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PROBLEMS OF BACKGROUND CONTAMINATION IN THE ANALYSIS OF OPEN OCEAN BIOTA FOR CHLORINATED HYDROCARBONS

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SUMMARY

Contamination from chemicals, materials and equipment used in the analysis of open ocean samples for chlorinated hydrocarbons in the parts per billion range is a serious problem. Where applicable, heating the materials, equipment and chemicals at 300–350° overnight was effective in removing the contaminants responding to the electron capture detector. With this and other precautions, background contamination can be reduced to an extremely low level and this permits more accurate determination of the chlorinated hydrocarbons in open ocean samples.

INTRODUCTION

With the introduction of tritium and then ^{63}Ni electron capture detectors in gas chromatographs, residue analysts have been pushing the limits of detectability from parts per million (p.p.m.) to parts per billion*** (p.p.b.) level. In the past few years, persistent chlorinated hydrocarbons, mainly DDT, its metabolites and polychlorinated biphenyls (PCBs) have been detected in fish and wild life[1–5]. The distribution of these compounds is world-wide. Thus, the monitoring of these contaminants in open ocean biota is of great importance. From the data gathered, the distribution pattern, transport mechanism and food chain accumulation of these chlorinated hydrocarbons can be formulated.

The residue levels of these persistent chlorinated hydrocarbons in open ocean biota, especially those occupying lower positions in the food chain, are expected to be low, generally in the p.p.b. range. Due to the limitations of sample availability and handling problems, composite samples for analysis are usually in the range of 25–100 g. Thus, sample extracts have to be concentrated to a small volume (often from about 500 ml to less than 5 ml); also the gas chromatograph has to be operated at very high sensitivity in order to detect such low levels of chlorinated hydrocarbons. At these levels, the background contamination from chemicals, materials and equipment used becomes a major problem, especially for the identification and quantification of PCBs,

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*** Throughout this article the American (10^{-9}) p.p.b. is used.

because any extraneous peaks introduced into the chromatogram during the manipulation will interfere with the identification of PCBs, or even give false identification.

This paper describes the solution to some of the problems of background contamination which arise in the analysis of open ocean biota samples. Some of these background contamination problems have been reported in the literature[6,7]; however, to our knowledge, detailed discussions of the problems of low level background contamination, as applied to the analysis of open ocean biota, are scarce.

EXPERIMENTAL

A Tracor gas chromatograph (Model MT 220) equipped with electron capture (^{63}Ni) detector and U-shaped glass column (6 ft. × 1/4 in. O.D.) packed with 5% DC-200 on HP Chromosorb W (80–100 mesh) was used. Nitrogen was used as the carrier gas, at a flow-rate of 60 ml/min. The injector, oven and detector temperatures were 225°, 200°, and 275°, respectively. The operating sensitivity was about 0.05 ng heptachlor epoxide giving 50% full scale deflection (f.s.d.).

In all the following experiments, except where otherwise indicated, duplicate runs were performed. Prior to use, all glassware and other equipment were thoroughly rinsed with nanograde solvents (acetone and petroleum ether, b.p. 30–60°) and the final rinse concentrated to 2–3 ml, of which 10 μl was injected into gas–liquid chromatographic (GLC) column. If any peak greater than 2.5% f.s.d. occurred, the whole rinsing procedure was repeated until the final rinsing concentrate showed no interfering peak.

Solvents

Commercial nanograde solvents for pesticide analysis are generally of sufficient purity for routine pesticide analysis without further purification. However, trace amounts of impurities are still present in these solvents, *e.g.* Mallinckrodt Chemical Company's nanograde solvents were specified at maximum GLC interferences of 10 ng heptachlor epoxide per liter of solvent. A simple redistillation through a fractionating column in an all glass system will not normally remove all the remaining contaminants, for some of the organic contaminants will codistil over with the solvent.

Fig. 1 shows the chromatograms of nanograde petroleum ether which had been doubly-distilled through a 30-cm fractionating column in an all glass system. The solvent thus obtained was of very high purity, and a 200-fold (100 ml to 0.5 ml) concentration did not give any interfering peak at the operating instrumental sensitivity of 0.05 ng heptachlor epoxide for 50% f.s.d. (Fig. 1a). However, very minute traces of interfering organic contaminants were still present. This was evidenced by Fig. 1b, when a 1000-fold (100 ml to 0.1 ml) concentration was reached. The level of contaminants was estimated at 1–2 ng of heptachlor epoxide per liter of solvent. It is obvious therefore that more elaborate solvent purification methods have to be used if a concentration factor greater than 500-fold is required.

Distilled water

In the method of PORTER *et al.*[8] for the analysis of fish tissue for chlorinated pesticide residues, about 900 ml of distilled water is used in the extraction of a sample. The final sample extract is generally concentrated to about 5 ml, giving a concentra-

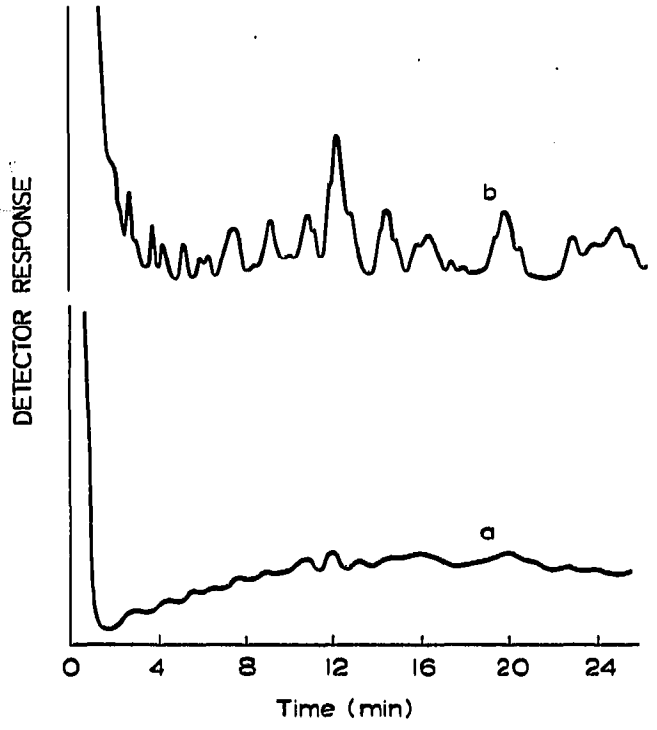


Fig. 1. Gas chromatograms of doubly-distilled petroleum ether. (a) 100 ml concentrated to 0.5 ml, 10 μl injected; (b) 100 ml concentrated to 0.1 ml, 10 μl injected.

tion factor of 180, and the purity of the distilled water used becomes a factor of prime importance. BEVENUE *et al.*[6] have discussed the contamination problems associated with the use of distilled water. We found that multiple distillations (up to four times, with the first 200 ml being discarded each time) through a 30-cm fractionating column in an all glass system did not remove all the contaminants. In order to remove the minute traces of organic contaminants, the water has to be triply-distilled in the presence of potassium permanganate (about 0.1–0.2 g of potassium permanganate for every 3 l of distilled water).

Glassware and Teflon equipment

The contamination problems associated with glassware and Teflon equipment have been discussed in the literature[7]. We found that we could remove most of the contaminants from glassware by washing it thoroughly with detergent and tap water, then rinsing with distilled water and acetone, and finally heating at 200° (method 3 in ref. 7) or 300° (we preferred 300°) overnight. Then, prior to use, we rinsed the glassware two or three times, first with acetone and then with the extraction solvent (petroleum ether or hexane) to remove the last traces of contaminants. Periodic washing of the polytetrafluoroethylene (PTFE) stoppers and stopcocks with chromic acid ensures the removal of organic contaminants from the PTFE surfaces. We also recommend soaking the PTFE material in acetone and then in petroleum ether or hexane for 10 min just prior to use.

Blender

The decontamination of the Waring stainless-steel high-speed blender posed

some problems. Due to the delicate fluorosilicone or PTFE gaskets the blender could not be heat-treated. Repeated washings with detergent and water could not generally remove the last traces of contaminants. However, we found that treatment of the blender with 100-ml portions of solvents (first acetone, then extraction solvent) at high speed several times (for about 1–2 min each time) effectively removed the last traces of contaminants.

Florisil

Florisil gave several early emerging peaks in the chromatogram that would interfere with the identification of PCBs, especially PCBs with low chlorine content, *e.g.* Aroclor 1242 and 1248. The contaminants could be removed by heating the Florisil at 300–350° for several hours or by washing the Florisil with 150 ml of petroleum ether prior to use. Fig. 2 shows the Florisil contaminants present in the 150-ml petroleum ether wash of 30 g of Florisil. No extraneous peak was observed after the 150-ml petroleum ether elution.

Sodium sulfate and sodium chloride

Reagent grade sodium sulfate and sodium chloride contain some volatile electron capturing contaminants. 30 g of reagent grade sodium sulfate (or sodium chloride) was extracted with 100 ml of petroleum ether in a 250-ml mixing cylinder

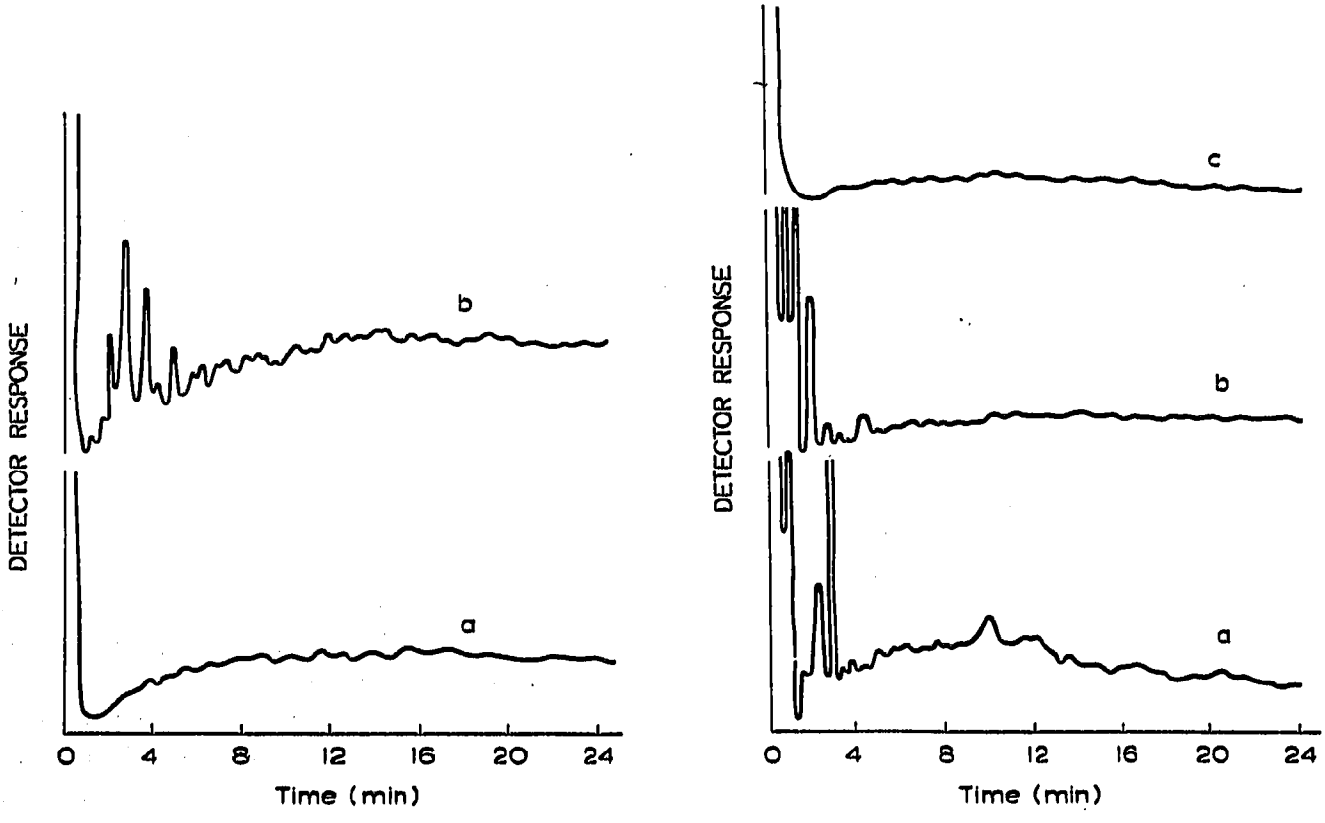


Fig. 2. (a) Gas chromatogram of petroleum ether extract of heat-treated Florisil; (b) gas chromatogram of impurities in Florisil.

Fig. 3. Gas chromatograms of petroleum ether extracts of sodium sulfate and sodium chloride. (a) Original sodium sulfate; (b) original sodium chloride; (c) heat-treated sodium sulfate.

for 1 h (with intermittent shaking). The petroleum ether extract was then concentrated to 4 ml, of which 10 μl was injected into the column. The resulting chromatogram showed several interfering peaks, as shown in Fig. 3a (or 3b). All of the contaminants in the petroleum ether extract could be removed by elution through a Florisil column (gas chromatogram not shown, but similar to Fig. 3c). Another procedure used to remove contaminants involved the heating of sodium sulfate (or sodium chloride) in a 300–350° oven overnight, prior to use. Fig. 3c shows the petroleum ether extract of 30 g of heat-treated sodium sulfate. No interfering peak was observed.

Sharkskin filter paper

Three different boxes of sharkskin papers were examined. Four pieces of filter paper were randomly selected from each box, and separately extracted with 100 ml of petroleum ether in 250-ml mixing cylinders with intermittent shaking for 1 h. Each extract was then concentrated to 4 ml, of which 10 μl was injected into the GLC column. All filter papers from the three boxes examined contained electron capturing contaminants. However, the composition and the level of contaminants seemed to vary from box to box. Thus the filter papers from one box gave only four minor interfering peaks, while filter papers from another box had a complex array of contaminants, as shown in Fig. 4a. Unlike sodium sulfate and sodium chloride, some of the contaminants in filter paper could not be removed by a Florisil column. Fig. 4b shows the chromatogram of a 6% diethyl ether in petroleum ether eluate from the Florisil column of the filter paper extract of Fig. 4a.

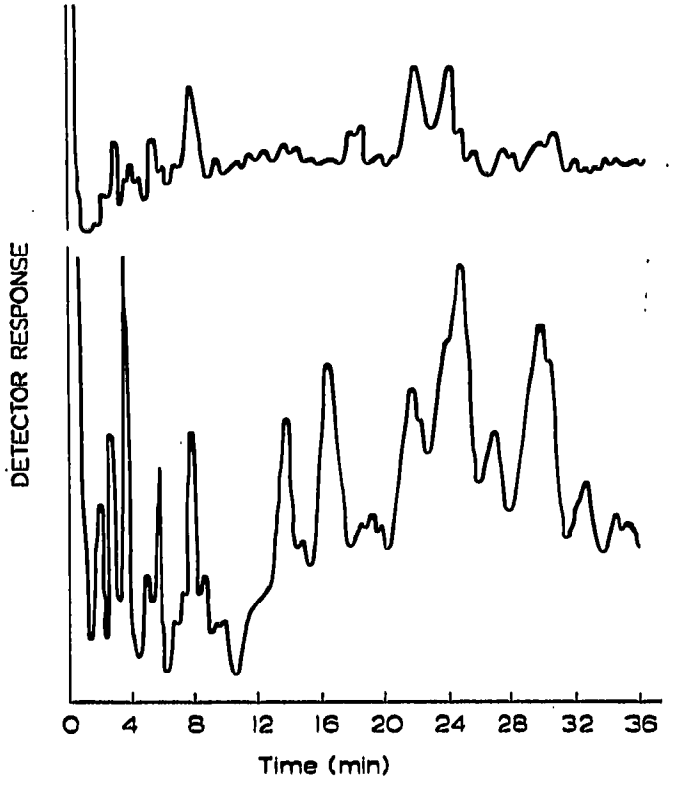


Fig. 4. Gas chromatograms of petroleum ether extracts of sharkskin papers. (a) Original petroleum ether extract concentrate; (b) 6% diethyl ether in petroleum ether eluate from Florisil column of extract concentrate from (a).

Most of the contaminants in the filter paper could be eliminated by extraction with petroleum ether or hexane. This could be done with a Soxhlet extractor or by soaking the filter papers in the solvent in a beaker for 3–4 h, and changing the solvent every hour.

Glasswool

Glasswool is used to plug the end of the Florisil and anhydrous sodium sulfate columns[8]. On some occasions it is substituted for sharkskin paper in the filtration process. An amount of glasswool equivalent to that normally used in analyzing a sample was extracted with 100 ml of petroleum ether in a 250-ml mixing cylinder and then concentrated to 4 ml, of which 10 μl was injected into the GLC column. A huge peak and several smaller peaks were observed in the resulting chromatogram (Fig. 5).

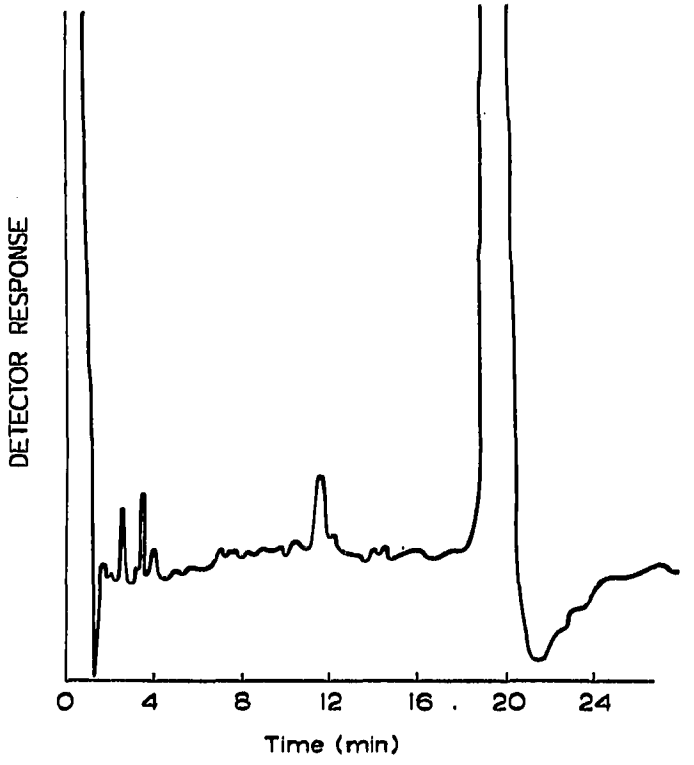


Fig. 5. Gas chromatogram of petroleum ether extract of untreated glasswool.

Heating the glasswool at 300–350° overnight was effective in removing all the electron capturing contaminants. The contaminants were also removed by passing the petroleum ether extract of the glasswool through a Florisil column.

Aluminum foil

Aluminum foil is used to line the caps of glass jars for storing samples. If a rubber stopper is used for the buchner funnel in the filtration process, it is also advisable to wrap the rubber stopper in aluminum foil, so as not to let the rubber stopper come in contact with the neck of the filtration flask or the organic solvent.

Three different boxes of aluminum foil were examined for electron capturing

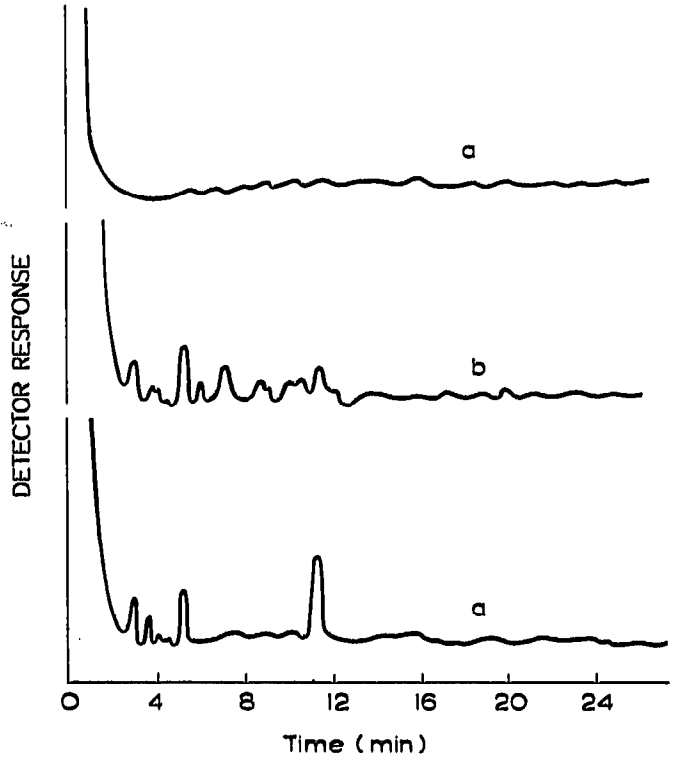


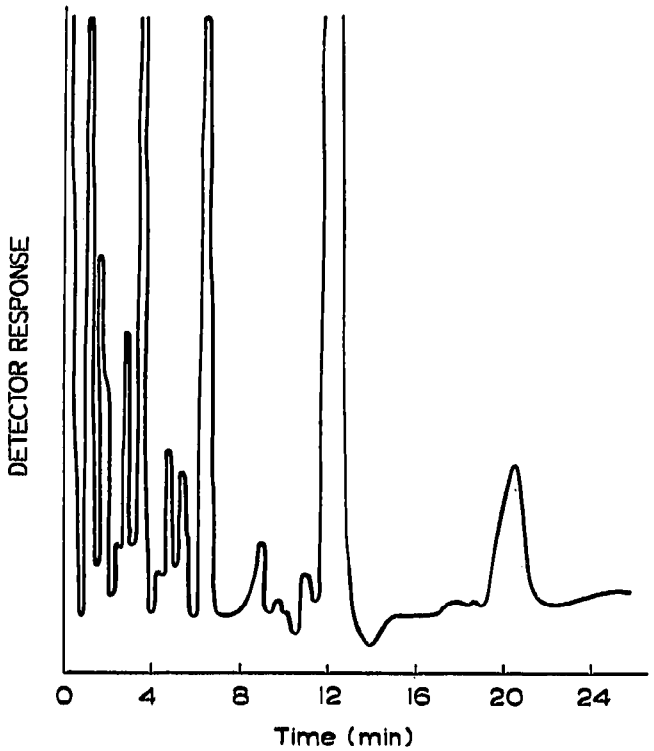
Fig. 6. Gas chromatograms of petroleum ether extracts of aluminum foil. (a) Original aluminum foil from box A; (b) original aluminum foil from box B; (c) heat treated aluminum foil, boxes A and B combined.

contaminants. A rectangular piece of approximately 5 in. × 7 in. was taken from each box and separately extracted with 100 ml of petroleum ether in a 250-ml mixing cylinder for about 1 h. Each of the extracts was then concentrated to 4 ml and 10 μl of this concentrate was injected into the column. As in the case of the filter papers, the composition and level of contaminants of the aluminum foil from each of the three boxes were different. However, only minor peaks were observed in all three cases. Two of the chromatograms are shown in Fig. 6 (a and b). Heating the aluminum foil in a 300–350° oven overnight effectively removed all the contaminants. Fig. 6c shows the petroleum ether extract of heat-treated aluminum foil.

PTFE–rubber laminated discs

The concentrated extract of a sample is usually stored in a 8–10 ml vial with the screw cap lined with a PTFE–rubber laminated disc (PTFE side facing the solution). The disc minimizes evaporation loss and thus the sample extract can be kept for some time without appreciable change in concentration. The PTFE surface is generally regarded as inert, and does not have any contaminant. However, caution is also needed in using the PTFE–rubber laminated discs.

We tested thirty of the PTFE–rubber laminated discs. The glass vials were first thoroughly washed with detergent, then chromic acid, tap water, and then rinsed with distilled water and heated overnight at 200°. The vials were then equipped with screw caps lined with PTFE–rubber laminated discs, and rinsed first with acetone and then twice with petroleum ether (by shaking about 4 ml of solvent in the vial for 1 min).



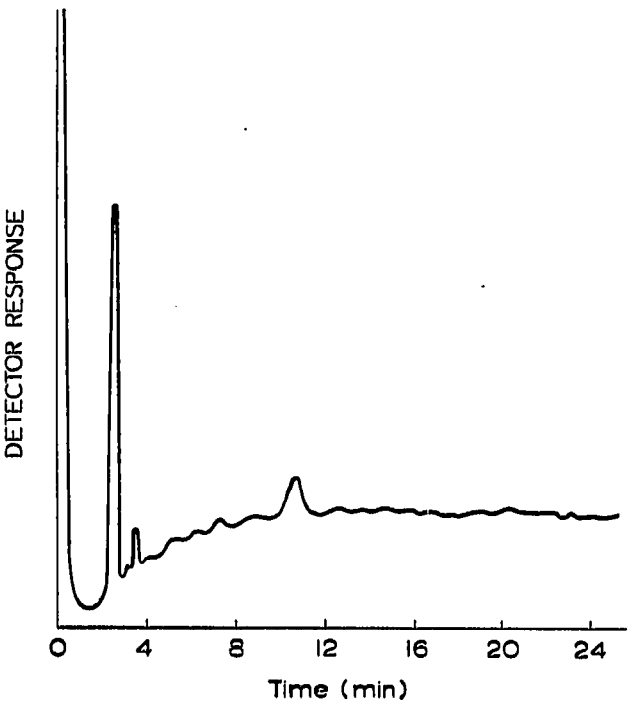


Fig. 7. Gas chromatogram of electron capturing contaminants from PTFE–rubber laminated disc.

Fig. 8. Gas chromatogram of electron capturing contaminants from screw cap with paper liner.

Then the vials were filled 3/4 full (about 6 ml) with petroleum ether. After two days 10 μl of the solvent in each vial were separately injected into the GLC column. No peak was detected in the solvent from any of the vials. The injections were repeated after six weeks. No peak was detected in the solvent from most of the vials. However, the solvent from two vials exhibited sizable amounts of contaminants, one was especially high, as shown in Fig. 7. The contaminants were not from the screw caps, as evidenced by observations from two control vials. In the two control vials, the original screw caps with paper liner were used. After six weeks, identical chromatograms were obtained from the solvents in the two vials. Only three peaks were observed (Fig. 8), indicating that the peaks originated from the cap.

The two PTFE–rubber laminated discs that gave off the electron capturing substances were observed to have some pinkish colorations on the PTFE sides. The contaminants may be from the PTFE, or it may be that there are some cracks in the PTFE layer, and that solvent vapor was able to penetrate through the PTFE and extract the electron capturing substances from the rubber. Although the majority of the PTFE–rubber laminated discs were good, the possible presence of a few defective discs would warrant some caution in their use. The problem could be minimized by running GLC measurements a few days after extraction of the sample.

RESULTS AND DISCUSSION

In the analysis of persistent chlorinated hydrocarbons (DDT, its metabolites

and PCBs) in open ocean biota, all the equipment and chemicals used are potential sources of contamination. If not properly eliminated or minimized, contaminants from these sources can contribute to false identification of certain chlorinated hydrocarbons, or the values obtained are higher than the actual residue levels. Such errors could lead to false alarms or conclusions. On the other hand, the low level of chlorinated hydrocarbons in open ocean biota should be accurately determined so that meaningful environmental control or improvement measures can be formulated. To this end, the analyst must be aware of the many problems associated with the analysis, the limitations imposed by the analytical method, and the meaningful detectability limits for the samples.

It is expected that the type and level of electron capturing contaminants in a given chemical (or material) will be different from batch to batch or from different

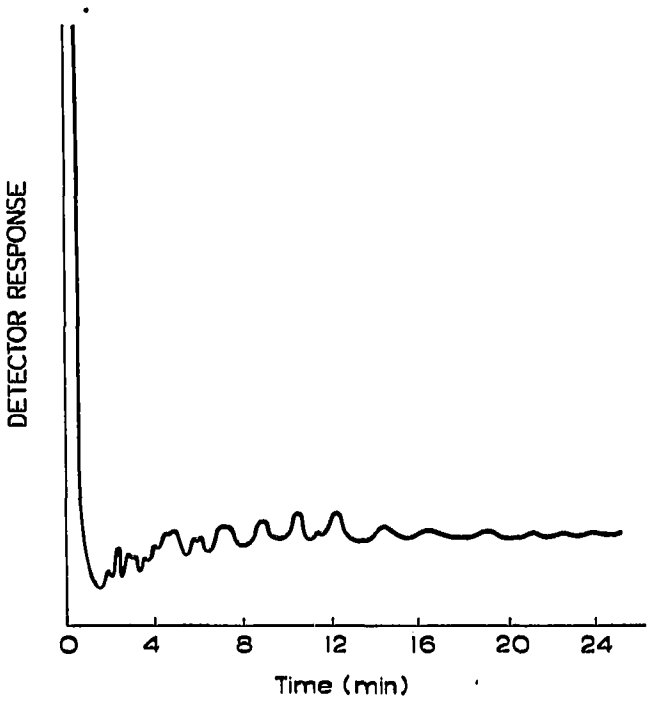


Fig. 9. Gas chromatogram of a blank run extract. 6% diethyl ether in petroleum ether eluate, concentrated to 5 ml, 10 μl injected.

sources. Although practically all the contaminants in the chemicals or materials can be removed by solvent extraction, we found that if the substance could be heated, heating in a 300–350° oven overnight was the most economical and convenient method.

Applying the precaution and the clean-up methods outlined in the EXPERIMENTAL section, background contamination can be reduced to very low levels. Fig. 9 shows the chromatogram of a typical blank run using the method of PORTER *et al.*[8] for the analysis of fish tissue. The background level of DDTs and PCBs for this particular blank run, based on a 50-g sample weight, is estimated at less than 0.05 p.p.b. for *p,p'*-DDE, less than 0.1 p.p.b. for *p,p'*-DDD and *p,p'*-DDT, and less than 1 p.p.b. for PCBs.

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