CHARACTERISATION OF ESTROGENIC COMPOUNDS IN UK ESTUARIES

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Endocrine Disrupters in the Marine Environment (EDMAR) is a multi-agency funded programme assessing the effects of endocrine disrupting compounds on many aspects of the UK marine environment. One objective of this study is to identify compounds demonstrating estrogenic activity in the marine environment. This has been achieved by the application of toxicity identification evaluation (TIE) procedures modified for use with an in vitro yeast-based screen for estrogenic activity.

Toxicity identification evaluation (TIE) of surface waters

As a first step to establish which estuarine waters showed estrogenic activity, water samples collected from six estuaries (Figure 1) were extracted by C8 solid phase extraction and tested using the yeast estrogen assay (YES) (Figure 2). Water samples collected from the Tyne and Tees estuaries showed high activity and were chosen for TIE characterisation.

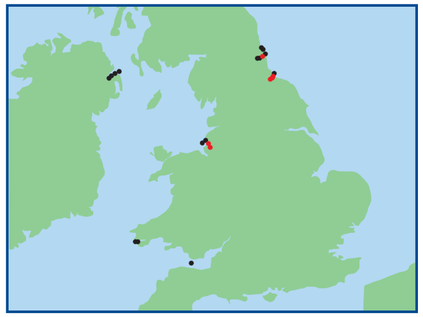

Figure 1. UK estuaries screened for estrogenic activity.

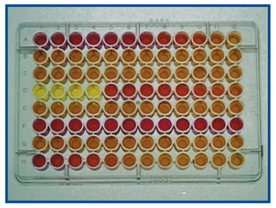

Figure 2a. Yeast-based estrogen screen.

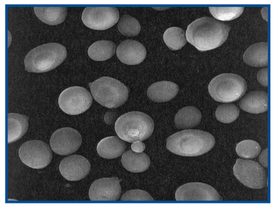

Figure 2b. Yeast-based estrogen screen.

The TIE characterisation process involved the solid phase extraction of bulk water samples using a layered Teflon SPE system (Figure 3). Extracts that showed estrogenic activity were fractionated by reverse phase HPLC and once again tested using the YES assay. Candidate estrogens were then identified in each group of fractions by full-scan gas chromatography-mass spectrometry (GC-MS) (Figure 4).

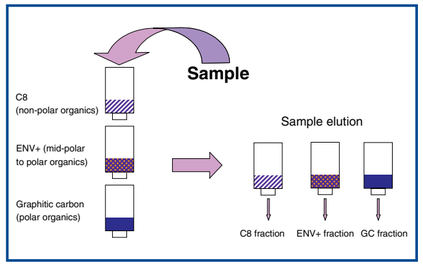

Figure 3. Teflon layered SPE system.

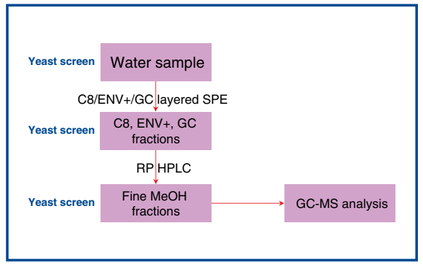

Figure 4. TIE scheme for the identification of estrogens in surface waters.

The majority of the estrogenic activity in the samples collected from the Tees and Tyne was isolated to a single group of fractions (Figure 5, Fractions 21-23, Tyne 90%, Tees 84%). GC-MS analysis identified 17β-estradiol as the major contributor of estrogenic activity. Other compounds were identified as having a minor contribution to the overall activity of the samples characterised (Table 1).

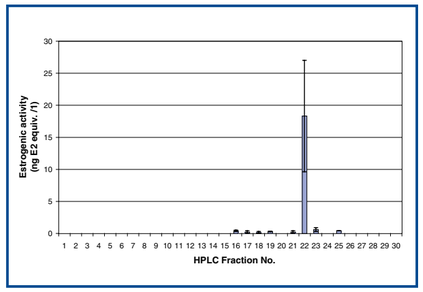

Figure 5a. Estrogenic activity of HPLC fractions from the Tyne represented as E2 equivalents.

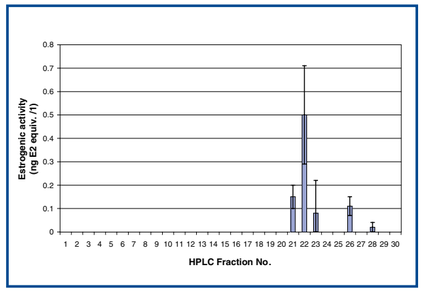

Figure 5b. Estrogenic activity of HPLC fractions from the Tees represented as E2 equivalents.

Table 1. Summary of estrogens identified in Howdon STW and Dabholm Gut effluent and associated chemical data

Effluent/ Fraction	Compound	Chemical structure	Chemical Abstract Service (CAS)	Log K_{ow}	Activity (ng E2 equiv. l^{-1})	Estrogenic potency†	Estimated conc. in effluent (μg l^{-1})	Source
Howdon STW: 16-19	Unknown	-	-	-	1.0	-	-	-
Howdon STW: 21-23	17β-estradiol		50-28-2	3.1	19.1	x1	0.0191	Natural steroid hormone
Howdon STW: 25	androsterone		53-41-8	-	0.4	x5 x10^{-4}	0.8	Testosterone metabolite
Dabholme Gut: 21-23	17β-estradiol	As above	50-28-2	3.1	0.73	x1	0.00073	Natural steroid hormone
Dabholm Gut: 26	bis (2-ethylhexyl)phthalate		117-81-7	4.9	0.11	x2.8 x10^{-7}*	393	Placticizer
Dabholm Gut: 28	nonylphenol		60-57-1	4.5	0.02	x2 x10^{-5}	0.77	Surfactant metabolite

† As compared to 17β-estradiol using the YES assay.
* Tentative quantification since plasticware is used in the extraction procedure, however blanks do not show a phthalate induced response.

Acknowledgements

This work is funded by contributions from the UK Department of the Environment, Transport and the Regions (DETR), UK Environment Agency (EA), The European Chemical Industry Council (CEFIC), UK Ministry of Agriculture, Fisheries and Food (MAFF), and the Scotland and Northern Ireland Forum for Environmental Research (SNIFER).

Toxicity identification evaluation (TIE) of sediments

Sediment samples were collected from the same locations on the Tyne and Tees (Figs 6a and 6b) estuaries and characterised using the procedures described in Fig 7.

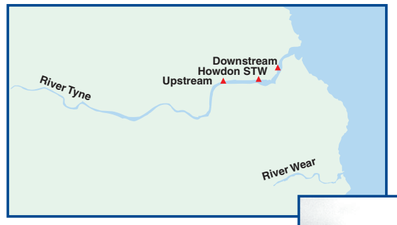

Figure 6a. Sample locations on the river Tyne.

Howdon STW is situated on the lower reaches of the river Tyne and discharges treated STW effluent.

Dabholm Gut is a discharge on the lower Tees estuary that receives treated and untreated sewage and industrial effluent.

Figure 6b. Sample locations on the river Tees.

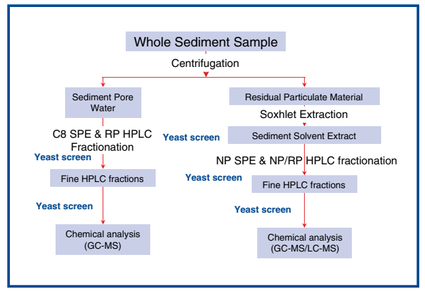

Figure 7. Protocol for the identification of estrogenic compounds in sediments.

The C8 SPE extract obtained from the Tees had an estrogenic effect equivalent to 7ng E2 l^{-1}. No activity was detected in pore waters collected from the Tyne. Fine fractionation of the active extract by HPLC produced four consecutive estrogenic fractions (Figure 8). GC-MS analysis tentatively identified estra-1,3,5-trien-3-ol as a potential candidate for the cause of this effect.

Estrogenic activity was detected in the dichloromethane extracts of sediment particulate material collected from both the Tyne and Tees. Coarse normal phase SPE fractionation of these extracts produced two estrogenic fractions (Table 2, Fig 9). Fine HPLC fractionation of these coarse fractions produced a number estrogenic fine fractions (Figure 10) which were analysed by GC-MS in an attempt to identify the cause of estrogenic activity (Table 3).

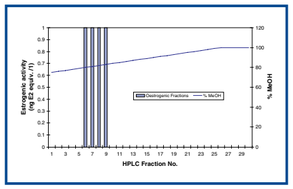

Figure 8. Estrogenic activity of the fine fractions from the HPLC fractionation of pore water collected from the Tees.

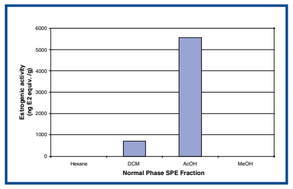

Figure 9a. Estrogenic activity of coarse sediment extract fractions isolated from sediments collected from the Tyne

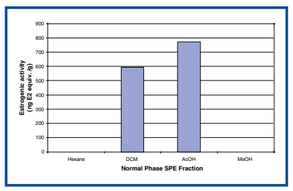

Figure 9b. Estrogenic activity of coarse sediment extract fractions isolated from sediments collected from the Tees

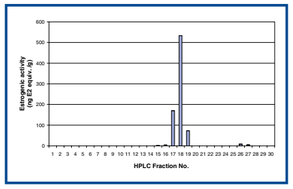

Figure 10. Estrogenic activity of the fine fractions from the HPLC fractionation of extracts of sediments collected from the Tees

Table 2. Summary of estrogens identified in Howdon STW and Dabholm Gut sediment and their associated chemical data

Effluent/ Fraction	Compound	Chemical structure	Chemical Abstract Service (CAS)	Log K_{ow}	Activity (ng E2 equiv. l^{-1} or ng E2 equiv. g^{-1})	Estrogenic potency†	Estimated conc. in effluent (μg l^{-1} or μg g^{-1})	Source
Dabholm Gut pore water:6-9	Estratrien-1,2,5-trien-3-ol		53-63-4	-	4	x0.3	0.0013	Unknown
Howdon STW sediment extract: DCM 20-21	Nonylphenol		60-57-1	4.5	-	x2.6 x10-5	-	Surfactant metabolite
Howdon STW sediment extract: AcOH 30	Nonylphenol		60-57-1	4.5	-	x2.6 x10-5	-	Surfactant metabolite

† As compared to 17β-estradiol using the YES assay. – data not yet acquired

This approach allows estrogenically active compounds to be identified in complex environmental samples and allows the contribution of each compound to the overall activity to be determined. This data will then be used to help identify the source of these compounds and assess the risk posed by environmental estrogens in UK estuaries.

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