DEVELOPMENT OF QSAR'S IN SOIL ECOTOXICOLOGY: EARTHWORM TOXICITY AND SOIL SORPTION OF CHLOROPHENOLS, CHLOROBENZENES AND CHLOROANILINES

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Abstract. Soil adsorption and the toxicity of four chloroanilines for earthworms were investigated in two soil types. The toxicity tests were carried out with two earthworm species, *Eisenia andrei* and *Lumbricus rubellus*. LC_{50} values in mg kg^{-1} dry soil were recalculated towards molar concentrations in pore water using data from soil adsorption experiments. An attempt has been made to develop Quantitative Structure Activity Relationships (QSAR's) using these results and data on five chlorophenols and dichloroaniline in four soils and five chlorobenzenes in two soils published previously (Van Gestel and Ma, 1988, 1990; Van Gestel *et al.*, 1991). Significant QSAR relationships were obtained between 1) adsorption coefficients (log K_{om}) and the octanol/water partition coefficient (log K_{ow}), and 2) LC_{50} values (in μmol L^{-1} soil pore water) and log K_{ow}. It can be concluded that both earthworm species tested are equally sensitive to chlorobenzenes and chloroanilines, *E. andrei* is more sensitive than *L. rubellus* to chlorophenols.

1. Introduction

In aquatic ecotoxicology, the equilibrium partitioning theory has been developed to assess the toxicity of sediment associated chemicals for organisms living in the sediment and in the overlaying water. This theory assumes that organisms are exposed to chemicals in the sediment via the water phase, and that water concentrations can be calculated from sediment concentrations by means of sorption data (Ziegenfuss *et al.*, 1986; Giesy and Hoke, 1990).

Recently, Van Gestel and Ma (1988, 1990) have demonstrated that the acute toxicity of five chlorophenols, 1,2,3-trichlorobenzene and 2,4-dichloroaniline for earthworms is primarily governed by the concentration in the soil solution, which can be estimated using adsorption data. In fact, a three-phase model involving soil-to-pore water and pore water-to-earthworm partitioning can be applied. This model is similar to the equilibrium partitioning theory, and has recently been shown to be valid for the bioaccumulation of stable lipophilic compounds from the soil by earthworms (Connell and Markwell, 1990). Based on this model, attempts have been made to develop quantitative structure-activity relationships (QSAR's) for bioaccumulation and acute toxicity of chemicals for earthworms (Van Gestel and

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Ma, 1988, 1990; Van Gestel *et al.*, 1991; Connell and Markwell, 1990). Toxicity and bioaccumulation of organic chemicals are related to their lipophilicity (octanol/water partition coefficient: log K_{ow}).

In the present study, data on chlorophenols, dichloroaniline and chlorobenzenes used in the development of QSAR's for acute earthworm toxicity and sorption (Van Gestel and Ma, 1990; Van Gestel *et al.*, 1991) are extended by the addition of data on four chloroanilines.

2. Materials and Methods

2.1. CHEMICALS

The following compounds were used: 3-chloroaniline (MCA), 2,4,5-trichloroaniline (TCA), 2,3,5,6-tetrachloroaniline (TeCA) and pentachloroaniline (PCA). MCA was obtained from J. T. Baker, TeCA from Aldrich and TCA and PCA from Riedel de Haen, and all chemicals were > 98%.

The octanol/water partition coefficients (log K_{ow}) of MCA, TCA, TeCA and PCA were obtained from De Bruijn *et al.* (1989), and were 1.91, 3.69, 4.46 and 5.08, respectively. Water solubility data were found for MCA (3630–5443 mg L^{-1}) (Nirmalakhandan and Speece, 1989) and TCA (16.7 mg L^{-1}) only (QSAR-estimate; QSAR-System of the US-EPA, Duluth).

2.2. SOILS

During the first stage of our research four soil types were used to validate the three-phase model: soil – pore water – earthworm (Van Gestel and Ma, 1988, 1990).

The second stage of our research, concentrated on the further development of QSAR's, and two soil types were used. KOBG is a sandy soil (pH 1N KCl 4.8; 3.7% organic matter), and was chosen as a representative of natural soils. It was collected from the top 20 cm layer of agricultural field plots. OECD (pH 1N KCl 5.9; 8.1% organic matter) is an artificial soil, chosen because of its international use in earthworm toxicity tests of new and existing chemicals (OECD, 1984; EEC, 1985). OECD was made up in the laboratory and is a homogeneous mixture of 10% peat (2 mm mesh-sieved), 20% kaolin clay, c. 69.5% quartz sand and c. 0.5% $CaCO_3$.

The properties of the two soils used have been described in Van Gestel *et al.* (1991). The soils were air-dried, sieved (4 mm mesh) and stored in the laboratory in a dry place at ambient temperatures.

2.3. TOXICITY ASSESSMENT

Methods used to determine 14–d LC_{50} values for the earthworms *Lumbricus rubellus* and *Eisenia andrei* followed those described by Van Gestel and Ma (1988). Test chemicals were mixed homogeneously through the soils. MCA was added to the soils as an aqueous solution; TCA, TeCA and PCA, 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. Toxicity tests were carried out in duplicate, with at least five concentrations and a control. Twenty adult earthworms were used for each test concentration. For the estimation of LC_{50} values the trimmed Spearman-Kärber method (Hamilton *et al*, 1977/1978) was applied.

At the start and at the end of the tests with *E. andrei* soil samples were extracted using toluene and analysed by GC-FID (MCA) or GC-ECD (TCA, TeCA and PCA). Detection limits were 0.8–1.0 mg kg^{-1} for MCA and 2.7–5.4 mg kg^{-1} for TCA, TeCA and PCA. All data were corrected for recovery by using a base-line correction.

2.4. ADSORPTION STUDIES

For the calculation of $LC_{50}(w)$ values (expressed as mg L^{-1}) in soil pore water, Freundlich's adsorption isotherm was applied:

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

or

$$\log q = \log K_f + 1/n * \log C_e \tag{2}$$

in which

q = concentration in solid phase in mg kg^{-1},
K_f = Freundlichs adsorption coefficient in dm^3kg^{-1},
C_e = concentration in soil water in mg L^{-1}
$1/n$ = degree of non-linearity.

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

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

C_e values (= $LC_{50}(w)$ 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 by Van Gestel and Ma (1988). For the adsorption tests, five concentrations of the test substance in 0.01 M $CaCl_2$ were used, ranging from 1.0 to 100 mg L^{-1} for MCA, from 0.3 to 30 mg L^{-1} for TCA and PCA, and from 0.1 to 10 mg L^{-1} for TeCA, and a control. Test suspensions were shaken with soil as described by Lagas (1988). Because chloroanilines have a low solubility in water, a solvent had to be added to the test solutions: for MCA, TCA and TeCA methanol (max. 1 ml L^{-1}) was used, while for PCA DMSO was used (max. 3 ml L^{-1}). Adsorption solutions were extracted with toluene, and analysed as described for soil samples.

Detection limits were 370, 40, 30 and 10 μg L^{-1} for MCA, TCA, TeCA and PCA, respectively. All data were corrected for recovery.

Concentrations in soil (q) were 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, the determination of K_f and $1/n$ by linear regression was thus based on 10 data points.

2.5. DATA ON PREVIOUSLY TESTED CHEMICALS

For the development of QSAR's in this paper, previously published data on 11 chemicals were used (Van Gestel and Ma, 1988, 1990; Van Gestel *et al.*, 1991). These chemicals are: 3-chlorophenol (MCP), 2,4-dichlorophenol (DCP), 2,4,5-trichlorophenol (TCP), 2,3,4,5-tetrachlorophenol (TeCP), pentachlorophenol (PCP), 2,4-dichloroaniline (DCA), 1,2,3-trichlorobenzene (TCB), chlorobenzene (MCB), 1,4-dichlorobenzene (DCB), 1,2,3,4-tetrachlorobenzene (TeCB) and pentachlorobenzene (PCB). Except for TeCP, the first 7 chemicals were tested in four different soils: KOBG, OECD, HOLT (sand, 6.1% organic matter, pH 1N KCl 5.6) and WAPV (peaty sand, 15.6% organic matter, pH 1N KCl 3.6). TeCP was tested in the soils KOBG and HOLT, whereas the latter four chlorobenzenes were tested in the soils KOBG and OECD. In Table I some physicochemical properties and the Freundlich adsorption coefficients K_f for these chemicals are summarized. Table II shows the LC$_{50}$(w) values for the toxicity for both earthworm species on a pore water concentration base.

3. Results and discussion

3.1. ADSORPTION

The results of the adsorption experiments with the two test soils are presented in Table III. For the adsorption of MCA and TCA on both soils Freundlich adsorption isotherms were fitted with $r^2 \geq 0.95$. In case of TeCA no Freundlich isotherm could be fitted, as concentrations in the test solutions had dropped below detection limit for the four lower concentrations. The K_f value given in Table III is therefore based on only one or two data points, obtained for the highest test concentration. In case of PCA, the following values were obtained in KOBG: $K_f = 272$ dm^3 kg^{-1}; $1/n = 0.70$; $r^2 = 0.92$, and OECD: $K_f = 83$ dm^3 kg^{-1}; $1/n = 0.60$; $r^2 = 0.90$. These values are too low, when compared to the ones for the other chloroanilines. The fact that K_f for OECD has a much lower value than for KOBG does not correspond with the expectation that adsorption is stronger in soil with a higher organic matter content.

Considering the fact that DMSO had to be added to dissolve PCA, and that test concentrations by far exceeded water solubility, these adsorption data must be considered unreliable. The data for PCA will therefore not be used in this paper. Except for TCA in KOBG, adsorption of MCA and TCA deviated strongly from linearity, showing low $1/n$ values in both OECD and KOBG. As can be seen in

TABLE I

Some physicochemical properties and Freundlich adsorption coefficients (K_f of chemicals tested previously (after Van Gestel and Ma, 1988, 1990; Van Gestel *et al.*, 1991)

Chemical	low K_{ow} [a]	pKa[b]	K_f (dm^3 kg^{-1}) for soil			
			KOBG	HOLT	OECD	WAPV
MCP	2.5	9.37	6	12	5	13
DCP	3.2	8.62	15	30	19	53
TCP	3.9	6.72	43	78	56	218
TeCP	4.5	5.64	85	95	–	–
PCP	5.0	4.74	120	125	57	714
DCA	2.9	–	16	17	14	63
MCB	2.9	–	5	–	9	–
DCB	3.4	–	8	–	18	–
TCB	4.1	–	48	84	63	206
TeCB	4.6	–	252	–	774	–
PCB	5.2	–	5133	–	10586	–

[a] for chlorophenols after Xie and Dyrssen (1984); for chlorobenzenes after De Bruijn *et al.* (1989).
[b] for chlorophenols after Ugland *et al* (1981).

TABLE II

Toxicity of chlorophenols, dichloroaniline and chlorobenzenes for *Eisenia andrei* and *Lumbricus rubellus* in four soils, based on the estimated concentration in the soil pore water phase (after Van Gestel and Ma, 1990; Van Gestel *et al.* (1991)

Chemical	LC_{50} (μmol L^{-1})							
	Eisenia andrei				*Lumbricus rubellus*			
	KOBG	HOLT	OECD	WAPV	KOBG	HOLT	OECD	WAPV
MCP	152	179	315	292	343	603	654	445
DCP	83	80	64	63	263	191	125	104
TCP	5.5	4.9	5.6	3.8	38	29	35	21
TeCP	6.4	8.6	–	–	39	67	–	–
PCP	2.4	4.4	5.7	2.6	59	48	29	13
DCA	114	152	103	127	180	164	65	84
MCB	1453	–	797	–	4281	–	2243	–
DCB	121	–	347	–	178	–	1556	–
TCB	16	17	12	15	14	14	17	15
TeCB	1.6	–	1.2	–	2.3	–	1.1	–
PCB	0.47	–	0.25	–	0.43	–	–	0.22

Table III, adsorption of the chloroanilines onto the OECD artificial soil is stronger compared to that onto KOBG; this corresponds with the difference in the organic matter content of the two soils. From Table III it can be concluded that for each

TABLE III
Freundlich adsorption coefficients (K_f and 1/n) for three chloroanilines in two different soils

Chemical	soil			
	KOBG		OECD	
	K_f	1/n	K_f	1/n
MCA	15	0.63	51	0.48
TCA	43	0.89	81	0.66
TeCA	244	–[a]	664	–[a]

[a] 1/n could not be calculated.

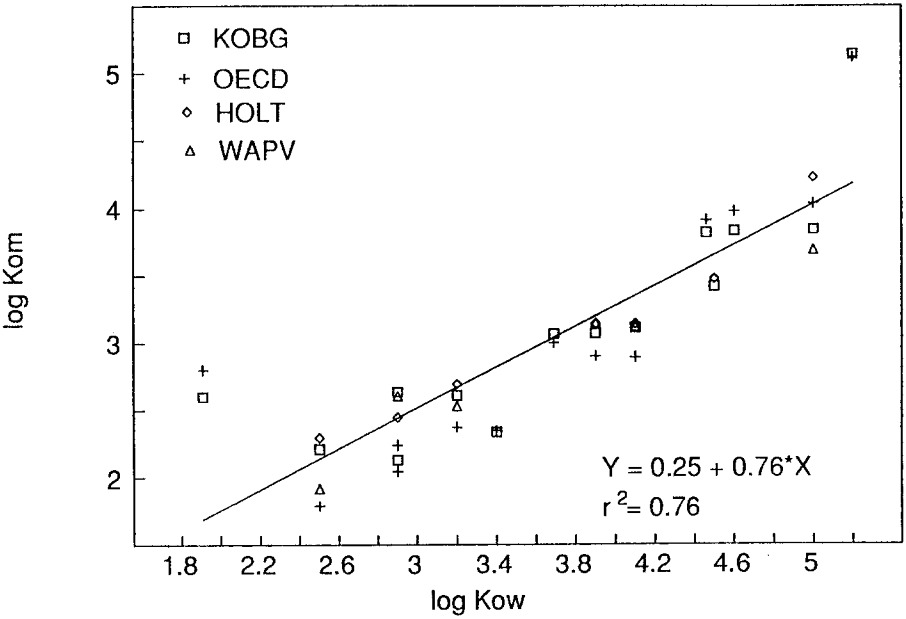

Fig. 1. Adsorption (log K_{om}) of chlorophenols, chlorobenzenes and chloroanilines on four soils, in relation to log K_{ow} values; with correction of chlorophenol data for soil pH.

soil, the adsorption coefficient K_f increased with increasing lipophilicity. As has been demonstrated by Van Gestel *et al.* (1991), this holds for the chlorophenols when sorption is corrected for dissociation. When the K_f presented in Tables I and III were recalculated towards K_{om} values (with $K_{\mathrm{om}} = K_f * 100/\%$ organic matter, and assuming 1/n values to be equal to unity), the following relationship could be derived (Figure 1):

$$\log K_{\mathrm{om}} = 0.76 * \log K_{\mathrm{ow}} + 0.25 \qquad (r^2 = 0.76;\, n = 40). \tag{4}$$

From Figure 1, it can be concluded that adsorption of MCA and PCB was much

TABLE IV
Toxicity of four chloroanilines for *Eisenia andrei* and *Lumbricus rubellus*, based on concentrations in dry soil

Chemical	LC_{50} (mg kg^{-1}) in soil			
	Eisenia andrei		*Lumbricus rubellus*	
	KOBG	OECD	KOBG	OECD
MCA	220(200– 241)[a]	448(403–499)	195(185– 207)	332(302–364)
TCA	134(>100<180)	233(220–247)	174(152– 199)	213(180–253)
TeCA	116(104–130)	133(>100<180)	159(138– 183)	179(153–198)
PCA	1014(420–2448)	>3200	825(665–1023)	825(700–972)

[a] ( ) = 95% confidence interval.

stronger than would be expected. Rippen *et al.* (1982) found a strong influence of soil pH on the adsorption of 4-MCA: at low pH (3.9–5.8) K_{om} was 56 to 65 dm^3 kg^{-1}, whereas at high soil pH (>7.0) K_{om} was much higher: 725–887 dm^3 kg^{-1}. Hodson and Williams (1988) determined a K_{oc} of 120 dm^3 kg^{-1}, corresponding to a K_{om} of about 200 dm^3 kg^{-1} under the assumption that organic matter contains c. 58% organic C. The K_{om} values found in this study for 3-MCA (400 and 630 dm^3 kg^{-1} for KOBG and OECD, respectively) are higher than would be expected on the basis of these literature data. This may also explain the outlier position of MCA in Figure 1. The outlier position of PCB remains unexplained at present.

The relationship described above is in agreement with relationships reported by Karickhoff *et al.* (1979), Karickhoff (1981), Means *et al.* (1982) and Hodson and Williams (1988) for the adsorption of organic chemicals onto soils and sediments.

3.2. TOXICITY ASSESSMENT

Table IV shows 2-wk LC_{50}(s) values (soil solids) and corresponding 95% confidence intervals for *E. andrei* and *L. rubellus* in experiments with two different soils. In some cases dose-response relationships were too steep to allow the calculation of 95% confidence intervals. For MCA and TCA LC_{50}(s) values were always higher in OECD compared to KOBG. For TeCA and PCA the LC_{50}(s) values for both test soils were not significantly different.

LC_{50} values (mg kg^{-1} dry soil) were transformed to concentrations in the soil water phase (Table V), using the adsorption coefficients of Table III. This procedure was not applied to PCA, because no reliable adsorption coefficients were available. Generally, the variation in LC_{50}(w) between soils was reduced by this procedure, remaining limited to less than a factor of 2. The only exception was TeCA, which yielded a significantly higher LC_{50}(w) in KOBG, for both species. This may be explained by fact that the difference in LC_{50} values (in mg kg^{-1} dry soil) was small, while the adsorption coefficients were lacking $1/n$ values.

TABLE V

Toxicity of three chloroanilines for *Eisenia andrei* and *Lumbricus rubellus*, based on the predicted concentration in the soil water phase

Soil	LC_{50} (μmol L^{-1}) for					
	Eisenia andrei			*Lumbricus rubellus*		
	MCA	TCA	TeCA	MCA	TCA	TeCA
KOBG	568	18	2.1	469	24	2.8
OECD	725	25	0.87	388	22	1.2

TABLE VI

Comparison of earthworm toxicity data on chloroanilines (based on pore water concentrations) with fish toxicity data[a]

Chemical	LC_{50} (μmol L^{-1}) for	
	earthworms	fish[b]
MCA	469 –725	105
DCA	60 –180	39-72
TCA	18 – 25	10
TeCA	0.87– 2.8	1.2(96 h)

[a] Literature data derived from: Hermens *et al.* (1984); Könemann (1981); Veith and Broderius (1987).

[b] 14 days LC_{50} values.

3.3. COMPARISON WITH AQUATIC TOXICITY DATA

As indicated before, the three-phase model applied in this study is similar to the equilibrium partitioning theory developed in aquatic ecotoxicology for sediment toxicity testing (Ziegenfuss *et al.*, 1986; Giesy and Hoke, 1990). Considering this similarity, and the fact that our data show that the toxicity of the chemicals for earthworms tested is predominantly governed by pore water concentrations (Van Gestel and Ma, 1988, 1990), our results were compared with literature data on toxicity for aquatic organisms. As has been indicated earlier by Van Gestel and Ma (1988) data for the toxicity of chlorophenols for earthworms based on pore water concentrations were in close agreement with data on toxicity for fish. A similar conclusion has been drawn for chlorobenzenes by Van Gestel *et al.* (1991). In Table VI data on the toxicity of the three chloroanilines and DCA for earthworms are compared with toxicity data for fish. From Table VI it can be concluded that LC_{50} values for the toxicity of chloroanilines for fish and earthworms are of the same order of magnitude. When making this comparison, it has to be realized that test procedures for fish and earthworms differ considerably. Such a comparison can therefore only be indicative.

Results of soil analysis at the start of the toxicity tests with *E. andrei* showed

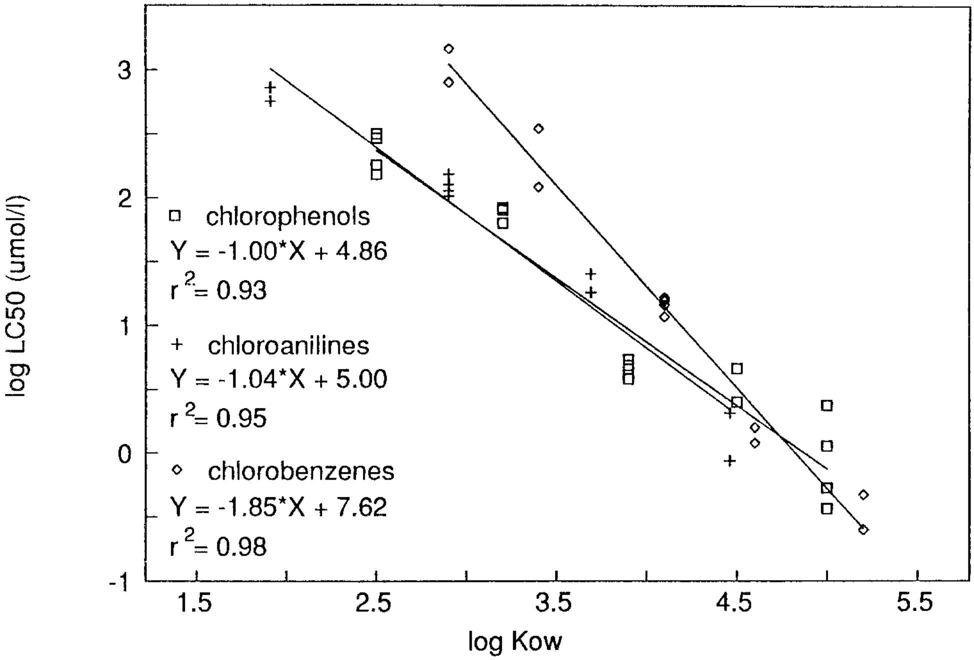

Fig. 2. Log LC_{50} (μmol L^{-1} soil pore water) of chlorophenols and chloroanilines for *Eisenia andrei*, in relation to low K_{ow} values.

that actual concentrations were 78 to 89, 88 to 114, 97 to 129 and 87 to 136% of nominal ones for MCA, TCA, TeCA and PCA, respectively. MCA, TCA, and PCA disappeared from the soils with calculated half-lives of <2.3–5.1, 82–125, and 57 to 83 days, respectively. In case of TeCA concentrations did not decline during the 14 days test period. This demonstrates that, except for MCA, exposure concentrations remained fairly constant during the test period. The half-life calculated for MCA is much shorter than the values of 23 to 52 and 15 days, which can be calculated from the data of Thompson and Corke (1969) and Fletcher and Kaufman (1980), assuming first order degradation kinetics.

3.4. QSAR RELATIONSHIPS

Figure 2 shows the relationships between log $LC_{50}(w)$ values (μmol L^{-1}) and the log K_{ow} of each test compound for *E. andrei*. In Figure 3 similar relationships are drawn for *L. rubellus*. In these figures toxicity data on the three chloroanilines (Table V) and the chemicals tested before (Table I) are used. For the chlorophenols toxicity is corrected for dissociation as described by Van Gestel *et al.* (1991). It is shown that the data fitted a straight-line relationship, for each group of chemically related compounds. The lines for chlorobenzenes and chloroanilines are almost similar for both species. When data for both species were lumped together, the

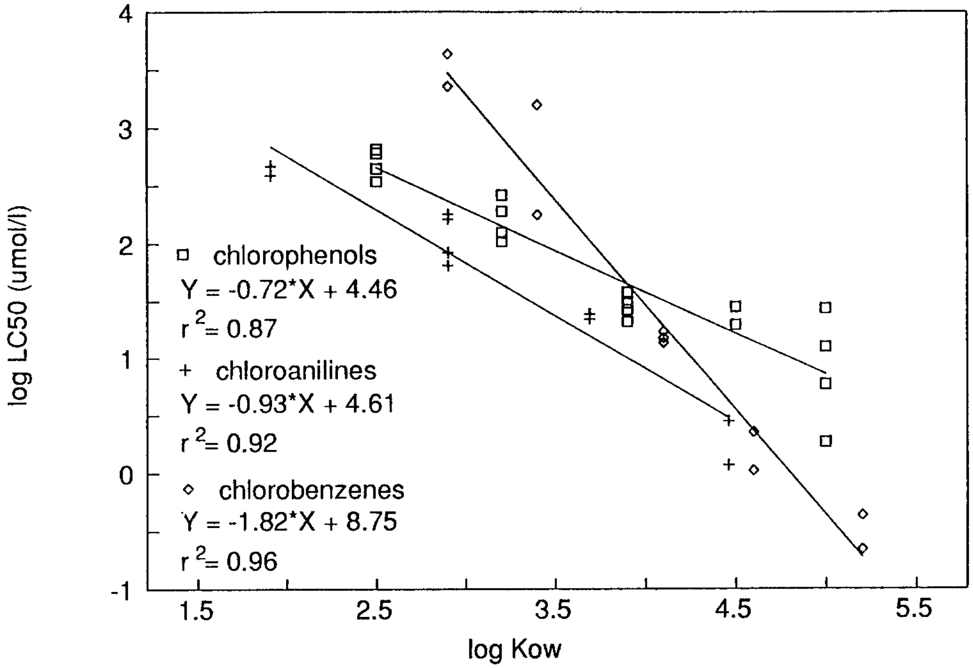

Fig. 3. Log LC_{50} (μmol L^{-1} soil pore water) of chlorophenols, chlorobenzenes and chloroanilines for *Lumbricus rubellus*, in relation to low K_{ow} values.

following relationships were found for chlorobenzenes:

$$\log LC_{50}(\mu\text{mol}L^{-1}) = -1.70 * \log K_{ow} + 8.19 \quad (r^2 = 0.96; n = 24). \qquad (5)$$

and for chloroanilines:

$$\log LC_{50}(\mu\text{mol}L^{-1}) = -0.98 * \log K_{ow} + 4.80 \quad (r^2 = 0.94; n = 20). \qquad (6)$$

The slope of the relationship found for chloroanilines is almost equal to the one found by Hermens *et al.* (1985) for the toxicity of some chloroanilines for guppies:

$$\log LC_{50}(\mu\text{mol}L^{-1}) = -0.92 * \log K_{ow} + 3.72 \quad (r^2 = 0.89; n = 11). \qquad (7)$$

Comparison of these relationships demonstrates that LC_{50} values for the toxicity of chloroanilines for earthworms expressed as soil pore water concentrations are about ten times higher than those for fish. This indicates that earthworms are less sensitive to chloroanilines than fish. For chlorophenols, lumping together of data for both species resulted in a correlation coefficient of $r^2 = 0.77$. From these results, it can be concluded that it is not possible to obtain a reliable QSAR for all chemicals with both species. The toxicity of chlorophenols and chloroanilines for *E. andrei* might be described by one relationship:

$$\log LC_{50}(\mu\text{mol}L^{-1}) = -1.01 * \log K_{ow} + 4.90 \quad (r^2 = 0.94; n = 28). \qquad (8)$$

The following relationship can be derived from Veith and Broderius (1987), for the toxicity of some chlorophenols and chloroanilines for fish with lipophilicity:

$$\log LC_{50}(\mu\mathrm{mol\,l}^{-1}) = -0.65 * \log K_{\mathrm{ow}} + 4.30 \quad (r^2 = 0.90; n = 39). \tag{9}$$

A comparison of these relationships demonstrates that the differences between *E. andrei* and fish are relatively small. In case of *L. rubellus*, the toxicity of chloroanilines and chlorophenols cannot be described by one QSAR.

3.5. SPECIES-RELATED TOXICITY

Van Gestel and Ma (1988, 1990) concluded that *E. andrei* was more sensitive to chlorophenols than *L. rubellus*, and that the difference between the two species increased with increasing lipophilicity of the test compounds. From Figures 2 and 3 and the relationships described above, it can be concluded that for the chlorobenzenes and chloroanilines both species are equally sensitive. This confirms our earlier conclusion (Van Gestel and Ma, 1990) that the difference found for chlorophenols has to be attributed to the specific mode of action of these compounds.

4. Conclusions

This study confirms our earlier findings, that the development of QSAR's for the adsorption and the toxicity of organic chemicals in the soil for earthworms is a feasible option also in terrestrial ecotoxicology.

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