Research Articles

Modelling the Accumulation of Hydrophobic Organic Chemicals in Earthworms

– Application of the Equilibrium Partitioning Theory

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Abstract

In this paper a method is developed which can be used to estimate the body burden of organic hydrophobic chemicals in earthworms. In contrast to the equilibrium partitioning theory, two routes of uptake are incorporated: uptake from interstitial water and dietary uptake. Although many uncertainties still remain, calculations show that for earthworms steady state body burdens are mainly determined by uptake from interstitial water. Under most circumstances, the contribution of dietary uptake is small, except for hydrophobic chemicals (log $K_{OW} > 5$) in soils with a high organic matter (OM) content of $\approx$ 20 %. Under those conditions, estimates of the steady state body burden calculated with the equilibrium partitioning model, in which only uptake from interstitial water is taken into account, might result in a small underestimation of the real body burden of chemicals in earthworms.

Key words: Hydrophobic organic chemicals; accumulation of chemicals in earthworms; equilibrium partitioning theory; biomonitoring; earthworms, uptake of chemicals from interstitial water; earthworms; dietary uptake of chemicals; exposure models; soil ecosystems; risk assessment; toxicity tests

1 Introduction

Earthworms are able to accumulate numerous different hydrophobic organic chemicals from soil, which may affect their survival, reproduction and development. The accumulation of chemicals in earthworms also implies a risk for various vertebrate species feeding on earthworms (COOKE et al. 1992). To assess the potential risk of chemicals for soil ecosystems and for the establishment of soil quality criteria, single species toxicity tests are carried out. This strategy is followed for several **new** and **existing chemicals**. However, as the number of existing chemicals on the European Inventory (EINECS) is estimated at around 100,000, it is unrealistic to expect that necessary data for risk assessment will become available in the near future. Estimation methods, such as by Quantitative Structure Activity Relationships (QSARs), may be used to fill these data gaps if they are used with great care and within their limitations (HERMENS 1990; VERHAAR et al. 1992; Van LEEUWEN et al. 1992).

The equilibrium partitioning method (SHEA 1988; DiTORO et al. 1991; Van Der KOOIJ et al. 1991; OECD 1992) has been developed to estimate the steady state body burdens of organic hydrophobic chemicals in organisms in sediment. The model assumes that the concentration of a chemical in an organism, and therefore toxicity, is solely determined by the concentration in the pore- or **interstitial water**. Using the concentration in the interstitial water calculated from adsorption data, the concentration in the organism can be predicted. Since adsorption to sediment particles is strongly determined by the hydrophobicity (log K_{OW}) of the compound, the estimation of the concentration in the organism is largely based on log K_{OW}.

The equilibrium partitioning model is based on the assumption that only the **dissolved fraction** of the chemical in interstitial water is available for uptake by passive diffusion. In **soil**, this hypothesis is supported by results of Van GESTEL and MA (1988, 1990). They observed a considerable variation in LC_{50} values of a chemical in the earthworms *Eisenia andrei* and *Lumbricus rubellus* after exposure in different types of soil. This variation could be reduced by normalization of the LC_{50} values to the concentration in interstitial water. This result indicates that the concentration of chemicals in soil interstitial water determines the toxicity for earthworms, suggesting that this route of uptake is of major importance. The relevance of the equilibrium partitioning model was also shown in the sediment water system for *Chironomus tentans, Daphnia magna,* oligochaete worms and several other organisms (DERR and ZABIK 1974; ZIEGENFUSS et al. 1986; KNEZOVICH and HARRISON 1988; OLIVER 1987; DiTORO et al. 1991).

However, our results showed that earthworms are able to **accumulate chemicals from food** indicating the existence of **dietary uptake** as another route of exposure (BELFROID et al. 1994a). For a few aquatic species, as the oligochaete worm *Lumbriculus variegatus* and the polychaete *Abarenicola pacifica*, it was even shown that dietary uptake of hexachlorobenzene and benzo(a)pyrene, respectively, is of major importance (SCHUYTEMA et al. 1988; WESTON 1990). It is not known whether dietary uptake is an important route

of exposure for earthworms. Consequently, to evaluate the applicability of the equilibrium partitioning theory for the estimation of body burdens in earthworms, it is important to determine and quantify the role of dietary uptake in the uptake process.

In this paper a model for the prediction of the accumulation of inert hydrophobic organic chemicals in earthworms is presented. Although the estimation of the parameters necessary for the model is based on only few experimental data, we believe that this approach can be a valuable tool for the evaluation of the equilibrium partitioning model. Unlike the equilibrium partitioning model, two routes of uptake are incorporated: 1) uptake by passive diffusion from the interstitial water and 2) dietary uptake. The model can be used to estimate steady state concentrations of chemicals in earthworms, but also to determine the relative contribution of the two routes of uptake to the total body burden. It is shown that the relative contribution depends on the **hydrophobicity** of the chemical and on the **type of soil**.

2 Modelling of Accumulation

2.1 Model

Terrestrial species like earthworms are able to accumulate and eliminate hydrophobic organic chemicals from soil. Two main routes of **uptake** exist:
1. Uptake of chemicals may take place by **passive diffusion** through the body wall of the earthworm (LORD et al. 1980; Van GESTEL and MA 1988, 1990 and BELFROID et al. 1993a). Only the dissolved fraction of the chemical in the interstitial water layer of the soil is taken up via this route.
2. Hydrophobic chemicals are also taken up through the gut wall when soil (particles) pass the gut (BELFROID et al. 1994a, b), which is called **dietary uptake**.

Several **elimination routes** exist:
— Most important, probably, is the elimination of chemicals by passive diffusion through the body wall and the gut of the earthworm.
— Another important route of elimination is biotransformation. Many chemicals are subject to biotransformation in earthworms (STENERSEN 1984), resulting in a loss of the original chemical.
— Growth dilution also accounts for a decrease in body burden. Young and subadult worms, but sometimes also adult worms, can grow. Growth results in a dilution and therefore in a decrease of the concentration of the compound in the worm.
— The last route of elimination discussed here is elimination because of reproduction. As all species, earthworms reproduce, resulting in offspring. In fish, transfer of PCBs from parent guppies to their offspring was observed by SIJM et al. (1992). To our best knowledge, however, the possible transfer of hydrophobic chemicals from the parent earthworm to the offspring has not been investigated.

It is assumed that all these uptake and elimination processes follow first-order one-compartment kinetics, except for the
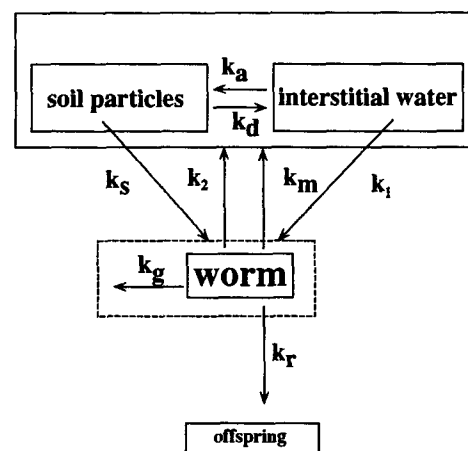

Fig. 1: Schematic representation of the relations between soil particles, soil interstitial water and the earthworm. (k_d is the desorption rate constant, k_a is the adsorption rate constant, k_s the dietary uptake rate constant, k_1 the uptake rate constant from interstitial water, k_2 the elimination rate constant, k_m the metabolic rate constant, k_g the growth rate constant and k_r the reproduction rate constant)

reproduction. The several uptake and elimination routes are visualized in **Fig. 1** and can be described with Equation 1:

$$\frac{dC_{worm}}{dt} = k_s C_p + k_1 C_{iw} - (k_g + k_2 + k_m)C_{worm} - R k_r \qquad (1)$$

in which C_{worm} is the concentration in the worm ($g.g^{-1}$), k_s the uptake rate constant from soil particles ($g.g^{-1}.day^{-1}$), C_{iw} the concentration in the interstitial water ($g.ml^{-1}$), k_g the growth rate constant (day^{-1}), k_2 the elimination rate constant (day^{-1}), k_m the metabolic rate constant (day^{-1}), R a trigger value which is 1 or 0, depending on the occurrence of reproduction and k_r the zero order reproduction rate. Reproduction will not be taken into consideration in this model and R is assumed to be zero.

The uptake rate constant from soil particles k_s is determined by the amount of soil consumed by the animal per time span, the feeding rate (f in $g.g^{-1}.day^{-1}$), and by the efficiency of the extraction of the compound from the food, the uptake efficiency (E):

$$k_s = E * f \qquad (2)$$

Substituting Equation 2 in Equation 1 results in:

$$\frac{dC_{worm}}{dt} = EfC_p + k_1 C_{iw} - (k_g + k_2 + k_m)C_{worm} \qquad (3)$$

When steady state is reached, $dC_{worm}/dt = 0$ and uptake from the soil and interstitial water equals elimination:

$$EfC_p + k_1 C_{iw} = (k_g + k_2 + k_m)C_{worm} \qquad (4)$$

which can also be described as:

$$C_{worm}(ss) = \frac{EfC_p}{(k_g + k_2 + k_m)} + \frac{k_1 C_{iw}}{(k_g + k_2 + k_m)} \qquad (5)$$

Assuming an one compartment model and first order kinetics, the three elimination rate constants $(k_g + k_2 + k_m)$ can be substituted by the total elimination rate constant k_e resulting in Equation 6:

$$C_{worm}(ss) = \frac{EfC_p}{k_e} + \frac{k_1C_{iw}}{k_e} \qquad (6)$$

The distribution of a chemical in soil between interstitial water and soil particles at steady state is described with the soil water partition coefficient K_p.

$$K_p = \frac{C_p}{C_{iw}} \qquad (7)$$

Substituting Equation 7 in Equation 6 results in:

$$C_{worm}(ss) = \frac{EfC_p}{k_e} + \frac{k_1C_p}{K_pk_e} \qquad (8)$$

The parameter C_p is calculated from the concentration in soil C_s minus the amount in the interstitial water C_{iw}. For hydrophobic compounds with log $K_{ow} > 2$, the C_{iw} will be small compared to C_s (BRIGGS 1990). Therefore it is stated that $C_p \approx C_s$:

$$C_{worm}(ss) = \frac{EfC_s}{k_e} + \frac{k_1C_s}{K_pk_e} \qquad (9)$$

first term describes dietary uptake of the chemical from soil while the second term describes uptake from interstitial water. When all parameters in Equation 10 are known, including C_s, it is possible to estimate body burdens of the chemical in the earthworm. When C_s is unknown, the relative contribution of the two routes of uptake to the total body burden can be calculated, since C_s appears in both terms of Equation 10.

2.2 Bioconcentration Factor

The bioconcentration factors on a wet weight basis (BCF_w) are calculated by dividing the concentration of the chemical in the organism by the concentration of the chemical in water. BELFROID et al. (1993a) studied the bioconcentration of chlorobenzenes in earthworms in water (→ Table 1) and calculated a QSAR describing the relationship between BCF_w and log K_{ow}:

$$logBCF = 1.05 \ (\pm 0.15) \ logK_{ow} - 2.36 \ (\pm 0.20)$$
$$(n = 5, r = 0.97) \qquad (11)$$

This QSAR applies for the log K_{ow} range between 4.2 and 5.7. As was already shown by BELFROID et al. (1993a), the regression coefficient of this QSAR is comparable to that of other QSARs describing the relationship between BCF_w and

Table 1: Bioconcentration factors (BCF_w), uptake efficiencies (E) and elimination rate constants (k_e) determined for several chemicals in the earthworm *Eisenia andrei*

Chemical	log K_{ow}[a]	BCF $\pm$ se(ml · g^{-1})[b]	E $\pm$ se(%)[c]	$k_e \pm$ se(day^{-1})[d]
1,3,5-trichlorobenzene	4.19	72 $\pm$ 11	n.d.[e]	n.d.
1,2,3-trichlorobenzene	4.14	132 $\pm$ 16	n.d.	n.d.
1,2,3,4-tetrachlorobenzene	4.64	567 $\pm$ 62	2.5 $\pm$ 0.5	3.4 $\pm$ 0.6
pentachloroobenzene	5.18	1733 $\pm$ 253	12.2 $\pm$ 1.5	1.31 $\pm$ 0.39
				1.16 $\pm$ 0.49
hexachlorobenzene	5.73	4085 $\pm$ 415	15.1 $\pm$ 1.6	0.479 $\pm$ 0.034
				0.372 $\pm$ 0.050
PCB153	7.53	n.d.	8.4 $\pm$ 1.1	0.044 $\pm$ 0.011
				0.0364 $\pm$ 0.0077[f]
octachloronaphthalene	>8	n.d.	4.7 $\pm$ 0.6	n.d.

[a] DE BRUIJN et al. (1989)
[b] Data from BELFROID et al. (1993a)
[c] Data from BELFROID et al. (1994a)
[d] All values shown are derived from the initial rapid elimination phase of the bi-phasic curves observed for the elimination from earthworms in soil, unless stated otherwise. For PCB153 the mono-phasic elimination rate constant is shown, because no bi-phasic elimination was observed during the experiment. Data from BELFROID et al. (1994b, 1995b)
[e] Not determined
[f] Elimination rate constant of PCB153 from earthworms in soil observed after exposure to contaminated food. Data from BELFROID et al. (1995a)

In aquatic ecotoxicology, k_1/k_e in the second term is also described as the bioconcentration factor BCF. So, Equation 9 can be written as:

$$C_{worm}(ss) = \frac{Ef}{k_e}C_s + \frac{BCF}{K_p}C_s \qquad (10)$$

Equation 10 can be used to calculate the steady state concentration of organic hydrophobic chemicals in earthworms. Both routes of uptake are represented in the equation. The

log K_{ow} in aquatic species like fish, molluscs, daphnids and oligochaetes (→ Table 2). The log K_{ow} range for which these QSARs apply ranges from 2 to 6 or 7. The intercept of the QSAR established for earthworms is different from the other QSARs. However, the intercept of a log BCF_w-log K_{ow} relationship is determined by the lipid content of the organism (1 % in case of earthworms) and therefore the difference in intercept is no indication of dissimilar log BCF_w-log K_{ow} relationships.

Table 2: Characteristics of Quantitative Structure Activity Relationships (QSARs) between the bioconcentration factor of a chemical in organisms (log BCF) and log K_{ow}: log BCF = a log K_{ow} + b

Organism	a	b	n[a]	r	log K_{ow}	Reference
Fish	1.00	-1.32	36	0.97	1 – 6	MACKAY 1982
Fish	0.98	-1.30	20	0.90	1 – 6.4	DAVIES and DOBBS 1984
Molluscs	0.86	-0.81	16	0.96	1.5 – 5.5	GEYER et al. 1982
Molluscs	0.84	-1.23	34	0.83	3.4 – 7.8	HAWKER and CONNELL 1986
Daphnids	0.90	-1.32	22	0.96	2 – 6.2	HAWKER and CONNELL 1986
Daphnids	0.85	-1.10	52	0.96	0.9 – 6.7	GEYER et al. 1991
Earthworms	1.05	-2.36	5	0.97	4.2 – 5.7	BELFROID et al. 1993a
Earthworms	1.26	-1.9	40	0.92	1.0 – 6.4	CONNELL and MARKWELL 1990

[a] Number of tested chemicals used for the QSAR

For the estimation of BCF_w values of chemicals in earthworms, a QSAR developed for earthworms is most appropiate. However, the log K_{ow} range from 4.2 to 5.7 is only small and an extension to the log K_{ow} range 2 to 7 is preferrable. Since the small-range QSAR calculated for earthworms (Equation 11) corresponds with the larger-range QSARs computed for aquatic species (→ Table 2), it is assumed that extending the log K_{ow} range of Equation 11 to the range 2 – 7 will not result in large deviations of the relationship between BCF_w and log K_{ow}. In this way BCF_w values for organic chemicals in earthworms can be calculated using Equation 11 that was developed for earthworms while a large log K_{ow} range is maintained.

BCF_w values will be overestimated for chemicals that are subject to biotransformation and for superhydrophobic large-sized chemicals. Biotransformation results in lower BCF_w values than expected based on the log K_{ow} value (DE WOLF et al. 1992; SIJM et al. 1989). For some superhydrophobic chemicals, it has been suggested that the uptake may be limited by its molecular size. For fish, indications are that chemicals with a cross section of > 0.95 mm cannot pass the gill membrane (OPPERHUIZEN et al. 1985), although discussion on this point is still going on (GEYER et all, 1994). It is not known whether a similar limit on molecular size is relevant for uptake in earthworms. BELFROID et al. (1994a, 1995a) showed that two large sized chemicals (hexabromobenzene with a cross section of 0.96 nm and octachloronaphthalene with 0.98 nm) were taken up in earthworms after dietary exposure. The uptake efficiencies, however, were smaller than for the other text chemicals.

In conclusion, Equation 11 does apply for chemicals that are not subject to biotransformation and for which a limited uptake because of its molecular size is not to be expected.

2.3 Soil Adsorption Coefficient

The soil adsorption coefficient K_p describes the distribution of a chemical between soil particles and soil pore water or interstitial water. Theoretically, K_p can be calculated by dividing the concentration of the chemical in soil by the concentration in interstitial water. However, experimentally the determination of K_p is difficult, especially in case of strong sorption or low water solubility of the chemical. The interstitial water fraction in soil is small and can not be separated from the soil particles in sufficient quantities to allow analysis. This means that the concentration of a chemical in interstitial water can not be determined.

To overcome this practical problem, K_p is measured with the shake-method, in which the ratio interstitial water/soil particles is increased compared to natural soil and in which high concentrations of the chemical, that bear no relation with concentrations in the soil, are used. In this system the interstitial water layer can be separated from the soil particles and the concentration of the chemical can be determined. The influence of the increased solid/liquid ratio on K_p is not yet clear. BOESTEN (1990) discussed that the influence of this ratio will be negligible for chemicals with log K_p > 1, which, according to BRIGGS (1990) correspond to log K_{ow} > 2 in soils with an organic matter content > 2,6 %. However, the discussion of BRIGGS was based on natural fluctuations in soil moisture content and not representative for the large differences in water/soil ratios. A second problem when determining K_p is the influence of the "third phase". For the adsorption of organic hydrophobic chemicals to soil, the organic matter fraction is of major importance. Part of this fraction is dissolved in the interstitial water and this fraction is generally referred to as the third phase (SCHRAP et al. 1992). Until now, no techniques are available to separate the third phase completely from the interstitial water. Because of adsorption to the third phase, the concentration of the chemical in interstitial water is overestimated resulting in an underestimation of the K_p (GSCHWEND and WU 1985; SCHRAP and OPPERHUIZEN 1992). An additional problem is the influence of the third phase in the shake-method compared to soil, which is unlikely to be similar.

In spite of all these drawbacks, K_p values are generally determined with the shake-method, because no alternatives are available at this moment.

VAN GESTEL et al. (1991) determined K_p values for several chlorobenzenes, chlorophenols and 1,4-dichoroaniline in 3 different types of natural soil and in OECD artificial soil (OECD 1984), assuming a linear adsorption process. The determined K_p values were normalized to the organic matter content (%OM) in soil (K_{om}) using Equation 12:

$$K_{om} = K_p * 100/(\%OM) \tag{12}$$

A QSAR describing the relationship between K_{om} and log K_{ow} was established:

$$\log K_{om} = 0.89 \ (\pm 0.06) \ \log K_{ow} - 0.32 \ (\pm 0.24)$$
$$(n = 34, r = 0.93) \tag{13}$$

which applies for chemicals with log K_{ow} between 2.4 and 5.2 in soil with an organic matter content between 3.7 and 15.6 %. Several QSARs describing the partitioning of chemicals in soil and sediment have been established for a much larger log K_{ow} range. These QSARs, including Equation 13 normalized to organic carbon, are summarized in **Table 3**. The regression coefficients of all these relationships are more or less similar. This implies that for all these types of soils and sediments and for all these different chemicals, the adsorption coefficients increase in the same degree with increasing K_{ow}. Therefore we anticipate that extending the log K_{ow} range of Equation 13 to the range 2 to 7 will not generate the introduction of large errors.

For the conversion of K_{om} into K_p values necessary for the model calculations, assumptions have to be made with respect to the organic matter content of soils. An overview of Dutch soil types shows that organic matter contents range from $1-5$ % in sandy soils to > 45 % in peaty soils (KUIPERS 1977). As earthworms are rarely found in such high organic soils (EDWARDS and LOFTY 1977), for the model calculations organic matter contents between 3 and 20 % are used.

2.4 Uptake Efficiency and Feeding Rate

Dietary uptake of organic chemicals was observed in different species living in sediment and soil (BELFROID et al. 1994a; SCHUYTEMA et al. 1988; WESTON 1990). For the earthworm *Eisenia andrei* uptake efficiencies (E), defined as the fraction of the ingested chemical passing the gut that is taken up into the organism, have been determined (BELFROID et al. 1994a) and the results are presented in **Table 1**. No relationship with log K_{ow} was observed. E values for penta- and hexachlorobenzene and 2,2',4,4',5,5'-hexachlorobiphenyl (PCB153) varied between 8 and 15 %, while E values for octachloronaphthalene and 1,2,3,4-tetrachlorobenzene were lower. For octachloronaphthalene, a decreased E was expected, because the uptake and membrane passage of this compound is probably limited by its large molecular size (BRUGGEMAN et al. 1984; OPPERHUIZEN et al. 1985, OPPERHUIZEN and SIJM 1990). For 1,2,3,4-tetrachlorobenzene, no explanation for the low E value could be furnished, although an experimental artefact can not be ruled out. For the model calculations, E values of penta- and hexachlorobenzene and PCB153 will be used.

The feeding rate (f) also determines dietary uptake. BELFROID et al. (1994a) observed a feeding rate of 0.288 (± 0.015 s.e.) $g \cdot g^{-1} \cdot day^{-1}$ in the earthworm *Eisenia andrei* when studying the dietary uptake of chemicals. This rate is not very different from feeding rates determined for earthworms in the field situation. HENDRIKSEN (1991) observed feeding rates of 0.08 and 0.15 $g \cdot g^{-1} \cdot day^{-1}$ for *Lumbricus festivus* and *Lumbricus castaneus*, respectively.

Table 3: Characteristics of Quantitative Structure Activity Relationships (QSARs) between the adsorption coefficient of a chemical in soil or sediment normalized to organic carbon content (log K_{oc}) and log K_{ow}: log K_{oc} = a log K_{ow} + b

Chemicals	a	b	n[a]	r	log K_{ow}	Reference
Triazines and subst. dinitrotoluenes	0,94	-0.01	9	0.97	2 – 5	BROWN and FLAGG 1979
Hydrophobic chemicals	1.00	-0.21	10	1	1.5 – 6.5	KARICKHOFF et al. 1979
Hydrophobic chemicals	0.54	1.38	45	0.86	0 – 6	KENAGA and GORING 1980
Substituted aromatic hydrocarbons	1.00	0.32	13	0.99	1.5 – 6.5	HASSET et al. 1980
Hydrophobic chemicals	0.99	-0.35	5	1	1.5 – 6.5	KARICKHOFF 1981
Hydrocarbons	0.72	0.49	13	0.97	0 – 6	SCHWARZENBACH & WESTALL 1981
Hydroph. hydrocarbons	1.00	-0.32	22	0.99	1.5 – 6.5	MEANS et al. 1982
Hydrophobic chemicals	1.04	-0.88	33	0.95	1 – 6.5	ABDUL et al. 1987
Hydrophobic chemicals	0.83	0.29	20	0.95	1.5 – 6	HODSON & WILLIAMS 1988
(Halogenated) aromatic hydrocarbons	0.90	0.82	5	0.97	4 – 7	EADIE et al. 1990
Chlorophenols and chlorobenzenes	0.89	-0.09	34	0.92	1.5 – 5.5	recalculated from VAN GESTEL et al. 1991[b]

[a] Number of tested chemicals used for the QSAR
[b] 1 % OM = 0.58 % OC

The total amount of a chemical taken up from food is determined by the feeding rate f and the uptake efficiency E. These two parameters, however, are not completely independent. OPPERHUIZEN (1991) suggested that the uptake efficiency of a chemical from food is mainly dictated by the digestibility of the food. When the digestibility of food is high, E will be high and the feeding rate f will be low. However, when food is poorly digested, E is low and the feeding rate f will be high to satisfy the nutrient and energy requirement of the animal. Results of CLARK and MACKAY (1991) confirm this hypothesis. They studied dietary uptake of four hydrophobic chemicals in fish varying the feeding rate f by feeding different amounts of food. They observed that fisch fed with twice the amount of food, accumulated less than twice as much chemical compared to control fish. This result shows that E and f are not completely independent of each other. On the other hand, it is not expected that E and f are completely inversely related. Since no information about the relation between E and f is available, we presume that for our model calculations E and f must be derived from the same experiment.

For the model calculations a mean E * f of 0.034 ± 0.006 (s.e.) g.g^{-1}.day^{-1} was used based on results with penta- and hexachlorobenzene and PCB153 in the earthworm *Eisenia andrei* (BELFROID et al. 1994a). It is assumed that this value accounts for the log K_{ow} range of 2 to 7. For less hydrophobic chemicals, like 1,2,3,4-tetrachlorobenzene, this might imply an overestimation of the dietary uptake route. However, by selecting relatively high E values a worst case situation concerning the contribution of dietary uptake is represented.

2.5 Elimination Rate Constant

In earthworms in soil, a bi-phasic elimination was observed for chlorobenzenes (BELFROID et al. 1993a, 1995b), suggesting two-compartment elimination. When elimination follows a bi-phasic pattern, mathematical contants β_1 and β_2 are calculated instead of elimination rate constants. For the calculation of elimination rate constants it is important to have a complete understanding of the relation and rate constants between the compartments causing the bi-phasic elimination (SCHRAP et al. 1994; DE WOLF et al. 1994, BELFROID et al. 1994b). Therefore, no elimination rate constants could be calculated. However it was argued that the initial rapid phase of the elimination is of major importance, because this phase is responsible for the larger part (90 – 99 %) of decrease in the body burden of the worms (BELFROID et al. 1995b). Therefore, elimination rate constants were calculated assuming one-compartment kinetics using data of the first elimination phase, ignoring the biphasic appearance of the curves (→ *Table 1*).

A QSAR was calculated based on mono-phasic elimination rate constants (PCBs) and 'initial phase' elimination rate constants (chlorobenzenes) determined in earthworms:

$$\log k_e = -0.662 \log K_{ow} \ (\pm 0.036) + 3.47 \ (\pm 0.15)$$
$$(n = 13, r = 0.98) \tag{14}$$

This QSAR applies for the log K_{ow} range between 4.6 and 8.1. For the model calculations this QSAR is extrapolated

to log K_{ow} of 2. It is assumed that this extrapolation to chemicals with low log K_{ow} values will not result in large deviations between the calculated and actual elimination rate constant. Equation 14 does not apply for chemicals that are subject to biotransformation.

3 Testing of the Model

All parameters necessary for the model calculations can be estimated. However, as is already discussed, the parameter estimation is based on only few experimental results and some uncertainties remain. In summary, these concern the extension of the log K_{ow} range of the QSARs for BCF_w, K_p and k_e, the relevance of the K_p determination for the soil environment, and the relationship between E and f. Because of these uncertainties, it is important to assess the correspondence between calculated and observed concentrations of chemicals in worms.

The model was tested by comparing calculated (according to Equation 10) and observed steady state concentrations of three chlorobenzenes in earthworms in OECD artificial soil. BELFROID et al. (1994b) studied the accumulation of 1,2,3,4-tetra-, penta- and hexachlorobenzene. The mean concentrations (± s.e.) in artificial soil were 10.05 ± 0.28, 12.08 ± 0.29 and 13.15 ± 0.34 mg/kg, respectively, while the organic matter content in artificial soil is determined at 8.1 % (VAN GESTEL and MA, 1990). Based on the log K_{ow} of these compounds of 4.64, 5.18 and 5.73 (DE BRUIJN et al., 1989), the parameters of Equation 10 are estimated and the steady state concentrations in the earthworm calculated. The standard error in the parameters and the propagation of the errors was calculated according to SOKAL and ROHLF (1981). Results of the calculations are shown in **Table 4**, together with the observed steady state concentrations. It can be concluded that in spite of all uncertainties in the parameter calculations, the model gives a fair estimation of the steady state concentrations to be expected in earthworms. For more hydrophobic chemicals, like hexachlorobenzene, the deviation between the calculated and observed concentration increases, but the discrepancy between these two is still smaller than a factor 2. The difference between calculated and measured values increases with increasing log K_{ow}.

Table 4: Calculated and observed steady state concentrations of 1,2,3,4-tetra-, penta- and hexachlorobenzene in the earthworm *Eisenia andrei* in OECD artificial soil in ng/mg (data from BELFROID et al., 1994b)

Chemical	Calculated C_{worm}[a]	Observed C_{worm}
1,2,3,4-tetrachlorobenzene	6.4 ± 0.3[b]	8.9 ± 0.3[b]
pentachlorobenzene	9.6 ± 0.5	16.1 ± 0.6
hexachlorobenzene	13.2 ± 0.7	24.1 ± 0.8

[a] Calculations based on the model presented in this paper
[b] ± Standard error

The model testing was also carried out with LC_{50} values, the concentration in soil resulting in death among 50 % of the population, of four chlorobenzenes (log K_{ow} 3.4 – 5.2)

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in earthworms. LC_{50} values for earthworms were determined in 14-day acute toxicity tests in 4 different soils (VAN GESTEL et al. 1991), and are shown in **Table 5**. Based on these concentrations in soil, the steady state concentration in earthworms was calculated. The results of the calculation are compared with lethal body burdens (LBBs) of the same chemicals measured in artificial soil, in water, after feeding and on filter paper (BELFROID et al. 1993b). Details of this validation are summarized in **Table 6**. Predicting LBB values with measured LC_{50} values using the model presented in

this paper shows that a fair estimation of the concentration in earthworms can be made. An exact estimation is not to be expected, because the model presumes steady state. It is not unlikely that steady state was not reached in all LC_{50} toxicity tests. Also the time until death differs. LC_{50} values were determined in 14-day acute toxicity tests, while for two chlorobenzenes the LBB was determined in short-term experiments. As was already discussed (BELFROID et al. 1993b), LBB can be influenced by time, especially for the less hydrophobic chemicals.

Table 5: LC50 values for the toxicity of four chlorobenzenes to *Eisenia andrei* in four different types of soil (data from VAN GESTEL et al. 1991)

Chemical	LC50 (in mg/kg)			
	Sandy soil I	Sandy soil II	Peaty soil	OECD artificial soil
% OM[a]	3.7	6.1	15.6	8.1
1,4-dichlorobenzene	128	n.d.[b]	n.d.	229
1,2,3-trichlorobenzene	134	240	596	134
1,2,3,4-tetrachlorobenzene	75	n.d.	n.d.	223
pentachlorobenzene	134	n.d.	n.d.	238

[a] Organic matter
[b] Not determined

Table 6: Calculated steady state body burdens in earthworms at soil concentrations equal to LC50 values (→ *Table 5*) and observed lethal body burdens (LBB) (data from BELFROID et al. 1993b), both in μmol/g

Chemical	Calculated C_{worm}[a]	Observed LBB	
1,4-dichlorobenzene	0.63 – 0.76		(0.33)[b]
1,2,3-trichlorobenzene	0.39 – 0.92	0.51 – 0.69	(2.1)
1,2,3,4-tetrachlorobenz.	0.48 – 0.66		(3.2)
pentachlorobenzene	0.75 – 0.91	1.46 – 2.34	(1.29)

[a] Calculations based on the model presented in this paper
[b] Values in parenthesis represent LBBs observed in earthworms that died within 2 days in the contact paper test, other LBBs are from experiments in water, soil and with food

4 Model Calculations

4.1 Experimental Data

In the previous section a model is described that can be used to calculate the absolute and relative contribution of two routes of uptake, uptake from interstitial water and dietary uptake, to the total steady state concentration of a hydrophobic organic chemical in the earthworm. The calculations can be based both on estimated data and on experimental data. However, experimentally determined parameters that could be used for the model calculations are scarce. Only for penta- and hexachlorobenzene, all parameters necessary are known (→ *Table 1*). With these data, and K_p values calculated using Equation 12 and 13, the relative contribution of both routes of uptake to the total

body burden is calculated. The standard error in K_p and the propagation of the errors was calculated according to SOKAL and ROHLF (1981). The results are summarized in **Table 7**. Bioconcentration of chemicals from interstitial water is the most important route of uptake. The contribution of dietary uptake to the total body burden is comparatively small for both penta- and hexachlorobenzene in OECD artificial soil. This seems to be in contrast with observations of BELFROID et al. (1994a). They conluded that for hexachlorobenzene dietary uptake is important, because the bioaccumulation factor BAF measured in soil was 2 – 3 times higher than the bioconcentration factor BCF measured in water. However, als already stated, such a comparison between BAF and BCF_w should not be made, since the kinetics of the compounds tested in both test systems were proven to be different, while they should be equal to allow for a comparison. In conclusion, these experimental data show that the contribution of dietary uptake to the total body burden for these two compounds is small compared to the contribution of uptake by passive diffusion from interstitial water.

Table 7: Relative contribution of two routes of uptake to the total body burden of the contaminant in the earthworm *Eisenia andrei* in OECD artifical soil. Calculations based on the model presented in this paper

Chemical	dietary uptake	uptake from interstitial water
pentachlorobenzene	2.7 % (1.2)[a]	97,3 % (14.4)
hexachlorobenzene	12.2 % (2.2)	87.8 % (9.2)

[a] Standard error in parenthesis

4.2 Estimated Data (log K_{ow} 2 – 7)

The model can also be used to predict the relative contribution of the two routes of uptake to the total steady state concentration for hydrophobic organic chemicals based on an estimation of the necessary parameters. Three chemicals with log K_{ow} of 2, 4.5 and 7 and three different types of soil, a sandy soil with 3 % organic matter, an average soil with 8 % organic matter and a soil with a high organic matter content of 20 %, are selected for the calculations. The standard error in the parameters and the propagation of the errors was calculated according to SOKAL and ROHLF (1981). Results are shown in **Table 8**. In **Fig. 2** this is visualized for the complete K_{ow} range. Both from the table and the figure it can be concluded that the major route of uptake is by passive diffusion from the interstitial water. Under most circumstances, dietary uptake contributes for less than 10 %. In soils with a high organic matter content the contribution

of this route becomes more important. Especially for extremely hydrophobic chemicals, the contribution of dietary uptake can increase up to $\approx$ 50 % in soils with a high organic matter content, while in a sandy soil this is only $\approx$ 10 %. This increased contribution is not due to an increased dietary uptake, but to the decreased uptake from interstitial water. The availability of a chemical in interstitial water, which is mainly determined by the organic matter content in soil, is reduced in these high organic soils. On the other hand, the availability of a chemical in soil with a low organic matter content, will be high, resulting in a diminished contribution of the dietary uptake route. In summary, with decreasing organic matter contents, the significance of the interstitial water route increases, while, on the other hand, with increasing organic matter contents, the contribution of dietary uptake increases because of decreasing interstitial water uptake.

Table 8: Relative contribution of two routes of uptake to the total body burden of three contaminants with log K_{ow} = 2, 5 or 7 in the earthworm *Eisenia andrei* in soil with a low and high organic matter content (3 and 20 %). Calculations based on the model presented in this paper

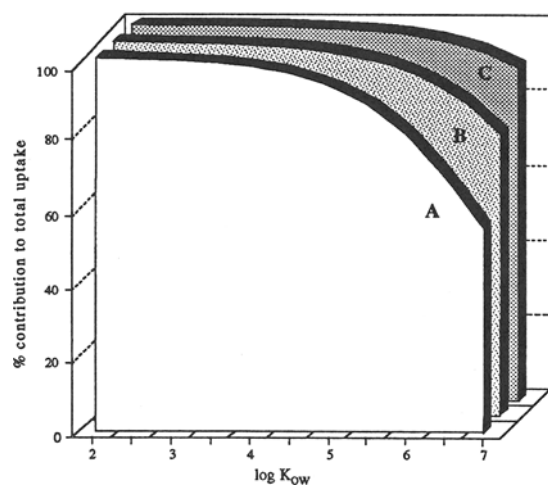| log K_{ow} of chemical	Accumulation in sandy soil		Accumulation in „rich" soil	
	dietary	interstial water	dietary	interstitial water
2	0.0 % (0.0)[a]	100 %　(160)	0.25 % (0.05)	99.7 % (159.6)
5	1.2 % (0.2)	98.8 % (3.9)	7.6 %　(1.4)	92.4 % (3.7)
7	11.0 % (2.0)	89.0 % (6.8)	45.2 % (8.3)	54.8 % (4.2)

[a] Standard error in parenthesis

Fig. 2: Calculated relative contribution of uptake from interstitial water to the total uptake in earthworms for organic hydrophobic chemicals with log K_{ow} between 2 and 7 in three different types of soil with A) 20 %, B) 8 % and C) 3 % organic matter

Extrapolation of the model to log K_{ow} > 7 may not be allowed. With increasing log K_{ow} both E and k_e decrease, until E is zero at log K_{ow} 8 – 9 (GOBAS et al. 1988; OPPERHUIZEN et al. 1985). For these chemicals dietary uptake is completely inhibited. Because of the low solubility in interstitial water and the high adsorption to soil of these

chemicals, uptake from interstitial water will also be very small.

5 Discussion

5.1 Application of the Equilibrium Partitioning Theory

The equilibrium partitioning model (VAN GESTEL and MA 1988, 1990; DITORO et al. 1991; ZIEGENFUSS 1986) is based on the hypothesis that only the fraction of the chemical dissolved in interstitial water is available and that only this fraction determines the concentration of the chemical in the worm. Dietary uptake is assumed to be absent, or a least to be negligible. Calculations based on our model clearly show that the contribution of dietary uptake can be significant under certain circumstances. However, for most chemicals in most soils, the relative contribution of dietary uptake is only small. This small contribution of the dietary route might explain why experiments meant to study the suitability of the equilibrium partitioning model, such as carried out by VAN GESTEL and MA (1988, 1990) and OLIVER (1987) for terrestrial and aquatic worms, always resulted in a confirmation of the model. As our approach shows, scientifically this is incorrect, especially for the more hydrophobic chemicals in soils with a high organic matter content.

5.2 Uncertainties

Some uncertainties still remain. For example, the feeding behaviour of earthworms might influence the exposure. For benthic organisms it is known that fine sediment particles are favoured above bulk sediment. Since hydrophobic chemicals preferably sorb to this fine organic fraction, the dietary exposure concentration may be higher than the bulk concentration (LANDRUM and ROBBINS 1990).

The extrapolation of lab results to the field situation also involves uncertainties. With increasing contact time between soil and chemical the bioavailability decreases, probably because during time the chemical will penetrate deeper into soil particles. The chemical can become available only after diffusion, which is a very slow process because of the small concentration difference within the soil particle. VERMA and PILLAI (1991) showed that accumulation of hexachlorocyclo-hexane (HCH) in worms in one-year exposed soil was lower than in freshly contaminated soil. BELFROID et al. (1995b) observed that accumulation in worms of chemicals from Volgermeerpolder soil, in which the chemicals have been in contact with the soil for several decades, was also less than anticipated. This means that the model presented here would overestimate the chemical concentration in the earthworm in soils contaminated several years ago.

Another uncertainty is the conversion of k_1/k_e in BCF (Equation 10). It can be argued that the interstitial water fraction will be depleted from its chemicals within hours. Evidence for this depletion is given by BRIGGS and LORD (1983) who showed that initial uptake of a compound in soil with a water content of 22 % was larger than in a soil containing 8 % water. Results of LANDRUM (1989) also suggested that uptake from interstitial water is kinetically controlled by desorption from sediment particles. To assess the consequences of this phenomenom, it is important to know in what form the chemical is present in soil: For example, sorbed to soil particles or in crystalline form. In the first case, since desorption is a slow process, this step will be rate limiting and BCF is determined by k_d/k_e which is smaller than k_1/k_e. This means that uptake from interstitial water is smaller than anticipated based on the estimation of the model. When the chemical is present in crystalline form, the uptake process will be limited by the rate of solubilization of the chemical, or, when this is a rapid process, by the diffusion across the membranes of the earthworm. All these uncertainties which apply for this model, but partially also for the equilibrium partitioning theory, might involve a risk when applying the model.

6 Conclusion

In this paper a model is presented which can be used to estimate body burdens of a range of inert chemicals with log K_{ow} 2 – 7 in earthworms. The model incorporates two routes of uptake: uptake from interstitial water and dietary uptake. Although many uncertainties still remain, calculations show that under most circumstances the contribution of dietary uptake to the total body burden is small. Steady state body burdens are mainly determined by uptake from

interstitial water. In conclusion, estimates of the steady state body burden calculated with the equilibrium partitioning model, in which only uptake from interstitial water is taken into account, might result in a small underestimation of the real body burden of a chemical in the earthworm.

7 References

ABDUL, A. S.; GIBSON, T. L.; RAI, D. N.: Statistical correlations for predicting partition coefficient for nonpolar organic contaminants between aquifer organic carbon and water. Hazardous Waste and Hazardous Materials 4, 211 – 222 (1987)

BELFROID, A.; VAN WEZEL, A.; SIKKENK, M.; VAN GESTEL, K.; SEINEN, W.; HERMENS, J.: The toxicokinetic behaviour of chlorobenzenes in earthworms (Eisenia andrei): Experiments in water. Ecotox. Environ. Safety 25, 154 – 165 (1993a)

BELFROID, A.; SEINEN, W.; VAN GESTEL, K.; HERMENS, J.: The acute toxicity of chlorobenzenes for earthworms (Eisenia andrei) in different exposure systems. Chemosphere 12, 2265 – 2277 (1993b)

BELFROID, A.; MEILING, J.; SIJM, D.; HERMENS, J.; SEINEN, W.; VAN GESTEL, K.: Uptake of lipophilic organic chemicals from food by earthworms (Eisenia andrei). Arch. Environ. Contam. Toxicol. 27, 260 – 265 (1994a)

BELFROID, A.; SIKKENK, M.; VAN GESTEL, K.; SEINEN, W.; HERMENS, J.: The toxicokinetic behaviour of chlorobenzenes in earthworms (Eisenia andrei), experiments in soil. Environ. Toxicol. Chem. 13, 93 – 99 (1994b)

BELFROID, A.; MEILING, J.; DRENTH, H.; HERMENS, J.; SEINEN, W.; VAN GESTEL, K.: Dietary uptake of super-lipophilic compounds by earthworms (Eisenia andrei). Ecotox. Environ. Safety, in press (1995a)

BELFROID, A.; SEINEN, W.; VAN DEN BERG, M.; HERMENS, J.; VAN GESTEL, K.: Uptake, bioavailability and elimination of lipophilic compounds in earthworms (Eisenia andrei) in field contaminated soil. Environ. Toxicol. Chem., in press (1995b)

BOESTEN, J. J. T. I.: Influence of the solid/liquid ratio on the experimental error of the sorption coefficients in pesticide/soil systems. Pestic. Sci. 30, 31 – 41 (1990)

BRIGGS, G. G.; LORD, K. A.: The distribution of aldicarb and its metabolites between Lumbricus terrestris, water and soil. Pestic. Sci. 14, 412 – 416 (1983)

BRIGGS, G. G.: Predicting the behaviour of pesticides in soil from their physical and chemical properties. Phil. Trans. R. Soc. Lond. B 329, 375 – 382 (1990)

BROWN, D. S.; FLAGGE. W.: Emperical prediction of organic pollutant sorption in natural sediment. J. Environ. Qual. 10, 382 – 386 (1979)

BRUGGEMAN, W. A.; OPPERHUIZEN, A.; WIJBENGA, A.; HUTZINGER, O.: Bioaccumulation of super-lipophilic chemicals in fish. Toxicol. Environ. Chem. 7, 173 – 189 (1984)

CLARK, K. E.; MACKAY, D.: Dietary uptake and biomagnification of four chlorinated hydrocarbons by guppies. Environ. Toxicol. Chem. 10, 1205 – 1217 (1991)

CONNELL, D. W.; MARKWELL, R. D.: Bioaccumulation in the soil to earthworm system. Chemosphere 20, 91 – 100 (1990)

COOKE, A. S.; GREIG-SMITH, P. W.; JONES, S. A.: Consequences for vertebrate wildlife of toxic residues in earthworm prey. In P. W. GREIG-SMITH, H. BECKER, P. J. EDWARDS and F. HEIMBACH, Ecotoxicology of earthworms, Athenaeum Press Ltd, Newcastle upon Tyne, UK 1992, pp. 139

DAVIES, R. P.; DOBBS, A. J.: The prediction of bioconcentration in fish. Water Res. 18, 1253 – 1262 (1984)

DE BRUIJN, J.; BUSSER, F.; SEINEN, W.; HERMENS, J.: Determination of octanol/water partition coefficients for hydrophobic organic chemicals with the "slow-stirring" method. Environ. Toxicol. Chem. 8, 499 – 512 (1989)

DE WOLF, W.; DE BRUIJN, J. H. M.; SEINEN, W.; HERMENS, J. L. M.: Influence of biotransformation on the relationship between bioconcentration factors and octanol-water partition coefficients. Environ. Sci. Technol. 26, 1197 – 1201 (1992)

DE WOLF, W.; YEDEMA, E.; SEINEN, W.; HERMENS, J.: Bioconcentra-
tion kinetics of some chlorinated anilines in guppy *Poecilia reticulata*.
Chemosphere 28, 159 – 167 (1994)

DERR, S. K.; ZABIK, M. J.: Bioactive compounds in the aquatic environ-
ment: studies on the mode of uptake of DDE by the aquatic midge,
Chironomus tentans (Diptera: Chironomidae). Arch. Environ. Con-
tam. Toxicol. 2, 152 – 164 (1974)

DI TORO, D. M.; ZABRA, C. S.; HANSEN, D. J.; BERRY, W. J.; SWARTZ,
R. C.; COWAN, C. E.; PAVLOU, S. P.; ALLEN, H. E.; THOMAS,
N. A.; PAQUIN, P. R.: Technical basis for establishing sediment
quality criteria for nonionic organic chemicals using equilibium par-
titioning. Environ. Toxicol. Chem. 10, 1541 – 1583 (1991)

EADIE, B. J.; MOREHEAD, N. R.; LANDRUM, P. F.: Three-phase parti-
tioning of hydrophobic organic compounds in Great Lakes water.
Chemosphere 20, 161 – 178 (1990)

EDWARDS, C. A.; LOFTY, J. R.: Biology of earthworms. 2nd ed. Chap-
man & Hall, London 1977

GEYER, H.; SHEEHAN, D.; KOTZIAS, D.; FREITAG, D.; KORTE, F.:
Prediction of ecotoxicological behaviour of chemicals: relationship
between physicochemical properties and bioaccumulation of organic
chemicals in the mussel. Chemosphere 11, 1121 – 1134 (1982)

GEYER, H.; SCHEUNERT, I.; BRUGGEMANN, R.; STEINBERG, C.; KORTE,
F.; KETTRUP, A.: QSAR for organic chemical bioconcentration in
Daphnia, algae and mussels. Sci. Total Environ. 109/110, 387 – 394
(1991)

GEYER, H.; MUIR D. C. G.; SCHEUNERT, I.; STEINBERG, C.; KETTRUP,
A.: Bioconcentration of superlipophilic persistent chemicals. Environ.
Sci. Pollut. Res. 1, 75 – 80 (1994)

GOBAS, F. A. P. C.; MUIR, D. C. G.; MACKAY, D.: Dynamics of
dietary bioaccumulation and faecal elimination of hydrophobic
organic chemicals in fish. Chemosphere 17, 943 – 962 (1988)

GSCHWEND, P. M.; WU, S.: On the constancy of sediment-water par-
tition coefficient of hydrophobic organic pollutants. Environ. Sci.
Technol. 19, 90 – 96 (1985)

HASSET, J. J.; MEANS, J. C.; BANWART, W. L.; WOOD, S. G.: Sorption
properties of sediments and energy related pollutants.
EPA-600/3-80-041 pp. 1 – 133 (1980)

HAWKER, D. W.; CONNELL, D. W.: Bioconcentration of lipophilic com-
pounds by some aquatic organisms. Ecotoxicol. Environ. Safety 11,
184 – 197 (1986)

HENDRIKSEN, N. B.: Gut load and food retention time in the earth-
worms *Lumbricus festivus* and *L. castaneus*: A field study. Biol. Fer-
til. Soils 11, 170 – 173 (1991)

HERMENS, J. L. M.: Quantitative Structure-Activity Relationships for
predicting fish toxicity. In: W. KARCHER and J. DEVILLERS, Prac-
tical application of Quantitative Structure-Activity Relationships
(QSAR) in Environmental Chemistry and Toxicology. ECSE, EEC,
EAEC, Brussels and Luxembourg 1990, pp 263 – 280

HODSON, J.; WILLIAMS, N. A.: The estimation of the adsorption coef-
ficients (Koc) for soils by high performance liquid chromatography.
Chemosphere 17, 66 – 77 (1988)

KARICKHOFF, S. W.; BROWN, D. S.; SCOTT, T. A.: Sorption of
hydrophobic pollutants on natural sediments. Water Res. 13,
241 – 248 (1979)

KARICKHOFF, S. W.: Semi-emperical estimation of sorption of
hydrophobic pollutants on natural sediments and soils. Chemosphere
10, 833 – 846 (1981)

KENAGA, E. E.; GORING, C. A. I.: Relationship between water
solubility, soil sorption, octanol water partitioning, and concentra-
tion of chemicals in biota. In J. C. EATON, P. R. PARRISH and A. C.
HENDRICKS, Aquat. Toxicology. STP 707, (Third Symposium) Am.
Soc. for Testing and Mat., Philadelphia 1980, pp. 78 – 115

KNEZOVICH, J. P.; HARRISON, F. L.: The bioavailability of sediment-
sorbed chlorobenzenes to larvae of the midge, *Chironomus decorus*.
Ecotoxicol. Environ. Safety 15, 226 – 241 (1988)

KUIPERS, S. F.: Bodemkunde. Tjeenk-Willink, Culemborg 1977, the
Netherlands.

LANDRUM, P.F.: Bioavailability and toxicokinetics of polycylic aromatic
hydrocarbons sorbed to sediments for the amphipod *Pontoporeia
hoyi*. Environ. Sci. Technol. 23, 588 – 595 (1989)

LANDRUM, P. F.; ROBBINS, J. A.: Bioavailability of sediment associated
contaminants to benthic invertebrates. In: R. BAUSO, J. P. GIESY and
H. MUNTAU, Sediments: Chemistry and toxicity of in-place
pollutants, Lewis publishers. Ann Arbor 1990

LORD, K. A.; BRIGGS, G. G.; NEALE, M. C.; MANLOVE, R.: Uptake of
pesticides from water and soil by earthworms. Pestic. Sci. 11,
401 – 408 (1980)

MACKAY, D.: Correlation of bioconcentration factors. Environ. Sci.
Technol. 16, 274 – 276 (1982)

MEANS, J. S.; WOOD, S. G.; HASSETT, J. J.; BANWART, W. L.: Sorption
of amino- and carboxy-substituted polynuclear aromatic hydrocar-
bons by sediments and soils. Environ. Sci. Technol. 16, 93 – 98
(1982)

OECD: Guideline for testing of chemicals no 207. Earthworm acute
toxicity tests. Adopted 4 april 1984.

OECD: Report of the OECD workshop on effects of chemicals in sedi-
ment (Copenhagen 13 – 15 May 1991). Environment monographs
no 60. Paris 1992

OLIVER, B. G.: Bio-uptake of chlorinated hydrocarbons from
laboratory-spiked and field sediments by oligochaete worms. En-
viron. Sci. Technol. 21, 785 – 790 (1987)

OPPERHUIZEN, A.; VAN DERVELDE, E. W.; GOBAS, F. A. P. C.; LIEM,
A. K. D.; VAN DER STEEN, J. M. D.; HUTZINGER, O.: Relationship
between bioconcentration of hydrophobic chemicals in fish and steric
factors. Chemosphere 14, 1871 – 1896 (1985)

OPPERHUIZEN, A.; SIJM, D. T. H. M.: Bioaccumulation and
biotransformation of polychlorinated dibenzo-p-dioxins and diben-
zofurans in fish. Environ. Toxicol. Chem. 9, 197 – 186 (1990)

OPPERHUIZEN, A.: Bioaccumulation kinetics: Experimental data and
modelling. In: Organic micropollutants in the aquatic environment.
Proceedings of the sixth European symposium held in Lisboa, Por-
tugal, 22 – 24 may 1990. Eds. G. Angeletti and A. Bjorseth. Kluwer
Academic Publishers 1991

SCHRAP, S.; SLEIJPEN, G.; SEINEN, W.; OPPERHUIZEN, A.: Sorption
kinetics of chlorinated hydrophobic organic chemicals: I. The use
of first-order kinetic multicompartment models. Environ. Sci. Poll.
Res. 1, 21 – 28 (1994)

SCHRAP, S.; OPPERHUIZEN, A.: On the contradictions between ex-
perimental sorption data and the sorption partitioning model.
Chemosphere 24, 1259 – 1282 (1992)

SCHUYTEMA, G. S.; KRAWCZYK, D. F.; GRIFFIS, W. L.; NEBEKER, A. V.;
ROBIDEAUX, M. L.; BROWNAWELL, B. J.; WESTALL, J. C.: Com-
parative uptake of hexachlorobenzene by fathead monnows, am-
phipods and oligochaete worms from water and sediment. Environ.
Toxicol. Chem. 7, 1035 – – 1045 (1988)

SCHWARZENBACH, R. P.; WESTALL, J.: Transport of nonpolar organic
compounds from surface water to groundwater. Laboratory sorp-
tion studies. Environ. Sci. Technol. 15, 1360 – 1367 (1981)

SHEA, D.: Developing national sediment quality criteria. Environ. Sci.
Technol. 22, 1256 – 1261 (1988)

SIJM, D. T. H. M.; WEVER, H.; OPPERHUIZEN, A.: Influence of
biotransformation on the accumlation of PCDDs and PCDFs from
fly-ash in fish. Chemosphere 19, 475 – 480 (1989)

SIJM, D. T. H. M; SEINEN, W.; OPPERHUIZEN, A.: Life-cycle
biomagnification study in fish. Environ. Sci. Technol. 26,
2162 – 2174 (1992)

SOKAL, R. R.; ROHLF, F. J.: Biometry. Second edition, W. H. Freeman
and Company, New York 1981

STENERSEN, J.: Detoxification of xenobiotics by earthworms. Comp.
Biochem. Physiol 78C, 249 – 252 (1984)

VAN DER KOOIJ, L. A.; VAN DE MEENT, D.; VAN LEEUWEN, C. J.;
BRUGGEMAN, W. A.: Deriving quality criteria for water and sedi-
ment from the results of aquatic toxicity tests and product standards:
Application of the equilibrium partitioning method. Water. Res. 6,
697 – 705 (1991)

VAN GESTEL, C. A. M.; MA, W.: Toxicity and bioaccumation of
chlorophenols in earthworms in relation to bioavailability in soil.
Ecotox. Environ. Safety 15, 289 – 297 (1988)

VAN GESTEL, C. A. M.; MA, W.: An approach to quantitative structure-activity relationships (QSARs) in earthworm toxicity studies. Chemosphere 21, 1023 – 1033 (1990)

VAN GESTEL, C. A. M.; MA, W.; SMIT, C. E.: Development of QSARs in terrestrial ecotoxicology: earthworm toxicity and soil sorption of chlorophenols, chlorobenzenes and dichloroaniline. Sci. Total Environ. 109/110, 589 – 604 (1991)

VAN LEEUWEN, C. J.; VAN DER ZANDT, P. T. J.; ALDENBERG, T.; VERHAAR, J. J. M.; HERMENS, J. L. M.: Application of QSARs, extrapolation and equilibrium partitioning in aquatic effects assessment. I. Narcotic industrial pollutants. Environ. Toxicol. Chem. 11, 267 – 282 (1992)

VERHAAR, H. J. M.; VAN LEEUWEN, C. J.; HERMENS, J. L. M.: Classifying environmental pollutants. I: structure-activity relationships for prediction of aquatic toxicity. Chemosphere 25, 471 – 491 (1992)

VERMA, A.; PILLAI, M. K. K.: Bioavailability of soil-bound residues of DDT and HCH to earthworms. Current Sci. 61, 840 – 843 (1991)

WESTON, D. P.: Hydrocarbon bioaccumulation from contaminated sediment by the deposit-feeding polychaete *Abarenicola pacifica*. Marine Biology 107, 159 – 169 (1990)

ZIEGENFUSS, P. S.; TENAUDETTE, W. J.; ADAMS, W. J.: Methdology for assessing the acute toxicity of chemicals sorbed to sediments: Testing the equilibrium partitioning theory. in: POSTON, T. M. and R. PURDY. Aquatic toxicology and environmental fate: 9th vol, ASTM STP 921. Am. Soc. for Testing and Materials. Philadelphia 1986

Monitoring Behavioural Responses to Metals in *Gammarus pulex* (L.) (Crustacea) with Impedance Conversion

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Abstract

An impedance conversion technique was used to study the behaviour of *Gammarus pulex* (L.) exposed to acutely toxic concentrations of Pb (0.01, 0.05, 0.1 and 0.5 mg Pb l^{-1}) and to field concentrations of Cu ($\leq$ 0.05 mg Cu l^{-1}). Initial stress responses were studied during short-term exposure (1 h) and sublethal toxic effects were monitored during 7 (Pb) and 35 days (Cu), respectively.

Exposure to Pb caused 30 % mortality and resulted in a bioconcentration factor (BCF) of 2700 at 0.5 mg Pb l^{-1} after 168 h. Exposure to Cu polluted stream water caused no mortality within 35 days and uptake was low (BCF 5.8).

Gammarus pulex reacted with initial stress responses to metal exposure within 30 min. (Cu) or 1 h (Pb). The reactions consisted of increased ventilation and decreased locomotion.

Sublethal concentrations of Pb and Cu caused toxic effects on the behaviour of G. *pulex* after several days of exposure, consisting of increased ventilation and decreased locomotion.

Impedance conversion is an appropriate method for detecting stress responses to metals and can be used in "early warning" biomonitoring systems as well as for acute and chronic behavioural toxicity testing.

Key words: Biomonitoring; exposition; *Gammarus pulex;* crustacea; metals: Cu, Pb; impedance-conversion technique; Pb (NO$_3$)$_2$

1 Introduction

Behaviour is the final outcome of a sequence of neurophysiological events including stimulation of sensory and motor neurons, muscular contractions and release of chemical messages (LAGADIC et al. 1994). According to the **Stimulus-Integration-Response model (SIR)** any overt behavioural response may be seen as a result of integrating external abiotic and biotic stimuli (SCHERER 1992). Behavioural responses integrate many cellular processes vital to an organism's survival and reproduction, thus reflecting both biochemical and ecological consequences of toxic impact (JANSSEN et al. 1994). Changes in behaviour appear to be among the most sensitive indicators of environmental alterations (WARNER 1967; ATCHISON et al. 1987; BEITTINGER 1990).

Behavioural responses to pollutants can generally be of two different characters:

1) A trial to maintain homeostasis in the body by compensatory responses due to "loading stress", which increases the cost of maintenance,
2) an overt effect at toxic concentrations above the regulative capacity, caused by "limiting" stress.

These two categories of environmental stress have been defined in terms of oxygen transport and aerobic metabolism (WILSON et al. 1994), but might be used in a broader sense. This makes behavioural parameters to come into focus in aquatic toxicity testing (toxic effects) and biomonitoring of water pollution (compensatory initial stress responses).

Biomonitoring has several advantages over chemical monitoring in the continuous survey of pollutants in streams. Biomonitoring results are more relevant than chemical monitoring because

(1) it takes the whole mixture of toxicants into account, including synergistic or antagonistic effects,
(2) it puts the organism in focus instead of giving concentration levels, which do not say anything about toxic effects at the organism-, population- or ecosystem level and