Distribution and Characteristics of Bound Residues of Prometryn in an Organic Soil

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Uniformly ^{14}C-ring-labeled prometryn [2-(methylthio)-4,6-bis(isopropylamino)-s-triazine] was incubated with an organic soil under laboratory conditions for 1 year. After exhaustive solvent extraction, the soil containing bound (nonextractable) ^{14}C-labeled residues was fractionated into humic substances by alkali extraction followed by acid precipitation. A considerable proportion of ^{14}C-labeled residues in humin and humic acid was comprised of the parent herbicide and its mono-N-dealkylated metabolite. However, the soluble fulvic acid fraction contained an appreciable amount of the 2-hydroxy analogue of prometryn. Thermoanalytical methods were used to obtain information on the nature and location of ^{14}C-labeled bound residues in soil and humic materials. Exhaustive methylation of humin and humic acid released some of the bound ^{14}C-labeled residues from these fractions. Bound ^{14}C-labeled residues in aqueous suspensions of humin or humic acid were stable to UV light. However, UV irradiation of the soil suspension resulted in the release of some of the bound ^{14}C-labeled residues which were subsequently decomposed to hydroxy analogues.

To assess the environmental significance of pesticide residues in soils, one must distinguish between two types of residues: those that are extractable with solvents and those that are not extractable with solvents. Bound pesticide residues are, basically those residues remaining in soil after exhaustive solvent extraction. Increasing evidence suggests that bound pesticide residues are not excluded from environmental interactions. A number of studies have demonstrated the potential availability of bound pesticide residues to plants (Führemann and Lichtenstein, 1978; Helling and Krivonak, 1978; Führ and Mittelstaedt, 1980; Khan, 1980a) and earthworms (Führemann and Lichtenstein, 1978). A recent study from our laboratory demonstrated that bound pesticide residues are susceptible to biodegradation by soil microbes (Khan and Ivarson, 1981).

Previously we reported that the formation of bound residues of radiolabeled prometryn [2-(methylthio)-4,6-bis(isopropylamino)-s-triazine] in an organic soil was enhanced with an increase in incubation time (Khan and Hamilton, 1980). Furthermore, a considerable portion of the bound ^{14}C-labeled residues in the incubated organic soil was identified as prometryn. The present work extends these investigations to determine the distribution of bound ^{14}C-labeled residues among the soil organic matter fractions. The release of ^{14}C as a function of temperature was also studied to obtain some clue as to the location and nature of bound residues. Finally, experiments were conducted on the photochemical release of bound ^{14}C-labeled residues under UV irradiation.

EXPERIMENTAL SECTION

Soil. Bound soil residues were produced as described in an earlier study (Khan and Hamilton, 1980). Briefly, moist soil samples treated with ^{14}C-ring-labeled prometryn (0.047 μCi/g) were incubated for 1 year at about 23 °C in the dark. The soil was then exhaustively extracted with solvents, and the extracts were dried and pooled together. The soil was again moistened with water, incubated, and extracted with methanol (Khan and Hamilton, 1980). Residual methanol in 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 total bound ^{14}C-labeled residue was determined by combustion of the air-dry soil to ^{14}CO$_2$.

Fractionation of Soil. The method of extraction, separation, and purification of humic materials outlined in Figure 1 is analogous to that described by Schnitzer and Khan (1972). The humic acid (HA), fulvic acid (FA), and humin (base-insoluble) fractions were freeze-dried. The yield of humin, HA, and FA amounted to 0.67, 0.04, and 0.12 g/g of soil, respectively.

Isothermal Thermoanalytical Study. (1) Soil. The high temperature distillation apparatus described earlier was used for isothermal heating (Khan and Hamilton, 1980). An air-dried soil sample (200–300 mg) containing ^{14}C-labeled bound residues was placed in a porcelain boat and inserted in the middle of the quartz tube preheated to the desired temperature. Helium or air was used as purge gas at a flow rate of 40–50 mL/min. The sample was heated at the desired temperatures isothermally for a period until no further release of ^{14}C was observed in the methanol and CO$_2$ traps (Khan and Hamilton, 1980). In preliminary experiments it was observed that 45 and 60 min of heating were adequate for a maximum release of ^{14}C from the soil under a stream of air and helium, respectively. In some cases samples were also heated under oxygen stream and covered by a combustion catalyst (CuO–Al$_2$O$_3$). At the end of the heating period, the radioactivity of the trapping solutions was determined by liquid scintillation counting.

(2) *Humic Materials.* Freeze-dried humin sample (50 mg) containing ^{14}C-labeled bound residues was heated isothermally under a stream of air, nitrogen, or helium as described above for a fixed period of 60 min. Preliminary experiments demonstrated that heating for a longer period did not increase the ^{14}C release. At the end of the experiment, the char resulting from heating was removed and analyzed for C, H, N, and S. Oxygen was calculated by difference. The radioactivity of the trapping solutions was also determined. The yields of HA and FA obtained from soil by the fractionation procedure used in this study were not adequate to allow for the thermal analysis of these materials.

UV Irradiation. (1) Soil. Soil samples (1 g) containing ^{14}C-labeled bound residues were irradiated with UV light by using a 15-W General Electric 45-cm germicidal lamp (253.7 nm). The suspension (soil–water, 1:600) was placed

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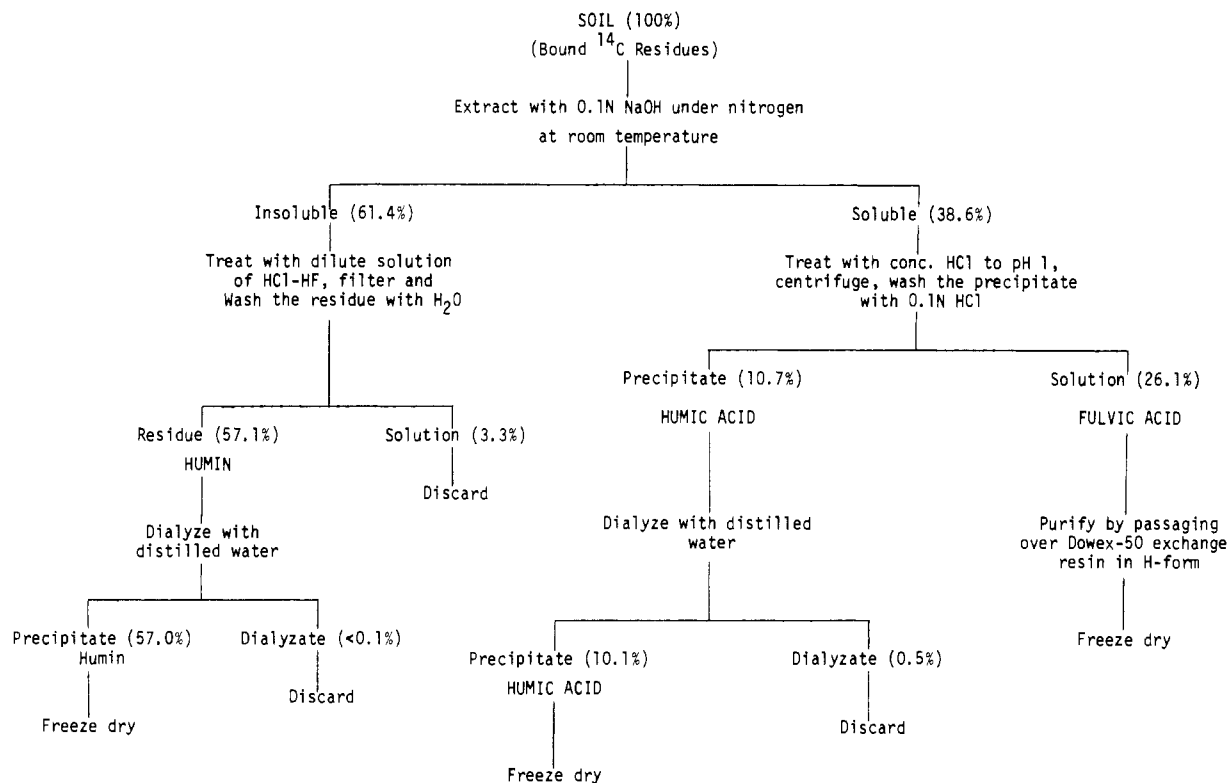
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                                        SOIL (100%)
                                  (Bound ¹⁴C Residues)
                                           │
                              Extract with 0.1N NaOH under nitrogen
                                    at room temperature
                                           │
              ┌────────────────────────────┴────────────────────────────┐
        Insoluble (61.4%)                                          Soluble (38.6%)
              │                                                          │
     Treat with dilute solution                              Treat with conc. HCl to pH 1,
        of HCl-HF, filter and                                 centrifuge, wash the precipitate
     Wash the residue with H₂O                                     with 0.1N HCl
              │                                                          │
              │                                       ┌──────────────────┴──────────────────┐
              │                                 Precipitate (10.7%)                   Solution (26.1%)
              │                                    HUMIC ACID                         FULVIC ACID
       ┌──────┴──────┐                                  │                                  │
  Residue (57.1%)  Solution (3.3%)                      │                                  │
      HUMIN              │                       Dialyze with distilled                Purify by passaging
              │       Discard                          water                        over Dowex-50 exchange
       Dialyze with                                     │                             resin in H-form
     distilled water                                    │                                  │
              │                                         │                                  │
     ┌────────┴────────┐                     ┌──────────┴──────────┐                  Freeze dry
Precipitate (57.0%)  Dialyzate (<0.1%)  Precipitate (10.1%)   Dialyzate (0.5%)
    Humin                  │                HUMIC ACID               │
       │               Discard                   │               Discard
  Freeze dry                                 Freeze dry
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Figure 1. Flow diagram of the fractionation of soil containing bound ¹⁴C-labeled residues. Numbers in parentheses show the percentage of ¹⁴C relative to the total ¹⁴C-labeled bound residues in soil.

15 cm below the lamp, continuously stirred with a magnetic stirrer, and irradiated for 216 h. Preliminary experiments indicated that under the experimental conditions described UV irradiation of soil suspension for a longer period or at higher water contents did not result in any further release of ¹⁴C in solution. At the end of the experiment, the soil suspension was centrifuged and the soil residue was exhaustively extracted with methanol. The aqueous supernatant was combined with the methanol extract and concentrated to a small volume on a rotary evaporator. An aliquot of this was analyzed by liquid scintillation counting while the remaining portion was evaporated to dryness. The material was then dissolved in chloroform and processed for gas chromatographic and mass spectrometric analyses as described in a previous publication (Khan and Hamilton, 1980). Residual methanol from the extracted soil was allowed to evaporate by air-drying the soil sample, and a portion of it was combusted to ¹⁴CO₂ to determine the remaining total ¹⁴C. The other portion was analyzed by the high temperature distillation (HTD) technique (Khan and Hamilton, 1980). The control, consisted of soil containing ¹⁴C-labeled bound residues, was also processed as described above except the suspension was kept in dark.

(2) Humic Materials. HA or humic sample (10 mg) was suspended in 10 mL of distilled water, stirred, and irradiated with UV light for 96 h as described before. Concurrently, samples were also run in the dark. The suspension was centrifuged, the solid residue washed with water, and the aqueous phases were combined. The solid residue after air-drying was combusted to ¹⁴CO₂ for determining the ¹⁴C remaining in the bound form. The aqueous phase was concentrated to a small volume and analyzed by liquid scintillation counting.

Exhaustive Methylation of HA and Humin. The HA or humin sample was suspended in methanol and methylated with freshly prepared diazomethane repeatedly for several days until the ¹⁴C released in solution remained constant. The mixture was filtered and the sample residue

washed with methanol several times. The combined filtrate was concentrated to small volume and analyzed by liquid scintillation counting. The dried solid residue was combusted to ¹⁴CO₂ for determining the remaining total ¹⁴C-labeled residues.

Determination of Radioactivity. Combustion of dried soil, HA, or humin was done in a Packard sample oxidizer, Model 306, to produce ¹⁴CO₂. Aliquots of various extracts or solutions (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 using [¹⁴C]prometryn.

Gas Chromatography (GC). The gas chromatograph was equipped with an alkali flame ionization detector and a scot quartz capillary column. The operating conditions were similar to those described earlier (Khan, 1980a). 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

Distribution of ¹⁴C in Humic Materials. The soil contained 57.4% bound ¹⁴C of the total applied radioactivity following an incubation period of 1 year. The bound residues became distributed among the various soil organic matter fractions. Thus, proportions of total bound ¹⁴C in humin, HA, and FA fractions were 57.0%, 10.1%, and 26.1%, respectively (Figure 1). The incorporation of ¹⁴C-labeled bound residues into humic materials observed in this study is consistent with those reported recently for other pesticides (Helling and Krivonak, 1978; Harvey and Han, 1978; Murthy et al., 1979; Spillner et al., 1979; Smith and Muir, 1980). The ¹⁴C-labeled residues were concentrated in the humin fraction (Figure 1). Furthermore, only a small amount of ¹⁴C (3.3%) was released in solution by solubilizing the mineral fraction from humin by the

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Table I.   Bound Residues in Humic Materials

fraction	prometryn, ppm	hydroxy-propazine, ppm	deisopropyl-prometryn, ppm
humic acid	0.3	<0.1	0.2
fulvic acid	0.3	0.9	<0.1
humin	1.9	ND[a]	1.1

[a] Nondetectable; <0.01 ppm.

HCl–HF treatment. Average molecular weight of the bound ^{14}C-labeled residues associated with humin or humic acid appeared to be greater than $\simeq 12000$ as very little ^{14}C was dialyzable. The presence of ^{14}C-labeled bound residues in the FA fraction (26.1%) is of special interest. The latter is a naturally occurring water-soluble low molecular weight polyelectrolyte and is considered to be the dominant soluble organic fraction present in the soil solution under field conditions. FA is known to be present in many surface waters and imparts a yellow to brown color in natural water (Christman and Minear, 1971; Schnitzer and Khan, 1972). Thus, FA-bound residues would be expected to become bioavailable to both plants and exposed aqueous or soil fauna.

Soil-bound residue has been defined as "that unextractable and chemically unidentifiable pesticide residue remaining in FA, HA, or humin fractions after exhaustive sequential extraction with non-polar organic and polar solvents" (*Fed. Regist.*, 1975). The general consensus appears to be that the bound pesticide residue becomes an integral part of the matrix without recognizable relationship to the original compound. In our study the freeze-dried humin, HA and FA samples (Figure 1) were analyzed by a high temperature distillation technique (Khan and Hamilton, 1980). The material released in various traps was analyzed by GC and GC–MS. A considerable proportion of ^{14}C-labeled residues in humic materials was comprised of the parent herbicide and its monodealkylated product, namely, 2-(methylthio)-4-amino-6-(isopropylamino)-*s*-triazine (Table I). However, the FA fraction contained an appreciable amount of the 2-hydroxy analogue of prometryn [2-hydroxy-4,6-bis(isopropylamino)-*s*-triazine]. The distillates from humin and humic acid also contained trace amounts of the dideal-kylated analogue of prometryn [2-(methylthio)-4,6-diamino-*s*-triazine] as evidenced by GC analysis. However, their presence could not be confirmed by GC–MS due to the lack of sufficient material in the sample. Alkaline extraction with 0.1 N NaOH under nitrogen, widely used for the extraction of humic materials from soil, was thought to hydrolyze or chemically degrade the herbicide residues in the soil. However, our preliminary experiments revealed nearly quantitative recoveries of unchanged prometryn from aqueous solutions when the herbicide was extracted with 0.1 N NaOH (pH $\simeq 13$). Hitchings and Roberts (1979) used 1.25 N aqueous NaOH solution at 80 °C for 2 h for extraction of humic materials containing bound residues and were able to identify small amounts of the total radioactivity in HA.

Thermoanalytical Study. Isothermal heating has been used for investigating the mechanism of thermal decomposition of organic matter (Schnitzer and Hoffman, 1965). Thermoanalytical methods applied to soil and humic materials containing bound residues appeared to provide useful information on the nature of pesticides binding. The thermal profiles of ^{14}C-labeled bound residues of soil in air and helium streams are shown in Figure 2. The principal reactions occurring under air and helium during the isothermal heating of soil containing ^{14}C-labeled
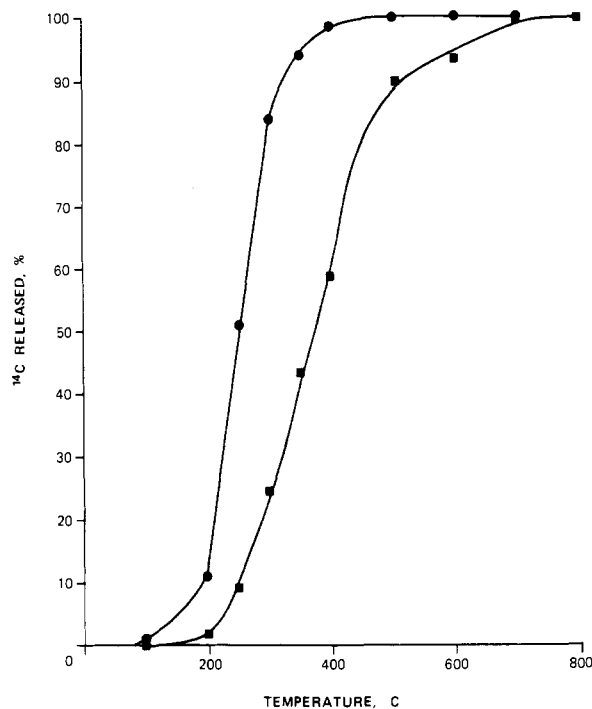

Figure 2. Release of bound ^{14}C from soil on isothermal heating under (●) air and (■) helium.

bound residues differed in that (1) ^{14}C was released over a broader temperature range under helium, (2) the release of ^{14}C under helium occurred and complete decomposition took place at temperatures that were 100–200 °C higher than those under air, and (3) all the bound ^{14}C released was decomposed to $^{14}CO_2$ in the air stream, whereas in the helium stream a portion of bound ^{14}C released (up to 26%) remained undecomposed as evidenced by the presence of radioactivity in the methanol trap. From these observations the release of bound ^{14}C-labeled residues appeared to be associated with the thermal decomposition of the organic matter fractions of the soil. Under oxygen (data not shown) or air the conditions were more conducive for a faster organic matter decomposition than under helium. Thus, the release of bound ^{14}C on heating under helium was considerably slower than that under air.

The thermal profile of bound ^{14}C-labeled residues in humin under air, helium, or nitrogen was similar to those obtained for soil. The amount of ^{14}C released from humin as a function of temperature is shown in Figure 3. In an air stream nearly all the radioactivity was found in the CO_2 trap. However, heating under helium or nitrogen resulted in the release of ^{14}C, part of which (up to 35%) was also trapped in methanol. While very little ^{14}C was released under air at 150 °C, extensive release occurred between 200 and 300 °C ($\simeq 90\%$), reaching 100% at 500 °C or higher (Figure 3). On the other hand, while under helium or nitrogen <1% of the radioactivity was released at 150 °C, about half of the total bound ^{14}C was released at 325–350 °C, and by 700 °C the recovery of ^{14}C in the trapping solutions was nearly quantitative. Differential thermogravimetry of humic materials showed elimination of all COOH and OH groups between 200 and 400 °C and decomposition of humic "nuclei" at 450–550 °C (Schnitzer and Khan, 1972). Helling and Krivonak (1978) stated that the bound butralin stability in their study could be linked with phenolic –OH and –COOH groups of soil organic matter or humic materials. Furthermore, chemically stabilizing reactions between phenolic hydroxyls and free aromatic amino groups from the metabolites of herbicides were postulated. Thus, under these conditions the pres-
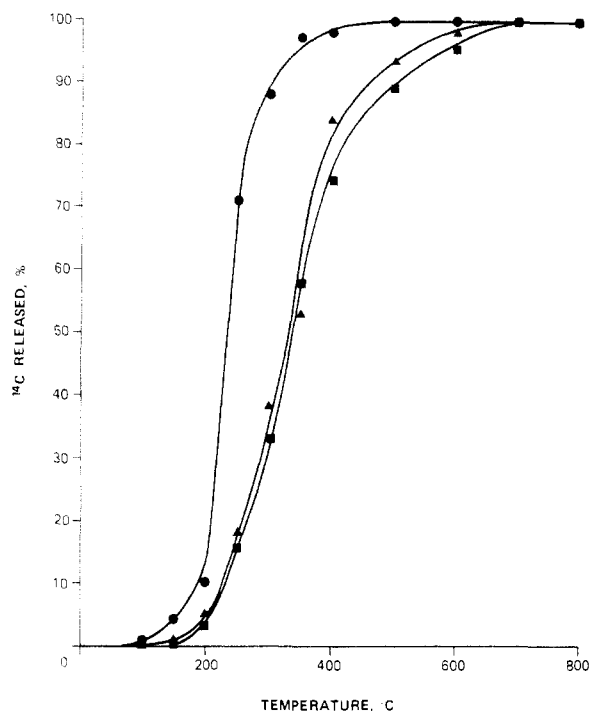
Figure 3. Release of bound ^{14}C from humin on isothermal heating under (●) air, (■) helium, and (▲) nitrogen.

Figure 4. Atomic H/C vs. O/C ratios for humin containing ^{14}C-labeled bound residues heated under nitrogen. Numbers in parentheses show the percentage of ^{14}C released at different temperatures.

ence of unchanged parent molecules of pesticides in the bound residues is not expected. However, our earlier study demonstrated that in addition to small amounts of hydroxy and dealkylated analogues, a considerable portion of the bound residues in the incubated soil was present in the form of prometryn (Khan and Hamilton, 1980). Similar results were obtained with humic materials in the present study (Table I). Thus, conceivably, in addition to the chemical binding of the metabolized pesticide, physical binding of the parent molecule also play an important role in the formation of soil-bound residues. Schnitzer and Khan (1972) suggested that humic materials are made of phenolic and benzenecarboxylic acids joined by hydrogen bonds to form a molecular sieve type polymeric structure of considerable stability. One of the characteristics of the structure is that it contains voids or holes of different molecular dimensions which can trap or fix organic molecules, such as pesticides. This may be considered analogous to the "clathrate compounds". The application of this structural concept to the pesticide bound residues in humic materials is still a matter of conjecture. However, judging from the thermoanalytical data, the thermal decomposition of organic matter or humic substances appears to weaken the structure by eliminating the functional groups and eventually decomposing the nuclei. This in turn permits the release of bound residues that are trapped in the structure. Further support for such a mechanism comes from the methylation study discussed later.

Schnitzer and Hoffman (1965) used the atomic H/C vs. O/C graphical–statistical relationship of Van Krevelen (1950) to study the principal reactions occurring during the thermogravimetry of soil organic matter under nitrogen. This approach may yield some valuable information on the relationship between the release of bound ^{14}C and reaction processes occurring during heating of the humic materials. In Figure 4 atomic H/C vs. O/C ratios are plotted for humin heated under nitrogen. The main reaction of humin up to 350 °C appeared to be dehydration (Schnitzer and Hoffman, 1965) which was accompanied by the elimination of about half of the bound ^{14}C. The next reaction up to 500 °C could be ascribed to dehydro-
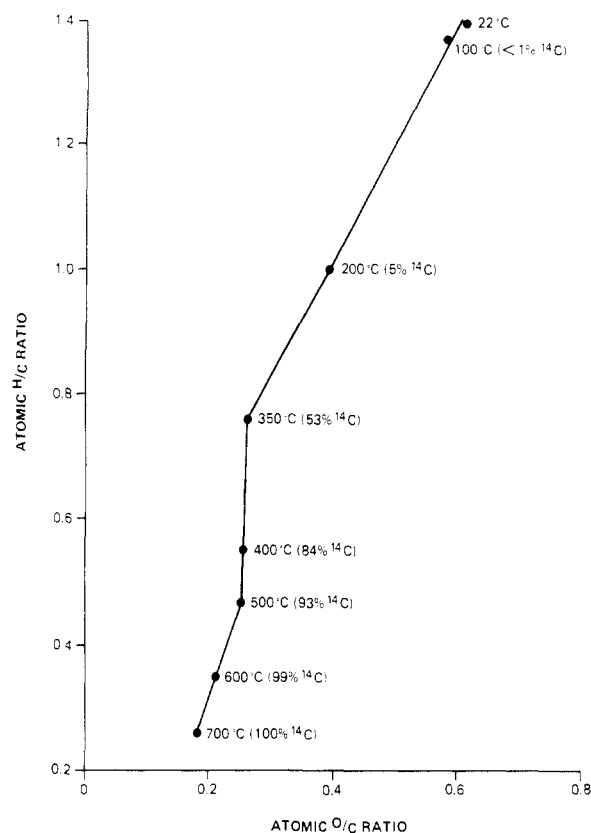
genation which resulted in another 40% release of bound ^{14}C. Finally, between 500 and 700 °C the reaction processes involving both dehydration and dehydrogenation (Schnitzer and Hoffman, 1965) could have taken place accompanied by the release of the remaining ^{14}C.

Methylation of Humic Materials. Exhaustive methylation of humin and humic acid resulted in about 25–30% release of the initially bound ^{14}C. Methylation reduces hydrogen bonding between the "binding units" of humic materials (Schnitzer and Khan, 1972). Thus, the molecular sieve like structural arrangement in humic material is weakened. The bound pesticide residues, adsorbed on its surface and trapped in internal voids, may then be released as indicated in our study.

UV Irradiation. (*1*) *Soil.* Preliminary experiments indicated that the decomposition of prometryn in water when exposed to UV light was complete in about 3 h. The herbicide was converted quantitatively to its hydroxy analogue. The stability of bound ^{14}C-labeled residues in soil suspension was affected by UV light. Thus, at the conclusion of the exposure period of 216 h, the extractable radioactivity accounted for about 30% of the total bound ^{14}C initially present in the soil. UV irradiation for a longer period did not increase the amounts of extractable ^{14}C. Only up to 3–5% or less of the total bound ^{14}C was recovered as extractable in the dark. GC and GC–MS analyses indicated that methanol extract of the UV irradiated soil suspension contained mainly hydroxypropazine. Thus, UV light appears to cause the release of some of the bound prometryn residues from soil which are subsequently decomposed to the hydroxy analogues. Burkhard and Guth (1979) demonstrated the photodecomposition of some organophosphorus insecticides on soil surfaces. They observed that the main photolytic breakdown products were identical with the terminal hydrolysis

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products obtained in aqueous solution. For comparative purposes, soil samples fortified with prometryn (24 ppm) were also processed under the experimental conditions described above. The herbicide in the soil suspensions was readily decomposed under the influence of UV light. Thus, at the end of a 72-h exposure, 75% of the material initially used to fortify soil sample was extractable in the form of hydroxypropazine. Although 70% of the bound ^{14}C in soil remained unextractable even after prolonged exposure, the fact that UV light caused the release of 30% bound ^{14}C should be of concern in determining the environmental stability of bound residues.

(2) *Humic Materials.* Exposure of aqueous solutions of humic acid or humin containing bound ^{14}C-labeled residues to UV light for 96 h resulted in 6.1 and 1.2% extractable ^{14}C, respectively. The relatively small release of ^{14}C from the bound form was unexpected since humic materials have been shown to act as photosensitizers for nonabsorbing pesticides (Khan, 1980b). Prolonged exposure of humin or humic acid to UV light did not significantly increase the release of bound ^{14}C. The concentration of extractable ^{14}C after UV irradiation was too low to be analyzed by GC. These observations therefore suggest that bound residues in humin or humic acid fractions of organic matter undergo to very little photolytic degradation.

CONCLUSION

Data presented herein indicate that the nonextractable or bound ^{14}C-labeled residues in an organic soil (45.4% C), incubated with [^{14}C]prometryn for 1 year, were associated with humin, humic acid, and fulvic acid fractions. The humin fraction contained more than half of the total ^{14}C-labeled residues in soil. The bound ^{14}C present in soluble low molecular weight FA fraction (26%) may be considered as potentially bioavailable to both plants and exposed aqueous and soil fauna. Contrary to the general belief that bound residues become an integral part of the polymolecular structure of the organic matter without a recognizable relationship to the original pesticide, our data demonstrate that a considerable proportion of such residues in humic materials was comprised of the parent molecule and its monodealkylated analogue. In addition to chemical binding, the pesticide or its metabolites also may be firmly retained by humic materials by a mechanism that most likely involves adsorption on external surfaces and in internal voids of a molecular sieve type structural arrangement. The structure appeared to be weakened by heat or methylation, resulting in the release of ^{14}C-labeled bound residues. UV irradiation of soil in aqueous suspension may release bound residues. However, bound residues in insoluble humin and humic acid fractions or organic matter appear to be stable to photodecomposition.

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