Plant Uptake of Unextracted (Bound) Residues from an Organic Soil Treated with Prometryn

Shahamat U. Khan

Soil-bound ^{14}C residues were absorbed by the oat plants grown in an organic soil treated with ^{14}C-ring-labeled prometryn [2-(methylthio)-4,6-bis(isopropylamino)-*s*-triazine]. The roots contained more extractable ^{14}C residues (75.0%) then did shoots (51.1%). The majority of extractable ^{14}C residues in the plant tissue was present in the form of conjugates. Plant-bound ^{14}C unextractable residues were lower in roots (19.9%), but greater in shoots (40.2%). Mono- and di-N-dealkylated metabolites of prometryn were present in the plant bound ^{14}C residues. A major portion of bound ^{14}C residues in plant tissues was associated with lignin.

Bound residues of pesticides in soils and plants have been the subject of numerous investigations. Several workers have demonstrated the release and plant uptake of soil bound residues (Fuhremann and Lichtenstein, 1978; Helling and Krivonak, 1978; Führ and Mittelstaedt, 1980). It has been suggested that a portion of these residues may again become bound within the plant tissues (Fuhremann and Lichtenstein, 1978; Führ and Mittelstaedt, 1980). Thus, once the soil-bound residues are absorbed and translocated in plant tissues, they may be present in three possible forms: (i) freely extractable residues, (ii) extractable conjugates bound to natural components of plants, and (iii) unextractable or bound residues incorporated into plant constituents. The latter may be considered as analogous to the bound residues in soil.

A recent report from our laboratory described a high-temperature distillation (HTD) technique for determining and chemically identifying the soil-bound residues of the *s*-triazine herbicide prometryn [2-(methylthio)-4,6-bis-(isopropylamino)-*s*-triazine] (Khan and Hamilton, 1980). It was observed that a considerable portion of the bound ^{14}C residues in the incubated soil treated with ^{14}C-ring-labeled prometryn was present in the form of the parent compound, whereas the remainder constituted some unidentifiable products. The investigation reported here is an extension of this study. The potential uptake of soil bound ^{14}C residues by oat plants was investigated. To obtain additional insight into the phenomenon of plant availability of soil bound residues, attempts were also made to characterize the ^{14}C residues in plant tissues.

EXPERIMENTAL SECTION

Soil. Bound soil residues were produced as described in an earlier study (Khan and Hamilton, 1980). Briefly, the ^{14}C-ring-labeled prometryn-treated soil samples were incubated for 1 year, exhaustively extracted with solvents, dried, and pooled together. The soil was again moistened, incubated, and extracted with methanol (Khan and Hamilton, 1980). Residual methanol from the extracted soil was allowed to evaporate by air-drying the sample. The soil was then exhaustively extracted with water and again air-dried. The bound ^{14}C residues was determined by combustion of the air dry soil to $^{14}CO_2$.

Growth Chamber Experiment. Soil containing ^{14}C-bound residues mixed with washed silica sand (soil:sand = 1:1.25) were used to grow oat plants. The soil–sand mixture was moistened and placed in 7.5 cm diameter, 9.5 cm high plastic pots. Ten oat seeds which had been germinated for 3 days between wet filter papers in Petri dishes were then planted in each pot. The pots were watered each day as necessary. During the growing period the pots were also fertilized with standard Hoagland's nutrient solution. The plants were grown in a controlled environmental chamber at a day/night temperature of 22/18 °C and 18 000 lux of fluorescent-incandescent light for 16 h/day. After 21 days the plants were harvested by cutting approximately 1 cm above the soil surface. Roots were removed from the soil and washed with cold tap water. The shoots and roots were stored at −20 °C until analyzed. Aliquots of the dried (24 h at 30 °C) plant tissues were combusted to $^{14}CO_2$ for determining the total ^{14}C residues.

Determination of Radioactivity. Combustion of dried soil or plant tissues was done in a Packard sample oxidizer, Model 306, to produce $^{14}CO_2$. Aliquots of various extracts (described later) and $^{14}CO_2$ released by combustion were analyzed by liquid scintillation counting (Khan and Hamilton, 1980).

Analysis. The analytical procedure used for the extraction, isolation, and determination of ^{14}C residues in plant tissues is shown in Figure 1. Both root and shoot samples were thawed at room temperature and blended at a high speed with dried chloroform (1:100 w/v) for 5 min. The mixture was filtered under suction and the sample residue washed with chloroform. The combined filtrate was concentrated to small volume and analyzed by gas chromatography (GC) after cleanup on an aluminum oxide column (Khan and Hamilton, 1980).

The insoluble material from root or shoot tissue was then blended at high speed with methanol (1:100 w/v) for 5 min. The mixture was filtered under suction, and the insoluble sample residue was blended again with methanol (1:100 w/v) and finally with water (1:100 w/v). Methanol was removed from the combined filtrate and the water phase concentrated to a small volume on a rotary evaporator. The insoluble shoot or root sample was set aside for determining the bound residues as described later. An XAD-2 column (20–50 mesh) was prepared and cycled between methanol and water three times and finally washed exhaustively with water. The aqueous extract of plant tissue was transferred onto the XAD-2 column and washed with one column volume of water. The nonionic polymeric adsorbent XAD-2 adsorbed most of the polar metabolites. The column was eluted with methanol and the eluate was concentrated to a small volume. The concentrate was divided into two parts for determining free and conjugated metabolites. One portion was methylated with diazomethane and analyzed by GC. The other part was hydrolyzed by heating at 100 °C for 4 h with concentrated HCl. The mixture was then evaporated to

Chemistry and Biology Research Institute, Research Branch, Agriculture Canada, Ottawa, Ontario, Canada K1A 0C6.

0021-8561/80/1428-1096$01.00/0

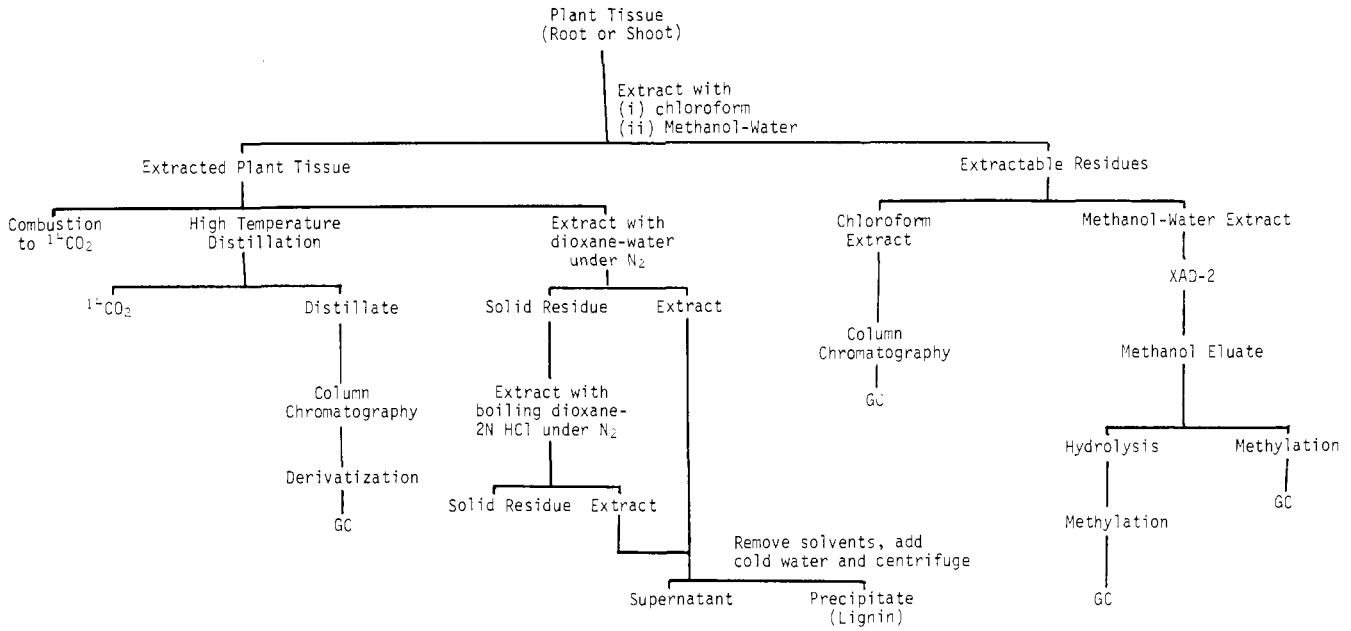

Figure 1. Schematic diagram for the analysis of extractable and bound residues.

Table I. Uptake of Bound ^{14}C Residues from Soil-Sand Mixture by Oat Plants

plant tissue	extractable residues, % ^{14}C of the total in the plant tissue			bound residues, % ^{14}C of the total in the plant tissue								
	chloroform soluble (nonpolar)	methanol-water soluble (polar)		determined by HTD[a]			determined as shown in Fig 1				determined by combustion to $^{14}CO_2$
		free	conjugate	total	distillate	thermal decomp ($^{14}C_2$)	total	lignin fraction	solid residue	supernatant	total	
roots	1.7	–[b]	73.3	75.0	15.5	2.5	18.0	10.5	4.7	2.3	17.5	19.9
shoots	2.1	–	49.0	51.1	31.9	8.0	39.9	28.5	7.5	2.4	38.4	40.2

[a] High-temperature distillation (Khan and Hamilton, 1980). [b] Not detected.

dryness under vacuum, redissolved in a small volume of methanol, methylated with diazomethane, and analyzed by GC.

The residual extracted roots or shoots containing bound residues were dried at 30 °C for 24 h and each tissue was then divided into three parts. One part of shoot or root sample was combusted to $^{14}CO_2$ to determine the total ^{14}C. Another part was analyzed by the HTD technique as described in our earlier publication (Khan and Hamilton, 1980). The third portion of plant tissue was used for the extraction of free and bound lignin as described by Balba et al. (1979). Briefly, the free lignin from roots and shoots was extracted with dioxane–water (9:1) under N_2. The solid residue was then extracted with dioxane–2 N HCl (9:1) at 70 °C under N_2. The two extracts were combined, and the solvents were removed by a rotary evaporator till a brown viscous material was obtained. Cold water was added with continuous stirring to the brown viscous material. The precipitate (lignin) was separated by centrifugation, dried, and combusted to $^{14}CO_2$ to determine the total ^{14}C. The solid plant residue after lignin extraction was then combusted to $^{14}CO_2$ for determining the ^{14}C remaining in the plant tissue (Figure 1).

Gas Chromatography (GC). The gas chromatograph and the alkali flame ionization detector (AFID) used was similar to that described in a previous publication (Khan and Hamilton, 1980), except a Scot quartz capillary column (30 m × 0.5 mm) coated with 3% Carbowax 20M was used. The operating conditions were on column injections: injection port, column, and detector temperatures, 260, 176, and 300 °C, respectively. The helium carrier gas, makeup gas, hydrogen, and air flow rates were 5, 30, 4, and 150 mL/min, respectively. The identity of the compounds was confirmed by comparing the retention times with those of the authentic samples, cochromatography, and finally gas chromatography–mass spectrometry (GC–MS).

RESULTS AND DISCUSSION

Following a soil incubation period of 1 year and exhaustive methanol–water extraction as described, bound residues remaining in the soil amounted to 57.4% of the total applied ^{14}C (0.47 μCi/g of soil). More than half (54%) of the total bound residue in soil was in the form of prometryn as determined by the HTD technique (Khan and Hamilton, 1980). The remainder constituted hydroxypropazine (8%) and unidentifiable methanol-soluble material (18%), and 20% was thermally decomposed to $^{14}CO_2$ during distillation.

It was found necessary to fertilize the extracted soil containing the bound residues with Hoagland's nutrient solution during the growing period. Under these conditions the plants showed normal growth in the extracted soil when compared with those grown in the unextracted (control) soil. The crop of oat plants removed 0.53% of the previously soil bound ^{14}C in the soil–sand mixture used for growing the plants in this experiment.

The shoots of the oat plants contained 69.8% of the total plant radioactivity whereas the remaining radioactivity

(30.2%) was present in the roots. Table I shows the extractable and unextractable (bound) ^{14}C residues in the plant tissues as determined according to the scheme depicted in Figure 1. Amounts of total extractable ^{14}C residues were larger in roots, but were smaller in shoots accounting for 75.0% and 51.1%, respectively, of the total ^{14}C residue. The chloroform extracts (nonpolar residues) contained a very small proportion of ^{14}C, which constituted only a trace amount of unchanged prometryn (<0.05 ppm) as indicated by GC. Most of the methanol–water-soluble ^{14}C residues (polar residues) were present in the shoot and root samples in the conjugated form. This was indicated by the absence of any free metabolites in the eluate obtained by eluting XAD-2 column with methanol (Figure 1). However, hydrolysis of this eluate resulted in the release of a compound which after methylation with diazomethane was identified as the methoxy derivative of hydroxypropazine [2-methoxy-4,6-bis(isopropylamino)-*s*-triazine].

The data demonstrate that some of the radioactivity absorbed by plants from the soil containing bound ^{14}C residues became again bound (unextractable) in the plant tissues. Plant-bound ^{14}C residues were smaller in roots and greater in shoots amounting to 19.9% and 40.2%, respectively, of the total ^{14}C recovered from each tissue (Table I). GC analyses of the HTD distillates indicated the presence of a mono-N-dealkylated compound namely, 2-(methylthio)-4-amino-6-(isopropylamino)-*s*-triazine, and traces of 2-(methylthio)-4,6-diamino-*s*-triazine.

The extracted plant tissue containing bound ^{14}C likely contained mainly lignin, carbohydrate, and denatured protein. It became of interest to determine the distribution of plant bound ^{14}C in these fractions (Table I). The lignin extracted by the procedure described in Figure 1 comprises free and bound lignin. The latter is bound to the carbohydrate of the cell wall and could only be extracted by boiling with dioxane–HCl under N_2. The data show that the total lignin (free + bound) from roots and shoots contained 10.5% and 28.5%, respectively, of the total ^{14}C bound residues in the plant tissue. The remaining solid residue of roots and shoots which presumably consists of mainly carbohydrate and protein contained relatively smaller amounts of ^{14}C residues (4.7% and 7.5% of the total ^{14}C bound residues in roots and shoots, respectively). About 2% of the total ^{14}C was present in the aqueous supernatant, which may be due to the hydrolysis products and can be regarded as extractable residues. It is likely that most of the ^{14}C associated with the lignin or solid residues fraction was comprised of 2-(methylthio)-4-amino-6-(isopropylamino)-*s*-triazine. The latter was identified as the major product in the HTD distillates of plant tissues. In a similar study, Balba et al. (1977) observed that the isolated lignin fraction from rice plant roots contained a considerable proportion of 3-chloroaniline and 3,4-dichloroaniline residues. It has been suggested that lignin formation in plants, in addition of being a support material, also serves as an excretory system for plants to store toxic or unwanted foreign compounds by incorporating them into insoluble lignin (Fundersberg and Neish, 1968).

In accordance with the earlier studies (Fuhremann and Lichtenstein, 1978; Helling and Krivonak, 1978; Führ and Mittelstaedt, 1980), our data also support the view that bound pesticides residues can be released from soil and absorbed by the plants. These residues in plants may then become partially bound and may constitute a significant fraction of the total residue. Special attention should be given to this form of bound residue in plants as it would escape detection in the routine analysis. It is possible that the plant bound residue might become released on digestion of the contaminated food or it might significantly accumulate in crops growing in soil containing bound residues. The latter will not be detected in the routine analysis involving solvent extraction of soil.

ACKNOWLEDGMENT

The skilled technical assistance of W. R. McDowall and H. Lie and mass spectrometric analysis by S. I. M. Skinner are much appreciated.

LITERATURE CITED

Balba, H. M.; Still, G. B.; Mansager, E. R. 174th National Meeting of the American Chemical Society, Chicago, IL, 1977; American Chemical Society; Washington, DC, 1967.

Balba, H. M.; Still, G. G.; Mansager, E. R. *J. Assoc. Off. Anal. Chem.* **1979**, 237.

Führ, F.; Mittelstaedt, W. *J. Agric. Food, Chem.* **1980**, *28*, 122.

Fuhremann, W.; Lichtenstein, E. P. *J. Agric. Food Chem.* **1978**, *26*, 605.

Fundersberg, K.; Neish, A. C. "Constitution and Biosynthesis of Lignin"; Springer-Verlag: New York, 1968; p 129.

Helling, C. S.; Krivonak, A. E. *J. Agric. Food Chem.* **1978**, *26*, 1163.

Khan, S. U.; Hamilton, H. A. *J. Agric. Food Chem.* **1980**, *28*, 126.

Received for review March 27, 1980. Accepted July 14, 1980. Contribution No. 1163. This study was part of a coordinated program of research under the sponsorship of the International Atomic Energy Agency.