

**Bioavailability and effects of non-ionic organic pesticides in
soil**

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Report 110



DLO Winand Staring Centre, Wageningen (The Netherlands), 1995

1 8 APR. 1996

15h 920500

ABSTRACT

Ronday, R., 1995. *Bioavailability and effects of non-ionic organic pesticides in soil*. Wageningen (The Netherlands), DLO Winand Staring Centre. Report 110, 44 pp.; 7 Figs; 2 Tables; 29 Refs; 1 Annex.

In soil contamination studies the extent of contamination is usually described in terms of the content of the chemical on a dry soil mass basis. However, it has been found that a particular content of a chemical in soil exhibits divergent bioavailability, and thus toxicity, in different soils. Measuring the chemical concentration in the soil pore water is a better method. For evaluating the biological risk of pesticides in soil, concentrations measured in pore water should be compared with soil quality criteria based on the saline-water effect concentrations for soil organisms derived in the laboratory.

Keywords: exposure, invertebrates, risk assessment, soil contamination, toxicity

ISSN 0927-4537

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The DLO Winand Staring Centre is continuing the research of: Institute for Land and Water Management Research (ICW), Institute for Pesticide Research, Environment Division (IOB), Dorschkamp Research Institute for Forestry and Landscape Planning, Division of Landscape Planning (LB), and Soil Survey Institute (STIBOKA).

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Preface

In 1991 the Dutch Ministry of Agriculture, Nature Management and Fisheries presented a Long-Term Crop Protection Plan, with the aim of using pesticides in a way that is ecologically more acceptable (Ministerie, 1991). In this plan it was recognized that knowledge of adverse effects of modern pesticides on flora and fauna is often lacking. The Ministry therefore stimulated scientific research in this field. To assist regulators in making decisions concerning persistence and side-effects of pesticides in soil, the DLO Winand Staring Centre started research into the toxicological significance of pesticide residues in topsoil. This study provides an overview and discussion of relevant concepts from the literature that could be used for this purpose. Some of the results of the laboratory research carried out by our group are integrated in this study.

I would like to thank A.M.M. van Kammen-Polman and A. Dekker for carrying out the chemical analyses described in Chapter 5 and M. Leistra and N.W.H. Houx for their useful critical comments on the manuscript.

Summary

In soil contamination studies usually the extent of contamination is described in terms of the content of the chemical in the soil. However, this does not account for the phenomenon of bioavailability. In soils containing pesticides, a considerable fraction of the chemical may be sorbed to the soil organic matter. This fraction is assumed to be not bioavailable; only the freely dissolved fraction of the chemical in the soil pore water is supposed to reach toxicological targets in organisms. That is why these total content values are not predictive of biological effects.

Bioavailability of pesticides in soil differs with soil composition. This complicates the development of soil quality criteria, which should preferably be based on concentrations of the chemical that are applicable across the range of soils encountered in practice.

Therefore a better method is needed to determine the biological risk of pesticides. It has been found by researchers that if the concentrations of the chemicals in pore water are used, instead of total contents in soil, the biological effect occurs at similar concentrations for the different soils. Apparently, only the freely dissolved state of contaminants is bioavailable and relevant to the biological effect on invertebrates in soil.

If the concentrations of chemicals in pore water could be estimated instead of measured, costly chemical analyses would be avoided. The 'equilibrium partitioning theory' has been proposed in the literature to estimate the dissolved (bioavailable) fraction of the chemical in pore water. It assumes that the partitioning of chemical is at equilibrium between the soil and its pore water. For non-ionic organic chemicals (like many pesticides), partitioning between soil and pore water is determined primarily by the organic matter content of the soil. Therefore, differences in biological effect concentrations for a particular chemical between different soils should disappear if they are expressed as mass of chemical per mass of soil organic matter. This method is called organic matter normalization.

In the present study it is evaluated whether organic matter normalization is able to predict biological risk to organisms in soil. It is also checked whether measurement of the concentration of chemical in the pore water provides a better explanation and prediction of the effects. The relevance of these methods was tested in laboratory toxicity tests by exposing the springtail *Folsomia candida* to three natural soils (differing in organic matter content) treated with parathion or carbofuran. These tests were carried out also after prolonged chemical-soil contact time. Attempts were made to quantify the improvement of both approaches as compared to risk assessment based on the total content of chemical in soil.

It could be shown that by normalizing total contents for organic matter, the soil-to-soil differences are significantly reduced. However, the bioavailable pore water concentration appeared to be not a constant over time. Until weeks after pesticide application equilibria shift and the bioavailability of the chemical decreases. The

method of organic matter normalization does not account for this, which prevents a confident prediction of risk. However, organic matter normalization could be useful for a first estimation of risk of pesticides in soil.

As the concentrations in pore water cannot be calculated accurately, the pesticide concentrations in the soil pore water should preferably be measured. They can be compared with the saline water effects concentration for soil organisms, derived in the laboratory. However, saline water effects concentrations for soil organisms are scarce. In the meantime possibly the large acute toxicity database for aquatic organisms could be used.

1 Introduction

1.1 Problem definition

Soil is an important production factor in agriculture and horticulture. Large-scale application of the results of agricultural research induced agricultural production systems of very high intensity over many areas of the European Community. This intensive use may be characterized by high yields and products of good quality, but may also have drawbacks.

Addition of chemicals to the soil has become common practice in present-day agriculture. Main purposes are the improvement of nutrient supply to the crop, as in the case of fertilizer applications, or crop protection and disease control, as in the case of the use of pesticides. These additions may cause accumulation of compounds and toxicological effects at undesirable levels.

To develop rules and measures for soil protection, a quantitative evaluation of soil quality is needed. During the EC Conference on 'Scientific basis for soil protection in the European Community' in 1987 (Barth & L'Hermite, 1987), agreement was reached that such evaluation should preferably be based on effects that can be expected from the presence and behaviour of contaminants in soil. This requires insight into the different soil functions we want to protect and a quantitative assessment of the risk of contaminants to soil functioning. Some of the most important functions of the soil are (De Haan et al., 1993):

- the bearing function (building of houses, playground for children)
- the plant growth function (natural vegetation, crop production)
- the function of purifying water
- the ecological function (contribution to element cycling)

1.2 Approach and objective

Proper functioning of the soil in different ways requires a different set of quality criteria for each single function. This strongly hampers the introduction of a single quality assessment methodology with general applicability and validity. A possibility to reduce the number of guidelines is to state that protection of the most vulnerable function also protects the other functions (De Haan et al., 1993). As pesticides were developed to control the population growth of plant pathogens, it is likely that the ecological function is the most vulnerable in this respect. Target as well as non-target plants and invertebrates should therefore be object of study when evaluating the quality of soil containing pesticides.

In soil contamination studies usually the extent of contamination is described in terms of the content of the chemical in the soil. However, this does not account for the phenomenon of bioavailability. In soils containing pesticides, a considerable fraction of the chemical may be sorbed to the soil organic matter. This fraction is assumed

to be not bioavailable; only the freely dissolved fraction of the chemical in the soil pore water is supposed to reach toxicological targets in organisms (Graham-Bryce, 1968; Van Gestel & Ma, 1990).

Bioavailability of pesticides in soil differs with soil composition. This complicates the development of soil quality criteria, which should preferably be based on concentrations of the chemical that are applicable across the range of soils encountered in practice. Therefore it is essential that the reasons for the divergent bioavailability are understood.

Boesten (1991, 1993) reviewed several studies on the bioavailability of organic chemicals in soil, mainly dealing with herbicide/plant systems. The present study focuses on the bioavailability and effects of neutral organic chemicals to invertebrates in soil. Concepts on bioavailability encountered in the literature on aquatic sediment toxicology are incorporated and the suitability of the approaches for risk assessment of pesticides in soil will be discussed.

The aim is to test the relevance of two approaches in laboratory toxicity tests. These tests are carried out by exposing the springtail *Folsomia candida* to three natural soils (differing in organic matter content) to which parathion or carbofuran was applied. The improvement of both approaches as compared to risk assessment based on the total content of chemical in soil is to be measured.

1.3 Reading guide

In Chapter 2 efforts are made to define 'bioavailability' and to show its importance for the evaluation of the biological risk of pesticides in soil. Two major concepts on bioavailability and risk evaluation derived from the literature are presented: the pore water hypothesis and the equilibrium partitioning theory.

In Chapter 3 some principles on the partitioning of chemicals between soil and pore water are described. This helps to understand the essence and the preassumptions of the equilibrium partitioning theory. The limitations of the equilibrium partitioning theory are discussed and proposals are made to test the relevance of the equilibrium partitioning theory through experiments.

In Chapter 4 it is evaluated whether the equilibrium partitioning theory is useful for predicting the biological risk of pesticides in soils and sediments. It is checked whether measurement of the concentration of chemical in the pore water provides a better explanation and prediction of the effects on organisms.

In Chapter 5 the suitability of the equilibrium partitioning theory to predict biological effects is assessed through experiments. In particular the influence of time on bioavailability and effects is investigated.

In the General Discussion the practical implications of the findings for soil quality assessment are discussed.

2 Concepts on bioavailability and toxicity of chemicals in soil or sediment

2.1 Defining bioavailability

In evaluating soil quality it is of interest what quantity of a particular compound in soil is the maximum acceptable in order to protect soil organisms. It has been found that a particular content of a chemical in soil or sediment, expressed in mass of chemical per mass of soil, exhibits divergent toxicity in different soils or sediments (Boesten, 1991, 1993; DiToro et al., 1991). The reason for this phenomenon is that a certain fraction of the total mass of the chemical contained by the soil or sediment, does not come into contact with organisms. It is sorbed to soil organic matter or has diffused into remote stagnant pores. This fraction is considered to be not bioavailable. The bioavailable fraction depends on soil or sediment composition and structure and on the properties of the chemical.

It is hard to give an exact definition of bioavailability, but it commonly refers to the concentration of the chemical which can easily reach and hit the toxicological target in a specific organism. Examples of states of organic chemicals in soil are: dissolved in the liquid phase, sorbed to dissolved organic matter in the liquid phase, sorbed to the solid phase in soil and present in the gas phase. The bioavailable fraction of the total concentration of a chemical in a system is related to the non-bioavailable fraction by coefficients describing the partitioning of the chemical over these phases.

2.2 Pore water hypothesis

The observation, first encountered in the literature on aquatic sediment toxicology, that provided insight into the concept of bioavailability is that if different sediments are compared, there is no relationship between the content of a chemical (mass per dry mass) and biological effect. However, if the concentrations of the chemicals in pore water were used, the biological effect occurred at similar concentrations for the different sediments. Moreover, the effect concentrations in pore water were found to be the same as the effect concentration determined in saline water exposures. This was found by Adams et al. (1985), who exposed the midge *Chironomus tentans* to the insecticide chlordecone added to different sediments and to saline water. It led to the hypothesis, that only the freely dissolved state of contaminants in pore water is bioavailable and relevant to the toxicological effect on invertebrates in soil or sediment. This hypothesis, which will be referred to as the 'pore water hypothesis', could be validated for the effects of heavy metals (Kemp & Swartz, 1986) and PAHs (Swartz et al., 1990) to the amphipod *Rhepoxynius abronius* in sediments.

Houx & Aben (1993) could support the pore water hypothesis for soils. They exposed the nematode *Globodera rostochiensis* in three different soils and in saline water

to the pesticides chlorpyrifos or pentachlorophenol. The organic matter content of the soils ranged from 2.4 to 8.7% and the pH (1M KCl) from 4.7 to 6.7. If the $LC50$ values were expressed on the basis of dry soil mass (mg/kg), the difference for the test soils was a factor of 2.6 (chlorpyrifos) and 4.9 (pentachlorophenol). However, if the $LC50$ values were based on the concentration measured in the pore water, they differed only a factor of 1.1 (chlorpyrifos) and 1.5 (pentachlorophenol). Moreover, they found that on a pore water basis the soil $LC50$ s for pentachlorophenol were at the same level as the saline water $LC50$: the maximum difference was only a factor of 1.9. (The experimental variation in the soil $LC50$ s on a pore water basis for chlorpyrifos was too large to justify comparison with the saline water $LC50$).

It can be concluded from the study of Houx & Aben (1993) that the concentration of chemicals in the soil pore water corresponds strongly to the biological effect on soil organisms. This suggests that for the purpose of risk evaluation of pesticides in soil, the concentration measured in the pore water should be used. However, if this parameter can be estimated instead of measured, costly chemical analyses are avoided. In order to estimate the dissolved (bioavailable) fraction of the chemical in pore water and thus to predict biological effects, the so-called 'equilibrium partitioning theory' has been proposed.

2.3 Equilibrium partitioning theory

The equilibrium partitioning theory assumes (i) that the partitioning of a contaminant between the soil or sediment and the pore water is at equilibrium, (ii) that concentrations are related by a partition coefficient K_s (Fig. 1) and (iii) that the fugacity or activity of the chemical, which is the same in each phase at equilibrium, produces the biological effect. This chemical activity is equivalent to the concentration of the chemical in the pore water.

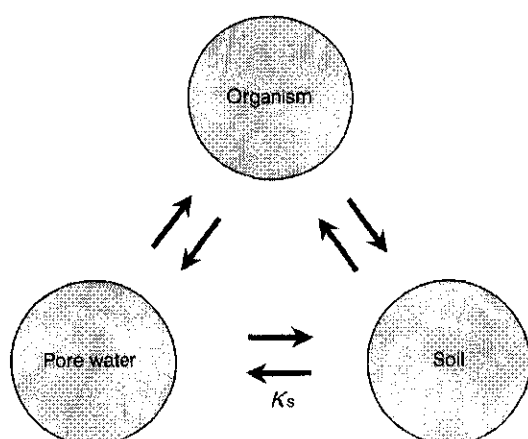


Fig. 1 The equilibrium partitioning theory assumes that equilibrium exists between the content of chemical sorbed to soil and the concentration in pore water. The sorption coefficient is K_s .

These assumptions do not preclude uptake from food, provided that the food consists only of contaminated particles that are in equilibrium with the concentration of the chemical in the pore water. Under these conditions, the relative importance of the

food and water uptake pathways can only affect the overall rate of uptake, not the final equilibrium state.

The equilibrium partitioning theory has been proposed in the field of sediment toxicology (Ziegenfuss et al., 1986). It was meant to explain the relationship between the concentration of a chemical in pore water and the biological effect, from the partitioning of organic chemicals among relevant sediment phases. Secondly, it provides a tool for estimating the (bioavailable) concentration of a chemical in pore water. Therefore, the equilibrium partitioning theory was proposed as a basis for development of threshold limit values for contaminants in sediment (Shea, 1988; see DiToro et al. 1991 for a review).

If we consider the equilibrium partitioning theory to be applicable to soil, the calculation of soil quality criteria (*SQC*) is as follows (after DiToro et al., 1991). The *EC* (mg/L) is the effect concentration in water for the chemical of interest. Then the *SQC* (mg/kg) is computed using the coefficient K_s (L/kg) for sorption on soil from pore water:

$$SQC = K_s EC \quad (1)$$

The utility of this equation depends on the existence of a methodology for quantifying the sorption coefficients K_s . This is the subject of the next chapter.

3 Partitioning of non-ionic organic chemicals

3.1 Assuming linear sorption

In soil contamination studies usually the content of the chemical in the soil, assessed by extraction with organic solvent, is known. For nonvolatile and moderately strong sorbing chemicals ($K_s > 10 \text{ L/kg}$) it can be assumed that the total content (expressed on a soil dry mass basis) is approximately equal to the content of chemical sorbed to the solid phase (X_s).

In the calculation procedure for establishing soil quality criteria on the basis of the equilibrium partitioning theory, as proposed by DiToro et al. (1991), it is assumed that sorption to the solid phase can be described with a linear sorption isotherm. The sorption coefficient can be described as:

$$K_s = X_s / c_L \quad (2)$$

where

K_s is the soil/water partition coefficient (L/kg)

X_s is the content of chemical sorbed to the solid phase (mg/kg)

c_L is the concentration of chemical in the liquid phase (pore water) (mg/L)

For non-ionic organic chemicals (like many pesticides), partitioning between soil and water is determined primarily by the organic matter content of the soil (Karickhoff, 1981). The sorption coefficient (K_s) can therefore be estimated from the organic-matter/water partition coefficient K_{om} (which describes the sorption to soil organic matter) and the mass fraction of organic matter in the soil:

$$K_s = f_{om} K_{om} \quad (3)$$

where

f_{om} is the mass fraction of organic matter in the soil (kg/kg)

K_{om} is the organic-matter/water partition coefficient (L/kg)

If for a particular compound no K_{om} is available, the octanol/water partition coefficient (K_{ow}) is often used to calculate a first approximation of K_{om} (see section 3.3).

Using Equations 1 and 3, a soil quality criterion (SQC) is calculated from

$$SQC = f_{om} K_{om} EC \quad (4)$$

If we define

$$SQC_{om} = SQC / f_{om} \quad (5)$$

as the organic-matter normalized SQC (mg/kg), then

$$SQC_{om} = K_{om} EC \tag{6}$$

So for a particular chemical having a specific K_{om} , the organic-matter normalized SQC_{om} of the chemical should be independent of soil properties, which is an advantage. This method of organic matter normalization was proposed by DiToro et al. (1991) for sediments and requires the determination of K_{om} .

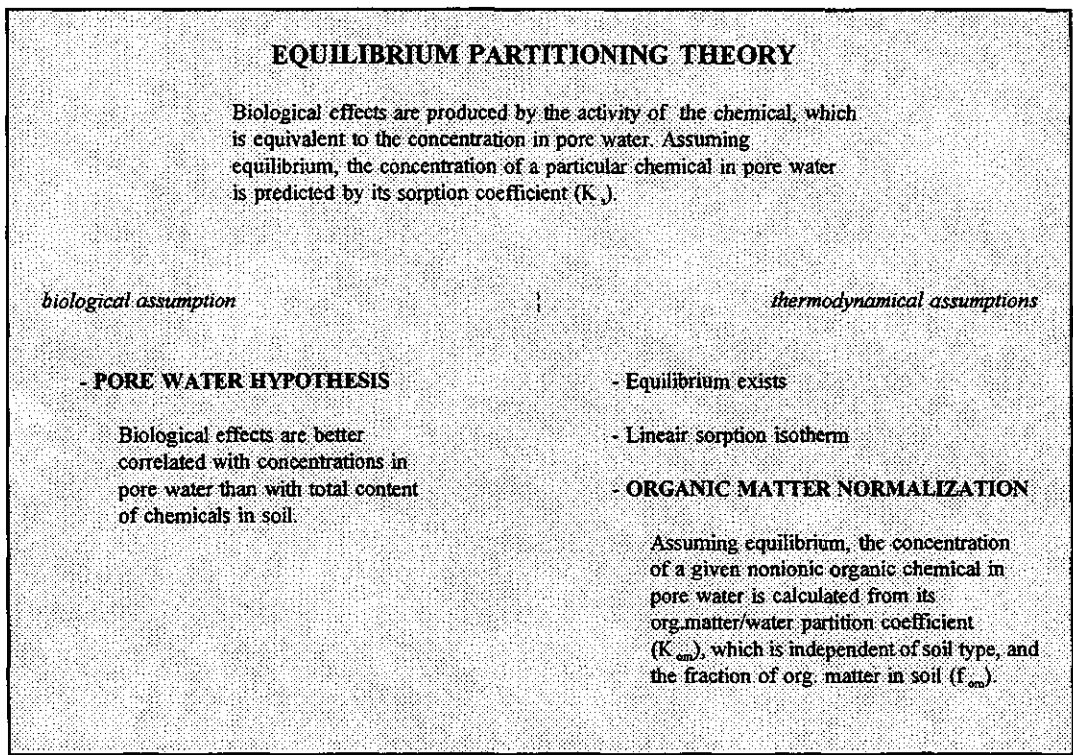


Fig. 2 Relationship between the concepts ‘equilibrium partitioning theory’, ‘pore water hypothesis’ and ‘organic matter normalization’

Organic matter normalization is often referred to in the literature as the ‘equilibrium partitioning theory’ or the ‘equilibrium partitioning approach’. In fact, the equilibrium partitioning theory applies to all contaminants that partition between soil phases (including ionic organic chemicals and metals). Organic matter normalization only applies to non-ionic organic chemicals (Fig. 2). Compared to the equilibrium partitioning theory, organic matter normalization requires additional assumptions, e.g. about the sorption of non-ionic organic chemicals to soil.

3.2 Experimental method for measuring K_{om}

The organic-matter/water partition coefficient, K_{om} , is commonly calculated from K_s values determined in batch equilibration experiments. Batch experiments are carried out by shaking the soil or sediment with an aqueous solution containing the chemical. After a certain equilibration period, typically ranging from a few hours to a few days, the aqueous phase is separated from the soil by centrifugation or filtering and then analysed. The adsorbed fraction is calculated from the decrease of the concentration of the chemical in the aqueous phase. To derive an adsorption isotherm, the experiment is carried out at a fixed temperature, preferably at three or more concentration levels.

3.3 Estimation methods for K_{om}

The organic-matter/water partition coefficient, K_{om} , is frequently predicted from the octanol/water partition coefficient, K_{ow} , because the availability of K_{ow} values is better than that of K_{om} values. Their relationship is often described using regression analysis on various data sets. In many cases, the organic-carbon/water partition coefficient (K_{oc}) is reported instead of K_{om} . K_{oc} values can be converted to K_{om} values by using the conversion factor 1.723 (Gerstl, 1990):

$$K_{om} = K_{oc} / 1.723 \quad (7)$$

The relationships between K_{oc} and K_{ow} are usually expressed in the form:

$$\log K_{oc} = a \log K_{ow} + b \quad (8)$$

where a and b are constants.

DiToro et al. (1991) simplified the relationship by proposing values for a and b close to 1 and 0 respectively, which yields:

$$K_{oc} \approx K_{ow} \quad (9)$$

For eleven pesticides, differing six orders of magnitude in hydrophobicity, they showed the relationship between K_{oc} and K_{ow} to be approximately log linear. So their simplification is very rough.

Gerstl (1990) derived different values for the constants a and b for different chemical groups. The idea behind this is that for chemicals with a similar structure the same type of molecular interactions determine sorption. After studying sorption data on more than 400 chemicals he found that equations for chemical groups are preferred over a general equation for all chemicals. However, he also stated that the presently-used methods for predicting sorption are limited in accuracy and that even experimentally derived K_{om} values are limited in their reliability to describe actual

field behaviour of pesticides. Therefore Gerstl (1990) recommended to measure the sorption whenever possible.

3.4 Phenomena not accounted for in the equilibrium partitioning theory

3.4.1 Nonequilibrium sorption

An important issue is the assumption of sorption equilibrium. Boesten (1991, 1993) reviewed several studies that showed that sorption of pesticides does not reach equilibrium on a time scale of a few days. Sorption coefficients after months or years of chemical-soil contact may be up to 100 times as high as expected from short-term sorption experiments. This may result from an increase in sorption strength on sorption sites or from diffusion of the chemical into soil aggregates. These aggregates, are destroyed during the extraction procedure, thus making the chemical inside available for solvent extraction. If soil invertebrates are not small enough to enter the aggregates, the chemical inside is not bioavailable. The chemical is buried within the soil particles and has become physically inaccessible (Karickhoff & Morris, 1987) for these organisms.

It would be useful if sorption kinetics of organic chemicals in soil could be predicted by mathematical models. Karickhoff & Morris (1985) proposed a two-site model and Boesten (1987) proposed a three-site model to describe sorption kinetics of non-ionic organic chemicals in sediment and soil, respectively. In these concepts, sites of different classes equilibrate at different time scales. Wu & Gschwend (1986) proposed a diffusion model for sorption kinetics of organic chemicals in soil or sediment. They estimated diffusion into particles from the measured particle size distribution and the effective intraparticle diffusion coefficient.

3.4.2 Nonlinear sorption

In many experiments it has been found that adsorption data are described better by using Freundlich's isotherm:

$$X_s = K_f c_L \quad (10)$$

where,

K_f is the Freundlich coefficient ($\text{mg}^{1-N} \text{L}^N / \text{kg}$)

N is a dimensionless exponent (sometimes indicated as $1/n$) describing deviation from linearity.

Reported values for N are mostly in the range from 0.7 to 1.2. Although the Freundlich isotherm has been used as an empirical relationship, it is now supposed to account for heterogeneity of the sorption sites in the soil.

An example of the application of Freundlich's isotherm to estimate the dissolved (bioavailable) fraction of the chemical in pore water is the study of Van Gestel & Ma (1990). They exposed the earthworms *Eisenia andrei* and *Lumbricus rubellus* to chlorophenols in four different soil types for two weeks. The organic matter content of the soils ranged from 3.7 to 15.6% and the pH(H₂O) from 4.3 to 6.2. The *LC50* was expressed on a dry soil mass basis (mg/kg) and on the estimated molar concentrations in the soil pore water (μmol/L). When the *LC50* values were expressed on a soil mass basis, the differences in *LC50* across the different test soils were large. The maximum difference was found for pentachlorophenol: here the *LC50* values for both species differed a factor of 6. Concentrations in the pore water were estimated using Freundlich isotherms. For all combinations of chemicals and soils K_f and N values were derived from batch equilibration experiments. The isotherms were fitted with $r^2 \geq 0.945$, with the N exponent sometimes substantially deviating from unity (range from 0.77 to 1.11). In most cases variation coefficients for both K_f and N values were less than 10%. The *LC50* values based on calculated concentrations of pentachlorophenol in the pore water differed by a factor of 2.3 (*E. andrei*) and 4.4 (*L. rubellus*).

It can be concluded from the study of Van Gestel & Ma (1990) that Freundlich's isotherm accurately describes the sorption of chlorophenols in batch equilibration experiments. By using the isotherms to calculate the pore water effect concentrations in toxicity tests, differences in *LC50* values across the different soils were reduced. However, the difference of a factor of 4.4 in *LC50* values for *L. rubellus* based on calculated concentrations of pentachlorophenol in the pore water is rather large. Possibly, the calculated concentration of the chemical in the pore water did not occur in the soils. The concentrations in the pore water were not checked in this study.

3.4.3 Differences in organic matter

Establishing soil quality criteria according to the ideas described above does not account for the observation that the K_{om} of a particular chemical is not constant for different soils. Gerstl (1990) reported large differences in K_{om} -value for a particular chemical in the literature. The inherent variability of soils and soil organic matter has consequences for their sorption properties.

3.5 Testing the validity of the equilibrium partitioning theory

The equilibrium partitioning theory neglects the contribution of the factors complicating the adsorption of chemicals in soils. Whether this is a problem should be tested in studies on the validity of the equilibrium partitioning theory. If it proves to be valid, it eliminates differences in toxicity across different soils by relating the toxicity to the mass of chemical per mass of soil organic matter (organic matter normalization). Basic data on the intrinsic sensitivity of soil organisms to a certain chemical could be determined by carrying out toxicity experiments in a standard soil

with known organic matter content. Also it would be possible to calculate the bioavailable pore water concentration from the total content of a chemical in soil. This would make a link to the extensive data base available on aquatic toxicity and criteria.

If the assumption of organic matter normalization is not justified, then the concentration of the chemical in the soil pore water should be measured. This requires a pore water isolation technique and subsequent analysis of the isolated pore water. The intrinsic sensitivity of soil organisms to a certain chemical can possibly be determined by carrying out toxicity experiments in a solution of the chemical in saline water.

In the next chapter it is evaluated whether organic matter normalization is able to predict risk of effects on organisms in soils and sediments. It is checked whether measurement of the concentration of chemical in the pore water provides a better explanation and prediction of the effects. Attempts are made to quantify the improvement of these approaches as compared to risk assessment based on the total content of chemical in soil.

4 Evaluating three quality criteria by using literature data

Several studies address the relationship between toxicity at one side and certain criteria indicating biological risk of pesticides on the other side. These criteria (described in chapters 2 and 3) are (i) the total content expressed as mass of chemical per mass of dry soil as determined by solvent extraction, (ii) the organic-matter normalized total content (mass of chemical per mass of soil organic matter) and (iii) the concentration of the chemical in the pore water. In this chapter, studies on exposure to pesticides in both soil and sediment are described. Differences in *LC50* values for different soils were calculated for each criterion by dividing the maximum value by the minimum value, yielding a difference factor. In the same way, soil or sediment *LC50* values based on measured concentrations in pore water are compared to the saline water *LC50* of the chemical. If they are similar, this indicates that the concentration of the chemical in the isolated pore water is relevant to the toxicological effect on the test organism.

It is discussed whether these studies are suitable to validate the method of organic matter normalization. For this purpose, the compiled *LC50* values are presented in a uniform way in Table 1. If the author did not report the *EC50* or *LC50* values, these were calculated from the published mortality data using the modified trimmed Spearman-Kärber method (Hamilton et al., 1977).

Adams et al. (1985) exposed the midge *Chironomus tentans* in three different sediments and in saline water to the pesticide chlordane, for 14 days. Only the larvae of the test species, which was thought to be representative of aquatic benthic invertebrates in North America, were used in the test. The sediment material, collected from Missouri River flood plain (USA), had an organic carbon content of 1.5%. Two other sediment types were derived from this natural sediment by combustion (yielding 0.09% organic carbon content) or adding sheep manure (yielding 12.3% organic carbon content). Before use, the sediments were sieved (710 µm opening) to remove the large particles.

Table 1 shows that if the *LC50* values are expressed on a dry sediment mass basis (mg/kg), the difference across the three test sediments is a factor of 71. If the *LC50* values are normalized for organic matter content, the difference across the test sediments is reduced to a factor of 1.9. If the sediment *LC50* values are based on concentrations measured in the pore water, they differ by a factor of 1.8 across the different sediments. For the exposure in saline water they found an *LC50* that is similar to the pore water effect concentrations: the maximum difference was only a factor of 1.4 (found for the sediment containing 12.3% organic carbon).

It can be concluded from the study of Adams et al. (1985) that the use of organic matter normalized contents as well as the use of concentrations measured in the pore water, considerably reduce the difference in *LC50* values for different sediments, as compared to the *LC50* values on a sediment mass basis.

Table 1 LC50s for pesticides in soil or sediment expressed on the basis of dry mass, organic matter and pore water. LC50s for isolated pore water and water-only exposures are also given. Confidence intervals are in parentheses. Values are based on measured concentrations

Chemical	Soil / sediment	OM ³⁾ (%)	Equil. time (d)	Exposure time (d)	LC50 values for exposure via					Ref ⁷⁾			
					soil/sediment, expressed on the basis of			Pore water ⁴⁾ (µg/L)	Saline water				
					Dry mass (µg/g)	OM ³⁾ (µg/g)	Pore water (µg/L)		(µg/L)		(µg/L)		
chlordecone ¹⁾	sediment	0.0 ₉	-	14	0.58	(0.48-0.70)	644 (533-778)	21 (18-24)	not measured	27 (23-32)	[1]		
		1.5			7.6	(6.7-8.7)	507 (447-580)	35 (30-41)					
		12.3			41	(35-47)	333 (285-383)	19 (16-22)					
DDT	sediment	3.0 7.2 10.5	60	10	11 20 50	(10-13) (16-24) (44-56)	367 (337-423) 272 (217-338) 473 (421-536)	0.9 (0.8-1.0) 1.5 (1.3-1.8) 0.8 (0.7-0.9)	not measured	0.46 ⁶⁾ 0.48 ⁶⁾ 0.52 ⁶⁾	(0.4-0.6) (0.4-0.5) (0.5-0.6)	[2]	
endrin	sediment	3.0 6.1 11.2	60	10	4.4 4.8 6.0	(3.9-5.2) (3.7-6.3) (4.7-7.4)	147 (130-173) 79 (61-103) 54 (42-66)	2.1 (1.8-2.5) 1.9 (1.6-2.4) 1.8 (1.4-2.2)	not measured	4.8 ⁶⁾ 4.4 ⁶⁾ 4.2 ⁶⁾	(4.5-5.2) (3.8-5.1) (3.8-4.8)	[2]	
chlorpyrifos ¹⁾	soil	2.4 8.7	2	2	1.1 2.9	(0.8-1.3) (2.0-3.8)	46 (33-54) 33 (23-44)	4.9 (0.6-9.2) 5.2 (0.7-9.7)	1.6 ⁵⁾ 1.4 ⁵⁾	(1.4-1.6) (1.3-1.6)	0.9	(0.8-1.0)	[3]
PCP ^{1) 2)}	soil	2.4 8.7	2	2	(mg/g) 0.13 0.64	(0.11-0.15) (0.47-0.82)	(mg/g) 5.42 7.36	(mg/L) 2.7 4.1	(mg/L) 3.9 ⁵⁾ 2.4 ⁵⁾	(mg/L) (2.8-5.1) (2.0-2.9)	(mg/L) 2.2	(1.3-3.2)	[3]

1) LC50 values computed from the published data using the modified trimmed Spearman-Kärber method (Hamilton et al., 1977)

2) pentachlorophenol

3) Organic matter content; for the chlordecone, DDT and endrin studies determined as organic carbon

4) Isolated pore water

5) Chemicals added after isolating pore water

6) Done concurrently with sediment tests

7) References:[1] Adams et al., 1985; [2] Nebeker et al., 1989; [3] Houx & Aben, 1993

However, their experimental set-up did not allow to detect any change of sorption (and thus bioavailability) with time. Furthermore it should be noted that the relevance of their results to the actual field situation may have been affected by the method of eliminating the large particles before the tests and by using sheep manure to increase the organic matter content of their sediment.

Nebeker et al. (1989) exposed the amphipod *Hyaletta azteca* to the pesticides DDT or endrin in three different sediments and in a solution in saline water for ten days. The test species is widely distributed in the United States and is an important link in many aquatic food chains. Only the young adults were used in the test. Sediment material was collected from two lakes in Oregon (USA), having organic carbon contents of 3% and 10.5%. The other sediment type was obtained from these natural sediments by mixing (1:1).

Nebeker et al. (1989) found (Table 1) for DDT that if the *LC50* values are expressed on a dry sediment basis (mg/kg), the difference between the different test sediments is a factor of 4.6. If the *LC50* values are normalized for organic matter in the sediment, the difference between the different test sediments is a factor of 1.7. The *LC50* values based on concentrations measured in the pore water differed by a factor of 1.9. The maximum difference found between the *LC50*s based on pore water concentrations and the *LC50* for the exposure in saline water was a factor of 3.3.

For DDT it can be concluded that the use of organic matter normalized *LC50* values as well as the use of *LC50* values on the basis of concentrations measured in the pore water, reduce the difference in *LC50* value, as compared to the *LC50* values on a dry sediment basis. It can also be concluded that *LC50*s based on pore water concentrations are higher than the *LC50* for the exposure in saline water.

For endrin, Nebeker et al. (1989) found (Table 1) that, if the *LC50* values are expressed on a dry sediment basis (mg/kg), the difference between the different test sediments is only a factor of 1.4. If the *LC50* values are normalized for organic matter in the sediment, the difference between the different test sediments is a factor of 2.7. The *LC50* values based on concentrations measured in the pore water differed by a factor of 1.2. The maximum difference found between the pore water effect concentrations and the *LC50* for the exposure in saline water was a factor of 2.7. In this case the *LC50*s for pore water were lower than those for exposure in saline water.

The *LC50*s of endrin on a dry sediment basis do not indicate differences due to sediment organic matter content. Remarkably, organic matter normalization increased the differences in *LC50*s of endrin for the different sediments. Nebeker et al. (1989) concluded that the endrin data do not justify organic matter normalization. It should be stated, however, that the difference found for the organic-matter normalized *LC50*s of endrin is comparatively small: a factor of 2.7. Differences of this size (factor of 3) are commonly encountered in toxicity tests carried out at different times. For the same reason, also the *LC50*s based on pore water concentration and the *LC50* for the exposure in saline water can be denoted as similar.

Nebeker et al. (1989) did not carry out experiments with other equilibration times in order to detect changes in bioavailability with time.

Houx & Aben (1993) exposed the nematode *Globodera rostochiensis* in three different soils and in saline water to the pesticides chlorpyrifos or pentachlorophenol. The organic matter content of the soils ranged from 2.4 to 8.7% and the pH (1M KCl) from 4.7 to 6.7. Also, Houx & Aben (1993) exposed their test organisms in isolated pore water. However, they added the test chemicals *after* isolating pore water from untreated soil. Possibly this was done to check whether the concentration measured in pore water is fully bioavailable to the organisms exposed in it.

If the *LC50* values for chlorpyrifos are expressed on the basis of dry soil mass (mg/kg), the difference between the two soils was a factor of 2.6 (Table 1). If organic matter normalization is applied to their data, the difference in *LC50s* for the test soils is reduced to a factor of 1.4. If the *LC50* values are based on the concentration measured in the pore water, they differed a factor of 1.1. The experimental variation in the soil *LC50s* on a pore water basis for chlorpyrifos was too large to justify comparison with the saline water *LC50*. The *LC50s* for the exposure in isolated pore water was at the same level as the saline water *LC50*: the maximum difference was only a factor of 1.8.

For pentachlorophenol Houx & Aben (1993) found that if the *LC50* values are expressed on the basis of dry soil mass (mg/kg), the difference for the test soils was a factor of 4.9 (Table 1). If organic matter normalization is applied, the difference in *LC50s* for the test soils is reduced to a factor of 1.4. If the *LC50* values are based on the concentration measured in the pore water, they differed a factor of 1.5. Moreover, they found that on a pore water basis the soil *LC50s* for pentachlorophenol were at the same level as the saline water *LC50*: the maximum difference was only a factor of 1.9. Also the *LC50s* for the exposure in isolated pore water was at the same level as the saline water *LC50*: the maximum difference was only a factor of 1.8.

It can be concluded from the study of Houx & Aben (1993) that, for both compounds, the use of organic matter normalized *LC50* values as well as the use of *LC50* values on the basis of concentrations measured in the pore water, reduce the difference in *LC50* value, as compared to the *LC50* values on a dry soil basis. For pentachlorophenol it can be concluded that *LC50s* based on pore water concentrations and the *LC50* for the exposure in saline water are similar. They showed also that the concentration measured in pore water is fully bioavailable to the organisms exposed in it. They did not continue their experiments in order to detect changes in bioavailability with time.

It seems that there are shortcomings in the studies mentioned so far.

(i) The equilibration times were not varied; therefore changes in bioavailability with time could not be traced so the validity of the assumption of equilibrium could not be checked.

(ii) It was not checked whether the isolated fraction of pore water is representative of the fraction that is available to the test organisms in soil. This could be done by comparing the *LC50* for exposure in soil (or sediment) to the *LC50* found for the organisms exposed to the isolated pore water: they should be at the same level if the isolated pore water is representative.

(iii) Except for the study of Houx & Aben (1993), the full bioavailability of the chemical in the isolated pore water was not checked. This could be done by comparing the *LC50* values for the organisms exposed in saline water to the *LC50* values for organisms exposed to isolated pore water: If the chemical in the isolated pore water is fully bioavailable they would be at the same level.

(iv) Except for the study of Adams et al. (1985) the differences between soil or sediment types were too small. The organic matter contents should differ by at least a factor of ten and the materials should be from different origins in order to let the test be representative for the field situation. Adams et al. (1985) artificially increased the organic matter content of their sediment, which may have affected its representability.

In the next chapter a study is described, which meets the requirements for a proper test of soil or sediment risk assessment methods.

5 The influence of time on bioavailability

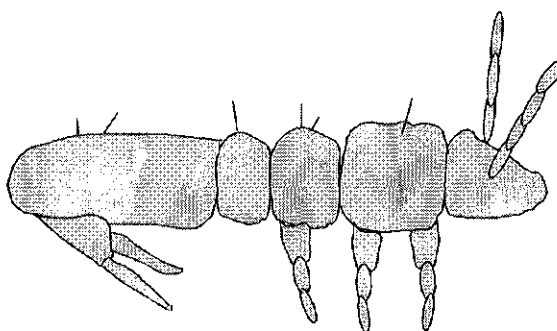
In the studies mentioned in Table 1 the influence of time on the bioavailability, and thus on the *LC50* value, has not been assessed. Bioavailability may be reduced as sorption of pesticides to the soil increases during prolonged chemical-soil contact time (Section 3.4). In order to investigate the influence of time on bioavailability and to assess the suitability of the equilibrium partitioning theory to predict toxicological effects, we carried out a study with increasing chemical-soil contact time (Ronday et al., in prep)

5.1 Experimental set-up

We applied the pesticides parathion or carbofuran to batches of three natural soils differing in organic matter content and texture: a loamy soil, a humic sandy soil and a peat soil. The soil material was collected from 5-30 cm depth at three different grassland sites near Wageningen (The Netherlands) and sieved through a 5 mm sieve. The organic matter contents were 1.6, 4.0 and 23.3%, respectively. The soil batches were incubated for at least 150 d at 15 °C. About once every month during the time of incubation, samples were taken for chemical analyses and toxicity tests.

Parathion was extracted from the soil samples using acetone as a solvent. The acetone was removed by evaporation at reduced air pressure and water was added to the residue. After the aqueous solution had been added to a C18 reversed-phase column, parathion was eluted using methanol. Water was added to the methanol eluents and the solution was analysed using HPLC. The same method was used for extracting carbofuran, except for the use of methanol instead of acetone.

Fig. 3 *Folsomia candida*



We exposed the springtail *Folsomia candida* (Fig. 3) to the pesticides in the soil samples, in isolated pore water and in a solution in saline water for two days. *Folsomia candida* has been selected because this species was found to be sensitive to a range of pesticides (Houx et al., in prep.) and it has proven to be a suitable test species for soil quality assessment using soil pore water (Ronday & Houx, in prep). Only juvenile animals (three weeks old) were used in the test.

A method was needed to isolate soil pore water in sufficient volumes for subsequent toxicity tests and chemical analysis using HPLC. The isolated solution must closely approximate soil solution composition in situ. In general, methods for recovery of relatively large volumes of pore water from relatively dry soils, without dilution, are limited to centrifugation (Annex). However, if pore water is collected by centrifugation and subsequent filtration, the effect on the composition of the solution (and thus the toxicity) need to be considered. Filtration with glass fibre, plastic or paper filters could remove pesticides by adsorption. In our study centrifuge tubes and filters were developed entirely made out of stainless steel and suitable for high speed centrifugation (Ronday, in prep.) (see Annex).

5.2 Results

The calculated *EC50* values and 95% confidence intervals of the soil exposures of *Folsomia candida* are summarized in Table 2. They are expressed on the basis of dry soil, organic matter and pore water, respectively. The *EC50*s of the isolated pore water and saline-water exposures are also given in Table 2.

The parathion data show that a high soil organic matter content induces high *EC50* values on the basis of parathion content in soil. If the *EC50* values are normalized for organic matter in the soil, the difference between the test soils is reduced (Table 2).

The comparison of *EC50*s for *Folsomia candida* exposed in soil and exposed in the isolated pore water (Table 2) indicates that both methods yield similar pore water effect concentrations. The soil *EC50*s on a pore water basis for parathion (0.8-2.6 µg/L) and carbofuran (51-131 µg/L) are similar to the pore water *EC50*s of 1.1-2.0 µg/L and 46-58 µg/L respectively. Apparently, the fraction of pore water that was isolated using the centrifugation technique is representative of the fraction that is available to the test organisms in soil.

Table 2 EC50, 96h (immobility) of parathion and carbofuran for juvenile *Folsomia candida* in soil, pore water and saline water exposures. The soil EC50s are expressed on the basis of dry soil, soil organic matter and pore water, respectively. Confidence intervals are in parentheses. The values were computed using the modified trimmed Spearman-Kärber method (Hamilton et al., 1977)

Chemical	Soil	OM1 (%)	Equil. time (d)	Exposure time (d)	EC50 values for exposure via					
					soil, expressed on the basis of			Pore water (µg/L)	Pore water 2) (µg/L)	Saline water (µg/L)
					Dry mass (µg/g)	OM1 (µg/g)	Pore water (µg/L)			
parathion	loam	1.6	2	4	0.039	(0.036-0.042)	2.44	(2.26-2.64)	1.0	(1.0-1.1)
			37		0.116	(0.104-0.130)	7.27	(6.50-8.14)	0.8	(0.7-0.9)
			55		0.205	(0.195-0.216)	12.8	(12.2-13.5)	-	
			83		> 0.150		> 9.38		> 0.3	
	sand	4.0	2	4	0.252	(0.185-0.244)	6.30	(5.44-7.30)	1.6	-
			26		0.171	(0.151-0.194)	4.27	(3.77-4.84)	1.3	(1.2-1.4)
			81		0.183	(0.158-0.212)	4.58	(3.95-5.31)	1.6	(1.4-1.8)
			144		0.496	(0.439-0.561)	12.4	(11.0-14.0)	1.2	(1.0-1.5)
			248		0.304	(0.270-0.342)	7.59	(6.74-8.55)	2.2	(1.8-2.7)
	peat	23.3	2	4	0.343	(0.302-0.389)	1.47	(1.29-1.67)	1.4	(1.3-1.5)
			19		1.688	(1.526-1.867)	7.24	(6.55-8.01)	1.2	(1.0-1.4)
			34		1.714	(1.535-1.913)	7.35	(6.59-8.21)	2.3	(2.0-2.6)
			98		1.262	(1.130-1.410)	5.42	(4.85-6.05)	2.6	(2.3-2.8)
			124		1.542	(1.368-1.738)	6.62	(5.87-7.46)	2.3	(2.0-2.8)
			153		1.819	(1.574-2.102)	7.81	(6.76-9.02)	2.4	(2.1-3.0)
carbofuran	sand	4.0	2	4	0.091	(0.074-0.111)	2.26	(1.85-2.77)	131	(111-154)
			29		0.057	(0.051-0.064)	1.43	(1.28-1.60)	78	(67-92)
			103		0.072	(0.061-0.085)	1.80	(1.52-2.14)	105	(82-133)
			159		0.053	(0.047-0.060)	1.33	(1.18-1.50)	51	(41-62)
			194		0.079	(0.071-0.088)	1.97	(1.77-2.19)	78	(68-90)
1) Organic matter content; 2) Isolated pore water										

Isolated pore water *EC50* values also correlate well to the saline-water *EC50*s of 2.5 µg/L for parathion and 63 µg/L for carbofuran (Table 2). This indicates that the concentration measured in the isolated pore water is available for uptake by the organisms and that sorption onto dissolved organic material is low.

The differences in the range of toxicity per soil type are visualized in Fig. 4. Here the *EC50*s for parathion in soil on a dry soil mass basis, on an organic matter basis and on a pore water basis, all derived from Table 2, are presented. It demonstrates the efficacy of using effect concentrations based on the concentration of the chemical measured in the pore water to reduce (i) soil-to-soil differences and (ii) the large variation within soils. On a dry soil mass basis the biological response of parathion as measured by *EC50* differs by approximately a factor of 50, whereas on an organic matter basis the *EC50*s exhibit a tenfold range. On a pore water basis the biological response of parathion between different soils and different points of time differs by only a factor of three.

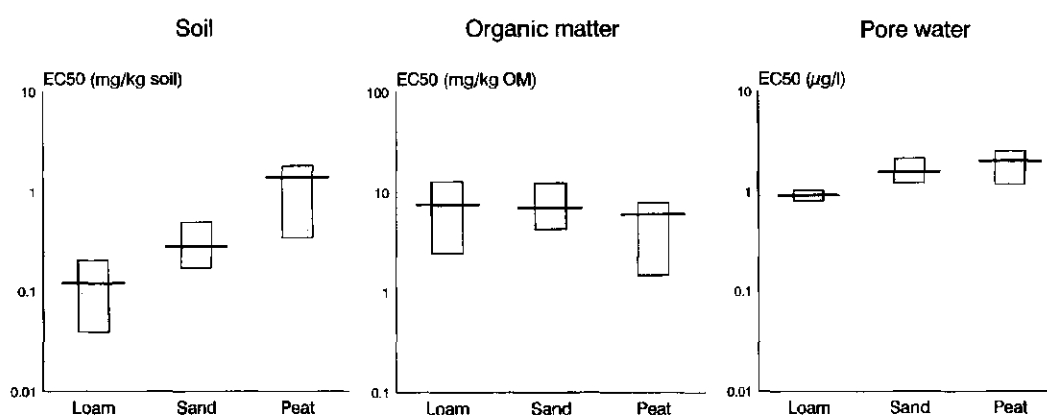


Fig. 4 Soil type dependency of the effect concentration (*EC50*) of parathion. The *EC50*s are expressed on the basis of dry soil (left), soil organic matter (OM) (middle) and the concentration measured in the isolated pore water (right). Boxes represent highest and lowest values, horizontal lines represent mean values measured by tests between 2 and 248 d after application of parathion

The greater within-soil variation of parathion *EC50*s on a dry soil mass basis, which cannot be removed by organic matter normalization, is to a great extent caused by reduced bioavailability of the chemical with time. A decrease in bioavailability of a factor of five was found in two of the four soil-chemical combinations investigated. This occurred within days (peat soil) or months (loamy soil) after application (Table 2, *EC50* dry mass). The influence of time on bioavailability is visualized in Fig. 5, in which the persistence of parathion and carbofuran in the four chemical-soil combinations is plotted. The persistence is expressed here in three ways, in view of to the soil quality assessment methods under test in this study:

- (i) the decrease in the content of the chemical with time, expressed on a dry soil mass basis;
- (ii) the decrease in the concentration of the chemical in the pore water with time;
- (iii) the decrease of the toxicity to the test organisms exposed to the treated soil.

The remaining quantity of the chemical in the soil and the pore water (i and ii) is expressed as percentage of the initial concentration. Both methods of expressing persistence are compared with the decrease in the toxicity to *Folsomia candida* with time. The soil EC_{50} values found for the exposures from one to four days after application (referred to in Table 2 as 2 d after application) were set at 100%. Persistence expressed on an organic matter basis would yield a line identical to the 'soil dry mass' line (i). So in persistence studies, organic matter normalization can not be used to improve the performance of the solvent extraction data.

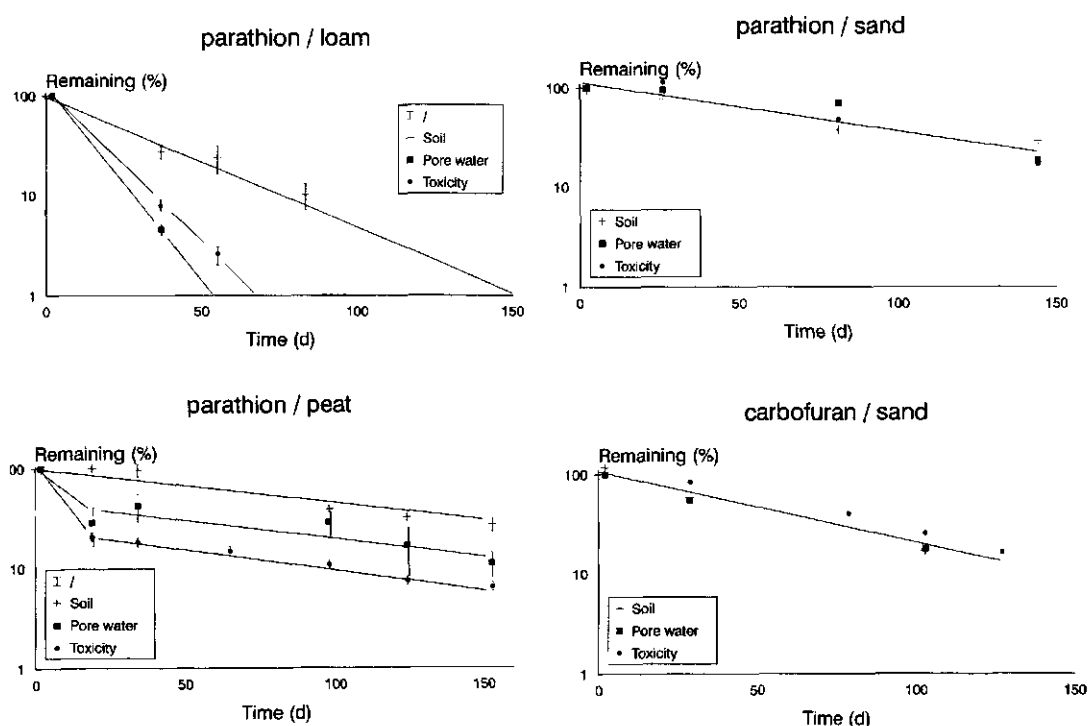


Fig. 5 Persistence measured by tests between 2 and 150 d after application of parathion and carbofuran to selected soil types, on total soil and pore water basis. Persistence is compared with the decrease in soil toxicity (EC_{50} , immobility) to *Folsomia candida*. High/low values are standard errors (soil and pore water; $n=4$) or 95% confidence intervals (toxicity)

It shows from Fig. 5 that in the case of parathion-sand and carbofuran-sand all methods described persistence in the same way.

For the parathion-loam combination the decrease in toxicity corresponds most to the decrease in the parathion concentration in the pore water. There is a considerable difference in decline between the parathion concentration in the pore water and its toxicity to *Folsomia candida* on the one hand, and parathion content in soil on the other. This phenomenon is probably caused by diffusion of parathion into soil aggregates, where it is still available to solvent extraction but not to the test organisms and to the pore water to be isolated.

For the combination of parathion with peat soil, a fast decrease in toxicity is observed during the first few days followed by a slower decrease. A fast initial decrease is also found in the pore water concentration, but not in the total soil content. It seems that here the rate of diffusion of parathion into soil particles is higher than in the case of the loamy soil.

The decrease in the pore water concentration resembles best the decrease in toxicity. However, the fast initial decrease in the parathion concentration in the pore water, is not quite as large as the decrease in toxicity. Consequently, although disappearance rates are the same after some time, a difference in level remains. This could be explained by the fact that toxicity cannot be measured on a certain point of time, whereas the other two parameters can. Organism are exposed over a period of time, in this study 96 h. Probably the concentration of parathion in the pore water during the first day of exposure was much higher than during the second day, when the pore water was isolated and analysed. This could have enhanced the occurrence of toxicological effects, resulting in a relatively high initial (100%) toxicity level.

It seems that persistence is best determined by measuring the decrease in the concentration of the chemical in pore water rather than measuring total content of the chemical in soil by solvent extraction, if toxicological effects are import.

6 General discussion and conclusions

6.1 The influence of soil type on bioavailability

The effect concentration of a chemical on a dry soil basis showed a large variation between different soils. This leads to a range of effect concentrations found for a specific chemical across different soils, which makes it difficult to set threshold limit values with general applicability and validity. So the content of the chemical in soil, expressed on a dry soil basis, does not seem to be a suitable parameter for determining biological risk.

Differences in biological effect concentrations for a particular chemical between different soils may be caused by a varying bioavailability, the crucial factor which links chemical content in soil and biological effects. These differences were considerably reduced if the biological effect concentrations were expressed as mass of chemical per mass of soil organic matter. This method is called organic matter normalization. The relevance of the partitioning of chemicals between soil organic matter and pore water to bioavailability, as suggested by the equilibrium partitioning theory, was demonstrated by this study.

Differences in biological effect concentrations for a particular chemical between different soils were further reduced if they were expressed as the pesticide concentration measured in the soil pore water. This parameter was found to correspond strongly with toxicological effects on organisms irrespective of soil composition.

It can be concluded that the equilibrium partitioning theory is useful for a first estimation of risk of pesticides in soil. It accounts for differences in bioavailability between soils and is therefore to be preferred to the traditional method of describing the extent of contamination in terms of the total content of the chemical in the soil.

6.2 The influence of time on bioavailability

Bioavailability was found to be not only a function of soil composition but also a function of time. A distinct decrease (a factor of five) in bioavailability was found in two of the four soil-chemical combinations investigated. This occurred within days (peat soil) or months (loamy soil) after application. So the assumption of the equilibrium partitioning theory that the bioavailable fraction of the chemical is constant with time and can be calculated using one particular partition coefficient, could not be justified by studying the dynamic situation shortly after pesticide application. As a consequence, concentrations in pore water calculated from measurements of chemical content and from the soil to pore water partition coefficient obtained in short-term (24 h) laboratory experiments, may overestimate real concentrations on a longer term. This prevents a confident assessment of risk. Until

better methods for predicting concentrations in pore water are developed, these concentrations should preferably be measured.

6.3 Practical implication for soil quality criteria

For evaluating the risk of pesticides in soil, concentrations measured in pore water should be compared with soil quality criteria based on the saline-water effect concentrations for soil organisms derived in the laboratory. Besides springtails, also other species of soil-inhabiting invertebrate like earthworms, potworms and probably mites can be used for this purpose (Ronday & Houx, in prep).

A threshold limit value for a specific chemical could be derived by:

threshold limit value (mg/L in pore water) = saline-water *EC50* (mg/L) * safety factor

The *EC50* used in the equation could be the lowest of the *EC50*s for three organisms from different taxonomic groups. A first safety factor could be used to extrapolate *EC50* values to *NOEC* values. A second safety factor could be used to extrapolate from the laboratory organisms to more sensitive organisms in the field. Of course other approaches based on saline-water effect concentrations are also possible.

Saline-water effect concentrations for soil organisms are scarce. A full record is needed on the saline-water toxicity of pesticides to at least one sensitive soil organism, like for instance the springtail *Folsomia candida*. In the meantime possibly the large acute toxicity database for aquatic organisms could be used if equal sensitivity of terrestrial and aquatic species can be assumed. This assumption should be tested in further studies.

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Annex

Obtaining soil pore water

Soil pore water can be sampled under field conditions as well as in the laboratory. Wolt (1993) reviewed the use of pore water isolation techniques for contaminant fate analysis. He concluded that reproducibility in the laboratory is often greater than in the field. The principle of the laboratory techniques is that the pore water is displaced from soil by another phase. Displacing phases that have been employed for obtaining soil pore water are water, immiscible liquid (like CCl_4) or air. However, immiscible liquid can not be used for nonpolar chemicals like many pesticides, because they dissolve in the liquid. Pore water is isolated by exerting force on the pore water in-situ. This can be done using gravity, using a vacuum extractor, by squeezing or by centrifugation.

Column displacement

Gravity is used in column displacement, which was employed by Adams et al. (1980). They poured moist soil into a 60 by 3 cm glass column. The column had a drain at the bottom, plugged with glass wool. After filling, the soil was compacted and a collection tube was attached to the column. Then, about 100 mL of displacing solution was added atop the column. Adams et al. (1980) concluded in their study that the column displacement technique requires some skill for its successful application and that it is not well-suited for sands and loamy sands. Sands could not be packed tight enough to prevent early break-through of the displacing solution. As water flow will be often uneven, the obtained pore water is easily mixed with the displacing solution.

Vacuum displacement

Wolt & Graveel (1986) used vacuum displacement as a method for obtaining soil pore water. They packed 75 g of moist soil in a syringe barrel containing an triacetate filter membrane. A volume of 9 mL of displacing solution was put on top of the packed soil. Pore water was obtained using a mechanical vacuum extractor. The average volume of pore water isolated was 7 mL per 100 g of moist soil. The technique used by Wolt & Graveel (1986) is rapid and simple. However, the method is not suitable for nonpolar organic pesticides. They will easily adsorb to the syringe and filter. Furthermore, the obtained pore water is easily mixed with the displacing solution: an inherent risk of this method.

Squeezing and centrifugation

In general, methods for recovery of relatively large volumes of pore water, without dilution, are limited to either squeezing or centrifugation. If the soil volume can be significantly reduced (e.g. peat), the efficiency of squeezing will be high. However, squeezing methods are not appropriate for sandy or relatively dry soils.

Using a centrifugation technique, the efficiency depends on capillary forces and thus on pore size distribution of the soil. The efficiency is relatively large in sandy soils: here the capillary forces are weak. Adams et al. (1980) successfully used a centrifugation method for obtaining pore water from soils ranging in texture from loamy sand to clay. Their centrifuge tubes consisted of a soil container and a collection cup divided by a filter paper. Soils were centrifuged for 2 h at 1070 g force. The method required no special skills and it worked for all soils. The pore water volume obtained was rather low: 0.1 to 3 mL per 100 g of moist soil. This is probably due to the relatively low speed of centrifugation. Their centrifuge tubes were made of Plexiglass, which easily adsorbs nonpolar organic pesticides.

Development of an improved centrifugation technique

If pore water is collected by centrifugation and subsequent filtration, the effect on the composition of the solution (and thus the toxicity) needs to be considered. Filtration with glass fibre, plastic or paper filters could remove pesticides by adsorption. In our study centrifuge tubes and filters were made entirely of stainless steel (Ronday, in prep.). They are suitable for high speed centrifugation and they make it possible to recover a relatively large volume of water with minimum loss of pesticide. These large volumes are necessary if the pore water has to be tested for its toxicity or if it has to be concentrated to lower the detection limit in chemical analysis.

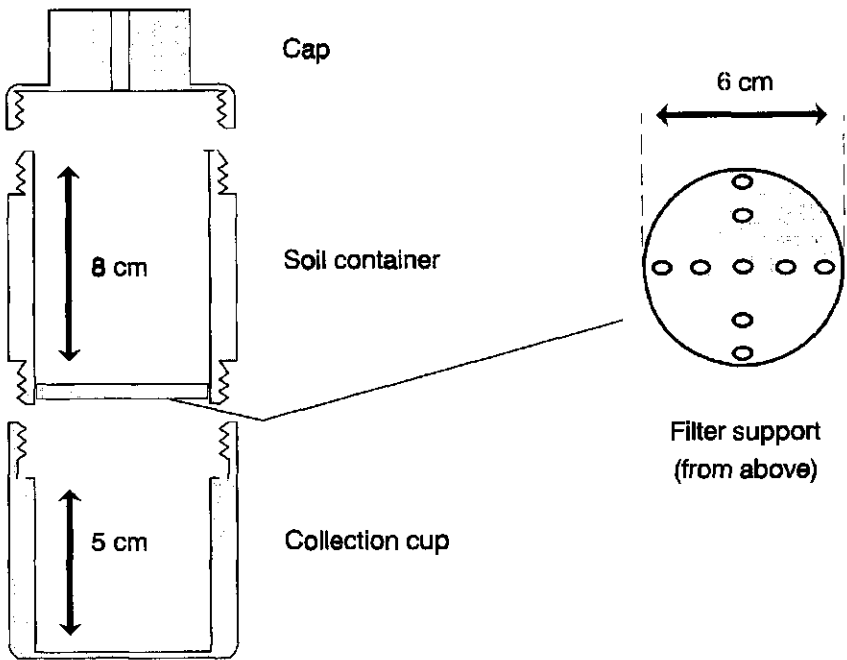


Fig. A1 All stainless steel centrifugation tube to obtain soil pore water. After a stainless steel filter is fitted in the filter support, the soil container is filled with 120 g of moist soil. The tube is centrifuged typically at 9000 rotations min⁻¹ (13 000 g force at filter). The pore water is collected from the collection cup

The centrifuge tubes (Fig. A1) consisted of two compartments divided by a stainless steel filter (mesh-width 17.6 µm). The top compartments or soil containers (volume 200 mL) were filled with 120 g of moist soil and centrifuged at 13 000 g force (at

the filter) for 50 min. Soil pore water was collected from the bottom compartments or collection cups. The yield was improved by lining the inner surface of the tube with a stainless steel small-mesh wire-netting to facilitate water flow. The procedure recovered 15-30% of the pore water contained by the moist (pF 2) soils, this is 6-10 mL pore water per tube, depending on the soil type (Fig. A2).

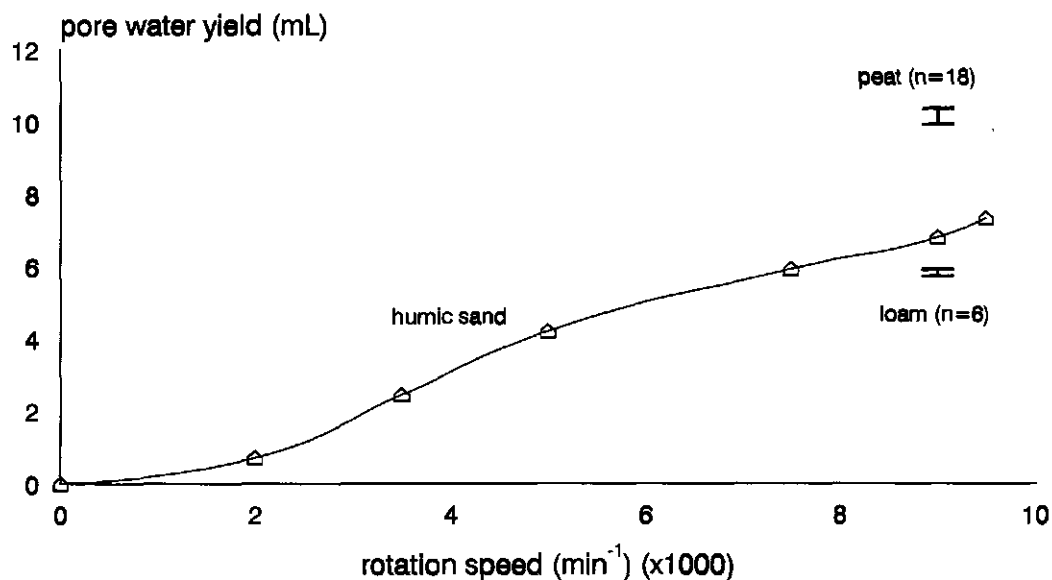


Fig. A2 Volume of pore water isolated from moist soil (pF 2) using the improved centrifugation method, as a function of rotation speed and soil type