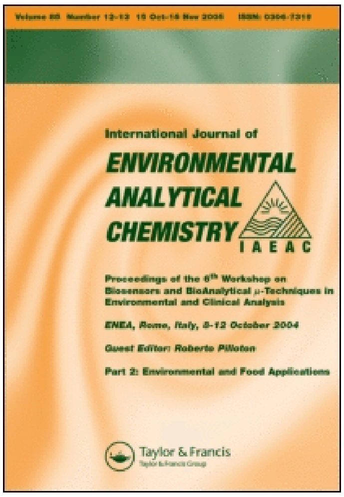

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

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To cite this Article Larsen, B. , Pelusio, F. , Skejø, H. and Paya-perez, A.(1992) 'Bio Availability of Polychlorinated Biphenyl Congeners in the Soil to Earthworm (*L. rubellus*) System', International Journal of Environmental Analytical Chemistry, 46: 1, 149 — 162
To link to this Article: DOI: 10.1080/03067319208027006
URL: http://dx.doi.org/10.1080/03067319208027006

Intern. J. Environ. Anal. Chem., Vol. 46, pp. 149–162
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BIOAVAILABILITY OF POLYCHLORINATED BIPHENYL CONGENERS IN THE SOIL TO EARTHWORM (*L. rubellus*) SYSTEM

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(Received, 13 March 1991; in final form, 23 August 1991)

The earthworm's (*Lumbricus rubellus*) uptake of polychlorinated biphenyls (PCBs) from a soil contaminated with a commercial PCB formulation (Askarel at 150 $\mu g\ g^{-1}$) and their elimination of PCBs into a low contaminated soil (1.5 $\mu g\ g^{-1}$) has been studied. 17 individual congeners were monitored. The uptake and the elimination rate were similar for all PCB congeners notwithstanding their different chloro-substitution pattern which suggested that bioaccumulation of PCBs in earthworms is governed by passive, possibly diffusion controlled processes. The equilibrium state in the three-phase system, soil/soil water/earthworm was reached with a half-time around 3–4 days. The soil to earthworm bioconcentration factor ranged from 4 to 20 for tetra- to octa-chlorinated biphenyls and was weakly depending on the octanol-water partition coefficient: $BCF = -(1.3\text{–}1.8) \times K_{OW}^{(0.35\text{–}0.40)}$.

KEY WORDS: PCB, earthworm, soil, uptake and elimination.

INTRODUCTION

Polychlorinated biphenyls (PCBs) are thermally and chemically stable chlorinated hydrocarbons with high dielectric constants. These properties have led to their application in a wide range of industrial products such as dielectric fluids in transformers and capacitors, and as components of hydraulic fluids. Some of these properties have also been responsible for the widespread and lasting environmental contamination problems associated with PCBs.

The world production since 1929 of PCBs is estimated to be around 1.5 metric tons of which one third is believed to have leached into the environment[1]. Technical formulations of PCBs have been produced by catalytic chlorination of biphenyl whereby approx. 140 out of 209 theoretically possible congeners are formed. The environmental behavior and the toxicity vary considerably between the different congeners and depend to a large extent on the chloro-substitution pattern.

PCBs enter the environment as long range air-transported pollutants[2] originating from scattered dumps and waste incineration[3]. For the terrestric ecosystem an important source may be the agricultural spreading of sewage sludge (contaminated with PCBs) used for improvements in soil fertility[4].

It has been shown that certain PCB congeners can be metabolized in the marine food web which leads to a selective bioaccumulation of resistant congeners. The presence of vicinal non-chlorinated *para-* and *meta-*positions in the biphenyl skeleton favours metabolism by facilitating (enzymatic) epoxidation of these positions. The epoxy-PCB will easily hydrolyze and the formed hydroxy-PCB will be eliminated from the organism due to its higher aqueous solubility[5].

Also in the terrestrial food web selective bioaccumulation of PCB congeners may occur. However, while the uptake and metabolism of PCBs in aquatic fauna have been studied extensively, little attention has been paid to terrestrial fauna and only a limited number of investigations on PCB uptake and metabolism in terrestrial fauna have been reported; these have been mostly confined to single PCB congeners or single technical PCB formulations. Many isomers, especially highly chlorinated biphenyls, have not been studied in this respect[5].

The principal animal biomass in soil is earthworms. They constitute a major food source for birds and terrestrial fauna. Earthworms ingest enormous quantities of soil and have shown to bioaccumulate organic trace pollutants such as PCBs. In the literature only scarce data are published on the importance of earthworms being the first step in the bioaccumulation of PCBs in the terrestrial food web[6]. A part of the reason for this lies in the experimental difficulties associated with PCB analysis of soil and biological tissue. The quantifications have primarily been carried out relative to commercial PCB formulations, thus obscuring any correlations which might exist between the physico-chemical properties of individual congeners and their behavior in the soil/soil water/earthworm system. The present study was designed to overcome these limitations through quantification of individual congeners. The study was facilitated by the previous characterization of PCB congeners in the Askarel PCB formulation by several gaschromatographic columns[7,8] and by taking into account the knowledge of the soil sorptive behavior of PCB congeners obtained from previous experiments[8,9].

EXPERIMENTAL

Setup

The uptake of PCB congeners in earthworms (*Lumbricus rubellus*) was studied after the earthworms had been kept for 48 hours in a contaminated soil (150 $\mu g\,g^{-1}$ Askarel). The earthworms were transferred into a low-contaminated soil (1.5 $\mu g\,g^{-1}$ total PCB) and their elimination of PCB congeners was monitored during 60 days.

Soil

To insure a uniform distribution of PCBs in the soil for the accumulation study (A_1) a commercial PCB formlation (Askarel) was dissolved in acetone and mixed with residue-free clean sand. After 24 hours respite the solvent had evaporated and the sand was then mixed with soil (1:4) by 48 hours mechanic shaking. The soil was obtained from the Ispra Joint Research Center experimental site as the A_1 horizon of a Dystric Gleysol[8].

The soil for the elimination study was a composted 0-horizon which was naturally contaminated with PCBs at a low level.

Table 1 *Soil characteristics*

Soil 0 *Composted agricultural soil*		*Soil A_1* *Dystric Gleysol*	
Sand	90%	Sand	85%
Silt	7%	Silt	11%
Clay	3%	Clay	4%
pH	7.4	pH	4.6%
f_{oc}	0.71	f_{oc}	0.042

The soil characteristics are shown in Table 1 and the concentration of PCB congeners in Table 2.

48 hours prior to the experiments the water content of the soils was adjusted to approx. 80% of the field capacity and then maintained during the experiments by frequent irrigation. The temperature was kept at 15°C and the air humidity at 90% RH. The soil surface was continuously illuminated with approx. 800 Lux in order to avoid that any earthworms escaped during the two-month experiment.

Earthworms

The species *L. rubellus* is one of the most common European earthworms. It lives in superficial soils and feed on partially degraded organic matter and microorganisms. Adult individuals normally weigh 0.3 to 0.5 g wet weight. Adults were breaded in soil 0 and rinsed thoroughly with distilled water before use. After the desired contact time with the soil the earthworms were prepared for analysis by allowing them to empty their digestive tract on wet filter paper for 48 hours. The used concentration of 150 $\mu g\, g^{-1}$ Askarel had no toxic effect on the earthworms.

Extraction and clean-up

Each individual was weighed and placed in a 10 ml teflon-capped glass tube containing a 4 ml 2 N KOH in methanol for one week. The dehydrated tissue was

Table 2 Concentrations (*$\mu g\ g^{-1}$ dw*) of PCB congeners in soils used in the accumulation (A_1) and the elimination (0) study

PCB #	*Soil A_1*	*Soil 0*	*PCB #*	*Soil A_1*	*Soil 0*
52	1.9[a]	0.036[b]	153	11.0	0.158[b]
95	7.3	0.108	170	4.0	0.044
101	8.0	0.132	171	1.4	0.048
110	7.0	0.106	177	1.5	0.156
118	4.0	0.064	180	7.0	0.078
138	9.7	0.130	183	1.9	0.022
146	1.1	0.020	194	0.9	0.008
149	9.7	0.122	201	0.7	0.006
151	5.5	0.056			

[a] Mean of three determinations. MEAN CV = 11%.
[b] Mean of three determinations. MEAN CV = 10%.

mechanically disintegrated and the obtained slurry was extracted with 3 x 5 ml hexane. The combined hexane phase was cleaned-up by acid-silica/silica open column chromatography[10]. The clean extract was roto-evaporated to approx. 1 ml, transferred into a tared autosampler vial, and then, by using a gentle stream of N_2, evaporated to almost dryness. The final volume was adjusted (gravimetrically) to approx. 2 ml with *iso*-octane including 2,4-dichlorobenzylheptyl ether and 2,4-dichiorobenzyltetradecanyl ether as internal (syringe) standards[11]. The vial was sealed with a teflon lined crimping cap and stored at 5°C until analysis.

The soils (10 g) were extracted with acetone-hexane 1:1 (50 ml) for 3 days. Hexane (50 ml) was added and an aliquot of the coloured extract (50 ml) was roto-evaporated to approx. 2 ml and prepared for analysis as described above.

Analysis

Aliquots (0.7 μl) of the final extract were automatically splitless-injected (290°C) into a DANI 8520 gaschromatograph (GC) equipped with an electron capture detector (300°C) and a 25 m x 0.22 mm HT5 column (0.1 μm film of siloxane-carborane co-polymer, Scientific Glass Engineering). The following GC conditions were used: Linear helium carrier gas velocity 23 cm s^{-1}; oven temperature program: 2 min at 90°C, then at 20°C min^{-1} to 200°C, hold 7.5 min, then at 3°C min^{-1} to 280°C.

In order to operate the ECD in the linear range, it was necessary to re-analyse some congeners of the most concentrated samples in diluted form.

Data were acquired and processed on a Chromstation/2 system (Spectra Physics, Milan, Italy).

The PCB congeners (Table 2) were quantified by comparison to standards obtained from the Community Bureau of Reference of the EC (Bruxelles) and from Promochem (Wessel, Germany). All standards were checked for identity and purity.

PCB congeners were selected for quantification based on their chromatographic behavior and ecotoxicological importance. They represent different degrees of chlorination (tetra- to octa-) with different substitution patterns (Figure 1).

The GC column used (HT5) allowed for the accurate analysis of 17 major congeners of the Askarel PCB mixture. Problems with peak interferernce (PCB 46/PCB 52, PCB 66/PCB 95, PCB 84/PCB 101, PCB 118/PCB 149, PCB 105/PCB 132/PCB 153, PCB 163/PCB 138 and PCB 170/PCB 190) as reported for the column phase 5% phenyl, methylsiloxane[7,12–14] were overcome with the HT5 column[15–16]. The interference on the HT5 column[15–16] from PCB 56/PCB 60 with PCB 101, PCB 102 with PCB 95, PCB 77 with PCB 149 and PCB 193 with PCB 180 is insignificant for the present experiments due to the low concentrations in Askarel of these potentially interfering compounds compared to the target compounds.

RESULTS AND DISCUSSION

Accumulation

After 48 hours in soil A_1 the earthworms had taken up considerable amounts of PCB and the concentration approached that of the soil for several congeners.

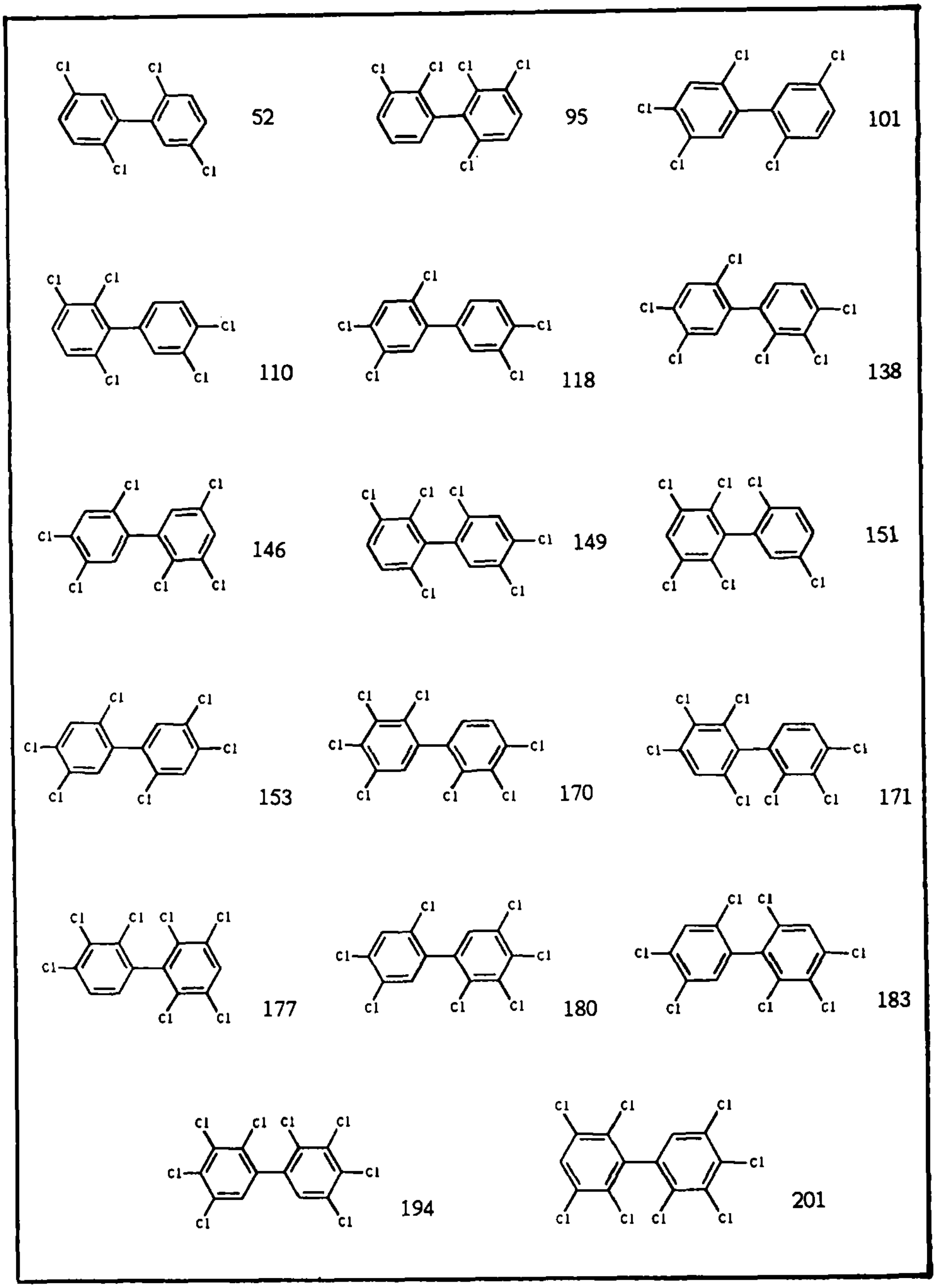

Figure 1 Chemical structures and IUPAC numbers of the studied PCB congeners.

Table 3 Uptake of PCB congeners from soil A_1 by *L. rubellus*

	Bio-concentration[a] *after 48 hours*	*Soil-water distribution* log K_{oc}
TETRACHLORO		
di-*ortho*		
PCB 52	0.74 (0.16)[b]	5.34[c]
PENTACHLORO		
tri-*ortho*		
PCB 95	0.71 (0.15)	5.90
di-*ortho*		
PCB 101	0.66 (0.14)	6.11
PCB 110	0.66 (0.13)	6.10
mono-*ortho*		
PCB 118	0.62 (0.14)	6.31
HEXACHLORO		
tri-*ortho*		
PCB 149	0.64 (0.14)	6.40
PCB 151	0.54 (0.12)	6.36
di-*ortho*		
PCB 138	0.55 (0.11)	6.56
PCB 146	0.57 (0.10)	(6.57)
PCB 153	0.58 (0.11)	6.58
HEPTACHLORO		
tri-*ortho*		
PCB 171	0.52 (0.11)	(6.74)
PCB 177	0.51 (0.11)	6.79
PCB 183	0.52 (0.10)	6.74
di-*ortho*		
PCB 170	0.45 (0.09)	(6.93)
PCB 180	0.49 (0.10)	6.83
OCTACHLORO		
tri-*ortho*		
PCB 201	0.42 (0.10)	(6.96)
di-*ortho*		
PCB 194	0.36 (0.08)	(7.20)

[a] Concentration in earthworm relative to soil.
[b] MEAN (SD) of four replications.
[c] Experimental (predicted) values from correlation studies[18].

The relative enrichment of PCBs from soil to earthworms (bioconcentration factor, BCF) after 48 hours of contact was calculated based on a 16.6% dry matter concentration of *L. rubellus* (Table 3). It appears that accumulation (BCF) is increasing with increasing degree of chlorination and with a decreasing number of chlorine atoms substituted in the *ortho*-positions. This may be due to a slow diffusion for such congeners. This explanation is in accordance with previously obtained data for soil sorption kinetics of PCB congeners[8].

The higher interstitial water concentration in the soil of the lower chlorinated PCBs (due to their lower soil sorption) may also explain their higher uptake. For chlorinated phenols recent data have strongly indicated that the concentration in the soil solution plays an important role in the bioaccumulation in earthworms (*Eisenia fetida Andrei and L. rubellus*) of lipophilic compounds[17].

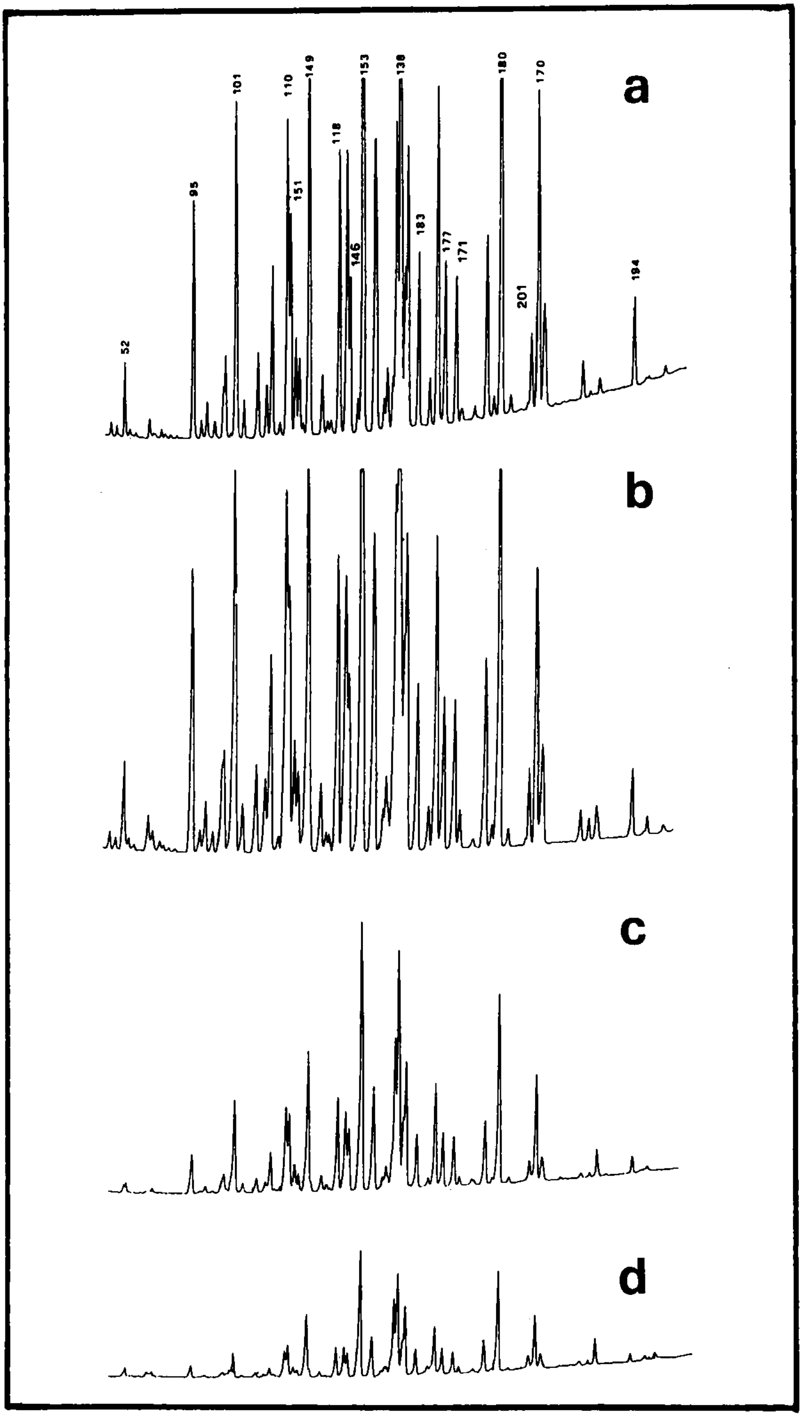

Figure 2 Chromatograms of PCBs from: (a) Soil A_1 used for the accumulation study; (b), (c) and (d) Earthworm after 48 hours in soil A_1, 35 days in soil 0 and 60 days in soil 0, respectively.

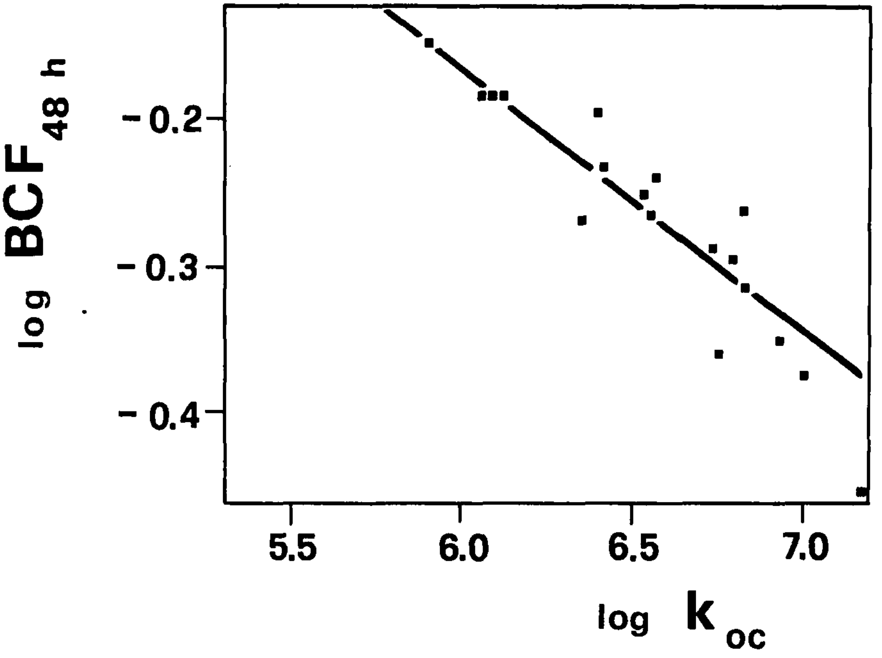

Figure 3 Short term bioconcentration factor (BCF) vs. soil-water distribution constant K_{oc} (log forms).

The soil-water distribution coefficient normalized to organic carbon (K_{oc}) of the PCB congeners is shown in Table 3. Data were obtained as mean values of predictions from correlation studies between K_{oc} and PCB structure, octanol-water partition coefficient (K_{ow}), water solubility and chromatographic behavior. The predicted values were derived from soil sorption data of 50 PCB congeners in three Dystric Gleysols[18].

The log BCF after 48 hours is plotted versus log K_{oc} in Figure 3. A reverse proportionality can be observed indicating that strongly soil-sorbed PCB congeners are not readily desorbed and accumulated in earthworms. Whether this is due to a slower desorption kinetic or due to a slower diffusions of the strongly soil-sorbed PCB congeners in the soil-water-earthworm system remains uncertain. It has been demonstrated that more than 20 days are needed for obtaining equilibrium between earthworms and soil[6]. It is quite possible that given sufficient time for the equilibrium to be reached a reverse relationship between log BCF and log K_{oc} may not exist any longer. However, with the design of the present experiment this question cannot be addressed.

Elimination

During the stay in the low contaminated soil PCBs were eliminated from the earthworms. The chromatographic profiles changed only quantitatively during the elimination study (Figure 2). All PCBs studied were present in measurable quantities after 60 days and no single congener behaved significantly different from the bulk.

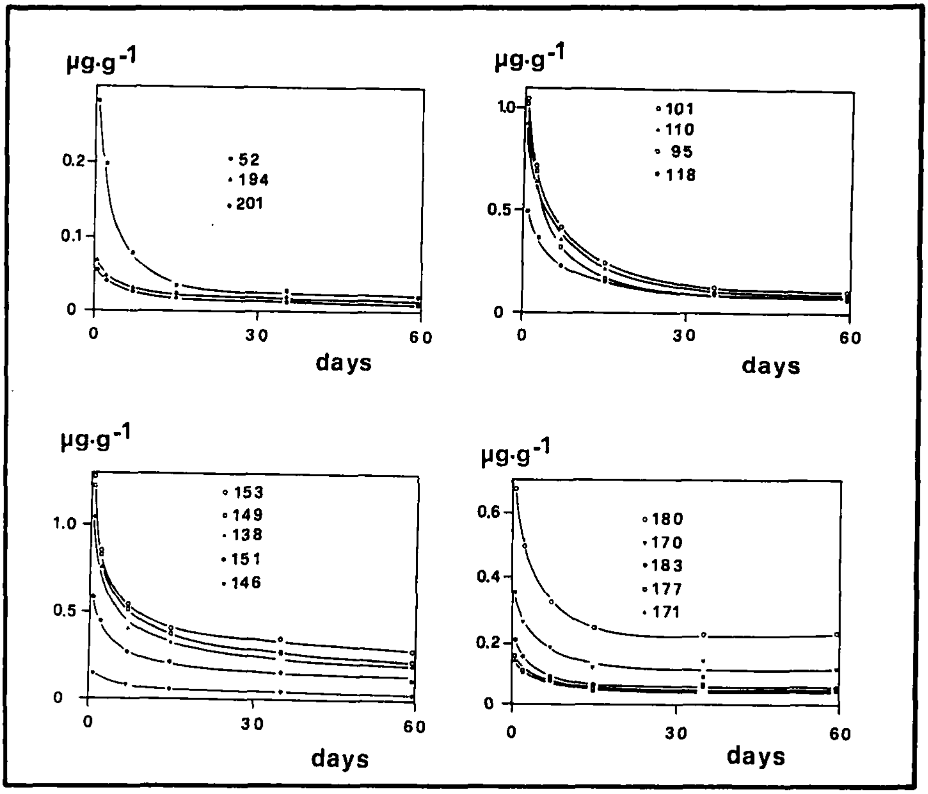

Figure 4 Time dependency of the PCB concentration in earthworms during 60 days of elimination. (a) Tetra- and octa-; (b) penta-; (c) hexa-; (d) hepta-chlorinated isomers.

No selective elimination was evident after the visual examination of all peaks in the chromatographic profiles.

The concentration of PCB congeners in earthworms in relation to the decontamination time is shown in Figure 4 for tetra- plus octa- (4a), penta- (4b), hexa- (4c) and hepta- (4d) chloro-biphenyls, respectively. The shapes of the curves are similar for all congeners and resemble an exponential degradation towards an equilibrium concentration, $C_{\mathrm{WORM,\ eq}}$. The major variation between congeners seems to be for the value of C_{WORM}, achieved after 15–20 days. A mathematical formulation of the elimination curves is given in Eq. (1):

$$C_{\mathrm{WORM}}(t) = C_{\mathrm{WORM,eq}} + (C_{\mathrm{WORM,day\,0}} - C_{\mathrm{WORM,eq}}) \times \mathrm{EXP}(-kt) \tag{1}$$

where the time constant k (days^{-1}) is a measure of how fast the equilibrium state is reached.

The data of Figure 4 were fitted to Eq. (1). The values of the variables $C_{\mathrm{WORM,\ eq}}$ and k were estimated for each congener by iterative least squares approximation based on a Taylor progression of k. Half-times were derived from the half-concentrations between $C_{\mathrm{WORM,day\,0}}$ and $C_{\mathrm{WORM,eq}}$ (Table 4). There was no statistically signifi-

Table 4 Kinetic of elimination of PCBs from *L. rubellus*

	k^a $days^{-1}$	$C_{WORM,eq}$ ng/g ww	$T_{1/2}$ $days$
TETRACHLORO			
di-*ortho*			
PCB 52	0.21 (0.01)[b]	0.024 (0.003)[b]	3.3 (2.9–3.6)[c]
PENTACHLORO			
tri-*ortho*			
PCB 95	0.21 (0.01)	0.10 (0.01)	3.3 (2.9–3.8)
di-*ortho*			
PCB 101	0.18 (0.02)	0.13 (0.03)	3.9 (2.9–6.0)
PCB 110	0.20 (0.03)	0.13 (0.02)	3.5 (2.4–6.0)
mono-*ortho*			
PCB 118	0.18 (0.03)	0.10 (0.02)	3.9 (2.7–6.7)
HEXACHLORO			
tri-*ortho*			
PCB 149	0.22 (0.03)	0.28 (0.03)	3.2 (2.3–5.2)
PCB 151	0.23 (0.05)	0.14 (0.02)	3.0 (1.9–7.1)
di-*ortho*			
PCB 138	0.28 (0.05)	0.27 (0.03)	2.5 (1.6–5.2)
PCB 146	0.26 (0.05)	0.050 (0.005)	2.7 (1.7–5.0)
PCB 153	0.26 (0.03)	0.35 (0.03)	2.7 (1.9–4.9)
HEPTACHLORO			
tri-*ortho*			
PCB 171	0.21 (0.05)	0.049 (0.005)	3.3 (2.1–9.0)
PCB 177	0.24 (0.04)	0.055 (0.003)	2.9 (2.0–5.1)
PCB 183	0.21 (0.05)	0.060 (0.005)	3.3 (2.1–9.0)
di-*ortho*			
PCB 170	0.23 (0.03)	0.12 (0.01)	3.1 (2.2–5.3)
PCB 180	0.24 (0.02)	0.26 (0.01)	2.9 (2.4–3.5)
OCTACHLORO			
tri-*ortho*			
PCB 201	0.25 (0.03)	0.018 (0.002)	2.8 (1.9–4.5)
di-*ortho*			
PCB 194	0.26 (0.04)	0.021 (0.001)	2.7 (1.9–4.6)

[a] $C_{WORM}(t) = C_{WORM,eq} + (C_{WORM,0} - C_{WORM,eq}) \times \exp(-kt)$.
[b] Estimate (standard error of estimates).
[c] Half-time (95% confidential range).

cant difference between the time constant (k) of each congener. The elimination occurred with a half-time of 3.3 ± 0.4 days (mean of all congeners ±SD).

The lack of selective elimination of certain PCB congeners strongly points to the conclusion that metabolism is not important in earthworms (*L. rubellus*). Several of the studied isomers have vicinal non-chlorinated *meta-* and *para*-positions (PCB 52, PCB 95, PCB 101, PCB 110, PCB 149 and PCB 151). They are usually selectively eliminated in the higher levels of the food web[2]. In the present experiment with earthworms (*L. rubellus*) these isomers showed elimination rates not different from the isomers which are resistant to metabolism in the food web[2].

The percentage of PCB eliminated after 60 days was calculated for each congener relative to day 0 (Table 5). There was a weak (statistically non-significant but consistent) tendency of the lower chlorinated congeners being eliminated to a greater

Table 5 Elimination of PCB congeners from *L. rubellus* after 60 days in soil 0

	% Eliminated	*Bio-concentration factor*		*Octanol-water distribution*[c]
		$BCF_{60\,days}$	BCF_{eq}	$\log K_{ow}$
TETRACHLORO				
di-*ortho*				
PCB 52	85 (7)[a]	3.6 (0.4)[a]	4.1[b]	5.84
PENTACHLORO				
tri-*ortho*				
PCB 95	84 (4)	5.1 (0.3)	5.6	6.13
di-*ortho*				
PCB 101	85 (5)	4.6 (0.3)	5.9	6.38
PCB 110	84 (6)	6.2 (0.8)	7.3	6.48
mono-*ortho*				
PCB 118	83 (6)	7.5 (0.6)	9.7	6.74
HEXACHLORO				
tri-*ortho*				
PCB 149	81 (11)	11.2 (1.4)	13.8	6.67
PCB 151	64 (13)	12.6 (2.0)	15.4	6.64
di-*ortho*				
PCB 138	77 (7)	10.2 (1.0)	12.3	6.83
PCN 146	79 (9)	11.0 (1.4)	13.2	6.89
PCB 153	76 (8)	10.4 (1.0)	13.3	6.92
HEPTACHLORO				
tri-*ortho*				
PCB 171	75 (5)	19.4 (1.7)	18.7	7.11
PCB 177	76 (5)	15.7 (1.6)	22.2	7.08
PCB 183	72 (6)	14.4 (1.5)	16.5	7.20
di-*ortho*				
PCB 170	72 (10)	15.6 (1.4)	16.8	7.27
PCB 180	70 (8)	17.2 (1.6)	17.4	7.36
OCTACHLORO				
tri-*ortho*				
PCB 201	75 (9)	18.1 (1.8)	18.0	7.62
di-*ortho*				
PCB 194	77 (6)	16.8 (1.5)	14.7	7.80

[a] MEAN (SD) of four replications.
[b] From the estimated equilibrium state by equation (1) of the text.
[c] Data from Hawker and Connell[19].

extent than the higher chlorinated congeners. However, no difference between isomers with or without vicinal non-chlorinated *meta-* and *para-* positions was discernible. The same tendency (statistically significant) was evident for the BCF at 60 days ($p < 0.001$). From the estimated $C_{WORM,\ eq}$ (Table 4) the bioconcentration factor at equilibrium (BCF_{eq}) was calculated (Table 5). A linear correlation between the log-forms of BCF_{eq} (Figure 5) or BCF_{60} and the octanol-water partition coefficient (K_{ow}) was derived (data on log K_{ow} from Hawker and Connel[19]):

$$\log BCF_{eq} = -1.30\ (\pm 0.12) + 0.35\ (\pm 0.06) \times \log K_{ow} \tag{2a}$$

$$r = 0.84,\ n = 17.$$

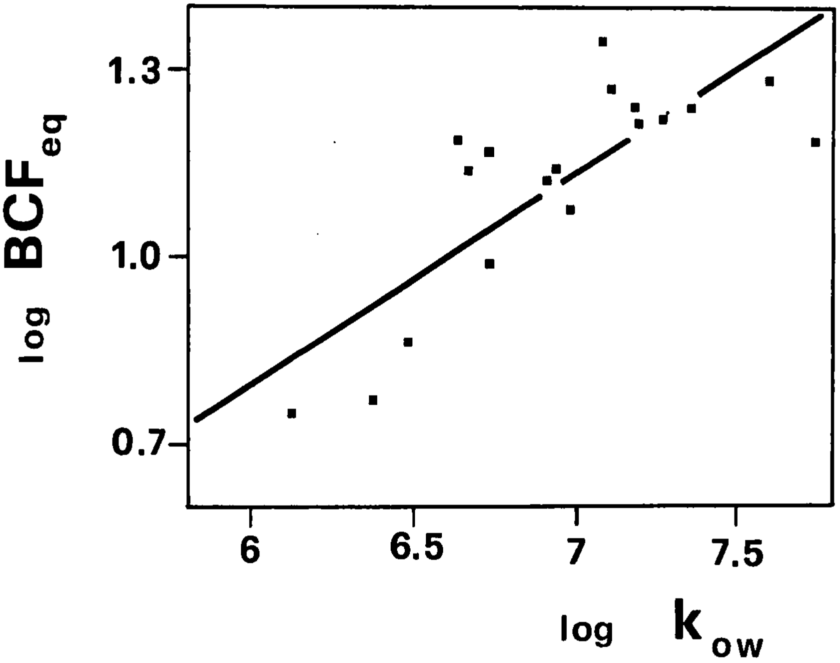

Figure 5 Equilibrium bio-concentration factor vs. octanol-water partition coefficient (K_{ow}) of PCBs (log forms).

$$\log \mathrm{BCF}_{60} = -1.73\ (\pm 0.10) + 0.40\ (\pm 0.05) \times \log K_{ow} \tag{2b}$$

$$r = 0.90,\ n = 17.$$

A theoretical relation between BCF and K_{ow} for stable compounds in the soil to earthworm system has recently been suggested^{20}:

$$\mathrm{BCF} = k_1 \times f_{\text{lipid}} \times f_{oc}^{-1} \times K_{ow}^{b-a} \tag{3}$$

where k_1 is a proportionality factor, f_{lipid} is the lipid fraction of the earthworms and f_{oc} is the organic carbon fraction of the soil. The non-linearity constant (a) originates from the empirical relations between soil-water partition coefficients (K_d) and K_{ow} of non-ionic compounds:

$$K_d = k_1 \times f_{oc} \times K_{ow}^{a} \tag{4}$$

and the non-linearity constant (b) originates from the empirical relations between biotic concentration/water concentration (K_B) and K_{ow} for stable lipophilic compounds:

$$K_B = f_{\text{lipid}} \times K_{OW}^{b} \tag{5}$$

Connel and Markwell (1990)^{20} concluded that since a and b are both often close to unity the dependency of BCF on K_{ow} is generally limited. By combining literature data on K_B and f_{lipid} from earthworms and other Oligochaete obtained for a variety of compounds with K_{ow} in the range of 1 to 6 the authors derived a value of 1.26 for b in Eq. (5). However for compounds with $K_{ow} > 6.4$ the non-linearity constant (b) decreases with K_{ow}[21]. For the soil sorption of 50 PCB congeners correlations have recently been developed between K_{oc} and K_{ow} resulting in values of the non-linearity constant (a) in Eq. (4) between 0.68 and 0.79 (depending on the origin of the K_{ow} data)[18]. Given the fact, that most of the studied PCBs have K_{ow} values greater than 6.4 and taking the substantial sources of variation into account when combining data from experiments conducted under different conditions, the values of b–a (0.47–0.58) which can be derived from earlier earthworm investigations[18,19,20] is in remarkable agreement with that of the present study (0.35–0.40; Equations 2a and 2b).

CONCLUSION

The earthworm's (*L. rubellus*) uptake and elimination of PCB congeners in the three-phase system soil/soil water/earthworms seem governed by passive, possibly diffusion controlled processes.

The elimination rate of PCB from earthworms (*L. rubellus*) was similar for all studied congeners ($T_{1/2}$: 3–4 days) notwithstanding their different chloro-substitution pattern. This strongly suggests that metabolism is of minor importance.

The soil to earthworm bioconcentration factor for PCB congeners demonstrates a weak dependence of the octanol-water partition coefficient: $\mathrm{BCF} = -(1.3\text{–}1.7) \times K_{ow}^{(0.35-0.40)}$.

Acknowledgements

The authors thank Dr. S. Kephalopoulos for assistance with computer calculations of the elimination kinetics.

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