

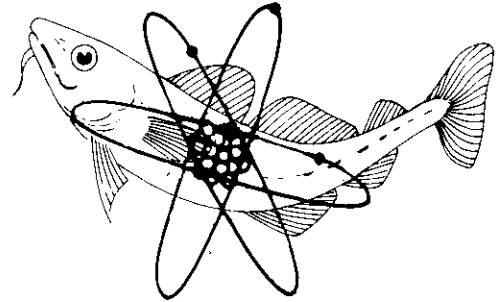
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MINISTRY OF AGRICULTURE FISHERIES AND FOOD  
DIRECTORATE OF FISHERIES RESEARCH

**AQUATIC ENVIRONMENT PROTECTION:  
ANALYTICAL METHODS**



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Methods of analysis for chlorinated  
hydrocarbons in marine and other samples

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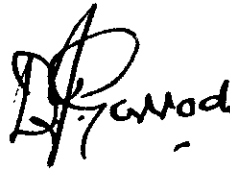
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## Foreword

As part of its responsibilities under various Acts of Parliament, MAFF Directorate of Fisheries Research has a duty to carry out a substantial programme of monitoring, surveillance and research in relation to the quality of the aquatic environment in and around the United Kingdom. In the course of that programme, a wide variety of methods of analysis are used for a wide variety of contaminants, both inorganic and organic, stable and radioactive. This series of publications describes the main methods used in the course of this work and parallels the existing Aquatic Environment Monitoring Report series, in which much of the resulting data is published. Regardless of whether the analytical procedure relates to a radionuclide or a non-radioactive contaminant, each report contains a step-by-step guide to analytical procedures and an explanation of the calculation of results.

A handwritten signature in black ink, appearing to read 'D. J. Garrod', is centered on the page. The signature is stylized and somewhat cursive.

D. J. Garrod

Director of Fisheries Research  
Ministry of Agriculture, Fisheries and Food

## Background

The Burnham-on-Crouch Laboratory of the Directorate of Fisheries Research (DFR) of the Ministry of Agriculture, Fisheries and Food has well-equipped analytical chemistry facilities and is called upon to provide a comprehensive range of analytical services. Although some of the requirements arise from customers elsewhere in the DFR, these are usually single or small-volume requests. The vast majority of the analyses undertaken stem from programmes generated in-house by DFR's Aquatic Environment Protection Division 2, which is responsible for assessing the distribution and the impact, or potential impact, of many types of discharge other than radioactive discharges, on both marine and freshwater fisheries. Some of this work arises from the Division's special responsibility for the licensing, enforcing and monitoring of the impact of wastes dumped at sea. In addition, there is a major programme of general environmental quality monitoring which is used to back-up pollution prevention and environmental protection activities.

Over the years, analyses have been conducted on a wide variety of sample types, both freshwater and marine, environmental and non-environmental (effluents, mine tailings, drill-muds, dredgings) for a large range of contaminants. Many of the analyses are of a routine or semi-routine nature and set procedures have been developed for their conduct. These procedures have been thoroughly tested and proven by the DFR and in most cases have been subjected to intercomparison tests with other laboratories and to quality assurance schemes involving the use of standards and reference materials.

It is believed that other laboratories may find details of the methods used at the Burnham-on-Crouch Laboratory of interest. Consequently, a suite of publications has been prepared. The present publication relates to methods for analysis of pesticides. Others in the series give details of the methods used for the determination of hydrocarbons, metals and organotin compounds. Each publication gives details of the procedures followed for the analyses of a wide variety of sample types. Although fairly complete details are provided, the intention of the publication is to inform other analysts of the general procedures, apparatus and analytical instrumentation required. If difficulties in applying the methods are encountered, contact should be made with the leading author from whom further details can be obtained.

Generally, details are provided of any special sampling or sample preservation procedures. However, where the necessary steps have been laid down by international agreement and apply in common to all of the determinands, details are not provided in the individual method section. This applies particularly to the analyses of fish and shellfish for either temporal or spatial trend purposes or for human health risk assessment purposes. For these objectives, the International Council for the Exploration of the Sea (ICES) has laid down guidelines and these are reproduced in Appendix 1.

## 1. Introduction

Interest in organochlorine pesticides at the Burnham-on-Crouch Laboratory historically focused on the residue levels found in commercially-exploited marine species. Consequently, the methods have been designed primarily for the analysis of fish and shellfish tissues. They have, however, been used on a variety of other sample types including water, sediments, sewage sludge and marine mammalian tissues.

Two basic methods are described here. The first was routinely used by the Laboratory for a number of years prior to June 1987. Since June 1987, as a result of the need for additional determinands to be incorporated into the schema, a second, similar, method was developed. The methods differ only in the alumina "clean-up" and silica fractionation stages and full details are provided below. For clarification, the two methods have been designated as Method A and Method B. It has been decided to include both methods in this paper, because Method A is still valid for those analyses with a more restricted determinand list or of a simpler sample matrix.

As described here, the methods are suitable for the determination of the following compounds: alpha-, beta- and gamma-HCH; chlorinated benzenes; dieldrin; pp'DDT; pp'TDE; pp'DDE and chlorobiphenyls. The methods need some modification for the analysis of sewage sludge and sediments. Details of these modifications are detailed in the appropriate sections as additions to the description of the procedure used for fish and shellfish.

The methods have also been used for the analysis of cis- and trans-permethrin, chlorinated nitrobenzenes, Endrin, heptachlor and heptachlor epoxide, cis- and trans-nonachlor, oxychlordane and hexachlorobutadiene. They can be used with some modification for a wide range of non-polar compounds, but for compounds other than those specifically mentioned it is up to the analyst to evaluate the methods to assess their suitability for a particular sample type and determinand.

The methods have been designed to be used with high-resolution capillary columns. They are not recommended for use with packed columns, because the inferior resolution of such columns may not be adequate for some of the more complex fractions.

## 2. General procedure

The procedure used has six stages. These are outlined briefly below, but more detail on each stage is provided in the subsequent Sub-sections.

The organochlorine pesticides (OCP's) and chlorinated biphenyls (CB's) are extracted into n-hexane from a tissue homogenate using a Soxhlet apparatus (Sub-section 2.1).

The percentage concentration of n-hexane extractable lipid is determined (Sub-section 2.2).

Lipids and other co-extracted materials are removed from the n-hexane extract using column chromatography with alumina as an absorbent and, in the case of Method B, some degree of class fractionation is also achieved (Sub-section 2.3).

Group separation is achieved by further column chromatography on silica gel (Sub-section 2.4).

Residues are quantified using capillary gas chromatography with electron capture detection (Sub-section 2.5).

Residues are confirmed using a separate capillary column with a different liquid phase, or by GC-MS or wet chemical methods (Sub-section 2.6).

## 2.1 Extraction

Samples of homogenised fish tissue (10 g muscle or 5 g liver) weighed into clean 60 ml Beatson jars are mixed with sufficient anhydrous sodium sulphate to produce a free-flowing mixture and stored in a freezer until required. Although the samples can be extracted as soon as they have been "sulphated" it is preferable to store them at least overnight to ensure that the tissues are completely dehydrated.

### 2.1.1 Biological material

The sulphated and dehydrated tissue samples are transferred to glass Soxhlet extraction thimbles into which has been added a layer of sodium sulphate (1 cm) to ensure that the n-hexane extract remains dry. The samples are then exhaustively extracted with approximately 120 ml of n-hexane, for a minimum of 4 h at roughly 20 cycles per hour. Once cool, each sample extract is quantitatively transferred to a 100 ml volumetric flask and the volume adjusted to exactly 100 ml.

### 2.1.2 Sediments and sewage sludges

After collection, sediment samples are stored in 500 ml Beatson jars and frozen until needed. After being allowed to come to room temperature, the samples are mixed well and 50 g subsamples are weighed into glass Petri dishes and air dried to constant weight. Each sample is then ground with 25 g of granular anhydrous sodium sulphate and stored in a 60 ml Beatson jar and allowed to stand overnight. The addition of sodium sulphate, in this instance, aids the passage of solvent through the sample during the extraction stage, as well as ensuring that the sample remains dry. The sediment samples are then transferred to glass extraction thimbles in a similar way to the fish tissue samples.

For samples of sediment and sewage sludge, the extraction time should be increased to ensure complete recovery of any residues present. The need for the increase in extraction time is because the residues are more tightly bound to the smaller-sized particles of most sediments and sewage sludges and the normal extraction time is thus less efficient. It has been found in extreme cases, when extracting sediment fines, that an extraction period of 24 h may be necessary for complete recovery of all of the residues. The addition of some copper turnings to the extraction flask (approximately 5 g, but variable depending on the sample matrix) will remove any elemental sulphur often present in marine sediments. If

the copper turns completely black during the extraction period, the process can be halted and more copper should be added.

## 2.2 Lipid determination

The total n-hexane-extractable lipids are determined gravimetrically after evaporation of the solvent. Fifty-millilitre aliquots of a sample are transferred to pre-weighed 100 ml beakers and the solvent is evaporated on a steambath in a fumehood. Once the solvent has been evaporated, the beakers are further dried to constant weight in an oven at 105°C. The beakers are then reweighed and the lipid concentrations determined as percentages of the original tissue sample weights.

The lipid concentrations are determined for two reasons. The first is to enable the final results to be expressed on a lipid weight basis, if desired. The second stems from the fact that the alumina micro-columns used for the "clean-up" have a finite capacity for the removal of lipids; if this capacity is exceeded, the resolution of the columns will be lost, with consequent inadequate "clean-up" of the samples. It is therefore essential that the concentrations of lipids in the sample are determined.

For the purpose of expressing results, lipid determination is normally only carried out on samples of biota. However, for the secondary reason given above it is also advisable to perform lipid determination on sewage sludges, because they often contain significant levels of fat which could interfere with the "clean-up" stage.

It should be noted that the term "lipid", as used in this paper, refers to that fraction of lipid which is hexane extractable, and it is accepted that this may not represent the "total lipid" concentration.

## 2.3 Lipid removal "clean-up"

### 2.3.1 Preparation of alumina - Methods A and B

The alumina and level of deactivation is the same for Methods A and B. The alumina columns used have capacities of approximately 60 mg of lipid. If it is necessary to remove more than that amount, the samples can either be "cleaned-up" twice, on two separate alumina columns, or a supplementary 1 g alumina column can be used. The alumina used is a neutral aluminium oxide (Merck 1077 70-230 mesh ASTM). It is prepared for use by firing in a muffle furnace in a quartz dish at 800°C for 4 h. This has the effect of removing any lightweight organic compounds which may interfere with the analysis at a later stage, and also serves to completely dehydrate (activate) the alumina. The activated alumina is transferred to a desiccator and allowed to come to room temperature. To deactivate the alumina to the 5% level, 95 g of the activated alumina are weighed into a round-bottomed flask and 5 g of double distilled water (that has previously been extracted with an equal volume of n-hexane) is added dropwise with a Pasteur pipette. The flask is then stoppered and shaken on a standard laboratory shaker for 30 min, its weight recorded and the flask and alumina stored in a desiccator cupboard. Each batch of alumina is used or discarded within seven days of its preparation.

### 2.3.2 Alumina fractionation "clean-up" - Method A

Note: The compositions of all of the various fractions are detailed in Table 1.

To clean up a sample, borosilicate glass chromatography columns (150 mm x 6 mm i.d.) with solvent reservoirs at the top and 2 mm bore tips are each plugged with n-hexane-washed cotton wool and 3 g of 5% deactivated alumina is poured in (the columns are dry packed) and the sides gently tapped to settle the alumina. The alumina is topped with a small quantity of anhydrous sodium sulphate to ensure that the sample is completely dry and to maintain the correct level of deactivation of the alumina. A suitable aliquot (based on its lipid content and expected OCP and CB content) of a sample extract is evaporated to 1 ml under a stream of dry air or oxygen-free nitrogen, in a dri-block evaporator heated at 40°C and then quantitatively transferred to the top of the alumina column with a Pasteur pipette. The sample is allowed to just drain into the bed of the column, 20 ml of n-hexane is added to the solvent reservoir and the first 16 ml of n-hexane from the column is collected in a graduated centrifuge tube. Provided that the lipid-holding capacity has not been exceeded, this 16 ml of n-hexane has been shown experimentally to contain all of the compounds of interest but none of the unwanted lipid. Once the eluant from the column has been collected, it is evaporated back to 1 ml and is then further fractionated on silica gel.

### 2.3.3 Alumina fractionation "clean-up" - Method B

The alumina columns are prepared and the sample transferred to the column as in Method A. However, whereas in Method A a single 16 ml fraction is collected, in Method B a total of three fractions can be collected. These fractions are nominated as AF1, AF2 and AF3. AF1 is made up of 2 ml of n-hexane and AF2 consists of 10 ml of n-hexane. After the collection of AF2, any n-hexane remaining in the solvent reservoir is removed with the aid of a Pasteur pipette and replaced with approximately 10 ml of 20% diethyl ether in n-hexane and a third fraction of 6 ml is collected (AF3). Fraction AF1 is evaporated back down to 1 ml for further fractionation on silica gel. Fraction AF2 is retained to be combined with a silica fraction (SF2) and AF3 is reduced to near dryness, to remove the ether and taken back up in n-hexane to a final volume of 1 ml.

## 2.4 Silica gel fractionation

### 2.4.1 Method A

Columns, similar to those used for the alumina stage, are also used for this stage. Silica gel is used (Merck 7734 70-230 ASTM) that has been activated to 5% in a similar fashion to the alumina, after firing at 600°C for 4 h. The columns are made-up of 2 g of silica and topped with sodium sulphate, as with the alumina columns; the silica columns are also packed dry. The 1 ml sample of extract from the alumina column is quantitatively transferred to the silica column and allowed to drain into the bed of the column. The top of the column should not be allowed to dry out. Approximately 20 ml of n-hexane containing 2% tetrahydrofuran is added to the solvent reservoir and the eluant is collected in graduated centrifuge tubes. Two fractions are collected: the first fraction (SF1 4.5-5.5 ml) contains hexachlorobenzene, pp'DDE and the CB's; the

Table 1 Composition of various fractions from alumina and silica column chromatography

| Method | Fraction                                    | Components   |
|--------|---|--|
| A      | AF1 (0-16 ml)<br>(n-hexane)                 | Alpha-, beta- and gamma-HCH<br>Chlorobiphenyls<br>pp'DDE, pp'TDE and pp'DDT<br>Dieldrin<br>Hexachlorobenzene |
| "      | SF1 (4.5-5.5 ml)<br>(2% THF in<br>n-hexane) | Chlorobiphenyls<br>Hexachlorobenzene<br>pp'DDE   |
| "      | SF2 (8.5-9.5 ml)<br>(2% THF in<br>n-hexane) | Alpha-, beta- and gamma-HCH<br>pp'TDE and pp'DDT<br>Dieldrin   |
| B      | AF1 (0-2 ml)<br>(n-hexane)                  | Chlorobiphenyls<br>Chlorobenzenes<br>pp'DDT<br>pp'DDE  |
| "      | AF2 (2-12 ml)<br>(n-hexane)                 | Alpha-, beta- and gamma-HCH<br>Endrin<br>Dieldrin<br>pp'TDE<br>Trans-permethrin                              |
| "      | AF3 (0-6 ml)<br>(20% ether<br>in n-hexane)  | Cis-permethrin<br>Cypermethrin<br>Deltamethrin   |
| "      | SF1 (0-7 ml)<br>(n-hexane)                  | Chlorobiphenyls<br>Chlorobenzenes  |
| "      | SF2 (8-24 ml)<br>(n-hexane)                 | pp'DDT   |

second fraction (SF2 8.5-9.5 ml) contains alpha-HCH, beta-HCH, gamma-HCH and pp'TDE and pp'DDT. After silica fractionation, the separate eluants are reduced back to 1 ml and a suitable internal standard is added. Various internal standards can be used; this laboratory commonly uses Mirex and 1,2,3,4-tetrachloronaphthalene. Because of the wide range of sample types and determinand concentrations found, it is not always possible to use a set concentration of internal standard. However, the addition of internal standard to give a final concentration of 50  $\mu\text{g } \mu\text{l}^{-1}$  is often satisfactory.

#### 2.4.2 Method B

For Method B, the silica gel is prepared as in Method A, except that it is deactivated to the 3% level and a total of 3 g of silica is used in 300 mm x 6 mm i.d. glass columns. Instead of using the 2% tetrahydrofuran, as in Method A, 100% n-hexane is used. Two fractions are

collected, the first fraction (SF1) is made up of 7 ml and the second fraction (SF2) consists of 16 ml. SF2 and AF2 are combined, then reduced to 1 ml after the addition of an internal standard; SF1 is also reduced to 1 ml after addition of an internal standard.

Method B silica fractionation has the advantage over Method A in using only n-hexane as the eluting solvent and, although this requires greater elution volumes, the pp'DDT split from the CB's has been found to be easier to achieve.

The precise volumes of the individual fractions for Methods A and B can vary from batch to batch of silica gel and alumina. Accordingly, the volumes which are used must be determined for each batch by subjecting a prepared standard solution to fractionation, followed by analyses of the fractions to determine the best "splitting" points.

## 2.5 Gas liquid chromatography and quantification

Several different gas chromatographs and capillary columns are used in this laboratory, depending upon the precise analysis being undertaken. All columns which are used are of the chemically-bonded type and have shown themselves to be ideally suited to routine pesticide analysis. The following lists of chromatographic instrumentation, column specifications and operating conditions are used at the Burnham-on-Crouch Laboratory, but it should be remembered that precise operating conditions will vary according to the particular characteristics of each individual capillary column and its precise application.

### Instrumentation:

Hewlett Packard Models: HP5880, HP5890, HP5700 gas chromatographs  
HP7671, HP7672, HP7673 automatic samplers  
HP3357 Laboratory Automation System (for the control of the automatic samplers and data acquisition and reduction)

|                        |  |                |
|------------------------|--|----------------|
| Column specifications: | 25 m x 0.31 mm HP-5 0.17 $\mu\text{m}$     | film thickness |
|                        | 25 m x 0.31 mm HP-1 "                      | " "            |
|                        | 25 m x 0.2 mm HP-5 0.33 $\mu\text{m}$      | " "            |
|                        | 25 m x 0.2 mm HP-1 "                       | " "            |
|                        | 50 m x 0.2 mm HP-5 0.5 $\mu\text{m}$       | " "            |
|                        | 25 m x 0.25 mm CP-Sil-19 0.2 $\mu\text{m}$ | " "            |
|                        | 50 m x 0.25 mm CP-Sil-19 0.2 $\mu\text{m}$ | " "            |

Operating conditions: As previously stated, the exact conditions will of course vary, depending upon the precise application. However, certain conditions are common to most analyses.

- a) The detector auxiliary gas is 5% methane in argon, used at a flow-rate of 50-60 ml min<sup>-1</sup>. The electron capture detectors are held at a temperature of 300°C.
- b) The injection port temperatures are held at 250°C. Injection is in the splitless mode with the split valve being opened 1-2 min into the run. The injection volume is 1-2  $\mu\text{l}$ .
- c) High-purity hydrogen is used as the carrier gas, the exact flow being dependent on the type of analysis (typically 2.5-5 ml min<sup>-1</sup>).

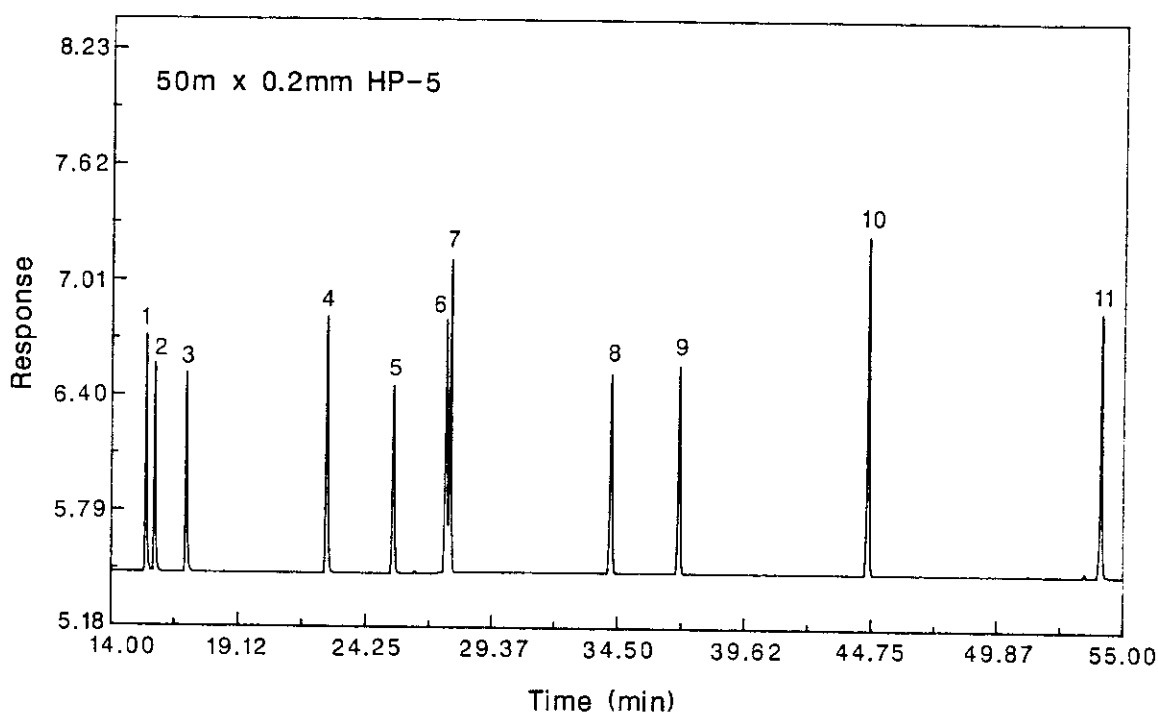
d) The oven temperature programmes vary, but two common programmes are as follows:

Inject at 60°C, hold for 1 min, programme at 30°C min<sup>-1</sup> to 150°C, then at 2.5°C min<sup>-1</sup> to 285°C, then hold for 15 min.

Inject at 90°C, hold for 1 min, then programme at 2.5°C min<sup>-1</sup> to 300°C, and hold for 15 min.

For routine analysis of pesticides, the 25 m columns are adequate, but for really high-resolution work, such as on individual CB's, then the 50 m columns are used. Examples of chromatograms are reproduced in Figures 1-4.

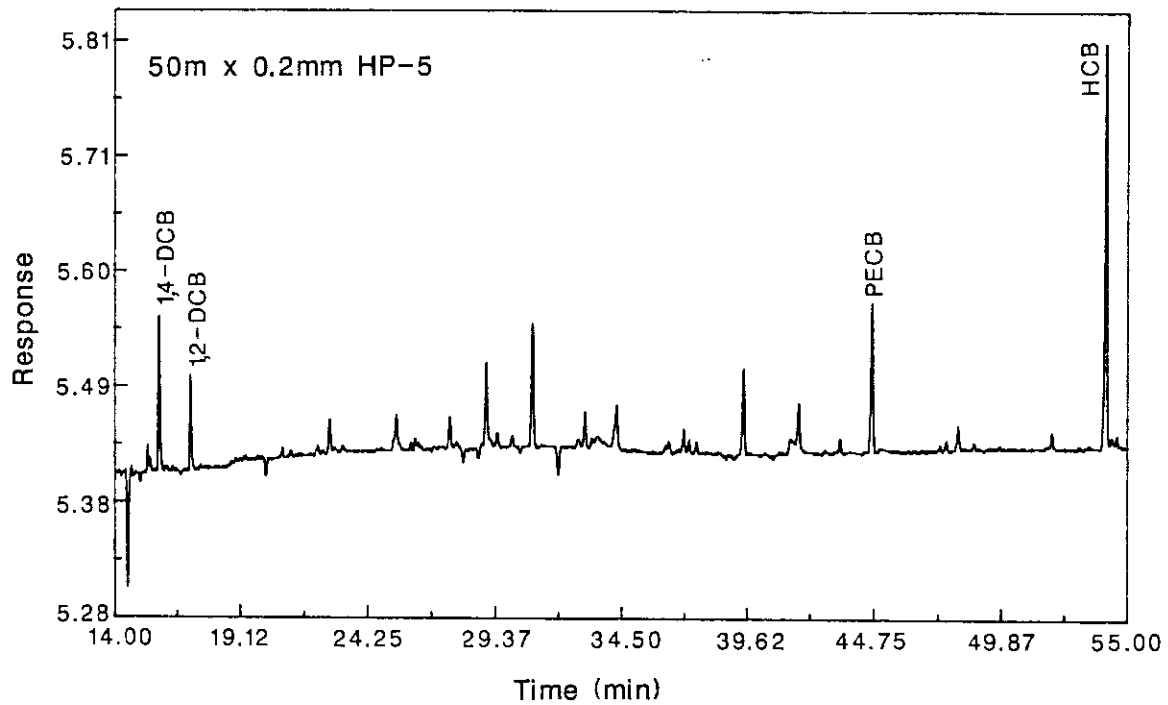
Modern gas chromatographs, especially when used in conjunction with capillary columns, are capable of producing large volumes of raw analytical data. This is particularly so when environmental samples are being analysed which, because of their very nature, even after the most efficient "clean-up" procedures, may still produce complex chromatograms. Most modern chromatographic integrators and microcomputing systems,



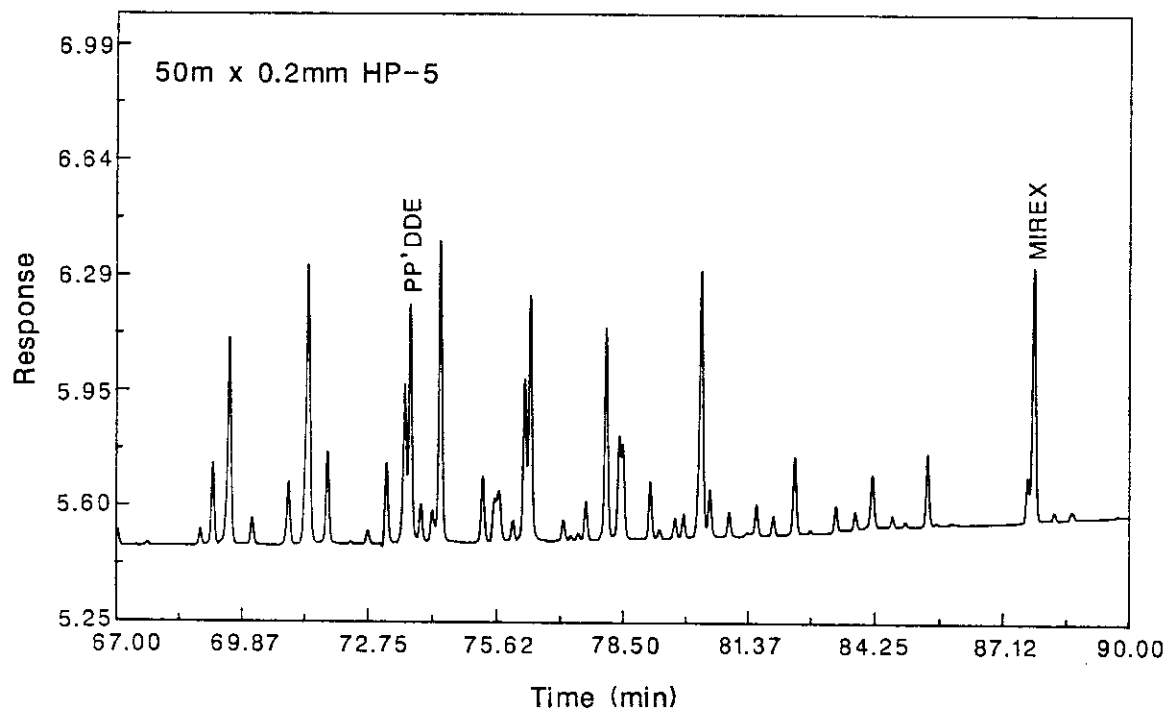
**Figure 1** Chlorobenzene standard including hexachloro-1,3-butadiene. Chromatographic instrumentation, column specifications and operating conditions are as follows: oven programme 60°C (1 min) then at 2.5°C min<sup>-1</sup> to 300°C.

Peak identification (in order of elution):

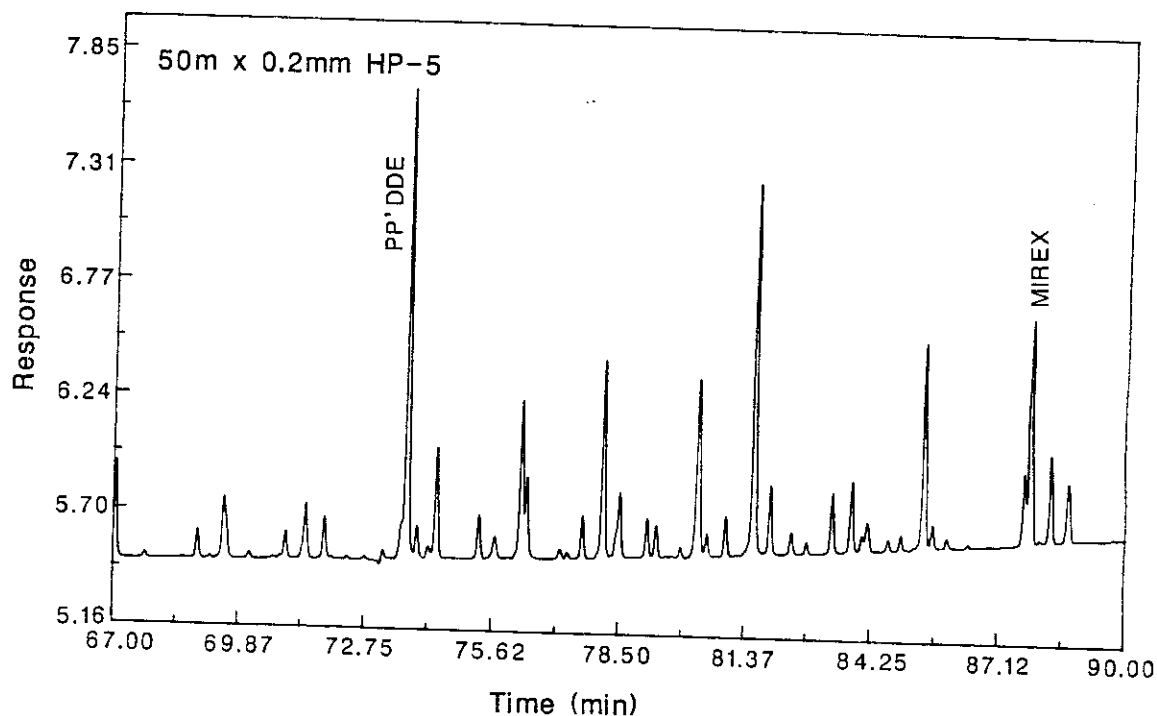
- (1) 1,3-dichlorobenzene; (2) 1,4-dichlorobenzene;
- (3) 1,2-dichlorobenzene; (4) 1,3,5-trichlorobenzene;
- (5) 1,2,4-trichlorobenzene; (6) 1,2,3-trichlorobenzene;
- (7) hexachloro-1,3-butadiene; (8) 1,2,4,5-tetrachlorobenzene;
- (9) 1,2,3,4-tetrachlorobenzene; (10) pentachlorobenzene;
- (11) hexachlorobenzene.



**Figure 2** Chlorobenzene fraction from a sample of eel muscle showing residues of 1,4-dichlorobenzene, 1,2-dichlorobenzene, penta- and hexachlorobenzene. (Chromatographic conditions as in Figure 1.)



**Figure 3** Chlorobiphenyl standard (Aroclor 1254) with pp'DDE and Mirex (internal standard). (Chromatographic conditions as in Figure 1.)



**Figure 4** Chlorobiphenyl fractions from the same sample as in Figure 2. (Chromatographic conditions as in Figure 1.)

running specialised chromatography software, will have little difficulty in reducing raw chromatograms to meaningful data, provided that the analyst is confident of the identification of each peak and has available suitable standards. The problems arise when suitable standards are not available or the peak profile of the chromatogram has been changed by some physical, chemical or biological process, such that it no longer matches that of the standard.

A prime example of this occurs during the quantification of chlorinated biphenyls (CB's). CB's are a ubiquitous group of chemicals in the aquatic environment and they are probably unique, in that no other group of compounds has occupied the minds of so many analytical chemists for so long while searching for the ultimate solution to quantitation. As a group, CB's are a mixture of up to 209 individual compounds, many of which occur in each of the commercial formulations which were widely used up to the mid-1970's. Of the theoretically possible 209 individual compounds, few were available until recently, as pure analytical standards. Even now, only about a third are available as individual CB standards and, although interest in these compounds is growing, they will remain expensive to procure. Previously, the analyst had been forced to use technical formulations, or mixtures of formulations, in an attempt to simulate the pattern found in environmental samples. This approach was the only reasonable solution to the problem before the advent of the routine use of capillary columns which, because of their much higher efficiency, can resolve groups of CB's into individual peaks and thus achieve a separation that was not possible when packed columns were all that were generally available. The combined use of very high-resolution capillary columns, coupled with the use of all of the available individual CB standards, plus extrapolation from structurally similar CB's to give effective relative response factors for those CB's for which authentic standards are not available, would seem to be the best approach.

Very few laboratories are equipped to do this on a routine basis however and, certainly, sample throughput would be low. This idealistic approach would also negate the mass of historic data based on formulations of PCBs (such as Aroclor 1254) that is available for the concentration of these products in the aquatic environment. Whilst these data are unlikely to be perfect, they do have considerable usefulness. The compromise situation, which this laboratory favours, is a combined use of formulations, to maintain a comparability with historic data, coupled with the careful use of well-chosen individual CB's that will best reflect the CB's commonly found in most marine environmental samples. The Marine Chemistry Working Group of the International Council for the Exploration of the Sea (ICES) has nominated a number of CB's for use in monitoring programmes. The primary list contains seven CB's:

- CB 28: 2,4,4'-trichlorobiphenyl;
- CB 52: 2,5,2',5'-tetrachlorobiphenyl;
- CB 101: 2,4,5,2',5'-pentachlorobiphenyl;
- CB 118: 2,4,5,3'4'-pentachlorobiphenyl;
- CB 138: 2,3,4,2',4',5'-hexachlorobiphenyl;
- CB 153: 2,4,5,2',4',5'-hexachlorobiphenyl;
- CB 180: 2,3,4,5,2',4',5'-heptachlorobiphenyl.

At least two inter-laboratory studies have been conducted using this primary list and the results have been reported (Tuinstra, Roos and Werdmuller, 1985; Tuinstra et al., 1985). ICES has also stressed that this is not a definitive list by any means, and that additional CB's should be added to this core according to the aims of any particular monitoring programme and, of course, the occurrence of toxicologically important CB's. One of the aims of the ICES primary list is to ensure that there is some degree of inter-comparability between different laboratories producing CB data. For this reason, this laboratory uses the ICES primary list as the basis for its quantitation of CB's.

## 2.6 Confirmation of residue identity

Gas chromatography does not give an absolute identification of OCP's or CB's, because any organic compound that passes through the extraction and "clean-up" stages of the procedure, and has the same retention characteristics as the compounds of interest, may be misidentified. Even though the "clean-up" procedure is quite specific and the use of capillary columns lessens the possibility of coinciding retention times, (especially when using a selective detector), it is still possible that interferences and/or misidentification may occur. It is, therefore, recommended that extracts are run on more than one capillary column and that occasional samples (especially those that give unexpected results) are subjected to further tests such as chemical confirmation or GC-MS. Because of the constraints of time and money it is not feasible to check each sample that is run, and it is not felt necessary to do so. For a full list of appropriate chemical confirmation techniques, the reader should refer to a Department of the Environment Standing Committee of Analysts publication (DOE, 1978).

## 3. Quality control

### 3.1 General considerations

As with any analytical method, quality control is of paramount importance. The use of certified reference materials, containing a known

concentration of the specific determinands, to which the method can be applied to enable experimental error to be determined, is clearly of great significance. However, such materials are not always available in the matrix type and at the concentration level that the analysis requires and their cost may inhibit routine use. In this situation, it is necessary to use some form of in-house quality control standard material and, preferably, invite other analysts from outside organisations to analyse this material to enable some form of certification and level of random and systematic error to be determined. The approach used by this laboratory when analysing fish tissue (for which the method has been primarily designed) is to obtain a suitably large volume of a fish liver oil (normally cod since this is most readily available) containing the range of determinands at the concentration level expected in "real samples".

Cod liver oil has several advantages as a quality-control material:

- a) it is cheap and readily available;
- b) it is available in bulk quantities, so that once a suitable batch has been found, an amount sufficient to last for some considerable time can be purchased;
- c) it is easy to obtain a homogeneous sample, which may not be the case with a fish tissue homogenate;
- d) it can represent a "worst case" sample as far as the analyst is concerned, in that it can combine low pesticide concentration with high lipid levels and thus provide an exacting test of methodology;
- e) it is convenient to sub-sample and send to other laboratories for intercomparison purposes;
- f) it can readily be spiked with additional concentrations of determinands to further check recoveries of pesticides. It has at least one disadvantage, in that it does not give an estimate of any error that may be associated with the Soxhlet extraction stage, but it is generally accepted that this stage is quantitative and that the recovery of non-polar compounds is complete.

A recent publication of the Commission of the European Communities gives details of a range of certified reference materials suitable for use in chlorobiphenyl analysis. (CEC, 1988).

### 3.2 Preparation of glassware

To avoid the possibility of contamination arising from unclean glassware, all glassware is subjected to the following cleaning procedures. An overnight soaking in a solution of a laboratory detergent (e.g. Pyroneg) at 40°C in a circulating bath. The detergent is then rinsed off under running tap water and the glassware transferred to a bath of 5% nitric acid in tap water. After an overnight soaking, the glassware is well rinsed, again in running tap water, and allowed to dry at 40°C and then stored. Immediately prior to use, all glassware is rinsed with n-hexane.

### 3.3 Blanks

As well as performing routine blank determinations on each batch of solvents, reagents and glassware that come into the laboratory, prior to their use, every sixth sample run through the method is a total method blank.

