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A Comparative Study of the Persistence, Movement, and Metabolism of Six Carbon-14 Insecticides in Soils and Plants

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A comparative study was conducted to investigate the fate of six insecticides in two soil types and oat plants grown in these soils. All systems were incubated under identical environmental conditions. The insecticides used, in order of increasing water solubility were [^{14}C]DDT, [^{14}C]lindane, [^{14}C]fonofos, [^{14}C]parathion, [^{14}C]phorate, and [^{14}C]carbofuran. Total amounts of ^{14}C residues recovered from insecticide-treated loam soils plus oats grown in these soils were similar with DDT and carbofuran. They were also higher than those observed with the other insecticides. While most of the [^{14}C]DDT residues remained in the soils, most of the [^{14}C]carbofuran residues were recovered from oat leaves in the form of carbofuran and 3-hydroxycarbofuran. ^{14}C residues of all insecticides were more persistent in loam than in sandy soil and sand-grown oats took up more ^{14}C insecticide residues than loam-grown oats. The more water-soluble insecticides [^{14}C]phorate and [^{14}C]carbofuran were more mobile and were metabolized to a greater extent than insecticides of lower water solubilities. Unextractable (bound) ^{14}C residues in loam soil ranged from 2.8 to 29.1% of the applied doses of [^{14}C]DDT and [^{14}C]parathion, respectively. Bound ^{14}C residues were lower in the sandy soil than in the loam soil; however, plant-bound ^{14}C residues were higher in oats grown in the sandy soil than in loam-grown oats. Insecticide metabolites recovered from soils and plants were identified and quantitated whenever possible. The oxygen analogue metabolites of the organophosphorus insecticides were most abundant in the sandy soil and in oats grown therein. Data illustrate the importance of chemical structure, water solubility, and soil type in predicting the comparative environmental behavior of pesticides.

Insecticides are an indispensable part of modern agriculture. On the basis of environmental considerations, restrictions in their usage have become necessary, yet their production and use is expected to increase (Berg, 1975). Responsible use of these toxicants requires knowledge of their persistence, transport, and transformation in the environment.

There are currently over 60 000 synthetic chemicals in common use which are potential environmental pollutants (Maugh, 1978). Since it would be nearly impossible to gather all the pertinent data on the environmental fate of each of these chemicals, it has been proposed to utilize the physical and chemical properties of these materials in order to predict their environmental behavior (Howard et al., 1978). Since many environmental studies have been conducted with pesticides, this technology is a natural starting point for investigating other potential pollutants. In order to determine which properties of a chemical can be used to predict its environmental behavior, it is first necessary to use existing data for the design and testing of predictive models. Most studies of pesticide behavior have been conducted using only one or two compounds at a given time. Thus, numerous investigations have been performed with various types of soil or plants and the experiments have been conducted under a variety of environmental conditions. While these studies have provided a large volume of data on the environmental fate and behavior of individual pesticides, the *comparative* behavior of different compounds is difficult to assess since the experimental conditions under which these data were obtained differed considerably.

Table I. Water Solubility and Vapor Pressure of the Insecticides Used

insecticide	water solubility, ppm	vapor pressure, mmHg
DDT	0.001[a]	1.9×10^{-7}/20° [b]
lindane	10[b]	9.4×10^{-6}/20° [b]
fonofos	15.7[c]	2.0×10^{-4}/25° [d]
parathion	12.4[c]	3.8×10^{-5}/20° [b]
phorate	17.9[c]	8.4×10^{-4}/20° [b]
carbofuran	320[c]	8.3×10^{-6}/25° [e]

[a] Bowman et al. (1960). [b] Spencer (1973). [c] Bowman and Sans (1979). [d] Menn (1969). [e] Caro et al. (1976).

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Therefore, the present study was conducted to compare the persistence, translocation, and metabolism of six different insecticides in and from two different soil types under identical environmental conditions. The six insecticides included organochlorine, organophosphorus, and carbamate compounds, with water solubilities ranging from 0.001 to 320 ppm (Table I). This range was felt to be important because water solubility has been shown to affect insecticide mobility in soils (Lichtenstein et al., 1967) and uptake and movement in plants (Finlayson and McCarthy, 1965). The insecticides utilized were labeled with carbon-14 so that both extractable and unextractable (bound) ^{14}C residues could be quantitated.

MATERIALS AND METHODS

Chemicals. [U-*phenyl*-^{14}C]DDT (sp act., 0.71 mCi/mM) and [U-^{14}C]lindane (sp act., 0.58 mCi/mM) were purchased from Amersham Corp. [*methylene*-^{14}C]Phorate (sp act., 1.30 mCi/mM) was prepared by Amersham Corp. and obtained through the courtesy of American Cyanamid Co. [2,6-*phenyl*-^{14}C]Parathion (sp act., 0.58 mCi/mM) was purchased from ICN Corp. [U-*phenyl*-^{14}C]Fonofos (sp act., 0.64 mCi/mM) and [U-*phenyl*-^{14}C]carbofuran (sp act., 0.44 mCi/mM) were obtained through the courtesy of Stauffer Chemical Co. and FMC Corp., respectively. The indicated specific activities were obtained by dilution with nonradioactive insecticide. These insecticides were determined to be at least 97% pure by thin-layer chromatography and autoradiography. Nonradioactive insecticides and their potential metabolites were obtained through the courtesy of the following companies: DDT (Ciba Geigy Corp.), lindane (Hooker Chemical Co.), phorate (American Cyanamid Co.), parathion (Farbenfabriken-Bayer), fonofos (Stauffer Chemical Co.), and carbofuran (FMC Corp.). Water solubilities and vapor pressures of these insecticides are shown in Table I.

Soils. A Plano silt loam soil (4.7% organic matter, 5% sand, 71% silt, 24% clay with a pH of 6.0), free of insecticide residues, was collected at the University of Wisconsin Experimental Farm near Madison and stored at 22 ± 2 °C in a moist condition prior to use. A Plainfield sand (0.6% organic matter, 93.4% sand, 3.6% silt, 3.0% clay, and a pH of 5.6), free of insecticide residues, was collected in Adams County, WI, and stored as described above.

Application of Insecticides to Soils. The loam soil was treated with 4 ppm of the insecticides, while the sandy soil was treated at 2 ppm. Since loam has a greater adsorptive capacity for pesticides than sandy soil (Edwards et al., 1957), it was treated at the higher concentration so that the amount of insecticide residues taken up by the plants would be sufficient for accurate analyses. For this purpose six 2400-g portions of loam soil were each treated with 80 mL of acetone containing 9.6 mg (19–48 μCi) of one of the six ^{14}C insecticides. Six 3000-g portions of sandy soil were each treated with 50 mL of acetone containing 6.0 mg (12–30 μCi) of one of the six ^{14}C insecticides. Treated loam or sandy soil were each thoroughly mixed, while the acetone was removed with a gentle stream of air (Lichtenstein and Schulz, 1959). Immediately following treatment, two 100-g aliquots of each treated soil were removed for extraction and analyses to determine the actual treatment levels. All data are reported as percentages of the initially determined doses.

After insecticide application, the remaining 2200 g of loam soil treated with one of the six insecticides were divided equally among four cartons (9 × 8.5 cm diameter), lined with polyethylene bags. Similarly, four cartons were prepared with treated loam soil for each of the five remaining insecticides, thus resulting in a total of 24 cartons. With the sandy soil, the larger weight of 700 g/carton was used in order to obtain the same volume (depth) of the two soil types in the cartons. As described above, four replicate containers with sandy soil were also prepared for each of the six insecticides, thus resulting in 24 additional cartons.

Planting and Growing Oats. Oat seeds (*Avena sativa*) were germinated for 72 h between moist paper towels. One day after soil treatment, 25 germinated seeds with ca. 1-cm long roots were planted to a depth of 1 cm in each of the 48 cartons containing insecticide-treated loam or sandy soil. Each carton was then watered with 150 mL of modified Hoagland's nutrient solution (Hoagland and Arnon, 1950) and weighed. This initial weight was maintained by daily addition of water or by adding nutrient solution on every third day. To maintain high humidity around the emerging oat plants, polyethylene bags were placed over each carton for 3 days. After that, plants which had not emerged from the soil were replaced to insure an equal number of plants (25) in each carton. The oat plants were grown at the University of Wisconsin Biotron at a temperature of 28 °C, relative humidity of ca. 70% and 3000 ft-candles of mixed incandescent and fluorescent light on a 15-h light, 9-h dark cycle. The carton positions were rotated daily within the growth chamber to minimize any minor fluctuations in growing conditions during the 13-day growing period.

Harvesting Plants and Soils. *Tops* of oat plants were cut 1 cm above the soil surface, rinsed with cold tap water, cut into 1-cm pieces, and immersed in appropriate extraction solvents.

Roots were separated from soils after the soils had dried for 2 days. For this purpose the soils were sieved through a 2-mm screen and any root pieces not retained by the screen were removed from the soil with a forceps. The roots were then washed with a forceful stream of cold tap water, cut into 1-cm pieces, and immersed in extraction solvents.

Sand or loam soils were thoroughly mixed after sieving and a 100-g aliquot was removed for moisture determination while a second 100-g aliquot was immersed in 200 mL of extraction solvents.

Extraction. *Soils.* All soils except those treated with [^{14}C]carbofuran were extracted by blending with a mixture of acetone–methanol–benzene (1:1:1), followed by solvent evaporation and partitioning of the extracted residues between benzene and water as previously described (Lichtenstein et al., 1973). Soils containing [^{14}C]carbofuran residues were refluxed for 1 h with 0.25 N HCl. This reflux mixture was quantitatively filtered under vacuum and the filtrate was partitioned three times with dichloromethane (Markus and Puma, 1973), resulting finally in dichloromethane and (acid) water extraction phases plus the extracted soil.

Plants. Oats from all ^{14}C-insecticide-treated soils were extracted with acetone–methanol–benzene as described above. However, those from [^{14}C]carbofuran-treated soils were further extracted by combining the extracted plant pulp and the water extraction phases, adjusting them to 0.25 N with concentrated HCl, and then refluxing and partitioning them with dichloromethane as described above. These procedures resulted in benzene, dichloromethane, and (acid) water extraction phases and the acid-hydrolyzed plant pulp. Cleanup of organic extraction phases of oat tops was necessary prior to analysis by thin-layer chromatography. For this purpose benzene extraction phases containing [^{14}C]phorate, [^{14}C]fonofos, [^{14}C]parathion, and [^{14}C]carbofuran or dichloromethane phases containing [^{14}C]carbofuran and its degradation

Table II. Thin-Layer Chromatography of Insecticides and Metabolites on E. Merck Silica Gel "60" Plates with UV Indicator

p,p'-[^{14}C]DDT and Metabolites; developing system: 2% diethyl ether in pentane; visualization: $AgNO_3$ reagent[a]							
	p,p'-DDE	o,p'-DDT	p,p'-DDT	DBP	TDE	dicofol	DDA
R_f	0.81	0.70	0.62	0.42	0.24	0.10	0.00
[^{14}C]Lindane and Metabolites; developing system: 1% acetone in hexane[b]; visualization: $AgNO_3$ reagent[a]							
	1,2,4,5-TCB 1,3,5-TCB	1,2,3,4-TCB 1,2,4-TCB	1,2,3-TCB	lindane	2,4,5-TCP		
R_f	0.92	0.82	0.74	0.22	0.10		
[^{14}C]Fonofos and Metabolites; developing system: 5% diethyl ether in pentane then chloroform-ethyl acetate-hexane (2:2:1); visualization: with UV (254 nm)							
	thiphenol and diphenyl disulfide	fonofos	methyl phenyl sulfone ($MPSO_2$)	oxon	2-OH-$MPSO_2$ 3-OH-$MPSO_2$	4-OH-$MPSO_2$	
R_f	0.78	0.70	0.39	0.32	0.25	0.17	
[^{14}C]Parathion and Metabolites; developing system: benzene-chloroform-methanol (6:3:1); visualization: 0.5% $PdCl_2$ in 0.25 N HCl then 5 N NaOH[c,d]							
	parathion	oxon	aminoparathion	p-nitrophenol	aminoparaoxon	aminophenol	
R_f	0.91	0.78	0.70	0.44	0.34	0.22	
[^{14}C]Phorate and Metabolites; developing system: nitromethane-acetonitrile-toluene (15:40:45)[d]; visualization: 5% $PdCl_2$ in 0.25 N HCl then 5 N NaOH[d]							
	phorate	sulfone	oxon	oxon sulfone	sulfoxide	oxon sulfoxide	
R_f	0.92	0.85	0.68	0.56	0.48	0.10	
[^{14}C]Carbofuran and Metabolites; developing system: diethyl ether-benzene (1:3); visualization: 1.5 N KOH in methanol then 2% p-nitrobenzenediazonium fluoroborate in acetone-methanol (1:1)[e]							
	7-phenol	3-keto-7-phenol	carbofuran	3-ketocarbofuran	3-hydroxy-7-phenol	3-hydroxycarbofuran	
R_f	0.74	0.55	0.44	0.36	0.24	0.12	

[a] Mitchell (1957). [b] Talekar and Lichtenstein (1971). [c] Lichtenstein and Schulz (1964). [d] Blinn (1963). [e] Metcalf et al. (1968).

products were purified using charcoal as described by Lichtenstein et al. (1973). Benzene extracts containing residues of [^{14}C]DDT or [^{14}C]lindane were purified using Florisil as described by Mills (1961).

Analyses. *Liquid scintillation counting* (LSC) was used to determine the amounts of radiocarbon in each organic solvent or water extraction phase (Fuhremann and Lichtenstein, 1978a). LSC vials containing 1.0 mL of (acid) water extraction phases were neutralized with 25 μL of 5 N NaOH, while dichloromethane extraction phases were evaporated to dryness before addition of scintillator to prevent excessive quenching. Extracted soils and plant pulp samples were pelleted and combusted in an automatic oxidizer to determine $^{14}CO_2$ derived from bound (unextractable) ^{14}C residues as described by Fuhremann and Lichtenstein (1978a). Unpurified aliquots (0.5 mL) of organic solvent extraction phases of oat tops were pipetted onto 7-cm disks of filter paper, and the solvents were allowed to evaporate at room temperature. These filter paper disks were then pelleted and combusted. To insure that no losses of ^{14}C residues occurred during the cleanup procedure, purified organic solvent extraction phases of oat tops used for TLC were also analyzed by LSC.

Thin-Layer Chromatography (TLC). The insecticides and their metabolites in the organic solvent extraction phases were characterized and quantitated by TLC as shown in Table II. To detect the presence of radioactive compounds on the unsprayed TLC plates, autoradiography with Kodak "No-Screen" X-ray film was performed. Areas of silica gel containing radiocarbon were removed from the TLC plates, suspended in methanol by sonication, and then counted by LSC (Fuhremann and Lichtenstein, 1978b). The distribution of radiocarbon in each thin-layer plate was used to calculate the amounts of each insecticide and its metabolites in the organic solvent extraction phases. These data were finally expressed in percent of the amounts of ^{14}C insecticide applied to the soils.

Gas–liquid chromatography (GLC) was used to confirm the identity of organic-soluble compounds isolated by TLC. A chromatograph equipped with a detector specific for nitrogen and phosphorus was used as previously described (Fuhremann and Lichtenstein, 1978b). A 122 cm × 2 mm i.d. Pyrex column packed with 10% OV-3 silicone grease on 80/100 mesh Chromosorb W-HP operated at 180 °C was used for the separation of parathion, paraoxon, and aminoparathion; fonofos and its oxygen analogue; or carbofuran, 3-hydroxycarbofuran, and 3-ketocarbofuran. A similar column packed with 3% DEGS (1BP) on 80/100 mesh Chromosorb W-HP was operated at 160 °C for separation of phorate and at 195 °C for the separation of phorate sulfoxide and phorate sulfone.

A gas chromatograph equipped with a ^{3}H electron-capture detector was used for the separation of DDT or lindane and their potential metabolites as previously reported (Lichtenstein and Schulz, 1970).

In general, three of the four plant or soil replicates were extracted and analyzed by LSC. The fourth replicate was held in reserve in case unforeseen difficulties occurred during the processing or analytical procedures described above. Aliquots of organic solvent extraction phases from one replicate of each treatment were used for qualitative and quantitative determination of metabolites as described.

RESULTS AND DISCUSSION

Persistence of Insecticides in Soils and Their Uptake by Oat Plants. Results shown in Figure 1 represent

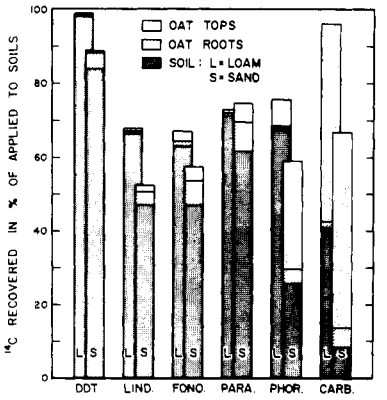

Figure 1. Persistence of ^{14}C insecticides in loam or sandy soil and the translocation of ^{14}C residues into oats grown in these soils. Data include extractable and unextractable (bound) ^{14}C residues. DDT = *p,p'*-[^{14}C]DDT; LIND. = [^{14}C]lindane; FONO. = [^{14}C]-fonofos; PARA. = [^{14}C]parathion; PHOR. = [^{14}C]phorate; CARB. = [^{14}C]carbofuran.

the total radiocarbon (extractable and bound) recovered from ^{14}C-insecticide-treated soils and oat plants which had grown in these soils for 13 days. Largest and similar amounts of total ^{14}C residues were recovered from loam soils plus plants with the organochlorine insecticide, [^{14}C]DDT, or the carbamate insecticide, [^{14}C]carbofuran, resulting in recoveries of 99 and 96%, respectively, of the amounts applied to the soil. For all insecticides, the total amounts of ^{14}C residues remaining in loam soil were higher than in sandy soil. However, oats grown in sandy soil contained more radiocarbon than those grown in loam soil. Because sandy soils have a lower adsorptive capacity for insecticides than loam soils (Edwards et al., 1957), it is likely that the ^{14}C residues in the sand were more available for volatilization and plant uptake. While only 0.7–4.3% of the applied [^{14}C]DDT, [^{14}C]lindane, [^{14}C]fonofos, and [^{14}C]parathion residues were taken up by loam-grown oats, 4.6–12.6% were taken up by sand-grown oats. The greatest uptake of radiocarbon by plants occurred with the more water-soluble insecticides. Thus, the two most water-soluble compounds, phorate and carbofuran, had 33 and 69% of the applied ^{14}C residues, respectively, removed from the sandy soil by oat plants. Therefore, the total amounts of these ^{14}C-insecticide residues in the plants were higher than the amounts remaining in the sandy soil in which these plants had grown. In [^{14}C]DDT- and [^{14}C]-lindane-treated systems most of the total radiolabeled plant residues were associated with the roots. However, with the more water-soluble insecticides [^{14}C]phorate and [^{14}C]carbofuran, the largest portion of the total ^{14}C plant residues were in the oat tops. It is likely that these more water-soluble compounds moved into the aerial parts of the plants with water via the transpiration stream.

Data presented in Figure 1, therefore, indicate that insecticide residues are more persistent in soils of higher organic matter (loam) than in sandy soils. Moreover, compounds with greater water solubility are more mobile and are translocated to a larger extent into the aerial portions of plants as shown in particular with [^{14}C]-carbofuran.

Metabolism of ^{14}C Insecticides in Soils and Oat Plants. Results obtained after analyses of the organic solvent and water extraction phases of soils and plant tissues provided information on insecticide metabolism to more polar, less toxic or detoxified water-soluble metabolites (Table III). Combustion of the extracted soils or plant pulp residues to $^{14}CO_2$ indicated the amounts of bound (unextracted) residues. In loam soils, the total amounts of organic-soluble residues, a good indicator of the persistence of a particular compound, ranged from 95% of the loam-applied dosage for [^{14}C]DDT to only 29% with [^{14}C]carbofuran. Only small amounts of water-soluble degradation products of the six ^{14}C insecticides were present in loam soils, ranging from none ([^{14}C]DDT) to 2.7% with [^{14}C]phorate. The amounts of bound ^{14}C residues remaining in the loam soil after extraction were small with the organochlorine insecticides ([^{14}C]DDT and [^{14}C]lindane) but comprised a rather large proportion of the recovered soil residues of [^{14}C]fonofos (25% = 15.6% of applied), [^{14}C]parathion (41% = 29% of applied), [^{14}C]phorate (19% = 13% of applied), and [^{14}C]carbofuran (25% = 10% of applied). These bound soil residues, whose nature in most cases is unknown, would increase the insecticide residues determined by analyses of extraction phases.

In oat roots residues of the six ^{14}C insecticides constituted only a minor proportion (0.4–1.7%) of the soil-applied doses. Most of the radiocarbon detected in oat roots from [^{14}C]fonofos-, [^{14}C]parathion-, and [^{14}C]carbofuran-treated soils were bound to plant components and could therefore not be extracted or identified.

^{14}C residues which had been translocated into the tops of oat plants from [^{14}C]DDT-, [^{14}C]lindane-, and [^{14}C]-parathion-treated loam soils amounted to less than 1% of the soil-applied doses (Table III). However, larger amounts of organic-soluble, water-soluble, and bound ^{14}C were present in oat tops grown in [^{14}C]fonofos- (2.7% of applied), [^{14}C]phorate- (7.4%), and [^{14}C]carbofuran- (53.4%) treated loam soils. While most of the [^{14}C]fonofos and [^{14}C]phorate residues in oat tops were in the form of water-soluble degradation products, most of the [^{14}C]-carbofuran residues were organic-soluble compounds. Bound residues in oat tops amounted to less than 1% of the applied dose for all insecticides except carbofuran where 3% of the applied dose was still bound after acid hydrolysis and extraction as described.

Comparable results obtained with sandy soils are presented in the right-hand portion of Table III. They indicate that, similar to loam soil, most of the ^{14}C residues in the sandy soil were also organic soluble. However, they were not as large as in the loam soil, with the exception of parathion. Total [^{14}C]parathion residues were, however, higher in the loam soil because of the large proportion of soil-bound residues.

For all insecticides, soil-bound residues were lower in sandy soil than in loam soil as was previously shown by Katan et al. (1976). With [^{14}C]parathion, for example, 29% of the soil-applied radiocarbon was recovered as bound residues in loam as compared to only 6% recovered as bound residues in the sandy soil.

Roots of oats grown in the sandy soil contained larger amounts of ^{14}C insecticide residues than those from treated loam soils (Table III). In oat tops, most of the [^{14}C]fonofos- and [^{14}C]parathion-derived residues were water-soluble degradation products, while most of radiocarbon derived from [^{14}C]phorate and [^{14}C]carbofuran were in the form of organic-soluble compounds. Bound residues of [^{14}C]-parathion in oat tops comprised 25% of all the radiocarbon recovered from the tops, but amounted to only 11% with [^{14}C]phorate and [^{14}C]fonofos and to 4% with [^{14}C]-carbofuran.

^{14}C-Insecticide Derivatives Produced in Soils and Plants. ^{14}C metabolites recovered from the organic extraction phases of soils and of oat plants were also calculated in percent of the soil-applied radiocarbon (TLC, Table III). In loam and sandy soils, *p,p'*-[^{14}C]*DDT* ac-

Table III. Uptake, Translocation, Distribution, and Metabolism of Six ^{14}C Insecticides in Oat Plants Grown in Insecticide-Treated Soils

extraction phases[a]		radicarbon recovered in percent of ^{14}C insecticides applied to soils from loam soil[b] soil	loam soil[b] oat roots	loam soil[b] oat tops	sandy soil[c] soil	sandy soil[c] oat roots	sandy soil[c] oat tops
			p,p'-DDT				
organic	LSC	95.4 ± 0.3	0.2 ± 0.0	0.1 ± 0.1	83.3 ± 3.0	4.1 ± 0.7	0.1 ± 0.0
	TLC						
	DDT	90.4[d]	0.2[d]	NA[e]	80.4[d]	4.2[d]	NA
	DDE	3.4[d]	0.0	NA	2.2[d]	0.2[d]	NA
	other[f]	1.7	0.0	NA	1.4	0.2	NA
	total	95.5	0.2	0.0	84.0	4.6	0.0
water	LSC	0.0 ± 0.0	0.2 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.0
bound[g]	LSC	2.8 ± 0.4	0.0 ± 0.0	0.1 ± 0.0	0.7 ± 0.3	0.1 ± 0.0	0.1 ± 0.0
			Lindane				
organic	LSC	61.8 ± 1.5	0.4 ± 0.2	0.3 ± 0.0	45.4 ± 3.6	2.2 ± 0.9	1.2 ± 0.1
	TLC						
	lindane	53.5[d]	0.5[d]	0.3[d]	33.8[d]	2.5[d]	1.2[d]
	unknown[h]	6.8	0.0	0.0	5.5	0.4	0.0
	other	1.7	0.0	0.0	2.0	0.3	0.0
	total	62.0	0.5	0.3	41.3	3.2	1.2
water	LSC	0.3 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.1	0.6 ± 0.1
bound	LSC	4.6 ± 1.2	0.1 ± 0.0	0.1 ± 0.0	1.3 ± 0.2	1.0 ± 0.2	0.2 ± 0.0
			Fonofos				
organic	LSC	46.8 ± 3.9	0.1 ± 0.0	0.4 ± 0.0	35.4 ± 4.1	0.7 ± 0.4	0.4 ± 0.1
	TLC						
	fonofos	42.8[d]	0.1[d]	0.0	29.4[d]	0.8[d]	0.1[d]
	-oxon	0.5[d]	0.0	0.0	1.1[d]	0.1[d]	0.2[d]
	other	5.4	0.0	0.4	1.7	0.2	0.1
	total	48.7	0.1	0.4	32.2	1.1	0.4
water	LSC	0.7 ± 0.1	0.3 ± 0.0	2.1 ± 0.1	2.5 ± 0.1	1.2 ± 0.0	2.8 ± 0.1
bound	LSC	15.6 ± 0.3	1.2 ± 0.1	0.2 ± 0.0	9.3 ± 0.8	4.7 ± 0.8	0.4 ± 0.0
			Parathion				
organic	LSC	41.1 ± 2.0	0.1 ± 0.0	0.1 ± 0.0	55.3 ± 4.3	1.5 ± 0.0	1.4 ± 0.2
	TLC						
	parathion	40.0[d]	0.1[d]	0.1[d]	50.8[d]	1.1[d]	0.8[d]
	-oxon	0.5[d]	0.0	0.0	2.4[d]	0.1[d]	0.6[d]
	other	1.6	0.0	0.0	0.4	0.4	0.1
	total	42.1	0.1	0.1	53.6	1.6	1.5
water	LSC	1.1 ± 0.2	0.1 ± 0.0	0.3 ± 0.0	0.5 ± 0.0	1.0 ± 0.1	2.2 ± 0.2
bound	LSC	29.1 ± 1.0	0.7 ± 0.1	0.3 ± 0.0	6.0 ± 1.7	5.3 ± 0.7	1.2 ± 0.1
			Phorate				
organic	LSC	52.1 ± 3.9	0.2 ± 0.1	2.1 ± 0.6	20.4 ± 1.2	0.4 ± 0.0	15.0 ± 1.0
	TLC						
	phorate	2.4	0.0	0.0	3.7	0.0	0.0
	-sulfoxide	24.0[d]	0.1[d]	0.4[d]	12.1[d]	0.2[d]	7.3[d]
	-sulfone	26.1[d]	0.1[d]	0.5[d]	3.5[d]	0.2[d]	3.6[d]
	other	1.2	0.0	0.9	1.7	0.0	4.4
	total	53.7	0.2	1.8	21.0	0.4	15.3
water	LSC	2.7 ± 0.1	0.0 ± 0.0	4.5 ± 0.7	1.0 ± 0.1	0.4 ± 0.1	11.4 ± 0.6
bound	LSC	13.1 ± 0.8	0.3 ± 0.1	0.8 ± 0.1	4.5 ± 0.1	2.7 ± 0.1	3.2 ± 0.4
			Carbofuran				
organic	LSC	29.4 ± 0.6	0.3 ± 0.1	39.0 ± 3.4	6.1 ± 3.0	0.7 ± 0.3	47.1 ± 2.4
	TLC						
	carbofuran	23.5[d]	0.1[d]	5.1[d]	5.7[d]	0.3[d]	7.9[d]
	3-keto-	2.5	0.0	1.6	0.8	0.1	2.7
	3-hydroxy-	1.0	0.1[d]	24.9[d]	1.6[d]	0.3[d]	27.1[d]
	other	1.8	0.0	8.7	0.9	0.3	8.4
	total	28.8	0.2	40.3	9.0	1.0	46.1
water	LSC	1.2 ± 0.1	0.4 ± 0.0	11.4 ± 1.7	1.4 ± 0.2	1.8 ± 0.3	14.0 ± 1.6
	LSC	10.4 ± 1.0	1.0 ± 0.2	3.0 ± 0.2	0.8 ± 0.1	2.8 ± 0.1	2.5 ± 0.1

[a] Analyses of the extraction phases were conducted by LSC and TLC. For metabolism studies, compounds were separated by TLC, eluted, and quantitated by LSC. [b] Applied 4 ppm (2.2–5.5 μCi) of ^{14}C insecticides to 550 g of silt loam soil. Results are means ±SD for three replicates. [c] Applied 2 ppm (2.8–7.0 μCi) of ^{14}C insecticides to 700 g of Plainfield sand. Results are means ±SD for three replicates. [d] Identity confirmed by GLC. [e] Not analyzed. [f] Compounds included in "other" are described in text. [g] Unextracted radiocarbon remaining in soils or plant pulp determined by combustion to $^{14}CO_2$. [h] Unknown; suspected to be γ-PCCH based on report by Yule et al. (1967). No authentic reference compound available for comparison.

counted for 94% (90% of applied) or 95% (80% of applied), respectively, of the total organic-soluble [^{14}C]DDT residues in these soils. The major metabolite detected was *p,p'*-DDE, but *o,p'*-DDT, TDE, and dicofol were also recovered as organic-soluble soil residues. In loam-grown oat roots only *p,p'*-[^{14}C]DDT could be detected, while oat

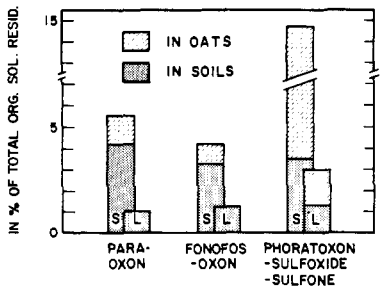

Figure 2. Recoveries of insecticide oxygen analogues from loam (L), sandy soil (S), and oat plants (roots plus tops) after growing for 13 days in [^{14}C]parathion-, [^{14}C]fonofos-, or [^{14}C]phorate-treated soils.

tops did not contain enough radiocarbon for analyses by TLC.

[^{14}C]*Lindane* was the major constituent of the organic-soluble soil residues. A major metabolite representing 11% of the organic-soluble loam soil ^{14}C residues was visualized at R_f 0.33 on the TLC plate but did not cochromatograph with any of the available potential lindane metabolites (Table II). Yule et al. reported in 1967 that the major soil metabolite of lindane was γ-pentachlorocyclohexene (γ-PCCH). By using a TLC developing system of 5% acetone in hexane they obtained R_f values of 0.31 (lindane) and 0.55 (γ-PCCH). We obtained R_f values of 0.22 (lindane) and 0.33 (unknown) using 1% acetone in hexane. On the basis of these results we suspect that the unknown compound is γ-PCCH. Another metabolite, amounting in loam soil to 2.7% of the recovered organic-soluble soil residues, was identified as 2,4,5-trichlorophenol (TCP) by TLC and GLC. No compounds other than lindane were detected in loam-grown oat roots or tops.

[^{14}C]*Carbofuran* was the major constituent of the organic-soluble loam soil residues. Major metabolites were 3-ketocarbofuran and 3-hydroxycarbofuran, amounting to 8.7 and 3.5%, respectively, of the recovered organic-soluble soil residues. Other metabolites totaling 6.2% of the organic-soluble soil residues included carbofuranphenol, 3-ketocarbofuranphenol, and 3-hydroxycarbofuranphenol, each of which amounted to less than 1% of the applied dose of carbofuran. Organic-soluble oat root residues were small (0.2% of applied) and consisted of equal amounts of carbofuran and 3-hydroxycarbofuran.

The majority of organic-soluble [^{14}C]carbofuran residues recovered were located in the oat tops, the major constituent being 3-hydroxycarbofuran. Partitioning of these residues before and after acid hydrolysis indicated that about two-thirds of the 3-hydroxycarbofuran residues were present as conjugates in the oat tops. Although the [^{14}C]carbofuran metabolites are not as toxic to rats as carbofuran (LD_{50} = 11 mg/kg), they still retain appreciable toxicity; thus Schoenig (1967) determined acute oral rat LD_{50}'s of 17.9 and 69.0 mg/kg for 3-hydroxy- and 3-ketocarbofuran, respectively. Additional organic-soluble radiocarbon recovered from oat tops grown in loam soil was distributed between several metabolites, including carbofuranphenol (0.4% of applied), 3-ketocarbofuranphenol (3.6%), 3-hydroxycarbofuranphenol (1.4%), and other unidentified compounds (3.2%).

As shown in Table III, most of the radiocarbon recovered by TLC from [^{14}C]*parathion*-, [^{14}C]*fonofos*-, and [^{14}C]-*phorate*-treated loam soils was still associated with the soils, while only 0.1–1.8% of the applied ^{14}C was recovered from oat tops. Metabolites of parathion were only found in soils and consisted of paraoxon, aminoparathion, *p*-nitrophenol, and *p*-aminophenol. In [^{14}C]fonofos-treated loam soil, the major metabolites recovered were methyl phenyl sulfone ($MPSO_2$) and its 2,3 and 4 hydroxy analogues, amounting to 4% of the loam-applied residues, while unknown compounds accounted for the remaining 1.4%. Oat tops grown in loam soil contained some $MPSO_2$ (0.4% of applied ^{14}C). In [^{14}C]phorate-treated loam, 93% (50% of applied) of all the recovered residues were in the form of phorate sulfoxide and phorate sulfone. The presence of phorate in soil could not be confirmed by GLC since phorate sulfoxide was detected in extracts of TLC isolates which had the same R_f value as phorate. [^{14}C]-Phorate was obviously converted to its sulfoxide on the TLC plate during the 2-week autoradiographic period. None of the other ^{14}C compounds present in the soil exceeded 1% of the applied dose. Oat tops contained phorate sulfoxide, phorate sulfone, and the oxygen analogues of these two compounds.

As shown in Figure 1, total recoveries of radiocarbon from sandy soil and from oats grown therein were, with the exception of [^{14}C]parathion residues, lower than in loam soils. Residue analyses by TLC (Table III), however, indicated similar trends to those observed with loam soils. With [^{14}C]parathion, 51% of the applied dose remained in the sandy soil as parathion, but only 40% in the loam. Also, a higher proportion of paraoxon was found in the sand than in the loam. This was probably related to the fact that the sandy soil is of a more aerobic nature. As shown in Figure 2, more fonofosoxon and also more of the oxygen analogues of phorate were recovered from the sandy soil. Another major difference in results due to different soil types was noticed with oats and in particular with [^{14}C]phorate. Thus the aerial portions (tops) of the sand-grown oat plants contained 15.3% of the applied [^{14}C]phorate in the form of metabolites as opposed to 1.8% in loam-grown oats. The distribution of these ^{14}C compounds in sand-grown oat tops, calculated in percent of recovered by TLC, was phorate sulfoxide (48%), phorate sulfone (24%), phoratoxon sulfoxide (11%), and phoratoxon sulfone (15%). Other ^{14}C compounds in oat tops could not be identified.

In conclusion, we would like to emphasize that the comparative data presented here illustrate the importance of three major factors which determine to some extent the environmental fate of insecticides. These factors are the insecticide itself, its water solubility, and the type of soil to which it is applied. The chemical nature of the insecticide determines its susceptibility to degradation processes, its affinity for soils, its volatility, and its water solubility. Thus compounds with greater water solubility were more mobile, were taken up by plants to a greater extent, and appeared to be more susceptible to degradation than compounds with lower water solubilities. In soils with low organic matter, insecticide residues are more mobile and hence more susceptible to volatilization, plant uptake, and degradation than in more adsorbent soils, such as a loam. It is apparent, however, that no single factor can be used to predict the environmental fate of insecticides and that only knowledge of the interaction of multiple parameters would suffice to predict the environmental behavior of a specific chemical.

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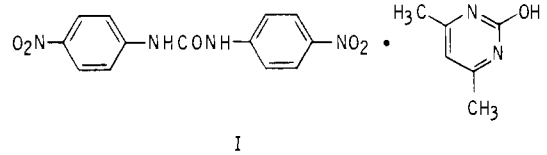
Modified Pulse Polarographic Determination of Nicarbazin in Chicken Tissue at the 0.1-Ppm Level

James S. Wood, Jr.,* and George V. Downing

The method described was devised to extend the assay of nicarbazin residues in chicken tissues by pulse polarography down to the 0.1-ppm level generally required by regulatory agencies. The 4,4′-dinitrocarbanilide portion of the complex is extracted with ethyl acetate. After removal of solvent, kidney and liver samples are cleaned up by a series of hexane washes of acetonitrile and acetonitrile/water solutions containing a small amount of dimethyl sulfoxide (Me_2SO), followed by extraction into methylene chloride. The methylene chloride is removed and pulse polarograms obtained on the residue dissolved in Me_2SO electrolyte after washing with hexane/toluene. For skin–fat and muscle, the acetonitrile/methylene chloride cleanup is unnecessary. The resulting polarograms were essentially clean for tissues from nonmedicated chickens, and recoveries of added drug at the 0.1–0.4-ppm level averaged 73% for liver (range 65–87), 76% for kidney (58–84), 85% for muscle (77–102), and 94% for skin–fat (79–106).

Nicarbazin (I) has been recognized as an effective coc-



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cidiostat for almost 25 years (Cuckler et al., 1956). The active component is an equimolar complex of 4,4′-dinitrocarbanilide (DNC) and 2-hydroxy-4,6-dimethylpyrimidine (HDP). Chickens dosed with nicarbazin have been shown to excrete the DNC portion of the complex more slowly than the HDP portion (Porter and Gilfillan, 1955) and consequently all residue analyses for nicarbazin are based on methods for the DNC moiety. The official FDA method for nicarbazin residues in chicken currently involves pulse polarography of DNC (Michielli and Downing, 1974).

The polarographic method involves reduction of the aromatic nitro groups. Dimethyl sulfoxide (Me_2SO) is used as the solvent largely due to solubility considerations, as nicarbazin and DNC are both extremely insoluble entities in most common solvents. In Me_2SO DNC yields two reduction peaks (potentials of ~−1.0 and −1.5 V vs. aqueous SCE). As protons are added to the solvent, the two waves merge until finally a single peak remains at about −0.98 V. The pulse polarographic system was designed to use an acid strength that generates a single wave.

The original polarographic method was estimated to have a lower limit of reliable measurement of about 2 ppm. In the United States a tolerance of 4 ppm has been established for nicarbazin and, hence, the method was satisfactory to determine compliance with FDA regulations. Recent experiments have demonstrated reliability at 1 ppm. At least for liver and kidney the method fails at levels much lower than that. For this study improvements

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