Bioavailability in Rats of Bound Residues from Radishes Treated with either Radiolabeled Dieldrin or Carbofuran

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The bioavailability of bound residues from radishes treated with [^{14}C]dieldrin and [^{14}C]carbofuran was investigated by feeding the rats ^{14}C material obtained after exhaustive solvent extraction. For comparison, nonextracted radishes were also fed to rats. The ^{14}C residues were predominantly excreted in feces. Urinary excretion of ^{14}C from rats fed nonextracted material was relatively greater than from those fed extracted radishes. The excreted material from rats fed dieldrin-treated radishes contained mainly parent compounds as residue. However, carbofuran and two of its metabolites, 3-hydroxycarbofuran and 3-ketocarbofuran, were present in feces and urine samples of rats fed carbofuran-treated radishes. These data demonstrated that bound residues in radishes treated with dieldrin and carbofuran have a low degree of bioavailabilty in rats. The results also show that bound residues in dieldrin-treated radishes would be more bioavailable than in the carbofuran-treated samples.

The fate of bound (nonextractable) pesticide residues in soil and plants has been the subject of numerous studies in recent years. However, relatively little attention has so far been given to the fate of these residues when consumed by animals in their diet. From the limited information available in the literature, it would appear that the majority of bound residues are excreted through the feces (Paulson et al., 1975; Sutherland, 1976; Dorough, 1976; Marshall and Dorough, 1977). This has been demonstrated by the measurement of excreted radioactivity from the animals fed a diet containing unidentified bound radiolabeled residues. However, in order to assess properly the question of bioavailability and/or toxicological significance, it is important to know whether the bound ^{14}C residues in the diet fed to animals, as well as the ^{14}C products excreted, are present as the parent compound or in the form of their metabolites. Such information was reported in a previous publication from our laboratory on the bioavailability in rats of bound [^{14}C]atrazine residues from corn plants (Khan et al., 1985). As a continuation of this work we now report the results of our investigations concerned with the bioavailability of bound ^{14}C residues in radishes treated with [^{14}C]dieldrin and [^{14}C]carbofuran.

MATERIALS AND METHODS

Chemicals. Uniformly ring-labeled [^{14}C]dieldrin (Amersham Corp., 85 mCi/mmol) was used to fortify a shell emulsifiable concentrate formulation containing 18% technical dieldrin. Uniformly ring-labeled [^{14}C]carbofuran was used to fortify an FMC commercial formulation (Furadan-4-Flowable) containing 40% technical carbofuran. The fortified formulations of dieldrin and carbofuran had specific activities of 0.19 and 0.15 mCi/mmol, respectively. The radiolabeled chemicals were purified by thin-layer chromatography. Dieldrin (99.7%), carbofuran (99.7%), 3-hydroxycarbofuran (manufacturer's standard), and 3-ketocarbofuran (manufacturer's standard) reference standards were obtained from the Environmental Protection Agency (Research Triangle Park, NC).

Plant Growth, Treatment, and Extraction. Red Globe radishes were grown in an environmental chamber, treated with the radiolabeled pesticide at rates of 11.1 kg/ha, and then harvested 21 days postapplication as described earlier (Khan et al., 1984). Untreated controls were maintained in the same growth chambers. Aliquots of edible portions of radishes were extracted with solvents to generte bound ^{14}C residue material (Khan et al., 1984).

Determination of Bound ^{14}C Residues. A portion of the insoluble radishes sample was used to determine the nature and amounts of bound ^{14}C residues as described earlier (Khan et al., 1984). The other portion of extracted radishes was used for feeding to rats as described below.

Treatments of Rats and Collection of Samples. Sprague–Dawley male rats weighing 170–230 g were housed, 1 animal/cage, in metabolism cages that permitted the separate collection of urine and feces. They were conditioned for 3 days to a daily diet consisting of a mixture of rat feed (Purina Laboratory Chow) and either nonextracted or extracted control radishes (5:1 w/w). The latter in the mixture was then replaced for 2 days with radishes containing ^{14}C residues (nonextracted, dieldrin 0.10×10^6 dpm/g, carbofuran 0.11×10^6 dpm/g; extracted, dieldrin 0.27×10^5 dpm/g, carbofuran 0.64×10^5 dpm/g). Four rats were used in each treatment group. The control rats continued to feed on the initial mixture. Food consumption was approximately 18 g/day per rat. The animals were then maintained on regular rat feed (Purina Laboratory Chow) for an additional 3 days, the rats were sacrificed by decapitation, and selected organs were excised, weighed, and frozen for subsequent analysis. The urine and feces were collected once daily after the treatment and radioassayed for ^{14}C. Portions of liver, kidney, and brain were used to determine radioactivity.

Analysis of Residues in Feces and Urine. (*i*) *Feces.* The flow diagrams in Figures 1 and 2 show the procedures used to analyze the extractable and nonextractable residues in feces.

(*ii*) *Urine.* Aliquots of urine samples were lyophilized, extracted with methanol–water (9:1), subjected to column chromatography, and finally analyzed as shown in Figures 1 and 2. A portion of urine sample was also radioassayed by scintillation counting.

Determination of Radioactivity. Liquids (e.g., urine, solvent extracts) were assayed in a Packard Model 3320 scintillation spectrometer and solids (plant material, feces, animal tissues) were assayed by combustion in a Packard

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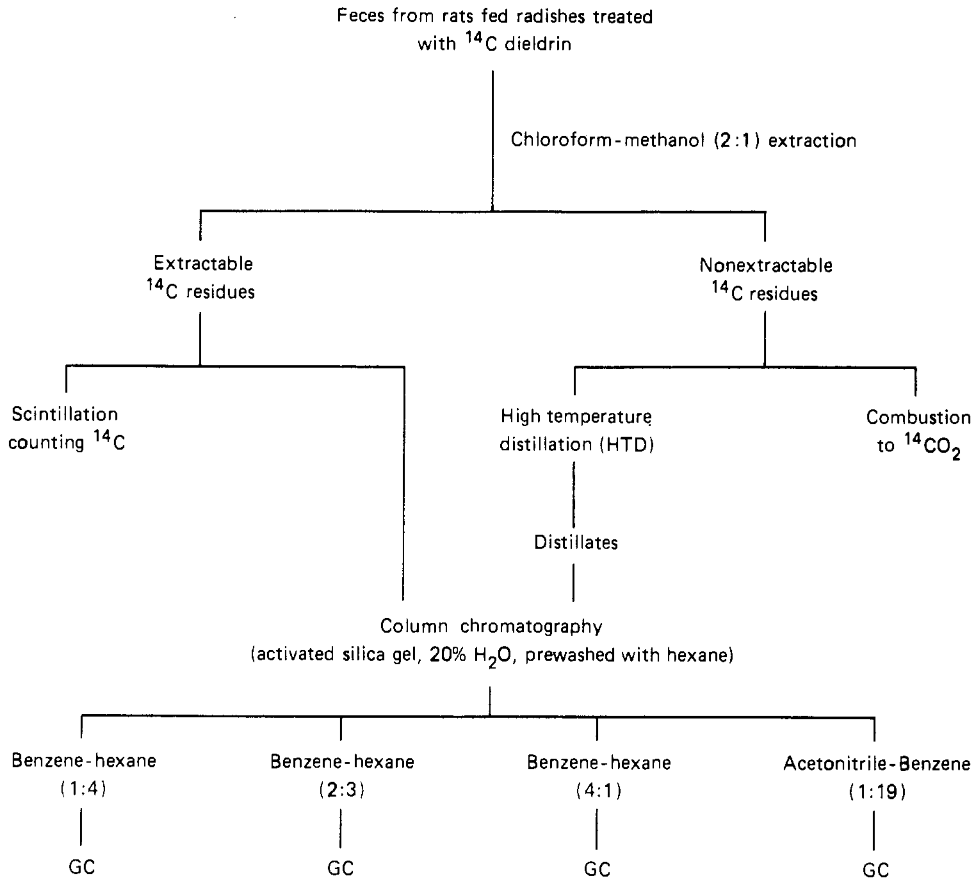

Figure 1. Schematic diagram for the analysis of feces from rats fed radishes treated with [^{14}C]dieldrin.

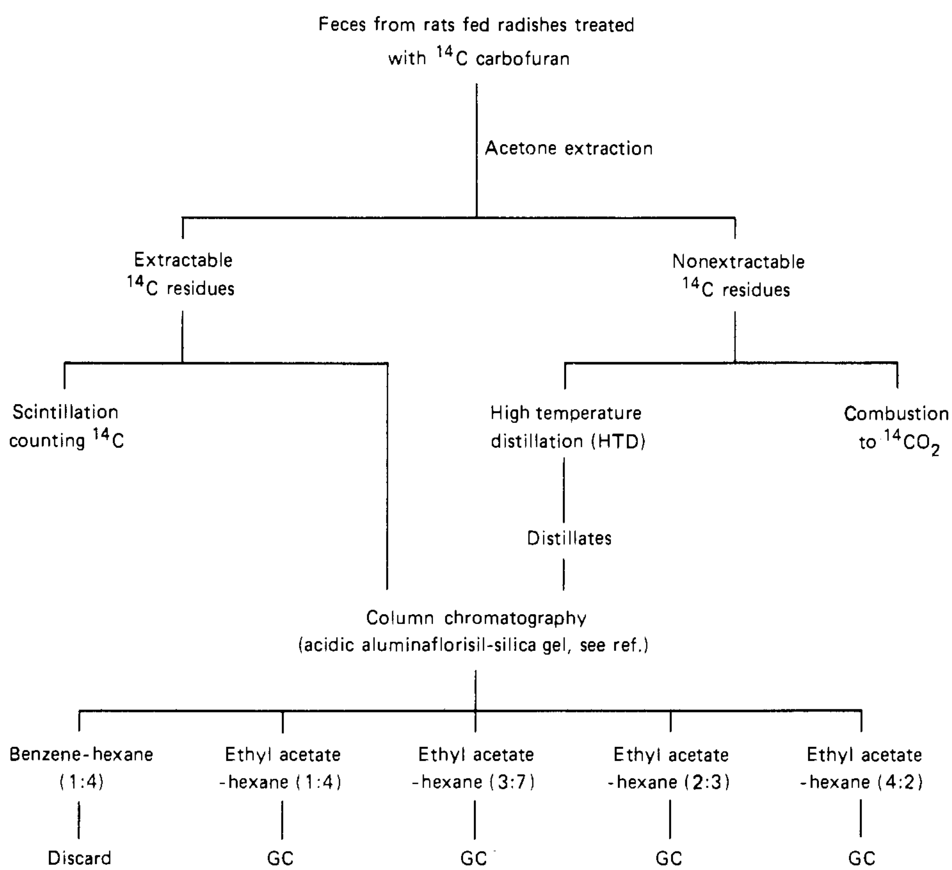

Figure 2. Schematic diagram for the analysis of feces from rats fed radishes treated with [^{14}C]carbofuran.

sample oxidizer, Model 306.

Gas Chromatography. The gas chromatograph was a Varian Model 6000 equipped with a thermionic detector (TD) and electron-capture detector (ECD) and connected to a Varian Vista 402 data system. Dieldrin was determined by using the ECD and carbofuran; 3-ketocarbofuran

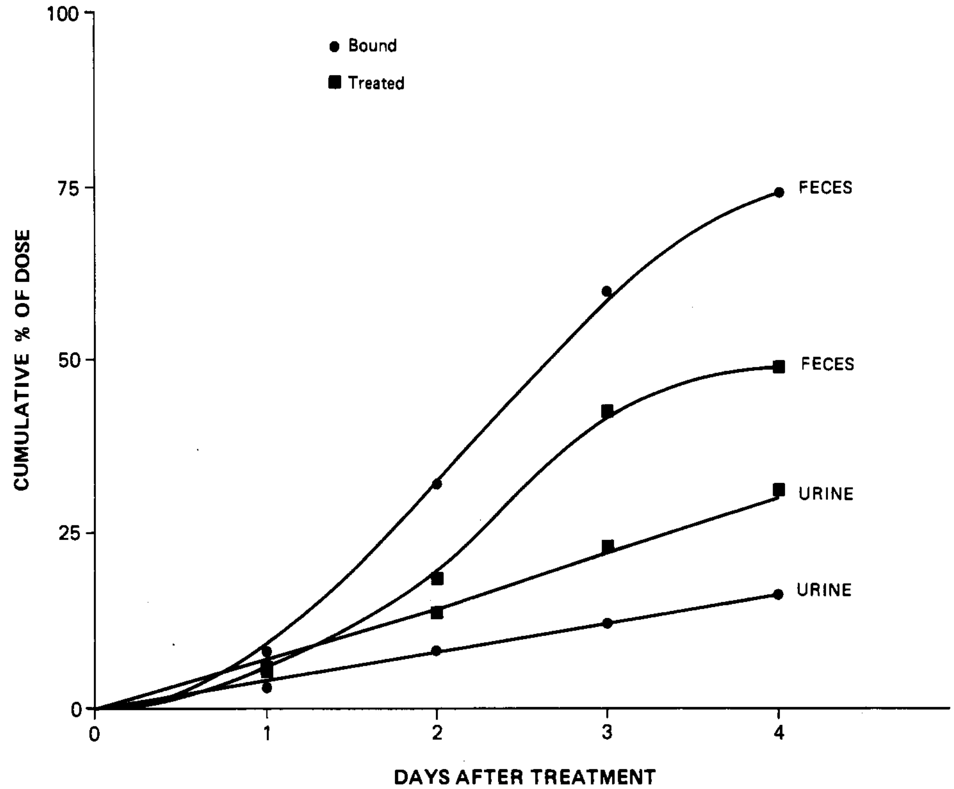

Figure 3. Elimination of ^{14}C from rats fed radishes treated with $[^{14}C]$dieldrin.

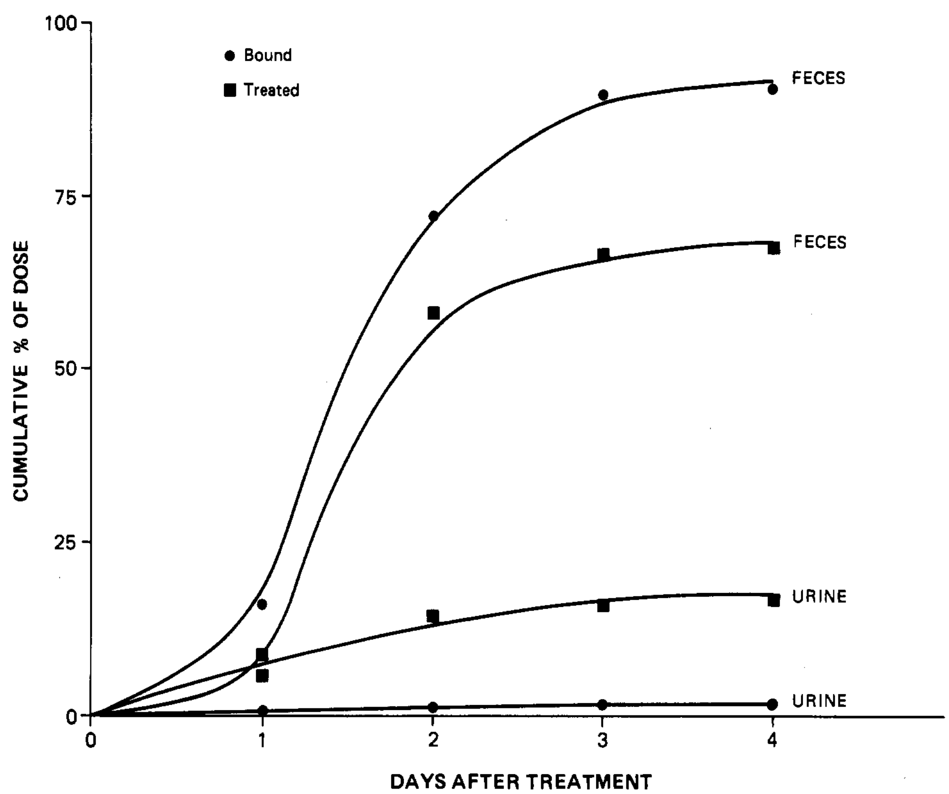

Figure 4. Elimination of ^{14}C from rats fed radishes treated with $[^{14}C]$carbofuran.

and 3-hydroxycarbofuran were analyzed by using the TD. Glass columns (1.8 m × 0.2 cm) packed with 3% SE-30 (dieldrin) or 4% SE-30 plus 6% QF-1 (carbofuran and metabolites) were operated at 240 °C. Nitrogen was the carrier gas and had a flow rate of 30 mL/min.

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

RESULTS AND DISCUSSION

Extraction of radishes with solvents removed 74% and 43% of the total ^{14}C present in the dieldrin- and carbofuran-treated samples, respectively. Thus, bound ^{14}C residues remaining in the dieldrin- and carbofuran-treated

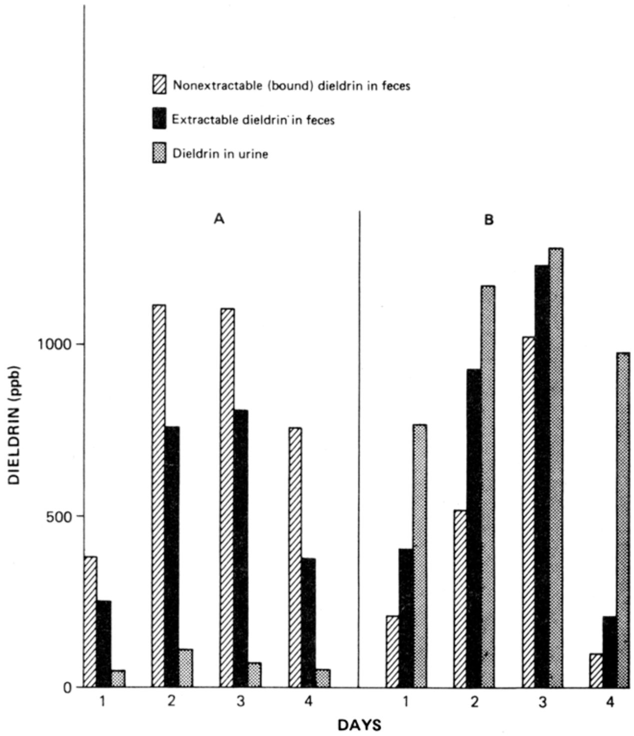

Figure 5. Elimination of dieldrin from rats fed extracted (A) and nonextracted (B) radishes.

radishes constituted 26% and 57%, respectively, of the total ^{14}C in the edible portions. The extractable fractions from both treatments were found to contain primarily the parent compounds. By the high-temperature distillation (HTD) technique it was shown earlier that bound residues in radishes treated with dieldrin and carbofuran consisted of 8.1 ppm dieldrin and 0.1–0.2 ppm carbofuran metabolites (Khan et al., 1984). However, in a later study using a supercritical methanol extraction technique, higher bound residues levels of dieldrin (11.3 ppm) and 3-hydroxycarbofuran (0.3 ppm) were reported (Capriel et al., 1986). In addition, the presence of carbofuran (0.5 ppm) in the form of bound residues was also observed in the radishes (Capriel et al., 1986).

None of the rats showed any sign of toxic manifestation during the course of the experiment, and macroscopic examination displayed no pathological conditions.

It has been assumed that urinary and/or biliary excretion of the radioactivity from animals fed radiolabeled compound signifies that the material is bioavailable. However, quantitative excretion of radioactivity in feces indicates that the material is not bioavailable. Figures 3 and 4 show the cumulative excretion of ^{14}C in feces and urine following feeding to rats of nonextracted and extracted samples of radishes treated with radiolabeled pesticides. In all the experiments, the ^{14}C excreted during the 4-day period was higher in feces than urine. After 4 days, cumulative excretion of radioactivity in the urine was about 16% of the total administered ^{14}C in the extracted dieldrin-treated radishes, or approximately one-fifth of the amount excreted in feces (Figure 3). However, rats given the nonextracted radishes diet eliminated approximately 32% of the total administered dose in urine, or about two-thirds of the amount excreted in feces (Figure 3). The pattern of excretion of ^{14}C from rats fed [^{14}C]dieldrin-treated radishes indicates a low degree of bioavailability of bound ^{14}C residues from the unextracted radishes. The low degree of bioavailability of bound ^{14}C residues in rats fed [^{14}C]carbofuran-treated extracted radishes was also apparent in that they were excreted predominantly in the feces (90% ^{14}C of the total dose), with less than 2% ^{14}C excreted in urine at the end of 4-day period (Figure 4). However, rats fed on the nonextracted radishes eliminated a relatively high percentage of the administered ^{14}C resi-

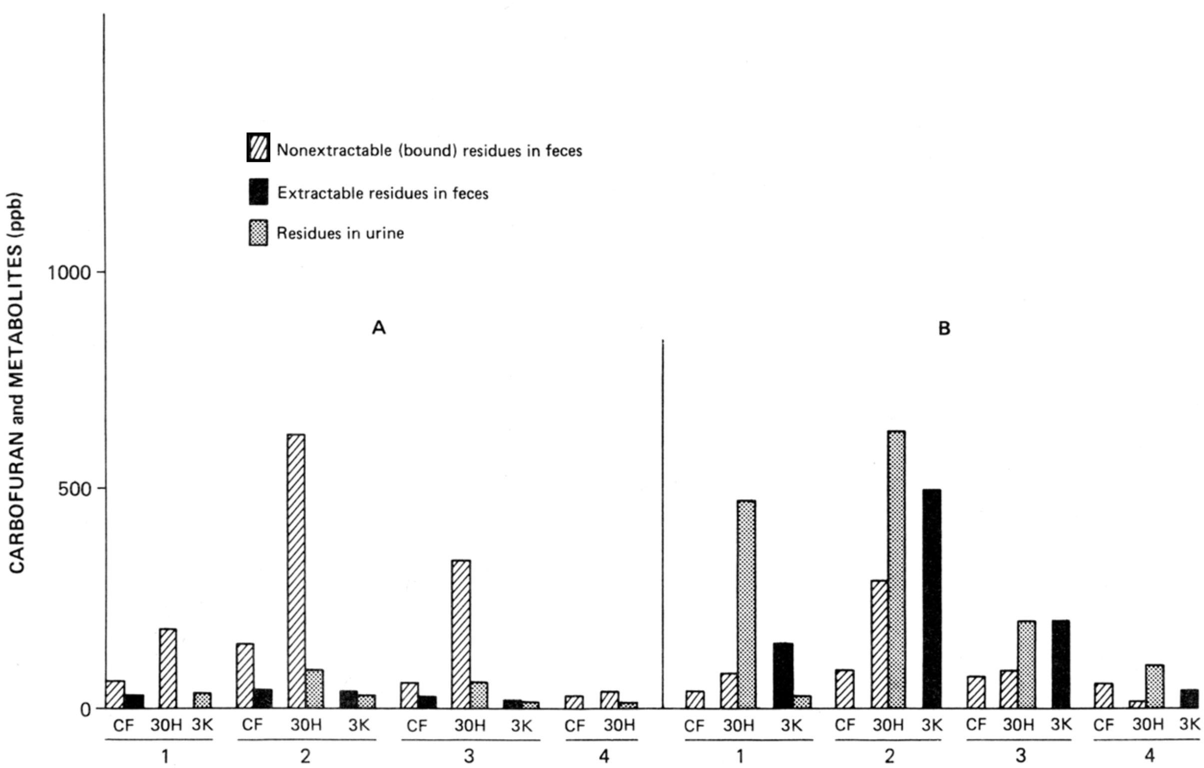

Figure 6. Eliminaton of carbofuran (CF), 3-hydroxycarbofuran (3OH), and 3-ketocarbofuran (3K) from rats fed extracted (A) and nonextracted (B) radishes.

dues in urine, 17%, while about 67% was voided via the feces (Figure 4).

Residual radioactivity in the brain, kidney, and liver was measured following the sacrifice of rats fed with radishes containing [^{14}C]dieldrin residues. The ^{14}C content in the brain, kidney, and liver of rats fed nonextracted radishes was 0.5%, 3.3%, and 5.2%, respectively, of the total administered radioactivity in diet. The corresponding values for rats fed extracted radishes were 0.2%, 1.1%, and 2.3%, respectively. The highest residues in both instances were in the liver. Very low ^{14}C residues (<0.1%) were detected in tissues from rats fed radishes treated with carbofuran.

Extraction of feces from rats fed nonextracted and extracted radishes treated with [^{14}C]dieldrin removed 63% and 38% ^{14}C, respectively (Figure 5). Similarly, about 23% and 2% radioactivity was extractable from feces of rats fed nonextracted and extracted [^{14}C]carbofuran-treated radishes, respectively (Figure 6). It appears that most of the ^{14}C remained in the bound form particularly in feces from rats that consumed a diet containing extracted radishes.

The nature of the excreted radioactivity in feces and urine is shown in Figures 5 and 6. The feces and urine samples from rats fed extracted and nonextracted dieldrin-treated radishes contained mainly the parent compound. Dieldrin was predominantly excreted in the bound form in the feces from rats fed extracted radishes, and only a small amount was excreted in the urine. However, a reverse pattern of dieldrin excretion was observed from rats fed nonextracted radishes. These observations further suggest that dieldrin residues in nonextracted radishes were far more readily bioavailable in rats than in extracted material. Similar results were obtained for carbofuran-treated radishes. The excreted material comprised mainly carbofuran and its two metabolites namely 3-hydroxycarbofuran and 3-ketocarbofuran. It was observed that 3-hydroxycarbofuran was the predominant metabolite in the feces and urine of rats fed extracted or nonextracted radishes. In the latter case, 3-ketohydroxycarbofuran was also present in relatively higher amounts in the feces extracts.

In earlier studies, it was observed that the extractable ^{14}C from dieldrin-treated radishes was primarily dieldrin and a very small amount of photodieldrin (Stratton and Wheeler, 1983). It was also reported that the compound present in the form of bound ^{14}C residues in radishes was the parent pesticide dieldrin (Khan et al., 1984). In the present study, only unchanged dieldrin was detected in the excreted urine and feces, which indicated that the pesticide did not undergo any bioalteration after it was consumed by rats. It has been shown that, for carbofuran-treated radishes, the extractable ^{14}C fraction was primarily parent compound (Stratton and Wheeler, 1986). Analysis of bound ^{14}C residues in the extracted radishes revealed the presence of carbofuran, 3-hydroxycarbofuran, and 3-ketocarbofuran (Khan et al., 1984; Capriel et al., 1986). The presence of these three compounds was also noted in the excreted material from rats fed extracted and nonextracted radishes. Although bound 3-hydroxycarbofuran in extracted radishes was present in a relatively small amount, this metabolite appears to be the dominant residue in the excreted feces and urine. It is likely that residues in the carbofuran-treated radishes underwent bioalteration when administered to rats. It appears that some of the altered material became strongly absorbed by feces.

Carbofuran and dieldrin have been shown to behave differently in the formation of bound residues in radishes (Stratton and Wheeler, 1986). Carbofuran was rapidly bound, and the proportion of material that was lignin bound also increased rapidly. However, dieldrin was bound slowly, and the proportion that was lignin-bound remained relatively small. The observation that hydrolytic processes were more effective in solubilizing ^{14}C from dieldrin-treated than from carbofuran-treated radishes (Stratton and Wheeler, 1986) suggests that bound residues in dieldrin-treated radishes would be more biologically available than in carbofuran-treated radishes. This is substantiated by our data as it was shown that, after 4 days, total excretion of ^{14}C in urine from rats fed extracted radishes treated with dieldrin and carbofuran was 16.4% and 1.2%, respectively. It is likely that the acid conditions in stomach released hydrolytically more ^{14}C from dieldrin-treated than carbofuran-treated samples.

Although the data presented in this study deomonstrate a low degree of bioavailability in rats of bound residues in radishes treated with dieldrin and carbofuran, it appears that a certain proportion of such residues, not detectable by standard analytical techniques, is potentially bioavailable. Furthermore, knowledge of the quantity and chemical identity of bound residues in crop material will assist in assessing properly their toxicological significance.

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Registry No. Dieldrin, 60-57-1; carbofuran, 1563-66-2; 3-hydroxycarbofuran, 16655-82-6; 3-ketocarbofuran, 16709-30-1.

LITERATURE CITED

Capriel, P.; Haisch, A.; Khan, S. U. *J. Agric. Food Chem.* **1986**, *34*, 70–73.

Dorough, H. W. In *Bound and Conjugated Pesticide Residues*; ACS Symposium Series 29; American Chemical Society: Washington, DC, 1976; p 11.

Khan, S. U.; Stratton, G. D.; Wheeler, W. B. *J. Agric. Food. Chem.* **1984**, *32*, 1189.

Khan, S. U.; Kacew, S.; Molnar, S. J. *J. Agric. Food Chem.* **1985**, *33*, 712.

Marshall, T. C.; Dorough, H. W. *J. Agric. Food Chem.* **1977**, *25*, 1003.

Paulson, G. D.; Jackobsen, A. M.; Still, G. G. *Pestic. Biochem. Physiol.* **1975**, *5*, 523.

Stratton, G. D.; Wheeler, W. B. *J. Agric. Food Chem.* **1983**, *31*, 1076.

Stratton, G. D.; Wheeler, W. B. Presented at the 3rd FAO/IAEA Research Coordination Meeting on Isotopic Tracer-Aided Studies of Bound Pesticide Residues in Soil, Plants and Food, Gainsville, FL, March 25–29, 1985.

Sutherland, M. L. In *Bound and Conjugated Pesticide Residues*; ACS Symposium Series 29; American Chemical Society: Washington, DC, 1976; p 153.

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