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PHYTOTOXICITY OF SOME CHLOROANILINES AND CHLOROPHENOLS, IN RELATION TO BIOAVAILABILITY IN SOIL

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Abstract. Soil adsorption and the effect of four chlorophenols and three chloroanilines on the growth of lettuce (*Lactuca sativa*) were determined in two soil types differing in organic matter content and pH. Adsorption increased with increasing organic matter content of the soils. Phytotoxicity, based on dosed amounts, was significantly higher in the soil with the low level of organic matter. This difference could be reduced by recalculating the EC_{50} values for the effect of the test substances on plant growth in mg kg^{-1} dry soil towards concentrations in mg L^{-1} pore water using data from soil adsorption experiments. For pentachlorophenol only this recalculation increased rather than decreased the difference between the two soils, however, when the EC_{50} values for pentachlorophenol were corrected for the difference in soil pH, almost the same values resulted for both soils. Calculated EC_{50} values on the basis of pore water concentrations appeared to be in good agreement with values determined in nutrient solution tests. These results indicate that, for plants, the toxicity and therefore the bioavailability of organic chemicals in soil mainly depend on the concentration in the soil solution, and can be predicted on the basis of sorption data. Attempts to develop QSARs relating log EC_{50} values in μmol L^{-1} pore water with lipophilicity (expressed as the octanol/water partition coefficient: log K_{ow}) of the test substances resulted in a statistically significant relationship. This relationship was further improved by correcting the chlorophenol data for dissociation effects.

Key words: chloroanilines, chlorophenols, phytotoxicity, structure activity relationships, equilibrium partitioning, soil sorption

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 concentrations in water can be calculated from concentrations in the sediment by means of sorption data (Ziegenfuss *et al.*, 1986; Giesy and Hoke, 1990; DiToro *et al.*, 1991).

Van Gestel and Ma (1988, 1990) have demonstrated that the acute toxicity for earthworms of five chlorophenols, 1,2,3-trichlorobenzene and 2,4-dichloroaniline is primarily governed by the concentration in the soil solution, which can be

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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 also been shown to be valid for the bioaccumulation of stable lipophilic compounds from soil by earthworms (Connell and Markwell, 1990).

Van Gestel (1992) concluded that the equilibrium partitioning theory opens the way to extrapolate acute toxicity data for earthworms from one soil type to another on the basis of adsorption data. For most organic chemicals sorption is related to organic matter content, and toxicity may therefore be extrapolated on the basis of differences in organic matter content. This conclusion is very important for the risk assessment of new chemicals, as the internationally accepted toxicity test with earthworms (OECD, 1994b) uses only one artificial soil type.

Higher plants, as primary producers, are of invaluable importance for the functioning of terrestrial ecosystems. Furthermore, the inclusion of higher plants in ecotoxicological test systems may allow for the evaluation of potential effects of chemicals on agricultural production. For these reasons, OECD (1984a) described a phytotoxicity test, using a number of different monocotyledonous and dicotyledonous plant species. Like for earthworms, only one soil type is used in these tests again leaving open the question how to translate results obtained in this test soil to other soil types.

Recently, Hulzebos *et al.* (1993) determined the phytoxicity of a number of chlorophenols and chloroanilines in soil and in nutrient solution, using *Lactuca sativa* (lettuce) as the test species. They tried to relate EC_{50} values (= concentrations of the test substances causing 50% inhibition of plant growth) determined in soil with those determined in nutrient solution, by recalculating the values determined for soil to soil pore water concentrations using estimated adsorption coefficients. Despite the differences in test design, EC_{50} values in soil pore water appeared to be in agreement with those in nutrient solution. Differences between both values could mainly be attributed to differences in test design and the resulting difference in exposure conditions.

In the present paper, further research to assess the validity of the equilibrium partitioning theory for phytotoxicity in soil is presented. Therefore, the data-set published by Hulzebos *et al.* (1993) was expanded by determining phytotoxicity of a number of chlorophenols and chloroanilines in an additional soil and measuring sorption of these chemicals in both soil types.

2. Materials and Methods

2.1. Plants

Lactuca sativa (lettuce), Ravel R2 was used as test species. Seeds were obtained from a local supplier. Plants were cultured in climatic chambers to maintain constant

TABLE I

Physicochemical properties of the test compounds (at 20 °C). It is referred to the text for data sources

Chemical	Mol. wt.	Solubility (mg L^{-1})	log K_{ow}	pK_a
MCP	128.5	26000	2.50	9.37
DCP	163.0	4600[a]	3.20	8.62
TCP	197.4	1200	3.90	6.72
PCP	266.3	14	5.00	4.74
MCA	127.5	5500	1.88	10.54[b]
TCA	196.5	16.7	3.45	–
TeCA	231.0	–	4.10	–

[a] 2,4-DCP.
[b] pK_b.

test temperatures and air humidity, and to provide a photoperiod of 16 hours light and 8 hours dark at a light intensity of 6500 lux under fluorescent tubes. In the studies on chlorophenols, the test temperature was 22 ± 6 °C and humidity varied between 35 and 95%, the temperature being lowest and humidity highest during the dark period. In the tests with chloroanilines, conditions showed smaller fluctuations, with a temperature of 22 ± 4 °C and a humidity of 50–65%.

2.2. Chemicals

The following compounds were used: 3-chlorophenol (MCP), 3,4-dichlorophenol (DCP), 2,4,5-trichlorophenol (TCP), pentachlorophenol (PCP), 3-chloroaniline (MCA), 2,4,5-trichloroaniline (TCA) and 2,3,5,6-tetrachloroaniline (TeCA). Chemicals were obtained from EGA Chemie, Aldrich Chemie, Fluka AG and Merck, and purity was ≥ 98% except for PCP which had a purity of 95%.

Table I lists some physicochemical properties of the test compounds. Log K_{ow} values for the chlorophenols were obtained from Xie and Dyrssen (1984), aqueous solubilities from Verschueren (1983), and pK_a values from Ugland *et al.* (1981). Log K_{ow} values for the chloroanilines were taken from the US-EPA ClogP data base, and solubility and pK_b values for MCA from Isnard and Lambert (1989) and Hodgman *et al.* (1962), respectively. No data on the aqueous solubility of TeCa were found, and the solubility value for TCA was based on a QSAR estimate (QSAR-System of the US-EPA, Duluth).

2.3. Soils

Two soil types were chosen for this study: a natural loam soil (LOAM), and an artificial soil (OECD). LOAM was the soil prescribed by OECD (1984a) for

TABLE II

Selected physical and chemical properties of the soils used in the toxicity and adsorption tests

Parameter	LOAM	OECD[a]
pH (1N KCl)	7.5	6.2
% organic matter	2.0	8.1
% clay	15.0	8.1
% silt	26.0	7.4
% sand	59.0	72.1

[a] Taken from Van Gestel and Ma (1990).

phytotoxicity studies, and was collected from an orchard. Before use, this soil was sieved (4 mm mesh) and K_2HPO_4 added to achieve good plant growth. The moisture content of the soil was raised to 25–30% (w/w) (80% of the water holding capacity). The OECD artificial soil was chosen because of its international use in earthworm toxicity tests of new and existing chemicals (OECD, 1984b). OECD was made up in the laboratory by mixing homogeneously 10% peat (2 mm mesh-sieved), 20% kaolin clay, c. 69.5% quartz sand and c. 0.5% $CaCO_3$. To support plant growth in OECD, the water content of this soil during the tests (c. 90% (w/w), corresponding with field capacity) was made up with a nutrient solution following Steiner (1968).

Some properties of the two soils used are shown in Table II. Properties of the OECD artificial soil were taken from Van Gestel and Ma (1990).

2.4. Adsorption Studies

Experimental adsorption studies were performed according to methods described by Van Gestel and Ma (1988), and Freundlich's adsorption isotherm was applied to calculate K_f and $1/n$ values:

$$q = K_f\,(C_e)^{1/n} \quad (1$$

or

$$\log q = \log K_f + 1/n \log C_e \quad (2$$

in which

q = Concentration in solid phase in mg kg^{-1}

K_f = Freundlich's adsorption coefficient in dm^3 kg^{-1}

C_e = Concentration in soil water in mg L^{-1}

$1/n$ = Degree of non-linearity

For the adsorption tests with chlorophenols in LOAM five concentrations of the test substance in 0.01 M $CaCl_2$ were used, ranging from 3 to 300 mg L^{-1} for MCP, DCP and TCP and from 1 to 15 mg L^{-1} for PCP, and a control. In case of PCP only four concentrations were used. Test suspensions were shaken with soil as described by Lagas (1988). Because TCP and PCP are slightly soluble in water, some drops of ethanol were added to the test solutions. After centrifugation, adsorption solutions were analysed as described below.

The same procedures were applied to determine the adsorption of the chloroanilines in LOAM and OECD. For this purpose, concentrations in 0.01 M $CaCl_2$ ranging from 3.2 to 320 mg L^{-1} for MCA, from 2 to 50 mg L^{-1} for TCA and from 0.5 to 5 mg L^{-1} for TeCA were used. In the case of TeCA only four concentrations were used, for the other two chloroanilines five concentrations were tested. No solvent was added. After centrifugation, adsorption solutions were analysed directly as described below.

To minimize loss by volatilization, adsorption tests were carried out in stoppered centrifuge tubes. Tests with chlorophenols were carried out in duplicate, the determination of K_f and $1/n$ by linear regression was thus based on 10 data points (8 data points for PCP in LOAM). For chloroanilines only single determinations were performed, resulting in 5 data points for MCA and TCA and 4 for TeCA for the determination of K_f and $1/n$.

K_f and $1/n$ values for the chlorophenols in OECD were taken from Van Gestel and Ma (1990).

2.5. Phytoxicity Assessment

The method used to determine 7 and 14 day EC_{50} values for *L. sativa* followed that described by OECD (1984a). Test chemicals were mixed homogeneously through the soils. MCP and DCP were added to the soils as an aqueous solution in demineralized water (LOAM) or nutrient solution (OECD); TCP and PCP, which are not readily soluble in water, were mixed with a small amount of dry soil (LOAM) or sand (OECD) and then added to the rest of the soil. The chloroanilines were dosed to some sand as a solution in acetone. The acetone was evaporated and the coated sand was mixed with the soil. Nominal concentrations in mg kg^{-1} dry soil were spaced by a factor of 3.2. For each exposure time (7 and 14 days), duplicate trays were used for the control and at least five test concentrations. The 0.25 L plastic trays contained 400 g of moist LOAM or 250 g of moist OECD. In each tray 10 seeds were sown. The trays were covered with glass plates until germination of the seedlings. After removal of the glass plates, evaporation of water was compensated for daily by adding demineralized water. Only the first 5 germinated seedlings were required, any additional germinated seedlings were discarded. After 7 and 14 days shoots were harvested by cutting them off at soil level and fresh weight of each plant was determined immediately after harvesting.

Water content and pH of the soil were checked at the start and the end of th experiment, and appeared to be rather constant.

Results of phytoxicity studies with MCP and PCP in LOAM were taken fron Hulzebos *et al.* (1993).

2.6. Chemical Analysis

Adsorption solutions of the studies with chlorophenols were extracted with methan after acidification to pH 4.0, and extracts were analysed by HPLC with UV detection at 254 nm. Detection limits were 0.5 mg L^{-1} for all chlorophenols, an recoveries were about 100% for all chlorophenols. The solutions of the adsorp tion studies with chloroanilines were analysed directly by reversed phase HPL with UV-detection (248 nm). TeCA in OECD, however, was analysed by GC ECD after extraction with toluene (pH 12). Detection limits were 10, 20 and 5 μg L^{-1} for MCA, TCA and TeCA, respectively for LOAM, and 150, 150 an 0.005 μg L^{-1}, respectively for OECD. Recoveries were about 100% for all thre chloroanilines.

At the start and at the end of the phytotoxicity tests, soil samples were take For the analysis of chlorophenols, soil samples were extracted with toluene methanol after acidification to pH 4.0, and analysed by GC-ECD (MCP and PC in LOAM) or HPLC with UV-detection (254 nm; see above). Upon GC-analysi detection limits were 0.011 and 0.005 mg kg^{-1} for MCP and PCP, respectivel For HPLC analysis, detection limits were 2 mg kg^{-1} for MCP and 0.2 mg kg^{-1} f the other chlorophenols. Recovery ranged between 89 and 102%, and all data we corrected for recovery. For the analysis of the chloroanilines, soils were extracte with hexane by steam distillation at pH 11–12, and analysed by GC-ECD (TC and TeCA) or GC-FID (MCA). Detection limits were 0.04 mg kg^{-1} (MCA), 0.0 μg kg^{-1} (TCA) and 0.008 μg kg^{-1} (TeCA). Recovery was $\geq$ 80%, and all da were corrected for recovery.

2.7. Statistics

The concentration at which growth was 50% of the control (EC_{50}) was calculat as a parameter in a log-logistic model using a least square approximation. EC was based on harvested shoots only; dead plants or non-germinated seeds were n taken into account.

3. Results and Discussion

3.1. Adsorption

The results of the adsorption experiments with the two test soils are present in Table III. For all combinations of chemicals and soils Freundlich adsorpti

TABLE III

Freundlich adsorption coefficients (K_f and $1/n$) for chlorophenols and chloroanilines in two different soils

Chemical	Soil			
	LOAM		OECD	
	K_f	$1/n$	K_f	$1/n$
MCP	3.0	0.59	5.0[a]	0.88[a]
DCP	8.6	0.59	19[a]	0.99[a]
TCP	4.0	0.81	56[a]	0.97[a]
PCP	6.2	0.62	57[a]	0.91[a]
MCA	1.4	0.75	5.5	0.82
TCA	6.9	0.80	56	1.10
TeCA	22	0.80	299	0.98

[a] Data taken from Van Gestel and Ma (1990).

isotherms were fitted with $r^2 > 0.95$, except for TCA in OECD ($r^2 = 0.91$). For MCP, DCP, PCP and MCA in LOAM the $1/n$ values were < 0.8. These low $1/n$ values may be due to the relatively high solution concentrations used. As was demonstrated by Lagas (1988), at low solution concentrations (in the order of μg L^{-1} rather than the mg L^{-1} used here) $1/n$ will approach unity.

Adsorption of the chlorophenols and chloroanilines was significantly stronger in the high organic OECD artificial soil compared to the low organic LOAM. When the K_f values in Table III are recalculated towards K_{om} values (with $K_{om} = K_f * 100/\%$ organic matter, and assuming $1/n$ values to be equal to unity or sorption to be linear), resulting values for the two soils differed by no more than a factor of 1.1–3.1. It should be noted that, strictly speaking, such an excercise is only allowed when $1/n$ approaches unity, which will only be the case at very low solution concentrations.

From Table III it can be concluded that for OECD, adsorption coefficient K_f of the chlorophenols and chloroanilines increased with increasing lipophilicity, however, this was not the case for the chlorophenols in LOAM. Most likely, the high pH of the latter soil is responsible for this. At the pH of about 7.5 of this soil, both PCP and TCP will mainly be present as the phenolate anions. Adsorption of these ions is much weaker compared to uncharged phenols (Lee *et al.*, 1990). This also explains why K_f for PCP in OECD is somewhat lower than expected on the basis of its lipophilicity. To correct for the influence of the pH/pK_a effect on the adsorption of the phenols, the K_{om} values were divided by the fraction non-dissociated ($f_{nd} = 1/(1 + 10^{pH-pKa})$) (Lee *et al.*, 1990). After this, the following relation is derived (Figure 1):

$$\log K_{om} = 0.91 \log K_{ow} - 0.22 \quad (r^2 = 0.85;\ n = 14) \tag{3}$$

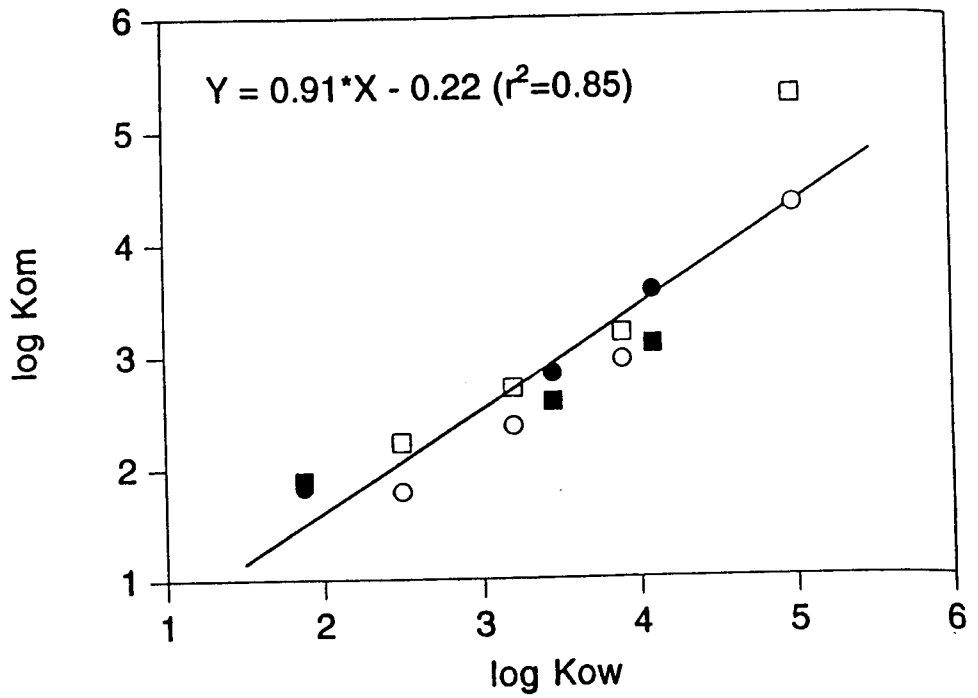

Fig. 1. Adsorption (log K_{om}) of chlorophenols and chloroanilines on two soils, in relation to l K_{om} values; with correction of chlorophenol data for soil pH. Open symbols: chlorophenols; sol symbols: chloroanilines; squares: LOAM; circles: OECD.

Correction for dissociaton of the chlorophenols significantly improved tl QSAR for adsorption of the test chemicals. It should also be noted, that aft correction for dissociation differences between K_{om} values for TCP in the tv soils was reduced by a factor of 2, whereas for PCP it was strongly increased (b factor of 4.7). Apparently, pH is not the only factor affecting sorption of chloroph nols in the tests soils. It should be noted, that the structure and properties of t organic matter in both soils differ. In the OECD artificial soil, peat is used a substitute for organic matter, whereas LOAM contains natural organic matter. T difference in organic matter will no doubt have affected sorption of the chemic to both soils.

The QSAR found in this study agrees rather well with the relationships repor by Hodson and Williams (1988), Karickhoff (1981), Karickhoff *et al.* (197 Means *et al.* (1982) and Van Gestel and Ma (1993).

3.2. Toxicity Assessment

Table IV shows the 7 and 14 day EC_{50}(s) values (soil solids) and correspond 95% confidence intervals for *L. sativa* in experiments with two different soils

TABLE IV

Toxicity of chlorophenols and chloroanilines for *Lactuca sativa*, in loam soil (LOAM) and artificial soil (OECD), as based on concentration in dry soil

Chemical	EC_{50} (mg kg^{-1} dry soil) in			
	LOAM		OECD	
	7 d	14 d	7 d	14 d
MCP	21 (12–37)[a,b]	7 (3–15)[a]	c. 60[c]	c. 80[c]
DCP	25 (24–25)	32 (31–34)	> 100	90 (–)
TCP	22 (22–23)	4.3 (–)	> 150 (148–152)	160 (–)
PCP	7 (4–15)[a]	8 (4–15)[a]	3.4 (3.2–3.6)	3.0 (3.0–3.1)
MCA	28 (24–33)	23 (20–27)	126 (88–181)	60 (45–81)
TCA	35 (27–46)	23 (19–28)	223 (157–317)	149 (112–198)
TeCA	50 (34–73)	17 (14–20)	549 (271–1110)	106 (69–163)

[a] Data taken from Hulzebos *et al.* (1993).
[b] ( ) = 95% confidence interval.
[c] EC_{50} could not be calculated using the logit model, and had to be estimated by graphical interpolation.

some cases dose-response relationships did not allow for the calculation of 95% confidence intervals, while in some cases growth of the plants was somewhat stimulated at the lower test concentrations. For all chemicals EC_{50}(s) values were higher in OECD compared to LOAM, except for PCP, where EC_{50}(s) was higher in the LOAM soil.

Little data on the phytotoxicity of chlorophenols and chloroanilines in soil were found in the literature. Adema and Henzen (1989) performed tests with 2,4,6-TCP and 2,4-DCA in two different soils, using methods comparable to those applied in this study. For 2,4,6-TCP, they found EC_{50} values of 144, 398 and 514 mg kg^{-1} dry soil for *L. sativa*, *Lycopersicum esculentum* and *Avena sativa*, respectively, in a sandy loam soil containing 3.7% organic matter and having a pH of 5.1. When using a soil (1.4% organic matter, pH 7.5) comparable to the LOAM we used, EC_{50} values of 180, 398 and 514 mg kg^{-1} dry soil, respectively were found for the three plant species. These values are higher than those found for 2,4,5-TCP in this study.

Hulzebos *et al.* (1993) reported 14-day EC_{50} values for a number of chlorophenols and chloroanilines in a LOAM soil, similar to the one used in this study. Besides the data on 3-MCP and PCP used in this study, they reported values of 43, 32, 16, 9 and 3.2 mg kg^{-1} for 2-MCP, 3,5-DCP, 2,4,6-TCP, 2,3,5-TCP and PCP, respectively, in a soil similar to the LOAM used in this study. For 3-MCA, 2,4,5-TCA, 2,4,6-TCA, 2,3,4,5-TeCA and 2,3,5,6-TeCA they found EC_{50} values of 15, 17, 23, 24 and 15 mg kg^{-1}, respectively.

TABLE V

Toxicity of chlorophenols and chloroanilines for *Lactuca sativa*, as based on the concentration in the soil pore water phase, and comparison with data on the toxicity in nutrient solution also obtained within our own laboratories (Hulzebos *et al.*, 1993)

Chemical	14 days EC_{50} (μmol L^{-1}) for		
	LOAM soil pore water	OECD soil pore water	Nutrient solution
MCP	25	154	44
DCP	50	29	15[a]
TCP	5.1	9.7	9.1[b]
PCP	5.2	0.15	0.11
MCA	195	120	46
TCA	21	12	6.6
TeCA	3.1	1.5	2.7

[a] Data for 2,4-DCP.
[b] Data for 2,4,6-TCP.

Casterline *et al.* (1985) determined EC_{50} values of 10 mg kg^{-1} dry soil for th toxicity of PCP for soybeans and spinach in a soil containing 2% organic matte Günther and Pestemer (1990) determined EC_{50} values of 10–20 mg kg^{-1} dry sc for the toxicity of PCP for *Brassica rapa* var. *rapa* and *A. sativa* in a sandy loa soil (2.2% organic matter; pH 6.1). These values are of the same order of magnituc as those found for PCP in this study (Table IV).

The 14 day EC_{50}(s) values (mg kg^{-1} dry soil) (Table IV) were transformed concentrations in the soil water phase (Table V), using the adsorption coefficien K_f and $1/n$ of Table III. For DCP, TCP, MCA, TCA and TeCA the variation EC_{50}(w) between soils was reduced by this procedure, from a factor of 2.6–: before transformation to a factor of 2.1 or less after it. In case of MCP reductic of this difference between the two soils was less, from a factor of 11 befo transformation to a factor of 6.2 after it. The only exception was PCP, for whic the difference between the two soils was only a factor of 2.7 before transformatic and increased to a factor of 35 after it.

Data for the chlorophenols were corrected for dissociation, assuming that on the non-dissociated fraction will be readily taken up by organisms and therefore responsible for the toxic action (Table VI). This significantly reduced the differen for PCP (to a factor of 1.1), but increased the difference for TCP (to a factor 11).

TABLE VI

Toxicity of chlorophenols for *Lactuca sativa*, as based on the concentration in the soil pore water phase, and comparison with data on the toxicity in nutrient solution also obtained within our own laboratories (Hulzebos *et al.*, 1993). All data are corrected for dissociation

Chemical	14 days EC_{50} (μmol L^{-1}) for		
	LOAM soil pore water	OECD soil pore water	Nutrient solution
MCP	25	154	44
DCP	46	29	15[a]
TCP	0.73	8.1	7.6[b]
PCP	0.009	0.008	0.006

[a] Data for 2,4-DCP.
[b] Data for 2,4,6-TCP.

3.3. Chemical Analysis

Results of soil analysis at the start of the toxicity tests showed that actual concentrations were 97–225, 101–102, 121–141 and 84–200% of the added ones for MCP, DCP, TCP and PCP, respectively. The highest actual concentrations were found in LOAM, the lowest ones in OECD. The high deviations from nominal concentrations found for MCP, TCP and PCP may be due to inhomogeneity of the soil. Van Gestel and Ma (1990) demonstrated that it is very difficult to establish a homogeneous distribution of PCP through soil, resulting in a maximal difference of a factor of 2 in concentrations measured in sub-samples taken from a freshly prepared batch of soil. MCP, DCP, TCP and PCP dissappeared from the soils with calculated (assuming 1st order biodegradation kinetics) half-lives of 3.4 – <3.5, 15–30, 2.5–64 and 2.8 – >>14 days, respectively.

In case of the studies with chloroanilines, initial concentrations in soil were 73–75, 99–116 and 95–110% of nominal ones for MCA, TCA and TeCA, respectively. Half-lives for the disappearance of these chemicals from the test soils were calculated to be 7.8–22, 41–54 and 46–102 days, respectively.

3.4. Comparison with Toxicity Data from Tests in Nutrient Solutions

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; DiToro *et al.*, 1991). Considering this similarity, and the fact that our data show that the phytotoxicity of the chemicals tested is at least partly governed by pore water concentrations, our results were compared with data on the toxicity of these chemicals in nutri-

ent solutions to *L. sativa*. These data were also determined in our laboratori (Table V).

From Table V it can be concluded that there is some agreement for all th chemicals between the pore water EC_{50}(w) values calculated in this study ar the nutrient solution values determined by Hulzebos *et al.* (1993), with diffe ences being less than a factor of 4.5; only for PCP in LOAM was the differenc rather large. Regression analysis only resulted in a significant correlation betwee OECD pore water EC_{50}'s and nutrient solution values, not for LOAM. When th EC_{50}(w) values were corrected for dissociation (Table VI), differences becam smaller except for TCP in LOAM. Again, only OECD pore water EC_{50}'s showed significant correlations with pore water values, whereas LOAM pore water EC_{50} did not. This again suggests that pH is not the only factor affecting sorption ar toxicity of chlorophenols.

3.5. QSAR RELATIONSHIPS

A significant relationship was found between log EC_{50}(w) values (μmol L^{-1}) ar the log K_{ow} of each test compound, which could be described by:

$$\log EC_{50}(\mu\text{mol}\, L^{-1}) = 3.72 - -0.76 \log K_{ow} \quad (r^2 = 0.78,\ n = 14)($$

The goodness of fit of this relationship was, however, rather low. When th data for chlorophenols were corrected for dissociation as described above, th relationship shown in Figure 2 resulted. It appears that the QSAR is significant improved by this correction.

4. Conclusions

The results described in this paper demonstrate that the equilibrium partitionir theory may also be applicable for phytotoxicity, although the variation betwe soils may be large. This was especially the case for MCP and PCP; when correct for dissociation this situation improved significantly for PCP, but led to a great difference for TCP.

There was a striking agreement between toxicity data based on pore wat concentrations calculated in this study and results of studies in nutrient solutio performed earlier in our laboratories. Again, correction for dissociation improv the situation for PCP, whereas it disimproved it for TCP. These findings stron ly suggest that pH is not the only factor determining sorption and toxicity chlorophenols in soil. Further research on this point is recommended.

It can also be concluded from these results that the OECD artificial soil c be used for phytotoxicity studies. This has several advantages, firstly this enabl comparison of results obtained with different test organisms (plants, earthworm etc.) in the same soil type. Secondly, the LOAM soil prescribed by OECD (1984

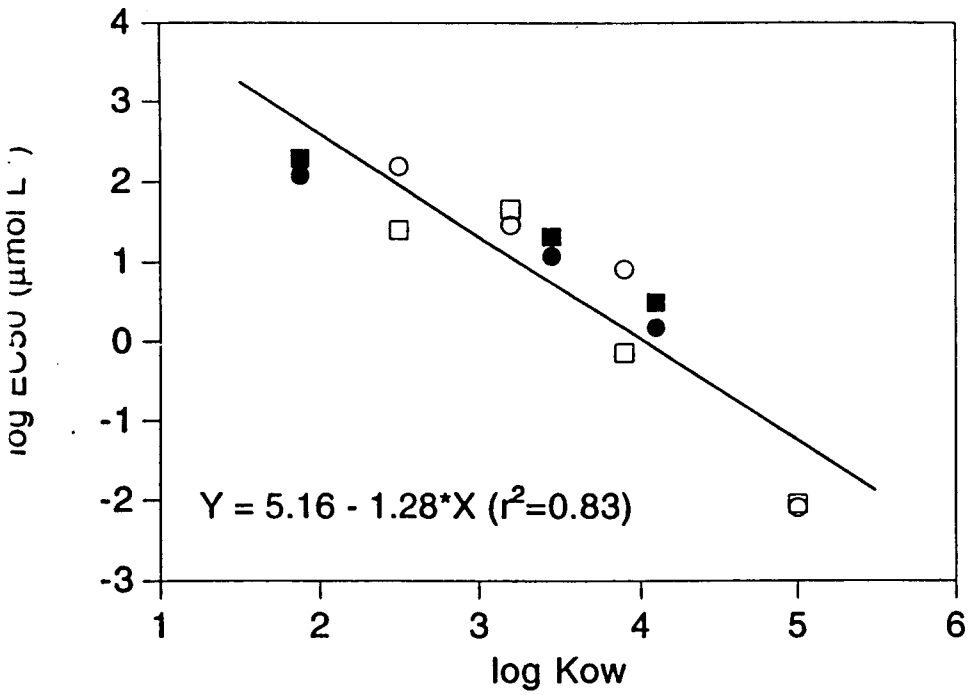

Fig. 2. Log EC_{50} (μmol L^{-1} soil pore water) of chlorophenols and chloroanilines for *Lactuca sativa*, in relation to log K_{ow} values; with correction for dissociation of chlorophenol data. Open symbols: chlorophenols; solid symbols: chloroanilines; squares: LOAM; circles: OECD.

for phytotoxicity studies has a rather rare composition; therefore, only one location in the Netherlands could be found to contain this soil type. For the ease of standardization, a universal soil type is preferred. The OECD artificial soil seems to meet this requirement.

Results obtained in different soils can be compared by using sorption data. This was demonstrated for earthworms by Van Gestel and Ma (1988, 1990). Van Gestel (1992) argued that for uncharged organic chemicals, comparison on the basis of differences in organic matter content seems to be possible. The results obtained in this study support this conclusion with respect to phytotoxicity data.

Finally, it can be concluded that results described in this paper seem to offer opportunities for the development of QSARs for phytotoxicity in soil.

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References

Adema, D. M. M. and Henzen, L.: 1989, *Ecotox. Environ. Safety* **18**, 219.
Casterline, J. L. Jr., Barnett, N. M. and Ku, Y.: 1985, *Environ. Res.* **37**, 101.
Connell, D. W. and Markwell, R. D.: 1990, *Chemosphere* **20**, 91.
DiToro, D. M., Zarbra, C. S., Hansen, D. J., Berry, W. J., Swartz, R. C., Cowan, C. E., Pavlou, Allen, H. E., Thomas, N. A. and Paquin, P. R.: 1991, *Environ. Toxicol. Chem.* **10**, 1541.
Giesy, J. P. and Hoke R. A.: 1990, 'Freshwater sediment quality criteria: toxicity bioassessment', Baudo, J. Giesy and H. Muntau (eds.), *Sediments: chemistry and toxicity of in-place pollut* Lewis Publishers, Inc., Ann Arbor, p. 265.
Günter, P. and Pestemer, W.: 1990, *Environ. Manag.* **14**, 381.
Hodgman, C. D., Weast, R. C., Shankland, R. S. and Shelby, S. M. (eds.): 1962, *Handbo Chemistry and Physics*, 44 ed., The Chemical Rubber Publishing Co.
Hodson, H. and Williams, N. A.: 1988, *Chemosphere* **17**, 67.
Hulzebos, E. M., Adema, D. M. M., Dirven-van Breemen, E. M., Henzen, L., Van Dis, W. A., Her H. A., Hoekstra, J. A., Baerselman, R. and Van Gestel, C. A. M.: 1993, *Environ. Toxicol. C* **12**, 1079.
Isnard, P. and Lambert, S.: 1989, *Chemosphere* **18**, 1837.
Karickhoff, S. W.: 1981, *Chemosphere* **10**, 833.
Karickhoff, S. W., Brown, D. S. and Scott, T. A.: 1979, *Water Research* **13**, 241.
Lagas, P.: 1988, *Chemosphere* **17**, 205.
Lee, L. S., Rao, P. S. C., Nkedi-Kizza, P. and Delfino, J. J.: 1990, *Environ. Sci. Technol.* **24**, 65
Means, J. C., Wood, S. G., Hassett, J. J. and Banwart, W. L.: 1982, *Environ. Sci. Technol.* **16**, 9
OECD: 1984a, *Guideline for testing of chemicals no. 208, Terrestrial plants, growth test.* Ad April 1984.
OECD: 1984b, *Guideline for testing of chemicals no. 207, Earthworm acute toxicity tests.* Ad April 1984.
Steiner, A. A.: 1968, 'Soilless culture', in *Proc. of the 6th Colloqium of the International P Institute*. Florence, Italy, p. 324.
Ugland, K., Lundales, E., Greibokk, T. and Bjorseth, A.: 1981, *J. Chromatogr.* **213**, 83.
Van Gestel, C. A. M. and Ma, Wei-chun: 1988, *Ecotox. Environ. Safety* **15**, 289.
Van Gestel, C. A. M. and Ma, Wei-chun: 1990, *Chemosphere* **21**, 1023.
Van Gestel, C. A. M., and Ma, Wei-chun: 1993, *Water, Air, and Soil Pollut.* **69**, 265–276.
Van Gestel, C. A. M.: 1992, 'The influence of soil characteristics on the toxicity of chemical earthworms: a review', in P. W. Greig-Smith, H. Becker, P. J. Edwards and F. Heimbach, F. (*Ecotoxicology of earthworms*. Intercept Press, Andover, Hants, p. 44.
Verschueren, K.: 1983, *Handbook of environmental data on organic chemicals*, 2nd edition. Nostrand Reinhold Inc., New York.
Xie, T. M. and Dyrssen, D.: 1984, 'Simultaneous determination of partition coefficients and a constants of chlorinated phenols and guaiacols by gas chromatography', in T. M. Xie *Investigation of chlorophenolic compounds from the paper and pulp industries*. Dissert Department of Analytical and Marine Chemistry, Göteborg.
Ziegenfuss, P. S., Renaudette, W. J. and Adams, W. J.: 1986, 'Methodology for assessing the toxicity of chemicals sorbed to sediments: testing the equilibrium partitioning theory', in Poston and R. Purdy (eds.), *Aquatic toxicology and environmental fate: Ninth volume*. A STP 921, American Society for Testing and Materials, Philadelphia, p. 479.