Extractable and Bound (Nonextractable) Residues of Prometryn and Its Metabolites in an Organic Soil

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A high-temperature distillation technique was developed for determining and chemically identifying the bound (nonextractable) residues in an organic soil treated with prometryn [2-(methylthio)-4,6-bis(isopropylamino)-*s*-triazine]. A steady decrease of extractable ^{14}C residues was accompanied by a corresponding increase of bound ^{14}C residues over a 150-day incubation period in the laboratory incubated soils treated with [^{14}C]prometryn. Similarly a decrease of extractable prometryn residues over a 345-day period in the field treated soil was accompanied by an increase of bound residues. Hydroxypropazine [2-hydroxy-4,6-bis(isopropylamino)-*s*-triazine] and deisopropylprometryn [2-(methylthio)-4-amino-6-(isopropylamino)-*s*-triazine] were the only solvent-extractable metabolites in the field samples, whereas the former was the only extractable metabolite in the laboratory incubated soil. Carrots grown in the field treated soil had very low residue levels of prometryn. A considerable portion of the bound radiolabeled residue in the incubated soil was identified as prometryn, whereas the remainder constituted unidentifiable products and $^{14}CO_2$. The latter was formed by the thermal decomposition of bound residues during high-temperature distillation. The bound residues will not be detected in the routine analysis involving solvent extraction of soil. Data demonstrate that special attention should also be given to bound residues in assessing the persistence or disappearance of pesticides in the organic soil.

It is a common observation that a portion of pesticide residues remains in soil after solvent extraction. This has been recently shown by using radiolabeled pesticides, indicating that a portion of ^{14}C residues are not extracted following incubation of pesticide-treated soils. The unextracted ^{14}C residues, referred to as "bound", are usually detectable by combustion of the extracted soil to yield $^{14}CO_2$. The significance of bound residues and their bioavailability is recently addressed by Lichtenstein and his co-workers (Lichtenstein et al., 1977; Katan and Lichtenstein, 1977; Fuhremann and Lichtenstein, 1978). Helling and Krivonak (1978a,b) recently reported on the physicochemical and biological characteristics of bound herbicides in soils. In view of these reports it appears that the total pesticide residues in soil may be underestimated as the bound residues will not be detected in routine analysis. There is no data available on the measurements of bound residues in the field treated soils. Therefore, development of an analytical method for determining bound residues is warranted to assess the total residue levels and their identity in field treated soil.

The herbicide, prometryn [2-(methylthio)-4,6-bis(isopropylamino)-*s*-triazine] is recommended for control of broad-leaf weed and grasses in vegetable crops. It has been observed that organic soils required higher rates of prometryn for weed control than those recommended for mineral soils. Our earlier studies have demonstrated the potential of some pesticide residues to persist in the organic soil and their uptake by vegetable crops (Khan et al., 1976a,b). The objective of the studies reported herein was to examine, under field and laboratory conditions, the persistence of prometryn and its metabolites in an organic soil and their uptake by carrots grown in the field treated plots. We also sought to develop an analytical method for measuring and chemically identifying the potential bound residues in soils. Laboratory soil incubation study was conducted with [^{14}C]prometryn in order to make it possible to detect bound ^{14}C-labeled residues by combusting or high-temperature distillation of the soil. The technique was then applied to field treated soil samples.

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EXPERIMENTAL SECTION

Field Experiment. The field experiments were conducted at the Ste. Clotilde experimental farm on a Humic Mesisol soil. The soil has 45.4% carbon, 2.4% nitrogen, 15.1% mineral matter, a bulk density of 0.34, and a pH of 5.2. The experimental plots were 1.4 × 6.1 m established on a previously untreated field. Prometryn (Gesagard 80 WP) was applied on May 18, 1977, as spray treatment to plots at a rate of 2.24 and 4.48 kg/ha. The control plots located adjacent to each of the treated plots received no herbicide. The plots were seeded to carrots (*Daucus carota* L. cv. Gold Pak) 1 day before treatment.

Soil and Crop Sampling. The plots were subdivided widthwise into three equal subplots from each of which six 10-cm deep cores of 10-cm diameter were taken at random and composited. The first set of samples was taken immediately after the herbicide was applied. Additional soil samples were taken on May 25, June 3, June 21, July 26, 1977, and May 2, 1978, representing 7, 16, 34, 64, and 345 days, respectively, after application of the herbicide. The samples were stored in sealed plastic bags in moist condition at −20 °C until analyzed. The water content of these samples ranged from 159 to 178% of oven-dry weight basis.

Representative samples of carrots were taken from each subplot after harvesting the crop on July 26, 1977, 65 days after the seeding. The tops and roots were separated and the tops discarded. The samples were washed with cold water to remove any adhering soil particles. Each carrot root was divided longitudinally into four parts resulting in four subsamples. One subsample was diced into small pieces and mixed thoroughly, and about 0.5 kg was stored in sealed plastic bags at −20 °C until they were analyzed.

Laboratory Experiment. Moist soil samples from control plots (20 g oven-dry weight basis) were placed in Erlenmeyer flasks (300 mL) to which 2 mL of acetone containing 164 μg of [^{14}C]prometryn (0.94 μCi) and 84 μg of unlabeled prometryn was added to give a herbicide concentration of 12.4 mg/kg. The solvent was evaporated

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with a gentle air stream and the soil was thoroughly mixed. The flasks were loosely stoppered with cotton wool and incubated at about 23 °C in the dark. Distilled water was added as necessary to maintain the initial moisture content of the soil samples. At intervals during a period ranging from 0 to 150 days, flasks were removed and soil samples analyzed.

Chemicals. All solvents were of pesticide grade and used as received. Reference standards of prometryn and metabolites, namely, hydroxypropazine [2-hydroxy-4,6-bis(isopropylamino)-*s*-triazine] and deisopropylprometryn [2-(methylthio)-4-amino-6-(isopropylamino)-*s*-triazine], and [^{14}C]prometryn were gifts from Ciba-Geigy Limited, Switzerland. The uniformly ring-labeled [^{14}C]prometryn (sp act., 5.7 μCi/mg) was examined by gas chromatography and was found to be 96% pure.

Determination of Extractable Residues. (1) Soil. Preliminary experiments indicated that maximum extraction efficiency was obtained when the soil had moisture level ranging from 150 to 200% prior to extraction with solvent. Therefore, in this study no attempt was made to air-dry the soil before extraction. The frozen soil in sealed plastic bags was thawed and brought to room temperature. Fifty grams of moist soil from field plots (moisture content predetermined) was placed in a 300-mL Erlenmeyer flask, extracted with 150 mL of methanol in a mechanical shaker for 2 h, and filtered under suction. The extraction was repeated two more times and filtered under suction into the same flask. Flasks removed at intervals from soil incubation experiment were also processed in a similar manner. Since extraction of field treated or [^{14}C]prometryn-treated laboratory incubated soil with methanol for more than three times removed no prometryn or ^{14}C residues, further extraction of the soil was considered unwarranted. The unextracted residues in the field treated soil or ^{14}C residues remaining in the incubated soil are hereafter referred to "bound". The sample residue (soil) was washed with methanol (3 × 75 mL), and the combined filtrate was concentrated to a small volume on a rotary evaporator at room temperature. An aliquot of this was analyzed by liquid scintillation counting while the remaining portion was evaporated to just dryness. Residual methanol from the extracted soil was allowed to evaporate by air-drying the sample and stored for determining the bound residues as described later. An aliquot of the 150-day air-dry extracted soil (incubation study) was mixed with enough water to bring the moisture content to the original level, allowed to stand overnight, and then exhaustively extracted with methanol. Analysis of the methanol extract revealed that ^{14}C residues were still bound to the soil and no radioactivity could be extracted. Thus, the procedure employed in this study was effective in removing all the extractable ^{14}C residues from the soil. Extraction of soil with other solvent systems such as acetone, acetone–methanol (1:1), methanol–HCl (9:1), acetone–hexane (1:1), or methylene chloride did not increase the extraction efficiency. Extraction of moist soil with methanol by mechanical shaking gave a better recovery of prometryn than blending for 10 min or Soxhlet extraction. The latter also extracted, similar to some of the solvent systems mentioned above, appreciable amounts of coextractive from soil and a considerable difficulty in cleanup of the sample was encountered. In addition, some of the dark extracts quenched the scintillation cocktail too strongly for analysis.

The dried methanol extract was dissolved in several portions of chloroform (5–10 mL) and placed on an acidic alumina column (24 × 70 mm, 20 g of acidic alumina oxide, Woelm, activity I) topped with 10 mm of anhydrous Na_2SO_4 and prewashed with chloroform. The column was first eluted with 300 mL of dried (anhydrous Na_2SO_4) chloroform (eluate I) and then with 300 mL of methanol (eluate II).

Eluate I was concentrated to about 10 mL on a rotary evaporator at room temperature and finally taken to dryness with a stream of dry air. The residue was dissolved in methanol, and an aliquot of solution was injected into the gas chromatograph.

Eluate II was concentrated to about 5 mL on a rotary evaporator at room temperature and methylated with diazomethane (Khan et al., 1975). The mixture was taken to near dryness in a gentle stream of dry air, the residue dissolved in hexane, and an aliquot analyzed by gas chromatography.

(2) *Carrots.* Chopped carrot samples (20 g) were extracted by blending at a high speed with dried chloroform (200 mL) for 5 min. The mixture was filtered under suction and the sample residue washed with 100 mL of chloroform. The combined filtrate (extract I) was evaporated to near dryness on a rotary evaporator, dissolved in several portions of hexane (5–10 mL), and transferred on an acidic alumina column topped with 10 mm of anhydrous Na_2SO_4 and prewashed with hexane, chloroform, and then hexane again. The column was first eluted with 25 mL of hexane and discarded. The column was then eluted with 300 mL of chloroform (eluate Ia) and finally with 300 mL of methanol (eluate IIa). The eluates were processed as described in the previous section for eluate I and II.

The insoluble residue of carrots was transferred into the blender and blended at a high speed first with 150 mL of methanol and then with 150 mL of water, filtering the mixture each time under suction. The combined filtrate was then processed for the determination of free and conjugated residues (Khan and Marriage, 1977). The solid carrot residue was air-dried and stored for determining the nonextractable or bound residues by the high-temperature distillation technique described later.

Determination of Bound Residues. A Lindberg Tube Furnace (Sola Basic S/B) was used for high-temperature distillation (HTD) of extracted soil (Figure 1). Air-dried soil sample (200–300 mg) containing bound residues was placed in a porcelain boat (0.2-cm width, 0.5-cm depth, 4 cm-length) and inserted in the middle of the quartz tube (30-cm length, 0.9-cm i.d.). One end of the tube was closed with a swagelok, while the other end was connected with a series of traps (Figure 1). The furnace was heated from room temperature to 800 °C (ca. 15 °C/min) and maintained at this temperature for about 15 min. Helium was used as the sweep gas at a flow rate of 45–50 mL/min. At the end of the experiment, the collection U-tube (trap II) was thoroughly washed with methanol. The quartz tube was also washed with methanol and washing combined with the first trapping solution (trap I). The material in different traps was then processed as depicted in Figure 2.

Soil samples containing bound ^{14}C residues were also combusted in a Packard sample oxidizer, Model 306, to produce $^{14}CO_2$. The latter was absorbed in and admixed with appropriate volumes of Carbo-Sorb and Permafluor V (Packard Instrumentation Canada Ltd.) and the radioactivity measured as described below.

Determination of Radioactivity. The radioactivity of aliquots of extracts was determined in a Packard Tri-Carb liquid scintillation spectrometer, Model 3320, using an external standard and correcting the data for quench-

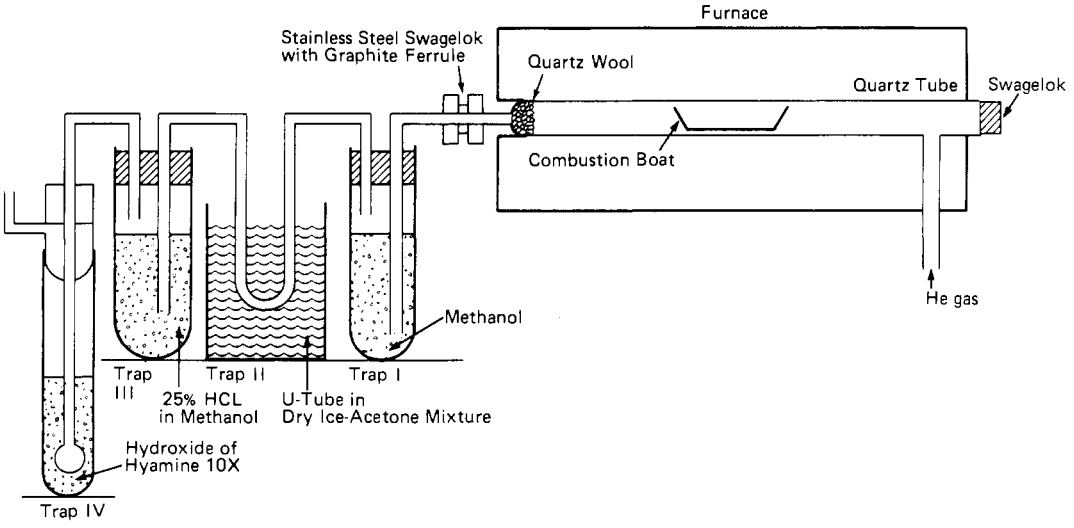

Figure 1. Apparatus used for high-temperature distillation of samples.

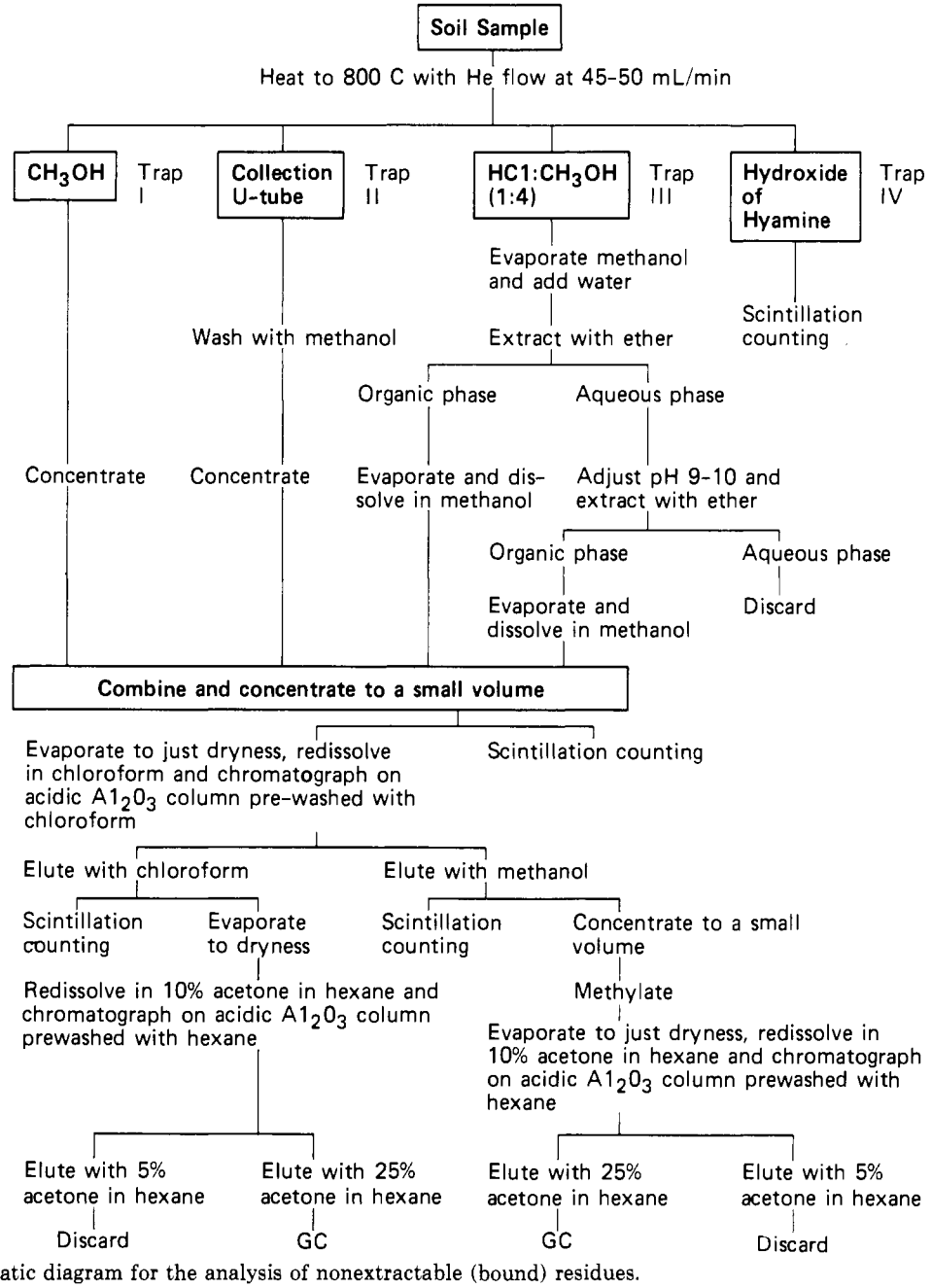

Figure 2. Schematic diagram for the analysis of nonextractable (bound) residues.

ing. The activity was measured in a scintillation solution containing PPO and POPOP in toluene (5 g, 50 mg, 1000 mL).

Gas Chromatography (GC). The gas chromatograph and its operating conditions were similar to those described earlier (Khan and Marriage, 1977). The retention times of prometryn, prometone (2-methoxy derivative of hydroxypropazine) and deisopropylprometryn were 7.1, 3.7, and 15.9 min, respectively. The compounds gave a 50% full-scale deflection in the 6–10-ng range.

Gas Chromatography–Mass Spectrometry (GC–MS). A GC–MS system similar to that described earlier was used for the identification of compounds (Khan and Marriage, 1977). A synthetic mixture of the suspected metabolites of prometryn was prepared from the reference compounds, and the mass spectra were obtained. The experimental samples were analyzed under identical conditions and the mass spectra of prometryn and/or metabolites were compared with those of the reference compounds.

Performance of the Methods. The recoveries of the residues by the methods used were determined by adding a mixture of prometryn and the metabolites at 0.01, 0.1, and 1.0 mg/kg and 0.01, 0.05, and 0.1 mg/kg levels, to control soil and carrot samples, respectively. The solvent was allowed to evaporate and the sample mixed thoroughly. Further processing of the samples for determining extractable residue was done as described above.

The recoveries of the residues by HTD technique were determined by adding prometryn to the air-dry control soil at 0.5 and 1.0 mg/kg, hydroxypropazine at 5.0 and 10.0 mg/kg levels, and prometryn to carrot samples at 0.5 and 1.0 mg/kg levels. The samples were processed as described earlier.

In this study all samples were extracted and analyzed in duplicate or triplicate, and average values are reported. Residue levels in soils are reported on an oven-dry basis and in carrots on fresh weight basis. The results reported here are not corrected for recovery.

RESULTS

Identification of Compounds. The identities of the compounds in extracts of samples represented by GC peaks were confirmed by cochromatography with authentic standards and finally by GC–MS analysis. The predominant ions in the mass spectra of materials represented by GC peaks were $(M)^{+\cdot}$, $(M - CH_3)^{+\cdot}$, $(M - C_3H_6)^{+\cdot}$, and/or $[M - (C_3H_6 + SCH_3)]^{+\cdot}$ with further fragmentation analogous to the spectra obtained for authentic samples. Thus, the compounds represented by peaks at retention times 3.7, 7.1, and 15.9 min were identified as the 2-methoxy derivative of hydroxypropazine, prometryn, and deisopropylprometryn, respectively.

Performance of the Methods. The recoveries of extractable prometryn, hydroxypropazine, and deisopropylprometryn added to the control soil ranged from 90 to 97%, 56 to 69%, and 80 to 85%, respectively. The corresponding recoveries for carrot samples were 72 to 85%, 33 to 39%, and 60 to 68%, respectively. Preliminary experiments showed that hydroxypropazine was converted to its methoxy analogue in 60–80% yield. Thus, the data reported for residues for hydroxypropazine should only be regarded as qualitative. However, it should be noted that in preliminary experiments, little loss of prometone (methoxy analogue of hydroxypropazine) was observed during the working procedure.

HTD of air-dried control soil to which prometryn and hydroxypropazine was added resulted in 80–81% and 50–55% recoveries of the compounds, respectively. Similarly, HTD of carrots fortified with prometryn at 0.5 and 1.0 ppm levels gave a recovery of 76–78%. It should be pointed out that in preliminary experiments, HTD of 30 μg of reference standards of prometryn or hydroxypropazine resulted in a recovery of 70–82%. It was observed that air or nitrogen used as the sweep gases resulted in a considerable lower recovery of the compounds. In general, better recoveries were obtained for prometryn by the HTD technique than for hydroxypropazine. This may be due to the greater volatility of the former and its more rapid removal from the quartz tube before substantial thermal decomposition occurred. It was also observed that trap I contained 60–88%, trap II 10–25%, and trap III only 2–4% of the total prometryn or hydroxypropazine recovered by HTD (Figure 1).

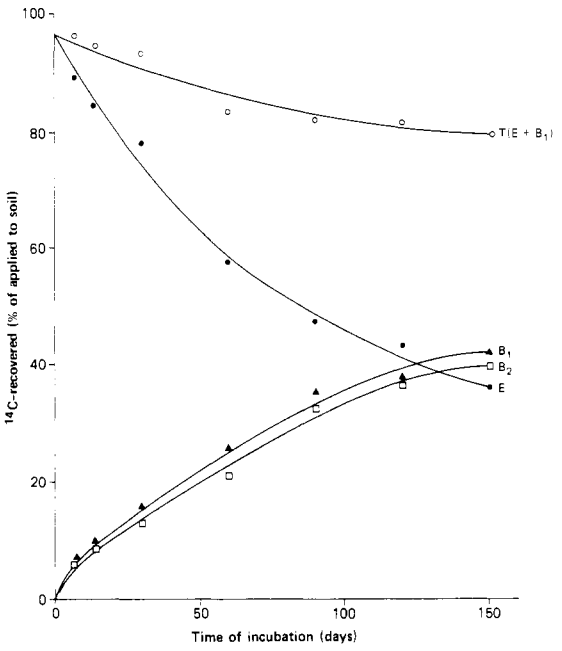

Figure 3. Extractable and bound residues of ^{14}C-labeled prometryn in an organic soil during a 150-day incubation period, after soil treatment at 12.4 mg/kg (0.94 μCi). Curve E, extracted ^{14}C; curve B_1, bound ^{14}C determined by combusting soil to $^{14}CO_2$; curve B_2, bound ^{14}C determined by high-temperature distillation technique; and curve T, total of extractable (E) and bound (B_1).

Soil Incubation Study. The amounts of the extractable ^{14}C residues recovered from soil decreased over an incubation period of 150 days (Figure 3). This in turn, corresponded to an increase in the formation of soil bound ^{14}C residues. Thus, by the end of the incubation period, extractable ^{14}C residues decreased to 36.5% while the bound ^{14}C residues (determined by combustion to $^{14}CO_2$) increased to 43.0% of the initially added ^{14}C. The total radioactivity recovered at the end of 150 days amounted to about 80% of that initially applied. Similarly, the radioactivity recovered by HTD of samples increased with incubation time and by the end of 150 days amounted to about 40% of the initially added ^{14}C. However, the amounts of ^{14}C recovered by HTD were slightly lower than those obtained by combustion to $^{14}CO_2$ (Figure 3).

The amount of radioactivity in the combined material from traps I, II, and III (Figure 1 and 2) was 73.9–80.9% of the total ^{14}C released by HTD. The remaining radiocarbon was thermally decomposed to $^{14}CO_2$ as evidenced by the analysis of hyamine hydroxide (trap IV). Analysis of the combined material (traps I, II, and III) according to the scheme depicted in Figure 2 revealed that over an incubation period of 150 days the radioactivity of the chloroform-soluble material decreased from 88.8 to 62.1%

Table I. Persistence of Prometryn and Metabolites in an Organic Soil (mg/kg)[a]

application rate, kg/ha	days after application					
	0	7	16	34	64	345
			Prometryn (extractable)			
2.24	9.7 ± 1.8	6.2 ± 1.0	3.7 ± 0.6	3.0 ± 0.8	2.5 ± 0.6	1.6 ± 0.2
4.48	17.8 ± 2.1	11.7 ± 1.4	6.2 ± 0.5	5.3 ± 1.9	3.9 ± 0.2	2.4 ± 0.7
			Prometryn (nonextractable or bound)			
2.24	0.08 ± 0.03	0.4 ± 0.1	0.5 ± 0.1	1.1 ± 0.3	1.3 ± 0.3	*b*
4.48	0.20 ± 0.05	1.2 ± 0.2	1.3 ± 0.2	2.3 ± 0.3	2.9 ± 0.4	*b*
			Hydroxypropazine (extractable)			
2.24	0.10 ± 0.03	0.3 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.8 ± 0.1	0.8 ± 0.2
4.48	0.21 ± 0.10	0.5 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	1.1 ± 0.1	0.9 ± 0.3
			Deisopropylprometryn (extractable)			
2.24	ND[c]	0.07 ± 0.01	0.05 ± 0.01	0.06 ± 0.02	0.06 ± 0.01	ND[c]
4.48	ND[c]	0.12 ± 0.01	0.14 ± 0.01	0.12 ± 0.04	0.14 ± 0.01	0.13 ± 0.01

[a] Mean values with standard errors for duplicate samples from each of the three plots. [b] Sample lost in storage due to freezer breakdown. [c] Nondetectable, less than 0.001 mg/kg.

of the total in the three traps. A corresponding increase in radioactivity from 16.9 to 25.9% was observed in the methanol-soluble material. GC and GC–MS analyses indicated that the chloroform eluate contained mainly prometryn. However, we were unable to confirm the identity of the ^{14}C material in the methanol eluate because of the low levels and unavailability of some reference standards. Contrary to our expectation, 2-hydroxy analogues of prometryn were not detected in the methanol eluate.

GC examination of the methanol-extractable residues from the incubated soil indicated that the total amounts of prometryn decreased from 95 to 23% of the initially applied herbicide, whereas that of hydroxypropazine (calculated in terms of prometryn) increased from 1.7 to 9.4% over the incubation period (Figure 4). Furthermore, analyses of the high-temperature distillates by GC indicated that the amounts of bound prometryn increased from 0.2 to 19.4% of the initially applied herbicide. Thus, the total recovery of residues (extractable + bound) at the end of an incubation period of 150 days was about 52% of the applied prometryn.

Field Study. Table I shows the decline of extractable prometryn residues over 345 days after its application. The higher rate of application of prometryn (4.48 kg/ha) resulted in higher initial amounts of the extractable and bound residues in the field treated soil. During the first week after application at the two different rates, the extractable residues of prometryn in the organic soil declined by about 35%. The corresponding bound prometryn concentrations after 7 days were about 4 and 7% of the initial extractable amounts from the lower and high application rates, respectively. During the first 2 weeks after application of the herbicide, a substantial loss of the extractable prometryn residues (more than 60% of the initial amounts recovered) occurred. After this rapid loss the disappearance of the extractable prometryn was slow. While a parallel exists between the disappearance of extractable prometryn residues in the laboratory incubated soils (Figures 3 and 4) and field treated soils (Table I), the loss was nearly doubled in the field during the first 2 months. Thus, at the end of the first growing season (64 days after application), an extractable residue concentration of 2.5 and 3.9 mg/kg or 26 and 22%, respectively, of the initial extractable amounts of prometryn from the two application rates remained in the soil (Table I). The corresponding bound residue concentrations of prometryn were 1.3 and 2.9 mg/kg, which represent an increase in bound residues and amounted to about 13 and 16%, respectively, of the initial extractable amounts recovered. It should be noted that although at higher rates of application (4.48 kg/ha) the bound residues in terms of percent of the initial extractable amounts were only slightly higher than the corresponding amounts at lower rates (2.24 kg/ha), the absolute amounts of the bound residues were 50–60% greater at the higher application than at lower rates.

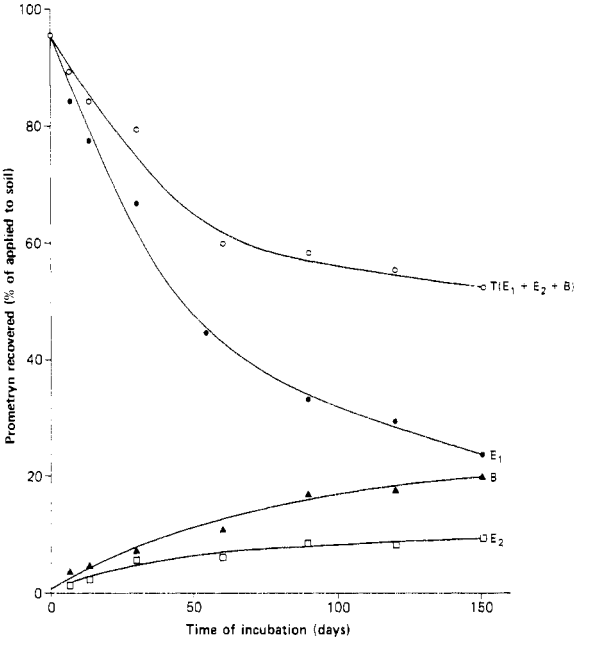

Figure 4. Extractable and bound residues of prometryn and hydroxypropazine in an organic soil during a 150-day incubation period, after soil treatment at 12.4 mg/kg. Curve E_1, extractable prometryn; curve E_2, extractable hydroxypropazine (values are converted to prometryn by multiplying with 1.14); curve B, bound prometryn determined by high-temperature distillation technique; and curve T, total of extractable prometryn (E_1), hydroxypropazine (E_2) and bound (B).

DISCUSSION

Soil Incubation Study. The analytical methods employed in earlier investigations for quantitating nonextractable or bound ^{14}C residues involved combustion of the soil to release $^{14}CO_2$. However, this technique results in the destruction of bound residue identity. The bound residues are not detected in routine analysis, but they can be released from soils and absorbed by plants (Fuhremann and Lichtenstein, 1978). Thus, development of an analytical technique for identifying the bound residues was apparently warranted to determine whether or not the bound residues consisted of intact pesticides or some

degradation products which may have been adsorbed, incorporated, or entrapped in the soil matrix. In the present investigation, a technique was developed involving high-temperature distillation (HTD) in order to release bound ^{14}C residues from the incubated soil samples. The released ^{14}C was collected in suitable solvents, purified, and analyzed by GC and GC–MS (Figures 1 and 2). Whereas some of the ^{14}C bound residues were thermally decomposed to $^{14}CO_2$ during high-temperature distillation, the remainder (74–81%) constituted parent compound and some unidentifiable products. The latter can be identified and quantitated by suitable techniques.

Bound residues in soils and plants have been the focal point of several recent investigations (Van Alfen and Kosuge, 1976; Katan et al., 1976; Katan and Lichtenstein, 1977; Lichtenstein et al., 1977; Helling and Krivonak, 1978a,b). Lichtenstein et al. (1977) observed that the binding of insecticide residues to soil was increased with incubation time. They found that methyl [*ring*-2,6-^{14}C]-parathion was rapidly bound to loam soil, where up to 41% of the applied ^{14}C insecticide residue could not be extracted after a 7-day incubation period. They also observed that [*ring*-^{14}C]fonofos, another organophosphorus insecticide, was bound to the soil amounting to 35.3% of the applied insecticide at the end of 28 days incubation period. These observations are in agreement with our data for [^{14}C]-prometryn in organic soil (Figure 3).

The disappearance of radiocarbon added to the soil could not be accounted for only by the extractable and bound ^{14}C residues (Figure 3). Thus, the loss of radiocarbon due to volatilization, possibly due to evolution of $^{14}CO_2$ and volatile degradation products, indicates mineralization or ring cleavage of prometry in the incubated soil. Liberation of $^{14}CO_2$ from the ring-labeled *s*-triazine-treated soils has been observed in other studies (Esser et al., 1975). The cumulative amount of $^{14}CO_2$ liberated varied with the soil type. Thus, in a sandy loam soil treated with ^{14}C-ring-labeled atrazine it was shown that after a long phase of about 1 month, approximately 18% of the incubated ^{14}C was liberated as $^{14}CO_2$ within 18 months (Esser et al., 1975). Our data suggest that $^{14}CO_2$ and/or volatile degradation products were evolved from [^{14}C]prometryn up to about 20% of the applied radioactivity within 0–150 days. It may be pointed out that mineralization occurs after the advent of hydrolysis for *s*-triazine herbicides (Kaufman and Kearney, 1970). Furthermore, as the number of hydroxyl groups on the *s*-triazine ring increased, as in the series from ammeline, ammelide to cyanuric acid, the percentage of mineralization increases (Hauck and Stephenson, 1964).

The data presented in Figure 4 indicate that the observed decrease in the extractable prometryn concentration in the laboratory-incubated organic soil with time was the result of degradation, binding to soil (bound residues), and dissipation via volatilization processes. The degradation of prometryn in the aerobically moist organic soil samples was likely due to a combination of chemical and microbiological processes. Hydroxypropazine was the only extractable degradation product observed in the laboratory experiment and its amounts increased with time of incubation (Figure 4). Formation of hydroxypropazine in prometryn treated soil incubation studies has also been demonstrated in earlier studies by Kearney and Plimmer (1970) and Plimmer et al. (1970). In addition, these workers also observed the occurrence of trace amounts of transient oxidation products of prometryn such as the sulfone and sulfoxide derivatives. In the present study we were unable to detect the presence of transient sulfoxide and sulfone intermediates in the methanol extracts of the organic soil. It should be noted that these oxidized forms of prometryn are quite unstable and readily hydrolyze to hydroxypropazine in the presence of water (Kaufman and Kearney, 1970).

Over an incubation period of 150 days the percent recovery of the extractable ^{14}C residues from the soil was slightly higher than that of the total extractable prometryn and hydroxypropazine (Figures 3 and 4). This indicates that either a small proportion of the applied prometryn had been metabolized to unidentified extractable products, or the material was lost during the workup procedure for GC analysis. Similarly, the percent of the total bound radiocarbon recovered by HTD (curve B_2, Figure 3) was considerably higher (0.3–39.8%) than the percent of prometryn recovered in the high-temperature distillates (curve B, Figure 4) as determined by GC (0.2–19.4%). This suggests that in addition to thermal decomposition of the bound residues to CO_2 during high-temperature distillation, some of the bound material was also released and collected in the traps which could not be identified by the techniques employed in this study.

Field Study. Table I shows the decline of extractable prometryn residues in the field soil over 345 days after its application. An initial relatively rapid loss of prometryn from the soil surface during the first 3–40 days has been observed by Walker (1976) on a sandy loam soil. Volatilization has been shown to account for a significant loss of atrazine from soil under certain conditions (Kearney et al., 1964). Since the vapor pressure of prometryn is greater than that of atrazine (Jordon et al., 1970), such losses might have also occurred under the field conditions in the present study. Significant injury to cotton seedlings by prometryn vapors in closed containers following application of the herbicide to the surface soils have been observed (Talbert et al., 1971). It appears unlikely, however, that the rapid loss of extractable prometryn observed in the field treated soils could be accounted for by volatilization alone. Photodecomposition of prometryn in the field treated soil appears to be unlikely as the herbicide absorbs in the region 220 nm much below the natural sunlight radiant energy at 295 nm (Kearney, 1970). In view of the foregoing, it appears that the decrease in extractable prometryn residues from field soils was due to a combination of factors such as volatilization, degradation, and possibly rapid elimination of some degradation products, formation of breakdown products that are not detectable by the technique employed, leaching, binding to soil to form bound residues, and/or plant uptake. The disappearance of prometryn in the field treated soil samples due to degradation and formation of bound residues warrants further discussion.

The presence of extractable metabolites of prometryn in the field treated soil was checked by GC and GC–MS. We were able to identify in the methanol extracts of soils only two metabolites, namely hydroxypropazine and deisopropylprometryn (Table I). The decrease in the extractable prometryn residues with time corresponded to an increase in extractable hydroxypropazine content in the field soil. Thus, at the end of 345 days after application at 2.24 and 4.48 kg/ha, a residue concentration of 0.8 and 0.9 mg/kg, respectively, was observed. However, unlike hydroxypropazine, the contents of deisopropylprometryn remained nearly unchanged during this period. The data shown in Table I suggest that in the field treated soils both hydrolysis and partial N-dealkylation were active as evidenced by the presence of extractable residues of metabolites containing both 2-hydroxy and 2-methylthio moie-

ties. Furthermore, metabolic dealkylation occurred on one of the isopropylamino groups. It was thought that both dealkylated and hydroxylated prometryn would be subjected to further degradation to give rise to the common metabolite, namely, 2-hydroxy-4,6-diamino-*s*-triazine. However, we were unable to confirm the identity of this compound in the methanol extracts of the field treated soil.

The application of HTD technique to the field treated soil samples made it possible to release the bound prometryn residues. The latter were collected in suitable solvents, purified, and analyzed by GC and GC–MS. The bound prometryn residues in the field samples (Table I) will not be detected in the routine residue analysis involving exhaustive solvent extraction of soil samples. This would result in an underestimation of the total prometryn residues in soil. It is of interest to note that 64 days after treatment (harvest time) with the herbicide at 2.24 and 4.48 kg/ha, the total prometryn concentration determined in soils constituted about 66 and 57% extractable and 34 and 43% bound residues, respectively. We were unable to detect any bound identifiable degradation products in the field soil by the technique employed.

Residue in Crop. A very low concentration of prometryn residues (0.012 ± 0.002 mg/kg) was found at the harvest time in the whole carrots grown in soil that received the herbicide at 4.48 kg/ha. However, at the lower rate of application (2.24 kg/ha) only a trace amount of prometryn was found by GC, the identity of which could not be confirmed by GC–MS. The ratio of the herbicide concentration in the whole carrots to prometryn residue at the harvest time was about 0.003. Our results suggest that the residues of prometryn were absorbed by carrots grown in the soil which received a high rate of application (4.48 kg/ha). The herbicide was present in the form of free state. No residue of prometryn or metabolites was detected in the form of any conjugates. HTD of the extracted carrots did not indicate the presence of any bound or conjugated residues.

CONCLUSION

The results of this study show that prometryn is moderately persistent in the organic soil under study. The metabolism of prometryn in the field soil involves hydrolysis and partial N-dealkylation. However, under laboratory conditions the latter reaction was not observed. Carrots grown in the organic soil treated at twice the recommended rate will have little residue of prometryn which is very likely to be classified negligible. However, the long-term safety from the repeated use of prometryn on organic soils should be considered when the latter are used for vegetable production. The high-temperature distillation technique developed in this study enables the identification and measurement of bound residues in the field treated soils. Contrary to the general consensus that the unextractable or bound pesticide becomes an integral part of the matrix without recognizable relationship to the original compound, our data demonstrate that a considerable proportion of such residues in soil was comprised of the parent molecule. Since the bound prometryn constituted a significant part of the total herbicide residues in soils, special attention should also be given to this form of residues in assessing the disappearance of pesticides in soil. Release of soil-bound residues and their uptake by plants have been demonstrated in recent studies (Fuhremann and Lichtenstein, 1978; Helling and Krivonak, 1978b). Thus, it is conceivable that soil-bound residues may continue to build up, may affect soil organisms, and/or may be released by changes in cultural practices, thereby reentering into the soil solution and subsequent uptake and translocation into crops.

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