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RESIDUES IN SOILS

Exploratory Studies on Occurrence of Organochlorine Insecticide Residues in Agricultural Soils in Southwestern Ontario

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Analysis of soil samples collected on 31 farms throughout southwestern Ontario indicated the presence of organochlorine insecticide residues in a number of cases. DDT and its metabolites were present in amounts in excess of 0.1 p.p.m. in 24 of 31 soil samples. Dicofol was present in three of four orchard soils and endosulfan in three of four greenhouse soils. Aldrin and/or dieldrin were found in amounts in excess of 0.1 p.p.m. in 16 of 31 samples. Heptachlor and/or its epoxide were found in three samples. Gamma-chlordan was present in all cases where there was a history of heptachlor treatment. On the average, the highest residues occurred in tobacco, vegetable, and orchard soils. The pattern of development of cyclodiene resistance by soil insects in southwestern Ontario can be correlated with the levels of cyclodiene insecticide residues in the soil.

The organochlorine insecticides have been used to control agricultural insects since 1946, initially with the introduction of DDT and BHC and, subsequently, the cyclodiene insecticides such as aldrin, dieldrin, endrin, and heptachlor. In the interval there has been considerable concern over the possibility that organochlorine insecticide residues will accumulate in the soil, either as a result of drift or runoff from foliage applications or from direct application of these materials to the soil for insect control. Considerable effort, particularly in the United States, has been devoted to defining the factors influencing persistence and degradation of organochlorine insecticide residues in soil and a considerable amount of information has been obtained, primarily in laboratory and field experiments conducted under carefully controlled conditions. Studies are now needed to determine to what extent residues of organochlorine insecticides are occurring in agricultural soils as a result of commercial applications of these materials for insect control.

In the past, surveys to determine levels of organochlorine residues in soil have been hampered by the lack of simple, yet accurate, analytical procedures. Colorimetric procedures, while adequate in some cases, were highly specific and time-consuming. Nevertheless, a number of surveys have been conducted, with particular reference to DDT in orchard soils (*2, 3, 14*). Comprehensive surveys on DDT in orchard, field crop, and vegetable soils were conducted by Ginsburg (*5*), Ginsburg and Reed (*6*), and Lichtenstein (*10*). More recently Wheatley, Hardman, and Strickland (*15*) surveyed DDT residues in 21 farm soils in Great Britain, while Murphy, Fahey, and Miles (*12*) studied DDT residues in farm soils in Indiana. The results of these studies have indicated that, generally, residues of DDT are high in orchard soils and considerably lower in field crop soils. Less effort has been devoted to determining levels of cyclodiene insecticide residues in soil resulting from commercial applications. However, Wheatley *et al.* (*15*) determined aldrin and dieldrin residues in soil, as well as DDT, and reported residues of dieldrin in 17 of 21 fields in amounts ranging from 0.01 to 0.41 p.p.m. Recently Decker, Bruce, and Bigger (*4*) found residues of aldrin and dieldrin ranging from 0.12 to 1.22 p.p.m. in 35 Illinois corn belt soils.

In Canada there is little information regarding the occurrence of organochlorine insecticide residues in soils. Wilkinson, Finlayson, and Morley (*16*) found residues of aldrin-dieldrin and heptachlor–heptachlor epoxide in soils 9 years after a single treatment, and recently Stewart, Chisholm, and Fox (*13*) reported on the persistence of aldrin and heptachlor in soils treated at a rate of 5 and 10 pounds per acre in 1958, 1959, and 1960. Virtually no information is available on residues in soils resulting from commercial applications of insecticide other than two studies on DDT residues in orchard soils. Herne and Chisholm (*9*) found DDT residues ranging from 2.5 to 7.1 p.p.m. in a soil in an Ontario peach orchard and MacPhee, Chisholm, and MacEachern (*11*) recorded residues of DDT in a Nova Scotia soil amounting to 136 p.p.m. in 1954 and 76 p.p.m. in 1958.

Southwestern Ontario is an area of intensive agriculture with a broad spectrum of soil types and crops. DDT has been used extensively since its introduction, and, in addition, since soil insects are a particularly serious problem in this area, the cyclodiene insecticides were utilized to a considerable extent between 1954 and 1960 and to a lesser extent since that time. It therefore seemed logical to select this area as ideal for an exploratory investigation of the occurrence of organochlorine residues in agricultural soils as a result of commercial applications of insecticide for insect control. This report summarizes the results obtained.

Methods and Materials

Soil samples were collected on 31 farms located between Windsor to the west, Parry Sound to the north, and Stouffville to the east (Figure 1). The sampling sites were carefully selected to obtain a comprehensive picture from a relatively small number of samples. The soil types ranged from sand to muck; the crops included sugar beets, forage and pasture, corn, cereals, tobacco, greenhouse vegetables, field vegetables, and fruit (Table I). Particular care was taken to include several farms where insecticide application had been minimal in order to ensure that the study would not become prejudiced toward exorbitantly high residue levels. Each cooperating farmer was interviewed and, as much as possible, a history of the cropping practices, insect problems, and insecticide treatments during the previous 5 years was obtained. By agreement, the names of the cooperators have been kept confidential and their anonymity has been retained throughout this report. A soil sample from the experimental plots at the Chatham laboratory (sample 32) was included as a check, since repeated tests on this area have shown that no insecticide residues are present in the soil. A number of the sampling sites were located in areas where soil insect resistance to the cyclodiene insecticides has developed.

Sampling Procedure. An area of approximately 5 acres was selected in a field and five subareas were sampled within this 5-acre site; the subareas, 4 feet square, were placed diagonal to the field perimeter; 25 6-inch cores were taken from each subarea and cores from all five subareas were pooled in order to obtain a representative sample from the field; the pooled sample (approximately 10 pounds of soil) was sealed in a plastic bag and stored in a refrigerated room. In orchards, samples were taken both beneath and between the trees and analyzed separately. Samples were collected in October and November 1964, and April 1965.

The insecticidal residues were extracted from the soil within 2 weeks of sampling. Three hundred grams of moist soil were extracted with a 1 to 1 acetone–petroleum ether solvent mixture. The petroleum ether extract was then freed of acetone by washing with water, dried, and stored in a freezer until required. The samples were analyzed on a Wilkens Hi-Fi gas chromatograph using an electron capture detector equipped with a Dow Silicone 11 chromatographic column. In order to separate pesticides which eluted simultaneously through this column, preliminary separations were first performed by liquid-solid fractionation using procedures developed at this laboratory (unpublished data). If additional identification was required, a QF-1 column was utilized and some special chemical conversion methods were developed. The procedures developed will be reported later. Quantitative determinations were made on residues greater than 0.1 p.p.m. All calculations were based on the oven-dry weight of the soil (105° C. for 24 hours).

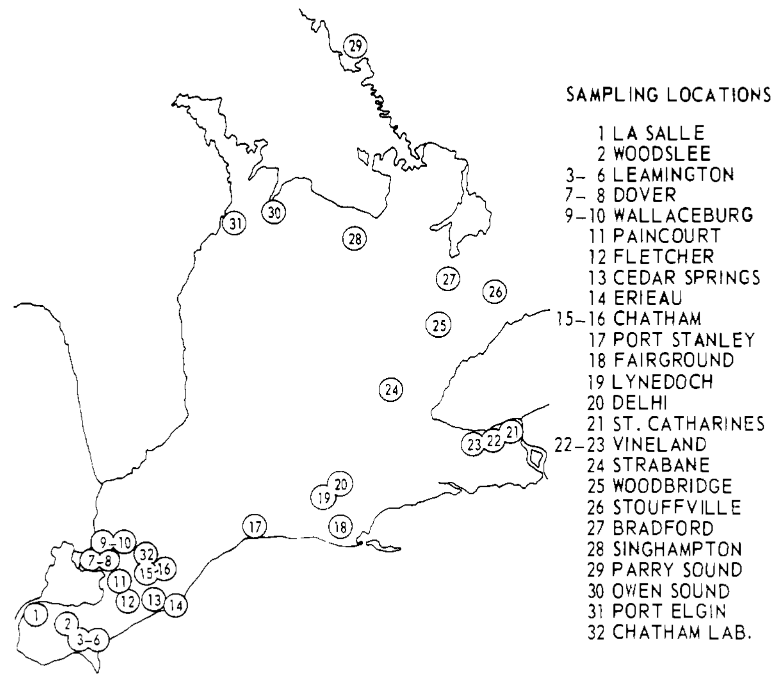

Figure 1. Sampling locations throughout southwestern Ontario

Assays of the bioactivity of the residues in the soil were conducted using first-instar nymphs of the common field cricket, *Gryllus pennsylvanicus* (Burmeister). Two 50-gram aliquots of each moist soil sample were weighed into waxed paper cups and 1-day-old cricket nymphs placed on the soil surface. Sources of food and water were provided. Mortality counts were made at 24 and 48 hours. Corrections for natural mortality were made using Abbott's formula (*1*).

Results and Discussion

DDT was found in amounts exceeding 0.1 p.p.m. in 24 of 32 soil samples (orchard samples between and under the trees counted as one), with the highest being 118.9 p.p.m. (Table II). Traces of DDT (less than 0.1 p.p.m.) were found in two additional samples. DDE was present in all cases where DDT was detected and DDD was found in 15 of the 26 samples containing DDT. Dicofol was present in three orchard soils in amounts ranging from 2.4 to 6.9 p.p.m. Aldrin and/or dieldrin were found in amounts exceeding 0.1 p.p.m. in 16 of 32 samples, with the highest concentration of aldrin being 2.1 p.p.m. and dieldrin 1.6 p.p.m. Trace amounts of aldrin and/or dieldrin were found in five additional samples. Heptachlor and heptachlor epoxide occurred in amounts in excess of 0.1 p.p.m. in three samples with traces in four other samples. The highest of either material was 0.2 p.p.m. Gamma-chlordan was found in seven samples, all of which contained heptachlor and heptachlor epoxide, and also in one sample which contained no detectable amounts of heptachlor or its epoxide. Stewart *et al.* (*13*) consider that gamma-chlordan is present as a contaminant of technical heptachlor. Endrin was detected at a concentration of 3.8 p.p.m. in one sample and in trace amounts in two other samples. Endosulfan was detected in amounts exceeding 0.1 p.p.m. in three samples—all greenhouse soils—with a trace occurring in a fourth sample taken from a vegetable growing area.

The presence of DDT, endosulfan, and, in one case, dieldrin (samples 3a, b and 4a, b) in greenhouse soils was somewhat surprising, since interviews with the growers had indicated no serious insect problems in greenhouse crops during the past 5 years, other than mites and white fly, for which dicofol, Dibrom, maneb, and zineb had been utilized. In addition, samples 3b and 4b were new greenhouses, no crops yet having been planted in 4b and only three crops in 3b. Further inquiry determined that the greenhouses had been built on land previously devoted to growing field vegetables, most recently cabbages, and that the same soil was being utilized in the greenhouses. Apparently the soils were contaminated with insecticidal residues prior to building the greenhouses.

The highest residue of DDT, 118.9 p.p.m. (sample 21), occurred under the

Table I. Cropping History and Insecticide Usage (Organochlorine Insecticides Only)

Sample No.	Soil Type		History Pre-1960	1960	1961	1962	1963	1964
1	Sand	Crop	Onions	Onions	Onions	Onions	Lettuce	Lettuce
		Insecticides	Aldrin	Aldrin	...	...	DDT (foliar)	...
2	Clay loam	Crop		Fallow	Corn	Fallow	Corn	Fallow
		Insecticides		...	DDT (foliar)	...	Aldrin	...
3	Sandy loam	Crop					Cabbage	Greenhouse
		Insecticides					...	Dicofol
4	Sandy loam	Crop			Greenhouse vegetables			
		Insecticides			Dicofol			
5	Muck	Crop			Continuous onions			
		Insecticides			Aldrin, dieldrin, heptachlor prior to 1960			
6	Muck	Crop			Continuous radishes			
		Insecticides			Endrin and DDT (foliar) applied annually			
7	Loam	Crop		Corn	Beets	Corn	Corn	Carrots
		Insecticides		...	...	...	...	DDT (foliar)
8	Loam	Crop	Onions					
		Insecticides	Aldrin					
9	Clay	Crop			Barley	Corn	Corn	Sugar beets
		Insecticides			...	...	...	Endrin (surface)
10	Clay loam	Crop		Corn	Oats	Corn	Sugar beets	Corn
		Insecticides		...	...	...	Endrin (surface)	DDT (foliar)
11	Loam	Crop		Sugar beets	Corn	Corn	Soya beans	Sugar beets
		Insecticides		...	...	...	...	Aldrin
12	Clay loam	Crop	Alfalfa	Alfalfa	Alfalfa	Corn	Corn	Corn
		Insecticides	...	...	...	Aldrin	Aldrin	Aldrin
13	Loam	Crop			Apple orchard			
		Insecticides			DDT (foliar) applied annually			
14	Muck	Crop	Onions	Onions	Celery	Celery	Onions	Onions
		Insecticides			DDT (foliar) five applications per year			
15	Loam	Crop			Corn	Corn	Beans	Corn
		Insecticides			...	...	...	Heptachlor (seed treatment)
16	Sandy loam	Crop			Corn	Oats	Alfalfa	Sugar beets
		Insecticides			Heptachlor (seed treatment)	...	...	...
17	Sandy loam	Crop			Tobacco-rye-potatoes			
		Insecticides			No history available			
18	Sand	Crop	Tobacco-rye	Rye	Tobacco	Rye	Tobacco	Wheat
		Insecticides	Aldrin	...	DDT	...	DDT	
19	Sandy loam	Crop	Tobacco-rye	Tobacco	Rye	Tobacco	Wheat	Tobacco
		Insecticides	Aldrin	Aldrin-DDT	...	DDT	...	DDT
20	Sandy loam	Crop	Tobacco-rye	Tobacco	Rye	Tobacco	Rye	Tobacco
		Insecticides	Aldrin-heptachlor			DDT-endrin (foliar)		
21	Sandy loam	Crop			Apple orchard			
		Insecticides			DDT from 1950 to 1961			
22	Clay loam	Crop			Apple orchard			
		Insecticides			No history available			
23	Sandy loam	Crop			Apple orchard			
		Insecticides			No history of organochlorine insecticides			
24	Loam	Crop	Turnips	Fallow	Turnips	Corn	Turnips	Wheat
		Insecticides	Aldrin	...	Aldrin	...	Aldrin	...
25	Sandy loam	Crop			Continuous radishes			
		Insecticides			Aldrin applied annually			
26	Clay	Crop	Turnips-clover	Turnips	Clover	Fallow	Oats	Turnips
		Insecticides	Aldrin	Aldrin	...	...	...	Aldrin
27	Muck	Crop		Carrots	Carrots	Lettuce	Onions	Carrots
		Insecticides			DDT (foliar) four to six applications per year			
28	Loam	Crop			Hay	Hay	Oats	Oats and barley
		Insecticides				No insecticide treatment		
29	Clay loam	Crop			Hay	Oats	Hay	Hay
		Insecticides				No insecticide treatment		
30	Silt loam	Crop			Pasture			
		Insecticides			No insecticide treatment			
31	Sandy	Crop			Tobacco	Rye	Tobacco	Corn
		Insecticides			Aldrin	...	DDT	...
32	Sandy loam	Crop			All crops			
		Insecticides			No insecticide treatment			

Table II. Residues of Organochlorine Insecticides Found in Soil

Sample No.	Insecticide Residues in Soil, P.P.M. Based on O.D. Weight of Soil										
	Heptachlor	Heptachlor Epoxide	Gamma-Chlordan	Aldrin	Dieldrin	Endrin	Endo-sulfan	DDT	DDE	DDD	Dicofol
1	Trace[a]	Trace	0.2	Trace	1.3	...	...	3.4	0.2	Trace	...
2	...	...	...	Trace	Trace	...	...	1.5	Trace	...	...
3a[b]	...	...	...	...	...	...	0.9	1.3	0.5	Trace	...
b[c]	...	...	...	...	...	...	1.4	2.0	0.6	Trace	...
4a[b]	...	...	...	...	0.4	...	0.6	Trace	0.2	0.2	...
b[d]	...	...	...	...	Trace	...	...	1.0	0.2	Trace	...
5	0.2	Trace	0.6	Trace	1.1	...	...	4.6	0.4	...	...
6	...	...	...	2.1	1.6	3.8	...	13.8	0.8	0.4	...
7	...	...	...	...	...	...	...	3.7	Trace	...	...
8	...	...	Trace	Trace	0.5	...	...	0.9	0.2	...	...
9	...	...	...	...	...	Trace	...	...	...	...	...
10	...	...	...	...	...	Trace	...	...	...	...	...
11	...	...	...	Trace	Trace	...	...	1.2	0.3	...	...
12	...	...	...	0.5	0.4	...	...	...	...	...	...
13a[e]	...	...	...	...	...	...	...	22.9	1.9	0.5	...
b[f]	...	...	...	...	...	...	...	6.9	1.0	0.2	...
14	...	...	...	Trace	Trace	...	...	22.6	1.0	0.3	...
15	...	...	...	0.1	...	...	...	0.7	Trace	...	...
16	...	...	...	Trace	Trace	...	...	...	...	...	...
17	...	...	...	0.2	0.6	...	...	1.0	0.1	...	...
18	Trace	Trace	Trace	Trace	0.3	...	...	4.1	0.3	...	...
19	Trace	Trace	0.1	Trace	0.3	...	...	4.6	0.5	Trace	...
20	Trace	0.2	0.2	Trace	0.2	...	...	2.1	0.3	0.1	...
21a[e]	...	...	...	...	...	...	...	118.9	9.1	3.1	6.9
b[f]	...	...	...	...	...	...	...	71.8	7.7	2.3	3.0
22a[e]	...	...	...	...	...	...	...	66.9	15.7	3.1	3.8
b[f]	...	...	...	...	...	...	...	66.5	9.6	3.5	2.4
23a[e]	...	...	...	...	...	...	...	28.2	5.9	0.3	5.0
b[f]	...	...	...	...	...	...	...	19.9	3.4	0.2	3.7
24	...	...	...	0.5	0.9	...	...	0.3	Trace	...	...
25	Trace	Trace	Trace	0.8	0.7	...	...	0.6	0.1	Trace	...
26	...	...	...	0.2	1.1	...	...	2.6	Trace	...	...
27	...	...	...	Trace	0.8	...	Trace	45.4	1.6	0.6	...
28	...	...	...	...	...	...	...	Trace	Trace	...	...
29	...	...	...	...	...	...	...	...	...	...	...
30	...	...	...	...	...	...	...	...	...	...	...
31	Trace	0.1	0.1	Trace	0.5	...	...	2.3	0.6	Trace	...
32	...	...	...	...	...	...	...	...	...	...	...

[a] "Trace" denotes less than 0.1 p.p.m.
[b] Old greenhouse.
[c] New greenhouse, only three crops planted.
[d] New greenhouse, no crops planted.
[e] Under trees.
[f] Between trees.

trees in an orchard where DDT had been utilized extensively from 1950 to 1961. DDT residues were also high in other orchard soils, particularly sample 22 which contained 66.9 and 66.5 p.p.m. of DDT under and between the trees, respectively. Generally residues of DDT in orchard soils are higher under the trees than between the trees and in three of the four orchards checked (samples 13, 21, and 23) this was the case. The highest concentration of cyclodiene insecticide residues (sample 6) occurred on a farm where four crops of radishes were grown each year and endrin was applied for cabbage maggot control with each new crop. This sample, a muck soil, contained 2.1 p.p.m. of aldrin, 1.6 p.p.m. of dieldrin, and 3.8 p.p.m. of endrin. In five other cases residues of cyclodiene insecticides in the soil exceeded 1.0 p.p.m. (samples 1, 5, 24, 25, and 26). In all cases vegetables, largely root crops, were the major crop.

It is apparent from Tables I and II that the insecticide residues in the soil are dependent on both the cropping practice and the method of insecticide application. Seed treatments would not be expected to result in any large accumulation of residues in the soil and, as can be seen with samples 15 and 16, this is the case. In addition, when insecticides are applied annually to tall, dense crops, such as endrin on tobacco (sample 20), residues do not appear in the soil. Applications of endrin to the soil surface for cutworm control in sugar beets (samples 9 and 10) resulted in only traces of endrin in the soil. The most serious residue problems occur when the insecticides are applied directly to and immediately incorporated into the soil. It is because of this type of treatment that cyclodiene insecticides are present in corn (sample 12), tobacco (samples 17, 18, 19, 20, and 31), and vegetable soils (samples 1, 5, 6, 8, 24, 25, 26 and 27). In addition, when DDT is applied as a foliar spray for flea beetle, leafhopper, or looper control, as many as four to six applications of DDT are made in a single summer. Such frequent applications, combined with frequent cultivation, have resulted in the soils becoming highly contaminated with DDT, as illustrated in Table II (samples 6, 14, and 27 which contain 13.8, 22.6, and 45.4 p.p.m., respectively).

When the over-all residue picture is considered it is obvious, from the data given in Table I, that crop rotation must be taken into consideration. For example, tobacco is normally rotated with rye, which is ploughed down in order to build up the soil. However, winter wheat (samples 18 and 19) or corn (sample 31) is often included as an alternate. Similarly, turnips are usually rotated with clover and either cereals or corn (samples 24 and 26). Consequently, since insecticides are applied for insect control on tobacco and turnips, the remaining crops in the rotation patterns are grown on contaminated soils, and some of these crops are able to absorb residues of cyclodiene insecticides from the soil.

An attempt has been made to summarize the residue picture in relation to cropping practices (Table III). Because of crop rotation certain sampling sites are included under more than one heading. Eight categories have been included: sugar beets, forage and pasture, corn, cereals, greenhouse vegetables, tobacco, field vegetables, and orchards. However, with the small number of samples taken, such an interpretation, while helpful for the purposes of this report, is not statistically sound. Since sugar beets, forage and pasture crops, corn, and cereals are grown largely on heavy mineral soils, the total residue levels of 0.4 to 1.8 p.p.m. do not appear to be sufficiently high to merit concern. However, in tobacco soils, both the DDT and cyclodiene insecticide residues are higher and, since these soils are such light mineral soils, the degree of insecticide inactivation would be slight.

By far the most serious residue problem is in vegetable soils where the average values of 9.5 p.p.m. of DDT and 1.6 p.p.m. of cyclodiene insecticides are high, and many of the major crops are root crops. The problem is mitigated to some extent by the fact that these soils are often high in organic content or are heavy mineral soils where inactivation of the residues is proportionately greater than in sandy tobacco soils. The average value of 61.8 p.p.m. of total residue in orchard soils is excessive, although in agreement with the numerous studies which have been conducted in the United States.

Data on the bioactivity of the residues in soil are given in Table IV. Harris (7) has shown that soil type has a pronounced influence on insecticide bioactivity, and recently data have been obtained at this laboratory on bioactivity in relation to a broad spectrum of soil types (unpublished data). These studies have established that, with the exception of two orchard soils (samples 21 and 22), the DDT residues are not sufficiently high to cause toxicity to first-instar cricket nymphs. Therefore, with the exception of samples 21 and 22, all mortality can be attributed to the presence of cyclodiene insecticide residues in the soil. Gas chromatographic analysis (Table IV) indicated that 16 of 32 samples contained significant amounts of the cyclodiene insecticides. However, in only 8 out of 16 cases were the materials sufficiently active to cause mortality. The importance of soil type is obvious—all of these soils were mineral soils low in organic matter (samples 1, 17, 19, 20, 24, 25, 26, and 31). Sample 6, a muck soil which contained 7.5 p.p.m. of cyclodiene insecticides, caused no mortality, thus indicating the degree to which these insecticides are inactivated in organic soil. Similar results were obtained with samples 5, 9, and 27, all of which were high in organic content.

Table III. Average Organochlorine Insecticide Residues (P.P.M.) in Soil in Relation to Major Crop(s) Grown

Crop	Samples	DDT and Related[a] Materials	Cyclodiene Insecticides[b] and Related Materials	Total Organochlorine Residue
Sugar beets	9, 10, 11, 16	0.4	. . .	0.4
Forage and pasture	16, 26, 28, 29, 30	0.5	0.3	0.8
Corn	2, 7, 9, 10, 11, 12, 15, 16, 24, 31	1.2	0.2	1.4
Cereals	9, 10, 16, 18, 19, 24, 26, 28, 29	1.4	0.4	1.8
Greenhouse vegetables	3a, b, 4a, b	1.5	0.8	2.3
Tobacco	17, 18, 19, 20, 31	3.2	0.6	3.8
Vegetables	1, 5, 6, 7, 8, 14, 17, 24, 25, 26, 27	9.5	1.6	11.1
Orchards	13a, b, 21a, b, 22a, b, 23a, b	61.8	. . .	61.8

[a] DDT, DDE, DDD, and dicofol.
[b] Heptachlor, heptachlor epoxide, gamma-chlordan, aldrin, dieldrin, endrin, and endosulfan.

Table IV. Bioactivity of Insecticidal Residues in Soil to First-Instar Nymphs of Common Field Cricket, *Gryllus pennsylvanicus* (Burmeister)

Sample No.	Soil Type	DDT in Soil, P.P.M.	Cyclodiene Insecticides in Soil, P.P.M.	Corrected % Mortality 24 hr.	Corrected % Mortality 48 hr.
1	Sand[a]	3.4	1.5	68	100
2	Clay loam	1.5	Trace	0	0
3a	Sandy loam	1.3	. . .	0	0
b	Sandy loam	2.0	. . .	0	0
4a	Sandy loam	Trace	0.4	0	0
b	Sandy loam	1.0	Trace	0	0
5	Muck[a]	4.6	1.9	0	0
6	Muck	13.8	7.5	0	0
7	Loam	3.7	. . .	0	0
8	Loam[a]	0.9	0.5	0	0
9	Clay loam	. . .	Trace	0	0
10	Clay loam	. . .	Trace	0	0
11	Loam	1.2	Trace	0	0
12	Clay loam	. . .	0.9	0	0
13a	Loam	22.9	. . .	0	0
b	Loam	6.9	. . .	0	0
14	Muck	22.6	Trace	0	0
15	Loam	0.7	0.1	0	0
16	Sandy loam	. . .	Trace	5	5
17	Sandy loam[a]	1.0	0.8	11	90
18	Sand[a]	4.1	0.3	0	0
19	Sandy loam[a]	4.6	0.4	5	95
20	Sandy loam[a]	2.1	0.6	0	11
21a	Sandy loam	118.9	. . .	45	83
b	Sandy loam	71.8	. . .	55	75
22a	Clay loam	66.9	. . .	0	17
b	Clay loam	66.5	. . .	0	33
23a	Sandy loam	28.2	. . .	0	0
b	Sandy loam	19.9	. . .	0	0
24	Loam[a]	0.3	1.4	84	100
25	Sandy loam	0.6	1.5	100	100
26	Clay[a]	2.6	1.3	11	100
27	Muck[a]	45.4	0.8	0	0
28	Loam	Trace	. . .	0	0
29	Clay loam	. . .	. . .	0	0
30	Silt loam	. . .	. . .	0	0
31	Sand	2.3	0.7	10	22
32	Sandy loam	. . .	. . .	0	0

[a] Areas where root maggots have developed resistance to cyclodiene insecticides.

The residue picture is of particular interest in relation to the development of cyclodiene insecticide resistance throughout southwestern Ontario. The most serious resistance problems have arisen in the vegetable-growing areas (onion maggot, cabbage maggot) and in the tobacco-growing areas (seed maggots, cutworms). In ten of the areas sampled (Table IV) resistance has been a serious problem, and the data in Table II establish the significance of the levels of cyclodiene insecticide residues in soils, their bioactivity, and the resistance problem. A separate publication (*8*) deals with the relationship between cyclodiene residues in the soil and the development of seed maggot resistance.

The data given in this report should not be misinterpreted. The study is exploratory, and, with the small numbers of samples taken, the data cannot be considered as statistically representative of the picture throughout southwestern Ontario. However, organochlorine insecticide residues are present in agricultural soils, especially tobacco, vegetable, and orchard soils. Work in the United States, particularly by Lichtenstein and his coworkers, has shown that certain crops will absorb residues of the cyclodiene insecticides from soils in amounts dependent on both climate and soil type. To establish the significance of these residues in soil, a logical extension of this program would be to investigate, under practical agricultural conditions, the absorption of pesticides from different soil types by root crops. In addition, because of the rotation pattern utilized in tobacco- and turnip-growing areas, which involves both forage crops and cereals, studies on the amount of absorption should also be initiated in this area. The former study was initiated in 1965; the latter is being undertaken in 1966.

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RESIDUE DETERMINATION

Residues in Tissues of Fish Killed by Antimycin

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Highly radioactive antimycin was obtained by reformylation of deformylantimycin with H^3-formic acid, and was used to kill trout and carp at 5 and 10 p.p.b. water concentrations. Antimycin levels in the fish tissues were estimated from their H^3 content. This method probably gives high results because of degradation and protein binding of the toxicant. Tissue concentrations so observed ranged from 30 to 950 μg. per kg. of fresh weight. Edible portions averaged 76 to 201 μg. per kg., while heart, liver, and kidney averaged 736, 683, and 388 μg. per kg., respectively. Whole body levels averaged 203 μg. per kg. Concentrations in carp were 2 to 3 times higher than in trout, probably because of greater resistance and longer survival times. The levels found are so low that no harmful effects would be anticipated from use of antimycin-poisoned fish in animal feeds. Preliminary evidence suggests that such fish probably are also safe for human consumption.

Antimycin (*14*) is a potent fish poison (*2*) and has other properties which give it promise as a fish control agent (*17*). The possibility of its widespread use for this purpose raised the question of whether fish killed in this manner are safe for animal or human consumption. A determination of the residual antimycin levels in the tissues of such fish was therefore undertaken.

Since the antimycin concentrations to be anticipated were so low (*2*) that they probably could not be estimated by available assay procedures (*12*, *13*), it was decided as a first attempt to rely on radioactively labeled antimycin, provided a product with sufficiently high specific activity could be obtained. This objective was accomplished by reformylation of deformylated antimycin with H^3-formic acid. The preparation obtained was used for the studies reported in this paper.

Experimental

Materials and Methods. Determination of Radioactivity. All counting was done in a Packard Tri-Carb liquid scintillation spectrometer, Model 3003, equipped with a Packard automatic standardizer, Model 3951. The phosphor solution was made from 15.0 grams of 2,5-diphenyloxazole (PPO), 0.19 gram of 1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP), 3000 ml. of toluene, and 750 ml. of absolute ethanol. All scintillation vials were filled with 18 ml. of the solution to be counted.

Deformylantimycin A_3 Hydrochloride. This compound was obtained by acid hydrolysis of blastmycin [antimycin A_3 containing a small proportion of A_4 (*7*)] as described previously (*16*). After recrystallization from ethanol and concentrated hydrochloric acid, it gave an infrared spectrum identical with that of an authentic specimen (*11*). Further confirmation of the identity of the deformylated compound was obtained by reformylation (as described below) with nonradioactive formic acid. The product, obtained in 75% yield, showed melting point of 168–172° C.;