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Characterization of Bound (Nonextractable) Residues of Dieldrin, Permethrin, and Carbofuran in Radishes

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[^{14}C]Dieldren (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-*exo*-1,4-*endo*-5,8-dimethanonaphthalene), [^{14}C]permethrin [3-phenoxybenzyl (±)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate], and [^{14}C]carbofuran (2,3-dihydro-2,2-dimethylbenzofuran-7-yl methylcarbamate) were applied in commercial formulations to radishes (*Raphanus sativus*) at 11.1 kg/ha and maintained in environmental growth chambers. Edible portions of the radishes were sampled 21 days postapplication, chopped, and exhaustively extracted with solvents. The amounts of nonextractable (bound) ^{14}C residues formed in the dieldrin-, permethrin-, and carbofuran-treated radishes were 23.5%, 28.6%, and 92.6%, respectively, of the total plant ^{14}C. The compounds that were present in the form of bound ^{14}C residues in radishes were identified as the parent pesticide (dieldrin, permethrin, or carbofuran) or metabolites of similar chemical structure [3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid, 3-ketocarbofuran, or 3-hydroxycarbofuran].

Tracer studies have shown that a considerable portion of pesticide residues may become bound (nonextractable) in plants. These residues are difficult to extract without chemical modification, are not detected in routine residue analysis, and are difficult to identify. Huber and Otto (1982) have reviewed bound residues of a number of pesticides in various crops.

During the course of a study on the fats of dieldrin (Stratton and Wheeler, 1983), permethrin (Stratton et al., 1981), and carbofuran (Forbes et al., 1980) in radishes (*Raphanus sativus*), it was observed that as much as 24%, 30%, and 70%, respectively, remained bound after exhaustive solvent extraction. The present study was undertaken to investigate the nature of these bound residues in radishes.

MATERIALS AND METHODS

Chemicals. Uniformly ring labeled [^{14}C]dieldrin was used to fortify a Shell Chemical Co. emulsifiable concentrate containing 18% technical dieldrin. Labeled [^{14}C]-*cis*-permethrin and [^{14}C]*trans*-permethrin was used to fortify an ICI emulsifiable concentrate containing 25% technical permethrin (a mixture containing 34% cis and 66% trans isomers). [^{14}C]Carbofuran, uniformly labeled in the benzene ring, was used to fortify an FMC commercial formulation (Furadan-4-Flowable) containing 40% technical carbofuran. The fortified formulations of dieledrin, permethrin, and carbofuran had specific activities of 0.19, 0.095, and 0.15 mCi/mmol, respectively. Dieldrin (99.7%), permethrin (99.8%), carbofuran (99.7%), 3-hydroxycarbofuran (manufacturer's standard), and 3-ketocarbofuran (manufacturer's standard) standards were obtained from the Environmental Protection Agency (Research Triangle Park, NC).

Treatment and Extraction of Plants. Red Globe radish seeds were germinated in flats containing soil and then transplanted at 1.5–2 weeks of age to 14.6-cm pots (four per pot) containing Hoagland nutrient solution and sand. The flats and pots were maintained in an environmental growth chamber (Scherer-Gillet Model CEL 512-37), with 10-h light periods and 14 h of dark. Light and dark temperatures were 27 and 16 °C, respectively; light and dark relative humidities were 80% and 60%, respectively. The radishes were treated at 5–6 weeks after germination by pipetting a known volume of the ^{14}C fortified formulated pesticide onto the roots and surrounding sand of each radish at rates of 11.1 kg/ha. An untreated control was maintained in the same growth chamber. The sand was covered with paraffin shavings immediately after the pesticide application to reduce volatilization of the insecticide.

The radishes were harvested 21 days postapplication by pulling them from the sand, rinsing them with water to remove adhering sand, and removing the tops. They were chopped with a hand-operated food chopper to particles of 0.5–1.0 mm in size prior to extraction in a Soxhlet extractor for 24 h with the solvents listed in Table II. The solvent-extracted radish tissues were dried and subjected to the Association of Official Analytical Chemists indirect lignin analysis (AOAC, 1970). Both the solvent-extracted radish tissues and resulting lignin were analyzed for bound residues.

Analysis of Bound ^{14}C Residues. The dried radish samples were subjected to the high-temperature distillation (HTD) technique (Khan and Hamilton, 1980). Six solvent

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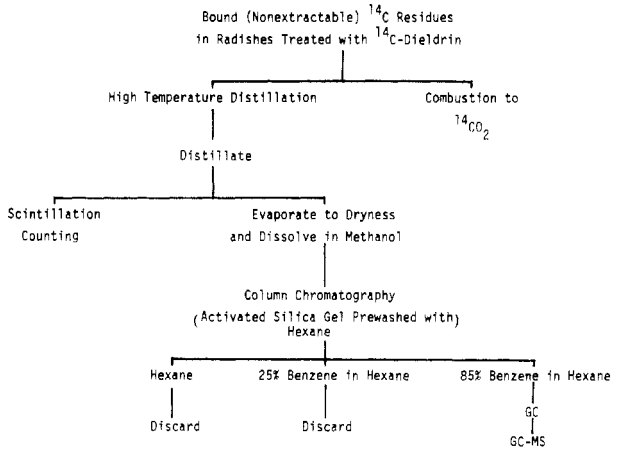

Figure 1. Schematic diagram for the analysis of bound ^{14}C residues in radishes treated with [^{14}C]dieldrin.

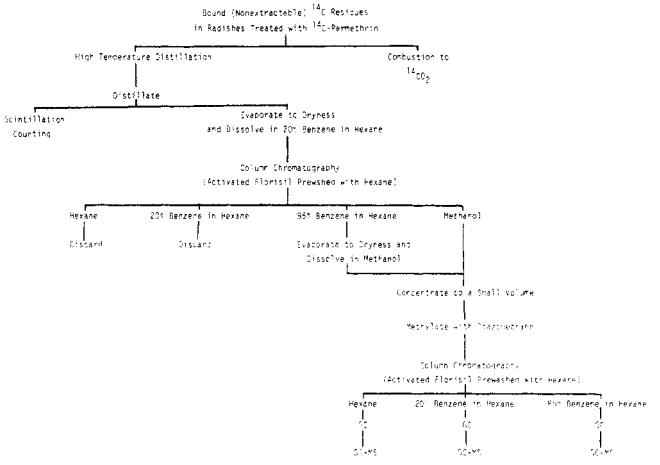

Figure 2. Schematic diagram for the analysis of bound ^{14}C residues in radishes treated with [^{14}C]permethrin.

traps were used for each sample in the HTD experiment. The first four traps contained 25% acetone in hexane (dieldrin), 20% hexane in ethyl acetate (carbofuran), or acetone (permethrin), the fifth contained methanol, and the last trap contained Oxisorb (New England Nuclear) to trap $^{14}CO_2$. The distillates were then processed as shown in Figures 1–3.

The dried radish samples containing bound ^{14}C residues were also combusted in a Packard sample oxidizer, Model B306, to produce $^{14}CO_2$. The $^{14}CO_2$ was absorbed in an admixed with appropriate volume of Carbo-Sorb and Permafluor V (Packard Instrumentation Canada, Ltd.), and the radioactivity was determined as described below.

Determination of Radioactivity. The radioactivity of aliquots of solutions was determined in a Packard Tri-carb liquid scintillation spectrometer, Model 3320, using an external standard and correcting the data for quenching. The activity was measured in a scintillation solution containing PPO and POPOP in toluene.

Gas Chromatography. The gas chromatograph was a Varian Model 6000 fitted with a thermionic (TD) and an electron capture detector (ECD). Cabofuran, 3-ketocarbofuran, and 3-hydroxycarbofuran were analyzed by using the TD, and dieldrin, permethrin, and its acid metabolites *cis*-Cl_2CA and *trans*-Cl_2CA [3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid] were determined by using the ECD. Glass columns (1.8 m × 0.2 cm) packed with 3% SE-30 (dieldrin and permethrin, Cl_2CA) and 4% SE-30 plus 6% QF-1 (carbofuran and metabolites) were operated at 240 °C except for Cl_2CA the column was operated at 105 °C. Nitrogen was the carrier

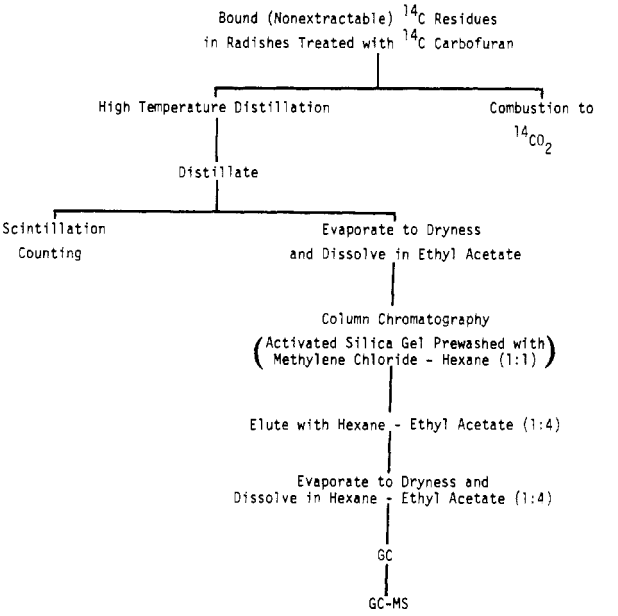

Figure 3. Schematic diagram for the analysis of bound ^{14}C residues in radishes treated with [^{14}C]carbofuran.

Table I. Recovery of Pesticides and Metabolites from Fortified Samples (5 ppm) of Extracted Control Radishes by the High-Temperature Distillation Techniques

compd	recovery, %	compd	recovery, %
dieldrin	90.1	carbofuran	55.6
permethrin	71.9	3-ketocarbofuran	56.8
Cl_2CA	58.3	3-hydroxycarbofuran	51.1

gas and had a flow rate of 30 mL/min.

Confirmation. The identity of the compound was confirmed by comparing the GC retention times with those of authentic samples, cochromatography, and finally by gas chromatography–mass spectrometry (GC–MS). A high-resolution mass spectrometer, Model VG 2AB-2F, connected to a Varian GC Model 3700 was used. The mass spectra were recorded at 70 eV.

Performance of the Methods. The recoveries of the residues by the HTD technique were determined by adding a known amount of the compound to the extracted and dried control radish samples. The samples were then processed as described earlier.

RESULTS AND DISCUSSION

Preliminary experiments indicated that HTD of reference radiolabeled standards of dieldrin, permethrin, and carbofuran resulted in ^{14}C recoveries of 95.3%, 67.4%, and 57.2%, respectively. Furthermore, GC analysis of the distillates showed the presence of only parent compounds. HTD of the extracted dried control radish samples to which the pesticide and metabolites were added and processed as shown in Figures 1–3 resulted in 51–90% recoveries of the compounds (Table I). The bound (nonextractable) ^{14}C residues in the treated radishes are shown in Table II. The total amount of ^{14}C residues (dpm/g) recovered by HTD were slightly lower than those obtained by combustion in a sample oxidizer to yield $^{14}CO_2$. The amount of ^{14}C residues in the combined distillates of four traps was 95.5%, 69.0%, and 65.6% of the total ^{14}C recovered by HTD from the dieldrin, permethrin–, and carbofuran-treated radishes, respectively. The remaining ^{14}C residues were thermally decomposed to $^{14}CO_2$ during distillation and were found in the last trap.

The distillates from the HTD experiment of the treated radishes containing bound residues were processed as shown in Figures 1–3 and examined by GC. The presence

Table II. Bound (Nonextractable) ^{14}C Residues in Radishes Treated with Labeled ^{14}C Pesticide

treatment	extracting solvent	bound ^{14}C residues, dpm/g HTD[b]			
		combustion to $^{14}CO_2$	solvent traps	CO_2 trap	total
dieldrin	chloroform–methanol (2:1)	56 800 (23.5)[a]	51 290	2 410	53 700
permethrin	hexane–2-propanol (2:1)	65 500 (28.6)	45 160	20 258	65 418
carbofuran	acetone	25 400 (92.6)	15 780	8 263	24 043

[a] Parentheses indicate percentage of bound (unextractable) ^{14}C of the total plant ^{14}C. [b] High-temperature distillation.

Table III. Bound Residues in Radishes Treated with Dieldrin, Permethrin and Carbofuran

treatment	compd identified	bound residues, ppm[a]
dieldrin	dieldrin	8.1 (0.28)
permethrin	*trans*-permethrin	0.19 (0.007)
	cis-permethrin	trace[b]
	Cl_2CA	trace
carbofuran	carbofuran	
	3-ketocarbofuran	0.2 (0.007)
	3-hydroxycarbofuran	0.1 (0.004)

[a] Calculated on the basis of air-dried weight of the solvent-extracted material. Parentheses indicate fresh weight in ppm. [b] <0.05 ppm.

of the parent compounds and/or metabolites was observed as shown in Table III. Traces of *cis*-permethrin and acid metabolite Cl_2CA were also detected, but their presence could not be confirmed by GC–MS because of their low concentration.

The yield of lignin from the dieldrin, permethrin; and carbofuran-treated radishes was 29.8%, 28.9%, and 22.5%, respectively, of the extracted dry tissue. Furthermore, 79.9%, 54.4%, and 44.3% radiocarbon was released from the extracted dry radish tissue in the soluble fractions from the hydrolytic treatments after the AOAC indirect lignin analysis. The bound ^{14}C residues remaining in lignin were subjected to HTD, and the distillates were processed as shown in Figures 1–3. GC analysis indicated the presence of trace amounts of dieldrin, permethrin, carbofuran, and 3-ketocarbofuran. 3-Hydroxycarbofuran was not detected. Due to the very low concentrations of these residues, quantitation was not possible. However, it is significant to note that bound residues of these compounds were still present in the isolated lignin and were not released or destroyed by the rigorous AOAC indirect procedure.

In a previous study it was observed that pepsin plus 0.1 N HCl or H_2SO_4 digestions (AOAC indirect lignin analysis) of the extracted dry radish tissue released bound radiocarbon (Stratton and Wheeler, 1983). Both procedures degrade plant macromolecules into smaller subunits. The results of our study showed that the bound residues in radish tissue and lignin were present as the parent pesticide or metabolites of similar chemical structure. Thus, it is likely that bound residues in radishes were formed by a process involving chemical entrapment of the pesticide or metabolites by lignin and/or other plant macromolecules. These bound residues may still possess toxicological character and may become released on digestion of the contaminated food crops. Whether these bound pesticide residues in radishes will be a hazard to human health will remain a matter of conjecture until more data are available. It has been assumed that the present methods of assessing the risks of pesticides are adequate for the detection of any adverse effects associated with their bound form. However, in view of our findings that bound pesticide residues may be present as the parent compound and/or its metabolites and constitute a considerable portion of the total residues in plant tissue, we believe that there is a need to develop more information for a better assessment of their toxicological significance.

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Registry No. Dieldrin, 60-57-1; *trans*-(±)-permethrin, 52341-32-9; *cis*-(±)-permethrin, 52341-33-0; carbofuran, 1563-66-2; *trans*-(±)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid, 55701-07-0; *cis*-(±)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid, 55701-06-9; 3-ketocarbofuran, 16709-30-1; 3-hydroxycrbofuran, 16655-82-6.

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