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MECHANISTIC APPROACH FOR ESTIMATING BIOCONCENTRATION OF ORGANIC CHEMICALS IN EARTHWORMS (OLIGOCHAETA)

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Abstract—Earthworms (Oligochaeta) represent an important food source for many vertebrates and as a result, predators may encounter toxic effects via the food chain (secondary poisoning) from consumption of contaminated worms. Therefore, including an assessment of secondary poisoning in risk assessment procedures is advisable. In this study, a mechanistic model is presented for estimating bioconcentration of organic chemicals in earthworms. It is assumed that bioconcentration can be described by a thermodynamic partitioning between soil solids, soil water, and the resident organism's tissues. For most chemicals, the lipid phase is the dominant site for sorption in the earthworm, but for more hydrophilic compounds, the water phase may also play a role. Model predictions are compared to literature data that were derived from experiments with earthworms in water, laboratory experiments with various soils, and from field experiments. Without calibration, the model was able to accurately predict bioconcentration factors (BCFs) from experiments in water, indicating the applicability of this theoretical approach. However, BCFs in soil were consistently overestimated by the model (on average a factor of 5.6), which may be due to the absence of true equilibrium conditions in the soil–pore water–earthworm system. The collected experimental data reveal no net influence of uptake via soil ingestion, growth dilution, or sorption to dissolved organic carbon. Field data were more variable, but were generally consistent with the model. Nevertheless, before field data can be accurately predicted, the influences of chemical sorption, sorption kinetics, and earthworm behavior must be quantified under field conditions.

Keywords—Earthworms Oligochaeta Bioconcentration Accumulation Organic chemicals

INTRODUCTION

Risk assessment of chemical substances is traditionally concerned primarily with direct effects of chemicals on organisms: the concentration in an environmental compartment is compared to a relevant effect or no-effect level for organisms living in or in close contact with that compartment. Chemicals can, however, be passed on in the food chain and predators may be largely exposed via their food. This accumulation in the food source is in itself no reason for concern when levels remain below toxic thresholds, but may eventually lead to adverse effects in the organisms preying on them (secondary poisoning). Earthworms (Oligochaeta) play a central role in terrestrial food chains because of their abundance, their relatively large size compared to other soil invertebrates, and the fact that they comprise a large part of the diet of many vertebrate species (e.g., moles, badgers, and thrushes). For certain pesticides, incidents of wildlife poisoning could be directly attributed to feeding on earthworms from treated agricultural areas [1]. The importance of earthworms in secondary poisoning is reflected in the implementation of this specific pathway in the derivation of environmental quality criteria [2] and European risk assessment guidances [3]. For these purposes, a short food chain is modeled to indicate a chemical's potential to cause secondary poisoning: soil → worm → predating bird or mammal. In these procedures, it is assumed that this food chain also protects more intricate food webs.

The assessment of secondary poisoning requires so-called bioconcentration or bioaccumulation factors (BCFs or BAFs), defined as the steady-state ratio between the concentration of a pollutant in the organism and the concentration in its environment. Although the difference between bioconcentration and bioaccumulation is more or less semantic, the first term is generally used to describe uptake from a water phase whereas the second describes the net result of all routes of exposure. In contrast with aquatic organisms, measured data and estimation routines for BCFs or BAFs are scarce for soil organisms. For the soil compartment, a clear need for descriptive and transparent estimation routines exists. Estimation routines in the form of empirical regressions with the octanol–water partition coefficient (K_{ow}) are useful for obtaining a first impression of the accumulating behavior of a chemical. Several authors have reported log-linear regressions for earthworm BCFs on a soil-solution basis (Table 1). Large discrepancies exist among the various regressions (both in slope and intercept), although these studies are not comparable because of radically different experimental designs. The slope and intercept depend heavily on the selected data (the training set) and therefore on the type of chemicals used and the experimental conditions. Extrapolating regressions beyond their domain can lead to serious errors and, clearly, care must be taken when applying these estimation routines in risk assessment procedures. Furthermore, regressions provide little insight into the mechanism of bioaccumulation and the role of organism and soil properties in this process. A more mechanistic approach is therefore preferred, as long as it is sufficiently descriptive for a broad range of chemicals and earthworm species.

For organic chemicals, the main route of exposure for earthworms is uptake from the soil solution through the outer skin [4] and the porewater concentration should therefore be an important factor determining bioavailability. This is supported

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Table 1. Regressions of earthworm bioconcentration factors (BCFs) to the octanol–water partition coefficient (K_{ow}). The BCFs are expressed on a solution basis (L/kg) either on a fresh or dry weight basis (Fwt and Dwt, respectively). The earthworm genus is abbreviated as *E.* = *Eisenia* and *L.* = *Lumbricus*

log BCF =	Basis	Species	Compounds	Log K_{ow} range	Steady state obtained	Experimental design	Reference
$1.06 \log K_{ow} - 2.36$	Fwt	*E. andrei*	Chlorobenzenes	4.2–5.7	Yes	Whole worms in water	[42]
$0.398 \log K_{ow} + 0.724$	Dwt	*L. rubellus*	Chlorophenols	2.5–5.0	No	Worms in soil in laboratory	[6]
$0.547 \log K_{ow} - 0.405$	Dwt	*E. andrei*	Chlorophenols	2.5–5.0	No	Worms in soil in laboratory	[6]
$0.476 \log K_{ow} + 1.04$	Dwt	*L. terrestris*	Pesticides	1–7.5	Unknown	Macerated worms in water	[28]
$1 \log K_{ow} - 0.6$	Dwt	Combined	Mainly pesticides	1–6.5	Unknown/no	Data of [6] and [28] combined with field data	[10]

by studies where bioaccumulation and toxicity for earthworms were found to be related to organic matter in the soil (the main sorption site for organic chemicals) [5,6]. Bioavailability is a rather vague term but is defined here as the fraction of the bulk amount of chemical in soil that can potentially be taken up into the organism's tissues during its lifespan [4]. A mechanistic approach should distinguish between sorption processes in soil and uptake by the earthworm (Fig. 1). When the solid phase, pore water, and organism are in thermodynamic equilibrium, the concentration in the organism is determined by the concentration in the solid phase and the steady-state partition coefficients (K_p and BCF in Fig. 1). This equilibrium partitioning (EP) concept is widely applied in soil and sediment risk-assessment procedures but has several shortcomings (extensively reviewed by Belfroid et al. [4]). Some processes that may reduce the applicability of the EP approach are also shown in Figure 1.

The first process in this three-phase equilibrium is the partitioning of soil solids and soil water. Sorption of neutral organic chemicals in soil is dominated by hydrophobic interactions with soil organic matter and, therefore, good correlations are observed between the partition coefficient normalized to organic carbon (K_{oc}) and the K_{ow} [7]. The second process is the actual bioconcentration process from soil water to earthworm. For aquatic organisms, lipid tissue is the main dissolving medium for organic chemicals [8,9] and relationships between BCFs and K_{ow}s provide satisfactory descriptions. In its simplest form, a mechanistic model for BCF depends on the lipid fraction of the organism and the K_{ow} of the chemical [9]. The same approach was proposed by Connell and Markwell [10] for the soil solution–earthworm system but, unfortunately, their empirical regression is not consistent with the lipid fraction in worms (their data suggest a lipid phase of 25% of the dry weight, whereas 4–6% is more realistic [11]).

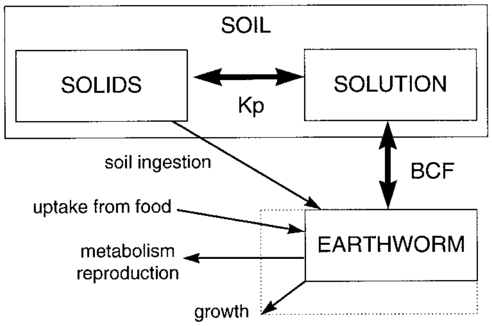

Fig. 1. Processes affecting the concentration of xenobiotics in earthworms. Thick lines represent the equilibrium partitioning theory, thin lines represent processes that may influence the validity of this theory.

The purpose of this study is to evaluate the mechanistic approach for the accumulation of organic chemicals in earthworms. The role of other phases in the worm (water and protein) will be investigated, as well as the possible influence of sorption to dissolved organic carbon (DOC) in soil. Subsequently, this mechanistic approach will be compared to evaluated experimental data from the literature and the applicability for risk assessment will be discussed.

METHODS

Theoretical model

Bioconcentration in aquatic organisms can be regarded as the result of a chemical's distribution between water and the phases inside the organism [9,12]. With this assumption, the organism is reduced to an inanimate container that is seeking a thermodynamic equilibrium with its medium. To reach equilibrium, the organism must be in close contact with the bioavailable phase in its environment to allow for sufficient diffusive exchange of chemicals. For organisms such as fish, this precondition is easily satisfied, as large amounts of water are pumped over the large surface of the gills. For earthworms, this assumption needs further consideration, although the earthworm's physiology provides clues to its appropriateness. Earthworms likely lose 10 to 20% of their body weight in moisture each day because of their respiratory system, which requires the maintenance of a moist outer surface, and because of their nitrogen excretion mechanism, which requires water to dilute ammonia and urea to more hypotonic urine [13]. This water loss can only be replenished by ingestion or direct contact with free water.

To estimate the equilibrium BCF, the chemical's affinity to each of the phases inside the organism must be defined relative to the surrounding medium. With the lipid pool as the main sorbing medium for organic chemicals, a partition coefficient between earthworm and water in equilibrium ($K_{\text{worm–water}}$) can be defined as a function of K_{ow} and the fraction of lipids in the organism (F_{lipid}) as proposed by Connell and Markwell [10]

$$K_{\text{worm–water}} = \frac{[\text{worm}]\ (\text{mg/L})}{[\text{soil solution}]\ (\text{mg/L})} = F_{\text{lipid}} K_{ow}\ (\text{L/L}) \qquad (1)$$

For the affinity of the lipid phase, the K_{ow} is used as a surrogate parameter. Even though octanol and natural lipids are structurally dissimilar, octanol was found to be an appropriate model for sorption of neutral compounds to biomembranes consisting of phospholipids [14]. It should be noted that K_{ow} is not truly dimensionless but is defined as the concentration in the octanol phase (mol/L octanol) divided by the concentration in the water phase (mol/L water), that is, a volume ratio. This implies that the fraction of the lipid phase must be a volume fraction also. This fact is easily overlooked [10,15,16], which

Table 2. Selected standard properties for earthworms

Parameter	Values (range)		Sources
Bulk density worm	1 kg/L		Assumed equal to water
Bulk density lipids	0.83 kg/L		Assumed equal to octanol
Dry to fresh weight ratio	0.16 (0.14–0.20) kg/kg		[2, 19, 29, 41]
	Wet-weight basis	Volume basis	
Fraction water	0.84 (0.80–0.86)	0.84	[2, 19, 29, 41]
Fraction lipids	0.01 (0.006–0.02)	0.012	[11, 29, 40–42]

stresses the relevance of conscientiously specifying units of an equation. A BCF with the general unit of L/kg is derived by dividing the partition coefficient $K_{\text{worm–water}}$ by the bulk density of the organism (ρ_{worm} in kg wet weight/L)

$$\text{BCF} = \frac{K_{\text{worm–water}}}{\rho_{\text{worm}}} \text{ (L/kg worm)} \qquad (2)$$

Because the bulk density is defined here on a wet-weight basis, the BCF is now also expressed on a worm wet-weight basis. This facilitates comparison of concentrations in worms with results of bird or mammalian toxicity tests [2].

In a situation of thermodynamic equilibrium, the water phase inside the organism can be expected to reach the same concentration as the external water (i.e., a partition coefficient of 1). When this phase is added (F_{water}), Equation 1 is extended to

$$K_{\text{worm–water}} = F_{\text{water}} + F_{\text{lipid}}K_{\text{ow}} \text{ (L/L)} \qquad (3)$$

Quantitatively, the internal water phase will be less important than the lipid phase for most hydrophobic chemicals. Equations of this form were successfully applied to describe bioconcentration in fish [15] and uptake of chemicals from air by plants (in that case also including an air phase) [17]. Similar equations are also used in environmental chemistry to describe chemical partitioning between the different phases in abiotic media (soil, sediment, water).

For aquatic organisms, lipid is thought to be the dominant sorbing medium for hydrophobic organic chemicals and other phases are usually ignored. Earthworms have a low lipid and high protein content compared to aquatic organisms (approximately 10 times more protein than lipid [11]) and the protein phase may therefore also play a role in bioconcentration. However, data on the affinity of proteins for organic chemicals are scarce. Neely et al. [18] observed a relationship between bioconcentration in trout muscle (a tissue rich in protein) and K_{ow}. Nevertheless, compared to the combined lipid and water phases, the affinity of these proteins is insufficient to exert influence on bioconcentration. Based on these considerations, the protein phase is ignored as a significant binding site in the model.

Parameters of the general model

Earthworm properties seem to be quite variable among various studies or various species [11,19]. Furthermore, the water and lipid content of the animals may also vary as a result of climatic conditions and nutritional status. In particular, the water content may vary because earthworms do not have efficient mechanisms for water conservation and can survive the loss of up to 70 to 80% of their water content [13,19].

For the model definition, parameter values for a standard worm were selected from literature sources (Table 2). Typical ranges are included for water and lipid contents. Volume fractions are required in the theoretical model, as discussed previously, and weight fractions of a phase were recalculated to a volume fraction by using the densities of the phase and the organism

$$F_{\text{phase}} \text{ (volume)} = F_{\text{phase}} \text{ (weight)} \frac{\rho_{\text{worm}}}{\rho_{\text{phase}}} \qquad (4)$$

Data collection and evaluation

Experimental bioaccumulation data were collected from the literature by on-line search in recent literature and tracing through references. A distinction is made between different experimental designs: earthworms exposed in aqueous medium under laboratory conditions, exposure in soil under laboratory conditions (either spiked or field-contaminated soil), and exposure in soil under field conditions. These exposure situations all provide valuable information but are very different and difficult to compare. Going from water to laboratory soil to the field not only increases the relevance for risk assessment but also the number of variables, thereby diminishing the possibilities for validation of the process mechanisms. The data from these categories are therefore presented separately. The following criteria were applied to evaluate the literature studies. Only data were used with exposure via soil or aqueous medium (thereby discarding food-only or filter paper exposure). In the selected field studies, however, uptake from contaminated food and/or soil ingestion may also have occurred. Organic matter content of the soil must be specified. At very high or very low organic matter contents, sorption is no longer linearly related to organic matter and, therefore, a range in organic matter content of 1 to 30% is taken as acceptable. Preferably, a steady-state situation between soil and earthworm must be achieved in the experiment. In several studies, whether steady state was actually achieved was not determined. These studies were still included when the exposure duration was at least 10 d (this period seems sufficient for most chemicals to reach steady state [20,21]). In other studies, chemicals disappeared from the exposure medium, thereby excluding steady-state situations. Although less valuable for validating the mechanistic model, these studies still provide relevant information for its applicability for risk assessment purposes because the same nonequilibrium conditions will also occur under field conditions. The exposure basis used to calculate the BCF is also listed as additional information in Table 3. Data for dissociating substances were included as long as the fraction in the neutral form (calculated from pKa of the chemical and pH of the soil) was at least 5%. This percentage was taken because it is around this value that the ionic species start to influence uptake in biomembranes (calculated from [14]). Because the anionic species is more polar than the neutral form, sorption and uptake are likely dominated by the latter.

Sorption is therefore calculated from K_{ow} of the neutral form and experimental BCFs are also related to the K_{ow} of the neutral species. In several studies evidence was found of biotransformation by the earthworms. This will usually result in more polar metabolites that are more readily excreted. These studies were still included in the data set to test if metabolism will seriously affect the steady-state BCFs.

Table 3 lists the BCFs as well as important characteristics from all selected studies. All BCF data were recalculated to fresh weight of worms (using the standard worm defined in Table 2) and expressed on soil-solution basis. The soil solids–water distribution in soil (K_p) is calculated from the partition coefficient normalized to organic-carbon (K_{oc}) and the reported fraction organic carbon (F_{oc}) in the study (a fixed ratio of 1.7 between organic matter and organic carbon is assumed [2])

$$K_p = \frac{[\text{soil dry weight}]}{[\text{soil solution}]} = F_{oc}K_{oc} \text{ (L/kg solids)} \qquad (5)$$

The K_{oc} values were estimated using quantitative structure–activity relationships (QSARs) as advised for risk assessment of new and existing chemicals [3]. The QSAR for the group of ‘‘predominantly hydrophobic’’ chemicals was used for most chemicals in this study [7] (for several polychlorinated biphenyls [PCBs], this QSAR was applied outside its log K_{ow} domain of 1–7.5). For chlorophenols and pesticides containing nitrogen groups, QSARs were used as derived for the chemical groups ‘‘phenols and benzonitriles’’ and ‘‘agricultural chemicals,’’ respectively [7]. The K_{ow} values were obtained from the MedChem database (version 3.55; Pamona College, Claremont, CA, USA). The advised measured value (logP*) was preferred, otherwise a calculated value was used (ClogP version 2.10, as given by MedChem). All curve fits and statistical analyses were performed with the software package GraphPad Prism™ (version 2.00; GraphPad Software, San Diego, CA, USA).

Dissolved organic carbon

Dissolved humic and fulvic acids can enhance the apparent water solubility of hydrophobic organic chemicals [22]. Chemicals associated with DOC are generally assumed to be unavailable for diffusive uptake by organisms. This was experimentally confirmed in fish [23] and it is therefore reasonable to assume that DOC-bound chemicals are also not taken up by earthworms through their skin. The association with DOC can be described as a partitionlike process and the truly dissolved fraction (F_{diss}) can be calculated from the DOC concentration (in kg/L) and the normalized partition coefficient with DOC (K_{doc} in L/kg) [22]

$$F_{diss} = \frac{\text{solubility}}{\text{apparent solubility}} = \frac{1}{1 + [\text{DOC}]K_{doc}} \qquad (6)$$

The K_{doc} for soil-derived humic acids was roughly a factor of two lower than K_{oc} (for fulvic acids, this factor was 6–8) [22]. The difference between bulk and dissolved organic matter was attributed to the size of the DOC, its polarity, and molecular configuration. The DOC concentrations in several typical soils were between 20 and 150 mg/L [24]. When only the truly dissolved fraction is bioavailable to the worm, the apparent BCF will be the true BCF multiplied with F_{diss} (Eqn. 6).

RESULTS AND DISCUSSION

General model behavior

Figure 2 shows the general behavior of the model and the effect of adding the water phase in the earthworm as described by Equation 3. The BCF is shown as log-transformed values on a wet-weight basis using Equation 2. For most of the K_{ow} range, the lipid fraction dominates the BCF. The water phase affects the modeled BCF for hydrophilic compounds only (log $K_{ow} < 2$), resulting in a minimum BCF equal to the fraction of water in the worm. Figure 2 also shows the predicted minimum and maximum effect of DOC on apparent BCFs (Eqn. 6). When chemicals associated with DOC are not available for uptake by earthworms, slopes less than unity at log K_{ow} greater than 5 or 6 will result.

The predictions of the theoretical model (as defined by Eqns. 2 and 3) are compared to experimental data from the literature (Table 3). The different exposure situations (water, soil in the laboratory, and field sampling) are treated separately.

Exposure in water

The theoretical model describes the data for earthworms in water very well without calibration (Fig. 3). As shown in Table 4, the fit is good ($R^2 = 0.90$) and improves when only experiments are included in which steady state was attained ($R^2 = 0.97$). This supports the assumption of earthworms as inanimate containers of water and lipids in the model. For chemicals with a log $K_{ow} > 2$, the slope of the experimental data is very close to 1 (Table 4), which means that K_{ow} is an appropriate descriptor for the affinity of these chemicals to worm lipids (at least up to a log K_{ow} of 6). When isoproturon is removed from the data set, the correspondence with linearity of the model is almost perfect. This chemical can be considered an outlier as it was intensively degraded in the solution and in the worm tissue [25] so that steady-state conditions were not achieved.

The data for aldicarb and oxamyl are above the predicted BCF for the lipid phase only (dotted line in Fig. 3), which supports the inclusion of the earthworm's water phase in the model for hydrophilic compounds. The experimental BCF for oxamyl is nevertheless lower than the predictions including the water phase. This BCF is likely to be erroneously low because it was based on the initial water concentration (in a similar experiment with carbofuran in the same study, the concentration in water decreased by 32% during the experiment) and degradation products of oxamyl were detected in the worm [26]. A lack of steady state is a more probable cause for deviation from the model than is metabolism because metabolites are unlikely to have very different BCFs (oxamyl lies in the K_{ow} range where hydrophobicity is no longer expected to affect the BCF). Furthermore, aldicarb was also intensively metabolized [27] but fits the model quite well. However, for pesticides containing nitrogen groups, such as isoproturon and oxamyl, K_{ow} is possibly not as good a predictor of bioconcentration as for the more simple hydrophobic substances such as chlorobenzenes.

Exposure to soil in the laboratory

The laboratory data for earthworms exposed via soil show that residues in earthworms are consistently lower than expected from the model (Fig. 4). On average, this difference is a factor of 5.6 (Table 4). Many factors can mediate the uptake of chemicals from soil or sediment matrices, as reviewed by Belfroid et al. [4]. These factors can be abiotic (e.g., the composition of the soil matrix, sorption kinetics) as well as biotic (e.g., feeding strategies, avoidance, burrowing activity) or a combination of both. Earthworms may deplete the chemical

Table 3. Bioconcentration factors (BCFs) for earthworms on a soil-solution basis (L/kg wet weight). The tested species and whether steady state is achieved are given for each study. For chlorophenols, octanol–water partition coefficients (K_{ow}) and BCFs are given for the neutral species only

Reference	Chemical[a]	Log K_{ow}	Log BCF	Species[b]	Steady state achieved	Exposure concentration
Water						
[42]	1,3,5-Trichlorobenzene	4.19	1.86	Ea	Yes	Constant
[42]	1,2,3-Trichlorobenzene	4.14	2.12	Ea	Yes	Constant
[42]	1,2,3,4-Tetrachlorobenzene	4.64	2.75	Ea	Yes	Constant
[42]	Pentachlorobenzene	5.18	3.24	Ea	Yes	Constant
[42]	Hexachlorobenzene	5.73	3.61	Ea	Yes	Constant
[25]	Isoproturon	2.87	0.05	Lt	No	24 h
[25]	Linuron	3.20	1.24	Lt	Yes	24 h
[25]	Lindane	3.72	1.85	Lt	Yes	24 h
[27]	Aldicarb	1.13	−0.16	Lt	Yes	6 h
[26]	Carbofuran	2.32	0.22	Ef	No	8 h
[26]	Oxamyl	−0.47	−1.06	Ef	Unknown	Initial
Soil						Exposure basis
[20]	1,2,3,4-Tetrachlorobenzene	4.64	2.64	Ea	Yes	Constant
[20]	Pentachlorobenzene	5.18	3.25	Ea	Yes	Constant
[20]	Hexachlorobenzene	5.73	3.84	Ea	Yes	Constant
[21]	1,2,3,4-Tetrachlorobenzene	4.64	1.50	Ea	Yes	Constant
[21]	Pentachlorobenzene	5.18	1.71	Ea	Yes	Constant
[21]	Hexachlorobenzene	5.73	3.52	Ea	Yes	Constant
[21]	PCB 101	6.50	3.86	Ea	Yes	Constant
[21]	PCB 118	7.12	4.51	Ea	Yes	Constant
[21]	PCB 138	7.25	4.49	Ea	Yes	Constant
[21]	PCB 153	7.16	4.51	Ea	Yes	Constant
[21]	PCB 156	7.57	4.83	Ea	Yes	Constant
[21]	PCB 167	7.50	4.69	Ea	Yes	Constant
[21]	PCB 180	8.04	5.10	Ea	Yes	Constant
[33]	1,3,5-Trichlorobenzene	4.19	1.21	Lt	Yes	Constant
[33]	1,2,4-Trichlorobenzene	4.02	1.08	Lt	Yes	Constant
[33]	1,2,3-Trichlorobenzene	4.14	1.26	Lt	Yes	Constant
[33]	1,2,3,5-Tetrachlorobenzene	4.66	1.50	Lt	Yes	Constant
[33]	1,2,4,5-Tetrachlorobenzene	4.60	1.50	Lt	Yes	Constant
[33]	1,2,4,5-Tetrachlorobenzene	4.64	1.52	Lt	Yes	Constant
[33]	Pentachlorobenzene	5.18	2.30	Lt	Yes	Constant
[33]	Hexachlorobenzene	5.73	2.94	Lt	Yes	Constant
[25]	Linuron	3.20	0.27	Lt	No	Average
[25]	Lindane	3.72	2.12	Lt	No	Average
[5]	Dieldrin	5.20	2.43	Ac	Unknown	Final
[5]			2.36	Ac	Unknown	Final
[5]			2.44	Ac	Unknown	Final
[5]			2.74	Ac	Unknown	Final
[5]			2.67	Ac	Unknown	Final
[5]	DDT	6.91	3.41	Ac	Unknown	Initial
[5]			3.61	Ac	Unknown	Initial
[5]			3.58	Ac	Unknown	Initial
[5]			3.66	Ac	Unknown	Initial
[5]			3.71	Ac	Unknown	Initial
[43]	Imazalil	3.82	0.57	Ac	Unknown	Initial
[43]	Triadimenol	3.08	1.05	Ac	Unknown	Initial
[43]			1.25	Lt	Unknown	Initial
[30]	2,3,7,8-TCDD	6.42	4.76	Ac	No	Maximum BCF
[30]			3.81	Ac	No	Maximum BCF
[30]			3.72	Ac	No	Maximum BCF
[30]			3.43	Ac	No	Maximum BCF
[29]	Phenanthrene	4.46	1.60	Lr	No	Maximum BCF
[29]			1.23	Lr	No	Maximum BCF
[29]			0.94	Lr	No	Maximum BCF
[29]	Fluoranthene	5.16	2.49	Lr	No	Maximum BCF
[29]			1.88	Lr	No	Maximum BCF
[6]	3-Chlorophenol	2.50	0.09	Ea	No	Average
[6]			0.41	Ea	No	Average
[6]			1.26	Lr	No	Average
[6]			1.27	Lr	No	Average
[6]	3,4-Chlorophenol	3.33	0.78	Ea	No	Average
[6]			0.64	Ea	No	Average
[6]			0.63	Lr	No	Average
[6]			0.88	Lr	No	Average
[6]	2,4,5-Chlorophenol	3.72	0.92	Ea	No	Average

Table 3. Continued

Reference	Chemical[a]	Log K_{ow}	Log BCF	Species[b]	Steady state achieved	Exposure basis
[6]			0.56	Ea	No	Average
[6]			1.14	Lr	No	Average
[6]			1.88	Lr	No	Average
[6]	2,3,4,5-Chlorophenol	4.21	0.72	Ea	No	Average
[6]			0.84	Ea	No	Average
[6]			1.63	Lr	No	Average
[6]			1.60	Lr	No	Average
[6]	Pentachlorophenol	5.12	2.26	Ea	No	Average
[6]			2.29	Ea	No	Average
[6]			2.14	Lr	No	Average
[6]			2.66	Lr	No	Average
[40]	2,3,4,6-Tetrachlorophenol	4.45	2.36	Ac	No	Average
[40]			2.82	Lr	No	Average
[40]			2.79	Ac	No	Average
[40]	Pentachlorophenol	5.12	2.67	Ac	No	Average

Field-collected earthworms

Reference	Chemical	Species	Description of study
[44]	PCBs and pesticides	Lr	Polluted floodplains in the Rhine delta (The Netherlands) at two locations (5 and 9% om); DDT residues summed.
[45]	Pesticides	Several	Treated study plots followed for 2 years (3.2–4.6% om); DDT residues summed.
[46]	Chlorobenzenes, dioxins, furans	Several	Earthworms sampled at contaminated refuse dump (21–24% om).
[47]	Pesticides	Several	Study site in a hayfield followed for 11 years (3–5% om); DDT residues summed.

[a] PCB = polychlorinated biphenyl; 2,3,7,8-TCDD = 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

[b] Ea = *Eisenia andrei,* Ef = *Eisenia fetida,* Lt = *Lumbricus terrestris,* Lr = *Lumbricus rubellus,* Ac = *Aporrectodea caliginosa.*

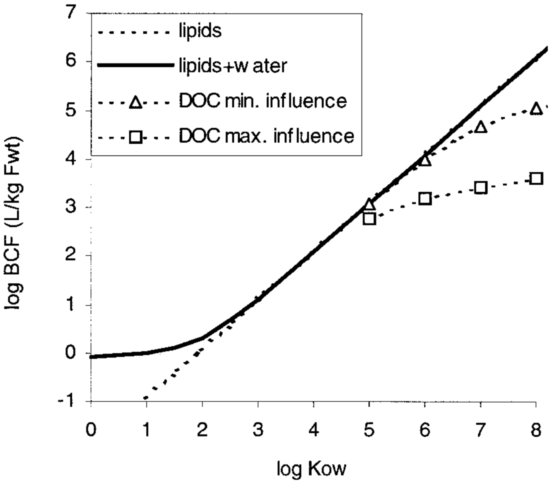

Fig. 2. Behavior of the theoretical bioconcentration model (bioconcentration factor [BCF] on solution basis in L/kg wet weight). The potential influence of dissolved organic carbon (DOC) on the apparent BCF when DOC-associated chemicals are not available to the organism. The maximum effect is obtained assuming $K_{doc} = 0.5K_{oc}$ and [DOC] = 150 mg/L, the minimum effect assumes $K_{doc} = 0.125K_{oc}$ and [DOC] = 20 mg/L. K_{doc} is the normalized partition coefficient with DOC and K_{oc} is the partition coefficient normalized to organic carbon.

pool in the pore water in their immediate surroundings (especially for very hydrophobic chemicals, which will have low concentrations in the water phase). Desorption from soil solids or diffusion through soil can subsequently become the rate-limiting processes [4]. Evidence for this depletion is found in studies where stirring of the soil increased concentrations in worms [28] and where wet soil resulted in higher uptake rates compared to soil with a lower moisture content [27]. Furthermore, in several studies, the residues in earthworms

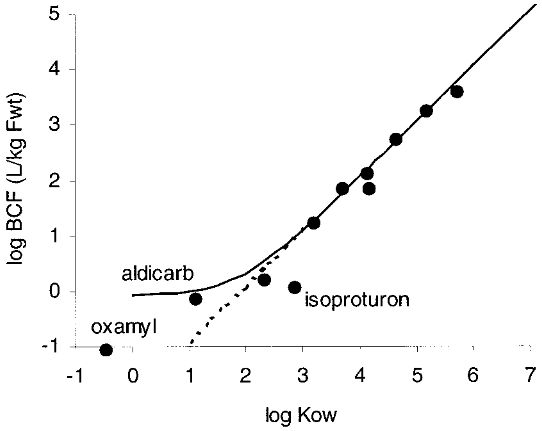

Fig. 3. Comparison of the theoretical bioconcentration model with experimental bioconcentration factor (BCF) data from experiments in water (BCF expressed in L/kg wet weight). The dotted line represents the behavior of the lipid phase only.

Table 4. Model fitting (Eqns. 2 and 3) and log-linear regressions of the experimental data against the octanol–water partition coefficient (K_{ow}). The modifying factor M represents a constant factor through which the model bioconcentration factor (BCF) must be divided to provide the best fit to the data

	Fitting the theoretical model			
Data sets	M	95% confidence interval	R^2	n
Water exposure[a]	1	—	0.90	11
Only steady-state data[b]	1	—	0.97	8
Laboratory soil data	5.6	3.9–7.2	0.83	69
Neutral compounds	7.1	4.7–9.6	0.86	45
Chlorophenols	3.5	1.5–5.5	0.54	24
PCBs[c] in field	38	32–44	0.81	55

	Linear regressions (log K_{ow} − log BCF)				
	Slope	95% confidence interval	R^2	n	Intercept
Water exposure[d]	1.09	0.86–1.32	0.95	9	−2.49
Excluding isoproturon	1.00	0.86–1.14	0.98	8	−2.03
Laboratory soil data	0.87	0.78–0.96	0.84	69	−2.00
Neutral compounds	0.94	0.82–1.05	0.86	45	−2.43
Chlorophenols	0.74	0.46–1.01	0.58	24	−1.42
PCBs in field	0.93	0.81–1.05	0.82	55	−3.01

[a] No curve fitting performed.

[b] See Table 3.

[c] PCB = polychlorinated biphenyl.

[d] Only data for log $K_{ow} > 2$ included.

reached a maximum in time, after which they declined [25,29,30] (even when the bulk concentrations in soil remained relatively constant).

The earthworm's behavior may also affect bioavailability. With regard to feeding habits, earthworms can be roughly divided into two groups, the humus formers or litter feeders, which eat slightly decomposed plant materials (e.g., *Lumbricus terrestris* and *Lumbricus rubellus*), and humus feeders or soil feeders (geophageous species), which consume organic materials dispersed in the soil (e.g., *Aporrectodea caliginosa*) [31]. The popular test species *Eisenia fetida* and *Eisenia andrei* can be considered litter feeders although they are not typical soil-dwelling species. They prefer accumulations of organic matter such as rotten vegetation and compost and manure heaps [32]. Within these groups, several subgroups of species can be identified according to their vertical distribution. Litter feeders will only ingest large amounts of soil when burrowing through compact soil [13] or possibly when driven by hunger stress [29]. In two studies, strong indications were found that behavior influenced BCFs. Beyer [33] found much lower BCFs for chlorobenzenes than did other authors [20] (both in artificial soil). Furthermore, he observed a cyclic trend in the residues of hexachlorobenzene (HCB) in earthworms, coinciding with transferring the earthworms to fresh soils. The experiments were performed with *L. terrestris*, a species that constructs permanent burrows, so the construction of new burrows in the fresh soil could have temporarily increased exposure to HCB. Ma et al. [29] found higher BCFs for *L. rubellus* when kept without a food source, probably because the worms were ingesting more soil when driven by hunger stress. This is supported by the experiments of Martin [34], who found that lack of food increased burrowing and soil ingestion.

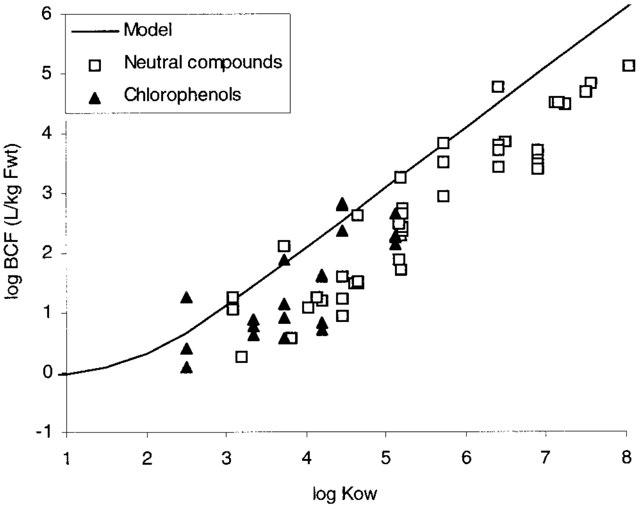

Fig. 4. Comparison of the theoretical bioconcentration model with experimental bioconcentration factor (BCF) data from laboratory experiments in soil. All BCFs are expressed on soil-solution basis (L/kg wet weight).

The present data set reveals no significant differences between different species or between natural soil and artificial medium. Even the data for field-contaminated soil are not particularly deviating from laboratory-spiked soils. This does not imply that these factors are unimportant as the effect may be largely disguised by the combination of these factors and differences in experimental design. For example, when only studies with chlorobenzenes are examined, a clear difference between two species in artificial soil can be seen (*E. andrei* [20] has higher BCFs than *L. terrestris* [33], $p < 0.0001$), as well as a slight difference between artificial [20] and field-contaminated soil [21] for *E. andrei* ($p < 0.1$). In a direct comparison, a difference between two species was found for chlorophenols [6]: BCFs for *L. rubellus* were generally higher than those for

E. andrei. This difference may be explained by their difference in size, differences in behavior, or by the different incubation temperatures. However, determining the cause of the difference is impossible because steady state was not achieved (the chemicals disappeared from the soil) and the worms were only measured at the end of the experiment.

The slope of the laboratory BCFs is very close to unity, as expected from the model, and, when chlorophenols are excluded, not significantly different from 1 (Table 4). For chlorophenols [6], the low slope in Table 1 may originate from the fact that BCFs were expressed by the original author on a total soil-solution basis, thereby including the neutral as well as the anionic species. The anionic form is more polar and the apparent hydrophobicity of the chemical will therefore decrease with increasing pH. The K_{ow} in the regression should therefore be corrected for the degree of dissociation, leading to steeper curves. It is also possible that interactions other than hydrophobic ones play a role in the bioconcentration process for these compounds. The BCFs for chlorophenols are quite variable and show little relation with K_{ow} (Table 4). Sorption of chlorophenols also shows less dependence on K_{ow} than for neutral compounds [7]. Furthermore, these BCFs are also on average closer to the model than those of neutral compounds ($p < 0.05$). However, drawing firm conclusions from these data is not possible because steady-state conditions were not achieved (see Table 3).

Slopes slightly less than unity are commonly found for aquatic bioconcentration data [35] and plant tissues [16] and are usually explained by structural differences between octanol and natural lipids or hindered transport over membranes. Interestingly, very low regression slopes (0.4–0.55) were reported for earthworms by several other authors (Table 1). For the pesticide QSAR [28] the low slope cannot be entirely explained although the experimental setup is in this case disputable. These data were excluded from this study because macerated worms were used, and only the concentration in water was measured. Steady state was unlikely to have been achieved, and for most chemicals indications were found of substantial chemical loss from the containers (by volatilization, metabolism, and/or degradation). For one chemical (aldicarb), BCFs were a factor 10 lower in a comparable study where whole worms were used [27].

No loss of linearity of the K_{ow}–BCF relationship could be discerned for very hydrophobic compounds, as is commonly observed for aquatic organisms [35]. This implies that the collected experimental data do not support a net effect of soil ingestion or sorption to DOC on BCF. Increased BCFs due to soil ingestion at log $K_{ow} > 5$ were predicted from an uptake model by Belfroid et al. [36]. However, it should be noted that the expected effect was at maximum only a factor of two. This kind of influence cannot easily be discerned on a log scale, as applied in this study. Worms are able to take up organic chemicals through feeding [37] but, nevertheless, this route does not seem to lead to increased body burdens. Apparently, the equilibrium-partition hypothesis still holds. Furthermore, DOC is unlikely to play a role in the bioconcentration process because a profound loss of linearity is expected when DOC-bound chemicals are not taken up (Fig. 2). However, it is still possible that DOC-bound chemicals cannot pass the outer skin but are remobilized during gut passage of soil when organic materials are digested.

Field-collected earthworms

In the field, the soil environment is much more heterogeneous than in the laboratory and further variability can be expected due to the earthworm's ecology. Earthworms in a field situation can express more of their natural behavior than in a laboratory jar, which may affect exposure to chemicals (e.g., due to feeding habits, burrowing, diapause, surface activity) [38]. Furthermore, additional variation can be expected because chemical sorption may increase with time (aging of the contamination). Four different field studies were selected (described in Table 3) and are shown in Figure 5A to D.

The BCFs found for PCBs are much lower than expected from the model (Fig. 5A); the difference is on average a factor of 38 (Table 4), which is also lower than observed for these chemicals in a field-contaminated soil tested in the laboratory [21]. Therefore, aging is unlikely to be solely responsible for this deviation. Species differences may have contributed to a different exposure (*L. rubellus* sampled from the polluted site, *E. andrei* in the laboratory study), although both species are litter feeders. Another difference between the field and the laboratory study is that in the latter the soil is homogenized before earthworms are introduced, thereby enhancing bioavailability. Furthermore, laboratory conditions diminish the possibilities for the earthworms to avoid the contamination (actively or unintentionally) [39]. The soil concentration in the field study was reported as an average of the top 20 cm but the worms may confine their activity to only a small part of this range (depending on the soil moisture content) and thereby may modify their exposure. Despite the deviations from the model, the slope of the data was very close to 1 (Table 4).

Figure 5B shows data for some chlorobenzenes, dioxins, and furans in earthworms from a refuse dump. In contrast with the PCBs in Figure 5A, the data are quite close to the model and some are even somewhat higher than predicted. Although the contamination was at least 15 years old, no aging effects were visible and bioavailability was quite high. The soil samples were taken from the top 10 cm and the earthworms sampled were all shallow-living species, including the soil feeder *Aporrectodea rosea*. These factors may have led to a closer correspondence with the model predictions than in the study with PCBs (Fig. 5A), where the litter-feeding *L. rubellus* and the top 20 cm of soil were sampled.

Figure 5C and D show data for surface-applied pesticides. The short-term average BCFs in Figure 5C are quite close to the model (data points indicate the geometric average over 0.5–4 months after application). The large variability in time indicated by the error bars is, however, striking. Generally, the BCFs decreased in the 2 years following application. Surface application will inevitably lead to higher concentrations in the upper soil layers, thereby increasing the exposure of surface-feeding or surface-active species in the initial period after treatment. In time, this effect will disappear as the chemical distributes more evenly through the soil layers and, furthermore, aging may result in lower apparent BCFs. The variability is especially large for heptachlor and its metabolite heptachlor epoxide. This transformation process was faster in the worms than in soil, leading to high initial concentrations of heptachlor epoxide in worm compared to soil (Fig. 5C). The pesticide BCFs in Figure 5D resulted from surface application, followed up to 11 years after treatment. These BCFs are also quite comparable to the model. The BCFs of heptachlor epoxide and dieldrin declined only a factor of 4 and 1.5 over this period,

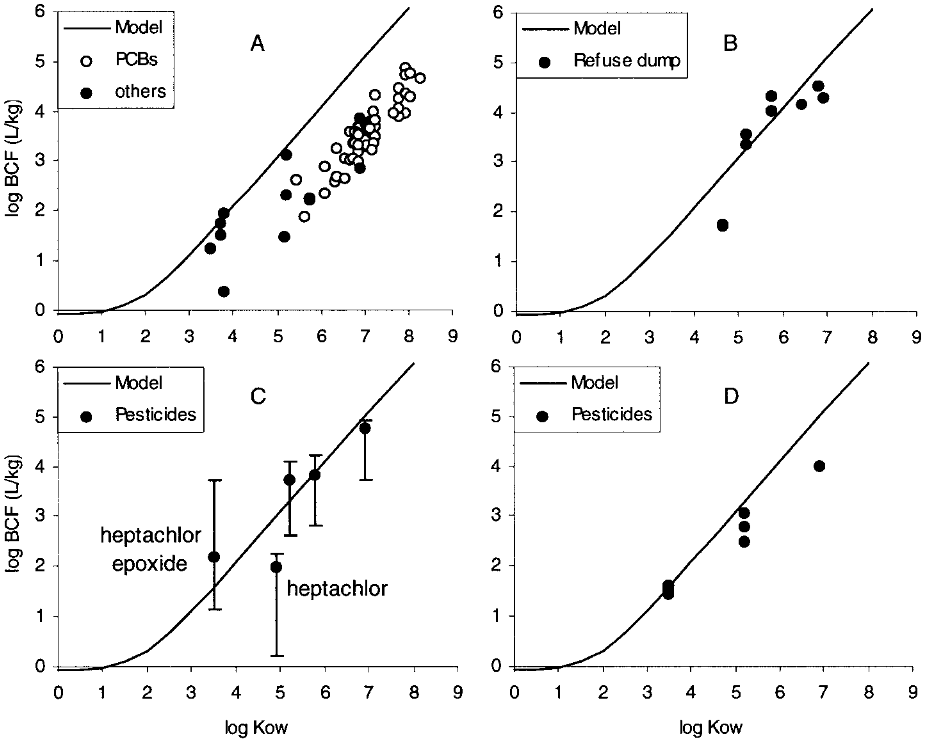

Fig. 5. Bioconcentration factors (BCFs) expressed on a soil-solution basis (L/kg wet weight) for field-collected earthworms. (**A**) Sampling from polluted floodplains. (**B**) Sampling from a refuse dump. (**C**) Sampling from agricultural soil with surface-applied pesticides. Points represent geometric averages over 0.5 to 4 months postapplication; range represents minimum and maximum over the entire 2-year period. (**D**) Sampling from agricultural soil with surface-applied pesticides. Points represent samples taken at 0, 5.5, and 11 years after treatment (BCFs decreasing in time).

respectively. The BCFs for total DDT residues remained remarkably stable in time and no aging effect could be discerned.

In summary, field data are reasonably consistent with the model, although they seem to be more variable than the laboratory data. This can be expected as the exact exposure concentration that the earthworms encounter in their microhabitat within the heterogeneous soil environment is not known. The interpretation of the field BCFs therefore strongly depends on the species sampled and the method and depth of soil sampling. Nevertheless, the slope of the field data remains close to 1, as predicted by the model, and shows no net result of uptake through food or sorption to DOC.

CONCLUSIONS

The collected experimental data are generally consistent with the theory that bioconcentration in earthworms is governed by thermodynamic partitioning of the chemical between surrounding water and the water and lipid phases inside the organism. Data from worms exposed to hydrophobic compounds in aqueous media are especially well predicted by this model. Generally, soil exposure leads to lower BCFs than expected. This is probably caused by nonequilibrium conditions because of slow desorption from soil solids, slow diffusion in soil, and/or earthworm behavior. Differences in experimental design and lack of direct comparisons preclude conclusions on differences between species and soil types. Uptake from ingested soil, dilution by growth, and sorption to DOC do not seem to exert a net influence on the body residues. Treating dissociating substances like neutral compounds by ignoring the ionic form in the calculation of sorption and bioconcentration (at least when the degree of dissociation is below 95%) seems possible. Nevertheless, indications exist that the mechanism of bioconcentration differs from neutral compounds. The theoretical model also seems to work for field situations, although field BCFs can only be reproduced when bioavailability is adequately quantified in terms of sorption kinetics and earthworm behavior.

For risk assessment purposes, Equations 2 and 3 can be used to predict BCFs for earthworms in the log K_{ow} range 0 to 8. This estimate should be regarded as a maximum BCF that is not always reached in soil situations. The theoretical model also seems sufficiently protective to cover most field situations but special care must be taken in case of pesticide spraying. The heterogeneous vertical distribution of the chemical in soil or the specific contamination of food sources may result in high exposure for specific species. As an example, *L. terrestris,* a litter feeder that constructs semipermanent burrows, is more susceptible to chemicals present in litter and granular pesticide formulations, but less susceptible to chemicals incorporated in the top soil layer, compared to shallow-living soil feeders such as *A. caliginosa* [5,39,40]. The ecology of individual species can thus be a dominant factor influencing body residues in the field.

Uptake and sorption to soil solids are inversely related to hydrophobicity of the compound. On a total soil basis, BAFs will therefore show little dependence on K_{ow}. Instead, concentration factors will be low and depend mainly on organism properties and organic matter content of the soil. Low concentration factors, however, do not imply that no danger exists to vertebrates feeding on earthworms, because the risk will also depend on the absolute concentrations in soil and the sensitivity of the predator. Furthermore, despite the relative ease of modeling uptake by earthworms from pore water, this approach may not apply to other soil organisms that live in less direct contact with the soil solution [41].

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