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CHARACTERISING HAZARDOUS SUBSTANCES IN THE UK MARINE ENVIRONMENT

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ABSTRACT

In order to assess the risk posed by hazardous substances present in the marine environment, investigations are required to characterise the most toxicologically significant substances present. This task can represent finding the proverbial needle in a haystack, since, in many industrially impacted areas, estuarine sediments and surface waters contain a complex mixture of natural and synthetic substances. To help the process, toxicity identification evaluation (TIE) procedures have been developed to isolate and characterise the biologically active compounds present. The TIE procedures have been combined with an acute bioassay using a marine copepod (*Tisbe battagliai*), mutagenic assay (Mutatox™) and an *in vitro* yeast-based screen for oestrogenic activity. A wide range of compounds, including pesticides, surfactant metabolites, natural steroids and industrial chemicals, have been identified and used to inform monitoring programmes and risk assessments.

Keywords: Toxicant characterisation; toxicity identification evaluation (TIE); UK; estuaries, surface waters, sediments.

INTRODUCTION

The majority of biologically active compounds that are present in the aquatic environment exist as a complex cocktail. Within such complex mixtures, certain compounds will exert a greater harmful effect on the environment than others. Identifying which compounds represent a hazard within these complex environmental samples is therefore necessary, before any risk posed can be assessed.

Over the past five years, a number of cases have arisen where there has been a need to characterise marine samples collected from within the United Kingdom (UK). There has been a need to characterise 1. estuarine waters that have periodically exhibited toxicity unexplained by the concentrations of compounds routinely measured in monitoring programmes (*e.g.* North Sea Task Force Monitoring Master Plan (NSTFMMP) and UK National Monitoring Programme (NMP)); 2. estuarine sediments that potentially represent a hazard to benthic organisms; and, 3; compounds that are causing oestrogenic responses in wild male flounder.

The approach that has been adopted to characterise these complex environmental samples is toxicity identification evaluation (TIE). Often referred to as bioassay directed fractionation, TIE techniques use a biological response to direct the simplification of a complex environmental sample in order to identify the biologically active compounds present. Developed by the United States Environmental Protection Agency (US EPA), to identify toxicants in effluent discharges (Mount and Anderson-Carnahan, 1988), these procedures have been applied to identify a broad range of active compounds (*i.e*. toxic, oestrogenic and mutagenic) in a variety of environmental matrices using a number of acute and sub-lethal bioassays (See Burgess, 2000 for full review of marine applications, whilst Burgess *et al*., 1996 provides technical guidance). More importantly, the process of TIE is considered a cost-effective and scientifically sound method for the characterisation and identification of toxic agents within an environmental sample (Burgess, 2000). It is therefore a particularly appropriate technique for characterising hazardous substances in marine environment.

Outside of the US, the application of marine TIEs is limited (Thomas *et al*., 1999a; Thomas *et al*., 1999b; Thomas *et al*., In press). In this paper we present an overview of the application of TIE in characterising marine samples collected within the UK in order to identify which compounds present in these samples are responsible for the observed biological effects.

METHODS

Water and Sediment Sampling
Typically, surface water samples (40 L) were collected from an aluminium boat using a stainless steel bucket and alloy churn (50 L). Surface sediment samples (10 L) were collected by van Veen grab and stored in 10 L stainless steel containers. Water samples were extracted within 48h of collection, whilst sediment samples were separated into pore water and residual sediments by centrifugation within 48h. The pore water extracted (and or tested) and the residual sediments stored in glass jars at -20ºC. Sampling regimes were particularly focused on the UK NMP sample stations and up- and downstream of point discharge sites (Table 1).

Extraction of toxicants from estuarine waters
The method used for the extraction of water samples has been previously described (Thomas *et al*., 1999a; Thomas *et al*., 1999b). Typically, a 20 L water sample was forced, using compressed air, through a Teflon column packed with glass wool (previously rinsed with acetone and dried) and two polystyrene-based polymer Isolute ENV+ solid phase extraction cartridges (1 g; IST, Hengoed, UK) connected in series. The ENV+ columns were then dried under vacuum, extracted using methanol, and combined.

Extraction of environmental oestrogens from estuarine waters
In order to extract as many organic compounds as possible from each sample a purpose designed layered SPE system was developed. Each extraction column was comprised of a C8 (Isolute, IST, 5 g), ENV+ (IST, 2 g) and graphitic carbon (GC; 5 g) PTFE columns placed in series and activated by flushing with methanol (10 ml) and water (10 ml). The sample (2 L) was passed through at a flow rate of <20 ml/min. Once all the sample had passed through, each column was dried by vacuum aspiration. Each layer was then separated and eluted individually with methanol (10 ml) to give three fractions for each sample; C8 extract, ENV+ extract and GC extract.

Extraction of sediment pore water
A 2 L pore water sample was drawn through an ENV+ column (IST, Hengoed, UK), previously solvated with 10 ml methanol followed by 10 ml water. After a 100 ml flushing period, a 250 ml sample was collected into a clean 500 ml amber glass Winchester to assay for toxicity in water passing through the column. Once extraction was complete the column was dried under vacuum for 20 min and the organic load recovered by the addition of methanol (2 x 5 ml). The sample was then reduced in volume to 5 ml using N_2 (40°C for 2 h) and stored at -20ºC.

Particulate material extraction
Sediment particulate material was dried in a fume cupboard at room temperature. The sample was then finely ground (< 0.45 mm) and soxhlet extracted using dichloromethane (DCM). The sample, capped by anhydrous Na_2SO_4, was placed into a pre-extracted system for 24 h. The DCM extract was then reduced in volume by rotary film evaporation to ~10 ml, transferred into hexane, and stored at -20ºC.

Normal phase SPE
Sediment extracts shown to induce a response in any assay were initially fractionated by normal phase (NP) SPE using silica glass SPE columns (1 g, IST, Hengoed, UK). Samples (total extract in hexane, 100 ml) were passed through the column and the post column hexane collected. The column was then eluted with hexane (10 ml) which was added to the original post column hexane. The column was then consecutively eluted with 10 ml of dichloromethane (DCM), acetone and methanol and each individual fraction reduced in volume to 5 ml for assay.

High performance liquid chromatography fractionation
All SPE extracts exhibiting biological activity were fractionated into fine fractions by HPLC. For surface, pore water, and, acetone and methanol NP SPE extracts, this was done by reverse phase HPLC using an Econo-Prep C18 semi-preparative HPLC column (30 cm x 10 mm x 5 µm; Phenomenex, Cheshire, UK). The column was fitted with a guard (5 cm x 10 mm x 5 µm) at a flow rate of 5 ml min^{-1}, with a UV detector (210 nm) using HPLC grade methanol and water as a mobile phase. Gradient elution was used over 30mins. Typically a linear 25 min gradient of 40:60 methanol:water v/v. to 100% methanol was used, ending with a 5 min 100% methanol flush. Thirty 5 ml fine fractions were collected each minute. For hexane and DCM NP SPE extracts, this was by normal phase HPLC using a Phenosphere semi-preparative HPLC column (25 cm x 10 mm x 10 µm; Phenomenex, Cheshire, UK). This was fitted with a guard column (Phenosphere, 5 cm x 10 mm x 10 µm) at a flow rate of 5 ml min^{-1}, using HPLC grade hexane, DCM and isopropylalcohol (IPA) as a mobile phase. Gradient elution was used over 30 min with the composition of the mobile phase dependent on the toxicity profile observed following coarse fraction. Typically, an isocratic 10 min of hexane, followed by a 15 min gradient of 100% hexane to 100% DCM, and ending with a 100% IPA flush. Thirty 5ml fine fractions were collected and stored at -20°C.

Compound identification
Fractions demonstrating biological activity were analysed by gas chromatography-mass spectrometry (GC-MS) using a Finnigan GCQ. Gas chromatography used a DB5 column (J&W Scientific; 60 m x 0.25 mm x 0.25 µm) with a temperature gradient 40-280°C at 5°C min^{-1}, and held isothermally at 280°C for 10 min. Splitless injection was used (2 µl; 270°C) with the mass spectrometer operating in full scan mode (50-500 AMU). Mass spectra of major peaks were compared to reference spectra in the NIST mass spectral database for

tentative identification. Co-injection with authentic reference standards was used to confirm identifications. Bracketing fractions were extracted and analysed to confirm the absence of any possible toxicant in non-toxic adjacent fractions. Concentrations of individual compounds were semi-quantified against the response of an external standard.

Bioassays
A small scale aquatic bioassay using the marine copepod *Tisbe battagliai* (Thomas *et al*., 1999a) and two *in vitro* bioassays capable of identifying oestrogens (YES assay; Desbrow *et al*., 1998) and mutagens (Mutatox™ (*Vibrio fisheri*); Ho and Quinn, 1993) were used to direct the separation processes described.

RESULTS

Identification of toxicants in estuarine surface waters
Very few estuarine surface water samples were acutely toxic to *T. battagliai* and as a matter of routine, samples were pre-concentrated 80-200 times to bring contaminants up to toxic concentrations. Samples found to be toxic at 200 times ambient concentrations were subjected to the TIE process and potential toxicants identified (Figure 1). Chlorinated phenols (pentachlorophenol, trichlorophenol, tetrachlorophenol and 4-chloro-3,5 dimethylphenol) were shown to be present in a sample isolated from down stream of Howdon STW on the Tyne estuary, whilst nonylphenol was present in the toxic fractions isolated from samples collected at Redcar Jetty on the Tees estuary (Table 2). Atrazine was also found in fractions isolated from samples collected from the Tees estuary. No effects were observed in samples collected from the Wear estuary even after pre-concentrating samples 200 times. The only location on the Mersey where sample concentrates gave a toxic response was at Seacombe Ferry. Fractionation of the samples collected here identified dieldrin and dodecylphenol as candidate toxicants.

A simple risk assessment of the identified compounds suggests that a number of the compounds were present at concentrations above ‘safe’ acute concentrations (Table 3). Overall it would appear that organic contaminants are not a major contributor of toxic effect in these estuaries and that in terms of toxic effect the overall quality of UK estuaries is good. The compounds identified, which traditionally have not been included in monitoring programmes, have been considered for inclusion in future surveys.

Identification of hazardous substances in estuarine sediments
Sediment pore water collected from Dabholm Gut was shown to be toxic *to T. battagliai.* Phase 1 characterisation of the pore water showed that the toxicity was caused by organic compounds (*i.e*. removal of toxicity by ENV+ SPE), cationic metals (*i.e*. removal of toxicity by ion exchange SPE) and ammonia (Figure 2). Testing of the thirty fine fractions, produced by HPLC, showed that three groups of fine fractions were toxic to *T. battagliai* (fractions #11-12, 14-15, 21-26, Figure 3). No compounds have yet been identified as the cause of toxic effect.

A C8 sediment pore water extract obtained from Dabholm Gut (Tees) had an oestrogenic effect equivalent to 7 ng E2 l^{-1}. No activity was detected in the pore waters collected from the Tyne. Fractionation of the active extract by HPLC produced five consecutive oestrogenic fractions (Figure 3; fractions 19-23). GC-MS analysis of these fractions failed to identify any candidate oestrogens. In an attempt to isolate the active compounds, fractions 19-23 were

fractionated further by HPLC using a shallower methanol:water gradient. No compound could be identified as the cause of oestrogenic activity.

The complex mixture extracted from the sediment particulate material was shown to contain compounds with oestrogenic and mutagenic activity (Table 4). Coarse fractionation of this extract produced four fractions (F1-F4) which were screened for both mutagenic and oestrogenic activity. Fractions F1 and F4 were shown to be mutagenic whilst fractions F2 and F3 were shown to be strongly oestrogenic (5950 to 5575 ng E2 g^{-1}; Table 4). Fine fractionation of coarse fraction F1 produced thirty fractions of which six demonstrated mutagenic activity (#4-6 & #8-10; Figure 4). GC-MS analysis of fine fractions #4-6 identified a broad range of alkyl substituted aromatics and an unresolved complex mixture (UCM) often characteristic of petrogenic inputs (Gough and Rowland, 1990). Fine fractionation of coarse fraction F4 produced three separate fractions that demonstrated mutagenic activity (#8, #12, #19; Figure 4). YES screening of the thirty fine fractions produced from coarse fractions F2 and F3 produced five separate areas of oestrogenic activity (Figure 4).

A sample collected from down stream of Howdon sewage treatment works (STW) was also characterised using the above protocols. The isolated pore water gave negative results when tested using all three assays, indicating that no toxic, mutagenic or oestrogenic chemicals were present in this sample. Testing of the coarse sediment extract fractions, obtained following normal phase SPE, gave the same positive results as for the Tees sample (Table 4).

Identification of oestrogens in estuarine water samples

Direct testing of marine surface waters for *in-vitro* oestrogenic activity suggested showed a < 15 ng l^{-1} 17β-oestradiol (E2) equivalent response. SPE of water samples, using a layered approach, allowed these waters to be concentrated prior to assay. All the oestrogenic activity detected in the samples was found in the C8 extract, suggesting that the oestrogenic compounds present were mid-polar to non-polar compounds (log K_{OW} > 3) (Table 5). The highest amount of activity was determined in a sample collected at Howdon STW (24 ng E2 equivalents l^{-1}) with lesser activity detected up- and downstream of the discharge point. Less activity was detected in a sample collected from Dabholm Gut with very little activity in the samples collected up- and downstream.

The C8 extracts of effluent samples collected from Dabholm Gut and Howdon STW were then fractionated by HPLC with oestrogenic activity detected in a number of fractions (Figure 5). In both samples the majority of the oestrogenic activity was isolated to a single group of fractions (Figure 5; Fractions 21-23; Howdon STW 90 %; Dabholm Gut 84 %). GC-MS characterisation of all oestrogenic fractions was used to identify candidate oestrogens (Table 6). The majority of the activity in both sample extracts (Fractions 21-23) was shown to be due to the presence of 17β-oestradiol. Bis(2-ethylhexyl)phthalate and nonylphenol were identified by GC-MS as present in oestrogenic fractions 26 and 28 respectively isolated from Dabholm Gut effluent. In the extracts isolated from Howdon STW effluent, GC-MS analysis failed to identify any oestrogenic compounds in fractions 16-19, however, androsterone was identified as present in fraction 25.

DISCUSSION

All three investigations described used flexible TIE protocols to successfully characterise different marine toxicants in a variety of matrices. A combination of modern SPE technology combined with a robust marine bioassay allowed large volumes of surface waters to be

extracted, concentrated and tested in order to identify the compounds responsible for unexplained toxicity in samples collected from UK estuaries. This toxicity was attributed to a number of compounds including, chlorinated phenols, nonylphenol and atrazine. A simple risk assessment indicated which compounds were considered to represent a hazard to marine life and were recommended for inclusion in future monitoring programmes.

The approach adopted to characterise sediments has involved separately characterising sediment pore waters and particle bound material. This assumes that the dissolved phase represents the primary bioavailable forms (DiToro *et al*., 1991), whilst the particle bound material offers a potential effect. In this investigation, a variety of bioassays were used in order to detect substances that may potentially cause acute and sub-lethal effects to benthic organisms. It is apparent that different substances are responsible for the different effects observed. Ammonia, cationic metals and organic compounds appear to be responsible for acute effects, whist organic compounds are responsible for oestrogenic and mutagenic activity. The compounds responsible for much of this oestrogenic activity remain to be identified.

Marine oestrogens were characterised using a protocol modified from that used for estuarine organics. The specificity of the YES assay, combined with a layered SPE system, allowed the organic loading of surface water samples to be isolated and concentrated prior to TIE assessment. The assay was successful at identifying 17β oestradiol as the main source of in vitro oestrogenic activity (80-90% of total activity), with minor contributions from Bis(2-ethylhexyl)phthalate, nonylphenol, and androsterone.

In the cases described within this paper, we have demonstrated that TIE is particularly suited to characterising hazardous substances in the marine environment. TIE is a flexible technique adaptable to the needs of a particular study. It can be applied to a broad range of environmental problems where there is a need to characterise and/or identify the causes of a particular effect. This adaptability is dependent on the selection of a suitable bioassay, and as more sophisticated bioassays are developed TIE will become an even more powerful tool. The three bioassays described within this report emphasise this. The *T. battagliai* bioassay is particularly suitable for TIE studies since it possesses a short lifecycle, requires low sample volumes (<20 ml), needs minimal space and equipment for testing, is sensitive to industrial effluent toxicity, has an easily determined acute endpoint and given its habitat will be exposed to organics present in marine waters. The YES assay offers an *in vitro* screen that detects both natural hormones (17β oestradiol, oestrone), medicinal pharmaceuticals (ethynyloestradiol) and all known xenoestrogens (alkylphenols, *bis*-phenol A, DDT, phytoestrogens and PCBs), whilst requiring very low sample volumes (<500 µl) with an easily determined calorimetric endpoint. The Mutatox™ assay is a commercially available, cost-effective, low volume genotoxicity assay that has been fully evaluated by the USEPA. The development of more sophisticated assays has been called for, and it is suggested that they should be able to quantify sensitive sub-lethal effects, population disturbances, community impacts and ecosystem malfunctions (Burgess *et al*., 2000).

Another area that has aided the progress of TIE is the development of modern SPE technology. Modern SPE allows the extraction of large volumes of water that in turn allows samples to be concentrated prior to bioassay. This facilitates the characterisation process by ensuring that there is a significant response from the bioassay when isolating the component of an environmental mixture. This approach may also be used when the assay is not

compatible with a seawater matrix. This is best illustrated by the isolation of surface water oestrogens by SPE prior to testing using the YES assay.

The value of TIE to the environmental manager is that it is a flexible technique capable of identifying the substance responsible for a detrimental effect within an environmental sample. This in turn may suggest a source for the compound identified (*e.g.* 17β oestradiol from STW effluent) which when combined with remedial techniques, such as toxicity reduction evaluation (TRE), may prove invaluable in truly assessing and protecting the quality of our seas.

CONCLUSIONS

A series of methods have been described to characterise toxic, mutagenic and oestrogenic compounds in both marine surface waters and sediments. These methods have been validated using a broad range of known environmental contaminants. These examples help demonstrate that TIE is particularly suitable for the characterisation of hazardous substances in marine samples. The benefits of the approach for marine environmental protection and management are that it is scientifically sound, cost effective, flexible and can be modified to the requirements of an individual study. Additionally, as advanced biological assays are developed, TIE will become an even more powerful tool in assisting with managing the marine environment.

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Table 1. Sample Stations where marine TIEs have been performed in the UK

Estuary	Location	Position	Dates	Sample	Assay
Thames	Rainham	51° 29.53'N 00° 10.76'E	1995	Surface water	*T. battagliai*
Tyne	Down stream Howdon STW	54° 59.30'N 01° 28.36'W	1995, 1996, 1998 & 1999	Surface water and sediment	*T. battagliai*, Mutatox™ and YES
	St. Anthony's	54°57.57'N 01° 32.94'W	1995 & 1996	Surface water	*T. battagliai*
	Team Mouth	54° 57.51'N 01° 38.01'W	1995 & 1996	Surface water	*T. battagliai*
Tees	The Gares	54° 33.52'N 01° 18.33'W	1995 & 1996	Surface water	*T. battagliai*
	Redcar Jetty	54° 37.40'N 01° 09.34'W	1995 & 1996	Surface water	*T. battagliai*
	Dabholm Gut	54° 36.27'N 01° 09.40'W	1998 & 1999	Surface water and sediment	*T. battagliai*, Mutatox™ and YES
	Phillips Approach Buoy	54° 37.79'N 01° 09.72'W	1995 & 1996	Surface water	*T. battagliai*
	Transporter Bridge	54° 35.07'N 01° 13.57'W	1995 & 1996	Surface water	*T. battagliai*
	Bamlett's Bight	54° 35.53'N 01° 15.03'W	1995 & 1996	Surface water	*T. battagliai*
Mersey	Seacombe Ferry	53° 24.56'N 03° 00.48'W	1995 & 1996	Surface water	*T. battagliai*
	Machester Ship Canal Lock	53° 19.47N 02° 56.67'W	2000	Sediment	*T. battagliai*, Mutatox™ and YES
Milford Haven	Popton Point	51° 41.80'N 05° 03.00' W	1996	Surface water	*T. battagliai*
Belfast Lough	Buoy 6	54° 40.10'N 05° 48.40' W	1996	Surface water	*T. battagliai*

Table 2. Summary of candidate toxicants identified in UK estuaries

Estuary/Station	Compound	Use
	pentachlorophenol	wood preservative
Tyne: St Anthonys	trichlorophenol	bactericide/ fungicide
	tetrachlorophenol	fungicide
	4-chloro-3,5 xylenol	disinfectant and pesticide metabolite
Tyne: Down stream of Howdon STW	pentachlorophenol	wood preservative
	tetrachlorophenol	fungicide
	trichlorophenol	bactericide/fungicide
	nonylphenol	surfactant metabolite
	triphenylphosphine sulphide	
	naphthaleneamine	
	methyl pyridiene amine	
	chlorobenzeneacetonitrile	
	methyl acridine	
	alkyl substituted naphthalenes	
	fluorenes (monomethyl-trimethyl)	
Tees: Gares	nonylphenol	surfactant metabolite
Tees:Transporter Bridge	4-chloro-3,5-dimethylphenol	pesticide metabolite and disinfectant
	dimethyl benzoquinone, dimethy	
	naphthalenecarboxamide, diethyl	
	naphthalenecarboxamide	
Tees:No. 23 Buoy	Atrazine	herbicide
	nonylphenol	surfactant metabolite
Tees: Redcar Jetty	atrazine	herbicide
	nonylphenol	surfactant metabolite
	4-chloro-3,5-dimethylphenol	pesticide metabolite and disinfectant
	carbophenothion methylsulphoxide	pesticide metabolite
Tees:Phillips Approach Buoy	nonylphenol	surfactant metabolite
Mersey: Seacombe Ferry	dieldrin	insecticide
Milford Haven: Popton Point	bis(2-ethylhexyl)phthalate	

Reproduced in part from Thomas *et al*., 1999a; 1999b.

Table 3. Aquatic toxicity data for the compounds identified in UK estuaries

Chemical	CAS	Fish		Invertebrates		Algae	Estimated chronic 'safe' level for aquatic life (μgL^{-1})
		Lowest acute LC_{50} value (μgL^{-1})	Lowest chronic EC_{50} value or LOEC (μgL^{-1})	Lowest acute LC_{50} value (μgL^{-1})	Lowest chronic EC_{50} value or LOEC (μgL^{-1})	Lowest acute EC_{50} value (growth) (μgL^{-1})	
pentachlorophenol	87-86-5	32	25	120	10	0.09	0.3-2.5
trichlorophenol	933-78-8	450	-	500	-	290	4.5-5
tetrachlorophenol	58-90-2	170	-	110	-	1300	1.1-1.7
4-chloro-3,5-xylenol	88-04-0	-	-	200	-	-	2
nonylphenol	2514-52-3	900	22	1310	100	-	2.2-13
dieldrin	60-57-1	0.2	0.75	13	-	-	$2x10^{-3}$-0.13
atrazine	1912-24-9	220	120	640	-	3	1.2-12

Reproduced from Thomas *et al*., 1999a

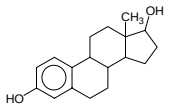
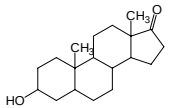
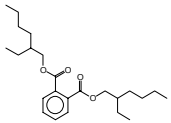
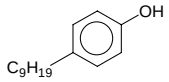
Table 4. Biological activity of sediment particulate extract fractions

Sample	**Si SPE Fraction**	**YES assay result**	**Mutatox Result**
Tees *(Dabholm Gut)*	F1 (hexane)	negative	positive
	F2 (DCM)	positive (595 ng E2 g^{-1})	negative
	F3 (acetone)	positive (775 ng E2 g^{-1})	negative
	F4 (methanol)	negative	positive
Tyne *(Howdon STW)*	F1 (hexane)	negative	positive
	F2 (DCM)	positive (5575 ng E2 g^{-1})	negative
	F3 (acetone)	positive (719 ng E2 g^{-1})	negative
	F4 (methanol)	negative	positive

Table 5. Oestrogenic activity of estuarine water samples

Location	Position	Oestrogenic activity (17β-oestradiol equivalent concentration, ng E2 l^{-1})
Howdon STW	54° 59.34'N 01° 28.18' W	23.6
Downstream of Howdon STW	54° 59.30'N 01° 28.36' W	4.4
Upstream of Howdon STW	54° 59.30'N 01° 28.31' W	3.1
Dabholm Gut	54° 37.47'N 01° 09.12' W	6.3
Downstream Dabholm Gut	54° 36.92'N 01° 09.21' W	0.5
Upstream Dabholm Gut	54° 36.80'N 01° 09.18' W	0.3
Alde	52° 05.86'N 01° 33.98' W	<0.15

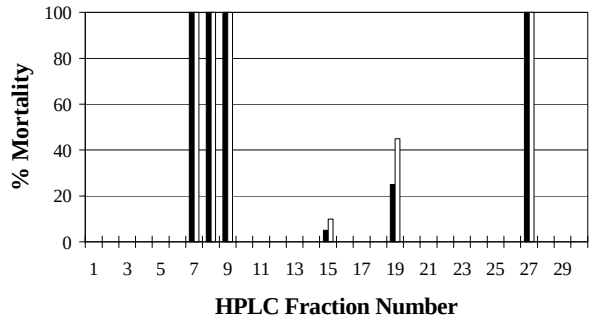
Table 6. Summary of oestrogens identified in Howdon STW and Dabholm Gut water samples and associated chemical data

Effluent/Fraction	Compound	Chemical structure	Chemical Abstract Service (CAS)	Log K_{OW}	Activity (ng E2 equiv. l^{-1})	Oestrogenic potency†	Estimated conc. in effluent ($\mu g\ l^{-1}$)	Source
Howdon STW: 16-19	Unknown	-	-	-	1.0	-	-	-
Howdon STW: 21-23	17β-oestradiol		50-28-2	3.1	19.1	x 1	0.0191	Natural steroid hormone
Howdon STW: 25	androsterone		53-41-8	-	0.4	x $5x10^{-4}$	0.8	Testosterone metabolite
Dabholm Gut: 21-23	17β-oestradiol	As above	50-28-2	3.1	0.73	x 1	0.00073	Natural steroid hormone
Dabholm Gut: 26	*bis*(2-ethylhexyl)phthalate		117-81-7	4.9	0.11	x $2.8x10^{-7*}$	393	Placticizer
Dabholm Gut: 28	nonylphenol		60-57-1	4.5	0.02	x $2.6x10^{-5}$	0.77	Surfactant metabolite

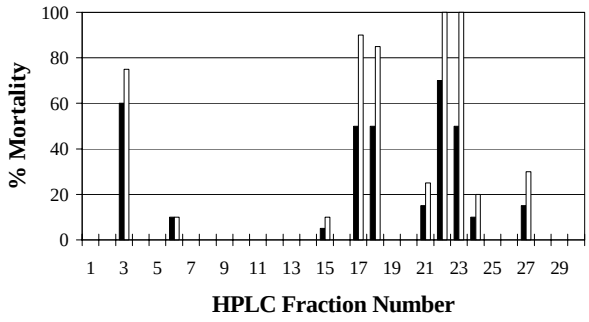
†As compared to 17β-oestradiol using the YES assay.

* Tentative quantification since plastic ware is used in the extraction procedure, however blanks do not show a phthalate induced response.

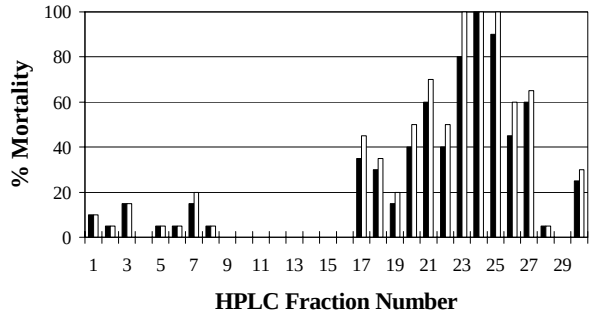
A. Down stream of Howdon STW



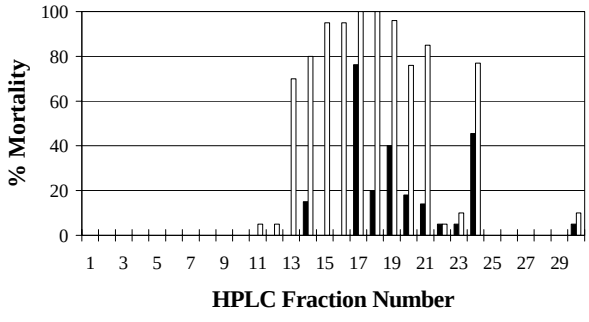
B. St Anthonys



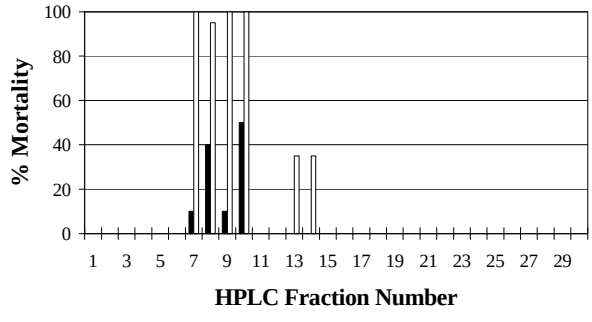
C. Gares



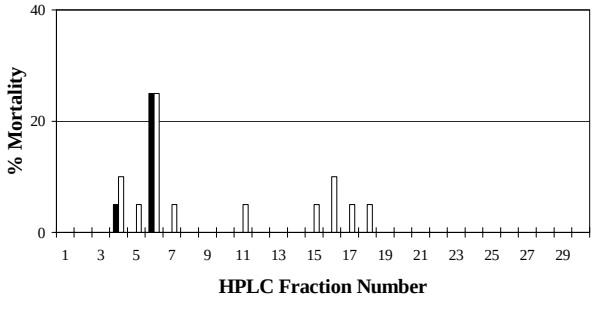
D. Redcar Jetty



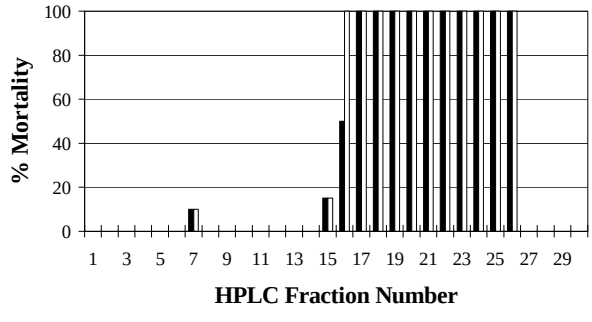
E. Seacombe Ferry



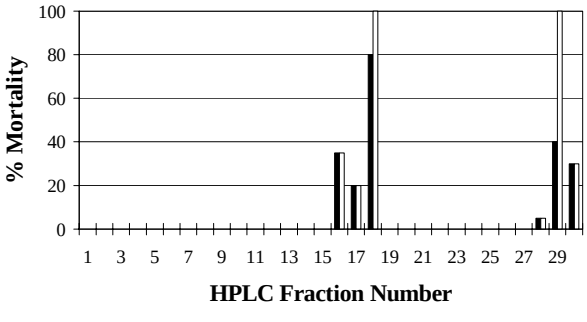
F. Rainham



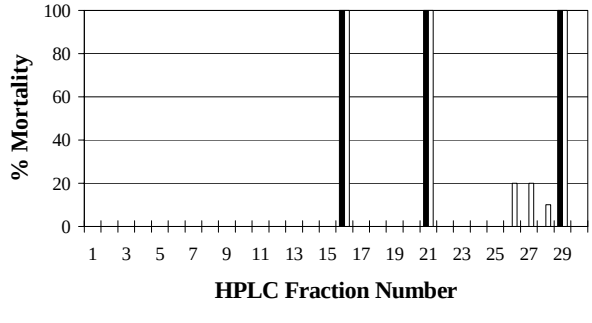
G.Transporter Bridge



H. Buoy 23



I. Redcar Jetty



J. Phillips Approach Buoy

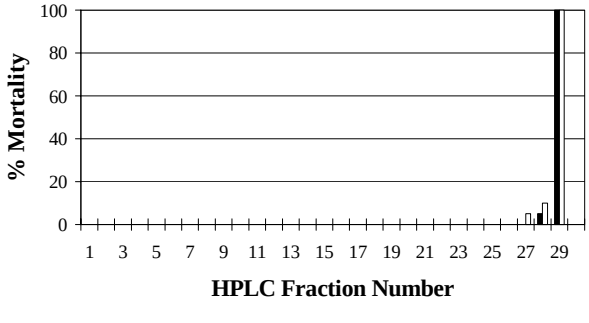


Figure 1. *T. battagliai* Toxicity profiles of a selection of water samples collected from UK estuaries (Reproduced from Thomas *et al.*, 1999a)

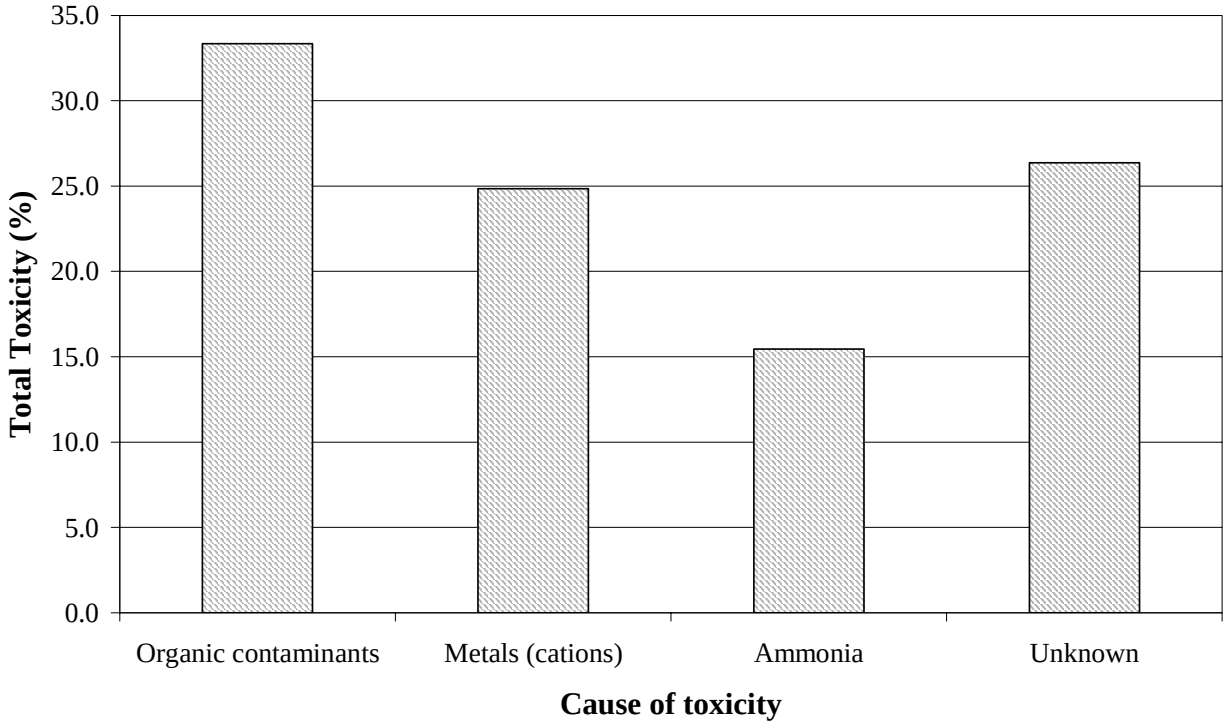
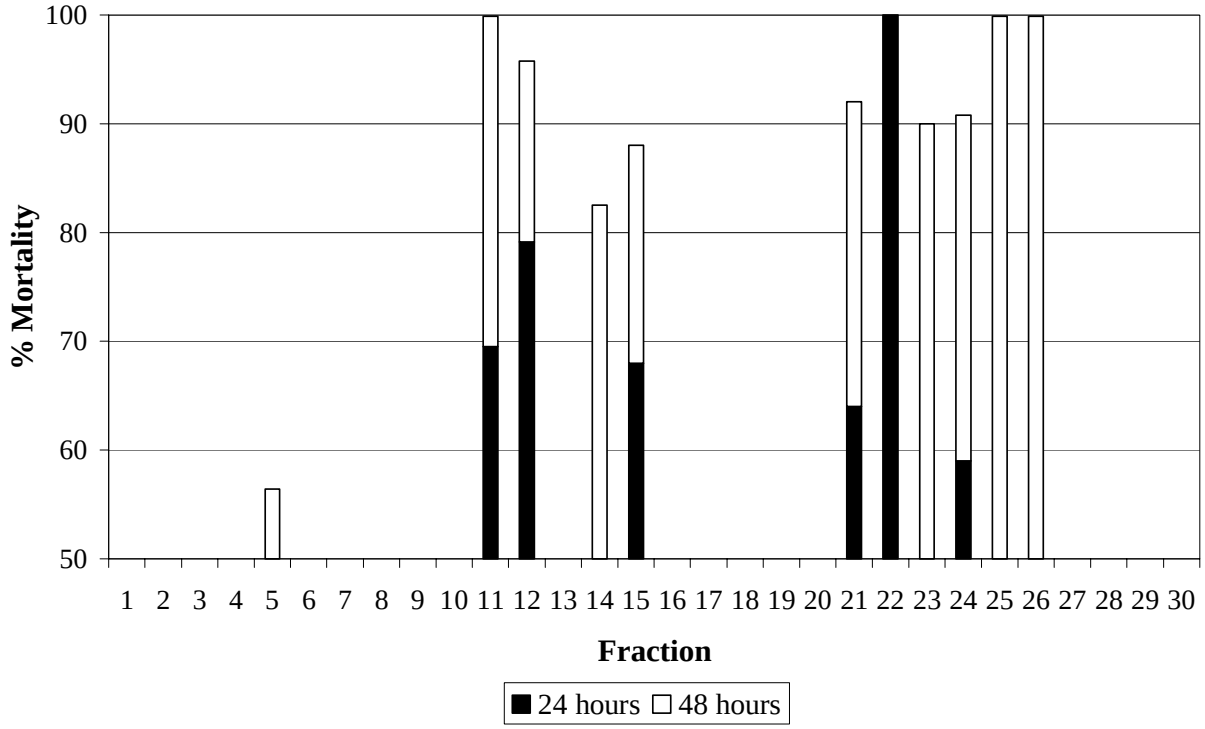


Figure 2. Cause of toxicity to *T. battagliai* in Tees sediment pore water

A. *T. battagliai* toxicity



B. Oestrogenic activity

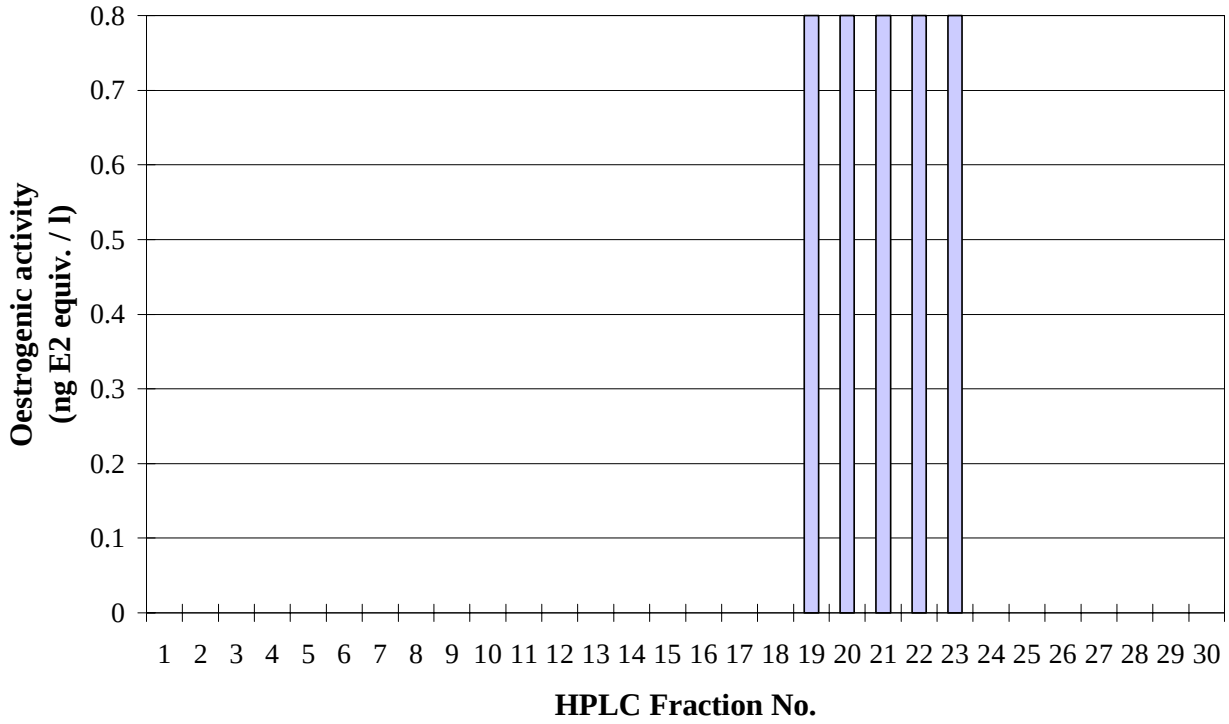
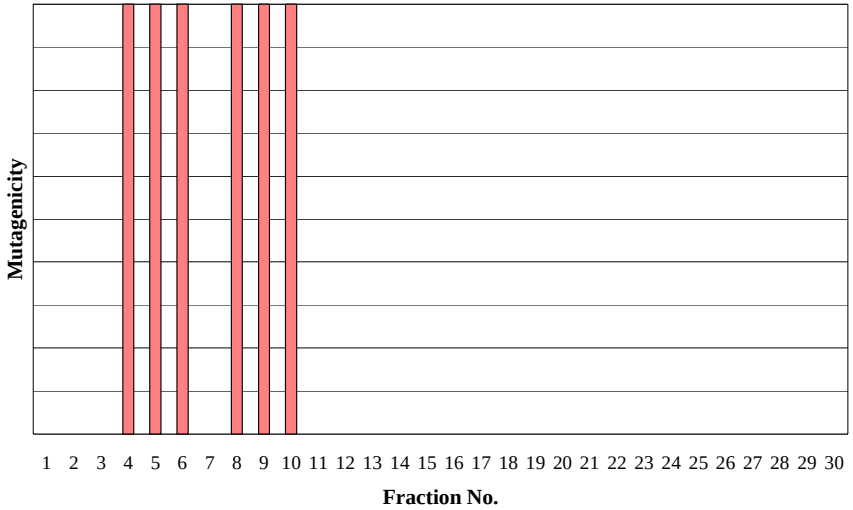
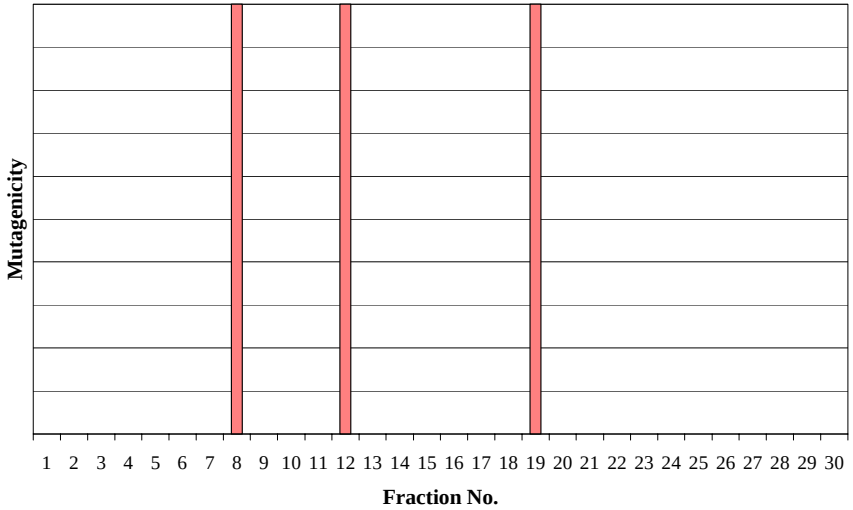


Figure 3. *T. battagliai* toxicity and oestrogenic activity of Dabholm Gut (Tees) sediment pore water

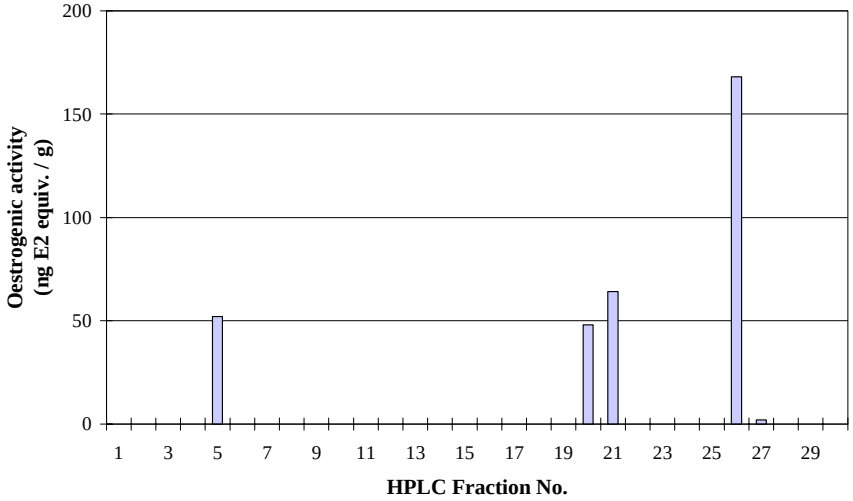
A Mutagenic Coarse F1



B Mutagenic Coarse F4



C Oestrogenic Coarse F2



D. Oestrogenic Coarse F3

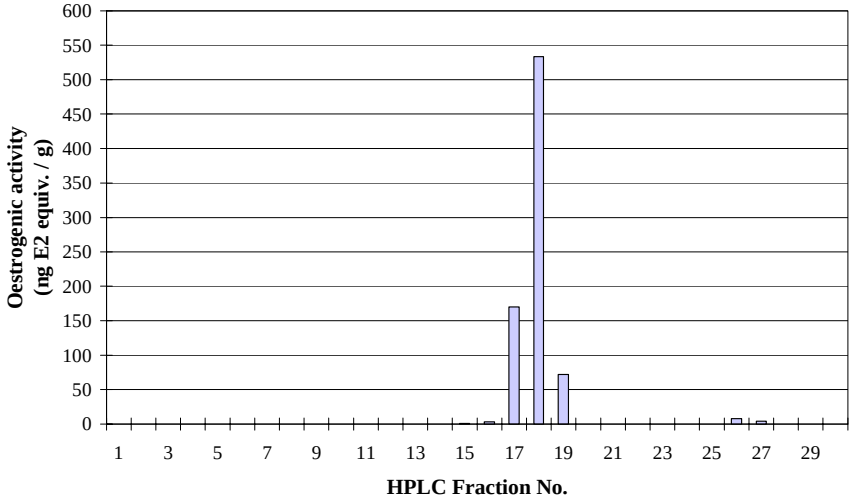
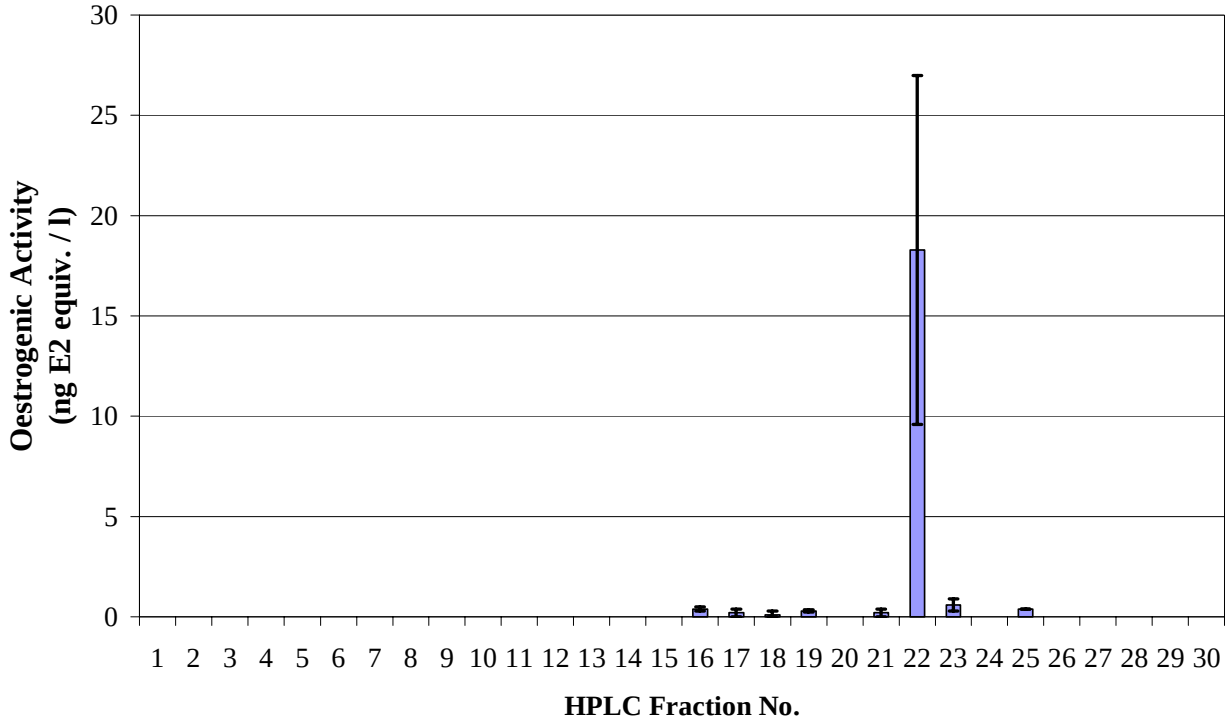


Figure 4. Mutagenic and oestrogenic activity of the fine fractions obtained from Tees sediment particulate material.

A. Howdon STW



B. Dabholm Gut

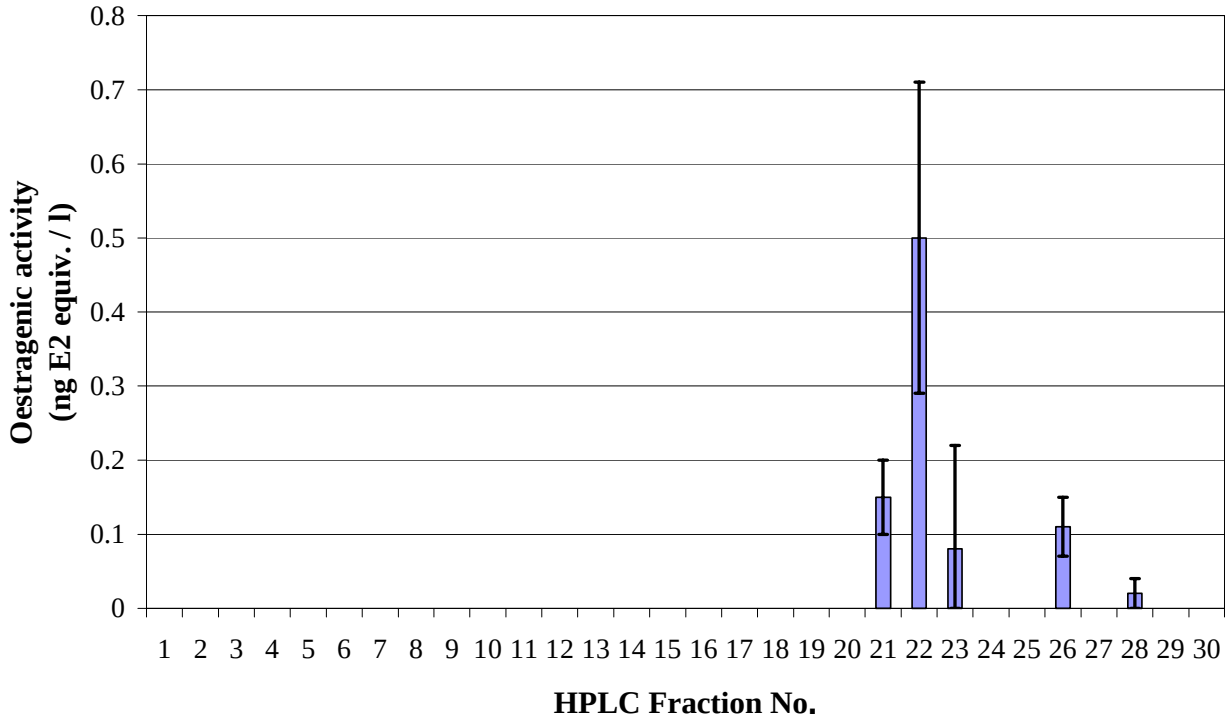


Figure 5. Oestrogenic activity of HPLC fractions from, A. Howdon STW (Tyne) water and B. Dabholm Gut (Tees) water, represented as 17β-oestradiol equivalents