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Review

# Developments in the use of chromatographic techniques in marine laboratories for the determination of halogenated contaminants and polycyclic aromatic hydrocarbons

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**Abstract**

Chromatography has been an important tool in marine laboratories. Since the 1960s, marine laboratories have been involved in the analysis of polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), polycyclic aromatic hydrocarbons (PAHs), and brominated flame retardants (BFRs). Column chromatography and liquid chromatography (LC) techniques have been used, mainly in the clean-up phase, while gas chromatography (GC) has been used extensively in the final determination of these contaminants. Developments have been observed from the use of packed GC columns, via capillary columns to the use of heart-cut multi-dimensional GC and comprehensive multi-dimensional GC. The progress made in interlaboratory studies and the availability of certified reference materials are discussed.
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## 1. Introduction

The interest of authorities in analysing marine water and other marine matrices goes back to the 19th century, when the first marine laboratories were established [1,2]. These laboratories could be divided into two categories: oceanographic laboratories and fisheries laboratories, sometimes combined within a single institute. The oceanographic laboratories initially focussed on the analysis of the seawater for oxygen, pH, temperature, seawater composition (salts) etc. The fisheries laboratories worked in a similar way, but this part was only supportive to the fish biologists in their tasks in fish stock assessment, primary production measurement and in algal bloom studies.

No studies of the contamination of the marine environment are known until after World War II. Because of growing population numbers and inadequate measures for treatment of domestic and industrial sewage, local discharges of large quantities of nutrients into coastal waters created the first noticeable cases of eutrophication. The public concern raised by these activities has contributed measurably to the emergence of aquatic pollution as a political issue [3]. Nutrient measurements were generally based on wet chemical methods, later followed by the use of auto-analyzers.

The second activity of marine laboratories in the field of contamination was focussed on the analysis of trace metals. The first instance of a public health impact resulting from aquatic contamination by a trace metal occurred at Minimata Bay, Japan, commencing in 1953 [3]. Trace element analyses were generally carried out by spectrometric methods. Later, analyses of organotin compounds were carried out by GC–MS or LC–MS [4].

The third group of contaminants, which initiated major activities in marine laboratories, was the group of organochlorine compounds. This group of contaminants created concern because of its adverse effects on wildlife, while, in addition, long-term effects on human health were predicted. DDT was one of the first insecticides, and was found to be present in the environment [5]. Other pesticides, such as dieldrin, hexachlorocyclohexane and chlordane followed later. Gas chromatography (GC) proved to be an ideal method for the analysis of these compounds, in particular, because the electron capture detector (ECD) with a high sensitivity for halogens had just been developed [6–8]. Soon after organochlorine pesticides were established in the analytical programmes of environmental laboratories, interferences were discovered in their chromatograms. Jensen [9,10] discovered that these interfering peaks were caused by the presence of industrial chemicals, known as polychlorinated biphenyls (PCBs), in the same samples. The presence of PCBs in the marine environment caused a major public concern. The combination of a high volume production (cumulative world production ca. 1.2 million tonnes [11]), a high persistency, strong bioaccumulative properties and obvious toxic effects resulted in political action and finally in a world-wide ban of this group of chemicals. Although PCBs have been banned since the early 1980s, monitoring in marine laboratories continues and will continue in future, as these chemicals will only disappear from the environment only very slowly. GC–ECD is the preferred technique for the analysis of PCBs. The development of

capillary columns [12] enabled a congener-specific determination to be undertaken. Multi-dimensional GC techniques and the use of mass spectrometric (MS) detection enabled the detection of the more toxic non-*ortho* substituted (planar) chlorobiphenyls (CBs). Techniques for the analysis of other halogenated compounds, such as brominated flame retardants (BFRs), have only recently been introduced into marine laboratories [13]. For these compounds GC–MS is the most important technique.

Finally, a large number of marine laboratories have been involved in monitoring of polycyclic aromatic hydrocarbons (PAHs), both in general monitoring and investigative studies, and following oil spills. During the 1950s the carcinogenic PAHs were reported to be present in food and cigarette smoke. Later, PAHs were also found to be present in air, due to traffic exhaust gases. The first large oil spill from a tanker occurred in March 1967. The Torrey Canyon, carrying 119,000 tonnes of Kuwait crude oil, ran aground near Land's End, UK. All of the oil cargo was released from the ship, heavily contaminating the coasts on both sides of the English Channel. During the period 1990–1999, there were 346 recorded spills from vessels totalling 1.1 million tonnes of oil, but 75% of the oil spilt derived from only 10 incidents. These included the well-known accidents involving the Braer in Scotland in 1993, and the Sea Empress in Wales in 1996 [14]. The recent accident with the Prestige near the North of Spain shows that these oil spills continue to occur. Monitoring programmes for PAHs were initiated as a result of many of these accidents and monitoring programmes concerning background levels of PAHs also involved a large number of marine laboratories. These analyses are carried out using GC–MS and LC techniques.

Many activities of marine laboratories in the field of monitoring of OCPs, PCBs, BFRs and PAHs, and related QA/QC work are coordinated by the International Council for Exploration of the Sea (ICES), through its Marine Chemistry Working Group (MCWG) which began its activities in 1979. For the past decade laboratory proficiency testing for marine laboratories has been coordinated within the QUASIMEME programme (Quality Assurance of Information for Marine Environmental Monitoring in Europe). The developments in the chromatographic techniques used for OCPs, PCBs, BFRs and PAHs in marine laboratories and some of the related QA/QC activities of the ICES MCWG and QUASIMEME are described in this review.

## 2. Organochlorine pesticides

The environmental presence of DDT was only discovered in the late 1950s [5], in spite of the fact that production began in 1929. Similar delays in awareness of environmental problems related to chemicals have also observed for other contaminants such as PCBs, toxaphene and BFRs. Apparently, 10–20 years of production can pass before persistent and bioaccumulative contaminants are observed by environmental laboratories. This is of course dependent on the production rate, and, to some extent, also of the availability of suitable and sensitive methods and standard materials of high purity. In total, ca. $4\times10^6$ tonnes of DDT have been produced [15].

Soon, other pesticides as the hexachlorocyclohexanes (HCHs), dieldrin, endrin and chlordanes were also discovered in environmental samples. Hexachlorobenzene (HCB) used as a fungicide [16], but also occurring as a by-product of the manufacture of tri- and tetrachloroethylene and chlorine production [17], was also detected. Because the character of these OCPs is strongly hydrophobic ($\log K_{ow} \geq 6$), determinations of these compounds in water have rarely been carried out [18,19]. The OCP concentrations in marine waters were in the range of pg/kg, which puts very high demands on blank values and the sensitivity of detectors. Therefore, monitoring programmes for OCP compounds in marine waters have not been conducted. Also, interlaboratory studies have not been carried out for OCPs in water, apart for γ-HCH, which has a $\log K_{ow}$ of ca. 4 and can therefore also be detected in water samples [20]. Certified reference materials (CRMs) for OCPs in marine water do not exist. The same is true for PCBs, BFRs and PAHs. Therefore, sediments and biota have been the main matrices analysed in marine laboratories.

### 2.1. Extraction

The extraction and clean-up methods for OCPs have been described in detail by various authors

[21–23]. The pre-treatment of the samples [24], as well as most extraction and clean-up techniques are rather similar for sediments and most biota. Extraction can successfully be carried out by Soxhlet extraction [25]. The use of mixed polar/non-polar solvents, e.g. dichloromethane/*n*-pentane or acetone/*n*-hexane is essential to extract all OCPs, including those which are being stored in other lipid layers than those only containing triglycerides. These techniques which have been applied successfully are ultrasonic extraction [26,27], liquid–liquid extraction [28], and supercritical fluid extraction (SFE) [29–31]. More recently two new techniques have been introduced: accelerated solvent extraction (ASE) [32] and microwave assisted solvent extraction (MASE) [33]. ASE is particularly useful when the extraction can be combined with a fat retainer, which leads to a reduction of further clean-up steps. For some samples with low contaminant concentrations, the cell volume may be a limitation. Recently, larger cells have become available. Both ASE and MASE reduce the extraction time by at least an order of magnitude. MASE offers the extra advantage of a sample carrousel, which allows extraction of a series of samples. Because of the more harsh conditions encountered during extraction, the recovery of the analytes should be thoroughly tested before use, as degradation may occur more easily than during Soxhlet extraction. Provided that these techniques have been optimized and tested before use, all of them can be used and will result in satisfactory recoveries [25,34]. More aggressive methods such as saponification and sulphuric acid treatment should not be applied for the extraction and clean-up of a wide range of OCPs as some compounds will be degraded. Dieldrin, endrin and *p,p′*-DDE are sensitive to sulphuric acid treatment and degrade immediately. Also, DDT and DDD are converted to DDE during saponification. These techniques and others [35,36] have also been applied in the past as confirmatory reactions in case of doubt regarding the identity of a peak, e.g. due to a possible co-elution. The recoveries of the various techniques can be checked by spiking (although there is always uncertainty to which extent the spike will represent real-life conditions), by exhaustive extraction with different solvents, or by comparison with other validated methods.

### *2.2. Clean-up*

Clean-up of extracts of biota and sediments is necessary because extraction methods are normally not sufficiently selective (SFE is an exception). This means that the final determination can be affected by (i) lipids, which can cause a deterioration of the column and contamination of the injector and detector, (ii) co-elution with other contaminants such as PCBs, toxaphene, polychlorinated naphthalenes (PCNs), etc., and (iii) other compounds such as sulphur and oil which can cause interfering peaks or an erratic response to the determinand, particularly when using ECD [22].

Frequently used techniques for fat separation are column chromatography over alumina or Florisil columns, and gel permeation chromatography (GPC) [37,38]. GPC is the most elegant technique and can easily be automated because the same column can be used repeatedly for hundreds of samples. A drawback of GPC is that a small remnant of lipids is left in the eluent containing the target analytes. Placing a second GPC column in series is normally the solution, although the use of higher quality gels may also help to solve this problem.

Silica gel and Florisil columns are often used for the fractionation of OCPs and PCBs or other compounds prior to their determination. A combination of alumina and silica gel columns is able to provide lipid-free extracts and complete separation between PCBs, HCB and *p,p′*-DDE in one fraction and the other OCPs in a second fraction [39]. Good results have also been obtained by using tandem solid-phase extraction (SPE) clean-up [40]. The required volumes of organic solvents are much lower with SPE, but the fat capacity of the columns may sometimes be a limitation. Sediments require an extra treatment to remove the sulphur [41]. Appropriate methods for sulphur removal are shaking with tetrabutylammoniumsulfide [28], or treatment with copper [42]. When applying GPC for fat removal, extra treatment for sulphur removal is redundant as sulphur will already have been removed by GPC clean-up. Possible losses of target analytes during these sulphur removing procedures should be tested. Clean-up methods should be tested thoroughly. Shifts in elution patterns may occur between standard solutions and samples, because remaining lipids may

affect the elution profile. Losses resulting from adsorption and evaporation should be checked by recovery experiments. The rotary evaporator is a source of contamination and should regularly be thoroughly rinsed with pentane or hexane. Solvent blanks, glassware and chromatographic material should all be checked for contamination.

### *2.3. GC analysis*

OCPs are sufficiently volatile to be analysed by GC. Also, they are inert and relatively apolar compounds, which allows direct injection without derivatisation.

#### *2.3.1. Detection*

The ECD detector allows a sufficiently sensitive detection, with absolute detection limits of ca. 100 fg. Initially $^{3}H$ (tritium gas) detectors were used, but within a few years these were replaced by the more robust $^{63}Ni$ (foil) detector [43]. The major drawback of the ECD is its poor linearity. Although in the past GC companies have claimed several decades of linearity, this appeared at best to be true only at higher concentrations. In the pg range, where normally most analyses are carried out, the linearity is certainly less than one decade [44]. To solve this problem of poor linearity, it was initially recommended to determine the linear range of the ECD and dilute or concentrate the extract in such a way that it was bracketed by two standards which marked both ends of the linear range [45,46]. As the various OCPs could often be present in samples at different concentrations, this method could sometimes result in a substantial amount of work because several dilutions and injections had to be made. Later, it appeared to be more effective and more precise to work with multi-level calibration [23]. Five or six different dilutions were randomly spread over a series of samples and injected. Several options exist to fit the calibration curve (quadratic, exponential, point-to-point). The differences resulting from the use of various curve-fitting methods were, however, generally small [47]. This method of multi-level calibration is now generally accepted, and has also been used with MS detection, although the linearity of MS detection is generally greater than for ECD. Both electron impact (EI) and electron capture negative ion (ECNI) have been used as ionisation methods in low resolution GC–MS for OCP detection. EI is the more selective method. ECNI is more sensitive, but only for molecules which contain five or more chlorine atoms [48]. Occasionally, and dependent on the substitution pattern, molecules with four chlorine atoms can also be detected by ECNI–MS. High resolution EI–MS has also been applied for OCP determination, but on a relatively small scale as the costs associated with the use of this technique are high compared to the use of ECD and low resolution MS (LRMS). Since the late 1990s an improved ECD (micro-ECD) has been commercially available, which has a very small cell volume and therefore yields both a higher sensitivity and a faster response [49].

#### *2.3.2. Injection*

Splitless injection is the most frequently used technique for the detection of OCPs [46]. On-column injection may be used as an alternative [50], in particular for more labile compounds, which could be degraded in the hot injector. A pre-requisite for on-column injection is that the injected extracts are very clean because otherwise the GC column may deteriorate rapidly. Split injection is not recommended because strong discrimination effects can occur [23]. Large-volume injectors (LVI) are becoming more popular, in particular because OCP concentrations in the marine environment are tending to decrease [51–53]. Disadvantages of LVI are the stronger influence of solvent-impurities in the chromatogram and contamination of the column and detector. The use of ECNI–MS is in particular sensitive to contamination due to LVI. Frequent cleaning of the ion source may be necessary as a result.

#### *2.3.3. GC separation*

OCPs are normally analysed within multi-residue schemes. Following extraction and fat separation, a silica gel or Florisil column separates the extract in two fractions: one in a 100% non-polar solvent, e.g. *iso*-octane, with PCBs, HCB and *p,p′*-DDE, and one in a mixed non-polar/polar solvent, e.g. *iso*-octane with diethylether, with the remaining OCPs such as HCHs, dieldrin, endrin, most relevant chlordanes (*cis*- and *trans*-chlordane, *trans*-nonachlor, oxychor-

dane) and *p,p′*-DDT and *p,p′*-DDD. Most of the *o,p*-metabolites of DDT usually also elute in the second fraction, but the size of the column and composition of the eluents may cause slight changes in the elution pattern. The analysis of the first fraction is normally a relatively easy task. In spite of the presence of PCBs, HCB and *p,p′*-DDE can be determined on various capillary columns without co-elution problems. The analysis of the second fraction is more difficult. Depending on the matrix it can contain a larger number of interfering compounds, which are eluted with the more polar solvent from the silica column. When using an ECD these compounds can cause both positive and negative peaks. Mussels, for example, contain many volatile compounds which interfere with the determination of the HCHs. Treatment with sulphuric acid improves the quality of the chromatograms substantially, but will destroy dieldrin and endrin. Because of these difficulties interlaboratory studies and proficiency tests have always resulted in larger coefficients of variation (C.V.) for OCPs than for PCB (see Section 2.5). These problems are exacerbated by the decreasing OCP concentration in the marine environment with time, following restrictions of their use. Errors due to column adsorption and co-elution have more influence on the final result when the concentration of the target compound is low. Secondly, the combined analysis of a range of OCPs is in fact a poor compromise. A targeted analysis of HCHs at a slower temperature programme would result in a more precise determination of these OCPs. Obviously, more injections would be required, resulting in higher costs, which is apparently not acceptable for most laboratories.

Both polar and non-polar GC columns can be used for the determination of the OCPs, although some OCPs may show a tendency for adsorption to polar columns. Until the late 1970s packed columns were used successfully. Stationary phases used were NPGS (neopentylglycolsuccinate), DEGS (diethylglycolsuccinate), OV-17 (50% methyl 50% phenylpolysiloxane), and mixed phases such as OV-225 (50% cyanopropyl 50% phenylpolysiloxane), with OV-17, and others. Soon after the introduction of the glass and fused-silica capillary columns [12], these were used for the determination of OCPs. Because fewer peaks are present in the OCP fraction than in the PCB-fraction, columns with a shorter length than 50 m may be used, provided the internal diameter is $<0.25$ mm. However, in case the duration of the analysis is less critical, 50-m columns as recommended by Wells et al. [54] for PCB analysis may be also beneficial for the OCP analysis, as such columns offer a better separation, e.g. between the HCHs and early eluting interferences. Suitable stationary phases are e.g. CP Sil8 (95% methyl, 5% phenyl polysiloxane), CP Sil19 (85% methyl, 7% phenyl, 7% cyanopropyl, 1% vinyl polysiloxane) or comparable phases [55–58]. The selection is not critical, but the separation characteristics should of course be tested prior to use.

### *2.4. Toxaphene*

An OCP that merits specific comment is toxaphene. Toxaphene is a mixture of primarily chlorobornanes, with lower concentrations of chlorobornadienes, chlorobornenes, chlorocamphenes, and chlorodihydrochamphenes. The technical mixture is extremely complex with 32,767 theoretically possible chlorobornane congeners [59,60]. In practice ca. 600 compounds are found in industrial formulations, with maybe 20–300 congeners present in environmental samples [60]. Several nomenclature systems have been developed for these compounds [60–66]. Muir and de Boer [67] and de Geus et al. [60] have made detailed overviews on the analysis of toxaphene. Toxaphene is a high volume chemical (cumulative world production $1.3\times10^6$ tonnes [68]), which has primarily been used as a pesticide in cotton production [69]. It is now found worldwide, with high concentrations in the USA and Canada [60,70,71], and arctic regions [60,72] in particular. Although toxaphene was introduced as an insecticide in the late 1940s [60,69], it was not until the late 1970s that toxaphene concentrations were reported in environmental samples [73–75]. This was not so much due to the actual levels of toxaphene, but more to the initial lack of suitable methods for their analysis (packed GC columns caused broad peaks and the ECD was not very sensitive for the aliphatic chlorobornanes), and to the reluctance of analysts who were initially deterred from undertaking these analyses by the complexity of the chemical.

The introduction of capillary columns helped, as it

did for PCBs, to enable a congener-specific analysis. However, the higher degree of complexity of the toxaphene pattern caused doubt regarding the purity of the selected peaks for this congener-specific analysis. Multi-dimensional GC (MDGC) analysis, using a heart-cut technique showed that in particular chlorobornane 26 (Parlar nomenclature [63]) co-eluted on a single capillary column with other compounds [76,77]. MDGC appeared to be a very useful technique for the analysis of such complex mixtures. Nevertheless its acceptance in marine laboratories was a slow process and even now it is not used routinely, mainly because analysts are deterred by possible technical difficulties in routine applications. However, many MDGC systems are successfully being used in the control of industrial processes [78]. In the 1990s, comprehensive MDGC or GC×GC was developed. The advantage of this technique was that the entire first-dimension chromatogram could be sent to the second dimension, resulting in a true three-dimensional chromatogram [79–82]. This technique is very suitable for complex mixtures such as toxaphene. A number of chlorobornanes have been analysed in narwhal blubber without interferences [83,84].

More complex mixtures of environmental contaminants, such as polychlorinated alkanes (paraffins) [85,86], polychlorinated naphthalenes (PCNs) [87,88] may find a place on the future list of interest of marine laboratories. It may therefore be expected that GC×GC, now it has become much more robust, will find its way to the marine laboratories to serve for semi-routine analyses of complex mixtures of environmental contaminants.

#### *2.4.1. Chiral compounds*

A large number of toxaphene congeners show chiral activity. Since bioaccumulation and metabolism in biota are often different for different enantiomers, a change in the enantiomeric ratio can be expected during disposition in the food chain. The determination of enantiomer ratios of chlorinated bornane congeners in biota can give an indication whether a specific biological mechanism changes the enantiomer ratio, If a ratio of one is found (as in the technical mixture), a high biological persistency is likely. In this way comparison of the enantiomer ratio of different congeners in combination with their chemical structures can provide an insight into the metabolism of these compounds. Some enantiomers may also be more toxic than others, so additional information on the occurrence of specific toxic enantiomers is also obtained. The occurrence of enantiomers is also known for some other OCPs (chlordane, HCHs) [89,90]. Heart-cut MDGC, and possibly in the future also GC×GC, may enable the determination of enantiomers among many other congeners. De Geus et al. [91] combined a non-polar GC column (Ultra2-55 phenyl, 95% methylsilicone) with an enantioselective column containing a mixture of OV-1701 and heptakis(2,3,6-*O*-*test*-butyldimethylsilyl)-β-cyclodextrin (10:1, v/v). In all hake and dolphin samples analysed a significant deviation from the racemic ratio was found for the chlorobornanes 38 and 65 [63]. Until now these enantiomer-specific determinations have only been carried out by specialized groups and have not been applied routinely in most marine laboratories.

### *2.5. Interlaboratory studies and CRMs*

Interlaboratory studies have been conducted for OCPs since 1969 [92]. The between-laboratory coefficients of variation (C.V.) together with the mean concentration of the target analyte are given in Table 1. Over a period of more than 30 years, one would maybe expect to see a positive time trend in the C.V. values, because the techniques which have been used have been improved gradually. Such a trend is not visible in Table 1, and a number of reasons can be given to explain that. In the initial interlaboratory studies fish oil was often used, because (i) it contained measurable concentrations of OCPs, and (ii) other materials such as freeze-dried materials or wet fish tissues had disadvantages in, respectively, their use or in the dispatch to participants. These fish oils, however, normally contained very high concentrations of OCPs, and were in addition often spiked [92–94]. This facilitated the analysis, but resulted in uncharacteristically low C.V. values for that period. Later sterilized fish tissue became available, which solved this problem [97,106]. Fig. 2 shows that indeed a trend can be seen for the OCPs (and CBs) with high C.V. values for low concentrations and lower C.V. values for high concentrations. Secondly, the environmental concentrations of

Table 1
Between-laboratory C.V. values (%) for $p,p'$-DDE, γ-HCH, dieldrin, CB52 and CB153 and related concentrations (μg/kg) over the period 1969–2002

| Study | DDE | | γ-HCH | | Dieldrin | | CB52 | | CB153 | | Matrix | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Conc. | C.V. | Conc. | C.V. | Conc. | C.V. | Conc. | C.V. | Conc. | C.V. | | |
| OECD, 1969 | ? | 14 | – | – | – | – | – | – | – | – | Spiked fish oil | [92] |
| ICES 2, 1973 | 450 | 30 | 80 | 70 | 115 | 55 | – | – | – | – | Fish oil | [93] |
| ICES 4, 1979 | 80 | 38 | 11 | 71 | 79 | 37 | – | – | – | – | Fish oil | [94] |
| ICES 5, 1983 | 189 | 69 | 113 | 32 | 95 | 55 | 61 | 22 | 87 | 45 | Herring oil | [95] |
| ICES 6, 1985 | – | – | – | – | – | – | 84 | 68 | 93 | 40 | Herring oil | [96] |
| ICES/IOC/OSPARCOM, 1994 | – | – | – | – | – | – | 0.24 | 115 | 1.5 | 51 | Herring oil | [106] |
| QUASIMEME 4/5, 1996 | 0.54 | 27 | 0.23 | 90 | 0.52 | 30 | 0.43 | 44 | 3.5 | 22 | Mussels | [97] |
| QUASIMEME 4/5, 1996 | 1.8 | 29 | 0.79 | 43 | 1.2 | 46 | 0.62 | 45 | 5.4 | 30 | Plaice | [97] |
| QUASIMEME 4/5, 1996 | 4.1 | 27 | 1.5 | 60 | 4.4 | 65 | 0.99 | 48 | 3.6 | 30 | Mackerel | [97] |
| QUASIMEME 4/5, 1996 | 4.9 | 39 | 0.9 | 69 | 7.2 | 62 | 27 | 28 | 13 | 29 | Sediment | [97] |
| QUASIMEME 4/5, 1996 | 1.1 | 26 | 0.09 | 77 | 0.49 | 36 | 1.4 | 23 | 6.1 | 21 | Sediment | [97] |
| QUASIMEME 4/5, 1996 | 1.1 | 29 | 0.14 | 79 | 0.59 | 57 | 1.4 | 25 | 5.7 | 24 | Sediment | [97] |
| QUASIMEME 4/5, 1996 | 7.6 | 37 | 0.6 | 77 | 1.2 | 93 | 32 | 31 | 45 | 29 | Cod liver oil | [97] |
| QUASIMEME 4/5, 1996 | 493 | 20 | 17 | 52 | 46 | 41 | 21 | 22 | 174 | 14 | Cod liver oil | [97] |
| QUASIMEME 4/5, 1996 | 760 | 16 | 8.0 | 24 | 86 | 41 | 37 | 19 | 345 | 10 | Cod liver oil | [97] |
| QUASIMEME 10, 1997 | 2.9 | 38 | 0.07 | 119 | 0.58 | 67 | 2.3 | 45 | 21 | 34 | Mackerel | [98] |
| QUASIMEME 10, 1997 | 6.5 | 27 | 3.1 | 49 | 6.8 | 47 | 1.6 | 46 | 6.4 | 40 | Herring | [98] |
| QUASIMEME 12, 1998 | 1.7 | 29 | 0.35 | 49 | 0.96 | 32 | 0.69 | 38 | 4.8 | 25 | Plaice | [99] |
| QUASIMEME 12, 1998 | 2.8 | 32 | 0.16 | 73 | 0.85 | 62 | 1.9 | 28 | 18 | 30 | Mussels | [99] |
| QUASIMEME 16, 1999 | 6.3 | 27 | 2.1 | 40 | 8.4 | 45 | 1.6 | 23 | 5.6 | 20 | Herring | [100] |
| QUASIMEME 16, 1999 | 0.92 | 29 | 0.22 | 70 | 0.89 | 62 | 0.52 | 38 | 5.4 | 17 | Mussels | [100] |
| QUASIMEME 16, 1999 | 0.34 | 60 | 0.13 | 53 | 0.17 | 41 | 0.56 | 29 | 1.8 | 21 | Sediment | [100] |
| QUASIMEME 16, 1999 | 0.19 | 71 | 0.25 | 88 | 0.22 | 76 | 0.32 | 53 | 0.70 | 21 | Sediment | [100] |
| QUASIMEME 18, 2000 | 1.0 | 44 | 0.26 | 60 | 0.56 | 45 | 1.28 | 41 | 5.7 | 32 | Sediment | [101] |
| QUASIMEME 18, 2000 | 0.29 | 40 | 0.13 | 69 | 0.30 | 77 | 0.53 | 39 | 1/63 | 26 | Sediment | [101] |
| QUASIMEME 18, 2000 | 4.6 | 32 | 0.29 | 46 | 2.9 | 52 | 3.0 | 35 | 6.8 | 28 | Dab | [101] |
| QUASIMEME 18, 2000 | 2.7 | 35 | 0.22 | 82 | 1.0 | 53 | 2.1 | 29 | 19 | 37 | Mussels | [101] |
| QUASIMEME 20, 2000 | 0.61 | 20 | 0.45 | 48 | 0.73 | 100 | 1.0 | 37 | 3.7 | 30 | Sediment | [102] |
| QUASIMEME 20, 2000 | 1.0 | 53 | 0.35 | 74 | 0.69 | 66 | 3.9 | 39 | 4.7 | 36 | Sediment | [102] |
| QUASIMEME 20, 2000 | 1.3 | 28 | 0.17 | 93 | 0.90 | 30 | 0.85 | 31 | 9.0 | 25 | Mussels | [102] |
| QUASIMEME 20, 2000 | 155 | 30 | 1.5 | 132 | 46 | 64 | 26 | 28 | 359 | 31 | Cod liver | [102] |
| QUASIMEME 22, 2001 | 0.57 | 33 (31)* | 0.29 | 67 (66)* | 0.25 | 35 (39)* | 0.6 | 42 (45)* | 2.8 | 18 (19)* | Sediment | [103] |
| QUASIMEME 22, 2001 | 0.7 | 54 (53)* | 0.9 | 63 (65)* | 7.3 | 61 (38)* | 21 | 44 (46)* | 11 | 36 (24)* | Sediment | [103] |
| QUASIMEME 22, 2001 | 0.9 | 30 (29)* | 0.06 | 133 (114)* | 0.6 | 49 (40)* | 0.12 | 77 (64)* | 1.0 | 28 (30)* | Plaice | [103] |
| QUASIMEME 22, 2001 | 2.2 | 23 (16)* | 0.14 | 139 (104)* | 1.7 | 51 (45)* | 0.40 | 39 (34)* | 6.4 | 15 (11)* | Mussel | [103] |
| QUASIMEME 26, 2002 | 1.0 | 25 (16)* | 0.10 | 105 (125)* | 0.54 | 30 (17)* | 0.14 | 62 (58)* | 1.1 | 26 (20)* | Plaice | [104] |
| QUASIMEME 26, 2002 | 2.3 | 19 (16)* | 0.15 | 104 (116)* | 1.6 | 48 (39)* | 0.44 | 43 (25)* | 6.6 | 26 (20)* | Plaice | [104] |
| QUASIMEME 28, 2002 | 0.59 | 38 (16)* | 0.22 | 40 (15)* | 0.45 | 89 (21)* | 0.52 | 60 (13)* | 2.7 | 29 (15)* | Sediment | [105] |
| QUASIMEME 28, 2002 | 0.65 | 35 (17)* | 0.11 | 52 (17)* | 0.16 | 82 (14)* | 0.23 | 58 (21)* | 1.2 | 31 (15)* | Sediment | [105] |

* Values in brackets based on new statistical model of Cofino [109].

OCPs have decreased, particularly during the last 10 years [107]. Therefore, possible lower C.V. values due to the availability of better techniques have been compensated by higher C.V. values due to lower OCP concentrations in the interlaboratory study test materials. The γ-HCH values in Table 1 illustrate this effect nicely. Thirdly, the influence of the use of different statistical techniques has not been unimportant. C.V. values shown in Table 1 have been generated by at least three or four different statistical techniques. The first reports were normally based on the use of the mean and relative standard deviation per component [92–94]. Later, multi-variate techniques have been used to generate C.V. values [46,96,106,108]. In the QUASIMEME programme robust statistics have been used [97–105], which were later replaced by the Cofino model [109]. Robust statistics were supposed to downweight outliers, in that way avoiding the use of relatively arbitrary outlier tests. The Cofino model offers a better understanding of the data distribution and calculates C.V. values for a first and possible second mode. The values obtained in the QUASIMEME programme by the Cofino model are shown in brackets in Table 1. They have not been used in the calculations for Fig. 1. Table 1 shows that, dependent upon the settings for resolution, the Cofino model generally results in lower C.V. values. Although it should be kept in mind that there is often a second group of laboratories with a poorer performance, not included in the calculated first C.V. value, it seems that the use of this model offers more realistic performance data. The downweighting effect of the robust statistics has been overestimated. The analytical performance of marine laboratories for OCPs (and CBs) is therefore better than was believed during the last five years. However, it should also be stated that the OCP analysis in marine laboratories has always lacked the quality that it could have had. The use of the ECD with many negative peaks in the polar silica gel fraction was one reason, the poor compromise of a multi-residue analyses for a series of OCPs is a second reason. A targeted analysis for just HCHs or DDTs, or dieldrin and endrin, preferably carried out by GC–MS would have improved the overall quality.

Table 2 shows the available certified reference materials (CRMs) for OCPs in marine matrices. Unfortunately, wet sterilised materials are not available yet as CRM for OCPs. The available oils have the disadvantage of unrealistically high OCP concentrations, although the BCR CRM 598 cod liver

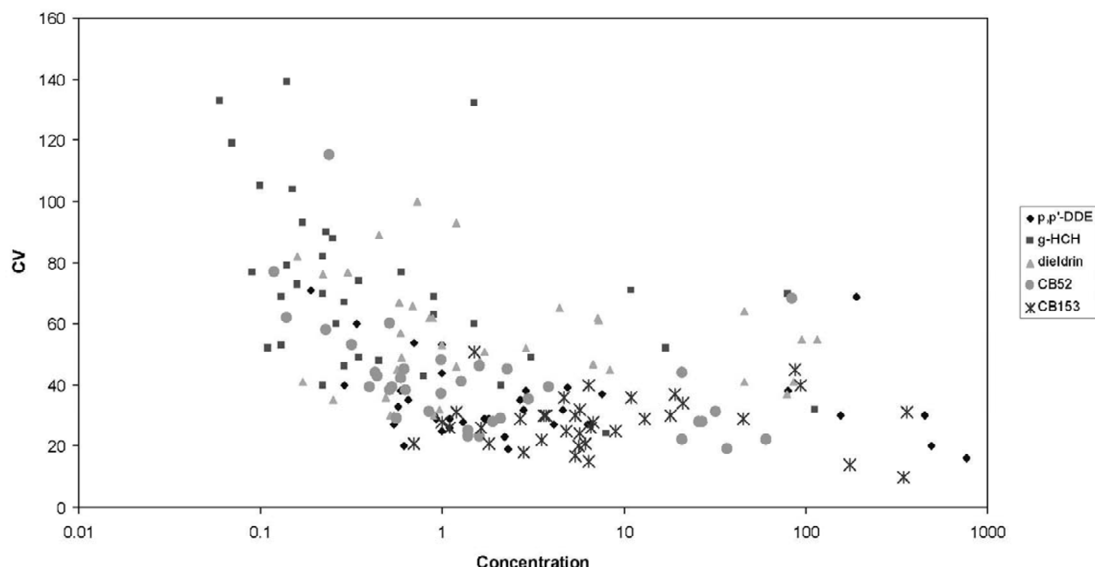



Fig. 1. Correlation between between-laboratory C.V. and concentration of analyte, for $p,p'$-DDE, γ-HCH, dieldrin, CB 52 and CB 153 in biota and sediments.

Table 2
Certified reference materials for OCPs

| CRM | Material | Form | Producer | Country | Number of certified OCPs |
|---|---|---|---|---|---|
| SRM 1974a | Mussel | Frozen | NIST | USA | 7 |
| SRM 1588a | Cod liver | Oil | NIST | USA | 14 |
| SRM 1945 | Whale blubber[a] | Frozen | NIST | USA | 15 |
| SRM 2974 | Mussel | Freeze-dried | NIST | USA | 7 |
| SRM 2977 | Mussel | Freeze-dried | NIST | USA | 7 |
| SRM 2978 | Mussel | Freeze-dried | NIST | USA | 12 |
| 140/OC | Fucus (plant) | Freeze-dried | IAEA | Monaco | 5 |
| BCR 598 | Cod liver | Oil | BCR | EU | 13 |
| SRM 1944 | Sediment | Freeze-dried | NIST | USA | 4 |
| SRM 1939a | Sediment | Air-dried | NIST | USA | 3 |
| IAEA 383 | Sediment | Freeze-dried | IAEA | Monaco | 1 |
| IAEA 408 | Sediment | Freeze-dried | IAEA | Monaco | 4 |

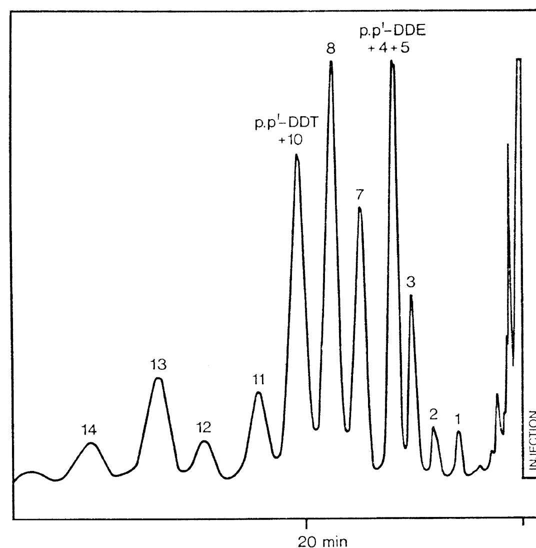
NIST: National Institute for Standards and Technology; BCR: Bureau Communautaire de Reference, EU; IAEA: International Atomic Energy Agency.

[a] Whale blubber cannot be transported overseas due to restrictions in transporting marine mammal materials.

seems to be relatively suitable. Dried materials also contain higher OCP concentrations than wet materials and have the additional disadvantage that the matrix may behave different during extraction.

## 3. PCBs

Over the last three decades marine laboratories have invested major quantities of energy in developing and carrying out PCB analyses. The amount of work was justified by the fact that PCBs caused visible damage to the environment. The most striking example was that of decreasing seal populations in the North Sea [110]. Although thin layer and column chromatography techniques were tried initially [111], PCBs can best be analysed by GC, after an extraction and clean-up. As mentioned previously, PCBs were actually discovered as interferences in an OCP analysis [9]. The extraction and cleanup procedures are similar to those for OCPs (see Section 2) and will therefore not be discussed separately. PCBs are eluted from silica or Florisil columns by non-polar solvents such as *iso*-octane, in a first fraction, often together with HCB and *p,p'*-DDE. In the early 1970s the analysis was complicated and prone to relatively large errors. Perchlorination of PCBs, resulting in one decachlorobiphenyl peak, often led to false positive results [112]. GC analysis, using packed columns was not able to provide sufficient resolution for the separation of the 209 possible congeners (Fig. 2a). The same stationary phases were used as for OCP (see Section 2.3.3). At least 50% of these 209 congeners were expected to be present at measurable concentrations in the technical mixtures, such as Aroclor 1248, Aroclor 1254, Aroclor 1260 or similar mixtures from other manufacturers (Kanechlor, Clophen, Phenochlor) [113]. Environmental samples, in particular biological matrices from higher levels in the food chain, contained lower numbers of congeners due to weathering effects and biotransformation. However, even in these samples, the individual congeners could not be completely separated. The most important source of error was the mismatch between the PCB pattern in the technical mixture and those in the environmental samples. Various methods were developed to improve the quantification of the PCBs [111,114]. Sometimes the entire areas of all peaks of the patterns were used, whilst sometimes one or more characteristic peaks were selected. Another drawback was that changes in patterns could not be observed distinctly, which hindered the establishment of a relationship between the environmental sample and a possible PCB source. Finally, when individual chlorobiphenyl (CB) congeners became commercially available, it appeared that they also all had their own specific toxicity. A congener-specific analysis was, therefore,



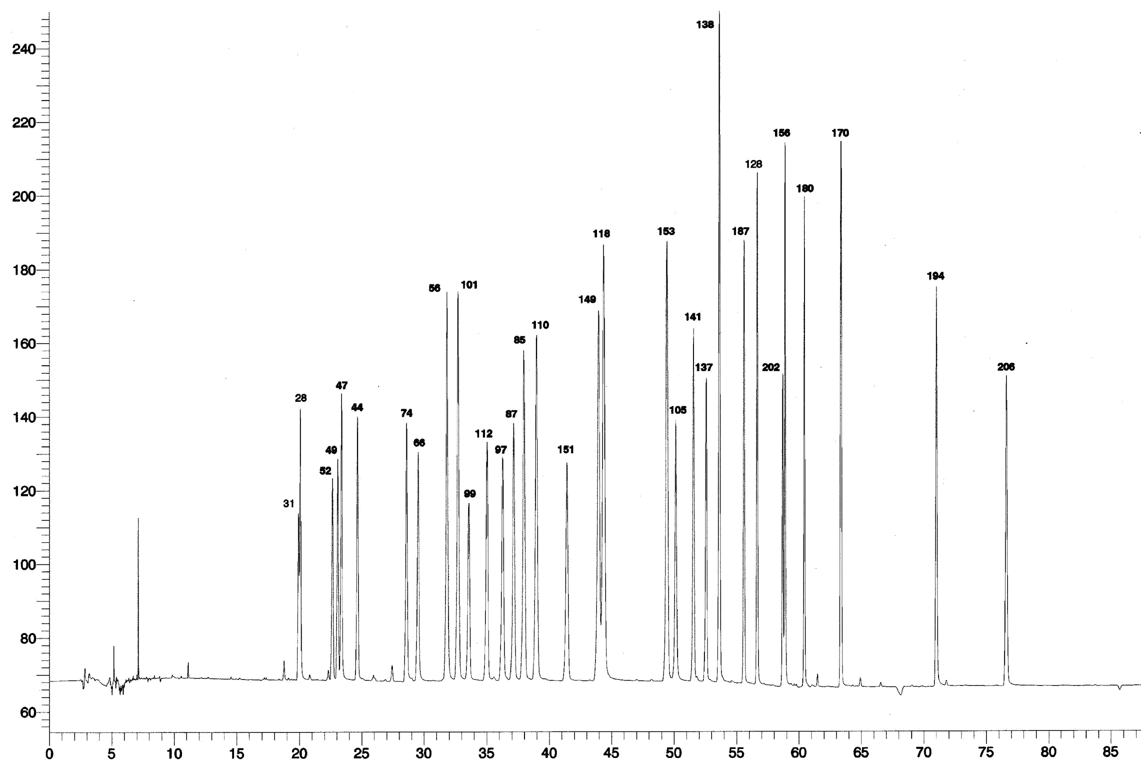




Fig. 2. (a) Progress in PCB analysis: packed column GC–ECD chromatogram of PCBs in white-tailed eagle; 4% SF 96 on Gas Chrom P (HMDS treated) [9]. (b) Progress in PCB analysis: capillary column GC–ECD chromatogram of PCBs standard solution on a 50 m×0.15 mm, 0.3 μm I.D., CP Sil 19 column. (c). Progress in PCB analysis: part of GC×GC chromatogram of mixture of 90 CBs and 17 PCDDs/PCDFs. HP 1 (30 m, 0.25 mm, 0. 25 μm)–HT 8 (1 m, 0.1 mm, 0.1 μm) column combination [140].

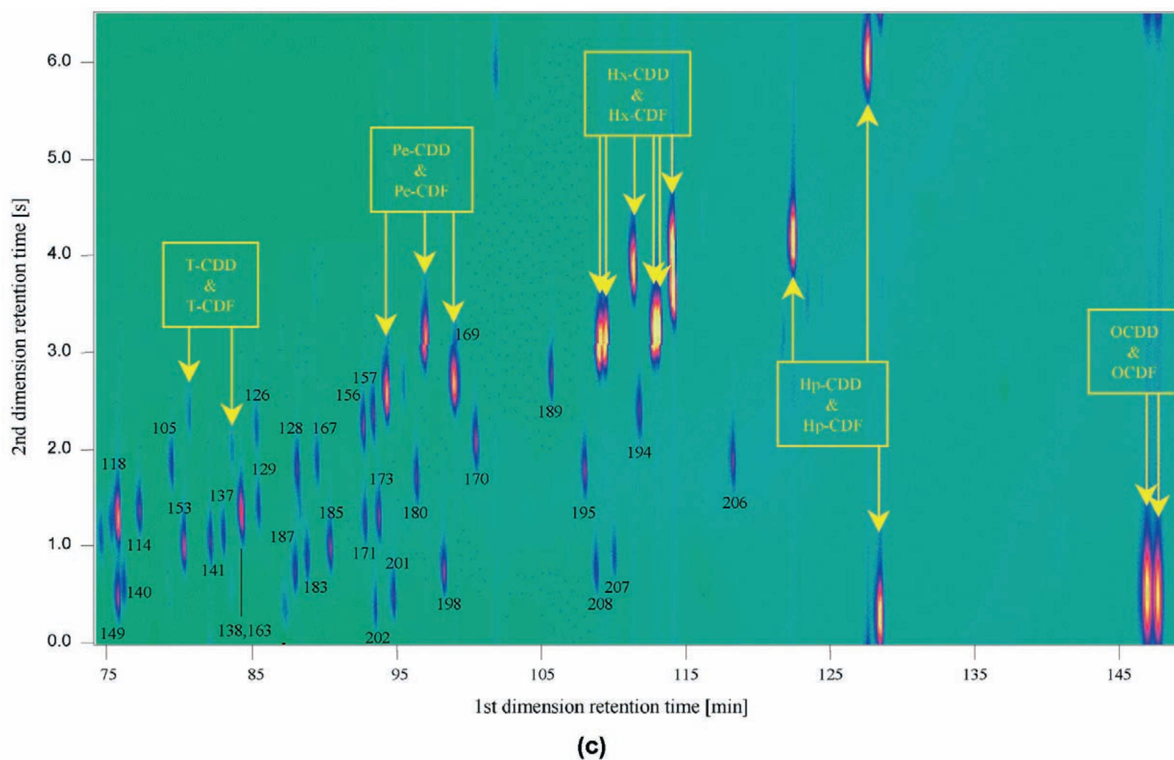



Fig. 2. (*continued*)

essential, when their effects were to be understood. The availability of the (fused-silica) capillary column [12] was therefore a breakthrough for PCB analysis. Suddenly, congener-specific analyses were possible (Fig. 2b). The stationary phases used were similar to the one used for OCP (see Section 2.3.3). Normally, both fractions from the silica column were injected, but some laboratories analysed all PCBs and OCPs together in a single run [47]. Detection and injection was not different from that for OCPs (see Sections 2.3.1 and 2.3.2). However, spitless injection required an even more careful optimization than for OCPs, as the volatility range was much larger for PCBs, which made discrimination effects easy to occur [47]. Lang [21], Law and de Boer [24], Hess [115], and de Boer [116] have produced extensive reviews on PCB analysis.

Mullin et al. [117] have given retention times for all 209 PCB congeners. Unfortunately, not all CBs could be separated on a single capillary column, even one with a length of 50 m and an internal diameter of only 0.15 mm [118]. Greater lengths and smaller diameters would improve the separation further, but would result in problems as the pressure regulators of most, if not all commercial GC instruments would be unable to deliver carrier gas at high enough pressure to achieve an optimum flow-rate [117]. Also, as a result of these higher pressures, leakages would easily occur. More useful information on retention times of PCB congeners on different GC columns was made available by Larsen and co-workers [119,120] and de Boer et al. [121]. Other GC techniques, such as the use of parallel GC [122], and serial GC [123], were developed for PCB analysis, but never found their way to routine PCB monitoring in marine laboratories.

### 3.1. Dioxin-like PCBs and dioxin and furans

During the 1980s it became clear that some of the PCB congeners showed a type of toxicity that was comparable to that of dioxins [124–126]. The non-

*ortho* substituted CBs were able to bind with the Ah receptor and in that way, although to a lesser extent, cause dioxin-like effects. These 3,4- or 3,4,5-substituted CBs (77, 81, 126, 169) [127], were, however, only present at very low concentrations, often ca. 1000 fold lower than the so-called indicator PCBs, such as 52, 101, 138, 153 and 180 [127]. The toxic effect of these CBs was, however, thought to be more than 1000-fold higher than that of the indicator CBs, which made a specific analysis of the non-*ortho* CBs essential. The situation became more complicated when it appeared that mono-*ortho* substituted CBs could also show a dioxin-like effect, although with a smaller effect than the non-*ortho* substituted CBs [128]. The concentrations of some mono-*ortho* substituted CBs, 118, 105, 156, were comparable to those of the indicator (di-*ortho* substituted) CBs, but concentrations of other mono-*ortho* substituted CBs (CB 114, 123, 156, 167, 189) were low. The European Commission has indicated that in the future all these non- and mono-*ortho* PCBs should be included within maximum residue limits for food, including fish and shellfish [129]. This challenge to the chromatographer was solved in the following way. It appeared that carbon columns were able to adsorb non- and mono-*ortho* PCBs for a longer period than di-*ortho* CBs, this because non- and mono-*ortho* CBs could adopt a planar structure, which, because of steric hindrance of the di-*ortho* CBs, was not possible for the di-*ortho* CBs. By changing the eluent from pentane/hexane (for di-*ortho* CBs) to toluene for the non- and mono-*ortho* CBs it was possible to collect different fractions. This type of separation was possible by using traditional column chromatography with carbon columns [130] and by using graphitzed carbon columns in LC [131–133]. In addition to carbon, it was also possible to use 2-(1-pyrenyl) ethyldimethylsilylated (PYE) LC columns [134]. The di-*ortho* and the mono-*ortho* CBs 105, 118, 156 were normally analysed by GC/ECD, while the non- and other mono-*ortho* CBs were analysed by GC–MS, using EI or ECNI and $^{13}$C-labelled standards [135,136]. The latter determination was often combined with that of dioxins and furans [130,137].

MDGC has also been used for the determination of non-*ortho* and mono-*ortho* CBs, as it was used in some specialized laboratories to obtain a better separation of the indicator CBs [138]. MDGC was in particular useful for a pure determination of a number of mono- and di-*ortho* CBs during the certification of reference materials [139]. Meanwhile interesting applications for a combined PCB and dioxin analysis have been shown by the use of GC×GC [140,141]. Ninety CBs and 17 relevant dioxins and furans could be separated in one GC run (Fig. 2c). The availability of better quality cryogenic modulators will further improve this type of separations, and may help to make alternative, cheaper and reliable dioxin analyses available.

A number of marine laboratories have carried out non-*ortho* PCB analyses. Dioxin and furan analyses in marine biota and sediments have generally been carried out by laboratories specializing in dioxin analysis in a range of matrices, as the high costs for the required high resolution MS and isotope-labelled standards could not normally be afforded by marine laboratories. Now new and stricter MRLs for dioxins in fishery products have been established by the European Commission [129], and alternative methods are expected to become available, it may well be that dioxin analyses may be carried out on a more routine basis in the near future in marine laboratories.

### *3.2. Interlaboratory studies and CRMs*

Table 1 shows the between-laboratory C.V. values and related concentrations of the CBs 52 and 153, obtained in various interlaboratory studies. Results expressed as total PCBs (from packed column GC) that were obtained during earlier studies have not been included. The C.V. values for total PCB in the period 1970–1985 were generally around 50% [93,94]. Fig. 1 shows a similar trend for CBs as for OCPs: higher C.V. values with lower concentrations and lower C.V. values with higher concentrations. However, in contrast with OCPs, at least some improvement in C.V. values is seen with time. This is mainly due to a lot of effort, which has been spent on improving the analysis of PCBs. Many exercises, studies, and workshops in ICES and QUASIMEME, and also in the EU–BCR working group on PCBs, have been devoted to the improvement of the PCB analysis [23,45–47,97,106,108]. This has finally resulted in CB analyses with between-laboratory C.V.

Table 3
Certified reference materials for PCBs

| CRM | Material | Form | Producer | Country | Number of certified congeners |
|---|---|---|---|---|---|
| SRM 1974a | Mussel | Frozen | NIST | USA | 20 |
| SRM 1588a | Cod liver | Oil | NIST | USA | 24 |
| SRM 1945 | Whale blubber[a] | Frozen | NIST | USA | 27 |
| SRM 2974 | Mussel | Freeze-dried | NIST | USA | 20 |
| SRM 2977 | Mussel | Freeze-dried | NIST | USA | 25 |
| SRM 2978 | Mussel | Freeze-dried | NIST | USA | 22 |
| 140/OC | Fucus (plant) | Freeze-dried | IAEA | Monaco | 12 |
| CARP-1 | Carp | Slurry | NRC | Canada | 9 |
| BCR 349 | Cod liver | Oil | BCR | EU | 6 |
| BCR 350 | Mackerel | Oil | BCR | EU | 6 |
| BCR 682 | Mussels | Wet, sterilised | BCR | EU | 8 |
| BCR 618 | Herring | Wet, sterilised | BCR | EU | 12 |
| BCR 619 | Chub | Wet, sterilised | BCR | EU | 4[b] |
| EDF 2524 | Fish | Slurry | CIL | USA | 2 |
| EDF 2525 | Fish | Slurry | CIL | USA | 2 |
| CS-1 | Sediment | Freeze-dried | NRC | Canada | Total-PCB |
| HS-1 | Sediment | Freeze-dried | NRC | Canada | 10 |
| HS-2 | Sediment | Freeze-dried | NRC | Canada | 10 |
| SRM 1944 | Sediment | Freeze-dried | NIST | USA | 28 |
| SRM 1939a | Sediment | Freeze-dried | NIST | USA | 20 |
| IAEA 383 | Sediment | Freeze-dried | IAEA | Monaco | 17 |
| IAEA 408 | Sediment | Freeze-dried | IAEA | Monaco | 14 |
| BCR 536 | Sediment | Dried | BCR | EU | 13 |

[a] Whale blubber cannot be transported overseas due to restrictions in transporting marine mammal.
[b] Non-ortho CBs; NIST: National Institute for Standards and Technology; BCR: Bureau Communautaire de Reference, EU;, IAEA: International Atomic Energy Agency; CIL: Cambridge Isotope Laboratories; NRC: National Research Council.

values of 15–20%. The higher chlorinated CBs (hexa-hepta, e.g. 153, 180) are normally somewhat easier to determine than the lower chlorinated ones (tri-tetra, e.g. 28, 52). Fig. 1 shows that CB 52 as γ-HCH is more sensitive for concentration differences than CB 153.

CRMs for PCBs are now widely available (Table 3). Most materials, including one for non-*ortho* CBs (BCR CRM 619), have realistic PCB levels and can be well compared with other samples as regards behavior during extraction and clean-up.

## 4. Brominated flame retardants (BFRs)

Flame retardants are substances used in plastics, textiles, electronic equipment and other materials in order to prevent them catching fire. Some of the technical flame retardant products contain brominated compounds such as polybrominated diphenylethers (PBDEs), polybrominated biphenyls (PBBs), hexabromocyclododecane (HBCD) and tetrabromobisphenol-A (TBBP-A). The BFRs may reach the marine environment through leaching from landfills, in effluents from sewage treatment plants, by evaporation from waste incinerators, and by other pathways. A number of BFRs have been shown to bioaccumulate in environmental biota and humans [13,142]. PBBs and PBDEs are complex mixtures with the same number of possible congeners (209) and can be described using the same numbering system that commonly used for PCBs [127].

PBBs were manufactured in the early 1970s and drew the attention of the public when, in 1973, they were mixed with cattle feed in Michigan, which led to a number of casualties [143]. Soon after that disaster the production ceased. Therefore, PBBs have rarely been determined in marine laboratories. PBDEs have also been produced since the early 1970s. There are three main products: the Penta-mix,

Octa-mix and Deca-BDE formulations [13]. Penta-mix related congeners were found in the aquatic environment, including the marine environment, since the late 1970s [143,144]. However, initially they did not draw much attention, and were only determined in a few marine laboratories. This situation changed completely in 1998 when it was shown that PBDEs were present in sperm whales and because of that could be considered as global pollutants [145]. At the same time exponentially increasing time trends of Penta-mix related PBDEs were reported in Swedish human milk [146]. These two observations have led to an enormous increase in activity in environmental laboratories, including marine laboratories in developing analytical methods for PBDEs and other BFRs [147].

The pre-treatment, extraction and clean-up for most PBDEs is very similar to that for PCBs (see Section 3). Most PBDEs elute together with most OCPs in the second (polar) fraction of a silica gel column. PBDEs can also be analysed by GC/ECD but co-elutions with OCPs or, when no fractionation is applied, with PCBs easily occurs, although the number of BDE congeners present in technical mixtures and biological and sediment samples is substantially lower than that of PCBs. The major congeners are BDEs 47 (tetra), 99 and 100 (pentas). BDE 183 (hepta) is considered indicative of the Octa-mix, but does not generally occur at high concentrations. GC–ECNI–MS or GC–EI–LR–MS are the two techniques most frequently used for PBDE analysis. ECNI–MS is a very sensitive method but it is less selective as often only the Br-mass fragment ($=m/z$ 79,81) can be seen. An advantage to ECD is of course the possibility to detect unknown bromine compounds. EI–MS is more selective, but has a much lower sensitivity. Combination of EI–MS with large volume injections may prove to be fruitful [148]. HRMS can be used but is, obviously, much more expensive than LRMS due to the increased capital and running costs of the instrumentation [149].

In addition to Penta- and Octa-mix PBDE congeners, it was found that in sediments deca-BDE (BDE 209) could be present, occasionally at relatively high concentrations [150]. The determination of deca-BDE is, however, much more complicated than that of the other PBDEs. Deca-BDE is a thermally labile compound that degrades when exposed for too long periods to temperatures above 300–320 °C [13,147,149]. This means that splitless injection is critical and can only be applied successfully when combined with a pressure pulse or by using a short splitless time. The latter may, however, cause some discrimination as a result. Fig. 3 shows a comparison of a splitless injection of BDEs and a septum equipped temperature programmable injection [53]. These conditions should thoroughly be tested and optimized. On-column injection may of course be a suitable alternative.

Deca-BDE is also sensitive to (UV) light. Therefore, possible incoming UV light should be blocked by placing UV filters at windows and below fluorescent lights. Short GC columns have to be used ($<15$ m) with a relatively thin film (0.1 μm) to reduce the residence time in the GC oven as much as possible. Fig. 4 shows a chromatogram in which decaBDE has been degraded due to too long exposure to high column temperatures [53]. The injection and maximum GC oven temperature should be kept below 320 °C.

During clean-up special attention should be paid to evaporation steps. In case an extract containing deca-BDE is evaporated to dryness, deca BDE may be only partially redissolved. This could easily lead to losses [151]. Coolers used for Soxhlet extraction and rotary evaporation should be rinsed frequently to avoid cross-contamination. Blanks do not correct for contamination of selective parts of glassware. GC columns used for PBDE (including deca BDE) analysis are generally non- or medium polar columns, comparable to those used for PCBs. As indicated above the analysis of Penta- and Octa-mix related congeners requires good resolution, which can be obtained by 50-m columns with an internal diameter of $<0.25$ mm.

In addition to PBDEs, the presence of HBCD and TBBP-A in environmental samples has been reported [151,152]. HBCD can be analysed by GC–MS, but the accuracy of that analysis will never reach that of some PBDEs. HBCD consists of three diastereomers, which are converted into each other at temperatures above 160 °C [153,154]. As GC cannot separate these diastereomers from each other, the change in pattern caused by the transformation is not visible. The HBCD peak is just somewhat broader than

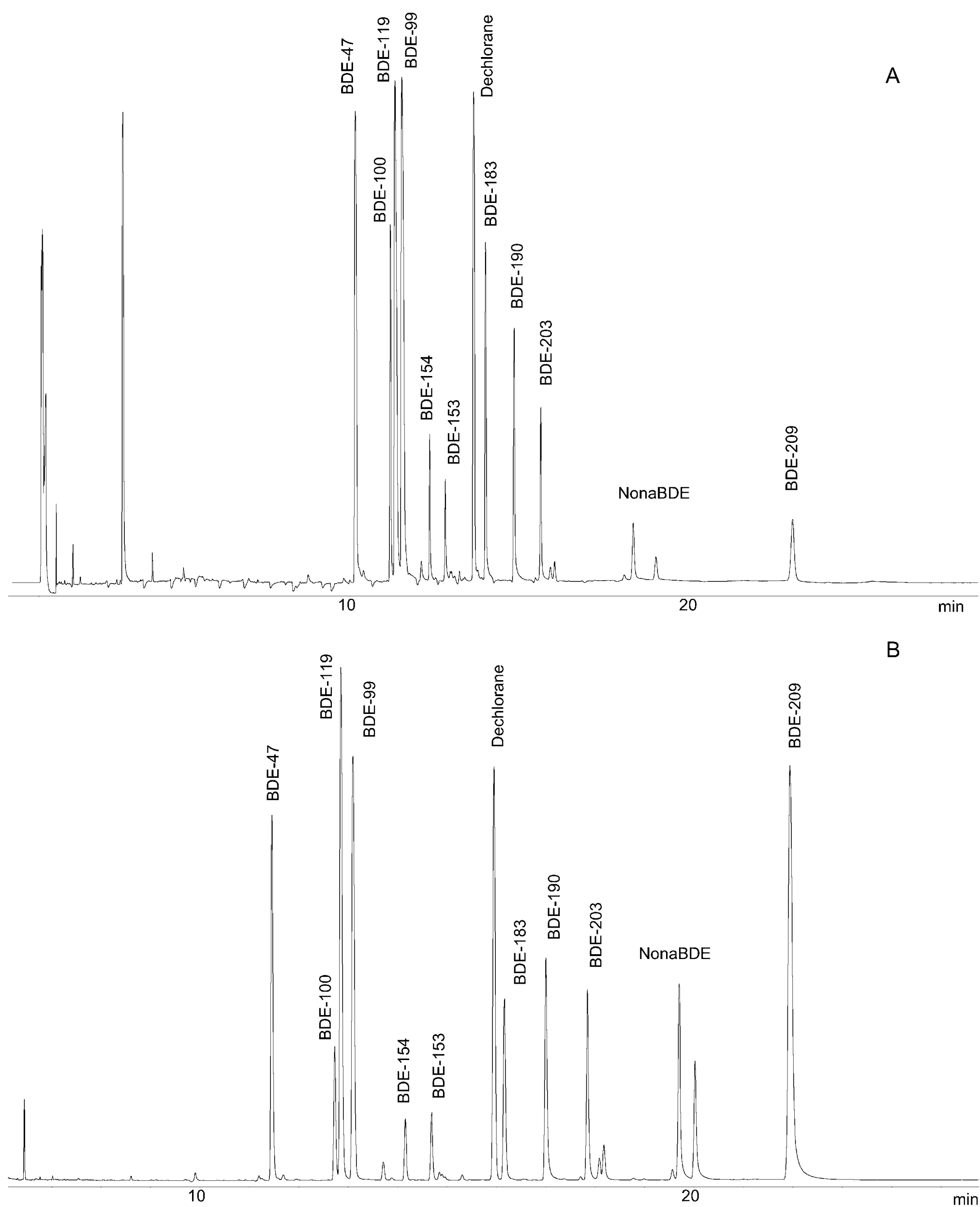



Fig. 3. A PBDE standard mixture injected using (a) splitless injection (no pressure pulse), and (b) SPI [53].

closely eluting PBDE peaks. A better result is obtained when applying LC/MS. The α, β and γ-HBCD can easily be separated on a $C_{18}$ column, using a methanol–water eluent. Electrospray ionisation is recommended [155]. This LC analysis can in the same run be used for the determination of TBBP-A. A TBBP-A analysis is also possible by GC, but adsorption to the column may occur.

Derivatisation (to the di-ethyl derivatives) may be a solution. Marine laboratories are now in the phase of setting up methods for these BFRs. Two international laboratory studies have been organized to date

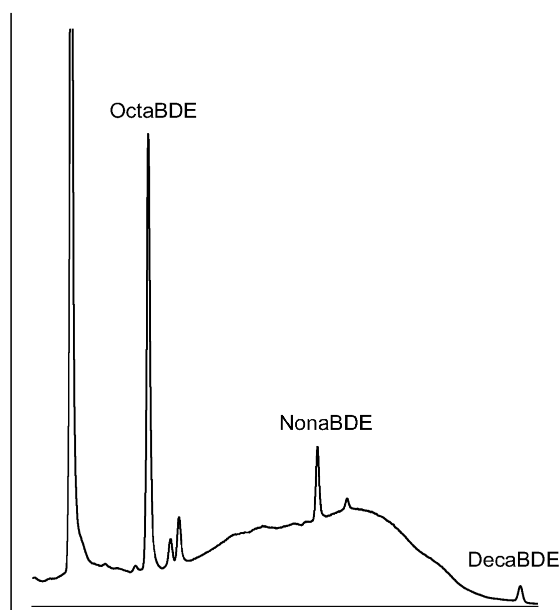



Fig. 4. The degradation of decabromodiphenyl ether shown as a hump in front of the deca BDE peak on a 15 m, 0.25 mm Agilent HP-1 column [53].

[149,151]. The results show that most laboratories are able to carry out reliable analyses of BDE47, and for some of the other BDEs. C.V. values for BDE47 and sediments were between 13 and 23%, for BDE 100 between 19 and 27% [149,151]. However, deca BDE can only be analysed by a few specialized laboratories. Between-laboratory C.V. values varied between 48 and 78% for sediments, while in biota (probably with very low deca BDE levels) the between-laboratory C.V. values were >100%. CRMs for PBDEs are not available at this moment.

## 5. PAHs

Research which led to the identification of certain PAH as chemical carcinogens began in 1775, when the British surgeon Sir Percival Pott [156] noted an association between the incidence of scrotal cancer in chimney sweeps and their exposure to soot. In 1915, Japanese workers induced skin tumors in rabbits by application of coal tar [157], and in 1933 the principal carcinogenic component of coal tar pitch was identified as benzo[*a*]pyrene [158]. Because of the complexity of the PAH mixtures found in the environment and the limitations of analytical techniques, this was the only PAH determined in older investigations, although other carcinogenic PAH were identified at around the same time [159]. There have been dramatic developments in the chromatographic analysis of PAH, especially within the last 50 years. Methods such as column chromatography, paper chromatography and TLC allowed the determination of a small number of very important parent PAH to be carried out. The advent of packed column GC extended the range of compounds, which could be identified and quantified, and this was further extended with the development of HPLC and capillary GC techniques. Capillary GC–MS in the electron-impact mode is the analytical method of choice if minor as well as major PAH must be quantified, and if alkylated PAH are to be determined. When only a restricted suite of the major parent PAH are to be determined, LC provides a cost-effective alternative, e.g. in compliance testing or screening applications.

### *5.1. Column chromatography*

During the 1950s, concern over human exposure to carcinogenic PAH in food, in industrial and ambient atmospheres, in cigarette smoke and in other media prompted the development of analytical methods for these compounds [160]. The analytical method most widely used for the determination of PAH compounds in this decade entailed a column chromatographic separation, followed by analysis using absorption and/or fluorescence spectrophotometry. The methods required relatively large samples, and a complete analysis took several days [161,162].

### *5.2. Thin-layer chromatography*

In a review published in 1970, Schaad [163] concluded that the most frequently used methods for PAH analysis were column and paper chromatography, but that the most promising methods for further application were thin-layer chromatography (TLC) and GC. In 1967 Sawicki et al. [164] compared a number of techniques for the determination of benzo[*a*]pyrene in airborne particulate material. They concluded that the best separation of ben-

zo[*a*]pyrene from other PAH present was achieved using two-dimensional TLC, although the recoveries obtained using this TLC material were variable (40–75%), and took 3 h. Even though the benzo[*a*]pyrene was not separated from other isomeric compounds (benzo[*e*]pyrene, benzo[*k*]fluoranthene and perylene), judicious selection of excitation and emission wavelengths for the quantification by fluorescence spectrometry made the method specific for benzo[*a*]pyrene, and 3 ng could be detected. Analysis of complex samples could be conducted using these methods, but the procedures were extremely time-consuming [165–168]. During the early- to mid-1970s, TLC was still in use as a fraction separation technique in order to simplify complex hydrocarbon mixtures prior to analysis by GC–FID and GC–MS.

The difficulties inherent in the determination of more than a few individual PAH using column chromatography or TLC techniques led to the establishment of limited suites for routine analyses. An example is the ''six of Borneff'', for which a standard method was established in the Federal Republic of Germany using TLC with fluorescence detection [169]. The six PAH determined were fluoranthene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, benzo[*ghi*]perylene and indeno[1,2,3-*cd*]pyrene, and this suite is still cited today. A more recent example would be the set of 16 priority pollutant PAH developed by the USEPA. These are all parent compounds, from naphthalene to indeno[1,2,3-*cd*]pyrene, and are primarily intended to address PAH from combustion sources. Such is their standing as a routine set of determinants that they have also been determined even when oil sources being studied [170], a task for which they are wholly inadequate as crude oil and its refined products are generally dominated by alkylated PAH compounds.

### *5.3. Extraction and clean-up for GC and LC analysis*

Ehrhardt et al. [171] reviewed the methods currently in use in marine laboratories in 1991. They noted that both liquid–liquid and solid-phase extraction (SPE) were being used for water samples, and that Soxhlet extraction with non-polar solvents and direct alkaline saponification under reflux were the most commonly used techniques for sediment and biota samples. Ultrasonication with solvents was also frequently used for extraction of sediment samples, and had been successfully validated alongside the other techniques [172,173]. A later study though reported a decrease in efficiency of the ultrasonic extraction due to aging of the sonication probe, necessitating frequent replacement if performance was to be maintained [174]. Freeze-drying of sediment and tissue samples is problematic for PAH, and an oil-filled pump usually produces the necessary vacuum, and backstreaming of oil vapour into the sample stage can contaminate the samples during the drying process [171]. The ability of alkaline saponification to handle wet samples directly is also an aid in the analysis of the more volatile PAH, such as naphthalene. Subsequently, other techniques such ASE and MASE have also been applied to samples for PAH analysis [175], and most recently the successful application of microwave-assisted alkaline saponification (MAAS) has been described [176].

Column chromatography using silica and/or alumina has been routinely used in clean-up and fractionation of PAH extracts [extra 2]. In more recent years there has been a growth in the use of LC and GPC techniques, particularly as these are readily automated [177,178]. Simple column techniques are still routinely applied following alkaline saponification, as so little interfering material remains after this vigorous technique has been applied to samples (mainly pigments) [179].

### *5.4. Gas chromatography*

GC has been used in the separation of hydrocarbons and PAH since the late 1950s and early 1960s, with practically all of the early work having been conducted using packed columns [180]. Early high resolution analyses of ''polynuclear arenes'' were performed on 60 m×0.5 mm I.D. stainless steel capillary columns by Wilmshurst [181]. CEFAS began to use a coupled (packed column) gas chromatograph–mass spectrometer (GC–MS) in 1975, a quadrupole instrument capable of scanning in 1s with a minimal reset time before the next scan. Experiments with glass capillary columns began in January 1976. The addition of a data system and the

additional selectivity of mass chromatography gave us the basis of the technique used to this day [179].

The PAH fraction of environmental samples is generally complex, with hundreds of compounds (or more) spread over a wide concentration and volatility range. Some compromise is usually necessary if the aim is to analyse "as many PAH as possible" in a single chromatographic run, rather than a small set of target compounds, even using capillary GC–MS and mass chromatography to aid selectivity. However, if alkaline saponification is used as the digestion/extraction step in the analytical procedure, very little further clean-up is then required prior to GC–MS analysis [179]. Most separations nowadays are made on crosslinked fused-silica capillary columns, often with non-polar (e.g. methyl silicone) or slightly polar (e.g. 5% phenyl methyl silicone) phases, as these columns are readily available commercially, are robust and give good service in routine applications. Cold on-column injection is preferred, as this both improves the resolution obtained for the first-eluting, low MW compounds, and reduces discrimination against the high MW compounds which is difficult to avoid entirely when using splitless injection.

Several analysts have developed selective stationary phases for special applications, although few of these, if any, have found widespread use. The use of nematic liquid crystal phases has been reported by a number of authors. Of two isomers, the one with the larger length-to-breadth ratio should have the longer retention on these columns. They are not well suited to routine determination of a wide range of PAH as they are generally of low separation efficiency, and operate within a restricted temperature range. Borwitsky et al. [182] used a polyphenyl ether sulphone phase to separate PAH in coal tar, and successfully resolved chrysene and triphenylene, a separation, which is usually not achieved using capillary GC columns. The working range of these columns was wider than that obtained with liquid crystal columns, 190–390 °C, which allowed the analysis of PAH from acenaphthene to coronene. Peaden et al. [183] produced a cross-linked phenylpolysiloxane column which could be programmed up to 400 °C, and on which PAH with MW >400 from carbon black were analysed. The number of applications of multidimensional GC to the analysis of PAH is few [184].

Although flame-ionisation detection was widely used with capillary GC in the 1980s, the reducing cost of GC–MS instruments has led to its replacement as a routine technique. MS analysis is usually conducted in electron impact (EI) mode as most PAH yield a reasonably strong molecular ion in this mode, with little fragmentation compared to many other organic contaminants. Conventional chemical ionisation (CI) MS using $CH_4$ as reagent gas produces mass spectra that appear quite similar to those produced by EI. Negative ion CI has not been generally accepted as a useful tool because of the low yield of negative ions usually obtained, with a corresponding decrease in sensitivity.

Ion-trap benchtop GC–MS systems are particularly well suited to the analysis of PAH and hydrocarbons. This is because, unlike quadrupole instruments, there is no sensitivity advantage to be gained by limiting the number of ions detected in ion-trap instruments. Collecting and archiving of full-scan EI data (e.g. on CD-ROM) means that all samples can potentially be available for biomarker/fingerprinting studies for little or no extra effort. Additionally, when new contaminants arise, there is the possibility of looking back through historic samples to determine the time at which its first appearance was seen.

The use of mass chromatography allows the signal due to internal (surrogate) standards—usually fully deuterated analogues of some of the parent PAH to be determined—to be distinguished from those due to the analytes, even where complete resolution is not achieved (Fig. 5). Experience gained during routine analysis with fully developed QA/QC procedures in place also indicate that the use of a larger number of these deuterated PAH standards spread throughout the run can help to significantly reduce errors due to e.g. volatility, or discrimination due to injection technique [179].

### *5.5. Liquid chromatography*

Since its development in the 1970s, liquid chromatography (LC) has been used extensively in the analysis of PAH, generally with fluorescence detection. Currently, LC does not offer the high resolution attainable with capillary gas chromatography, although the use of fluorescence detection provides sensitive and selective detection for parent

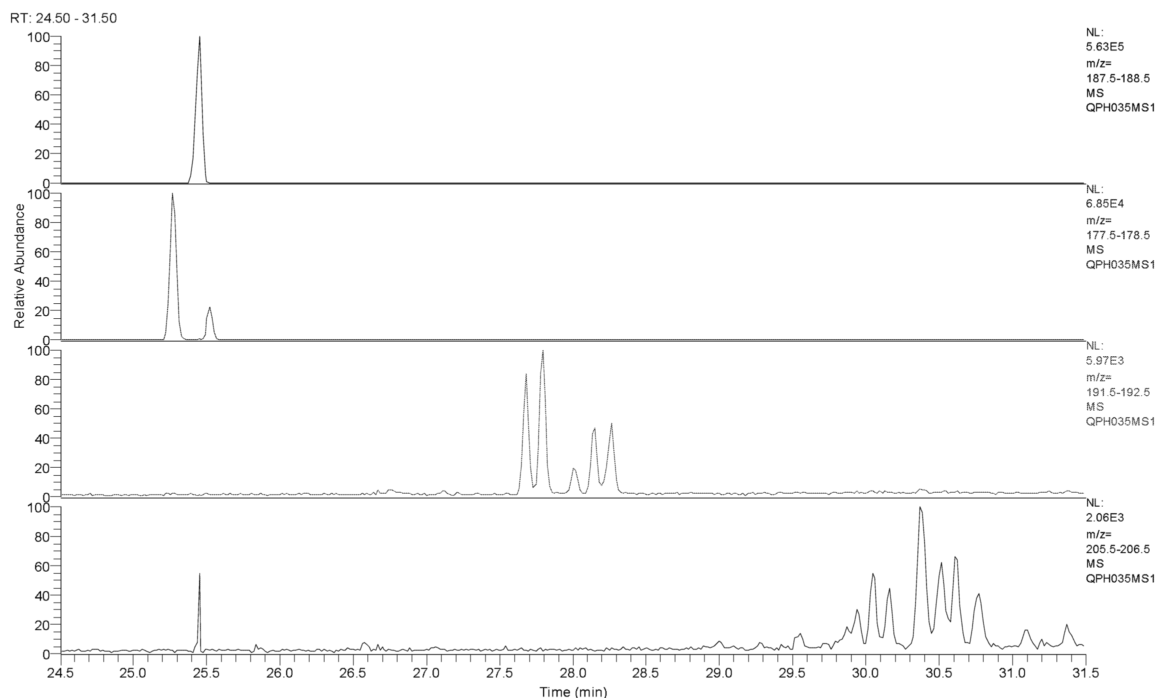



Fig. 5. Selected mass chromatograms from an extract of QPH035MS, a sediment sample collected in the Wadden Sea and circulated in Exercise 534 of the QUASIMEME PAH laboratory proficiency scheme. The four plots represent: 188 dalton, anthracene-$d_{10}$ (internal standard for this group of compounds); 178 dalton, phenanthrene and anthracene; 192 dalton, $C_1$-phenanthrenes and $C_1$-anthracenes; 206 dalton, $C_2$-phenanthrenes and $C_2$-anthracenes.

PAH in a relatively straightforward instrument. The advent of benchtop GC–MS systems has introduced these instruments to routine laboratories and applications on a wide scale, whilst in contrast, coupled LC–MS instruments are only now beginning to make the transition from research to routine status. However, LC does have some advantages over GC. LC can provide additional selectivity and yield separations of PAH isomers, which are difficult to separate using GC; heat-sensitive compounds are not degraded during analysis; and LC can provide a useful fractionation technique for the isolation of PAH for subsequent analysis by other techniques. An example of this latter approach, as applied to the detailed characterisation of a complex PAH mixture derived from an air particulate sample, was described by Wise et al. [185]. Higher resolution can be obtained in a single run, but at the cost of extremely long run times. In 1980, Hirata et al. [186] published a chromatogram of the aromatic fraction of a coal tar sample analysed on a reversed-phase packed microcapillary column using LC. Good resolution was achieved, but with a run time around 1 day. Long analysis times are typical of this technique [187].

Adsorption chromatography on alumina or silica was used initially, essentially transferring separations directly from classical column chromatography [188]. The development of reversed-phase packing materials, in which hydrocarbons with chain lengths of $C_2$, $C_8$, or $C_{18}$ are chemically bonded to silica particles, provided a unique selectivity for the separation of PAH isomers. In reversed-phase LC the mobile phase (usually mixtures of water with an organic solvent) is more polar than the stationary phase. In combination with gradient-elution techniques and fluorescence detection, this is now an established and popular method for the determination of parent PAH. Care must be taken, though, to deoxygenate solvents used in LC as the fluorescence of some PAH (e.g. pyrene) can be dramatically reduced in the presence of oxygen [189]. Other PAH (e.g. chrysene and benzo[*a*]pyrene) are photo-labile,

and so light must be excluded from extracts and standard solutions containing these compounds. Normal phase LC on polar stationary phases such as amino-, cyano- and nitro-derivatives of silica has been used extensively for the separation of PAH based on the number of aromatic carbon atoms in the molecule. The most commonly used phase has been the amino-phase, with hexane or pentane as mobile phase. This provides a class fractionation of the PAH present [190].

LC is inherently more suited to the analysis of high MW PAH than GC, due to their limited volatility. Peaden et al. showed a good separation for high molecular mass PAH derived from carbon black on a 25-cm $C_{18}$ reversed-phase column with mass spectrometric and spectrofluorimetric detection in collected fractions in which compounds up to 448 dalton could be distinguished [191]. Recently, the analysis of a range of high MW PAH (of 300, 326, 350 and 374 dalton) using LC–MS with an atmospheric pressure chemical ionisation (APCI) source has been reported, in a range of matrices including zebra mussels. This offers enhanced selectivity relative to fluorescence detection, and was used in source identification in the study reported, however, the resolution obtained during the LC separation is still much less than that available using GC–MS. In addition, this instrumentation is, as yet, only rarely available in routine monitoring laboratories [192].

Although they are not usually determined using LC, a number of authors have reported the analysis of alkyl-substituted PAH using this technique. Sleight [193] suggested that alkyl substitution results in a significant increase in retention time due to decreased solubility in the polar mobile phase, proportional to the number of aliphatic carbons in the side-chain. Subsequently, Wise et al. [194] investigated the effect of the site of methyl substitution on the retention of PAH on a polymeric $C_{18}$ column, and concluded that it was the shape of the PAH molecules, particularly their length-to-breadth ratios, which had the major influence.

It seems, therefore, that alkylated PAH can be analysed using LC, but in extracts from samples known to contain a preponderance of alkyl-substituted PAH only the parent compounds are usually seen. Whether this is a function of the limited resolution and the large number of isomers within many of these groups, so that they form a sort of ''unresolved complex mixture'' on which the resolved parent compounds sit is not known. Another possibility is that, as the wavelength pairs are optimized for a small number of parent compounds and switched automatically as these elute, possibly the fluorescence of the alkyl-substituted PAH is reduced in intensity relative to the parent PAH. To the best of our knowledge, these aspects have not yet been studied in detail.

### *5.6. Interlaboratory studies and CRMs*

Writing in 1983, Dunn [195] indicated that the analysis of PAH in marine samples represented one of the more difficult tasks in the field of PAH determination. This was because concentrations of individual compounds are often small, and, in some cases, such as organisms or portions of dated sediment cores, amounts of sample are very limited. Especially in organisms, the PAH are associated with a complex mix of natural and biogenic hydrocarbons, necessitating extensive sample clean-up before separation and quantification of PAH can be attempted. He recognised that these difficulties led to problems in ensuring adequate and consistent recovery of the compounds to be determined.

Intercomparisons concerning the determination of hydrocarbons and PAH in specifically marine samples began in 1978, on a bilateral basis. Two European institutes, the Institute of Marine Research in Bergen, Norway, and the MAFF Fisheries Laboratory in Burnham-on-Crouch, UK (the laboratory which is now the CEFAS Burnham Laboratory) had collaborated in the previous year on studies of the environmental impact of the blow-out on the Ekofisk Bravo oil production platform in the North Sea. Analysis of both aliphatic and PAH compounds in water, sediments and fish from the vicinity of the platform had been undertaken using capillary GC–MS [196]. The results of the bilateral intercomparison, involving the determination of aliphatic and aromatic compounds in crude oil, were presented within ICES [197]. The authors noted that the exercise was ''not a success story'', but it began to highlight some of the difficulties in obtaining agreement across laboratories, and stimulated further work

within a wider group of analysts which has continued within ICES and QUASIMEME.

The first ICES hydrocarbon international intercomparison exercise took place in 1981, including mussel and sediment samples. This demonstrated that the poor interlaboratory comparability for PAH determination seen in the initial bilateral exercise was replicated across a wider group of laboratories, and so that there was indeed a problem which needed to be resolved before collaborative monitoring could be undertaken [198]. Additional exercises conducted under ICES auspices demonstrated unacceptable losses of volatile PAH during the freeze-drying of mussel tissues, and also that resulting changes in the tissue matrix reduced both the extraction efficiencies obtained for PAH and the recovery of added surrogate standards (fully deuterated parent PAH) [199]. Poor intra- and interlaboratory precision were found for the analysis of PAH in lobster tissue using both GC–MS and LC techniques, and performance was judged to be poorer than that seen in other studies on foodstuffs, including smoked fish, and recommended the adoption of a standard methodology including an alkaline saponification step [200]. Two exercises conducted by the IAEA also demonstrated poor agreement for sediment and mussel samples [201,202]. Frequent errors in calculation, transcription and reporting of data were also noted. From 1994 onwards, the QUASIMEME programme began to include PAH within its programme, with the difference that this set out to provide a continuous laboratory proficiency scheme (LPS) with two exercises each year rather than occasional interlaboratory intercomparison rounds [203]. The first round involved 10 PAH and 25 laboratories [204]. Seven laboratories reported satisfactory data, and problems were identified with data reporting and the preparation and preservation of standard solutions. In 1994–1995 sediment extracts were added to the LPS scheme, with a similar outcome and the same problems limiting comparability [205]. From 1996 onwards, sediment and biota samples were circulated for analysis, and the number of PAH determined was increased to 17. Both GC–MS and LC were shown to be capable of generating good data in laboratories with experience and expertise, and the performance of laboratories remaining within the scheme generally improved with time [206]. By study of an extremely large number of datasets derived from intercomparison studies (almost 10,000 interlaboratory datasets to 1997), Horwitz [207] derived a simple, empirical relationship between the concentration of analytes within a matrix and the reproducibility attainable between laboratories. This is usually referred to as the ''Horwitz curve'' and shows as a straight line when plotted on a log–log scale.

Fig. 6 shows all the data from the QUASIMEME PAH LPS studies plotted in this way. Although some of the higher values in particular are close to the Horwitz curve (shown plotted as a dotted line), on average the decreasing trend with concentration in

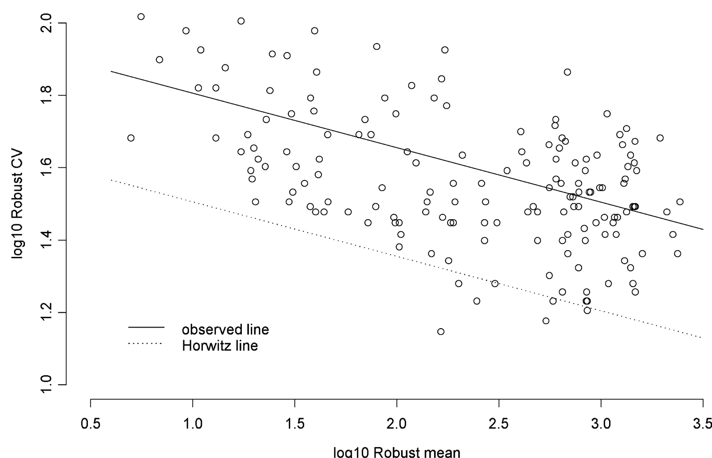



Fig. 6. Correlation between between-laboratory C.V. and concentration of analyte, for PAHs in biota and sediments.

Table 4
QUASIMEME proficiency test results (C.V. values, %) of selected PAHs in sediments [191–193]

| Test material | Naphthalene | Phenanthene | Pyrene | Benzo[*a*]pyrene |
|---|---|---|---|---|
| QPH010MS | – | 26 | 26 | 36 |
| QPH011MS | – | 31 | 30 | 37 |
| QPH012MS | – | 35 | 17 | 25 |
| QPH013MS | – | 28 | 19 | 23 |
| QPH014MS | – | 21 | 20 | 22 |
| QPH015MS | – | 22 | 26 | 29 |
| QPH016MS | 45 | 30 | 23 | 35 |
| QPH017MS | 44 | 19 | 15 | 29 |
| QPH018MS | 84 | 31 | 27 | 37 |
| QPH019MS | 73 | 34 | 29 | 36 |
| QPH020MS | 73 | 56 | 28 | 31 |
| QPH021MS | 81 | 56 | 40 | 39 |
| QPH022MS | 40 | 23 | 18 | 26 |
| QPH023MS | 47 | 50 | 19 | 24 |
| QPH024MS | 43 | 27 | 23 | 28 |
| QPH025MS | 45 | 59 | 46 | 50 |
| QPH026MS | 44 | 38 | 31 | 25 |
| QPH027MS | 59 | 35 | 35 | 24 |
| QPH028MS | 40 | 31 | 17 | 20 |
| QPH029MS | 34 | 24 | 15 | 16 |
| QPH030MS | 34 | 17 | 23 | 14 |
| QPH031MS | 77 | 36 | 33 | 30 |
| QPH032MS | 36 | 52 | 47 | 36 |
| QPH033MS | 45 | 50 | 53 | 52 |

the whole QUASIMEME dataset is summarized by the solid line. This indicates that at the present level of intercomparability the analysis of PAH within QUASIMEME is underperforming on average by a factor of two relative to the Horwitz curve. This suggests that there is the potential for a 50% improvement in the variability between laboratories if further method improvements are undertaken. Tables 4 and 5 shows that as for OCPs and PCBs, no clear improvement with time is apparent in the between-lab C.V. values for PAH, as this progress has been masked by the effect of the concentration of the target analyte on the C.V. value.

Specific recommendations from recent intercomparison studies have been made. Law et al. [206] recommended, in studies of the data generated within the QUASIMEME laboratory proficiency scheme, that further method development should be concen-

Table 5
QUASIMEME proficiency test results (C.V. values, %) of selected PAHs in biota [191–193]

| Material | Naphthalene | Phenanthene | Pyrene | Benzo[*a*]pyrene |
|---|---|---|---|---|
| QPH08BT | 99 | 43 | 40 | 55 |
| QPH09BT | 34 | 45 | 45 | 64 |
| QPH010BT | 77 | 46 | 24 | 44 |
| QPH011BT | 59 | 19 | 21 | 69 |
| QPH012BT | 85 | 45 | 28 | 32 |
| QPH013BT | 49 | 34 | 20 | 34 |
| QPH019BT | 45 | 10 | 15 | 45 |
| QPH020BT | 79 | 16 | 18 | 40 |
| QPH027BT | 36 | 33 | 31 | 40 |
| QPH028BT | 81 | 17 | 26 | 29 |

Table 6
Certified reference materials for PAHs

| CRM | Material | Form | Producer | Country | Number of certified PAHs |
|---|---|---|---|---|---|
| SRM 1974a | Mussel | Frozen | NIST | USA | 15 |
| SR2974 | Mussel | Freeze-dried | NIST | USA | 14 |
| SRM 2977 | Mussel | Freeze-dried | NIST | USA | 14 |
| SRM 2978 | Mussel | Freeze-dried | NIST | USA | 9 |
| 140/OC | Fucus (plant) | Freeze-dried | IAEA | Monaco | 14 |
| EC-1 | Sediment | Freeze-dried | NWRI | Canada | 11 |
| EC-2 | Sediment | Freeze-dried | NWRI | Canada | 10 |
| EC-3 | Sediment | Freeze-dried | NWRI | Canada | 6 |
| EC-6 | Sediment | Freeze-dried | NWRI | Canada | 2 |

NIST: National Institute for Standards and Technology; IAEA: International Atomic Energy Agency; NWRI: National Water Research Institute.

trated on GC–MS techniques, with LC used primarily as a screening method and in compliance monitoring for a restricted range of parent PAH [206]. Also, Law and Klungsøyr [208] noted that there is a need for CRMs certified for a wider range of PAH compounds than at present (Table 6), and for the provision of homogeneous but uncharacterised materials for use as LRMs (Laboratory Reference Materials). Many laboratories prepare these as needed, but others are uncertain of their ability to do so [208].

Developments in quality assurance/quality control have meant that laboratories are now able to monitor and document the performance of their analytical methods on a routine basis, and data quality has undoubtedly improved as a result. The determination

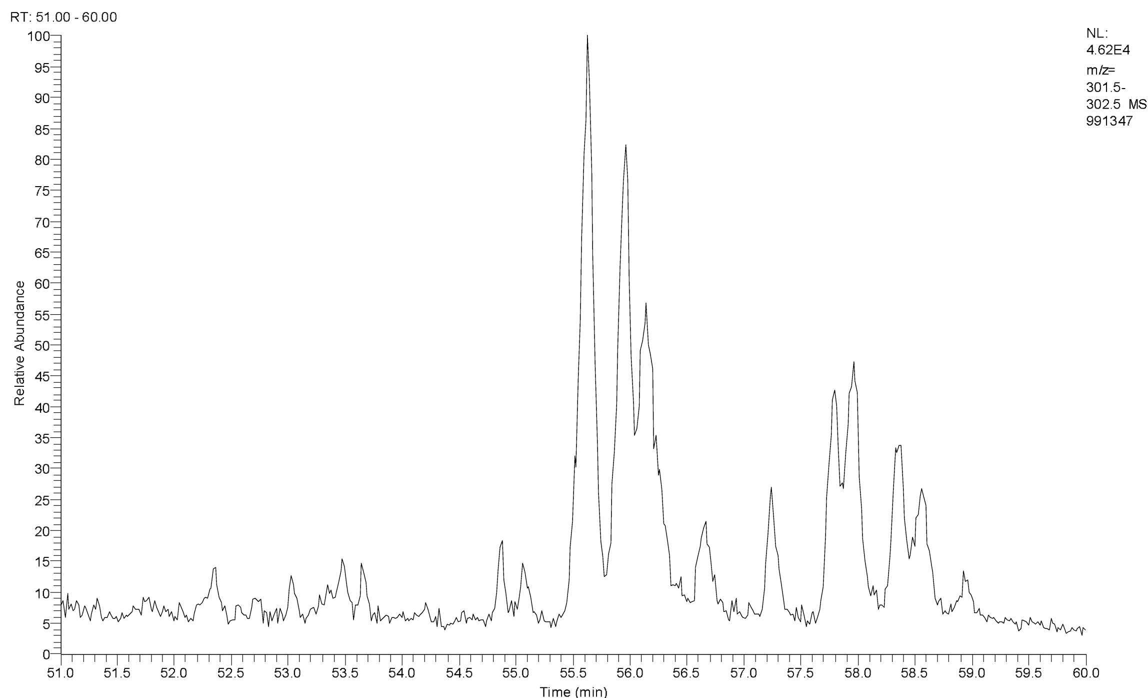



Fig. 7. PAH of molecular mass 302 dalton in whole soft tissue of mussels taken from a beach below a former gasworks site in Shoreham, UK in 1999.

of the concentrations of additional PAH compounds in marine samples (such as shellfish tissues) is likely to be required in the future as marine monitoring programmes develop. This will be driven by concern over the possible impact on both wildlife and human consumers of a wider range of PAH recognized for their toxicity and environmental distribution. Marvin et al. [209], for example, have recently highlighted the significant contribution that PAH of molecular mass 302 dalton (such as naphtho[2,1-$\alpha$]pyrene and naphtho[2,3-$\alpha$]pyrene) make to the biological activity of coal tar-contaminated sediments. These compounds are certainly present in environmental samples, as Fig. 7 illustrates for mussels from Shoreham in the UK, below a derelict gasworks site [210]. Most of these higher molecular mass PAH are available as standards, either in pure form or as solutions, and so their determination by GC–MS is feasible. Gas chromatographic methods have also now been developed for the analysis of isomers of PAH of higher molecular mass, up to 450 dalton [211,212], essentially the same range as that currently amenable to analysis by LC.

Another recent development has followed the development of GC detectors which allow carbon isotope ratios to be determined in individual PAH compounds eluting from GC columns [213,214]. This has provided additional useful information for source identification, always a complicated process as PAH have a multiplicity of sources, as observed following the Exxon Valdez oil spill in 1989 [215,216].

## 6. Concluding remarks

Marine laboratories have gratefully used various chromatographic techniques for the determination of a range of organic contaminants. GC was by far the most popular technique, although useful applications of LC, in particular for PAH analysis have been seen. Partly because of their workload, marine laboratories have focused on the practical implementation of existing methods for their purpose: the analysis of marine matrices. Only a few marine laboratories have invested in the development of new methods. However, marine laboratories have been strong in adapting existing sampling, extraction and clean-up techniques for the analysis of marine samples [217]. Also, and in particular, they have contributed to a better quality of organic contaminant analysis, through the organisation of various international interlaboratory studies, the development of a proficiency testing programme for marine laboratories (QUASIMEME) [218], and the organisation of many workshops and and seminars on QA/QC for marine environmental analysis. Future trends within marine laboratories will include the analysis of more compounds and compound classes (more PAHs, more BFRs, fluorinated compounds, chlorinated alkanes), possible inclusion of dioxin analysis, use of more advanced chromatographic techniques such as GC×GC, more intensive use of LC (fluorinated compounds, some BFRs), wider availability of more CRMs and continued interlaboratory studies. Therefore, marine laboratories will require robust chromatographic techniques and trained and skilled staff to work with these techniques.

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## References

[1] J. van Bennekom, Geschiedenis van het Zoölogisch Station en het Nederlands Instituut voor Onderzoek der Zee, NIOZ, Den Burg, Texel, The Netherlands, 2001.
[2] S.J. de Groot, Een eeuw visserijonderzoek in Nederland 1888–1988, RIVO, IJmuiden, The Netherlands, 1988.
[3] D.J.H. Phillips, P.S. Rainbow, Biomonitoring of Trace Aquatic Contaminants, Elsevier, London, 1993.
[4] F. Smedes, A.S. de Jong, I.M. Davies, J. Environ. Monit. 2 (2000) 541.
[5] E.G. Hunt, A.I. Bischoff, Calif. Fish Game 46 (1960) 91.
[6] C.H. Deal, J.W. Otvos, V.N. Smith, P.S. Zucco, Anal. Chem. 28 (1958) 456.
[7] H. Boer, in: H. Desty (Ed.), Vapour Phase Chromatography, Butterworths, London, 1957, p. 169.
[8] J.E. Lovelock, J. Chromatogr. 1 (1958) 35.
[9] S. Jensen, Ambio 1 (1972) 123.
[10] S. Jensen, A.G. Johnek, M. Olssen, G. Otterkind, Nature 224 (1969) 247.

[11] C.R. Pearson, in: O. Hutzinger (Ed.), The Handbook of Environmental Chemistry, Anthropogenic Compounds, Vol. 3, Springer, Heidelberg, 1982, p. 90, Part B.
[12] R.D. Dandenau, E.H. Zerrener, J. High Resolut. Chromatogr. 6 (1979) 351.
[13] J. de Boer, K. de Boer, J.P. Boon, in: J. Paasivirta (Ed.), Handbook of Environmental Chemistry, Vol. 3, Springer, Heidelberg, 2000, p. 61, Part K.
[14] http://www.itopf.com/stats.html
[15] R.L. Metcalf, Crit. Rev. Environ. Contam. 3 (1972) 25.
[16] W. de Jonckheere, W. Steurbaut, R.H. Krips, Bull. Environ. Contam. Toxicol. 13 (1975) 720.
[17] Anon, Environmental Contamination From Hexachlorobenzene, National Technical Information Service Report PB-251 874, US Department of Commerce, Washington, DC, 1976.
[18] Z. Shigun, Z. Zhanxia, S. Guoying, M. Yushun, F. Jiamo, Environ. Monit. Assess. 44 (1997) 563.
[19] A.H. Knap, K.S. Binkley, W.G. Deuser, Nature 319 (1986) 572.
[20] D. Dannenberger, Selected Chlorinated Microcontaminants in Baltic Waters, 1992 to 1994, Marine Environment Committee Report CM 1995/E:9, International Council for Exploration of the Sea (ICES), Copenhagen, Denmark, 1995.
[21] V. Lang, J. Chromatogr. 595 (1992) 1.
[22] D.E. Wells, in: D. Bacelo (Ed.), Environmental Analysis Techniques, Applications and Quality Assurance, Elsevier, Amsterdam, 1993, p. 79.
[23] F. Smedes, J. de Boer, Trends Anal. Chem. 16 (1997) 503.
[24] R.J. Law, J. de Boer, in: P. Quavauviller (Ed.), Quality Assurance in Environmental Monitoring, VCH, Weinheim, 1995, p. 129.
[25] J. de Boer, Chemosphere 17 (1988) 1803.
[26] T.A. Bellar, J.J. Lichtenberg, S.C. Larneman, in: R.A. Baker (Ed.), Recovery of Organic Compounds From Environmental Contaminated Bottom Material, Ann Arbor Press, Ann Arbor, MI, 1980.
[27] R. Fuoco, M.P. Colombini, E. Samcova, Chromatographia 36 (1993) 65.
[28] P. Jensen, L. Renberg, L. Reutergårdh, Anal. Chem. 49 (1977) 316.
[29] S.B. Hawthorne, P.J. Miller, J.J. Langenfeld, M.D. Burford, in: P. Sandra, G. Devos (Eds.), Proceedings 15th International Symposium on Capillary Chromatography, Riva del Garda, Italy, May 240-27, Huething, Heidelberg, 1993, p. 1701.
[30] J.J. Langerfeld, S.B. Hawthorne, D.J. Miller, J. Pawliszyn, Anal. Chem. 6 (1993) 338.
[31] S. Bøwadt, B. Johansson, P. Fruekilde, M. Hansen, D. Zilk, B. Larsen, J. de Boer, J. Chromatogr. A 675 (1994) 189.
[32] G. Kernick Carvalhaes, P. Brooks, C. Gama Marques, G. Carneiro Azevedo, J.A. Teixeira Azevedo, C. Silva Machado, Organohalogen Comp. 50 (2001) 225.
[33] J.R. Jocelyn Paré, J.M.R. Bélanger, B. Lesnik, R. Turpin, R. Singhvi, Soil Sedim. Contam. 10 (2001) 375.
[34] F. van der Valk, Q.T. Dao, Chemosphere 17 (1988) 1735.
[35] B. Baker, Bull. Environ. Contam. Toxicol. 10 (1973) 279.
[36] W.W. Wiencke, J.A. Burke, J. Assoc. Off. Anal. Chem. 52 (1969) 1277.
[37] N. Furusawa, K. Okazaki, S. Iriguchi, H. Yamaguchi, M. Saitoh, J. Assoc. Off. Anal. Chem. 81 (1997) 1033.
[38] A. Pauwels, F. David, P. Schepens, P. Sandra, Int. J. Anal. Chem. 73 (1999) 171.
[39] J. de Boer, Chemosphere 17 (1988) 1811.
[40] F. Schenck, R. Wagner, M.K. Hennessy, J.L. Okrasinski Jr., J. Assoc. Off. Anal. Chem. 77 (1994) 102.
[41] L.M. Smith, D.L. Stalling, J.L. Johnson, Anal. Chem. 56 (1984) 1830.
[42] M.W. Jacobs, J.J. Delfino, G. Bitton, Environ. Toxicol. Chem. 11 (1992) 1137.
[43] H.M. McNair, C.J. Bonelli, Basic Gas Chromatography, Varian Aerograph, Walnut Creek, CA, 1965, p. 86.
[44] L. Reutergårdh, Identification and distribution of chlorinated organic pollutants in the environment, PhD thesis, Stockholm University, Stockholm, Sweden, 1988, p. 42.
[45] D.E. Wells, E.A. Maier, B. Griepink, Int. J. Environ. Anal. Chem. 46 (1992) 255.
[46] J. de Boer, J.C. Duinker, J.A. Calder, J. van der Meer, J. Assoc. Off. Anal. Chem. 75 (1992) 1054.
[47] Anon, Report QUASIMEME Workshop on PCB analysis, Roskilde, Denmark, June, FRS Marine Laboratory, Aberdeen, UK, 1995.
[48] P.G. Wester, J. de Boer, U.A.Th. Brinkman, Environ. Sci. Technol. 30 (1996) 473.
[49] P. Korytar, P.E.G. Leonards, J. de Boer, U.A.Th. Brinkman, J. Chromatogr. A 958 (2002) 203.
[50] R.P. Snell, J.W. Danielson, G.S. Oxborrow, J. Chromatogr. Sci. 25 (1987) 225.
[51] J. Staniewski, H.-G. Jansen, J.A. Rijks, C.A. Cramers, J. Microcol. Sep. 5 (1993) 429.
[52] A. Covaci, J. de Boer, J.J. Ryan, S. Voorspoels, P. Schepens, Anal. Chem. 74 (2002) 790.
[53] J. Björklund, Gas chromatography and mass spectrometry of polybrominated diphenyl ethers, PhD thesis, Stockholm University, Stockholm, Sweden, 2003.
[54] D.E. Wells, J. de Boer, L.G.M.Th. Tuinstra, L. Reutergårdh, B. Griepink, Fresenius J. Anal. Chem. 332 (1988) 591.
[55] M. Cleemann, F. Riget, G.B. Paulsen, J. de Boer, R. Dietz, Sci. Total Environ. 245 (2000) 103.
[56] J. de Boer, Analysis and biomonitoring of complex mixtures of persistent halogenated micro-contaminants, PhD thesis, Free University, Amsterdam, The Netherlands, 1995.
[57] G.S. Durrell, T.S. Sauer, Anal. Chem. 62 (1990) 1867.
[58] L. Hopper, J. Assoc. Off. Anal. Chem. 74 (1991) 974.
[59] P. Vetter, Chemosphere 26 (1993) 1079.
[60] H.-J. de Geus, H. Besselink, A. Brouwer, J. Klungsøyr, B. McHigh, E. Nixon, G.G. Rimkus, J. de Boer, Environ. Health Perspect. 107 (1999) 115.
[61] P.G. Wester, H.-J. de Geus, J. de Boer, U.A.Th. Brinkman, Chemosphere 35 (1997) 1187.
[62] P.G. Wester, H.-J. de Geus, J. de Boer, U.A.Th. Brinkman, Chemosphere 35 (1997) 2857.
[63] J. Burhenne, D. Hainzl, L. Xu, B. Vieth, L. Alder, H. Parlar, Fresenius J. Anal. Chem. 346 (1993) 779.
[64] V.A. Nikiforov, V.G. Tribulovitch, V.S. Karavan, Organohalogen Comp. 26 (1995) 393.

[65] M. Oehme, R. Kallenborn, Chemosphere 30 (1995) 1739.
[66] P. Andrews, W. Vetter, Chemosphere 31 (1994) 3879.
[67] D.C.G. Muir, J. de Boer, Chemosphere 27 (1993) 1827.
[68] E.C. Voldner, L. Smith, Report of the Workshop On the Estimation of Atmosphere Loadings of Toxic Chemicals To the Great Lakes, October, Scarborough, Ontario, Canada, International Joint Commission, 1986, Appendix II.
[69] M.A. Saleh, Rev. Environ. Contam. Toxicol. 118 (1991) 1.
[70] S.T. Glassmeyer, D.S. De Vault, T.R. Meyers, R.A. Hites, Environ. Sci. Technol. 31 (1997) 84.
[71] G.A. Stern, D.C.G. Muir, C.A. Ford, N.P. Grift, E. Dewailly, F.F. Bidleman, M.D. Walla, Environ. Sci. Technol. 26 (1992) 1838.
[72] M. Cleemann, F. Riget, G.B. Paulsen, J. de Boer, J. Klungsøyr, P. Aastrup, Sci. Total Environ. 245 (2000) 173.
[73] C.J. Musial, J.F. Uthe, Int. J. Environ. Anal. Chem. 14 (1983) 117.
[74] B. Jansson, U. Widequist, Int. J. Environ. Anal. Chem. 13 (1983) 309.
[75] B. Jansson, R. Vaz, G. Blomkvist, S. Jensen, M. Olssen, Chemosphere 8 (1979) 181.
[76] J. de Boer, H.-J. de Geus, U.A.Th. Brinkman, Environ. Sci. Technol. 31 (1997) 873.
[77] J. de Boer, Comparative tests to improve the analysis of chlorobornanes, J. Assoc. Off. Anal. Chem. (2003) in press.
[78] J. Blomberg, Multi-dimensional GC-based separations for the oil and petrochemical industry, PhD thesis, Free University, Amsterdam, The Netherlands, 2002.
[79] Z. Liu, J.B. Phillips, J. Chromatogr. Sci. 29 (1991) 227.
[80] J. Blomberg, P.J. Schoenmakers, J. Beens, R. Thijssen, J. High. Resolut. Chromatogr. 20 (1997) 539.
[81] R.B. Gaines, G.S. Frysinger, M.S. Hendrick-Smith, J.D. Stuart, Environ. Sci. Technol. 33 (1999) 2106.
[82] E.B. Ledford, C. Billesbach, J. High. Resolut. Chromatogr. 23 (2000) 202.
[83] H.-J. de Geus, A. Schelvis, J. de Boer, U.A.Th. Brinkman, J. High Resolut. Chromatogr. 23 (2000) 189.
[84] H.-J. de Geus, PhD thesis, Free University, Amsterdam, The Netherlands, 2002.
[85] W. Kraemer, K. Ballschmiter, Fresenius J. Anal. Chem. 327 (1987) 47.
[86] P.O. Darnerud, Å. Bergman, B.O. Lund, I. Brandt, Chemosphere 18 (1989) 1821.
[87] J. Falandysz, L. Strandberg, P.-A. Bergqvist, S.E. Kulp, B. Strandberg, C. Rappe, Envrion. Sci. Technol. 30 (1996) 3266.
[88] R. Ishaq, K. Karlson, C. Näf, Chemosphere 41 (2000) 1913.
[89] H. Karlsson, M. Oehme, S. Skopp, I.C. Burkow, Environ. Sci. Technol. 34 (2000) 2126.
[90] J. Moisey, A.T. Fisk, K. Hobson, R.J. Norstrom, Environ. Sci. Technol. 35 (2000) 1920.
[91] H.-J. de Geus, R. Baycan-Keller, M. Oehme, J. de Boer, U.A.Th. Brinkman, J. High Resolut. Chromatogr. 21 (1998) 39.
[92] A.V. Holden, in: F. Coulston, F. Karte (Eds.), Environmental Quality and Safety, Pesticides, Thieme, Stuttgart, 1975, p. 40, Suppl. III.
[93] Anon, Report of the working group for the international study of the pollution of the North Sea and its effects on living resources and their exploitation. Cooperative Research Report 39, International Council for the Exploration of the Sea (ICES), Copenhagen, Denmark, 1974.
[94] A.V. Holden, Report on the fourth ICES organochlorines intercalibration exercise, CM 1980/E:40, ICES Marine Environmental Quality Committee, Copenhagen, Denmark, 1980.
[95] J.F. Uthe, C.J. Musial, R.K. Misra, J. Assoc. Off. Anal. Chem. 71 (1988) 369.
[96] R. Misra, J.F. Uthe, C.J. Musial, Analyst 117 (1992) 1085.
[97] J. de Boer, D.E. Wells, Mar. Pollut. Bull. 35 (1997) 52.
[98] Anon, QUASIMEME laboratory performance studies round 10, FRS Marine Laboratory, Aberdeen, UK, 1997.
[99] Anon, QUASIMEME laboratory performance studies round 12, FRS Marine Laboratory, Aberdeen, UK, 1998.
[100] Anon, QUASIMEME laboratory performance studies round 16, FRS Marine Laboratory, Aberdeen, UK, 1999.
[101] Anon, QUASIMEME laboratory performance studies round 18, FRS Marine Laboratory, Aberdeen, UK, 2000.
[102] Anon, QUASIMEME laboratory performance studies round 20, FRS Marine Laboratory, Aberdeen, UK, 2000.
[103] Anon, QUASIMEME laboratory performance studies round 22, FRS Marine Laboratory, Aberdeen, UK, 2001.
[104] Anon, QUASIMEME laboratory performance studies round 26, FRS Marine Laboratory, Aberdeen, UK, 2002.
[105] Anon, QUASIMEME laboratory performance studies round 28, FRS Marine Laboratory, Aberdeen, UK, 2002.
[106] J. de Boer, J. van der Meer, U.A.Th. Brinkman, J. Assoc. Off. Anal. Chem. 79 (1996) 83.
[107] Anon, North Sea Quality Status Report 1993, Ministry of Environment and Energy, Danish Environmental Protection Agency, Copenhagen, Denmark, 1996.
[108] J. de Boer, J. van der Meer, L. Reutergårdh, J.A. Calder, J. Assoc. Off. Anal. Chem. 77 (1994) 1411.
[109] W.P. Cofino, I.H.M. van Stokkum, D.E. Wells, F. Ariese, J.-W.M. Wegener, R.A.L. Peereboom, Chemometr. Intell. Lab. Syst. 53 (2000) 37.
[110] P.J.H. Reijnders, Nature 324 (1986) 456.
[111] L. Fishbein, J. Chromatogr. 68 (1972) 345.
[112] J. Armour, J. Assoc. Off. Anal. Chem. 56 (1973) 987.
[113] N. Kannan, D.E. Schulz Bull, G. Petrick, J.C. Duinker, Int. J. Environ. Anal. Chem. 47 (1992) 201.
[114] V. Zitko, Bull. Environ. Contam. Toxicol. 6 (1971) 464.
[115] Ph. Hess, D.E. Wells, J. de Boer, W.P. Cofino, P.E.G. Leonards, J. Chromatogr. 1 (1995) 417.
[116] J. de Boer, in: W. Kleiböhmer (Ed.), Environmental Analysis, Handbook of Analytical Separations, Vol. 3, Elsevier, Amsterdam, 2001, p. 237.
[117] M.D. Mullin, C. Pochini, S. McGrundle, M. Rrambes, S. Safe, H. Safe, Environ. Sci. Technol 18 (1984) 468.
[118] J. de Boer, Q.T. Dao, J. High Resolut. Chromatogr. 12 (1989) 755.
[119] B. Larsen, S. Bøwadt, R. Tilio, Int. J. Environ. Anal. Chem. 47 (1992) 47.
[120] B. Larsen, S. Bøwadt, R. Tilio, S. Faccetti, Chemosphere 25 (1992) 1343.

[121] J. de Boer, Q.T. Dao, R. van Dortmund, J. High. Resolut. Chromatogr. 15 (1992) 249.
[122] E. Storr-Hansen, J. Chromatogr. 558 (1991) 375.
[123] S. Bøwadt, B. Johansson, Anal. Chem. 66 (1994) 667.
[124] N. Kannan, S. Tanabe, R. Tatsukawa, Arch. Environ. Health 43 (1988) 11.
[125] S. Safe, Crit. Rev. Toxicol. 21 (1990) 51.
[126] D.E. Tillitt, J.P. Giesy, G.T. Ankley, Environ. Sci. Technol. 25 (1991) 87.
[127] K. Ballschmiter, R. Bacher, A. Mennel, R. Fischer, U. Riehle, M. Swerev, J. High Resolut. Chromatogr. 15 (1992) 260.
[128] F.W. Kutz, D.G. Barnes, D.P. Bottimore, H. Greim, E.W. Bretthauer, Chemosphere 20 (1990) 751.
[129] F. Verstraete, Organohalogen Comp. 55 (2002) 1.
[130] A.K.D. Liem, R.M.C. Theelen, PhD thesis, University of Utrecht, The Netherlands, 1997.
[131] J.H. Knox, B.J. Kaur, J. Chromatogr. 352 (1986) 3.
[132] C.S. Creaser, A. Al Haddad, Anal. Chem. 61 (1989) 1300.
[133] J. de Boer, C.J.N. Stronck, F. van der Valk, P.G. Wester, M.J.M. Daudt, Chemosphere 25 (1992) 1277.
[134] P. Haglund, L. Asplund, U. Jårnberg, B. Jansson, Chemosphere 20 (1990) 887.
[135] J. de Boer, C.J.N. Stronck, W.A. Traag, J. van der Meer, Chemosphere 26 (1993) 1823.
[136] D.W. Kuehl, B.C. Butterworth, J. Libal, P. Marquis, Chemosphere 22 (1991) 849.
[137] L.M. Smith, T.R. Schwarz, K. Feltz, T.J. Kubiak, Chemosphere 21 (1990) 1063.
[138] J. de Boer, Q.T. Dao, P.G. Wester, S. Bøwadt, U.A.Th. Brinkman, Anal. Chim. Acta 300 (1995) 155.
[139] J. de Boer, Q.T. Dao, Int. J. Environ. Anal. Chem. 43 (1991) 245.
[140] P. Korytár, P.E.G. Leonards, J. de Boer, U.A.Th. Brinkman, J. Chromatogr. A 958 (2002) 203.
[141] H.-J. de Geus, J. de Boer, U.A.Th. Brinkman, Chromatographia 55 (2002) 339.
[142] C. de Wit, Chemosphere 46 (2002) 583.
[143] Ö. Andersson, G. Blomkwist, Chemosphere 10 (1981) 1051.
[144] J. de Boer, Chemosphere 18 (1989) 2131.
[145] J. de Boer, P.G. Wester, H.J.C. Klamer, W.E. Lewis, J.P. Boon, Nature 394 (1998) 28.
[146] K. Norén, D. Meyronité, Chemosphere 40 (2000) 1111.
[147] A. Covaci, S. Voorspoels, J. de Boer, Chemical analysis of brominated flame retardants, Environ. Int. (2003) in press.
[148] A. Covaci, J. de Boer, J.J. Ryan, S. Voorspoels, S. Schepens, Anal. Chem. 74 (2002) 790.
[149] J. de Boer, W.P. Cofino, Chemosphere 46 (2002) 625.
[150] J. de Boer, A. van der Horst, P.G. Wester, P.E.G. Leonards, Environ. Pollut. 122 (2003) 63.
[151] J. de Boer, D.E. Wells, K. Norén, Organohalogen Comp. 58 (2002) 197.
[152] Y. Lind, M. Aune, S. Atuma, W. Becker, R. Bjerselius, A. Glynn, P.O. Darnerud, Organohalogen Comp. 58 (2002) 181.
[153] M. Peled, R. Scharia, D. Sondack, in: Advances in Organic Chemistry, Vol. II, Elsevier, New York, 1995, p. 92.
[154] F. Barontini, V. Cozzani, L. Petarca, Ind. Eng. Chem. Res. 40 (2001) 3270.
[155] C. Allchin, in: QUASIMEME report of the Workshop on the analysis of BFRs, Barcelona, 21–23 October, 2002.
[156] P. Pott, Chirurgical Observations Relative to the Cataracts, the Polypus of the Nose, the Cancer of the Scrotum, the Different Kinds of Ruptures, and the Mortification of the Toes and Feet, Hawes, Clarke, and Collins, London, 1775.
[157] K. Yamigawa, K. Ichiwawa, Uber die kunstliche Erzeugung von Papillom, V. Jap. Path. Ges. 5 (1915) 142.
[158] J.W. Cook, C.L. Hewett, I. Hieger, J. Chem. Soc. (1933) 395.
[159] R.G. Harvey, Polycyclic Aromatic Hydrocarbons: Chemistry and Carcinogenicity, Cambridge University Press, Cambridge, 1991.
[160] A. Bjørseth (Ed.), Handbook of Polycyclic Aromatic Hydrocarbons, Marcel Dekker, New York, 1983, p. 397.
[161] J. Borneff, R. Fischer, Arch. Hyg. Bakteriol. 146 (1962) 183.
[162] J. Borneff, R. Fischer, Arch. Hyg. Bakteriol. 146 (1962) 334.
[163] R.E. Schaad, Chromatogr. Rev. 13 (1970) 61.
[164] E. Sawicki, T.W. Stanley, W.C. Elbert, J. Mecker, S. McPherson, Atmos. Environ. 1 (1967) 131.
[165] E. Sawicki, T.R. Stanley, J.D. Pfaff, W.C. Elbert, Chemist-Analyst 53 (1964) 6.
[166] R.C. Pierce, M. Katz, Anal. Chem. 47 (1975) 1743.
[167] M. Katz, T. Sakoma, A. Ho, Environ. Sci. Technol. 12 (1978) 909.
[168] M. Katz, C. Chan, Environ. Sci. Technol. 14 (1980) 838.
[169] H. Kunte, J. Borneff, Z. Wasser Abwasser 9 (1976) 35.
[170] F. Telli-Karakoç, L. Tolun, B. Henkelmann, C. Klimm, O. Okay, K.-W. Schramm, Environ. Pollut. 119 (2002) 383.
[171] M. Ehrhardt, J. Klungsøyr, R.J. Law, ICES Times Series 12 (1991) 1.
[172] G.M. Brilis, P.J. Marsden, Chemosphere 21 (1990) 91.
[173] E. Manoli, C. Samara, Chromatographia 43 (1996) 135.
[174] R.J. Woodhead, R.J. Law, P. Matthiessen, Mar. Pollut. Bull. 38 (1999) 773.
[175] B. Beliaeff, T.P. O'Connor, C. Munschy, B. Raffin, D. Claisse, Environ. Toxicol. Chem. 21 (2002) 1783.
[176] C. Kelly, R. Law, K. Baker, P. Roberts, The extraction of polycyclic aromatic hydrocarbons (PAH) from sediments and biota using microwave-assisted alkaline saponification (MAAS), in: Poster presented at the QUASIMEME Conference ''Measurement in the Marine Environment: Challenges and Achievement'', Barcelona, Spain, 23–26 October 2002, 2002.
[177] L. Webster, R.J. Fryer, E.J. Dalgarno, C. Megginson, C.F. Moffat, J. Environ. Monit. 3 (2001) 591.
[178] H.A. Bamford, D.Z. Bezabeh, M.M. Schantz, S.A. Wise, J.E. Baker, Chemosphere 50 (2003) 575.
[179] C.A. Kelly, R.J. Law, H.S. Emerson, CEFAS Sci. Ser., Aquat. Environ. Prot.: Anal. Methods 12 (2000) 1.
[180] A. Bjørseth (Ed.), Handbook of Polycyclic Aromatic Hydrocarbons, Marcel Dekker, New York, 1983, p. 257.
[181] J.R. Wilmshurst, J. Chromatogr. 17 (1965) 50.

[182] H. Borwitsky, B. Schomburg, J. Chromatogr. 170 (1979) 99.
[183] P.A. Peaden, B.W. Wright, M.L. Lee, Chromatographia 15 (1982) 335.
[184] A. Bjørseth (Ed.), Handbook of Polycyclic Aromatic Hydrocarbons, Marcel Dekker, New York, 1983, p. 290.
[185] S.A. Wise, C.F. Allen, S.N. Chesler, H.S. Hertz, L.R. Hilpert, W.E. May, R.E. Rebbert, C.R. Vogt, NBSIR-82-2595, National Bureau of Standards, Washington, DC, 1982.
[186] Y. Hirata, M. Novotny, J. Chromatogr. 186 (1980) 521.
[187] M.L. Lee, M.V. Novotny, K.D. Bartle, Analytical Chemistry of Polycyclic Aromatic Compounds, Academic Press, New York, 1981.
[188] A. Bjørseth (Ed.), Handbook of Polycyclic Aromatic Hydrocarbons, Marcel Dekker, New York, 1983, p. 190.
[189] F. Ariese, S.J. Kok, M. Verkaik, C. Gooijer, N.H. Velthorst, J.W. Hofstraat, Aquat. Toxicol. 26 (1993) 273.
[190] J.W. Kicenuik, S. Ray (Eds.), Analysis of Contaminants in Edible Resources, VCH, Weinheim, 2000, p. 439.
[191] P.A. Peaden, M.L. Lee, Y. Hirata, M. Novotny, Anal. Chem. 52 (1980) 2268.
[192] C.H. Marvin, R.W. Smith, D.W. Bryant, B.E. McCarry, J. Chromatogr. A 863 (1999) 13.
[193] R.B. Sleight, J. Chromatogr. 83 (1973) 31.
[194] S.A. Wise, W.J. Bonnett, F.R. Guenther, W.E. May, J. Chromatogr. Sci. 19 (1981) 457.
[195] A. Bjørseth (Ed.), Handbook of Polycyclic Aromatic Hydrocarbons, Marcel Dekker, New York, 1983, p. 439.
[196] R.J. Law, Mar. Pollut. Bull. 9 (1978) 321.
[197] O. Grahl-Nielsen, R. Law, K. Palmork, J.E. Portmann, S. Wilhelmsen, CM 1978/E:22, ICES, Copenhagen, Denmark, 1978.
[198] R.J. Law, J.E. Portmann, ICES Cooperative Research Report 117, ICES, Copenhagen, Denmark, 1982.
[199] J.W. Farrington, A.C. David, J.B. Livramento, C.H. Clifford, N.M. Frew, A. Knap, ICES Cooperative Research Report 141, ICES, Copenhagen, Denmark, 1986.
[200] J.F. Uthe, C.J. Musial, G.R. Sirota, ICES Cooperative Research Report ICES 141, ICES, Copenhagen, Denmark, 1986.
[201] J.P. Villeneuve, L.D. Mee, IAEA Marine Environment Laboratory Report No. 51, IAEA, Monaco, 1992.
[202] J.P. Villeneuve, M. Horvat, C. Cattini, IAEA Marine Environment Laboratory Report No. 59, IAEA, Monaco, 1996.
[203] R.J. Law, J.L. Biscaya, Mar. Pollut. Bull. 29 (1994) 235.
[204] R.J. Law, J. Klungsøyr, Mar. Pollut. Bull. 32 (1996) 667.
[205] R.J. Law, J. Klungsøyr, I.L. Freriks, Mar. Pollut. Bull. 35 (1997) 64.
[206] R.J. Law, C.A. Kelly, M.D. Nicholson, J. Environ. Monit. 2 (2000) 517.
[207] R. Albert, W. Horwitz, Anal. Chem. 69 (1997) 789.
[208] R.J. Law, J. Klungsøyr, Int. J. Environ. Pollut. 13 (2000) 262.
[209] C.H. Marvin, B.E. McCarry, J.A. Lundrigan, K. Roberts, D.W. Bryant, Sci. Total Environ. 231 (1999) 135.
[210] R.J. Law, C.A. Kelly, K.L. Baker, K.H. Langford, T. Bartlett, Mar. Pollut. Bull. 44 (2002) 903.
[211] A. Bemgård, A. Colmsjö, B.-O. Lundmark, J. Chromatogr. 595 (1992) 247.
[212] A. Bemgård, A. Colmsjö, B.-O. Lundmark, J. Chromatogr. 630 (1993) 287.
[213] L. Mazeas, H. Budzinski, Environ. Sci. Technol. 36 (2002) 130.
[214] W. Wilcke, M. Krauss, W. Amelung, Environ. Sci. Technol. 36 (2002) 3530.
[215] P.D. Boehm, D.S. Page, W.A. Burns, A.E. Bence, P.J. Mankiewicz, J.S. Brown, Environ. Sci. Technol. 35 (2001) 471.
[216] W.A. Burns, P.J. Mankiewicz, A.E. Bence, D.S. Page, K.R. Parker, Environ. Toxicol. Chem. 16 (1997) 1119.
[217] D.E. Wells, A. Allan, Quality Assurance of Sample Handling (QUASH), EU report STM4-CT96-2100, EU DG12, Brussels, Belgium, 2001.
[218] D.E. Wells, A. Aminot, J. de Boer, W.P. Cofino, D. Kirkwood, B. Pedersen, Mar. Pollut. Bull. 35 (1997) 3.