Chromatographic clean-up methods for the determination of persistent organic compounds in aqueous environmental samples.

de Voogt, P.

Published in:
Trends in Analytical Chemistry

DOI:
10.1016/0165-9936(94)85010-0

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: https://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Chromatographic clean-up methods for the determination of persistent organic compounds in aqueous environmental samples

Pim de Voogt
Amsterdam, The Netherlands

A review is presented of clean-up methods for the determination of persistent organic compounds. Basic techniques and principles are discussed and recent developments indicated. It is concluded that, although classical methods still predominate, several new techniques which combine extraction and clean-up are promising, particularly for aqueous matrices. These will allow faster, cheaper, and more easily automated methods of determination.

1. Introduction

Halogenated aromatic compounds (HACs) have been manufactured since the early twentieth century. As a result of the commercial production, subsequent consumption and of unwanted side products, these compounds have entered the environment. The majority are rather persistent. Concentrations in the environment have therefore been increasing steadily between the 1930s and 70s. Despite many regulations, including bans on several products, such as the PCBs, the concentrations of several HACs continue to increase. Model calculations have shown that this increase will continue on a global scale in the coming decades [1,2], even when significant amounts of the chemicals which are in use at the moment will have been destroyed.

The presence of these chemicals in the environment has led to exposure of animals and man, sometimes resulting in adverse effects. There is consequently great interest in the analysis of the HACs, both in scientific circles and from many regulatory and controlling bodies.

The quantitation of xenobiotics in samples from the environment is difficult, because of the complex nature of natural matrices and the similarity of many of the analytes. The clean-up of extracts and the isolation of determinants from various matrices presents an ongoing challenge for the environmental analytical chemist. Chromatographic methods have been ubiquitously used for this purpose ever since environmental analysis started, and continue to maintain their central position. Specificity for trace HACs can be achieved either by efficient clean-up, i.e., isolation of the analytes from the matrix and interferences, or by a less efficient purification in combination with highly specific detection methods.

This paper focuses on the isolation of HACs from aqueous samples, subsequent purification (clean-up) of extracts, and the group separation (fractionation) of determinants prior to instrumental analysis. Analytical separations accomplished during the final detection step, for example with high resolution capillary gas chromatography, will not be included. Rather than trying to be complete, we shall discuss some principles and review the most important developments of the last decade. Since clean-up and fractionation of extracts from aqueous samples is usually similar to, or even simpler than from other types of sample, experience with some of these will be discussed also.

2. Extraction

Usually, the first step in sample treatment is the extraction of the compounds of interest. Extraction in itself is not the subject of this review; however, since modern techniques can combine extraction and clean-up steps, it will be dealt with briefly. Normally, extraction involves separation of the bulk materials constituting the sample (i.e., water) from the analytes, using suitable solvent. Liquid–liquid extraction is still used most frequently but, particularly for aqueous samples, solid-phase extraction (SPE) is becoming increasingly valua-
ble (as will be shown below), and supercritical fluid extraction (SFE) has also been shown to be applicable.

2.1. Membranes

Membrane interfaces can separate analytes from solvent or bulk molecules as a result of their differences in transport rates through the membrane. In dialysis, the transport rate is governed by the influence of a concentration gradient on the diffusion ability. The relative size of different molecules largely determines the permeation selectivity of a membrane (when other specific interactions are absent). Other examples of membrane processes used for separations are ultra filtration and reverse osmosis. Dialysis has been used to isolate apolar analytes from co-extracted lipids [3]. A drawback is the long dialysis times involved. Very recently, lipid-containing semipermeable membrane sampling devices have been developed. These devices consist of a thin film of neutral lipid (e.g., triolein) enclosed in thin-walled layflat tubing made of low-density polyethylene or another polymer. They were able to extract PAHs and PCBs directly from water [4], thereby combining extraction and isolation in one step.

2.2. Solid-phase extraction

Initially, SPE was a miniaturized version of adsorption chromatography (AC). In principle this consists of a column containing a sorbent which will trap the analytes. Sample and solvents pass through the column under gravity or applied pressure [5]. After activation of the sorbent, the sample is applied, interferences are removed, and finally the concentrated analytes are eluted with an appropriate solvent. SPE serves not only as a separation technique, but also for trace-enrichment. For aqueous samples this is a particularly useful application of SPE, since large volumes of water can be passed through the column before breakthrough of organic solutes occurs, and only a few ml of organic solvent are necessary to desorb the analytes afterwards.

With the development of solid-phase materials, many different types of separation mechanisms could be employed. Thus, as well as mechanisms such as adsorption and bonded phase partition (both in normal and reversed-phase modes), ion-exchange chromatography and size exclusion became available for the analyst. Prepacked SPE columns are commercially available in many sizes and shapes with a wide selection of packing materials. Combinations of such materials are easily achieved using the cartridge columns. These and other aspects of SPE have been reviewed by, for example Nielen et al. [5].

A recent development which is relevant for organic pollutants, including more polar pesticides, is the SPE modification in which chromatographic particles are enmeshed in a network of teflon fibrils to form strong sheets or ‘membranes’ [6,7]. These devices are known under the name of membrane disks or Empore disks and can be used in standard filtration equipment. Efficient adsorption results from: (a) reduction in linear velocity of the water flow; (b) reduction in particle size as compared to SPE; (c) dense packing of the particles so that the mean-free-path of the dissolved analyte to the adsorptive surface is small. A comparison of characteristics for SPE columns and membrane disks has been given recently by Hendriks [8]. Most importantly, membrane disks have a higher sample capacity, but need at least 80 ml of sample, whereas SPE columns can cope with much smaller amounts.

Interestingly, Sirén et al. [9] compared SPE with normal-phase HPLC and concluded that a combination of three different types of SPE adsorbents (silica, alumina and carboxpack C) achieved a better separation of planar from non-planar compounds, but that the LC separation was faster and more effective for the separation of the compounds of interest from matrix interferences.

2.3. Supercritical fluid extraction (SFE)

The development of instrumentation for supercritical chromatography and extraction has made SFE a modern choice of extraction technique, in particular when analytes have to be isolated from solid or bulky samples. SFE techniques have been reviewed recently [10–12]. Applications to aqueous samples usually include a concentration of the analytes onto an adsorbent, such as polyurethane foam, SPE cartridges or Empore disks (see above). Subsequently, the analytes are extracted by placing the adsorbent in the extraction chamber of an SFE instrument [13–15]. Alternatively, the supercritical fluid (CO₂) can be passed directly through an extraction vessel containing the sample, and then analyzed on-line by SFC [16]. The extraction efficiency of SFE can be improved by the use of more polar fluids than CO₂ (e.g., CHClF₂), giving excellent recoveries for PCBs, PAHs and nitro-PAHs [11], or by addition of organic modifiers to CO₂.
Alternatively, the use of pure CO₂ at higher temperatures may greatly improve extraction efficiency, as has been demonstrated for PCBs in sediments and PAHs in air-particulates [17].

2.4. Solid-phase microextraction

One of the most recent developments in aqueous sampling is the so called solid-phase microextraction technique. Basically, this technique uses a fused-silica optical fibre glued to the plunger of a normal injection syringe. By pushing down the plunger, the fibre is exposed and can be put into the water to be sampled. After a typical equilibration time of 2–15 min, analytes are adsorbed onto the fibre or its coating and the fibre is pulled up into the needle of the syringe where it is protected from damage during storage and transport. The sorbed analytes can be analysed directly by using the syringe in a conventional GC injector. Typical lifetimes of the fibre are about 6 weeks. Depending on the expected distribution constants of analytes between the coating and the water, thick or thin films can be selected.

Uncoated silica–poly(dimethylsiloxane) fibre has been used for analysis of PCBs and chlorobenzenes [18]. A polyimide coated silica has been used for chlorinated hydrocarbons and liquid crystal coatings proved successful for the analysis of PAHs and PCDDs [19,20]. The suitability of the technique has been demonstrated even for polar compounds such as phenols, using acrylic coatings [21].

3. Further treatment of the extract

Since an apolar solvent or solvent mixture is used most frequently in extraction, lipids and fat soluble compounds are co-extracted. In aqueous samples the lipids are usually a minor problem. Further treatment of the lipid-containing extract involves one or more steps in which these co-extractants are separated from the analytes, followed by group separations of the remaining analytes. These separations can be achieved by many different processes, e.g., chemical reaction (acid, base, desulfurisation), molecular interactions (H-bond, charge transfer, dipole–dipole), or chromatography based on differences in polarity, shape or size.

The first step can be accomplished either by chemical reaction (such as, saponification, chromic or sulfuric acid destruction, or dechlorination) [22,23], physical methods [22] (e.g. further liquid–liquid partition or sweep co-distillation) or chromatographic methods. Chemical methods, of course, change the constituents of the extract and this may include some analytes of interest. Hence, low recoveries may be obtained — as was reported for PCDDs [24] and PCBs [25,26] when saponification was used to destroy lipids. Physical methods tend to be difficult to control in terms of repeatability. Both the chemical and physical methods are difficult to miniaturise and automate. Non-destructive techniques for removing lipid material have centred around gel permeation chromatography (GPC [27]) and adsorption chromatography (AC) using magnesium silicate, alumina and/or silica, and combinations of the last two [28]. They also include high performance liquid chromatography (HPLC) and thin-layer chromatography (TLC). These methods will be the subjects of the following paragraphs.

3.1. Adsorption chromatography

Open-column adsorption chromatography is still by far the most widely used technique for the clean-up of extracts prior to the detection of HACs. Various types of adsorbents have been used, including silica, alumina and magnesium silicates (e.g. Florisil). These materials adsorb polar substances via interactions with the –OH and =O moieties present at their surfaces. Non-polar analytes will not be retarded and, as a consequence, elute earlier than polar analytes. Since the bulky lipid material present in environmental samples contains many polar functional groups, it will be retained on such columns.

AC columns are usually made by the user to suit the nature of the extract, the analytes of interest and the amount of lipids present in the extract. The columns can be dry or slurry packed. Prior to use, the materials are normally heated at appropriate temperatures to remove water. After cooling, a specific amount of water, base or acid is added. This amount can be varied to slightly change the elution characteristics of the column: for example the addition of more water results in earlier elution of polar analytes, whereas when no water is added to the adsorbent all analytes, including the non-polar ones, are retained on the column. The degree of deactivation is critical and difficult to keep under control. Elution profiles need to be established regularly, and certainly from batch to batch since dif-
ferences may strongly influence the elution pattern.

Examples of specific applications in PCB, PCDD and PCDF analysis can be found in articles by Lamparsky et al. [29], Smith et al. [30,31] and Tanabe et al. [32], who have used silica columns impregnated with aqueous sulfuric acid (40%, w/w) in order to mimic the effect of chemical destruction in a more reproducible way. In current PCDD/F analysis, combinations of acid, base and other chemical reagent (e.g. AgNO₃) impregnations are commonly applied [33]. Reviews of such techniques [22,34] mostly cover the work-up of modest amounts of tissue (<20 g) or sediment (<50 g) extract. In many applications the lipid co-extractant loading of the column will be the limiting factor, and some modification may be required to cope with any increase in sample size necessary for, e.g., planar PCB determination. Although for aqueous samples the low analyte concentrations require large sample volumes, this will not pose a particular problem in cleaning up the extract.

3.2 Gel permeation chromatography

Basically, a GPC system consists of a solvent delivery system, a sample introduction device, a column containing the gel and a detector (ultraviolet or refractive index). The separation mechanisms in GPC involve adsorption, partition and size exclusion [35]. Which of these processes dominates in the final separation depends on factors such as the mobile phase composition, the type of gel, and the pore size of the material used. Size exclusion and adsorption predominate when large pore sizes (1000–2000 molecular weight exclusion) are used with poorly solvating mobile phases (e.g. cyclohexane, toluene-ethyl acetate). Highly polar solvents (e.g. tetrahydrofuran) and smaller pore sizes (M, 400) will result in size exclusion as the sole process governing separation. The most common materials used in GPC are Sephadex and BioBeads, but other types such as Lipidex have been shown to be successful as well. Size exclusion is of minor importance in gels such as the Lipidex gel, where the distribution of substances is mainly based on the polarity of compounds and solvents. This mechanism has been referred to as liquid gel chromatography [36].

The advantages of GPC over AC have been summarized recently by Liem et al. [23]. Large series of samples can be run using the same column and monitoring of the efficacy of the procedure can be accomplished by the detector that is usually part of the system. The technique can be automated easily.

A variety of applications in the field of HAC analysis show the success of GPC in this type of analysis. Usually the function of GPC is to separate lipids from the analytes of interest [24,27,37,38]. Its use in water analysis is therefore still limited. However, GPC can be used for group separation (e.g., the separation of more polar metabolites from unconjugated parent compounds [39] or the separation of chlorinated paraffins from other HACs [40]), or can serve as an alternative to unwanted destruction by wet chemical methods. Additional clean-up by AC is necessary to remove remaining biogenic compounds which may interfere in low level determinations [24]. Lipidex gels have been applied for the determination of DDT, TCDD and PCP in water [39].

GPC can be used either in atmospheric pressure or in high-performance or high-pressure (HP) modes. The latter requires a high-pressure solvent delivery system. Using the currently available solvent delivery systems, modern HPGPC has become a highly reliable and repeatable clean-up technique.

Limitations of GPC pertain to the maximum amount of lipid that can be applied to the column to (typically 1–2 g) and, therefore, pose no problems for water analysis.

3.3 HPLC

Although HPLC has been used quite often for clean-up purposes its application in HAC analysis has been relatively rare, presumably owing to the abundance of suitable adsorbents for AC.

The use of HPLC with a aminopropylsilica column has recently been shown to be able to separate HACs from halogenated aliphatics [41]. The mechanism is supposed to involve an electron donor–acceptor complex forming between the π-electrons in the analyte and the lone pair electrons in the amino group. Hydrocarbons are separated according to the number of fused aromatic rings. Aliphatic and olefinic compounds elute before aromatic compounds. Zebühr et al. [42,43] coupled an aminopropyl silica column with a porous graphitic carbon column in an automated mode to separate PCDD/Fs, planar and non-planar PCBs and PAHs in sediments, fish and electrostatic filter precipitates after Soxhlet extraction. The method also appeared to remove efficiently the sulfur present in sediments.

Other recent developments are the (group) separation of planar molecules by the use of 2-(1-
4. Sample pretreatment for and separation of planar HACs

4.1. Carbon chromatography

The separation of planar PCBs, PCDDs and PCDFs and their methoxy derivatives [44] from non-planar HACs (e.g., PCRs) has been achieved mostly using various crystalline forms of active carbon as the column material in column liquid (adsorption) chromatography. In PCDD/F analysis, activated carbon has played a central role in clean-up and fractionation [30,33]. Activated carbon has a number of specific advantages over common adsorbents, viz. a greater selectivity for planar molecules (resulting from the graphitic structure) and a more reproducible behaviour, which is determined by the activity of the adsorbent. It is also less prone to contamination by sample constituents since it combines a high capacity with a low sorption for endogenous co-extracted material. However, other types of adsorbents can also be used in AC for separation of planar PCBs from other congeners [45].

The literature on the separation of planar PCBs has been reviewed recently by De Voogt et al. [46] and Lang [47], and is briefly summarized here. Recent improvements in carbon chromatography have led to greater resolution between classes of planar compounds, such as PCDDs, PCDFs and planar PCBs, at the ultra-trace level. Carbon chromatography is now part of many routinely applied methods, including multi-residue methods [40]. The isolation of planar PCBs from environmental samples by carbon AC has become more and more feasible [31,48–50].

The methods exhibit acceptable recoveries and relatively large sample capacities. However, they are laborious and require much solvent (for example, the elution scheme used by Tanabe et al. [32] requires more than 1200 ml of solvent per sample) and expensive equipment (e.g., multi-column switching valves, backflushing apparatus, and columns). Miniaturisation of the activated carbon clean-up has been reported [51].

Creaser and Al-Haddad [52] have reported the use of porous graphite carbon (PGC) in an HPLC system. This is a commercially available graphitic material which can withstand the back-pressure generated by HPLC and was specifically developed by Knox et al. [53]. The main disadvantage of PGC seems to be its relatively low capacity [52], which necessitates prior removal of the bulk of co-extractants, for example by adsorption chromatography. The same type of column has been used in the analysis of horse fat [54] and of food [55].

In recent years, HPLC methods for carbon chromatography have improved significantly, resulting in enhanced repeatability and potential for automation. The activated carbon can be dispersed on common HPLC supporting materials, e.g., LiChrospher RP-18 [56], thereby enabling high pressure to be applied, which results in better performance.

To summarize, we can say that activated carbon and other types of graphitic carbon have specific selectivity towards planar molecules, and this can be used for the separation of planar chlorinated aromatics from non-planar congeners and related products. The greater selectivity of graphitized carbon compared to, e.g., C18-cartridges has been demonstrated in the analysis of pentachlorophenol [57]. The potential of the material is promising, which is substantiated by the work of Di Corcia et al. [57]. For example, specific treatment of the carbon can make it serve simultaneously as a reversed-phase and anion exchanger.

Typical elution patterns are given in Fig. 1. The selectivity is governed by:

- the interaction between molecules which have a planar configuration (e.g., PCDFs, PCDDs, polychlorinated biphenyls) or can easily adopt a planar configuration (such as the planar PCBs), with the (planar) graphitic surface [52,53];
- the number of electronegative substituents on the biphenyl skeleton, [27,52].

These specific properties of carbon allow the separation of planar PCBs from non-planar PCBs and also from related compounds such as PCNs, PCDDs and PCDFs.

Recently, SPE has been shown to be a possible alternative to the traditional clean-up/fractionation schemes for the determination of PCDD/Fs [15,58].

4.2. HPLC

Another recent development in the (group) separation of planar molecules is the use of 2-(1-pyr-
Fig. 1. Carbon chromatographic separation of halogenated aromatic hydrocarbons. Top: HPLC column packed with porous graphitic carbon; eluent, n-hexane, 5 ml min⁻¹, back-flushed from 100–300 ml. Detection: UV, 254 nm. Bottom: open-column active carbon chromatography; for eluents, see ordinate. Adapted from Refs. [32] and [52].

enyl)ethyldimethylsilylated (PYE) silica HPLC columns for the isolation of PCNs, planar and mono-ortho PCBs from other PCB congeners [59–61]. The selectivity of the PYE phase may be explained by a charge-transfer mechanism, in which electron density-acceptor and donor regions of the compounds induce a change in the localization of the π-electron cloud of the pyridine moieties of the PYE phase, so that an electron donor-acceptor complex is formed [59]. The same material has been used to isolate TCDD isomers from synthetic mixtures [62]. Several variants of this material are now available commercially. They can be used more generally to separate planar compounds from non planar ones, as in the separation of aromatics from aliphatics.

5. Hyphenated techniques

Coupled chromatographic techniques permit sensitive and selective methods for the determination of HAC. Perhaps the most important ones today are LC–LC, LC–GC and GC–GC. In these techniques the sample is introduced into the first, LC or GC, column. In LC–LC and LC–GC, this involves introduction of a large volume, in order to enhance the sensitivity of the method. In GC–GC or multi-dimensional GC (MDGC), clean-up is necessary prior to introduction of the extract on the first column. After the separation on the first column, fractions containing the analytes of interest are directed towards the second column where final separation and detection is effected. Interfacing of the two columns is achieved by peak focusing and column switching (LC–LC), cold trapping (LC–GC) or by heart-cutting (GC–GC). Sample pretreatment may be much shorter when such systems are employed. These systems have been used successfully for the enrichment of PCBs and pesticides from aqueous and sediment samples [63,64] at the ppt level, since they allow one to use injection volumes of up to 1 ml. The separation of, e.g., planar PCBs from other PCB congeners can be achieved using a SE-54 and a OV-210 capillary column in a MDGC system [65]: this allows identification of a planar congener even when its relative contribution to the non-separated peak from the first column is as low as 0.01%.

6. Multiresidue methods

For organochlorines, the best-known example of a multiresidue technique is the separation of non-polar and polar organochlorines, which was already practised in the 1950s. With the use of adsorbents such as silica, alumina and florisil, separations were achieved of compounds including the drins, mirex and hexachlorobenzene (and later PCBs) from polar pesticides such as DDTs, chlordane and HCBs. Both fractions were then analysed by GC–ECD. Such methods have been described in detail, [28–30] and incorporated into handbooks and manuals [66,67]. Here, we no longer consider these to be multiresidue methods, although essentially they are.

One of the major extensions of these methods was the introduction of different detectors to increase the selectivity for a single sample. Thus, PAHs could be analysed in the early-eluting non-polar fraction from a given analytical scheme using FID, with analysis by ECD yielding results for the non-polar organochlorines [68]. The development of GC–MS into a routine analytical laboratory
instrument was another great step which enormously enhanced the selectivity.

Nowadays, several sophisticated multiresidue methods are available. Jansson et al. [40] published a method which enables one to fractionate one biological sample into six different fractions. In these it is possible to recover polychlorinated phenols and guaiacols (fraction 1), chlorinated benzenes and drins (2), chlorinated paraffins (3), chlorinated pesticides and brominated diphenyl ethers (4) DDTs and \textit{ortho}-substituted PCBs (5), and finally planar PCBs, polychlorinated naphthalenes and hexahalogenated benzenes (6). Recently, this method was extended by further treating the sulfuric acid residue, resulting in a seventh fraction containing methylsulfonyl-metabolites of PCBs and DDE [69].

With the increasing demand for data on PCDDs and PCDFs, efforts have been made to include these compounds in residue methods. In principle, most of the methods aimed at isolating chlorinated furans and dioxins offer the possibility of determining other organic micropolllutants, and particularly related compounds such as PCBs, since several fractionation steps are incorporated in the analytical schemes. Smith et al. [30] developed the analytical scheme to isolate PCDD/Fs from PCBs using carbon chromatography as described above. Many modifications of this method have been published since. Cuiu et al. [70] described a method to determine phenols, PAHs, chlorobenzenes, PCBs and PCDD/Fs in an extract of a stack train. The extract was divided into two. One part was back-extracted to give an aqueous layer containing phenols, which could be detected after derivatization, and an organic layer containing PAHs. The second half of the extract was fractionated over acid- and base-loaded alumina, using silver nitrate to eliminate sulfur, to give fractions containing chlorobenzenes, PCBs and PCDD/Fs.

When carbon chromatography is applied in the methods above it leads to several fractions containing non-planar or planar compounds. Zebühr et al. [43] developed an automated system for the determination of PAH, PCBs and PCDD/Fs, using two HPLC columns (see the section on Further treatment, with HPLC) in a column-switching system. The method gives five fractions, containing aliphatic and monoaromatic hydrocarbons (1), polycyclic aromatics (2), PCBs with two or more \textit{ortho}-chlorines (3), mono-\textit{ortho} PCBs (4) and non-\textit{ortho} PCBs and PCDD/Fs (5). The method, which uses five switching valves, was successfully applied to extracts of sediments, biological samples and electrostatic filter precipitates.

7. Conclusions

For persistent chlorinated aromatic compounds, clean-up and fractionation are usually performed by the more classical methods. Since a water matrix, in general, presents fewer problems than most other environmental matrices, several new techniques which combine extraction and clean-up through on line adsorption using SPE materials — and selective desorption mechanisms are promising. They will enable faster, cheaper, and more easily automated methods of determination. However, additional group separation will often be required as a consequence of the complex nature and number of congeners that must be handled.

References

[34] M.D. Erickson, Analytical Chemistry of PCBs, Ann Arbor Science, Ann Arbor, MI, 1986.
The use of fish as biomonitors for the determination of contamination of the aquatic environment by persistent organochlorine compounds

J. de Boer *
IJmuiden, The Netherlands

U.A.Th. Brinkman
Amsterdam, The Netherlands

The determination of persistent organic micro-contaminants such as chlorinated biphenyls (CBs) and organochlorine pesticides is hampered by their extremely low solubility in water. Because they are strongly lipophilic these compounds are concentrated in the fat of aquatic organisms and it is much easier to determine them in these lipid fractions. Aquatic organisms can be used as biomonitors if they can reflect the contaminant levels at a certain location and their variations over a period of time. Non-migratory species are, therefore, essential. During the last fifteen years two monitoring programmes, one of Dutch freshwater using eel, and one of the North Sea using cod liver, have been carried out where the potential of fish as biomonitors was demonstrated. In these programmes, spatial differences and temporal trends of contaminant levels have been identified. Correlations with contaminant levels in sediments and water confirmed the suitability of the fish species used as biomonitors. Sampling of a class of restricted length is essential, in order to reduce the contribution of biomagnification to the contaminant levels in fish. No elimination of strongly hydrophobic compounds ($\log K_{ow} > 6.5$) was observed from larger fish. Nevertheless, yellow eel and cod liver can serve as biomonitors, because the reductions in concentration in the water body are reflected by these fish through growth-dilution.

* Corresponding author.