Identification of Nanogram Amounts of Certain Organochlorine Insecticides

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Individual insecticides, eluting from a gas chromatographic column, were detected with an electron-capture detector and concurrently trapped in a Teflon capillary tube. Fifty microliters of solvent was added, and the trapped component was irradiated with ultraviolet (uv) light. Following irradiation periods of 15 to 120 sec, the contents of the Teflon tube were reinjected into the gas chromatograph and the uv-induced degradation pattern was noted. Characteristic "fingerprint" degradation patterns were obtained for nanogram quantities of heptachlor, heptachlor epoxide, aldrin, dieldrin, and the *p,p'* and *o,p'* isomers of DDT, DDE, and DDD. Analysis of a number of foods, soil, and osprey egg demonstrated the utility of the method to aid in the identification of the above insecticides in actual samples, even in the presence of coextracted materials or artifacts. The latter, which have retention times identical to those of certain insecticides, do not give the characteristic "fingerprint" degradation pattern of the insecticide upon irradiation.

Various instrumental methods of analysis (glc–ms, glc–ir, and nmr) have been used for the identification of organochlorine insecticides isolated from environmental samples, but microgram (or larger) quantities are ordinarily required for identification. With the ever-increasing limitations placed on the use of certain of the organochlorine insecticides, a simple method for the identification of nanogram quantities has become increasingly important. There are methods available which can be applied to nanogram amounts, but most of them are either time-consuming (chemical modification or derivatization or tlc followed by gc) or ambiguous (relative retention times on several columns of different polarity). The determination of *p* values, as outlined by Beroza and Bowman (1965), is simple and rapid and can provide data to aid in the identification of relatively small quantities of certain insecticides.

Banks and Bills (1968) reported the formation of characteristic degradation products when certain organochlorine insecticides were irradiated with a laboratory ultraviolet (uv) light. The "fingerprint" glc pattern of the degradation products facilitated insecticide identification. The objective of the current study was to devise a means for the manipulation and rapid identification of nanogram quantities of certain organochlorine insecticides occurring simultaneously in a sample.

EXPERIMENTAL

Glc Operating Conditions. An F&M gas chromatograph (Model 810), equipped with a tritium electron capture (ec) detector, was used in all analyses. A glass column (4-mm i.d. × 122-cm), containing an equal-weight mixture of 4% SE-30 and 4% QF-1 on 70/80 mesh Anakrom ABS (Analabs, Inc.), was used to separate and identify the insecticides and their degradation products, except for heptachlor epoxide. The degradation products of heptachlor epoxide were separated using an additional glass column (4-mm i.d. × 61-cm) containing 5% OV-225 on 70/80 mesh Anakrom ABS. Temperatures of the injector, column, and detector were 205, 180, and 205°C, respectively. The flow rate of the carrier gas (95:5 argon:methane) was 60 ml per min.

Teflon Splitter. The splitter was constructed as illustrated in Figure 1. Two pieces of AWG size 19 standard-wall Teflon tubing (Trimflex, Inc., Dover, N.J.), each 40-cm long, were cemented into a Teflon reducing ferrule (Chemical Research Services Inc., Addison, Ill.) with a high-temperature epoxy resin, Chemgrip HT (Chemplast, Inc., Wayne, N.Y.). The assembly was then attached to the effluent end of the 0.25-in. o.d. glc glass column by means of a 0.25-in. Swagelok union. The union was drilled out to 0.25-in i.d. throughout its length so that the end of the column and the Teflon ferrule butted together inside. One piece of tubing was connected to the detector using another Teflon reducing ferrule. The other piece of tubing was cemented into a Hoke Gyrolok $^1/_8$ to $^1/_{16}$-in. stainless steel union that had been drilled out with a No. 50 drill to accommodate the tubing. This assembly was mounted in the outer wall of the oven. By means of a $^1/_{16}$-in. Swagelok cap, the splitter could be closed off to route the entire flow to the ec detector. With the splitter uncapped, a 1:1 split (ec detector:atmosphere) of the effluent from the glass column was obtained. A similar splitter constructed of stainless steel tubing appeared to degrade DDT, DDE, and DDD, but did not affect the other insecticides.

A heating cartridge controlled by a variable transformer was installed in the space between the inner and outer oven walls to maintain a temperature equal to the oven temperature throughout the length of the splitter.

Teflon Trap. Seven-centimeter lengths of AWG size 16 thin-wall Teflon tubing (Trimflex, Inc., Dover, N.J.) were used for trapping the individual components in a sample as they eluted from the column. The Teflon trapping tube fitted friction-tight into the Gyrolok union and butted against the Teflon splitter tubing. In this way, a component did not contact metal at any point in the system. A small piece of Dry Ice was used to condense a component in the trap which was supported on a small platform attached to the chromatograph. The use of Teflon tubing as a trap made possible irradiation of the sample in the trapping tube since sufficient uv light is transmitted by Teflon to easily degrade the sample.

Procedure. Degradation patterns were obtained for the following organochlorine insecticides: heptachlor, heptachlor epoxide, aldrin, dieldrin, DDT, DDD, and DDE. Insecticide standards (1.4 to 4.2 ng) dissolved in 5.0 μl of *n*-hexane were chromatographed, trapped, and irradiated for preliminary evaluation of the method.

After a component was trapped, the trap was handled as shown in Figure 2. The tubing was detached from the outlet (1), the effluent end folded over, flattened with pliers, and placed in a spring loaded No. 1 paper clip (2). Fifty microliters of hexane was added slowly to the tube, taking care to

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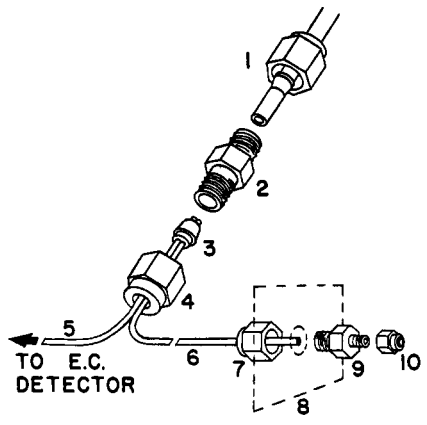

Figure 1. Splitter assembly for trapping insecticides. (1) Gas chromatographic column; (2) 0.25-in. Swagelok union; (3) reducing ferrule; (4) 0.25-in. Swagelok nut; (5) detector leg; (6) atmospheric leg; (7) 1/8-in. Hoke Gyrolok nut; (8) outer wall of gas chromatograph; (9) 1/8 to 1/16-in. reducing union; (10) 1/16-in. Swagelok cap

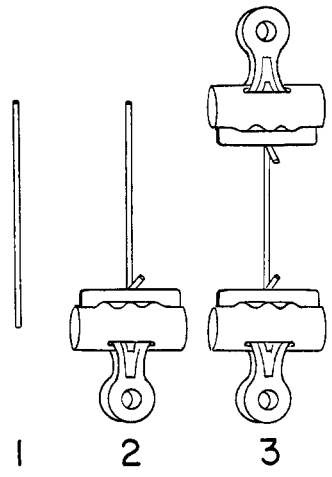

Figure 2. Preparation of trapped sample for irradiation

rinse down the interior walls. The tube was then folded over about 1 cm from the open end, flattened, and placed in a second paper clip (3).

The Teflon tube containing the trapped sample was positioned 14 cm from a 100-W, medium-pressure uv lamp (Hanovia, Model 616A) in an irradiation chamber constructed with a sliding, light-tight door for a shutter. Rubber bands attached to hooks in the irradiation chamber and to the paper clips at the ends of the trap held the tube perpendicular to the uv source. The lamp was warmed-up for 4 min before the shutter was opened. An "optimum irradiation time" (the shortest time of exposure required to yield approximately equal areas for the largest degradation glc peak and the remainder of the parent glc peak following irradiation) was determined for each insecticide. This time is a function of several variables which can be controlled, with the exception of the strength of the lamp. As the source becomes weaker with use, the "optimum irradiation time" becomes somewhat longer if other variables are held constant. The temperature in the irradiation chamber ranged from 30°C following a 20-sec irradiation to 38°C following a 5-min irradiation. After irradiation, the entire sample (or any desired portion thereof) was injected into the glc with a 50-μl syringe, and the degradation products were chromatographed.

It was possible to externally close off the atmospheric leg of the splitter with a Swagelok cap so that the entire effluent could be routed to the ec detector. This procedure was usually desirable when chromatographing irradiated samples due to the decrease in parent compound and the formation of small amounts of degradation products.

Sample Analysis. Food and other samples were prepared for analysis according to the method of Mills (1959). The Florisil column cleanup technique of Bills and Sloan (1967) was employed with slight modifications. The eluting solvent was methylene chloride–petroleum ether (1:3 v/v), and the sample was concentrated by means of a rotary evaporator. An aliquot of the sample extract was analyzed by glc for the presence of insecticides. A second aliquot was fortified with a mixture of the organochlorine insecticides under study. The fortified extract was injected into the chromatograph and the insecticides were trapped, irradiated, and reinjected to determine if substances present in the sample extracts would interfere with the consistent degradation pattern obtained with the standards.

Table I. Quantities, Relative Retention Times, and Optimum Irradiation Times of Insecticide Standards Used for Trapping and Irradiation Studies

Insecticide	Quantity (ng) per injection	Retention time relative to aldrin	Optimum irradiation time, sec
Heptachlor	3.2	0.81	90
Aldrin	1.4	1.00	90
Heptachlor epoxide	3.4	*a*	60
o,p'-DDE	2.6	1.66	15
p,p'-DDE	3.4	2.00	15
o,p'-DDD	2.8	2.14	60
Dieldrin	3.0	2.24	60
o,p'-DDT	3.2	2.68	45
p,p'-DDD	3.2	2.87	120
p,p'-DDT	4.2	3.58	30

[a] Not analyzed on same column as aldrin.

The following food samples were analyzed: butterfat, bacon fat, hamburger, chicken eggs, turnips, cucumbers, beets, spinach, and carrots. Samples of soil and osprey egg were also analyzed.

RESULTS AND DISCUSSION

The quantities, relative retention times, and optimum irradiation times of the insecticide standards used for trapping and irradiation studies are shown in Table I. The relative retention times (RRT) reported are an average of six determinations. Irradiation of greater and lesser concentrations of the insecticides included in this study revealed that the optimum irradiation time is not detectably concentration-dependent in the range of 0.2 to 5.0 ng of insecticide per μl of hexane solution irradiated. At higher concentrations, a lesser degradation of the parent compound was generally observed when irradiation time was held constant. By using size 16 Teflon tubes, which are only 1.3-mm o.d., for trapping and irradiation, it was possible to shorten the irradiation time considerably from those previously reported in the literature (Wichmann *et al.*, 1946; Robinson *et al.*, 1966; Banks and Bills, 1968).

Trapping Efficiency. The trapping efficiency of this system, as shown in Table II, ranged from 86–98% and was reproducible. Greater than 90% of the trapped residue was condensed in the half of the trap nearest the glc column.

It was not possible to determine exactly the efficiency with which *o,p'*- and *p,p'*-DDT were trapped, since these com-

Table II. Trapping Efficiency

Insecticide	Quantity (ng) per injection	Percent efficiency[a]
Heptachlor	1.6	90
Aldrin	1.4	93
Heptachlor epoxide	1.7	91
o,p'-DDE	5.1	87
p,p'-DDE	3.4	86
o,p'-DDD	5.7	89
Dieldrin	3.0	98
o,p'-DDT	6.4	*b*
p,p'-DDD	3.2	89
p,p'-DDT	4.2	*b*

[a] Range for all values obtained from at least three replications falls between ±6%. [b] See text.

Table III. Samples Analyzed

Sample	Weight per injection (mg)	Insecticide present	Concentration in sample (ppm)
Bacon fat	2.8	Dieldrin	0.05
Beets	10	*p,p'*-DDE	0.24
		p,p'-DDT	0.34
Hamburger	20	*p,p'*-DDE	0.012
		p,p'-DDT	0.014
		Dieldrin	0.007
Turnips	20	Dieldrin	0.002
Cucumbers	17	Dieldrin	0.024
Eggs	40	Dieldrin	0.002
Carrots	46	Aldrin	0.008
		Dieldrin	0.013
		p,p'-DDE	0.013
		p,p'-DDT	0.024
Osprey egg	*a*	*p,p'*-DDE	*a*
		p,p'-DDD	
		p,p'-DDT	
Soil	40	*p,p'*-DDE	0.052
		p,p'-DDD	0.065
		p,p'-DDT	0.090
		Dieldrin	0.580

[a] Sample received for verification only; sample weight or dilution was not received.

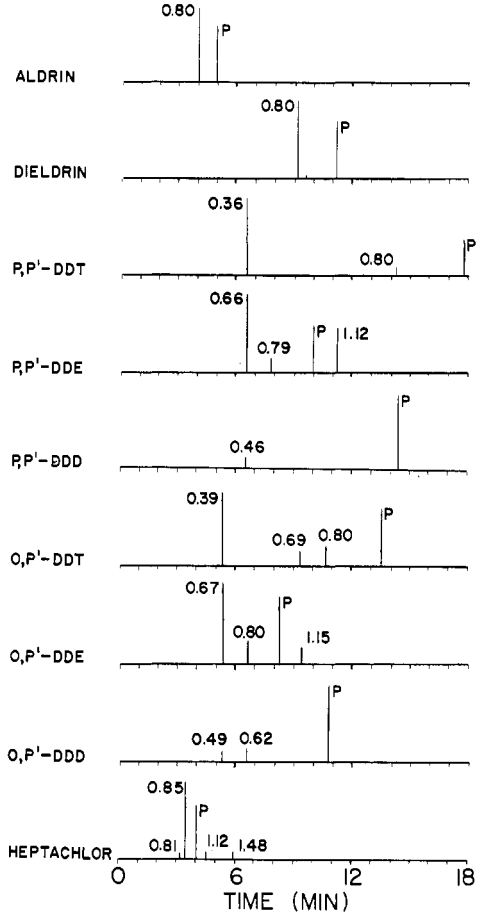

Figure 3. Uv degradation patterns of certain insecticides presented as bar graphs of peak heights *vs.* retention times. The parent compound is labeled P. The relative retention time of degradation peak to parent compound is indicated by the number adjacent to the bar. Separation carried out on a column packed with an equal-weight mixture of 4% SE-30 and 4% QF-1 on 70/80 mesh Anakrom ABS

pounds were slightly degraded by the glc system. Two peaks in addition to DDT were apparent upon reinjection of the trapped sample. The combined area of the two peaks was equivalent to about 10% of the area of the DDT peak, and their retention times agreed with those of DDE and DDD. Degradation was not eliminated by lowering the temperature of the system. This minor degradation of DDT in the system did not interfere, however, with its analysis by the uv irradiation method outlined herein.

Irradiation Results. Degradation patterns of the insecticide standards are presented in Figures 3 and 4. For the sake of clarity and convenience, the chromatographic patterns of the degradation products are presented in the form of bar charts. The RRT are relative to the parent peak and are indicated by the numbers adjacent to the peaks.

The degradation patterns were confirmed by repeated determinations and were qualitatively reproducible with respect to the number of peaks and their glc retention times. When subnanogram amounts of insecticides were irradiated, some of the minor degradation peaks were not detected, but at least one major degradation peak that could be used for identification purposes was always detected.

Four degradation peaks were obtained for heptachlor (Figure 3), but three of them were comparatively small. When heptachlor was irradiated in subnanogram quantities, the peak at 0.85 RRT was the only degradation peak that could be observed. The major degradation peak for heptachlor probably represents one or both of the monodechlorinated derivatives which McGuire *et al.* (1970) reported as being the only products formed when low concentrations of heptachlor are irradiated in hexane. With the SE-30/QF-1 column, the heptachlor epoxide degradation peaks were not separated from the parent peak. Using the more polar OV-225 column, two degradation peaks were resolved (Figure 4).

The degradation peaks for aldrin and dieldrin (Figure 3) are most probably pentachloroaldrin and pentachlorodieldrin, respectively, as reported by Henderson and Crosby (1967).

The major uv degradation product of DDT, DDD, and DDE is 4,4'-dichlorobenzophenone, the component shown in Figure 3 at RRT of 0.66 for *p,p'*-DDE, 0.46 for *p,p'*-DDD, and 0.36 for *p,p'*-DDT.

Tests were conducted to determine the stability of the samples after irradiation. Samples of *p,p'*-DDE were irradiated and a sample was injected at 2, 16, 29, and 42 min after the irradiation period. The RRT did not change during this interval and the ratios of the degradation peak areas to the parent peak area remained constant.

Analysis of Soil, Food, and Biological Samples. All of the samples tested proved to be responsive to the analytical procedure reported herein. A 10-μl portion of each of the food extracts was chromatographed before it was spiked. These 10-μl portions represented from 2.8 to 4.6 mg of the sample

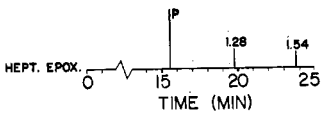

Figure 4. Uv degradation pattern of heptachlor epoxide. Separation carried out on a column packed with 5% OV-225 on 70/80 mesh Anakrom ABS

before clean-up (see Table III). Peaks having a retention time similar to that of an authentic insecticide were trapped and irradiated. The successful identification of insecticides in unspiked samples by this method is illustrated in Table III.

In some cases, the 10-μl portion of the unspiked food extract contained an insufficient amount of insecticide to yield a detector response when the irradiated sample was injected. In such instances, the desired component was collected in the same trap from as many as three successive chromatographic runs before irradiation. Successive trapping improved the clarity of the insecticide degradation pattern, especially for DDT, DDE, and DDD. With some of the insecticides, particularly aldrin and dieldrin, it was possible to obtain clear degradation patterns with very small quantities of material. A good "fingerprint" degradation pattern was obtained with 0.07 ng of aldrin. It is suggested that successive trappings might also prove useful in some instances for obtaining quantities large enough for mass spectrometry or infrared spectroscopy.

Extracts of the samples of food and soil were fortified with heptachlor, aldrin, heptachlor epoxide, *p,p'*-DDE, dieldrin, *p,p'*-DDD, and *p,p'*-DDT at concentrations approximating those shown in Table I. Each component was then trapped and the identification procedure previously described was followed to test the effect of any coextracted material on the degradation patterns obtained. In each case, the added insecticide degraded exactly as the reference standards.

Coextracted materials or artifacts are known to give peaks with the same retention times as some insecticides (Sans, 1967; Glotfelty and Caro, 1970; Deubert, 1970). Pearson *et al.* (1967) reported that elemental sulfur was such an artifact for aldrin. Some samples were spiked with either aldrin or sulfur or mixtures of the two. From the irradiation pattern, it was possible to determine whether the peak was entirely aldrin, sulfur, or a mixture of aldrin and sulfur. Since sulfur does not degrade upon irradiation, it cannot be mistaken for aldrin.

Sans (1967) reported finding an aldrin artifact in turnips. A number of the vegetable samples analyzed in the current study contained a component which yielded a symmetrical peak having exactly the same retention time as aldrin. When this peak was trapped and irradiated, the "fingerprint" degradation pattern of aldrin was obtained in only one case. Coextracted materials or artifacts were apparently responsible for most of the "aldrin" peaks in these samples.

The trapping and irradiation of a component can provide a quick means for the verification of the identity of certain insecticides. It is suggested that the analyst irradiate both the known and the unknown compound concurrently during the course of an analysis. When the irradiated known and unknown are chromatographed, the resulting degradation patterns should be nearly identical if the two compounds are indeed the same.

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