CHARACTERISATION OF HAZARDOUS SUBSTANCES IN MARINE SEDIMENTS

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The UK's commitment to the OSPAR Hazardous Substances strategy requires that information is available on the toxicity of hazardous substances present in the marine environment, particularly in marine sediments. In this study, toxicity identification evaluation (TIE) techniques were used to characterise the toxic substances present in sediments collected from six UK estuaries that have been historically impacted by primary industry; Tyne, Tees, Mersey, Humber, Thames and Southampton Water.

Spatial survey

As a first step, sediment pore water extracts and sediment solvent extracts were screened using a battery of bioassay tests. Pore water samples were tested for the presence of lethal toxicants by using the *Tisbe battagliai* bioassay, *in vitro* oestrogen receptor (ER) agonists using the yeast oestrogen screen (YES) and for *in vitro* mutagenic activity using the Mutatox® assay (Figure 1; Table 1). Sediment solvent extracts were also tested for ER agonists and mutagens, whilst *in vitro* aryl hydrocarbon receptor (AhR) agonists were also tested for by using the DR-CALUX® assay (Figure 1; Table 1). Lethal toxicity was only measured in 10% of the sediment pore water samples tested. ER agonists were detected in 66% of the porewater samples and 91% of the sediment solvent extracts tested. Potential mutagens were not detected in any of the pore water samples, while 25% of sediment solvent extracts scored positive. All of the samples tested using the DR-CALUX® assay scored positive.

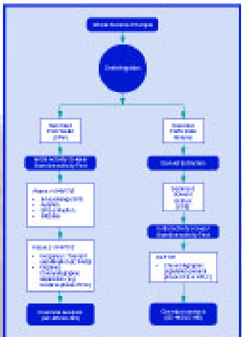

Figure 2: Fractionation scheme for the characterisation of marine sediments

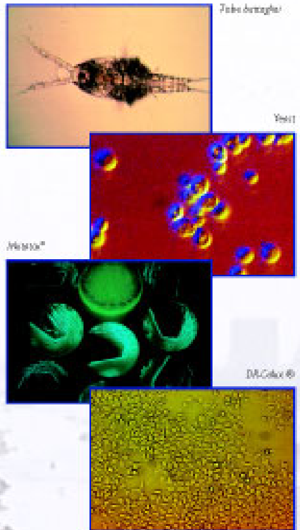

Figure 1: Bioassays used in this study

Table 1: Summary of the bioassay testing of sediment pore waters and extracts

Estuary	Location	Position	PORE WATER: T. battagliai (48h mortality) (95% CI)[a] LC50 (%)	TU	ER agonist potency[b] ng E2 l⁻¹	Mutagenic activity[c] + or -	SEDIMENT SOLVENT EXTRACT: ER agonist potency[b] µg E2 kg⁻¹	Mutagenic activity[c] + or -	AhR agonist potency (6h total)[d] µg BaP g⁻¹	AhR agonist potency (24h total)[d] ng TCDD TEQ g⁻¹	AhR agonist potency (24h dioxin)[e] pg TCDD TEQ g⁻¹
Tyne	Team Confluence	54° 57.46'N 01° 36.22'W	>100	<1	61	-	1.44	-		31	30
	Tyne Bridge	54° 58.25'N 01° 35.63'W	>100	<1	10	-	3.96	-	250	21	13
	Hebburn	54° 58.90'N 01° 31.04'W	>100	<1	23	-	1.86	+	740	60	32
	Howdon STW	54° 59.26'N 01° 27.73'W	>100	<1	25	-	3.13	+	93	90	33
	D/S Howdon STW	54° 58.78'N 01° 26.57'W	>100	<1	NS	-	1.28	+	52.5	19	47
Tees	British Steel	54° 37.64'N 01° 00.28'W	>100	<1	60	-	3.42	-	555	180	15
	Dabholm Gat	54° 36.86'N 01° 00.04'W	0.78 (0.5-1.1)	128 (88-200)	7	-	13	+	96	76	6
	Tees Port Authority	54° 38.20'N 01° 09.63'W	>100	<1	56	-	5.3	-	160	89	27
	Mid Channel Buoy	54° 35.10'N 01° 11.59'W	>100	<1	10	-	1.66	-	195	150	42
	Transporter Bridge	54° 34.81'N 01° 12.76'W	>100	<1	33	-	1.68	-	360	185	85
	Billingham	54° 35.45'N 01° 14.72'W	>100	<1	66	-	1.52	-	3133	160	35
	D/S Tees Lock	54° 33.90'N 01° 16.78'W	>100	<1	38	-	0.04	-			
Mersey	Opposite Port Radar	53° 27.80'N 03° 02.68'W	>100	<1	<2	-	<0.2	-	68.5	17	9
	Opposite Canada Dock	53° 26.16'N 03° 00.78'W	18.2	5.5	<2	-	0.04	+	135	14	40
	Seacombe Ferry	53° 24.93'N 03° 00.69'W	>33	<3	<2	-	0.04	-	84	13	24
	Tranmere Oil terminal	53° 22.72'N 02° 59.38'W	>100	<1	<2	-	<0.2	-	31.8	8	19
	Bromborough	53° 20.10'N 02° 57.29'W	>100	<1	<2	-	<0.2	-			
	Cutty Sark	51° 28.96'N 00° 00.60'W	>100	NS	NS	-	0.87	-			
Thames	Gravesend	51° 26.59'N 00° 22.74'W	>100	<1	7	-	1.43	-	62	13	1
	Greenhythe	51° 27.34'N 00° 16.48'W	>100	<1	4	-	1.12	-	18	8.9	2
	Erith	51° 28.94'N 00° 11.03'W	>100	<1	3	-	2.38	-	51.5	17	2
	Thames barrier	51° 30.03'N 00° 04.78'W	10.45	9.6	3	-	0.63	+			
Southampton Water	Fawley A	50° 49.67'N 01° 19.58'W	>100	<1	<2.12	-	0.79	-	71	10	2
	D/S Hythe STW	50° 52.06'N 01° 23.08'W	>100	<1	4	-	1.18	-	105	70	4
	Docks E	50° 53.32'N 01° 26.71'W	>100	<1	<2.12	-	0.79	-	11	7.4	16
	Docks W	50° 53.78'N 01° 24.73'W	>100	<1	3	-	0.65	-	0.8	1.1	2
	D/S Woolston STW	50° 53.52'N 01° 23.25'W	>100	<1	<2.12	-	0.67	-	24.5	13	3
	Hythe Marina	50° 52.46'N 01° 23.91'W	>100	<1	68	-	2.15	+	32.5	65	4
Humber	Immingham East Jetty	53° 37.75'N 00° 26.51'W	>100	<1		NA		NA	570	18	39
	Grimsby Dock	53° 35.16'N 00° 04.28'W	>100	<1		NA		NA	460	20	36
	Pyewipe Flats	53° 35.74'N 00° 07.58'W	38.9	2.6		NA		NA	250	16	27
	Humber Sea Port	53° 48.97'N 00° 15.38'W	>100	<1		NA		NA	310	10	39

[a]Lethal toxicity to T. battagliai after 48 h. [b]In vitro ER agonist potency determined using the yeast oestrogen screen (YES) and expressed as the equivalent response to 17β-oestradiol (ng E2 l⁻¹). [c]Mutagenic activity determined using the Mutatox assay. [d]In vitro AhR agonist potency determined using the DR-CALUX assay and expressed as benzo[a]pyrene (BaP) equivalent activity of the total extract over 6 and 24 h. [e]In vitro AhR agonist potency determined using the DR-CALUX assay and expressed as 2,3,7,8-tetrachlorodibenzo[b,e][1,4]dioxin (TCDD) TEQ equivalent activity of the dioxin fraction over 24 h. NA - not analysed.

Toxicity identification evaluation (TIE)

TIE techniques, using the above bioassays to direct the fractionation of the complex environmental samples, were then applied in an attempt to identify the compounds responsible for the observed effects (Figure 2). Ammonia was responsible for much of the lethal toxicity to *T. battagliai*, although cationic metals, unknown hydrocarbons and unknown substances also contributed to the toxicity of one sample. TIE techniques were successfully applied to identify polycyclic aromatic hydrocarbons (PAH), polycyclic aromatic ketones (PAKs), oxy-PAH and nitro-PAH as potentially contributing to the mutagenic signal measured in certain sediments. Unknown polar and non-polar mutagens were also shown to be present. Nonylphenol, cinnarizine and cholesta-4,6-dien-3-one were shown to contribute to the in vitro ER agonist potency of sediment solvent extracts, however, the identity of the compounds responsible for the majority of the measured ER agonist activity in both sediment solvent extracts and pore waters remain unknown. Applications of the DR-CALUX® bioassay showed that dibenzo-p-dioxins, dibenzofurans and PAH were acting as AhR agonists in certain samples (Table 2).

Comparison of the identified substances with OSPAR lists of substances for priority action and possible concern showed a number not to be included. Where available, occurrence, persistency, bioaccumulation and toxicity data indicated that certain of these compounds should be considered for further assessment.

Table 2: Summary of compounds identified

Substance	CAS No.	OSPAR listing category: Priority action	Possible concern	No listing	Function or use category	Persistent (t½)	Bioaccumulative (log K_{ow})	Toxic	Source
Nonylphenol	104-40-5	✓	✓		Phenol				Surfactant metabolite
Cinnarizine	298-57-7			✓	Drug	?	?	In vitro ER potency*	Drug
Cholesta-4,6-dien-3-one	566-93-8			✓	Cholesterol oxidation product	?	?	In vitro ER potency*	Human waste
Biphenyl	92-52-4	✓¹			PAH				
Petrogenic/ pyrogenic									
Napthalene	91-20-3	✓¹			PAH				
1-Methylnaphthalene	90-12-0	✓¹			PAH				
1,6-Dimethylnaphthalene	575-43-9	✓¹			PAH				
1,8-Dimethylnaphthalene	569-41-5	✓¹			PAH				
1,4-Dimethylnaphthalene	571-58-4	✓¹			PAH				
1,2-Dimethylnaphthalene	573-98-8	✓¹			PAH				
1,3,6-Trimethylnaphthalene	3031-08-1	✓¹			PAH				
Acenaphthylene	208-96-8	✓¹	✓¹		PAH				
Fluorene	86-73-7	✓¹			PAH				
9,9-Dimethyl-9H-fluorene	4569-45-3	✓¹			PAH				
9-Methylene-9-H-fluorene		✓¹			PAH				
Phenanthrene	85-01-8	✓¹	✓¹		PAH				
3-Methylphenanthrene	832-71-3	✓¹			PAH				
4-Methylphenanthrene	1576-69-8	✓¹			PAH				
2,7-Dimethylphenanthrene	832-64-4	✓¹			PAH				
2-Methylanthracene	613-12-7	✓¹			PAH				
Aromatic unresolved complex mixture (UCM)		✓¹			PAH				
Fluoranthene	206-44-0	✓¹	✓¹		PAH				
Pyrene	129-00-0	✓¹	✓¹		PAH				
1-methylpyrene	2381-21-7	✓¹			PAH				
4-Methylpyrene	3353-12-8	✓¹			PAH				
1,3-Dimethylpyrene	64401-21-4	✓¹			PAH				
11H-Benzo[a]fluorene	243-17-4	✓¹			PAH				
Benz[a]anthracene	56-55-3	✓¹	✓		PAH				
9-Methylbenz[a]anthracene	2381-31-9	✓¹			PAH				
5-Methylbenz[a]anthracene	2319-96-2	✓¹			PAH				
1-Methylchrysene	3351-28-8	✓¹			PAH				
2-Phenylphenanthrene	4325-77-3	✓¹			PAH				
2,2-Binaphthalene	612-78-2	✓¹			PAH				
Fluoranthene	206-44-0	✓¹	✓¹		PAH				
Benzo[b]fluoranthene	205-99-2	✓¹			PAH				
Benzo[k]fluoranthene	207-08-9	✓¹	✓¹		PAH				
Benzo[a]pyrene	50-32-8	✓¹	✓¹		PAH				
Benzo[e]pyrene	192-97-2	✓¹	✓¹		PAH				
Perylene	198-55-0	✓¹	✓¹		PAH				
Dibenzo-1,2,7,8-anthracene	224-41-9	✓¹	✓¹		PAH				
Benzo[b]chrysene	214-17-5	✓¹			PAH				
Indeno[1,2,3-cd]pyrene	193-39-5	✓¹			PAH				
Benzophenone	119-61-9			✓	PAKs	26 d*	3.18*	Mutagen†	
Pyrogenic									
Anthrone	90-44-8			✓	PAKs	65 d*	3.66*	Mutagen†	
9,10-Anthracenedione	84-65-1			✓	PAKs	10 d*	3.39*	Mutagen†	
7H-Benz[de]anthracen-7-one	82-05-3		✓¹		PAKs				
9-Fluorenone	486-25-9			✓	PAKs	?	3.58*	Mutagen†	
2H-Phenanthro[9,10-b]pyran	217-67-4			✓	Oxy-PAH	?	?	Sus mut*	
Benzo[k]xanthene	203-29-7			✓	Oxy-PAH	?	?	Sus mut*	
2,4-Dinitroaniline	97-02-9			✓	Nitro-PAH	?	1.84*	Mutagen	Industrial intermediate
Diethyldinitroaniline				✓	Nitro-PAH	?	?	Sus mut*	
2-methylcarbazole	3652-91-3			✓	Nitro-PAH	?	?	Mutagen†	
Petrogenic									
4-Methylcarbazole	3770-48-7			✓	Nitro-PAH	?	?	Mutagen†	
Methylcarbazole	27323-29-1			✓	Nitro-PAH	?	?	Mutagen†	
1,3-Dimethylcarbazole	26542-39-7			✓	Nitro-PAH	?	?	Sus mut*	
3,6-Dimethylcarbazole				✓	Nitro-PAH	?	?	Sus mut*	
2,7-Dimethylcarbazole				✓	Nitro-PAH	?	?	Sus mut*	
1,5-Dimethylcarbazole				✓	Nitro-PAH	?	?	Sus mut*	
Trimethylcarbazole	64944-51-8			✓	Nitro-PAH	?	?	Sus mut*	
4H-Benzo[def]carbazole	203-65-6			✓	Nitro-PAH	?	?	Sus mut*	
Benzo[b]carbazole	243-28-7			✓	Nitro-PAH	?	?	Mutagen†	
Methylbenzo[c]carbazole	64839-54-7			✓	Nitro-PAH	?	?	Sus mut*	
1-Amino-9,10-anthracenedione	82-45-1			✓	Nitro-PAH	?	?	Mutagen†	
Triclosan	3380-34-5			✓	PCP	?	4.76	Mutagen†	PCPs
Chlorophene	120-32-1			✓	PCP	<1*	3.64	Mutagen†	Disinfectant
Dibenzo-p-dioxins		✓			Dioxins				Pyrogenic
Dibenzofurans		✓			Furans				Pyrogenic
Unknown toxicants				✓	?			Toxic	
Unknown in vitro ER agonists				✓	?	?	?	In vitro ER potency	?
Unknown in vitro mutagens				✓	?	?	?	Mutagen†	?
Unknown in vitro AhR agonists				✓	?	?	?	In vitro AhR potency	?

✓¹ Included under the general heading of polycyclic aromatic hydrocarbons (PAHs). *In vitro oestrogenic potency = 3.3×10⁻⁸ when compared to E2. *In vitro oestrogenic potency = 1.5×10⁻⁶ when compared to E2. *Health and Safety Database and references therein. *RTECS. *Found in S9 mediated mutagenic fraction in this study. †Mutagenic when tested using the S9 mediated Mutatox assay. *Vallo & Salkinoja-Salonen, 1986. PCP: personal care product. Vallo, R. & Salkinoja-Salonen, M.L. (1986) J. Gen. Appl. Microbiol. 32, 505-517.

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