

Effect of Dairy Manure and Sewage Sludge on ^{14}C -Pesticide Degradation in Soil

The degradation of 14 ^{14}C pesticides in sewage sludge and dairy manure amended Matapeake silt loam was investigated. The dairy manure and sewage sludge (60% primary–40% secondary) were applied at rates of 0, 50, and 100 ton/ha, leached to remove excess soluble salts, and incubated (30 °C) for 2 weeks prior to application of ^{14}C pesticides. The pesticides were selected as representative of the major pesticide degradation pathways in soil. Total CO_2 and $^{14}\text{CO}_2$ evolution were monitored regularly throughout the 60-day incubation period, and ^{14}C -product distribution was determined by soil extraction and thin-layer chromatographic analysis at the end of the incubation period. Altered rates of pesticide degradation were observed in sludge and/or manure amended soils for a number of structurally unrelated pesticides. ^{14}C -product distribution varied with soil amendments. The degradation of pesticides metabolized by dealkylation reactions (e.g., *s*-triazines, phenylureas, etc.) was inhibited by sewage sludge, but enhanced by manure.

Disposal of sewage sludge and feedlot wastes is a pivotal question in their processing. "Recycling" sludge nutrients to agricultural land is considered feasible (Keeney et al., 1975) and desirable. Application of animal manure to agricultural lands has been a common worldwide practice for centuries. One of the most actively investigated areas of environmental research has been the decomposition of agricultural, industrial, and municipal wastes in various types of water processing systems. The oxidation of individual organic chemicals in activated sludge has been examined in everything from simple bench-type systems to full-scale treatment plants. The results of these investigations are obvious in that we now commonly use waste treatment facilities which are dependent upon microbiological processes.

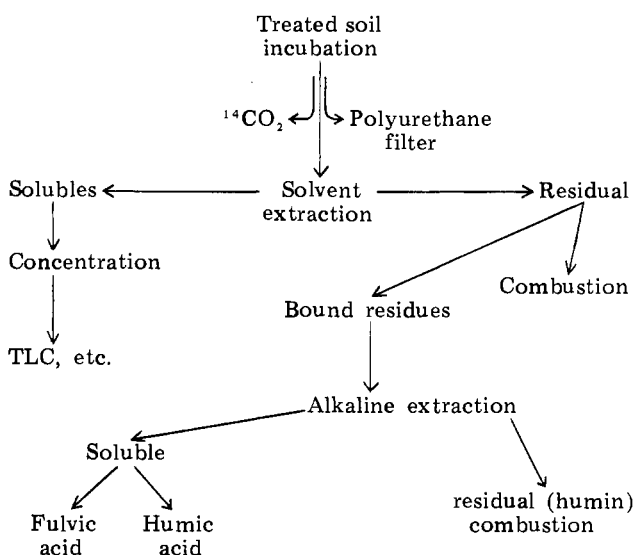
Some of the major problems which have been considered with land application of sludge include public acceptance, possible surface and ground water contamination, pathogens, crop yield reductions caused by accumulation of heavy metals, and food chain contamination by toxic elements. The authors are aware of no information concerning the possible effects of sewage or feedlot waste applications on the fate and behavior of pesticides in agricultural soils. It is conceivable that the fate and behavior of pesticides in soil may be altered by the addition of sewage sludge or manure. Thus, the purpose of this investigation was to examine the degradation of selected pesticides in sludge and manure amended soils.

MATERIALS AND METHODS

Soil, Sludge, and Manure Collection and Treatment. The soil used in these experiments was a Matapeake silt loam collected at the USDA's Agricultural Research Center, Beltsville, Md. Matapeake silt loam has a pH of 5.3, and organic matter, sand, silt, and clay contents of 1.5, 38.4, 49.4, and 12.2%, respectively. The soil was collected, partially air-dried ($\approx 10\%$ moisture) to permit sieving (2 mm), and stored moist in large polyethylene bags at 7 °C until used. The sewage sludge (60% primary and 40% secondary) was obtained from the Blue Plains Sewage Treatment Facility of Washington, D.C. The sludge was lyophilized, ground to a fine powder in a Wiley mill, and stored at -15 °C in polyethylene bags. The dairy manure was obtained from the University of Maryland Dairy-Forage Research Farm and was processed and stored by the same procedures. The manure contained no straw or other bedding materials.

Prior to initiating all experiments the soil was amended with CaCO_3 , watered, and incubated at 30 °C for 14 days to allow pH equilibration to approximately pH 6.2. Manure or sludge was then added to the soil to establish application rates of 0, 50, and 100 metric ton/ha. The soils

Scheme I. General Extraction Scheme for Characterizing ^{14}C -Pesticide Residue Distribution in Manure- and Sludge-Treated Soils



were then leached with water to remove excess soluble salts until the conductivity of the leachate was ≤ 2 mmhos and incubated for an additional 14 days at the 30 °C prior to addition of the pesticides. The rates of sewage sludge application rate used in this investigation are in accordance with those rates which have been considered for possible use in Maryland agricultural soils.

Pesticide Application and Soil Incubation. Common chemical names, ^{14}C -label position, specific activity, and application rates of the pesticides used in this investigation are listed in Table I. All pesticides were applied dropwise in appropriate solvents. Pesticide-treated soils were thoroughly mixed in the incubation flask after solvent evaporation, watered to 26.25% moisture content, and attached to a flow-through incubation unit. Each treated soil sample was aerated with CO_2 -free air. Air removed from the incubated soils initially passed through a polyurethane filter (Kearney and Kontson, 1976) to trap any volatile products other than CO_2 , which was subsequently trapped in 0.25 N NaOH. Pesticide-treated soils were incubated at 25 °C for 60 days. CO_2 traps were replaced at regular intervals and subsampled for liquid scintillation counting of evolved $^{14}\text{CO}_2$. Portions of the remaining trapping solution were used for determining total evolved CO_2 .

Soil Extraction and Product Identification. At the conclusion of the incubation period, the treated soils were frozen (15 °C) and stored prior to extraction. The basic

Table I. Common and Chemical Names, ^{14}C -Label Position, and Specific Activity of Pesticides Examined

Common name	Chemical name	^{14}C -label position	Sp act., $\mu\text{g}/\mu\text{Ci}$	Appli. rate, ppm
Atrazine	2-Chloro-4-(ethylamino)-6-(isopropylamino)- <i>s</i> -triazine	Ethyl	151.5	2.0
Chlorpropham	Isopropyl <i>m</i> -chlorocarbanilate	Isopropyl	82.0	3.0
2,4-D	(2,4-Dichlorophenoxy)acetic acid	Acetate	58.2	1.0
Dalapon	2,2-Dichloropropionic acid	Carboxyl	943.4	5.0
Dinoseb	2- <i>sec</i> -Butyl-4,6-dinitrophenol	Ring	79.3	5.0
Diuron	3-(3,4-Dichlorophenyl)-1,1-dimethylurea	Carbonyl	242.8	2.5
EPTC	<i>S</i> -Ethyl dipropylthiocarbamate	Ethyl	781.2	2.0
Linuron	3-(3,4-Dichlorophenyl)-1-methoxy-1-methylurea	Carbonyl	146.0	1.0
MH, Maleic hydrazide	1,2-Dihydro-3,6-pyridazinedione	Ring-3,6	373.8	2.5
PCMC, PPG-124	<i>p</i> -Chlorophenyl methylcarbamate	Carbonyl	185.0	2.0
PCNB	Pentachloronitrobenzene	Ring	20.0	5.0
Permethrin	<i>m</i> -Phenoxybenzyl <i>cis,trans</i> -(±)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate	Carbonyl	6.7	1.0
Propachlor	2-Chloro- <i>N</i> -isopropylacetanilide	Carbonyl	105.9	2.0
Trifluralin	α,α,α -Trifluoro-2,6-dinitro- <i>N,N</i> -dipropyl- <i>p</i> -toluidine	Ring	452.5	1.0

procedure for extracting soils is outlined in Scheme I. Solvents appropriate for each pesticide were used in the extraction and thin-layer chromatographic (TLC) procedures. Tentative product identification was by TLC comparisons with authentic standards. A more positive confirmation of PCNB and its degradation products was obtained by gas-liquid chromatographic comparisons with authentic standards.

RESULTS AND DISCUSSION

Numerous biochemical reactions have been associated with the microbial decomposition of pesticides: dehalogenation, dehydrohalogenation, dealkylation, amide and ester hydrolysis, oxidation, reduction, ether fission, aromatic ring hydroxylation, ring cleavage, condensation, conjugation, and alkylation. The pesticides and ^{14}C -labeling patterns used in this investigation were each selected to yield information on how sludge and manure may affect these mechanisms.

Various types of sludge and manure effects were observed on pesticide degradation in the amended soils. These effects were observed in terms of microbial respiration, $^{14}\text{CO}_2$ evolution, and distribution of ^{14}C in degradation products. Both sludge and manure increased total (titratable) CO_2 evolution with all pesticides. This would indicate that the pesticides had no inhibitory effect on the overall microbial activity in amended soils and that both amendments stimulated microbial activity. $^{14}\text{CO}_2$ evolution from manure-treated soils was equal to or greater than that from sewage sludge with all but one of the pesticides tested. Increased rates of pesticide degradation were observed in sludge and/or manure amended soils for a number of structurally unrelated pesticides. Pesticide losses by volatilization from all treated soils were negligible.

Sludge and manure increased the rate of $^{14}\text{CO}_2$ evolution from chlorpropham treated soil (Table II). Similar results were observed with dalapon, EPTC, and permethrin. Only sludge stimulated the degradation of 2,4-D, whereas linuron and trifluralin degraded most rapidly in manure-treated soil. Both manure and sludge partially inhibited the degradation of maleic hydrazide. Initially the inhibitory effect of sludge on maleic hydrazide was very pronounced, but decreased rapidly with time.

Neither sludge nor manure had any effect on $^{14}\text{CO}_2$ evolution in PCNB-treated soil. Significant differences were observed in extractable and residual ^{14}C activity and in product distribution from these soils, however. Lower ^{14}C extractions and higher residual ^{14}C activities were obtained from sludge and manure treated soil (Table III). ^{14}C -product distribution differed markedly (Table IV).

Table II. Percent ^{14}C Evolved as $^{14}\text{CO}_2$ from ^{14}C Pesticides in Control, Sludge-, and Manure-Amended Soils^a

Chemical	% ^{14}C evolved as $^{14}\text{CO}_2$				
	Control	Sludge		Manure	
		50	100	50	100
Atrazine	13.8	6.7	6.2	25.6	33.1
Chlorpropham	41.3	44.0	49.0	50.8	49.7
2,4-D	21.7	29.1	38.0	21.7	20.0
Dalapon	50.7	52.3	55.4	57.4	59.0
Dinoseb	36.0	17.8	19.8	35.1	31.1
Diuron	3.6	1.5	1.4	15.7	15.3
EPTC	63.7	65.1	65.8	65.3	61.8
Linuron	8.6	8.6	13.4	31.5	33.6
MH	73.1	46.4	59.4	68.8	66.2
PCMC	86.0	89.6	106.2	94.0	98.2
PCNB	0.4	0.4	0.1	0.6	0.4
Permethrin	0.1	3.8	5.8	2.0	5.7
Propachlor	20.3	8.9	6.0	24.2	24.9
Trifluralin	0.6	0.3	0.3	1.1	1.7

^a Sludge and manure amendments were made at 50 and 100 metric tons/ha; pesticides were applied at normal recommended rates.

Table III. Percent Recovery of ^{14}C from Ring-Labeled Pentachloronitrobenzene (PCNB) after 60 Days Incubation in Soil Amended with Sewage Sludge and Dairy Manure

Amendment	Rate, ton/ha	% ^{14}C recovered as			
		$^{14}\text{CO}_2$	Extraction	Residual	Total
Control		0.4	72.8	17.0	90.2
Sludge	50	0.4	61.9	23.6	85.9
	100	0.1	67.5	20.4	88.0
Manure	50	0.6	50.3	40.8	91.7
	100	0.4	57.7	34.1	92.2

Table IV. Percent Distribution of [^{14}C]Pentachloronitrobenzene Metabolites in Soil Extracts after 60 Days Incubation in Soil Amended with Sewage Sludge and Dairy Manure

Amendment	Rate, ton/ha	% ^{14}C recovered as ^a			
		PCNB	PCA	PCTA	Origin
Control		67.2	10.3	8.2	14.3
Sludge	50	59.5	8.2	22.1	10.2
	100	67.1	9.9	17.2	5.7
Manure	50	11.8	24.2	9.2	54.2
	100	38.8	19.5	10.7	31.0

^a PCNB, pentachloronitrobenzene; PCA, pentachloroaniline; PCTA, pentachlorothioanisole; origin, unknown.

Lower levels of pentachloroaniline and higher levels of pentachlorothioanisole were obtained from sludge-treated

Table V. Percent of Original ^{14}C Present in Atrazine and Propachlor Metabolites after 60 Days Incubation in Soil Amended with Sewage Sludge and Dairy Manure

Amend- ment	Rate, ton/ha	% ^{14}C recovered as			
		Atrazine	HO-Atrazine	Pro- pachlor	HO-Pro- pachlor
Control		39.1	7.9	8.6	2.4
Sludge	50	34.7	10.7	3.9	10.7
	100	38.3	10.4	2.5	12.9
Manure	50	25.0	7.7	3.7	0.6
	100	17.7	6.6	5.1	0.6

soil, whereas a more extensive conversion of PCNB to pentachloroaniline occurred in manure-treated soils.

The degradation of pesticides whose decomposition involves dealkylation appeared to be the most dramatically affected by sludge amendments. $^{14}\text{CO}_2$ evolution from [carbonyl- ^{14}C]diuron treated soil was stimulated by manure, but inhibited by sludge amendments. Extractable and residual ^{14}C activity did not differ greatly except in manure-treated soil where more $^{14}\text{CO}_2$ had been evolved and greater diuron degradation had occurred. Differences were observed, however, in the distribution of ^{14}C in degradation products. Both mono- [3-(3,4-dichlorophenyl)-1-methylurea] and didealkylated (3,4-dichlorophenylurea) degradation products of diuron were present in manure-treated soil. The principal products in sludge-treated and control soils were the parent diuron and the monodealkylated product.

Sludge also inhibited evolution of $^{14}\text{CO}_2$ from atrazine-, dinoseb-, and propachlor-treated soils. The principal degradation product isolated from propachlor sludge treated soil was 2-hydroxy-*N*-isopropylacetanilide (hydroxypropachlor) (Table V). Considerably more hydroxypropachlor was present in sludge-amended soil than in any of the other soils. This suggested that while dehalogenation of propachlor to hydroxypropachlor was not inhibited by sludge, its further degradation with liberation of the ^{14}C -carbonyl carbon was inhibited. Similar results were obtained with atrazine. Sludge inhibited the degradation and subsequent evolution of $^{14}\text{CO}_2$ from ^{14}C -chain-labeled atrazine (Table II). Manure, however, stimulated this reaction. Extractable ^{14}C activity was reduced from manure-treated soil. 2-Chloro-4-amino-6-isopropylamino-*s*-triazine and 2-hydroxy-4-ethylamino-6-isopropylamino-*s*-triazine (hydroxyatrazine) were identified as atrazine degradation products. The concentration of these products varied with each soil amendment. The concentration of several additional products, as yet unidentified also varied with the soil amendment.

The distribution of ^{14}C in atrazine and hydroxyatrazine is shown in Table V. Care must be taken in interpreting the data from this table, however. Microbial degradation of atrazine proceeds via dealkylation (Kaufman and Blake, 1970), with the ethyl group being removed most readily. Percentage figures indicating ^{14}C distribution in chain-labeled products are somewhat misleading since 2-chloro-4-amino-6-isopropylamino-*s*-triazine, the major dealkylation product, no longer contains the ^{14}C -ethyl group. Inability to record radioactivity within this metabolite would distort the significance of other metabolites

appearing from chain-labeled atrazine. Results obtained with ring-labeled atrazine would not lend this distortion. Of particular interest, however, is the somewhat higher level of hydroxyatrazine in sludge-treated soils. The conversion of atrazine to hydroxyatrazine immediately inactivates this pesticide, whereas dealkylation only leads to a progressive loss of phytotoxicity (Kaufman and Blake, 1970). These results suggest, therefore, that while residual phytotoxicity of atrazine or propachlor may not necessarily be increased in sludge-amended soils, the nature of the residual products may be altered. Additional work with ^{14}C -ring-labeled atrazine is needed to further clarify these effects.

The effects of sewage sludge on dealkylation reactions described herein have been observed consistently in all four subsequent experiments designed with various modifications to further confirm and elucidate the mechanism and significance of this apparent interaction in sludge-treated soils.

The results of this investigation indicate that sludge and manure amendments to soil can alter the degradation rate and persistence of pesticides in soil. This effect is realized through stimulation or inhibition of pesticide degradation, or alteration of the concentrations of residual degradation products. Additional work is in progress to: (1) elucidate the causal mechanisms of the effects observed, (2) assess the significance of these effects under actual field conditions, (3) determine the effects of composting and sludge source on these phenomena, (4) determine the effects of lower sludge application rates on the dealkylation mechanism, and (5) to determine the effect of prolonged preincubation of sludge-treated soil on subsequent pesticide degradation. In regard to the last aspect (no. 5), results obtained to date indicate that the inhibition of atrazine dealkylation reactions is as strong or even more pronounced in sludge-treated soils incubated 120 days prior to introduction of the pesticide. Longer preincubation periods are also being considered.

LITERATURE CITED

- Kaufman, D. C., Blake, J., *Soil Biol. Biochem.* **2**, 73 (1970).
 Kearney, P. C., Kontson, A., *J. Agric. Food Chem.* **24**, 424 (1976).
 Keeney, D. R., Lee, K. W., Walsh, L. M., "Guidelines for the Application of Waste Water Sludge to Agricultural Land in Wisconsin", Department of National Resources, Madison, Wis., Technical Bulletin No. 88, 1975, 36 pp.

Richard C. Doyle¹
 Donald D. Kaufman²
 Gordon W. Burt¹

¹Agronomy Department
 University of Maryland
 College Park, Maryland 20142
²Beltsville Agricultural Research Center, West
 Agricultural Research Service
 U.S. Department of Agriculture
 Beltsville, Maryland 20705

Received for review December 14, 1977. Accepted April 20, 1978.
 Presented at the Pesticide Division, 174th National Meeting of the American Chemical Society, Chicago, Ill., Aug 28, 1977.
 Computer time for data processing was funded by the University of Maryland Computer Science Center.