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A Method for Confirming Organochlorine Pesticide Residues in Wildlife

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Methods involving chemical reactions have been used frequently for the confirmation of organochlorine pesticide residues. Dehydrochlorination or dechlorination in alkaline solution is one such method. KLEIN *et al.* (1963), HAMMENCE *et al.* (1965), SANS (1967), and HOLDEN and MARSDEN (1967) used alcoholic alkali to convert DDT and similar compounds to their corresponding olefins. YOUNG and BURKE (1972) and KRAUSE (1972) found that treatment of DDT and DDD with alcoholic KOH converted the DDT and DDD to olefinic derivatives. The authors reported that the hexachlorocyclohexane (HCH) isomers ($\alpha,\beta,\gamma,\delta$) were transformed into unidentified compounds. The former authors also noted that dieldrin and polychlorinated biphenyls (PCBs) were unchanged by this treatment. MENDOZA *et al.* (1968) treated residues from plant extracts with sodium methylate followed by gas-liquid chromatography (GLC) and thin layer chromatography (TLC) to confirm the presence of certain pesticides. TAYLOR and KEENAN (1970) detected unchanged hexachlorobenzene (HCB) and one unidentified product in samples containing HCB that had been treated with alcoholic KOH or sodium methoxide. COLLINS *et al.* (1972) found that HCB produced pentachlorophenyl propyl ether when it was treated with KOH in 1 - propanol. The derivative was found to have a longer retention time (GLC) than HCB. HOLDRINET (1974) refluxed HCB with 0.2N KOH in ethylene glycol, to produce pentachlorophenol (PCP). The PCP was esterified with diazomethane to give the methyl ether derivative. BAKER (1973) demonstrated that HCB was converted to a monoethoxypentachlorobenzene (MEPCB) derivative when it was refluxed with sodium ethoxide. The present study deals with the confirmation of HCB and other organochlorine residues in wildlife samples by reactions involving the use of sodium ethoxide.

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EXPERIMENTAL

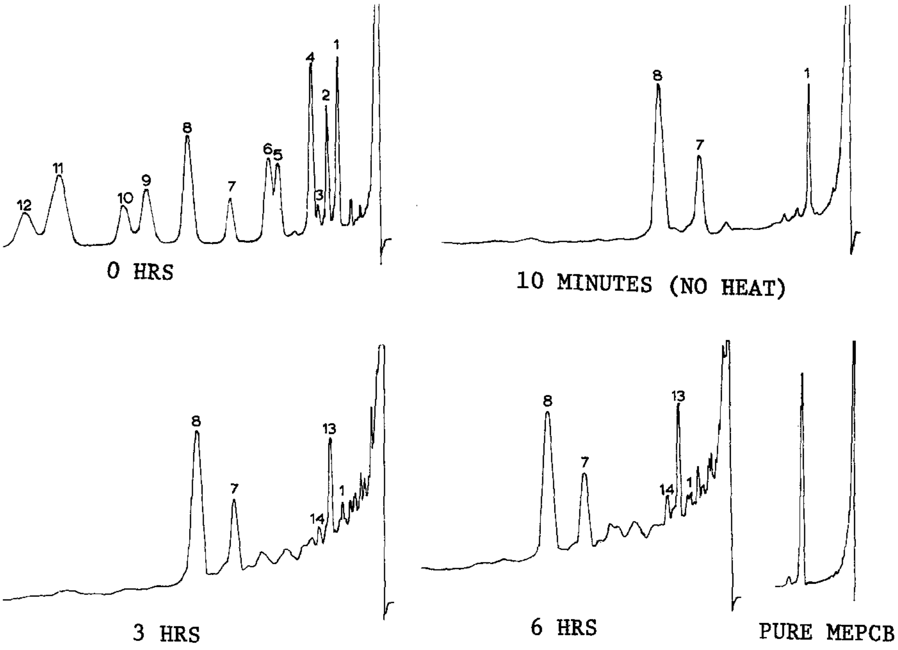
Conversion of HCB to MEPCB in the Presence of Certain Other Organochlorine Compounds

A solution (6 ml) containing HCH isomers (α,β,γ,δ), p, p'-DDT, o, p'-DDT, p, p'-DDD, o, p'-DDD, p, p'-DDE, DDMU and HCB was placed in a flask (₷) along with 34 ml of freshly prepared sodium ethoxide (5 g Na/100 ml absolute ethanol). The reaction mixture was allowed to stand for 10 min (room temperature) with occasional mixing and then a sample (1 ml) was removed. The reaction mixture was heated under reflux and samples (1 ml) were taken after 3 and 6 hr of heating. Each sample was placed in a separatory funnel along with hexane (30 ml). The mixture was cooled, mixed thoroughly, and then extracted with 30 ml portions of glass-distilled water until the aqueous washings were free (litmus) of alkali. The hexane extracts were passed through pre-washed (5 ml acetone plus 5 ml hexane) cotton plugs and their volume was adjusted to give concentrations suitable for gas chromatographic (Varian 600D) analysis. The instrument was operated under the following conditions: 1.68 m x 3 mm O.D. Pyrex tube packed with 6% QF - 1 + 4% OV - 1(1.38 m) and 6%OV - 225 + 3%OV - 1 (0.3m) on Chromosorb W - HP; N_2, 30 ml/min; oven, 210°C; detector, 225°C. The results of this experiment are shown in Figure 1. The sodium ethoxide treatment (10 min; no heat) eliminated all of the GLC peaks except those corresponding to HCB, DDMU and p, p'-DDE. The two latter peaks increased in height because of the dechlorination of DDT and DDD to give their respective olefin derivatives, DDE and DDMU. After 3-hr heating, the HCB peak was virtually eliminated and a new peak, whose retention time corresponded to that of MEPCB, was observed. No change was observed, however, in connection with the relative heights of the DDE and DDMU peaks after the 3-hr period. Heating for 6 hr produced major GLC peaks that corresponded to DDE, DDMU and MEPCB. An unidentified peak (Peak No. 14, Figure 1) that emerged after MEPCB, was also observed. Further heating of the reaction mixture resulted in a decrease in the MEPCB peak height and a concurrent increase in the height of the unknown peak.

Similar experiments with PCBs (25.8 mg of Aroclor 1260) were also performed. No substantial change in the number of GLC peaks or their relative peak heights were observed even after a 6-hr treatment with sodium ethoxide.

Identification of Diethoxytetrachlorobenzene

An experiment was performed to identify the unknown substance obtained in the previous experiment. HCB (0.5 g) was placed in a flask (500 ml) fitted with a reflux condenser, along with 75 ml of hot absolute ethanol. Freshly prepared sodium ethoxide (125 ml; 5 g sodium/100 ml absolute ethanol) was added to the HCB solution and the mixture was heated under reflux.



0 HRS

10 MINUTES (NO HEAT)

3 HRS

6 HRS

PURE MEPCB

Fig. 1. Chromatograms illustrating the conversion of HCB to MEPCB in the presence of certain other organochlorine compounds. Peak 1, HCB; Peak 2, α-HCH; Peak 3, heptachlorocyclohexane isomers; Peak 4, γ -HCH; Peak 5, β-HCH; Peak 6, δ -HCH; Peak 7, DDMU; Peak 8, p,p'-DDE; Peak 9, p,p'-DDD; Peak 10, o,p'-DDT; Peak 11, p,p'-DDD; Peak 12, p,p'-DDT; Peak 13, MEPCB; Peak 14, unknown.

Samples (1 ml) were removed after 1, 6, 21 and 41 hr. Each sample was placed in a 125-ml separatory funnel containing glass-distilled water (25 ml). The samples were shaken, neutralized with HCl (6 N) and then extracted with three 25 ml portions of hexane. The combined hexane extracts were then passed through a pre-washed cotton plug. The samples were analyzed by gas chromatography (Varian 600D). The instrument was operated under the following conditions: 1.38 m x 3 mm O.D. Pyrex tube packed with 5% QF - 1 on chromosorb W - HP; N_2, 50 ml/min.; oven, 150°C; detector, 175°C. The results of this experiment indicated that HCB was converted first to MEPCB and then to an unidentified substance as the heating continued (Figure 2). To confirm the identity of the unknown substance,

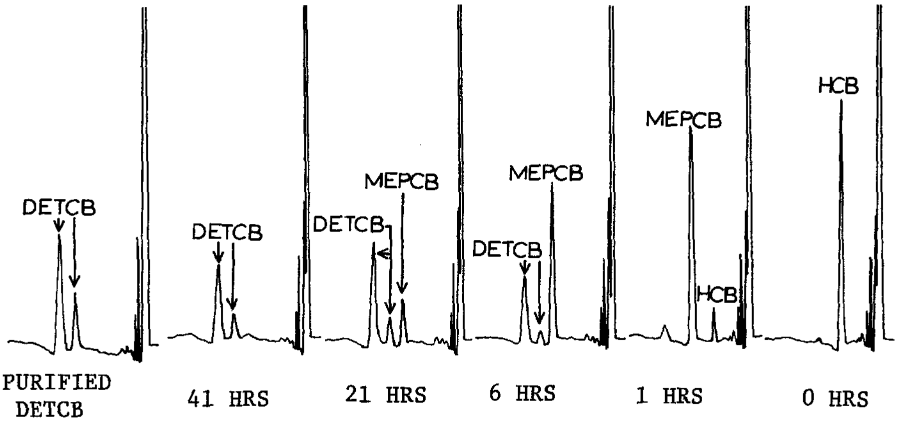


Fig. 2. Chromatograms showing the conversion of HCB to MEPCB and diethoxytetrachlorobenzene (DETCB).

the remaining reaction mixture was diluted to a volume of 200 ml with glass-distilled water, neutralized with HCl (6N) and then extracted several times with hexane. The combined extracts were dried over sodium sulfate and then evaporated to dryness using a rotary vacuum evaporator (no heat). The crystalline product that was obtained was recrystallized from ethanol. The resultant recrystallized product (m.p. 53-55°C) was analyzed by mass spectroscopy and the results were as follows: m/e 302 [$C_6{}^{35}Cl_4(OC_2H_5)_2$]; m/e 274 [i.e., 302-28, which corresponds to $C_6Cl_4OC_2H_5OH$ by the loss of C_2H_4 in the parent ion]; m/e 246 [i.e., 302-56, which corresponds to $C_6Cl_4(OH)_2$ by the loss of $2(C_2H_4)$ in the parent ion]. These results confirmed that the unknown substance was diethoxytetrachlorobenzene (DETCB). A chromatogram of the purified DETCB is included in Figure 2. The pure DETCB gave two major chromatographic peaks, which might suggest that the DETCB consisted of a mixture of isomers.

Confirmation of HCB and Other Organochlorine Compounds.

Procedure:- The following method was developed for the confirmation of organochlorines using sodium ethoxide for dechlorination. Standard solutions (hexane) of HCB (0.01 to 0.1 µg) were placed in screw-cap tubes (16 x 150 mm). The solutions were concentrated at room temperature using nitrogen, to a volume of 0.5 ml. Freshly prepared sodium ethoxide (2ml; 5 g Na/100 ml absolute ethanol) was added to each solution. The tubes were tightly capped, shaken, and then heated in a water bath (90-95°C) for 15 min. Each tube (capped) was cooled and the contents were mixed thoroughly with glass-distilled water (10 ml). Hexane (5 ml) was added to each tube which was then shaken for about one min. The lower aqueous phase was removed using a disposable pipette, and the hexane layer was washed with glass-distilled water (10 ml portions) until the aqueous layer was free (litmus) from alkali. The hexane extract was then analyzed by gas chromatography (Varian 1400). The instrument was operated under the following conditions: 1.83 m x 3 mm I.D. Pyrex tube packed with 4% SE-30+6% QF-1 on Chromosorb W-HP; N_2, 60 ml/min; oven temperature, 215°C; detector, 240°C. Hexane was treated with sodium ethoxide using the same conditions as are outlined above, in order to check possible artifacts. The overall recovery of HCB ranged between 64 and 81% based on the MEPCB derivative.

Confirmation of Residues in Wildlife Samples:- Eight samples of Harp seal tissues that had been analyzed for organochlorine residues using hexane extraction, Florisil column cleanup, and charcoal column separation (HOLDRINET 1974), and that were presumed to contain HCB residues (toluene eluate), were subjected to dechlorination using sodium ethoxide. Reagent blanks were also treated with sodium ethoxide. Seven extracts (acetone-diethyl ether eluate) of Harp seal tissues (blubber, liver, brain) that were presumed to contain DDT, DDD, DDE and dieldrin residues, were subjected to the sodium ethoxide treatment (25°C; 15 min) along with reagent blanks and several standard solutions containing 0.08 to 0.8 µg of DDT, 0.02 to 0.2 µg of DDD, 0.15 to 1.5 µg of DDE, and 0.01 to 0.1 µg of dieldrin. Seven extracts of Harp seal tissues (blubber, liver, brain) along with standard solutions of Aroclor 1254 were treated with sodium ethoxide for the confirmation of PCB residues. Reagent blanks were subjected to the same procedure.

Analysis (GLC) of the treated eluates showed that p,p'-DDT and p,p'-DDD were dechlorinated to their corresponding olefin derivatives, p,p'-DDE and DDMU. Figure 3 illustrates the increase in the peak height of the p,p'-DDE and DDMU peaks following the dechlorination (sodium ethoxide) procedure. It was possible to confirm the presence of p,p'-DDE by this procedure since it was stable to sodium ethoxide treatment and its peak height actually increased as a result of the dechlorination of p,p'-DDT (Figure 3). It was also possible to confirm (McCULLY 1969) dieldrin because of its stability in the alkaline sodium ethoxide (Figure 3). The PCBs did not react with the sodium ethoxide. It will be noted from Figure 4 that the reten-

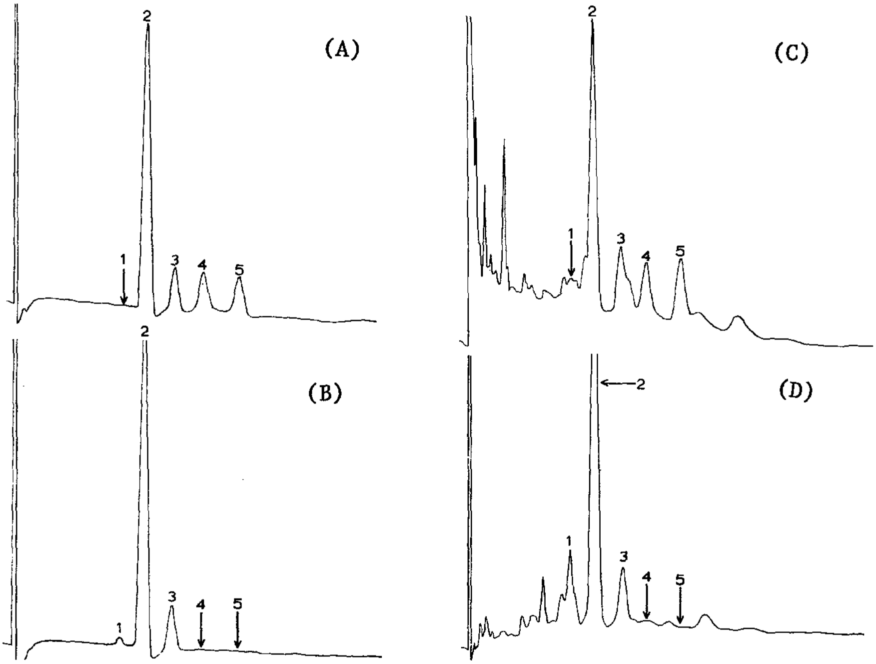


Fig. 3. Chromatograms illustrating the confirmation of certain organochlorine compounds in standard solutions and in seal tissue extracts using sodium ethoxide. (A) Mixture of pesticide standards, (B) Mixture after sodium ethoxide treatment (25°C, 15 min). (C) Seal liver extract. (D) Seal liver extract after sodium ethoxide treatment (25°C, 15 min). Peak 1, DDMU; Peak 2, p,p'-DDE; Peak 3, dieldrin; Peak 4, p,p'-DDD; Peak 5, p,p'-DDT.

tion times and the relative peak heights of the major PCB peaks remained virtually unchanged following the sodium ethoxide treatment. The recoveries of unreacted PCBs in standard solutions and in seal tissue extracts averaged 102% (91 to 108%) and 91% (74 to 111%), respectively. HCB was confirmed by the formation of the MEPCB derivative with sodium ethoxide (BAKER 1973). The longer retention time of the MEPCB derivative as compared to that of HCB is illustrated in Figure 5.

It was concluded that this dechlorination procedure can be used for rapid, routine confirmation of certain organochlorine compounds in wildlife samples.

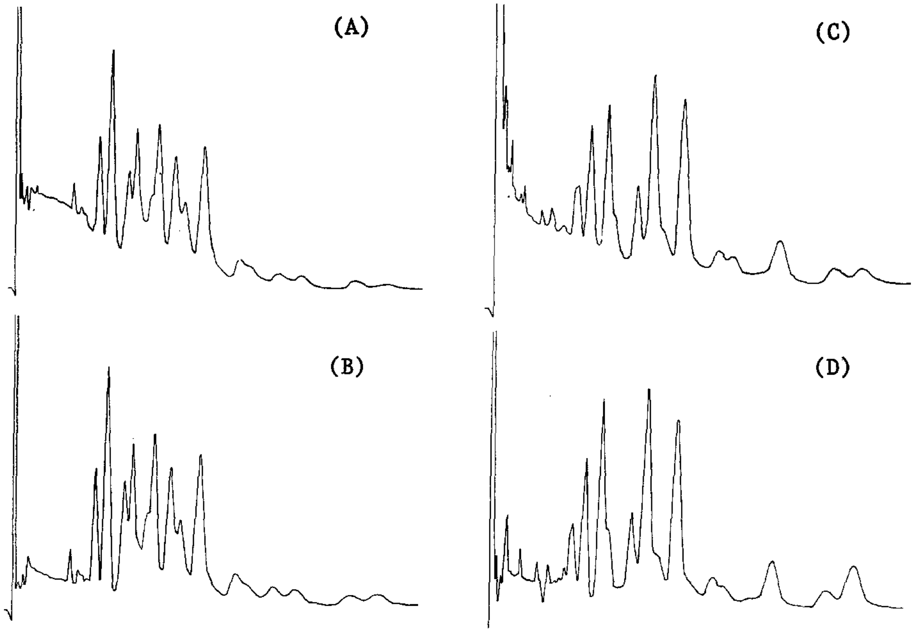


Fig. 4. Chromatograms illustrating the confirmation of PCBs in standard solutions and in seal tissue extracts. (A) Aroclor 1254 solution. (B) Aroclor 1254 solution after sodium ethoxide treatment (25°C, 15 min). (C) Seal blubber extract. (D) Seal blubber extract after sodium ethoxide treatment (25°C, 15 min).

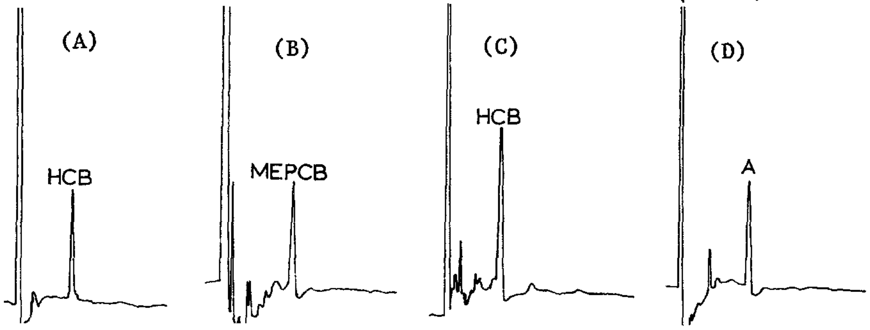


Fig. 5. Chromatograms illustrating the confirmation of HCB in standard solutions and in seal tissue extracts. (A) HCB solution. (B) HCB solution after sodium ethoxide treatment (90-95°C, 15 min). (C) Seal blubber extract. (D) Seal blubber extract after sodium ethoxide treatment (90-95°C, 15 min). Peak A has a retention time that corresponds to that of MEPCB.

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