Distribution and Nature of Bound (Nonextractable) Residues of Atrazine in a Mineral Soil Nine Years after the Herbicide Application

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Uniformly ^{14}C ring-labeled atrazine was applied to a mineral soil under field conditions. Nine years after application of the herbicide soil samples were collected for analysis. The soil contained about 50% ^{14}C residues in the bound (nonextractable) form. The bound ^{14}C residues were distributed among the various soil humic fractions. In addition to the parent herbicide a considerable proportion of these residues was comprised of the hydroxy analogues of atrazine and their dealkylated products.

INTRODUCTION

In recent years bound residues of pesticides have been the subject of numerous investigations which suggest that these residues are not excluded from environmental interactions. A number of studies have demonstrated the potential availability of soil bound pesticide residues to plants (Fuhremann and Lichtenstein, 1978; Helling and Krivonak, 1978; Fuhr and Mittelstaedt, 1980; Khan, 1980) and to earthworms (Fuhremann and Lichtenstein, 1978), and their biodegradation by soil microbs (Khan and Ivarson, 1981, 1982).

Earlier studies in our laboratory with radiolabeled atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine] have shown that under outdoor conditions nine years after the herbicide application the soil contained about 83% ^{14}C of the initially applied radioactivity (Capriel and Haisch, 1983a). Furthermore, a considerable portion of the ^{14}C residues (50%) in soil remained in the bound (nonextractable) form even after exhaustive solvent extraction. These bound residues will not be detected in routine residue analysis thereby underestimating the soil burden of the total herbicide or metabolites residues. Our subsequent study demonstrated that some of the ^{14}C residues in soil (extractable + nonextractable) were absorbed and translocated in oat plants under field conditions (Capriel and Haisch, 1983b). The present study is a continuation of our investigations and reports on the nature and distribution of bound ^{14}C residue in soil and its organic matter fractions.

MATERIALS AND METHODS

Soil. Soil samples were obtained in summer of 1982 from field plots treated with ^{14}C ring-labeled atrazine in 1973 (Capriel and Haisch, 1983a). The samples were stored in sealed plastic bags in moist condition at −20 °C until analyzed.

Extraction of Soil. The air-dried, pulverized, and screened soil samples were Soxhlet extracted with methanol for 2 h in a hot extractor. Control soil (untreated) was similarly extracted. Residual methanol in the extracted soil was allowed to evaporate by air drying the sample. The methanol extract was analyzed for ^{14}C by direct addition to a liquid scintillation cocktail and the nonextractable ^{14}C in soil (bound ^{14}C) by combustion of the air-dried sample to $^{14}CO_2$.

Fractionation of the Extracted Soil. The method of extraction, separation, and purification of humic materials outlined in Figure 1 is analogous to that described by Matsuda and Schnitzer (1972). The humic acid (HA), fulvic acid (FA), and humin (base-insoluble) fractions were freeze-dried. The high ash content of humic fractions was removed as described earlier (Khan, 1982a).

Analysis of Bound ^{14}C Residues in Soil and Humic Materials. A Lindberg Tube Furnace (Sola Basic S/B) was used for high temperature distillation (HTD) of the extracted soil and humic materials in order to release the ^{14}C residues. The released ^{14}C material was collected in various traps containing suitable solvents. The details of the HTD procedure for the bound ^{14}C residues analysis are described in earlier publiciations (Khan and Hamilton, 1980; Khan, 1982a). At the end of the HTD experiment the ^{14}C content of the trapping solutions was determined by liquid scintillation counting. The soluble FA fraction was also dissolved in methanol and analyzed for total ^{14}C content. The solutions from each trap and the methanolic solution of FA were subjected to column cleanup, derivatization, and finally analyzed by gas chromatography (Figure 2).

Chemicals. All solvents were of pesticide grade and used as received. Uniformly ^{14}C ring-labeled atrazine, reference standards of atrazine, and metabolites were gifts from Ciba-Geigy, Ltd., Switzerland.

Determination of Radioactivity. Combustion of air-dried soil and humic material samples was done in a Packard sample oxidizer, Model 306, to produce $^{14}CO_2$. The later was absorbed in and admixed with appropriate volumes of Carbo-Sorb and Permafluor-V. Aliquots of various solutions or extracts obtained as described above were analyzed by liquid scintillation counting (Khan and Hamilton, 1980). For dark-colored solutions of humic materials, it was found necessary to prepare quench correction curves by internal standardization with [^{14}C]atrazine.

Gas Chromatography (GC). The gas chromatograph was a Varian Model 6000 fitted with a thermionic-specific detector. The column was a 1.8 m × 0.2 cm i.d. glass tube packed with 3% Carbowax 20 M coated on 100–200 mesh Supelcoport. The operating conditions were as follows: column, detector, and injector port temperatures were 190, 300, and 220 °C, respectively; the nitrogen carrier gas, hydrogen, and air flow rates were 20, 4, and 150 mL/min, respectively.

Confirmation. The identity of the compounds was confirmed by comparing the GC retention times with those of authetic 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 ^{14}C residues by combustion or HTD technique were de-

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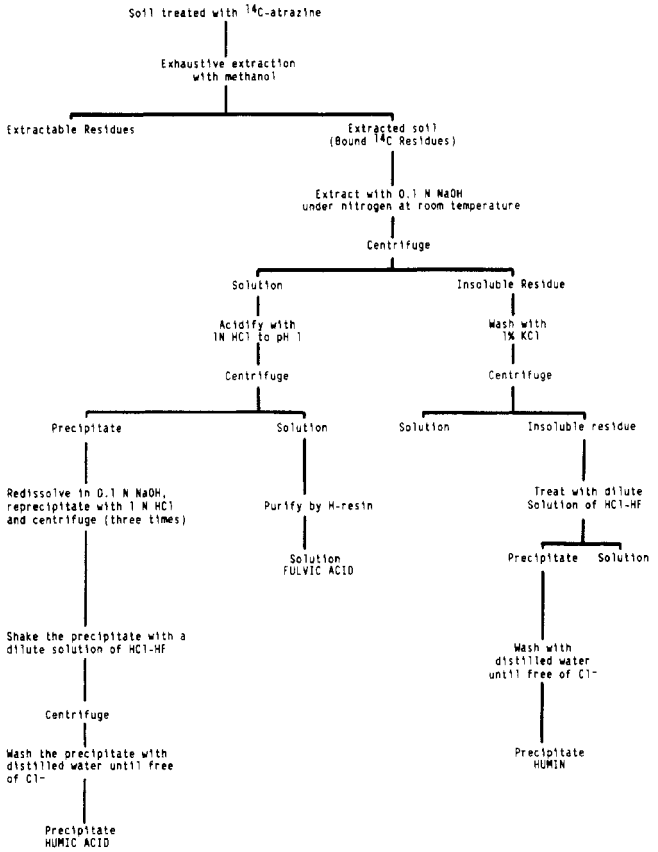

Figure 1. Fractionation of soil containing bound ^{14}C residues.

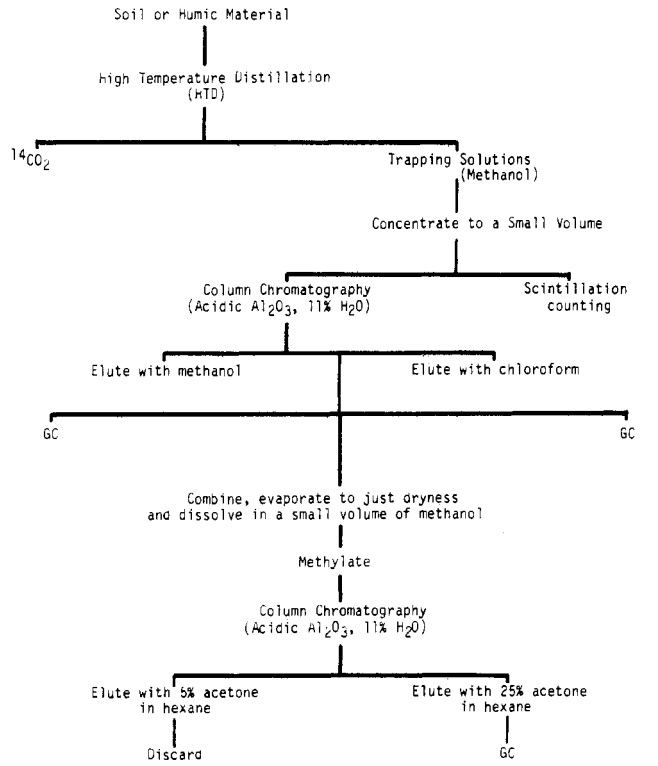

Figure 2. Schematic diagram for the analysis of bound (nonextractable) residues.

termined by adding [^{14}C]atrazine to the air-dried control soil or humic materials extracted from control soil at 1.0 μg/g level. The samples were processed as described earlier.

In this study all samples were analyzed in duplicate and average values are reported. Residue levels in soils are reported on an oven-dry basis and in humic materials on dry ash-free basis. The results reported here are not corrected for recovery.

Table I. Bound ^{14}C Residues in Soil and Humic Materials

sample	bound ^{14}C residues, dpm/g
soil	13 198[a]
humic acid	275 974[b]
fulvic acid	4 545 454[b]
humin	116 444[b]

[a] Oven-dry basis. [b] Oven-dry ash-free basis.

RESULTS AND DISCUSSION

Combustion of control soil, HA, FA, and humin samples to which [^{14}C]atrazine was added resulted in 91%, 95%, 84%, and 95% recovery of radioactivity, respectively. Similarly, HTD of soil, HA, or humin fortified with [^{14}C] atrazine gave a recovery of ^{14}C in the combined trapping solutions in the range of 90–92%. In preliminary experiments it was observed that 92–99% of radioactivity was recovered by the HTD of ^{14}C ring-labeled atrazine reference standard. However, some of the compound (15–20%) was thermally decomposed to $^{14}CO_2$ during distillation. GC analyses of the trapping solutions (methanol) showed that 81% of the compound was prresent in the form of atrazine. It was also observed that the thermal decomposition of atrazine to CO_2 during distillation was considerably greater in the presence of mineral soil or humic materials. This resulted in low recoveries (40–50%) of atrazine (determined by GC) in the trapping solutions. In our studies the release of the parent pesticide or metabolites by HTD was always found lower from mineral than organic soil.

The yield (dry ash-free basis) of HA, FA, and humin amounted to 0.006, 0.001, and 0.050 g/g of soil, respectively. The amounts of bound ^{14}C in soil and humic materials is shown in Table I. The proportions of total bound ^{14}C in HA, FA, and humin were 13%, 33%, and 44%, respectively, whereas 10% ^{14}C remained in nonhumic and mineral components of soil. The distribution of bound ^{14}C in humic materials was somewhat similar to that reported for other pesticides (Khan, 1982a; Helling and Krivonak, 1978; Smith and Muir, 1980). In terms of the initial radiolabeled atrazine applied in the field in 1973 the distribution of bound ^{14}C in HA, FA, and humin was 7%, 17%, and 22%, respectively. Thus, it appears that a considerable proportion of ^{14}C residue was present in the bound form in humic materials 9 years after the application of the herbicide under outdoor conditions. This implies that incorporation of ^{14}C residues in humic materials may limit the extent to which the herbicide or metabolites may be further metabolized. Whether formation of bound pesticide residues is accompanied by a permanent loss or decrease in their phytotoxicity or toxicity is not known. However, both direct and circumstantial evidence indicates that humus-bound residues may constitute a source of crop contamination (Still and Mansager, 1969; Still et al., 1980).

The methanolic solutions in traps after the HTD of soil or humic materials (HA and humin) were combined and processed as depicted in Figure 2. The methanolic solution of FA was also subjected to column chromatography and derivatization (Figure 2). GC and GC–MS analysis of the various eluates (Figure 2) revealed the presence of a number of compounds shown in Table II. It is noteworthy that the parent herbicide was still present in soil and HA in the bound form. In general the dominant compounds present in the bound form were atrazine, hydroxyatrazine, and the dealkylated analogues of the latter. Furthermore, these compounds were present in the highest concentration in HA.

Table II. Bound Residues in Soil and Humic Materials

compound	ppm[a] soil	humic acid	fulvic acid	humin
atrazine	0.11	0.30	ND	ND
deethylatrazine	T	T	ND	ND
deisopropylatrazine	T	0.12	ND	ND
hydroxyatrazine	0.10	0.30	T	0.11
deethylhydroxyatrazine	0.13	0.20	T	T
deisopropylhydroxyatrazine	0.07	0.20	ND	T

[a] ND = nondetectable; <0.01 ppm. T = trace amount; <0.05 ppm.

Our previous studies have shown a long-term persistence of atrazine and its metabolites in soil under field conditions (Capriel and Haisch, 1983a). Furthermore, it was demonstrated that these residues could be absorbed by oat plants (Capriel and Haisch, 1983b). Data presented in this study indicate that about 50% of the initially applied radioactivity was present in soil in the bound (nonextractable) form even after 9 years of the herbicide application and this bound radioactivity still contains the parent herbicide in addition to other metabolites. This raises the question whether analysis of the extractable residues alone is a valid criteria to determine the persistence of the herbicide under the outdoor conditions described in this study. The observations that soil bound residues are absorbed by plants (Khan, 1982b) and the data presented in this paper should prompt further research to determine whether the long-term accumulation of bound residues could affect sensitive crops grown in rotation or contribute to the contamination of the environment.

Registry No. Atrazine, 1912-24-9.

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Composition and Photochemical Reactions of a Dimethylamine Salt Formulation of (4-Chloro-2-methylphenoxy)acetic Acid (MCPA)

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A commercial formulation of MCPA ((4-chloro-2-methylphenoxy)acetic acid, dimethylamine salt) was found to be over 95% pure, with (2-methylphenoxy)acetic acid as the principal impurity. Sunlight irradiation of a spray mixture and the semisolid residue remaining after its evaporation on a glass surface resulted in >80% loss of MCPA within 6 days and formation of 4-chloro-*o*-cresol and 13 other identified products as well as an unresolved mixture of polycarboxylic acid salts. Unexpected products included (4-chloro-2-methylphenoxy)-*N*,*N*-dimethylacetamide and 4-chloro-2-methylanisole; amine salts of other phenoxy herbicides also were converted photochemically to corresponding amides. After 31 days of outdoor exposure, only 10% of the original MCPA remained. MCPA would be expected to undergo chemical degradation as long as it remained airborne as spray drift.

MCPA ((4-chloro-2-methylphenoxy)acetic acid) has been applied for weed control in California rice fields since 1951. In 1979, 80% of the state's half-million acres of rice were sprayed with the herbicide, primarily as the dimethylamine (DMA) salt, and in 1982, 200 000 kg of the herbicide was used (CDFA, 1983). Earlier work (Soderquist and Crosby, 1975) indicated that a significant proportion of the aerially applied spray did not reach its intended target, and concern over the environmental fate of possible spray drift prompted the present project.

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The objectives of our work were (1) to determine the chemical composition and physical characteristics of MCPA/DMA concentrate and spray and (2) to investigate changes in the spray composition due to evaporation, photodegradation, and other environmental factors as they might affect airborne droplets.

MATERIALS AND METHODS

Materials. "MCP Amine" herbicide (Dow Chemical Co.) was the commercial product used in California rice fields. The label correctly stated that it contained 4 lb/gal of MCPA acid equivalent as the dimethylamine salt (52.1%, or 589 g/L, of the salt). Spray mixtures were prepared, according to common practice, by dilution of this

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