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Biological Effects of Contaminants in Marine Pelagic Ecosystems
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IN VITRO ECOTOXICOLOGICAL ASSESSMENT OF PELAGIC ECOSYSTEMS

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ABSTRACT

The ICES biological effects monitoring in pelagic ecosystems (BECPELAG) workshop is a multi-national, multi-disciplinary workshop aimed at establishing suitable techniques for monitoring the effects of contaminants on pelagic ecosystems. One of the many activities that have been performed concurrently is the extraction of water samples using semi-permeable membrane devices (SPMDs) and large volume solid phase extraction (SPE) followed by *in vitro* testing and targeted chemical analysis of the concentrated extracts. The following *in vitro* assays were used: DR-CALUX, yeast oestrogen and androgen screen (YES & YAS), blue mussel (*Mytilus edulis*) embryo, *Tisbe battagliai*, and *Skeletonema costatum*. Data from the study are presented along with recommendations on procedures for the use of *in vitro* in the monitoring of the pelagic environment.

Keywords: *In vitro* bioassays, solid phase extraction, contaminant concentration, semi-permeable membrane devices, produced water.

INTRODUCTION

High quality data on the health of marine pelagic ecosystems are required for the successful environmental management of any activity that has the potential to have an adverse effect. The ICES biological effects monitoring in pelagic ecosystems (BECPELAG) workshop is a multi-national, multi-discipline workshop aimed at establishing suitable techniques for monitoring the effects of contaminants on pelagic ecosystems. The main objective of the workshop is to assess the ability of selected methods to detect biological effects of xenobiotics in pelagic systems. In addition, the results from the workshop will be used as a basis to suggest methods for future monitoring of biological effects in pelagic systems.

One of the many activities that have been performed concurrently is the extraction of water samples using semi-permeable membrane devices (SPMDs) and large volume solid phase extraction (SPE) followed by *in vitro* testing and targeted chemical analysis of the concentrated extracts. The following *in vitro* bioassays were used: DR-CALUX (aryl hydrocarbon receptor), yeast oestrogen and androgen screen (YES & YAS), blue mussel

(*Mytilus edulis*) embryo, *Tisbe battagliai*, and *Skeletonema costatum*. All these assays are suitable for the testing of concentrated extracts since they require low sample volumes (< 20 ml), need minimal space and equipment for testing, are sensitive and have easily determined end-points.

In this paper we describe the sampling of produced and surface water samples from the North Sea, their extraction and subsequent testing using the *in vitro* bioassays described above.

METHODS

Sample locations

During the workshop eight sample locations were used along two transects; one in the German Bight and one in the Statfjord area within the Norwegian sector of the North Sea (Table 1).

Water Sampling

Water samples (50 L) were collected in an alloy churn sampler designed to take discrete water samples at a selected depth (1 m for GB and 10 m for the Statfjord area). Samples were collected from both areas during June 2001. SPMDs were placed at the same locations for 6 weeks during the same period.

Solid phase extraction (SPE)

The general approach used for the extraction of water samples has been previously described (Thomas *et al*., 1999a; Thomas *et al*., 1999b). Typically, a 100 L water sample was forced, using compressed air, through a Teflon column packed with glass wool (previously rinsed with acetone and dried) and a octylsilane (C18; 5 g; IST, Hengoed, UK) and polystyrene-based polymer Isolute ENV+ solid phase extraction cartridges (1 g; IST, Hengoed, UK) connected in series. The SPE columns were then dried under vacuum, extracted using methanol (10 ml) and then dichloromethane (DCM; 10 ml), combined, reduced in volume to 5 ml in methanol and stored at -20ºC.

Semi Permeable Membrane Devices (SPMDs)

SPMDs were deployed and extracted as described by Huckins *et al*. (1995). Extracts in DCM were stored at -20ºC prior to testing.

Bioassays

A suite of small scale bioassays was employed using published methods: *Tisbe battagliai* (mortality; Thomas *et al*.,1999a), blue mussel (*Mytilus edulis*) embryo (larval formation; ASTM, 1993), *Skeletonema costatum* (growth inhibition; ISO, 1994), DR-CALUX (rat aryl hydrocarbon receptor, AhR; Murk *et al*., 1996), yeast oestrogen and androgen screen (YES & YAS, human oestrogen and androgen receptor, Routledge & Sumpter, 1996; Sohoni and Sumpter, 1998).

Chemical analyses

SPMD and SPE extracts were analysed for alkylphenols (APs) and polycyclic aromatic hydrocarbons (PAHs) by the Norwegian Institute for Marine Research (Bergen, NO).

RESULTS

Results from the bioassay testing of the SPE and SPMD extracts are presented in Table 2 and 3 respectively.

SPE extracts of surface waters
None of the surface water SPE extracts, even when concentrated x 10^3, were lethally toxic to the marine copepod *T. battagliai*. Only one SPE extract inhibited the growth of the diatom *S. costatum* (SF03), whilst blue mussel larval deformities were observed in extracts obtained from GB02, GB04, SF02, SF04 and UK01. These samples had to be concentrated between 268 and 793 times before abnormalities were observed in 50% of the organisms tested. With the exception of the sample collected at GB02 the presence of *in vitro* oestrogen receptor (ER) agonists were detected in all of the extracts tested (0.007-0.3 ng E2 L^{-1}). No response from *in vitro* androgen receptor (AR) agonists was determined in any of the extracts tested (< 0.01 ng DHT L^{-1}). With the exception of the sample collected at GB04 *in vitro* AhR agonists were determined in all of the surface water samples collected.

SPE extracts of produced water
A positive response was obtained in all the bioassays used to test produced water extracts apart for the YAS assay (*in vitro* AR agonists) which the response was below the limit of detection of the method (Table 2).

SPMD extracts
Only aryl hydrocarbon receptor (AhR) agonist activity was determined in the SPMD extracts tested (Table 3).

Chemical analysis
A summary of the targeted chemical analysis of PAHs and APs, as performed by IMR, NO, is presented in Table 4.

DISCUSSION

The concentration of contaminants in offshore environments is generally below the detection limits of standard bioassays. The extraction and concentration of organic contaminants allows the contaminant burden of a sample to be tested, and both spatial and temporal trends to be established. In this study, none of the surface water samples tested were toxic to *T. battagliai* and only a few inhibited *S. costatum* growth even when concentrated x 10^3. However, previous CEFAS studies in coastal and offshore areas have demonstrated that the *T. battagliai* survival and *S. costatum* growth inhibition tests are suitable for use in this context with temporal and spatial data obtained around the UK over the period 1999-2002 (CEFAS, Unpublished data). Testing the toxicity of the extracts to blue mussel embryos resulted in a response in all but one of the samples tested. This suggests that the combination of sample extraction and testing using bivalve embryos may be a promising tool for marine pelagic monitoring. Testing surface water extracts with the YES and YAS assays was successful in determining the *in vitro* ER, AR and AhR activities of offshore surface waters. Unsurprisingly, low levels of ER activity were measured, whilst the levels of AR activity were below that of the method. The *in vitro* AhR activity of the total SPE extracts were determined after 24h using the DR-CALUX assay and show measurable activities in all of the samples measured except the sample collected at GB04. Data from the YES and DR-CALUX assays will therefore allow an assessment of likely trends to be made when applied on a much broader scale.

Toxicity assessment of the produced water from two oil production platforms in the Northern North Sea allowed the toxic contribution from these effluents to be determined. Extraction and concentration of the produced water collected was necessary before effects could be measured using the assays deployed. The mussel embryo bioassay was the most sensitive of the acute bioassays used with samples collected from SFC demonstrating levels of toxicity of between 0.25 and 1 TU representing toxicity at concentrations observed in the original produced water. The most sensitive *in vitro* receptor based assay was for ER activity, followed by AhR activity, whilst the AR activity was below the detection limit of the method.

Since produced water contains a complex mixture of chemicals, it is not clear as to which chemicals are likely to be causing the effects measured. The most likely compounds to be responsible for ER and AhR activities are APs & PAHs respectively (Table 4). However, further confirmatory studies are necessary, such as the application of toxicity identification evaluation (TIE) procedures, since there is always the possibility of other unknown compounds contributing to the overall activity.

A limitation of the sampling design adopted for the SPE extraction as part of this study is that the samples only represent a 'snapshot' at the time of the sampling event. The SPMDs deployed for a six-week period at the same locations offer a time-integrated sample that can be tested using the same assays. From the bioassay data it appears that the SPMDs accumulated only AhR agonists during this period (Table 3). For all the benefits that integrated sampling SPMDs offer, further work is required to ensure that they accumulate the target compounds of choice (e.g. a system suitable for the accumulation of ER agonists) and that performance reference compounds (PRCs) are used in order to calculate the average concentration of accumulated contaminants in the environment that they are deployed. An *in situ* integrated approach to SPE may also yield benefits in this area.

CONCLUSIONS

- The combination of sample extraction and concentration using SPE/SPMDs with *in vitro* testing is a technique that is suitable for determining the ecotoxicological effects of marine pelagic ecosystems and point source discharges.
- *In vitro* ER and AhR agonist activity can be detected using these techniques in offshore surface waters.
- The produced waters sampled contain compounds that are toxic to blue mussel embryos.
- The produced waters sampled contain ER and AhR agonists.
- It is recommended that integrated SPE is combined with SPMDs in future applications of this approach.

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Table 1. Positions of stations used for ICES BECPELAG workshop

Station	Position (Lat Long)
GB01	54.083 °N, 7.833 °E
GB02	54.178 °N, 3 7.43°E
GB03	5.00 °N, 6.325 °E
GB04	55.38 °N, 4.50 °E
SF01	61,12,3 °N, 01,50,6 °E
SF02	61,11,8 °N, 01,52,1 °E
SF03	61,10,0 °N, 01,59,5 °E
SF04	60,07,0 °N, 03,03,0 °E

Table 2. Summary of the bioassay tests conducted on solid phase extraction (SPE) extracts as part of the BECPELAG workshop

Station	*Tisbe battagliai*		*Skeletonema costatum*		*Mytilus edulis*		*In vitro* ER agonist potency[2]	*In vitro* AR agonist potency[3]	*In vitro* AhR agonist potency[4]
	LC50	TU[1]	EC50	TU[1]	EC50	TU[1]	ng E2 L^{-1}	ng DHT L^{-1}	TCDD TEQ (pg L^{-1})
GB01	>1000	< 0.001	>1000	< 0.001	>1000	<0.001	0.007	<0.01	12 (1)
GB02	>1000	< 0.001	>1000	< 0.001	408	0.002	<0.0007	<0.01	9 (0.3)
GB04	>1000	< 0.001	>1000	< 0.001	730	0.001	0.01	<0.01	< 1
SF02	>1000	< 0.001	>1000	< 0.001	793	0.001	-	<0.01	30 (1)
SF03	>1000	< 0.001	288	0.003	-	-	0.07	<0.01	170 (6)
SF04	>1000	< 0.001	>1000	< 0.001	325	0.003	0.3	<0.01	13 (1)
SFC PW1	67	0.015	31	0.032	4	0.25	23	<0.01	630 (19)
SFC PW2	75	0.013	13	0.077	2	0.5	24	<0.01	34 (1)
SFC PW3	54	0.019	-	-	1	1	28	<0.01	88 (1)
UK 01	>1000	< 0.001	>1000	< 0.001	-	-	0.04	<0.01	135 (4)
UK PW 1	945	0.001	>1000	< 0.001	-	-	5	<0.1	65 (3)
UK PW 2	518	0.002	>1000	< 0.001	268	0.004	3	<0.1	38 (0.5)

[1] 1 TU (Toxic Unit) = 1/EC50 concentration factor. [2] Determined using the yeast oestrogen screen (YES) and expressed as the equivalent concentration of 17β-oestradiol to give the same response. [3] Determined using the yeast androgen screen (YAS) and expressed as the equivalent concentration of 5α-dihydrotestosterone to give the same response. [4] Determined using the DR-CALUX assay and expressed as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) TEQ.

Table 3. Bioassay test results conducted on the SPMD extracts as part of the BECPELAG workshop

Station	*In vitro* ER agonist potency (ng E2 Kg $lipid^{-1}$)[1]	*In vitro* AR agonist potency (ng DHT Kg $lipid^{-1}$)[2]	*In vitro* AhR agonist potency (pg TCDD TEQ mg $lipid^{-1}$)[3]
GB01	<149	<394	2
GB02	<168	<447	1
GB03	<89	<236	1
GB04	<172	<458	<0.4
SF01	<70	<188	4
SF04	<41	<108	0.5

[1] Determined using the yeast oestrogen screen (YES) and expressed as the equivalent concentration of 17β-oestradiol to give the same response. [2] Determined using the yeast androgen screen (YAS) and expressed as the equivalent concentration of 5α-dihydrotestosterone to give the same response. [4] Determined using the DR-CALUX assay and expressed as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) TEQ.

Table 4a. Alkylphenol concentrations present in the SPE samples collected as part of the BECPELAG workshop.

Station	Total sum of alkylphenols (μg L^{-1})									
	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	Total (C_{1-9})
GB01	1.2	2.8	1.9	0.9	0.7	0.2	0.0	0.3	0.4	8.4
SF02	0.7	0.8	0.7	0.3	0.2	0.1	0.0	0.1	0.2	3.0
SF03	0.7	0.9	0.5	0.2	0.1	0.0	0.3	0.1	0.0	2.8
SF04	1.2	2.1	2.1	0.9	0.8	0.2	0.3	0.2	0.3	8.2
SFC01	37.4	67.0	26.9	18.6	13.0	3.7	0.2	6.0	9.3	182.2
SFC02	68.6	1103.4	73.7	64.7	88.2	21.5	0.8	15.4	32.7	1468.9
SFC03	2.1	2.4	1.1	0.6	0.5	0.1	0.0	0.2	0.4	7.3

Table 4b. Polycyclic aromatic hydrocarbon concentrations in the SPE samples collected as part of the BECPELAG workshop

	Polycyclic aromatic hydrocarbons (μg L^{-1})													
	Naphthalene & Alkyl Na				Phenanthrene & Alkyl Phe				Dibenzothiophene & Alkyl Dthio				Flu	Pyr
Sample	N	C_1	C_2	C_3		C_1	C_2	C_3		C_1	C_2	C_3		
GB01	0.08	0.12	0.19	0.18	0.08	0.10	0.05	0.02	0.04	0.03	0.13	0.01	0.01	0.02
GB02	0.12	0.14	0.12	0.10	0.04	0.06	0.06	0.01	0.02	0.01	0.05	0.01	0.01	0.01
SF02	0.16	0.26	0.85	0.75	0.42	1.66	2.26	1.33	0.07	0.27	0.17	0.32	0.28	1.05
SF03	0.10	0.08	0.10	0.01	0.05	0.07	0.01	0.01	0.02	0.02	0.02	0.00	0.00	0.00
SF04	0.15	0.31	1.01	0.37	0.45	1.60	1.98	1.12	0.08	0.25	0.23	0.29	0.30	1.00
SFC01	0.22	0.25	0.23	0.16	0.05	0.08	0.05	0.02	0.03	0.02	0.04	0.01	0.01	0.02
SFC02	252	81.5	59.4	20.5	5.27	12.6	10.5	3.02	2.19	4.29	1.60	0.17	0.40	0.73
SFC03	790	834	356	99.0	13.4	11.5	9.09	2.20	5.90	5.37	6.74	4.25	0.48	0.75

Na- napthalene, Phe- phenanthrene, Dthio- dibenzothiophene, Flu- fluoranthene, Pyr- pyrene.

Table 4b (cont.)

	Polycyclic aromatic hydrocarbons (μg L^{-1})												
Sample	Ay	Ace	Flu	Anth	BaA	Chr	BbjkF	BeP	BaP	Per	IdP	DbA	Bghi
GB01	-	-	0.04	0.01	-	-	-	-	-	-	-	-	-
GB02	-	-	0.01	-	-	-	-	-	-	-	-	-	-
SF02	0.01	0.01	0.12	0.01	0.02	0.18	0.06	0.20	0.11	0.01	0.22	0.02	
SF03	-	-	-	-	-	-	-	-	-	-	-	-	-
SF04	0.01	0.01	0.15	0.01	0.01	0.18	0.06	0.20	0.12	0.01	0.34	0.01	
SFC01	-	-	0.03	-	-	-	-	-	-	-	-	-	-
SFC02	0.85	0.19	3.07	0.04	0.01	0.13	0.01	0.01	-	-	0.01	-	0.01
SFC03	1.81	0.69	8.15	0.08	0.01	0.13	-	0.01	-	-	-	-	-

Ay- acenaphthylene, Ace- acenaphthene, Flu- fluorene, Anth- anthracene, BaA- benzo[*a*]anthracene, Chr- chrysene/triphenylene, BbjkF- benzo[*bjk*]fluoranthene, BeP- benzo[*e*]pyrene, BaP- benzo[*a*]pyrene, Per- perylene, IdP- indeno[1,2,3-*cd*]pyrene, DbA- dibenz[*a,h*]anthracene, Bghi- benzo[*ghi*]perylene.