IDENTIFICATION OF PERSISTENT, BIOACCUMULATIVE AND TOXIC (PBT) SUBSTANCES IN NORTH SEA PRODUCED WATER DISCHARGES

by J. L. Balaam, R. A. Dyer, W. J. Reynolds, P. H. Roberts and K. V. Thomas

CEFAS The Centre for Environment, Fisheries & Aquaculture Science

Introduction

Various OSPAR strategies require that monitoring data are available in order to establish the environmental impact of offshore oil and gas activities (e.g. Hazardous substances, Environmental Goals and Management Mechanisms for Offshore Activities). The largest discharge from these activities is produced water (PW). Knowing which compounds to monitor for is key when establishing/focusing any monitoring plan. The overall purpose of this project is to identify biologically active substances in PW in order to inform and focus monitoring plans and risk assessment procedures.

Bioassay-directed fractionation techniques were used to attempt to identify the biologically active compounds present in produced water discharges from offshore oil and gas installations. This study was performed on produced water samples from 20 installation outlets from the UK Continental Shelf. Installations were selected from the results of previous studies [1-3], based on the highest discharge volume, highest oestrogen and aryl hydrocarbon receptor agonist potency. Samples were tested using a suite of bioassays covering several levels of biological organisation:

YES assay (for estrogen receptor agonist potency);

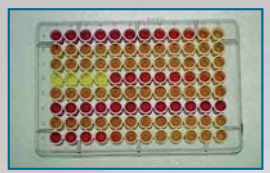
DR-CALUX assay (for aryl hydrocarbon receptor agonist potency);

Tisbe battagliai assay (for acute toxicity);

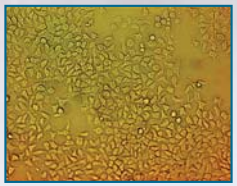
Skeletonema costatum assay (for algal growth);

and oyster (*Crassostrea gigas*) embryo bioassay (for larval development).

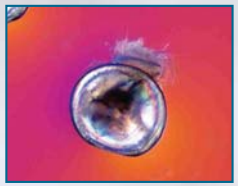
A bioassay directed fractionation approach was employed in order to elucidate the substances responsible for the observed effects. Several GC-MS systems were used to reveal unknown compounds. Several compounds have been tentatively identified.



Yeast plate



Calux cells



Oyster embryo

Skeletonema costatum

Tisbe battagliai

Phase 1 methods

Samples were collected in 30 l barrels. *In vivo* assays (*Tisbe battagliai*, *Skeletonema costatum* and Oyster Embryo Bioassay) were carried out on raw produced water samples. *In vitro* assays (YES and DR-CALUX) were carried out on concentrated organic extracts of produced water. Samples were extracted using large volume SPE extraction with C8 and ENV+ columns, and eluted with DCM and methanol.

Large volume extraction

Phase 1 – Bioassay results

Figures 1-3 Show results of Initial bioassay testing. Figure 1 shows the results of the *in vivo* bioassays. It is clear that the assays respond differently to the samples, with the oyster embryo bioassay being the most sensitive assay.

Figure 2 shows the response in the YES assay. Results range from <LOD to 91 ng E2 l^{-1}. Figure 3 shows the aryl hydrocarbon receptor response. The total response (which includes PAHs and PCBs), is measured in ng l^{-1} and ranges from <LOD to 417 ng TCDD l^{-1}, and the dioxin-like activity, measured in pg l^{-1}, ranged from <LOD to 1004 pg TCDD l^{-1}.

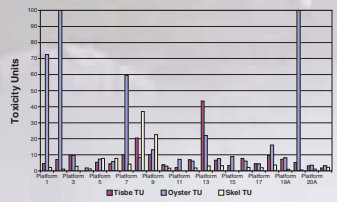


Figure 1

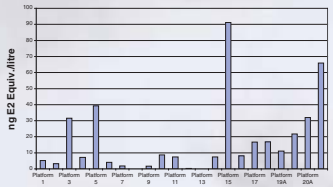


Figure 2

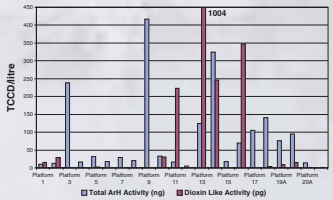
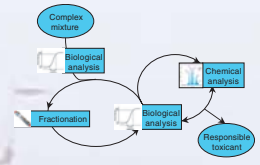


Figure 3

Phase 2 methods

The sample with the highest response in each assay was then investigated further, using a bioassay directed fractionation approach. These samples are shown in Table 1. Samples were fractionated using HPLC on a partisil PAC column, using hexane, DCM and IPA on a gradient elution programme over 30 minutes.



Bioassay DIR Fractionation

HPLC

Table 1- Samples for further investigation

Effect/Bioassay	Samples for further investigation
Acute Toxicity (T. Battaglia)	Platform 13
Larval Development (OEB)	Platform 1 & 20
Oestrogenic Activity (YES)	Platform 15
Algal Growth (S. Costatum)	Platform 8
Total Aryl Hydrocarbon (CALUX)	Platform 9
Dioxin Like (CALUX)	Platform 13 & 16

Phase 2 – Results

None of the *in vivo* samples showed any toxic activity after extraction and fractionation. This would indicate the PBT organics are not responsible for the activity in these samples. Further Phase 1 type TIE investigations of these samples led to the conclusion that ionic imbalance was responsible for the observed toxicity.

The results of the fractionation of platform 15, which showed high oestrogenic activity, can be seen in Figure 4. All of the oestrogenic activity is found in fractions 22 to 29, the highest activity being in fraction 28. These fractions were analysed by GC(EI) -ion trap MS and by GCxGC-TOF MS. The GCxGC 3D chromatogram for fractions 22-28 (combined) can be seen in Figure 5.

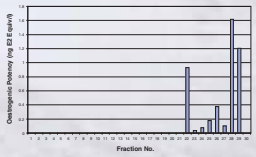


Figure 4

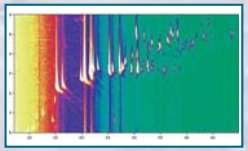


Figure 5

The results of the fractionation of platforms 9, 13 and 16, can be seen in Figure 6 A, B and C respectively. The total aryl hydrocarbon response can be seen from fraction 7 to 28, with the highest in fraction 19-21. The samples investigated for dioxin-like response had the majority of activity in fractions 7-9. These samples were analysed by GC(EI)-ion trap MS, GCxGC –TOF MS and GC(Cl)-MS. The GCxGC 3D chromatogram for platform 9 can be seen in Figure 7.

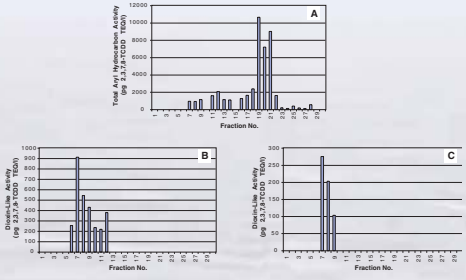


Figure 6

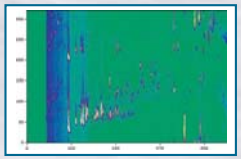


Figure 7

Compounds Identified

Activity	Compounds Responsible
Oestrogenic	Alkylphenols
Aryl Hydrocarbon (total)	PAHs, Carbazoles
Dioxin-like Activity	Chlorinated Naphthalenes, other chlorinated PAHs

Summary

- Produced Water Samples have been analysed in a battery of assays
- All samples showed activity in at least one assay
- The sample showing highest response in each assay was further investigated using bioassay directed fractionation
- *In vivo* toxicity was assigned to ionic imbalance
- Work is ongoing, using several GC-MS techniques, to identify substances causing observed *in vitro* oestrogenic and aryl hydrocarbon activity
- Once identified, PBT assessment of this data will be used to compile a list of priority substances.

References

1. Thomas, K.V., Balaam, J., Collins, K., Hurst, M., Reynolds, W., Thain J.E. (2003) In vitro ecotoxicological assessment of pelagic ecosystems. SETAC BECPELAG Publication.
2. Balaam, J.L., Thomas, K.V., Hurst, M.R., Thain J.E. Assessment of in vitro oestrogen receptor agonist potency and alkylphenol content of produced water discharges. SETAC Europe 13th Annual Meeting, 28th April - 1st May 2003, Hamburg, Germany
3. Balaam, J.L., Thomas, K.V., Thain J.E. Toxicity Identification Evaluation (TIE) of produced water discharges into the North Sea. SETAC Europe 14th Annual Meeting, 19th – 22nd April 2004, Prague, Czech Republic.

The Centre for Environment, Fisheries and Aquaculture Science (CEFAS), Burnham Laboratory, Remembrance Avenue, Burnham on Crouch, Essex CM0 8HA, UK.

http://www.cefas.co.uk.