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Incorporation of Fumigants into Soil Organic Matter

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Halogenated fumigants are some of the most heavily used pesticides worldwide. A number of studies have shown that fumigant transformation in soil is correlated with soil organic matter content. However, relatively little is known about the mechanisms of fumigant interaction with soil organic matter. In this study, we used ^{14}C-labeled 1,3-dichloropropene (1,3-D) and methyl bromide (MeBr) to characterize their incorporation into soil organic matter and the association of bound radioactivity with the different organic matter components. The ^{14}C activity of bound residues increased with time and reached 38−49% for 1,3-D and 37−42% for MeBr after 72 d of incubation at 25°C. More bound residues were produced for 1,3-D than for MeBr in the same soils. The distribution of ^{14}C activity in soil humic substances followed the order of fulvic acids ≫ humin > humic acids. These observations suggest that incorporation into soil organic matter is the predominant pathway for transformation of halogenated fumigants in soil and that fulvic acids are likely the most significant sink of all soil organic matter fractions. It is further speculated that bound residues formed as a result of alkylation of organic matter by the fumigants through nucleophilic replacement.

Introduction

Halogenated hydrocarbons form the majority of soil fumigants. In particular, methyl bromide (bromomethane, MeBr) has been heavily used for several decades because of its broad spectrum of activity against nematodes, arthropods, weeds, fungi, and bacteria. However, the potential contribution of MeBr to stratospheric ozone depletion will result in a complete MeBr phase-out in 2005 in the United States and other industrialized countries (*1*). Most of the probable chemical alternatives are also halogenated fumigants. These include 1,3-dichloropropene (1,3-D) and chloropicrin, which are already in widespread use, and methyl iodide (iodomethane, MeI) and propargyl bromide (bromopropyne, PBr), which are being considered as potential alternatives (*2*−*4*). All of these compounds have very high vapor pressures and have been shown to quickly volatilize after soil incorporation (*5*−*8*). Atmospheric emission of fumigants is an environmental or health hazard because many fumigants are acutely toxic and potentially carcinogenic (*7*). Evaluation of fumigant emissions and mechanisms has attracted great research interest over the past decade. Transformation in soil has been identified as the most important process in reducing fumigant emissions (*9*).

Fumigant transformation can be mediated by both chemical and microbial processes (*10*−*12*). For halogenated fumigants, the rate of fumigant transformation has been shown to depend closely on soil organic matter content (*10*, *13*−*16*). For instance, transformation of MeBr, 1,3-D, and MeI was more rapid in soils with higher organic matter content or after amendment of organic materials such as compost, and the enhanced transformation occurred also in sterilized soils (*13*, *14*, *17*). All these observations suggest that soil organic matter is involved in the abiotic transformation of fumigants in soil. In separate studies, Papiernik et al. (*15*, *16*) showed that transformation of PBr or MeBr proceeded at similar rates in sterile and nonsterile soils, and little propargyl alcohol or methanol was produced from the transformation. This implies that abiotic processes predominated fumigant transformation and that hydrolysis was not the main transformation pathway. In a recent study using solid-state ^{13}C NMR, Tao and Maciel (*18*) observed bond interactions between MeBr and whole soil samples or soil organic matter components. While the study offered direct evidence to the hypothesis that MeBr alkylated organic matter in soil, the actual contribution by this pathway to the overall abiotic transformation was not known.

The main objective of this study was to evaluate the role of organic matter in fumigant transformation in soil by following the formation and distribution of nonextractable or bound residues. Bound residues are defined as the fraction of pesticides that is nonextractable after exhaustive extraction (*19*). Bound residues commonly occur as a result of irreversible interactions between pesticides and soil organic matter. The fumigants 1,3-D and MeBr were used as the test compounds in this study. The same mechanisms should apply also to the other halogenated fumigants of similar structures, including MeI, PBr, and chloropicrin.

TABLE 1. Selected Properties of the Soils Used in This Study

	Waukegan silt	Chualar loam
organic carbon (%)	1.80	0.80
sand (%)	41.0	55.4
silt (%)	38.0	28.0
clay (%)	21.0	16.6
pH	5.5	8.4
WHC[a] (%)	44.7	29.6

[a] WHC, maximum water holding capacity.

Materials and Methods

Soils. Two different types of soil were used in this study, a Chualar loam (fine-loamy, mixed, thermic Argixerol) from Salinas, CA, and a Waukegan silt loam (fine silty, over sandy or sandy-skeletal, mixed, mesic Typic Hapludoll) from Rosemont, MN. The basic soil properties were analyzed before the study (Table 1). The soil organic carbon content was determined using the Walkley−Black method (*20*), and particle sizes were determined using the hydrometer method (*21*). These soils were passed through a 2-mm sieve without air-drying and stored at 4 °C before use.

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Chemicals. Methyl bromide (>99% purity) was obtained from the Great Lakes Chemical Company (West Lafayette, IN). ^{14}C-labeled MeBr with a specific activity of 3.1 mCi mmol^{-1} and radiochemical purity of >97% was synthesized by the New England Nuclear Co. (Boston, MA). The standard of 1,3-D (48% cis isomer and 49% trans isomer) was purchased from Chem Service (West Chester, PA). ^{14}C-labeled 1,3-D with specific activity of 1.11 mCi mmol^{-1} and radiochemical purity of 98.6% was provided by Dow AgroSciences, LLC (Indianapolis, IN).

Incubation Experiments. The moist soil samples were preincubated for 1 week at room temperature to revive soil microbial activity. To prepare the spiking solutions, both ^{14}C-labeled and unlabeled MeBr or 1,3-D were dissolved in ethyl acetate to give a solution containing 0.25 mmol mL^{-1} of MeBr or 1,3-D. A total of 10 g of soil (oven-dry weight equivalent) was placed in 50-mL serum bottles (Supelco Inc., Bellefonte, PA), and the soil water content was adjusted to 40% of the soil maximum water holding capacity by addition of distilled water. Each soil sample was then treated with 20 μL of the spike solution, which gave an initial fumigant concentration of 0.5 mmol kg^{-1} soil. The treated vials were immediately sealed with aluminum caps and Teflon-faced butyl rubber septa and incubated at 25 ± 0.5 °C in an incubator. Because of the high volatility of MeBr and 1,3-D, it was expected that the fumigant had quickly vaporized and redistributed evenly in the soil. On day 0, 1, 3, 7, 14, 21, 42, and 72 after the treatment, three replicates of each soil were removed, and the bottles were stored in a −20 °C freezer until extraction.

Preparation of ^{14}C-Bound Residue Samples. The soil samples were thawed at room temperature and transferred to 50-mL centrifuge tubes, followed by aeration in a fume hood overnight to remove any untransformed parent compound or volatile transformation products. The soil samples were shaken with 20 mL of methanol for 1 h on a mechanical shaker. The mixture was then centrifuged for 10 min at 10 000 rpm, and the supernatant was discarded. The same extraction step was repeated for a total of five times. Preliminary experiments showed that, after five consecutive extractions, no radioactivity could be detected in the extract following additional extractions. After the exhaustive solvent extraction, the soil tubes (with soil) were kept open in a fume hood overnight to allow evaporation of any residual methanol from the soil. An aliquot (0.6 g) of the extracted soil was combusted on a biological oxidizer, and the evolved $^{14}CO_2$ was recovered in 15 mL of scintillation cocktail. The radioactivity of the recovered $^{14}CO_2$ was measured by liquid scintillation counting (LSC). The fraction of bound residues was defined as the percentage of the radioactivity that was associated with the extracted soil.

Fractionation of ^{14}C-Bound Residues. Bound residues derived from the previous incubation experiments were fractionated into fulvic acids (FA), humic acids (HA), and humin using the procedures given by Schnitzer (*22*). Briefly, the extracted soil samples (8.0 g) were mixed with 20 mL of 1.0 M NaOH solution by shaking overnight on a platform shaker at room temperature. The soil slurry was centrifuged at 10 000 rpm, and the supernatant was decanted. The extraction was repeated for a total of three times, and all supernatants were combined. The combined NaOH extract was then acidified to pH 2 with HCl, adjusted to 100 mL with distilled water, and allowed to stand overnight to precipitate the HA fraction. The samples were further centrifuged at 10 000 rpm to separate HA (the precipitated phase) and FA (the soluble phase). The radioactivity associated with FA was determined by measuring 0.5 mL of the soluble phase on LSC. The HA precipitate was redissolved in 50 mL 0.5 M NaOH solution, and a 0.25-mL aliquot was used for measurement of radioactivity. The residual soil (humin) in centrifuge tubes was left in a fume hood to dry, and an aliquot (0.6 g) of the dried material was combusted on a biological oxidizer to determine the radioactivity associated with humin.

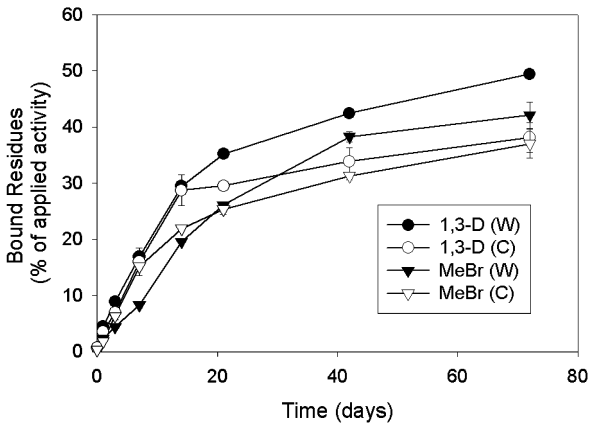

FIGURE 1. Total ^{14}C-bound residues formed during incubation of methyl bromide (MeBr) and 1,3-dichloropropene (1,3-D) in a Waukegan silt loam (W) and a Chualar loam (C).

Radioactivity Measurement. The radioactivity in HA and FA fractions was measured directly on a Packard Tri-Carb 1600TR liquid scintillation analyzer (Packard Instrument Company, IL) after mixing with 5 mL of Ultima Gold Cocktail (Packard, CT). Combustion of solid samples was carried out on an OX-500 biological oxidizer (R. J. Harvey Instrument Corp., Hillsdale, NJ). The total time of oxidation was 4 min, and the temperature was 900 °C. The $^{14}CO_2$ evolved from combustion was trapped in 15 mL of Carbon-14 Cocktail (Harvey, Hillsdale, NJ), and the radioactivity was determined by LSC. The efficiency of $^{14}CO_2$ recovery, as determined by combusting ^{14}C standards, was determined to be >97%. A 5-min interval was used for all samples for radioactivity counting by LSC.

Results and Discussion

Mass Balance. In this study, the recovery of ^{14}C activity after organic matter fractionation ranged from 86 ± 4.0% to 112.7 ± 7.9% for 1,3-D-derived bound residues and from 82.5 ± 2.8% to 109.9 ± 7.2% for MeBr-derived bound residues. The average recovery was 95.4 ± 3.6% and 108.4 ± 3.9% for 1,3-D in the Waukegan and Chualar soils, respectively, and 91.3 ± 2.6% and 104.9 ± 3.8% for MeBr in the two soils, respectively. This indicates that a good mass balance was maintained during the fractionation of soil bound residues into HA, FA and humin. The good recovery also suggests that ^{14}C-bound residues of 1,3-D or MeBr were nonvolatile once they became associated with the soil organic matter.

Total Bound Residues in Soil. Figure 1 shows accumulation of bound residues in the Waukegan silt loam and the Chualar loam after treatment of ^{14}C-labeled 1,3-D or MeBr. The ^{14}C activity in the form of bound residues rapidly increased during the initial 14 d for both MeBr and 1,3-D, and continued to increase, although at more gradual rates, until 72 d after the treatment. At the end of incubation, 49.4% and 38.1% of the initially applied radioactive 1,3-D were present as bound residues in the Waukegan and Chualar soils, respectively. The corresponding values were 42.1% and 37.1% for MeBr. Between the two test soils, the fraction of bound residues was consistently greater in the Waukegan silt loam than in the Chualar loam for both fumigants. This may be attributed to the higher organic matter content in the Waukegan soil (1.80%) than in the Chualar soil (0.80%). In previous studies, the rate of MeBr or 1,3-D transformation in soil was found to increase with increasing soil organic matter content (*13*). Close dependence between fumigant

transformation and soil organic matter content was seen in soils of differing OM (*16*), along soil profiles where soil organic matter content generally decreased with increasing soil depth (*13*) and in soils amended with organic matter such as biosolids and composted manure (*14*, *17*). However, in these studies, because the fumigants were not labeled, the role of organic matter could only be inferred through regression analysis. It was further speculated that fumigants became associated with soil organic matter by nucleophilically alkylating organic matter (*14*). More direct evidence was shown in a recent study in which ^{13}C-solid-state NMR was used to study bond interactions between MeBr and soil samples (*18*). Formation of methoxy (CH_3-O-) linkage between MeBr and nucleophilic structures such as -COO^- and aromatic O^- in whole soil samples and soil-derived HA and humin was observed. Our finding that a significant fraction of 1,3-D or MeBr was transformed into bound residues provided yet another evidence that organic matter plays an important role in the transformation of halogenated fumigants in soil. In soil, fumigants quickly become incorporated into soil organic matter, and the incorporation causes apparent fumigant disappearance because the residues were not recovered even after exhaustive extraction. Previous studies showed that fumigant transformation at high concentrations, as used in this study, proceeded abiotically. Thus, although the soil microbial activity was not suppressed in this study, it was likely that the formation of bound residues was a result of chemical interactions, through mechanisms such as the S_N2 nucleophilic substitution reactions in which the dehalogenated fumigant fragment became attached to nucleophilic sites on the soil organic matter.

Between the two fumigants, the fraction of bound residues was slightly greater for 1,3-D than for MeBr in the same soils at each incubation time. This was consistent with the observation that MeBr was generally more persistent in water or soil than 1,3-D isomers under similar conditions. The hydrolysis half-life of MeBr in water was about 50 d (*23*) and that of 1,3-D was only 11 d under comparable conditions (*24*). In the same soils, the half-life of MeBr was found to be longer than that of 1,3-D (*13*, *25*). With a boiling point of 4 °C and a vapor pressure of 1800 mmHg, MeBr is also more volatile than 1,3-D isomers (114−124 °C boiling point and 34−43 mmHg vapor pressure). In a closed soil system such as the serum bottles used in this study, MeBr might be present predominantly in the headspace, further limiting its interaction with soil organic matter. Because of its high volatility and limited transformation in soil, emission of MeBr was found to be greater than 1,3-D under similar conditions (*26*). Our study also suggests that practices to increase soil organic matter content (e.g., amendment with organic wastes into soil) may be more effective in accelerating the transformation and thus reducing the offsite movement potential of 1,3-D than for MeBr.

Composition of Bound Residues. Bound residues of ^{14}C-labeled 1,3-D and MeBr were fractionated into FA, HA, and humin using the standard procedures for organic matter fractionation (Figures 2-4). Bound residues of 1,3-D and MeBr were associated predominantly with the FA fraction and only marginally with the HA and humin fractions. For example, at the end of incubation, 32.4−34.6%, 2.5−3.4%, and 4.5−7.4% of applied ^{14}C-labeled 1,3-D were found with the FA, HA, and humin fractions, respectively. For MeBr, 31.3−32.9%, 2.7−2.9%, and 4.1−6.2% were distributed in the FA, HA, and humin fractions, respectively. For the entire incubation period, the FA-associated activity accounted for 70−81% and 82−92% of the total bound activity for 1,3-D in the Waukegan soil and in the Chualar soil, respectively (Figure 4). The respective contributions were 67−80% and 78−95% for MeBr in the two different soils. While more bound residues were formed in the Waukegan soil than in the Chualar soil (Figure 1), a higher proportion of bound residues were in the FA fraction in the Chualar soil as compared to the Waukegan soil (Figure 4). This may be caused by the different makeup of the fulvic acids in these soils.

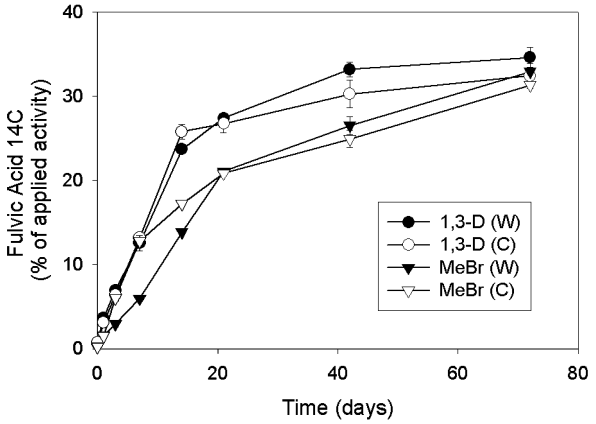

FIGURE 2. Accumulation of ^{14}C activity in the fulvic acid fraction of soil organic matter during incubation of methyl bromide (MeBr) and 1,3-dichloroprene (1,3-D) in a Waukegan silt loam (W) and a Chualar loam (C).

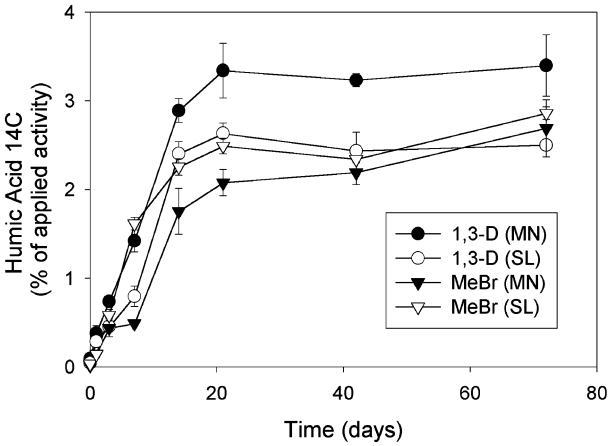

FIGURE 3. Accumulation of ^{14}C activity in the humic acid fraction of soil organic matter during incubation of methyl bromide (MeBr) and 1,3-dichloroprene (1,3-D) in a Waukegan silt loam (W) and a Chualar loam (C).

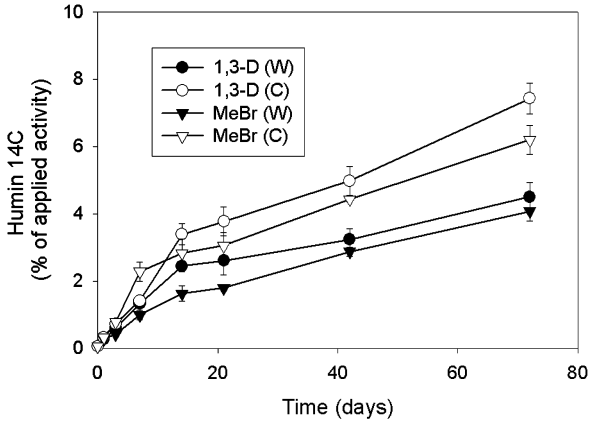

FIGURE 4. Accumulation of ^{14}C activity in the humin fraction of soil organic matter during incubation of methyl bromide (MeBr) and 1,3-dichloroprene (1,3-D) in a Waukegan silt loam (W) and a Chualar loam (C).

The ^{14}C bound residues in humin fraction was apparently higher in the Chualar soil than in the Waukegan soil

throughout the incubation period. The activity with humin appeared to increase continuously throughout the incubation period. Humin represents the last stage in soil organic matter turnover. Therefore, the continued increase in humin-associated bound residues suggests that fumigant bound residues were transformed progressively and became stabilized in soil. In contrast, the fraction of HA-associated bound residues reached a plateau after 21 d of incubation and remained unchanged for the rest of the incubation period (Figure 3).

The more dominant distribution of bound residues in the FA fraction over the HA or humin fraction was inconsistent with the observation by Tao and Maciel for MeBr (*18*). Using soil components isolated prior to MeBr exposure, Tao and Maciel (*18*) showed that there was formation of $^{13}CH_3O$ moieties between MeBr and HA or humin but not between MeBr and FA. The lack of interaction between MeBr and FA, as suggested by the authors, could have been caused by the fact that the fractionation prior to MeBr exposure might have resulted in the loss of reactivity of FA. In addition, as observed by the authors, the reactivity of HA or humin toward MeBr was substantially weaker than that of whole soil samples, probably because of the separation of humic substances from clay minerals. In this study, the fractionation of organic matter was performed after the whole soil samples were exposed to the fumigant vapor, and the effect of sample preparation on the reactivity of soil organic matter was therefore eliminated. Previous analysis of purified HA and FA extracted from various types of soils have shown that HA and FA contain approximately the same concentrations of phenolic OH, total C═O, and OCH_3 groups but that FA is richer in total acidity, COOH, and alcoholic OH groups than HA. Fulvic acids also contain approximately 10% more O than HA. The characteristics of humin are identified to be similar to those of HA (*27*). Therefore, FA contains more nucleophilic groups potentially capable of being alkylated with alkyl halides than HA or humin. This may explain the greater tendency of fulvic acids to associate with MeBr and 1,3-D and provide additional support to the hypothesis that alkylation may have caused the irreversible interaction between soil organic matter and halogenated fumigants such as MeBr and 1,3-D.

The rapid incorporation of fumigants into soil organic matter suggests that fumigants may be inactivated through this process. Previous studies have shown that the biological activity of most halogenated fumigants is caused by their ability to alkylate essential biological macromolecules such as proteins and DNA (*28*). Therefore, interaction of fumigants with soil organic matter (e.g., alkylation of FA and other organic matter components) should render the fumigant fragment inactive due to the loss of the nucleophile (i.e., Br in MeBr and Cl at the saturated carbon in 1,3-D). The increase in radioactivity with the humin fraction over time further suggests that the ^{14}C was no longer associated with the original compound but became an integral part of the soil organic matter. Therefore, incorporation into soil organic matter may be considered a detoxification process for soil fumigants such as MeBr and 1,3-D.

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