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Toxicity and Bioaccumulation of Chlorophenols in Earthworms, in Relation to Bioavailability in Soil

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The acute toxicity of five chlorophenols for two earthworm species was determined in two sandy soils differing in organic matter content and the results were compared with adsorption data. Adsorption increased with increasing organic matter content of the soils, but for tetra- and pentachlorophenol was also influenced by soil pH. Earthworm toxicity was significantly higher in the soil with a low level of organic matter. This difference disappeared when LC_{50} values were recalculated to concentrations in soil solution using adsorption data. *Eisenia fetida andrei* showed LC_{50} values lower than those of *Lumbricus rubellus* although bioaccumulation was generally higher in the latter species. Toxicity and bioaccumulation based on soil solution concentrations increased with increasing lipophilicity of the chlorophenols. The present results indicate that the toxicity and bioaccumulation and therefore the bioavailability of chlorophenols in soil to earthworms are dependent on the concentration in soil solution and can be predicted on the basis of adsorption data. Both the toxicity of and bioaccumulation data on chlorophenols in earthworms demonstrated surprisingly good agreement with those on chlorophenols in fish.
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INTRODUCTION

The toxicity and bioaccumulation of chemicals in soil organisms are a function of the physicochemical properties of the substance and of the soil. Adsorption has a strong influence on bioavailability (Böttger, 1976) and for organic chemicals is correlated with the lipophilicity (expressed as the octanol/water partition coefficient P_{oct}) of the individual chemical and the organic matter content of the soil (Brown and Flagg, 1981). The purpose of this study was to establish relationships between toxicity and bioaccumulation of chemicals in earthworms on the one hand and lipophilicity and adsorption to soil on the other.

MATERIALS AND METHODS

Chemicals. 3-, 3,4-Di-, 2,4,5-tri-, and 2,3,4,5-tetrachlorophenol were obtained from Fluka AG and were >97% pure; 2,3,4,6-tetrachlorophenol was supplied by Aldrich Chemie and appeared on analysis to contain a large amount of pentachlorophenol as impurity. Pentachlorophenol was obtained from Merck and was 95% pure.

Table 1 shows the pK_a and log P_{oct} values of these chemicals. By mistake, the 2,3,4,6-isomer of tetrachlorophenol was used in adsorption experiments, whereas the toxicity and bioaccumulation studies were carried out with the 2,3,4,5-isomer.

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TABLE 1

PHYSICOCHEMICAL PROPERTIES OF THE CHLOROPHENOLS USED IN THIS STUDY

	Chlorophenol					
	3-	3,4-	2,4,5-	2,3,4,5-	2,3,4,6-	Penta-
pK_a[a]	9.37	8.62	6.72	5.64	5.22	4.74
$\log P_{oct}$[b]	2.5	3.2	3.9	4.5	4.4	5.0

[a] After Ugland *et al.* (1981).
[b] After Xie and Dyrssen (1984).

Soils. Soil was collected from the top 20 cm of agricultural fields, sieved, air-dried, and stored in the laboratory until used. Table 2 shows the most important characteristics.

Earthworms. Earthworms of the ("compost") species *Eisenia fetida andrei* were grown in the laboratory on horse manure at an ambient temperature of 20–22°C. The worms used in this study were adults with a well-developed clitellum (more than 6 weeks old) and their average weight ranged between 324 and 486 mg. Earthworms of the soil-inhabiting species *Lumbricus rubellus* were collected from the field and preincubated in breeding boxes at 15°C. Clitellated adult worms weighing on average between 1314 and 1816 mg were used. To assess the influence of smaller body weight, the effect of 2,4,5-trichlorophenol was additionally determined for smaller adults weighing between 544 and 696 mg.

Toxicity assessment. Chemicals were mixed homogeneously through the soils. 3-, 3,4-Di-, and 2,4,5-trichlorophenol were dissolved in water under the addition of some drops of ethanol and added to the soils as aqueous solutions. 2,3,4,5-Tetra- and penta-chlorophenol, which are not readily soluble in water, were mixed with a small amount of dry soil and then added to the rest of the soil. Soils were mixed thoroughly and the moisture content was raised to the desired level of 16% (w/w).

Glass jars intended for *E. fetida andrei* received about 0.5 kg soil (dry wt) and 10 worms. For *L. rubellus*, about 0.65 kg soil (dry wt) and 5 worms were put into each jar. The jars were placed in an incubator set at the optimal temperature for each species, i.e., 23 and 15°C, respectively. Mortality was determined after 7 and 14 days. Earthworms were considered dead if they did not respond to gentle mechanical pressure on their anterior parts.

TABLE 2

MAIN CHARACTERISTICS OF THE SOILS USED IN THIS STUDY

Characteristic	Holten soil (very humic sand)	Kooyenburg soil (moderately humic sand)
pH (0.01 *N* KCl)	5.6	5.2
pH (1 *N* KCl)	5.6	4.8
% organic matter	6.1	3.7
% clay	2.4	1.4
CEC (meq · 100 g^{-1})	10.0	6.6

TABLE 3

FREUNDLICH ADSORPTION COEFFICIENTS FOR FIVE CHLOROPHENOLS IN TWO SOILS

	K_f for chlorophenol				
Soil	3-	3,4-	2,4,5-	2,3,4,6-	Penta-
Holten	12	30	78	95	125
Kooyenburg	6	15	43	85	120

A range-finding test with chemical concentrations of 0, 0.1, 1, 10, 100, and 1000 $mg \cdot kg^{-1}$ dry soil was used to determine the concentrations causing 0 and 100% mortality, and the definitive test was performed with five selected concentrations and a control. Ten earthworms were used for each concentration in the range-finding test and 30 in the definitive test. For the definitive test earthworms were preconditioned by keeping them under the same conditions but in untreated soil for 24 hr.

Bioaccumulation tests. Bioaccumulation was determined from analysis of samples of soil and earthworms containing a nonlethal concentration of one of the chemicals under study taken on Days 0 and 14. The earthworms were placed on wet filter paper for 24 hr in the dark at 23 ± 2°C to allow the gut to empty and were then frozen at −23°C. Soil samples were stored at 4°C before analysis. For the determination of dry weights, samples of frozen earthworms were dried at 105°C for 16 hr before they were reweighed.

Adsorption studies. Adsorption to soil was assessed by shaking solutions of the chlorophenols in 0.01 *M* $CaCl_2$ with air-dried soil at a water:soil ratio of 5:1 for 16 hr. After centrifugation, the supernatants were analyzed and adsorption coefficients were calculated according to Freundlich's equation.

Statistics. LC_{50} values based on 14 days' mortality data were calculated according to a logit model. The effect of soil type on the toxicity was assessed by comparing an adapted form of the individual logit curves with that of a common curve for both soils (McCullagh and Nelder, 1983). Results of all tests with both earthworm species were combined for an analysis of variance on the log LC_{50} values with four factors: earthworm species, chlorophenol, soil type, and the two-way interaction among these factors. With this analysis of variance we also wished to determine whether the effect of the chlorophenols and their log P_{oct} could be properly described by a linear relationship (Montgomery, 1976).

Chemical analysis. Samples (water, soil, or earthworms) were acidified and extracted with toluene. The toluene was then extracted with a K_2CO_3 solution. Chlorophenols were acetylated by addition of acetic anhydride and petroleum ether to this K_2CO_3 extract. The acetates obtained were analyzed by GLC with ECD.

RESULTS

Table 3 shows the Freundlich adsorption coefficients (K_f); $1/n$ values lay between 0.77 and 0.85. It is clear from this table that for mono-, di-, and trichlorophenol, differences in adsorption between the two soils reflect the difference in organic-matter content. Adsorption increases with increasing chlorination of the compounds. This

TABLE 4

TOXICITY OF CHLOROPHENOLS FOR THE EARTHWORM *Eisenia fetida andrei* IN TWO SOILS (LC_{50} AND 95% CONFIDENCE INTERVAL)

	LC_{50} ($mg \cdot kg^{-1}$ dry soil)		
Chlorophenol	Kooyenburg soil	Holten soil	Difference
3-	>56, <100	>100, <180	Not tested
3,4-	>100, <180	>180, <320	Not tested
2,4,5-	52 (45–61)	90 (77–104)	$P < 0.01$
2,3,4,5-	116 (104–129)	176 (163–191)	$P < 0.01$
Penta-	94 (81–109)	143 (119–172)	$P < 0.01$

increase is lower for tetra- and pentachlorophenol because of dissociation; for these substances the pK_a values are lower than the soil pH, indicating that they were predominantly present as the negatively charged phenolate ions.

Tables 4 and 5 show the LC_{50} values for the toxicity of the five chlorophenols under study for the two earthworm species and the two soils under study. For the smaller individuals of *L. rubellus*, LC_{50} values for the toxicity of 2,4,5-trichlorophenol were 185 (95% confidence interval: 156–220) and 278 (248–313) $mg \cdot kg^{-1}$ for the Kooyenburg and Holten soils, respectively, indicating that in comparison with the data shown in Table 5 the sensitivity does not differ much between large and small individuals.

Bioaccumulation factors (BCF) were estimated by dividing the concentration in earthworms at the end of the 14-day exposure period by the average soil concentration during the same period. The average soil value was estimated from the 0- and 14-day soil concentrations on the basis of a first-order degradation rate. Table 6 shows the DT_{50} values for the disappearance of chlorophenols from the soil and the BCF values.

DISCUSSION

The literature offers little information about the toxicity of chlorophenols for earthworms. Only pentachlorophenol has been studied frequently, e.g., as a reference sub-

TABLE 5

TOXICITY OF CHLOROPHENOLS TO THE EARTHWORM *Lumbricus rubellus* IN TWO SOILS (LC_{50} AND 95% CONFIDENCE INTERVAL)

	LC_{50} ($mg \cdot kg^{-1}$ dry soil)		
Chlorophenol	Kooyenburg	Holten	Difference
3-	150 (145–156)	296 (264–332)	$P < 0.01$
3,4-	303 (245–375)	486 (427–554)	$P < 0.01$
2,4,5-	201 (165–246)	290 (247–341)	$P < 0.01$
2,3,4,5-	514 (474–558)	828 (664–1033)	$P < 0.01$
Penta-	1094 (977–1225)	883 (775–1033)	n.s.

TABLE 6

DT_{50} VALUES FOR THE DISAPPEARANCE OF CHLOROPHENOLS FROM SOIL AND BCF VALUES FOR THE ACCUMULATION IN THE EARTHWORMS *Eisenia fetida andrei* AND *Lumbricus rubellus*

		DT_{50} (days) in soil		BCF in soil	
Species	Chlorophenol	Kooyenburg	Holten	Kooyenburg	Holten
E. fetida andrei	3-	2.5	5.4	1.1	1.4
	3,4-	10.1	11.2	1.8	0.8
	2,4,5-	3.4	6.6	1.5	0.4
	2,3,4,5-	43.4	29.1	0.5	0.4
	Penta-	23.2	47.9	5.3	3.4
L. rubellus	3-	2.6	2.1	16.3	10.1
	3,4-	24.7	49.5	1.3	1.4
	2,4,5-	39.6	13.9	2.5	8.4
	2,3,4,5-	26.8	42.5	4.1	2.3
	Penta-	27.4	31.8	4.0	8.0

stance in an EEC ring test. In the present study the LC_{50} for *E. fetida* in an artificial soil substrate was 75.1 ± 40.6 mg·kg^{-1} ($n = 18$). In another ring test, the average LC_{50} amounted to 68.9 mg·kg^{-1} ($n = 32$) (Edwards, 1983). Heimbach (1984) reported an LC_{50} of 87 mg·kg^{-1} for the toxicity of pentachlorophenol to *E. fetida andrei* in an artificial soil. Van Gestel and van Dis (1988) determined the toxicity of pentachlorophenol for the same species in three soils and found that toxicity increased with decreasing organic-matter content and increasing soil pH; LC_{50} values lay between 16 and 52 mg·kg^{-1}. According to Goats (1983), a field application of 12.5 kg·ha^{-1} pentachlorophenol is toxic to *L. terrestris* and *Allolobophora longa*. Neuhauser *et al.* (1986) determined the toxicity of 2,4,6-trichlorophenol in an artificial soil for four earthworm species; LC_{50} values ranged between 58 and 100 mg·kg^{-1}, and *E. fetida* appeared to have the highest sensitivity.

No data on the bioaccumulation of chlorophenols in earthworms were found in the literature. The uptake of DDT and dieldrin by earthworms was found to increase with decreasing organic-matter content of the soil (Davis, 1971), and Briggs and Lord (1983) found the same effect of organic matter for the uptake of aldicarb by *L. terrestris*.

As Tables 4 and 5 show, toxicity proved to be correlated with adsorption. Conversions of the LC_{50} values in these tables to concentrations in soil solution with the use of the adsorption coefficients given in Table 3 are shown in Table 7. From Table 7 it is evident that the differences in toxicity between the two soils completely disappear after correction for adsorption. An analysis of variance on the log LC_{50} values expressed as μmol·liter^{-1} showed that the difference between the two soils is not significant ($P > 0.9$). This indicates that the toxicity of chlorophenols for earthworms is correlated with the concentration in soil solution and can be predicted on the basis of adsorption coefficients.

From Tables 4, 5, and 7 it is clear that *E. fetida andrei* is more sensitive than *L. rubellus* to all of the chemicals tested and in both soil types. It should be realized, however, that the experimental temperature differed for the two species tested, being

TABLE 7

TOXICITY OF CHLOROPHENOLS FOR THE EARTHWORMS *Eisenia fetida andrei* AND *Lumbricus rubellus* ACCORDING TO LC_{50} VALUES BASED ON CONCENTRATIONS IN SOIL SOLUTION

	LC_{50} as $\mu mol \cdot liter^{-1}$ soil solution			
	E. fetida andrei		*L. rubellus*	
Chlorophenol	Kooyenburg soil	Holten soil	Kooyenburg soil	Holten soil
3-	>71, <126	>65, <115	159	159
3,4-	>40, <73	>37, <65	103	83
2,4,5-	6.1	5.8	20	16
2,3,4,5-	5.9	8.0	22	32
Penta-	2.9	4.3	29	22

lower for *L. rubellus* than for *E. fetida andrei*. Comparison of log LC_{50} values based on soil concentrations shows only a weak correlation ($r = 0.495$), but when log LC_{50} values expressed as $\mu mol \cdot liter^{-1}$ soil solution are compared for both species, the correlation is much better ($r = 0.952$). Heimbach (1985) found a correlation factor of $r = 0.81$ for the toxicity of 23 pesticides for the earthworm species *E. fetida andrei* and *L. terrestris*. With respect to this comparison between two species it should, however, be noted that good correlation says nothing about the real difference in sensitivity.

An analysis of variance on the log LC_{50} values expressed as $\mu mol \cdot liter^{-1}$ soil solution showed a significant difference between the two species ($P < 0.01$). The difference in sensitivity becomes greater with increasing lipophilicity of the test substances ($P < 0.01$). This can also be seen in Fig. 1, where the LC_{50} in $\mu mol \cdot liter^{-1}$ is depicted as a function of the log P_{oct} for both earthworm species. For 3-chlorophenol the LC_{50} for *L. rubellus* is 1.6–2.1 times higher than that for *E. fetida andrei*, whereas for pentachlorophenol this difference increases to 5.1–11.6. From Heimbach's (1985) data it can be derived that for 4 chemicals *L. terrestris* is 5 to 9 times more sensitive

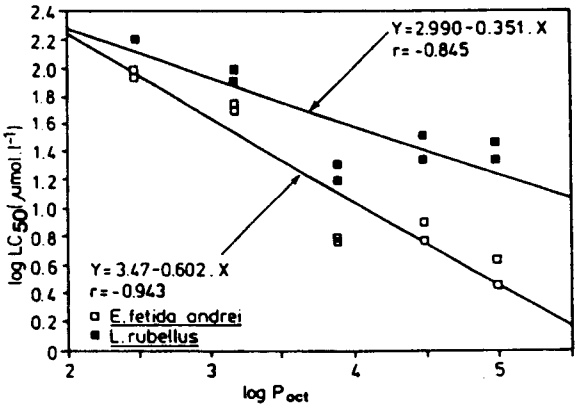

FIG. 1. Relationship for earthworms between toxicity (LC_{50} in $\mu mol \cdot liter^{-1}$ soil solution) and lipophilicity (log P_{oct}) of five chlorophenols in two soil types.

TABLE 8

BCF VALUES FOR THE BIOACCUMULATION OF CHLOROPHENOLS IN THE EARTHWORMS *Eisenia fetida andrei* AND *Lumbricus rubellus* BASED ON CONCENTRATIONS IN SOIL SOLUTION (BCF = CONCENTRATION IN EARTHWORM DIVIDED BY AVERAGE CONCENTRATION IN SOIL SOLUTION)

		BCF (based on soil solution concn) for chlorophenol				
Species	Soil	3-	3,4-	2,4,5-	2,3,4,5-	Penta-
E. fetida andrei	Kooyenburg	7	27	64	41	634
	Holten	17	24	34	38	426
L. rubellus	Kooyenburg	102	20	110	351	476
	Holten	123	41	659	213	996

than *E. fetida andrei*, for 3 chemicals the former species is 3.5–8 times less sensitive than the latter, and for the remaining 16 chemicals these two species differ by no more than a factor of 1.6.

From Fig. 1 it can be concluded that toxicity increases with increasing lipophilicity of the chlorophenols. The regression coefficients (±SE) for log LC_{50} on log P_{oct} for *E. fetida andrei* and *L. rubellus* are -0.59 ± 0.025 and -0.35 ± 0.028, respectively. A similar increase of toxicity with increasing lipophilicity was found for chlorophenols in fish by Schultz *et al.* (1986).

The difference in toxicity among the five chlorophenols studied cannot, however, be completely explained by differences in lipophilicity. The regression lines showed systematic deviations ($P < 0.01$). This holds especially for 2,4,5-trichlorophenol, which upon regression analysis was more toxic than expected for both earthworm species. Probably, this compound acts as a decoupling agent. But since the same holds for penta- and possibly also for 2,3,4,5-tetrachlorophenol (Schultz *et al.*, 1986), this cannot explain the increased toxicity.

Another explanation for the extreme toxicity of 2,4,5-trichlorophenol may be found in its behavior in soil and its response to the influence of pH. This can be understood better by comparing the pK_a value with the soil pH (0.01 *N* KCl), which shows that 2,4,5-trichlorophenol must be present in both soils as the uncharged phenol, which adsorbs strongly to soil and has a marked tendency for bioaccumulation in living organisms. Inside the body of an earthworm the pH will reach values as high as about 7.0. At this pH, 2,4,5-trichlorophenol will dissociate at least partially and be present as a negatively charged phenolate anion that is more toxic than the phenol. The fact that also 2,3,4,5-tetra- and pentachlorophenol will be present as phenolate ions within earthworms (as well as in fish) could explain why these substances are much more toxic than 3- and 3,4-dichlorophenol.

In the same way, the bioaccumulation data in Table 6 can be recalculated with the use of adsorption data to obtain values based on concentrations in soil solution. The results of this recalculation are shown in Table 8, from which it is clear that differences in BCF values between the two soils are also reduced after correction for adsorption, which indicates once again that the concentration in soil solution plays an important role in the uptake of chemicals from soil by earthworms.

LC_{50} and BCF values based on concentrations in soil solution can be compared with data for aquatic organisms. Table 9 shows some data from the literature on fish.

TABLE 9

SUMMARY OF PUBLISHED DATA ON CHLOROPHENOL TOXICITY AND BIOACCUMULATION IN FISH (AFTER VERSCHUEREN, 1983; SCHULTZ *et al.*, 1986)

Chlorophenol	LC_{50} (μmol·liter^{-1})	BCF
3-	23–47	—
3,4-	25–49	34[a]
2,4,5-	6.6–8.6	62
2,3,4,5-	1.8–3.3	93[b]
Penta-	0.7–6.5	475

[a] Data on 2,4-dichlorophenol.
[b] Data on 2,3,4,6-tetrachlorophenol.

Comparison of the data in this table with the earthworm toxicity data in Table 7 reveals substantial agreement. This is rather surprising, because although the sensitivity of fish and earthworms could be expected to be more or less the same, there are great differences in uptake and exposure. It should be kept in mind that fish toxicity studies have spanned at most 4 days, whereas earthworm studies have covered 14 days. Another difference is that in fish studies concentrations are often kept at a constant level, but the concentration in soil decreases steadily.

In Fig. 2 the log BCF values based on soil solution concentrations are shown as a function of log P_{oct}. The log BCF values of 3,4-dichlorophenol and pentachlorophenol were quite similar for *E. fetida andrei* and *L. rubellus*. For 3-, 2,4,5-tri-, and 2,3,4,5-tetrachlorophenol, however, log BCF values were much greater for *L. rubellus* than for *E. fetida*. There is fairly good correlation between bioaccumulation and lipophilicity (r = 0.856 and 0.702 for *E. fetida andrei* and *L. rubellus*, respectively). Lord *et al.* (1980) found a relationship between lipophilicity and the adsorption of pesticides to earthworm cell components. For aquatic organisms, too, bioaccumulation generally increases with increasing lipophilicity, as shown by the data in Table 8 and a review by Van Gestel *et al.* (1985).

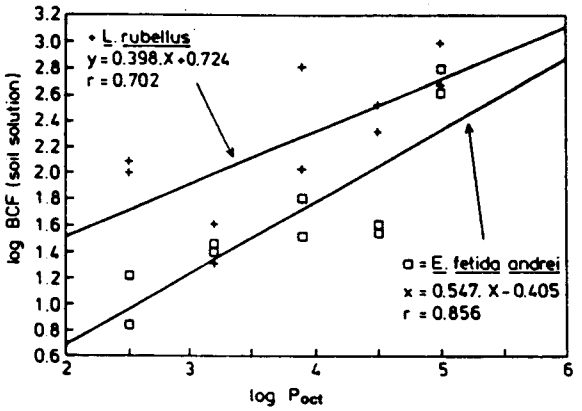

FIG. 2. Relationship between the bioaccumulation of five chlorophenols in the earthworms *Eisenia fetida andrei* and *Lumbricus rubellus* (log BCF based on soil solution concentrations) and lipophilicity (log P_{oct}).

CONCLUSIONS

From the results reported here, it can be concluded that for earthworms the toxicity and bioaccumulation and, therefore, the bioavailability of chlorophenols in soil are dependent on the concentration in soil solution and can be predicted by using adsorption coefficients.

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