Chemical Release and Nature of Soil-Bound DDT Residues

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Soil was treated with ^{14}C-labeled and unlabeled *p,p'*-DDT and allowed to age under field conditions. Soil samples were extracted by refluxing with methanol for 24 h; further extraction did not yield additional radioactivity. The bound (unextractable) residues increased gradually to about 8% after 1 year and then declined to 4.5 and 3.3% after 1.5 and 2 years, respectively. About 81% of the bound residues could be released from the soil by sulfuric acid treatment without affecting the chemical nature of the residues. The residues released consisted mainly of *p,p'*-DDT and smaller proportions of *p,p'*-DDE and *p,p'*-DDD.

INTRODUCTION

Pesticides, once released into the environment, eventually reach the soil, which acts as a major sink for pesticide residues. Formation of conjugated and bound residues is an important mechanism for pesticide retention in soil (Kaufman, 1976). Bound residues constitute only a small fraction of the total pesticides present in soil and may represent residues bound to the soil organic matter (Meikle et al., 1976). A very small proportion of these residues may be released into the environment by the action of microorganisms (Khan and Ivarson, 1981; Racke and Lichtenstein, 1985) and may be taken up by plants (Khan, 1980; Verma and Pillai, 1991) and earthworms (Fuhremann and Lichtenstein, 1978). About 35% of the DDT applied to sandy loam soil persisted 1 year after application in Delhi. Approximately 25% of this residual DDT, i.e., about 8% of the DDT applied initially, was present in the form of soil-bound residues (Samuel et al., 1988). The rate of dissipation of DDT residues decreased with time and was extremely slow from 180 days onward. Presumably the presence of residues in the bound form was responsible for reduced dissipation of the total DDT left in the soil. Therefore, the formation of bound pesticide residues may be very important in regulating the rate and extent of dissipation of the pesticides from the soil. The bound residues may also account for the reduction in the toxic effects of the pesticide residues in the soil because their availability to biota is very small (Kaufman, 1976). DDT, being one of the most persistent insecticides in the soil, is admirably suited for studying the nature of soil-bound residues. Hence the present study.

MATERIALS AND METHODS

Materials. Uniformly phenyl ring labeled [^{14}C]DDT [1,1,1-trichloro-2,2-bis(*p*-chloro[^{14}C]phenyl)ethane; Amersham International, Amersham, U.K.; sp act. 104 mCi/mmol] was added to unlabeled DDT (25% EC). In another experiment, [^{14}C]DDT (Du Pont, Boston, MA; sp act. 27.95 mCi/mmol) was added to unlabeled DDT (99+% pure, HPLC) for field applications. All glassware was silanized before use.

Methods. *Field Treatment and Sampling.* A plot (3 m × 4 m) at the campus of the University of Delhi was prepared for field experiments. The soil was a sandy loam (59.27 ± 0.33% sand, 25.85 ± 0.43% silt, 14.82 ± 0.1% clay) rich in organic matter. The pH of the soil was 7.7 for the first experiment and 8.1 for the second experiment. Temperature of the soil was 40 °C maximum in summer and 9 °C minimum in winter. The relative humidity was high during the monsoon season (July–September). The ambient photoperiod varied from 14 h in June to 10 h in December.

Hollow poly(vinyl chloride) (PVC) cylinders (17.5 cm long and 10 cm i.d.) open at both ends were pushed down into the soil, leaving a 3-cm rim above the ground to prevent run off. The cylinders were left undisturbed for about 2 months under natural conditions for equilibration. In the first experiment, the soil in the cylinders was treated on June 20, 1989, with a mixture of 4.67 μCi of [^{14}C]-*p,p'*-DDT and 1.91 mg of unlabeled *p,p'*-DDT (ai, 25% EC) in 10 mL of *n*-hexane. In the second experiment, the soil in PVC cylinders was treated with 8 μCi of [^{14}C]-*p,p'*-DDT mixed with 10 mg of unlabeled *p,p'*-DDT in 10 mL of *n*-hexane on February 7, 1990. Samples dug out immediately after application of DDT were taken as zero-time control. The remaining cylinders were taken out at various time intervals ranging from 7 to 730 days after treatment (see Table I for sampling schedule). Each cylinder was immediately covered with aluminum foil, sealed in polythene bags, and stored at −20 °C. Each sample consisted of three cylinders selected at random.

Extraction, Cleanup, and Combustion of Soil. Soil was removed from each cylinder up to a depth of 10 cm and allowed to air-dry at room temperature. The samples were ground in a mortar and pestle, mixed thoroughly, and weighed. Five-gram portions in triplicate from each sample were dried at 110 °C for 18 h and reweighed to determine the moisture content (Head, 1980). Three 50-g samples of the air-dried soil from each cylinder were extracted by refluxing with 3 volumes of methanol in a Soxhlet apparatus for 24 h (72 cycles). Pilot experiments showed that longer extractions do not yield any further radioactivity. Soxhlet extraction of soil with three different solvents (hexane–acetone, 1:1; hexane–acetone–methanol, 1:1:1; methanol only) showed that the methanol extraction gave the maximum recovery of 98 ± 1.7% (Samuel et al., 1988). The total ^{14}C radioactivity of unextracted and extracted soil was estimated by combusting three replicates of 500 mg of soil each from every cylinder in a Harvey biological oxidizer Model OX-400. The efficiency of the oxidizer was 99%.

Chemical Release of Bound Residues. Fifty grams of methanol-extracted soil was placed in a 500-mL stoppered conical flask. Sulfuric acid was added to cover the soil. After thorough mixing, the flask was allowed to stand at room temperature. After 48 h, 100 mL of methanol was added and the flask shaken vigorously for 30 min. The contents were then partitioned three times with 50 mL of hexane. The hexane fractions were pooled and washed three or four times with water to remove the acid. The extract was filtered through anhydrous sodium sulfate and dried in vacuo at 40 °C. The residue was dissolved in HPLC grade methanol for analysis by HPLC.

High-Performance Liquid Chromatography (HPLC). An HPLC method was developed for separation, identification, and quantification of *p,p'*-DDT, *p,p'*-DDE, and *p,p'*-DDD. A Shimadzu LC-4A liquid chromatograph attached to a C-R3A Chromatopac and an SPS-2AS UV detector with variable wavelength was used. A C_{18} RP Zorbax ODS column (25 cm long and 4.6 mm i.d.) was found to be the most suitable when methanol was used as the eluting solvent at a flow rate of 0.5 mL/min. The absorbance was recorded at 240 nm at 0.16 AUFS sensitivity. The

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Table I. Release of Soil-Bound Residues of DDT by Sulfuric Acid Treatment in the First Experiment

days after DDT application	ng/g of soil dry wt		
	bound (tissue oxidizer)	release by sulfuric acid	% recovery
0	83.8 ● 8.70	71.8 ● 6.02	84.80
7	151.2 ± 11.43	131.9 ± 6.60	87.24
14	154.5 ± 11.13	131.7 ± 11.36	85.24
21	171.2 ± 11.36	137.0 ± 18.35	80.70
28	191.8 ± 22.72	171.4 ± 5.25	89.33
35	189.6 ± 24.37	166.1 ± 20.63	87.59
50	201.0 ± 12.23	152.9 ± 36.90	76.08
65	210.8 ± 20.18	177.3 ± 27.87	84.08
80	243.1 ± 17.22	174.6 ± 5.95	71.80
95	255.6 ± 11.26	179.8 ± 5.99	70.36
125	237.2 ± 33.34	192.2 ± 1.00	81.03
185	260.5 ± 7.90	228.1 ± 12.34	87.56
245	272.9 ± 4.27	185.6 ± 4.79	68.01
305	302.1 ± 20.48	266.0 ± 5.60	88.37
365	318.4 ± 9.45	207.5 ± 5.09	65.80
545	186.5 ± 21.68	142.1 ± 16.58	76.17
730	103.7 ± 13.18	94.9 ± 8.03	91.51

resolution of *p,p'*-DDD, *p,p'*-DDT, and *p,p'*-DDE is shown in Figure 3. The retention times for *p,p'*-DDD, *p,p'*-DDT, and *p,p'*-DDE were 7.65 ± 0.02, 9.22 ± 0.04, and 10.42 ± 0.03 min, respectively. The reliability of the HPLC method in identification and quantification was verified by different tests. The individual peaks were collected as they eluted from the HPLC column. They were reinjected individually on the HPLC column. Only a single peak was obtained in every case with the original retention time. In addition, DDT and its metabolites were subjected to thin-layer chromatography (TLC) on precoated plates using hexane as the mobile phase. The spots corresponding to authentic standards were eluted from the plate and subjected to HPLC as described above. Each of the extracts contained predominantly only one compound as identified by TLC. Dehydrochlorination of DDT by alcoholic alkali (Metcalf, 1955) followed by HPLC revealed the absence of the DDT peak and the appearance of the DDE peak. The peak area of each peak was utilized for quantification of the compound giving this peak. Appropriate standard curves were prepared from authentic standards. The HPLC method used in the present investigation was found to be quite satisfactory for qualitative and quantitative analyses of *p,p'*-DDT, *p,p'*-DDD, and *p,p'*-DDE. An advantage of this method is that DDT and its metabolites can be recovered from the column in sufficiently pure form for further analyses. Methanolic extracts of residues in the soil samples were analyzed by HPLC as described above. Fractions from the column were collected at 0.25-min intervals, and radioactivity in the fractions was estimated. The radioactive peaks coincided with the corresponding peak of the respective unlabeled compounds. In subsequent HPLC analyses the individual peaks were pooled and analyzed again by HPLC.

Recovery Experiments. The efficiency of the different analytical procedures used was determined at every step. The efficiency of Soxhlet extraction of soil with methanol was 98%. Drying the samples in vacuo by flash evaporation reduced the recovery by 3%.

Radioassay. A Packard 2000 CA liquid scintillation spectrometer with automatic quench correction facility was used. The scintillation cocktail was prepared according to the modification of Chaudhari and Yadav (1969) of the method of White (1968).

Analysis of Data. The data were corrected for recovery. The zero-time samples were taken as 100%. Each value represents a mean of nine replicates. The quantities of residues of DDT and its metabolites were estimated from the disintegrations per minute recovered and the specific activity of the parent compound. Unlabeled residues were also estimated from the peak areas in HPLC analyses. Each sample was chromatographed twice, and each value was an average of 18 analyses. Total and bound residues were estimated by combusting the unextracted soil and the methanol-extracted soil, respectively.

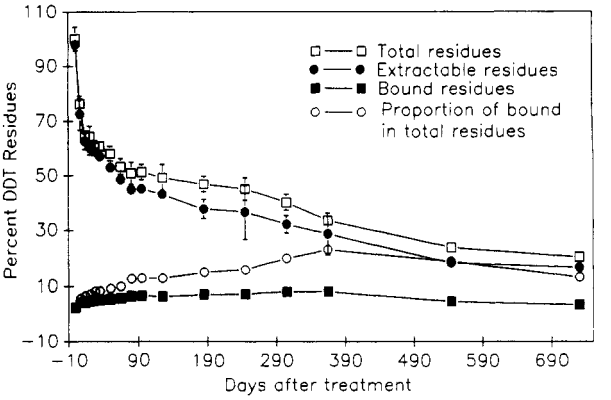

Figure 1. Persistence of ^{14}C-labeled and unlabeled DDT in soil under field conditions.

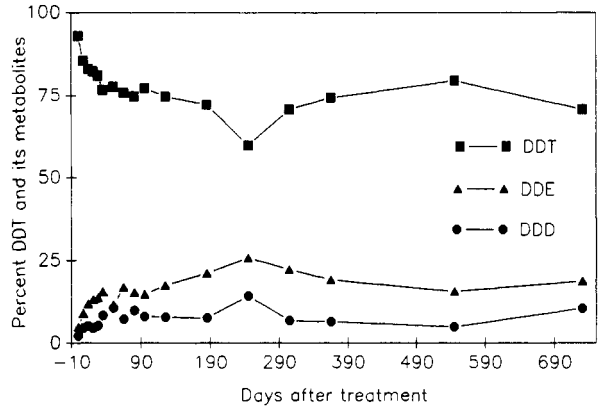

Figure 2. Composition of methanol-extractable DDT residues in soil.

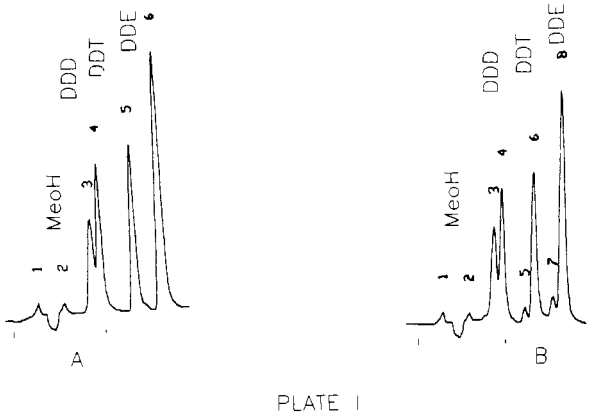

Figure 3. Resolution of DDD, DDT, and DDE by HPLC. (A) Before sulfuric acid treatment; (B) after sulfuric acid treatment.

RESULTS AND DISCUSSION

Nearly all of the DDT applied to the soil could be extracted by methanol from the zero-time soil samples and represented 19 790 ± 851 dpm/g of dry weight and 3.63 ± 0.16 μg/g of dry weight. This initial value has been taken as 100%. With time, the amount of DDT extracted declined rapidly at first and slowly at later time points. The dissipation exhibited a typical triphasic pattern (Figure 1). The methanol-unextractable residues of DDT were very low initially but increased gradually to a maximum of 7.9% 1 year after treatment and reduced thereafter (Figure 1). Those residues which could not be extracted by methanol have been considered to be the soil-bound residues of DDT. The proportion of bound residues in the total DDT residues present in the soil increased gradually with time (Figure 1). At the end of

Table II. Effect of Sulfuric Acid Treatment on DDT and Its Metabolites[a]

	ng		%		ratio		%
	B	A	B	A	B	A	recovery
DDD	0.61 ± 0.003	0.54 ± 0.01	33.36 ± 0.34	33.51 ± 0.20	0.91 ± 0.01	0.94 ± 0.005	88.5
DDT	0.67 ± 0.005	0.58 ± 0.01	36.09 ± 0.32	35.50 ± 0.08	1	1	86.6
DDE	0.58 ± 0.005	0.51 ± 0.008	30.87 ± 0.15	31.97 ± 0.68	0.85 ± 0.01	0.89 ± 0.02	87.9

[a] A, after acid treatment; B, before acid treatment.

1 year, 34.5% of the initially applied DDT was present in the soil, of which more than 25% was in the bound form. Essentially similar results were obtained in the second experiment. The maximum value of bound residues was found to be 7.79% 305 days after the application. These residues declined subsequently, as in the first experiment. Thus, the results of the present investigation are in accord with our earlier findings in the same locality (Samuel et al., 1988). The results of the present experiment suggest that the bound form of DDT constitutes a significant portion of the total residues present in the soil. This may be an important causative factor in the prolonged persistence of DDT in the soil. It has been reported that DDT has greater affinity for hydrophobic sites of the organic matter such as fats, waxes, resins, aliphatic side chains on humic and fulvic acids (Wershaw et al., 1969; Ballard, 1971; Stevenson, 1976) and lignin-derived materials, and a few polar groups (Walker and Crawford, 1968). Pierce et al. (1971) proposed that binding of nonpolar pesticide such as DDT to the soil organic matter is a peptide–lipid interaction. Another reason for persistence of residual compounds in the soil may be trapping in soil micropores (Steinberg et al., 1987). It is possible that after long periods of time or under suitable soil conditions, this bound form may be released in the environment and may adversely affect the biota. The pesticide release as well as binding may be considered to be a reversible process under appropriate conditions, and the pesticides may be lost to the environment due to volatilization and decomposition (Hamaker and Goring, 1976). In the first experiment there was a decline in the bound residues to 4.47% in 1.5 years and to 3.35% in 2 years. In the second experiment, the decline in bound residues was noticed after 1 year. It was 6.35 and 5.45% after 1 and 1.5 years, respectively. Soil microorganisms are believed to play an important role in the release and further degradation of bound pesticide residues (Khan and Ivarson, 1981; Racke and Lichtenstein, 1985). The bound residues so released can now be considered available for degradation and uptake by the biota. It is therefore very important to know the chemical nature of the bound residues of DDT in the soil.

Chemical Release of Bound Residues. Sulfuric acid treatment of the soil was found to be a suitable procedure as it released up to 91.5% of the bound residues (Table I). Bartha and Hsu (1976) reported that in some cases hydrolysis by strong acid and alkali can release the bound residues by irreversibly altering the binding sites.

To be able to use the sulfuric acid treatment for releasing the soil-bound residues of DDT, it is essential to show that this treatment does not alter the nature of the residues. Results presented in Table II and Figure 3 reveal that the recoveries after sulfuric acid extraction were 86.6% for DDT, 87.9% for DDE, and 88.5% for DDD. The ratio of DDD and DDE with respect to DDT did not change as a result of acid treatment (Table II). Furthermore, the retention time of the three compounds did not change as a result of acid treatment, suggesting that their chemical identity was unaltered. Singh and Chawla (1982) have also reported that use of sulfuric acid for cleanup of DDT residues from lipid and nonlipid foods does not affect the nature of the residues.

Table III. Composition of the Soil-Bound Residues of DDT Released by Sulfuric Acid Treatment[a]

days after DDT application	ng/g of soil dry wt		
	DDD	DDT	DDE
0	2.95 ± 1.07	66.2 ± 24.64	6.26 ± 2.47
7	8.97 ± 2.93	112.5 ± 22.0	11.9 ± 3.58
14	5.35 ± 0.7	92.6 ± 0.6	7.26 ± 0.68
21	5.80 ± 0.7	106.4 ± 15.65	10.3 ± 0.96
28	13.2 ± 3.5	134.9 ± 12.0	18.0 ± 2.4
35	6.27 ± 0.74	138.5 ± 1.1	27.3 ± 1.7
50	10.0 ± 0.37	102.2 ± 29.4	12.8 ± 1.0
65	4.0 ± 0.37	124.6 ± 4.4	26.3 ± 5.25
80	12.8 ± 1.1	120.1 ± 36.8	34.7 ± 1.59
95	30.6 ± 2.3	92.2 ± 6.8	36.1 ± 5.5
125	18.0 ± 0.36	116.2 ± 0.69	46.1 ± 1.18
185	14.3 ± 1.01	138.5 ± 6.35	74.2 ± 1.55
245	18.4 ± 1.12	103.5 ± 4.7	62.9 ± 2.4
305	13.3 ± 3.78	167.0 ± 29.0	86.1 ± 16.53
365	35.6 ± 7.79	133.9 ± 20.7	78.6 ± 21.3
545	17.1 ± 10.8	83.7 ± 0.36	52.4 ± 14.7
730	23.5 ± 5.94	57.7 ± 11.2	25.6 ± 7.66

[a] First experiment.

Table IV. Percent DDT and Its Metabolites in Bound Residues Extracted from Soil by Sulfuric Acid Treatment[a]

days after application	DDD	DDT	DDE
0	3.96 ± 1.2	87.8 ± 32.75	8.3 ± 3.3
7	7.7 ± 2.05	84.4 ± 16.6	8.9 ± 2.9
14	5.1 ± 0.5	87.94 ± 5.6	6.94 ± 0.7
21	4.7 ± 1.06	86.8 ± 12.8	8.4 ± 0.8
28	7.94 ± 2.2	81.2 ± 7.2	10.8 ± 1.2
35	4.65 ± 0.4	80.5 ± 0.7	15.9 ± 1.0
50	8.03 ± 0.4	81.7 ± 2.6	10.3 ± 0.9
65	3.6 ± 0.3	80.4 ± 28.7	17.0 ± 3.4
80	7.7 ± 1.6	71.4 ± 1.2	20.7 ± 0.9
95	19.3 ± 1.2	58.0 ± 4.3	22.8 ± 3.5
125	10.1 ± 1.2	64.4 ± 2.03	25.7 ± 0.7
185	6.3 ± 0.6	61.1 ± 2.8	32.6 ± 0.7
245	9.95 ± 0.7	56.0 ± 2.6	34.0 ± 1.3
305	5.03 ± 1.5	62.6 ± 10.9	32.3 ± 6.2
365	14.3 ± 3.25	54.0 ± 8.3	31.7 ± 0.8
545	11.2 ± 0.1	54.6 ± 0.3	34.2 ± 1.3
730	22.0 ± 5.8	54.0 ± 10.5	24.0 ± 8.1

[a] First experiment.

Residue Analysis by HPLC. When the cleaned sulfuric acid-released bound residues were subjected to HPLC analyses, radioactivity was localized only in the three peaks identified as *p,p'*-DDD, *p,p'*-DDT, and *p,p'*-DDE. The peaks for unlabeled compounds coincided precisely with those of the labeled compounds, thereby showing that *p,p'*-DDT was metabolized to *p,p'*-DDE and *p,p'*-DDD. The major proportion of the bound residues consisted of *p,p'*-DDT followed by *p,p'*-DDE and *p,p'*-DDD (Table III). The zero-time bound residue samples contained 66.16 ng of DDT followed by 6.26 ng of DDE and 2.95 ng of DDD per gram of soil dry weight. The *p,p'*-DDT accounted for about 88% of the total residues

Table V. Release of Soil-Bound Residues of DDT by Sulfuric Acid Treatment[a]

days after DDT application	ng/g of soil dry wt		
	bound (tissue oxidizer)	relase by sulfuric acid	% recovery
0	53.9 ± 1.35	45.4 ± 4.8	84.23
95	851.4 ± 259.2	767.7 ± 27.6	82.40
185	1042.4 ± 11.6	783.3 ± 85.6	75.13
245	1039.9 ± 82.9	802.6 ± 35.5	77.18
365	989.9 ± 21.5	908.7 ± 21.2	91.5
545	718.9 ± 21.1	665.8 ± 1.2	92.6

[a] Second experiment.

Table VI. DDT and Its Metabolites in Bound Residues Released from the Soil by Sulfuric Acid Treatment[a]

days after DDT application	ng/g of soil dry wt		
	DDD	DDT	DDE
0	2.2 ± 0.25	39.4 ± 1.2	3.9 ± 0.2
95	37.5 ± 2.65	574.5 ± 73.9	120.0 ± 13.4
185	84.5 ± 3.2	561.5 ± 20.4	138.2 ± 33.7
245	76.6 ± 4.7	527.3 ± 5.9	198.7 ± 17.0
365	67.1 ± 6.8	663.2 ± 5.8	238.0 ± 50.9
545	30.2 ± 0.13	276.3 ± 4.15	121.7 ± 1.5

[a] Second experiment.

(Table IV). With time, the proportion of DDT gradually decreased to about 54% 2 years after application. The major metabolite was *p,p'*-DDE, which gradually increased with time to about 34% 245 and 545 days after treatment. The proportion of DDD also increased gradually but its amount was much less than that of DDE. However, by 2 years, DDD (22%) was almost equal to DDE. The second experiment also showed similar results (Tables V and VI).

The above results show that the composition of the soil-bound DDT residues very closely reflects the composition of the extractable residues with the exception that the bound residues contain slightly more DDE (Figure 2). DDT is known to be metabolized to DDE and DDD in soil due to various mechanisms including microbial action (Kaufman, 1974). It has also been reported that soil-bound residues of DDT can be taken up by plants (Verma and Pillai, 1991). An important mechanism for release may be the microbial action in the soil (Racke and Lichtenstein, 1985). It is, therefore, quite likely that *p,p'*-DDT, *p,p'*-DDE, and *p,p'*-DDD may be released gradually from the soil and in time increase the insecticide load of the soil. Thus, binding of DDT residues with the soil may at best lead only to a temporary unavailability of these residues to the biota.

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