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

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HIGHLIGHTS

- PAHs and PCBs were determined in a rural catchment using passive sampling.
- Semi-polar pesticides and herbicides were detected using silicone rubber samplers.
- Coupling passive sampling with *in vitro* tests enabled evaluation of mixture effects.
- No cytotoxicity was observed in RTL-W1 cells exposed to passive sampler extracts.
- EROD activity was measured; only 12–21% of this was attributed to PAHs and PCBs.

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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 ($\sum PAH_{40}$, parent and branched) in the water column of the catchment varied from 38 to 69 ng L^{-1}, whilst PCBs ($\sum PCB_{32}$) 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.

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).

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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. Materials and methods

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. 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.

2.3. Sampling locations and passive sampling

2.3.1. 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. 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.

2.3.2. 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 d at sites 1, 2, 3 and 4, and for 58 d 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.

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

2.4.1. 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

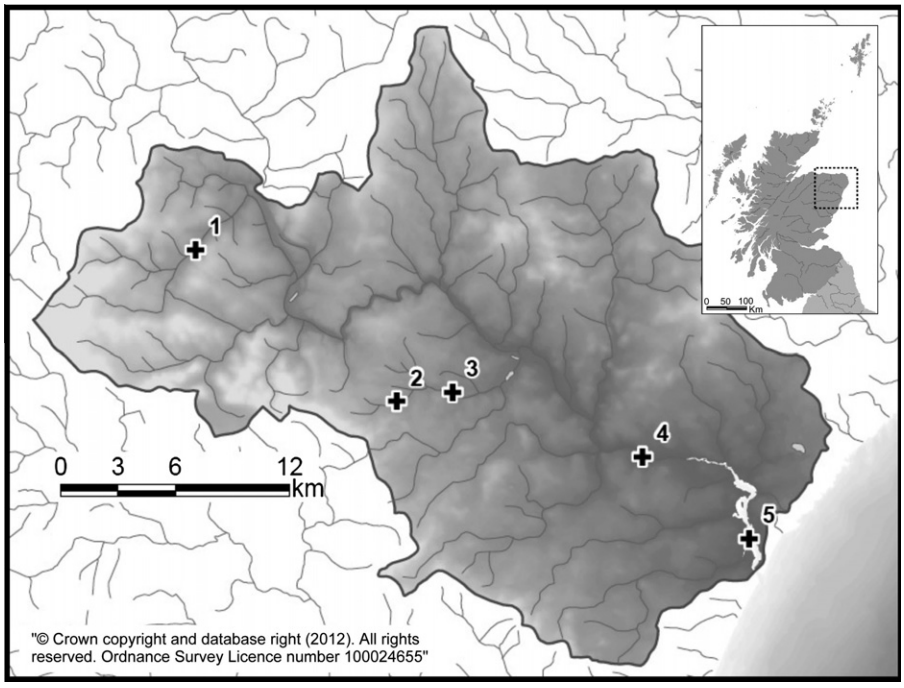

Fig. 1. River Ythan catchment (showing River Ythan and main tributaries) in NE Scotland. Sample sites 1–5 are indicated. Inset shows map of Scotland and location of the catchment.

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.

2.4.2. 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 2.4.1 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 °C 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.

2.5. 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 Supporting information.

2.5.1. 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:

$$R_s = FAM^{-0.47} \tag{1}$$

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:

$$f = \frac{N_t}{N_0} = \exp\left[-\frac{R_{st}}{mK_{SW}}\right] = \exp\left[-\frac{FAM_t^{-0.47}}{mK_{SW}}\right] \tag{2}$$

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 (NLSs) 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 (Fig. SI 1).

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

$$C_W = \frac{N_t}{mK_{SW}} \frac{1}{1 - \exp\left(\frac{-R_S t}{mK_{SW}}\right)} \tag{3}$$

where N_t is amount (ng) of target compound absorbed by the SR sampler during exposure; by combining Eqs. (2) and (3):

$$C_W = \frac{N_t}{mK_{SW}\left[1 - \exp\left(-\frac{FAM^{0.47}t}{mK_{SW}}\right)\right]} \tag{4}$$

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. (4) a correction for production blank can be included (Smedes and Booij, 2012):

$$C_W = \frac{N_t - N_0 \exp\left(-\frac{FAt}{mK_{SW}M^{0.47}}\right)}{mK_{SW}\left(1 - \exp\left(-\frac{FAt}{mK_{SW}M^{0.47}}\right)\right)} \tag{5}$$

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.

2.6. Biological effects analysis of silicone rubber extracts

2.6.1. 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).

2.6.2. 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).

2.6.3. 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).

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

2.7.1. 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).

2.7.2. 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. (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).

$$Chem - \mathrm{TEQ} = \sum[\mathrm{PAH}_i \times \mathrm{TEF}_i] + \sum[\mathrm{PCB}_i \times \mathrm{TEF}_i]n \quad (6)$$

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. (7):

$$\text{Bio-TEQ} = \frac{\mathrm{TCDDEC}_{25}}{\mathit{extract}\ \mathrm{EC}_{25}\mathrm{TCDD}} \quad (7)$$

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).

3. Results and discussion

3.1. Chemical analysis

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 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 1). Similar composition profiles where 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). 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.

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). 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).

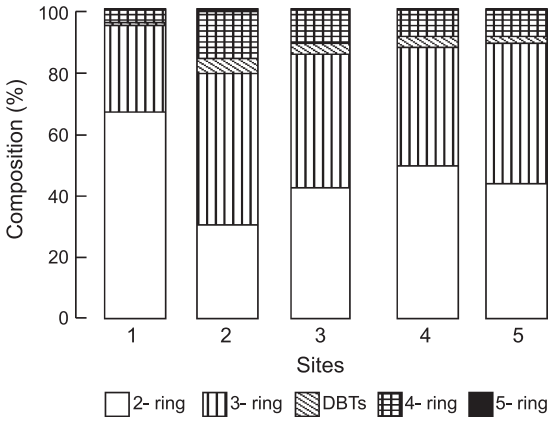

Fig. 2. Percentage composition of PAH groups in water at the various sampling locations. Term description: 2-ring = ∑naphthalenes (parent and C1-C4); 3-ring = ∑acenaphthene; acenaphthylene; fluorene; phenanthrene and anthracene (parent and C1–C3); DBTs = ∑dibenzothiophenes (parent and C1–C3); 4-ring = ∑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).

Table 1
Sum of dissolved concentrations of PAHs and of PCBs and the concentrations of pesticides and acid/urea herbicides absorbed by silicone rubber passive sampling devices (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[c]	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.
N.D.-Not detected.
SR-PSDs were deployed for 65 d at sites 1, 2, 3 and 4 and for 58 d at site 5 (November 2010–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.
[c] 2,4-dichlorophenoxy acetic acid (2,4-D).

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 2). The PCB extract for site 4 was lost during the clean-up process. Sum-concentrations of the seven Indicator-PCBs (∑PCB_7) followed the same pattern as the sum of all measured PCBs (∑PCB_{32}), with about 3 times higher concentrations at site 2 than at the other sites (Table 1). Unlike for PAHs, the highest total concentrations of the PCBs were found at sites 2 and 3. Total concentrations of PCBs (∑PCB_{32}) measured in this study ranged 0.02–0.06 ng L^{-1} (Table 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. 3). The overall data obtained for PCBs in the present study did not indicate any specific point source inputs in the catchment.

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 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

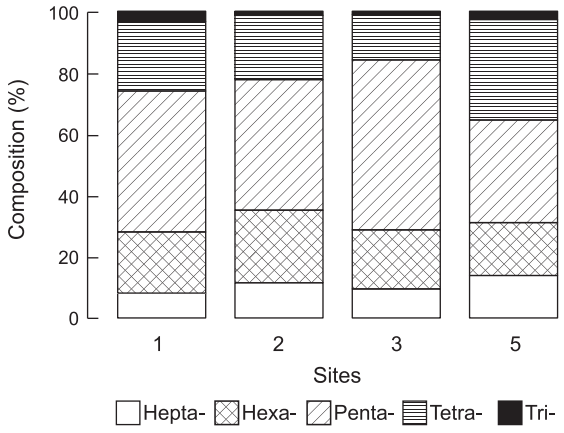

Fig. 3. Percentage composition of PCB congeners in water at the various sampling locations. Term descriptions; Tri = ∑CBs 28 and 31; Tetra = ∑CBs 44, 49, 52, 70, and 74; Penta = ∑CBs 97, 99, 101, 105, 110, 114, 118, and 123; Hexa = ∑CBs 149,132,153,137,138,158,128,167,156, and 157; Hepta = ∑CBs 170, 180,183, 187, and 189; Octa and Deca = ∑CBs 194 and 209. Data for site 4 not available.

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).

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 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 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 ($\log K_{OW}$ = 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 $\log K_{OW}$ 3–8. 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.

3.2. Biological effects analysis

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. 4A). In contrast, cytotoxicity was observed with the positive control (DCP; Fig. 4B) indicating that the cells were indeed responsive and any

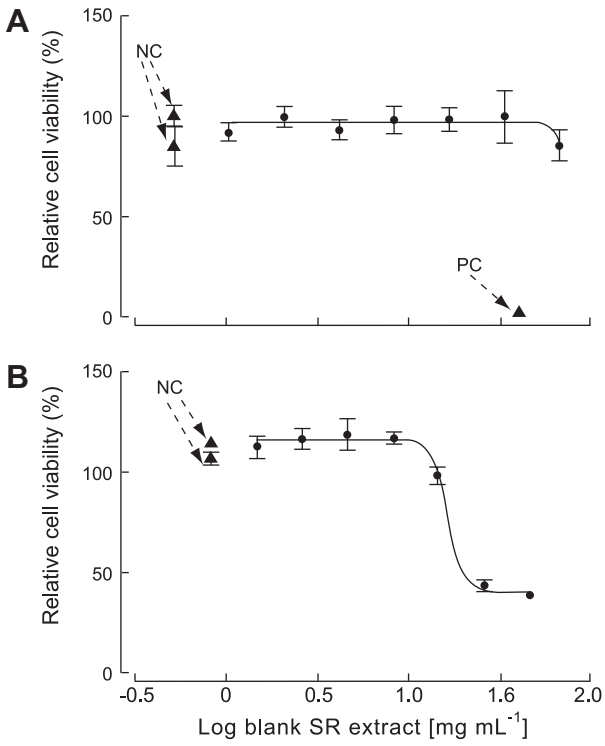

Fig. 4. Concentration–response curves following 48 h exposure of (A) blank SR sampler extracts and (B) 3,5 DCP to RTL-W1 cells using neutral red retention assay. Results of extracts from deployed SR-PSDs were analogous to that of the blank. Cell viability expressed as percentage of unexposed controls (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.

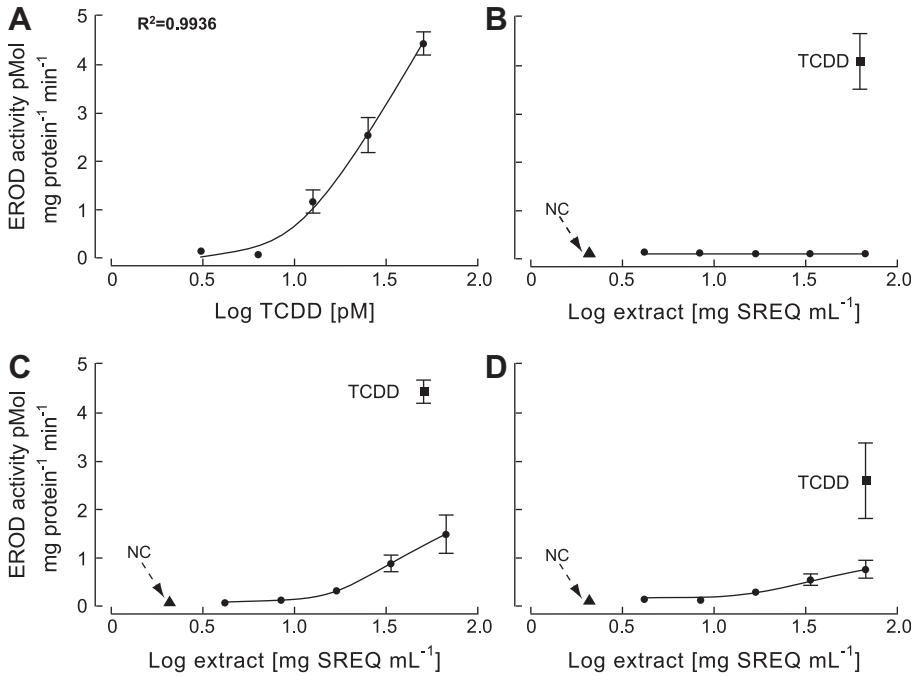

Fig. 5. Concentration–response curves for EROD induction in the RTL-W1 cells by (A) TCDD, (B) SR sampler extracts-blank (C) SR sampler extract-site 2 and (D) SR sampler extract -site 4. Data points represent the mean ± standard deviation (SD) of four replicates at each exposure concentration. NC = negative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

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).

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. 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. 5C and D). Complete EROD activity concentration–response curves were obtained for most of the SR extracts and for the TCDD positive control (Fig. 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. 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).

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. 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

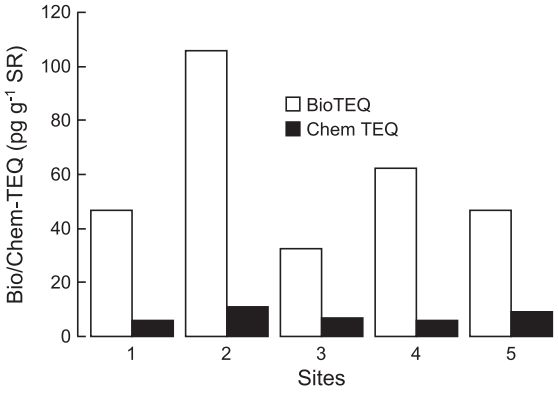

Fig. 6. Comparison of Bio-TEQ and Chem-TEQ values obtained from *in vitro* RTL-W1 assay and concentrations of PAHs and PCBs measured with silicone rubber (SR) passive sampling technique in water of the study locations.

(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.

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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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2012.06.041.

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