

Figure 2. Infrared spectrum of 2-chloro-4-amino-6-isopropylamino-*s*-triazine isolated from shoots of mature pea plants

The compound was collected on KBr as the effluent from the gas chromatographic column and pressed into 1.5-mm. diameter pellets

altering the original atrazine molecule that resulted in the accumulation of 1.2 to 2.0 times as much dealkylated product as atrazine within 48 hours. Therefore, susceptibility of the pea plant to atrazine was not due to its inability to metabolize atrazine. If this is true, the amount of unchanged atrazine found in some species may not necessarily be correlated with its tolerance. Although some herbicidal activity has been attributed to 2-chloro-4-amino-6-isopropylamino-*s*-triazine (4), no tolerance study of peas to this compound has been reported. Apparently, an alternate pathway other than the degradation of 2-chlorotriazine to the

2-hydroxy analog exists in some higher plants. The importance of this pathway with regard to the resistance or susceptibility of a species will require further investigation.

Results indicate that in mature pea plants the principal degradation reaction of atrazine is the dealkylation of the ethyl group at the 4-position of the triazine ring. The second possible product, 2-chloro-4-amino-6-ethylamino-*s*-triazine, was not detected. Subsequent metabolism of the dealkylated product may follow closely the proposed pathway for simazine degradation in the soil fungus *A. fumigatus* (8).

## RESIDUES IN SOYBEANS

### Insecticide Residues in Soybeans Grown in Soil Containing Various Concentrations of Aldrin, Dieldrin, Heptachlor, and Heptachlor Epoxide

SEVERAL investigations have reported on the translocation of aldrin, dieldrin, heptachlor, and heptachlor epoxide in crops grown on treated soil. Lichtenstein (5-8), working with vegetable crops, found the highest residues in root crops such as carrots and radishes.

Peanuts grown in soil treated with aldrin and heptachlor contained significant amounts of dieldrin, heptachlor, and heptachlor epoxide, as reported by Beck (1) and Bruce (2). Soybeans grown on heptachlor-treated soil contained heptachlor and heptachlor epoxide according

## Acknowledgment

The authors gratefully acknowledge the receipt of authentic benzoxazinone from R. H. Hamilton, and compound II and labeled and nonlabeled atrazine from the Geigy Chemical Corp, Ardsley, N. Y.

## Literature Cited

- (1) Castelfranco, P., Foy, C. L., Deutsch, D. B., *Weeds* **9**, 580 (1961).
- (2) Davis, D. E., Funderburk, H. H., Jr., Sansing, N. G., *Ibid.*, **7**, 300 (1959).
- (3) Funderburk, H. H., Jr., Davis, D. E., *Ibid.*, **11**, 101 (1963).
- (4) Gysin, H., Knüsli, E., in "Advances in Pest Control Research," R. L. Metcalf, ed., Vol. **III**, p. 319, Interscience, New York, 1960.
- (5) Gysin, H., Knüsli, E. (to J. R. Geigy Akt. Ges.), Swiss Patent **342,784-5** (Jan. 15, 1960).
- (6) Hamilton, R. H., *J. AGR. FOOD CHEM.* **12**, 14 (1964).
- (7) Hamilton, R. H., Moreland, D. E., *Science* **135**, 373 (1962).
- (8) Kearney, P. C., Kaufman, D. D., Sheets, T. J., *J. AGR. FOOD CHEM.* **13**, 369 (1965).
- (9) Montgomery, M. L., Freed, V. H., *Ibid.*, **12**, 11 (1964).
- (10) Montgomery, M. L., Freed, V. H., *Res. Prog. Repts. Western Weed Control Conf.*, **71**, (1960).
- (11) Negi, N. S., Funderburk, H. H., Jr., Davis, D. E., *Weeds* **12**, 53 (1964).
- (12) Roth, W., *Compt. Rend.* **245**, 942 (1957).
- (13) Roth, W., Knüsli, E., *Experientia* **17**, 312 (1961).
- (14) Smith, I., ed., "Chromatographic and Electrophoretic Techniques," Vol. **I**, p. 324, Interscience, New York, 1960.
- (15) Stambach, K., Kilchher, H., Friedrich, K., Larsen, M., Szekeley, G., *Weed Res.* **4**, 64 (1964).
- (16) Thurston, J. T., Dudley, J. R., Kaiser, D. W., Heckenbleikner, I., Schaefer, F. C., Holm-Hansen, D., *J. Am. Chem. Soc.* **73**, 2981 (1951).

Received for review December 23, 1965.  
Accepted March 13, 1966.

W. N. BRUCE and G. C. DECKER  
Illinois Natural History Survey,  
Urbana, Ill.

to Eden and Arthur (4). Bruce (2) established a direct relationship between oil content of seeds and residue content of crops grown on aldrin- and heptachlor-treated soil.

Because of the economic importance of soybeans in Illinois and the large

A 4-year residue study of soybeans grown on soil treated with aldrin and heptachlor establishes a basis for computing the amount of pesticides translocated into soybeans from soils of known residues. Aldrin and heptachlor granules were applied and soil was analyzed during each growing season to find the average residue levels. The level of insecticide in soybeans from each plot was related to the concentration of pesticide in the soil. Through the 4 years of study the residues decreased in both the soybeans and the soil—e.g., soybean residues from the 2-pound-per-acre treatment decreased from 0.066 p.p.m. of heptachlor plus heptachlor epoxide in 1961 to 0.007 p.p.m. in 1964 and from 0.043 p.p.m. of aldrin plus dieldrin in 1961 to 0.006 p.p.m. in 1964. Soil residues were 10 to 15 times as great as the soybean residues.

**Table I. Recovery of Heptachlor Epoxide and Dieldrin from 500-Gram Samples of Soybeans Fortified with Pesticide**

P.P.M. Added	P.P.M. Recovered <sup>a</sup>	
	Heptachlor epoxide	Dieldrin
0.050	0.046	0.045
0.024	0.021	0.022
0.012	0.010	0.009
0.006	0.003	0.004

Probability	Heptachlor Epoxide, Md	Dieldrin, Md
	0.05	0.0248
0.01	0.0343	0.0290

<sup>a</sup> Average of six replicate analyses by specific methods.

number of acres of soil treated with aldrin and heptachlor for the control of soil insects, experiments were initiated in 1961 to establish the relationship between residues found in soil and those found in soybeans.

#### Method of Study

Clay loam soil plots measuring 20 × 100 feet were treated once April 14, 1961, with 5% aldrin and 5% heptachlor granules at rates of 2, 5, 10, and 20 pounds of chemical per acre. A small 10-foot broadcast applicator calibrated to deliver 40, 100, 200, and 400 pounds of granules per acre was used to treat the soil, which was disked just after application. Soybeans were grown on these plots for 4 successive years. The harvested beans were stored in a refrigerated room until they were analyzed. Soil samples, consisting of 50 6-inch cores, were collected twice during each growing season from each plot and frozen until analysis. Analyses of soil and soybeans were replicated five times.

**Methods of Extraction, Cleanup, and Analysis.** For each analysis, 500-gram replicates of soybean seed were first rinsed with 1000 ml. of hexane to remove possible external residue. Less than 1% of the total seed residue was found in the hexane rinse. The beans were then finely ground and extracted with 2000 ml. of 10% acetone in hexane. From the 500-gram sample of soybeans, approximately 85 to 90 grams of soybean

**Table II. Comparison of Residues in Soybeans with Soil Residues and Soybean Residues Calculated from Soil Concentrations of Aldrin and Dieldrin, P.P.M.**

Treatment, Lb. Aldrin per Acre	Year	Soil Residue		Soybean Residue, Aldrin + Dieldrin	
		Aldrin	Dieldrin	Found	Calcd. <sup>a</sup>
2	1961	0.206	0.184	0.043	0.038
	1962	0.039	0.214	0.026	0.020
	1963	0.017	0.157	0.017	0.014
	1964	0.011	0.081	0.006	0.007
5	1961	0.280	0.490	0.066	0.069
	1962	0.121	0.513	0.052	0.052
	1963	0.056	0.529	0.044	0.046
	1964	0.019	0.214	0.020	0.018
10	1961	0.960	0.717	0.176	0.164
	1962	0.235	1.089	0.105	0.108
	1963	0.116	0.869	0.075	0.078
	1964	0.032	0.445	0.034	0.037
20	1961	2.535	1.042	0.330	0.371
	1962	0.645	1.402	0.147	0.178
	1963	0.398	1.425	0.113	0.152
	1964	0.054	0.942	0.066	0.076

<sup>a</sup> Calculated soybean residue = p.p.m. aldrin in soil × 0.116 + p.p.m. dieldrin in soil × 0.074.

**Table III. Comparison of Residues in Soybeans with Soil Residues and Soybean Residues Calculated from Soil Concentrations of Heptachlor and Heptachlor Epoxide, P.P.M.**

Treatment, Lb. Heptachlor per Acre	Year	Soil Residue		Soybean Residue, Hept. + Hept. Epoxide	
		Heptachlor	Hept. epoxide	Found	Calcd. <sup>a</sup>
2	1961	0.421	0.027	0.066	0.042
	1962	0.186	0.141	0.021	0.025
	1963	0.095	0.140	0.018	0.016
	1964	0.030	0.039	0.007	0.005
5	1961	1.140	0.045	0.121	0.112
	1962	0.536	0.337	0.074	0.068
	1963	0.237	0.230	0.038	0.034
	1964	0.045	0.132	0.012	0.011
10	1961	1.961	0.100	0.196	0.194
	1962	0.872	0.667	0.119	0.116
	1963	0.539	0.525	0.076	0.077
	1964	0.177	0.228	0.031	0.028
20	1961	3.321	0.178	0.308	0.329
	1962	1.548	1.169	0.198	0.206
	1963	0.962	0.801	0.110	0.131
	1964	0.512	0.399	0.066	0.069

<sup>a</sup> Calculated soybean residue = p.p.m. heptachlor in soil × 0.0964 + p.p.m. heptachlor epoxide in soil × 0.0483.

oil were obtained for analysis. Oils extracted from the beans were saponified with 200 ml. of 50% KOH and 400 ml. of 95% redistilled ethyl alcohol. Oil extracts containing heptachlor and heptachlor epoxide residue were saponified with warm KOH and alcohol for 15 minutes to prevent degradation of the

pesticides. Oils containing aldrin and dieldrin residue were heated 1 hour on the steam bath with the saponification mixture. The pesticides were recovered by adding 800 ml. of water to the saponified mixture and extracting with four successive 200-ml. portions of hexane. After combined hexane extracts were

washed several times with distilled water, cleanup procedures continued in the usual manner, as described in the Shell Method Series (73). Replicate soil samples, 500 grams each, were extracted with 2000 ml. of 10% acetone in hexane and cleaned up by column chromatography. Two chromatographic columns, 330 × 19 mm., containing MgO-Celite and Florisil were found adequate for the three kinds of analyses used. The Florisil was partly deactivated by adding 5% water, in order that dieldrin and heptachlor epoxide could be quantitatively eluted with 250 to 300 ml. of 10% ether in hexane.

Aldrin and dieldrin samples were analyzed according to the specific colorimetric method of O'Donnell (71, 72). Heptachlor and heptachlor epoxide were determined by the Davidow (3) colorimetric method.

Along with the colorimetric methods of analyses, tests using Mills' (10) method of paper chromatography and gas chromatography with electron-capture detection as described by Lovelock and Lipsky (9) were employed especially to establish residue levels below 0.04 p.p.m. The gas chromatograph was fitted with a 1/4-inch stainless steel column 24 inches long packed with 60- to 80-mesh Chromosorb W containing 2.0% Epon 1001, 0.5% Viton A, and 0.2% silicon 710 fluid. The column and detector temperature was 172° C. with a port temperature of 210° C. Fifty milliliters of nitrogen per minute effected excellent resolution of pesticides peaks.

Chemical calculations were based upon recoveries from soybeans and soil fortified with known amounts of insecticides. Analytical standards run with each set of tests indicate a recovery of 80 to 95% in samples fortified with more than 0.025 p.p.m. Recovery data are shown in Table I.

### Results and Discussion

As in previously reported experiments, most of the residues found in soybeans were epoxides of aldrin and heptachlor. On an average 90% of the total heptachlor residue was heptachlor epoxide and 97 to 99% of the aldrin residue in soybeans was dieldrin.

As shown in Figures 1 and 2, total residues in soybeans through the 4 years decreased faster than the total residues of the plots. This trend is explained by the fact that aldrin and heptachlor were converted into dieldrin and heptachlor epoxide during this time, and that these epoxides are not absorbed by the plants so rapidly as the parent compounds. A contributing factor may be the relative penetration rates of the pesticides through the plant membrane as influenced by the polarity of the compound. Possibly the less polar compounds, aldrin and heptachlor, may be absorbed and translocated more readily than the more polar dieldrin and heptachlor epoxide. Whether the pesti-

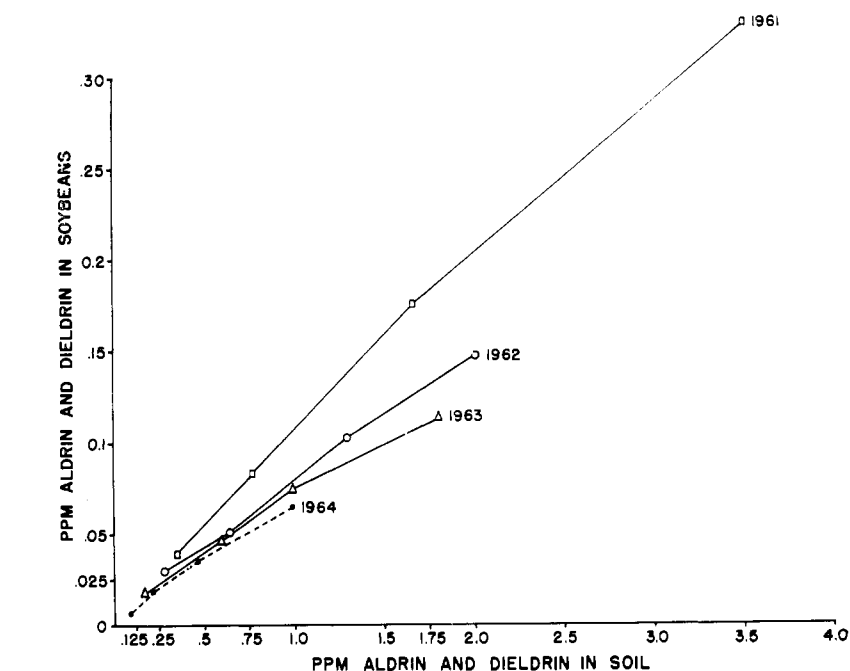


Figure 1. Residues in soybeans grown 4 successive years on soil treated once with 2, 5, 10, and 20 pounds of aldrin per acre

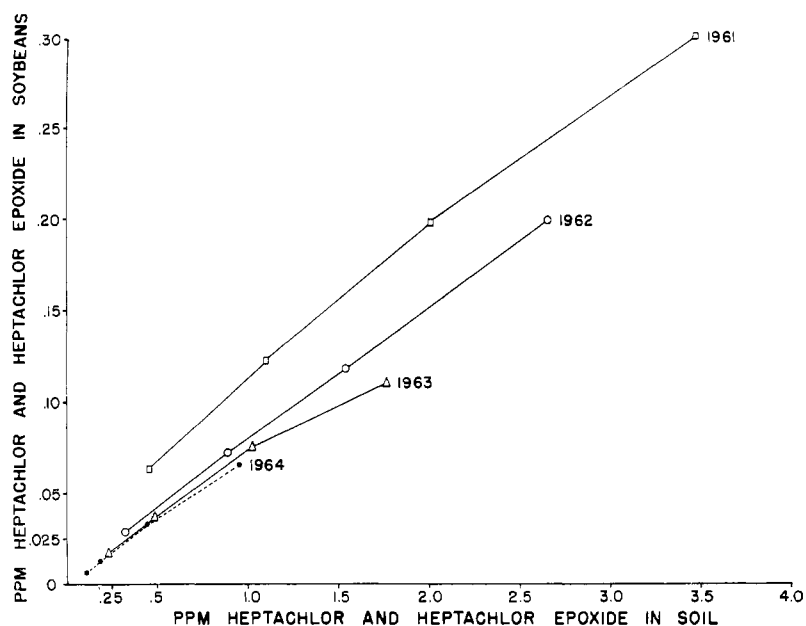


Figure 2. Residues in soybeans grown 4 successive years on soil treated once with 2, 5, 10, and 20 pounds of heptachlor per acre

cide enters the plant through a dissolved, gaseous, or solid contact phase is not known. Additional studies involving soybeans grown in nutrient solutions without soil would help to resolve this problem.

The obvious conclusion is that aldrin and heptachlor contribute more to soybean contamination than do dieldrin and heptachlor epoxide. To support this conclusion, Tables II and III show the actual residues and residues calculated by a formula obtained by equating soil residue values to soybean residue values. The aldrin residue in soybeans (97 to 99% dieldrin) is equal to the sum

of 0.116 times the soil aldrin and 0.074 times the soil dieldrin. The heptachlor residue in soybeans (90% heptachlor epoxide) is equal to the sum of 0.0964 times the soil heptachlor and 0.0483 times the soil heptachlor epoxide. There is a high degree of agreement between the found and calculated residues, as shown in Tables II and III.

Figures 1 and 2 graphically illustrate the direct relationship between the concentration of pesticides in the soil and residues in the soybean seed. The parts per million of total residue in the soil was 10 to 15 times the residue found in soybeans.

## Literature Cited

- (1) Beck, E. W., Dawsey, L. H., Woodham, D. W., Leuck, D. B., Morgan, L. W., *J. Econ. Entomol.* **55**, 953 (1962).
- (2) Bruce, W. N., Decker, G. C., Wilson, J. G., *Ibid.*, **59**, 179 (1966).
- (3) Davidow, Bernard, Radomski, J. L., *J. Pharmacol. Exptl. Therap.* **107**, 259 (1953).
- (4) Eden, W. G., Arthur, B. W., *J. Econ. Entomol.* **58**, 161 (1965).
- (5) Lichtenstein, E. P., *J. Agr. Food Chem.* **7**, 430 (1959).
- (6) *Ibid.*, **8**, 448 (1960).
- (7) Lichtenstein, E. P., Myrdal, G. R., Schulz, K. R., *J. Econ. Entomol.* **57**, 133 (1964).
- (8) Lichtenstein, E. P., Schulz, K. R., *J. Agr. Food Chem.* **13**, 57 (1965).
- (9) Lovelock, J. E., Lipsky, S. R., *J. Am. Chem. Soc.* **82**, 431 (1960).
- (10) Mills, P. A., *J. Assoc. Offic. Agr. Chemists* **42**, 734 (1959).
- (11) O'Donnell, A. E., Johnson, W., Jr., Weiss, F. T., *J. Agr. Food Chem.* **3**, 757 (1955).
- (12) O'Donnell, A. E., Neal, M. M., Weiss, F. T., Bann, J. M., DeCino, T. J., Law, S. C., *Ibid.*, **2**, 573 (1954).
- (13) Shell Chemical Co., Shell Method Series, *MMS-2/59* (1959).

Received for review October 11, 1965. Accepted April 21, 1966. Division of Agricultural and Food Chemistry, 150th Meeting, ACS, Atlantic City, N. J., September 1965. Contribution to North Central Regional Project NC-33.

## RESIDUES IN SOILS

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

C. R. HARRIS, W. W. SANS,  
and J. R. W. MILES

Entomology Laboratory, Canada  
Department of Agriculture, Chatham, Ontario, Canada

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. Dicrofol 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 pro-

cedures. 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