = predicted value; i = interval; t = timesteps. SIMULAT was run with (+) and without (–) the macropore flow option.

	VARLEACH	LEACHP	MACRO	PELMO	PESTLA	SIMULAT+	SIMULAT–
$ME = \frac{\Sigma(P_i - O_i)}{t}$	-30.4	19.3	40.7	35.2	-30.6	0.51	3.1
$RTP = \left[\frac{\Sigma P_i}{\Sigma O_i}\right]^t; t = \max$	1.02	1.18	1.15	1.14	0.95	1.03	1.04

water are distinguished into two components: the macro- and micropore systems. Within the micropore regime, the water and solute movement is described using the classical Richards and CDE descriptions (the same approach is used by SIMULAT). MACRO can be run in this mode alone, and so reduces to the standard solutions equivalent to that offered by other models such as LEACHP. Within the macropore region, it is assumed that water flows under a unit hydraulic gradient using Poiseulle's law. Applications of MACRO for a cracked clay field plot were described by Harris *et al.* (1984), Goss *et al.* (1993) and Jarvis (1995). To fit the model, it is necessary to set the macro-micropore interaction parameters α' and β to low values, e.g., 0.05, typical for clay soils (Armstrong *et al.*, 1995) and to attribute to the soil matrix a very low conductivity. In this way, the majority of the water and solute movement is concentrated in the macropore component. Manipulation of this intrinsic hydraulic tool often enables calibration of both saturated and unsaturated water flow. However, the procedure does not necessarily promote the generation of predictive statements that are required in a large-scale agricultural management.

Figure 4 shows the results of the leaching experiment in combination with the exercises carried out with the six pesticide leaching models. The differences between calculations of bromide, aldicarb and simazine are large, given the fact that soil properties, hydraulic characteristics and pesticide properties are determined directly from the used soil. In spite of the fact that water flow may be well approximated, leaching of bromide may be ill-matched. This indicates that the water balance is fair, but that the flow routes along which water and solutes move are not described properly. Subsequent addition of pesticides to the solute may either override (e.g., MACRO) or superimpose (e.g., VARLEACH) this error.

According to calculations of VARLEACH,

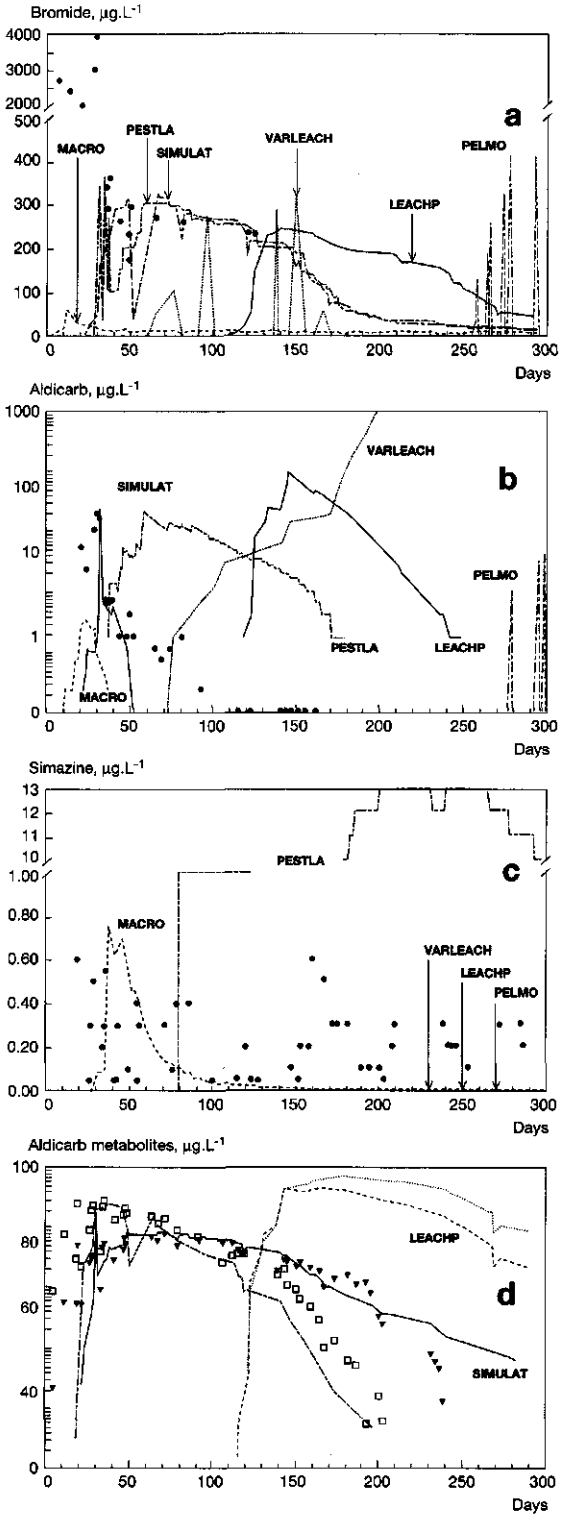

Fig. 4. Measured [●] and simulated concentrations of: a) bromide; b) aldicarb; c) simazine; d) aldicarb- sulfoxide [□ = measured, - - - - = LEACHP, – – – – = SIMULAT] and aldicarb-sulfone [▼ = measured, ····· = LEACHP, —— = SIMULAT].

LEACHP and PELMO, simazine concentrations in the leachate are zero during the entire period, and the compound is retarded and sorbed in the top 0.35 m of the soil. PESTLA predicts continuous leaching of simazine over a long period, as was observed, but overestimated concentrations. SIMULAT predicted four non-observed peak concentrations. When the model was run without the assumption of macropore flow, these peaks did not appear, and concentrations became more constant (0.1-0.2 $\mu g \cdot L^{-1}$ during days 50 till 280). However, the calculated leached fraction (0.03%) was underestimated in that case. For the prediction of aldicarb concentrations, the differences between the two runs were not significant.

The occurrence of aldicarb's metabolites, calculated by SIMULAT and LEACHP, is shown in Fig. 4d. SIMULAT gives an accurate prediction of the breakthrough curves of both metabolites. Although LEACHP makes a fair prediction of maximum metabolite concentrations in the leachate, two major inaccuracies occur: i) The appearance time of aldicarb sulfoxide and aldicarb sulfone in the leachate is delayed, which may be attributed to ill-matching water flow in combination with the underlying chemical mechanisms; ii) The chain-reaction type mechanism, i.e. breakdown of the parent compound and sequential formation of the metabolites, is not reflected in the concentration pathways of the individual metabolites. The underlying interactive mechanism is based on metabolite formation rates that depend on the concentrations of the previous compound (Vink and Van der Zee, 1997). Since aldicarb sulfone cannot be formed until aldicarb sulfoxide is transformed, concentrations of aldicarb sulfoxide initially exceed those of sulfone. This is followed by a period in which the formation of aldicarb sulfoxide becomes dominant, thus exceeding concentrations of aldicarb sulfoxide. Nevertheless, the steepness of the leaching curves prior and after breakthrough, which reflects interactive formation and transformation rates of the metabolites, are fairly well approximated.

4 Discussion

It is shown that water retention and conductivity, which are difficult to determine for a cracked clay soil in an experimental set-up, may be approximated by easily measurable soil characteristics such as texture, bulk density and organic carbon content. In this way, tedious and generally poorly reproducible experiments were avoided. We have also shown that the leaching behaviour of two pesticides, mediated by their chemical properties, impose different risks to ground and surface water. Pronounced peak exposure of aldicarb and its aerobic metabolites sulfoxide and sulfone were observed in concentrations far exceeding norm-levels for water. In contrast, simazine was found in relatively low concentrations, with small variations during the leaching period of 300 days. This absence of a pronounced, time dependent 'breakthrough behaviour' (peak exposure) implies long term delivery of the compound from the soil. Although simazine concentrations never reached levels that may be acute toxic to aqueous organisms, the period of exposure is long (chronic exposure). Still, very little is known about the persistence, and residence time, of pesticides in aqueous systems. Especially clay soils, in which preferential flow of water and solutes may occur, may contribute to the risk of temporal high concentrations in drainage water, and consequently dictate the implications for these aqueous environments. In a management point of view,

Table 5. Measured and calculated losses (%) of initial aldicarb and simazine doses. SIMULAT was run with the macropore flow option.

	Measured	VARLEACH	LEACHP	MACRO	PELMO	PESTLA	SIMULAT
Aldicarb	0.35% (0.104 kg · ha^{-1})*	27.7	46.1	0.002	0.08	0.18	0.40
Simazine	0.11% (0.008 kg · ha^{-1})	0	0	0.01	0	0.31	0.15

* active ingredient

better understanding on the behaviour of pesticides in the altered environment are needed.

Measured and calculated losses of the initial pesticide dose from the soil are compared in Table 5. The presented values give a fair indication of the individual performances, but do not provide the necessary information to evaluate environmental risks of the compound. We observed that approximately 0.35% of the initial aldicarb dose was leached. However, including its metabolites in the mass balance, this amount increases dramatically to almost 20%. The occurrence of metabolites is, obviously, of great importance when assessing environmental risks to the parent pesticide. LEACHP and SIMULAT are one of the very few models that have incorporated the possibility to calculate the formation and transformation of metabolites. Although some conceptual inaccuracies may occur regarding the time of formation and leaching, and the lack of interactive, chain-reaction type descriptions, the ability to predict the occurrence of metabolites is essential in risk evaluations of the parent compounds.

It is concluded that none of the models were able to describe both water percolation and pesticide leaching to a complete satisfying degree. Even in this case where model parameters are determined directly from the studied soil, as opposed to being derived from literature, the variety in results is large. Since input data were identical, these variations in predictive performance may be directly attributed to the conceptual differences of the models. Water movement was initially well described by LEACHP, but the model ill-matched in wet periods in the early season. In the first 170 days of leaching, the mean error between observed and predicted percolation was very small (ME = -3.4), somewhat underestimating percolation from the lysimeter. Frequent rain events during the months of February and March resulted in a serious overestimation of water flow, and increased the ME to +19.3 (Table 4). This phenomenon was also observed by Khakural *et al.* (1995), who reported inaccurate performance of LEACHP in relatively wet years. PELMO, VARLEACH and PESTLA have comparable and relatively low ME values for water flow, but PELMO and VARLEACH showed larger maxima in specific periods. Over the entire period of the experiment, SIMULAT and PESTLA delivered the most satisfying results of both water and tracer percolation. However, when models are rated by the predicted time period in which maximum concentrations occur in drain water, the macropore-models SIMULAT and MACRO delivered the best results for the tracer as well as pesticides. It is emphasized that these conclusions are derived from the interpretation of leaching data, and do not *a priory* apply to the accuracy in which the models are able to approximate time-depth-distribution of pesticides in soil. Although these models all recognise the effect of moisture and temperature on transformation rates, it should be noted that these parameters are only two of many that (indirectly) determine microbial activity and subsequent biotransformation of organic compounds in soil.

Disregarding formation of metabolites, it is generally suggested that the risk that is imposed by a pesticide on its environment is stipulated by i) the (maximum) concentrations that may occur, and ii) the time of exposure. The results have shown that variation in predictive behaviour of water, tracer and pesticides is large. Of much concern is the fact that the agreement between the models of maximum concentrations and the time period in which these occur, is very small. Gottesbüren *et al.* (1995) exercised with five leaching models on a different soil and pesticide, and derived similar conclusions. However, the predictive performance of these pesticide leaching models change (for better or for worse) when using different soils and pesticides. Extrapolation of model results to various soil types and/or pesticides should be interpreted with great caution. In our view, attempts to further improve pesticide leaching models by incorporating detailed, physically based descriptions, may not necessarily result in an improved risk evaluation of the studied pesticide. The implementation of an important biochemical process may very well override the disadvantage of a simple water flow concept (Van der Zee and Boesten, 1991; Gottesbüren *et al.*, 1994). The applicability of leaching models in risk assessments, e.g., for legislature purposes, may be enhanced by introducing a variety of conceptual descriptions which may be applied on a site-specific basis, including the formation and fate of relevant, toxic metabolites.

Acknowledgement

The authors wish to thank Dr. Jos J.T.I. Boesten for his

useful suggestions. Karin Aden is acknowledged for her contribution to the model computations.

Notation

E_{ACT}	=	Activation energy ($KJ \cdot K^{-1} \cdot mole^{-1}$)
H	=	Henry constant
h	=	Hydraulic pressure head (m)
K	=	Hydraulic conductivity ($m \cdot d^{-1}$)
K_f	=	Freundlich distribution coefficient ($L \cdot kg^{-1}$)
K_s	=	Saturated conductivity ($m \cdot day^{-1}$)
K_{OC}	=	Organic carbon/water distribution coefficient ($L \cdot kg^{-1}$)
K_{OW}	=	Octanol/water distribution coefficient
n	=	Freundlich parameter
P_v	=	Vapour pressure (Pa)
Q10	=	Increase in decay rate at a temperature increase of 10 °C
S	=	Solubility ($mg \cdot L^{-1}$)
S_e	=	Effective saturation constant
T	=	Temperature, C
T_{ref}	=	Reference temperature
$\alpha' \beta$	=	Mass transfer coefficients for macropore and micropore regions
ρ	=	Density ($g \cdot cm^{-3}$)
Φ	=	Porosity ($cm^3 \cdot cm^{-3}$)
θ	=	Volumetric water fraction ($cm^3 \cdot cm^{-3}$)
θ_{ref}	=	Reference θ
θ_F, θ_{WP}	=	θ at field capacity, wilting point
θ_r, θ_s	=	Residual, respectively saturated θ
μ	=	Transformation rate (day^{-1})
n,m,α,L,γ	=	Empirical parameters
τ, b, f_z	=	Transformation correction factor for temperature, moisture and depth

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Chapter 4

Mathematical Descriptions of Accelerated Transformation of 1,3-Dichloropropene in Soil; a Microbiological Assessment

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Published in *The Science of the Total Environment* **123/124** (1992) 591-603.

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Mathematical descriptions of accelerated transformation of 1,3-dichloropropene in soil; a microbiological assessment

Abstract - The rate of transformation of the soil fumigant (Z)-and (E)-1,3-dichloropropene in moist soil layers was measured at incubation temperatures of 5 °C, 10 °C, and 20 °C. 1,3-D was added to four characterized soil layers, amounts corresponding to field realistic contents after fumigation. Rapid transformation immediately after application was observed in layers with low initial contents (30-300 $\mu g \cdot kg^{-1}$ dm), and could well be described with a first-order rate model. Incubation at higher doses (5-15 $mg \cdot kg^{-1}$ dm respectivily) showed distictly different transformation pathways. Degradation curves could well be computed using a microbiological intrinsic compitition (MIC) model for moist soil. The transformation rate is inversely correlated to microorganism population size and growth. Transformation curves described by MIC are characterized by a lag-time, a period of accelerated transformation and a period of decreasing transformation rates. At low temperatures, DT50 values of more than 20 days could be observed. Half lives computed with the first-order rate model did not exceed 8 days. The use of different mathematical discriptions for various soil layers and soil temperatures permits more reliable simulation of 1,3-D transformation by microorganisms in the soil profile throughout the growing season.

1 Introduction

The soil fumigant 1,3-dichloropropene (DD95 Shell registrated trademark) is widely used in agricultural systems for the control of plant parasitic nematodes in soil. Its main constituents are (Z)-1,3-dichloropropene (*'cis'-isomer*) and (E)-1,3-dichloropropene (*'trans'-isomer*). The compound 1,2-dichloropropane, which is a by-product during fabrication of 1,3-D, amounts approximately 3%. 1,3-D is usually applied by injection into the soil at 0.15-0.2 m depth. The surface is sealed by rolling, in order to reduce excessive volatilization.

Numerous authors (Van Dijk, 1974; Van Dijk, 1980; Roberts and Stoydin, 1976; Smelt *et al.*, 1989a) have reported on degradation mechanisms in soil under field and laboratory conditions. In most cases, observed parameters affecting degradation is limited or insufficient in order to give a full seasonal description throughout the soil profile.

Field studies, carried out in 1989 and 1990 under flower bulb cultivation in the North East polder, The Netherlands, showed large variety in total content of 1,3-dichloropropene in each characterized soil layer. Considering the injected toplayer and the subsoil to 0.8 m depth and a total dose of 150 l 1,3-D per ha, total contents varied up to a factor 10,000. Accelerated degradation was monitored at approximately one week after fumigation. This phenomenon was also reported by Smelt *et al.* (1989a). Transformation rates increased drastically when initial contents decreased. Leached amounts, periodically measured with a proportional automatic drain discharge sampler, did not exceed 0.001 % of the total dose (mean daily temperature ± 18 °C). These findings suggest that the soil contained microorganisms that can transform 1,3-dichloropropene effectively.

Observations from field studies prompted further study on transformation rates under pre-set conditions. These conditions were derived from meteorological data and field measurements. At temperatures of 5 °C, 10 °C and 20 °C, and initial contents ranging from 30 $\mu g \cdot kg^{-1}$ dm to 15 $mg \cdot kg^{-1}$ dm, transformation of 1,3-dichloropropene was monitored in time. Moisture content was kept at approximately 75% of field capacity,

Table 1. Soil properties.

Soil layer depth (cm)	< 2μm (%)	S ($m^2 \cdot 100\ g^{-1}$)	Org.C (%)	$CaCO_3$ (%)	pH_{H_2O} (air-dry)
5-15	6.4	3.39	1.1	5.90	7.8
25-35	6.6	4.19	1.0	5.20	7.8
45-55	6.3	5.47	0.9	5.45	7.8
65-75	4.8	5.56	0.9	6.85	7.8

corresponding to a moisture pressure of approximately -10 kPa (0.1 bar). Transformation curves were computed and optimised with the aid of mathematical models. Results were used to compute transport and dissipation rates of the compound in the soil profile for a seasonal temperature range.

2 Materials and methods

2.1 Incubation series and analyses

On an experimental field plot nearby the city of Creil in the North East polder in The Netherlands, a detailed soil survey was carried out in order to characterize the soil profile on a morfological basis. The soil is classified as a calcaric fluvisol of the Formation of Duinkerke. The topsoil is fine textured loamy sand to a depth of 0.4 m. The subsoil is fine layered sandy loam and loamy sand. At 0.8 m. below surface, an impermeable layer of detritus-gyttja is found. Based on morfological and chemical characteristics, soil samples were taken at 5-15 cm, 25-35 cm, 45-55 cm and 65-75 cm depth at ten sites and were mixed thoroughly. In Table 1, some physical and chemical soil characteristics of these layers are presented. Note the increase of the specific surface area ($m^2 \cdot 100\ g^{-1}$ dry soil) in depth, determined for the 16 μm - 2 mm fraction.

The plot was last treated with 1,3-D in 1987. Initial contents of (Z)- and (E)-1,3-dichloropropene were determined in each mixed layer. In preliminary tests, best results for the analyses of 1,3-D were obtained using 500 cm^3 glass flasks (ground glass stoppers) containing 100 gr soil, 100 ml demineralized water and 10.0 ml hexane. This mixture is heated to 100 °C, trapping hexane and sorbed 1,3-dichloropronene in a distillation column. The extraction method proved a recovery of approximately 70%.

Moist soil in quantities of 100 g (corresponding to 75-85 g of dry soil) were weighed into three temperature series of 28 flasks to a total of 84 flasks. Moisture content was, if necessary, adjusted. To each flask within the series, specific amounts of the isomers (Z)- and (E)-1,3-dichloropropene were added with microlitre syringes in order to obtain initial contents of approximately 30 $\mu g \cdot kg^{-1}$ to 15 $mg \cdot kg^{-1}$ dry soil, as shown in Table 2. The flasks were sealed and stored in the dark at 5 °C, 10 °C and 20 °C respectively. At intervals of 1 hour, 1 day, 3, 7, 14, 35, and 70 days during incubation, four flasks were taken for extraction. Analyses were carried out in a HP 5880A gas chromatograph using the Purge and Trap-method. Helium was used as a carrier gas, and 2,6-diphenyl-p-phenyloxide as an adsorbent.

2.2 Mathematical descriptions - first-order degradation

Pesticide degradation in soil is often described by the

Table 2. Initial contents ($\mu g \cdot kg^{-1}$ dm) of (Z)-('*cis*') and (E)-('*trans*') 1,3-dichloropropene in various incubation series, measured at one hour after addition.

Temperature	5 °C		10 °C		20 °C	
Soil layer Depth (cm)	*cis*	*trans*	*cis*	*trans*	*cis*	*trans*
5-15	13980	12180	12000	10700	15526	14672
25-35	4664	4056	4120	3530	5127	5092
45-55	245	213	195	165	270	261
65-75	23	23	20	18	29	23

first-order differential equation:

$$\frac{dC}{dt} = - kC$$

where C = concentration of the compound, t = time, and k is the first-order rate constant. Upon integration and natural logarithmic transformation, the resulting equation is a simple linear model that is easily tested by regression analysis. The first-order model adequately describes concentration/time products, and permits calculation of DT50 from the slope. The use of this model was tested under conditions previously mentioned.

2.3 A microbiological approach - adaption and intrinsic competition

Linear and non-linear models have been used to describe the initial rapid degradation and slow subsequent degradation for pesticides in soil (Shaaban and Elprince, 1989; Reyes and Zimdahl, 1989; Walker, 1974). In case of 1,3-dichloropropene, it was reported by several authors (Van Dijk, 1980; Van der Pas and Leistra, 1987) that degradation does not always seem to follow first-order kinetics. Smelt *et al.* (1989b) noted a remarkable effect of comparatively small differences in the initial content in soils on the transformation rate, and concluded a non-linear relationship of the concentration-time products. Furthermore, accelerated transformation after one week of fumigation was also confirmed by the last authors. These findings suggest that degradation of 1,3-dichloropropene in soil is mainly a micobiological process. Hence, concentrations are dependent on *in-situ* population size and development of degrading microorganisms.

If substrate is abundant, it is assumed that the microbial population size (A) increases in time according to a first order function:

$$\frac{dA}{dt} = - rA \qquad (1)$$

with t = time since incubation and r = rate coefficient. Simple integration and transformation leads to:

$$A_t = A_0 \cdot \exp(rt) \qquad (2)$$

Where A_t is the total population size and A_0 is the initial population size at t = 0. This accelerated growth however, will be reduced in the course of time due to intrinsic compitition for the available substrate, i.e. within the present strains of degrading microbial populations. Therefore, a reduction factor is introduced:

$$f = 1 - \frac{A}{A_m}$$

with A_m being the maximum value of A. Combination with equation 1 leads to:

$$\frac{dA}{dt} = rA\left(1 - \frac{A}{A_m}\right) \qquad (3)$$

$$\rightarrow \int \frac{1}{A} \cdot \frac{1}{\left(1 - \frac{A}{A_m}\right)} dA = \int r\, dt$$

$$\rightarrow \int \frac{A_m}{A(A_m - A)} dA = \int r\, dt \qquad (4)$$

Equation 4 is now manipulated mathematically in order to divide the left term into two convenient terms:

$$\frac{A_m}{A(A_m - A)} = \frac{\alpha}{A} + \frac{\beta}{(A_m - A)}$$

$$\rightarrow \quad A_m = \alpha (A_m - A) + \beta (A)$$

Which leads to $\alpha = 1$ and $\beta = 1$. Equation 4 may now be written as:

$$\int \frac{1}{A} dA + \int \frac{1}{(A_m - A)} dA = \int r\, dt$$

$$\rightarrow \qquad \ln A_t - \ln (A_m - A_t) + \ln W = rt \qquad (5)$$

In equation 5, the population size of microorganisms at time t is represented by A_t. The constant W equals:

$$W = \ln \frac{A_m - A_0}{A_0} \qquad (6)$$

Equation 5 can now be solved as follows:

$$\ln \frac{A_t W}{A_m - A_t} = rt$$

$$\rightarrow \quad \frac{A_t}{A_m - A_t} = \left(\frac{1}{W}\right) \cdot \exp(rt)$$

$$\rightarrow \quad A_t = \frac{A_m}{1 + W \cdot \exp(-rt)} \qquad (7)$$

The size of the microorganism population at time t is a resultant of the relative speed of growth, the maximum value of A and the initial population size at t = 0. In figure 1, relationships between relative speed of growth (dA/dt), maximum amount of microorganisms (A_m) and time (t) are presented.

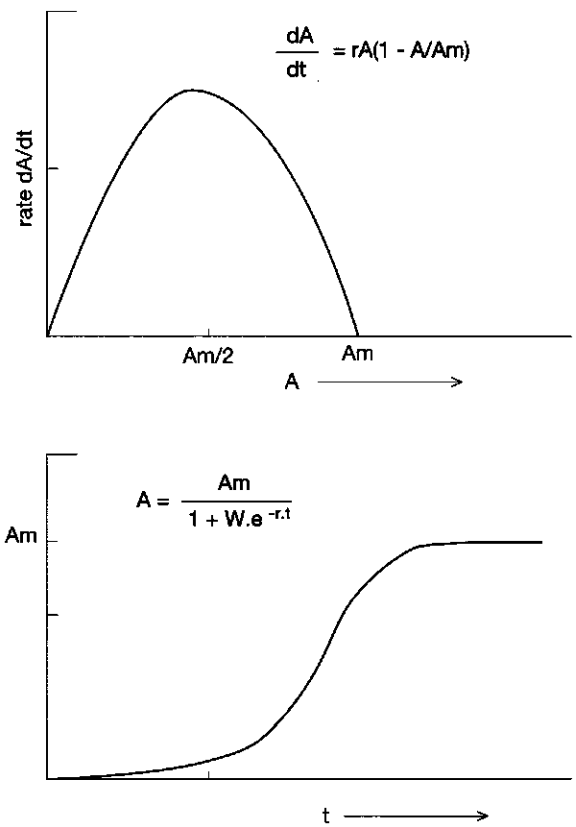

Fig. 1. Relationship between microbial growth rate and population size (top) and population size in time (below).

In order to link population size and degradation rate of 1,3-dichloropropene, an inverse proportional correlation is assumed. Hence, consumption (CON) of 1,3-D is a function of the amount of microorganisms A, or $CON = f(A)$. Including a linear relationship in this function, consumption at time t may be written as:

$$CON_t = b \cdot A_t \qquad (8)$$

which leads equation 7 to:

$$CON_t = \frac{b \cdot A_m}{1 + W \cdot \exp(-rt)} \qquad (9)$$

The amount of 1,3-dichloropropene available at time t equals the initial content C_i minus total consumption: C_t - CON_t expressed in $\mu g \cdot kg^{-1}$ dm, for example. A small amount of the compound, strongly bound by organic matter, is not susceptable to micobiological breakdown. Maximum residues (C_r) were found in the toplayer and were as high as 78 $\mu g \cdot kg^{-1}$ dm. In the subsoil, these values reached to a maximum of 4.2 $\mu g \cdot kg^{-1}$.

In order to describe degradation in the course of time, the following conditions have to be applied:

1) For t = 0 : $C_i = C_{max}$ and $C_i = b \cdot A_m$;
2) for t $\rightarrow \infty$: $C_t = C_r$.

which leads to:

$$C_t = C_{Tot} - \frac{C_i}{1 + W \cdot \exp(-rt)} \qquad (10)$$

in which $C_{Tot} = C_r + C_i$. The right term in the denominator is now transformed as follows:

$$W \cdot \exp(-rt) = \exp(x) \cdot \exp(-rt) = \exp(x - (rt)) = \exp(r(D - t))$$

In which $D = x/r$. Simple transformation of equation 10 now leads to the following equation which describes the content of 1,3-dichloropropene at time = t in soil:

$$C_t = C_{Tot} - \frac{C_i}{1 + \exp(r(D - t))} \qquad (11)$$

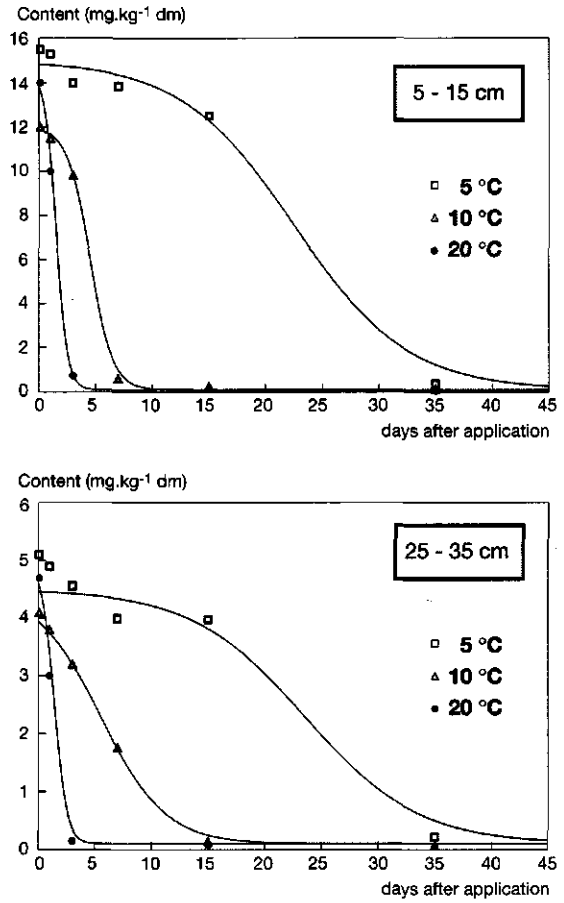

Fig. 2a. Transformation computations carried out with microbiological intrinsic compitition-approach (MIC) for (Z)-1,3-dichloropropene in topsoil layers 5-15 cm (top) and 25-35 cm (below) at 5, 10 and 20 °C.

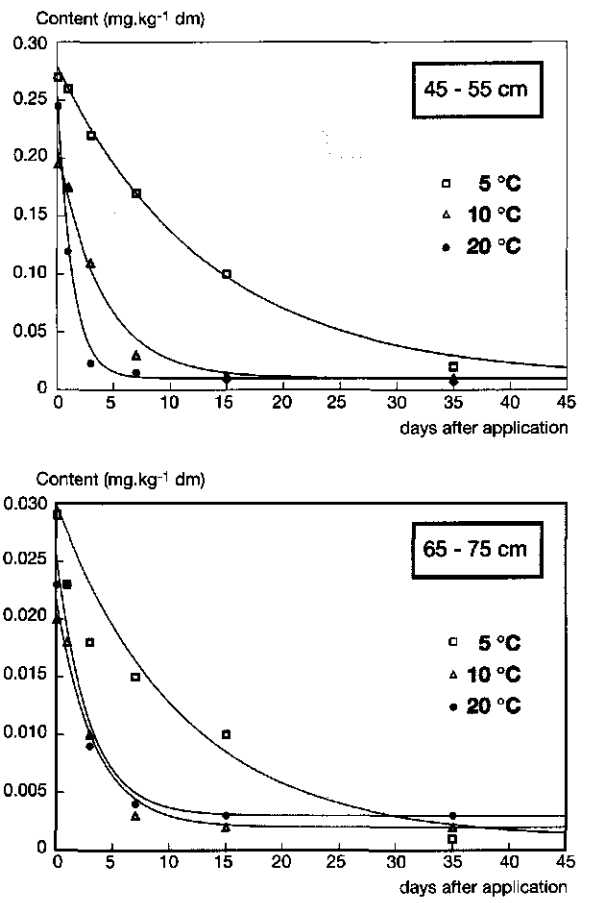

Fig. 2b. Transformation computations carried out with a first-order rate approach (FOR) for (Z)-1,3-dichloropropene in subsoil layers 45-55 cm (top) and 65-75 cm (below) at 5, 10 and 20 °C.

C_t = Content at time t ($\mu g \cdot kg^{-1}$ dm)
C_{Tot} = Total content at t = 0 ($\mu g \cdot kg^{-1}$ dm)
C_i = Initial content at t = 0 ($\mu g \cdot kg^{-1}$ dm)
r = Constant ($days^{-1}$)
D = Disappearance time 50% (days)
t = Time (days)

For $t \rightarrow 0$, $C_0 = C_{Tot} - C_i / (1 + exp\ (r.D))$. If the product of $r.D$ has a high value, the term $C_i / (1 + exp\ (r.D))$ is negligible, resulting in $C_0 = C_{Tot}$. Hence, equation 11 does meet condition 1.

For $t \rightarrow \infty$, the term $exp\ (r(D\text{-}t)) = 0$, and consequently $C_t = C_{Tot} - C_i = C_r$. Hence, equation 11 does meet the second condition.

Disappearance time 50%, which is a known parameter in order to characterize degradation of pesticides, is also incorporated in equation 11. $Exp\ (r(D\text{-}t)) = 1$ if $D = t$, allowing equation 10 to be transformed in $C_{t \rightarrow 1/2} = Cr + 0.5\ .\ Ci$, which seems to be an acceptable assumption.

3 Results

Table 2 shows initial contents of (Z)- and (E)-1,3-

dichloropropene in the observed soil layers at one hour after incubation. Degradation curves, obtained from incubation series at 5 °C, 10 °C en 20 °C were subjected to statistical analysis. Data were optimised to best-fit for the models described before. No significant differences between the degradation pathways of the (Z)- and (E)-isomers could be observed. This is in agreement with findings of Roberts and Stoydin (1976).

Results of (Z)-1,3-dichloropropene are represented in Fig. 2a and 2b. In the two topsoil layers (5-15 cm and 25-35 cm; Fig. 2a), where initial contents were as high as 15 mg · kg^{-1} dm and 5 mg · kg^{-1} dm respectivily, degradation curves could well be described using the microbiological intrinsic compitition-approach (MIC) for moist soil. Computations carried out with MIC are characterized by three different stages: 1) a lag-time; 2) a period of accelerated transformation; 3) decreasing transformation rates. During lag-time (stage 1), the amount of microorganisms may well be a limiting factor to rapid transformation. During decreased transformation (stage 3), the amount of substrate may be the limiting factor. Subsoil layers (45-55 and 65-75 cm; Fig. 2b) however, with initial contents of 300 $\mu g.kg^{-1}$ dm and 30 $\mu g.kg^{-1}$ dm respectivily, show distincly different shapes of the degradation curves. Immediately after incubation, the amount of 1,3-dichloropropene is rapidly decreased. A lag-time is absent. Apparently, the amount of microorganisms nor the substrate are limiting accelerated transformation. Table 3 summerizes experimental constants and parameters for topsoil and subsoil layers derived from model calculations.

In incubation tests, carried out at 5 °C, there appears to be a remarkable relationship between DT50 values and initial content of 1,3-dichloropropene in soil. At identical moist contents and incubation temperatures, the 50% dissapearance time in the '*µg/kg*-range' (top-

Table 3. Experimental parameters derived from incubation series at 5, 10 and 20 °C. For topsoil layers, the experimental constant r, first-order rate coefficient k and corresponding disappearance time 50% (DT50) were derived from computations carried out with MIC (Microbiological Intrinsic Competition). For subsoil layers, first-order rate computations were applied.

(Z)-1,3-dichloropropene

Temperature	5 °C			10 °C			20 °C		
Soil layer depth (cm)	r (d^{-1})	k (d^{-1})	DT50 (d)	r (d^{-1})	k (d^{-1})	DT50 (d)	r (d^{-1})	k (d^{-1})	DT50 (d)
5-15	0.20	0.031	22.5	1.00	0.154	4.5	2.00	0.462	1.5
25-35	0.20	0.029	23.5	0.35	0.126	5.5	1.80	0.495	1.4
45-55	-	0.086	8.1	-	0.301	2.3	-	0.630	1.1
65-75	-	0.080	8.7	-	0.301	2.3	-	0.408	1.7

(E)-1,3-dichloropropene

Temperature	5 °C			10 °C			20 °C		
Soil layer depth (cm)	r (d^{-1})	k (d^{-1})	DT50 (d)	r (d^{-1})	k (d^{-1})	DT50 (d)	r (d^{-1})	k (d^{-1})	DT50 (d)
5-15	0.26	0.035	19.8	1.00	0.173	4.0	2.00	0.462	1.5
25-35	0.22	0.036	19.0	0.35	0.110	6.3	1.80	0.495	1.4
45-55	-	0.055	12.5	-	0.158	4.4	-	0.630	1.1
65-75	-	0.082	8.5	-	0.198	3.5	-	0.408	1.7

soil) is approximately three times as high as in the '*mg/kg*-range'. This is also expressed by an increase of the constant r described in equation 11. At 10 °C, topsoil DT50 values are about two times as high as compared to subsoil measurements. In the case of incubation at 20 °C, there seems to be no significant correlation between DT50 and initial content.

4 Discussion

Transformation by microorganisms of the soil fumigant 1,3-dichloropropene seems to be a proces that can be discribed mathematically if intrinsic competition of microorganisms is assumed. Transformation rates are inversely correlated to size and growth of microorganisms. At high contents (5 and 15 mg · kg^{-1} dm) of 1,3-D, the population of organisms that can transform 1,3-dichloropropene effectively in soil is a limiting factor to maximum transformation rates. Accelerated transformation only takes place at optimum growth of the population of microorganisms. However, transformation rates decline as a result of decreasing substrate availability, giving rise to competition within the population. At low substrate content (30-300 μg · kg^{-1} dm), accelerated transformation was observed immediately after incubation. Transformation pathways could well be described by first-order rate models.

For all soil layers, DT50 values decrease rapidly at increasing temperatures. This is shown in figure 3. Incubation series at 15 °C with similar soils were carried out by Van der Pas and Leistra (1987). An initial content of 12 mg · kg^{-1} dm was used in this study. Disappearance time 50% was 3.5 days.

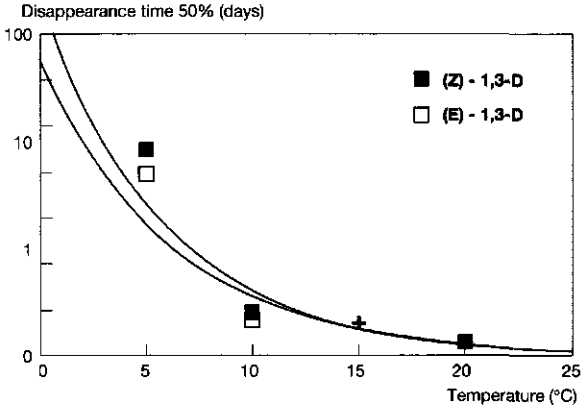

Fig. 3. Disappearance time 50% versus temperature for the 5-15 cm topsoil layer. One data point (+) is adopted from Van der Pas and Leistra (1987) for a similar moist soil, at an initial 1,3-D content of 12 mg · kg^{-1} dm.

DT50 values measured at 5 °C exceed values measured at 20 °C by a factor 5 to 15. It should be noted however, that under field conditions this effect might be somewhat smoothed. Incubation tests were carried out at comparable moisture contents, giving rise to one-parameter interpretations. Under field conditions, moisture content may show significant variations throughout the soil profile. Especially at higher temperatures (e.g., > 15 °C), the soil hydraulic gradient may show great variety in depth, and maximum transformation rates may likely not be reached.

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Chapter 5

Modelling the Microbial Breakdown of Pesticides in Soil Using a Parameter Estimation Technique

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Published in *Pesticide Science* **40** (1994) 285-292.

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Modelling the microbial breakdown of pesticides in soil using a parameter estimation technique

Abstract - Results of field measurements and laboratory experiments were used to simulate the behaviour of 1,3-dichloropropene (1,3-D) in a loamy sand soil. Microbial activity was described using pre-set conditions to compute biotransformation rates as dependent on compound concentrations and temperature. These kinetics could only be analysed using non-linear transformation rates. To link the development of microbial populations and the consumption of the compound over time, an iterative technique was used to estimate the necessary parameters.

1 Introduction

The environmental fate of an organic chemical depends on both physical and biological processes. The determining physico-chemical processes include convective transport, diffusion, dispersion, sorption and desorption, and non-biological transformations such as hydrolysis or photolysis. In soil, most organic chemicals undergo biological transformations, mediated by microorganisms. For many compounds, the details of the underlying biochemical mechanisms are not yet identified. It is obvious that these processes depend in a complex manner on soil properties, environmental conditions and the population dynamics of the microorganisms.

In many publications focusing on transport, simple kinetic terms (usually first-order) are used (Jury and Sposito, 1985; Wagenet and Rao, 1985), whereas in those publications focusing on non-linear kinetics, transport phenomena are often totally neglected (Soulas, 1982). Only few papers combine both aspects (Nietfeld *et al.*, 1992; Richter *et al.*, 1992a). The behaviour of pesticides needs to be simulated by mathematical models that comprise both biological processes, with their inherent non-linearities, and physical transport processes.

In previous studies (Roberts and Stoydin, 1976; Smelt *et al.*, 1989; Vink and Groen, 1992; Richter *et al.*, 1992b), field observations of pesticides in soil showed appreciable variation of transformation rates with time and depth. Transformation rates could not always be described according to first-order models. The rate of transformation of pesticides into other compounds is generally ascribed to initial microbial activity and potential growth. Provided no physico-chemical impediments in soil layers occurs, the effect of microbial activity and development is regulated by substrate availability (i.e. pesticide concentrations) and temperature. It should be noted however, that this may depend on whether the microorganisms are using the pesticide as a sole or major source of energy.

Richter *et al.* (1992a,b) promotes to use a non-linear approach in pesticide degradation, and reports the consequences for parameter estimation and model application. In general, the non-linear differential equations that describe non-linear degradation cannot be solved analytically. Therefore, the solutions cannot be written in the form of known functions such as exponentials. In recent years, advanced numerical tools (Bock, 1987; Dixon, 1987) have been developed to estimate parameters in such systems. This paper demonstrates further applications of these methods, using the behaviour of the nematicide 1,3-dichloropropene (1,3-D) in a sandy soil together with earlier data on 2,4-D (2,4-dichlorophenoxy acetic acid) as a comparison.

2 Methods

2.1 Experimental design

2.1.1 Field measurements

The soil fumigant 1,3-dichloropropene is widely used

as a pre-planting treatment for the control of plant parasitic nematodes in potato and flower bulb systems. To minimise volatilisation, the compound is injected into the soil and the surface is sealed by rolling. 1,3-D consists of two isomers, *CIS-(Z)*-1,3-dichloropropene and *TRANS-(E)*-1,3-dichloropropene, present in approximately equal amounts.

On the experimental 1.6 ha field plot (North East polder, The Netherlands), the soil profile (calcaric Fluvisol) has to a depth of 0.4 m a topsoil of fine textured loamy sand, and a subsoil of fine layered loamy sand interlayered by sandy loam. At 0.8 m below the surface, an impermeable layer of detritus-gyttja occurs. Individual soil layers were determined on morphological and chemical bases and samples were taken at 0.05-0.15 m, 0.25-0.35 m, 0.45-0.55 m and 0.65-0.75 m depth at ten locations and mixed thoroughly. Some physical and chemical characteristics of these soil layers are presented in Table 1.

In September 1990, 1,3-D (150 litre · ha^{-1}) was applied by injection to this field plot, and an intensive monitoring programme was started. Over a period of 40 days, precipitation was measured and amounts of 1,3-D, water content and soil temperature were measured periodically in four consequetive soil layers. These data were used in combination with laboratory experiments to calibrate simulation of transport and persistence.

2.1.2 Laboratory incubations

To determine realistic concentrations for incubations, the results of previous field measurements (Vink *et al.*, unpublished results) were used as a guideline. Concentrations of 1,3-D decreased with depth by a factor of up to 10,000. Therefore, it is realistic to use decreasing concentrations for samples from increased depths. The initial concentrations assigned to the four depths were 15, 5, 0.3 and 0.03 mg · kg^{-1} (dry weight) respectively.

Mixed samples of the four soil layers were weighed (100 g, representing 75-85 g dry soil) into four series (concentrations and soil layers) of seven (time steps) 250 ml flasks. Required amounts of 1,3-D were added to the soil with microlitre syringes. This procedure was carried out three times, and the flasks, stoppered with glass wool, were stored in the dark at 5 °C, 10 °C and 20 °C. If necessary, water was added by calculating the loss of weight for each sample from approximately 75% of field capacity (0.4%). Extraction and analyses were done after 1 hour and 1, 3, 7, 14, 35 and 70 days after addition. 1,3-D was extracted from the soil by heating to 100 °C. This method appeared to be more reliable than conventional extraction with hexane. Analyses were performed with a Perkin-Elmer ATD-400 (automatic thermic desorption) gas chromatograph. Linear Helium gas (0.25 m · s^{-1}) was used to displace the volatile compound, which was then trapped by the adsorbent 2,6-diphenyl-p-phenyloxide. Two desorption columns (CPsil5/13CB) of 25 m length, 3.2 · 10^{-4} m internal diameter, were used in combination with an ECD Nickel-63 (300 °C) detection system. A step-wise temperature programme lead to an oven temperature of 250 °C. Analytical recovery amounted to approximately 75%.

2.2 Estimation of the parameters

Deviations from linearity of transformation rates can be analysed using experiments with a large variation of

Table 1. Soil properties of the field plot.

Depth (m)	< 2 μm (%)	Specific surface area[1] ($m^2 \cdot 100g^{-1}$)	Bulk density ($kg \cdot m^{-3}$)	Saturated conductivity K_s ($m \cdot d^{-1}$)	Org.C[2] (%)	$CaCO_3$ (%)	pH_{H_2O}
0.05-0.15	6.4	3.39	1430	nd	1.1	5.90	7.8
0.25-0.35	6.6	4.19	1500	0.281	1.0	5.20	7.8
0.45-0.55	6.3	5.47	1470	nd	0.9	5.45	7.8
0.65-0.75	4.8	5.56	1430	0.028	0.9	6.85	7.8

[1] Calculated from particle radius of fraction distribution

[2] Determined via heating and subsequent oxidation by an oxygen transmitter

concentrations. The parameters can then be estimated from a least squares criterion built up accross all experiments (Richter *et al.*, 1992b).

The rate of transformation is determined by the concentration and a set of parameters which evolve in time according to a system of ordinary differential equations. The system is given in general form by:

$$\frac{dC_i}{dt} = f(C, p_1 \ldots p_n) \qquad (1)$$

where C is the concentration and $p_1, \ldots, p_n$ are n parameters.

The available data are measured concentrations C_{ij} at j time points in i experiments. The parameters are estimated by minimising the difference between measured and estimated concentrations using the method of least squares. However, to do this involves solving equation 1 at each step of the interactive minimisation procedure. The most commonly methods in use are the initial value method, which is, among others, realised in the statistical programme package BMDP (Dixon, 1987), and a recently developed boundary value method (Diekkrüger, 1992). The use of these methods is described by several authors (Richter and Söndgerath, 1990; Nörtersheuser, 1993).

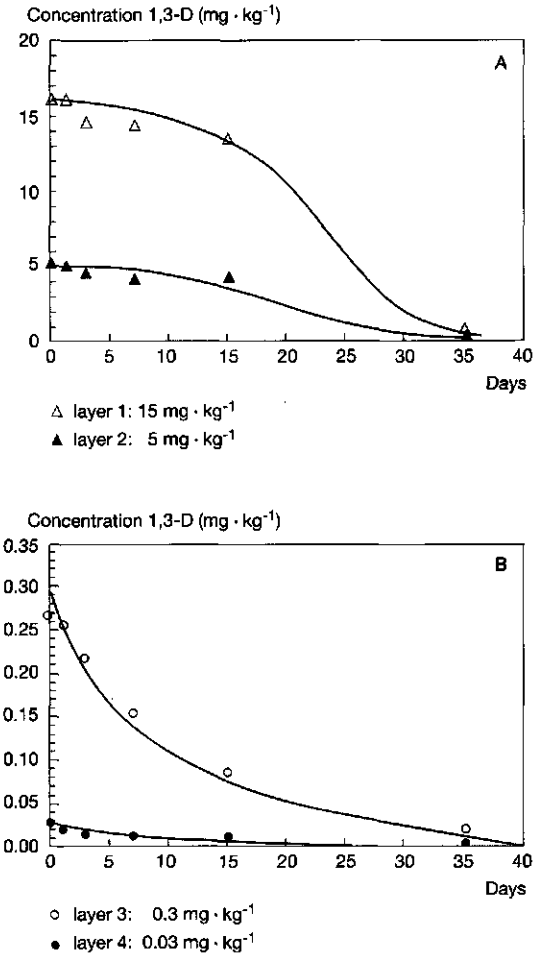

Fig. 1. Fit of equations 4 and 5 to observed time courses of 1,3-D concentration at 5 °C and different initial concentrations. Note the difference in transformation pathways at high (A) and low (B) concentrations.

3 Results

3.1 Experimental results

3.1.1 Transformation in moist soil

Results of incubation series at 5 °C, 10 °C and 20 °C with initial concentrations of 15, 5, 0.3 and 0.03 mg · kg^{-1} showed considerable differences in transformation pathways and, hence, rates. At high initial concentrations (Figure 1a), up to 10% loss of compound after 1 day, most probably due to volatilisation, was observed at all temperatures, followed by a lag-time of approximately 10 days at 5 °C. After this lag-time, during which concentration levels remained constant, transformation of 1,3-D accelerated. Eventually, transformation rates decreased again, resulting in constant low (residual) concentrations. Unlike transformation at low initial concentrations (0.3 and 0.03 mg · kg^{-1}, Figure 1b), the transformation curve at high contents cannot be described by a first-order model. This non-linear pattern of transformation can be ascribed to the size of the microbial population, its development and subsequent competition within the population. Only marginal differences in degradation pathways could be detected between *CIS-(Z)-* and *TRANS-(E)-*1,3-D isomers.

3.2 Coupling of transformation and microbial population dynamics

The transformation rate of biologically transformed

pesticides reflects both microbial activity and microbial population density. In the case of metabolic degradation, microbial growth depends on pesticide concentration. At high concentrations, inhibition of microbial activity frequently occurs. The link between microbial growth dM and consumption (attended by concentration decrease dC) of a substrate with time is given by equations 2 and 3. A density- dependent mortality rate β is added to prevent unlimited growth in the case of excess of substrate. This phenomenon was recognised by Vink and Groen (1992) as *microbial intrinsic competition.* Microbial growth is linked to substrate concentration by Monod's approach, modified by a factor that takes substrate inhibition into account. This inhibition factor becomes zero at high substrate concentrations. Transformation of a compound and microbial growth can be represented by the formulae:

$$\frac{dC}{dt} = -\frac{1}{\gamma} \cdot \frac{\mu_{max} C}{K_M + C} \cdot \frac{1}{1 + \left(\frac{C}{K_I}\right)^a} \cdot M \qquad (2)$$

$$\frac{dM}{dt} = \frac{\mu_{max} C}{K_M + C} \cdot \frac{1}{1 + \left(\frac{C}{K_I}\right)^a} \cdot M - \alpha(1 + \beta M)M \qquad (3)$$

C = Concentration of compound;
M = Microbial concentration;
μ_{max} = Maximum rate of growth;
K_M = Saturation constant of Monod's formula;
K_I = Critical concentration level for microbial growth;
a = Constant;
α,β = Microbial mortality rate constants;
γ = Gain factor for biomass production.

The first term on the right hand side of equation 3 is derived from the Michaelis-Menten enzyme kinetic law; the second term represents substrate inhibition, and the third term incorporates microbial mortality rates to prevent unlimited population growth.

In a previous study (Richter *et al.*, 1992a), this approach was applied to describe the transformation of the herbicide 2,4-D at various initial concentrations. The parameters of equations 2 and 3 can best be estimated using a large range of concentrations, in the neighbourhood of K_M and the critical concentration threshold K_I. At concentrations far below the critical pesticide concentration for microbial development (K_I), equations 2 and 3 reduce to:

$$\frac{dC}{dt} = -\frac{1}{\gamma} \cdot \frac{\mu_{max} C}{K_M + C} \cdot M \qquad (4)$$

$$\frac{dM}{dt} = \frac{\mu_{max} C}{K_M + C} \cdot M - \alpha (1 + \beta M) M \qquad (5)$$

Figure 1 and Table 2 show the results of the simultaneous fit of equations 4 and 5 to the observed transformation of 1,3-D at 5 °C and at different initial concentrations using the statistical package BMDP. The microbial parameters γ, α, β cannot be estimated exactly, due to the lack of explicit data about microbial activity after application of 1,3-D. Because of the short term nature of the experiments, the microbial mortality parameters α and β were fixed to zero. Starting from an estimated initial population size M_0 (Table 2), microorganisms grow with maximum rate (μ_{max}) at concentrations far above the K_M value and far below the K_I value. A primary adaptation to the new substrate is probably the reason for the apparent lag-phase in experiments having high initial concentrations of 1,3-D. In this stage, the microbial biomass is yet too small to perform maximum transformation.

Appreciable transformation occurs when the microbial biomass achieves a sufficient population density. At concentrations far below K_M, 1,3-D can persist for a

Table 2. Parameter estimation by simultaneously fitting equations 4 and 5 to the observed transformation of 1,3-D at 5 °C (s = standard deviation, M_0 = initial microbial concentration x 10^5).

Parameter	Estimate	[s]
μ_{max}	0.098	[0.014]
K_M	0.642	[0.269]
γ	0.560	[fixed]
M_0	0.349	[0.179]
α	0	[fixed]
β	0	[fixed]
r^2	0.95	

long time in soil layers with low microbial biomass content, because growth of microbial biomass cannot be stimulated. This phenomenon has been observed in several studies (Boethling and Alexander, 1979; Amrein *et al.*, 1981; Hurle, 1982; Vink and Groen, 1992).

The dependence of the growth rate of microorganisms able to degrade 1,3-D on the concentration of 1,3-D is simulated in Figure 2. The fit is based on the product of Monod's kinetic and the inhibition term presented in equation 2. Considering the simple molecular structure and the high use rates of 1,3-D, it appears that a close relationship between concentration and microbial population size is valid. It is likely that the responsible microorganisms use the compound as a sole source of energy.

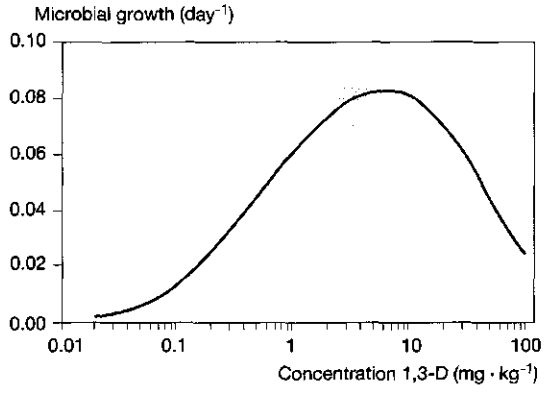

Fig. 2. Relationship at 5 °C between growth rate of microorganisms able to transform 1,3-D and the 1,3-D concentration.

3.3 Temperature dependence of 1,3-D transformation rates

Transformation rates of pesticides are functions of climatic factors (e.g., temperature, rainfall) and soil factors (e.g., soil microbial biomass, and strenght of sorption). The dependence of transformation rates on soil temperature and soil moisture has been modelled in several ways (Walker and Barnes, 1981; Nörtersheuser, 1993). The influence of temperature on the transformation of substances which are primarily degraded by microorganisms is not appropriately described by classical physico-chemical response functions such as the Arrhenius function. Biological activity depends upon temperature in a specific way; below a threshold temperature, T_{min}, no activity occurs. Above the threshold, activity is enhanced with increasing temperature until a maximum rate is reached. At even higher temperatures, activity decreases again until an upper - lethal - temperature is reached. Temperature influences basic life processes at the level of biochemistry in two ways. Firstly, elementary reaction rates increase with temperature. Secondly, biochemical reactions are mediated by enzymes which function only if a complex secondary structure is maintained; a rise in temperature leads to an enhanced decay rate of secondary structure, and ultimately the enzymes are denatured. The two effects are superimposed, and a suitable empirical equation was proposed by O'Neill (1969; O'Neill *et al.*, 1972):

$$\mu(T) = \begin{cases} \mu_{\max}\left(\dfrac{T_{max}-T}{T_{max}-T_{opt}}\right)^{a} \cdot e^{\frac{a(T-T_{opt})}{T_{max}-T_{opt}}} & \text{for } T > T_{min} \wedge T < T_{max} \\ 0 & \text{for } T \le T_{min} \wedge T \ge T_{max} \end{cases} \quad (6)$$

$$\text{with } a = \frac{\left[b\left(1+\sqrt{1+\frac{40}{b}}\right)\right]^2}{400} \text{ and } b = (Q_{10}-1)(T_{max}-T_{opt}) \quad (7)$$

$\mu(T)$ = Rate of a microbial temperature dependent reaction;
μ_{max} = Maximum rate;
T_{max} = Lethal temperature for microorganisms (°C);
T_{min} = Minimum temperature for microbial activity (°C);
T_{opt} = Optimum temperature for maximum microbial activity (°C);
T = Temperature at time t (°C);
Q_{10} = Increase of decay rate by increase of temperature of 10 °C.

Figure 3 shows the fit of equation 6 to observed transformation rates of 1,3-D, compared to the herbicide 2,4-D (1.4 mg · kg^{-1} dry weight) observed by Nash (1989). Table 3 summarises the results of parameter estimation. This difference in optimal temperatures for both compounds, 24.3 °C for 2,4-D and 15.6 °C for 1,3-D, may be a strong indication that different microbial

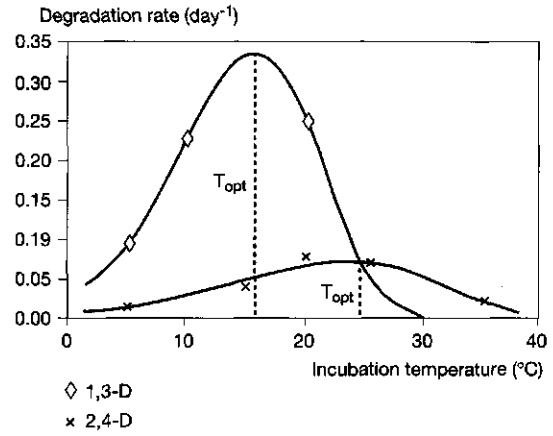

Fig. 3. Fit of the O'Neill function (Equation 6) to observed transformation rate of 1,3-D and 2,4-D.

Table 3. Parameter estimation of fitting the O'Neill function to the observed transformation rates of 2,4-D and 1,3-D (values in brackets denote standard deviations).

Parameter	2,4-D		1,3-D	
μ_{max}	0.074	[0.019]	0.339	[0.001]
Q_{10}	3.601	[0.021]	3.22	[0.001]
T_{opt}	24.30	[1.770]	15.64	[1.324]
T_{max}	40.00	[fixed]	40.00	[fixed]
T_{min}	0	[fixed]	0	[fixed]
r^2	0.94		1.00	

species are responsible for the occuring metabolism and subsequent transformation of each compound, reflecting their different molecular structures.

3.4 Coupling microbial dynamics and transport

The kinetic model for 1,3-D defined in Section 3.2 is coupled with the transport model as presented in Richter *et al.* (1992a). Solute transport in soil is modelled by the classical convection-dispersion equation. Description of the water flux is based on Richards' equation. Under the assumption that the movement and transport of microorganisms is negligible compared to the movement of the substance, it is straightforward to extend the kinetic and population dynamic equations by a spatial component. The state variables of equations 5 and 6 are formally written as a function of time and depth coordinate z. Hence, $M = M(t,z)$ and $C = C(t,z)$. The combination of transport processes and population dynamic models generates complex spatio-temporal patterns as demonstrated by the following examples. The kinetic models for these are derived from experiments with 1,3-D in the laboratory.

Water retention and hydraulic conductivity functions were computed from one-step pressure outflow experiments of undisturbed samples from each layer. The used method, which is described (Kool *et al.*, 1985) and analysed (Hopmans and Overmars, 1986) by several authors, is based on the simultaneous fit of water retention characteristic (Equation 8) and the $K(h)$ relationship (Equation 9):

$$\theta = \theta_r + \frac{\theta_s - \theta_r}{(1 + (\alpha h)^n)^m} \tag{8}$$

$$K(h) = Ks \; \frac{((1 + (\alpha h)^n)^m - (\alpha h)^{n-1})^2}{(1 + (\alpha h)^n)^{m(L+2)}} \tag{9}$$

θ = moisture content (%);
r,s = residual, respectively saturated θ;
h = hydraulic pressure head (m);
n,m,α,L = empirical parameters;
K_s = saturated conductivity (m d^{-1});

This resulted in: θ_s = 0.415-0.457, θ_r = 0.000, α = 0.0102-0.0039, L = 1.000-1.123 and n = 1.577-1.443.

Freundlich sorption isotherms of both 1,3-D isomers were determined for a wide concentration range (1-10,000 μg litre^{-1}) at four depths at 10 and 20 °C. Sorption proved to be low (K_f = 0.75-1.27; n = 0.88-0.68; $r^2 > 0.990$ for *CIS-(Z)*-1,3-D and slightly lower for the *TRANS-(E)* isomer). A kinetic formulation of Freundlich sorption can easily be incorporated in equation 2, as was done by Richter *et al.* (1992a). Here, this term is left out of consideration since it is assumed that the bonding strength to the soil matrix is weak and that most of the compound is available to 1,3-D-transforming microorganisms.

Figure 4 shows measured and computed time courses of 1,3-D concentration and microbial activity in this sandy soil (Table 1) at depths of 0.10 and

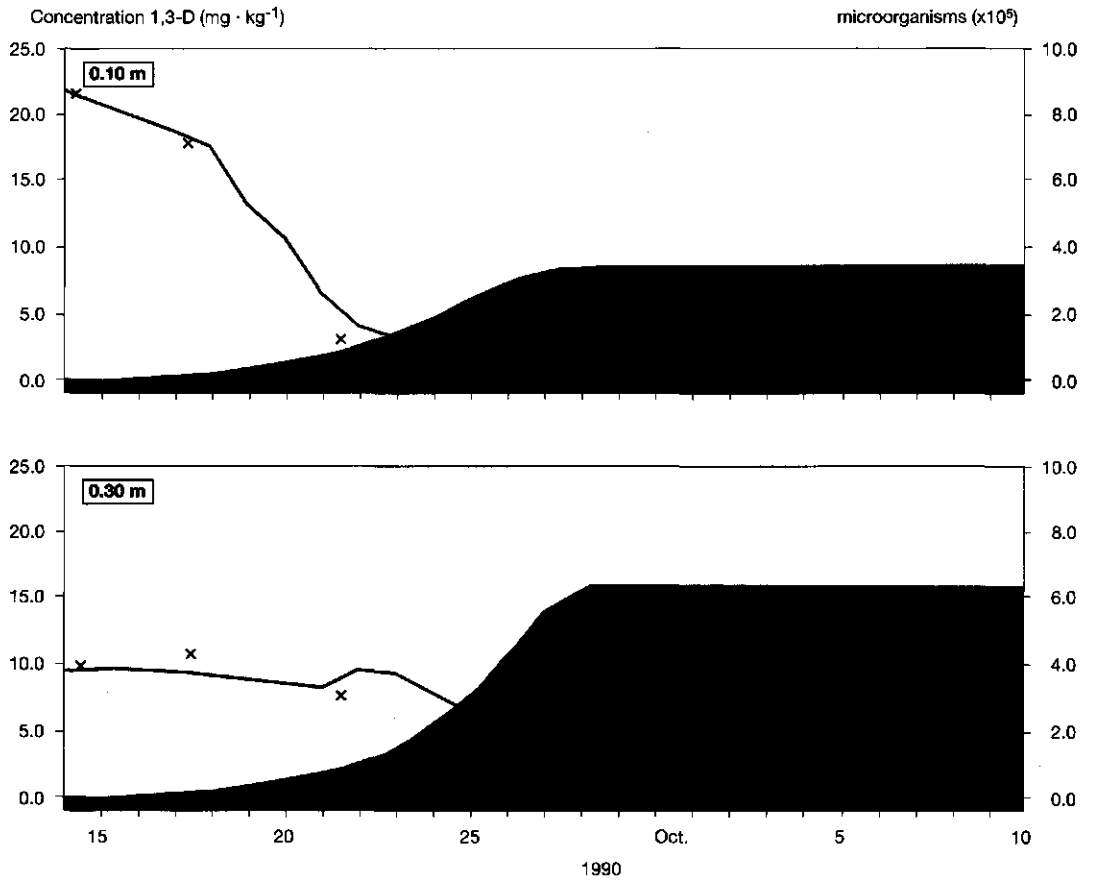

Fig. 4. Time courses of 1,3-D concentrations at various depths of a sandy soil. The shaded areas represent computed microbial activity.

0.30 m. The simulation was carried out with the programme SIMULAT (Diekkrüger, 1992). During this period, the mean soil temperature was 13.9 ± 1.2 °C at 0.10 m depth, and 13.3 ± 1.0 °C at 0.30 m depth. The total precipitation was 86.2 mm, intensities varying from 0-14.9 mm · day^{-1}, while the ground water level did not rise above 0.87 cm below surface. For this field simulation, the parameters from Table 2 were used, with the exception of M_0, which was calibrated at 0.1. Note the translocation of compound after 3 days. It is assumed that microbial concentrations are equally distributed over the soil profile at the beginning of the simulation. The duration of the time lag of 1,3-D transformation depends on the growth rate of degrading microorganisms.

Figure 5 shows profiles of 1,3-D concentration at 3, 7 and 13 days after application. The agreement between observed and predicted values is relatively good, considering the limited data available. The microbial activity profiles run nearly parallel to the concentration profiles. It is important to note that the parameter estimation is based only on studies carried out under controlled conditions in the laboratory.

4 Discussion

The transformation kinetics of pesticides are the result of complex biological and physico-chemical processes. These comprise microbial activity patterns as

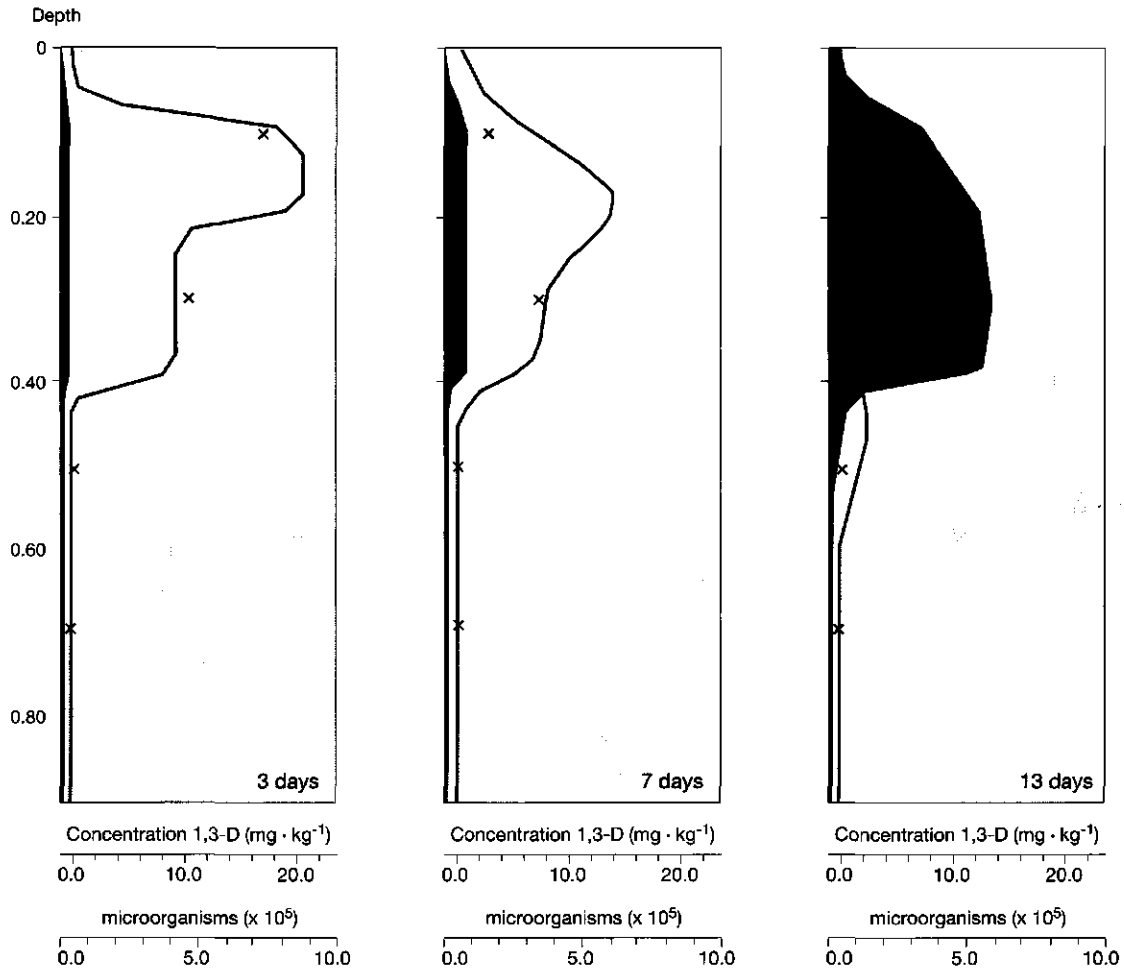

Fig. 5. Computed and observed concentration profiles of 1,3-D and of microbial activity with depth at 3, 7 and 13 days after application.

influenced by the concentration and properties of the pesticide itself and by environmental covariables, sorption to the soil matrix and transport in the solute phase. A multi-layered approach, and the assignment of realistic concentrations to various soils layers, will improve approximations of microbial inhibition or growth in depth; transformation rates are tailored to concentrations in depth.

The resultant kinetics are in general non-linear and first order kinetics are only crude approximations which are valid at low concentrations. The analysis of non-linear transformation and the establishment of a mathematical model necessitate experimental designs involving experiments under a variety of initial conditions.

It was shown that a simple population dynamic model, coupled with a pesticide transformation model, is able to explain the dynamic patterns characteristic of non-linear transformation, including a time lag and a dependence on the initial concentrations. Furthermore, it was demonstrated that parameter estimation is feasible even if analytical solutions do not exist. This result indicates that complex dynamic models are amenable to a thorough statistical analysis; the experimenter

need not restrict his analysis only to simple, mostly linear, kinetic models on the grounds of the availability of statistical estimation procedures for explicit regression functions. With regard to temperature dependence of the transformation kinetics, it has been demonstrated that the model can fit the data adequately. A comparison between the O'Neill approach and the Arrhenius equation was done by Nörtersheuser (1993). He concluded that the Arrhenius equation can only be applied at temperatures below 20 °C and at very low pesticide concentrations. Biological reactions appear to have a different temperature sensitivity than pure chemical reactions.

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Chapter 6

Some Physico-chemical and Environmental Factors Affecting Transformation Rates and Sorption of the Herbicide Metamitron in Soil

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Published in *Pesticide Science* **46** (1996) 113-119.

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Some physico-chemical and environmental factors affecting transformation rates and sorption of the herbicide metamitron in soil

Abstract - Beside the molecular structure of a pesticide, environmental conditions may influence its persistence by putting a strain on growth and activity of pesticide degrading microorganisms. As a result, transformation rates may decrease rapidly when a compound is leached into subsoil. Metamitron sorption isotherms were determined and incubation series were set up for a sandy loam soil, simulating single and combination effects that occur during transport of metamitron into subsoils. K_{OC}-values increased with increasing depth from 185 to 700 L · kg^{-1}. The combination of conditions that are unfavourable for microbial activity, such as low temperature (5 °C), low concentrations (0.5 mg · kg^{-1}) and a large sorbed fraction (K_{OC} =700) resulted in half-lives of over one year. Oxygen inhibition decreased the transformation rate of metamitron from 0.058 to 0.019 day^{-1}. In order of significance, the transformation of metamitron appears to be a function of temperature, oxygen availability and sorption to organic carbon. Increasing doses did not change transformation rates significantly, although different transformation pathways were observed.

1 Introduction

The transformation rate of a pesticide in the environment is related to the molecular structure and chemical properties of this compound. The complexicity or length of its structure may be expressed numerically by the molecular connectivity index (MCI), described by Sabljic (1984), and requires the summation of the number of bonding sites within the non-hydrogen part of the molecular skeleton, according to $\Sigma(n \cdot P_i)^{-0.5}$ in which P is a pair of adjacent bonding sites and n the occurrence of this pair. Based on the assumption that a large, complex molecule generally has a larger number and more diverse arsenal of bonding types, it is expected that the overall sorption strength is larger than for simple molecules like alkanes. Hence, the bioavailability in pore water may be lower. Based on this assumption, Shaaban and Elprince (1989) used the MCI as a substitute for K_{OC}. If a reliable value of K_{OC} is not available, the MCI may be a useful tool to estimate the persistency of a pesticide.

For the chemical breakdown of pesticides, an activation energy is required to seperate the bondings. A classic method to describe the speed of chemical reactions is expressed by the Arrhenius function:

$$\mu = A \cdot e^{-E_a / RT} \tag{1}$$

in which μ is the reaction rate, A is the frequency factor of chemical interactions, E_a is the activation energy in kJ · K^{-1} · mole^{-1}, R is the gas constant and T is the absolute temperature. The variable A can be eliminated mathematically if μ is determined for at least two temperatures:

$$for\ T_2 > T_1 : \begin{cases} \ln \mu_1 = \ln A - \dfrac{E_a}{RT_1} \\ \ln \mu_2 = \ln A - \dfrac{E_a}{RT_2} \end{cases} \tag{2}$$

In which μ_1 and μ_2 are transformation rates at temperature T_1 and T_2 (K). This results in:

Table 1. Molecular connectivity index and activation energy for several pesticides.

Compound	MCI	E(act) ($kJ \cdot K^{-1} \cdot mole^{-1}$)	reference
1,3-Dichloropropene	2.41	12.2	Vink *et al.*, 1994
Mecoprop	5.10	25	Estimated
Aldicarb	5.51	22.4	Bromilow *et al.*, 1980
2,4-D	5.68	34.4	Vink *et al.*, 1994
Simazine	6.25	45; 57.4	Smith and Walker, 1977; Walker, 1976
Metribuzin	6.37	59.0	Walker and Smith, 1979
2,4,5-T	6.50	85.0	Walker and Smith, 1979
Atrazine	6.61	44.0	Smith and Walker, 1989
Oxamyl	6.70	67.3	Bromilow *et al.*, 1980
Metamitron	7.19	86.7; 127.3	Bond and Roberts, 1976; This work
DDT	8.87	>300	Estimated

$$\ln \frac{\mu_2}{\mu_1} = -\frac{E_a}{R}\left(\frac{1}{T_2} - \frac{1}{T_1}\right) \qquad (3)$$

which enables the calculation of E_a. The activation energy is proportionally related to a compound's persistency (DT_{50}). Aromatic type structures, as found among others in triazine pesticides, are generally characterized by large MCI values as well as a high activation energy. Table 1 gives for metamitron and some other widely applied pesticides a comparison between the characteristic molecular connectivity index and the activation energy. Although there is some uncertainty in the intermediate area, induced by length rather than complexicity of the molecule, there appears to be an increasing concave relationship.

Besides these structure related conditions, the overall transformation of pesticides is highly regulated by environmental factors. In this study, we consider the herbicide metamitron (4-amino-4,5-dihydro-3-methyl-6-phenyl-1,2,4-triazin-5-one (IUPAC), tradename Goltix, Fig. 1). Metamitron is a selective systemic herbicide (photosynthesis inhibitor) which is widely used for the control of broad-leaved weeds and grasses in beet and flower bulbs. Application rates vary between 2 to 6 $kg \cdot ha^{-1}$ (70% active ingredient; 1.8 $g \cdot L^{-1}$ solubility). The compound is characterized by its relative complex molecular structure and large activation energy, and may thus be potentially persistent to transformation under certain conditions. To microorganisms, metamitron may act as a nitrogen source. The main metabolite of metamitron is formed after the separation of the amino group, giving 3-methyl-6-phenyl-1,2,4-triazin-5(4*H*)-one. Transformation rates may decrease when an alternative nitrogen source is readily available, or when physico-chemical impediments occur. This is likely to happen when the compound is leached into other, low oxygeneous systems like subsoils or ground waters and surface waters. It may be stated that environmental conditions that inhibit the growth and activity of microorganisms may decrease transformation rates of metamitron. However, there is little agreement on the quantitative effects of environmental factors of metamitron transformation. Bond and Roberts (1976) concluded that temperature may be more important than soil moisture in limiting degradation of metamitron. Walker and Brown (1981) concluded that transformation rates were correlated with microbial biomass and not with soil respiration.

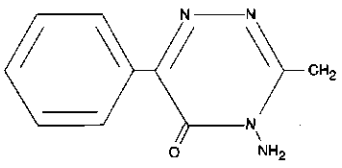

Fig. 1. Metamitron.

Allen and Walker (1987) found that the availability of metamitron in the soil solution is of particular importance. Also, the rate of loss may be related to adsorption rate and strength of the herbicide by soil solids, thus protecting it from being transformed by microorganisms. This study focusses on the microbial transformation and sorption sensitivity of metamitron, as regulated by temperature, oxygen availability, sorption to soil colloids and concentration of the compound. Incubations of samples of a sandy loam soil were used to simulate single effects and combination effects on transformation rates.

2 Material and methods

2.1 Soil samples

From a calcareous Fluvisol (sandy loam) in a field in the North-east polder, The Netherlands, soil samples were taken of four layers (5-15 cm, 25-35 cm, 45-55 cm and 65-75 cm) from ten locations, using a stainless steel auger, and homogenized to achieve a representative bulk sample of the various layers. These layers reflect the geomorphological horizons of the soil profile (marine, brackish and fresh water deposits). Soil properties are summarized in Table 2. In previous years, the soil had been treated periodically with metamitron, and it is likely that a population of microorganisms able to transform metamitron has developed. Residual metamitron concentrations in the samples were below the detection limit of 0.01 mg $\cdot$ kg^{-1} dry weight.

2.2 Adsorption series

Partitioning of metamitron over the solid and liquid phases for the four layers was determined for a wide concentration range, emphasizing the lower concentration range that is field-realistic. Standard solutions contained 1, 5, 10, 100, 500 and 1000 $\mu g \cdot L^{-1}$ metamitron (active ingredient) in 0.01 M $CaCl_2$. Standard solutions, soil samples and batch mixtures were kept at 5 °C to minimise transformation during the experiment. The soil/liquid ratio was 0.5, which is in agreement of the recommendations made by Boesten (1990). A large solid/liquid ratio has the additional advantage that sorption kinetics proceed more rapidly (Boesten and Van der Pas, 1988). The samples were shaken linearly (192 movements per minute, 6.5 cm amplitude) for 6 hours, left overnight for 16 h and shaken again for 1 hour, all at 5 °C. After centrifugation at 1500 g for 10 min., an aliquot of 60 ml extract was pipetted, extracted with dichloromethane and purified over florisil. A two column HP 5880-GC was used for duplicate analyses.

2.3 Incubation series

To simulate oxygen inhibition during microbial transformation of the compound, as would occur after leaching into subsoils, open and closed incubation series were set up. To 50 g samples of each layer ($\theta \approx 0.25\%$), a quantity was added that would reflect a realistic concentration decrease in depth, based on field observations in previous years. This resulted in a dose of 2 mg $\cdot$ kg^{-1} for the first layer, 1 mg $\cdot$ kg^{-1} for the second layer and 0.5 mg $\cdot$ kg^{-1} for the third and fourth layer. Seperate vials were used for each observation (1 h, 1 day, 3, 7, 15, 35 and 70 days) and were tightly sealed with a ground glass stopper and stored at 5 °C. An identical series of samples was stored at 15 °C. This resulted in a total of 56 incubation vials. The available oxygen in the incubation vials was $7.4 \cdot 10^{-4}$ mole O_2, which corresponds to an oxidation energy of approximately 0.2 kJ.

Table 2. Soil properties and Freundlich parameters.

Soil layer (cm)	< 2 μm (%)	Org.C (%)	$CaCO_3$ (%)	pH_{H_2O}	Freundlich parameters			
					n	K_f	K_{OC}	r
1: 5-15	6.4	1.1	5.90	7.8	0.90	2.04	185	0.959
2: 25-35	6.6	1.0	5.20	7.8	0.87	2.29	229	0.959
3: 45-55	6.3	0.9	5.45	7.8	0.84	3.72	413	0.979
4: 65-75	4.8	0.9	6.85	7.8	0.81	6.30	700	0.985

A similar experiment was done with 7 samples of the top soil layer (5-15 cm) and four concentrations (10, 4, 2, 0.5 mg · kg^{-1}). In this test, the 28 flasks were loosely stoppered with glass wool to allow diffusive exchange of oxygen, and stored at 15 °C. If necessary, water was added corresponding to the loss of weight in random checks.

3 Results and discussion

Sorption isotherms were described with the Freundlich equation:

$$q = K_f \cdot C_e^n \qquad (4)$$

in which q is the sorbed amount in mg · kg^{-1}, C_e is the equilibrium concentration of the water phase in mg · L^{-1}. Kf and n are experimental constants, which represent the intercept and the slope of the liniarized logarithmic isotherm when presented as log q = log K_f + n · log C_e. Freundlich parameters and corresponding measurements/curve-fit correlation coefficients are presented in Table 2. Note the gradual but significant increase of K_{OC} as depth increases, which is possibly a result of a higher degree of humification and reactivity of the soil organic phase.

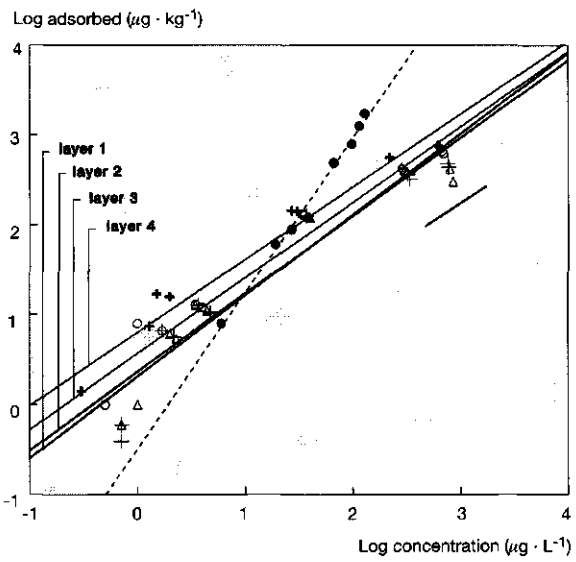

Fig. 2. Sorption isotherms of metamitron in soil layers 1-4 and as reported by Allen and Walker, 1987 (——), and Goicolea *et al.*, 1991 (---). Sample properties and corresponding Freundlich parameters are:

	< 2 μm	Org.C	pH	n	K_f	K_{OC}
Allen and Walker	15	0.6	6.3	0.76	1.5	250
Goicolea *et al.*	25	1.9	7.6	1.71	0.3	16
Layers 1-4: Table 2						

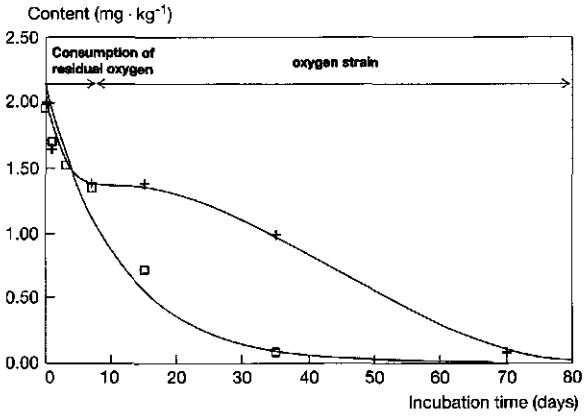

Fig. 3. Metamitron transformation in the 5-15 cm soil layer at open (□) and closed (+) incubations. Experimental conditions, such as temperature (15 °C) soil properties (< 2 μm = 6.4%, org. C = 1.1%, pH = 7.8), dose (2 mg · kg^{-1} dw) and moisture content (0.25% wght) are identical.

In Fig. 2, measurements on similar soils carried out by Allen and Walker (1987) and Goicolea *et al.* (1991) were used for comparison. Organic carbon contents range from 0.6 to 1.9. The fact that Goicolea *et al.* find K_f = 0.3 and n = 1.71 may be the effect of a relativily high organic carbon content with a low sorption capacity. Consequences of the observed concentration ranges (1000-5000 mg · L^{-1} by Allen and Walker, 100-1000 μg · L^{-1} by Goicolea *et al.*, and 1-1000 μg · L^{-1} in this study) is clearly expressed by the (un)certainty of the exact intercept value, resulting in K_f, and the representativity of the slope (n) for a wide concentration range.

The consumption of oxygen is expressed in the two pathways in Fig. 3. It shows that transformation rates during the first ten days do not differ much for both

incubation series, and transformation at oxygen saturation is well described ($r = 0.997$) by a simple first order equation:

$$\frac{d}{dt}(P) = -\mu \cdot P \qquad (5)$$

In which P is the total amount of metamitron in soil and soil solution and μ is the specific transformation rate.

However, differences in DT_{90}-values at saturated and low oxygen concentrations prove to be significant, which implies high persistency of residual concentrations at low oxygen concentrations. This type of transformation could not be described with Eqn. (5). Under limiting O_2-concentrations, we assume that the specific growth rate of strictly aerobic microorganisms depends on the dissolved oxygen concentration $[O_2]$ according to the Monod-equation (Owens and Legan, 1987):

$$\mu = \frac{\mu_{max}\,[O_2]}{(K_s + [O_2]} \qquad (6)$$

In which μ_{max} is the maximum specific growth rate and K_s is the Monod half-saturation constant. By further assuming that transformation of the compound mainly occurs for the dissolved fraction, Eqn. 5 may be modified if combined with Eqn. 4, and defining P as:

$$\begin{cases} \dfrac{d}{dt}(P) = -\mu \cdot P \\ P = \theta C(t) + \varrho K_f [C(t)]^n \end{cases} \qquad (7a,b)$$

In which θ is the water fraction, $C(t)$ is the time-dependent concentration and ϱ is the soil bulk density ($g \cdot cm^{-3}$). A major obstacle for the quantification of the inhibitory effect of O_2 on the growth of microorganisms is that the concentration of O_2 in the solution has to be monitored, if detectable, under these low oxygen conditions. Furthermore, slowly decreasing oxygen concentratations, as was simulated in this experiment, requires dynamic coupling of Eqn. (6) and (7a,b).

Boesten *et al.* (1991) concluded from experiments with water saturated sediments that certain compounds may be very stable in aqueous, low oxygenous systems. Ashley and Leigh (1963) and Gerstl *et al.* (1977) reported similar findings. These results indicate, that in low-oxygeneous conditions the microbial contribution in the total transformation is slowed down or eliminated, and that transformation in low-oxygeneous environments is likely to be of a chemical rather than of a microbiological nature.

Table 3. Dose, org. C sorption coefficient and half-lives for metamitron at two temperatures.

Soil layer	Dose ($mg \cdot kg^{-1}$)	K_{OC}	Half-lives (days) 5 °C	15 °C
1	2.0	185	61	9
2	1.0	229	140	5
3	0.5	413	> 1 yr	41
4	0.5	700	> 1 yr	68

Results of the first incubation series are presented in Table 3, in which a relation is made between soil layer, its specific sorption coefficient related to organic carbon, dose and temperature. Note the significant decrease of transformation rates at 5 °C at a combination of low concentrations and high sorption. The difficulty to determine causal relationships for field conditions is obvious. One-parameter interpretations of results of incubation tests may not be justified, since physico-chemical properties of both soil and compound, and the specific population dynamics of microorganisms may be (inversely) correlated (Vink and Groen, 1992; Vink *et al.*, 1994).

A formulation discribing the influence of temperature on the specific transformation rate is given by O'Neill (1969), and assumes a maximum transformation rate at the temperature with the highest microbial activity. This formulation was used by many authors (Richter *et al.*, 1992; Vink and Groen, 1992; Vink *et al.*, 1994) and resulted in the determination of optimum temperatures for rapid transformation of several pesticides. Other formulae (Logan *et al.*, 1976), including the Arrhenius function (Eqn. 1) appeared to be less accurate.

Fig. 4 shows the dynamic patterns of transformation pathways as a function of concentration (incubation series II). At low concentrations, transformation can be expressed by a first order model (Eqn. 5, Fig. 4b). At

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Chapter 7

Effect of Oxygen Status on Pesticide Transformation and Sorption in Undisturbed Soil and Lake Sediment

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Published in *Environmental Toxicology and Chemistry* **4** (1997) 608-616.

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Effect of oxygen status on pesticide transformation and sorption in undisturbed soil and lake sediment

Abstract - The behaviour of four chemically distinctly different pesticides (aldicarb, simazine, mecoprop and MCPA) was investigated under simulated redox conditions that occur at the terrestrial-aqueous interface. Sorption was measured in soil and lake sediment and in pure and combined pesticide solutions to study effects of competition. Transformation was investigated in undisturbed soil micro columns and in anaerobic lake sediment. Using an air-tight nitrogen incubator, oxygen concentrations were varied from 20.8% (ambient) to less than 0.01% v/v O_2, while redox potentials varied from +330 to -120 mV. Aldicarb was transformed more rapidly under anaerobic than under aerobic conditions. Under low oxygen conditions, both reductive and oxidative metabolites were formed. Simazine showed some reductive transformation, but the overall transformation rate decreased with decreasing O_2-concentrations. A pronounced redox effect was shown for both mecoprop and MCPA. With decreasing oxygen concentrations, transformation rates decreased from 0.173 to less than 0.001 day^{-1}, monitored over 200 days. These compounds, which are considered improbable leachers based on their short aerobic half lifes, appear to be more persistent in low-oxygenous conditions. It is shown that a period of oxygen inhibition could be survived by the responsible soil microorganisms, and was followed by accelerated transformation of the compound when oxygen became abundant again. No relationship was observed between sorption affinity and transformation rate. The effect of redox conditions that occur along the pesticide's emission route appears to be an important screening parameter to assess environmental risks.

1 Introduction

Transformation plays a crucial role in transport behaviour of pesticides and movement from soil layers. The involved kinetics can usually be described with non-linear models based on microbial growth and competition, substrate availability and temperature (Soulas, 1982; Richter *et al.*, 1992a,b; Vink *et al.*, 1994) . The impact of conventional and new pesticides on the environment is generally tested with such models, using parameters that are derived from and apply to terrestial conditions. However, when leached into environments with lower oxygen concentrations (e.g., subsoils, surface waters and saturated sediments), transformation pathways of these compounds may change drastically as a result of altered, mostly unfavourable redox conditions for aerobic microorganisms. Many publications report on compounds that are highly stable in aqueous systems (Ashley and Leigh, 1963; Reese *et al.*, 1972; Edwards, 1973; Gerstl *et al.*, 1977; Boesten *et al.*, 1991). In cracked clay soils that are found in polders in The Netherlands, leaching along preferential routes is common. By-pass flow may allow pesticide leaching without significant transformation. These soils are in general artificially drained and discharge directly to surface waters. Lake Markermeer, a large water body in the central part of The Netherlands, is considered to be the final sink of pesticides. In the anaerobic sediments of this lake, a variety of pesticides have been detected, among which are compounds that are considered improbable leachers because of their high, aerobic transformation rates in soils (Vink, 1993). Similar observations of the occurence of generally unstable organochlorine pesticides in fresh water sediments have been reported by many authors (Stickel, 1968; Tan and Vijayaletchymy,

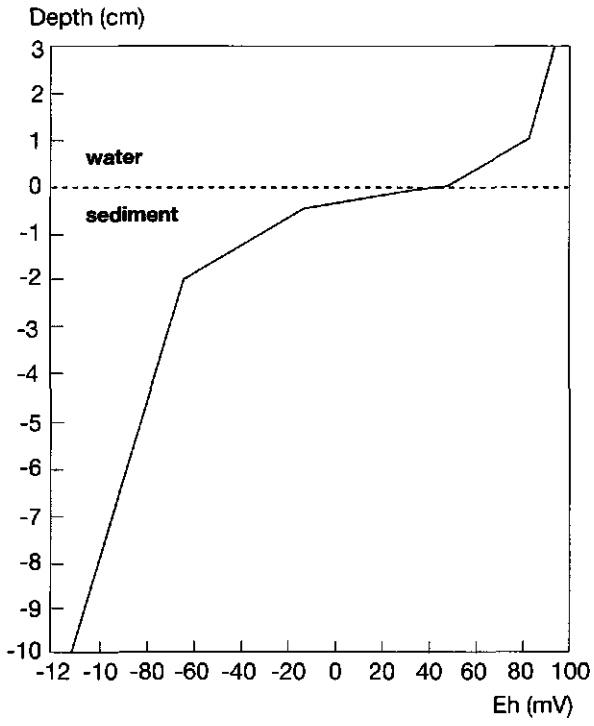


Fig 1. Redox conditions at the water/sediment interface of a lake sediment.

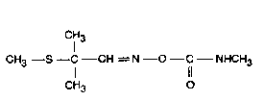
1994; Zaranyika, 1994; Donald and Syrgiannis, 1995).

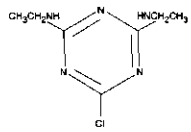
Toplayers of sediments in water courses and lakes can become anaerobic during the summer months, allowing the overall transformation to proceed along different pathways (Wolfe *et al.*, 1986; Lehmann *et al.*, 1993). Figure 1 shows the prevailing redox conditions at the water/sediment interface of a lake sediment bulk sample, inundated with 3 cm surface water and stored at ambient air for three months. Positive redox potentials (Eh) are restricted to the top 5 mm of the sediment. Under reduced conditions, some pesticides may undergo reductive transformation. Paris and Wolfe (1980) found a correlation between microbial transformation and chemical structure of the compound. Anderson (1995) suggests that the routes of transformation, and the produced metabolites, may not differ between topsoils, subsoils or groundwater. However, the lack of fungi in deeper soil layers, or water course sediments, may lead to slower mineralization of the metabolites of some compounds. Still, only limited information is available on pesticide transformation rates or pathways in low-oxygen environments. Therefore, the influence of transformation in the low-oxygen environments on the overall fate of pesticides has not sufficiently been evaluated.

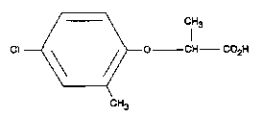
The environmental implications caused by the combined action of pesticides has been evaluated by some authors (Koneman, 1981; Faust *et al.*, 1993; Faust *et al.*, 1994). Canna and Piera (1994) concluded that aldicarb and its aerobic metabolites, when present in mixtures, lead to a 50% higher toxicity to some bacteria than when exposed to the individual compounds. The increase in toxicity was attributed to synergistic toxic effects. This is an important conclusion, considering the probable co-existence of aldicarb and its aerobic metabolites in the environment.

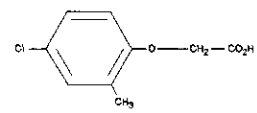
In view of these knowledge gaps on transformation pathways, rates, and the formed metabolites at low oxygen conditions, we performed transformation and sorption experiments on aldicarb, simazine, mecoprop and MCPA. Sorption was studied for single and mixed pesticide solutions in soil and fresh water sediment. Aldicarb, simazine and mecoprop were chosen as representatives of their chemical group. These compounds all occur in the first ten of a list of pesticides presented by Bailey *et al.* (1995) which are most likely to be detected in water. The herbicide MCPA was added for comparison with mecoprop. Transformation in soil was studied in undisturbed micro columns. Walker *et al.* (1995) reported 60% lower sorption for isoproturon in undisturbed ('static') samples, compared to values found in disturbed ('shaken') samples. Kookana *et al.* (1993) found that sorption of simazine under preferential flow conditions was much slower than occurs under shaking conditions, employed in the batch method. A lower sorption may consequently lead to a higher transformation rate in the undisturbed soil matrices, due to a higher (bio)availability in the pore solution. Hence, the use of undisturbed soil columns better reflects the structure-related physico-chemical impediments and the corresponding microbial dynamics when studying transformation in soil than disturbed or mixed samples.

Table 1. Pesticide molecular structures and properties (Bol *et al.*, 1992; Waugope *et al.*, 1992).









	Aldicarb	Simazine	Mecoprop	MCPA
Solubility ($g.L^{-1}$)	6.16	$3.5 \cdot 10^{-3}$	$6.2 \cdot 10^{-1}$	$8.3 \cdot 10^{-1}$
Vapour Pressure (Pa)	$1.3 \cdot 10^{-2}$	$8.1 \cdot 10^{-7}$	$2.7 \cdot 10^{-2}$	$2 \cdot 10^{-4}$
Henry ($Pa \cdot m^3 \cdot mole^{-1}$)	$3.2 \cdot 10^{-4}$	$3.3 \cdot 10^{-5}$	$9.3 \cdot 10^{-3}$	$3.9 \cdot 10^{-5}$
log K_{ow}	1.13	2.18	2.99	1.41
GUS[1)]	2.34	3.25	1.71	1.48

[1)] Groundwater ubiquity score (Gustafson, 1988).

2 Materials and methods

2.1 Soil and lake sediment samples

Samples were collected from a clayey, calcareous fluvisol in a polder in The Netherlands (Southern Flevoland). A total of 88 undisturbed samples were taken from the top soil by driving stainless steel rings, 6.2 cm diameter, 100 cm^3, into the 5-15 cm soil layer. This volume represented approximately 115 g dry soil.

From the top layer of the anaerobic, fresh water sediment of Lake Markermeer, a bulk sample was taken at a depth of 4 m below the water surface. A stainless steel auger was used to sample the upper 0.05 m of the lake sediment. To minimize oxidation, the sediment was maintained with in-situ surface water and stored in an air-tight incubator. Redox potentials (Eh) of soil and lake sediment were measured in the laboratory with platinum electrodes and an Ag/AgCl reference electrode, connected to a saturated KCl salt bridge.

2.2 Pesticides

To study transformation behaviour of chemically distinctly different compounds, aldicarb (carbamate group; 2-methyl-2-(methylthio)propionaldehyde *O*-methylcarbamoyloxime [tradename Temik]), simazine (*s*-triazine group; 6-chloro-N^2,N^4-diethyl-1,3,5-triazine-2,4-diamine [Luxan 500 FC]) and mecoprop (phenoxic acid; (*RS*)-2-(4-chloro-*o*-2-tolyloxy)propionic acid [Luxan liquid]) were chosen as representatives of their chemical group. Additionally, MCPA (phenoxic acid; (4-chloro-2-methylphenoxy) acetic acid [Luxan 500]) was selected. Unlike Mecoprop, MCPA does not posses a propionic acid side chain. This lack of a methyl group may affect the susceptibility of the aromatic herbicide to microbial degradation (Alexander and Aleem, 1961). Molecular structures and some properties are shown in Table 1.

2.3 Sorption with pure and mixed pesticide solutions

Two types of sorption experiments were conducted. In the first experiment, we determined characteristic Freundlich sorption parameters for soil and lake sediment for each pesticide separately. In the second experiment, we studied the effect of competition on Freundlich constants when these compounds occur in the solution simultaneously. For a proper comparison of pure and mixed solutions, the ionic strengths and total pesticide concentrations of these solutions were kept constant. Of each pesticide, standard solutions were made that contain 5, 10, 100, 500, 1000 and 5000 $\mu g \cdot L^{-1}$ active ingredient in 0.01 M $CaCl_2$. Solutions were pre-chilled to 5 °C to avoid transformation and volatilization of the compounds as much as possible. An aliquot of 100 ml of these standard solutions were added to 70 g moist soil (≈ 50 g dry weight) and 120 g lake sediment (≈ 50 g dry weight). The soil/liquid ratio was 0.5, which is in agreement of the recommendations made by Boesten (1990). A large solid/liquid ratio has the additional advantage that sorption kinetics proceed more rapidly (Boesten and Van der Pas, 1988).

In a second series, 25 ml of each pesticide standard solution were added to soil and lake sediment samples. The concentration of each individual compound is therefore one fourth of the concentration of the first series, so that the total pesticide concentration in all solutions would be constant.

The suspensions were shaken linearly (192 movements per minute, 6.5 cm amplitude) for 6 hours, left overnight for 16 hours and shaken again for two hours. This procedure was carried out at 5 °C. After centrifugation at 2000 g for 15 minutes, 80 ml of supernatant was sampled. For the second series, this amount was split up in equal amounts for further treatment and analyses of the various compounds.

2.4 Incubations with soil and sediment

Incubation series were set up to monitor transformation of the four pesticides at eleven time intervals. These series were set up separately for soil (4 x 11 samples under aerobic and 4 x 11 samples under anaerobic conditions) and for lake sediment (4 x 11 samples).

Standard solutions were made, containing 500 mg · L^{-1} aldicarb (active ingredient), 250 mg · L^{-1} simazine, 250 mg · L^{-1} mecoprop and 250 mg · L^{-1} MCPA respectively in 0.01 M $CaCl_2$. An aliqout of 2.0 ml of standard solution of each pesticide series was distributed uniformly over the soil column surfaces with a microlitre syringe. This resulted in doses of 9 mg (aldicarb) and 4.5 μg (simazine, mecoprop and MCPA) per gram dry weight, which corresponds to common field application rates. A total of 88 micro-columns were treated this way.

For incubations with the lake sediment, 50 g (21.5g dry weight) was weighed into 250 ml flasks. To achieve very low, but reliably detectable concentrations, doses were attuned to the analytical detection limit and recovery, and the dry weight in the samples. To each sediment sample, 1.0 ml of the standard solution was added for each pesticide series. The samples were then carefully homogenized with a stainless steel stirrer, and inundated with approximately 5 mm of degassed (O_2-free), demineralized water. This resulted in an approximate 1:1.5 solid to liquid ratio. A total of 44 sediment samples were treated this way.

Half (44) of the total amount of the undisurbed soil columns were stored in the dark at ambient atmosphere

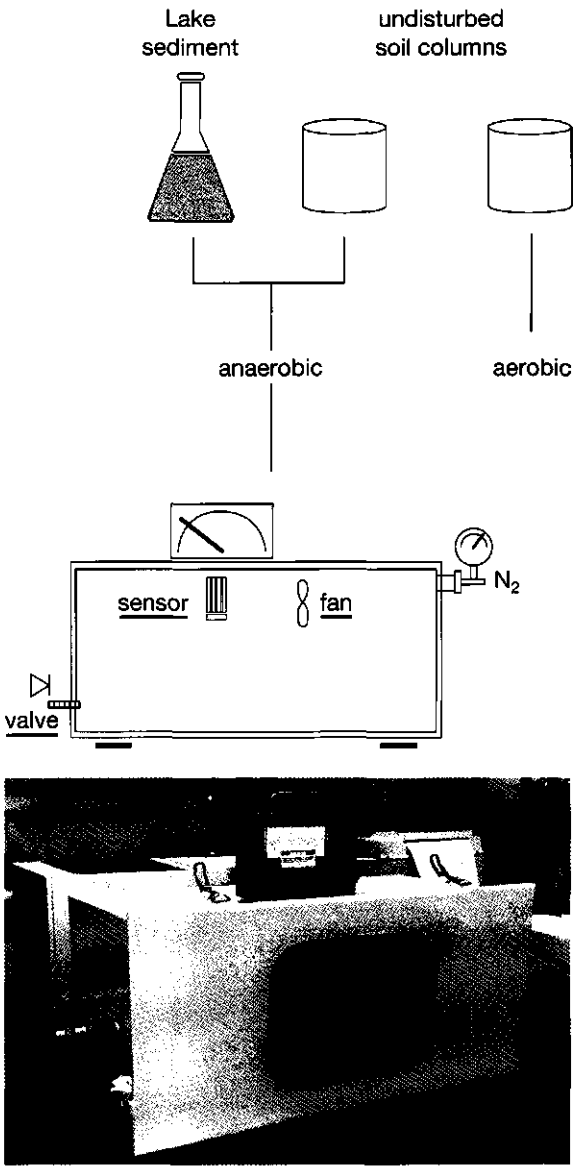


Fig 2. Experimental set-up: nitrogen incubator to study transformation behaviour at low oxygen atmospheres.

(20.8 % v/v $O_2 \approx 8 \cdot 10^{-3}$ mole · L^{-1}). The other half, and all of the lake sediment samples (44), were stored under low oxygen conditions in an air-tight, insulated chamber with a capacity of 100 L (Fig. 2). Nitrogen gas (99.99% purity) was used in combination with a diffuse-tight faucet to expel oxygen from the system

(flow rate 75ml · min^{-1}. continuous). A valve avoided excessive air pressures (≤ 0.2 bar) in the chamber. Samples were subjected to very low oxygen levels between zero and 0.01 % v/v $O_2 \approx 3 \cdot 10^{-6}$ mole · L^{-1}. Photodecomposition was avoided by the use of black paint in the interior and dark stained glass for a window. The oxygen concentration in the chamber was monitored with a Rosemount 715 polarographic (Au/Ag) sensor. Measurements were done in combination with a small fan which could be operated from the exterior. This fan provided sufficient gas turbulence in the system to warrant accurate, continuous oxygen measurements. During the incubations, the temperature was maintained at 18 ± 1 °C.

Prior to addition of the pesticides, the sediment samples and half of the soil samples were left in the incubator under low oxygen conditions for six weeks. This time was considered to be sufficient for the oxygen to diffuse out of the soil and sediment matrix. The soil samples were weighed once every week, and loss of moisture was corrected by adding small amounts of de-gassed, demineralised water. In these six weeks, the redox potential of the soil samples decreased from +330 mV to +180 mV. The redox potential of the lake sediment samples remained -120 mV, and was apparently not affected by the atmosphere of the incubator.

In a parallel test, two undisturbed soil columns were stored in the incubator at $3 \cdot 10^{-6}$ M O_2 for six weeks. After this period, 0.25 mg MCPA was added, which corresponds to an application of 4.5 mg · kg^{-1}, and the samples were put back in the incubator. The moisture content was monitored periodically by weighing. After 67 days, one column was taken out of the incubator and stored in the dark in ambient air. After ten days, both samples were analysed. The measurements were corrected for analytical recovery with a standard.

Analyses

Aldicarb was extracted from soil with acetone, shaken, and centrifuged for 15 minutes at 2000 *g*. The extract was dried under a continuous (125 ml per minute) nitrogen flow and delivered in 1 ml acetonitrile (ACN, 10%). After filtration (0.22 μm), the supernatant was shaken with dichloromethane (DCM), and dried with Na_2SO_4. This was done twice, and the two DCM-phases were joined. The supernatant was dried using a rotary evaporator at 50 °C to 2 ml. The residue was then treated with ACN and filtered. Analyses of aldicarb, aldicarb-sulfoxide and aldicarb-sulfone was done with a High Performance Liquid Chromatography (HPLC) system, using a Waters 510 pump and an WISP712B auto-injector, in combination with a Waters 490E UV-detector and a Vydac 201TP54m 250 x 4.6 mm column packed with C18 (5 μm) adsorbent. Metabolites were identified by comparing fluorescence characteristics with known standards.

Simazine was extracted with dichloromethane (DCM) and centrifuged for 15 minutes at 2000 *g*. The DCM-phase was dried with 5 g Na_2SO_4 and filtered into a 50 ml flask, where it was dried under a continuous (125 ml per minute) nitrogen flow to 5 ml supernatant. This was supplemented with DCM, 1:1, and 250 μl of the solution was evaporated. The residue was further treated with 500 μl iso-octane/ethylacetate (9:1). Analyses were performed with a Perkin Elmer 8500 gas chromatograph using a CP sil 5 CB column (50 m length, 320 mm diameter, 0.42 μm film thickness) in combination with an NP-detector. The temperature was increased gradually to 300 °C. Helium was used as a make up gas at 80 kPa.

Mecoprop and MCPA were extracted from the soil samples with 100 ml acetone, centrifuged for 15 minutes at 2000 *g*, and filtered. This was done twice, and the solutions were joined. The acetone was evaporated under a rotary evaporator at 65 °C, 5 ml 0.05 M NaOH was added and supplemented with demineralized water. The solution was transferred into a separation funnel, 20 g NaCl was added and dissolved, and extracted twice with 25 ml DCM. The eluate was acidified with 3.25 ml 1.0 M H_2SO_4 to pH = 2. Extraction with DCM was done twice, the DCM-phases were joined, dried with 5 g Na_2SO_4 and filtered. The supernatant was dried under a rotary evaporator at 55 °C to 2 ml. 5ml acetone, 25.0 mg caesium carbonate and 100 μl PFBB-solution (3% acetone) was added. The solution was heated to 80 °C for 30 minutes. After the solution had cooled down, 5 ml iso-octane was added and concentrated to 1 ml under a continuous (125 ml per minute) nitrogen flow at 55 °C. The supernatant was added to a column, containing 1 g silica gel (MN 70-270 mesh), covered with 100 mg Na_2SO_4. The column was rinsed

Table 2. Sample properties.

	Soil	Lake sediment	
Redox potential (Eh)	+330	-120	mV
Fraction <2 μm	35.2	22.6	%
Fraction <16 μm	55.8	32.4	%
Fraction <63 μm	84.3	66.4	%
Organic C	2.8	1.6	%
$CaCO_3$	8.0	16.5	%
pH-H_2O	8.1	8.8	-
Dry matter	74.3	42.8	%
Bulk density	1.2	1.1	$g \cdot cm^{-3}$
P_2O_5-Total	nd	1200	$\mu g \cdot g^{-1}$
P_2O_5-citr. acid	367	418	$\mu g \cdot g^{-1}$
Total N	0.24	nd	%
NH_4	200	301	$\mu g \cdot g^{-1}$
NO_3+NO_2	<2	3	$\mu g \cdot g^{-1}$
Chloride	530	40	$\mu g \cdot g^{-1}$

nd = not determined.

with a 9:1 hexane/toluene solution, and eluated with a 1:1 hexane/toluene solution. Gas chromatographic analyses were carried out with a Perkin Elmer 8500, using two capillary CP sil 5/13 CB columns (50 m length, 250 μm diameter, 0.12 μm film thickness). Helium was used as a carrier gas (0.3 $m \cdot s^{-1}$) and nitrogen as a make up gas.

3 Results

Table 2 summarizes some physical and chemical properties of the soil and lake sediment samples. The matrices are clayey and calcareous, and the pore solutions are slightly basic. Nutrient concentrations are moderately high. Sixty percent of the phosphorus in the lake sediment is present as poorly-soluble complexes and is not readily bio-available. The ratios of reduced (NH_4) to oxidized (NO_X) nitrogen species do not appear to reflect the prevailing redox conditions.

3.1 Sorption

The distribution of the pesticides over the solid and liquid phase was described with the Freundlich equation:

$$q = K_f \cdot C_e^{\,n} \qquad (1)$$

In which q is the sorbed fraction [$\mu g \cdot kg^{-1}$], C_e is the equilibrium concentration of the dissolved fraction [$\mu g \cdot L^{-1}$] of the pesticide, and K_f [$L \cdot kg^{-1}$] and n are constants. Because generally sorption increases with an increasing amount of organic matter, a normalization according to

$$K_{OC} = K_f / f_{OC} \qquad (2)$$

is often used to compare different soils using K_{OC}, [$L \cdot kg^{-1}$] (f_{OC} is the fraction of organic carbon in soil or sediment). In Table 3, sorption parameters for pure and mixed pesticide solutions with soil and lake sediment samples are given. Correlation coefficients (r) of the non-linear sorption isotherms were 0.95 or larger.

From Table 3, we find that the sorption of aldicarb to the organic matter is larger in the lake sediment than in soil. Aldicarb K_{OC}-values in lake sediment are up to a factor 9 larger than those determined in soil.

The retention of simazine to the organic matter in

Table 3. Organic carbon partitioning coefficient K_{OC} ($L \cdot kg^{-1}$) and Freundlich constant n in pure and mixed solutions for four pesticides, determined in soil and lake sediment.

	Soil				*Lake sediment*			
	Pure		Mixed		Pure		Mixed	
	K_{OC}	n	K_{OC}	n	K_{OC}	n	K_{OC}	n
Aldicarb	27	0.90	31	0.88	261	0.89	263	0.88
Simazine	171	0.89	170	0.88	250	0.92	252	0.90
Mecoprop	73	0.58	78	0.50	38	1.01	55	0.91
MCPA	36	0.83	76	0.84	30	1.07	81	0.98

the lake sediment is slightly higher than in the soil samples. K_{OC}-values agree with those reported in literature: 189 ± 56 L · kg^{-1} (Kenaga, 1980; Rao and Davidson, 1980; Brown and Flagg, 1981; Glotfelty *et al.*, 1984).

In soil, the Freundlich constant n for mecoprop is significantly lower than was found for the lake sediment samples. Apparently, the sorption capacity of the organic phase in the sediment is higher than for soil. An explanation might be that the organic carbon in the lake sediment has reached a higher state of degradation and has therefore a higher reactive surface area.

Measurements were statistically analysed with paired T-tests to determine whether the use of pure or mixed solutions affects the value of K_{OC}. For aldicarb, simazine and mecoprop, no effects (0.05 confidence interval) were found. For MCPA, a significant effect could be shown. Apparently, the occurence of other compounds in the solutions has a synergistic effect on the retention of MCPA to organic matter. This phenomenon may be explained by assuming that one or more pesticides compete for bonding sites on the highly hydrophobic components in the system (e.g., dissolved organic carbon, DOC), thus forcing MCPA to bond with the soil particulate organic carbon (POC). Mecoprop, having the highest octanol/water distribution ratio of the four pesticides, may likely have a large preference to these hydrophobic components. A variety of highly hydrophobic compounds, like hydrocarbons, were found in this lake sediment in concentrations that are relatively high compared to concentrations in which the pesticides occur (Vink and Winkels, 1991; Vink, 1993). The masking effect of differences in the Freundlich constant n may explain why mecoprop does not show decreasing K_{OC}-values in the mixed solutions.

In a successive test, a small quantity (6 mmole) of octanol, $CH_3(CH_2)_6CH_2OH$, was added to a mixture of 50 μg · L^{-1} mecoprop, 50 μg · L^{-1} MCPA and 50 g (dw) of soil to study this effect of increasing hydrophobicity. An identical mixture, where no octanol was added, was used as a comparison. After centrifugation at 2000 g for 15 minutes, the supernatants were treated with 10M NaOH (pH = 12) to minimize the interference of octanol during analysis. The results showed that the distribution of mecoprop over solid and liquid phase was not significantly affected by the addition of this small quantity of octanol, the distribution coefficients being 0.63 and 0.71 L · kg^{-1} respectively. For MCPA however, this was 0.57 and 1.62 L · kg^{-1}, which is an increase of almost 300%. These results indicate that in solutions with similar acting agents, a competitive effect over bonding sites in DOC and POC may occur. The presence of readily accessible dissolved carbon may immobilize the less competitive component, in this case MCPA.

3.2 Transformation in soil and sediment

Figure 3 shows the results of the aerobic and low-oxygen incubations of aldicarb. In soil, aldicarb is transformed principally by oxidation and hydrolysis (Ou *et al.*, 1985). Aldicarb is rapidly oxidized to aldicarb sul-

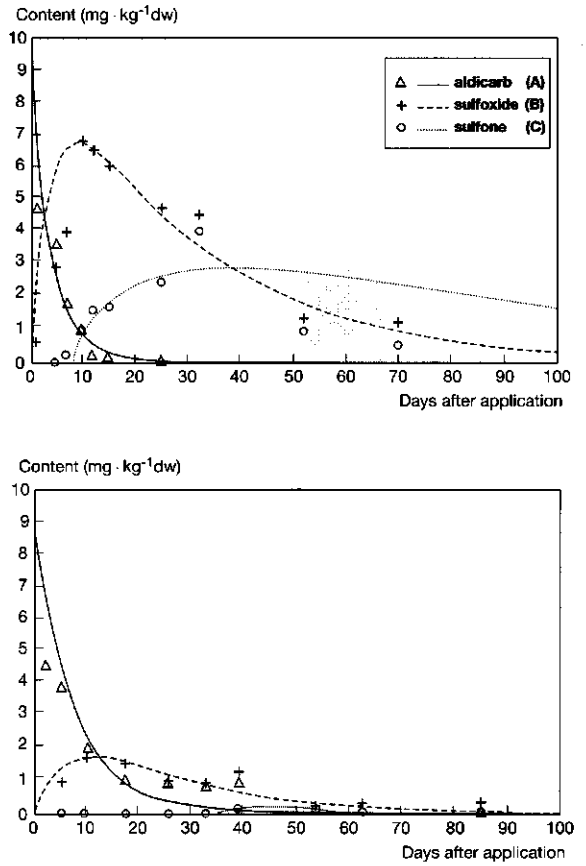


Fig 3. Transformation of aldicarb into its sulfoxide and sulfone metabolites at oxygen concentrations of 8 · 10^{-3} M O_2 (20.8% v/v, top) and 3 · 10^{-6} M O_2 (< 0.01% v/v, below). Model lines correspond with calculations of equations 3 through 6.

Table 4. Transformation rates (day^{-1}).

	Soil (aerobic)	Soil (low oxic)	Lake sediment (anaerobic)
Eh (mV)	+ 330	+ 180	- 120
Aldicarb	0.250	0.150	0.277
- sulfoxide	0.035	0.055	not formed
- sulfone	0.015	-	not formed
Simazine	0.046	0.019	0.007
Mecoprop	0.173	0.017	< 0.001
MCPA	0.178	0.018	< 0.001

- = Concentrations were too low to determine a reliable transformation rate.

foxide, which in turn is slowly oxidized to aldicarb sulfone. We assume that concentrations of the individual compounds in this chain reaction are mutually related and depend on formation and transformation rates by:

$$\begin{cases} \dfrac{dA}{dt} = -k_A A \\ \dfrac{dB}{dt} = r_B A - k_B B \\ \dfrac{dC}{dt} = r_C B - k_C C \end{cases} \quad (3)$$

A = aldicarb
B = aldicarb sulfoxide
C = aldicarb sulfone
r = formation rate
k = transformation rate

With the assumption of first order reactions, and for $A_{(0)} = 9\ mg \cdot kg^{-1}$, $B_{(0)} = C_{(0)} = 0$, we find:

$$A_{(t)} = A_{(0)} e^{-k_A t} \quad (4)$$

$$B(t) = \frac{r_B \cdot A_{(0)}}{k_A - k_B} e^{-k_B t} - e^{-k_A t} \quad (5)$$

$$C(t) = \frac{r_C \cdot r_B \cdot A_{(0)}}{(k_A - k_B)(k_A - k_C)} (e^{-k_C t} e^{-k_B t} - e^{-k_A t}) \quad (6)$$

For aldicarb, the transformation rate was 0.250 d^{-1} in aerobic soil, 0.150 d^{-1} in anoxic soil and 0.277 d^{-1} in anaerobic sediment. Under aerobic conditions, the formation rate of aldicarb sulfoxide was 0.300 d^{-1} with a transformation rate of 0.035 d^{-1}. For aldicarb sulfone, this was 0.200 d^{-1} and 0.015 d^{-1} respectively. Under anoxic conditions in soil, the formation rate of aldicarb sulfoxide was 0.080 d^{-1} and its transformation rate 0.055 d^{-1}. Aldicarb sulfone concentrations were too small ($\leq 0.13\ mg \cdot kg^{-1}$) to determine reliable rates.

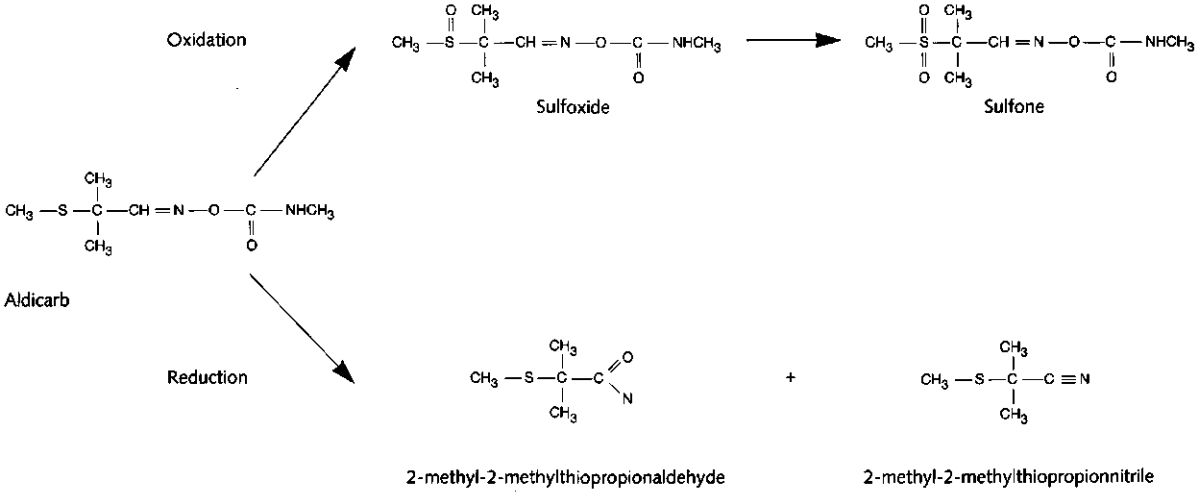


Fig 4. Oxidative and reductive transformation products of aldicarb.

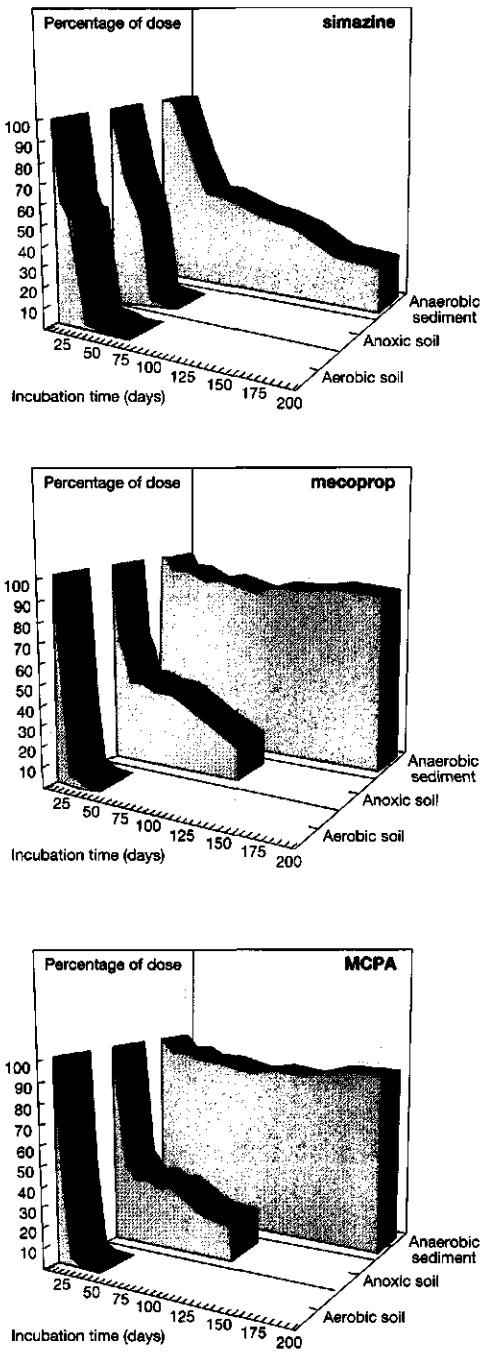


Fig 5. Recovery in time of simazine (top), mecoprop (middle) and MCPA (below) in oxic soil (Eh = +330 mV), low-oxic soil (Eh = +180 mV) and in anaerobic lake sediment (Eh = -120 mV).

In the anaerobic lake sediment, aldicarb was transformed very rapidly. Within 5 days, over 95% of the initial concentration was transformed. No sulfoxide and sulfone could be detected during a 180 day incubation period. This rapid anaerobic transformation has been reported previously (Smelt *et al.*, 1983; Ou *et al.*, 1985; Bromilow *et al.*, 1986; Jones *et al.*, 1986). The complexity of the possible redox reactions is illustrated in Figure 4, in which a distinction is made between strictly aerobic and strictly reductive transformation. Besides aldicarb sulfoxide and small quantities of aldicarb sulfone, relatively large amounts of 2-methyl-2-methylthiopropionnitrile were detected in the low-oxic soil. Apparently, both aerobic and anaerobic conditions occur in the aggregates of these undisturbed samples. This compound was also detected in the lake sediment, where the oxidation products were absent. These observations show that variations in oxygen availability not only affects the rate of transformation of the primary compound, but also determines the types and concentrations of transformation products.

Transformation rates of simazine in the aerobic and anoxic soil columns are 0.046 and 0.019 day^{-1}, respectively (Fig. 5, top). In the anaerobic lake sediment, over 30% of the initial concentration remained after 200 days.

The effect of oxygen on the transformation rate of mecoprop is pronounced (Fig. 5, middle). While transformation in the aerobic, undisturbed soil columns is rapid (0.173 d^{-1}), mecoprop is persistent in anaerobic lake sediment. Little loss could be observed over 200 days of incubation. At low oxygen concentrations ($3 \cdot 10^{-6}$ M O_2), a transformation rate of 0.017 d^{-1} was found in the undisturbed soil, which is approximately ten times smaller than for aerobic soil. In all incubation samples, the metabolite 4-chloro-2-methylphenoxy acetic acid was identified.

The behaviour of MCPA is similar to mecoprop (Figure 5, below). In the aerobic soil columns, rapid transformation was observed with a half life of less than 4 days. The transformation curve showed no lag or adaptation period and could well be described with a first order equation. In the low-oxic soil samples, the half life was approximately 38 days. In the anaerobic sedi-

ment, only 5% of the initial compound was transformed over an incubation period of 200 days.

In the first soil column from the parallel test, which was stored in the incubator ($3 \cdot 10^{-6}$ M O_2) for 109 days, 18% of the initial MCPA concentration remained. In the second column, which was taken out of the incubator and stored in ambient air for ten days, only 5% of the applied amount could be detected. In a ten day aerobic period, accelerated transformation of MCPA had taken place. These results suggest that the involved microorganisms can survive a severe oxygen deficiency, and are able to recuperate and enhance their activity in a very short period of time.

4 Discussion

It is shown that redox conditions have a large impact on the behaviour of the pesticides used in this study. The quantitative effects of a variation in redox conditions on transformation rates are shown in Figure 6. With decreasing oxygen availability, aldicarb is transformed more rapidly, whereas mecoprop and MCPA become persistent at a redox potential of -120 mV. Simazine shows less sensitivity to anaerobicity than mecoprop and MCPA. Despite the higher sorption of aldicarb in the anaerobic lake sediment as compared to soil, transformation rates were higher. Hence, the often made suggestion that bioavailability is inversely related with sorption may not always hold. This accelerated transformation of aldicarb under reductive conditions was attributed by some authors (Bromilow *et al.*, 1986; Jones *et al.*, 1986) to the catalytic effect of Fe^{2+}. Reduced iron is abundant in environments of which the reductive status is beyond denitrification (e.g., sulfur-reducing or methanogenic conditions).

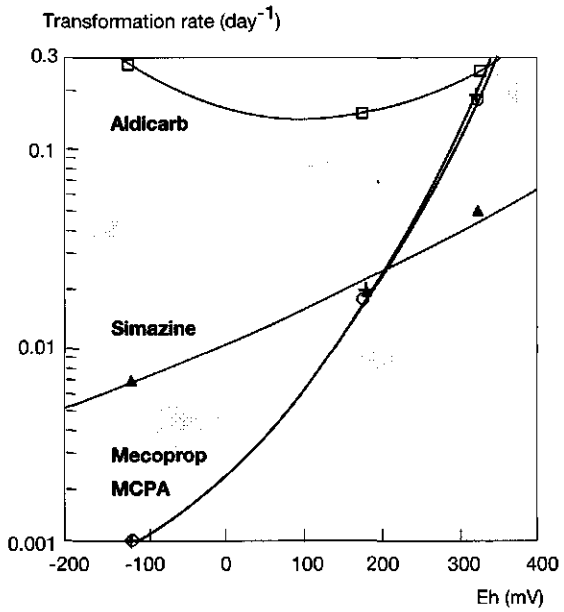


Fig 6. Redox (Eh) dependency on transformation rates.

It is apparent that anaerobic transfomation of simazine occurs. The possible reaction patterns for the transformation of simazine is shown in Figure 7. Dealkalinization of simazine is a biotic process in which aerobic microorganisms are involved, whereas hydrolysis is an abiotic process. The formation of a variety of intermediate products (Cook, 1987; Erickson and Lee, 1989; Lai *et al.*, 1995; Sisodia *et al.*, 1996), which includes deaminization and ring cleavage, may occur in aqueous systems.

Transformation rates of mecoprop and MCPA are mainly regulated by the activity of oxygen-utilizing microorganisms. The competitive effect over dissolved and solid organic matter bonding sites of pesticides that are applied simultaneously, such as mecoprop and MCPA, needs further attention. It was shown that the mobility of MCPA decreases when other hydrophobic compounds are present.

Little is known about the mechanisms of mecoprop biotransformation. It may be utilized as a primary substrate (Agertved *et al.*, 1992). At low concentrations, the substrate might be insufficient as an energy source and decomposition may stop (McCarty *et al.*, 1981). If mecoprop is not completely metabolized as a primary substrate, it may be transformed as a secondary substrate if a chemically simpler or better available carbon source is present (McCarty *et al.*, 1981; Mackay *et al.*, 1985). This phenomenon is known as cometabolism: the compound undergoes microbial transformation without supplying the microorganisms with energy, carbon or other nutrients (Alexander, 1981). Kilpy (1980) has described degradation of mecoprop by mixed cultures isolated from soil, but only when ben-

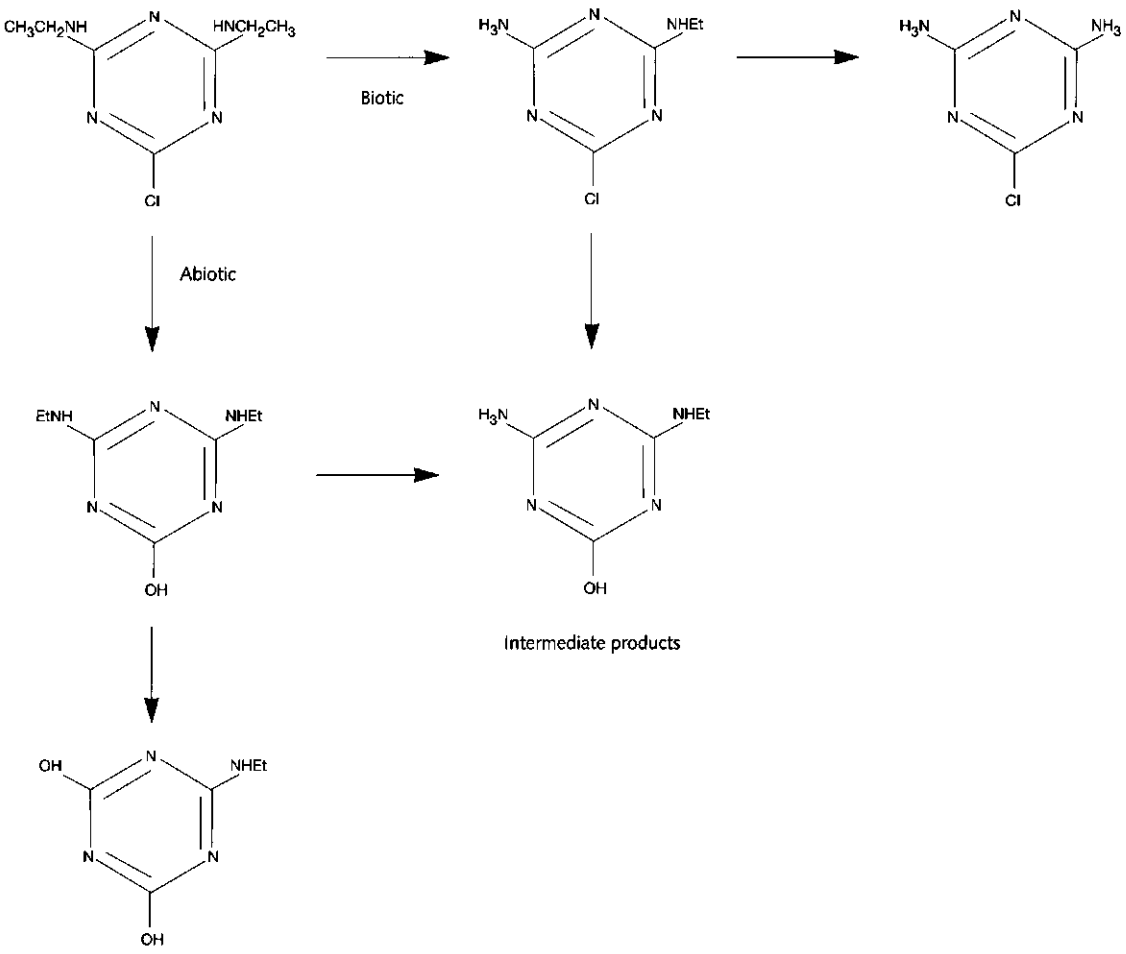


Fig 7. Possible biotic and abiotic transformation pathways of simazine.

zoic acid was added as a co-substrate. Recently, Tett *et al.* (1994) reported that no single member of a consortium of bacteria was able to degrade mecoprop as a pure culture, but after prolonged incubation, *A. denitrificans* was able to grow on the herbicide as a sole source of carbon and energy. In natural systems, the complete degradation of mecoprop can only occur through the synergistic activities of a consortium of microorganisms (Bull, 1985; Tett *et al.*, 1994).

There is still some uncertainty about the biotransformation pathways of mecoprop in soil (Amrein, 1982; Lappin *et al.*, 1985; Smith, 1985). One possibility is decarboxylation and formation of MCPA [4-chloro-2-methylphenoxy acetic acid] (Lindholm *et al.*, 1982). Another possible mechanism is cleavage of the side chain, giving the corresponding phenol [4-chloro-*o*-crexol] (Lindholm *et al.*, 1982). Kelly *et al.* (1991) reported that 4-chloro-methylphenol was an intermediate in the aerobic biotransformation of mecoprop and that additional phenolic compounds were also produced. In our transformation studies, decarboxylation of mecoprop was observed. In the aerobic as well as the low-oxygen incubations, considerable amounts of MCPA were formed as mecoprop concentrations

decreased. Apparently, the methyl side chain of mecoprop can be utilized by microorganisms as a carbon source, which confirms the suggestion made by Lindholm *et al.* (1982).

The data reveal that a temporal but severe oxygen inhibition (e.g., due to seasonal changes or movement into anaerobic environments) can be survived by the microbial population. Apparently, the involved microorganisms can temporarily decrease their activity and can recover within some days from a 109 days stress period. This recuperating mechanism has been described by Brock and Madigan (1988). For strictly aerobic bacteria, O_2 is an essential terminal electron acceptor for membrane energization and for ATP-synthesis. Aerobics may adapt, temporarily, to low oxygen concentrations by boosting their O_2-affinity. This can be accomplished by raising the concentration of components of the respiratory chain and/or by synthesising alternative cytochrome oxidases with lower half-saturation constants for O_2 (Harrison, 1976; Rice and Hempling, 1978). When the O_2-concentration drops to such low levels that insufficient O_2 can be captured to match the supply of carbon substrates, the internal concentration of NADH increases. The metabolism becomes choked and carbon substrates can no longer be converted entirely into structural biomass, carbon dioxide and water (Gerritse, 1993). As a result, the transformation rate decreases.

To evaluate environmental risks of pesticides, a better understanding is needed on the specific dynamics of chemical transformation processes and microbial mineralization in low-oxygen habitats. From our experiments, we could not observe an inverse relationship between sorption affinity and transformation rates of the studied pesticides. Besides using sorption parameters (e.g., K_{OC}-value) as an evironmental risk indicator, the prevailing redox conditions that occur along the pesticide's emission route appears to be an important parameter to assess environmental risks for both conventional and new pesticides. Results of a previous study with metamitron (Vink and Van der Zee, 1996), in which the relative importance of various environmental factors on transformation rates was investigated, confirm this suggestion. Eco(toxico)logical risk assessments for habitats should primarily include and distinguish the chemical group and molecular structure of the pesticide, since it was shown that this largely determines the compound's behaviour when redox conditions change. Systems need to be characterized in terms of microbial potential, and prevailing and potential redox conditions.

Acknowledgement

The research was supported by the Directorate General Science, Research Development of the Commission of the European Community via the ECP Environment, contract nr. EV5V-CT94-0536.

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Appendix

ABC chain reaction

A = Parent compound
$A_{(0)}$ = Dose of parent compound in mg·kg^{-1} / mg·L^{-1}
$A_{(t)}$ = Concentration parent compound at time t
B = Transformation product 1
$B_{(0)}$ = Concentration compound B at t=0
$B^{*}_{(0)}$ = Help parameter
$B_{(t)}$ = Concentration compound B at time t
C = Transformation product 2
$C_{(0)}$ = Concentration compound C at t=0
$C_{(t)}$ = Concentration compound C at time t
k_A = Transformation rate compound A (day^{-1})
k_B = Transformation rate compound B (day^{-1})
k_C = Transformation rate compound C (day^{-1})
r_B = Formation rate compound B (day^{-1})
r_C = Formation rate compound C (day^{-1})
t = Time (days)

Decreasing concentrations of compound A in time as a result of transformation into other products may be written in its basic form as a first order function:

$$\frac{dA}{dt} = -k_A A$$

Compound A acts as a sourse of B. As a result of mass continuity, the formation of compound B in time must be written as a function of both its own formation rate and the transformation rate of compound A. Hence:

$$\frac{dB}{dt} + k_B = r_B A$$

It is assumed that both functions follow first order kinetics.

$$\frac{dB}{dt} + k_B B = r_B A_{(0)} e^{-k_A t}$$

$$-k_B B_{(0)} e^{-k_B t} + B^{*}_{(0)} e^{-k_B t} + k_B B_{(0)} e^{-k_B t} = r_B A_{(0)} e^{-k_A t}$$

Eliminate:

$$B^{*}_{(0)} = r_B A_{(0)} e^{(k_B - k_A)t}$$

Rewrite:

$$B_{(t)} = B_{(0)} e^{-k_B t} + \frac{r_B A_{(0)}}{k_A - k_B} A_{(0)} e^{-k_A t}$$

$$\frac{B_{(t)}}{B_{(0)}} = (1 - \frac{A_{(0)}}{k_B - k_A} e^{-k_B t} + \frac{A_{(0)}}{k_B - k_A} e^{-k_A t}$$

Boundary condition: $B_{(0)} << A_{(0)}$, since the initial concentration of the parent compound is much larger than the concentration of B. Rewrite:

$$B_{(t)} = \frac{r_B A_{(0)}}{k_A - k_B} (e^{-k_B t} - e^{-k_A t})$$

k_A and $A_{(0)}$ are known, r_B and k_B are calculated. Transformation product 2 can be described in an analogous way:

$$C_{(t)} = C_{(0)} e^{-k_C t} + \frac{r_C B_{(0)}}{k_C - k_B} e^{-k_B t} + \frac{r_C r_B A_{(0)}}{(k_B - k_A)(k_C - k_A)} e^{-k_A t}$$

Following:

$$C_{(t)} = \frac{r_C r_B A_{(0)}}{(k_A - k_B)(k_A - k_C)} (e^{-k_C t} - e^{-k_B t} - e^{-k_A t})$$

Chapter 8

Pesticide Biotransformation in Surface Waters: Multivariate Analyses of Environmental Factors at Field Sites

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Published in *Water Research* **11** (1997)

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Pesticide biotransformation in surface waters: multivariate analyses of environmental factors at field sites

Abstract - Transformation rates of four widely used pesticides were determined in surface waters that were characterized on the basis of hydrological status and physico-chemical, biochemical and chemical composition. Large variations in transformation rates were observed, ranging from 0.004-0.01 day^{-1} (half life = 70-173 days) for aldicarb, 0.005-0.57 day^{-1} (half life = 1-139 days) for simazine, 0.002-0.43 day^{-1} for MCPA (half life = 2-347 days) and 0.0005-0.24 day^{-1} (half life = 3-1400 days) for mecoprop. Principal component analyses and stepwise multiple regression analyses were carried out, combining field data and laboratory observations, to reveal the discriminating environmental variables that determine transformation rates of aldicarb, simazine, MCPA and mecoprop in various aqueous systems. A large set of environmental variables (286 observations) was reduced to three underlying components, explaining 84% of the total variance in the data set. The first component contains variables that promote biorespiratory processes, in which a relationship appears between sorption potential, N-sources and microbial activity. The second component is the macro/micro nutrient group. The third component is the phosphorus group. Rapid transformation of these pesticides generally occurs in small hydrological systems like field ditches and channels. Large water bodies like main discharge channels or lakes seem to enhance the persistency of all four pesticides. Besides the hydrological status of the water course, historical application of the pesticide and subsequent adaptation of biorespiratory processes appears to be the most discriminating environmental factor that determines transformation rates of the studied pesticides.

1 Introduction

The occurrence of pesticides and derivatives in deep ground water and surface water has been reported by many authors (Stickel, 1968; Reese *et al.*, 1972; Edwards, 1973; Klint *et al.*, 1990; Agertved *et al.*, 1992; Schnoor, 1992; Ruangwises *et al.*, 1994; Tan and Vijayaletchumy, 1994). Various phenoxyacetic pesticides such as MCPA (≤ 50 ng/g) and mecoprop (10-80 ng/g) were detected at ten locations in the sediments of lake Markermeer, a large water body in the central part of The Netherlands (Vink, 1993). These compounds are considered as not very susceptible to leaching into ground water and surface waters because of their rapid biotransformation in aerobic soils. Taking into account the long transport route, both in time and distance, and the often high dilution factors for water and suspended solids, the occurrence of such compounds in aquatic sediments gives reason for concern.

There is only partial understanding about the actual mechanisms how pesticides move between the aerobic, terrestrial soil and aquatic environments, and only little progress has been made on predicting biotransformation in the environment. The significance of individual environmental properties in the overall transformation of pesticides has much been debated. It is generally believed that the dissolved fraction of a compound, as opposed to the sorbed fraction, is much better available to microorganisms and is therefore degraded rapidly. For surface waters however, it has been suggested that sorption may in fact enhance biodegradation by concentrating the target compound (Olmstead and Weber, 1991; Voice *et al.*, 1992), by concentrating nutrients (Tranvik and Jørgensen, 1995), and by providing a

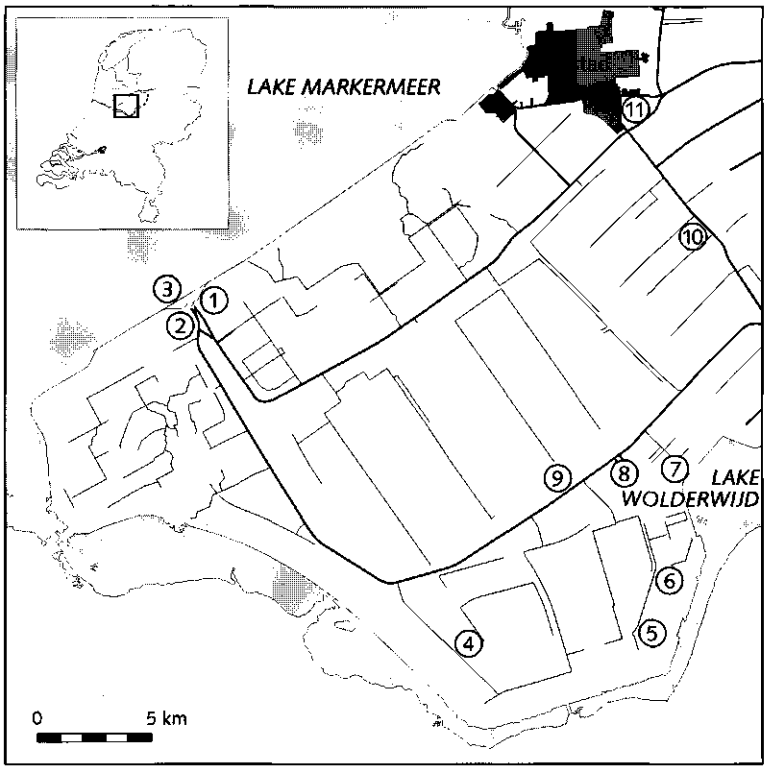

Fig. 1. Research area, water courses and sampling sites.

large surface area for the attachment of bacteria which are then protected from shear forces by water movement (Shimp and Pfaender, 1987). The enhanced biodegradation in the presence of activated carbon has been demonstrated by Feakin *et al.* (1994). They showed for simazine that biodegradation ceased after 14-17 days, and was not increased by prolonged incubation. They suggest that organic carbon and/or mineral nutrients that can be assimilated and are required for the biodegradation process, became limiting. Meakins *et al.* (1994) found in their efforts to enhance transformation of simazine in waste waters, that varying the total suspended solid concentration had no effect on the degree of removal. Kuhlmann *et al.* (1995) found a slightly lower MCPA transformation rate in the absence of phosphorus. Lewis *et al.* (1986) noted that phosphorus is frequently limiting in surface waters. Tett *et al.* (1994) showed that no single member of a consortium of bacterial cultures could initially use mecoprop as a sole source of carbon and energy, but that they need additional nutrients. Cook and Hutter (1981) and Feakin *et al.* (1994) demonstrated that the availability of alternative nitrogen sources, tested in a $NH_4^+NO_3^-$-medium, may decrease transformation rates of simazine.

Many carbamate pesticides will not readily hydrolyse in natural waters. Only in the case of N-substituted carbamates, hydrolysis is likely to be fast enough to be an important degradation pathway (Wolfe *et al.*, 1987). Thus, kinetic studies of other pathways must be considered in evaluating the fate of many carbamates in aquatic systems.

We conclude that the composition of surface waters may be very relevant for the overall transformation rate of a specific compound. Little is known about the quantitative contribution of surface water characteristics and possible synergistic or antagonistic effects in combinations of these characteristics. The main goal in

this study is to identify the discriminating variables that may dictate the fate of various organic compounds in surface water. Next to molecular properties, pesticide transformation rates are largely mediated by the physico-chemical, biological and chemical characteristics of surface waters.

2 Methods

2.1 Surface Water Sampling

The research area is Southern Flevoland, a 44.000 ha polder in the central part of the Netherlands. This polder is artificially drained by a large number of water courses which discharge on lake Markermeer. We made an inventory of these water courses, which were categorised on the basis of depth, with, and water receiving and discharging potential. This resulted in four main classes: (I) ditches, (II) channels, (III) main channels and (IV) lakes. Per system, representative locations were selected for a sampling program of eleven sites (Fig. 1) which was carried out in August 1995. Characteristics of these sites are listed in Table 1. Surface water was collected with an electrical pump and Teflon™ tubing. The inlet tube was connected to a measuring pole which carried a temperature sensor and a polarographic sensor to measure dissolved oxygen concentrations *in-situ*. The measurements were automatically corrected for temperature. The instantaneous reading enables a quick characterization, and determination, of the sampling site and depth. Water was stored after sampling in dark, one-litre bottles and closed. Air entrapment was avoided. The samples were analysed for a total of 50 physico-chemical, chemical and biochemical properties according to the appropriate NEN-procedures.

2.2 Pesticides

To study transformation behaviour of four distinctly different compounds, aldicarb [Carbamate group; 2-methyl-2-(methylthio)propionaldehyde *O*-methylcarbamoyloxime (Temik™)], simazine [S-triazine group; 6-chloro-N^2,N^4-diethyl-1,3,5-triazine-2,4-diamine (Luxan 500 FC™)] and mecoprop [Phenoxic acid; (*RS*)-2-(4-chloro-*o*-2-tolyloxy)propionic acid (Luxan liquid™)] were chosen as representatives of their chemical group. Additionally, MCPA [Phenoxic acid; (4-chloro-2-methylphenoxy) acetic acid (Luxan 500™)] was selected to serve as a comparison with mecoprop. Unlike mecoprop, MCPA does not possess a propionic acid side chain. This lack of a methyl group may affect the susceptibility of the aromatic herbicide to microbial degradation (Alexander and Aleem, 1961).

2.3 Incubations

Standard solutions of aldicarb, simazine, MCPA and mecoprop, containing 100 mg · L^{-1} active ingredient were made. Incubation series of 100 ml unfiltered samples were set up for each pesticide (4x), for each location (11x), and for five time intervals. This resulted in 220 incubation vessels. To each pesticide series, a 1.0

Table 1. Characteristics of the sampled water systems.

Location , type (class)	Sampling depth (m)	Hydrological origin and water depth (m)
1 Basin (IV)	1.0	Discharge water of Southern and Eastern Flevoland polders. Clear, 2 m.
2 Wide channel (III)	1.0	Polder discharge water, main water-way; clear, running, 4 m.
3 Lake (IV)	0.75	Lake Markermeer; clear, 4-6 m.
4 Narrow channel (II)	0.75	Discharge water, cattle and pasture area; turbid, brown, running, water plants, 1 m.
5 Narrow channel (II)	0.75	Discharge water, forest and pasture area; clear, slowly running, water plants and reed, 1 m.
6 Narrow channel (II)	0.50	Discharge water, urban area Zeewolde; clear, slowly running, reed, 0.6 m.
7 Lake (IV)	0.75	Lake Wolderwijd; clear, 2-3 m.
8 Channel (III)	0.50	Water purifying plant Zeewolde; stagnate, turbid, brown. Fauna: e.g., *Paramecium Aurelia*; 1.5 m.
9 Field ditch (I)	0.75	Artificial drainage, orchard area; duck-weed and water plants; slowly running, 0.7 m.
10 Field channel (II)	0.50	Artificial drainage, agriculture area; running, 0.7 m, turbid.
11 Wide channel (III)	0.50	Discharge water, urban area Lelystad; clear, running, water plants, 0.7 m.

ml aliquot of the matching standard solution was added with a microlitre syringe. The samples were stored in the dark to avoid photolysis (Ross and Crosby, 1985). Analyses of aldicarb and its metabolites aldicarb sulfoxide and aldicarb sulfone, and simazine, mecoprop and MCPA were performed as described by Vink and Van der Zee (1997). Bacterial colony forming units (CFU) were determined on plate count agar, incubated at 22 °C. Dilution factors of 10, 100 and 1000 were used in duplicate to obtain reliable counts. Bacterial colonies were counted after 3 and 5 days of incubation.

2.4 Multivariate Analyses

To test whether relationships between the surface water parameters can be expressed in terms of a smaller number of components, we subjected the data to Principle Component Analyses (PCA). PCA describes the variation of a set of multivariate data as a reduced set of uncorrelated variables, each of which is a particular linear combination of the original variables. The first principal component is the combination of variables that accounts for the largest part of variance in the sample. It is described as the linear combination, y_1, of the original variables:

$$y_1 = a_{11} x_1 + a_{12} x_2 = \ldots a_{1p} x_p \qquad (1)$$

of which the total variance is maximised for all vectors $a_{11} \ldots a_{1p}$. The second component accounts for the next largest amount of variance and is uncorrelated with the first, etc. To decide how many components are needed to represent the data, the percentage of total variance explained by each component is examined. Only components that account for variances greater than the variances of all variables are included. Hence, the sum of vectors that represent the variables (Eigenvalue) should be larger than 1.

PCA is useful when a relatively large part of the total variance is explained by only a few components. Knowledge about the significance of each variable that is included in the analyses is necessary, since each variable contributes in the total variance of the data set and therefore influences the interpretation of results. We excluded variables from the data set that 1) display very little variation over the locations and were therefore expected to be non-discriminating, and 2) revealed too small concentrations (< d) to inhibit microbial activity and affect biotransformation processes. Hence, we excluded the heavy metals, As and Se, and pH from the data set. A selection of 26 variables (286 observations) were used in PCA.

When processing a large set of data in PCA, many variables may score high within one component. For a useful interpretation of the component matrix, it should be attempted to minimise the number of variables that have high loadings onto one component. To achieve this, we carried out an orthogonal rotation of the component matrix, so that the variables score high on the individual, non-correlated components. Since rotation does not influence the Eigenvalue, it does not affect the goodness of fit of a component solution (Massart, 1988; Hair, 1992).

Since one of the goals in PCA is to reduce the set of variables to a smaller number of components, it may be useful to estimate component scores for each location. A component score F_{jk} is estimated by multiplying the standardised value of each variable with its corresponding component loading:

$$F_{jk} = \sum_{i=1}^{n} X_{ij} F_{ik} = X_{1j} F_{1k} = X_{2j} F_{2k} + \ldots X_{nj} F_{nk} \qquad (2)$$

in which X_{ij} is the standardised value of the i-th variable for case j and F_{ik} is the component loading for the k-th component and the i-th variable. Component scores can be used in subsequent analyses to represent the values of the components and to detect unusual observations.

To reveal the relative impact of each variable on the transformation rates of each pesticide, we subjected the data to a stepwise multiple regression analysis. While PCA was used to explore correlation patterns in data matrices, stepwise multiple regression is used to find association between variables that may have mechanistic significance. Transformation rates of the pesticides ('r-pesticide') were fixed as dependent linear variables. Step-by-step analyses of the total variance and comparison with the significance level ($p = 0.05$) leads to entering or deleting variables after each run. The result reveals the variable, or combination of variables, that significantly explain the variance of transformation rates of the individual pesticide. Calculations were performed with the SPSS/PC+ software package.

To test whether a relationship exists between the hydrological class of the water course and the specific transformation rates of all pesticides, we used a non-parametric correlation method which is based on the Spearman ranking test. Pairs of the dependent variables c (hydrological class) and r (transformation rate) are compared according to:

$$d^2 = \sum_{i=1}^{n} (c_i - r_i)^2 \qquad (3)$$

in which d is the ranking correlation coefficient. Transformation rates of all four pesticides were ranked, assigning rank 1 to the smallest and rank 11 to the highest value, and were added. In the final ranking, the highest value (fastest overall transformation rates) is ranked 1, etc. The method is appropriate in cases where a phenomenological relationship may occur that is based on ranks of the data, rather than the actual value.

3 Results and discussion

3.1 Surface Water Characterization

Table 2 shows the results of physico-chemical, biochemical and chemical analyses. The variation of the individual properties over the locations is in many cases very large. Ratios are introduced to compare or quantify interrelated properties. For example: at location 8, 91% of the total iron is present as Fe^{2+} (dissolved Fe^{2+} + weekly bound Fe^{2+}-organic complexes). With an almost identical total iron content, this ratio is only 7% at location 11. At location 5, 99% of the total phosphorus content is available as ortho-P, which probably originates from artificial fertilizers that are used in this pasture area. A very large number of microbial colonies was counted at location 8. In spite of the large variety in surface water characteristics and properties, the pH-values are remarkably equal for all locations.

3.2 Incubations

Results of incubations are presented in Fig. 2 and Table 3. None of the transformation pathways show a lag-phase, which indicates that first-order transformation may be assumed (Vink *et al.*, 1994). Some variation in analytical recovery occurs over time but is well within

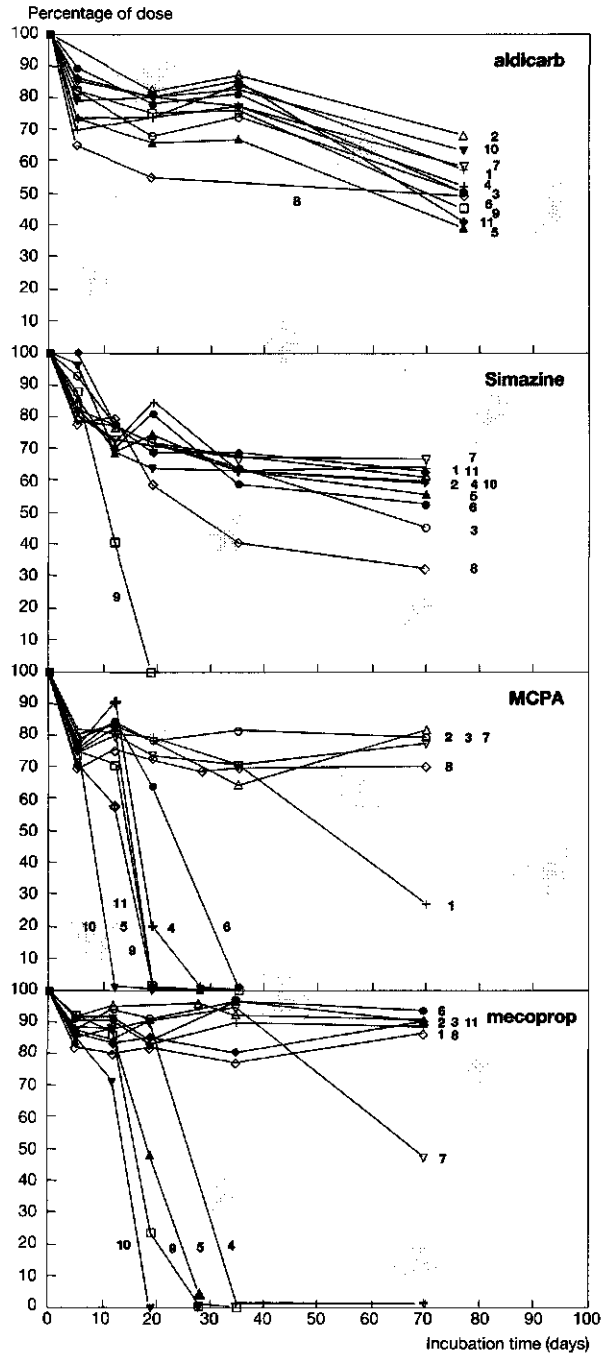

Fig. 2. Transformation in time of aldicarb, simazine, MCPA and mecoprop in surface waters of eleven field sites.

Table 2. Characterization of surface waters.

PARAMETER	LOCATION										
mg · L^{-1}	1	2	3	4	5	6	7	8	9	10	11
PHYSICO-CHEMICAL											
Suspended solids	8.3	12.0	19.3	25.0	8.8	6.1	16.9	49.6	11.6	12.9	7.1
POC	<3.0	<3.0	3.0	<3.0	<3.0	<3.0	3.6	9.6	<3.0	<3.0	<3.0
DOC	7.0	7.0	6.4	14	24	4.7	6.7	14	11	6.5	6.6
Dissolved oxygen	8.6	9.3	10.1	4.7	1.7	9.3	10.8	4.1	3.6	2.4	7.1
BIOCHEMICAL											
Microbial colonies x 10^6 per ml	0.2	0.4	0.3	19	10	1.2	0.6	1600	100	3	1.4
Biochemical oxygen demand, 20 °C, 5 days	1.8	2.4	1.9	2.4	2.5	2.5	2.7	10.0	4.6	3.1	1.1
CHEMICAL											
pH (-)	7.9	7.9	8.0	7.3	7.4	8.0	8.2	7.5	7.2	7.2	7.9
(Bi)carbonates	140	140	135	450	505	240	150	355	250	335	295
Chloride	150	150	145	380	445	65	135	100	140	850	95
As ($\mu g \cdot L^{-1}$)	1.5	1.4	1.4	<1.0	<1.0	<1.0	1.4	<1.0	<1.0	<1.0	<1.0
Co ($\mu g \cdot L^{-1}$)	14	26	15	15	39	31	26	35	44	<10	<10
Cr ($\mu g \cdot L^{-1}$)	9.4	8.5	8.6	8.6	9.6	7.6	7.5	7.1	7.3	5.5	6.8
Cu ($\mu g \cdot L^{-1}$)	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	10	<5.0	<5.0	<5.0
Pb ($\mu g \cdot L^{-1}$)	<5.0	<5.0	<5.0	<5.0	54	<5.0	<5.0	6.7	<5.0	<5.0	<5.0
Zn ($\mu g \cdot L^{-1}$)	<5	15	<5	9	5	9	<5	10	63	10	7
Ca	63	66	61	190	120	81	80	37	180	290	200
K	8.4	9.5	9.2	12	12	5.0	8.5	24	13	9.1	11
Mg	16	17	16	43	30	13	16	4.6	37	49	30
Mn	<0.05	0.061	0.056	0.83	0.69	0.28	0.062	0.084	0.34	0.96	0.27
Fe-total	0.14	0.37	0.4	1.24	0.89	0.17	0.15	0.23	0.27	1.16	0.28
Fe^{2+}	0.04	0.05	0.05	0.40	0.27	0.06	0.05	0.21	0.03	0.11	0.02
(Fe^{2+}/Fe-total) · 100 (%)	*29*	*14*	*13*	*32*	*30*	*35*	*33*	*91*	*11*	*9*	*7*
N-total	1.2	1.2	1.3	12	8.8	0.9	1.5	52	4.0	2.4	0.7
NH_4^+	0.12	0.06	<0.05	10.0	6.5	0.10	<0.05	47	1.6	1.3	<0.05
$NO_2^- + NO_3^-$	0.08	0.11	0.12	0.94	0.06	0.09	0.12	0.08	0.84	0.80	0.09
NH_4^+ / NO_x^- ratio	*5*	*0.5*	*<0.4*	*11*	*108*	*1.1*	*<0.4*	*588*	*1.9*	*1.6*	*<0.6*
P-total	0.12	0.09	0.09	0.35	2.1	0.18	0.88	2.0	0.51	0.15	<0.05
Ortho-P	0.018	0.022	0.017	0.037	2.08	0.060	0.005	1.44	0.295	<0.005	0.014
(Ortho-P/P-total) · 100 (%)	*15*	*24*	*19*	*11*	*99*	*33*	*6*	*72*	*58*	*<3*	*>28*
Simazin ($\mu g \cdot L^{-1}$)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.2	<0.01	<0.01
Mecoprop ($\mu g \cdot L^{-1}$)	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	8.5	<0.1
MCPA ($\mu g \cdot L^{-1}$)	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	2.2	<0.1
γ-HCH ($\mu g \cdot L^{-1}$)	<0.005	<0.005	<0.005	<0.005	0.009	0.040	<0.005	0.021	1.8	0.014	0.011

Organochlorine pesticides (heptachlor, c-heptachlorepoxide, t-heptachlorepoxide, α-HCH, β-HCH, HCB, telodrin, isodrin, aldrin, dieldrin, α-endosulphan and DDE/DDD/DDT derivatives) all below 0.005 $\mu g \cdot L^{-1}$. Cd all <0.20 $\mu g \cdot L^{-1}$. Se all <0.10 $\mu g \cdot L^{-1}$. Ni all <0.20 $\mu g \cdot L^{-1}$

Table 3. First-order transformation rates ($x10^{-3}$ day^{-1}).

Location	Aldicarb	Simazine	MCPA	Mecoprop
1	4.9	5.2	17.2	1.2
2	4.4	5.4	2.2	0.9
3	7.4	10.6	1.7	0.5
4	6.5	6.0	229.2	111.1
5	10.4	7.1	293.7	100.8
6	8.2	8.1	191.3	0.05
7	6.2	4.6	2.0	9.5
8	6.5	15.6	7.5	0.9
9	9.25	69.4	231.9	184.9
10	4.6	6.6	430.9	244.2
11	10.5	6.3	259.5	0.6

tolerance. Transformation of aldicarb appears to be relatively consistent in all water samples. The mean transformation rate is 0.0071 ± 0.002 day^{-1}. The initial rapid transformation at location 8 ceases after some days. Concentrations of the main metabolites aldicarb sulfoxide and aldicarb sulfone are significantly higher (up to a factor 5) than in other samples (Fig. 3). Because the biochemical oxygen demand in this sample is large, and the dissolved oxygen concentration is small, the available oxygen concentration may drop to levels that inhibit biorespiratory processes during biotransformation or even inactivate microorganisms. Also, the two main metabolites, aldicarb sulfoxide and aldicarb sulfone, bind one and two oxygen atoms per unit formed, respectively. Besides the oxygen demand for microbial metabolism, approximately 6 mM (≈ 0.1 mg $\cdot$ L^{-1} O_2) of oxygen is immobilised in the samples in this way.

The approximate 30% reduction of simazine concentrations after 3 weeks of incubation are well in agreement with findings of Feakin *et al.* (1994), who performed incubation tests with low simazine concentrations. Location 9 however shows a deviant transformation behaviour. Location 9 is the only field site where simazine has been detected in the water course (0.2 $\mu g \cdot L^{-1}$). Very rapid transformation is also observed for both MCPA and mecoprop at location 10, where both compounds were detected in the water course (8.5 and 2.2 μg , L^{-1} respectively). Possibly, the microbial communities within these surface waters have adapted to these compounds, and are capable to accelerate their transformation when concentrations

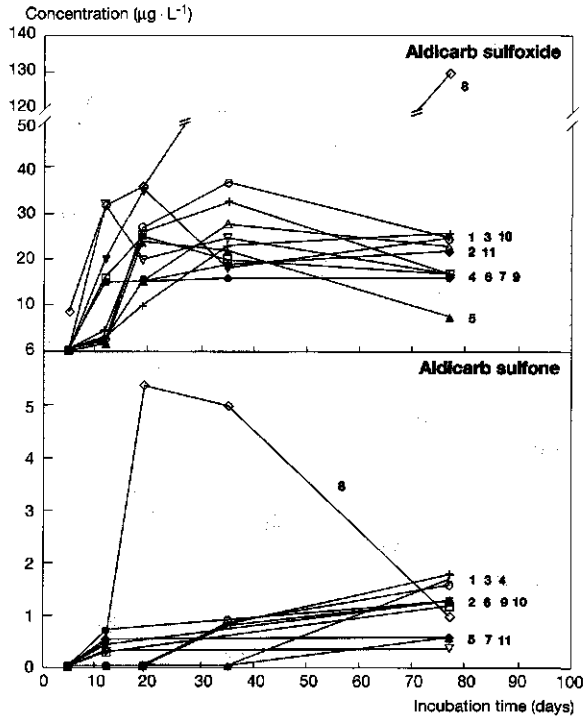

Fig. 3. Formation of aldicarb metabolites in time. Numbers indicate sampling locations. Note the high metabolite concentrations on location 8.

increase. Such adaptation, which improves the ability of a microbial community to degrade a compound after prolonged exposure, is known to occur in soil (Fournier *et al.*, 1981; Soulas *et al.*, 1983; Robertson and Alexander, 1994), but no incontrovertible references are found reporting on this effect occurring in surface waters. The mechanism of how microorganisms are immobilised in surface waters is not quite clear. It is generally suggested that the relevant microbial communities are attached to sediments, or are sorbed to cellular tissue of hydrophytes. The quantitative contribution of each mechanism is yet unknown.

MCPA and mecoprop transformation rates show a high sensitivity to the composition of the water samples. At locations 4, 5, 9 and 10, both phenoxy-acids are transformed completely within 35 days, whereas at locations 1, 2, 3 and 8 the compounds are virtually persistent. A remarkable difference occurs at locations 6

and 11, which are both discharge waters from urban areas. Rapid transformation occurs for MCPA (0.191 and 0.260 day^{-1} respectively), but mecoprop is almost persistent. Water samples from these locations show the lowest nitrogen concentration of all samples (0.9 and 0.7 mg $N \cdot L^{-1}$). In comparison to MCPA, the additional methyl group of mecoprop may attribute to a higher carbon-to-nutrient ratio in the incubation system. Apparently, relevant biotransformation of mecoprop in water requires a minimum threshold of readily available N-nutrients.

Results of the ranking correlation test between the hydrological class and transformation rates of all pesticides are presented in Table 4. Summarized:

Class I: rank 1
Class II: rank 2, 3, 5, 6
Class III: rank 4, 6, 11
Class IV: rank 8, 9, 10

With a positive correlation coefficient of 0.803 (two-tailed 0.003 significance), it appears that the low rankings are represented by field sites of class I and II, whereas high rankings are clustered in classes III and IV. This indicates that transformation rates generally are relatively high in small hydrological systems like ditches and small channels, whereas large water bodies such as wide channels and lakes may enhance the pesticide's persistency. Consequently, the fate of a pesticide in the aqueous environment is mediated by its residence times in certain types of water courses. In view of risk management, this is an important conclusion. A possible explanation for the relationship between hydrology and transformation rates is offered by Lewis and Gattie (1988). They suggest that the relevant microorganisms, able to metabolise pesticides, occur in the water column as suspended colonies and as attached communities (biofilms) to objects such as stones, sediment and macrophytes. Transformation by attached communities correlate well with the external biofilm surface, without considering biofilm volume. These biofilms dominate in ditches and other shallow systems. They concluded that the contribution of attached communities in the overall transformation may be insignificant in deep or turbulent ecosystems. Also, higher concentrations of nutrients, which are essential to support microbial development, may exceed minimum threshold values for optimum microbial growth, and thus contribute to the effect.

Table 4. Ranking of transformation rates.

Location	Hydrol. class	Sum of rankings	Final rank
1	IV	16	9
2	III	11	11
3	IV	19	8
4	II	25	5
5	II	35	2
6	II	23	6
7	IV	14	10
8	III	23	6
9	I	38	1
10	II	30	3
11	III	28	4

3.3 Multivariate Analyses

Table 5 reveals that the data set can be reduced to a set of three components, which account for 84% of the total data set variance. Component 1 consists of N-nutrients (total-N, ammonium), microbial activity (MIC, BOD) and sorption sites (SS, POC). These variables are interrelated. As was suggested by some authors (Cook and Hutter, 1981; Morris and Lewis, 1992; Feakin *et al.*, 1994), the presence of sufficient and readily available N-sources are necessary for maintaining biorespiration processes at an optimum rate, and are therefore correlated with microbial activity. The positive relationship between microbial activity and the availability of sorption sites was reported by Olmstead and Weber (1991) and Voice *et al.* (1992). Considering the high component loadings of these variables in this group, there appears to be an analogy between sorption potential, N-sources and microbial activity. This analogy was recently confirmed by Tranvik and Jørgensen (1995) in their studies on bacterial substrates in surface waters.

A large part of the total variance can be explained by redox-indicators as NH_4^+/NO_X^- ratio (N-ratio) and Fe^{2+}/Fe-total ratio (Fe-ratio), and the biochemical oxygen demand. At non-equilibrium, dissolved Fe^{2+} may be temporarily maintained when favourable electron donors (e.g., NO_X) are present. The effects of redox conditions and indicators on transformation rates have

Table 5. Results of principle component analyses after Varimax rotation with Kaiser normalization. Components have Eigenvalues > 1 and at least two significant loadings onto each component. Component loadings < | 0.50 | are indicated by X.

	Comp. 1	Comp. 2	Comp. 3
Eigen value	10.60	8.27	2.89
% of variance	40.8	31.8	11.1
BOD	0.956	X	X
MIC	0.964	X	X
POC	0.927	X	X
Susp. solids	0.952	X	X
Ca	X	0.868	X
Mg	X	0.919	X
Mn	X	0.945	X
Cl	X	0.860	X
Fe-Tot	X	0.884	X
Fe^{2+}	X	X	X
Fe-ratio	0.867	X	X
N-ratio	0.938	X	X
N-tot	0.969	X	X
NH_4^+	0.967	X	X
P-Tot	X	X	0.701
P-ortho	X	X	0.833
P-ratio	X	X	0.917
O_2 dissolved	X	-0.770	X
CO_3^{2-}	X	0.657	0.620
DOC	X	X	0.803
NO_X^-	X	0.819	X
K^+	0.903	X	X
r-aldicarb	X	X	0.795
r-MCPA	X	0.914	X
r-mecoprop	X	0.825	X
r-simazine	X	X	X

BOD = Biochemical oxygen demand;
MIC = Microbial population;
POC = Particulate organic carbon;
DOC = Dissolved organic carbon;
r-pesticide = Transformation rate.

been reported by Vink and Van der Zee (1996, 1997). Component 1 is referred to as the 'Biorespiration-group' The relative contribution of K in this group is yet unexplained.

Component 2 represent the 'Macro/micro nutrient-group'. Macro nutrients Mg, Fe and Ca and micro nutrients Mn and Cl show high component loadings. Transformation rates of both mecoprop and MCPA are also represented in this group and have high component loadings. Macro and micro nutrients play a role in a variety of primary or secondary bioassimilation processes. Bacterial demand for inorganic nutrients was discussed by Zweifel *et al.* (1993).

Component 3 is the 'Phosphorus-group'. The influence of phosphorus is discussed by Kuhlmann *et al.* (1995) and Lewis *et al.* (1986). Transformation of aldicarb is represented in this group. The presence of Dissolved Organic Carbon (DOC) in this group cannot be fully explained. A fourth component, in which the transformation of simazine is presented, consists of a rest variance of 7.8% from many variables.

Component scores of all locations are shown in Fig. 4. Locations 1, 2, 3 and 7 score low on all components.

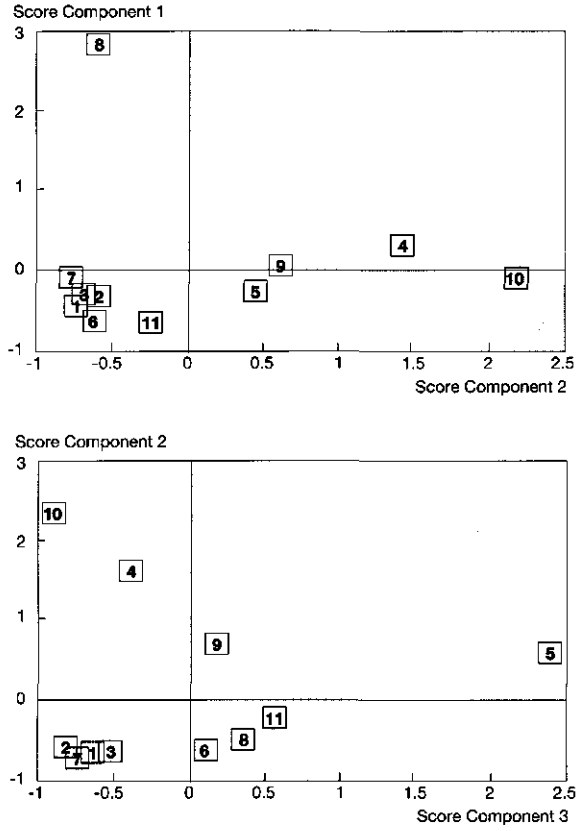

Fig. 4. Principle Component scores of variables from locations 1 to 11 (indicated in boxes). High component scores may either indicate a large values of the environmental variables, or may represent a strong relationship with variables from the component group.

Locations 4, 5, 9 and 10 score high on component 2, while location 5 also scores high on component 3. Location 8 scores high on component 1. A location-specific liability to score on a certain component may either indicate irregularities of variable-values, or may represent a high dependency for a certain variable within this group.

Results of the stepwise multiple regression analyses show that by selecting the most discriminating variables in the linear equations, significant regression coefficients between environmental parameters and transformation rates can be obtained. If more than one discriminating parameters are found, the regression coefficient is adjusted for the amount of independent variables. For aldicarb, we found a maximum regression coefficient (R^2) of 0.53 with P-ratio (Component 3). For MCPA, an $R^2_{adj} = 0.72$ was found for Mg (Component 2), which was increased till $R^2_{adj} = 0.92$ when variables of component 1 (BOD, MIC) were entered in the stepwise analyses. For mecoprop, $R^2_{adj} = 0.77$ was found for Ca (Component 2), and increased till 0.85 by entering variables of component 3 (P-ortho). Simazine transformation appeared to be distributed over the components. No discriminating variables could be entered in the analyses, even when the significance level was increased to 0.10.

The high Mg and Mn loadings on the second component group and their expected large impact on biotransformation rates may be explained by their biochemical functions. Mg plays an important role in the activation of the numerous enzymes that split and transfer ATP phosphate groups. ATP is required in the synthesis of proteins, nucleic acids and co-enzymes, and participates in the utilisation of glucose. In its activation of phosphate transfer from ATP to phosphate receptors, Mg usually can be replaced by Mn. Mn is believed to be required for oxidative phosphorylation in mitochondria (Scott, 1972). Mn metabolism is believed to be involved in amino acid metabolism, not only because of its activation of some of the hydrolysing enzymes, but also because it forms chelates with amino acids (Christensen *et al.*, 1956). This links protein metabolism to Mn turnover. Wakil *et al.* (1957) and Tietz (1957) have demonstrated that Mn is an activator in the synthesis of fatty acids. In both chemical behaviour and geological occurrence, Mn is remarkably similar to Fe (Krauskopf, 1972).

Both MCPA and mecoprop transformation seem to be affected by the occurrence of Mg and Mn. Aldicarb transformation shows a more pronounced response to phosphate concentrations, whereas simazine is affected mainly by variables of component groups 2 and 3, but not by component 1 (loading = 0.006). The N-substituted ring structure of simazine is apparently not co-metabolised parallel to the consumption of other N-sources (presented by component group 1).

This deviant behaviour of the individual pesticides to the various environmental variables may also indicate that the microorganisms, able to transform the individual pesticides, differ in kind. Tett *et al.* (1994) stated that a consortium of bacteria may be needed in the overall biotransformation of pesticides. Following these findings, it is plausible to assume that the presence and activity of (a consortium of) microorganisms, including fungi, tailored to a specific pesticide, directly determines the pesticide's fate, but only when environmental conditions for the entire bacterial consortium are favourable.

Acknowledgements

The research was partly funded by Directorate General Science, Research Development of the commission of the European Community via the ECP Environment, contract nr. EV5V-CT94-0536. The authors thank Mr. Hans van Twuiver and Mrs. Marca Schrap of the RIZA Water Systems division for their suggestions.

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Chapter 9

Nutrient Effects on Microbial Transformation of Pesticides in Nitrifying Surface Waters

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Nutrient effects on microbial transformation of pesticides biotransformation in nitrifying surface waters

Abstract - Multivariate analyses of a large set of physical, chemical and biological measurements have indicated possible effects that specific surface water properties may have on biotransformation rates of pesticides. In this study, we investigated the roles of Mg, Mn and P in the biotransformation processes as it occurs under nitrifying conditions. The pesticides aldicarb [Carbamate group (Temik™)], simazine [S-triazine group (Luxan 500 FC™)] and MCPA [Phenoxy acetic acid (Luxan 500™)] were selected as representatives of chemical groups. We observed an increase in the total microbial population in the surface water samples in the presence of aldicarb or simazine. No such increase was observed with MCPA, which was probably cometabolized. Selective bacterial growth was observed on DES, a transformation product of simazine. Large phosphorus concentrations not only favoured bacterial growth, but also increased the residence time of dissolved Mn which may under certain conditions promote biotransformation. Furthermore, PO_4 enrichment may decrease the concentrations of aldicarb metabolites. Simazine was persistent over a period of at least 80 days, except for a short period that coincides with the period in which NH_4 dissipates and NO_2 and NO_3 are formed during nitrification. Relationships of Mg/Mn concentrations to MCPA transformation rates, and of P/PO_4 concentrations to aldicarb transformation rates, are presented. These relationships may be used to assess these elements as environmental indicators for potential biotransformation, but only under conditions that warrant the development and growth of a degrading population over a prolonged period.

1 Introduction

The general agreement to include soil properties and soil type characterization in pesticide behavior assessments has not yet been implemented in surface water risk assessments. Only a few authors (e.g., Cook and Hutter, 1981; Lewis *et al.*, 1986; Feakin *et al.*, 1994; Kuhlman *et al.*, 1995) report on surface water characteristics and their effect on biodegradability of individual pesticides. Models correlating chemical structure with biodegradation potential have been proposed to address the fate of chemicals in the environment (Larson, 1984). A major weakness in these models is that they do not account for the diversity of environmental factors affecting biotransformation (Davis and Madsen, 1996). Little is known about the quantitative contribution of surface water characteristics on transformation rates, and the possible synergistic or antagonistic effects from combinations of characteristics. In a previous study (Vink and Van der Zee, 1997b), we determined transformation rates of some commonly used pesticides in surface waters that were characterized on the basis of hydrological status, and physico-chemical, biochemical and chemical composition. By combining field data, laboratory observations and statistical analyses, some discriminating environmental variables were identified that may dictate transformation rates in aqueous systems. A large set of environmental parameters was reduced to three underlying components (1:biorespiration; 2:macro/micro-nutrients; 3:phosphorus), each of which may have an effect on the individual pesticide. The lifetime of aldicarb, simazine and MCPA are related to the initial level and activity of suspended microorganisms, but only in combination with conditions that warrant the development and growth of a degrading population

over a prolonged period. These conditions are provided mainly by the availability of inorganic nutrients such as nitrogen, with NH_4 in particular, phosphorus, and metals, with Mn and Mg in particular. Suspended solids may support both nutrients and microorganisms, and may therefore have a stimulating effect on biotransformation processes.

Although microbial activity usually functions optimally at a carbon:nitrogen:phosphorus ratio of approximately 100:5:1 (Water Pollution Control Federation, 1977), the effect of a limiting micro-nutrient may be significant. To better predict the fate of pesticides in surface waters, it is necessary to develop a better understanding of the environmental variables which regulate microbial transformation. In this study, we consider the effects of surface water properties under conditions that favor nitrification, as would occur in most actual surface waters. Furthermore, oxygen concentrations do not limit the rate of biotransformation. We focus on the role of magnesium, manganese, and phosphorus in the overall transformation of aldicarb, simazine and MCPA. Results were related with nitrogen species and availability, and to microbial development.

2 Methods

Water courses of a 44,000-ha polder in the central part of the Netherlands have been classified on the basis of water receiving and discharging potential (Vink and Van der Zee, 1997b). Surface water was collected from a representative channel, using an electrical pump and Teflon™ tubing. The inlet tube was connected to a measuring pole which carried a temperature sensor and a polarographic sensor to measure dissolved oxygen concentrations *in-situ*. The measurements of dissolved oxygen were automatically corrected for temperature. Water was collected in dark, one-litre bottles and closed. Air entrapment was avoided. The collected water was passed through a paper filter and analysed for a set of properties. Part of the sample was passed through a ceramic filter (1mm pore size, 1 bar suction) to determine the effect of suspended particles on the distribution of microbial colonies. The paper-filtered water was transferred to ten sterile 1 L-incubation vessels, each of which had two openings. One opening was used for sample collection and was closed with a stopper. The second opening carried a stainless steel tube, 1 mm diameter, which was connected to an air cylinder, and was stoppered with glass wool. The vessels were then covered with aluminum foil to prevent photolysis. To study transformation behavior of three distinctly different compounds, aldicarb [Carbamate group; 2-methyl-2-(methylthio)propionaldehyde *O*-methylcarbamoyloxime (Temik™)], simazine [S-triazine group; 6-chloro-N^2,N^4-diethyl-1,3,5-triazine-2,4-diamine (Luxan 500 FC™)] and MCPA [Phenoxic acid; (4-chloro-2-methylphenoxy) acetic acid (Luxan 500™)] were chosen as representatives of their chemical group.

Incubation series were set up for each pesticide as follows:

Series 1:	Surface water + pesticide (A = aldicarb, S = simazine, M = MCPA)
Series 2:	Surface water + pesticide + Mg, Mn
Series 3:	Surface water + pesticide + P
Blank:	Surface water

Aldicarb, simazine and MCPA were applied in a dose of 0.5 mmole in all series. Assuming that these pesticides act as a carbon and energy source to bacteria, each incubation vessel received comparable equivalents. To vessels of series 2, we added 20 mg Mg as $MgCl_2 \cdot 6H_2O$ (final Mg-concentrations 69 ± 1 mg $\cdot L^{-1}$) and 2.0 mg Mn as $MnCl_2 \cdot 4H_2O$ (final Mn-concentrations 2.6 ± 0.1 mg $\cdot L^{-1}$). Series 3 was enriched with 3.0 mg P as $NaHPO_4$ (final P-concentration 2.4 ± 0.2 mg $\cdot L^{-1}$). The vessels were mechanically aerated for four hours per day. The air flow rate was equivalent to 25 ml $O_2 \cdot min^{-1}$, at an ambient temperature of 18 °C. pH and redox potential were measured every two or three days. Sampling from each incubation vessel was done with sterile pipettes, and Mn, Mg, N-organic, NH_4, NO_2, NO_3, P-total, PO_4 and pesticides were analyzed periodically. Since sampling consequently leads to a decrease in volume of the incubation medium, which may affect the observations, we kept the necessary amounts as small as possible: for pesticide analysis, aliquots of 6 ml (aldicarb) and 10 ml (simazine, MCPA) were used, and 5 to 20 ml for other characteristics. At the end of the experiments, volumes of 450 ml (A,S series) to 550 ml (M series) remained in the vessels.

Bacterial colony forming units (CFU) were determined on plate count agar, which consisted of 2.5‰ bacto-yeast extract, 5‰ bacto-Trypton (pancreatic carcein digest used as an N-source in calcium medium), 1.0‰ glucose and 12-20‰ agar. The pH was 7.0 ± 0.2 after sterilization. Incubation was done at 22 °C (Standard procedure NEN 6560). We used three dilution series (10x, 100x and 1000x), prepared in duplicate, to obtain reliable counts. Colonies were counted after 3 and 5 days of incubation.

Analyses of aldicarb, aldicarb-sulfoxide and aldicarb-sulfone were done with a High Performance Liquid Chromatography (HPLC) system, using a Waters 510 pump and an WISP712B auto-injector, in combination with a Waters 490E UV-detector and a Vydac 201TP54 m 250 x 4.6 mm column packed with C18 (5 mm) adsorbent (detection limit 0.1 $\mu g \cdot L^{-1}$). Simazine analyses were performed with a Perkin Elmer 8500 gas chromatograph using a CP sil 5 CB column (50 m length, 320 mm diameter, 0.42 mm film thickness) in combination with a NP-detector, using helium as a make-up gas at 80 kPa. Simazine metabolites were analysed separately on an LC-TSP-Mass spectrometer (detection limits 0.1 $\mu g \cdot L^{-1}$). MCPA analyses were carried out with a Perkin Elmer GC 8500, using two capillary CP sil 5/13 CB columns (50 m length, 250 mm diameter, 0.12 mm film thickness). Helium was used as a carrier gas (0.3 $m \cdot s^{-1}$) and nitrogen as a make-up gas (detection limit 0.1 $\mu g \cdot L^{-1}$). Extraction procedures for these pesticides were described in detail by Vink and Van der Zee (1997a).

3 Results

3.1 Medium Properties

Surface water properties are listed in Table 1. Concentrations are within normal ranges, except for phosphorus concentrations and biochemical oxygen demand, which are relatively low. Filtration through a ceramic 1 mm-filter led to dissipation of iron from the solute, which indicates that Fe is present as a relatively large 'solid' phase. Moreover, ceramic filtration led to an approximate 70% reduction in the concentration of suspended solids. This may explain the decreasing numbers of bacterial CFU. The association between suspended solids and bacterial colonization has been suggested by several authors (Shimp and Pfaender, 1987; Olmstead and Weber, 1991; Voice *et al.*, 1992; Tranvik and Jørgensen, 1995). Large particles appear to be more effective at supporting bacteria than the small particles[1], which may be attributed to an increased ability of larger particles to support organic matter and nutrients (Tranvik and Jørgensen, 1995). The predominant contribution of attached bacteria in the overall degradation process has been discussed by Hoppe (1984), Lewis and Gattie (1984), Berger *et al.* (1996) and others.

Concentration in which nutrients are present in surface waters may show large gradients over time (Houx and Dekker, 1987). This may have direct implications for bacterial development and, consequently, biotransformation rates. In most surface waters, the nitrogen status is dominated by nitrification. In the incubation vessels, initial NH_4, NO_2 and NO_3 (i.e. NO_m) concentrations changed as a result of oxidation of NH_4 (Fig. 1). The reaction may be written as:

$$nNH_4 + n(1 + 1/2m)O_2 \rightarrow nNO_m + 2nH_2O \qquad (1)$$

The rate of oxidation varies within the incubation series. The largest oxidation rates were observed in simazine series, the smallest in aldicarb series, and MCPA series and the blank are intermediate. This suggests a slight inhibition for nitrifying microorganisms by aldicarb, and some stimulus by simazine. Consumption of O_2 by microorganisms as a result of reac-

[1] To investigate the relative distribution of bacterial colonies over different particle size classes, the CFU's were compared of the samples that were filtered through two size class filters (Table 1). To do so, it is assumed that particles are spherical shaped, and have a mean diameter of 5 μm in the paper filtered samples and 0.5 μm in the ceramic filtered samples. The total number of particles that are present in the solute are therefore approximately 2 x 10^4 and 7 x 10^6 L^{-1}, respectively. One 5 μm-particle associates with approximately 420 bacteria, whereas for the 0.5 μm size class, this is approximately 0.6 bacteria per particle, which is 700 times smaller. Hence, the number of bacteria seems to increase when the particle size increases. Assuming that approximately 50% of the total number of bacteria are attached to solids (Berger *et al.*, 1996), the relatively large particles are colonized by approximately 5.3 bacteria per μm^2, whereas the small particles are colonized by approximately 0.8 bacteria per μm^2.

Table 1. Characteristics of surface water incubation samples, filtered through paper or a 1 μm-mesh ceramic filter. Properties that showed a significant filtering effect are shown in the second column. Concentrations of aldicarb, simazine and MCPA were below detection limits.

Parameter		Paper filter 4-7 μm	Ceramic filter 1 μm
Suspended solids	(mg · L^{-1})	3.8	1.2
DOC†	(mg · L^{-1})	8.2	
(bi)carbonates	(mg · L^{-1})	250	
Ca	(mg · L^{-1})	300	
Mg	(mg · L^{-1})	49	
Mn	(mg · L^{-1})	0.64	
Fe	(mg · L^{-1})	0.27	< 0.05
CFU‡	(mg · L^{-1})	8400	4500
BOD§	(mg · L^{-1})	1.4	< 1.0
N-organic	(mg · L^{-1})	0.80	
NH_4	(mg · L^{-1})	0.98	
NO_{2+3}	(mg · L^{-1})	1.58	
P-total	(mg · L^{-1})	< 0.05	
PO_4	(mg · L^{-1})	0.025	
Cl^-	(mg · L^{-1})	790	
pH	(-)	7.2	
Eh	(mVolts)	+274	

† Dissolved Organic Carbon.

‡ Colony Forming Units, 5d, 22 °C.

§ Biochemical Oxygen Demand.

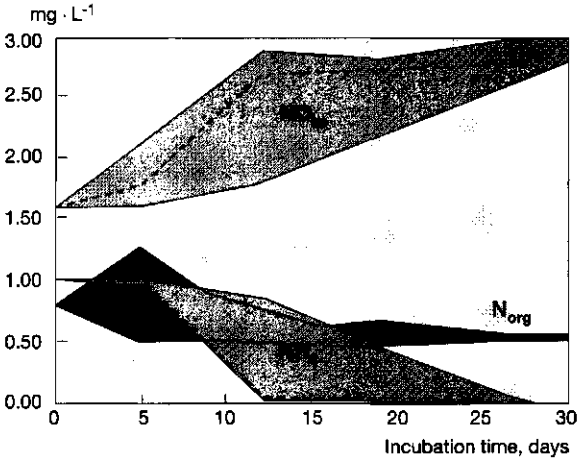


Fig 1. Concentrations of nitrogen species in the incubation vessels, dominated by nitrification. For surveyability reasons, the concentrations in the 10 vessels are shown by the shaded areas and include the overall variation. The production and consumption rates of the involved substances are related via molar conversion terms, leading equation 1 to:

$$-\frac{\delta[NH_4]}{\delta t} = \frac{\delta[NO_m]}{\delta t} = -\frac{n}{(n+1/2m)}\frac{\delta[O_2]}{\delta t}$$

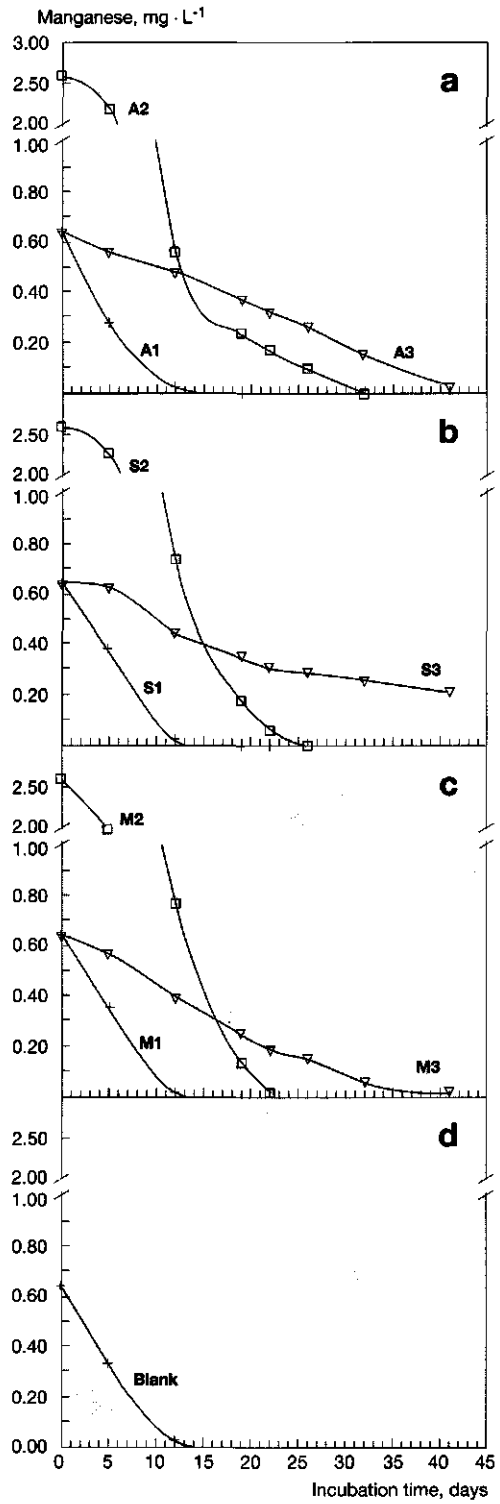
which is the dynamic relation between $[NH_4]$, its oxidation products $[NO_2] + [NO_3]$, and the necessary oxygen for the reaction to occur. Concentrations in the blank are represented by the dotted line.

tion (1) was 0.40 μmole · day^{-1} in the aldicarb vessels, and 0.56 μmole · day-1 in the simazine vessels. Concentrations of organically bound-N were not affected by aeration.

In aerobic surface waters, the oxidation of NH_4 to NO_2 and NO_3 may be considered unfavourable to N-utilizing bacteria. The synthesis of amino-acids and proteins with NO_2 and NO_3 not only requires more energy than the synthesis with NH_4, but also demands additional compounds which do not contribute to the assimilation process (Clark and Rosswall, 1981). This is supported by the relationships between available N-species in the studied water samples and the biochemical oxygen demand (BOD). BOD correlates much better to NH_4 concentrations ($r^2 = 0.84$; n = 12) than to NO_2/NO_3-concentrations ($r^2 = 0.001$). In surface waters where oxygen is not limiting, nitrification is the predominant process. NH_4 concentrations may therefore gradually decrease, which may lead to an inhibition of bacterial activity and, consequently, lead to decreasing transformation rates.

Besides altered nitrogen species, and the availability over time, gradual decreasing concentrations were observed for the micronutrient Mn. Dissolved Mn concentrations in vessels A1, S1, M1 drop to zero within 15 days. Mn-disappearance rates in vessels A2, S2, M2 approximate these rates, in spite of the elevated initial Mn concentrations. Figure 2 shows concentrations of Mn in the vessels over a period of 42 days. In all vessels, it is suggested that Mn concentrations decrease as a result of co-precipitation with $CaCO_3$:

$$Ca^{2+} + Mn^{2+} + CO_2(g) + H_2O + 2e \rightarrow CaMnCO_3\,(s) + 2H^+ \qquad (2)$$



or, as a result of slow diffusion and substitution from the solid phase:

$$CaCO_3\,(s) + Mn^{2+} \rightarrow MnCO_3\,(s) + Ca^{2+} \qquad (3)$$

Stumm and Morgan (1996) presented a stability relation for Mn compounds as a function of pH:

$$\log \frac{[MnCO_3(s)]}{[Mn^{2+}]} = -0.2 + pH + \log[HCO_3^-] \qquad (4)$$

At the current pH (7.2), Mn^{2+} precipitated to form approximately 30 g of $MnCO_3$.

In vessels A1, S1, M1, precipitation rates equaled the rate observed in the blank. An interesting effect was observed in vessels A3, S3, M3, where the residence time of dissolved Mn is significantly increased by the addition of PO_4. This resulted in a prolonged residence time of 40 days or longer. This effect, which may be considered as a co-solvent mechanism, is the result of the reaction:

$$MnCO_3\,(s) + HPO_4^{2-} \rightarrow MnHPO_4 + CO_3^{2-} \qquad (5)$$

followed by dissociation:

$$MnHPO_4 \rightarrow Mn^{2+} + HPO_4^{2-} \qquad (6)$$

and

$$Mn^{2+} \rightarrow \text{Mn-cell} \qquad (7)$$

In which Mn-cell represents bacterial uptake, synthesis, and immobilization of Mn (Sunda and Huntman, 1985). The equilibrium reactions (5) and (6) are very sensitive to small changes of pH, induced by net nitrification (1) and precipitation reactions (2) in the system. With $[CO_3] \approx [pCO_2]$ and $[Ca] = 1.10^{-3}$M, we find that $[Mn^{2+}]$ concentrations may drop 50% when the pH

Fig. 2. Manganese concentrations in time in: **a)** aldicarb series; **b)** simazine series; **c)** MCPA series and **d)** blank. In the phosphorus-enriched vessels, the residence time of dissolved Mn is significantly increased as a result of reactions 5 and 6.

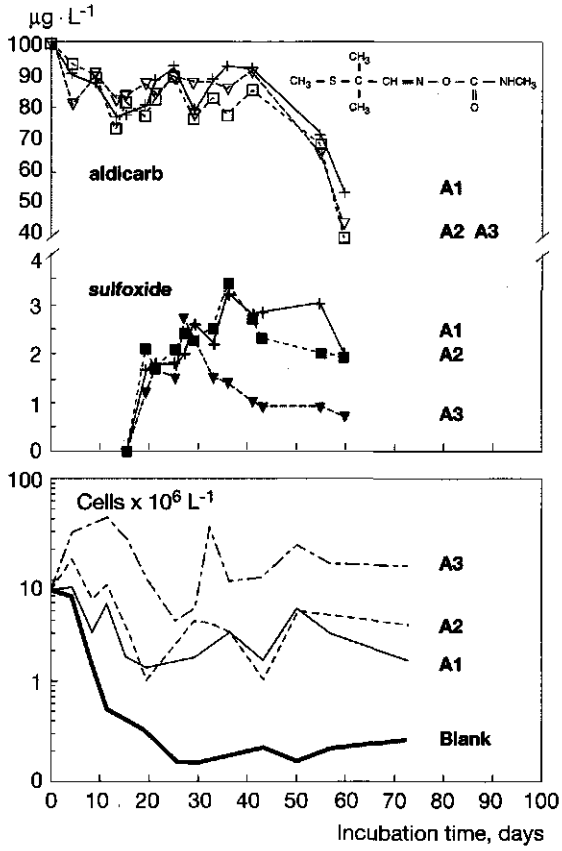


Fig. 3. (Top) Aldicarb, aldicarb-sulfoxide concentrations and (below) bacterial cells in time.

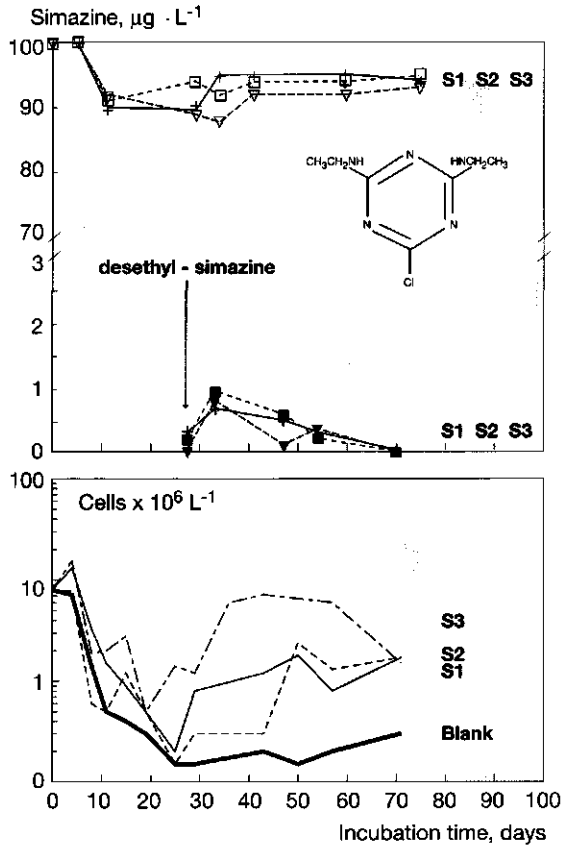


Fig. 4. (Top) Simazine concentrations and (below) bacterial cells in time.

decreases from 7.2 to 7.0. In PO_4-dominated systems however, 95% of total Mn will still be present as soluble $MnHPO_4$ at this pH. Mn will therefore continue to be available to bacteria for a prolonged period. Dissolved Mg concentrations remained constant during the entire incubation experiment, and did not reach concentrations that would inhibit (potential) bacterial development.

3.2 Bacterial Potential and Pesticide Transformation

Compared with zero addition, the addition of aldicarb and simazine to the incubation vessels resulted in elevated bacterial cell numbers in the incubation vessels (Figure 3 and 4). The effect was profound in the aldicarb series, where the number of bacterial cells were up to a factor 10 ($\pm$ 2 x 10^6 cells L^{-1}) larger. This suggests that these bacteria, or specific bacteria, benefit from the addition of aldicarb and, to a lesser extent, simazine. Addition of MCPA did not lead to an increase of bacterial cells (Figure 5). Apparently, MCPA is not an essential source of carbon and energy to these bacteria.

Addition of PO_4 to the incubation vessels A3, S3 and M3 showed a significant elevated number of bacterial cells compared to the blank, which was most profound in the aldicarb series. Up to 4.3 x 10^7 cells L^{-1} were counted after only 10 days of incubation. Apparently, in view of bacterial development, phosphorus was initially a limiting element. As a result, optimum bacterial growth was not reached. This effect may actually occur at field sites, since phosphorus concen-

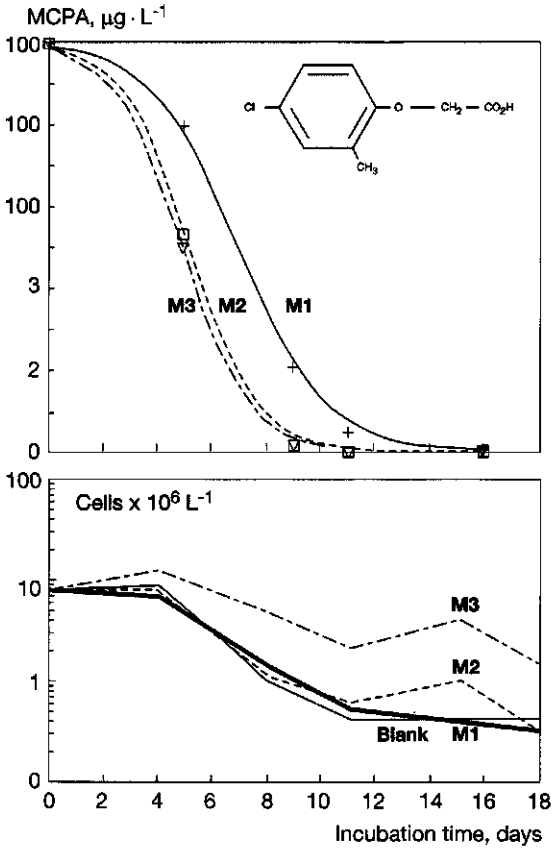


Fig. 5. (Top) MCPA concentrations and (below) bacterial cells in time.

trations may be limiting in many inland surface waters (Lewis *et al.*, 1986; Stumm and Morgan, 1996).

Time-dependent concentrations of Aldicarb, simazine and MCPA are presented in Fig. 3,4,5 respectively. Some transformation of simazine occured within the first 20 days. This period coincided with the period in which NH_4 was still present in solution, and had not been completely oxidized to NO_2 and NO_3. After this period, simazine concentrations were virtually unchanged for at least 80 days. No treatment effects between S1, S2 and S3 could be observed.

The vessels were periodically screened for metabolites on a LC-TSP-Mass spectrometer. From day 27 till 54, small amounts ($\leq 1 \mu g \cdot L^{-1}$) of 2-chloro-4/6-ethylamino-1,3,5-triazine (desethyl-simazine, DES) were detected in all three vessels. This degradate is the result of dealkalinization of simazine, which therefore acted as a carbon source. The occurrance of DES coincided with a significant increase of bacterial cells. The effect was most profound in the P-enriched vessel (S3). In this vessel, P-concentrations did not impose any limitations on rapid bacterial growth. Increased bacterial activity while simazine was not being consumed (concentrations remain constant) may be explained by assuming preferential metabolism of DES. Donati *et al.* (1994) and Bottoni *et al.* (1996) found at DES is slightly less hydrophobic than simazine, and is therefore more soluble. This difference may contribute to an increased availability to suspended bacteria, but this has not yet been established.

Aldicarb and MCPA transformations could be described by first order kinetics, but then transformation rates during the first days were overestimated in all cases. The Exponential Growth Model (EGM) presented by Vink and Groen (1992) gave better results. EGM relates pesticide concentration with growth of the microbial population. Microbial development is described in three phases: i) an initial lag-phase, in which the microbial population adapts to the available substrate; ii) a period of accelerated transformation, in which microbial growth matches the supply of substrate; iii) a period of decreasing transformation rates as a result of substrate limitation in relation to the microbial population size. We observed that transformation of aldicarb in the vessels was slower than was observed in the previous study with open, but not mechanically aerated flasks. Nitrification rates may have been smaller under those conditions, but this was not measured. Also, aldicarb-sulfoxide concentrations were up to a factor 10 smaller (20-35 $\mu g \cdot L^{-1}$ over a period of 15 days in the previous study). Aldicarb-sulfone was not detected at all ($< 0.01 \mu g \cdot L^{-1}$). As was also observed for simazine, it appears that not aldicarb, but mainly its oxidation products aldicarb-sulfoxide and aldicarb-sulfone, were being consumed. These compounds are less hydrophobic than aldicarb, and are probably more available to and co-metabolized by suspended bacteria. Although no significant differences in aldicarb transformation rates were observed among the three vessels, we found that transformation of aldicarb-sulfoxide was faster in the PO_4-enriched environment.

MCPA transformation in vessels M1, M2 and M3 was rapid, although prolonged persistence has been

observed in other surface water samples (Vink and Van der Zee, 1997b). Within 18 days, MCPA was transformed to concentrations below 0.01 $\mu g \cdot L^{-1}$. Both the addition of phosphorus and Mg/Mn slightly increased transformation rates of MCPA. The effect of PO_4 is reflected in an increased number of bacterial cells, which was up to 8 times larger in M3 than in M1, M2, and the blank. Kuhlman *et al.* (1995) also reported a slightly larger transformation rate of MCPA in the presence of phosphorus.

4 Discussion

This and previous studies show that properties and composition of surface waters largely determine the fate of pesticides. The effect is not restricted to the rate of breakdown, but also determines specific transformation routes, and therefore the occurrence of metabolites.

Sorption of pesticides to suspended particles may enhance biodegradation by concentrating nutrients and by providing a large surface area for the attachment of bacteria. Large particles (5 mm) appear to be more effective in doing so than small particles (0.5 μm). It is hypothesized that large particles are colonized by bacteria in a larger density than occurs with smaller particles. Suspended particles may bind NH_4, but not NO_2 and NO_3. In contrast to NO_2 and NO_3, NH_4 was found to correlate well with the biochemical oxygen demand of the water samples, which implies a preference of NH_4 as a nitrogen source to bacteria.

Selected to represent their chemical groups, aldicarb, simazine and MCPA showed different reactions to system conditions. Although the addition of phosphorus does significantly affect bacterial growth, aldicarb breakdown was not directly affected by the amount of phosphate in the system. However, the distribution of phosphate species appears to be of more importance. Fig. 6 shows the relative distribution of ortho-phosphorus (PO_4) to total phosphorus, measured in 12 surface water samples, and the effect on aldicarb transformation rates. It appears that a large fraction of less available phosphates, e.g., $Ca_nH_n(PO_4)_n$, may inhibit transformation rates of aldicarb. Moreover, PO_4 enrichment may slightly decrease aldicarb-sulfoxide concentrations as a result of enhanced bacterial activity

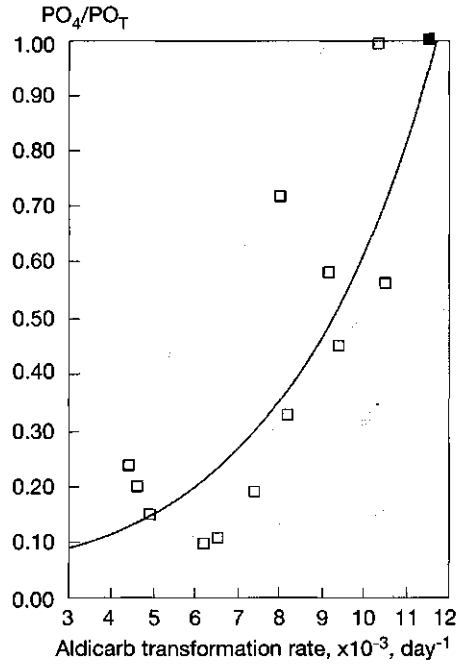


Fig. 6. Aldicarb transformation rates (μ) as a function of the distribution of phosphorus-species in surface waters. Open markers are derived from a previous study (Vink and Van der Zee, 1997b), the closed marker (■) represents vessel A3. The line represents the optimised exponential function, which may be written as

$$\mu_{aldicarb} = \frac{\ln[PO_4] / [P_T]) + 3.2}{0.3}$$

and metabolism. It is unclear whether this may be attributed to the abundance of PO_4 itself, which is utilized as a nutrient, or to its synergistic effect on dissolved Mn concentrations as was described by equations 5 and 6.

Simazine is virtually persistent over a period of at least 80 days in this surface water sample. The fact that no differences could be observed among the three treatments confirms the findings from a previous study that transformation rates are not affected by particular environmental variables from either of the three major discriminating groups. The period in which some breakdown of simazine takes place seems to coincide with the period in which NH_4 dissipates and NO_2 and NO_3 is formed by oxidation. In respect to the need for nitrogen for bacterial growth, we expected that

decreasing concentrations of easily available NH_4 in the vessels would enhance simazine metabolism by N-utilizing bacteria, that use simazine as an alternative source, but this did not occur. A possible explanation may be given by assuming that not simazine, but its metabolites (e.g., desethyl-simazine) are utilized as an N-source. This may explain the low concentrations of desethyl-simazine (DES) in the vessels. The suggestion that the degrading bacteria may switch to the energetic less favourable NO_2 and NO_3 could not be tested. Cook and Hutter (1981) and Feakin *et al.* (1994) investigated simazine transformation in an NH_4NO_3-medium, but did not elaborate on the transition of N-species in solutions over time. Hence, any suggestions made on the availability of alternative nitrogen sources and its effect on (cometabolic) simazine transformation may be questioned.

Fig. 7 shows the relationship between MCPA transformation rates and concentrations of Mg and Mn in solution. The observed effect of Mg and Mn on biotransformation rates of MCPA may be explained by their biochemical function. Magnesium plays an important role in the activation of the numerous enzymes that split and transfer ATP phosphate groups. ATP is required in the synthesis of proteins, nucleic acids and co-enzymes, and participates in the utilization of glucose. In its activation of phosphate transfer from ATP to phosphate receptors, Mg usually can be replaced by Mn. Mn is believed to be required for oxidative phosphorylation in mitochondria (Scott, 1972) and to be involved in amino acid metabolism, not only because of its activation of some of the hydrolyzing enzymes, but also because it forms chelates with amino acids (Christensen *et al.*, 1956). This links protein metabolism to Mn turnover. Wakil *et al.* (1957) and Tietz (1957) have demonstrated that Mn is an activator in the synthesis of fatty acids. Also, some abiotic Mn-mediated transformation of MCPA has been reported (McBride, 1987; Stone, 1987; Ulrich and Stone, 1989; Pizzigalo and Ruggiero, 1992).

It is noted that the relations in Fig. 7 do not imply that high Mg and Mn concentrations warrant rapid breakdown of the pesticide. They merely show the effect that may occur when Mg and Mn concentrations decrease from a certain threshold value, at which maximum transformation rates occur if no other essential elements are limiting. Within these concentration ranges, Mg and Mn may be assessed as environmental indicators for possible MCPA breakdown. Moreover, observed behaviour of the studied pesticides is not restricted to the individual compound, but may represent analogies for similar compounds. It is evident that laboratory breakdown tests, which are mostly conducted in a batch-type set up, should include system characterization, and should recognize the influence of alterations in these characteristics. Co-precipitation, which is a common phenomenon in heterogeneous solutions, may lead to deficiencies of essential elements that are utilized by pesticide degrading bacteria, and therefore affect the interpretation of such experiments.

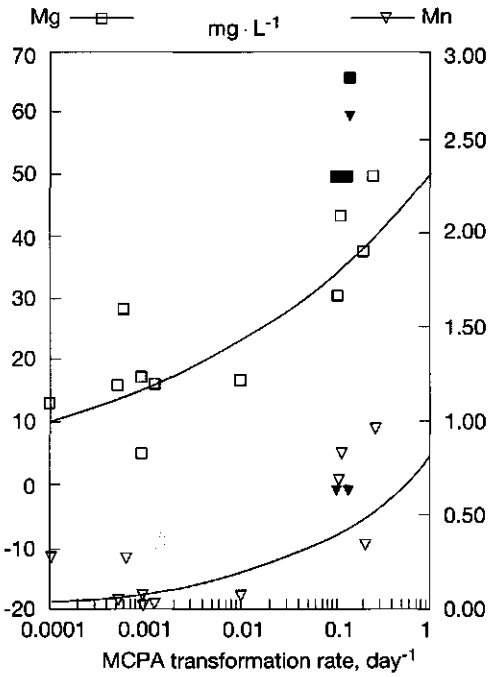


Fig. 7. MCPA transformation rates (μ) as a function of Mg/Mn concentrations in surface waters. Open markers are derived from a previous study (Vink and Van der Zee, 1997b), the closed markers (■,▼) represent vessels M1, M2, M3. The lines represent optimised exponential functions, which may be written as

$$\mu_{MCPA} = \frac{\ln[Mg] - 2.7}{5.6} \quad \text{and} \quad \mu_{MCPA} = \frac{\ln[Mn] + 2.5}{11.6} \quad \text{respectively.}$$

Acknowledgment

The research was partly funded by Directorate General Science, Research Development of the commission of the European Community via the ECP Environment, contract nr. EV5V-CT94-0536. Erwin Temminghoff is acknowledged for performing calculations on chemical equilibria.

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Chapter 10

Summary and concluding remarks

Summary and concluding remarks

The effects and relative impacts of environmental variables on the behaviour of pesticides, through the effect on pesticide-degrading microorganisms, was studied in a broad spectrum and covered the most relevant emission routes. It is shown that the effect of landscape geochemistry, which is a pre-set condition in an agricultural management, may be significant (chapter 2). Adjoining soil types, which occur within short distance in an agricultural unit, were characterized and tested on their pesticide leachability and potential risk to groundwater. The use of a ^{14}C radio-labelled pesticide enabled accurate study of its movement in soil layers. Distinctly different pesticide behaviour in topsoil and subsoil layers were observed within this soil series sequence. An alternative of conventional (i.e. uniform) pesticide application rates, which generally results in over-treating some sites and under-treating others, is site specific treatment. In such managements, doses are tailored to individual soil types or soil properties. This may be beneficial from both an economical as an environmental point of view. A prerequisite in such pest managements is the accuracy and reliability in which the leaching potentials of pesticides in various soils are predicted. Of much concern is the fact that there appears to be little agreement between the existing pesticide leaching models in predicting the residence time in soil and subsequent leaching to groundwater (chapter 3). This may be especially valid in soils that display preferent flow patterns, in which solutes by-pass the soil matrix without being subjected to significant sorption and degradation. It was concluded that the introduction of a variety of conceptual descriptions, thus covering ranges in specific soil properties, may improve the applicability of such models. From an environmental point of view, it is necessary to approximate concentrations not only in terms of magnitude but also at the time and the duration at which these occur. With conventional application rates, distinctly different leaching behaviour was observed in a clay lysimeter for aldicarb, a carbamate nematicide, and simazine, an s-triazine herbicide. Aldicarb leached almost instantaneously, but its aerobe metabolites were found in very high concentrations which did not meet the EC-norm level for water over a period of 300 days. A mass balance for aldicarb showed that 0.35% of the initial dose had leached from the soil. However, when the two isosteric metabolites aldicarb-sulfoxide and aldicarb-sulfone were included in the mass balance, this percentage increased dramatically to almost 20%. The concentrations of both metabolites far exceeded those of the parent compound over a long period. Aldicarb is isosteric to acetylcholine, which plays an important role in the nerve system of organisms, and is therefore capable of inhibiting the performance of acetylcholine-esterase. Since aldicarb's metabolites are oxidation products, in which the critical molecular length is not altered, it may be assumed that aldicarb-sulfoxide and aldicarb-sulfone are capable of doing the same. Therefore, the toxicity of these metabolites may be at least additive. In contrast, simazine leaches in relatively low concentrations - only 0.11% of the initial dose was recovered - but these concentrations were measured over a very long period. The absence of a 'breakthrough behaviour' (peak exposure to aqueous environments), as was observed for aldicarb, implies long term delivery (chronic exposure) of simazine from the soil. To better quantify the effects on intrinsic pesticide behaviour, the relative impact of individual soil characteristics and environmental properties was discussed (chapter 6). For the herbicide metamitron, it is shown that transformation rates were dictated, in order of significance, by temperature, oxygen availability and sorption to organic carbon. Since these variables show large variation in depth of the soil column, the resulting biodegradation rates may change dramatically. Half lives increased from several days in the topsoil to over

one year in subsoil layers. The underlying dynamics of microbial growth and development that are proposed (chapter 4 and 5) show that a multi-layered approach and the assignment of realistic concentrations and conditions to soil layers may improve approximations of microbial inhibition and growth in depth. These non-linear dynamics can be linked, simultaneously, to pesticide metabolism and disappearance rates, using parameter estimation techniques.

Transformation rates and pathways that occur over the soil-aqueous transition zone (i.e. aerobic topsoil-subsoil-anearobic sediment) were tested extensively for four distictly different pesticides that represent their chemical group (chapter 7). It is shown that the prevailing redox conditions have a large impact on pesticide transformation rates. Some phenoxy-acetic compounds, which are considered improbable leachers based on their short aerobic half lives, appear to be persistent in low-oxygeneous conditions. The opposite effect was observed for aldicarb, in which chemical catalysis increased transformation rates when redox potentials decreased. It is shown that a temporal but severe period of oxygen inhibition can be survived by the microbial population. The involved microorganisms can temporarily decrease their activity and can recover within some days from a 109 day stress period. A dynamic chain reaction model is presented, which describes the formation of metabolites from the parent compound, and subsequent transformation, as an interactive, concentration-dependent process.

An attempt to identify the major discriminating variables that determine the fate of pesticides in surface waters was undertaken (chapter 8). A large set of environmental parameters, composed of physico-chemical, bio-chemical and chemical characteristics, was reduced to three major component groups, explaining the majority of variance of transformation rates of four pesticides that were observed in a variety of surface waters. The first component contains variables that promote biorespiratory processes. The second component is a macro/micro-nutrient group. The third component is the phosphorous group. It is shown that small, lotic systems such as field ditches have a larger potential to degrade specific compounds than large, lentic systems, such as channels and lakes. This effect is largely attributed to microbial activity and the possibility of a relevant community to develop. The specific role of Mg/Mn and phosphorus concentrations in nitrifying surface waters on biotransformation rates is identified (chapter 9). Large phosphorus concentrations favour bacterial growth, but a large fraction of less available phosphates may inhibit transformation rates of aldicarb. Addition of orthophosphate increased the residence time of dissolved Mn, which may under certain conditions promote biotransformation rates. Furthermore, PO_4 enrichment may decrease concentrations of aldicarb's metabolites. In a mechanically aerated batch experiment, it was shown that simazine is virtually persistent except for a short period that coincides with the nitrification process in which NH_4 dissipates and NO_2 and NO_3 are formed. Respectively, relationships of Mg/Mn concentrations with MCPA transformation rates, and P/PO_4 concentrations with aldicarb transformation rates, are presented. These relationships may be used to assess these elements as environmental indicators for potential biotransformation of these compounds, or members of the chemical group, but only in combination with conditions that warrant the development and growth of a degrading population over a longer period of time. An illustration of the effects of the individual properties, that were identified in chapter 8 and 9, is given in figure 1. The relationships between these properties were previously discussed in detail. It is generally believed that the dissolved fraction of a compound, as opposed to the sorbed fraction, is much better available to microorganisms and is therefore degraded rapidly. For surface waters however, it is likely that sorption may in fact enhance biodegradation by concentrating the target compound, by concentrating nutrients, and by providing a large surface area for the attachment of bacteria.

It should be emphasised that the observed behaviour of the studied pesticides is not restricted to the individual compound, but may represent analogies for compounds within their chemical group. It is evident that laboratory breakdown tests, which are mostly conducted in a batch-type set up, should include system characterisation, and should recognise the influence of alterations in these characteristics. Co-precipitation, which is a common phenomenon in heterogeneous solutions, may lead to deficiencies of essential elements that are utilised by pesticide degrading microorganisms, and therefore affect the interpretation of such experiments.

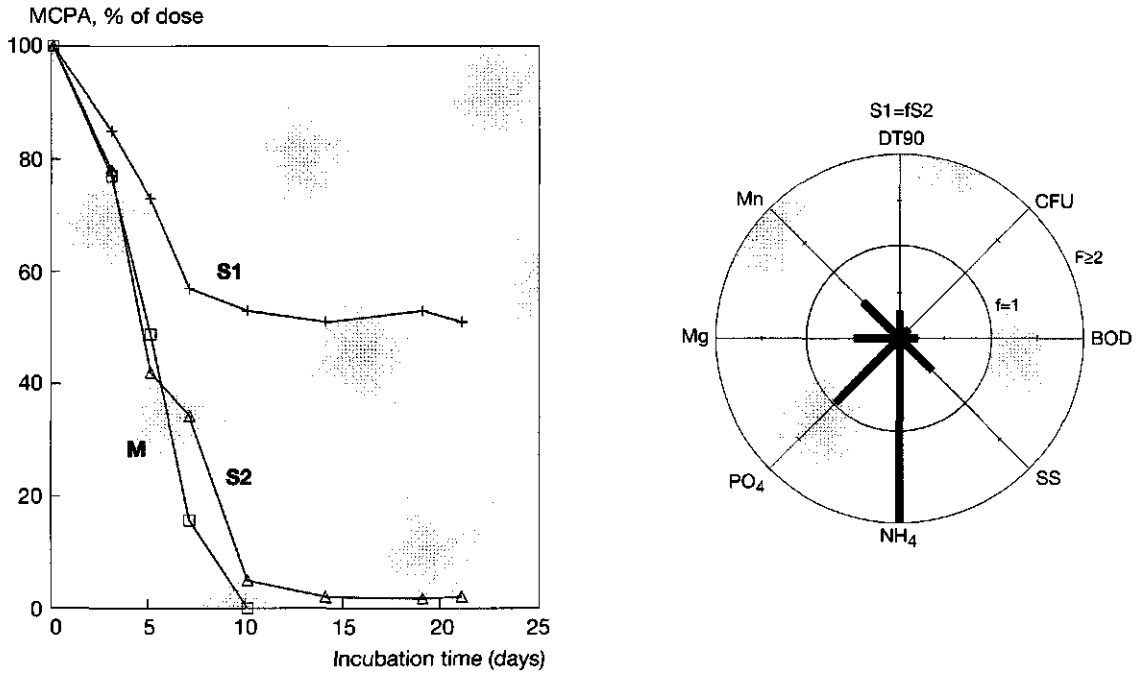

Fig. 1. Transformation of (4-chloro-2-methylphenoxy)acetic acid (MCPA) in two surface water samples (S1, S2) and in a 1:1 mixture (M). Sample S2 is considered a rapid degrading medium, whereas the conditions in S1 do not allow equal transformation rates. Mixing evidently results in an increased transformation rate in S1, due to the effect of canceling out inhibitory effects. The individual effects of sample properties may be illustrated with the aid of 'fraction circles', which compare the relative concentrations of environmental properties in two samples. The inner circle (f = 1) represents equal values of these properties. Relative to S2, the slow transformation rate observed in S1 may be attributed mainly to the smaller values of CFU, BOD, SS and Mn/Mg concentrations. Ammonium and orthophosphorus concentrations do not impose any limitations on transformation rates in S1.
(DT_{90} = Disappearance Time 90%; CFU = bacterial Colony Forming Units; BOD = Biochemical Oxygen Demand; SS = Suspended Solids).

A key issues in pesticide risk assessments is the fact that many compounds are readily transformed to compounds which are toxic to target and non-target organisms throughout the environment. Organophosphate and organosulfur insecticides commonly have initial transformation products with well-established insecticidal activity, often of greater potency than the parent compound. A common reaction observed in many sulfide-containing pesticides is oxidation to sulfoxides and sulfones which are usually active on a spectrum of pests similar to the parent compound. The formation of aldicarb sulfoxide and sulfone, which is described in chapter 7, is an example of this. The simultaneous occurrence of parent compound and oxides may even lead to an increased toxicity. Of much concern is the fact that these toxicologically active transformation products tend to be more mobile than the respective parent compound (Chapter 3). Thus, there is an underestimation of the environmental risks when the parent compound rather than the residue concentrations are used. It may well be stated that metabolite formation must be considered a key issue in pesticide risk evaluations that consider the terrestrial-aquatic emission route. However, there is much hesitation to study pesticide transformation products. This is due to mainly four arguments:

1. High costs are associated with the analyses of the numerous possible compounds.
2. The increase in polarity makes the isolation and

analysis of metabolites often more difficult than the analysis of the parent compound.
3. If a metabolite standard is not available, synthesis may be required.
4. The problem of which metabolites to identify, to prioritise, and to assign as important, environmental risk indicators.

The last argument is of particular importance, and future scientific efforts should focus on this issue. The recent advances in solid phase extraction (SPE) techniques, which have increased the ability to isolate metabolites from water, have attributed significantly to the feasibility of this goal.

A second key issue in risk assessments originates from the fact that the environment is subjected to a range of pesticides that occur simultaneously. This is true for soil, but may be of particular importance for surface waters and aquatic sediments. According to the published literature, the toxicity of many pesticide combinations is at least additive. In some cases, pesticide mixtures - particularly those involving insecticides - have been shown to be synergistic. The bilateral effects in pesticide mixtures on sorption has also been discussed in chapter 7. The most appropriate approach to minimising risks for pesticide mixtures appears to be to assume additive toxicity in all cases, which may include the possible formation of specific metabolites. Still, the problem remains of identifying the environmental compartment that dictates the bottle-necks in risk assessments of a specific pesticide or chemical group.

Chapter 11

Samenvatting en slotopmerkingen

Samenvatting en slotopmerkingen

De effecten en relatieve bijdragen van omgevingsfactoren op het gedrag van pesticiden in het heterogene milieu werd uitvoerig bestudeerd voor de meest relevante emissieroutes. In dit gedrag speelt de dynamiek van micro-organismen in de verschillende milieucompartimenten een belangrijke rol. Er is aangetoond dat het effect van landschappelijke, geochemische variatie groot kan zijn (hoofdstuk 2). Deze variatie is inherent aan het voorkomen van verschillende bodemtypen binnen een agrarisch systeem, soms op zeer korte afstand. Enkele aangrenzende bodemtypen, deel uitmakend van een bodemkundige sequentie, werden gekarakteriseerd en vervolgens getest op hun uitloogbaarheid van pesticiden en potentieel risico voor grondwater. Door het gebruik van een ^{14}C radio-isotopisch gelabeld pesticide was het mogelijk om het gedrag in bodemlagen accuraat te bestuderen. Binnen deze bodemkundige sequentie waren grote verschillen waar te nemen in het gedrag van het pesticide. Als direct gevolg van conventionele (uniforme) toepassingen van pesticiden worden lokaties over- of ondergedoseerd ten opzichte van zowel het economisch als het milieukundig optimum. Een alternatief is bodemspecifieke behandeling, waarbij de dosering is aangepast op de karakteristieken van het bodemtype. Een voorvereiste in dergelijke systemen is de nauwkeurigheid en betrouwbaarheid waarmee het uitspoelingsrisico van pesticiden in verschillende bodemtypen kan worden voorspeld. De overeenstemming tussen de bestaande rekenkundige modellen met betrekking tot het voorspellen van de verblijftijd en uitloogsnelheid is echter gering (hoofdstuk 3). In het bijzonder geldt dit voor bodemtypen die preferentiële stomingspatronen vertonen en waarbij de stoffen de bodemmatrix passeren zonder onderhevig te zijn aan significante sorptie of transformatie. De toepasbaarheid van deze rekenmodellen zou verbeterd kunnen worden door een variatie aan conceptuele beschrijvingen te introduceren die de variatie in bodemkundige karakteristieken bestrijken. Vanuit milieukundig oogpunt is het noodzakelijk om de concentraties waarin pesticiden in grond- en oppervlaktewater kunnen voorkomen niet uitsluitend te schatten naar grootte-orde, maar ook het tijdstip waarop en de duur waarover deze te verwachten zijn. Naast bodemspecifieke eigenschappen speelt het stofspecifieke gedrag van het pesticide hierbij een grote rol. Uit een lysimeterstudie, uitgevoerd met een zware kleibodem en conventionele dosering, bleek dat aldicarb, een nematicide uit de carbamaat-groep, zich redelijk snel kan verplaatsen en uitspoelt met een korte piek-belasting op het grondwater. De metabolieten aldicarb-sulfoxide en aldicarb-sulfon, de aërobe transformatieprodukten van de moederstof, werden echter in zeer hoge concentraties gemeten over een periode van 300 dagen, hierbij de EG-normering continu overschrijdend. Uit de massabalans van aldicarb bleek dat 0,35% van de aanvankelijke dosis was uitgespoeld. Door het meenemen van de twee metabolieten in deze massabalans stijgt deze waarde naar 20%. De concentraties van deze transformatieprodukten overschreden ruimschoots die van de moederstof over een lange periode. Aldicarb is isosterisch aan acetylcholine, een stof die een belangrijke rol speelt in het zenuwstelsel van organismen. Hierdoor is aldicarb in staat om de werking van choline-esterase te remmen. Aangezien de twee genoemde metabolieten oxidatie-produkten zijn, waarbij de kritische moleculaire lengte voor de systemische werking niet wordt aangetast, mag worden aangenomen dat aldicarb-sulfoxide en aldicarb-sulfon in staat zijn om dezelfde remmende werking op dit enzym uit te oefenen. De toxiciteit van deze produkten is derhalve additief. Contrasterend is het gedrag van simazin, een s-triazine herbicide. Concentraties in het effluent waren weliswaar relatief laag - slechts 0,11% van de dosering werd teruggevonden - maar werden over een lange tijdsperiode gemeten. De

afwezigheid van een duidelijke doorbraak-curve (piekbelasting op aquatisch milieu), zoals was waargenomen voor aldicarb, impliceert lange nalevering (chronische belasting op aquatisch milieu) van simazin vanuit de bodem.

Om het intrinsieke gedrag van pesticiden in de bodem beter te kwantificeren werden de effecten van individuele bodemeigenschappen en omgevingscondities bestudeerd (hoofdstuk 6). Voor het herbicide metamitron werd aangetoond dat de transformatiesnelheid werd gedicteerd, in mate van significantie, door temperatuur, zuurstof beschikbaarheid en sorptie aan organische materie. Deze condities vertonen een hoge ruimtelijke variatie, met name naar de diepte, waardoor de omzettingssnelheden sterk variëren. Halfwaarde tijden namen toe van enkele dagen in toplagen tot langer dan een jaar in diepere bodemlagen. De achterliggende dynamische processen van microbiële groei en ontwikkeling (hoofdstukken 4 en 5) laten zien dat een multi-lagen model benadering, inclusief de toekenning van realistische concentraties en karakteristieken, de voorspelling van de microbiële ontwikkeling *in-situ* kan verbeteren. Met behulp van parameter schatting-technieken werden de niet-lineaire groeivergelijkingen simultaan gekoppeld aan transformatiesnelheden in individuele bodemlagen.

Interessant is de dynamiek van omzettingsroutes en -snelheden in de terrestrische-aquatische overgangszone. Voor vier - moleculair van elkaar verschillende - pesticiden is de omzettingsdynamica gesimuleerd voor de emissieroute aërobe bodem, laag-oxische bodem en anaëroob waterbodem sediment (hoofdstuk 7). Met behulp van een daartoe ontwikkelde stikstof incubatiekamer werd aangetoond dat de heersende *in-situ* redox-condities bepalend kunnen zijn voor de routes en snelheden waarin transformatie plaatsvindt. De fenoxyzuren mecoprop en MCPA, die worden beschouwd als onwaarschijnlijke uitlogers op basis van hun - aërobe - transformatie-karakteristieken, blijken in milieus met lage zuurstofconcentraties nagenoeg persistent te zijn. Deze middelen konden worden aangetoond in het sediment van waterbodems van het Markermeer, het hydrologisch eindpunt van het Flevolandse afwateringssysteem. Gezien de lange transportroute, zowel in afstand als in tijd, is de aanwezigheid van deze stoffen in het aquatisch systeem opmerkelijk te noemen. Er werd aangetoond dat de verantwoordelijke populatie van micro-organismen in staat was om een periode van ernstig zuurstoftekort te overleven. Door tijdelijk de metabolische activiteit te verlagen kon een stress-periode van 109 dagen worden overleefd, waarna de populatie zich na enkele dagen herstelde. De kritieke duur van deze stress-periode is echter niet bekend.

Het tegengestelde gedrag werd waargenomen bij aldicarb, waarbij de omzetting werd geïnduceerd door chemische katalyse met reductief ijzer. Bij lage redoxpotentialen werden transformatiesnelheden waargenomen die vergelijkbaar zijn met die onder aërobe condities.

Om de onderlinge wisselwerkingen tussen moederstof en transformatieprodukten te voorspellen werd een dynamisch ketting-reactie model beschreven. Hierin wordt de vorming en daaropvolgende omzetting van (tussen)produkten beschreven als een interactief, concentratie-afhankelijk proces.

Er is een poging ondernomen om de discriminerende variabelen te identificeren die het gedrag van pesticiden bepalen in oppervlaktewateren (hoofdstuk 8). Een grote gegevensset met omgevingsvariabelen, bestaande uit fysisch-chemische, biochemische en chemische karakteristieken van verschillende typen watersystemen, werd met behulp van verschillende statistische technieken gereduceerd naar drie dominante component-groepen die het overgrote deel van de spreiding in transformatiesnelheden verklaren. De eerste component-groep bevat variabelen die de biorespiratie van micro-organismen promoten. De tweede component-groep is de macro/micro-nutriënt groep. Als derde component-groep is de fosfor-groep onderkend. Aangetoond werd dat relatief kleine, lotische watersystemen zoals kavelsloten een groter potentieel herbergen om pesticiden versneld te transformeren dan grotere, lentische systemen zoals kanalen en meren. Dit effect wordt voornamelijk bepaald door de initiële microbiële activiteit en de mogelijkheden voor ontwikkeling van een relevante microbiële populatie.

Onder nitrificerende omstandigheden wordt een specifieke, kwantitatieve rol toegekend aan de elementen magnesium en mangaan enerzijds en fosfor anderzijds (hoofdstuk 9). Hoge fosfaat concentraties in oppervlaktewateren stimuleren microbiële groei, maar een hoge fractie laag-beschikbaar fosfor remt de transformatie van aldicarb. Additie van ortho-fosfaat doet

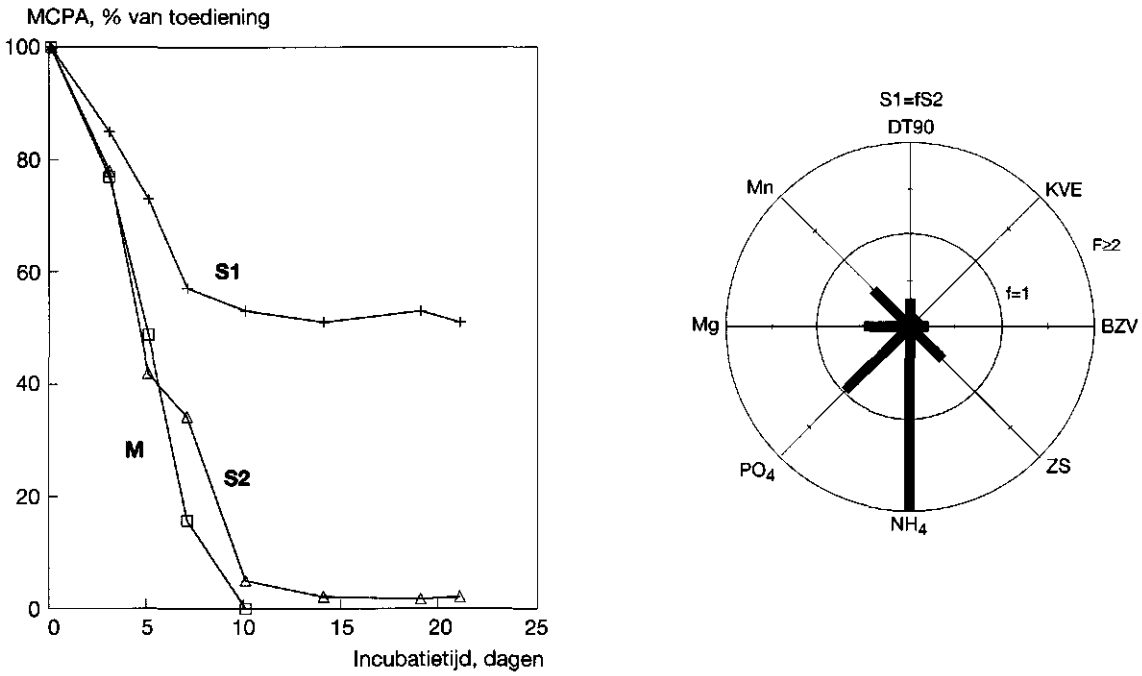

Fig. 1. Transformatie van (4-chloor-2-methylfenoxy)azijnzuur (MCPA) in twee oppervlaktewater monsters (S1, S2) en in een 1:1 mengsel (M). Monster S2 wordt beschouwd als een medium waarin versnelde omzetting optreedt. In S1 zijn de omstandigheden dusdanig dat de omzetting wordt gehinderd. Menging resulteert in versnelde omzetting ten opzichte van S1 vanwege het uitsluiten van remmende factoren. De individuele effecten van de eigenschappen van de monsters kan worden geïllustreerd aan de hand van 'fractie-cirkels'. Hierin worden de relatieve concentraties van milieuvariabelen in twee monsters vergeleken. De binnenste cirkel (f = 1) representeert gelijke waarden van deze variabelen. Vergeleken met S2 kan de vertraagde omzetting in S1 worden toegeschreven aan met name de lagere KVE, BZV, ZS en Mg/Mn concentraties. De concentraties aan ammonium en orthofosfaat leggen geen restricties op op de transformatiesnelheid van S1.
(DT_{90} = Tijd waarover 90% van de moederstof is omgezet; KVE = microbiële Kolonie Vormende Eenheden; BZV = Biochemisch Zuurstof Verbruik; ZS = Zwevend stof).

de verblijftijd van opgelost Mn toenemen. Dit opgelost Mn kan, onder voorwaarden, de transformatiesnelheid stimuleren. Bovendien kan fosfaatverrijking de vorming van aldicarb-sulfoxide en aldicarb-sulfon terugdringen. Met behulp van metingen in een mechanisch geaëreerd reactievat werd aangetoond dat simazin nagenoeg persistent gedrag kan vertonen, met uitzondering van de periodes die samenvallen met de nitrificatieperiode. In deze periode wordt ammonium omgezet in nitriet en nitraat. Er werden kwantitatieve relaties getoond die het verband geven tussen Mg/Mn-concentraties en de omzettingssnelheid van MCPA en voor P/PO_4-concentraties en de omzettingssnelheid van aldicarb. Deze relaties kunnen gebruikt worden als omgevings-indicatoren voor potentiële biotransformatie van deze stoffen (of representanten van de chemische groep), doch uitsluitend in combinatie met de condities die de groei van een relevante microbiële populatie over een langere periode toestaan. Een illustratie van de effecten van individuele milieu-indicatoren, zoals beschreven in de hoofdstukken 8 en 9, is weergegeven in figuur 1. De onderlinge relaties tussen deze variabelen werd uitvoerig beschreven. Er wordt aangenomen dat de opgeloste fractie van een stof, in tegenstelling tot de geadsorbeerde fractie aan zwevend materiaal, beter beschikbaar is voor micro-organismen

en derhalve sneller wordt getransformeerd. Voor oppervlaktewateren echter lijkt adsorptie aan zwevend materiaal de transformatie juist te stimuleren. De onderliggende processen hiervoor zijn het concentreren van de stof, het concentreren van voor metabolische activiteit noodzakelijke nutriënten en de beschikking over een aanhechtingsoppervlak voor micro-organismen.

Benadrukt moet worden dat het waargenomen gedrag van de bestudeerde pesticiden niet beperkt hoeft te zijn tot uitspraken over de individuele stof, maar analogieën kan vertegenwoordigen voor de representatieve chemische groep. Het is evident dat transformatie-studies, die als batch-experimenten worden uitgevoerd in het laboratorium, het medium waarin deze worden onderzocht dienen te karakteriseren. Veranderingen in deze karakteristieken dienen te worden onderkend en gekwantificeerd. In heterogene oplossingen kan (co)precipitatie leiden tot een afname van essentiële elementen die door micro-organismen tijdens het metabolisch proces worden gebruikt. Hierdoor wordt de interpretatie van dergelijke experimenten beïnvloed.

Aandachtspunt in de risicobeoordeling van pesticides is het feit dat veel moederstoffen worden omgezet naar stoffen met een toxische werking voor doel- en niet-doel organismen. Dit geldt met name voor organofosfor- en organosulfaat insecticides, waarbij de toxische werking van de transformatieprodukten hoger is dan die van de moederstof. Sulfoxiden en sulfonen hebben een breed toxisch spectrum. Het simultaan voorkomen van moederstof en metaboliet kan zelfs leiden tot een toename in toxiciteit. Bovendien zijn deze metabolieten vaak mobieler dan de moederstof (hoofdstuk 3). Geconcludeerd wordt dat er in veel gevallen een onderschatting kan plaatsvinden van de potentiële milieurisico's van een pesticide indien er geen inzicht is in de vorming en het gedrag van de transformatieprodukten. In risicobeoordelingen dient derhalve de gehele terrestrische-aquatische emissieroute beschouwd te worden. De aarzeling om uitvoerig onderzoek te doen naar metabolieten kan herleid worden tot de volgende oorzaken:

1. De analyses van transformatieprodukten gaan gepaard met hoge kosten;
2. De toename in polariteit bemoeilijkt de isolatie en analyse van deze produkten;
3. Indien er geen standaard beschikbaar is dient een metaboliet gesynthetiseerd te worden;
4. Het probleem van welk metaboliet te identificeren, te prioriteren en te onderkennen als indicator.

Met name de laatste reden stagneert het gestructureerde onderzoek naar de vormingsvoorwaarden en het gedrag van transformatieprodukten in het milieu. Voortgang op het gebied van vaste fase extractie technieken (SPE), waarbij hydrofobe stoffen geïsoleerd kunnen worden uit water, kan bijdragen aan de haalbaarheid van dit doel.

Een tweede aandachtspunt in de risicobeoordeling van pesticiden komt voort uit het feit dat in het milieu een scala aan pesticiden simultaan voorkomt. Dit geldt niet alleen voor landbodem, maar in het bijzonder voor grond- en oppervlaktewateren en sediment van waterbodems. Onderzoek heeft synergetische toxiciteit van een combinatie van stoffen aangetoond. Ook de onderlinge beïnvloeding op het fysische gedrag is aangetoond (hoofdstuk 7). De meest aangewezen methode voor risicobeoordeling is derhalve door te veronderstellen dat de risico's van pesticidecombinaties, inclusief relevante transformatieprodukten, optelbaar zijn. Het identificeren van de discriminerende milieucompartimenten en de daartoe behorende omgevingsfactoren blijft echter de risicobeoordeling van pesticiden bemoeilijken.

Nawoord

Het beoordelen van risico's van bestrijdingsmiddelen in het milieu ondervindt nog steeds grote problemen vanwege het gebrek aan inzicht in de basale processen. Het vergroten van dit inzicht is een moeizaam proces, dat in de regel een fundamenteel karakter draagt. Hoewel ik mij realiseer dat de hier beschreven materie niet tot in detail voor iedereen even toegankelijk zal zijn, meen ik dat niet alleen de echte insiders baat hebben bij de resultaten.

In de loop van de tijd zijn veel mensen betrokken geweest bij dit onderzoek, die ieder op verschillende manieren een bijdrage hebben geleverd. Een aantal wil ik graag in het bijzonder bedanken.
Dankzij de ondersteuning van de leiding van Directie IJsselmeergebied, en later van het RIZA, is het onderzoek gestart waarvan een deel van de resultaten in dit werk is verzameld. Bart, je hebt me geholpen in mijn keuze tussen een OIO-aanstelling aan een universiteit en een functie als onderzoeksmedewerker bij Rijkswaterstaat. Bedankt voor je steun gedurende het onderzoek.
Sjoerd van der Zee, mijn co-promotor, je hebt mij steeds weer geïmponeerd met jouw inhoudelijke en inzichtelijke talenten. Het was erg prettig om met jou te discussiëren en te publiceren, en hopelijk blijven wij dit doen in andere verbanden.

Frans de Haan, je hebt mij veel vertrouwen geschonken toen ik je benaderde om op te treden als promotor. Hiervoor, en voor je openheid, bedank ik je hartelijk.
My stay at the University of Minnesota has been a pleasure and a tremendous inspiration to explore the field of organic chemistry and soil science. Pierre, many thanks for the opportunities and faith you gave me so graciously.
Mein herzlicher Dank gilt Otto, Bernd, Peter und weiteren Mitarbeitern von der Forschungsgruppe Geographie und Geoökologie von der Technischen Universität Braunschweig für die hervorragende Zusammenarbeit und die inspirierenden Gespräche.
Bijzondere dank gaat uit naar de mensen van het laboratorium die met mij meeleefden, plezier beleefden aan de vele experimenten ondanks de werk- en tijdsdruk, en die het werken daardoor zo prettig maakten.

Het bieden van de organisatorische en technische mogelijkheden zijn slechts enkele voorwaarden geweest voor het afronden van deze onderneming. Ik heb het aan mijn ouders te danken dat ik ook de overtuiging en kracht heb gevonden om er überhaupt aan te beginnen.

Jos Vink
Amersfoort, juli 1997

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Curriculum Vitae

Joseph Pièrre Marie Vink werd geboren op 25 september 1963 te Moordrecht. Na zijn MAVO, HAVO en VWO examens begon hij in september 1984 zijn studie Regionale Bodemkunde aan de Landbouwuniversiteit te Wageningen. In 1989 woonde en werkte hij acht maanden aan de University of Minnesota in St.Paul/Minneapolis, Verenigde Staten, aan een voor hem zeer inspirerend onderzoek naar de grootschalige toepassing van pesticiden en de invloed van de variatie in de bodemgesteldheid. Hier haalde hij het certificaat voor het werken met radio-gelabelde stoffen. Met vier doctoraal specialisaties behaalde hij op 30 maart 1990 zijn graad. Hierna begon hij als sectorleider Bodemkwaliteit bij Rijkswaterstaat, Directie IJsselmeergebied, waar hij het onderzoek heeft opgezet en uitgevoerd dat heeft geleid tot dit proefschrift. Sinds 1 januari 1996 is hij werkzaam als bodemkundig ingenieur bij het Rijksinstituut voor Integraal Zoetwaterbeheer en Afvalwaterbehandeling (RIZA) te Lelystad.