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Carbofuran. Comparative Toxicity and Metabolism in the Worms *Lumbricus terrestris* L. and *Eisenia foetida* S.

Andrew P. Gilman[1] and Alexander Vardanis*

Eisenia foetida, a manure worm, showed characteristic symptoms of carbofuran poisoning (coiling and random muscle contractions). There appeared to be, however, a wide difference between this species and *Lumbricus terrestris,* the common dew worm, in susceptibility to this carbamate insecticide. Compared to the toxicity toward the dew worm this compound was one-sixth as toxic to *Eisenia* when injected and approximately half as toxic when applied in the soil. The insecticide appeared to repel *Eisenia* but not *Lumbricus.* Both worms took up carbofuran in quantities proportional to their size; however, *Eisenia* excreted 95% of this material in 48 hr compared to only 10% excreted by *Lumbricus.* Approximately half of the excreted material in the case of *Eisenia* was unchanged insecticide. Of the insecticide broken down by the worms in a 48-hr period, *Eisenia* retained only 5% as metabolites whereas *Lumbricus* retained 87%. This comparative study emphasized the importance of selecting truly representative species for the evaluation of new insecticides.

Stenersen *et al.* (1973) have recently reported on the severe toxicity of the carbamate insecticide carbofuran (Furadan) to the earthworm *Lumbricus terrestris.* However, apparently conflicting reports on the effects of this relatively new carbamate on earthworms have been released by the manufacturer (FMC Corporation, Niagara Chemical Division) in a technical review paper in June 1972. This discrepancy was not real, however, since FMC Corporation used as their experimental animal a species of worm that is not strictly an earthworm. This species, *Eisenia foetida,* inhabits animal dung and manure and feeds by ingesting organic debris without ever surfacing (Satchell, 1967). It can tolerate colder temperatures than *Lumbricus* and can mate the year round. Although this manure worm has the highest nitrogen excretion rate of any worm species (0.4 mg/g of worm per day), it is of little direct significance to field fertility since it lives only in excreta.

The wide difference in susceptibility of these two species presented an excellent opportunity for a comparative study in an effort to better understand the reasons for the high toxicity of carbofuran toward the agriculturally important species *Lumbricus.*

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METHODS

Five hundred *Eisenia foetida* were purchased from Brazos Worm Farms (Waco, Tex. 76705) and 500 were supplied by the FMC Corporation (Niagara Chemical Divison, Middleport, N. Y.). These worms, referred to by the dealer as "red-gold hybrids," were kept under conditions identical with those for *Lumbricus.* The latter were purchased and maintained as described by Stenersen *et al.* (1973). All worms used were adults and weighed either 3-5 g (*Lumbricus*) or 0.5-0.7 g (*Eisenia*).

Toxicants were obtained, prepared, and administered as outlined by Stenersen *et al.* (1973).

All worms to be injected were prechilled on ice for 15 min. The maximum injection for *Lumbricus* was 5 μl and the maximum for *Eisenia* was 1 μl.

Selection of carbofuran-treated *vs.* untreated soils by the two species was tested by presenting both soil types in the same container. Round plastic buckets (20-cm diameter) were used. Carbofuran (recrystallized) was incorporated into the treated soils at 1.5-2.0 ppm. The soil types

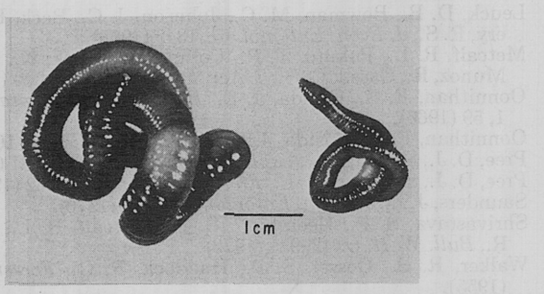

Figure 1. Coiling reaction of *Lumbricus* and *Eisenia* (right) to carbofuran treatment.

were placed in two separate sections, a central core and a periphery in the following manner. A glass cylinder was filled with soil and inverted in the bucket. The latter was filled to the same level and the cylinder carefully withdrawn. Approximately 10 *Lumbricus* or 25 *Eisenia* were used per experimental replicate. The worms were always placed beneath the surface of the soil. After 2 weeks the number of worms in the two zones was recorded.

Uptake of ring-labeled [^{14}C]carbofuran by *Eisenia* was tested at 25° over a 6-hr period. Three groups of five worms were used for uptake at each concentration. Aliquots (0.1 ml) of the uptake solution were removed for counting at several time intervals.

The methods used to study the rates of excretion of carbofuran and its metabolites by the two species were identical with those outlined by Stenersen *et al.* (1973). The quantitative determination of radioactivity in all tissue and sand samples was made as follows. Extracts to be separated by tlc were streaked on plates and run consecutively three times in 4:1 ether–petroleum ether solvent. The silica gel in the area of 1 cm above the origin to the top of the plate was then scraped and extracted four times with 1:1 acetonitrile–95% ethanol. This extract was concentrated by evaporation and spotted and chromatographed using 0.5-mm silica gel plates and 4:1 ether–petroleum ether. The chromatography chamber was presaturated for 30–45 min. The radioactive spots were located with a scanner and scraped and the gel was added to vials for counting. Determination of the amount of unknown I present (Figure 4C) was made from the total radioactivity remaining at the origin of the streaked plates and the origin of the subsequently spotted plates.

Significance of results in some experiments was determined at both 5 and 1% levels of confidence from a *t* test (Snedecor and Cochran, 1967).

RESULTS

Gross Effects. Upon injection *Eisenia* began to coil violently and released a yellow-green substance predominantly from the injected area. This reaction to injection subsided within minutes. Initial symptoms of poisoning with carbofuran did not appear as severe as those observed in injected *Lumbricus* by Stenersen *et al.* (1973). Whereas *Lumbricus* remained on the soil surface after injection, 83% of the manure worms had moved beneath the surface within 1 hr.

When placed in soil treated with carbofuran (4 ppm) coiling was always observed in both species (Figure 1). Eighty per cent of the *Lumbricus* were found coiled at the surface within 24 hr. *Eisenia,* on the other hand, while showing some coiling and random muscle contractions, remained buried while in treated soils. Both species continued to show symptoms of poisoning for the duration of their contact with the treated soil.

Although two types of swellings were consistently observed in carbofuran-treated dew worms (Stenersen *et al.,* 1973) few growths were found on *Eisenia.* (Although tissue proliferation and swelling in *Eisenia* were the exception rather than the rule three specimens were sent to the Smithsonian Institute, Washington, D. C., for examination.) Both *Lumbricus* and the manure worm were often observed with body constrictions, probably caused by contraction of the circular muscle layer. In time these constrictions appeared to result in removal of posterior portions of the worm from the rest of the animal. There was no noticeable increase in mortality in worms undergoing this removal.

Table I. Movement of Earthworms into and out of Carbofuran-Treated Soils[a]

Worm	No. at start	Starting zone	Alternate zone	% migration out of starting zone
Lumbricus	32	C–T	P–UT	18.3 ± 6.2[c]
	52	C–UT	P–T	65.5 ± 6.2
	30[b]	C–UT	P–UT	66.7 ± 4.7
Lumbricus	20	P–T	C–UT	5.0 ± 5.0[d]
	25	P–UT	C–T	23.0 ± 7.0
	20[b]	P–UT	C–UT	25.0 ± 5.0
Eisenia	71	C–T	P–UT	84.3 ± 5.4
	73	C–UT	P–T	43.0 ± 16.9[d]
	40[b]	C–UT	P–UT	83.5 ± 8.5

[a] C, center; P, periphery; T, treated soil; UT, untreated soil. [b] Controls. [c] 1% level of confidence. [d] 5% level of confidence.

The results of experiments designed to study the ability of the two species to detect carbofuran-treated soils and their movements in treated and untreated areas are shown in Table I. *Lumbricus* was placed in both the central (C) and the peripheral (P) soils some of which were treated (T) and others untreated (UT). When placed in the center of the bucket *Lumbricus* appeared to migrate to outer treated soils in numbers similar to those of control experiments where neither the center nor periphery was treated. However, when the center soils contained carbofuran only 18% migrated out of the center. Similar observations were made when *Lumbricus* was started in peripheral soils. Worms placed in the untreated periphery demonstrated a percentage migration to the central, treated areas similar to the control. However, if the peripheral zone was treated, only 5% were able to migrate into the untreated center.

Eisenia showed a similar control pattern to that of *Lumbricus, i.e.,* 83.5% migrated to the outer circumference from the center. However, unlike *Lumbricus,* when the periphery was treated few worms entered the area. Furthermore, there was no significant reduction in migration out of the treated zones.

Toxicity. The toxicity of carbofuran for both species of worms is summarized in Table II. Since *Eisenia* is approximately one-tenth the weight of *Lumbricus* each LC_{50} value is also expressed per gram of worm tissue to facilitate comparison. It can be seen that when injected, carbofuran is nearly six times more toxic for the larger worm than for *Eisenia.* When the insecticide is applied in the soil the difference between the toxicities is approximately twofold. The toxicities of the carbofuran metabolites 3-hydroxycarbofuran and 3-hydroxycarbofuran phenol were determined for *Lumbricus* only. The former had less than half the toxicity of the parent compound and the latter no toxicity whatsoever (up to 57 μg injected).

Uptake and Excretion. The concentrations of carbofuran used for uptake from solution by *Eisenia* were the same as those used earlier by Stenersen *et al.* (1973) for *Lumbricus.* The total amount of carbofuran taken up by *Eisenia* after 6 hr was similar to that taken up by *Lumbricus,* on a microgram per gram of worm basis (Figure 2 compared with Figure 6 in Stenersen *et al.,* 1973). The initial rate of uptake was slightly lower in the case of *Lumbricus.*

Table II. Toxicity of Carbofuran and Its Metabolites to *Lumbricus* and *Eisenia*

Species	Toxicant	No. of worms	Method of application	LC_{50}
L. terrestris	Carbofuran	144	Injected (5 days)	12 μg/worm 2.43 μg/g of worm
		100	Injected (15 days)[a]	5.2 μg/worm 2.08 μg/g of worm
		100	In soil (5 days)	13.2 ppm
	3-Hydroxycarbofuran	125	Injected (5 days)	35 μg/worm 7.2 μg/g of worm
	3-Hydroxycarbofuran phenol	65	Injected (5 days)	>57 μg/worm
E. foetida	Carbofuran	104	Injected (5 days)	4 μg/worm 13 μg/g of worm
		140	In soil (5 days)	24.5 ppm

[a] Value of Stenersen *et al.* (1973).

Excretion of radioactivity subsequent to 6 hr of uptake of the insecticide was drastically different in the two species (Figure 3 compared with Figure 7 in Stenersen *et al.*, 1973). After 48 hr *Eisenia* had excreted over 95% of the ^{14}C-labeled material whereas *Lumbricus* had excreted less than 10%. There did not appear to be any reabsorption of excreted material by *Eisenia*, in contrast with *Lumbricus*.

Metabolism. Separation of carbofuran and its metabolites was improved by presaturating the chromatography chamber for 30–45 min. The R_f values of the various compounds were: unknown I, 0.02; unknown II, 0.30; 3-hydroxycarbofuran, 0.36; 3-ketocarbofuran, 0.52; carbofuran, 0.58; 3-hydroxycarbofuran phenol, 0.76; and carbofuran phenol, 0.94.

Although similar metabolites were produced by both *Eisenia* and *Lumbricus* the quantities found and the rates at which these were excreted differed markedly. The percentage excretion of unmetabolized carbofuran by *Eisenia* is 20 times that of *Lumbricus* (Figure 4A). The majority of the metabolized insecticide produced by *Eisenia* was 3-hydroxycarbofuran (Figure 4B) and all of this was excreted within 48 hr. *Lumbricus*, however, primarily produced and maintained metabolite unknown I (Figure 4C). Figure 4D illustrates the increase of unextractable ^{14}C-labeled material in the worm tissue residues. This accounts for 2% of the total activity for *Eisenia* and 50% for *Lumbricus*. The amounts of 3-hydroxycarbofuran phenol and unknown II produced by the two species were negligible: less than 0.5 and 1.5% for *Lumbricus* and 1.5 and 1.0% for *Eisenia*, respectively.

DISCUSSION

Clearly large differences exist between *Eisenia* and *Lumbricus* in their reactions to carbofuran and their relative abilities to metabolize and excrete this insecticide. *Eisenia* may be repelled by carbofuran as outlined by the FMC Corporation (1972). However, *Lumbricus*, which comprises a large per cent of the detritus feeder biomass in Ontario, seems to be immobilized by this carbamate, as demonstrated by a marked inability to leave treated soils. The large dew worms did not show any reluctance to enter treated areas. This aspect of carbofuran's effects on *Lumbricus* has far-reaching implications. Even though applied on the topsoil (in rows or broadcast) the insecticide would render this surface feeding species immobilized and susceptible to predation and dehydration. Kring (1969) has already reported on the large numbers of earthworms found dead and dying on the soil surface of a tobacco field row treated with granular carbofuran (0.5–4.0 g of A.I./acre). He observed little mortality with several organophosphorus compounds, and found Bux, another carbamate insecticide, to be only half as toxic to earthworms as carbofuran.

The relative toxicities of carbofuran for both species when injected and when present in the soil are interesting.

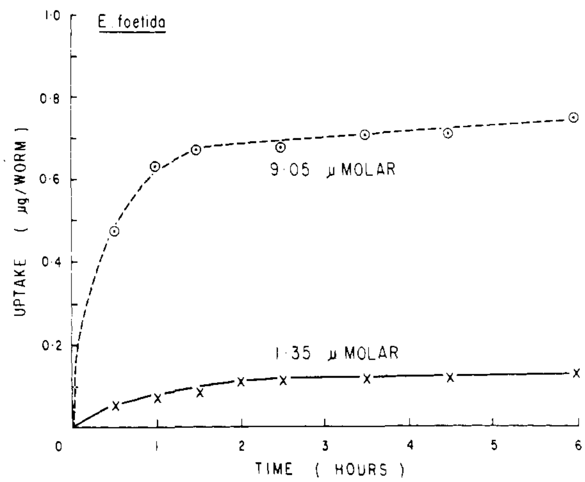

Figure 2. Uptake of [^{14}C]carbofuran from water by *Eisenia*.

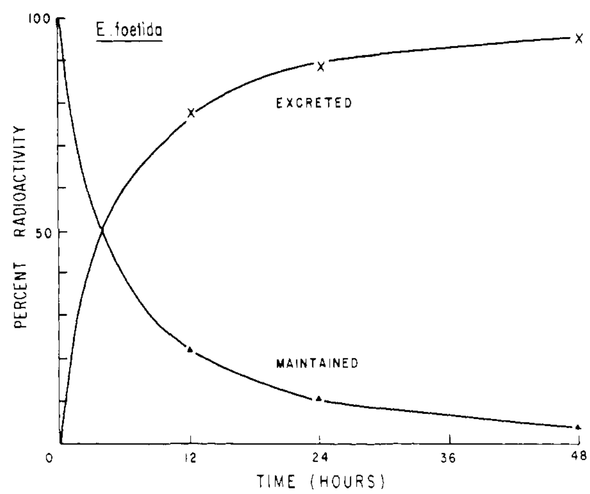

Figure 3. Excretion and maintenance of radioactive material by *Eisenia* after uptake of [^{14}C]carbofuran from solution.

As shown in Table II, carbofuran is approximately six times more toxic to *Lumbricus* when injected but only twice as toxic when applied in the soil. This discrepancy cannot be explained by uptake of insecticide since on a microgram per gram of worm basis *Eisenia* removes as much carbofuran as *Lumbricus* from experimental solutions (Figure 2). In fact, the more rapid metabolism and excretion of carbofuran by *Eisenia*, that can be assumed to be taking place during gradual uptake of insecticide from the soil, should permit *Eisenia* to be much more tolerant than *Lumbricus*. The tendency of *Lumbricus* to

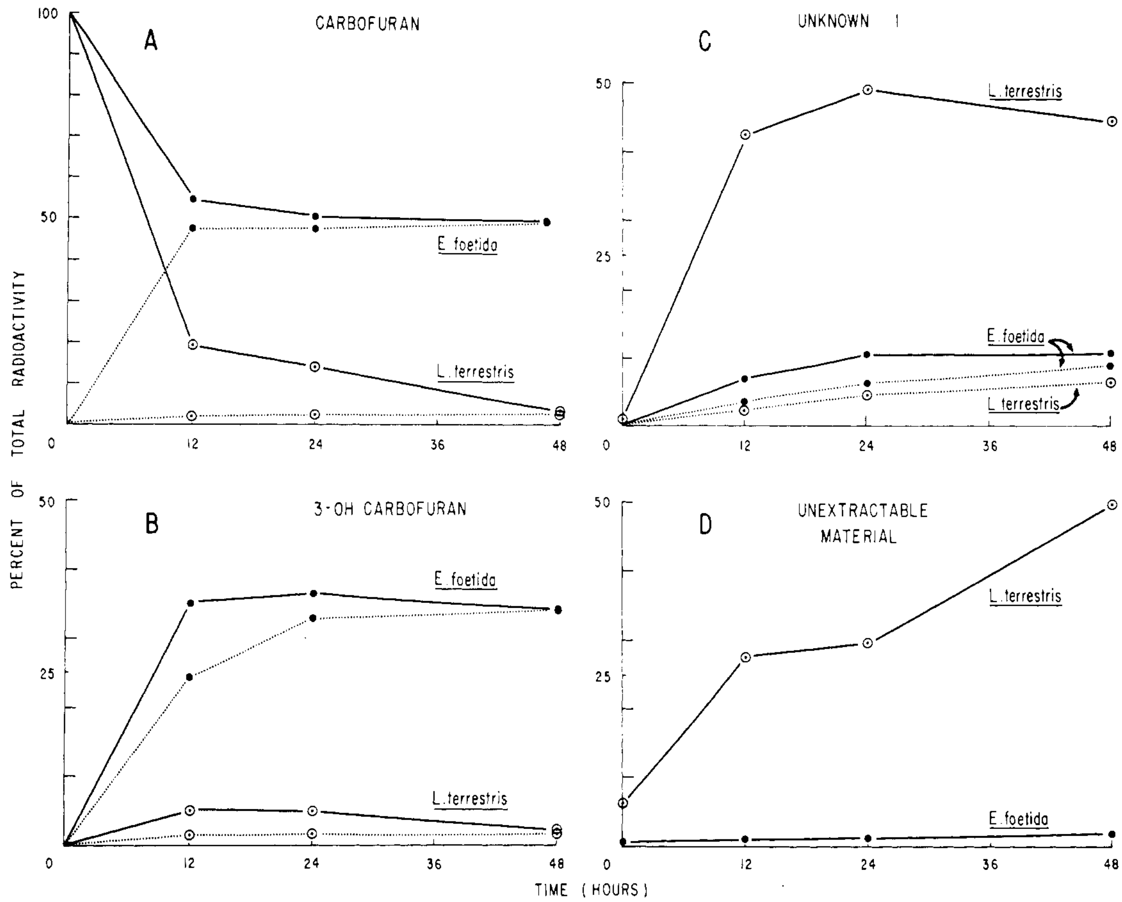

Figure 4. Total amounts of carbofuran and metabolites found in the two worm species and their sand habitat: (A–C) solid line represents the total amount found in sand and worms; dotted line represents the amount found in sand only; the area between the solid and dotted lines for each species represents the amount maintained by the worms; (D) total amount of "unextractable" material found in ground sandworm residues.

reabsorb excreted carbofuran or its metabolites certainly adds to the large quantity of material maintained. However, reabsorption in itself is not likely to account for the large difference in the quantities of material excreted after 2 days.

Most animals appear to metabolize and excrete up to 90% of administered carbofuran in 2 days (Dorough, 1968; Hicks *et al.*, 1970; Krishna and Casida, 1966; Leeling and Casida, 1966; Ivie and Dorough, 1968). Metcalf *et al.* (1968) report that *Estigmene acrea,* the salt marsh caterpillar, had excreted 88% of the total administered carbofuran (found as polar conjugates) in the feces after 4 days. The excretion of carbofuran by *Lumbricus* would appear to be significantly different from other organisms as well as from *Eisenia*.

Metcalf *et al.* (1968) have separated and identified the major metabolites of carbofuran using up to seven solvents. Since good separation of the known metabolites in our study was obtained using 4:1 ether-petroleum ether and cochromatography was employed, no further identification of the metabolites was undertaken. *Eisenia* was found to metabolize carbofuran similarly to *Lumbricus* as described by Stenersen *et al.* (1973). Two quantitative differences are observed, however. *Eisenia* metabolized only half of the carbofuran taken up, the other half it excreted directly as unchanged insecticide. Second, of the carbofuran broken down by the two species, *Eisenia* maintained only 5% as metabolites whereas *Lumbricus* maintained 87%.

Unknown I which builds up in *Lumbricus* probably corresponds to the polar conjugates found by Metcalf *et al.* (1968) and Dorough (1968). Perhaps the high toxicity of carbofuran for *Lumbricus* is associated with the large amount of polar material bound in the worm's tissue. This hypothesis remains untested, however, since unknown I could not be isolated in "clean" enough form for injection.

This comparative study has shown wide differences in the effects of carbofuran in two species of Annelid worms. It again emphasizes the great care that must be taken when selecting a representative species for evaluation of the ecological effects of a new insecticide, and underscores a recommendation made 10 years ago that the use of *Eisenia foetida* as a test species is unwise if the results are to be applied to other earthworms (Davey, 1963).

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