tissues of the pigs decreased only slowly during the 14 days after dosing is consistent with these findings. So is the intermediate increase of ^{14}C in both mesenteric and subcutaneous fat, which reflects the relatively slow turnover of fatty acids in this tissue.

In summary, the absence of dichlorvos and of the known metabolites of dichlorvos, the high concentrations of ^{14}C in tissue as compared to the detection limits for dichlorvos and its metabolites, and the presence of ^{14}C in expired air suggest that the ^{14}C residues in tissues of growing pigs reported in this paper are due to normal tissue constituents formed from the vinyl carbon of dichlorvos.

ACKNOWLEDGMENT

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Carbofuran: Its Toxicity to and Metabolism by Earthworm (*Lumbricus terrestris*)

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The toxicity of the carbamate insecticide carbofuran to *L. terrestris* has been tested. The LD_{50} of injected material was 1.3 mg/kg, and when mixed in soil the LC_{50} was 12.2 ppm (5-day test period). Cholinesterase depression was less severe and recovery was faster for carbofuran-treated worms than for worms treated with paraoxon or Dasanit, although the latter two were less toxic. Characteristic symptoms of carbofuran poisoning were rigidity, immobility, sores, and segmental swellings, while only rigidity and immobility were observed after treatment with organophosphorus anticholinesterases. The toxicity of this insecticidal carbamate to earthworms may therefore be due to factors other than cholinesterase inhibition. Earthworms excreted carbofuran, mainly as the unchanged insecticide, its hydroxylated analog (3-hydroxycarbofuran), and at least two unidentified products. The earthworms were found to reabsorb excreted insecticide and its metabolites from a sand medium. The ^{14}C-labeled material was ultimately bound to some tissue component, not extractable with acetonitrile.

The importance of insecticidal carbamates has recently increased as a result of the discontinued use of DDT and other organochlorine insecticides. It is essential, therefore, that more information about possible environmental side effects of carbamates be obtained.

Working along these lines and as a part of a more general study, Thompson (1970) has recently reported on the effects of several insecticides on earthworms when the chemicals are applied to pasture plots. Application of the carbamate carbofuran (2,3-dihydro-2,2-dimethyl benzofuran-7-yl *N*-methylcarbamate) was found to reduce the total number of earthworms by 83% and the total biomass by 60%.

Preliminary studies in our laboratory indicated that treatment of the earthworm (*Lumbricus terrestris* L.) with carbofuran under controlled conditions caused high mortality and the appearance of segmental swellings. Aspoeck and an der Lan (1963) described a similarly high toxicity of the carbamate carbaryl to earthworms. In their studies worms painted with a suspension of carbaryl (between 0.1 and 0.8%) rapidly developed swellings that burst into bleeding sores. Sepsis and death subsequently occurred.

In this paper we describe the effects of carbofuran treatment on *L. terrestris* (mortality, development of swellings, cholinesterase depression, and recovery) and report on the metabolism and excretion of the insecticide. Experiments have been carried out with other carbamates and some known cholinesterase inhibitors to gather information about the mode of action of carbofuran. Work on species other than *Lumbricus terrestris* is underway and will be reported in a subsequent paper.

MATERIALS AND METHODS

Earthworms. Earthworms (*L. terrestris* L.) were purchased in lots of 300–500 from a live bait dealer in London, Ontario. Information concerning exact age and possible exposure to chemicals prior to the investigation is lacking, although the majority collected by the dealer came from area golf courses where they had presumably been in contact with many insecticides and herbicides. The worms used for experiments were all sexually mature (showed well developed clitella) and weighed between 3 and 5 g. The physiological condition and susceptibility of

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the worms to toxicants varied slightly throughout the study period. The earthworms were stored in a mixture of 50% sphagnum floral moss and 50% sterilized loam at 10° under a 12-hr day/night cycle. *In vivo* experiments were usually run at 15°.

Toxicants. Carbofuran (90% pure), several of its derivatives, and ring-labeled [^{14}C]carbofuran (98% pure, 2.2 Ci/mol) were gifts of F.M.C. Corp. (Niagara Chemical Division), Middleport, N. Y., U.S.A. The unlabeled carbofuran was recrystallized from an ethanol–water mixture before use. All other toxicants were obtained either commercially or as samples from the producer.

Soil Treatment. Sterilized moistened loam (organic content approximately 20%, water content 10%) with added peat moss (1:5 by volume) was used for *in vivo* experiments requiring a soil type environment. The previously mentioned light cycle was maintained throughout these experiments. Soil treatment was done as follows. The required amount of pesticide was dissolved in a small volume of ethanol. The solution was diluted with water and thoroughly mixed with the soil–peat moss medium described above. For the more insoluble compounds three drops of Triton X-100 were used to maintain a homogeneous water suspension of the chemical prior to incorporation into the soil

Injections of Toxicants. Injections of toxicants were done with 10-μl Hamilton syringes. Test chemicals were carried in 95% ethanol (5 μl/worm). Worms were chilled and injected in the pseudocoel dorsally and anteriorly.

Cholinesterase Measurements. Homogenates for cholinesterase measurements were prepared in ice cold phosphate buffer (20 ml per worm, pH 7.5, 0.04 *M*) with a Virtis R 45 homogenizer for 30 sec. The crude homogenate was centrifuged for 15 min at 12,000 × *g* (0°) and the supernatant was diluted 1:5 with the same buffer. Cholinesterase activity was measured using the following modification of the method reported by Ellman *et al.* (1961). The reaction mixture contained 0.13 m*M* of phosphate buffer (pH 7.5), 0.1 ml of earthworm homogenate, and 0.4 m*M* of DTNB [5,5-dithio-bis(2-nitrobenzoic) acid]. After all nonspecific reactions ceased, 3.0 μ*M* of substrate, acetylthiocholine iodide, was added and readings were taken each minute. The total volume of the reaction mixture was 3.2 ml. All determinations were carried out in a quartz cuvette at 24° using an Hitachi spectrophotometer.

Determinations of cholinesterase activity of worms from insecticide-treated soil were made using the following procedure. Four-percent homogenates of worms (posterior half of worms only) prepared in the Virtis mixer at half speed for 3 min were centrifuged at 4500 × *g*. From this supernatant (87% of the total activity) 0.05 ml was removed and added to 0.13 m*M* of phosphate buffer (pH 7.6), 0.3 μ*M* of substrate, and 0.1 m*M* of DTNB. The reaction, carried out at 25° in a test tube, was stopped after 5 min by addition of 64 m*M* of TPA (tetra-*n*-propyl ammonium bromide), a competitive inhibitor of cholinesterase. The final volume was 3.57 ml and the absorbance was read at 512 nm after 10 min.

Studies of *in vitro* inhibition of cholinesterase were carried out as outlined above, except that the insecticide was preincubated at 25° for 10 min with the enzyme prior to addition of the substrate. For these experiments which determined the pI_{50} for four pesticides, the enzyme was initially prepared as a 4% homogenate as outlined above. However, in order to concentrate and store the enzyme's activity for later use, it was recentrifuged for 30 min at 45,000 × *g* and the pellet was kept at −20°. When required, the pellet (79% of the total activity) was resuspended in 3 ml of buffer (pH 7.5) and added to the reaction mixture at 25°. A 7% loss in activity occurred over a 3-week storage period.

Thin-Layer Chromatography. Thin-layer chromatography was carried out on 0.5-mm silica gel (Camag DF-5) plates. Good separation of carbofuran, its metabolites, and compounds of similar structure was obtained using ether–petroleum ether (4:1) as solvent similar to that used by Metcalf *et al.* (1968). Another system of acetonitrile–water (88:12) (Stenersen, 1971) was found to aid separation of a more polar metabolite(s) left at the origin by the previous solvent. Chromatographs of radioactive substances were scanned with a panax-R thin-layer scanner RTLS-1A. Nonradioactive carbofuran derivatives containing the benzofuran ring structure were located by spraying the plates with 1% ethanolic NaOH solution, heating for 30 min at 110°, and spraying again with 1% (w/v) 2,6-dibromobenzoquinone-4-chloroimide in ethanol (Nurnberg, 1961). A blue spot indicated the presence of a phenolic compound.

Determinations of Radioactivity. Quantitative determinations of radioactivity were made using a scintillation counter and a toluene–methyl cellosolve (10:8) cocktail. Toluene labeled with ^{14}C was used as an internal standard to obtain counting efficiency.

Extraction of radioactive carbofuran and its metabolites from earthworms was carried out by grinding each worm (pestle and mortar) with 20 g of sea sand and extracting four times with 25-ml lots of acetonitrile. An aliquot of the pooled extract was removed and counted. The remainder was evaporated to near dryness (at 40° and reduced pressure), redissolved in acetonitrile, and streaked or spotted on tlc plates.

Uptake and Excretion Studies. To study excretory products from metabolism of the carbofuran, the worms were either injected or placed in 125-ml Erlenmeyer flasks containing various concentrations of the radioactive insecticide in 10 ml of distilled water. The uptake by the noninjected worms was allowed to occur at 25° for 4 hr. Aliquots of the uptake solution were taken before and after the 4-hr period. The worms were washed and placed in 100-ml jars containing 50 g of sea sand and 10 ml of water. A representative number were extracted immediately after washing to determine the amount of [^{14}C]carbofuran picked up. Sample aliquots of the sand (1–2 g) were removed from the jars and counted to obtain the rate of excretion data. At time intervals, worms were removed and analyzed as described above. The sand was extracted with 100 ml of acetonitrile. Anhydrous sodium sulfate was used to remove the water present. The sand extracts were taken to near dryness and redissolved in acetonitrile, an aliquot was removed for counting, and the rest was spotted on tlc plates and chromatographed. The total recovery of radioactivity from the earthworms and sand was 75 ± 2%.

Residues of material found in the sand–sodium sulfate mixture after extraction were estimated by liquid scintillation, as were residues in the ground sand–worm mixture. The latter mixture required solubilization in Soluene prior to counting. An alternate procedure described by Davidson *et al.* (1970), requiring combustion in oxygen, was also used in some determinations of radioactive residues after extraction.

RESULTS

Toxicity of Carbofuran and Other Insecticides. Worms injected with dosages of carbofuran between 1.5 and 5.0 μg (0.5–1.55 mg/kg) showed very predictable symptoms. Heavy secretion of slime was immediately observed and after several minutes the specimens became swollen, rigid, and immobile. Upon tactile stimulation the earthworms curled tightly but demonstrated an inability to move away from the stimulus. The rate of both circular and longitudinal muscle contraction remained rapid and

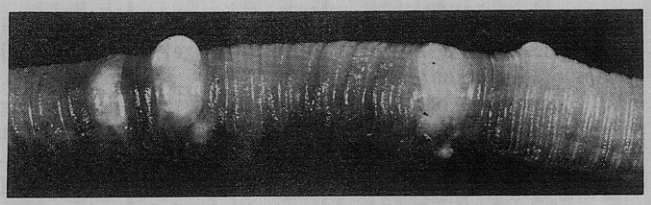

Figure 1. Photograph of dorsolateral surface of the posterior half of an earthworm *(L. terrestris)* showing segmental swellings (3-day exposure to carbofuran-treated soil, 12 ppm).

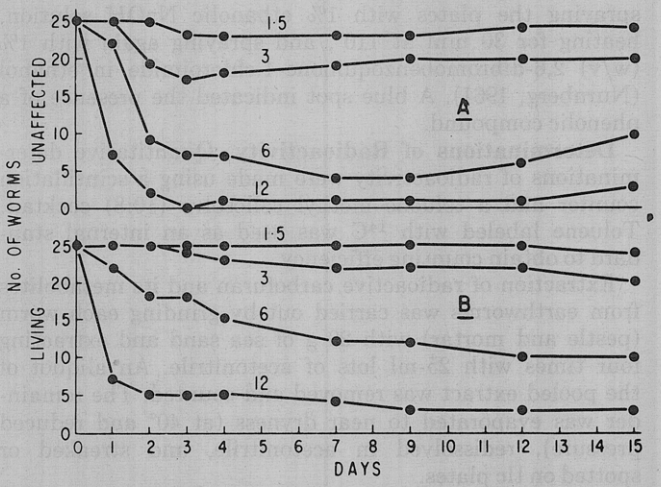

Figure 2. Dose response relationships for groups of 25 *L. terrestris* after injection with 1.5, 3, 6, and 12 μg of carbofuran. A: Numbers of living worms without swellings. B: Total number of live worms at different times.

equal. Escape from repeated stimuli was never observed, only increased random contractions.

If placed on the soil after injection, the worms were seldom observed to burrow and if placed in the soil they often appeared at the surface or tightly coiled close to the surface. Dehydration of the worms at the surface was prevalent. During the second and third days after injection, those worms still alive appeared to regain their orientation and ability to contract segments sequentially.

Injected doses of over 2 mg/kg of carbofuran caused similar responses but resulted in over 50% mortality after 3 days. Large swellings involving four to five segments appeared, especially in the region of the esophagus, crop, and gizzard. The clitellum was also usually swollen. The swollen areas most often burst, creating bleeding sores. The gut frequently protruded as a loop from lesions in the posterior half of the animal.

Those worms surviving 2 days often showed a second type of swelling (Figure 1), an epithelial growth of a single segment. These proliferations seldom ruptured or appeared to cause death. All symptoms of poisoning observed in injected worms were apparent in specimens placed in carbofuran-treated soils at a level recommended for use in the field. Carbaryl has been shown by Aspoeck and an der Lan (1963) to have similar effects.

A quantitative dose–mortality relationship was observed with the injection technique and is shown in Figure 2B (LD_{50} 1.3 mg/kg). A comparison of the number of unaffected living worms (no swellings) to total number of living worms is shown in Figures 2, A and B. These graphs indicate that mortality is highest during the first 2 days after injection and that worms can recover from the induced swellings after 2 weeks. A parallel study of toxicity of carbofuran when it is mixed into the soil showed the LC_{50} value to be 12.2 ppm (5 days of exposure).

The toxicity of several other substances was also tested.

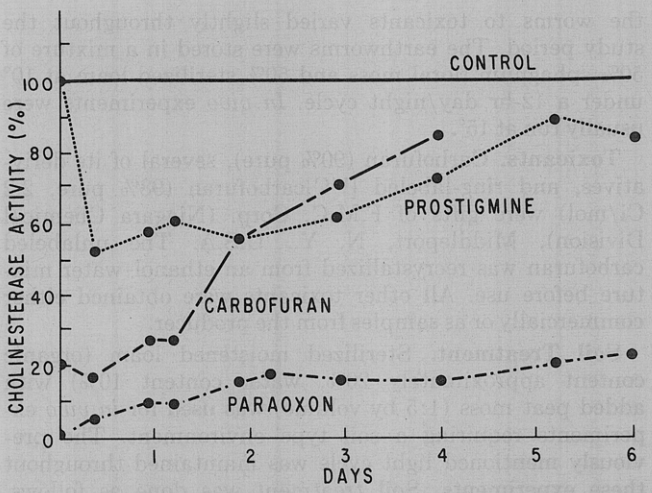

Figure 3. Mean cholinesterase activity levels determined over a 6-day period in three groups of earthworms (four worms/group) injected with (a) 50 μg of prostigmine, (b) 10 μg of carbofuran, and (c) 50 μg of paraoxon. Zero time data represent activity from worms injected and immediately homogenized.

Injections of only 0.5 mg/kg of either Dasanit {*O,O*-diethyl *O*-[*p*-(methylsulfinyl)phenyl] phosphorothionate} or paraoxon (a very potent inhibitor of cholinesterase) caused severe symptoms of poisoning similar to those observed for carbofuran (*i.e.*, sporadic contraction, coiling, and rigidity). However, levels as high as 30 mg/kg appeared to be well below the LD_{50} of these compounds. Prostigmine, an ionic anticholinesterase carbamate, caused no deaths at dosages of 30 mg/kg, while the symptoms of poisoning were again observed, lasting over a week at this concentration. Parathion (30 mg/kg) produced similar symptoms which were apparent 2 days after injection. Triorthotolyl phosphate and Azak [2,6-bis(2-methylprop-2-yl)-4-methylphenyl *N*-methylcarbamate], a herbicidal carbamate, had no observable effect at 30 mg/kg.

Cholinesterase Inhibition. *In vitro* studies of the inhibition of earthworm cholinesterase by paraoxon, malaoxon, carbofuran, and carbaryl gave the following pI_{50} values, respectively, 7.52, 5.16, 6.31, and 5.00.

The depression and recovery of cholinesterase activity observed in specimens injected with prostigmine, paraoxon, and carbofuran are shown in Figure 3. Paraoxon, at an essentially nontoxic dosage (15 mg/kg) for worms, gave immediate and total depression with slow recovery. However, with only 20% of the control enzyme activity present, the intoxication symptoms had, by the third day, disappeared. Ten micrograms of carbofuran (3 mg/kg) caused the cholinesterase level to drop to 20% of the normal activity, but in this case recovery was rapid. Prostigmine depressed the enzyme's activity only moderately and showed maximum depression several hours after injection. Recovery to normal levels in the latter case occurred after 5 days.

The results of studies of cholinesterase activity found in worms placed in soils treated with several insecticides are shown in Figure 4. All organophosphorus compounds used, with the exception of malathion, caused the enzyme activity level of the worms to drop to less than 0.01% of the control value within 15 days. Only 5 of the 69 specimens were found dead. Disorientation and sporadic contractions lasted only temporarily, usually ending after 5 days, at which time normal burrowing and fecal deposition were observed. The carbamates tested, however, did not cause high enzyme depression, but the expected high mortality was present (26 of 46 specimens), as well as swelling and sore development.

Figure 5 illustrates the gradual disappearance of carbo-

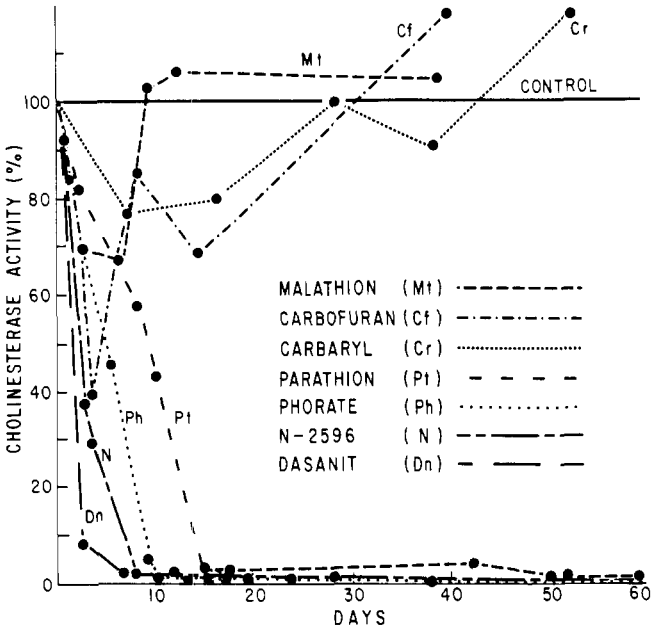

Figure 4. Cholinesterase activity of earthworms kept for up to 60 days in insecticide-treated soils. Concentrations used were: (a) malathion, 1.3 ppm; (b) carbofuran, 4.1 ppm; (c) carbaryl, 4.1 ppm; (d) parathion, 2.0 ppm; (e) phorate, 8.2 ppm; (f) N-2596, 4.1 ppm; and (g) dasanit, 6.1 ppm.

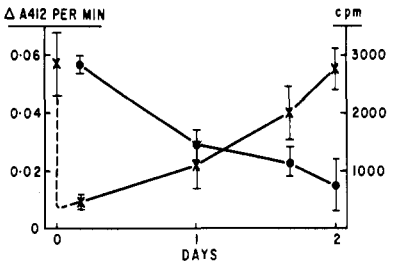

Figure 5. Disappearance of radioactivity (●) and the restoration of cholinesterase activity (X) in the supernatant of individually homogenized worms after injections of 5 μg of [^{14}C]carbofuran (three to eight worms/point).

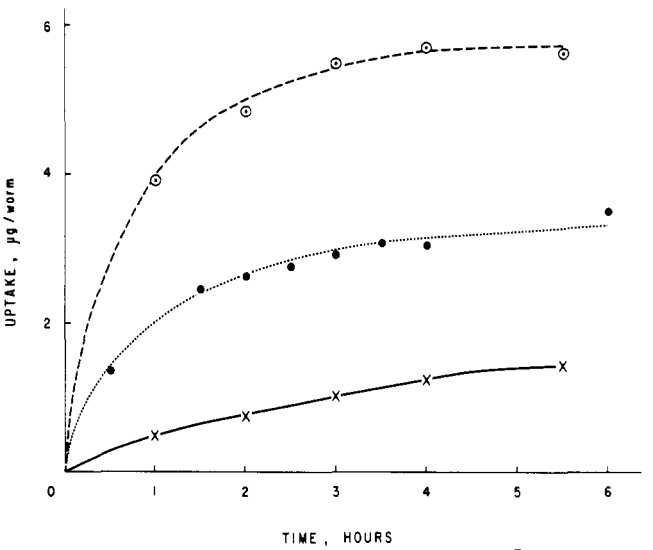

Figure 6. Uptake of [^{14}C]carbofuran (μg) by *L. terrestris* at three concentrations of the insecticide [20 μg (⊙), 10 μg (●), 3 μg (X)] all dissolved in 10 ml of water (each point mean of three worms).

furan from injected worms and its coicidence with restoration of normal cholinesterase activity. The symptoms of poisoning also tended to disappear over the 2-day period.

Uptake, Metabolism, and Excretion of Carbofuran. When placed in 10 ml of water containing radioactive carbofuran, the worms took up the radioactivity as illustrated in Figure 6. At the highest dosage of 20 μg (9.05×10^{-6} *M*), the specimens were close to death after 4 hr and showed little subsequent uptake. The lowest concentration allowed higher percentage uptake but fewer total μmoles of the material. After 6 hr at this latter concentration, uptake appeared to be continuing. No detectable metabolites were found in the worms during this period of uptake except for a small quantity of material remaining at the origin when extracts were developed with the ether petroleum ether solvent system. The bathing solution contained only radioactive carbofuran after 6 hr.

To study the excretion of carbofuran and its metabolites, experiments were performed using the previously described sand jar method. Figure 7 shows that the amount of excreted radioactive material increases up to a maximum level at 30 hr and then steadily decreases (lower curve). The upper curve shows the radioactivity found in the worms over the same period of time. These data suggest that reabsorption of excreted materials is taking place. The curve for rate of excretion of carbofuran and its metabolites for injected worms is also shown in Figure 7. Excretion is more rapid in this latter instance and 30–40% of the total injected radioactive carbofuran is found outside the animal after 24 hr, either as carbofuran or a metabolite. Reabsorption is again prevalent.

The results of quantitative analysis of the metabolites excreted by *L. terrestris* after both injection of radioactive carbofuran and uptake of this insecticide from solution appear in Table I. An example of a scanned chromatogram from which scrapings were made for quantitative analysis is also shown (Figure 8). The results obtained indicated that it is the excreted carbofuran which is primarily reabsorbed by the earthworms. A large percentage of the excreted material is the polar origin metabolite(s) of unknown composition indicated in Figure 8.

Injected worms appear to excrete similar proportions of the same metabolites. Preliminary results indicate that after 96 hr only 15% of the radioactive materials is found outside the worm (as compared to 34% after 24 hr), indicating reabsorption of excreted chemicals. Decreases in the amounts of both carbofuran and 3-hydroxycarbofuran were also apparent.

Experiments were performed in an effort to evaluate the role played by microorganisms and/or spontaneous conversion in the breakdown of excreted carbofuran and its metabolites. Sand into which worms had excreted [^{14}C]carbofuran and its metabolites for 24 hr was equally divided and one half was extracted immediately, as described above. The second half was incubated at room temperature for a further 48 hr, at which time it too was extracted. After thin-layer chromatography and scanning, quantitative determinations were made by liquid scintillation. The results showed that there was no significant difference in the amounts of carbofuran and its metabolites found in the two samples.

The breakdown of carbofuran in the worm and the metabolites present has not yet been analyzed quantitatively, however, all five of the excreted chemicals previously observed were also found inside the worm after 24 hr. Further studies of the residues in the worms after 72 and 96 hr indicated the presence of the polar metabolite(s) (unknown I) alone. Analysis of the earthworm residue after extraction has shown that increasing amounts of radioactive material were unextractable. These data are shown in Table II, together with the percentages of radioactive ma-

Table I. Mean Quantities (ng) of Carbofuran and Its Metabolites Found Excreted at Various Times after (A) Injection of 5 μg and (B) Uptake of 2.78 μg from Solution

Excreted products	A,[a] hr		B, hr	
	24	12	24	48
3-OH carbofuran phenol	35 ± 15	5 ± 1	3 ± 1	2 ± 1
Carbofuran	840 ± 85	39 ± 3	9 ± 1	3 ± 1
3-OH carbofuran	340 ± 25	57 ± 16	52 ± 10	49 ± 4
Unknown II	170 ± 155	23 ± 1	48 ± 3	23 ± 6
Unknown I	130 ± 95	85 ± 2	104 ± 29	109 ± 8

[a] A, mean of four worms; B, mean of five worms.

Table II. Mean Percentage Quantities of Carbofuran and Its Metabolites Found Maintained and Excreted by *L. terrestris* (Four Groups of Four Worms) after Uptake of [^{14}C]Carbofuran from Solution

Presence of carbofuran or metabolites	Time, hr			
	0	12	24	48
% extracted from worm	93.88 ± 0.85	65.03 ± 11.71	51.35 ± 0.50	47.59 ± 0.71
% unextractable from worm	6.11 ± 0.85	27.43 ± 10.02	40.77 ± 0.70	46.70 ± 2.05
% excreted by worm	0	7.54 ± 1.68	7.85 ± 1.10	6.70 ± 0.35

terial found in the worm and in the sand over a 2-day period.

DISCUSSION

The earthworm's effective breakdown of leaf litter and aeration of the soil make him a most valuable asset to land fertility, particularly in noncultivated soils. Since *L. terrestris* is a surface-feeding species, it must come in contact with treated soils in order to reach its food source. Thus the previously described symptoms of disorientation, muscle spasms, and coiling could potentially be induced, exposing the worms to heavy predation and excessive dehydration. For this reason, any consideration of prolonged use of this insecticide should take into account the possible deleterious effects upon the earthworm population in that area.

The presence of epithelial swellings and growths observed in *L. terrestris* treated with carbofuran has also been found after applications of carbaryl (Aspoeck and an der Lan, 1963). These authors considered the swellings to be cancer-like and of an irreversible nature. Since the epithelial swellings (Figure 1) observed in this study appeared usually in worms that survived (Figure 2, A and B), our results indicate that these are secondary. They may indeed be repair responses of damaged tissue. Many worms that did develop these single segment swellings were able to recover completely. Samples of effected worms have been sent to the Smithsonian Institute, Registry of Tumors in Lower Animals, Washington, D.C., at

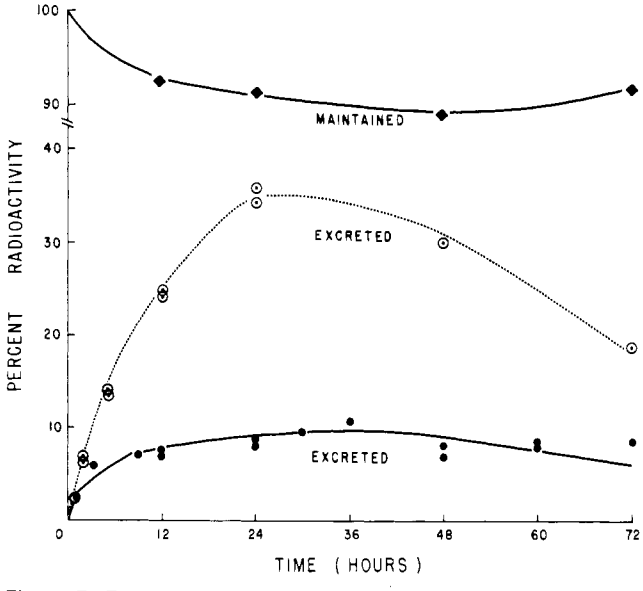

Figure 7. The percent radioactivity excreted and maintained by earthworms after taking up 2 μg of [^{14}C]carbofuran from solution (solid line). Dotted line shows amount of radioactivity excreted by earthworms injected with 5 μg of the ^{14}C-labeled insecticide.

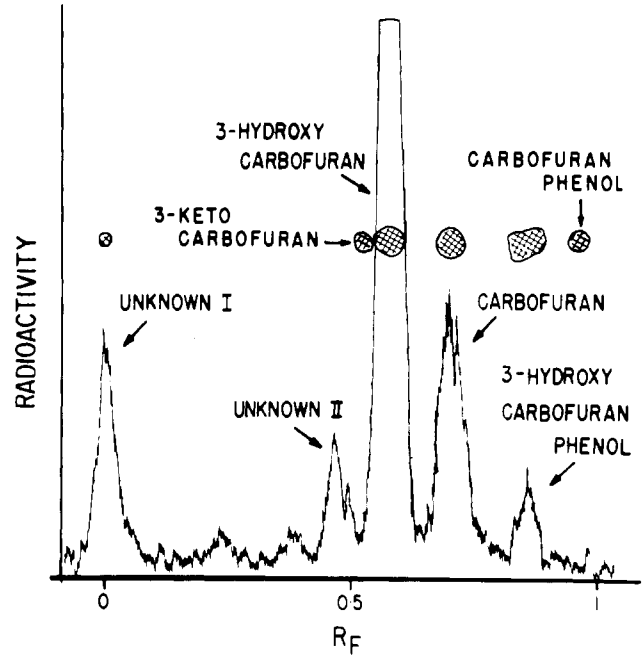

Figure 8. A tracing of a scan of a radioactive tlc showing the separation of [^{14}C]carbofuran and its metabolites excreted by three worms after 48 hr. Each worm was injected with 5 μg of the insecticide. The hatched spots show the position of reference compounds.

their request, for analysis and placement in the Institute's permanent collection.

The depression of cholinesterase activity by carbofuran is not excessive, as shown in both Figures 3 and 4. Organophosphorus compounds used caused severe inhibition of this enzyme's activity down to 0.01% of the control value for over 50 days. Using these compounds, however, mortality was only 7%. It would appear that the ability of carbofuran to inhibit cholinesterase is not the reason for its toxicity to *L. terrestris*. It is obvious, however, that the enzyme activity depression promotes many of the reactions described. Worms given both small (0.5 mg/kg) and severe doses of paraoxon (15 mg/kg) showed coiling and rigidity. Even with the highest dose, these symptoms seldom persisted more than 2 days, although cholinesterase levels remained below normal throughout the experimental period of 6 days (Figure 3).

Carbofuran metabolism has been studied by several authors, including Metcalf *et al.* (1968), Dorough (1968a,b), Ivie and Dorough (1968), and Ashworth (1969). *Lumbricus terrestris* would appear to metabolize the insecticide initially in a similar fashion to both plants and animals. Hydroxylation at the 3 position causes formation of 3-hydroxycarbofuran (2,3-dihydro-2,2-dimethyl-3-hydroxybenzofuran-7-yl *N*-methylcarbamate) and subsequent cleavage of the carbamine group produces 3-hydroxycarbofuranphenol (2-3-dehydro-2,2-dimethyl-3,7-dihydroxybenzofuran). Traces of other allied compounds normally found after metabolism of carbofuran by insects were detected but not qualified or quantified. The metabolite(s) "Unknown I" found excreted was very polar and was not further separated, but moved as a single spot in the acetonitrile–water solvent system.

Excretion of less than 10% of the total amount of insecticide taken up originally would appear to be connected with the reabsorption of excreted materials by the worms. The nature of the tissue bound radioactive material probably derived from previously excreted metabolites of [^{14}C]carbofuran requires further study. Reabsorption and maintenance of excreted material, especially carbofuran and 3-hydroxycarbofuran, will increase the effectiveness of this insecticide as a toxicant to the earthworm. Efforts to find the rates of production of metabolites and a quantitative analysis of these compounds in the worm will be made.

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Method for High-Speed Liquid Chromatographic Analysis of Benomyl and/or Metabolite Residues in Cow Milk, Urine, Feces, and Tissues

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Residues of benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate] and/or methyl 2-benzimidazolecarbamate, methyl 5-hydroxy-2-benzimidazolecarbamate, and methyl 4-hydroxy-2-benzimidazolecarbamate may be simultaneously determined in cow milk, tissues, urine, and feces. The first step in the method consists of hydrolyzing the sample in aqueous acid to convert benomyl to methyl 2-benzimidazolecarbamate and to free the metabolites from conjugates. The freed materials are then extracted into an organic solvent, the extract is cleaned up by a solvent–solvent partitioning process, and the components are determined in a single scan by high-speed strong cation exchange liquid chromatography. Recoveries of the various components average about 80% in cow milk and urine, with average recoveries of about 50–80% obtained from tissue samples and feces. Recoveries for the various compounds have been demonstrated at the 0.01–0.02-ppm level in cow milk, at the 0.05–0.1-ppm level in tissues and feces, and at the 0.2-ppm level in urine. No interference with the method was found from a number of other pesticides with tolerances in milk and meat tissues.

Analytical methods have previously been given for determining residues of benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate], the active ingredient in DuPont Benlate benomyl fungicide (Pease and Gardiner, 1969; Pease and Holt, 1971). These procedures involve the quantitative conversion of benomyl to methyl 2-benzimidazolecarbamate, sometimes called MBC, then to 2-aminobenzimidazole (2-AB) by a two-stage acid–base hydrolysis procedure. Hence, benomyl, its principal degradative product, MBC, and a minor component of the residue in plants, 2-AB, are measured as a composite value by fluorometry or an alternate colorimetric procedure.

Methyl 2-benzimidazolecarbamate has been identified as a major benomyl breakdown product in aqueous solution and within plants (Clemons and Sisler, 1969; Fuchs *et al.*, 1970; Peterson and Edgington, 1969; Sims *et al.*, 1969). This compound has also been proposed as a possible intermediate in the conversion of benomyl to methyl

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