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ASSESSING THE EFFECTS OF MODIFYING FACTORS ON PENTACHLOROPHENOL TOXICITY TO EARTHWORMS: APPLICATIONS OF BODY RESIDUES

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Abstract—This study examined the toxicokinetic relationship between critical body residues (CBRs) of pentachlorophenol (PCP) at lethality, soil PCP concentrations, and modifying factors of toxicity such as body size, species, temperature, and soil type during 28-d bioassays with earthworms. *Eisenia fetida* (0.2–0.4 g) were exposed to PCP at 15 and 24°C in artificial soil and at 24°C in Brookston clay. *Lumbricus terrestris* (3.3–4.6 g) and *Eudrilus eugeniae* (1.5–2.7 g) were exposed in artificial soil at 15 and 24°C, respectively. Soil PCP concentrations ranged from 0 to 6.75 mmol PCP/kg soil (dry weight), and body residues were measured by gas chromatography following cryoextraction of freeze-dried earthworms. Comparisons of toxicity were made using incipient lethal levels (ILLs) and CBRs at lethality. The equations estimated by nonlinear curve fitting of a one-compartment, first-order kinetics model provided a good fit to the observed data for all species, temperatures, and soil types at which tests were conducted. Incipient lethal levels for *L. terrestris* (0.72 mmol/kg soil) and *E. eugeniae* (0.63 mmol/kg soil) were similar and significantly higher than those for all *E. fetida* treatments. Incipient lethal levels for *E. fetida* exposed to PCP in artificial soil at 15 and 24°C were similar and lower than the ILL for *E. fetida* exposed to PCP in Brookston clay. The toxicity half-lives were similar for all *E. fetida* treatments but were higher than for the larger worms, *L. terrestris* and *E. eugeniae*, which were similar. Mean CBRs at lethality for *E. fetida* tested in artificial soil at 24°C ranged from 0.33 to 0.74 mmol/kg (wet weight) over the range of soil PCP exposure concentrations from 0.12 to 3.75 mmol PCP/kg. Mean CBRs for *E. fetida* exposed to PCP at 24°C in Brookston clay ranged from 0.51 to 1.59 mmol PCP/kg (wet weight) over the same range of soil PCP concentrations and were not significantly different. Critical body residues for *E. fetida* (15°C), *L. terrestris*, and *E. eugeniae* tested in artificial soil at 2.10 mmol PCP/kg ranged between 0.34 and 0.80 mmol PCP/kg. Residues in surviving worms were either similar to or lower than those in dead worms. Similar CBRs in three species of earthworms differing 10-fold in body size, and for *E. fetida* exposed to PCP concentrations ranging over more than two orders of magnitude in two different soil types, demonstrate that body residues can act to more effectively integrate the relationship between test organism, exposure, and mortality than soil toxicant concentrations alone.

Keywords—Earthworms Body residues Soil Pentachlorophenol Toxicity

INTRODUCTION

A basic tenet of toxicology is that the dose makes the poison. A number of toxicity tests have been proposed to examine the potential impacts of the toxic dose of contaminants on soil organisms, particularly earthworms [1–3]. One of the principal difficulties with interpreting and applying the results of these tests has been the variability in contaminant bioavailability arising from differences in soil type and test organisms. Two main strategies have been employed to account for bioavailability of toxicants in soils. First, artificial soil formulations have been used to standardize the test matrix with respect to particle size, organic matter content, pH, and chemical constituents with species-specific tests [4]. While this approach is useful for comparing the dose response of chemicals for a single test species, it is compromised when comparing results to natural soils that differ in chemical and physical characteristics from artificial soil and support biologically diverse communities. The second approach has involved investigations of the physical and chemical heterogeneity of natural soils and to what extent these factors modify toxicity. The basic premise with this approach assumes that if the relationships between soil constituents and toxicity can be understood, then models relating the toxicity of chemicals to soil attributes can be developed. Unfortunately, the relationships are complex and interactive, and it is difficult to incorporate the spatial and temporal heterogeneity of the soil medium, as well as the behavior and physiology of soil organisms, into a model.

Several soil bioassay protocols in the last decade have identified *Eisenia fetida* (Sav.) as the preferred test species, although *Lumbricus terrestris* (L.) has also been suggested as a suitable species [3–5]. Numerous other species have also been used in toxicity tests (*Perionyx excavatus, Aporrectodea caliginosa, Eudrilus eugeniae, Lumbricus rubellus,* and *Allolobophora tuberculata*), and comparisons of toxicity have always been based on soil concentrations of toxicants, often for soils of varying composition [5–8]. The use of critical body residues (CBRs) [9] may reduce some of the variability in relating bioassay end points to measures of chemical exposure for test results obtained with different worm species in various soil types. This toxicokinetic approach assumes that the toxicant concentration at the site(s) of toxic action in an organism is relatively constant for an overt biological response such as mortality and that uptake follows Fick's Law of Diffusion [9]. In order for the approach to be applied, measures of toxicity (e.g., LC50s) are linked with pharmacokinetic models of toxicant uptake, such as a one-compartment, first-order kinetics (1CFOK) model. It is essential that toxicity comparisons are made at steady state, when both mortality and tissue residues have reached threshold values. In practical terms, bioassays must be continued for a period of time sufficient for the concentration causing 50% mortality (LC50) to have reached a time-independent LC50 or incipient lethal level (ILL).

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Our study examined the relationship between ILLs and CBRs for pentachlorophenol (PCP), a xenobiotic which uncouples oxidative phosphorylation [10], in three species of earthworms (*L. terrestris*, *E. eugeniae*, and *E. fetida*). These test species have distinct temperature requirements and encompass a 10-fold difference in body size. Tests were also conducted with *E. fetida* at one temperature in two soil types. The general hypothesis examined in these experiments was that at lethality, body residues or CBRs were similar regardless of test species, body size, test temperature, soil type, or soil PCP concentration.

MATERIALS AND METHODS

Toxicity tests were conducted with *E. fetida*, *L. terrestris*, and *E. eugeniae* exposed to nominal PCP concentrations ranging from 0 to 6.75 mmol/kg for 28 d in artificial soil. A similar toxicity test was also conducted with *E. fetida* in a natural clay soil. Tests with *E. fetida* were conducted at 15 and 24°C, while those with *L. terrestris* and *E. eugeniae* were conducted at 15 and 24°C, respectively. Comparisons of toxicity were made using ILLs derived from nonlinear regressions of time–toxicity data using nominal soil PCP concentrations or with whole-body PCP concentrations.

Adult *E. fetida* (0.3–0.4 g) and *E. eugeniae* (1.5–2.7 g) were purchased from a commercial grower (Early Bird Ecology and Bait Ltd., Smithville, ON, Canada), while adult *L. terrestris* (3.3–4.6 g) were purchased from a live bait wholesaler (Hogans Live Bait Ltd., Alma, ON). All earthworms were monitored in the laboratory for mortality and general health for a minimum of 2 weeks before use. *Eisenia fetida* and *E. eugeniae* were maintained at 22 ± 0.5°C, while *L. terrestris* were refrigerated (4°C).

Artificial soil (clay, ~20%; organic matter [OM], ~10%; sand, 69%; $CaCO_3$, 1%; pH, ~7.5) [3] was prepared in 10-kg batches and allowed to equilibrate for at least 3 d prior to the addition of PCP. Brookston clay (OM, ~13%; pH, ~6.1) was obtained from the Agriculture Canada Research Station (Harrow, ON), sieved through a 2-mm stainless-steel mesh until it appeared uniform in color and texture, and stored at 4 ± 0.5°C pending use. The collection site had a known land-use history and was considered to be free of pesticide and other contamination.

Testing was conducted in environmental chambers (Model E-7, Conviron, Winnipeg, MB, Canada) with controlled humidity, photoperiod, and temperature. *Eudrilus eugeniae* were tested at 24 ± 0.5°C; *L. terrestris*, at 15 ± 0.5°C; and *E. fetida*, at both temperatures. Earthworms and test soils were acclimated to test conditions in the environmental chambers for a minimum of 24 h prior to testing. Test soils (*E. fetida* and *E. eugeniae*, 300 g dry weight; *L. terrestris*, 400 g) were weighed and placed into clear glass jars which had been washed according to Greene et al. [11]. A stock solution was prepared by dissolving PCP (Aldrich Chemical Co., St. Louis, MO, USA) in a sodium hydroxide (NaOH) solution. Appropriate volumes of stock solution were added to the soil aliquot and mixed manually until the soil appeared uniform in color, texture, and moisture content. Each test used a logarithmic series of 10 PCP concentrations covering the range of 0.038 to 6.75 mmol/kg plus a distilled water control and a solvent control containing the maximum amount of NaOH solution used in dispensing PCP to the soil. Soil PCP concentrations are reported as nominal values throughout. The tests were conducted in triplicate with 10 worms per jar and lasted 28 d, with mortality observations at 1, 2, 4, 8, and 16 h, and on days 1, 2, 4, 5, 7, 14, 21, and 28. Some additional mortality observations were also made during individual bioassays. Worms were presumed dead when no contractile response was observed upon light prodding with a blunt probe. All mortalities were frozen at −80°C for PCP residue analysis.

Whole-body PCP concentrations were determined for *E. fetida* at 24°C in both soil types for all exposure concentrations. Residues for *E. fetida* exposed to PCP at 15°C and for other earthworm species were determined for worms from the distilled water control, from soil PCP concentrations associated with the estimated ILLs, and from one other concentration (2.10 mmol PCP/kg). Pentachlorophenol concentrations were measured by gas chromatography (GC) following cryoextraction of the earthworms. The complete method for residue and lipid analysis is described elsewhere [12]. Earthworms were freeze-dried, then digested in hot sulphuric acid with a hexane overlay. This digest was mixed, centrifuged, and then cooled on dry ice. Once the lower aqueous layer had solidified, the hexane was decanted, and the procedure was repeated twice more. The final hexane volume was adjusted to 10 ml by either evaporation or topping up. A 1-ml aliquot of the sample was spiked with an internal standard (dibromobenzene) and derivatized with diazomethane [13] in preparation for GC analysis. Pentachlorophenol was quantified with a Varian 3400 gas chromatograph equipped with an electron capture detector using nitrogen as the make-up gas. Recovery of PCP from the tissue samples was calculated to be >90%, and reported values were not corrected for recovery. Lipid determination followed the vanilline method of Meyer and Walther [14], using oleic acid for constructing the standard curve.

Initial nominal PCP concentrations in soils were used to determine LC50s from mortality data by trimmed Spearman-Karber analysis [15] for each time where >50% mortality occurred in at least one concentration. Incipient lethal levels were estimated by fitting a ICFOK model to the LC50 time data using nonlinear regression analysis and calculating the asymptotic value of the curve. Residue and ILL data were analyzed by analysis of variance, and means were compared using Tukey's Honestly Significant Difference test ($\alpha = 0.05$) for *E. fetida* at 24°C in both soil types. Data normality and homogeneity of variance were determined by analysis of residuals [16]. If these assumptions were violated, $\log_{10}$ transformations were applied to the data to satisfy those assumptions prior to statistical analysis.

RESULTS

A significant ($p < 0.0001$) treatment effect on ILLs and toxicity half-lives ($t_{1/2}$, time required to reach half the time-independent threshold for lethality, i.e., the ILL) was evident (Table 1). Incipient lethal levels for *L. terrestris* and *E. eugeniae* were similar and significantly higher than those for all *E. fetida* treatments. The ILLs for *E. fetida* exposed to PCP in artificial soil at 15 and 24°C were similar, while the ILL for *E. fetida* exposed to PCP in Brookston clay was significantly higher. A sevenfold difference was observed between the highest and lowest ILLs. The half-lives for all *E. fetida* treatments were similar, as were half-lives for the two larger species of worms, *L. terrestris* and *E. eugeniae*. However, $t_{1/2}$ values for the larger species were significantly lower than those for *E. fetida*.

Critical body residues at lethality for *E. fetida* tested in artificial soil at 24°C ranged from 0.33 to 2.65 mmol PCP/kg

Table 1. Toxicological end points for earthworms exposed to PCP in different experimental regimes

Species	Size[a] (g)	Soil type	Temperature (°C)	ILL[a,b] (mmol PCP/kg)	$t_{1/2}$[a,b] (h)
E. fetida	0.23 (0.03)	Natural	24	0.27A (0.08)	72A (21.5)
	0.36 (0.06)	Artificial	24	0.14B (0.001)	138A (31)
	0.36 (0.06)	Artificial	15	0.10B (0.002)	129A (5.7)
E. eugeniae	2.25 (0.35)	Artificial	24	0.63C (0.12)	23B (5.7)
L. terrestris	3.81 (0.30)	Artificial	15	0.72C (0.12)	17B (6.4)

[a] Average measurement (n = 3) with SD in parentheses below.
[b] Values in columns with a common letter are not significantly different ($p > 0.05$).

(wet weight) over the entire range of soil PCP exposure concentrations, from 0.12 to 6.75 mmol PCP/kg (dry weight) (Table 2). Only CBRs in worms exposed to 6.75 mmol PCP/kg were significantly higher than those measured at other PCP concentrations (Table 2). Residues of PCP in surviving worms were either similar to or lower than those in dead worms (Table 2). Body residues of worms from the two control treatments did not differ from one another and were significantly lower than residues in all PCP-exposed worms (Table 2). Mean CBRs for *E. fetida* exposed to PCP at 24°C in Brookston clay ranged from 0.51 to 1.59 mmol PCP/kg (wet weight) over the same range of soil PCP concentrations and were not significantly different (Table 2). Critical body residues for *E. fetida* (15°C), *L. terrestris*, and *E. eugeniae* tested in artificial soil at PCP concentrations near their respective ILLs and 2.10 mmol PCP/kg ranged from 0.47 to 2.18 mmol PCP/kg (wet weight). The ranges for lipid content in the earthworms were as follows: *E. fetida* tested in Brookston clay was 3.6 to 6.8% and in artificial soil was 3.2 to 7.2%; *L. terrestris* was 1.7 to 5.5%, and *E. eugeniae* was 2.7 to 7.6%.

No mortality of *E. fetida* occurred at either 15 or 24°C in either soil type for treatment groups exposed to 0.038 or 0.068 mmol PCP/kg or in the artificial soil controls. Additionally, no mortalities occurred in *E. fetida* exposed to 0.12 mmol PCP/kg in Brookston clay. For all other *E. fetida* treatments, as well as for all tests with *E. eugeniae* and *L. terrestris*, all exposure concentrations showed some mortality over the 28-d duration of the tests. In tests with *E. eugeniae* and *L. terrestris*, no mortalities were observed at exposure concentrations below 0.12 mmol PCP/kg or in controls groups over the first 14 d of the test. However, mortalities consistently occurred after 14 d of exposure, by which time ILLs were clearly established. As a result of these mortalities, only the data up to 14 d were used to model toxicity and estimate an ILL. The equations estimated by nonlinear curve fitting of the 1CFOK model to the toxicity data provided a good fit to the observed data for all species, temperatures, and soil types at which tests were conducted (Figs. 1 and 2).

DISCUSSION

The results of this study generally support the hypothesis that regardless of earthworm species, test temperature, or soil

Table 2. Body residues of earthworms exposed to PCP in different experimental regimes

Soil concn.		Body residue[a,b] (mmol PCP/kg)				
		Brookston clay	Artificial soil			
			E. fetida		*E. eugeniae*	*L. terrestris*
(mg/kg)	(mmol/kg)	(24°C)	(24°C)	(15°C)	(24°C)	(15°C)
1,800	6.75	1.39A (0.384)	2.65A (1.89)			
1,000	3.75	1.19A (0.277)	0.74BC (0.70)			
560	2.10	1.35A (0.395)	0.62BC (0.53)	0.80 (0.26)	0.41 (0.10)	0.47 (0.18)
320	1.20	1.16A (0.882)	0.56BC (0.22)			
180	0.68	1.58A (0.531)	0.59BC (0.026)		0.61 (0.18)	2.18 (1.07)
100	0.38	0.51A (0.235)	0.80BC (0.028)			
56	0.21	0.84A (0.306)	0.33DE (0.064)			
32	0.12	1.16A[c] (0.387)	0.79BC (0.055)	1.29 (0.15)		
18	0.068	1.29A[c] (0.911)	0.44CD[c] (0.12)			
10	0.038		0.21E[c] (0.057)			
NaOH	0.0	0.04B (0.015)	0.006F (0.003)			
H_2O	0.0	0.02B (0.006)	0.005F (0.002)	0.006 (0.001)	0.002 (0.001)	0.002 (0.0003)

[a] Average measurements with standard deviation in parentheses below.
[b] Values with a common letter in columns are not significantly different ($p > 0.05$).
[c] Body residues measured in live worms exposed to PCP.

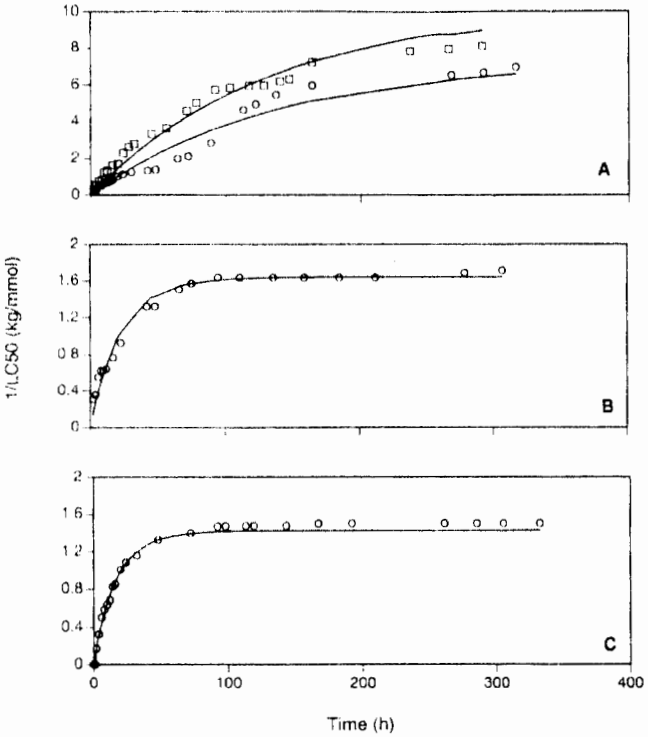

Fig. 1. Toxicity curves for three species of earthworm exposed to PCP in artificial soil for 14 d (336 h). (**A**) *Eisenia fetida* at 15°C (□) and 24°C (○). (**B**) *Eudrilus eugeniae* at 24°C. (**C**) *Lumbricus terrestris* at 15°C. Each value is the mean of three replicates; the curve is the ICFOK model line of best fit.

type, CBRs of PCP for lethality were similar. This phenomenon also occurred over a range of soil-based PCP concentrations spanning more than two orders of magnitude. This suggests that despite the various abiotic and biotic modifying factors present in the different toxicity tests, the internal dose of chemical in the earthworm that results in lethality is the same. Earthworms in this study were exposed to soil concentrations of PCP which ranged more than two orders of magnitude, 0.038 to 6.75 mmol PCP/kg. However, when body residues were measured at all exposure concentrations (*E. fetida*, 24°C Brookston clay or artificial soil; Table 2), CBRs varied less than threefold, excluding CBRs of earthworms exposed to 6.75 mmol/kg in artificial soil. Critical body residues in these worms were significantly elevated, likely due to the continued diffusion of PCP into earthworms after death due to the high concentration gradient present between the soil and the worm. A reduced concentration gradient may also account for the lack of elevated CBRs at lower exposure concentrations. Since mortalities were first monitored at 1 h, it is not known at exactly what time after the commencement of exposure these worms died and how much additional time was available for continued diffusion. A similar increase in PCP body burdens was not observed in worms exposed to the same concentrations of PCP in Brookston clay. This may have been due to the differences in uptake kinetics due to variations in pH, particle size, and organic matter content between the different soils. A similar range of CBRs was observed in other species of worms, but the comparison is tenuous due to the incomplete sets of CBR values.

The test organisms used in this study represent not only three distinct taxonomic genera from two separate families (Lumbricidae and Eudrilidae) but also three separate size classes which range 10-fold in weight (Table 1). Body size has long been known to affect metabolic processes and to alter toxicant kinetics [17]. Smaller organisms are usually thought to be more sensitive to contaminants. *Eisenia fetida* were observed to be about five to seven times as sensitive to PCP in artificial soil compared to the larger test species based on soil PCP concentrations. However, when CBRs are compared at a single exposure concentration higher than ILLs for all species, the difference in PCP residues is less than twofold (Table 1, 2.10 mmol/kg exposure). In other words, the differences in sensitivity to PCP observed when comparing ILLs are simply an artifact of expressing toxicity as soil concentrations rather than the actual dose in the organism. Other studies have also compared the toxicity of various chemicals to different species of earthworms. Neuhauser et al. [18] observed that the four earthworm species tested (*E. fetida*, *E. eugeniae*, *A. tuberculata*, and *P. excavatus*) varied two- to 10-fold in their sensitivity to a given contaminant based on soil concentrations of the chemicals expressed as time-dependent LC50s. However, the

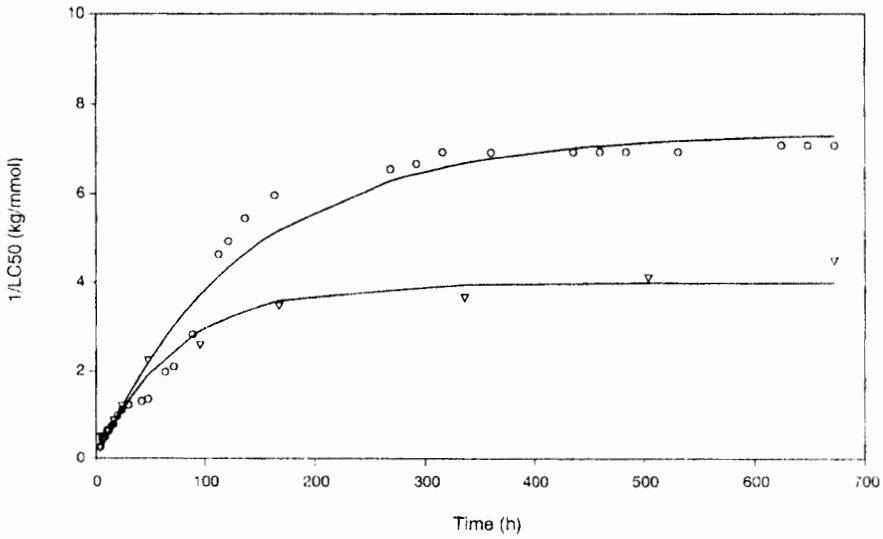

Fig. 2. Toxicity curves for *Eisenia fetida* exposed to PCP in artificial soil (○) or Brookston clay (▽) for 28 d (672 h) at 24°C. Each value is the mean of three replicates; the curve is the ICFOK model line of best fit.

correlations among the LC50s of the earthworm species for the 10 chemicals tested in artificial soil was high, with correlation coefficients ranging from 0.974 to 0.998. Similarly, van Gestel and Ma [8] found up to a 10-fold difference in the toxicity of various chlorophenols between two species of earthworms (*E. fetida andrei* and *L. rubellus*). Two different sizes (1.3–1.8 and 0.54–0.70 g) of *L. rubellus* were also exposed to 2,4,5-trichlorophenol to investigate the effect of body size on toxicity. No differences were observed, likely due to the small (two- to threefold) difference in body size.

Since different species of worms exhibited similar CBRs in artificial soil, it follows that the same worm species should have similar CBRs if exposed in different soils. The ILL for *E. fetida* exposed to PCP in Brookston clay at 24°C (0.27 mmol PCP/kg) was approximately twofold higher than the ILL for those exposed at the same temperature in artificial soil (0.14 mmol PCP/kg, Table 1). A difference in ILLs would be expected since toxicity is expressed as a function of soil PCP concentration and the two test soils differed in physical and chemical properties. The pH of the clay ranged between 5.7 and 6.5, while the pH of the artificial soil ranged between 6.9 and 8.1. The amount of organic matter and its composition, as well as the particle size distribution, also varied between the two soil types. The toxicity of PCP to *E. fetida andrei* has been reported to be influenced by soil pH and organic matter content [19]. The 14-d LC50 for worms exposed in a natural soil (Gilze; pH, 4.1; OM, 1.7%) was 0.20 mmol/kg, about twofold higher than the LC50 of 0.11 mmol/kg observed in worms exposed in an artificial soil (pH, 7.0; OM, 7.7%). It is apparent that the interplay between modifying factors will determine the bioavailability and hence the uptake kinetics of PCP from the soils, resulting in statistically different ILLs or LC50 values. Although differences in ILLs were observed in the current study, comparisons of CBRs at the ILLs for *E. fetida* exposed in Brookston clay and in artificial soil did not appear to provide any additional resolution of relative toxicity in these two soils (Table 2). Additionally, a valid statistical comparison of individual CBRs between studies is difficult to make due to the effects of latent variables since tests were conducted by separate researchers using different batches of earthworms at different times. By contrast, end points like an ILL or t_c are based on summary data derived from bioassays conducted over 14 or 28 d that allow for statistical comparisons that minimize the variability associated with latent variables as they have reached time-independent thresholds.

Another modifying factor which can influence toxicity is test temperature. As ambient temperature increases, the toxicity of chemicals to ectothermic organisms often increases due to increased activity, both metabolic and behavioral, as well as to enhanced diffusion across epithelial surfaces. The results of our study showed no differences in the toxicity of PCP to *E. fetida* at 24°C compared to 15°C based on ILLs (Table 1), and CBRs supported this observation (Table 2). Either temperature is not a major modifying factor of toxicity in *E. fetida* or the temperature differential in this experiment was too small to act as a significant modifying factor of toxicity.

If no differences in CBRs exist between species, why do ILLs differ between species exposed in the same test medium? Since modifying factors associated with the test matrix (artificial soil) and the test chemical (PCP) were held constant, observed differences in ILLs may have been due to modifying factors associated with the test organisms. Differences in the physiology, body size, or behaviour of the test species may lead to altered toxicant uptake kinetics and hence different ILLs. Body size has been observed to alter toxicant kinetics in poikilotherms [20], and in this study, the t_c was significantly shorter in the larger worms (*L. terrestris* and *E. eugeniae*), indicating that they reached an ILL with respect to soil PCP concentrations about four to five times faster than the smaller worm species (*E. fetida*) (Table 1 and Fig. 1). Conversely, van Gestel and Ma [8] found no statistical differences in the toxicity of 2,4,5-trichlorophenol to *L. rubellus* despite a threefold difference in body weight of test worms. During the toxicity tests reported here, *E. fetida* were often found clumped together near the bottom of the test chamber during mortality checks. The two larger species were usually found throughout the test chamber, perhaps indicating greater activity. Although the nature of the testing medium and concentrations of PCP were held constant, the soil mass/worm biomass ratios differed (*E. fetida*, 83; *E. eugeniae*, 13; *L. terrestris*, 10). Additionally, the larger worms may have found the organic matter in the test soil (*Sphagnum* peat moss) more suitable for consumption and thus had an increased rate of dietary uptake. The importance of the dietary route of uptake in toxicity is unknown in worms, but van Gestel and Ma [8] inferred the cutaneous route of uptake to be dominant when they found that differences in the toxicity of chlorophenols in different natural soils, expressed on a milligram per kilogram soil basis, disappeared once exposure was expressed as milligrams per liter of pore water using sorption coefficients. However, differences in sorption would not be encountered in the current study, since the test soil was similar for all three species. It is possible that physiological differences exist between species in their ability to regulate contaminant uptake through mechanisms such as mucous production. During this study *E. eugeniae* produced more yellowish mucus on contact with elevated levels of PCP than did *E. fetida*, while *L. terrestris* produced the least mucus which was qualitatively different in that it was black in color. Belfroid et al. [21] exposed *E. fetida* to different concentrations of chlorobenzenes via different routes of exposure: water, soil, contaminated food, and filter paper. Except for the contact filter paper toxicity test, body residues at lethality were similar for a given chemical. The authors suggested that the differences in lethal body burdens for worms exposed on filter paper may have been related to survival time and/or to the metabolism or biotransformation of the test chemical. Species differences in PCP metabolism may also have contributed to the differences in ILLs observed in this study. Earthworms appear to have the ability to bind xenobiotics or their metabolites in unextractable forms [22]. Specifically, Haque and Ebing [23] documented the formation of unextractable compounds in worms exposed to ^{14}C-PCP. Differences between species in the ability to bind PCP may have contributed to the observed differences in ILLs yet similar body residues.

Whole-body PCP residues of *E. fetida* surviving the 28-d exposure in either artificial soil or Brookston clay were similar to those of worms that perished during the test (Table 2). Similar results were observed by Belfroid et al. [21] with *E. fetida* exposed to chlorobenzenes in artificial soil. Since these worms are exposed to lower soil PCP concentrations (i.e., below the ILL), the amount of PCP available for uptake may be low enough that the animals have established some physiological equilibrium between uptake, metabolism, and depuration that would allow nonlethal adaptation to residues sim-

lar to those found in worms exposed to higher concentrations of PCP.

Similar CBRs for *E. fetida* exposed at PCP concentrations ranging over more than two orders of magnitude in two different soil types and between three species of earthworms differing in body size demonstrate that body residues can act to more effectively integrate the relationship between test organisms, exposure, and mortality than soil toxicant concentrations alone. The CBR approach quantifies the amount of toxicant partitioning into the organism and provides an estimate of dose which is less dependent on external modifying factors than measures such as ILLs. Since body residues explicitly account for the bioavailability of a chemical, this expression of the internal dose of contaminant received by an organism has distinct advantages over the traditional expression of dose as a concentration of contaminant in the test medium. Expression of toxicity in this manner accommodates the classic problem of modifying factors in toxicity testing and provides a route for comparing toxicity between different media [24].

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