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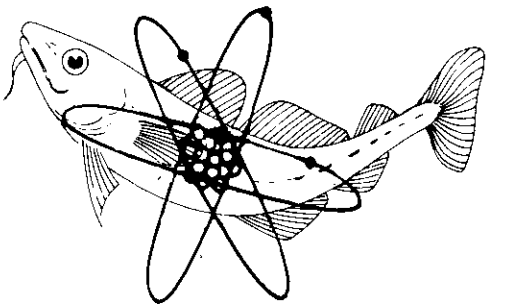
MINISTRY OF AGRICULTURE FISHERIES AND FOOD

DIRECTORATE OF FISHERIES RESEARCH

AQUATIC ENVIRONMENT PROTECTION: ANALYTICAL METHODS



Number 6

Methods of analysis for chlorinated hydrocarbons in marine and other samples

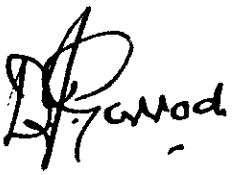
C. R. Allchin, C. A. Kelly and J. E. Portmann

Lowestoft 1989

CONTENTS

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.



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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 quantitively 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) (n-hexane)	Alpha-, beta- and gamma-HCH Chlorobiphenyls pp'DDE, pp'TDE and pp'DDT Dieldrin Hexachlorobenzene
"	SF1 (4.5-5.5 ml) (2% THF in n-hexane)	Chlorobiphenyls Hexachlorobenzene pp'DDE
"	SF2 (8.5-9.5 ml) (2% THF in n-hexane)	Alpha-, beta- and gamma-HCH pp'TDE and pp'DDT Dieldrin
B	AF1 (0-2 ml) (n-hexane)	Chlorobiphenyls Chlorobenzenes pp'DDT pp'DDE
"	AF2 (2-12 ml) (n-hexane)	Alpha-, beta- and gamma-HCH Endrin Dieldrin pp'TDE Trans-permethrin
"	AF3 (0-6 ml) (20% ether in n-hexane)	Cis-permethrin Cypermethrin Deltamethrin
"	SF1 (0-7 ml) (n-hexane)	Chlorobiphenyls Chlorobenzenes
"	SF2 (8-24 ml) (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-tetrachloronapthalene. 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 pg μl^{-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 µm	film thickness	
25 m x 0.31 mm HP-1 "	"	"
25 m x 0.2 mm HP-5 0.33 µm	"	"
25 m x 0.2 mm HP-1 "	"	"
50 m x 0.2 mm HP-5 0.5 µm	"	"
25 m x 0.25 mm CP-Sil-19 0.2 µm	"	"
50 m x 0.25 mm CP-Sil-19 0.2 µm	"	"

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^{-1}. 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 µl.

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^{-1}).

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^{-1} to 150°C, then at 2.5°C min^{-1} to 285°C, then hold for 15 min.

Inject at 90°C, hold for 1 min, then programme at 2.5°C min^{-1} 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,

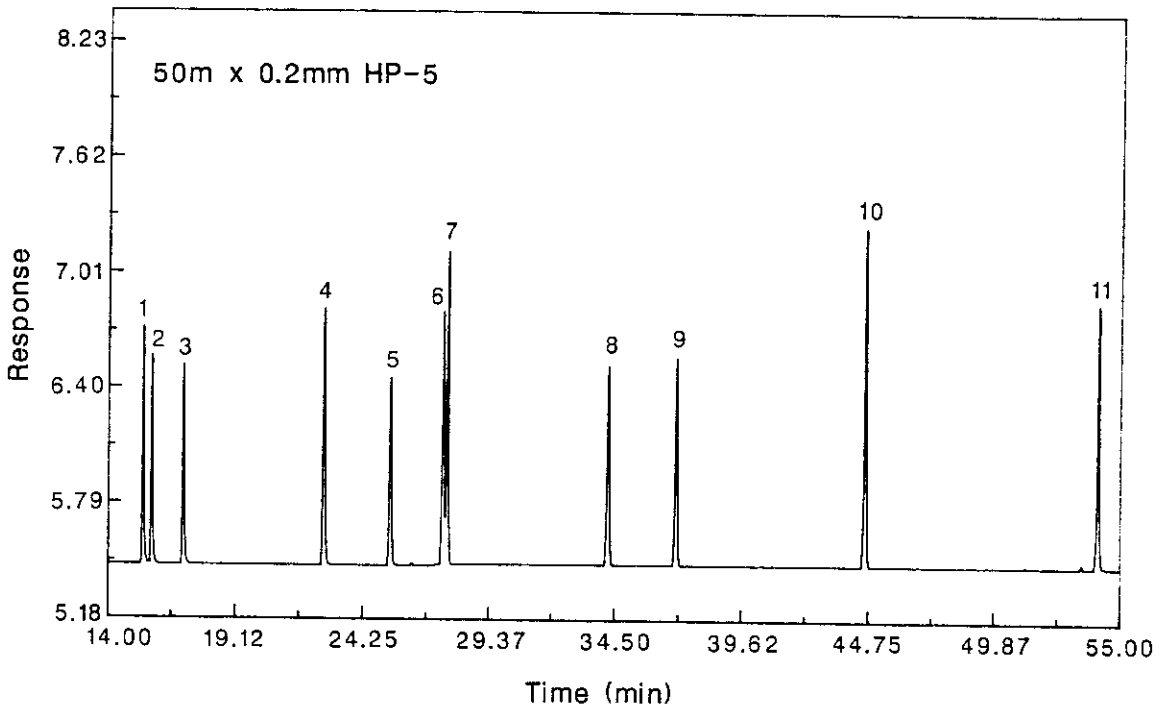

Figure 1 Chlorobenzene standard including hexachloro-1,3-butadiene. Chromatographic instrumentation, column specifications and operating conditions are as follows: oven programme 60°C (1 min) then at 2.5°C min^{-1} to 300°C.

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.

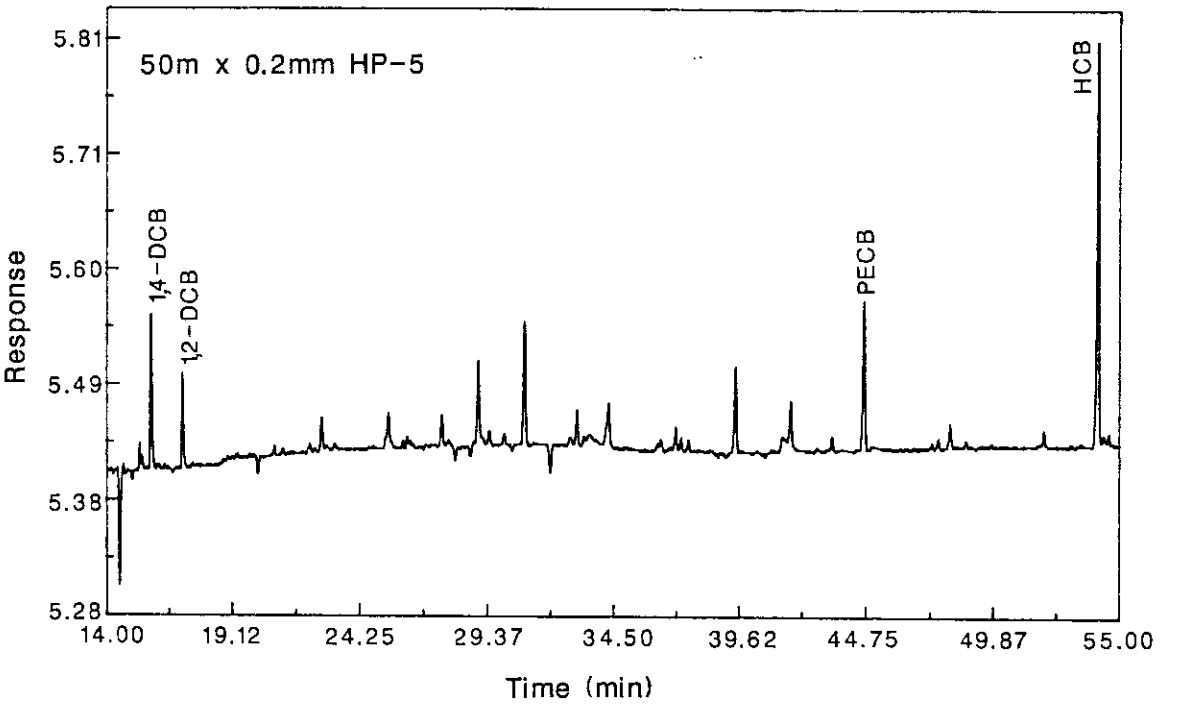

Figure 2 Chlorobenzene fraction from a sample of eel muscle showing residues of 1,4-dichlorobenzene, 1,2-dichlorobenzene, penta- and hexachlorobenzene. (Chromatographic conditions as in Figure 1.)

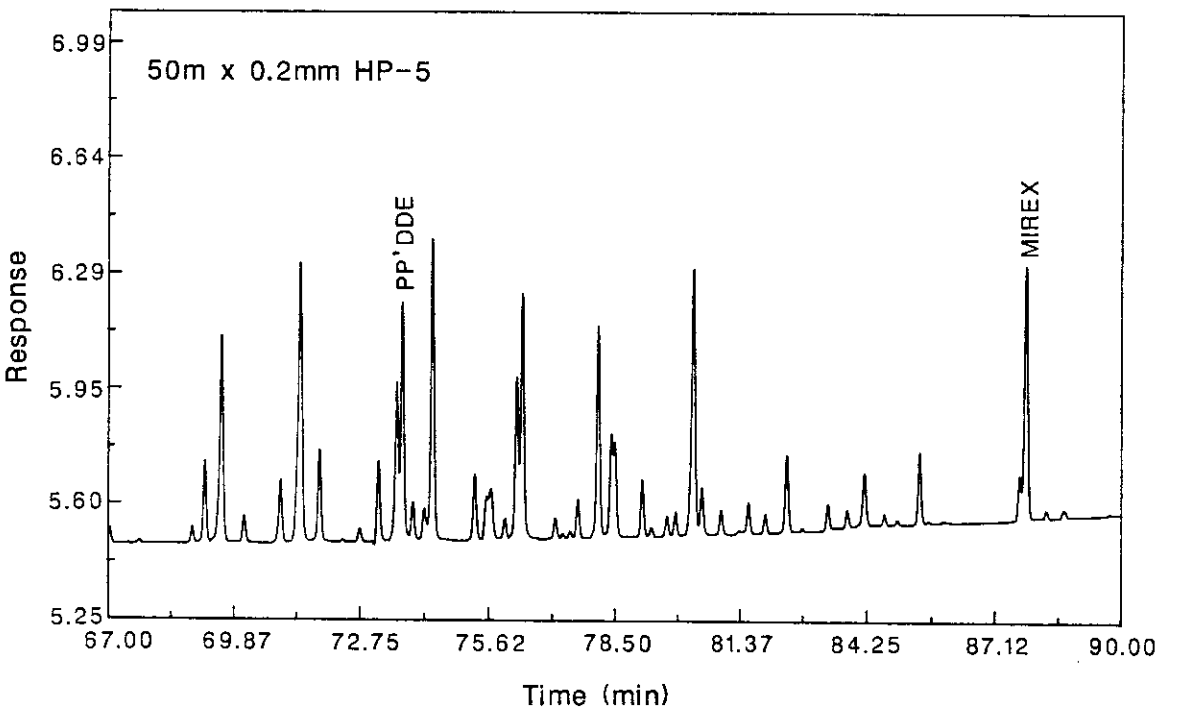

Figure 3 Chlorobiphenyl standard (Aroclor 1254) with pp'DDE and Mirex (internal standard). (Chromatographic conditions as in Figure 1.)

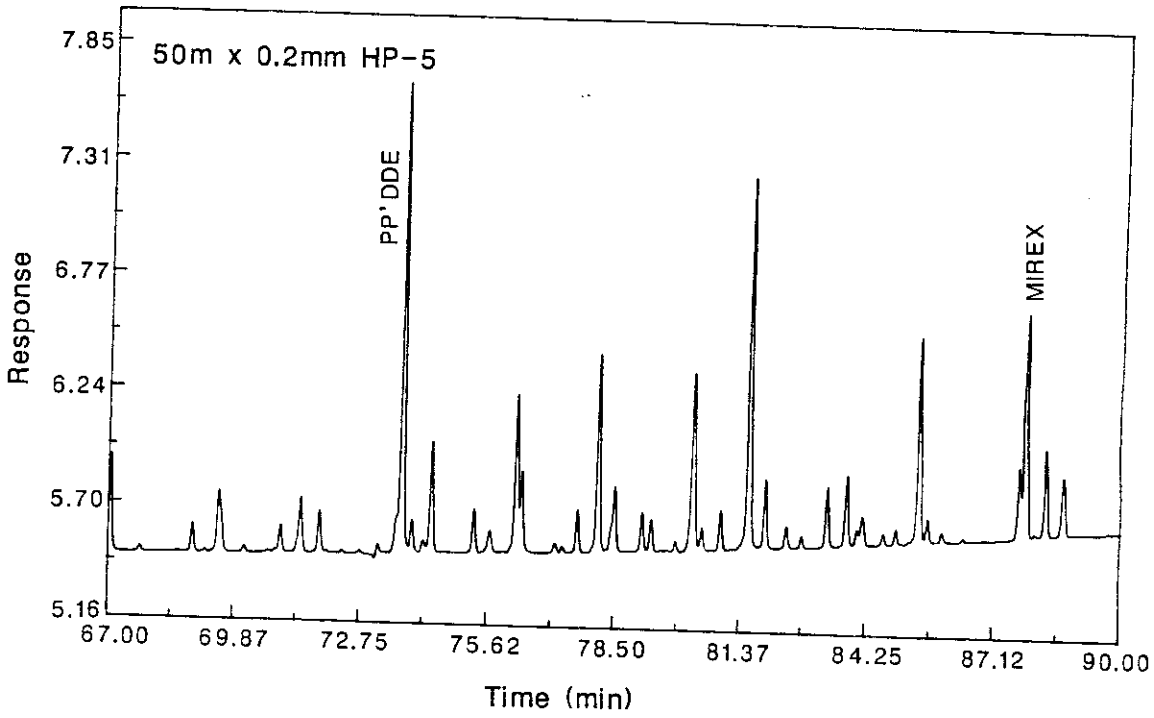

Figure 4 Chlorobiphenyl fractions from the same sample as in Figure 2. (Chromatographic conditions as in Figure 1.)

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.

4. Accuracy and precision

Because of the range of concentrations encountered and the numerous determinands covered, it is not possible to give a single indication of error that would be applicable to all samples. It is perhaps better to indicate the tolerance which we aim to achieve with this method. Accordingly, as an example at the 1 mg kg^{-1} level, for Aroclor 1254 in fish liver we would aim to achieve a relative standard deviation of 3-5%, where n = 10. At lower concentrations, we would expect this error to increase to 5-10% at the 0.1-0.01 mg kg^{-1} level and to 10-15% at the 0.001 mg kg^{-1} level.

5. Chemicals

5.1 Solvents

n-Hexane glass distilled grade		Rathburn Chemicals Ltd., Walkerburn, Scotland
Acetone " " "		" " " "
Tetrahydrofuran glass distilled grade		" " " "
Diethyl ether	Analar	BDH Chemicals Ltd., Poole, England

5.2 Reagents

Sodium sulphate (anhydrous)	Analar	BDH Chemicals Ltd., Poole, England
Silica gel	(Merck no. 7734)	" " " "
Aluminium oxide	(Merck no. 1077)	" " " "

5.3 Standards

All OCP standards are obtained from the U.S. EPA Pesticides and Industrial Chemicals Repository, Research Triangle Park, NC 27711, USA.

Solutions of CB's are obtained from the National Research Council of Canada, 1411 Oxford Street, Halifax, N.S., Canada B3H 3Z1.

Pure CB standards are obtained from the Community Bureau of Reference, Commission Communities, 200 Rue de la Loi, B-1049 Brussels, Belgium.

The reference to proprietary products, in this report, should not be construed as an official endorsement of these products, nor is any criticism implied of similar products which have not been mentioned.

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6. References

CEC, 1988. The certification of the purity of polychlorinated biphenyl isomers BCR Nos 289-298. Commission of the European Communities, Brussels, 59 pp.

DOE, 1978. Organochlorine insecticides and polychlorinated biphenyls in waters. Her Majesty's Stationery Office, London, 20 pp.

TUINSTRA, L. G. M. Th., ROOS, A. H. and WERDMULLER, G. A., 1985. BCR ring test of individual chlorobiphenyls, summary of results. Internal BCR-report. State Institute for Quality Control of Agricultural Products, Wageningen, The Netherlands.

TUINSTRA, L. G. M. Th., ROOS, A. H., GRIEPINK, B. and WELLS, D. E., 1985. Interlaboratory studies of the determination of selected chlorobiphenyl congeners using splitless- and on-column injection techniques. J. High Resolut. Chromatogr. and Chromatogr. Communic., 8: 475-480.

Appendix - Details to be followed for sample collection, preparation and analysis in the conduct of cooperative monitoring

Monitoring, using fish or shellfish as indicator species, may be conducted for one of the following three purposes:

(1) The provision of a continuing assurance of the quality of marine foodstuffs with respect to human health.

(2) The provision over a wide geographical area of an indication of the health of the marine environment in the entire ICES North Atlantic area.

(3) The provision of an analysis of trends over time in pollutant concentrations in selected areas, especially in relation to the assessment of the efficacy of control measures.

Sampling

Samples to meet Objective 1 (to be collected every second year; started in 1982)

(a) A sample should consist of 25 fish or large crustaceans such as crabs or lobsters, 50 mussels or other molluscs, and 100 small crustaceans such as shrimps.

(b) The sample should be selected in a way that reflects the size distribution of the commercially exploitable portion of the catch of that species within that particular geographical area. This distribution may be determined from previous data or on board the sampling vessel but, having been established, should only be amended if a significant change in the distribution can be demonstrated.

(c) Sampling should be conducted prior to spawning of the species concerned.

(d) Samples should be collected from at least the following areas: the estuaries of the Forth, Thames, Rhine, Scheldt and Clyde, the Skagerrak, Kattegat and Oslo Fjord, the Irish Sea, German Bight and Southern Bight of the North Sea, certain parts of the Gulf of St Lawrence and the US middle Atlantic Bight, and the area off Portugal.

Samples to meet Objective 2 (to be collected every 5 years; started in 1985)

(a) A sample should consist of 25 fish or 50 mussels.

(b) Selected fish samples should be representative of the area in question, i.e. they should not be very recent immigrants to the area or on passage through it. Each sample should consist of the same or similar sized fish.

(c) Mussels should be between 20 and 50 mm in size and preferably as close as possible to the lower end of this range.

(d) Sampling should take place prior to spawning of the species concerned.

(e) Samples should be collected from as many locations as is practicable throughout the ICES area.

Samples to meet Objective 3 (to be collected every year; started in 1982)

(a) A sample of fish should consist of at least 25 individuals, and preferably more than 25 individuals. The sample should be collected in a length-stratified manner, i.e. the sizes of the fish should span a length range which is as wide as possible and there should be an equal number of individuals in each length grouping.

The stratification should be based upon an equidistant logged length interval, i.e. the log (upper bound) minus log (lower bound) should be equal for each length interval. The length range of the entire sample should be selected so that the individuals in the lower bound yield sufficient tissue for the chemical analyses, while the upper bound should be selected so that at least 5 fish can readily be found in the sampled catch. The length range should be divided into 5 (or more) length intervals of equal size (after log transformation). Once the length stratification for a particular species and area has been agreed, this stratification should be strictly adhered to for a number of years. No length interval should be less than 2-3 cm. If the length range is smaller than 2-3 cm, the species is not ideally suited to the proposed analysis.

(b) A sample of mussels should span a size range which is as wide as possible and should consist of sufficient individuals to provide material for analysis in groups of the different sizes. The number of individuals in each length range should be recorded for each site and, thereafter, this distribution should be used for that site each year.

(c) Sampling should be conducted annually from the same areas, from the same stock and at the same time each year; mussel samples should be collected at the same position in relation to tidal height each year.

(d) Samples should be collected in such a way that at least the following areas are adequately covered: the estuaries of the Forth, Thames, Rhine, Scheldt and Clyde, the Skagerrak, Kattegat and Oslo Fjord, the Irish Sea, German Bight and Southern Bight of the North Sea, certain parts of the Gulf of St Lawrence and the US middle Atlantic Bight and the area off Portugal.

(e) The species of interest can only be selected in the light of information on fish stock composition and history and the known or perceived problems which define national priorities. It is preferable to use a fish species which continues to grow throughout its life. Species which are of particular interest in an ICES context are: cod or hake; plaice; flounder; mackerel (Scomber scombrus); mussels; shrimps; but data relating to other species are also required.

Storage and pretreatment of samples prior to analyses

To meet all three objectives:

(a) Fish samples should be collected ungutted and preserved (deep frozen) as soon as practicable after collection; length and weight should be determined before freezing.

(b) Mussels should be held live in clean (settled) sea water from the area of collection for 12-14 h to allow discharge of pseudo-faeces. The length of each individual, even if used as part of a composite, should be measured as a maximum value regardless of direction or orientation.

(c) After cleaning and measuring the mussels, the individual animals should be carefully freed from their shells by cutting the adductor muscle. The shell cavity liquor can then be drained and discarded by placing the opened shells vertically in a filter funnel for 5 min. The remaining shell contents may then be preserved either individually or as pooled samples.

(d) Since a wide variety of factors can affect the total body burden of a pollutant in shrimps, the only useful objective in analysing shrimps is Objective 1. For this purpose, the shrimps should be boiled whole in sea water from the area of collection for 10 min. The tails should then be removed, peeled and thoroughly homogenised in preparation for storage or analysis.

To meet Objectives 1 or 2

(a) In order to reduce the number of analyses which have to be performed, pooled samples may be used. These should be prepared as described below and analysed in duplicate.

(b) An equivalent quantity of muscle tissue must be taken from each fish, e.g. a whole fillet of every fish. If the total quantity of yielded tissue is too large to be handled conveniently, the tissue may be sub-sampled, but a fixed proportion of each tissue must then be taken, e.g. 10% of each whole fillet or 10% of each whole liver, the sub-sample being taken after homogenisation of the whole fillet/liver or in the form of complete longitudinal sections.

To meet Objective 3

(a) Each fish should be analysed individually and the following biological variables should always be recorded when sampling for time trend analysis purposes: age; total weight; total length; liver weight when contaminants in liver are determined (if another fatty organ is used, the weight should be recorded); sex; and degree of sexual maturation, where applicable.

(b) Mussels may be pooled in small groups to provide enough tissue for analysis but different sized groups should be analysed separately.

Reporting of results

For Objective 1

Results should be reported on a wet weight basis along with details of the size range of the sample and details of site, date and method of collection, preservation details (if appropriate) and brief details of the methods of analysis used; if PCBs were analysed for, these details should include the formulation or isomer(s) and the method of quantification used.

For Objective 2

(a) Results should be reported as for Objective 1. In addition, results of analyses of mussels for metals should also be reported on a dry weight basis. All results of analyses for organochlorine compounds must be reported also on an extracted fat weight basis or as a minimum be accompanied by a fat weight determination result.

(b) Dry weight determinations should be carried out in duplicate by air-drying, to constant weight at 105°C, sub-samples of the material analysed for the contaminants.

(c) Fat weight should be determined on a sub-sample of the extract used for the organochlorine compound analyses. The results should be accompanied by a brief description of the method used for extraction.

For Objective 3

Results should be reported as for Objective 2, but the individual analytic figures should be given together with full details of the size, age, weight, sex, etc., of the individual fish analysed.

In reporting these data to ICES, the ICES Interim Reporting Format for Contaminants in Fish and Shellfish must be used for such data so as to allow machine handling and statistical analysis of the data.

General details

Results should be submitted to the ICES Environment Officer not later than 30 June of the calendar year following collection of the sample. These results should be accompanied by the name of the contributing laboratories and the name of an individual contact in the event of queries. The contributors should specify the most recent ICES intercalibration exercise in which they took part. A brief commentary on the data is also required, at least in relation to that supplied for the purposes of Objectives 1 and 3.

Notes on length stratification

The main finding from the statistical analyses of data on contaminants in fish tissue is the gain in precision which can be obtained from stratification using biological variables. Although several biological parameters

have been shown to be significant as stratification variables in different materials, length appears to be the only parameter which is simple to apply at sea and which shows up as being significant in most analyses.

Much discussion has been devoted to whether simple linear or log-linear (multiplicative) models give the better fit. General experience with other fish and other types of data indicate preference for the log-normal model at least for the present. As the length dependence of the contaminant level is not well understood, sampling should keep the length-contaminant relationship under constant surveillance, i.e. the entire length range should be covered evenly. The length range should be defined from practical considerations, the lower bound ensuring that enough tissue is available for chemical analysis and the upper bound such that at least 5 fish in the largest length interval can readily be found. The length stratification should be determined in such a way that it can be maintained over many years. The length interval should be at least 2-3 cm in size.

It is suggested that the length range be split into 5 length intervals which are of equal size after log transformation. For example, if the length range is 20-70 cm, then the interval boundaries could be (rounded to 0.5 cm) as follows:

cm	No. of fish	Log upper - log lower
20.0-25.5	5	0.243
25.5-33.0	5	0.258
33.0-42.5	5	0.253
42.5-54.5	5	0.249
54.5-70.0	5	0.250
Total	25	

Care should be taken to ensure that samples are not unduly clustered within each stratum (length interval). More length intervals could be used and the test of the hypothesised contaminant-length relationship becomes stronger if the lengths are evenly distributed. But the item of major importance is to keep the length stratification identical from one year to the next.

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