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Carbon-14 Balance and Residues of Dichlorvos and Its Metabolites in Pigs Dosed with Dichlorvos-^{14}C

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One dose of dichlorvos-^{14}C (2,2-dichlorovinyl-*1*-^{14}C dimethyl phosphate) formulated as slow-release polyvinylchloride (PVC) pellets was fed as a top dressing to each of nine young male Yorkshire pigs. After treatment, three of the pigs were killed at each of the following intervals: 2 days, 7 days, and 14 days. In the 14-day trial, 61.8% of the ^{14}C administered was found in the pellets recovered from the feces, 5.6% was found in the remainder of the feces, 3.6% was found in the urine, 14.1% was recovered from the expired air, and 9.6% remained in the carcass. The ^{14}C content of the tissues from the pigs in all the treatments ranged from 2 to 33 ppm equivalents of dichlorvos, but no dichlorvos, demethyl dichlorvos, dichloroacetaldehyde, and dichloroacetic acid were found in the tissues of the pigs. It is concluded that the ^{14}C present in the tissues is the result of incorporation of C-1 and C-2 fragments from the vinyl moiety of dichlorvos into normal tissue constituents.

In vivo and *in vitro* studies have shown that the insecticidal and anthelmintic compound dichlorvos (2,2-dichlorovinyl dimethyl phosphate) is degraded enzymatically by animal tissue, and in whole animals is degraded to dichloroacetaldehyde (DCA), dichloroethanol (DCE), dichloroacetic acid (DCAA), demethyl dichlorvos (DMD), dimethyl hydrogen phosphate, and methyl dihydrogen phosphate (Bull and Ridgway, 1969; Casida *et al.*, 1962; Hodgson and Casida, 1962). Rats dosed with dichlorvos labeled with ^{14}C in the 1 position of the vinyl group excreted part of the ^{14}C as carbon dioxide in the expired air.

Because no information was available on the fate of the dichlorovinyl part of the molecule after dechlorination other than the evolution of carbon dioxide, a study was initiated to determine the degradative fate of dichlorvos in young pigs, one of the animals in which dichlorvos is effective as an anthelmintic.

Dichlorvos labeled with ^{14}C on the 1 position of the vinyl group was prepared as described by Burton (1971) and formulated as a slow-release formulation in polyvinylchloride (PVC) pellets. The pellets were fed to pigs and the residues of the ^{14}C, dichlorvos, and known dichlorvos metabolites were measured.

MATERIALS AND METHODS

Preparation of PVC Pellets. The PVC pellets were prepared by extruding a mixture of PVC resin (63.75 wt %), plasticizer and stabilizer (13.25 wt %), and dichlorvos (23 wt %) into a strand 0.06-in. in diameter and by cutting off ⅛-in. long segments. The conditions employed have been given by Menn *et al.* (1965) and Folckemer *et al.* (1967). Analysis of the pellets showed that they contained ^{14}C equivalent to 21.3 wt % of dichlorvos. Tlc analysis of the pellet extracts gave only one radioactive spot, which had an R_f value corresponding to that of a dichlorvos standard.

Feeding Experiment. Nine Yorkshire cross male pigs were employed. Three days prior to the start of the test the pigs were placed in individual closed metabolism cages that allowed the separate collection of the urine and feces. The carbon dioxide in the expired air was collected by countercurrent extraction with 8% sodium hydroxide. After a 48-hr acclimatization period, the pigs were fasted for 24 hr and then given a standard pig mash containing 19.85% dehydrated alfalfa meal, 2.48% meat and bone, 2.48% fish meal, 74.4% ground milo and wheat, 0.50% salt, and 0.25% Microfac supplement. (The Microfac supplement was supplied by Dawes Laboratories, Chicago, Ill.) A single dose of PVC pellets containing 21.3% dichlorvos-^{14}C was given to each pig as a top dressing at a rate of approximately 40 mg of dichlorvos per kilogram, the therapeutic dose of dichlorvos. The specific activity of the dichlorvos was 205 dpm per microgram. After the pigs consumed the initial portion of feed and top dressing, they were given water and feed *ad libitum* for the duration of the experiment. Three of the pigs were killed 2 days after treatment, three were killed 7 days after treatment, and three were killed 14 days after treatment.

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Table I. Correction Factor for Pellets

Pig no.	Sampling interval, days	Pellet count method: Weight of 100 pellets, mg	Pellet count method: Correction factor	Dichlorvos analysis method: ^{14}C content of dichlorvos equivalents, %	Dichlorvos analysis method: Correction factor
43	3	781.7	0.9218	11.86[a] ± 0.71	0.8929
43	4	758.6	0.8945	11.29[a] ± 0.95	0.8935
45	2	797.8	0.9480	14.45[a] ± 0.35	0.9200
45	3	784.0	0.9245	12.50[a] ± 0.93	0.8994
Mean		780.5	0.9204 ± 0.0096[b]	12.68 ± 0.61[b]	0.9014 ± 0.0063[b]
Original pellets		848		21.3	

[a] Mean of triplicate ± S.E.M. [b] Mean ± S.E.M.

Table II. Recovery of ^{14}C from Pigs Dosed with Dichlorvos-^{14}C

Pig no.	Dosage,[a] mg/kg	Wt, kg	Collection period, days	Recovery of ^{14}C, % of dose: Carcass	Expired air	Feces	Voided pellets	Urine	Total recovered[b]
258	42.4	28.2	2	8.4	N.D.[c]	3.4	38.0	3.0	
259	41.0	27.6	2	10.0	N.D.	1.2	37.0	2.4	
261	42.6	24.7	2	11.1	N.D.	2.6	31.8	3.8	
5	42.6	27.3	7	13.2	N.D.	3.9	46.6	3.3	
6	42.6	27.2	7	11.8	N.D.	6.9	45.6	3.3	
260	42.6	20.0	7	9.6	N.D.	6.4	46.6	2.8	
4	42.6	26.3	14	8.7	16.2	5.1	64.9	3.3	98.2
43	42.6	32.6	14	9.4	13.9	6.3	62.7	2.4	94.8
45	42.6	31.2	14	10.8	12.3	5.3	57.8	5.0	91.2

[a] Dichlorvos formulated as PVC pellets containing 21.3% dichlorvos. [b] Mean recovery of ^{14}C, 94.73%. [c] Not determined.

The carbon dioxide in the expired air (14-day trial only) and the urine and feces were collected and weighed after 0.5, 1.0, 1.5, 2.5, and 3.0 days and every day thereafter for the duration of the test. At the end of the experiment the pigs were killed and samples of tissues were collected. All samples were stored at −10 to −15° until analyzed.

Separation of PVC Pellets from Feces. To separate a small portion of fecal material for packed tube combustion, a 10% aliquot of the fecal sample was spread on a glass plate in a layer 1–2 mm thick. The pellets were then picked out with a pair of tweezers. The rest of the feces–pellets mixture was placed in a 1 gal Waring blender and covered with tap water, and the blender was operated at full speed for 1 min. An equal volume of water was added and the slurry was poured through a 16-mesh wire screen. Additional water was then used to transfer the pellets and fecal matter to the screen and to wash the fecal matter through the screen. The pellets were then transferred to a vial, rinsed with a small volume of water, and blotted dry. With the aid of tweezers, the pellets were then separated from any remaining fecal material and stored in a glass vial at −10°.

Determination of ^{14}C. The ^{14}C was counted with either a Model 3380 or 3023 Packard Tri-Carb liquid scintillation counter. The optimum gains for the different liquid scintillators and counters were determined by the internal standard method by the use of a toluene-^{14}C standard.

Urine (0.1 to 0.25 ml) was counted in 20 ml of liquid scintillation solution containing 5 g of PPO and 0.1 g of dimethyl POPOP per liter of toluene–absolute ethanol (75:25, v/v) solution. Because the fecal and tissue samples might have contained volatile ^{14}C-labeled material, a packed-tube combustion method (Bastin and Gordon, 1969) was employed instead of the Schoniger flask combustion method (Kalberer and Rutschmann, 1961; Schoniger, 1955). Caustic solution containing sodium carbonate-^{14}C was counted by adding 1 ml of the caustic solution to a counting vial containing 15 ml of a solution made by mixing 579 ml of toluene, 204 ml of absolute ethanol, 33 ml of concentrated liquid scintillation solution (Packard Instrument Company), 150 ml of Bio-Solve Solubilizer BBS-2 (Beckman Instrument, Inc.), 10 ml of freshly prepared stannous chloride in 0.1 *N* HCl, and 25 ml of ethanolamine.

Determination of ^{14}C in PVC Pellets. PVC pellets (100 ± 4 mg) were weighed to the closest 0.1 mg into a 25 × 100 mm test tube containing 25 ml of reagent grade chloroform. The pellets were then ground for 1 min with a Polytron homogenizer. Aliquots (250 μl) of the chloroform extract were then counted in 20 ml of ethanol liquid scintillation solution (LSS).

Glpc Analysis of Tissues. The tissues were analyzed for dichlorvos, DCA, DCAA, and DMD by glpc (Schultz *et al.*, 1971).

Recovery analyses were made on samples fortified at levels of 0.2 to 0.5 ppm with the following results: dichlorvos, 90 ± 12 (standard deviation); dichlorovinyl methyl phosphate, 93 ± 11; dichloroacetaldehyde, 96 ± 11; and dichloroacetic acid, 102 ± 17.

^{14}C Balance Calculation. The masses of pellets recovered from pigs 4, 43, and 45 (14-day trial) were 5.628, 4.925, and 4.945 g, respectively, which corresponds to 107, 76, and 79% of the mass of the pellets administered. The low recovery of pellets from pigs 43 and 45 is probably caused by incomplete recovery of the pellets from the feces. To correct the total ^{14}C balance for the low recovery, the total ^{14}C found in the excreted pellets was divided by the fractional recovery of pellets (0.76 for pig 43 and 0.79 for pig 45) and the quotients were multiplied by a correction factor, *f*, to correct for the change in mass of the pellets on passage through the gastrointestinal tract of the pigs. Two methods which gave essentially the same results were used to estimate the value of *f*. The first method is based on pellet counts or the mass of 100 pel-

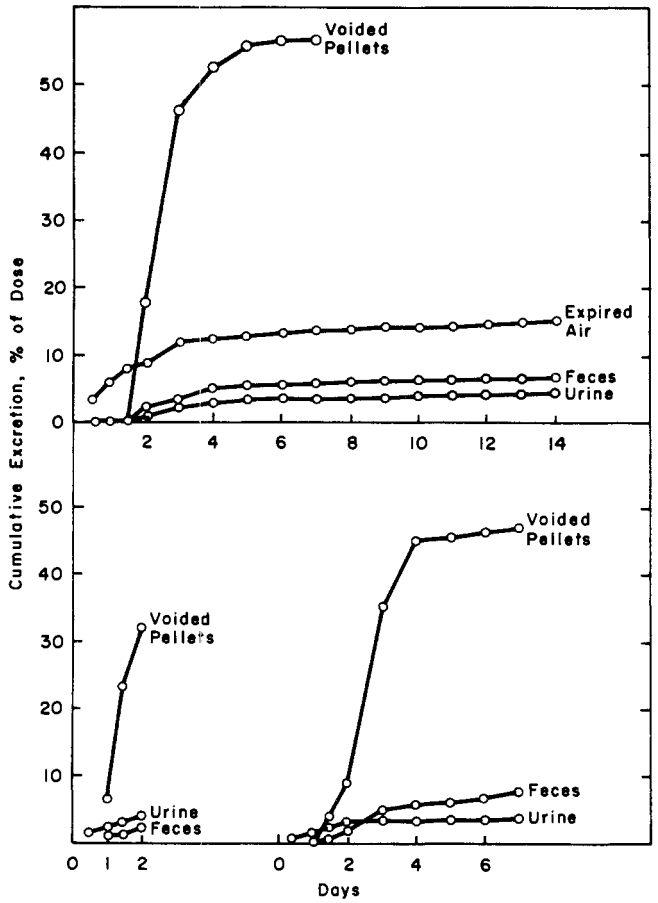

Figure 1. Excretion of radioactivity from pigs; 2, 7, and 14-day experiments (no pellets voided in 14-day experiment after day 7).

lets. The weight of the pellets recovered from the feces was 780.5 mg (Table I). Thus, if 100 pellets or 848 mg of pellets were fed to a pig, assuming that none of the pellets are lost, 100 pellets or 780.5 mg of pellets should be recovered in the feces, or for every gram of pellets fed 0.9204 (780.5/848) g of pellets should be recovered.

An alternative method of calculation is based on the assumption that the change in mass of the pellets on passage through the pigs is caused by the loss of dichlorvos from the pellets. Based on this assumption, the correction factor, f, can be calculated by the formula $f = (1 - CF)/(1 - CR)$, where CF = ^{14}C content of pellets fed to pigs in gram-equivalents of dichlorvos-^{14}C per gram of pellets, and CR = ^{14}C content of pellets recovered from feces in equivalents of dichlorvos-^{14}C per gram of pellet.

Table I summarizes the comparison of the two methods of calculating the correction factor f.

Because the values of f estimated by the two methods, 0.9204 and 0.9014, agree closely, the mean value 0.9110 was used to correct for the low recovery of pellets from feces for pigs 43 and 45.

RESULTS

Carbon-14 Measurements. The proportion of ^{14}C recovered in the expired air (14-day trial), excreta, and carcass is given in Table II. The correction for the low recovery of pellets was only made in the 14-day trial. The mean total recovery of ^{14}C in the 14-day trial was 94.7%. Most of the ^{14}C administered, 61.8%, was found in the pellets recovered from the feces. Therefore, only 38.2% of the dichlorvos-^{14}C escaped from the pellets while they were in the gastrointestinal tract. Of the total amount of radioactivity lost from the pellets, 37% was found in the expired air, 25% was retained in the carcass and tissues, 9% was excreted in the urine, and 15% was found in the feces.

Table III. ^{14}C Residues in Tissues from Pigs Dosed with Dichlorvos-^{14}C

Tissue	^{14}C residues,[a,b] interval, days 2	7	14
Adrenals	8.7 ± 0.18	6.5 ± 0.72	4.6 ± 0.85
Bladder	8.1 ± 0.56	8.0 ± 0.31	5.6 ± 1.26
Blood	6.5 ± 0.95	3.7 ± 0.35	2.8 ± 0.14
Brain	2.5 ± 0.03	1.9 ± 0.11	1.9 ± 0.34
Carcass	5.2 ± 0.91	5.1 ± 0.38	4.2 ± 0.27
Duodenum	12.3 ± 0.46	5.6 ± 0.44	3.1 ± 0.74
Femur	11.7 ± 2.67	7.4 ± 0.70	5.4 ± 0.63
Gastrocnemius muscle	6.3 ± 2.10	4.6 ± 0.44	4.8 ± 0.77
Kidney	12.2 ± 0.91	7.6 ± 0.60	4.0 ± 0.69
Liver	32.9 ± 4.27	30.9 ± 1.89	9.7 ± 1.33
Lungs	8.6 ± 0.56	5.3 ± 2.61	3.8 ± 0.54
Mesenteric fat	2.6 ± 0.60	4.2 ± 0.24	2.5 ± 0.20
Pancreas	9.6 ± 0.86	5.7 ± 0.03	3.5 ± 0.47
Quadriceps muscle	4.7 ± 0.69	4.8 ± 0.53	4.3 ± 0.60
Salivary gland	10.0 ± 0.89	5.6 ± 0.13	3.7 ± 0.51
Spiral colon	8.1 ± 0.85	3.8 ± 0.15	3.5 ± 0.97
Spleen	11.5 ± 0.53	7.0 ± 0.19	4.3 ± 0.56
Stomach	7.1 ± 0.97	4.7 ± 0.28	3.0 ± 0.72
Subcutaneous fat	1.6 ± 0.14	4.0 ± 0.31	2.2 ± 0.42
Thyroid	5.0 ± 0.71	5.1 ± 0.49	5.5 ± 0.62

[a] Mean from three pigs ± S.E.M. [b] Although no dichlorvos was found in the tissues, the ^{14}C residues were calculated for simplicity as ppm equivalents of dichlorvos-^{14}C.

The mean cumulative proportions of the dose excreted are plotted in Figure 1. These results are not corrected for the low recovery of pellets isolated from the feces. The greatest rate of excretion of the pellets occurred between days 2 and 4.

The concentration of ^{14}C in the tissues is summarized in Table III. Although no dichlorvos was found in the tissues, the ^{14}C residues were calculated for simplicity as ppm equivalents of dichlorvos-^{14}C. All of the tissues contained ^{14}C. The lowest levels, 1.9 to 2.5 ppm equivalents, were found in the brain. The highest levels, 9.7 to 32.9 ppm equivalents, were found in the liver.

DICHLORVOS AND METABOLITE RESIDUES BY GLPC

The minimum detectable concentrations of dichlorvos and its suspected metabolites in ppm are as follows: dichlorvos, 0.03; DMD, 0.07; DCA, 0.05; and DCAA, 0.11. None of the tissue samples were found to contain residues of any of these compounds.

DISCUSSION AND CONCLUSIONS

A large proportion (37%) of the no. 1 carbon atom in the vinyl group of the dichlorvos-^{14}C which diffused from the pellets was oxidized to carbon dioxide and eliminated in the expired air.

No residues of dichlorvos, demethyl dichlorvos, dichloroacetic acid, and dichloroacetaldehyde were found in the tissues.

The concentration of ^{14}C in all 20 tissues analyzed was 60 to 1000 times higher than the detection limit of dichlorvos. Information presented by Page *et al.* (1971), Hutson *et al.* (1971), and Loeffler *et al.* (1971) showed that the liver and muscle of pregnant sows and rats treated with dichlorvos-^{14}C contained such labeled compounds as glycine, serine, creatine, glucose, glycogen, fatty acids, cholesterol, choline, lecithin, and ribonucleic acids. The observation that the ^{14}C content of most

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.

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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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