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Confirmation of Mirex and *cis*- and *trans*-Chlordane in the Presence of other Organochlorine Insecticides and Polychlorinated Biphenyls

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The ability of certain pesticides to withstand strong acid treatment was utilized in developing a confirmation procedure for mirex and *cis*- and *trans*-chlordane. SCHECHTER *et al.* (1945) applied intensive nitration to DDT residues from plant extracts using a 1:1 mixture of concentrated sulfuric and fuming nitric acid and subsequently determined the nitrated DDT's colorimetrically. ERRO *et al.* (1964) modified this procedure to determine toxaphene in the presence of DDT.

Mirex analysis is often complicated by the presence of PCB's and other organochlorine insecticides. On most GLC columns mirex co-elutes with a heptachlorobiphenyl compound, necessitating some form of separation before quantitation. REYNOLDS (1969), ARMOUR and BURKE (1970), and HOLDRINET (1972) modified existing adsorption chromatography systems to allow PCB and organochlorine separation from mirex before GLC determination. These methods require careful control of the adsorbant activity and are subject to non-reproducibility. Additional confirmation, especially for regulatory purposes, is required. HALLET *et al.* (1976) used perchlorination, NORSTROM (1976) described the use of the Hall detector, and KAISER (1974) and HALLET *et al.* (1976) described a GLC-MS system for mirex confirmation. Mass spectrometric determination of mirex presents some difficulties; the major fragment C_5Cl_6+ can arise from a variety of compounds, particularly from PCB components, and care in the interpretation of the mass spectrometric data must be exercised.

Plant and animal co-extractives often interfere with the electron capture determination of *cis*- and *trans*-chlordane. CHAU and COCHRANE (1969) and COCHRANE (1969) developed a confirmation procedure based on dehydrochlorination. This procedure is lengthy and co-extractives present may produce a confusing chromatogram.

METHODS AND MATERIALS

After extraction and preliminary column clean-up (HOLDRINET 1974), evaporate the extract to dryness in a 125-ml boiling flask using rotary vacuum evaporation. Add 5 ml of a freshly prepared mixture of concentrated sulfuric:fuming nitric acid 1:1 (v/v). Swirl the flask to insure acid contact with the sides, stopper and place in a waterbath at 70°C for ½ hr. Transfer the acid mixture quantitatively with 3 X 10 ml of dichloromethane to a 500-ml separatory funnel containing 200 ml of tap water. Add a further

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30 ml of dichloromethane, shake the funnel 1 min, allow the phases to separate and drain the dichloromethane layer into a second 500-ml separatory funnel containing 100 ml of tap water. Re-extract the original 200 ml of tap water with an additional 60 ml of dichloromethane, and add the organic layer to the second separatory funnel and discard the water. Shake the second separatory funnel 1 min, allow the phases to separate and drain the dichloromethane back into the first 500-ml separatory funnel. Add 60 ml of 5% $NaHCO_3$, shake 1 min, allow the phases to separate and filter the organic layer over Na_2SO_4 into a 250-ml boiling flask. Rinse the Na_2SO_4 with 3 X 10 ml of dichloromethane and collect the rinsings into the flask. Evaporate to dryness on a rotary evaporator, make up in hexane (10 ml) and transfer an aliquot to a Florisil clean-up column (MILLS *et al.* 1972). Collect the first fraction, evaporate on a rotary evaporator, make up with a suitable volume of hexane and proceed to GLC determination.

Gas chromatographic instrumentation and conditions used to carry out the analyses: Tracor MicroTek 550 equipped with Ni^{63} EC detector; Pyrex column, 2 m x 4 mm i.d. packed with 2.5% OV-17 plus 2.5% OV-210 on Gas Chrom Q, 100-120 mesh, at 180°C; detector temperature 310°C; nitrogen carrier flow 60 ml/min; all injections were 5 µl.

RESULTS AND DISCUSSIONS

Figure 1 illustrates a mixture of organochlorine insecticides before nitration (A) and after nitration (B). Amounts of insecticides injected were: HCB and lindane, 0.05 ng each; oxychlordane, heptachlor-epoxide, *trans*-chlordane, *cis*-chlordane, p,p'-DDE and dieldrin, 0.1 ng each; o,p'-DDT, p,p'-TDE and mirex, 0.25 ng each. After nitration and clean-up only lindane, *trans*-chlordane, *cis*-chlordane and mirex remain (same amount injected). Figures 2 and 3 show chromatograms of unnitrated (A) and nitrated (B) Aroclor 1254 and Aroclor 1260 respectively; 1 ng injected. Figure 4 shows chromatograms of a fish sample, fortified with *trans*- and *cis*-chlordane at 0.02 ppm and mirex at 0.1 ppm before nitration (A) and after nitration (B). Mirex recoveries ranged from 90% to 100% (30 determinations) and *cis*- and *trans*-chlordane recoveries ranged from 80% to 95% (10 determinations).

To insure complete nitration of PCB's a reaction time of ½ hr at 70°C is required. The column clean-up procedure is necessary to separate the nitrated products from mirex and *cis*- and *trans*-chlordane. Because of the small acid mixture volume, boiling flasks larger than 125 ml should be avoided. Care in transferring the acid to the water should be exercised.

The nitration procedure has been used successfully in this laboratory to confirm mirex, and *cis*- and *trans*-chlordane in such substrates as water, sediment, sludge, shearwaters, fish, seals, milk and human fat. Interferences were not encountered except in one sample of industrial sludge.

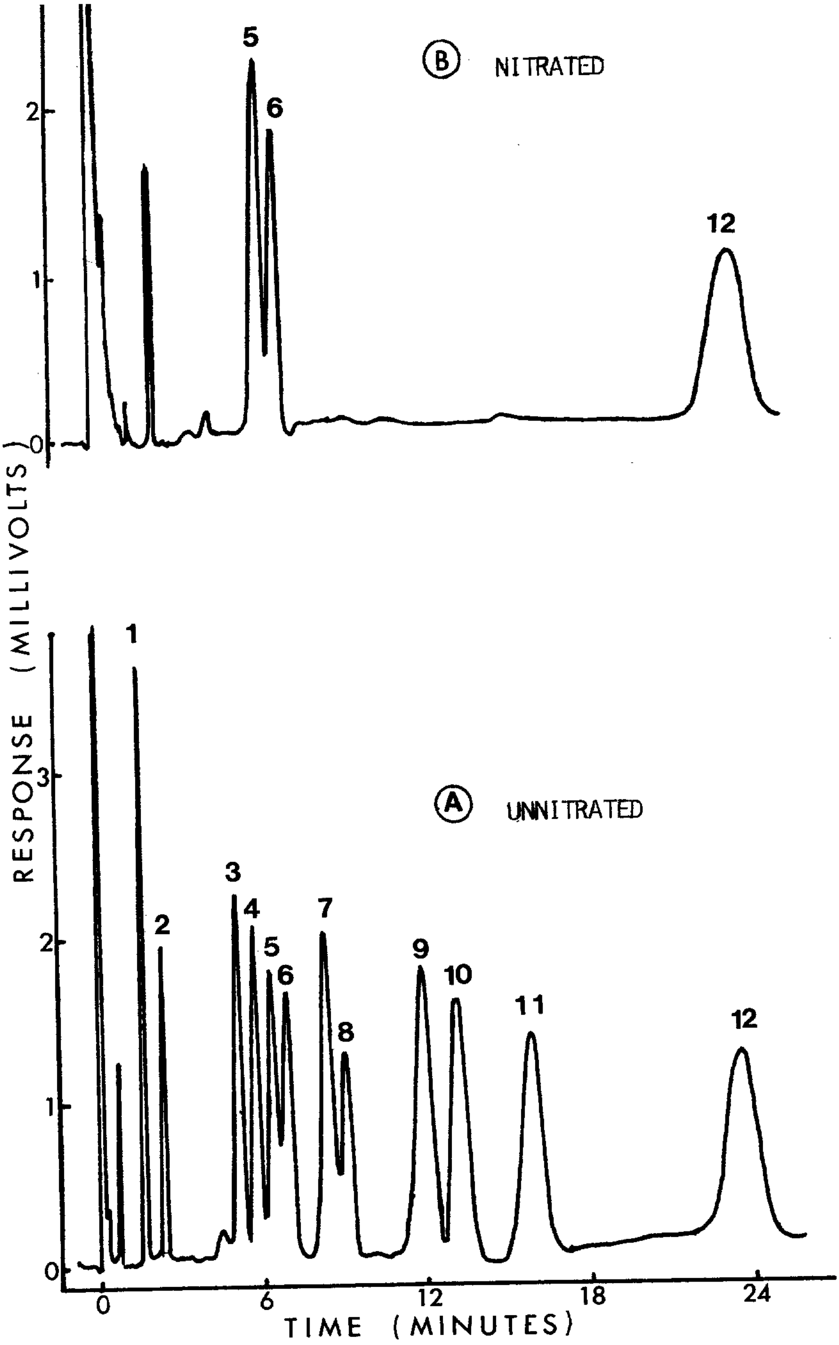

Figure 1. Gas chromatogram of an organochlorine mixture before (A) and after (B) nitration, 1-HCB, 2-lindane, 3-oxychlordane, 4-heptachlorepoxide, 5-*trans*-chlordane, 6-*cis*-chlordane, 7-p,p'-DDE, 8-dieldrin, 9-o,p'-DDT, 10-p,p'-TDE, 11-p,p'-DDT, 12-mirex.

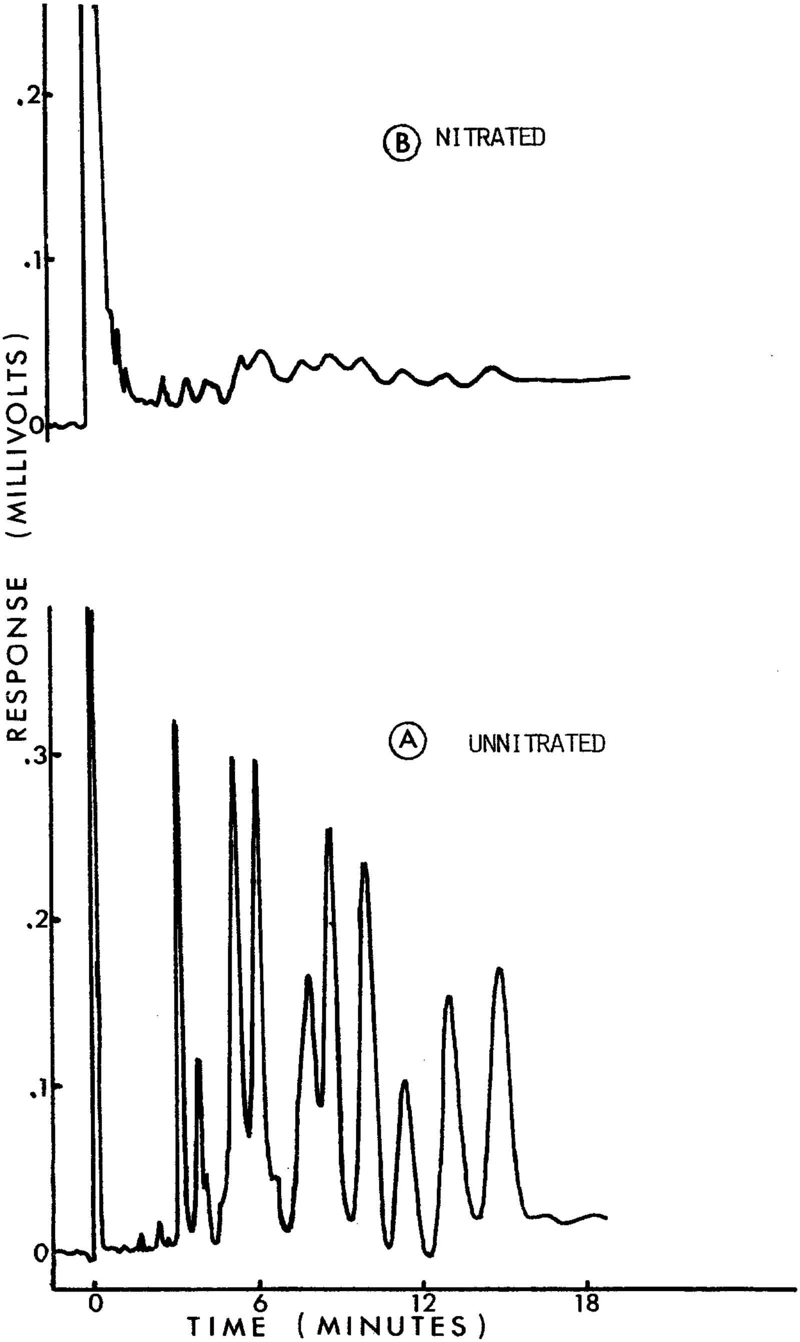

Figure 2. Gas chromatogram of Aroclor 1254 before (A) and after (B) nitration.

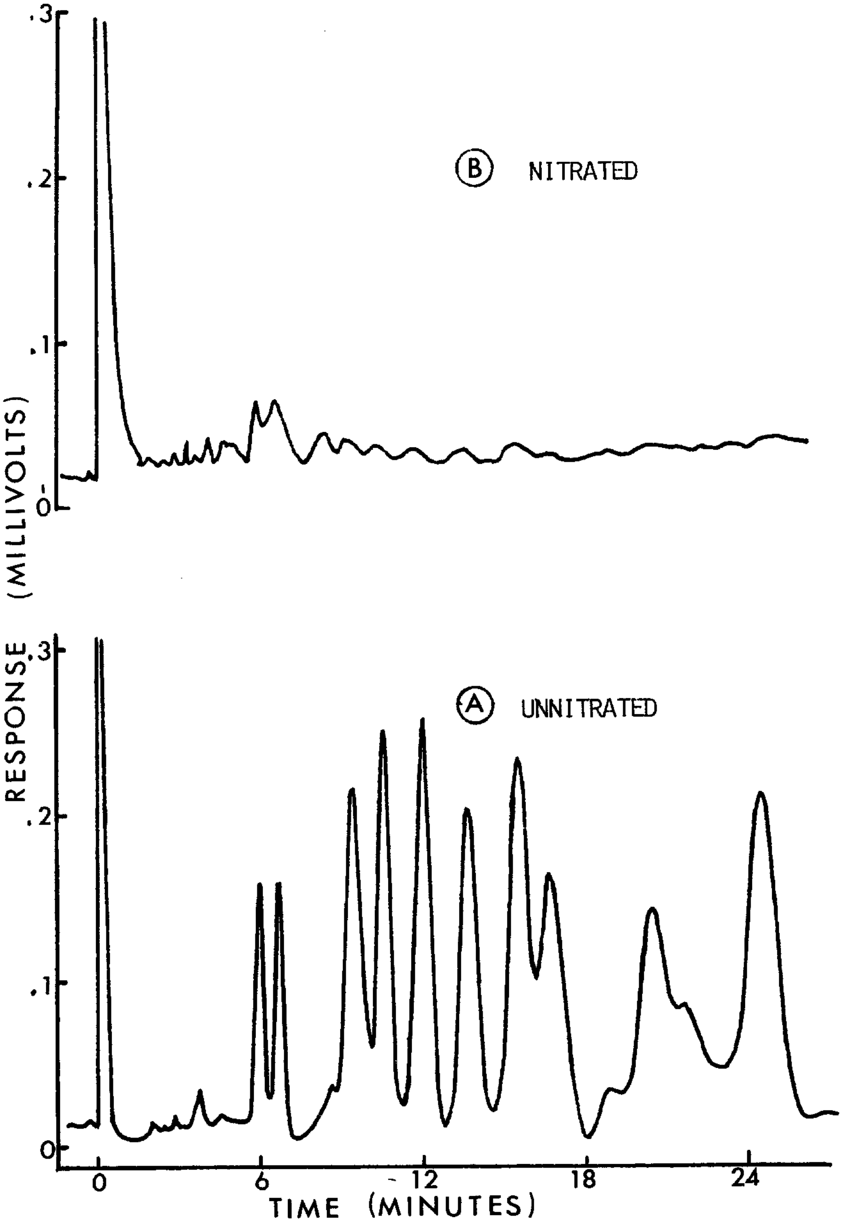

Figure 3. Gas chromatogram of Aroclor 1260 before (A) and after (B) nitration.

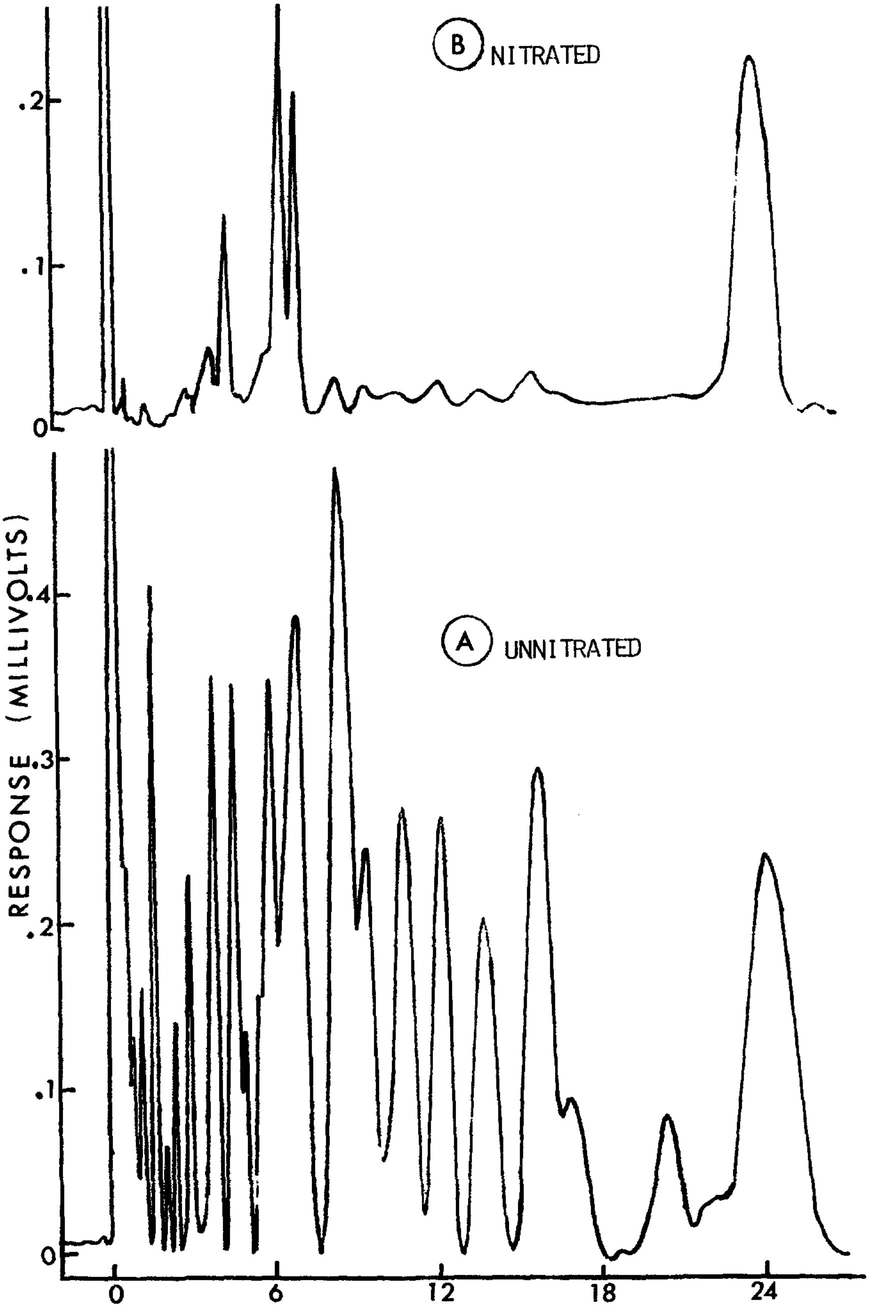

Figure 4. Gas chromatogram of a fish sample fortified with *trans*- and *cis*-chlordane and mirex (1 g fish tissue/ml) before (A) and after (B) nitration.

The method is simple, reasonably rapid and the nitrated extract can be analysed by electron capture, Hall or Coulson GLC detection systems, or by GLC-mass spectrometry without problems associated with interferences.

Lindane remains unnitrated (Fig. 1) but recoveries are erratic. Before lindane is incorporated in this procedure, further investigation is required.

This procedure may be used as a simplified analytical method when only mirex and/or *cis*- and *trans*-chlordane are to be determined.

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