Nature of Chlorine Interferences in Total Halogen Methods of Analysis of Organochlorine Pesticide Residues in Plants

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The source of a widely distributed background-organochlorine compound, which interferes with the determination of organochlorine pesticide residues in plant parts by any total halogen method, has been tentatively identified as being due to quaternary chloride salts of lecithins. The degree and extent of interference vary from species to species of plant, among samples of the same species, and among parts of the same plant. These differences are due largely to variations in concentrations of certain plant constituents at the time of sampling but, also, reflect some effects due to sample storage and processing procedures that may cause lecithin degradation.

THE COMBUSTION and the several other total halogen methods for determining total organically bound chlorine have been used extensively in pesticide residue research despite their lack of specificity. For in lieu of other residue data, or as a supplemental procedure, results are often acceptably interpretable as maximum possible loads of parent compound of interest present. From the earliest reports of results obtained by this method (*1*, *12*, *20*), evidently, a variable interfering background organochlorine was present, almost always, in the plant materials studied. These interferences commonly are in the fractional parts-per-million range but may reach 35 p.p.m. and more, as illustrated in Table I. With careful sample preparation, selection of solvent, and cleanup, these interferences often can be reduced, but usually not below the 0.2 to 3.0 p.p.m. level, and the more sensitive the over-all procedure, the more apparent the interference. Where a specific analytical method is not available for a particular organochlorine pesticide, or for some types of residue screening, the total combustion procedure is the method of choice for various reasons (*12*, *20*). The advent of an automated process (*13*) now makes determination of the total chlorine content of plant extractives possible at the rate of eight or more an hour; the speed, wide applicability, sensitivity, and utility of this method made desirable the determination of the nature of its chief drawback, the variable background organochlorine contents of plant parts.

Experimental

Materials. PLANTS. Native wild plants were collected in the desert (Coachella Valley, Calif.) and from an uncultivated virgin field at the University of California, Riverside, prior to planting crops. Cultivated plants were then grown in this same field after the addition of various soil amendments and fertilizers; these additions were necessary to improve the fertility of the field and were analyzed and selected for their minimum of known amounts of organically bound chlorine. Soil samples were analyzed, also, periodically for hexane-extractable chlorine during the 5 years of this study to assure noncontamination of the crops by added organically bound chlorine; contamination of the soil was not demonstrable, and therefore the irrigation water was absolved, also. Lots of all seeds were analyzed before planting for the same assurance; seed organochlorine values ranged from nil to 0.02 p.p.m. No special attempt was made to exclude chloride ion from the crops because the ion would have to be the source for any organically bound chlorine a plant might produce. In a few instances, ammonium chloride was used as a fertilizer in the attempt to stimulate organochlorine production, but this effect was not demonstrable with any of the several crops produced. The chloride content of the Coachella Valley soil is several hundred milliequivalents per liter of soil solution in the unleached areas, yet apparent organochlorine contents of plants from this soil were not consistently higher than those from leached areas as collated in Tables I and II.

RADIOISOTOPES. Chlorine-36 was obtained commercially as a hydrochloric acid solution, which was converted to calcium chloride by addition of the calculated amount of calcium carbonate. Radioactive calcium chloride was administered by injection into the developing roots of beet plants grown in the glasshouse either in soil or in sand nutrient culture. Radioactive plant parts were either lyophilized or blanched with steam at 100° C. for 20 minutes then dried in a forced draft oven at 70° C.

PHOSPHOLIPASE D. This was prepared and assayed as described by Kates (*17*).

Table I. Illustrative Background-Organochlorine Residues in Some Reported Residue Investigations of Edible Plant Parts

Edible Plant Part	Extracting Solvent	Organochlorine, P.P.M.	References
Alfalfa	Benzene	10–1800[a]	(*12*, *20*)
Black currants	*n*-Hexane	0.0–0.1	(*5*)
	Acetone	0.0–0.1	(*5*)
Cabbages	Petroleum ether	0.0–0.3	(*24*)
	Acetone	0.0–0.3	(*24*)
Carrots	Various	0.1–1.0	(*8*, *12*, *24*)
Citrus fruits	Various	0.0–0.7	(*6*)
Field crops (10)[b]	Benzene	0.2–20.0[c]	(*11*)
Field crops (6)[b]	*n*-Hexane	0.0–4.0[c]	(*11*)
Fruits (10)[b]	Benzene	0.1–3.0[c]	(*11*)
Fruits (15)[b]	*n*-Hexane	0.0–1.5[c]	(*11*)
Grains (several)	Acetone	0.1–2.0	(*7*, *22*)
Potatoes	Various	0.0–0.2	(*7*, *25*)
Strawberries	Benzene	3–35	(*12*)
Tomatoes	Petroleum ether	0–1	(*7*, *24*)
	Acetone	0–1	(*7*, *24*)
Vegetables (10)[b]	Benzene	0.1–3.0[b]	(*11*)
Vegetables (25)[b]	*n*-Hexane	0.0–2.0[b]	(*11*)

[a] From edge of a salt flat; analyzed in 1950.
[b] Number refers to number of different crops analyzed over a 15-year period.
[c] From hundreds of determinations over a 15-year period.

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Table II. Hexane-Extractable Chlorine in Some Wild and Cultivated Plants

Wild Plants[a]	Average Chlorine Contents[b] in P.P.M. by Combustion	Neutron activation
Amaranthus graecizans	0.1 (5)	0.1 (1)
Artemisia californica		
Leaves	2.0 (2)	...
Roots	0.3 (1)	...
Whole plants	3.5 (4)	3.4 (1)
Atriplex lentiformis		
Leaves	4.0 (5)	2.8 (2)[c]
Stems	0.5 (2)	...[c]
Roots	0.7 (2)	...
Brassica incana		
Tops	0.8 (3)	0.9 (1)
Roots	0.1 (1)	...
Whole plant	2.2 (4)	1.9 (1)
Encilia farinosa		
Tops	0.8 (2)	...
Roots	0.5 (2)	...
Whole plants	0.2 (8)	0.1 (2)
Eremocarpus setigerus	1.4 (9)	1.7 (2)
Erigonium fasiculatum		
Tops	0.9 (3)	0.5 (1)
Roots	0.3 (2)	...
Whole plants	0.9 (9)	1.6 (1)
Nicotiana glauca		
Tops	0.2 (2)	0.2 (1)
Roots	0.3 (1)	...
Whole plants	0.4 (8)	...
Opuntia basilasis	0.4 (7)	0.6 (2)
Pulchea sericea		
Tops	2.5 (5)	2.2 (1)
Roots	0.3 (1)	...
Schimus molle		
Tops	0.5 (2)	0.3 (1)[c]
Roots	0.8 (1)	...
Whole plants	1.0 (8)	1.3 (4)
Sueada spp.	8.0 (3)	5.5 (1)[c]
Tamarix gallica		
Tops	1.3 (4)	1.8 (1)
Cultivated plants[a]		
Alfalfa	1.1 (19)	0.8 (8)
Apple fruits	0.5 (2)	0.6 (2)
Artichoke leaves	0.9 (2)	1.0 (2)
Beans, lima	0.9 (2)	0.6 (2)[c]
Beans, pole		
Seeds	0.4 (3)	0.4 (1)
Pods	0.2 (2)	...
Whole plants	0.6 (6)	0.6 (1)[c]
Beets, table		
Seeds	6.8 (2)	...
Leaves	1.9 (14)	1.5 (3)
Whole plants	0.3 (1)	...
Broccoli		
Seeds	Nil (2)	...
Whole plants	Nil (10)	Nil (1)[c]
Brussels sprouts	0.1 (4)	Nil (1)
Cabbages	Nil (13)	Nil (1)
Cantaloupe leaves	Nil (7)	...
Carrots		
Seeds	26[d] (7)	22 (1)
Roots	0.2 (2)	0.3 (3)
Tops	0.5 (2)	0.6 (1)

Cultivated Plants[a]	Average Chlorine Contents[b] in P.P.M. by Combustion	Neutron activation
Cauliflowers		
Seeds	Nil (2)	...
Whole plants	0.4 (8)	0.4 (3)
Celery		
Seeds	14 (2)	...
Whole plants	0.4 (2)	0.4 (1)
Chard, Swiss	0.2 (17)	0.4 (5)[c]
Cowpeas	2.6 (2)	...
Corn, sweet		
Kernels	2.0 (2)	...
Cobs	Nil (4)	...
Whole young plants	0.2 (10)	0.2 (7)[c]
Cucumbers		
Seeds	1.1 (11)	1.2 (3)[c]
Fruits	0.2 (8)	0.5 (3)
Whole plants	0.6 (3)	...
Date palms		
Fruits	Nil (4)	...
Fronds	1.0 (4)	0.9 (1)
Date palms[e]		
Fruits	Nil (3)	...
Fronds	0.8 (4)	0.9 (1)
Lettuce		
Seeds	1.9 (2)	...
Tops	0.1 (6)	0.2 (2)
Onions		
Seeds	1.2 (2)	1.1 (1)
Bulbs	0.1 (16)	0.1 (3)
Parsley		
Seeds	7.5 (2)	...
Tops	0.2 (6)	...
Peas	0.6 (14)	0.7 (2)
Peppers, bell	0.4 (2)	0.3 (1)[c]
Potatoes, sweet	0.3 (2)	0.4 (1)
Potatoes, white		
Seed tubers	Nil (2)	...
Crop tubers	Nil (10)	0.1 (2)
Whole young plants	Nil (6)	...
Radishes		
Seeds	1.8 (3)	1.3 (1)
Roots	0.9 (12)	0.8 (6)
Tops	Nil (6)	...[c]
Whole plants	0.2 (5)	...
Squash, banana	0.5 (2)	0.4 (1)
Squash, zucchini		
Seeds	3.6 (2)	...
Fruits	0.2 (8)	...[c]
Whole plants	0.2 (36)	0.2 (8)
Tomatoes		
Seeds	26 (2)	...
Fruits	0.4 (4)	0.3 (1)
Whole young plants	0.4 (2)	0.3 (1)
Turnips		
Seeds	5.4 (2)	...
Roots	0.1 (12)	Nil (5)
Tops	0.4 (12)	0.3 (1)
Whole young plants	Nil (5)	Nil (1)

[a] Portions of whole plants analyzed unless noted otherwise.

[b] Numbers of replicates are in parentheses. Many replicates were of crops from different years and different parts of the field. Analytical variations were usually <0.1 p.p.m. chlorine content by either method. The detection minimum for combustion is determined by sample size represented by the analytical aliquot, percentage recovery, and the minimum amount (4 μg.) of chloride detectable; for neutron activation it is the standard deviation estimated from counting statistics.

[c] Traces of organobromine also were found (*14*); organoiodine compounds were not sought.

[d] Contained 12 p.p.m. *p,p'*-DDT by microcoulometric gas chromatography.

[e] Coachella Valley, high chloride ion content in soil.

LECITHINS. Lecithin-Soy-Refined from Nutritional Biochemicals Corp., Cleveland, Ohio and Lecithin, Soya bean from a health food store.

Methods. All sampling, sample preparation, and replicated analyses for chlorine by combustion were performed as described by Gunther and Blinn (*12*) and by Gunther, Miller, and Jenkins (*13*). Either 100- or 500-gram subsamples were used, with 2 ml. of stripping solvent per gram of substrate.

Neutron-activation analyses for chlorine (*9, 10*) were performed under contract by the General Atomic Division of General Dynamics Corp., San Diego, Calif. Replicated concentrates of stripping solution were analyzed, with dieldrin-fortified controls as reference standards.

The procedure of Hirsch and Ahrens (*15*) was used for lipid fractionation by silicic acid chromatography. Thin-layer chromatography of phospholipids was performed according to the methods of Wagner, Hörhammer, and Wolff (*26*).

A Beckman DK-2 spectrophotometer equipped with a hydrogen-flame photometric attachment was used for emission spectrophotometry.

Radioactivity measurements were made with a Nuclear Chicago scaler equipped with a thin-window gas-flow counter having an efficiency of 35% for chlorine-36.

Inorganic chloride was determined coulometrically, by direct potentiometry (*12, 13*), or titrimetrically with 0.0025*N* silver nitrate solution using an amperometric detection system similar to that of Cotlove, Trantham, and Bowman (*2*). Choline was estimated by the method of Wheeldon and Collins (*28*).

Phosphorus was determined by the manual method of King (*18*) and by the automated procedure of Weinstein *et al.* (*27*).

Results and Discussion

The distribution of background organochlorine in more than 300 samples of wild and cultivated plants was determined by two-method analysis of the *n*-hexane extracts of 100- or 500-gram portions of 47 plant species and their parts: seeds, leaves, stems, fruits, and roots. The cultivated plants were grown from noncontaminated seed in soil never before used for agricultural purposes and, therefore, were free of direct pesticide contamination. Representative results of this survey of crops over several years are presented in Table II; note the excellent agreement between the two methods. Samples with unusually high values were confirmed by microcoulometric gas chromatography. Tables I and II show that one or more unidentified chlorine-containing substances are indeed present in repeatedly distilled, water-washed, hexane extracts of a wide variety of plants and plant parts.

Some pertinent properties of this background organochlorine were:

Widely distributed throughout the plant kingdom.

Extractable in variable amounts from plant materials by hexane or hexane–2-propanol mixtures.

Not significantly removed from its hexane solution by repeated washings with tap, distilled, or conductivity water, but usually removable by dilute acidified silver nitrate solution.

Unstable to acids and bases, giving rise to chloride salts insoluble in hexane.

Nonsedimentable on prolonged high-speed centrifugation of its solutions in organic solvents.

Not eluted from gas chromatographic columns equipped with electron-capture or coulometric detectors, or else not transferred from block to column—i.e., not volatile.

Afforded chloride ion upon combustion.

In view of these properties, the authors considered the possibility unlikely that an unknown, widely distributed, carbon compound containing covalently bound chlorine was responsible for the observed background. This premise was not supported by the long known (*3*) fact that chloride ion is an essential plant nutrient (*16*).

The properties listed above do favor the interpretation that the background in question results from chloride salts of some inorganic or organic cations. To estimate this presumed importance of inorganic salts, 10 hexane extracts, known to contain from 0.3 to 1.5 p.p.m. of organochlorine by analysis, were analyzed by flame spectrophotometry to detect inorganic cations. Sodium and calcium ions were found in these extracts in concentrations of 0.01 to 0.1 p.p.m. These quantities of inorganic cations are sufficient to account for only a small portion of the background chloride observed, however. Therefore, organic cations were suspect, particularly the widely distributed phosphatidyl cholines, or lecithins. The amounts of background chloride present in the samples studied were so small as to preclude direct isolation of the chloride salt of lecithin from plant extracts. However, labeling the salts in the plant with chlorine-36 made possible the fractionation of the radioactivity extracted from the plants with hexane. Beet plants were selected for this fractionation since they showed a consistent and relatively high background organochlorine (see Table II) and could be grown conveniently both in soil and in sand nutrient culture in the glasshouse. Various amounts of chlorine-36 as calcium chloride were injected into developing sugar and table beet roots growing in sand culture and, after 3 and 7 days, the leaves were removed, dried, and extracted exhaustively (Table III). Fractionation of the concentrated hexane extracts was achieved on a silicic acid column. The results of several such fractionations are presented in Table IV. The methanol and acid eluates contained most of the radioactivity, and the methanol fraction also contained the phospholipids, including the lecithins, as established by parallel experiments and phosphorus assays of all fractions (Table V).

Radioactivity eluted by the acids almost certainly results from dissociation of the organic chloride salts on the silicic acid although some may be due to inorganic chlorides actually dissolved or suspended colloidally in the extracts. Thus, an aliquot of the methanol solution was evaporated to dryness in vacuo, and the residue was dissolved in hexane. This hexane solution was washed three times with an equal volume of water each time and then with one volume of 0.1*N* silver nitrate solution. The residual hexane solution and the water washes were evaporated on planchets for radioactivity determination. The silver nitrate solution was treated with 1 ml. of 0.1*N* potassium chloride solution, and the resulting precipitate was collected, washed, dried, and counted. The results of this procedure, shown in Table VI, indicate that all of the chloride in the methanol eluate was in a very loosely bound form—e.g., the salt of an organic cation.

Another aliquot of the methanol fraction was fractionated by thin-layer chromatography on silica gel. The two developing-solvent mixtures of chloroform and methanol differed only in that one contained an ionic component, acetic acid, and the other the same volume of water. As can be seen from Figure 1, development in the nonionic solvent caused the radioactivity to migrate with an R_f identical with the R_f for authentic plant (soybean) lecithins (0.14), but quite different from the R_f of chloride ion (0.33). Conversely, in the ionic acidified solvent, the radioactivity in the methanol extract migrated with an R_f identical to that of chloride ion (0.26) but quite different from the R_f of lecithins (0.14). From these results, the background organochlorine compound is really the chloride salt of the lecithins present. Phosphatidyl choline, or lecithin, is widely distributed in nature and is probably present in every higher plant and animal (*4*, *21*). Associated with the phospholipids in higher plants is an enzyme, phospholipase D, which catalyzes the hydrolysis of choline from lecithin (*17*). This enzyme is activated by organic solvents such as hexane and ether (*17*). If the radioactive chloride, in fact, is present as the salt of lecithins, then this enzyme should also cause, in the process of catalyzing the hydrolysis of the choline moiety, the radioactivity to be transferred from the hexane solution to the aqueous phase. The results of an

Table III. Distribution of Chlorine-36 in Extracts of Table Beet Tops

Source	Radioactivity in D.P.M.[a] after 3 days	7 days
Injected into beet root	8.8×10^6	4.4×10^6
Recovered in extracts		
n-Hexane	7.2×10^4	3.9×10^4
Methanol	3.4×10^6	1.1×10^6
Residue	7.8×10^4	3.9×10^4

[a] Disintegrations per minute.

Table IV. Distribution of Chlorine-36 in Eluates from Silicic Acid Chromatography of Hexane Extracts of Beet Leaves

Elutants	Recovery of Radioactivity in Column Eluates: Table Beets[a] C.P.M.[d]	%	Table Beets[b] C.P.M.[d]	%	Sugar Beets[c] C.P.M.[d]	%
Hexane–4% ether	106	1.9	103	0.1	1	0.1
Ethyl ether	9	0.1	168	0.1	19	1.8
Methanol	2760	34.0	47200	45	370	35.2
0.1*N* HNO_3	1881	23.3	45882[e]	44	268	25.5
0.1*N* HCl	...	...	117	0.1	410	39.0
Total	4756	58.6	93470	99	1068	101.5
Counts applied to column	8120		95800		1050	

[a] Six weeks old; tops harvested 10 days after administration of 4 μc. of $CaCl_2^{36}$.
[b] Ten weeks old; tops harvested 3 days after administration of 4 μc. of $CaCl_2^{36}$.
[c] Tops harvested 10 days after administration of 4 μc. of $CaCl_2^{36}$.
[d] Counts per minute.
[e] 1.0*N* HNO_3 used in place of 0.1*N* HNO_3.

Table V. Phospholipid Contents of Eluates from Silicic Acid Chromatography of Hexane Extracts of Beet Leaves

Elutant	Phosphate Content of Eluates in μmoles: Table beets[a]	Table beets[b]	Sugar beets[c]
Hexane–4% ether	Nil[d]	0.1	Nil
Ethyl ether	Nil	Nil	0.2
Methanol	3.2	10.6	21.7
0.1*N* HNO_3	Nil	Nil	Nil
0.1*N* HCl	Nil	Nil	Nil

[a] Six weeks old; tops harvested 10 days after administration of 4 μc. of $CaCl_2^{36}$.
[b] Ten weeks old; tops harvested 3 days after administration of 4 μc. of $CaCl_2^{36}$.
[c] Tops harvested 7 days after administration of 4 μc. of $CaCl_2^{36}$.
[d] Nil means less than 0.05 μmole of phosphate.

Table VI. Extraction of Hexane-Soluble Chlorine-36 by Several Reagents

Reagent	Radioactivity: C.P.M.[a]	Recovery, %
Hexane solution	788	100
Water washes	10	1
AgCl precipitate	768	97
Hexane residue	18	2

[a] Counts per minute.

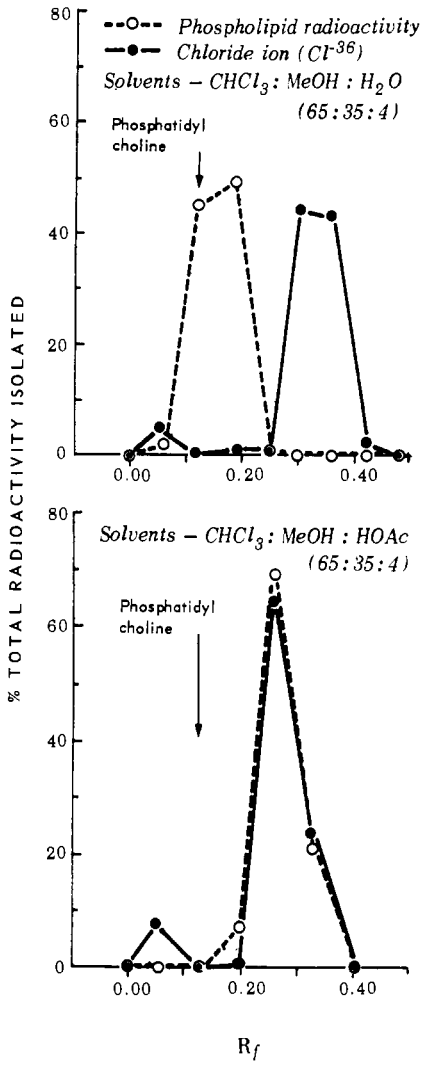

Figure 1. Thin-layer chromatography of radioactive phospholipids and chlorine-36 on silicic acid

Table VII. Release of Hexane-Soluble Chlorine-36 by Phospholipase D

Enzyme	Radioactivity in Hexane Extracts of Incubation Mixtures,[a] C.P.M.[b] Unheated	Heated
Absent	170 ± 10	165 ± 11
Present	6 ± 3	145 ± 7

[a] Incubation mixtures consisted of 0.5 ml. of hexane containing chlorine-36 phospholipids from silicic acid chromatography, suspended in 4 ml. of water; 100 mg. of table beet chloroplasts were used as the enzyme source. Heated samples, with and without enzyme, were boiled for 2 minutes and then cooled to room temperature. At zero time, 3 ml. of hexane was added with vigorous shaking, and incubation continued at room temperature for 30 minutes. The mixtures then were extracted with three 10-ml. portions of hexane, and the radioactivity in the combined extracts was determined for each incubation mixture with three replications.

[b] Counts per minute.

Table VIII. Partial Analysis of Plant Constituents Extracted by Hexane

Sample	Amounts in Extract from 10 Grams of Plant Material: Solids, mg.	Phosphate, μmoles	Choline, μmoles	Chloride,[a] μmole: Comb.	NAA	Titr.
Alfalfa	115.6	37.2	2.40	0.23	0.24	0.26
Table beet (leaves)	45.9	12.7	1.84	0.53	0.41	0.45
Cucumber (whole plant)	10.7	2.3	0.26	Nil	...	0.01
Egg plant (leaves)	10.0	1.7	0.20	0.19	...	0.18

[a] Chloride was determined by combustion (Comb.), neutron-activation analysis (NAA), and direct titration (Titr.) with silver nitrate.

experiment to test this hypothesis are presented in Table VII. Boiled enzyme preparation was used as a control for nonenzymatic dissociation of the lecithin chloride. The release of lipophilic radioactivity is virtually quantitative in the complete, unboiled system used.

Four randomly selected hexane extracts were analyzed for phosphate, choline, and chloride to determine if adequate amounts of lecithin phospholipid were present in these extracts to account for all the chloride ion as lecithin chloride. Examination of the results in Table VIII shows that from one to 10 times as much hydrolyzable choline was present as required for the observed background chloride contents.

Conclusions

The above results support the conclusion that background organochlorine observed in hexane extracts of plant parts is, in fact, due to the quaternary chloride salts of lecithins. During maceration and extraction of plant materials, the integrity of the cells is destroyed and an intermingling of the cell wall, cytoplasmic, and vacuolar constituents results. As a consequence, the extracted phospholipids are brought into intimate contact with various anions, including chloride ion. Subsequent removal of the hydrophilic phase leaves some of the chloride in the organic layer in the form of quaternary chloride salts of lecithins. Washing with water does not remove these substances since they are amphiphilic and, presumably, preferentially oriented with respect to the organic phase. The enzyme, phospholipase D, also is released during extraction and comes into contact with the lecithins. In the presence of many organic solvents, its catalytic activity is increased and appreciable amounts of lecithin are degraded. Nevertheless, even if 99% of the choline is liberated, sufficient lecithin remains in most cases to account for all the background chloride detected in hexane extracts (Table II). In addition, small amounts of calcium and sodium salts actually are dissolved in the extracting solvent, possibly as the undissociated chlorides. Thus, the factors affecting the concentration of these chloride salts in hexane extracts of plant materials are: the concentrations of lecithins, chloride, sodium, and calcium in the plant material; the length and temperature of storage of the raw samples prior to stripping; the duration and possibly the temperature of stripping equilibration (*12*); the temperature and duration of storage of stripping solutions; the concentration of phospholipase D; and, undoubtedly, other undetermined factors.

Identification of the interfering substance as lecithin chloride permits consideration of possible modes of cleanup which may remove this interference. Acetone precipitation of phospholipids was used by Onley and Mills (*23*) to remove interfering halogenated substances from eggs, but this technique is ineffective in the present case because of the small amounts of phospholipids present. Koblitsky, Adams, and Schechter (*19*) and Gunther (*11*) sometimes have utilized 0.01 *N* silver nitrate solution to reduce the level of inorganic chloride in fat and some plant-part samples prior to analysis; because of the staining qualities, silver nitrate is not desirable for use in the quantities necessary for routine residue sample preparation. An alternative suggested by the present work is dilute nitric acid solution, which is under investigation.

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SOIL METHODS

Methods for Extracting Insecticides from Soil

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Three separate extraction procedures for DDT- and endrin-contaminated soils were compared for reproducibility, as well as sample size and equipment needed. Two of the methods were developed by the authors; the third came from the Shell Development Manual of Method. The Immerex extractor is recommended because of its reproducibility, rugged equipment, and capability of handling large samples.

THE MILLIONS of pounds of insecticides used since the advent of DDT have mostly been used in agriculture. Because of their low water solubility and low vapor pressure, the majority of the chlorinated insecticides tend to persist in the soil—although sometimes as a metabolite or oxidation product, as in the case of DDE or dieldrin. These materials have been detected in surface waters (*4*).

Since the land is the major reservoir of these chemicals once they are applied, the determination of persistent insecticides in soils is of considerable interest from the standpoint of environmental health, as well as agriculture and wildlife conservation. Only comparatively recently, however, has general interest been shown in the amounts and types of pesticidal chemicals which tend to accumulate in the soil.

The big problem in soil residue studies is the collection of a truly representative sample of soil following such practices as tillage, crop rotation, and nonuniform application of chemicals. Major variations in soil type or soil series within a field would have obvious effects upon the analytical results of an insecticide study of the field.

The taking of representative soil samples has been discussed in detail by Lykken (*3*).

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From the analytical standpoint, the efficient extraction of the insecticides from the soil samples is a major problem. Soil samples, as submitted for analysis, may vary widely in moisture content; thus, it is usual to report results on an air-dried basis. Since many of the chlorinated organic insecticides are volatilized at temperatures as low as 50° C., the attempted removal of all moisture risks the loss of some of the insecticide content.

General methods for extracting insecticides from soil are not plentiful in the literature. The method of the Agricultural Division, Shell Development Co. (*5*) was used in this study. It has been compared to two other methods used by the authors during the past 3 years.

Methods

Reagents. All organic solvents are distilled, using all-glass distilling apparatus. The first 10% cut is discarded and the next 80% collected for use.

Petroleum ether, 30°–60° b.p. range.

Florisil, 60- to 100-mesh preactivated at 1200° F. Heat in 135° C. oven for 5 hours. Store in glass-stoppered bottles at 135° C. prior to use.

Apparatus. Gas chromatographic, Dohrmann Microcoulometric, Model C100 with a T-200S titration cell, and Micro Tek 2500R column oven.

Gas chromatographic column, 4-foot × 1/4-inch o.d., packed with 5% DC-200 (12,500 centistokes) on 80- to 90-mesh Anakrom ABS. Column temperature 180° C., gas flow N_2 at 100 cc. per minute.

Preparation of Sample. Air-dry the sample in a 9 × 9 × 2 inch, 2-quart borosilicate glass baking dish. When the soil is dry to the touch, reduce to a fine powder, using a grinding mill. Mix thoroughly and withdraw 100 grams for analysis.

EXTRACTION. Shell Development Co. Method. Weigh a representative sample (100 grams) into a 1000-ml. Erlenmeyer flask. Add enough distilled water to effect a slurry. Add 2 ml. of extraction solvent (*n*-hexane–isopropyl alcohol, 3+1) per gram of sample and shake vigorously for 20 minutes, using a wrist action shaker. Decant and collect the hexane phase into a separatory funnel. Repeat extraction of the mud-aqueous phase twice more, quantitatively decanting the hexane portions each time into the separatory funnel. Wash any remaining alcohol from the combined hexane extracts with water, dry over anhydrous sodium sulfate, and concentrate to 10 ml. or less.

Soxhlet Extractor Method. Weigh 100 grams of soil in an extraction thimble (Fisher, 123 × 43 mm.). Add 250 ml. of solvent (*n*-hexane–acetone, 9+1). Connect the extractor, and extract sample for 4 hours. Transfer the extracting solvent to a 500-ml. Kuderna-Danish evaporator with 3-ball Snyder column. Evaporate to 10 ml. or less.

Immerex Extractor Method. Weigh