Ref ID 0276 59288

Comp. Biochem. Physiol. Vol. 83C, No. 1, pp. 197–200, 1986
Printed in Great Britain

0306-4492/86 $3.00 + 0.00
© 1986 Pergamon Press Ltd

COMPARATIVE TOXICITY OF TEN ORGANIC CHEMICALS TO FOUR EARTHWORM SPECIES

EDWARD F. NEUHAUSER,* PATRICK R. DURKIN,† MICHAEL R. MALECKI* and MICHELE ANATRA†

*Department of Agricultural Engineering, Cornell University, Ithaca, NY 14853, USA and †Center for Chemical Hazard Assessment, Syracuse Research Corporation, Syracuse, NY 13210, USA

(Received 24 April 1985)

Abstract—1. Ten organic chemicals were tested for toxicity to four earthworm species: *Allolobophora tuberculata*, *Eisenia fetida*, *Eudrilus eugeniae* and *Perionyx excavatus*, using the European Economic Community's (EEC) earthworm artificial soil and contact testing procedure.

2. The phenols were the most toxic chemicals tested, followed by the amine, substituted benzenes, halogenated aliphatic hydrocarbon, polycyclic aromatic hydrocarbon and phthalate as the least toxic chemical tested.

3. Correlations among species within each type of test for a given chemical were extremely high, suggesting that the selection of earthworm test species does not markedly affect the assessment of a chemical's toxicity.

4. The correlation between the two tests was low for all test species. The contact test LC_{50} for a given chemical cannot be directly correlated to an artificial soil test LC_{50} for the same earthworm species.

INTRODUCTION

The recent development of two toxicity testing methodologies using earthworms, the artificial soil test and the contact test (Goats and Edwards, 1982; Edwards, 1983) has concentrated on using one earthworm species, *Eisenia fetida*. Currently, work is being done using these tests (Callahan, 1985) and related tests (Drewes *et al.*, 1984) to evaluate the potential toxicity of soils from hazardous waste sites containing unknown mixtures of chemicals. Since earthworm species other than *E. fetida* may be present in hazardous waste sites, it is important to know something about the comparative toxicity of chemicals of *E. fetida* and other earthworm species.

With the development of the two different earthworm tests (Edwards, 1983) it is important to evaluate if the results from one test can be used to predict the results for the other test. This study evaluates the comparative toxicity of 10 organic chemicals to four species of earthworms and also the predictive ability of contact test results for the artificial soil test results for each earthworm species.

MATERIALS AND METHODS

General

The worms used in these tests, *Allolobophora tuberculata* (Eisen), *Eisenia fetida* (Savigny), *Eudrilus eugeniae* (Kinberg) and *Perionyx excavatus* (Perrier), were grown in the authors' (E.F.N. and M.R.M.) laboratory at room temperature (20–22°C) on a consistent organic food source, horse manure. Care was taken to ensure that manure from medicated animals was not used.

These earthworm species represent three different families: the Eudrilidae, Lumbricidae and Megascolecidae. The Eudrilidae species used in this study, *E. eugeniae*, is native to Africa. The two Lumbricidae species, *A. tuberculata* and *E. fetida* are native to the northern temperate regions, while the Megascolecidae species, *P. excavatus* is Asian in origin.

The purity of the chemicals used in this evaluation was at least 98%. The chemicals were purchased from the Aldrich Chemical Co., Eastman Kodak Co., and Fisher Scientific Co. The chemicals were selected from the EPA list of Priority Pollutants (U.S. EPA, 1979).

Contact test

The contact filter paper test, developed by the EEC to evaluate chemical toxicity (Edwards, 1983), was followed. The glass vials used (Wheaton, 3 oz. snap cap) were 8 cm long and 3 cm in diameter. Strips of filter paper 12 × 6.7 cm (Whatman No. 1) lined and completely covered the sides of the vial.

If possible, the organic chemical was applied to the filter paper using distilled water as the solvent. If the chemical was not soluble in water, other solvents (acetone or chloroform) were used. The organic solvent used to add the chemical should volatilize readily and be evaporated from the vial before the distilled water and the worm are added to the vial. The loss of organic solvents was evaluated and did occur in all cases before the worms were added. In addition, it was determined that the organic chemicals added to the vials with the organic solvents did not volatilize with the organic solvent. One milliliter of water was added to the filter paper to provide enough moisture for earthworm survival. One adult earthworm was added per vial and the vials were kept on their side at 20°C in a darkened incubator for 48 hr. Only one worm was added per vial because the death of one worm in the vial could adversely affect the well-being of other worms present. After the 48 hr, mortality was determined. Earthworms were considered dead if they did not respond to a gentle mechanical touch. Death was related to the concentration of the chemical to which the worms were exposed.

The LC_{50} concentration for each chemical tested was obtained by first using a series of tests to determine the range of chemical concentrations that had an adverse impact. The range finding test included concentrations that results in mortalities from 0 to 100%. This was accomplished by using chemical concentrations that varied over four logs. A narrow, more definitive range of chemical

9400

concentrations to determine the more specific LC_{50} value was then used.

At least five concentrations were evaluated in the definitive test, with 10 or more replicates used for each concentration tested. Controls containing everything except the test chemical were part of each series of experiments.

The LC_{50}-value for each chemical tested was calculated using the method of Litchfield and Wilcoxon (1949). The LC_{50} values are reported as μg of test chemical cm^{-2} of filter paper.

Artificial soil test

The EEC artificial soil test method (Edwards, 1983) was followed. This test uses a medium which consists by weight of 10% finely ground sphagnum peat, 20% kaolinite clay, 69% fine sand and 1% pulverized calcium carbonate. The pH was adjusted to 6.0 ± 0.5 and the water content raised to 35% of the dry wt of the four components. The test chemical was dissolved in water and then added to the test medium. If the test chemical was not water soluble, it was mixed with a small volume of suitable organic solvent (acetone or chloroform) and then mixed with the artificial soil. Tests evaluated the amount of time needed to completely evaporate the organic solvent from the test medium. After the organic solvent evaporated, the artificial soil was then brought to a moisture content of 35%. Controls, which consisted of all components except the test chemical, were included in each set of experiments.

Ten adult earthworms were added to 400 g (dry wt) of the test mixture in covered glass dishes 6.5 cm high and 12.5 cm in diameter. The dishes were incubated at 20°C and mortality was assessed after 2 weeks. Earthworms were considered dead when they did not respond to a gentle mechanical stimulus.

Concentrations causing mortalities of 0–100% were determined using a range finding test that included chemical concentrations that varied over four logs. After the chemical range had been narrowed, a definitive test was performed. This test had at least five chemical concentrations, with four repetitions for each test concentration and four control dishes, all containing 10 worms per dish.

The LC_{50} values for each chemical were calculated using the method of Litchfield and Wilcoxon (1949) and are reported as mg chemical/kg dry wt of artificial soil.

RESULTS AND DISCUSSION

The results of the earthworm contact soil test (Table 1) and the earthworm artificial soil test (Table 2) show the values for the chemicals listed, in order of relative toxicity to the four earthworm species. The phenols were the most toxic chemicals tested, followed by the amine and substituted benzenes. The halogenated aliphatic hydrocarbons, polycyclic aromatic hydrocarbon, and phthalates were the least toxic chemicals tested. These results agree with the contact test results of Roberts and Dorough (1983).

In analyzing the data presented in Tables 1 and 2, the major points of interest were the consistency among earthworm species in both the contact test and the artificial soil test but lack of consistency between the two types of tests. In assessing these relationships, univariate and multivariate analyses were performed using the canonical correlation program on the Statistical Analysis System (1982), a computerized statistical package.

As indicated in Tables 3 and 4 for the contact test and artificial soil test, respectively, correlations among species within each type of test were extremely high, suggesting that the selection of the test species does not markedly affect the assessment of a compound's toxicity. However, as shown in Table 5, the correlations between the two types of tests were extremely low in all test species. Thus, the results obtained from the contact test using a single species cannot be used to directly assess the toxicity of a compound under field conditions. However, using multiple correlation analysis, results of the soil test for each species were highly correlated ($r^2 = 0.93$, $P < 0.005$) with the LC_{50} values for all four of the test species in the contact test.

All previous work on the earthworm artificial soil and contact tests (Edwards, 1983; Roberts and Dorough, 1983) used only the earthworm species

Table 1. Contact test results—LC_{50} and 95% confidence interval (μg cm^{-2})

CHEMICAL	EARTHWORM SPECIES			
	A. tuberculata	*E. fetida*	*E. eugeniae*	*P. excavatus*
4-Nitrophenol	2.7 (2.0-3.7)	0.7 (0.6-0.8)	0.8 (0.6-1.0)	2.7 (2.0-3.7)
N-nitrosodiphenylamine	3.5 (3.0-3.9)	2.4 (2.2-2.6)	2.5 (2.0-3.1)	3.5 (3.0-3.9)
2,4,6-Trichlorophenol	6.4 (5.1-8.0)	5.0 (4.1-6.1)	5.5 (4.4-6.9)	2.5 (2.0-3.1)
Phenol	10.6 (8.7-12.8)	5.0 (4.2-5.8)	2.4 (1.6-3.8)	6.1 (4.6-8.2)
Carbaryl	0.5 (0.3-0.9)	14 (10-19)	0.5 (0.3-0.9)	9.5 (6.9-13.0)
Nitrobenzene	11.6 (10.0-13.4)	16 (15-17)	5.5 (4.6 6.5)	10.4 (9.5-11.4)
1,2,4-Trichlorobenzene	23 (16-32)	27 (24-32)	23 (17-32)	25 (20-31)
1,2-Dichloropropane	84 (65-110)	64 (59-70)	44 (38-51)	63 (56-72)
Fluorene	124 (102-150)	171 (128-224)	47 (43-52)	78 (37-162)
Dimethyl phthalate	773 (376-1591)	550 (440-690)	576 (436-760)	400 (230-670)

Table 2. Artificial soil test results—LC_{50} and 95% confidence interval (mg/kg)

CHEMICAL	EARTHWORM SPECIES			
	A. tuberculata	*E. fetida*	*E. eugeniae*	*P. excavatus*
4-Nitrophenol	56 (34-93)	38 (32-45)	40 (28-58)	44 (31-62)
2,4,6-Trichlorophenol	108 (73-160)	58 (54-62)	85 (39-182)	78 (43-143)
Carbaryl	22 (16-30)	106 (81-138)	119 (43-326)	263 (179-385)
N-nitrosodiphenylamine	155 (117-204)	151 (136-167)	109 (75-159)	128 (97-171)
Fluorene	206 (164-260)	173 (150-201)	197 (100-384)	170 (118-245)
1,2,4-Trichlorobenzene	251 (167-378)	197 (137-282)	127 (82-197)	180 (150-220)
Nitrobenzene	362 (230-569)	319 (243-419)	226 (169-303)	343 (227-518)
Phenol	450 (296-684)	401 (347-463)	188 (89-419)	258 (201-330)
Dimethyl phthalate	3335 (2124-5235)	3160 (2450-3820)	2000 (1500-2800)	1064 (581-1947)
1,2 Dichloropropane	4272 (3140-5825)	4240 (3830-4680)	5300 (4200-6800)	3880 (3241-4647)

Table 3. Correlations among the LC_{50}s of the earthworm species in the contact test for the 10 organic chemicals tested

	A. tuberculata	*E. fetida*	*E. eugeniae*	*P. excavatus*
A. tuberculata	1.000			
E. fetida	0.999	1.000		
E. eugeniae	0.941	0.950	1.000	
P. excavatus	0.895	0.907	0.992	1.000

Table 4. Correlations among the LC_{50}s of the earthworm species in the artificial soil test for the 10 organic chemicals tested

	A. tuberculata	*E. fetida*	*E. eugeniae*	*P. excavatus*
A. tuberculata	1.000			
E. fetida	0.989	1.000		
E. eugeniae	0.997	0.974	1.000	
P. excavatus	0.998	0.991	0.992	1.000

Table 5. Correlation among the LC_{50}s of the earthworm species between the contact test and artificial soil test

Contact Test	Artificial Soil Test			
	A. tuberculata	*E. fetida*	*E. eugeniae*	*P. excavatus*
A. tuberculata	0.069			
E. fetida	0.590	0.558		
E. eugeniae	0.320	0.577	0.300	
P. excavatus	0.204	0.618	0.355	0.242

E. fetida. Work is also being done on evaluating soils from hazardous waste sites using laboratory tests (Callahan, 1985). Mixing different proportions of hazardous site soil with artificial soil can be used to evaluate the actual toxicity of the site soil. A water leachate of the site soil can be tested for potential toxicity with the contact test.

Acknowledgement—This work was supported by a grant from the U.S. Environmental Protection Agency, Project CR-810006 to Cornell University. Although the research described in this article has been funded by the U.S. EPA, it has not been subjected to the Agency's required peer and policy review and, therefore, does not necessarily reflect the views of the Agency; no official endorsement should be inferred.

REFERENCES

Callahan C. A. (1985) Earthworms as ecotoxicological assessment tools. Proceedings International Conference on Earthworms in Waste and Environmental Management. Cambridge, UK, July (1984).

Drewes C. D., Vining E. P. and Callahan C. A. (1984) Non-invasive electrophysiological monitoring: a sensitive method for detecting sublethal neurotoxicity in earthworms. *Environ. Toxicol. Chem.* **3**, 599–607.

Edwards C. A. (1983) Development of a standardized laboratory method for assessing the toxicity of chemical substances to earthworms. Report EUR 8714EN. Commission of the European Community.

Goats G. and Edwards C. A. (1982) Testing the toxicity of industrial chemicals to earthworms. Rothamsted Report 1982, pp. 104–105.

Litchfield J. T. Jr. and Wilcoxon F. (1949) A simplified method of evaluating dose–effect experiments. *J. Pharmac. exp. Ther.* **96**, 99–118.

Roberts B. L. and Dorough H. W. (1983) Relative toxicities of chemicals to the earthworm *Eisenia foetida*. *Environ. Toxicol. Chem.* **3**, 67–78.

SAS (1982) *SAS Users Guide: Statistics*. SAS Institute, Cory.

U.S. Environmental Protection Agency (1979) Water-related environmental fate of 129 priority pollutants. EPA-440/4-79-029. Washington, DC.