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Differences in Toxicity of the Insecticide Chlorpyrifos to Six Species of Earthworms (Oligochaeta, Lumbricidae) in Standardized Soil Tests

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The choice of the earthworm species for use in the laboratory screening of chemicals remains a matter of controversy. The earthworm *Eisenia fetida* has been specified as a test species in current international standards for testing the acute lethality of chemicals to earthworms (OECD 1984; EEC 1985). *E.fetida* is a compost-dwelling species convenient for captive breeding. However, its ecological representativeness for soil-dwelling species may be questioned. From an ecotoxicological point of view it is important to know how the susceptibility of *Eisenia fetida* to chemicals compares to that of other species of earthworms.

The aim of the present study was to compare the laboratory toxicity of the pesticide chemical chlorpyrifos to six species of earthworms. Acute lethality tests were conducted as well as tests assessing the effect of chlorpyrifos on earthworm reproduction. Additional control tests were carried out with chloracetamide as a toxic reference standard. The combined results suggest that chlorpyrifos shows a considerable and specific species-related variation in toxicity to earthworms.

MATERIALS AND METHODS

Chlorpyrifos [O,O-diethyl-O-(3,5,6-trichloro-2-pyridyl)phosphorothioate] is a broad spectrum insecticide. Its toxicity was assessed as a 99% pure form obtained from Riedel-de-Haën. The reference standard chemical chloracetamide ($ClCH_2CONH_2$) was obtained from Aldrich Chemical Company in a 98.5% pure form.

Acute lethality tests were performed according to the Artificial Soil Test Guidelines (OECD 1984; EEC 1985). Ten sexually mature worms were placed in glass containers with 500 g dry mass of a soil-like substrate

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composed of a mixture of sphagnum peat, kaolinite clay, and industrial quartz sand in a dry-weight ratio of 1:2:7. The pH (1 N KCl) was adjusted to 6.0±0.5 with calcium carbonate. The water content was kept at 55% on a dry mass basis. A constant temperature of 15 °C was maintained in the experiments instead of the higher temperature of 20 °C prescribed by the OECD/EEC guidelines. The former temperature level, however, is more in agreement with the optimum soil conditions required by earthworms from temperate climatic zones (Ma 1983).

Aporrectodea caliginosa (Savigny), *Aporrectodea longa* (Ude), *Lumbricus rubellus* (Hoffmeister), and *Lumbricus terrestris* (Linnaeus) were collected in the field. *Eisenia fetida* (Savigny) and *Eisenia veneta* (Rosa) were bred in laboratory cultures on organic waste materials. Tests with the large-sized *Lumbricus terrestris* were conducted with five instead of ten worms per container. Forty worms were tested at each test concentration.

The experimental conditions applied in the reproduction tests were similar to those applied in the lethality tests. However, some modifications were applied which consisted of: a) the replacement of the artificial soil with a natural sandy soil (Kooyenburg) containing 3.7% organic matter, 1.4% clay, and pH (1 N KCl) 4.8, and b) the supply of coarsely ground air-dried leaves of alder, *Alnus glutinosa*, for food.

Mortality was expressed as 14d-LC50, the fourteen-day median lethal concentration added to the test medium. Reproduction effects were expressed as 14d-EC50, the fourteen-day effective concentration causing a 50% reduction in the number of cocoons produced compared to the control. Earlier studies have shown that there is no particular advantage in applying a longer exposure duration than two weeks in earthworm fecundity tests (Ma 1983). A third toxicity endpoint which was included in the present study consisted of the calculation of the NOEC, the no-observed-effect-concentration or the highest test concentration at which no lethal or adverse reproduction effect can be observed.

LC50 values were calculated according to the trimmed Spearman-Kärber method (Hamilton et al. 1977). To calculate EC50 values the statistical software package GENSTAT 5 was used, following a logit model. NOEC values were determined by applying analysis of variance.

RESULTS AND DISCUSSION

The results obtained with the acute lethality tests are summarized in Table 1. The toxicity of chlorpyrifos appears to vary greatly among the six species of earthworms tested. *Lumbricus rubellus* was the most sensitive species showing a LC50 of an order of magnitude lower than

Eisenia veneta, the least sensitive species. *Lumbricus rubellus* was somewhat more sensitive than the closely related species *Lumbricus terrestris*, but both were the most sensitive compared with species belonging to the genera of *Aporrectodea* or *Eisenia*.

The sensitivity of the earthworms to chlorpyrifos showed a striking correspondance with the taxonomical classification. According to an increasing level of sensitivity, the various genera can be arranged as follows:

Eisenia sp. < *Aporrectodea sp.* < *Lumbricus sp.*

Table 1. 14d-LC50 with 95% confidence intervals (mg/kg) and NOEC values (mg/kg) of chlorpyrifos for six species of earthworms in OECD/EEC artificial soil.

Species	LC50	95% conf.int.	NOEC
Aporrectodea caliginosa	755	674 - 846	486
Aporrectodea longa	778	690 - 877	486
Eisenia fetida	1077	951 - 1221	486
Eisenia veneta	1174	1095- 1259	875
Lumbricus rubellus	129	113 - 148	83
Lumbricus terrestris	458	403 - 521	270

The acute lethality test was repeated for the most susceptible species, *L.rubellus*, in order to check the reproducibility of the LC50 value shown in Table 1. In addition, parallel tests were run with chloracetamide as a positive toxic reference control. The chloracetamide toxicity standard has been recommended by the international OECD/EEC test guidelines as an additional quality assurance for the laboratory tests performed. According to these guidelines, the LC50 value of chloracetamide *Eisenia fetida* in OECD/EEC medium should be between 20 and 80 mg/kg (OECD 1984; EEC 1985).

The results shown in Table 2 indicate that the LC50 value of chlorpyrifos for *L.rubellus* given in Table 1 is highly reproducible. Table 2 also shows that the LC50 of the chloracetamide reference compound for *E.fetida* was well within the range specified by the OECD/EEC test guidelines, which can be taken as an indication of the satisfactory quality of the laboratory tests performed. It further appears from Table 2 that the LC50 value of chloracetamide for *L.rubellus* is similar to the value for *E.fetida*. This suggests that the observed species difference in sensitivity to chlorpyrifos is not a general phenomenon. The two species of *Eisenia*

showed a similar LC50 for the chloracetamide standard. Although the test with *E.fetida* yielded a slightly lower value than with *E.veneta*, both species were clearly among the least sensitive to chlorpyrifos.

A separate series of tests was run with another soil type (Kooyenburg) in order to compare the LC50 obtained in the artificial OECD/EEC medium with values obtained in a natural humic sandy soil. The results are given in Table 2 as well. It is shown that the influence of the type of soil substrate on the toxicity of chlorpyrifos and chloracetamide depends on the chemical concerned, although differences remained limited to a factor of about two. Thus, chlorpyrifos was more toxic to *L.rubellus* when tested in OECD/EEC medium than in Kooyenburg soil, whereas the opposite was true for the toxicity of chloracetamide.

Table 2. 14d-LC50 with 95% confidence interval (mg/kg) and NOEC values (mg/kg) of chlorpyrifos and chloracetamide (reference control).

Chemical	Substrate	LC50	95% conf.int.	NOEC
Chlorpyrifos				
Lumbricus rubellus	OECD/EEC	104	85-126	46
Lumbricus rubellus	Kooyenburg	262	228-302	150
Chloracetamide				
Lumbricus rubellus	OECD/EEC	48	>36-<65	36
Lumbricus rubellus	Kooyenburg	27	>20-<36	20
Chloracetamide				
Eisenia fetida	OECD/EEC	41	34-48	20
Eisenia veneta	OECD/EEC	58	49-68	36

The large difference in LC50 between *Lumbricus rubellus* and *Eisenia veneta* (Tables 1 and 2) was further examined by comparing the effect of chlorpyrifos on the reproduction of the two species. The reproduction was studied as the fecundity (cocoon formation) of the earthworms. Under the experimental conditions applied, the normal average rate of the production of cocoons within a period of fourteen days was found to be 1.5 cocoons for *L.rubellus* and 2.1 cocoons for *E.veneta*.

The effect of chlorpyrifos on earthworm reproduction is summarized in Table 3. It can be seen that the reproduction EC50 for *L.rubellus* was more than an order of magnitude lower than for *E.veneta*, which thus confirmed the much larger sensitivity of the former species as observed

in the LC50 tests.

The reproducibility of the reproduction EC50 value shown in Table 3 was checked by repeating the test for *L.rubellus*. This yielded essentially the same result as the previous test, i.e., an EC50 value of 8.4 mg/kg with a 95% confidence interval of 3.5 to 21 mg/kg and a NOEC value of 2.6 mg/kg. The results showed that the EC50 value for the effect of chlorpyrifos on reproduction was also highly reproducible.

It is of interest to note from a comparison of the data shown in Tables 1 and 3 that the effect on earthworm reproduction is a much more sensitive toxicity endpoint in ecotoxicity tests than mortality when applying the same duration of exposure of fourteen days.

Table 3. 14d-EC50 (reproduction) with 95% confidence interval (mg/kg) and NOEC values (mg/kg) of chlorpyrifos in Kooyenburg soil.

Species	EC50	95% conf.int.	NOEC
Eisenia veneta	121	74 - 199	49
Lumbricus rubellus	9.5	5.3 - 17	4.6

In conclusion, this study demonstrates that the toxicity of chlorpyrifos to earthworms depends greatly on the species concerned and that the extent of the difference in toxicity is much greater than with the chloracetamide standard. The question may be asked whether such species-related variation in susceptibility is due to some physiological property of the earthworms or to factors governing the exposure to chlorpyrifos. An important factor determining the level of exposure is body size, which affects the surface/volume ratio and thus may influence the intensity of exposure of earthworms when the route of chemical entry is via the outer skin.

In order to examine the possible influence of body size, data on both toxicity and body size are given in Fig.1. The figure clearly shows that there is no correlation between the toxicity of chlorpyrifos and the average body weight of the worms. For instance, *L.rubellus* and *E.veneta* both are virtually similar in body size but yet they show the largest difference in susceptibility to chlorpyrifos. Body size therefore can be ruled out as a possibility to explain the large variation in susceptibility to this chemical. It seems more likely that some physiological factor is involved which would render certain taxonomic species or genera of earthworms more tolerant or sensitive than others.

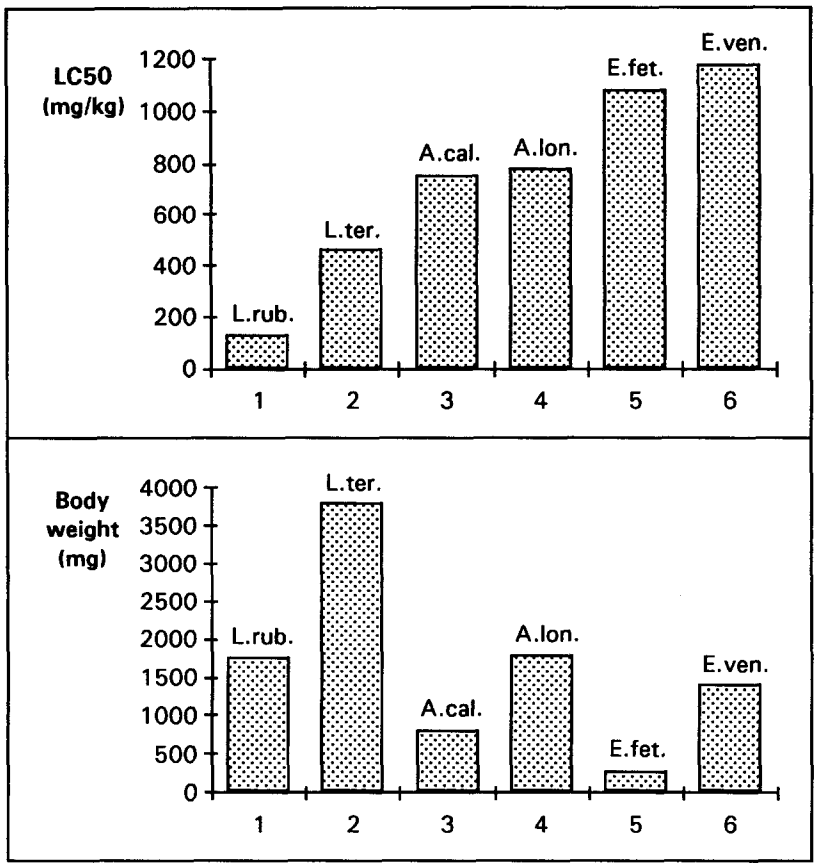

Figure 1. Comparison of 14d-LC50 values of chlorpyrifos to six species of earthworms with the average fresh body weight of each species. L.rub.= *Lumbricus rubellus*, L.ter.= *Lumbricus terrestris*, A.cal.= *Aporrectodea caliginosa*, A.lon.= *Aporrectodea longa*, E.fet.= *Eisenia fetida*, E.ven.= *Eisenia veneta*.

A final remark concerns the application of the current international OECD/EEC guidelines for the testing of new chemicals on earthworms. These guidelines specify the use of *Eisenia fetida* as a test species, merely because of its convenient breeding. However, the results of this study show that the toxicity of chemicals may be an order of magnitude greater in species other than *E.fetida*. Studies on the effect of benomyl and carbofuran on earthworms have similarly shown that the toxicity is much greater to *Lumbricus terrestris* than to *Eisenia fetida* when tested under standardized conditions in soil substrates (Haque and Ebing 1983). These observations emphasize the risk of scoring false negatives when using protocols for screening new chemicals with *E.fetida* as the sole test species.

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