Combined Effects of Bioavailable Organic Contaminants in the Aquatic Environment

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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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This research programme was carried out in collaboration with Marine Scotland Science (MSS), Aberdeen

DECLARATION

I declare that the work presented in this thesis is my own, except where otherwise acknowledged, and that this thesis was composed by me. All cited references are listed in each chapter of the thesis. No part of this thesis has been presented for any degree or qualification at any other academic institution.

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Emmanuel Steven Emelogu **Date**

ABSTRACT

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Passive sampling, as opposed to the conventional spot or bottle water sampling technique, has shown to be reliable and efficient in monitoring the toxicologically relevant, freely dissolved (e.g. bioavaialable) concentrations of a wide range of organic contaminants in water. At the same time, partitioning controlled delivery (passive dosing; PD) techniques promise to overcome many of the challenges associated with toxicity testing of hydrophobic substances that may bias the interpretation of toxicity data. The present study investigated the feasibility of coupling silicone rubber passive sampling devices (SR-PSDs) with bioassay techniques for both chemical and ecotoxicological assessment of complex mixtures of organic contaminants in the aquatic environment. SR-PSDs were deployed in water at various locations within the Ythan catchment (north east, Scotland, UK), Forth estuary and the Firth of Forth (east coast of central Scotland, UK) for 7 to 9 weeks. Following retrieval, extracts from the SR-PSDs were analysed for dissolved concentrations of a variety of organic contaminants including PAHs and PCBs using GC-MS and GC-ECD respectively and were screened for a wide range of pesticides using GC-MS/MS and LC-MS/MS. The extracts were further evaluated for acute cytotoxicity (i.e. neutral red uptake assay) and EROD induction potential using rainbow trout liver cell line (*Oncorhynchus mykiss*; RTL-W1) and for phytotoxicity and developmental toxicity potential using algal growth inhibition test (with a marine phytoplankton, *Diacronema lutheri)* and fish embryo toxicity test (with embryos from zebrafish *Danio rerio*) respectively. Overall, the individual and total dissolved concentrations of PAHs ($\sum PAH_{40}$; parent and branched) and PCBs ($\sum PCB_{32;}$ *ortho* and mono-*ortho*) measured in water from the Ythan, Forth estuary and Firth of Forth were relatively low compared with other studies using PSDs. A number and level of pesticides, including insecticides, herbicides and fungicides of varying hydrophobicity (log K_{OW}s ~2.25 to ~5.31) were detected in the silicone rubber (SR) extracts from the Ythan catchment, the Forth estuary and the Firth of Forth, suggesting input mainly from agricultural run-off and possibly from direct discharges. No statistically significant (p<0.05) acute cytotoxicity was observed following 48 h exposure of RTL-W1 cells to SR extracts from the Ythan catchment. But, on a sublethal level, for every site, statistically significant EROD activity was observed to some degree following 72 h exposure. In addition, developmental and algal toxicities on embryos of *D. rerio* and *D. lutheri* respectively, were measured in all the deployed samples compared with the procedural controls (undeployed samples). Interestingly, extracts of SR-PSDs from the Forth estuary and the Firth of Forth exhibited growth inhibitions on *D. lutheri* that were similar to those of extracts from the Ythan, even though, fewer numbers of pesticides were detected in the Forth estuary and Firth of Forth than the Ythan. This suggests that pesticides were not solely responsible for the observed effects in the Ythan catchment. To further improve data from toxicity testing of hydrophobic substances, the study identified the use of SR O-rings as a suitable passive dosing format in *in vitro* toxicity tests and was partially validated through their use in dosing RTL-W1 cells with two individual PAHs and subsequently determining cytotoxicity and EROD-activity.

Keywords: Silicone rubber passive sampling (SR-PS), Passive dosing, Hydrophobic organic contaminants (HOCs), Fish embryotoxicity test (FET), Bioassays, Bioavailable, Water quality monitoring, Toxicity testing, , Pesticides, Algal growth inhibition (AGI).

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To the only wise God our Saviour,
be glory and majesty, dominion and power,
both now and ever.
Amen.

Jude 1:25

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This thesis is dedicated to the loving memory of my parents, Mr and Mrs Steven Okezie Emelogu who guided my early years and made me to believe in myself no matter the circumstances.

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Presentations related to this study

Selected Platform Presentations:

Emelogu ES, Heger S, Pollard P, Robinson CD, Webster L, McKenzie C, Seiler TB, Hollert H, Moffat CF. Using passive dosing to assess the cytotoxicity and cytochrome induction potentials of polycyclic aromatic hydrocarbons (PAHs) on fish cell line. To be presented at the 23rd Annual Meeting of the *Society of Environmental Toxicology and Chemistry (SETAC Europe).12th-16th May 2013*.

Emelogu ES, Heger S, Pollard P, Robinson CD, Webster L, Napier F, McKenzie C, Seiler TB, Hollert H, Moffat CF. Linking complex mixtures of organic contaminants to biological responses via passive sampling and embryonic zebrafish assays. *5th International Passive Sampling Workshop (IPSW 2012) September 11-12, 2012, Columbia, MO, USA*.

Emelogu ES, Robinson CD, Pollard P, Webster L, Fiona Napier, McKenzie C, and Moffat CF. Field application of silicone rubber passive samplers in monitoring aqueous freely-dissolved concentrations of organic contaminants in the Ythan catchment area of Scotland. *4th International Passive Sampling Workshop (IPSW 2011), 11th to 14th of May 2011 Krakow, Poland*

Selected Poster Presentations:

Emelogu ES, Pollard P, Dymond P, Robinson C, Webster L, McKenzie C, Dobson J, Bresnan E, Moffat C. Linking algal toxicity with freely dissolved concentrations of organic contaminants in the Firth of Forth Scotland. To be presented at the *23rd Annual Meeting of the Society of Environmental Toxicology and Chemistry (SETAC Europe).12th-16th May 2013.*

Emelogu ES, Pollard P, Robinson CD, Webster L, McKenzie C, Napier F, Moffat CF. Identification of selected organic contaminants in streams associated with agricultural activities and comparison between autosampling and silicone rubber passive sampling. *5th International Passive Sampling Workshop (IPSW 2012) September 11-12, 2012, Columbia, MO, USA.*

Emelogu ES, Robinson CD, Napier F, Webster L, Moffat L, McKenzie C and Pollard P. Biological effects assessment of freely-dissolved hydrophobic organic contaminants (HOCs) in the Ythan catchment area of Scotland using silicone rubber passive sampling (PS) and dosing (PD) techniques. *4th International Passive Sampling Workshop (IPSW 2011), 11th to 14th of May 2011 Krakow, Poland*

Emelogu ES, Robinson CD, Webster L, Moffat CF, Pollard P.Development of passive sampling/passive dosing protocols for in vitro toxicological assessments of hydrophobic organic contaminants in environmental matrices. *Society of Environmental Toxicology and Chemistry UK Branch Meeting 2010*, 13 - 14 Sep 2010, SETAC.40.

Contributions of others and co-authors to thesis

Contributions of others to study

Other than candidate's primary institutions i.e. the Robert Gordon University, Aberdeen and Marine Scotland Science, Aberdeen, this thesis includes a number of collaborative research works with individuals from other institutions.

All pesticide analysis was performed by staff members of the Trace Organic Chemistry Unit of the Scottish Environment Protection Agency (SEPA), East Kilbride, Scotland, UK.

Fish embryo toxicity tests (FET) were performed by staff members at the Department of Ecosystem Analysis, Institute for Environmental Research (Biology V), RWTH University, Aachen, Germany.

Dr. N.C. Bols and Dr. L. Lee; University of Waterloo, Canada provided the permanent fish cell line, RTL-W1 cells used for the *in vitro* bioassays (Chapter 2).

Foppe Smedes provided the mathematical model used in the calculation of water concentrations of PAHs and PCBs and assisted in the calculation of water concentrations of PAHs and PCBs in Chapter 2.

Contributions of co-authors to published works

This thesis incorporates published articles i.e. Chapters 2, 3, 4 and 5 (submitted) and appendix 1 (extended abstract, SETAC) written primarily by the candidate with co-authorship granted to supervisors and other individuals in recognition of their significant contribution to the respective studies. In all cases, the entire manuscript, major ideas, primary contributions, data analysis and interpretation, responses to reviewers and editor's comments were performed by the candidate as primary author, and the contributions of co-authors were mainly through the provision of constructive critique, suggestions, corrections to typographical errors and grammar, where necessary.

Abbreviations

A	Surface area
AA-EQS	Annual Average Maximum Allowable Concentration
AHH	Aryl Hydrocarbon Hydroxylase
AHR	Aryl hydrocarbon receptor
ANOVA	Analysis of Variance
ATCC	American Type Culture Collection
BED	Biological effective dose
C_{TWA}	Time-weighted average concentration
CV	Coefficient of Variation
*C*w	aqueous concentration
DCM	Dichloromethane
D_F	Dilution factor
DGT	Diffusive gradients for thin films
DLCs	Dioxin-like compounds
DMSO	Dimethyl sulfoxide
*D*w	Diffusion coefficient in water
EC	European Commission
EC_{50}	Median effective concentration
ECACC	European Collection of Animal Cell Cultures
EINECS	European Inventory of Existing Commercial Chemical Substances
EQC	Environmental quality criteria
EQS	Environmental Quality Standards
EROD	Ethoxyresorufin-*0*-deethylase
EU	European Union
GC-ECD	Gas Chromatography-electron capture detection
GC-MS	Gas Chromatography-Mass Spectrometry
GES	Good Environmental Status (as described within the Marine Strategy Framework Directive, EC Directive 2008/56/EC
GSTs	Gluthathione-S-transferases
HMW	High Molecular Weight
HOCs	Hydrophobic Organic Contaminants
HPLC	High Performance Liquid Chromatography
HTD	Highest Tolerated Dose
ISO	International Standards Organisation
IUPAC	International Union of Pure and Applied Chemists
*k*e	exchange rate coefficient
kg	Kilogram
K_{OW}	Octanol-water partition coefficient
K_{SW}	Sampler-water partition coefficient
LC_{50}	Median lethal concentration
LC-MS	Liquid Chromatography Mass Spectrometry
LDPE	Low Density Polyethylene
LMW	Low molecular weight
LoD	Limit of Detection
LoQ	Limit of Quantification
MAC-EQS	Maximum Allowable Concentration-Environmental Quality Standards
MDL	Method Detection Limit
MeOH	Methanol
MFO	Mixed Function Oxidase
MRM	Multiple reaction mode
Mrna	Messenger ribonucleic acid
M*s*	mass of the sampler
MS	Mass spectrometry
MSFD	Marine Strategy Framework Directive
MSS	Marine Scotland Science
MTT	Tetrazolium salt reduction assay
NR	Neutral red

OCPs	Organochlorinated pesticides
OECD	Organisation for Economic Co-Operation and Development
OSPAR	OSPAR Convention for the Protection of the Marine Environment of the North-East Atlantic
PAHs	Polycyclic aromatic hydrocarbons
PBT	Persistence bioaccumulation and toxicity
PCA	Principal Component Analysis
PCBs	Polychlorinated biphenyls
PCDDs	Polychlorinated dibenzodioxins
PCDFs	Polychlorinated dibenzofurans
PD	Partition controlled delivery
PD	Partitioning controlled delivery (passive dosing)
PDMS	Polydimethylsiloxane
PHS	Priority hazardoussubstances
POCIS	Polar organic integrative sampler
POM	Polycyclic organic matter
PoM	Polyoxymethylene
ppm	Parts per million
PPPs	Plant protection products
PRCs	Performance Reference Compounds
PS	Passive sampler
PSDs	Passive sampling devices
QC/QA	Quality control/assurance
REACH	Registration, Evaluation, Authorization and Restriction of Chemicals
R_S	Sampling rate
RTL-W1	Rainbow trout liver cell line
SEPA	Scottish Environment Protection Agency
SPM	Suspended particulate matter
SPMDs	Semi-Permeable Membrane Devices
SR	Silicone rubber
SR-PSDs	Silicone rubber passive sampling devices
STs	Sulphotransferase
TBT	Tributyltin
TEF	Toxic Equivalency Factor
TEQ	Toxic Equivalent concentration
UGT	UDP-glucuronosyltransferases
UK	United Kingdom
UKAS	United Kingdom Accreditation Service
UNEP	United Nations Environment Programme
US EPA	United States Environmental Protection Agency
USA	United States of America
WFD	Water Framework Directive
WHO	World Health Organisation
WWTPs	Waste Water Treatment Plants

1 CHAPTER ONE: Introduction

1.1 Organic contaminants in aquatic environment

An enormous quantity of organic compounds of natural and anthropogenic origin enters the aquatic environment through point and non-point (diffuse) sources. Aqueous concentrations of most organic contaminants, including the polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and a variety of pesticides, are typically at trace or ultra-trace levels. The low concentrations are mainly due to incomplete partitioning between the water column (e.g. freely dissolved), suspended particulate matter (SPM) and dissolved organic carbon (DOC) (Booij et al., 2003; Huckins, 2006). A good number of organic contaminants, particularly, the hydrophobic organic contaminants (HOCs) are persistent and due to their lipophilicity, they bioaccumulate in organisms and, in some instances, biomagnify (e.g. PCBs). Generally, organic contaminants have the potential to elicit adverse effects on aquatic organisms and, ultimately, on humans, even at low concentrations. There is a large body of evidence that shows that several organic contaminants in aquatic environments are responsible for a range of adverse effects on aquatic organisms. These effects include, but are not limited to, immunotoxicity, genotoxicity, endocrine disruption, reproductoxicity, carcinogenicity and, in extreme cases, death (Eljarrat and Barceló, 2003; Van der Oost et al., 2003). Further, these effects have led to a sudden decline in the population of some aquatic organisms and/or contributed to impoverished communities (e.g. Matthiessen and Law, 2002). Indeed, all organic contaminants exhibit a level of baseline or narcotic toxicity on organisms due to their bioaccumulative tendencies (Johansen, 2003).

The occurrence of organic contaminants in the aquatic environment is not of itself an indicator of adverse effects. It is the freely dissolved concentrations of the total contaminants load that are directly related to the contaminants' chemical activity (Mayer et al., 2003), thus controls the bioavailability in any media, as a result, the bioaccumulation in organisms, and consequently, the toxicological effects (Escher and Hermens, 2004; Reichenberg et al., 2006). Therefore, it is essential to measure the freely dissolved concentrations for the evaluation of adverse effects. Importantly, environmental and ecological exposures to organic contaminants do not occur as single compounds, but as complex mixtures, hence, their toxicological effects may be altered by synergistic, cumulative or additional interactions.

1.2 Overview of selected organic contaminants relevant in this study

1.2.1 Polycyclic aromatic hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) are a diverse group of organic compounds with two or more fused benzene rings in linear, angular or clustered arrangements. Generally, most environmental studies have focused essentially on two major groups: the low molecular weight (LMW) and high molecular weight (HMW) PAHs. The LMW PAHs are composed of two or three rings e.g. naphthalene ($C_{10}H_8$; MW = 128.16) and the HMW PAHs are composed of four or more rings e.g. benzo(*g,h,i*)perylene ($C_{22}H_{12}$; MW = 276.34).

The European Union Water Framework Directive (EU-WFD; 2000/60/EC; EC, 2000), which provides a legislative framework for the protection of inland and coastal waters within the EU countries, has established a list of priority substances and substances identified as priority hazardous substances (PHSs). Some PAHs, including anthracene, benzo[*a*]pyrene, benzo[*b*]fluoranthene, benzo[*g,h,i*]perylene, benzo[*k*]fluoranthene and indeno[1,2,3-*cd*]pyrene are identified as PHSs and naphthalene as priority substances (EC, 2011). WFD aims to protect all transitional and coastal waters, prevent further deterioration and enhance the status of aquatic ecosystems. The Scottish Environment Protection Agency (SEPA) undertakes the majority of WFD related monitoring in Scotland. PAHs are also included on the OSPAR (Oslo-Paris convention for the protection of the marine environment of the North-East Atlantic) List of Chemicals for Priority Action (OSPAR Commission, 2011). The United States Environmental Protection Agency (US-EPA) and World Health Organisation (WHO) have identified sixteen PAHs as priority pollutants due to their potential toxic effects (e.g. carcinogenic). Figure 1-1 shows the molecular structures of the sixteen US-EPA and WHO priority PAHs, which are also of interest in this study.

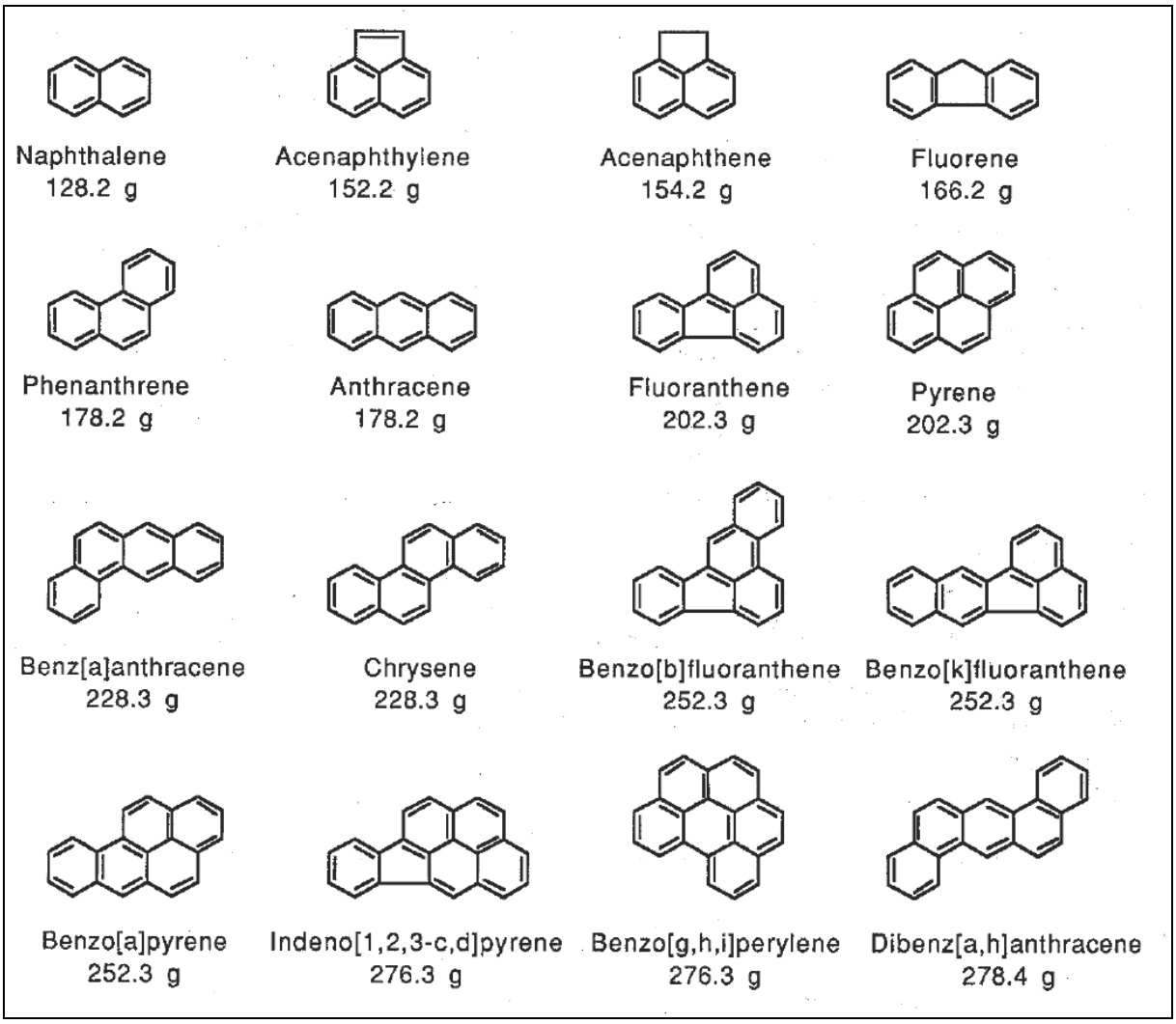


Figure 1-1 Molecular structures, nomenclature and molecular weight of the sixteen US Environmental Protection Agency (US-EPA) and World Health Organisation (WHO) polycyclic aromatic hydrocarbons (PAHs).

The physicochemical properties of individual PAHs differ with their respective molecular weights. PAHs are generally non-polar, hydrophobic compounds; they exhibit high affinity for organic matter and preferentially partition on sediment or soil particles and accumulate in the lipid-rich tissues of organisms. The volatility and aqueous solubility of PAHs decreases with increasing molecular weight, whilst their ability to resist oxidation and reduction increases (Mackay et al., 2006). Hydrophobicity is often expressed on the basis of the octanol-water partition coefficient (log K_{OW}). The log K_{OW} of a compound describes the relative solubility of a chemical, with high values indicating insolubility in water but solubility in non-polar solvent e.g. octanol (Sangster, 1997; Hawker and Connell, 1988; Borja, et al., 2005). Generally, the log K_{OW} values of a compound is inversely related to the aqueous solubility and directly proportional to the molecular weight. The log K_{OW} of a compound has proven to be a very useful parameter in predicting its environmental behaviour (Farrington, 1991). Table 1-1 presents some physicochemical

properties of the 16 US-EPA priority PAHs. It can be seen that naphthalene has the lowest molecular weight, lowest log $K_{OW,}$ but the highest aqueous solubility.

Table 1-1 Summary of the physicochemical properties of 16 US-EPA PAHs that are of interest in this project (Bojes and Pope, 2007).

Polycyclic aromatic hydrocarbons (PAHs)	No. of rings	Log K_{OW}	Molecular weight (g $mole^{-1}$)	Aqueous Solubility (mg L^{-1})	Vapour pressure (mm Hg)
Naphthalene	2	3.37	128.17	31	8.89E−02
Acenaphthylene	3	4.00	152.20	16.1	2.90E−02
Acenaphthene	3	4.07	154.21	3.8	3.75E−03
Fluorene	3	4.18	166.22	1.9	3.24E−03
Phenanthrene	3	4.45	178.23	1.1	6.80E−04
Anthracene	3	4.45	178.23	0.045	2.55E−05
Pyrene	4	4.88	202.26	0.132	4.25E−06
Fluoranthene	4	4.90	202.26	0.26	8.13E−06
Chrysene	4	5.16	228.29	0.0015	7.80E−09
Benzo[a]anthracene	4	5.61	228.29	0.011	1.54E−07
Benzo[*b*]fluoranthene	5	6.04	252.32	0.0015	8.06E−08
Benzo[*a*]pyrene	5	6.06	252.32	0.0038	4.89E−09
Benzo[*k*]fluoranthene	5	6.06	252.32	0.0008	9.59E−11
Benzo[*g,h,i*]perylene	6	6.84	276.34	0.00026	1.00E−10
Indeno[*1,2,3-cd*]pyrene	6	6.58	276.34	0.062	1.40E−10
Dibenz[*a,h*]anthracene	6	6.50	278.35	0.0005	2.10E−11

PAHs are formed from both natural (e.g. volcanic eruptions and forest fires) and anthropogenic processes. However, anthropogenic activities including oil and gas activities, exhaust emissions, heating, energy generation, industrial emissions and refuse incineration are responsible for most PAHs that enter the environment (Readman et al., 2002). Principally, there are two major mechanisms by which anthropogenic PAHs are formed i.e. petrogenic and pyrolytic processes. Petrogenic source PAHs are formed by the gradual degradation of sedimentary organic materials at moderate to low temperature to produce various petroleum products e.g. crude oil. Petrogenic PAHs are characterised by the homologue groups of 2- to 3- ring PAHs e.g.

naphthalenes, chrysenes, fluorenes but with high proportions of alkylated PAHs compared to the respective parent PAHs. Most PAHs of petrogenic origin are thermodynamically stable. Pyrolytic PAHs are formed through high temperature combustion of fossil fuels and organic matter and are dominated by the parent compounds of 4-, 5- and 6-ring PAHs. The dominant isomers are formed quickly and as a result, are not thermodynamically stable. PAHs are also formed by direct biosynthesis e.g. microbes and other organisms. Perylene is synthesised by organisms including bacteria, fungi and algae (Readman et al., 2002; Witt, 2002; Neff et al., 2005; Haritash and Kaushik, 2009).

PAHs are found everywhere in the atmospheric, terrestrial and aquatic environments, with elevated concentrations mostly in urban and industrialised areas. In the aquatic environment they have been detected in rivers, lakes, coastal waters, groundwater, sediments, soils, and biota (Witt, 2002; Zakaria et al., 2002; Baumard et al., 2009). There are a variety of routes by which PAHs enter the aquatic environment. These include atmospheric deposition, spillage and disposal of oil and petroleum products, and industrial and urban discharges. Air to water deposition plays a major role since PAHs are adsorbed to small particles (<1 μm) and can therefore be transported through the atmosphere as gases or airborne particles over long distances (Manoli et al., 2000; Li et al., 2009). PAHs are ultimately removed from the air through dry and wet deposition; rain-water can also wash-out PAHs from road surfaces and industrial and household sewage or oil contamination are other potential sources of PAHs to surface waters.

The distribution and fate of PAHs in the aquatic environment are largely influenced by the physicochemical properties of the compounds including aqueous solubility, log K_{OW} and vapour pressure (Burgess et al., 2003). PAHs tend to associate with particulate material and bioaccumulate in biota, due to their low aqueous solubility and more lipophilic nature, particularly, the HMW PAHs. Over time, PAHs may undergo various chemical, physical, biological or photochemical processes, including volatilisation, photolysis, hydrolysis, oxidisation, microbial degradation, and adsorption and subsequent sedimentation. The degradation processes occur at various rates, depending on the physical-chemical properties of each compound and nature of the environment in which it is present.

Molecular indices based on the ratios of various selected PAHs are widely used to characterise the possible origins of PAHs, particularly in sediment samples (e.g. Soclo et al., 2000, Yunker et al., 2002; Rocher, 2004; Webster et al., 2004). This involves the differences in the physicochemical properties (e.g. thermodynamic stability) of some PAH structural isomers. Petroleum products contain lower molecular weight PAHs such as phenanthrene and anthracene; but phenanthrene is a more thermodynamically stable than the later. Therefore, for

PAHs contamination of petrogenic origin, the ratio of phenanthrene (P) to anthracene (A) is usually >10 but is lower when contamination originates from pyrolytic sources (Neff et al., 2005). Fluoranthene (Fl) to pyrene (Py) ratio is also another commonly used source distribution ratio; where Fl/Py values greater than one indicate pyrolytic source and less than unity suggest petrogenic input (Yunker et al., 2002). However, when contamination arises from a combination of both petrogenic and pyrolytic processes, it has been observed that the ratios of phenanthrene to anthracene and fluoranthene to pyrene will typically indicate pyrolytic processes as being the dominant source, thus making specific source apportionment more complex (Webster et al., 2000). Other ratios such as methylphenanthrene (Mp) to phenanthrene (P) and fluoranthene + pyrene to methylfluoranthene (MFl) + methylpyrene (Fl+Py)/(MFl+MPy), chrysene to benz[*a*]anthracene (Chry/BaA) and benz[*a*]pryene to benzo[*ghi*]perylene (B[*a*]P/B[*ghi*]P) can also be used to differentiate between sources (Webster et al., 2001; Yunker et al., 2002).

PAHs are toxic, carcinogenic and in some cases mutagenic to aquatic organisms and humans. PAHs differ in their behaviour, uptake, metabolism, and their biological effects on systems based on their molecular weights. The LMW PAHs exact significant acute toxicity and other deleterious effects to some organisms, but are not particularly carcinogenic, i.e. the 2- and 3-ring compounds can cause tainting of fish and shellfish (Davies et al., 2002). HMW PAHs are significantly less acutely toxic than LMW PAHs on organisms; however, many of them may become toxic in the presence of solar ultraviolet radiation. On the other hand, majority of the HMW PAHs e.g. four-, five- and six-ring PAHs are known to be both mutagenic and carcinogenic to a wide variety of aquatic organisms and humans compared with LMW PAHs (Neff, 1979). In most cases, the substituted alkylated PAHs have conspicuously greater biological effects than the parent compounds (Schirmer et al., 1998; Smith et al., 2000), as the parent compounds require a higher metabolic activation to the reactive electrophilic metabolites (Neff, 1979).

1.2.2 Polychlorinated biphenyls (PCBs)

Polychlorinated biphenyls (PCBs) are a group of 209 anthropogenic organic compounds, each of which consists of the biphenyl structure (two linked benzene rings; Figure 1-2) containing from one to ten chlorine atoms. PCBs are produced by chlorination of biphenyl which has 10 substituent positions (i.e. 2-6 and 2'-6'). The empirical formula of PCBs is $C_{12}H_{10-n}Cl_n$, where n=1-10 and corresponds to the number of chlorine atoms.

Each individual PCB is known as a congener, while a group of PCBs with the same level of chlorination is termed homologue. Table 1-2 shows the composition of chlorinated biphenyls by

a homologue. PCBs of the same homologue but with different positions of chlorine substitution are called isomers. Although, 209 different congeners are theoretically possible, only 130 have been discovered in industrial mixtures (Safe, 1990).

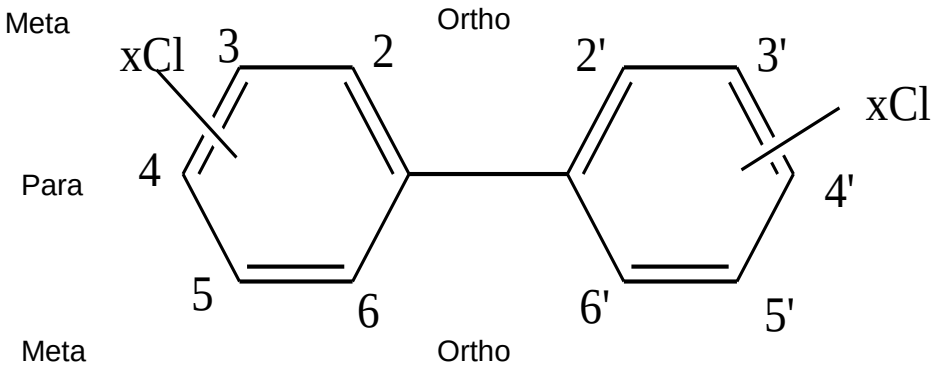
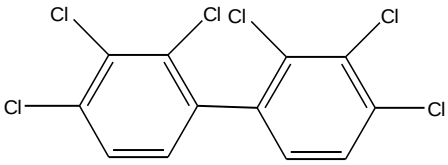


Figure 1-2 General structure of PCBs with the conventional numbering of the substituent positions; 2-6 and 2'-6' =10 possible chlorination positions of the biphenyl; positions C2 and C6=*Ortho*; C4=*Para*; and C3 and C5=*Meta*.

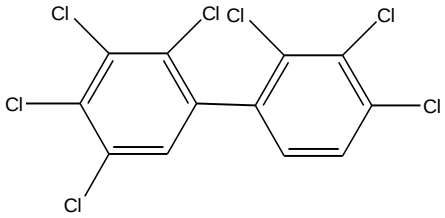
The nomenclature of PCB congeners are cumbersome, as a result, Ballschmiter and Zell (1980) proposed a numbering system which has been adopted by the International Union of Pure and Applied Chemists (IUPAC). The number of substituent positions according to this arrangement is taken as an abbreviation for the PCB structure. The structures and the corresponding IUPAC numbers of all 209 (possible) PCB congeners are shown in Table 1-3.

The bond joining the two benzene rings of PCBs can rotate to give varying configurations of the molecule, such as planar configurations (when the two rings sit in the same plane), or non-planar configurations (when the two rings are at right angle to each other). Planarity of the molecule depends largely on the level of chlorination at the *ortho* positions. In PCBs with one (mono-*ortho*) or no chlorine substitution (*non-ortho*) in the *ortho* positions (i.e. only in the *meta* and *para* positions), the atoms of the congener are able to line up in a single plane (i.e. coplanar) and assume 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) like configuration. The most toxic PCB congeners are the so called “dioxin-like” PCBs, which include four non-*ortho* PCBs (CBs 77, 81,126 and 169) and eight mono-*ortho* substituted PCBs (CBs 114, 105,123,118, 156, 157,167 and 189). Figure 1-3 shows some mono-*ortho* and *ortho* PCBs.

CB118 (2,3',4,4' 5-Pentachlorobiphenyl)



CB156 (2,3,3',4,4',5-Hexachlorobiphenyl)



CB128 (2,2',3,3',4,4'- Hexachlorobiphenyl)

CB170 (2,2,3,3,4,4,5 Heptachlorobiphenyl)

Figure 1-3 Substitution pattern of some selected mono-*ortho* (co-planar) PCB congeners (PCB 118 and CB156) and *ortho* (non-planar) PCB congeners (CB128 and CB 170).

Table 1-2 Composition of PCBs by homologue

Molecular Formula	Name: -(Chlorobiphenyl)	Number of Isomers	IUPAC-No.	Molecular Mass	% of Chlorine[1]	No. of Isomers Identified
$C_{12}H_9Cl$	Mono	3	1-3	188.65	18.79	3
$C_{12}H_8Cl_2$	Di	12	4-15	233.1	31.77	12
$C_{12}H_7Cl_3$	Tri	24	16-39	257.54	41.3	23
$C_{12}H_6Cl_4$	Tetra	42	40-81	291.99	48.65	41
$C_{12}H_5Cl_5$	Penta	46	82-127	326.43	54.3	39
$C_{12}H_4Cl_6$	Hexa	42	128-169	360.88	58.93	31
$C_{12}H_3Cl_7$	Hepta	24	170-193	395.32	62.77	18
$C_{12}H_2Cl_8$	Octa	12	194-205	429.77	65.98	11
$C_{12}HCl_9$	Nona	3	206-208	464.21	68.73	3
$C_{12}Cl_{10}$	Deca	1	209	498.66	71.1	1

[1] On the basis of molecular mass

The physical and chemical characteristics of individual PCB congeners are dependent upon the number of chlorines and their positions around the biphenyl rings. The molecular weight and percent chlorine for PCB homologue groups are shown in Table 1-2. Generally, PCBs are highly resistant to biological, photochemical, chemical and thermal degradation. However, under certain conditions, they can be destroyed by chemical, biological and thermal processes e.g. via incineration. Generally, PCBs are hydrophobic and their hydrophobicity increases with increasing degree of chlorination. The log Kow for PCBs ranges from 4.5 for monochlorobiphenyls to greater than 8 for higher chlorinated PCBs e.g. deca-chlorobiphenyl (Fiedler, 2001).

Table 1-3 Nomenclature of PCB-congeners according to IUPAC (Ballschmiter and Zell, 1980).

No.	Structure	No.	Structure	No.	Structure	No.	Structure	No.	Structure
Monochlorobiphenyls		**Tetrachlorobiphenyls**		**Pentachlorobiphenyls**		**Hexachlorobiphenyls**		**Heptachlorobiphenyls**	
1	1	42	2,2',3,4'	87	2,2',3,4,5'	132	2,2',3,3',4,6'	177	2,2',3,3',4',5,6
2	2	43	2,2',3,5	88	2,2',3,4,6	133	2,2',3,3',5,5'	178	2,2',3,3',5,5',6
3	3	44	2,2,3,5'	89	2,2',3,4,6'	134	2,2',3,3',5,6	179	2,2',3,3',5,6,6'
		45	2,2',3,6	90	2,2',3,4',5	135	2,2',3,3',5,6'	180	2,2',3,4,4',5,5'
Dichlorobiphenyls		46	2,2',3,6'	91	2,2',3,4',6	136	2,2',3,3',6,6'	181	2,2',3,4,4',5,6
4	2,2'	47	2,2',4,4'	92	2,2',3,5,5'	137	2,2',3,4,4',5	182	2,2',3,4,4',5,6'
5	2,3	48	2,2',4,5	93	2,2',3,5,6	138	2,2,3,4,4',5'	183	2,2',3,4,4',5',6
6	2,3'	49	2,2',4,5'	94	2,2',3,5,6'	139	2,2',3,4,4',6	184	2,2',3,4,4',6,6'
7	2,4	50	2,2',4,6	95	2,2',3,5',6	140	2,2',3,4,4',6'	185	2,2',3,4,5,5',6
8	2,4'	51	2,2',4,6'	96	2,2',3,6,6'	141	2,2',3,4,5,5'	186	2,2',3,4,5,6,6'
9	2,5	52	2,2',5,5'	97	2,2',3',4,5	142	2,2',3,4,5,6	187	2,2',3,4',5,5',6
10	2,6	53	2,2',5,6'	98	2,2',3,4,6	143	2,2',3,4,5,6'	188	2,2',3,4',5,6,6'
11	3,3'	54	2,2',6,6'	99	2,2',4,4',5	144	2,2',3,4,5',6	189	2,3,3',4,4',5,5'
12	3,4	55	2,3,3',4	100	2,2',4,4',6	145	2,2',3,4,6,6'	190	2,3,3',4,4',5,6
13	3,4'	56	2,3,3',4'	101	2,2',4,5,5'	146	2,2',3,4',5,5'	191	2,3,3',4,4',5',6
14	3,5	57	2,3,3',5	102	2,2',4,5,6'	147	2,2',3,4',5,6	192	2,3,3',4,5,5',6
15	4,4'	58	2,3,3',5'	103	2,2',4,5',6	148	2,2',3,4,5,6'	193	2,3,3',4',5,5',6
		59	2,3,3',6	104	2,2',4,6,6'	149	2,2',3,4',5'6		
Trichlorobiphenyls		60	2,3,4,4'	105	2,3,3',4,4'	150	2,2',3,4',6,6'	**Octachlorobiphenyls**	
16	2,2',3	61	2,3,4,5	106	2,3,3',4,5	151	2,2',3,5,5',6	194	2,2',3,3',4,4',5,5'
17	2,2',4	62	2,3,4,6	107	2,3,3',4',5	152	2,2',3,5,6,6	195	2,2',3,3',4,4',5,6
18	2,2',5	63	2,3,4',5	108	2,3,3',4,5'	153	2,2',4,4',5,5'	196	2,2',3,3',4,4',5',6
19	2,2',6	64	2,3,4',6	109	2,3,3',4,6	154	2,2',4,4,5,6'	197	2,2',3,3',4,4',6,6'
20	2,3,3'	65	2,3,5,6	110	2,3,3',4',6	155	2,2',4,4',6,6'	198	2,2',3,3',4,5,5',6
21	2,3,4	66	2,3',4,4'	111	2,3,3',5,5'	156	2,3,3',4,4',5	199	2,2,3,3',4',5,5',6
22	2,3,4'	67	2,3',4,5	112	2,3,3',5,6	157	2,3,3',4,4',5'	200	2,2',3,3',4,5,6,6'
23	2,3,5	68	2,3',4,5'	113	2,3,3',5',6	158	2,3,3',4,4',6	201	2,2',3,3',4,5',6,6'
24	2,3,6	69	2,3',4,6	114	2,3,4,4',5	159	2,3,3',4,5,5'	202	2,2',3,3',5,5',6,6'
25	2,3',4	70	2,3',4',5	115	2,3,4,4',6	160	2,3,4',4,5,6	203	2,2',3,4,4',5,5',6
26	2,3',5	71	2,3,4',6	116	2,3,4,5,6	161	2,3,3',4,5',6	204	2,2',3,4,4',5,6,6'
27	2,3',6	72	2,3',5,5'	117	2,3,4',5,6	162	2,3,3',4',5,5'	205	2,3,3',4,4',5,5',6'
28	2,4,4'	73	2,3',5',6	118	2,3',4,4',5	163	2,3,3',4',5,6		
29	2,4,5	74	2,4,4',5	119	2,3,4,4',6	164	2,3,3',4',5',6	**Nonachlorobiphenyls**	
30	2,4,6	75	2,4,4',6	120	2,3',4,5,5'	165	2,3,3',5,5',6	206	2,2',3,3',4,4',5,5',6
31	2,4',5	76	2',3,4,5	121	2,3',4,5',6	166	2,3,4,4',5,6	207	2,2',3,3',4,4',5,6,6'
32	2,4',6	77	3,3',4,4'	122	2',3,3',4,5	167	2,3',4,4',5,5'	208	2,2',3,3',4,5,5',6,6'
33	2',3,4	78	3,3',4,5	123	2',3,4,4',5	168	2,3',4,4',5',6		
34	2',3,5	79	3,3',4,5'	124	2',3,4,5,5'	169	3,3',4,4',5,5'	**Decachlorobiphenyl**	
35	3,3',4	80	3,3',5,5'	125	2',3,4,5,6'			209	2,2',3,3',4,4',5,5',6,6'
36	3,3',5	81	3,4,4',5	126	3,3',4,4',5	**Heptachlorobiphenyls**			
37	3,4,4'			127	3,3',4,5,5'	170	2,2',3,3',4,4',5		
38	3,4,5	**Pentachlorobiphenyls**				171	2,2',3,3',4,4',6		
39	3,4',5	82	2,2',3,3',4	**Hexachlorobiphenyls**		172	2,2',3,3',4,5,5'		
		83	2,2',3,3',5	128	2,2',3,3',4,4'	173	2,2',3,3',4,5,6		
Tetrachlorobiphenyls		84	2,2',3,3',6	129	2,2',3,3',4,5	174	2,2',3,3',4,5,6'		
40	2,2',3,3'	85	2,2',3,4,4'	130	2,2',3,3',4,5'	175	2,2',3,3',4,5',6		
41	2,2',3,4	86	2,2',3,4,5	131	2,2',3,3',4,6	176	2,2',3,3',4,6,6'		

PCBs were first manufactured as technical mixtures in the USA by Monsanto Corporation in 1929 under the trade name Aroclor; subsequently, they were manufactured in other countries with various trade names i.e. Clophen (Germany), Chlorofen (Poland), and Sovol (USSR). Aroclors are designated by a four digit number, of which the first two digits are 1 and 2, representing the 12 carbons of the biphenyl skeleton. The second 2 digits in the code indicate the average percent weight of the chlorine. Codes for the PCB Arochlor for instance include 1242, 1254, and 1260, in which chlorine constitutes 42, 54 and 60% of the total weight respectively. PCBs were acclaimed as an industrial breakthrough due to their high stability, inertness and good insulating properties. This resulted in their wide commercial applications in electric generating industries as a coolant and lubricants in transformers and capacitors; also as flame retardants, and in the manufacture of paints, plastics, coatings and pressure-sensitive copying paper etc (Ramu et al., 2006). Production and use of PCBs have been banned or severely restricted in several developed countries since the 1980s due to their bioaccumulation and toxicity (Breivik et al., 2007); this is discussed further in this chapter. However, owing to their high persistency, low volatility and extensive applications in various industries over the years, significant quantities have been spilled, dumped, or leaked into the environment, persisting in air, water, land and biota (Baars et al., 2004; Falandysz et al., 2004; Fu et al., 2009).

It was not until 1966 that the distribution of PCBs in the environment was documented (Jensen, 1966). Since then, PCBs have been detected in almost every environmental compartment or matrix, including in rural and pristine areas of the world such as the Antarctic, Canadian Arctic and Albatross of the Pacific, upper layers of top mountains, even the Penguins in Antarctica have been found to contain trace amounts of PCBs. (Harrison, 2001; Holoubek, 2001; Weber and Goerke 2003; Tanabe et al., 2004). In addition, Webster et al (2011) identified a range of PCBs in deep water fish collected from the seas to the west of Scotland. These demonstrate the disposition of PCBs to long-range transport from their initial source.

PCBs are dispersed rapidly through both atmospheric processes and watercourses. The aquatic environment is often the final destination of PCBs, through long-range global atmospheric transport and point sources. Hence, aquatic organisms are subject to an increased risk of exposure to PCBs, particularly via their diet. Exposure to PCBs has been linked to a wide range of effects, including reproductive toxicity, immunotoxicity, hepatotoxicity, neurotoxicity, teratogenic, apoptosis, necrosis, and endocrine abnormalities (Safe, 1994; Kucewicz, 2004; Carpenter, 2006). The toxic effects of PCBs are extensive. Studies showed that they could activate genes involved in generating carcinogenic compounds from PAHs (Robertson and Hansen, 2001). Further, 2,3,7,8-tetrachlorodibenzo-*p* dioxin (TCDD), a combustion product of

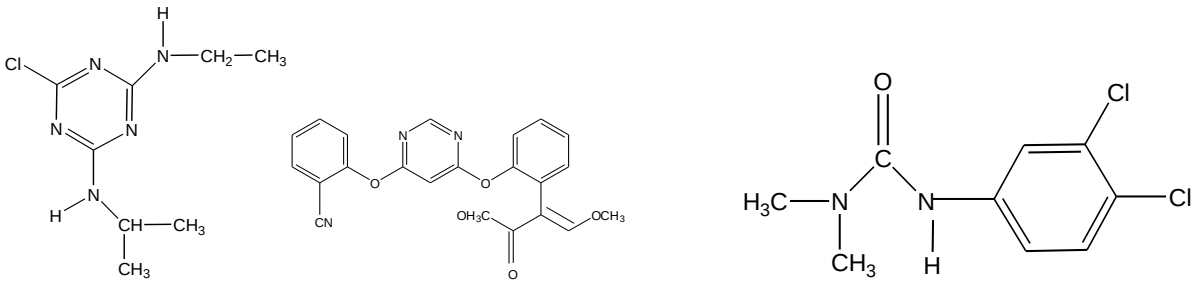
PCBs and a by-product of many industrial activities is one of the most toxic chemical compounds identified (Johansen, 2003). Individual PCB congeners and the commercial mixtures exert different categories of biochemical and toxic responses, some of which are analogous to those caused by dioxins. For example, some PCBs display a high binding attraction to hepatic cytosolic receptor protein (Ah receptor) and induction potency of hepatic microsomal enzymes (Yu, 2005). This may lead to an increase in the metabolism of endogenous substances including some hormones such as estradiol. Further, it has been shown that in biota, PCBs could initiate the metabolism of vitamin A; Yu (2005) showed that rats fed with 20 ppm of PCBs exhibited decreased storage of vitamin A. Ecological exposures to trace concentrations of individual PCBs may seem insignificant, but, with continuing exposures, the trace concentrations may magnify, due to their bioaccumulation tendency.

Whilst, PCBs are not in the list of WFD priority substances, they are included in OSPAR's List of Chemicals for Priority Actions due to their persistence, bioaccumulation and toxicity (PBT). PCBs are integral aspect of the Scottish marine monitoring programme i.e. monitored by both Marine Scotland Science (MSS) and SEPA. Although, aqueous concentrations of PCBs, as with most other trace organic contaminants in Scottish marine waters are often below the analytical limit of detection using conventional sampling techniques, there is still a need to develop appropriate methodologies in respect of sampling and biological effects measurements of these contaminants and their mixtures.

1.2.3 Pesticides

Pesticides are chemicals or mixtures that are specifically produced to prevent, destroy, repel, attract or reduce pests. Some of the well-known pesticides include insecticides, herbicides, and fungicides. Pesticide usage cuts across different spheres of life, including health, agriculture, forestry and the home. In the last two decades, there has been a worldwide increase in pesticide usage both for agricultural and non-agricultural purposes (Carvalho, 2006; Grube et al., 2011). There are more than 800 chemicals registered as pesticides in the European Union (Carvalho, 2006), there are in excess of 450 active chemical ingredients that are approved for use in pesticide formulations in the UK alone, and the use of pesticides worldwide is estimated to be approximately 11.2 billion kg annually (Grube et al., 2011). Until now, little is known about the environmental fate and ecological impacts of a number of these pesticides in the environment. The large number of active ingredients used in manufacturing of pesticides in different countries worldwide makes their classification rather challenging. Generally, pesticides are grouped or

classified on the basis of their chemical structures, functions (target pests), mode of action, formulation or hazard/toxicity. Figure 1-4 shows the structures of some selected pesticides that are of interest in this study.



Atrazine Azoxystrobin Diuron

Figure 1-4 Chemical structures of some selected pesticides relevant in this study.

Pesticides can also be grouped according to their mode of action i.e. the primary sites of action. The largest classes of pesticides based on the number of active ingredients and the quantities used are the herbicides (e.g. atrazine), insecticides (e.g. carbaryl, aldicarb), and fungicides (e.g. azoxystrobin, chlorothalonil). Irrespective of the complexity in classification of pesticides, their overall characteristics are largely influenced by their physicochemical properties e.g. water solubility, polarity, and volatility of each compound. Pesticides enter the aquatic environment through a variety of processes including agricultural and urban run-off, leaching, spray drift, wind drift, waste water from agricultural product store houses and green houses, illegal dumping and indiscriminate disposal of pesticide containers etc (Cerejeira et al., 2003; Konstantinou et al., 2006). The transport pathways are highly influenced by the physicochemical properties of each pesticide and the local climatic and geological conditions.

Pesticides can be degraded by a variety of processes such as photodecomposition, thermal degradation, biological action (microbial decay) and soil conditions (pH). However, similar to other contaminants, some pesticides are resistant to degradation and may persist in the environment and accumulate in aquatic ecosystems (Weigmann et al., 2009). The fate of pesticides in the aquatic environment is largely influenced by their natural affinity for any of the four environmental compartments: solid matter, including mineral matter and particulate organic carbon; water (solubility), gaseous form (volatilisation) and biota. The partitioning behaviour of pesticides in environmental compartments can be determined by assessing the organic carbon

sorption coefficient (K_{OC}), Henry's Constant (H) and the octanol/water partition coefficient (K_{OW}; Ongley, 1996).

As a result of their wide applications and relative persistence, pesticides and their transformation products have been detected in various environmental and biological matrices (da Silva et al., 2003; Belmonte Vega et al., 2005). Despite the large amounts and varieties of pesticides that are released into the environment, a limited number are routinely monitored in European water bodies. As a result, there is insufficient knowledge regarding the fate and impacts of varieties of pesticides released into marine and freshwater ecosystems. Owing to concern on their ecological impacts, some pesticides have been banned or have several restricted uses within Europe as set down by various European Commission Regulations and Directives on the placing on the market and use of plants protection products (PPPs; e.g. EC, 1978; EC, 1991; EC, 2009). In recent times, there has been an increased demand for environmental monitoring of pesticides, as some of them, including atrazine, chlorpyrifos, diuron, endosulfan, isoproturon, pentachlorophenol, simazine, trifluralin have already been identified as priority substances or priority hazardous substances (PHSs) under the EU-WFD. Some are currently under review and may become priority substances in the future (EC, 2000; EC, 2011).

The extensive usage of pesticides can have unpremeditated adverse effects on the aquatic environment. The majority of sprayed pesticides are transferred to destinations other than their target species, including non-target species (Warren et al., 2003). The various types of pesticides have diverse toxicological impacts on aquatic organisms; hence it is difficult to make a generalised statement on their biological effects. However, the effects on organisms at various ecological levels are considered as an early warning indicator of the potential impacts of pesticides at the human level. Some pesticides are extremely toxic to aquatic organisms and to humans. Pesticide poisoning of aquatic animals can lead to their immediate death, for example, chlorpyrifos and 1,2 diazinon are widely used pesticides and are commonly detected in most urban streams and rivers (Bailey et al., 2000; Relyea, 2009). These pesticides have been shown to be acutely toxic to fish. It should be noted, however, that not all pesticide poisoning leads to the immediate death (acute toxicity) of an organism; the gradual accumulation of small “sublethal” concentrations of some pesticides can lead to various morphological and behavioural changes including weight loss low level of physiological response and impaired reproduction etc. Other chronic effects of pesticides include suppression of the immune systems, disruption of (hormonal) endocrine systems, cellular and DNA damage and inter-generational effects (i.e. effects that are not apparent until subsequent generations of the organism) (Ongley, 1996; Weston et al., 2004). Importantly, these effects may not be caused by the action of one particular pesticide but by a mixture of the pesticides, through additional or synergetic effects

with other organic contaminants and/or environmental stressors. Pesticides can also be transformed by chemical, physical, and biological processes into one or more metabolites, and some of the metabolites can be more persistent and toxic than the parent compound.

1.3 Bioavailability, bioaccumulation, exposure, and metabolism of selected organic contaminants

There is still some ambiguity around the definition of bioavailability and the concept is sometimes interchanged with bioaccessibility (Peijnenburg and Jager, 2003). However, in this study, we align with Semple et al. (2004) and define bioavailable as the fraction of the total concentrations of contaminants that are readily available for uptake by organisms (i.e. free to pass through biological membranes) at a given time. Similarly, bioaccessibility refers to the fraction of total contaminants that are within reach or accessible to organisms but not readily available e.g. may be bound to sediments. Paustenbach (2000) explained that the biologically effective dose (BED), or the amount of contaminants that actually reaches the cells, sites or membranes where adverse effect occur may represent just a fraction of the delivered dose, but this fraction is actually the most useful in estimating adverse effects. Accumulation of xenobiotics (foreign compounds) in biological systems in the aquatic environment requires the compounds to be available in the dissolved state. The estimation of the actual concentration of a contaminant at the target site in an organism is very challenging. Basic information of the freely dissolved concentration in the system is fundamental in respect of obtaining a more precise estimate of the concentration at the target (Joop et al., 2007). To a large extent, bioavailability is controlled by factors including the physicochemical characteristics of the compound (e.g. octanol-water partition coefficient; log K_{OW}, vapour pressure), and environmental factors (e.g. pH, temperature, salinity, dissolved organic carbon).

Bioaccumulation refers to the process in which the concentration of a contaminant in an organism reaches a level higher than the concentration in water as a result of contaminant uptake through all possible routes including dietary absorption, transport across respiratory surfaces and dermal absorption (Gobas and Morrison, 2000). Bioaccumulation can be regarded as comprising both bioconcentration (e.g. contaminants uptake by organisms through non dietary route) and food uptake (Mackay and Fraser 2000).

Aquatic organisms are exposed to organic contaminants in polluted waters via direct and/or indirect uptake. Direct uptake of contaminants by the organisms dissolved in the water maybe through absorption e.g. the skin, ingestion of contaminated food and through the respiratory systems (Landrum and Fisher, 1998; Van der Oost et al., 2003; Taylor, 2009). Indirect exposure may involve the transfer of contaminants from parents to offspring. Walker (2001) explained that the transfer of organic contaminants into organisms is primarily through passive diffusion across natural boundaries e.g. through gills, epithelial tissues, or the gastrointestinal tract. Exposure of organisms to contaminants constitutes the first stage in the manifestation of toxicity. For

contaminants to elicit an effect on an aquatic organism there has to be contact between the organism and the contaminant at a suitable concentration over a period of time. For most organic contaminants, exposure to organisms is highly dependent on the degree of lipophilicity i.e. log K_{OW}. Biological membranes are composed of mainly lipids and majority of organic contaminants, particularly the hydrophobic organic contaminants (HOCs) are lipophilic. Therefore, the relatively high log K_{OW} of HOCs enables them to easily penetrate cellular membranes of aquatic organisms (Pritchard, 1993; Escher and Hermens, 2004).

After entering into the organism, the contaminant is stored, excreted or metabolised (biotransformed). Most organisms have a suite of biotransformation enzymes required to metabolise xenobiotics to a certain extent. The liver plays an essential role in this regard in vertebrates, whilst, the tissues responsible for processing of foods are responsible in invertebrates. Generally, biotransformation involves a series of enzyme-catalysed processes in which toxic hydrophobic compounds are converted to less toxic water-soluble compounds that are easily excreted. However, biotransformation may also lead to formation of toxic metabolites which may elicit toxicological effects on the host organism by interfering with physiological or biochemical activities (Livingstone, 1998; Yu, 2005). Therefore, the biotransformation of an organic contaminant in an organism is vital in predicting toxicity, distribution and ability to be excreted. These activities are tissue-specific functions and metabolism (activation) is often needed to initiate the toxic activities of the contaminants (Taylor, 2009).

Biotransformation processes consist of two major phases, Phase I involves the introduction of a reactive polar functional group (e.g. −OH, −COOH, $-NO_2$) into the hydrophobic compound through oxidation, reduction, or hydrolysis, which leads to the formation of a primary metabolite. Phase II processes involve direct conjugation of the activated water-soluble Phase 1 metabolites to endogenous enzymes e.g. the transferase enzymes including sulphotransferase (STs), gluthathione-S-transferases (GSTs) and UDP-glucuronosyltransferases (UGTs). These two phases lead to a progressive formation of more water soluble compounds, from a lipophilic contaminant to a polar metabolite then to a more polar metabolite, which is easily excreted (Walker, 2001; Mothersill and Austin, 2003). The microsomal monooxygenase enzymes or mixed-function oxidase (MFO), particularly, the cytochrome P450 (CYP450), are the major Phase 1 enzymes involved in biotransformation of organic contaminants in aquatic organisms including invertebrates and fish (Thies et al., 1996; Cao et al., 2000; Stegeman et al., 2001; Van der Oost et al., 2003).

Cytochrome P450 enzymes are predominantly found in the endoplasmic reticulum and mitochondria of liver and specific tissues in fish and other vertebrates as well as in aquatic

invertebrates (Arinç et al., 2000; Snyder, 2000). The most widely studied cytochrome P450 protein is cytochrome P4501A (CYP1A), which is regulated by the aryl hydrocarbon receptor (AhR) (Stegeman et al., 2001). Whilst Cytochrome P450 enzymes are principally responsible for the oxidative metabolisms of fatty acids, biogenic amines, leukotrienes, pheromones, and endogenous steroids, etc (Lewis, 2001), they are also involved in the metabolism and activation of various xenobiotic compounds (Mothersill and Austin, 2003). In fish and other vertebrates, the CYP1A gene is highly inducible (bioactivation) by various organic contaminants, including PAHs, PCBs and some pesticides (Londono et al., 2004; Široká and Drastichova, 2004; Hahn et al., 2005). The induction of CYP1A by several xenobiotics is mediated through the binding of xenobiotics to the cytosolic AhR. Evaluation of the CYP1A induction in aquatic organisms and cellular systems has proven to be a sensitive biomarker of exposure to organic contaminants in the aquatic environment. This can be evaluated in various ways including measuring the catalytic activity of 7-ethoxyresorufin-*O*-deethylase (EROD) in various organisms and test systems (Fent, 2001).

The level of metabolism of xenobiotics varies from one organism to the other and also on the type and concentration of the xenobiotic compounds. The highly polar chemical compounds are easily metabolised and eliminated from biological systems; whereas, the more non-polar compounds require some level of transformation in order that they can be eliminated from the organism. Most aquatic organisms particularly fish, crustacea, and polychaetes have active enzyme systems that can transform substantial portions of bioaccumulated xenobiotics including PAHs, PCBs and pesticides (Farrington, 1991; Van der Oost et al., 2003). In some aquatic organisms, e.g. fish, the PAHs are rapidly metabolised, transformed and excreted unlike the PCBs. The metabolism of PAHs in vertebrate organisms involves Phase 1 and Phase 11 systems (Mothersill and Austin, 2003).

Most PCB congeners are resistant to metabolism in fish; however, studies have shown that generally, overtime, some PCBs are metabolised to certain extent in most organisms (Stapleton et al. 2001, Borja et al. 2005). The overall metabolic pathway is identical for all congeners; it involves oxidation through the cytochrome P450 (CYP) enzyme system. The addition of oxygen across or onto an aromatic ring of a PCB yields either the epoxide or the hydroxylated metabolite. The rate of metabolism of PCBs is highly dependent on the number of chlorine atoms present, planarity of the PCB, and the CYP isozyme with which it interacts. Consequently, the majority of highly chlorinated PCBs, particularly the non-planar or *ortho* substituted PCBs that are not substrates to CYP1A are not only very slow to metabolise but, their metabolites are not easily excreted. Further, several PCB metabolites are more lipophilic than the parent

compound, hence more difficult to excrete, and therefore have a higher potential to exert a biological effect.

Pesticides undergo oxidation in which the cytochrome P450 enzymes plays major role in the transformation. The major metabolites, produced from this initial enzymatic action, are further conjugated with natural components such as carbohydrates, and this assists in their removal from the organisms. Some pesticides are also susceptible to abiotic hydrolysis, and are generally also biotically metabolised by various esterases to from hydrophilic conjugates (Katagi, 2010).

In general, the assessment of environmental and ecological exposure to organic contaminants including PAHs, PCBs and pesticides and their biotransformation products require information on their bioavaiable concentrations.

1.4 Chemical analytical techniques in water quality monitoring

Due to their persistence, tendency to bioaccumulate and cause adverse effects on biota, monitoring of a variety of organic contaminants in the aquatic environment has become a major activity under a number of obligatory monitoring programmes including the European Union (EU) Water Framework Directive (WFD; EC, 2000), Marine Strategy Framework Directive (MSFD; EC, 2008) and the OSPAR Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR Commission, 2011).

Sampling and reliable analysis of the wide range of organic contaminants in the aquatic environment represents an ongoing challenge in water quality and wider environment monitoring. Conventional techniques in water quality monitoring involve the collection of discrete spot or bottle samples of water at a particular point in time. The subsequent chemical analysis of "priority" contaminants and comparison with available environmental quality standards (EQS) provide a moment in time total concentration of the individual compounds. No information on the toxicologically relevant concentrations (i.e. freely dissolved) is generally available and the contributions of mixture effects to any overall effect are ignored. Further, measuring the truly dissolved concentrations of organic compounds from spot or bottle water sampling may require challenging phase separations (e.g. filtrations) and at low environmental concentrations, large volumes of water are often collected to achieve the desired limit of detection (LOD). It is now increasingly being recognised that data from intermittent spot or bottle water sampling techniques may not be a representative of the actual environmental situation. Episodic pollution events can be missed, for example, during a flood event, seasonal pesticide applications or random discharges of industrial and domestic waste.

As a result of these limitations in the spot or bottle water sampling technique, alternative sampling techniques including the use of biota (biomonitoring), automatic water samplers (autosamplers) and passive sampling, have gained considerable attention in recent years (Vrana, 2005; Greenwood et al., 2009; MacGregor et al., 2010). Most of these novel sampling techniques have been developed to replace or compliment the use of spot or bottle water sampling in monitoring water quality.

1.4.1 Application of biological monitoring technique (biomonitors) in water quality monitoring

Due to the increasing awareness of the importance of biologically relevant concentrations of contaminants in water quality monitoring, most environmental monitoring programmes employs the use of sentinel organisms (biomonitors). Biomonitors are aquatic organisms such as the blue mussel (*Mytilus edulis*) or fish that are used to assess environmental quality and monitor organic contaminants present in a particular environment. They are usually native to the assessed environment or are deployed to the environment for an extended period of time; during which they passively bioaccumulate contaminants from the deployed environment (Vrana et al., 2005). Determination of the contaminant burdens in the tissues of biomonitors after exposure compared to an appropriate reference can reveal the environmental quality and health risk of the locations where they have been deployed or collected (see Lauenstein et al., 2002; Zhou et al., 2008). The application of biomonitors to environmental monitoring is appealing due to the sensitivity of some of the biomonitors and the fact that they can function as early biological warning signals of potential ecological change (Holt and Miller, 2011). Nevertheless, the use of biomonitors in environmental monitoring of HOCs has some drawbacks. Environmental (e.g. temperature, salinity and wind/wave action) and physiological (e.g. reproductive, feeding and disease status) factors affect the accumulation of contaminants in biomonitoring organisms (Leung et al., 2002). In addition, at lower concentrations, depuration and biodegradation of some contaminants is very rapid in some biomonitors (Guieysse et al., 2001, Fung et al., 2004).

1.4.2 Application of automatic water samplers (autosamplers) in water quality monitoring

For an increased frequency of monitoring so as to collect larger volumes of water samples and to track episodic input, increasing numbers of environmental monitoring programmes often rely on continuous automatic samplers (Tran et al., 2007; Holvoet et al., 2007; Meyer et al., 2011). Autosamplers are designed to collect several water samples at specified intervals over an extended period and thus increasing the frequency of sampling over the sampling period (Vrana et al., 2005). Some limitations of this approach include the high costs of the equipment, on-site security, and the burden of frequent inspections, together with operational instability during adverse weather conditions. Further, samples collected with autosamplers may suffer from problems associated with analyte volatilisation, sorption to container walls and microbiological degradation during storage at ambient temperatures in the field prior to analysis (Jeannot, 1994). Figure 1-5 shows a typical automatic water sampler at a sampling site.

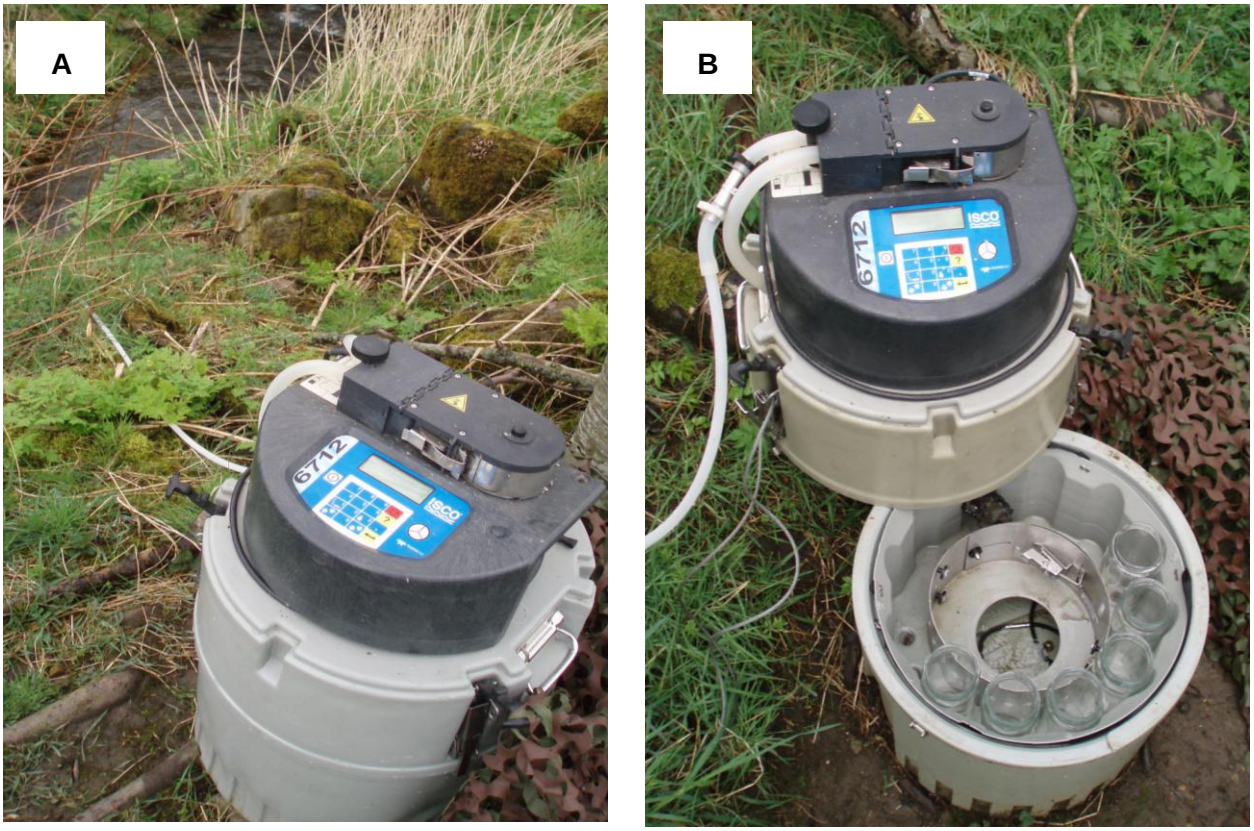


Figure 1-5 Automatic water samplers (autosamplers) at sample sites; Figure 1.5 B shows the tubing and bottles used to collect the waters samples.

1.4.3 Application of passive sampling techniques in water quality monitoring

A passive sampler is any manmade device that collects chemicals passively, without the need for any external source of energy. Passive sampling techniques provide an alternative approach to traditional grab and spot sampling, in monitoring the freely dissolved concentrations of a wide range of contaminants in a variety of environmental matrixes including water, air and soil. Passive sampling devices (PSDs) sequester and pre-concentrate the freely dissolved contaminants over extended sampling periods (days to months), as a result, overcoming the challenge of achieving very low detection limits through sampling larger volumes of water. This has been found to reduce the cost and time of sample preparation, particularly the cost of analyte isolation and pre-concentration as passive sampling combines sampling, analyte isolation and preconcentration all in one step. Passive samplers also allow the detection of trace concentrations of contaminants in the environment, which are usually beyond the capabilities of conventional techniques. This is achieved through the time integrated exposures which preconcentrate the contaminants over the specific period of deployment. Further, in passive sampling, the gradual accumulation of analytes into the samplers lead to stabilisation, hence,

the effect of decomposition of the analytes during the transport, storage and the transformation of the analytes during sample enrichment are significantly reduced (Namieśnik et al., 2005). Losses of contaminants through sorption and evaporation are also greatly reduced with passive samplers, since neither tubing nor containers are used, unlike spot sampling and autosamplers. The driving force behind the uptake of substances by passive samplers is the differences in chemical potential between the concentrations of the substance in the passive sampler and the deployed environment. The exchange of substances between the sampler and the sampling environment continues until equilibrium is reached or the sampling process stopped.

Passive sampling techniques are applicable to a wide range of contaminants including the non-polar organics (e.g. PAHs and PCBs), polar organics (e.g. pharmaceuticals and pesticides) and metals (e.g. copper, zinc, nickel) in the aquatic environment. Diffusive gradients in thin films (DGT) are the major PSD used for monitoring metals, mostly the transistion and heavy metals in soils, sediments and water (Zhang and Davison, 1995; Dahlqvist et al., 2002). For the organic compounds, PSDs are distinguished into two major classes: samplers for the non-polar compounds and those for the polar compounds. The semi-permeable membrane devices (SPMDs), a biphasic passive sampler, containing a lipid (triolein) phase in a low density polyethylene (LDPE) membrane were the first non-polar PSD to be introduced (Huckins et al., 1990; Huckins, 2006). Simpler, single phase non-polar PSDs are also available including low density polyethylene (LDPE; Adams et al., 2007), polyoxymethylene (POM; Jonker and Koelmans, 2001) and silicone rubber (SR); polydimethylsiloxane (PDMS) (Rusina et al., 2007; Smedes, 2007). For polar organic compounds, commonly used passive samplers include the polar organic chemical integrative sampler (POCIS; Alvarez et al., 2009) and polar Chemcatcher (Kingston et al., 2000).

Despite the wide varieties of non-polar PSDs, the principles of contaminant uptake are the same. Most non-polar passive samplers are made of a polymeric membrane which is permeable to varieties of non-polar organic chemicals. At deployment of the passive samplers in water, the target contaminants diffuse freely from the sampled medium (e.g. water) to the receiving medium (e.g. the passive sampler) as a result of difference in chemical activities of the target compounds between the two media. The analytes are sequestered and held in an appropriate medium including a chemical reagent (e.g. resin), solvent or porous adsorbents, within the sampler. Contaminant uptake into passive samplers can either be through partitioning (i.e. absorption) or through adsorption (i.e. surface bonding) processes. Generally, the theory of the uptake of contaminants into non-polar samplers is well established (Huckins et al., 2006; Booij,

2007; Mills, et al., 2011; Lohmann et. al., 2012). The uptake of target compounds from water to non-polar passive samplers typically follows three stages: linear or kinetic, curvilinear or intermediate and equilibrium as illustrated in Figure 1-6.

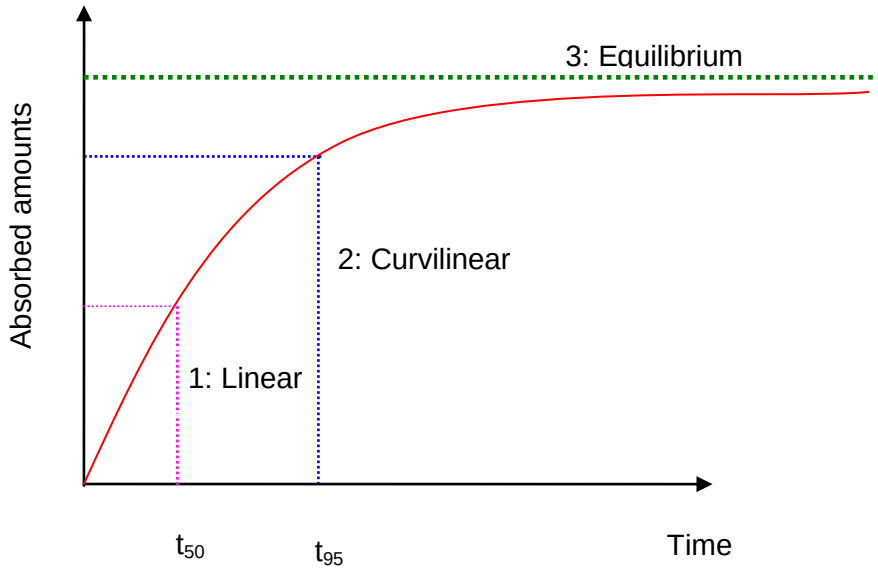


Figure 1-6 Amounts of contaminants absorbed by passive samplers expressed as a function of time. Uptake generally follows: linear, curvilinear and equilibrium model.

In the initial stage of deployment, the uptake of the target compounds (i.e. absorbed amounts; N) by the samplers increases in a linear manner (i.e. kinetic sampling) proportional to the water concentration with time (t), as long as the aqueous concentration (Cw) remains constant (i.e. stage 1; Fig.1-6). At this initial stage, the absorbed amounts do not diffuse back into the water and can be related to the aqueous concentration (C_W) using the sampling rate (R_S; L day^{-1}) of each target compound and the deployment period (t; days) as shown in Equation 1.1:

$$N_t = C_w R_s t \qquad \text{Equation 1.1}$$

During the initial stage, the product $R_S t$ is conceptualised as the volume of water that was extracted by the passive sampler during deployment. As sampling continues, i.e. long exposure times, the contaminant gradually approaches its equilibrium concentration in the sampler (equilibrium sampling). At this stage, (equilibrium sampling), the amount of contaminants absorbed by the passive sampler becomes:

$$N_t = C_w K_{sw} M_s \qquad \text{Equation 1.2}$$

where K_{SW} is the sampler-water partition coefficient

M_S is the mass of the sampler

The product $K_{SW}M_S$ can be interpreted as the volume of water that was extracted by the passive sampler at equilibrium.

Equation 1.3 shows the general equation that controls the uptake of non-polar compounds to passive samplers with time (Huckins et al., 2006; Booij et al., 2007)

$$N_t = K_{sw} M_s C_w \left[1 - exp\left(- \frac{R_s t}{K_{sw} M_s} \right) \right] \qquad \text{Equation 1.3}$$

The use of passive sampling techniques in monitoring aqueous dissolved organic contaminants requires the calibration of data to convert the amount (ng) of contaminants absorbed by the samplers to the water concentrations ($C_{W;}$ ng L^{-1}) of target compounds (Rusina et al., 2009). To achieve this, accurate K_{SW} values and *in situ* sampling rates (R_S) of each target compound should be known. Log K_{SW} values are measured from laboratory calibration studies (e.g. Smedes et al., 2009). Other than the sampler material and the water boundary layer (WBL), Sampling rates (R_S) are also influenced by a variety of environmental conditions such as temperature, water flow, turbulence, the degree of biofouling etc (Booij et al., 2002; Huckins, 2006).

Performance or permeability reference compounds (PRCs) are often used to correct for the large differences in environmental conditions and to determine *in situ* R_S of target compounds. PRCs are analytically non-interfering compounds that are not found in the environment and are added to passive samplers prior to deployment in order to calibrate sampling rates (R_S). Addition of PRCs to sampler prior to deployment can correct for this site to site variability of the uptake rates and allows for the accurate determination of *in situ* R_S of the target compounds(Huckins et al., 2002). The rate of dissipation of PRCs from the samplers is proportional to the uptake of analytes. Hence, the rate of loss of PRCs during deployment can be used to estimate *in situ* sampling rates (R_S) of the target analytes, which can then be used to calculate the water concentrations of the analytes. From the absorbed amount, the aqueous concentration of each target compound with known log K_{SW} and *in situ* R_S can be calculated.

Equation 1.4 is valid for the linear uptake, the curve linear (transition), and the equilibrium stage. The accuracy of the measured C_W depends largely on the accuracy of the log K_{SW} and R_S used.

$$C_W = \frac{N_t}{K_{sw} m \left[1 - \exp\left(-\frac{R_s t}{K_{sw} M_s}\right)\right]}$$ Equation 1.4

1.4.3.1 Silicone rubber passive samplers

Silicone rubber passive sampling devices (SR-PSDs) are single phase polymers that are increasingly applied in monitoring environmental exposures and contamination of trace organic contaminants in the aquatic environment (Schafer et al., 2010; Yates et al., 2011). SR-PSDs are relatively low costs, simple to handle and to analyse; it can be re-used and does not have the complication of an additional trioline phase e.g. in SPMDs. Figure 1-7 shows a typical SR-PSDs mounted on a cage prior to deployment.

Figure 1-7 Silicone rubber passive sampling sheets mounted on a sampler frame

Like other non-polar passive samplers, SR-PSDs absorb freely dissolved organic contaminants from water through a partitioning process. This imitates the uptake of contaminants by aquatic organisms and biomonitors, and hence, are said to “mimic” the bioaccumulation process observed for biota. They do not require the collection, transport or storage of large volumes of aqueous samples, they are also very easy to handle, and hence, samplers can be easily deployed in remote places including turbulent waters.

Whereas most PSDs are relatively selective to contaminants of a particular group, silicone rubber (SR)-PSDs have been shown to be effective in monitoring organic contaminants across a wide range of polarity i.e. octanol-water partition coefficient (log K_{OW}), ~2.3 to ~7.5 (Smedes et al., 2007). However, most studies using SR-PSDs have focused primarily on classical non-polar organic contaminants i.e. PAHs and PCBs in the marine environment. Polar to semi-polar organic compounds including some pesticides and herbicides attain equilibrium with single phase PSDs within hours to days due to their low log K_{OW} values and the sampler's physical dimensions (e.g. large surface area and thin). Therefore, the possibility exist to apply single phase PSDs in monitoring polar to semi-polar organic contaminants at least, for preliminary identification purposes (Lohmann et al., 2012). There is also the need to widen the range of substances that can be assessed using silicone rubber passive samplers. For example, under current monitoring programmes, evaluations of impacts are frequently limited to substances for which agreed environmental quality standards (EQS) are available (e.g. for WFD priority substances, Decision No 2455; EC, 2001).

1.5 Biological effects analytical techniques in water quality monitoring

The vast numbers of anthropogenic and naturally occurring organic contaminants with diverse polarities that are released into the aquatic environment present a considerable challenge in water quality monitoring. The use of chemical analytical techniques in their identification is expensive and may not be realistic. Therefore, most water quality monitoring programmes focus on measuring just a portion of these contaminants e.g. the priority contaminants. It is now widely recognised that the use of chemical analytical techniques alone in environmental water quality monitoring are often inadequate in identifying all the compounds with the potential to impact our environment and pose an ecological hazard. In addition to these, contaminants exist as complex mixtures with possibilities of interactions e.g. addition and synergy. Additional or alternative techniques are therefore required to compliment the use of chemical analytical methods for a reliable and cost effective water quality monitoring. Further, the combined toxicity of the known and unknown contaminants may still compromise water quality even when the total concentrations of the individual compounds are within the limit of EQSs (Schwarzenbach, et al., 2006).

For these reasons biological analytical techniques (bioassays) have become an essential tool for evaluating the potential damage of contaminants on organisms. Generally, biological effects technique assesses whether or not a compound has the potential to be toxic to organisms, and if so to what degree. This technique is very relevant in water quality monitoring and in environmental regulatory programmes since it can, on occasion, be used to demonstrate a link between contaminants and their ecological responses in biota. Biological effects techniques can also be focused on specific effects e.g. endocrine disruption, and can be used to identify exposure to a group of substances with common biological effects, therefore, direct the use of more specific chemical analytical techniques. It can also be used to indicate the presence of substances, or combination of substances, that hitherto were not identified as being of ecological concern using chemical analytical techniques. In addition, unlike chemical analytical techniques, biological effects analytical technique can provide a measure of the combined effects of mixture of toxic compounds present in the aquatic environment. However, they do not usually provide the identity of the compounds that are responsible for the biological effects. Many biological analytical techniques have been derived from conventional toxicity testing with whole organisms (*in vivo)* or using cells or molecular (*in vitro*) bioassays.

1.5.1 *In vivo* toxicity testing

Conventional techniques for the toxicity assessment of chemicals often involve the use of live aquatic organisms such as the water flea (*Daphnia magna)* or the zebra fish (*Danio rerio)*. Known numbers of the live aquatic organism *are* exposed to increasing concentrations of an individual test chemical. The endpoints that are often measured include mortality, growth impairments and reproductive abnormalities on the test organisms, through determination of the median lethal concentration (LC_{50}) or median effective concentration (EC_{50}) (OECD, 1992a; Braunbeck et al., 2005). This testing approach, which forms the basis of regulatory decisions in environmental and chemical risk assessment, has been widely criticised, on the basis of ethical concerns, high cost and usage of resources e.g. time, test species, and chemical (Castaño et al., 2003). The new European Union REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) initiative requires toxicity testing of thousands of previously untested substances (EC, 2007); this entails more animals will be used to evaluate the risk of chemicals to human health and the environment. Further, the environmental significance of death of organisms after short-term exposure to high concentrations of chemicals is questionable (Nagel, 2002). Extrapolation of *in vivo* toxicity data from aquatic organisms to humans is not realistic. There are major differences in toxicokinetics (i.e. absorption, metabolism and excretion) between the two organisms. *In vivo* toxicity testing is whole organism dependent, the mode and mechanisms of the effects of contaminants on the cells of the test organisms are unknown (Hoffman et al., 2003). Importantly, it fails to address the fact that in the real environment, aquatic organisms and humans are often exposed to lower concentrations of the freely dissolved contaminants in complex mixtures.

1.5.2 *In vitro* toxicity testing

Cells are the basic building blocks of all life and they represent a vital level of organisation for identifying and understanding general and distinctive mechanisms of toxicity (Castaño et al., 2003, Davoren et al., 2005). In *in vitro* toxicity tests, specific responses are examined directly on cells or molecules (e.g. enzymes) following exposure to the test chemical. Therefore, it is very suitable for the assessment of cellular biochemistry and cellular mechanisms in the overall toxic response. Chemical interactions within an organism occur at the cellular level. Therefore, cellular responses are the first manifestation of biological effects and are the suitable tools for the early and sensitive detection of chemical exposure and effects (Fent, 2001).

Two types of cell cultures are routinely used for *in vitro* bioassays: primary and continuous cell lines. The primary cultures are isolated and cultured directly from the animals and they retain the

differentiated characteristics of the parent animal for a short period e.g. days to weeks. The major advantage of a primary culture is their ability to retain characteristics of the more complex *in vivo* condition of the donor organism. However, isolation and maintenance of the cells is laborious and challenging. There are also often differences in the physiological performance between cells isolated from different donor animals (Mothersill and Austin, 2003). Permanent or continuous cell lines are cells that have acquired the ability to divide and reproduce indefinitely through random mutation or systematic modification. They are produced by initially preparing primary cultures; if the primary cultures can be successfully reproduced then a cell line is obtained. Most fish cell lines are available from cell banks, such as the European Collection of Animal Cell Cultures (ECACC) or the American Type Culture Collection (ATCC).

In vitro toxicity testing with primary or permanent cell cultures are a very valuable, time and cost efficient and effective alternative to the use of whole living animals (*in vivo* procedures). Cell cultures can be exposed to large numbers of test compounds in miniature cell culture plates and assessed in high throughput systems. In addition, *in vitro* toxicity assays enhances standardisation of toxicity assessment systems. Fish and mammalian derived cell cultures have been extensively applied in the study of short term totoxicity of chemicals and environmental contaminants. They offer numerous advantages including higher throughput and standardisation e.g. using multititre plates (Castaño et al., 2003; Mothersill and Austin, 2003). However, whole organisms have some level of defence and metabolism mechanisms against xenobiotics, which cell cultures do not have. This may result in the manifestation of biological effects at lower concentrations in *in vitro* than in *in vivo*.

1.5.3 Toxicity testing of organic contaminants

A number of toxicity testing methods have been developed to assess the toxicity potential of chemicals. However, most toxicological assessment studies have often focused on the acute effects of individual chemicals or class of chemicals (e.g. OECD, 1992a) and not the complex mixtures or dissolved concentrations, therefore, may lack environmental relevance. Toxicity testing for organic contaminants is often biased due to poorly defined and declining exposure concentrations. It is often difficult to dissolve hydrophobic organic compounds (HOCs; e.g. PAHs and PCBs) in water or culture medium due to their low aqueous solubility; it is also challenging to maintain a defined and stable concentration in the assay system due to volatilisation, adsorption and absorption to the plastic surfaces and biological matrices thereby leading to poorly defined exposure and low sensitivity (Schirmer et al., 2008; Smith et al., 2009). Interpretation of toxicity test results may be hampered when there are doubts about the actual exposure concentrations (Brown et al., 2001). Further, toxicity test of HOC mixtures give rise to

another difficulty, as the concentrations of individual compounds decreases variably, hence the mixture composition can change during the test. Co-solvents including methanol or dimethyl sulfoxide (DMSO) may increase the solubility of poorly soluble compounds. However, as illustrated by Avdeef et al. (2007), during toxicity tests, co-solvents may interact with the test compounds and interfere with the biological activity of assay systems (Hutchinson et al., 2006; Tanneberger et al., 2010). Thus, toxicity profiles such as LC_{50} and EC_{50} obtained from these procedures are frequently subject to controversy (Malins and Ostrander, 1994). For realistic *in vivo* or *in vitro* toxicity testing of organic contaminants, particularly, HOCs and their mixtures, novel partitioning controlled delivery (PD) systems promise a stable and better defined exposure concentrations in such tests.

1.5.3.1 Passive dosing systems

Passive dosing involves the partitioning of chemical compounds from a dominating source, usually a biologically inert polymer phase (such as silicone rubber), to the test medium. This ensures a gradual and stable release of accurately controlled concentrations of the test compound(s) to the testing system and compensates for compound losses, resulting in constant freely dissolved exposure concentrations. In addition, PD also helps to eradicate the need of dosing with co-solvents e.g. methanol or dimethyl sulphoxide (DMSO). The working principle of passive dosing is similar to the passive sampling technique, instead of sequestering the substances into the sampling material over a time period, passive dosing material acts as storage and source of the test substance(s) during toxicity testing. It then gradually and steadily releases the test substance(s), as a result, maintaining a known and stable concentration of test substance throughout the test period.

Various passive dosing procedures and formats have been investigated in recent years to overcome the limitations and challenges in toxicity testing of HOCs (Brown et al., 2001; Kiparissis et al., 2003; Mayer and Holmstrup 2008). Smith et al. (2009) have shown that silicone rubber O-rings (Fig. 1-8), a special format of polymethyl siloxane (PDMS) silicone rubber, can be used as passive carriers and regulators of stable dissolved concentrations of HOCs in toxicity assays. This passive dosing system can serve as an effective means to maintain a stable and accurate concentration of HOCs during biological effects assessments in a miniature system.

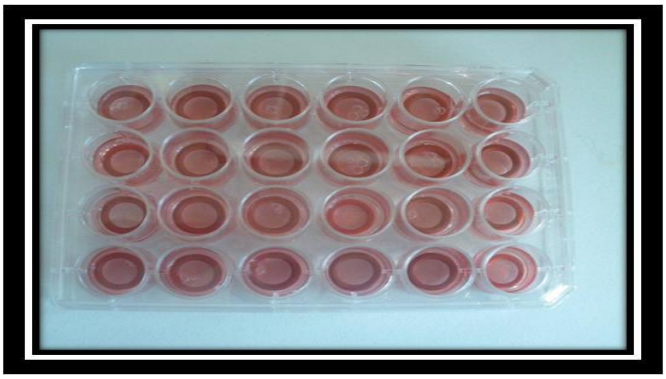


Figure 1-8 Silicone rubber O-rings in a 24 well multititre plate and cell culture medium

1.6 Aims and structure of study

The above literature review has demonstrated that conventional techniques in water quality monitoring and biological effects testing of organic contaminants do not reflect *in situ* conditions: i.e. exposures to complex mixtures of freely dissolved concentrations of a wide range of compounds at trace or ultra-trace concentrations. Silicone rubber passive sampling devices (SR-PSDs) have shown to be reliable and efficient in monitoring the freely dissolved concentrations of a variety of organic contaminants in water. While passive dosing techniques promise to overcome many of the challenges in toxicity testing of organic contaminants and their mixtures. Despite their numerous advantages, applications of passive sampling and dosing techniques in routine and regulatory water quality monitoring and toxicity testing of organic contaminants are still scant. The techniques require further validation studies and refinement so as to gain wider acceptance. Studies are still urgently required to broaden the range of applications of passive sampling techniques e.g. in the evaluation of the toxicity of mixtures of chemicals at environmentally relevant concentrations and expansion of the range of substances that can be monitored using PSDs. The overall aim of this study was to investigate the feasibility of silicone rubber passive sampling devices (SR-PSDs) as an alternative monitoring technique for a wide range of freely dissolved organic contaminants in streams, rivers, estuaries and coastal waters within Scotland. The study also aimed to circumvent the challenges associated with identifying the risks posed by mixtures of contaminants by coupling the enrichment of contaminants through SR-PSDs with the biological effects assessment of the extracts using a variety of *in vitro* and *in vivo* bioassays. In line with these aims, seven selected tasks undertaken during the course of this study are presented herewith in the chapters.

This chapter (i.e. **Chapter 1**) introduces and underpins the rationale behind the study and includes the overall aims of the study. It also provides an overview of the major aspects of the study including selected organic contaminants, bioavailabilty, techniques in water quality monitoring, passive sampling and passive dosing techniques.

Chapter 2 presents the first field application of SR-PSDs in determining environmental exposures of dissolved organic contaminants (i.e. pesticides and acid/urea herbicides, PAHs and PCBs) in water at five sites within the River Ythan catchment in North East Scotland, UK. In addition to chemical analysis, extracts from the SR-PSDs deployed in water at the five sites were screened for their potential to elicit combined cytotoxicity and cytochrome induction on a permanent fish cell line (RTL-W1) obtained from rainbow trout liver (*Oncorhynchus mykiss*). This has been published as a paper, *Chemosphere 2013,* 90, 210–219.

Chapter 3 details the utility of SR-PSDs as an alternative technique to conventional water sampling in routine environmental monitoring and screening for a wide range of organic contaminants in streams associated with agricultural activities. The study involved the comparison of the field application of the silicone rubber passive sampling technique with conventional automated composite sampling for a range of pesticides and herbicides, and also presents data for PAHs and PCBs in the same passive sampler extracts. This has been published as a paper, *Science of the Total Environment 2013,* 445–446, 261–272

Chapter 4 presents the use of SR-PSDs and an algal growth inhibition assays in evaluating the impacts of anthropogenic activities in the Forth estuary and coastal waters of the Firth of Forth Scotland, UK. The study presents the dissolved concentrations of selected organic contaminants in water from the Forth estuary and Firth of Forth and their combined toxicity on sensitive native organisms. This has been published in the journal of *Science of the Total Environment* 2013, 461-4620, 230-239

Chapter 5 describes the reproductive, developmental and algal toxicity of dissolved mixtures of organic contaminants in water from the Ythan catchment using *in vivo* bioassays i.e. fish embryo toxicity test (FET) and algal growth inhibition assays. This has been submitted for publication, *Environmental Science and pollution research*

Chapter 6 provides the summary of the results and overall discussion. It also includes suggestions for way forward that are indicated by the results presented here.

Chapter 7 is the appendices which include mostly the supporting information for most of the chapters and outlines the preliminary use of silicone rubber O-rings as a passive dosing format in testing the cytotoxicity and cythochrome induction potentials of some selected PAHs on a fish cell line. This study was presented as a platform presentation at the 23rd Annual Meeting of the Society of Environmental Toxicology and Chemistry (SETAC) Europe, Glasgow, UK, 12-16 MAY 2013.

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2 CHAPTER TWO: Investigating the significance of dissolved organic contaminants in aquatic environments: coupling passive sampling with in vitro bioassays

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Abstract

We investigated the feasibility of coupling passive sampling and *in vitro* bioassay techniques for both chemical and ecotoxicological assessment of complex mixtures of organic contaminants in water. Silicone rubber passive sampling devices (SR-PSDs) were deployed for 8–9 weeks in four streams and an estuary of an agricultural catchment in North East (NE) Scotland. Extracts from the SR-PSDs were analysed for freely dissolved hydrophobic organic contaminants (HOCs) and screened for wide range of pesticides. The total concentrations of dissolved PAHs (PAH_{40}, parent and branched) in the water column of the catchment varied from 38 to 69 ng L^{-1}, whilst PCBs (PCB_{32}) ranged 0.02–0.06 ng L^{-1}. A number and level of pesticides and acid/urea herbicides of varying hydrophobicity (log K_{OW}s ~2.25 to ~5.31) were also detected in the SR extracts, indicating their occurrence in the catchment. The acute toxicity and EROD induction potentials of SR extracts from the study sites were evaluated with rainbow trout liver (*Oncorhynchus mykiss*; RTL-W1) cell line. Acute cytotoxicity was not observed in cells following 48 h exposure to the SR extracts using neutral red uptake assay as endpoint. But, on a sublethal level, for every site, statistically significant EROD activity was observed to some degree following 72 h exposure to extracts, indicating the presence of compounds with dioxin-like effect that are bioavailable to aquatic organisms in the water bodies of the catchment. Importantly, only a small fraction of the EROD induction could be attributed to the PAHs and PCBs that were determined. This preliminary study demonstrates that the coupling of silicone rubber passive sampling techniques with *in vitro* bioassays is feasible and offers a cost effective early warning signal on water quality deterioration.

Keywords

Passive sampling
Silicone rubber
Hydrophobic organic contaminants
Water
Monitoring
Toxic equivalency (TEQ)

2.1 Introduction

Hydrophobic organic contaminants (HOCs), alongside other environmental stressors, are major pressures on the ecological and chemical status of many European freshwater and marine water bodies. Numerous HOCs are persistent, bioaccumulative, and have the potential to induce both acute and chronic toxicological effects on aquatic organisms and humans (Warren et al., 2003). Monitoring of HOCs such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and pesticides of varying chemical composition is a major activity under a number of obligatory monitoring programmes including the EU Water Framework Directive (WFD; EC, 2000); Marine Strategy Framework Directive (MSFD Directive *2008/56*; EC, 2008) and the Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR Commission, 2011).

The limitations of traditional water monitoring techniques have been widely recognised (e.g. Booij et al., 2003; Vrana et al., 2005) and many statutory monitoring programmes are now embracing or investigating other methods of assessing HOCs in the aquatic environment, including biota monitoring and the use of passive sampler devices (Covaci et al., 2005; Greenwood et al., 2009; MacGregor et al., 2010). Much work is still required on the integration and refinement of these alternative methods of monitoring HOCs. Work is also urgently required to widen the range of substances that can be assessed, as under current monitoring regimes evaluations of impacts are frequently limited to substances for which agreed environmental quality standards (EQSs) are in place (e.g. for WFD priority substances, Decision No 2455; EC, 2001).

The combination of accurate chemical data with biological effects measurement can improve risk assessment for aqueous organic contaminants; this is particularly true where complex mixtures of widely varying compounds occur and where interactions amongst the components are possible. Further, understanding the biological availability and interaction of complex mixtures of HOCs in the environment and in biological systems is crucial in predicting their toxicological impacts in those systems.

As an alternative to traditional bottle sampling and monitoring techniques, passive sampling techniques can provide time-weighted average concentrations (C_{TWA}) of freely dissolved aqueous contaminants (i.e. bioavailable). Essentially, passive sampling involves the free flow of contaminants from water, to the receiving medium, e.g. the passive sampler, which is driven by differences between the two media in terms of chemical activities. The exchange of the contaminants continues until equilibrium is reached in the system or the sampling is discontinued. Compared to spot sampling, much lower limits of detection (LOD) are attained with passive sampling techniques through sampling a large volume of water over the extended deployment period i.e. days to months.

A variety of passive sampling devices (PSDs) exist, including the semi-permeable membrane device (SPMD; Huckins, 2006), polar organic chemical integrative sampler (POCIS; Alvarez et al., 2004), low density polyethylene (LDPE; Adams et al., 2007) and silicone rubber (Smedes, 2007). Silicone rubber (SR) passive sampling devices (PSDs), have been shown to be effective in monitoring organic contaminants across a wide range of polarity, i.e. octanol-water partition coefficient (log K_{OW}) over the range 3-8 (Smedes, 2007) and the extraction and clean-up steps are straightforward compared to bi-phasic PSDs, e.g. SPMDs. These properties, in addition to their low cost and relative ease of handling and analysis, makes them ideal for application in an integrated chemical-biological effect analysis of environmental samples.

Polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) as well as some planar PCBs and PAHs (referred to as dioxin-like compounds, DLCs) exert their toxic effects on aquatic organisms by the same mechanism of action; mainly through the initial binding to the soluble receptor protein known as the aryl hydrocarbon receptor (AhR). This initiates several biochemical effects, including the induction of cytochrome P450 1A (CYP1A) (Stegeman et al., 2001).

Evaluation of CYP1A induction in aquatic organisms has proven to be a sensitive biomarker of organic contaminants in the aquatic environment and can be routinely assessed in various ways, such as immunoblotting or measuring the activity of 7-ethoxyresorufin-*O*-deethylase (EROD) in various organisms and test systems (Hahn et al., 1996; Hallare et al., 2011). DLCs usually occur in the environment as complex mixtures with several other potentially toxic compounds. The dioxin-like toxic potencies of complex mixtures can be expressed in terms of toxic equivalency (TEQ) to the reference compound; 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). The TEQ can be estimated directly from bioassays but is more routinely calculated from the individual compounds in the complex mixtures and their toxic equivalent factors (TEFs) or relative potency (REP) (Van den Berg et al., 2006; Olsman et al., 2007). When using the TEQ approach for risk assessment the process is still restricted to the suite of chemicals analysed. Using bioassays with EROD as the endpoint may determine the joint effects of all DLCs present in complex environmental samples, although the presence of some compounds may inhibit the induction of EROD activity. Importantly, bioassay is often a cheaper and more rapid estimate of contaminant exposure than chemical analysis, and can be used to detect the presence of DLCs at concentrations below the LODs of chemical methods (Thain et al., 2008).

Possibly, the quantified contaminant concentrations in PSDs deployed in water can be linked to biological effects determined via concurrent toxicity assays and/or measurement of CYP1A induction *in vitro*. This is supported by a previous work, as Bauer (2008) applied SR-PSD extracts in *umu*C (DNA damage) bioassays and assessed the genotoxicity of the water in which the sampler had been deployed. Unlike the SPMDs and POCIS (e.g. Muller et al., 2006; Rastall

et al., 2006; Alvarez et al., 2008), to date, there is still a paucity of studies that have integrated SR-PSD with *in vitro* toxicity testing of environmental samples as part of water quality assessment.

The specific objectives of this preliminary study were: (1) to investigate the feasibility of integrating SR-PSD and *in vitro* bioassays for chemical and biological effect analysis of aqueous organic contaminants; (2) to quantify the dissolved concentrations in water (C_W) of PAHs and PCBs and to investigate if selected pesticides and acid/urea herbicides could be detected in four streams and an estuary draining an agricultural catchment; (3) to evaluate the toxicological effects of complex mixtures extracted from deployed SR-PSDs on a fish cell line using neutral red uptake (NR) and EROD assays as endpoints; (4) to estimate the TEQ values of the mixtures.

2.2 Materials and methods

2.2.1 Chemicals and materials

HPLC grade solvents (acetone, methanol, dichloromethane, ethyl acetate, *iso*-hexane, toluene and acetonitrile) were purchased from Rathburn Chemicals Ltd., Scotland, United Kingdom (UK). Certified custom made solutions of PAHs (including deuterated PAHs) and PCBs were obtained from QMX Laboratories, Essex, UK. All chemicals and biological reagents used for the neutral red and EROD induction assays were obtained from Sigma-Aldrich, Deisenhofen, Germany unless stated otherwise. AlteSil® translucent food grade SR sheet with a thickness of 0.5 mm and a dimension of 30 × 30 or 60 × 60 cm was purchased from Altec Products, Ltd., Cornwall, UK. The SR sheets were cut to a dimension of 6 × 9 cm and pre-extracted in hot ethyl acetate for >100 h using a Soxhlet apparatus (Laboratory Glass Specialists BV, Ubenna, Netherlands). This removed low molecular weight silicone SR oligomers that might affect instrumental analysis and bioassays. Glass solid-phase extraction (SPE) C8 columns were supplied by Mallinckrodt Baker, London, UK. Ultra-pure water (18.2 MΩ cm) was used throughout the experiment.

2.2.1.1 Preparation of silicone rubber passive samplers

Pre-extracted SR sheets were split into two batches, one for chemical analysis and the other for biological effect assessments (bioassays). The SR sheets for bioassays were thoroughly rinsed with ultra-pure water to remove any trace of chemical solvent. The SR sheets for chemical analysis were spiked with a mixture of performance reference compounds (PRCs)

including deuterated PAHs (D12-chrysene, D12-benzo[*e*]pyrene, D10-fluorene and D10-fluoranthene) and chlorinated biphenyl congeners (CBs 10, 14, 21, 30, 50, 55, 78, 104, 155, and 204) by equilibrating in a methanol/water spiking solution (Booij et al., 2002). PRCs are a group of non-environmentally occurring compounds and their release during deployment enables determination of *in situ* sampling rates (R_S). The SR sheets for chemical and bioassay analyses were kept in amber coloured jars with lids lined with aluminium foil and stored at -20 °C until required.

2.2.2 Sampling locations and passive sampling

2.2.2.1 Sampling locations and site descriptions

The study was conducted in the Ythan catchment in north east (NE) Scotland, which has an area of ~675 km^2. Approximately 85% of the catchment is utilised for agriculture (mixed farming). The area is sparsely populated and there are no known major industrial facilities in the vicinity. Four stream locations and an estuary site were targeted for study (Fig. 2-1). Site 1 was at the headwaters of the River Ythan (the main river within the catchment); sites 2 and 3 were on a small tributary and were approximately 3.3 km apart; site 4 was on the River Ythan just above the tidal limit; and site 5 was located in the estuary of the river. The catchment was selected for this study on the basis of preliminary ecology and chemical assessments at sites 2 and 3, which indicated a degree of pesticide influence. Site 4 was close to the largest town in the catchment and all of the other study sites were in the vicinity of moderate, rural vehicular road usage. At sites 2 and 3, the minimum and maximum temperature and pH values during the sampling period where 1.31 and 7.10 °C and 6.70 and 7.01; at the estuary, the mean salinity was 15 PSU, mean temperature was 4.1 °C, and mean pH was 7.5.

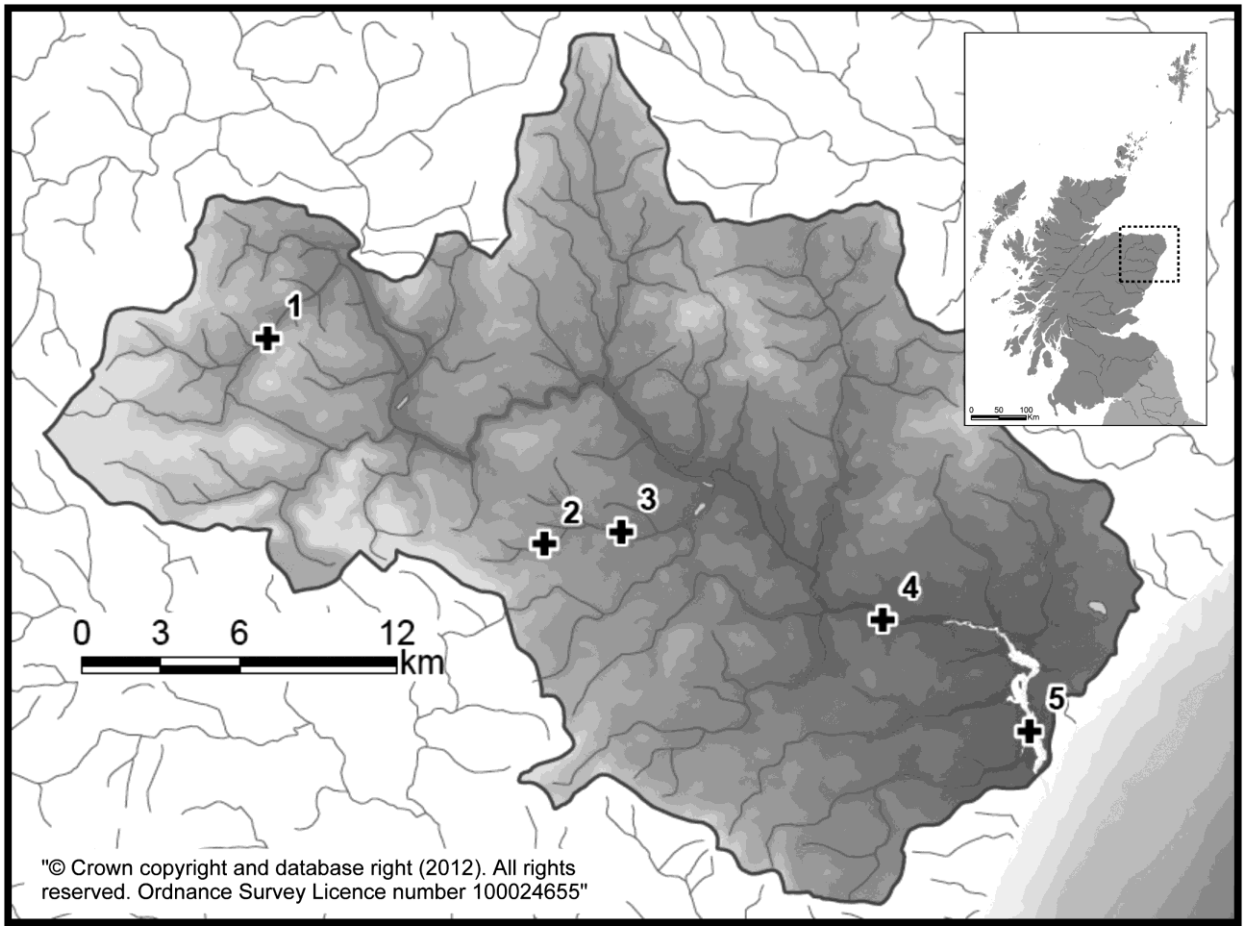

Figure 2-1 River Ythan catchment (showing River Ythan and main tributaries) in NE Scotland. Sample sites 1-5 are indicated. Inset shows map of Scotland and location of the catchment.

2.2.2.2 Passive sampling

The procedures for the preparation, deployment, retrieval and extraction of SR-PSDs in this study followed Smedes (2007). A SR sampler consisted of six sheets weighing ~20 g in total and had a surface area of 600 cm^2. One PRC-spiked and one un-spiked sampler were deployed simultaneously at the five sites from November 2010 to January 2011. Samplers were deployed for 65 days at sites 1, 2, 3 and 4, and for 58 days at site 5. Upon retrieval, sampler surfaces were gently and rapidly wiped using solvent-free household cleaning pads and water from the study sites in order to remove any biofouling. Sets of PRC-spiked SR samplers served as field and production control blanks and for time zero determination of PRC loss. The field blanks were similarly taken to the study sites during deployment and retrieval; but were only exposed to air (i.e. not submerged in water) and the production blanks were kept in the laboratory. A separate set of SR samplers for bioassay controls were kept in ultra-pure water in the laboratory during the entire sampling period. This served as control blank for the bioassays. Once

retrieved, SR samplers for chemical analysis and bioassays were kept separately in amber coloured jars with lids lined with aluminium foil and stored at -20 °C until required.

2.2.3 Extraction of silicone rubber passive sampling devices (SR-PSDs)

2.2.3.1 Extraction of SR-PSDs for chemical analysis

Extractions of SR samplers were performed with Soxhlet apparatus for 24±4 h in hot mixture of acetonitrile (ACN):methanol (MeOH; 2:1 v/v). The design of this apparatus ensures that the sheets are continuously submerged in sub-boiling solvent throughout the extraction period. Prior to extraction, known amounts of deuterated internal standards (D8-naphthalene, D10-biphenyl, D8-dibenzothiophene, D10-anthracene, D10-pyrene, D12-benzo[*a*]pyrene and D14-dibenzo [a,h]anthracene), a PCB recovery standard (CB112) and pesticide internal standards (azobenzene and diphenamid) were added to each Soxhlet apparatus containing each set of the SR samplers. The choice of azobenzene and diphenamid as internal standards for pesticides/herbicides was because they are not prevalent in the environment and they are stable and similar to other components in the suite of analysis. Inclusion of low molecular weight PAH internal standard (i.e. D8-naphthalene) to the Soxhlet extraction system was to correct for possible loss of low molecular weight HOCs e.g. naphthalene. After extraction, samples were reduced to ~2 mL via Kuderna-Danish evaporation apparatus (Laboratory Glass Specialists BV, Ubenna, Netherlands). Subsequently, extracts were added to glass solid-phase extraction (SPE) C8 columns and eluted with ACN to remove any co-extracted SR oligomers. The samples in ACN were further concentrated to ~2 mL and were solvent exchanged into *iso*-hexane. The extracts were aliquoted into three equal fractions for determination of (1) PAHs, (2) PCBs, and (3) selected pesticides and acid/urea herbicides.

2.2.3.2 Extractions of SR-PSDs samplers for bioassays

Two sheets from each set of six deployed at each site for bioassay use, together with the laboratory process blanks, were extracted using the hot Soxhlet apparatus as explained in section 2.2.3.1 but without any added internal or recovery standards. The other four sheets from each set were preserved for future toxicological assessment. Following extraction, extracts were purified using the glass solid-phase extraction (SPE) C8 column, solvent exchanged into MeOH and concentrated to ~1 mL with Kuderna-Danish evaporation apparatus and activated carbon purified nitrogen blow down. Bioassay results are expressed relative to the equivalent mass of SR per mL extract (mg SREQ mL^{-1}). The extracts were stored at -20 ºC until needed for

bioassays. Extracts from blank samplers were utilised to confirm that the extraction procedure and extraction solvents were not inherently toxic to cell systems during bioassays.

2.2.4 Chemical analysis of silicone rubber extracts

Extracts of SR samplers were analysed for 40 PAHs (parent and branched), 32 *ortho* and mono-*ortho* PCBs and several selected pesticides and acid/urea herbicides using a combination of GC and liquid chromatography (LC). The detectors applied include mass spectrometry (MS), electron capture detector (ECD), and MS-MS. Detailed procedures for the analysis, including the complete list of pesticides and acid/urea herbicides selected for this study are provided in the Supporting information i.e. appendix 3 (supporting information for chapter two).

2.2.4.1 Calculation of sampling rate and freely dissolved concentrations of HOCs in water

To calculate the dissolved concentrations (C_W; ng L^{-1}) of the analytes from the amounts absorbed by the samplers during deployment, the sampler water partition coefficients (K_{SW}) and sampling rates (R_s, L d^{-1}) are required. The K_{SW} values for most of the compounds used in the current study were obtained from Smedes et al. (2009). The uptake of organic compounds and release of PRCs by passive samplers is principally controlled by the resistance to transport in the water boundary layer (WBL) and the sampler material (Huckins, 2006). However, compared to the WBL, the resistance to transport of the sampler material was found negligible on SR samplers for compounds with log K_{ow} >3 (Rusina et al., 2007). As the molecular weight of the sampled compounds increases, the diffusion and transport through the WBL decreases and consequently the R_S decreases. Hence, in this situation, where uptake is entirely dependent on the thickness of the WBL, Rusina et al. (2010) proposed a model which expresses R_S as a function of molecular mass (M) of the compounds

$$R_S = FAM^{-0.47} \quad (2.1)$$

where *A is* the surface area (m^2) of the sampler and *F* is the flow proportionality constant, which includes the flow dependence sampling rate and factors to fit the units. This flow proportionality (F) constant is derived from the release of PRCs loaded to the samplers prior to deployment. The fraction (f) of PRCs retained in the SR samplers after deployment is related to the sampling rate (R_S) through:

$$f = \frac{N_t}{N_0} = exp\left[-\frac{R_{st}}{mK_{SW}}\right] = exp\left[-\frac{FAM^{-0.47}t}{mK_{SW}}\right] \quad \text{.....} \quad (2.2)$$

where N_0 is the initial amount (ng) of PRC in the sampler, N_t the final amount (ng) remaining in the deployed SR sampler, t is the exposure time (d), and m is the mass (kg) of the SR sampler. By applying non-linear least-squares (NLS) regression with f as function of $FAM^{-0.47}$, the modelled f values can be fitted with the experimental values using the proportionality constant (F) as the adjustable variable as detailed in Booij and Smedes (2010).

Using the spreadsheet supplied by the authors, an estimate of the R_S and the standard error of the target compounds were obtained by applying the deviations of the experimental value from the model. The PRC derived R_S values for an average compound of mass 300 in L d^{-1} were 47±5.7 for site 1, 14±2.0 for site 2, 29±4.2 for site 3; 32±14 for site 4, and 36±5.2 for site 5. Graphs of the obtained fits can be found in Supporting information (SI; Fig. 3.1).

Conventionally, the C_W can be calculated by applying the uptake model that is valid for equilibrium and linear uptake situations (Huckins, 2006):

$$C_W = \frac{N_t}{mK_{SW}} \frac{1}{1-\exp\left(-\frac{R_s t}{mK_{SW}}\right)} \quad (2.3)$$

Where N_t is amount (ng) of target compound absorbed by the SR sampler during exposure; by combining Eqs. (2.2) and (2.3):

$$C_W = \frac{N_t}{mK_{SW}\left[1-exp\left(-\frac{FAM^{0.47}t}{mK_{SW}}\right)\right]} \quad (2.4)$$

In this study, the uptake of PAHs were sufficiently high, so production and field blanks were insignificant (<10%), hence, no corrections were needed. However, the absorbed amounts of PCBs were very low and consequently closer to amounts in the production and field blanks. Dealing with blanks is not a straight forward subtraction in passive sampling, for example a production blank will not influence the amount on the sampler for compounds that attained equilibrium, but needs to be fully subtracted for compounds that stay in the linear uptake phase during the whole deployment (Booij et al., 2007). Further, production blanks dissipate like PRCs during sampling, hence, from Eq. (2.4) a correction for production blank can be included (Smedes and Booij, 2012):

$$C_W = \frac{N_t - N_0 \exp\left(-\frac{FAt}{m K_{SW} M^{0.47}}\right)}{m K_{SW}\left(1 - \exp\left(-\frac{FAt}{m K_{SW} M^{0.47}}\right)\right)} \quad (2.5)$$

where N_0 is the initial amount (ng) of target compound in the production blank. Calculated concentrations (ng g^{-1} SR) that were less than twice the production blank were considered below the detection limit and marked accordingly. The whole data were included when summing the compounds.

2.2.5 Biological effects analysis of silicone rubber extracts

2.2.5.1 Cell culture

The fibroblast-like permanent fish cell line, RTL-W1 cells was used in bioassays. The choice of RTL-W1 cell line was due to its high potential to express CYP1A-based EROD activity on exposure to dioxin-like compounds (Lee et al., 1993; Hallare, et al., 2011). RTL-W1 cells (Drs. N.C. Bols and L. Lee; University of Waterloo, Canada) were cultured in 75 cm^2 plastic culture flasks (TPP, Trasadingen, Switzerland) without additional gassing at 20 °C in Leibowitz's L15 medium supplemented with 9% foetal bovine serum (FBS) and 1% penicillin/streptomycin solution (10,000 U/10,000 µg mL^{-1}) in 0.9% sodium chloride (NaCl).

2.2.5.2 Neutral red uptake assay

Acute cytotoxicity of SR (deployed and controls) extracts and the vehicle control on RTL-W1 cells were assessed with neutral red uptake assay as detailed in Borenfreund et al. (1988) and Seiler et al. (2006). In this study, the procedure was adapted to a 24-well microtitre plate (TPP, Trasadingen, Switzerland). Each SR extract (1.04-66.7 mg SREQ per mL) and vehicle control (methanol; highest concentration = 1% v/v) were tested on individual plates. Each sample and dilution was tested in duplicate. The positive control used for each test was 3,5-dichlorophenol (DCP; highest concentration = 40 mg L^{-1} of medium) in each test plate. DCP was also tested separately on an individual plate at concentrations in the range 0.63-40 mg L^{-1} of medium. Duplicate negative control wells (with cells but without test extracts/vehicle control) were located both near the samples with the highest concentrations and those with the lowest concentrations. Four further wells were left blank with neither solvent nor cells. Neutral red uptake (cell viability) was determined photometrically at 540 nm with a reference wavelength of 690 nm using Infinite M200 multiwell plate reader (Tecan, Crailsheim, Germany).

2.2.5.3 EROD induction assay

The CYP1A induction potentials of SR extracts from each of the study sites were assessed using the EROD assay. The details of the procedure have been previously described in Gustavsson et al. (2004) and Wölz et al. (2011); in this study, the procedure was modified and optimised to a 24 well microtitre plate. Two plates were used for each test, one plate for the samples and positive control (2,3,7,8-tetrachlorodibenzo-*p*-dioxin, TCDD; Promochem, Wesel, Germany) and the other plate for the measurement of resorufin and protein calibration standard curves. Each test plate had five concentration levels of SR extracts (4.17-66.7 mg SREQ per mL) or vehicle control (methanol; highest concentration 1% v/v) and TCDD (3.13 - 50 pM). Wells with cells but without solvent or extracts were used as negative controls (NC) and another two blank wells contained neither cells nor test compound. All tests and dilutions were conducted in duplicate. EROD activity was measured fluorimetrically at an excitation and emission wavelength of 544 and 590 nm using an Infinite M200 plate reader (Tecan, Crailsheim, Germany). The protein concentrations were determined fluorimetrically in parallel using the fluorescamine method at excitation and emission wavelengths of 360 and 465 nm (Lorenzen and Kennedy, 1993), according to the protocol detailed in Hollert et al. (2002). No cytotoxicity was observed in response to methanol (vehicle control; maximum 1% v/v).

2.2.6 Data analysis of biological effect assessment and calculation of Chem-TEQ and Bio-TEQ values

2.2.6.1 Data analysis of neutral red and EROD assays

In both the neutral red uptake and EROD assays, the average readings for blank wells were subtracted from the values obtained for the test wells. With the neutral red uptake assay, each test was considered valid if the two sets of negative controls did not differ by more than 20% from each other. Statistical analyses were performed using a one-way analyses of variance (ANOVA) followed by Dunnett's and Tukey's multiple comparison tests. Extracts were considered cytotoxic if the ANOVA and the multiple comparison tests with mean values for two or more consecutive concentrations were significantly ($p < 0.05$) higher than the two negative controls and the lowest concentration imposed. Where possible, Boltzmann sigmoidal concentration-response curves (with variable slopes) were fitted to the mean (±SD) viability of four replicates at each exposure concentration using Graphpad Prism 5.0 (GraphPad, San Diego, USA). Viability of the exposed cells was expressed relative to the NC and the cytotoxic potential of the test samples were calculated as EC_{50} values.

For the SR extracts and vehicle control to be evaluated as capable of inducing EROD activity, procedures described in Bols et al. (1999) were followed. Concentration-response curves for the EROD induction response to each sample were computed by non-linear regression using the Boltzmann sigmoid curve as a model equation and the concentration of each sample causing 25% of the TCDD-induced maximum EROD activity (defined as extract EC_{25} TCDD) was calculated (Seiler et al., 2006).

2.2.6.2 Calculation of Toxicity Equivalent concentrations (TEQ)

Chemically derived toxic equivalent (Chem-TEQ) concentrations for each SR extract were calculated by the sum of the products of the measured PAH and PCB concentrations with their corresponding toxic equivalent factors (TEF) values as shown in Eq. (2.6) while assuming additive effect (Eadon et al., 1986). TEF values for some PAHs including benzo[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*a*]pyrene, dibenzo[*a,h*]anthracene, and indeno[1,2,3-*cd*]pyrene and some mono *ortho* PCBs including CBs 105, 118 and 156 have been derived from EROD assay using RTL-W1 cells (Clemons et al., 1998; Bols et al., 1999). Chem-TEQs were calculated as concentrations in picogram TCDD per gram SREQ (pg TCDD g^{-1} SREQ).

$$\text{Chem}-\text{TEQ}=\sum\left[\text{PAH}_i\times\text{TEF}_i\right]+\sum\left[\text{PCB}_i\times\text{TEF}_i\right]_n \qquad (2.6)$$

where TEF_i is the TEF for the individual PAH or PCB congener and *n* is the number of compounds in each extract.

Subsequently, the EC_{25} TCDD values calculated for each SR extract were used for calculations of the bioassay TCDD-equivalents (expressed as Bio-TEQ; pg TCDD g^{-1} SR) in each SR extract as shown in Eq. (2.7):

$$\text{Bio}-\text{TEQ}=\frac{\text{TCDD}\,\text{EC25}}{\text{extract}\,\text{EC25}\,\text{TCDD}} \qquad (2.7)$$

where TCDD EC_{25} (pg mL^{-1}) is the concentration of the TCDD positive control in each SR extract causing 25% of EROD induction and extract EC_{25}TCDD (g mL^{-1}) is the concentration of the SR extract equivalent causing 25% of EROD induction (Engwall et al., 1999).

EC_{25} TCDD was considered more appropriate than EC_{50} in this study because in several cases the EC_{50} was not well defined by the dose response curve. In addition, interactions are less likely to occur at lower extract concentrations, therefore the lower concentration portion of the curves were considered more appropriate to calculate the EROD inducing potencies of the extracts (Hollert et al., 2002).

2.3 Results and discussion

2.3.1 Chemical analysis

2.3.1.1 PAHs

The concentrations of the environmental analyte PAHs were above the limit of detection (LOD) in most of the samples, except for the field blank in which most compounds were below the LOD. The freely dissolved concentrations of the individual PAH compounds are provided in Supporting information (Table SI 3-1). The total concentrations (sum of 40 parent and branched) of freely dissolved PAHs in water at each of the study locations varied from 38 to 69 ng L^{-1}, being highest at sites 1 and 5 and lowest at site 2 (Table 2-1). Similar composition profiles were observed for all study locations, with the low to medium molecular weight PAHs (2- to 3-rings), particularly naphthalene and phenanthrene, dominating the overall PAH compositions and accounting for more than 70% of the total PAHs measured (Fig. 2-2). Naphthalene accounted for more than 46% and 22% of the total PAH concentrations at sites 1 and 2 respectively. Using SPMDs, concentrations in the range of 79-540 ng L^{-1} were reported for individual PAHs including fluoranthene, fluorene and pyrene in surface waters in an area with intensive agricultural activities in the United States (Alvarez et al., 2008). Schafer et al. (2010) used silicone passive samplers to estimate a total concentration of 0.1-10 ng L^{-1} for 16 PAHs in 9 streams in Victoria, Australia after a wildfire.

Table 2-1 Sum of freely dissolved concentrations (ng L^{-1}) of PAHs and of PCBs and the concentrations (ng g-1 SR sampler) of pesticides and acid/urea herbicides absorbed by silicone rubber passive sampling (SR-PSDs) deployed in water at the sampling locations.

Site	1	2	3	4	5
PAHs and PCBs (ng L^{-1})					
∑ $PAHs_{40}$	69	38	41	59	69
∑ $PCBs_{32}$	0.03	0.06	0.04	N.A	0.02
∑ Indicator-PCBs (∑PCB_7)	0.01	0.03	0.01	N.A.	0.01
Chem-TEQ [pgTCDD g^{-1} SR sampler]	6	11	7	6	9
Pesticides (ng g^{-1} SR sampler)					
Chlorpyrifos ethyl	8	34	287	9	11
Cyprodinil	8	3	19	9	11
Diazinon	20	3	8	31	43
Epoxiconazole	26	205	201	37	50
Fenpropimorph	1200	93	150	280	44
Flusilazole	27	460	301	54	71
Metazachlor	36	330	230	35	50
Pendimethalin	31	76	740	250	180
Hexaconazole	N.D.	7	N.D.	12	15
Propiconazole	18	34	44	32	23
Diflufenican	27	106	440	190	160
Tebuconazole	11	120	120	47	23
Others[a]	15	27	31	27	27
Acid/urea herbicides (ng g^{-1} SR sampler)					
2,4-D*	4.0	8.7	3.8	1.40	4.0
Mecoprop (MCPP)	6	30	15	10	12
Diuron	N.D	N.D	290	N.D	290
Isoproturon	50	80	275	340	220
Linuron	7600	3200	1500	900	1500
Chlorotoluron	180	90	4000	3000	2100
Others[b]	3	4	5	14	11

N.A. not available; ND = not detected. SR-PSDs were deployed for a total of 65 days at sites 1, 2, 3 and 4 and 58 days at site 5 from November 2010 to January 2011.

[a]Others =atrazine, chlorfenvinphos, disulfoton, iprodione, malathion, pentachlorobenzen, pirimicarb, pirimiphos methyl, terbuthylazine, terbutryn, triadimenol, trifloxystrobin, fenpropidin and trifluralin.

[b]Others = 4-(2, 4-dichlorophenoxy) butyric (2, 4-DB), dichlorprop, bromoxynil, loxynil, 4-(4-chloro-2-methylphenoxy) butyric acid (MCPB), triclopyr, monolinuron, and fenuron. *=2, 4-dichlorophenoxy acetic acid (2, 4-D).

A high proportion of the heavier parent PAHs (5-and 6-rings) implies predominately pyrolytic source, whilst a high proportion of alkylated 2- and 3-ring PAHs suggests a petrogenic origin, hence concentration ratios can identify possible sources (Witt, 2002; Neff et al., 2005). At all of the study sites 5- and 6-ring PAHs comprised <5% of the total (Fig. 2-2). The ratio of methylphenanthrene / phenanthrene sequestered by SR-PSDs was 1.2 at site 1, 0.9 at site 2, 0.8 at site 3, 1.7 at site 4 and 1.1 at site 5, suggesting a higher petrogenic input at site 4 than at the other sites. This may reflect the proximity of this site to the largest town in the catchment. The freely dissolved concentrations of individual PAHs in the streams and estuary of Ythan catchment were 3 - 4 orders of magnitude lower than their annual average environmental quality standards (AA-EQS) for surface waters under the WFD (EC, 2006).

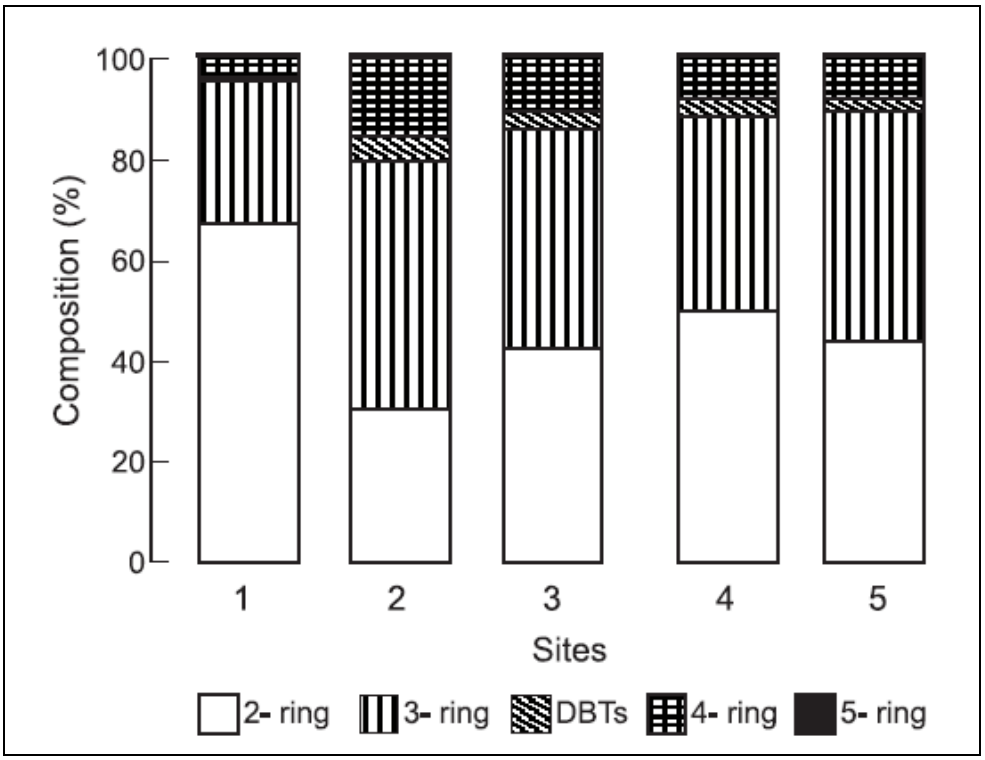

Figure 2-2 Percentage composition of PAH groups in water at the various sampling locations.

Term description: 2-ring = ∑naphthalenes (parent and C1-C4); 3-ring = ∑acenaphthene; acenaphthylene; fluorene; phenanthrene and anthracene (parent and C1-C3); DBTs =∑dibenzothiophenes (parent and C1-C3); 4-ring = ∑fluoranthene and pyrene (parent and C1-C3); benzo[c]phenanthrene; benz[a]anthracene; benz[b]anthracene and chrysene (parent and C1-C2); 5-ring = ∑benzofluoranthene, dibenz[ah]anthracene, benzo[a]pyrene, benzo[e]pyrene and perylene (parent and C1-C2). 6-ring=∑indenopyrene, benzoperylene (parent and C1 C2).

2.3.1.2 PCBs

Freely-dissolved concentrations of thirty-two *ortho and* mono *ortho* PCB congeners, including the Indicator-PCBs (CB28, 52, 101, 118, 153, 138, and 180) are listed in Supporting information (Table SI 3-2). The PCB extract for site 4 was lost during the clean-up process. Sum-concentrations of the seven Indicator-PCBs ($\sum PCB_7$) followed the same pattern as the sum of all measured PCBs ($\sum PCB_{32}$), with about 3 times higher concentrations at site 2 than at the other sites (Table 2-1). Unlike for PAHs, the highest total concentrations of the PCBs were found at sites 2 and 3. Total concentrations of PCBs ($\sum PCB_{32}$) measured in this study ranged 0.02-0.06 ng L^{-1} (Table 2-1) and were consistent with a previous survey in Scottish waters which concluded that values of PCBs in most surface waters were less than 1 ng L^{-1} for the sum of all the congeners (SOAEFD, 1996). In other studies, a sum concentration of 0.12-1.47 ng L^{-1} of 20 PCBs were measured in spot water samples from eight major riverine runoff outlets of the Pearl River Delta (PRD), South China (Guan, et al., 2009). Similarly, a study using SPMDs to monitor 12 dioxin-like PCBs in Port Jackson (Sydney Harbour), Australia estimated sum concentrations ranged from 0.021 to 0.54 ng L^{-1} (Roach, et al., 2009).

Most of the PCB congeners were present at each of the four successfully analysed locations and a predominance of the moderately to relatively highly chlorinated PCBs, i.e. tetra, penta, and hexa PCBs, was apparent (Fig. 2-3). The overall data obtained for PCBs in the present study did not indicate any specific point source inputs in the catchment.

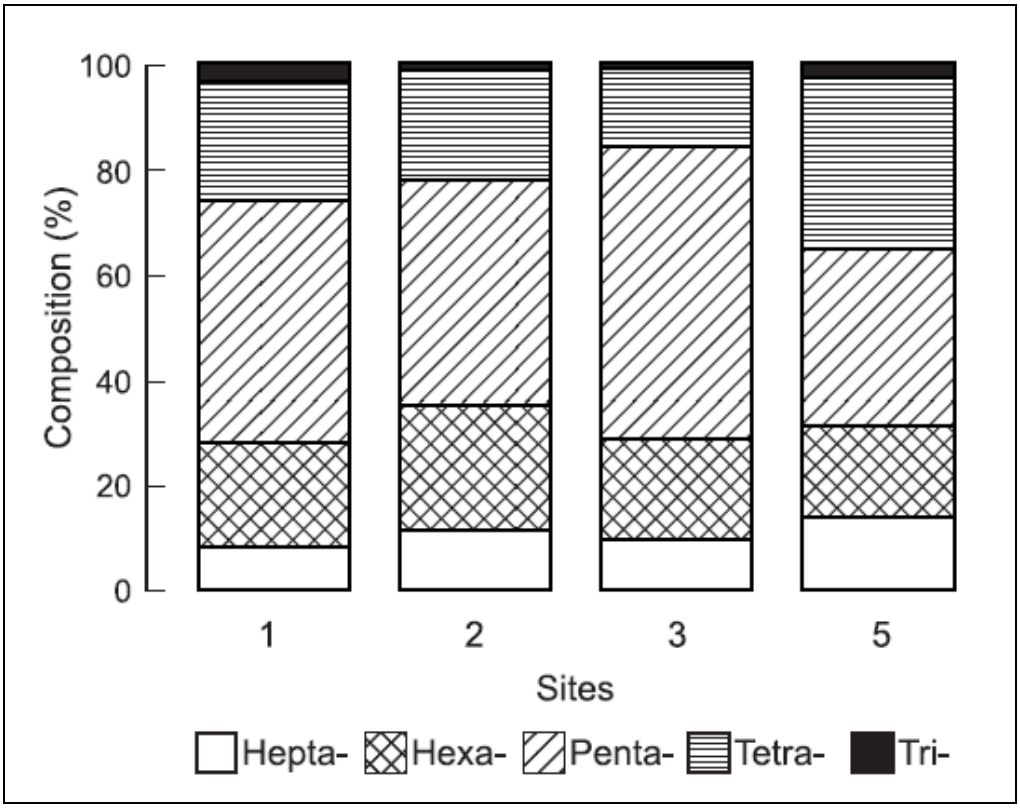

Figure 2-3 Percentage composition of PCB congeners in water at the various sampling locations.

Term descriptions; Tri=∑CBs 28 and 31; Tetra= ∑CBs 44, 49, 52, 70, and 74; Penta =∑CBs 97, 99, 101, 105, 110, 114, 118, and 123; Hexa=∑CBs 149,132,153,137,138,158,128,167,156, and 157; Hepta = ∑CBs 170, 180,183, 187, and 189; Octa and Deca= ∑CBs 194 and 209. Data for site 4 not available.

2.3.1.3 Chem-TEQ from chemical analysis

The Chem-TEQ (based on concentrations in silicone rubber) values measured in this study varied considerably among the study locations and ranged from 6 to 11 pg TCDD g^{-1} SREQ (Table 2-1). The lowest and highest concentrations were measured for sites 1 and 2 respectively, and reflected the dominant contribution of PAHs to the Chem-TEQ values (i.e. PAHs contributed >80% of Chem-TEQ). Chrysene, benzofluoranthenes and benz[*a*]anthracene were the predominant PAH compounds, while CBs 105 and 118 were the highest PCB contributors. The measured Chem-TEQ values in each of the study locations would enable comparison to Bio-TEQ values, so as to evaluate the contributions of the analysed freely dissolved PAHs and PCBs to the overall biological effects measured with the EROD assays. It has been suggested that the presence of PAHs in environmental complex mixtures can dominate the contributions of other DLCs such as PCBs, PCDF and PCDD in the estimation of TEQ (Eljarrat and Barcelo, 2003).

2.3.1.4 Pesticides and acid/urea herbicides

Unfortunately, SR-PSDs K_{SW} and diffusion coefficients (D_P) are not currently available for these compounds and consequently aqueous concentrations of pesticides and acid/urea herbicides could not be calculated. However, SR-PSDs samplings could be used to assess their occurrence and absorbed amounts allowed a relative comparison.

There was a downward trend in the absorbed amounts of pesticides towards the estuary, indicating the possible influence of agricultural activities in the upper parts of the catchment and increasing water dilutions in the lower parts (Table 2-1). The distribution pattern of pesticides in the catchment was variable, for example, fenpropimorph, a cereal fungicide, was dominant at sites 1 and 4, accounting for more than 84% and 27% of the sum detected components respectively, while pendimethalin, a selective herbicide used for the control of broadleaf and grassy weeds, was dominant at sites 3 and 5, accounting for over 28% and 24% of the sum components in the two sites, respectively; flusilazole, a systematic fungicide for broad spectrum disease control, was dominant (over 31%) at site 2. The occurrence and dimension of the detected pesticides in the catchment may be influenced by a number of factors including agricultural runoff potential and the physico-chemical properties of the individual components. Using POCIS, Alvarez et al. (2008) reported up to 3400 ng $POCIS^{-1}$ of individual pesticides (atrazine) in surface waters of an area with intensive agricultural activities in United States.

A number of acid and urea herbicides were also detected in the SR extracts at all sites, with linuron, isoproturon, chlortoluron and Mecoprop (MCPP) being predominant (Table 2-1). Linuron accounted for more than 90% of the sum amount of acid/urea herbicides absorbed to the samplers at sites 1 and 2, while chlorotoluron dominated the profile of the herbicides at study sites 3, 4 and 5, accounting for >65% of the components in sites 3 and 4, and >50% in site 5. Linuron is widely used in vegetable production, while chlorotoluron has applications in barley and wheat production. Non-agricultural uses of acid/urea herbicides also exist, with their application to control weed growth on hard surfaces, particularly roads, railways, airport runways, golf courses and public parks, being an important example (Lapworth and Gooddy, 2006). Although their environmental fate in the aquatic environment is yet to be fully defined, most herbicides are transformed by both biotic and abiotic processes and can be biodegraded to their metabolites which in some instances may be more toxic than the parent compounds (Virkutyte et al., 2010).

Relatively polar pesticides and acid/urea herbicides, e.g. metazachlor (log Kow=2.49) and diuron (log K_{OW}=2.68), were adequately sequestered by the SR-PSDs alongside non-polar compounds, i.e. PAHs and PCBs with log K_{OW} 3 to 8. This demonstrates the great utility of SR-PSDs, as this level of sensitivity in detection would have been extremely challenging using

conventional sampling and analytical techniques (e.g. Kuster et al., 2009). This study has demonstrated that SR-PSDs can be employed to monitor occurrence and distribution of these pesticides in catchment waters and also provides insights into future prospects of using SR-PSDs quantitatively (for measurement, source identification and establishing environmental fate) once the required D_P and K_{SW} data become available.

2.3.2 Biological effects analysis

2.3.2.1 Cytotoxic effects of SR passive sampler extracts

Following 48 h exposure to SR extracts and MeOH (vehicle), no statistically significant cytotoxicity was measured in RTL-W1 cells due to any of the extracts from the study sites (e.g. Fig. 2-4A). In contrast, cytotoxicity was observed with the positive control (DCP; Fig. 2-4B) indicating that the cells were indeed responsive and any toxicity present would have been measured. Considering that cytotoxicity was not measured with the blank SR extracts, it shows that the extraction process and solvents used were not inherently toxic to the cells in this study. The measured concentrations of contaminants sequestered by the SR-PSDs were relatively low, hence did not elicit measureable cytotoxic effects in the neutral red cell viability assays. This result agrees with a previous study in which no toxicity was observed using Microtox assay in extracts from POCIS (herbicides) and SPMDs deployed in surface water that was affected by large pesticide inputs and with estimated PAH concentrations three orders of magnitudes higher than observed in this study (Alvarez et al., 2008). Reduction of cell numbers due to exposure to xenobiotic compounds is often compensated by a parallel lysosomal proliferation which could, to some degree, mask cytotoxic effects (Hollert et al., 2000).

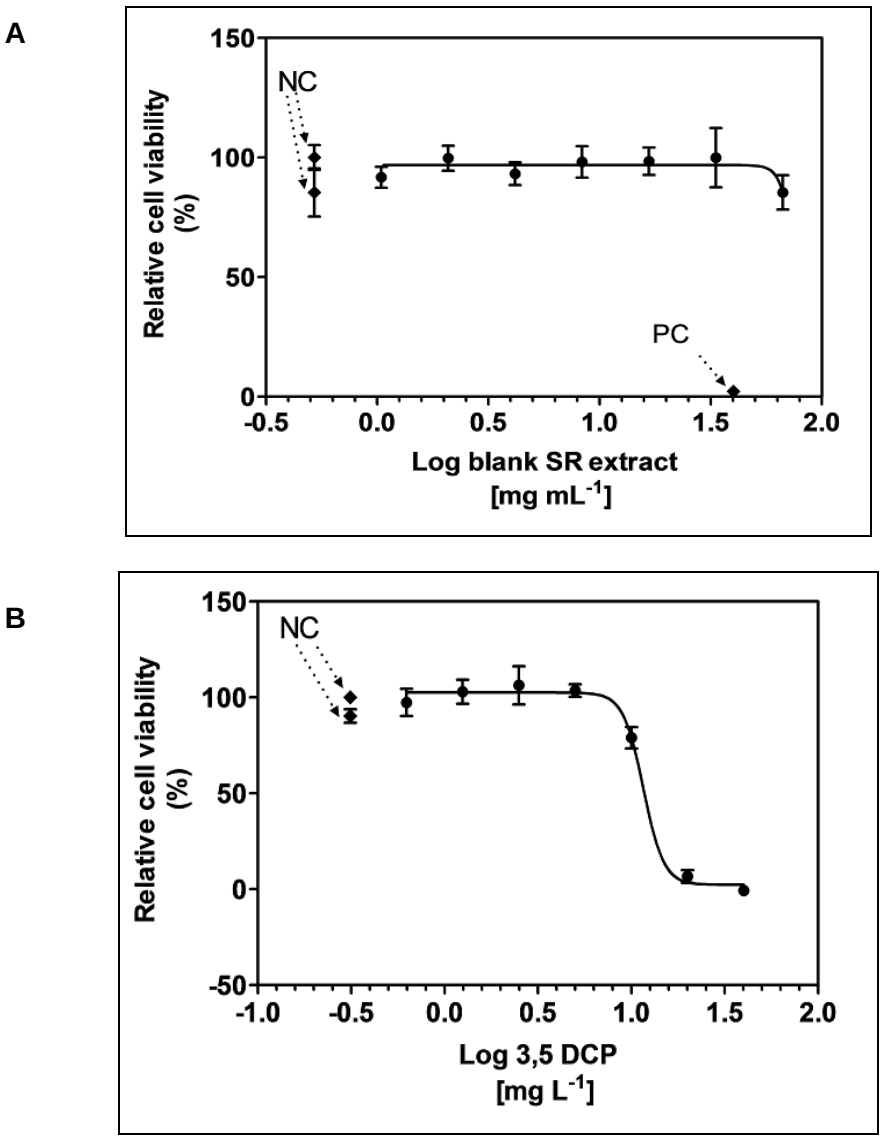

Figure 2-4 Concentration-response curves following 48 h exposure of (A) blank SR sampler extracts and (B) 3,5 DCP to RTL-W1 cells using neutral red retention assay. Results of extracts from deployed SR-PSDs were analogous to that of the blank. Cell viability expressed as percentage of unexposed controls (negative control, NC). Data points are mean with ± standard deviation (SD) of four replicates at each exposure concentration. NC=negative control; PC=positive control (1.6 mg L-1 3,5 DCP). PC and NC are not plotted in the indicated units.

2.3.2.2 EROD assays

In vitro bioassays were applied in this study as an alternative chemical detector, with EROD activity used to determine the overall dioxin-like activity of organic compounds in water at each of the study sites. EROD activity was not observed with either the blank SR extract (Fig. 2-5B), or with the vehicle control (methanol, maximum 1% v/v; not shown). Contrastingly, SR extracts from the five study sites of the catchment induced statistically significant EROD activity (e.g. Fig. 2.5C-D). Complete EROD activity concentration-response curves were obtained for most of the SR extracts and for the TCDD positive control (Fig. 2-5A). The potencies, i.e. the maximal level

of EROD activity (EC_{25} TCDD), were different in each sample and generally lower than the TCDD positive control (maximum 50 pM) in each assay (e.g. Fig. 2.5B-D). At site 2 the highest extract concentration (dose equivalent to 66.67 mg SREQ mL^{-1}) reduced the EROD activity compared to the peak induction (data not shown). This should not be attributed to cytotoxicity considering the NR results, but was a result of sublethal inhibitory effects. Extracts from site 2 displayed high EROD induction, while sites 1 and 4 were significantly lower. It is curious to note that site 3 showed low EROD induction in comparison to site 2, despite their near proximity. At site 3, higher concentrations of pesticides and acid/urea herbicides were detected in the SR-PSD and could be causing EROD inhibition as has been postulated by other studies (Babín and Tarazona, 2005; Han et al., 2007).

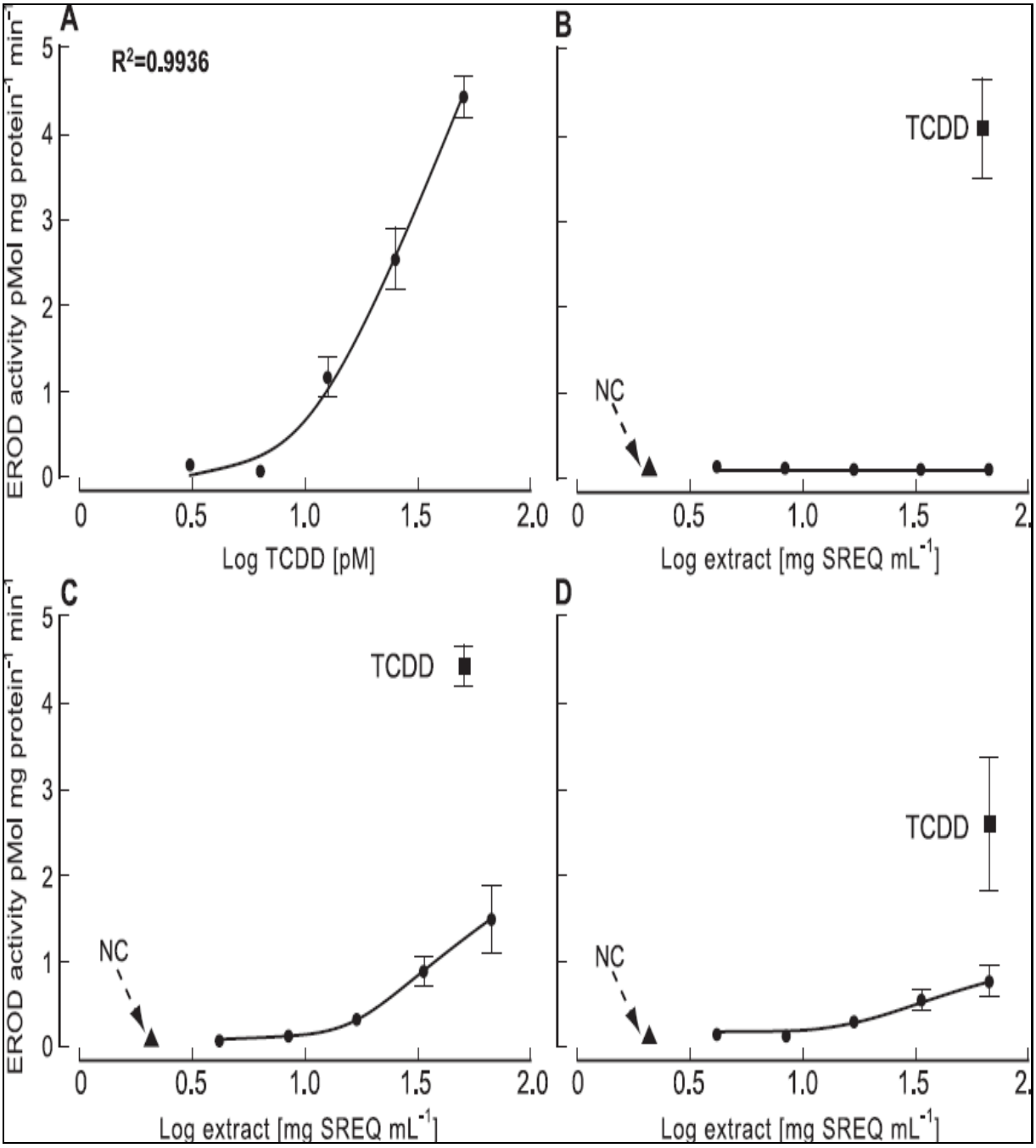

Figure 2-5 Concentration-response curves for EROD induction in the RTL-W1 cells by (A) TCDD, (B) SR sampler extracts-blank (C) SR sampler extract -site 2 and (D)SR sampler extract -site 4. Data points represent the mean ± standard deviation (SD) of four replicates at each exposure concentration. NC=negative control; within plot, B-D the TCDD (50 pM) is marked. TCDD and NC are not plotted in the indicated units.

2.3.2.3 Bio-TEQ from EROD bioassay

The EC_{25} values derived for the positive control TCDD run with each extract (including the vehicle control) were approximately the same for each bioassay and were used to calculate Bio-TEQ values for each site. The Bio-TEQ values were then compared to the Chem-TEQ values (Fig. 2-6). Chem-TEQ values were significantly lower than Bio-TEQ values in SR extracts from all the study sites, indicating a higher sensitivity (detection) by the bioassay method, and/or the presence of other dioxin-like compounds not captured by the PAH and PCB TEQs (e.g. pesticides or other chemicals). Previous studies have shown similar discrepancies between chemically calculated TEQ values and bioassay induction values (Willett et al., 1997; Brack et al., 2007). Keiter et al. (2008) illustrated that combinations of chemical analysis, fractionation techniques and various *in vitro* assays do not necessarily explain inductions, even when the concentrations of priority PAHs were very high. It is possible that other environmental contaminants including polybrominated diphenyl ethers (PBDEs), PCDD/Fs and polychlorinated naphthalenes (PCNs) that were not measured in this study might have contributed to the Bio-TEQ values. Applying chemical and effects directed fractionation techniques to SR sampler extracts prior to and after chemical analysis and bioassays could help to identify the compounds present in the complex mixtures responsible for the observed EROD responses and help to bridge the gap between the Chem-TEQ and Bio-TEQ values. Further, HOCs may volatilise and adsorb to the walls of microplate, leading to reduced sensitivity during bioassays. This may contribute, positively or negatively, to disparity between Chem-TEQ and Bio-TEQ values depending upon whether EROD inducers or inhibitors are preferentially lost from the exposure system. The application of partition controlled delivery for bioassays (passive dosing; e.g. Smith et al., 2010) should provide more stable exposure conditions and eliminate the use of carrier solvents during aqueous toxicity assays.

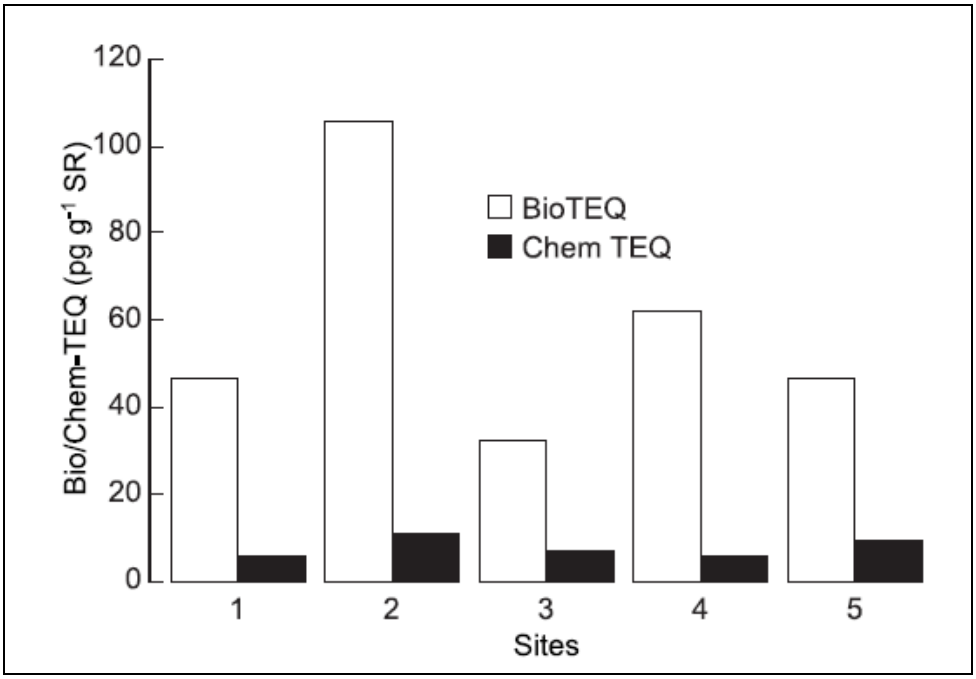

Figure 2-6 Comparison of Bio-TEQ and Chem-TEQ values obtained from in vitro RTL-W1 assay and concentrations of PAHs and PCBs measured with silicone rubber (SR) passive sampling technique in water of the study locations.

2.4 Conclusions

The study demonstrates that extracts of SR-PSDs deployed in surface water can be applied with minimal preparation to *in vitro* cell line bioassays. These can be used to rapidly and economically measure the potential impact of complex mixtures of organic contaminants and also to detect the presence of toxic compounds not routinely analysed for. The concentration data of organic contaminants presented in this study are significant from the ecotoxicological perspective since SR-PSDs samples reflect the contaminant level aquatic organisms are exposed to. SR samplers absorbed relatively polar pesticides/herbicides, as well as non-polar compounds extending the potential application of the SR-PSD technique in regulatory monitoring programmes, particularly in relation to the challenging LODs set for some compounds under the WFD.

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Supplementary material in appendix 3 Chapter seven

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3 CHAPTER THREE: Identification of selected organic contaminants in streams associated with agricultural activities and comparison between autosampling and silicone rubber passive sampling

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Abstract

This study evaluates the potential of silicone rubber passive sampling devices (SR-PSDs) as a suitable alternative to automatic water samplers (autosamplers) for the preliminary identification of a wide range of organic contaminants in freshwater systems. The field performance of SR-PSDs deployed at three sites on two streams of an agricultural catchment area in North East (NE) Scotland, United Kingdom (UK) was assessed concurrently with composite water samples collected from two of the sites using autosamplers. The analytical suite consisted of selected plant protection products (PPPs; commonly referred to collectively as 'pesticides'), including 47 pesticides and a separate sub-category of 22 acid/urea herbicides. Of these, a total of 54 substances, comprising 46 pesticides and 8 urea herbicides were detected in at least one of the SR samplers. All but 6 of these SR-PSD detected substances were quantifiable. By comparison, a total of 25 substances comprising 3 pesticides and 22 acid/urea herbicides were detected in the composite water samples, of which only 8 acid/urea herbicides were quantifiable. The larger number and chemical classes of compounds detected and quantified via passive sampling reflect the lower limits of detection achieved by this device when compared to autosamplers. The determination of dissolved concentrations of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) added to the information on contaminant pressures at each site, allowing assessment of the reliability of SR-PSDs in freshwater systems and the identification of possible contaminant sources. The study demonstrated the utility of SR-PSDs for detecting and semi-quantifying low concentrations of analytes, including those which hitherto have not been measured in the catchment area and also some pesticides that are no longer approved for agricultural use in the UK and EU. The SR-PSD approach can thus provide a better understanding and clearer picture of the use and presence of organic contaminants within catchments.

Keywords

Plants protection products (PPPs)
Pesticides
Polychlorinated biphenyls (PCBs)
Freshwater
Polycyclic aromatic hydrocarbons (PAHs)
Water quality monitoring

3.1 Introduction

The most significant policy challenge presented by the implementation of the European Union Water Framework Directive (WFD; EC, 2000) is the management and control of diffuse water pollution from agriculture (SEPA, 2007; Kay et al., 2008; Volk et al., 2009). Plant protection products (PPPs; commonly referred to collectively as 'pesticides') often contribute significantly to the ecological risks posed to freshwater ecosystems in agricultural areas, exhibiting both acute and chronic toxicological effects even at their typically low aqueous concentrations (Warren et al., 2003; Schäfer et al., 2011). Pesticides are also amongst the major groups of organic compounds that are ultimately transferred to the marine environment via river system discharges. Knowledge of the identity, quantity, sources and modes of inputs of these substances into the aquatic environment is essential to facilitate the control of diffuse source pollution and to help reduce or manage their ecological impact.

The conventional use of monthly spot (bottle or grab) sampling in water quality monitoring is often not adequate to identify specific sources and fully assess loadings of diffuse pollutants in freshwater systems. Intermittent pulses of contaminants can easily be missed or incompletely captured and quantified, particularly for agricultural chemicals such as plant protection products that are applied episodically. Moreover, when contaminants are present at ultra-trace concentrations, large volumes of water are often needed in order to reach the analytical limits of detection (LOD) required to assess potential exposure to organisms. Sampling of large volumes of water may also increase the presence of other interfering compounds (matrix effects). Consequently, increasing numbers of environmental monitoring programmes are making use of continuous automatic water samplers, also known as 'autosamplers' (Tran et al., 2007; Holvoet et al., 2007; Meyer et al., 2011). However, some limitations of this approach include the high costs of the equipment, on-site security and the burden of frequent inspections, together with operational instability during adverse weather conditions. Further, samples collected with autosamplers may suffer from problems associated with volatilisation, sorption to container walls and microbiological degradation during storage at ambient temperatures in the field prior to analysis (Jeannot, 1994). These limitations motivated our search for a robust, efficient, and cheaper alternative sampling technique to these autosamplers.

Passive sampling devices (PSDs) sequester and pre-concentrate contaminants over extended sampling periods, overcoming the challenge of achieving very low detection limits through selectively sampling larger volumes of water. The sorption of target analytes to the passive samplers can also in some cases minimise the extent of matrix interferences by

excluding potential interfering compounds from the analysis. When applied in a quantitative way, PSDs can provide time-weighted average concentrations (C_{TWA}) of target analytes and an estimate of the dissolved (and, therefore, readily bioavailable) fractions of the total contaminant loading. It should be noted however, that TWA concentrations are only determined when sampling occurs in the kinetic phase. If sampling is in the equilibrium phase, then analytes collected by the samplers can also be lost if environmental concentrations reduce. Therefore, while transient peaks in environmental concentrations are more likely to be captured by passive sampling than by spot sampling, their magnitude can be diluted and dissipated depending upon the polarity of the analyte, rates of diffusion within the sampler, and the duration of the sampling period. The principles and theories of passive sampling techniques for aquatic system applications have been previously described (Huckins et al., 2006; Booij et al., 2007) and a variety of reviews on passive sampling devices (PSDs) have been published (Stuer-Lauridsen 2005; Vrana et al., 2005; Allan et al., 2010).

Passive sampling technology has been shown to be a robust and reliable monitoring tool for range of contaminant types (e.g. non-polar and polar organic compounds) present in the aquatic environment, hence, it is being considered as part of emerging strategy for monitoring a variety of priority and emerging pollutants (Mills et al., 2011; Lohmann, 2012). Essentially, two types of PSDs are widely used for environmental monitoring of trace organic contaminants. Non-polar PSDs, such as the lipid-filled semipermeable membrane devices (SPMDs; Huckins et al. 1990; Huckins, 2006), silicone rubber (SR; Smedes 2007) and low-density polyethylene (LDPE; Adams et al., 2007) monitor compounds with log K_{OW} values over the range of ~3.5 to ~8; whereas, polar PSDs including polar organic chemical integrative sampler (POCIS; Alvarez et al., 2009) and polar Chemcatcher (Schäfer et al., 2008) target compounds with log K_{OW} values <4. Due to their relatively low costs, ease of handling and analysis, single phase, silicone rubber passive sampling devices (SR-PSDs) are increasingly applied to assess environmental exposures and contamination of trace organic contaminants in the aquatic environment (Schäfer et al., 2010; Yates et al., 2011; Emelogu et al., 2012). However, most studies using SR-PSDs have focused primarily on classical non-polar organic contaminants i.e. polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs), in the marine environment. Polar organic compounds attain equilibrium with single phase PSDs within hours to days due to their low log K_{OW} values and the sampler's physical dimensions (e.g. large surface area and thin), hence, it is possible to apply SR-PSDs in monitoring polar to semi-polar organic contaminants at least, for preliminary identification purposes (Lohmann et al., 2012).

In this study, we evaluated the potential of SR-PSDs for determining the presence/absence and relative abundance (i.e. semi-quantitative assessment) of a wide range of important organic contaminants (such as PPPs) in the streams within an agricultural catchment. Selected PPPs were assessed and we refer to these collectively throughout simply as pesticides (including insecticides, fungicides, and herbicides), with a separate sub-category of acid/urea herbicides examined as a distinct group. For the pesticides, comparison was made on the effectiveness of SR-PSDs relative to conventional sampling techniques using autosamplers. In addition, we determined the dissolved concentrations of PAHs and PCBs in the streams using SR-PSDs to provide fuller information on contaminant pressures at each site and to provide comparison with data from our previous study at the same sites (Emelogu et al., 2013).

3.2 Materials and methods

3.2.1 Site selection

The sampling locations chosen for the study (sites 1, 2 and 3; Fig. 3-1) are in the Ythan catchment in North East (NE) Scotland, United Kingdom. Detailed descriptions of the catchment and the sites have been provided in a previous study (Emelogu et al., 2013). Briefly, the catchment is sparsely populated and covers a total surface area of ~67,500 ha with arable agriculture and grazing as the major land use and some small areas of forestry (SEPA, 2011). The River Ythan (the main river within the catchment) is 64 km long, of which ~9 km are tidal; the mean annual discharge is 8.1 $m^3 s^{-1}$. Site 1 was at the headwaters of the River Ythan, while sites 2 and 3 were on a small tributary and approximately 3.3 km apart. This catchment was chosen because it has been identified as at risk of failing to meet WFD good ecological status due to pressures from agricultural diffuse pollution. Previous study using the pesticide specific trait-based indicator “SPEcies At Risk” (SPEARpesticides) model (Liess et al., 2008) indicated ecological impact on aquatic invertebrates due to pesticides on some streams within the catchment, including sites 2 and 3; site 1 was not impacted according to the SPEAR tool (SEPA, 2010). However, conventional water quality monitoring demonstrated no failures of existing freshwater environmental quality standards (EQSs). An alternative and improved approach was therefore needed to assess whether chemical pressures not observed by spot sampling may be contributing to the observed ecological conditions in the catchment. It was therefore an ideal candidate in which to conduct a comparative study using SR-PSDs and autosamplers. Furthermore, this catchment is being used by the Scottish Environment Protection Agency (SEPA) to investigate pesticide occurrence and their impacts in mixed farming catchments. Continuous multi-parameter data

sondes (YSI 6920 or equivalent) for water quality monitoring deployed by SEPA in some selected sites within the catchment including sites 2 and 3 measures water temperature and pH (at 15 min intervals). The average water temperature and pH at sites 2 and 3 during the sampling period were 6.8 and 6.4 °C and 7.71 and 7.66 respectively.

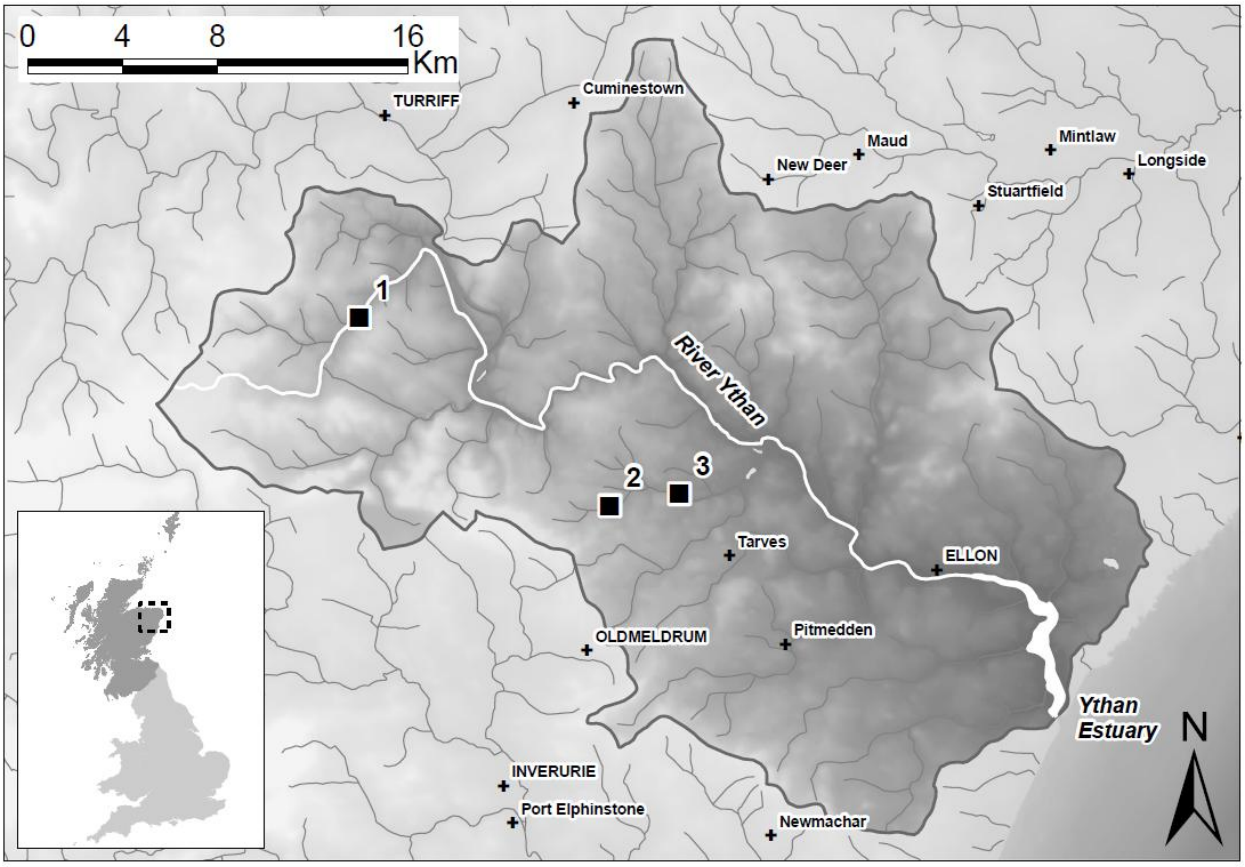

Figure 3-1 River Ythan catchment (showing River Ythan and main tributaries) in NE Scotland. Sample sites 1-3 are indicated. The inset shows a map of United Kingdom (UK) with the approximate location of the Ythan catchment.

3.2.2 Sampling and sample handling

3.2.2.1 Passive sampling and extraction of silicone rubber sheets

Prior to deployment, SR-PSDs were pre-extracted and loaded with performance reference compounds (PRCs). PRCs are analytically non-interfering compounds that are not found in the environment and are added to passive samplers prior to deployment in order to calibrate sampling rates (R_S). Uptake rates are sensitive to a variety of exposure conditions including temperature, salinity, flow rate and biofouling. Addition of PRCs to samplers prior to deployment can correct for this site to site variability of the uptake rates and allows for the accurate determination of R_S (Huckins et al., 2002). The rate of dissipation of PRCs from the samplers is proportional to the uptake of analytes. Hence, the rate loss of PRCs during

deployment can be used to estimate *in situ* sampling rates (R_S) of the target analytes, which can then be used to calculate the water concentrations of the analytes.

A detailed description of pre-extraction, loading with PRCs, deployment, retrieval and extraction of the silicone rubber (SR) sheets has been provided elsewhere (Emelogu et al., 2012). Briefly, the pre-cleaned and PRC-spiked SR samplers (each comprising 6 sheets) with a dimension of 6 x 9 cm each and a total surface area of ~ 600 cm^2, were attached to stainless steel cages and deployed in water at the three streams for 54 days during the period October to December 2011. During deployment and retrieval, another set of PRC-spiked SR samplers was exposed to air; this served as a field control sampler and was also used for the determination of the initial concentrations of the fourteen PRCs (Table 3.1) that were loaded on the samplers prior to deployment. A suite of recovery and internal standards, including surrogate and deuterated compounds (Table 3.1) were added to the SR samplers prior to extraction.

Extraction of deployed and undeployed SR samplers, including process blanks (i.e. procedural/field blanks and samples spiked with target pesticide/acid urea herbicide secondary calibration standards) was performed using Soxhlet apparatus with hot acetonitrile (ACN):methanol (MeOH; 150 mL; 2:1; v/v) solution for 24±4 h. After the extraction and clean-up processes, the extracts were subsequently aliquoted into three equal volumes for the determination of PRCs, pesticides, PAHs and PCBs. The aliquots targeted for the analysis of selected pesticide and acid/urea herbicide were further split into two equal volumes and solvent exchanged to appropriate solvents for the analysis of selected pesticide and acid/urea herbicide. Prior to analysis, known amounts of injection standards (Table 3.1) were added to the extracts targeted for the analysis of selected pesticide, acid/urea herbicide and PCB.

Table 3-1 List of target compounds; the performance reference compounds (PRCs) spiked on the silicone rubber samplers before deployment; the recovery/spiking standards; internal/injection standards used and the analytical techniques applied.

Target compounds	Recovery standards	Internal standards	Injection standards	Analytical technique
Selected pesticides (47 compounds, see Table 3-2A)	Secondary calibration pesticide standards; see Table 3.2A[1]		Azobenzene and diphenamid	GC-MS/MS;
Acid herbicides and urea herbicides (22 compounds, see supporting information, SI)	**A**: Secondary calibration acid/urea herbicide standards; see Table 3-2B[1] **B**: 2,4-dichloro-phenyl acetic acid (2,4-DPA)		2-bromo-4-cyanophenol, 4-chlorophenyl acetic acid and neburon	LC-MS/MS;
Polycyclic aromatic hydrocarbons (PAHs; 40 parent and branched compounds or groups of compounds)		Deuterated PAHs: Naphthalene-d8, biphenyl-d10, dibenzothiophene-d8, anthracene-d10, pyrene-d10, benzo[*a*]pyrene-d12 and dibenzo[*a,h*]anthracene-d14		GC-MS;
Polychlorinated biphenyls (PCBs; 32 *ortho*- and mono *ortho*- PCBs)	CB112		2,4-dichloro-benzyl alkyl ether with C16 alkyl chains (DCBE 16)	GC-ECD
PAH PRCs: fluorene-d10, fluoranthene-d10, chrysene-d10, benzo[*e*]pyrene-d10	Same as PAHs		Same as PAHs	Same as PAHs
PCB PRCs: congeners 10, 14, 21,30, 50, 55, 78,104,155, and 204	Same as PCBs		Same as PCBs	Same as PCBs

[1] Added to blank samples as independent process check standard (IPCS)

3.2.2.2 Autosampling and handling of composite water samples

To aid direct comparison of silicone rubber passive sampling with the conventional sampling procedure, autosamplers (Model 6712; Isco, Inc; Lincoln, NE, USA) equipped with six 400 mL glass bottles (non-cooled), batteries and a peristaltic pump were deployed concurrently with passive samplers at sites 2 and 3 (Fig. 3-1). The autosamplers were programmed to run continuously from October to December 2011 and collected 40 mL discrete water sample every 2 h 45 min via vinyl tubing into the 6 bottles. Once deployed, autosamplers were visited weekly (except for two weeks in December) in order to collect the water samples and to change batteries if required. At the laboratory, the water samples from each site were shaken manually and composited (~2.4 L per site per week) prior to being mixed thoroughly using a magnetic stirrer (IKA Color Squid IKAMAG®) at ~ 800 rpm for 1 min. Approximately 1 L of the unfiltered and thoroughly mixed composite water sample was transferred into a Pyrex glass bottle fitted with a PTFE lined screw cap and stored in the dark at 4 °C and extracted for pesticide/herbicide analysis within 48 h of collection. Water samples remained unfiltered to represent the total pesticide and acid/urea herbicide input in both the aqueous and particulate phases.

3.2.2.2.1 Extraction of water samples

Each of the 1 L weekly water samples and process blanks (including blank samples spiked with known amount of target pesticide standards) was thoroughly mixed by manual shaking and an aliquot of 500 mL was transferred into a 1 L glass Duran bottle for the extraction of selected pesticides. Approximately, 50 mL dichloromethane (DCM) was added to the sample and shaken manually for 1±0.1 min, taking care to release any pressure build up. The sample was further shaken for 5±2 min on a rotary shaker with the setting at 180±10 shakes min^{-1} and then transferred to a 1 L separating funnel to allow separation of the phases. The lower, DCM, layers were run-off directly into a funnel packed with anhydrous sodium sulphate (Na_2SO_4) held on filter paper and was collected in a glass Zymark flask. The water sample was subjected to a second DCM extraction and the aqueous layer discarded. The combined DCM extracts were reduced to approximately 0.5 mL using a Zymark TurboVap system and solvent exchanged into toluene. Known amount of pesticide internal standard (Table 3-1) was added to the extract in a 2 mL GC vial and the prepared extracts were stored at 5±3 °C until required for analysis.

For the acid/urea herbicides, an aliquot of 25 mL of each thoroughly mixed 1 L water sample and process blanks (including blank sample spiked with known amount of target acid/urea herbicide), was spiked with known amounts of acid/urea herbicide surrogate spiking and

internal standard solutions (Table 3-1) and 10 mL of this filtered through a 25 mm, 0.2 µm CHROMAFIL® Syringe filters with polyester PET membrane (Type PET 20/25; Macherey-Nagel) using a vacuum manifold (IST-VacMaster) in order to remove suspended particles. The suspended particles on the filter were then washed with 0.85 mL of acetonitrile (ACN) to extract the acid/urea herbicides on them and both the filtered samples and the ACN extracts were combined. The filtered water samples and ACN were mixed thoroughly using a Pasteur pipette and aliquots were transferred to vials for analysis.

3.2.3 Sample analysis

Detailed descriptions of all the analytical procedures and instruments employed are provided in Emelogu et al. (2013). Briefly, analyses of extracts of both SR-PSDs and water samples for selected pesticides were performed using gas chromatography with tandem triple quadrupole mass spectrometry (GC-MS/MS) operated in positive electron ionisation (EI+) mode. For increased sensitivity and selectivity, the triple quadrupole MS was operated in multiple reaction monitoring (MRM) mode. Target selected pesticides were identified by comparison of their retention times and MRM transitions to the standard solutions. Instrument control, data acquisition and processing were performed using the Varian MS Workstation version 6.3.

The optimised MRM transitions for the 47 pesticides monitored and the internal standards used are presented in Table 3-2A. Extracts of passive samplers and water samples were analysed for 22 acid/urea herbicides by direct aqueous injection into liquid chromatography coupled to electrospray ionisation (ESI) tandem mass spectrometry (LC-ESI-MS/MS). The electrospray interface was operated in both positive and negative ion modes. Due to the differing LC and MS conditions of the target compounds, each suite of herbicides was analysed separately; negative ESI ionisation was applied on the 14 acid herbicides (Table 3-2B), while positive ionisation ESI was applied on the 8 urea herbicides (Table 3-2B). Two MRM transitions were used to monitor all target compounds and thus allowed reliable confirmation of positive detections. The first, most sensitive and more abundant MRM transition was used for quantitation and the second MRM transition was used for confirmation using ion ratio determination. The components were identified and quantified by comparison with standards using retention time and MRM mode. Table 3-2B shows the 22 acid/urea herbicides, internal/recovery standards and the MRM transitions with the optimal detector acquisition parameters for each target compound. Dionex Chromeleon 6.5 software was used to acquire the data and instrument control.

The composition and concentration of 40 PAH compounds (2- to 6-ring; parent and branched; Table 3-4) and the 4 PAH-PRCs (Table 3-1) in SR extracts were determined by GC-MS using calibration standards and with deuterated PAHs (Table 3-1) as internal standards. SR extracts for PCB analysis were analysed for 32 PCB congeners (*ortho*- and mono *ortho*-PCBs; Table 3-5) and 10 PCB-PRCs (Table 3-1) using GC with an electron capture detector (ECD). Quantification was achieved using recovery and injection standards and external calibration standards.

Table 3-2 A Summary of the GC-MS/MS optimised multiple reaction monitoring (MRM) acquisition parameters for the determination of 47 pesticides; the collision energy (CE) is shown in parenthesis.

Pesticides	**MRM 1 Transition**	**LOD (ng L^{-1})**	**Precision (n=11)**
Atrazine	200>122 (10V)	8.1	12.4
Azoxystrobin	344>183 (25)	14.3	17.9
Boscalid	342>140 (20)	20.2	12.7
Carbofuran	221>164 (15V)	12.5	14.2
Chlorfenvinphos (Z)	267>159 (35V)	9.5	13.1
Chlorothalonil	264>229 (25V)	5.6	12.7
Chlorpyrifos ethyl	314>258 (10V)	11.6	14.3
Chlorpyrifos methyl	286>271 (25V)	8.5	13.5
Cyanazine	225>189 (15V)	6.5	12.7
Cyproconazole	222>125 (30V)	18.9	11.3
Cyprodinil	224>208 (35V)	9.4	9.3
Diazinon	304>179 (10V)	5.2	9.7
Dichlobenil	171>136 (15V)	25	17.8
Dichlorvos	109>79 (5V)	9.2	22.2
Diflufenican	266>218 (25V)	7.6	11.9
Dimethoate	125>79 (10V)	7.4	13.2
Epoxiconazole	192>138 (15V)	14.8	9.3
Famoxadone	224>196 (10V)	19.1	14.9
Fenoxycarb	116>88 (30V)	41.4	23.4
Flusilazole	233>165 (10V)	10.2	13.2
Heptenophos	250>124 (10V)	23.4	17.9
Kresoxim methyl	116>89 (15V)	14.6	12.8
Malathion	173>99 (10V)	7.1	9.5
Metalaxyl	249>190 (15V)	35.6	23.4
Metazachlor	277>132 (40V)	37.6	21
Methiocarb	168>153 (15V)	21.8	13.6
Metribuzin	198>182 (20V)	5.7	9.8
Mevinphos	127>109 (10V)	7.0	15.2
Oxadixyl	163>132 (15V)	7.7	15.1
Parathion ethyl	291>109 (10V)	5.2	16.6
Pendamethalin	252>162 (10V)	7.0	22.3
Picoxystrobin	335>173 (10V)	21	14.4
Pirimicarb	166>96 (10V)	4.5	13.3
Pirimphos methyl	290>125 (20V)	15.8	20.2
Prometryn	226>184 (10V)	8.2	11.8
Propetamphos	236>194 (10V)	5.8	10.7
Propiconazole	259>173 (20V)	21.4	13.4
Simazine	201>173 (5V)	7.9	11.8
Tebuconazole	250>125 (20V)	11.4	12.7
Terbuthylazine	214>132 (10V)	6.5	11.9
Terbutryn	241>170 (10V)	7.0	10.4
Triademefon	208>181 (10V)	10.6	10.4
Triademenol	168>70 (10V)	17.7	13.1
Trietazine	200>122 (10V)	5.5	8
Trifloxystrobin	116>89 (15V)	8.4	12.9
Trifluralin	306>264 (5V)	5.2	15.2
Vinclozolin	285>212 (10V)	5.2	16
Azobenzene ((I.S)	182>77 (15V)		
Diphenamid (I.S)	167>152 (10V)		

I.S=internal standard

Table 3-2 B LC-MS/MS multiple reaction monitoring (MRM) acquisition parameters

Acid herbicides	**Retention Time (min)[a]**	**Precursor Ion m/z**	**Product Ion m/z**	**DP (V)**	**EP (V)**	**CE (V)**	**CXP (V)**	**Dwell (ms)**	**LOD (ng L-1)**	**Precision (n=11)**
2,4-D	8.68	218.9	161.1	-40	-7.5	-20	-4	30	3	4.2
Dicamba	8.87	218.9	175.1	-20	-7.5	-8	-3	30	2	8.2
2,4-DB	8.48	246.9	161	-20	-8	-18	-4	30	4	9.2
Dichlorprop	8.85	232.9	161.1	-45	-7.5	-18	-4	30	4	4.1
Bromoxynil	8.27	275.77	81	-70	-9.5	-45	0	30	5	4.9
Ioxynil	8.89	369.7	127	-75	-8.5	-50	0	30	1	5.6
Bentazone	10.31	239	132	-70	-7.5	-36	-2	30	2	3.9
MCPA	8.39	199	141.1	-55	-9	-20	-4	30	9	4.6
MCPB	8.51	227.1	141.1	-35	-2.5	-25	-4	30	4	3.8
MCPP	8.61	213	141.1	-30	-8	-22	-4	30	2	5
Triclopyr	8.7	253.9	196	-40	-7.5	-16	-4	30	4	6.5
Clopyralid	6.36	189.9	146	-20	-5.5	-12	-4	30	1	6.4
Fluroxypyr	7.41	253	195	-35	-4	-20	-4	30	2	3.7
Benazolin	7.66	242	170.1	-25	-7	-20	-2	30	4	5.4
2,4-DPA (R.S)	7.87	203.1	159.1	-35	-3	-12	-6	30		
4-Chlorophenyl acetic acid (I.S)	7.36	169	125	-20	-10	-12	-2	30		
2-Bromo-4-Cyano phenol (I.S)	7.24	195.9	78.9	-45	-12	-32	-1	30		
Urea herbicides										
Diuron	12.07	233	72	71	12	35	10	40	4	6.5
Isoproturon	12.02	207	72	56	12	23	6	40	4	3.8
Monolinuron	12.05	215	126	56	9	25	12	40	3	5.6
Metoxuron	11.42	229	72	55	6	30	6	40	4	5.5
Fenuron	10.88	165.2	72	86	6	27	6	40	5	7.9
Pencycuron	13.24	329	124.8	90	7	39	10	40	3	5.2
Linuron	12.5	249	159.9	51	6	27	14	40	2	4.6
Chlorotoluron	11.91	213	72	51	6	30	6	40	4	6.8
Neburon (I.S)	12.95	275	88	76	8	25	8	40		

DP=declustering potential; EP=entrance potential; CE=collision energy; CXP=collision cell exit potential. I.S=internal standard; R.S=recovery standard. [a]Expected retention time may vary with column.

3.2.4 Quality assurance and quality controls (QA/QC)

All solvents used in this study were HPLC grade and the water was ultra-pure (18.2 MΩ.cm). The glassware used for the SR samples was washed in a CAMLAB GW glassware washer and Decon ® 180 detergent, rinsed in clean water and dried in an oven at 100±5 ºC. Before use, the glassware was rinsed with a mixture of ACN: MeOH (1:1 v/v) and then with *iso*-hexane, with the latter allowed to evaporate to dryness.

All analytical procedures were batched and each batch included procedural/process blanks; the SR samples also included field blanks, which accompanied the deployed samplers during transportation, deployment and retrieval from the sampling sites and were also used for the determination of sampling rates (R_S). Known amounts of deuterated PAH standards (Table 3-1) of the same polarity with the target PAH compounds were added to the SR samples as internal standards prior to extraction and to compensate for loss during the exhaustive Soxhlet extractions and processing. The percent recoveries of the spiked CB 112 on the SR samplers used as recovery standard for the PCBs ranged from 68 to 81%. In this study, the amounts of PAHs sequestered by the SR-PSDs were sufficiently high (i.e. 10 times higher than amounts in the procedural and field control blanks); hence, blank corrections were not necessary. However, the sequestered amounts of PCBs were very low and were close to the amounts in the procedural blanks, consequently, blank corrections were performed for the PCBs using a correction factor included in the passive sampling general uptake model as described in Emelogu et al. (2013) and Smedes and Booij, (2012).

Analyses of all pesticide and acid/urea herbicide samples were performed at the trace organic chemistry Unit of the Scottish Environment Protection Agency (SEPA), East Kilbride, Scotland, UK. The laboratory is accredited to ISO 17025 standards for the determination of pesticide and acid/urea herbicides in freshwaters. During method development, validation procedures were performed using river water and groundwater samples and matrix effects were assessed in the appropriate spiked matrices. Internal quality control procedures include preparation and analyses of process blank, calibration standards, independent process check standards (IPCS), independent calibration check standards and calibration drift check standards alongside the samples. Process blanks and IPCS (i.e. blank samples spiked with secondary calibration standards containing all target pesticides and acid/urea herbicides) were processed as samples. The mean recoveries and RSD (n=8) for the 47 pesticides were in the range of 64-117% and 5.6-29% respectively, except for boscalid that had a recovery and RSD of 54 and 56% respectively. While, the mean recoveries for the acid/urea herbicides range 90-108% and the %RSDs (n=6) were all less than 9%, except for pencycuron which was 16%. In addition, surrogate acid/urea herbicides standard (Table 3-1) was also used as an indicator of performance of the LC-MS/MS procedure during the

analyses of acid/urea herbicides. The recovery for the herbicide surrogate standard at the time of the analysis was 98%. The choice of 10 mL as sample volume in the analysis of acid/urea herbicide using LC-ESI-MS/MS was also to further minimise any potential matrix effects in the LC-MS/MS analysis. This approach has been shown to be effective in this study and elsewhere (e.g. Kuster et al., 2008).

The limit of detection (LOD), limit of quantification (LOQ) and precision of each selected pesticide and acid/urea herbicide studied in the water samples were determined through method validation, by analysing 11 independent procedural blank samples. The LOD was defined as the mean value of the blank samples plus 4.65 times the within-batch standard deviation (S.D; n=11) of the mean values. The LOQ was calculated as 3 times the LOD. The method LOD and LOQ for pesticides and acid/urea herbicides in the water samples were considered adequate for the silicone rubber samples. The LOD and precision for the selected pesticides and acid/urea herbicides are provided in Tables 3-2A and B. The LOD for PAHs and PCBs sequestered by the SR samplers were defined as two times the area count in the field blank; expressed as aqueous concentration (ng L^{-1}), the aqueous LOD varied at each sampling site, depending upon deployment duration and sampling rates (R_S).

3.2.5 Data analysis and handling

3.2.5.1 Water samples

The concentration of individual selected pesticides and acid/urea herbicides analysed in the samples were calculated using Eqns. 3.1 and 3.2 respectively:

$$X_1 = \frac{[Y_1 * D_1] - B_1}{R * \left(\frac{C}{1000}\right)} \quad (3.1)$$

$$X_2 = \frac{[Y_2 * D_2] - B_2}{R} \quad (3.2)$$

Where X_1 and X_2 are the concentrations (ng L^{-1}) of substance in the samples using GC-MS/MS and LC-MS/MS respectively; Y_1 and Y_2 = raw result from quantification (ng mL^{-1} and ng L^{-1} respectively); D_1 and D_2 = are the dilution factors, if applicable (i.e. $V_{final\ extract}$ [µL]) /

$V_{original\ extract}$ [µL]); B_1 and B_2 = process blank value (ng mL^{-1} and ng L^{-1} respectively); R= the % mean process recovery / 100; and C = the concentration factor (initial volume/final volume). The mean (n=6) concentration of pesticides and acid/urea herbicides from each weekly water samples was calculated. Where concentrations of samples fell below the LOQ, half of the value of LOQ was used for the calculation of the mean as recommended by the EU Directive 2009/90/EC (EC, 2009a).

3.2.5.2 Passive samplers

Passive sampling requires calibration data to convert the amount (ng) of target compounds absorbed by the samplers to the freely dissolved aqueous concentrations (e.g. ng L^{-1}). To achieve this, sampler-water partition coefficients (log K_{SW}) and the sampling rate (R_S) values for the target compounds are essential for fully quantitative assessments. The log K_{SW} values of SR-PSDs have not yet been developed for the pesticides and acid/urea herbicides examined here. It is possible, although with a level of uncertainty, to estimate the log K_{SW} values from empirical correlations with physicochemical properties of the compound e.g. from log K_{OW} values (e.g. Adams et al., 2007) or preferably, from aqueous solubility values (Lohmann, 2011). Alternatively, the polyparameter linear free energy relationships can also be used to estimate log K_{SW} values (Endo et al., 2011; Lohmann et al., 2012). However, estimation of R_S of target compounds without knowledge of *in situ* exchange kinetics through the use of PRCs can lead to errors. In addition, other than log K_{SW} values, knowledge of the sampler-water diffusion coefficient (D_P) of the analytes is equally essential so as to estimate the water concentrations to a level of accuracy. For the majority of the polar and semi-polar pesticides, uptake may be limited by diffusion into the sampler membrane. Hence, in this study, the results of the pesticides and acid/urea herbicides from the samplers are presented on a semi-quantitative basis as the amount absorbed by the sampler (i.e. ng g SR $sampler^{-1}$). The log K_{SW} values and sufficient knowledge of the D_P in SR-PSDs are available for PAHs and PCBs; hence full quantification was possible for these chemicals. By using the dissipation rates of the PRCs from the samplers during deployment and the available log K_{SW} values obtained from Smedes et al. (2009), the site-specific sampling rates (R_S; L d^{-1}) of each PAH and PCB compound with known log K_{SW} was calculated by applying an empirical model (Huckins et al., 2006; Smedes et al., 2009). The recently improved non-linear least squares (NLS) model for estimating *in situ* compound-specific R_S and uncertainties (Booij and Smedes, 2010) was used in this study. From the amount of PAHs and PCBs sequestered by the SR samplers and the site specific R_S, the dissolved time weighted average concentration (C_{TWA}) of PAHs and PCBs were calculated using an empirical uptake

model that is valid for both equilibrium, transitional and linear uptake system (Smedes and Booij, 2012). A more detailed description on how these methods were applied to derive the C_{TWA} of PAHs and PCBs, including the quality control procedures, are provided in Emelogu et al. (2013). The accuracy of the estimated dissolved concentrations of the PAHs and PCBs is highly dependent on the values of log K_{SW} and R_S used. Sums of PAHs and of PCBs were calculated using zero as the concentration for analytes that could not be detected.

3.3 Results and discussions

3.3.1 Performance reference compounds (PRCs) and sampling rates (R_S)

All the deployed SR samplers were successfully retrieved after 54 days and visual inspection of the samplers did not indicate either membrane damage or excessive biofouling. Similarly, the autosamplers functioned effectively throughout the sampling period and weekly samples were collected from sites 2 and 3, except for two weeks in December when samples were not collected for logistical reasons. PRC data suggested relatively slow transfer kinetics of the compounds during the sampling period as even the low K_{ow} compounds (e.g. D10-fluorene) could be quantified (minimum 4.5% recovery compared to un-deployed sheets). There were significant differences in the dissipation rates of PRCs between the three study sites, with site 1 showing the lowest loss of compounds (i.e. lowest dissipation rate). The PRC derived R_S for an average compound of molecular mass 300 at the three streams in this study and the previous year using SR-PSDs (Emelogu et al., 2013) are similar as illustrated in Figure 3-2.

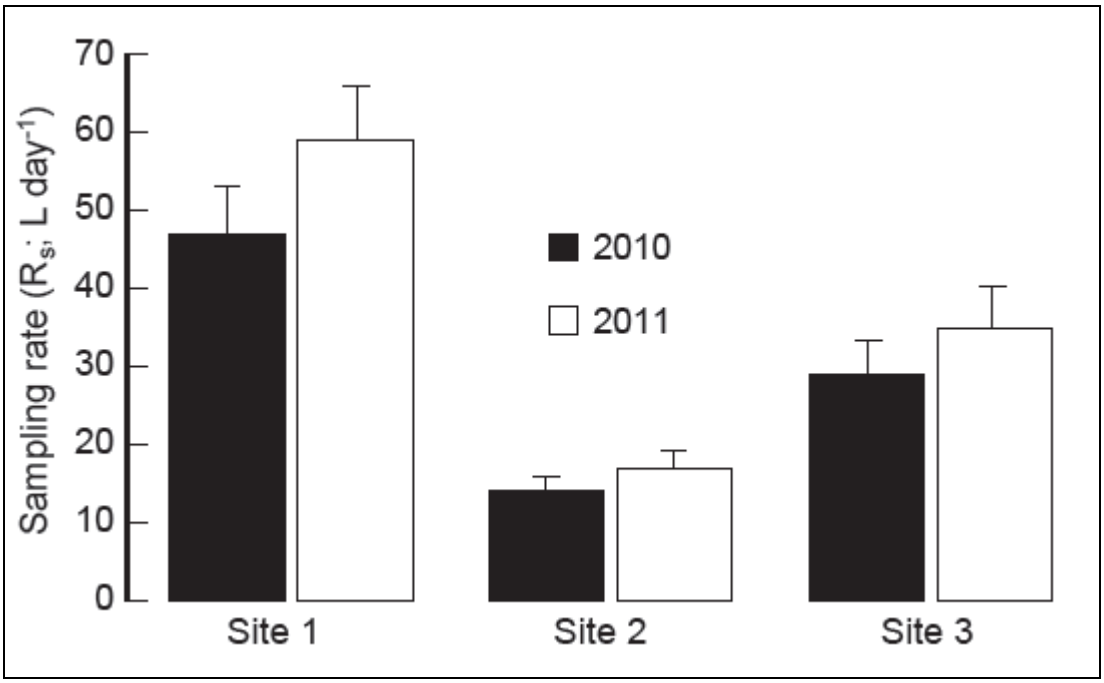

Figure 3-2 Comparison of PRC derived in situ sampling rates in this study (2011) and previous study (2010) for a compound of mass 300. Error bars shows standard deviation.

The % RSD of the PRC derived *in situ* R_S at the three sites in both studies range 9.4-11%. High temperature values were recorded in water in this present study in comparison with the previous study and this might have contributed to the slightly higher PRC derived in situ R_S in this study. An increase in environmental temperature increases the water solubility of compounds and decreases partitioning to waterborne particulate matter (see e.g. Söderström

et al., 2009). The similarity of the PRC derived *in situ* R_S for a compound of molecular mass 300 at the three streams in this study and the previous year using SR-PSDs indicates the reproducibility and robustness of these devices in freshwater systems.

Table 3-3 Summarised concentrations of (A) selected pesticides and (B) acid/urea herbicides measured in silicone rubber (SR) samples (ng g^{-1} SR sampler) and water samples (ng L^{-1}). Water sample concentrations are mean ± S.D; *n*=6. The numbers of weekly detections in the water samples are shown in parenthesis. Note, no water samples taken from site 1.

		Site 1	Site 2		Site 3	
	Log K_{OW} [a]	SR-PSD (ng g^{-1} SR sampler)	SR-PSD (ng g^{-1} SR sampler)	Water (ng L^{-1})	SR-PSD (ng g^{-1} SR sampler)	Water (ng L^{-1})
A						
Pesticides						
Atrazine	2.7	2.7	2.1	N.D	2.5	N.D
Azoxystrobin	2.5	<LOD	<LOD	N.D	1.2	N.D
Boscalid	2.96	<LOD	<LOD	N.D	6.7	N.D
Carbofuran	1.8	3.5	1.6	N.D	2.1	N.D
Chlorfenvinphos (Z)	3.8	1.87	1.3	N.D	1.4	N.D
Chlorothalonil	2.94	<LOQ	<LOQ	N.D	<LOQ	N.D
Chlorpyrifos ethyl	4.7	4.6	6.3	N.D	6.9	N.D
Chlorpyrifos methyl	4.0	2.4	1.4	N.D	1.3	N.D
Cyanazine	2.1	1.5	0.74	N.D	1.1	N.D
Cyproconazole	3.09	<LOD	<LOD	N.D	1.5	N.D
Cyprodinil	4.0	3.9	2.2	N.D	3.7	N.D
Diazinon	3.69	4.1	2.7	N.D	3.2	N.D
Dichlobenil	2.7	2.0	1.3	N.D	1.4	N.D
Dichlorvos	1.9	1.7	1.04	N.D	1.0	N.D
Diflufenican	4.2	6.7	6.5	N.D	13	N.D
Dimethoate	0.704	2.5	1.9	N.D	2.2	N.D
Epoxiconazole	3.3	<LOQ	<LOQ	N.D	<LOQ	N.D
Fenoxycarb	4.07	<LOD	<LOD	N.D	2.2	N.D
Flusilazole	3.87	4.5	2.3	<LOD(1)	25	N.D
Heptenophos	2.39	2.6	1.8	N.D	2.0	N.D
Kresoxim methyl	3.4	1.6	1.0	N.D	1.7	N.D
Malathion	2.75	2.4	1.2	N.D	1.5	N.D
Metalaxyl	1.65	2.2	1.7	N.D	1.8	N.D
Metazachlor	2.49	12	25	N.D	25	N.D
Methiocarb	3.18	<LOQ	<LOD	N.D	<LOQ	N.D
Metribuzin	1.65	2.0	0.89	N.D	1.2	N.D
Mevinphos	0.127	2.5	1.6	N.D	1.8	N.D
Oxadixyl	0.65	0.94	0.42	N.D	1.3	N.D
Parathion-ethyl	3.83	2.5	1.4	<LOD(1)	1.2	N.D
Pendimethalin	5.2	16	12	<LOD(1)	25	N.D
Picoxystrobin	3.6	2.7	4.2	N.D	6.9	N.D
Pirimicarb	1.7	2.5	1.8	N.D	2.2	N.D
Pirimiphos methyl	3.9	2.8	1.8	N.D	1.8	N.D
Prometryn	3.34	1.3	1.3	N.D	1.6	N.D
Propetamphos	-	<LOQ	<LOQ	N.D	<LOQ	N.D
Propiconazole	3.72	3.1	3.2	N.D	7.1	N.D
Simazine	2.3	2.4	1.6	N.D	1.5	N.D
Tebuconazole	3.7	1.9	1.9	N.D	6.8	N.D
Terbuthylazine	3.4	<LOQ	<LOQ	N.D	<LOQ	N.D
Terbutryn	3.66	2.5	6.4	N.D	4.3	N.D
Triadimefon	3.18	2.4	2.7	N.D	2.4	N.D
Triadimenol	3.18	<LOQ	<LOQ	N.D	<LOQ	N.D

Trietazine	3.34	2.0	1.4	N.D	1.7	N.D
Trifloxystrobin	4.5	1.31	0.65	N.D	1.6	N.D
Trifluralin	5.27	4.0	11	N.D	4.9	N.D
Vinclozolin	3.02	2.38	1.58	N.D	1.65	N.D
B						
Acid herbicides						
Bentazone	1.67	N.D	N.D	<LOD(6)	N.D	7±1(6)
Mecoprop	-0.19	N.D	N.D	17.1±11(6)	N.D	7±1(6)
MCPB	1.32	N.D	N.D	<LOD(6)	N.D	6±1
MCPA	-0.81	N.D	N.D	<LOD(6)	N.D	<LOD(6)
2,4 D	-0.83	N.D	N.D	<LOD(6)	N.D	<LOD(6)
2,4 DB	1.35	N.D	N.D	<LOD(6)	N.D	<LOD(6)
Benazolin	1.34	N.D	N.D	<LOD(6)	N.D	<LOD(6)
Bromoxynil	1.04	N.D	N.D	<LOD(6)	N.D	<LOD(6)
Clopyralid	-2.63	N.D	N.D	<LOD(6)	N.D	<LOD(6)
Dicamba	-1.88	N.D	N.D	<LOD(6)	N.D	<LOD(6)
Dichloroprop	2.29	N.D	N.D	<LOD(6)	N.D	<LOD(6)
Fluoroxpyr	0.04	N.D	N.D	<LOD(6)	N.D	<LOD(6)
Ioxynil	2.2	N.D	N.D	<LOD(6)	N.D	<LOD(6)
Triclopyr	4.62	N.D	N.D	<LOD(6)	N.D	<LOD(6)
Urea Herbicides						
Diuron	2.87	17	25	6±9(6)	19	<LOD(6)
Isoproturon	2.5	267	78	13±16(6)	26	33±41(6)
Monolinuron	2.2	14	18	<LOD(6)	21	<LOD(6)
Metoxuron	1.6	1.1	1.0	N.D	5.1	N.D
Fenuron	0.98	2.9	2.0	<LOD(6)	1.1	<LOD(6)
Pencycuron	4.68	<LOQ	2.0	10±9	3.0	<LOD
Linuron	3.0	654	658	<LOD(6)	826	7±3(6)
Chlorotoluron	2.5	443	456	45±22(6)	117	89±60(6)

[1] The log K_{OW} values were obtained from PPDB (2012). LOD= limit of detection (mean blank values + 4.65 X S.D); LOQ=limit of quantification (3 X LOD).N.D=not detected

Concentrations of pesticides and herbicides measured in silicone rubber passive samples are not directly comparable to the mean individual concentrations in the water samples.

MCPB=4-(4-chloro-2-methylphenoxy) butanoic acid; MCPA= 4-chloro-o-tolyloxyacetic acid; 2,4 D= (2,4-dichlorophenoxy)acetic acid; 2,4 DB=4-(2,4-dichlorophenoxy)butyric acid.

3.3.2 Pesticides and acid/urea herbicides

None of the 47 pesticides and 22 acid/urea herbicides analysed for were detected in the process blank passive sampler extracts. A wide range of pesticides and acid/urea herbicides were detected in all the three streams using SR-PSDs and autosamplers. Results for individual pesticides and acid/urea herbicides measured in the SR-PSDs and weekly water samples during sampling period are provided in Table 3.3 A-B. Amongst the pesticides and acid/urea herbicides sequestered by the SR samplers, the largest amounts were for pendimethalin, diflufenican, metazachlor, chlorpyrifos ethyl, trifluralin, linuron, chlorotoluron, isoproturon and diuron (Table 3.3 A-B). With the weekly water samples, acid and urea herbicides (particularly, chlorotoluron, isoproturon, diuron, bentazone and mecoprop) were dominant both in terms of frequency of weekly detection and concentration in water. Only three selected pesticides (flusilazole, parathion-ethyl and pendimethalin) were detected in water with the weekly water samples over the 6 weeks sampling period.

Some of the acid herbicides including, bentazone, MCPB and mecoprop, which were detected in the water samples but were not detected via SR samplers, have very low octanol-water partition coefficients (e.g. log K_{OW} <2) and this may partially explain the observed lack of detection by SR-PSDs. However, this does not provide a full explanation because there were instances where lower log K_{OW} chemicals were detected in the SR-PSD samples. Similarly, in our previous study, mecoprop and 2,4-D were detected in water samples taken at the same sites and other sites in the Ythan catchment using SR samplers (Emelogu et al., 2012). The sorption of pesticides with acidic or neutral properties in water is influenced by a variety of environmental conditions including the pH level of the water since partitioning to organic matter is considerably lower with charged species than the neutral species (Helweg et al., 2003). For the acid herbicides, a combination of physicochemical properties, such as dissociation constant (pKa) and log K_{OW} values would influence their absorption by the samplers from water. Acid herbicides most often exist in ionised form at environmental pH values (Wells and Yu, 2000); the average water pH values at sites 2 and 3 during the deployment period were 7.71 and 7.66. This could mean that most of the acidic herbicides were in the charged and therefore more water-soluble form during the sampling period, not being detected in the SR-PSD samples might suggest possible limitations due to their low log K_{OW}. In addition to sorption and water solubility, degradation is another vital factor that influences the fate of pesticides in the environment. Most pesticides breaks down over time as a result of chemical (e.g. photolysis, hydrolysis, oxidation and reduction) and microbiological reactions in soils and majority of these factors are determined by specific local conditions. Further probing on the limitations of SR-PSDs for such chemicals will feature in future work.

In the weekly water samples from sites 2 and 3, the urea herbicides, particularly, chlorotoluron exhibited a relatively high concentration and frequency of detection in comparison with other acid/urea herbicides. Similarly, the amounts of chlorotoluron sequestered by the SR-PSDs at the three sites were also high in comparison with other acid/urea herbicides. This may reflect the increasingly common usage of chlorotoluron as a soil-acting herbicide *in lieu* of isoproturon, which has been banned from use within the EU since June 2009. Another urea herbicide, linuron, exhibited the highest amount of acid/urea herbicide sequestered by the SR-PSDs from all three sites during the 8 weeks of deployment. In the water samples, however, linuron was measured at < LOQ at site 2 and was only marginally quantifiable at site 3 (Table 3.3 B).

Highest concentrations and frequencies of pesticide and acid/urea herbicide detections in the weekly water samples from autosamplers were observed in November, particularly for the acid and urea herbicides. This can be attributed to their use in farming activities in the catchment, especially during the winter cropping season. Generally, and consistent with the known activities in the catchment, the pesticides and acid/urea herbicides identified in water in this study appear to be related to agricultural practices. This is also in agreement with Skark et al. (2004), who concluded that the occurrence of herbicides such as chlorotoluron, isoproturon and terbuthylazine in surface water could be due to their broad agricultural applications.

Amongst the pesticides measured in the streams during this study, some are banned or have severely restricted uses within Europe as set down by various European Commission Regulations and Directives on the placing on the market and use of PPPs (e.g. EC, 1978; EC, 1991; EC, 2009b). Such substances include diuron, isoproturon, chlorpyrifos, monolinuron, atrazine, trifluralin, dichlorvos, diazinon, fenuron, diflufenican, mevinphos and parathion ethyl. The sources of their environmental occurrence in the catchment cannot be established with certainty from this study, but their presence might reflect a legacy from previous legitimate agricultural use and/or more recent, post-restriction, use. The fate of pesticides in the environment is complex and cannot be exclusively predicted using sorption capability e.g. log K_{OW} values. Pesticides that are adequately resistant to degradation and relatively soluble in water can easily be transported to surface waters and detected months after application. The relatively long persistence of some of these pesticides including isoproturon, atrazine, trifluralin, and diuron in soil-water after last application have been demonstrated in both under controlled conditions and in some field studies (Buhler et al., 1993; Johnson et al., 1996; Kreuger, 1998). Nevertheless, the sources of their environmental occurrence and potential ecological impact warrant further investigation. Importantly, the

majority of these banned or tightly restricted compounds detected in the streams were detected using the SR samplers and were not detected in the weekly water samples.

3.3.3 Freely dissolved PAHs and PCBs

The freely dissolved aqueous concentrations of individual and total PAHs ($\sum PAH_{40}$; parent and branched) and ∑16 US-EPA PAHs determined following use of the SR passive samplers are shown in Table 3. 4 with the lowest concentration measured at site 1 and the highest at site 2. These concentrations are comparable with those measured in 2010 at the three sampling sites and elsewhere in the Ythan catchment. In the previous study, Emelogu et al. (2013) measured sum dissolved concentrations of 40 PAHs in the range of 38 to 69 ng L^{-1}. Similarly, the individual and total dissolved concentrations of PAHs measured in water at the sampling sites were comparable to those reported in a recent study using passive samplers. Prokeš, et al. (2012) used SR-PSDs to measure a total mean concentrations (sum of 16 US-EPA PAHs) in the range of 48 to 93 ng L^{-1} at five sites on the River Morava, an industrial and agricultural region in the Czech Republic. The profile patterns of freely-dissolved PAHs at the three sampling sites in the Ythan catchment are shown in the supplementary information Fig. SI; 4-1. It is evident that the 2- and 3-ring, low molecular weight (LMW), PAHs were the most abundant, whereas the high molecular weight (HMW) PAHs (5- and 6-ring) were minor components of the total PAHs. By plotting PAH concentration ratios, it is possible to identify the likely source(s) of the PAHs (Yunker et al., 2002; Webster et al., 2004). In sediments and mussels, fluoranthene/pyrene (Fl/Py) ratios >1 and methylphenanthrene/phenanthrene ratios (Mp/P) of <2 are indicative of pyrolytic sources (incomplete combustion). In contrast, Fl/Py ratios of <1 and Mp/P ratios of >2 are indicative of petrogenic sources. Comparing the composition of the PAHs sequestered by the SR samplers with these diagnostic ratios suggests a mainly pyrolytic source at sites 1 and 3 and mixed sources (pyrolytic and petrogenic) of PAHs at site 2 (see supplementary information; SI; Fig 4-2).

Table 3-4 Time weighted average (TWA) concentrations (ng L-1) of freely dissolved PAHs detected in water using silicone rubber passive samplers.

	SITE		
	1	**2**	**3**
Naphthalene	6.89	12.10	11.1
2-Methyl Naphthalene	1.91	2.80	2.90
1-Methyl Naphthalene	1.99	3.02	2.75
C2 Naphthalenes	3.02	4.50	3.40
C3 Naphthalenes	0.90	1.38	1.38
C4 Naphthalenes	0.31	1.36	0.76
Phenanthrene	3.20	4.90	5.90
Anthracene	0.01	0.32	0.26
C1 178	1.11	2.29	2.54
C2 178	0.52	1.43	1.12
C3 178	0.26	0.83	0.65
Dibenzothiophene	0.13	0.91	0.32
C1 Dibenzothiophenes	0.14	0.62	0.29
C2 Dibenzothiophenes	0.08	0.38	0.28
C3 Dibenzothiophenes	0.14	0.79	0.98
Fluoranthene	0.92	1.56	1.93
Pyrene	0.68	1.59	1.37
C1 202	0.51	0.83	0.95
C2 202	0.11	0.30	0.25
C3 202	0.05	0.20	0.15
Benz[*a*]anthracene	0.02	0.04	0.04
Chrysene/Triphenylene	0.14	0.38	0.35
Benz[*b*]anthracene	<LOD	0.01	0.01
C1 228	0.07	0.20	0.15
C2 228	0.03	0.15	0.15
Benzofluoranthenes *	0.03	0.12	0.11
Benzo[*e*]pyrene	<LOD	0.09	0.07
Benzo[*a*]pyrene	0.01	0.05	0.01
Perylene	0.02	0.03	0.02
C1 252	<LOD	<LOD	<LOD
C2 252	<LOD	0.47	0.04
Indenopyrene	<LOD	0.01	0.01
Benzoperylene	<LOD	0.01	<LOD
C1 276	<LOD	<LOD	0.01
C2 276	<LOD	<LOD	<LOD
Acenaphthylene	1.10	1.70	1.40
Acenaphthene	1.30	4.30	2.90
Fluorene	1.30	3.90	2.90
Dibenz[*a,h*]anthracene	<LOD	0.01	<LOD
∑PAHs	27	54	47
∑16 USEPA PAHs	16	31	28

LOD =limit of detection, 2 X the concentrations (ng g-1 SR sampler) in the field blank sample; expressed as aqueous concentration (ng L^{-1}), the aqueous LOD varied at each sampling site, depending upon deployment duration and sampling rates (R_S). * Benzofluoranthenes is the sum of benzo[*b*]fluoranthene and benzo[*k*]fluoranthene.

All target PCB compounds were detected in water at the three sites; generally, the freely dissolved concentrations of the individual PCBs were very low and varied from <0.1 to 2.4 pg L^{-1}. The individual concentrations of the 32 PCBs; sum concentrations of the seven Indicator-PCBs (CB 28, 52,101,118,153,138 and 180) and the sum concentrations of 32 PCBs

measured in water at the three sites are presented in Table 5. The measured freely dissolved PCB concentrations were very low in comparison with some other studies in freshwater systems using passive samplers. Verweij et al. (2004) measured freely dissolved concentrations of individual PCBs up to 595 pg L^{-1} in 10 freshwater sites within the city of Amsterdam using SPMDs. In another study using SR passive samplers, a total mean concentration (sum of 7 indicator PCBs) in the range of 140-440 pg L^{-1} were measured at five sites on the River Morava within an industrialised and agricultural region in the Czech Republic (Prokeš, et al., 2012). The higher concentrations in these other studies likely reflect the greater urban and industrial pressures in those areas compared to the agricultural catchment investigated here.

A predominance of the less highly chlorinated PCBs (e.g. tri-, tetra- and penta-) in surface waters commonly reflects atmospheric depositions. Where particulate bound waterborne sources dominate (i.e. particularly in estuaries) a higher proportion of the more hydrophobic, highly chlorinated hexa- and hepta-PCBs is often found (Webster et al., 2011). At all three in-land freshwater sites examined in this study the predominance of tetra-, penta- and hexa-chlorinated PCBs was apparent (supplementary information; Fig. SI 4-3), indicating possible contributions from particulate bound waterborne and atmospheric sources. This may also reflect the historical usage of these PCB congeners as tetra-, penta- and hexa-chlorinated PCBs were major constituents of Aroclors 1254 and 1260 PCB mixtures (Breivik et al., 2002).

Table 3-5 Time weighted average (TWA) concentrations (pg L^{-1}) of freely dissolved PCBs detected in water using silicone rubber passive samplers.

	SITE		
	1	**2**	**3**
		pg L^{-1}	
CB31	0.38	0.81	0.56
CB28	0.34	0.98	0.60
CB52	2.50	<LOD	<LOD
CB49	0.46	0.18	0.26
CB44	0.77	0.47	0.01
CB74	0.35	0.53	0.36
CB70	1.30	2.10	0.98
CB 101	2.40	1.99	1.10
CB 099	0.33	<LOD	<LOD
CB 097	0.26	0.17	0.01
CB 110	0.80	2.40	0.54
CB 149	0.54	0.11	<LOD
CB 123	0.87	2.80	1.39
CB 118	0.60	0.21	<LOD
CB 114	0.20	0.52	0.22
CB 132	0.34	0.39	<LOD
CB 153	0.52	0.80	0.30
CB 105	0.42	0.80	0.37
CB 137	0.13	0.33	0.11
CB 138	0.95	1.30	0.42
CB 158	0.17	0.48	<LOD
CB 187	0.20	0.94	0.23
CB 183	0.16	0.43	0.24
CB 128	0.31	0.89	0.36
CB 167	0.19	0.59	0.29
CB 156	0.08	0.43	0.20
CB 157	<LOD	0.56	<LOD
CB 180	0.29	0.47	0.33
CB 170	0.10	0.23	0.09
CB 189	0.03	0.49	0.37
CB 194	0.36	<LOD	0.32
CB 209	0.14	0.17	0.21
∑ $PCBs_{32}$	16.5	22.4	9.87
∑7 Indicator-PCBs	7.60	5.80	2.80

LOD = limit of detection, 2 X the concentrations (ng g-1 SR sampler) in the field blank sample; expressed as aqueous concentration (ng L^{-1}), the aqueous LOD varied at each sampling site, depending upon deployment duration and sampling rates (R_S). The bold PCBs are the 7 congeners used as indicators by the International Council for the Exploration of the Sea (ICES).

The use of SR passive samplers in this study has allowed the quantification of PCBs in the Ythan catchment, which has not previously been possible using conventional water or biota monitoring. The Scottish Environment Protection Agency (SEPA) investigated the concentrations of selected persistent organic pollutants (POPs), including PCBs, at 30 sites across Scotland subject to industrial, population and agricultural pressures over a four year period using freshwater eels (*Anguilla anguilla*) as the biomonitor species (Macgregor et al., 2010). In contrast to the results obtained here with SR-PSDs, in the eel study, PCBs were not detected above the LOD in samples collected from the River Ythan (Macgregor et al., 2010), but were detected in eels sampled elsewhere in Scotland. The PCB congener profile reported here is consistent with those reported previously in the Ythan (Emelogu et al., 2012) and in Scottish eels (Macgregor et al., 2010). Concentrations of individual and total PCBs in this study are also comparable with those measured in 2010 at the three sampling sites and elsewhere in the Ythan catchment. In the previous study, the measured sum concentration for 32 PCBs varied from 20 to 60 pg L^{-1}. The similarity of results in both studies indicates that the inputs of PAHs and PCBs to the Ythan catchment are stable and the sources are not changing significantly in the area.

3.3.4 Comparison of silicone rubber passive sampling and autosamplers

The comparison between SR passive sampling and automatic sampling techniques in this study was based on semi-quantitative and quantitative determinations of the presence or absence of pesticides and acid/urea herbicides in the samples. A total of 54 substances, comprising 46 selected pesticides and 8 acid/urea herbicides, were detected in at least one extract of the SR-samplers deployed in water at the three sites during the 8 weeks of sampling. Of these, 40 pesticides and all 8 urea herbicides (diuron, isoproturon, monolinuron, metoxuron, fenuron, pencycuron, linuron, and chlorotoluron) were quantifiable (Table 3-3 A-B); six selected pesticides (chlorothalonil, propetamphos, epoxiconazole, terbuthylazine, triadimenol and methiocarb) were identified but were below the analytical LOQ and a further 14 acid/urea herbicides analysed for were not detected. By comparison, a total of 25 substances comprising 3 pesticides and 22 acid/urea herbicides were detected in the weekly water samples collected from the two streams using autosamplers. The 8 acid/urea herbicides (diuron, isoproturon, pencycuron, linuron, chlorotoluron, bentazone, mecoprop and MCPB) could be quantified (Table 3-3 A-B). All the pesticides and acid/urea herbicides except mecoprop, bentazone and MCPB measured in the water samples were also measured in the SR samples, which may indicate a current limitation of SR-PSDs for these

chemicals. Similarly, there were 14 acid herbicides that were not detected in the SR samples but were detected in the water samples, albeit at concentrations lower than LOQ.

As depicted in Table 3-3 A-B, most of the pesticides and acid/urea herbicides sequestered by the SR-PSDs were of varied hydrophobicity whereas the majority of the compounds measured in the weekly water samples were of low log K_{OW} (e.g. the acid and urea herbicides). This reflects the high sensitivity and utility of the SR samplers to sample both semi-polar to non-polar pesticides. SR-PSDs showed greater potential than autosamplers in providing evidence of diffuse input of a wide range of pesticides in the streams of the Ythan catchment as evidenced by the far greater number of compounds quantified above LOQ. This reflects, and affirms, that SR-PSDs achieve lower LODs/LOQs through sampling a larger volume of water compared to the weekly water samples from autosamplers. However, partition and diffusion coefficients are required in order to determine dissolved concentrations of compounds from use of passive sampling and this remains an information gap that needs filling in order to allow wider use of passive sampling in water quality monitoring.

3.3.5 Potential environmental and ecotoxicicological implications of study

The EU Water Framework Directive (2000/60/EC) and its daughter Directive (2008/105/EC) have established EQS values for a set of priority and priority hazardous substances (EC, 2000; EC, 2011), while other non-statutory chemical thresholds are also in use in the UK (SEPA, 2011). EQS values are expressed as annual average (AA) and maximum allowable concentration (MAC) to protect against long and short term exposures respectively. In this study, the dissolved concentrations of the individual PAHs determined by SR-PSDs were 2-3 orders of magnitude lower than the criteria defined by the WFD EQS-AA for PAHs (Table 3-6). Caution is required, however, when making this comparison because EQS values are defined for total (unfiltered) water samples while passive sampling produces time weighted average concentrations (C_{TWA}) of freely-dissolved compounds only. The true comparability of PSD results and current EQS still needs to be determined. It should also be kept in mind that this passive sampling study was conducted over a 2 month period, whereas EQS-AA values are set for 12 month periods. There are currently no EQS set for PCBs under the WFD, although this might change in future.

Table 3-6 Water Framework Directive (WFD) proposed environmental quality standard (EQS) values for select pesticides and polycyclic aromatic hydrocarbons (PAHs) in freshwater (EC, 2011).

	Chemical Abstrac Service number (CAS No.)	Annual average (AA)-EQS µg L^{-1}	Maximum allowable concentrations (MAC)- EQS
Substances			
Atrazine	1912-24-9	0.6	2
Chlorpyrifos-ethyl	2921-88-2	0.03	0.1
Chlorfenvinphos	470-90-6	0.1	0.3
Dichlorvos	62-73-7	6×10^{-4}	7×10^{-4}
Diuron	330-54-1	0.2	1.8
Isoproturon	34123-59-6	0.3	1
Terbutryn	886-50-0	0.065	0.34
Trifluralin	1582-09-8	0.03	not applicable [a]
Anthracene	120-12-7	0.1	0.1
Benzo[a]pyrene	50-32-8	1.7×10^{-4}	0.27
Benzo[*b*]fluoranthene	205-99-2	1.7×10^{-4}	0.017
Benzo[*k*]fluoranthene	207-08-9	1.7×10^{-4}	0.017
Benzo[*g,h,i*]perylene	191-24-2	1.7×10^{-4}	8.2×10^{-3}
Fluoranthene	206-44-0	0.0063	0.12
Indeno[1,2,3-*cd*]pyrene	193-39-5	1.7×10^{-4}	not applicable*
Naphthalene	91-20-3	2	130

[a] The EQS-AA is applied where no MAC concentration for a substance is defined.

The mean concentrations of pesticides and acid/urea herbicides obtained in water samples collected over 6 weeks were assessed against the EQS-AA and the weekly individual results against EQS-MAC. Although EQSs are not available for all pesticides and acid/urea herbicides measured in this study, observed concentrations did not exceed any available EQS-AA/MAC values as shown in Table 3-6. The highest mean concentration of 89 ng L^{-1} was recorded for chlorotoluron at site 2, which was well below the non-statutory EQS-AA of 2,000 ng L^{-1} and the highest weekly individual result was 136 ng L^{-1} which was equally below the non-statutory EQS-MAC of 20,000 ng L^{-1} (SEPA, 2011). This indicates that, on an individual basis, the chemicals measured in the catchment are not at levels associated with any known environmental harms based on WFD. Nevertheless, this does not completely safeguard against potential mixture effects, which is a recognised knowledge gap. Diffuse source contamination of agriculturally applied pesticides is widely recognised as one of the most important anthropogenic stressors in stream ecosystems (Schulz, 2004). Such diffuse contamination is known to exert a variety of effects on all aquatic ecosystems at different trophic levels. For example, on a sublethal level, our previous study showed that extracts of SR samplers deployed in the Ythan catchment had greater potential to induce characteristic enzyme activity (cytochrome P450 activity, EROD) in a 72 h exposure of rainbow trout liver (*Oncorhynchus mykiss;* RTL-W1) cell line than could be attributed to the measured individual concentrations of PAH and PCBs (Emelogu et al., 2013). Similarly, some recent studies have demonstrated that pesticide mixtures, albeit at low environmental concentrations can have remarkable impacts on the biodiversity of the aquatic community. A study that assessed the impact of runoff-related pesticide contamination, amongst other environmental factors, that contributes to differentiation in the macroinvertebrate communities in six streams in Braunschweig, Lower Saxony, Germany concluded that the existing pesticide concentrations affect the invertebrate community structure in the field (Liess and Ohe, 2009). In another study, Relyea, (2009) showed that a mixture of five insecticides (malathion, carbaryl, chlorpyrifos, diazinon, and endosulfan) and five herbicides (glyphosate, atrazine, acetochlor, metolachlor, and 2,4-D) at low concentrations (2-16 µg L^{-1}) affected aquatic

communities composed of zooplankton, phytoplankton, periphton and larval amphibians. Further, Hayes et al. (2006) demonstrated that nine pesticide mixtures including four herbicides, two fungicides and three insecticides at low ecologically relevant concentrations (0.1 µg L^{-1}) could have dramatic effects on amphibian development and growth, and ultimately survivorship. Similar indirect effects have been demonstrated in other studies using pesticide mixtures at environmentally relevant concentrations (Schäfer et al., 2007; Ricart et al., 2010). The use of SR-PSDs in this study has further facilitated the identification of additional contaminants in the River Ythan catchment particularly at sites 2 and 3, which have been missed in previous studies using conventional water sampling and biota techniques.

3.4 Conclusions

This study further demonstrates the higher utility and sensitivity of silicone rubber passive sampling over conventional water sampling for the reliable identification of a wide range of relevant pesticides (plant protection products) in freshwater systems. The PRC derived *in situ* sampling rates for a compound of mass 300 and the dissolved concentrations of PAHs and PCBs measured in water in this study using SR-PSDs were comparable with those measured in the previous year at the same sites. Evidence was presented that a broad range of pesticides and acid/urea herbicides are entering the streams of the Ythan catchment as a possible result of agricultural and related land use practises. Numerous compounds which hitherto have not been detected in the catchment using conventional spot sampling techniques, including some plant protection products that are not currently authorised for agricultural use in the EU, were identified in this study using silicone rubber passive sampling. The individual compounds or their mixtures are possibly contributing to the observed poor ecological indicators in some streams within the catchment.

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Disclaimer

The opinions and conclusions expressed in this paper are solely those of the authors and do not necessarily represent the views of the host institutions.

Supplementary material in appendix 4

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4 CHAPTER FOUR: Occurrence and potential combined toxicity of dissolved organic contaminants in the Forth estuary and Firth of Forth, Scotland assessed using passive samplers and an algal toxicity test

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Abstract

As an alternative procedure to conventional water quality assessment, the presence and combined toxicity of dissolved organic contaminants in water at five sites in the Forth estuary and the Firth of Forth, Scotland, United Kingdom was investigated using silicone rubber passive sampling devices (SR-PSDs) and an algal growth inhibition bioassay. SR-PSDs were deployed in water at the five sites for ~2 months. Following retrieval, extracts from the deployed SR-PSDs were assessed for both algal growth inhibition and the occurrence of a wide range of organic contaminants, including polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and a variety of plant protection products (PPPs; commonly referred to collectively as 'pesticides'). The 72 h algal growth inhibition test was performed using a native marine phytoplankton (*Diacronema lutheri*) in 24 well microplates. Freely dissolved (e.g. bioavailable) concentrations of PAHs and PCBs were determined using performance reference compounds (PRCs). The algal toxicity tests exhibited varied effects at the five sites indicating the presence of, and exposure to, phytotoxic compounds and their potential toxicity in the Forth. The individual and total dissolved concentrations of 40 PAHs and 32 PCBs measured in the study were relatively low and showed input of petrogenic, atmospheric and sewage-related sources. Several pesticides of diverse polarities were identified in the water suggesting sources from both riverine input and direct discharges. The study thus illustrates the value of combining bioassays and chemical analysis (with effective sampling technique) for a realistic and rapid assessment of organic contaminants in the aquatic environment.

Keywords

Estuary
Silicone rubber passive sampling
Algal growth inhibition
Firth of Forth
Water quality
Organic contaminants

4.1 Introduction

The Forth estuary and Firth of Forth (Fig. 4-1) located on the east coast of central Scotland, UK are important resources for humans, plants and wildlife, but they are under pressure from a variety of human activities. The waters receive direct discharges from petrochemical, agro-chemical and pharmaceutical industries and discharges of urban waste water. Other possible sources of contaminant inputs into the estuary include refineries, a coal-fired power station and a number of shipping ports. Diffuse input from agricultural and urban run-off further adds to the contaminant pressure in the estuary. The major constituents of discharges into most estuaries are complex mixtures of heavy metals and organic compounds (Villars and Delvigne, 2000; Kennish, 2002). Amongst the organic constituents of direct discharges and diffuse inputs are priority and historic contaminants e.g. polycyclic aromatic hydrocarbons (PAHs) and some emerging contaminants e.g. plant protection productions (PPPs; commonly referred to collectively as 'pesticides'). Many organic contaminants are persistent, bioaccumulate and in some instances, biomagnify (e.g. the polychlorinated biphenyls; PCBs) and generally result in adverse effects on aquatic organisms.

Water quality in the Forth estuary and Firth of Forth is compromised by discharges of industrial effluent and treated sewage. Although, due to contemporary legislation, effluent treatment has improved and direct input of trace organic contaminants into the Forth estuary and Firth of Forth has reduced considerably in recent years. This has led to returning populations of residential and migratory fish and reducing environmental concentrations of contaminants (Baxter et al., 2011). As a result, in addition to the low aqueous solubility of most organic contaminants and dilution factors, the concentrations of the majority of the priority organic contaminants monitored in the water phase using conventional sampling techniques are often low or below instrumental detection limit (Dobson et al., 2001; Matthiessen and Law, 2002). This could lead to an underestimation of environmental exposure and risk owing to insufficient knowledge of their freely dissolved concentrations (e.g. bioavailability) as well as the mixture effects on sensitive aquatic organisms. Further, whilst, there is increasing evidence of environmental exposure to multiple contaminants at low environmental concentrations, the potential ecotoxicological and human risks remain poorly understood.

Phytoplanktons are the major primary producers in the marine ecosystems; any alteration in the phytoplankton community would affect the entire ecosystem (Burkiewicz et al., 2005). Phytoplanktons are also very sensitive bioindicators, they are often the first targets to be

affected by environmental exposure to some anthropogenic contaminants (Eklund and Kautsky, 2003; Fränzle, 2006). Knowledge of the chronic effects of sublethal exposure to environmentally relevant concentrations of organic contaminants on the phytoplankton estuarine community and a method to assess these effects are essential. In addition, algal growth inhibition tests have been shown to be rapid cost effective and sensitive to a variety of chemicals (Geis et al., 2000; Radix et al., 2000; Okamura et al., 2002; Sbrilli et al., 2005; and Levy et al., 2007). However, the majority of these studies have focused on single toxicants, and not the toxicologically relevant environmental mixtures; therefore, they lack environmental realism.

Passive sampling devices (PDSs) are now widely acknowledged as suitable monitoring tools for a wide range of ultra-trace concentrations of the freely dissolved fractions of the total contaminants in a variety of environmental matrices (Vrana et al., 2005; Allan et al., 2006; Huckins, 2006; and Greenwood et al., 2009). They absorb and pre-concentrate contaminants during an extended sampling period, as a result, much lower limit of detection is achieved, through sampling a large volume of water. The uptake of non-polar organics to non-polar passive samplers typically follows three stages: linear or kinetic, curvilinear or intermediate and equilibrium. In equilibrium sampling phase, dissipation of absorbed analytes from the sampler may occur, when concentration in water fluctuates, however, pollution events are not likely to be missed, as opposed to conventional spot or bottle water sampling. This not only improves the effective analytical detection limits, but also provides equilibrium or time-weighted average concentrations of the bioavailable compounds, which are the toxicologically relevant components (Lohmann et al., 2012). In addition to chemical analysis, extracts of PSDs which have been deployed in an environmental matrix can be evaluated for their toxicity potential and this linked to the sequestered contaminants. This can also help to identify if some toxicologically relevant contaminants are being missed by routine environmental monitoring and therefore guide the direction of future environmental assessments.

The present study is aimed at assessing the presence and combined toxicity of dissolved organic contaminants in water from the Forth estuary and the Firth of Forth using silicone rubber (SR-PSDs) and algal growth inhibition tests. Chemical analyses were focused on PAHs (parent and branched), PCBs (*ortho* and mono-*ortho*) and selected PPPs (including insecticides, fungicides, and herbicides), with a separate sub-category of acid/urea herbicides examined as a distinct group. To the best of our knowledge, no study has linked algal toxicity with freely dissolved concentrations of organic contaminant mixtures derived

from SR-PSDs. Another dimension that was added to the study was the use of principal component analysis (PCA) to further evaluate the possible sources of the freely dissolved PAHs and PCBs in the estuary and the Firth of Forth. This paper presents the current status of dissolved concentrations and combined toxicity of organic contaminants in water of Forth estuary and Firth of Forth.

4.2 Materials and methods

4.2.1 Description of study area and sampling sites

Five sites (sites 1 to 5; Fig.4-1) along the Forth estuary and Firth of Forth were selected for sampling in this study. The Forth estuary is the area between the tidal limit at Stirling bridge to the Forth rail bridge at South Queensferry, whilst, the area to the east is the coastal waters of the Firth of Forth. The first three sampling sites, Alloa (AL), Grangemouth West (GW) and Crombie (CB) were located in the Forth estuary, whilst the fourth and fifth sampling sites, Braefoot (BT) and Gunnet Ledge (GL) were situated in the Firth of Forth. The major rivers draining into the Forth estuary include the Forth, Teith, Alan, Devon, Black Devon, Carron and Avon (Villars and Delvigne, 2000) with an average yearly freshwater input of 63 m^3 s^{-1} (Leatherland, 1987). The actual river discharge varies, depending on the season, ranging from less than 10 m^3 s^{-1} in summer to over 300 m^3 s^{-1} in winter (Balls, 1992). The rivers Almond, Esk, Leven and Water of Leith drain into the Firth of Forth in addition to discharges from the Forth estuary, accounting for a yearly average runoff of 87 m^3 s^{-1} (Leatherland, 1987). There are also several direct discharges of “freshwaters” from municipal sewages and industrial effluents into the estuary and Firth of Forth (see Fig.4-1). The five sites were chosen to incorporate contributions from some of the major discharge points in the Forth estuary and the Firth of Forth, the shipping ports and contributions from tributaries. Generally, the Forth estuary is turbid and the turbidity level is highest at Alloa and decreases with distance to the Firth of Forth. Other major characteristics of the Forth estuary and the Firth of Forth have been reviewed by Webb and Metcalfe (1987). Table 4-1 presents some of the characteristics of the sites during the sampling period. The data were gathered by the Scottish Environment Protection Agency (SEPA) using continuous remote monitoring devices deployed at Alloa and Gunnet Ledge.

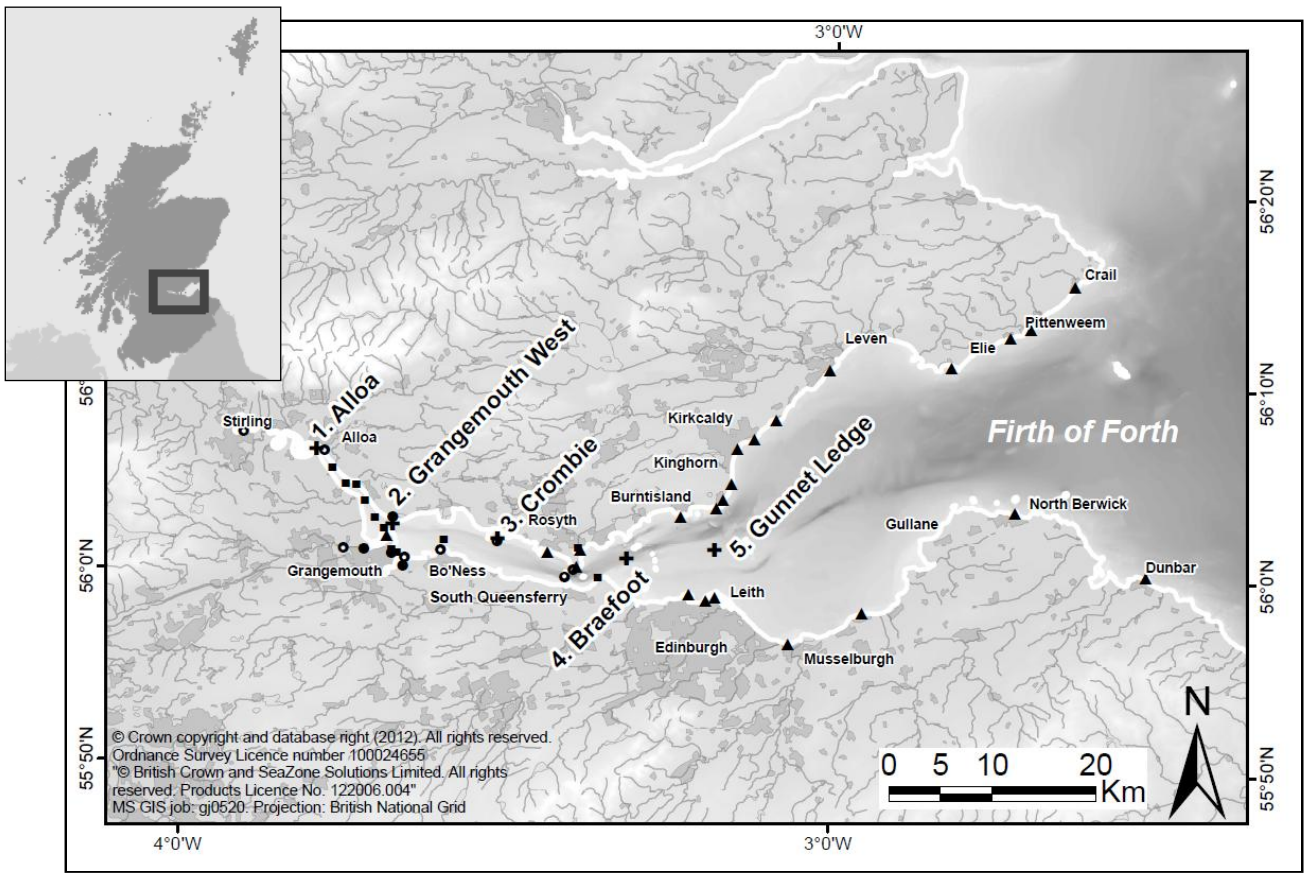

Figure 4-1 Firth of Forth showing the five sampling stations (+; 1 to 5), some selected discharge points (■ industrial outfalls; O sewage outfalls) and shipping ports (▲); with longitude (x-axis) and latitude (y-axis). Inset is a map of Scotland, showing the approximate location of the Forth estuary and Firth of Forth (within the black box).

4.2.2 Passive sampling and extraction of silicone rubber passive samplers

The procedures for the preparation and processing of the SR-PSDs, including, pre-extraction, spiking of performance reference compounds (PRCs; Huckins, 2006), deployment, retrieval and extraction followed Emelogu et al. (2013a). Briefly, two sets (one set for the toxicologic al assessment and the other for chemical analysis) of food grade Altesil® translucent SR-PSDs were pre-extracted for ~100 h in boiling ethyl acetate using a Soxhlet extraction apparatus. The SR-PSDs to be used for chemical determination were spiked with fourteen PRCs (4 deuterated PAHs and 10 PCBs) having water-octanol partition coefficients (log K_{OW}s) in the range of 4.14-7.3. Both sets (6 sheets per set, total 20±2 g) of SR-PSDs of SR-PSDs were attached to stainless steel cages and deployed in water at depths of 2-5 m by attaching the cages to navigation buoys at each of the five sampling sites (Fig. 4-1) for a total of 44-51 days between February and April 2011 (see Table 4-1). During deployment, a set of pre-extracted, PRC-free, SR-PSDs were kept in ultra-pure water, to

serve as procedural blanks for the algal growth inhibition tests. A further set of PRC-spiked SR samplers were exposed to ambient air during deployment and retrieval and served as field blanks for the chemical analysis, and for the determination of the initial concentrations of the PRCs spiked to the SR-PSDs.

Table 4-1. General characteristics of the sampling sites in the Forth estuary and the Firth of Forth during the sampling period. (The physicochemical data are the average from 30 min interval readings throughout the sampling periods).

Sampling Site	Location		Total Deployment	Water Depth	Deployment depth	Temp	pH	Salinity	Dissolved oxygen (DO)	Turbidity
	Latitude	Longitude	Time (d)	(m)	(m)	(° C)		(ppt)	(mg L^{-1})	(NTU)
AL	56.0901°N	3.7775°W	44	4	2	6.7	7.4±0.3	4±5.2	10±1.4	215.8±275.5
GW	56.0388°N	3.6638°W	51	6	5					
CB	56.0265°N	3.5304°W	51	13	5					
BT	56.0204°N	3.3293°W	51	28	5					
GL	56.0234°N	3.1770°W	51	30	5	6.2	N.M	34±0.8	10±1.2	N.M

AL=Alloa, GW=Grangemouth West, CB=Crombie, BT=Braefoot and GL=Gunnet Ledge. N.M=not measured

After retrieval, two out of the six SR sheets at each site for toxicological assessment (equivalent to 6.6 g of SR sampler) and all the SR samplers for chemical analysis, including the field blanks, were extracted separately for algal growth inhibition tests and chemical analysis respectively. Extraction was performed for 24±4 h using a Soxhlet apparatus with an azeotropic mixture of boiling acetonitrile (ACN) and methanol (MeOH; 2:1 v/v). The extracts were cleaned-up (in order to remove any co-extracted SR oligomers) by passing through glass solid phase extraction (C8 SPE; Mallinckrodt Baker, London, UK) columns and eluting with ACN. The column eluents were pre-concentrated with a Kuderna-Danish evaporation apparatus followed by activated carbon purified nitrogen blow down. Prior to algal growth inhibition and chemical analysis, extracts were solvent exchanged to appropriate solvents. In this study, each SR sampler extract for the algal growth inhibition tests was solvent exchanged into 1 mL of dimethyl sulfoxide (DMSO; 99.9% purity, Sigma-Aldrich Dorset, UK) before bioassay, rather than being tested in methanol as previously described (Emelogu et al., 2013a). DMSO is a more suitable solvent to use in algal toxicity bioassays (Ma and Chen, 2005). The extracts targeted for chemical analysis were aliquoted into three equal

volumes for the determination of PAHs and PCBs (including the PRCs) and pesticides and acid/urea herbicides. All extracts were stored at -20 ºC for less than 2 months prior to chemical analysis or the algal growth inhibition bioassays. Algal toxicity of the silicone rubber samples was expressed relative to the equivalent mass of SR per mL of DMSO extract (e.g. mg SREQ mL^{-1}).

4.3 Analytical procedures

4.3.1 Chemical analysis

The individual concentrations of PRCs (D12-chrysene, D12-benzo[*e*]pyrene, D10-fluorene and D10- fluoranthene and CBs 10, 14, 21, 30, 50, 55, 78, 104, 155, and 204), 40 individual and groups of PAHs covering both parent and branched compounds including the 16 US-EPA (Environmental Protection Agency) PAHs, 32 PCBs (*ortho* and mono-*ortho*) including the seven International Council for the Exploration of the Sea (ICES) indicator PCBs and pesticides (47 selected pesticides and 22 acid/urea herbicides) were analysed using a combination of gas chromatography (GC) and liquid chromatography (LC) techniques. The detectors applied include mass spectrometry (MS) for PAHs, an electron capture detector (ECD) for PCBs and tandem MS for pesticides and acid/urea herbicides. The detailed descriptions of the analytical procedures including the quality control and assurance procedures and the limits of detection and quantification (LOD and LOQ) are described in previous studies by Emelogu et al. (2013a,b). Tables 1 A, B and C (supporting information; S.I) show the summary of the PAHs, PCBs and pesticides and acid/urea herbicides targeted in this study.

4.3.2 Algal strain and culture

A unicellular flagellated marine prymnesiophyte, *Diacronema lutheri* (Bendif et al., 2011, synonym, *Pavlova lutheri* Droop; Green, 1975; CCAP 931/6) was used as the test organism in this study. *D. lutheri,* originally isolated from the Firth of Clyde, Scotland (~ 55.667°N 5°W) was obtained from the Culture Collection of Algae and Protozoa (CCAP), Scottish Marine Institute, Argyll Scotland and was grown at 20±2°C at a constant light illumination (cool-white fluorescent lights) with a photosynthetic active radiation (PAR) of ~150 µMol m^{-2} s^{-1}. The

growth medium used was 0.45 µm filtered natural sea water (pH 8.2±0.2; salinity of 33±2) obtained from the Scottish area of the North Sea and supplemented with f/2 medium (Guillard, 1975). Prior to the addition of supplements, the filtered seawater and all glassware used in the algal toxicity testing were sterilised in an autoclave at 121°C for 40 min. The culture flasks were gently shaken by hand at least once daily. Cultures were renewed weekly by aseptically transferring stock (5 mL) to new culture media (150 mL) for a continuous supply of exponentially growing cells for the algal toxicity tests.

4.3.3 Algal toxicity tests of passive sampler extracts

Algal growth inhibition tests are traditionally conducted in Erlenmeyer flasks with a 50 to 500 mL test volume using freshwater algae e.g. *Scenedesmus subspicatus* or *Pseudokirchneriella subcapitata* and often involve cell counting with microscopes (ISO, 2000). However, in this study, for cost and time effective purposes as well as environmentally relevant testing, the combined toxicity of dissolved organic contaminants in the Forth estuary and Firth of Forth was assessed on a native marine phytoplankton *D. lutheri,* using a standard 72 h exposure (ISO, 2012), but using modified test plates and measurement procedures.

The test plates were untreated 24-well microplates with round-bottomed wells (Corning, Sigma-Aldrich, Dorset UK). Briefly, algal culture in the exponential growth phase (5–7 days old) was adjusted to 1 x 10^4 (± 10%) cells *per* mL of test medium. The cultures were inoculated to the wells in the microtitre plates prior to exposure to extracts of SR-PSDs (deployed and undeployed) at concentrations of ~6.66, 3.33, 1.66, 0.83 and 0.42 mg SREQ mL^{-1} of final test medium. The highest concentration of DMSO in all the tests was kept at 0.1% (v/v). A preliminary range finding test showed that 0.1% DMSO had no significant algal toxicity on *D. lutheri* cells. During each batch test, the reference chemical 3,5-dichlorophenol (DCP; Sigma-Aldrich, Dorset, UK) was added to a separate plate at concentrations of ~10, 7.5, 5, 2.5 and 1.25 mg L^{-1} as a positive control and used to ensure the validity of the test method.

For all samples, each test concentration and blank control (containing only algal growth media with no phytoplankton or DMSO) was assayed in triplicate wells, while growth controls (containing phytoplankton and test media only*)* had six replicates. Three of the growth controls were located on the wells next to the samples with the highest concentration and the other three were close to the samples with lowest extract concentration. Further details of the exposure format on the 24 well microtitre plates are provided in Eisentraeger et al. (2003). The final test

volume in each well was 2 mL and each sample was tested in duplicate on two different plates. To minimise evaporation inter-well spaces of the microtitre plate were filled with water (Lukavský, 1992) and the wells were covered with lids that have condensation rings to reduce cross contamination. The microtitre plates were put into a heat sealed transparent polyethylene bag to further prevent water evaporation and were incubated at 20±1ºC under constant illumination (cool-white fluorescent lights) with PAR of ~150 µMol m^{-2} s^{-1} at the surface of the microtitre plate lid and continuous shaking at 100 rpm. Positions of the microplates with respect to the lamp were changed daily in order to reduce light inclination. Algal growth was measured using a fluorometric data acquisition system (PerkinElmer VICTOR™ 3 Multilabel Counter plate reader, Bucks, UK) at intervals of 24 h over a period of 72 h. The plates were shaken (double orbital) before measurement for 2 s to resuspend cell pellets. Each microtitre plate was read twice on each occasion using excitation and emission wavelengths of 450 nm and 680 nm respectively. The fluorescence reading was converted to cell density by multiplying with a pre-determined conversion factor; this was estimated through direct counting of cells in control cultures with a haemocytometer using a fluorescence microscope (Zeiss Axiovert 200 M; Carl Zeiss GmbH, Jena, Germany). The pH value in the growth control test solutions was measured at the start and at the end of the test with an MP-220, pH meter (Metler-Toledo, GmBH, Schwerzenbach, Switzerland). The measured pH values of the growth control replicates ranged from 7.99 to 8.40 during the 72-hour exposure, thus, meeting the test validity criteria (ISO, 2012).

4.4 Data handling

4.4.1 Passive sampling data

Detailed descriptions of the procedures used in the calculation of the *in situ* sampling rates (R_S; L d^{-1}) from the performance reference compounds (PRCs) spiked on the silicone rubber passive sampling devices (SR-PSDs) prior to deployment and the concentrations (ng L^{-1} and ng g^{-1} SR sampler) of target compounds are provided elsewhere (Emelogu et al., 2013a,b). Briefly, a non-linear least squares (NLS) model for estimating PRC derived *in situ* compound-specific R_S was applied (Booij and Smedes, 2010). The amounts (ng) of individual PAHs and PCBs absorbed by the SR-PSDs were converted to their freely dissolved concentrations (C_W; ng L^{-1}) using the determined *in situ* R_S and an empirical uptake model that is valid for linear, transition and equilibrium uptake situations (Smedes and Booij, 2012). The estimation of the water concentrations (ng L^{-1}) of PAHs and PCBs from the absorbed amount was possible in this study because of the availability of the SR sampler-water partition coefficients (log K_{SW}s), which were obtained from Smedes et al. (2009). However, the log K_{SW}s for pesticides and acid/urea herbicides analysed in water in this study have not yet been established. Hence the analysis was semi-quantitative (see Emelogu et. al., 2013b) and the concentrations of individual pesticides and acid/urea herbicides sequestered by the SR samplers were normalised to their respective sampler weight and presented in ng g of SR $sampler^{-1}$. Dissolved concentrations (ng L^{-1}) of individual PAHs and PCBs were summed to produce total freely dissolved concentrations and are denoted as the $\sum PAH_{40}$ and $\sum USEPA_{16}$ for the PAHs and $\sum PCB_{32}$ and $\sum ICES\text{-}PCB_7$ for the PCBs.

4.4.2 Algal toxicity data

For each sample, the mean fluorescence reading from the blank control wells (i.e. wells with culture medium alone) was subtracted from the readings in all the other wells. The average specific growth rate (cell division; μ) for the individual test concentrations in each sample compared to the growth control was calculated for each concentration using Equation 1:

$$\mu = \frac{\ln N_{72} - \ln N_0}{t} \qquad \text{Equation 4.1}$$

Where:

N_{72} is the measured algal density at the end of the test
N_0 is the measured algal density at the start of the test
t is the period of test (72 h)

The percentage growth inhibition *Ii* (%) at each test concentration (of average specific growth rate relative to growth controls) was calculated for each sample using:

$$I_i(\%) = \frac{\mu_c - \mu_i}{\mu_c} \times 100 \qquad \text{Equation 4.2}$$

Where:
I_i *(%)* is the percent algal growth inhibition for test concentration *i*
μ_C= is the mean growth rate for the control wells
μ_i= is the growth rate for test concentration *i*

Each I_i (%) value of the test concentrations was plotted against the respective test concentration on a logarithmic scale. A sigmoidal concentration-inhibition model with variable slopes i.e. four-parameter logistic nonlinear regression analysis using GraphPad Prism 5.0, software (GraphPad, San Diego, California USA) was used to calculate the median effective concentration (EC) values (with 95% confidence limits) for each sample using:

$$Y = \text{Bottom} + \frac{(\text{Top} - \text{Bottom})}{1 + 10^{(\text{LogEC}50 - X) \cdot \text{Hillslope}}} \qquad \text{Equation 4.3}$$

Where:
Bottom is the Y value at the bottom plateau;
Top is the Y value at the top plateau;
LogEC50 is the X value when the response is halfway between Bottom and Top.
The HillSlope describes the steepness of the curve.
The toxicity result of each tested sample was expressed as EC_{10}-72 and/or EC_{50}-72 value.

4.4.3 Source apportionment (ratios and principal component analysis; PCA)

Concentration ratios of some specific PAHs are often used to identify either their petrogenic or pyrolytic sources, particularly in sediments (Webster et al., 2001; Yunker et al., 2002). Fluoranthene/pyrene (Fl/Py) ratios <1 and methylphenanthrene/ phenanthrene (Mp/Ph) ratios >2 are indicative of a petrogenic PAH source. Further, the predominance of alkylated 2- and 3-ring PAHs indicates a predominantly petrogenic input, while the dominance of heavier parent PAHs is indicative of mainly pyrolytic input. Although some level of caution must be exercised due to the variable of hydrophobicity of the PAHs and thus the impact on their uptake by the SR-PSDs, concentration ratios of the PAHs sequestered by SR-PSDs from water were used to discriminate their possible sources in the Forth estuary and the Firth of Forth.

Further, a multivariate statistical technique using principal component analysis (PCA) was applied to the data of freely dissolved concentrations of PAHs and PCBs to further assess their similarities and differences and to evaluate their possible sources at the Forth estuary and the Firth of Forth. PCA was performed using Minitab version 15 Statistical software. The use of PCA facilitates the reduction of the original dataset to fewer factors (i.e. principal components; PCs) without significant disruption of the relationships present in the original dataset (Reid and Spencer, 2009). The dissolved concentrations of PAHs were normalised to the total PAH concentrations at each site. The dissolved concentrations of PCBs were normalised to CB153 (which is a predominant PCB) so as to remove the variance and produce relative contaminant patterns (Echarri et al., 1998).

4.5 Results

4.5.1 Dissolved concentrations of selected organic contaminants in the Forth estuary and Firth of Forth

All target PAHs and PCBs were detected in water in the Forth estuary and Firth of Forth using silicone rubber passive sampling devices (SR-PSDs) after 6-7 weeks of sampling. However, from the semi-quantitative analysis, only a total of 8 selected pesticides and 5 acid/urea herbicides were detected in the extracts of SR-PSDs from the five sites. The PRC derived *in situ* sampling rates (R_S; L day^{-1}) for a compound of average mass 300 ranged from 17.4 (±.3.7) L day^{-1} to 21.0 (± 4.0) L day^{-1} in water at the three sites of Forth estuary and 8.2 (±1.4) L day^{-1} to 11.1(±1.9) L day^{-1} in the Firth of Forth. From Figure 4-2, it can be seen that the R_S at the three sites in the Forth estuary (i.e. Alloa, Crombie and Grangemouth West) were similar and slightly greater than those in the Firth of Forth. This shows that the sampling kinetics at the three sites in the Forth estuary were faster than the sites in the Firth of Forth and this can be attributed to the strong tidal currents in the Forth estuary.

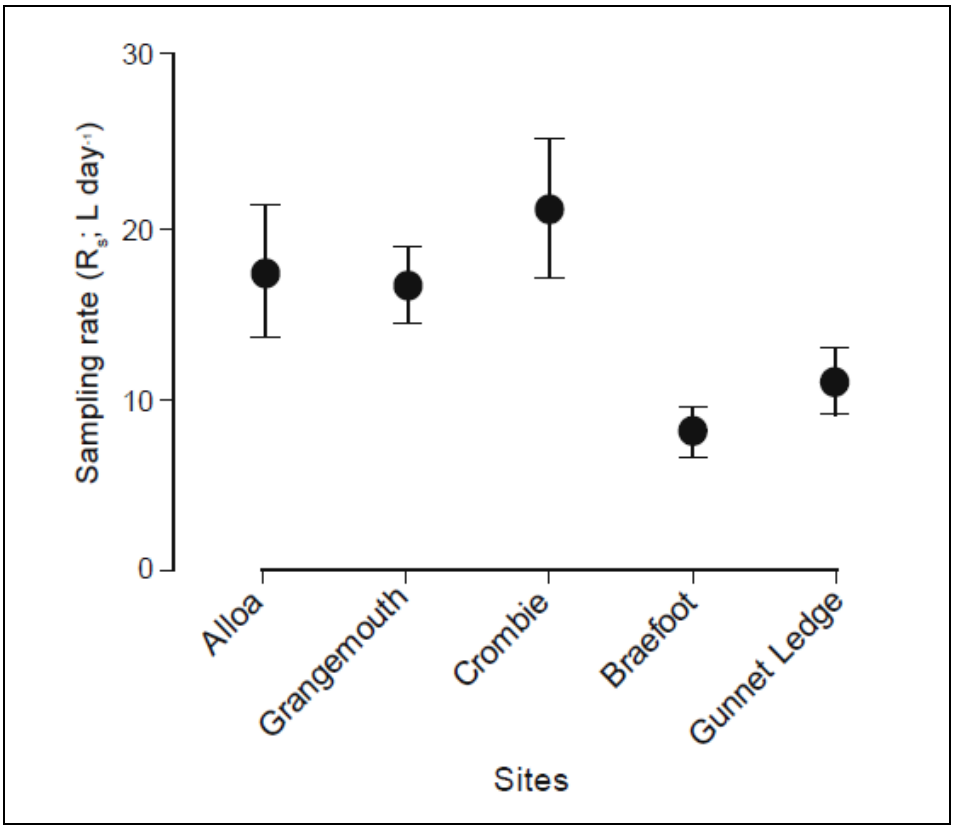

Figure 4-2 The *in situ* derived sampling rates (R_S) of a compound of mass 300 in water at the Forth estuary and Firth of Forth after 6-7 weeks of sampling using silicone rubber passive sampling devices (SR-PSDs). Error bars indicate the standard deviation (±SD).

The measured R_S of each target PAH and PCB with known log K_{SW} was used to derive their respective water concentrations (ng L^{-1}) at the various sites. The summary of the total freely dissolved concentrations of PAH ($\sum PAH_{40}$) and PCB ($\sum PCB_{32}$) including the 16 US-EPA PAH ($\sum$16 US-EPA PAHs) and the ICES indicator PCBs ($\sum$ICES-PCB_7) and the concentrations (ng g SR $sampler^{-1}$) of pesticides and acid/urea herbicides are presented in Table 4-2. The individual dissolved concentrations of PAHs and PCBs are shown in the supporting information (SI 5; Table 4-2 and 4-3;). The total dissolved concentration of PAHs ($\sum PAH_{40}$) at the five sites ranged from 48.6 to 69.5 ng L^{-1} (Table 4-3) with the lowest concentration measured at Gunnet Ledge. The highest concentrations were observed in the Forth estuary, particularly at Grangemouth West, which is closest to the potential sources of pollution.

The total concentrations of freely dissolved $\sum PCB_{32}$ ranged 0.16 to 0.30 ng L^{-1} (Table 4-2), while the total concentrations of ICES indicator PCBs ($\sum$ICES-$PCBs_7$) were similar at the five sites and were all less than or equal to 0.13 ng L^{-1} (Table 4-2). From the semi-quantitative analysis of the extracts for pesticides and acid/urea herbicides, a total of 13 pesticides and acid/urea herbicides (Table 4-2) of varying hydrophobicities were detected in water at the

Forth estuary and Firth of Forth using SR-PSDs. This further highlights the utility of this devices and it ability to sequester a wide range of contaminants including the semi-polar compounds (e.g. pesticides and acid/urea herbicides), other than the non-polar compounds (e.g. PAHs and PCBs) that they were originally used for.

Table 4-2: The total concentrations of PAHs ($\sum PAH_{40}$), ∑16 USEPA PAHs and PCB_S $\sum PCB_{32}$, $\sum$ICES-PCB_7 and pesticides and acid/urea herbicides measured in water at five sites within the Forth estuary and Firth of Forth over six-seven weeks using silicone rubber passive samplers.

	Site				
Parameter	Alloa	Grangemouth	Crombie	Braefoot	Gunnet
PAHs(ng L^{-1})					
$\sum PAH_{40}$	67.3	69.5	54.1	50.6	48.6
∑16 USEPA PAHs *	21.6	26.1	22.0	20.4	26.0
Fl/Py[1]	0.64	0.64	0.68	0.79	0.97
Mp/Ph[2]	2.19	2.79	4.18	4.15	2.69
PCBs (ng L^{-1})					
$\sum PCB_{32}$	0.30	0.22	0.16	0.25	0.19
$\sum$ICES-PCB_7	0.13	0.09	0.07	0.10	0.08
(ng g SR $sampler^{-1}$)					
Chlorpyrifos ethyl	8.75	6.67	4.68	5.35	2.48
Dimethoate	<LOD	<LOD	<LOD	1.61	0.93
Flusilazole	0.81	<LOD	0.84	0.61	<LOD
Pendimethalin	11.0	8.07	11.4	13.1	4.90
Pirimicarb	<LOD	0.37	<LOD	<LOD	<LOD
Propiconazole	3.69	10.78	5.40	5.12	2.77
Terbutryn	1.52	<LOD	0.94	<LOD	<LOD
Triadimefon	<LOD	<LOD	<LOD	0.72	<LOD
Diuron	<LOD	0.93	<LOD	6.94	4.30
Isoproturon	<LOD	0.66	<LOD	0.72	4.05
Pencycuron	1.02	<LOD	<LOD	<LOD	<LOD
Linuron	878	957	766	796	742
Chlortoluron	<LOD	168	<LOD	144	155

[1] and [2] Ratios of fluoranthene/pyrene (Fl/Py) and methylphenanthrene/phenanthrene (Mp/Ph) sequestered from water in the Forth estuary and Firth of Forth. LOD = limit of detection

4.5.2 Algal toxicity of extracts of silicone rubber passive samplers

D. lutheri exhibited dose responsive growth inhibition following 72 h exposure to 3,5-DCP, with an average EC_{50}-72 of 5.0 mg L^{-1} with 95% confidence limit of 3.15 mg L^{-1} to 6.84 mg L^{-1}. In other studies, using freshwater algae in microtitre plates, Eisentraeger et al. (2003) reported a higher EC_{50}-72 value (10.70–11.00 mg L^{-1}) for 3,5-DCP using *Desmodesmus subspicatus*, while, Paixao, et al. (2008) reported 1.96 (1.70–2.11) mg L^{-1} using *Pseudokirchneriella subcapitata*. In this study, statistically significant (p<0.05) growth inhibition in comparison to the growth control, using one-way ANOVA, Dunnett's post hoc test (GraphPad Prism 5.0) was not observed after 72 h exposure of *D. lutheri* to extracts of undeployed SR samplers (i.e. procedural blank). As a result, EC was not calculated for extracts of procedural blank SR-PSDs (undeployed) due to insufficient growth inhibition data. In contrast, all samples from the five sites of the Forth estuary and the Firth of Forth exhibited algal toxicity after 72 hour exposure of *D. lutheri*, particularly at concentrations of 3.4-6.7 mg SREQ mL^{-1} extract. However, it was not possible to derive an EC_{50}-72 from this data as the dose–response was below maximum levels. This indicates that chronic exposure to complex mixtures of dissolved organic contaminants in water from the Forth estuary and Firth of Forth has potential to exhibit moderate growth inhibition to *D. lutheri*. The EC_{10}-72 values with 95% confidence limits (95% CI) for the extracts of SR-PSDs deployed in water at the Forth and Firth of Forth following a 72 hr exposure to *D. lutheri* are shown in Table 4-3, while, the concentration-response curves are shown in Figure 5-3 (supporting information, SI).

Table 4-3: Median effective concentrations (EC) of dissolved mixtures of organic contaminants from the Forth estuary and the Firth of Forth on *D. lutheri* following 72 h exposure

	Algal growth inhibition	
	EC_{10} (mg SREQ L^{-1})	CI (95%)
Samples		
Blank SR extract (undeployed)	NC[1]	NC[1]
Alloa	3.68	1.41 to 5.94
Grangemouth West	4.53	2.54 to 6.51
Crombie	4.87	3.27 to 6.47
Braefoot	4.59	3.75 to 5.43
Gunnet Ledge	2.29	1.24 to 3.33

[1]NC= not calculated, due to insufficient effect data

4.6 Discussion

The Forth estuary and the Firth of Forth are major centres for agrochemical, petrochemical and refining industries in Scotland and receive high inputs of urban and industrial wastes. In this study, the individual and total dissolved concentrations of PAHs and PCBs measured in water using SR-PSDs in the Forth estuary and Firth of Forth were similar to those measured in some other marine waters using passive sampling devices (PSDs). For example, Harman et al. (2009) reported a $\sum PAH_{27}$ (parent and alkylated; excluding naphthalene) in the range of 32 to 49 ng L^{-1} in water around an oil production platform in the Norwegian section of the North Sea using semi-permeable membrane devices (SPMDs). Similarly, Emelogu et al. (2013a) reported a sum total concentration ($\sum PAHs_{40}$) of 69 ng L^{-1} in the Ythan estuary, North-East Scotland, UK, using SR-PSDs. However, total concentrations of $\sum PAH_{15}$ (excluding naphthalene) in the range of 3.9 and 170 ng L^{-1} were reported by Monteyne et al. (2013) in water from seven monitoring stations along three major Belgian coastal harbours.

Table 4-4 Factor loadings of dissolved concentrations of (A) PAHs (normalised to the total concentrations) and (B) dissolved concentrations of PCBs (normalised to CB153) in the Forth estuary and Firth of Forth. The main correlations between variables are in bold.

A	Factors			
Variable	PC1	PC2	PC3	PC4
Naphthalene	-0.175	**0.120**	-0.179	-0.058
2-Methyl Naphthalene	-0.045	**0.348**	0.089	0.164
1-Methyl Naphthalene	-0.087	**0.262**	0.245	0.143
C2 Naphthalenes	0.09	0.196	**0.279**	**0.279**
C3 Naphthalenes	0.178	0.01	**0.196**	-0.153
C4 Naphthalenes	**0.201**	0.027	0.062	-0.051
Phenanthrene	-0.091	-0.225	-0.301	0.008
Anthracene (178)	**0.193**	-0.035	0.13	0.064
C1-178	-0.118	-0.263	**0.163**	-0.147
C2-178	**0.182**	-0.15	0.069	-0.093
C3-178	**0.200**	-0.068	-0.01	-0.057
Dibenzothiophene	-0.091	-0.119	0.307	**0.352**
C1 Dibenzothiophene	0.192	0.031	0.021	**0.225**
C2 Dibenzothiophene	0.025	-0.186	**0.381**	-0.131
C3 Dibenzothiophene	0.153	0.019	**0.279**	-0.171
Fluoranthene	-0.07	-0.349	-0.034	-0.084
Pyrene (202)	0.06	-0.30	**0.232**	-0.085
C1 202	**0.189**	-0.088	0.123	0.063
C2 202	**0.198**	-0.05	0.092	0.019
C3 202	**0.198**	-0.05	0.092	0.019
Benz[a]anthracene	**0.184**	-0.156	0.019	0.05
Chrysene (228)	**0.139**	-0.267	-0.062	0.064
Benz[b]anthracene	**0.203**	0.005	0.047	0.009
C1 228	**0.198**	-0.06	-0.045	0.104
C2 228	**0.189**	0.046	-0.13	0.147
Benzofluoranthenes	**0.181**	-0.043	-0.184	0.135
Benzo[e]pyrene	**0.185**	-0.088	-0.149	0.072
Benzo[a]pyrene	**0.201**	0.002	-0.074	0.026
Perylene (252)	**0.196**	0.023	-0.084	0.128
C1 252	**0.192**	0.01	-0.131	0.128
C2 252	**0.188**	0.061	-0.135	0.137
Indenopyrene	**0.180**	0.111	-0.094	-0.211
Benzoperylene (276)	0.137	**0.163**	0.03	-0.425
C1 276	**0.198**	0.043	-0.089	-0.028
Acenaphthylene	-0.141	-0.139	-0.007	0.443

Acenaphthene	-0.109	-0.282	0.143	0.161
Fluorene	-0.105	-0.274	-0.203	-0.011
Dibenz[a,h]anthracene	**0.186**	-0.059	-0.166	0.072
Eigenvalue	24.1	7.09	4.85	1.96
Variance (%)	63.4	18.7	12.8	5.20

B

Variable	Factors PC1	PC2	PC3	PC4
CB 031	**0.209**	-0.03	-0.301	-0.022
CB 028	**0.232**	-0.066	0.048	-0.093
CB 052	**0.194**	-0.189	0.093	0.128
CB 049	**0.239**	-0.021	0.014	0.032
CB 044	**0.184**	0.001	-0.392	-0.089
CB 074	**0.217**	-0.074	0.211	-0.096
CB 070	**0.233**	0.059	0.037	0.113
CB 101	0.224	0.017	0.021	**0.268**
CB 099	**0.191**	0.064	**0.352**	0.073
CB 097	**0.152**	-0.096	0.136	-0.524
CB 110	**0.233**	0.039	-0.05	0.138
CB 149	**0.238**	0.036	0.011	-0.024
CB 123	-0.158	-0.200	0.034	**0.366**
CB 118	**0.226**	0.006	0.157	0.155
CB 114	-0.148	-0.158	**0.314**	**0.304**
CB 132	0.192	0.162	**0.232**	-0.043
CB 105	0.106	**0.299**	0.147	-0.08
CB 137	-0.152	**0.266**	-0.056	-0.042
CB 138	0.125	**0.279**	0.184	0.029
CB 187	-0.094	**0.319**	-0.003	-0.057
CB 183	-0.135	**0.286**	-0.026	-0.062
CB 128	0.126	**0.263**	-0.186	0.193
CB 167	-0.175	-0.184	-0.132	**0.286**
CB 156	-0.144	**0.269**	0.061	0.135
CB 157	-0.178	**0.225**	-0.106	-0.005
CB 180	-0.054	**0.32**	0.089	0.222
CB 170	0.173	0.190	-0.137	**0.278**
CB 189	**0.157**	0.087	-0.444	-0.008
CB 194	**0.178**	-0.200	-0.158	**0.173**
CB 209	**0.229**	0.093	0.006	0.100
Eigenvalue	17.5	8.3	2.6	1.7
Variance (%)	58.1	27.5	8.6	5.8

The compositional profile of dissolved PAHs by ring size was dominated by the 2-, 3- and 4-ring PAHs at all sites, which on average accounted for 45, 26 and 22% of the total dissolved PAH concentrations in the water from the Forth estuary and Firth of Forth (Fig. 5-1; SI). As shown in Table 4-2, the (Fl/Py) ratio was less than one at all sites and relatively consistent at Alloa, Grangemouth West and Crombie. The (Mp/Ph) ratios were greater than 2 at all sites (Table 4-2) with values greater than 4 at Crombie and Braefoot. This data is consistent with a possible petrogenic input of PAHs to the Forth estuary and Firth of Forth. Principal components were obtained from the normalised data with PAHs and PCBs as the variables. PCA of the dissolved PAHs showed that four major principal components (PCs) i.e. with an eigenvalue > 1, accounted for about 99.9% of the total variability in the dataset. The first group, PC1, accounted for about 63.4% (Table 4-4A) of the total variations. It was the most important principal factor and was largely dominated mainly by the alkyl substituted PAHs and heavy parent PAHs. This may indicate a mixture of both petrogenic and pyrolytic source input, because, the dominance of alkylated PAHs and heavy parent PAHs are generally associated with petrogenic and pyrolytic source input respectively (Yunker et al., 1996). Possible sources of petrogenic PAHs in the study area are discharges of contaminated wastewater from the petrochemical industries and discharges of bilge water from ships. Any pyrolytic source inputs could be as a result of exhaust fumes from ships and other combustion activities within the Forth, particularly, during the cold sampling period (i.e. February to April). The second major principal factor, PC2 explains about 18.7% of the variance in the dissolved PAH data and are characterised by factor loadings of both parent and alkylated naphthalenes (Table 4-4A). Considering that naphthalene and alkyl-naphthalenes degrades easily in the marine environment, their high loadings in this study may indicate a recent or continuous petrogenic input. Further, from the PCA of the distribution patterns of the dissolved PAHs, the samples were classified into groups, where possible, at the different sampling sites (Fig. 4-3A). Gunnet Ledge, Crombie and Grangemouth West clustered in the same group, suggesting possible similar source. The group is characterised mainly by negative PC1 scores (except Grangemouth West, which has a positive PC1) and positive PC2 scores, indicating a higher petrogenic input at those sites. However, Alloa and Braefoot were not grouped, suggesting dissimilar sources of PAHs; the loading plot revealed that both sites were characterised by a high proportion of 4- to 6-ring PAH content, indicative of a pyrolytic source.

For the PCBs, the $\sum PCB_{32}$ (0.16 to 0.30 ng L^{-1}) and the $\sum ICES\text{-}PCB_7$ (0.07 to 0.13 ng L^{-1}) measured in water at the Forth estuary and Firth of Forth (Table 2) were an order of magnitude higher than the $\sum PCB_{32}$ (0.02 ng L^{-1}) and $\sum ICES\text{-}PCB_7$ (0.01 ng L^{-1}) measured in water at the Ythan estuary using SR-PSDs (Emelogu et al., 2013a). However, Monteyne et

al. (2013) reported sum dissolved concentrations of $\sum PCB_{14}$ in the range of 0.030 to 3.1 ng L^{-1} in water from seven monitoring stations along three Belgian coastal harbours using SR-PSDs, with the highest concentrations reported in stations within the marinas and the lowest at the offshore stations. Despite the continual direct discharges of urban and industrial wastes into the Forth estuary and the Firth of Forth, the relatively low dissolved individual and total concentrations of PAHs and PCBs measured in water at the five sites in comparison to those in Belgian coastal harbours maybe in part due to the turbid nature of the estuary which may facilitates the transfer of the hydrophobic compounds from the water column to particulate matters, therefore, depositing and accumulating in the sediments (Vuksanovic et al., 1996; Eggleton and Thomas, 2004). It should be noted however, that the dissolved concentrations of organic contaminants measured in this study are the biologically available fractions, hence, toxicologically relevant.

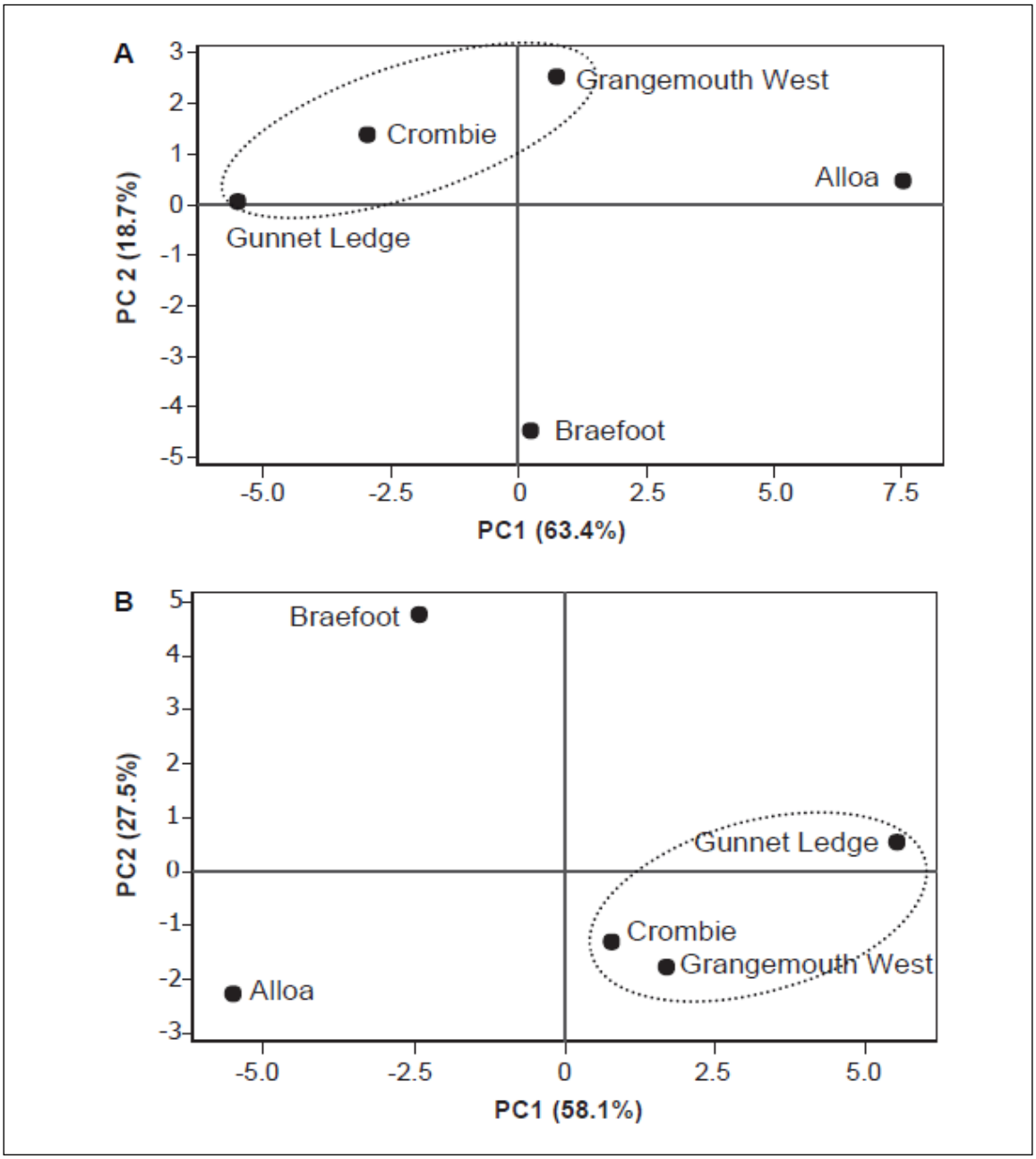

Figure 4-3 A and B: Principal Component Analysis (PCA) score plots showing the pattern distribution of dissolved concentrations of (A) 40 PAHs individual and groups of PAHs (expressed as proportions of the total PAH concentration; ($\sum PAH_{40}$) and (B) 32 PCBs (normalised to CB153) in water from the Forth estuary and Firth of Forth.

At the five sites, the relative abundance of PCB congeners in the dissolved phase was similar. The distributions were dominated by the more water soluble, less chlorinated PCB congeners e.g. tri-, tetra-, and penta-chlorinated biphenyls (CBs), as well as the higher chlorinated, hexa-CBs. On average, the tri-, tetra-, and penta-CBs accounted for 17.1%, 30.3%, and 28.2 % of the total PCB concentrations respectively, while hexa-, hepta- and octa/nona-CBs accounted for 20.5%, 2.7% and 1.7% of the total dissolved PCBs respectively

(Fig. 5-2; SI). A predominance of the less chlorinated PCBs indicates possible sources to be atmospheric deposition, urban run-off and waste discharges, whilst, the dominance of heavy, more chlorinated PCBs i.e. hexa-, hepta- and octa-PCBs, suggests a near source input through sediments and particulate matters remobilisation (e.g. historic or discharges). Therefore, the main sources of PCBs in water at the five sites maybe related to atmospheric deposition, urban run-off and some remobilisation from sediments (historic).

Further, PCA distinguished the individual dissolved PCBs (normalised to CB153) into four major PCs with an eigenvalue > 1 and explained 100% of the total variance of the dissolved PCB data (Table 4-4B). However, most of the variance in the dissolved PCB input in the Forth estuary and Firth of Forth can be explained by the first and second principal components (i.e. PC1 and PC2). PC1 and PC2 accounted for about 58.1% and 27.5% respectively of the total variations. PC1 loading was dominated by the low chlorinated, more volatile PCB congeners, e.g. tri-, tetra-, and penta-CBs and few heavy chlorinated hepta- and octa/nona-CBs (Table 4-4B), suggesting a higher atmospheric source input. PC2 had higher loadings of the heavier, hexa- and hepta-CBs indicating likely sediment and particulate matter remobilisation and input from sewage outfalls. Generally, the less chlorinated PCBs are transported over longer distances and deposit at remote sites owing to their longer residence time in the atmosphere; whilst, the heavier chlorinated PCBs are more likely to be adsorbed on particulate matters, as a result, accumulating and depositing in the sediments near the pollution sources. Further, the PCB score plots grouped Grangemouth West, Crombie and Gunnet Ledge sites together with a positive PC1 and PC2 (Fig. 4-3B). This indicates that these sites exhibit similar PCBs sources with higher proportion of the lower chlorinated PCBs, suggesting a higher input from atmospheric sources. However, similar to the PAHs, Alloa and Braefoot sites were not grouped, with negative PC1 and a positive and negative PC2, indicating likely sources from sediment remobilisation, urban runoff, and discharges from sewage outfalls.

A total of 47 selected pesticides and 22 acid/urea herbicides (Table 4-1C, SI) were targeted in the extracts of SR-PSDs deployed in water at the Forth estuary and Firth of Forth. As shown in Table 4-2, only eight pesticides (chlorpyrifos ethyl, dimethoate, flusilazole, pendimethalin, pirimicarb, propiconazole, terbutryn and triadimefon) and five acid/urea herbicides (diuron, isoproturon, pencycuron, linuron, and chlorotoluron) were detected above the instrumental limits of detection in the extracts from SR-PSDs deployed in the Forth, although not at all sites. Amongst the pesticides and acid/urea herbicides detected, chlorpyrifos ethyl, pendimentalin, linuron and chlorotoluron showed the highest concentrations (ng g SR sampler^{-1}). While, this should be considered with caution since

environmental conditions were not corrected for, these compounds, and others, were also detected in water at the Ythan estuary, albeit at higher concentrations (Emelogu et al., 2013a). The concentrations and profile of pesticides and acid/urea herbicides detected in SR-PSDs deployed in water in Forth estuary and Firth of Forth are consistent with the industrial nature of these areas compared to the rural agricultural catchment in our previous study.

There is a discharge of agrochemical effluents at Grangemouth West, it was interesting and significant to note that pirimicarb was detected and indeed quantified in water exclusively at this site. This, in addition to the overall data from this study thus supports the suggestion that the occurrence of dissolved pesticides and acid/urea herbicides in the Forth estuary and Firth of Forth was as a result of both riverine input related to some agricultural activities along the coastline and discharges from agrochemical industries. Generally, the differences in the concentrations and profile of the pesticides and herbicides at each site can be attributed to a variety of factors including the agricultural practices, the differences in their physicochemical characteristics and hydrodynamics at the sites. Compared to freshwaters, there is still paucity of literature data for marine water contamination by pesticides and herbicides using passive sampling devices (PSDs). In some recent studies, Shaw et al. (2010) and Munaron et al. (2011) have reported a variety of pesticides and herbicides including diuron and isoproturon in marine waters using various passive sampling devices (PSDs) including SPMDs, chemcatchers and Polar Organic Contaminants Integrative Samplers (POCIS). This study has therefore, further demonstrated the potential of SR-PSDs as effective and sensitive preliminary monitoring tools for organic contaminants of diverse polarities in estuarine and marine waters.

All algal growth inhibition bioassays in this study were conducted in accordance with ISO 8692:2012 (ISO, 2012). The modified procedure, using fluorometric detection and 24 well microplates with a total volume of 2 mL, proved effective in assessing the potential combined toxicity of the low volumes of environmental extracts. Extracts from the procedural blank SR-PSDs (undeployed) showed no significant (P<0.05) growth inhibition on *D. lutheri,* thus, confirming that the extraction procedure and extraction solvents were not inherently phytotoxic to *D. lutheri*. Overall, the use of 24 well microplates in this study instead of the Erlenmeyer flasks that are often used in algal toxicity tests, the lower volume of sample extracts tested and a greater number of replicates combined to give a rapid, sensitive and statistically relevant experiment. The results from the algal toxicity tests indicate that combined dissolved concentrations of organic contaminants in water at the Forth estuary and

Firth of Forth sampled with silicone rubber passive sampling devices (SR-PSDs) are phytotoxic to marine phytoplankton (*D. lutheri*).

A number of organic compounds, of diverse hydrophobicities, detected in the extracts of SR-PSDs deployed in water at the Forth estuary and Firth of Forth would have contributed to the observed algal growth inhibition on *D. lutheri.* Pesticides and acid/urea herbicides in particular, are well known to interfere with microorganism's metabolism processes, in addition to cell growth, division and molecular composition (DeLorenzo et al., 2001). Pennington and Scott (2001) reported a 96 h growth rate EC_{50} of 147 µg L^{-1} (95% CI = 116.4–178.7 µg L^{-1}) for atrazine on a phytoplankton *Pavlova* sp. Some other studies have also demonstrated the phytotoxic effects of several pesticides and acid/urea herbicides, including isoproturon, pendimenthalin, chlorotoluron, and diuron to both marine and freshwater algae (DeLorenzo and Serrano, 2003; Sbrilli et al., 2005; and Ma et al., 2006). Tien and Chen (2012) reported EC_{50} of 2.56 and 1.68 mg L^{-1} for chlorpyrifos in open and closed systems respectively, using a marine diatom, *Nitzschia* sp. Other laboratory studies have also revealed the phytotoxic characteristics of some of the other organic contaminants detected in water in this study, for example, Chung et al. (2007) showed that PAHs including naphthalene, phenanthrene and pyrene were toxic to some marine microalgae in a 4-day solid-phase microalgal bioassay exposure tests. In their study, phenanthrene showed to be the most phytotoxic compound with an EC_{50} of 9.4 mg kg^{-1} on *Selenastrum capricornutum.* Although, the water concentrations of the pesticides and acid/urea herbicides in this study were not calculated, the overall data of the organic contaminants measured in the Forth estuary and Firth of Forth suggested that mixture of the contaminants might have induced the observed growth inhibition of *D. lutheri* more easily than the single compounds.

4.7 Conclusions

A realistic and effective evaluation of potential impacts of trace organic contaminants in the marine environment requires knowledge of their identity, sources, environmental exposure and their combined toxicity on sensitive aquatic organisms. A major objective of this study was to determine whether dissolved mixtures of organic contaminants in the Forth estuary and Firth of Forth posed a significant risk to aquatic organisms. This study has shown that extracts of SR-PSDs from the five sites investigated exhibited potential for growth inhibition on *D. lutheri*, indicating the presence of, and exposure to, phytotoxic compounds in the estuary and the Firth of Forth.

Whilst, the dissolved concentrations of PAHs and PCBs measured in the estuary and Firth of Forth were relatively low and may not present an acute threat to resident aquatic organisms, a number of pesticides and herbicides that are known to be phytotoxic were detected in extracts of SR-PSDs deployed in the estuary and Firth of Forth. These compounds, in addition to other known e.g. PAHs and PCBs and unknown contaminants, likely contributed to the algal toxicity observed on the native marine phytoplankton *D. lutheri* in this study.

The study further revealed the complexities in the accurate identification of the origin of contaminants in estuarine and coastal systems, due to possible coexistence of several different sources. However, using multivariate principal component analysis (PCA) and other parameters, petrogenic input and combustion activities were found to be the most significant sources of individual PAHs in water at the Forth estuary and Firth of Forth, while, historical use, through, sediments and particulate matters remobilisation, sewage discharges and atmospheric deposition are amongst the possible sources of PCBs in the study area. Pesticides and acid/urea herbicides detected in the study area exhibited possibilities of riverine and direct discharge type inputs. Generally, the application of a silicone rubber passive sampling technique coupled with algal growth inhibition tests (in microplates) and chemical analysis proved to be efficient, sensitive and a highly suitable techniques for assessing the occurrence and combined toxicity of a variety of dissolved organic contaminants in estuarine and coastal waters.

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Supplementary material in appendix 5

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5 CHAPTER FIVE: Evaluations of combined embryo and algal toxicity of dissolved organic contaminants in the Ythan catchment using zebrafish (*Danio rerio*) and marine phytoplankton (*Diacronema lutheri*)

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Abstract

A wide variety of organic contaminants including pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) have previously been detected in surface waters in the river Ythan catchment, North East Scotland UK. Whilst, the concentrations detected were below Water Framework Directive Environmental Quality Standards (EQSs), environmental exposures to the diverse mixtures of contaminants, known and unknown, may pose chronic and/or sublethal effects to non target organisms. The present study assessed the embryo and algal toxicity potential of freely dissolved organic contaminants from the Ythan catchment using silicone rubber passive sampling devices (SR-PSDs) and miniaturised bioassay techniques. Zebrafish (*Danio rerio*) embryos' and a marine phytoplankton (*Diacronema lutheri*) were exposed to extracts from SR-PSDs deployed at different locations along the river Ythan and an undeployed (procedural blank). Statistically significant developmental and algal toxicities were measured in all tests of extracts from deployed samples compared with the procedural blanks. This indicates environmental exposure to, and the combined toxicity potential of, freely dissolved organic contaminants in the catchment. The present and previous studies in the Ythan catchment, coupling SR-PSDs and bioassay techniques, have both helped to understand the interactions and combined effects of dissolved organic contaminants in the catchment. They further have revealed the need for improvement in the techniques currently used to assess environmental impact.

Keywords

Passive sampling
Algal growth inhibition test
Fish embryo toxicity test (FET)
Algal growth inhibition
Zebrafish (*Danio rerio*)
Diacronema lutheri
Toxicity tests
Water quality
Organic contaminants

5.1 Introduction

Contamination by a variety of organic contaminants, particularly pesticides through diffuse sources has been identified as a cause for concern in the Ythan catchment of north east (NE) Scotland, UK (SEPA 2011; Emelogu et al. 2013a, Emelogu et al. 2013b). The total concentrations in water of a number of individual priority organic contaminants monitored in the catchment using automatic water samplers (autosamplers) seldom exceed the environmental quality standards (EQSs; EC 2000; SEPA 2011; Emelogu et al. 2013b). However, the consequence of long-term ecological exposure and the combined toxicity of the multiple arrays of the known and unknown chemical stressors still remain a cause for ecological concern. Further, it is the freely dissolved concentrations of organic contaminants, that are directly proportional to the compounds' chemical activity e.g. bioavailability and bioaccumulation in organisms (Mayer et al. 2003; Reichenberg and Mayer 2006). Therefore, the freely dissolved concentrations of organic contaminants are more ecologically relevant in water quality monitoring and the evaluation of potential adverse effects on organisms than the total environmental concentrations.

Traditional discrete spot or bottle water sampling, followed by standard chemical analytical methods in water quality monitoring cannot directly measure the bioavailability of organic contaminants or account for the unknown contaminants, transformation products and their toxicological effects on organisms. This is more so where the sources of the contaminants are diffuse in nature, coupled with possible spatial and temporal complexities. Passive sampling offers an alternative to traditional spot or water bottle sampling techniques in water quality monitoring. It involves the continued exposure of the passive sampling devices (PSDs) in the environment, during which, sampling of the freely dissolved concentrations of contaminants occur passively, overcoming most of the challenges of traditional spot or bottle water sampling (Vrana et al. 2005; Greenwood et al. 2007). Silicone rubber passive sampling devices (SR-PSDs), in particular, have shown to be a valuable tool for investigating the toxicologically relevant, freely dissolved fractions of a wide range of organic contaminants in water (Smedes 2007; Emelogu et al. 2013b; Monteyne et al. 2013). In addition to chemical analysis, extracts of SR-PSDs deployed in water can also be evaluated for the toxicity potential of all the accumulated organic compounds using *in vivo* (whole organisms) and/or *in vitro* (cells) bioanalytical methods (bioassays) (Bauer 2008; Emelogu et al. 2013a).

The applicability of coupling SR-PSDs and bioassay techniques has been successfully demonstrated in a previous study in the Ythan catchment (Emelogu et al. 2013a). The

extracts of SR-PSDs deployed in water at five sites at the catchment were assessed (*in vitro*) for potential acute cytotoxicity and cytochrome induction potentials on a rainbow trout liver (*Oncorhynchus mykiss*) cell line (RTL-W1; Lee et al., 1993). Owing to the diverse modes of action of the different organic contaminants detected in water from the catchment, the use of organisms from different trophic levels (e.g. algae and fish) should provide additional understanding of the potential combined effects of the chemical stressors on resident non-target organisms.

The fish embryo toxicity test (FET; e.g. OECD 1992b) is a simple, cost effective, high-throughput test, which is more sensitive when compared to the use of juvenile or adult fish in conventional ecotoxicity testing, it measures mostly acute toxicity or lethality of individual chemicals (e.g. OECD 1992a). The use of fish embryos *in lieu* of adult or juvenile fish presents fewer ethical concerns, since embryos are not considered as whole organisms (Nagel 2002; Braunbeck et al. 2005) and their use does not require license under the European Directive 2010/63/EU on the use of animals in scientific procedures (EC 2010). In addition, the FET test gives comparable data to acute fish toxicity tests using whole organisms (Braunbeck et al. 2005; Lammer et al. 2009). In Germany, the FET test has become a mandatory part of whole effluent testing by the German Institute for Standardisation (DIN 2001) and the FET test with embryos of zebrafish (*Danio rerio*) has been standardised at the international level (ISO 2007).

The phytoplankton community, as the dominant primary producers in aquatic ecosystems, can directly reflect the water quality. Since, phytoplankton cells typically divide once per day. Toxicity tests using phytoplankton are chronic, involving several generations of phytoplankton cells over a few days and they typically measure sublethal effects, e.g. inhibition of growth rate, rather than mortality. Algal growth inhibition tests are very sensitive and a good indicator of environmental stressors and internationally standardised algal toxicity tests using freshwater and/or marine algae are available (e.g. ISO 1989; 1SO 1995; ISO 2000; ISO 2012).

The present study evaluated further the combined toxicity potential of the freely dissolved organic contaminants to which aquatic organisms in the Ythan catchment are exposed. This was done using SR-PSD extracts in FET tests with zebrafish embryos and in an algal growth inhibition test using a unicellular flagellated marine prymnesiophyte, *Diacronema lutheri*. The overall data and insights from the present and previous studies in the Ythan catchment using SR-PSDs and bioassay techniques were used to evaluate the suitability of the techniques and to identify areas for possible improvement.

5.2 Materials and Methods

5.2.1 Study area and passive sampling

To evaluate the combined embryo and algal toxicity of freely dissolved organic contaminants in water, four riverine sites and one estuarine site in the Ythan catchment in the NE of Scotland were selected for this study (Fig.1). The rationale for the choice of the catchment, sampling locations, and detailed description of the SR-PSDs, preparation, sampling, extraction, clean-up and analysis procedures are provided in Emelogu et al. (2013a) and Emelogu et al. (2013b). Briefly, the Ythan catchment is sparsely populated, with high intensity agricultural activities (mixed farming) and low industrial activity. As a result, there is evidence of low level plant protection products (PPPs; including selected pesticides and a separate sub-category of acid/urea herbicides) commonly referred to collectively as 'pesticides' in this study, in addition to other organic contaminants, including variety of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) (SEPA 2011; Emelogu et al. 2013a; Emelogu et al. 2013b).

The study involved two sampling campaigns; the first campaign was from November 2010 to January 2011 and targeted two sites on the main river, two sites on a tributary at risk of failing its ecological status (SEPA 2010), and one site in the estuary (i.e. sites 1, 2, 3, 4, and 5; Fig.1). The second campaign was from October to December 2011 and targeted only sites 1, 2 and 3 (Fig.1). During each campaign, sampling was performed using two sets of 6 translucent food grade silicone rubber (SR) passive sampler sheets (one set for chemical analysis and the other for bioassay use). Both sets of SR-sheets were pre-extracted for >100 h using a Soxhlet apparatus (Laboratory Glass Specialists BV, Ubenna, Netherlands).
The set of SR samplers targeted for chemical analysis were spiked with a set of non-naturally occurring compounds i.e. 4 perdeuterated PAHs and 10 PCBs (referred generally as performance reference compounds; PRCs; Booij et al. 2002). The rate of dissipation of PRCs from the samplers allows for the calculation of sampling rates (R_S) for the targets compounds, which can then be used to calculate the water concentrations of the compounds (Huckins et al. 2002).

In both campaigns, the two sets of SR passive sampler sheets, (i.e. for bioassay and PRC-spiked, for chemical analysis) were deployed in water simultaneously. In the first campaign, deployments were for 65 d at sites 1-4 and 58 d at site 5. In the second year, samplers were deployed for 54 d at each of the three sites (sites 1, 2 and 3). Each SR passive sampler

comprised of six sheets weighing ~ 20 g in total and had a total surface area of 600 cm^2. During the period of deployment, a set of pre-extracted, PRC-free, SR sheets were kept in ultra-pure water (18.2 MΩ cm) in the laboratory so as to serve as the procedural blank during the bioassays. Another set of PRC-spiked SR sampler sheets were exposed to air at both deployment and retrieval to serve as field blank during chemical analysis and time zero for the PRC dissipation.

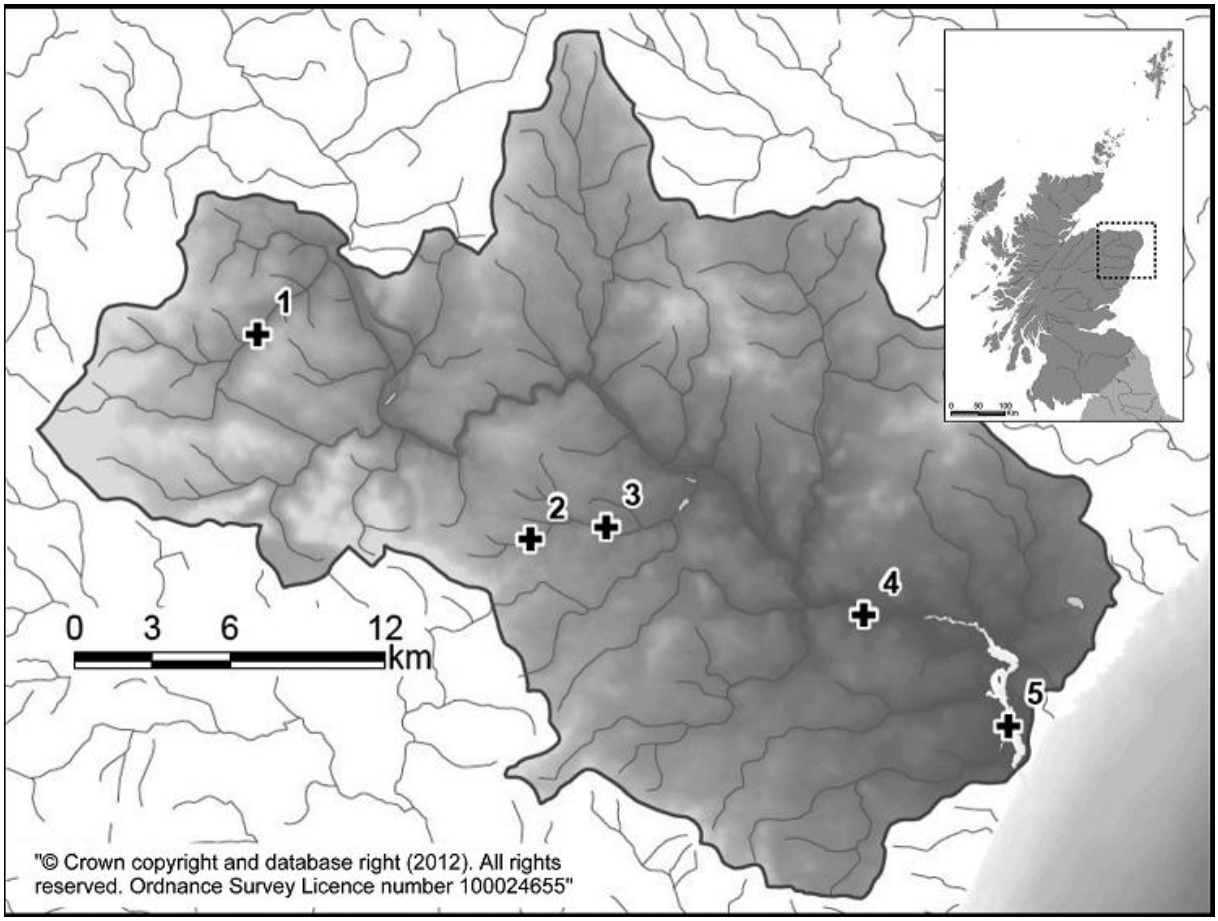

Figure 5-1 The Ythan catchment in north east (NE) Scotland, UK, showing the approximate locations of the five sampling sites. (Inset is the map of Scotland with the approximate location of the catchment).

After retrieval of the SR passive samplers, all six SR sheets targeted for chemical analysis from each location and the field blank were Soxhlet extracted in boiling acetonitrile (ACN):methanol (MeOH) mixture; (2:1 v/v) for 24 ± 4 h. Prior to chemical analysis, the extracts were purified using glass solid-phase extraction (SPE) C8 columns (Mallinckrodt Baker, London, UK) so as to remove any co-extracted SR oligomers. The extracts were subsequently split into three aliquots and analysed for a variety of pesticides, PAHs and PCBs using gas chromatography (GC) and liquid chromatography (LC) respectively, with different detectors including mass spectrometry (MS), an electron capture detector (ECD), and MS–MS (Emelogu et al. 2013a; Emelogu et al. 2013b).

Only two sheets out of the six SR sheets for bioassay use from each site, including the procedural blank (undeployed SR samplers), were extracted in the first instance. In the first sampling campaign, the extracts from each site were evaluated for acute cytotoxicity and potential to induce cytochrome P4501A enzyme activity (Emelogu et al. 2013a). The remaining four sheets from each site were preserved in amber coloured flasks at – 20 °C for future evaluations of toxicity.

In the present study, the remaining (four) SR sheets from the first sampling campaign and the undeployed SR sheets (i.e. procedural blank) were extracted, two at a time to produce duplicate extracts, and the extracts solvents exchanged into dimethylsulphoxide (DMSO; 0.25 mL, of 99.9% purity, Sigma-Aldrich Dorset, UK). The SR sheets were extracted separately so as to facilitate two independent FET tests. From the second deployment, two each of the six SR sheets for bioassay use from the three sites and the procedural blank were extracted, cleaned-up and solvent exchanged into ~1 mL of DMSO for the algal toxicity test. All extracts were transferred to amber coloured GC vials and stored at -20 °C until bioassay. The bioassay results are expressed relative to the equivalent mass of SR extracted per mL of extract (mg SREQ mL^{-1}).

5.2.2 Fish embryo toxicity test (FET) with zebrafish (*Danio rerio*)

5.2.2.1 Maintenance and egg production of zebrafish

A breeding stock of male and female zebrafish at a ratio of 3:2 was maintained at the Department of Ecosystem Analysis, Institute for Environmental Research (Biology V), RWTH, Aachen University, Aachen, Germany. The zebrafish were maintained in glass aquarium tanks with charcoal-filtered tap water with manual exchange conditions at a temperature of 26 ± 1 °C under a 14/10 hour day/night light regime. Additional characteristics of the charcoal filtered water were: hardness 196.3 mg L^{-1} $CaCO_3$, conductivity 744 S; pH 7.8 ± 0.5 and dissolved oxygen 10.5 ± 0.5 mg L-1 (95 ± 5% saturation). The fish were fed twice daily with commercially available artificial diets (TetraMin™ dry flakes; Tetra, Melle, Germany) and *Artemia* nauplii. Metal trays with a stainless steel mesh net covering and artificial green flowers attached to the net were placed on the base of the aquaria on the evening prior to the day of spawning. The artificial green flowers help to simulate a natural water environment so as to further stimulate spawning, and the net prevents the fish from

reaching and feeding on their own offspring. Spawning is initiated within half an hour after the lights are turned on in the morning. Prior to the fish embryo toxicity test, the eggs were rinsed several times with artificial water. Further details of the maintenance of the zebrafish and egg production are provided in (Hollert et al. 2003; Lammer et al. 2009). Zebrafish, although not ecologically relevant for Scotland, belong to the same carp family (*Cyprinidae*) as the Scottish roach (*Rutilus rutilus*) and tench (*Tinca tinca*) were chosen due to being a model test organism, with well characterised embryo development and reproductive behaviour that can be readily manipulated to provide eggs when required (Strähle, et al., 2012) and due to previous experience (e.g. Heger et al. 2012).

5.2.2.2 Test procedure for fish embryo toxicity test (FET) with zebrafish

The embryo toxicity potential of extracts from the first deployment of SR-PSDs in the Ythan catchment was assessed in 24-well microtitre plates (TPP, Trasadingen, Switzerland) using zebrafish embryos, according to the FET procedure described by Nagel (2002), with modifications by Hollert et al. (2003) and Seiler et al. (2006). The FET test was started as soon as possible after fertilisation of the eggs and no later than 3 hours post fertilisation (hpf; 128-cell stage). Briefly, the DMSO extracts from the five sites, together with an extract from the procedural blank, (undeployed SR samplers) were serial diluted in standardised dilution water (294.0 mg L^{-1} $CaCl_2$ x 2H2O; 123.3 mg L^{-1} MgSO4 x 7H2O; 63.0 mg L^{-1} NaHCO3; 5.5 mg L^{-1} KCl, diluted in double distilled water) prepared a day prior to the beginning of the test to give five serial concentrations of 133, 66.7, 33.3, 16.7, and 8.3 mg SR EQ mL^{-1} in 24-well microtitre plates. The highest concentration of DMSO in the test solutions was 0.5%. Hallare et al. (2006) have shown that 0.5% DMSO or less has little or no adverse effects on the development of zebrafish embryos. The positive control was 3,4-dichloroaniline (DCA; 3.7 mg L^{-1}, > 98%; Sigma–Aldrich GmbH, Steinheim, Germany) dissolved in Millipore water. The negative control was the standardised dilution water (with no chemical or DMSO added). Ten eggs were then transferred to each well of the 24-well microtitre plate containing 2 mL of the freshly prepared test solutions and controls (positive and negative). Each sample of SR-PSDs,(deployed and undeployed), was tested in two independent replicates. The plates were covered with adhesive film (Renner, Darmstadt, Germany) and incubated for 48 h at 25 ± 1 °C. After 48 h of exposure, the developing embryos were evaluated using an inverted microscope (Nikon Eclipse TS100; Nikon, Japan). Four lethal effects were determined: coagulation of the embryos, non-detachment of the tail, non-formation of somites and non-detection of a heartbeat. The test was considered valid if the mortality of the negative control (dilution water) did not exceed 10% and the positive control (3.7 mg L^{-1} of DCA) induced

effects in more than 20% of the embryos. Results (percent mortalities) of the mean of two independent replicates were plotted with GraphPad Prism as a function of the logarithm of concentrations of the extract of SR-PSD (i.e. log mg SREQ mL^{-1}).

5.2.3 Algal growth inhibition bioassay

5.2.3.1 Algal strain and culture

To evaluate the phytotoxicity potential of freely dissolved organic contaminants at the three sites (i.e. sites 1, 2 and 3; see Fig. 1), sampled in the second sampling campaign, a 72 h algal growth inhibition assay was performed using a unicellular flagellated marine prymnesiophyte, *Diacronema lutheri* (Bendif et al. 2011), synonym, *Pavlova lutheri* Droop (Green 1975); CCAP 931/6 as outlined in (ISO 2012) with slight modifications in the exposure and measurement procedures. *D. lutheri* was originally isolated from the Firth of Clyde in Scotland and was obtained from the Culture Collection of Algae and Protozoa (CCAP), Scottish Marine Institute, Argyll Scotland. *D. lutheri* is routinely cultivated in our laboratory under standardised conditions, e.g. medium f/2 (Guillard and Ryther 1962), prepared from water from the Scottish area of the North Sea, temperature 20±2°C at a constant light illumination (cool-white fluorescent lights) with a photosynthetic active radiation (PAR) of ~150 µMol m^{-2} s^{-1}. All glassware and media were previously sterilised by autoclave at 121°C for 40 minutes. We previously used *D. lutheri* for a study of the Forth estuary and Firth of Forth in the East coast of central Scotland (Emelogu et al. 2013c submitted), and its use here allowed a comparison to be made between the two studies. We are not aware of any major differences reported between the sensitivity of marine and freshwater flagellate phytoplankton to contaminants.

5.2.3.2 Algal toxicity tests of passive sampler extracts

A detailed description of the culture and algal toxicity test procedures followed in this study are provided in Emelogu et al. (2013c submitted). The tests were performed in untreated 24-well microtitre plates (Corning, Sigma-Aldrich, Dorset UK) with a phytoplankton cell density of 1 x 10^4 (± 10%) cells/well. Five serial dilutions (i.e. 6.66, 3.33, 1.66, 0.83, and 0.42 mg SREQ mL^{-1}) of the DMSO extract of SR-PSDs from each of the three sites (i.e. sites 1, 2 and 3) and the SR procedural blank were made using algal growth medium. Each test concentration and a medium control (containing only algal growth media with no phytoplankton or DMSO) was tested in triplicate wells, while growth controls (containing phytoplankton and algal growth media only) were tested in six wells. During each test, the reference chemical, 3,5-dichlorophenol (DCP;

Sigma-Aldrich, Dorset, UK) was tested on separate plates at concentrations of 10, 7.5, 5.0, 2.5, 1.25 mg L^{-1} (triplicate wells) as a positive control and used to ensure the validity of the test method. The final volume in each well was 2 mL (i.e. sterilised culture medium, test solution and *D. lutheri* culture). The concentration of DMSO in all the tests was kept at 0.1% (v/v) or less and each sample was tested in duplicate on two different plates. Algal growth was measured at intervals of 24 h over a period of 72 h using a fluorometric data acquisition system (PerkinElmer VICTOR™ 3 Multilabel plate reader, Bucks, UK) with excitation and emission wavelengths of 450 nm and 680 nm respectively. Growth inhibition was calculated as a reduction in the specific growth rate relative to the control cultures (no DMSO) grown under identical conditions. For each SR-PSD extract from the three sites, and the procedural blank, the calculation of the mean percentage growth inhibition of the specific growth rate was performed according to ISO 2012 and the percentage reduction in specific growth rate for each extract concentration compared to the growth controls plotted against the logarithm of the concentration.

5.3 Data analysis

The percentage FET mortality and algal growth inhibition were expressed as a function of the logarithm of concentration of each SR-PSD extract (log mg SREQ mL^{-1}) using GraphPad Prism 5.0, software (GraphPad, San Diego, California USA) and concentration-response curves were created using a sigmoidal dose response regression model (variable slopes) i.e. four-parameter logistic nonlinear regression analysis. The median effective concentration (EC_{50}) values with 95% confidence limits (CL) for each SR-PSD extract were calculated where possible using Equation 1 in GraphPad Prism:

$$Y = Bottom + \frac{(Top - Bottom)}{1 + 10^{(LogEC50 - X) * HillSlope}} \qquad \text{Equation 1}$$

Where:
Bottom is the Y value at the bottom plateau;
Top is the Y value at the top plateau;
LogEC50 is the X value when the response is halfway between Bottom and Top.
The Hill Slope describes the steepness of the curve.

The statistical differences in percent mortalities and algal growth inhibition among the different samples were evaluated using one-way analysis of variance (ANOVA) followed by

Tukey's multiple comparison test or Dunnett's test comparing mean percent mortalities of the highest concentrations in the procedural blank SR-PSDs extracts with the mean percent mortalities in the highest concentrations of other samples (deployed) using GraphPad Prism Software. Accepted significance level of statistical analyses was p=0.05.

5.4 Results and discussions

5.4.1 Fish embryo toxicity tests

The embryo toxicity potential of extracts of silicone rubber passive sampling devices (SR-PSDs) deployed in water at five sites of the Ythan catchment was assessed through 48 h of exposure of the embryos of zebrafish to the extracts. With each sample tested, the negative and positive controls caused embryo mortalities of less than 10% and greater than 20% respectively, indicating that all the test procedures were valid. No significant embryo toxicity was observed from procedural blank SR extracts, indicating that the extraction procedure and the solvents used did not induce embryo toxicity (see Fig. 5-2). In contrast, all the extracts from SR-PSDs deployed at the five sites showed embryo toxicity, but to varying degrees. Extracts of SR-PSDs from sites 1, 2 and 4 exhibited low to moderate embryo toxicity. As a result, it was not possible to calculate the EC_{50} values from those samples. However, extracts from sites 3 and 5 exhibited high embryo toxicity compared to other sites and EC_{50} values were calculated (Table 5-1).

Table 5-1 Median effective concentration (EC_{50}) of embryo toxicity of extracts of silicone rubber passive sampling devices (SR-PSDs) on zebrafish (*Danio rerio*) from five sites in the Ythan catchment following 48 h of exposure.

Zebrafish (*Danio rerio*) embryo toxicity test		
	Median effective concentration	
Samples	**EC_{50}**	**95% CI[3]**
Procedural blank [1]	NA [2]	NA
Sample 1	NA	NA
Sample 2	NA	NA
Sample 3	37.8	13.1 to 109.0
Sample 4	NA	NA
Sample 5	88.5	63.0 to 124.0

[1] Extracts from silicone rubber passive sampling devices (SR-PSDs) that were not deployed at a test site. [2] NA= Not applicable; [3]confidence interval.

The highest embryo mortalities were recorded in the samples at the three highest tested concentrations i.e. 133, 66.67 and 33.33 mg SR EQ mL^{-1}. At the lower concentrations little or no mortality was recorded. Figure 5-2 shows the mean percentage mortality of the first three concentrations of each SR-PSD's extract and the undeployed (procedural blank) in the 48 h FET test using zebrafish embryos from two independent tests. There were no statistical differences between the mean percent mortalities of the procedural blank extract (undeployed SR-PSDs) compared with sites 1, 2 and 4, but there were significant differences in the toxicity of SR-PSD extracts from sites 3 and 5 compared to the procedural blank.

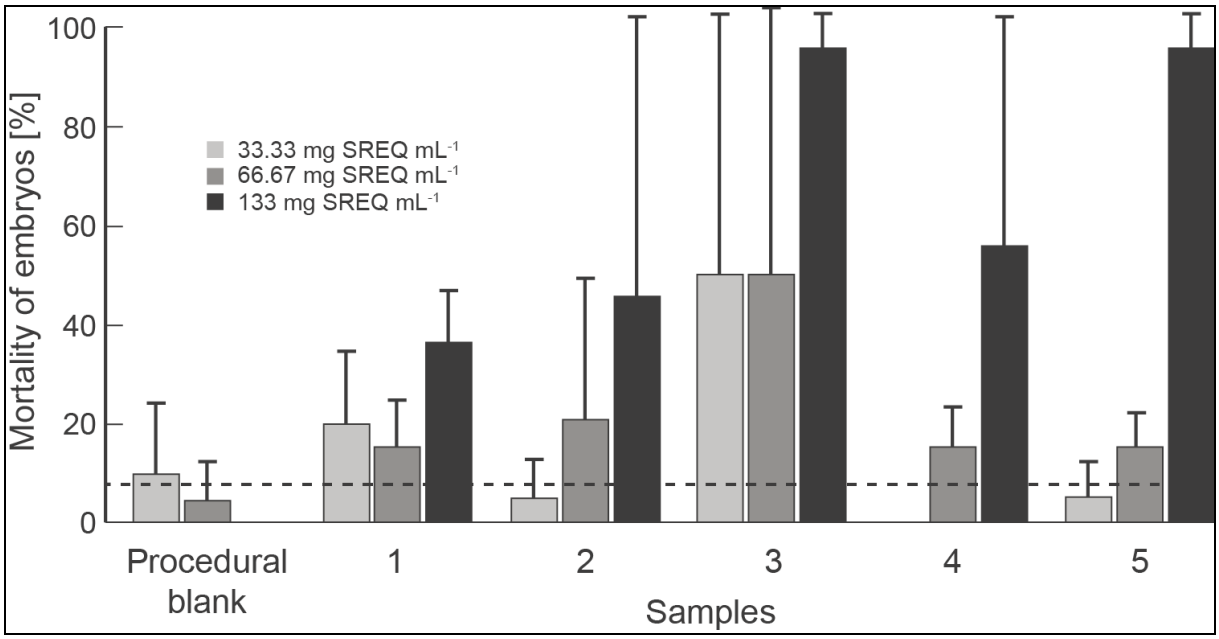

Figure 5-2 Embryo toxicity of the first three highest concentrations of extracts of silicone rubber passive sampling devices (SR-PSDs) in water at five sites in the Ythan catchment and the undeployed SR-PSDs (procedural blank), following exposure of the embryos of zebrafish (*Danio rerio)* for 48 hours. Error bars indicate standard deviations (SD) of two independent replicate tests. The dotted line represents the point of 10% emryotoxicity.

5.4.2 Algal growth inhibition

Following 72 h of exposure of *D. lutheri* to extracts of SR-PSDs deployed in water at the three sites of the Ythan catchment and the procedural blank (undeployed), statistically significant growth inhibition was measured for all of the samples. However, none of the samples exhibited a maximum response to facilitate the derivation of complete dose-response curves. Therefore, it was not possible to calculate the EC_{50} values of any of the samples. Significant growth inhibitions of *D. lutheri* occurred mostly with the three highest concentrations of extracts from the SR-PSDs (i.e. 6.66, 3.33 and 1.66 mg SREQ mL^{-1}), whilst the two lowest concentrations (i.e. 0.83, and 0.42 SREQ mL^{-1}) resulted in little or no effect. Figure 5-3 shows the percentage growth inhibition of the three highest concentrations for each extract. Extracts of SR-PSDs from all the three sites showed significant differences compared with the procedural blank SR-PSDs extracts, although, the inhibition was not significantly different among the three sites.

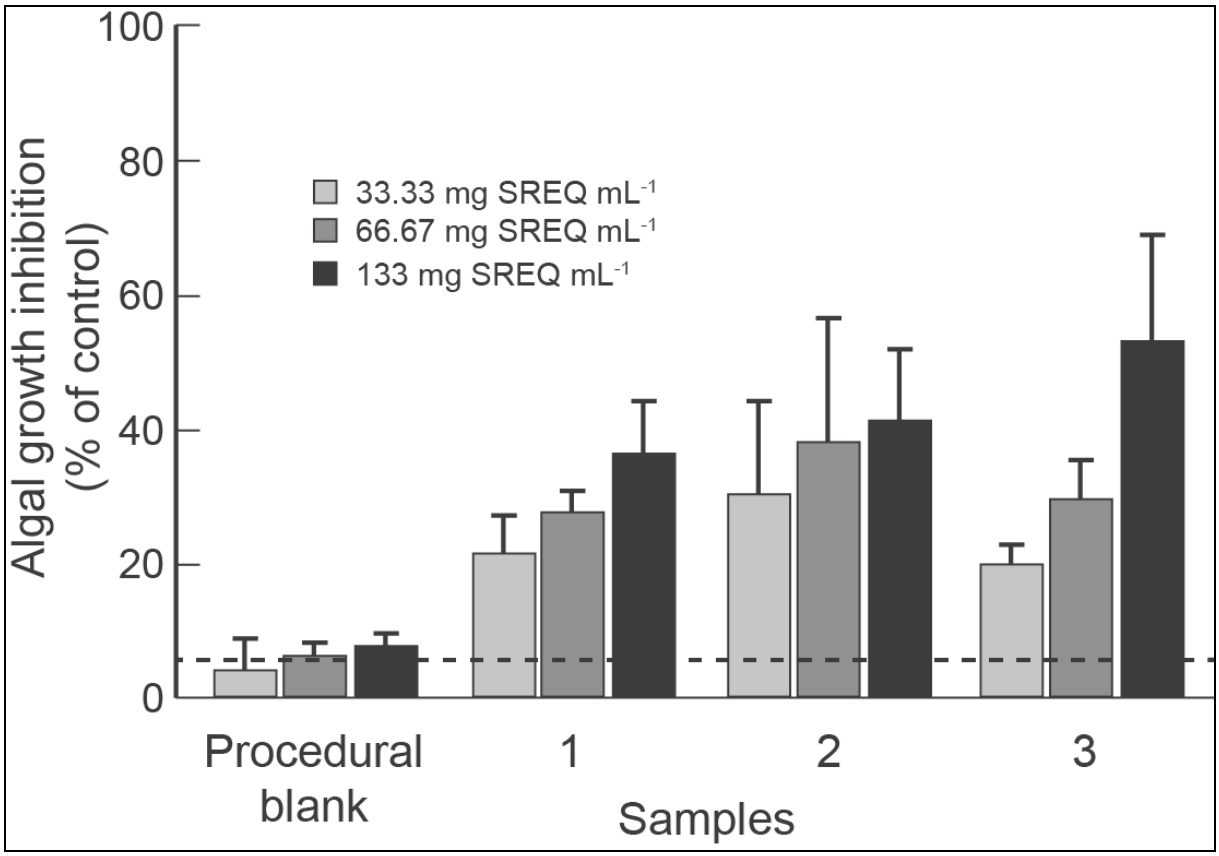

Figure 5-3 Algal growth inhibition of *Diacronema lutheri* resulting from the three highest concentrations of extracts of silicone rubber passive sampling devices (SR-PSDs) obtained following deployment at three sites in the Ythan and the undeployed SR-PSDs (procedural blank) following exposure of *Diacronema lutheri* for 72 h. Error bars indicate standard deviations (SD) of two replicate tests of the same extracts. The dotted line represents the point of 10% algal toxicity.

5.4.3 Discussions

The application of both passive sampling and bioassays in water quality monitoring, as used in this study permits simultaneous detection of a range of organic contaminants which can be related to a given end-point. Extracts of SR-PSDs deployed in the Ythan, exhibited developmental inhibition of the embryos of zebrafish and growth inhibition of *D. lutheri* respectively (Figs. 5-2 and 5-3 respectively). This indicates the presence and bioavailability of organic contaminants, with the potential to cause algal and fish developmental toxicity in the catchment.

It was interesting to observe that the highest concentrations of SR-PSDs extracts from site 3 and site 5 (the estuary) caused 90% and 100% embryo mortalities respectively in the two independent FET tests, whilst, extracts of SR-PSDs from site 1 (which is at the headwaters of the River Ythan) showed no significant mortality on the embryos. Relatively high concentrations (ng g SR $^{-1}$) of pesticides were detected in the SR-PSD from site 3 during the first sampling campaign. Most of the compounds detected in the Ythan including atrazine, isoproturon, chlorotoluron, malathion, linuron, diuron, PCBs and PAHs have been shown in other field and laboratory studies to exhibit embryo and developmental toxicity to organisms (Barron et al. 2004; Bellas et al. 2005; Farwell et al. 2006; Sawasdee and Köhler 2009).

Extracts from the second sampling campaign i.e. sites 1, 2 and 3 exhibited growth inhibition on *D. lutheri* population. However, none of the extracts caused maximum growth inhibition to *D. lutheri* following 72 h of exposure. A wide variety of pesticides including pendimethalin, diflufenican, metazachlor, chlorpyrifos ethyl, trifluralin, linuron, chlorotoluron, isoproturon and diuron were detected in the extracts of SR-PSDs deployed in water at the three streams (Emelogu et al. 2013b). Pesticides, generally, can interfere with a number of biochemical activities in organisms, including, respiration, photosynthesis, biosynthetic reactions, cell growth, division, and cytochrome P450 induction whilst herbicides target mainly photosynthesis pathways (DeLorenzo et al. 2001; Trimble and Lydy 2006). The growth inhibition of *D. lutheri* populations by SR-PSD extracts from the three sites were similar to those measured in extracts from SR-PSDs deployed in water within the Forth estuary and the Firth of Forth in Scotland, UK, using SR-PSDs and *D. lutheri* (Emelogu et al. 2013c submitted). Considering the higher concentrations (ng g SR^{-1}) and greater number of pesticides detected in the Ythan compared to the Forth estuary and Firth of Forth, it was expected that, a more significant algal inhibition of *D. lutheri* population would be observed compared to the Forth estuary and the Firth of Forth following 72 h exposure to the SR-PSDs

extracts. This indicates that pesticide were probably not solely responsible for the effects seen on the ecology in the Ythan.

The result from the present study corroborate the previous study in the Ythan using SR-PSDs and bioassays, in which none of the extracts from the five sites elicited any significant acute cytotoxicity to a rainbow trout liver cell line (RTL-W1) following a 48 h exposure. However, significant low-level cytochrome P450 1A (CYP1A) induction was measured in RTL-W1 cells following 72 h exposure of the extracts of SR-PSDs from the five sites to RTL-W1 cells (Emelogu et al. 2013a). This may suggest that the toxicity of the freely dissolved organic contaminants in the extracts of SR-PSDs from the Ythan may be metabolism related e.g. biotransformation products of the detected parent compounds. A variety of the PAHs and PCBs detected in the Ythan catchment during first and second sampling campaign are known to be toxic e.g. Schirmer et al. (1998) and Van der Oost et al. (2003). The observed low CYP1A activity and lack of acute cytotoxicity correspond to the low concentrations of freely dissolved PAHs and PCBs measured in the catchment.

As noted, the application of SR-PSDs provides advantages over spot water sampling. Passive sampling mimics the bioaccumulation of organic contaminants in water by biota; therefore, the contaminants accumulated by the SR-PSDs are substances that are potentially available to organisms in water at the catchment. Temporal trends in contaminant concentrations are more likely to be captured by passive sampling than the spot sampling technique. This is useful in the Ythan catchment, where episodic contamination events are expected to occur due to seasonal and intermittent pesticide applications. Overall, the present and previous studies have helped to identify the classes of contaminants in the Ythan with the same modes of action and provided better understanding of the interactions of dissolved organic compounds and their effects on non target organisms.

The present and previous studies in the Ythan catchment, using SR-PSDs combined with bioassay techniques have revealed the potential of these integrated methodologies in environmental quality assessments. There are still significant areas for improvement and further research. Consideration must be given to the uptake by the SR-PSDs of the contaminants, especially where the compounds present have a wide range of log K_{OW} values. At the initial stage of deployment of SR-PSDs in water, the uptake (i.e. sampling rate, R_S) of compounds to PSDs is time integrated, as uptake is linearly proportional to the aqueous concentrations i.e. kinetic sampling. As the sampling continues, the sampler gradually reaches equilibrium with the water (Huckins 2006; Greenwood et al. 2009).

Whereas, compounds with low log K_{OW} values e.g. most pesticides attain equilibrium in SR-PSDs within hours to days, it would take months or years for compounds with high log K_{OW} values e.g. most PCBs and some PAHs to attain equilibrium in SR-PSDs (e.g. Rusina et al. 2009; Lohmann et al. 2012). The differences in R_S of the varieties of contaminants sequestered by SR-PSDs during deployment, might affect the composition in the extracts for the bioassays.

Further, accurate estimation of water concentrations of compounds sequestered by the PSDs either in the kinetic or equilibrium uptake phase requires sampler-water partition coefficient (log K_{SW}) and R_S of the target compounds. In the uptake of hydrophobic compounds e.g. log K_{OW} >4, the transport through the water boundary layer (WBL) is the rate limiting factor, as a result, controlled by the *in situ* hydrodynamic conditions including water flow, temperature, biofouling. The rate of dissipation of the PRCs spiked to samplers prior to deployment can correct for this site to site variability of the uptake rates and allows for the accurate determination of R_S, and then the water concentrations (Huckins et al. 2002; Booij et al. 2007). There are still a number of uncertainties in the uptake of organic compounds of low log K_{OW} to SR-PSDs. There might be transport resistance within the sampler, and the diffusion rate may be low, therefore, the diffusion coefficients (D_F) in addition to log K_{SW} and R_S values might be required for a reliable estimation of the water concentrations. Laboratory calibration studies are therefore needed for a variety of organic contaminants including pesticides so as to expand the range of compounds that can be monitored using such SR-PSDs.

Toxicity testing of low water soluble organic contaminants is challenging due to possibility analytes of loss through volatilisation and sorption processes, leading to inaccurate and declining exposure concentrations and bias in the interpretation of toxicity data (Bandow et al. 2009). Although, the use of co-solvents (e.g. methanol or DMSO) may increase the solubility of organic compounds in the culture medium during bioassays, co-solvents may also interact with the test compounds and may interfere with the biological activity (Kramer et al. 2010; Tanneberger et al. 2010). Partitioning controlled delivery (passive dosing; PD), which involves a solvent-free dosing system and provides a known and stable amount of the test compounds to the test system, promises to overcome the highlighted challenges and improve the exposure of organic contaminants during bioassays (Mayer and Holmstrup 2008; Smith et al. 2010). Passive dosing is recommended for future studies using passive sampling and bioassay techniques for increased reliability of the estimation of the combined toxicities of multiple arrays of freely dissolved contaminants in the aquatic environment.

5.5 Conclusion

Overall, this preliminary study has shown that extracts of SR-PSDs deployed in water, are adaptable to evaluation of combined toxicity of the freely dissolved organic contaminants, using fish embryo toxicity (FET) with zebrafish and algal growth inhibition using *D. lutheri* in multi-well plates. Extracts of SR-PSDs deployed in water at the selected sites in the Ythan catchment during the first and second sampling campaigns exhibited varying levels of both embryo and algal toxicity indicating environmental exposures to and combined toxicities of multiple arrays of organic contaminants in the catchment. The present and previous studies in the Ythan catchment using SR-PSDs and bioassay techniques have shown that the organic contaminants in the Ythan catchment have the potential to pose chronic and/or sublethal effects to non target organisms.

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6 CHAPTER SIX: General discussions, conclusion and future work

6.1 General discussions and conclusion

Sampling is a vital aspect in water quality monitoring, and a reliable assessment of environmental and ecological exposure to organic contaminants and their potential impacts requires a sensitive and cost effective sampling technique. Passive sampling is a developing methodology with considerable advantages over the conventional spot or bottle water sampling technique and promises to circumvent most of the challenges in water quality monitoring. The first study (Chapter 2) investigated the feasibility of coupling passive sampling and *in vitro* bioassay techniques for chemical and ecotoxicological assessment of complex mixtures of organic contaminants in water at four riverine sites and one estuarine site in the Ythan catchment, north east (NE) of Scotland. Extracts of SR-PSDs deployed in water at the five sites for 8–9 weeks were analysed for freely dissolved PAHs, PCBs and a variety of pesticides. Unfortunately, SR-PSDs K_{SW} and diffusion coefficients (D_P) are not currently available for pesticides and acid/urea herbicides and consequently aqueous concentrations of pesticides and acid/urea herbicides could not be calculated. However, SR-PSDs could be used to assess their occurrence and the absorbed amounts allowed a relative comparison and screened for a wide range of pesticides and acid/urea herbicides in the catchment. The acute cytotoxicity and cytochrome P450 1A (EROD) activity potentials of each of the extracts were assessed on the RTL-W1 fish cell line in 24-well multititre plates. Chemical and biological toxic equivalent concentrations (Chem-TEQ and Bio-TEQ) of each SR-PSDs extract were also calculated and compared, indicating the wider application of the procedure. Whilst no acute cytotoxicity was observed, extracts from all five sites in the Ythan catchment exhibited greater EROD-inducing potential than was predicted based upon the determined concentrations of PAHs and PCBs. Overall, the study demonstrated that extracts of SR-PSDs deployed in surface water can be applied with minimal preparation to both chemical and ecotoxicological evaluations. The procedure can also be used to rapidly and economically measure the potential impact of complex mixtures of organic contaminants and also to detect the presence of toxic compounds not routinely analysed for using chemical analytical techniques.

In the second study (Chapter 3), the field performance of SR-PSDs deployed in water at three sites was assessed concurrently with composite water samples collected from two of the sites using autosamplers. The comparison between SR passive sampling and automatic sampling techniques was based on semi-quantitative and quantitative determinations of the presence or absence of pesticides and acid/urea herbicides in the samples. Overall, the use of SR-PSDs detected many more compounds than the use of autosamplers, and the sites

with the number and highest concentrations were those where ecology was known to be poor and failing. This reflects the higher utility and lower limits of detection achieved by this device when compared to autosamplers. In addition, a number of compounds which hitherto have not been detected in the catchment using conventional spot water sampling and autosamplers, including some pesticides that are not currently authorised for agricultural use in the EU, including diuron, isoproturon, chlorpyrifos, monolinuron, atrazine, trifluralin, dichlorvos, diazinon, fenuron, diflufenican, mevinphos and parathion ethyl (EC, 1979, 1991, 2009b) were identified in this study using silicone rubber passive sampling. It was suggested that, the individual banned compounds or their mixtures may be responsible or contributed to the observed poor ecological indicators and biological effects measured in some streams within the catchment. However, it was not conclusive from the study if the banned compounds were as a result of historic useage or present irregularities in the catchment. Further, the use of SR passive samplers in this study allowed the quantification of some PCBs in the catchment, which has not previously been possible using conventional water or biota monitoring techniques (Macgregor et al., 2010). It was concluded that the silicone rubber sampling approach can thus provide a better understanding and clearer picture of the use and presence of organic contaminants within catchments than existing techniques. However, a number of the acid/urea herbicides, with very low log K_{OW}s including bentazone, MCPB and mecoprop, were detected in the water samples, albeit at concentrations lower than limit of quantification (LOQ), but were not detected via SR-PSDs, which maybe a major drawback for this technique. However, this does not provide a full explanation because there were instances where lower log K_{OW} chemicals were detected in the SR-PSD samples. Other factors, including the acidic or neutral properties of the compounds in water and a variety of environmental conditions of the water including the pH level of the water since partitioning to organic matter is considerably lower with charged species than the neutral species (Helweg et al., 2003). For the acid herbicides, a combination of physicochemical properties, such as dissociation constant (pKa) and log K_{OW} values would influence their absorption by the samplers from water.

Having established and successfully applied silicone rubber passive sampling twice in this study, as well as coupling SR-PSDs with *in vitro* bioassays in evaluating the combined toxicity of organic contaminants in the Ythan catchment, it was concluded that *in vivo* bioassays might provide additional understanding of the potential combined effects of the chemical stressors on resident non-target organisms. The first application of an *in vivo* bioassay in this study was performed with extracts of SR-PSDs deployed in water at the Forth estuary and Firth of Forth Scotland United Kingdom. The presence and combined

toxicity of dissolved organic contaminants in water at five sites in the Forth estuary and Firth of Forth, was investigated using SR-PSDs and an algal growth inhibition bioassay with *D. lutheri* in a 24 well microtitre plates (Chapter 4). The Forth estuary and the Firth of Forth are major centres for agrochemical, petrochemical and refining industries in Scotland and receive high inputs of urban and industrial waste water. However, due to contemporary legislation and treatment of discharges, direct input of trace organic contaminants into the Forth estuary and Firth of Forth has reduced considerably in recent years (Baxter et al., 2011). As a result, in addition to the low aqueous solubility of most organic contaminants and dilution factors, the concentrations of the majority of the priority organic contaminants monitored in the water phase using conventional sampling techniques are often low or below instrumental detection limit (Dobson et al., 2001; Matthiessen and Law, 2002). Further, whilst there is increasing evidence of environmental exposure to multiple contaminants at low environmental concentrations in the Forth estuary and Firth of Forth, the potential ecotoxicological and human risks remain poorly understood. An alternative and improved approach was therefore needed to assess whether chemical pressures not observed by chemical analysis may be having impacts on sensitive aquatic organisms in the estuary and Firth of Forth. Another dimension that was added to the study was the use of source apportionment (ratios and principal component analysis; PCA) to further evaluate the possible sources of the freely dissolved concentrations of PAHs and PCBs in the Forth estuary and the Firth of Forth. PCA of dissolved PAH and PCB concentrations effectively clustered sites and the similarities in sources for these sites were obtained. The individual and total dissolved concentrations of PAHs and PCBs measured in the study were relatively low and showed input of petrogenic, atmospheric and sewage-related sources. Several pesticides of diverse polarities were identified in the water suggesting sources from both riverine input and direct discharges. Overall, the study demonstrated that extracts of SR-PSDs from the five sites in the Forth estuary and Firth of Forth exhibited potential for growth inhibition in *D. lutheri*, indicating the presence of, and exposure to, phytotoxic compounds in the estuary and the Firth of Forth.

To further evaluate the causes of poor and failing ecological status in some sites within the Ythan catchment, extracts of SR-PSDs from the first and the second sampling campaign were evaluated for embryo and algal toxicity potentials of the organic contaminant mixtures in the catchment. The extracts of SR-PSDs deployed in the Ythan, exhibited developmental toxicities to embryos of zebrafish and growth inhibition to *D. lutheri* indicating the presence and environmental exposure to toxic compounds in the catchment. The results from the algal growth inhibition tests from the Forth estuary were similar to those of Firth of Forth, even though, fewer number of pesticides and acid/urea herbicides were detected in the Forth

estuary and Firth of Forth than the Ythan. This indicates that pesticides and acid/urea herbicides were probably not solely responsible for the effects observed on the ecology in the Ythan catchment.

Prior to the onset of this project, the challenges (e.g. possibilities of losses through volatilisation and sorption processes) of toxicity testing of organic contaminants in multititre plates were recognised. Part of the initial objectives was to investigate the possible application of partition controlled delivery (passive dosing; PD) in toxicity testing of environmental extracts. In line with this objective, there was a need to identify, validate and apply a suitable passive dosing format in the toxicity testing of environmental mixtures collected using SR-PSDs. This study has achieved a part of the set objectives, silicone rubber O-rings were identified as a suitable PD format, compatible with our scale of investigations in 24 well multititre plates. The use of SR O-rings in *in vitro* toxicity tests was partially validated through their use in dosing a fish cell line with two PAHs and subsequently determining cytotoxicity and EROD-activity in a rainbow trout cell line (see Appendix 1; Chapter 7). This preliminary PD study was published as an extended abstract and presented at the 23rd Annual Meeting of the Society of Environmental Toxicology and Chemistry (SETAC) Europe, Glasgow, UK, 12th -16th MAY 2013 Emelogu et al., 2013d). Further work is required to adapt the protocols for use with environmental extracts, and for demonstrating use of the PD approach in whole organism bioassays.

Overall, the mixtures of chemical contaminants that are present in our aqueous ecosystems have the potential to cause impact on the biota. Man has long recognised that there is a need for appropriate legislation to ensure that such impacts are minimal or non-existent. Over time, studies have allowed an improved understanding of the effects of contaminants. However, there remain gaps which require to be filled. One of such gaps is the appropriate sampling of our aquatic environment and passive sampling is a developing methodology with considerable advantages. At the same time, passive dosing can also provide improved toxicity testing. Ultimately, there is a need to provide clear information in respect of the presence and impact of hazardous substances such that appropriate legislation can be introduced to reduce, and ultimately, eliminate hazardous substances form our aquatic ecosystems. We need to be able to determine the impact of legislation and the cessation of use and application of compounds. Lower concentrations require new methodologies and assessments should be as close to the situation experienced by the plants and animals that inhabit our rivers and seas. Other than the requirements of water framework directive (WFD) in achieving good environmental status in coastal and transitional waters by 2015 and OSPAR Convention, the marine strategy framework directive (MSFD; EC, 2008) requires Scotland to demonstrate that concentrations of some selected

hazardous substances including PAHs and which may include PCBs in the marine environment, do not result in pollution effects or are a risk to human health. As such, there is a need to develop appropriate methodologies in respect of biological effects measurements. The methodologies described in this thesis are part of the development process to ensure that the objectives of good environmental status can be met for hazardous substances.

In conclusion, this research has facilitated a better understanding of sources, fate, bioavailability and potential biological effects of multiple arrays of organic contaminants in the Scottish aquatic environment. The study also demonstrated that SR-PSDs can be employed to monitor the occurrence and distribution of a wide range of semi-polar organic compounds, including a variety of pesticides and acid /urea herbicides in addition to the non-polar organic compounds that they are already often employed for. Therefore, the study has provided insights into the future wider application of using SR-PSDs quantitatively for measurement, source identification and establishing the environmental fate of pesticides and herbicides once the required diffusion coefficient (D_P) of the compounds in the sampler material and sampler-water partition coefficient (K_{SW}) data become available.

6.2 Recommendations and future work

The study also identified some aspects in coupling passive sampling and bioassay techniques that could do with some improvement. Alternative techniques in the extraction of passive samplers should be explored, including the use of dialysis or accelerated solvent extraction (ASE). This would allow faster and cost effective (i.e. reduced energy and solvent) extractions of the samplers.

Reliable estimation of water concentrations of contaminants absorbed by passive sampling requires calibration data to convert the amount (ng) of target compounds absorbed by the samplers to the freely dissolved aqueous concentrations (e.g. ng L^{-1}) (Huckins, 2006). To achieve this, the log K_{SW} and the sampling rates (R_S) values for the target compounds are essential for fully quantitative assessments. Further, the uptake of organic compounds by non-polar passive samplers is principally controlled by a number of factors including the resistance to transport in the water boundary layer (WBL), nature of the target compound and the diffusion in the sampler material (Huckins, 2006). However, compared to the WBL, the resistance to transport of the sampler material was found negligible on SR samplers for compounds with log K_{OW} >3 (Rusina et al., 2007). As the molecular weight of the sampled

compounds increases, the diffusion and transport through the WBL decreases and consequently the R_S decreases (Rusina et al., 2010). Therefore, for most of the pesticides and acid/urea herbicides monitored in this study, the diffusion and transport in the membrane may be the limiting factor. So, other than log K_{SW} values, knowledge of the D_P of the analytes is equally essential so as to estimate the water concentrations to a level of accuracy. Future work should focus on laboratory studies for a variety of organic contaminants including pesticides and acid/urea herbicides so as to expand the range of compounds that can be monitored using SR-PSDs.

From the preliminary studies on passive dosing (PD), further work is required to adapt the protocols for use with environmental extracts, and for demonstrating the use of the PD approach in whole organism bioassays. SR O-rings could be loaded/spiked with environmental extracts by a variety of methods including solvent evaporation, however, this can lead to evaporative loses and crystallisation at the silicone layer-air interface (Mayer et al., 1999). The SR O-rings could be loaded through partitioning of the compounds from a methanol standard solution as described by Booij et al. (2002). Possibility also exists for the SR O-rings to be deployed in water alongside SR samplers during routine sampling. The challenge, however, is to sterilise the surface of the O-rings prior to in vitro toxicity test without affecting the composition of the absorbed substances. Another option is to cut SR-PSD that have been deployed in water to suitable shapes and exposed directly to test organisms in appropriate test systems.

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7 CHAPTER SEVEN: Appenidices

7.1 Appendix 1: Using passive dosing to assess the cytotoxicity and cytochrome induction potentials of polycyclic aromatic hydrocarbons (PAHs) on fish cell line

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7.1.1 Introduction

In vitro toxicity testing of hydrophobic organic contaminants (HOCs) is challenging due to their low aqueous solubility, compound losses through volatilisation, adsorption and absorption to plastic well plates and serum constituents. This leads to inaccurate and declining exposure concentrations and bias in the interpretation of toxicity data. Whilst, the use of co-solvents (e.g. methanol or dimethyl suphoxide; DMSO) may increase the solubility of HOCs in cell culture medium, co-solvents may also interact with the test compounds and therefore interfere with the biological activity [1]. For a realistic *in vitro* toxicity evaluation of HOCs, partitioning controlled delivery (passive dosing; PD) promises to overcome the highlighted challenges. PD involves the continual partitioning of test compounds e.g. HOCs, spiked into a biologically inert polymer that acts as a storage compartment and source. This eliminates test compound loses, provides known and stable amount of the freely dissolved concentrations of the test compounds and eradicate the need of dosing with co-solvents. The simplicity, cost effectiveness and sensitivity of using silicone rubber (SR) O-rings as the PD polymer during *in vitro* toxicity test of HOCs have been demonstrated [2]. However, preparation of the SR O-rings including pre-extraction and loading with test compounds can still be improved and the dosing system can be extended to assays with adherent fish cell lines. In this study we assessed the feasibility of using SR O-rings for the toxicity testing of fluoranthene and chrysene with a fibroblast-like permanent fish cell line derived from rainbow trout liver (*Oncorhynchus Mykiss;* RTL-W1) in 24-well microtitre plates. The study is intended to set a foundation and provide insight to the assessment of environmental extracts in future studies.

7.1.2 Materials and methods

7.1.2.1 Preparation of SR O-rings

Food-grade silicone rubber O-rings [2] were pre-extracted in hot ethyl acetate for > 100 h using Soxhlet apparatus so as to remove low molecular weight SR oligomers that might affect the toxicity test. Following pre-extraction, the SR O-rings were thoroughly rinsed with ultra-pure water to remove adhering chemical solvents and loaded separately to saturation with fluoranthene and chrysene compounds through partitioning in methanol/water [3]. Loading to saturation implies water concentrations of the test compounds during testing were at the limit of aqueous solubility. The surface of the SR O-ring was thoroughly wiped using lint-free tissue paper to remove any adhering suspension and quickly rinsed with ultra-pure

water to remove any residual methanol. A set of SR O-rings were treated the same way as above, but in this case without any PAH compound; these served as the process control (ProC), while another pre-extracted but not spiked SR O-rings were used as negative control (NC).

7.1.3 Acute cytotoxicity and induction of cytochrome P450 1A (CYP1A

RTL-W1 cells used in this study were provided by Drs. N.C. Bols and L. Lee; University of Waterloo, Canada. The details of cell culture and handling have been described previously [4]. Cells were exposed to chrysene and fluoranthene spiked and ProC SR O-rings to assess the acute cytotoxicity and cytochrome P450 1A (CYP1A) induction potential of test compounds using neutral red uptake (NRU) and EROD induction as endpoints. Each well had 1 mL of fresh L15 medium to completely immerse the SR O-rings. As positive control (PC) in the NRU and EROD assays, 3, 5-dichlorophenol (DCP) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) was used respectively. Each test was conducted in replicate with n=4 SR O-rings. During the exposure, the plates were incubated at 20 °C with constant but gentle shaking at ~ 80 rpm. Neutral red uptake and EROD induction assays were performed after 24 and 72 h respectively as described in a previous study [4].

7.1.3.1 Results and discussions

In the present study, there were no significant cytotoxic effects of chrysene and fluoranthene on RTL-W1 cells (Fig. 1 A); this is contrary to literature data on these compounds. It might be that in previous studies the vehicle control solvents used e.g. methanol or DMSO increases exposure to the test compounds due to their ability to increase cell membrane permeability. However, chrysene loaded on SR O-rings exhibited dioxin-like activity while fluoranthene did not at their aqueous concentrations as illustrated in Fig 1 B. It should be noted that the introduction and removal of SR O-rings to 24 well microtitre plates containing RTL-W1 cells is uncomplicated using suitable tweezers, however, adequate care must be observed to minimise disruption of the adherent cells.

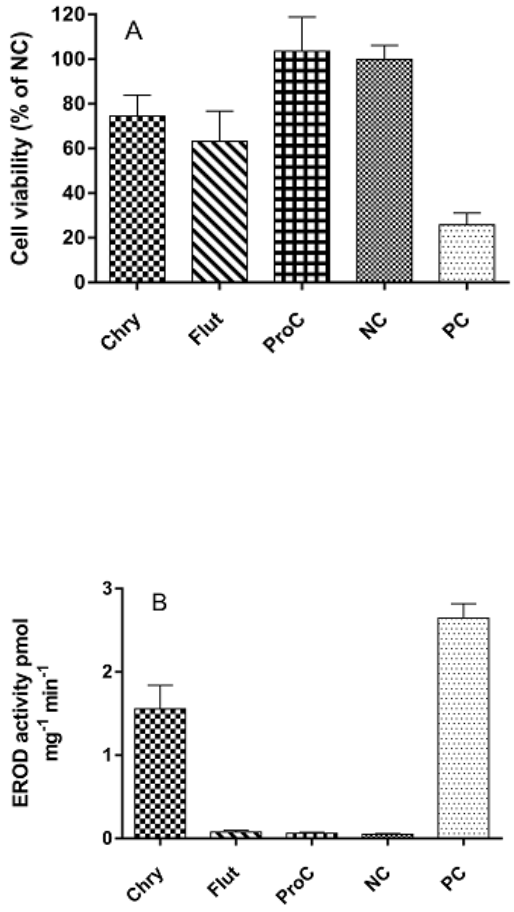

Fig.1 A and B Cytotoxicity and EROD induction of chrysene and fluoranthene loaded on SR O-rings on RTL-W1 cells. Cell viability given as means of standard deviation (±SD) of negative control; n=4. EROD induction given as mean ± SD; n=4. Chry=chrysene; Flu =fluororathene, ProC=process control, NC= negative control and PC=positive control

7.1.4 Conclusion

This study has demonstrated the feasibility of applying SR O-rings as the donor phase for exposure control of RTL-W1 cells within 24-well microtitre plates using neutral red retention and EROD assays. The study has also provided useful knowledge and insights on the advantages and limitations of the procedure. Pragmatic knowledge has been gained on preparation of SR O-rings including pre-extraction, spiking with test compounds and exposure to RTL-W1 cells. Future work is focusing on applying passive dosing in the assessment of mixture toxicity of freely dissolved organic contaminants obtained in water using silicone rubber passive samplers.

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7.2 Appendix 3: Supporting information for chapter two

Investigating the significance of dissolved organic contaminants in aquatic environments: coupling of passive sampling with *in vitro* bioassays

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Supporting material for the paper:

7.2.1 Chemical analysis

7.2.1.1 Analysis of PAHs

The PAH fractions of SR extracts were determined following standard procedures within a laboratory quality system accredited to ISO17025 by the United Kingdom Accreditation Service (UKAS). Briefly, extracts were purified and aromatic PAHs separated from aliphatic hydrocarbons using isocratic high performance liquid chromatography (HPLC, normal phase Genesis SIL 4 µm HPLC column, 25 cm x 4.6 mm, Jones Chromatography, Mid Glamorgan, UK). The aromatic fractions were reduced to ~100 µL using a rotary evaporation apparatus and activated carbon purified nitrogen blow down. Quantitative analysis of forty PAH compounds, 2 to 6 ring (parent and branched) were performed by GC with mass spectrometry (MS) as described in Webster et al. (2009) using the seven deuterated PAHs (section 2.4.1) as internal standards (recoveries were greater than 90%).

7.2.1.2 Analysis of PCBs

The PCBs fractions of SR extracts were cleaned-up to separate the PCBs from organochlorine pesticides (OCPs) by passing through a 3% deactivated silica column (with a pre-determined split volume) using *iso*-hexane as the carrier solvent. The OCP fractions were discarded. The PCB eluent was reduced to ~300 µL. An injection standard containing ~130.6 µg μL^{-1} 2, 4-dichlorobenzyl alkyl ether with C16 and C6 alkyl chains (DCBE6 and DCBE16) were added and transferred to pre-weighed GC vials and concentrated to ~100 µL. The concentrations of thirty-two *ortho and* mono *ortho* PCBs were determined by GC-electron capture detector (ECD) as detailed in Webster et al. (2009) however an HT-8 column (60 m x 0.25 mm, 0.25 µm film thickness; Agilent, Stockport, UK) was used due to co-elution and interferences of CBs 31, 105 and 153 using an HT-5 column.

7.2.2 Analysis of pesticides and acid/urea herbicides

Pesticides fractions of SR extracts were aliquoted into two equal portions for the analysis of selected pesticides and acid/urea herbicides. Selected pesticides (toluene extract) were

analysed using a Varian CP-3800 GC with a Varian 1079 injector (CTC Analytics CombiPal) interfaced to a triple quadrupole MS (Varian 1200 L) both from Varian, Walnut Creek, CA, USA. Separation of the analytes was made using a DB-5ms deactivated fused silica column (30 m × 0.25 mm I.D., 0.25 µm film thickness; Agilent Technologies, Stockport, England). On-column split injection was applied and the split ratio was 1:10 at an injection volume of 2 µL; the inlet temperature was 220 °C. Helium (99.99% purity) was used as a carrier gas at a constant flow rate of 2 mL min^{-1}. The GC temperature program was 50 ºC held for 2 min, then raised at 25 ºC min^{-1} to 150 ºC, increased at 25 ^{o}C min^{-1} to 300 ^{o}C and held for 8.0 min (total run time 20 min). Electron ionisation (EI) mode was applied and components were identified and quantified by comparison with standards using retention time and in multiple reactions monitoring (MRM) mode (two masses per compound).

The acid/urea herbicide fractions of SR extracts (in ACN) were analysed using a Dionex UltiMate 3000 LC system (Dionex, Sunnyvale, CA, USA) coupled to a triple quadrupole Sciex 4000 MS (Applied Biosystems, CA, USA). The MS-MS system was equipped with a Turbo Ion Spray source (Electrospray ionisation; ESI) operating in both negative (acid herbicides) and positive (urea herbicides) ionization modes. Separation in the negative and positive modes was performed with C_{18} (2) Luna (150 mm x 3 mm I.D; 3 µm film thickness) and C_{18} Gemini (150 mm x 2 mm I.D; 3 µm film thickness) analytical columns respectively (Phenomenex, Cheshire, UK). The mobile phase for both acid and urea herbicides was a gradient of ACN and water (both containing 0.1% acetic acid), at a flow rate of 0.4-0.7 and 0.2-0.3 mL min^{-1} respectively. The injection volume was 200 µl and the total run time (both positive and negative ionisation modes) was 16 min. Each compound was detected and confirmed by two MRM transitions.

7.2.3 Sampling rates

All SR-PSDs deployed at the five sites were successfully retrieved after the exposure period without any loss or punctures of the SR sheets. Significant level of biofouling was not observed on the SR sheets in all sites despite the relatively long exposure time. The fourteen PRCs used in this study cover a wide range of log K_{OW} hence were appropriate for determining the *in situ* site specific sampling rates (R_S) for the analytes. In all sites, the percentage release of the PRCs from the SR passive samplers decreased with an increasing log K_{OW} and exhibited significant differences in the dissipation rates between the study sites. D10-Fluorene dissipated almost completely from the SR passive samplers in all sites, while CB155 and 204 showed the least dissipation. All PRC data were used to derive the R_S of the target compounds in all the sampling sites. The PRC derived sampling rates (R_S) and the

standard error for an average compound of mass 300 at each of the sampling site are shown in Figure 1 (1-5) .

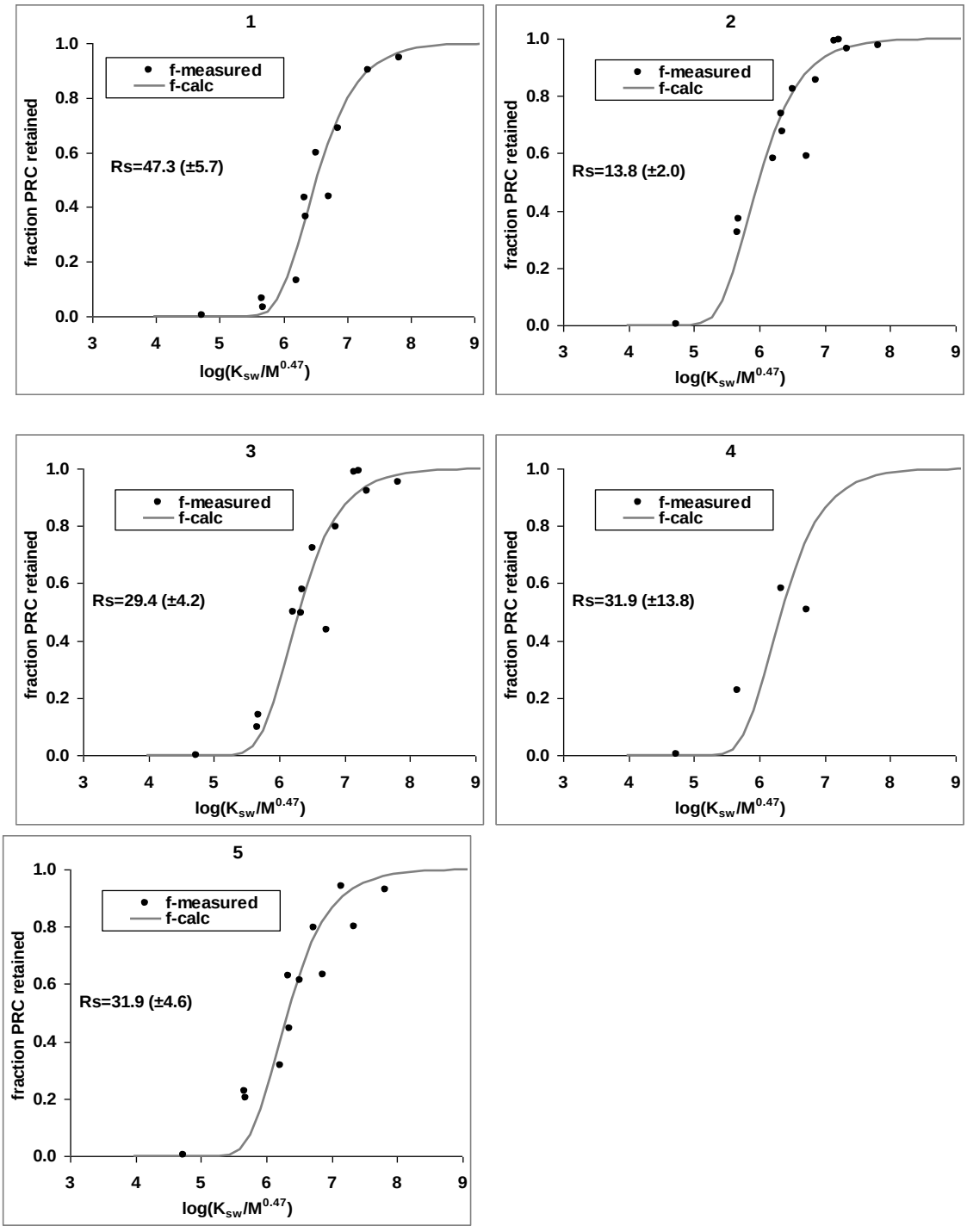

Figure 1: Plot of f versus compound properties and the fitting of measured dissipation of PRCs versus the modelled value. The PRC derived sampling rate ($R_{S;}$ L d^{-1}) values shown in the graphs were derived for an average compound of mass 300.

Table SI 3:

Concentrations of PAHs derived from silicone rubber (SR) passive samplers deployed in water at the five study sites

Site	**1**	**2**	**3**	**4**	**5**
PAHs	**ng L^{-1}**				
Naphthalene	32	5.5	7.1	10.5	17
2-Methyl Naphthalene	6.7	2	3.4	8	5.3
1-Methyl Naphthalene	4.9	1.8	2.9	5.9	3.7
C2 Naphthalenes	1.34	1.11	2.8	3.4	2.5
C3 Naphthalenes	0.64	0.7	0.7	0.75	1.04
C4 Naphthalenes	0.18	0.37	0.21	0.41	0.48
Phenanthrene (178)	8.4	8	7.3	9	17
Anthracene (178)	0.114	0.34	0.33	0.43	0.44
C1 178	2.9	2.7	1.8	4.7	5.5
C2 178	0.46	0.45	0.31	0.58	0.52
C3 178	0.34	0.42	0.26	0.41	0.34
Dibenzothiophene	0.26	0.63	0.54	0.38	0.46
C1 Dibenzothiophenes	<LOD	<LOD	<LOD	<LOD	<LOD
C2 Dibenzothiophenes	0.16	0.78	0.42	0.98	0.66
C3 Dibenzothiophenes	0.072	0.29	0.25	0.36	0.21
Fluoranthene (202)	1.52	2.2	2	1.8	2.6
Pyrene (202)	0.61	2.5	1.6	2.5	2.4
C1 202	0.24	0.37	0.26	0.18	0.2
C2 202	<LOD	<LOD	<LOD	<LOD	<LOD
C3 202	0.0025	0.0147	0.0010	0.00124	0.0064
Benzo[c]phenanthrene (228)	<LOD	<LOD	<LOD	<LOD	<LOD
Benz[a]anthracene (228)	0.019	0.097	0.068	0.054	0.067
Chrysene/Triphenylene (228)	0.31	0.55	0.35	0.29	0.34
Benz[b]anthracene (228)	0	0.023	0.0118	0.0125	0.0084
C1 228	0.0146	0.051	0.022	0.017	0.019
C2 228	0.0044	0.00082	<LOD	0.0024	0.00027
Benzofluoranthenes (252)[1]	0.116	0.149	0.107	0.088	0.19
Benzo[e]pyrene (252)	0.062	0.117	0.059	0.059	0.106
Benzo[a]pyrene (252)	0.0031	0.028	0.0128	0.016	0.0151
Perylene (252)	0.00103	0.018	0.0139	0.019	0.02
C1 252	0.00115	0.0068	0.0019	0.0022	0.0022
C2 252	0	0	0	0	0
Indenopyrene (276)	0.0051	0.0085	0.0053	0.004	0.0106
Benzoperylene (276)	0.0021	0.0113	0.006	0.0069	0.0115

C1 276	<LOD	<LOD	<LOD	<LOD	<LOD
C2 276	0.000095	<LOD	<LOD	<LOD	<LOD
Acenaphthylene (152)	0.57	0.56	0.95	1.7	1.6
Acenaphthene (154)	2	2.4	3.7	3.6	2.1
Fluorene (166)	4.7	3.8	2.9	1.9	4
Dibenz[a,h]anthracene (278)	0.00129	0.0031	0.0013	0.00158	0.0022
∑PAHs	69	38	41	59	69

[1] Benzofluoranthenes is sum of benzo[*b*]fluoranthene and benzo[*k*]fluoranthene

LOD = the limit of detection. The LOD was 2 x the concentrations (ng g^{-1} SR) in the field blank; expressed as aqueous concentration (ng L^{-1}) the LOD would vary at each site, depending upon duration and sampling rate.

Table SI 4: Concentrations of PCBs derived from silicone rubber (SR) passive samplers deployed in water at the four study sites

Site	**1**	**2**	**3**	**5**
PCBs	**ng L^{-1}**			
CB 31	0.00061	0.0027	0.0014	0.00068
CB 28	0.0011	0.003	0.0015	0.0011
CB 52	<LOD	0.0054	0.0028	<LOD
CB 49	0.00098	0.0021	0.001	0.0006
CB 44	0.00081	0.0029	0.0013	<LOD
CB 74	0.00034	0.00097	0.00052	<LOD
CB 70	0.0012	0.0032	0.0016	<LOD
CB 101	0.0022	0.0059	0.0026	<LOD
CB 99	0.00087	0.0022	0.0011	0.00061
CB 97	0.00073	0.002	0.00085	0.00049
CB 110	0.0023	0.0064	0.0028	0.0014
CB 149	0.0013	0.0031	0.0014	0.00097
CB 123	0.0026	0.0035	0.0090	<LOD
CB 118	0.0018	0.0051	0.0022	0.0014
CB 114	0.0002	0.0004	0.00019	0.00019
CB 132	0.00068	0.0018	0.00075	0.00046
CB 153	0.0015	0.0031	0.0014	0.0012
CB 105	<LOD	<LOD	<LOD	<LOD
CB 137	0.00011	<LOD	<LOD	<LOD
CB 138	0.0014	0.0037	0.0016	<LOD
CB 158	0.00017	0.00041	0.00019	<LOD
CB 187	0.00027	0.00044	0.00022	0.00018
CB 183	0.000094	0.00014	0.00006	0.000072
CB 128	0.00045	0.0012	0.0005	0.00034
CB 167	<LOD	<LOD	<LOD	<LOD
CB 156	0.00012	<LOD	<LOD	<LOD
CB 157	<LOD	<LOD	<LOD	<LOD
CB 180	<LOD	<LOD	<LOD	<LOD
CB 170	0.00011	0.00023	0.00011	0.000072
CB 189	0.00022	<LOD	<LOD	0.00019
CB 194	<LOD	<LOD	<LOD	0.000043
CB 209	<LOD	<LOD	<LOD	<LOD
∑PCBs	0.03	0.06	0.04	0.02

LOD = the limit of detection. The LOD was 2 x the concentrations (ng g^{-1} SR) in the field blank; expressed as aqueous concentration (ng L^{-1}) the LOD would vary at each site, depending upon duration and sampling rate.

Table SI 5

List of selected pesticides measured in this study and the MS/MS detector acquisition parameters

Analyte	Quantification	Analyte	Quantification Transitio
Atrazine	200>122 (10V)	Metalaxyl	249>190 (15V)
Azinphos ethyl	160>132 (5V)	Metaldehyde	89>45 (10V)
Azinphos methyl	160>132 (5V)	Metazachlor	277>132 (40V)
Azoxystrobin	344>183 (25)	Methiocarb	168>153 (15V)
Bitertanol	170>141 (20V)	Metribuzin	198>182 (20V)
Boscalid	342>140 (20)	Mevinphos	127>109 (10V)
Carbofuran	221>164 (15V)	Molinate	187>126 (5V)
Carbophenothion	157>45 (10V)	Omethoate	156>110 (15V)
Chinomethionate	206>148 (15V)	Oxadixyl	163>132 (15V)
Chlorfenvinphos (Z)	267>159 (35V)	Parathion ethyl	291>109 (10V)
Chlorothalonil	264>229 (25V)	Pendamethalin	252>162 (10V)
Chinomethionate	206>148 (15V)	Pentachlorobenzene	250>214 (15V)
Chlorpyrifos ethyl	314>258 (10V)	Picoxystrobin	335>173 (10V)
Chlorpyrifos methyl	286>271 (25V)	Pirimicarb	166>96 (10V)
Cyanazine	225>189 (15V)	Pirimphos ethyl	318>166 (25V)
Cyproconazole	222>125 (30V)	Pirimphos methyl	290>125 (20V)
Cyprodinil	224>208 (35V)	Prochloraz	308>70 (10V)
Diazinon	304>179 (10V)	Prometryn	226>184 (10V)
Dichlobenil	171>136 (15V)	Propamocarb/	188>58 (10V)
Dichlorvos	109>79 (5V)	Propazine	229>58 (10V)
Dicofol	251>139 (10V)	Propetamphos	236>194 (10V)
Difenoconazole	323>265 (10V	Propiconazole	259>173 (20V)
Diflufenican	266>218 (25V)	Propoxur	152>110 (10V)
Dimethoate	125>79 (10V)	Pyraclostrobin	132>77 (15V)
Disulfoton	142>81 (10V)	Simazine	201>173 (5V)
Epoxiconazole	192>138 (15V)	Spiroxamine	100>72 (10V)
Etrimfos	266>133 (40V)	Tebuconazole	250>125 (20V)
Famoxadone	224>196 (10V)	Terbuthylazine	214>132 (10V)
Fenchlorphos	285>240 (25V)	Terbutryn	241>170 (10V)
Fenitrothion	277>260 (5V)	Thiabenzadole	201>174 (30V)
Fenoxycarb	116>88 (30V)	Tralkoxydim	137>57 (35V)
Fenpropidin	273>98 (10V)	Triademefon	208>181 (10V)
Fenpropomorph	128>70 (10V)	Triademenol	168>70 (10V)
Fenthion	278>109 (20V)	Trietazine	200>122 (10V)
Fluazinam	418>372 (20)	Trifloxystrobin	116>89 (15V)
Flusilazole	233>165 (10V)	Trifluralin	306>264 (5V)
Furathiocarb	163>107 (20V)	Vinclozolin	285>212 (10V)

Heptenophos	250>124 (10V)		
Hexaconazole	175>111 (30V)	Azobenzene (I.S.)	182>77 (15V)
Imazalil	215>173 (10V)	Diphenamid (I.S.)	167>152 (10V)
Iodofenphos	377>362 (15V)		
Iprodione	314>245 (10V)		
Isodrin	193>157 (25V)		
Isofenphos	224>208 (35V)		
Kresoxim Methyl	116>89 (15V)		
Malathion	173>99 (10V)		

I.S. = internal standards

Table SI 6 List of selected acid/urea herbicides measured in this study and the MS/MS Detector acquisition parameters

Analyte	R/T**	Q1	Q3	DP	EP	CE	CXP	Dwel
2, 4-dichlorophenoxy acetic acid (2,4-D)	8.68	218.9	161.1	-40.0	-7.50	-20.0	-4.00	30
Dicamba	8.87	218.9	175.1	-20.0	-7.50	-8.00	-3.00	30
4-(2,4-dichlorophenoxy) butyric acid	8.48	246.90	161.0	-20.0	-8.00	-18.0	-4.00	30
Dichlorprop	8.85	232.9	161.1	-45.0	-7.50	-18.0	-4.00	30
Bromoxynil	8.27	275.77	81.0	-70.0	-9.50	-45.0	0.00	30
Ioxynil	8.89	369.7	127.0	-75.0	-8.5	-50.0	0.00	30
Bentazone	10.31	239.0	132.0	-70.0	-7.50	-36.0	-2.00	30
2-Methyl-4-chlorophenoxyacetic acid	8.39	199.0	141.1	-55.0	-9.00	-20.0	-4.00	30
4-(4-Chloro-2-methylphenoxy) butanoic acid (MCPB)	8.51	227.1	141.1	-35.0	-2.50	-25.0	-4.00	30
2-(2-Methyl-4-chlorophenoxy) propionic acid (MCPP)	8.61	213.0	141.1	-30.0	-8.00	-22.0	-4.00	30
Triclopyr	8.7	253.9	196.0	-40.0	-7.50	-16.0	-4.00	30
Clopyralid	6.36	189.9	146.0	-20.0	-5.50	-12.0	-4.00	30
Fluroxypyr	7.41	253.0	195.0	-35.0	-4.00	-20.0	-4.00	30
Benazolin	7.66	242.0	170.1	-25.0	-7.00	-20.0	-2.00	30
4-Chlorophenyl acetic acid	7.36	169.0	125.0	-20.0	-10.0	-12.0	-2.0	30
2-Bromo-4-Cyano phenol	7.24	195.9	78.9	-45.0	-12.0	-32.0	-1.00	30
Diuron	12.07	233.0	72.0	71.0	12.0	35.0	10.0	40
Isoproturon	12.02	207.0	72.0	56.0	12.0	23.0	6.0	40
Monolinuron	12.05	215.0	126.0	56.0	9.0	25.0	12.0	40
Metoxuron	11.42	229.0	72.0	55.0	6.0	30.0	6.0	40
Fenuron	10.88	165.2	72.0	86.0	6.0	27.0	6.0	40
Pencycuron	13.24	329.0	124.8	90.0	7.0	39.0	10.0	40
Linuron	12.5	249.0	159.9	51.0	6.0	27.0	14.0	40
Chlortoluron	11.91	213.0	72.0	51.0	6.0	30.0	6.0	40
Neburon	12.95	275.0	88.0	76.0	8.0	25.0	8.0	40

**Expected retention time: may vary with column age.

References

Webster, L., Russell, M., Walsham, P., Phillips, L., Packer, G., Hussy, I., Scurfield, J., Dalgarno, E., Moffat, C., 2009. An assessment of persistent organic pollutants (POPs) in wild and rope grown blue mussels (Mytilius edulis) from Scottish coastal waters. J. Environ. Monitor. 11, 1169-1184.

7.3 Appendix 3: Supporting information for chapter three

Identification of selected organic contaminants in streams associated with agricultural activities and comparison between autosampling and silicone rubber passive sampling

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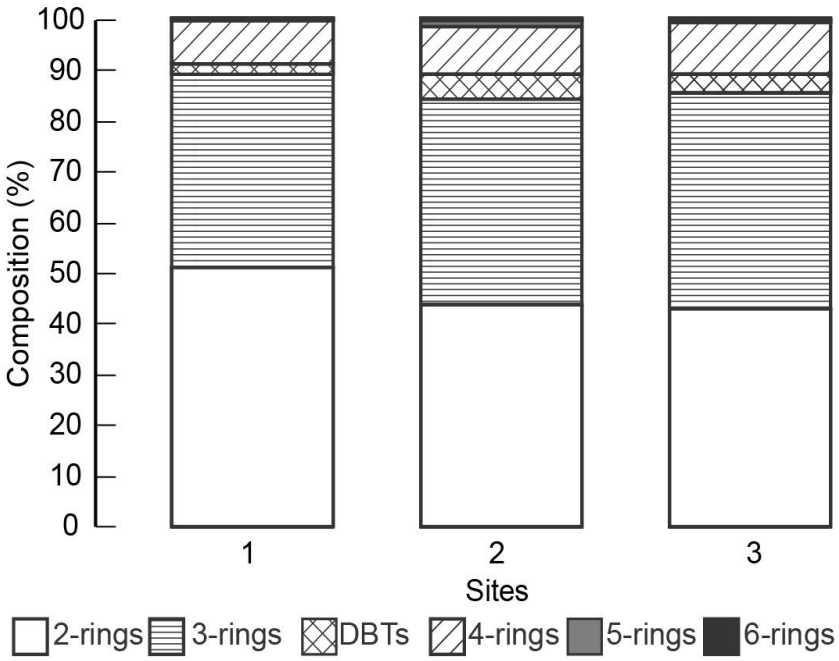
Fig.S1

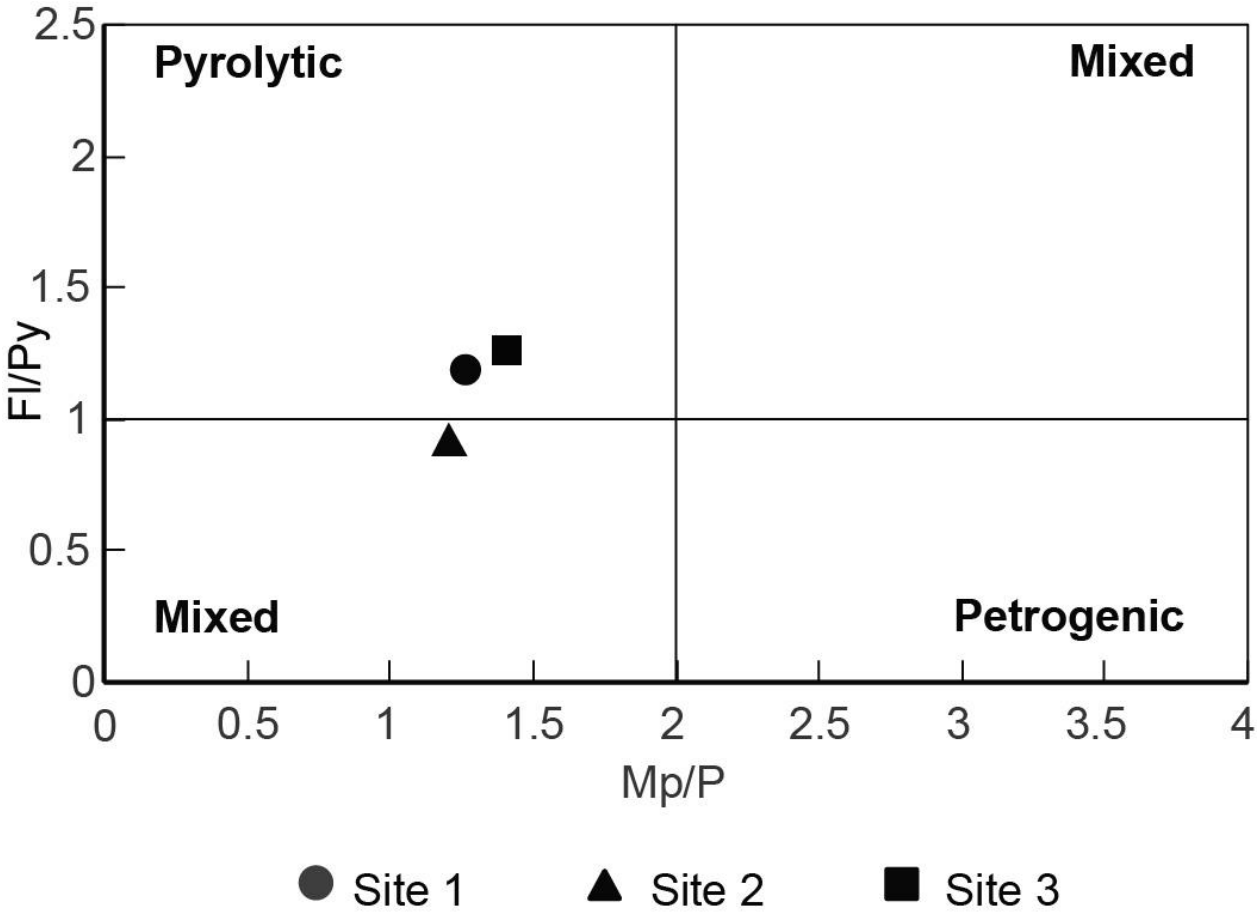
Composition of polycyclic aromatic hydrocarbons (PAHs) in water at the three sites.



Term description**:** 2-ring = ∑naphthalenes (parent and C1-C4); 3-ring = ∑acenaphthene; acenaphthylene; fluorene; phenanthrene and anthracene (parent and C1-C3); DBTs =∑dibenzothiophenes (parent and C1-C3); 4-ring = ∑fluoranthene and pyrene (parent and C1-C3); benzo[*c*]phenanthrene; benz[a]anthracene; benz[b]anthracene and chrysene (parent and C1-C2); 5-ring = ∑benzofluoranthene, dibenz[a,h]anthracene, benzo[*a*]pyrene, benzo[*e*]pyrene and perylene (parent and C1-C2). 6-ring=∑indenopyrene, benzoperylene (parent and C1-C2).

Fig. S2

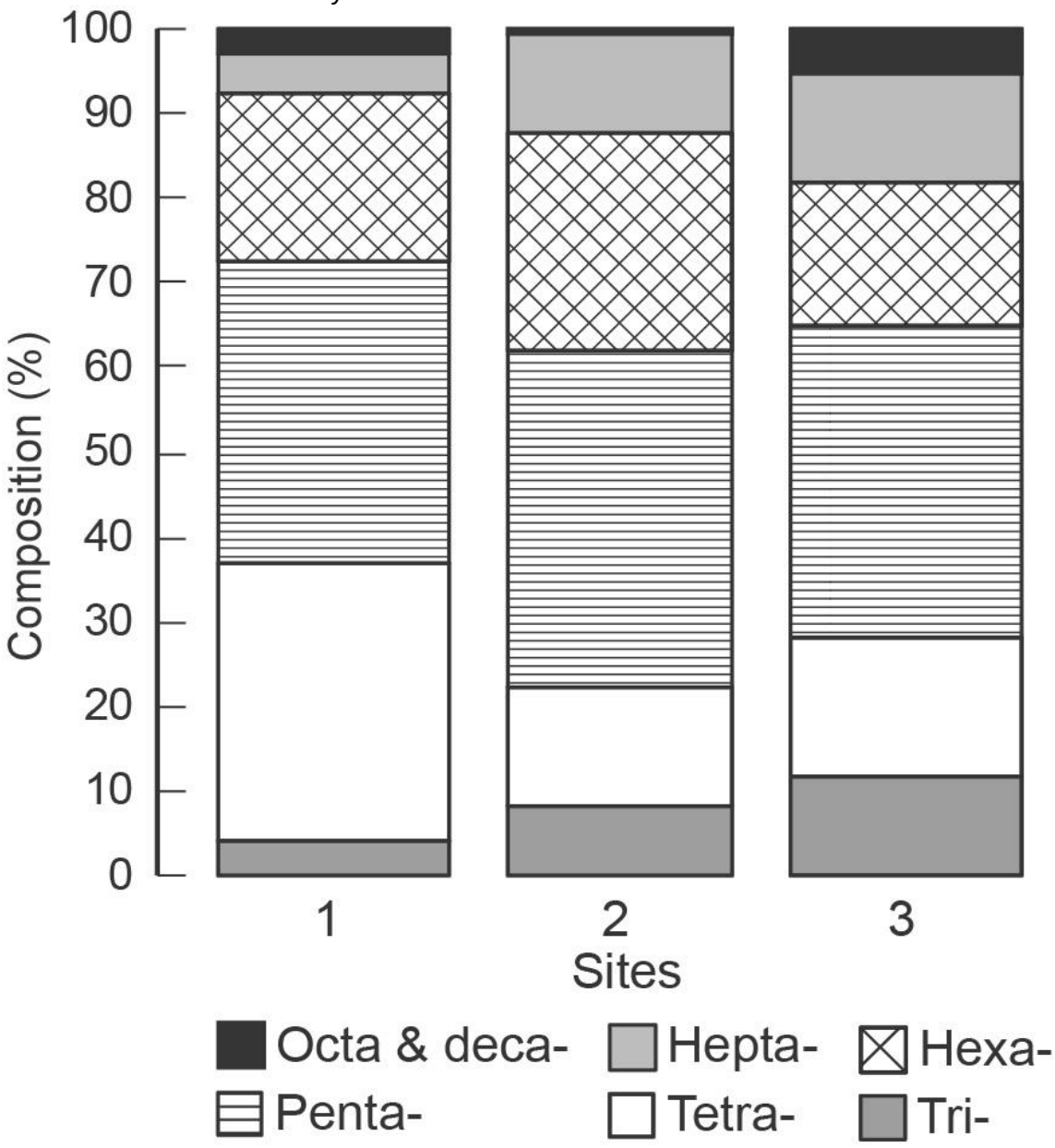
Silicone rubber (SR) concentration ratio plots to aid source identification of polycyclic aromatic hydrocarbons (PAHs) in water



Term description**:** Plot of fluoranthene/pyrene (Fl/Py) against methylphenanthrene/phenanthrene (Mp/P). Sites 1 and 3 were situated in the pyrolytic region, whereas site 2 was situated in the mixed region.

Fig. S3

Percentage composition of polychlorinated biphenyls (PCBs) in water by degree of chlorination at the three study sites.



Term description: Tri=∑CBs 28 and 31; Tetra= ∑CBs 44, 49, 52, 70 and 74; Penta =∑CBs 97, 99, 101, 105, 110, 114, 118 and 123; Hexa=∑CBs 149, 132, 153, 137, 138, 158, 128, 167, 156, and 157; Hepta = ∑CBs 170, 180,183, 187 and 189; Octa and Deca= ∑CBs 194 and 209

7.4 Appendix 4: Supporting information for chapter four

Occurrence and potential combined toxicity of dissolved organic contaminants in the Forth estuary and Firth of Forth, Scotland assessed using passive samplers and an algal toxicity test

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Table 1
A
Summary of (A) (individual and groups of) polycyclic aromatic hydrocarbons (PAHs), (B) 32 PCBs (ortho and mono-ortho), (ICES-7 indicator PCBs, highlighted in bold) and (C) pesticides, acid herbicides and urea herbicides analysed in this study grouped according to their structure and classification.

2-ring	3-ring	Dibenzothiophenes (DBTs)	4-ring	5-ring	6-ring
Naphthalenes	Acenaphthene	DBT	Fluoranthene	Benzofluoranthenes [1]	Indenopyrene
2-Methyl Naphthalene	Acenaphthylene	C1-DBT[2]	Pyrene 202	Bibenz[*a,h*]anthracene	Benzoperylene 276
1-Methyl Naphthalene	Fluorene	C2-DBT	C1-202	Benzo[*a*]pyrene	C1-276
C2 Naphthalenes	Phenanthrene	C3-DBT	C2-202	Benzo[*e*]pyrene	C2-276
C3 Naphthalenes	Anthracene 178		C3-202	Perylene 252	
C4 Naphthalenes	C1 178		Benzo[*c*]phenanthrene	C1-252	
	C2 178		Benz[*a*]anthracene	C2-252	
	C3 178		Benz[*b*]anthracene		
			Chrysene [3]		
			C1-228		
			C2-228		

B

Degree of chlorination	Tri-	Tetra-	Penta-	Hexa-	Hepta-	Octa&Nona-
Congeners	CB 031	**CB 052**	**CB 101**	CB 149	CB 187	CB 194
	CB 028	CB 049	CB 099	CB 132	CB 183	CB 209
		CB 044	CB 097	**CB 153**	**CB 180**	
		CB 074	CB 110	CB 137	CB 170	
		CB 070	CB 123	**CB 138**	CB 189	
			CB 118	CB 158		
			CB 114	CB 128		
			CB 105	CB 167		
				CB 156		
				CB 157		

C

Pesticides		Acid herbicides	Urea herbicides
Atrazine	Metalaxyl	2,4-D	Diuron
Azoxystrobin	Metazachlor	Dicamba	Isoproturon
Boscalid	Methiocarb	2,4-DB	Monolinuron
Carbofuran	Metribuzin	Dichlorprop	Metoxuron
Chlorfenvinphos (Z)	Mevinphos	Bromoxynil	Fenuron
Chlorothalonil	Oxadixyl	Ioxynil	Pencycuron
Chlorpyrifos ethyl	Parathion ethyl	Bentazone	Linuron
Chlorpyrifos methyl	Pendamethalin	MCPA	Chlorotoluron
Cyanazine	Picoxystrobin	MCPB	
Cyproconazole	Pirimicarb	MCPP	
Cyprodinil	Pirimphos methyl	Triclopyr	
Diazinon	Prometryn	Clopyralid	
Dichlobenil	Propetamphos	Fluroxypyr	
Dichlorvos	Propiconazole	Benazolin	
Diflufenican	Simazine		
Dimethoate	Tebuconazole		
Epoxiconazole	Terbuthylazine		
Famoxadone	Terbutryn		
Fenoxycarb	Triademefon		
Flusilazole	Triademenol		
Heptenophos	Trietazine		
Kresoxim methyl	Trifloxystrobin		
Malathion	Trifluralin		
	Vinclozolin		

[1]Benzofluoranthenes is the sum of benzo[*b*]fluoranthene and benzo[*k*]fluoranthene. [2]C1-C4 (alkylated). [3]Chrysene is Chrysene/Triphenylene.. MCPB=4-(4-chloro-2-methylphenoxy) butanoic acid; MCPA= 4-chloro-o-tolyloxyacetic acid; 2,4 D--(2,4-dichlorophenoxy)acetic acid; 2,4-DB, 4-(2,4-dichlorophenoxy)butyric acid.

Table 2
Freely dissolved concentrations (ng L^{-1}) of PAHs detected in water using silicone rubber passive samplers.

	Sites				
	Alloa	Grangemouth	Crombie	Braefoot	Gunnet Ledge
	ng L^{-1}				
PAHs					
Naphthalene	4.09	9.83	8.30	4.07	12.32
2-Methyl Naphthalene	3.80	4.60	3.86	2.07	2.83
1-Methyl Naphthalene	2.32	3.08	2.67	1.69	1.85
C2 Naphthalenes	5.37	5.40	4.70	3.22	2.61
C3 Naphthalenes	5.44	5.47	3.63	3.77	2.68
C4 Naphthalenes	5.94	5.03	3.10	3.35	2.19
Phenanthrene	2.41	1.98	1.75	2.28	2.57
Anthracene	0.53	0.43	0.31	0.33	0.19
C1 178	2.54	3.27	2.68	3.07	2.45
C2 178	3.44	3.03	2.06	2.49	1.71
C3 178	3.35	2.61	1.60	2.03	1.33
Dibenzothiophene	0.21	0.23	0.25	0.21	0.16
C1 Dibenzothiophenes*	1.54	0.98	0.79	0.72	0.46
C2 Dibenzothiophenes*	1.37	1.67	1.28	1.43	0.85
C3 Dibenzothiophenes*	1.73	1.91	1.14	1.23	0.57
Fluoranthene	2.98	2.99	2.51	3.46	2.77
Pyrene	4.35	4.41	3.42	4.18	2.69
C1 202	4.24	3.51	2.58	2.85	1.79
C2 202	1.38	1.02	0.66	0.81	0.37
C3 202*	0.54	0.36	0.22	0.30	0.12
Benzo[*c*]phenanthrene	<LOD	<LOD	<LOD	<LOD	<LOD
Benz[*a*]anthracene	0.33	0.24	0.17	0.22	0.14
Chrysene/Triphenylene	0.85	0.63	0.48	0.64	0.45

Benz[*b*]anthracene	0.09	0.06	0.03	0.04	0.02
C1 228	0.64	0.40	0.28	0.34	0.21
C2 228	0.78	0.34	0.20	0.21	0.16
Benzofluoranthenes[1]	0.68	0.32	0.21	0.29	0.22
Benzo[*e*]pyrene	0.42	0.23	0.15	0.22	0.15
Benzo[*a*]pyrene	0.16	0.09	0.04	0.06	0.03
Perylene	0.17	0.07	0.04	0.05	0.02
C1 252	0.27	0.11	0.06	0.08	0.05
C2 252	0.09	0.03	0.01	0.01	0.01
Indenopyrene	0.05	0.04	0.01	0.02	0.01
Benzoperylene	0.08	0.10	0.02	0.03	0.02
C1 276	0.02	0.01	<LOD	0.01	<LOD
C2 276	<LOD	<LOD	<LOD	<LOD	<LOD
Acenaphthylene	1.19	1.08	1.33	1.12	1.14
Acenaphthene	1.80	1.89	1.73	1.76	1.49
Fluorene	2.13	2.07	1.74	1.99	1.96
Dibenz[a,h]anthracene	0.02	0.01	0.01	0.01	0.01

LOD =limit of detection, 2 X the concentrations (ng g^{-1} SR) in the field blank sample; expressed as aqueous concentration (ng L^{-1}), the aqueous LOD varied at each sampling site, depending upon deployment duration and sampling rates (R_S).

[1]Benzofluoranthenes is the sum of benzo[*b*]fluoranthene and benzo[*k*]fluoranthene.

Table 3

Freely dissolved concentrations (pg L^{-1}) PCBs detected in water using silicone rubber passive samplers.

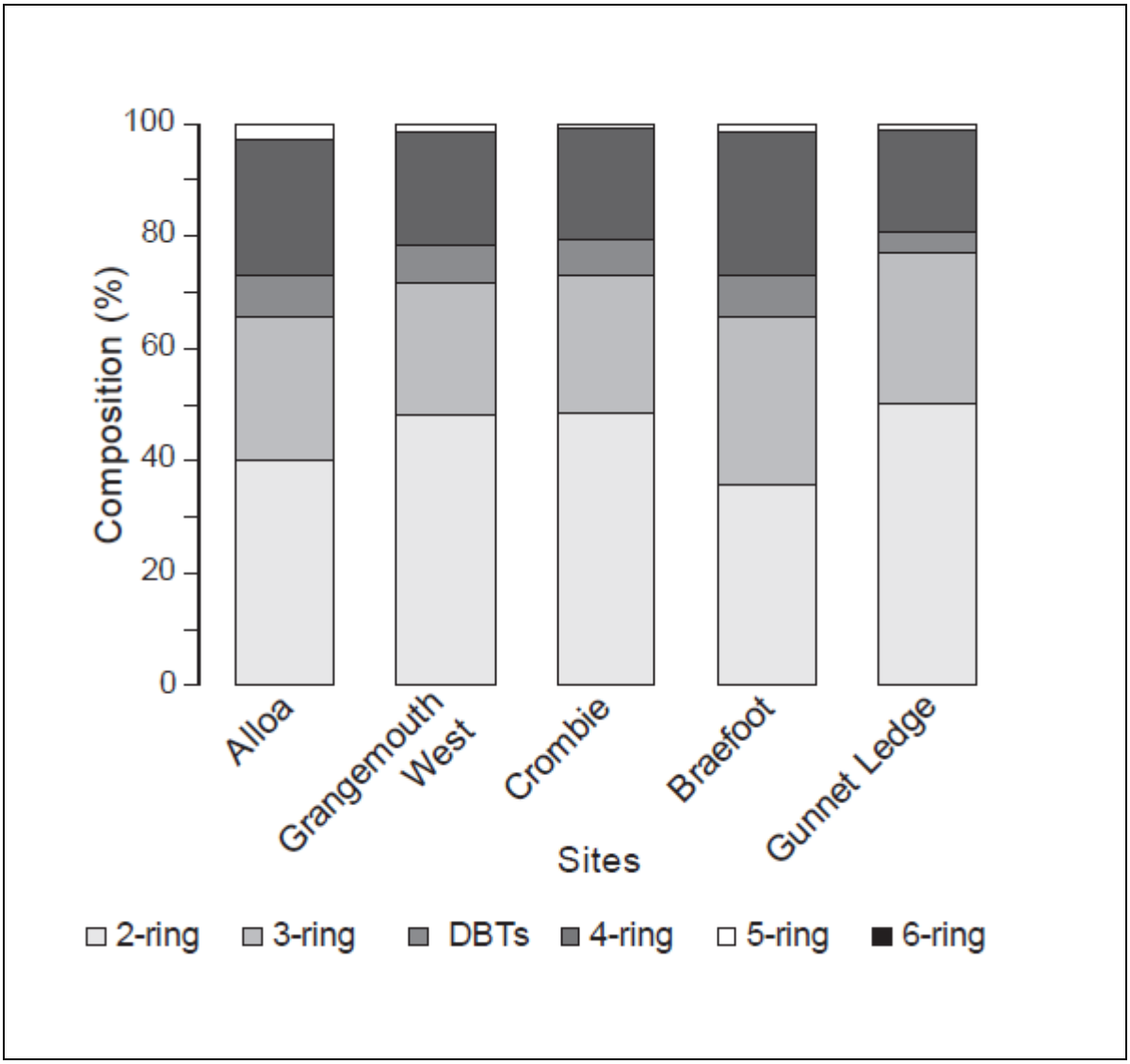
	Sites				
	Alloa	Grangemouth	Crombie	Braefoot	Gunnet Ledge
			pg L^{-1}		
PCBs					
CB 031	27.9	17.1	11.6	19.3	14.1
CB 028	31.1	21.0	14.9	22.6	16.4
CB 052	32.1	22.4	17.7	18.4	18.8
CB 049	18.1	14.1	10.2	14.3	12.5
CB 044	23.9	15.5	9.20	16.7	13.6
CB 074	6.16	5.64	4.12	5.03	4.28
CB 070	12.9	11.4	9.0	13.0	11.6
CB 101	21.9	15.2	12.9	17.7	15.8
CB 099	7.21	8.20	7.72	9.7	7.19
CB 097	3.30	13.9	5.16	5.07	5.44
CB 110	23.8	16.7	12.5	19.2	15.8
CB 149	17.6	14.3	10.1	15.4	12.5
CB 123	0.74	<LOD	0.18	<LOD	<LOD
CB 118	8.70	9.14	8.2	9.22	9.44
CB 114	3.46	1.66	1.44	2.11	1.19
CB 132	4.65	4.42	3.48	5.80	3.78
CB 153	23.8	13.2	9.73	16.5	10.0
CB 105	2.37	2.35	1.81	4.28	2.13
CB 137	0.59	<LOD	<LOD	1.01	<LOD
CB 138	6.77	6.66	5.86	12.47	6.68
CB 158	<LOD	<LOD	<LOD	1.44	<LOD
CB 187	2.09	0.8	0.63	3.88	0.76
CB 183	0.78	<LOD	<LOD	1.8	<LOD
CB 128	3.13	1.84	1.41	3.08	2.02
CB 167	1.72	<LOD	0.2	<LOD	<LOD

CB 156	3.92	0.9	1.32	4.64	0.99
CB 157	1.11	<LOD	<LOD	1.29	<LOD
CB 180	3.10	1.45	1.38	3.12	1.41
CB 170	1.59	1.01	0.87	1.70	1.34
CB 189	1.06	0.73	0.32	0.86	0.84
CB 194	0.72	0.55	0.39	<LOD	0.66
CB 209	2.33	2.24	1.73	2.68	2.40

LOD=limit of detection, 2× the concentrations (ng g^{-1} SR sampler) in the field blank sample; expressed as aqueous concentration (ng L^{-1}), the aqueous LOD varied at each sampling site, depending upon deployment duration and sampling rates (Rs).

Figure 1

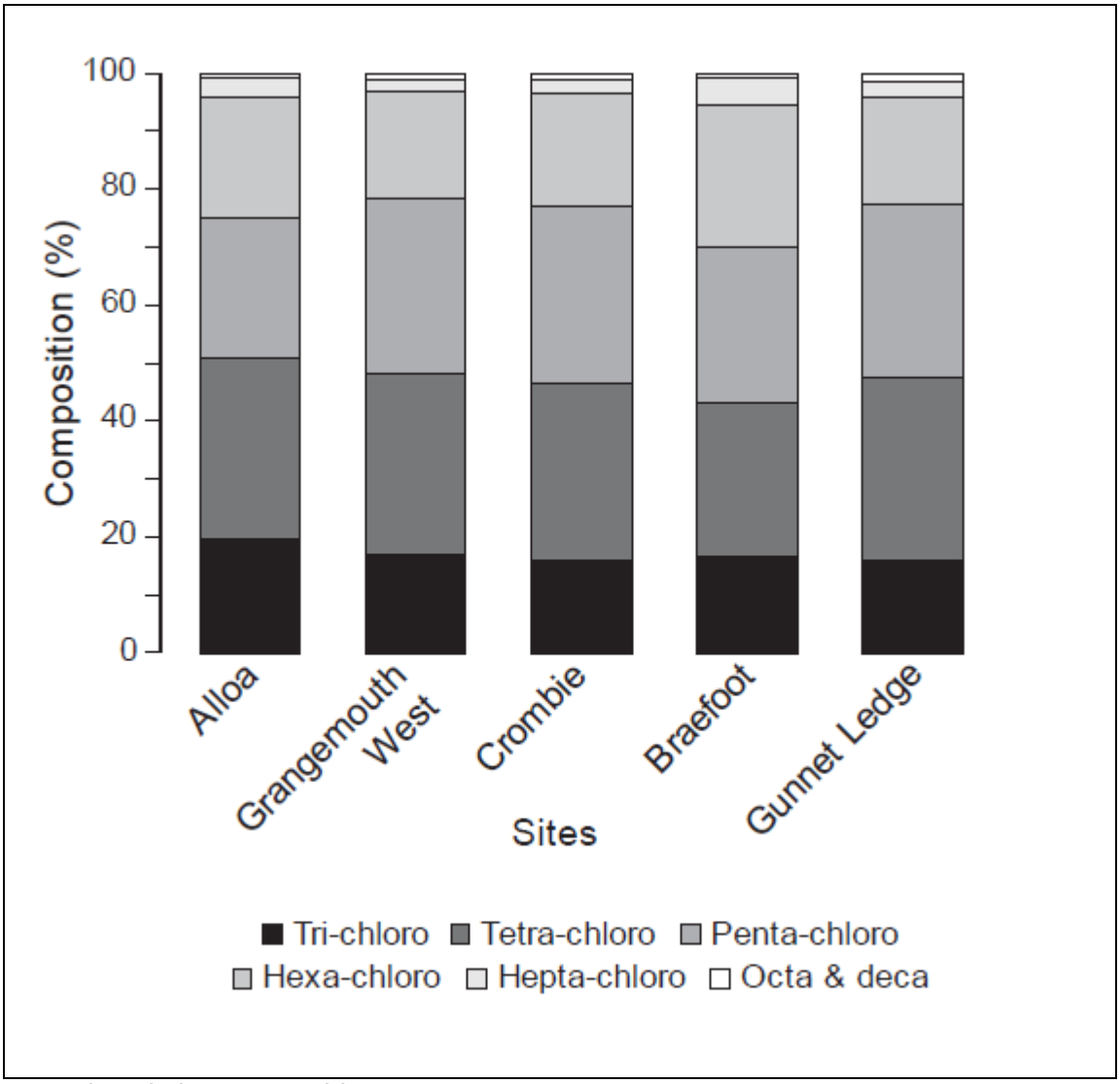
Percentage compositions of PAH profiles measured in water from the Forth estuary and Firth of Forth using silicone rubber passive sampling devices (SR-PSDs)



Term description: See Table 1A

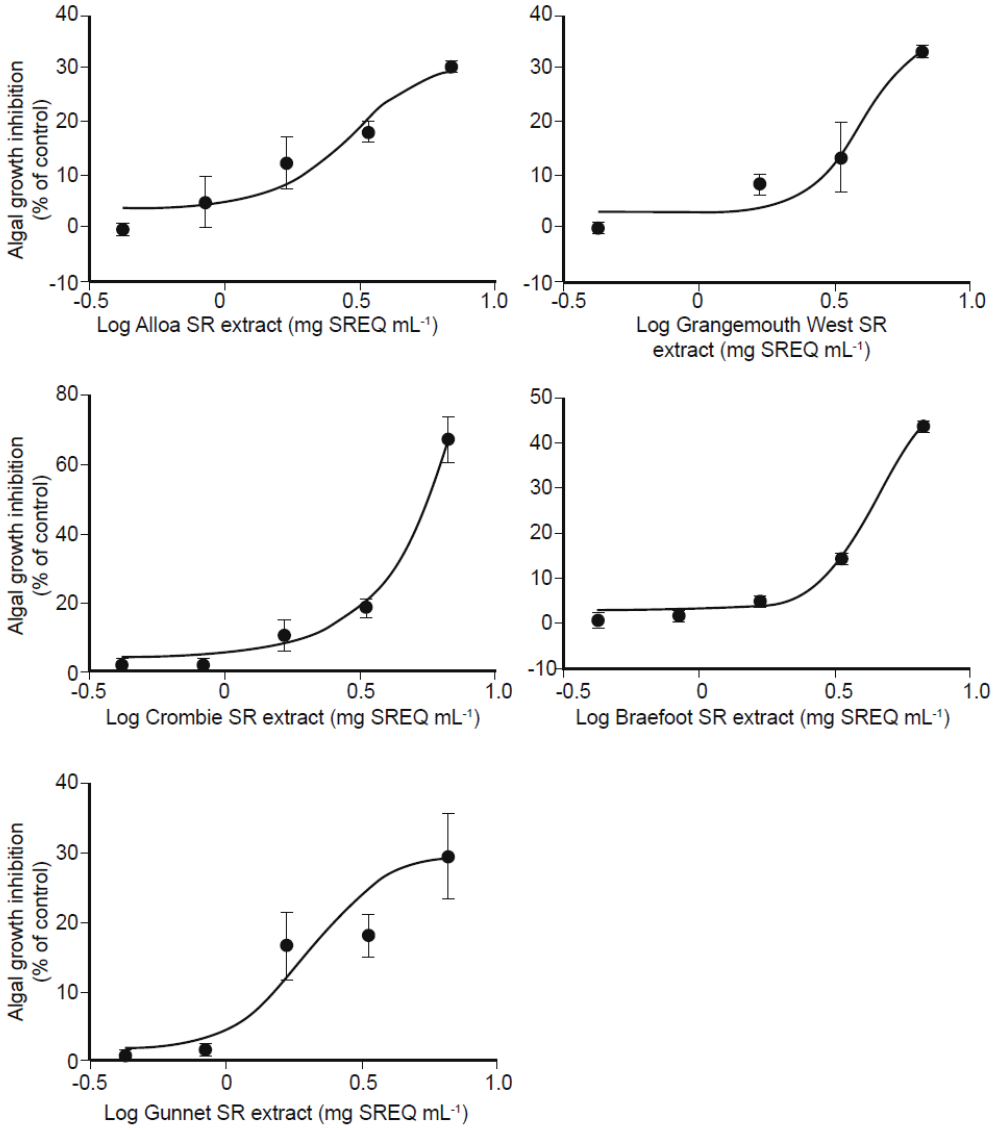
Figure 2

Percentage compositions of PCB profiles measured in water from the Forth estuary and the Firth of Forth using silicone rubber passive sampling devices (SR-PSDs)



Term description: See Table 1B

Figure 3: Concentration-response curves for extracts of silicone rubber passive sampling devices (SR-PSDs) deployed in water at the Forth estuary and Firth of Forth following 72 h exposure to *D.lutheri*.



Data are presented as mean ± standard deviations (±SD).

7.5 Appendix 5: Marine Scotland Science (Marine Laboratory), selected Standard Operating Procedures (SOPs)

See attached CD