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AN APPROACH TO QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIPS (QSARs) IN EARTHWORM TOXICITY STUDIES

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ABSTRACT

Two-wk LC_{50} values for earthworms in soil were determined for a homologous series of seven organic chemicals, i.e., 1,2,3-trichlorobenzene, 2,4-dichloroaniline and five chlorophenols. The toxicity tests were conducted with two species, i.e., *Eisenia andrei* and *Lumbricus rubellus*, and four different soil types, i.e., two sandy soils, a peaty soil, and an artificial soil. The LC_{50} values were recalculated towards molar concentrations in pore water using data from soil adsorption experiments. Significant relationships were obtained between LC_{50} values (in μmol/l pore water) and the octanol/water partition coefficient (log P_{oct}) of the compounds. The described approach will be useful for developing QSARs for the toxicity of chemicals in soil for earthworms.

INTRODUCTION

The relationship between chemical properties and biological activity has drawn the attention of many scientists for a long time. Many parameters, like the octanol/water partition coefficient (log P_{oct}), the acid-dissociation constant (pK_a), steric and electronic properties (expressed by the Hammett σ constant and the Taft E_s constant, resp.) and structural or topological indices (e.g., molecular connectivity), can be used to predict the biological activity of a chemical (1,2). In aquatic toxicology, most often log P_{oct} is used to predict toxicity or bioaccumulation of chemicals in aquatic organisms. In terrestrial ecotoxicology, however, research on quantitative structure-activity relationships (QSARs) is only beginning. Studies on the relationship between the adsorption of chemicals onto soil organic matter and log P_{oct} have been published (3), and more recently a relationship between soil sorption and molecular connectivity indices has been described (4). QSARs for soil invertebrates are still lacking, but relations have been found between physico-chemical properties of chemicals and effects on soil microbial respiration and biodegradation rates in soil (5,6).

In a previous study (7) we determined the acute toxicity for earthworms of five chlorophenols in two soils and recalculated LC_{50} values, expressed in mg/kg dry soil, towards molar

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concentrations in pore water using the Freundlich adsorption coefficient K_f. The st that toxicity was dependent on soil type and correlated with organic matter contant the soils. Differences in toxicity between soils disappeared when correcting LC_{50} v adsorption, suggesting that the toxicity is primarily governed by the soil concentration. The corrected LC_{50} values correlated with the compounds' log P_o seemed to offer possibilities for the development of QSARs for soil.

Since we used sandy soils with most probably a similar organic matter type, th needed further validation as to different soil types. The general validity of ou with chlorophenols also needed to be further validated as to a broader range of compounds. As shown also in our study the Freundlich's intensity factor 1 adsorption of the chlorophenols differed considerably from unity (7). The intensi was, however, not used in calculating the soil pore water concentration and its si in this regard still remains to be established.

In the present study, a peaty soil and an artificial soil recommended by OECD (8) a for earthworm toxicity testing were added to the range of experimental soils. In 2,4-dichloroaniline and 1,2,3-trichlorobenzene were included as test substances. method is presented to calculate the concentration of the chemicals in the pore wa of the soil using soil adsorption data, while correcting for the value of the Fr intensity factor. Finally, a more accurate method is presented to calculate LC_{50} va

MATERIALS AND METHODS

The following compounds were used: 3-chlorophenol (MCP), 3,4-dichlorophenol (DCP) trichlorophenol (TCP), 2,3,4,5-tetrachlorophenol (TeCP), pentachlorophenol (P dichloroaniline (DCA), and 1,2,3-trichlorobenzene (TCB). The sources, and chemical (pK_a and log P_{oct}) of each of the chlorophenols have been described previously (7). molecular weight of 162 and TCB of 181. Log P_{oct} values are 2.9 for DCA and 4.1 for Both compounds were obtained from Merck as 99% pure. Table 1 lists the aqueous sol of the test compounds.

TABLE 1. Aqueous solubilities (S) of the test compounds at 20°C (after 11,12)

S (mg/l)						
MCP	DCP	TCP	TeCP	PCP	DCA	TCB
26,000	4,600*	1,200	140#	14	91@	.12

*2,4-DCP #2,3,4,6-TeCP @3,4-DCA

Table 2 shows some properties of the soils used in the experiments. Kooyenburg (KOBG) and Holten (HOLT) are two different sandy soils, Wanneperveen (WAPV) is a peaty soil, and OECD an artificial soil. The latter has been described in internationally accepted guidelines on earthworm ecotoxicity tests (8,9) and consists of a mixture of 10% peat (2mm mesh-sieved), 20% kaolin clay, c. 69.5% quartz sand and c. 0.5% $CaCO_3$. KOBG, HOLT and WAPV were collected from the top 20 cm layer of agricultural field plots; the soils were air dried, sieved (4 mm mesh) and stored in the laboratory at a dry place and ambient temperatures.

TABLE 2. Selected physical and chemical properties of the test soils

Parameter	WAPV	OECD	HOLT	KOBG
pH (1N KCl)	3.6	5.9	5.6	4.8
pH (0.01N KCl)	3.9	6.1	5.6	5.2
pH (H_2O)	4.3	6.2	6.2	5.8
% organic matter	15.6	8.1	6.1	3.7
% clay	9.0	8.1	2.4	1.4
% silt	11.2	7.4	7.6	7.5
% fine sand	55.4	17.6	52.6	57.3
% coarse sand	4.4	54.5	31.2	29.2

Methods used to determine 2-wk LC_{50} values for the earthworms *Lumbricus rubellus* and *Eisenia andrei* followed those of (7). Toxicity tests were carried out at least in duplicate, with at least 5 concentrations and a control. In most tests 20 adult earthworms were used per concentration. The toxicity tests on DCA with *L. rubellus* were performed with only 10 worms per concentration. Tests in KOBG and HOLT were carried out with 30 worms per concentration, and in some cases (PCP/WAPV; tests on other chlorophenols with *E. andrei* in OECD) 40 worms were used per concentration. For the estimation of LC_{50} values the trimmed Spearman-Karber method (13) was used; this method yielded more consistent results compared to the logit model used in our previous study, especially in case of steep dose-response relationships (14).
To test whether the concentration-response curves were equal in different soil types, the following procedure was followed. Two logit models were fitted to the mortality data: the first model had the same intercept and slope for all soil types, the second model allowed for different slopes and intercepts. Under the null hypothesis that all soil types have the same slope and intercept, the difference in adequacy between the two models, as measured by the reduction in residual deviance when going from the simple to the more complex model, follows a χ^2 distribution. The number of degrees of freedom equals 2 * (the number of soil types - 1) (15). A measure of the adequacy of a model as such is:

$$\left(1 - \frac{\text{Residual deviance}}{\text{Total deviance}}\right) * 100\%$$

For the calculation of LC_{50} values in soil pore water, Freundlich's adsorption isotherm was applied:

$$q = K_f * (C_e)^{1/n} \qquad (1)$$

$$\text{or: } \log q = \log K_f + 1/n * \log C_e \qquad (2)$$

in which: q = concentration in solid phase in mg/kg,
C_e = concentration in soil water in mg/l, and
1/n = intensity factor

Considering the relatively high values of the adsorption coefficients in soil, it may be assumed that $q = q + C_e$ by approximation, and, hence, $q = LC_{50}$ (soil solid phase). Equation (2) can then be rewritten as :

$$\log C_e = (\log LC_{50} - \log K_f) / 1/n \qquad (3)$$

C_e values (= LC_{50} soil pore water) are thus obtained by substituting the values of K_f and 1/n in equation (3). K_f and 1/n values were determined experimentally according to methods described previously (7). For each adsorption test five concentrations of the test substance ranging from 0.3 to 30 mg/l (3.0 to 300 mg/l for DCA) and a control were prepared in 0.01 M $CaCl_2$. In case of poor water solubility of test substances (DCA, TCB, PCP) some drops of ethanol were added to the test solutions. Test suspensions were shaken with soil as described by (16). Concentration in soil (q) was calculated from the concentration decrease in the aqueous phase. To minimize loss by volatilization, adsorption tests were carried out in stoppered centrifuge tubes. Tests were carried out in duplicate, so determination of K_f and 1/n by linear regression was based on 10 data points.

At the start and end of the toxicity experiments with *E. andrei* soil samples were analysed to get some insight into the stability of the test substance during the 14 days test period. Methods for the analysis of chlorophenols in soil and water samples have been described previously (7). TCB was extracted from water with hexane, and analysed by GC-ECD. Soil samples were extracted three times with petroleum ether, extracts were dried over Na_2SO_4, and analysed by GC-ECD. DCA was extracted from both water and soil samples with toluene and analysed by GC-ECD.
Detection limits for the analysis of water samples ranged between 0.01 (TeCP, PCP and TCB) and 0.4 (MCP) µg/l; in case of DCA detection limit was much higher (50 µg/l). Detection limits for soil were between 0.1 and 11 µg/kg for all chemicals. Recoveries varied between 75 and 111% for water samples and between 90 and 106% for soil samples. All data were corrected for recovery.

RESULTS AND DISCUSSION

Adsorption

Table 3 shows the results of the adsorption experiments with the four test soils. For all combinations of chemicals and soils Freundlich adsorption isotherms were fitted with $r^2 \geq 0.945$. In most cases variation coefficients for both K_f and 1/n values were less than 10%, in many cases even less than 5%. Variation exceeded 10% in case of K_f values for MCP/KOBG (27%), MCP/OECD (33%) and DCP/KOBG (11%), and in case of the 1/n values for MCP/OECD (12%) and DCP/KOBG (12%).

Adsorption onto the OECD artificial soil appeared to be much weaker than could be expected from its organic matter content. Most of the test chemicals showed a more or less similar adsorption onto OECD and KOBG soils, although the former had a much higher organic matter content (Table 2). The peat-derived organic matter of OECD apparently had a lower adsorption capacity than organic matter derived from soil humus. This implies that adsorption of organic chemicals onto artificial soil cannot be estimated using QSARs describing the relationship between adsorption onto organic matter (K_{om}) and log P_{oct}, such as presented by (3). As was argued before (7), the adsorption of TeCP and PCP does not fully correlate with the soil's organic matter content, because for these substances pK_a is close to the soil pH. For such chlorophenols soil pH will have a relatively large influence on adsorption.

TABLE 3. Freundlich adsorption coefficients (K_f and 1/n) for chlorophenols, dichloroaniline, and trichlorobenzene in four different soils

	MCP		DCP		TCP		TeCP		PCP		DCA		TCB	
Soil	K_f	1/n	K_f	1/n	K_f	1/n	K_f	1/n	K_f	1/n	K_f	1/n	K_f	1/n
KOBG	6	0.85	15	0.84	43	0.84	85	0.82	120	0.84	16	0.75	48	0.98
HOLT	12	0.77	30	0.81	78	0.81	95	0.81	125	0.82	17	0.88	84	0.96
OECD	5	0.88	19	0.94	56	0.97	-	-	57	0.91	14	1.11	63	1.00
WAPV	13	0.96	53	0.90	218	0.98	-	-	714	0.92	63	0.85	206	1.01

Toxicity assessment

Table 4 shows the 2-wk LC_{50} values (soil solids) and corresponding 95% confidence intervals for *L.rubellus* and *E.andrei* in experiments with four different soils. In some cases dose-response relationships were too steep to allow the calculation of 95% confidence intervals. It appears that the toxicity of the test compounds, as expressed on a dry soil basis, varied considerably among soils. Tests carried out in WAPV soil consistently yielded the highest LC_{50} values, with lowest values found with the KOBG and OECD soils. As was already described earlier (7), LC_{50} values for chlorophenols were significantly different ($p<0.01$) between KOBG and HOLT. The only exception was the similar LC_{50} for PCP in KOBG and HOLT for *L. rubellus*.

The results in table 4 show that also for DCA and TCB LC_{50} values for KOBG and HOLT were significantly different between soils. LC_{50} values for WAPV were consistently higher than those for the other three soils; differences were significant at $p<0.01$. LC_{50} values for OECD were mostly between those for KOBG and HOLT, showing no significant differences in most cases. The LC_{50} of PCP in OECD for *L. rubellus*, however, differed significantly ($p<0.01$) from the LC_{50} in KOBG and HOLT.

TABLE 4. Toxicity of chlorophenols, dichloroaniline and trichlorobenzene for *Eisenia andrei* and *Lumbricus rubellus*, as based on concentrations in dry soil.

Species	Chemical	LC_{50} (mg/kg) in soil			
		KOBG	HOLT	OECD	WAPV
E. andrei	MCP	75 (> 56 <100)#	134 (>100 <180)	130 (125- 136)	423 (>320 < 560)
	DCP	134 (>100 <180)	240 (>180 <320)	172 (157- 188)	423 (>320 < 560)
	TCP	46 (43- 49)	76 (> 56 <100)	63 (57- 68)	164 (145- 186)
	TeCP	117 (105- 131)	166 (150- 184)	-	-
	PCP	84 (76- 93)	142 (121- 167)	83 (74- 93)	502 (462- 545)
	DCA	142 (132- 154)	285 (253- 320)	319 (281- 362)	824 (>560 <1000)
	TCB	134 (>100 <180)	240 (>180 <320)	133 (>100 <180)	547 (482- 621)
L. rubellus	MCP	150 (131- 171)	342 (316- 370)	247 (218- 280)	633 (598- 671)
	DCP	352 (332- 372)	486 (421- 561)	322 (275- 377)	680 (616- 751)
	TCP	235 (203- 272)	316 (288- 346)	362 (>270 <486)	875 (-)
	TeCP	515 (444- 599)	875 (-)	-	-
	PCP	1206 (1122-1295)	1013 (900-1141)	362 (>270 <486)	2298 (1909-2767)
	DCA	201 (>150 <270)	304 (256- 360)	190 (170- 212)	580 (495- 679)
	TCB	115 (104- 127)	200 (>150 <270)	195 (184- 207)	563 (497- 637)

( ) = 95% confidence interval

For *E. andrei* the difference between lowest and highest LC_{50} values in the four soils tested ranged between a factor of 3.2 (DCP) and 6.0 (PCP), and for *L. rubellus* between 2.1 (DCP) and 6.3 (PCP).

LC_{50} values (mg/kg dry soil) were transformed to concentrations in the soil water phase (Table 5). The variation in LC_{50} between soils was considerably reduced by this procedure, remaining limited to approximately a factor of 2 or less. Therefore, the results appeared to substantiate our earlier conclusion, i.e., that it is the concentration in the soil water phase which largely determines the exposure of earthworms to chemicals in soil.

Both *E. andrei* and *L. rubellus* showed a highly significant difference ($p<<0.01$) in concentration-response curves between the four different soil types, when concentrations were

ssed as mg/kg (χ^2=202-326 in case of *E. andrei* and 74-233 in case of *L. rubellus*; df=6, 01), which accounted for the quite different LC_{50} values shown in table 4. When ssed as µmol/l, LC_{50} values of MCP and DCP for *E. andrei* showed similar concentration-nse curves between soils (χ^2=0.129 and df=6, n.s. in both cases). In all other cases rences in concentration-response curves remained significant ($p<0.01$) between the soils d after transformation to pore water concentrations (χ^2=25-123 in case of *E. andrei* and 3 in case of *L. rubellus*, df=6).

greater similarity of the concentration-response curves between soils when the ntration was expressed as µmol/l could be concluded from the much greater adequacy of odel having a single slope and intercept for all soils than when concentrations were ssed as mg/kg. For *E. andrei* adequacies ranged between 37% (PCP) and 72% (TCP) when LC_{50} s were expressed as mg/kg and between 78% (PCP) and 100% (MCP and DCP) after culation to µmol/l. For *L. rubellus* adequacies ranged between 50% (PCP) and 81% (DCP) tween 63% (PCP) and 92% (TCB) when LC_{50} values were expressed as mg/kg and µmol/l, ctively.

dlich's intensity factor 1/n had a relatively large influence on the lower chlorinated s, when compared with previously published uncorrected data (7). Correction for this in the calculations will generally affect compounds with a high LC_{50} and with 1/n substantially deviating from unity. It thus appears that the intensity factor 1/n d be taken into account when calculating concentrations of organic chemicals in the soil water phase.

BLE 5. Toxicity of chlorophenols, dichloroaniline and trichlorobenzene, as based on the concentration in the soil pore water phase

ecies	Soil	LC_{50} (µmol/l) MCP	DCP	TCP	TeCP	PCP	DCA	TCB
senia andrei	KOBG	152	83.4	5.47	6.38	2.44	114	15.7
	HOLT	179	79.8	4.91	8.58	4.39	152	16.5
	OECD	315	63.8	5.62	-	5.67	103	11.7
	WAPV	292	63.2	3.80	-	2.55	127	14.5
mbricus rubellus	KOBG	343	263	38.2	38.8	58.6	180	13.5
	HOLT	603	191	28.5	66.8	48.1	164	13.6
	OECD	654	125	34.7	-	28.6	65	17.1
	WAPV	445	104	20.9	-	13.4	84	15.0

s further interesting to note that the results obtained with the OECD soil did not ite substantially from data determined with the natural soils (KOBG, HOLT and WAPV). This

indicates the usefulness of the OECD artificial soil as a standard test medium in future QSAR studies. It also indicates that results obtained with this soil can be translated to other soils by using experimental adsorption data; this is of importance for the extrapolation of results obtained with this soil towards other soil types.

Chemical analysis: persistence of test substances

The analyzed concentrations of MCP, DCP, TeCP, and PCP directly after application were more than 73% of the applied concentrations. Analyzed concentrations of PCP in OECD and WAPV were rather high (149-203%) compared to the applied concentrations. For TCP this was also the case in KOBG and HOLT, but in OECD and WAPV analyzed concentrations were lower (58-67%), while for DCA they were low in the first two soils (53-59%). For TCB after 7 days a higher concentration was determined than at the start: analyzed concentrations were 40-65% of applied at start and 58-144% after 7 days.

This variation in analyzed concentrations may be due to an inhomogenous distribution of the chemicals in soil. To check this, a batch of soil (HOLT) was treated with PCP (56 mg/kg), and divided into 10 subsamples which were analyzed separately. Concentrations in the subsamples varied between 39.8 and 73.4 mg/kg, with an average of 54.8±12.6 mg/kg. So, concentrations in subsamples taken from one batch of soil may vary by a factor of 2.

From the analytical results for t=0 and t=14 days DT_{50} values (time needed for 50% decrease of concentrations) in soil were calculated as described earlier (7). Table 6 gives the results.

TABLE 6. DT_{50} values for the disappearance from soil of chlorophenols, dichloroaniline and trichlorobenzene from soil in toxicity experiments with *Eisenia andrei*.

Soil	DT_{50} (days)						
	MCP	DCP	TCP	TeCP	PCP	DCA	TCB
KOBG	2.5	10	3.4	43	23	6.1	11
HOLT	5.4	11	6.6	29	48	6.1	-
OECD	1.6	24	48	-	9	-	3.1
WAPV	3.7	13	21	-	13	44	-

From table 6 a large difference in DT_{50} values can be observed between KOBG/HOLT a OECD/WAPV in case of TCP, PCP, DCA and TCB. Comparison of the LC_{50} values in tables 4 and with DT_{50} values does not show any correlation. Since mortality occurred rather rapidly aft introduction of the worms into the soil, such a correlation would also be unlikely. There no evidence that the compounds are degrading faster in high organic soil than in low organ

soil, so that degradation rates cannot be taken to explain the observed differences in toxicity between soils.

QSAR relationships

Fig. 1 shows the relationships between log LC_{50} values (μmol/l) averaged for all four soils and the octanol/water partition coefficient of each test compound. It is shown that the data fitted a relationship of the general form:

$$\log LC_{50} = a * \log P_{oct} + b$$

It appears from Fig.1 that the LC_{50} values of DCA, TCB and the chlorophenols, which represent a homologous series of compounds can be described by a QSAR relation using log P_{oct} as a predictor. The somewhat outlier position of TCP in the relationship, for which an explanation has been proposed in an earlier study (7), was retained in the present study.

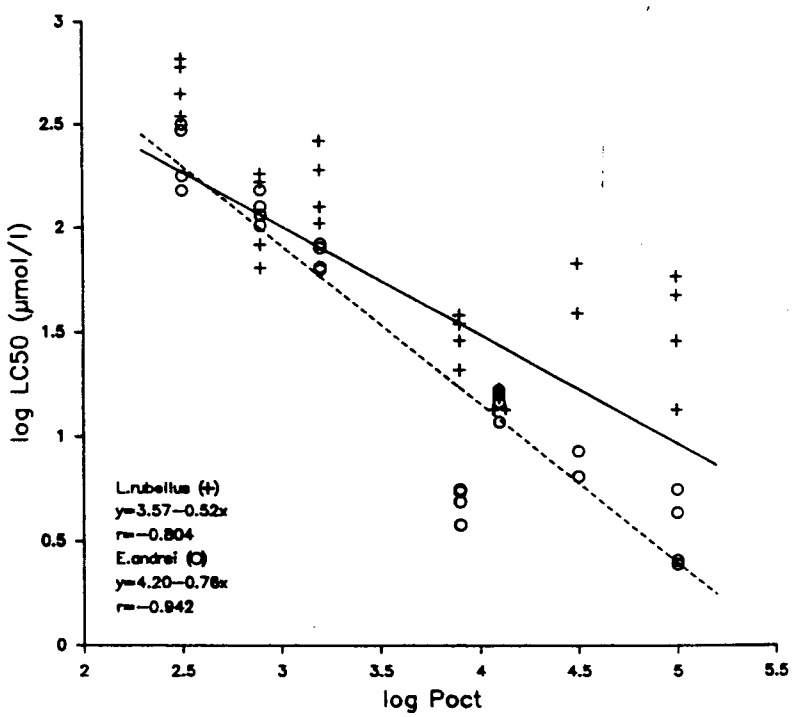

FIG.1. Log LC_{50} (μmol/l soil pore water) of seven organic chemicals for *Lumbricus rubellus* and *Eisenia andrei*, in relation to log P_{oct} values.

Species-related toxicity

As shown in Fig.1 and Table 5, the LC_{50} values of the chlorophenols were lower for *E.andrei* than for *L.rubellus*. The difference increased with increasing log P_{oct}, i.e., from a factor of 2 for MCP to a factor of 10 for PCP. The differences in toxicity seemed to be specific for

the group of chlorophenols, as they were neither observed for DCA nor for TCB. Studies to explain the observed species difference in toxicity of chlorophenols for earthworms are in progress.

CONCLUSIONS

- The soil pore water concentration is of primary importance for the exposure of lumbricid earthworms to organic chemicals in soil.
- The OECD artificial soil is a potential standard soil for use in QSAR studies with soil organisms, and results obtained with this soil can be extrapolated towards other (natural) soils by using adsorption data.
- The intensity factor 1/n in Freundlich's equation has to be taken into account when calculating pore water concentrations of chemicals in soil from adsorption data.
- Chlorophenols show a difference in toxicity for the earthworms *Eisenia andrei* and *Lumbricus rubellus*, which is specific to this group of compounds.
- The approach described in the present study renders QSAR studies for organic chemicals in soil a feasible option in terrestrial ecotoxicology.

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