BIOAVAILABILITY OF ORGANIC CONTAMINANTS IN SEDIMENTS

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A thesis submitted in partial fulfilment of the requirements of The Robert Gordon University Aberdeen for the degree of Doctor of Philosophy

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Declaration

I hereby declare that the work or any portion of it, referred to in this thesis has not been submitted in support of an application for another degree or qualification of this, or any other university or institute of learning. This is an original piece of work undertaken by myself. All results and work other than my own are clearly cited and acknowledged.

Kyari Yates

BIOAVAILABILITY OF ORGANIC CONTAMINANTS IN SEDIMENTS

The bioavailability of polycyclic aromatic hydrocarbons (PAHs) and chlorobiphenyls (CBs) in sediments is largely dependent on the freely dissolved concentration of these pollutants. However, measuring these is challenging, due to the low concentrations of lipophilic contaminants in the environment and their strong affinity for particles and for traditional sampling (filtration and centrifugation) equipment. An equilibrium passive sampling device made of silicone rubber was developed in this research to measure the freely dissolved concentrations of lipophilic contaminants and other parameters (water extractable proportions and sediment-water partition coefficients) that describe the availability of these contaminants in the environment.

Equilibration between sampler and sediment for PAHs and CBs was found to be adequately achieved after 20 days shaking of a silicone rubber sampler in sediment slurry on an orbital shaker at 200 rpm. The reproducibility of uptake was better than 5 %.

Silicone rubber-water partition coefficients for 34 PAHs and 32 CBs were measured in the laboratory using a co-solvent method using methanol as co solvent. Strong linear correlations of $\log K_{sr,w}$ with octanol-water partition coefficients ($\log K_{ow}$) ($\log K_{sr,w} = 0.97 \log K_{ow} - 0.01; r^2 = 0.94$ & $\log K_{sr,w} = 1.17 \log K_{ow} - 1.82; r^2 = 0.90$) were found for PAHs and CBs, with a systematic difference in correlations observed for the different classes of compounds which was attributed to structural differences of the compounds.

The silicone rubber samplers were then used to measure concentrations of PAHs in the pore water of sediments from the Fladen Ground of the North Sea, Loch Shell, Firth of Forth, Firth of Clyde, Loch Etive and Aberdeen Harbour in Scotland and the Vefsn fjord, Norway. A proportion of the PAHs were found to be unavailable for exchange into the aqueous phase, and this was reflected in the high $\log K_{oc}$ measured in all the sediments studied. The sediment-water partition coefficients also correlated positively with the octanol-water partition coefficients. Accumulation of PAHs in *Nereis virens* from sediments was better predicted from literature bio concentration factors and pore water concentrations obtained using the silicone rubber samplers than from sediment concentrations traditionally used in risk assessments.

Participation in an International Council for the Exploration of the Seas (ICES) passive sampling trial survey using silicone rubber in sediments and water is described, and demonstrated the potential of passive sampling in monitoring environmental pollution. The log BCF (bio concentration factor) for PAHs in mussels increased with increasing $\log K_{ow}$ at both Loch Etive and Aberdeen Harbour locations, and could be used to estimate concentrations in mussels directly. The survey data also showed the use of silicone rubber in assessing the diffusive exchange of PAHs across sediment-water interfaces.

Acknowledgements

I can do all things through Christ who strengthens me… Phil. 4 v 13!

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Finally to the Petroleum Technology Development Fund of Nigeria, thanks for making my dreams come true.

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Glossary

AHH	Aryl Hydrocarbon Hydroxylase
AhR	Aryl hydrocarbon receptor
ANOVA	Analysis of Variance
ASE	Accelerated Solvent Extraction
BAT	Best Available Techniques
BAC	Background Assessment Concentration
BAF	Bioaccumulation Factor
BC	Background Concentrations
BCF	Bio concentration Factor
BHT	Butylated hydroxytoluene
BRC	Background Reference Concentrations
BSAF	Biota Sediment Accumulation Factor
CBs	Polychlorinated biphenyls
CO_2	Carbon dioxide
CRM	Certified Reference Material
CV	Coefficient of Variation
DCBE	Dichlorobenzyl alkyl ethers
DCM	Dichloromethane
DF	Distribution Factor
DNA	Deoxyribonucleic acid
EAC	Ecotoxicological Assessment Criteria
EAF	Exposure Adjustment Factor
EC	European Commission
EHC	Environmental Health Criteria
EROD	Ethoxyresorufin-*0*-deethylase
ESD	Equilibrium Sampling Devices
EU	European Union
FRS ML	Fisheries Research Services Marine Laboratory
GC-ECD	Gas Chromatography Electron Capture Detection
GC-FID	Gas Chromatography Flame Ionisation Detection
GC-MSD	Gas Chromatography Mass Selective Detection

GST	Glutathione –*S*- transferase
H_2O	Water
HCB	Hexachlorobenzene
HCl	Hydrochloric acid
HOC	Hydrophobic Organic Contaminant
HPCD	Hydroxypropyl-β-cyclodextrin
HPLC	High Performance Liquid Chromatography
IARC	International Agency for Research on Cancer
ICES	International Council for the Exploration of the Seas
ISO	International Standards Organisation
JMP/JAMP	Joint Monitoring Programme/ Joint Assessment Monitoring Programme
K_{ow}	Octanol-water partition coefficient
LDPE	Low Density Polyethylene
LMW	Low Molecular Weight
LOD	Limit of Detection
LOQ	Limit of Quantification
LRM	Laboratory Reference Material
LRTAP	Long Range Trans-boundary Air Pollutants
LSD	Least Significant Difference
MAC-EQS	Maximum Allowable Concentration-Environmental Quality Standards
MCWG	Marine Chemistry Working Group
MeOH	Methanol
MFO	Mixed Function Oxidase
MTBE	Methyl *t*-butyl ether
N_2	Nitrogen
NMBAQC	National Marine Biological Analytical Quality Control
NO_3	Nitrates
NRC	National Research Council
OCPs	Organochlorinated pesticides
OSPAR	Oslo and Paris Commission, OSPAR
PAHs	Polycyclic aromatic hydrocarbons
PBDEs	Polybrominated diphenyl ethers
PCA	Principal Component Analysis

PCDD/Fs	Polychlorinated dibenzo-*p*-dioxins and furans
PCTs	Polychlorinated terphenyls
PDA	Partitioning driven administration
PDMS	Poly (dimethyl)siloxane
PISCES	Passive *in situ* concentration /extraction sampler
POM-SPE	Polyoxymethylene-solid phase extraction
POPs	Persistent Organic Pollutants
PPC	Pollution Prevention Control
PRC	Performance/ Permeability Reference Compound
PSA	Particle Size Analysis
PSD	Passive Sampling Devices
PSTS	Passive Sampling Trial Survey
PTFE	Polytetrafluoroethylene
QUASIMEME	Quality Assurance of Information for Marine Environmental Monitoring in Europe
SARA	Soil (or sediment) availability ratio
SIM	Selected Ion Monitoring
SLM	Supported liquid membrane
SOP	Standard Operating Procedure
SPM	Suspended Particulate Matter
SPMD	Semi permeable membrane devices
SPME	Solid Phase Microextraction
TCDD	2, 3, 7, 8- tetrachlorodibenzo-*p*-dioxin
TEF	Toxicity Equivalency Factor
TEQ	Toxicity Equivalency Quotient
TOC	Total organic carbon
UKAS	United Kingdom Accreditation Service
UK NMMP	United Kingdom National Marine Monitoring Programme
UNEP	United Nations Environment Programme
USA	United States of America
USEPA	United States Environmental Protection Agency
WGMS	Marine Sediment Working Group in relation to pollution
WHO	World Health Organisation
WTSB	Water Science and Technology Board

CHAPTER ONE

Introduction

1.0 Background

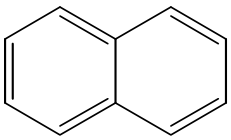
Persistent Organic Pollutants (POPs) are pollutants that are poorly soluble in water, mostly persistent and have high affinity for particles. Aquatic/marine sediments are known to serve as both repositories and long term sources of POPs to water and biota, therefore posing both ecological and human health risks for a prolonged period of time. Polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (CBs), polychlorinated dibenzo-*p*-dioxins and furans (PCDD/Fs) and organochlorinated pesticides (OCPs) are among the important POPs in contaminated sediments which are of concern due to their long life and toxicity. Due to this repository/reservoir-serving nature, fish and other aquatic organisms can accumulate these toxic compounds which are then likely to be passed up the food chain. As a consequence they have been identified as priority pollutants by United States environmental protection agency, USEPA (Keith and Telliard, 1979) and are included on the Oslo and Paris Commission, OSPAR (OSPAR, 2000) List of Chemicals for Priority Action.

1.1 Occurrence

PAHs are a class of organic compounds characterised by fused aromatic rings made up of carbon and hydrogen atoms. They occur mostly in mixtures which are complex and vary with generation process. Environmental PAHs arise from natural (mainly volcanic eruptions, forest and prairie fires (Manoli *et al*., 2000)) or anthropogenic sources (as a result of human influence on the environment) such as aluminium smelters, creosote, automobile exhaust, the combustion of fossil fuels (Law and Biscaya, 1994) - pyrolytic origin and are also present in crude oils, coal, coal tar, or various refinery products (petrogenic origin) (Webster *et al*., 2001).

The pyrolytic PAHs are characterised by the dominance of the parent, non-alkylated species (Fig **1.0**), such as pyrene, benzo[*a*]pyrene, and coronene (the highly peri-condensed compounds). Petrogenic sources are dominated by 2- and 3-ring alkylated PAHs. PAHs have been detected in a variety of environmental samples, including air, soil, sediments, water, oils, tars and foodstuffs (Juhasz and Naidu, 2000). Pyrolytic PAHs are widespread in sediments post-dating the industrial revolution and demonstrate the extent of human influence on the environment (Dahle *et al*., 2003). They have been found in motor vehicle exhausts (Wenchuan *et al*., 2001), tobacco smoke (Lee *et al*., 2002), and in a study of indoor PAH levels in 16 family houses in Sweden (9 used wood burning as domestic heating while 7 used either electrical or heating pumps), Strandberg *et al*., 2004 found ~ 450 ng m^{-3} as highest concentration in the vapour phase of indoor air in houses using wood against 50 ng m^{-3} in houses using other heating systems, with phenanthrene being the most abundant compound. Webster *et al*. (2003) determined PAH concentrations from sediments collected from the Moray Firth axis and environs, Scotland, where the main economic activity is oil exploration and production and having had an aluminium smelting plant. They found PAHs of both pyrolytic and petrogenic sources. Pyrolytic input dominated the inner Moray Firth while the middle section of the transect studied was predominantly petrogenic. However, the most likely source of the petrogenic contamination was from shipping activity and not oil exploration activity in the area. In a similar study of twelve sea lochs in west coast of Scotland, Webster *et al.* (2004) found the highest total PAH concentration to be in Loch Linnhe and close to an aluminium smelter at Loch Leven, with only sediments from Loch Clash showing evidence of petrogenic input. McIntosh *et al*. (2002) have also reported PAHs in Loch Leven and attributed these to discharges from the aluminium smelting process in Kinlochleven.

A few PAHs can also be produced by natural processes such as the relatively rapid recombination or rearrangement of constituents (diagenesis such as that of sedimentary organic material to form fossil fuels (Wenchuan *et al*., 2001)) and from direct biosynthesis by organisms and plants (biogenic PAHs). Venkatesan (1988) in a review of occurrence and possible sources of perylene in marine sediments concluded that perylene originates in sediments fed by both terrestrial and aquatic organic debris, and diatoms appear to be the major potential precursors for perylene in the aquatic regime.



Naphthalene

$C_{10}H_8$
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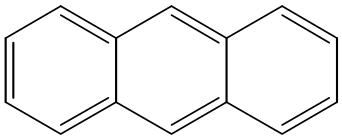
Mol. Wt.: 128.17
C, 93.71; H, 6.29



Anthracene

$C_{14}H_{10}$
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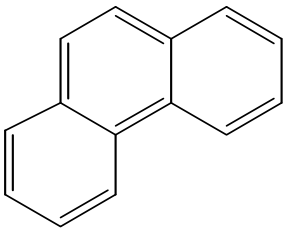
Mol. Wt.: 178.23
C, 94.34; H, 5.66



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$C_{14}H_{10}$
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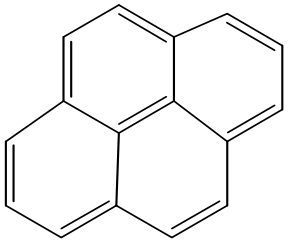
Mol. Wt.: 178.23
C, 94.34; H, 5.66



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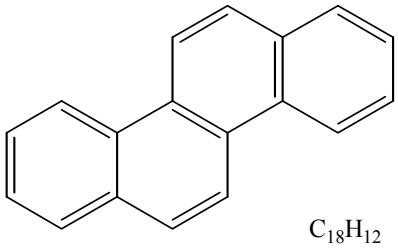

Chrysene

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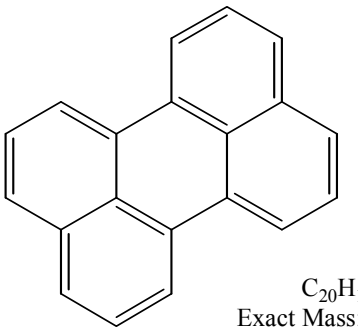
Mol. Wt.: 228.29
C, 94.70; H, 5.30



Perylene

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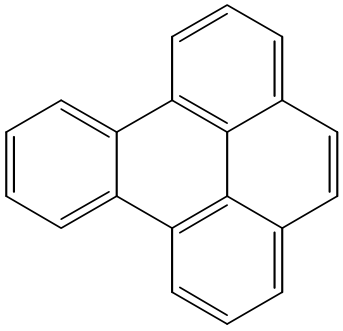
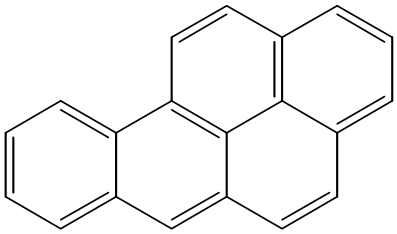
Mol. Wt.: 252.31
C, 95.21; H, 4.79



Benzo[*e*]pyrene

$C_{20}H_{12}$
Exact Mass: 252.09
Mol. Wt.: 252.31
C, 95.21; H, 4.79



Benzo[*a*]pyrene

$C_{20}H_{12}$
Exact Mass: 252.09
Mol. Wt.: 252.31
C, 95.21; H, 4.79

Fig **1.0:** Examples of PAHs in the USEPA priority list

While studying surface sediments of the Yalujiang estuary of North China, Wu *et al*. (2003) found that perylene accounted for 20 % or more of the total PAHs in the sediments, indicative of biogenic origin. High perylene levels in Kara Sea samples with highest levels found in areas of strong terrigenous influence was attributed to decaying peat products being transported to the Sea by both large and small rivers (Dahle *et al*., 2003).

Each source of PAH is characterised by a specific molecular pattern and it is therefore possible, to a degree, to determine which source generated these compounds (Baumard *et al*., 1999). A summary of PAH concentration ratios commonly used to distinguish between petrogenic and pyrolytic sources has been presented by Webster *et al*. (2001). The ratios include; Phenanthrene: Anthracene (P/A), Fluoranthene: Pyrene (Fl/Py), Methylphenanthrene: Phenanthrene (MP/P) and Fluoranthene+Pyrene: Methylfluoranthene+Methylpyrene (Fl+Py)/ (MFl+MPy). A high proportion of parent compounds (> 40 %) and a (Fl+Py)/ (MFl+MPy) ratio of close to 3 indicates a pyrolytic contribution. An MP/P ratio > 2 suggests a petrogenic source while P/A ratios < 10 and Fl/Py > 1 suggests pyrolytic source of PAHs.

PAHs in sediments are mainly associated with organic matter, so sediments with a high organic carbon content have the potential to accumulate higher concentrations of hydrophobic compounds such as PAHs, with the PAH concentration also affected by the particle size (Webster *et al*., 2001).

Polychlorinated biphenyls (CBs) have been widely studied as a result of their wide production and use, transport capability, persistence, bioaccumulation and risks to the environment and human health. They are mainly of anthropogenic origin and are not naturally occurring compounds but are produced from the chlorination of a biphenyl ring.
CBs are two biphenyl rings linked by a single carbon bond (Fig **1.1**). The two biphenyl rings are free to rotate unless there are ortho chlorine substitutions at the 2, 2'- or 6, 6' positions. A number of chlorine atoms can be substituted to each ring. The CBs are a group of 209 congeners, with ten identifiable homolog groups (mono-, di-, to deca-CBs) although only about 130 congeners are likely to occur in commercial products (WHO: EHC 140, 1993).

The position of the chlorine substitution, ability of the molecule to rotate about the bridging carbon bond, and the reactivity of the chlorine atoms determine the toxicity of the compounds. CBs are basically non planar, although one of the conformations is a situation where the two phenyl groups exist in the same plane (coplanarity).

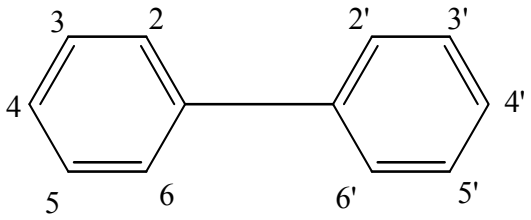


Fig **1.1**: CB general structure showing the numbered positions for possible substitution of chlorine atoms

Historically, CBs entered the environment during the manufacture and use but they can enter the sea through processes like riverine discharges and water runoffs and get bound in sediments or diluted and transported across the water body. Due to their wide use and production, they have been found in different areas of the world (UNEP, 1998), sometimes as a result of atmospheric transport and deposition. They had been recognised as significant contaminants since 1966 (Hawker and Connell, 1988) and were sold as mixtures (example, trade name Aroclor, by Monsanto Inc., USA) that was based on percent chlorination, for example, Aroclor 1248, 1254 and 1260, representing relative 48, 54 and 60 % chlorination respectively in each mixture.

It is estimated that in 2002, 68 % of UK CB emission to the atmosphere was from old CB-containing appliances (Dore *et al.*, 2004). Estuarine input into the sea is considered important as estuaries are a major interface between the land and sea (Hong *et al*., 1999). Possible routes of entry of CBs into the environment (Nisbet and Sarofim, 1972) include:

- Leaks from sealed transformers and heat exchangers
- Leaks of CB-containing fluids from hydraulic systems which are only partially sealed
- Spills and losses in the manufacturing of either CBs or CB-containing fluids
- Vaporisation or leaching from CB-containing formulations
- Improper disposal of waste CBs or CB-containing fluids

93.1 % of the contemporary UK environmental burden of total CBs was estimated to be associated with soils, 3.5 % with sea water and 2.1 % in marine sediments. Freshwater sediments, vegetation, humans and sewage sludge collectively account for 1.4 % while CB loadings in air and freshwater are insignificant (Harrad *et al*., 1994).

1.2 Properties of PAHs & CBs

PAHs are hydrophobic compounds. Hydrophobicity is expressed by the octanol-water partition coefficient (K_{ow}) which estimates solubility in both aqueous and organic phases (n-octanol being commonly used) and the tendency for a compound to move from water (polar liquid) into a non-polar liquid which does not mix with water. K_{ow} is the ratio of C_{org} to C_{aq}, i.e.

$$K_{ow} = \frac{C_{org}}{C_{aq}} \qquad \textbf{1.0}$$

C_{org} and C_{aq} are concentrations in organic (n-octanol) and aqueous (water) phase respectively.

Values of K_{ow} vary by several orders of magnitude and this parameter is usually expressed in the logarithmic form. The $\log K_{ow}$ is essential for understanding transport mechanisms and distributions of compounds into the environment. The $\log K_{ow}$ increases with increasing molecular weight while solubility decreases along the same trend. PAHs of different molecular weights or structures vary substantially in their behaviour and distribution in the environment and their effects on biological systems (Wenchuan *et al*., 2001). The PAH compounds found in the US EPA priority list (Mastral and Callén, 2000; Keith and Telliard, 1979) were selected on the basis of their potential effects on human health and risks posed to the environment, persistence and degradability of the pollutant (see Table **1.0**).

Table **1.0:** Overview of PAH compounds showing the 16 US EPA PAHs and the 9 PAH agreed by OSPAR[a]

PAH	US EPA	OSPAR
Naphthalene	X	
Acenaphthylene	X	
Acenaphthene	X	
Fluorene	X	
Phenanthrene	X	X
Anthracene	X	X
Fluoranthene	X	X
Pyrene	X	X
Chrysene	X	X
Benzo[*a*]Anthracene	X	X
Benzo[*b*]fluoranthene	X	
Benzo[*k*]fluoranthene	X	
Benzo[*a*]pyrene	X	X
Indeno[1,2,3-*cd*]pyrene	X	X
Dibenz[*a,h*]Anthracene	X	
Benzo[*g,h,i*]perylene	X	X

[a] (OSPAR, 2000)

They generally exist as colourless, white or pale yellow-green solids at ambient temperatures with high melting- and boiling points, and low vapour pressure. They are soluble in many organic solvents e.g. *iso*-hexane, acetone, methanol, toluene, acetonitrile, e.t.c. and are highly lipophilic. They are relatively chemically inert with photodecomposition reactions and reactions with nitrogen oxides, nitric acids, sulphur oxides, sulphuric acid, ozone and hydroxyl radicals being those of interest with respect to their environmental fate and possible sources of loss during atmospheric sampling. When they react, they undergo either electrophilic substitution (such as nitration, sulfonation, halogenation and alkylation reactions) in which they tend to form derivatives rather than electrophilic addition in which the aromatic character of the affected benzene ring is destroyed. Different PAHs have considerably varying reactivity with atmospheric oxidants such as OH radicals, ozone or NO_3 (Kalberer *et al*., 2004).

Huang *et al*., (2004) in their study found that the degree of alkylation is a critical factor controlling the rate of biodegradation of reservoired oil, with the rate decreasing with increasing number of alkyl substituents in most cases. Their study showed that the thermally most-stable isomers of the studied PAHs (alkylated naphthalenes and phenanthrenes as well as their isomers) are generally more susceptible to biodegradation than thermally less-stable ones, suggesting that biodegradation and selective depletion is not controlled by thermodynamics but is related to the stereo chemical structure of individual compounds.

The physical and chemical properties are largely determined by the conjugated alpha-electron systems, which vary fairly regularly with the number of rings and molecular mass, giving rise to a more or less wide range of values for each parameter within the whole class (Table **1.1**). Most do not dissolve easily in water, but some readily evaporate into the air and most do not burn easily. The volatility however decreases with increasing number of fused rings with their structure making them stable in the environment especially under reducing conditions (Wilcock and Northcott, 1995). The breakdown of PAHs generally occurs by biological or geochemical degradation, or by photolysis (PAHs strongly absorb in the UV range). Vapour pressure and aqueous solubility decrease almost logarithmically with increasing molecular weight (Yu, 2004). The aqueous solubilities of some PAHs have been shown to increase with increase in temperature. For example, May and Wasik (1978) showed an increase in the aqueous solubility of pyrene from 0.132 ± 0.002 mg kg^{-1} at 25 ^{o}C to 0.161 ± 0.001 mg kg^{-1}at 29 ^{o}C, while Miller and Hawthorne (1998) also found a 275 000-fold increase in solubility of benzo[*a*]pyrene in water from 0.004 µg g^{-1} at 25 ^{o}C to 1100 µg g^{-1} at 250 ^{o}C. The structural chemistry of the compounds control their solubility, bioavailability, susceptibility to degradation and capacity for depuration by an organism (Elder and Dresler, 1988).

Table **1.1**: Selected properties of PAHs from Martinez *et al*., (2004)

Compound	Rings	Molecular mass	Solubility (mmol/L)	log K_{ow}	Vapour pressure 25 °C (Pa)
Naphthalene	2	128	2.4×10^{-1}	3.37	10.9
Acenaphthylene[(1)]	3	152	1.1×10^{-1}	4.00	9.0×10^{-1}
Acenaphthene	3	154	2.9×10^{-2}	4.07	5.96×10^{-1}
Fluorene	3	166	1.2×10^{-2}	4.18	8.81×10^{-2}
Phenanthrene	3	178	7.2×10^{-3}	4.45	$(1.8 \pm 0) \times 10^{-2}$
Anthracene	3	178	3.7×10^{-4}	4.45	$(7.5 \pm 0) \times 10^{-4}$
Fluoranthene	4	202	1.3×10^{-3}	4.90	2.54×10^{-1}
Pyrene	4	202	7.2×10^{-4}	4.88	8.86×10^{-4}
Benz[*a*]anthracene	4	228	4.8×10^{-5}	5.61	$(7.3 \pm 1.3) \times 10^{-6}$
Chrysene	4	228	5.7×10^{-7}	5.16	1.3×10^{-5}
Benzo[*b*]fluoranthene	5	252	6.0×10^{-6}	6.04	1.2×10^{-7}
Benzo[*k*]fluoranthene	5	252	3.2×10^{-6}	6.06	5.5×10^{-8}
Benzo[*a*]pyrene	5	252	8.4×10^{-7}	6.06	1.5×10^{-5}
Indeno[1,2,3-*cd*]pyrene	6	276	6.9×10^{-7}	6.58	n.f.
Dibenz[*a*, *h*]anthracene	6	278	$(3.7 \pm 1.8) \times 10^{-10}$	6.50	0.8×10^{-6}
Benzo[*ghi*]perylene	6	276	6.0×10^{-8}	6.84	2×10^{-5}

CBs are oily liquids or solids, clear to yellow in colour with no distinct smell or taste. The CB congeners show distinct physicochemical and toxicological properties that allow them to be studied as well defined individual chemical entities (Camacho-Ibar and McEvoy, 1996). The congener pattern of samples from different environments is influenced by both original composition of pollutants and sources of pollution, but also varies with the properties of the

CBs (Konat and Kowalewska, 2001). Generally, melting point, vapour pressure, water solubility and biodegradability decrease with increasing number of chlorine atoms though the decrease is not always uniform (Nisbet and Sarofim, 1972). The properties vary widely and are dependent on the number and position of chlorine atoms in the biphenyl ring. The lower chlorinated congeners such as CB 28 and 52 possess comparatively greater vapour pressures and water solubilities than the higher chlorinated CBs such as CB 138 and 180, whilst the higher chlorinated congeners are more lipophilic (Harrad *et al.*, 1994).

CB mixtures may contain polychlorinated dibenzofurans (PCDFs) and chlorinated quarterphenyls as impurities which are relatively stable and resistant to chemical reactions under normal conditions (WHO EHC 140, 1993). They are lipophilic with low water solubility and thus accumulate in fatty tissues for long periods of time, once they enter the food chain and can biomagnify. They are also able to dissolve in polar matrix to a degree and are highly soluble in organic solvents of low polarity (Walker *et al*., 1996). They have been measured in various environmental matrices like air, water, soil and sediments.

CBs have very low volatility, do not crystallise even at low temperatures but turn to solid resins, and are fire resistant with high flash points (170-380 ^{o}C). They have low electrical conductivity, rather high thermal conductivity and high resistance to thermal break down (the basis for their use in electrical and heat exchange equipments). They are very stable under normal conditions, but when heated, some toxic compounds such as PCDFs can be produced (WHO EHC 140, 1993). They are very hydrophobic with $\log K_{ow}$ ranging from 4.09 to 8.18 (see Table **1.2**) with high density (1.182-1.566 kg L^{-1}) due to the presence of the chlorine atoms.

Table **1.2:** Octanol-water partition coefficients of selected CBs and other pollutants in this study (Hawker and Connell, 1988)

Congener	Log K_{ow}	Congener	Log K_{ow}	Congener	Log K_{ow}	Congener	Log K_{ow}
CB 31	5.67	CB 70	6.20	CB118	6.74	CB 198	7.62
CB 28	5.67	CB 101	6.38	CB 153	6.92	CB 170	7.27
CB 53	5.62	CB 99	6.39	CB187	7.17	CB 189	7.71
CB 52	5.84	CB 112	6.45	CB 183	7.20	CB 194	7.80
CB 49	5.85	CB 97	6.29	CB 128	6.74	CB 209	8.18
CB 35	5.82	CB 110	6.48	CB 156	7.18	HCB	5.70
CB 44	5.75	CB 151	6.64	CB 157	7.18	Heptachlor	5.86
CB 74	6.20	CB 149	6.67	CB 180	7.36		

1.3 Uses

Most of the PAHs have no known use but a few are used in medicines, making dyes, plastics and pesticides. Naphthalene for example is used in making dyes, explosives, plastics, lubricants and is used as a household fumigant against moths. Anthracene is used in dyes, insecticides and wood preservatives; acenaphthene and acenaphthylene are used in the production of intermediates for pigments and resins respectively; pyrene and fluoranthene are also used in production of dyes (perinon pigments for pyrene and fluorescent and vat dyes for fluoranthene). They are mostly used in research, and hardly have any commercial use.

CBs are mainly used as dielectric fluids for capacitors and transformers, industrial fluids for hydraulic gas turbines and in vacuum pumps, heat transfer fluids, in plasticizers (Broadhurst, 1972) and other miscellaneous uses such as in paints, printing inks, sealants, adhesives, and carbonless copy paper (OSPAR, 2001). Between 1929 and 1989, the total world production of CBs, excluding the Soviet Union was in excess of 1 million tonnes and despite the restrictions on the production and use of CBs, very large amounts are found in the environment either in use or as waste, most especially in South America and Africa (UNEP, 1998).

1.4 Environmental fate, distribution and transportation

Contaminants tend to move from source of input to end up in one or more environmental media depending on their toxicity, physicochemical properties, persistence and mobility and in the process get transformed as a result of biological, physical and chemical processes into different breakdown products (Shaw and Chadwick, 1998). PAHs are released into the aquatic environment through a range of processes either through atmospheric deposition directly on water surfaces or indirectly via land run off and sewage waste water (Kirso and Irha, 1998). PAHs are easily transported atmospherically into the environment and over long distances as they bind to atmospheric particles. The transport and distribution of PAHs in the environment depend on their physico-chemical properties of very low solubility in water and low vapour pressure, and high partition coefficients for n-octanol: water ($\log K_{ow}$) and organic carbon: water ($\log K_{oc}$).

Organisms and detrital organic matter residing near the air-water interface may be exposed to high levels of more volatile PAHs as a result of gas phase deposition (Countway *et al*., 2003). Pereira *et al*. (1996) for example, suggested that the factors responsible for the transport of the hydrophobic compounds studied in the San Francisco Bay included biological processes such as bioturbation and microbial degradation of the sediment organic coatings, wind and tidal action, and ship traffic in and out of the canal cause mixing and re-suspension of contaminated particulate matter, with an observed diffusion of the studied PAHs from the source (where total PAHs concentration of ~ 30 ppm was found) down the canal (where ~ 5 ppm was measured).

Environmental contamination by CBs has arisen exclusively from human activities and therefore heavily contaminated areas tend to be located around industrial areas (Edgar *et al*., 1999) and then may cycle through atmospheric, aqueous, or biotic pathways (Grundy *et al*., 1996). Although there are restrictions in most countries on the use of CBs, their persistence, bioaccumulation and toxicity has created the need to understand their transport and distribution in the environment, as sediment which is a major sink for CBs now acts as a potential source for CBs by releasing the bound contaminants (Hong *et al*., 1995). As is the case with other contaminants, the physical and chemical properties; volatility, aqueous

solubility and lipophilicity in particular (Harrad *et al*., 1994) determine the transport and distribution of CBs. The fate and distribution of CBs in the environment has been studied (Hong *et al*., 1995; Hong *et al*. 1999; Sweetman *et al*., 2002; Sweetman *et al*., 2005; deBruyn and Gobas, 2004; Edgar *et al*., 1999, Webster *et al*., 2000a; Webster *et al*., 2000b; Webster and Campbell, 2002b), for example, Edgar *et al*. (1999) suggests that salinity and residence time in water may play an important role in the distribution patterns of CBs.

Vaporised CBs can be partially absorbed on particulates, transported with prevailing winds and deposited on land or water by particle sedimentation or rain-out (Nisbet and Sarofim, 1972). POPs of different volatilities migrate through the global atmosphere at varying velocities with the highly volatile ones remaining airborne and migrating faster while the low volatility POPs partition into water, snow, ice, soil or vegetation creating a variation in composition/concentration of POPs with temperature and latitudinal gradients (Wania and Mackay, 1996). Wania and Mackay suggest that the chemical fate of a POP is controlled by the point of discharge into the global environment, movements of the atmosphere and the oceans, rate of exchange processes between the atmosphere and earth's surface, and the rate of chemical loss from various environmental phases. Grundy *et al*., (1996), and Dushenko *et al*., (1996) concluded that vegetation (living and detrital plant materials) play a significant role in the environmental partitioning of CBs and probably other organic contaminants. A similar conclusion was made by Konat and Kowalewska (2001) on the role of algae and algal detritus on transport and distribution of CBs in the Southern Baltic. Hutzinger *et al*., (1972) showed that photolysis of CBs revealed a number of degradative reactions when irradiated in sunlight and a number of laboratory conditions such as dechlorination, formation of polymers and carboxylic products as well as hydroxylation. Thence a reduction in proportion of at least some higher congeners as a result of photolysis is expected (Nisbet and Sarofim, 1972) concluding that most CB isomers with 4 or fewer chlorine atoms have been degraded in the environment, possibly by microbial action.

Generally the persistence of CBs increase with increasing degree of chlorination, with the mono-, di-, and trichlorinated biphenyls biodegrading relatively rapidly, the tetrachlorinated biphenyls biodegrading slowly and the higher chlorinated biphenyls being resistant to

biodegradation; biodegradation possibly being the ultimate degradation process in water and soil (US EPA Fact sheet, 2005).

1.4.1 PAHs and CBs in sediments

The physico-chemical properties of PAHs tend to promote their accumulation in the solid phases of the terrestrial environment and specifically in marine sediments. Sediments in marine and freshwater systems are complex matrices composed of organic matter in various stages of decomposition, particulate mineral material that varies both in size and chemical composition, and inorganic material of biogenic origin e.g. diatom frustules and calcium carbonate (Chen and White, 2004).

Viguri *et al*., (2002) found that higher amounts of PAHs mainly occur in sediments with higher total organic carbon (TOC) and thus concluded that PAH concentration is strongly dependent on the organic matter, with the dependence being both in amount and in nature (Reid *et al*., 2000a; Borglin *et al*., 1996). In aquatic ecosystems organic matter has been reported to determine the partitioning of PAHs in sediments to a large extent (King *et al*., 2004; deBruyn and Gobas, 2004). These organic matters interact with organic chemicals by various modes of binding and adsorption, such as ion exchange, hydrogen bonding, charge transfer, covalent bonding and hydrophobic adsorption and partitioning. These interactions have been shown to enhance the dissolution of pollutants in water (through a partition-like interaction between the pollutant and the humic molecules, largely being controlled by the dissolved organic matter molecular size and polarity (Chiou *et al*., 1986)), reduce volatilisation, increase photolysis rates, alter bio concentration and affect toxicity of organic compounds (Haitzer *et al*., 1998). POP associations and cycling with natural organic matter therefore influence their fate, transport, and bioavailability in aquatic environments (Mitra *et al*., 1999). Similarly in the same study they concluded that sources of contaminants such as PAHs to sediments, and particle geochemistry play significant roles in determining their distribution, bioavailability and biogeochemical cycling. For example, Bouloubassi and Saliot (1993), found that the distributions of natural and anthropogenic PAH were different, suggesting that the carrier particles of natural PAH were coarser and settled more rapidly than

those carrying anthropogenic PAHs. Sediments may thus be viewed as reservoirs for PAH accumulation. There has been a lot of research on PAHs in sediments (Baumard *et al*., 1999; Webster *et al*., 2001, Webster *et al*., 2004; Macrae and Hall, 1998) and the concentrations of PAHs found in sediments may range over several orders of magnitude (from a few μg kg^{-1} up to g kg^{-1}) depending on the proximity of the waterway to industrial activity, water currents and water usage (Juhasz and Naidu, 2000).

Marine sediments can be regarded as an important reservoir of hydrophobic and persistent organochlorines including CBs and the absorption of these compounds by suspended particulate matter and subsequent sedimentation are important factors that affect the transport, diffusion and fate of these compounds within the estuary (Hong *et al*., 1999; OSPAR, 2000) mainly due to the low water solubility and high density of CBs. Studies on the occurrence of CBs in sediment has received a lot of attention (Webster *et al*., 2000a, Webster *et al*., 2002b; Edgar *et al*., 1999; Eljarrat *et al*., 2005; Johan Persson *et al*., 2005; Rose and Rippey, 2002; Tyler and Millward, 1996; Zhang *et al*., 2003) mainly because of its persistence and inherent risks. The dependence of CB concentration on grain size has been reported (Camacho-Ibar and McEvoy, 1996) as is the case with other hydrophobic contaminants. They concluded that particle dispersion associated with intense hydrodynamic conditions in the coast studied (Liverpool Bay) controlled the distribution of CB concentrations after disposal. However in a study of the Clyde estuary, UK, (Edgar *et al*., 2003), no significant correlation was observed between total CB and total organic carbon or particle size although separation into grain size fractions and subsequent analysis suggested there is an influence by both variation in organic matter source and mineralogical composition on congener distribution with implications for mobility of CBs within intertidal sediments. CB levels in sediment classified as contaminated or highly contaminated will typically be found in coastal environment close to an industrial estuary (Miller *et al*., 2000).

The release of CB compounds from sediment to the water column during low flow conditions in a river system can be facilitated through physical-chemical processes (that include desorption of CBs from sediment and diffusion of the CBs through pore water to the sediment-water interface), microbial activity (transforming the CBs from highly chlorinated compounds

to less chlorinated and hence more mobile compounds) and bioturbation (McDonough and Dzombak, 2004). The uptake of CBs into aquatic food chains generally begins with sediments, planktons or films (which the CBs associate with due to their lipophilicity) being incidentally ingested by fish and invertebrates or taken up by aquatic macrophytes (Hope *et al*., 1997).

In UK sediments, the highest concentration of PAHs are generally found in estuaries, or around oil and gas platforms which have in the past drilled (mainly developmental) wells with diesel based muds (Law and Biscaya, 1994). The fate and behaviour of organic contaminants in the environment is governed by many different factors including soil/ sediment characteristics, compound properties and environmental factors such as temperature and precipitation (Reid *et al*., 2000a), as well as biotic and abiotic processes including volatilisation, photooxidation, chemical oxidation, bioaccumulation and microbial transformation (Abbondanzi *et al*., 2005). A number of factors such as pH, ionic strength, and temperature could affect the release of pollutants from sediments (Chen *et al*., 2000).

1.4.2 PAHs and CBs in marine organisms

PAHs may accumulate in lipid-rich tissues of marine organisms. Organism size, ingestion rate, growth rate, membrane permeability, ventillatory rate, gut residence time and osmoregulation are biological processes that influence the organism's rate of uptake of PAHs as well as changes in the organisms' behaviour, seasonal rhythms, nutritional quality and stress (Juhasz and Naidu, 2000). There have been many studies of the occurrence of PAHs in marine organisms. For example, in assessing the long term effect of the 'Nakhodka' oil spill, Koyama *et al*. (2004) monitored the accumulation of PAH from the oil in the spiny top shell (*Turbo cornutus*- a commercial snail in Japan), the blue mussel (*Septifer virgatus*), goose barnacle (*Capitulum mitella*), and two species of limpet (*Cellana toreuma* and *Cellana grata*) found 44 ng g^{-1} wet weight total PAH concentration (fluorene, dibenzothiophene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene, and benzo[*a*]pyrene) in the spiny top shell in the first month which rapidly decreased to less than 5.4 ng g^{-1} wet weight from the second month. They also found total PAH concentrations (fluorene, dibenzothiophene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene, and benzo[*a*]pyrene) in the other organisms that

ranged from 20-30, 5.3-17.0, 6.3-32.7 and 5.7-14.5 ng g^{-1} wet weight for the blue mussel, goose barnacle, *C. toreuma* and *C. grata* respectively. Thus they concluded that the spiny top shell accumulates PAHs because of its low ability to metabolise these compounds, but seem to excrete parent PAHs very fast, like fish.

PAHs have been shown to accumulate in fish tissues, such as whole gall bladders and livers (Pointet and Milliet, 2000) and mussels (Baumard *et al*., 1998a; Baumard *et al*., 1999). Baumard *et al*. (1998a) reported that caged mussels exposed to higher levels of sediment contamination accumulated more PAHs than those exposed to moderate or lower contaminated sediments which they postulated to be likely due to higher bioavailability at the former locations or differences in equilibrium between uptake and depuration at those sites.

Aquatic organisms, because of their filtering activities, absorb xenobiotics either by absorption of compounds from the water phase through the gills or indirectly from those adsorbed on the small grain size fraction of particles through the digestive system (Baumard *et al*., 1998b). PAHs can undergo biotransformation once taken in and may also be eliminated from organisms by passive diffusion. Environmental factors such as temperature, oxygen content, pH and salinity can also influence the uptake of PAHs by marine organisms due to their effect on the bioavailability of the compounds.

The uptake of CBs in aquatic or marine organisms is generally determined by the lipophilic nature of the compounds (expressed as the $\log K_{ow}$) and is either through the direct uptake from water via the gills or body surface, through food or contact with contaminated sediments. CBs have been detected in fish, birds and ultimately in humans (being part of the food chain). CBs found their way into man either directly through contaminated food like rice oil, from animal tissue or aquatic animals eaten by man like fish (Cook, 1972). They are not easily metabolised, can bioaccumulate in living organisms, and be further biomagnified in food webs raising a potential risk for high trophic level predators (Danis *et al*., 2005). The contamination level in organisms is usually affected by distance between living areas and the major source of pollution (Tanabe, 1988), although not always the case and in a study using sea stars and sea urchins (Danis *et al*., 2005), it was shown that the bioaccumulation depends on the

contamination source and CB congener being considered. The occurrence of CBs in marine organisms, such as Mediterranean mussel, Norway lobster, red mullet, common cuttle-fish (a cephalopod) and a host of other organisms (Perugini *et al*., 2004) and in various organs of monk fish (*Lophius piscatorius*) and black scabbard (*Aphanopus carbo*) from the continental slope of the Rockall Trough, west of Scotland (Mormede and Davies, 2001), have been studied. The study by Perugini *et al*., (2004) concluded that in marine organisms, habitat, physiological factors, lipid content, geographical origin and feeding behaviour are factors that explain storage and elimination of pollutants. CBs have also been studied in the filter feeder, *Mulinia lateralis* and the deposit feeder *Yoldia limatula* (Burgess and Mckinney, 1999) and in other marine fish, birds and mammals (Nendza *et al*., 1997). Harrad *et al*. (1994) concluded that CB levels in biota are likely to diminish at a far slower rate than that observed for soils and vegetation, partly due to the cross-generational transfer from parent to offspring via breast feeding and transfer during pregnancy for mammals, or via eggs for avian species and also to the persistence of CBs within biotic tissues.

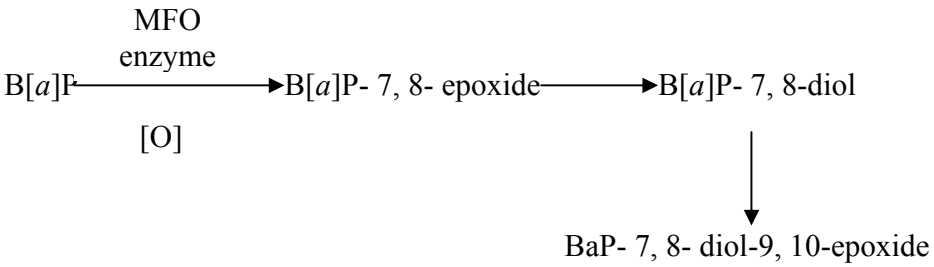
CB concentrations in fish are related to factors such as the size and fat content of the fish and the food web structure (Yu, 2004) with the slower-growing fish accumulating higher levels of contaminants than faster growing fish, this being because faster-growing fish gain more body mass for each unit mass of contaminant they consume than the slow-growing fish. Equilibrium partitioning is believed to control the concentration of CBs in gill breathing aquatic animals and determines the biomagnification of lipophilic pollutants. Therefore, CB concentrations (on lipid weight- related data basis) is not dependent on age or body size in lower trophic animals as uptake is primarily via the water phase (Tanabe, 1988) although, in higher trophic level animals, an age dependent-accumulation of CBs is often found. The lactation process in mammals has likewise been viewed with concern as it serves as a means of transfer of long term residues of CBs and their consequent biological impact to subsequent generations (Walkowiak *et al*., 2001; Reddy *et al*., 2001; Sudaryanto *et al*., 2006)

1.5 Regulations, toxicity and effects of PAHs and CBs

The concern from PAH are mainly because:

- the low molecular weight PAHs can be directly toxic to marine animals,
- metabolites of some of the high molecular weight PAH (e.g. benzo[*a*]pyrene) are potent animal and human carcinogens and
- the low molecular weight PAH can cause taint in fish and shellfish, consequently impacting the fish and shellfish industries (Law *et al*., 2002).

PAHs can enter the human body either by inhalation, contact with the skin or ingestion, and their lipophilic nature makes it easy for them to penetrate cellular membranes.
The metabolism (biotransformation) of PAHs tends to be a series of oxidative processes producing progressively more polar metabolites, and conjugative reactions by enzymes which will make them more readily excretable (Solè, 2000). The oxidative processes are initiated by the cytochrome P450- dependent monoxygenase or mixed function oxygenase (MFO) system referred to as Phase I which catalyses the insertion of an oxygen atom into a substrate molecule, forming hydroxylated derivatives. Exposure of fish to a diverse range of planar molecules, such as polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (CB's), dioxins and dibenzofurans, induce specific forms of P450 that catalyse aryl hydrocarbon hydroxylase (AHH) and ethoxyresorufin-0-deethylase (EROD) activities (Stagg *et al*., 1995). The Phase I by-products may further undergo metabolism by conjugative enzymes (Phase II) such as glutathione –*S*- transferase (GST), which will make them more readily excretable, for example through bile. However, the PAHs are sometimes transformed or metabolised into intermediates that are highly toxic or carcinogenic to the host, for example, the oxidative metabolism of benzo[*a*]pyrene (B[*a*]P) by the MFO system converts it to a dihydroxy epoxide (an electrophilic reactant) believed to be a carcinogen that can covalently interact with DNA (Yu, 2004) as shown in the reaction below,



The carcinogenic potential of PAHs is generally expressed only when they have been metabolised to forms in which they can bind to DNA.

There are existing environmental requirements set out in various OSPAR documents and directives of the European Community on emission and control of PAHs and CBs. UK (including Scottish) releases of PAHs are controlled under the surface water pollution prevention control (PPC) regulations (Scottish Statutory Instrument, 2005); the European Air Quality Framework Directive 96/62/EC (OJ L 296, 1996); and the Water Framework Directive 2000/60/EC (OJ L 327, 2000). The United Kingdom is also a signatory to the UNECE Convention on Long Range Trans-boundary Air Pollutants (LRTAP) which includes a protocol (which came into effect in 2003) to develop a legally binding global agreement to reduce risks to health and the environment posed by Persistent Organic Pollutants (POPs) such as PAHs. The 1998 Aarhus protocol (UNECE, 1998) also requires signatories to reduce emission of POPs like dioxins, furans, PAHs, CBs etc.

The International Agency for Research on Cancer (IARC) has designated some PAHs as probable carcinogens as they may be found in coal tar, and other materials known to be carcinogenic to man. These identified PAHs have been shown to produce tumors in mice and some other animals tested, with similarities of metabolism of benzo[*a*]pyrene in human and mouse cells cultured *in vitro* having been reported (IARC, 1973). The US EPA has classified seven PAHs (benzo[*a*]pyrene, benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, dibenz[*a,h*]anthracene, and indeno[1,2,3-*cd*]pyrene) as Group B2 substances, i.e. probable human carcinogens.

Most of the high molecular mass PAHs have been suggested to be probable or possible human carcinogens (Farmer *et al.*, 2003). For example, the resulting guidelines for benzo[*a*]pyrene in drinking-water (by the World Health Organisation) corresponding to excess lifetime risks for gastric cancer of 10^{-4}, 10^{-5}, and 10^{-6} are 7, 0.7, and 0.07 μg l^{-1} (WHO: EHC 202, 1998). Therefore, the use of potencies of individual PAHs in mixtures relative to that of a standard (benzo[*a*]pyrene being the reference standard used) to express quantitatively the risks posed by individual PAHs has been proposed, referred to as toxicity equivalency factor (TEF) approach which is a weighting system with benzo[*a*]pyrene given a weighting factor of 1. A summation of the TEFs yields the toxicity equivalency quotient (TEQ) and both TEF and TEQ are used to express the different toxicities of individual compounds. Law *et al*. (2002) compiled different PAH toxic equivalency factors as shown in Table **1.3**

In similar regard as to the PAHs, there exist legislations and requirements for use of CBs. The phase out and destruction of identifiable CBs was first agreed at the third international North Sea conference in 1990 and a subsequent OSPAR Decision (OSPAR, 1992) in 1992 (PARCOM Decision 92/3) affirming 1999 as the date for phase-out in terms of existing uses by North Sea countries and 2010 for non North Sea countries signatory to the Paris Convention. In Scotland, the mechanism for compliance is covered by the Environmental Protection (Disposal of polychlorinated biphenyls and other dangerous substances) (Scotland) Regulations 2000. These regulations are in response and give effect to the EC Directive 96/59/EC on disposal of polychlorinated biphenyls and polychlorinated terphenyls (PCTs). The UNECE protocol on POPs (UNECE, 1998) requires destruction or decontamination of equipment containing more than 50 ppm CBs by 2015 and the use of best available techniques (BAT) whilst also banning CB production and use.

Table **1.3:** Compiled Toxic Equivalency Factors for PAH based on their carcinogenic potential, from Law *et al*. (2002)

Compound	USFDA[a]	KSIR[b] Carcino-genic	KSIR Mutagenic	OEHHA[c]	USEPA[d]	Nisbet & Lagoy (1992)
Dibenz[*a,h*]Anthracene	1.05				1.00	5.00
Benzo[*a*]pyrene	1.00	1.00	1.00	1.00	1.00	1.00
Indeno[*1,2,3-c,d*]pyrene	0.25			0.1	0.1	0.1
Pyrene	0.13	0.081	0.2			0.001
Benzo[*b*]fluoranthene	0.11			0.1	0.1	0.1
Benzo[*k*]fluoranthene	0.07					0.01
Benzo[*g,h,i*]perylene	0.03					0.001
Fluoranthene	0.02					
Benzo[*a*]anthracene	0.014	0.145	0.62	0.1	0.1	0.1
Chrysene	0.013	0.0044	0.37	0.01	0.001	0.01
Anthracene		0.32	0.06			0.01
Acenaphthene						0.001
Acenaphthylene						0.001
Fluorene						0.001
2-Methylnaphthalene						0.001
Naphthalene						0.001
Phenanthrene						0.001

[a] USFDA- United States Food and Drug Administration; [b] KSIR- Kuwait Institute for Scientific Research; [c] OEHHA- Office of Environmental Health Hazard Assessment (of the California EPA); [d] USEPA- United States Environmental Protection Agency

The IARC has designated some CBs as being of possible or probable carcinogenic risks (Group 2A) to humans based on studies of occupational populations, populations accidentally exposed to the compounds and tests on rats and mice. Some of the studies showed cancers of the digestive system and of lymphatic and haematopoietic tissues (IARC monograph, 1983).

As a consequence, monitoring of these POPs is being undertaken under many national monitoring programmes like the UK NMMP, and some "mussel watch" programmes, showing trends in pollutant levels. CBs are also included in the OSPAR list of Chemicals for Priority Action and background concentration values of zero have been adopted for man made chemicals such as CBs. CB results are mostly reported with emphasis on the ICES 7 (CB 28, 52, 101, 118, 153, 138, and 180) which were chosen as indicators of contamination level due to their relatively high concentrations in technical mixtures, wide chlorination range and persistence (Webster *et al*., 2005).

CBs have been shown to have effects on animals such as on the endocrine system (Colborn *et al*., 1993; Murphy *et al*., 2005); reproductive systems (Toft *et al*., 2004), the neurotoxicological consequence of developmental exposure to CBs (Jacobson and Jacobson, 1996; Stewart *et al*., 2000); estrogenic effects of CBs which may be mediated by the hydroxylated CB-metabolites (Kester *et al*., 2000; Xie and Zhang, 2004); immunotoxic effects (Ross *et al*., 1996; Hall *et al*., 1997; Beckmen *et al*., 2003; Hammond *et al*., 2005). The four non-ortho coplanar CBs, CB 77, 81 126, and 169 which are approximate isostereoisomers of the toxic 2, 3, 7, 8- tetrachlorodibenzo-*p*-dioxin (TCDD) are considered to have similar toxicity and bioaccumulation properties to the TCDD and are the most toxic CBs (Tanabe, 1988; Yu, 2004) although they are not as toxic as the 2, 3, 7, 8- TCDD. The toxic effects of CBs to wildlife appear to be correlated more with total TCDD toxicity equivalents than with absolute CB concentrations and TEF values can be used to normalise CB concentrations to their TCDD equivalents (Hope *et al*., 1997). However they may exert other forms of toxicity that are not expressed by TCDD-equivalency. Table **1.4** gives World Health Organisation (WHO) proposed TEF values for dioxin-like (Van den Berg *et al.*, 1998).

Table **1.4:** World Health Organisation-proposed TEF[1] values for CBs

Congener	**TEF**		
	Humans/ Mammals	**Fish**	**Birds**
CB 81	0.0001	0.0005	0.1
CB 77	0.0001	0.0001	0.05
CB126	0.1	0.005	0.1
CB 169	0.01	0.00005	0.001
CB 105	0.0001	<0.000005	0.0001
CB 114	0.0005	<0.000005	0.0001
CB 118	0.0001	<0.000005	0.00001
CB 123	0.0001	<0.000005	0.00001
CB 156	0.0005	<0.000005	0.0001
CB 157	0.0005	<0.000005	0.0001
CB 167	0.00001	<0.000005	0.00001
CB 189	0.0001	<0.000005	0.00001

[1]TEF: Toxicity Equivalency Factor

The dioxin-like CBs exert their toxicities by activating the aryl hydrocarbon receptor (AhR), a ligand-dependent transcription factor, in a similar way to the toxic 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (Pan *et al.*, 2004). It is suspected that the PCDFs contained in the commercial CB mixtures as co-contaminants account for the toxicity of such mixtures.

In a similar regard, the commission regulation (EC) 208/2005 (OJ L 34, 2005) Directive has suggested the use of B[*a*]P as a marker for the occurrence and effect of carcinogenic PAH in food and have listed maximum concentrations of B[*a*]P in different matrices/products ranging from 1.0 ng g^{-1} wet weight in foods for infants and young children to 10.0 ng g^{-1} wet weight in bivalve molluscs.

Ecotoxicological Assessment Criteria (EACs) which are the concentrations of specific substances in the marine environment below which no harm to the environment or biota is

expected, can be used in the assessment of chemical monitoring data and /or joint chemical/biological effects monitoring data and are based on toxicity tests for individual substances (OSPAR, 2004). EACs have also been suggested by OSPAR as a means of identifying possible areas of concern, indicate which substances to be considered as priority and determine likelihood of effects occurrence on biota.

Table **1.5**: Overview of Ecotoxicological Assessment Criteria of some PAHs/CBs (OSPAR, 2004)

PAH	Water (mg/l)	Sediment (mg/kg dw)	Mussel (mg/kg dw)
Naphthalene	5-50*	0.05-0.5*	0.5-5†
Phenanthrene	0.5-5†	0.1-1*	5-50†
Anthracene	0.001-0.01†	0.05-0.5*	0.005-0.05†
Fluoranthene	0.01-0.1†	0.5-5†	1-10†
Pyrene	0.05-0.5†	0.05-0.5†	1-10†
Benzo[*a*]Anthracene	nd	0.1-1†	nd
Chrysene	nd	0.1-1†	nd
Benzo[*a*]fluoranthene	nd	nd	nd
Benzo[*a*]pyrene	0.01-0.1†	0.1-1†	5-50†
Benzo[*g,h,i*]perylene	nd	nd	nd
Indeno[*1,2,3-cd*]pyrene	nd	nd	nd
$\sum CB_7$	0.00001-0.0001*	0.001-0.01†	0.005-0.058

* Firm † Provisional: The assessment criteria have no legal significance and are used only for preliminary assessment of OSPAR JMP/JAMP chemical monitoring data with the aim of identifying potential areas of concern. nd – no data available.

Once taken up by an organism, the fate and effects of PAHs are especially dependent on their susceptibility to biotransformation (Selck *et al.*, 2005) and the accumulation of PAHs to in organisms will remain limited in case of rapid biotransformation (Ma *et al.*, 1998). Sorption-desorption behaviour of organic pollutants in marine sediments does not only affect concentrations and fates of these pollutants in the sediment but also the toxicity of these contaminants toward benthic communities in the receiving environment (Zhang *et al.*, 2000).

There has been many studies of the effects of PAHs on animals (Juhasz and Naidu, 2000; EHC 202, WHO1998; Shailaja and D'Silva, 2003; Bejarano *et al.*, 2004) and on embryonic and early larval development in fish (Geffard *et al.*, 2003; Incardona *et al.*, 2004, Luckenbach *et al.*, 2003). Exposure to dibenzothiophene and phenanthrene was found to induce defects such as oedema, spinal curvature, cardiac dysfunction, etc and pyrene inducing a different syndrome of anaemia, peripheral vascular defects and neuronal cell death on the Zebra fish (*Danio rerio*) (Incardona *et al.*, 2004). These effects by PAHs include their genotoxicity (White, 2002; Du Four *et al.*, 2004 and Chen and White, 2004); carcinogenicity (Jacob, 1996), immunogenicity (Oh *et al.*, 2005); hepatotoxicity (Hakkinen *et al.*, 2004); mutagenicity (Du Four *et al.*, 2004); reproductive effects (Miller *et al.*, 2004) such as the embryotoxicity of naphthalene and teratogenicity of benzo[*a*]pyrene. The enhanced toxicity of certain PAHs (e.g., retene, benzo[*a*]pyrene) when exposed to UV radiation to a number of aquatic organisms has been highlighted (Hakkinen *et al.*, 2004; Lyons *et al.*, 2002 and Pelletier *et al.*, 1997). Similarly effects on plants have been studied to include: decreased chlorophyll production (Southerland and Lewitus, 2004) and reduction in seedling growth (Sverdrup *et al.*, 2003). Djomo *et al.*, (2004) in an exposure study of the green alga *Scenedemus subspicatus* to naphthalene, anthracene, phenanthrene, pyrene and benzo[*a*]pyrene (B[*a*]P), concluded that the toxicity of PAHs is strongly influenced by their physicochemical properties (such as K_{ow}, aqueous solubility, coefficient of volatilisation) and conditions of exposure (light, presence of nitrate ions, etc) with B[*a*]P being the most toxic. However during the course of the metabolism reactions of PAHs, reactive intermediates can be formed which are more toxic, mutagenic or carcinogenic than the initial PAH, for example benzo[*e*]pyrene and benzo[*a*]pyrene (Baumard *et al.*, 1999). A review of some of the effects of PAHs on marine organisms can be found in Knutzen, (1995) and Næs *et al.* (1995).

1.6 Bioavailability

Manifestation of the toxic effects of POPs require uptake into organisms, which often bio concentrate these contaminants in their lipids to relatively high levels (ppm) and therefore the concentrations available to the organisms (bioavailability) needs to be assessed. Knowledge of the bioavailable fraction is thus essential for environmental risk assessments (Ehlers and Luthy, 2003).

Bioavailability/availability has been defined in various ways and context:

- Semple and Doick (2003) describe it as the fraction of a chemical that is freely available to cross an organism's (cellular) membrane from the medium the organism inhabits.
- Measure of how available a toxic pollutant is to the biological processes of an organism (Anonymous- www.seagrant.umn.edu/pubs/ggl/b.html).
- The proportion of the ingested amount available for metabolic processes (Brouwer *et al*., 2001).
- To environmental scientists, bioavailability/availability represents the accessibility of a chemical for assimilation and possible toxicity (Alexander, 2000).

Due to varying definitions, a United States NRC report (WSTB, 2003) used and defined "bioavailability processes" as 'the individual physical, chemical and biological interactions that determine the exposure of organisms to chemicals associated with soils and sediments - see Figure **1.2** below. More definitions and concepts of bioavailability are given in the NRC report. Bioavailability is thus a complex process which includes all kind of relationships between the concentration in the ambient environment and the portion of that concentration an organism experiences with regard to uptake (Sijm *et al*., 2000). It is essentially the total concentration of chemicals in soils or sediments that is or will potentially be taken up by an organism.

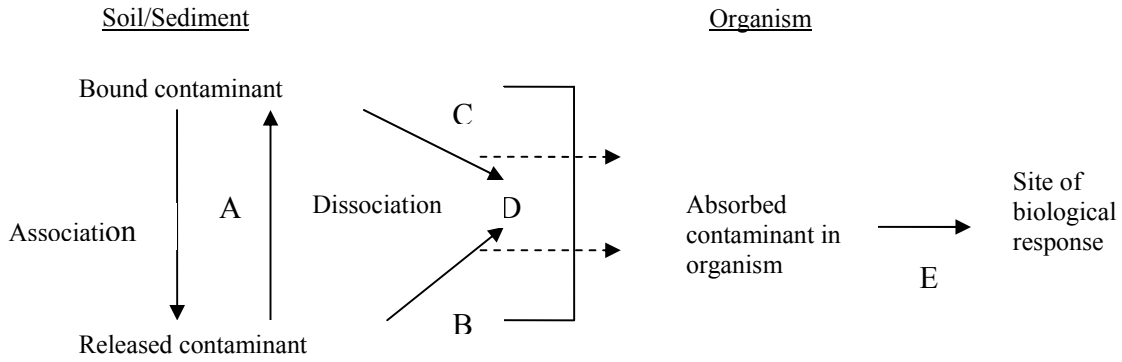


Fig **1.2**: Bioavailability processes (culled from WSTB, 2003)

A- physical, chemical and biochemical phenomena that bind, unbind, expose, or solubilise a contaminant associated with soil or sediment

B- involves movement of a released contaminant to the membrane of an organism

C- Involves movement of contaminant still bound to solid phase. Note: B& C are integral fate and transport processes that control an organisms overall response

D- Involves movement from the external environment through a physiological barrier and into a living system. One common factor among all organisms is the presence of a cellular membrane that separates cell interior from the external environment through which most contaminants must pass before deleterious effects on the cell or organism occur

E- Refers to paths taken by the chemical following uptake across a membrane, e.g. metabolic processing/exerting toxic effect within a particular tissue. Note: Sediment and soil no longer play a role here so this may not be considered a bioavailability process.

The bioavailability of PAHs in soils/sediments varies with the contamination history and the characteristics of the soils or sediments. An extraction procedure that predicts bioavailability would thus be highly useful in predicting the actual exposure to sequestered compounds and to provide a more toxicologically relevant basis for establishing clean up goals for molecules that are only partially available to living organisms (Kelsey and Alexander, 1997a).

In a study to assess the significance of PAH contamination from the Alcan aluminium smelter and the extent of any adverse effects on biota of Kitimat Arm, Canada, (Paine *et al.*, 1996)

high concentrations of about 10,000 mg kg^{-1} total PAH were found within 1 km of the smelter although most were < 150 mg kg^{-1}, yet these did not show significant effects on toxicity test responses and benthic communities. Thus the authors concluded that high contaminant concentrations may not give rise to biological effects due to limited bioavailability. A similar conclusion was arrived at by Knutzen (1995) in his review of effects of PAHs and other constituents of waste water from aluminium smelter discharges on marine organisms.

Assessments of POPs and their risks have been primarily based on total concentrations of contaminants in the sediment, which are obtained from harsh extractions, and the accumulation of these POPs in organisms (Alexander, 2000; Ehlers and Luthy, 2003; Escher and Hermens, 2004; Simpson *et al*., 2006). These have been found to be inappropriate for predicting toxicity and bioavailability as it is the free dissolved contaminant content that is responsible for uptake by organisms (Smedes and Luszezanec, 2001). Since the standard analytical methods measure total and not bioavailable concentrations, they may overestimate the magnitude of the environmental and societal problems/risks from these pollutants (Alexander, 2000).

Sequestration (an ageing process whereby a compound becomes more firmly bound in a matrix over a long period of time in such a way that though it can only be recovered by vigorous extraction, it is less accessible to living organisms) has been shown to have significant influence on bioavailability of contaminants (Luthy *et al*., 1997; Kraaij *et al*., 2003). Sequestration is mostly attributed to slow migration of the aromatic molecules into condensed organic matter and inaccessible micro sites.

Reichenberg and Mayer (2006) identified two complementary parameters of bioavailability as the accessible quantity and the chemical activity of the POP. They identified the accessible quantity as the portion of the total concentration that can be mobilised and made available for processes like bio degradation and digestive uptake, and is determinable by harsh extractions or POP sediment concentration-depletive extraction. The chemical activity, however, refers to the energetic state of the chemical and determines processes such as partitioning and diffusion

and encapsulates concepts such as freely dissolved concentration and fugacity which are measurable using equilibrium sampling devices.

Kelsey and Alexander (1997a), found atrazine, phenanthrene and naphthalene to be less available to earthworms, and naphthalene less available to bacteria, after the compounds remained in soil for extended periods of time. Their findings demonstrated that persistent compounds undergo some type of slow sequestration in soil, one that results in a reduction in the quantity of some organic chemicals that are available to earthworms, bacteria, fruit fly (*Drosophila*), and plants. They similarly concluded that vigorous extraction appreciably overestimates the quantity of a compound that is bioavailable, thus raising the need to assess whether current analytical methods are appropriate indicators of exposure to risk from toxic chemicals that have persisted in soil. Sequestration and decline in bioavailability of a compound are greatly affected by various environmental factors and differ among soils (White *et al*., 1997). In addition, the extent of contaminant retention is directly correlated with the octanol-water (K_{ow}) partitioning coefficient and the percentage or organic material in the soil or sediment, e.g., benzo[*a*]pyrene (characterised by a large K_{ow}) tends to sorb onto the organic soil fraction and become highly unavailable (Juhasz and Naidu, 2000).

The microbially bioavailable concentration of soil-associated phenanthrene were best predicted using an optimised hydroxypropyl-β-cyclodextrin (HPCD) extraction technique (Reid *et al*., 2000b), in contrast to dichloromethane Soxhlet extraction and butan-1-ol shake extraction which both overestimated phenanthrene bioavailability by an average greater than 60 %.

1.6.1 Methods for predicting/ measuring bioavailability

Simple methods which are improvements over the harsh chemical extractions of soils and sediments are required to measure available fractions to aid in the assessments of POPs and their risks to the environment.

1.6.1.1 Biological methods

Bioavailability can be estimated by measuring amounts of target contaminants and/or more polar metabolites of contaminants in biota and sediments. However, different physico-chemical properties of the contaminants (e.g. lipophilicity and recalcitrance against biological degradation) and the sediments (e.g. particle size and organic content) or biota (e.g. lipid content, age) can affect the bioavailability (Ruus *et al*., 2005). Nevertheless, there are different means of determining bioavailability; which include use of organisms such as earthworms (Kelsey and Alexander, 1997a; White *et al*., 1997), the ragworm "*Nereisa diversicolor*" (polychaeta) and the netted dogwhelk "*Hini reticulata*" (gastropoda) (Ruus *et al*., 2005); mussels (Boehm *et al.*, 2005; Page *et al.*, 2005; Hellou *et al*., 2005, Utvik and Johnsen, 1999) and caged carp (Verweij *et al*., 2004), etc.

Different organisms tend to give different results whilst assessing bioavailability, therefore, choice of organism is quite important. For example, Ruus *et al*. (2005) found higher pyrene biota sediment accumulation factor (BSAF) in *Nereis diversicolor* than in the *Hini reticulata* exposed to the same sediment which they speculated could be as a result of poorer capability to metabolise and eliminate the pyrene by the polychaete compared to the gastropoda. Similarly, higher CB concentrations were measured in *Nereis virens* compared to the *Mercenaria mercenaria* and *Palaemonetes pugio* exposed to the same sediment by Rubinstein *et al*. (1983). Factors such as sediment ingestion or interaction, feeding behaviour and metabolism potential, easy culturing or collection, high bioaccumulation potential, sufficient biomass for chemical analysis, etc are considered important in the selection of test species (Lee, 1998). Some of these studies had also set out to assess the toxicity of the organic pollutants as a measure of the bioavailability. The use of *in vitro* extraction of contaminated sediments using the digestive fluid of a deposit-feeding polychaete (*Arenicola brasiliensis*) has been reported (Weston and Mayer, 1998a; 1998b) to study bioaccumulation mechanisms and to attempt to quantify the bioavailable fraction of the contaminant burden. They suggested that extraction by digestive fluid is controlled by fugacity-driven partitioning between particulate and dissolved phases. Mayer *et al*. (1996) also reported a similar biomimetic approach to *in vitro* measurement of bioavailability by measuring the solubilisation of coastal

sedimentary contaminants by digestive fluids extracted from the gut lumens of adult deposit feeders (*Arenicola marina*- lugworm and *Parastichopus californicus*- Sea cucumber). This approach is essentially a chemical extraction but with a biologically-derived extractant.

Steady-state body burdens, when expressed as ratio between contaminant in the tissue and the surrounding media e.g. sediment or water (BSAF, bio concentration factor (BCF), etc) are a measure of bioaccumulation. Differences relate to bioavailability and also to contaminant biotransformation rates. Toxicokinetic measures (specifically uptake clearance-which represents the rate of increase in tissue contaminant concentration normalised to the sediment concentration) and absorption efficiency (determined by direct measurement or indirect estimation of contaminant loss between ingested material and faeces) are other traditional measures of bioavailability (Weston & Mayer, 1998b).

These living organisms are used either as biomonitors or bioindicators to quantify and indicate contamination of the aquatic environment by hydrophobic organic pollutants (Kot *et al.*, 2000). Another technique being used is bioluminescence-based microbial biosensors in which the impact of environmental pollutants on the activity of the lux-marked microorganisms (Mowat and Bundy, 2001; Steevens *et al.*, 1999) is measured. For example, luminescent bacteria emit light as a by-product of their metabolism. If toxic substances are present, less light is emitted. This reduction of light emission is measured and reported as bioluminescence inhibition compared to a nontoxic control (Loibner *et al.*, 2004; O'Neill *et al.*, 2003).

1.6.1.2 Chemical methods

There has been a great emphasis recently on developing less vigorous or harsh extraction techniques that will better reflect the bioavailable fractions of contaminants in sediment. Some of the methods used include rapid persulfate oxidation (Cuypers *et al*., 2000), use of hydroxypropyl –β-cyclodextrin (HPCD) and Triton X-100 aqueous solutions (though Triton X-100 was concluded to be unfit for the prediction of PAH bioavailability (Cuypers *et al*., 2002)), non exhaustive cyclodextrin-based extraction technique (Reid *et al*., 2000b), chemical assay using different extractants (solvents) such as n-butanol, ethanol-water, acetonitrile-

water, methanol-water (Kelsey *et al*., 1997b). Another biomimetic approach that has been used is the soil (or sediment) availability ratio (SARA) which uses organisms to estimate the bioavailable concentrations by comparing the concentration in the organism to that in the soil or sediment (Sijm *et al*., 2000).

Other approaches use a reference phase which is exposed to the water phase or a sediment-water system such as desorption from soil onto a resin (Tenax) and polyethylene tube dialysis (Macrae and Hall, 1998; Cornelissen *et al*., 2001); polyoxymethylene-solid phase extraction (POM-SPE) (Jonker and Koelmans, 2001); partitioning driven administration (PDA)- solid phase (Mayer *et al*., 1999); use of poly (dimethyl)siloxane (PDMS)- coated glass fibers [SPME also referred to as matrix-SPME] (Mayer *et al*., 2000a; Mayer *et al*., 2000b); use of liposome-water systems (Escher and Schwarzenbach, 1996); kinetic solid phase extraction using C-18 disks (Freidig *et al*., 1998); and use of semipermeable membrane devices- SPMD's (Macrae and Hall, 1998; Petty *et al*., 2000; Huckins *et al*., 1993; Huckins *et al*., 1999; Booij *et al*., 1998; Utvik and Johnsen, 1999; Verweij *et al*., 2004).

In using SPMDs, any pollutants that are attached to particles or are associated with colloidal material will be unable to pass through the sampler membrane (or through biological membranes). POPs thus become concentrated in the lipid relative to the water phase according to their lipid-or octanol-water partition coefficients, as they might in fish lipids or the tissues of other organisms (Macrae and Hall, 1998). SPMDs, consist of low density polyethylene (LDPE) lay-flat membrane tubing containing a thin film of high molecular weight lipid, typically triolein (usually 20 % by mass) and have been used to sample PAHs, CBs, OCPs, dioxins and furans, organotin compounds (Namieśnik *et al*., 2005; Booij *et al.*, 1998), chlorophenols-anisoles-veratoles (Booij *et al.*, 1998).

Most of these chemical methods assume the contaminant must pass through the dissolved phase to be taken up by organisms, be biodegraded or to exert any toxic effects; and equilibrium and kinetic sampling can be distinguished. In equilibrium sampling, the exposure time is sufficiently long enough to permit the establishment of thermodynamic equilibrium between the water phase and the reference phase, thus knowledge of the reference phase-water

partition coefficients allows the determination of the dissolved contaminant concentration. However with kinetic sampling, the rate of mass transfer to the reference phase is assumed to be linearly proportional to the difference in chemical activity of the contaminant between the water- and reference phase. Therefore knowing the proportionality constant allows for the calculation of the dissolved contaminant concentration (Booij *et al.*, 1998). They work on the laws of diffusion and the rate at which the contaminant is adsorbed and retained on the sampling material is a fixed constant referred to as the uptake rate. Most of these tools referred to act as equilibrium sampling devices (ESDs).

1.7 Passive sampling

Some of these chemical methods vary principally in the area of sampling and are generally seen as passive sampling as "they usually combine sampling, analyte isolation and preconcentration into a single step" (Gòrecki & Namieśnik, 2002). Gorecki defined passive sampling as any sampling technique based on free flow of analyte molecules from the sampled medium to a collecting medium, as a result of a difference in chemical potential of the analyte between the two media. The passive sampler is designed to mimic lipid pools in animals (Kot *et al.*, 2000) therefore serving as good estimators of bioavailability and can also be used for long term monitoring. Kot *et al.*, (2000) reviews various membrane-based passive samplers such as solvent-filled devices, SPMDs, passive *in situ* concentration /extraction sampler (PISCES), supported liquid membrane (SLM) technique and sorbent filled devices which are all based on the process of passive partitioning of a compound between water and a lipophilic material enclosed in a semipermeable polymeric membrane. A similar detailed review of passive sampling and samplers is given by Namieśnik *et al*. (2005) and Stuer-Lauridsen (2005).

Passive sampling devices (PSD) have a number of advantages over traditional sampling methods, including:

- Simplicity and low cost,
- No need for expensive and sometimes complicated equipment,
- No power requirements and unattended operation,

- The ability to produce accurate results,
- Ease of transportation, and minimises decomposition of the sample during transport and storage
- Wide applicability to various media (soils, sediments and water) and contaminants,
- Samples only readily available contents and provides time weighted average concentrations of environmental media,
- Ability to assess integrated concentration levels over long time periods

However, they are limited by the possible effect of environmental conditions like temperature, air movement; biofouling and humidity on the analyte uptake rate (Gòrecki and Namieśnik, 2002) though the use of performance/ permeability reference compounds (PRCs) can circumvent these limitations. They also do not account for biomagnification (though this may be viewed as an advantage as it will give an a true concentration of contaminants in the water phase) when effects of POPs or metabolites may be of interest, and when carrying out aqueous exposures, there is the likelihood of vapour phase contamination as well as the photodegradation of certain PRCs without shading (Lu *et al*., 2002; Huckins *et al*., 2002).

Passive sampling devices for water monitoring are classed either diffusive or membrane. They are mainly devices that consist of an organic polymer strip, or polymer tubing filled with a high molecular weight organic liquid (e.g. octanol or triolein). Contaminants can pass through the membrane, much like the membranes of biota, and accumulate in the organic liquid phase. Samplers are typically exposed in water for a period of time to gather or concentrate the hydrophobic contaminants and are then collected and analysed for the contaminants. The principle behind some of these samplers (e.g. SPMDs), is that they absorb the contaminants at a rate that is linearly proportional to the aqueous concentration of the sampling medium and would thus give time weighted concentrations or episodic information.
Examples of materials used are polyoxymethylene, silicone rubber, LDPE, polypropylene, polyethylene. Other materials used in passive samplers include glass coated fibers, Empore disks; polydimethylsiloxane (PDMS), etc.

1.7.1 Permeability/performance reference compounds

A performance reference compound (PRC) is an analytically non-interfering compound, such as perdeuterated PAHs, and certain CBs, which has moderate-to-fairly high SPMD fugacity (escaping tendency) or $K_{ow}{}'s$ that is added to the SPMD lipid before field studies and calibration exposures. Measurements of the loss/dissipation rates of PRCs are used to account for any differences in sampling rate between field and calibration study (i.e., sampling rates measured under a specific set of conditions) exposure conditions such as biofouling, water turbulence or flow velocity and temperature differences can be assessed with PRCs (Huckins *et al*., 2002; Booij *et al*., 2002, Bartkow, *et al*., 2004). These compounds can be used only if they do not interfere with the analysis and if their losses can be measured. One approach for use of PRCs for *in situ* calibration of exchange kinetics is to determine exchange rate constants, k_e from release of these exposure standards (PRCs) and use them to predict uptake rate constant, k_e of environmental contaminants (Booij *et al*., 1998), on the assumption that the rate of contaminant loss is proportional to the rate of contaminant uptake. Another approach is the use of exposure adjustment factors (EAFs) calculated from exposure studies and based upon loss of PRCs spiked into the SPMD prior to deployment (Huckins *et al*., 2002). The EAFs are then used to determine the uptake rate constants of analytes based on the assumption of that the same effects of environmental factors on the chemicals uptake rates apply to the PRC.

The PRCs are chosen to cover a similar range of $K'_{ow}s$ as those shown by the contaminants of interest, with both the uptake and loss rates of the native and perdeuterated forms of the compounds assumed identical (Fig **1.3**). The PRCs are also used to determine if equilibrium has been attained. A PRC that has been completely dissipated implies that compounds of similar $\log K_{ow}$ or lower have already attained sorption equilibrium (Booij *et al*., 2002).

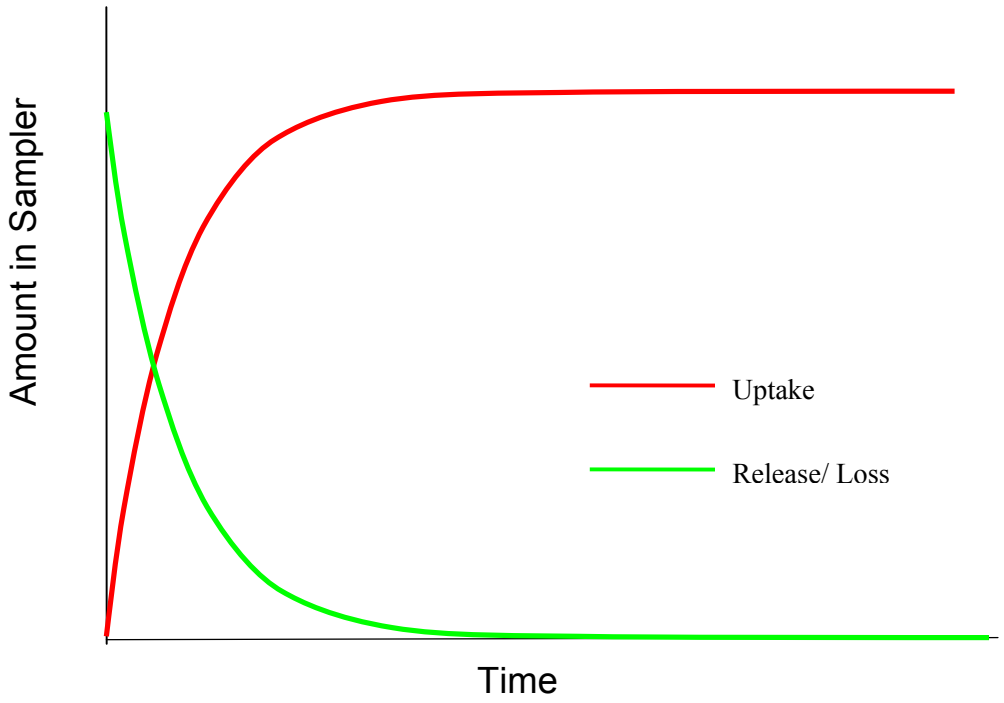


Fig **1.3**: Uptake of compounds and release of PRC curve with similar rates under the same environmental conditions

Examples of compounds used as PRCs include CB004, CB029, CB 112, CB155, CB204, and the perdeuterated PAHs. However, their use is not recommended when the rate limiting step in analyte uptake is desorption of residues/ contaminants from sediment particles e.g. they cannot be used in sediment sampling for contaminants with high K_{ow}. The use of PRCs can also be extended to other passive samplers, such as silicone rubber. The term Permeability Reference Compound is used when the membrane layer controls the uptake rates, while Performance Reference Compound is used when aqueous boundary layer controls uptake.

1.7.2 Freely dissolved concentration

The concentration of the pollutant in the sampled phase (commonly referred to as "freely" or "truly" dissolved concentration), and the quantity of pollutant that in time can become available in the dissolved form (the water-extractable concentration) are more realistic criteria of measuring availability (ICES, WGMS 2003). The water extractable concentration in the solid phase can be viewed as the fraction that can potentially go into the freely dissolved phase

under changeable environmental conditions (accessible quantity referred to by Reichenberg and Mayer, (2006)). The freely dissolved concentration is that fraction of the whole chemical concentration that is not bound to the dissolved- or particulate- organic matter and is available for uptake into an organism. The "total" concentrations of contaminants obtained by traditional extraction methods such as sonication, Soxhlet, ASE, etc, when compared with the freely dissolved or available fraction will indicate the amount of the contaminant that is sediment-bound.

Uptake by most organisms is either through the pore water, food (dietary) or through the soil or sediment. Measuring these availability parameters is a great challenge, as differentiating the dissolved form from the sorbed form is quite difficult. In determining the freely dissolved concentration (and therefore the bioavailability), most of the methods make use of the equilibrium partitioning model which relates the concentration of the contaminant in soil or sediment to an organism, although deviations may be observed due to biological or physico-chemical factors (Sijm *et al*., 2000) especially if equilibrium is not attained and has led to the development of alternative models like the probabilistic model of Thomson *et al*. (2000).

Equilibrium partitioning is based on the premise that the distribution of the contaminant in an environmental phase (e.g. sediment) is controlled by a continuous exchange with other environmental phases (water and biota) and equilibrium partitioning models are considered adequate to describe transport phenomena especially where long (days to months) contact times are used (Wu and Gschwend, 1986). These biogeochemical exchange processes must be sufficiently rapid and reversible to have reached equilibrium (or a steady state) at the time of sample collection (Shea, 1988).

Therefore, an equilibrium sampling strategy has been developed in which the concentration in a reference phase that has been brought into equilibrium with the measuring medium is determined (Mayer *et al*., 2003). This involves the use of an equilibrium sampling device which senses either the chemical potential or fugacity (logarithmically related). Fugacity is a measure of the escaping tendency of a substance from one media to another (mostly from high concentrations or chemical potential to low). It is normally considered in relation to gases and

has the units of pressure (Pa) and gives information on the degree to which a gas deviates from the behaviour of an ideal gas and is essentially the partial pressure exerted by the substance or chemical in each medium. If a chemical attains concentrations in various media that are in equilibrium, its fugacity is equal in these media (Clark *et al*., 1988). The chemical potential or fugacity is linearly related to the freely dissolved concentration in a medium, hence its use in assessing availability of contaminants. In equilibrium sampling, the sampling device is required to sample a volume which is typified by the uptake rate constant that is much greater than the equilibrium partition coefficient of the contaminant (Mayer *et al*., 2003). The equilibrium partition theory also assumes that the fugacity in the pore water and biota are equal, hence, at steady state, accumulation from sediment into the organism can be modelled as accumulation from pore water only.

Most of the models (Huckins *et al*., 1993; Huckins *et al*., 1999; Kraaij *et al*., 2003; Jonker and Koelmans, 2001) have shown that the uptake by the reference phase is governed by the aqueous concentration, surface area (of reference phase), exposure time, uptake rate constant (as well as the resistances to mass transfer), and the physicochemical properties of the contaminants. The uptake rate constant depend on the sampling scenario when diffusion through the aqueous boundary layer is the rate limiting step; therefore the thinner the boundary layer, the faster the exchange between the reference phase and the bulk environmental medium.

When using an equilibrium sampler, knowledge of the partition coefficient of the contaminant and its concentration in the sampler yields the concentration in the sampling medium (e.g. water) using equation **1.1**

$$C_{medium} = \frac{C_{sampler}}{K_{sampler,medium}} \qquad \textbf{1.1}$$

where C_{medium} and $C_{sampler}$ are concentrations of POP in medium and sampler respectively, while $K_{sampler,medium}$ is the equilibrium partition coefficient between the sampler and the

medium. The accuracy of the partition coefficient to a large extent determines the accuracy of the calculated environmental POP concentration.

Mayer *et al*. (2003) gives a generalised uptake profile (Fig **1.4**) for a passive sampling device, in which there are three sampler-operation phases: kinetic (linear), intermediate (curvilinear) and the near equilibrium, with a first order one-compartment model used to fit experimental results in most cases (equation **1.2**).

$$C_{sampler}(t) = C_{medium}\frac{k_1}{k_2}\left(1 - e^{-k_2 t}\right) \qquad \textbf{1.2}$$

where $C_{sampler}(t) =$ concentration of contaminant in the sampler at time 't'; k_1 and k_2 are the uptake and elimination/clearance rate constants respectively.

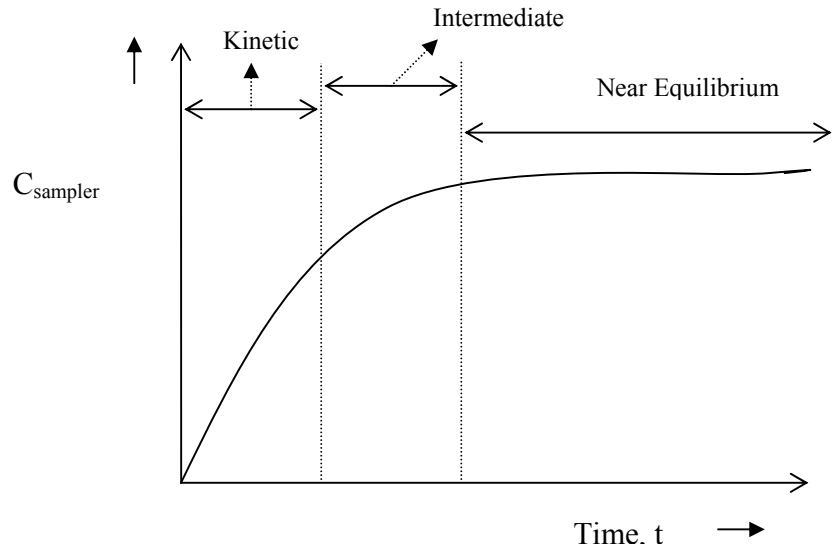


Fig **1.4**: Generalised uptake profile: The linear range (kinetic) is usually within short sampling times whereas relatively long sampling times can be required to attain the near equilibrium (Mayer *et al*., 2003)

The three sampling scenarios (kinetic, intermediate and equilibrium) are determined by the physicochemical properties of the contaminant and the duration of the exposure.

1.8 Passive sampling theory

The use of equation **1.2** is valid for samplers with short (hours-days) equilibration times such as SPME's and Empore disks without a diffusion limiting boundary. However for samplers (and in some situations compounds) with long equilibration times such as SPMDs, the use of equation **1.2** may not be valid. To determine the dissolved concentration in water, Huckins *et al*., (1999) has presented the equation **1.3** below for the uptake by a sampler. Booij *et al*. (2003) applied the same equation in a similar regard when desorption from the sediment particles is not rate limiting (i.e. scenarios where excess sediment is used)

$$N_s = C_w K_{s,w} V_s \left[1 - e^{(-k_e t)}\right] \qquad \textbf{1.3}$$

Where $k_e = \dfrac{k_o A}{K_{s,w} V_s}$ **1.4**

$$= \frac{R_s}{K_{s,w} V_s} \qquad \textbf{1.5}$$

$k_e =$ Exchange rate constant determined by curve fitting equation **1.3**

$k_o =$ Overall mass transfer coefficient

$K_{s,w} =$ SPMD/sampler-water partition coefficient

$C_w =$ Concentration in water/ aqueous concentration

$V_s =$ Volume of SPMD/ sampler

$A =$ Sampler surface area

$N_s =$ Amount in SPMD/ sampler

$R_s =$ Volume of water cleared per time unit, litre/day referred to as the sampling rate and t is time. The R_s values are normally calculated.

The time required for the concentration of the compounds in the sampler to reach steady state levels, referred to as the equilibration time or residence time- the mean length of time that a molecule spends in a passive sampling device, where exchange follows first-order kinetics (Huckins *et al*., 2006) is given as

$$\tau = {}^{1}\!/_{k_e} = \frac{K_{s,w}V_s}{R_s} \qquad \textbf{1.6}$$

The equilibration time which can be days to months depends on the geometry of the sampler, with smaller samplers attaining equilibrium or steady state faster than larger ones as well as the properties of the compound such as K_{ow} or molar volume.

Under kinetic sampling mode (short term exposures), equation **1.3** approximates to

$$N_s = C_w R_s t \qquad \textbf{1.7}$$

While under equilibrium mode, equation **2.3** approximates to

$$N_s = C_w K_{s,w} V_s \qquad \textbf{1.8}$$

These equations can be analogously written for silicone rubber samplers, and generally for equilibrium samplers as

$$N_s^{\infty} = C_w K_s M_r \qquad \textbf{1.9}$$

Where N_s^{∞} = Amount in sampler or reference phase at equilibrium

K_s = Partition coefficient of sampler or reference phase (e.g. silicone rubber)

M_r = Mass of sampler or reference phase

However, many compounds will not attain equilibrium easily (especially in water sampling) and may require impractically long time periods to do so, and therefore the sampling rate

(dependent on kinetics or dynamics at a specific site) is usually estimated. In field applications of SPMDs, R_s is quite difficult to control and R_s calibration made in the laboratory generally do not always apply in the field (Booij and van Drooge, 2001), therefore the use of PRCs added to the sampler prior to exposure is recommended (Huckins *et al*., 2002; Booij *et al*., 2002; Booij *et al.*, 1998) as a means to calibrate exchange rates *in situ.*

The release of these PRCs is governed by the equation:

$$N_t = N^o \cdot e^{(-k_e t)} \qquad \textbf{1.10}$$

$N_t =$ Amount of PRC in sampler or reference phase at time 't', N^o is initial amount of PRC added to sampler or reference phase

The release rate of PRCs is also boundary layer controlled and the first order exchange rate constant is equal for uptake and dissipation. Solving for k_e yields

$$k_e = - \ln\left[\frac{N_t}{N^o}\right]_{prc} \Big/ t \qquad \textbf{1.11}$$

The subscript 'prc' is the performance reference compound. Therefore,

$$R_{s,prc} = k_{e,prc} M_r K_{s,prc} \qquad \textbf{1.12}$$

Based on the equilibrium partitioning concept (EqP); in a multi-compartment aqueous system (Fig **1.5**), the possibility exist of measuring the pollution level in the other compartments, once that of the water phase is known, i.e.,

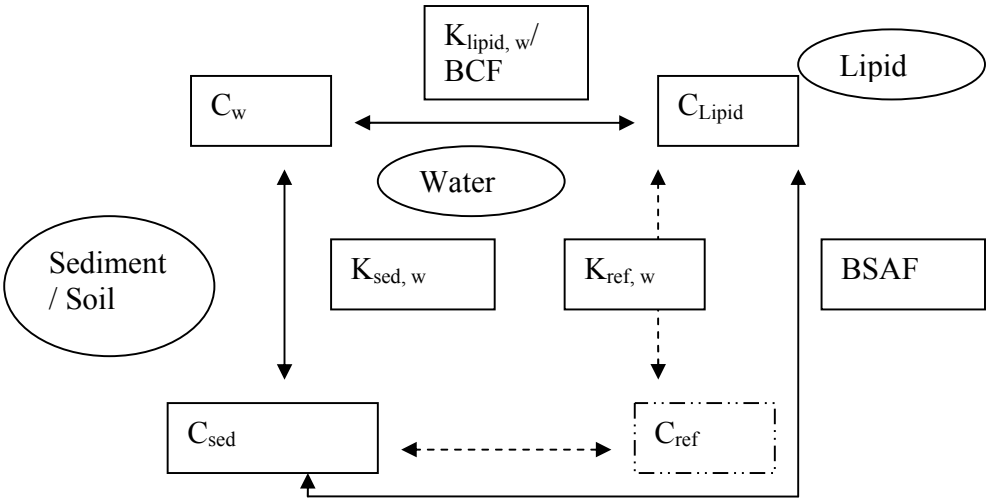


Fig **1.5**: Contaminant distribution in a multi-compartment system as described by the equilibrium partitioning concept

Where;

C_w = Concentration in aqueous or water phase (kg L^{-1})

C_{sed} = Concentration in sediment (kg kg^{-1})

C_{lip} = Concentration in biota/ lipid or organism (kg kg^{-1})

C_{ref} = Concentration in reference phase (kg kg^{-1})

$K_{sed,w}$ = Partition coefficient between sediment and water (L kg^{-1})

$K_{ref,w}$ = Partition coefficient between introduced reference phase and water (L kg^{-1})

BSAF = Partition coefficient between sediment and organism or biota/ lipid or the biota-sediment accumulation factor (BSAF) in L kg^{-1} lipid

BCF= Bio concentration factor

Mathematically,

$$\frac{C_{sed}}{A_{sed}} = \frac{C_w}{S_w} = \frac{C_{lip}}{S_{lip}} = \frac{C_{ref}}{S_{ref}} = \frac{C_{soil}}{A_{soil}} \text{ (if soil is used)} \quad \textbf{1.13}$$

A_{sed}, A_{soil} are uptake capacities of sediment and soil phases respectively

S_w, S_{lip}, S_{ref} represent the solubility of contaminants in water, biota and reference phase

If only a negligible amount of pollutant is extracted from the system, the free concentration in the solution will not change significantly and the equilibrium between the bound and free form will remain undisturbed, with the extracted amount being proportional to the free concentration (Herringa and Hermens, 2003).

Taking an overall mass balance of sediment-water-reference phase system;

$$N_{tot} = C_{sed}M_{sed} + C_{ref}M_{ref} + C_wV_w \qquad \textbf{1.14}$$

C_{ref}, C_{sed} = Concentration in reference/ sampler and sediment respectively (kg kg^{-1})

M_{ref}, M_{sed} = Mass of reference phase and sediment respectively in kg; C_w = Concentration in the water/aqueous phase (kg L^{-1}), V_w = Volume of water; N_{tot} = total analyte amount in system (kg)

However, because the volume of water normally used is very negligible, the term $C_wV_w \to 0$ and becomes negligible compared to the other terms in equation **1.14**, which then transforms to

$$N_{tot} = C_{sed}M_{sed} + C_{ref}M_{ref} \qquad \textbf{1.15}$$

Assuming steady state, $N_{tot} = C^o_{sed}M_{sed}$ **1.16**

C^o_{sed} = exchangeable concentration of analyte in sediment at $t = 0$ and C_{sed} = exchangeable concentration after exposure to reference phase;

Substituting equation **1.16** into **1.15**,

$$C^o_{sed}M_{sed} = C_{ref}M_{ref} + C_{sed}M_{sed} \qquad \textbf{1.17}$$

Since $K_{sed,w} = \frac{C_{sed}}{C_w} \Rightarrow C^o_{sed} = K_{sed,w} C^o_w$ and

$C_{sed} = K_{sed,w} C_w$. Similarly, $K_{ref,w} = \frac{C_{ref}}{C_w} \Rightarrow C_{ref} = K_{ref,w} C_w$

Substituting all these into **1.17** yields

$$C^o_w K_{sed,w} = C_w K_{ref,w} \frac{M_{ref}}{M_{sed}} + C_w K_{sed,w} \qquad \textbf{1.18}$$

Rearranging gives,

$$\frac{1}{C_w} = \frac{1}{C^o_w} + \frac{K_{ref,w} M_{ref}}{C^o_w K_{sed,w} M_{sed}} \qquad \textbf{1.19}$$

The introduction of a reference phase tends to affect the distribution of pollutants between the sediment and water phase by decreasing the amount of compounds from sediment via absorption into the reference phase (silicone rubber). Therefore, use of a reference phase with known amount of sediment at different phase ratios (mass of reference phase/mass of sediment) and subsequently extrapolating to a situation of zero mass of reference phase should give the best estimate of the concentration in the water phase, i.e. which is closest to the freely dissolved concentration.

Equation **1.19** is of the form $y = a + bx$ where; $y = 1/C_w$; $a = 1/C^o_w$;

$b = K_{ref,w} / C^o_w K_{sed,w}$ and $x = M_{ref} / M_{sed}$, which is the phase ratio, and varying this quantity gives different C_w values. Plotting equation **1.19** and extrapolating to get the intercept gives the value of the dissolved concentration, C^o_w while the slope of the graph yields $K_{sed,w}$.

Another approach is to divide equation **1.17** by M_{sed} yields

$C^o_{sed} = \left(\frac{C_{ref}}{M_{sed}}\right) M_{ref} + C_{sed}$. Rearranging yields

$$C_w = C^o_w - \left(\frac{C_{ref}}{M_{sed}}\right)\left(\frac{M_{ref}}{K_{sed,w}}\right) \qquad \textbf{1.20}$$

From equation **1.20** C_w can be plotted (Fig **1.6**) against the term $C_{SedEx} = {}^{C_{ref}}\!/_{M_{sed}}$ referred to as the amount of contaminant extracted by the silicone rubber or reference. This was the format or plot used mostly in the thesis as both equations **1.19** and **1.20** yielded similar results for pore water concentrations C^o_w and the maximum water exchangeable concentration C^o_{SedEx}

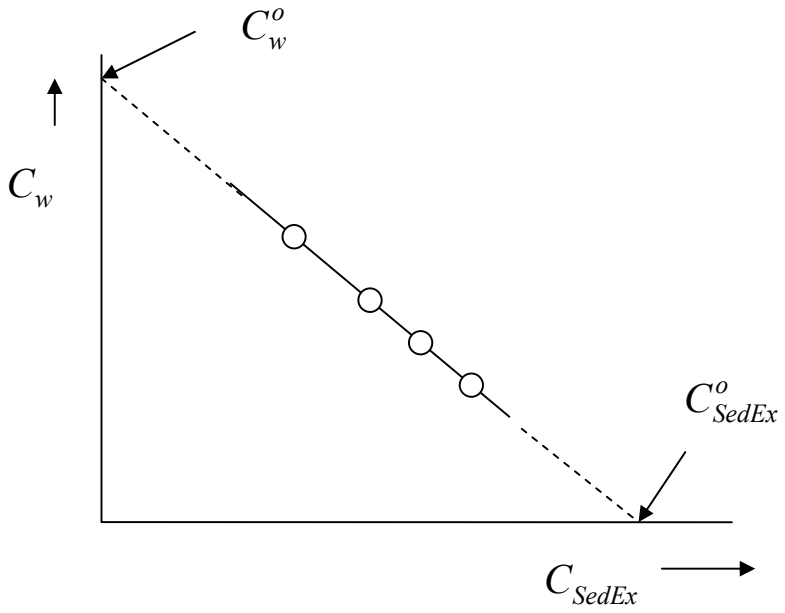


Fig **1.6**: Example plot used to determine C^o_w and C^o_{SedEx} by extrapolation to both axes

Similarly, at equilibrium,

C_w is constant and therefore

$$C_{sed} = \frac{K_{sed,w}}{K_{ref,w}} C_{ref} \qquad \textbf{1.21}$$

Then, substituting into equation **2.17** gives,

$$C_{ref}M_{ref} = C^{o}_{sed}M_{sed} - \frac{K_{sed,w}}{K_{ref,w}}C_{ref}M_{ref} \qquad \textbf{1.22}$$

Dividing by $C_{ref}M_{sed}$ yields

$$\frac{M_{ref}}{M_{sed}} = C^{o}_{sed}\frac{1}{C_{ref}} - \frac{K_{sed,w}}{K_{ref,w}} \qquad \textbf{1.23}$$

Which is in the form of $y = a + bx$ where $y = \frac{M_{ref}}{M_{sed}}, x = \frac{1}{C_{ref}}, a = -\frac{K_{sed,w}}{K_{ref,w}}$ and

$b = C^{o}_{sed}$

Equation **1.23** can be used to calculate $K_{sed,w}$ from the intercept. An alternative approach is to plot C_w against a term referred to as residual content $C_{res} = C_{tot} - C_{SedEx}$ (Fig **1.7**) which was used in this thesis. The inverse of the slope of such a plot yields $K_{sed,w}$ of the POP.

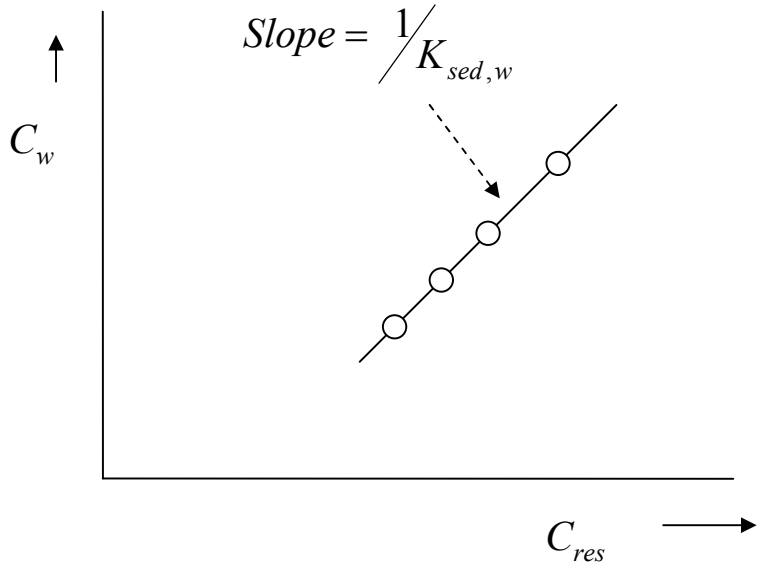


Fig **1.7**: Example plot of a "sorption isotherm" used to calculate $K_{sed,w}$

C_w^o, C_{SedEx}^o and $K_{sed,w}$ are the parameters that are generally required to describe the availability of POPs in sediments.

1.9 Aims and Objectives of this work

The use of passive samplers made from silicone rubber film will be developed and used to measure the freely dissolved concentrations of the POPs in sediment with emphasis on PAHs and CBs.

At equilibrium, following from section **1.8.1**, with the introduction of the silicone rubber reference phase, either equations **1.19** or **1.20** can be used to determine the free dissolved concentrations as well as the sediment-water partition coefficients. These equations aid in determining the parameters required to evaluate or reflect the bioavailability of PAHs and CBs in sediment-water systems.

The objectives of the research are outlined in the following milestones:

MPhil Milestones:

- Familiarisation with some analytical techniques to be used e.g. HPLC, GC-FID, GC-MSD, Soxhlet (total) extraction of contaminant from sediments
- Investigate the period or time to reach equilibrium for the PAHs as well as evaluating distribution or partition coefficients
- Investigate the influence of suspension density, phase ratio, dissolved organic matter and temperature on equilibrium
- Determination of "total" concentration by traditional methods, e.g sonication and Soxhlets extraction
- Determination of freely dissolved concentration and extractable concentration of PAHs from sediment samples and comparison with concentration from "total" extraction

Final PhD Milestones:

- Validation of uptake of POPs by silicone rubber using bio-monitoring organism, *Nereis virens* (a ragworm of the polychaeta family).
    - Investigate the time required to attain steady state concentrations by the rag worms
    - Collect a range of sediments and expose the rag worms to these
    - Compare results obtained with the pore water concentrations calculated from the same sediments.
- Validation of the silicone rubber samplers through participation in an ICES passive sampling trial survey

CHAPTER TWO

General analytical methods

2.0 Analytical methods

The sections below describe the standard analytical methods used in this thesis. These methods are well established at FRS Marine laboratory (FRS ML) and are accredited by the United Kingdom Accreditation Service (UKAS) to ISO 17025. Details of the methods are given in the FRS ML standard operating procedures in Appendix **1** (see attached CD). The development of method for passive samplers is described in Chapter **3**.

Environmental trace analysis of POPs requires considerations for quality control and assurance which are equally applicable to passive samplers such as the silicone rubber. Consequently to avoid issues such as contamination, cleaning of glass ware, checks on solvents and proper handling of the samples and equipment was ensured and where necessary, work was carried out using dedicated environments and equipment. Chemical standards used in the preparation of calibration solutions are of high quality and concentrations were adjusted for purity where necessary. The procedures described in this chapter are UKAS accredited under ISO 17025 (Webster *et al*., 2005) and in cases where the method was just being optimised, UKAS procedures were followed.

Samples were kept in designated freezers and properly logged into the FRS quality system for traceability and also to maintain their integrity. Procedural, field blanks and laboratory reference materials were included in analyses and where necessary, monitored using control charts as a check for recovery and contamination as well as the use of internal standards. As indicated in earlier sections, Shewart control charts (Fig **2.0**) are used to monitor the performance of a method (either a laboratory reference material- LRM or a blank) for individual compounds or parameters by updating the data after each analysis with warning and action limits drawn at ± 2 × and ± 3 × the standard deviation of results obtained. As part of the quality assurance, the laboratory participates successfully in the external, UK National Marine Biological Analytical Quality

Control (NMBAQC) quality assurance scheme for the Particle Size Analysis and the Quality Assurance of Information for Marine Environmental Monitoring in Europe (QUASIMEME) laboratory performance study scheme for PAHs and CBs (Webster *et al*., 2005).

Procedures were risked assessed at the FRS ML.

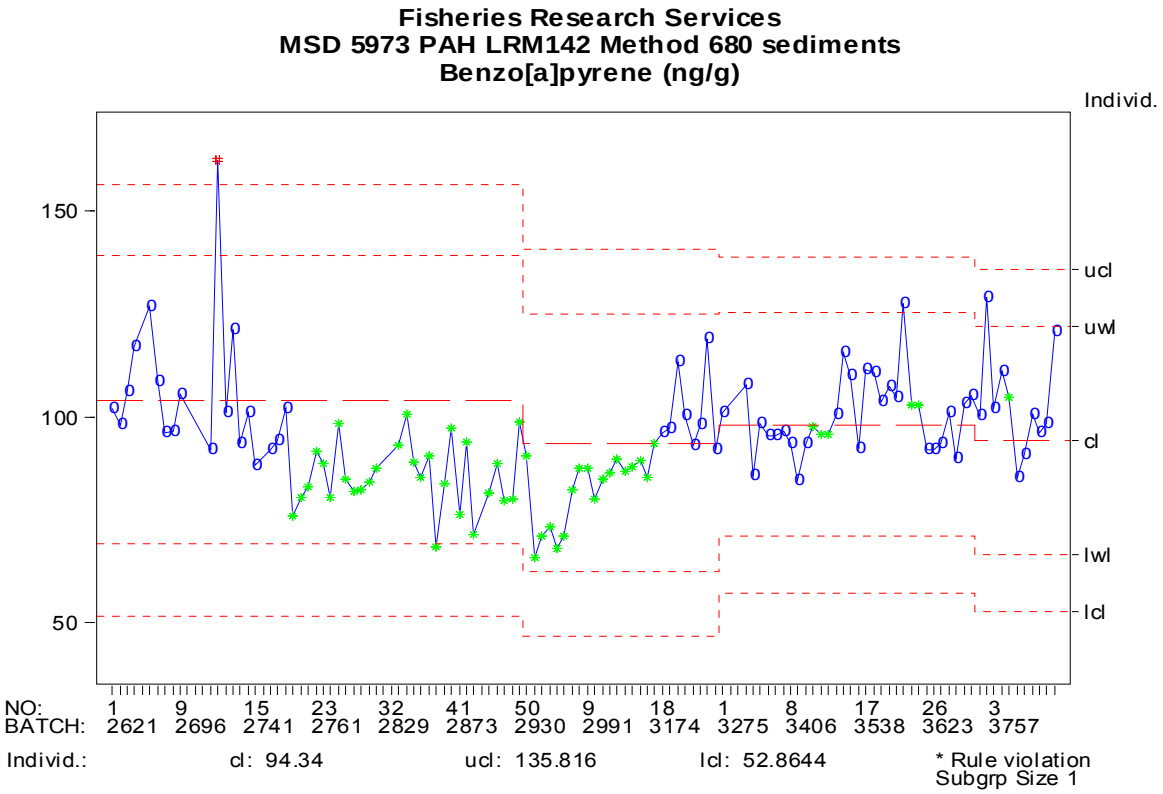


Fig **2.0**: Example of a Shewart control chart for Benzo[*a*]pyrene showing warning and action control limits, based on ± 2 × and ± 3 × the standard deviation of results obtained with each data point on the plot representing a value from a single analysis of an LRM in a batch. The green points show instances where there are seven points in a row in above or below the mean

2.1 Bulk sediment properties

2.1.1 Freeze drying of sediments

Freeze drying involves the removal of water from frozen samples without heating. This was carried out based on the FRS ML SOP 0110 using an LTE scientific freeze drier to remove ice by sublimation from frozen samples. The underlying principle being that at low pressures (using

vacuum pump) and temperatures (-30 ^{o}C), water in the form of ice is converted directly into water vapour and by avoiding the liquid phase of water, boiling is inhibited and the sample remains intact during the entire process. Frozen samples, stored in plastic bags or aluminium cans were slightly opened to allow escape of water vapour generated from sublimation and placed in the freeze drier to remove any moisture.

2.1.2 Laser granulometry

Laser granulometry was used to determine the particle size distribution of sediment samples over the 0.02-2000 microns range. The analysis was performed using a Malvern Mastersizer 2000 instrument (Worcestershire, UK). Essentially, the Mastersizer is a light scattering based particle sizer comprised of an optical measurement unit and computer (with Mastersizer 2000 software V5.1 installed). The angle of light scattered by the particle (inversely proportional to the particle size) in a sample is measured and used to determine the size distribution of the samples.

Briefly, the freeze dried samples were sieved (providing a more representative sub sample) using a rough 2000 micron sieve, introduced into a tank of water and stirred at 1000 rpm in order to keep the particles in suspension. The tank and sieve were cleaned after each sample. The sample was then pre-treated for 50 s by ultrasound to break up any conglomerates and measurement effected for 12 s using a red laser and another 12 s using a blue laser at 1000 measurements per second. Data output is in the form of percentage amounts by volume of the sample lying within specified size bands (size bands are based on the 'PHI' unit scale) and as percentage of particles with different diameter (for this thesis, only the percent ≤ 20 and 63 μm were used). Two LRM's (glass beads) were analysed prior to analysis of samples and data quality was monitored by Shewart quality control chart. The precision of the method based on 7 replicate measurements of the LRM C100 on the D (4, 3) mean weighted volume fraction equals 2.50 %.

2.1.3 Total Organic Carbon (TOC)/ Elemental (Carbon, Nitrogen) analysis of sediment

The FRS UKAS accredited method describes the determination of organic carbon and nitrogen in sediment samples using a ThermoQuest Flash EA 1112 elemental analyser. The machine was

calibrated on start up using acetanilide and system suitability checks were carried out using the CRM Mess-2 (LGC Promochem, Middlesex, UK) for carbon and nitrogen. Prior to analysis, the freeze dried samples are acidified with hydrochloric acid (HCl) in silver cups to remove the inorganic carbon fraction. The samples are then combusted in the analyser with the aid of an oxidation catalyst (platinumised alumina) in a pure oxygen environment and to break down the sample elements to simple gases (CO_2, H_2O, and N_2) and through a reduced copper reactor column where nitrogen oxides possibly formed are converted into elemental nitrogen and any excess oxygen retained. After column separation, the resulting gases were measured (peak areas) under steady state conditions as a function of thermal conductivity. "Clean" homogenised sediment from Raasay Sound was used as LRM and LRM values monitored using Shewart quality control charts. The limit of detection for both organic carbon and nitrogen is 0.005 mg although the limit of quantification (LOQ) calculated as 0.005 divided by the sample weight analysed × 100 % is normally reported. E.g. for a sample weight of 16.50 mg, the LOQ is 0.03 %.

2.2 Moisture Content of sediment and invertebrates

Frozen samples were first defrosted at room temperature, thoroughly mixed with metal spoons or spatula before the determination of moisture content. 10 ± 1 g wet sediment was accurately weighed into aluminium weighing boats and dried in an oven at 80 ± 5 ^{o}C for 22 ± 2 h and subsequently reweighed to determine weight loss which gives the moisture content of the sediment. Dry weights of sediments are thus calculated using the moisture content.

Biota samples (mussel) were shucked, homogenised and weighed into pre-weighed plastic bags and frozen. The samples were removed from the freezer, and freeze-dried as in **2.1.1**. The bags were slightly opened to allow escape of water vapour generated from sublimation during the freeze drying operation. The weight loss from freeze drying was then calculated.

2.3 PAH and CB analysis of sediment and biota

2.3.1 Treatment of Glassware and Solvents

Hydrocarbon analysis was carried out in a clean environment avoiding contamination of samples and reagents. All glassware used was either washed in Decon® 180 solution and rinsed with distilled water or washed in a CAMLAB GW 4050 glassware washer and dried in an oven at 100 ± 5 ^{o}C. Before use, the glassware were rinsed twice each in dichloromethane and *iso*-hexane with the latter allowed to evaporate to dryness to avoid carry over of contamination from previous samples. HPLC grade solvents (dichloromethane, ethyl acetate, acetone, methanol and *iso*-hexane) were purchased from Rathburn Chemicals Ltd, Scotland, UK. Certified solid standards for PAHs (including deuterated PAHs) and CBs were obtained from QMX Laboratories, Essex, UK and dissolved in *iso*-hexane (PAHs) and *iso*-octane (CBs) to obtain required concentrations of spiking solutions.

2.3.2 Anhydrous sodium sulphate

The anhydrous sodium sulphate used for drying of organic extracts from biota, water and sediment extractions was prepared by washing the sodium sulphate filled 3/4 in a 500 ml conical flask with dichloromethane (DCM) covering the sodium sulphate using a sonic bath for 15 min. The DCM was then decanted to waste and the washing procedure repeated using *iso*-hexane covering the sodium sulphate and the washings also decanted to waste. This was done twice and the washed anhydrous sodium sulphate dried in an oven at 10 ^{o}C for 16 ± 2 h.

2.3.3 Extraction of sediments and biota

Extractions of sediment and biota for organic contaminants were carried out using UKAS accredited methods (ML M 680, 690 and 0645, Appendix **1**).

2.3.3.1 Sediment

Sediment sample were defrosted at room temperature, ensuring that exposure to direct sunlight or heat was minimised and the samples thoroughly mixed before sub sampling for analysis. Sediment samples for CB analysis were freeze dried before extraction; PAHs were extracted from wet sediment

2.3.3.1.1 Analysis of sediments for PAHs

Sediment sub samples of 1-20.5 g, depending on expected hydrocarbon content, were weighed into a centrifuge tube. Deuterated PAH aromatic internal standard containing D_8-naphthalene, D_{10}-biphenyl, D_8-dibenzothiophene, D_{10}-anthracene, D_{10}-pyrene and D_{12}-benzo[*a*]pyrene (100 or 200 µl; approximately 1 µg ml^{-1} each) was added dependent on the estimated concentration of hydrocarbons. 200 ± 10 µl of aliphatic standard (containing approximately 3.2 µg each of heptamethylnonane and squalane) was then added to the sample. 20 ± 2 ml of DCM and methanol respectively were then added to the centrifuge tube and the solution thoroughly mixed by swirling to break up the sediment. The sample was then sonicated for 5 min, followed by centrifugation at 1800 rev/s for 10 min at 5 ± 0.5 ^{o}C. The liquid layer was the decanted into a separating funnel containing 18 ± 2 ml of water and thoroughly shaken. The bottom DCM layer was consequently transferred to a 100 ml flask containing 10 ± 1 g of anhydrous sodium sulphate. The sediment was re-extracted by sonication for 5 min with fresh 20 ± 2 ml of DCM, centrifuged and the solvent layer decanted into the separating funnel. This was thoroughly mixed, allowed to separate and the DCM layer combined with the first DCM extract in the 100 ml conical flask. The extract was then dried over the anhydrous sodium sulphate for ~ 10 min, concentrated by rotary evaporation and exchanged into *iso*-hexane by the addition *iso*-hexane and further reduced by rotary evaporation before a final concentration to 500 ± 100 µl in a 2 ml glass vial under a stream of purified (to avoid contamination) nitrogen. An aliquot of the concentrated extract was fractionated using an isocratic, normal phase Genesis metal-free high performance liquid chromatograph (HPLC) column and the aromatic fraction collected and concentrated before Gas Chromatography –mass selective detection (GC-MSD) analysis.

An LRM (Aberdeen Harbour sediment) and a procedural blank were analysed with each batch of sediment samples and data obtained from the LRM monitored on a Shewart quality control chart with standard warning and action limits drawn. Sediment samples spiked with 1, 10, and 100 ng g^{-1} PAH standard solutions were passed through the procedure above to validate the method and yielded recoveries ≥ 85 % with precision ≤ 17 % for individual PAH compounds.

2.3.3.1.2 Analysis of biota samples for PAHs

The isolation of hydrocarbons from biota (mussel and *N. virens*) was as described in Webster *et al*. (1997). Biota (~ 10 g) was accurately weighed into a 250 ml round bottom flask and 200 ± 10 µl of aliphatic standard (containing approximately 3.2 µg each of heptamethylnonane and squalane) and 100 ± 10 µl deuterated PAH aromatic internal standard containing D_8-naphthalene, D_{10}-biphenyl, D_8-dibenzothiophene, D_{10}-anthracene, D_{10}-pyrene and D_{12}-benzo[*a*]pyrene (100 µl; approximately 1 µg ml^{-1} each) were then added to the sample. Sodium hydroxide (10 %; 40 ± 4 ml) in methanol/H_2O (90:10 $^{v}/_{v}$) and a few anti-bumping granules were added to the sample and a cleaned reflux condenser fitted to the flask and lowered onto a heated (maintained at 75 ± 5 ^{o}C, monitored using a calibrated thermometer in a beaker of water) sand bath and saponified for 3 h 45 min. Water (10 ± 0.1 ml) was then added and further heated for 15 min.

The hot solution was transferred to a 250 ml separating funnel containing *iso*-hexane (40 ± 4 ml) and methanol: water (4:1 $^{v}/_{v;}$ 40 ± 4 ml) was used to rinse the round bottom flask and added to the separating funnel. The mixture was thoroughly shaken and the lower aqueous layer transferred to a second separating funnel containing *iso*-hexane (80 ± 5 ml) and the solution thoroughly mixed. The first *iso*-hexane extract was washed with 40 ± 4 ml methanol: water (1:1 $^{v}/_{v}$) by shaking vigorously and allowed to separate. The aqueous layer from the second *iso*-hexane extraction was run-off to waste and the methanol/water layer from the first separating funnel added to the second separating funnel. This was shaken, allowed to settle and the aqueous layer drained to waste. The extracts from the two separating funnels were then combined and washed thrice with 40 ± 4 ml water, each time draining the bottom water layer to waste and finally passed through anhydrous sodium sulphate columns to remove any water in the extracts. 50 ± 5 ml of *iso*-hexane was subsequently passed through the columns and the eluate concentrated by rotary evaporation

followed by nitrogen blow down to 500 ± 100 µl. The PAHs were isolated from the aliphatic hydrocarbons by isocratic normal-phase HPLC and prepared for GC-MSD analysis. Recoveries of ≥ 82 % with precision ≤ 9 % were obtained for mussel samples spiked with 1, 10 and 100 ng g^{-1} PAH solution, for individual PAHs.

2.3.3.1.3 Gas chromatography-mass selective detection (GC-MSD)

The concentrations and composition of the PAHs were determined by GC-MSD using an HP6890 Series Gas Chromatograph interfaced with an HP5973 MSD fitted with a cool on-column injector (Webster *et al.*, 2005). Briefly, a non-polar HP5 (30 m × 0.25 mm id, 0.25 µm film thickness; Agilent Technologies, Stockport, England) column was used for the analyses with helium as the carrier gas, controlled using the constant flow mode at 0.7 ml min^{-1}. The MSD was set for selective ion monitoring (SIM) with a dwell time of 50 ms. Injections were made at 50 ^{o}C and the oven temperature held constant for 3 min. Thereafter, the temperature was raised at 20 ^{o}C min^{-1} up to 100 ^{o}C, followed by a slower ramp of 4 ^{o}C min^{-1} up to a final temperature of 270 ^{o}C. A total of 29 (later 36 with the addition of extra performance reference compounds, Table **2.0**) ions plus the six internal standard ions were measured over the analysis period, thus incorporating 2- to 6-ring, parent and branched PAHs. Limits of detection based on multiplying the standard deviation of the mean of the lowest standard (0.005 ng ml^{-1}) by 4.65 were found to be < 0.2 ng g^{-1} for chrysene and < 0.1 ng g^{-1} for benzo[*a*]pyrene. The GC-MSD is calibrated using seven different concentrations of a solution containing 33 PAHs.

Table **2.0**: List of ions measured using the GC-MSD in SIM mode

PAH	Molecular Weight/ Da				
	Parent PAH	Alkylated PAH			
		C1	C2	C3	C4
Naphthalene	128	142	156	170	184
Phenanthrene	178	192	206	220	
Dibenzothiophene	184	198	212	226	
Fluoranthene/ Pyrene	202	216	230	244	
Benzo[*c*]phenanthrene/Benz[*a*]anthracene/ Benz[*b*]anthracene/Chrysene+Triphenylene	228	242	256		
Benzofluoranthene/Benzo[*e*]pyrene/ Benzo[a]pyrene/ Perylene	252	266	280		
Benzo[*g,h,i*]perylene/ Indeno[*1,2,3-c,d*]pyrene	276	290	304		
Acenaphthylene	152				
Acenaphthene	154				
Fluorene	166				
Dibenz[*a,h*]anthracene	278				
D_8- Naphthalene*	136				
D_{10}- Biphenyl*	164				
D_{10}- Anthracene*	188				
D_8- Dibenzothiophene*	192				
D_{12}- Fluoranthene** / D_{10}- Pyrene*	212				
D_{12}-Benzo[*a*]pyrene* / D_{12}-Benzo[*e*]pyrene**	264				
D_{10}- Fluorene**	176				
D_{12}- Chrysene**	240				

* Deuterated PAHs used as internal standards

** Deuterated PAHs used as performance reference compounds

2.3.3.2 Analysis of sediment and biota for CBs

Concentrations of CB congeners (Table **2.1**) were determined in sediment and biota by Soxhlet extraction, column chromatography clean-up, and gas chromatography electron capture detection (GC-ECD).

Table **2.1**: List of CB congeners and Hexachlorobenzene (HCB) analysed in sediments and biota

S/No	Congener	S/No	Congener
1	HCB	19	CB 153
2	CB 31	20	CB 132
3	CB 28	21	CB 105
4	CB 53*	22	CB 137
5	CB 52	23	CB 138
6	CB 49	24	CB 158
7	CB 35*	25	CB187
8	CB 44	26	CB 183
9	CB 74	27	CB 128
10	CB 70	28	CB 156
11	CB 101	29	CB 157
12	CB 99	30	CB 180
13	CB 112*	31	CB 198*
14	CB 97	32	CB 170
15	CB 110	33	CB 189
16	CB 151*	34	CB 194
17	CB 149	35	CB 209*
18	CB118		

* CBs used as recovery standards

2.3.3.2.1 Analysis of sediments for CBs

Freeze-dried sediment was weighed into a cellulose thimble, previously cleaned by pre-extracting in methyl t-butyl ether (MTBE) for four hours in a Soxhlet apparatus. A 1 ml-weighed 5 µg g^{-1} recovery standard containing CB 35, 53, 112, 151, 198, and 209 (Promochem, Herts, UK) was then added to the thimble and placed into a Soxhlet apparatus connected to a round bottom flask containing MTBE (180 ± 10 ml), 15 g of activated copper powder and a few anti-bumping granules. The Soxhlet and round bottom flask were fitted to a condenser and extracted by heating on a heating mantle overnight (at least 8 h). The flask was allowed to cool and the remaining

solvent from the Soxhlet collected into the flask and stoppered. The extract was reduced by means of a Turbovap system, exchanged into hexane by addition of 10 ± 2 ml hexane and reduced to 0.5 ± 0.1 ml. The extract was then passed through an alumina column and eluted with hexane to remove any lipids and separate the extracts into CB and organochlorinated pesticide (OCP) fractions. The CB fraction was further separated into 2 fractions by means of a silica column, collecting only the first fraction that contains only CBs using hexane whilst allowing the remaining solvent, containing OCPs, to go to waste. The eluate was evaporated to 0.5 ± 0.1 ml, made up in *iso*-octane and 500 ± 50 µl 2, 4- dichlorobenzyl alkyl ethers (DCBE 6 and DCBE 16) internal standard added and reduced using a Turbovap system to 0.5 ± 0.1 ml. This was finally vialled for Gas Chromatography-electron capture detection (GC-ECD) analysis. Sediment samples (n = 9) analysed by the same procedure as above but analysed on a Varian GC, yielded recoveries ≥ 83 % for individual CBs with precisions ≤ 16 %.

2.3.3.2.2 Analysis of biota samples for CBs

The method describes the determination of CBs using accelerated solvent extraction (ASE). Briefly, mussels (8 ± 0.5 g) were mixed with anhydrous sodium sulphate (30 ± 5 g) to dehydrate the sample prior to solvent extraction in a glass jar and stored in a refrigerator overnight. Sodium sulphate (10 ± 1g), followed by, 5 % deactivated alumina (30 ± 1 g) were added to an extraction cell *via* a funnel. A filter paper was placed at the bottom of the cell and at the top after adding the alumina. The dried tissue was removed from the fridge, ground with a pestle for ~ 2 min and turned into an extraction cell followed by the addition of 200 µl of 5 µg g^{-1} CB recovery standard containing CB 35, 53, 112, 151, 198, and 209 using a calibrated syringe. The sample jar was then rinsed with 5 ml *iso*-hexane and decanted into the cell, filling the cell with sodium sulphate where there was void volume before a final filter paper was placed at the top and the cell lids tightened. The sample was then extracted on the Dionex ASE 300 using *iso*-hexane (Fig **2.1** shows the ASE extraction cell set up) and the settings below:

Pressure	: 1500 psi
Temperature	: 60 ^{o}C
Heat	: 5 minutes
Static Time	: 5 minutes
Flush %	: 50
Purge	: 120 seconds
Number of cycles	: 2

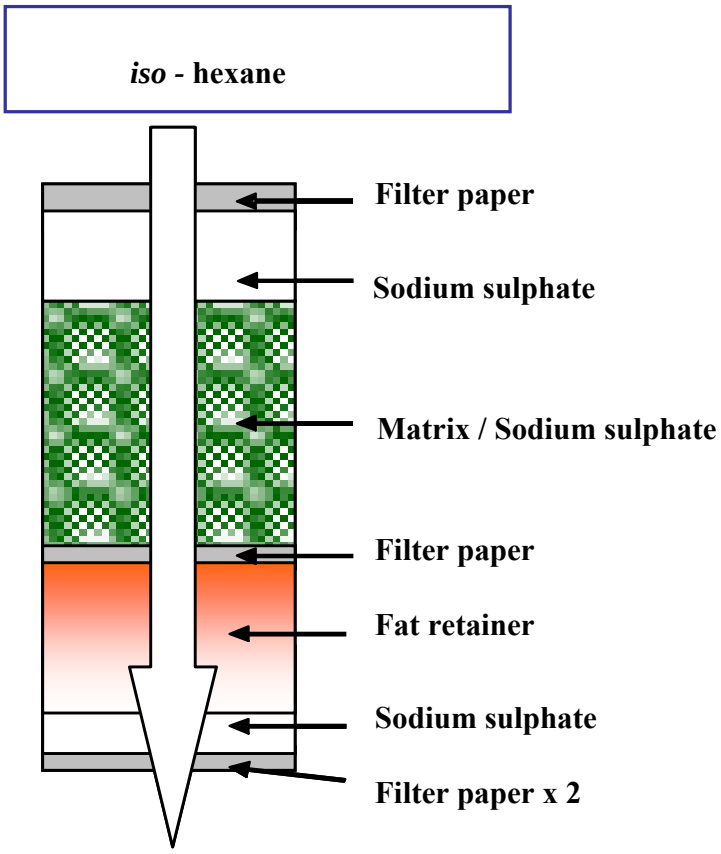


Fig **2.1**: Schematic of the ASE extraction cell set-up for the extraction of CBs in biota

The cells were allowed to cool after the extraction for at least 15 min before being emptied to waste. The extracts were collected in ASE bottles, transferred with 2 x 10 ± 2 ml washings of the bottles to Syncore tubes and concentrated to 0.5 ± 0.2 ml using the syncore evaporation system. The reduced extracts were then cleaned up by column chromatography using alumina, and silica

and eluting in both cases with 25 ± 2 ml *iso*-hexane. The eluates were concentrated to 0.5 ± 0.2 ml and 100 ± 10 µl of DCBE internal standard added using a calibrated syringe. The samples were then weighed and vialled for GC-ECD analysis. Mean percent recoveries on a Varian GC from mussel samples spiked with 100 ng of CB solution were ≥ 74 % for individual CBs with precision ≤ 15 %.

2.3.3.2.3 Gas chromatography-electron capture detection (GC-ECD)

The concentration and composition of CB congeners were determined by GC-ECD using a PE GC Clarus 500 system (Perkin Elmer, Beaconsfield, UK) fitted with a cool on-column injector. A non-polar column was used for the analyses (HP 5 column, 60 m × 0.25 mm id, 0.25 µm film thickness; Agilent Technologies, Stockport, England) along with an uncoated pre-column (2.5 m × 0.53 mm id). The carrier gas was hydrogen (1–3 ml min^{-1}) and make-up gas nitrogen (30 ± 5 ml min^{-1}). At the start of the thesis the temperature programming was set at an initial oven temperature of 80 ^{o}C and held for 1 min after which it was ramped at 3 ^{o}C min^{-1} to a final temperature of 280 ^{o}C and held at this temperature for 12 min. Subsequently the programme was changed and the initial oven temperature was 80 ^{o}C which was held for 1 min. The temperature was raised at 5 ^{o}C min^{-1} up to 150 ^{o}C and raised at 2 ^{o}C min^{-1} to 250 ^{o}C then at the rate of 3 ^{o}C min^{-1} to a final temperature of 280 ^{o}C and held at this temperature for 12 min. A PerkinElmer PreVent™ was included to benefit from the time-saving mode in terms of preventing unwanted, low volatility materials reaching the detector, thus shortening analysis times, facilitating isothermal chromatography and protecting the detector from contamination by means of a back flush of the column. The pre-vent also helped to obtain good separation between CB153 and 132. The chromatograph was calibrated using a series of external standards and the two 2, 4-dichlorobenzyl alkyl ethers. The data were quantified using a Client Server Turbochrom data system (Perkin Elmer, Beaconsfield, UK). The limit of detection was < 0.03 ng g^{-1} for all the CBs. The gas chromatograph is calibrated by a series of seven external CB standards that include two internal standards, 2, 4- dichlorobenzyl alkyl ethers (DCBE 6 and DCBE 16).

2.4 Determination of extractable lipid concentration in biota samples

POPs tend to accumulate in the lipids of biota samples and lipid concentrations vary with season, reproductive state, etc. Therefore, organic contaminant concentrations are often normalised to the lipid content of marine organisms (Smedes, 1999). The sections below describe the determination of extractable lipid concentrations in samples of mussels and *Nereis virens*.

2.4.1 Mussels

Mussel tissue was extracted using a method developed by Smedes (1999) for the determination of total lipid content. Briefly, 5 ± 0.1 g of sample, containing ≤ 1 g of lipid was weighed into a 100 ml centrifuge tube, then *iso*-propanol (18 ± 1 ml) and cyclohexane (20 ± 1 ml) were added and mixed by UltraTurrax® for 2 min at 13500 rpm. Water (17 ± 1 ml) was added and the mixture further mixed for 1 min using the UtraTurrax®. The sample was centrifuged at 1800 rpm for 10 min and 10 ± 0.5 ml of the organic phase transferred using a glass pipette and a measuring cylinder to a pre-weighed 100 ml round bottom flask, ensuring no tissue particles were included. The remaining organic phase was removed by means of a glass pipette fitted to a vacuum pump and discarded. A second extraction was carried out by adding 20 ± 1 ml of 13 % $^{w}/_{w}$ *iso*-propanol in cyclohexane and mixed with an UtraTurrax® for 1 min. This was centrifuged at 1800 rpm for 10 min and 10 ± 0.5 ml of the upper organic phase pipetted into the round bottom flask containing the first extract by means of a measuring cylinder. The solvent was evaporated to dryness at 75 ± 1 ^{o}C using a rotary evaporator and further dried in an oven at 80 ± 1 ^{o}C for 1 h, after which it was cooled at room temperature in a dessicator and weighed. From the mass residue and intake mass, the lipid content was calculated.

LRMs (0.30 ± 0.05 g of fish oil and 4 ± 0.5 g freeze dried mussel homogenate) were extracted in same manner as the samples, replacing the 17 ml of water with 22 ml, since the LRMs are water free and the LRM data monitored on a Shewart control chart.

2.4.2 *Nereis virens*

To determine the lipid content in *N. virens*, an accredited method for copepod lipid extraction was modified due to the low sample size available. Homogenised *N.virens* (0.5 ± 0.2 g) was weighed into a glass 2 ml vial and 1200 µl of 2, 6- di- tert- butyl- *p*- cresol (butylated hydroxytoluene [BHT]) in chloroform-methanol solution (2:1 $^{v}/_{v}$) added and shaken thoroughly to mix the solvent ensuring the sample is fully suspended in the solvent. The vial was placed in a refrigerator for 24 ± 2 h allowing for lipid extraction into the solvent. Afterwards, 300 µl of potassium chloride solution (8.8 g L^{-1}) was then added to the vial and the contents mixed gently but thoroughly. This was centrifuged at 1800 rpm and 0 ^{o}C for 20 min and the lower layer of lipid (in solvent), ~ 500 µl, removed and transferred to a clean pre-weighed 2 ml glass vial using a glass Pasteur pipette avoiding collection of suspended particles. The solvent was evaporated until dryness with a gentle stream of charcoal scrubbed nitrogen and placed in a dessicator with for 15 ± 3 h to remove any remaining water. From the initial mass of sample taken and the final residue mass, the lipid content was calculated.

CHAPTER THREE

Development of a silicone rubber passive sampling device for PAHs and CBs

3.0 Introduction

POPs such as PAHs and CBs are persistent, poorly soluble in water and pose a risk to the environment due to their long life, toxicity and tendency to bioaccumulate (Keith and Telliard, 1979). The availability of organic pollutants in sediment has mostly been described or measured by the uptake or accumulation of such compounds by sediment dwelling organisms (Sijm *et al*., 2000; Simpson *et al*., 2006). Some studies (McElroy *et al*., 1990; Granberg and Selck, 2007) have addressed the ability of the organisms to metabolise these POPs. Total POP concentrations, usually determined following harsh and exhaustive extractions have been used in risk assessments of these contaminants and can lead to overestimating the available fraction (Alexander, 2000).

The free dissolved concentration in pore water (see section **1.7.2**) is an important parameter in understanding the availability of organic contaminants in sediments. However, due to the low concentrations of the free dissolved compounds and small sample volumes that can be available, determination of such free dissolved fraction is complicated. Large volumes of pore waters would need to be isolated to determine the concentrations (Booij *et al*., 2003) in sediment pore waters. Due to these complications and limitations associated with using biomonitoring organisms, such as their tendency to metabolise some organic contaminants (McElroy and Means, 1988), the potential for species-dependent availability (Reid, 2000a) and influence of organism physiology and sediment characteristics (Juhasz and Naidu, 2000; Ruus *et al*., 2005) on availability, surrogate methods such as passive sampling devices (Huckins *et al*., 2006) have been developed to mimic the accumulation of POPs by organisms by utilising polymer membranes or lipids enclosed in membranes to sequester the POPs.

ICES Working Group on Marine Sediments in Relation to Pollution (ICES WGMS, 2002) supported the development of passive sampling devices to measure free dissolved concentrations, which might later be introduced to complement monitoring programmes within member states. Consequently, FRS Marine Laboratory decided to develop a passive sampling device made of silicone rubber in conjunction with other members of ICES WGMS to estimate free dissolved concentration in sediment as well as other availability parameters such as the water exchangeable fraction and sediment-water partition coefficients (see section **1.8**). Silicone rubber has been shown to be a suitable material (polymer) for use as a passive sampler due to its low transport resistances and high partition coefficients (Rusina *et al*., 2007).

The uptake of the compounds into the silicone rubber from sediment is a function of exposure time, exposure surface and freely dissolved concentration (provided temperature and flow regime around the sampler are kept constant).

$$Q_{ref} = f(C_w \cdot A_{ref} \cdot t \cdot k) \qquad \textbf{3.1}$$

Where Q_{ref} is the amount in the reference phase, C_w is the concentration in the water phase, A_{ref} is the reference phase-surface area, 't'is the time and 'k' is the rate constant. The rate constant 'k' depends on the flow regime (e.g. induced by shaking or stirring), temperature, and suspended matter content. This is mainly applicable to low sediment depletion scenarios achieved through the use of excess sediment. In a reference phase (silicone rubber in this case) / water / sediment system (Fig **3.0**), compounds moving from the bulk sediment into the reference phase via the water phase have to pass though a boundary layer that separates the water phase from the sampler surface.

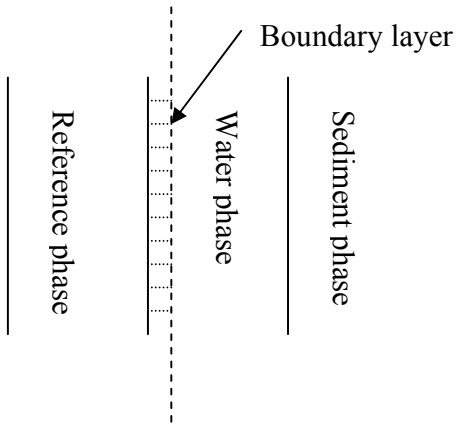


Fig **3.0**: Sediment-reference phase- water system

Diffusion through the aqueous boundary layer is considered important for uptake of the compounds (Booij *et al*., 1998; Huckins *et al*., 2006) and the thickness of the boundary layer, as well as physicochemical properties of the compounds, affects the rate at which compounds are absorbed by the silicone rubber. Shaking reduces the thickness of the layer and enhances interaction between the reference phase and particulates in the water phase. More particulates (high sediment content) in the test mixture tend to enhance the uptake rate as more particulates encroach into the boundary layer and the mean diffusion distance decreases. The increase in analyte concentrations in SPMDs during exposure has been shown by Huckins *et al*. (1993) to follow first order kinetics and this has been extended to silicone rubber samplers, although to avoid misinterpretation of uptake, specific sampling scenarios for the PAHs and CBs (analogous to Fig **1.4**) need to be determined and the equilibrium time deduced. The first order kinetic model, referred to as the chemical reaction kinetic model by Huckins *et al.* (2006), is considered highly empirical and other models are needed to more fully describe the uptake and release rate constants in terms of fundamental processes such as the mass transfer coefficient model which is essentially a mathematical description of solute-mass transfer through sequential but distinct physical phases.

This chapter aims to present the development of the silicone rubber sampling device as a monitor of organic contaminants in the environment, with a specific emphasis on its suitability to measure free dissolved concentrations in sediment pore waters. Initial experiments were carried out as detailed in the following sections:

3.1 Materials

3.2. Pre-extraction of silicone rubber sheets to remove potentially interfering compounds

3.3. Investigation of the partitioning or sorption of known amounts of POPs (PAHs at the start of the study) into silicone rubber sheets using a spiking method described in Booij *et al*., (2002) and calculation of the reproducibility of the spiking procedure. The preferred solvent to use in extracting the PAHs from the silicone rubber sheets was also determined.

3.4. Having established the sorption capability of the silicone rubber and the efficacy of the extraction solvent, attempts were made to optimise the loading procedure of the PAHs into the silicone rubber by comparing sonication and shaking of sheets in jars containing methanol-water mixtures spiked with test PAHs.

3.5. Further experiments were carried out to determine the storage time of sheets (which could be spiked in batches of several sheets) in the freezer at - 18 ^{o}C before they are used, specifically the compounds to be used as performance reference compounds (PRCs). At this point, PRCs for the CBs were obtained and introduced.

3.6. Pilot experiments were then carried out using silicone rubber sheets that had been loaded with PRCs which were then exposed to sediment collected from the Firth of Forth at varying time interval to determine the sampling region, i.e. if kinetic or equilibrium sampling was occurring (Huckins *et al*., 1993), and reproducibility of uptake from sediment. Consequently, the time to attain equilibrium by the POP between the silicone rubber and the pore water was determined. The effect of sediment content (suspension density) on uptake was also investigated. Experiments at this point were carried out in a light and temperature controlled room at 20 ^{o}C to reduce any effect of temperature variation on partition coefficients, and photodegradation of compounds of interest such as PAHs.

3.7. Finally, optimised conditions for use of the silicone rubber sampling device are presented which were applied to sediments in subsequent chapters.

3.1 Materials

HPLC grade solvents (dichloromethane, ethyl acetate, acetone, methanol and *iso*-hexane) were purchased from Rathburn Chemicals Ltd, Scotland, UK. Certified solid standards for PAHs (including deuterated PAHs) and CBs were obtained from QMX Laboratories, Essex, UK and dissolved in *iso*-hexane (PAHs) and *iso*-octane (CBs) to obtain required concentrations of spiking solutions. AlteSil™ Silicone rubber sheet manufactured from translucent, food grade silicone rubber, with a hardness of 60 Shore A, (600 × 600 mm, 0.5 mm thick) was purchased from Altec Products, Ltd, Cornwall, UK. To avoid contamination of samples, all glassware, stainless steel forceps, was either washed in Decon® 180 solution and rinsed with distilled water or washed in a CAMLAB GW 4050 glassware washer and dried in an oven at 100 ^{o}C. Before use, the glassware were rinsed twice each in dichloromethane and *iso*-hexane, with the latter allowed to evaporate to dryness. Glass Duran® or Schott® (VWR, Leicester, UK) bottles were used and always capped with aluminium-lined PTFE caps to reduce or prevent sorption of POPs by the caps.

3.2 Pre-extraction of silicone rubber sheets

The silicone rubber sheets used were cut into required dimensions (6 × 4 cm, or 4 × 4 cm). Gruber *et al*. (2000) has shown that Soxhlet extraction of silicone elastomers for 72 h has been shown to remove any residues from the curing process of such elastomers such as silicone oils and uncured oligomers. The silicone rubber sheets were therefore pre-extracted in hot ethyl acetate using a Soxhlet apparatus for ~ 4 days before use to remove any low molecular weight oligomers or residues that may be co-extracted with the analytes and could affect instrumental analysis. After pre-extraction and cooling, the sheets were removed from the solvent and stored in bulk in glass jars containing methanol prior to use. The weight of the sheets used was determined after extraction of exposed sheets to avoid contamination from the environment. During pre-extraction/ extraction, the silicone rubber sheets were observed to elongate or swell and subsequently shrink back after they have been removed from the hot solvent.

3.3 Loading of test compounds into silicone rubber

For application in passive sampling the silicone rubber sheets would need to be able to absorb the POPs.

3.3.1 Initial loading experiment

To test the applicability, homogeneity and magnitude of the uptake by the silicone sheets, sheets were loaded with between 102 and 137 ng of naphthalene, dibenzothiophene, pyrene, chrysene and indeno[1,2,3-*c,d*]pyrene using a spiking method described in Booij *et al.* (2002). Briefly, methanol in an amber glass jar was spiked with known concentrations of the PAHs listed above and silicone rubber sheets were added. The glass jar was shaken for 2 h on an orbital shaker at 200 rpm followed by addition of water to obtain 80 % methanol solution and further shaken for 6 h with a subsequent addition of water to obtain 50 % methanol solution and followed by a final shaking overnight at room temperature. After the loading the silicone rubber sheets were removed, dabbed with tissue to dry any water on the surface, and extracted as in **3.3.1.1**. The water-methanol solution was also extracted as in **3.3.1.2** below.

3.3.1.1 Extraction of silicone rubber

The silicon rubber sheets were Soxhlet extracted using 100 ± 5 ml of *iso*-hexane: acetone (3:1 $^{v}/_{v}$) mixture or methanol for 6 h and 200 ± 10 µl of aliphatic standard (containing heptamethylnonane and squalane) and 100 ± 10 µl deuterated PAH aromatic internal standard containing D_8-naphthalene, D_{10}-biphenyl, D_8-dibenzothiophene, D_{10}-anthracene, D_{10}-pyrene and D_{12}-benzo[*a*]pyrene were then added to the sample, followed by concentration by rotary evaporation followed by nitrogen blow down of the extract to 1 ml. The extract was then split into 2 fractions by weight for PAHs and CBs (in cases where CBs were included or of interest), otherwise the extract was reduced to 0.5 ± 0.2 ml if PAHs only are being analysed. For PAHs an aliquot (150 ± 10 µl) of the concentrated extract was fractionated using an isocratic, normal phase Genesis metal-free high performance liquid chromatograph (HPLC) column (25 cm x 4.6 mm) and eluted with *iso*-hexane at a flow rate of 2 ± 0.1 ml/min into

aliphatic and aromatic fractions. The aliphatic fraction was discarded and the aromatic fraction was collected in 100 ml flasks, concentrated by rotary evaporation and further reduced under nitrogen to 50 ± 10 µl for Gas Chromatography – Mass Selective Detection (GC-MSD) analysis (see **2.3.3.1.3**).

The CB fraction was passed through an alumina column and eluted with hexane to separate the extracts into CB and organochlorinated pesticide (OCP) fractions. The CB fraction was further separated into 2 fractions by means of a silica column, collecting only the first fraction that contains only CBs using hexane whilst allowing the remaining solvent to go to waste. The eluate was evaporated to 0.5 ± 0.1 ml, made up in *iso*-octane and 500 ± 50 µl 2, 4-dichlorobenzyl alkyl ethers (DCBE 6 and DCBE 16) internal standard added and reduced using a Turbovap system to 0.5 ± 0.1 ml. This was finally vialled for Gas Chromatography-Electron Capture Detection (GC-ECD) analysis (see **2.3.3.2.2**).

3.3.1.2 Extraction of PAHs and CBs from methanol: water mixture

Deuterated PAH internal standards (100 µl; approximately 1 µg ml^{-1} each) D_8-naphthalene, D_{10}-biphenyl, D_{10}-anthracene, D_8-dibenzothiophene, D_{10}-pyrene, D_{12}-benzo[*a*]pyrene) were added to the methanol-water mixtures from the silicone rubber loading experiments (**3.3**) and these were liquid-liquid extracted twice in separating funnels using dichloromethane and dried over anhydrous sodium sulphate. The extracts were then exchanged into *iso*-hexane, concentrated by rotary evaporation followed by nitrogen blow down to 1 ml and split into 2 parts (PAHs and CBs). The PAH fraction was cleaned up and isolated by isocratic, normal phase HPLC while the CB fraction was cleaned up and isolated using alumina and silica columns (followed by addition of DCBE internal standard) and both fractions concentrated prior to chromatographic analysis, as above.

The recoveries per silicone rubber sheet (Table **3.0**, Appendix **2**) are calculated from the sum of the amounts remaining in the methanol-water mixture and in the silicone rubber relative to the initial nominal amount spiked into the experimental system. The mean percent recovery was found to be between 57 and 107 % when using the *iso*-hexane: acetone mixture as

extraction solvent while it ranged from 51-112 % when methanol was used. The percent coefficient of variation (% CV) was < 7 % in both cases except for the more volatile naphthalene which was 26 and 12 % for *iso*-hexane: acetone and methanol solvents respectively. An F-test (95 % confidence level; e.g. pyrene; $F_{calc} = 1.38$ and $F_{critical} = 19$) showed no significant difference between the variances of the extraction using *iso*-hexane and methanol except for indeno[1, 2, 3 - *c, d*]pyrene ($F_{calc} = 0.07$ and $F_{critical} = 0.05$) and subsequently a *t*-test (95 % confidence level: e.g. pyrene; $t_{calc} = 1.96$ and $t_{critical} = 2.77$) also showed no significant differences between the recovery means. The silicone rubber absorbed between 45-88 % of the spiked PAHs per sheet with between 28-42 % left in the methanol-water solution. However, due to the time taken to rotary evaporate the methanol extract and the need to exchange that into *iso*-hexane, losses of the more volatile components may occur, and therefore it was concluded that the *iso*-hexane: acetone mixture offered a better choice and was chosen as the extraction solvent.

3.3.2 Further loading test

Preliminary loading experiments (**3.3.1**) showed the silicone rubber absorbs PAHs. The loading was repeated as before (**3.3.1**) but with more PAHs in the spiking solution (Table **3.1**, Appendix **2**), while extraction was carried out using the *iso*-hexane: acetone mixture only.

Mean percent recoveries calculated ranged from 50-101 % for the individual compounds (with only 5 out of the 32 PAHs showing recoveries < 70 %; Table **3.1**, Appendix **2**) with < 5 % CV in most PAHs except acenaphthylene where it was 18 %. The results showed the silicone rubber absorbed between 50-95 % of the spiked individual PAHs with a median value of 81 % and mean value of 80 % across PAHs. The % CV between amounts in duplicate sheets was calculated to be < 5 % for the PAHs studied (Table **3.1**, Appendix **2**). Lower recoveries were found mostly with the high K_{ow} compounds like benzo[*a*]pyrene ($\log K_{ow} = 6.35$, mean % recovery = 59 %). Diffusion coefficients have been shown to be inversely related to increasing molecular weight (K_{ow}) in non-porous polymers (Huckins *et al*., 1990; Williamson *et al*., 2002; Rusina *et al*., 2007). Longer equilibration times may thus be required for such compounds to be absorbed more into the silicone rubber sheets. High K_{ow} compounds (PAHs

and CBs) have low solubility in water and high affinity for glass surfaces making their experimental determination difficult thus leading to lower recoveries.
As with other synthetic polymers, silicone polymers appear to have cavities or pores and have large free volumes formed by thermal motion of polymer chains in rubbery regions of the polymer matrix (Huckins *et al*., 1990; Jiang and Kumar, 2005; Zhao *et al*., 2006; Huckins *et al*., 2006) which allow for transport or absorption of only dissolved molecules (< 600 Da, molecular size exclusion) which can fit into or through the pores. High partition coefficients (Rusina *et al*., 2007; chapter **4**) of silicone rubber make partitioning of PAHs into the silicone rubber quite easy and possible.

The results obtained indicate that silicone rubber can absorb, and have affinity for PAHs with very good reproducibility in terms of amounts absorbed by the sheets and can subsequently be used in the sampling of PAHs.

3.4 Optimisation of PAH loading

In an attempt to reduce the time required for spiking of compounds (PAHs of interest, Table **3.2**, Appendix **2**) and yet maintaining the reproducibility and accuracy of spiking, the above spiking procedure (**3.3**) was modified slightly, where the shaking was replaced with sonication for 2, 4, 5 and 7.5 h to determine the optimum time for leaving the jar in the sonic bath based on high (~ 100 %) loading percent recovery calculated and shorter exposure times. The times for addition of water were varied by taking into account the total exposure time. The water in the sonic bath was renewed intermittently to prevent overheating of the bath. After the exposures, the silicone rubber sheets and methanol- water solutions were extracted as described in **3.3.1** and **3.3.2** respectively above.

The mean recovery for individual PAHs per sheet was calculated as 88 ± 11, 87 ± 10, 77 ± 14, 71 ± 11 and 84 ± 13 % for sonication for 2, 4, 5, 7.5 h and shaking for 24 h respectively of the spiked amount for the different compounds (Tables **3.3** and **3.4**, Appendix **2**). The less than 100 % recovery in some cases could be due to losses from evaporation. ANOVA single factor ($p \geq 0.05$) showed that there is a significant difference between the mean percent recoveries of

the different sonication times in almost all cases, and subsequently calculating the least significant difference (LSD) showed that for most of the compounds there was no significant difference between the sonication for 2 h and 4 h. Consequently the 2 h method could be chosen due to time consideration. Comparing results of sonication for 2 h with the shaking for ~ 24 h showed no significant differences in mean percent recovery between the two methods ($p \leq 0.05$) for 18 PAHs, therefore the 2 h-sonication method was initially chosen. For all other loading of the PRCs- D_{12}-chrysene and D_{12}- benzo[*e*]pyrene for PAHs and later CB 35, 53, 112, 198 and 209 for the CBs, a similar procedure using ultrasonic bath for 2 h was initially adopted using known amounts of PRCs. The spiking or loading procedure appears to be homogeneous and reproducible within and between batches. Reproducibility of the spiking method was always better than 5 % with some few cases where it was ≤ 22 %.

3.5 Storage Experiment

Typically to be able to use several sheets for specific exposures, the silicone rubber sheets would need to be spiked in batches, and left in the spiking solution until required for use. Therefore, to determine the time to store spiked sheets to avoid loss of the spiked POPs, silicone rubber sheets were spiked with ~ 350 ng (CBs) and ~ 550 ng (PAHs) of each performance reference compounds per sheet of silicone rubber using the Booij *et al*. (2002) method (both by sonication for 2 h and shaking for ~ 24 h while varying the methanol percentage), stored at -20 ± 2 ^{o}C and sampled after 0, 8, 14, 21, and 30 days. The sheets were then extracted as in **3.3.1** to check the reproducibility after storage as well as potential storage time of spiked sheets before use.

The silicone rubber sheets absorbed > 75 % of the spiked PRCs irrespective of the method of spiking and only the amounts absorbed by the sheets were determined as this can be used in the calculation of the sampling rates and in modelling the exchange of the PRCs in the sediment. Lower variation between days was observed when spiking was by shaking (< 9 %) as against spiking by sonication (< 22 %) shown in Table **3.5**, Appendix **2**.

The percent coefficient of variation in mean PRC uptake per sheet was found to be higher after storage period when the spiking was by sonication (30, 35, 12, 40 and 7 % on 0, 8, 14, 21 and 30 days respectively) than when it was by shaking (15, 3, 3, 2 and 4 % on day 0, 8, 14, 21 and 30 respectively). Figures **3.1-3.4** show the amounts absorbed in the silicone rubber sheets over the study period.

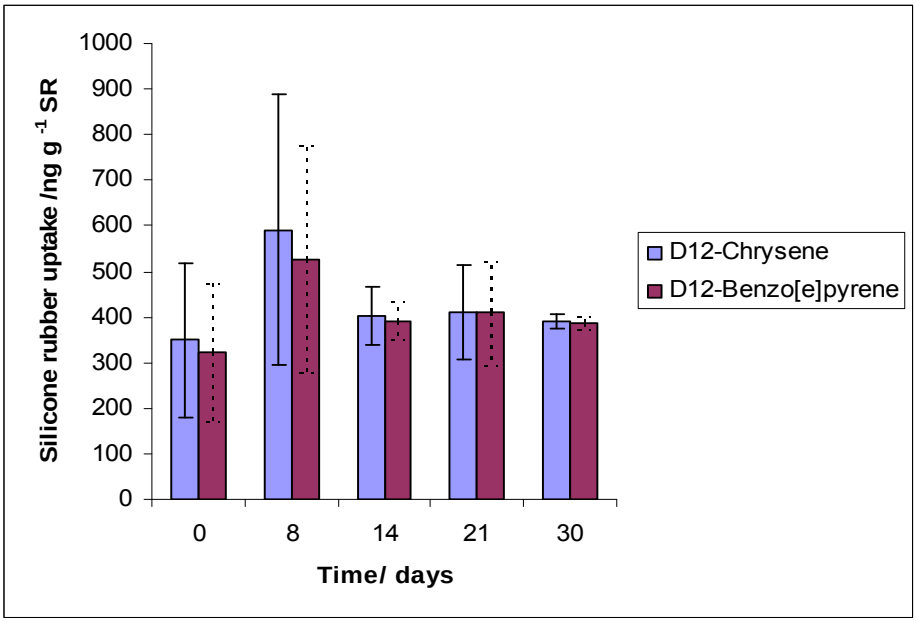


Fig **3.1**: Mean amount of D_{12}-chrysene and D_{12}-benzo[*e*]pyrene in silicone rubber sheets spiked by sonication in glass jars for ~ 2 h and stored for 0, 8, 14, 21 and 30 days. Error bars are standard deviations of triplicate measurements. Error bars are standard deviations of triplicate measurements

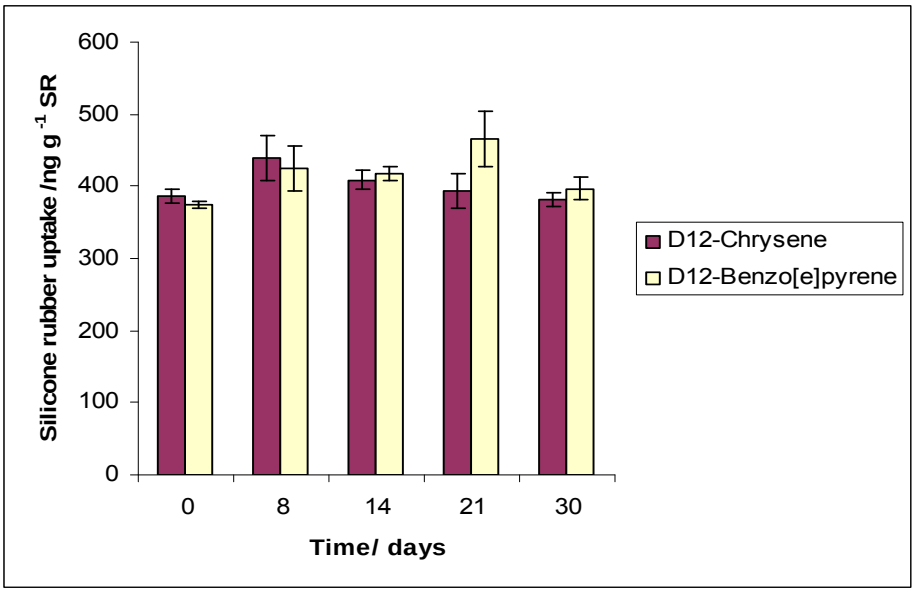


Fig **3.2**: Mean amount of D_{12}-chrysene and D_{12}-benzo[*e*]pyrene in silicone rubber sheets spiked by shaking in glass jars for ~ 24 h and stored for 0, 8, 14, 21 and 30 days. Error bars are standard deviations of triplicate measurements. Error bars are standard deviations of triplicate measurements

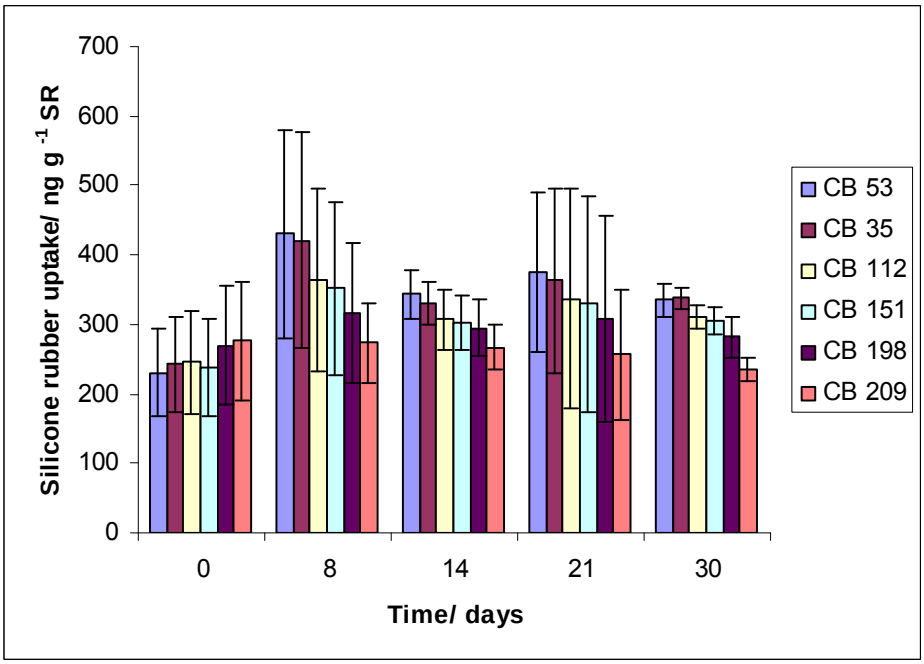


Fig **3.3**: Mean amount of CB PRCs in silicone rubber sheets spiked by sonication in jars for 2 h and stored for 0, 8, 14, 21 and 30 days. Error bars are standard deviations of triplicate measurements

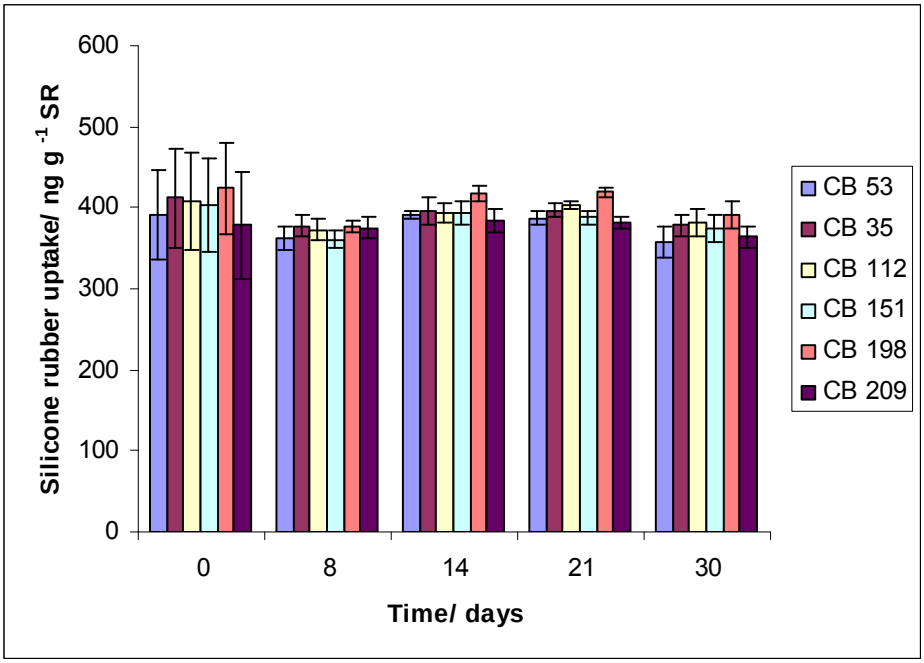


Fig **3.4**: Mean amount of CB PRCs in silicone rubber sheets spiked by shaking in jars for ~ 24 h and stored for 0, 8, 14, 21 and 30 days. Error bars are standard deviations of triplicate measurements

Spiking using the shaker appears to be preferable due to the wider variation observed with sonication. Field application of sheets requires that the amounts of PRCs initially present in the sheets are accurately known. The high variation observed when spiking by sonication is probably due to uneven distribution of ultrasonic energy across the sheets which lie on each other (even though diffusion through the sheets is expected) in the jar, as against the shaking which ensures the sheets move around within the jar. ANOVA ($p \leq 0.05$) showed there was no significant difference in the mean amounts absorbed between the spiking-method and also on the days, implying that the sheets can be stored in the methanol-water solution for the 30 days studied. However, due to overheating of the glass jar during sonication it was necessary to revert to spiking by shaking. Sonication does not offer much advantage except in terms of reduced time but the shaking offsets that by the improved reproducibility over the storage period.

3.6 Uptake of PAHs and CBs by silicone rubber from sediment

In order to minimise errors in the estimations of the pore water concentrations in sediment samples, it is essential to determine the region of uptake (see Fig **1.4**) by the silicone rubber, i.e., if equilibrium sampling is the case and if indeed, the silicone rubber does absorb the PAHs and CBs of interest from an environmental matrix. The sections below describe the determination of the region and reproducibility of the uptake by and analysis of the silicone rubber sheets from sediments, as well as the equilibrium time.

3.6.1 Equilibration time and effect of sediment content

The proposed silicone rubber passive sampler is hypothesised to operate as an equilibrium sampler (Mayer *et al*., 2003; Rusina *et al*., 2007). To determine the time needed to attain equilibrium, and the influence of sediment content on equilibrium time and on the uptake of the compounds by the silicone rubber, 100 g of Firth of Forth sediment (moisture content of 65 %) was weighed into 200, 250, 500 and 1000 ml bottles, and 30, 117, 334 and 550 g of distilled water added to provide a range of suspension densities/ sediment contents of 0.27, 0.16, 0.08 and 0.05 g g^{-1} respectively. Sediment content (sometimes referred to as suspension density, generally a measure of how dilute sediment slurry is) is given as the ratio of dry weight of sediment to the sum of wet sediment weight and water added. Similarly, the time required for the analytes from the sediment to attain equilibrium between the silicone rubber and the aqueous phase is referred to as the equilibration time.

A silicone rubber sheet that had been loaded with PRCs: D_{12}-chrysene and D_{12}-benzo[*e*]pyrene (550 ± 50 ng each) for PAHs and CB 35, CB 53, CB 112, CB 198 and CB 209 (400 ± 50 ng each) for the CBs was then added to each bottle and placed on a shaker at 250 rpm for different periods (1, 3, 9, 14 and 27 days). Only singlet measurements were carried out and a procedural blank was included containing only 117 g of sea water and an un-spiked silicone rubber sheet. After each period, the silicone rubber sheet was retrieved from the bottle and rinsed with distilled water to remove any adhering sediment particles and wiped dry with

clean tissue before being extracted as described in **3.3.1.1**. The sediment in the bottle was discarded.

Uptake profiles were therefore generated for the different sediment contents (Fig **3.5a** & **3.5b** show examples for pyrene and CB110). These uptake curves are necessary for the determination of the time required for an equilibrium extraction, with most of the compounds appearing to plateau after 9-14 days. The larger compounds have higher partition coefficients and thus the uptake rate of such compounds is expected to be lower, probably due to slower diffusion in both the water and the silicone rubber membrane.

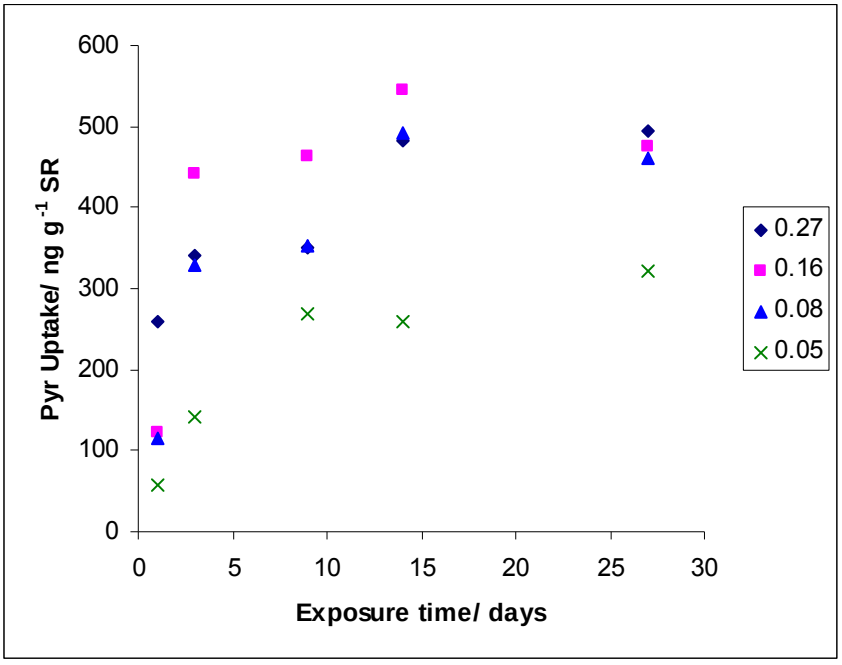


Fig **3.5a**: Pyrene uptake at varying sediment contents

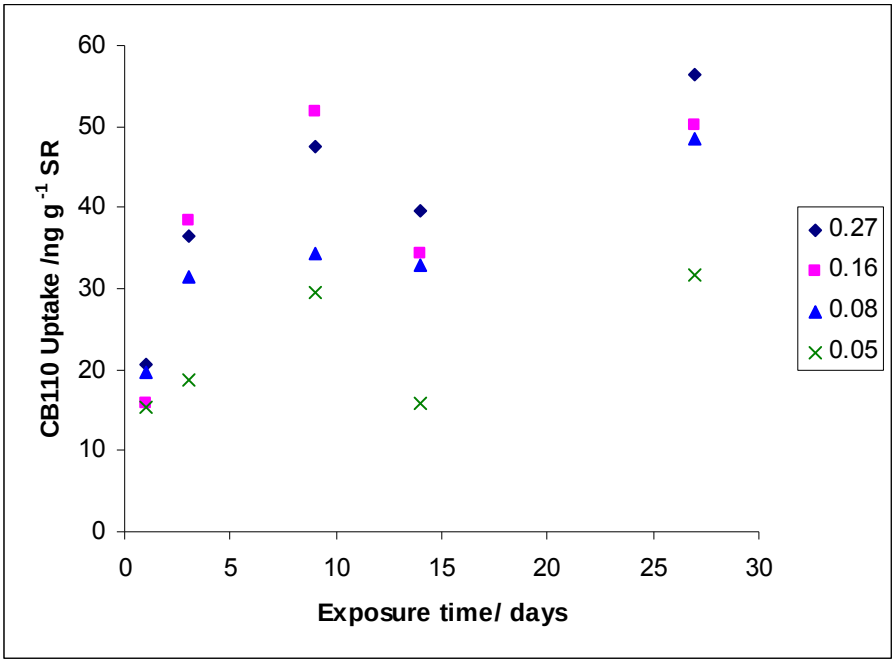


Fig **3.5b**: CB 110 uptake at different sediment contents

The typical uptake profiles generated (see Fig **3.5a** and **3.5b** as examples) showed an initial rapid uptake on day 1 followed by a linear increase until day 9 when it begins to curve before reaching equilibrium amounts which is similar with the uptake profiles described by Mayer *et al*. (2003).

The data obtained from the exposure were fitted by equation **3.2** (Booij *et al*., 1998) which is essentially the mass transfer coefficient model described by Huckins *et al.* (2006) and plots as Fig **3.6 a-c** obtained.

$$N(t) = N^0 + \left(N^\infty - N^0\right)\cdot\left[1 - \exp(-k_e t)\right] \qquad \textbf{3.2}$$

Where, $t =$ time, $N(t)$ is the amount at time 't', $N^o =$ amount at $t = 0$, $N^\infty =$ amount at equilibrium, and $k_e =$ overall exchange rate constant (preferable to use of uptake rate constant because the uptake and release processes are characterised by the same rate constant). The fitting to the equation above was to enable the calculation of the exchange rate constant, k_e.

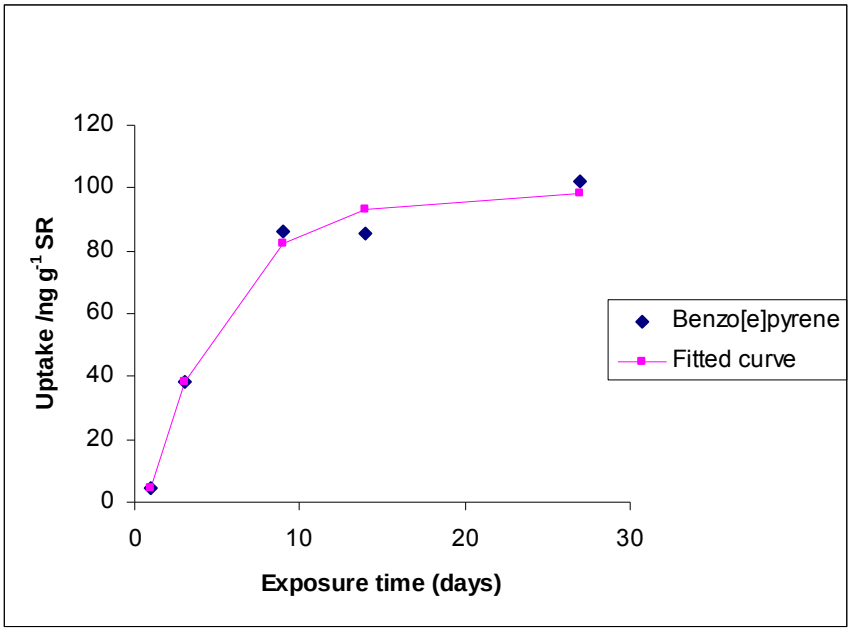


Fig **3.6a**: Benzo[*e*]pyrene uptake data at sediment content "0.16 g g^{-1}"fitted to equation 3.2 using Solver, in Excel, k_e calculated = 0.22 d^{-1}

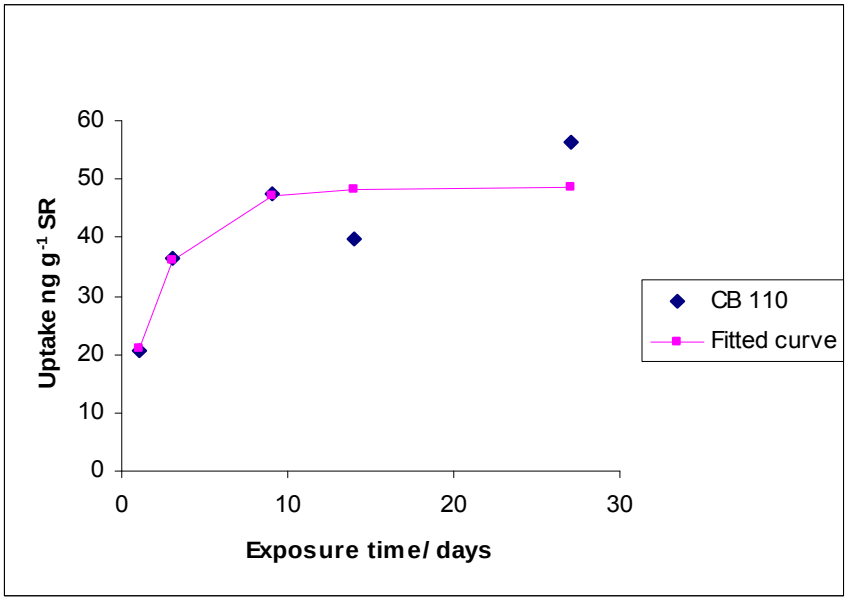


Fig **3.6b**: CB110 uptake data at sediment content "0.16 g g^{-1}"fitted to equation **3.2** using Solver, in Excel, k_e calculated = 0.749 d^{-1}

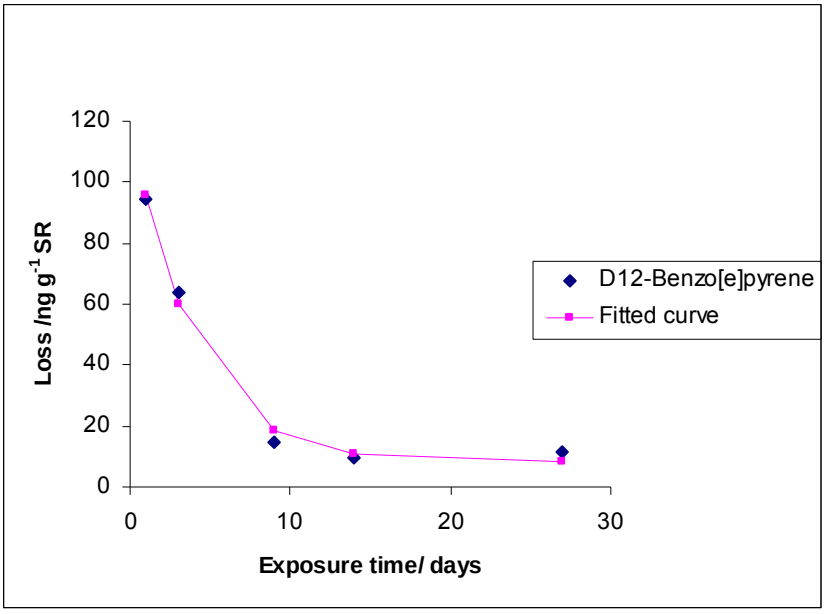


Fig **3.6c**: D_{12}-Benzo[*e*]pyrene loss data at sediment content "0.16 g g^{-1}" fitted to equation **3.2** using Solver, in Excel, k_e calculated = 0.27 d^{-1}

The exchange rate constant can then be used to calculate the sampling rate (R_s) especially for the more hydrophobic compounds, for which equilibrium may not be attained within the sampling or exposure time. Sampling rates are given as volume of medium cleared of chemical, per unit time, or L d^{-1} (Petty *et al*., 2000) and are mostly useful in water sampling. Similarly, the uptake rate constant controls the time required to attain equilibrium, with compounds having higher k_e values tending to reach apparent asymptotes at steady state sampler concentrations in shorter times (Richardson *et al.*, 2005).

The time required for a compound to attain 90 % of the steady state equilibrium concentration ($t_{90\%}$) can be calculated from equation **3.3**

$$t_{90\%} = \ln 10 / k_e \qquad \textbf{3.3}$$

This was calculated for the PAHs and CBs (Table **3.6**, Appendix **2**) studied and ranged from 0.6 (acenaphthylene) to 48 (naphthalene) days. Most of the PAH compounds had $t_{90\%}$ less than 10 days with a median at ~ 7 days, and 0.5 - 70 days, median at ~ 14 days for the CBs

with most attaining $t_{90\%}$ within 20 days. However, the deviation from the expected pattern of equilibrium time increasing with increasing $\log K_{ow}$ was found for the naphthalenes, which showed longer than expected equilibration times. This may have been due to the volatility of the compounds and attendant losses during the analytical process. Variable behaviour of naphthalenes is a general feature of several experiments, and the problem could perhaps be addressed by including another PRC, e.g. D_8 naphthalene or D_{10} acenaphthene, and compare the behaviour of the native naphthalenes with that of the PRC. However, this was not possible in this instance, as D_8 naphthalene is being used in FRS ML as an analytical internal standard and any further addition of this compound would complicate quantification through interference with the internal standard peak. The CBs had similar $t_{90\%}$ to the PAHs (Table **3.6**, Appendix **2**) although there were a couple of CBs that showed very long equilibration times (e.g. 69 days for CB 138) which could have been due to the low CB levels in the Firth of Forth sediment used (22.12 ng g^{-1} dry weight total CB content) leading to high uncertainty in calculations at concentrations close to detection limits. It was thus concluded that an exposure time of 20 days is sufficient to attain equilibrium and this was subsequently used in further studies.

The $t'_{90\%}s$ (Table **3**.**7**, Appendix **2**) calculated for the 4 different sediment contents, suggest that the 20 days adopted for the exposure of silicone rubber to sediments for compounds to attain equilibrium is sufficient with a few exceptions where the times were longer. The sediment content appears to strongly influence the uptake of the compounds by the silicone rubber, with higher sediment content favouring higher uptake up to 0.16 g g^{-1}. The observed decrease in uptake at 0.27 g g^{-1} could be as a result of the dense mixture not allowing for adequate mixing/ interactions during shaking and may require more vigorous shaking to allow for better contact between the silicone rubber and the water phase. Shorter times for the PAHs to attain 90 % steady state concentrations were observed with increasing sediment content from 0.08 to 0.16 g g^{-1} as more particulates are available for contact with the silicone rubber sheets at the exchange interface with increased sediment content, although the times then increased at 0.27 g g^{-1} possibly due to poor mixing leading to uncertainty in the times at 0.27 g g^{-1} sediment content. Since the 0.16 g g^{-1} sediment content gave consistent shorter times across

the PAHs, it may be necessary to liquefy some sediment samples by the addition of "local" or distilled water for effective exchange and a final sediment content of 0.16 g g^{-1} would be ideal.

A linear increase of k_e was observed (Fig **3.7a & b**) from sediment content of 0.05 to 0.16 g g^{-1} but then decreased at 0.27 g g^{-1} with very poor correlation coefficients (example chrysene with $r^2 = 0.003$). However if the last point (0.27 g g^{-1}) is removed, r^2 improves to 1.

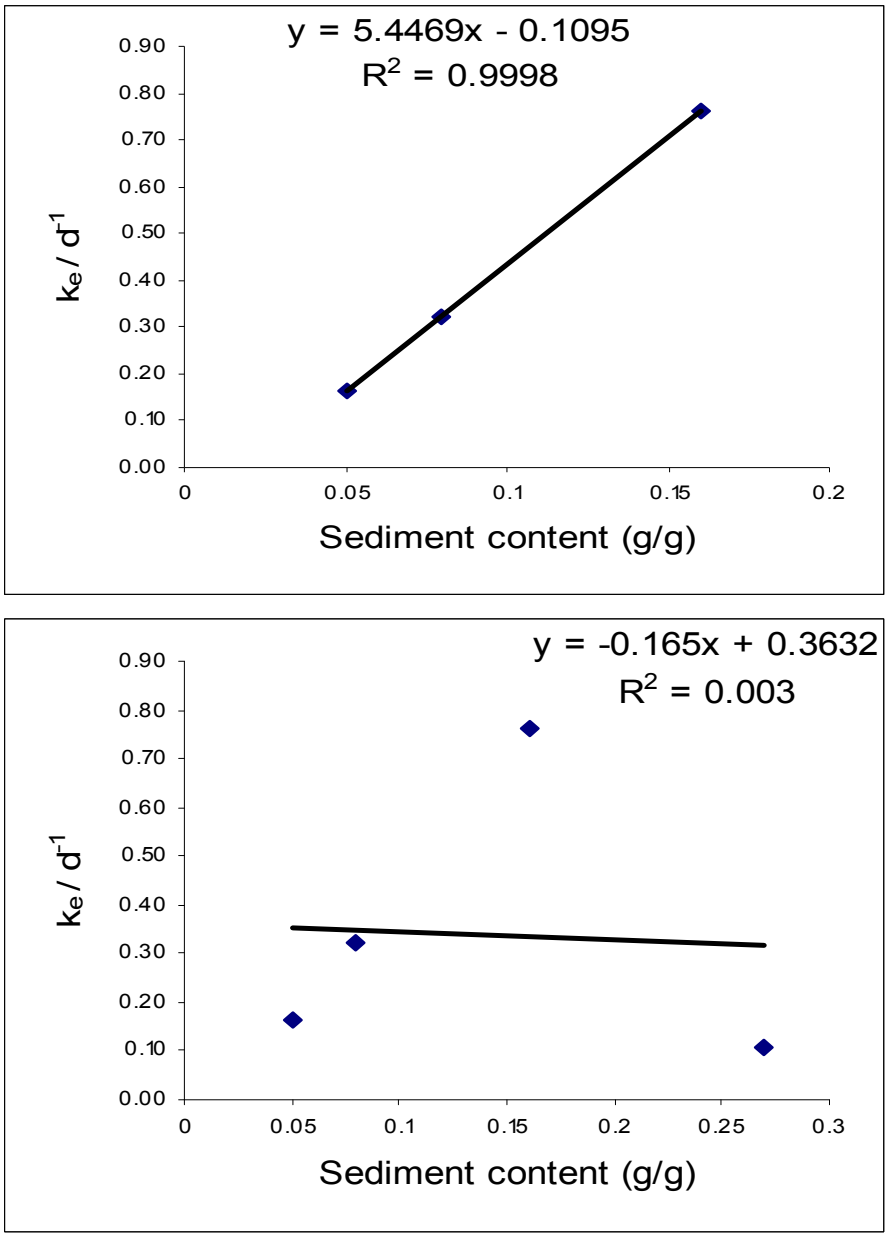


Fig **3.7a** & **b**: Plots of k_e calculated for chrysene with varying sediment content

The plot of log $k_e's$ calculated for PAHs with corresponding log K_{ow} (Fig **3.8**) showed a decrease in the exchange rate coefficients with increasing K_{ow} similar to the trend shown by Booij *et al*. (2003) and Tixier *et al*. (2007). The PAH $k_e's$ from dissipation of PRCs were similar to those of their analogues (e.g. D_{12}-chrysene $k_e = 0.721$ d^{-1} and chrysene $k_e = 0.763$ d^{-1}).

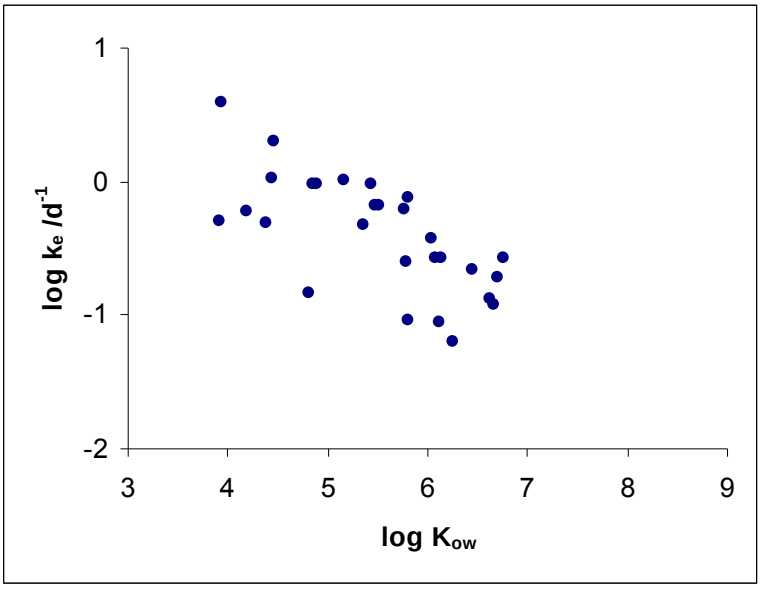


Fig **3.8**: Exchange rate coefficients ($\log k_e$) of the PAHs studied, estimated from equation **3.2** as a function of $\log K_{ow}$ for silicone rubber exposed to Firth of Forth sediment. Due to the uncertainty in the CB k_e estimates, these are not presented

Fig **3.9** show the loss or dissipation profiles for the performance reference compounds spiked into the silicone rubber sheets before exposure to the sediment slurry, which reflect the uptake of the compounds. Both PRCs appear to have reached equilibrium after 14 days at which point only about 5 % of D_{12}-chrysene and 13 % of D_{12}-benzo[*e*]pyrene is left in the silicone rubber. Fig **3.10** an analogous plot to Fig **1.3** (see chapter one, **1.7.1**), shows the uptake of benzo[*e*]pyrene and the loss of its deuterated analogue.

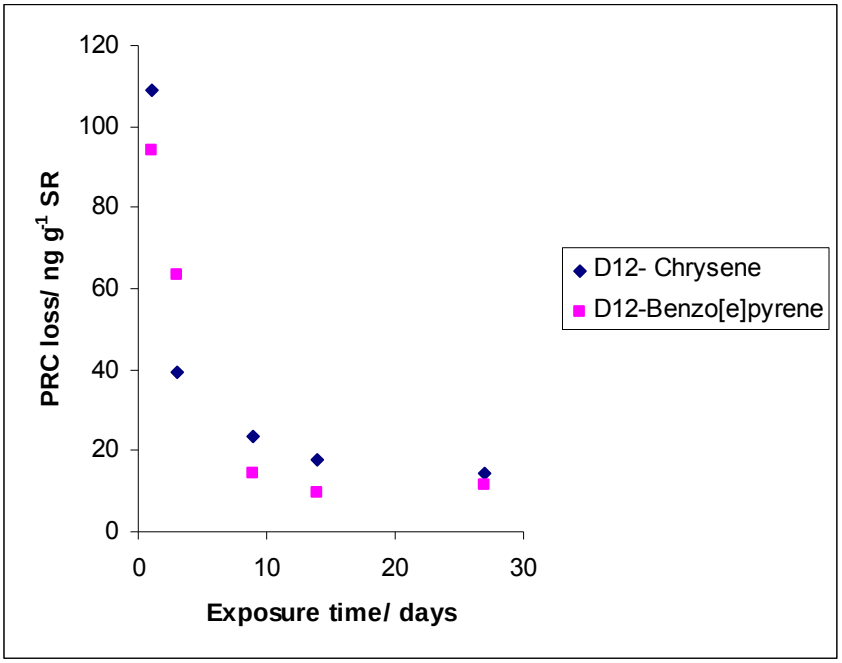


Fig **3.9**: PRC dissipation /loss profiles for PAHs, sediment content = 0.16 g g^{-1}

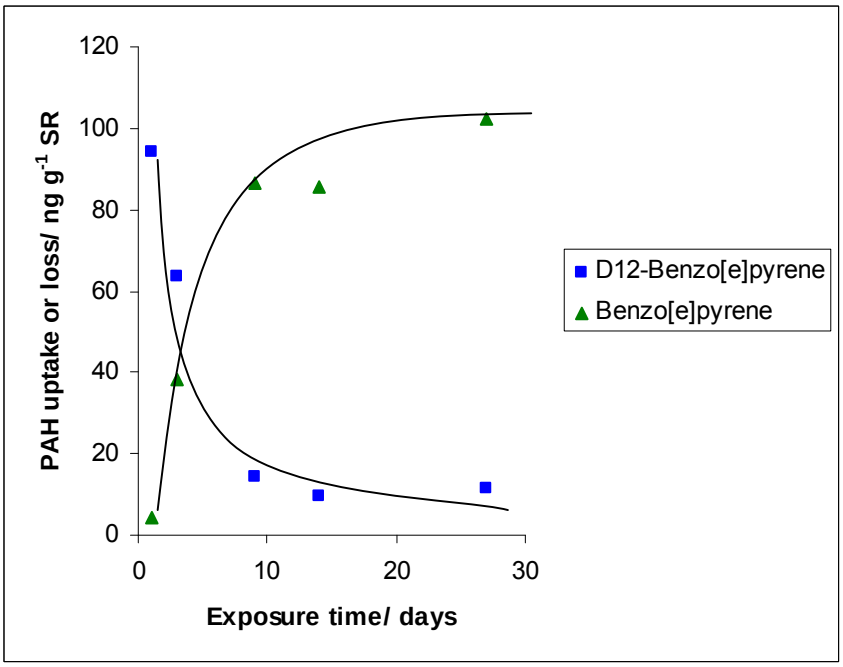


Fig **3.10**: PRC (D_{12}-benzo[*e*]pyrene) loss mirroring PAH (benzo[*e*]pyrene) uptake

3.6.2 Optimisation of equilibrium time

In an attempt to reduce the equilibration time by pre-treating the sediment in the bottle by sonication or shaking before adding the silicone rubber sheets (to release more compounds into the water phase for more rapid uptake and possibly reduce the time to reach equilibrium), fresh 250 ml bottles containing 100 g of Firth of Forth sediment slurry at a sediment content of 0.16 g g^{-1} were pre-treated by sonication for 6 min, shaking for 3 h and shaking for 2 days before introducing PRC-spiked silicone rubber sheets. The bottles were sampled after 1, 4, 10 and 20 days in triplicates and the silicone rubber sheets extracted.

The result showed poorer reproducibility (high % CVs) for the CBs than for the PAHs (Tables **3.8 & 3.9**, Appendix **2**) which were probably due to the low concentrations of CBs in the Firth of Forth sediment being close to detection limits. The three different pre-treatment methods used showed good precision (<10 % in almost all PAHs), especially the shaking for 2 days and 3 h before exposure of the silicone rubber to the sediment compared to sonication for 6 min (< 35 % in most cases) particularly after 20 days.

The data obtained from the study using three different pre-treatment methods were fitted to equation **3.2**. The exchange coefficients, and subsequently the time required for a compound to attain 90 % steady state concentration ($t_{90\%}$), were calculated for the different treatments. From the exchange rate constants calculated, the shaking for 3 h and 2 days showed $t_{90\%}$ of generally between 4 – 60 days for the PAHs with only indeno[1,2,3-*c,d*]pyrene, benzo[*ghi*] perylene and dibenz[*a,h*]anthracene giving impractical $t_{90\%}$ of 19029, 177 and 83427 days respectively when shaking for 3 h. The sonication yielded $t_{90\%}$ ranging from 2 – 30 days with about 12 other compounds within 230< $t_{90\%}$ > 8000 days. For the CBs, the results are quite variable as some compounds were detected in some treatments and not in others, possibly due to the low individual CB concentrations (between 0.2 – 1.9 ng g^{-1}) found in the Firth of Forth sediment.

For the CBs, between 20 to >100 % (Table **3.10**, Appendix **2**) depletion of the sediment amounts in terms of uptake by the silicone rubber sheets after 20 days was observed compared to the < 5 %

calculated for the PAHs (Table **3.11**, Appendix **2**), and this probably increased the uncertainty in the CB results.

Generally, higher uptake in terms of amount in silicone rubber per gram of sediment (dry weight) used was observed using the sonication pre-treatment while the shaking (both 2 days and 3 h) showed similar amounts (Fig **3.11**). An F-test on the day 1 uptake using the different methods showed no significant differences in the variances between shaking for 2 days and shaking for 3 h in most of the PAH compounds analysed. Subsequently a t-test showed no significant difference in the means of the two pre-treatment methods for the day 1 uptake data. Consequently due to the time advantage involved and low % CV, shaking for 3 h seems a better option. However, considering that the point of interest in the silicone rubber method is attainment of equilibrium, the day 1 uptake may not be required.

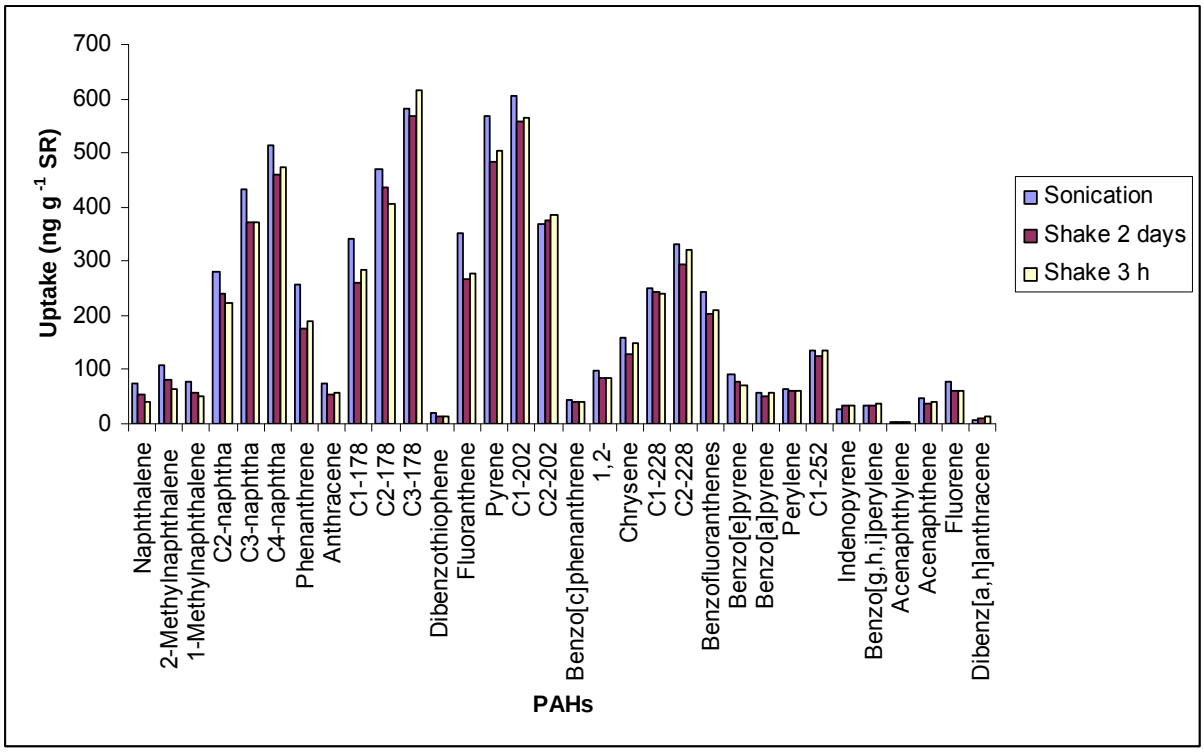


Fig **3.11**: Uptake after 20 days using different pre-treatments before exposure to silicone rubber sheets

3.7 Method development conclusion

Generally, the summary of the method is presented below (see also Fig **3.12**). A draft standard operating procedure is attached in Appendix **3** for the exposure of silicone rubber sheets to sediment.

Silicone rubber sheets (0.5 mm thickness; 6 × 4 or 4 × 4 cm sizes) are pre-extracted in a Soxhlet apparatus for 4 days to remove any residues that may interfere with instrumental analysis before use.

Methanol is then added to a 500 ml amber glass jar and spiked with ~ 350 ng g^{-1} silicone rubber of CBs 35, 53, 112, 151, 209 and D12-chrysene, D12-benzo[*e*]pyrene as performance reference compounds. The sheets of silicone rubber (10-20 per batch) are added to the jar and shaken for 2 h on an orbital shaker at 200 rpm followed by the addition of Milli-Q water to obtain 80 % methanol solution and further shaken for 6 h with a subsequent addition of Milli-Q water to obtain a 50 % methanol solution. The glass jar and its contents are finally shaken overnight at room temperature in a light and temperature controlled room. The spiked silicone sheets in the methanol-water solution can be stored for 30 days before being used.

The silicone rubber sheets can then be exposed to sediment samples at a sediment content of 0.16 g g^{-1} in glass Duran® bottles with aluminium-lined caps. In instances where the sediment is not fluid enough, distilled, sea or 'local' water can be added to obtain the 0.16 g g^{-1} sediment content. The exposure is carried out on an orbital shaker in a light and temperature controlled room at 250 rpm for 20 days within which equilibrium between the silicone rubber sheets and the sediment pore water would have been attained. The silicone rubber sheets are removed from the bottles, rinsed with distilled water and dried with tissue paper. They are then extracted in a Soxhlet apparatus with *iso*-hexane: acetone mixture (3:1 $^{v}/_{v}$; 100 ml) for 6 h. The extract is allowed to cool and deuterated PAH internal standard (containing 100 or 200 µl; approximately 1 µg ml^{-1} each of D_8-naphthalene, D_{10}-biphenyl, D_8-dibenzothiophene, D_{10}-anthracene, D_{10}-pyrene and D_{12}-benzo[*a*]pyrene) added. The extract is concentrated by rotary evaporation and nitrogen blow down to 1 ml (split into 2 fractions; PAHs and CBs, if CBs are to be analysed). An aliquot is

fractionated by isocratic normal phase HPLC and the aromatic eluate collected and concentrated prior to analysis by GC-MS while the CB fraction is cleaned by column chromatography using alumina and silica prior to the addition of D6D16 internal standard and analysis by GC-ECD.

With each exposure of silicone rubber sheets to sediment, triplicate spiked sheets would be analysed to determine the initial PRC concentrations in the sheets.

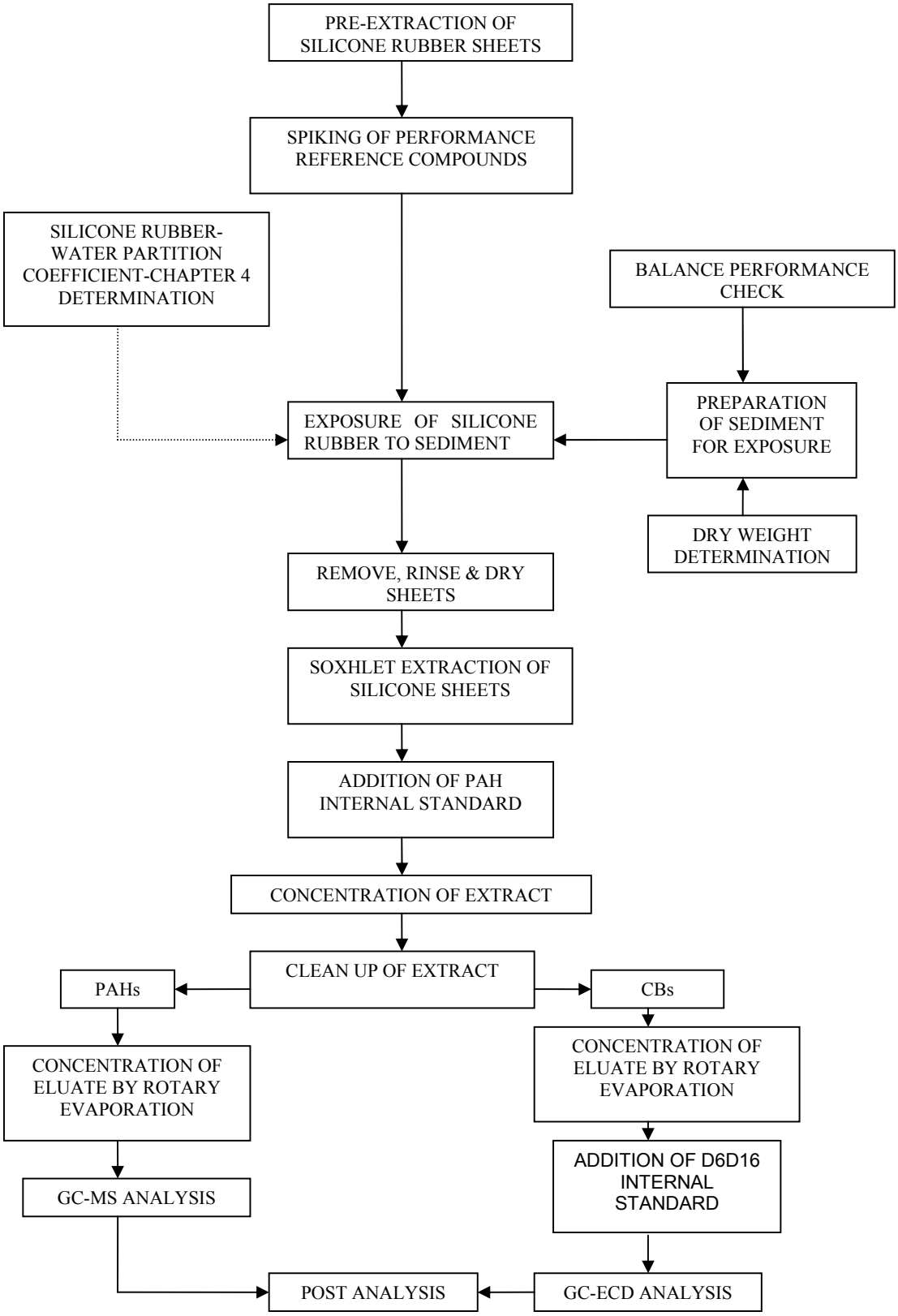


Fig **3.12**: Procedure for the exposure of silicone rubber sampler to sediment-water slurry

CHAPTER FOUR

Passive sampling: Partition coefficients for a silicone rubber reference phase

4.0 Introduction

The determination of the dissolved concentrations of priority hydrophobic organic contaminants (HOCs) such as polycyclic aromatic hydrocarbons (PAHs) and chlorobiphenyls (CBs) is a difficult task due to their low concentrations and the problem of incomplete phase separation between particle-bound and dissolved analytes (Booij *et al*., 2003). The problems are even more pronounced when the determination is required in sediment pore waters, as large volumes of pore water are required. The development of passive sampling methods, using solid or liquid reference phases, allows direct measurement of exposure to dissolved hydrophobic organic contaminants. Single phase sampling devices, such as low density polyethylene (LDPE strips; Booij *et al*., 2003), polyoxymethylene strips (POM; Jonker and Koelmans, 2001), Tenax (Cornelissen *et al*., 2001), polydimethylsiloxane (PDMS; Mayer *et al*., 2000), and silicone rubber (Booij *et al*., 2002; Rusina *et al*., 2007; Wennrich *et al*., 2003) have been developed in addition to the two-phase semi-permeable membrane devices (SPMDs; Huckins *et al*., 1990; Huckins *et al*., 1993) developed earlier to determine the free dissolved HOC concentrations. They are easy to construct, re-usable and of low cost (Rusina *et al*., 2007). Rusina *et al*. (2007) discussed the properties of materials for passive samplers and proposed that silicone rubbers can be attractive reference phases due to their high partition coefficients and low transport resistances.

Using reference (sampling) phases, such as silicone rubber, that equilibrate with the surrounding medium, the partition coefficient can be used, together with the concentration in the sampler, to determine the freely dissolved concentration in the environmental medium (Mayer *et al*., 2003), to estimate the sampling rates of added performance reference compounds (Huckins *et al*., 2006) that have dissipated from the passive sampling device and

subsequently the equilibration rate constants which are used to determine the sampling scenario (kinetic or equilibrium), as not all compounds would have attained equilibrium. Consequently, passive sampling using a reference phase that equilibrates with the dissolved concentration in the sampling medium is attractive. The ratio of the concentration in the sampler to that in the surrounding water at equilibrium yields the sampler-water partition coefficient as described in equation **4.1**:

$$K_{s,w} = \left(\frac{C_s}{C_w} \right) \qquad \textbf{4.1}$$

where $K_{s,w}$ is the sampler-water partition coefficient, C_S and C_W is the concentration in the sampler and water respectively. The sampling rate is calculated from equation **4.2** (Booij *et al*., 2003):

$$R_s = k_e K_{s,w} M_s \qquad \textbf{4.2}$$

where R_s is the sampling rate, k_e is the exchange coefficient (assuming first order kinetics during sampling) and M_S is the sampler mass. The k_e can be estimated from the release of performance reference compounds, spiked onto the sampler using

$$k_e = -\frac{\ln\left(N^t / N^0\right)}{t} \qquad \textbf{4.3}$$

N^t = Amount of PRC left in sampler at the end of exposure time t, N^0 = Amount of PRC spiked onto the sampler at the start of the exposure

It is therefore necessary to determine the partition coefficients that accurately describe partitioning in multi-compartment systems (Booij *et al*., 2002; Mayer *et al*., 2003; Huckins *et al*., 2006; Doong and Chang, 2000; Jabusch and Swackhamer, 2005), particularly those between the reference sampling phase (e.g. silicone rubber) and water. The dissolution of PAHs and CBs in water is quite difficult due to their hydrophobic nature, and as a consequence may adhere to glass surfaces used in the experimental set up and yield variable water concentrations, which complicates the determination of partition coefficients. The partitioning of HOCs between environmental media is mainly determined by the aqueous solubility of the HOCs which may be modified (increased) by the addition of organic solvents (Li *et al*., 1996). Therefore, we have measured the silicone rubber-water partition coefficients

of a series of HOCs (PAHs and CBs) with log K_{ow} values for the compounds studied ranging from 3.3 to 8.2 using the cosolvent method, with methanol as co solvent. This was carried out by determining log partition coefficients at different co-solvent-water volume percentages and extrapolating the > 20 % $^{v}/_{v}$ methanol portion of the linear curve of log partition coefficient versus percent methanol to zero percent methanol which yields the true partition coefficient of the HOC between silicone rubber and water (Jonker and Smedes, 2000; Hegeman *et al*., 1995; Smedes, 2007). The estimation of partition coefficients over a wide range of methanol percentages reduces some of the errors normally associated with measurements of partition coefficients.

4.1 Materials and methods

4.1.1 Materials

AlteSil™ Silicone rubber sheet manufactured from translucent, food grade silicone rubber, with a hardness of 60 Shore A, (600 × 600 mm, 0.5 mm thick) were purchased from Altec Products, Ltd, Cornwall, UK. HPLC grade solvents (ethyl acetate, acetone, methanol and 2-methylpentane) were purchased from Rathburn Chemicals Ltd, Scotland, UK. Certified solid standards for PAHs (including deuterated PAHs) and CBs were obtained from QMX Laboratories, Essex, UK and dissolved in 2-methylpentane (PAHs) and 2, 2, 4-trimethylpentane (CBs) to obtain required concentrations of spiking solutions. The silicone rubber sheets were cut into 6 × 4 cm pieces and pre-extracted with ethyl acetate in a Soxhlet apparatus for 4 days to remove any low molecular weight oligomers or residues that may interfere with subsequent analyses. Milli-Q water (18.2 MΩ.cm) was used throughout.

4.1.2 Loading of Compounds on silicone rubber

Test compounds (PAHs and CBs) were loaded onto the silicone rubber sheets using the spiking method described by Booij *et al*. (2002). Briefly, 100 ml of methanol in an amber glass jar was spiked with known concentrations of the PAHs and CBs of interest (Table **4.0**) and silicone rubber sheets added. The glass jar was shaken for 2 h on an orbital shaker at 200 rpm followed by addition of water to obtain 80 % $^{v}/_{v}$ methanol solution and further shaken for 6 h with a subsequent addition of water to obtain 50 % $^{v}/_{v}$ methanol solution. This was followed by a final shaking overnight at room temperature. Reproducibility of the spiking method was always better than 5 % within each batch of spiked silicone rubber.

4.1.3 Partition coefficient determination

The co solvent method (Jonker and Smedes, 2000; Hegeman *et al*., 1995) was used with methanol as co-solvent. The silicone rubber sheets, loaded with appropriate amounts of test PAHs and CBs, were introduced into 1 L glass bottles (one sheet per bottle) containing 900 ml of the water-methanol mixture at a range of 7 fractions (20-50 % $^{v}/_{v}$ methanol). An un-spiked sheet was added to a bottle containing 900 ml of 20 % $^{v}/_{v}$ methanol solution as procedural blank. Duplicate bottles were then placed on an orbital shaker at 150 rpm for 15 days (Smedes, 2007-used 20 days in his work; Li and Andren, 1994) during which time equilibrium can be assumed to have been attained. Other studies have shown organic compounds, e.g. CB29, D_{10}-phenanthrene, phenol, benzene, dichlorobenzene, etc to equilibrate in silicone membranes within hours (Booij *et al*., 2002; Brookes and Livingston, 1995). Sorption of analytes to the glass container is considered negligible (Hegeman *et al*., 1995; Smedes, 2007) and the concentrations in the water phase were generally measurable. The bottle caps were lined with solvent-washed aluminium foil to prevent sorption onto the plastic caps. The silicone rubber sheets were removed from the bottles and gently wiped dry with paper rolls before extraction.

Deuterated PAH internal standards (D_8-naphthalene, D_{10}-biphenyl, D_{10}-anthracene, D_8-dibenzothiophene, D_{10}-pyrene and D_{12}-benzo[*a*]pyrene) were added to the methanol-water mixtures in the 1 L bottles and transferred into separation funnels. These were liquid-liquid

extracted in separating funnels using 2 × 60 ml dichloromethane and the extracts dried over anhydrous sodium sulphate. The extracts were rotary evaporated to ~ 2 ml and 2 × 25 ml 2-methylpentane added followed by concentration, first by rotary evaporation, then by nitrogen blow down to 1 ml and split into 2 parts: 1 fraction was analysed by gas chromatography-mass selective detection (GC-MSD) for PAHs and the other by gas chromatography-electron capture detection (GC-ECD) for CBs. The silicone rubber sheets were Soxhlet extracted using a 2-methylpentane: acetone (3:1 $^{v}/_{v}$) mixture for 6 h and the same suite of deuterated PAH internal standards added, then exchanged into 2-methylpentane by the addition of 2 × 25 ml of 2-methylpentane. The extract was concentrated by rotary evaporation followed by nitrogen blow down to 1 ml which was then split into 2 fractions by weight for PAHs and CBs. An aliquot of the PAH fraction was fractionated by isocratic, normal phase high performance liquid chromatography using a Genesis silica column and eluted with 2-methylpentane into aliphatic and aromatic fractions. The aliphatic fraction was discarded and the aromatic fraction collected and concentrated before analysis for PAHs. Similarly an aliquot of the CB fraction was fractionated as above and the eluate from the first 6 min (predetermined using a solution of CBs) collected and 2, 4-dichlorobenzyl alkyl ethers internal standard added, then concentrated before analysis for CBs. Recoveries calculated on the basis of the sum of the amounts determined in the methanol-water mixtures and amounts in the sheets relative to the amount loaded onto the sheets were 86 ± 20 % for the CBs and 101 ± 16 % for PAHs.

Confirmatory data were obtained using silicone rubber sheeting (Vizo, Technirub, Netherlands) for which partition coefficients had been independently determined (Smedes, 2007). Vizo and Altec sheeting were equilibrated together by loading them with the test compounds, in triplicate, in amber glass bottles as previously described. As the two materials were equilibrated in the same methanol-water phase, measurements of the concentrations in the methanol-water phase were not necessary since:

$$\left(\frac{C_{sr}}{K_{sr,w}}\right)_1 = \left(\frac{C_{sr}}{K_{sr,w}}\right)_2 = C_w \qquad \textbf{4.4}$$

Implying $\left(K_{sr,w}\right)_2 = \left(\frac{(C_{sr})_2}{(C_{sr})_1}\right) \cdot \left(K_{sr,w}\right)_1$ **4.5**

where the subscripts sr- denotes silicone rubber, w-water and 1, 2 refer to Vizo, and Altec sheeting respectively.

4.1.4 Chromatographic Analysis

The concentrations and composition of the PAHs were determined by gas chromatography mass selective detection (GC-MSD) using an HP6890 Series Gas Chromatograph interfaced with an HP5973 MSD fitted with a cool on-column injector as described by Webster *et al*. (2005). Briefly, a non-polar HP5 (30 m × 0.25 mm id, 0.25 μm film thickness; Agilent Technologies, Stockport, England) column was used for the analyses with helium as the carrier gas, controlled using the constant flow mode at 0.7 ml min^{-1}. Injections were made at 50 ^{o}C and the oven temperature held constant for 3 min. Thereafter, the temperature was raised at 20 ^{o}C min^{-1} up to 100 ^{o}C, followed by a slower ramp of 4 ^{o}C min^{-1} up to a final temperature of 270 ^{o}C. The MSD was set for selective ion monitoring (SIM) with a dwell time of 50 ms. A total of 30 ions plus the six internal standard ions were measured over the analysis period, thus incorporating 2- to 6- ring, parent and branched PAHs.

The concentration and composition of CB congeners were determined by gas chromatography with electron capture detection (GC-ECD) using a Perkin Elmer Gas Chromatograph Clarus 500 auto system (Perkin Elmer, Beaconsfield, UK) fitted with a cool on-column injector. A non-polar HP 5 column (60 m × 0.25 mm id, 0.25 μm film thickness; Agilent Technologies, Stockport, England) was used for the analyses along with an uncoated pre-column (2.5 m × 0.53 mm id). The carrier gas was hydrogen (1–3 ml min^{-1}) and make-up gas was nitrogen (30 ± 5 ml min^{-1}). The initial oven temperature was 80 ^{o}C and held for 1 min after which it was ramped at 3 ^{o}C min^{-1} to a final temperature of 280 ^{o}C and held at this temperature for 12 min. The chromatograph was calibrated using a series of external standards and the two 2, 4-dichlorobenzyl alkyl ethers. The data were quantified using a Client Server Turbochrom data system (Perkin Elmer, Beaconsfield, UK).

4.1.5 Octanol-water partition coefficients

Octanol-water partition coefficients were obtained from literature. Sangster (2005) compiled $\log K_{ow}$ values reviewed from available literature and gave recommended values for some compounds which were adopted in this study. For compounds where no recommended value was given, average value of the $\log K_{ow}$ data presented was chosen and where only one value was given this was adopted.

4.2 Results

4.2.1 Co solvent method

Silicone rubber-water partition coefficients were calculated (Eq. **4.1**) at each methanol percentage and log linear curves plotted for $K_{sr,w}$ as a function of the methanol volume percent (Fig. **4.0**). The partition coefficients by the co-solvent method were estimated from the intercept of the regression lines at 100 % water (0 % methanol) thus eliminating the need to measure the partition coefficients directly in pure water. Addition of the cosolvent increases the hydrophobicity of the resulting solvent (water/co-solvent mixture), which increases solubility of the target compounds in the solvent mixture (Li *et al*., 1996; Hegeman *et al*., 1995; Chawla *et al*., 2001; Spurlock and Biggar, 1994). The solubility increases exponentially while the logarithm of partition coefficients will decrease linearly with increasing mole fraction of methanol.

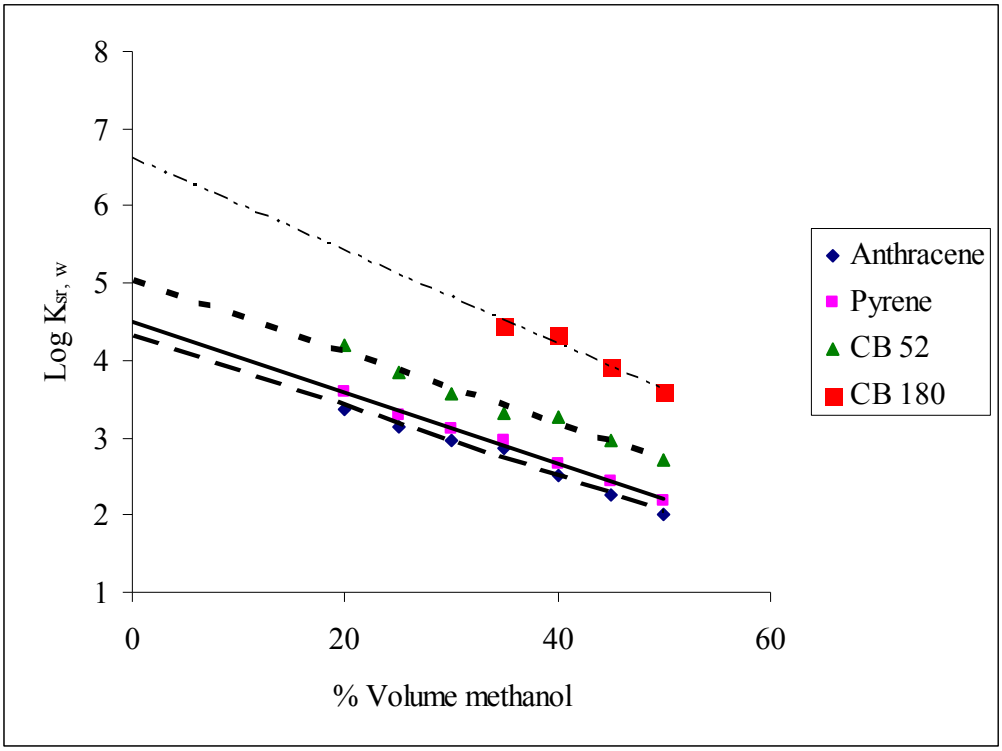


Fig **4.0**: Log $K_{sr,w}$ as a function of volume percentage methanol for anthracene (diamonds), pyrene (squares), CB 52 (triangle) and CB 180 (large squares). Only four points were used for the regression of CB 180, as it was not detected in 20-30 % volume methanol. Linear regression was used to obtain estimates of intercept

Linear regressions were good descriptors of the relationship between log $K_{sr,w}$ and percent methanol with the intercept representing log $K_{sr,w}$ at 0 % methanol. Log $K_{sr,w}$ values, along with the 95 % confidence interval of the intercept of log $K_{sr,w}$ - methanol volume percentage are shown in Table **4.0**. For the more hydrophobic compounds, the number of data points used in the regression analysis is often less than 7 due to problems in the accurate measurement of concentrations of HOCs at low percentages of methanol in water. Inclusion of some of these data of relatively high uncertainty would not have improved the quality of the regression analysis or the estimates of partition coefficients. At least five partition coefficients are recommended for the regression, because if fewer partition coefficients are used for extrapolation, the statistical errors in the estimate of the intercept (log $K_{sr,w}$) will tend to be large (Hegeman *et al*., 1995).

Table **4.0:** Log Partition coefficients and 95 % confidence interval of estimate of intercept from regression of test compounds

PAHs	Log K_{ow}	Log $K_{sr,w}$	C.I.[a]	n[b]	CBs	Log K_{ow}	Log $K_{sr,w}$	C.I.	n
Naphthalene	3.35	3.53	0.40	7	CB 28	5.67	4.79	0.33	7
Acenaphthylene	3.61	3.39	0.20	7	CB 31	5.67	4.66	0.30	7
Acenaphthene	3.92	3.84	0.35	7	CB 35	5.82	4.56	0.40	7
Fluorene	4.18	3.89	0.33	7	CB 44	5.75	5.21	0.32	7
2-Methylnaphthalene	4.00	4.06	0.48	7	CB 49	5.85	5.22	0.40	7
1-Methylnaphthalene	3.87	4.00	0.50	7	CB 52	5.84	5.04	0.29	7
C2-naphthalene	4.31	4.33	0.40	7	CB53	5.62	5.02	0.39	7
C3-naphthalene	4.73	4.64	0.41	7	CB 70	6.2	5.17	0.35	7
C4-naphthalene	5.17[d]	5.17	0.44	7	CB 74	6.2	5.29	0.34	7
Phenanthrene	4.52	4.18	0.31	7	CB 97	6.29	5.49	0.32	7
Anthracene	4.50	4.31	0.28	7	CB 99	6.39	5.68	0.29	7
2-Methylphenanthrene	5.24	4.89	0.37	7	CB 101	6.38	5.93	0.31	6
3,6-Dimethylphenanthrene	5.25[d]	5.15	0.25	7	CB 105	6.65	5.60	0.33	7
2,6,9-Trimethylphenanthrene	5.99[d]	5.34	0.30	7	CB 110	6.48	5.74	0.32	6
Dibenzothiophene	4.38	4.04	0.31	7	CB 112	6.45	5.59	0.31	7
Fluoranthene	5.20	4.45	0.21	7	CB 118	6.74	6.16	0.33	6
Pyrene	5.00	4.49	0.17	7	CB 128	6.74	6.10	0.42	7
1-Methylfluoranthene	5.48[d]	5.01	0.19	7	CB 132	6.58	5.79	0..29	7
2,7-Dimethylpyrene	6.03[d]	6.30	0.27	7	CB 137	6.83	6.10	0.35	6
Benzo[*c*]phenanthrene	5.76[h]	5.38	0.18	7	CB 138	6.83	6.52	0.31	6
Benz[*a*]anthracene	5.91	5.42	0.16	7	CB 149	6.67	6.17	0.40	6
Chrysene	5.86	5.23	0.15	7	CB 151	6.64	6.07	0.37	7
2-Methylchrysene	6.88[g]	6.15	0..20	7	CB 153	6.92	6.30	0.25	4
Benzo[*b*]fluoranthene	5.78	6.33	0.09	7	CB 156	7.18	7.26	0.34	5
Benzo[*k*]fluoranthene	6.11	6.25	0.05	7	CB 157	7.18	6.06	0.38	6
Benzo[*e*]pyrene	6.44	6.12	0.06	7	CB 158	7.02	6.20	0.30	7
Benzo[*a*]pyrene	6.35	6.27	0.14	7	CB 170	7.27	6.56	0.82	4
Perylene	6.25	6.02	0.12	6	CB 180	7.36	6.61	0.67	4
7-Methylbenzo[*a*]pyrene	nf	6.97	0.18	6	CB 183	7.2	6.67	0.42	4
Indeno[*1,2,3-cd*]pyrene	7.66	7.48	0.17	7	CB 187	7.17	6.61	0.36	4
Benzo[*ghi*]perylene	6.90	6.63	0.14	6	CB 189	7.24	6.45	0.40	4
Dibenz[*a,h*]anthracene	6.75	6.76	0.23	6	CB 209	8.18	7.81	0.28	3
D_{12}-Chrysene	5.80[e]	5.15	0.15	7					
D_{12}-Benzo[*e*]pyrene	6.29[f]	6.29	0.14	7					

Log $K_{sr,w}$ values are means of 2 replicates. [a] C.I. = Confidence interval; [b] n = number of data points making up plot; [c] log K_{ow} of PAHs are from Sangster (2005), [d] from Booij *et al*. (2006), [e] from Booij *et al*. (2002), [f] adopted the value of Benzo[*e*]pyrene and those of CBs from Hawker and Connell (1988), nf = no log K_{ow} value found, [g] value adopted from (www.nature.nps.gov/hazardssafety/toxic/chrys_c1.pdf), [h] value for naphthacene is used.

4.2.2 Batch incubation of reference phases

The co solvent method can however be laborious and so an attempt was made to estimate the $\log K_{sr,w}$ from a material of known partition coefficients. Therefore using data obtained from the co solvent method for Vizo sheets (Smedes, 2007), $\log K_{sr,w}$ values were predicted for the Altec sheets using Eq. **4.5**. Results obtained by incubating the Vizo and Altec silicone rubbers are presented in Table **4.1** for those compounds for which partition coefficients for Vizo rubber were available.

Table **4.1:** $\text{Log } K_{sr,w}$ obtained by equilibration of 2 different silicone rubbers

Compounds†	ALTEC Mea[1]	VIZO[2]	ALTEC Est[3]	Compound	ALTEC Mea	VIZO	ALTEC Est
1	4.18	3.89	4.06	CB 28	4.79	5.22	5.36
2	4.31	4.00	4.18	CB 31	4.66	5.23	5.33
3	4.45	4.38	4.59	CB 44	5.21	5.56	5.71
4	4.49	4.44	4.66	CB 49	5.22	5.66	5.78
5	5.42	5.06	5.29	CB 52	5.04	5.57	5.70
6	5.23	4.97	5.20	CB 101	5.93	6.03	6.13
7	6.33	5.51	5.71	CB 105	5.60	6.17	6.31
8	6.25	5.51	5.71	CB 118	6.16	6.20	6.30
9	6.12	5.45	5.65	CB 138	6.52	6.53	6.64
10	6.27	5.52	5.71	CB 153	6.30	6.45	6.54
11	7.48	5.99	6.20	CB 156	7.26	6.58	6.65
12	6.63	5.92	6.12	CB 170	6.56	6.90	6.98
13	6.76	6.04	6.26	CB 180	6.61	6.84	6.90
14	5.15	4.91	5.14	CB 187	6.61	6.77	6.84

† 1- Phenanthrene; 2- Anthracene; 3- Fluoranthene; 4- Pyrene; 5- Benz[*a*]anthracene; 6- Chrysene; 7- Benzo[*b*]fluoranthene; 8- Benzo[*k*]fluoranthene; 9- Benzo[*e*]pyrene; 10- Benzo[*a*]pyrene; 11- Indeno[1,2,3-*cd*]pyrene; 12- Benzo[*ghi*]perylene; 13- Dibenz[*a*,*h*]anthracene; 14- D_{12}- Chrysene

[1] Experimentally measured in this study;

[2] $\log K_{sr,w}$ values for the Vizo rubber from Smedes (2007);

[3] estimated using Eq. **4.4**

4.3 Discussion

In the absence of direct measurements, estimates of partition coefficients for passive samplers are often obtained from $\log K_{ow}$ values. The measured Altec silicone rubber-water partition coefficients were plotted against corresponding $\log K_{ow}$ values and good linear relationships were found (Fig. **4.1**a & b, Table **4.2**). This confirms that partitioning into the silicone rubber is strongly determined by compound hydrophobicity, as has been reported for other materials, such as POM (Jonker and Koelmans, 2001) and PDMS fibres (Mayer *et al*., 2000). No significant differences were found (F-test; $p > 0.05$) between the $\log K_{sr,w}$ - $\log K_{ow}$ relations depending on the source of the $\log K_{ow}$ data for either the PAHs or CBs. The regressions using different sources of $\log K_{ow}$ differ in their intercepts for PAHs while the slope did not differ significantly (slope = 0.97 ± 0.11; slope = 1.13 ± 0.19, Table **4.3**) when the intercepts are ignored. Deviations from linearity could arise from some uncertainty in available K_{ow} values (Jabusch and Swackhamer, 2005; Meylan and Howard, 2005) and other factors, such as possible transport resistance of silicone rubber to large molecules (large K_{ow}s), as had been observed for various tissues and membranes (Chiou, 1985).

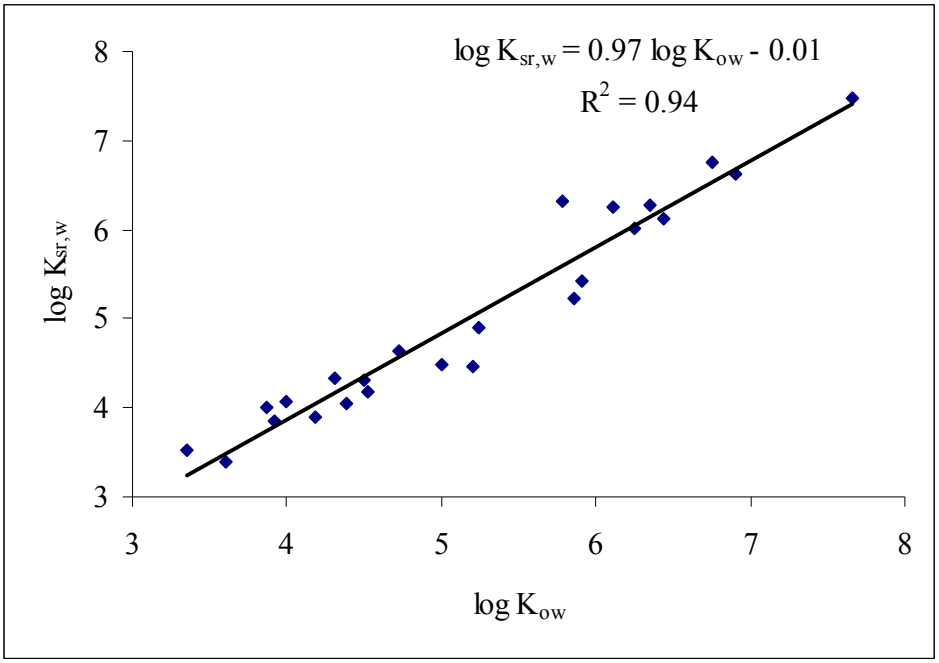


Fig **4.1a:** Correlation of log $K_{sr,w}$ with log K_{ow} for 24 PAH compounds. Log $K_{sr,w}$'s are mean values of duplicate measurements and log K_{ow} are from Sansgter (2005)

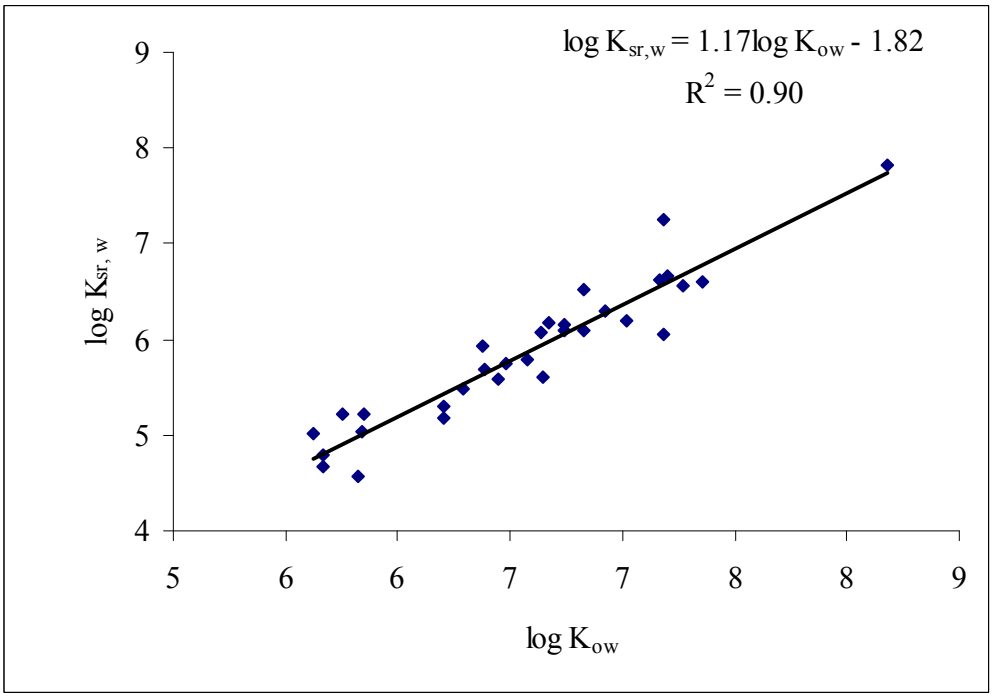


Fig **4.1b:** Correlation of log $K_{sr,w}$ with log K_{ow} CBs. Log $K_{sr,w}$ are mean values of duplicate measurements and log K_{ow} are from Hawker and Connell (1988)

Combining both classes of compounds also yields a linear relationship (Table **4.3**) but the decrease in the quality of the regression indicates systematic differences in the relationship between $\log K_{sr,w}$ and $\log K_{ow}$ for different classes of compound. (*c.f.* Vrana *et al*. (2001)). The measured partition coefficients were on average, 0.70 units less than the octanol-water partition coefficients for the CBs and 0.18 units for the PAHs. The regressions of $\log K_{POM}$ of CBs and PAHs with $\log K_{ow}$ have been reported (Jonker and Koelmans, 2001) not to differ significantly which allow for pooling of both classes of compounds while in our study the regressions differed significantly (F-test; $p< 0.001$). The observed differences in the regressions may be due to structural differences between PAHs and CBs. Differences have been shown (Booij *et al*., 2003; Jonker and Smedes, 2000; Booij *et al*., 1998) between log K_{oc} (organic carbon normalised partition coefficients) -$\log K_{ow}$ regression lines for PAHs and CBs which have been attributed to differences in molecular structure between the two classes of compounds. Similarly, caution has been suggested with regards to estimation of bio concentration factors (BCF) from log BCF-$\log K_{ow}$ correlations (which are essentially partitioning models between biological membranes and water) for different classes of compounds (Mackay, 1982; Barron, 1990) due to uncertainties in the measurement of both BCFs and $\log K_{ow}$ (especially at $\log K_{ow} > 6$) .

SPMD-water partition coefficients (reviewed from literature) are available in Huckins *et al*. (2006), and the average $\log K_{spmd,w} - \log K_{ow}$ values from the various data was described by a quadratic fit with deviations from linearity observed from $\log K_{ow} > 5$ possibly due to solute-triolein incompatibility. This was not the case in the good linear relationships found in this study over a $\log K_{ow}$ range of 3 – 8, suggesting that such complications do not occur with silicone rubber and that equilibrium partitioning between the silicone rubber and aqueous phases is almost entirely a function of the hydrophobicity of the target contaminants. The need to determine partition coefficients for both the lipid and the membrane as is the case for SPMDs is eliminated as only a single partition coefficient is sufficient to describe partitioning in the silicone rubber sampler.

Table **4.2**: Selected estimated $\log K_{sr,w}$ values from the $K_{sr,w}$ - log K_{ow} regressions

Compound	**log $K_{sr,w}$**	**S. E.**[a]	**log K_{ow}**
PAHs			
Acenaphthene	3.78	0.09	3.92
Fluorene	4.04	0.08	4.18
Phenanthrene	4.36	0.07	4.52
Fluoranthene	5.02	0.06	5.20
Chrysene	5.66	0.07	5.86
Benzo[*a*]pyrene	6.14	0.08	6.35
Benzo[*ghi*]perylene	6.67	0.11	6.90
Dibenz[*a,h*]anthracene	6.52	0.10	6.75
CBs			
CB 31	4.81	0.08	5.67
CB 52	5.01	0.07	5.84
CB 101	5.64	0.05	6.38
CB 110	5.76	0.05	6.48
CB 118	6.07	0.05	6.74
CB 105	5.96	0.05	6.65
CB 183	6.60	0.07	7.20
CB 170	6.69	0.07	7.27

[a]S.E. = Standard error of predicted value, $\log K_{ow}$ of PAHs are from Sangster (2005) and those of CBs from Hawker and Connell (1988).

In view of the uncertainties inherent in the measurements of partition coefficients by either the co-solvent or batch incubation method, it is suggested that use can be made of the linear regressions between $\log K_{sr,w}$ and $\log K_{ow}$ to obtain estimates of $\log K_{sr,w}$. The standard errors in the experimental data in Table **4.0** are on average 2.35 (CBs) and 1.69 (PAHs) times the errors from the predicted values (Table **4.3**) from the log $K_{sr,w}$ - log K_{ow} regressions.

The $\log K_{sr,w}$ values obtained by incubation (referred to as estimated $K_{sr,w}$, Table **4.1**) correlated well (r^2 = 0.93, PAHs and 0.90, CBs) with the co solvent method values (Table **4.0**), however, there was an observed increase in deviation of log $K_{sr,w}$ between the two methods with increasing $\log K_{ow}$. Uncertainties in the silicone rubber-water partition coefficients for the Vizo rubber will have added to overall uncertainties in the partition coefficients estimated by this method for the Altec rubber. It may be noted that a quick

estimate of $\log K_{sr,w}$ values can be obtained by, equilibrating the silicone rubber of known partition coefficient with an unknown rubber, for example to confirm the quality of a new batch of rubber.

Table **4.3**: Summary of linear regression analysis of $\log K_{sr,w}$ versus $\log K_{ow}$

	$\log K_{ow}$	*Slope*	*Intercept*	r^2	s^a	n^b
PAHs	Sangster (2005)	0.97	-0.01	0.94	0.29	24
	US EPA (2005)	1.13	-0.79	0.94	0.38	17
CBs	Hawker and Connell (1988)	1.17	-1.82	0.9	0.25	31
	Mackay *et al*. (1992)	1.2	-1.98	0.91	0.28	15
Pooled (PAHs & CBs)	**	0.87	0.30	0.89	0.35	55

[a] standard deviation of the fit
[b]sample size
** Sangster (2005) & Hawker and Connell (1988)

4.4 Conclusions

The co solvent method offers a practical way to estimate partition coefficients for passive sampling materials, such as silicone rubber. The estimated partition coefficients showed strong linear relations with published values for $\log K_{ow}$ confirming that partitioning into the silicone rubber is largely a function of compound hydrophobicity, and that absorption into the silicone rubber is the main mechanism governing retention of analytes in the polymer. It is suggested that best estimates of partition coefficients for silicone rubber may be obtained from regressions between $\log K_{ow}$ and $\log K_{sr,w}$. The fitted values can be used to estimate sampling rates of passive samplers and in the determination of free dissolved concentrations of PAHs and CBs in water and sediment pore water. In cases where no silicone rubber-water partition coefficients are available, estimations can be made using the octanol-water partition coefficients, although measured values are always better. It is also possible to attempt to

measure $\log K_{sr,w}$ in pure water, with any agreement of the co solvent values with water-only values greatly enhancing the reliability of the final values, although there always will be some uncertainty with the individual methods.

CHAPTER FIVE

Preliminary application of the silicone rubber passive sampler to determine pore water and water exchangeable concentrations of PAHs in sediments from the Fladen Ground of the North Sea

5.0 Introduction

The Fladen Ground (North Sea) is well known for offshore oil and gas production activities, resulting in discharges of hydrocarbons during drilling, production and flaring operations. These can accumulate in the predominantly fine muddy sediments of the area and due to fishing activities in the area, the implications of hydrocarbon contamination could be important (Russell *et al*., 2005). The freely dissolved concentrations of persistent organic pollutants (POPs) such as CBs and PAHs determine their environmental impact with a general acceptance that only the dissolved concentrations are available for uptake or accumulation by biota and which may ultimately cause biological effects in biota (Booij *et al*., 2003, Kraaij *et al*., 2003). The bioavailability of a contaminant is related to the sediment-water column partitioning because for contaminant partition coefficient above the corresponding octanol-water partition coefficient (K_{ow}), contaminants in the sediment are strongly bound and not readily released to the pore waters or to the overlying waters for subsequent uptake by aquatic organisms (Thomann and Komlos, 1999). Passive samplers have been used to determine the bound fractions of contaminants in sediments and the fractions that could potentially become available for uptake-accessible fractions (Macrae and Hall, 1998; Cornelissen *et al*., 2001; Reichenberg and Mayer, 2006) in sediments.

Following the confirmation of use of the silicone rubber sheet as an equilibrium passive sampling device (assuming first order accumulation kinetics) and the determination of the silicone rubber-water partition coefficients for individual CBs and PAHs in chapters **3** and **4** respectively, the draft protocol (see Appendix **3**) developed was applied to sediments collected

from the Fladen area of the North Sea, Scotland (Fig **5.0**). This was done with the aim of measuring availability parameters (ICES, WGMS 2003) such as the freely dissolved concentration, water-exchangeable (extractable) concentrations, and sediment-water partition coefficients of PAHs only. Most equilibrium samplers assume non- or negligible depletion of the sediment phase (Jonker and Koelmans, 2001; Mayer *et al*., 2002; Mayer *et al*., 2003; Heringa and Hermens, 2003). However, in this study, no such assumption was made; rather measurements of pore water concentrations were made at different degrees of sediment depletion (or phase ratios-see Appendix **3**), construction of isotherms, and extrapolating as described in Chapter **1**; section **1.8.1**.

The objective of this work was to determine the availability of PAHs in sediments from the Fladen Ground of the North Sea by measuring pore water and water extractable (exchangeable) concentrations in the sediment slurries and the sediment-water partition coefficients using the silicone rubber passive sampler discussed in Chapters **3** and **4**.

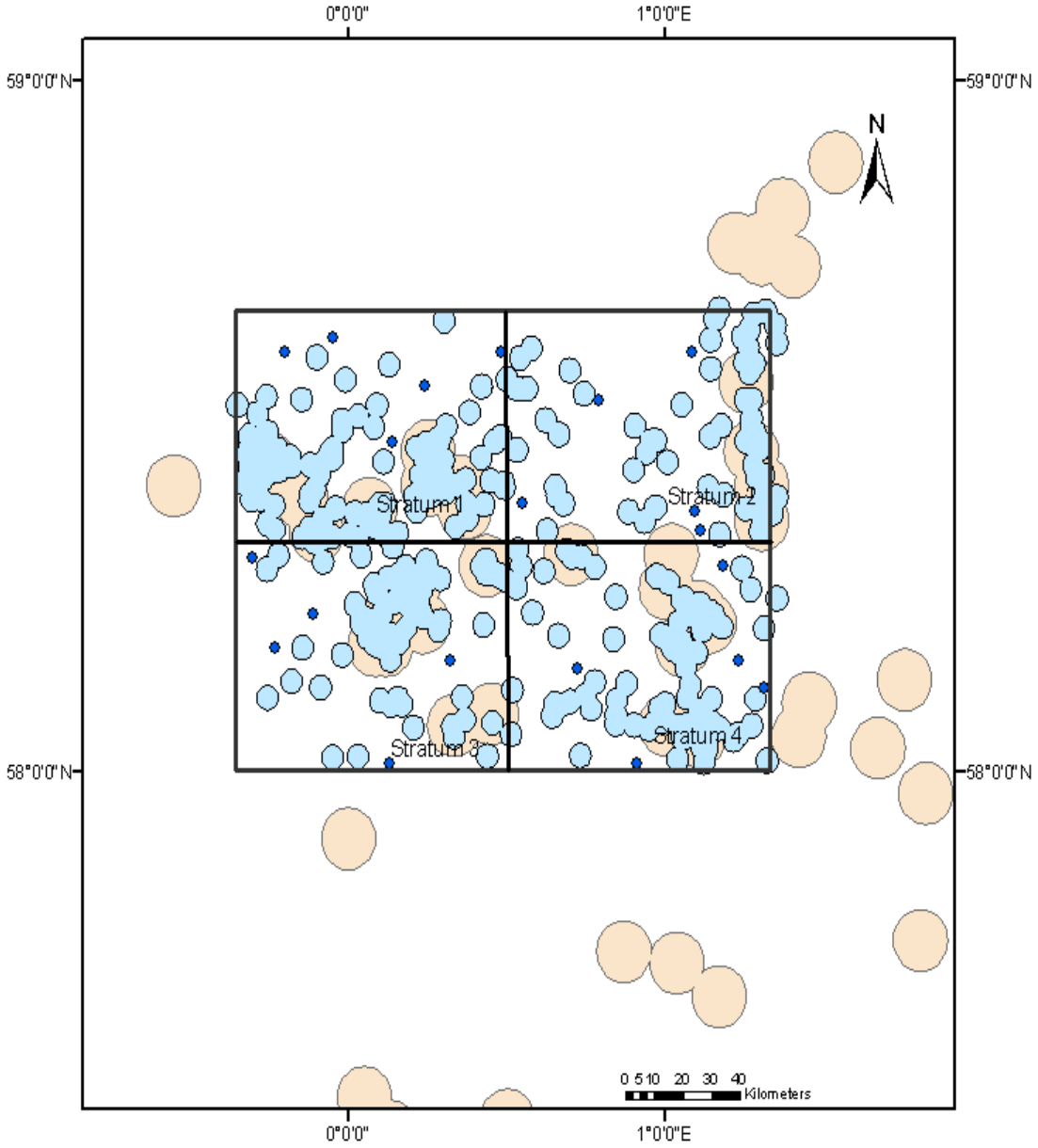


Fig **5.0**: Sampling points within the Fladen Ground during the 0106S cruise in 2006. FL1/4 & 5 were collected from Stratum 1 while FL3/2 & 3 were from Stratum 3

5.1 Materials and Methods

5.1.1 Materials

HPLC grade solvents (dichloromethane, ethyl acetate, acetone, methanol and *iso*-hexane) were purchased from Rathburn Chemicals Ltd, Scotland, UK. Certified solid standards for PAHs (including deuterated PAHs) were obtained from QMX Laboratories, Essex, UK and dissolved in *iso*-hexane (PAHs) to obtain required concentrations of spiking solutions. To avoid contamination of samples, all glassware, stainless steel forceps, was either washed in Decon® 180 solution and rinsed with distilled water or washed in a CAMLAB GW 4050 glassware washer and dried in an oven at 100 ^{o}C. Before use, the glassware were rinsed twice each in dichloromethane and *iso*-hexane, with the latter allowed to evaporate to dryness. Glass Duran® or Schott® (VWR, Leicester, UK) bottles were used and always capped with aluminium-lined PTFE caps to reduce or prevent sorption of POPs by the caps.

AlteSil™ Silicone rubber sheet manufactured from translucent, food grade silicone rubber, with a hardness of 60 Shore A, (600 × 600 mm, 0.5 mm thick) was purchased from Altec Products, Ltd, Cornwall, UK. The silicone rubber sheets used were cut into required dimensions (6 × 4 cm, or 9 × 4 cm) and pre-extracted in hot ethyl acetate using a Soxhlet apparatus for ~ 4 days before use to remove any low molecular weight oligomers or residues that may be co-extracted with the analytes and could affect instrumental analysis. After pre-extraction and cooling, the sheets were removed from the solvent and stored in bulk in glass jars containing methanol prior to use. The weight of the sheets used was determined after extraction of exposed sheets to avoid contamination from the environment.

5.1.2 Methods

5.1.2.1 Sediment Sampling

Stratified random sediment samples were collected from FRV *Scotia*, using a 0.1 m^2 Day Grab at depths between 123 and 155 m from four strata of the Fladen Ground in January 2006. Two samples from two of the stratum (Stratum 1 & 3) were used for this study. The top 2 cm of the sediment was transferred to a solvent washed aluminium can, thoroughly mixed, labelled and stored at -20 ± 5 ^{o}C until required for analysis.

5.1.2.2 Hydrocarbon analysis of sediment

Hydrocarbon analysis of sediments was as described in chapter **2**, section **2.3.3.1**, **2.3.3.1.1** and **2.3.3.1.3**

5.1.2.3 Determination of availability parameters

Four sediment samples from the Fladen Ground (10/2006KY≡ FL 3/2; 11/2006KY≡ FL 3/3; 27/2006KY≡ FL 1/4 and 28/2006KY≡ FL 1/5) were defrosted at room temperature, ensuring that exposure to direct sunlight or heat was minimised and the samples thoroughly mixed before sub sampling for analysis using the protocol developed in Chapter **3**.

The samples were weighed into a 50 ml and 3 × 250 ml glass Duran® bottles, and water added to liquefy the sediment where necessary so as to ensure proper interaction between the sediment slurry and the silicone rubber sheets that would be added to the bottles. Pre-extracted silicone rubber sheets were loaded with the PAH performance reference compounds (PRCs) as described in chapter **3** and a single sheet added to each bottle to obtain different phase (g silicone rubber per g sediment) ratios (Table **5.0**). The bottles were placed on an orbital shaker horizontally and shaken at 200 rpm for 20 days. After this time, the sheets were removed from the bottles, rinsed with distilled water and gently wiped dry with paper tissue to remove any

adhering water. The sheets were then extracted as described in chapter **3** (section **3.3.1.1**) and the weight of the silicone rubber sheet recorded after the extraction to avoid contamination from external sources. A procedural blank (an un-spiked sheet exposed to only the distilled water used to liquefy the sediments) was included and analysed in the same manner as the other samples. The result from the procedural blank was subsequently subtracted from the results of samples. Similarly a spiked sheet was also analysed to obtain the initial amounts of PRCs in the sheets.

Table **5.0**: Exposure of silicone rubber sheets to samples from the Fladen Ground, showing the weights of silicone rubber and phase ratios

Sample ID	Sediment wet wt/ g	Moisture content/ %	Sediment dry wt/ g	Water added/ g	SR weight/ g	Phase ratio
10/2006KY/A	200.14	35.71	128.68	89.97	1.47	0.011
10/2006KY/B	100.17	35.71	64.40	48.29	1.53	0.024
10/2006KY/C	100.45	35.71	64.58	52.85	2.73	0.042
10/2006KY/D	30.48	35.71	19.60	13.61	1.50	0.077
11/2006KY/A	200.38	39.03	122.18	75.11	1.48	0.012
11/2006KY/B	100.12	39.03	61.05	39.06	1.49	0.024
11/2006KY/C	100.28	39.03	61.15	39.28	2.91	0.048
11/2006KY/D	30.59	39.03	18.65	10.78	1.51	0.081
27/2006KY/A	200.04	39.70	120.63	75.13	1.64	0.014
27/2006KY/B	105.45	39.70	63.59	41.34	1.51	0.024
27/2006KY/C	100.70	39.70	60.72	37.86	2.79	0.046
27/2006KY/D	30.13	39.70	18.17	30.25	1.51	0.083
28/2006KY/A	200.17	36.63	126.85	89.97	1.48	0.012
28/2006KY/B	100.16	36.63	63.47	48.06	1.61	0.025
28/2006KY/C	100.06	36.63	63.41	49.45	2.81	0.044
28/2006KY/D	30.24	36.63	19.16	13.71	1.57	0.082
Blank	-	-	-	40.00	1.54	-

5.2 Results and Discussion

5.2.1 Sediment PAH concentration

The total sediment PAH concentration (2- to 6-ring parent and alkylated PAHs, Table **5.1** and Fig **5.1**) varied between 43 ng g^{-1} dry weight (FL3/2) to 173 ng g^{-1} dry weight (FL1/4)), with the 4- to 6-ring compounds accounting for ~ 77 % of the total PAH concentration.

Provisional Oslo and Paris Commission (OSPAR) Background Concentrations (BCs) and Background Assessment Concentrations (BACs) have been established for ten parent PAHs in sediments (OSPAR, 2006). The Background Concentration has been defined as the concentration of a contaminant at a "pristine" or "remote" site based on contemporary or historical data. Observed concentrations are said to be 'near background' if the mean concentration is statistically significantly below the corresponding Background Assessment Concentration (BAC). Only single measurements of PAH concentrations were made in this study, therefore the individual PAH concentrations for each station were normalized to 2.5 % organic carbon and an approximate 95 % confidence interval calculated using uncertainty values from method validation data at Fisheries Research Services Marine Laboratory, Aberdeen (FRS ML). The normalized concentrations for individual PAHs (Table **5.1**) exceeded the corresponding BCs and BACs at the 5 % significance level except anthracene in the two samples from stratum 3.

Table **5.1**: Available OSPAR Background Concentrations and provisional Background Assessment Concentration (both ng g^{-1} dry weight) normalised to 2.5 % organic carbon (OSPAR, 2006) and corresponding concentrations in the sediment samples from the Fladen Ground, also normalised to 2.5 % organic carbon with 95 % confidence interval (C.I) of concentrations at the individual stations expressed as a percentage

	Concentration (ng g^{-1} dry weight)						
			Station				
	***BC*[1]**	***BAC*[2]**	**C.I. (%)**	**FL3/2**	**FL3/3**	**FL1/4**	**FL1/5**
Naphthalene	5	8	30	8	16	196	180
Phenanthrene	17	32	28	28	48	96	72
Anthracene	3	5	24	TR	TR	8	8
Fluoranthene	20	39	25	32	56	84	60
Pyrene	13	24	25	24	40	60	48
Benz[*a*]anthracene	9	16	33	16	28	48	36
Chrysene	11	20	38	36	60	92	60
Benzo[*a*]pyrene	15	30	22	36	60	120	80
Indeno[1,2,3-*cd*]pyrene	50	103	19	208	368	832	496
Benzo[*ghi*]perylene	45	80	19	172	284	600	360

[1]Background Concentration; [2]Background Assessment Criteria

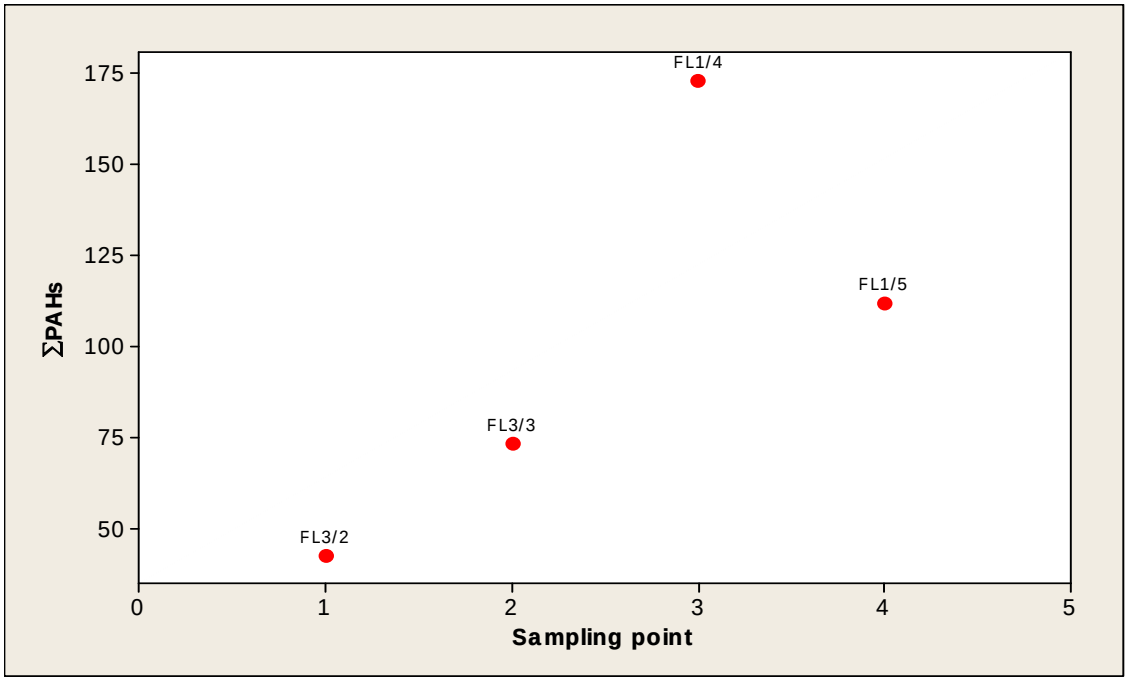


Fig **5.1**: Total PAH concentration (ng g^{-1}) in the 4 samples used from the Fladen Ground

The percent organic carbon (% OC) was on average 0.59 % while the mean percent particle size for the < 63 μm fraction was 60 % (Table **5.2**). The individual PAH concentrations normalised to % OC showed a similar trend across the different samples (Fig **5.2**) although the naphthalenes were higher in the 2 samples from stratum 1 (FL1/4 and FL1/5) than in the 2 from stratum 3 (FL3/2 and FL3/3). Low PAH concentrations are normally associated (Webster *et al*., 2004) with sediments with low total organic carbon and proportion of fine material. The sample (FL1/4) with the highest % OC had the highest total PAH concentration (173.1 ng g^{-1} dry weight) while the sample (FL3/2) with the lowest % OC contained the lowest total PAH concentration (42.5 ng g^{-1} dry weight). The sediment % OC and particle size values were within the range previously found for the Fladen Ground by Ahmed *et al*., (2006).

Table **5.2**: Sediment PAH concentration (ng g^{-1} dry weight) and bulk sediment properties of samples collected from the Fladen Ground in January 2006

UKAS ID*	10/2006KY	11/2006KY	27/2006KY	28/2006KY
Field ID	FL3/2	FL3/3	FL1/4	FL1/5
%OC	0.47	0.64	0.67	0.56
PS: % < 63μm	54.31	67.54	66.08	51.20
Naphthalenes[1]	3.2	5.6	36.9	28.0
3-ring[2]	3.3	5.6	10.1	7.6
DBTs[3]	0.2	0.8	1.5	1.5
4-ring[4]	7.8	14.2	23.2	14.7
5-ring[5]	16.5	26.9	55.8	33.3
6-ring[6]	11.5	20.4	45.6	26.9
∑PAHs	42.5	73.5	173.1	112.0

[1]Sum of naphthalene; 1 & 2-methylnaphthalene; C2-, C3- and C4- naphthalenes
[2]Sum of phenanthrene; anthracene, C1-178, C2-178, and C3-178; acenapthylene; acenaphthene and fluorene
[3]Sum of Dibenzothiophene; C1-DBT, C2-DBT, and C3-DBTs
[4]Sum of fluoranthene; pyrene; C1-202, C2-202, and C3-202; benzo[*c*]phenanthrene; benz[*a*]anthracene; benz[*b*]anthracene; chrysene; C1-228; C2-228
[5]Sum of benzofluoranthenes; benzo[*e*]pyrene; benzo[*a*]pyrene; perylene; C1-& C2-252
[6]Sum of indeno[*1, 2, 3-cd*]pyrene; benzo[*ghi*]perylene; C1-276 and C2-276
* Sample identification number

Acenaphthylene, acenaphthene, anthracene and DBT were either not detected in the samples or were found in trace levels.

Smedes *et al*. (2006) in a draft guideline on equilibrium passive sampling of sediments (www.passivesampling.net) recommended the use of sediments (non-sandy) which contain > 1 % OC so as to ensure the capacity of the sediment phase is high enough to avoid deviation from the original concentrations.

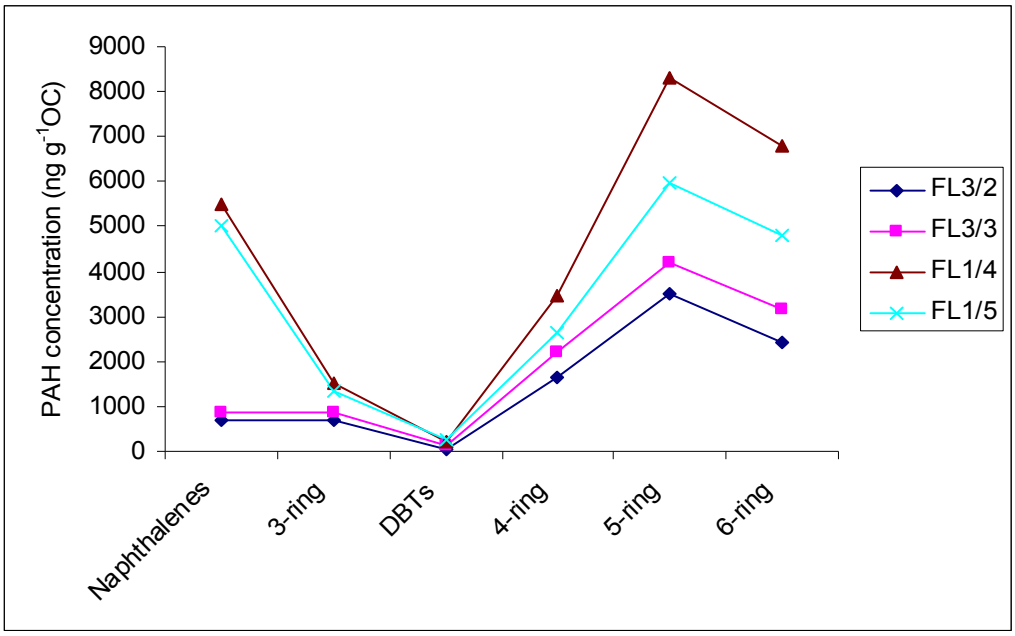


Fig **5.2**: Sediment PAH concentration normalised to percent organic carbon content (ng g^{-1} OC) from Fladen Ground

5.2.2 PAH concentration in silicone rubber

Silicone rubber exposed to the sediments accumulated PAHs from the sediments as was also shown in Chapter **3**. A high proportion (~ 73 %) of the 4- and 5-ring PAHs was absorbed into the individual silicone rubber sheets from the sediments (Fig **5.3**).

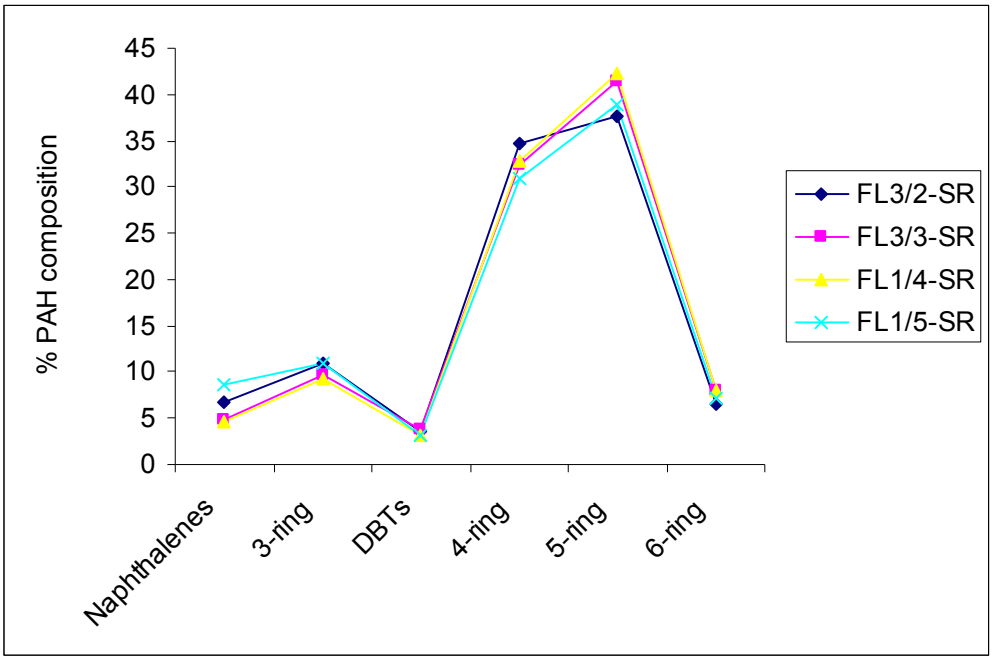


Fig **5.3**: Percent PAH composition in silicone rubber (SR) strips exposed to all four sediments

5.2.3 Pore water and water extractable concentrations

The pore water (freely dissolved) concentrations were calculated as described in Chapter **1** using isotherms (Fig **1.6**, chapter **1**) created from concentrations in the silicone rubber and the phase ratios (Table **5.0**).

Briefly, pore water concentration (C_w) is calculated from the concentration in the silicone rubber using equation **5.0** below

$$C_w = \frac{C_{sr}}{K_{sr,w}} \qquad \textbf{5.0}$$

where, C_{sr} = concentration in the silicone rubber / ng g^{-1} SR (sr and SR denoting silicone rubber), $K_{sr,w}$ = silicone rubber-water partition coefficient / l kg^{-1}

$$C_{sr} = \frac{N_{sr}}{M_{sr}} \qquad \textbf{5.1}$$

N_{sr} = Amount of PAH in the silicone rubber, M_{sr} = mass of silicone rubber

Similarly the concentration extracted from the sediment (C_{SedEx}) is calculated from equation **5.2** below

$$C_{SedEx} = \frac{N_{sr}}{M_{sed}} \qquad \textbf{5.2}$$

M_{sed} = Mass of sediment / ng g^{-1} dry weight

Sorption isotherms (examples in Fig **5.4** – **5.7**) were plotted for the four sediments and only plots that yielded positive linear relations (in this case taken as $r^2 \geq 0.5$) were used to estimate the dissolved concentrations (C_w^0) in pore water and water extractable fractions by extrapolation to both axes. The data obtained from the extrapolation are presented in Table **5.2**. However for some PAHs, the plots yielded poor correlations ($p > 0.05$) especially for the 2- to 3-ring PAHs (plots not shown). This could be as a result of the low concentrations found in the sediments or due to poor interactions between the silicone rubber and the aqueous phase (sandy sediments tend to settle into a solid phase at the base of the bottle), as desorption from the sediment into the aqueous phase then becomes a rate limiting step (Huckins *et al*., 2006) leading to uncertainty in the results obtained. Ahmed *et al*. (2006) had also found low total PAH concentrations in sediments from the Fladen Ground.

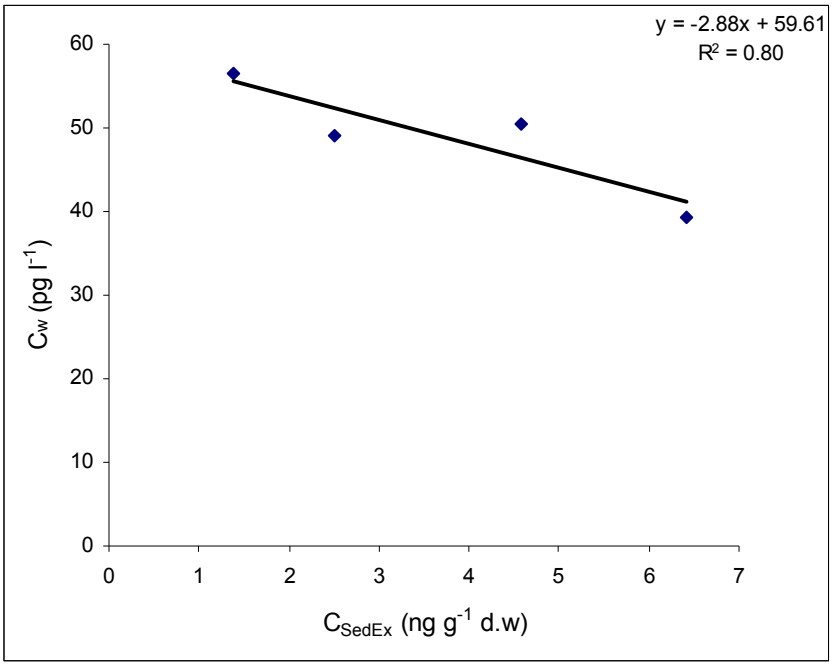


Fig **5.4**: Sorption isotherm for benzofluorathenes in FL3/2, showing the obtained regression equation

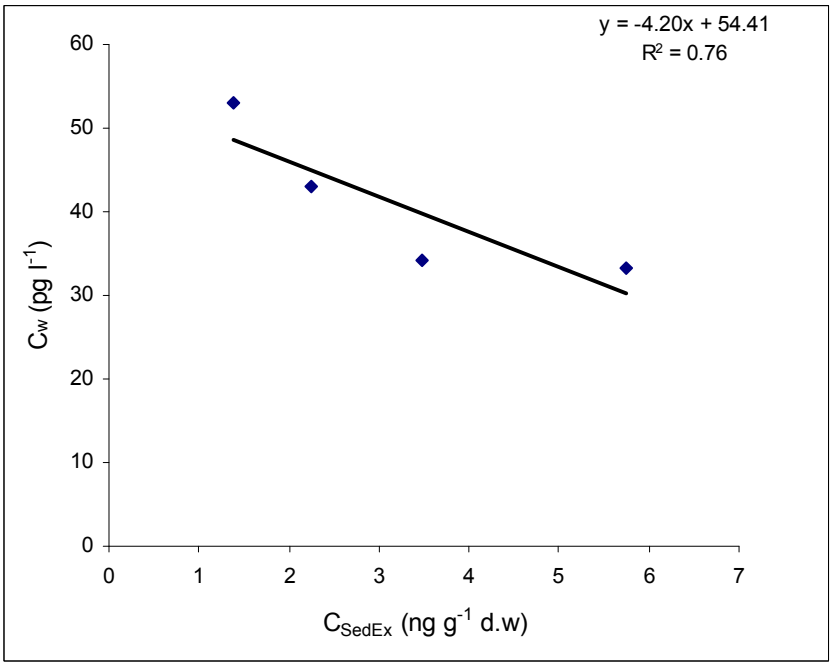


Fig **5.5**: Sorption isotherm for benzofluoranthenes in FL3/3, showing the obtained regression equation

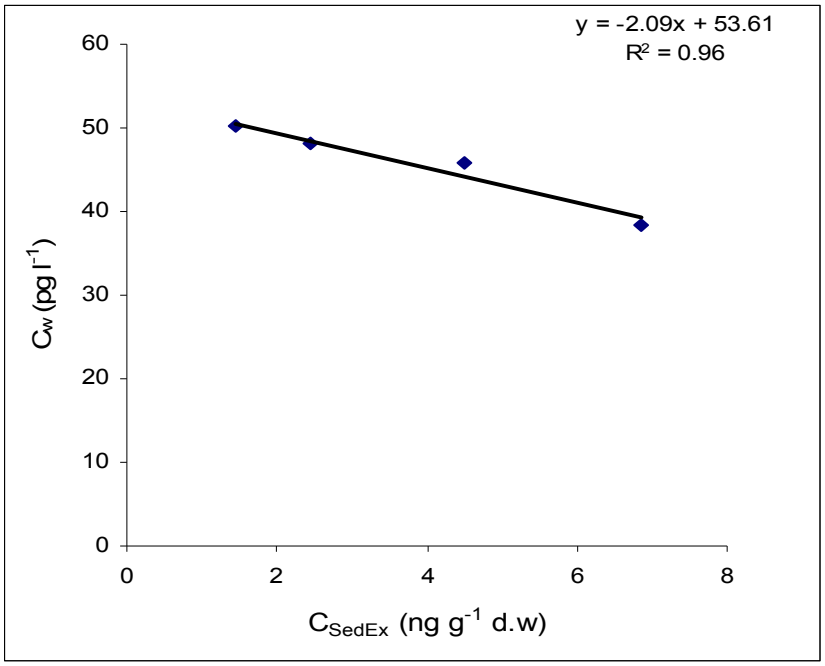


Fig **5.6**: Sorption isotherm for benzofluoranthenes from FL1/4, showing the obtained regression equation

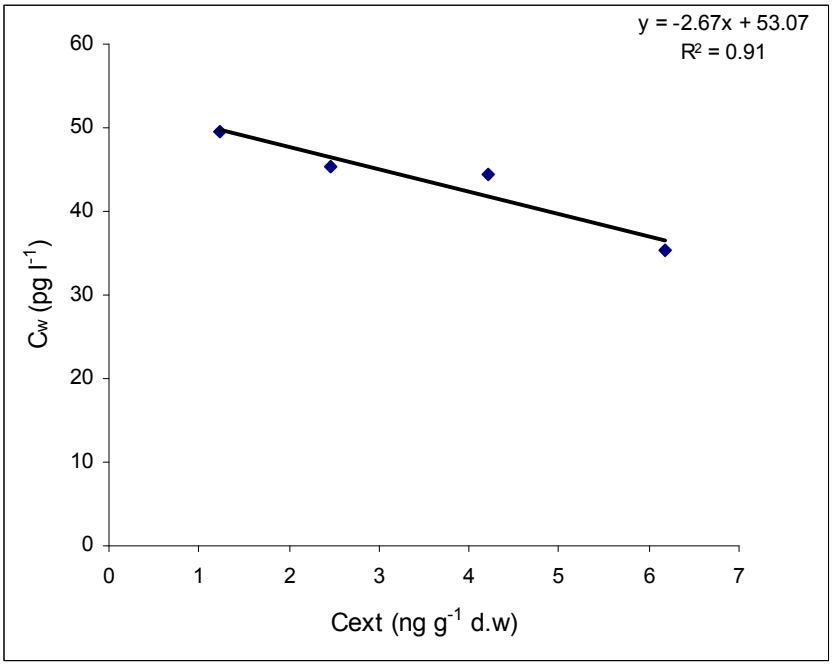


Fig **5.7**: Sorption isotherm for benzofluoranthenes from FL1/5, showing the obtained regression equation

Smedes *et al.* (2006) defined a term called "distribution factor (DF)" as the ratio of the amount of PRC left in the silicone rubber sheets to that absorbed by the sediment (equation **5.3**) which helps in assessing whether the silicone sheets have depleted the sediment of the contaminants. A DF value of ≤ 0.1 implies the measured pore water concentration will not be affected by depletion for PAHs although the test value may be higher for CBs.

$$DF = \frac{N_{sr}}{N_{sr}^{0} - N_{sr}} \qquad \textbf{5.3}$$

N_{sr}^{0} = Initial amount spiked onto the silicone rubber sheet; N_{sr} = Amount left in the silicone rubber

Calculating the DF for the PRCs used (deuterated chrysene and benzo[*e*]pyrene) for each of the phase ratios showed a gradual increase in DF with increasing phase ratios (e.g. for FL3/2, the DF's were 0.07, 0.17, 0.38 and 0.39 for bottles A, B, C and D respectively), which indicates a varying degree of depletion used in creating isotherms for estimation of the availability parameters.

The water exchangeable concentrations have been defined earlier as the concentration that could potentially be mobilised into the water phase and become available for exchange/ partitioning. These were calculated and are given in Table **5.3**. In most cases, the C_{SedEx} values were either close to or in a few cases, exceeded the sediment PAH concentration. For most of the PAHs that had $r^2 > 0.5$, the water exchangeable fraction was mostly > 50 % of the total sediment concentration, suggesting the remaining fraction are tightly bound to the sediment, although for a few PAHs such as benzofluoranthenes and benzo[*e*]pyrene (especially in FL3/2 and FL3/3) the fraction exceeded 100 %. This may be due to measurements being made at concentrations that were close to detection limits thus overestimating the amounts extracted.

Table **5.3**: Pore water concentrations (pg L^{-1}), water exchangeable concentration (ng g^{-1} dry weight) and water exchangeable fraction (F_{SedEx}; as proportion of sediment concentration) determined from sorption isotherms for the four Fladen Ground sediments

	FL3/2				**FL3/3**			
	C_w	C_{SedEx}	F_{SedEx}	r^2	C_w	C_{SedEx}	F_{SedEx}	r^2
C2-202	13.39	0.89	1.11	0.72	8.98	1.13	0.81	0.48
Chrysene	96.83	1.06	1.18	0.58	nr[1]	nr	nr	<0.5
Benzofluoranthenes	59.61	20.70	3.04	0.80	54.41	12.94	1.27	0.76
Benzo[*e*]pyrene	30.20	10.16	4.62	0.67	28.39	5.13	1.60	0.82
Benzo[*a*]pyrene	5.81	1.80	2.00	0.73	nr	nr	nr	<0.5
C1-252	7.87	12.56	3.06	0.68	7.68	10.37	1.48	0.59
Dibenz[*a,h*]anthracene	nr	nr	nr	<0.5	1.29	0.52	0.65	0.80
	FL1/4				**FL1/5**			
	C_w	C_{SedEx}	F_{SedEx}	r^2	C_w	C_{SedEx}	F_{SedEx}	r^2
C2-178	110.50	0.58	0.17	0.80	171.65	0.36	0.13	0.51
Fluoranthene	715.87	1.37	0.65	0.81	896.91	0.77	0.51	0.75
Pyrene	433.60	1.04	0.70	0.76	500.87	0.61	0.51	0.63
C1-202	188.17	1.32	0.51	0.84	241.05	0.71	0.38	0.79
C2-202	10.62	0.88	0.46	0.95	12.02	0.60	0.50	0.92
Benzo[*c*]phenanthrene	17.89	0.12	0.29	0.47	20.18	0.10	0.50	0.72
Benz[*a*]anthracene	21.84	0.71	0.59	0.90	23.22	0.39	0.44	0.92
Chrysene	80.41	1.33	0.58	0.89	92.51	0.82	0.54	0.87
Benzofluoranthenes	53.61	25.66	1.23	0.96	53.07	19.90	1.57	0.91
Benzo[*e*]pyrene	26.75	11.26	1.73	0.89	27.05	8.63	2.16	0.89
Benzo[*a*]pyrene	4.86	2.04	0.66	0.95	4.90	1.32	0.66	0.78
C1-252	7.83	14.35	1.01	0.87	7.37	9.35	1.11	0.72
Benzo[*g,h,i*]perylene	8.38	14.15	0.94	0.59	nr	nr	nr	<0.5
Dibenz[*a,h*]anthracene	1.20	1.49	0.65	0.56	1.03	1.14	0.95	0.48

[1]not reported ($p > 0.05$)

A profile of the freely dissolved concentrations and water exchangeable concentrations is shown in Fig **5.8** for benzo[*e*]pyrene as an example, with no apparent difference in the pore water concentrations observed. Fluoranthene and pyrene showed higher freely dissolved concentrations compared to high molecular weight compounds like benzofluoranthenes and benzo[*e*]pyrene that were more abundant in the sediment, which is likely due to strong sorption of the high molecular (high K_{ow}) compounds to the sediment. The pore water concentrations estimated in this study (PAHs that yielded positive correlations) were well below the European Union Council (EU)- proposed Maximum Allowable Concentration-

Environmental Quality Standards (MAC-EQS) for PAHs in surface waters such as anthracene (0.4 µg L^{-1}); benzo[*a*]pyrene (0.1 µg L^{-1}); 0.03 µg L^{-1} for benzofluoranthenes and 0.002 µg L^{-1} for both benzo[*g,h,i*]perylene and indeno[1,2,3-*cd*]pyrene (EU, 2007).

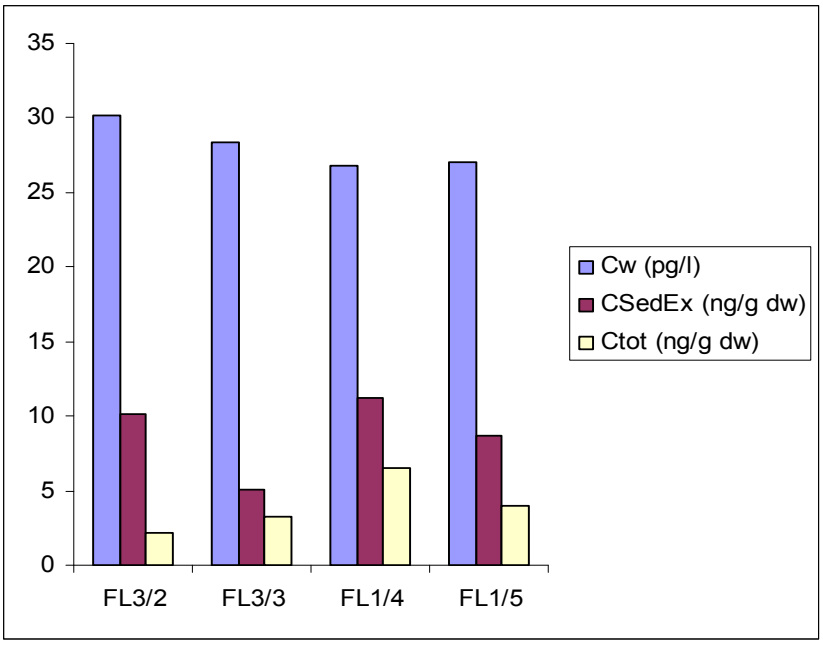


Fig **5.8**: Benzo[*e*]pyrene profile of sediments from Fladen Ground, showing the pore water (C_w), water exchangeable (C_{SedEx}) and sediment PAH (C_{tot}) concentration

5.2.4 Sediment-water partition coefficients

Sediment-water partition coefficients ($K_{sed,w}$) describe the partitioning of POPs between sediment and water phases, and to a large degree determine the distribution of the POPs in the environment. The importance of methodology in determining these coefficients was highlighted by Harkey *et al*. (1994); where they used four methods to determine the partition coefficients. Other methods have been used in determining these coefficients such as from the Free-energy relationship of Karickhoff *et al*. (1979), co-solvent method (Jonker and Smedes, 2000), the use of polyoxymethylene strips (Jonker and Koelmans, 2001), etc, with most of the methods dependent on attainment of equilibrium. At equilibrium, the ratio of the concentration

in the sediment to the concentration in the water phase gives the partition coefficients (equation **5.4**) although the use of equilibrium partitioning as a good approximation of real situations is being questioned (Borglin *et al*., 1996).

$$K_{sed,w} = \frac{C_{sed}}{C_w} \qquad \textbf{5.4}$$

The dependence of sorption of POPs to components of sediment with sorbent properties such as organic material has been shown (Karickhoff *et al*., 1979; Borglin *et al*., 1996 and Chen *et al*., 2000) and mostly the partition coefficients are expressed relative to the organic carbon content as:

$$K_{oc} = \frac{K_{sed,w}}{f_{oc}} = \frac{C_{sed}}{f_{oc} \cdot C_w} \equiv \frac{C_{oc}}{C_w} \qquad \textbf{5.5}$$

$f_{oc} =$ Organic carbon fraction, $C_{oc} =$ Organic carbon normalised sediment concentration, $K_{oc} =$ partition coefficient normalised to organic carbon content.

Having established equilibrium conditions in the exposure of sediments to silicone rubber in this study, K_{oc} were calculated from the plots (Fig **5.9** and **5.10)** of C_w at versus C_{res} ($C_{Sed} - C_{SedEx}$) at each phase ratio (see chapter **1**, Fig **1.7**). The slope of such a plot equals $1/K_{sed,w}$.

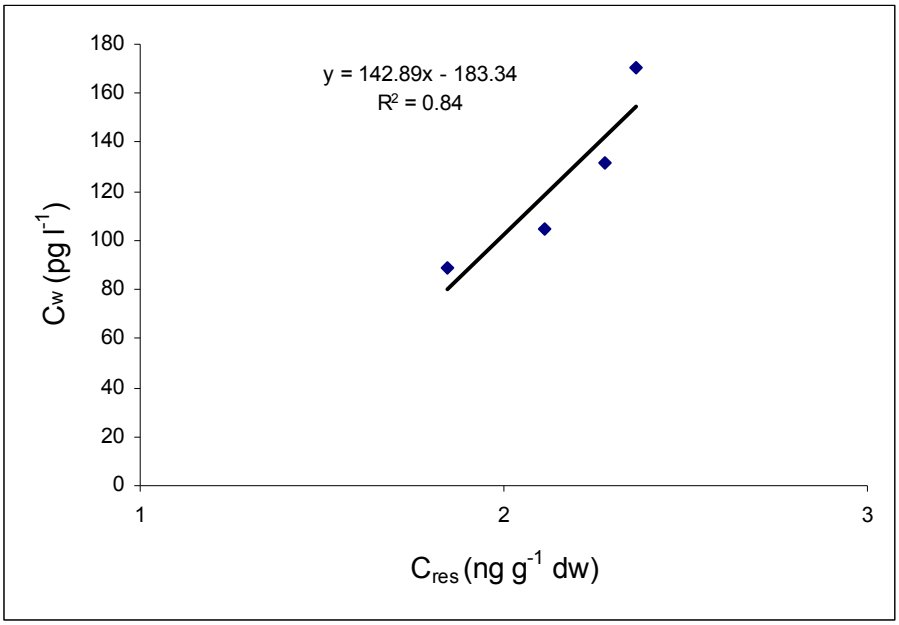


Fig **5.9**: Plot to determine $K_{sed,w}$ from FL1/4 for C1-202

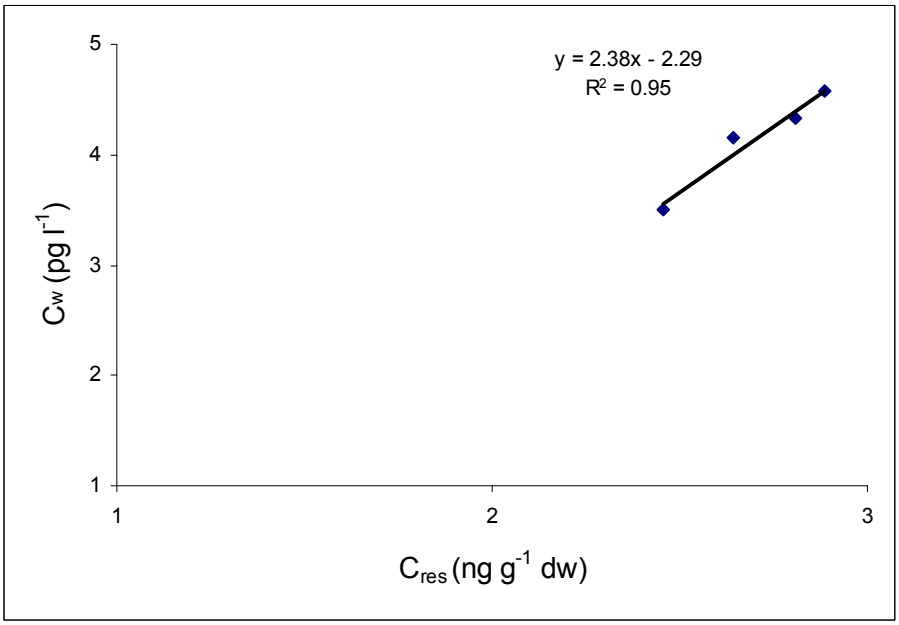


Fig **5:10**: Plot to determine $K_{sed,w}$ from FL1/4 for benzo[*a*]pyrene

Sediment-water partition coefficients normalised to total organic carbon content (K_{oc}) are presented (Table **5.4**) for PAHs that yielded positive correlations (basis of r^2 >0.5). It should be noted also that such estimations have a high level of uncertainty (high standard deviations of the fits) as any uncertainty in the regression line causes a significant uncertainty in the partition coefficients.

The estimated log K_{oc} , however, show some consistency across the locations sampled, and correlated positively with log K_{ow} (e.g. Fig **5.11**) which has been shown to be the case (Karickhoff *et al*., 1979; Jonker and Smedes, 2000; Jonker and Koelmans, 2001 and Booij *et al*., 2003) for sediments.

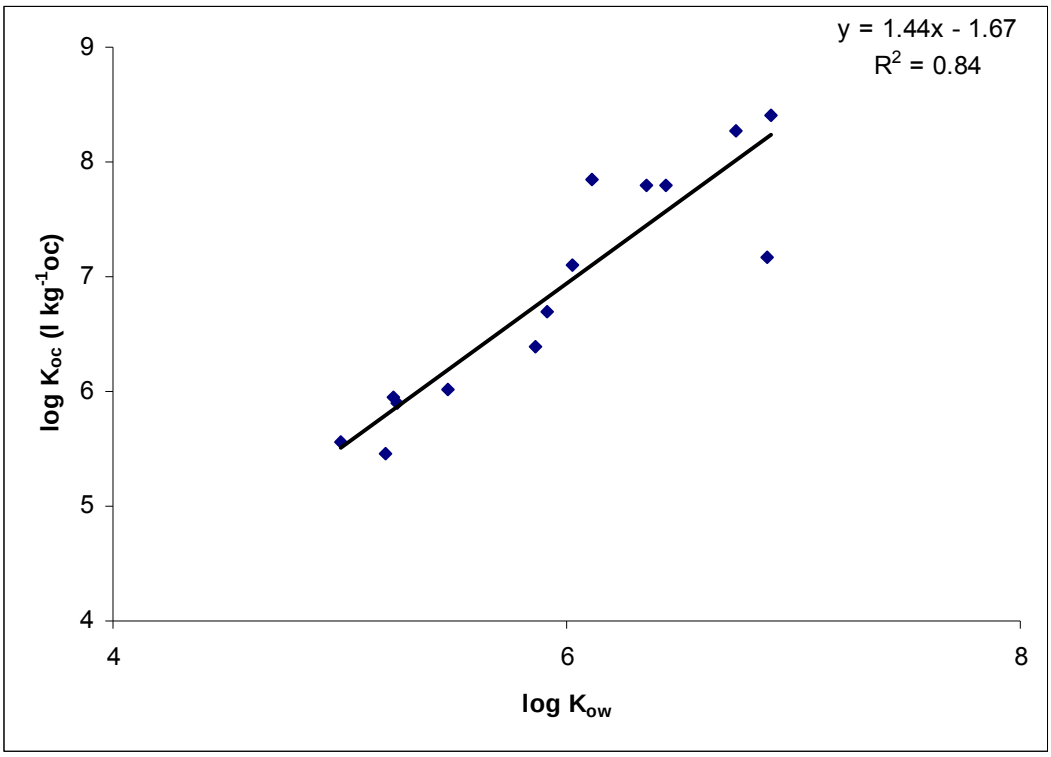


Fig **5.11**: Log K_{oc} plotted against log K_{ow} (Sangster, 2005) for sediment from FL1/4 where there were more values determined

When all the Log K_{oc} values (from the 4 stations) are plotted, the relationship did not change though the correlation slightly worsened ($\log K_{oc} = 1.43 \log K_{ow} - 1.69$; $r^2 = 0.77$).

Table **5.4**: Sediment partition coefficients normalised to organic carbon content estimated from plots of C_w versus C_{res}

	log K_{oc} (L kg^{-1} oc)					
PAH	FL3/2	FL3/3	FL1/4	FL1/5	A†	B‡
2-Methylphenanthrene (C1-178)	nd	nd	5.95	nr	5.80	5.03
3,6-Dimethylphenanthrene (C2-178)	nd	nd	5.90	5.58	5.82	5.04
Fluoranthene	nd	nd	5.46	5.19	5.75	4.99
Pyrene	nd	nd	5.56	5.34	5.46	4.79
1-Methylfluoranthene (C1-202)	nd	nd	6.02	5.72	6.15	5.27
2,7-Dimethylpyrene (C2-202)	7.15	7.29	7.09	6.95	6.93	5.82
Benzo[*c*]phenanthrene	nd	nd	nd	5.95	6.55	5.55
Benz[*a*]anthracene	nd	nd	6.69	6.48	6.76	5.70
Chrysene	6.37	nd	6.39	6.20	6.69	5.65
2-Methylchrysene (C1-228)	7.16	7.17	7.16	7.03	8.15	6.67
Benzofluoranthene	7.87	7.57	7.85	7.83	7.05	5.90
Benzo[*e*]pyrene	7.86	7.45	7.80	7.76	7.52	6.23
Benzo[*a*]pyrene	7.82	7.68	7.80	7.68	7.39	6.14
7-Methylbenzo[*a*]pyrene (C1-252)	8.53	8.32	8.43	8.35	8.32	6.79
Benzo[*g,h,i*]perylene	nd	nd	8.40	nd	8.18	6.69
Dibenz[*a,h*]anthracene	nd	7.80	8.27	8.29	7.96	6.54

nd- not determined ($p > 0.05$); † Log K_{oc} estimated from the combined equation $\log K_{oc} = 1.43 \log K_{ow} - 1.69$; ‡ Estimated Log K_{oc} from Karickhoff *et al*. (1979)

Log K_{oc} calculated for individual PAHs (for C2-202, C1-228, benzofluoranthene, benzo[*e*]pyrene, benzo[*a*]pyrene and C1-252 which were the only PAHs that were calculated in all the stations) only differed by a maximum of 0.4 log units across the four stations. Log K_{oc} calculated from the combined relation ($\log K_{oc} = 1.43 \log K_{ow} - 1.69$) differed from that predicted by the Karickhoff relation ($\log K_{oc} = \log K_{ow} - 0.21$) by ~ 0.7-1.5 log units. However they were 1-2 log units lower than those calculated from the $\log K_{oc} - \log K_{ow}$ relationship reported for CBs and PAHs by Jonker and Smedes (2000) in

sediments (0-30 cm layer) from Lake Ketelmeer, Netherlands (determined using the cosolvent method). The high partition coefficients for PAHs found by Jonker and Smedes were attributed to extremely high soot-water partition coefficients of the PAHs.

5.3 Conclusions

Using the silicone rubber sampling protocol developed in Chapters **3** - **4** for pore water, freely dissolved concentrations of some PAHs were estimated from sorption isotherms (those with $r^2 > 0.5$) in the pg L^{-1} levels for sediments sampled within the Fladen Ground of the North Sea. The pore water concentrations did not exceed the Maximum Allowable Concentration-Environmental Quality Standard (MAC-EQS) values proposed under the European Union environment council for PAHs that could be compared.

A proportion of some of the PAHs in the sediments appear to be unavailable for exchange into the aqueous phase and therefore unavailable for uptake although for others the method appeared to have overestimated this proportion (> 100 %) which is attributed to the low sediment PAH concentrations.

The method also allowed for the estimation of sediment partition coefficients normalised to organic carbon which correlated positively with $\log K_{ow}$ and would be useful in sediment risk assessments for the PAHs. The study confirmed the unreliability of measurements made with sandy sediments containing low organic carbon content, thus subsequent applications will focus on sediment samples that meet the criteria set in the draft guidelines. Further exposures of sediment to silicone rubber sheets would be in light and temperature controlled room.

CHAPTER SIX

Availability of PAHs in pore waters and sediments from Loch Shell, Scotland

6.0 Introduction

Persistent organic pollutants (POPs) such as polycyclic aromatic hydrocarbons (PAHs) are highly ubiquitous contaminants due to their hydrophobic nature and low water solubility and tend to be associated with particulate organic matter with their persistence in sediments well documented (Webster *et al*., 2001; Webster *et al*., 2004). Availability of organic contaminants has been recognised as an important tool in the risk assessment of sediments (Di Toro *et al*., 1991, Webster *et al*., 1997) and that the pore water (freely dissolved) concentrations of these POPs reflects availability (for uptake/accumulation in biota) as it represents the concentration that is key to biological effects of the POPs such as PAHs. However, it is difficult to distinguish between the dissolved form of contaminants and their sorbed forms (Smedes, 1994; Mayer *et al*., 2000), as large volumes of sediment pore waters would be required to determine the dissolved concentration due to their low levels. This necessitated the development of techniques and tools to measure the available concentrations from sediment pore waters and water such as solid phase micro-extraction (SPME) fibers (Mayer *et al*., 2000; Heringa and Hermens, 2003); low density polyethylene (LDPE) strips (Booij *et al*., 2003; Vinturella *et al*., 2004); semi permeable membrane devices (SPMD) developed by Huckins *et al*. (1990) and used by Booij *et al*. (1998) and the silicone rubber passive sampler (ICES WGMS, 2003) being used in this work. The silicone rubber sampler operates as an equilibrium sampling device on the premise of existing or attaining equilibrium between the sediment and pore water (equilibrium partitioning theory) and the silicone rubber sampler. The equilibration period is dependent on the physicochemical properties of the contaminants and exposure conditions (Vinturella *et al*., 2004), and has been determined in Chapter **3**.

Therefore the developed method was applied to sediment samples collected from Loch Shell to estimate the pore water (freely dissolved), water extractable (accessible) concentrations as well as the sediment-water partition coefficients of PAHs by creating sorption isotherms as described in chapter **1**.

6.1 Materials and Methods

6.1.1 Materials

HPLC grade solvents (dichloromethane, ethyl acetate, acetone, methanol and *iso*-hexane) were purchased from Rathburn Chemicals Ltd, Scotland, UK. Certified solid standards for PAHs (including deuterated PAHs) were obtained from QMX Laboratories, Essex, UK and dissolved in *iso*-hexane to obtain required concentrations of spiking solutions. To avoid contamination of samples, all glassware, stainless steel forceps, was either washed in Decon® 180 solution and rinsed with distilled water or washed in a CAMLAB GW 4050 glassware washer and dried in an oven at 100 ^{o}C. Before use, the glassware were rinsed twice each in dichloromethane and *iso*-hexane, with the latter allowed to evaporate to dryness. Glass Duran® or Schott® (VWR, Leicester, UK) bottles were used and always capped with aluminium-lined PTFE caps to reduce or prevent sorption of POPs by the caps.

AlteSil™ Silicone rubber sheet (600 × 600 mm, 0.5 mm thick) used as passive sampling material was purchased from Altec Products Ltd, Cornwall, UK. The silicone rubber sheets used were cut into required dimensions (6 × 4 cm, or 9 × 4 cm) and pre-extracted in hot ethyl acetate using a Soxhlet apparatus for ~ 4 days before use to remove any low molecular weight oligomers or residues that may be co-extracted with the analytes and could affect instrumental analysis. After pre-extraction and cooling, the sheets were removed from the solvent and stored in bulk in glass jars containing methanol prior to use. The weight of the sheets used was determined after extraction of exposed sheets to avoid contamination from the environment.

6.1.2 Methods

6.1.2.1 Sediment sampling

Loch Shell is located in the East coast of the Isle of Lewis (Western Isle) of Scotland and is known to support some fish farms. Samples of surface sediment (12-14 cm of mud) were collected from FRV *Clupea*, using a 0.1 m^2 Day Grab at depths between 39 and 42 m. The top 2 cm of the sediment was transferred to a solvent washed aluminium can, thoroughly mixed, labelled and stored at -20 ± 5 ^{o}C until required for analysis. The samples were collected at 25, 90, 150 m distances from the fish farm (Fig **6.0**). The sediment bulk properties were also determined using the methodology in section **2.1**.

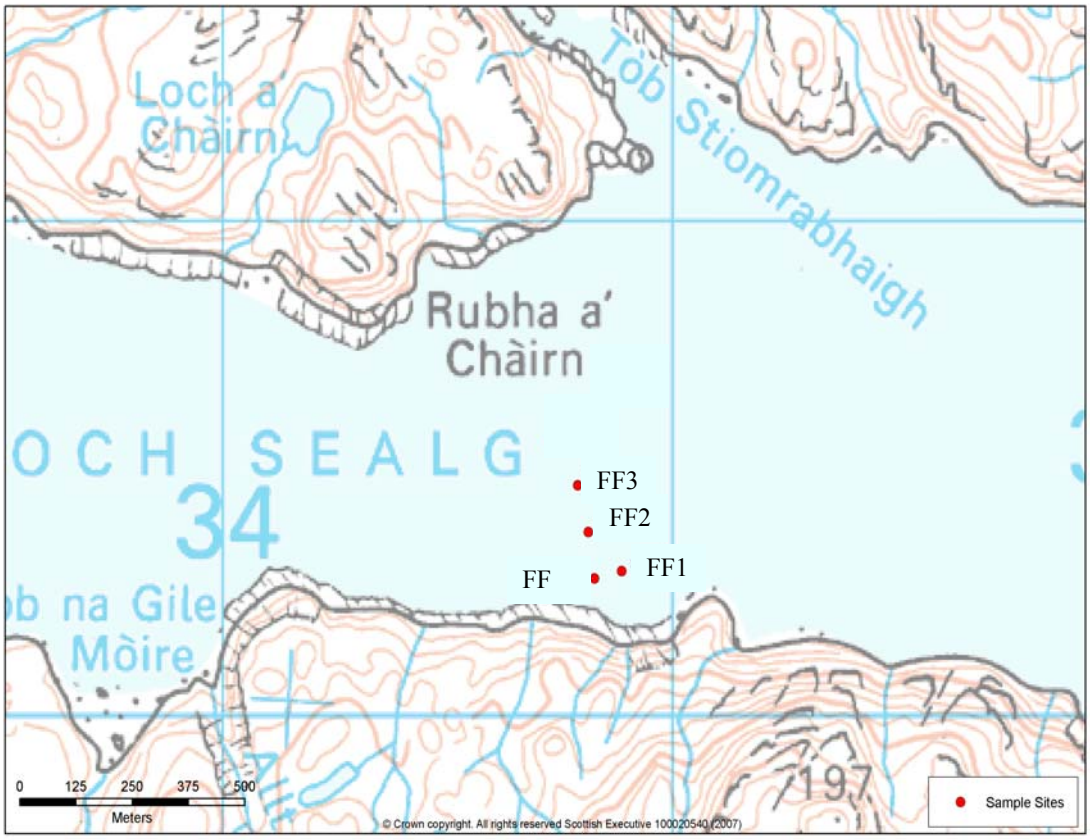


Fig **6.0**: Sampling location- Loch Shell, Scotland close to a fish farm on the South shore of the loch. FF1, FF2 and FF3 represent sampling points 25, 90 and 150 m away from the fish farm at FF

6.1.2.2 Hydrocarbon analysis

Hydrocarbon analysis of PAHs was as described in chapter **2**, section **2.3.3.1**, **2.3.3.1.1** and **2.3.3.1.3.**

6.1.2.3 Determination of availability of PAHs in sediments

Sediment samples from the loch were defrosted at room temperature, ensuring that exposure to direct sunlight or heat was minimised and the samples thoroughly mixed before sub sampling for analysis using the protocol developed in chapter **3** for the determination of pore water concentrations, available proportions and sediment-water partition coefficients of PAHs.

The samples were weighed into glass Duran® bottles, and water added to liquefy the sediment where necessary so as to ensure proper interaction between the sediment slurry and the sheets. A single pre-extracted silicone rubber sheets was added to each bottle to obtain different phase (g silicone rubber per g sediment) ratios. The bottles were placed on an orbital shaker horizontally and shaken at 200 rpm for 20 days in a light and temperature controlled room. The sheets were then removed from the bottles, rinsed with distilled water and gently wiped dry with paper tissue to remove any adhering water.
The sheets were extracted as described in chapter **3** (section **3.3.1.1**) and the weight of the silicone rubber sheet recorded after the extraction to avoid contamination from external sources. A procedural blank (an un-spiked sheet exposed to only the distilled water used to liquefy the sediments) was included and analysed in the same manner as the other samples. The result from the procedural blank was subsequently subtracted from the results of samples. No performance reference compounds were available at the time of exposure and were therefore not used in this study.

6.2 Results and Discussion

6.2.1 PAH concentration in sediment samples

The total sediment PAH concentration (2- to 6-ring parent and alkylated PAHs, Table **6.0** and Fig **6.1**) was 632.8, 700.3 and 271.9 ng g^{-1} dry weight from FF1 (25 m), FF2 (90 m) and FF3 (150 m) respectively, with the 2- to 3-ring PAHs accounting for ~ 24 % while the 4- to 6-ring compounds dominated with ~ 74 % of the total concentrations. The sediment total PAH concentration at 25 and 90 m were on average a factor of 2.5 higher than the concentration from 150 m. The total PAH concentrations (2- to 6-ring parent and alkylated PAHs) in the samples from Loch Shell were similar to total PAH concentrations (2- to 6-ring parent and alkylated PAHs) found in lochs classed as having moderate concentrations (Loch Clash, Laxford, Glendhu and Torridon) by Webster *et al*. (2004).

Table **6.0**: Sediment PAH concentration (ng g^{-1} dry weight) and bulk sediment properties of samples collected from the Loch Shell in 2006

Field ID	FF1	FF2	FF3
% OC	2.561	2.344	2.24
PS: % < 63µm	89.853	92.856	93.168
Naphthalenes[1]	80.5	92.5	40.3
3-ring[2]	66.0	74.1	31.7
DBTs[3]	9.4	11.0	5.3
4-ring[4]	162.0	182.5	69.6
5-ring[5]	207.9	228.9	84.6
6-ring[6]	107.0	111.3	40.4
∑PAHs	632.8	700.3	271.9

[1]Sum of naphthalene; 1 & 2-methylnaphthalene; C2-, C3- and C4- naphthalenes
[2]Sum of phenanthrene; anthracene, C1-178, C2-178, and C3-178; acenapthylene; acenaphthene and fluorene
[3]Sum of Dibenzothiophene; C1-DBT, C2-DBT, and C3-DBTs
[4]Sum of fluoranthene; pyrene; C1-202, C2-202, and C3-202; benzo[*c*]phenanthrene; benz[*a*]anthracene; benz[*b*]anthracene; chrysene; C1-228; C2-228
[5]Sum of benzofluoranthenes; benzo[*e*]pyrene; benzo[*a*]pyrene; perylene; C1-252, C2-252 and dibenz[*a,h*]anthracene
[6]Sum of indeno[*1, 2, 3-cd*]pyrene; benzo[*ghi*]perylene; C1-276 and C2-276
Note: Acenaphtylene was found only at trace levels in the sediment

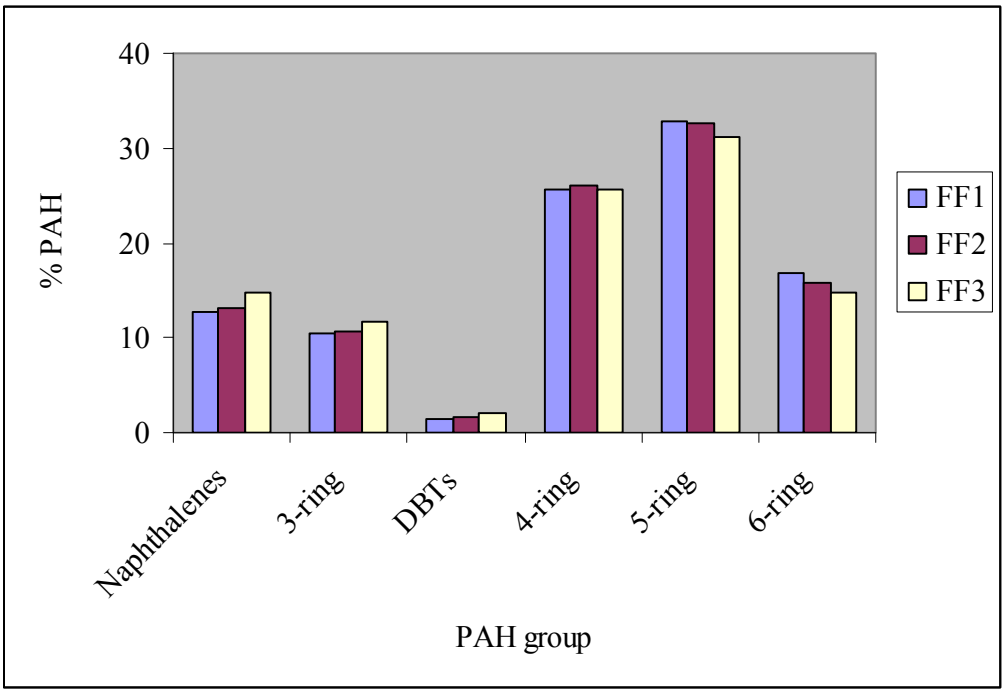
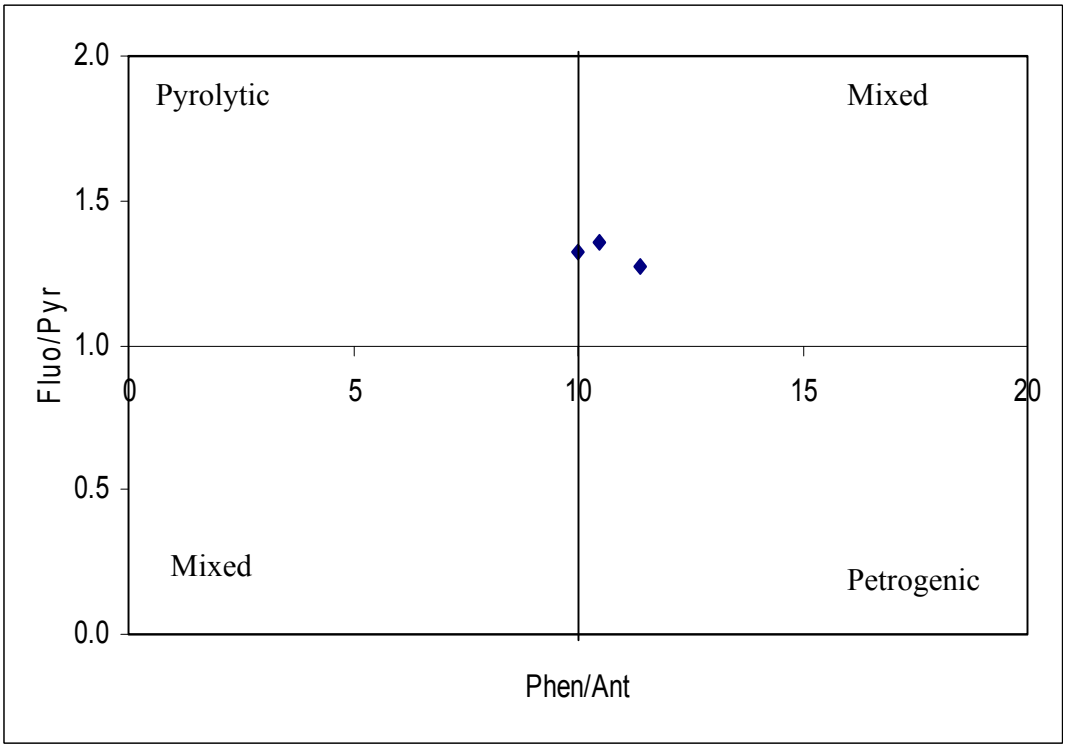


Fig **6.1**: PAH composition in sediment samples from Loch Shell. FF1, FF2 and FF3 represent samples collected at 25, 90 and 150 m away from the position of a fish farm

PAH concentration ratios have been used (Webster *et al*., 2001; Webster *et al*., 2005) to aid identification of PAH sources. A Fluo/Pyr ratio > 1 and Phen/Ant ratio less than 10 or C1-178 (MP)/Phen ratio < 2 indicates a pyrolytic source while contrastingly; a petrogenic source is indicated by a Fluo/Pyr ratio < 1, a Phen/Ant ratio > 10 and C1-178/Phen ratio > 2. Plotting these ratios (Figure **6.2**) suggests the source of the PAHs to be from a mixed source (top panel) while the lower panel showed the source to be mainly pyrolytic, although the low amounts of anthracene in the sediments could have led to the observed high Phen/Ant ratio thus overestimating this ratio. Low anthracene proportions at remote sites may be linked to atmospheric deposition (Ahmed *et al*., 2006). Loch Shell is known to have some fish farms that use fish feed which contains large amounts of lipids and associated contaminants such as PAHs (Easton *et al*., 2002) and would normally experience boat activity or traffic. Sather *et al*. (2006) found similar PAH concentrations (511-2736 ng g^{-1} dry weight, sum of 54 parent and alkylated PAHs) in sediments from around fish farms located in New Brunswick, Canada.



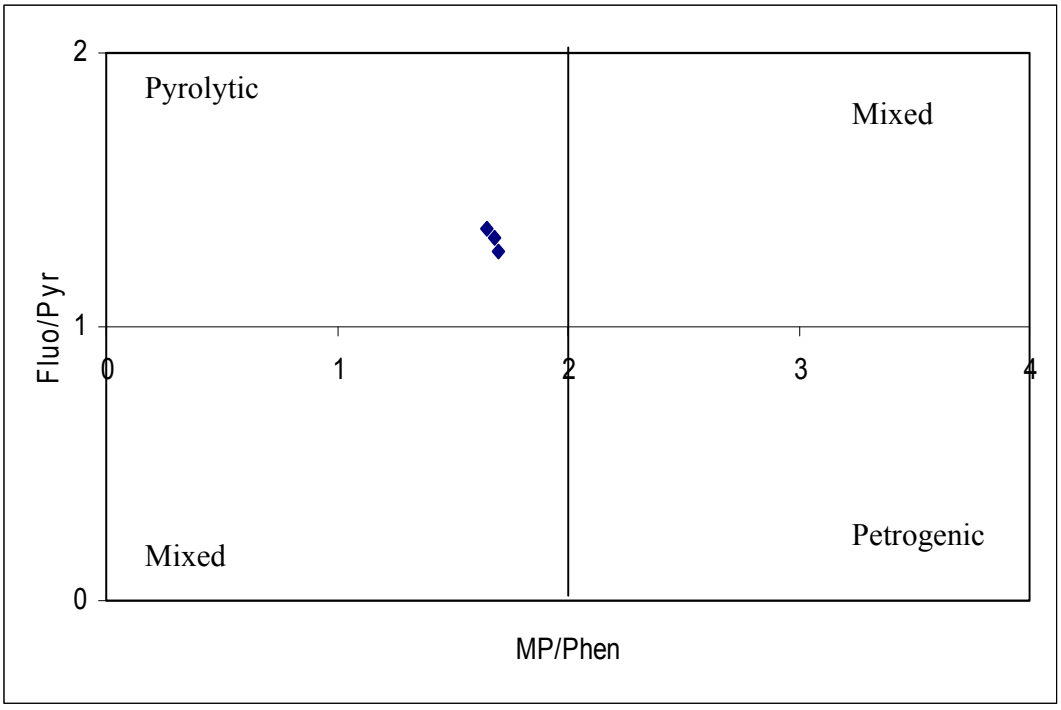


Fig **6.2**: PAH concentration ratios in sediment from Loch Shell to aid source identification

Provisional Oslo and Paris Commission (OSPAR) Background Concentrations (BCs) and Background Assessment Concentrations (BACs) have been established for ten parent PAHs in sediments (OSPAR, 2006). The Background Concentration has been defined as the concentration of a contaminant at a "pristine" or "remote" site based on contemporary or historical data. Observed concentrations are said to be 'near background' if the mean concentration is statistically significantly below the corresponding Background Assessment Concentration (BAC). Only single measurements of PAH concentrations were made in this study, therefore the individual PAH concentrations for each station were normalized to 2.5 % organic carbon and an approximate 95 % confidence interval calculated using uncertainty values from method validation data at Fisheries Research Services Marine Laboratory, Aberdeen (FRS ML). The normalized concentrations for individual PAHs exceeded the BACs at the 5 % significance level (Table **6.1**)

Table **6.1**: Available OSPAR Background Concentrations and provisional Background Assessment Concentration (both ng g^{-1} dry weight) normalised to 2.5 % organic carbon (OSPAR, 2006) and corresponding concentrations in the sediment samples from Loch Shell, also normalised to 2.5 % organic carbon with 95 % confidence interval of concentrations at the individual stations expressed as a percentage

	(ng g^{-1} dry weight)					
			Station			
	***BC*[1]**	***BAC*[2]**	**C.I. (%)**	**FF1**	**FF2**	**FF3**
Naphthalene	5	8	30	224	252	132
Phenanthrene	17	32	28	480	544	228
Anthracene	3	5	24	48	52	20
Fluoranthene	20	39	25	624	732	296
Pyrene	13	24	25	472	540	232
Benz[*a*]anthracene	9	16	33	300	344	132
Chrysene	11	20	38	448	496	200
Benzo[*a*]pyrene	15	30	22	568	616	244
Indeno[1,2,3-*cd*]pyrene	50	103	19	1664	1880	680
Benzo[*ghi*]perylene	45	80	19	1448	1532	568

[1]Background Concentration; [2]Background Assessment Criteria

6.2.2 Concentration of PAHs in silicone rubber

Silicone rubber exposed to the sediments accumulated PAHs from the sediments as was shown in chapter **3**. The amounts of PAHs (grouped by ring number) reflected the profile found in the sediments (Fig **6.3**) with the 4- to 6-ring PAHs dominating (average ~ 78 %) in the silicone rubber exposed to the sediments and the 2- to 3-ring PAHs accounting for an average of ~ 18 % in the silicone rubber.

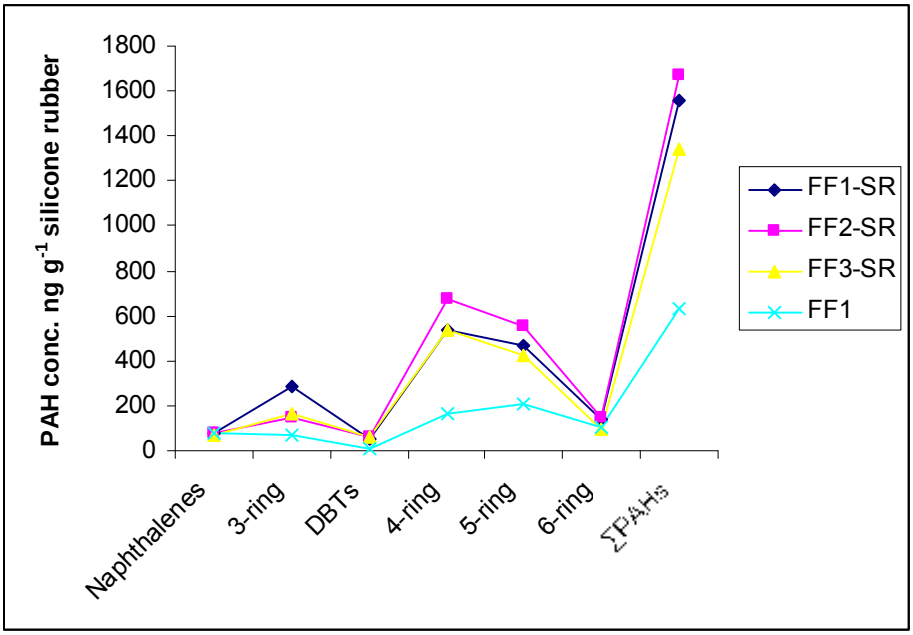


Fig **6.3**: PAH concentration accumulated by silicone rubber exposed to sediments from Loch Shell. FF1 represents the sediment profile while FF1, FF2 and FF3-SR are silicone rubber samplers exposed to sediment at a phase ratio of 0.01

6.2.3 Freely dissolved and water extractable concentration

The freely dissolved (C_w^o) and water extractable concentration (C_{SedEx}^o) for each PAH were determined by extrapolation from sorption isotherms of the pore water concentration versus concentration of sediment extracted by the silicone rubber. The dissolved concentrations (C_w) were calculated at each phase ratio from the concentrations determined in the silicone rubber

and the corresponding silicone rubber-water partition coefficient ($K_{sr,w}$) of the PAH determined in chapter **4** using Eq **6.0** below:

$$C_w = \frac{C_{sr}}{K_{sr,w}} \qquad \textbf{6.0}$$

where, C_{sr} = concentration in the silicone rubber / ng g^{-1} SR (sr and SR denoting silicone rubber) and is given by

$$C_{sr} = \frac{N_{sr}}{M_{sr}} \qquad \textbf{6.1}$$

N_{sr} = Amount of PAH in the silicone rubber, M_{sr} = mass of silicone rubber

Similarly the concentration extracted from the sediment (C_{SedEx}) is calculated from equation **6.2** below

$$C_{SedEx} = \frac{N_{sr}}{M_{sed}} \qquad \textbf{6.2}$$

M_{sed} = Mass of sediment

Smedes (2007a) discussed the form of sorption isotherms commonly encountered during passive sampling using silicone rubber samplers in sediments. PAH compounds often show nonlinear isotherms, but the departure from linearity occurs at phase ratios that are not readily accessible to the silicone rubber samplers. The phase ratios used in the current work were therefore assumed to lie within the linear portion of the isotherms. Free dissolved concentrations were therefore determined from plots (e.g. Fig **6.4**) that had significant correlations ($p < 0.05$) and are presented in Table **6.2**.

PAH pore water concentration (C_w^o) was found to be similar between FF1 and FF2, while these were on average a factor of 2.3 greater than C_w^o from FF3 which corresponded with what was observed in the sediment PAH concentrations. Pore water concentrations of PAHs decreased in the order FF2 > FF1 > FF3, which is essentially a decrease away from the location of a fish farm.

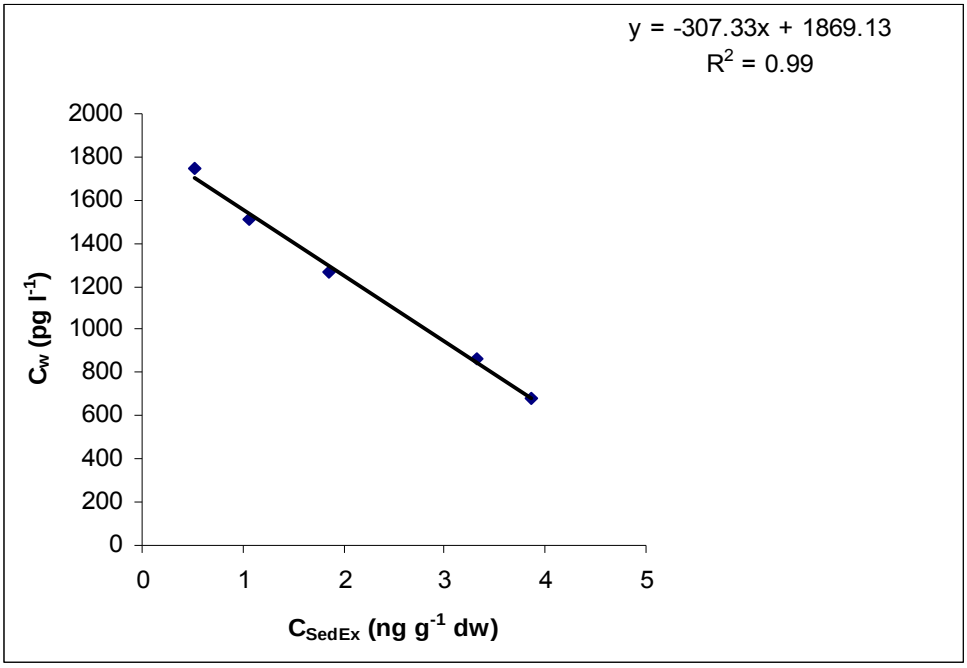


Fig **6.4**: Pyrene pore water and water exchangeable concentration determination plot from FF2. Extrapolation to both axes yields the desired parameters

The pore water concentrations were compared with available proposed Maximum Allowable Concentration-Environmental Quality Standards (MAC-EQS) for PAHs (Table **6.2**) in the draft European Union Council Directive document (EU, 2007) and were found to be well below (2 to 4 order of magnitude) these standards and may therefore not pose any significant risks to biota.

Table **6.2**: Pore water concentrations estimated from sorption isotherms

	Pore water concentration / ng L^{-1}			
PAHs	**MAC-EQS**	**FF1**	**FF2**	**FF3**
Naphthalene		nd*	1.559	0.566
2-Methylnaphthalene		nd	0.271	0.123
1-Methylnaphthalene		nd	0.200	0.093
C2-naphthalene		nd	0.480	0.204
C3-naphthalene		0.625	0.808	0.284
C4-naphthalene		0.207	0.394	0.188
Acenaphthylene		nd	0.036	0.054
Acenaphthene		0.127	0.135	0.073
Fluorene		0.540	0.478	0.237
Phenanthrene		nd	nd	0.119
Anthracene	400	0.085	0.063	nd
C1-178		nd	0.182	0.193
C2-178		0.449	0.486	0.233
C3-178		1.490	0.550	0.225
Dibenzothiophene		0.312	0.266	0.122
Fluoranthene		2.361	2.902	1.273
Pyrene		1.598	1.869	0.824
C1-202		0.735	0.869	0.387
C2-202		0.032	0.038	0.018
Benzo[*c*]phenanthrene		0.060	0.075	0.035
Benz[*a*]anthracene		0.076	0.081	0.037
Chrysene		0.157	0.173	0.077
C1-228		0.056	0.067	0.029
Benzofluoranthenes	30	0.095	0.112	0.047
Benzo[*e*]pyrene		0.048	0.057	0.024
Benzo[*a*]pyrene	100	0.011	0.012	0.005
Perylene		0.016	0.018	0.007
C1-252		0.014	0.016	0.006
Dibenz[*a,h*]anthracene		0.002	0.002	0.001
Indeno[1,2,3-*cd*]pyrene	2	0.002	0.002	0.001
Benzo[*ghi*]perylene	2	0.011	0.012	0.005

* not determined; $p > 0.05$

Only dissolved concentrations of pollutants are generally expected to pass through biological membranes to exert any toxic effects. The PAH profile in the sediments was dominated by the high molecular weight PAHs (high K_{ow}) where as the low molecular weight (LMW) PAHs (2- to 3-rings) dominated in the pore water concentrations which is due to the low partition coefficients of the LMW PAHs to sediment particulate matter. The low molecular weight PAHs such as naphthalene and substituted naphthalenes are known to cause taint in fish and shellfish (Law *et al*., 2002 and Craig *et al*., 2005). Only those PAHs which were detected and measured at all the 3 sampling points are subsequently reported. Figure **6.5** shows the pore water concentrations from the 3 locations.

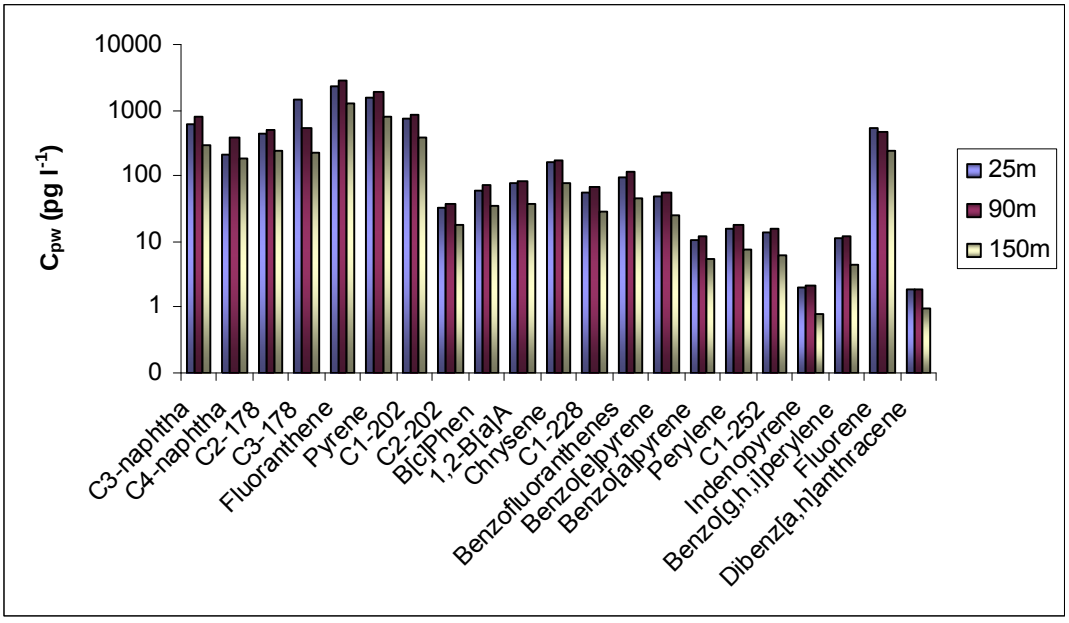


Fig **6.5**: Pore water concentrations determined from 25, 90 and 150 m away from the fish farm. Note that the C_w axis is on a log scale due to the ~ 4 order of magnitude difference between fluoranthene and dibenz[*a*,*h*]anthracene C_w for example

6.2.4 Water extractable proportion

Water extractable concentration (C^{o}_{SedEx}) for each PAH were determined by extrapolation from sorption isotherms of the pore water concentration versus concentration of sediment extracted by the silicone rubber (Fig **6.3** and **6.4**) to the sediment extractable concentration axis. These concentrations represent the proportion of the PAH concentration in sediment that

could potentially be mobilised into the pore water and subsequently interact with biota (ICES WGMS, 2003).

Figure **6.6** shows the percentage that is water extractable from each sediment sample. Only a proportion of the PAHs would become available for uptake with time and thus there is a percentage of the sediment concentration that is tightly bound to the sediment.
Traditionally risk assessments are based on total sediment concentration; however this may overestimate the risks due to reduced availability.

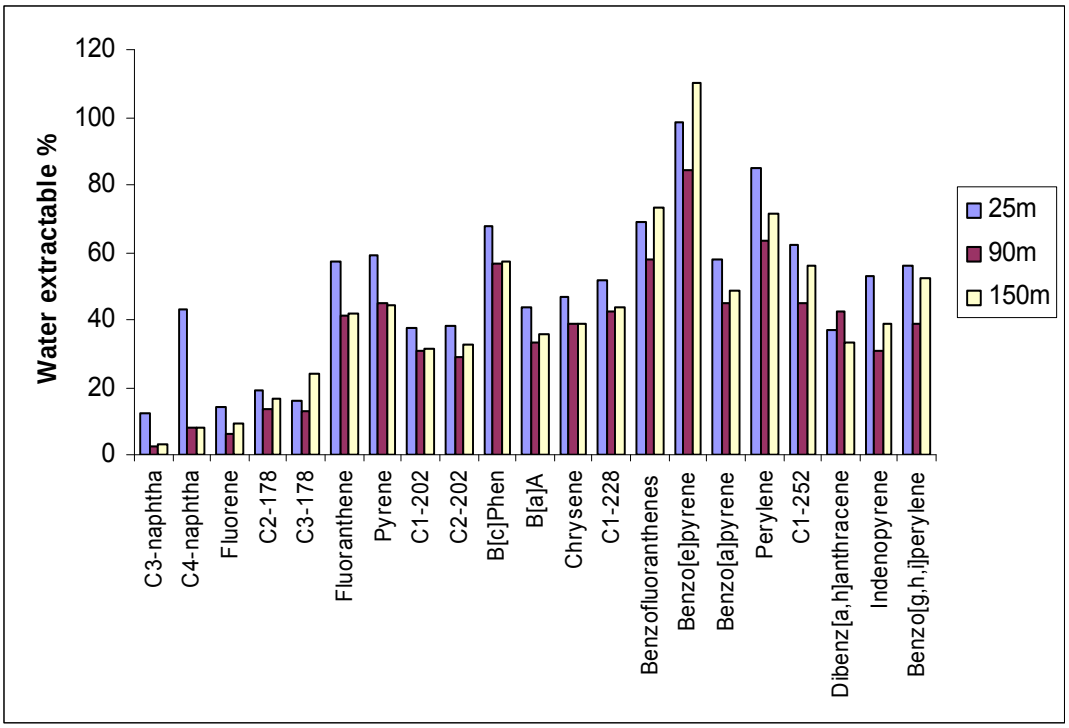


Fig **6.6**: Water extractable concentrations determined from 25, 90 and 150 m away from the fish farm expressed as percentages of corresponding sediment concentration

Similar water extractable proportions of the LMW PAHs were observed in sample FF1 and FF2 (up to C1-228) and it appears a relatively large proportion of the high molecular weight PAHs (up to 100 %) is available for exchange into the aqueous phase.

6.2.5 Sediment-water partitioning

At equilibrium, the ratio of the concentration in the sediment to the concentration in the water phase gives the partition coefficients (equation **6.3**). These partition coefficients determine the availability and fate of contaminants in the environment (Booij *et al*., 1997 and Smedes, 1994) and are useful for risk assessments. Lack of adequate methodology for isolating and measuring the freely dissolved concentration has often hindered accurate determination of these partition coefficients.

$$K_{sed,w} = \frac{C_{sed}}{C_w} \qquad \textbf{6.3}$$

The dependence of sorption of POPs to components of sediment with sorbent properties such as organic carbon content has been shown (Karickhoff *et al*., 1979; Di Toro *et al*., 1991; Borglin *et al*., 1996; Chen *et al*., 2000) and the partition coefficients are mostly normalised to the organic carbon content as

$$K_{oc} = \frac{K_{sed,w}}{f_{oc}} = \frac{C_{sed}}{f_{oc} \cdot C_w} \equiv \frac{C_{oc}}{C_w} \qquad \textbf{6.4}$$

f_{oc} = Organic carbon fraction, C_{oc} = Organic carbon normalised sediment concentration, K_{oc} = partition coefficient normalised to organic carbon content.

Having established equilibrium conditions in the exposure of sediments to silicone rubber in this study, $K_{sed,w}$ were calculated from the plots (see Fig **6.7** as an example) of C_w at versus C_{res} ($C_{Sed} - C_{SedEx}$) with the slope of such a plot equalling $1/K_{sed,w}$.

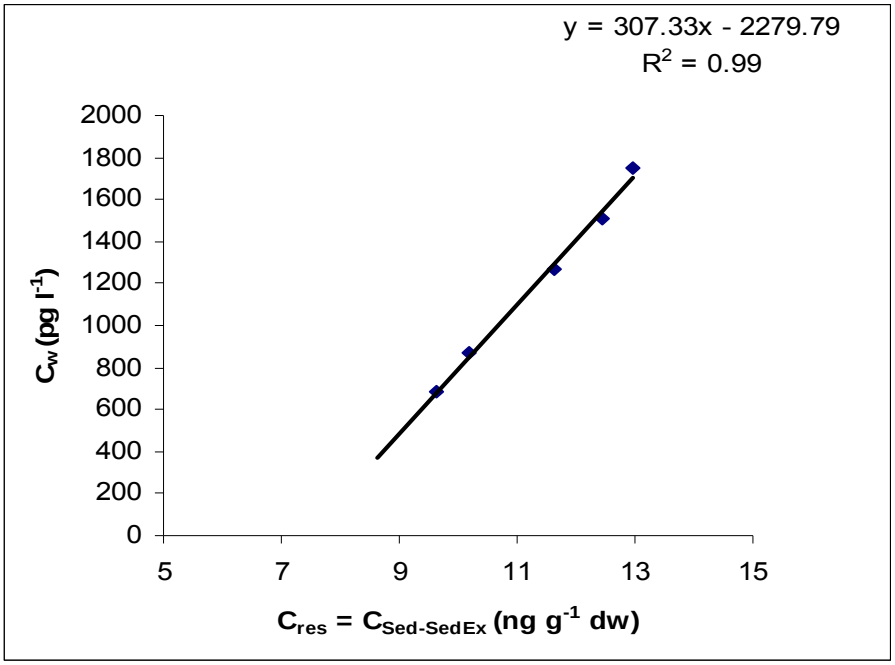


Fig **6.7**: Determination of sediment-water partition coefficients for pyrene from a sorption isotherm

The calculated sediment-water partition coefficients were normalised to the percent total organic carbon from each sampling point and these are presented in Table **6.3** for the plots that were significantly correlated in all locations. There was no observable trend in the $\log K_{oc}$ across the locations for individual PAHs implying any differences observed in the pore water is very likely due to differences in the sediment concentrations as the K_{oc} is broadly similar across the locations.

Table **6.3**: Log partition coefficients normalised to organic carbon content (Log K_{oc}) with values predicted from the Karickhoff relation (Karickhoff *et al*., 1979)

	log K_{oc}				log K_{ow}[a]
	25m	**90m**	**150m**	**Predicted**	
% OC	**2.56**	**2.34**	**2.24**		
C3-naphthalene	5.18	4.49	4.59	4.52	4.73
C4-naphthalene	5.94	5.01	4.97	5.09	5.30
Fluorene	4.33	4.10	4.23	3.97	4.18
C2-178	5.44	5.36	5.40	5.39	5.60
C3-178	4.74	5.17	5.49	5.64	5.85
Fluoranthene	5.17	5.04	5.04	4.99	5.20
Pyrene	5.23	5.14	5.14	4.79	5.00
C1-202	5.66	5.59	5.57	5.27	5.48
C2-202	6.90	6.79	6.77	5.82	6.03
Benzo[*c*]phenanthrene	5.99	5.91	5.86	5.60	5.81
Benz[*a*]anthracene	6.23	6.18	6.15	5.70	5.91
Chrysene	6.11	6.08	6.05	5.65	5.86
C1-228	6.89	6.83	6.79	5.99	6.20
Benzofluoranthenes	7.36	7.29	7.36	5.90	6.11
Benzo[*e*]pyrene	7.28	7.23	7.30	6.23	6.44
Benzo[*a*]pyrene	7.48	7.37	7.40	6.14	6.35
Perylene	7.28	7.18	7.21	6.04	6.25
C1-252	7.94	7.83	7.92	-	nf
Dibenz[*a,h*]anthracene	7.71	7.91	7.64	6.54	6.75
Indeno[1,2,3-*cd*]pyrene	8.64	8.45	8.57	7.45	7.66
Benzo[*ghi*]perylene	7.85	7.72	7.86	6.69	6.90

nf- no log K_{ow} value found in literature; [a]- obtained from Yates *et al*. (2007)

The log K_{oc} values were correlated to the log K_{ow} in Figure **6.8** and strong linear relationships were obtained which are statistically different ($p < 0.05$) from the prediction of log K_{oc} from the Karickhoff relation but similar (F-test, $p > 0.001$) across the sediment samples. Similar relationships with log K_{ow} have been reported (see Table **6.4**).

Table **6.4**: Summary of linear regression analysis of $\log K_{oc}$ versus $\log K_{ow}$ [a] with 95 % confidence interval of intercept and slope and regressions reported in literature for PAHs

	Slope	*Intercept*	r^2	s^b	n^c
FF1	1.36 ± 0.30	-1.61 ± 1.81	0.83	0.50	20
FF2	1.48 ± 0.25	-2.47 ± 1.49	0.90	0.41	20
FF3	1.47 ± 0.23	-2.38 ± 1.37	0.91	0.38	20
Booij *et al*., 2003 [d]	1.32	-0.39	0.95	0.26	39
Jonker and Koelmans, 2001[e]	1.07 ± 0.44	-0.77 ± 2.57	0.77	0.38	10
Jonker and Smedes, 2000[f]	1.14 ± 0.32	1.40 ± 1.89	0.86	0.38	12

[a] obtained from Yates *et al*. (2007) [b] standard deviation of the fit; [c] sample size; [d] determined from the ratio of concentrations in sediment to that in pore water measured using low density polyethylene (LDPE) samplers; [e] estimated from data provided; [f] determined using the co-solvent method

The difference between the log partition coefficients obtained and that predicted is more pronounced from $\log K_{ow} > 6$. Booij *et al*. (1997) suggest that slopes equal to one in $\log K_{oc}$ versus $\log K_{ow}$ relations should be considered as exceptions rather than the rule due to possible changes in the effect of dissolved octanol on the activity coefficient in the water phase with the hydrophobicity of the contaminants and non-proportionality in the non-ideality of the solution in the organic matter to the non-ideality of the solution in octanol. Differences in the slope and intercept of these relationships could also be due to inaccurate measurements of K_{ow}. The close to unity slopes with comparable width of 95 % confidence interval of the relationships obtained suggest the sorption is strongly determined by hydrophobicity.

More of the higher K_{ow} PAHs appear to be more available (Fig **6.6**) although they have higher K_{oc} which is a consequence of the measurement method (see Fig **6.7**). The partition coefficients are obtained from the slopes of Fig **6.7** while C^{o}_{SedEx} is obtained from the extrapolation to the x-axis of the same plot. The likelihood of wrongly estimating C^{o}_{SedEx} has been discussed by Smedes (2007a). Log K_{oc} for PAHs have been shown (Gustafsson *et al*., 1997) to be larger than those predicted using the Karickhoff relation due to the interaction of PAHs with soot present in sediments.

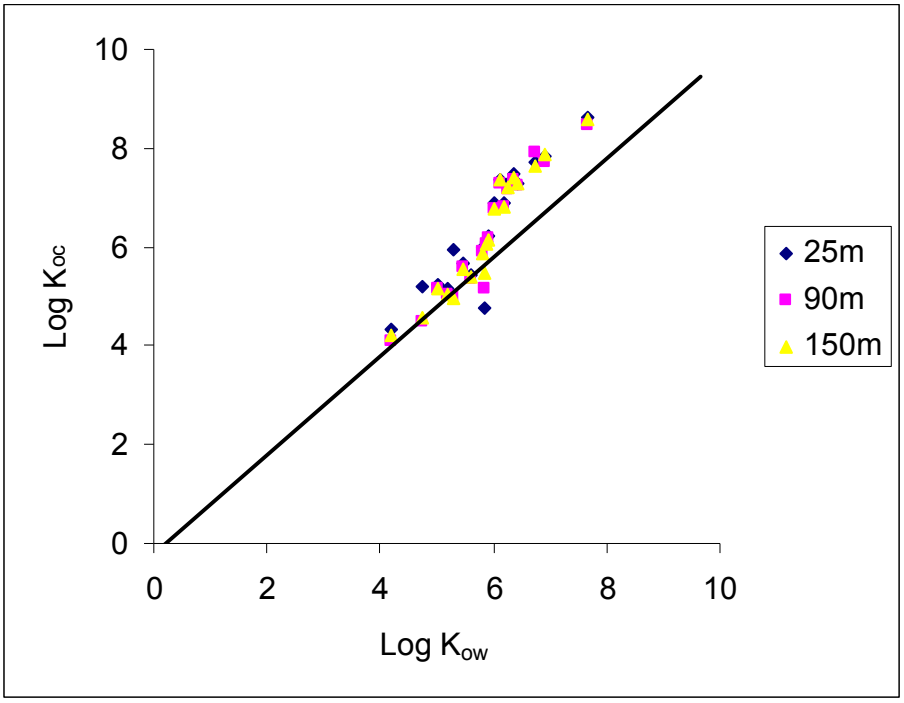


Fig **6.8**: Log K_{oc} from the sorption isotherms versus log K_{ow}. Drawn line represents the relation from Karickhoff *et al*., 1979 which was used for comparison

6.3 Conclusions

Pore water concentrations were determined from equilibrations of silicone rubber with sediment from Loch Shell, in the pg L^{-1} range together with water exchangeable concentrations which give a measure of the concentrations of PAHs that will become available with time. Fluoranthene and pyrene were found to be in high concentrations (> 1500 pg L^{-1} in FF1 and FF2 and > 800 pg L^{-1} in FF3). The pore water concentrations mirrored the concentrations in the sediments with concentrations at FF1 and FF2 being a factor of 2 higher than concentrations in FF3. Nevertheless, the pore water concentrations at the three sampling points were below the proposed EU Council directive MAC-EQS for surface waters.

Varying proportions (3-100 %) of the individual PAH sediment concentration appears to be available for exchange with the sediment pore water. The sediment-water partition coefficients normalised to organic carbon (log K_{oc}) increased with increasing hydrophobicity

($\log K_{ow}$). Silicone rubber passive samplers can be used to measure dissolved concentrations and sediment-water partition coefficients that hitherto have been difficult to determine.

CHAPTER SEVEN

Measurement of the availability of PAHs in marine sediments and pore waters from Loch Leven, Scotland using silicone rubber passive samplers

7.0 Introduction

The sediments of Loch Leven, Scotland contain high concentrations of polycyclic aromatic hydrocarbons (PAHs) and have been monitored by Fisheries Research Services since 1999. The contamination has been attributed to the effluent discharged from an aluminium smelter at Kinlochleven, which ceased operation in June 2000 (McIntosh *et al*., 2002; McIntosh *et al*., 2004; Webster *et al*., 2004) and enhanced concentrations of PAHs may persist for a considerable time. Loch Leven is also known to support a number of fish farms and elevated PAH concentrations have been found (McIntosh *et al*., 2004) in farmed mussels close to Kinlochleven, with a dominance of the 5-ring PAHs compared to mussels from a reference site in Loch Etive, where 2- to 3-ring compounds were dominant. Electrodes used in some aluminium smelting processes include coal tar pitch, which contains PAHs. However, Naes *et al*. (1999) found that PAHs generated from aluminium production appeared not to have adverse or severe effects on benthic biota, even in areas of very elevated concentrations of PAHs in sediment. This observation supported their hypothesis that PAHs associated with soot-like materials had limited biological availability.

The availability of persistent organic pollutants (POPs) such as PAHs for uptake by organisms has been linked to the freely dissolved concentration in sediment pore waters through equilibrium partition theory (Smedes, 1994; Mayer *et al*., 2000). The pore water concentration is important in the assessment of biological effects as only dissolved concentrations tend to partition into biological membranes (Macrae and Hall, 1998; Huckins *et al*., 1990). Reichenberg and Mayer (2006) identified two complementary parameters of bioavailability as the accessible quantity and the chemical activity of the POP. They identified the accessible quantity as the proportion of the total concentration that can be mobilised and made available

for processes such as biodegradation and digestive uptake, and is determinable by strong extractions of sediment, or POP sediment concentration-depletive extraction. The chemical activity, however, determines processes such as partitioning and diffusion and encapsulates concepts such as freely dissolved concentration and fugacity which are measurable using equilibrium sampling devices. Consequently, passive sampling using a reference phase that equilibrates with the dissolved concentration in the sampling medium is attractive.

The availability of PAHs in sediments from Loch Leven was therefore studied using a silicone rubber passive sampler, following protocols developed through the ICES Working Group on Marine Sediments (ICES WGMS 2003). Determinations were made of the pore water and water extractable (accessible) concentrations of PAHs in the sediments, and also the partition coefficients of PAHs between sediment and water.

7.1 Sampling Theory

Most equilibrium samplers assume non- or negligible depletion of the sediment phase (Jonker and Koelmans, 2001; Mayer *et al*., 2003; Heringa and Hermens, 2003). However, in this study, no such assumption was made; rather measurements of the pore water concentrations were made at different degrees of sediment depletion (or phase ratios) using equation **7.1** and also calculating the concentration extracted by the silicone rubber from the sediment from equation **7.3**.

The pore water concentration (C_w) is calculated from the concentration in the silicone rubber using equation **1** below

$$C_w = \frac{C_{sr}}{K_{sr,w}} \qquad \textbf{7.1}$$

where, C_{sr} = concentration in the silicone rubber / ng g^{-1} SR (sr and SR denoting silicone rubber), $K_{sr,w}$ = silicone rubber-water partition coefficient / L kg^{-1}. Silicone rubber-water

partition coefficients for the silicone rubber sampler used were independently determined in an earlier study (Yates *et al*., 2007).

$$C_{sr} = \frac{N_{sr}}{M_{sr}} \qquad \textbf{7.2}$$

N_{sr} = Amount of PAH in the silicone rubber, M_{sr} = mass of silicone rubber

Similarly the concentration extracted from the sediment (C_{SedEx}) is calculated from equation **7.3** below

$$C_{SedEx} = \frac{N_{sr}}{M_{sed}} \qquad \textbf{7.3}$$

M_{sed} = Mass of sediment

Subsequently, a plot of C_w versus C_{SedEx} is made and extrapolated to the axes to yield the freely dissolved concentrations in pore water, and the water extractable concentrations. In a similar regard, sediment-water partition coefficients, $K_{sed,w}$ were calculated from the plots of C_w versus C_{res} ($C_{Sed} - C_{SedEx}$), referred to as residual sediment concentration at each phase ratio with the slope of such plots being equivalent to $1/K_{sed,w}$.

7.2 Materials and Methods

7.2.1 Materials

HPLC grade solvents (dichloromethane, ethyl acetate, acetone, methanol and *iso*-hexane) were purchased from Rathburn Chemicals Ltd, Scotland, UK. Certified solid standards for PAHs (including deuterated PAHs) were obtained from QMX Laboratories, Essex, UK and dissolved in *iso*-hexane to obtain the required concentrations of spiking and calibration solutions. To avoid contamination of samples, all glassware and stainless steel forceps were either washed in Decon® 180 solutions and rinsed with distilled water or washed in a CAMLAB GW 4050 glassware washer and dried in an oven at 100 ^{o}C. Before use, the glassware was rinsed twice

each in dichloromethane and *iso*-hexane, with the latter allowed to evaporate to dryness. Glass Duran® or Schott® (VWR, Leicester, UK) bottles were used for passive extraction of contaminants from sediment, and were always capped with aluminium-lined PTFE caps to reduce or prevent sorption of POPs by the caps.

AlteSil™ Silicone rubber sheet (600 × 600 mm, 0.5 mm thick) used as the passive sampling material was purchased from Altec Products, Ltd, Cornwall, UK. The silicone rubber sheets used were cut into required dimensions (6 × 4 cm, or 9 × 4 cm) and pre-extracted in hot ethyl acetate using a Soxhlet apparatus for ~ 4 days before use to remove any low molecular weight oligomers or residues that might subsequently be co-extracted with the analytes and could affect instrumental analysis (Gruber *et al*. 2000; Rusina *et al*., 2007). After pre-extraction and cooling, the sheets were removed from the solvent and stored in bulk in glass jars containing methanol until required for use. The weight of the sheets used was determined after extraction of exposed sheets to avoid contamination from the environment.

7.2.2 Methods

7.2.2.1 Sediment Sampling

Samples of sediment (12-14 cm depth of mud) were collected from RV *Seol Mara* using a 0.1 m^2 Day Grab and Van Veen grab at depths between 15 and 40 m at five stations within Loch Leven (Fig **7.0**). The top 2 cm of the sediment was transferred to a solvent washed aluminium can, thoroughly mixed, labelled and stored at -20 ± 5 ^{o}C until required for analysis. Particle size analysis of sediment samples was determined by laser granulometry using a Malvern Mastersizer E Particle Size Analyser, after freeze drying of the sediments. The precision of the method based on 7 replicate measurements of a laboratory reference material (LRM C100) on the D (4, 3) mean weighted volume fraction equals 2.50 %. Total organic carbon (TOC) was determined on freeze dried sediment using a ThermoQuest Flash EA 1112 elemental analyser, following removal of inorganic carbon by acidification using hydrochloric acid. The method limit of detection is 0.005 mg although the limit of quantification (LOQ) calculated as 0.005

divided by the sample weight analysed × 100 % is normally reported. E.g. for a sample weight of 16.50 mg, the LOQ is 0.03 %.

Fig **7.0**: Sediment sampling stations in Loch Leven, September, 2006. The closed Smelter is closer to Station 8. Stations 6-8 lie within the upper basin of the loch while Stations 4 and 5 are in the lower basin. The closed aluminium smelter is towards the right (landwards) of station 8

7.2.2.2 Hydrocarbon analysis of sediment

Sediment samples were thoroughly mixed after thawing and ~ 10 g per sample was removed for determination of moisture content by drying in an oven at 80 ± 5 ^{o}C for 22 ± 2 h (Webster *et al*., 1997). PAHs were determined as described by Webster *et al*. (2004). Briefly, aliphatic hydrocarbon internal standards, heptamethylnonane and squalane and deuterated aromatic internal standards (D_8-naphthalene, D_{10}-biphenyl, D_8-dibenzothiophene, D_{10}-anthracene, D_{10}-pyrene and D_{12}-benzo[*a*]pyrene (100 or 200 µl; approximately 1 µg ml^{-1} each)) were added to 0.3 ± 0.1 g sub samples of wet sediment. The hydrocarbons were extracted using

dichloromethane/methanol with sonication and the halogenated solvent isolated and dried over sodium sulphate prior to solvent exchange into *iso*-hexane. The aliphatic and aromatic hydrocarbons were separated by isocratic high performance liquid chromatography (HPLC). The aromatic fraction collected and concentrated prior to chromatographic analysis. A laboratory reference material (LRM) and procedural blank were also included in the analyses. Recoveries of ≥ 85 % and precision of ≤ 17 % for individual PAH compounds were obtained with spiked sediment samples.

7.2.2.3 Determination of availability parameters

The sediment samples were weighed into 50 ml and 3 × 250 ml glass Duran® bottles, and water added, where necessary, to liquefy the sediment so as to ensure proper interaction between the sediment slurry and the silicone rubber sheets that would be added to the bottles. Pre-extracted silicone rubber sheets were loaded with PAH performance reference compounds (PRCs) as described by Booij *et al*. (2002) and a single sheet added to each bottle to obtain different phase ratios (g silicone rubber per g sediment). The bottles were placed on an orbital shaker horizontally and shaken at 200 rpm for 20 days in a light and temperature controlled room. In a preliminary developmental study (data not reported), this time was found to be sufficient for the PAHs to attain equilibrium. After this time, the sheets were removed from the bottles, rinsed with distilled water and gently wiped dry with paper tissue to remove any adhering water.

7.2.2.4 Extraction of silicone rubber sheets

The silicone rubber sheets were Soxhlet extracted using 100 ± 5 ml of *iso*-hexane: acetone ($3{:}1^{v}/_{v}$) mixture or methanol for 6 h. Aliphatic hydrocarbon standard (containing heptamethylnonane and squalane) and deuterated PAH aromatic internal standard containing D_8-naphthalene, D_{10}-biphenyl, D_8-dibenzothiophene, D_{10}-anthracene, D_{10}-pyrene and D_{12}-benzo[*a*]pyrene were then added to the cooled extract, concentrated by rotary evaporation followed by nitrogen blow down of the extract to 1 ml. The extract was reduced to 0.5 ± 0.2 ml and an aliquot of the concentrated extract was fractionated using an isocratic, normal phase

Genesis metal-free high performance liquid chromatograph (HPLC) column (25 cm x 4.6 mm) and eluted with *iso*-hexane at a flow rate of 2 ± 0.1 ml/min into aliphatic and aromatic fractions. The aliphatic fraction was discarded and the aromatic fraction collected in 100 ml flasks, concentrated by rotary evaporation and further reduced under nitrogen to 50 ± 10 µl for gas chromatography – mass selective detection (GC-MSD) analysis.

The weights of the silicone rubber sheets were recorded after the extraction to avoid contamination from external sources. A procedural blank (an un-spiked sheet exposed to only the distilled water used to liquefy the sediments) was included and analysed in the same manner as the other samples. The result from the procedural blank was subsequently subtracted from the results of samples. Similarly a spiked sheet was also analysed to obtain the initial amounts of PRCs in the sheets.

7.2.2.5 Gas chromatography-mass selective detection (GC-MSD)

The concentrations and composition of the PAHs were determined by GC-MSD using an HP6890 Series Gas Chromatograph interfaced with an HP5973 MSD fitted with a cool on-column injector (Webster *et al.*, 2007). Briefly, a non-polar HP5 (30 m × 0.25 mm id, 0.25 µm film thickness; Agilent Technologies, Stockport, England) column was used for the analyses with helium as the carrier gas, controlled using the constant flow mode at 0.7 ml min^{-1}. The MSD was set for selective ion monitoring (SIM) with a dwell time of 50 ms. Injections were made at 50 ^{o}C and the oven temperature held constant for 3 min. Thereafter, the temperature was raised at 20 ^{o}C min^{-1} up to 100 ^{o}C, followed by a slower ramp of 4 ^{o}C min^{-1} up to a final temperature of 270 ^{o}C. A total of 29 (later 36 with the addition of extra performance reference compounds) ions plus the six internal standard ions were measured over the analysis period, thus incorporating 2- to 6- ring, parent and branched PAHs. Limits of detection based on multiplying the standard deviation of the mean of the lowest standard (0.005 ng ml^{-1}) by 4.65 were found to be < 0.2 ng g^{-1} for chrysene and < 0.1 ng g^{-1} for benzo[*a*]pyrene. The GC-MSD is calibrated using seven different concentrations of a solution containing 33 PAHs.

7.3 Results and Discussion

7.3.1 Sediment PAH concentration

The total PAH concentrations in sediment (2- to 6-ring parent and alkylated PAHs, Table **7.0** and Fig **7.1**) varied between 13282 ng g^{-1} dry weight (Station 4 which had the lowest % TOC) to 95158 ng g^{-1} dry weight (Station 7 with the highest % TOC). The 4- to 6-ring compounds accounting for ~ 96 ± 0.98 % of the total concentrations (Fig **7.1**) and there was a strong dominance of the 5-ring compounds, as has been reported for sediments around aluminium smelters elsewhere (Naes *et al*., 1995 and Naes *et al*., 1999).

Table **7.0**: Sediment bulk properties and total PAH (2- to 6-ring parent and alkylated compounds) concentration from the five sampling points

Field ID	% TOC	PS % < 63 µm	Total PAH (ng g^{-1} dw)
Station 4	4.9	85.26	13282
Station 5	5.0	78.39	15244
Station 6	7.3	77.50	64305
Station 7	7.8	78.18	95158
Station 8	5.2	54.41	68099

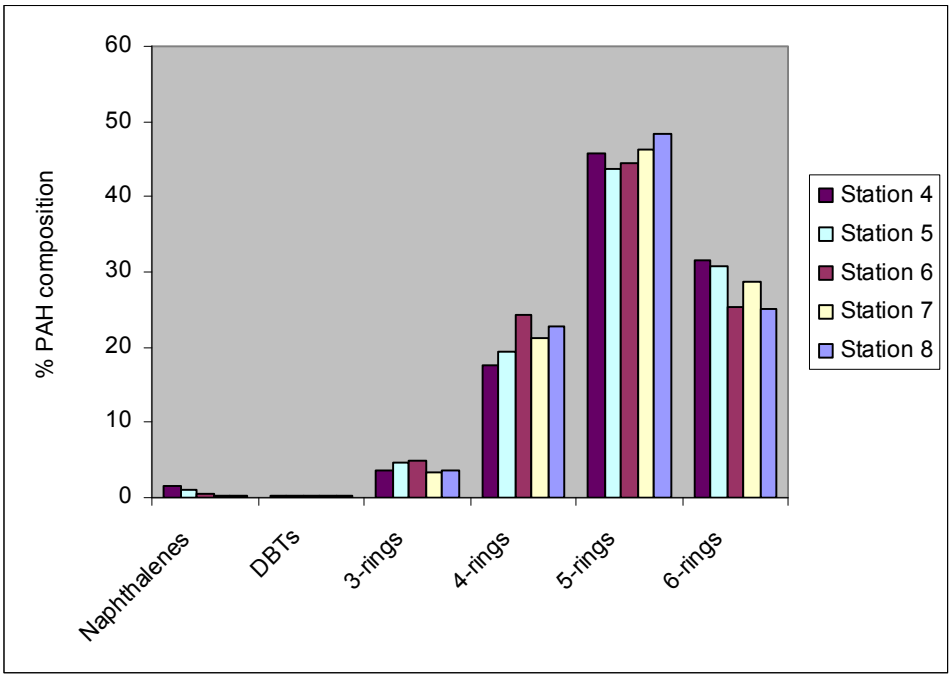


Fig **7.2**: Sediment PAH composition determined in sediments collected from Loch Leven in September, 2006. Naphthalenes = sum of naphthalene; 1 & 2-methylnaphthalene; C2-, C3- and C4- naphthalenes. 3-rings = sum of phenanthrene; anthracene, C1-178, C2-178, and C3-178; acenapthylene; acenaphthene and fluorene. DBTs = sum of Dibenzothiophene; C1-DBT, C2-DBT, and C3-DBTs. 4 –rings = sum of fluoranthene; pyrene; C1-202, C2-202, and C3-202; benzo[*c*]phenanthrene; benz[*a*]anthracene; benz[*b*]anthracene; chrysene; C1-228; C2-228. 5-rings = sum of benzofluoranthenes; benzo[*e*]pyrene; benzo[*a*]pyrene; perylene; C1-252 and C2-252. Benzofluoranthene is the summation of benzo[*b*] and [*k*]fluoranthene. 6-rings = sum of indeno[1, 2, 3-*cd*]pyrene; benzo[*ghi*]perylene; C1-276 and C2-276

Provisional Oslo and Paris Commission (OSPAR) Background Concentrations (BCs) have been established for ten parent PAHs in sediments (OSPAR, 2006). The Background Concentration has been defined as the concentration of a contaminant at a "pristine" or "remote" site based on contemporary or historical data. Observed concentrations are said to be 'near background' if the mean concentration is statistically significantly below the corresponding Background Assessment Concentration (BAC). In this study, only single measurements of PAH concentrations were made, therefore the individual PAH concentrations for each station were normalized to 2.5 % organic carbon and an approximate 95 % confidence interval calculated using uncertainty values from validation data at FRS. The normalized concentrations for individual PAHs far exceeded the BACs at the 5 % significance level (Table **7.1**)

Table **7.1**: Available OSPAR Background Concentrations and provisional Background Assessment Concentration (both ng g^{-1} dry weight) normalised to 2.5 % organic carbon (OSPAR, 2006) and corresponding concentrations in the sediment samples from Loch Leven, also normalised to 2.5 % organic carbon with 95 % confidence interval expressed as a percentage

				Station				
	BC[1]	*BAC*[2]	C.I. (%)	4	5	6	7	8
Naphthalene	5	8	30	440	536	2180	2268	1636
Phenanthrene	17	32	28	7572	10756	60448	60536	45392
Anthracene	3	5	24	1480	2536	12836	13944	11324
Fluoranthene	20	39	25	12780	16244	99776	106060	77652
Pyrene	13	24	25	12352	15488	94428	103616	76144
Benz[*a*]anthracene	9	16	33	7316	9284	52520	68704	53600
Chrysene	11	20	38	12628	15020	102024	156336	140460
Benzo[*a*]pyrene	15	30	22	30976	34072	173256	257692	196292
Indeno[1,2,3-*cd*]pyrene	50	103	19	61012	66612	236480	381444	255576
Benzo[*ghi*]perylene	45	80	19	67920	72748	258724	430504	277232

[1]Background Concentration; [2]Background Assessment Criteria

7.3.2 Pore water concentrations

Sorption isotherms (example in Fig **7.2**) were plotted for the sediments and only plots that yielded significant linear relations ($p < 0.05$) were used to estimate the dissolved concentrations (C_w^0) in pore water and water extractable fractions by extrapolation to both axes.

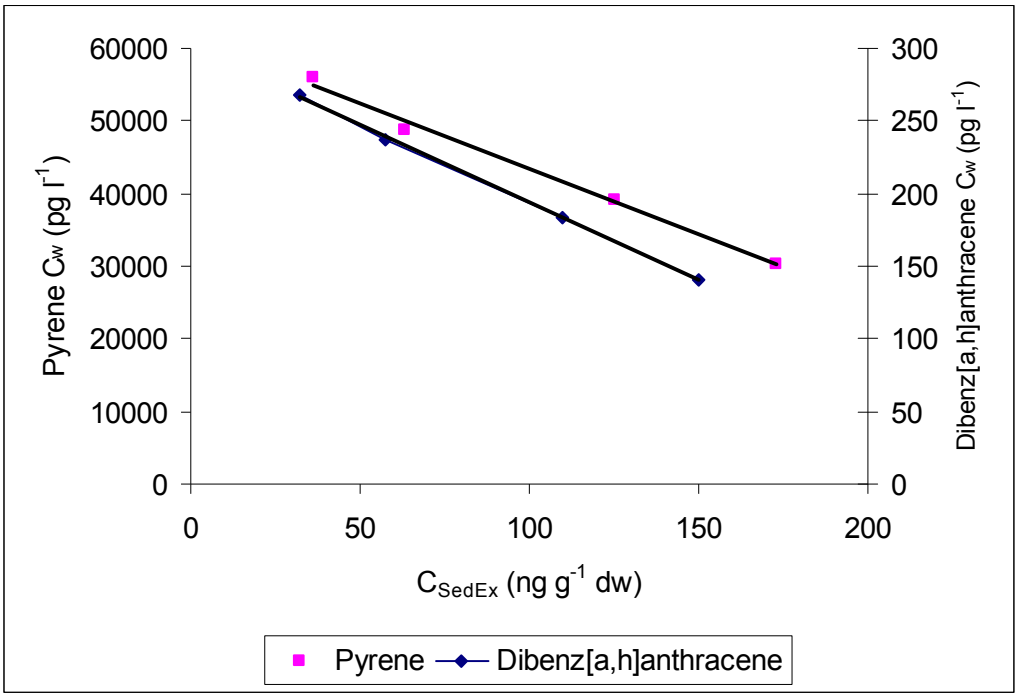


Fig **7.2**: Sorption isotherm to determine pore water concentration of pyrene (squares) and dibenz[*a,h*]anthracene (diamonds) from station 8 in loch Leven

The pore water concentrations obtained from the extrapolation are presented in Fig **7.3**. However for some PAHs (~ 10 %), the plots yielded poor correlations ($p > 0.05$), especially from station 5 (plots not shown).

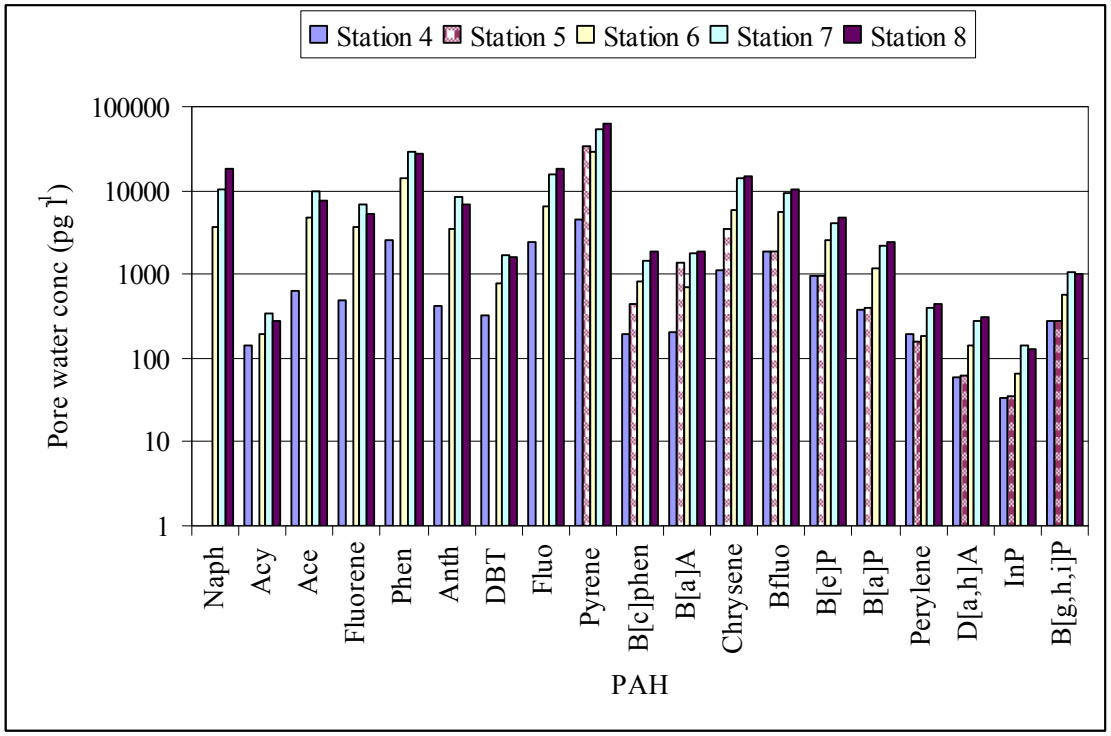


Fig **7.3**: Pore water concentrations obtained from sorption isotherms similar to Fig **7.2** for the stations sampled and PAHs for which statistically significant regressions were obtained ($p < 0.05$). Naph-Naphthalene; Acy- Acenaphthylene; Ace-Acenaphthene; Phen-Phenanthrene; Anth-Anthracene; DBT-Dibenzothiophene; Fluo-Fluoranthene, B[*c*]phen-Benzo[*c*]phenanthrene; B[*a*]A-Benz[*a*]anthracene; Bfluo-Benzofluoranthenes; B[*a*]P-Benzo[*a*]pyrene; D[*a,h*]A-Dibenz[*a,h*]anthracene; InP-Indeno[1,2,3-*cd*]pyrene; B[*g,h,i*]P-Benzo[*ghi*]perylene

The pore water concentrations were dominated by the 2- to 4- ring PAHs (like naphthalenes, phenanthrene, fluoranthene, pyrene and chrysene) with benzofluoranthenes and benzo[*e*]pyrene as exceptions from the higher ring PAHs. Generally though, the pore water concentrations tended to decrease in the order station 8> 7> 6> 5> 4 (e.g. chrysene in Fig **7.4**), i.e. generally decreases away from the smelter (see Fig **7.0**), with a decrease by factors ranging from 2 to 14 for individual PAHs. PAH concentration in the pore water also followed the general trend seen in the sediments

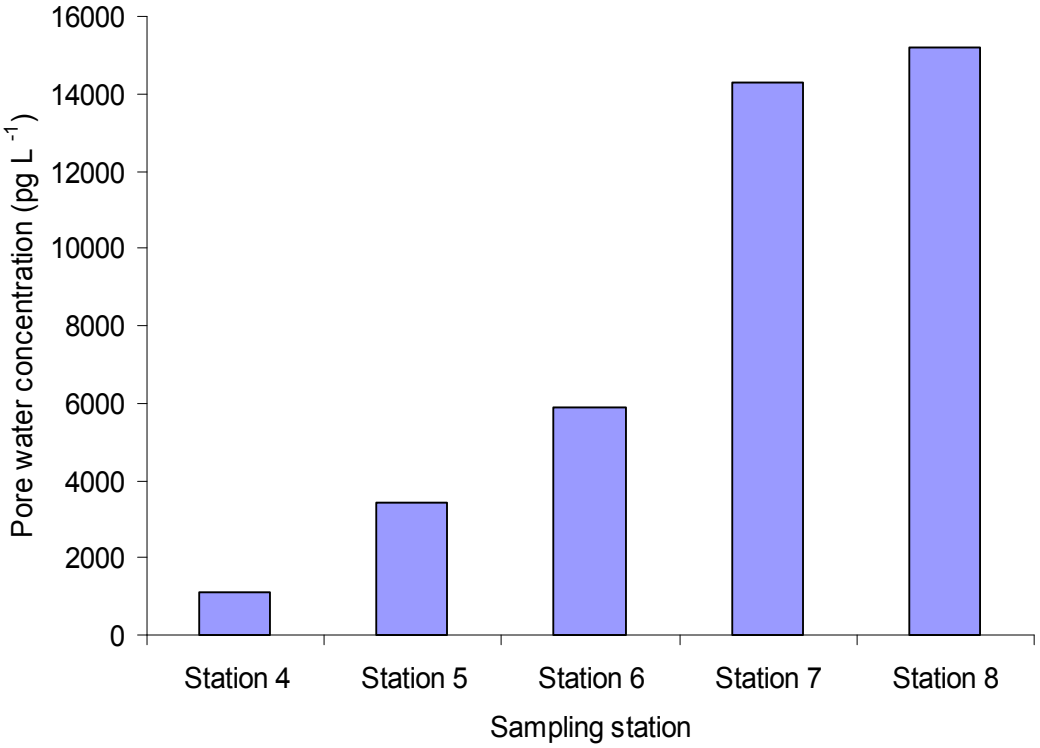


Fig **7.4**: Chrysene pore water concentrations profile along the sampling gradient

The pore water concentrations estimated in this study were well below the draft European Union Council Directive proposed Maximum Allowable Concentration-Environmental Quality Standards (MAC-EQS) for PAHs (Table **7.2**) in surface waters (EU, 2007).

Table **7.2** Maximum allowable concentration – Environmental Quality Standards for PAHs in surface waters

PAH	MAC-EQS	Pore water concentration (ng L^{-1})				
		Station				
	ng L^{-1}	4	5	6	7	8
Anthracene	400	0.42	ND*	3.48	8.31	6.93
Benzofluoranthene	30	1.92	1.92	5.60	9.13	10.34
Benzo[*a*]pyrene	100	0.38	0.40	1.16	2.21	2.47
Indeno[1,2,3-*cd*]pyrene	2	0.03	0.04	0.07	0.14	0.13
Benzo[*ghi*]perylene	2	0.28	0.28	0.58	1.07	1.00

* not determined

7.3.3 Water extractable proportions

Water exchangeable concentrations were determined from extrapolation of the sorption isotherms to the sediment-extractable concentration axes of plots such as Fig **7.2**. These concentrations represent the fraction of the sediment PAH concentration that could potentially be mobilised into the pore water and consequently interact with biota, and are presented as a percentage of the total concentration of each PAH in the sediment (Fig **7.5)**. The exchangeable proportions varied between station and compounds, generally between 5 and 40 % of the total concentration. The remaining percentage is tightly (irreversibly) bound to the sediment. Thus any risk assessment using the total concentration of PAHs in the sediment may overestimate the risks. The presence of a soot phase in sediments (Naes and Oug, 1998; Jonker and Koelmans, 2001) and ageing processes (Reid *et al*., 2000) has also been shown to reduce availability of PAHs.

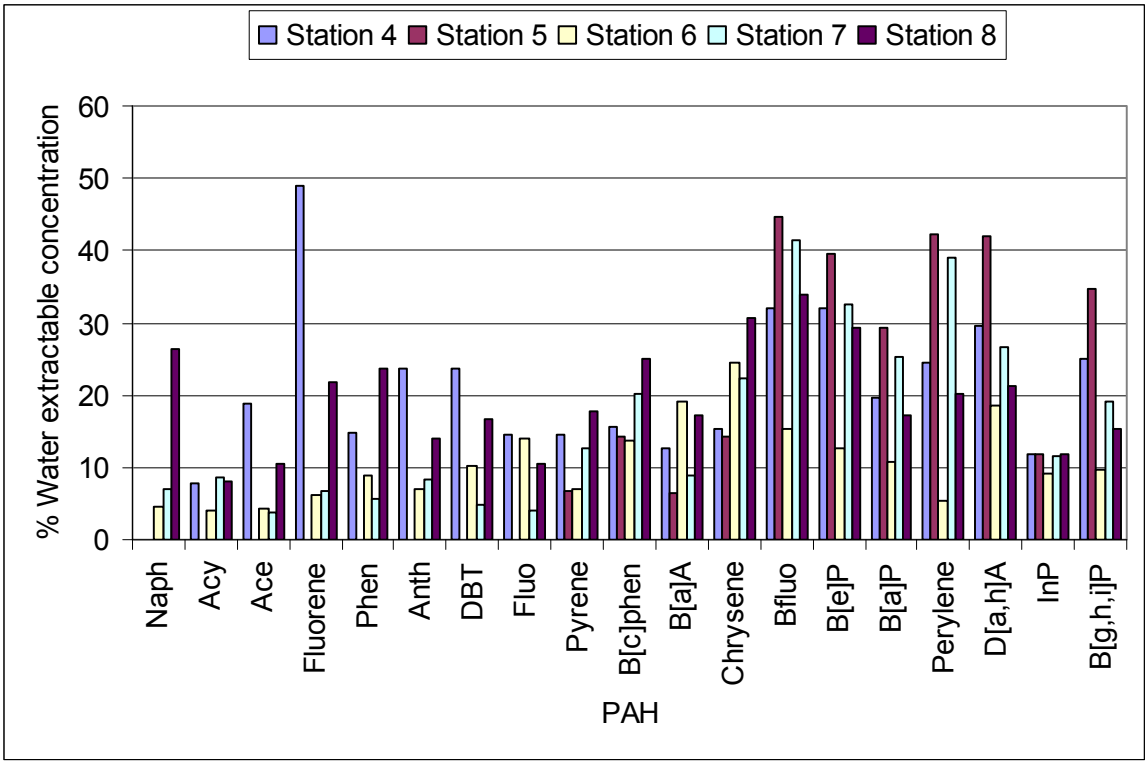


Fig **7.5**: Water extractable concentration as a percentage of sediment PAH concentration from the sampling stations. Data are not shown where the sorption isotherm plots (Fig **7.2**) yielded poor correlations ($p > 0.05$). PAHs as defined in Fig **7.3**

A relatively high proportion of the 5- to 6- ring PAHs (up to 40 %) is more available for exchange into the water phase, with the exception of indeno[1,2,3-*cd*]pyrene (~12 %). There was no general observable trend across the stations of the water extractable proportions.

7.3.4 Sediment-water partition coefficients of PAHs

Sediment-water partition coefficients ($K_{sed,w}$) describe the partitioning of POPs between sediment and the water phase, and determine the activity (and hence availability and mobility) of contaminants in the environment (Booij *et al*., 1997; Smedes, 1994) and are useful for risk assessments. Lack of adequate methodology for isolating and measuring the freely dissolved concentration has often hindered accurate determination of these partition coefficients. The importance of methodology in determining these coefficients was highlighted by Harkey *et al*. (1994); where they used four methods to determine the partition coefficients. Other methods have been used in determining these coefficients such as from the free-energy relationship of Karickhoff *et al*. (1979), co-solvent method (Jonker and Smedes, 2000), the use of polyoxymethylene strips (Jonker and Koelmans, 2001), etc, with most of the methods dependent on attainment of equilibrium.

At equilibrium, the ratio of the concentration of a contaminant in the sediment to the concentration in the water phase gives the partition coefficients (equation **7.4**)

$$K_{sed,w} = \frac{C_{sed}}{C_w} \qquad \textbf{7.4}$$

The dependence of sorption of POPs to components of sediment with sorbent properties, such as organic material, has been shown (Karickhoff *et al*., 1979; Borglin *et al*., 1996 and Chen *et al*., 2000) and mostly the partition coefficients are expressed relative to the organic carbon content as

$$K_{oc} = \frac{K_{sed,w}}{f_{oc}} = \frac{C_{sed}}{f_{oc} \cdot C_w} \equiv \frac{C_{oc}}{C_w} \qquad \textbf{7.5}$$

f_{oc} = Organic carbon fraction, C_{oc} = Organic carbon normalised sediment concentration, K_{oc} = partition coefficient normalised to organic carbon content.

In a similar way, having established equilibrium conditions in the exposure of sediments to silicone rubber in this study, $K_{sed,w}$ were calculated from the plots of C_w at versus C_{res} ($C_{Sed} - C_{SedEx}$) at each phase ratio (Fig **7.6**). The slope of such a plot equals $1/K_{sed,w}$.

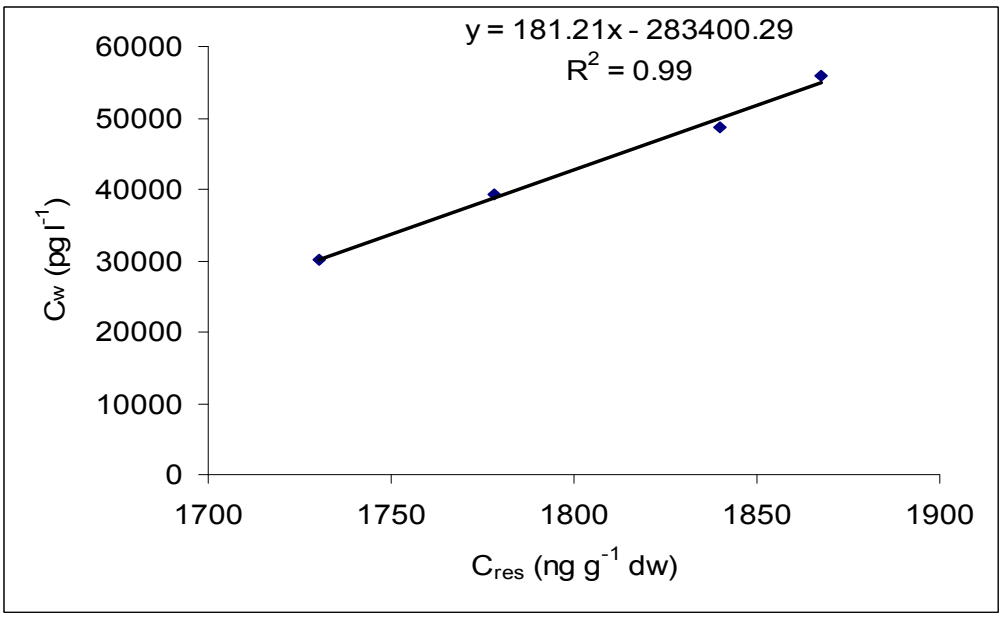


Fig **7.6**: Sorption isotherm of pyrene to determine sediment-water partition coefficient from the slope of the plot

The calculated sediment-water partition coefficients were normalised to the total organic carbon for each sampling point and these are given in Table **7.3**. There was no observable trend in the log K_{oc} for individual PAHs along the contamination gradient, implying that changes in the pore water concentrations with distance from the smelter observed are probably due to differences in the concentrations of contaminants in the sediment as the K_{oc} is broadly similar across the locations. The log K_{oc} values were correlated to the log K_{ow} in Figure **7.7** and strong linear relationships were obtained which were similar (F-test, p> 0.001) across the stations with similar relationships with log K_{ow} having been reported (Table **7.4**).

Table **7.3** Log partition coefficients normalised to organic carbon content ($\text{Log}\,K_{oc}$) with values obtained from the Karickhoff relation shown as predicted log K_{oc} (Karickhoff *et al*., 1979)

PAH	log K_{ow}[1]	Log K_{oc} (L kg^{-1} oc)					
		Predicted Karickhoff, 1979	Station				
			4	5	6	7	8
Naphthalene	3.35	3.14	nd	nd	3.97	3.68	4.06
Acenaphthylene	3.61	3.40	3.93	nd	3.93	3.99	4.06
Acenapthene	3.92	3.71	4.94	nd	4.23	3.85	4.49
Fluorene	4.18	3.97	5.47	nd	4.30	4.00	4.73
Phenanthrene	4.52	4.31	5.34	nd	5.12	4.56	5.28
Anthracene	4.50	4.29	5.62	nd	4.94	4.64	5.04
Dibenzothiophene	4.38	4.17	5.09	nd	5.02	4.34	4.98
Fluoranthene	5.20	4.99	5.58	nd	5.86	4.92	5.33
Pyrene	5.00	4.79	5.30	4.14	4.88	4.88	5.03
Benzo[*c*]phenanthrene	5.76	5.55	5.74	5.39	5.69	5.64	5.72
Benzo[*a*]anthracene	5.91	5.70	6.35	5.29	6.69	6.04	6.37
Chrysene	5.86	5.65	5.94	5.45	6.16	5.88	6.14
Benzofluoranthenes	6.11	5.90	6.91	7.04	6.65	7.01	6.95
Benzo[*e*]pyrene	6.44	6.23	6.82	6.90	6.50	6.83	6.82
Benzo[*a*]pyrene	6.35	6.14	6.89	7.04	6.74	6.96	6.82
Perylene	6.25	6.04	6.67	6.92	6.33	7.08	6.74
Dibenz[*a,h*]anthracene	6.75	6.54	7.55	7.62	7.32	7.43	7.26
Indeno[1,2,3-*cd*]pyrene	7.66	7.45	8.01	7.99	8.06	7.98	8.06
Benzo[*ghi*]perylene	6.90	6.69	7.47	7.61	7.17	7.38	7.31

nd- not determined due to poor regressions. [1] Obtained from Sangster, 2005.

$\text{Log}\,K_{oc}$ values were in most cases higher than those predicted by the Karickhoff equation ($\log K_{oc} = \log K_{ow} - 0.21$) which has also been shown for PAHs (Gustafsson *et al*., 1997) and attributed to the presence of a soot phase in sediments. Similar high partition coefficients have been reported for field sediments elsewhere (Jonker and Smedes, 2000; Hawthorne *et al*., 2006) which suggests that the use of $\log K_{oc}$ in risk assessments to assess potential for transfer of contaminants from sediment to water or biota may over-estimate the true risk. Therefore experimental measurements of $\log K_{oc}$ in field sediments should improve the reliability of risk assessments.

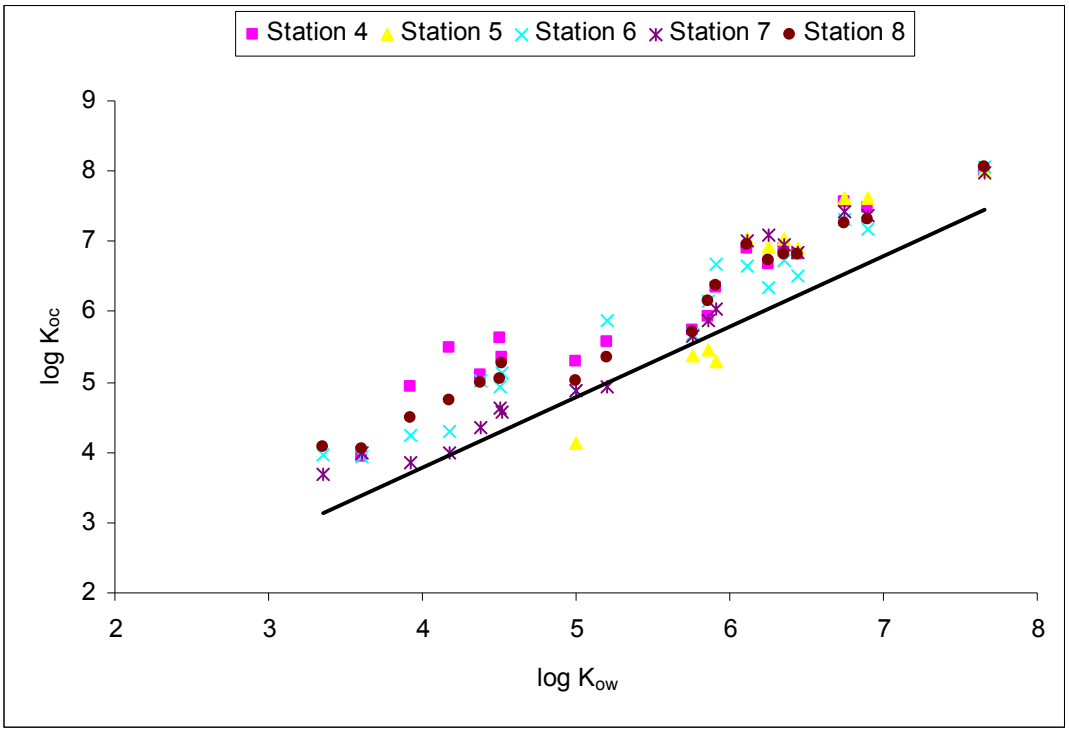


Fig **7.7**: Log K_{oc} as a function of log K_{ow} (Sangster, 2005) for sediments from Loch Leven. Drawn line is the Karickhoff relation $\log K_{oc} = \log K_{ow} - 0.21$ (Karickhoff *et al*., 1979)

Estimates of log K_{oc} increased with increasing log K_{ow} values, indicating the partitioning of the PAHs is strongly determined by the hydrophobicity of the compounds and implies that the higher log K_{ow} PAHs are more strongly bound to the sediments than the low log K_{ow} PAHs. This is supported by the high concentrations of the low log K_{ow} PAHs in pore water. Booij *et al*. (1997) suggest that slopes equal to one in log K_{oc} versus log K_{ow} relations should be considered as exceptions rather than the rule due to possible differences in the effect of dissolved octanol on the activity coefficient in the water phase related to the hydrophobicity of the contaminants, and non-proportionality of the non-ideality of the solution of the contaminants in the organic matter to the non-ideality of the solution in octanol. Differences in the slope and intercept of these relationships could also be due to uncertainty in the measurements of K_{ow}. The close to one slopes with similar widths of 95 % confidence interval of the relationships obtained suggest the sorption is strongly determined by hydrophobicity.

Table **7.4** Summary of linear regression analysis of log K_{oc} versus log K_{ow} [a] with 95 % confidence interval of intercept and slope with regressions reported in literature for PAHs

Station/ Source	*Slope*	*Intercept*	r^2	s^b	n^c
4	0.88 ± 0.15	1.24 ± 0.84	0.91	0.33	18
5	1.62 ± 0.54	-3.62 ± 3.39	0.84	0.52	11
6	0.98 ± 0.11	0.50 ± 0.60	0.96	0.27	19
7	1.14 ± 0.13	-0.49 ± 0.71	0.95	0.32	19
8	0.96 ± 0.09	0.69 ± 0.51	0.97	0.23	19
Booij *et al*., 2003 [d]	1.32	-0.39	0.95	0.26	39
Jonker and Koelmans, 2001[e]	1.07 ± 0.44	-0.77 ± 2.57	0.77	0.38	10
Jonker and Smedes, 2000[f]	1.14 ± 0.32	1.40 ± 1.89	0.86	0.38	12

[a] log K_{ow} values from Sangster (2005)
[b] standard deviation of the fit
[c] sample size
[d] determined from the ratio of concentrations in sediment to that in pore water measured using low density polyethylene (LDPE) samplers
[e] estimated from data provided
[f] Determined using the co-solvent method

7.4 Conclusions

Concentrations of PAHs in pore water in sediments from Loch Leven contaminated by waste from an aluminium smelter were dominated by acenaphthene, fluorene, fluoranthene, pyrene, chrysene, benzofluoranthenes and benzo[*e*]pyrene. The pore water concentrations decreased down the loch (Station 8> 7> 6> 5> 4); with higher concentrations being found close to the former smelter plant (Station 8). A similar spatial pattern was seen in the concentrations of PAHs in sediments. Concentrations in pore water were below the MAC-EQS for surface waters proposed under the draft European Union Council directive. Water extractable concentrations of individual PAHs were below 50 % of their corresponding total concentrations in sediment. Log K_{oc} values were found to be significantly correlated with hydrophobicity expressed as log K_{ow} and can be useful in understanding the partitioning of PAHs in field sediments. This study has shown the use of silicone rubber membranes as an equilibrium sampler to measure pore water and water extractable concentrations as well as sediment-water partition coefficients of PAHs in sediments. These data are useful tools to assist in assessing the availability and consequently risks of these contaminants.

CHAPTER EIGHT

Pore water concentrations of PAHs in dredge spoils from Scotland

8.0 Introduction

Measurements of the concentrations of persistent hydrophobic organic pollutants (POPs) in sediment pore waters is difficult due to their very low concentrations, the small sample volumes that are normally available, and their affinity for sampling tubes, bottles, filters, and adsorption onto particulate matter (Smedes, 1994). Passive samplers have been shown to be effective and useful in the sampling of organic contaminants from water and sediment pore water, as they accumulate only the freely dissolved concentrations of POPs, in a similar manner as biological membranes and lipid pools. The importance of free dissolved concentrations in assessing the availability (and consequently environmental risks) of POPs, such as polycyclic aromatic hydrocarbons, to biota is therefore clear. Passive samplers include the two-phase semipermeable membrane devices (SPMDs) introduced by Huckins *et al*. (1990) which consist of a low density polyethylene layflat tubing containing a thin film of triolein; solid phase microextraction (SPME) fibers (Mayer *et al*., 2000; Heringa and Hermens, 2003; Kraaij *et al*., 2003, Yang *et al*., 2007), low density polyethylene strips (Booij *et al*., 2003) and silicone rubber sheets (Smedes, 2007). Silicone rubber (a single phase sampler), has been shown to possess low transport resistance and high partition coefficients making it a suitable alternative to SPMDs (Rusina *et al*., 2007).

Passive sampling using a reference phase that equilibrates with the dissolved concentration in the sampling medium is therefore attractive as a mechanism of studying the availability of contaminants in sediment. The equilibrium sampling approach relies on the use of sampler-water partition coefficients to translate the concentration in the sampler to concentrations in other media (Mayer *et al*., 2003).

FRS Marine Laboratory, Aberdeen, is responsible for licensing the deposition of some wastes, such as dredged spoils, in UK waters adjacent to Scotland, under Part II of the Food and Environment Protection Act 1985. Their assessment process includes chemical

analysis of material being considered for disposal at sea, as well as assessment of various factors influencing the suitability of the proposed disposal locations. This can be supported by field monitoring programmes before and after the deposit has been made. Dredged harbours sediments being considered for disposal at sea are traditionally analysed for total concentrations of polycyclic aromatic hydrocarbons (PAHs), chlorobiphenyls (CBs) and metals and the results are assessed in relation to sediment quality criteria or guidelines.

As part of the progressive development of the licensing process, FRS has measured pore water concentrations of PAHs in groups of sediment samples collected from 6 locations where dredging occurs in the Firth of Clyde, Scotland, UK (which, due to current and historical industrial and domestic inputs, is arguably Scotland's most contaminated large estuary) using passive samplers with silicone rubber as the reference phase. Additional samples of dredged material were obtained from the Firth of Forth, and from Aberdeen harbour, both in the east of Scotland (Fig **8.0**).

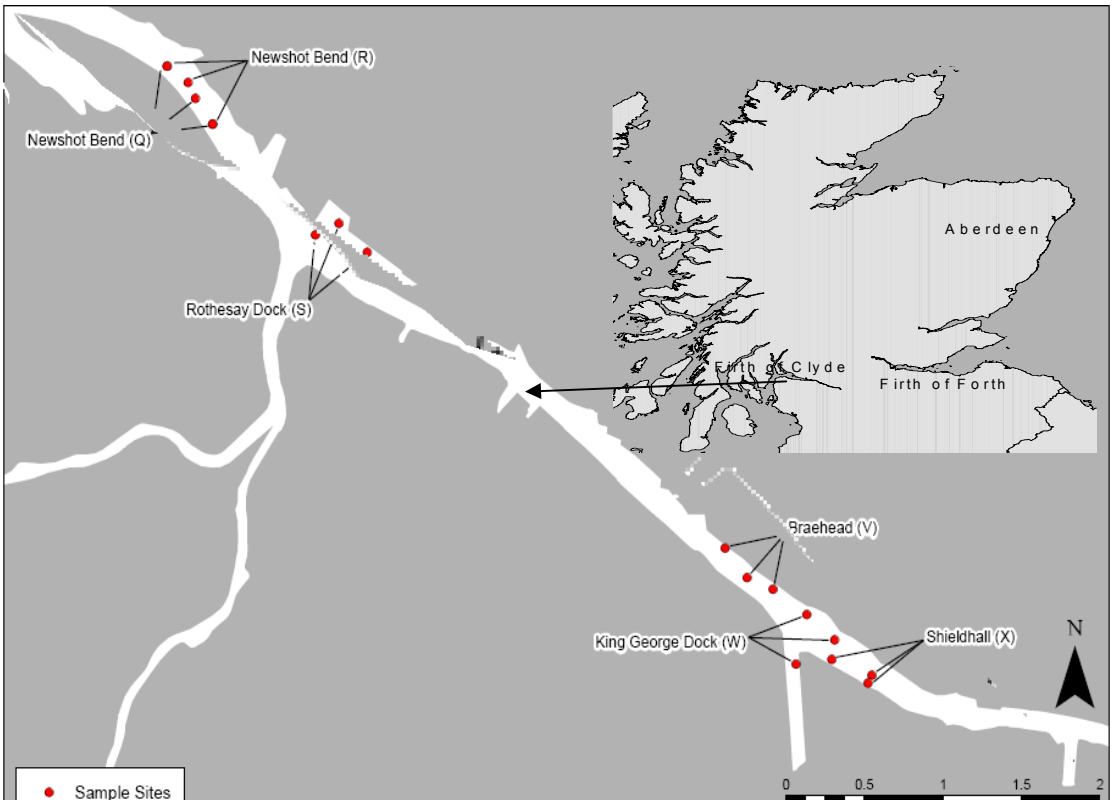


Fig **8.0**: Sampling locations in the Firth of Clyde during dredging operations in 2006 and an inset map showing the Firth of Forth and Aberdeen Harbour

8.1 Theory

Introducing the reference phase (silicone rubber sampler in this case) to a sediment-water system, measurements of the pore water concentrations can be made at different phase ratios (reference phase: sediment), which lead to different degrees of depletion of the contaminants held in the sediment, following equation **8.1**

$$C_w = \frac{C_{sr}}{K_{sr,w}} \qquad \textbf{8.1}$$

where, C_{sr} = concentration in the silicone rubber / ng g^{-1} SR (sr and SR denoting silicone rubber), $K_{sr,w}$ = silicone rubber-water partition coefficient / L kg^{-1}. Silicone rubber-water partition coefficients for the sampler used were independently determined in another study (Yates *et al*., 2007).

$$C_{sr} = \frac{N_{sr}}{M_{sr}} \qquad \textbf{8.2}$$

N_{sr} = Amount of PAH in the silicone rubber, M_{sr} = mass of silicone rubber

Similarly the concentration extracted from the sediment (C_{SedEx}, ng g^{-1} dry weight) can be calculated from equation **8.3**

$$C_{SedEx} = \frac{N_{sr}}{M_{sed}} \qquad \textbf{8.3}$$

M_{sed} = Mass of sediment

Taking an overall mass balance of sediment-water-reference phase system therefore;

$$N_{tot} = C_{sed}M_{sed} + C_{ref}M_{ref} + C_wV_w \qquad \textbf{8.4}$$

C_{ref}, C_{sed} = Concentration in reference/ sampler and sediment respectively (μg kg^{-1})

M_{ref}, M_{sed} = Mass of reference phase (silicone rubber) and sediment respectively in kg;

C_w = Concentration in the water/aqueous phase (μg L^{-1}), V_w = Volume of water (L) and

N_{tot} = total analyte amount in system (μg).

However, because the volume of water normally used is very negligible, the term $C_wV_w \to 0$ and becomes negligible compared to the other terms in equation **8.4**, which then transforms to

$$N_{tot} = C_{sed}M_{sed} + C_{ref}M_{ref} \qquad \textbf{8.5}$$

Assuming steady state,

$$N_{tot} = C^{o}_{sed} M_{sed} \qquad \textbf{8.6}$$

C^{o}_{sed} = total sediment concentration of analyte in sediment at $t = 0$ and C_{sed} = concentration after exposure to reference phase;

Substituting equation **8.6** into **8.5**,

$$C^{o}_{sed} M_{sed} = C_{ref} M_{ref} + C_{sed} M_{sed} \qquad \textbf{8.7}$$

Dividing equation **8.7** by M_{sed} yields

$$C^{o}_{sed} = \left(\frac{C_{ref}}{M_{sed}} \right) M_{ref} + C_{sed} \qquad \textbf{8.8}$$

However, $K_{sed,w} = \frac{C_{sed}}{C_w} \Rightarrow C^{o}_{sed} = K_{sed,w} C^{o}_{w}$ and

$C_{sed} = K_{sed,w} C_w$. Similarly, $K_{ref,w} = \frac{C_{ref}}{C_w} \Rightarrow C_{ref} = K_{ref,w} C_w$ and

substitution and rearrangement yields

$$C_w = C^{o}_{w} - \left(\frac{C_{ref}}{M_{sed}} \right) \left(\frac{M_{ref}}{K_{sed,w}} \right) \qquad \textbf{8.9}$$

Note that $\left(\frac{C_{ref}}{M_{sed}} \right) . M_{ref} = \frac{N_{ref}}{M_{sed}} = C_{SedEx}$ **8.10**

Therefore equation **8.9** becomes

$$C_w = C^{o}_{w} - C_{SedEx} \left(\frac{1}{K_{sed,w}} \right) \qquad \textbf{8.11}$$

Equation **8.11** is of the form $y = a + bx$ where; $y = C_w$; $a = C^{o}_{w}$; $b = {}^{1}\!/\!_{K_{sed,w}}$ and

$x = C_{SedEx}$. Plotting equation **8.11** and extrapolating to both axes gives the value of the pore water concentrations C^{o}_{w} and the maximum water exchangeable (extractable) concentration C^{o}_{SedEx} .

8.2 Materials and Methods

8.2.1 Materials

HPLC grade solvents (dichloromethane, ethyl acetate, acetone, methanol and *iso*-hexane) were purchased from Rathburn Chemicals Ltd, Scotland, UK. Certified solid standards for PAHs (including deuterated PAHs) were obtained from QMX Laboratories, Essex, UK and dissolved in *iso*-hexane to obtain required concentrations of spiking and calibration solutions. To avoid contamination of samples, all glassware, stainless steel forceps, were either washed in Decon® 180 solution and rinsed with distilled water or washed in a CAMLAB GW 4050 glassware washer and dried in an oven at 100 ^{o}C. Before use, the glassware were rinsed twice each in dichloromethane and *iso*-hexane, with the latter allowed to evaporate to dryness. Glass Duran® or Schott® (VWR, Leicester, UK) bottles were used and always capped with aluminium-lined PTFE caps to reduce or prevent sorption of PAHs by the caps.

AlteSil™ Silicone rubber sheet (600 × 600 mm, 0.5 mm thick) was purchased from Altec Products, Ltd, Cornwall, UK. The silicone rubber sheets used were cut into required sizes (6 × 4 cm, or 9 × 4 cm) and pre-extracted in hot ethyl acetate using a Soxhlet apparatus for ~ 4 days before use to remove any low molecular weight oligomers or residues that may be co-extracted with the analytes and could affect instrumental analysis (Gruber *et al*. 2000; Rusina *et al*., 2007). After pre-extraction and cooling, the sheets were removed from the solvent and stored in bulk in glass jars containing methanol prior to use. The weight of the sheets used was determined after extraction of exposed sheets to avoid contamination from the environment.

8.2.2 Methods

8.2.2.1 Sediment Sampling

Samples of sediment were collected from FRV *Clupea*, using a 0.1 m^2 Day Grab at six sites within the Firth of Clyde (Fig **8.0**). The top 2 cm of the sediment was transferred to a solvent washed aluminium can, thoroughly mixed, labelled and stored at -20 ± 5 ^{o}C until

required for analysis. Due to insufficient sample mass, the sediment samples were pooled for each site.

8.2.2.2 Bulk properties of sediment

Total organic carbon (TOC) was determined on freeze dried sediment using a ThermoQuest Flash EA 1112 elemental analyser, following removal of inorganic carbon by acidification using hydrochloric acid. The method limit of detection is 0.005 mg although the limit of quantification (LOQ) calculated as 0.005 divided by the sample weight analysed × 100 % is normally reported. E.g. for a sample weight of 16.50 mg, the LOQ is 0.03 %. Particle size (PS) analysis of sediment samples was carried out by laser granulometry using a Malvern Mastersizer E Particle Size Analyser, after freeze drying of the sediments. The precision of the method based on 7 replicate measurements of a laboratory reference material (LRM C100) on the D (4, 3) mean weighted volume fraction equals 2.5 %.

8.2.2.3 PAH analysis of sediment

Sediment samples were thoroughly mixed after thawing and ~ 10 g per sample was removed for determination of moisture content by drying in an oven at 80 ± 5 ^{o}C for 22 ± 2 h (Webster *et al*., 1997). PAHs were determined as described by Webster *et al*. (2004). Briefly, aliphatic hydrocarbon internal standards, heptamethylnonane and squalane and deuterated aromatic internal standards (D_8-naphthalene, D_{10}-biphenyl, D_8-dibenzothiophene, D_{10}-anthracene, D_{10}-pyrene and D_{12}-benzo[*a*]pyrene (100 or 200 µl; approximately 1 µg ml^{-1} each)) were added to 0.3 ± 0.1 g sub samples of wet sediment. The hydrocarbons were extracted using dichloromethane/methanol with sonication and the halogenated solvent isolated and dried over sodium sulphate prior to solvent exchange into *iso*-hexane. A laboratory reference material (LRM) and procedural blank were also included in the analyses. Recoveries of ≥ 85 % and precision of ≤ 17 % for individual PAH compounds were obtained with spiked sediment samples.

8.2.2.4 Exposures of sediments to silicone rubber samplers

The sediment samples were weighed into 50 ml and 250 ml glass Duran® bottles and pre-extracted silicone rubber sheets (one each) that had been loaded with the PAH performance reference compounds (PRCs) as described by Booij *et al*. (2002) were added to each bottle to obtain different phase (g silicone rubber per g sediment) ratios. Duplicate bottles were placed on an orbital shaker horizontally and shaken at 200 rpm for 20 days in a dark room at 20 ± 2 ^{o}C. In a preliminary developmental study, this time was found to be sufficient for the PAHs to attain equilibrium. After this time, the sheets were removed from the bottles, rinsed with distilled water and gently wiped dry with paper tissue to remove any adhering water before extraction. The weights of the silicone rubber sheets were recorded after the extraction to avoid contamination from external sources. A procedural blank (an un-spiked sheet exposed to only the distilled water used to liquefy the sediments) was included and analysed in the same manner as the other samples. The result from the procedural blank was subsequently subtracted from the results of samples. Similarly, a spiked sheet was also analysed to obtain the initial amounts of PRCs in the sheets.

8.2.2.5 Extraction of silicone rubber sheets

The silicone rubber sheets were Soxhlet extracted using 100 ± 5 ml of *iso*-hexane: acetone (3:1$^{v}/_{v}$) mixture or methanol for 6 h. Aliphatic hydrocarbon standard (containing heptamethylnonane and squalane) and deuterated PAH aromatic internal standard containing D_8-naphthalene, D_{10}-biphenyl, D_8-dibenzothiophene, D_{10}-anthracene, D_{10}-pyrene and D_{12}-benzo[*a*]pyrene was then added to the cooled extract, concentrated by rotary evaporation followed by nitrogen blow down of the extract to 1 ml.

8.2.2.6 Clean up

The extracts from the sediments and silicone rubber were reduced to 0.5 ± 0.2 ml and an aliquot of the concentrated extract was fractionated using an isocratic, normal phase Genesis metal-free high performance liquid chromatograph (HPLC) column (25 cm × 4.6 mm) and eluted with *iso*-hexane at a flow rate of 2 ± 0.1 ml min^{-1} into aliphatic and aromatic fractions. The aliphatic fraction was discarded and the aromatic fraction collected

in 100 ml flasks, concentrated by rotary evaporation and further reduced under nitrogen to 50 ± 10 µl for analysis by gas chromatography – mass selective detection (GC-MSD).

8.2.2.7 Gas chromatography-mass selective detection (GC-MSD)

The concentrations and composition of the PAHs were determined by GC-MSD using an HP6890 Series Gas Chromatograph interfaced with an HP5973 MSD fitted with a cool on-column injector (Webster *et al.*, 2007). Briefly, a non-polar HP5 (30 m × 0.25 mm id, 0.25 µm film thickness; Agilent Technologies, Stockport, England) column was used for the analyses with helium as the carrier gas, controlled using the constant flow mode at 0.7 ml min^{-1}. The MSD was set for selective ion monitoring (SIM) with a dwell time of 50 ms. Injections were made at 50 ^{o}C and the oven temperature held constant for 3 min. Thereafter, the temperature was raised at 20 $^{o}C\ min^{-1}$ up to 100 ^{o}C, followed by a slower ramp of 4 $^{o}C\ min^{-1}$ up to a final temperature of 270 ^{o}C. A total of 29 ions plus the six internal standard ions were measured over the analysis period, thus incorporating 2- to 6-ring, parent and branched PAHs. Limits of detection based on multiplying the standard deviation of the mean of the lowest standard (0.005 ng ml^{-1}) by 4.65 were found to be < 0.2 ng g^{-1} for chrysene and < 0.1 ng g^{-1} for benzo[*a*]pyrene. The GC-MSD is calibrated using seven different concentrations of a solution containing 33 PAHs.

8.3 Results and Discussion

8.3.1 Sediment PAH concentration

All the sediments including those from Aberdeen Harbour and the Firth of Forth were muddy, containing 56 – 86% of particles < 63 µm diameter. The organic carbon concentrations were correspondingly high at 4.2 – 8.6%. The total sediment PAH concentration (2- to 6-ring parent and alkylated PAHs, Table **8.0**) averaged 33,073 ± 4964 ng g^{-1} dry weight with similar concentration profiles across the Clyde estuary sites (Fig **8.1**). The 3- to 5-ring compounds accounted for ~ 80 % of the total concentration. The sediment from the Firth of Forth had a higher percentage of naphthalenes (20 %) compared to the other sites (mean value of ~ 8.3 %). The concentrations in the Clyde sediments on average a factor of 14 times higher than sediment total PAH concentration found in Telford

Dock, Aberdeen Harbour, UK, which is a busy harbour for vessels linked with the North Sea oil and gas and fishing industries, as well as ferries to and from the Northern Isles.

Table **8.0**: Sediment bulk properties and total PAH (2- to 6-ring parent and alkylated compounds) concentrations from the eight sampling points[#]

Location[#]	Field ID	% TOC	PS % < 63 μm	Total PAH (ng g^{-1} dw)
1	King George Dock	8.6	69.7	35,968
2	Shieldhall	7.0	72.2	38,437
3	Braehead	5.7	70.8	32,169
4	Newshot Bend Q	5.8	63.4	25,361
5	Newshot Bend R	4.2	55.8	29,667
6	Rothesay	7.6	72.9	36,834
7	Aberdeen Harbour	2.8	64.0	2,358
8	Firth of Forth	4.8	85.9	12,128

[#] The first 6 samples were from the Firth of Clyde while the seventh is from Telford dock, Aberdeen Harbour, and the last sample from the Firth of Forth

The UK is currently developing a system of action levels to aid in the management of the disposal of dredge spoil. Action levels are based on concentrations of contaminants in the < 2 mm fraction of sediment. Contaminant levels in dredged material below Action level 1 are of no concern and will be typical of concentrations found in estuarine or coastal sediments. Action level 2 also measures sediment concentrations but incorporates ecotoxicological information thereby describing the concentrations above which biological effects are likely to be observed. The use of action levels typically results in three categories:

Category 1: Concentrations < Action level 1

Category 2: Concentrations > Action level 1 but < Action level 2

Category 3: Concentrations > Action level 2

Conventionally, Category 1 material would be suitable for disposal to sea. Category 2 material might require further consideration, including possible additional analysis, before a decision can be made and may require mitigating licence conditions before being disposed to sea. Category 3 would not normally be accepted for sea disposal unless further testing, mitigative measures or impact assessment showed that this was acceptable.

Provisional Action level 1 values (Table **8.1**) have been proposed for PAHs, but Action Level 2 values have not yet been developed.

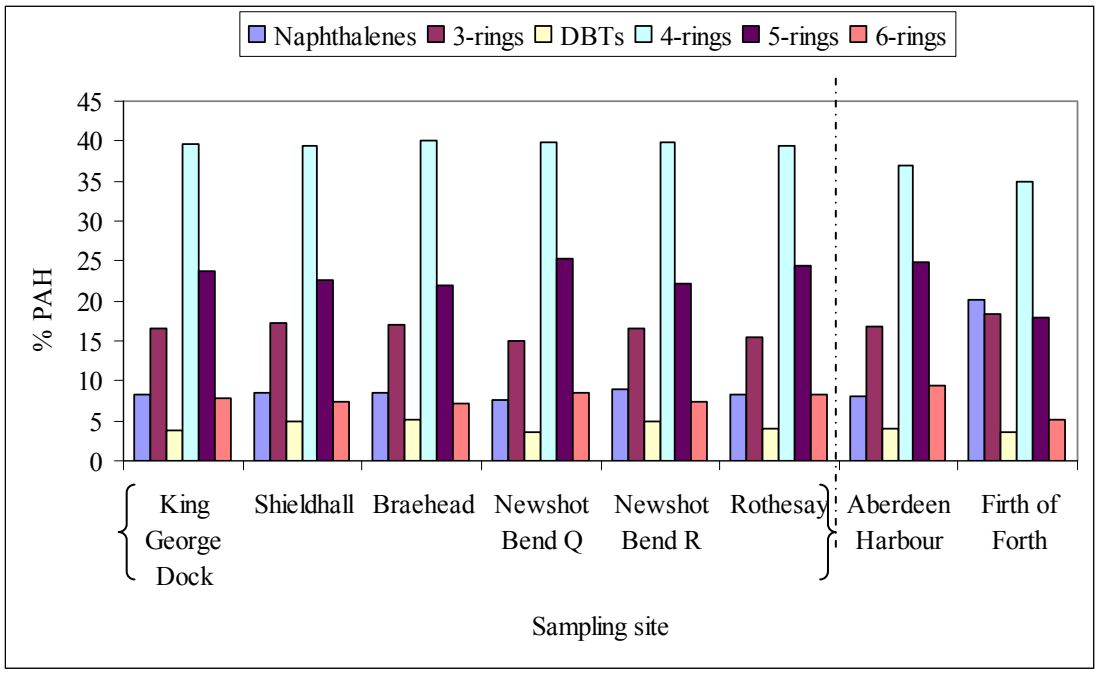


Fig **8.1**: Sediment PAH composition determined in sediments collected from the Clyde estuary (in double brace), Aberdeen harbour and Firth of Forth in 2006.
Naphthalenes = sum of naphthalene; 1 & 2-methylnaphthalene; C2-, C3- and C4-naphthalenes
3-rings = sum of phenanthrene; anthracene, C1-178, C2-178, and C3-178; acenaphthylene; acenaphthene and fluorene
DBTs = sum of Dibenzothiophene; C1-DBT, C2-DBT, and C3-DBTs
4 –rings = sum of fluoranthene; pyrene; C1-202, C2-202, and C3-202; benzo[*c*]phenanthrene; benz[*a*]anthracene; benz[*b*]anthracene; chrysene; C1-228; C2-228.
5-rings = sum of benzofluoranthenes (benzo[*b*] and [*k*]fluoranthene); benzo[*e*]pyrene; benzo[*a*]pyrene; perylene; C1-252 and C2-252
6-rings = sum of indeno[*1, 2, 3-cd*]pyrene; benzo[*ghi*]perylene; C1-276 and C2-276

Table **8.1**: Comparison of dredge spoil analyses for PAHs with Provisional UK Action Level 1 concentrations for PAHs in use at FRS Marine Laboratory for dredge spoils assessment

PAH	Concentration (ng g^{-1} dry wieght)								
	Action Level 1[†]	Locations[#]							
		1	2	3	4	5	6	7	8
Naphthalene	100	201.8	224	183.8	130.6	159.8	233.5	16.0	175.0
Acenaphthylene	100	10.0	16.0	11.4	8.1	8.6	13.2	0.8	3.1
Acenaphthene	100	162.2	164.5	162.1	107.0	150.7	157.3	15.2	31.0
Fluorene	100	182.0	198.2	157.0	98.2	153.0	158.9	11.2	70.9
Phenanthrene	100	1097.6	1147.8	900.5	655.7	854.5	934.0	77.7	410.5
Anthracene	100	329.0	401.9	309.7	211.3	293.1	313.0	22.4	135.4
Fluoranthene	100	2074.7	2212	1851.0	1401.0	1727.7	1999.6	132.0	438.2
Pyrene	100	1926.6	2029.9	1719.2	1304.4	1600.4	1870.8	125.5	559.6
Benz[*a*]anthracene	100	1100.1	1198.5	1008.7	805.5	915.4	1147.3	66.7	208.5
Chrysene	100	1496.6	1530.7	1267.0	1003.3	1151.0	1480.6	72.6	378.1
Benzofluoranthenes	100	2943.5	3013.2	2437.1	2205.3	2254.5	3059.8	193.9	694.1
Benzo[*a*]pyrene	100	1263.7	1297.8	1063.6	963.7	987.3	1331.4	82.4	248.6
Dibenz[*a,h*]anthracene	10	179.8	186.6	151.3	136.6	137.5	190.4	13.5	20.3
Indenopyrene	100	1132.6	1146.3	929.9	871.7	888.5	1238.1	82.6	174.6
Benzoperylene	100	1058.6	1049.9	851.0	785.2	794.8	1132.1	69.6	229.0

[†]Action Levels from Hayes *et al*. (2005)
[#]Locations 1, 2, 3, 4, 5, 6, 7 and 8 represent King George Dock, Shieldhall, Braehead, Newshot Bend Q, Newshot Bend R, Rothesay, Aberdeen Harbour (Telford Dock), and Firth of Forth respectively. Highlighted values are below the set Action Levels

8.3.2 Pore water concentrations

Due to the low amounts of sediment available, sorption isotherms created in this study were limited to data for only two (high and low) phase ratios (Fig 8.2). Smedes (2007a) discussed the form of sorption isotherms commonly encountered during passive sampling using silicone rubber samplers in sediments. PAH compounds often show nonlinear isotherms, but the departure from linearity occurs at phase ratios that are not readily accessible to the silicone rubber samplers. The phase ratios used in the current work were therefore assumed to lie within the linear portion of the isotherms.

Total PAH pore water concentration (sum of 31 PAHs) was an average of 492 ng L^{-1} across the sites from the Firth of Clyde, which was a factor of 2.2 and 3.5 higher than the total pore water concentrations found in Aberdeen Harbour and Firth of Forth (207.5 and

128.7 ng L^{-1} respectively). There was no significant difference in the pore water concentrations across the sites from the Clyde ($p > 0.05$, ANOVA). Pore water concentrations at Telford Dock, Aberdeen Harbour were determined during the ICES Passive Sampling Trial Survey, 2006-2007 (Smedes *et al*., 2007b).

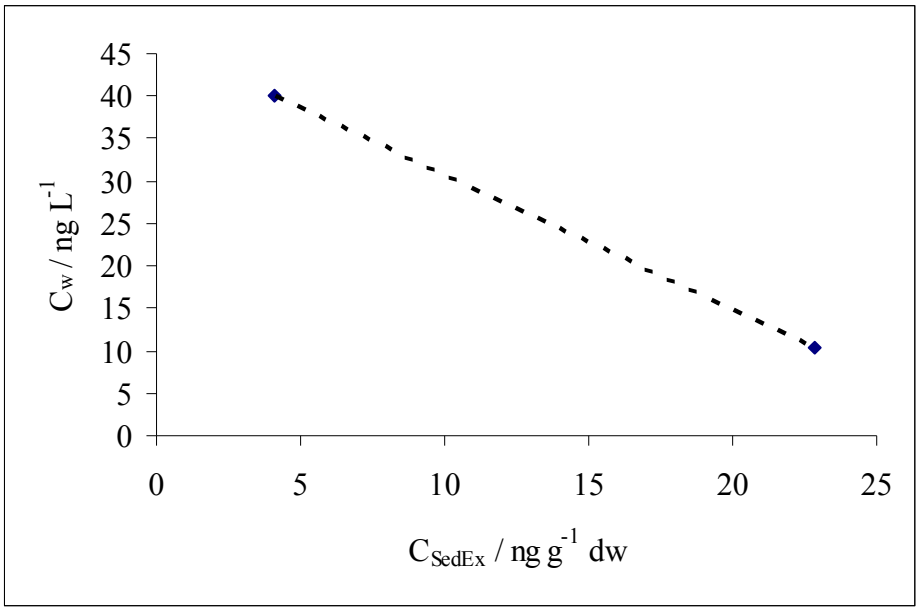


Fig **8.2**: Plot of C_w versus C_{SedEx} to determine pore water (freely dissolved) concentrations C_w^o by extrapolation to the C_w axis. Isotherms were assumed linear even though only 2 phase ratios were used

A profile of the pore water concentrations across the 8 study sites (Fig **8.3)** shows the 2- to 3-ring PAHs dominating in this instance. The dominance of the 2- to 3-ring PAHs may be due to their higher solubility and lower affinity for the sediment. The high proportion of the alkylated 2- and 3-ring PAHs suggests a petrogenic source of the PAHs in the Clyde sediments (Webster *et al*., 2005). The pore water concentration profiles found in Telford Dock, Aberdeen Harbour and the Firth of Forth (Fig **8.3**) appeared similar to those found in the Clyde samples, even though the Firth of Forth had a higher percentage of 4-ring PAHs.

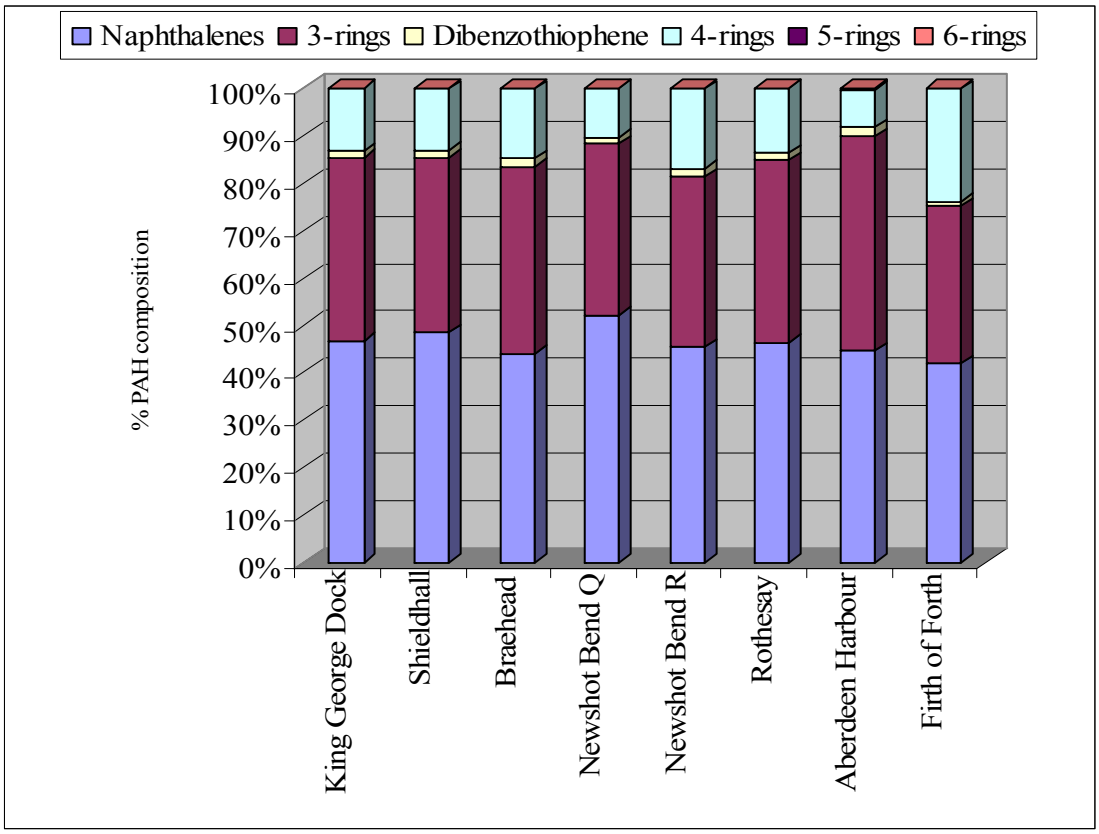


Fig **8.3**: Pore water composition in sediment sampled using the silicone rubber sampler. Only the result for the exposure using a large amount of sediment (where no depletion is expected) was reported for indeno[1,2,3-*cd*]pyrene, as the amounts were quite low in the other scenario leading to uncertainty in the analyses and subsequent data processing

8.3.3 PAH concentration ratios

PAH concentration ratios have been used (Webster *et al*., 2001; Webster *et al*., 2005) to aid identification of PAH sources. A Fluo/Pyr ratio > 1 and Phen/Ant ratio less than 10 or C1-178 (Methylphenanthrene)/Phen ratio < 2 indicates a pyrolytic source while contrastingly, a petrogenic source is indicated by a Fluo/Pyr ratio < 1, a Phen/Ant ratio > 10 and C1-178/Phen ratio > 2. Applying these ratios to the data on PAHs in sediment showed (Fig **8.4**) most of the sites to be of a predominately pyrolytic source, which has similarly been shown from other sediment data by Webster *et al*. (2005). The Firth of Forth sediment showed ratios consistent with a mixed zone. However much greater variance is observed for the concentration ratios calculated from the pore water data suggesting the assessment based on these ratios, which was primarily designed for use with sediment data, may not be immediately applicable to pore water data.

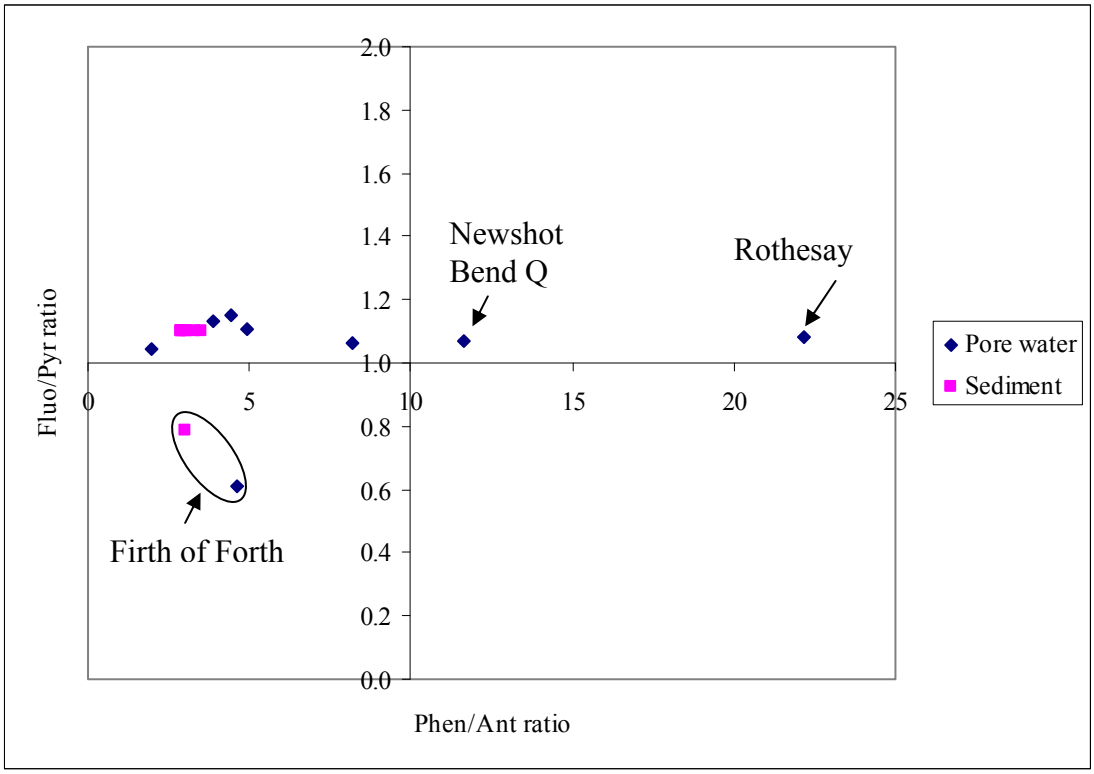


Fig **8.4**: PAH concentration ratios in sediments and pore waters to aid source identification. Rothesay and Newshot Bend Q ratios suggest a mixed source

8.3.4 Principal component analysis

Differences in pore water concentration profiles between the sites were investigated using principal component analysis (PCA) in Minitab 14. PCA is a data reduction technique that aims at summarising information in a multivariate data set by a linear combination of the original variables referred to as principal components (Webster *et al*., 2005). The factor-loading plot (Fig **8.5**) shows the contribution of each variable (PAHs) to the variation observed in the data, with the first three components explaining 65.7 %, 20.1 % and 7.9 % of the variation (cumulatively 93.7 %) respectively with corresponding variance of 20.38, 6.23 and 2.45. Factor 1 distinguishes between the predominantly heavier PAHs (indeno[1,2,3-*cd*]pyrene, benzofluoranthenes, benzo[*ghi*]perylene, benzo[*a*]pyrene, dibenz[*a,h*]anthracene, and perylene) and acenaphthylene which have negative scores, and the other lighter PAHs with positive scores (which however also includes the relatively heavy benzo[*e*]pyrene and 7-methylbenzo[*a*]pyrene). The perylene is entirely from Aberdeen Harbour and Firth of Forth as this PAH was not determined in the Clyde pore-waters.

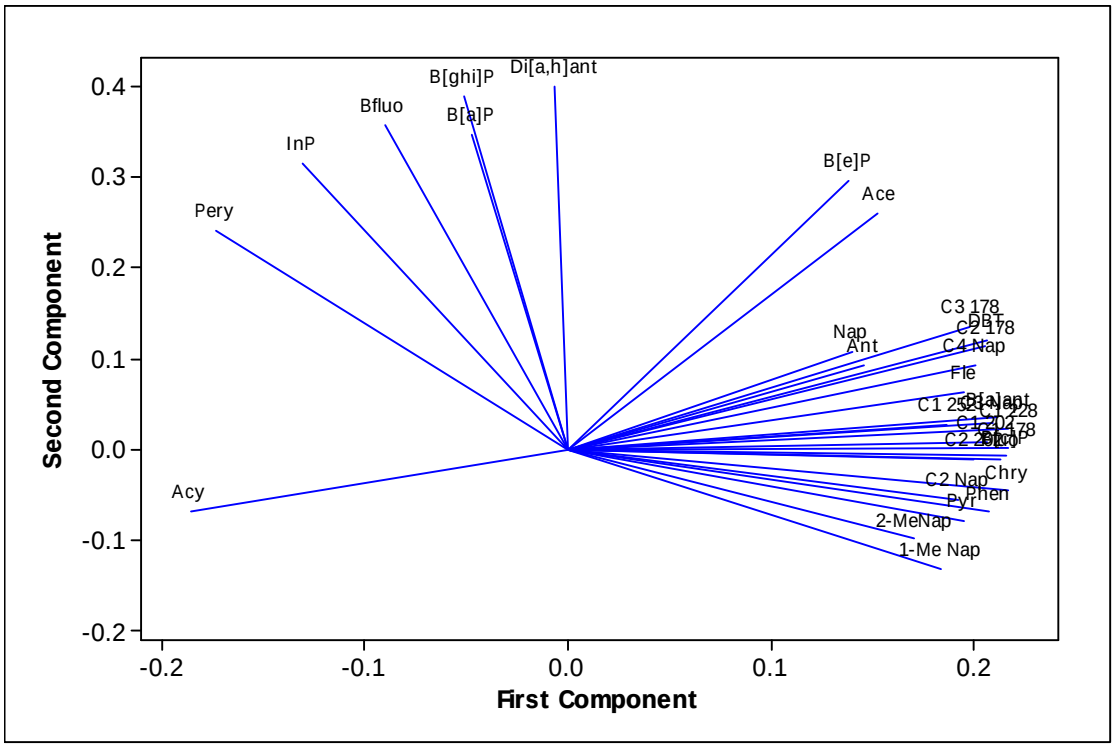


Fig **8.5**: Loading plot for the PCA of PAH pore water concentrations of sediments from the Firth of Clyde, Aberdeen Harbour and Firth of Forth. Nap-Naphthalene; 1- and 2-MeNap= 1- and 2-methylnaphthalene; C2- to C4-Nap = C2- to C4- naphthalene; Acy-Acenaphthylene; Ace-Acenaphthene; Fle- Fluorene; Phen-Phenanthrene; Ant-Anthracene; DBT-Dibenzothiophene; Fluo-Fluoranthene, Pyr- Pyrene; B[*c*]P-Benzo[*c*]phenanthrene; B[*a*]ant-Benz[*a*]anthracene; Chry-Chrysene; Bfluo-Benzofluoranthenes; B[*a*]P-Benzo[*a*]pyrene; B[*e*]P-Benzo[*e*]pyrene; Pery- Perylene; Di[*a,h*]ant-Dibenz[*a,h*]anthracene; InP-Indeno[1,2,3-*cd*]pyrene; B[*ghi*]P-Benzo[*ghi*]perylene

The score plot (Fig **8.6**) distinguished between the Aberdeen Harbour, Firth of Forth and the Firth of Clyde sediment pore water concentrations with the Clyde test sites positively correlated with component 1, even though the Rothesay site of the Clyde was slightly negatively correlated. The Aberdeen Harbour was also positively correlated with the second component, while the Firth of Forth was negatively correlated by this component. These preliminary data analyses suggest that the heavier PAHs are relatively more important in pore waters from Aberdeen harbour than from the Clyde estuary.

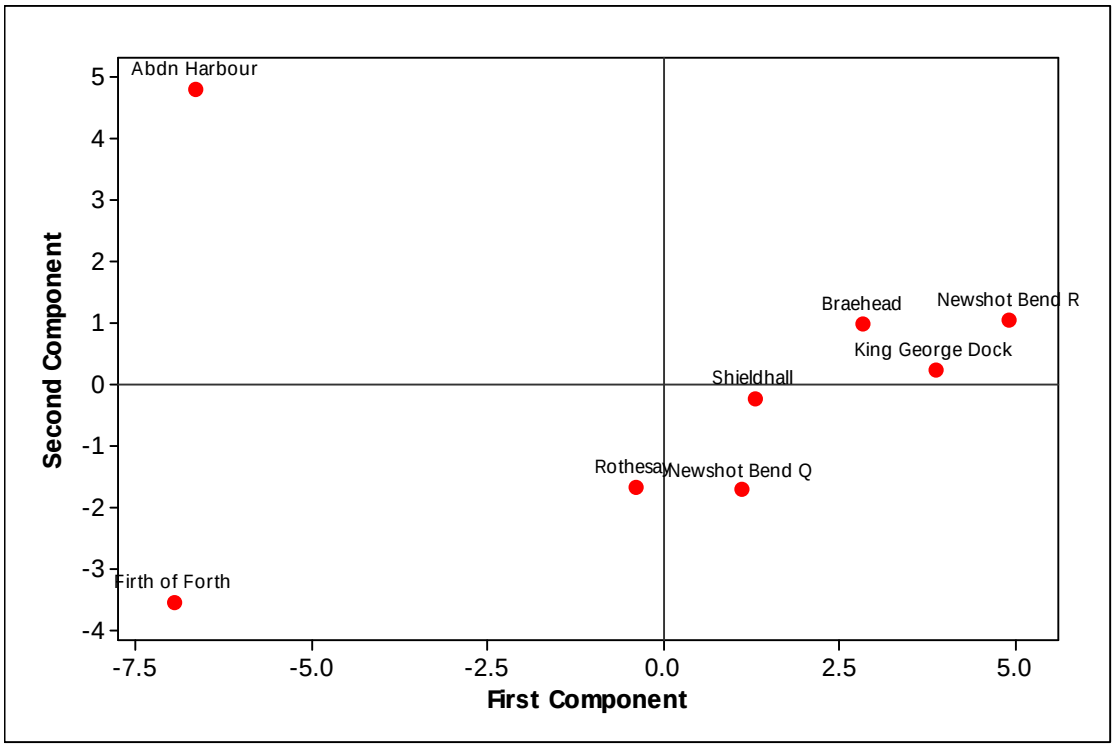


Fig **8.6**: Score plot for the PCA of PAH pore water concentrations of sediments from the Firth of Clyde and Aberdeen Harbour

8.3.5 Comparisons with quality standards

Draft Maximum Allowable Concentration - Environmental Quality Standards (MAC-EQS) for PAHs in surface waters (Table **8.2**) are being discussed in relation to environmental quality assessments under the European Union Council Directive (EU, 2007). The pore water concentrations in all the sediment samples were very much (1 – 3 orders of magnitude) less than the draft MAC-EQS values for the 5 PAHs for which draft standards have been developed. This suggests that the concentrations in the pore waters may not present a significant risk to sediment-dwelling organisms, even though the total concentrations in the sediments from the Clyde and Forth are generally much greater than the Provisional UK Action Levels 1 for dredge spoils (Table **8.1**).

Table **8.2** Maximum allowable concentration – Environmental Quality Standards for PAHs in surface waters and corresponding PAH pore water concentrations in this study

PAH	MAC-EQS	Concentration (ng L^{-1}) Location							
		1	2	3	4	5	6	7	8
Anthracene	400	3.582	14.250	8.401	12.398	4.588	6.081	2.045	2.220
Benzofluoranthenes	30	0.430	0.144	0.103	0.144	0.085	0.180	0.113	0.090
Benzo[*a*]pyrene	100	0.083	0.050	0.035	0.050	0.010	0.030	0.004	0.022
Indenopyrene	2	0.043	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Benzoperylene	2	0.048	0.020	0.019	0.021	0.013	0.022	0.013	0.007

[#]Locations 1, 2, 3, 4, 5, 6, 7 and 8 represent Aberdeen Harbour (Telford Dock), King George Dock, Shieldhall, Braehead, Newshot Bend Q, Newshot Bend R, Rothesay and Firth of Forth respectively.

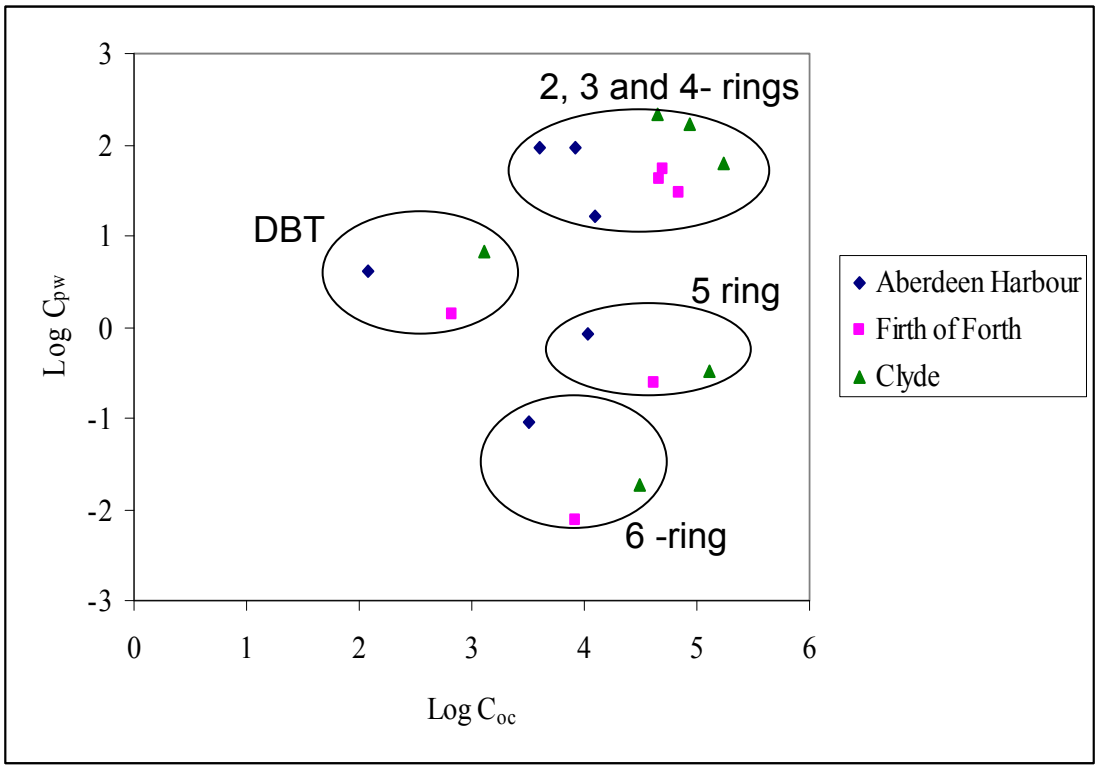


Fig **8.7**: Relationship between the log pore water concentrations (ng L^{-1}) with the log organic carbon normalised sediment PAH concentration (ng g^{-1} oc) for the different sampling points and locations. Individual points represent PAH group based on ring size. The PAH groups are: **Naphthalenes** = sum of naphthalene, 1 & 2-methylnaphthalene C2-, C3- and C4- naphthalenes; **3-rings** = sum of phenanthrene, anthracene, C1-178, C2-178, C3-178, acenaphthylene, acenaphthene and fluorene; **DBTs** = Dibenzothiophene; **4 –rings** = sum of fluoranthene, pyrene, C1-202, C2-202, benzo[*c*]phenanthrene, benz[*a*]anthracene, chrysene and C1-228; **5-rings** = sum of benzofluoranthenes, benzo[*e*]pyrene, benzo[*a*]pyrene, perylene, and C1-252; **6-rings** = sum of indeno[*1, 2, 3-cd*]pyrene and benzo[*ghi*]perylene.
Benzofluoranthene is the summation of benzo[*b*] and [*k*]fluoranthene

The relationship between the pore water concentrations and the concentrations (grouped based on PAH ring size) in sediment normalised to organic carbon is shown in Fig **8.7**. The three sampling areas show considerable similarities, and the data points for each of the 6 groupings of PAHs used generally group together. Within each group, the consistently higher concentrations in sediment from the Clyde than from the Forth, and from Aberdeen, can be clearly distinguished. Pore water concentrations for the 2- to 4-ring PAHs were higher in the Firth of Clyde than in Aberdeen Harbour or Firth of Forth samples (Clyde > Aberdeen Harbour > Firth of Forth), while the 5- to 6-ring PAH concentrations in pore water were higher in Aberdeen Harbour than in samples from the other two areas (Aberdeen Harbour > Clyde > Firth of Forth).

Simple partitioning theory would indicate that concentrations in pore water should be proportional to the normalised concentrations in sediment. The constant of proportionality is known as K_{oc}. Within the groupings of PAHs used in Figure **8.7**, the compounds show generally similar K_{ow} and K_{oc} values. Therefore, within PAH groupings, a correlation between the pore water and normalised sediment concentrations would be expected. However, no such correlations are apparent in Figure **8.7**, i.e. this simple application of partitioning theory is not an adequate model for these data.

Smedes (2007a), Yates *et al*. (2007a) and others have noted that sorption isotherms for PAHs in sediment are generally not linear, and that a large proportion of the PAH burden in sediment may be unavailable to passive samplers, i.e. be much more firmly bound to sediment than would be described by K_{oc} values modelled from literature values of K_{ow}. Even the K_{oc} value for the available component can be significantly different from the predicted values.

These processes will lead to the apparent behaviour of PAHs deviating from that described by simple partitioning theory, generally leading to lower than expected concentrations in pore water, and consequently a lower environmental risk than would be predicted from total concentrations in the sediment. One possible implication of these observations for dredge spoil management is that total concentrations of PAHs in sediment may provide a poor expression of the environmental risk posed by their disturbance during dredging and disposal. Additional site specific investigations are currently undertaken in cases that raise

concern. These may be extended to cover pore water concentrations, the proportion of the total concentration that may be potentially mobilisable into the aqueous phase, and the K_{oc} values that control this partitioning.

8.4 Conclusions

Pore water concentrations of PAHs in dredge sediments from three areas in Scotland were determined using a passive sampling procedure. Concentrations in pore waters from sediment collected from 6 sites in the Clyde area were similar, but greater than those in sediment pore water from the Forth and from Aberdeen harbour. The sediment PAH concentrations in the Clyde and Forth were mainly well above the Provisional Action Level 1 concentrations being used currently for assessment of PAH concentrations in dredge spoils, but the pore water concentrations were well below the draft MAC-EQS values being proposed in the context of the European Union Council Directive.

The data suggest that simple partitioning theory does not adequately describe the relationships between concentrations of PAHs in sediment and pore water. It is suggested therefore that, in support of the current regulatory view, simple chemical assessment methods may not provide a reliable indication of the environmental risk presented by dredged materials. A more comprehensive site-specific chemical approach addressing concentrations in pore water, extractable fractions and K_{oc} values could be considered to strengthen the current suite of assessment tools.

CHAPTER NINE

Method validation: Bioaccumulation of PAHs in *Nereis virens*

9.0 Introduction

Persistent organic pollutants (POPs) with low water solubility like the PAHs and CBs have been shown to partition into particulates and accumulate in sediment (King *et al.*, 2004; deBruyn and Gobas, 2004; Juhasz and Naidu, 2000) from where they can be bioaccumulated by benthic organisms. It has also been recognised that the exchange or uptake into the organism is mainly via direct absorption of compounds in the water phase or indirectly from those adsorbed on the small grain size fraction of particles through the digestive system (Baumard *et al.*, 1998b). Patterns of bioaccumulation can be affected by reproductive condition and / or seasonality, temperature, tidal cycle, nutritional state and species feeding habits. Tendency to bioaccumulate increases with increasing hydrophobicity of the chemical and increasing levels of tissue lipids as well as chemical structure of the compound (Livingstone, 1992) and also depends on contamination source (Ruus, *et al.*, 2005 and Danis *et al.*, 2005). The mode of exposure and route of contaminant uptake also have a major influence on tissue concentration and on uptake kinetics in the organisms (Ciarelli *et al.*, 2000). Other characteristics of the organisms may affect accumulation kinetics such as general mode of living, gut fluid components, metabolic activity, etc (McElroy *et al*., 1990). As a result of the possible toxic effects of these POPs, the concentrations that are likely to cause harm need to be assessed and established methods exist for measuring the availability of the POPs. These methods can either be biological (estimated by measuring amounts of target contaminants and/ or polar metabolites of contaminants in organisms) or chemical (less vigorous techniques that aim to mimic the conditions in the digestive systems of organisms and estimate the bioavailable fractions). The biological methods can be viewed as a measurement of bioaccumulation in the organisms and two basic approaches to assess bioaccumulation have been identified (Lee, 1998). The first approach includes methods that directly measure bioaccumulation using either laboratory-exposed or field-exposed organisms; and methods that directly measure bioaccumulation at a particular site using either natural populations or transplanted populations. The second approach uses methods that model bioaccumulation

which involves either an empirical approach using laboratory or field data to calculate bioaccumulation factors (BAF), biota-sediment accumulation factor (BSAF), and bio concentration factors (BCF) or a deterministic modelling approach usually referred to as food web models (Lee, 1998; Di Toro *et al*., 1991). Due to the persistence and hydrophobicity of POPs, they accumulate in benthic deposit feeders and the equilibrium partitioning theory is frequently used for assessment of bioaccumulation of POPs from sediments (Kraaij *et al.*, 2003).

Laboratory experiments/ studies provide detailed information on uptake and metabolism of contaminants which may not be easily extrapolated to field situations but nevertheless provides useful scenarios for actions (Burkhard, 2003).

The empirical approach (equilibrium models, e.g. BAF, BSAF and BCF) assume steady state conditions between the organism and the surrounding environment and are essential parameters that are usually calculated and used in the assessments of environmental risks. BAF can be calculated as

$$BAF = \frac{C_{org}}{C_{sed}}, \qquad \textbf{9.0}$$

C_{org} = Concentration of PAH in organism (μg kg^{-1} wet wt) and C_{sed} = Concentration of PAH in sediment (μg kg^{-1} dry wt)

$$BSAF = \frac{C_{org,lipid}}{C_{sed,oc}} = \frac{\frac{C_{org}}{f_l}}{\frac{C_{sed}}{f_{oc}}} \qquad \textbf{9.1}$$

$C_{org,lipid}$ = Lipid normalised PAH concentration (μg kg^{-1} lipid), $C_{sed,oc}$ is organic carbon-normalised sediment PAH concentration (μg kg^{-1} oc), f_l and f_{oc} are lipid and organic carbon fractions (kg lipid kg^{-1} organism wet wt and kg organic carbon kg^{-1} dry wt) respectively and BSAF is the biota-sediment accumulation factor.

$$BCF = \frac{C_{org}}{C_w} \qquad \textbf{9.2}$$

C_w is water or aqueous phase PAH concentration (ng L^{-1}) ≡ pore water concentration BCF (L kg^{-1} lipid) is analogous to the lipid-water partition coefficient.

There have been a few studies in recent times that looked at the relationship between bioaccumulation and aqueous (free dissolved) water concentrations (Kraaij *et al.*, 2003; Vinturella *et al.*, 2004; Cornelissen *et al.*, 2006 and Oen *et al.*, 2006) while others have aimed at linking bivalve data to passive sampler (SPMD) data (Booij *et al.*, 2006; Utvik and Johnsen, 1999) and some that studied bioaccumulation in marine organisms only (McElroy and Means, 1988; Ciarelli *et al.*, 2000; and Rubinstein *et al.*, 1983). Due to the problems of extrapolating results from one contaminant to other contaminants, it is always good practice to measure bioaccumulation of contaminants of interest in sediment dwelling organisms such as polychaetes.

The availability of PAHs to a marine polychaete (*Nereis virens*) was studied by exposing the rag worm to contaminated marine sediments and relating the obtained results with pore water concentrations from the application of the silicone rubber passive samplers to the same contaminated sediments as well as testing the hypothesis that silicone rubber passive samplers can mimic POP uptake by marine organisms.

9.1 *Nereis virens*

Nereis virens (*N. virens*), a large marine polychaete, typically called the rag worm is known to construct deep vertical, well irrigated and discrete burrows lined with mucus (Ciarelli *et al.*, 2000; McElroy *et al.*, 1990). It is a shallow water, free-living marine worm which typically inhabits muddy sand of the littoral and sub littoral areas of marine and estuarine habitats (Bass and Brafield, 1972) whilst maintenance of the burrows generates intense perturbations of the sediment column (Ouelette *et al.*, 2004). *Nereis virens* have been shown to accumulate PAHs from sediments (McElroy *et al.*, 1990; Rubinstein *et al.*, 1983 and Ciarelli *et al.*, 2000) and are significant prey items for bottom feeding fish (Rubinstein *et al.*, 1984; McElroy *et al*., 1989) which makes the dietary transfer through the food very important. Uptake of contaminants into the rag worm is primarily via interstitial water, ingestion and absorption from sediments as it is known to feed on detritus and organically rich sediments (Rubinstein *et al.*, 1983).

9.2 Materials and Methods

9.2.1 Materials

HPLC grade solvents (dichloromethane, ethyl acetate, acetone, methanol and *iso*-hexane) were purchased from Rathburn Chemicals Ltd, Scotland, UK. Certified solid standards for PAHs (including deuterated PAHs) were obtained from QMX Laboratories, Essex, UK and dissolved in *iso*-hexane to obtain the required concentrations of spiking and calibration solutions. To avoid contamination of samples, all glassware and stainless steel forceps were either washed in Decon® 180 solutions and rinsed with distilled water or washed in a CAMLAB GW 4050 glassware washer and dried in an oven at 100 ^{o}C. Before use, the glassware was rinsed twice each in dichloromethane and *iso*-hexane, with the latter allowed to evaporate to dryness. Glass Duran® or Schott® (VWR, Leicester, UK) bottles were used for passive extraction of contaminants from sediment, and were always capped with aluminium-lined PTFE caps to reduce or prevent sorption of POPs by the caps. *Nereis virens*, 2-2.5 g size (of the polychaeta family) were purchased from Seabait Limited, Northumberland, UK, and acclimated in tubs containing ~3.5 cm control sediment (obtained from Ythan river) and flowing seawater that was continuously aerated (>75 % saturation) for 8 days in constant temperature (CT) room.

9.2.2 Methods

9.2.2.1 Sediment sampling

Sediment samples were collected from the Firth of Forth, Scotland using a Day Grab and from the vicinity of Elkem Aluminium smelter, Mosjøen (3 locations in the Vefsn fjord, Fig **9.0**), Southern Norway using a van Veen Grab (0.1 m^{2}).

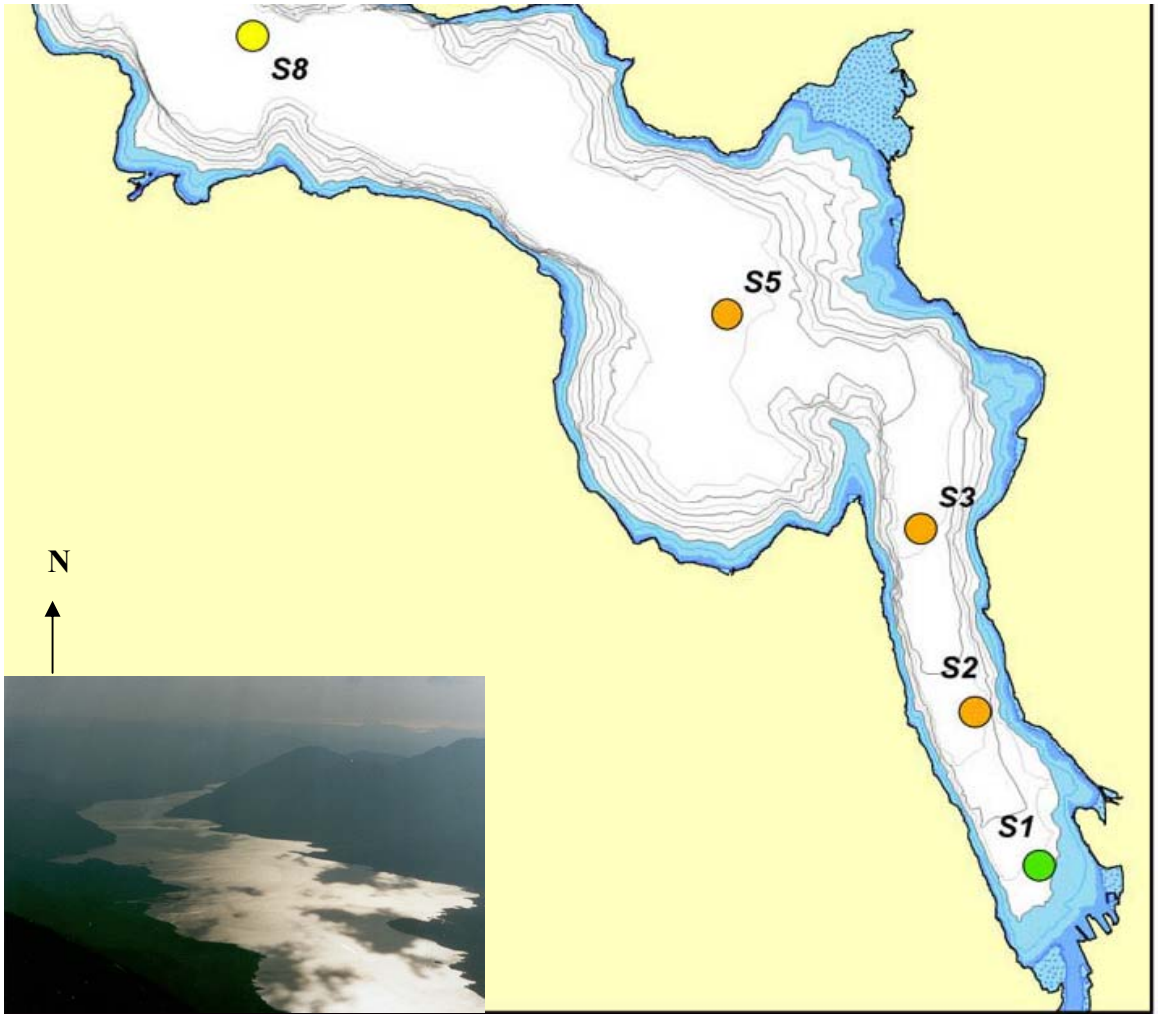


Fig **9.0**: Sediment sampling stations within the Vefsn fjord, 2006. Samples for this study were collected only at S1= St 1, S3= St 3 and S8= St 8. The Elkem Aluminium Smelting Co. is upstream of S1. Inset is Loch Etive

Unpolluted surface sediment was also collected from a "clean" site- the Ythan River near Ellon, Scotland collected using a shovel, as control/acclimation sediment and was subjected to the same treatment as the Vefsn fjord sediment. Sediment samples were similarly collected from Loch Etive and Telford Dock, Aberdeen Harbour as part of the ICES Passive Sampling Trial Survey (Smedes *et al*., 2007b). The top 2 cm of the sediment was transferred to a solvent washed aluminium can, thoroughly mixed, labelled and stored at -20 ± 5 ^{o}C until required for analysis. TOC, particle size distributions and water content of individual sediment samples were determined using standard accredited methods as described in Chapter **2**.

9.2.2.2 Hydrocarbon analysis of sediment

The sediments were analysed as in **3.8.1.1** for sediment PAH concentrations. Sediment samples were also collected at different times from Aberdeen Harbour and Loch Etive in Scotland. Pore water concentrations were determined as described in Chapters **6**-**8** for sediments from the Vefsn fjord while the pore water concentrations of sediments from Aberdeen Harbour (AH) and Loch Etive (LE) were determined as part of the ICES passive sampling trial survey (PSTS) 2006-2007.

9.2.2.3 Sediment pore water concentrations

Concentrations in pore waters of sediments from the different locations (with the exception of the Ythan sediment) were determined using the protocol developed in this thesis and as described in Yates *et al*. (2007a) and Smedes *et al*. (2007b). Pore water concentrations were determined for 31 PAHs for which silicone rubber-water partition coefficients (Chapter **4**) were available.

9.2.2.4 Equilibrium/ Exposure period determination/Uptake

Test sediment used in this exposure is a mixture of Firth of Forth, Tyne sediments and sand (referred to as Forth & Tyne mix). ~ 400 ml of well mixed sediment was added to each test beaker with flowing seawater passing through each beaker. Rubinstein *et al*. (1984), had observed that flow through set-ups are more representative of external conditions where there is movement and mixing of water over the bottom and they aid in meeting the life support requirements of test organisms that are in direct contact with anaerobic sediments for extended periods of time.

The worms were exposed for 0, 1, 4, 7, 14, 28 and 42 days in triplicate to determine steady state conditions, with each beaker containing five worms. Similarly five worms were added to 3 beakers containing ~ 400 ml of well mixed control sediment at the same depth as the test sediment. After exposure, the polychaetes were retrieved from the beakers, transferred into beakers containing seawater and held for an hour to remove sediments (purging) as suggested by Ruus *et al.* (2005); though Vinturella *et al.* (2004), postulated that gut sediment would not likely inflate PAH concentrations in worm extracts as at least 50 mg of

sediments in the gut of an individual worm is needed to account for 25 % PAH mass in the worm. The worms burrowed into the sediment when introduced and no worm mortality was observed in any of the beakers analysed. The worms were then homogenised using a chopper and stored frozen in aluminium cans, until analysed using the standard extraction and analysis method in FRS ML for biota (see **2.3.3.1.2**).

9.2.2.5 Accumulation of PAHs in *N. virens* from test sediments

700 ± 50 g of wet sediment was weighed out into 1 L beakers and a flow through exposure set up (Fig **9.1**), which was allowed to stand for a day before 6 rag worms were added to each individual beaker for a period of 15 days which was determined previously (**9.2.2.4**). The seawater temperature remained at 11 ± 2 ^{o}C, while the worms were not fed throughout the exposure period. Lee (1998) showed from a review of literature that, PAHs attained ~ 70 % steady state after 10 days and 90 % after 28 days, thus recommended 28 days for bioaccumulation study.

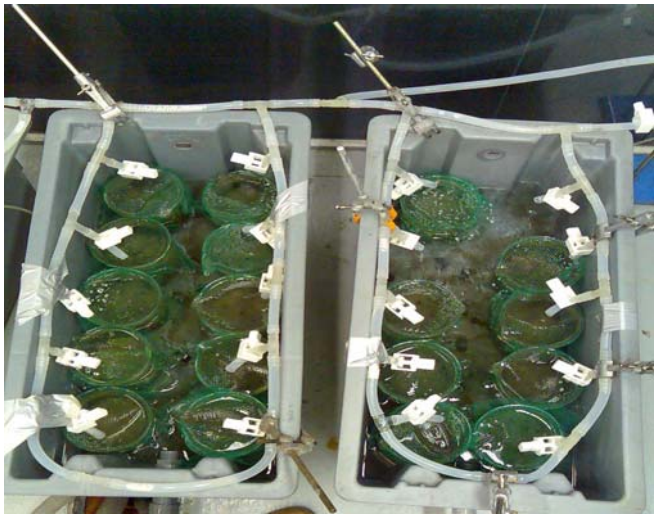


Fig **9.1**: Experimental set-up showing exposure of *N.virens* to test sediments in 1 L glass beakers with flowing artificial sea water

The test organisms (*N. virens*) were retrieved from the beakers after the 15 days and rinsed in artificial sea water used during the exposure. These were then transferred into beakers containing the seawater and left to stand for a further 1h to reduce remnants of sediments from the body surface as well as the intestines (Ruus *et al.*, 2005). The collected worms were then homogenised using an ultra Turrax® and stored in aluminium cans in the freezer

until analysis as described earlier. Lipid content of homogenised worms was determined as described in Chapter **2**.

9.3 Results and Discussion

9.3.1 PAH concentration in sediments

Total PAH concentrations in sediment (2- to 6-ring parent and alkylated PAHs) varied (Table **9.0**) between 1053 ng g^{-1} dry weight (Loch Etive) to 4364 ng g^{-1} dry weight (Vefsn St 8). The sediment PAH profile showed the 4- to 6-ring PAHs accounted for ~ 94 % of the total PAH concentration (Fig **9.2**) in sediments from the Vefsn fjord and Ythan while the 3- to 6-ring PAHs dominated in Loch Etive and 91 % of the total PAH concentration in the Forth and Tyne sediment was mainly the 2- to 5-ring PAHs with more of the 2- and 3-ring PAHs compared to other sediments suggesting a greater petrogenic influence. A similar dominance of the 4- to 6-ring PAHs was found in sediments from Loch Leven (Chapter **7**) which was attributed as with earlier studies to proximity of these locations to aluminium smelting plants.

Table **9.0**: Sediment PAH concentration (ng g^{-1} dry weight) and bulk sediment properties of samples collected from the Vefsn fjord (St 1, 3 & 8), Loch Etive, Aberdeen harbour, Forth/Tyne and Ythan sites

Field ID	Location	PS % < 63 µm	% TOC (f_{oc})	% Lipid (f_l)	Total PAH (ng g^{-1} dw)
St 1	**Vefsn, Norway**	45.1	1.2	1.6	2489
St 3		67.7	1.1	1.7	3865
St 8		91.7	1.6	1.8	4364
Loch Etive	**Scotland, UK**	58.3	2.9	1.8	1053
Aberdeen Harbour		64.0	2.8	1.5	2358
Ythan		55.0	1.4	1.3	1235
Forth & Tyne	**UK**	58.1	2.5	-	2051

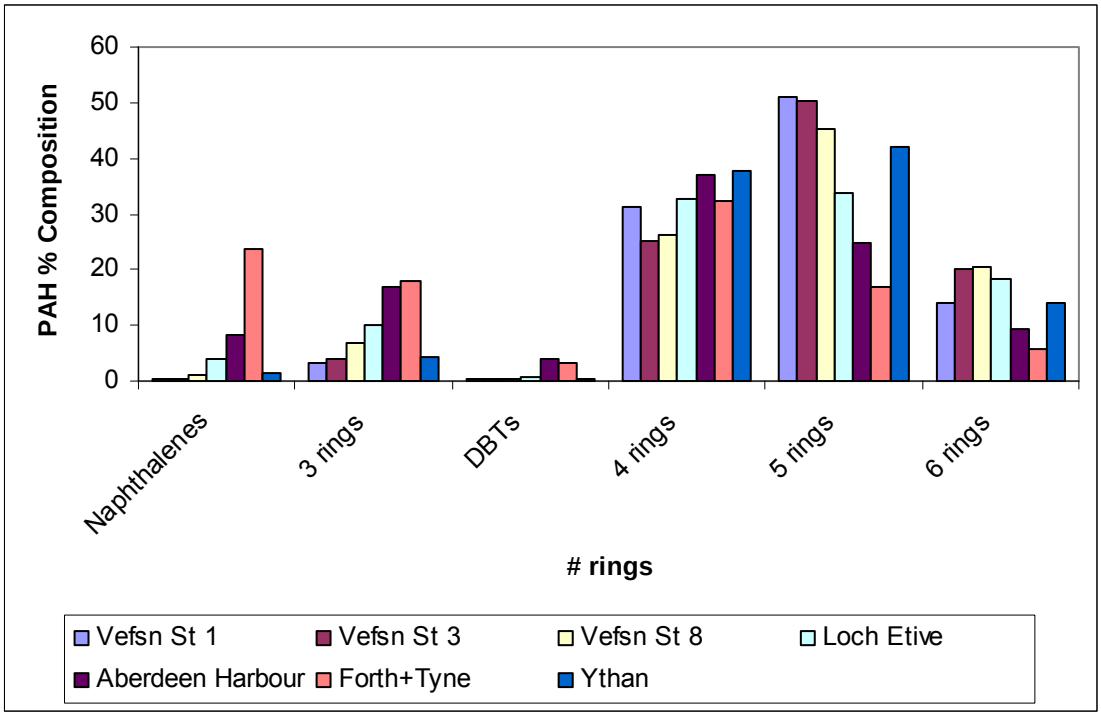


Fig **9.2**: Sediment PAH composition determined in sediments collected from the Vefsn fjord (St 1, 3 & 8), Loch Etive, Aberdeen harbour, Forth/Tyne and Ythan sites. Naphthalenes (2-rings) = sum of naphthalenes (parent and C1-C4). 3-rings = sum of acenaphthene; acenaphthylene; fluorene; phenanthrene and anthracene (parent and C1-C3). DBTs = sum of dibenzothiophenes (parent and C1-C3). 4 –rings = sum of fluoranthene and pyrene (parent and C1-C3); benzo[*c*]phenanthrene; benz[*a*]anthracene; benz[*b*]anthracene and chrysene (parent and C1-C2). 5-rings = sum of benzofluoranthene, dibenz[*a,h*]anthracene, benzo[*a*]pyrene, benzo[*e*]pyrene and perylene (parent and C1-C2). 6-rings = sum of indenopyrene, benzoperylene (parent and C1-C2).

PAH concentration ratios have been used (Webster *et al*., 2005) to aid identification of PAH sources. A Fluo/Pyr ratio > 1 and Phen/Ant ratio less than 10 or C1-178 (Methylphenanthrene)/Phen ratio < 2 indicates a pyrolytic source while contrastingly, a petrogenic source is indicated by a Fluo/Pyr ratio < 1, a Phen/Ant ratio > 10 and C1-178/Phen ratio > 2. Applying these ratios to the data on PAHs in sediment showed (Fig **9.3**) most of the sites to be of a predominately pyrolytic source. The only exception was the sample from Ythan River which was in a mixed zone having a Fluo/Pyr ratio less than 1. A higher proportion (47-83 % across the test sediments) of the parent PAHs is also generally associated with pyrolysis (Ahmed *et al*., 2006) with the Vefsn fjord (smelter-site) sediments exhibiting the highest proportion (~83 %).

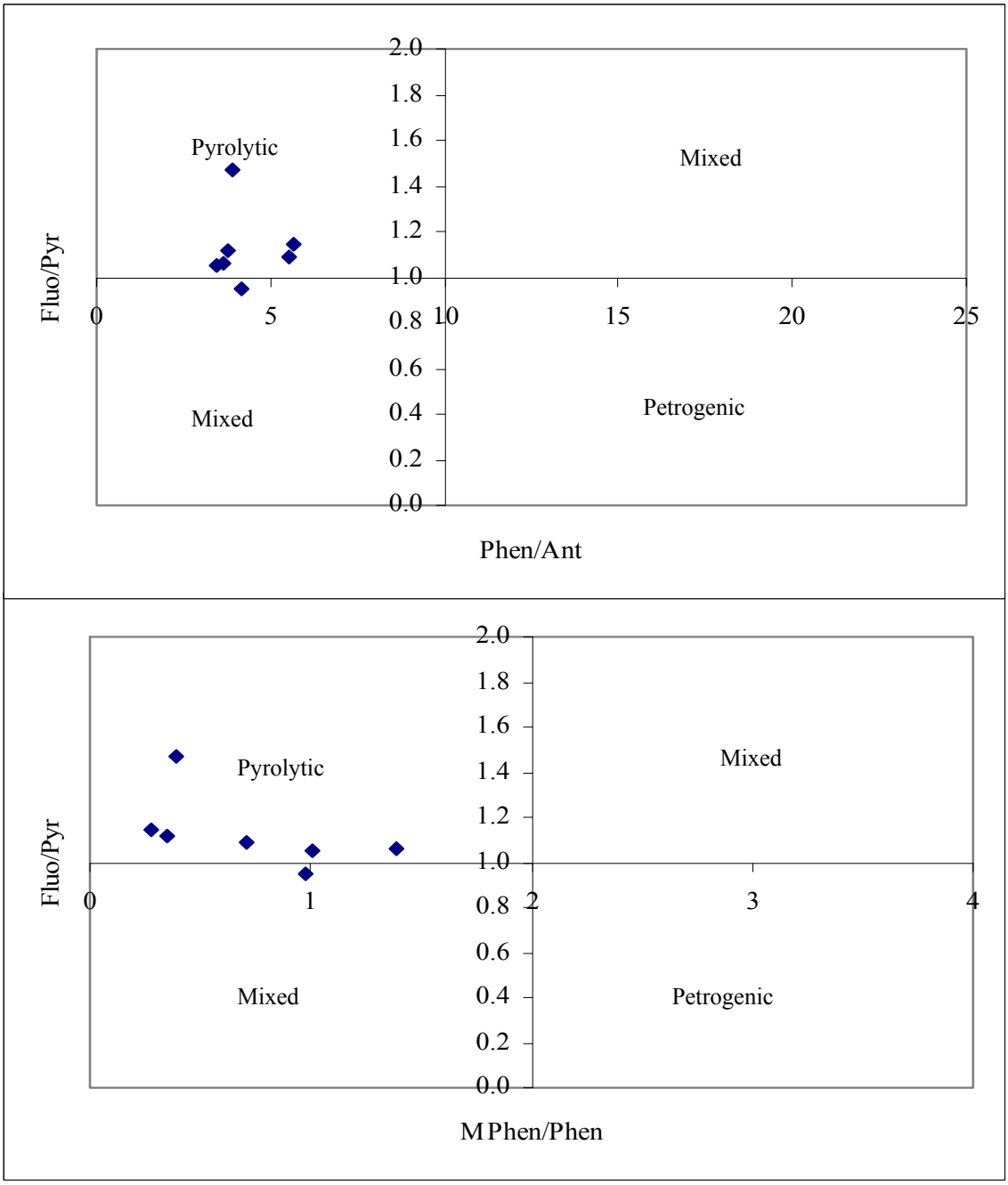


Fig **9.3**: PAH concentration ratios in sediments from the different locations

Furthermore, principal component analysis (PCA) was used to investigate differences in PAH concentration across the sites after reducing the data based on the proportion of the parent to alkylated components with respect to the total PAH concentration. The first three components cumulatively accounted for 93 % of the variance in the data (first 2 components explained 64.7 and 18.6 % respectively of the variation). The score plot (Fig **9.4**) distinguished between the sediments dominated by the 4- to 6-ring PAHs (Vefsn fjord

and Ythan sediments) which were negatively correlated by the first component, and the other locations (Loch Etive, Aberdeen Harbour and Forth & Tyne).

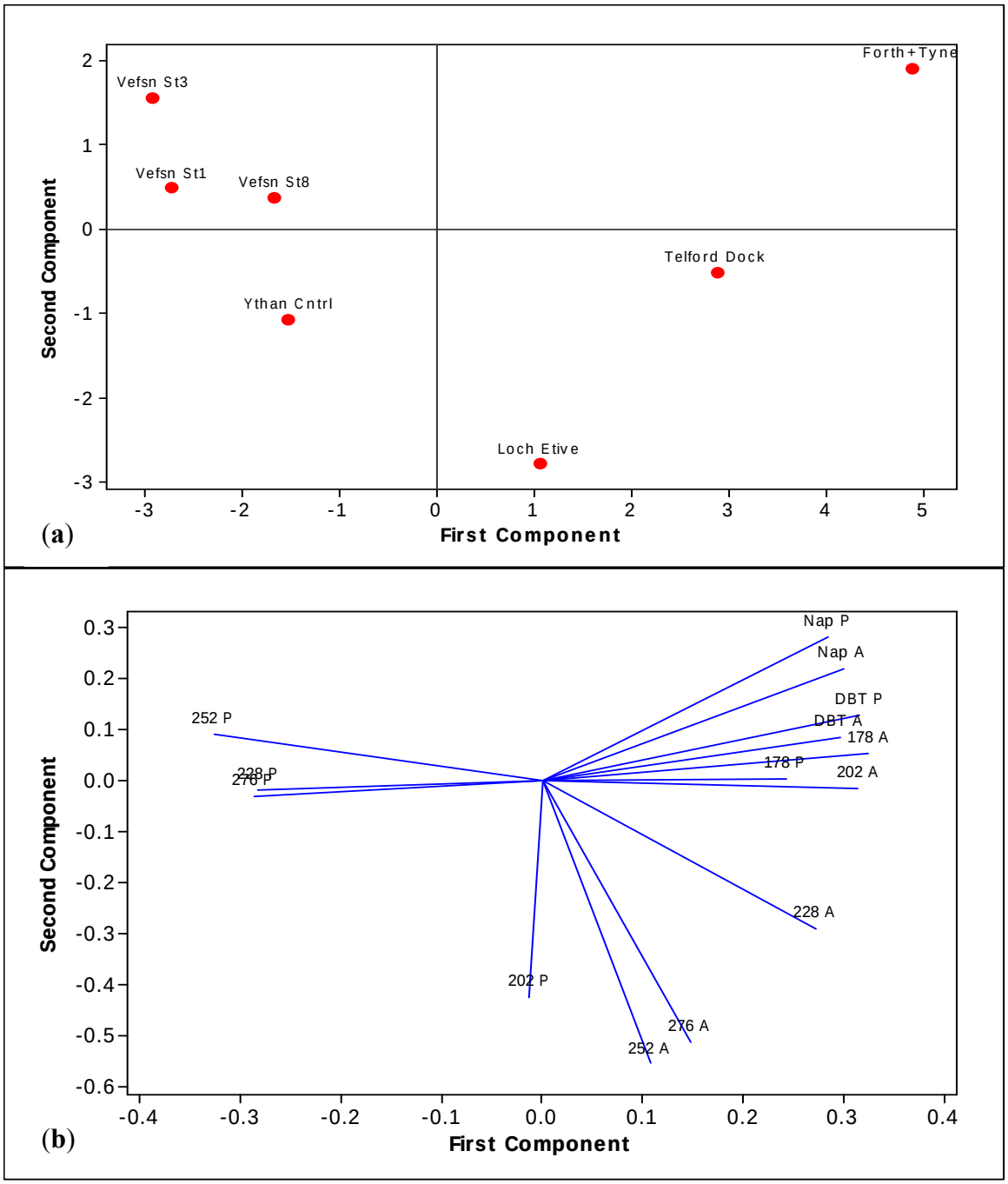


Fig **9.4**: Score plot (a) and Loading plot (b) for the PCA of PAH concentrations in sediments from the test locations

Similarly the score plot distinguished the samples based on the locations with the Vefsn samples grouped together and the Loch Etive and Aberdeen Harbour samples also grouped together. The Forth & Tyne mix sample was placed in a separate region, possibly due to it being a mixture of sediments from the Firth of Forth in Scotland and the Tyne River in

England. The first component (Fig **9.4b**) separated the parent heavier PAHs (negatively correlated) from the lighter parent PAHs and alkylated analogues of both heavy and light PAHs (positively correlated).The second component however distinguished the heavier PAHs (4- to 6-ring parent and alkylated) from the lighter PAHs (2- to 3-ring, DBT parent and alkylated) which however included the parent-252. The PCA suggests the lighter PAHs are relatively more important in the sediments from the Forth & Tyne sample than from the other locations while the heavier parent components are more important in the Vefsn fjord and Ythan sediments.

9.3.2 Pore water concentrations

Total PAH pore water concentration (sum of 31 PAHs, with the exception of Vefsn St 8 and Forth & Tyne Mix that was sum of 30 PAHs, see Table **9.1** below) was 7.4 ng L^{-1} (Loch Etive), 207.5 ng L^{-1} (Aberdeen Harbour), 84.9 ng L^{-1} (Vefsn St 8), 474.8 ng L^{-1} (Vefsn St 1), 542.9 ng L^{-1} (Vefsn St 3) and 152.7 ng L^{-1} (Forth & Tyne mix). The 2- to 4-ring PAHs dominated (cumulatively > 87 %) in the pore water concentrations found in sediments from all the sites with only Loch Etive showing a relatively (Q test, 90 %) higher percentage of the 5-ring PAHs (Fig **9.5**). The lower ring PAHs are more soluble in water and have a lower affinity for sediments. Table **9.1** shows the concentrations of the 31 individual PAHs in pore waters measured using the silicone rubber sampler.

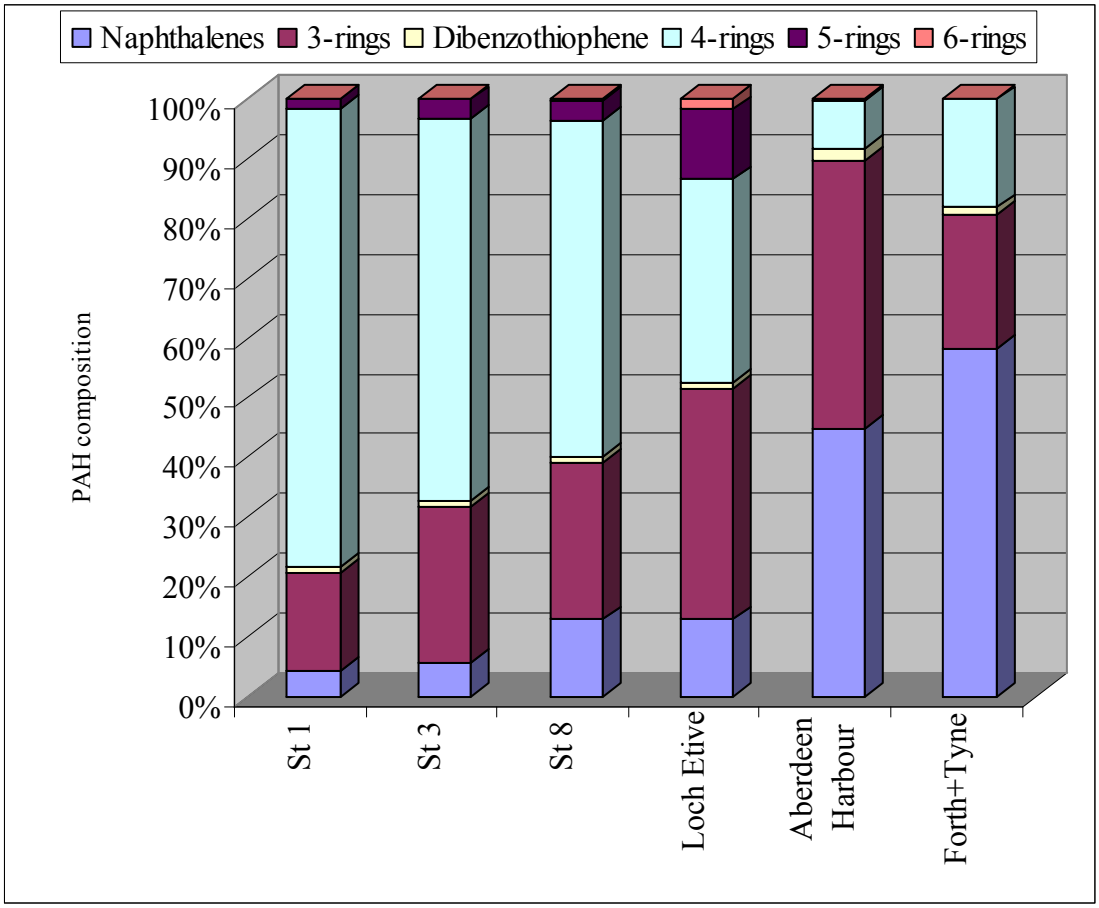


Fig **9.5**: Pore water composition in sediment sampled using the silicone rubber sampler

Table **9.1**: PAH concentrations (ng L^{-1}) measured in the pore waters of test sediments

PAH	**Pore water concentration (ng L^{-1})**					
	Vefsn St 1	**Vefsn St 3**	**Vefsn St 8**	**Loch Etive**	**Aberdeen Harbour**	**Forth & Tyne mix**
Naphthalene	15.12	22.09	8.78	0.56	38.46	22.10
2-Methyl Naphthalene	1.60	3.21	1.05	0.04	4.43	6.58
1-Methyl Naphthalene	1.02	1.98	0.58	0.16	2.44	27.98
C2 Naphthalenes	2.13	2.93	nc	0.13	13.38	15.64
C3 Naphthalenes	0.97	0.63	0.51	0.06	21.08	10.77
C4 Naphthalenes	0.38	0.16	0.02	nd	12.94	5.76
Acenaphthylene	0.24	0.15	0.17	0.32	3.95	1.71
Acenaphthene	11.10	22.08	6.08	0.59	40.70	9.09
Fluorene	8.85	12.75	2.88	0.06	15.93	5.38
Phenanthrene	33.98	72.27	9.95	0.90	7.07	7.50
Anthracene	10.75	19.65	1.56	0.21	3.58	1.66
C1 178	6.54	9.55	0.99	0.39	4.64	2.84
C2 178	3.01	2.70	0.32	0.18	9.15	2.85
C3 178	2.43	2.01	0.24	0.21	8.46	3.13
Dibenzothiophene	5.43	5.45	0.99	0.07	4.12	1.90
Fluoranthene	196.15	117.15	13.89	0.54	5.59	9.96
Pyrene	89.36	118.24	19.73	1.12	5.35	11.77
C1 202	47.58	56.70	6.64	0.53	4.12	4.47
C2 202	0.47	0.68	0.10	0.01	0.15	0.16
Benzo[*c*]phenanthrene	1.65	2.66	0.42	0.03	0.11	0.15
Benz[*a*]anthracene	7.22	13.34	1.65	0.09	0.24	0.30
Chrysene	20.16	35.94	4.84	0.15	0.50	0.66
C1 228	1.21	2.56	0.39	0.04	0.12	0.13
Benzofluoranthenes	4.33	10.03	1.76	0.42	0.43	0.10
Benzo[*e*]pyrene	1.94	4.84	0.84	0.16	0.15	0.07
Benzo[*a*]pyrene	0.52	1.21	0.17	0.09	0.08	0.03
Perylene	0.25	0.49	0.07	0.17	0.17	nc
C1 252	0.15	0.46	0.09	0.02	0.01	0.02
Dibenz[*a,h*]anthracene	0.06	0.19	0.04	0.01	0.01	<0.005
Indeno[1,2,3-*cd*]pyrene	0.02	0.09	0.02	0.05	0.04	<0.005
Benzo[*ghi*]perylene	0.20	0.65	0.17	0.09	0.05	0.01

nd- not detected; nc- not determined ($p > 0.05$)

9.3.3 Equilibration time study

The rag worms accumulated PAHs to different degrees (Fig **9.6**) with the lighter PAHs (2- to 4-ring PAHs) accumulated the most. Typical uptake curves are shown in Fig **9.7** for some PAHs. There was an initial rise in the PAH concentration in the worms with increasing time until day 14 after which it began to decline as has been shown for other polychaetes (Ferguson and Chandler, 1998). All the ring groups showed a similar uptake pattern, with an outlier observed at day 14 which can skew the data. Therefore, that data point was ignored in further analysis after carrying out the least significant difference (LSD) test for individual PAHs. There was no significant difference in the mean worm concentrations from day 8 to 42 (ANOVA, $p > 0.05$), thus 14 days appears to be suitable to attain steady state concentrations in the exposed worms (although due to work plans, 15 days was used in further exposure studies). Ciarelli *et al*. (2000) found concentrations of fluoranthene in *N.virens* to have reached steady state after 5 days.

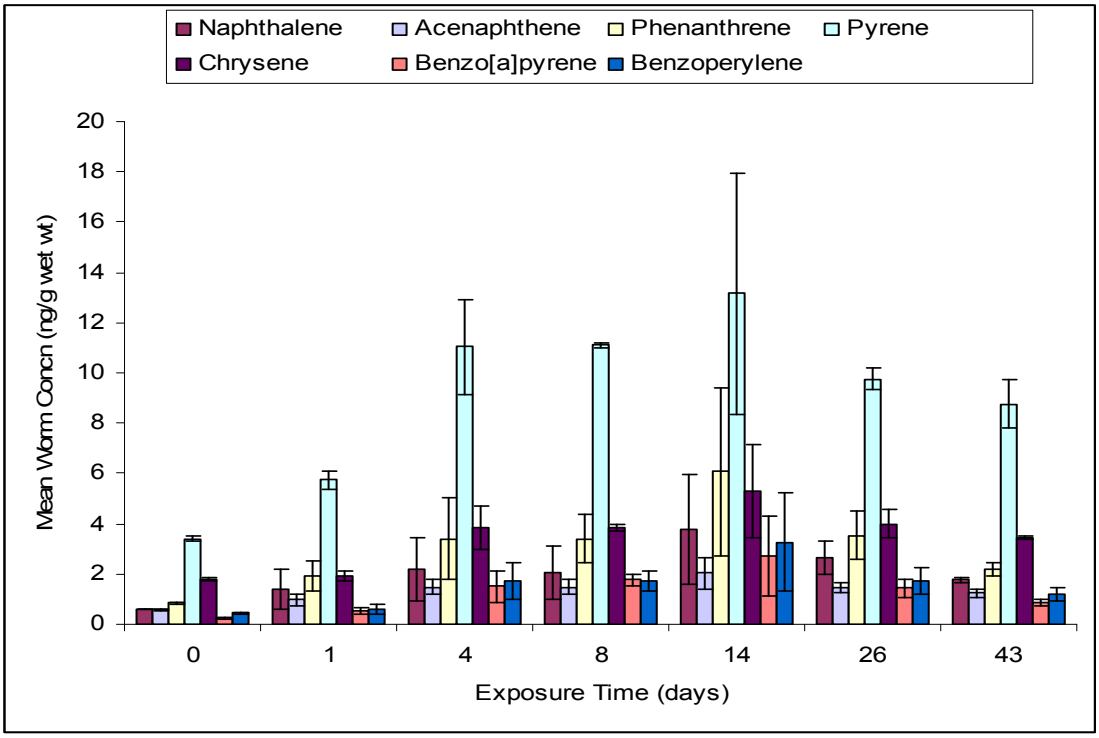


Fig **9.6**: Selected individual PAHs in *N. virens*. Error bars represent standard deviation of triplicate measurements

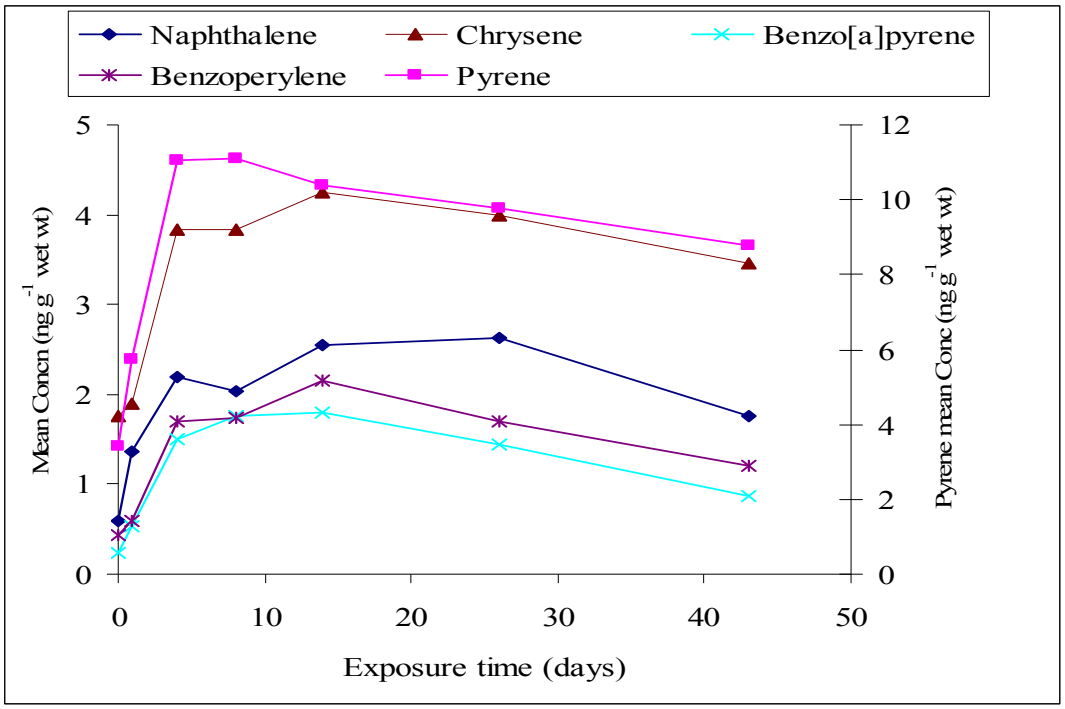


Fig **9.7**: Accumulation of PAHs in *N. virens* over time from Forth & Tyne sediment mix. Data are means of triplicate measurements with the exception of day 14 where only the mean duplicate data is used due to the reasons given in the text above. Note that pyrene data is on a different axis so as not to skew the plot.

The reduction in tissue (worm) concentrations of PAHs after the steady rise (up to 14 days) has been suggested to be due to an inducible enzyme system (mixed function oxidases; MFO) that is capable of transforming the hydrocarbons into more water-soluble forms for easy excretion (Augenfeld *et al*., 1982) which has been seen in *Nereis* species (McElroy *et al*., 1990).

9.3.4 Accumulation of PAHs in *N.virens* from sediments

The PAH profile in the rag worms reflected what was observed in the sediments with a dominance of the 4- to 6-ring PAHs (> 85 %) in those exposed to sediments from the Vefsn fjord. Worms exposed to sediments from Loch Etive and Aberdeen Harbour absorbed more of the 2- to 4-ring PAHs (68 and 77 % respectively). Uptake by *Nereis virens* is mainly through ingestion of sediments and the water phase (Ciarelli *et al*., 2000); however at equilibrium the uptake route does not play a significant role.

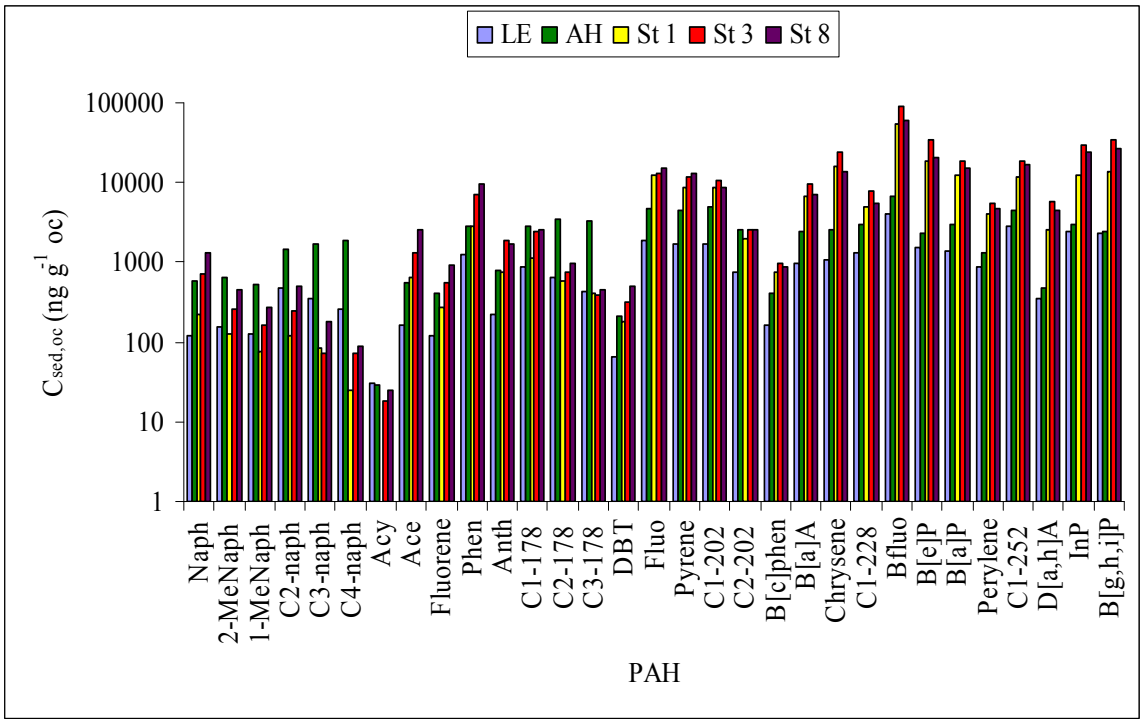


Fig **9.8a**: Sediment PAH normalised concentration (ng g^{-1} organic carbon) at the different locations

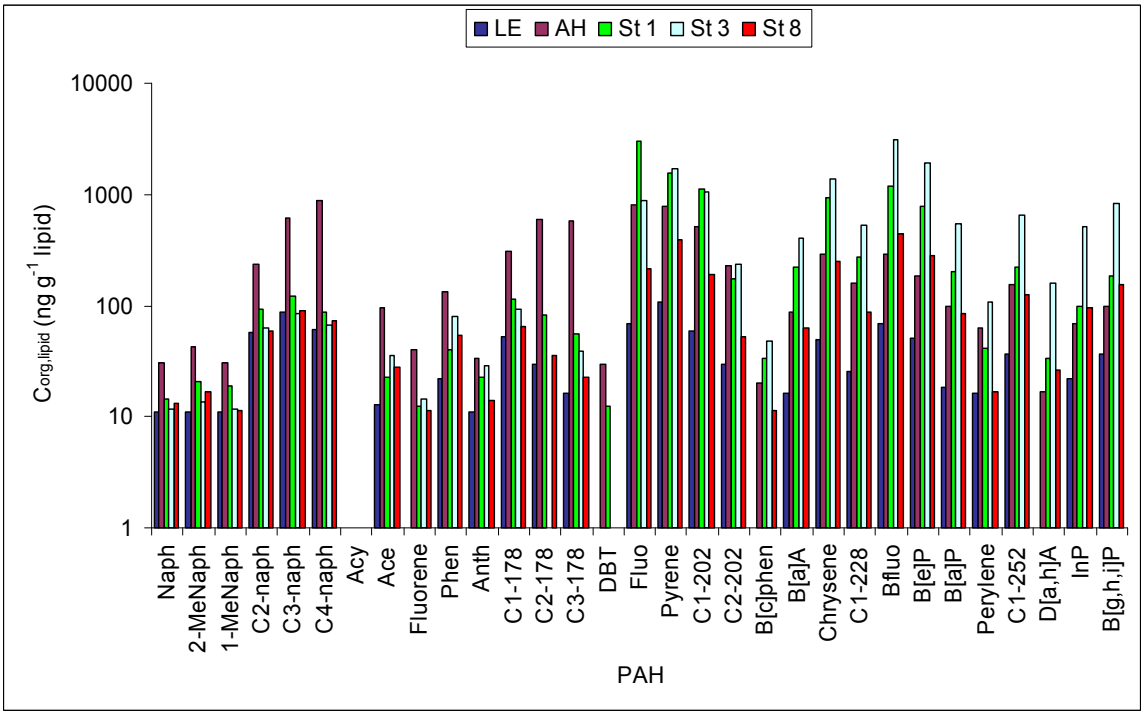


Fig **9.8b**: Biota (worm) mean PAH normalised concentration (ng g^{-1} lipid) from the different exposed sediments

9.3.4.1 Biota-sediment accumulation factors

The lipid content was determined to be 1.82, 1.51, 1.60, 1.71, and 1.78 % in the rag worms exposed to sediments from Loch Etive, Aberdeen Harbour, Vefsn St 1, Vefsn St 3 and Vefsn St 8 respectively. The data obtained from sediment and worm concentrations were normalised to organic carbon and lipid content (Table **9.0**; Fig **9.8a** & **b**) as discussed in the introduction (section **9.0**) so as to calculate and compare biota-sediment accumulation factors (BSAF). Normalisation (organic carbon and lipid content) tends to reduce the variability normally associated with BSAFs between sediments. Measured BSAF values (computed from equation **9.1**) had median values of 0.02 (Loch Etive); 0.07 (Aberdeen Harbour); 0.04 (Vesfn St 1); 0.03 (Vefsn St 3) and 0.01 (Vefsn St 8) which are a factor of 1 to 227 lower than the lower theoretical BSAF value of approximately one (Di Toro *et al*., 1991) calculated on the assumption of equilibrium partitioning (for both organisms and sediments). Average PAH concentrations in *N. virens* and sediments were normalised to average lipid and organic carbon content and used in the calculations of BSAF shown in Fig **9.9**.

Low BSAF values as compared to the theoretical value have also been reported for gastropods and polychaetes (Oen *et al*., 2006; Cornelissen *et al*., 2006). BSAFs were plotted against the $\log K_{ow}$ in Fig **9.10** and these increased for PAHs with $\log K_{ow}$ up to 5, but then gradually decreased thereafter which suggests a systematic variation with $\log K_{ow}$ and stronger sorption to sediments with increasing hydrophobicity. Such low BSAF values may be due to biotransformation in worms (McElroy *et al*., 1990; Sijm *et al*., 2000; Ruus *et al*., 2005).

With the exception of the naphthalenes, measured BSAF values mostly decreased in the order Aberdeen Harbour > St 1 > St 3 > St 8 ≡ Loch Etive. The Vefsn station samples reflect the decrease away from the smelter (which is a converse situation to the sediment concentration trend). Simpson *et al*. (2006) also reported higher BSAF values for a benthic bivalve, *Tellina deltoidalis*, at sites with the lowest total-PAH sediment concentration.

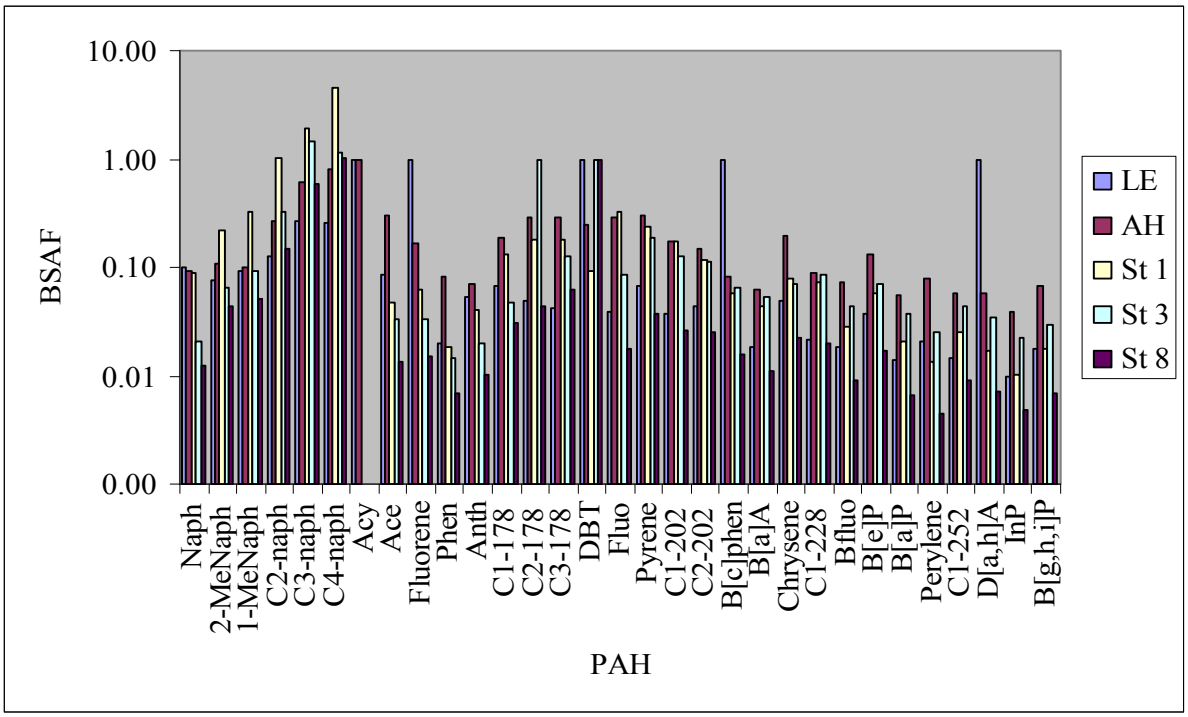


Fig **9.9**: Measured BSAF values on a log scale for the PAHs studied

BSAFs have also been measured on the basis of the freely dissolved concentration (Cornelissen *et al*., 2006; Oen *et al*., 2006) with the authors explaining that it is not an attempt to correct the observed BSAFs to the theoretical value of ~ 1 - 2 but rather offers an approach to account for strong sorption of compounds to total organic carbon. This was adopted in this work using equation **9.3** below:

$$BSAF_{free} = \frac{K_{lip}.C_w}{C_{sed,oc}} \qquad \textbf{9.3}$$

Where $BSAF_{free}$ = BSAF calculated using free dissolved (pore water) concentrations (C_w); K_{lip} = lipid-water partition coefficient (L kg^{-1}); approximated to be equal to K_{ow} (DiToro *et al*., 1991 and log K_{ow} values adopted from Yates *et al*., 2007); $C_{sed,oc}$ = sediment concentration, normalised to total organic carbon content.

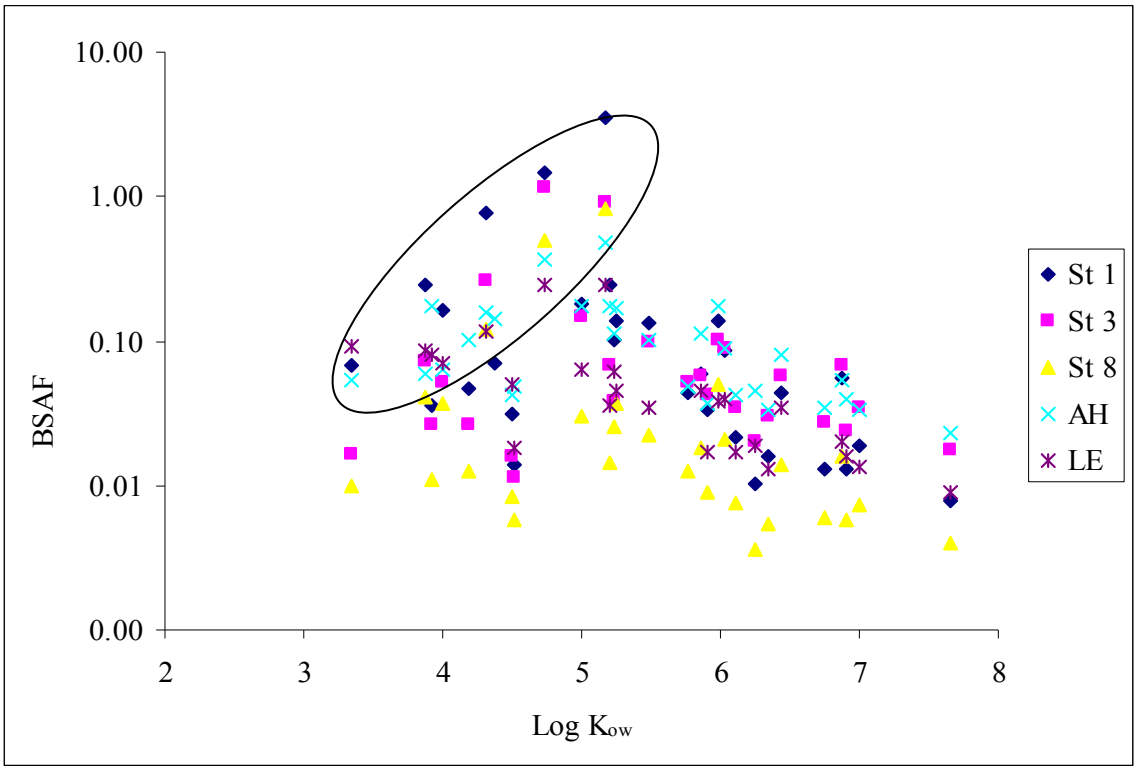


Fig **9.10**: Measured BSAF for the different sediments on a log scale as a function of $\log K_{ow}$. St 1, 3 and 8 are sediment samples from the Vefsn fjord; AH is from Aberdeen Harbour and LE is from Loch Etive. Enclosed in the circle are the naphthalenes (naphthalene, 1 & 2-methylnaphthalene, and C2- to C4-naphthalene) that increased with increasing $\log K_{ow}$

$BSAF_{free}$ showed median values of 0.06, 0.29, 0.56, 0.43 and 0.06 for Loch Etive, Aberdeen Harbour, Vefsn St 1, Vefsn St 3 and Vefsn St 8 sediments respectively which were a factor of 0.2 to 354 lower than the theoretical value of ~ 1 across individual PAHs and sediments but more comparable to $BSAF_{measured}$. The ratio $BSAF_{free} : BSAF_{measured}$ from the 5 locations showed median values of 1.30 (Loch Etive); 2.26 (Aberdeen Harbour); 8.53 (Vefsn St1); 6.85 (Vefsn St 3) and 5.01 (Vefsn St 8) suggesting that free dissolved concentrations can reasonably predict availability of PAHs from sediments into *N. virens*. The assumption of $K_{lip} \cong K_{ow}$ may not be completely valid as Vinturella *et al*. (2004) have found the relationship to be:

$$\log K_{lip} = 0.76 \log K_{ow} - 0.96 \qquad \textbf{9.4}$$

in *N.virens* which would have led to an overestimation of $BSAF_{free}$ by up to an order of magnitude.

9.3.4.2 Bio concentration factors (BCF)

Based on the equilibrium partition theory, the equilibrium relationship between the organism and pore water can be described by the BCF (Sijm *et al*., 2000) as given in equation **9.2**. Measured $C_{org,lip}$ in *N. virens* was thus compared with $C^{p}_{org,lip}$ (predicted worm concentrations) estimated using equation **9.5** (Markwell *et al*., 1989) below for worms and pore water concentrations determined in **9.3.2**. It is worth noting that the Markwell *et al*. relationship was for chloro-hydrocarbons:

$$\log BCF = 1.11 \log K_{ow} - 1.0 \qquad \textbf{9.5}$$

The predicted worm concentrations were a factor of 0.01 to 23.04 (with a median value of 2) higher than the measured concentrations in the worms (across all the PAHs and sediments). The values found showed reasonably good agreement (with the exception of the naphthalenes) particularly for the sediments from the Vefsn fjord (Fig **9.11**). A similar close correlation between accumulation of PAHs in worms and the predicted accumulation (from BCF) has been reported for deposit-feeders (Kraaij *et al*., 2003) which is in agreement with equilibrium partition theory.

The predicted $C_{org,lip}$ in rag worms exposed to the Aberdeen Harbour sediments were higher than the expected 1:1 relationship between $C_{org,lip}$ and $C^{p}_{org,lip}$ than in the sediments from the other locations which were lower than the expected concentrations.

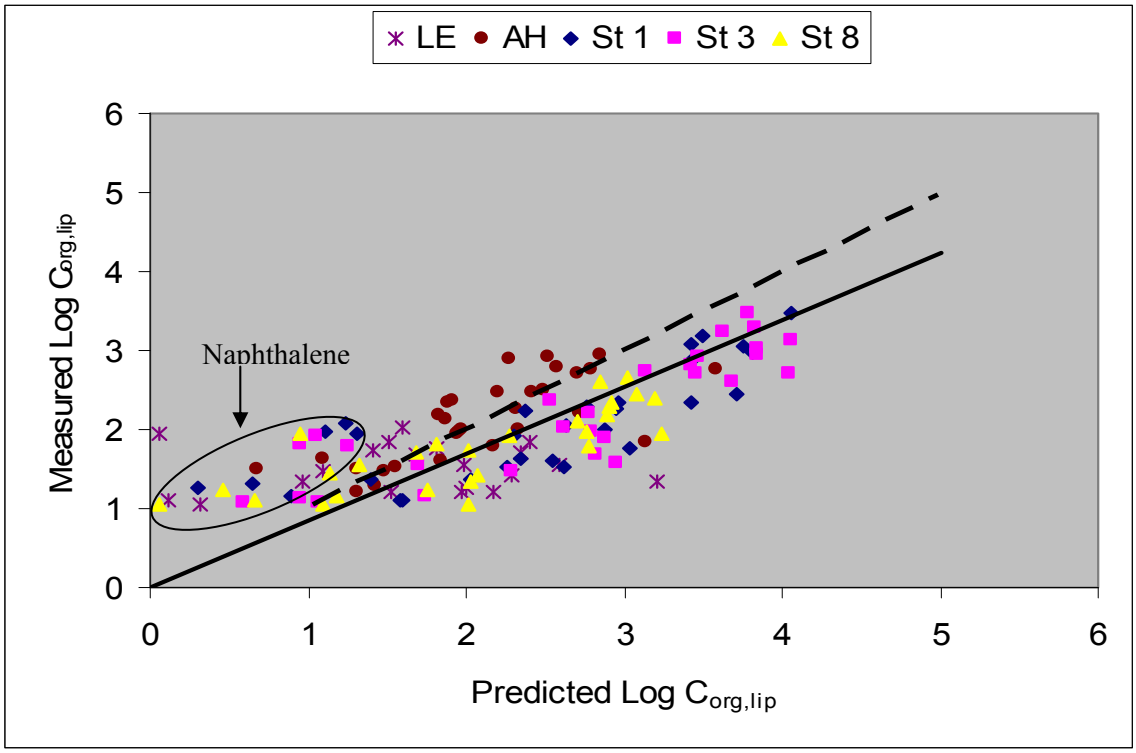


Fig 9.**11**: Measured log $C_{org,lip}$ versus log $C^{p}_{org,lip}$. The naphthalenes that deviated from a linear relationship are also shown but not included in the drawn observed relation (block line) while the broken line represents the expected 1:1 relationship

9.4 Conclusions

Pore water concentrations were 7.4, 207.5, 474.8, 542.9 and 84.9 ng L^{-1} in sediments from Loch Etive, Aberdeen Harbour, and Vefsn fjord stations 1, 3 and 8 respectively with a high proportion (> 87 %) of the 2- to 4-ring PAHs.

Nereis virens, a marine polychaete accumulated PAHs from the test sediments reaching steady state levels within 14 days. Measured BSAF on the basis of equilibrium partition theory (using total sediment concentrations) were lower than theoretical BSAF value of approximately 1 and varied between PAHs and sediments. $BSAF_{free}$ calculated on the basis of free dissolved (pore water) concentrations were more comparable to $BSAF_{measured}$ (ratios less than 9).

Worm concentrations predicted using BCF values and pore water concentrations were a median factor of 2 higher than measured worm concentrations. Equilibrium partition

models based on pore water concentrations measured with silicone rubber samplers appear to be better predictors of bioaccumulation in *N. virens* than the sediment-organic carbon normalised concentrations.

CHAPTER TEN

International Council for the Exploration of the Seas (ICES) Passive Sampling Trial Survey

10.0 Introduction

Passive sampling is used to determine the freely (truly) dissolved concentration of persistent organic pollutants (POPs) such as PAHs and CBs, which is related to the availability of these POPs to biota (Huckins *et al*., 2006). The validation of passive sampling methods is problematic, even though it yields a more environmentally relevant parameter (truly dissolved concentration) than classical sampling methods (Smedes, 2007). As part of the drive by ICES to support the development of passive samplers, a passive sampling trial survey project was organised by the ICES Working Group on Marine Sediments in Relation to Pollution (WGMS) and the Marine Chemistry Working Group (MCWG). The project covered water and sediment sampling across various locations within Europe (Smedes *et al*., 2007b, 2007c, and 2007d). The trial survey specifically focussed on the silicone rubber sampler. FRS Marine Laboratory participated in this survey through application of the passive sampling method at two locations where pollution levels were expected to be very different - Aberdeen Harbour (contaminated) and Loch Etive (reference site). The sampling point at Aberdeen Harbour was the Telford Dock, although within this study it is only referred to as Aberdeen Harbour. Of specific relevance to this thesis are these aims of the survey:

- Comparing concentrations in water derived from passive samplers with contaminant concentrations in mussels in order to demonstrate the environmental relevance of passive sampling data
- Gaining further information towards the validation of the use of passive samplers in sediment.

10.1 Materials and Methods

10.1.1 Materials

For the water sampling, a sampling cage and two sets of six silicone rubber sheets (each set referred to as a sampler) spiked with performance reference compounds (PRC) in glass jars (Fig **10.0**) for each station were received from the coordinating laboratory (RIKZ) in the Netherlands. A final glass jar with 6 silicone rubber sheets spiked with PRCs was also provided as a reference for the initial amounts of PRCs spiked onto the sheets. For the sediment sampling, 2 × 1 L bottles for each sampling station were also received from the coordinating laboratory. The bottles had aluminium foil lined caps and the inside wall was coated with ~ 300 mg polydimethylsiloxane (PDMS) spiked with PRC. These were stored in a freezer until use. Each bottle was engraved with an identification number. A third 1 L coated bottle (reference) that had been spiked with PRCs was also provided as a check of initial amounts of PRCs.

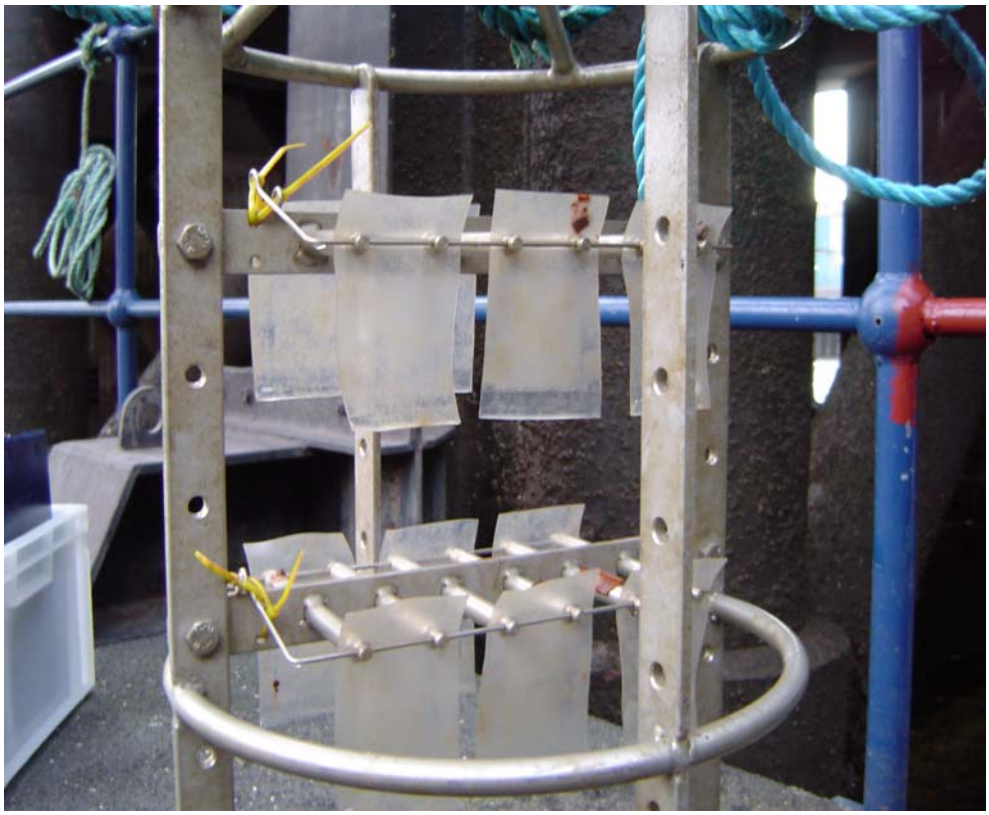


Fig **10.0**: Samplers in cage/frame deployed at Aberdeen Harbour

HPLC grade solvents (dichloromethane, ethyl acetate, acetone, methanol and *iso*-hexane) were purchased from Rathburn Chemicals Ltd, Scotland, UK. Certified solid standards for PAHs (including deuterated PAHs) and CBs were obtained from QMX Laboratories, Essex, UK and dissolved in *iso*-hexane to obtain the required concentrations of calibration solutions. To avoid contamination of samples, all glassware and stainless steel forceps were either washed in Decon® 180 solutions and rinsed with distilled water or washed in a CAMLAB GW 4050 glassware washer and dried in an oven at 100 ^{o}C. Before use, the glassware was rinsed twice each in dichloromethane and *iso*-hexane, with the latter allowed to evaporate to dryness.

10.1.2 Methods

10.1.2.1 Sediment and Mussel Sampling

At each of the stations (Aberdeen Harbour and Loch Etive), ~ 5 L of sediment was collected into plastic buckets for exposure in coated bottles, determination of total PAH and CB concentrations in sediment and for sediment bulk property (organic carbon and particle size distribution) determinations. The water depth at Aberdeen Harbour site was 7 m while at Loch Etive it was 80 m. The water temperature was between 6.5 – 7 ^{o}C during deployment in October 2006. Similarly water samples were taken in 2.5 L glass bottles for salinity and suspended particulate matter (SPM) measurements in the laboratory. SPM was measured by filtration using 0.45 μm filter papers and application of vacuum. Mussels (*Mytilus edulis*, the blue mussel) were also collected during recovery of the samplers from each station and those of ~ 5 cm in length were selected for analysis.

10.1.2.2 Sediment Bulk Properties

Total organic carbon (TOC), Particle size (PS) analysis, and moisture content of sediment samples was carried out as described in Chapter **2**, sections **2.1** - **2.2**.

10.1.2.3 PAH and CB analysis of Sediment and Biota

Sediment and biota (mussel) samples were analysed for PAHs and CBs as described in Chapter **2**, section **2.3**. For the biota samples, lipid concentration was also determined, as in Chapter **2** section **2.4.1**.

10.1.2.4 Exposure of sediments to and extraction of samplers

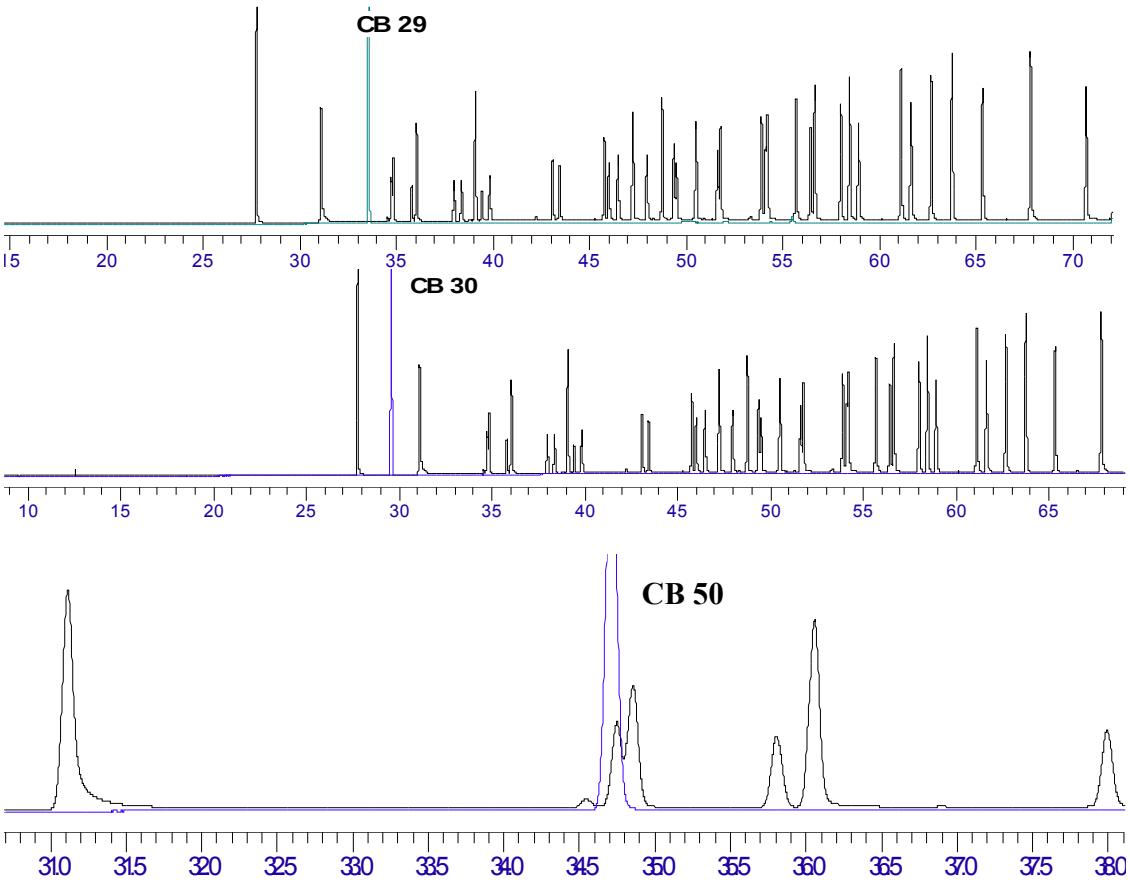
Exposures were according to the guidelines (www.passivesampling.net) provided by the coordinating group. Briefly, the 1 L coated glass bottles were filled with 920 ± 80 g of homogenised sediment samples and shaken on an orbital shaker at 175 rpm for 21 days (Loch Etive) and 23 days (Aberdeen Harbour). After the exposure period, the bottles were emptied of the sediments and rinsed with Milli-Q water with the water drained out of the bottles as much as possible. A bottle exposed to sediment from each station was sent to the coordinating laboratory, while the other bottles were extracted using 2 × 50 ml of methanol on an orbital shaker for 4 h with the bottles turned halfway through the extraction. The extracts were concentrated down, exchanged into *iso*-hexane and treated as in Chapter **3**, section **3.3.1.1**. The reference bottle and an uncoated glass 1 L bottle (procedural blank) were also extracted and treated in a similar way as the exposed bottles.

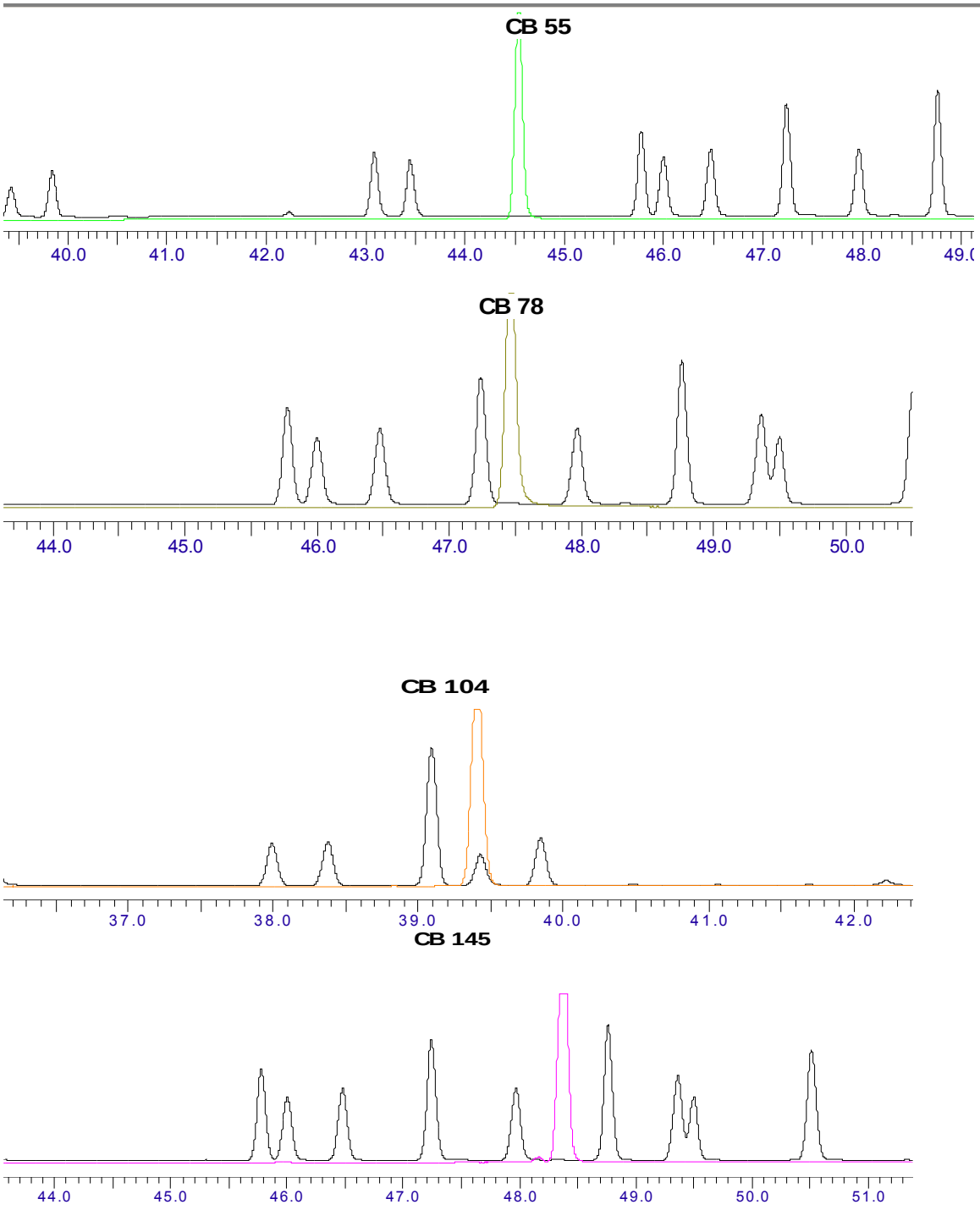
10.1.2.5 Exposure of silicone rubber samplers to water

The silicone rubber sheets provided by the coordinating laboratory were mounted on the samplers just before deployment. Two samplers on the sampler cage (Fig **10.0**) were deployed per site at a depth of 1.5 and 2 m for Loch Etive and Aberdeen Harbour respectively. The samplers were deployed, as described in the guidelines provided, for 35 days (for one sampler and 42 days for the other) at Loch Etive and for 42 days for both samplers at Aberdeen Harbour. After the exposure period, the samplers were retrieved and the silicone rubber sheets removed and wiped clean using paper rolls and 'local' water where there was any biofouling (which was observed to be very minimal at both locations) onsite. The sheets were placed back in the glass jars and returned to the laboratory where they were stored in the freezer until

analysed for PAHs and CBs as described in Chapter **3**, section **3.3.1.1**. The reference sheets were analysed in the laboratory, along with an un-spiked sheet of silicone rubber as a procedural blank. The six sheets that constitute each sampler were pooled and analysed together.

Due to the additional number of PRCs (specifically CB 29, 30, 50, 55, 78, 104, 145, 155, and 204) included by the coordinating laboratory which were not in use at FRS, these CBs were purchased pure and analysed on the GC –ECD to evaluate possible co-elution before further analysis (Fig **10.1**). Subsequently, only CB 29, 30, 55, 78, 145, 155 and 204 were determined as they did not co-elute with any CB of interest.




CB 55
40.0
41.0
42.0
43.0
44.0
45.0
46.0
47.0
48.0
CB 78
44.0
45.0
46.0
47.0
48.0
49.0
50.0
CB 104
37.0
38.0
39.0
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48.0
49.0
50.0
51.0

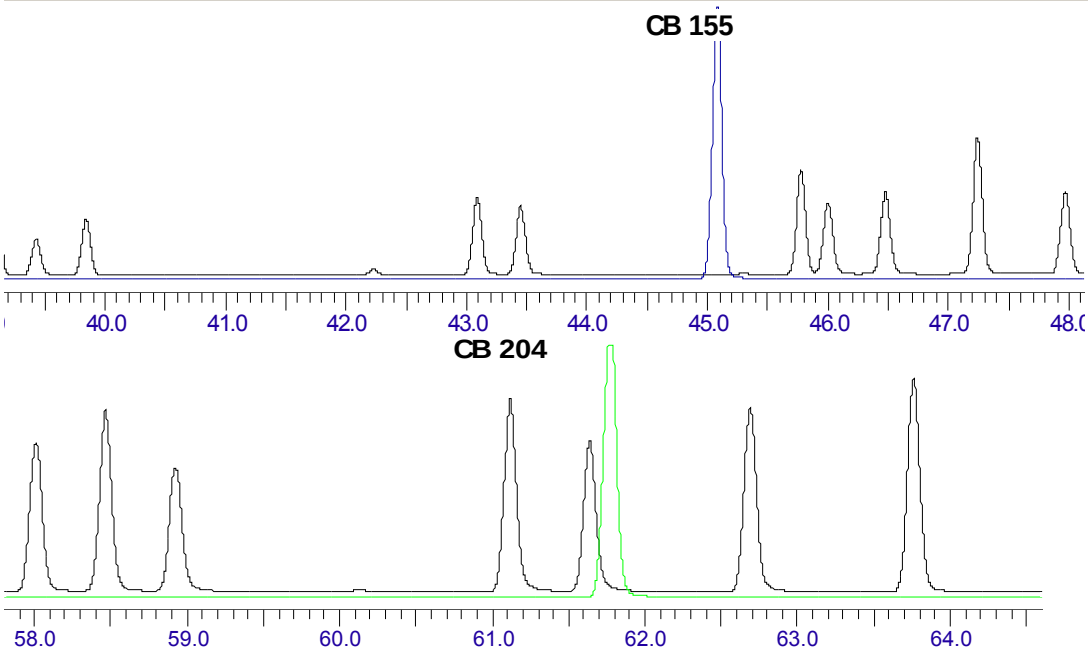

Fig **10.1**: Chromatograms used in screening additional PRCs

However, the additional PAH PRCs (D_8-naphthalene, D_{10}-phenanthrene, D_{12}-perylene and D_{12}-coronene) presented greater difficulties. D_8-naphthalene is used in FRS as an internal standard and could not be used for this purpose as it was added by the coordinating laboratory as a PRC. Therefore, compounds that are normally quantified on the basis of D_8-naphthalene were quantified using the D_{10}-biphenyl internal standard. D_{10}-phenanthrene was quantified using the calibration available for phenanthrene. D_{12}-perylene and D_{12}-coronene were not quantified due to coelution of D_{12}-perylene with an unknown peak and the absence of alternative calibration data at FRS ML for D_{12}-coronene. Some of the issues encountered that affected accurate measurements of the desired parameters are discussed in Smedes *et al*. (2007c).

10.3 Results and Discussion

The measured parameters of the sediments, water and mussels are presented in Table **10.0** below.

Table **10.0**: Sediment bulk properties and total PAH (2- to 6-ring parent and alkylated compounds) concentrations from the two sampling stations

	Loch Etive	**Aberdeen harbour**
Sediment		
% TOC[1]	2.9	2.8
PS[2] % < 63µm	58.3	64.0
% Moisture content	60.4	59.0
Total PAH[3] (ng g^{-1} dry weight)	1053	2358
Total CB[4] (ng g^{-1} dry weight)	6	9
Water		
SPM[5] on deployment (mg L^{-1})	1.0	13.1
SPM on recovery (mg L^{-1})	5.2	12.9
Salinity on deployment (o/oo)	4.6	24.1
Salinity on recovery (o/oo)	5.7	15.5
Biota (Mussels)		
Lipid Content (%)	0.85	0.84
% Moisture content	92.1	88.1
Total PAH (ng g^{-1} wet weight)	21	701
Total CB (ng g^{-1} wet weight)	0.6	2.0

1- Total organic carbon.
2- Particle size.
3- Sum of naphthalenes (parent and C1-C4), acenaphthene, acenaphthylene, fluorene, phenanthrene and anthracene (parent and C1-C3), dibenzothiophenes (parent and C1-C3), fluoranthene and pyrene (parent and C1-C3), benzo[*c*]phenanthrene, benz[*a*]anthracene, benz[*b*]anthracene and chrysene (parent and C1-C2), benzofluoranthenes, dibenz[*a,h*]anthracene, benzo[a]pyrene, benzo[e]pyrene and perylene (parent and C1-C2), indenopyrene, benzoperylene (parent and C1-C2).
4- Sum of CB 28, 31, 44, 49, 52, 70, 74, 97, 99, 101, 105, 110, 118, 128, 132, 137, 138, 149, 153, 156, 157, 158, 170, 180, 183, 187, 189 and 194.
5- Suspended particulate matter.

Figure **10.2** and **10.3** below gives PAH % composition in sediment and mussels used for the ICES trial survey, and the dominance of the 4- and 5-ring PAHs in sediments while the 3- to 4-ring PAHs dominated in the mussels.

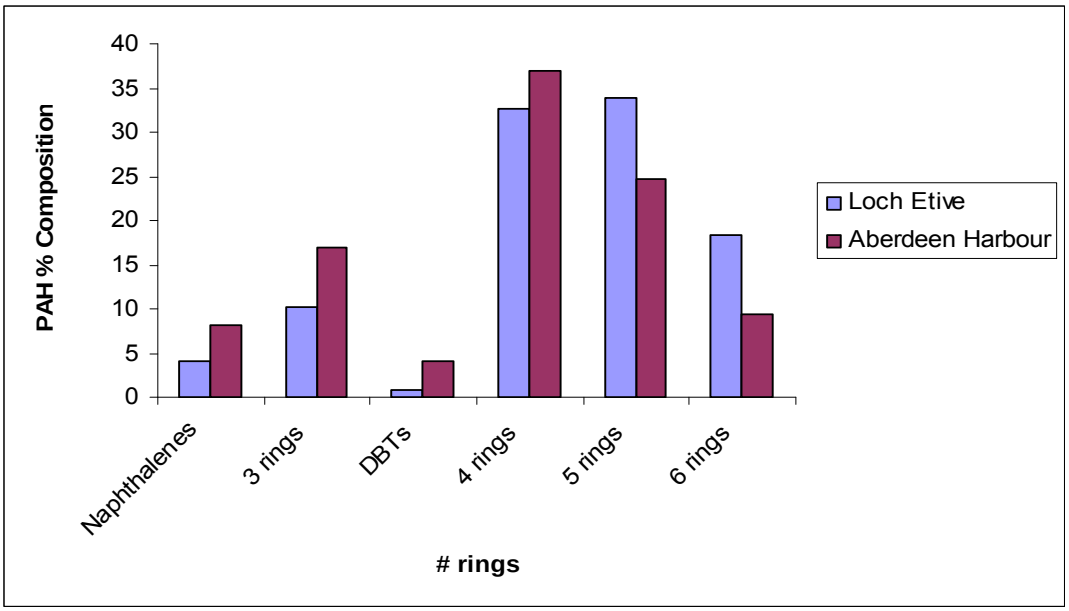

Fig **10.2**: PAH % Composition in ICES PSTS sediments. PAH groups (ring numbers) are as defined in earlier chapters

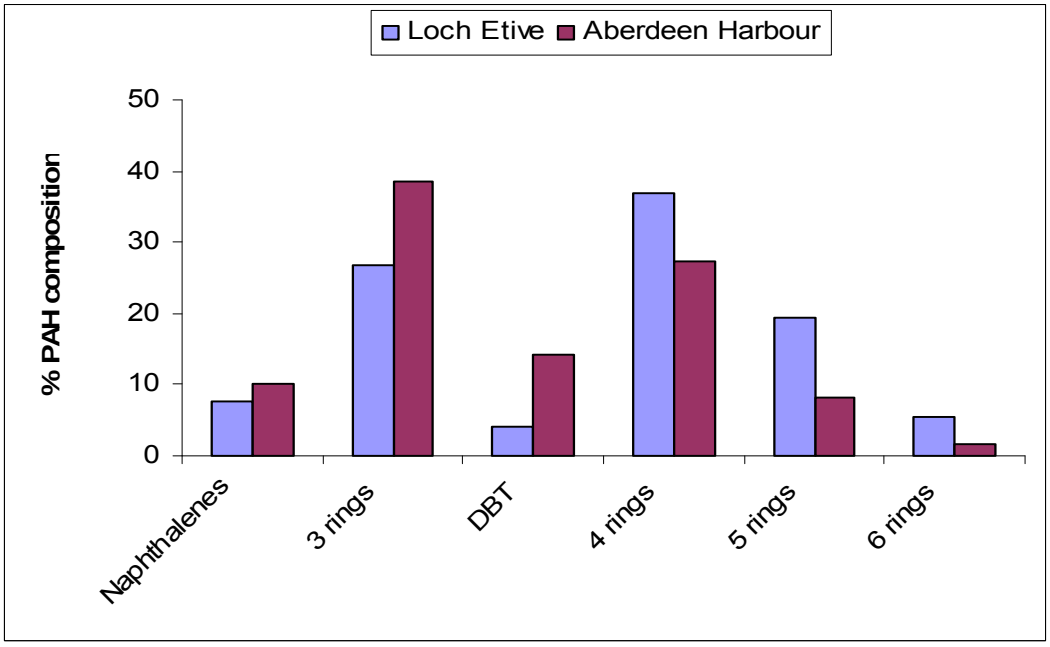

Fig **10.3**: PAH composition in mussels based on ring size (defined in earlier chapters)

10.3.1 Sediment pore water concentrations

The pore water concentration (C_{pw}) in the equilibrated sediment was calculated using the equation below

$$C_{pw} = \frac{(N_{sr})}{(m_{sr} * K_{sr,w})} \qquad \textbf{10.1}$$

N_{sr} = amount of PAH (ng) in exposed silicone rubber sheets respectively; m_{sr} = mass of silicone rubber (kg) and $K_{sr,w}$ = silicone rubber – water partition coefficient (L kg^{-1}). Silicone rubber-water partition coefficient values were measured and supplied by the coordinating laboratory for some of the compounds. No blank subtraction was effected in the calculations based on the guidelines provided by the coordinating group.

Total pore water concentrations of PAHs (sum of 31 PAHs parent and alkylated, Table **10.1**, for which silicone rubber-water partition coefficients are available) were found to be 207.5 and 7.4 ng L^{-1} in sediments from Aberdeen Harbour and Loch Etive respectively with the 2- to 3-ring PAHs dominating (90 %) at Aberdeen Harbour while the 3- to 4-ring PAHs dominated (73 %) at Loch Etive (Fig **10.4**). The high proportion of the alkylated 2- to 3-ring PAHs in Aberdeen Harbour suggests a petrogenic source of PAHs possibly from shipping traffic. PAH concentration ratios could not be used to confirm this based on the discussion on its use for pore waters in Chapter **8**, section **8.3.3**.

A parallel exposure of the rag worm, *Nereis virens* to the sediments from Loch Etive and Aberdeen Harbour is discussed in Chapter **9** as part of the validation of the use of silicone rubber passive sampler in sediment pore water measurements.

Table **10.1**: Pore water (C_{pw}) and free dissolved (C_w) concentrations calculated in sediment and water respectively for the two ICES sediment locations

Compound	$K_{sr,w}$ (L kg^{-1})	C_{pw} (pg L^{-1})		C_w (pg L^{-1})	
		AH	LE	AH	LE
Naphthalene	974	38462	557	3862	2314
2-Methyl Naphthalene*	11482	4432	41	806	730
1-Methyl Naphthalene*	10000	2441	163	496	585
C2 Naphthalenes*	21380	13376	126	3797	2036
C3 Naphthalenes*	43652	21076	64	17278	2299
C4 Naphthalenes*	147911	12941	0	21224	1196
Acenaphthylene	1709	3950	318	533	327
Acenaphthene	3860	40697	589	1057	604
Fluorene	5942	15929	57	976	791
Phenanthrene	12385	7069	897	1772	1977
Anthracene	15981	3582	209	366	94
C1 178*	77625	4643	386	12243	1850
C2 178*	141254	9150	185	16302	1362
C3 178*	218776	8457	210	7628	884
Dibenzothiophene*	10965	4123	66	261	19
Fluoranthene	40763	5588	540	3454	1422
Pyrene	44440	5346	1116	4633	807
C1 202*	102329	4124	533	3460	590
C2 202*	1995262	153	0	1144	203
Benzo[*c*]phenanthrene*	239883	109	29	102	45
Benz[*a*]anthracene	202520	243	91	229	75
Chrysene	175539	502	151	788	304
C1 228*	1412538	122	44	397	119
Benzofluoranthenes	524526	430	421	414	207
Benzo[*e*]pyrene	476948	147	155	203	117
Benzo[*a*]pyrene	480285	83	88	55	18
Perylene†	262531	173	174	47	58
C1 252*	9332543	11	16	110	66
Dibenz[*a,h*]anthracene	1698920	7	6	2	2
Indenopyrene	1175355	43	52	17	14
Benzoperylene	973944	48	86	22	16

* PAHs for which $K_{sr,w}$ values were adopted from Yates *et al*. (2007)

† $K_{sr,w}$ Value of D_{12}-perylene provided in the trial guideline adopted

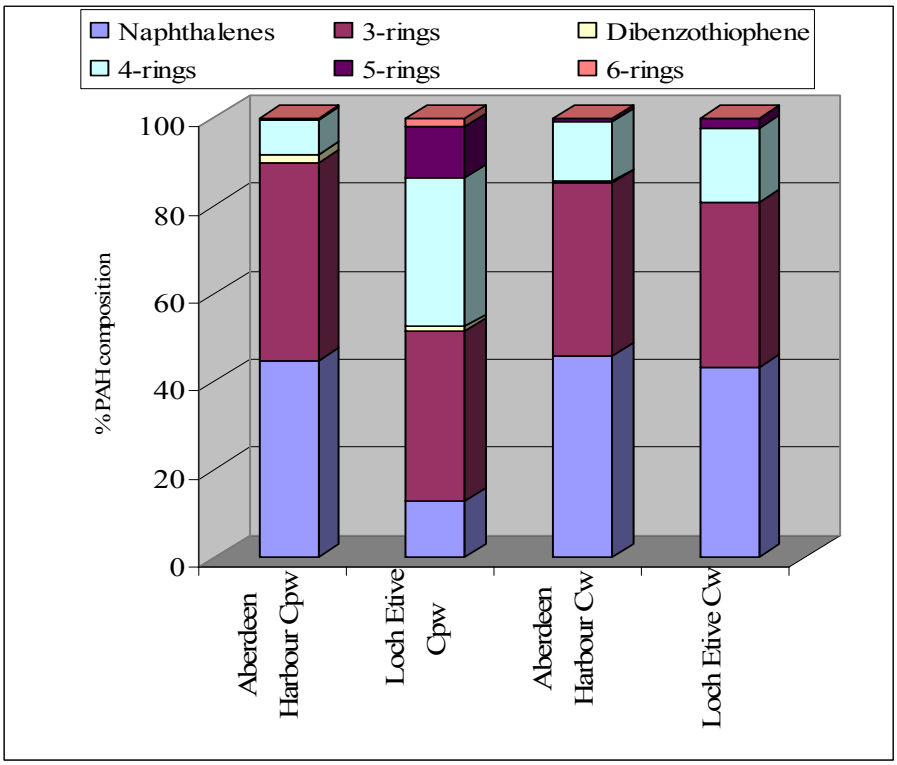

Fig **10.4**: Sediment pore water concentrations and free dissolved PAH concentrations in water-profile from Aberdeen Harbour and Loch Etive

Only CBs that were detected are reported in Table **10.2**.

Table **10.2**: Pore water (C_{pw}) and free dissolved (C_w) concentrations of CBs and hexachlorobenzene (HCB) in sediments and water at the 2 stations

Compound	$K_{sr,w}$ † (L kg^{-1})	C_{pw} (pg L^{-1})		C_w (pg L^{-1})	
		AH	LE	AH	LE
CB 52	562341	13.3	-*	229.16	0.09
CB 44	575440	40.8	-	93.84	0.02
CB 101	1659587	11.5	12.1	213.40	0.08
CB 118	2238721	5.0	5.0	107.70	0.03
CB 153	4570882	2.9	4.0	79.06	0.03
CB 138	4786301	3.0	3.5	84.03	0.02
CB 187	5754399	0.8	2.4	-	-
CB 180	8511380	0.4	0.6	-	-
HCB	107152	10.3	-	229.18	0.09

* not detected; † provided in the ICES PSTS guidelines

10.3.2 Dissolved concentrations in water

Aqueous (free dissolved) concentrations are valuable parameters in environmental risk assessments as it is only dissolved molecules that can pass through biological membranes of marine organisms (Huckins *et al*., 1990). To avoid any approximations due to non attainment of equilibrium, particularly by the more hydrophobic contaminants; free dissolved (aqueous) concentrations were determined using the full uptake model- equation **10.2** (Huckins *et al*., 2006; Booij *et al*., 2003).

$$C_w = \left(\frac{N^t}{m_{sr} * K_{sr,w}} \right) * \left(\frac{1}{\left(1 - \exp\left(-\left(\frac{R_s * t}{m_{sr} * K_{sr,w}} \right) \right) \right)} \right) \qquad \textbf{10.2}$$

Sampling rates (R_s) in L d^{-1} were calculated using:

$$R_s = -\left(\left(\frac{\left(\ln\left(\frac{N^t}{N^0} \right) \right)}{t} \right) * m_{sr} * K_{sr,w} \right) \qquad \textbf{10.3}$$

N^t and N^0 are amounts (ng) in the silicone rubber sampler at time‘ t ’ and zero, C_w is the aqueous concentration (sometimes referred to as water or free dissolved concentration) and other terms are as previously defined.

Sampling rates were calculated for all the PRCs (6 of the 8 PRCs added for PAHs) without taking into consideration the rule of thumb given in the draft trial guideline, which says: “*Prior to calculating the sampling rate, the PRC data are screened. If the PRC amount measured in the sampler is more than half the amount of the same PRC in the reference sampler the PRC is rejected and the remaining compounds used to determine the* R_s ”. Most of the PRCs would have failed the criteria, therefore sampling rates were calculated for the six and the median used (7.7 L d^{-1} at Aberdeen Harbour and 3.52 L d^{-1} at Loch Etive) in further calculations of C_w which are presented in Table **10.1** for PAHs. The sampling rate could only

be calculated for one chlorobiphenyl PRC (CB 30 = 0.65 L d^{-1}) as the amounts found after exposure were larger than the reported added amounts ($N^t > N^0$). This value was used to estimate aqueous concentrations (C_w) for CBs at both locations (Table **10.2**). There is a high uncertainty with this estimate of R_s as it is approximately a factor of 5 and 12 lower than that estimated for the PAH PRCs (7.7 and 3.5 L d^{-1}) at both Loch Etive and Aberdeen Harbour respectively thus making the estimates of freely dissolved CB water concentrations unreliable. A more comparable R_s value was expected for similar $\log K_{ow}$ compounds as Booij *et al*. (2003) have shown a relationship between R_s and $\log K_{ow}$.

Individual PAH free dissolved concentration, C_w in Aberdeen Harbour differed from that in Loch Etive by a median factor of 2.3, with the lighter PAHs (2- to 3-ring) dominating the profile in the water (see Fig **10.4**). Total PAH concentration (sum of 31 parent and alkylated PAHs) was determined to be 103.7 and 21.1 ng L^{-1} at Aberdeen Harbour and Loch Etive respectively.

C_{pw}/C_w ratios have been used to study the diffusive transport of PAHs in water bodies (Booij *et al*., 2003; Tixiěr *et al*., 2007), i.e. to determine the source of contaminants. A plot of these ratios for individual PAHs is shown in figure **10.5**. Booij *et al*. (2003), suggested that over saturation of the pore water (sediment as a source) is indicated by bars extending upward from the reference line $C_{pw}/C_w = 1$, whilst downward bars are indicative of sediment acting as a local sink for contaminants. The sediment at Loch Etive appears to be a sink for the PAHs especially for the 2- to 3-ring PAHs and a few other PAHs (benzo[*c*]phenanthrene, chrysene, C1-228 and C1-252). However, the sediment seems to be acting as a source of the PAHs at Aberdeen Harbour which is likely due to contamination from ships that ply the harbour and the relatively high suspended particulate matter found at the station which can adsorb PAHs. Some of the PAHs (pyrene, benz[*a*]anthracene, benzo[*e*]pyrene) have ratios close to 1 indicating approximation to equilibrium between the pore water and the overlying water. Aberdeen Harbour sediments have been shown to have higher total PAH contamination compared to Loch Etive.

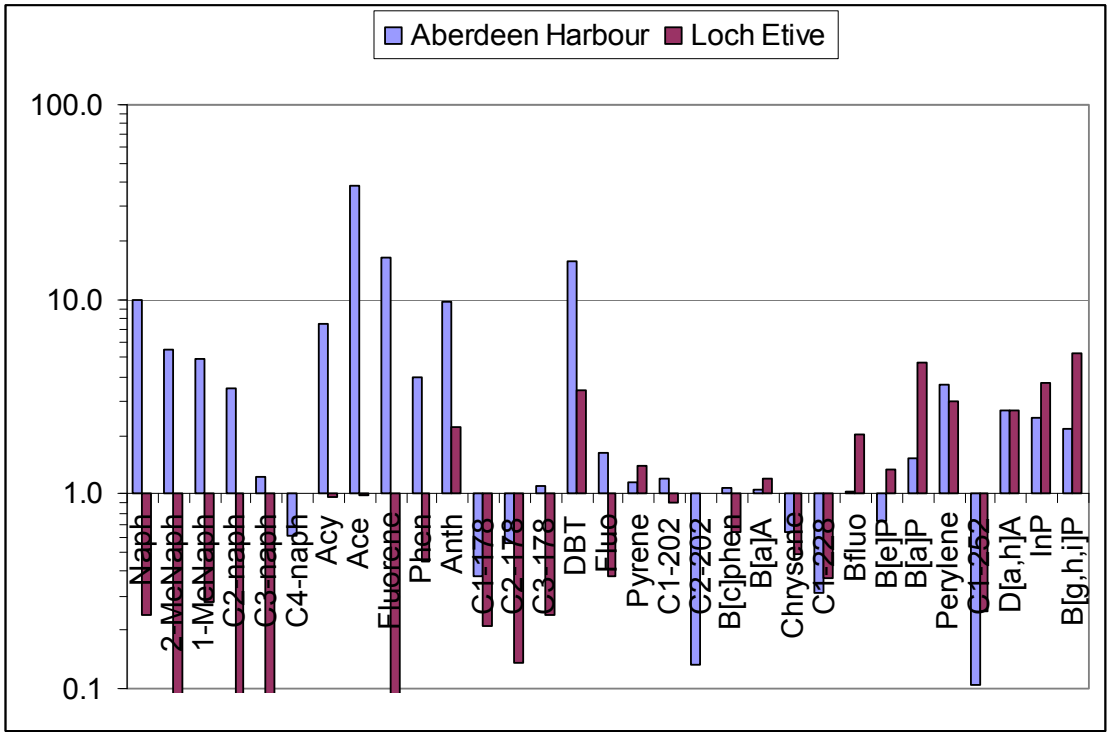

Fig **10.5**: Concentration ratios in pore water and overlying water for PAHs. X-axis legend: Naph= naphthalene; 2-MeNaph= 2-methylnaphthalene; 1-MeNaph= 1-methylnaphthalene; C2-naph= C2-naphthalene; C3-naph= C3-naphthalene; C4-naph= C4-naphthalene; Acy= acenaphthylene; Ace = acenaphthene; Phen= phenanthrene; Anth= anthracene; C1-C3 178= C1-C3 phenanthrenes/anthracenes; DBT= dibenzothiophene; Fluo= fluoranthene; C1-C2 202= C1-C2 fluoranthene/pyrenes; B[c]phen= benzo[*c*]phenanthrene; B[a]A= benz[*a*]anthracene; C1-228= C1- chrysene; Bfluo= benzofluoranthenes; B[e]P= benzo[*e*]pyrene; B[a]P= benzo[*a*]pyrene; C1-252= 7-methylbenzopyrene; D[a,h]A=dibenz[*a,h*]anthracene; InP= indeno[1,2,3-*cd*]pyrene; B[*g,h,i*]P= benzo[*ghi*]perylene

10.3.3 Bioaccumulation factors

Passive samplers have been developed to sample free dissolved concentrations in a way similar to marine organisms (Huckins *et al*., 2006; Gourlay *et al*., 2005) with passive samplers reported to yield more environmentally relevant and reliable exposure concentrations than mussels (Booij *et al*., 2006; Smedes, 2007) due to better control over passive samplers. Bioaccumulation or bio concentration factors can be calculated from the equation **10.4** when steady state equilibrium between both phases is attained which gives an estimate of a chemical's likelihood to accumulate in an aquatic animal (Barron, 1990).

$$BCF = \frac{C_{org}}{C_w} \qquad \textbf{10.4}$$

C_{org} = concentration in mussels (ng g^{-1} wet weight)

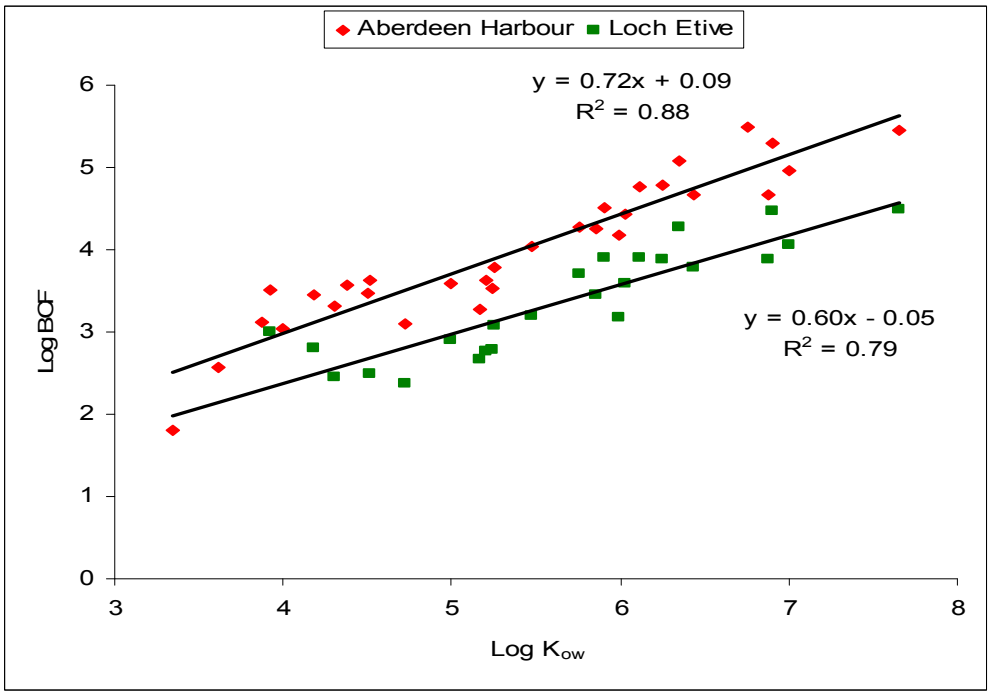

Fig **10.6**: Bio concentration factors calculated from free dissolved concentrations in water and concentrations in mussels as a function of log K_{ow}

Tendency to bioaccumulate increases with increasing hydrophobicity of the chemical and linear relationships (e.g. Fig **10.6**) have been observed between equilibrium BCFs and chemical hydrophobicity (Livingstone, 1992). Good correlations were observed for the data obtained, which are similar to that reported by Veith *et al*., (1979). Environmental factors such as pollution levels (~ 1 order of magnitude in dissolved concentrations) may account for the differences in log BCF-log K_{ow} correlations seen in Fig **10.6**. Values of BCF in the literature and correlations with log K_{ow} vary by several orders of magnitude (Booij *et al*., 2006; Huckins *et al*., 2006) possibly due to unreliable measurements of aqueous concentrations. Deviations from linearity of log BCFs with increasing log K_{ow} (> 6) have been observed (Baussant *et al*., 2001; EC TGD, 2003) mainly due to either biotransformation, reduced solubility of large

molecules to biotic lipids, non-equilibrium conditions and or reduced bioavailability. Mackay (1982) has also suggested that the less than unity slopes reported in log BCF-log K_{ow} correlations is due to poor data on K_{ow} values for high molecular weight compounds. The correlations obtained were used with dissolved concentrations to estimate PAH concentrations in mussels (e.g. Fig **10.7** for Aberdeen Harbour) and a reasonable agreement was found with the exception of some of the alkylated PAHs (C2- to C4-naphthalene). Passive samplers provide useful alternatives in the estimation of exposure concentrations to bio monitoring organisms, e.g. mussels, as they are not affected by factors such as metabolism, mortality and the validity of equilibrium conditions are easily confirmed by the use of PRCs.

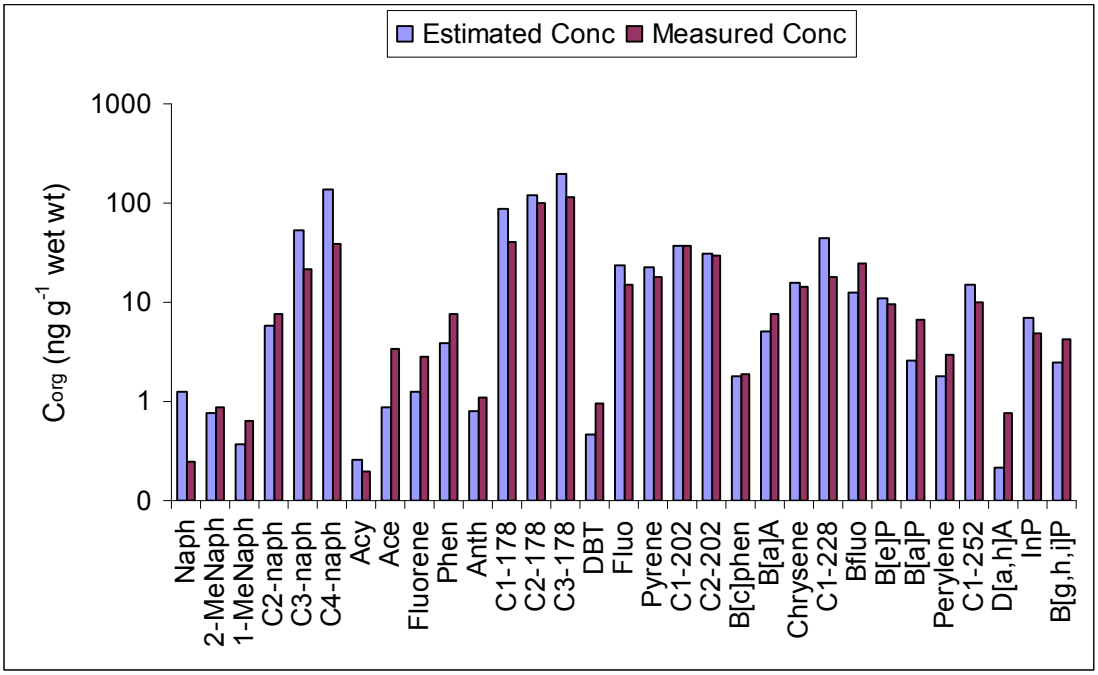

Fig **10.7**: A comparison of measured and estimated (from BCFs and C_w) concentrations in mussels from Aberdeen Harbour

10.4 Conclusions

The trial survey has shown the utility of the silicone rubber passive sampler in determining the environmentally relevant exposure concentration of PAHs and CBs (in some cases) in pore waters and waters at two locations in Scotland. Total pore water concentrations of PAHs at Telford Dock, Aberdeen Harbour was 2 orders of magnitude higher than the concentration

found at a relatively clean location (Loch Etive), with the 2- to 3-ring PAHs dominating (90 %) at Aberdeen Harbour while the 3- to 4-ring PAHs dominated (73 %) at Loch Etive. Similarly, the water (aqueous) concentration of PAHs was higher at Aberdeen Harbour than Loch Etive by a factor of 5. Further analysis of the results showed the sediment at Aberdeen Harbour to be a diffusive source of the PAHs to the overlying water, while the water at Loch Etive appeared to be a source of PAHs to the sediment, especially the lower ring PAHs.

Bio concentration factors calculated from parallel exposure of mussels and water concentrations determined using the silicone rubber samplers correlated well with log K_{ow} at the two locations studied. This correlation can be useful in predicting PAH levels in mussels from passive sampling data.

CHAPTER ELEVEN

Overall conclusions and future work

11.0 Background

The use of passive sampling techniques to measure exposure concentrations and availability of persistent organic pollutants (POPs) such as PAHs and CBs in environmental media has increased in recent years (Mayer *et al*., 2003; Huckins *et al*., 2006; Booij *et al*., 2006; Smedes, 2007). This is partly due to the low concentrations; adsorption to sampling equipment such as glass bottles and the problem of incomplete phase separation between particle-bound and dissolved POP analytes (Smedes, 1994; Booij *et al.*, 2003). The problems are even more pronounced when the determination is required in sediment pore waters, as large volumes of pore water are needed.

The availability of contaminants to organisms is described by the freely dissolved concentration (referring to the concentration that is bioavailable and not bound to dissolved or particulate organic carbon) in pore water and the water-extractable concentrations (the quantity that in time becomes available in its dissolved form) which is mainly the amount released by depletive processes like the action of deposit-feeding invertebrates and bacterial communities (Macrae and Hall, 1998; Cornelissen *et al*., 2001; ICES WGMS, 2003; Reichenberg and Mayer, 2006). Manifestation of the toxic effects of POPs require uptake into organisms, which often bio concentrate these contaminants in their lipids to relatively high levels. Therefore the concentrations available to cause harm needs to be assessed. However, contaminants that are very strongly adsorbed to particles are unlikely to be transferred from the particles into organisms, and therefore present little toxic risk to organisms. Knowledge of the available fraction is thus essential for environmental risk assessment.

Single phase sampling devices, such as low density polyethylene (LDPE strips), plastic poly-oxy-methylene strips (POM), Tenax, polydimethylsiloxane (PDMS), and silicone rubber have been used in addition to the two phase semi-permeable membrane devices (SPMDs) to

determine the free dissolved and available POP concentrations. These samplers are easy to construct, re-usable and of low cost (Rusina *et al.*, 2007) with high partition coefficients and low transport resistances.

An equilibrium passive sampling device made from silicone rubber film was developed and used to measure the freely dissolved, water extractable (accessible) concentrations and sediment-water partition coefficients of POPs in sediment with emphasis on PAHs.

The conclusions are based on the objectives/ milestones highlighted in Chapter **1** (section **1.9**).

11.1 Method development conclusions

In developing the method, milestones **1**-**4** were studied and covered in chapters **2**-**4**:

- Familiarisation with analytical techniques (Chapter **2**)
- Investigate the period or time to reach equilibrium for the PAHs as well as determining silicone rubber-water partition coefficients
- Investigate the influence of suspension density, phase ratio, dissolved organic matter and temperature on equilibrium
- Determination of 'total' concentration by traditional methods, e.g. sonication in DCM and methanol (PAHs) and Soxhlet extraction (CBs) in sediments and biota (Chapter **2**)

Pre-extraction of the silicone rubber sheets in a Soxhlet apparatus using ethyl acetate was sufficient to remove any residues that may interfere with subsequent instrumental analysis before use (Gruber *et al*., 2000). The uptake of PAHs and CBs into the silicone rubber was confirmed by spiking the compounds using a procedure developed by Booij *et al*. (2002) with reproducibility better than 5 %. Spiked sheets can be stored at – 20 ^{o}C for 30 days before use. Exposure of silicone rubber sheets to sediment slurry (Chapter **3**) from the Firth of Forth showed 20 days was sufficient for the POPs to attain equilibrium within the sediment-water system with good reproducibility of exposure. Consequently silicone rubber-water partition coefficients were determined (Chapter **4**) for PAHs and CBs using the co-solvent method with methanol as co-solvent. These showed strong linear correlations with hydrophobicity for both

CBs and PAHs and these values can be used to estimate water sampling rates as well as free dissolved (or pore water) concentrations in water and sediments. A draft operating procedure is presented in Appendix **3**.

11.2 Application of developed methods to field sediments and validation of silicone rubber samplers

The developed method was applied to sediment samples collected from the Fladen Ground of the North Sea (Chapter **5**), Loch Shell (Chapter **6**) and Loch Leven (Chapter **7**) to measure pore water concentrations of PAHs, proportions of PAHs in the sediment that are potentially mobilisable into the aqueous phase with time and the sediment-water partition coefficients of PAHs.

Pore water concentrations determined in Chapters **5-7** showed high proportions of the 2- to 4-ring (lighter) PAHs with the exception of sediments from Loch Leven (a site with a closed aluminium smelting plant) which also showed high proportions of benzofluoranthenes and benzo[*e*]pyrene. Concentrations in pore waters within the pg L^{-1} range were determined. The lighter PAHs are more water soluble and have shown lower partitioning into the sediments. Pore water concentration of PAHs was also determined as part of an MSc project (Kennedy, 2006) in dredge spoils sediments from the Firth of Clyde and Forth. These were compared with concentrations found in pore waters of sediments from Telford Dock, Aberdeen Harbour (Chapter **8**) as part of FRS's progressive development of its licensing operations of deposition of wastes such as dredge spoils in UK waters adjacent to Scotland. Higher pore water concentrations were found in sediments from the Firth of Clyde (collected during a dredging operation in 2006) than in those from Firth of Forth and Aberdeen Harbour. Even though the sediment PAH concentrations were mostly above the Provisional Action Level 1 concentrations for PAHs (Hayes *et al*., 2005) in use at FRS for the chemical assessment of dredge spoils, the pore water concentrations were below the proposed MAC-EQS values for PAHs in surface waters in the draft proposal of the European Union Council directive (EU, 2007)

Total PAH concentration in sediments from the Fladen Ground, Loch Leven and Loch Shell exceeded the OSPAR Background Concentrations and Background Assessment Concentrations. Sediments from Loch Leven showed PAH profiles consistent with contamination from aluminium smelters with a high dominance of 5- to 6-ring PAHs. Varying proportions of PAHs (dependent on location and PAH) were calculated to be available for exchange into the water/aqueous phase. The high and variable (uncertain; > 100 %) proportions found in the Fladen Ground sediments was attributed to the low sorption capacity and sandy (total organic carbon < 1 %) nature of the sediments. These varying proportions are indicative of the varying partition coefficients of the sediments and PAHs.

Similarly, sediment-water partition coefficients were also determined using the method developed in this research and when normalised to organic carbon content, indicated that the partitioning of PAHs in the studied sediments is strongly driven by hydrophobicity. Higher partition coefficients were found in the sediments compared to estimates from the Karickhoff relation (Karickhoff *et al*., 1979) which is traditionally used in environmental risk assessments. This suggests a likely overestimation of risks and stresses the importance of measuring partition coefficients for individual field sediments.

A parallel exposure of *Nereis virens* and silicone rubber samplers in sediments from the Vefsn fjord, Norway; Aberdeen Harbour and Loch Etive both in Scotland was carried out to validate the use of the silicone rubber samplers (Chapter **9**). Steady state concentrations of PAHs in *N. virens* were attained within 14 days. A good correlation was found between concentrations in *N. virens* predicted from $\log BCF - \log K_{ow}$ models and free dissolved concentrations determined from the silicone rubber samplers with measured concentrations in *N. virens*. Equilibrium partition models based on pore water concentrations predicted accumulation of PAHs into *N. virens* better than models that use the sediment organic carbon normalised concentrations.

As part of an inter-laboratory study (Smedes *et al*., 2007b) to validate the use of passive samplers in measuring exposure concentrations of PAHs and CBs in water and sediments (Chapter **10**), pore water and dissolved concentrations in sediments and water were measured

and found respectively to be higher in Aberdeen Harbour than in Loch Etive reflecting the expected pollution scenario. The results obtained also aided the assessment of diffusive transport of PAHs across the sediment-water system. Log BCF values of PAHs calculated from a parallel analysis of silicone rubber samplers and local mussels correlated positively with $\log K_{ow}$ at the 2 locations studied. The study also confirmed the viability of silicone rubber passive samplers as monitors of environmental contamination by PAHs and CBs.

11.3 Recommendations and future work

The use of silicone rubber passive samplers in measuring availability (free dissolved and accessible concentrations and sediment-water partition coefficients) has been demonstrated in this thesis. However, a major issue is "knowing what to measure and measuring what you know" (Smedes, 2005-Pers. Comm.).

The presence of plasticisers or fillers in membranes such as silicone rubber and temperature can affect the pore sizes (Huckins *et al*., 2006), and consequently the uptake of POPs (and sampling rates, diffusion and partition coefficients). Temperature, salinity and possibly pH have been shown to affect sampler-water partition coefficients. These effects would need to be studied with regards to silicone rubber-water membranes.

Generally, one of the desired attributes of any experimental method is high speed. Thus it would be attractive if the equilibration time of silicone rubber in sediment slurries found in this thesis (20 days) could be reduced, possibly by the modification of factors that enhance uptake such as size and geometry of samplers-using thinner sheets and addition of a solubilising solvent like methanol to enhance solubility and release of POPs into the aqueous phase. The use of bottles coated with thin layers of silicone adhesive has been reported (Gothard, 2007; Smedes *et al*., 2007b). However these samplers would also need to be calibrated before being used in environmental measurements. Another area that requires further optimisation is the selection of the phase ratios used in creating sorption isotherms from which availability parameters are extrapolated (see Chapter **5**) to ensure that varying degrees of depletion are always achieved, so as to optimise the isotherm plots. The behaviour

of naphthalene and its alkylated homologues have often been quite variable and difficult to understand, showing relatively poor correlations with biological uptake etc. This may be partly due to volatilisation, or decomposition, of naphthalenes, but method development is required to improve the performance on these compounds, which are important causes of taint in fish and shellfish.

When applying the method in the measurement of sediment-water partition coefficients, BSAF and BCF, the presence and nature of soot and other forms of black carbon (if any), have been linked with high partition coefficients of PAHs in sediments (Gustafsson *et al*., 1997; Jonker and Smedes, 2000), especially from around aluminium smelters. The form of carbon in sediment, and its consequences for partition coefficients should be investigated.

In terms of water sampling, the effect of temperature, hydrodynamics, and biofouling on exchange rates have been studied (Booij *et al*., 1998; Huckins *et al*., 2002) and this has led to the introduction of performance reference compounds to calibrate the exchange kinetics of POPs *in situ*. It is suggested that more PRCs, namely those not currently in use at FRS (particularly low K_{ow} PRCs for PAHs to cover the naphthalenes and a few higher K_{ow} PRCs for the higher K_{ow} PAHs) be added to silicone rubber samplers to estimate sampling rates and exposure concentrations. Any additional PRCs would need to be validated though.

The development of stringent quality control and assurance practices is recommended, considering the low concentrations being measured with passive samplers. These can include procedural blanks, transport blanks (water sampling), and a standard source of the silicone rubber membranes or sheets. Care must also be taken to prevent contamination of silicone rubber prior to deployment, after exposure and during laboratory analysis of samplers. Further participation in inter-laboratory comparisons and comparison with other equilibrium samplers such as LDPE, SPME and even SPMDs would improve the reliability of reported exposure concentrations.

Further applications of passive samplers (extracts) should include the maintenance of target concentrations in toxicity tests (Nipper *et al*., 2007) and the identification of other pollutants

such as polybrominated diphenyl ethers (PBDEs), polychlorinated dibenzo-*p*-dioxins and furans (PCDD/Fs) etc that may have been difficult to measure with traditional sampling methods due to very low concentrations. Passive sampling data from silicone rubber (and other samplers) can be used to support the development of environmental quality standards used in a legislative context, monitoring programmes and advice. A more comprehensive site-specific chemical approach addressing concentrations in pore water, extractable fractions and sediment-water partition coefficients could be considered to support the current assessment tools and in the measurement of availability of POPs.

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APPENDIX 1:

Fisheries Research Services, Marine Laboratory-Standard Operating Procedures

(See attached CD)

APPENDIX 2:

Tables from Chapter Three

Table **3.0**: Mean percent PAH recovery (n = 3) from silicone rubber sheets spiked with PAHs and extracted with 2 different extraction solvent (methanol and *iso*-hexane: acetone mixture)

PAH	Isohexane: Acetone (3:1 $^{v}/_{v}$)		Methanol	
	Mean % Recovery	**% CV**	**Mean % Recovery**	**% CV**
Naphthalene	56.69	25.20	51.20	11.76
Dibenzothiophene	78.10	5.15	73.62	2.92
Pyrene	79.84	3.85	75.27	3.47
Chrysene	85.59	3.04	80.37	2.55
Indeno[1,2,3-*c,d*]pyrene	106.74	1.85	112.97	6.20

Table **3.1**: Mean (n=2) amounts and % absorbed by silicone rubber sheets spiked with PAHs

PAH	Mean Amount (ng SR^{-1})	% CV	Spiked Amount (ng SR^{-1})	% Absorbed by sheet	% Recovery	
					Mean	% CV
Naphthalene	71.3	4.8	101.0	70.6	87.38	2.14
2-Methylnaphthalene	129.6	1.4	140.8	92.1	101.35	0.22
1-Methylnaphthalene	269.2	1.2	283.0	95.1	105.32	0.03
2,6-Dimethylnaphthalene	81.9	3.4	94.8	86.4	88.24	1.59
2,3,5-Trimethylnaphthalene	135.0	2.8	158.4	85.2	88.26	2.19
C4-naphthalene	118.4	4.8	133.4	88.7	90.03	5.18
Phenanthrene	86.5	3.3	104.8	82.5	90.83	1.68
Anthracene	72.8	2.4	95.6	76.2	82.26	1.41
2-Methylphenanthrene	80.3	2.2	84.8	94.7	99.37	1.42
3,6-Dimethylphenanthrene	85.4	0.2	99.6	85.7	87.71	0.02
2,6,9-Trimethylphenanthrene	75.5	0.7	87.2	86.6	87.94	1.09
Dibenzothiophene	112.8	1.5	159.6	70.7	77.32	0.08
Fluoranthene	75.1	0.4	95.2	78.9	83.96	0.34
Pyrene	231.7	0.3	248.4	93.3	98.97	0.44
1-Methylfluoranthene	98.0	0.9	112.4	87.2	90.44	1.15
2,7-Dimethylpyrene	84.2	0.0	84.6	99.5	101.31	0.03
Benzo[*c*]phenanthrene	108.5	1.9	122.8	88.3	89.45	0.12
Benz[*a*]anthracene	84.2	0.1	108.4	77.6	80.05	0.19
Chrysene	102.5	0.7	118.0	86.9	89.97	1.13
2-Methylchrysene	79.4	0.4	84.8	93.6	95.39	0.65
Dimethylbenz[*a*]anthracene	44.3	4.7	91.2	48.6	49.28	2.74
Benzofluoranthene[1]	163.9	0.2	202.0	81.1	83.16	0.55
Benzo[*e*]pyrene	71.4	0.8	90.4	79.0	81.41	1.64
Benzo[*a*]pyrene	54.6	1.2	93.4	58.4	59.21	2.99
Perylene	52.0	1.0	83.0	62.7	63.71	1.15
7-Methylbenzo[a]pyrene	60.1	2.4	87.4	68.8	69.46	2.00
Indeno[1,2,3-*cd*]pyrene	65.6	4.7	94.8	69.2	70.09	4.87
Benzo[*g,h,i*]perylene	68.5	0.1	88.0	77.8	78.50	1.33
Acenaphthylene	63.2	4.8	102.8	61.5	68.02	17.77
Acenaphthene	70.8	2.4	84.6	83.7	90.85	1.47
Fluorene	66.7	3.1	89.6	74.5	81.07	1.93
Dibenz[*a,h*]anthracene	58.0	0.7	89.8	64.6	64.91	1.30

[1]Benzofluoranthene is sum of benzo[*b*] and [*k*] fluoranthene

Table **3.2**: List of PAHs and CBs analysed in this thesis. In some experiments however, not all the listed compounds were analysed. The CBs were only introduced at a later point in the thesis

S/No	PAH	S/No	CB
1	Naphthalene	1	HCB
2	2-Methyl Naphthalene	2	CB 31
3	1-Methyl Naphthalene	3	CB 28
4	C2- Naphthalene	4	CB 53*
5	C3- Naphthalene	5	CB 52
6	C4- Naphthalene	6	CB 49
7	Phenanthrene	7	CB 35*
8	Anthracene	8	CB 44
9	2-Methylphenanthrene	9	CB 74
10	3,6-Dimethylphenanthrene	10	CB 70
11	2,6,9-Trimethylphenanthrene	11	CB 101
12	Dibenzothiophene	12	CB 99
13	C1- Dibenzothiophenes	13	CB 112*
14	C2- Dibenzothiophenes	14	CB 97
15	C3- Dibenzothiophenes	15	CB 110
16	Fluoranthene	16	CB 151*
17	Pyrene	17	CB 149
18	1-Methylfluoranthene	18	CB 118
19	2,7-Dimethylpyrene	19	CB 153
20	C3- 202	20	CB 132
21	Benzo[*c*]phenanthrene	21	CB 105
22	Benz[*a*]anthracene	22	CB 137
23	Chrysene/Triphenylene	23	CB 138
24	Benz[*b*]anthracene	24	CB 158
25	2-Methylchrysene	25	CB 187
26	Dimethylbenz[*a*]anthracene	26	CB 183
27	Benzofluoranthenes[1]	27	CB 128
28	Benzo[*e*]pyrene	28	CB 156
29	Benzo[*a*]pyrene	29	CB 157
30	Perylene	30	CB 180
31	7-Methylbenzo[*a*]pyrene	31	CB 198*
32	C2-alkylated benzopyrene/ perylenes	32	CB 170
33	Indeno[1,2,3-*cd*]pyrene	33	CB 189
34	Benzo[*ghi*]perylene	34	CB 194
35	C1- 276	35	CB 209*
36	C2- 276		

37	Acenaphthylene		
38	Acenaphthene		
39	Fluorene		
40	Dibenz[*a,h*]anthracene		
41	D_{12}-Chrysene*		
42	D_{12}-Benzo[*e*]pyrene*		

[1] sum of benzo[*b*] and [*k*] fluoranthenes; * CBs and PAHs that were used as performance reference compounds

Table **3.3**: Mean percent recovery for different sonication times and loading method

PAHs	Mean % Recovery				
	Sonication				Shaking
	2 h (n=3)	4 h (n=3)	5 h (n=3)	7.5 h (n=2)	~ 24 h (n= 2)
Naphthalene	88.54	88.07	71.57	68.47	87.38
2-Methylnaphthalene	97.67	104.19	88.44	75.46	101.35
1-Methylnaphthalene	98.84	106.31	91.47	80.32	105.32
2,6-Dimethylnaphthalene	91.36	93.25	82.19	74.25	88.24
2,3,5-Trimethylnaphthalene	84.13[†]	83.87	82.23	73.75	88.26
1,4,6,7-Tetramethylnaphthalene	89.63	89.76	82.83	77.96	90.03
Phenanthrene	88.96	89.19	84.73	77.73	90.83
Anthracene	82.97	82.07	71.41	67.45	82.26
2-Methylphenanthrene	101.04	99.43	92.93	85.47	99.37
3,6-Dimethylphenanthrene	89.66	88.82	83.90	76.61	87.71
2,6,9-Trimethylphenanthrene	92.48	91.10	84.55	75.75	87.94
Dibenzothiophene	90.77	91.10	71.20	63.73	77.32
Fluoranthene	85.70	85.22	79.79	71.95	83.96
Pyrene	98.73	98.54	93.56	84.38	98.97
1-Methylfluoranthene	90.43	91.34	84.13	77.09	90.44
2,7-Dimethylpyrene	103.21[‡]	104.37	95.72	87.96	101.31
Benzo[*c*]phenanthrene	93.09	92.28	87.10	78.21	89.45
Benz[*a*]anthracene	88.28	86.38	74.44	68.86	80.05
Chrysene	90.76	89.16	95.78	85.00	89.97
2-Methylchrysene	98.42	96.29	91.91	83.17	95.39
Benzofluoranthenes	89.87	92.61	76.72	71.30	83.16
Benzo[*e*]pyrene	85.95	86.88	75.62	69.13	81.41
Benzo[*a*]pyrene	71.27	70.19	49.47	50.66	59.21
Perylene	78.04	78.24	54.99	55.98	63.71
7-Methylbenzo[*a*]pyrene	81.27	80.02	59.32	59.70	69.46
Indeno[1,2,3-*cd*]pyrene	75.41	72.95	61.13	58.57	70.09
Benzo[*ghi*]perylene	80.86	80.61	68.58	66.43	78.50
Acenaphthylene	75.88	75.77	61.78	56.38	68.02
Acenaphthene	92.45	96.82	83.68	76.37	90.85
Fluorene	78.98	77.95	76.05	67.45	81.07
Dibenz[*a,h*]anthracene	70.91	68.14	57.60	54.74	64.91

[‡] Only one point is reported as the other runs yielded over recoveries (> 160 %)

Table **3.4**: % CV[a] on percent recovery of loading trials when optimising for sonication time and using shaking as described by Booij *et al*. (2002)

PAH	Sonication				shaking
	2 h	**4 h**	**5 h**	**7.5 h**	**~24 h**
Naphthalene	0.79	1.79	1.58	10.78	4.81
2-methylnaphthalene	1.51	9.29	9.68	9.24	1.36
1-methylnaphthalene	1.84	10.39	11.05	12.39	1.18
2,6-dimethylnaphthalene	3.08	2.42	2.26	9.36	3.38
2,3,5-trimethylnaphthalene	6.84	2.35	1.97	7.77	2.79
1,4,6,7-tetramethylnaphthalene	3.78	1.66	1.49	7.65	4.81
Phenanthrene	0.42	1.62	1.44	7.75	3.28
Anthracene	0.77	1.69	1.38	9.88	2.44
2-methylphenanthrene	3.31	2.11	2.10	8.36	2.16
3,6-dimethylphenanthrene	0.86	2.03	1.80	10.68	0.22
2,6,9-trimethylphenanthrene	2.78	1.50	1.37	11.41	0.74
Dibenzothiophene	0.52	1.06	0.97	8.22	1.49
Fluoranthene	0.66	1.55	1.32	10.67	0.40
Pyrene	0.26	0.90	0.89	10.26	0.30
1-methylfluoranthene	1.95	0.57	0.52	8.47	0.92
2,7-dimethylpyrene	17.09	0.43	0.45	8.96	0.03
Benzo[*c*]phenanthrene	1.43	0.89	0.82	8.05	1.87
Benz[*a*]anthracene	0.72	1.66	1.43	10.02	0.14
Chrysene	1.10	0.88	0.79	11.88	0.69
2-methylchrysene	1.34	1.60	1.54	9.44	0.45
Dimethylbenz[*a*]anthracene	3.32	0.75	0.49	6.63	4.69
Benzofluoranthenes[b]	0.73	1.25	1.16	8.57	0.19
Benzo[*e*]pyrene	0.44	1.39	1.21	9.53	0.78
Benzo[*a*]pyrene	0.58	1.25	0.88	4.21	1.24
Perylene	0.36	0.96	0.75	0.12	1.03
7-methylbenzo[a]pyrene	0.86	1.26	1.01	0.38	2.40
Indeno[1,2,3-*cd*]pyrene	0.75	0.36	0.27	7.97	4.72
Benzo[*ghi*]perylene	1.05	0.38	0.31	3.92	0.06
Acenaphthylene	0.45	5.05	3.83	4.00	4.75
Acenaphthene	1.90	4.79	4.64	9.52	2.41
Fluorene	5.45	2.32	1.81	9.37	3.07
Dibenz[*a,h*]anthracene	1.08	1.35	0.92	6.67	0.67

[a] % CV- percent coefficient of variation; [b] sum of benzo[*b*] and [*k*] fluoranthenes

Table **3.5**: Between days CV based on analysis of triplicate spiked silicone rubber sheets over 5 separate days using either sonication or shaking when spiking

Compound	% CV	
	Sonication	**Shaking**
D_{12}-Chrysene	21.86	5.72
D_{12}-Benzo[*e*]pyrene	18.39	8.30
CB 53	21.29	4.34
CB 35	19.03	3.64
CB 112	14.13	3.59
CB 151	13.96	4.27
CB 198	6.41	4.99
CB 209	6.29	2.08

Table **3.6**: k_e's and $t_{90\%}$ calculated from equation **3.2** and **3.3** using Solver for PAHs and CBs

Compounds	**k_e (day^{-1})**	**$t_{90\%}$ (days)**		**k_e (day^{-1})**	**$t_{90\%}$ (days)**
PAH			PAH		
Naphthalene	0.048	48.3	Benzo[*ghi*]perylene	0.134	17.2
2-Methylnaphthalene	0.084	27.4	Acenaphthylene	3.933	0.6
1-Methylnaphthalene	0.072	32.2	Acenaphthene	0.510	4.5
2,6-Dimethylnaphthalene	0.096	24.0	Fluorene	0.604	3.8
2,3,5-Trimethylnaphthalene	0.145	15.9	Dibenz[*a,h*]anthracene	0.272	8.5
1,4,6,7-Tetramethylnaphthalene	0.478	4.8	D_{12}-Chrysene	0.721	3.2
Phenanthrene	1.991	1.2	D_{12}-Benzo[*e*]pyrene	0.265	8.7
Anthracene	1.050	2.2	CBs		
2-Methylphenanthrene	0.946	2.4	HCB	0.777	3.0
3,6-Dimethylphenanthrene	0.947	2.4	CB 31	0.479	4.8
2,6,9-Trimethylphenanthrene	0.343	6.7	CB 28	0.759	3.0
Dibenzothiophene	0.497	4.6	CB 52	1.261	1.8
Fluoranthene	1.037	2.2	CB 49	1.351	1.7
Pyrene	0.972	2.4	CB 35	0.119	19.3
1-Methylfluoranthene	0.662	3.5	CB 44	4.915	0.5
2,7-Dimethylpyrene	0.379	6.1	CB 74	1.287	1.8
Benzo[*c*]phenanthrene	0.656	3.5	CB 70	0.670	3.4
Benz[*a*]anthracene	0.612	3.8	CB 101	0.078	29.5
Chrysene	0.763	3.0	CB 112	0.030	76.8
2-Methylchrysene	0.265	8.7	CB 110	0.749	3.1
Dimethylbenz[*a*]anthracene	0.091	25.4	CB 149	0.051	45.1
Benzo[*b*]fluoranthene	0.250	9.2	CB 118	0.391	5.9
Benzo[*e*]pyrene	0.221	10.4	CB 153	0.056	41.1
Benzo[*a*]pyrene	0.266	8.6	CB 132	0.068	33.9
Perylene	0.063	36.8	CB 138	0.033	69.8
7-Methylbenzo[*a*]pyrene	0.119	19.4	CB 128	0.133	17.3
Indeno[1,2,3-*cd*]pyrene	0.190	12.1	CB 209	0.132	17.4

Table **3.7**: $t_{90\%}$ calculated using equation **3.3** at the different sediment contents studied

PAH	Sediment content (ng g^{-1})			
	0.27	**0.16**	**0.08**	**0.05**
Naphthalene	48.6	48.3	298.3	4921.6
2-Methylnaphthalene	24.5	27.4	131.9	5481.0
1-Methylnaphthalene	23.2	32.2	213.2	137.7
2,6-Dimethylnaphthalene	35.9	24.0	54.6	50.8
2,3,5-Trimethylnaphthalene	7.9	15.9	58.3	27.3
1,4,6,7-Tetramethylnaphthalene	186.3	4.8	20.1	15.9
Phenanthrene	1.9	1.2	1.8	5.0
Anthracene	3.9	2.2	18.8	21.3
2-Methylphenanthrene	3.9	2.4	3.9	6.6
3,6-Dimethylphenanthrene	18.6	2.4	12.1	12.4
2,6,9-Trimethylphenanthrene	13.2	6.7	14.5	14.9
Dibenzothiophene	5.5	4.6	43.7	33.6
Fluoranthene	33.8	2.2	6.6	14.3
Pyrene	27.4	2.4	5.5	12.0
1-Methylfluoranthene	15.9	3.5	9.6	12.5
2,7-Dimethylpyrene	21.8	6.1	19.4	26.0
Benzo[*c*]phenanthrene	14.6	3.5	10.1	12.6
Benz[*a*]anthracene	14.9	3.8	10.7	14.6
Chrysene	21.1	3.0	7.2	13.9
2-Methylchrysene	17.7	8.7	17.4	21.0
Dimethylbenz[*a*]anthracene	52.6	25.4	130.8	28961.0
Benzo[*b*]fluoranthene	6.7	9.2	24.4	23.7
Benzo[*e*]pyrene	28.8	10.4	30.6	154.3
Benzo[*a*]pyrene	7.4	8.6	27.3	82.0
Perylene	268.1	36.8	136.2	12.5
7-Methylbenzo[*a*]pyrene	19.9	19.4	136.3	127.5
Indeno[1,2,3-*cd*]pyrene	24.2	12.1	28.6	260.7
Benzo[*ghi*]perylene	29.0	17.2	47.1	154.4
Acenaphthylene	3.4	0.6	0.6	2.7
Acenaphthene	19.9	4.5	36.4	28.8
Fluorene	4.5	3.8	40.4	2.6
Dibenz[*a,h*]anthracene	8.0	8.5	45.3	10593.8
D12-Chrysene	4.4	3.2	10.0	14.4
D12-Benzo[*e*]pyrene	8.5	8.7	15.0	20.1

Table **3.8**: Percent coefficient of variation (% CV) in PAH uptake after the pre-treating of the bottle containing Firth of Forth sediment before adding the spiked silicone rubber sheets

PAH	%CV (n=3)		
	SHAKE 2D	**SHAKE 3H**	**SONICATE 6MIN**
Naphthalene	5.10	7.80	11.44
2-Methylnaphthalene	1.95	7.50	9.47
1-Methylnaphthalene	1.20	8.69	8.75
2,6-Dimethylnaphthalene	3.11	8.29	10.25
2,3,5-Trimethylnaphthalene	3.24	6.98	12.72
1,4,6,7-Tetramethylnaphthalene	1.58	4.85	14.07
Phenanthrene	3.35	10.09	16.16
Anthracene	4.99	9.34	13.73
2-Methylphenanthrene	2.46	7.37	14.10
3,6-Dimethylphenanthrene	0.97	3.36	9.40
2,6,9-Trimethylphenanthrene	1.06	2.96	9.96
Dibenzothiophene	4.74	5.79	12.33
Fluoranthene	1.94	6.15	14.71
Pyrene	1.67	4.32	12.48
1-Methylfluoranthene	0.90	4.02	11.35
2,7-Dimethylpyrene	0.42	3.37	9.92
Benzo[*c*]phenanthrene	0.53	4.63	12.12
1,2-Benz[*a*]anthracene	0.64	4.79	13.64
Chrysene	0.56	4.22	10.79
2-Methylchrysene	0.89	3.40	9.02
Dimethylbenz[*a*]anthracene	6.43	4.47	7.53
Benzo[*b*]fluoranthene	32.90	3.77	6.36
Benzo[*k*]fluoranthene	39.64	3.83	3.16
Benzo[*e*]pyrene	5.91	4.23	19.22
Benzo[*a*]pyrene	5.65	2.63	3.81
Perylene	7.09	3.31	10.25
7-Methylbenzo[*a*]pyrene	6.12	3.61	7.98
Indeno[1,2,3-*cd*]pyrene	7.83	4.23	9.02
Benzo[*ghi*]perylene	6.44	53.42	9.10
Acenaphthylene	3.19	24.85	5.67
Acenaphthene	1.09	11.28	11.06
Fluorene	3.84	9.11	11.54
Dibenz[*a,h*]anthracene	6.96	3.19	13.14
D_{12}-Chrysene*	2.72	3.04	13.64
D_{12}-Benzo[*e*]pyrene*	11.29	8.39	13.56

* These are deuterated PAHs that were spiked onto the sheets as performance reference compounds

Table **3.9**: Percent coefficient of variation (% CV) in CB uptake after the pre-treatment of the bottle containing Firth of Forth sediment before adding the spiked silicone rubber

	%CV (n=3)		
CB	**SHAKE 2D**	**SHAKE 3H**	**SONICATE 6MIN**
HCB	2.62	11.26	19.14
CB 31	11.49	30.27	37.49
CB 28	7.56	9.56	18.59
*CB53	5.74	5.67	25.69
CB 52	10.99	9.36	14.00
CB 49	8.90	9.14	12.85
*CB 35	6.27	2.18	22.08
CB 44	4.70	4.73	17.38
CB 74	5.95	18.81	19.51
CB 70	4.26	76.28	13.93
CB 101	6.45	5.57	14.63
CB 99	2.72	9.49	15.26
*CB 112	4.94	3.32	27.87
CB 97	6.09	34.92	15.10
CB 110	5.27	5.01	14.80
*CB 151	7.34	5.59	28.99
CB 149	5.01	6.27	12.71
CB 118	8.14	7.44	14.18
CB 153	6.88	7.85	10.37
CB 132	9.18	9.06	12.84
CB 105	13.72	16.38	14.61
CB 137	-	-	-
CB 138	3.63	1.30	6.47
CB 158	17.88	-	20.84
CB 187	10.21	19.58	20.38
CB 183	-	104.22	14.94
CB 128	6.60	7.14	11.20
CB 156	-	173.21	47.96
CB 157	-	86.68	8.91
CB 180	5.61	73.63	16.24
*CB 198	7.60	6.46	27.11
CB 170	-	94.84	6.66
CB 189	39.50	-	-
CB 194	5.79	12.72	29.52
*CB 209	11.04	1.61	32.12

- Depicts instances for which no % CV could be calculated as the CBs were not absorbed from the sediment.

* These are CBs that were spiked onto the sheets as performance reference compounds

Table **3.10**: Percent CB depletion absorbed by silicone rubber sheets after 20 days

CB	% Depletion		
	Sonicate 6 min	**Shake 2d**	**Shake 3h**
HCB	20.11	19.88	20.66
CB 31	14.02	19.64	14.36
CB 28	27.73	23.57	24.90
CB 52	22.07	22.91	20.84
CB 49	23.09	17.78	20.75
CB 74	11.07	17.04	9.61
CB 70	23.32	21.43	40.27
CB 101	23.93	21.38	24.92
CB 99	23.69	32.87	27.23
CB 97	27.08	31.70	22.77
CB 110	106.63	123.18	139.76
CB 149	27.41	25.65	31.54
CB 118	18.90	14.61	17.99
CB 153	17.38	16.47	20.09
CB 132	47.37	51.45	50.09
CB 105	30.88	23.28	28.35
CB 138	17.31	14.68	18.43
CB 158	8.89	10.84	0.00
CB 187	10.88	10.13	10.15
CB 183	6.13	0.00	2.42
CB 156	10.82	0.00	83.72
CB 180	6.88	9.32	13.88
CB 170	9.52	0.00	2.99
CB 194	4.75	27.47	14.34

Table **3.11**: Percent depletion and fraction of PAHs absorbed by silicone rubber sheets after 20 days

PAH	% Depletion		
	Sonicate 6 min	**Shake 2d**	**Shake 3h**
Naphthalene	0.64	0.47	0.37
2-Methylnaphthalene	0.55	0.41	0.33
1-Methylnaphthalene	0.52	0.40	0.35
2,6-Dimethylnaphthalene	0.78	0.67	0.63
2,3,5-Trimethylnaphthalene	1.24	1.08	1.08
1,4,6,7-Tetramethylnaphthalene	2.13	1.92	1.98
Phenanthrene	1.60	1.11	1.20
Anthracene	1.29	0.94	1.01
2-Methylphenanthrene	1.41	1.08	1.18
3,6-Dimethylphenanthrene	2.30	2.13	2.01
2,6,9-Trimethylphenanthrene	3.46	3.41	3.72
Dibenzothiophene	1.37	1.01	0.97
Fluoranthene	2.21	1.67	1.76
Pyrene	2.71	2.33	2.43
1-Methylfluoranthene	2.03	1.88	1.92
2,7-Dimethylpyrene	1.90	1.94	2.01
Benzo[*c*]phenanthrene	2.47	2.32	2.32
Benz[*a*]anthracene	1.12	0.98	0.96
Chrysene	1.40	1.14	1.31
2-Methylchrysene	1.86	1.83	1.82
Dimethylbenz[*a*]anthracene	1.92	1.70	1.87
Benzo[*b*+*k*]fluoranthene	1.15	0.95	1.00
Benzo[*e*]pyrene	0.95	0.82	0.77
Benzo[*a*]pyrene	0.60	0.51	0.61
Perylene	0.89	0.87	0.89
7-Methylbenzo[*a*]pyrene	0.72	0.66	0.71
Indeno[1,2,3-*cd*]pyrene	0.41	0.48	0.52
Benzo[*ghi*]perylene	0.42	0.44	0.47
Acenaphthylene	2.33	1.09	1.67
Acenaphthene	3.32	2.62	2.89
Fluorene	2.43	1.89	1.97
Dibenz[*a,h*]anthracene	2.95	4.32	6.24

APPENDIX 3:

Draft Procedure for silicone rubber sampling of PAHs and CBs in sediments

Draft protocol for exposure of Silicone Rubber to sediments

All glassware is rinsed twice in dichloromethane and twice in *iso*-hexane before use.

1.0 Preparation of Silicone Rubber sheets:

1.1 Cut silicone rubber sheet (0.5 mm thickness) into pieces of 4 × 6 cm (~50 cm^2). For exposure to sediment at different phase ratios, the silicone rubber can be cut to different dimensions (weights).

1.2 Put six 4 × 6 cm sheets into a 200 ml Soxhlets and pre-extract the sheets using ~ 400 ml ethyl acetate in 500ml round bottom flasks by Soxhlet (setting 5½), for 100 h. The pre-extracted sheets are then collected and rinsed with methanol by soaking the pre-extracted sheets in a 250 ml beaker containing 100 ml methanol and swirling for 2 min. The sheets are then removed using pre-cleaned forceps and stored in a wide mouth 500 ml glass jar containing 100 ml or just enough methanol covering the sheets before use.
Note: Separate glassware is kept for the pre-extraction as the silicone rubber releases oligomers that leave residues and could coat the flasks.

2.0 Spiking Performance reference compounds (PRCs):

The PRCs to be used are D_{12}-Chrysene, D_{12}-Benzo[*e*]pyrene for the PAHs, and PCB 35, 53, 112, 151, 198 and 209 for the PCBs. Other PRCs can be added.

2.1 Using a calibrated syringe a known volume of PRCs is added to a known volume (the volume of methanol used is ~5 ml g^{-1} of silicone rubber) of methanol in a 500 ml amber glass jar with a lid/cover to obtain the required amount (350 ± 50 ng for PCBs and 550 ± 50 ng for PAHs) of PRC per sheet.

2.2 The silicone rubber sheets are then added and the jar sonicated for 30mins, followed by addition of water to obtain 80% methanol, and sonicated for a further 30 min. Water is again added to attain 50% methanol and sonicated for 1hr. The sheets are stored covered by the solution in the 50% methanol solution in a freezer prior to use.

3.0 Determination of Silicone rubber-water partition coefficients:

To calculate the concentration in the water phase of sediments, the partition coefficient of the sampler or reference phase being used has to be determined. This is done using the co-solvent method based on the concentration in the silicone rubber sheets attaining equilibrium with the methanol-water mixture.

3.1 The silicone rubber sheets were loaded as in section **2** with appropriate amount of test PAHs and PCBs (500 ± 50 ng for PAHs and 350 ± 50 ng for PCBs.

3.2 900 ml of methanol-water mixtures at 20, 25, 30, 35, 40, 45 and 50 % methanol is added to 1 L glass bottles. Milli-Q water (18.2 mΩ) is used.

3.3 Introduce a spiked sheet into each bottle and an un-spiked sheet is added to a bottle containing 900 ml of 20 % methanol as procedural blank. The exposure is carried out in duplicate- one sheet in each of two flasks.

3.4 The bottles are placed horizontally and shaken on an orbital shaker at 150 rpm for 15 days, within which equilibrium would have been attained. Sorption of analytes to the container wall is considered negligible and the concentration in the water phase remains measurable.

3.5 The silicone sheets are removed from the bottles and extracted using 100 ml of *iso*-hexane: acetone mixture (3:1 $^{v}/_{v}$) in Soxhlets at setting 4½, for 6 h. Add 100 µl of deuterated PAH internal standard using a calibrated syringe to the extracts after allowing cooling to room temperature.

3.6 The extracts are then rotary evaporated down to ~2 ml, exchanged into isohexane by the addition of 2 x 20ml isohexane and further reduced by rotary evaporation to ~2 ml. Blow down to 1ml (volume is checked against a calibrated vial) under gentle flow of nitrogen. The extract is then further split into two equal fractions of ~0.5 ml. The first fraction is treated as in **3.7** for PAHs and the 2nd fraction (for CBs) is further treated as in **3.8**

3.7 Using a syringe, a 150 µl aliquot of the sample is passed through a Genesis metal free HPLC column (25 cm x 4.6 mm) and eluted with isohexane at a flow rate of 2 ± 0.1 ml min^{-1}. The eluate is collected in a 100 ml round bottom flask for 20 min and reduced by rotary evaporation to ~1 ± 0.2 ml. Transfer the reduced extract to a GC vial with insert and reduce under a stream of scrubbed nitrogen to 50 ± 10 µl for GC-MS analysis.
Note: The HPLC use and clean up is based on SOP 1600

3.8 The CB fraction from 3.6 is treated as below:

3.8.1 The ~ 0.5 ± 0.1ml fraction for CBs is weighed, and transferred to the top of a 3 ± 0.2 g alumina column (SOP 0440) and adsorb. Add 1 ± 0.2 ml of *iso*-hexane to rinse the vial and pipette rinse to the top of the column and adsorb. Repeat using another 1 ± 0.2 ml of *iso*-hexane.

3.8.2 Dispense 20 ± 1 ml of *iso*-hexane into a measuring cylinder, a small volume of this is used to rinse the extract tube, which is then transferred to the top of the column; transfer the remaining isohexane to the top of the column also and collect the eluate (using the test split information given on the absorbent storage jar) into a 10 ml test tube. The remaining eluate is allowed to go to waste.

3.8.3 Concentrate the collected fraction from 3.8.2 along with washings using Turbovaps to ~0.5 ± 0.1 ml, add ~0.5 ± 0.1 ml (weighing) of D6D16 internal standard, followed by the addition of 6 ± 1 ml of *iso*-octane before reducing using Turbovaps to ~0.5 ± 0.1 ml in GC vials and weighed before GC-ECD analysis

3.9 100 µl of dilute PAH deuterated internal standard is then added using a syringe to the methanol-water mixture and extract the mixture twice with 60 ml DCM each time in separating funnels.

3.10 The DCM extract is dried over 40 ± 5 g anhydrous sodium sulphate in a 250 ml conical flask for at least 10 min and then transferred to a 250 ml round bottom flask together with washings (twice with DCM, 10 ± 1 ml each washing) from the sodium sulphate.

3.11 The DCM extracts are rotary evaporated down to 2 ml and exchanged into isohexane by two times addition of 50 ml isohexane, and further concentrated to 2 ml.

3.11.1 Concentrate down to 1 ml (checked against calibrated vial) and split into two fractions of 500 µl each for PCBs and PAHs respectively.

3.11.2 The PAH fraction is reduced under nitrogen to 50 ± 10 µl for GC-MS analysis.

3.11.3 Add 500 µl D6D16 internal standard to the PCB fraction and reduce to 500 µl under nitrogen for GC-ECD analysis (the weight of the sample and the D6D16 internal standard is noted).

4.0 Sediment exposure

The sediment is thoroughly mixed manually in 5 or 10 L containers or cans (preferably aluminium). Pre-extracted silicone rubber sheets (spiked with PRCs, section **2**) are added to sub samples of sediment taken in different quantities to obtain varying weight (phase) ratios (g silicone rubber/ g sediment).
Note: Although any range of phase ratios between sediment and silicone rubber can be used to study the sediment-water exchange it is necessary to have a range of mixtures that result in limited to high depletion scenarios.

4.1 Weigh sediment suspension into 250 ml, 500 ml or 1 L bottles as appropriate, add sea or distilled water (note weight) to liquefy the sediment and obtain a suspension density of 0.16 g dry weight sediment/ g (wet weight of sediment + weight of added water) and then introduce pre-extracted silicone rubber of known weights to the bottles. See Table **1** below as a guide:
The bottles are labelled appropriately and exposures in duplicates.

Table **1**: Phase ratio variation

Wt of silicone rubber g	Wt of wet sediment g	Moisture content %	Wt of dry sediment g	Phase ratio (g SR/ g dw)
1.60	200	65	130	0.012
1.60	100	65	65	0.025
3.20	100	65	65	0.049
1.60	30	65	20	0.082
3.20	30	65	20	0.164

4.2 Shake horizontally at 200 rpm on an orbital shaker for 20 days

4.3 After exposure, remove the sheets from the bottles and rinse with distilled water. Wipe/ dry each sheet gently with a tissue to remove any water or moisture

4.4 Roll each sheet and Soxhlet extract with 100 ml *iso*-hexane: acetone ($3{:}1^{v}/_{v}$) for 6 h. Add 100 µl of deuterated PAH internal standard using a calibrated syringe to the extracts after allowing cooling to room temperature. The extracts are then treated as in section **3.6** to **3.8**.

4.5 The weight of the silicone rubber sheet is recorded after the extraction to avoid contamination from external sources. A procedural blank is included and subtracted from the results obtained. A spiked sheet is also analysed as a reference for the PRCs spiked

1. Introduction and Scope

This method describes the determination of chlorobiphenyls and organochlorine pesticides in sediments. The chlorobiphenyls organochlorine and pesticides which can be measured by this method are listed below:

CB31*, 28, 35*, 52, 53*, 49, 44, 74, 70, 97*, 99*,101, 110, 112*, 149, 118, 153, 132* 105, 137*, 138, 158, 157* 187, 128, 156, 180, 170, 183*, 189*, 194, 198* and 209.

The following CBs are analysed for recovery measurement only : CB35, 53*, 112*, 151*, 198* and 209.

HCB*, α-HCH, γ-HCH, Heptachlor*, α-Chlordene*, γ-Chlordene*, α-Chlordane*, γ-Chlordane, Aldrin*, Oxychlordane, Trans-Nonachlor, Heptachlor, Heptachlor Epoxide, Dieldrin, Endrin*, o,p'-DDE, p,p' DDE, o,p'-DDD, o,p'-DDT, p,p'-DDD and p,p'-DDT.

The range of this method of 0.002µg/g to 0.200µg/g in solution.

The method can be applied to a range of sediments, from "clean" offshore sediments to sediments from dredge spoil sites.

Clients can request that the results of individual determinands are summed together into groups to provide the following data:

ICES7 - sum of CB28, 52, 101, 118, 138, 153 and 180

Total CB* - sum of all CBs analysed, excluding the recovery CBs 35, 53, 112, 151, 198 and 209

Total o,p' DDE - sum of the o,p' DDE concentrations found in both the CB and OCP fractions

Total Heptachlor* - sum of the Heptachlor concentrations found in both the CB and OCP fractions

Sum CDANE* - sum of OCPs α-chlordene, γ-chlordene, Heptachlor Epoxide, Oxychlordane, α-chlordane, γ-chlordane, Trans Nonachlor, Total Heptachlor*

Sum DDT - sum of p,p' DDE, o,p' DDD, p,p' DDD, o,p' DDT, p,p' DDT, Total o,p' DDE

* Congeners not UKAS accredited.

2. Principle of the Method

The chlorobiphenyls and organochlorine pesticides are extracted from the sediments by Soxhlet extraction using a mixed polarity solvent, methyl tertiary butyl ether (MTBE). The samples are reconstituted in hexane and subjected to a clean-up procedure involving column chromatography on alumina and silica.

The cleaned up extract is reconstituted in a known weight of 2,2,4-trimethylpentane and analysed by gas chromatography using a HP-5 (non-polar) column or equivalent. The chromatograph is calibrated by a series of external standards and two internal standards, 2,4 dichlorobenzyl alkyl ethers (DCBE 6 and DCBE 16).

3. Reference Materials

Laboratory Reference Material, LRM 140 (sediment).

4. Reagents

See SOP's for reagents used.

5. Major Equipment

Gas chromatograph with on column injector and electron capture detector.
HP-5 column or equivalent - 60 m x 0.25 mm, film thickness 0.25 µm.
Chromatography data collection system.

6. Environmental Control

See individual SOP's.

7. Interferences

Not relevant.

8. Sampling and Sample Preparation

8.1 Samples must be logged into the laboratory according to SOP 60.

8.2 Sediments are prepared for extraction by freeze drying and grinding, SOPs 110 and 120. Record in sediment preparation Worksheet B62.

9. Analytical Procedure

9.1 Sample Extraction and Clean-up

9.1.1 A method blank and at least one appropriate reference material must be analysed per batch.

9.1.2 A weighed amount of recovery standard is added to the sample prior to extraction, SOP 345.

9.1.3 Extraction of chlorobiphenyls is carried out as detailed in SOP 365. The addition of activated copper powder, SOP 490 ensures that any sulphur is removed during the extraction.

9.1.5 The sample is prepared for clean-up using SOP 368 and cleaned up using alumina and silica, SOP 440.

9.1.6 After clean up, a weighed amount of the internal standard, DCBE 6 and 16 is added to each sample, SOP 350.

9.1.7 The sample is vialled for chromatographic analysis, SOP 590.

9.2 **Setting up and Testing of Gas Chromatograph**

Note: Varian refers to the Varian 3500 GC (Varian 4) and Perkin Elemer refers to the Autosystem and Clarus 500 GCs

9.2.1 Under normal circumstances the chromatograph will have been set up. If not, procedures for the set up of the GC-ECD are given in SOP 1020 (Perkin Elmer) / SOP 1000 (Varian).

9.2.2 If the gas chromatograph has analysed samples within the previous 24 hours, or is currently analysing samples, check that the standards meet the QC criteria SOP 1100.

9.2.3 If the QC criteria are met, samples can be analysed, following the method from 9.3.

9.2.4 If the QC criteria are not met, then maintenance may be required. The individual responsible for GC maintanance should be contacted. Maintenance procedures are outlined in SOP 980 (Perkin Elmer) / SOP 960 (Varian).

9.2.5 If 9.2.2 does not apply, then a solvent blank, CB 0.200µg/g and 0.200µg/g p,p DDT standards are analysed and checked against the QC criteria, SOP 1100.

9.2.6 Refer to 9.3.1 for autosampler loading.

9.2.7 If the QC criteria are met, samples can be analysed, following the method from 9.3.

9.2.8 If QC criteria are not met, see 9.2.4.

9.3 **Calibration and Quality Control**

9.3.1 Sample vials are loaded onto the autosampler carousel according to SOP 1060 (Perkin Elmer) / SOP 1040 (Varian). For instructions on the autosampler and running samples on the GCs, see the Users Manual.

9.3.2 A full set of chlorobiphenyl or organochlorine pesticide calibration standards (prepared previously in SOP 330) must be analysed with each batch of analysis or at least one full set for every 30 samples.

9.3.3 Check that the standards meet the QC criteria given for chlorobiphenyls in SOP 1100.

9.3.4 If the standards do not meet the required QC criteria, then maintenance may be required. The individual responsible for GC maintenance should be contacted. Maintenance procedures are detailed in SOPs 980 (Perkin Elmer) and 960 (Varian).

9.3.5 If QC criteria are met, proceed with calculation of results.

9.4 Entering Results, Test Report and Archiving of Data

9.4.1 Enter sample information and data into CB OCP quantification worksheets B568 as per SOP595.

9.4.2 Enter LRM data into relevant QC charts as per SOP595. When copying the calculated LRM data from the CB OCP Sediment Template to the control chart, delete the concentrations of the recovery CBs in the chart to leave an *. The recovery CBs only appear in the control chart as an aid for copying and pasting.

9.4.3 Produce Test Report and archive relevant batch paperwork and electronic copies of finalised spreadsheet and test report in NTS2/shared/Chem_Dat (SOP595 and SOP1350).

9.4.4 All chromatography data is archived and stored as stated in SOP905.

10. Calculation of Results

10.1 The results are calculated and the GC-ECD calibrated using the Chromatography Data System SOP 1242.

10.2 Results for the LRM should be compared with the appropriate Shewart Control Charts SOP 1380.

10.3 Method and solvent blanks are evaluated SOP 1230.

11. Precision, Accuracy and Practical Detection Limits

Precision and bias for the method are defined in SOP 1310.

Target Precision - 25%
Target Bias - 25%

Actual Precision - this is derived from the Shewart Charts and is expressed as the percentage standard deviation
Actual Bias- To be determined using a spiked sediment from Raasay Sound.

Detection Limits: To be determined according to SOP 1310. See Performance Data (15).

12. **Reports**

Test report and relevant documentation, SOP 1350 should be submitted to the technical manager for checking and issue.

13. **Safety**

Safety for all relevant procedures are provided in appropriate SOPs detailed above with reference to Procedure Risk Assessments.

14. **Literature References**

Not relevant.

15. **Performance Data**

For precision of the working range a low (10kg/g) and high (200ug/kg) were used.

Individual Limits of Detection (LOD) are calculated for each individual sample from the concentration of the lowest calibration solution i.e.

LOD = (2ug/kg x sample multiplier)/ Weight of Sample Extracted

Note: That the Perkin Elmer and Clarus 500 are not accredited. Refer to Section 1 for the list of accredited determinands.

Chlorobiphenyls in Sediment

	Varian 4 Precision % CV of Sediment n=9	Varian 4 % Recovery for Sediment n=9
HCB	12.00	57.06
CB31	5.88	82.91
CB28	5.14	83.05
Heptachlor	7.25	NA
CB52	10.69	123.85
CB49	5.90	93.31
CB44	15.71	95.86
CB74	5.36	98.02
CB70	6.96	108.73
o,p DDE	6.60 (comb.)	97.18 (comb.)
CB101	11.85	124.75
p,p DDE	4.09	102.02
CB110	10.09	111.75
CB149	6.66	107.51
CB118	8.72	113.73
CB153	5.50	110.34
CB105	7.66	90.58
CB138	7.25	108.63
CB158	7.53	99.13
CB187	4.68	100.34
CB128	9.56	84.23
CB156	9.87	99.57
CB157	7.48	97.05
CB180	3.58	102.23

CB170	4.52	95.06
CB189	9.47	96.96
CB194	4.21	98.96
CB209	9.02	101.66

Precision data from Batch 266 and Recovery Data from Batch 269

	Varian 4	
%CV at Lower End of Working Range n=7	%CV at Higher End of Working Range n=7	%CV at 1ug/kg n=8

HCB	10.87	9.26	2.87
CB 31	6.24	4.81	5.09
CB 28	8.01	4.52	2.19
CB53	4.68	5.97	NA
heptachlor	10.76	6.81	3.67
CB 52	6.65	4.70	5.97
CB 49	5.16	4.64	4.14
aldrin	5.62	5.09	NA
CB 35	9.68	4.91	NA
CB 44	8.71	5.49	2.03
CB 74	6.31	4.85	1.32
CB 70	7.18	4.07	4.46
opDDE	5.84	4.91	3.86
CB 101	6.49	2.82	3.88
CB 99	5.16	4.64	NA
CB 112	4.34	4.23	NA
CB 97	4.76	5.71	NA
ppDDE	4.20	5.12	2.71
CB 110	4.63	5.10	3.82
CB 151	7.61	4.07	NA
CB 149	4.38	3.64	3.96
CB 118	8.71	5.47	4.61
CB 153	12.20	9.47	3.46
CB 132	6.05	6.05	NA
CB 105	4.31	3.93	3.85
CB 137	5.57	3.86	NA
CB 138	6.56	4.28	3.97
CB 158	8.39	5.73	1.90
CB 187	17.96	15.25	2.78
CB 183	13.82	10.58	NA
CB 128	4.67	3.49	2.37
CB 156	10.43	7.75	2.38
CB 157	7.64	4.34	NA
CB 180	15.64	11.51	2.79
CB 198	12.76	10.70	NA
CB 170	8.20	6.78	2.60
CB 189	12.59	7.87	2.38
CB 194	11.31	9.77	2.15
CB 209	18.73	2.64	2.16

Precision data from Batch 2824

Organochlorine Pesticides in Sediment

	Varian 4 Precision %CV for	Varian 4 % Recovery for

	Sediment n=9	Sediment n=9
α-HCH	3.44	54.28
γ-HCH	2.14	66.15
Heptachlor	33.14 (combined)	NA
α-Chlordene	15.82	57.29
γ-Chlordene	NA	67.70
Heptachlor Epoxide	NA	75.31
Oxychlordane	NA	75.47
γ-Chlordane	6.32	81.08
o,p' DDE	7.78	88.47
α-Chlordane	5.51	83.11
Trans nonachlor	2.37	84.69
Dieldrin	2.35	88.88
o,p' DDD	1.67	92.39
Endrin	NA	94.84
p,p' DDD	3.83	103.37
o,p' DDT	8.37	78.21
p,p' DDT	5.83	94.80

Precision data from Batch 266 and Recovery Data from Batch 269

Varian 4

	%CV at Lower End of Working Range n=7	%CV at Higher End of Working Range n=7	%CV at 1ug/kg n=8
AHCH	5.33	10.08	2.16
GHCH	5.76	8.20	2.44
Hept	8.23	5.66	2.71
ACdene	4.10	6.18	2.24
GCdene	3.53	4.50	1.98
HeptEpox	4.12	6.06	2.77
Oxychlordane	3.87	4.53	2.98
GCdane	4.10	4.36	2.31
o,p' DDE	6.37	7.02	2.70
ACdane	5.60	4.23	2.65
Transnonachlor	3.50	3.34	1.95
Dieldrin	5.13	6.29	1.66
o,p' DDD	6.48	4.20	1.88
Endrin	9.05	9.35	1.87
p,p' DDD	10.27	4.29	2.32
o,p' DDT	14.38	9.70	2.52
p,p' DDT	23.83	15.20	2.46

Precision data from Batch 2824

Validation of Clarus 500 without PreVent for Chlorobiphenyls

	%CV at 0.01ug/g (Low Standard) n = 7	%CV at 0.2ug/g (High Standard) n = 7	%CV at 1ug/kg (LOD) n=7	%CV of Sediment LRM 140 n = 6
HCB	8.46	8.38	9.24	14.50
CB 31	7.38	8.34	13.16	10.80
CB 28	6.78	6.77	13.17	16.23
CB53	8.28	8.57	4.41	14.55
Heptachlor	10.13	7.50	5.84	NA
CB 52	7.88	7.59	6.43	12.93
CB 49	9.60	7.54	4.70	9.91
Aldrin	10.80	6.92	6.53	NA
CB 35	11.94	6.43	27.27	17.76
CB 44	8.41	8.70	6.22	16.38
CB 74	5.43	6.87	12.11	9.64
CB 70	6.29	6.34	12.23	11.26
o,p' DDE	9.27	8.28	8.63	18.00
CB 101	7.69	7.22	4.57	10.55
CB 99	9.02	7.18	4.71	8.51
CB 112	9.08	7.77	5.67	5.16
CB 97	9.21	7.50	2.80	8.56
p,p' DDE	8.67	7.62	2.99	8.43
CB 110	8.48	7.34	2.73	11.66
CB 151	10.05	7.71	2.68	6.77
CB 149	9.89	7.73	6.39	10.41
CB 118	5.16	7.08	8.20	10.07
CB 153	8.69	7.09	3.04	6.35
CB 132	8.57	8.62	3.90	8.88
CB 105	6.07	7.02	5.14	10.97
CB 137	8.45	5.92	3.01	9.58
CB 138	8.48	7.26	4.17	8.43
CB 158	8.26	7.18	3.15	8.25
CB 187	10.02	7.73	6.00	7.02
CB 183	10.44	7.83	7.02	7.77
CB 128	8.13	6.36	4.53	12.84
CB 156	7.10	6.46	2.47	8.23
CB 157	7.55	6.91	2.56	6.84
CB 180	9.58	6.32	6.04	6.46
CB 198	9.67	7.40	5.83	3.81
CB 170	9.94	5.92	6.66	7.15
CB 189	9.66	7.38	3.08	10.91
CB 194	10.60	6.95	4.42	5.89
CB 209	11.55	10.20	6.27	8.22

Data from Batch 2828

Validation of Clarus 500 without PreVent for Organochlorine Pesticides

	%CV at 0.01ug/g (Low Standard) n = 7	%CV at 0.2ug/g (High Standard) n = 7	%CV at 1ug/kg (LOD) n=7	%CV of Sediment LRM 140 n = 6
alpha HCH	7.47	9.45	5.51	
gamma HCH	6.72	11.95	6.00	
Heptachlor	9.92	9.59	4.86	
alpha Chlordene	7.50	7.85	6.01	
gamma Chlordene	7.70	8.94	4.95	
Heptachlor Epoxide	9.59	14.01	5.09	
Oxychlordane	10.12	8.17	4.43	
gamma Chlordane	10.25	9.56	6.10	
o,p' DDE	8.43	9.03	5.55	
alpha Chlordane	9.71	9.23	5.54	
Transnonachlor	8.71	8.80	6.49	
Dieldrin	7.79	7.66	5.60	
o,p' DDD	4.83	6.83	7.76	
Endrin	7.02	9.02	5.53	
p,p' DDD	4.72	5.92	11.01	
o,p' DDT	8.51	8.31	4.63	
p,p' DDT	6.79	8.91	5.08	

Data from Batch 2828

Validation of Clarus 500 in PreVent Mode for Chlorobiphenyls

	%CV at 0.01ug/g (Low Standard) n = 7	%CV at 0.2ug/g (High Standard) n = 7	%CV at 1ug/kg (LOD) n=7	%CV of Sediment LRM 140 n = 6
HCB	5.90	4.43	4.70	10.51
CB 31	6.08	3.93	8.79	11.07
CB 28	7.04	4.54	3.88	8.08
CB53	7.96	5.77	4.14	16.07
Heptachlor	6.30	8.60	4.44	NA
CB 52	8.43	9.09	5.42	8.84
CB 49	7.21	9.77	5.29	13.17
Aldrin	6.51	6.58	6.00	NA
CB 35	8.60	8.35	6.40	17.64
CB 44	7.41	9.12	7.03	19.18
CB 74	6.01	4.20	5.64	10.82
CB 70	6.99	3.98	4.15	5.00
o,p' DDE	5.96	5.26	4.09	32.12
CB 101	8.53	7.23	3.94	9.41
CB 99	7.31	13.11	5.41	12.75
CB 112	5.38	9.42	5.36	9.76
CB 97	6.58	7.66	2.34	10.38
p,p' DDE	9.52	5.80	4.11	9.83
CB 110	7.18	6.09	5.22	9.51
CB 151	5.34	5.98	4.55	6.02
CB 149	6.85	6.50	3.74	8.57
CB 118	6.99	5.06	3.25	6.40
CB 153	6.52	4.72	3.60	7.17
CB 132	6.20	6.34	3.60	13.03
CB 105	6.80	4.24	4.35	15.45
CB 137	6.59	4.82	3.94	8.48
CB 138	6.42	4.25	5.42	6.72
CB 158	6.66	3.70	4.20	10.02
CB 187	5.58	5.62	4.25	7.54
CB 183	5.47	2.96	4.63	11.22
CB 128	6.70	4.23	5.45	17.35
CB 156	6.75	5.31	4.93	8.03
CB 157	7.22	4.10	4.63	9.14
CB 180	7.04	3.63	6.30	7.52
CB 198	3.82	11.43	2.97	6.79
CB 170	6.55	4.65	3.98	10.09
CB 189	5.74	4.13	3.73	15.63
CB 194	5.91	7.09	4.96	11.34
CB 209	5.49	7.65	4.62	5.96

Data from Batch 2823

Validation of Clarus 500 in PreVent Mode for Organochlorine Pesticides

	%CV at 0.01ug/g	%CV at 0.2ug/g	%CV at 1ug/kg	%CV of Sediment

	(Low Standard) n = 7	(High Standard) n = 7	(LOD) n=7	LRM 140 n = 6
alpha HCH	8.18	7.54	3.82	
gamma HCH	10.22	8.60	4.97	
Heptachlor	11.83	11.63	8.15	
alpha Chlordene	9.30	7.52	6.10	
gamma Chlordene	7.41	7.12	4.64	
Heptachlor Epoxide	11.12	10.99	6.98	
Oxychlordane	11.45	9.65	8.11	
gamma Chlordane	8.70	8.98	4.72	
o,p' DDE	9.01	9.13	5.16	
alpha Chlordane	8.26	8.31	5.27	
Transnonachlor	9.65	9.83	6.57	
Dieldrin	12.56	10.96	7.63	
o,p' DDD	9.91	8.04	4.68	
Endrin	12.09	16.08	6.98	
p,p' DDD	14.02	8.95	9.62	
o,p' DDT	12.62	10.42	5.50	
p,p' DDT	13.67	10.46	8.30	

Data from Batch 2823

16. Uncertainty

Main Steps of Method:

Sediment sample is received frozen, this is freeze-dried, sieved and ground prior to sub sampling. Recovery standard is added to the sample, prior to extraction with solvent. Extract is cleaned by adsorption chromatography. Internal standard is added to resultant CB and OCP fractions. These fractions along with calibration standards are analysed by Gas Chromatography. Software calculates the concentrations of each determinand according to the calibration curves, and blank corrections are performed using an Excel spreadsheet. Method is monitored by the use of a Laboratory Reference Material. The instrument is checked using the response of the 0.2ug/g calibration standard and a standard checking for determinand breakdown. The laboratory participates in the QUASIMEME laboratory intercomparison trials for these determinands.

Sources of Uncertainty:

- **Sampling:**
  Samples are analysed and results reported on the samples as received – out with uncertainty calculations

- **Sub-sampling:**
  Sediment sample is ground and mixed prior to sub-sampling and considered homogenous so that sub-sampling does not contribute to the error – negligible contribution to uncertainty

- **Storage Conditions:**
  Samples are stored in solvent washed aluminum cans to minimise contamination. Prior to freeze drying samples are stored at -20°C – negligible contribution to uncertainty

- **Reagent Purity:**
  Solvents are of at least HPLC grade quality – uncertainty accounted for in the validation data
  Sodium Sulphate are of at least Analar quality - uncertainty accounted for in the validation data
  Adsorbents are of unknown purity – uncertainty accounted for in the blank correction
  Chemical standards used in the preparation of calibration solutions are of the highest purity available at the time of purchase. Final concentrations of the calibration solutions have not been corrected for purity - uncertainty accounted for in the validation data

- **Instrument Effects:**
  Weight: Balance check weight tolerances 0.05% and 0.002%, 2, 3 and 4 decimal places used, sufficient for accuracy required - uncertainty accounted for in the validation data
  Volume: pipettes are calibrated to within required limits, tolerances stated in SOPs for measuring cylinders are sufficient for purpose - uncertainty accounted for in the validation data
  Temperature: settings of Turbovaps calibrated against calibrated thermometers, variations accounted for by control chart data - uncertainty accounted for in the validation data
  Gas Chromatographs: quality control criteria set out in SOPs determine when maintenance is required. Between sample and calibration variations are monitored by quality control charts - uncertainty accounted for in the validation data

- **Environmental Conditions:**
  Contamination is minimised by the use of dedicated accommodation, equipment and glassware for organic analysis. Glassware is also separated during cleaning - uncertainty accounted for in the validation data

- **Operator Effects:**
  All measurement methods are described in fully documented Standard Operating Procedures (SOPs) to limit inconsistencies between operators - uncertainty accounted for in validation data

- **Matrix Effects:**
  Final sample fractions to be analysed are in 2,2,4 trimethylpentane as are the calibration standards - uncertainty accounted for in validation data

- **Computation Errors:**
  Concentrations are calculated by chromatography software. Manual check of calculation has been carried out and acceptable – negligible contribution to uncertainty

- **Blank Correction:**
  Method blank run alongside each batch of samples and is subtracted from each sample in the batch by use of an Excel spreadsheet. Manual check of calculation has been carried out and acceptable - uncertainty accounted for in the validation data

- **Random Effects:**
  Uncertainty accounted for by validation and control chart data

Summary Validation Data

	% Recovery of Spiked Sample	Precision of Sediment Sample	Low Standard 50ug/kg	High Standard 450ug/kg	Mean	SD	Variance from Shewart Chart %
HCB	57.06	12.00	1.86	1.91	0.20	0.04	21.50
CB 31	82.91	5.88	1.67	2.72	1.88	0.52	27.66
CB 28	83.05	5.14	0.93	3.10	2.52	0.60	23.81
HEPTACHLOR		7.25	1.10	2.30	0.19	0.04	18.42
CB 52	123.85	10.69	1.36	2.38	3.83	1.01	26.37
CB 49	93.31	5.90	2.42	3.60	1.67	0.38	22.75
CB 44	95.86	15.71	1.27	2.40	2.03	0.64	31.53
CB 74	98.02	5.36	2.33	2.73	1.64	0.21	12.80
CB 70	108.73	6.96	2.42	3.22	3.53	0.54	15.30
o,p'-DDE	97.18	6.60	1.35	2.23	0.48	0.17	35.42
CB 101	124.75	11.85	2.11	3.09	5.05	1.09	21.58
p,p'-DDE	102.02	4.09	1.43	1.65	2.57	0.61	23.74
CB 110	111.75	10.09	1.16	2.29	4.64	0.79	17.03
CB 149	107.51	6.66	1.88	1.94	5.34	0.56	10.49
CB 118	113.73	8.72	2.29	2.47	4.41	0.63	14.29
CB 153	110.34	5.50	1.05	2.66	7.33	0.65	8.87
CB 105	90.58	7.66	2.00	1.93	1.82	0.18	9.89
CB 138	108.63	7.25	1.01	2.29	6.75	0.66	9.78
CB 158	99.13	7.53	1.81	2.02	0.68	0.08	12.21
CB 187	100.34	4.68	1.88	2.90	3.99	0.29	7.27
CB 128	84.23	9.56	1.99	2.36	1.01	0.19	18.81
CB 156	99.57	9.87	1.90	2.56	0.68	0.09	13.53
CB 157	97.05	7.48	1.49	2.04	0.30	0.04	13.33
CB 180	102.23	3.58	1.66	3.49	5.91	0.27	4.57
CB 170	95.06	4.52	1.55	2.00	2.46	0.17	6.91
CB 189	96.96	9.47	2.54	2.44	0.06	0.02	29.51
CB 194	98.96	4.21	1.92	2.23	1.83	0.12	6.56
CB 209	101.66	9.02	1.66	2.20	3.46	0.87	25.14

	% Recovery of Spiked Sample	Precision of Sediment Sample	Low Standard 50ug/kg	High Standard 450ug/kg	Mean	SD	Variance from Shewart Chart %
A HCH	57.22	6.74	2.10	1.98	0.13	0.03	20.00
G-HCH	71.22	8.28	3.44	0.81	0.43	0.07	16.28
HEPTACHLOR		7.25	1.55	1.50			
A-CHLORDENE	66.35	47.04	2.13	1.65	0.29	0.13	44.83
G-CHLORDENE	76.39		2.37	1.50	0.19	0.18	94.74
HEPT.EPOXIDE	89.03	9.04	2.54	1.51	0.08	0.02	25.64
OXYCHLORDANE	86.34	7.74	1.80	0.91	0.23	0.05	20.43
G-CHLORDANE	97.13	12.68	2.28	1.94	0.15	0.04	25.33
o,p' DDE	97.18	6.60	2.54	1.65	0.23	0.12	52.17
A-CHLORDANE	99.87	10.83	2.70	1.42	0.33	0.14	42.42
T-NONACHLOR	102.71	7.22	2.17	1.64	0.24	0.06	25.83
DIELDRIN	103.79	5.19	2.40	1.51	0.61	0.83	136.07
o,p'-DDD	108.81	6.55	1.36	1.70	2.16	0.40	18.52
ENDRIN	99.01	12.51	2.61	1.83	2.10	1.07	50.95
p,p'-DDD	116.97	4.24	1.79	0.94	7.99	1.05	13.14
o,p'-DDT	90.52	8.49	2.45	1.59	0.19	0.09	47.37
p,p'-DDT	108.20	9.26	1.02	1.52	0.51	0.83	162.75

Uncertainty

Systematic Component (Recovery of Spiked Sample, Perkin Elmer) : e.g. CB153 = 10.34/2%
Random Component (Shewart Chart S.D.): e.g. CB153 = (S.D.)0.65/(mean)7.33*100= 8.87%

Assume linear summation and a value of K=2

Combined Standard Uncertainty **= (**$C_s^2 + C_r^2)^{0.5}$ = X%

Expanded Uncertainty = 2 * $C_s^2 + C_r^2)^{0.5}$ = X%

Expanded Uncertainty = e.g. CB153 = 2*$(5.17^2 + 8.87^2)^{0.5}$ ug/kg = 20.53%

The reported expanded uncertainties are based on uncertainties multiplied by a coverage factor of k=2, providing a level of confidence of approximately 95%

	Systematic Component %	Random Component %	Expanded Uncertainty %
HCB	21.47	21.50	60.77
CB 31	8.55	27.66	57.90
CB 28	8.48	23.81	50.55
HEPTACHLOR	50.00	18.42	106.57
CB 52	-11.93	26.37	57.88
CB 49	3.35	22.75	46.00
CB 44	2.07	31.53	63.19
CB 74	0.99	12.80	25.69
CB 70	-4.37	15.30	31.82
o,p'-DDE	1.41	35.42	70.89
CB 101	-12.38	21.58	49.76
p,p'-DDE	-1.01	23.74	47.51
CB 110	-5.88	17.03	36.02
CB 149	-3.76	10.49	22.28
CB 118	-6.87	14.29	31.70
CB 153	-5.17	8.87	20.53
CB 105	4.71	9.89	21.91
CB 138	-4.32	9.78	21.38
CB 158	0.44	12.21	24.43
CB 187	-0.17	7.27	14.54
CB 128	7.89	18.81	40.80
CB 156	0.22	13.53	27.06
CB 157	1.48	13.33	26.83
CB 180	-1.12	4.57	9.41
CB 170	2.47	6.91	14.68
CB 189	1.52	29.51	59.09
CB 194	0.52	6.56	13.16
CB 209	-0.83	25.14	50.32
A HCH	21.39	20.00	58.57
G-HCH	14.39	16.28	43.45
HEPTACHLOR	50.00		
A-CHLORDENE	16.83	44.83	95.76
G-CHLORDENE	11.81	94.74	190.94
HEPT.EPOXIDE	5.49	25.64	52.44
OXYCHLORDANE	6.83	20.43	43.09
G-CHLORDANE	1.44	25.33	50.75
o,p' DDE	1.41	52.17	104.39
A-CHLORDANE	0.06	42.42	84.85
T-NONACHLOR	-1.36	25.83	51.74
DIELDRIN	-1.90	136.07	272.16
o,p'-DDD	-4.41	18.52	38.07
ENDRIN	0.49	50.95	101.91
p,p'-DDD	-8.49	13.14	31.29
o,p'-DDT	4.74	47.37	95.21
p,p'-DDT	-4.10	162.75	325.59

1. Introduction and Scope

This method describes the determination of chlorobiphenyls and organochlorine pesticides in biota. The following determinands can be measured:

CB31*, CB28, CB35*, CB52, CB53*, CB44, CB49, CB70, CB74, CB97*, CB99*, CB101, CB 110, CB112*, CB149, CB118, CB153, CB132*, CB105, CB137*, CB138, CB158, CB187, CB128, CB156, CB157*, CB180, CB183*, CB170, CB189*, CB194, CB198* and CB209.

The following CBs are analysed for recovery measurement only :
CB35*, 53*, 112*, 151*, 198* and 209

HCB*, α-HCH, γ-HCH, α-Chlordene*, γ-Chlordene*, α-Chlordane, γ-Chlordane, Aldrin*, Oxychlordane, Trans-Nonachlor, Heptachlor*, Heptachlor Epoxide, Dieldrin, Endrin, o,p'-DDE, p,p' DDE, o,p'-DDD, o,p'-DDT, p,p'-DDD and p,p'-DDT.

The range of this method is 0.002µg/g to 0.200µg/g in solution.

Clients can request that the results of individual congeners are summed together into groups to provide the following data:

ICES7 - sum of CB28, 52, 101, 118, 138, 153 and 180

Total CB* - sum of all CBs analysed, excluding the recovery CBs 35, 53, 112, 151, 198 and 209

Total o,p' DDE - sum of the o,p' DDE concentrations found in both the CB and OCP fractions

Total Heptachlor* - sum of the Heptachlor concentrations found in both the CB and OCP fractions

Sum CDANE* - sum of OCPs a-chlordene, g-chlordene, Heptachlor Epoxide, Oxychlordane, g-chlordane, a-chlordane, Trans Nonachlor, Total Heptachlor*

Sum DDT - sum of p,p' DDE, o,p' DDD, p,p' DDD, o,p' DDT, p,p' DDT, Total o,p' DDE

* Congeners not UKAS accredited.

2. Principle

The chlorobiphenyls and organochlorine pesticides are extracted from the tissue by Soxhlet extraction using a mixed polarity solvent, methyl t-butyl ether (MTBE). Samples are reconstituted in hexane and subjected to a clean-up procedure involving column chromatography on alumina and silica.

The cleaned-up extract is reconstituted in a known weight of 2,2,4 trimethylpentane and analysed by gas chromatography using a HP-5 (non polar) column or equivalent. The gas chromatograph is calibrated by a series of eight external chorobiphenyl standards that include two internal standards, 2,4 dichlorobenzyl alkyl ethers (DCBE 6 and DCBE 16).

3. Reference Materials

LRM 110, contaminated cod liver oil, contains all determinands.

LRM 134, a 'blank' fish oil suitable for recovery experiments using this method.

4. Reagents

See SOP's for reagents used.

5. Major Equipment

Gas chromatograph with on column injector and electron capture detector.
Chromatography data collection system.
HP-5 column or equivalent, 60 m x 0.25 mm, film thickness 0.25 µm.

6. Environmental Control

See individual SOPs.

7. Interferences

Samples are stored either wrapped in aluminium foil, before storing in a labelled plastic bag or a labelled aluminium can.

8. Sample Preparation

8.1 Samples are logged into the laboratory according to SOP 60.

8.2 Biota samples are sub-sampled and homogenised as in SOP 130.

8.3 Biota samples are ground with anhydrous sodium sulphate, to dehydrate the sample prior to solvent extraction, SOP 150.

9. Analytical Procedure

9.1 Sample Extraction and Clean-Up

9.1.1 A method blank and at least one appropriate LRM is analysed per batch.

9.1.2 A weighed amount of recovery standard is added to the sample prior to extraction, as in SOP 345.

9.1.3 The extraction of chlorobiphenyls in biota is carried out as detailed in SOP 360.

9.1.4 A lipid determination is carried out on each extract, M880.

9.1.5 The extracts are prepared for clean-up, SOP 368.

9.1.6 The extracts are cleaned up using alumina and silica column chromatography, SOP 440.

9.1.7 A weighed amount of the internal standard, DCBE 6 and 16 is added to each sample, SOP 350.

9.1.8 Sample is vialled for chromatographic analysis, SOP 590.

9.2 **Setting Up and Testing of Gas Chromatograph**

Note: Varian refers to the Varian 3500 GC (Varian 4) and Perkin Elemer refers to the Autosystem and Clarus 500 GCs

9.2.1 In normal circumstances the gas chromatograph will be set up. If not, the procedures for set up is given in SOP 1020 (Perkin Elmer), SOP 1000 (Varian), and SOP 1280 (HP6890).

9.2.2 If the gas chromatograph has analysed samples within the previous 24 hours, or is currently analysing samples, check that the standards meet the QC criteria, SOP 1100 (Perkin Elmer and Varian) and SOP 1285 (HP6890).

9.2.3 If the QC criteria are met, samples can be analysed, following the method from 9.3.

9.2.4 If the QC criteria are not met, then maintenance may be required. The individual responsible for GC maintenance should be contacted. Maintenance procedures are outlined in SOP 980 (Perkin Elmer), SOP 960 (Varian) and SOP 1275 (HP6890).

9.2.5 If 9.2.2 does not apply, then a solvent blank, CB 0.200µg/g and 0.200µg/g p,p' DDT standards are analysed and checked against the QC criteria, SOP 1100 (Perkin Elmer and Varian) and SOP 1285 (HP6890).

9.2.6 Refer to 9.3.1 for autosampler loading.

9.2.7 If the QC criteria are met, samples can be analysed, following the method from 9.3.1.

9.2.8 If QC criteria are not met, see 9.2.4.

9.3 **Calibration and Quality Control**

9.3.1 Sample vials are loaded onto the autosampler carousel SOP 1060 (Perkin-Elmer), SOP 1040 (Varian GCs) and SOP 1280 (HP6890). Refer to GC Users Manual, for instructions on the autosampler and running samples.

9.3.2 A full set of chlorobiphenyl or organochlorine pesticide calibration standards,

prepared previously using SOPs 290, 320 and 330 are run first followed by a solvent blank. A quality control standard (in order to check the calibration calculations), the method blank, the reference material and the samples are run along with 200ug/kg p,pDDT (DDT breakdown).

9.3.3 Check that the standards meet the QC criteria, SOP 1100 (Perkin-Elmer and Varian GCs) and SOP 1285 (HP6890).

9.3.4 If the QC criteria are not met, then maintenance may be required. The individual responsible for GC maintenance should be contacted. Maintenance procedures are outlined in SOP 980 (Perkin-Elmer), SOP 960 (Varian GCs) and SOP 1275 (HP6890).

9.3.5 If QC criteria are met, proceed with calculation of results.

9.4 **Entering of Results, Test Report and Archive of Data**

9.4.1 Enter sample information and data into CB OCP quantification worksheets B569 as per SOP595.

9.4.2 Enter LRM data into relevant QC charts as per SOP595. When copying the calculated LRM data from the CB OCP Biota Template to the control chart, delete the concentrations of the recovery CBs in the chart to leave an *. The recovery CBs only appear in the control chart as an aid for copying and pasting.

9.4.3 Produce Test Report and archive relevant batch paperwork and electronic copies of finalised spreadsheet and test report in NTS2/shared/Chem_Dat (SOP595 and SOP1350).

9.4.4 All TotalChrom data is archived and stored as stated in SOP905.

10. **Calculation of Results**

10.1 The GC-ECD is calibrated and results calculated using the Chromatography data system, SOP 1242.

10.2 Results for the LRM should be compared with the appropriate Shewart Control Chart data SOP 1380.

10.3 Evaluate method and solvent blanks, SOP 1230.

11 **Precision, Accuracy and Practical Detection Limits**

11.1 Precision and bias for the method are defined in SOP 1310.

Target Precision - 25%
Target Bias - 25%

11.2 Actual Precision - derived from the Shewart charts and is expressed as the percentage standard deviation.

Actual Bias - to be calculated.

11.3 Detection Limit - to be determined according to SOP 1310. See Performance Data (15).

12. **Reports**

Test report and relevant documentation, SOP 1350 should be submitted to the technical manager for checking and issue.

13. **Safety**

Safety for all relevant procedures are provided in appropriate SOP's detailed above with reference to Procedure Risk Assessments.

14. **Literature References**

Not relevant.

15. **Performance Data**

For precision of the working range a low (10kg/g) and high (200ug/kg) were used.

Individual Limits of Detection (LOD) are calculated for each individual sample i.e.

LOD = (2ug/kg x sample multiplier)/Weight of Sample Extracted

Note: That the Perkin Elmer and Clarus 500 are not accredited. Refer to Section 1 for the list of accredited determinands.

Chlorobiphenlys in biota

	Varian 4	
	Precision* % of CV of Biota n=10	**% Recovery for Biota n=6/7**

HCB	15.85	23.7
CB31	34.24	35.6
CB28	20.56	39.0
Heptachlor	N/A	51.3
CB52	22.45	41.2
CB49	22.16	43.9
CB44	23.17	39.8
CB74	14.78	56.3
CB70	22.10	54.2
o,p DDE	7.0	62.5
CB101	10.17	59.4
p,p DDE	7.35	67.0
CB110	8.42	61.3
CB149	9.83	68.1
CB118	7.58	73.9
CB153	8.94	73.3
CB105	10.11	73.8
CB138	7.90	78.8
CB158	8.03	77.5
CB187	9.31	78.5
CB128	10.76	81.5
CB156	11.55	82.3
CB157	19.30	80.1
CB180	8.68	87.5
CB170	7.18	82.9
CB189	25.36	86.8
CB194	9.64	86.8

- Precision based on last 10 results for LRM 110 (fish oil).
- Precision and recovery data from Batch 648

	Varian 4		
	%CV at Lower End of Working Range n=7	%CV at Higher End of Working Range n=7	%CV at 1ug/kg n=8
HCB	10.87	9.26	2.87
CB 31	6.24	4.81	5.09
CB 28	8.01	4.52	2.19
CB53	4.68	5.97	NA
heptachlor	10.76	6.81	3.67
CB 52	6.65	4.70	5.97
CB 49	5.16	4.64	4.14
aldrin	5.62	5.09	NA
*CB 35	9.68	4.91	NA
CB 44	8.71	5.49	2.03
CB 74	6.31	4.85	1.32
CB 70	7.18	4.07	4.46
opDDE	5.84	4.91	3.86
CB 101	6.49	2.82	3.88
CB 99	5.16	4.64	NA
CB 112	4.34	4.23	NA
CB 97	4.76	5.71	NA
ppDDE	4.20	5.12	2.71
CB 110	4.63	5.10	3.82
CB 151	7.61	4.07	NA

CB 149	4.38	3.64	3.96
CB 118	8.71	5.47	4.61
CB 153	12.20	9.47	3.46
CB 132	6.05	6.05	NA
CB 105	4.31	3.93	3.85
CB 137	5.57	3.86	NA
CB 138	6.56	4.28	3.97
CB 158	8.39	5.73	1.90
CB 187	17.96	15.25	2.78
CB 183	13.82	10.58	NA
CB 128	4.67	3.49	2.37
CB 156	10.43	7.75	2.38
CB 157	7.64	4.34	NA
CB 180	15.64	11.51	2.79
CB 198	12.76	10.70	NA
CB 170	8.20	6.78	2.60
CB 189	12.59	7.87	2.38
CB 194	11.31	9.77	2.15
CB 209	18.73	2.64	2.16

Organochlorine Pesticides in biota

	Varian 4	
	Precision %CV of Biota n=	**% Recovery for Biota n=8**
α-HCH	N/A	43.87
γ-HCH	9.4	51.81
Heptachlor	N/A	N/A
α-Chlordene	N/A	31.49
γ-Chlordene	N/A	46.60
Heptachlor Epoxide	N/A	67.19
Oxychlordane	8.9	64.54
γ-Chlordane	N/A	67.89
o,p' DDE	12.8	60.4
α-Chlordane	6.5	79.86
Trans nonachlor	6.1	79.09

Dieldrin	7.8	76.84
o,p' DDD	N/A	87.92
Endrin	8.3	105.06
p,p' DDD	6.9	104.57
o,p' DDT	8.2	49.80
p,p' DDT	6.2	112.19

- Precision based on last 10 results for LRM 110 (fish oil)
- Recovery data calculated from Batch 277

	Varian 4		
	%CV at Lower End of Working Range n=7	%CV at Higher End of Working Range n=7	%CV at 1ug/kg n=8
AHCH	5.33	10.08	2.16
GHCH	5.76	8.20	2.44
Hept	8.23	5.66	2.71
ACdene	4.10	6.18	2.24
GCdene	3.53	4.50	1.98
HeptEpox	4.12	6.06	2.77
Oxychlordane	3.87	4.53	2.98
GCdane	4.10	4.36	2.31
o,p' DDE	6.37	7.02	2.70
ACdane	5.60	4.23	2.65
Transnonachlor	3.50	3.34	1.95
Dieldrin	5.13	6.29	1.66
o,p' DDD	6.48	4.20	1.88
Endrin	9.05	9.35	1.87
p,p' DDD	10.27	4.29	2.32
o,p' DDT	14.38	9.70	2.52
p,p' DDT	23.83	15.20	2.46

HP6890

Congeners	% Recovery	Precision	% CV at 10%	% CV at 90%	% CV at
		%CV of Biota	Working Range	Working Range	1ug/kg
	n = 8	n=7	n=8	n=8	n=8
Chlorobiphenyls					
HCB	31.1	17.27	2.13	2.59	N/A
CB 31	41.2	7.34	3.65	3.34	N/A
CB 28	44.3	6.43	8.74	3.73	N/A
HEPTACHLOR	51.1	13.53	8.6	3.73	N/A
CB 52	45.7	5.98	2.38	2.15	N/A
CB 49	42.8	35.61	1.63	2.55	N/A
CB 44	46.8	6.55	2.77	2.45	N/A
CB 74	61.0	4.03	4.76	2.32	N/A
CB 70	62.0	5.09	9.32	2.79	N/A
o,p-DDE	88.9	11.85	2.61	3.28	N/A
CB 101	64.9	6.07	3.22	2.12	N/A
p,p'-DDE	73.6	4.22	2.50	3.27	N/A
CB 110	74.5	4.62	2.12	2.66	N/A
CB 149	75.6	3.95	2.99	2.35	N/A
CB 118	75.6	3.12	4.63	2.34	N/A
CB 153	80.3	3.28	2.51	2.79	N/A
CB 105	81.5	3.73	2.95	2.76	N/A
CB 138	86.7	2.85	2.31	2.42	N/A
CB 158	85.5	3.14	1.64	2.43	N/A
CB 187	85.8	1.84	2.67	2.46	N/A
CB 128	78.8	5.05	1.08	3.21	N/A
CB 156	95.2	2.51	3.28	2.04	N/A
CB 157	86.8	2.31	2.02	3.94	N/A
CB 180	92.6	1.96	0.92	2.87	N/A
CB 170	88.6	2.60	3.31	2.22	N/A
CB 189	94.1	2.74	2.22	3.29	N/A
CB 194	88.9	1.96	2.31	3.43	N/A
CB 209	91.0	1.71	1.55	2.72	N/A
Organochlorine Pesticides					
A HCH	49.5	13.64	N/A	N/A	N/A
G-HCH	61.2	9.50	N/A	N/A	N/A
HEPTACHLOR	35.0	24.97	N/A	N/A	N/A
A-CHLORDENE	37.5	12.45	N/A	N/A	N/A

G-CHLORDENE	50.4	6.90	N/A	N/A	N/A
HEPT.EPOXIDE	71.7	6.04	N/A	N/A	N/A
OXYCHLORDANE	66.6	6.57	N/A	N/A	N/A
G-CHLORDANE	68.2	5.56	N/A	N/A	N/A
0,P DDE	43.7	23.8	N/A	N/A	N/A
A-CHLORDANE	66.6	6.34	N/A	N/A	N/A
T-NONACHLOR	68.2	6.25	N/A	N/A	N/A
DIELDRIN	82.8	5.17	N/A	N/A	N/A
O,P-DDD	94.0	4.31	N/A	N/A	N/A
ENDRIN	103.0	3.91	N/A	N/A	N/A
P,P'-DDD	101.4	3.80	N/A	N/A	N/A
O,P-DDT	59.2	8.53	N/A	N/A	N/A
P,P'-DDT	67.9	2.62	N/A	N/A	N/A

* Precision and recovery data from Batch 648

Validation of Clarus 500 without PreVent for Chlorobiphenyls

	%CV at 0.01ug/g (Low Standard) n = 7	%CV at 0.2ug/g (High Standard) n = 7	%CV at 1ug/kg (LOD) n=7	%CV of Biota LRM 110 n = 6
HCB	8.46	8.38	9.24	15.99
CB 31	7.38	8.34	13.16	17.72
CB 28	6.78	6.77	13.17	11.81
CB53	8.28	8.57	4.41	12.79
***Heptachlor**	10.13	7.50	5.84	NA
CB 52	7.88	7.59	6.43	7.94
CB 49	9.60	7.54	4.70	9.26
Aldrin	10.80	6.92	6.53	NA
CB 35	11.94	6.43	27.27	14.83
CB 44	8.41	8.70	6.22	13.40
CB 74	5.43	6.87	12.11	8.15
CB 70	6.29	6.34	12.23	6.45
o,p' DDE	9.27	8.28	8.63	22.05
CB 101	7.69	7.22	4.57	5.02
CB 99	9.02	7.18	4.71	3.56
CB 112	9.08	7.77	5.67	2.91
CB 97	9.21	7.50	2.80	2.61
p,p' DDE	8.67	7.62	2.99	6.36
CB 110	8.48	7.34	2.73	5.47
CB 151	10.05	7.71	2.68	2.93
CB 149	9.89	7.73	6.39	3.82
CB 118	5.16	7.08	8.20	3.22
CB 153	8.69	7.09	3.04	5.30
CB 132	8.57	8.62	3.90	5.75
CB 105	6.07	7.02	5.14	6.74
CB 137	8.45	5.92	3.01	5.80
CB 138	8.48	7.26	4.17	3.79
CB 158	8.26	7.18	3.15	4.65
CB 187	10.02	7.73	6.00	5.47
CB 183	10.44	7.83	7.02	4.16
CB 128	8.13	6.36	4.53	9.45
CB 156	7.10	6.46	2.47	2.73
CB 157	7.55	6.91	2.56	4.12
CB 180	9.58	6.32	6.04	4.33
CB 198	9.67	7.40	5.83	4.57
CB 170	9.94	5.92	6.66	5.82
CB 189	9.66	7.38	3.08	7.48
CB 194	10.60	6.95	4.42	5.86
CB 209	11.55	10.20	6.27	3.05

Data from Batch 2828

Validation of Clarus 500 without PreVent for Organochlorine Pesticides

	%CV at 0.01ug/g (Low Standard) n = 7	%CV at 0.2ug/g (High Standard) n = 7	%CV at 1ug/kg (LOD) n=7	%CV of Biota LRM 110 n = 6

alpha HCH	7.47	9.45	5.51	15.38
gamma HCH	6.72	11.95	6.00	11.82
Heptachlor	9.92	9.59	4.86	12.54
alpha Chlordene	7.50	7.85	6.01	17.17
gamma Chlordene	7.70	8.94	4.95	18.54
Heptachlor Epoxide	9.59	14.01	5.09	13.58
Oxychlordane	10.12	8.17	4.43	12.45
gamma Chlordane	10.25	9.56	6.10	10.79
o,p' DDE	8.43	9.03	5.55	10.25
alpha Chlordane	9.71	9.23	5.54	12.22
Transnonachlor	8.71	8.80	6.49	8.26
Dieldrin	7.79	7.66	5.60	11.60
o,p' DDD	4.83	6.83	7.76	8.12
Endrin	7.02	9.02	5.53	13.23
p,p' DDD	4.72	5.92	11.01	6.93
o,p' DDT	8.51	8.31	4.63	11.05
p,p' DDT	6.79	8.91	5.08	13.35

Data from Batch 2828

Validation of Clarus 500 in PreVent Mode for Chlorobiphenyls

	%CV at 0.01ug/g (Low Standard) n = 7	%CV at 0.2ug/g (High Standard) n = 7	%CV at 1ug/kg (LOD) n=7	%CV of Biota LRM 110 n = 6
HCB	5.90	4.43	4.70	13.57
CB 31	6.08	3.93	8.79	7.41
CB 28	7.04	4.54	3.88	9.55
CB53	7.96	5.77	4.14	16.72

Heptachlor	6.30	8.60	4.44	NA
CB 52	8.43	9.09	5.42	14.68
CB 49	7.21	9.77	5.29	15.86
Aldrin	6.51	6.58	6.00	NA
CB 35	8.60	8.35	6.40	10.85
CB 44	7.41	9.12	7.03	16.31
CB 74	6.01	4.20	5.64	11.70
CB 70	6.99	3.98	4.15	7.39
o,p' DDE	5.96	5.26	4.09	21.88
CB 101	8.53	7.23	3.94	10.32
CB 99	7.31	13.11	5.41	12.44
CB 112	5.38	9.42	5.36	16.72
CB 97	6.58	7.66	2.34	12.08
p,p' DDE	9.52	5.80	4.11	10.51
CB 110	7.18	6.09	5.22	11.06
CB 151	5.34	5.98	4.55	9.91
CB 149	6.85	6.50	3.74	10.47
CB 118	6.99	5.06	3.25	8.45
CB 153	6.52	4.72	3.60	8.09
CB 132	6.20	6.34	3.60	11.50
CB 105	6.80	4.24	4.35	10.71
CB 137	6.59	4.82	3.94	13.18
CB 138	6.42	4.25	5.42	10.62
CB 158	6.66	3.70	4.20	9.80
CB 187	5.58	5.62	4.25	10.73
CB 183	5.47	2.96	4.63	11.70
CB 128	6.70	4.23	5.45	11.26
CB 156	6.75	5.31	4.93	8.65
CB 157	7.22	4.10	4.63	10.87
CB 180	7.04	3.63	6.30	8.36
CB 198	3.82	11.43	2.97	12.96
CB 170	6.55	4.65	3.98	10.38
CB 189	5.74	4.13	3.73	10.84
CB 194	5.91	7.09	4.96	7.05
CB 209	5.49	7.65	4.62	8.74

Data from Batch 2823

Validation of Clarus 500 in PreVent Mode for Organochlorine Pesticides

	%CV at 0.01ug/g (Low Standard) n = 7	**%CV at 0.2ug/g (High Standard) n = 7**	**%CV at 1ug/kg (LOD) n=7**	**%CV of Biota LRM 110 n = 6**
alpha HCH	8.18	7.54	3.82	8.12
gamma HCH	10.22	8.60	4.97	4.32
Heptachlor	11.83	11.63	8.15	22.55
alpha Chlordene	9.30	7.52	6.10	20.74
gamma Chlordene	7.41	7.12	4.64	22.98
Heptachlor Epoxide	11.12	10.99	6.98	7.56
Oxychlordane	11.45	9.65	8.11	16.41
gamma Chlordane	8.70	8.98	4.72	13.14
o,p' DDE	9.01	9.13	5.16	22.10

alpha Chlordane	8.26	8.31	5.27	9.33
Transnonachlor	9.65	9.83	6.57	10.38
Dieldrin	12.56	10.96	7.63	8.05
o,p' DDD	9.91	8.04	4.68	11.86
Endrin	12.09	16.08	6.98	8.76
p,p' DDD	14.02	8.95	9.62	6.70
o,p' DDT	12.62	10.42	5.50	9.75
p,p' DDT	13.67	10.46	8.30	5.21

Data from Batch 2823

16. Uncertainty

Main Steps of Method:

Biota samples are received frozen, these are homgenised prior to sub sampling. Samples are then dried, by grinding with sodium sulphate. Recovery standard is added to the sample, prior to extraction with solvent. Residual lipid content is calculated as per M880. An aliquot of sample extract is cleaned by adsorption chromatography. Internal standard is added to the resultant CB and OCP fractions. These fractions along with calibration standards are analysed by Gas Chromatography. Software calculates the concentrations of each determinand according to the calibration curves, and blank corrections are performed using an Excel spreadsheet. Method is monitored by the use of a Laboratory Reference Material. The instrument is checked using the response of the 0.2ug/g calibration standard and a standard checking for determinand breakdown. The laboratory participates in the QUASIMEME laboratory intercomparison trials for these determinands.

Sources of Uncertainty:

- **Sampling:**
  Samples are analysed and results reported on the samples as received – out with uncertainty calculations

- **Sub-sampling:**
  Biota sample is homogenised as far as possible prior to sub sampling - uncertainty accounted for in the validation data

- **Storage Conditions:**
  Samples are stored in aluminum foil to minimise contamination. Samples are stored at -20°C – negligible contribution to uncertainty

- **Reagent Purity:**
  Solvents are of at least HPLC grade quality – uncertainty accounted for in the validation data
  Sodium Sulphate are of at least Analar quality - uncertainty accounted for in the validation data
  Adsorbents are of unknown purity – uncertainty accounted for in the blank correction
  Chemical standards used in the preparation of calibration solutions are of the highest purity available at the time of purchase. Final concentrations of the calibration solutions have not been corrected for purity - uncertainty accounted for in the validation data

- **Instrument Effects:**
  Weight: Balance check weight tolerances 0.05% and 0.002%, 2, 3 and 4 decimal places used, sufficient for accuracy required - uncertainty accounted for in the validation data
  Volume: pipettes are calibrated to within required limits, tolerances stated in SOPs for measuring cylinders are sufficient for purpose - uncertainty accounted for in the validation data
  Temperature: settings of Turbovaps calibrated against calibrated thermometers, variations accounted for by control chart data - uncertainty accounted for in the validation data
  Gas Chromatographs: quality control criteria set out in SOPs determine when maintenance is required. Between sample and calibration variations are monitored by quality control charts - uncertainty accounted for in the validation data

- **Environmental Conditions:**
  Contamination is minimised by the use of dedicated accommodation, equipment and glassware for organic analysis. Glassware is also separated during cleaning - uncertainty accounted for in the validation data

- **Operator Effects:**
  All measurement methods are described in fully documented Standard Operating Procedures (SOPs) to limit inconsistencies between operators - uncertainty accounted for in validation data

- **Matrix Effects:**
  Final sample fractions to be analysed are in 2,2,4 trimethylpentane as are the calibration standards - uncertainty accounted for in validation data

- **Computation Errors:**
  Concentrations are calculated by chromatography software. Manual check of calculation has been carried out and acceptable – negligible contribution to uncertainty

- **Blank Correction:**
  Method blank run alongside each batch of samples and is subtracted from each sample in the batch by use of an Excel spreadsheet. Manual check of calculation has been carried out and acceptable - uncertainty accounted for in the validation data

- **Random Effects:**
  Uncertainty accounted for by validation and control chart data

Summary Validation Data:

	% Recovery of Spiked Sample	Precision of Biota Sample	Low Standard 50ug/kg	High Standard 450ug/kg	Mean	SD	Variance from Shewart Chart %
HCB	34.50	14.35	2.38	1.80	100.26	21.49	21.43
CB 31	46.30	7.33	1.76	1.73	11.01	3.90	35.42
CB 28	45.70	5.96	1.72	1.69	19.63	4.22	21.50
HEPTACHLOR	52.50	13.44	2.72	2.29			
CB 52	46.90	8.44	2.63	1.99	92.72	23.33	25.16
CB 49	49.90	7.89	1.59	2.10	24.20	5.28	21.82
CB 44	47.10	8.94	2.33	1.81	34.72	8.18	23.56
CB 74	62.90	8.61	1.68	2.20	31.67	3.10	9.79
CB 70	112.90	12.73	2.24	1.32	48.55	15.93	32.81
o,p'-DDE	101.30	12.86	2.42	2.12			
CB 101	73.70	5.41	3.08	2.04	282.66	44.68	15.81
p,p'-DDE	83.40	4.98	2.86	2.09	1297.37	451.52	34.80
CB 110	82.10	5.27	1.06	1.79	184.66	25.06	13.57
CB 149	80.70	6.79	2.23	2.70	215.19	23.58	10.96
CB 118	86.40	5.25	1.75	1.66	249.49	26.90	10.78
CB 153	86.80	5.94	2.26	1.64	560.04	56.94	10.17
CB 105	95.10	3.81	2.06	2.27	91.36	15.90	17.40
CB 138	94.90	4.97	1.67	1.52	500.34	50.88	10.17
CB 158	88.80	4.25	2.22	1.41	32.13	2.86	8.90
CB 187	92.60	4.42	1.34	2.03	107.14	10.11	9.44
CB 128	90.40	4.72	2.31	2.40	65.70	38.64	58.81
CB 156	96.80	4.08	1.42	1.69	42.48	4.77	11.23
CB 157	101.10	5.41	2.51	1.84	11.65	1.59	13.65
CB 180	100.70	4.29	3.37	2.88	171.49	16.83	9.81
CB 170	97.80	3.24	1.97	2.41	62.09	5.98	9.63
CB 189	100.60	3.18	1.87	2.15	2.69	0.57	21.19
CB 194	100.50	4.26	2.22	2.41	18.03	2.62	14.53
CB 209	104.90	3.98	1.74	1.25	71.85	12.89	17.94

	% Recovery of Spiked Sample	Precision of LRM	Low Standard 50ug/kg	High Standard 450ug/kg	Mean	SD	Variance from Shewart Chart %
A HCH	48.00	15.35	2.10	2.37	53.18	13.29	24.99
G-HCH	56.70	16.08	3.44	3.26	31.63	9.17	28.99
HEPTACHLOR	36.60	83.56	1.55	3.86	1.44	0.86	59.72
A-CHLORDENE	39.60	58.89	2.13	3.77	0.91	0.60	65.93
G-CHLORDENE	52.10	94.29	2.37	3.16	3.52	3.21	91.19
HEPT.EPOXIDE	77.50	15.67	2.54	3.91	14.75	4.23	28.68
OXYCHLORDANE	69.50	12.71	1.80	3.89	21.72	3.90	17.96
G-CHLORDANE	78.80	13.96	2.28	4.06	11.14	1.90	17.06
o,p' DDE	49.80	7.00	2.54	5.35	36.21	11.39	31.46
A-CHLORDANE	80.20	16.95	2.70	2.93	45.60	7.17	15.72
T-NONACHLOR	78.00	13.17	2.17	3.80	79.87	9.19	11.51
DIELDRIN	93.80	14.86	2.40	3.42	131.61	14.64	11.12
o,p'-DDD	99.30	9.50	1.36	3.40	62.02	11.30	18.22
ENDRIN	91.80	27.67	2.61	3.41	77.55	14.40	18.57
p,p'-DDD	98.90	5.80	1.79	3.53	1018.35	84.96	8.34
o,p'-DDT	63.40	37.82	2.45	3.03	29.30	8.16	27.85
p,p'-DDT	93.10	17.41	1.02	3.38	406.39	46.58	11.46

Uncertainty

Systematic Component (Recovery of Spiked Sample, Perkin Elmer) : e.g. CB153 = 13.2/2%

Random Component (Shewart Chart S.D.): e.g. CB153 = (S.D.)56.94/(mean)560.04 = 10.17%

Assume linear summation and a value of K=2

Combined Standard Uncertainty = $(C_s^2 + C_r^2)^{0.5}$ = X %

Expanded Uncertainty = 2 * $(C_s^2 + C_r^2)^{0.5}$ = X %

Expanded Uncertainty e.g. CB153 = $2*(6.60^2 + 10.17^2)^{0.5}$ ug/kg = 24.24%

The reported expanded uncertainties are based on uncertainties multiplied by a coverage factor of k=2, providing a level of confidence of approximately 95%

	Systematic Component %	Random Component %	Expanded Uncertainty %
HCB	32.75	21.43	78.28
CB 31	26.85	35.42	88.90
CB 28	27.15	21.50	69.26
HEPTACHLOR	23.75		
CB 52	26.55	25.16	73.16
CB 49	25.05	21.82	66.44
CB 44	26.45	23.56	70.84
CB 74	18.55	9.79	41.95
CB 70	-6.45	32.81	66.88
o,p'-DDE	-0.65		
CB 101	13.15	15.81	41.12
p,p'-DDE	8.30	34.80	71.56
CB 110	8.95	13.57	32.51
CB 149	9.65	10.96	29.20
CB 118	6.80	10.78	25.49
CB 153	6.60	10.17	24.24
CB 105	2.45	17.40	35.15
CB 138	2.55	10.17	20.97
CB 158	5.60	8.90	21.03
CB 187	3.70	9.44	20.27
CB 128	4.80	58.81	118.02
CB 156	1.60	11.23	22.68
CB 157	-0.55	13.65	27.32
CB 180	-0.35	9.81	19.64
CB 170	1.10	9.63	19.39
CB 189	-0.30	21.19	42.38
CB 194	-0.25	14.53	29.07
CB 209	-2.45	17.94	36.21
A HCH	28.50	24.99	75.80
G-HCH	23.32	28.99	74.41
HEPTACHLOR	23.75	59.72	128.54
A-CHLORDENE	34.48	65.93	148.81
G-CHLORDENE	24.54	91.19	188.87
HEPT.EPOXIDE	14.77	28.68	64.51
OXYCHLORDANE	15.62	17.96	47.60
G-CHLORDANE	10.42	17.06	39.97
o,p' DDE	18.93	31.46	73.42
A-CHLORDANE	10.80	15.72	38.15
T-NONACHLOR	9.01	11.51	29.22
DIELDRIN	9.81	11.12	29.66
o,p'-DDD	7.66	18.22	39.53
ENDRIN	8.09	18.57	40.51
p,p'-DDD	0.01	8.34	16.69
o,p'-DDT	27.66	27.85	78.50
p,p'-DDT	6.14	11.46	26.00

1. Introduction and Scope

This method describes the determination of chlorobiphenyls in sediment and biota and organochlorine pesticides in biota . The following determinands can be measured:

CB31 CB28, CB35 CB52, CB53, CB44, CB49, CB70, CB74, CB97,CB99,CB101, CB 110, CB112, CB149, CB118, CB153, CB132 CB105, CB137 CB138, CB158, CB187, CB128, CB156, CB157, CB180, CB183, CB170, CB189, CB194, CB198 and CB209 – Sediment and biota

The following CBs are not accredited for biota CB 97, 99, 132, 137, 183

The following CBs are analysed for recovery measurement only :
CB35, 53, 112, 151, 198 and 209

HCB, α-HCH, γ-HCH, α-Chlordene*, γ-Chlordene*, α-Chlordane*, γ-Chlordane, Aldrin*, Oxychlordane, Trans-Nonachlor, Heptachlor, Heptachlor Epoxide, Dieldrin, Endrin, o,p'-DDE, p,p' DDE, o,p'-DDD, o,p'-DDT, p,p'-DDD and p,p'-DDT - biota

The range of this method is 0.002 µg/g to 0.2 µg/g in solution.

Clients can request that the results of individual congeners are summed together into groups to provide the following data:

ICES7 - sum of CB28, 52, 101, 118, 138, 153 and 180

Total CB - sum of all CBs analysed, excluding the recovery CBs 35, 53, 112, 151, 198 and 209

Sum CDANE - sum of OCPs a-chlordene, g-chlordene, Heptachlor Epoxide, Oxychlordane, g-chlordane, a-chlordane, Trans Nonachlor, Total Heptachlor

Sum DDT - sum of p,p' DDE, o,p' DDD, p,p' DDD, o,p' DDT, p,p' DDT, Total o,p' DDE

* Congeners which will not be accredited

2. Principle

Biota - The chlorobiphenyls and organochlorine pesticides are extracted using 5% alumina fat retainer from the tissue by Accelerated Solvent Extraction (ASE) using *iso*-hexane. Samples are subjected to a clean-up procedure involving column chromatography on alumina and silica.
Sediment (**non-accredited**) - The chlorobiphenyls are extracted from the sediment by Accelerated Solvent Extraction (ASE) using *iso*-hexane. Samples are subjected to a clean-up procedure involving column chromatography on silica.

The cleaned-up extract is reconstituted in a known weight of *iso*-hexane and analysed by gas chromatography with electron capture detection (GC-ECD) using a HP-5 (non-polar) column or equivalent. The gas chromatograph is calibrated by a series of eight external chlorobiphenyl standards that include two internal standards, 2,4 dichlorobenzyl alkyl ethers (DCBE 6 and DCBE 16).

3. Reference Materials

Biota - LRM 110, contaminated cod liver oil, contains all determinands.
Sediment - LRM 140, contaminated sediment contains all determinads.

4. Reagents

See SOP's for reagents used.

5. Major Equipment

ASE 300 EN1241
Turbovap or Synchore for solvent evaporation
Gas chromatograph with on column injector and electron capture detector (Varian 4 EN 164, Perkin Elmer Clarus EN 1181.
Totalchrom Chromatography data collection system (see B 145 for current version)
HP-5 column or equivalent, 60 m x 0.25 mm, film thickness 0.25 µm.

6. Environmental Control

See individual SOPs.

7. Interferences

Biota samples are stored either wrapped in aluminium foil, before storing in a labelled plastic bag or a labelled aluminium can.
Sediment samples are stored in aluminium cans

8. Sample Preparation

8.1 Samples are logged into the laboratory according to SOP 60.

8.2 Biota samples are sub-sampled and homogenised as in SOP 130.

8.3 Biota samples are ground with anhydrous sodium sulphate, to dehydrate the sample prior to solvent extraction, SOP 0367.

9. Analytical Procedure

9.1 Sample Extraction and Clean-Up

9.1.1 Biota

9.1.1.1 A total lipid determination is carried out on each sample , M890

9.1.1.2 A method blank and at least one LRM is analysed per batch. Each batch consists of no more than 10 samples, a procedural blank and LRM.

9.1.1.3 Biota samples are ground with anhydrous sodium sulphate, to dehydrate the sample prior to solvent extraction, SOP 0367.

9.1.1.4 Recovery standard is added to the ground sample/sodium sulphate mixture in the ASE tube prior to extraction, SOP 0367

9.1.1.4 The extraction of chlorobiphenyls and OCPs in biota is carried out as detailed in SOP 0371.

9.1.1.5 The extracts are concentrated by Turbovap or Syncore SOP 560 or SOP 565 and are cleaned up using alumina and silica column chromatography (CBs and OCPs), SOP 435. Note if CBs alone are analysed and a 30g Alumina is used in the ASE only the alumina clean up is used (**non-accredited**)

9.1.2 **Sediment (non-accredited)**

9.1.2.1 Sediment samples are freeze dried and sieved as per SOPs (SOP 0110, SOP 0120). A method blank and at least one LRM is analysed per batch. Each batch consists of no more than 10 samples, a procedural blank and LRM.

9.1.2.2 The freeze dried sediment is mixed with sodium sulphate and transferred to extraction tubes and recovery standard added, SOP 0367

9.1.2.3 The extraction of chlorobiphenyls in sediment is carried out as detailed in SOP 0371.

9.1.2.4 The extracts are concentrated by Turbovap SOP 560 or Syncore SOP 565 and are cleaned up using silica column chromatography, SOP 435.

9.1.2.5 Sulphur is removed using copper as detailed in SOP 435

9.1.3 A weighed amount of the internal standard, DCBE 6 and 16 is added to each sample, SOP 435.

9.1.4 Sample is vialled for chromatographic analysis, SOP 435.

9.2 **Setting Up and Testing of Gas Chromatograph**

9.2.1 In normal circumstances the gas chromatograph will be set up. If not, the procedures for set up is given in SOP 1020 (Perkin-Elmer), SOP 1000 (Varian GCs).

9.2.2 If the gas chromatograph has analysed samples within the previous 24 hours, or is currently analysing samples, check that the standards meet the QC criteria, SOP 1100 (Perkin-Elmer and Varian GCs).

9.2.3 If the QC criteria are met, samples can be analysed, following the method from 9.3.

9.2.4 If the QC criteria are not met, then maintenance may be required. The individual responsible for GC maintenance should be contacted. Maintenance procedures are outlined in SOP 980 (Perkin-Elmer), SOP 960 (Varian GCs)

9.2.5 If 9.2.2 does not apply, then a solvent blank, CB 0.200 µg/g and 0.200 µg/g p,p' DDT standards are analysed and checked against the QC criteria, SOP 1100 (Perkin-Elmer and Varian GCs)

9.2.6 Refer to 9.2.7 for autosampler loading.

9.2.7 Sample vials are loaded onto the autosampler carousel SOP 1060 (Perkin-Elmer), , SOP 1040 (Varian GCs). Refer to GC Users Manual, for instructions on the autosampler and running samples.

9.2.8 A full set of chlorobiphenyl or organochlorine pesticide calibration standards, prepared previously using SOPs 290, 320 and 330 are run first followed by a solvent blank. Samples are followed by a solvent blank and a quality control standard CB 0.050 µg/g (in order to check the calibration calculations and account for drift).

9.4 Entering of Results, Test Report and Archive of Data

9.4.1 Enter sample information and data into CB OCP quantification worksheets B569 (biota) and B568 (sediment) as per SOP595. CBs are corrected for the recovery B569 and B568.

Biota - Recovery correction for liver samples if recovery standard recovery is 50-100% (CB 112). If the recovery of CB 112 is < 50% the sample is repeated.

Sediment - Recovery correction of sample if recovery standard is 50-100% (CB 112). If the recovery of CB 112 is < 50% the sample is repeated.

9.4.2 Enter LRM data into relevant QC charts as per SOP595. When copying the calculated LRM data from the CB OCP Biota Template to the control chart, delete the concentrations of the recovery CBs in the chart to leave an *. The recovery CBs only appear in the control chart as an aid for copying and pasting.

9.4.3 Produce Test Report and archive relevant batch paperwork and electronic copies of finalised spreadsheet and test report in NTS2/shared/Chem_Dat (SOP595 and SOP1350).

9.4.4 All TotalChrom data is archived and stored as stated in SOP905.

10 Calculation of Results

10.1 The GC-ECD is calibrated and results calculated using the Chromatography data system, SOP 1242.

10.2 Results for the LRM should be compared with the appropriate Shewart Control Chart data SOP 1380.

10.3 Evaluate method and solvent blanks, SOP 1230.

11 Precision, Accuracy and Practical Detection Limits

11.1 Precision and bias for the method are defined in SOP 1310.

Target Precision - 25%
Target Bias - 25%

11.2 Actual Precision - derived from the Shewhart charts and is expressed as the percentage standard deviation.

Actual Bias - to be calculated.

11.3 Detection Limit - to be determined according to SOP 1310. See Performance Data (15).

12. Reports

A hard copy of all data should be obtained, SOP 1242 and submitted to the technical manager along with other relevant documentation, SOP 1350.

13. Safety

Safety for all relevant procedures are provided in appropriate SOP's detailed above with reference to Procedure Risk Assessments.

14. Literature References

Not relevant.

15. Performance Data

15.1.1 Instrument Precision

	Varian 4 %CV at Lower End of Working Range n=7	%CV at Higher End of Working Range n=7	%CV at 1ug/kg n=8
HCB	10.87	9.26	2.87
CB 31	6.24	4.81	5.09
CB 28	8.01	4.52	2.19
CB53	4.68	5.97	NA
heptachlor	10.76	6.81	3.67
CB 52	6.65	4.70	5.97
CB 49	5.16	4.64	4.14
aldrin	5.62	5.09	NA
CB 35	9.68	4.91	NA
CB 44	8.71	5.49	2.03
CB 74	6.31	4.85	1.32
CB 70	7.18	4.07	4.46
opDDE	5.84	4.91	3.86
CB 101	6.49	2.82	3.88
CB 99	5.16	4.64	NA
CB 112	4.34	4.23	NA

CB 97	4.76	5.71	NA
ppDDE	4.20	5.12	2.71
CB 110	4.63	5.10	3.82
CB 151	7.61	4.07	NA
CB 149	4.38	3.64	3.96
CB 118	8.71	5.47	4.61
CB 153	12.20	9.47	3.46
CB 132	6.05	6.05	NA
CB 105	4.31	3.93	3.85
CB 137	5.57	3.86	NA
CB 138	6.56	4.28	3.97
CB 158	8.39	5.73	1.90
CB 187	17.96	15.25	2.78
CB 183	13.82	10.58	NA
CB 128	4.67	3.49	2.37
CB 156	10.43	7.75	2.38
CB 157	7.64	4.34	NA
CB 180	15.64	11.51	2.79
CB 198	12.76	10.70	NA
CB 170	8.20	6.78	2.60
CB 189	12.59	7.87	2.38
CB 194	11.31	9.77	2.15
CB 209	18.73	2.64	2.16

	Varian 4 %CV at Lower End of Working Range n=7	%CV at Higher End of Working Range n=7	%CV at 1ug/kg n=8
AHCH	5.33	10.08	2.16
GHCH	5.76	8.20	2.44
Hept	8.23	5.66	2.71
ACdene	4.10	6.18	2.24
GCdene	3.53	4.50	1.98
HeptEpox	4.12	6.06	2.77
Oxychlordane	3.87	4.53	2.98
GCdane	4.10	4.36	2.31
o,p' DDE	6.37	7.02	2.70
ACdane	5.60	4.23	2.65
Transnonachlor	3.50	3.34	1.95
Dieldrin	5.13	6.29	1.66
o,p' DDD	6.48	4.20	1.88
Endrin	9.05	9.35	1.87
p,p' DDD	10.27	4.29	2.32
o,p' DDT	14.38	9.70	2.52
p,p' DDT	23.83	15.20	2.46

Validation of Clarus 500 without PreVent for Chlorobiphenyls

	%CV at 0.01ug/g (Low Standard) n = 7	**%CV at 0.2ug/g (High Standard) n = 7**	**%CV at 1ug/kg (LOD) n=7**	**%CV of Biota LRM 110 n = 6**
HCB	8.46	8.38	9.24	15.99
CB 31	7.38	8.34	13.16	17.72
CB 28	6.78	6.77	13.17	11.81
CB53	8.28	8.57	4.41	12.79

Heptachlor	10.13	7.50	5.84	NA
CB 52	7.88	7.59	6.43	7.94
CB 49	9.60	7.54	4.70	9.26
Aldrin	10.80	6.92	6.53	NA
CB 35	11.94	6.43	27.27	14.83
CB 44	8.41	8.70	6.22	13.40
CB 74	5.43	6.87	12.11	8.15
CB 70	6.29	6.34	12.23	6.45
o,p' DDE	9.27	8.28	8.63	22.05
CB 101	7.69	7.22	4.57	5.02
CB 99	9.02	7.18	4.71	3.56
CB 112	9.08	7.77	5.67	2.91
CB 97	9.21	7.50	2.80	2.61
p,p' DDE	8.67	7.62	2.99	6.36
CB 110	8.48	7.34	2.73	5.47
CB 151	10.05	7.71	2.68	2.93
CB 149	9.89	7.73	6.39	3.82
CB 118	5.16	7.08	8.20	3.22
CB 153	8.69	7.09	3.04	5.30
CB 132	8.57	8.62	3.90	5.75
CB 105	6.07	7.02	5.14	6.74
CB 137	8.45	5.92	3.01	5.80
CB 138	8.48	7.26	4.17	3.79
CB 158	8.26	7.18	3.15	4.65
CB 187	10.02	7.73	6.00	5.47
CB 183	10.44	7.83	7.02	4.16
CB 128	8.13	6.36	4.53	9.45
CB 156	7.10	6.46	2.47	2.73
CB 157	7.55	6.91	2.56	4.12
CB 180	9.58	6.32	6.04	4.33
CB 198	9.67	7.40	5.83	4.57
CB 170	9.94	5.92	6.66	5.82
CB 189	9.66	7.38	3.08	7.48
CB 194	10.60	6.95	4.42	5.86
CB 209	11.55	10.20	6.27	3.05

Data from Batch 2828

Validation of Clarus 500 without PreVent for Organochlorine Pesticides

	%CV at 0.01ug/g (Low Standard) n = 7	**%CV at 0.2ug/g (High Standard) n = 7**	**%CV at 1ug/kg (LOD) n=7**	**%CV of Biota LRM 110 n = 6**
alpha HCH	7.47	9.45	5.51	15.38
gamma HCH	6.72	11.95	6.00	11.82
Heptachlor	9.92	9.59	4.86	12.54
alpha Chlordene	7.50	7.85	6.01	17.17
gamma Chlordene	7.70	8.94	4.95	18.54
Heptachlor Epoxide	9.59	14.01	5.09	13.58
Oxychlordane	10.12	8.17	4.43	12.45
gamma Chlordane	10.25	9.56	6.10	10.79
o,p' DDE	8.43	9.03	5.55	10.25

alpha Chlordane	9.71	9.23	5.54	12.22
Transnonachlor	8.71	8.80	6.49	8.26
Dieldrin	7.79	7.66	5.60	11.60
o,p' DDD	4.83	6.83	7.76	8.12
Endrin	7.02	9.02	5.53	13.23
p,p' DDD	4.72	5.92	11.01	6.93
o,p' DDT	8.51	8.31	4.63	11.05
p,p' DDT	6.79	8.91	5.08	13.35

Data from Batch 2828

Oxychlordane	11.45	9.65	8.11	16.41
gamma Chlordane	8.70	8.98	4.72	13.14
o,p' DDE	9.01	9.13	5.16	22.10
alpha Chlordane	8.26	8.31	5.27	9.33
Transnonachlor	9.65	9.83	6.57	10.38
Dieldrin	12.56	10.96	7.63	8.05
o,p' DDD	9.91	8.04	4.68	11.86
Endrin	12.09	16.08	6.98	8.76
p,p' DDD	14.02	8.95	9.62	6.70
o,p' DDT	12.62	10.42	5.50	9.75
p,p' DDT	13.67	10.46	8.30	5.21

Data from Batch 2823

15.2 Matrix Recovery

15.2.1 Salmon Liver

Varian 4
% Recovery from matrix spike, **Batch 3104**
Spike ; 100 µl of ~ 100 µg/Kg
CBs have been corrected for recovery.
No recovery correction for OCPs

Determinand	Mean	SD	CV%
CB 31	93.348	15.5	16.6
CB 28	93.960	6.7	7.2
CB 52	92.903	10.5	11.3
CB 49	103.122	6.0	5.9
CB 44	101.287	10.1	10.0
CB 74	98.205	1.6	1.6

CB 70	97.877	7.2	7.4
CB 101	96.615	2.9	3.1
CB 99	98.461	1.4	1.4
CB 97	97.811	5.3	5.4
CB 110	86.035	12.6	14.6
CB 149	95.909	4.9	5.1
CB 118	98.218	7.4	7.5
CB 153	89.032	7.6	8.6
CB 132	84.422	11.2	13.3
CB 105	78.830	10.5	13.4
CB 137	93.718	6.8	7.2
CB 138	88.466	6.5	7.4
CB 158	110.889	9.6	8.7
CB 187	99.429	5.0	5.0
CB 183	100.189	3.1	3.1
CB 128	76.775	14.8	19.3
CB 156	95.402	8.8	9.2
CB 157	97.503	4.8	4.9
CB 180	89.323	1.9	2.2
CB 170	99.158	2.3	2.4
CB 189	88.262	1.3	1.5
CB 194	98.671	3.3	3.4
HCB	56.451	3.7	6.5
aldrin	50.981	6.0	11.8
ppDDE	53.729	5.1	9.5
Alpha HCH	50.7	6.1	12.1
Gamma HCH	58.1	5.3	9.2
Heptachlor	52.9	3.6	6.8
Alpha Chlordene	50.2	10.2	20.3
Gamma Chlordene	52.6	10.8	20.5
Heptachlor Epoxide	60.5	9.2	15.3
Oxychlordane	67.6	5.4	8.0
Gamma Chlordane	55.1	12.3	22.3
o,p' DDE	64.1	6.5	10.2
Alpha Chlordane	67.4	5.9	8.8
Determinand	**Mean**	**SD**	**CV%**
Transnonachlor	66.2	7.2	10.9
Dieldrin	63.7	9.2	14.4
Endrin	64.4	2.7	4.2
o,p' DDD	63.2	5.7	9.0
p,p'-DDD	64.0	2.6	4.0
o,p'-DDT	55.9	5.0	9.0
p,p' DDT	62.5	5.1	8.2

15.2.2 **Mussels**

Varian 4

% Recovery from matrix spike **Batch 3104**
Spike; Spike ; 1.5 ml of ~ 100 µg/Kg
CBs have been corrected for recovery.
No recovery correction for OCPs

Determinand	**Mean**	**SD**	**cv%**
CB 31	74.2	9.0	12.2

CB 28	79.6	8.3	10.4
CB 52	83.4	9.7	11.6
CB 49	86.9	8.5	9.8
CB 44	93.8	4.5	4.8
CB 74	86.9	3.9	4.5
CB 70	87.6	7.3	8.3
CB 101	93.4	3.8	4.1
CB 99	81.4	7.8	9.6
CB 97	88.8	4.8	5.4
CB 110	84.4	10.8	12.8
CB 149	95.5	3.8	4.0
CB 118	97.1	3.6	3.7
CB 153	100.9	6.9	6.9
CB 132	84.0	12.9	15.3
CB 105	86.9	13.2	15.2
CB 137	103.2	4.1	3.9
CB 138	98.5	4.0	4.0
CB 158	113.7	4.4	3.9
CB 187	91.7	4.8	5.2
CB 183	98.1	4.7	4.8
CB 128	81.2	16.6	20.5
CB 156	101.8	5.8	5.7
CB 157	93.0	3.1	3.4
CB 180	92.8	5.8	6.2
CB 170	97.5	5.3	5.4
CB 189	82.8	9.1	11.0
CB 194	90.8	9.4	10.3
Determinand	**Mean**	**SD**	**CV%**
HCB	40.3	7.1	17.6
aldrin	46.7	7.2	15.4
ppDDE	48.5	5.8	12.1
Alpha HCH	46.3	6.8	14.8
Gamma HCH	50.0	7.3	14.5
Heptachlor	47.4	3.9	8.2
Alpha Chlordene	39.3	7.2	18.2
Gamma Chlordene	18.5	3.2	17.5
Heptachlor Epoxide	51.7	9.0	17.3
Oxychlordane	45.4	6.0	13.3
Gamma Chlordane	38.9	5.9	15.1
o,p' DDE	53.7	12.8	23.9
Alpha Chlordane	49.0	6.7	13.7
Transnonachlor	44.9	7.9	17.7
Dieldrin	47.8	5.6	11.7
Endrin	46.0	6.5	14.1
o,p' DDD	56.0	7.6	13.7
p,p'-DDD	43.2	6.0	13.9
o,p'-DDT	44.4	7.7	17.4
p,p' DDT	45.2	5.6	12.4

15.2.3 Sediment Recovery

Varian 4

% Recovery from matrix spike **Batch 3606**
Spike; Spike ; 400 ul of ~ 100 µg/Kg
Recovery correction

Determinand	Mean	Std	CV%	n
CB 31	103.5	3.1	3.0	7
CB 28	102.6	3.6	3.6	7
CB 52	113.9	7.9	6.9	7
CB 49	121.4	5.5	4.5	7
CB 44	94.6	3.4	3.6	7
CB 74	80.8	3.9	4.8	7
CB 70	79.5	3.4	4.3	7
CB 101	85.4	3.9	4.5	7
CB 99	92.1	4.2	4.5	7
CB 97	90.4	3.5	3.8	7
CB 110	87.8	1.6	1.8	7
CB 149	71.0	2.7	3.8	7
CB 118	98.7	4.2	4.3	7
CB 153	78.2	5.5	7.0	7
CB 132	67.2	2.4	3.5	7
CB 105	100.7	5.1	5.1	7
CB 137	87.6	8.3	9.5	7
CB 138	78.9	5.4	6.8	7
CB 158	102.5	8.1	7.9	7
CB 187	79.9	8.0	10.0	7
CB 183	87.4	5.8	6.6	7
CB 128	71.3	3.8	5.3	7
CB 156	94.2	6.7	7.1	7
CB 157	84.9	6.2	7.3	7
CB 180	85.1	8.8	10.4	7
CB 170	89.9	8.3	9.3	7
*CB 189	80.1	4.3	5.3	7
CB 194	87.5	5.0	5.8	7
HCB	49.9	3.8	7.5	7
aldrin	42.2	3.8	8.9	7
ppDDE	80.1	7.8	9.7	7

15.2.4 Limit of Detection (LOD) Mussels

Varian 4

Spike; Spike ; 40 µl (CB) & 50 µl (OCP) of ~ 100 µg/Kg
~ 8g sample
CBs have been corrected for recovery.
No recovery correction for OCPs
LOD = 4.65 x SD

Determinand	Batch	SD	LOD	n
CB 31	3104 O	0.01	0.05	6
CB 28	3104 O	0.02	0.07	6
CB 52	3104 O	0.10	0.48	6
CB 49	3104 O	0.03	0.13	6
CB 44	3104 O	0.04	0.17	6
CB 74	3104 O	0.03	0.14	6
CB 70	3104 O	0.06	0.26	6
CB 101	3104 O	0.16	0.76	6
CB 99	3104 O	0.04	0.20	6
CB 97	3104 O	0.05	0.22	6
CB 110	3104 O	0.15	0.68	6

Determinand	Batch	SD	LOD	n
CB 149	3104 O	0.07	0.32	6
CB 118	3104 O	0.08	0.39	6
CB 153	3104 O	0.07	0.34	6
CB 132	3104 O	0.06	0.26	6
CB 105	3104 O	0.05	0.23	6
CB 137	3104 O	0.02	0.07	6
CB 138	3104 O	0.07	0.33	6
CB 158	3104 O	0.02	0.10	6
CB 187	3104 O	0.02	0.11	6
CB 183	3104 O	0.02	0.07	6
CB 128	3104 O	0.04	0.20	6
CB 156	3104 O	0.02	0.08	6
CB 157	3104 O	0.02	0.10	6
CB 180	3104 O	0.04	0.18	6
CB 170	3104 O	0.02	0.07	6
CB 189	3104 O	0.01	0.05	6
CB 194	3104 O	0.02	0.08	6
HCB	3104 O	0.02	0.09	6
Aldrin	3104 O	0.02	0.07	6
a-HCH	3104 K	0.02	0.08	7
g-HCH	3104 K	0.02	0.11	7
Heptachlor	3104 K	0.06	0.28	7
a-Chlordene	3104 K	0.01	0.06	7
g-Chlordene	3104 K	0.01	0.07	7
Heptachlor Epoxide	3104 K	0.02	0.08	7
Oxychlordane	3104 K	0.02	0.10	7
g-Chlordane	3104 K	0.02	0.09	7
o,p'-DDE	3104 K	0.05	0.22	7
a-Chlordane	3104 K	0.02	0.08	7
T-Nonachlor	3104 K	0.02	0.08	7
Dieldrin	3104 K	0.02	0.10	7
o,p'-DDD	3104 K	0.03	0.12	7
Endrin	3104 K	0.03	0.15	7
p,p'-DDD	3104 K	0.02	0.09	7
o,p'-DDT	3104 K	0.03	0.15	7
p,p'-DDT	3104 K	0.04	0.17	7

15.2.5 **Limit of Detection Sediment.**

Varian 4
Spike; Spike ; 25 µl (CB) of ~ 100 µg/Kg
~ 21g sample
LOD = 4.65 x SD

Determinand	Batch	Stddev	LOD	n
CB 31	3606A	0.004	0.02	6
CB 28	3606A	0.004	0.02	6
CB 52	3606A	0.015	0.07	6
CB 49	3606A	0.004	0.02	6
CB 44	3606A	0.015	0.07	6

CB 74	3606A	0.010	0.05	6
CB 70	3606A	0.010	0.05	6
CB 101	3606A	0.020	0.09	6
CB 99	3606A	0.010	0.04	6
CB 97	3606A	0.008	0.04	6
CB 110	3606A	0.022	0.10	6
CB 149	3606A	0.012	0.06	6
CB 118	3606A	0.014	0.07	6
CB 153	3606A	0.011	0.05	6
CB 132	3606A	0.012	0.05	6
CB 105	3606A	0.011	0.05	6
CB 137	3606A	0.007	0.03	6
CB 138	3606A	0.012	0.06	6
CB 158	3606A	0.007	0.03	6
CB 187	3606A	0.010	0.05	6
CB 183	3606A	0.007	0.03	6
CB 128	3606A	0.010	0.05	6
CB 156	3606A	0.006	0.03	6
CB 157	3606A	0.006	0.03	6
CB 180	3606A	0.007	0.03	6
CB 170	3606A	0.006	0.03	6
CB 189	3606A	0.007	0.03	6
CB 194	3606A	0.006	0.03	6
HCB	3606A	0.01	0.04	6
Aldrin	3606A	0.004	0.02	6

15.3.1 **LRM 110**

Varian 4

µg/Kg wet weight
CBs have been corrected for recovery.
No recovery correction for OCPs

Determinand	Mean	SD	CV%	n
CB 31	9.4	2.5	27.2	7
CB 28	28.8	1.9	6.6	7
CB 52	87.5	5.2	5.9	7
CB 49	36.7	2.1	5.7	7
CB 44	38.4	2.8	7.3	7
CB 74	42.7	2.0	4.8	7
CB 70	44.9	1.8	4.0	7
CB 101	275.4	21.3	7.7	7
CB 99	137.5	5.5	4.0	7
CB 97	62.1	2.8	4.6	7
CB 110	197.3	17.0	8.6	7
CB 149	223.6	12.6	5.6	7
CB 118	267.3	20.2	7.5	7
CB 153	507.3	41.5	8.2	7
CB 132	74.9	4.3	5.7	7
CB 105	98.9	8.9	9.0	7
CB 137	20.2	1.0	4.9	7

CB 138	419.0	34.2	8.2	7
CB 158	41.7	2.2	5.4	7
CB 187	112.1	7.1	6.4	7
CB 183	58.2	4.0	6.8	7
CB 128	74.3	7.4	9.9	7
CB 156	49.9	3.2	6.3	7
CB 157	13.1	0.7	5.3	7
CB 180	181.9	15.0	8.2	7
CB 170	68.4	3.6	5.2	7
CB 189	3.5	0.3	9.0	7
CB 194	21.8	2.1	9.7	7
HCB	124.5	17.5	14.0	7
p,p'-DDE	445.0	63.6	14.3	7
-HCH	74.8	17.0	22.8	9
-HCH	45.8	6.3	13.7	9
Heptachlor	1.3	0.3	25.1	7
-Chlordene				
-Chlordene				
Heptachlor Epoxide	9.3	1.4	15.0	5
Oxychlordane	19.9	3.3	16.6	8
-Chlordane	5.3	1.2	23.0	4
o,p'-DDE	23.3	4.4	18.9	6
-Chlordane	44.5	5.5	12.4	9
T-Nonachlor	69.3	10.7	15.4	9
Dieldrin	102.4	6.0	5.8	9
o,p'-DDD	45.8	9.6	20.9	9
Endrin	71.7	15.5	21.7	9
p,p'-DDD	394.7	84.7	21.5	9
o,p'-DDT	31.2	7.9	25.3	9
p,p'-DDT	203.7	27.5	13.5	9

15.3.2 LRM 140
Varian 4

µg/Kg dry weight corrected for recovery.

Determinand	Mean	SD	CV%
CB 31*	1.46	0.25	17.05
CB 28	1.97	0.41	20.87
CB 52	2.55	0.50	19.55
CB 49	1.29	0.29	22.39
CB 44	1.11	0.15	13.97
CB 74	1.15	0.14	12.40
CB 70	2.37	0.43	18.11
CB 101	3.02	0.40	13.39
CB 99*	1.50	0.24	16.12
CB 97*	0.99	0.12	12.45
CB 110	2.88	0.69	24.08
CB 149	4.09	0.70	17.09
CB 118	2.94	0.61	20.78
CB 153	5.91	0.83	14.12
CB 132*	1.52	0.28	18.51

CB 105	1.11	0.23	20.86
CB 137*	0.19	0.04	19.67
CB 138	5.40	1.01	18.63
CB 158	0.54	0.06	10.64
CB 187	2.95	0.50	16.90
CB 183*	1.16	0.16	14.03
CB 128	0.75	0.18	23.82
CB 156	0.52	0.07	12.64
CB 157*	0.26	0.03	11.99
CB 180	4.61	0.48	10.34
CB 170	1.72	0.25	14.53
CB 189*	Tr	Tr	
CB 194	1.35	0.19	14.16

15.4 Reference Material data

15.4.1 Biota FAPAS T0620 (Quality Assurance Round Sample) Batch 3755

				FAPAS T0620 Cod Liver Oil		
Determinand	**Mean n=8**	**SD**	**CV%**	**Assigned value**	**Satisfactory range**	
CB 31	Tr	Tr	Tr			
CB 28	3.64	0.242	6.651	2.76	1.55-3.97	Pass
CB 52	10.66	1.105	10.365	8.31	4.65-11.96	Pass
CB 49	3.90	0.362	9.278			
CB 44	3.48	0.413	11.858			
CB 74	5.08	0.412	8.116			
CB 70	4.83	0.719	14.882			
CB 101	20.86	1.686	8.083	16.6	9.3-23.9	Pass
CB 99	12.44	0.884	7.106			
CB 97	5.00	0.683	13.660			
CB 110	11.15	1.235	11.077			
CB 149	14.01	1.149	8.200			
CB 118	20.34	1.236	6.077	16	9-23.1	Pass
CB 153	42.82	2.701	6.306	37.6	21-54.1	Pass
CB 132	4.53	0.511	11.266			
CB 105	6.61	0.536	8.111	5.19	2.91-7.47	Pass
CB 137	Tr	Tr	Tr			
CB 138	34.11	2.140	6.273	31.7	17.7-45.6	Pass
CB 158	3.33	0.258	7.741			
CB 187	11.64	0.763	6.551			
CB 183*	4.26	0.416	9.748			
CB 128	5.85	0.420	7.172			
CB 156	Tr	Tr	Tr	1.67	0.94-2.41	<LOQ
CB 157	Tr	Tr	Tr	0.468	0.262-0.674	<LOQ
CB 180	12.25	0.801	6.540	10.8	6-15.6	Pass
CB 170	5.04	0.460	9.112			
CB 189	Tr	Tr	Tr	0.159	0.089-0.229	<LOQ
CB 194	TR	Tr	Tr			

15.4.2 Biota NIST 2977 and 1946 (Certified Reference Material) Batch 3769 and 3730

	NIST 2977 Mussel	**NIST 1946 Fish Flesh**
	%	**%**

Determinand	Assigned Value	Assigned Value
CB 31	98.75	83.61
CB 28	109.76	78.52
CB 52	99.36	84.29
CB 49		89.05
CB 44	55.64	63.20
CB 74		94.85
CB 70		72.05
CB 101	90.65	104.04
CB 99	286.48	95.52
CB 97		
CB 110	105.68	94.31
CB 149		103.15
CB 118	88.61	94.45
CB 153	88.73	83.22
CB 132		112.93
CB 105	61.09	114.27
CB 137		
CB 138	68.27	90.68
CB 158		96.95
CB 187	91.33	97.22
CB 183	55.45	118.88
CB 128	69.48	112.30
CB 156	74.98	115.69
CB 157		
CB 180	86.91	111.67
CB 170	87.06	120.33
CB 189		
CB 194	102.42	135.12
Batch	3769	3730
	n=2	n=2

15.4.3 Sediment NIST 1941b (Certified Reference Material) Batch 3941

Determinand	NIST 1941b Sediment Mean n=10	SD	CV%	% CRM
CB 31	2.82	0.16	5.64	88.73
CB 28	3.09	0.16	5.08	68.30
CB 52	6.02	0.61	10.18	114.93
CB 49	4.16	0.34	8.26	95.92
CB 44	2.69	0.47	17.39	69.84
CB 74	2.03	0.09	4.24	*99.45*
CB 70	4.37	0.21	4.88	*87.58*
CB 101	5.29	0.22	4.24	103.45
CB 99	3.05	0.12	3.83	105.06
CB 97	1.72	0.11	6.13	
CB 110	4.00	0.33	8.18	86.50
CB 149	3.78	0.11	2.89	86.88
CB 118	3.70	0.16	4.42	87.42
CB 153	4.61	0.13	2.79	84.36

CB 132	1.31	0.12	9.15	*101.97*
CB 105	1.13	0.12	10.94	78.84
CB 137				
CB 138	3.77	0.16	4.26	104.83
CB 158	0.45	0.02	4.96	*69.36*
CB 187	2.41	0.07	3.03	110.92
CB 183	1.43	0.04	2.97	146.14
CB 128	0.71	0.08	11.45	101.65
CB 156	0.53	0.04	6.91	104.09
CB 157	0.24	0.01	3.29	
CB 180	3.19	0.10	3.01	98.54
CB 170	1.36	0.04	2.65	100.71
CB 189				
CB 194	1.24	0.03	2.69	119.50

reference value

16 Uncertainty

Main Steps of Method: Biota

Biota samples are received frozen, these are homgenised prior to sub sampling. Samples are then dried, by grinding with sodium sulphate, recovery standard is added to the sample and the sample left overnight in a fridge. Samples are exracted by ASE with *iso*-hexane. Residual lipid content is calculated as per M890. The sample sample extract is cleaned by adsorption chromatography. Internal standard is added to the resultant CB and OCP fractions. These fractions along with calibration standards are analysed by Gas Chromatography. Software calculates the concentrations of each determinand according to the calibration curves, and blank corrections are performed using an Excel spreadsheet. Method is monitored by the use of a Laboratory Reference Material. The instrument is checked using the response of the 0.2ug/g calibration standard and a standard checking for determinand breakdown. The laboratory participates in the QUASIMEME laboratory intercomparison trials for these determinands.

Sources of Uncertainty:

- **Sampling:**
  Samples are analysed and results reported on the samples as received – out with uncertainty calculations

- **Sub-sampling:**
  Biota sample is homogenised as far as possible prior to sub sampling - uncertainty accounted for in the validation data

- **Storage Conditions:**
  Samples are stored in aluminum foil to minimise contamination. Samples are stored at -20°C – negligible contribution to uncertainty

- **Reagent Purity:**
  Solvents are of at least HPLC grade quality – uncertainty accounted for in the validation data
  Sodium Sulphate are of at least Analar quality - uncertainty accounted for in the validation data
  Adsorbents are of unknown purity – uncertainty accounted for in the blank correction
  Chemical standards used in the preparation of calibration solutions are of the highest purity available at the time of purchase. Final concentrations of the calibration solutions

have not been corrected for purity - uncertainty accounted for in the validation data

- **Instrument Effects:**
  Weight: Balance check weight tolerances; balances within allowable tolerances for decimal places, sufficient for accuracy required - uncertainty accounted for in the validation data
  Volume: syringes are calibrated to within required limits, tolerances stated in SOPs for measuring cylinders are sufficient for purpose - uncertainty accounted for in the validation data
  Temperature: settings of Turbovaps calibrated against calibrated thermometers, variations accounted for by control chart data - uncertainty accounted for in the validation data
  Gas Chromatographs: quality control criteria set out in SOPs determine when maintenance is required. Between sample and calibration variations are monitored by quality control charts - uncertainty accounted for in the validation data

- **Environmental Conditions:**
  Contamination is minimised by the use of dedicated accommodation, equipment and glassware for organic analysis. Glassware is also separated during cleaning - uncertainty accounted for in the validation data

- **Operator Effects:**

All measurement methods are described in fully documented Standard Operating Procedures (SOPs) to limit inconsistencies between operators - uncertainty accounted for in validation data

- **Matrix Effects:**
  Final sample fractions to be analysed are in *iso*-hexane - uncertainty accounted for in validation data

- **Computation Errors:**

Concentrations are calculated by chromatography software. Manual check of calculation has been carried out and acceptable – negligible contribution to uncertainty

- **Blank Correction:**

Method blank run alongside each batch of samples and is subtracted from each sample in the batch by use of an Excel spreadsheet. Manual check of calculation has been carried out and acceptable - uncertainty accounted for in the validation data

- **Random Effects:**

Uncertainty accounted for by validation and control chart data

Main Steps of Method: Sediment

Sediment samples are received frozen, these are freeze dried prior to sub sampling. To the samples is added recovery standard. Samples are exracted by ASE with *iso*-hexane. The sample sample extract is cleaned by adsorption chromatography. Internal standard is added to the resultant CB fraction. The CB fraction along with calibration standards are analysed by Gas Chromatography. Software calculates the concentrations of each determinand according to the calibration curves, and blank corrections are performed using an Excel spreadsheet. Method is monitored by the use of a Laboratory Reference Material. The instrument is checked using the response of the 0.2ug/g calibration standard and a standard checking for determinand breakdown. The laboratory (will) participate in the QUASIMEME laboratory intercomparison trials for these determinands.

Sources of Uncertainty:

- **Sampling:**
  Samples are analysed and results reported on the samples as received – out with uncertainty calculations

- **Sub-sampling:**
  Sediment samples are homogenised as far as possible prior to sub sampling - uncertainty accounted for in the validation data

- **Storage Conditions:**
  Samples are stored in aluminum cans to minimise contamination. Samples are stored at -20°C prior to freeze drying – negligible contribution to uncertainty

- **Reagent Purity:**
  Solvents are of at least HPLC grade quality – uncertainty accounted for in the validation data
  Sodium Sulphate are of at least Analar quality - uncertainty accounted for in the validation data
  Adsorbents are of unknown purity – uncertainty accounted for in the blank correction
  Chemical standards used in the preparation of calibration solutions are of the highest purity available at the time of purchase. Final concentrations of the calibration solutions have not been corrected for purity - uncertainty accounted for in the validation data

- **Instrument Effects:**
  Weight: Balance check weight tolerances; balances within allowable tolerances for decimal places, sufficient for accuracy required - uncertainty accounted for in the validation data
  Volume: syringes are calibrated to within required limits, tolerances stated in SOPs for measuring cylinders are sufficient for purpose - uncertainty accounted for in the validation data
  Temperature: settings of Turbovaps calibrated against calibrated thermometers, variations accounted for by control chart data - uncertainty accounted for in the validation data
  Gas Chromatographs: quality control criteria set out in SOPs determine when maintenance is required. Between sample and calibration variations are monitored by quality control charts - uncertainty accounted for in the validation data

- **Environmental Conditions:**
  Contamination is minimised by the use of dedicated accommodation, equipment and glassware for organic analysis. Glassware is also separated during cleaning - uncertainty accounted for in the validation data

- **Operator Effects:**

All measurement methods are described in fully documented Standard Operating Procedures (SOPs) to limit inconsistencies between operators - uncertainty accounted for in validation data

- **Matrix Effects:**
  Final sample fractions to be analysed are in *iso*-hexane - uncertainty accounted for in validation data

- **Computation Errors:**

Concentrations are calculated by chromatography software. Manual check of calculation has been carried out and acceptable – negligible contribution to uncertainty

- **Blank Correction:**

Method blank run alongside each batch of samples and is subtracted from each sample in the batch by use of an Excel spreadsheet. Manual check of calculation has been carried out and acceptable - uncertainty accounted for in the validation data

- **Random Effects:**

Uncertainty accounted for by validation and control chart data

Summary Validation Data:

Biota

Determinand	% Recovery of spiked fish liver	Precision	% Recovery of Mussels	Precision	Low Standard	High Standard	LRM Mean	LRM SD	LRM Variance
CB 31	93.35	16.61	74.21	12.17	1.76	1.73	9.74	2.18	22.42
CB 28	93.96	7.16	79.56	10.39	1.72	1.69	29.11	1.64	5.64
CB 52	92.90	11.26	83.42	11.64	2.63	1.99	88.16	4.36	4.94
CB 49	103.12	5.87	86.87	9.77	1.59	2.10	36.19	1.94	5.36
CB 44	101.29	9.98	93.76	4.78	2.33	1.81	38.56	2.34	6.08
CB 74	98.20	1.64	86.87	4.52	1.68	2.20	42.52	1.73	4.08
CB 70	97.88	7.37	87.56	8.31	2.24	1.32	44.99	1.49	3.31
CB 101	96.61	3.05	93.41	4.10	3.08	2.04	276.19	17.49	6.33
CB 99	98.46	1.37	81.44	9.56			140.91	7.34	5.21
CB 97	97.81	5.38	88.84	5.36			63.89	3.88	6.07
CB 110	86.03	14.63	84.37	12.79	1.06	1.79	208.64	23.94	11.47
CB 149	95.91	5.14	95.55	3.99	2.23	2.70	225.66	11.01	4.88
CB 118	98.22	7.53	97.12	3.71	1.75	1.66	270.32	17.35	6.42
CB 153	89.03	8.56	100.86	6.86	2.26	1.64	511.56	34.86	6.81
CB 132	84.42	13.27	83.99	15.34			81.26	11.50	14.16
CB 105	78.83	13.35	86.94	15.16	2.06	2.27	104.45	11.94	11.43
CB 137	93.72	7.23	103.22	3.94			20.29	0.83	4.07
CB 138	88.47	7.36	98.54	4.02	1.67	1.52	425.39	30.11	7.08
CB 158	110.89	8.67	113.68	3.91	2.22	1.41	41.98	1.89	4.51
CB 187	99.43	4.99	91.68	5.20	1.34	2.03	111.37	6.02	5.41
CB 183	100.19	3.09	98.09	4.77			58.43	3.29	5.63
CB 128	76.77	19.29	81.20	20.49	2.31	2.40	80.31	11.99	14.93
CB 156	95.40	9.25	101.80	5.66	1.42	1.69	51.03	3.22	6.32
CB 157	97.50	4.88	92.98	3.37	2.51	1.84	13.10	0.58	4.43
CB 180	89.32	2.17	92.77	6.22	3.37	2.88	179.57	12.95	7.21
CB 170	99.16	2.36	97.53	5.44	1.97	2.41	69.83	3.87	5.54
CB 189	88.26	1.52	82.77	11.03	1.87	2.15	3.48	0.27	7.66
CB 194	98.67	3.37	90.81	10.34	2.22	2.41	21.73	1.74	8.00
HCB	56.45	6.47	40.29	17.59	2.38	1.80	116.79	19.48	16.68
aldrin	50.98	11.83	46.71	15.43					
ppDDE	53.73	9.52	48.47	12.05	2.86	2.09	426.22	61.17	14.35
Alpha HCH	50.69	12.08	46.32	14.79	2.10	2.37	74.76	17.04	22.79
Gamma HCH	58.14	9.20	49.96	14.54	3.44	3.26	45.80	6.28	13.70
Heptachlor	52.94	6.79	47.36	8.23	1.55	3.86	1.31	0.33	25.09
Alpha Chlordene	50.24	20.33	39.35	18.22	2.13	3.77			
Gamma Chlordene	52.64	20.47	18.51	17.50	2.37	3.16			
Heptachlor Epoxide	60.51	15.27	51.66	17.33	2.54	3.91	9.34	1.40	15.02
Oxychlordane	67.62	7.97	45.40	13.29	1.80	3.89	19.91	3.30	16.55
Gamma Chlordane	55.06	22.34	38.95	15.07	2.28	4.06	5.32	1.22	23.02
o,p' DDE	64.06	10.21	53.73	23.88	2.54	5.35	23.33	4.42	18.93
Alpha Chlordane	67.44	8.80	48.99	13.69	2.70	2.93	44.51	5.54	12.44

Transnonachlor	66.23	10.93	44.87	17.71	2.17	3.80	69.30	10.70	15.43
Dieldrin	63.72	14.39	47.83	11.67	2.40	3.42	102.38	5.99	5.85
Endrin	64.40	4.16	45.98	14.05	2.61	3.41	45.77	9.57	20.91
o,p' DDD	63.19	9.04	55.99	13.66	1.36	3.40	71.67	15.52	21.66
p,p'-DDD	63.96	4.00	43.24	13.92	1.79	3.53	394.66	84.71	21.46
o,p'-DDT	55.93	8.96	44.39	17.35	2.45	3.03	31.21	7.91	25.34
p,p' DDT	62.48	8.16	45.21	12.39	1.02	3.38	203.74	27.51	13.50

Sediment

Determinand	% Recovery of spiked sediment	Precision	Low Standard precision	High Standard precision	LRM Mean	LRM SD	LRM Variance
CB 31	103.46	2.99	6.24	4.81	1.46	0.25	17.05
CB 28	102.59	3.55	8.01	4.52	1.97	0.41	20.87
CB 52	113.89	6.90	6.65	4.70	2.55	0.50	19.55
CB 49	121.40	4.55	5.16	4.64	1.29	0.29	22.39
CB 44	94.63	3.55	8.71	5.49	1.11	0.15	13.97
CB 74	80.76	4.79	6.31	4.85	1.15	0.14	12.40
CB 70	79.45	4.33	7.18	4.07	2.37	0.43	18.11
CB 101	85.43	4.53	6.49	2.82	3.02	0.40	13.39
CB 99	92.12	4.52	5.16	4.64	1.50	0.24	16.12
CB 97	90.40	3.83	4.76	5.71	0.99	0.12	12.45
CB 110	87.77	1.82	4.63	5.10	2.88	0.69	24.08
CB 149	71.03	3.82	4.38	3.64	4.09	0.70	17.09
CB 118	98.68	4.26	8.71	5.47	2.94	0.61	20.78
CB 153	78.17	7.04	12.20	9.47	5.91	0.83	14.12
CB 132	67.15	3.50	6.05	6.05	1.52	0.28	18.51
CB 105	100.72	5.10	4.31	3.93	1.11	0.23	20.86
CB 137	87.57	9.51	5.57	3.86	0.19	0.04	19.67
CB 138	78.92	6.82	6.56	4.28	5.40	1.01	18.63
CB 158	102.53	7.92	8.39	5.73	0.54	0.06	10.64
CB 187	79.87	9.99	17.96	15.25	2.95	0.50	16.90
CB 183	87.40	6.62	13.82	10.58	1.16	0.16	14.03
CB 128	71.35	5.32	4.67	3.49	0.75	0.18	23.82
CB 156	94.20	7.07	10.43	7.75	0.52	0.07	12.64
CB 157	84.95	7.29	7.64	4.34	0.26	0.03	11.99
CB 180	85.06	10.39	15.64	11.51	4.61	0.48	10.34
CB 170	89.86	9.25	8.20	6.78	1.72	0.25	14.53
CB 189	80.11	5.33	12.59	7.87	Tr	Tr	
CB 194	87.45	5.77	11.31	9.77	1.35	0.19	14.16

16. Uncertainty

16.1 Biota

Systematic Component (Recovery of Spiked fish Sample, V4) : e.g. CB153 = (89.03-100)/2% = - 5.48

Random Component (LRM CV%.): e.g. CB153 = (S.D.)/(mean) x100 = (SD)34.86 / (mean)511.56 x 100 = 6.81

Assume linear summation and a value of K=2

Combined Standard Uncertainty = $(C_s^2 + C_r^2)^{0.5}$ = X %

Expanded Uncertainty = 2 * $(C_s^2 + C_r^2)^{0.5}$ = X %

Expanded Uncertainty e.g. CB153 = $2*(-5.48^2 + 6.81\ ^2)^{0.5}$ ug/kg = 17.50 %

The reported expanded uncertainties are based on uncertainties multiplied by a coverage factor of k=2, providing a level of confidence of approximately %

Determinand	Systematic Component %	Random Component %	Expanded Uncertainity%
CB 31	-3.33	22.42	45.34
CB 28	-3.02	5.64	12.79
CB 52	-3.55	4.94	12.17
CB 49	1.56	5.36	11.17
CB 44	0.64	6.08	12.22
CB 74	-0.90	4.08	8.35
CB 70	-1.06	3.31	6.94
CB 101	-1.69	6.33	13.11
CB 99	-0.77	5.21	10.53
CB 97	-1.09	6.07	12.34
CB 110	-6.98	11.47	26.86
CB 149	-2.05	4.88	10.58
CB 118	-0.89	6.42	12.96
CB 153	-5.48	6.81	17.50
CB 132	-7.79	14.16	32.32
CB 105	-10.58	11.43	31.16
CB 137	-3.14	4.07	10.28
CB 138	-5.77	7.08	18.26
CB 158	5.44	4.51	14.14
CB 187	-0.29	5.41	10.83
CB 183	0.09	5.63	11.26
CB 128	-11.61	14.93	37.84
CB 156	-2.30	6.32	13.44
CB 157	-1.25	4.43	9.20
CB 180	-5.34	7.21	17.95
CB 170	-0.42	5.54	11.10
CB 189	-5.87	7.66	19.30
CB 194	-0.66	8.00	16.06
HCB	-21.77	16.68	54.86
aldrin	-24.51		49.02
ppDDE	-23.14	14.35	54.45
Alpha HCH	-24.65	22.79	67.15
Gamma HCH	-20.93	13.70	50.04
Heptachlor	-23.53	25.09	68.79
Alpha Chlordene			
Gamma Chlordene			
Heptachlor Epoxide	-19.75	15.02	49.61
Oxychlordane	-16.19	16.55	46.31
Gamma Chlordane	-22.47	23.02	64.33
o,p' DDE	-17.97	18.93	52.20
Alpha Chlordane	-16.28	12.44	40.97
Transnonachlor	-16.88	15.43	45.75
Dieldrin	-18.14	5.85	38.12
Endrin	-17.80	20.91	54.91
o,p' DDD	-18.40	21.66	56.84
p,p'-DDD	-18.02	21.46	56.05
o,p'-DDT	-22.04	25.34	67.16
p,p' DDT	-18.76	13.50	46.23

16.2 Sediment

Systematic Component (Recovery of sediment Sample, V4) : e.g. CB31 = (103.46-100)/2% = 1.73

Random Component (LRM 140 CV%.): e.g. CB31 = (S.D.)/(mean) x100 = (SD)0.83 / (mean)5.91 = 17.05

Assume linear summation and a value of K=2

Combined Standard Uncertainty = $(C_s^2 + C_r^2)^{0.5}$ = X %

Expanded Uncertainty = 2 * $(C_s^2 + C_r^2)^{0.5}$ = X %

Expanded Uncertainty e.g. CB31 = 2*$(1.73^2 + 17.05^2)^{0.5}$ ug/kg = 34.27 %

The reported expanded uncertainties are based on uncertainties multiplied by a coverage factor of k=2, providing a level of confidence of approximately %

Determinand	Systematic Component %	Random Component %	Expanded Uncertainity%
CB 31	1.73	17.05	34.27
CB 28	1.29	20.87	41.82
CB 52	6.95	19.55	41.50
CB 49	10.70	22.39	49.64
CB 44	-2.69	13.97	28.44
CB 74	-9.62	12.40	31.39
CB 70	-10.27	18.11	41.64
CB 101	-7.29	13.39	30.49
CB 99	-3.94	16.12	33.20
CB 97	-4.80	12.45	26.68
CB 110	-6.11	24.08	49.68
CB 149	-14.49	17.09	44.81
CB 118	-0.66	20.78	41.58
CB 153	-10.92	14.12	35.70
CB 132	-16.42	18.51	49.49
CB 105	0.36	20.86	41.72
CB 137	-6.21	19.67	41.26
CB 138	-10.54	18.63	42.81
CB 158	1.27	10.64	21.44
CB 187	-10.06	16.90	39.33
CB 183	-6.30	14.03	30.76
CB 128	-14.33	23.82	55.59
CB 156	-2.90	12.64	25.93
CB 157	-7.53	11.99	28.31
CB 180	-7.47	10.34	25.51
CB 170	-5.07	14.53	30.78
CB 189	-9.94		
CB 194	-6.27	14.16	30.98

1. Introduction and Scope

This method describes the determination of polycyclic aromatic hydrocarbons (PAHs) in sediments. The analysis incorporates two- to six-ring, both parent and branched PAHs. This does not cover all of the many PAH compounds that exist. The concentration range of the method is from the limit of detection to 10 mg g^{-1}.

2. Principle of the Method

The hydrocarbons, including PAHs, are extracted from the sediment by sonication in dichloromethane and methanol. The extract is purified and the PAHs separated from the aliphatic hydrocarbons using high performance liquid chromatography.

Quantitative analysis is carried out by gas chromatography with mass selective detection (GC-MSD) using a CPSil 8 column or equivalent. Deuterated PAH standards (D_8-naphthalene, D_{10}-biphenyl, D_8-dibenzothiophene, D_{10}-anthracene, D_{10}-pyrene and D_{12}-benzo[*a*]pyrene) are used as internal standards, and are added to the sediment before the extraction. The GC-MSD is calibrated using seven different concentrations of a solution containing 33 PAHs.

3. Reference Materials

LRM 142, Aberdeen Harbour sediment.

4. Reagents

See SOPs (1600, 1605, 1620, 1630, 1635 and 1640) for reagents used

5. Major Equipment

Two gas chromatographs with on column injectors and mass selective detectors (EN GC-MSD1 - EN294; GC-MSD 2 - EN 751) SOP 1625. Isocratic HPLC pump, analytical column and Rheodyne injector, SOP 1600.

6. Environmental Control

See individual SOPs (1600, 1605, 1620, 1630, 1635 and 1640).

7. Interferences

Hydrocarbon analysis requires a stable environment, avoiding contamination of samples and reagents eg contact with fingers, dirty equipment, smoking, exhaust fumes, newly painted surfaces, external hydrocarbon sources. Samples known to contain high concentrations of hydrocarbons (eg cuttings samples and sediments from close to wellheads) must be stored separately and extracted separately from other samples.

All new batches of *iso*-hexane and dichloromethane are checked for contamination as outlined in SOP 1620 and analysed by gas chromatography with flame ionisation detection (GC-FID), as described in SOP1610.

8. Sampling and Sample Preparation

Samples are logged into the laboratory according to SOP 60. Sediments are sub-sampled and mixed according to SOP 1600.

9. Analytical Procedure

9.1 A procedural blank and the LRM are analysed with each batch of samples. Procedural blanks are rejected if compound abundances in the blank exceed the values stated in the appendix to this method. If this is the case the procedural blank will be HPLCed again and if the problem persists the batch will be repeated.

9.2 The extraction of hydrocarbons, including PAHs, is carried out as detailed in SOP 1600 and recorded on B 561. An electronic copy is archived to ChemDat/sediment dry weights, using the batch number as the file name, and a paper copy is kept with the batch paperwork.

9.3 The dry weight and moisture content of the each sediment is carried out as in SOP 1615 and recorded on B 235.

9.4 The prepared extracts are cleaned-up, and separated from the aliphatic hydrocarbons, by HPLC as outlined in SOP 1600. The split time for the separation of aliphatics and PAHs on the HPLC is as outlined in SOP 1600 and the results recorded on B241.

9.5 The cleaned up extracts are concentrated by rotary evaporation (SOP 1640) and transferred to a GC vial with insert prior to analysis.

9.6 Analysis is performed by GC-MSD as outlined in SOP 1625. Sequences are set up as in SOP 1265 and results are quantified using SOP 1260.

9.7 Internal standards and calibration standards, required for quantitative analysis, are prepared as described in SOP 1605 and 1630.

10. Calculation of Results

The GC-MSD is calibrated and results are calculated using the HP data analysis software as described in SOP1260 and SOP1625 using the internal standard method. The correlation coefficient should be greater than 0.996 for the calibration curves. A check is made on the continuing validity of the calibration by running two calibration check solutions with each batch of samples (see SOP 1630 for preparation). The results are monitored using set limits, which are recorded on B 582 (EN294) and B 583 (EN751), and a copy of which is filed in each of the calcheck folders, which are stored next to the GC-MSDs. These limits are updated when new calibration solutions are prepared. The retention times of compounds in the calibration checks are also used to confirm retention times and identities of peaks in the LRM and in the samples. LRM data are monitored by plotting results on Shewhart charts with limits at ±2x and ±3x S.D. The concentration of

components in the procedural blanks must be below set limits, a copy of which is kept in the current Internal Standard file folder for reference.

Results are calculated using the PAH template (B237). The dry weight of the sediment and the amount of each PAH in the procedure blank is inserted in the table. The software programme carries out the blank subtraction and calculates the concentration of each PAH to give a figure in ng g^{-1} dry weight of sediment. A macro is available to transfer the results of large numbers of samples from the MSD computer to office computers, where they can then be transferred to the PAH template for use with the macro (B570). Use of this macro is described in SOP 1260.

Corrections for calibration standards of less than 99% purity are also carried out on this spreadsheet by the application of correction factors. Compounds for which correction factors are applied will be recorded on Annex 1 to the test batch. Correction factors are listed on record sheet B 563, a hardcopy of which will be attached to each batch sheet. B 563 is updated with each new calibration.

11. Precision, Accuracy and Practical Detection Limits

Limits of detection are calculated by multiplying the standard deviation of the mean of the lowest standard (0.005 ng ml^{-1}) by 4.65. See Appendix I.

12. Reports

A hardcopy of all data should be obtained (SOP 1260) and submitted to the Technical Manager along with other relevant documentation (SOP 1350). Batches of results are electronically archived (SOP 030) onto duplicate CDs (*via* internal CD writer). CDs are labelled with the archive dates, group name and contents, one is given to the Quality Manager for archive and the other is stored in rm C125. Paper copies of chromatograms are kept for one year. Test reports are archived to ChemDat/PAH using the batch number as the file name.

13. Safety

Safety for all relevant procedures are provided in the appropriate SOPs detailed above, with reference to COSHH assessments.

14. Literature References

See relevant SOPs (1600, 1615 and 1625)

15. Uncertainty of Measurement

Sampling:
Sampling not part of method. Samples are analysed and results reported on the samples as received – outwith uncertainty calculations.

Subsampling:
Processing – Error due to inhomogeneity of sample is minimised by mixing thoroughly in sample container - negligible contribution to uncertainty.

Injection on Rheodyne – Assume sample in vial is homogenous - negligible contribution to uncertainty.
Injection on GC-MS – Assume sample in vial is homogenous – negligible contribution to uncertainty.

Storage:
Samples are stored deep frozen to minimise degradation.

Reagent purity:
All solvents are from Rathburn Chemicals and of at least HPLC Grade, considered sufficient – uncertainty accounted for in validation data.
Other chemicals are at least Analar quality, considered sufficient – uncertainty accounted for in validation data.
Chemical standards used in the preparation of calibration solutions are of the highest purity available at time of purchase. Final concentrations of the calibration solutions have been corrected for purity- uncertainty accounted for in the validation data.

Instrument effects:
All syringes are solvent washed between samples.
Weight – Tolerance of balance – balances check weight tolerances 0.05% and 0.002%, 2,3 and 4 decimal places used, sufficient for accuracy required. Uncertainty accounted for in validation data.
Volume – Pipettes used for calibration standards calibrated to <1% . Uncertainty accounted for in validation data.
Temperature – Thermometer to measure rotary evaporator water bath temperature calibrated to <1^{o}C. Uncertainty accounted for in validation data.
Timer – Timer for HPLC flow calibrated to < 2 sec. Uncertainty accounted for in validation data.

Environmental conditions:
Contamination is minimised by the use of dedicated accommodation, equipment and glassware for organic analysis. Glassware is also separated during cleaning – uncertainty accounted for in validation data.

Computational Effects:
Integration of peaks by means of instrument software. Concentrations calculated by means of internal standard using instrument integrations. Manual checks of peak integrations are made for each sample, negligible contribution.

Blank Correction:
A procedural blank is analysed with each batch of samples. No contribution to uncertainty.

Operator Effects: Only trained personnel may perform method unsupervised. Variations between operators are accounted for by control chart data. Uncertainty accounted for in validation data.

Random Effects: These will be accounted for by validation data.

Summary of validation data

GC-MSD1 (EN294)

Compound	Recovery of 1ng g^{-1} %	Recovery of 10ng g^{-1} %	Recovery of 100ng g^{-1} %	Precision of Sediment (CV%)	Low Std (CV%)	High Std (CV%)	Mean (Shewhart)	SD (Shewhart)	CV%
Napthalene	127	108	123	15.0	0.3	0.1	266.19	25.17	9.46
2-Methylnaphthalene	120	121	114	12.3	1.0	0.2	463.27	59.75	12.90
1-Methylnaphthalene	121	122	118	12.2	1.0	0.2	362.80	44.24	12.19
C2-Naphthalene	103	104	95	18.8	1.4	0.2	1300.46	129.81	9.98
C3-Naphthalene	98	102	85	11.0	0.6	0.4	1273.01	188.34	14.79
C4-Naphthalene	95	106	99	12.4	0.7	0.4	996.35	139.18	13.97
Phenathrene	115	108	87	8.0	0.4	0.3	1229.38	152.33	12.39
Anthracene	98	107	99	11.3	0.4	0.4	323.69	39.19	12.11
C1-178	123	122	112	10.1	0.9	1.4	1208.69	142.26	11.77
C2-178	117	110	100	12.5	1.0	0.3	1318.46	182.21	13.82
C3-178	105	104	101	13.1	1.0	0.3	1615.92	221.39	13.70
Dibenzothiophene	114	110	104	8.9	0.4	0.3	159.20	14.74	9.26
Fluoranthene	101	106	93	10.4	0.4	0.2	2194.08	263.69	12.02
Pyrene	114	105	95	10.6	0.5	0.4	2004.94	242.91	12.12
C1-202	99	104	115	10.5	0.8	0.3	1216.07	148.18	12.19
C2-202	99	105	93	13.3	1.4	0.4	1517.60	264.10	17.40
Benzo[c]phenathrene	99	107	94	9.1	0.7	0.4	122.86	14.33	11.66
Benz[a]anthracene	100	106	88	14.9	1.6	0.2	842.46	127.10	15.09
Chrysene	94	104	88	12.9	0.7	0.3	1031.94	187.34	18.15
C1-228	86	101	95	10.8	2.1	0.2	1005.74	104.47	10.39
C2-228	115	110	123	11.2	3.0	3.1	2527.60	200.53	7.93
Benzo[b]fluoranthene	94	125	94	10.7*	2.2	0.2	3233.05*	320.23*	9.90*
Benzo[k]fluoranthene	97	118	106	10.7*	1.8	1.1	3233.05*	320.23*	9.90*
Benzo[e]pyrene	108	124	113	8.7	0.4	0.1	1063.86	91.32	8.58
Benzo[a]pyrene	117	122	112	6.1	0.2	0.2	1100.40	96.54	8.77
Perylene	103	120	107	8.0	0.9	0.2	376.90	33.40	8.86
C1-252	103	124	114	12.7	1.3	0.3	977.70	211.31	21.61
Indenopyrene	86	122	108	8.1	3.6	0.4	969.62	82.51	8.51
Benzoperylene	108	120	110	9.1	0.9	0.6	1031.89	78.39	7.60
Acenaphthylene	100	104	101	8.3	1.0	0.2	9.47	3.09	32.65
Acenaphthene	104	107	102	16.7	0.7	0.3	122.96	13.01	10.58
Fluorene	97	102	90	10.0	0.4	0.5	100.97	18.34	18.16
Dibenz[a,h]anthracene	112	118	118	8.2	2.8	0.4	227.02	32.60	14.36

* Data calculated for combined Benzofluoranthenes

GC-MSD2 (EN757)

Compound	Recovery of 1ng g^{-1} %	Recovery of 10ng g^{-1} %	Recovery of 100ng g^{-1} %	Precision of Sediment sample (CV%)	Low Std (CV%)	High Std (CV%)
Napthalene	124	109	125	8.9	0.3	0.3
2-Methylnaphthalene	119	126	120	10.9	0.9	0.3
1-Methylnaphthalene	125	128	124	10.1	0.6	0.2
C2-Naphthalene	93	105	94	7.4	0.7	0.3
C3-Naphthalene	100	102	90	4.8	0.4	0.4
C4-Naphthalene	97	108	102	4.5	0.4	0.2
Phenathrene	107	105	90	4.2	1.0	0.2
Anthracene	95	109	108	4.4	0.2	0.2
C1-178	95	107	96	3.8	1.5	0.3
C2-178	92	113	121	3.5	2.2	0.3
C3-178	92	112	116	4.7	1.3	0.5
Dibenzothiophene	77	101	99	4.3	0.3	0.5
Fluoranthene	92	99	85	4.8	0.5	0.2
Pyrene	96	99	91	4.6	0.4	0.3
C1-202	99	105	98	5.1	1.1	0.3
C2-202	82	100	87	12.4	2.2	0.2
Benzo[c]phenathrene	63	102	89	4.9	1.5	0.4
Benz[a]anthracene	83	96	83	5.7	3.4	0.2
Chrysene	74	97	110	5.3	1.2	0.5
C1-228	89	103	92	5.5	3.3	0.2
C2-228	103	99	115	2.9	1.8	1.3
Benzo[b]fluoranthene	41	80	65	18.8	2.7	0.6
Benzo[k]fluoranthene	102	115	113	3.6	0.4	0.5
Benzo[e]pyrene	102	120	118	2.9	0.5	0.6
Benzo[a]pyrene	82	110	99	2.4	0.3	0.3
Perylene	92	115	95	2.6	1.0	0.3
C1-252	83	117	104	6.4	2.7	0.2
Indenopyrene	113	120	114	2.4	3.0	0.4
Benzoperylene	103	127	115	2.8	2.1	0.4
Acenaphthylene	100	105	97	5.7	0.6	0.1
Acenaphthene	101	110	103	4.6	0.6	0.3
Fluorene	96	101	91	4.9	0.4	0.3
Dibenz[a,h]anthracene	101	122	119	6.6	2.8	0.5

No Shewhart chart data available as no samples analysed.

Combined uncertainty:
Systematic component: recovery of spike (ng)/ spike added (ng) x 100/1= Y.
100-Y = Z/2% = C_r
Spike added is 100ng/g.

Random component (CV% Shewhart chart) = C_s
(for GC-MSD2 the CV% is the precision of the sediment sample as no Shewhart chart data available)

Assume linear summation and a value of K=2:

Combined standard uncertainty = $(C_r^{\,2} + C_s^{\,2})^{0.5}$ ng
Expanded uncertainty = $2*(C_r^{\,2} + C_s^{\,2})^{0.5}$ ng

The reported expanded uncertainty is based on an uncertainty multiplied by a coverage factor of K= 2, providing a level of confidence of approximately 95%.

GC-MSD1 (EN294)

Compound	Systematic component %	Random Component %	Expanded Uncertainty %
Napthalene	-11.50	9.46	29.78
2-Methylnaphthalene	-7.00	12.90	29.35
1-Methylnaphthalene	-9.00	12.19	30.31
C2-Naphthalene	2.50	9.98	20.58
C3-Naphthalene	7.50	14.79	33.17
C4-Naphthalene	0.50	13.97	27.96
Phenathrene	6.50	12.39	27.98
Anthracene	0.50	12.11	24.24
C1-178	-6.00	11.77	26.42
C2-178	0.00	13.82	27.64
C3-178	-0.50	13.70	27.42
Dibenzothiophene	-2.00	9.26	18.95
Fluoranthene	3.50	12.02	25.04
Pyrene	2.50	12.12	24.74
C1-202	-7.50	12.19	28.62
C2-202	3.50	17.40	35.50
Benzo[c]phenathrene	3.00	11.66	24.09
Benz[a]anthracene	6.00	15.09	32.47
Chrysene	6.00	18.15	38.24
C1-228	2.50	10.39	21.37
C2-228	-11.50	7.93	27.94
Benzo[b]fluoranthene	3.00	9.90*	20.69*
Benzo[k]fluoranthene	-3.00	9.90*	20.69*
Benzo[e]pyrene	-6.50	8.58	21.53
Benzo[a]pyrene	-6.00	8.77	21.26
Perylene	-3.50	8.86	19.06
C1-252	-7.00	21.61	45.44
Indenopyrene	-4.00	8.51	18.80
Benzoperylene	-5.00	7.60	18.19
Acenaphthylene	-0.50	32.65	65.30
Acenaphthene	-1.00	10.58	21.26
Fluorene	5.00	18.16	37.67
Dibenz[a,h]anthracene	-9.00	14.36	33.89

* Data calculated for combined Benzofluoranthenes

GC-MSD2 (EN757)

Compound	Systematic component %	Random Component %	Expanded Uncertainty %
Napthalene	-12.50	8.9	30.69
2-Methylnaphthalene	-10.00	10.9	29.58
1-Methylnaphthalene	-12.00	10.1	31.37
C2-Naphthalene	3.00	7.4	15.97
C3-Naphthalene	5.00	4.8	13.86
C4-Naphthalene	-1.00	4.5	9.22
Phenathrene	5.00	4.2	13.06
Anthracene	-4.00	4.4	11.89
C1-178	2.00	3.8	8.59
C2-178	-10.50	3.5	22.14
C3-178	-8.00	4.7	18.56
Dibenzothiophene	0.50	4.3	8.66
Fluoranthene	7.50	4.8	17.81
Pyrene	4.50	4.6	12.87
C1-202	1.00	5.1	10.39
C2-202	6.50	12.4	28.00
Benzo[c]phenathrene	5.50	4.9	14.73
Benz[a]anthracene	8.50	5.7	20.47
Chrysene	-5.00	5.3	14.57
C1-228	4.00	5.5	13.60
C2-228	-7.50	2.9	16.08
Benzo[b]fluoranthene	17.50	18.8	51.37
Benzo[k]fluoranthene	-6.50	3.6	14.86
Benzo[e]pyrene	-9.00	2.9	18.91
Benzo[a]pyrene	0.50	2.4	4.90
Perylene	2.50	2.6	7.21
C1-252	-2.00	6.4	13.41
Indenopyrene	-7.00	2.4	14.80
Benzoperylene	-7.50	2.8	16.01
Acenaphthylene	1.50	5.7	11.79
Acenaphthene	-1.50	4.6	9.68
Fluorene	4.50	4.9	13.31
Dibenz[a,h]anthracene	-9.50	6.6	23.14

Upper Limits (ngs) for Abundances in Procedural Blanks

Naphthalene	5
2-Methyl Naphthalene	5
1-Methyl Naphthalene	5
C2 Naphthalenes	10
C3 Naphthalenes	10
C4 Naphthalenes	10
Phenanthrene (178)	3
Anthracene (178)	3
C1 178	10
C2 178	10
C3 178	10
Dibenzothiophene	3
C1 Dibenzothiophenes	5
C2 Dibenzothiophenes	5
C3 Dibenzothiophenes	5
Fluoranthene (202)	5
Pyrene (202)	5
C1 202	5
C2 202	10
C3 202	10
Benzo[*c*]phenanthrene (228)	3
Benz[*a*]anthracene (228)	3
Chrysene/Triphenylene (228)	3
Benz[*b*]anthracene (228)	1
C1 228	10
C2 228	10
Benzofluoranthenes (252)	3
Benzo[*e*]pyrene (252)	3
Benzo[*a*]pyrene (252)	3
Perylene (252)	5
C1 252	10
C2 252	10
Indenopyrene (276)	5
Benzoperylene (276)	5
C1 276	10
C2 276	10
Acenaphthylene(152)	1
Acenaphthene(154)	1
Fluorene(166)	1
Dibenz[*a,h*]anthracene(278)	1

1. Introduction and Scope

This method describes the determination of polycyclic aromatic hydrocarbons (PAHs) in biota. The analysis incorporates two- to six-ring, both parent and branched PAHs. This does not cover all of the many PAH compounds that exists. The concentration range of the method is from the limit of detection to 10 mg g^{-1}.

2. Principle of the Method

The hydrocarbons, including PAHs, are extracted from the biota by saponification followed by a two phase separation. The extract is purified and the PAHs separated from the aliphatic hydrocarbons using high performance liquid chromatography.

Quantitative analysis is carried out by gas chromatography with mass selective detection (GC-MSD) using a CPSil 8 column or equivalent. Deuterated PAH standards (D_8-naphthalene, D_{10}-biphenyl, D_8-dibenzothiophene, D_{10}-anthracene, D_{10}-pyrene and D_{12}-benzo[*a*]pyrene) are used as internal standards, and are added to the biota before the extraction. The GC-MSD is calibrated using seven different concentrations of a solution containing 33 PAHs.

3. Reference Materials

LRM148, homogenised freeze dried Aberdeen Harbour mussel tissue..

4. Reagents

See SOPs for reagents used.

5. Equipment

Two gas chromatographs with on column injector and mass selective detector (GC-MSD 1 - EN 294; GC-MSD 2 - EN 751) SOP 1625. Isocratic HPLC pump, analytical column and Rheodyne injector.

6. Environmental Control

See individual SOPs.

7. Interferences

Hydrocarbon analysis requires a stable environment, avoiding contamination of samples and reagents eg contact with fingers, dirty equipment, smoking, exhaust fumes, newly painted surfaces, external hydrocarbon sources. Samples known to contain high concentrations of hydrocarbons must be stored separately and extracted separately from other samples

.

All new batches of *iso*-hexane and dichloromethane are checked for contamination as outlined in SOP 1620 and analysed by gas chromatography with flame ionisation detection (GC-FID), as described in SOP1610.

8. Sampling and Sample Preparation

Samples are logged into the laboratory according to SOP 60. Samples are sub-sampled and mixed according to SOP 1660.

9. Analytical Procedure

9.1 A procedural blank and the LRM are analysed with each batch of samples. Shewart charts for some PAHs have a negative action limit (ie - 3 x S.D.). In such cases an absolute limit is set and will remain in place until limits are re-calculated (as outlined in SOP 1380). Procedural blanks are rejected if compound abundances in the blank exceed the values stated in the appendix to this method. If this is the case the procedural blank will be HPLCed again and if the problem persists the batch will be repeated.

9.2 The extraction of hydrocarbons, including PAHs, is carried out as detailed in SOP 1660 and recorded on B 561.

9.3 The prepared extracts are cleaned-up, and separated from the aliphatic hydrocarbons, by HPLC as outlined in SOP 1660. The HPLC split time for aliphatics and PAHs is as outlined in SOP 1660, with the results being reported on worksheet B 241.

9.4 The cleaned up extracts are concentrated by rotary evaporation (SOP 1640) and transferred to a GC vial with insert prior to analysis.

9.5 Analysis is performed by GC-MSD as outlined in SOP 1625. Sequences are set up as in SOP 1265 and results are quantified using SOP 1260.

9.6 Internal standards and calibration standards, required for quantitative analysis, are prepared as described in SOPs 1605 and 1630.

10. Calculation of Results

The GC-MSD is calibrated and results are calculated using the HP data analysis software as described in SOP 1260 and SOP 1625 using the internal standard method. The correlation coefficient should be greater than 0.996 for the calibration curves. A check is made on the continuing validity of the calibration by running two calibration check solutions (see SOP 1630 for preparation details) with each batch of samples. The results are monitored using set limits, a copy of which is filed in each of the calcheck folders. These limits are updated when new calibration solutions are prepared (limits recorded on B 582 (EN294) and B 583 (EN751). The retention times of the compounds in the calibration checks are also used to confirm retention times and identities of peaks in the LRM and the samples. LRM data are monitored by plotting results on Shewhart charts with limits at ±2x and ±3x S.D. The concentration of components in the procedural blanks must be below set limits, a copy of which is kept in the current Internal Standard file folder for reference.

Results are calculated using the PAH template (B237). The wet weight of the sample and the amount of each PAH in the procedure blank is inserted in the table. The

software programme carries out the blank subtraction and calculates the concentration of each PAH to give a figure in ng g^{-1} wet weight of biota. A macro is available to transfer the results of large numbers of samples from the MSD computer to any computer with the macro installed, where they can then be transferred to the PAH template for data entry using a macro (B 570). Use of this macro is described in SOP 1260.

Corrections for calibration standards of less than 99% purity are also carried out on this spreadsheet by the application of correction factors. Compounds for which correction factors are applied will be recorded in Annex 1 to the test batch. Correction factors are listed on record sheet B 563, a hardcopy of which will be attached to each batch sheet. B 563 is updated with each new calibration.

11. Precision, accuracy and practical detection limits

Limits of detection are calculated by multiplying the standard deviation of the mean of the lowest standard (0.005 ng ml^{-1}) by 4.65. See Appendix I.

12. Reports

A hardcopy of all data should be obtained (SOP 1260) and submitted to the Technical Manager along with other relevant documentation (SOP 1350). Batches of results are electronically archived (SOP 030) onto duplicate CDs (*via* internal CD writer). CDs are labelled with archive dates, group name and contents, one is given to the Quality Managerfor archive and the other is stored in rm C125. Paper copies of chromatograms are kept for one year. Test reports are archived to ChemDat/PAH using the batch number as the file name.

13. Safety

Safety for all relevant procedures are provided in the appropriate SOPs detailed above, with reference to risk assessments.

14. Literature references

See relevant SOPs.

15. Uncertainty of Measurement

Sampling:
Sampling not part of method. Samples are analysed and results reported on the samples as received – outwith uncertainty calculations.

Subsampling:
Processing – Error due to inhomogeneity of sample is minimised by mixing thoroughly in sample container - negligible contribution to uncertainty.
Injection on Rheodyne – Assume sample in vial is homogenous - negligible contribution to uncertainty.

Injection on GC-MS – Assume sample in vial is homogenous – negligible contribution to uncertainty.

Storage:
Samples are stored deep frozen to minimise degradation.

Reagent purity:
All solvents are from Rathburn Chemicals and of at least HPLC Grade, considered sufficient – uncertainty accounted for in validation data.
Other chemicals are at least Analar quality, considered sufficient – uncertainty accounted for in validation data.
Chemical standards used in the preparation of calibration solutions are of the highest purity available at time of purchase. Final concentrations of the calibration solutions have been corrected for purity- uncertainty accounted for in the validation data.

Instrument effects:
All syringes are solvent washed between samples.
Weight – Tolerance of balance – balances check weight tolerances 0.05% and 0.002%, 2,3 and 4 decimal places used, sufficient for accuracy required. Uncertainty accounted for in validation data.
Volume – Pipettes used for calibration standards calibrated to <1% . Uncertainty accounted for in validation data.
Temperature – Thermometer to measure rotary evaporator water bath temperature calibrated to <1^{o}C. Uncertainty accounted for in validation data.
Timer – Timer for HPLC flow calibrated to < 2 sec. Uncertainty accounted for in validation data.

Environmental conditions:
Contamination is minimised by the use of dedicated accommodation, equipment and glassware for organic analysis. Glassware is also separated during cleaning – uncertainty accounted for in validation data.

Computational Effects:
Integration of peaks by means of instrument software. Concentrations calculated by means of internal standard using instrument integrations. Manual checks of peak integrations are made for each sample, negligible contribution.
Blank Correction:
A procedural blank is analysed with each batch of samples. No contribution to uncertainty.

Operator Effects: Only trained personnel may perform method unsupervised. Variations between operators are accounted for by control chart data. Uncertainty accounted for in validation data.

Random Effects: These will be accounted for by validation data.

Summary of validation data

GC-MSD1 (EN294)

Compound	Recovery of 1ng g^{-1} %	Recovery of 10ng g^{-1} %	Recovery of 100ng g^{-1} %	Precision of Biota Sample (CV%)	Low Std (CV%)	High Std (CV%)	Mean (Shewhart)	SD (Shewhart)	CV%
Napthalene	126	123	129	2.0	0.3	0.1	963.09	49.50	5.14
2-Methylnaphthalene	119	115	126	7.5	1.0	0.2	2637.25	137.15	5.20
1-Methylnaphthalene	121	116	129	8.2	1.0	0.2	2321.68	138.30	5.96
C2-Naphthalene	102	101	99	2.3	1.4	0.2	5867.70	578.69	9.86
C3-Naphthalene	88	88	82	4.5	0.6	0.4	5034.33	363.91	7.23
C4-Naphthalene	96	94	94	2.9	0.7	0.4	2203.74	309.62	14.05
Phenathrene	98	96	93	2.3	0.4	0.3	427.81	34.52	8.07
Anthracene	99	99	103	2.6	0.4	0.4	16.09	5.24	32.58
C1-178	117	114	105	2.2	0.9	1.4	1269.66	60.76	4.79
C2-178	112	105	96	2.7	1.0	0.3	1179.58	76.17	6.46
C3-178	94	98	95	3.0	1.0	0.3	772.09	47.97	6.21
Dibenzothiophene	104	102	109	2.8	0.4	0.3	156.26	6.36	4.07
Fluoranthene	101	100	97	3.9	0.4	0.2	18.24	6.15	33.71
Pyrene	99	101	97	3.8	0.5	0.4	33.64	4.34	12.90
C1-202	122	103	97	3.5	0.8	0.3	169.31	14.01	8.27
C2-202	92	96	97	4.0	1.4	0.4	223.51	71.94	32.19
Benzo[c]phenathrene	95	98	101	2.5	0.7	0.4	988.56	32.85	3.32
Benz[a]anthracene	92	97	95	3.4	1.6	0.2	9.95	2.59	26.04
Chrysene	92	96	96	4.2	0.7	0.3	32.70	4.74	14.50
C1-228	89	91	96	2.6	2.1	0.2	107.49	8.59	7.99
C2-228	130	126	135	3.4	3.0	3.1	190.93	37.94	19.87
Benzo[b]fluoranthene	114	99	84	7.2	2.2	0.2	*	*	
Benzo[k]fluoranthene	100	104	99	6.6	1.8	1.1	*	*	
Benzo[e]pyrene	96	102	103	4.0	0.4	0.1	6.80	0.73	10.74
Benzo[a]pyrene	106	105	110	3.3	0.2	0.2	6.27	0.91	14.51
Perylene	100	105	115	2.7	0.9	0.2	394.25	33.94	8.61
C1-252	108	108	104	3.4	1.3	0.3	34.47	11.86	34.41
Indenopyrene	92	99	88	3.1	3.6	0.6	784.02	80.57	10.28
Benzoperylene	97	101	104	2.6	0.9	0.4	785.95	161.94	20.60
Acenaphthylene	103	102	104	3.6	1.0	0.2	0.69	0.34	49.28
Acenaphthene	107	107	107	4.7	0.7	0.3	47.45	11.49	24.21
Fluorene	89	88	87	4.5	0.4	0.5	208.40	12.47	5.98
Dibenz[a,h]anthracene	89	98	88	3.5	2.8	0.4	797.52	128.62	16.13

* Data only available for combined Benzofluoranthenes

GC-MSD2 (EN751)

Compound	Recovery of 1ng g^{-1}	Recovery of 10ng g^{-1}	Recovery of 100ng g^{-1}	Precision of Biota Sample	Low Std	High Std

	%	%	%	(CV%)	(CV%)	(CV%)
Napthalene	112	121	127	2.2	0.3	0.3
2-Methylnaphthalene	126	122	129	7.2	0.9	0.3
1-Methylnaphthalene	127	122	133	8.0	0.6	0.2
C2-Naphthalene	103	103	97	2.4	0.7	0.3
C3-Naphthalene	89	90	89	4.2	0.4	0.4
C4-Naphthalene	93	92	103	3.0	0.4	0.2
Phenathrene	93	102	91	2.6	1.0	0.2
Anthracene	70	96	106	5.5	0.2	0.2
C1-178	92	104	102	2.2	1.5	0.3
C2-178	99	111	115	2.2	2.2	0.3
C3-178	85	111	119	2.5	1.3	0.5
Dibenzothiophene	98	97	100	1.8	0.3	0.5
Fluoranthene	92	96	92	4.7	0.5	0.2
Pyrene	93	96	93	4.0	0.4	0.3
C1-202	90	100	97	2.9	1.1	0.3
C2-202	86	89	104	3.5	2.2	0.2
Benzo[c]phenathrene	94	89	103	4.5	1.5	0.4
Benz[a]anthracene	80	87	92	10.8	3.4	0.2
Chrysene	86	92	97	15.8	1.2	0.5
C1-228	28	88	98	2.4	3.3	0.2
C2-228	72	115	118	2.7	1.8	1.3
Benzo[b]fluoranthene	42	71	57	4.4	2.7	0.6
Benzo[k]fluoranthene	107	95	104	11.9	0.4	0.5
Benzo[e]pyrene	99	103	98	4.4	0.5	0.6
Benzo[a]pyrene	89	90	95	5.4	0.3	0.3
Perylene	93	94	98	4.8	1.0	0.3
C1-252	46	94	96	4.1	2.7	0.2
Indenopyrene	77	97	89	3.7	3.0	0.4
Benzoperylene	94	81	100	3.6	2.1	0.4
Acenaphthylene	95	96	102	4.3	0.6	0.1
Acenaphthene	99	102	108	4.1	0.6	0.3
Fluorene	81	80	90	5.0	0.4	0.3
Dibenz[a,h]anthracene	46	96	93	4.2	2.8	0.5

No Shewhart chart data available as no samples analysed.

Combined uncertainty:
Systematic component: recovery of spike (ng)/ spike added (ng) x 100/1= Y.
100-Y = Z/2% = C_s
Spike added is 100ng g^{-1}

Random component (CV% Shewhart chart) = C_r
(random component of GC-MSD2 is from Cv% of precision of biota samples as no Shewhart chart data available)

Assume linear summation and a value of K=2:

Combined standard uncertainty = $(C_s^2 + C_r^2)^{0.5}$ ng

Expanded uncertainty = $2*(C_s^2 + C_r^2)^{0.5}$ ng

The reported expanded uncertainty is based on an uncertainty multiplied by a coverage factor of K= 2, providing a level of confidence of approximately 95%.

GC-MSD1 (EN294)

Compound	Systematic component %	Random Component %	Expanded Uncertainty %
Napthalene	-14.50	5.14	30.77
2-Methylnaphthalene	-13.00	5.20	28.00
1-Methylnaphthalene	-14.50	5.96	31.35
C2-Naphthalene	0.50	9.86	19.75
C3-Naphthalene	9.00	7.23	23.09
C4-Naphthalene	3.00	14.05	28.73
Phenathrene	3.50	8.07	17.59
Anthracene	-1.50	32.58	65.22
C1-178	-2.50	4.79	10.80
C2-178	2.00	6.46	13.52
C3-178	2.50	6.21	13.39
Dibenzothiophene	-4.50	4.07	12.14
Fluoranthene	1.50	33.71	67.49
Pyrene	1.50	12.90	25.98
C1-202	1.50	8.27	16.82
C2-202	1.50	32.19	64.44
Benzo[c]phenathrene	-0.50	3.32	6.72
Benz[a]anthracene	2.50	26.04	52.33
Chrysene	2.00	14.50	29.27
C1-228	2.00	7.99	16.48
C2-228	-17.50	19.87	52.96
Benzo[b]fluoranthene	8.00		
Benzo[k]fluoranthene	0.50		
Benzo[e]pyrene	-1.50	10.74	21.68
Benzo[a]pyrene	-5.00	14.51	30.70
Perylene	-7.50	8.61	22.84
C1-252	-2.00	34.41	68.94
Indenopyrene	6.00	10.28	23.80
Benzoperylene	-2.00	20.60	41.40
Acenaphthylene	-2.00	49.28	98.63
Acenaphthene	-3.50	24.21	48.93
Fluorene	6.50	5.98	17.67
Dibenz[a,h]anthracene	6.00	16.13	34.42

GC-MSD2 (EN751)

Compound	Systematic component %	Random Component %	Expanded Uncertainty %
Napthalene	-13.50	2.20	27.36
2-Methylnaphthalene	-14.50	7.20	32.38
1-Methylnaphthalene	-16.50	8.00	36.67
C2-Naphthalene	1.50	2.40	5.66
C3-Naphthalene	5.50	4.20	13.84
C4-Naphthalene	-1.50	3.00	6.71
Phenathrene	4.50	2.60	10.39
Anthracene	-3.00	5.50	12.53
C1-178	-1.00	2.20	4.83
C2-178	-7.50	2.20	15.63
C3-178	-9.50	2.50	19.65
Dibenzothiophene	0.00	1.80	3.60
Fluoranthene	4.00	4.70	12.34
Pyrene	3.50	4.00	10.63
C1-202	1.50	2.90	6.53
C2-202	-2.00	3.50	8.06
Benzo[c]phenathrene	-1.50	4.50	9.49
Benz[a]anthracene	4.00	10.80	23.03
Chrysene	1.50	15.80	31.74
C1-228	1.00	2.40	5.20
C2-228	-9.00	2.70	18.79
Benzo[b]fluoranthene	21.50	4.40	43.89
Benzo[k]fluoranthene	-2.00	11.90	24.13
Benzo[e]pyrene	1.00	4.40	9.02
Benzo[a]pyrene	2.50	5.40	11.90
Perylene	1.00	4.80	9.81
C1-252	2.00	4.10	9.12
Indenopyrene	5.50	3.70	13.26
Benzoperylene	0.00	3.60	7.20
Acenaphthylene	-1.00	4.30	8.83
Acenaphthene	-4.00	4.10	11.46
Fluorene	5.00	5.00	14.14
Dibenz[a,h]anthracene	3.50	4.20	10.93

Appendix

Upper Limits (ngs) for Abundances in Procedural Blanks

Naphthalene	5
2-Methyl Naphthalene	5
1-Methyl Naphthalene	5
C2 Naphthalenes	10
C3 Naphthalenes	10
C4 Naphthalenes	10
Phenanthrene (178)	3
Anthracene (178)	3
C1 178	10
C2 178	10
C3 178	10
Dibenzothiophene	3
C1 Dibenzothiophenes	5
C2 Dibenzothiophenes	5
C3 Dibenzothiophenes	5
Fluoranthene (202)	5
Pyrene (202)	5
C1 202	5
C2 202	10
C3 202	10
Benzo[*c*]phenanthrene (228)	3
Benz[*a*]anthracene (228)	3
Chrysene/Triphenylene (228)	3
Benz[*b*]anthracene (228)	1
C1 228	10
C2 228	10
Benzofluoranthenes (252)	3
Benzo[*e*]pyrene (252)	3
Benzo[*a*]pyrene (252)	3
Perylene (252)	5
C1 252	10
C2 252	10
Indenopyrene (276)	5
Benzoperylene (276)	5
C1 276	10
C2 276	10
Acenaphthylene(152)	1
Acenaphthene(154)	1
Fluorene(166)	1
Dibenz[*a,h*]anthracene(278)	1

1. Introduction and Scope

The method is used to determine particle size distributions of marine sediments in the range 0.02-2000 microns.

2. Principle of the Method

The Mastersizer is a light-scattering based particle sizer comprised of an optical measurement unit and computer. The angle through which light is scattered by a particle is proportional to the particle's size, and this property is used to determine the size distribution of the sample.

3. Reference Materials

Instrument LRMs:
Glass beads are used as an LRM see B 040 for details, The LRMs are analysed prior to analysis of samples on each day of use and at random every 25 samples the results are recorded on B 103 and a quality control chart. See SOP 1380 for use of quality control charts.

The machine is validated each year by Malvern instruments using a reticule. The Operational Qualification Certificate and the Certificate of Calibration and Traceability for the reference material used for this are available in the Mastersizer 2000 maintenance folder. The LRMs are analysed immediately after the validation has been performed.

4. Reagents

No reagents are required for marine sediments, as the sample is added directly into a water bath.

5. Equipment

Optical measurement unit - Malvern Mastersizer 2000 / EN 1185
Hydro 2000G EN 1186
Mastersizer 2000 Software V5.1
Computer EN 1187
Printer EN1188
Rough 1000 micron sieve EN 1205
Rough 1400 micron sieve EN 1206
Rough 2000 micron sieve EN 1207

Spatula
Tap water wash bottle
Lens cleaning tissues
Plastic Trays

Sieve brush
Calibrated balance
Microsoft Excel Software

6. Environmental Control

The Mastersizer laser mechanism is sited on an optical bench to reduce interference due to vibration. Sites experiencing extremes of light and heat should be avoided.
Freezer temperatures are set and monitored as in SOP 0280.

7. Interference's

The production of bubbles is limited by the use of ultrasonic treatment as the sample is introduced into the water bath.

Dust or smears on the lenses or cell windows should be removed by following the procedure outlined on page10.3 in the maintenance section of the manual.

If maintenance work has been carried out on the header tanks check the water is running clear from the other taps in the lab before starting any analysis.

8. Sampling and Sample Preparation

Samples should be logged according to SOP 0060 and all batch paperwork filled in according to SOP 0065 Sample pre-treatment should be noted on form B 062. The samples may be introduced either as a wet slurry or as a freeze-dried powder (see SOPs 0110 and 0120) freeze dried samples are preferred as it is easier to get a representative sub sample. Breaking up of the sample, e.g. by pressing sample firmly through sieve apertures, should be avoided.

9. Analytical Procedure

9.1 Switching On

The Mastersizer is normally kept switched on and warmed up; Ensure Mastersizer and PC are switched on. If for any reason it has been switched off the switch on procedure is;

Ensure Mastersizer is switched **off** at the right hand side of the unit. Switch mains power on - then switch on at right hand side of the unit. Allow at least five minutes for the laser to warm up. Switch on computer, in windows double click the Mastersizer 2000 icon. At the prompt enter your username and password, if your password has been forgotten or there are any other problems logging on see the Technical Manager.

9.2 Sample Logging

9.2.1 Each batch of samples should be saved in a separate sample file. Each "record" within a "sample file" corresponds to a "sample" in the Laboratory sense.

Once logged on go to **File** and select either **New** if starting a new batch or **Open** if continuing an existing one. If new selected then enter the batch number as the file name (Batch xxxx) then click **save**.

9.3 Default Settings

The Mastersizer has been set up so that the SOP settings are those which are most commonly used in routine analyses. The data output is in the form of percentage amounts by volume of the sample lying within specified size bands. The default size bands are based on a "PHI" units scale, commonly used within Geological literature.

Statistical descriptors such as mean, median %< 20 microns are also displayed.

9.4 Software Set-up

Go to **Measure** then select **Start SOP**, select either **LRM standard.sop** for LRM's. Or **Sediment.sop** for samples to be measured by the MS2000 only or **Sediment2.sop** for samples being measured by the MS2000 and sieved.

9.5 Sample Measurement

9.5.1 The analyser will then automatically align the laser and measure the background. If the background is higher than 150 stop the analyser by clicking the **Close** icon in the Sop window, then click **OK** in the prompt box. If the background is too high go to **Configure** then select **Accessories**, tick the **Degas at end of fill or clean sequence** box then select **Clean**. The analyser will then run a cleaning sequence when this has finished click on **Close** and return to 9.4.

9.5.2 If the background is still too high the lens windows may require cleaning. Return to **Configure** then select **Accessories** and select **Empty**. When the tank has emptied turn the handle on top of the instrument 90° clockwise and remove the sample cell. The cell windows should be removed one at a time using the window tool.

9.5.3 The window should be held by the edges to avoid getting fingerprints on it. It should be rinsed with de-ionised water; excess water can be blown off using the compressed air in lab 503a. The window should then be cleaned using a new lens cloth.

9.5.4 Replace the cell windows and sample cell and check the background again as in 9.4. If the problem persists consult the Technical Manager.

9.5.5 By visual inspection, ensure that the chamber is clear of any sediment or particulates - if any are found, run a clean sequence as in 9.5.1.

9.6 Daily Calibration

Run the LRMs at the start of each day, by selecting **LRM standard.sop** as in 9.4

When the analyser has aligned itself and carried out background measurements it will bring up a documentation screen.

Prior to any analysis the rough sieves (EN1205, EN1206, and EN1207) should be visually checked for any damage, scoring, blinding, or contamination likely to affect the performance of the sieve. A record of the inspection of the sieves and any other comments should be recorded on the documentation screen.

Enter the appropriate sample name, LRM……..ref.xx (see B 103 for next number)

After the documentation screen has been completed the analysis screen will be displayed.

The lrm should be added directly to the tank until a minimum obscuration of 2.5% is achieved. The glass beads give a good signal to noise ratio (this is the light energy value for the sample compared to that for the background check), so a lower obscuration value than is used for samples is acceptable.

The mean diameter D (4,3) should lie within the limits determined by the control chart.

Mean diameter for the day should be recorded on Form B 103.

9.7 Sample Details

If samples are being analysed by the MS2000 only run the **Sediment.sop,** if samples are being measured by the MS2000 and sieved then use **Sediment2.sop**, **as** in 9.6. When the documentation screen is brought up, enter the appropriate sample name using the UKAS ID for the sample. The Field ID into the **Source Name** box, any other observations about the sample and a note of any sieves used should be recorded in the **operator notes** section then click **OK**.

9.8 Sample Inspection

Ensure the sample has been well mixed by tumbling gently for at least 30 seconds, and remove a sub sample, by placing a small amount on the end of a spatula. Silty samples will require significantly less sample than sandy ones.

9.9 Sample Measurement

Place the appropriate sieve over the chamber, and wash the sample through the sieve with tap water. The particle size can be estimated using a grain size scale to help determine which sieve to use. If unsure which sieve to use start with the smallest, 1000 µm (EN1205).

Keep adding sample, washing through with tap water, until the obscuration is stable (± 2%) between 5 and 15%. There is now sufficient sample for a measurement to be made. Select **Start**.

If there is sediment left in the sieve after washing the sample should be repeated using either the rough 1400 µm (EN 1206) or 2000 µm (EN1207) sieve. If repeating a sample using a larger sieve this should be recorded in the **operator notes** section of the documentation screen.

A report will be printed out and saved automatically after the sample has been analysed if running **Sediment.sop**. If **Sediment2.sop** is being used the report will be saved but not printed out. Click **OK** at the prompt. A second prompt box will appear asking if you want to run the SOP again click **OK** if there are more samples to run or **NO** if analysis is finished or a different SOP is required.

9.9.1 If using **Sediment2.sop** the data should be exported when all the samples have been analysed. Select all the files to be exported then go to **File, Export Data** select the appropriate **Blended** template, ensure that **include header row** and **use commas as separators** are ticked. Check the option **export data to this file** and enter the appropriate batch number and drive e.g. **E:\Batchxxxx** and select **OK**.

9.10 Cleaning Between Each Sample

The analyser will clean itself automatically after each analysis. If the tank still contains sediment after the cleaning cycle, it can be cleaned again as in 9.5.1.

9.11 Measurement of the >2000 micron Fraction.

See M 0855.

9.11.1 The weights for each fraction should be entered into the **sieved weights** page of B 069 in Excel. The cumulative % volume for each size range from the MS2000 which were exported in 9.9.1 should be entered into the **raw data** worksheet. The file is opened in Excel by selecting **file, open, look in,** select the appropriate drive, ensure **files of type** is set to **all files**. Open the

appropriate file, select **delimited**, **next**, tick the **semicolon** box and select **finish.** The data can then be copied and pasted into B 069.

When all the data has been entered B 069 should be printed out and archived with the batch, it is **not** necessary to save worksheet B 069 The corrected volumes can then be blended using the Mastersizer 2000 software.

9.12 Blending

Select the record to be blended
Go to **tools**, **result emulation, generator wizard**, select **next, load 'blended.ext'**.

Enter the cumulative volumes for each size. For larger sizes not measured ensure volume is 100%, for smaller sizes measured for but not detected ensure a volume of 0% is entered.

Save as **'blended.ext'**, select **yes** to replace

Select **next** then **save factors**, save as **blended.rmu** and select **yes** to replace the file.

Select **finish**

Go to **edit, result**s ensure **"pick up settings from selected measurement"** is selected, click **OK**.

On materials screen tick **"specify new result calculation"** select **advanced, result emulation, "use new emulation from a file".** Browse for the **blended.rmu** saved above, click **OK** twice

Go to labels screen tick **"specify new sample identifier"** in sample name add blended, e.g. "1111/sed/04 blended". Click **OK**

In **report/saving** screen, tick **"specify new report"** box and ensure report page is **new2.pag.** Click **OK**

Repeat for all samples to be blended.

9.13 Saving

A back up of the measurement file must be made as soon as the batch has been completed.

When all the samples have been analysed and blended (if necessary) go to the **Records** screen then go to **File** then **Save As** and save to a CD-ROM, giving the batch number as the filename, and ensuring the file type is set to ***.mea.**

A record of which files have been backed up and when should be recorded in lab book 218. When the CD-ROM is full, or every 6 months, whichever is soonest, the CD-ROM should be write protected and a second copy of it made (see SOP 0030). The CD's or their cases labelled with Analytical Investigations, PSA and the batch numbers stored on them. Two random batches should be selected to open on the analyser to ensure the backup has been successful and a note of which batches were checked made on the CD or its case. One should be sent to the Quality Manager for storage 'off-site' the other is stored in lab 503.

9.14 Switch Off Procedure

The PC and Mastersizer unit normally remains on. When analysis is complete fill the chamber with a decon solution until next use and ensure water tap is switched off.

The software programme is normally kept open.
To close the software down click on File, Exit; the computer will ask you if you wish to save the configuration - click on "No".
To switch the PC off; exit from Windows and switch off the computer.
To switch the Mastersizer off use the power switch located on the right hand side of the instrument.

10. Calculation of Results - See above.

11. Precision, Bias and Limit of Detection

LRM C100

	Size um						
Date	**31.25**	**44.194**	**62.5**	**88.388**	**125**	**D 4,3 (um)**	**% < 63um**
11/06/2004	2.00	24.80	49.52	22.20	1.48	75.90	27.81
19/07/2004	2.08	22.66	47.01	25.49	2.74	78.20	25.66
20/07/2004	2.72	25.43	47.06	22.72	2.07	75.99	29.13
21/07/2004	2.67	25.36	47.14	22.76	2.07	76.03	29.01
22/07/2004	2.63	25.30	47.21	22.79	2.06	76.07	28.91
23/07/2004	2.76	25.63	47.10	22.51	2.00	75.82	29.38
26/07/2004	2.79	25.82	47.14	22.30	1.94	75.66	29.60
Mean	**2.52**	**25.00**	**47.45**	**22.97**	**2.05**	**76.24**	**28.50**
SD	**0.33**	**1.08**	**0.91**	**1.14**	**0.37**	**0.88**	**1.38**
CV	**13.24**	**4.32**	**1.92**	**4.95**	**17.98**	**1.15**	**4.83**

LRM 590/840

	Size um							
DATE	**250**	**353.553**	**500**	**600**	**710**	**1000**	**1400**	**D [4,3] (um)**
11/06/2004	0.23	12.78	21.46	25.54	34.14	5.68	0.17	690.46
21/07/2004	0.20	12.27	21.23	25.69	34.74	5.72	0.16	693.00
22/07/2004	0.22	12.22	20.67	25.00	34.96	6.66	0.28	700.33
23/07/2004	0.09	9.24	18.20	24.23	38.18	9.44	0.62	732.47
26/07/2004	0.23	12.29	20.58	24.75	34.78	7.00	0.36	702.59
27/07/2004	0.24	13.5	22.26	26.01	33.13	4.76	0.10	680.97
28/07/2004	0.16	11.24	20.10	25.03	36.03	7.11	0.32	707.91

Mean	**0.20**	**11.93**	**20.64**	**25.18**	**35.14**	**6.62**	**0.29**	**701.10**
SD	**0.05**	**1.37**	**1.28**	**0.61**	**1.60**	**1.50**	**0.17**	**16.42**
CV	**27.49**	**11.46**	**6.22**	**2.42**	**4.56**	**22.64**	**60.68**	**2.34**

See Batch 2709

12. Reports

The results should be exported using the appropriate template depending on what data the client requested. The templates are
63+20um – exports the % below these sizes
Percentiles – exports the percentile (5, 16, 25, 50, 75, 84, 95) sizes
Results Between Sizes – exports the % of sample in each size range
All – exports all of the above
NMMP exports sediment descriptors such as kurtosis, skewness etc

These reports are exported as in 9.9.1. They should be opened in Excel as in 9.11.2.2 and copied into B 580 . Where the appropriate worksheet should be printed out to use as the Client Test Report, depending on the type of analysis requested. An electronic copy of the worksheet should be saved as Batchxxxx in a folder also named Batchxxxx in Chemdat.

13. Safety

Refer to Risk Assessments
AI132. Automated Particle Size Analysis
The Mastersizer has an electrically operated safety shutter fitted to the optics within the transmitter module - this is failsafe in the closed position and will not allow laser light to emit.

14. Literature References

Mastersizer 2000 Operators Guide.
Hydro 2000S/G User Manual.
ISO 13320-1:1999

15. Uncertainty of the Automated Particle Size Determination of Sediments

Main Steps of Method:

Sediment sample is received and stored frozen until it is freeze dried. The sample is placed through a 1000 and/or 2000 μm sieve into a mixing chamber. Using a laser source measurements of the particle size and size distribution of the sample are calculated using the instrument software. The angle through which the light is scattered by the particle is proportional to the particle's size.

Sources of Uncertainty:

- **Sampling:**
  Samples are analysed and results reported on the samples as received – out with uncertainty calculations

- **Sub-sampling:**
  Sediment samples are homogenised at the time of sampling. Samples are broken up and mixed prior to analysis - negligible contribution to uncertainty

- **Storage Conditions:**
  Prior to freeze drying samples are stored at -20°C – negligible contribution to uncertainty

- **Reagent Purity:** Not Relevant

- **Instrument Effects:**
  Weight: Balance check weight tolerance 0.01%, 2 decimal places used, sufficient for accuracy required - uncertainty accounted for in the validation data

  Sieves: Quality control charts monitor variation - uncertainty accounted for in the validation data

  Instrument: Quality control criteria set out in method to determine when maintenance is required. Instrument is calibrated by manufacturer annually at which time in the LRM is analysed, this LRM is then analysed on day of use therefore calibration variations are monitored by quality control charts - uncertainty accounted for in the validation data

- **Environmental Conditions:**
  Measurement not affected by environmental conditions. Cross contamination is minimised by washing the chamber and a background measurement is made which is required to be within set limits - uncertainty accounted for in the validation data

- **Operator Effects:**
  All measurement procedures are described in a fully documented Method to limit inconsistencies between operators. Contributions from different operators are incorporated in the validation data and monitored through the use of control charts - uncertainty accounted for in validation data

- **Matrix Effects:** Not Relevant

- **Computation Errors:**
  Measurements and calculations are performed by the instrument software commercial software assumed correct. No manual check possible -negligible contribution to uncertainty

- **Blank Correction:**

A background measurement is made prior to each sample which is required to be within limits stated in the Standard Operating Procedure. This measurement is included in the calculation of the final results by the instrument software. Uncertainty accounted for in the validation data

- **Random Effects:**
  Uncertainty accounted for by validation and control chart data

Summary Validation Data:

Precision of D (4,3) of LRM (from shewhart charts) $= \sqrt{(1.19^2 + 2.20^2)}$
$= 2.50\%$

Combined Uncertainty:

Systematic Component (from reticule validation on commissioning) = -2.36%

Random Component, (from LRM C100) % <63 um = 4.83%
Random Component (from LRM 590/840) D (4,3) mean weighted volume = 2.34%
Assume linear summation and a value of K=2

Combined Standard Uncertainty:

Combined standard uncertainty = $(C_s^2 + C_r^2)^{0.5}$ = X %

For % <63um = $(-2.36^2 + 4.83^2)^{0.5} = 5.38\%$
For D 4,3 = $(-2.36^2 + 2.34^2)^{0.5} = 3.33\%$

Expanded uncertainty:

Expanded uncertainty = $2 * (C_s^2 + C_r^2)^{0.5}$ = X %

For % <63um = $2*(-2.36^2 + 4.83^2)^{0.5} = 10.75\%$

For D (4,3) = $2*(-2.36^2 + 2.34^2)^{0.5} = 6.65\%$

The reported expanded uncertainties are based on uncertainties multiplied by a coverage factor of k=2, providing a level of confidence of approximately 95.

1. Introduction and Scope

The method describes the determination of total and organic carbon and nitrogen in sediment samples using a ThermoQuest FlashEA 1112 elemental analyser.

The working range for carbon is 0.005 – 55.6 mg per sample.
The working range for nitrogen is 0.005 – 6.07 mg per sample.

2. Principle of the Method

The CHN analyser uses a combustion method to convert the sample elements to simple gases (CO_2, H_2O and N_2). For Total Organic Carbon (TOC) the samples are acidified with HCl in silver cups, prior to analysis to remove the inorganic carbon fraction. The sample is first oxidised in a pure oxygen environment; the resulting gases are then controlled at exact conditions of pressure, temperature and volume. Finally, the product gases are separated. Then, under steady state conditions, the gases are measured as a function of thermal conductivity.

3. Reference Materials

Acetanilide is used to calibrate the machine on start-up. Mess -2 is used as a system suitability check for C and N . "Clean" homogenised sediment from Raasay Sound is run at random every 15 samples and used as an LRM. The values for Mess 2 and Raasay are recorded in quality control charts, see SOP 1380 for use of quality control charts.

The Raasay reference material is prepared in the same manner as samples. However, acetanilide weights should be in the range 1-2.5 mg.

4. Reagents

Helium Prepurified 99.995 mole % minimum
Oxygen (Research Grade) 99.995 mole % minimum
Acetanilide Standard

5. Equipment

ThermoQuest FlashEA 1112 elemental analyser EN1027
Sartorious MC2106 Autobalance EN341
Crane PC EN1026
Hewlett Packard DeskJet 845c printer EN1024

Regulators: Helium: dual stage with stainless steel diaphragm, 5-60 psi (35-415 kPa) outlet pressure. Oxygen: single stage with stainless steel diaphragm, 5-60 psi outlet pressure.

Pressed tin capsules 8 x 5 mm
Micro forceps (2)
Micro spatula
Sample trays
Dessicator

6. Environmental Control

Freezer temperatures are set and monitored as in SOP 280

7. Interferences

Samples must be completely freeze-dried and ground (see SOP 110 and 120). Excess moisture within the sample affects the sample weight and hence the analysis results.

Care should be taken when preparing samples that no foreign material (dust, excess sample, etc) is collected on the outside of the sample cups, as this will affect the weight and hence the sample results. Any cups suspected of being contaminated should be discarded.

8. Sampling and Sample Preparation

Samples are logged into the laboratory according to SOP 060. Pre-analysis sample preparation and storage conditions should be recorded on form B 62.

The samples must be freeze-dried and ground (see SOPs 110 and 120) prior to analysis. The spatula, forceps and sample preparation tray used must be kept scrupulously clean to prevent contamination of the sample during preparation.

For TOC results, silver capsules should be used, and the sample treated according to SOP 170. An empty silver cup should be treated in the same way as the samples to use as a blank. If analysing for Total Carbon (TC) tin capsules are used. Acetanilide and Mess 2 samples are both weighed into tin capsules, the Raasay LRM is treated the same as the samples.

The sample weight taken for sediments is typically 10-20 mg. The Sartorious MC 2105 Autobalance is calibrated prior to sample weighings.

Tare the balance with the cup to be used then remove the capsule from the sample side and place on a clean surface. Using a metal spatula take some of the sample and place carefully into the capsule. Tap the capsule lightly to ensure all the sample particles have reached the bottom return the capsule to the balance. When the weight has stabilised, note the weight on form B68, ensuring sufficient Raasay lrms are prepared (a minimum of 1 per 15 samples)

If analysing for TC the sample can be run without further treatment. Remove the capsule and place on a clean surface. Then, with the forceps, pinch the centre of the capsule and fold it in half and flatten. Then fold in half again and make sure the there are no jagged edges. Reweigh the capsule to ensure no sample has been lost in folding.

If analysing for TOC the sample cups aren't folded until after they have been acidified as per SOP 170.

To prevent moisture ingress to the samples, they should be stored in a dessicator cabinet prior to analysis. The silica gel should be checked to ensure it is still active, if it has turned yellow it should be replaced.

9. Analytical Procedure

9.1 Setting up

9.2 The analyser is normally kept on and at operating temperature, but with reduced flow rates of gases. However if the analyser has been switched off, refer to section III chapter 9, "Instrument Start-up" in the manual.

9.3 To start the software select the "Eager 300" icon, then the EA1112 #1 icon which appears in the next window, then click OK in the following dialog box. The following steps should be carried out prior to beginning any analysis.

9.4 Open a new folder in the **N C Data** folder, found in the **Analysis** folder in the C drive and re-name with the batch number and date. The files **CN system.mth** and **NC.eam** should then be copied to this folder from the **Analysis** folder.

It is essential to visually inspect the adsorption filter every day of use, which is located behind the panel on the right hand side of the front of the machine. If there is obvious clumping of the filter material then remove and refill it according to section II chapter 5 "Preparation of reactors and adsorption filters" and chapter 6 "Connecting reactors and adsorption filters" of the manual.

9.5 In the Eager 300 software ensure the correct method is loaded by selecting File, then **Load Method,** browse for the folder created in **9.4.** Open it and select **CN system.mth** then **Open**. Ensure that the gas flow rates are at the correct setting for analysis. To do this go to **VIEW** menu, then **VIEW ELEMENTAL ANALYSER STATUS**, this should bring up the **GENERAL** screen where the set and actual flow rates can be compared in the **FLOW** section. If the set levels are too low, then go to **EDIT**, then **EDIT ELEMENTAL ANALYZER PARAMETERS**, then **FLOW TIMING**, then **GAS FLOW** and adjust the settings accordingly, (usually 130ml/min for carrier gas and 100ml/min for both oxygen and reference gases.)

9.6 When any adjustments have been made press **SEND** to send the new settings to the analyser then press OK, a prompt asking to either save the new method settings or not will appear , in order to keep any changes made the **YES** option should be chosen

9.6.1 A leak test should then be carried out, by going to the **VIEW** menu, **select VIEW ELEMENTAL ANALYSER STATUS**, then **SPECIAL FUNCTIONS**, then select **LEAK TEST**, and **START**. Select **YES** to the auto zero, and the leak test will be performed automatically. The reference flow should fall to 0 very quickly, the carrier flow should fall to <5ml/min after no more than 360 secs. If it doesn't then there is a leak somewhere in the system. Click **STOP** to end the leak test.

9.6.2 If the test failed then tighten all the screws at the gas inlet/outlet points, then repeat the leak test. If it still fails then check the O-rings and shaft of the auto-sampler, according to section IV, chapter 13 "Installing and servicing the MAS 200 auto-sampler" then repeat the leak test. If it still fails then the reactors O-rings need to be changed the analyser should be put in stand by mode in the **EDIT ELEMENTAL ANALYSER PARAMETERS** section, and allowed to cool overnight. The reactors should be removed as described in section II chapter 6 "Connecting reactors and adsorption filters" of the manual.

9.7 To input the samples and standards go to **EDIT**, then **SAMPLE TABLE**, followed by **EDIT SAMPLE**, then **FILL SAMPLE TABLE**. The sample name box should be left blank, in "chr.filename" enter the batch number and date followed by a minus sign i.e. B1939-090103-, tick the "unknown" box then enter the number of samples and standards to be analysed. The "Sample Name idx." And "filename idx." Should be left at 1, unless more samples are being added, in which case they should be the next number after that of the last sample in the table. The weight should be set to 0 then select **REPLACE**.

9.8 The names of the samples, lrms, crms and standards should be entered along with the weights into the appropriate columns. The type of sample to be run should also be selected, for samples, lrms and crms they should be entered as unknowns. An empty tin capsule should be run as a blank. Then three acetanilide samples should be run. The first as a bypass to condition the machine, the second as a standard to calibrate the machine, and the third as an unknown to check the calibration. (The weight of a bypass doesn't need to be entered into the table, as the results for it aren't recorded.). Followed by a Mess II then an empty line should be inserted using the **EDIT** menu.

9.9 After the empty line if analysing for TOC the acidified silver cup should be added as a blank (as the blank results are cumulative). The samples can then be entered into the table, Raasay lrms should be run a minimum of every 15 samples. When the sample table has been completed the auto-sampler can be loaded, starting in the position above the barrel.

9.10 Once the leak test has been completed, the filament level should be checked by going to the **DETECTOR** menu, the filament should be at 1000μV ±10. If it isn't press the **AUTO-ADJUST LEVEL AT 1000μV** button. Then when this has been done press
OK to close this menu.

9.11 The calibration figures and results table should be cleared, prior to starting analysis. To do this, go to **RECALCULATION**, then **RESET CALIBRATION FACTORS** and then answer **YES** to the next 3 prompts.

9.12 To start the analysis itself go to **RUN**, then **START SEQUENCE OF SAMPLES**, then **START NOW**

.

9.13 Once the analyser is operating go to the **VIEW** menu, then **VIEW SAMPLE BEING ACQUIRED**, this will bring up the chromatogram of the sample being analysed. This should be monitored to ensure the analysis is proceeding as expected. There should be a small sharp trough at the start of the chromatogram, followed by a peak at approximately 115 seconds for N, then one at around 260 seconds for C depending on the age of the columns. The first acetanilide, run as a bypass, is used to check the retention times. To change the retention times for the peaks go to **Edit**, then select **Component Table**, make any required adjustments to the retention times then select **OK.**

9.14 The analyser will run through the sample table until it reaches the empty row. When it has stopped go to **RECALCULATION**, and then **SUMARISE RESULTS** this will bring up a table of results if the values for the MessII are acceptable then the analysis can be continued. The values for the reference materials are entered into Shewart charts updated by the QC Chart Manager. Record the values on form B104. To restart the analysis return to the sample table (as in 9.7) then in click in the first column next to the sample to be analysed and set this as "**ACTUAL SAMPLE TO BE ANALYSED**". Exit the sample table and restart the analysis as in 9.12.

10. Calculation of Reports

The C and N percentages are calculated after each sample, taking into account any blanks and no further calculations are required. For details of how such values are obtained see chapter 10 "Guide to Run Analyses".

10.1.1 If an incorrect weight has been entered for any of the samples, or the baseline for the integration is in the wrong place, as can happen with low nitrogen values it is possible to recalculate the % of C and N for that sample.

10.1.2 Go to the **RECALCULATION** menu and, select **IDENTIFY PEAKS, AND REVIEW IDENTIFICATION**, click on **SINGLE SAMPLE** if only 1 sample is being recalculated or **SAMPLE SEQUENCE** for more than one sample. If only 1 sample is being recalculated then in the box underneath browse for the

sample number to be recalculated enter the correct weight of the sample then click OK. If a sample sequence is being recalculated then enter the first and last sample numbers in the boxes under **RECALCULATE SAMPLES FROM SAMPLE SEQUENCE**.

10.1.3 Select **REINTEGRATE, IDENTIFY PEAKS, REVIEW ID AND INTEGRATION, SAVE AFTER REINTEGRATION** then select **OK**

10.1.4 The first window allows adjustments to be made to the retention times for nitrogen and carbon as in **9.13**.

10.1.5 The second window allows changes to be made to the peak integrations. If the baseline needs adjusting select **PEAK**, then **MOVE PEAK START/END**, the baseline can now be adjusted using the mouse. When satisfied with any changes save the chromatogram and close the window down.

10.1.6 The third window previews a copy of the chromatogram report the PC will print out, check to make sure there are values both for nitrogen and carbon close this window and the report will print out.

11. Precision, Bias and Limit of Detection

See Batch 1965

Material	Determinant	Mean %	SD	% CV
Mess II	Carbon	2.06	0.05	2.5
Tibet	Nitrogen	0.11	0.01	5.86
Raasay	Organic Carbon	1.57	0.20	13.02
Raasay	Orgainic Nitrogen	0.18	0.02	12.12
Raasay	Total Carbon	4.16	0.08	1.90
Raasay	Total Nitrogen	0.20	0.01	5.22

Limit Of Detection (LOD)

A Quasimeme Test Material (QOR068MS) was used. 20 samples ranging in weight from 1.06 mg to 20.37mg (at approx. 1mg increments) were acidified then analysed.

The smallest sample to give an adequate response was 2.26 mg giving results of
0.211% N and 0.221% C. These percentages were converted to masses to obtain an
LOD.

Nitrogen (2.26/100)*0.211 = 0.005mg

Carbon (2.26/100)*0.221 = 0.005mg

LOD

Carbon	0.005mg
Nitrogen	0.005mg

Recovery

Raasay samples were spiked with acetanilide and half were treated with HCL to remove organic carbon SOP 0170. They were than analysed and the recoveries calculated the results are summarised below.

	Mean % Recovery	SD	%CV
Organic Carbon	89.79	4.88	5.44
Organic Nitrogen	94.6	4.51	4.77
Total Carbon	97.82	1.78	1.82
Total Nitrogen	101.38	1.76	1.74

12. Reports

Once all the samples have been analysed review the results as above. The results table are exported to an excel file using the **EXPORT TO** command. A hard copy of all data should be archived with analysis documentation as a control document (SOP1350). All hard copies should include the date of analysis and the signature of the analyst.

The Client Test Report is prepared using B 594. This allows a Limit of Quantification (LOQ) to be determined for each sample. The Limit of Detection (LOD) is the lowest weight which can be determined. The LOD is converted to a percentage, which is sample weight dependant (this is the LOQ)

For example with a sample weight 16.50mg
Carbon LOQ = (0.005/16.50)*100 = 0.03%

This results table is then saved in a network drive (chemdat). The raw data is saved in the folder created in section 9.4. This folder should be copied to a CD-ROM. A record of which files have been backed up and when should be recorded in lab book 218. When the CD-ROM is full, or every 6 months, whichever is soonest, the CD-ROM should be write protected and a second copy of it made (see SOP30). The CD's or their case labelled with Analytical Investigations, CHN and the batch numbers stored on them. Two random batches should be opened to check the backup has been successful and a note of which batches were checked should be made on the CD or its case. One should be sent to the Quality Manager for storage 'off-site' the other is stored in lab 503.

13. Safety

Lab coat, safety specs and gloves should be worn in the laboratory and when handling samples. See risk assessments AI003 AI009 AI070 AI077

14. Literature Reference

ThermoQuest FlashEA1112 Elemental Analyser Operating Manual. Part Number 317 082 41, Second Edition November 1999

Estimation of Uncertainty

Sources of uncertainty:

- Sampling: Samples are analysed and results reported on the samples as received – outwith uncertainty calculations;
- Sub-sampling: Samples are freeze dried and ground to ensure a homogenous sample. – negligible contribution to uncertainty;
- Storage conditions: Prior to analysis samples are frozen, after freeze drying they are stored in a dessicator - negligible contribution to uncertainty.
- Reagent purity: All gasses used are 99.995-mole % minimum quality, considered sufficient – uncertainty accounted for in validation data.
- Instrument effects: Typical standard curve r-value 0.9995. Uncertainty accounted for in validation data
- Weight: Tolerance of balance/decimal places – balances check weight tolerance generally <1%. 1-2 decimal places used, sufficient for accuracy required. Uncertainty accounted for in validation data
- Volume: Not applicable
- Time: Not applicable
- Computational Effects: Concentrations are calculated by instrument software.– negligible contribution to uncertainty.
- Blank Correction: Sn vials are run as blanks these values are taken into account during calculations. Uncertainty is accounted for in validation data
- Environment conditions: Contamination is minimised by the use of a dedicated laboratory and equipment for analysis, and a separate dedicated lab for sample preparation. The temperature controlled make-up air avoids gross temperature changes. Uncertainty accounted for in validation data

- Operator Effects: All measurement methods are described in fully documented standard operating procedures to limit inconsistencies between operators. Only trained personnel may perform method unsupervised. Variations between operators are accounted for by control chart data. Uncertainty accounted for in validation data.
- Matrix Effects: Uncertainty accounted for in validation data
- Random effects: These will be accounted for by validation and control chart data.

Summary validation data:
C
Precision of Raasay LRM: 1.68%
Recovery of Mess II Reference Material: 96.26%
Variance from Shewart Chart: (Std Dev: 0.052 /Mean: 2.06): 2.5%

Combined uncertainty:

Systematic component (Recovery on CRM): C_s R/2%
Random Component (Shewart chart S.D.): C_r y%

Assume linear summation and a value of k=2:

Combined standard uncertainty

Combined standard uncertainty = $(C_s^2 + C_r^2)^{0.5}$ = X %

Expanded uncertainty:

Expanded uncertainty = 2 * $(C_s^2 + C_r^2)^{0.5}$ = X %

The reported expanded uncertainty is based on an uncertainty multiplied by a coverage factor of k=2, providing a level of confidence of approximately 95%

	Component		Uncertainty	
Determinant	**Systematic**	**Random**	**Combined**	**Expanded**
Organic C	3.55	13.03	13.5	27
Organic N	3.34	12	12.46	24.91
Total C	1.87	2.5	3.12	6.24
Total N	7.81	5.55	9.58	19.16

1. Introduction and Scope

The following method describes a procedure for extracting lipids from both shellfish and fish matrices using the method developed by Smedes (1999). The lipid and water content of the sample mass should be ≤ 1g and ≤ 8g respectively. These limits can be controlled by adjusting the sample amount. The method is capable of detecting a lipid content of up to100%.

2. Principles of the Method

The sample is homogenised into a mixture of isopropanol and cyclohexane and a calculated volume of de-ionised water, followed by centrifugation, to extract lipid material. A second extraction is carried out with 13% isopropanol (w/w) in cyclohexane. The two extracts are combined, rotary evaporated and the resulting residue is dried in an oven. The residue weight is determined and the lipid content is calculated from the intake mass.

3. Reference materials

A Laboratory Reference Material (0.30 g ± 0.05 g fish oil or 0.50 g ± 0.05 g dried mussel homogenate, see B040) is extracted with each batch of samples. The LRM results are plotted on a Shewhart chart with warning and action limits set at ± 2 and ± 3 times the standard deviation of the mean respectively.

4. Reagents

Isopropanol (propan-2-ol) (HPLC Grade)
Cyclohexane (HPLC Grade)
Deionised water
Sodium chloride

5. Equipment

Blenders
Freeze drier EN1189
Ultra Turrax
Calibrated balance
Oven EN1041
Centrifuge EN287/EN325
100ml centrifuge tubes
Rotary evaporators with water baths
100ml round-bottom flasks
25ml measuring cylinders
10 ml measuring cylinders

Calibrated thermometer
Calibrated timer
Glass pipettes
Glass column and cotton wool

6. Environmental Control

All solvent handling is carried out inside a fume cupboard. Wet samples are stored in freezers monitored by the Woodley system (SOP0280), until they are required for analysis. Dry samples may be stored in a cool, dark cupboard prior to analysis.

7. Interferences

7.1 All glassware must be cleaned prior to use in accordance with SOP0220 and gloves should be used at all times to prevent contaminating glassware.

8. Sampling and Sample Preparation

Samples are logged in with a unique identification number in accordance with SOP060 and SOP065.

8.1 Prior to lipid determination the tissue is thoroughly homogenised by domestic blender.

8.2 Tissue for preparation may be frozen (-20°C) or cryogenically stored (-70°C). In such instances allow the tissue to thaw thoroughly before lipid extraction is carried out.

8.3 A dry-weight determination (to 3 decimal places) on a representative portion (eg. 10.000 g of fish flesh – see Table 1) of the test sample is carried out before analysis using a freeze drier (SOP0110). The details are recorded on B066. An aliquot of sample containing ≤ 8 g of water is then used for lipid determination.

8.4 The rotary evaporator water baths (SOP0285) and oven are switched on 1-2 hours before use to allow them to reach the required temperature and checked by calibrated (SOP0260) thermometer before use.

9. Analytical Procedure

Ensure that a balance performance check has been carried out (SOP0240) prior to weighing samples. All relevant weights are recorded on B624.

9.1 Weigh an appropriate amount of sample into a pre-tared, labelled 100 ml centrifuge tube.

- The maximum matrix weight should be no more than 10 g (± 0.5 g).

- The sample should not contain more than 1 g of lipid. If the lipid content is more than 1 g the extraction is repeated with less sample.
- The sample should not contain more than 8 g of water. The water content is determined from a sample of freeze dried material and the amount of sample to extract can be adjusted accordingly.

Examples of typical matrix weights are presented in Table 1.

9.2 Add 18 ml (± 1 ml) of isopropanol and 20 ml (± 1 ml) of cyclohexane using a measuring cylinder and mix by Ultra Turrax (13500 rpm) for 2 minutes using a calibrated (SOP0250) timer.

9.3 Add the appropriate volume of de-ionised water (by measuring cylinder) as calculated using the following formula (Table 1 lists some of the values used):

Water (ml) = 22 – ([Sample weight (g) * moisture content (%)] / 100)

Record the water volume on B624

Table 1 – Example of the volume (ml) of water required for various matrices

Matrix	Matrix weight (g)	Water added (ml)
LRM	0.3 ± 0.05 g	22
Freeze Dried LRM	0.5 ± 0.05 g	22
Whole scallops	5.0 ± 0.1 g	17 – 18
Mussels	5.0 ± 0.1 g	17 – 18
Plaice flesh	10.0 ± 0.5 g	13 – 15
Cod/plaice liver	0.25 ± 0.05 g	22

9.4 Mix with an Ultra Turrax (13500 rpm) for a further minute using a calibrated timer.

9.5 For fatty matrices such as liver extracts, add 0.5 – 2.0 g of powdered NaCl before centrifugation, to break down any emulsions formed. Centrifuge at the 1800 rpm and 10 minutes settings.

9.6 Remove 10 ml (± 0.5 ml) of the organic (upper) phase, by glass pipette, to a measuring cylinder and transfer to a pre-weighed, labelled 100 ml round bottom flask, rinsing the measuring cylinder with 1-2 ml cyclohexane. In some cases the organic phase may contain some tissue particles. When this occurs the extract should be filtered by passing the extract through a pipette plugged with ca. 2 cm of cotton wool which had previously been cleaned with cyclohexane.

Record the weight of the empty flask.

9.7 Remove as much of the remaining organic phase as possible, by means of a glass pipette fitted to a vacuum pump, and discard.

9.8 Prepare a solution of cyclohexane containing 13% (w/w) isopropanol.

9.9 Weigh 13 g ± 0.5 g of isopropanol into a conical flask.

9.10 Add cyclohexane, to make a final combined weighht of 100 g ± 1.0 g.

9.11 Label this solution with an expiry date of 3 months.
Other volumes of this solution may be prepared, using the same ratio of 13:100, isopropanol : final weight.

9.12 For the second extraction add 20 ml (± 1 ml) of this mixture, by measuring cylinder, to the sample and mix by Ultra Turrax for 1 minute using a calibrated timer. The Ultra Turrax dispersing shaft should be rinsed with de-ionised water and wiped down between samples. Use forceps to remove any sample particles caught in the blades. After use the Ultra Turrax is thoroughly cleaned following SOP0220.

9.13 Centrifuge at the 1800 rpm and 10 minute settings.

9.14 Remove 10 ml (± 0.5 ml) of the upper, organic, phase by pipette to a measuring cylinder and transfer to the round bottom flask containing the first extract. Rinse the measuring cylinder with 1-2 ml cyclohexane and evaporate the solvent at 75^{o}C (± 1^{o}C) by rotary evaporator (SOP1640).

9.15 The round bottom flask is further dried in an oven at 80^{o}C (± 1^{o}C) for one hour, removed and allowed to cool to room temperature in a desiccator before weighing.

9.16 The % lipid content of an LRM is calculated with each batch of samples and the result entered into the appropriate spreadsheet (maintained in NTS5\qalan\data\Analytical Investigations\Lipids) in accordance with SOP1380. A Shewhart chart is maintained to monitor the performance of the method.

10. Calculation of Results

10.1 A set volume of solvent (10 ml) is removed from each extraction step, (A and B) combined and rotary evaporated, therefore an aliquot factor is used in the lipid content calculation, as follows:

$$\text{Aliquot Factor} = (A + B) \div (C + D)$$

Where A = volume removed from first extract (10 ml), B = volume removed from second extraction (10 ml), C = volume of solvent used in first extraction (20 ml) and D = volume of solvent used in second extraction (17.4 ml*).

Aliquot factor = (10 + 10) ÷ (20 + 17.4) = 0.535

* The volume of solvent used is actually 20 ml but 13% of this is isopropanol which is 100% soluble in water (i.e. 13% of 20 ml = 2.6 ml).

10.2 The % lipid content in each sample is calculated by the following formula:

Lipid = [(Weight of residue (g) ÷ aliquot factor)/Weight of sample extracted (g)] * 100

11. Precision, Accuracy and Limits of Detection

Calculated according to SOP1310. See section 15 for method performance data. The precision and accuracy of the method are monitored by analysing an LRM with each batch of samples.

12. Reports

Batch reports and the associated paperwork are archived in accordance with SOP030 and an electronic copy of the results is also kept on NTS2/Chem_Dat/Biota lipids and moisture/Smedes.
All relevant weights and calculated lipid concentrations are recorded on B624 and archived with the batch cover sheet (B050) and test report cover sheet (B535).

13. Safety

General laboratory protective clothing is required. For further information see Procedure Risk Assessment No. AI021.

14. Literature References

F. Smedes (1999). Determination of total lipid using non-chlorinated solvents. *Analyst* 1999, **124**, 1711-1718.

15. Performance Data

Validation data is archived in the NTS5/Ukas/Method_Validation directory.

Cod liver homogenate

Sample ID	Batch	Weight (g) of sample	Weight (g) of residue	Lipid content (%)
1706/00 A	3369	0.279	0.168	60.295
1706/00 B	3369	0.280	0.170	60.748
1706/00 C	3369	0.264	0.161	60.889
1706/00 D	3369	0.273	0.164	60.251
1706/00 E	3369	0.285	0.168	59.026
1706/00 F	3369	0.287	0.176	61.220
1706/00 G	3369	0.291	0.179	61.663
			Mean	**60.585**
			SD	**0.848**
			%CV	**1.40**
			Bias*	**4.10**

* QUASIMEME assigned value = 58.10%

Plaice liver homogenate

Sample ID	Batch	Weight (g) of sample	Weight (g) of residue	Lipid content (%)
1729/06 A	3369	0.255	0.022	8.796
1729/06 B	3369	0.201	0.017	8.369
1729/06 C	3369	0.268	0.022	8.369
1729/06 D	3369	0.206	0.019	9.074
1729/06 E	3369	0.283	0.021	7.265
1729/06 F	3369	0.265	0.022	8.464
1729/06 G	3369	0.238	0.017	7.068
1729/06 H	3369	0.236	0.017	7.128
			Mean	**8.067**
			SD	**0.793**
			%CV	**9.84**

Mussel homogenate

Sample ID	Batch	Weight (g) of sample	Weight (g) of residue	Lipid content (%)
820/05 A	3436	5.092	0.065	1.285
820/05 B	3436	5.031	0.067	1.338
820/05 C	3436	5.089	0.064	1.249

820/05 D	3436	5.022	0.062	1.228
820/05 E	3436	5.016	0.062	1.230
820/05 F	3436	5.051	0.065	1.295
820/05 G	3436	5.059	0.064	1.256
			Mean	**1.269**
			SD	**0.040**
			%CV	**3.12**

16. Uncertainty

Main Steps of Method

Between 0.3 g (± 0.05 g) and 10 g (± 0.5 g) of sample is extracted, first with cyclohexane, isopropanol and water and then with 13% (w/w) isopropanol in cyclohexane to remove lipids. Fractions of the two extracts are combined and the solvents evaporated. The dried residue is weighed to determine the total extractable lipid concentration.

Sources of Uncertainty

- **Sampling:**

Samples are analysed and the results are reported on the samples as received – out with uncertainty calculations.

- **Sub-sampling:**

A sub-sample (0.25 – 10.5 g) of the homogenate is weighed out for lipid analysis. For some matrices it is difficult to obtain a homogeneous sample (notably mussels) and therefore there will be differences in the lipid concentrations between sub-samples. This will also contribute to levels of uncertainty and is accounted for in the precision data.

- **Storage Conditions:**

Samples may be stored in a freezer prior to homogenisation and sub-sampling. The lipid content of samples is determined on the day of extraction and therefore there is a negligible contribution to uncertainty.

- **Reagent Purity:**

Solvents are at least HPLC Grade and prepared solutions are labelled with expiry dates and any uncertainty is accounted for in the validation data.

- **Instrument Effects:**

Weight: A 3 decimal place balance is used which is sufficient for the accuracy required and any uncertainty is accounted for in the validation data.
Volume: Tolerances stated in method for measuring cylinders are sufficient for purpose and any uncertainty is accounted for in the validation data.
Temperature: Evaporating baths are calibrated against a calibrated thermometer

(SOP0260) and variations are accounted for by the control chart data.
Time: Any timer used is calibrated in accordance with SOP0250. Any uncertainty is accounted for in the validation data.
Centrifuge: Serviced annually and suitable for the method. Any uncertainty is accounted for in the validation data.

- **Environmental Conditions:**

Contamination is minimised by the use of dedicated laboratory space, equipment and glassware for organic analysis. Glassware is also separated during cleaning and any uncertainty is accounted for in the validation data. Cross contamination is minimised by rinsing the Ultra Turrax after each sample homogenisation. Freezer temperatures are monitored and connected to an alarm system. Any uncertainty is accounted for in the validation data.

- **Operator Effects:**

Only trained personnel can carry out the method unsupervised. The latest method, and SOPs associated with the method, are fully documented and the latest version readily available. Variations between operators are accounted for in the QC sample run with each batch of samples. Uncertainty is accounted for in the validation data.

- **Matrix Effects:**

Lipid is in solution in cyclohexane and isopropanol and any uncertainty is accounted for in the validation data.

- **Computer Errors:**

Results are calculated manually and entered into and electronic spreadsheet which is checked by Technical Management – negligible contribution to uncertainty.

- **Blank Correction:**

Results are not blank corrected – negligible contribution to uncertainty.

- **Random Effects:**

Uncertainty accounted for by validation and control chart data.

Summary Validation Data:

Low sample (ca. 1.3% lipid): 3.12%
High sample (ca. 60.6% lipid): 1.40%
Precision of LRM110: 3.06%
Recovery of QUASIMEME Reference Material (1706/00 A – G): 104.3%
Variance from LRM110 Shewhart chart: 2.77%

Uncertainty:

Systematic Component (Recovery on QUASIMEME reference material – 1706/00 A - G): 4.10% / 2
Random Component (LRM110 Shewhart Chart SD): 2.77%

Assume linear summation and a value of k = 2:

Combined Standard Uncertainty = $(C_s^2 + C_r^2)^{0.5}$ = X%
Expanded Uncertainty = 2 x $(C_s^2 + C_r^2)^{0.5}$ = X%

$2 \times (2.05^2 + 2.77^2)^{0.5}$ % = **6.88%**

The reported expanded uncertainty multiplied by a coverage factor of k = 2, providing a level of confidence of approximately 95%.

1. Introduction and Scope

This procedure describes the removal of water from a frozen sample without heating.

2. Principle of Method

Using the LTE Scientific Freeze Drier, frozen samples have the ice removed from them by sublimation. At low pressures and temperatures water in the form of ice can be converted directly into water vapour. By avoiding the liquid phase of water, boiling is inhibited, and the sample remains intact. It is essential that **the sample is frozen** before being placed in the freeze drier.

3. Reference Materials

Not relevant.

4. Reagents

Vacuum Oil – Javac V100 or equivalent
LF009 Oil filter – supplier: Javac

5. Equipment

LTE Scientific EN1189

6. Environmental Controls

All freeze drying is undertaken in laboratory 503A. Clean the soiled surfaces of the freeze drier after use with tap water and tissue.

7. Interferences

Not relevant.

8. Sampling and Sample Preparation

Samples for freeze-drying **must** be frozen.
To avoid possible loss of sample, do not freeze-dry previously dried sediments. If a sample may have absorbed moisture, the moisture content of a small portion (1-3 g) should be determined by oven drying as described in SOP1615. Afterwards, consult Technical Management to decide whether to analyse the sample and correct for moisure content, or if the sample should be re-dried, and how.

9. Analytical Procedure

9.1. The condenser should be kept dry when the freeze-drier is not in use. Leave the door to the condenser ajar when the instrument is switched off.

9.2. Remove the circular clear acrylic lid from the top of the freeze drier, and place it on a bench top. Remove acrylic cylinder (may require two people) and place on a bench top.

9.3. Switch on the freeze drier by depressing the small black button on the control panel on the front right of the machine.

9.4. Close the condenser door, ensuring the seal is fitted correctly and the black release valve under the chamber by turning the knob so it is pointing upwards. Switch on the condenser chamber freezer by pressing the top left hand blue button marked **Condenser Chamber**. The pump should start and a red light will show. To display the temperature press the bottom left blue button marked **Temperature**, a red light will come on and temperature will be displayed. After a few minutes this temperature should begin to fall.

9.5. When the chamber temperature has fallen below -30°C. transfer the frozen samples from the freezer, ensuring the sample is not completely sealed (i.e. open lids, unzip bags etc). Place the samples on the shelves in the freeze drier. The samples need to be unsealed to allow the water vapour generated from the sublimation of the ice to escape.

9.6 Replace the acrylic cylinder and circular lid carefully on top of the freeze drier. Switch on the vacuum pump by pressing the switch marked **vacuum pump** on the top right hand side of the display, a red light should now show and the pump will start pulling a vacuum. The vacuum display will now read **ON** but after a few minutes this should change to a number which will show the pressure in the freeze drier.

9.7. If this does not happen then there maybe a leak in the system. The acrylic lid and cylinder need to be removed and the seals checked for dirt, the seal in the condenser door should also be checked. They should be cleaned by wiping with absorbent roll and lightly coated with High Vacuum Silicone Grease.

9.8. The length of time required for sample to be freeze dried will vary depending on the number of samples and the sample size as well as their water content. The start date and time should be noted in the freeze drier log book on sheet B150.

9.9. To stop the operation or check the drying progress, switch off the vacuum pump and open the release valve very slowly. It will take between a minimum of 30-45 seconds for the vacuum to release. (Test by pushing the acrylic lid upwards after 2 minutes.)

9.10. Open the freeze drier by removing the acrylic lid and cylinder and check the samples for any remaining frozen parts.

9.11. If the samples have dried acceptably, remove the samples. Clean up any spillage's and complete the log sheet B150. If the accumulated running time

of the instrument has exceeded 1000 hours, bring the log to the attention of the Technical Manager, as the oil needs to be changed.

9.12. It is preferable to remove any built up ice from the condenser before further samples are freeze-dried. To do this press **Defrost**. The condenser will heat up, melting the ice. When melting is complete, drain by opening the valve under the condenser door (with bucket underneath.) Dry condenser fully using paper towel once excess water has been removed.

9.13 If the samples require further drying, or if more samples are to be freeze dried, check if the samples are at all wet, if so re-freeze before proceeding

9.14 Troubleshooting and Maintenance

General Maintenance should be recorded on the Maintenance sheet, B212

Trouble Shooting.
If at any stage samples are seen to be defrosting fully, release the vacuum, remove (9.9) and re-freeze samples . Some slight defrosting is normal at the start of the process.

Checking seals:
Remove acrylic lid and chamber. Clean thoroughly around the edges of the chamber, and vacuum/wipe the rubber seals the acrylic cylinder rests on to remove any dirt. If necessary remove the seals completely, clean the recesses which hold the seals and the seals using damp tissue and replace. The seals are a tight fit, so it may require some time to get the two ends to meet tightly.

9.15 Changing the Oil
Removing the pump and changing the oil requires two people.

The oil should be changed after a maximum of 1000 hours (cumulative time recorded on log sheet). Emsure freeze drier is off at the mains and disconnect. Remove front bottom left panel and diconnect hose from pump, lift the pump out and discoonect the power lead.

Stand pump over suitable waste container. Unscrew black stopper at front of pump using spanner, until it can be turned by hand. Remove stopper and allow oil to drain (last bits may require lifting the pump on end). Replace stopper and tighten with spanner, but do not distort o-ring. Refill with fresh vacuum oil within the limits on the glass at he front of the pump. Do not over-fill. Record oil change on freeze-drier log sheet. Ensure any spillages are cleaned up immediately.

To replace the pump remove the left hand panel from the freeze drier, lift the pump back into place and reconnect the power lead, tube and oil filter. Replace left hand panel and update B150.

9.16 Changing the oil filter.
When changing the oil check the condition of the oil filter. If it is soaked in oil, it requires changing. Unscrew the clear plastic casing and pull off the filter, then replace it with a fresh one. Dispose of the filter in a chemical waste bin.

10. Calculation of Results

Not relevant.

11. Precision Bias and Limit of Detection

Not relevant.

12. Reports

Use of the freeze-drier, and any oil changes, is recorded on the log sheet B150. Maintenance is recorded on the generic Maintenance record sheet B 212. All samples freeze-dried as part of a batch should have the sediment preparation sheet B 62 completed and archived with the batch paperwork.

13. Safety

See AI003.
GEN002.

14. Literature References

Lyotrap Ultra Freeze Drying Machine Operating Instructions

1. Introduction and Scope

This procedure describes the preparation of samples into a homogeneous condition.

2. Principle of Method

Sediments are sieved to remove the coarse fraction and then ground to homogenise the sample for further chemical analysis.

3. Reference Materials

NA

4. Reagents

Deionised water, hexane.

5. Equipment

Powder funnel
Stand and clamp
Sieve and collecting base
Pestle and mortar
Brush
Aluminium foil
Tissue
Fume cupboard
Sample pots/bags
Trulla spatula
Retsch Planetary Ball Mill PM100 (EN1216)

6. Environmental Controls

All sieving and grinding is to be undertaken in a fume cupboard to prevent contamination, and inhalation of the sediment.

7. Interferences

Not relevant.

8. Sampling and Sample Preparation

The sediment is freeze dried prior to sieving SOP 110.

9. Analytical Procedure

The extent of the sample preparation is dependent on the analysis to be undertaken (see request form B21). 2 mm fraction is removed prior to PSA

for some clients. For some chemical analyses, a proportion of the <2 mm is ground to a fine powder.

9.1 Particle Size Analysis

- Attach the funnel to the clamp and place the 2mm sieve inside the funnel. Locate one of the mortars beneath the funnel.
- Transfer the sample to a mortar and gently dissociate the sediment with a pestle into its constituent grains. It is essential not to damage the sediment grains so that the sample integrity is maintained.
- Place a labelled pot below this sieve.
- Pour the dissociate sample into the sieve, using the brush to remove any fine material adhered to the mortars surface.
- Assist the sieving using a brush gently.
- Clean the funnel, pestle and mortars between samples with a damp tissue or brush, ensuring all equipment is dry before preparing the next sample.
- The <2 mm fraction is retained for archive purposes.

9.2 CHN Analysis/Metal Analysis

9.2.1 Manual Method (CHN /metals (nitric acid) analysis)

- The sample may be sieved as described in section 9.1 prior to grinding.
- Using a clean mortar and pestle, a proportion of the sieved material is ground into a fine homogenous mixture and then transferred to a second labelled sample pot/bag.
- The ground fraction is retained for archive purposes.

9.2.2 Automated Method (CHN / TBT / metals (HF) analysis)

- The sample maybe sieved as described in section 9.1 prior to grinding. Two types of grinding vessels/balls are available. The agate grinding vessels/balls are used with samples for metals analysis, not the metal set.
- Place a proportion (up to 1/3 of the grinding bowl volume) of the sample in the grinding bowl. In order to ensure that the machine runs smoothly, the PM 100 must be balanced after the grinding bowl has been inserted and clamped in.
- Balance the machine by following the instructions on p 14 of the PM100 manual. Place three 20 mm balls within the grinding bowl (12 balls are required when using the agate equipment).
- Close the cup and insert and clamp down securely described in p 14 of MP100 manual.
- Set the menu for the running time as described on p 22 of the MP100 manual. Usually 3 minutes at 300 rpm is sufficient (10 minutes using the agate equipment).
- After the grinding cycle is complete, visually inspect the ground sample. If it does not appear homogenous, regrind for further 3 minutes, repeating until homogeneity is achieved.

- Empty the ground sample into a labelled pot/bag and clean the grinding cup with a tissue, ensuring the cup is completely dry before further samples are ground.
- Switch the instrument off after use.
- The milled fraction is retained for archive purposes.

9.3 Fish Farm Chemical, OCP and CB Analysis

- Transfer the freeze dried sediment into the 2 mm sieve, with the collecting base attached.
- Gently break up the sediment, using the back of a Trulla spatula, and allow it to pass though the 2 mm mesh.
- Transfer the sieved sediment to a mortar and grind to a powder with a pestle.
- Transfer the powder onto aluminium foil and then back into the original container.
- The use of the powder funnel is permitted for sample transfer, if required.
- Clean the sieve, pestle and mortars between samples with a tissue moistened with hexane.
- Ensure all equipment is dry before preparing the next sample.
- The ground <2 mm fraction is retained for archive purposes.

10. Calculation of Results

Not relevant.

11. Precision, Bias and Limits of Detection

Not relevant.

12. Reports

Not relevant.

13. Safety

See Risk assessments no AI005, AI010 and AI105. Gloves and dust mask are required.

14. Literature References

Operating Instructions Ball Mills Type PM100/PM200. Retsch GmBH. 35pp. Room C502A

1. Introduction and Scope

The procedure describes the method for removing carbonate from dried sediments using 15% hydrochloric acid. The sample can then be analysed for organic carbon content using the Thermoquest, EA Flash 1112 Elemental Analyser.

2. Principle of the Method

HCl reacts with carbonate in the sample to produce CO_2 and water, leaving only organic carbon to be analysed. The sample is dried to remove excess HCl which may interfere with the CHN analyser when the sample is analysed.

3. Reference Material

LRM - Raasay sediment, is analysed after every 10-15 samples.

4. Reagents

Hydrochloric Acid 15% v/v (approx)

Using a measuring cylinder, measure out 85 ml ± 5 ml of distilled water and pour into a beaker. In a fume cupboard, carefully dispense 15 ± 2 ml HCl (Merck, sp gr 1.18) into the distilled water and gently mix. Transfer to a labelled volumetric flask with an expiry date of 6 months.

5. Equipment

Sartorious MC2105 Autobalance EN341
100 ml glass volumetric flask
200ml beaker
100 ml measuring cylinder
P039 - 20 µl and P276 - 30 µl calibrated pipettes
Aluminium sample tray
Teflon sample tray
Hot plate EN69
Silver capsules 12.5 x 5 mm
Forceps
Plastic Sample Tray
Small glass beaker
Timer EN1162
Dessicator

6. Environmental Control

The acidification and drying should be carried out in a fume cupboard.

7. **Interferences –** Not Relevant

8. **Sampling and Sample Preparation**

Sample are logged into the laboratory according to SOP 60, sample treatment and storage should be recorded on form B 62.
The samples must be freeze-dried and ground, SOP 110 and 120, prior to analysis.

9. **Analytical Procedure**

Notes:

Transfer all capsules between trays in order using forceps.

Ensure that pipettes are within annual and monthly calibration checks before use.

To prevent moisture ingress to the samples, they should be stored in a dessicator cabinet, from when they are weighed until they have been analysed. The silica gel in the dessicator should be checked to ensure it is still active, if it has turned yellow it needs replacing.

The spatula, forceps and sample preparation tray used must be kept scrupulously clean to prevent contamination of the sample during preparation.

A new plastic sample tray is used per batch, labelled with batch number, date of acidification and analyst initials.

Weigh a few extra Rassay sediment reference samples in the eventuality that they are required for checking the instrument.

9.1 **Weighing of Samples and Reference Materials**

Check weigh the balance on the day of use if not already checked.

9.1.1 The sample weight taken for sediments and reference materials is between 10-30 mg.
9.1.2 Tap the capsule lightly to ensure all the sample particles have reached the bottom.
9.1.3 When the weight has stabilised, note the weight on form B104 for LRM's and B68 for samples. **LRMS ARE NOTED ON BOTH FORMS**

9.2 Switch on the hot plate in the fume cupboard checking it is set at mark 65, hotplate temperature sholud not exceed 120ºC.

9.3 Transfer some prepared 15% HCl into a small beaker and pipette 20 µl into each of the sample capsules standing in the plastic tray. A record of the acidification process should be recorded on form B68.

9.4 Depending on how vigorous the reaction with the first 20 µl of acid, after at least five minutes pipette a further 20 µl of acid into each capsule.

9.5 Transfer the capsules into a Teflon tray sitting on the hotplate in order, using forceps. Dry the samples for at least 15 minutes and transfer the capsules cups back to the plastic tray in order. **DO NOT REMOVE THE TEFLON TRAY FROM THE HOTPLATE.**

9.6 Repeat steps 9.3 – 9.5 above, ie 20ul of acid is added 4 times.

9.7 Pipette 30 µl of acid into each capsule and transfer to the Teflon tray on the hotplate to dry for at least 15 minutes. Transfer the capsules back to the plastic tray.

9.8 Repeat 9.7 a further three times until 200 µl (total) has been added to each sample i.e. (4 x 20ul) + (4 x 30ul) = 200ul. On transferring the capsules to the Teflon tray for the last time, leave samples to dry for at least 30 minutes.

9.9 Transfer capsules to the aluminium tray on the hotplate and leave to dry for at least one hour.

9.10 **DO NOT REMOVE THE ALUMINIUM TRAY FROM THE HOTPLATE.** Transfer the capsules into the labelled plastic tray.

9.11 Using forceps place the capsule on a clean surface. While holding just above the bottom of capsule squeeze together the top and centre of the capsule. Fold the capsule in half and flatten, then fold the capsule in two again and flatten, ensuring no jagged edges that can catch on the autosampler. If the capsule bursts and sample is lost the sample must be repeated.

9.12 Replace the capsules to their positions in the labelled plastic tray and store in a desiccator until ready for analysis, Method M885.

10. **Calculation of Results** - Not relevant.

11. **Precision, Bias and Limit of Detection** - Not relevant.

12. **Reports** - Not relevant.

13. **Safety**

See Risk Assessment AI 009.

Disposable gloves should be worn when handling silver cups, and all digestion should take place inside a fume cupboard. Acid is added to distilled water using an automatic dispenser.

14. **Literature References** - Not relevant.

1. Introduction and Scope

CB recovery standard is used as a measure of method efficiency.

2. Principle of the Method

The quantitative addition of recovery standard, to samples prior to Soxhlet extraction permits the calculation of recovery CBs lost during analysis as a percentage. This percentage loss of recovery CBs is taken as being indicative of the losses of other chlorobiphenyls determined by the method

It is assumed in the recovery calculations, that the CB used to calculate recovery is not present in environmental matrices or if present is at negligible concentrations.

Calculation of recovery can only be carried out on chlorobiphenyls.

3. Reference Materials

Not relevant.

4. Reagents

50 µg/kg Standard containing CB35, CB53, CB112, CB151, CB198 and CB209

5. Equipment

Analytical balance (3 decimal place).
1000 µl pipette.
Pipette tips.
Beaker
Test-Tube

6. Environmental Control

Not relevant.

7. Interferences

Not relevant.

8. Sampling and Sample Preparation

The CB recovery standard is prepared using (SOP 290),(SOP 310) and (SOP 320).

9. **Procedure**

9.1 Check if a balance performance check has been carried out for the day, if not, perform this check, SOP 240

9.2 Break open ampoule containing CB recovery standard, Pour into a 10ml test-tube and stopper.

9.3 Place a beaker containing the extraction thimble with sample onto the balance and tare.

9.4 Pipette 1 ± 0.1 ml of recovery solution into the sample, and record the weight of solution on record sheet B247 alongside the sample number.

9.5 Repeat 9.3 and 9.4 until recovery standard has been added to all the samples.

10. **Calculation of Results**

10.1 Cr = * x Wt of sample extracted/Wt of CB Recovery Standard added

* Concentration of Recovery CB from Injection Report

10.2 % Recovery = 100 x (C_R / C_{std})

C_r = concentration of CB recovered (from injection report)
C_{std} = concentration of CB in recovery standard (from standards weights book)

11. **Precision, Accuracy and Practical Detection Limits**

Not relevant.

12. **Results**

Not relevant.

13. **Safety**

General protective clothing is required. For more information refer to Procedure Risk Assessment no AI031.

14. **Literature References**

Not relevant.

1. **Introduction and Scope**

   An internal standard containing DCBE (dichlorobenzyl alkyl ether) homologues 6 and 16 is added to sample fractions, then the final volume of the sample is adjusted by evaporation in preparation for GC analysis.

2. **Principles of the Method**

   Not relevant.

3. **Reference Materials**

   Not relevant.

4. **Reagents**

   2,2,4 trimethylpentane - Rathburn, HPLC grade.
   2000 ug/kg DCBE 6 and 16 standard solution (ampouled).

5. **Equipment**

   Analytical balance (4 decimal place)
   Blowdown apparatus
   Turbovaps
   glass beaker
   500 µl pipettor
   Pasteur pipettes
   Pipette tips
   10ml test-tubes
   measuring cylinder

6. **Environmental Control**

   Blowdown operations are carried out in the fume cupboard.

7. **Interferences**

   Not relevant.

8. **Sampling and Sample Preparation**

   Standard prepared using SOP's 290, 320, 300.

9. **Analytical Procedure**

9.1 When the sample clean-up fractions, S1 and S2 have been obtained (SOP 440), the fractions are reduced using turbovaps or blowdown apparatus (SOP 560) to 0.5 ml ± 0.1 ml. Using a measuring cylinder add 6 ± 0.5ml of 2,2,4 trimethylpentane into each Turbovap tube.

9.2 If a balance performance check has not been carried out on the balance for this day, then refer to SOP 240 and carry out this check.

9.3 Open the ampoule containing the 2000ug/kg D6D16, and empty into a 10 ml test tube.

For SI fraction:

9.4 Place a beaker on the balance pan, place the turbovap tube or test-tube containing the reduced sample into the beaker and tare.

9.5 Pipette 500 ± 50 µl of the internal standard into the sample and record the weight of standard added on record sheet B247.

9.6 Remove the turbovap tube or test-tube from the balance and shake gently.

9.7 Repeat steps 9.4, 9.5 and 9.6 ensuring that internal standard has been added to each S1 fraction.

For S2 fraction as S1 fraction or:

9.8 Label 10 ml test tubes with sample identification.

9.9 Place a beaker on the balance pan, place a labelled 10 ml test tube into the beaker and tare.

9.10 Pipette 500 ± 50 µl of internal standard into the test tube and record the weight of standard added in B247.

9.11 Repeat steps 9.9 and 9.10 for all labelled test tubes.

9.12 Pipette internal standard from the appropriate test tube into the appropriate turbovap tube (S2 fraction). Rinse the test tube with 2 x 0.5 ± 0.2 ml washes of 2,2,4 trimethylpentane. Transfer the washes to the turbovap tube containing the S2 fraction. Repeat for all samples.

For all fractions:

9.13 The sample volumes are reduced to 0.5 ± 0.1 ml, using turbovap apparatus (SOP 560). The samples are then vialled following SOP 590.

10. Calculation of Results

Not relevant.

11. Precision, Bias and Limit of Detection

Not relevant.

12. Reports

Not relevant.

13. Safety

General protective clothing is required. For more information refer to Procedure Risk Assessment no AI031

14. Literature References

Not relevant.

1. Introduction and Scope

A sample of sediment is weighed into a cellulose thimble and extracted with methyl t-butyl ether.

2. Principles of the Method

The solvent in the flask is heated on a heating mantle, the pure solvent vapour rises through the soxhlet (joined to the flask) and cools on the condenser (joined to the Soxhlet). The solvent drips into the Soxhlet (containing the thimble and sample) until the Soxhlet capacity is reached, then the solvent siphons into the flask below. The reflux process is continued until all extractable compounds have been removed to the solvent.

3. Reference Materials

3.1 In each batch (maximum of 12) of samples/extractions for CB's and OCP's.

3.1.1 Laboratory Reference materials are included and treated as samples:

LRM 140 - 10±1g
Record the use of each LRM in the Reference Material Worksheets B102

3.1.2 For the procedural blank a blank thimble is extracted and treated as a sample.

3.2 In each batch (maximum of 8 sediment samples) of samples for fish farm chemical analysis:

A blank thimble is extracted and treated as a sample. A reference sediment is spiked with the determinand chemical and treated as a sample, sediment 0799C. Approximately 20 g ± 1 g of sediment is weighed into a thimble and to this is added 500 µl of the determinand (1000 ng/ml) by calibrated syringe.

4. Reagents

Methyl t-butyl ether - Rathburn, HPLC grade.
Anti bumping granules - Merck.
Copper powder - prepared as in SOP 490.

5. Equipment

Balance (2 decimal place).
Whatman cellulose thimbles (either 41 x 123 mm or 28 x 80 mm).
Glass measuring cylinder.
Glass beakers.
Round bottomed flasks (either 500 ml or 250 ml).
Soxhlets (large or small).

Condensers
Spatula.
Forceps.
Cork rings.
Aluminium foil.

6. Environmental Control

Extraction process is carried out on the bench in a spark proof room with fume cupboards in operation. Measuring and transfer of solvent operations are carried out in a fume cupboard. Cellulose thimbles are only handled using forceps.

7. Interferences

Not relevant.

8. Sampling and Sample Preparation

See SOP 120 - Preparation of freeze dried sediment samples for physical and chemical analysis.

Note: Record extraction conditions on B247, OCP's and CB's.
Record extraction conditions on Worksheet B81 for fish farm chemicals.

9. Analytical Procedure

9.1 Set up condensers, checking for water leaks. Water flows in the bottom and out the top. Into each flask a few anti bumping granules (4-20) are added using a spatula.

9.2 Using a dispenser on a winchester bottle, the required volume of methyl t-butyl ether is dispensed into a measuring cylinder and in turn transferred into a round bottomed flask and stoppered, see below.

For a 28 x 80 mm thimble dispense 3 x 60 ± 5 ml into a measuring cylinder and transfer to a 250 ml round bottomed flask with a small Soxhlet.

For a 41 x 123 mm thimble dispense 3 x 100 ± 5 ml into a measuring cylinder and transfer to a 500 ml round bottomed flask with a large Soxhlet.

9.3 Ensure balance performance check has been carried out, SOP 240.

9.4 Activated copper powder 10-20 g is added by spatula to each flask (weight will increase due to hexane). For preparation see SOP 490. During extraction activated copper will react with any sulphur extracted from the

sediment to produce a black coating of copper sulphide on the surface of the copper.

9.5 Prepared samples are weighed into a tared thimble (thimbles are pre-extracted see SOP 555). Weights are recorded in B247 (OCP's and CB's only), B81 (fish farm chemicals). Guidance as to the quantity of material to be extracted is listed below

Clean offshore marine sediment: 20-150 g.
Coastal or estuarine sediment: 20-50 g.
Sludges or spoils: 1-20g
Highly contaminated sediment sites: 1-20 g.
Fish farm chemical analysis: 20 g ± 1g.

9.6 Add weighed recovery standard to the samples contained in thimbles (OCP's and CB's only), SOP 345. The thimbles are inserted into the Soxhlets using forceps.

9.7 Fit Soxhlets to flasks and soxhlets to condensers. Ensure flasks are labelled with correct sample identification numbers. Cover the tops of the condensers with foil to minimise evaporation.

9.8.1 Switch on the main connection to the heating mantles. If the red indicator light on the flow controller unit is flashing proceed to 9.8.2. If the red indicator light does not flash, switch on cooling water and switch power off then on again. Light should flash proceed to 9.8.2.

9.8.2 Switch on the condenser cooling water at the taps, ensuring a good flow. Depress flow controller button, the red indicator light will stop flashing.

9.8.3 The controls on the individual mantles are adjusted setting is between 4-5.

9.8.4 The flow controller will cut off the power to the heating mantles if the water flow stops or is reduced to a trickle, and a light will flash indicating no power flow. If this happens increase the water flow and depress the button on the flow controller to restart extractions.

9.9 Samples are extracted overnight or staged for at least a total of eight hours. Each time the extractions are stopped, the power is switched off leaving the water flowing through the condensers until the samples have cooled. If extractions are to be restarted add fresh anti-bumping granules.

9.10.1 If unreacted copper is visible after the extraction process is complete, the extracted sulphur has been removed from the extract.

9.10.2 If the copper is completely black, ie no unreacted copper is visible then more activated copper powder is added to the extract. The extraction is continued for one hour.

9.10.3 The above step is repeated until some copper powder remains unreacted.

9.11 Remove the flasks and soxhlets from the mantles, draining the solvent from the soxhlets and thimbles into the flask. Remove the thimbles (when drained) from the soxhlets and place on aluminium foil to dry. Pour the remaining solvent from the soxhlets into the flasks and stopper.

9.12 When the thimbles are dry, dispose of into the chemical waste bins.

9.13 Prepare extracts for clean-up, SOP 368, CB's and OCP's only.

9.14 The copper powder can be easily removed from the flasks when dry and disposed of into the chemical waste bins.

9.15 Dismantle the condensers for cleaning.

10. Calculation of Results

Not relevant.

11. Precision, Bias and Limits of Detection

Not relevant.

12. Reports

Not relevant.

13. Safety

General protective clothing is worn. Gloves to be used in solvent and sediment handling procedure. Dust mask to be worn when handling sediments. For more information refer to Procedure Risk Assessment no (AI011)

14. Literature References

Not relevant.

1. Introduction and Scope

Weighed samples of sediment and biota are placed into extraction cells and extracted with *iso*-hexane for CBs (ortho and non-ortho), OCPs and PBDEs. Lipid removal from samples is achieved by the addition of 5% deactivated alumina to the extraction cells. 30 g alumina can be used to remove 300 mg of lipid of samples for PBDE analysis or CB analysis. If OCPs and or non-ortho CBs are also to be extracted less alumina should be used and further clean-up steps are needed.

2. Principles of the Method

Samples are extracted on the Accelerated Solvent Extractor (ASE 300) under elevated temperatures and pressures. Solvent is added to an extraction cell and heated before being flushed into a collection bottle. 5% Deactivated alumina can be added to the extraction cell to remove lipid from biota samples.

3. Reference Materials

In each batch of samples (maximum 12) a laboratory reference material and a procedural blank must be analysed.

LRM 110 Danish Cod Liver Oil is analysed with biota samples (CBs, OCPs and PBDEs).
LRM 110 is stored at room temperature in amber ampoules in the reference material cupboard located in room 506.

LRM 140 is analysed with sediment samples (CBs and PBDEs).
LRM 140 is stored at room temperature in amber jars in the reference material cupboard located in room 506.

The use of LRMs are recorded in organic reference material worksheet (B 102, CBs (ortho and non-ortho) and OCPs only)

4. Reagents

Anhydrous Sodium Sulphate (Analar, granular).
Copper wire – Elemental Microanalysis.
iso-Hexane (HPLC grade with bakelite caps, Rathburn Chemicals).
Acetone (HPLC grade with bakelite caps, Rathburn Chemicals).
Alumina (Aluminium oxide 9 standardised) – Merck 1.01097.1000 .
Distilled water – ultra pure..
50 µg kg^{-1} standard containing CB 35, CB 53, CB 112, CB 151, CB 198 and CB 209 (this standard is for CB or CB/PBDE split extractions).
100 ng ml^{-1} internal standard containing C^{13}CB 81, C^{13} CB 77, C^{13} CB 126 and C^{13}CB 169 (non-ortho CBs) as per SOP 0295.
CB198, approx 140 ng ml^{-1} (for extractions for only PBDEs).

If an alternative supplier is used, an equivalent grade of reagent is used

5. Equipment

Dionex Sample Cells (various sizes)
Dionex glass fibre filters
Dionex cell filter insertion tool
Sample collection bottles
Balance (set to read to 3 decimal place)
Glass Beakers (various sizes)
Glass jars and lids (250 ml)
Spatulas
Forceps
Aluminium foil
Solvent reservoir bottle
Dionex ASE 300 (EN 1241)
Calibrated syringe
Ultra sonic bath
Drying oven (EN 547)
Measuring cylinders (various sizes)
Glass stirring rod
Test tubes
Pasteur pipettes
Pipettors
Refrigerator
Pestle
Air Purifier
Kenwood mini chopper
Muffle Furnace

6. Environmental Control

The extraction process is carried out on the bench in the spark proof room (Room 505) with the fume cupboards in operation. Measuring and transfer of solvent is undertaken in the fume cupboard.
Labcoats must be worn at all times in labs 505 and 506.
The ASE is cleaned daily by both Acetone and *iso*-hexane before use as per SOP 0371.
If PBDEs are to be extracted then the window blinds must be down, the lights off and the ioniser switched on.

7. Interferences

PBDE and CB/OCP analysis requires a stable environment, avoiding contamination of samples and reagents eg. contact with fingers, dirty equipment. Glass fibre filters are only handled with forceps. All glassware/ extraction cells are solvent washed with either acetone or *iso*-hexane prior to use. Nitrile gloves are to be worn while working in the laboratory.
The addition and transfer of extracts and standards is by means of either glass pipette tips or calibrated glass syringe to prevent contamination.

PBDE extraction is carried out with as little light in the room as possible - with the window blinds down and the lights off. Ensure the air purifier is switched on to reduce possible contamination from particulates in the air.

8. Sampling and Sample Preparation

All biota samples should be defrosted at room temperature or in the fridge overnight and exposure to direct sunlight or heat should be avoided. Once defrosted samples are cut up finely with a knife and/or homogenised using a Kenwood Mini Chopper. Samples are then transferred glass jars using a solvent washed metal spatula. The remainder of the sample should be returned to the deep freeze as soon as weighing of all samples in the batch is completed.
Sediment samples are freeze dried and sieved as per SOPs
(SOP 0110,SOP 0120)

9. Analytical Procedure

9.1 Cleaning of equipment/materials

9.1.1 Glass fibre filters - Glass fibre filters are wrapped in aluminium foil and placed in a muffle furnace, set to 300°C. The filters are muffled for at least 12 hours. Date, time in/out of furnace, temperature and operator are recorded at the back of the ASE logbook, which is kept next to the instrument.

9.1.2 All extraction cells, caps and collection bottles are solvent washed with acetone or *iso*-hexane followed by *iso*-hexane, with the latter being allowed to evaporate. The collection bottles lids are fitted with ultra clean low bleed septa. Cells and caps are numbered 1 – 12 (s, small, m, medium and L, large). Ensure each cell is fitted with the appropriately numbered lid.

9.2 Sample preparation

9.2.1 Sediment sample Preparation

9.2.1.1 Sediment samples are freeze dried and sieved as per SOPs (SOP 0110, SOP 0120)

9.2.1.2 Label both the side and lid of a glass jar with the UKAS ID. Into a solvent washed glass jar weigh the appropriate amount of sediment: suggested weights for different sediment types Table 1

Clean offshore marine sediment:	20-40 g
Coastal or estuarine sediment:	15-25 g

Sludges or spoils:	5-15 g
Highly contaminated sediment sites:	5-15 g
LRM 140:	10 ± 1 g

Table 1: Suggested sediment weights

9.2.1.3 Solvent washed sodium sulphate (SOP 1643) should be added to the sediment in the ratio of ~ 1:1 (minimum 30g).

9.2.1.4 Record weights on the organic worksheet B 244 (CBs and OCPs) or B 314 (PBDEs –record on B 314 and subsequently transfer to electronic copy, both copies are kept with batch).

Note If the batch is to be split for determination of both PBDEs and CBs (ortho CBs only) from a single extract this must be recorded on the worksheet.

9.2.1.5 Mix the sample and sodium sulphate using a solvent washed spatula.

9.2.1.6 Each batch consists of a procedural blank, LRM and up to 10 samples. The procedural blank consists only of sodium sulphate. The LRM is treated as a sample. Steps 9.2.1.2 - 9.2.1.5 are repeated for all samples.

9.2.2 Biota sample preparation.

9.2.2.1 Ideally the % lipid of biota samples should be determined first using the Smedes method (M 0890). If not then use the indicative values in the Table 2, determined on various biota types.

9.2.2.2 For PBDE or CB/OCP analysis an appropriate amount of sample equivalent to 300 mg of lipid is used in the extraction. The weight of tissue required is determined in worksheet B 245 for CBs and B 314 (PBDEs).

Approximate weights and indicative % lipid for various tissue samples:

Biota type	weight	Indicative % lipid
Fish muscle, lean	10-15 g	3%
Fish muscle, fatty	0.5 - 2 g	15%
Fish liver	0.5-3 g	40%
Mussel	8-12 g	2.5%
Cod liver oil	0.1- 0.3 g	100%
LRM 110	≤ 0.3 g	100%

Table 2 – Indicative values to be used if % lipid has not first been determined.

Label both the side and lid of a glass jar with the UKAS ID. The sample is weighed in a glass jar and the weight recorded in the organic Worksheet B 244 for CBs/OCPs and B 314 for PBDEs. Individual samples are cut into small pieces using solvent washed, forceps, scalpel or scissors. Pooled samples may have been previously homogenised (SOP 130).

If the sample is for non-ortho CB analysis add 100 ul of the internal standard prepared as per SOP 0295.

Note If the batch is to be split for determination of both PBDEs and CBs (ortho CBs only) from a single extract this must be recorded on the worksheet.

9.2.2.3 Solvent washed sodium sulphate (SOP 1643) is added to the sample to allow drying. Use between 20 and 40 g of sodium sulphate. Mix the sample and sodium sulphate using a solvent washed spatula.

9.2.2.4 The spatula used to mix the sample with sodium sulphate should be rinsed down into the jar with *iso*-hexane (2 x1ml).

Each batch consists of a procedural blank, LRM and up to 10 samples. The LRM is treated as a sample. The procedural blank consists of only 30 ± 5 g sodium sulphate.

The forceps, scalpel and scissors are rinsed with acetone and dried with blue roll between samples. The waste acetone is emptied into a non-chlorinated solvent waste bottle.

Steps 9.2.2.2-9.2.2.3 are repeated for all samples.

9.2.2.5 Place lid on glass jar and store overnight in a refrigerator.

9.2.2.6 Remove from the fridge and grind with a pestle wrapped in aluminium foil for at least 2 minutes.

9.3 Preparation of recovery standard for ortho CBs and PBDEs

If samples are for non-ortho CBs proceed to 9.4 as an internal standard has been added (9.2.2.2).

9.3.1 CB/OCP recovery standard (ortho CBs only or ortho CB/PBDE split batch)

9.3.1.1 The recovery standard is prepared using (SOP 290),(SOP 310) and (SOP 320).

9.3.1.2 Break open ampoule containing recovery standard. Pour into a 10ml solvent washed test tube and stopper.

9.3.2 PBDE recovery standard (extraction of PBDEs only)

The recovery standard for the PBDEs is CB198. This is prepared according to SOP 290. The stock is a nominal 1400 ng.

9.4 Filling of extraction cells

9.4.1 Samples are typically extracted using the 100 ml extraction cells. **Note** It is permissible to use an alternative size of cell if necessary as long as the LRM and procedural blank are extracted in the same size cells.

9.4.2 Cells and caps are numbered, ensure cap and cell body numbers correspond. Cells are filled as per schematic Figure 1.

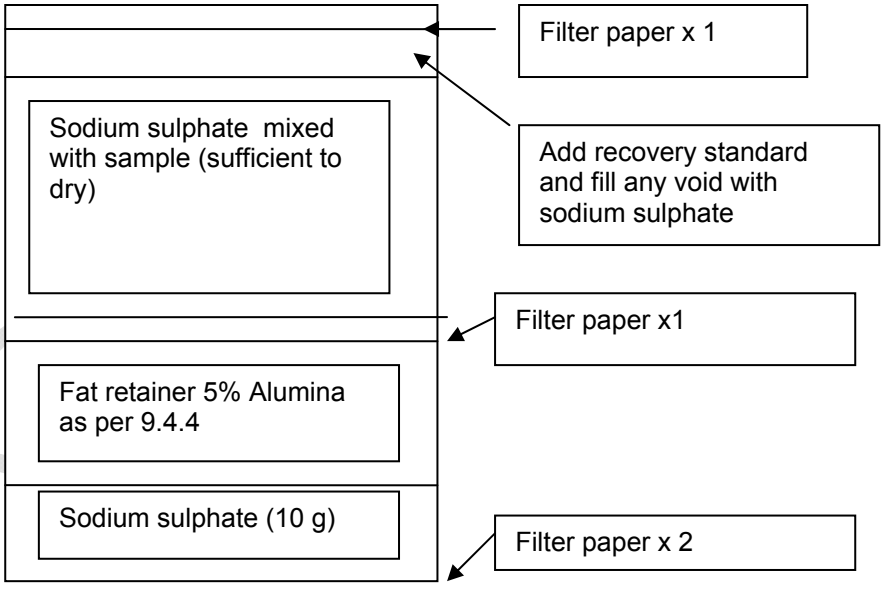


Figure 1 Schematic of ASE cell for the extraction of PBDEs, CBs and OCPs from biota and sediment.

9.4.3 Unscrew the top cap from the cell body. Place 2 filters in the cell at a slight angle (Dionex ASE 300 manual 3-6). Place the insertion tool over the filters and slowly push the insertion tool into the cell. Ensure the filter is in full contact with the cell.

Note: Do not place the filter in the bottom cap before installing the cap, this creates an improper seal and allows leaks.

9.4.4 To the cells add solvent washed sodium sulphate (SOP 1643), 10 ± 1g *via* a funnel.

- PBDEs and/or CBs (ortho) from biota only - Add 30 ± 1g, *via* a funnel, of 5% deactivated alumina (SOP 0430)

- If OCPs and/or non ortho CBs are to be extracted in addition to CBs from biota then use 15 ± 0.1g, *via* a funnel, of 5% deactivated alumina (SOP 0430).

- If sediment is being extracted for PBDEs and/or CBs then 15 ± 1g, *via* a funnel, of 5% deactivated alumina (SOP 0430). NB if extracting sediment a spatula full of activated copper wire is placed in the sample collection bottles.

Note If the samples are required for CBs (ortho and non ortho) or OCPs the weight of the alumina added to the cell must be recorded on B 244.

Place another filter in the cell and push down on to the alumina using the insertion tool.
Add the sample to the cell (see section 9.2.1 and 9.2.2) *via* a funnel.

Rinse sample jar with 5 ml of *iso*-hexane then add this carefully to top of the sodium sulphate.

Add the recovery standard as per **9.4.5** (ortho CBs and PBDEs only) and add more sodium sulphate to fill any void volume remaining.

The whole cell is tapped down using the insertion tool – top up with sodium sulphate if necessary before adding a further filter on top before hand tighten the top and bottom lids of the cell.

DO NOT USE A WRENCH OR OTHER TOOL TO TIGHTEN THE CAP.

9.4.5 Addition of Recovery Standard (CBs (ortho) and PBDEs)

A known volume of recovery solution (Table 3) is added by means of a calibrated syringe into the extraction cell containing the sample and sodium sulphate (9.4.4). Repeat for all samples. The volume of recovery standard added is recorded on record sheet B 244.

Determinand	Volume of recovery standard
CBs alone or with OCPs	1 ml CB/OCP recovery std
Combined CB and PBDE analysis	1ml CB/OCP recovery std
PBDE analysis	100 ul (conc standard) PBDE recovery std

Table 3 Recovery standard volumes

Note No PBDE recovery standard is added if CBs also being analysed (added in 9.3.1.3). No recovery standard is added if non-ortho CBs are to be extracted, internal standard already added (9.2.2.2).

9.4.6 Proceed to extraction by ASE 300 SOP 0371.

10. Calculation of Results

Not relevant

11. Precision, Accuracy and Practical Detection Limits

Not relevant.

12. Results

Not relevant

13. Safety

AI143

1. **Introduction**

Extracts are reduced in volume and changed into hexane in preparation for the clean-up procedure, SOP 440.

2. **Principle** - not relevant

3. **Reference Materials** - not relevant

4. **Reagents**

Methyl t-butyl ether - Rathburn, HPLC grade
Hexane - Rathburn, HPLC grade

5. **Equipment**

turbovap systems and tubes (SOP 560)
blowdown apparatus (SOP 570)
test-tubes
glass measuring cylinder
glass beaker

6. **Environmental Control**

All solvent manipulations are performed in a fume hood.

7. **Interferences** - not relevant

8. **Sampling and Sample Preparation**

The residual lipid content of biota extracts is calculated, see M880.

9. **Analytical Procedure**

9.1 The total sediment and blank extracts are transferred to Turbovap tubes rinsing the flasks three times with a few mls of MTBE.

9.2 The volume of biota extract for clean-up is calculated, see M880.

9.2.1 Measure the calculated volume for clean-up (Organic Worksheet E, B59) in a measuring cylinder and decant into a turbovap tube, along with three rinses of a few mls of MTBE.

Note: Rinse the measuring cylinder with several washes of solvent in between each sample.

9.3 Extracts are evaporated to 0.5 ± 0.1 ml, using either the turbovap systems (SOP 560) or blowdown apparatus (SOP 570).

9.4 Measure 10+/- 2ml of hexane into a measuring cylinder and add to each extract.

9.5 Evaporate extracts to 0.5 ± 0.1 ml using the turbovap systems.

9.6 Extracts are now ready for the clean-up procedure, SOP 440.

10. **Calculation of Results** - not relevant

11. **Precision, Accuracy and Practical Detection Limits** - not relevant

12. **Safety**

General protective clothing is required. Gloves are required during solvent manipulations. For more information refer to Procedure Risk Assessment nos AI034 and AI034A.

13. **Literature References** - not relevant

1. Introduction

Extracts are cleaned up and separated into CB and OCP fractions using alumina and silica column chromatography. Each column type serves a different purpose but their preparation and use is similar and is therefore described under the same heading.

2. Principle

During the alumina clean-up, lipid is removed and the CB's and OCPs are primarily separated between two fractions A1 and A2. The A1 fraction is further separated by a silica clean-up into a further two fractions S1 containing CB's and the S2 which when combined with the A2 fraction contains the OCPs.

3. Reference Materials

Not relevant.

4. Reagents

5% deactivated alumina (preparation SOP 430)
3% deactivated silica (preparation SOP 410)
Hexane - Rathburn, HPLC grade
Iso- Hexane, Rathburn HPLC grade
Anhydrous sodium sulphate (preparation SOP 140)

5. Equipment

12 space column rack.
10 ml conical glass test tubes
Clean glass rod.
Glass beaker.
Glass chromatography columns with solvent reservoir.
Calibrated glass tubes (stored with adsorbents).
200 ml and 50 ml Turbovap tubes.
MTBE washed cotton-wool balls.
Glass measuring cylinders

6. Environmental Control

Procedure is carried out in a fume hood.

7. Interferences

Not relevant.

8. Sampling and Sample Preparation

Not relevant.

9. **Procedure**

Note: Measure all solvent with volumes > 1ml into a measuring cylinder before transferring to the head of the chromatography columns. Pipette any volumes < or = to 1ml.

If clean up procedure is for CB anlysis only. Then the amount of hexane required for eluting the columns is as follows:
6g Alumina – 50ml
3g Alumina – 20ml
3g Silica – 20ml

9.1 Column Preparation

Note: Clean-up columns are prepared no more than two hours before use. Batch numbers, the volumes of solvent used and the split volumes are recorded in (B247).

9.1.1 Place the chromatography columns into the rack. Rinse the columns with either hexane or iso hexane and allow to dry before adding absorbent.

9.1.2 Plug the chromatography columns with a cotton-wool ball, pushed to the end of the column with the glass rod.

9.1.3 Measure the appropriate amount of absorbent into the calibrated glass tube tapping gently to settle the contents, see 9.2, 9.3 and 9.4.

9.1.4 Pour the absorbent into the column and tap down gently to pack the absorbent. A small amount (between 1 and 4cm in length) of anhydrous sodium sulphate is added the top of the alumina columns.

9.1.5 Column tops are covered with aluminium foil.

9.2 Biota Samples with 50 mg > Extractable Lipid < 200 mg

9.2.1 Prepare 6 ± 0.2 g alumina columns.

9.2.2 Using a pasteur pipette transfer the reduced hexane extract (SOP 368) to the top of the column and allow to adsorb.

9.2.3 Add 1 ± 0.2 ml of hexane to rinse the extract tube. Pipette rinse to the top of the column and adsorb. Repeat using another 1 ± 0.2 ml rinse of hexane.

9.2.4 Dispense 100 ± 2 ml of hexane into a measuring cylinder, a small volume of this is used to rinse the extract tube, which is then transferred to the top of the column.

9.2.5 Transfer the remaining hexane to the top of the column. Note: if the volume of hexane stated on the storage jar for a 6 g column is > 100 ml, add the remainder when solvent reservoir allows.

9.2.6 The total eluate is collected in a 200 ml turbovap tube.

9.2.7 Repeat steps 9.2.2 to 9.2.6 for all reduced hexane extracts.

9.2.8 The collected eluates are reduced in volume to approximately 0.5 ± 0.1 ml, using the turbovap systems (SOP 560).

9.2.9 Prepare 3 ± 0.1 g alumina columns.

9.2.10 Repeat steps 9.2.2 to 9.2.5, using the reduced extracts from the 6 g alumina columns. Replace the 100 ml in step 9.2.4 with the volume given on the storage jar.

9.2.11 Using the test split information given on the absorbent storage jar the first fraction, A1 eg 5 ml is collected in a 10 ml test tube. The remaining eluate, A2 is collected in a 200 ml Turbovap tube.

9.2.12 The A1 fractions are transferred to turbovap tubes with 3 x 3 ± 1 ml washes of hexane. They are reduced to 0.5 ± 0.1 ml using the turbovap systems (SOP 560).

9.2.13 Prepare 3 ± 0.2 g silica columns.

9.2.14 Repeat steps 9.2.2 to 9.2.5, using the reduced A1 fraction. Replace the 100 ml of hexane in step 9.2.4 with 25 ± 1 ml.

9.2.15 Using the test split information given on the absorbent storage jar the first fraction, S1 eg 5 ml is collected in a conical 10 ml test-tube and transferred to a turbovap tube with 3 x 3 ± 1 ml washes of hexane. They are reduced to 0.5 ± 0.1 ml using the turbovap systems (SOP 560). Internal standard is added see SOP 350.

9.2.16 The remaining eluate, S2 is collected in the 200 ml Turbovap tube used in step 9.2.11, or seperate 50ml TurboVap tube. The A2 and S2 fractions are combined to produce the final S2. Internal standard is added see SOP 350.

9.3 Biota Samples with < 50 mg of Extractable Lipid

9.3.1 Prepare 3 ± 0.2 g alumina columns.

9.3.2 Follow steps 9.2.10 to 9.2.11, using the reduced extracts. Replace the 100ml of hexane in step 9.2.4 with the volume on the storage jar for a 3 g column.

9.3.3 Follow steps 9.2.11 to 9.2.13.

9.4 Sediment Samples

9.4.1 As above for biota samples < 50 mg extractable lipid.

10. Calculation of Results

Not relevant.

11. Precision, Accuracy and Practical Detection Limits

Calibration and Precision of Absorbent Tubes

	Silica 3 g (3.0070 g)	Alumina 3 g (3.0012 g)	Alumina 6 g (6.0086 g)
1	2.9887	2.9809	5.9568
2	3.0190	2.9644	5.9753
3	2.9859	2.9426	5.9554
4	2.9916	3.0261	5.9898
5	3.0226	2.9982	5.9670
6	3.0306	3.0118	5.9797
7	3.0431	2.9497	6.0486
8	3.0098	3.0295	5.9716
9	3.0657	2.9985	6.0192
10	3.0547	3.0017	6.0694
11	3.0305	2.9482	5.9740
12	3.0018	3.0234	6.0242
Mean	**3.0203**	**2.9896**	**5.9926**
STD	**0.0249**	**0.0304**	**0.0372**
% CV	**0.82**	**1.02**	**0.62**

12. Safety

General protective clothing is required. Dust mask is required during column preparation. Gloves are required during solvent manipulations. For more information refer to Procedure Risk Assessments no AI023 and AI024.

13. Literature References

Not relevant.

1. Introduction and Scope

A set of 7 standards (0.002, 0.005, 0.010, 0.020, 0.050, 0.100 and 0.200µg/g – CB or OCP) are run before a batch of samples in order to verify GC performance and to set up a calibration file. If standards meet the required criteria, analysis can proceed.

A 0.200µg/g *p,p'*-DDT standard (containing 2.0µg/g D6/D16) is also run prior to a batch of samples in order to check for breakdown of *p,p'*-DDT to *p,p'*-DDE and *p,p'*-DDD.

2. Principles of the Method

2.1 Evaluation of organochlorine pesticide and chlorobiphenyl standards

Records of the 0.200µg/g standard data are kept in the GC injection book, and charts for D16/D6 ratios maintained on NTS5/qalan/data/Analytical Investigations/CBs and OCPs/GC D6 and D16. Also results of the 0.05ug/g standards analysed as samples (SOP1040 and SOP1060) are recorded and monitored for drift.

Lower and upper limits are set for the D16/D6 ratios and results are plotted on charts. If values fall outside these limits, action should be taken (see SOP960 (Varians) or SOP980 (Perkin Elmer) for maintenance). Different columns can give different ratios, and the D16/D6 charts assist in monitoring for any deterioration in column performance.

2.2 Evaluation of *p,p'*-DDT standard

Records of 0.200µg/g *p,p'*-DDT chromatograms are archived along with TURBOCHROM data files (see SOP905). The percent breakdown of *p,p'*-DDT to *p,p'*-DDE and *p,p'*-DDD is calculated before running a batch of samples, and this value is recorded in the appropriate GC injection book and Shewhart Charts as 2.1 above.

3. Reference Material - Not relevant.

4. Reagents - Not relevant.

5. Equipment - Not relevant.

6. Environmental Control - Not relevant.

7. Interferences - Not relevant.

8. **Sampling and Sample Preparation** - Not relevant.

9. **Analytical Procedure**

9.1 **Quality control for CB and OCP standards**

A set of standards (0.002, 0.005, 0.010, 0.020, 0.050, 0.100, 0.200µg/g, CB or OCP as required) must be run before a batch of samples and the following criteria met:

9.1.1 Peak tailing should be kept to a minimum. Peaks should be symmetrical (gaussian) not skewed.

9.1.2 Retention times and peak heights for D6 and D16 are taken from Turbochrom – **R**esults, **D**isplay, **P**eak Report.

Retention times and peak heights will vary depending on the temperature programme used in the GC method. Peak heights should be at least the values indicated in Table 1 and the D16/D6 ratio should fall within the limits in Table 2.

Table 1 – Minimum D6 and D16 peak heights (uV).

	Column	Method	HT D6	HT D16	AT
PE GC	HP5 or equivalent	1	20,000uV	15,000uV	64
V4 GC		1	25,000uV	20,000uV	16

PE = Perkin Elmer V4 = Varian 4

Table 2 – Retention time (RT) windows for D6 and D16 and D16/D6 ranges.

	Column	Method	RT D6	RT D16	Ratio D16/D6	AT
Perkin Elmer GC	HP5 or equivalent	1	34mins ± 3mins	64mins ± 3mins	0.50 – 1.00	64
Varian GC	HP5 or equivalent	1 (V4)	34mins ± 3mins	64mins ± 3mins	0.60 – 1.00 (V4)	16

V4 = Varian 4

9.1.3 The following check is not routine due to the inability of the Turbochrom Client software to provide the peak width.The peak width at half height can be roughly estimated using the mouse and a steady hand by the operator if there is doubt over peak resolution. Peak widths should be less than 5.2

seconds (width at half peak height), otherwise the response is compromised, and closely eluting peaks will be poorly resolved.

9.1.4 HP5 or equivalent - CB standards. Check for complete resolution between; CB52 and CB49; CB74 and CB70; CB101 and *o,p'*-DDE; CB153 and CB105; and CB138 and CB158.

9.1.5 The results of the 0.05ug/g standards analysed as samples to check for instrument drift (SOP1040 and SOP1060) are entered into a worksheet along with the associated sample results in either the CB OCP sediment template B568 or the CB OCP biota template B569. The individual calculated concentration of each congener is automatically checked for acceptability, this is ± 5µg/kg of the standard concentration in the calibration file. The concentrations are then copied to NTS5/qalan/data/Analytical Investigations/CBs + OCPs/GC Drift/ "instrument", so trends can be identified.

9.1.6 Check the baseline integrity. If a rising baseline or ghost peaks are apparent in the standards, either the column has deteriorated or contamination is present in the system.

9.1.7 If one or more of the criteria are not met then GC maintenance is required see SOP960 (Varian) or SOP980 (Perkin Elmer)

9.2 **Quality control for p,p'-DDT breakdown**

As active sites develop within the GC *p,p'*-DDT will break down to form *p,p'*-DDE and *p,p'*-DDD. This will result in lower levels of p,p'-DDT being recorded. To monitor for this a 0.200µg/g *p,p'*-DDT standard (containing 2.0µg/g D6/D16) should be run prior to a batch of samples to check for DDT breakdown.

9.2.1 The criteria set in 9.1.1-9.1.4 should be followed.

9.2.2 The chromatogram should produce three distinct peaks (D6, *p,p'*-DDT, and D16) if no adsorption of DDT occurs. If so then no maintenance is required and analysis can continue.

9.2.3 If more than three peaks (other than background peaks) appear on the chromatogram this indicates that *p,p'*-DDT has broken down to *p,p'*-DDE and *p,p'*-DDD. There are two areas where DDT may break down - (1) at the front end of the column, and (2) in the injection port.

If *p,p'*-DDT breaks down on the column the resultant chromatogram will show three "closely" eluting peaks (in addition to D6 and D16) These are *p,p'*-DDE, *p,p'*-DDD and *p,p'*-DDT. They will not have had sufficient time to separate fully during the temperature programme.

However, if *p,p'*-DDT adsorbs to the injection liner it will break down at this site and produce three "distinct" peaks for *p,p'*-DDE, *p,p'*-DDD and *p,p'*-DDT (see attached chromatogram A).

9.2.4 If the level of DDT breakdown exceeds 10% (see Section 10 below) then the appropriate maintenance should be carried out (ie change liner and trim the column - see SOP960 (Varian) or SOP980 (Perkin Elmer)).

10. Calculation of Results

Using Peak heights,
% Breakdown DDT = [*p,p'*-DDE + *p,p'*-DDD]/[*p,p'*-DDE + *p,p'*-DDD + *p,p'*-DDT] x 100

Peak heights of *p,p'*-DDE, *p,p'*-DDD and *p,p'*-DDT are obtained from the Peak Report in Turbochrom – **R**esults, **D**isplay, **P**eak Report.

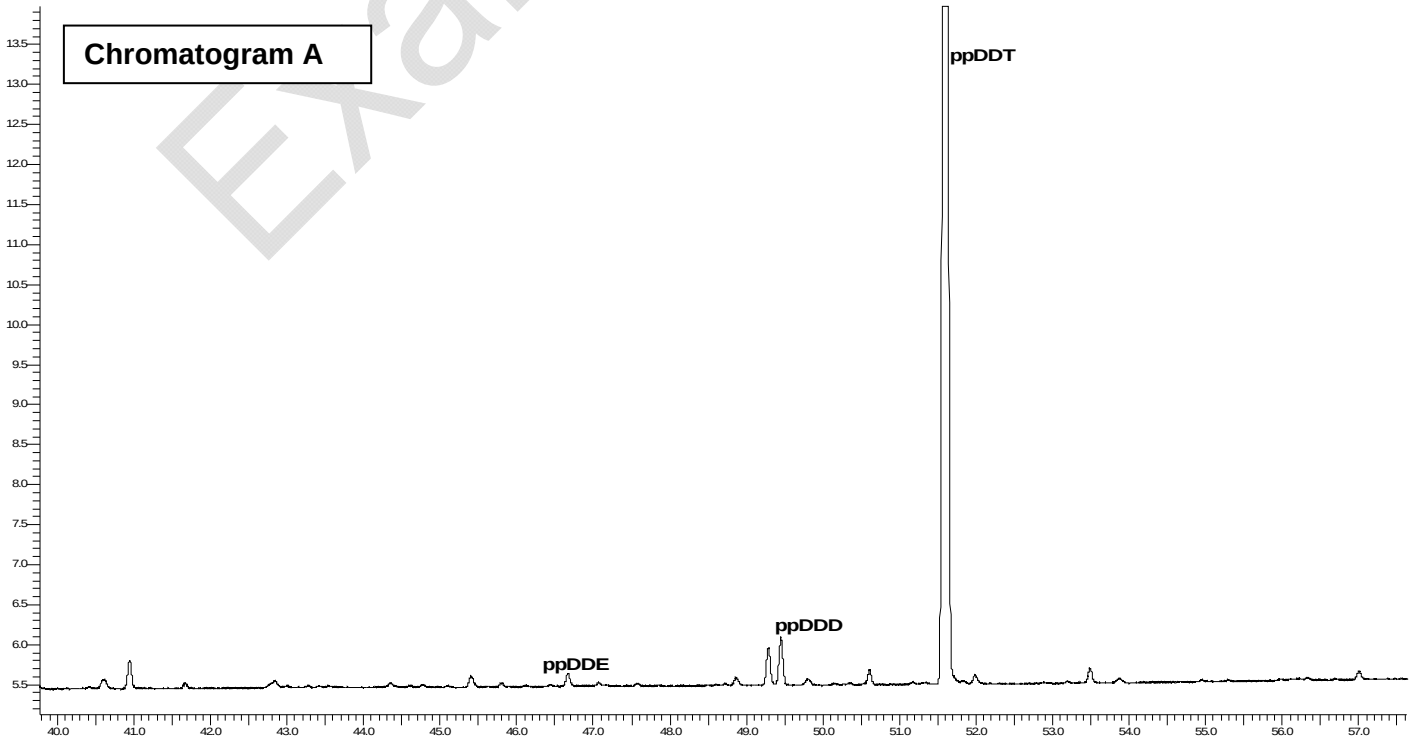
11. Precision, Bias and Limit of Detection - Not relevant.

12. Reports - Not relevant.

13. Safety - Not relevant.

14. Literature References - Not relevant.

Chromatogram A



1. Introduction and Scope

This method describes the extraction of sediments for determination of total hydrocarbons by fluorescence, aliphatic hydrocarbons by gas chromatography with flame ionisation detection (GC-FID) and polycyclic aromatic hydrocarbons (PAHs) by gas chromatography mass spectroscopy (GC-MS).

2. Principle of the Method

Hydrocarbons are extracted from the sediment using a polar solvent mixture, sonication and centrifugation. The chlorinated solvent is isolated from the methanol by partitioning with water. The chlorinated solvent is dried and then diluted to a standard volume from which a small aliquot is removed for fluorescence determination, if required. The remaining solution is solvent exchanged to *iso*-hexane and the hydrocarbons fractionated into aliphatic hydrocarbons and PAHs by normal phase HPLC. The final concentration is quoted on the basis of dry weight of sediment (SOP 1615).

3. Reference Materials

In each batch (maximum 24) of sample extractions a laboratory reference material is included and tested as a sample, (Aberdeen Harbour sediment, LRM 142 - kept in a cupboard in Laboratory 123/124). Approximately 1 g of sediment is weighed into a 250 ml centrifuge tube and the exact weight recorded on the hydrocarbon worksheet (B561). Hydrocarbons are then extracted from the LRM using the same procedure as for the samples.

4. Reagents

Dichloromethane (DCM), methanol, *iso*-hexane and water will all be HPLC grade with all bottles fitted with bakelite caps as supplied by Rathburn Chemicals Ltd, Walkerburn, Scotland. Each batch of DCM and *iso*-hexane is assessed as detailed in SOP 1620.

Washed analytical grade anhydrous sodium sulphate (Fisons, Loughborough, UK) prepared as per SOP 1643.

Heptamethylnonane, squalane, D_8-naphthalene, D_{10}-biphenyl, D_8-dibenzothiophene, D_{10}-anthracene, D_{10}-pyrene and D_{12}-benzo[*a*]pyrene will be used as internal standards.

5. Equipment

- 250 ml centrifuge bottles
- 250 ml round bottom flasks
- 250 ml separating funnels
- 10 µl syringes (2)
- 250 µl syringe for addition of internal standards

- 100 ml conical flasks
- Pasteur pipettes
- Socorex pipetter
- Aluminium foil
- Metal spatula
- Electronic balance accurate to 0.001 g
- Refrigerated centrifuge
- Balance
- Ultrasonic bath
- Rotary evaporator and water bath
- Nitrogen blow-down apparatus with carbon filter
- Isocratic HPLC pump (EN285, EN334 and EN725), analytical column and Rheodyne injector
- Tissues
- Calibrated Timer

6. Environmental Control

The solvent rinsing of glassware, liquid/liquid extraction and concentration by nitrogen flow should take place in a fume hood.

7. Interferences

Hydrocarbon analysis requires a stable environment, avoiding contamination of samples and reagents eg contact with fingers, dirty equipment, smoking, exhaust fumes, newly painted surfaces, external hydrocarbon sources. Samples known to contain high concentrations of hydrocarbons (eg cuttings samples and sediments from close to wellheads) must be stored separately and extracted separately from other samples.

All glassware must be thoroughly cleaned so as to avoid contamination from previous samples. **Before use all glassware is rinsed with DCM followed by iso-hexane**, the latter being allowed to evaporate before proceeding. Similarly, HPLC columns and Rheodyne injectors should be cleaned regularly. HPLC columns are cleaned every 7 samples, except if the pressure is 3000 psi when it is cleaned immediately (see Section 9.3 below). The aliphatic-aromatic split time is checked every 12 batches of 7 samples. A solvent blank is run through the HPLC after a sample of known high hydrocarbon concentration to avoid carry over. If more than two GC-FID chromatograms in succession show large unexplained peaks the Rheodyne injector and the HPLC column are cleaned.

The carbon filter on the nitrogen blowdown apparatus is changed annually and the new filter will be labelled with the expiry date.

8. Sampling and Sample Preparation

All samples should be defrosted at room temperature and, where practicable, using a continuous flow of cold water. Samples can also be defrosted overnight in the fridge. Exposure to direct sunlight or heat should be minimised.

Once defrosted, sub-samples for analysis should be removed after thorough mixing of the contents, in laboratory 123, using solvent washed metal spoons. The remaining sample should be returned to the deep freeze as soon as weighing of all samples in the batch is complete.

The contents of the can must be thoroughly mixed before a sub-sample is removed for analysis.

9. Analytical Procedure

The Hydrocarbon Worksheet (B561) will be used to record sample and LRM weights, standard amounts and expiry dates and equipment used. The number of the current LRM, e.g. LRM142, should be recorded on the worksheet. The position of the procedural blank and LRM should be randomised within the sample extraction order.

9.1 Extraction Methodology

The isolation of hydrocarbons from a sediment is based on solvent extraction with sonication.

9.1.1 A sample of sediment (1 - 20.5 g, see Table 1; 1g ± 0.1g for LRM) is accurately weighed into a 250 ml centrifuge bottle using a 3 figure electronic, using the metal spoons. To this is added the aliphatic standard (200 ±10 µl of dilute aliphatic standard; see SOP 1641) containing heptamethylnonane and squalane and dilute deuterated aromatic standard (100 ±5 µl; see SOP 1605) which contains D_8-naphthalene, D_{10}-biphenyl, D_8-dibenzothiophene, D_{10}-anthracene, D_{10}-pyrene and D_{12}-benzo[*a*]pyrene. The amount of internal standard added is dependent on the estimated concentration of hydrocarbons present in the sediment (see the Technical Manager and/or Table 1 and Table 2). Standards are stored in the freezer in room 126. Solutions are marked with an expiry date of three months from when the solutions were prepared. N.B. The amount of standard added and the standard preparation and expiry dates should be noted on the hydrocarbon worksheet (B561), a copy of which will be archived with the batch. Sediment wet weights are also recorded on this worksheet and the balance printout, with the batch number, date and initials of the analyst and the sample numbers will be stapled into the analysts notebook, and the page and notebook number recorded on worksheet B561. For more concentrated samples i.e. those requiring the use of the concentrated deuterated standard, two procedural blanks should be extracted, one with 100 µl of dilute deuterated standard, the other with the same volume and concentration of deuterated standard as has been added to the samples.

9.1.2 Dichloromethane (20 ±2 ml) and methanol (20 ±2 ml) are added after the internal standard using a 25 ml measuring cylinder. The mouth of the

centrifuge tube is covered with dichlormethane washed aluminium foil and the solution is thoroughly mixed by swirling such that the sediment is broken-up. If necessary, the sediment should be broken-up in the presence of the solvent with a solvent washed glass rod. The sample is ultrasonicated at setting five.

9.1.3 The sample is centrifuged at 1800 revs at 5 ±2 ºC at setting 10 and the liquid decanted into a separating funnel containing water (18 ±2 ml) and, after thorough shaking, the DCM (bottom layer) is transferred to a solvent rinsed, 100 ml conical flask.

9.1.4 The sediment is re-extracted by sonication at setting 5 with fresh DCM (20 ±2 ml), centrifuged and the solvent decanted into the separating funnel. After thorough mixing, the two layers are allowed to separate and the DCM layer is combined with the first DCM extract.

If the sediment is very muddy, or a clear DCM extract is not obtained, a second complete extraction (DCM/methanol followed by methanol only) may be required.

Table 1 - Basic Methodology Showing Concentration Specific Variations

Nominal [Hydro-carbon] (µg g^{-1})	Approx. Weight of sediment used (g)	Nominal amount of aliphatic Standard added (µg)	Nominal amount of aromatic standard added (ng)	Volume of extract prior to HPLC (ml)	Volume applied to HPLC (µl ±2 µl)	Final extraction volume for GC (ml)
≤5	20	5	100 (dilute)	0.5 ±0.1 [b]	150	0.05 ±0.01 [d]
100	10	250	100 (dilute)	1.0 ±0.2 [b]	150	0.25 ±0.04 [d]
500	10	1000	100 (dilute)	5.0 ±0.2 [c]	150	1.0 ±0.15[e]
5000	5	5000	200 (dilute)	20 ±0.5 [c]	150	2.0 ±0.2 [c]
10000	5	10 mg	200 (conc)	N/A	XX[a]	100 ±1 µl - 2 ±0.1 ml
>10000	1	20 mg	200 (conc)	N/A	XX[a]	50 ±10 µl - 2 ±0.1ml

[a]These samples are not subjected to HPLC and the extract is simply diluted as detailed
[b] 2 dram vial [c] volumetric flask [d] GC vial with inset [e]GC vial.

Table 2 - Summary of the Amount of Internal Standard That Should Be Added to Survey Samples

Sample Position from the oil platform (m)	[expected hydrocarbon] (µg g^{-1})	Volume dilute aromatic standard (µl)	Volume aliphatic standard (µl)	Nominal amount of aliphatic internal standard (µg)
>1000	<5	100 ±1	200 ±1 (dilute)	5
500 - 1000	100	100 ±1	50 ±0.5 (conc)	250
100 - 500	500	100 ±1	200 ±2 (conc)	1000
50 - 100	5000	200 ±2	1000 ±10 (conc)	5000
<50	10000	200 ±2	2000 ±20 (conc)	10 mg
Wellhead	>10000	200 ±2	XXX	20 mg

9.1.5 The organic solvent is dried over sodium sulphate (10 g ± 1 g) for at least 10 minutes. If after 10 minutes the sodium sulphate is not free flowing add a further 10 g (± 1g) of sodium sulphate.

9.1.6 If fluorescence is to be measured then the solvent is transferred to a solvent washed 100 ml volumetric flask. The sodium sulphate is thoroughly washed twice with DCM (10 ±1 ml each washing), the washings being added to the volumetric flask and the solution then diluted to volume with DCM.

A 10 ± 0.1ml aliquot is removed by use of a volumetric flask for fluorescence determination, if necessary, and stored in a vial at –18 ºC. The remaining aliquot is transferred, with washings (twice with DCM - 10 ±1 ml each washing), to a 250 ml round bottom flask.

9.1.7 If no fluorescence is required then the DCM is transferred to a 250 ml round bottom flask together with the washings (twice with DCM - 10 ±1 ml each washing), from the sodium sulphate.

The DCM present in the 250 ml round bottom flask is concentrated under reduced pressure using a rotary evaporator (water bath temperature ‹30 ºC).

At no time must the system be allowed to go dry. If this happens the sample should be discarded and a new sample of sediment extracted.

The DCM is replaced with *iso*-hexane by addition of the solvent (25 ml ± 2 ml) followed by rotary evaporation to a small volume (~0.5 ml). This process is repeated once more.

The final concentrated solution may be treated in a variety of ways depending on the estimated nominal hydrocarbon concentration.

a) If the method being applied is that for a sediment with a nominal hydrocarbon concentration of <5 µg g^{-1} then the concentrated solution is transferred, with washings (3 times with *iso*-hexane, approx 150µl each time), to a 2 dram vial where the solvent volume is reduced to 500 µl ± 100 µl under a stream of scrubbed nitrogen. Check the final volume against the calibrated vial.

b) If the method being applied is that for a sediment with a nominal hydrocarbon concentration of 100 µg g^{-1} then the concentrated solution is transferred, with washings (3 times with *iso*-hexane, approx 150µl each time), to a 2 dram vial where the solvent volume is altered to 1.0 ml ± 0.2 ml by either concentration under a stream of scrubbed nitrogen or dilution with *iso*-hexane as appropriate. Check the final volume against the calibrated vial.

c) If the method being applied is that for a sediment with a nominal hydrocarbon concentration of 500 µg g^{-1} then the concentrated solution is transferred, with washings (3 times with *iso*-hexane, approx 500µl each time), to a 5 ml volumetric flask where the solution is diluted with *iso*-hexane to the mark.

d) For all other samples the concentrated solution is transferred, with washings, to a 20 ml volumetric flask where the solution is diluted with *iso*-hexane to the mark.

9.2 Isocratic HPLC Fractionation of Aliphatic Hydrocarbons and PAHs

9.2.1 SYSTEM SET UP

a) the column is cleaned after every batch of 7 samples unless the back pressure increases and remains > 3000 psi following an injection. Under these circumstances the column should be cleaned immediately. It should also be cleaned after the injection of very dirty or concentrated samples.

b) the flow rate is checked each day of use by measuring the volume eluted from the column over 10 minutes following a settling period of at least 15 minutes. The eluate is collected in a designated 25 ml measuring cylinder. The volume is recorded B072. If the volume of solvent collected is within the range 20 ml ± 0.5 ml then the procedure is continued, otherwise remedial action must be taken until the above criterion is obtained.

c) the split time is determined after every 12 column cleanings by injecting 150 µl from a mixture comprising 200 µl of the concentrated aliphatic standard (SOP 1641), 200 µl of the concentrated deuterated aromatic standard (SOP 1605) and 200ul of the concentrated PAH internal check standard (SOP 1605). Collect the solvent eluted in the first 2 minutes and then at 15 second intervals up to 3 minutes 30 seconds in separate round-bottom flasks. Collect the column eluate from 3 minutes 30 seconds to 20 minutes and from 20 minutes to 30 minutes in separate flasks. Analyse each fraction by GC-FID (SOP 1610) and determine the fraction where all the aliphatic components have been eluted and no aromatic components are present to give the split time. The 20 minute to 30 minute fraction will be used to ensure that all aromatics have eluted before 20 minutes. The results of the split time are recorded on record sheet B241 and stored in box files labelled with the relevant HPLC EN number in laboratory C123/124. If the column is allowed to dry out or if a very dirty sample is analysed then the split time will be re-determined. The column will be cleaned (section 9.3) after every split time determination. The split time will be redetermined after any maintenance.

9.2.2 SAMPLE FRACTIONATION

HPLC fractionation is performed on an aliquot measured using a calibrated 250 µl syringe (150 ± 10 µl) for the concentrations detailed in Table 1 using a Genesis metal free HPLC column (25 cm x 4.6 mm) being eluted with hexane at a flow rate of 2 ± 0.1 ml min^{-1}. The split time used should be that determined as detailed in Section 9.2.1c above.

a) Aliphatic Fraction. The aliphatic fraction (0 min. to split time) is collected in 100 ml round bottom flask and the volume adjusted in accordance with section 9.2.3. If the aliphatic fraction is not required by the client it need not be collected – the analyst should cross check with the client request form before discarding this fraction.

b) Aromatic Fraction. The second fraction (the aromatic fraction) is collected from the split time to the final time of 20 minutes. It is collected in a separate 100 ml round bottom flask and the volume adjusted in accordance with section 9.2.3.

9.2.3 DILUTION/CONCENTRATION OF HPLC FRACTIONATION

The HPLC fraction is concentrated under reduced pressure and transferred, with washings, to a gas chromatography (GC) vial with insert (< 5 µg g^{-1} and 100 µg g^{-1}) or a GC vial (500 and 5000 µg g^{-1}). The volume of *iso*-hexane for the aromatic fraction is altered as required and is concentration dependent. The aliphatic fraction should be reduced to 25 ± 5 µl and the final volume checked against a calibrated vial. After final concentration the inside of the insert should be rinsed with approx 10 µl of the concentrated fraction. Vials are labelled with the UKAS number plus ali (for aliphatic fraction) or aro (for aromatic fraction). Any repeat extractions

should be labelled as above but with rpt (for repeat) added. The LRM and the procedural blank are labelled as LRM and Pr. Bl. respectively plus the date and the batch number.

a) If the method being applied is that for a sediment with a nominal hydrocarbon concentration of < 5 µg g^{-1} then the solvent volume is reduced to 50 µl ± 10 µl under a stream of scrubbed nitrogen, the final volume being checked against the calibrated vial.

Note: If the aromatic fraction is not immediately analysed then the sample should only be blown down to 250 µl +/- 40 µl and stored below –25 ºC. To prepare sample for analysis remove from deep freeze allow to come to room temperature and blow down to correct volume.

b) If the method being applied is that for a sediment with a nominal hydrocarbon concentration of 100 µg g^{-1} then the solvent volume is altered to 250 µl ± 40 µl by either concentration under a stream of scrubbed nitrogen of dilution with *iso*-hexane as appropriate, the final volume being checked against the calibrated vial.

c) If the method being applied is that for a sediment with a nominal hydrocarbon concentration of 500 µg g^{-1} then the solution is diluted in the GC vial with *iso*-hexane, to 1 ml ± 0.15 ml, the final volume being checked against the calibrated vial.

d) If the method being applied is that for a sediment with a nominal hydrocarbon concentration of 5000 µg g^{-1} then the solution is diluted in a 2 ml volumetric flask to 2 ml ± 0.2ml.

9.3 Cleaning HPLC column

The column is cleaned by first inverting it and then running a series of mobile phases through it at a rate of 2 ml min^{-1}. The initial mobile phase is acetone (run for 10 minutes ± 1 minute), followed by methanol (10 ± 1 min.), acetone (30 ± 2 min.) and finally *iso*-hexane (30 ± 2 min.). The column is then re-inverted and *iso*-hexane run for at least 1 hour before injecting a sample.

9.4 Calibration and Quality Control

Quality control is assured by participation in the PAH in sediments programme of QUASIMEME (Quality Assurance of Information for the Marine Environmental Monitoring in Europe).

10. Calculation of Results- not relevant

11. Precision, Accuracy and Practical Detection Limits

Recoveries- See Method M680

12. Reports - Not relevant

13. Safety

Refer to risk assessment number E013.

14. Literature References

Webster, L., Mackie, P.R., Hird, S.J., Munro, P.D., Brown, N.A. and Moffat, C.F. (1997) Development of analytical methods for the determination of synthetic mud base fluids in marine sediments. Analyst, 122, 1485-1490.

1. Introduction and Scope

This method describes the determination of the dry weight and moisture content of sediment.

2. Principle of the Method

The sediment is accurately weighed and oven dried for 22 ±2 h at 80±5^{o}C. The sediment is reweighed and the moisture content calculated and used for calculating the dry weight of the extracted sediment.

3. Reference Materials

Not relevant.

4. Reagents

Not relevant.

5. Equipment

Electronic balance accurate to 0.001 g
weighing boats
spatula
oven
desiccator
calibrated thermometer

6. Environmental Control

Not relevant.

7. Interferences

Not relevant.

8. Sampling and Sample Preparation

The sample for this determination is a sub-sample of that taken for extraction of sediments for fluorescence and hydrocarbon analysis, SOP 1600. The sample is defrosted at room temperature, where practicable, using a continuous flow of cold water.

Once defrosted, sub-samples should be removed after thorough mixing of the contents of the can. The sample should be weighed to an accuracy of 0.001 g using a 3 figure electronic balance. The remainder of the sample should be returned to the deep freeze as soon as weighing of all samples is completed.

9. Analytical Procedure

All weights will be recorded in the analysts laboratory notebook (as balance printouts and labelled appropriately) and transferred to the Sediment Dry Weight sheet (B235) when all weighings are complete. A copy of B235 is electronically archived to ChemDat/sediment dry weights using the batch number as the file name. The balance printouts will be stapled to the relevant page in the analysts notebook. A 10±1 g aliquot is removed from the well mixed sample in the container onto a weighing boat whose weight has been accurately recorded to 0.001 g using a 3 figure electronic balance. The combined weight of the boat and sediment is also recorded to 0.001 g. The boat and sediment are placed in the appropriate oven, which will be set at 80^{o}C and the temperature and time recorded (on the hydrocarbon worksheet B561) at the beginning and at the end of the drying period. The temperature of the oven will be checked for each batch using a calibrated thermometer. After an 22±2 h drying period, the boat and sediment are transferred to a desiccator until ambient temperature is achieved. One sample at a a time is removed from the dessicator and weighed. The weight is recorded to 0.001 g. The sample is discarded.

10. Calculation of Results

The Sediment Dry Weight sheet (B235) will be completed and a hardcopy kept with the batch. A copy will be electronically archived to the shared folder ChemDat, subfolder Sediment Dry Weights.

Weight of sediment extracted is copied from the Hydrocarbon worksheet (B561), other weights are copied from balance printouts.

Calculations are performed by the Excel spreadsheet (B235).

Sample	Weight of sediment extracted	weight of weighing dish	Weight of dish and wet sed.	Weight of wet sed.	weight of dish and dry sed.	weight of dry sed.	Moisture %	corrected dry weight

Sed. = sediment

% moisture content is the:
(wet weight of sediment- dry weight of sediment) / (wet weight of sediment) x 100

dry weight of extracted sediment is the :

extracted wet weight of sediment - [(extracted wet weight of sediment x % moisture/100]

11. Precision, Accuracy and Practical Detection Limits

Not relevant.

12. Reports

Not relevant.

13. **Safety**

Refer to COSHH assessment number E007.

14. Literature References

Webster, L., Mackie, P.R., Hird, S.J., Munro, P.D., Brown, N.A. and Moffat, C.F. (1997) Development of analytical methods for the determination of synthetic mud base fluids in marine sediments. Analyst, 122, 1485-1490

1. Introduction and Scope

This method describes the quantitative determination of polycyclic aromatic hydrocarbons (PAHs) in pathological and environmental samples by gas chromatography with mass selective detection (GC-MSD).

2. Principle of the Method

Polycyclic aromatic hydrocarbons are analysed by GC-MSD using an HP6890 Series GC interfaced with a 5973 MSD. Automated cool on-column injections are achieved using an HP7673 auto injector. Samples are chromatographed using a fused silica capillary column (30 m x 0.25 mm id) coated with a 0.25 µm film of an immobilised 5% phenyl 95% dimethylpolysiloxane, such as CPSil 8. (Phases with similar McReynold's constants include HP-5, DB-5, ZB-5 and Ultra 2).

3. Reference Materials

Internal deuterated standard solution and standard check solution (See SOP 1605). Two calibration check solutions are also run with each batch of samples (See SOP 1630 for preparation).

4. Reagents

iso-Hexane, ethyl acetate and acetone from Rathburn Chemicals Ltd, Walkerburn, Scotland.

5. Equipment

GC-MSD 1

HP6890 Series gas chromatograph, EN321
HP7673 Automatic injector, EN1256
Injector Controller, EN318
Sample Tray, EN319
HP5973 MSD, EN294
HP COMPAQ, EN1306
Hewlett Packard LaserJet printer

And:

GC-MSD 2

HP6890 Series gas chromatograph, EN757
HP7673 Automatic injector, EN1352
Sample Tray, EN1156,
HP5973 MSD, EN751
HP COMPAQ, EN1307

Hewlett Packard LaserJet printer

Prior to analysing any samples ensure that:

a) the ethyl acetate and *iso*-hexane in the injector wash bottles is replaced with fresh solvent
b) the solvent waste bottles on the injector are emptied and rinsed with acetone followed by *iso*-hexane
c) the injector screw is secure and the plunger is free moving
d) there is paper in the printer
e) the GC-MSD log book is completed

6. Environmental Control - Not relevant

7. Interferences

Hydrocarbon analysis requires a stable environment, avoiding contamination of samples and reagents eg. contact with fingers, dirty equipment, smoking, exhaust fumes, newly painted surfaces, external hydrocarbons sources. Samples known to contain high concentrations of hydrocarbons (eg cuttings samples, sediments from close to wellheads, tissue samples with a known high concentration of hydrocarbons) should be analysed separately from samples which are not contaminated or contain only biogenic hydrocarbons.

Once the hydrocarbon solution has been concentrated in the GC vial with insert it should be checked for any particulate material and viscosity by visual inspection.

8. Sampling and sample preparation

All samples for GC-MSD analysis should be clearly labelled. The vials must be securely capped and logged in the instrument specific log book.

9. Analytical procedure

9.1 GC conditions for PAH determination

Carrier gas	Helium
Carrier gas flow (constant)	0.7 ml min^{-1}
Injector temperature	tracks oven
Oven Temperature	50 ^{o}C, hold for 3minutes
Oven temperature ramp 1	20 ^{o}C min^{-1} to 100 ^{o}C
Oven temperature ramp 2	4 ^{o}C min^{-1} to 270 ^{o}C, hold for 30 minutes
Transfer line temperature	280 ^{o}C

The MSD is operated in selective ion monitoring mode at 70 eV with a dwell time of 50 msec The ions determined for deuterated standards are:

Compound	Ion (m/z)
D_8 Naphthalene	136
D_{10} Biphenyl	164
D_8 Dibenzothiophene	192
D_{10} Anthracene	188
D_{10} Pyrene	212
D_{12} Benzo[*a*]pyrene	264

The ions determined for the PAH analytes are presented below:

Polycyclic Aromatic Hydrocarbon (PAH)	Abbreviation	Molecular Wt/Da				
		Parent Compound	Branched PAH			
			C1	C2	C3	C4
Naphthalene	Nap	128	142	156	170	184
Phenanthrene/Anthracene	Ph/An	178	192	206	220	
Dibenzothiophene	DBT	184	198	212	226	
Fluoranthene/pyrene	Fl/Py	202	216	230	244	
Benzophenanthrene/Benzanthracene/ Chrysene+Triphenylene	Bph/BAn/ Ch+Tr	228	242	256		
Benzofluoranthene/benzopyrene/ Perylene	BFl/BPy/Pe	252	266	280		
Benzoperylene/Indenopyrene	BPe/IPy	276	290	304		
Acenaphthylene	Acy	152				
Acenaphthene	Ace	154				
Fluorene	Flu	166				
Dibenz[a,h]anthracene	D[a,h]A	278				

At the start of each week weekly maintenance is carried out and an autotune is performed Samples should be analysed only if there is no peak tailing, and response factor ratios for each of the five PAHs are within QC limits The retention gap (SOP 1635) should be changed if there is peak tailing or if the peaks are excessively broad (SOP 1250).

10. Calculation of Results

See SOP1260, SOP 1630 and SOP 2841.

11. Precision, Accuracy and Practical Detection Limits- See M680 and M680

12. Reports – SOP1260

13. Safety- Refer to COSHH assessment number E004

14. Literature references

Topping, G., Davies, J.M., Mackie, P.R. and Moffat, C.F. The impact of the *Braer* spill on commercial fish and shellfish. *In* The impact of an oil spill in turbulent waters:The *Braer* (Davies, J. M. and Topping, G. Eds), The Stationery Office, Edinburgh, 1997, pp 121 -143.

Whittle, K.J., Anderson, D.A., Mackie, P.R., Moffat, C.F., Shepherd, N.J. and McVicar, A.H. The impact of the *Braer* oil on caged salmon. *In* The impact of an oil spill in turbulent waters:The *Braer* (Davies, J. M. And Topping, G. Eds), The Stationery Office, Edinburgh, 1997, pp 144 - 160.

Webster, L., Angus, L., Topping, G., Dalgarno, E.J. and Moffat, C.F. Long term monitoring of polycyclic aromatic hydrocarbons in mussels (*Mytilus edulis*) following the Braer oil spill. *Analyst,* 1997, **122**, 1491 - 1495.

Moffat, C.F., McIntosh, A.D., Webster, L., Shepherd, N.J., Dalgarno, E.J., Brown, N.A and Moore, D.C. Determination and environmental assessment of hydrocarbons in fish, shellfish and sediments following an oil spill at the Captain Field. Fisheries Research Services Report, 1998.

1. Introduction and Scope

Rotary evaporation is used to reduce the volume of sample extracts by heat and rotation under a vacuum. The solvent vapour is condensed and collected in a separate flask.

2. Principle of the Method - Not relevant

3. Reference Materials - Not relevant

4. Reagents

iso-Hexane will be HPLC grade and the bottles fitted with Bakelite caps as supplied by Rathburn Chemicals Ltd, Walkerburn, Scotland.

5. Equipment

rotary evaporator consisting of:-
vacuum pump
heated water bath
condenser connected to cold water supply
waste flask
rotating motor attached to adjustable stand
round bottom flasks
quickfit adaptors
calibrated thermometer

6. Environmental Control - Not relevant

7. Interferences

Hydrocarbon analysis requires a stable environment, avoiding contamination of samples and reagents eg contact with fingers, dirty equipment, smoking, exhaust fumes, newly painted surfaces, external hydrocarbon sources.

All glassware must be thoroughly cleaned so as to avoid contamination from previous samples.

8. Sampling and Sample Preparation - Not relevant

9. Analytical Procedure

9.1 Switch on power to individual pieces of equipment

9.2 Set water bath to equilibrate at ‹30 º C, check with calibrated thermometer.

9.3 Start the cold water flowing through the condenser, check the flow periodically as water pressure may drop.

9.4 Rinse the adaptor with *iso*-hexane into the waste solvent bottle. This is repeated in between each sample.

9.5 Start the high pressure water flowing, open the vacuum tap on the condenser and attach the flask (containing the sample) to the adaptor on the rotary arm and still holding the flask close the vacuum tap.

9.6 Start rotating the flask increasing the momentum slowly. If bumping occurs (air bubbles in sample) stop rotation and release the vacuum, allow approximately 30 seconds before reapplying the vacuum. Lower the assembly so the flask enters the water bath, adjust as evaporation proceeds so that not all the sample is immersed in the water.

9.7 Evaporate the solvent slowly by adjusting the speed of rotation accordingly ie slowing the rotation will slow the evaporation.

Note: the loss of more volatile compounds will occur when the solvent is evaporated too quickly.

9.8 Allow the sample to evaporate to the approximate volume required. Note that evaporation to dryness results in the loss of more volatile compounds.

9.9 The flask is removed by stopping the rotation and raising the assembly so the flask is clear of the water. While holding the flask open the tap to release the vacuum slowly, when pressures have equalised, remove the flask from the adaptor.

9.10 The high pressure water should be left running between samples.

9.11 When evaporations are complete the waste solvent should be disposed of into the appropriate solvent waste bottle. Cover the mouth of the waste container with a piece of aluminium foil during transfer to fume cupboard.

10. Calculation of Results - Not relevant

11. Precision, Accuracy and Practical Detection Limits- Not relevant

12. Reports - Not relevant

13. Safety- Refer to COSHH assessment number AI 033.

14. Literature References

Bűchi Rotavapor R 110 Instructions – Room 123/124.

1. **Introduction and Scope**

This method describes the washing of sodium sulphate for use in the extraction of biota and edible oils for hydrocarbon analysis.

2. **Principle of the Method**

The sodium sulphate is required for sodium sulphate columns (SOP 1660) and for drying of organic extracts (SOP 1600 and SOP 1665 and is washed with dichloromethane and dried in an oven.

3. **Reference Material**

Not relevant.

4. **Reagents**

Dichloromethane (DCM) and *iso*-hexane will be HPLC grade and supplied by Rathburn Chemicals Ltd, Walkerburn, Scotland. Each batch of solvent will be assessed as detailed in SOP 1620.

5. **Equipment**

Oven
Sonic bath

6. **Environmental Control**

Not relevant.

7. **Interferences**

Not relevant.

8. **Sampling and Sample Preparation**

Not relevant.

9. **Analytical Procedure**

9.1 Anhydrous sodium sulphate is placed in a 500 ml conical flask until it is approximately 3/4 full.

9.2 DCM is added until the sodium sulphate is covered and the top of the flask covered with aluminium foil before placing in the sonic bath at setting 15.

9.3 The DCM is decanted, and disposed of in the chlorinated waste bottle.

9.4 The washing procedure is repeated with *iso*-hexane and again the washings decanted to waste.

9.5 The flask containing the sodium sulphate is then placed in the oven in Room 126 for 16 ± 2 hours with the fan at setting 10.

9.6 The expiry date of one month from the date of washing is written on the flask which is stoppered and kept in lab 123/124.

10. Calculation of Results

Not relevant.

11. Precision, Bias and Limit of Determination

Not relevant.

12. Reports

No relevant.

13. Safety

Refer to COSHH assessment number AI 121.

14. Literature References

Not relevant.

1. Introduction and Scope

This method describes the extraction of biota for the determination of aliphatic hydrocarbons by gas chromatography with flame ionisation detection (GC-FID) and polycyclic aromatic hydrocarbons (PAHs) by gas chromatography mass spectroscopy (GC-MS).

2. Principle of the Method

Hydrocarbons are isolated from the biota by saponification followed by a two phase separation. The organic solvent is isolated from aqueous solution and passed through an anhydrous sodium sulphate column. The dried solution is concentrated and the hydrocarbons fractionated into aliphatic hydrocarbons and PAHs by normal phase high performance liquid chromatography (HPLC). The final concentration is quoted on the basis of wet weight of sample.

3. Reference Material

In each batch (maximum 12) of sample extractions a laboratory reference material (LRM 148 – homogenised, freeze dried Aberdeen Harbour mussel tissue) is included and tested as a sample. Approximately 1.5 g of the freeze dried homogenate is weighed into a 250 ml round bottomed flask and the exact weight recorded on the hydrocarbon worksheet (B561). Hydrocarbons are then extracted from the LRM using the same procedure as for the samples.

4. Reagents

Methanol, dichloromethane (DCM), *iso*-hexane and water will all be HPLC grade with all bottles fitted with bakelite caps as supplied by Rathburn Chemicals Ltd, Walkerburn, Scotland. Each batch of DCM and *iso*-hexane will be assessed as detailed in SOP 1620.

Sodium hydroxide, sodium chloride and anhydrous sodium sulphate are analytical reagent grade reagents from Fisons (Loughborough, UK). Analytical grade nitric acid was purchased from BDH (Poole, Dorset, UK).

Heptamethylnonane, squalane, D_8-naphthalene, D_{10}-biphenyl, D_8-dibenzothiophene, D_{10}-anthracene, D_{10}-pyrene and D_{12}-benzo[*a*]pyrene will be used as internal standards.

The anhydrous sodium sulphate is washed as per SOP 1643.

Preparation of methanolic sodium hydroxide. 10% methanolic sodium hydroxide is prepared when required by weighing 50 g ± 1 g of sodium hydroxide into a tared beaker. Add 50 ml ± 5 ml of water to a Duran bottle and slowly add the sodium hydroxide while stirring. Then slowly add 450 ml ± 5 ml of methanol stirring constantly until the solution is completely mixed.

Solution is stable for 3 months. Bottle will be marked with expiry date 3 months from date of preparation.

5. Equipment

250 ml round bottom flasks
condensers
sand bath
beaker
measuring cylinders (100 ml and 10 ml)
250 ml separating funnels
glass columns with sinters (11 x 1.5 cm)
10 µl syringes (2)
100 and 250 µl syringe for addition of internal standards
Pasteur pipettes
Socorex pipetter
Aluminium foil
Metal spoon or knife
Electronic balance accurate to 0.001 g
Rotary evaporator and water bath
Nitrogen blow-down apparatus with carbon filter
Isocratic HPLC pump (EN285, EN334 and EN725), analytical column and Rheodyne injector
Calibrated thermometer
Tissues
Kenwood MiniChopper
Calibrated Timers
Magnetic stirrer

6. Environmental Control

The solvent rinsing of glassware, saponification and liquid/liquid extraction and concentration by nitrogen flow takes place in a fume hood.

7. Interferences

Hydrocarbon analysis requires a stable environment, avoiding contamination of samples and reagents eg. contact with fingers, dirty equipment, smoking, exhaust fumes, newly painted surfaces, external hydrocarbon sources. Samples known to contain high concentrations of hydrocarbons must be stored separately and extracted separately from other samples.

All glassware must be thoroughly cleaned so as to avoid contamination from previous samples. Before use **all glassware is rinsed with dichloromethane followed by *iso*-hexane**, the latter being allowed to evaporate before proceeding. Similarly, HPLC columns and Rheodyne injectors are cleaned regularly. HPLC columns are cleaned every 7 samples, except if the pressure is 3000 psi when it is cleaned immediately (see Section 9.3 below). The aliphatic-aromatic split time is checked every 12 batches of 7

samples. A solvent blank is run through the HPLC after a sample of known high hydrocarbon concentration to avoid carry over. If more than two GC-FID chromatograms in succession show large unexplained peaks the Rheodyne injector and the HPLC column are cleaned.

The carbon filter on the nitrogen blowdown apparatus is changed annually and the new filter will be labelled with the expiry date.

8. Sampling and Sample Preparation

All samples should be defrosted at room temperature and exposure to direct sunlight or heat should be minimised. Samples can also be defrosted in the fridge overnight. Once defrosted samples are cut up finely with a knife and/or homogenised using a Kenwood MiniChopper, in laboratory 123. Samples are then transferred, using a solvent washed metal spoon, to a 250 ml round bottom flask or to a solvent washed aluminium can for storage in a freezer if analysis does not take place immediately.

The remainder of the sample should be returned to the deep freeze as soon as weighing of all samples in the batch is completed.

The contents of the can must be thoroughly mixed before a sub-sample is removed for analysis.

9 Analytical Procedure

The Hydrocarbon Worksheet (B561) will be used to record sample and LRM weights, standard amounts and preparation and expiry dates, equipment used and expiry dates of methanolic NaOH anhydrous sodium sulphate. The current LRM number, e.g. LRM148, will be entered on the worksheet also.

9.1 Extraction Methodology

The isolation of hydrocarbons from biota is based on that of Grimmer and Böhnke.

9.1.1 A sample of fish muscle or other animal tissue (5-10.5 g) is accurately weighed into a solvent washed 250 ml round bottomed flask using a 3 figure electronic balance, using solvent washed metal spoons or spatulas. For adipose tissue the sample size is reduced to 0.3 - 1.0 g. The sample wet weights will be recorded on worksheet B561and the balance printout, with the batch number, date and initials of the analyst and the sample numbers, will be stapled into the analysts notebook, and the page and notebook number recorded on worksheet B561. The position of the procedural blank and LRM should be randomised within the sample extraction order.

9.1.2 To this is added the aliphatic standard (200 ± 10 µl of dilute aliphatic standard; see SOP 1641) containing heptamethylnonane and squalane and

deuterated aromatic standard (100 ± 5 µl; see SOP 1605) which contains D_8-naphthalene, D_{10}-biphenyl, D_8-dibenzothiophene, D_{10}-anthracene, D_{10}-pyrene and D_{12}-benzo[*a*]pyrene. The preparation and expiry dates of the standards and volume used should be recorded on the hydrocarbon worksheet B561, a copy of which will be archived with the batch.

9.1.3. To the biota/oil sample is added sodium hydroxide (10%) in methanol/water (90:10, 40 ± 4ml) and a few pre-washed anti-bumping granules, and a cleaned reflux condenser fitted to the flask. Flasks are lowered onto the sandbath (75°C ± 5°C, monitored by calibrated thermometer and beaker of water) at staggered intervals (approx. 2 per 20 minutes). The mixture is refluxed for 3 h 45 ± 5 min before the addition of water (10 ± 1 ml) via the top of the condenser and the refluxing continued for a further 15 ± 2 min. At regular intervals during the refluxing the experimental set up should be checked. Any anomalies should be recorded in the analysts lab notebook.

N.B. Condensers are dismantled and cleaned after each batch of extractions.

9.1.4. On completion of the saponification the hot solution is transferred to a solvent washed 250 ml separating funnel containing *iso*-hexane (80 ± 5 ml). Methanol/water (4:1, 40 ± 4 ml) is used to rinse round bottom flask before being added to the separating funnel. The mixture is thoroughly shaken, taking care to release the pressure at regular intervals, and then allowed to settle. The lower aqueous layer is transferred into a second 250 ml separating funnel containing *iso*-hexane (80 ± 5 ml) and the solutions thoroughly mixed. While the second extraction is settling the first *iso*-hexane extract is washed with methanol/water (1:1, 40 ± 4 ml). Vigorous shaking is required at this stage. The aqueous layer from the second *iso*-hexane extraction is run-off to waste and the methanol/water from the first extract mixed with the second *iso*-hexane extract. After thorough shaking the two layers are allowed to separate and the aqueous layer allowed to run off to waste.

NOTE: If the layers do not separate and emulsions are formed a sufficient amount of sodium chloride is added to the separating funnel by solvent washed spatula to aid the separation. If an emulsion is still present after addition of sodium chloride the Technical Manager should be consulted.

9.1.5. The *iso*-hexane extracts are combined and washed with water (3 x 40 ± 4ml). A sodium sulphate column is prepared by adding washed sodium sulphate (see SOP 1643) to a glass column with sinter (11 x 1.5 cm), up to where the column widens. The combined extracts, followed by 50 ± 5 ml of *iso*-hexane, are run through the column and collected in a 250 ml round bottomed flask. The solvent is concentrated, by rotary evaporation, to approximately 300 µl and transferred to a screw topped vial. The flask is washed with *iso*-hexane and the washings transferred to the vial to give a total volume of 500 µl, using a calibrated vial for comparison to give the correct volume.

At no time should the system be allowed to go dry. If this happens the sample must be discarded and repeated later.

N.B. The rotary evaporator adaptor is removed and solvent washed between samples to minimise cross-contamination.

Sinters are cleaned every three months by soaking in concentrated nitric acid. The columns are then washed in water, oven dried (at 105 ± 10ºC) and rinsed, as described in Section 7, before use.

9.2 Isocratic HPLC Fractionation of Aliphatic Hydrocarbons and PAHs

9.2.1 System Set up

a) The HPLC column is cleaned after every 7 samples unless the back pressure increases and remains > 3000 psi following an injection. Under these circumstances the column should be cleaned immediately.

b) the flow rate is checked each day of use by measuring the volume eluted from the column over 10 minutes, following a settling period of at least 15 minutes. The eluate is collected in a designated 25 ml measuring cylinder and the volume recorded on record sheet B72. If the volume of solvent collected is within the range 20 ml ± 0.5 ml then to proceed with the method, otherwise remedial action must be taken until the above criterion is obtained.

c) the split time is determined after every 12 column cleanings by injecting 150 µl from a mixture comprising 200 µl of the concentrated aliphatic standard (SOP 1641), 200 µl of the concentrated deuterated aromatic standard (SOP 1605) and 200ul of the concentrated PAH internal check standard (SOP 1605). Collect the solvent eluted in the first 2 minutes and then at 15 second intervals up to 3 minutes 30 seconds in separate round-bottom flasks. Collect the column eluate from 3 minutes 30 seconds to 20 minutes and from 20 minutes to 30 minutes in separate flasks. Analyse each fraction by GC-FID (SOP 1610) and determine the fraction where all the aliphatic components have been eluted and no aromatic components are present to give the split time. The 20 minute to 30 minute fraction will be used to ensure that all aromatics have eluted before 20 minutes. The results of the split time are recorded on record sheet B241and stored in box files labelled with the relevant HPLC EN number in laboratory C123/124. If the column is allowed to dry out or if a very dirty sample is analysed then the split time will be re-determined. The column will be cleaned (section 9.3) after every split time determination. Split time will be redetermined after any maintenance.

9.2.2 Sample Fractionation

HPLC fractionation is performed on an aliquot measured using a calibrated 250 µl syringe (150 ± 10 µl) using a Genesis metal free HPLC column (25 cm

x 4.6 mm). Elution is with *iso*-hexane at a flow rate of 2 ± 0.1 ml min^{-1}. The split time used should be that determined as detailed in Section 9.2.1c above.

Aliphatic fraction. The aliphatic fraction (0 min. to split time) is collected in a 100 ml round bottom flask and the solution concentrated to 50 µl as in accordance with 9.2.3. If the aliphatic fraction is not required by the client it need not be collected – the analyst should cross check with the client request form before discarding this fraction.

Aromatic fraction. The second fraction (the aromatic fraction) is collected from the split time to the final time of 20 minutes. It is collected in a separate round bottom flask and the solution concentrated to 50 µl as in accordance with 9.2.3.

9.2.3 Concentration of HPLC Fractionation

The HPLC fraction is concentrated under reduced pressure using the rotary evaporator (SOP1640) and transferred, with washings, to a gas chromatography (GC) vial with insert. The solvent volume for the aromatic fraction is reduced further to 50 µl ± 10 µl under a stream of scrubbed nitrogen, the final volume being checked against the calibrated vial. If samples are suspected to contain total PAH concentrations of > 2,000 ng g^{-1} wet weight then the solvent volume is reduced to 250 µl ± 40 µl, the volume being checked against the calibrated vial. The aliphatic fraction is reduced to 25 µl ± 5 µl, the final volume being checked against a calibrated vial. After concentration the inside of the insert should be rinsed with approx 10 µl of the concentrated fraction. Vials are labelled with the UKAS number plus ali (for aliphatic fraction) or aro (for aromatic fraction). Any repeat extractions should be labelled as above but with rpt (for repeat) added. The LRM and the procedural blank are labelled as LRM and Pr. Bl. respectively plus the date and the batch number.

9.3 Cleaning HPLC column

The column is cleaned by first inverting it and then running a series of mobile phases through it at a flow rate of 2 ml min^{-1}. The initial mobile phase is acetone (run for 10 minutes ± 1 minute), followed by methanol (10 ± 1 min.), acetone (30 ± 2 min.) and finally *iso*-hexane (30 ± 2 min.). The column is then re-inverted and *iso*-hexane run for at least 1 hour before injecting a sample.

9.4 Calibration and quality control

Quality control is assured by participation in the PAH in biota programme of Quasimeme (Quality Assurance of information for the Marine Environmental Monitoring in Europe).

10 Calculation of Results

Not relevant.

11 Precision, Bias and Limit of Determination

Recoveries- See Method M690.

12. Reports

Not relevant.

13. Safety

Refer to risk assessment number E0013.

14. Literature References

Grimmer, G and Böhnke, H. (1975), *J. Assoc. Offic. Anal. Chem*., **58**, 725.

Webster, L., Angus, L., Topping, G. Dalgarno, E.J. and Moffat, C.F. (1997) Long-term monitoring of polycyclic aromatic hydrocarbons in mussels (*Mytilus edulis*) following the *Braer* oil spill. *Analyst*, **122**, 1491-1495.

1. Introduction and Scope

This presents a technique for extracting total lipid from an individual and groups zooplankton copepods using a variation of the method described by Folch *et al*..

2. Principle of the Method

Zooplankton copepods are allowed to stand in a chloroform-methanol mixture (2:1 v/v) of approximately 20-fold its volume, for at least 24 hours in a refrigerator. Potassium chloride in water is added to form a final emulsified mixture of chloroform, methanol and water (8:4:3 v/v/v). Centrifugation is used to separate the organic and aqueous layers. The chloroform extract is transferred to a new vial and evaporated under a stream of nitrogen, desiccation then removes any water present. Lipid is stored frozen in *iso*-hexane until further analysis.

3. Reference Materials

Not applicable

4. Reagents

- 2,6-Di-tert-butyl-*p*-cresol [Butylated hydroxytoluene (BHT)]; GPR; VWR International
- Chloroform; HPLC grade Rathburn Chemicals Ltd.
- Methanol, dichloromethane, *iso*-hexane, water (aliphatic and aromatic hydrocarbon free), winchesters fitted with bakelite caps; Rathburn Chemicals Ltd.
- Charcoal scrubbed nitrogen. **Note**: The charcoal filter must be replaced annually.

If an alternative supplier is used, reagents must be of the same grade.

4.1 Preparation of Butylated hydroxytoluene (BHT)

Ensure balance performance check has been carried out for the day. If not refer to SOP 240 and carry out this check. Weigh BHT (1.8 mg ± 0.2 mg) to a conical flask. To a second conical flask add methanol (60 ml ± 1.0 ml, added by measuring cylinder) to chloroform (120 ml ± 1.0 ml, added by measuring cylinder). Stopper the flask immediately to prevent solvent evaporation and swirl to mix solvents. Decant the solvent into the first conical flask and gently shake until well mixed and no BHT crystals are visible. The solution is freshly prepared on the day of use, and any unused solution should be discarded into the chloroform waste bottle.

4.2 Preparation of potassium chloride in water

Ensure balance performance check has been carried out for the day. If not refer to SOP 240 and carry out this check. Potassium chloride (0.88g ± 0.08g) is weighed into a conical flask and the weight recorded in a laboratory notebook. To this is added water (100 ml ± 1.0 ml, added by measuring cylinder). The conical flask is stoppered and the solution shaken until it has dissolved completely. This solution is to be marked with an expiry date of 3 months from the date of preparation.

5. Equipment

Fine point, non serrated forceps.
Measuring cylinders
Balance to measure mg to 2 decimal places (EN 284)
Conical flask (25ml) with glass stopper
Conical flask (200ml) with glass stopper
Calibrated Socorex pipette (0.2 – 2.0 ml) with disposable glass pastuer pipette tips
Screw top glass vials (2 ml) with screw on lid
Screw top test tube (15 ml) with screw top lid
Temperature controlled centrifuge, EN 287 or EN 325
Dessicator with silica gel.

6. Environmental Control

6.1 All fridge and freezer temperatures are monitored according to SOP 280.

7. Interferences

7.1 Contamination from glassware surfaces is possible and re-useable glassware is to be cleaned in accordance with SOP 220.

7.2 Transferring the sample between glassware is to be kept to a minimum, as lipid will be lost this way. This procedure has reduced as far as practicable the number of glass vessels used.

7.3 To remove traces of lipids all glassware is solvent washed prior to use. **All glassware is rinsed with dichloromethane followed by *iso*-hexane**, the latter being allowed to evaporate before proceeding.

7.4 Always wear gloves when handling samples, as lipids are present on human skin.

8. Sampling and sample preparation

8.1 Zooplankton selected for lipid extraction will have been stored frozen or cryogenically.

9. Analytical procedure

9.1 Sample Preparation of Individual Copepods

9.1.1 Label with a sample ID reference a clean labelled glass 2 ml vial, with a screw top lid.
9.1.2 Add BHT solution (1200 μl, by means of calibrated pipette with glass pasteur pipette tip) to the vial.
9.1.3 Select an individual copepod using forcep and place in prepared glass vial. The copepod should not have any visible signs of perforation, which may result in loss of storage lipid. Forceps must be cleaned with methanol between samples.
9.1.4 Replace lid on vial to prevent solvent evaporation.
9.1.5 Shake vial gently to thoroughly mix solvent, ensure copepod is suspended in solvent and not stuck to the sides of the glass vial.
9.1.6 Place in a refrigerator for at least 24 hours allowing lipid extraction from copepod.

9.2 Sample Preparation of Groups (3-6) of Copepods

9.2.1 Label with sample ID reference, a clean glass test tube with a screw top lid.
9.2.2 Add BHT solution (6 ml ± 0.5 ml, added by means of measuring cylinder) to the test tube.
9.2.3 Select copepods using forceps and place in prepared glass test tube. The copepods should not have any visible signs of perforation, which may result in loss of storage lipid. Forceps must be cleaned with methanol between samples.
9.2.4 Replace lid on test tube to prevent solvent evaporation.
9.2.5 Shake test tube gently to thoroughly mix solvent, ensure copepod is suspended in solvent and not stuck to the sides of the test tube.
9.2.6 Place in a refrigerator for at least 24 hours allowing lipid extraction from copepods.

9.3 Separation, purification and storage of lipid from individual copepods.

9.3.1 Potassium chloride solution (300 μl, is added using a calibrated pipette and glass pipette tip), replace lid and shake test tube gently to thoroughly mix contents.
9.3.2 Set the centrifuge at 1800 rpm, 0°C and 20 minutes, and centrifuge.
9.3.3 Remove lower layer of lipid and solvent (approximately 500μl), and transfer by means of a pipette with glass pasteur pipette tip to a clean glass vial labelled with the sample ID reference. Care is taken to avoid collection of suspended copepod particulates.
9.3.4 Remove solvent by evaporating until dryness with a gentle stream of charcoal scrubbed nitrogen.
9.3.5 Place sample in a dessicator with silica gel for at least 12 hours, but no longer than 18 hours, to remove any remaining water present. To reduce the possible effects of lipid oxidation the dessicator should be

kept in a fridge and out of direct sunlight.

9.3.6 Resuspend lipid by adding 0.2 ml *iso*-hexane by means of a calibrated pipette fitted with a glass pasteur pipette tip. Blow nitrogen into vial to prevent oxidation.

9.3.7 Store extracted lipid in freezer.

9.4 Separation, purification and storage of lipid from groups (3-6) copepods.

9.4.1 Potassium chloride solution (1.5 ml) is added using a calibrated pipette and glass pasteur pipette tip, replace lid and shake test tube gently to thoroughly mix contents.

9.4.2 Set the centrifuge at 1800 rpm, 0°C and 20 minutes, and centrifuge.

9.4.3 Remove ½ the lower layer of lipid and solvent (approximately 2.0ml) from the test tube, and transfer to a clean 2ml glass vial labelled with the sample ID reference using a pipette fitted with a glass pasteur pipette tip. Care is taken to avoid collection of suspended copepod particulates.

9.4.4 Remove solvent by evaporating until dryness with a gentle stream of charcoal scrubbed nitrogen.

9.4.5 Remove the remaining ½ of the lower layer of lipid and solvent (approximately 2 ml) from the screw top test tube and combine with the previously solvent evaporated lipid by means of a pipette fitted with a glass pasteur pipette tip. Care is taken to avoid collection of suspended copepod particulates.

9.4.6 Remove the solvent by evaporating to dryness under a stream of charcoal scrubbed nitrogen.

9.4.7 Place sample in a dessicator with silica gel for at least 12 hours, but no longer than 18 hours, to remove any remaining water present. To reduce the possible effects of lipid oxidation the dessicator should be kept in a fridge and out of direct sunlight.

9.4.8 Resuspend lipid by adding 2.0 ml *iso*-hexane by means of a calibrated pipette fitted with a glass pasteur pipette tip. Blow nitrogen into vial to prevent oxidation.

9.4.9 Store extracted lipid in freezer.

10. Calculation of Results

Not applicable.

11. Precision, Accuracy and Practical Detection Limits

Not applicable.

12. Reports

Additional details or observations should be clearly detailed in a Laboratory notebook in accordance with SOP 1390.

13. Safety

Refer to procedure risk assessment AI148.

14. Literature references

Folch, J., Lees, M., Sloane Stanley, G. H., (1956), A Simple method for the isolation and purification of total lipids from animal tissues, *J. Biol. Chem*., **226**; 497.