CHARACTERISATION AND OCCURRENCE OF *in vitro* OESTROGEN RECEPTOR AGONISTS IN PRODUCED WATER DISCHARGES

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Introduction

Studies on reproductive endocrine disruption in the aquatic environment have to date been predominantly concerned with the effects of oestrogenic substances in rivers and estuaries. Until recently, the possible release of oestrogenic substances from offshore activities had received very little attention, however reports have suggested that discharges from offshore production platforms may contain oestrogen receptor (ER) agonists [1-2]. The total North Sea discharge of produced water has been estimated at 340×10^6 m^3 yr^{-1} [3] and will increase as North Sea wells get older [4,5]. The purpose of this study was to characterise and determine the occurrence of *in vitro* ER agonists in offshore produced water discharges.

Methods

ER agonists were characterised by using bioassay-directed fractionation techniques. Produced water samples (200 Litres) were collected and extracted *in situ* on two North Sea platforms. The sample was forced, using compressed air through a glass wool filter and the organic load extracted onto resin ENV+ and octadecylsilane SPE columns placed in series (Figure 1). The samples were then transported under dry ice to the CEFAS Burnham Laboratory where they were eluted with methanol and dichloromethane.

Figure 1: Large Volume Extraction Equipment

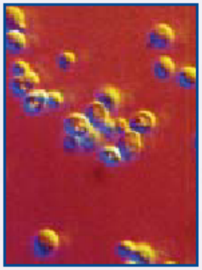


Figure 2: Yeast oestrogen screen

The samples were tested for oestrogenic activity using the yeast oestrogen screen (YES) (Figure 2), and then fractionated using a cyano-amino (PAC) bonded silica semi-preparative HPLC column fitted with a silica guard at a flow rate of 5 ml min^{-1}, using HPLC grade hexane, DCM and isopropanol as a mobile phase. Gradient elution was used over 30 min. Thirty 5 ml fine fractions were collected at one minute intervals. All HPLC fractions were tested in the YES assay and any fractions demonstrating ER agonist potency were analysed by gas chromatography-mass spectrometry (GC-MS) using the method of Thomas *et al.* [6]. Fractions were analysed using a Polaris Q ion trap mass spectrometer operated in full scan mode.

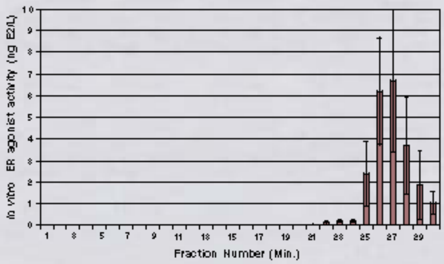


Figure 3: Oestrogen receptor agonist potency of produced water fractions

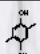
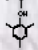
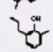
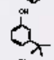
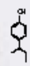
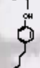
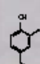
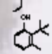
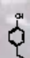
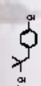
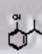
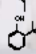
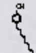
A further 81 produced water samples (2.5 L) were extracted and analysed using the method of Balaam and Thomas (in prep). Samples were passed through a 1 g C18 column and eluted with DCM. Samples were analysed for ER agonist potency and C_1-C_9 alkylphenol content.

Results and Discussion

ER agonist potencies of between 5 and 28 ng E2 L^{-1} were determined for the produced water samples collected. HPLC fractionation provided 30 fractions of which 10 tested positive for the presence of ER agonists (Figure 3). GC-MS analysis of these fractions identified a range of alkyl substituted phenols (Table 1).

Subsequent analysis of 81 produced water samples are shown in Table 2. ER agonist potencies ranged from below limits of detection to 91 ng E2 equivalents L^{-1} while total alkylphenol concentrations ranged from below limits of detection to 1600 µg L^{-1}.

Table 1: Compounds identified as potential ER agonists in produced water

Compound	Structure	Compound	Structure	Compound	Structure
2-methylphenol		2,4,5-trimethylphenol		2-methyl-5-(1-methylethyl)phenol	
4-methylphenol		2,4,6-trimethylphenol		2-methyl-6-propylphenol	
2,3-dimethylphenol		2-ethyl-5-methylphenol		3-(1,1-dimethylethyl)phenol	
2-ethylphenol		2-ethyl-6-methylphenol		4-(1-methylpropyl)phenol	
3,4-dimethylphenol		3,4,5-trimethylphenol		4-butylphenol	
3,5-dimethylphenol		4-ethyl-2-methylphenol		2-(1,1-dimethylethyl)-3-methyl-phenol	
4-ethylphenol		4-propylphenol		4-(1,1-dimethylpropyl)phenol	
2-(1-methylethyl)phenol		2-(1,1-dimethylethyl)phenol		4-(1,1,3,3-tetramethylbutyl)phenol	
2,3,5-trimethylphenol		2,3,5,6-tetramethylphenol		4-nonylphenol	
2,3,6-trimethylphenol		2-ethyl-4,5-dimethylphenol			

The samples contained isomers of C_1 to C_5 alkylphenols. These compounds are likely to be petrogenic in origin and are partitioning into the water phase during oil/water separation. Oil/water partition coefficients suggest that alkylphenols $>C_7$ are unlikely to partition into water. It is therefore unsurprising that only alkylphenols $<C_5$ were identified in these produced water samples.

If alkylphenols were exclusively responsible for the oestrogenic activity shown by the collected PW effluent samples, then it would be possible to correlate the two sets of data or components of the two sets. The correlation between oestrogenic activity and total alkylphenol concentrations is low. It can be concluded that either a few of the alkylphenol isomers are particularly active and are present at differing concentrations, or that there are other compounds present that are responsible for some of the activity.

Table 2: Summary of ER agonist potency and sum C_1 to C_5 alkylphenol concentrations

Platform	Oestrogenic Activity (ng E2 L^{-1})	GC-MS Analysis of C_1-C_5 alkylphenols (µg L^{-1})[a]					
		Sum C_1	Sum C_2	Sum C_3	Sum C_4	Sum C_5	Total
Balmoral	65	104	126	43	35	0.0	309
AH001	59	127	141	41	6	0.0	315
Andrew	60	98	156	41	4.0	0.0	299
Pierce	22	113	188	204	21	0.0	526
Janice	1	60	151	15	0.3	0.0	226
Ninian Central	35	124	84	35	0.0	0.0	243
Ninian North	91	65	626	174	10	0.0	876
Eider Alpha	50	48	356	196	99	0.0	700
Gannett A	28	81	92	31	0.0	0.0	204
Alwyn North	37	87	87	86	19	0.0	278

[a] C_6- C_9 <LOD (1 µg L^{-1})

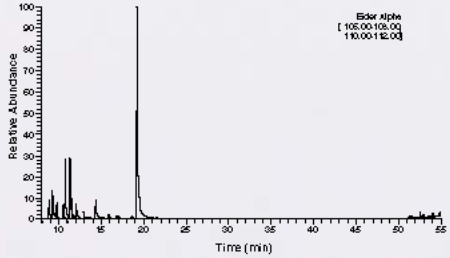


Figure 4: Gas Chromatogram of produced water extract

Conclusions

- The concentration of ER agonists in the sampled produced waters was equivalent to <LOD to 91 ng E2 L^{-1}.
- Bioassay-directed fractionation identified C_1 to C_5 and C_9 alkylphenols as responsible for the majority of the activity in one of the samples.
- Total alkylphenol (C_1 to C_5) concentrations of between <LOD and 1600 µg L^{-1} were determined in the samples collected.
- Little or no correlation exists between the ER agonist potency and total alkylphenol concentration suggesting that other compounds may be responsible for some of the activity.

References

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