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Comparative Toxicity in Earthworms *Eisenia fetida* and *Lumbricus* terrestris Exposed to Cadmium Nitrate Using Artificial Soil and Filter Paper Protocols

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Earthworms are ideal soil organisms for use in terrestrial ecotoxicology (Greig-Smith et al. 1992). As such, several earthworm protocols have been developed for testing toxic potential of chemicals and contaminated soils (Reinecke 1992). Of these, the 48-h filter paper contact (FP) and the 14-d artificial soil exposure (AS) protocols, using mortality (LC50) as the toxic endpoint and *Eisenia fetida* as the test species, have received the most attention, with the latter being adopted by both OECD (1984) and EEC (1985) in Europe and the Environmental Protection Agency (USEPA) (Greene et al. 1989) in the United States. Although the FP technique, adopted by EEC (1985), provides for inexpensive reproducible toxicity screening for chemicals (i.e. establishing relative toxicities), it has been criticized for lacking the ecotoxicological relevance of the AS protocol (Reinecke 1992). Choice of earthworm species for laboratory testing also has been controversial (Edwards and Coulson 1992). The manure worm, *E. fetida,* is criticized for not being sufficiently sensitive to chemicals or representative of "typical" earthworms. *Lumbricus terrestris* and *Apporectodea caliginosa* have been suggested as more sensitive and ecologically relevant earthworms by Dean-Ross (1982) and Martin (1985, 1986), respectively.

Since we have been using both exposure protocols, and *L. terrestris* and *E. fetida* for mortality tests and in developing earthworm biomarkers to assess sublethal toxicity of chemicals, complex mixtures of chemicals and hazardous waste site (HWS) materials (e.g. Fitzpatrick et al. 1990; Venables et al. 1992; Goven et al. 1994), we had interest in comparing both exposure protocols and species. Specifically, we wished to determine the comparative efficacy of the two exposure protocols and sensitivities of the two species using contact-based LC5Os and body burden-based LD50s as lethal toxic endpoints. Because the latter is based on actual tissue residues, it should provide for more direct and meaningful comparisons of

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chemical sensitivity, and delivery effectiveness (i.e. uptake) of the exposure protocols. We believe that some reported variability in toxic responses within and among earthworms, and low correlations between FP and AS tests (e.g. Heimbach 1984, 1988) may best be explained in terms of body burden concentrations of toxicants. FP exposure should be a reliable predictor of AS results when body burden-based LD50s are used and should be more reflective of a chemical's actual toxic potential than exposure-based LC5Os. Herein, we compare AS and FP protocols in assessing toxicity of cadmium (as $CdNO_3$), an important terrestrial contaminant, to *L. terrestris* and *E. fetida* using LC5Os and LD50s.

MATERIALS AND METHODS

Earthworms were purchased from Carolina Biological Supply (Burlington, N.C.). *L. terrestris* were maintained for 10 d prior to experiments in moistened peat moss within plastic containers (70 x 40 x 15 cm) in continuous darkness at 10° C in environmental chambers. *E. fetida* were maintained similarly, but in moistened cow manure at 20° C. Commercial dry powdered baby food, layered on the peat moss for *L. terrestris* and commercial rabbit food pellets for *E. fetida,* provided nutrition. Worms were observed daily and supplied food as required. Sexually mature adults with well-developed clitella and masses of 3 to 4 g were used for *L. terrestris,* and 0.2 to 0.5 g for *E. fetida.* Prior to experiments, general health of earthworms was assessed by determining the LC50 for randomly selected individuals using the standard reference toxicant 2-chloroacetamide test (OECD 1989; Greene et al. 1989) except exposure for *L. terrestris* was for 5 d instead of 2 d used for *E. fetida.* Our experience with *L. terrestris* has shown that more reliable and repeatable results are obtained with 5-d exposure.

Range finding tests were used to approximate the LC50 for each species. A single replicate of one 500-ml jar containing 10 worms for each of five concentrations (range = 1000 - 1.0 ppm) was used to bracket the LC50 for the AS protocol. A single replicate of five 85-ml glass vials containing one worm in each was used for the FP protocol. After bracketing the LC50, definitive test concentrations included one that produced no mortality, two above and two below the expected LC50, and the control. For both species, these were: 200, 100, 50, 25, 12.5 and 0 (control) ppm $CdNO_3$. Three replicate 500-ml jars containing 10 worms each and 400 g AS were used for each concentration and control. Definitive test results were used to calculate a 14-d LC50 and LD50 (Stephan 1977) after tissue analysis using atomic absorption spectrometry USEPA (1982). The artificial soil (AS) was composed of the following ingredients as percent dry mass: (1) 10% Canadian sphagnum moss; (2) 20% kaolinite clay; (3) 69% industrial quartz sand; and (4) 1-2% $CaCO_3$ (to bring pH to 6.5 ± 0.5%). Distilled deionized (DDI) water was added to bring moisture to 35%. Thus, each 400 g of AS

contained 140 ml H_2O for a total mass of 540 g. Preparation involved mixing sand and clay thoroughly by hand, adding moss and mixing until homogenous, after which, water containing dissolved $CdNO_3$ was mixed in. The AS was then divided into three nearly equivalent quantities, each representing one replicate for exposing worms to the same concentration of $CdNO_3$. Before worms were added, a 10 g sample was removed from one replicate at each concentration for pH measurement.

FP exposure used 85 ml capped glass vials lined with 12 x 7 cm Whatman No. 1 filter paper for individual worm exposure. Appropriate concentrations of $CdNO_3$, dissolved in 1 mL DDI water, were pipetted onto the filter paper. Ten replicates of five nominal $CdNO_3$ concentrations and controls were used for the definitive exposure: 12.5, 6.25, 3.125, 1.56, 0.78 and 0 (control) µg/cm^2 $CdNO_3$ for *L. terrestris* and 60, 30, 15, 7.5, 3.75 and 0 (control) µg/cm^2 $CdNO^3$ for *E. fetida.* Controls were worms exposed only to DDI water. Each vial, containing one worm for each species, was kept horizontal at 20°C for *E. fetida* and 10°C for *L. terrestris* under darkness for 2 and 5 d, respectively, in environmental chambers. Earthworms not responding to a gentle touch were considered dead.

RESULTS AND DISCUSSION

Although different exposure times were used (2 vs 5 d), *L. terrestris* had higher LC5Os and somewhat greater variability than *E. fetida* (Table 1). The average of the four 95 % confidence limits (CL) divided by their respective LC50s for *L. terrestris* was 2.5 times higher than for *E. fetida (0.57 vs 0.23).* Both species responded with sufficient consistency to the 2-chloroacetamide FP test to indicate that earthworms were suitable for definitive testing. Greater apparent resistance shown by *L. terrestris* conflicts with reports that *E. fetida* is a more resistant species (e.g. LC50 = 76 vs 54 mg/kg dry mass soil; Edwards and Coulson 1992). The 2-d LC50 for *E. fetida* compares very closely to results reported by Neuhauser et al. (1986) for *E. fetida (2* µg/cm^2; 95 % CL 1.9 - 2.1).

Table 1. LC5Os (95 % confidence limits) of earthworms *Eisenia fetida* and *Lumbricus terrestris* exposed on filter paper to the reference toxicant 2-chloroacetamide (µg/cm^2) for 2 and 5 d, respectively.

	L. terrestris		*E. fetida*	
	LC50	(95%CL)	LC50	(95%CL)
1.	11.0	(8.4 - 14.5)	1.8	(1.5 - 2.0)
2.	6.5	(4.6 - 8.8)	2.0	(1.8 - 2.2)
3.	8.8	(6.7 - 11.7)	2.1	(1.9 - 2.3)
4.	8.8	(6.7 - 11.7)	2.0	(1.8 - 2.3)
$\bar{x}$	9.6		2.0	

Comparisons of LC5Os and dry mass-based LD50s determined from both FP and AS exposures indicate that *L. terrestris* was more sensitive to $CdNO_3$ than *E. fetida* (Table 2). Difference between species is less clear using wet mass-based LD50s. Dry mass LD50s were remarkably close between FP and AS exposures for both *L. terrestris* (110 vs 145 ppm) and *E. fetida (273* vs 298 ppm). Except for the AS exposure LC50 for *L. terrestris,* variability measured as the ratio of each LC50 or LD50 to its respective 95 % CL was lower in AS than FP exposures. There was no clear pattern of variability among LC5Os or LD50s within either exposure protocol. However, dry mass-based LD50s for *L. terrestris* showed lower variability than for *E. fetida* in both exposure protocols. Controls showed 100% survival.

Table 2. LC50 and LD50 comparisons for earthworms *Eisenia fetida* and *Lumbricus terrestris* exposed for 2 and 5 d, respectively, to $CdNO_3$ via filter paper (FP) and 14 d via artificial soil (AS) protocols.

	L. terrestris		*E. fetida*	
	FP[a]	AS[b]	FP	AS
LC50	5 (4-7)[c]	256 (140- 414)	10 (7-15)	374 (317-444)
LD50 wet mass	18 (14-24)	28 (26-30)	16 (12-45)	40 (36-44)
LD50 dry mass	110 (86-147)	145 (127-164)	273 (88-656)	298 (212-419)

a. Based on nominal $\mu g/cm^2$ concentrations
b. Based on nominal mg/kg concentrations
c. 95 % confidence limits

Greater sensitivity to $CdNO_3$ shown by *L. terrestris* agrees with reports that it is, in general, more sensitive to chemicals than *E. fetida* (Edwards and Coulson 1992). In their comprehensive study, Neuhauser et al. (1986) reported FP and AS LC5Os 2.4 and 4.9 times higher, respectively, for *E. fetida* exposed to $CdNO_3$ than we found. They reported FP LC50s of 24 $\mu g/cm^2$ (95% CL = 12-45) and AS LC5Os 1843 mg/kg (95% CL = 1660-2045) for $CdNO_3$, and FP LC5Os of 20 (16-25), 18 (12-25) and 26 (17-39) $\mu g/cm^2$ for acetate, chloride and sulfate salts of Cd, respectively. Van Gestel and van Dis (1988) reported a lower FP LC50 (4.8 $\mu g/cm^2$; 95% CL = 3.8-6.1) for $CdCl_2$ and an AS LC50 > 1000 mg/Kg for $CdCl_2$. Comparative data were not found for *L. terrestris.*

Both exposure protocols, and LC5Os and dry mass-based LDSOs produced the same toxic rankings of the two species for CdNO3. Neuhauser et al. (1986) reported that FP and AS exposures produced the same toxic rankings in *E. fetida* exposed to Cd, Cu, Pb, Ni and Zn. *L. terrestris,* which showed lower variability than *E. fetida* in dry mass-based LD50s, appears to be a good species for laboratory toxicity testing. We have found this

especially to be true for coelomocyte (annelid immune cells) - based sublethal tests using both inorganic and organic toxicants. Our results indicate that dry mass-based LD50s enable direct comparison between exposure protocols as well as species. For example, we have shown elsewhere (Fitzpatrick et al. 1992) that PCB - toxicity ranking between *E. fetida* and *L. terrestris* differed between FP LC50 and LD50 endpoints. *E. fetida* appeared 10 times more sensitive than *L. terrestris* using LC50, but over 4 times more resistant when actual body burden-based LD50 was used. We believe that further research using a number of different chemical classes, as did Heimbach (1984,1988) and Neuhauser et al. (1986), but using dry mass-based LD50s instead of LC50s will show higher correlation (i.e. prediction) between FP and AS protocols, establishing the former as a cost-effective exposure protocol.

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