Biliary PAH metabolites and EROD activity in flounder (*Platichthys flesus*) from a contaminated estuarine environment

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The present study in the Firth of Forth, Scotland, emphasises the usefulness of biliary metabolite measurements in estuarine monitoring using the flounder (*Platichthys flesus*). The short time scale (a few hours) of response to polycyclic aromatic hydrocarbon (PAH) exposure, allowed clearer interpretation of trends and differences between sites. Such differences and trends in the metabolite data were not as apparent from 7-ethoxyresorufin O-deethylation (EROD) activity measurements, which were likely to have been blurred by the movements of flounder between sites. Statistical differences were not observed in EROD activity in flounder from the Firth of Forth (ANOVA, \( P = 0.065 \)). The biliary metabolites showed statistical differences between the uppermost site of Longannet and Port Edgar, in the outer Firth, for both the 1-OH pyrene and 2-OH naphthalene metabolites (ANOVA, Tukeys, \( P = 0.012 \) and \( 0.022 \) respectively). There was also a significant correlation between the concentrations of all three individual metabolites (1-OH pyrene, 2-OH naphthalene, 1-OH phenanthrene) and the log of the distance downstream from the major PAH input sources of Longannet power station and Grangemouth. Biliary PAH metabolites were shown to better reflect local contamination gradients than EROD activity. This is explained by the relative response times of the two biomarkers and local movements of the flounder in the estuary.

Introduction

The Firth of Forth on the east coast of Scotland has been the focal point for Scotland’s petrochemical and refining industries since 1924 (Fig. 1). Although, in recent years, the amounts of hydrocarbons discharged have dramatically decreased, the British Petroleum (BP) Chemicals, BP Refinery and BP Kinnell sites, around Grangemouth, are historically the dominant sources of oil related PAHs.\(^1\) The average daily loads of oil discharged into the Firth of Forth in 2000, were 84, 9 and 75 kg d\(^{-1}\) respectively from these three sites. The adjacent coal-fired power station at Longannet is likely to contribute to the PAH loading to the Firth via the release of combustion related PAHs, although these releases are more likely to have long range effects. Further minor inputs may arise from municipal discharges, such as, sewage outfalls. This long history of inputs, combined with the estuary’s physical characteristics, have created a large reservoir of PAH contamination in the sediments.\(^1\),\(^2\)

The measurement of PAHs in sediment does not provide direct information on their potential to cause biological effects. One contributing factor is a process termed sediment ageing, whereby prolonged contact between PAHs and sediment may result in strong bond formation and a subsequent reduction in bioavailability. Amongst the range of biological indicators of sub-lethal exposure to PAHs, the induction and activity of the cytochrome P450 monoxygenase system has been shown to be one of the most promising marine biomarkers, and has been used in numerous monitoring and research projects.\(^3\)–\(^5\) The activity of this system in fish exposed to PAHs is traditionally assayed as the catalytic rate of 7-ethoxyresorufin O-deethylation (EROD) activity.\(^6\)–\(^8\)

In fish, EROD activity is believed to represent the catalytic activity of a single cytochrome P450 (CYP) form, namely CYP1A.\(^9\) The induction mechanism of CYP1A1 is thought to occur via the high binding affinity of planar aromatic compounds to the aryl hydrocarbon (Ah) receptor complex.\(^10\) The CYP1A1 enzyme is induced by a range of planar organic contaminants including PAHs, and some polychlorinated biphenyls (PCBs), dioxins and dibenzofurans. Recent studies by Stagg et al.\(^11\) have shown induction of CYP1A1 in salmon (*Salmo salar*) by a variety of PAHs. CYP1A1 forms the first phase of the metabolism of PAHs, whereby the Ah-receptor mediated reaction of the CYP1A1 enzyme catalyses

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**Fig. 1** Map showing the geographical location of trawl sites in the Firth of Forth and the Tay, on the east coast of Scotland. The location of major industrial PAH sources are shown as are the major urban areas.
Material and methods

Chemicals

The metabolite standards 2-hydroxy (2-OH) naphthalene and 1-OH pyrene were supplied by Sigma-Aldrich Company Ltd, Dorset, UK. 1-OH Phenanthrene was supplied by Promocem Ltd, Herts, UK. Ethanol was purchased from BDH-Merck Ltd, Herts, UK. Dichloromethane, methanol, iso-hexane and acetonitrile were supplied by Rathburn Chemicals Ltd, Tweedale, UK. Ethanol was purchased from BDH-Merck Ltd, Herts, UK. Ethanol was purchased from BDH-Merck Ltd, Herts, UK. Ethanol was purchased from BDH-Merck Ltd, Herts, UK. Ethanol was purchased from BDH-Merck Ltd, Herts, UK.

Table 1 Mean (± SE for Yall) PAH concentrations (n = 3, ng g⁻¹ dry weight) from the start, middle and end of seven trawl sites in the Firth of Forth and the outer Tay, in September 1999

<table>
<thead>
<tr>
<th>Compound</th>
<th>Outer Tay (1)</th>
<th>Kingston Hudds (2)</th>
<th>Port Edgar (3)</th>
<th>Blackness (4)</th>
<th>Tancerd (5)</th>
<th>Bo’ness (6)</th>
<th>Longannet (7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΣNaph²</td>
<td>54</td>
<td>2766</td>
<td>5711</td>
<td>3903</td>
<td>3254</td>
<td>4588</td>
<td>5625</td>
</tr>
<tr>
<td>Σ178³</td>
<td>39</td>
<td>1355</td>
<td>2552</td>
<td>1618</td>
<td>1428</td>
<td>2058</td>
<td>2162</td>
</tr>
<tr>
<td>ΣDBT’s⁵</td>
<td>7</td>
<td>196</td>
<td>593</td>
<td>332</td>
<td>325</td>
<td>488</td>
<td>528</td>
</tr>
<tr>
<td>Σ228⁶</td>
<td>59</td>
<td>1981</td>
<td>3313</td>
<td>2029</td>
<td>1951</td>
<td>2724</td>
<td>2900</td>
</tr>
<tr>
<td>Σ252⁶</td>
<td>67</td>
<td>1169</td>
<td>2064</td>
<td>1198</td>
<td>1151</td>
<td>1615</td>
<td>1740</td>
</tr>
<tr>
<td>Σ276⁶</td>
<td>31</td>
<td>478</td>
<td>745</td>
<td>414</td>
<td>415</td>
<td>557</td>
<td>538</td>
</tr>
<tr>
<td>ΣTotal</td>
<td>300</td>
<td>9621</td>
<td>17724</td>
<td>11264</td>
<td>10067</td>
<td>14058</td>
<td>15782</td>
</tr>
</tbody>
</table>

as three standard deviations of the mean of six procedural blanks, were found to be: naphthalene 0.345, phenanthrene 0.194, anthracene 0.208, fluoranthene 0.275, pyrene 0.202, benzo(a)anthracene 0.141, chrysene/triphenylene 0.201, benzo- fluoranthenes 0.079, benzo(a)pyrene 0.150, indeno-pyrene 0.041, and benzo(p)erylene 0.039. Quality control was established through successful participation in the PAH laboratory performance study programme of QUASIMEME (Quality Assurance of Information for Marine Environmental Monitoring in Europe). FRS holds UKAS accreditation for this analysis.

**Determination of total organic carbon (TOC)**

TOC was determined by the method of Nieuwenhuize et al. Analysis was carried out on freeze dried sediments, following treatment to remove carbonate, by combustion in a Perkin Elmer CHN elemental analyser.

**Hydrolysis and HPLC-fluorescence analysis of bile samples for PAH metabolites**

Bile samples were hydrolysed by a modification of the method of Ariese et al. Bile (20 μl) was mixed with 200 μl of HPLC-grade water 4% ascorbic acid to which 20 μl of β-glucuronidase-arylsulfatase solution (5.5 and 2.6 U ml⁻¹, respectively) was added. The resultant solution was incubated for 1 h at 37 °C in a shaking water bath. The hydrolysed metabolites were diluted with ethanol-water (25 μl, 84% ethanol by weight) containing 4% (by weight) ascorbic acid. The final solution was centrifuged (10000g) at 4 °C for 5 min. One aliquot of 50 μl of the solution was removed for HPLC-fluorescence analysis.

The concentration of the individual hydroxylated PAHs was determined using a Hewlett Packard series 1050 HPLC system comprising a quaternary pump, de-gas unit, and autosampler. Samples were chromatographed on a Vydc 201TPSF (25 cm × 4.6 mm) reverse phase analytical column, non-endcapped with 0.5 μm x 3 mm Phenomenex filter. The hydroxylated compounds were detected using a Waters 470 Scanning fluorescence detector. Injections (20 μl) were made at 40 °C and the oven temperature remained constant throughout the run. The initial mobile phase was 20:80 v/v acetonitrile–water (water was acidified to pH 4 with acetic acid). The solvent composition progressively changed to 100% acetonitrile over 45 min. At the end of each run the column was allowed to re-equilibrate over 5 min.

The excitation: emission wavelength pairs (nm) for 2-OH naphthalene, 1-OH phenanthrene, 1-OH pyrene were 222–370, 246–370, 243–388, respectively. Bile samples were analysed individually and the results are expressed as the mean OH-metabolite (nmol) per ml of bile, ±SE for each site.

**EROD activity in liver tissue**

Partially defrosted liver samples were homogenised with a Potter-Elvejem type homogeniser in an ice cold phosphate buffer containing 0.1 M K₂HPO₄·3H₂O, 0.1 M KH₂PO₄, 0.5 mM CaCl₂, 1 mM EDTA, 0.1% BSA, pH 7.4. The homogenate was transferred into Tref-tubes and centrifuged at 10000g at 4 °C for 20 min. The resultant supernatant was snap frozen and stored at ~70 °C until analysis.

EROD activity was determined according to the method of Burke and Mayer as described by Stagg et al. Resorufin production was measured in a temperature controlled (20 °C) cuvette with continuous stirring of 1960 μl 100 mM pH 7.4 phosphate assay buffer (0.1 M K₂HPO₄·3H₂O, 0.1 M KH₂PO₄, 0.15 M KCl), 7-Ethoxyresorufin (10 μl, 0.4 mM) in DMSO solution and a sample supernatant (20 μl) was then added. The reaction was started by the addition of NADPH (10 μl, 100 mM) and calibrated by the addition of an internal spike of resorufin (10 μl, 12.5 μM). Resorufin was normalised on a daily basis using the molar absorptivity of 73.2 μM⁻¹ cm⁻¹. The increase in fluorescence with time was measured over a 2 to 10 min period using a Perkin Elmer LS50B luminescence spectrometer and analysed with Enzyme Activity Rev 3.0 (Biolight Ltd) software. The excitation and emission wavelengths were 535 nm and 585 nm, respectively. Reaction rates were expressed as pmol of resorufin produced per min per mg of protein.

The protein content was determined colorimetrically according to the method of Lowry et al. with bovine serum albumin (BSA) as the reference standard. The assay was performed using a Biorad Protein Assay kit, on an Argus plate reader at 595 nm. The reaction product was quantified against the response produced by the serial dilution (0.2–1.6 mg ml⁻¹) of the BSA standard.

**Statistical analysis**

The data were analysed for statistical significance by one-way ANOVA, EROD data were log transformed prior to analysis. EROD activity is presented as back-transformed values. Residuals were stored and tested for normality by the Ryan-Joiner Test. Where significant differences were found, a Tukey’s multiple comparisons test was applied to highlight where differences lay. Significant differences were determined at the 95% level. Linear regressions were calculated by a least squares approximation. Data were examined for correlations using Pearson product moment coefficients. The data analysis was performed using Minitab version 12 software.

**Results and discussion**

**Sediment PAH content**

The mean total measured PAH concentration (averaged across the 3 sediment samples taken for each trawl, Table 1) in sediment varied between 300 ng g⁻¹ dry weight at the outer Tay (site 1) and 17724 ng g⁻¹ at Port Edgar (site 3). All sites within the estuary (sites 2–7) were shown to have significantly elevated PAH burden in comparison to the outer Tay (ANOVA, P<0.001). There were, however, no statistical differences between PAH concentrations for any of the sites within the Firth of Forth estuary itself (sites 2–7, P>0.05).

The large within site variation in the PAH concentration across the trawl track (shown only for Yall PAHs, Table 1) suggests a non homogeneous sediment PAH burden in the Firth of Forth. Individual total sediment PAH values, for the start, middle and end of each trawl, at the Port Edgar site ranges between 4400 and 21800 ng g⁻¹ dry weight. High within site variations were also observed at the remaining sediment sites within the Firth of Forth. Such large local difference in sediment burden may relate to localised ‘hotspots’ of PAH contamination, which may have further implications for the interpretation of the biomarkers of PAH exposure.

Contamination of the sediment by hydrocarbons in the Firth of Forth, including PAHs, has been extensively studied and is reviewed by Elliot and Griffiths. Since previous studies measured different components or used differing techniques only general comparison between trends can be made. The findings highlighted by Elliot and Griffiths described a clear gradient of hydrocarbon contamination in sediment from enhanced levels at the inner Firth (Grangemouth area) to lower levels towards the open sea. This is in contrast to the present study where no clear gradient was observed. This can perhaps be explained by differences in sampling areas, since previous studies sampled on the intertidal areas in the Firth of Forth. The present study was confined to the shipping channel which undergoes continuous dredging leading to a high degree of sediment mixing. A decrease in the discharges of PAH from the Grangemouth area may also have a bearing on the results.
Further, although no contamination gradient was measured in the total PAHs or the individual parent PAHs in the present study, a gradient may exist in the bioavailable fraction of the sediment which cannot be measured by the extraction procedure used in this study. Normalisation of PAH concentration to total organic carbon concentration for the data in the present study did not alter the statistical differences between sites (results not shown).

The level of PAH contamination in the Firth of Forth estuary is within the range reported for other European estuarine areas. For example, Budzinski et al.\(^2\) reported total PAH concentrations between 18 and 5000 ng g\(^{-1}\) in the Gironde estuary, France. Studies in the western Mediterranean Sea (French, Spanish and Balearic coasts) showed PAH concentrations ranging from 1 to 8500 ng g\(^{-1}\) dry weight.\(^2\) Baumard et al.\(^2\) reported total PAH concentrations ranging from 3 to 30000 ng g\(^{-1}\) dry weight in the Western Baltic Sea.

### Biological parameters in fish

Length, weight, and GSI (gonad somatic index) are shown in Table 2. Fish caught from the outer Tay were significantly smaller in comparison with the fish caught at sites in the Firth of Forth (\(P<0.001\), ANOVA). The GSIs measured in the flounder from the outer Tay were significantly different from those at Longannet (\(P=0.027\), ANOVA).

<table>
<thead>
<tr>
<th>Site (number in Fig. 1)</th>
<th>EROD N</th>
<th>Bile N(^a)</th>
<th>Weight/g</th>
<th>Length/cm</th>
<th>GSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outer Tay (1)</td>
<td>5</td>
<td>0</td>
<td>67.4 ± 8.5</td>
<td>17.5 ± 0.8</td>
<td>1.10 ± 0.37</td>
</tr>
<tr>
<td>Kingston</td>
<td>4</td>
<td>1</td>
<td>150.3 ± 34.6</td>
<td>22.8 ± 1.6</td>
<td>0.49 ± 0.19</td>
</tr>
<tr>
<td>Hudds (2)</td>
<td>7</td>
<td>2</td>
<td>133.0 ± 12.9</td>
<td>22.6 ± 0.6</td>
<td>0.58 ± 0.16</td>
</tr>
<tr>
<td>Port Edgar (3)</td>
<td>6</td>
<td>2</td>
<td>123.7 ± 17.2</td>
<td>25.3 ± 10.5</td>
<td>0.72 ± 0.19</td>
</tr>
<tr>
<td>Blackness (4)</td>
<td>10</td>
<td>7</td>
<td>155.2 ± 11.6</td>
<td>23.6 ± 0.7</td>
<td>0.86 ± 0.25</td>
</tr>
<tr>
<td>Tancred (5)</td>
<td>7</td>
<td>7</td>
<td>168.1 ± 18.7</td>
<td>23.4 ± 1.1</td>
<td>0.46 ± 0.22</td>
</tr>
<tr>
<td>Bo’ness (6)</td>
<td>10</td>
<td>10</td>
<td>162.2 ± 16.0</td>
<td>23.5 ± 0.8</td>
<td>0.34 ± 0.09</td>
</tr>
</tbody>
</table>

\(^a\)Several flounder caught within the Firth of Forth area were void of bile, possibly reflecting recent feeding. \(^\text{Mean}±\) standard error.

The EROD activity in the male flounders (\(Platichthys flesus\)) caught from the Firth of Forth is shown in Fig. 2. Flounder from Blackness had the most elevated levels of activity being found at trawl sites in the vicinity of the Grangemouth refining and chemical works and the Longannet power station. Again no significant differences were found between these inner Firth of Forth trawl sites. It is notable that results in flounder contrast to those of juvenile plaice (\(Pleuronectes platessa\)) with those of juvenile plaice found (unpublished data).

The median EROD activity increased towards the upper Firth sites, i.e. Blackness, Tancred and Longannet, corresponding to the major industrial areas on the Firth.\(^1,2\) Sulaiman et al.\(^2\) found similar EROD activity patterns in flounder from the Firth of Forth with the highest levels of activity being found at trawl sites in the vicinity of the Grangemouth refining and chemical works and the Longannet power station. Again no significant differences were found between these inner Firth of Forth trawl sites. It is notable that results in flounder contrast with those of juvenile plaice (\(Pleuronectes Platessa\)) in the same study area, where significant differences between sites were found (unpublished data).

Fig. 3 shows the bile concentrations of three PAH metabolites, 1-OH pyrene, 1-OH phenanthrene and 2-OH naphthalene, in male flounder from each site. Each of the three metabolites show similar profiles along the length of the sampling transect, with the highest concentrations of metabolites (ANOVA, \(P=0.065\)). There were no correlations found between EROD activity or biliary metabolites and weights, lengths or the gonad somatic index (ANOVA, \(P>0.05\)).

**Table 2** Numbers of male flounder (\(Platichthys flesus\)) analysed, mean weights, mean lengths and the mean gonad somatic index (GSI) for each site in the Firth of Forth. Fish caught from the outer Tay were significantly smaller in comparison with the fish caught at sites in the Firth of Forth (\(P<0.001\), ANOVA). The GSIs measurements from the outer Tay were significantly different from those at Longannet (\(P=0.027\), ANOVA).

**Fig. 2** Box plot of log EROD activity (pmol min\(^{-1}\) mg\(^{-1}\) protein) in male flounder (\(Platichthys flesus\)) from the Firth of Forth showing median values, quartile range, upper and lower limits. No significant statistical differences were observed between groups (\(P>0.05\)).

**Fig. 3** Mean (± SE) biliary metabolite concentrations (nmol ml\(^{-1}\) bile) in male flounder (\(Platichthys flesus\)) from six sites in the Firth of Forth in September 1999. All fish from the Outer Tay had gallbladders void of bile. * Denotes significant differences from Port Edgar (\(P=0.012\)), ** denotes significant differences from Port Edgar (\(P=0.022\)). Note scale differences (×10) in the y-axis for 1-OH pyrene compared to 2-OH naphthalene and 1-OH phenanthrene.
being found in flounder from Longannet. Mean 1-OH pyrene, 2-OH naphthalene, and 1-OH phenanthrene concentrations at Longannet (mean ± standard error of the mean) were 5.2 ± 1.2, 0.67 ± 0.98, 0.14 ± 0.04 nmol m⁻¹ of bile respectively. There were significant statistical differences between Longannet and Port Edgar for both 1-OH pyrene and 2-OH naphthalene (P = 0.012 and 0.022 respectively, ANOVA, Tukey). The HPLC-chromatograms of the hydroxylated PAHs from bile were dominated by the 1-OH pyrene. Similar findings have been noted previously and attributed to the physico-chemical properties of pyrene resulting in the compound’s high bioavailability to aquatic organisms.\(^{18,29}\) The concentration of 1-OH pyrene in the present study were similar to those observed by Van der Oost et al.\(^{12}\) in a contaminated Dutch estuary.

The concentrations of all three biliary metabolites exhibited a significant linear relationship with the log₁₀ of the distance downstream from the major PAH input sources of Longannet power station and Grangemouth. The biliary metabolites therefore reflected the expected contaminant inputs in the Firth of Forth. The 1-OH pyrene concentration showed the strongest linear relationship with \(r = -0.58\) and \(P < 0.001\). 2-OH naphthalene and 1-OH phenanthrene had respective values of \(r = -0.49\) and \(P = 0.004\), and \(r = -0.51\) and \(P = 0.002\). There were no correlations between any of the biliary metabolites, EROD activity or any of the sediment PAH measurements both TOC normalised and non-TOC normalised (\(P > 0.05\)). The results show that flounder in the Firth of Forth are exposed to bioavailable PAHs from the Longannet area, despite the fact that the sediment concentrations measured at this site are not significantly different from the other area in the Firth of Forth (Table 1). This is likely to reflect fresh inputs from the Grangemouth oil refinery and petro-chemical complex.

The lack of observable differences between the site specific EROD activity when there is a clear difference in the exposure to PAHs as indicated by the biliary metabolite data needs to be reconciled. The biliary PAH metabolites measured in the present study are light molecular weight PAHs, and it is well documented that the principal inducing PAHs are higher molecular weight with a bay region.\(^{11,15}\)

An alternative explanation may be that the two measurements are independently influenced by the short-term (days) migratory movements of the flounder population within the Firth of Forth. Biliary metabolite formation has been shown to respond over a much shorter time scale than CYP1A1 induction. Varanasi et al.\(^{10}\) measured the presence of metabolites of \(^{14}\)C-labelled PAHs in the bile of flounder (Platichthys flesus) 12 h after administration. Similarly, authors have reported the presence of biliary metabolites between 4 and 24 h after exposure.\(^{31-34}\) In all these studies, the first sampling point was also the first detection time and its likely that the metabolites appear in bile within hours of exposure. This rapid response to exposure, coupled with the short persistence of metabolites in feeding fish,\(^{12}\) may give greater discrimination in estuarine monitoring than has previously been possible with other techniques. In contrast to metabolites, the time scale of maximum CYP1A1 induction under experimental exposure has been shown to be 2-5 d, and a further 4-7 d to return to control levels.\(^{11,19,36}\) Therefore, fish caught from specific sample sites may be more likely to reflect local contamination through their metabolite profile than through CYP1A1 induction.

Several other authors have noted such a range in EROD activity measurements. Kirby et al.\(^{37}\) suggested that EROD activity distribution patterns attributed to the Sea Empress oil spill might have been significantly blunted by the migration of the plaice and dab (Limanda limanda) between the spill date and the sampling date three months later. Cooreman et al.\(^{38}\) also suggested that large variations in EROD activity between individuals could be attributed to local migrations. A previous study in the Firth of Forth implicated short distance movements of flounder as the probable cause for a lack of correlation between sediment hydrocarbon burdens and the associated burdens in the lives, flesh, and gonads.\(^{39,40}\) Rijnsdorp et al.\(^{19}\) working on dab, suggest that, at any one time, the population at a sampling site is a transient aggregation of fish originating from a larger area. It is likely that flounder in the present study have moved between sites, possibly obscuring any interpretable trends in the EROD activity data.

Although the local movements of the flounder may begin to explain the observations in this study, other factors are also likely to influence the results. EROD activity, in particular, is influenced by numerous exogenous and endogenous factors, such as temperature, sex, season, maturity, and chemical inducers and inhibitors.\(^{3,6,37,39}\) For example, Sleiderink et al.\(^{41}\) showed a three fold increase in EROD activity in dab acclimated to 8°C compared to a separate group acclimated to 16°C. The effect of exposure to multiple xenobiotic compounds in the Firth of Forth is likely to influence the EROD activity in flounder. High levels of heavy metals, including cadmium, nickel, mercury, and lead, have been reported in both sediment and fish from the inner Firth of Forth by several authors.\(^{24,26}\) In particular cadmium has been shown to inhibit EROD activity in plaice.\(^{45}\) Beyer et al.\(^{36}\) however, showed only a slight suppression in the EROD activity of flounder exposed to benzo[a]pyrene and cadmium. Eggens et al.\(^{46}\) found a low EROD activity in male flounder chronically exposed under laboratory conditions to contaminated dredge spoil from Rotterdam Harbour containing high levels of PAHs, PCBs and heavy metals. Although the acute administration of pure inducing compounds routinely results in the rapid induction of CYP1A in laboratory exposed flounder, this may not be the case in the natural chronic exposure of a mobile population.

Biliary PAH metabolite concentrations in flounder from the Firth of Forth showed clear trends and statistically elevated levels close to the major inputs of hydrocarbons. This study has shown the usefulness of biliary PAH metabolites in flounder in distinguishing a gradient in the Firth of Forth. In this species EROD activity was less useful at identifying PAH exposure. Correlations between biomarkers were not observed, nor were there any correlations or any links between the sediment PAH burden and either of the biomarkers.

References

24 M. Burke and R. Mayer, *Drug Metab. Dispos.*, 1974, 2, 538.