Release of Soil-Bound Methyl [^{14}C]Parathion Residues and Their Uptake by Earthworms and Oat Plants

Tom W. Fuhremann and E. Paul Lichtenstein*

Experiments were conducted to study the release of unextractable, soil-bound residues of methyl [*ring*-2,6-^{14}C]parathion and the potential pick up of these ^{14}C residues by earthworms and oat plants. Data indicate that soil-bound insecticide residues are not entirely excluded from environmental interaction. After incubation of soil treated with methyl [^{14}C]parathion, and exhaustive solvent extractions, unextracted, bound residues remaining in this soil amounted to 32.5% of the applied insecticide. However, after worms had lived for 2 to 6 weeks in this previously extracted soil or several crops of oats had grown in it, sizable amounts of ^{14}C residues were found in these organisms. Once they had penetrated into the animal or plant tissue, they were translocated and found partially in an unextractable, bound form or as benzene and water-soluble ^{14}C compounds. The majority of previously soil-bound ^{14}C residues taken up by earthworms (58–66%) again became bound within these worms, while most (82–95%) of the ^{14}C residues in oat plants were extractable. Greens of oat plants contained 46–62% of the ^{14}C residues recovered from plants. Although most of the ^{14}C residues in oat greens were benzene soluble, most of the ^{14}C residues in the seeds and roots were water soluble. In the future it will be important to determine how soil-bound pesticide residues can become biologically available, thus potentially interacting in the environment with biological systems.

The use of radiolabeled pesticides applied to soils in laboratory studies has shown that a portion of the ^{14}C residues of these compounds are not extracted following incubation of the pesticide-treated soils. These unextracted ^{14}C residues are often referred to as "bound" and are usually detectable only after using destructive techniques such as strong hydrolysis or combustion of the soil. Soil-bound residues have been reported for the herbicide propanil (Bartha, 1971), the fungicide DCNA (Van Alfen and Kosuge, 1976), and for the insecticides parathion, methyl parathion, fonofos, and *p,p'*-DDT (Katan et al., 1976; Lichtenstein et al., 1977). Katan and Lichtenstein (1977) have demonstrated that parathion is first reduced to amino compounds by soil microorganisms, and these reduced compounds are then rapidly bound to soil irrespective of microorganism activity. They have also found that these bound residues are nontoxic to *Drosophilia melanogaster* Meigen (Lichtenstein et al., 1977). Binding of insecticide residues to soil was found to increase with incubation time. Methyl [*ring*-2,6-^{14}C]parathion was rapidly bound to loam soil, where up to 41% of the applied ^{14}C insecticide residues could not be extracted after a 7-day incubation period.

The question of the significance of these bound residues is certainly an important one. These residues will not be detected in routine residue analysis, thus underestimates of the soil burden of total pesticide residues would result. We cannot predict what effects these compounds might have if they should be released. There is practically no data at hand which would indicate whether these bound residues might be released from soil, again become available, and affect living organisms in the soil. In this respect, Süss and Grampp reported in 1973, that mustard plants could take up residues of [^{14}C]monolinuron which could not be removed from soil with five acetone extractions.

In our experiments we attempted to study the potential release of bound residues of methyl [^{14}C]parathion from soil in the presence of earthworms and oat plants and the potential pick up and possible metabolism of the ^{14}C residues by these organisms.

Department of Entomology, University of Wisconsin, Madison, Wisconsin 53706.

MATERIALS AND METHODS

Chemicals. Methyl [*ring*-2,6-^{14}C]parathion (sp act. 2.83 μCi/mg) was purchased from Amersham Corp. Arlington Heights, Ill. Solvents used were anhydrous methanol, isopropyl alcohol, and redistilled acetone, benzene, chloroform, and ethyl acetate.

Soils. The soil used was an agricultural Plano Silt Loam (organic matter 4.2%, sand 4.8%, silt 68%, clay 23%, pH 6.0) free of insecticide residues. This soil was collected at the University of Wisconsin Experimental Farms near Madison and was stored at room temperature in a moist condition prior to use. Insecticide free silica sand was also used, to prepare a 1:20 soil–sand mixture.

Oats and Earthworms. Oat seeds (*Avena sativa* var. *Lodi*) were purchased from a local seed dealer. Earthworms (*Lumbricus terrestris*), collected in southern Ontario, Canada, were purchased from a local bait shop. Their identity was confirmed by Dr. William Reeder, Department of Zoology, University of Wisconsin, Madison.

Production of Soil-Bound Residues and Experimental Design. Figure 1 depicts the way in which the experiments were conducted. Basically, soil was treated with methyl [^{14}C]parathion and handled in such a way that, after exhaustive extractions, it contained only bound ^{14}C residues. Earthworms or oats were then introduced into this soil, and the amounts of radiocarbon, their extractability, and their solvent partitioning behavior in these organisms were determined. For control purposes, soils containing bound ^{14}C residues were prepared as described, but no earthworms or oat plants were introduced into the soil. In addition, earthworms or oats were exposed to soil containing freshly deposited and initially extractable methyl [^{14}C]parathion residues.

To produce soil containing only bound ^{14}C residues, 500 g of moist loam soil were treated with 40 mL of acetone containing 3 mg (8.5 μCi) of methyl [^{14}C]parathion. The acetone was evaporated with a gentle air stream and the soil was thoroughly mixed. An aliquot of the treated soil was removed for determination of moisture content. Also, 50 g of moist soil were extracted in a Soxhlet apparatus for 6 h with 250 mL of acetone–methanol (1:1) to determine the initial insecticide concentration. The remaining

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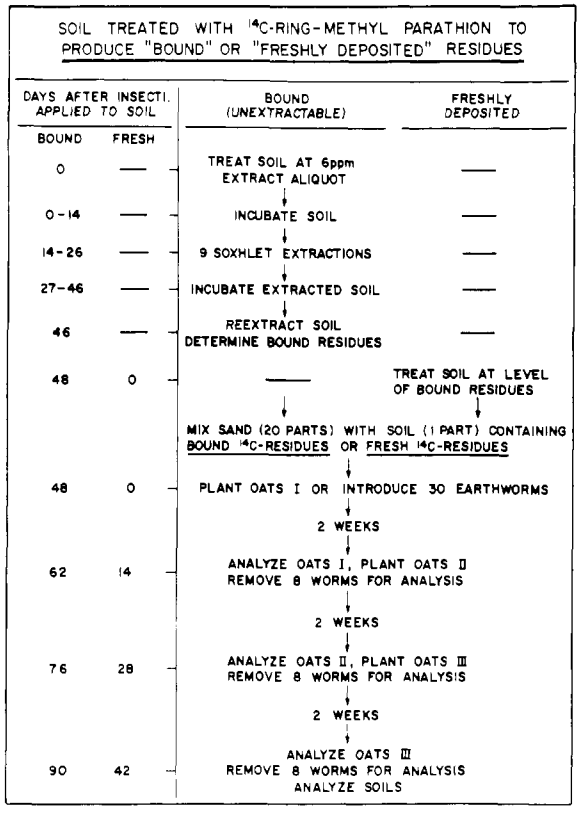

Figure 1. Flow sheet depicting the experimental design. Control soil was handled the same way except that no earthworms or oats were placed in it.

treated soil was then incubated in a cotton plugged, glass container for 14 days in the dark at 25 ± 2 °C (Figure 1, bound, 0–14 days). Distilled water was added as necessary to maintain soil moisture. Following the 14-day incubation period, the treated soil was extracted nine times with various solvents over a 12-day period (Figure 1, bound, day 14–26). For this purpose, the soil was mixed, divided into three (150 g) portions and each was extracted for 6 h in a Soxhlet apparatus with 250 mL of solvents. Residual solvents were allowed to evaporate from the soil between the daily extractions. Water was added to the soil between each extraction and allowed to equilibriate overnight to reestablish original moisture levels (ca. 15% water). This procedure resulted in four extractions with acetone–methanol (1:1), followed by three extractions with ethyl acetate–methanol (1:1), one with chloroform–methanol (1:1) and one with isopropyl alcohol. Each of these nine extracts was analyzed by liquid scintillation counting (LSC) to determine the extraction end point. Aliquots of this dried soil after nine extractions were then combusted to $^{14}CO_2$ for analyses by LSC as described below to determine the level of unextractable, bound residues of methyl [^{14}C]parathion.

To determine if the bound ^{14}C residues could be released upon further incubation (Figure 1, bound, day 27–46), the exhaustively extracted soil was remoistened and reincubated for 19 days as previously described. The soil was then (day 46) extracted (for the tenth time) with acetone–methanol (1:1) for 6 h. The unextracted residues of methyl [*ring*-^{14}C]parathion remaining in this soil are referred to as "bound". After all the solvents had been removed and the soil had been dried, aliquots of the soil were combusted to $^{14}CO_2$ to determine the level of the bound radiocarbon by LSC as described below. At that point (Figure 1, bound, day 48), earthworms or oat plants were introduced into this soil.

Exposure of Earthworms and Their Extraction. Earthworms were kept for various time periods in this soil to determine if they could affect a release of soil-bound ^{14}C residues and incorporate them into their own tissue. To this effect, 250 g of the dry loam soil containing bound residues of methyl [^{14}C]parathion were thoroughly mixed with 5000 g (1:20) dry, uncontaminated silica sand to increase the bulk volume of the soil. This loam–sand mixture was moistened and placed in a polyethylene bag. Thirty earthworms were then added (Figure 1, bound, day 48) and the bag was closed with a rubber band to prevent their escape. The soil–sand mixture containing these earthworms was incubated at 16 °C with a 12-h photoperiod. The earthworms avoided the light by burrowing into the soil–sand mixture during the photoperiod. Yellow corn meal served as food and was added once a week to the top of the soil.

After living for 2, 4, or 6 weeks in this soil, eight earthworms were removed each time and rinsed with three changes of cold tap water. These earthworms had soil with ^{14}C residues in their digestive tracts. To remove this soil the worms were purged by allowing them to feed for 5 days on wet macerated filter paper (Kükenthal et al., 1967). On each of these 5 days the earthworms were rinsed with cold tap water and placed into clean, wet, macerated filter paper. After the first three daily changes, the filter paper excreted by the earthworms appeared to be free of soil particles.

The eight purged earthworms which had been removed from the soil–sand mixture after 2 weeks, were lyophylized for 24 h in a Virtis Model 10-100 "Unitrap" freeze dryer at −55 °C and 10-μm Hg vacuum. They were then cut into approximately equal anterior, middle, and posterior sections and each was combusted to $^{14}CO_2$ for LSC analyses. In this way we intended to determine the amount and location of previously soil-bound ^{14}C residues of methyl parathion within these earthworms.

The eight purged earthworms which had been removed after 4 or 6 weeks were extracted by macerating them in a Waring blender with 3 × 75 mL chloroform–methanol–acetone (1:1:1). The solvents were then evaporated to near dryness and the residues were partitioned between benzene and water for LSC analyses. To determine the amount of unextractable, bound ^{14}C residues within the earthworm tissues, the previously extracted worms were air-dried and combusted to $^{14}CO_2$ for LSC analyses.

For control purposes, 30 earthworms were exposed to loam soil–sand mixtures, immediately after soil treatment with methyl [^{14}C]parathion, when no bound residues had yet been formed. These soils were treated with methyl [^{14}C]parathion at a level corresponding to the amount of bound radiocarbon remaining in the soil as determined on day 46 (Figure 1, bound). These freshly deposited ^{14}C residues are referred to as "fresh" in Figure 1 (second column, day 0). Earthworms were kept in these soils for 2, 4, or 6 weeks under identical conditions and were finally extracted the same way as described for worms exposed to soil containing bound ^{14}C residues. In addition, soils containing bound ^{14}C residues were diluted with silica sand and incubated without earthworms under identical conditions.

Growing of Oats and Their Extraction. Soil–sand mixtures containing either bound (Figure 1, bound, day 48) or fresh (Figure 1, fresh, day 0) methyl [^{14}C]parathion residues were also used to grow oat plants. This was done to investigate if oat plants could release ^{14}C residues from soil, absorb these chemicals through their roots, and then translocate them into their leaves. To that effect, 50 g of

Table I. Production of Soil-Bound Residues of Methyl [*ring*-^{14}C]Parathion by Removal of Extractable ^{14}C Residues[a]

Days after insecticide appl. to soil[c]		^{14}C residues recovered from soil[b]		
		Bound[d]	Extracted	Total (T)
0	dpm/g	140	43340	43480
	% T[e]	0.3	99.7	100
	% appl.[e]	0.3	99.7	100
14	dpm/g	14010 ± 237	8084 ± 86	22094 ± 320
	% T	63.4	36.6	100
	% appl.	32.2	18.6	50.8
46	dpm/g	14035 ± 173	65 ± 10	14100 ± 183
	% T	99.5	0.5	100
	% appl.	32.3	0.1	32.4
		^{14}C residues recovered from Soil-Sand Mixture (1:20)		
90[f]	dpm/g	567	22	589
	% T	96.3	3.7	100
	% appl.[g]	27.4	1.1	28.5

[a] See Figure 1 for details of soil handling procedures. [b] Data are expressed as dpm/g of dry soil. [c] Five hundred grams moist loam soil were treated with methyl [*ring*-^{14}C]parathion at 6 ppm (8.5 μCi). [d] Determined by combusting aliquots of previously extracted soil to $^{14}CO_2$. [e] % T = in percent of total dpm recovered; % appl. = in percent of radiocarbon originally applied to soil. [f] After earthworms had been kept in the soil-sand mixture for 6 weeks. [g] Based on 21 g of soil-sand mixture containing 1.0 g of loam soil.

dry loam soil containing bound residues or 50 g containing fresh residues were each mixed with 1000 g (1:20) of dry, insecticide-free, silica sand to increase the bulk volume of the soil. This soil–sand mixture was moistened and placed in a 8.5 cm diameter, 12 cm high paper carton lined with a polyethylene bag. Approximately 150 oats seeds which had been germinated for 48 h between wet paper towels were then planted in this soil. The plants (Oats I in Figure 1, day 48) were grown at 16 °C, 12 h light and were watered each day as necessary. After the oats had grown for 14 days in soil–sand mixtures containing bound or freshly deposited residues, they were harvested. Greens were cut 1 cm above the soil surface and weighed. Seeds and roots were removed from the soil and washed in a screen with a forceful stream of cold tap water. The seeds were then removed from the roots. The roots, seeds, and greens were dried for 18 h at 26 ± 2 °C. Aliquots of these dried plant tissues were then combusted to $^{14}CO_2$ for LSC analyses to determine the amounts of soil originating ^{14}C residues of methyl [^{14}C]parathion in roots, seeds, and greens of these plants.

The soils in which Oats I had grown were then planted with a second group of oat seeds (Oats II). These oats were also grown for 2 weeks and then harvested as described. The seeds, roots, or greens of this second crop of oats, however, were extracted twice with acetone–methanol (1:1) and a third time with acetone–methanol–benzene (1:1:1). The extracts were then evaporated and subsequently partitioned into benzene and water phases (Lichtenstein et al., 1973a) for LSC analyses. Extracted plant tissues were air-dried to remove solvent vapors and aliquots were combusted to $^{14}CO_2$ for LSC analyses. In this way, plant-bound ^{14}C residues could be quantitated. A third crop of oats (Oats III) was then planted in the same soils and grown for 2 weeks, when they were harvested, extracted, and analyzed as described for Oats II.

Since in previous experiments the fresh weight of oat greens from ten groups of 50 plants had been shown to amount to 4.06 ± 0.2 g each, the total number of oat plants per pot could be calculated based on the fresh weight of the oat greens.

Final Soil Extraction. Soils containing bound or fresh ^{14}C residues in which earthworms had been living for 6 weeks or soils containing bound residues but without earthworms were finally extracted on day 90 (Figure 1, bound) for 6 h in a Soxhlet apparatus with the acetone–methanol mixture as described. In this way we intended to determine if some of the soil-bound residues had been released while earthworms had lived in it.

Analyses. To determine the total amounts of radiocarbon in the various materials under investigation, combustion to $^{14}CO_2$ of lyophilized or extracted earthworms, air-dried or extracted plant material and extracted soils was performed in a Packard Model 305 Tri-Carb Sample Oxidizer (Flashinski and Lichtenstein, 1974). For this purpose 200-mg aliquots of plant or earthworm tissues or 200 mg of dry loam soil or 1.0 g of dry soil–sand mixtures were mixed with 100 mg of starch powder (to maintain combustion), wrapped in a 7 cm disc of Whatman No. 1 filter paper, and compressed into a solid pellet for combustion.

Organic solvent and water extraction phases and $^{14}CO_2$ released by combustion of extracted aliquots of worms, plants, or soils were analyzed by LSC. One-milliliter aliquots of organic solvent or water extraction phases were counted in 5 mL of a dioxane based scintillation solvent (Lichtenstein et al., 1973b) in 7-mL polyethylene vials. Benzene extracts of oat greens could not be counted directly because of excessive quenching due to the presence of extracted plant pigments. Therefore 0.5-mL portions of these extracts were evaporated on a 7-cm disc of filter paper which was also pelleted for combustion. All samples were counted in a Packard Model 3320 Tri-Carb Liquid Scintillation Spectrometer. Data were corrected for background, combustion, and counting efficiency and for total sample volume or weight.

RESULTS AND DISCUSSION

Methyl [^{14}C]Parathion Residues in Soils. Results summarized in Table I indicate that immediately following soil treatment, 99.7% of the applied methyl [^{14}C]parathion residues were extractable utilizing a single 6 h Soxhlet extraction with acetone–methanol (1:1). Following a soil incubation period of 14 days and nine exhaustive extractions as described, it was evident (Table I, bound, day 14) that 32.2% of the applied radiocarbon could no longer be extracted. These residues are designated as "soil-bound". Since 18.6% of the applied radiocarbon were extractable, a total of only 50.8% of the applied radiocarbon were recovered, possibly due to volatilization of ^{14}C

Table II. Release and Uptake of Bound or Freshly Deposited Soil Residues of Methyl [*ring*-^{14}C]Parathion by Earthworms (*Lumbricus terrestris*)

Days after insecticide appl. to soil		Worms exposed to soil for[a]	^{14}C residues recovered from earthworms (dpm/worm)				
Bound[b]	Fresh[c]	Weeks		Bound ^{14}C residues[b]		Fresh ^{14}C residues[c]	
62	14	2		855 ± 195[d]		10734 ± 2218[d]	
					% T[e]		% T
76	28	4	Total ^{14}C	2610	100	11179	100
			Bound[f]	1510	57.9	2067	18.6
			Benzene	300	11.5	1562	13.9
			Water	800	30.6	7550	67.5
90	42	6	Total ^{14}C	3143	100	10151	100
			Bound	2078	66.1	3383	33.4
			Benzene	225	7.2	888	8.7
			Water	840	26.7	5880	57.9

[a] Following the exposure periods to soil, earthworms were fed wet filter paper for 5 days to eliminate soil from their digestive tracts. [b] One gram of soil-sand mixture contained on day 48 (bound, Figure 1) 668 dpm of bound (unextractable) ^{14}C residues. [c] One gram of soil-sand mixture contained on day 0 (fresh, Figure 1) 518 dpm of extractable, freshly deposited methyl [^{14}C]parathion. [d] Determined by combustion of soil-free unextracted worms to $^{14}CO_2$. [e] % T = distribution of ^{14}C between extraction phases and bound residues in percent of total recovered. [f] Determined by combustion of soil-free worm tissue to $^{14}CO_2$ after solvent extraction of eight worms.

materials from the soil. Thus, after a 14-day incubation period, two-thirds of the total recovered ^{14}C residues were bound to the soil while only one-third were extractable. Eighty-five percent of the residues which could be extracted were removed with the first three acetone–methanol extractions and only 15% (2.9% of applied) were removed with the subsequent six 6 h Soxhlet extractions with the other solvent mixtures.

After removal of the extractable residues of methyl [*ring*-^{14}C]parathion, the extracted loam soil was moistened and incubated for an additional 19 days (Figure 1, bound, day 27 to 46). At that time (Figure 1, day 46), 99.5% of the recovered ^{14}C residues were still bound to the soil and could not be extracted (Table I). Because these ^{14}C residues amounted to 32.3% of those applied to the soil, no further loss of radiocarbon had occurred during this second additional incubation period.

The soil–sand mixtures (bound and fresh) in which earthworms had lived for 6 weeks, were also extracted and analyzed on day 90 (Figure 1). As shown in Table I, 96.3% of the radiocarbon recovered from soils with bound residues were still bound. However, it is interesting to note that 3.7% of the total radiocarbon recovered were extractable, while only 0.5% were extractable before the soil was mixed with sand and earthworms had been introduced into it (Table I, day 46).

After 90 days incubation of control soils containing bound ^{14}C residues but in the absence of earthworms, 98% of the recovered radiocarbon was still bound and only 2% could be extracted. Since on day 46, before the soil was diluted with sand, less radiocarbon was extractable (0.8% of total recovered), it appears that some release of bound residues had also occurred in the absence of earthworms, possibly due to mixing the soil with sand. Soils which had been treated immediately prior to the introduction of earthworms contained bound and extractable radiocarbon amounting to 90.5 and 9.5%, respectively, of the totally recovered ^{14}C residues.

Uptake and Metabolism by Earthworms of ^{14}C Bound and ^{14}C Fresh Residues from Soils. Results (Table II) indicate that worms which had lived for 2, 4, or 6 weeks in the soil–sand mixture containing bound ^{14}C residues of methyl parathion had indeed incorporated some of these residues into their body tissues. Worms which had been kept for 2 weeks in a soil–sand mixture with 668 dpm/g of bound ^{14}C residues contained a total of 855 ± 195 dpm/earthworm. These worms had been divided into three approximately equal sections for combustion. The anterior section contained 51.7 ± 11.7% of the recovered ^{14}C residues, while the middle and posterior sections contained 30.1 ± 8.6% and 18.2 ± 3.6%, respectively. Earthworms which had been living for 2 weeks in a soil–sand mixture containing 518 dpm/g of freshly deposited and initially extractable residues of methyl [*ring*-^{14}C]parathion, contained 12.6 times more radiocarbon than those which lived in soil with bound ^{14}C residues. These residues, however, were distributed differently within the worms. The anterior and posterior sections contained only 21.6 ± 4.1 and 22.0 ± 5.0%, respectively, while the middle section contained 56.4 ± 16.6% of the total ^{14}C residues recovered from the worms. While previously soil-bound ^{14}C residues were concentrated in the anterior portion of the worm, ^{14}C residues from freshly treated soil were concentrated in the middle portion of the worm, indicating a possible difference in the chemical nature of ^{14}C residues released from the soils and subsequently absorbed by the worms.

Worms living in soil containing only bound ^{14}C residues absorbed increasing amounts of radiocarbon with time; however, worms living in freshly treated soil contained similar amounts of radiocarbon in their tissues after 2, 4, and 6 weeks, although these amounts of ^{14}C residues were considerably larger than those in worms from soil containing bound residues. Thus, the greater availability of the fresh residues either saturated the worms during the first 2 weeks or these residues had also become bound to soil and hence less available to the worms. This appears to be in line with results obtained after analysis of this soil–sand mixture when all the worms had been removed (Figure 1, fresh, day 42). Of the ^{14}C residues which were recovered from this soil–sand mixture 90.5% had become bound and only 9.5% could be extracted.

Based on the partitioning behavior between benzene and water (Table II) differences in the chemical nature of the absorbed ^{14}C residues in worms were apparent: 58 to 66% of the ^{14}C residues from worms exposed to soil containing bound residues had also become bound to the worm tissue, but only 18 and 33% of the ^{14}C residues in worms exposed to soils containing initially fresh residues were bound after 4 and 6 weeks of exposure, respectively. In all cases, the majority of the extractable residues were water soluble. Because the amounts of benzene-extractable residues were

Table III. Release and Uptake of Bound or Freshly Deposited Soil Residues of Methyl [*ring*-^{14}C]Parathion by Three Crops of Oat Plants (*Avena sativa*) Grown Successively for 2 Weeks Each

Days after insecticide appl. to soil				^{14}C residues recovered from 100 oat plants (dpm)[a] after growing in soils containing			
Bound[b]	Fresh[c]			Bound ^{14}C residues[b]		Fresh ^{14}C residues[c]	
					% T[d]		% T
62	14	Oats I	Total ^{14}C	8042	100	39860	100
			Greens	5026	62.5	18455	46.3
			Seeds	1022	12.7	5580	14.0
			Roots	1994	24.8	15825	39.7
76	28	Oats II	Total ^{14}C	13765	100	21245	100
			Greens	6593	47.9	10240	48.2
			Seeds	3331	24.2	4993	23.5
			Roots	3841	27.9	6012	28.3
90	42	Oats III	Total ^{14}C	13892	100	21215	100
			Greens	7057	50.8	11350	53.5
			Seeds	3112	22.4	4900	23.1
			Roots	3723	26.8	4965	23.4

[a] Data resulting from combustion of plants to $^{14}CO_2$ (Oats I) or extraction and then combustion of extracted plant tissues (Oats II, III). [b] Oats (I) were grown in a soil-sand mixture which contained on day 48 (bound, Figure 1) 668 dpm/g of bound (unextracted) ^{14}C residues, while Oats II and III were planted in the same soil 2 or 4 weeks later. [c] Oats I were grown in a soil-sand mixture which contained on day 0 (fresh, Figure 1) 518 dpm/g of extractable freshly deposited methyl [^{14}C]parathion while Oats II and III were planted in the same soil 2 or 4 weeks later. [d] % T = distribution of ^{14}C between greens, seeds, and roots in percent of total recovered.

relatively small, they were not identified.

Based on data presented in Table II, we calculated that 30 worms placed in the soil containing only bound ^{14}C residues would have absorbed 94 290 dpm of ^{14}C residues if all of them would have remained in this soil for the entire 6-week period. This figure would represent 2.7% of all the bound ^{14}C residues present in the 5250 g of the soil–sand mixture. This soil–sand mixture contained before earthworms were placed into it, bound ^{14}C residues amounting to 32.3% of the originally applied radiocarbon (Table I, day 46), but only 27.4% of applied radiocarbon was bound after the earthworms had been living in it for 6 weeks. This represents a 15% loss of the soil-bound ^{14}C residues present on day 46. No loss of radiocarbon had occurred during this 6-week period in soils incubated without earthworms. Thus in the presence of earthworms in soil containing bound residues of methyl [^{14}C]parathion, a release of a portion of these soil residues had occurred, resulting in loss, increased extractability, and their absorption by the worms. These worm residues were then converted to either a soluble form or a form which again became strongly bound within the worms themselves.

It might be assumed that if the worms had remained in the soil for a longer period, or if the density of worms in the soil had been greater, an even larger amount of the bound residues would have been released and absorbed by these earthworms.

Uptake by Oat Plants of ^{14}C-Bound and ^{14}C-Fresh Residues from Soils. The number of oat plants grown per pot during the three 2 week periods (Figure 1, oats I, II, and III) varied from 90 to 150. For this reason, results were calculated and are expressed on the basis of 100 plants each period (Table III). Data indicate that 2 weeks after oat plants grew in soil containing bound ^{14}C residues of methyl [^{14}C]parathion, portions of these residues had penetrated into the plant roots and had been translocated into the plant greens. The first crop of oat plants (I) which grew for 2 weeks in the soil with bound ^{14}C residues contained a total of 8042 dpm/100 plants while five times more ^{14}C residues were present in oats (I) grown for 2 weeks in soil with freshly applied methyl [^{14}C]parathion. However, when these same soils were replanted 2 or 4 weeks later, oat plants from "freshly treated" soils contained after 2 weeks of growing time only 1.5 times more ^{14}C residues than those which had grown for 2 weeks in a previously extracted soil containing bound ^{14}C residues. Presumably some of the ^{14}C residues, after 2 weeks of soil incubation, had also become bound during the plant growing periods and thus less residues were absorbed by the second and third crops of oats. The presence of the first crop of oat plants (I) in soil containing bound ^{14}C residues may have been responsible for releasing some of these bound soil residues and making them more available to the second and third crops of oats. The three crops of oat plants (I, II, and III), grown successively for 2 weeks, removed a total of 5.1% of the previously soil-bound ^{14}C residues in the 1050 g (668 dpm/g) of soil–sand mixture in which they had grown.

The greens of all three crops of oat plants (I, II, and III) contained 46 to 62% of the total plant residues irregardless of whether they were grown in soil–sand mixtures containing bound or initially fresh ^{14}C residues. The roots from the first crop of oats (I) contained more ^{14}C residues than did the seeds; however, in the second and third crops (II, III) a similar distribution of ^{14}C residues between seeds and roots (23–28% of the total plant residues) was noticed.

Roots, seeds, and greens of the second and third crop of oats (II, III) were extracted and the residues were partitioned between benzene and water. The distribution (benzene soluble, water soluble, and bound) of ^{14}C residues in oats from soils containing bound or fresh residues were nearly identical. Results were therefore averaged and are presented in Figure 2. Plant-bound ^{14}C residues were smallest in greens and largest in roots, amounting to 5.0 ± 3.0 and 18.3 ± 2.3%, respectively, of the total ^{14}C residues recovered from the oat plants. Conversely, benzene–soluble ^{14}C compounds were largest in greens (60.3 ± 3.3% of total radiocarbon in plants). Amounts of water-soluble methyl [^{14}C]parathion residues were smallest in greens, but were considerably larger in roots and seeds where they accounted for over 50% of the total recovered ^{14}C residues. It is difficult to explain why the majority of residues in the greens were benzene soluble while the majority of residues in the roots and seeds were water soluble. It is unlikely that the residues became more apolar in moving from the roots to the greens. It is, however, possible that the benzene or lipid-soluble residues were preferentially translocated from the roots to the greens and

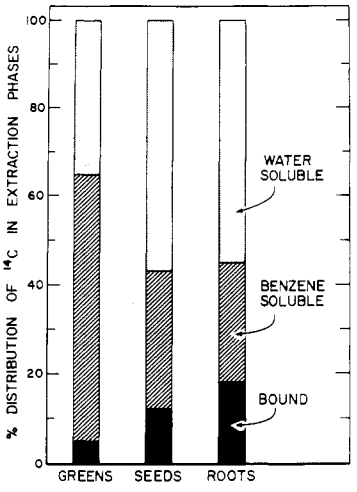

Figure 2. Distribution of extractable and bound residues in oat plants, after having grown in soils containing primarily bound residues of methyl [^{14}C]parathion.

thus, after the 2-week growing periods, a greater proportion of the residues in the greens were benzene soluble, while a greater proportion of the residues in the roots and seeds were water soluble.

It is interesting to note that the majority of soil-bound residues taken up by earthworms had again become bound in these worms, while most of the residues in the oat plants were extractable.

Data presented herein indicate that soil-bound insecticide residues are not excluded from environmental interaction. These residues could in fact be released from soils and were absorbed by both earthworms and roots of oat plants. Once they had penetrated into the animal or plant tissue, they were translocated and found partially in a bound form or as benzene and water-soluble ^{14}C compounds. It is, therefore, important to determine how soil-bound pesticide residues can be released and interact in the environment, thus potentially affecting biological systems.

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Fate of Dichlorodiphenyltrichloroethane and Its Metabolites during the Preparation of Fish Protein Concentrate

Virginia F. Stout

The partitioning of dichlorodiphenyltrichloroethane and its metabolites, dichlorodiphenyldichloroethane and dichlorodiphenyldichloroethylene (ΣDDT = the sum of the three compounds), between protein and lipid fractions during the laboratory production of fish protein concentrate (FPC) from Pacific herring (*Clupea harengus pallasi*) by isopropyl alcohol solvent extraction (IPA) and aqueous phosphate fractionation (APF) was determined. Analysis of the protein fractions of the raw herring flesh showed that myofibrillar proteins contained 0.06 ppm ΣDDT and sarcoplasmic proteins ~0.014 ppm. The FPC was essentially free of ΣDDT (<0.01 ppm) in both cases. The oil from the APF process contained more than three times as much ΣDDT (1.12 ppm) as the IPA oil (0.33 ppm).

The presence of organochlorine pesticides and industrial materials in fishery products (Stout, 1968; Henderson et al., 1969; Jensen, 1966; Risebrough et al., 1968; Jensen et al., 1969) suggested the need for information about the ultimate fate of these substances during the production of fish protein concentrate (FPC), a high-quality, nonperishable food prepared by separation of the water and lipids from the proteinaceous matter of fish. Species proposed for FPC production include many of the pelagic fatty species such as the herrings, menhaden, and anchovies utilized primarily for fishmeal and animal feed production. Chlorinated hydrocarbons are retained in fish to a variable extent during food preparation, that is, baking, poaching, frying, broiling, smoking, and canning (Smith et al., 1973; Reinert et al., 1972; Stout et al., 1970). In general, the concentration of chlorinated hydrocarbons in fish tissue changes in conjunction with the separation of lipids and the denaturation of the proteins. The degree of this change is related to the concentration and distribution of lipids in the animal, the size and the species of

U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Utilization Research Division, Seattle, Washington 98112.