Photochemical Confirmation of Mirex in the Presence of Polychlorinated Biphenyls

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A simple method for the determination of mirex in the presence of polychlorobiphenyls (PCB's) is reported. The procedure depends on diethylamine-assisted photodegradation of interfering PCB's prior to measurement of the mirex by electron capture gas chromatography. An inexpensive 275-W sunlamp (spectral output >280 nm) may be used as the irradiation source. Reductive dechlorination of the PCB results apparently through primary photoexcitation of the biphenyl, followed by hydrogen abstraction from both the alkyl and amino groups of the amine. The method has been successfully applied to human tissue extracts for the determination of mirex in the presence of Aroclor 1260 and other commonly occurring chlorinated pesticides.

The difficulties inherent in the identification and quantitative measurement of pesticide residues in biological and environmental samples are all too familiar to the residue chemist. Polychlorinated biphenyls are ubiquitous contaminants of our environment and the tissues of our bodies despite the restrictions on their use. Their continuing presence contributes to uncertainties in the analysis of pesticide residues, especially those of chlorinated insecticides.

Several different methods have been developed for the elimination of PCB interferences in the determination of organochlorine pesticides (e.g., see Armour and Burke, 1970; Mulhern et al., 1971; Dolan et al., 1972). However, these procedures add considerably to the time required for analysis and/or result in substantially higher limits of detection.

Chlorinated biphenyls have relatively poor stabilities to ultraviolet light. This property has been utilized by Leavitt et al. (1973) for the degradation of PCB's in the analysis of *p,p'*-DDT, *p,p'*-DDE, and dieldrin. Under the conditions employed by these authors, long exposure times were necessary and there was considerable photodegradation of the pesticides. It was noted that analysis of the photoproducts is helpful in confirming the identity of the pesticides from which they arise, a technique first utilized by Mitchell (1961) and further developed by Banks and Bills (1968). However, quantitative analysis by this method is necessarily complicated.

This effort sought to develop a simple, rapid, and inexpensive method for the confirmation and quantitative determination of the insecticide mirex in the presence of interfering PCB's. Such a method would be of considerable value to small laboratories lacking mass spectrometric capability.

EXPERIMENTAL SECTION

Irradiations. A Rayonet photochemical reactor equipped with a merry-go-round and 16 2537-Å lamps was used in the preliminary work. A GTE-Sylvania sunlamp housed in a ventilated chamber with a flat-black interior was used for all subsequent irradiations. The spectral distribution curve of this lamp is given in Figure 1. The major emission is at a wavelength of 366 nm and is said by the manufacturer to have an intensity of 4.65 μW/cm^2 at a distance of 70 cm. Emission intensities between 276 and 296 nm are said to be less than 0.25 μW/cm^2 at this distance. All irradiations were made at a distance of 10 cm from the sunlamp. The total light output in the region from 180 to 4000 nm was measured at 118 mW/cm^2 at this distance with a YSI Model 65A radiometer. Most photolyses were carried out in 14 × 130 mm cylindrical quartz tubes.

Gas Chromatography. Most analyses were performed on a Tracor 222 gas chromatograph equipped with a pulsed nickel-63 electron capture detector and a 138 cm × 4 mm i.d. glass column packed with 1.5% OV-17/1.95% OV-210 on 80–100 mesh Gas-Chrom Q. The injection port, column oven, and detector temperatures were maintained at 232, 200, and 275 °C, respectively. The carrier gas was nitrogen with a flow rate of 80 ml/min.

A Varian Aerograph 1520 preparative gas chromatograph was employed to isolate the heptachlorobiphenyl from Aroclor 1260. A 183 cm × 4 mm i.d. stainless steel column packed with 4% SE-30/6% OV-210 on 80–100 mesh Gas-Chrom Q at 200 °C was used and fractions were collected manually. The carrier gas was helium at a flow rate of 40 ml/min. A thermal conductivity detector was used to follow the elution.

Mass Spectrometry. Mass spectra were obtained with a Hewlett Packard 5930A mass spectrometer equipped with a Hewlett Packard 5932A data system. A 183 cm × 2 mm i.d. glass column packed with 1.5% OV-17/1.95% OV-210 on 80–100 mesh Gas-Chrom Q was used. The GC inlet temperature was 200 °C. Helium flow rate was 42 ml/min. Temperature programmed runs were made from 80 (2 min) to 210 °C at 8 °C/min. Isothermal runs were made at 210 °C. The transfer line to the mass spectrometer was maintained at 210 °C and the silicone membrane separator was kept at 190 °C.

Reagents and Solvents. Mirex (98+%) and Aroclor 1260 (100%) were obtained from the pesticide repository of the Quality Assurance Section, Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, N.C.

Diethylamine, bp 55–56 °C, and triethylamine, bp 88–89 °C, were fractionally distilled before use. *N*-Deuteriodiethylamine was prepared by stirring for 2 h a mixture of 10 ml of diethylamine and 20 ml of deuterium oxide to which 3 drops of concentrated hydrochloric acid was added to promote exchange. The amine was recovered from the mixture by fractional distillation. This process was repeated four times. Total deuteration of the amino group was indicated from the NMR spectrum. Other reagents were analytical grade or better and were used without further purification.

Except in preliminary studies, all hexane used as solvent was spectroscopic quality. Pesticide quality hexane is a

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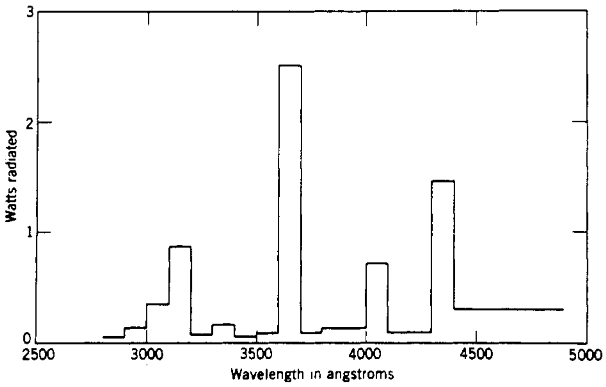


Figure 1. Spectral output of the GTE-Sylvania 275-W sunlamp.

satisfactory substitute for irradiations using the sunlamp, however. *n*-Hexane-d_{14} was obtained from Merck, Sharp and Dohme Canada, Ltd., and was used as received.

RESULTS AND DISCUSSION

Mirex (1,1a,2,2,3,3a,4,5,5,5a,5b,6-dodecachlorooctahydro-1,3,4-metheno-2*H*-cyclobuta[*cd*]pentalene) coelutes with higher molecular weight polychlorinated biphenyls on gas chromatographic columns commonly used for pesticide residue analysis. For example, at 200 °C on a column of 1.5% OV-17/1.95% OV-210 on Gas-Chrom Q, the retention time for mirex is the same or nearly the same as that for a heptachlorobiphenyl component of Aroclor 1260, depending on the carrier gas flow rate. The photostability of mirex is, however, considerably greater than that of the biphenyl. When a mixture of mirex and Aroclor 1260 in hexane was exposed to a short-wavelength ultraviolet light source (Rayonet 2537-Å lamps), the heptachlorobiphenyl component of the latter was totally photolyzed in 10 min, while only 15 to 20% of the mirex was destroyed. A mirex photolysis product, resulting from the loss of one chlorine atom, was observed with a GC retention time of 0.66 relative to that of mirex. It was found that the rate of photolysis of mirex was sensitized by traces of benzene in the hexane solvent, so that yields of the mirex photoproduct varied with solvent quality. While information derived from the short-wavelength photolysis was of value in establishing the identity of mirex, it was difficult to determine quantitatively the amount of mirex in the mixture. Therefore, attempts were made to find a photosensitizer that would selectively promote the photolysis of the interfering PCB's by long-wavelength ultraviolet light.

The light source selected was an inexpensive 275-W sunlamp of the type obtainable from retail drug outlets. The ultraviolet energy output of the lamp is greatest at 366 nm, with no output below 280 nm. Solutions were exposed to the lamp in quartz reaction tubes at a distance of 10 cm. Direct irradiation of mirex in hexane with this lamp for periods of up to 2 h produced little or no degradation. The heptachlorobiphenyl component of Aroclor 1260 undergoes a 25–30% reduction in this period.

The ultraviolet absorption spectrum of Aroclor 1260 shows maxima at 282 and 291 nm (ϵ 1540 and 1420, respectively, based on an average molecular weight of 395). The output of the 275-W sunlamp is relatively weak in this region of the spectrum. Mirex manifests no uv absorption above 230 nm at concentrations several times greater than those used in these studies. Conventional photosensitizers such as acetophenone and crystal violet did not facilitate the photolysis of PCB's, but caused appreciable degradation of the mirex.

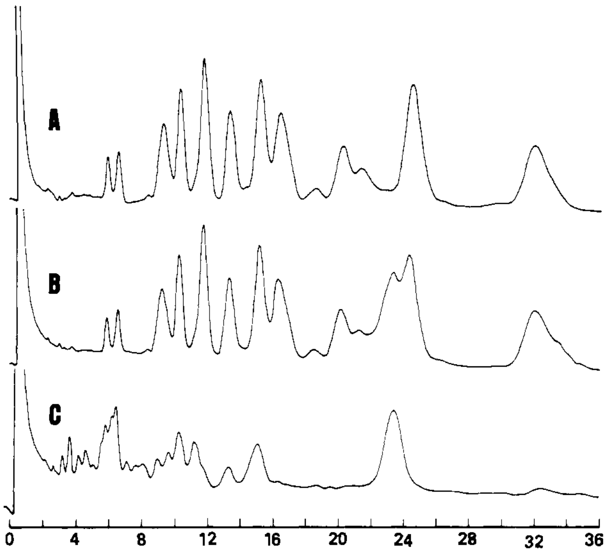


Figure 2. DEA-assisted photolysis of polychlorinated biphenyls in the presence of mirex: (A) Aroclor 1260 in hexane; 250 pg/μl; (B) mixture of 250 pg/μl Aroclor 1260 and 50 pg/μl mirex in 5% v/v diethylamine/hexane; (C) mixture B after 100-min exposure to 275-W sunlamp. Gas chromatograms obtained on 1.5% OV-17/1.95% OV-10 on Gas-Chrom Q at 200 °C; nitrogen carrier gas at 86 ml/min; pulsed Ni-63 detector.

Mirex has been shown by Alley et al. (1974) to undergo photochemical elimination of chlorine when irradiated in triethylamine with a 275-W sunlamp. Spectroscopic evidence was cited suggestive that the reaction was assisted by the formation of a charge-transfer (C-T) complex with the amine which absorbed wavelengths out to 320 nm. In their studies, the irradiation was carried out in a borosilicate tube for 50 h to achieve an 80% conversion of mirex at a concentration of 50 μg/μl. At the much lower concentrations normally encountered in residue analysis, reaction times are greatly reduced, especially when quartz reaction vessels are used. For example, we were able to effect 95–100% photodegradation of mirex at 100 pg/μl in hexane containing 10% by volume of triethylamine after only 15 or 20 min.

Under similar conditions, we found that diethylamine (DEA) selectively promotes the photodegradation of PCB's, while mirex is virtually unaffected. In a typical experiment, a mixture of 2.5 μg of Aroclor 1260 and 0.5 μg of mirex in 10 ml of hexane containing 5% by volume of DEA was irradiated in quartz tubes for 100 min with the sunlamp. There was total elimination of the interfering heptachlorobiphenyl component while only 0 to 5% of the mirex was lost. Gas chromatograms of the mixture before and after exposure are shown in Figure 2. When borosilicate reaction tubes were used, the degradation of the PCB was reduced to 50% in 100 min.

Purging the solution with nitrogen before and during irradiation had no apparent effect on the rate of photolysis. The air temperature at 10 cm from the lamp was measured as 59 °C after 100 min. When the mixture of mirex, PCB, and DEA was protected from light and held in a water bath at this temperature for 100 min, no reaction occurred.

Figure 3 shows the loss of the heptachlorobiphenyl component of Aroclor 1260 vs. exposure time. It is apparent that the reaction proceeds rapidly during the first 20 min of exposure and the rate decreases substantially after 40 min. The reaction is essentially complete in 100 min. When the concentration of DEA was reduced to 1.0% and 0.5% v/v, conversions were reduced to 90 and 85%,

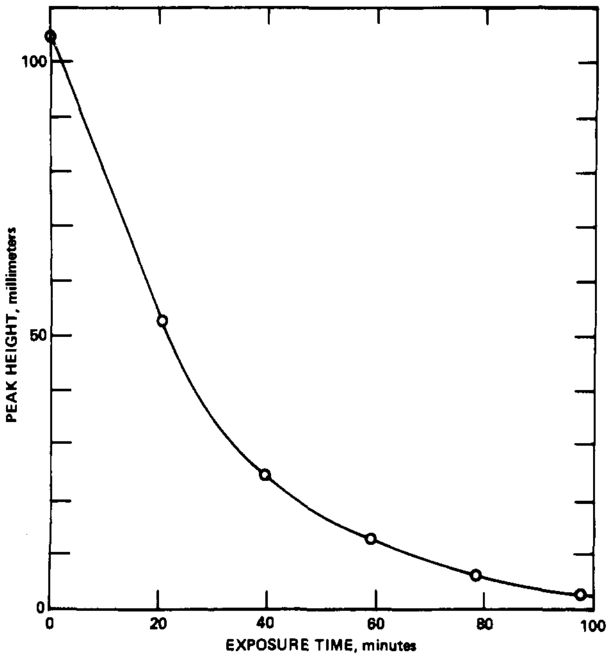


Figure 3. Rate of photodegradation of Aroclor 1260 in 5% v/v diethylamine in hexane. GC peak height of heptachlorobiphenyl component is plotted vs. time of exposure.

respectively, after 100 min. Irradiations in 10, 25, and 50% DEA solutions in 20-min intervals for a total of 100 min indicated no acceleration of the reaction rate relative to that obtained in 5% DEA.

The heptachlorobiphenyl component was essentially quantitatively destroyed after 100 min of exposure in solutions of up to 1 μg/ml of Aroclor 1260 in 5% v/v DEA in hexane. At a concentration of 100 μg/ml, the conversion was reduced to 84% and only 32% reaction was achieved after 100 min when the concentration was increased to 1 mg/ml.

The photolysis of Aroclor 1260 in the presence of DEA appears to be initiated by direct absorption of light by the biphenyls. No evidence of complex formation between Aroclor 1260 and DEA could be found in ultraviolet absorption spectra of the mixtures. Ground-state C-T complexation between PCB and DEA was further ruled out by ^{1}H NMR studies with 2,3,4,5,6,2′,5′-heptachlorobiphenyl (Analabs Catalog No. RCS-047). No measurable shifts in the proton resonance bands of the PCB were observed in mixtures having molar ratios of DEA to PCB ranging from 0:1 to 200:1.

Polychlorobiphenyls have been shown by Safe and Hutzinger (1971) and by Ruzo et al. (1972) to undergo slow photochemical elimination of chlorine in hexane when exposed to wavelengths greater than 280 nm. Nordblom and Miller (1974) demonstrated that reductive photodechlorination of PCB's could be accelerated when 2-propanol, methanol, acetic acid, or acetonitrile were used as solvents or additives. They concluded more than one mechanism was involved and that simple homolytic cleavage of aryl–chlorine bonds was not occurring. Aromatic amines have been shown to sensitize the photolysis of alkyl halides (Miller and Narang, 1970) and DDT (Miller et al., 1973). A mechanism involving photoexcitation of the amine and subsequent reaction with the halogenated compound was postulated.

Mechanistic Studies. The role of diethylamine in the photolysis of Aroclor 1260 was investigated through studies with the isolated heptachlorobiphenyl component. The component was separated by preparative GC and confirmed by combined gas chromatography–mass spectrometry (GC–MS) to be a nearly pure heptachlorobiphenyl or isomeric mixture of heptachlorobiphenyls. Irradiation of this component in 5% DEA in hexane resulted in its conversion to lower chlorinated biphenyls and minor PCB–DEA addition products. The reconstructed total ion and mass chromatograms presented in Figure 4 were obtained at approximately 50% conversion and show the major product to be a hexachlorobiphenyl. Other biphenyls containing 6, 5, and 4 chlorine atoms were also identified from the mass spectra. The photoproduct exhibiting the longest observed GC retention time was identified from its mass spectrum as a diethylaminohexachlorobiphenyl. At complete conversion (130 min for a solution of 2.7 ng/μl) small amounts of a trichlorobiphenyl and a diethylaminopentachlorobiphenyl were also formed.

When the photolysis was conducted with 5% DEA in n-hexane-d_{14}, no deuterium was incorporated into the photoproducts. This appears to eliminate mechanisms involving simple homolytic cleavage and hydrogen abstraction from the solvent. The extent of reaction after 100 min and distribution of photoproducts was not altered from those observed in hexane. However, when N-deuteriodiethylamine (DEA-d_1) was substituted for the normal amine, deuterated as well as normal PCB photolysis products were identified by GC–MS. The solution contained 140 ng/μl of the biphenyl and 5% by volume of DEA-d_1. It was estimated by GC–MS that only 25% of the major hexachlorobiphenyl photoproduct was deuterated. Of major penta- and tetrachlorobiphenyls produced, 40 and 60%, respectively, contained deuterium. These results are consistent with what would be expected if only 1 in 4 hydrogens abstracted by the biphenyl came from the amino group (ignoring any deuterium isotope effect). There also appeared to be a ca. 20–25% decrease in the extent of conversion after 100 min, although this was not rigorously confirmed. Hence, it appears that the source of hydrogen in the DEA-promoted photoreduction is the amine rather than the solvent and that hydrogen is abstracted from both the alkyl and amino moieties of the amine.

Triethylamine (TEA), which contains no amino hydrogen, also efficiently promotes the photolysis. When the photolysis was conducted in n-hexane-d_{14} with 1% TEA, little, if any, deuterium was incorporated into the photoproducts. On the other hand, diphenylamine, in which the only readily extractable hydrogens are those on the amino group, is at least as efficient in promoting the photolysis as DEA and TEA. In this case, however, the reaction may follow a different route. Perfluorotributylamine (PFTBA), which contains no hydrogen, assists the reaction, but to a lesser extent. Propanol-2 also is much less efficient than DEA, although the HO(CH$_3$)$_2$C–H bond energy is 4 kcal/mol lower than that for N–H in secondary amines and 13–20 kcal/mol less than for the C–H bonds in alkanes. All of these results are summarized in Table I.

It must be concluded from the results of this study that aliphatic amines assist the photolysis of PCB's by some mechanism involving more than simple hydrogen donation. Although the formation of a charge-transfer (C-T) complex between DEA and the PCB ground state appears to be ruled out from NMR and uv data, an excited-state C-T complex (exciplex) cannot be excluded. Whether by way of exciplex formation or through a simple bimolecular reaction, a plausible mechanism may involve the primary excitation of the PCB, followed by abstraction of an α-

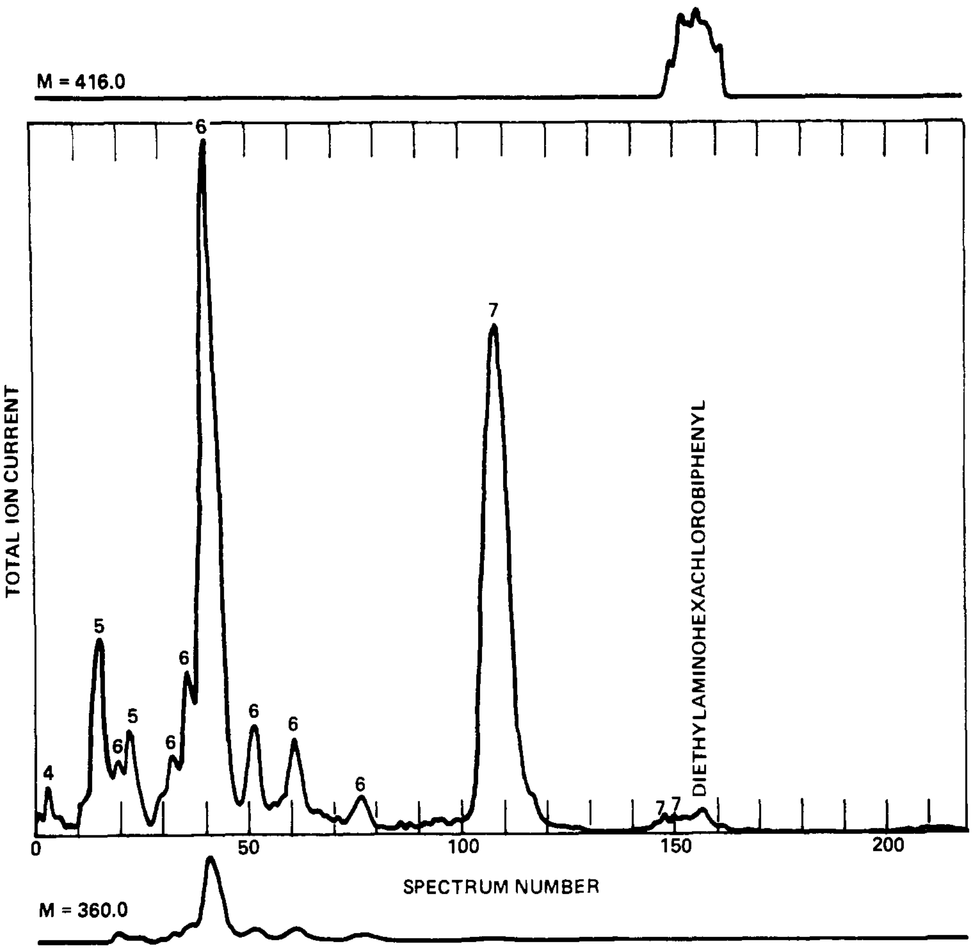
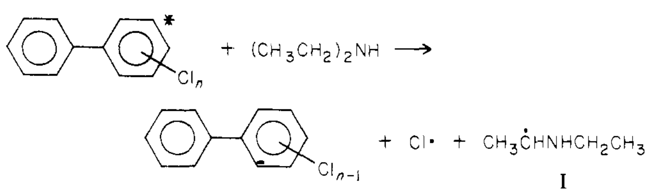


Figure 4. Reconstructed total ion and mass chromatograms of heptachlorobiphenyl at ca. 50% conversion by photolysis in 5% v/v diethylamine in hexane. Lower chromatogram is of hexachlorobiphenyl photoproducts. Upper chromatogram is of a diethylaminohexachlorobiphenyl addition product. Numerals indicate the numbers of chlorine atoms in the biphenyls.

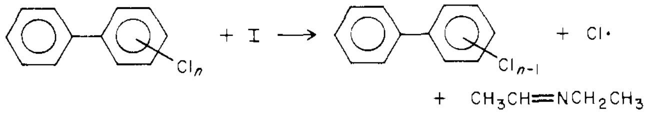
Table I. Photolysis of Aroclor 1260 and Mirex in Hexane

Additive	Exposure time, min	% loss 7Cl-PCB	% loss Mirex
None	20	5	0
None	100	26	0
0.1 M acetophenone	20	9	14
0.03 M crystal violet	20	10	20
5% diethylamine	20	51	0
5% diethylamine	100	100	2
10% triethylamine	20	34	98
1% triethylamine	100	93	
0.1% diphenylamine	100	100	
1% PFTBA	100	43	
5% propanol-2	100	46	5

hydrogen from DEA and elimination of a chlorine atom:



The amine free radical I may then react with a ground-state PCB molecule, resulting in transfer of an amino hydrogen:



This two-step process is very similar to that which is operative in the photoreduction of aromatic ketones by amines (Cohen et al., 1973). Evidence of the imine expected to be formed in the second step was not sought.

Application to Multiresidue Analysis in Tissues. The utility of the method for the determination of mirex in adipose tissue was successfully demonstrated. Spiked chicken fat supplied as a reference standard by the Quality Assurance Section, Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, N.C., was used in the study. The fat sample contained 0.3 ppm of β-BHC, 0.2 ppm of aldrin (for reference), 0.4 ppm of heptachlor epoxide, 10 ppm of *p,p'*-DDE, 0.5 ppm of dieldrin, 0.8 ppm of *o,p'*-DDT, and 2 ppm of *p,p'*-DDT. The reference fat was further fortified with mirex and Aroclor 1260, both separately and together, to levels of 1 and 5 ppm, respectively. The pesticides and PCB's were extracted from the fat and the extract cleaned up by means of the modified Mills–Onley–Gaither procedure (Thompson, 1974). The 6% diethyl ether in hexane fraction from the Florisil column contained all the PCB's and pesticides except dieldrin. This fraction was analyzed by electron capture gas chromatography before and after 100 min of irradiation in 5% DEA in hexane. It was not necessary to remove the excess DEA before analysis. The recovery of mirex from the sample which did not contain PCB's was 69–72%. When PCB's were present, they were eliminated by photolysis to afford mirex yields of 65 to 70%. Of the pesticides eluting in the 6% fraction, only heptachlor epoxide and *p,p'*-DDE were appreciably photolyzed. The 15% ether in hexane fraction, which

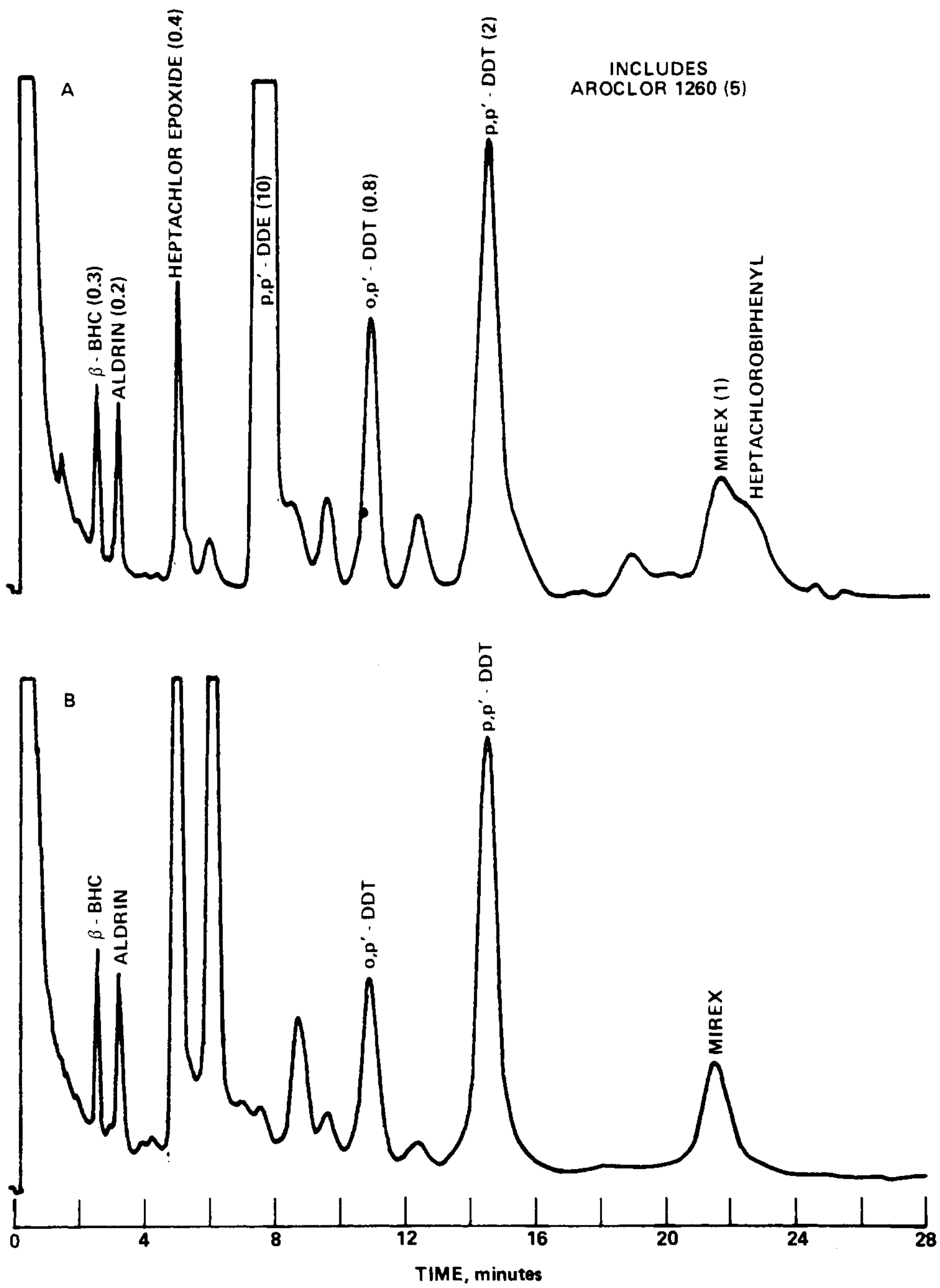


Figure 5. Gas chromatograms of the 6% ether in hexane fraction from Florisil cleanup of an adipose tissue sample containing a mixture of pesticides and Aroclor 1260: (A) before exposure; (B) after exposure for 100 min in 5% diethylamine in hexane. Column: 1.5% OV-17/1.95% OV-210 on 80/100 mesh Gas-Chrom Q at 200 °C; carrier gas, nitrogen at 86 ml/min; detector, nickel-63 electron capture at $10^2 \times 16$. Numbers in parentheses indicate levels in parts per million of compounds in tissue sample.

contained dieldrin, was not analyzed. Gas chromatograms of the 6% fraction before and after irradiation are shown in Figure 5. Some broadening of the solvent front is apparent and is due to the presence of DEA; however, this should not interfere with the determination of most pesticides.

CONCLUSION

Diethylamine-assisted photodechlorination has been demonstrated to be a rapid and efficient means of eliminating PCB interferences in the determination of mirex. The method is probably also applicable to many other chlorinated pesticides. The mechanism of the photochemical reaction appears to involve the direct photoexcitation of the PCB, followed by hydrogen abstraction from both the alkyl and amino groups of the amine.

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Determination of Zineb by Oxidation with Chloramine-T

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A simple volumetric procedure is described for the estimation of zineb in its formulations. This is based on its oxidation in an alkaline medium by chloramine-T after the removal of the oxidizable impurities.

Zineb (or zinc ethylene bis(dithiocarbamate)) is widely used in India as a protective fungicide for the control of plant diseases. It is available as a 78% water-dispersible powder formulation. The analysis of zineb formulation is therefore of great interest.

The most widely used method (Stevenson, 1964; AOAC, 1970) for the analysis of zineb is based on the quantitative evolution of carbon disulfide by digestion with sulfuric acid. The hydrogen sulfide that is evolved simultaneously is removed by passing the mixture of gases through lead acetate. The carbon disulfide is then absorbed in alcoholic potassium hydroxide when the xanthate is formed. The xanthate is determined by titration with iodine. A simple volumetric procedure for the determination of zineb based on its oxidation in an alkaline medium with chloramine-T is described.

It is well known that metallic salts of dithiocarbamic acid undergo degradation during storage and give rise to a variety of products. Thus, it is reported that zineb water-dispersible powder formulations contain (Fishbein and Fawkes, 1965) ethylenethiourea, ethylene thiuram-monosulfide, zinc sulfide, and sulfur as decomposition products. Since the degradation products are also susceptible to oxidation with chloramine-T, it is necessary to separate the parent compound from the contaminants. In the method worked out and described below, the organic impurities and sulfur are removed by washing the sample successively with ethyl alcohol, chloroform, and dilute acetic acid. The residue is dissolved in dilute sodium hydroxide when the zineb goes into solution leaving behind the insoluble zinc sulfide. The zineb is determined by taking an aliquot of the alkaline solution.

EXPERIMENTAL PROCEDURE

About 0.4 g of the sample was weighed accurately and transferred into a 100-ml beaker. Alcohol (10 ml) was added and the sample was stirred well. The solid was allowed to settle and the supernatant alcohol was decanted through a dry filter paper. Washing with 10-ml portions of alcohol was continued until the filtrate was colorless. The sample was similarly washed with 10-ml portions of chloroform and then with dilute acetic acid (6 N). With each solvent the washing was continued until a colorless filtrate was obtained. Three to four washings with each solvent were sufficient. The solid in the beaker was dissolved in about 50 ml of sodium hydroxide. The solution was poured through the filter paper and collected in a 250-ml volumetric flask. The beaker and the filter paper were washed with further quantities of sodium hydroxide solution so that the sample was quantitatively transferred into the measuring flask. The solution in the flask was then made up to the mark with 1 N sodium hydroxide.

Table I. Oxidation of Zineb with Chloramine-T

Sample no.	mol of zineb taken $\times 10^4$	equiv of chloramine-T consumed $\times 10^4$	equiv of chloramine-T per mol of zineb
1	0.228	6.845	30.02
2	0.228	6.896	30.25
3	0.230	6.845	29.73
4	0.231	6.896	29.81
5	0.221	6.640	30.05
6	0.230	6.850	29.79
7	0.226	6.780	29.93

Oxidation with Chloroamine-T. The sample solution (5 ml) was pipetted into a 250-ml stoppered conical flask. Sodium hydroxide (20 ml, 1 N) was added, followed by 25 ml of 0.1 N chloramine-T solution. The flask was stoppered, shaken well, and kept aside for 20 min. Then 40 ml of 4 N sulfuric acid was added and the flask shaken and kept aside for 10 min. Potassium iodide (10 ml, 10%) was added followed by 50 ml of water. The liberated iodine was titrated with standard 0.1 N sodium thiosulfate using starch as the indicator near the end point.

Simultaneously, the volume of sodium thiosulfate consumed by 25 ml of the chloroamine-T was determined. No blank correction for the reagents is necessary as it amounts to less than 0.05 ml. The zineb content of the sample is calculated from the following formula:

$$\%\ \text{zineb content of formulation} = [(a - b) \times \text{N} \times 0.919 \times 50 \times 1000]/w$$

where a = milliliters of standard sodium thiosulfate consumed by 25 ml of chloramine-T, b = milliliters of standard sodium thiosulfate consumed by the excess of chloramine-T remaining after reaction with zineb, N =

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