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Characterisation of Persistent, Bioaccumulative and Toxic Chemicals in Produced Water Discharges

Final report for Defra, Dti, Shell and UKOOA

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A final report for Defra, Dti, Shell and UKOOA

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Characterisation of Persistent, Bioaccumulative and Toxic Chemicals in Produced Water Discharges

Final Report

Report No. C1738

Report Approved by

Name: Dave Sheahan

Position: Project Sponsor

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Executive Summary

The UK's commitment to OSPAR strategies such as Hazardous substances, Environmental Goals and Management Mechanisms for Offshore Activities require that monitoring data are available in order to establish the environmental impact of offshore oil and gas activities. The largest discharge from these activities is Produced Water (PW). In this study, toxicity identification evaluation (TIE) techniques were used to enable an initial hazard assessment by characterising the acute and chronically toxic substances present in 22 PW samples from 16 offshore oil and gas installations in the UK sector of the North Sea.

Initially, PW samples were screened using a battery of bioassay tests. Samples were tested for acute (lethal) toxicity using the *Tisbe battagliai* bioassay, sub-lethal toxicity to algal growth using the *Skeletonema costatum* bioassay, toxicity to larval development using the Oyster Embryo bioassay, *in vitro* oestrogen receptor (ER) agonists using the yeast oestrogen screen (YES) and *in vitro* aryl hydrocarbon receptor (AhR) agonists using the DR-CALUX® assay. Of the whole organism assays, acute toxicity was measured in 21 of the 22 samples analysed, toxicity to algal growth was measured in 19, and toxicity to oyster embryos occurred in all samples. Taking into account dilution of produced water only the results of the Oyster Embryo bioassay indicated that some toxicity may occur beyond 500 metres from just one of the platforms surveyed. ER agonists were detected in all samples. Total AhR agonists were detected in all samples, while dioxin-like activity was measured in 13 samples.

TIE techniques, using the above bioassays to direct the fractionation of the complex PW samples, were then applied in an attempt to identify the compounds responsible for the observed effects in selected samples. Ionic imbalance is likely to be responsible for some of the observed toxicity to *T. battagliai*, *S. costatum* and oyster embryos, although further techniques need to be applied to fully characterise this activity. In this instance, the toxicity could not be assigned to PBT organics. TIE techniques were successfully applied to identify PAHs and substituted PAHs, as well as other aromatic hydrocarbon compounds, as contributing to the AhR signal measured in selected PW samples. Alkyl substituted phenols were identified as contributing towards the *in vitro* ER agonist potency of PW extracts.

In this study the authors were able to assign 23 % of the oestrogenic activity and 4 % of the aryl hydrocarbon activity to specific compounds. Comparisons of identified substances with OSPAR lists of substances for priority action and possible concern show that only 2 compounds identified as part of this programme are included on any OSPAR lists. Little experimental data on bioaccumulation or persistence of these compounds was available, but predictive models indicate that 3 other compounds identified may be candidates for assessment.

None of the compounds identified could be matched to substances known to be used in the offshore industry. This may be due to lack of background spectral library data on offshore chemicals.

This study provides an indication that some components of produced water are toxic and/or have other biological activities. Studies on the UK coastal shelf indicate that produced water will be mixed and diluted to at least 1/1000th of the discharge concentration within 500 metres of the discharge point (Karman and Reerink, 1998). Therefore, based on the toxicity of the samples measured in this study, acute toxic effects are very unlikely to be observed outside of this mixing zone. Further work is required to identify compounds that contribute the majority of the sub-lethal biological effects measured and to fully characterise the risk of effects occurring in the vicinity of offshore discharges and therefore level of concern due to the biological activity identified in produced water.

Acknowledgements

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1 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.

The total North Sea discharge of PW has been estimated at 340×10^6 m^3 yr^{-1}. The composition of PW is complex and typically contains small amounts of dispersed oil, dissolved organics (including hydrocarbons), organic acids, phenols, production chemicals and inorganic compounds. PW composition differs considerably between oil and gas production and between different production fields, but can also vary on a daily basis at any one location dependent upon different activities taking place on a platform or the wells tied back to that platform. PW also changes during the production life of an oil field: more water is produced from the formation as the oil is depleted and as a result of water re-injection to maintain formation pressure

The complex and variable composition of PW makes it difficult to assess which components have the potential to cause environmental harm. A conventional approach would be to determine the environmental risk by correlating toxicity and contaminant concentration within PW, to suggest causes of the observed effects. Although these methods are universally applied, they are severely limited; for instance, compounds causing toxicity may not be included in surveys of chemicals if they are reaction products formed from added treatment chemicals. In addition, concentrations of potentially toxic compounds may co-vary, and it may be difficult to assess the bioavailability of the contaminants measured or interactions may not be accounted for among potential toxicants (*e.g.* additivity). An approach that overcomes these limitations is bioassay-directed fractionation, also termed toxicity identification evaluation (TIE). TIE techniques are now widely applied and use the response from a bioassay to direct the simplification of

complex environmental mixtures to less complex extracts (i.e. by chromatographic techniques). The cause of toxicity can then be identified by broad-spectrum chemical analysis (using gas chromatography-mass spectrometry and liquid chromatography-mass spectrometry). The outcome of the procedure is a list of identified compounds with a demonstrated biological effect for each sample analysed. Following previous characterisation in the Norwegian and UK continental shelf (UKCS) of the North Sea (Thomas *et al*, 2004a,b), this study was performed on produced water samples from 16 installations from the UKCS. Installations were selected from the results of previous studies, based on the highest discharge volume and highest oestrogen and aryl hydrocarbon receptor agonist potency.

The range of bioassays that can be used with TIE techniques is also considerable and can be tailored to identify effects of interest and/or target particular groups of compounds. The assays selected for this study and their endpoints were:

I. Sub-lethal toxicity to bivalve embryos (larval development)
II. Sub-lethal toxicity to micro-algae (growth)
III. Acute toxicity to marine copepod (mortality)
IV. *in vitro* oestrogenic activity (Oestrogen receptor binding)
V. *in vitro* arylhydrocarbon (AhR) receptor activity (AhR receptor binding).

The above assays were selected for a number of reasons. Firstly, the whole organism bioassays integrate the effects of all compounds in complex mixtures (and their breakdown products). Furthermore, the 3 bioassay organisms selected are from different phyla and represent different levels of biological organisation. This provides a range of responses and helps to identify a range of potential compounds of interest. The receptor based assays target specific groups of compounds within PW that have demonstrable effects in the environment.

2 OBJECTIVES

I. Collect composite PW samples from up to ten UK production platforms and test using the battery of bioassays described above.

II. If activity is found using these screening assays then follow this up with TIE procedures to identify the causative agents.

III. Provide a list of potential chemicals of concern using the criteria persistent, bioaccumulative and toxic (PBT) to the offshore industry.

3 METHODS

Methods are described fully in appendix A. Briefly, samples were collected from selected oil and gas installations and shipped to the CEFAS Burnham on Crouch Laboratory, where they were assayed. For the whole organism tests, a logarithmic series of dilutions (including 100 % concentration) of PW samples were assayed using *Tisbe battagliai* (*T. battagliai*), *Skeletonema costatum* (*S. costatum*) and Oyster Embryo (OEB) Bioassays to derive EC_{50}'s and toxic units (TU). For the *in vitro* tests, samples were extracted using C8 and ENV+ solid phase extraction (SPE), and eluted with methanol and dichloromethane (DCM) prior to assay.

The most potent sample in each assay was taken for further investigation. These samples were fractionated using normal phase HPLC, and 30 fractions collected. These fractions were retested in the appropriate assay, giving a profile of activity by fraction number. For the *T. battagliai, S. costatum* and OEB Bioassays, none of the fractions were active after extraction and fractionation, and therefore a phase I type approach was used for these samples (see appendix A, section 1.2). This approach attempts to identify groups of compounds with similar physico-chemical properties contributing towards toxicity, by establishing in which compartment the toxicity resides. This is done by performing a number of manipulations such as filtration, aeration and EDTA addition. Samples are tested following each manipulation to establish whether toxicity is reduced.

Positive fractions in the YES and DR-CALUX® assays were analysed by a variety of analytical techniques (see appendix A, section 6.5) in order to identify causative compounds. Spectra were initially compared to reference libraries, and a subsequent QSAR analysis was carried out on suggested compounds. Compounds which showed a high probabiltity of toxic action were purchased and assessed for potential toxic action.

These analytical standards were also used to quantify the amount of compound present in each fraction. Chemicals identified were then evaluated using PBT criteria to establish the environmental significance of the toxicity.

4 RESULTS

4.1 Toxicity Screening

Tables of full results can be seen in Appendix C

4.1.1 Produced Water toxicity to T. battagliai

Results of PW toxicity to *T. battagliai* are shown in Fig 4.1 and are quoted in Toxic Units (TU). TU are calculated by dividing 100 % by the EC_{50}. Of the twenty-two samples tested, 21 gave a positive response when using *T. battagliai*. The mean toxicity is 7.5 TU with a range of below detection limits (<1) at Platform 3 to 43.5 TU at Platform 11.

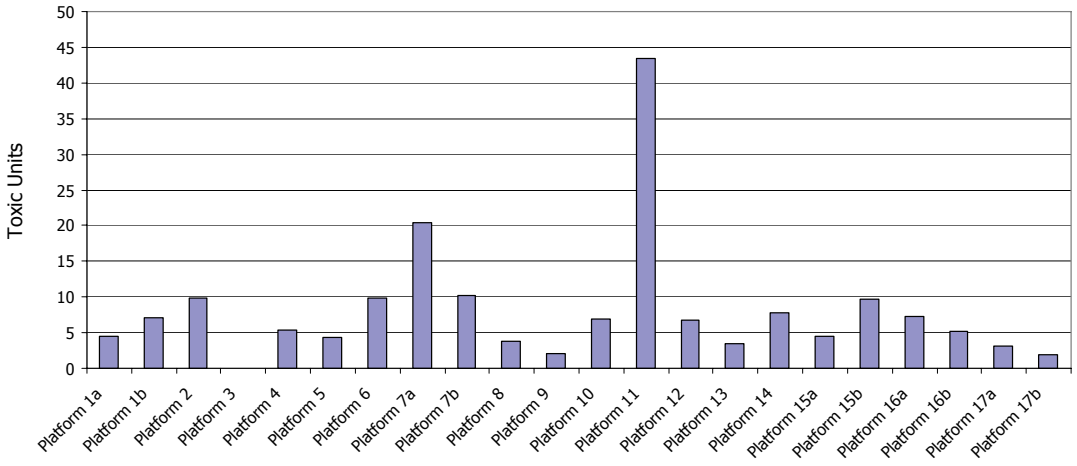


Figure 4.1 Produced Water Toxicity to *T. battagliai*

Due to a larger sample volume being obtained from the two Platform 15 discharge points, extraction of organics using C18 SPE columns was carried out. This reduces the toxicity of Platform 15b from 9.6 to 1.7 TU, while Platform 15a shows no toxicity to *T. battagliai* after extraction with C18. All of the toxicity to *T. battagliai* in the overboard sample can therefore be attributed to organic compounds, while some 17 % of toxicity remains after removal of organics from the Degasser sample.

4.1.2 Produced Water toxicity to oyster embryos

Results of PW toxicity to oyster embryos are shown in Fig 4.2. Of the twenty-two samples tested, all gave a positive response when using the oyster embryo bioassay. Toxicity ranged from 1.8 TU at Platform 3 to 13241 TU at Platform 16b, with a mean of 569 and a median of 7.6. Platform 16b samples a and b, which are from the same discharge point, show very different activity in this assay.

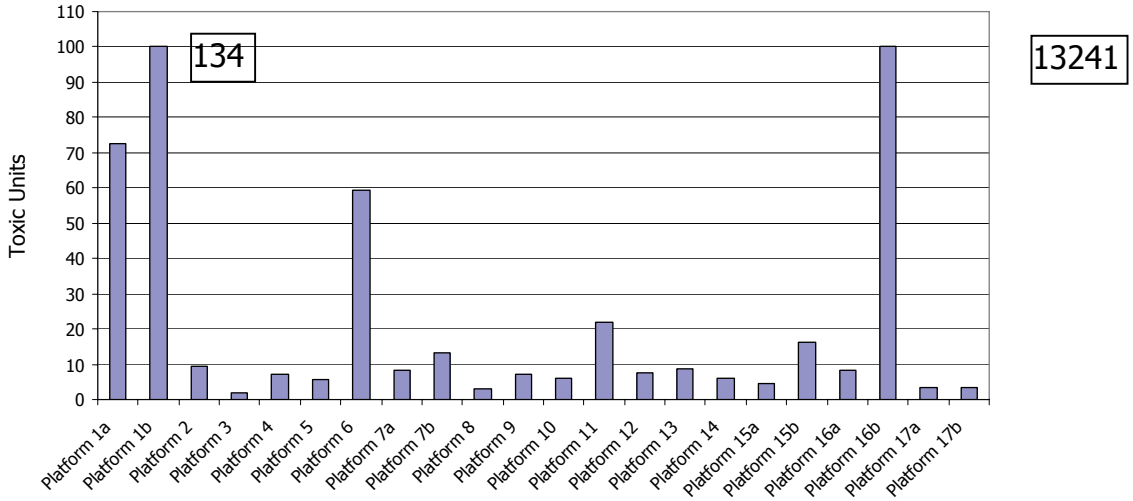


Figure 4.2 Produced Water Toxicity to Oyster Embryos

After extraction of organics using C18 SPE columns, approximately half of the toxicity is removed for both Platform 15b and Overboard samples. This differs from the result obtained in the *T. battagliai* assay and would suggest that the two assays respond to different organic compounds.

4.1.3 Produced Water toxicity to S. costatum

Results of PW toxicity to *S. costatum* are shown in Fig 4.3. Results are expressed as Toxicity Units (TU). Of the twenty-two samples tested, 19 gave a positive response when using *S. costatum*. The mean toxicity is 4.9 TU with a range of below detection limits (<1) to 37.1 TU at Platform 7b. No result could be obtained from the data from Platform 15a post C18 sample.

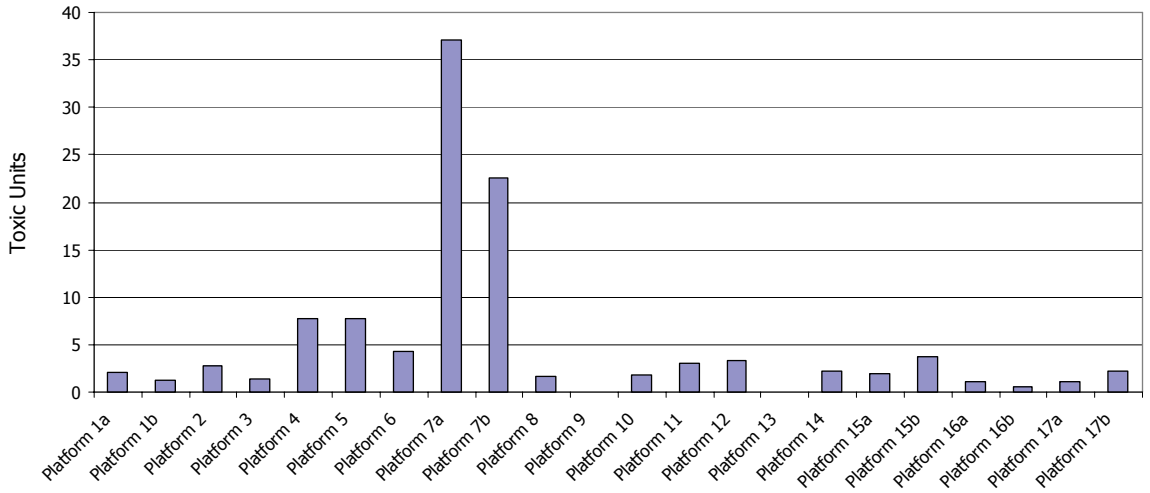


Figure 4.3 Produced Water Toxicity to *S. costatum*

4.1.4 Produced Water oestrogen receptor agonist potency

PW oestrogen receptor agonist potencies are shown in Fig 4.4. These potencies are calculated as 17ß-oestradiol (E2) equivalents as determined by the yeast oestrogen screen (YES). Of the twenty-two samples tested for oestrogenic activity, all gave a positive response. The oestrogenic potency ranged from 0.01 to 91.17 ng E2 equivalents l^{-1} with a mean of 2.3 ng E2 equivalents l^{-1}. The highest oestrogen receptor agonist potency was found in the sample collected from Platform 13, while the lowest was at Platform 11, although this sample was toxic to yeast at high concentrations, which may have masked potential oestrogenic effects.

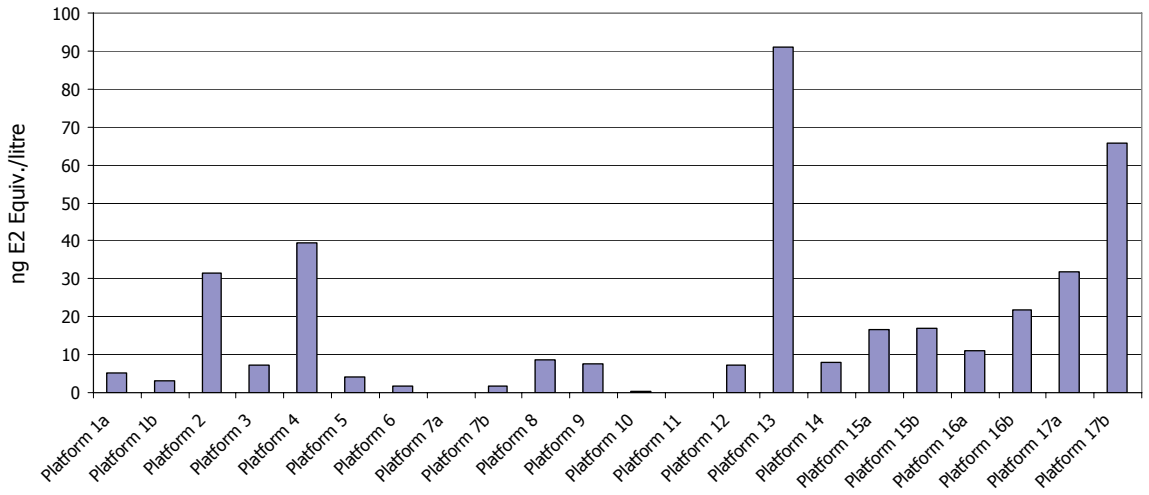


Figure 4.4 Produced Water Oestrogen Receptor Agonist Potency

4.1.5 Produced Water aryl hydrocarbon agonist potency

PW aryl hydrocarbon agonist potencies are shown in Fig 4.5. Potencies are calculated as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) equivalents as determined by the CALUX® assay. Of the twenty-two samples tested, all gave a positive response for total aryl hydrocarbon agonists, while 13 gave a positive response for dioxin-like activity (after clean-up). The highest total activity is seen in the samples taken from Platform 7a, while the highest dioxin-like activity is found in the sample from Platform 11, also the highest acutely toxic sample.

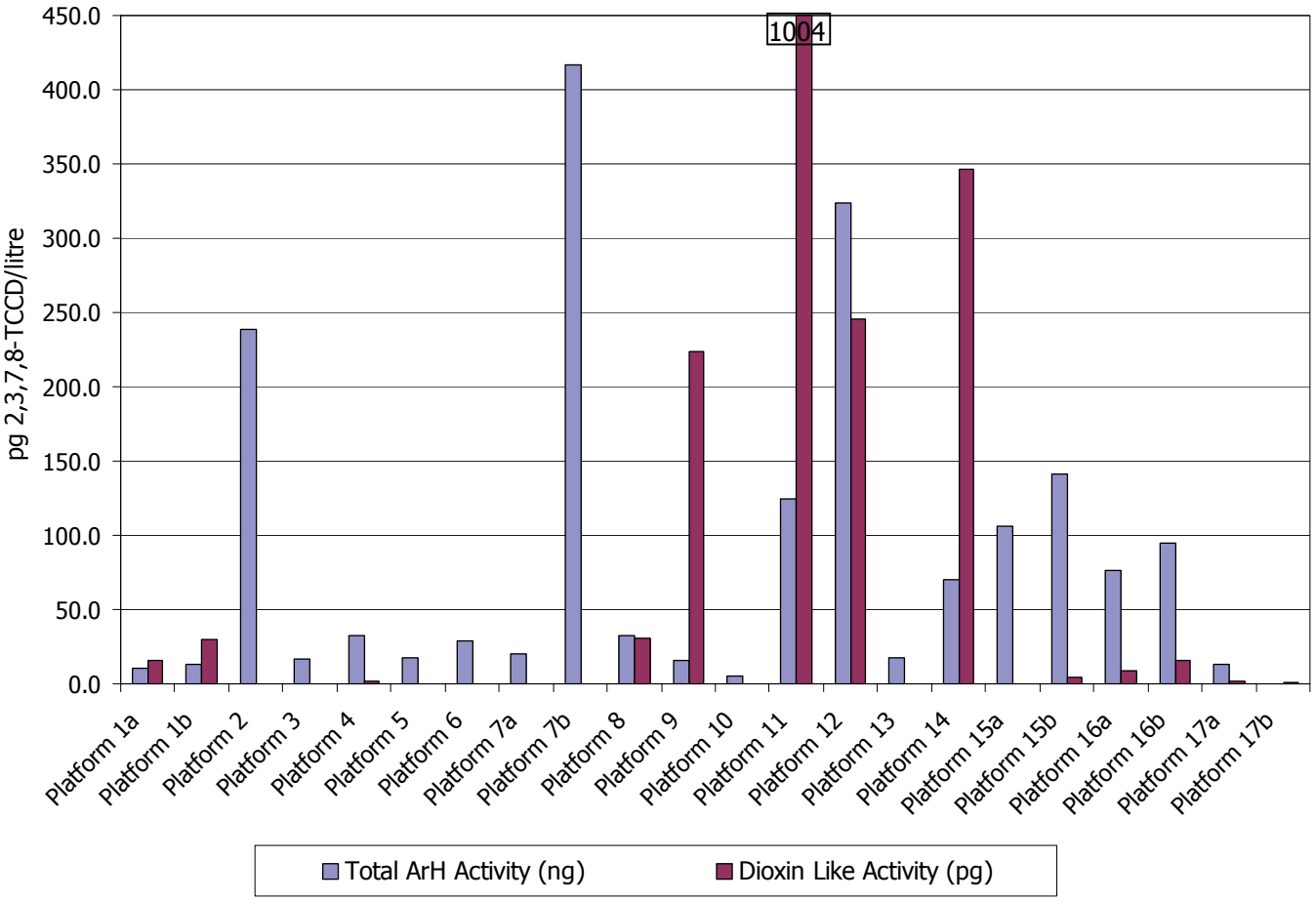


Figure 4.5 Produced Water Aryl hydrocarbon receptor agonist potency

4.2 Produced Water TIE

Since it was not possible to carry out TIE on all samples, the most potent sample in each assay was fractionated and retested in that assay, with positive samples subsequently being analysed using chemical techniques to elucidate compounds of interest. These samples are shown in table 4.1.

Table 4.1 Samples of interest for TIE studies

Effect/Bioassay	Sample(s) to be fractionated
Acute Toxicity (*T. Battagliai*)	Platform 11
Embryonic Development (OEB)	Platform 1b/Platform 16b
Algal Growth (*S. Costatum*)	Platform 7b
Oestrogenic Activity (YES)	Platform 13
Total Aryl Hydrocarbon (CALUX®)	Platform 7a
Dioxin Like (CALUX®)	Platform 11/Platform 14

4.2.1 Characterisation of in vivo toxicity to T. battagliai, oyster embryos and S. Costatum

The most toxic sample to *T. battagliai* was from Platform 11. This was therefore the sample chosen for investigation. Similarly, Platform 7b was the most toxic to *S. costatum*, and so was chosen for further investigation. The most toxic sample to oyster embryos was Platform 16b, although Platform 16a, taken from the same discharge point, was one of the least toxic samples. It was thought that the toxicity observed may be due to contamination in the barrel introduced during sampling, or some other outside influence such as the time interval between the two samples being taken. Platform 1b was the second most toxic sample analysed in this assay, and was thought more likely to return results associated with the PW than with other contaminants. For this reason, studies were not only carried out on Platform 16b, but also Platform 1b.

Samples, which had previously been tested as raw PW, were extracted by SPE, and fractionated using normal phase high performance liquid chromatography (NP HPLC). Following this procedure, none of the eluants showed any activity in the corresponding assay. This would indicate that the compounds of interest were not eluted from the SPE columns, and were therefore unlikely to be organic in origin. Despite the fact that Platform 15 samples has already been passed through an SPE column and shown to contain organic compounds, this was not chosen for further study as it did not show very high toxicity in the first phase of the project. It was considered that the samples which were

chosen were representative of the whole study and therefore these were the samples that manipulations were carried out on.

Further samples were obtained from Platform 1b, Platform 16b and Platform 11, and a phase 1 type TIE study was carried out in order to compartmentalise the activity. Samples were subjected to filtration, aeration, EDTA chelation, activated carbon addition, sodium thiosulphate addition, C18 SPE and cation exchange SPE. Following these manipulations, they were tested for acute toxicity use *T. battagliai* at only one concentration (100 %). Results are shown in table 4.2. Results are quoted in percentage survival of the *T. battagliai* in the test. Following all manipulations, samples were still toxic to 100 % of the *T. battagliai*, except for the Platform 16b sample following treatment with activated carbon.

Table 4.2 Survival rate of *T. battagliai* following phase 1 TIE manipulations

Manipulation	Removes	Survival (%)			
		Control	Platform 1b	Platform 16b	Platform 11
No manipulation		100	0	0	0
C18 SPE	Organics	93	0	0	0
CAX SPE	Metals	100	0	0	0
EDTA 1mg	Metals	100	0	0	0
EDTA 10mg	Metals	100	0	0	0
EDTA 100mg	Metals	93	0	0	0
$Na_2S_2O_3$	Oxidizers	100	0	0	0
Activated Carbon	Organics	100	0	100	0
Filtered	Particulates	100	0	0	0
N_2 Purged	Volatiles	93	0	0	0

Since these manipulations have very little effect on toxicity in these samples, it can be assumed that the toxicity in the samples which was causing the majority of the activity is not due to organics, metals or strong oxidisers. Further data on Platform 16b and Platform 11 suggests that the activity is not due to heavy metals, (Laurie, Pers. Comm.). Heavy metals were below limits of detection for almost all metals in both cases. The only exceptions were a small amount of Zinc at Platform 16b and Mercury at Platform 11.

Salinity is adjusted in these samples prior to assay, but other ion concentrations are not adjusted. It is well documented that ionic strength can have a profound effect on toxicity (Douglas and Grasso, 1996; Douglas and Horne, 1997; Ho and Caudle, 1997), and it is

thought that some of the toxicity observed in these samples may be due to ionic imbalance. For instance, Douglas and Horne (1996) found that there were toxic effects observed in *Mysidopsis bahia* at concentrations of Ca^{+}, K^{+}, Br^{+} and Mg^{2+} that were both above and below natural seawater concentrations. Since these ions concentrations will not be affected by the TIE manipulations used in this study (Ho and Caudle, 1997), they may occur at sufficient concentrations to cause the observed toxicity in certain samples. Further investigations into the exact ion concentrations could be carried out in the form of inductively coupled plasma mass spectrometry (ICP-MS) analysis, although this is a fairly costly technique and so not explored within the scope of this project. Following an ion scan, individual ion concentrations can be adjusted. If ion concentrations are too low, ions can be added. If too high, however, it becomes very difficult to adjust the concentration of any particular ion, so a weight of evidence approach is used. One of the methods used is elution of C18 SPE columns with organic solvents. These will be non-toxic in a situation of ionic imbalance, as shown in the SPE solvent extracts in the samples explored in this study. Since the purpose of this study was to determine persistent and bioaccumulative toxins (i.e. organics), this line of investigation was continued no further.

In the initial phase of the project, Platform 15 was shown to contain organic compounds, due to it's reduction in activity following SPE extraction. In the second phase of the project, the only sample to show a reduction in activity due to any of the manipulations carried out was Platform 16b, which showed 100 % survival of *T. Battagliai* following extraction with activated carbon. This would indicate that there were toxic organics present in the Platform 16b sample. However this sample was thought to be anomolous in some way, since a duplicate sample showed very little toxicity. No toxic organics could be eluted from the SPE columns following extraction, and therefore this activity could not be attributed to specific compounds.

4.2.2 Characterisation of oestrogen receptor agonists

The sample chosen for further investigation into oestrogenic potency was Platform 13. The sample was fractionated using NP HPLC and 30 fractions collected. Each fraction was subsequently tested in the yeast oestrogen screen, giving the profile shown in fig 4.6.

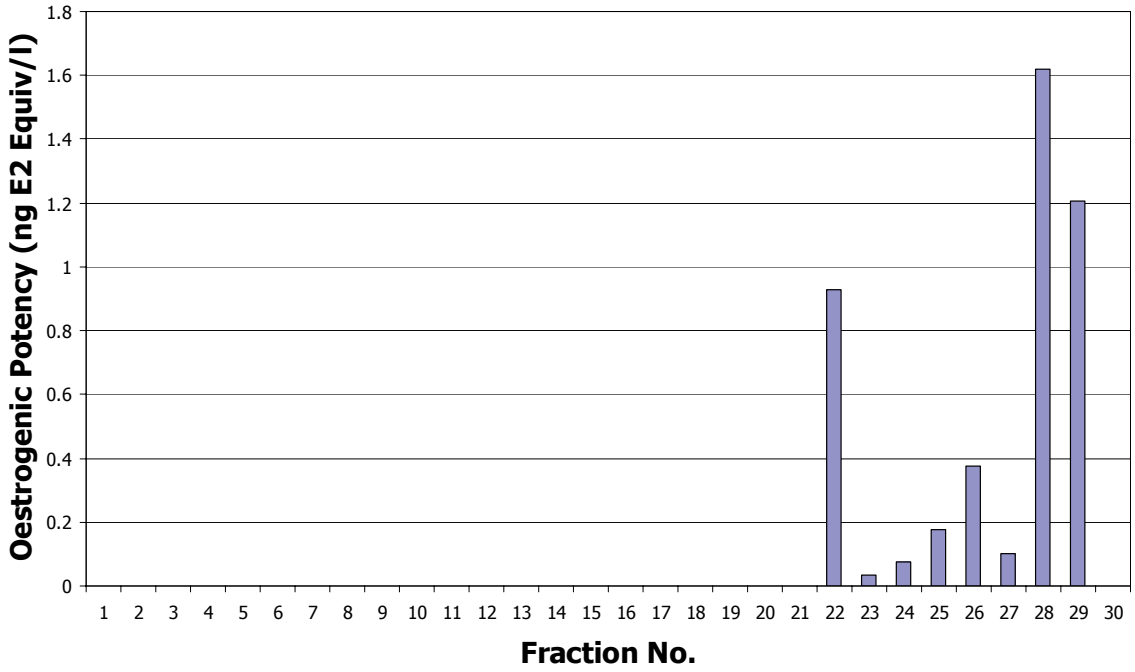
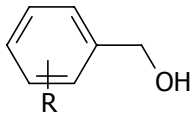
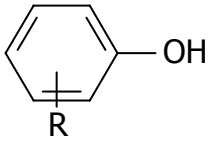
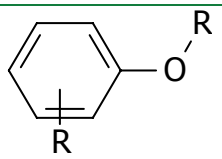
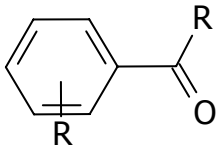
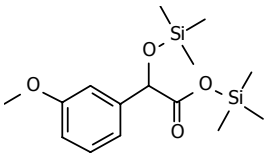
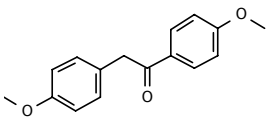
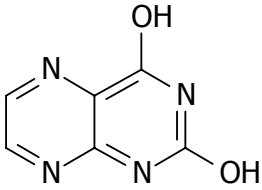
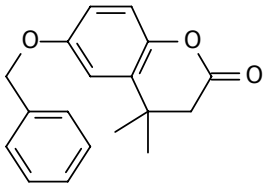
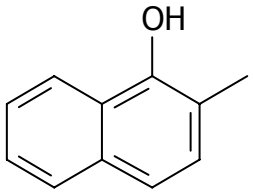
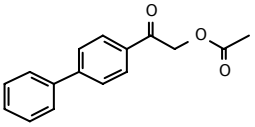


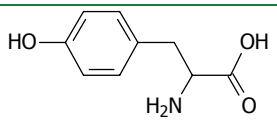
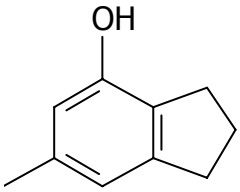
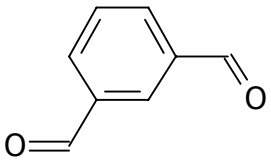
Figure 4.6 Oestrogenic potency of Platform 13 Fractions Following Normal Phase HPLC Fractionation

The activity in the Platform 13 sample is concentrated in fractions 22 – 29, with 22, 28 and 29 having the highest activity. These samples were analysed using GC-EI-MS and GC-TOF-MS. The positive fractions were combined and analysed using GC x GC-TOF-MS. Compounds of interest are listed in table 4.3. There are many alkylphenols, which have been identified previously as contributing towards oestrogenic activity of produced water samples (Thomas *et al.* 2004a,b). There are also a number of alkylated methoxybenzenes and other multi-ring compounds which have structures similar to those which are known to have oestrogenic activity. Most of the compounds identified are petrogenic in origin. The oestrogenic potency of all of these compounds could not be assessed within the scope of this project. A QSAR study was carried out on these substances of interest, in order to assess the likelihood of each compound to have oestrogenic activity, making it possible to potentially eliminate certain substances while focussing on those more likely to cause activity.

Table 4.3 Compounds identified as potential Oestrogen receptor agonists

CAS	Name	Class / Structure	Fraction
698-87-3	Benzeneethanol, à-methyl-	Alkylated benzene-	26,27
27129-87-9	Benzenemethanol, 3,5-dimethyl-	alcohol compounds	28
536-50-5	Benzenemethanol, à,4-dimethyl-		23
61967-11-1	Benzenemethanol, à-methyl-à-(1-methyl-2-propenyl)-		28
74685-13-5	Benzenemethanol, à-methyl-à-2,5,7-octatrienyl-		28
88-18-6	Phenol, 2-(1,1-dimethylethyl)-	Alkylated Phenols	26
88-69-7	Phenol, 2-(1-methylethyl)-		26,28,29
2631-40-5	Phenol, 2-(1-methylethyl)-, methylcarbamate		28
89-72-5	Phenol, 2-(1-methylpropyl)-		25,28
2416-94-6	Phenol, 2,3,6-trimethyl-		26,23
526-75-0	Phenol, 2,3-dimethyl-		26,28
496-78-6	Phenol, 2,4,5-trimethyl-		24
105-67-9	Phenol, 2,4-dimethyl-		26
90-00-6	Phenol, 2-ethyl-		23,25,27,28
1687-61-2	Phenol, 2-ethyl-5-methyl-		28
1687-64-5	Phenol, 2-ethyl-6-methyl-		26,28
95-48-7	Phenol, 2-methyl-		24
499-75-2	Phenol, 2-methyl-5-(1-methylethyl)-		26
618-45-1	Phenol, 3-(1-methylethyl)-		26,28
95-65-8	Phenol, 3,4-dimethyl-		23,26,29
1197-34-8	Phenol, 3,5-diethyl-		24
108-68-9	Phenol, 3,5-dimethyl-		24,27,28,29
620-17-7	Phenol, 3-ethyl-		28
698-71-5	Phenol, 3-ethyl-5-methyl-		26,28
108-39-4	Phenol, 3-methyl-		24,26,29
31143-55-2	Phenol, 3-methyl-6-propyl-		26
99-89-8	Phenol, 4-(1-methylethyl)-		26
99-71-8	Phenol, 4-(1-methylpropyl)-		28
1988-89-2	Phenol, 4-(1-phenylethyl)-		24,29
123-07-9	Phenol, 4-ethyl-		26,28,29
106-44-5	Phenol, 4-methyl-		23,26,27,29
14938-35-3	Phenol, 4-pentyl-		28
89-83-8	Thymol		26
645-56-7	Phenol, 4-propyl-		29
4074-46-8	4-Methyl-2-propylphenol		26
3520-52-3	2-Methyl-6-propylphenol		26
4013-37-0	Benzene, (1,2-dimethoxyethyl)-	Alkylated alkoxy	28
68705-86-2	Benzene, (1-methoxy-4-methyl-3-pentenyl)-	benzenes	28

CAS	Name	Class / Structure	Fraction
1515-95-3	Benzene, 1-ethyl-4-methoxy-		23
578-58-5	Benzene, 1-methoxy-2-methyl-		28
100-84-5	Benzene, 1-methoxy-3-methyl-		28
4132-48-3	Benzene, 1-methoxy-4-(1-methylethyl)-		23
104-46-1	Benzene, 1-methoxy-4-(1-propenyl)-		23
104-93-8	Benzene, 1-methoxy-4-methyl-		28
4685-47-6	3,4-Dimethylanisole		23
874-63-5	3,5-Dimethylanisole		28
59588-12-4	Benzene, (1-methoxypropyl)-		28
588-67-0	Benzene, (butoxymethyl)-		27
10519-06-9	p-Butoxytoluene		26
2040-07-5	Ethanone, 1-(2,4,5-trimethylphenyl)-	Alkyl phenyl ketones	23
89-74-7	Ethanone, 1-(2,4-dimethylphenyl)-		23
2893-05-2	2-Butanone, 3-methyl-1-phenyl-		27
6683-92-7	2-Pentanone, 1-phenyl-		27
37920-25-5	p-n-Butylacetophenone		23
55530-67-1	Benzeneacetic acid, 3-methoxy-à-[(trimethylsilyl)oxy]-		22
120-44-5	Desoxyanisoin		23
487-21-8	Lumazine		24
EPA-129261	Coumarin, 6-benzyloxy-3,4-dihydro-4,4-dimethyl-		24
7469-77-4	1-Naphthalenol, 2-methyl-		26
EPA-196488	Acetic acid, 2-oxo-2-(1,1'-biphenyl-4-yl)ethyl ester		27

CAS	Name	Class / Structure	Fraction
60-18-4	Tyrosine		28
20294-32-0	6-Methyl-4-indanol		29
626-19-7	Isophthalaldehyde		29

Of the 63 compounds tested in the ER QSAR, just one was considered to have high affinity (RBA > 0.1 %), 33 had medium affinity (0< RBA < 0.1 %), and 29 were not active. In order to enable early completion of the analysis a range of compounds were purchased for testing based on their similarity to compounds with known oestrogenic activity. Results were then compared with the QSAR.

4.2.2.1 Comparison of QSAR and Assay Data

Following QSAR, compounds were purchased and assayed to test actual activity. Results of QSAR analysis, and comparison with experimental data can be seen in Appendix C Tables 10.6 and 10.7 respectively.

There is little correlation between YES assay data and QSAR data for oestrogen receptor agonists. However, in all but one case, the inactive compounds in QSAR are also below limits of detection in the YES assay. For the medium affinity compounds, there is a range of activity, from below the limit of detection, to 1.19 x 10^{-3} E2 equivalents. This is not unreasonable, since the 'medium' affinity ranges from 0 to 0.1 %. The only anomalous result is for desoxyanision, which is the only compound to show activity above 0.1 % in the QSAR analysis, but shows no activity in the YES assay. The main conclusion to draw from these data is that the QSAR rarely gives false negatives, but may give some false positives.

4.2.2.2 Chemical Analysis

Chemical analysis was carried out on all positive fractions for which it was possible to obtain standards for the suggested compounds of interest. Results can be seen in Table 4.4. This table shows the results of the analysis of the Platform 13 fraction, along with oestrogenic potencies, and contribution to the overall activity observed. No data are shown if a compound was below the limit of detection in both the chemical analysis and the YES assay. Eight of the suggested compounds from the initial investigations are found both to be present in one or more fractions, and also to be active in the YES assay. None of the compounds identified are considered by OSPAR parameters to be persistent or bioaccumulative. These compounds contribute to approximately 35 % of the total activity observed. In some of the fractions more activity was observed in terms of compounds present than was originally observed in the YES assay. If these data are normalised to 100 %, then 23 % of the activity is observed. These data do not take into account any synergistic or antagonistic effects that may occur in a mixture such as produced water. Screening of a mixture of chemicals at the concentrations indicated in table 4.4 for Platform 13 fraction 27-29 showed no significant elevation in activity compared to that seen when testing individual compounds. It is unlikely, therefore, that the uncharacterised activity is accounted for by synergistic effects This indicates that there is 60 – 70 % of activity not accounted for. This may be due to the fact that those compounds that were not commercially available having high activity, although the QSAR data would suggest that this is not the case. What is more likely is that the compounds in question are not amenable to gas chromatography, as they are too polar, or that they are not present in chromatographic libraries since they are not widely used or known.

Table 4.4 Quantification of Platform 13 Fractions with oestrogenic potencies

Compound	Platform 13 Fractions (ng l^{-1})								E2 Equiv binding affinity	Contribution (E2 ng l^{-1})
	22	23	24	25	26	27	28	29		
2,3-dimethylphenol	**4327**	**9091**	**4575**	**99**	**90**	**<LOD**	**<LOD**	**<LOD**	**1.53E-05**	**0.28**
2,4,5-trimethylphenol	4134	2996	353	13	<LOD	<LOD	<LOD	<LOD	-	
2,4-dimethylphenol	<LOD	8355	9616	634	<LOD	<LOD	<LOD	<LOD	-	
2-Isopropylphenol	393	117	40	3	10	<LOD	<LOD	<LOD	-	
2-Methyl-6-propylphenol	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	9.82E-06	
2-methylphenol	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	123	10856		
2-sec-Butylphenol	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	8.46E-06	
2-tert-Butylphenol	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1.08E-05	
3,5-dimethylphenol	<LOD	<LOD	<LOD	<LOD	<LOD	6279	4964	8178		
3-Ethyl-5-methylphenol	2836	1159	122	3	<LOD	<LOD	<LOD	<LOD		
3-ethylphenol	**<LOD**	**<LOD**	**<LOD**	**<LOD**	**<LOD**	**3953**	**14730**	**<LOD**	**8.96E-06**	**0.17**
3-Isopropylphenol	**<LOD**	**<LOD**	**<LOD**	**3**	**3407**	**3777**	**<LOD**	**<LOD**	**6.61E-06**	**0.05**
3-metylphenol	<LOD	<LOD	<LOD	1345	669	23	167	<LOD		
4-butylacetaphenone	111	50	11	<LOD	<LOD	<LOD	<LOD	<LOD		
4-ethylanisole	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	9.43E-06	
4-ethylphenol	**<LOD**	**<LOD**	**<LOD**	**<LOD**	**<LOD**	**<LOD**	**<LOD**	**1487**	**1.71E-05**	**0.03**
4-Isopropylphenol	<LOD	<LOD	<LOD	<LOD	2240	2309	1600	1586		
4-pentylphenol	**<LOD**	**<LOD**	**<LOD**	**<LOD**	**<LOD**	**277**	**116**	**45**	**1.19E-03**	**0.52**
4-propylphenol	**<LOD**	**<LOD**	**<LOD**	**<LOD**	**<LOD**	**<LOD**	**442**	**753**	**3.32E-05**	**0.04**
4-sec-Butylphenol	**<LOD**	**<LOD**	**240**	**257**	**1880**	**2198**	**2678**	**2163**	**5.30E-05**	**0.50**
Thymol	1178	1923	283	<LOD	<LOD	<LOD	<LOD	<LOD		
									Total	1.58
E2 Equiv accounted for	0.07	0.14	0.08	0.02	0.12	0.51	0.43	0.22	1.58	
E2 Equiv from YES assay	0.93	0.03	0.08	0.18	0.38	0.10	1.62	1.21	4.51	
%	7.1	413	109	8.6	32	505	26	18	**35**	

4.2.3 Characterisation of aryl hydrocarbon receptor agonists

Platform 7a was the most potent aryl hydrocarbon receptor agonist, and so was further investigated using TIE techniques. Platform 11 was the most potent dioxin-like agonist, but is the only condensate installation in this study, and so was investigated along with Platform 14 to ensure that anomalous compounds solely associated with condensate installations were not mistakenly identified as problem compounds in all types of installation. Activity in each fraction is shown in fig 4.7. Samples from Platform 11 and Platform 14, which went through a clean-up procedure before fractionation, have aryl hydrocarbon activity concentrated in fractions 6-12 and 7-9 respectively. The Platform 7a sample also shows activity in the 7-9 fraction range, but also has many other positive peaks, the highest being fractions 19-21. The activity in the Platform 7a sample is expressed in ng TCDD l^{-1} whereas due to the much lower concentration present after clean-up in the Platform 11 and Platform 14 samples it is expressed in pg TCDD l^{-1}. All of these positive fractions were subjected to GC-IE-MS GC-NCI-MS, and GC-TOF-MS. Positive fractions were combined and analysed by GC x GC-TOF-MS. Compounds of interest are listed in table 4.4.

Many of the compounds identified as having the potential to bind to the aryl hydrocarbon receptor are PAHs which would be present in formation water. There are other compounds present with similar structures, which have not been documented as appearing in produced waters previously, such as anthraceneamine and clopidol. A QSAR study of these compounds in relation to the aryl hydrocarbon receptor was carried out in order to focus on the most likely agonists.

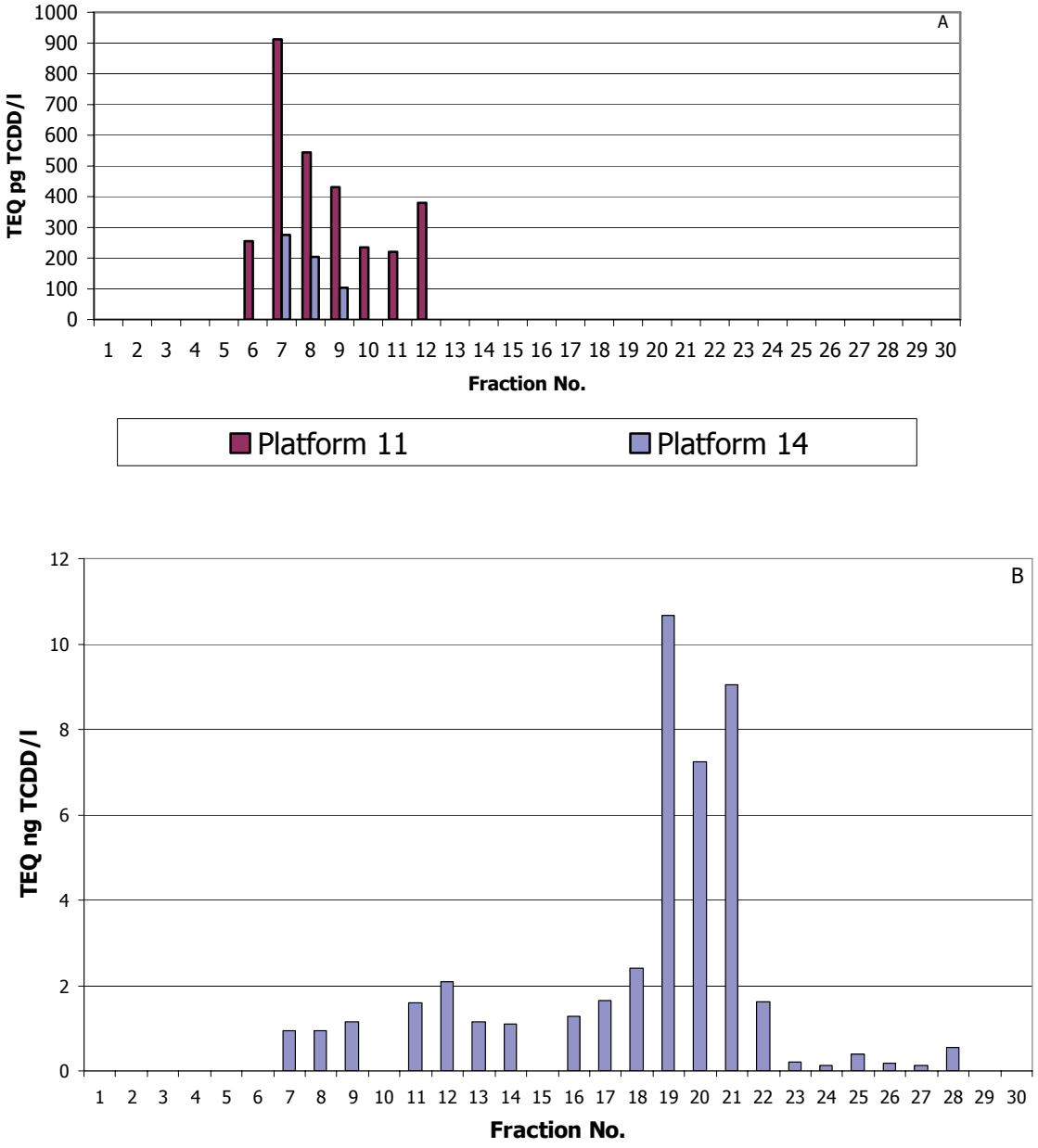
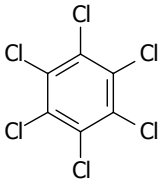
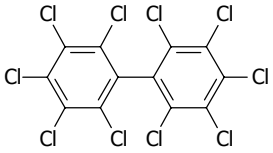
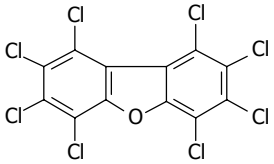
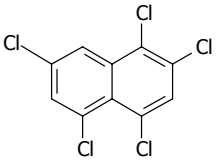
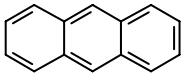
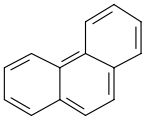
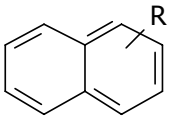
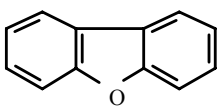
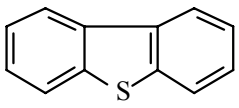
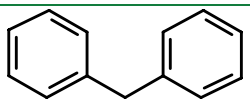
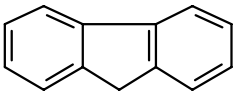
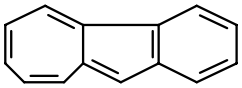
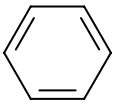
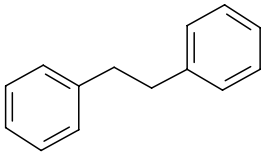
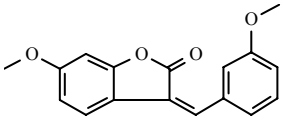
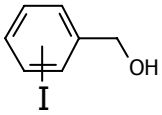
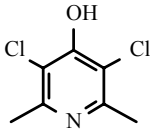
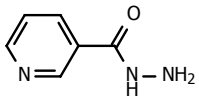
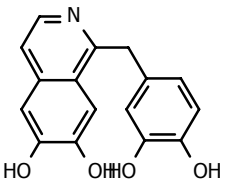
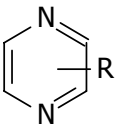
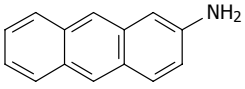
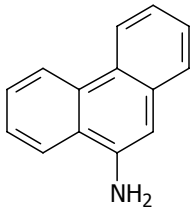
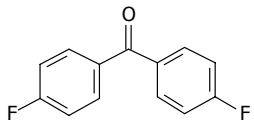
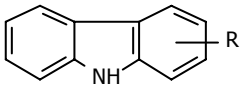


Figure 4.7 HPLC fractions, showing (A) dioxin-like response from Platform 11 and Platform 14 and (B) total aryl hydrocarbon response from Platform 7a

Table 4.5 Compounds identified as potential aryl hydrocarbon receptor agonists

CAS	Compound	Structure	Fraction No		
			Platform 7a	Platform 11	Platform 14
118-74-1	hexachlorobenzene				5
2051-24-3	Decachlorobiphenyl				5
39001-02-0	octachlorodibenzofuran				5
1321-64-8	Pentachloronaphthalene				5
120-12-7	Anthracene			6	
85-01-8	Phenanthrene			6,7	
91-20-3	Naphthalene			6	7
573-98-8	Naphthalene, 1,2-dimethyl-				6
569-41-5	Naphthalene, 1,8-dimethyl-				6
1127-76-0	Naphthalene, 1-ethyl-				6
827-54-3	Naphthalene, 2-ethenyl-				6
91-57-6	Naphthalene, 2-methyl-				6
90-12-0	Naphthalene, 1-methyl-				7
86-52-2	Naphthalene, 1-(chloromethyl)-				7
132-64-9	Dibenzofuran				7
132-65-0	Dibenzothiophene		7		

CAS	Compound	Structure	Fraction No		
			Platform 7a	Platform 11	Platform 14
101-81-5	Diphenylmethane			6	
86-73-7	Fluorene		7	6	
246-02-6	Benz[a]azulene		8	7	
71-43-2	Benzene		13,21	6,11,12	
103-29-7	Bibenzyl		11,21,23	8,9	7,8,9
77764-84-2	3',6-Dimethoxyaurone			11	
5159-41-1	Benzenemethanol, 2-iodo-			12	
57455-06-8	Benzenemethanol, 3-iodo-			12	
2971-90-6	Clopidol		12		
553-53-7	Nicotinic acid hydrazide		21		
574-77-6	Papaveroline		23		
290-37-9	Pyrazine		21		
4177-16-6	Pyrazine, ethenyl-		9		
613-13-8	2-Anthracenamine		13		

CAS	Compound	Structure	Fraction No		
			Platform 7a	Platform 11	Platform 14
947-73-9	9-Phenanthrenamine		13		
345-92-6	4,4-difluorobenzophenone		19,20		
86-74-8	Carbazole		19		
EPA-138137	Carbazole, 1,3,4-trimethyl-		16		
EPA-138124	Carbazole, 1,3-dimethyl-		18		
EPA-138125	Carbazole, 1,4-dimethyl-		18		
EPA-138126	Carbazole, 1,5-dimethyl-		18		
EPA-138133	Carbazole, 3,4-dimethyl-		18		
4630-20-0	3-Methylcarbazole		19		
3770-48-7	4-Methylcarbazole		18		
EPA-80557	N-Hydroxymethylcarbazole		20		

4.2.3.1 Comparison of QSAR and Assay Data

Aryl hydrocarbon QSAR analysis results and comparison with experimental data are shown in Appendix C (Tables 10.8 and 10.9 respectively). Only 5 of the 41 potential aryl hydrocarbon agonists were indicated by QSAR to have a high affinity for the aryl hydrocarbon receptor (defined as Log 1/EC50 >7), 17 were indicated as having medium affinity (4 < log 1/EC50 <7), and 19 to have low affinity (log 1/EC50 <4). A range of compounds across these affinities were purchased, in order to internally validate the QSAR against the DR-CALUX® assay, although concentrating on the more active compounds. Unfortunately, 2 of the 5 compounds indicated to be most active by QSAR could not be purchased, and so no assay or chemical analysis data are available for these.

The results of the comparison between QSAR and DR-CALUX® data for aryl hydrocarbon activity again shows that false positives can be assigned using QSAR analysis compared to the DR-CALUX® assay. There seems to be no correlation between the results for QSAR and DR-CALUX®.

In the sample from Platform 7a (Table 4.6), which was analysed for total aryl hydrocarbon activity, there are four compounds from the initial investigations which are present and are active in the DR-CALUX® assay These compounds are anthracene, 1-methyl naphthalene, dimethylnaphthalene and carbazole. These compounds only account for approximately 2.8 % of the observed activity. In the Platform 11 and Platform 14 fractions (Table 4.7), the only compounds which are present and also active are 1-methyl naphthalene and diemthyl naphthalene. Of the 4 compounds, anthracene appears on OSPAR lists. Anthracene and dimethylnaphthalene are considered to be bioaccumulative with log K_{ow}'s of 4.35 and 4.38 respectively. Anthracene is also considered to be bioaccumulative with a half life of approximately 58 days. These 4 compounds account for 3.8 and 1.9 % of the observed activity for Platform 11 and Platform 14 respectively. Similarly to the situation with ER agonists, there are compounds which are not readily available to purchase. In this case, 2 of the compounds showed a relatively high activity in the QSAR analysis and may contribute to the overall activity of the sample, although it is unlikely that they would represent 95 % of the activity. It may again be the case that the compounds causing this activity are not present in chromatographic libraries: Some compounds may be particularly associated with or produced by the unusual conditions in which produced water is created i.e high temperatures and pressures, with a mixture of man-made and naturally occurring compounds present in high concentrations.

Table 4.6 Quantification of Platform 7a Fractions with aryl hydrocarbon potencies

Compound	Platform 7a Fractions (ng l^{-1})												TEF	Contribution (TCDD ng l^{-1})
	7	8	9	11	12	13	16	18	19	20	21	23		
Decachlorobiphenyl													5.54×10^{-5}	
Naphthalene	247		5			2							-	
Phenanthrene	29		834										-	
Anthracene	**11**					**1**							**7.38×10^{-5}**	**0.0008**
2-methylnaphthalene	225		1			1							-	
2-ethenylnaphthalene	2												-	
1-ethylnaphthalene	29												-	
Dimethylnaphthalene	**28**												**1.14×10^{-3}**	**0.03**
1-methylnaphthalene	**139**		**2**			**2**							**5.19×10^{-5}**	**0.007**
Fluorene	821		2										-	
Dibenzothiophene	408		65										-	
9-Phenanthrenamine													7.22×10^{-3}	
2-Anthracenamine													2.61×10^{-3}	
Carbazole			**212**						**4130**	**2288**			**1.6×10^{-4}**	**1.035**
3-Methylcarbazole			1228						90347	3382			-	
2-Vinylpyrazine													3.9×10^{-5}	
TEQ accounted for	0.04	0.00	0.03	0.00	0.00	1×10^{-4}	0.00	0.00	0.64	0.36	0.00	0.00		**1.08**
TEQ in DR-CALUX® assay	0.95	0.94	1.15	1.60	2.09	1.14	1.29	2.40	10.67	7.23	9.05	0.21		**38.7**
%	4.19	0.00	2.88	0.00	0.00	0.01	0.00	0.00	6.04	4.94	0.00	0.00		**2.8**

Table 4.7 Quantification of Platform 11 and Platform 14 Fractions with aryl hydrocarbon potencies

Compound	Platform 11 Fractions (ng l^{-1})						TEF	Contribution (TCDD ng l^{-1})	Platform 14 Fractions(ng l^{-1})					TEF	Contribution (TCDD ng l^{-1})
	6	7	8	9	11	12			5	6	7	8	9		
hexachlorobenzene									0.03						
Decachlorobiphenyl							5.54E-05							5.54E-05	
octachlorodibenzofuran									1.20						
Naphthalene		514									823	29	13		
Phenanthrene													742		
Anthracene							7.38E-05							7.38E-05	
2-methylnaphthalene		658									429	6	2		
2-ethenylnaphthalene		6									4				
1-ethylnaphthalene		24									23				
Dimethylnaphthalene		**10**					**1.14E-03**	**0.01**			**14**			**1.14E-03**	**0.015**
1-methylnaphthalene		**210**					**5.19E-05**	**0.01**			**468**	**20**	**7**	**5.19E-05**	**0.025**
Fluorene		303									131	95			
Dibenzothiophene												155	95		
9-Phenanthrenamine							7.22E-03							7.22E-03	
2-Anthracenamine							2.61E-03							2.61E-03	
Carbazole							1.6E-04							1.6E-04	
2-Vinylpyrazine							3.9E-05							3.9E-05	
TEQ accounted for	0	0.02	0	0	0	0		**0.02**	0	0	0.04	0.001	0.0004		**0.04**
TEQ in DR-CALUX® assay		0.28	0.20	0.10				**0.58**		0.26	0.91	0.54	0.43		**2.14**
%		8.07	0	0				**3.8**		0	4.35	0.19	0.09		**1.9**

4.3 Assessment of Identified Substances

A full summary of the substances identified as present in PW collected from UK offshore installations is presented in Table 10.10 (Appendix C), with compounds of particular interest in Table 4.8.

All the substances identified have been cross-referenced with the OSPAR substances for priority action and substances of possible concern lists (OSPAR website). Under the OSPAR convention a compound is initially considered to be of increased concern when it meets the following criteria: toxic, persistent ($t_{½}$ > 50 days) and bioaccumulative (Log K_{OW} > 4 or BCF > 500). These criteria were applied to the compounds identified in this study that were shown to have toxic action in a particular assay by experiment or by QSAR (where analytical standards were not available). An initial assessment of available persistence and bioaccumulation data showed that experimental data could not be found for many of these compounds. Much of the persistence and bioaccumulation data presented in Appendix C Table 10.10 are therefore estimated using the US EPAs 'PBT profiler' (PBT profiler website), while the toxicity data is from the experimental data in this project. Table 4.8 shows those compounds of particular interest; there are 2 compounds which already appear on the OSPAR possible concern list, and 4 which are both bioaccumulative and toxic but not already listed by OSPAR.

The compounds in Appendix C Table 10.10 come under a number of function or use categories; drugs, organohalogen, phenols and personal care products (PCP). Many are used in the food, cosmetics and drug industries. A comparison with available data on chemicals used in the offshore industry did not give any positive results. The libraries used in this study for the analysis of spectra, however, may not contain spectra of chemicals used in the offshore industry. If this were the case, no offshore chemicals will be found in the samples when comparing to the library. In order to resolve this issue, it is recommended the fractions be analysed using high-resolution mass spectrometry (HR-MS), which gives exact formulae for compounds rather than using a library to suggest close matches. It would also be beneficial to analyse the samples by Liquid Chromatography mass spectrometry (LC-MS), since methods are currently being developed using this technique for the analysis of offshore chemical formulations.

Of the 5 compounds identified as being both bioaccumulative and toxic, all are aryl hydrocarbon receptor agonists; octachlorodibenzofuran, 2-Anthracenamine, 1,3,4-trimethyl Carbazole, anthracene and decachlorobiphenyl.

Table 4.8 Substances of possible concern

CAS	Substance	OSPAR Listing category	Sediment partition (%)	Persistent ($t_{½}$/d)[1]	Bioaccumulative (log Kow)[1]	Toxicity
2051-24-3	Decachlorobiphenyl	PC	49	579	8.27	AhR agonist
120-12-7	Anthracene	PC	8.5	73	4.45	AhR agonist
39001-02-0	octachlorodibenzofuran	-	50	1200	8.6	AhR agonist
EPA-138137	Carbazole, 1,3,4-trimethyl-	-	24	58	4.93	AhR agonist
613-13-8	2-Anthracenamine	-	12	64	4.3	AhR agonist

[1] Where experimental data is not available, predictions are taken from PBT predict. PA: Priority Action. PC: Possible Concern. – No OSPAR listing

Studies indicate that for platforms on the UK coastal shelf Produced water will be mixed and diluted to at least 1/1000th of the discharge concentration within 500 metres of the discharge point (Karman and Reerink, 1998). Taking the dilution factor into account only one sample tested with the most sensitive of the three species (oyster embryos), is likely to have >1 unit of toxicity beyond 500 metres of the platform (Platform 16b – 13 TU). Oestrogenic activity of produced water samples as measured in the yeast assay is likely in all cases to decrease to <0.1ng E2 equivalent/litre within 500 metres of the platform this is approximately 100 – 500 times less than many of the final effluents from sewage treatment works in the UK. Recent studies have measured changes indicative of exposure to oestrogenic chemicals in fish exposed to extracts of produced water (Tollefsen *et al*., 2006). However evidence from other work (Scott *et al*., 2006) indicates that contaminants from diffuse, possibly land-based sources may be making more significant contributions to oestrogenic activity in the marine environment and these need to be taken into account when interpreting data on produced water discharges. Based on a dilution factor of 1/1000, none of the platforms have total aryl hydrocarbon activity in produced water > 1 ng TCCD equivalent l^{-1} 500 metres from the platform and only one platform has an estimated dioxin-like activity > 1 pg TCCD l^{-1} at 500 metres from the platform. The concentration of the compounds measured in this study are therefore likely to be significantly reduced close to the discharging platform. However substances that contribute to any of the measured biological activity in produced water and which are persistent and can bioaccumulate in organisms or become associated with sediments are of concern as they may increase in

concentration with time. The predicted values for the percentage partitioning into the sediment for compounds identified in this study (PBT Profiler) are presented in Table 4.8. The values range from 8.5 to 50 %. The sediment in some cases can act as a source of contaminants to sediment dwelling organisms or compounds may be remobilised in the water column if the sediment is disturbed, long after discharge of that compound ceases. This may be particularly significant for those compounds such as octachlorodibenzofuran which have a long half life (1200 days), and also a relatively high sediment partition value (50 %). For this reason it would be beneficial to carry out targeted analysis or a similar TIE study on sediments from around offshore installations in order to establish whether the chemicals measured in produced water discharges become associated with sediments.

4.4 Results Summary

Whole organism bioassays

- Acute Toxicity to *T. battagliai* was observed in 21 of the 22 samples tested, although none are likely to result in > 1 unit of toxicity within 500 metres of a platform.
- Inhibition of growth in *S. costatum* was observed in 19 of the 22 samples, although none are likely to result in > 1 unit of toxicity within 500 metres of a platform.
- Toxicity to Larval development of oyster embryos was observed in all samples, although only one is likely to result in > 1 unit of toxicity within 500 metres of a platform..
- Platform 15 was the only sample which showed reduced toxicity after being passed through a C18 SPE column.
- SPE solvent extracts of PW were found to be non-toxic.
- Further investigation using type 1 TIE manipulations revealed that for the majority of the toxicity was not organic, metallic, or in the particulate or volatile compartments.
- Toxicity to whole organisms was not assigned to specific compounds. Ionic imbalance may play a role in the observed toxicity.

Oestrogen receptor agonists

- Oestrogenic response was observed in all samples tested, but none are likely to have activity >0.1 ng E2 equiv l^{-1} beyond 500 metres from the platform.
- Fractionation of the most highly oestrogenic sample led to eight positive fractions.
- Chemical analysis of these fractions led to tentative identification of 63 compounds.
- 63 compounds were tested in a QSAR for ER activity.
- 34 of these compounds were considered to be active by QSAR.
- 50 compounds were tested in the YES assay.
- 11 of these compounds were active in the YES assay.
- Correlation between YES and QSAR data was limited.
- 8 active compounds were present in the fractions from Platform 13.
- 23 % of the observed activity was assigned to these compounds.
- None of the compounds identified are considered to be bioaccumulative or persistent.

Aryl hydrocarbon receptor agonists

- Total Aryl hydrocarbon receptor activity was observed in all samples tested but none are likely to have > 0.1 ng TCCD equiv l^{-1} beyond 500 metres from the platform.
- Fractionation of the mostly active sample led to 20 positive fractions.
- Dioxin-like activity was observed in 13 samples but only one is likely to have >1pg TCCD equivalent/l at 500 metres from the platform.
- Fractionation of the 2 most potent samples gave rise to 3 and 7 positive fractions respectively.
- Chemical analysis of fractions from both total and dioxin like activity gave rise to 41 potential compounds of interest.
- 41 compounds were tested in a QSAR for AhR activity.
- 5 of these compounds were considered to be highly active.
- 17 were considered to be of medium activity.
- 19 were considered of low, or no, activity.
- 18 compounds were tested in the DR-CALUX® assay.
- 8 of these compounds were active in the DR-CALUX® assay.
- Correlation between DR-CALUX® and QSAR data was limited.
- 4 active compounds were quantified in the total AhR fractions from Platform 7a.

- 2 of these 4 active compounds were also found in the ‘dioxin-like’ fractions from Platform 11 and Platform 14.
- 3 % of the observed activity was assigned to the 4 active compounds from Platform 13.
- 1 % of the observed activity was assigned to the 2 dioxin –like compounds from Platform 11.
- 2 % of the observed activity was assigned to the 2 dioxin-like compounds from Platform 14.
- of the 4 compounds identified, the 2 which appear only in the total AhR fractions (anthracene and carbazole) are considered to be bioaccumulative. Anthracence is also considered to be persistent. For this reason anthracene is on the OSPAR possible concern list.

General

- No substances could be correlated with available data on chemicals used on offshore installations.
- Improved library information may lead to identification of further substances.

5 CONCLUSIONS

A suite of bioassays was applied to characterise the toxic, sub-lethally toxic, ER and AhR agonist activity associated with 24 PW samples from 16 installations. Toxicity Identification Evaluation was applied to seven of these PW samples and was partially successful in identifying the compounds responsible for the measured effects. Although a large amount of activity remained uncharacterised the predicted dilution of the produced water within 500 metres from the discharge indicates that it is unlikely that any effects will be observed beyond this distance. However further work to provide greater characterisation of produced water contaminants and focussed studies of potential bioaccumulation by organisms within and beyond the 500 metre mixing zone would increase confidence that cumulative effects of produced water discharges are unlikely.

6 RECOMMENDATIONS

Although this study indicates that the activity identified in the produced water is unlikely to persist beyond 500 metres from the discharge a number of recommendations are made to provide greater confidence in this conclusion.

- A limited modelling study is conducted to predict the fate and longer-term effects of the compounds identified in this study.
- Targeted analysis of existing samples for the occurrence of formulation products be carried out, in order that they may be eliminated from the investigation into the cause of observed activity.
- In the absence of suitable methods for the analysis of offshore chemicals, the activity of the most likely candidate products could be screened in order that they can be eliminated from the investigation.
- High-resolution mass spectrometry be employed in order to make more definite identification of substances.
- A study of sediments around a few selected offshore installations (based on the findings of the present work) be initiated, in an attempt to establish links between produced water discharge and sediment contamination.
- The toxicity to whole organisms may be assessed in order to fully characterise the observed effects.
- To assess the true bioaccumulation of the compounds discharged in produced water, a TIE study of mussels collected from around offshore installations could be carried out. The chemicals identified in this study could act as a starting point for targeted analysis.
- Further work to assess the variability of produced water over time, would give a more accurate indication of average and worst case scenarios.

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8 APPENDIX A: METHODS

8.1 Sample Collection

Clean stainless steel barrels (30 l) and a standard operating procedure (SOP) on sample collection were provided to each operator (Appendix B). Briefly, samples were to be collected at a sampling point immediately down stream of the last conventional water treating vessel or other designated sampling location. The lines were to be flushed for several minutes prior to sampling, the container rinsed with sample, filled to the brim and labelled. Samples were then to be dispatched to the CEFAS Burnham on Crouch laboratory as soon as possible.

8.2 Produced Water Manipulation

8.2.1 C8 and ENV+ Solid Phase Extraction

Samples were extracted within 24 h of receipt. Each sample (30 l) was transferred into a pressure vessel with a gas inlet and water outlet. Pressure from a gas line was used to pass the water through a Teflon tube filled with glass wool, acting as a filter, and then through C8 (5 g) and ENV+ (1 g) columns in series at a flow rate of approximately 40 ml min^{-1}. The C8 column was conditioned with methanol (10 ml) before use. The columns were then dried under vacuum and if necessary frozen until extraction.

Columns were eluted with DCM (5 ml) and methanol (5 ml). These extracts were combined and reduced to 5 ml using a turbovap (Zymark, USA) at 35 ˚C.

8.2.2 C18 Solid Phase Extraction

The C18 solid phase extraction (SPE) test is designed to determine the extent of sample toxicity caused by organic compounds. PW was passed through a solvated (methanol, 10 ml; water, 10 ml) 1 g C18 SPE column (IST, UK) and the eluent collected.

8.2.3 Filtration

The filtration experiment provides information on toxicants associated with particulate material. Filtration was carried out using Millipore Sterivex-GP (0.22 µm) filters. Typically, 50 ml was passed through each filter.

8.2.4 Aeration

The aeration test is designed to determine how much sample toxicity can be attributed to volatile compounds. PW samples (100 ml) were aerated in measuring cylinders (100 ml) using nitrogen for a period of 30 min.

8.2.5 Ion Exchange Solid Phase Extraction (SPE)

To determine the extent to which sample toxicity was caused by certain cationic metals, PW was passed through an ion exchange SPE column. PW was passed through a solvated (methanol, 10ml; water, 10ml) WCX ion exchange column (Supelco, USA) and the eluent collected.

8.2.6 EDTA Chelation

To determine any activity that may be due to metal ions, EDTA is added to the PW. EDTA chelates metal ions and will therefore reduce the toxic activity of samples containing metal ions. EDTA was added to PW (100 ml) in 3 quantities; 1 mg, 10 mg and 100 mg. Samples were then stirred for 10 minutes and filtered before assay.

8.2.7 Activated Carbon

Activated carbon is used to remove dissolved organic matter from the produced water and therefore gives an indication of the amount of toxicity in a sample that is due to organic compounds. Activated carbon was added to the PW samples, stirred for 10 minutes and then filtered off before testing.

8.2.8 Sodium Thiosulphate

Sodium thiosulphate is a mild reducing agent, and will therefore react with oxidizers such as chlorine and remove them from aqueous solutions. Sodium thiosulphate was added to PW samples and stirred for 10 minutes. Samples were then re-tested for toxic activity.

8.3 Bioassays

8.3.1 Assay for acute toxicity to T. battagliai

Where necessary, adjustments were made to bring salinity and pH levels into acceptable limits (salinity 31-34 $^{o}/_{oo}$, pH 7.6-8.2). Adjustments to salinity were made with the addition of de-ionised water to reduce salinity, or addition of sea salts to increase salinity. Adjustments to pH were made with the addition of 1M NaOH to increase pH. Each PW sample was tested at suitable logarithmic series of nominal concentrations. Dilutions were

made using aged seawater filtered to 0.2 µm. Solutions were made up on the day of the test to prevent any degradation of the PW in seawater before the test began.

Toxicity testing using *T. battagliai* (Copepoda; Harpacticoida) was performed as described by Thomas *et al.* (1999), without modification. Tests were conducted in 12 cell well plates. Each well contained 5 ml of test solution, 4 replicates were used for each dilution. At the start of the test 5 copepodites (4-6 days old) were introduced to each well. Mortality was recorded at 24 h and 48 h using a binocular microscope with dark-field illumination. Organisms were classified as dead if no appendage movement was detected after gentle agitation with a mounted needle. A positive control, using $ZnSO_4$, was run concurrently with each batch of samples for QA purposes. The raw data from these *T. battagliai* assays were analysed using the Toxcalc statistical package (Tidepool Scientific, USA). EC_{50}'s were calculated according to average observed mortality (%) in each test. EC_{50}'s were calculated using Maximum Likelihood Probits (MLP). When this method was not appropriate a Trimmed Spearman Karber (TSK) method was used. EC_{50}'s were then converted to Toxicity Units (TU) by dividing 100 % by the EC_{50}.

8.3.2 Assay for sub-lethal toxicity to oyster embryos

The oyster embryo bioassay (OEB) is a procedure for obtaining laboratory data concerning the acute effects of a test material (ASTM, 1995). The OEB is a procedure that is currently deployed under the OSPAR JAMP as a general water quality biological effects method and QA procedures with this assay have now been developed within the BEQUALM programme. The OEB was conducted according to published protocols (DTA, 2001). The only amendment to the protocol was the use of 5ml volumes of test solution opposed to the normally used 30 ml volumes for the bioassay procedure. This methodology is regularly conducted at the Environment Agency (EA) and Cefas laboratories for routine and research purposes.

A stock solution of the reference compound zinc sulphate was prepared at 1000 mg l^{-1} using Millipore water (0.2 µm filtered). Surface plating to the walls of the containers was reduced by the addition of 1 ml of 1M HCl. Working solutions (50 mg l^{-1}) and test solutions (suitable logarithmic series of nominal concentrations) were prepared using artificial seawater.

The pH and salinity of the un-diluted PW's were initially tested to ensure they were within acceptable limits for the tests. pH adjustment was made by the addition of 1M HCl or NaOH and salinity adjustment by dilution with test water or addition of sea salts (Sigma). Test solutions of the PW were made on the day of testing by mixing artificial sea water with the PW to give a suitable logarithmic series of nominal concentrations. (Artificial seawater was prepared from sigma sea salts at a concentration of 33 ‰ and was 'aged' over a period of 24 h by vigorous mixing and aeration). Conditioned, ripe, oysters for the oyster larval development bioassay were obtained from Guernsey Sea Farms.

The DTA (2001) methodology for the oyster larval development assay (practical laboratory work) statistical analysis was followed for all oyster bioassays. Briefly, gametes were obtained by the process of manual stripping. Gamete quality was assessed microscopically and the sexes of individuals identified. Gametes were placed into beakers of reference seawater at 24 °C for fertilisation and development to 16-32 cell stage. Embryo suspensions were then adjusted to the required density and transferred in set volumes to the test solutions. Embryo suspensions were added to the test solutions to achieve densities of approximately 50 embryos ml^{-1}. Test solutions were prepared as a logarithmic series of concentrations. The range of concentrations was selected so as to give abnormalities between 0 and 100 % in 2 or more treatments.

Test containers were incubated in the absence of light at 24 °C $\pm$ 2 °C. During each test pH, dissolved oxygen, temperature and salinity were monitored to ensure they were within threshold criteria (DTA 2001). The experiments were terminated after 24 h. At the end of the test samples were preserved by the addition of neutral buffered formalin (4 %) to preserve the larvae. A 1ml homogenous aliquot of each test solution containing embryos was taken and placed into a Sedgewick rafter cell. Normal D-shaped larvae (ASTM, 1995) were counted by microscopic examination at x4 magnification using an inverted microscope.

The bioassay results were analysed using the methods described by ASTM guidelines (1995). EC_{50}, NOEC and LOEC values were determined using Toxcalc software V5. The method used is based upon the number of abnormal 'D' shaped larvae per ml in each replicate. The number of abnormal larvae per ml is calculated as 50 minus the number of normal larvae, assuming 50 embryos have been added per ml at the initiation of the test. An egg count check was employed to assess whether the correct density of embryos had

been achieved. Where this was 5 % outside of this range the number of abnormal larvae was calculated as: the mean number of embryos in the egg count checks minus the number of normal larvae.

The proportion of abnormal larvae was transformed using the arc-sine square root transformation (to stabilise the variance and satisfy the normality and homogeneity of variance requirements). NOEC's and LOEC's were determined using appropriate hypothesis tests. Percentage net response values were calculated from percentage normal development and percentage abnormal development values. These values were subsequently used to consider an appropriate statistical methodology for the calculation of EC50s from the proportion abnormal data.

8.3.3 Assay for sub-lethal toxicity to S. costatum

The *S. costatum* assay was conducted in 96-well microplates. Each sample was run as a dilution series over 2 microplates with a control of clean seawater (Burnham on Crouch, UK). Each plate was incubated in continuous light (6000 Lux) at 20 °C whilst being shaken at 100 RPM. Algal growth was measured by fluorescence (excitation 430 nm, emission 670 nm) at 0 h, 24 h, 48 h and 72 h. A positive control, using $ZnSO_4$, was run concurrently with each batch of samples to check that the sensitivity of each batch did not vary significantly.

On arrival at the laboratory each PW sample was checked for salinity and pH levels. Where necessary adjustments were made to bring these levels into acceptable parameters (salinity 31-34 $^{o}/_{oo}$, pH 7.6-8.2). Adjustments to salinity were made with the addition of de-ionised water to reduce salinity, or addition of sea salts to increase salinity. Adjustments to pH were made with the addition of 1M NaOH to increase pH.

Each PW sample was tested at a suitable logarithmic series of nominal concentrations. Dilutions were made using aged seawater filtered to 0.2 µm. Solutions were made up on the day of the test to prevent any degradation of the PW in seawater before the test began.

The raw data generated were analysed using Microsoft Excel. Fluorescence units (FU) were converted to cell counts by means of external calibration. The growth of each treatment over 72 h was then calculated and transformed to a percentage of the growth of

the seawater control. The EC_{50} was calculated from the dose versus percentage growth inhibition data using Toxcalc statistical package (Tidepool Scientific, USA).

8.3.4 Assay for oestrogen receptor agonist potency

Samples were tested for oestrogenic activity using a yeast-based oestrogen screen (YES) that has been fully validated and used in similar TIE type investigations (Routledge and Sumpter, 1996; Desbrow *et al*., 1998). The bioassay detects all known oestrogens (e.g. 17β-oestradiol and oestrone) and xenoestrogens (e.g. alkylphenols and Bisphenol A). The bioassay was carried out using the method of Routledge and Sumpter (1996).

As an outline, the human oestrogen receptor (hER-α) has been integrated into the yeast genome, together with expression plasmids carrying oestrogen-responsive elements (ERE's) which control the expression of the reporter gene *Lac-Z*. In the presence of oestrogens or chemicals with oestrogenic activity (which bind to and activate the receptor), β-galactosidase is synthesised and secreted into the assay medium in which the yeast is grown. The β-galactosidase then breaks down the chromogenic substrate chlorophenol red β-galactopyranoside (CPRG). CPRG is initially yellow but breaks down into a red product, the concentration of which can be measured by absorbance spectrophotometry.

Samples were produced in methanol. Samples were added to the microtitration test plate at a range of concentrations and then allowed to evaporate to dryness at room temperature. An assay medium, which consisted of the chromogenic substrate and a growth medium that had been inoculated with yeast cells, was added to the plate. The plate was then incubated for 3 days at 32 °C and was shaken vigorously for 2 minutes on each day. On the third day any change in the colour of the chromogenic substrate was read colourimetrically using a UV/VIS plate-reader (Bio-Tek instruments, Inc.) at an absorbance of 540 nm for colour and 620 nm for the turbidity. A dilution series of 17β-oestradiol (E2) (as a positive control and oestrogenic standard) together with a solvent blank, were assayed alongside each batch of samples.

The oestrogenic activity of each sample was determined by comparing responses with that of the E2 standard. All equivalent E2 values of a sample were divided by their relevant concentration factors to produce equivalent E2 values for the original raw water sample. All values falling in the linear range of the sample response curve were averaged to produce the final equivalent E2 value (ng E2 l^{-1}) of the sample.

8.3.5 Assay for aryl hydrocarbon agonist potency (DR CALUX® assay)

The rat hepatoma H4IIE cell line stably transfected with luciferase reporter gene under the control of dioxin-responsive enhancers (pGudLuc 1.1) was used to screen for compounds eliciting AhR-mediated gene expression. Cells were cultured in a minimum volume of essential medium (α -MEM, GIBCO, UK), with 10 % Foetal bovine serum (GIBCO, UK) in 75 cm^3 disposable culture flasks, incubated at 37 °C in a humidified 5 % CO_2 atmosphere.

Cells for assay were seeded into the central 60 wells of a 96-well culture plate (100 µl per well, 90-100 % confluence), 24 h before exposure. Samples in DMSO were diluted in α-MEM, and 100 µl added to each well to give a maximum DMSO concentration of 0.4 %. Samples were assayed in triplicate and compared to a TCDD dose-response curve generated on the same plate. DMSO was used as a negative control. A significant response was defined as one that was more than three times the standard deviation of the solvent control response. The limit of quantification was defined as ten times the standard deviation of the measured solvent response.

8.3.6 Clean up for DR CALUX® assay

Samples were fractionated on a multi-layer silica column to remove PAHs, leaving PCDDs, PCDFs and PCBs. Glass columns (20 mm i.d.) were packed with 1 g anhydrous sodium sulphate (Na_2SO_4), 5 g 20 % sulphuric acid (H_2SO_4) on silica and 5 g 33 % H_2SO_4 on silica. After packing, the columns were rinsed with 20 ml hexane. The sample was then transferred to the top of the column and eluted with 40 ml hexane:diethyl ether (97:3 v:v). This extract was reduced to 5 ml by rotary film evaporation, and further evaporated to 1 ml at 35 °C under a gentle stream of N_2 and transferred to DMSO for assay.

8.4 Sample Fractionation

Selected samples were fractionated in order to separate active compounds from interferences. Normal phase HPLC was performed using a Partisil PAC semi-preparative HPLC column (25 cm x 10 mm x 10 µm; Phenomenex, Cheshire, UK) fitted with a guard column (Phenosphere, 5 cm x 10 mm x 10 µm; Phenomenex) at a flow rate of 5 ml min^{-1}, using HPLC grade hexane, DCM, and *iso*-propylalcohol (IPA) as a mobile phase. Gradient elution was used over 30 min, with isocratic elution for 5 min with hexane, followed by a 20 min gradient of 100 % hexane to 100 % DCM, and ending with a 100 % IPA flush for 5 min. Thirty 5 ml fine fractions were collected at 5 min intervals and stored at –20 °C.

8.5 Sample Analysis

8.5.1 GC-EI-MS

Fractions giving a positive when bioassay tested were analysed by gas chromatography-electron impact (EI) mass spectrometry (GC-MS). Separations were carried out using a DB5 column (J&W Scientific; 30 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 at the end of the chromatographic run. Splitless injection was used (2 μl; injector temperature 270 °C) with the mass spectrometer operating in full scan mode (50-500 Daltons). Mass spectra of major peaks were compared to reference spectra in the NIST mass spectral database using AMDIS software for tentative identification.

8.5.2 GC-NCI-MS

GC-NCI-MS was carried out on the hexane extracts using a Hewlett Packard 5973 GC-MSD. Chromatographic separations were carried out using a DB5 column (J&W Scientific; 50 m x 0.25 mm x 0.25 μm) with a temperature gradient of 60-300 at 5 ˚C min^{-1} and held isothermally at 300 ˚C for 20 min. Splitless injection was used (2 μl; injector temperature 270 ˚C). The GC column was directly coupled via a transfer line heated to 295 ˚C, to the source of the mass spectrometer, using methane as the reagent gas. The mass spectrometer was operated in full scan mode (50-650 Daltons).

The acquired mass spectra were then manually compared with reference spectra (Stemmler and Hites, 1988) for tentative identification.

8.5.3 GC × GC–TOF-MS

The GC × GC–TOF-MS system was built from a TRACE 2D (ThermoElectron, Milan, Italy) gas chromatograph coupled to a TEMPUS time-of-flight mass spectrometer (ThermoElectron, Austin, TX, USA). Separations are carried out using a DB1 fused-silica column (J&W Scientific; 30 m × 0.25 mm × 0.25 μm) as the first-dimension column, and an HT8 column (1 m × 0.1 mm × 0.1 μm) as the second-dimension column. One end of the second-dimension column was coupled directly to the first-dimension column and the other end to the 30 cm × 0.1 mm retention gap mounted in the GC–MS interface. Mini press-fits (Techrom) are used for the connections. Modulation is performed at the beginning of the second column with a modulation period of 6 s. Helium gas (Hoek Loos) is used as carrier gas at a constant flow of 1.2 ml min^{-1}. Samples were injected manually (1 μl) into a PTV inlet port operated in constant-temperature splitless mode at 280 °C with the split opening

2 min after injection. The mass range of 50–750 Da was acquired at a data acquisition rate of 40 Hz.

8.6 QSAR Analysis

QSAR analysis was carried out using the COREPA model for hER binding affinity and the COREPA-M model for AhR binding affinity, both developed and processed on our behalf by Prof. O. Mekenyan at the University “Prof. As. Zlatrov”, Bulgaria. All of the compounds suggested as aryl hydrocarbon or oestrogen receptor agonists were analysed, giving a probability of their binding to the receptor.

9 APPENDIX B: SAMPLING PROTOCOL

PROTOCOL FOR THE COLLECTION OF PRODUCED WATER

Jan Balaam, CEFAS Burnham Laboratory

26 November 2003

1.0 Purpose

This protocol will be used for the collection of produced water (PW) for the C1738 study.

2.0 Application

This procedure applies to produced water sample collection. The following instructions should be carefully followed.

3.0 Procedures

3.1 Sampling equipment

30 litre stainless steel barrels.

3.2 Labels

The attached labels must be completed.

3.3 Sampling

The sample is to be collected at a sampling point immediately down stream of the last conventional water treating vessel or other designated sampling location.
Flush the line for several minutes before sampling. Rinse each container with sample before filling. Fill barrel almost to brim, allow to cool before closing lid and then apply completed label. Ensure lid is securely fastened.

3.4 Carriage

Samples should be sent to:
Jan Balaam
CEFAS Burnham Laboratory
Remembrance Ave
Burnham on Crouch
Essex
CM0 8HA,
as soon as practicable after sampling (i.e. the same day, or the following morning). Samples should be chilled until they leave the platform. Please contact Jan Balaam or Rob Dyer to advise them of approximate arrival dates. Labels are provided.

5.0 Safety

Sampling procedures should be performed within the health and safety guidelines of the participating facility.

10 APPENDIX C: TABLES OF RESULTS

Table 10.1 Produced Water Toxicity to *T. battagliai*

Installation	EC_{50}[1] (MLP)	95% confidence limits		EC_{50} (TSK)	95% confidence limits		TU[2]
Platform 1b	20.59	12.54	28.24	14.10	9.61	20.70	7.1
Platform 1a	n/r	-	-	22.40	17.63	28.46	4.5
Platform 2	12.18	7.52	17.75	10.25	6.58	15.93	9.8
Platform 3	>100	-	-	n/r	-	-	<1
Platform 5	n/r	-	-	23.00	15.16	34.90	5.3
Platform 4	27.41	17.75	38.73	22.56	15.56	32.71	4.3
Platform 6	n/r	-	-	10.07	7.23	14.03	9.9
Platform 7a	n/r	-	-	9.78	6.64	13.95	10.2
Platform 7b	5.97	3.39	8.14	4.89	3.46	6.91	20.5
Platform 8	31.27	20.13	43.22	26.68	18.13	39.25	3.7
Platform 9	n/r	-	-	47.13	36.92	60.16	2.1
Platform 10	17.37	11.05	24.61	14.59	10.16	20.94	6.9
Platform 11	n/r	-	-	2.30	1.72	3.07	43.5
Platform 12	18.76	12.96	25.68	15.02	10.55	21.38	6.7
Platform 13	n/r	-	-	29.32	21.83	39.38	3.4
Platform 17b	63.62	22.59	83.16	53.00	39.08	71.88	1.9
Platform 17a	35.20	4.11	118.43	32.04	23.15	44.35	3.1
Platform 14	n/r	-	-	12.90	9.90	16.80	7.8
Platform 15b	16.24	9.31	19.74	10.49	8.23	13.36	9.6
Platform 15b post C18	n/r	-	-	61.29	51.36	73.14	1.7
Platform 15a	24.70	18.45	31.82	22.50	17.36	29.15	4.4
Platform 15a post C18	>100	-	-	>100	-	-	<1
Platform 16b	n/r	-	-	19.16	15.80	23.22	5.2
Platform 16a	n/r	-	-	13.76	10.92	17.34	7.3

[1] EC50s are the percentage concentration of PW that caused an effect in 50% of the organisms observed

[2] TU – Toxic Units are calculated as 100 % / EC50

Table 10.2 Produced Water Toxicity to oyster embryos

Installation	EC_{50}[1] (MLP)	95% confidence limits		EC_{50} (TSK)	95% confidence limits		TU[2]
Platform 1b	0.74	0.37	1.29	-	-	-	134.4
Platform 1a	1.38	0.87	2.00	-	-	-	72.5
Platform 2	n/r	-	-	10.42	9.39	11.55	9.6
Platform 3	n/r	-	-	56.57	-	-	1.8
Platform 5	n/r	-	-	17.46	17.24	17.67	5.7
Platform 4	n/r	-	-	13.66	12.83	14.55	7.3
Platform 6	n/r	-	-	1.68	1.54	1.83	59.5
Platform 7a	7.60	7.05	8.13	-	-	-	13.2
Platform 7b	n/r	-	-	12.09	11.45	12.77	8.3
Platform 8	n/r	-	-	33.47	30.79	36.38	3.0
Platform 9	n/r	-	-	13.90	13.25	14.57	7.2
Platform 10	n/r	-	-	16.30	15.80	16.82	6.1
Platform 11	n/r	-	-	4.55	4.24	4.89	22.0
Platform 12	n/r	-	-	13.22	12.34	14.15	7.6
Platform 13	n/r	-	-	11.31	10.28	12.43	8.8
Platform 17b	n/r	-	-	30.88	28.35	33.64	3.2
Platform 17a	n/r	-	-	28.00	26.44	29.64	3.6
Platform 14	n/r	-	-	16.52	15.99	17.07	6.1
Platform 15b	n/r	-	-	6.19	6.02	6.36	16.2
Platform 15b post C18	n/r	-	-	12.14	11.68	12.61	8.2
Platform 15a	n/r	-	-	22.93	21.99	23.90	4.4
Platform 15a post C18	n/r	-	-	48.87	46.71	51.13	2.0
Platform 16b	0.01	6 e-9	0.08	-	-	-	13241
Platform 16a	12.20	8.40	16.11	-	-	-	8.2

[1] EC_{50}'s are the percentage concentration of PW that caused an effect in 50% of the organisms observed

[2] TU – Toxic units are calculated as 100 % / EC_{50}

Table 10.3 Produced Water toxicity to *S. costatum*

Installation	EC_{50} (MLP)	95% confidence limits[1]		Toxic Units
Platform 1b	83.33	n/r	n/r	1.20
Platform 1a	47.84	n/r	n/r	2.09
Platform 2	36.18	n/r	n/r	2.76
Platform 3	75.56	n/r	n/r	1.32
Platform 5	12.78	9.27	16.01	7.82
Platform 4	12.78	8.28	16.18	7.82
Platform 6	23.31	n/r	n/r	4.29
Platform 7a	4.42	1.60	6.36	22.62
Platform 7b	2.70	1.62	4.48	37.04
Platform 8	60.16	n/r	n/r	1.66
Platform 9	>100	n/r	n/r	<1
Platform 10	55.70	n/r	n/r	1.80
Platform 11	32.53	n/r	n/r	3.07
Platform 12	30.29	24.73	35.03	3.30
Platform 13	>100	n/r	n/r	<1
Platform 17b	44.59	n/r	n/r	2.24
Platform 17a	91.97	n/r	n/r	1.09
Platform 14	45.92	n/r	n/r	2.18
Platform 15b	27.09	20.88	32.19	3.69
Platform 15b post C18	>100	n/r	n/r	<1
Platform 15a	52.33	n/r	n/r	1.91
Platform 15a post C18	n/r			
Platform 16b	>100	n/r	n/r	<1
Platform 16a	96.32	n/r	n/r	1.04

[1]95 % confidence limits not calculated where no maximum value is obtained

Table 10.4 Produced Water oestrogen receptor agonist potency

Installation	E2 Equiv. ng/l	Stdev
Platform 1b	3.24	0.51
Platform 1a	5.23	0.97
Platform 2	31.58	2.19
Platform 3	7.17	0.71
Platform 4	39.28	9.92
Platform 5	4.10	0.14
Platform 6	1.89	0.00
Platform 7a	0.02	0.01
Platform 7b	1.71	0.00
Platform 8	8.69	1.85
Platform 9	7.46	1.10
Platform 10	0.32	0.09
Platform 11	0.01	0.01
Platform 12	7.41	2.88
Platform 13	91.17	15.24
Platform 14	8.11	0.80
Platform 17b	65.89	6.91
Platform 17a	31.96	0.06
Platform 15b	16.87	5.11
Platform 15a	16.73	1.07
Platform 16b	21.76	0.01
Platform 16a	11.13	2.11

Table 10.5 Produced Water aryl hydrocarbon receptor agonist potency

Sample Site	Total		Dioxin - like	
	ng TCDD/l	stdev	pg TCCD/l	stdev
Platform 1b	13	1.0	29.6	2.6
Platform 1a	10	0.5	15.8	0.9
Platform 2	239	17.4	<LOD	<LOD
Platform 3	16	1.5	<LOD	<LOD
Platform 4	32	2.0	2.2	0.1
Platform 5	18	1.9	<LOD	<LOD
Platform 6	29	2.2	<LOD	<LOD
Platform 7a	417	19.4	<LOD	<LOD
Platform 7b	20	2.9	<LOD	<LOD
Platform 8	33	0.7	30.4	2.0
Platform 9	16	2.1	223.3	20.8
Platform 10	5	0.4	<LOD	<LOD
Platform 11	124	18.1	1004.2	117.4
Platform 12	324	15.1	245.5	7.0
Platform 13	18	1.1	<LOD	<LOD
Platform 14	70	7.1	346.9	14.8
Platform 17b	141	3.7	4.2	0.3
Platform 17a	106	9.4	<LOD	<LOD
Platform 15b	95	7.2	15.8	1.8
Platform 15a	76	3.0	8.6	0.7
Platform 16b	93	2.9	1.2	0.0
Platform 16a	13	0.1	1.4	0.0

Table 10.6 Oestrogen receptor activity as predicted by the COREPA QSAR model

CAS #	Chemical name	0.1<RBA<10%	0<RBA<0.1%	Not Active	Purchased
7469-77-4	2-Methyl-1-naphthol		0<RBA<0.1%		yes
2897-05-2	3-Methyl-1-phenyl-butanone			Not active	yes
3520-52-3	2-Methyl-6-propylphenol		0<RBA<0.1%		yes
6683-92-7	1-phenyl-2-pentanone			Not active	no
4685-47-6	3,4-Dimethylanisole			Not active	yes
874-63-5	3,5-Dimethylanisole			Not active	yes
4074-46-8	4-Methyl-2-propylphenol		0<RBA<0.1%		no
20294-32-0	6-Methyl-4-indanol		0<RBA<0.1%		no
EPA-196488	Acetic_acid,_2-oxo-2-(1,1'-biphenyl-4-yl)ethyl_ester			Not active	no
4013-37-0	1,2-dimethoxyethylbenzene			Not active	no
68705-86-2	1-methoxy-4-methyl-3-pentenylbenzene			Not active	no
59588-12-4	1-methoxypropylbenzene			Not active	no
588-67-0	butoxymethylbenzene			Not active	yes
1515-95-3	4-ethylanisole			Not active	yes
578-58-5	2-methylanisole			Not active	yes
100-84-5	3-methylanisole			Not active	yes
4132-48-3	4-Isopropylanisole			Not active	yes
104-46-1	4-Propenylanisole			Not active	yes
104-93-8	4-methylanisole			Not active	yes
55530-67-1	3-methoxy-a-[(trimethylsilyl)oxy]-Benzeneacetic acid			Not active	no
698-87-3	a-methyl-benzeneethanol			Not active	yes
27129-87-9	3,5-dimethylbenzyl alchol			Not active	yes
536-50-5	1-(p-tolyl)-ethanol			Not active	yes
61967-11-1	a-methyl-a-(1-methyl-2-propenyl)-benzenemethanol			Not active	no
74685-13-5	a-methyl-a-2,5,7-octatrienyl-benzenemethanol			Not active	no
EPA-129261	6-benzyloxy-3,4-dihydro-4,4-dimethylcoumarin			Not active	no
120-44-5	Desoxyanisoin	0.1<RBA<10%			yes
2040-07-5	2,4,5-Trimethylacetophenone			Not active	yes
89-74-7	2,4-dimethylacetophenone			Not active	yes
626-19-7	Isophthalaldehyde			Not active	yes
487-21-8	Lumazine		0<RBA<0.1%		yes
10519-06-9	p-Butoxytoluene			Not active	yes
88-18-6	2-tert-Butylphenol			Not active	yes
88-69-7	2-Isopropylphenol		0<RBA<0.1%		yes
2631-40-5	Isoprocarb			Not active	yes
89-72-5	2-sec-Butylphenol		0<RBA<0.1%		yes
2416-94-6	2,3,6-trimethylphenol		0<RBA<0.1%		yes
526-75-0	2,3-dimethylphenol		0<RBA<0.1%		yes
496-78-6	2,4,5-trimethylphenol		0<RBA<0.1%		yes
105-67-9	2,4-dimethylphenol		0<RBA<0.1%		yes
90-00-6	2-ethylphenol		0<RBA<0.1%		yes
1687-61-2	2-ethyl-5-methyl-phenol		0<RBA<0.1%		no
1687-64-5	2-Ethyl-6-methylphenol		0<RBA<0.1%		yes
95-48-7	2-methylphenol		0<RBA<0.1%		yes
499-75-2	5-Isopropyl-2-methylphenol		0<RBA<0.1%		yes
618-45-1	3-Isopropylphenol		0<RBA<0.1%		yes
95-65-8	3,4-dimethylphenol		0<RBA<0.1%		yes
1197-34-8	3,5-diethylphenol		0<RBA<0.1%		no

CAS #	Chemical name	0.1<RBA<10%	0<RBA<0.1%	Not Active	Purchased
108-68-9	3,5-dimethylphenol		0<RBA<0.1%		yes
620-17-7	3-ethylphenol		0<RBA<0.1%		yes
698-71-5	3-Ethyl-5-methylphenol		0<RBA<0.1%		yes
108-39-4	3-metylphenol		0<RBA<0.1%		yes
31143-55-2	3-methyl-6-propylphenol		0<RBA<0.1%		no
99-89-8	4-Isopropylphenol		0<RBA<0.1%		yes
99-71-8	4-sec-Butylphenol		0<RBA<0.1%		yes
1988-89-2	4-(1-phenylethyl) phenol		0<RBA<0.1%		yes
123-07-9	4-ethylphenol		0<RBA<0.1%		yes
106-44-5	4-methylphenol		0<RBA<0.1%		yes
14938-35-3	4-pentylphenol		0<RBA<0.1%		yes
645-56-7	4-propylphenol		0<RBA<0.1%		yes
37920-25-5	4-butylacetaphenone			Not active	yes
89-83-8	Thymol				yes
60-18-4	Tyrosine		0<RBA<0.1%		yes

RBA = relative binding affinity

Table 10.7 Comparison of QSAR and assay data for Oestrogen Receptor Agonists

Chemical name	QSAR Activity	ER YES ER Activity*
1-(p-tolyl)-ethanol	Not active	<LOD
2,4,5-Trimethylacetophenone	Not active	<LOD
2,4-dimethylacetophenone	Not active	<LOD
2-methylanisole	Not active	<LOD
2-tert-Butylphenol	**Not active**	**1.08×10^{-5}**
3,4-Dimethylanisole	Not active	<LOD
3,5-Dimethylanisole	Not active	<LOD
3,5-dimethylbenzyl alchol	Not active	<LOD
3-Methyl-1-phenyl-butanone	Not active	<LOD
3-methylanisole	Not active	<LOD
4-ethylanisole	**Not active**	**9.43×10^{-6}**
4-Isopropylanisole	Not active	<LOD
4-methylanisole	Not active	<LOD
4-Propenylanisole	Not active	<LOD
a-methyl-benzeneethanol	Not active	<LOD
butoxymethylbenzene	Not active	<LOD
Isophthalaldehyde	Not active	<LOD
Isoprocarb	Not active	<LOD
p-Butoxytoluene	Not active	<LOD
p-n-Butylacetophenone	Not active	<LOD
2,3,6-trimethylphenol	0<RBA<0.1%	<LOD
2,3-dimethylphenol	**0<RBA<0.1%**	**1.53×10^{-5}**
2,4,5-trimethylphenol	0<RBA<0.1%	<LOD
2,4-dimethylphenol	0<RBA<0.1%	<LOD
2-Ethyl-6-methylphenol	0<RBA<0.1%	<LOD
2-ethylphenol	0<RBA<0.1%	<LOD
2-Isopropylphenol	0<RBA<0.1%	<LOD
2-Methyl-1-naphthol	0<RBA<0.1%	<LOD
2-Methyl-6-propylphenol	**0<RBA<0.1%**	**9.82×10^{-6}**
2-methylphenol	0<RBA<0.1%	<LOD
2-sec-Butylphenol	**0<RBA<0.1%**	**8.46×10^{-6}**
3,4-dimethylphenol	0<RBA<0.1%	<LOD
3,5-dimethylphenol	0<RBA<0.1%	<LOD
3-Ethyl-5-methylphenol	0<RBA<0.1%	<LOD
3-ethylphenol	**0<RBA<0.1%**	**8.96×10^{-6}**
3-Isopropylphenol	**0<RBA<0.1%**	**6.61×10^{-6}**
3-metylphenol	0<RBA<0.1%	<LOD
4-ethylphenol	**0<RBA<0.1%**	**1.71×10^{-5}**
4-Isopropylphenol	0<RBA<0.1%	<LOD
4-methylphenol	0<RBA<0.1%	<LOD
4-pentylphenol	**0<RBA<0.1%**	**1.19×10^{-3}**
4-propylphenol	**0<RBA<0.1%**	**3.32×10^{-5}**
4-sec-Butylphenol	**0<RBA<0.1%**	**5.3×10^{-5}**
5-Isopropyl-2-methylphenol	0<RBA<0.1%	<LOD
Lumazine	0<RBA<0.1%	<LOD
Thymol	0<RBA<0.1%	<LOD
Tyrosine	0<RBA<0.1%	<LOD
Desoxyanisoin	0.1<RBA<10%	<LOD

*YES assay data relative to E2. E2 has an activity of 1

Table 10.8 Aryl Hydrocarbon activity as predicted by the COREPA-M QSAR model

CAS No.	Chemical Name	Log (1/EC50) >7	4<log (1/EC50) <7	Log (1/EC50) <4	Purchased?
71-43-2	benzene			<0	No
85-01-8	phenanthrene		4.92		Yes
86-52-2	1-(chloromethyl)naphthalene			2.20	No
86-73-7	9h-fluorene			3.50	Yes
86-74-8	9H-Carbazole		4.13		Yes
90-12-0	1-methylnaphthalene			1.29	Yes
91-20-3	naphthalene			0.68	Yes
91-57-6	2-methylnaphthalene			2.58	Yes
101-81-5	1,1'-methylenebisbenzene			<0	No
103-29-7	Benzene, 1,1'-(1,2-ethanediyl)bis-			<0	No
118-74-1	hexachlorobenzene		5.08		Yes
120-12-7	anthracene	8.76			Yes
132-64-9	Diphenylene_oxide			3.15	No
132-65-0	Dibenzothiophene		6.15		Yes
246-02-6	Benz[a]azulene		4.13		No
290-37-9	Pyrazine			<0	No
345-92-6	methanone,_bis(4-fluorophenyl)-			2.77	No
553-53-7	nicotinohydrazide			<0	No
569-41-5	1,8-dimethylnaphthalene			2.07	Yes
573-98-8	1,2-dimethylnaphthalene			2.97	Yes
574-77-6	papaveroline	>10			No
613-13-8	2-Anthracenamine	>10			Yes
827-54-3	naphthalene, 2-ethenyl-		5.50		Yes
947-73-9	phenanthren-9-amine		6.59		Yes
1127-76-0	1-ethylnaphthalene			2.94	Yes
1321-64-8	Naphthalene, pentachloro-		4.21		Yes
2051-24-3	decachloro-1,1'-biphenyl	7.85			Yes
2971-90-6	clopidol			<0	No
3770-48-7	4-methylcarbazole		4.29		No
4177-16-6	vinylpyrazine			<0	Yes
4630-20-0	3-Methylcarbazole		5.75		Yes
5159-41-1	2-iodobenzylic_alcohol			<0	No
39001-02-0	octachlorodibenzofuran		7.48		No
57455-06-8	3-iodobenzyl_alcohol			<0	No
77764-84-2	3',6-Dimethoxyaurone	>10			No
EPA-138137	Carbazole,1,3.4-trimethyl-		6.20		No
EPA-138124	Carbazole,1,3-dimethyle-		5.93		No
EPA-138125	Carbazole,1,4-dimethyle		4.78		No
EPA-138126	Carbazole,1,5-dimethyle		4.50		No
EPA-138133	Carbazole,3,4-dimethyle-		5.97		No
EPA-80557	N-Hydroxymethylcarbazole		4.30		No

Table 10.9 Comparison of QSAR and assay data for Aryl Hydrocarbon Receptor Agonists

Chemical Name	Log (1/EC50)	AhR Activity
Vinylpyrazine	<0	3.92×10^{-5}
Naphthalene	0.68	<LOD
1-methylnaphthalene	1.29	5.19×10^{-5}
dimethylnaphthalene	2.07	1.14×10^{-3}
2-methylnaphthalene	2.58	<LOD
1-ethylnaphthalene	2.94	<LOD
9h-fluorene	3.50	<LOD
9H-Carbazole	4.13	1.56×10^{-4}
phenanthrene	4.92	<LOD
hexachlorobenzene	5.08	<LOD
2-ethenylnaphthalene	5.50	<LOD
3-Methylcarbazole	5.75	<LOD
Dibenzothiophene	6.15	<LOD
Phenanthren-9-amine	6.59	7.22×10^{-3}
decachloro-1,1'-biphenyl	7.85	5.54×10^{-5}
Anthracene	8.76	7.38×10^{-5}
Pentachloronaphthalene	4.21	<LOD
2-Anthracenamine	>10	2.61×10^{-3}

Table 10.10 Summary of substances identified against PBT criteria

CAS	Substance	OSPAR Listing category			Persistent	Bioaccumulative	Toxic
		Priority Action	Possible Concern	No Listing	(t ½/d)[1]	(log Kow)[1]	
88-18-6	Phenol, 2-(1,1-dimethylethyl)-			✓	**55**	3.3	Sus ER agonist[2]
89-72-5	Phenol, 2-(1-methylpropyl)-			✓	22	3.3	Sus ER agonist[2]
526-75-0	Phenol, 2,3-dimethyl-			✓	21	2.5	Sus ER agonist[2]
1687-61-2	Phenol, 2-ethyl-5-methyl-			✓	22	3.1	Sus ER agonist[3]
618-45-1	Phenol, 3-(1-methylethyl)-			✓	22	3	Sus ER agonist[2]
1197-34-8	Phenol, 3,5-diethyl-			✓	22	3.6	Sus ER agonist[3]
620-17-7	Phenol, 3-ethyl-			✓	21	2.4	Sus ER agonist[2]
31143-55-2	Phenol, 3-methyl-6-propyl-			✓	22	3.6	Sus ER agonist[2]
99-71-8	Phenol, 4-(1-methylpropyl)-			✓	22	3.1	Sus ER agonist[2]
123-07-9	Phenol, 4-ethyl-			✓	22	2.6	Sus ER agonist[2]
14938-35-3	Phenol, 4-pentyl-			✓	20	**4.02**	Sus ER agonist[2]
645-56-7	Phenol, 4-propyl-			✓	22	3.2	Sus ER agonist[2]
4074-46-8	4-Methyl-2-propylphenol			✓	20	3.59	Sus ER agonist[3]
3520-52-3	2-Methyl-6-propylphenol			✓	20	3.59	Sus ER agonist[2]
1515-95-3	Benzene, 1-ethyl-4-methoxy-			✓	17	3.1	Sus ER agonist[3]
20294-32-0	6-Methyl-4-indanol			✓	**56**	3.5	Sus ER agonist[3]
2051-24-3	Decachlorobiphenyl		✓		**579**	**8.27**	Sus AhR agonist[2]
39001-02-0	octachlorodibenzofuran			✓	**1200**	**8.6**	Sus AhR agonist[3]
120-12-7	Anthracene		✓		**73**	**4.45**	Sus AhR agonist[2]
573-98-8	Naphthalene, 1,2-dimethyl-			✓	41	**4.31**	Sus AhR agonist[2]
569-41-5	Naphthalene, 1,8-dimethyl-			✓	42	**4.26**	Sus AhR agonist[2]
90-12-0	Naphthalene, 1-methyl-			✓	10	3.87	Sus AhR agonist[2]
86-52-2	Naphthalene, 1-(chloromethyl)-			✓	44	3.97	Sus AhR agonist[3]
132-64-9	Dibenzofuran			✓	19	**4.12**	Sus AhR agonist[3]
246-02-6	Benz[a]azulene			✓	18	**4.38**	Sus AhR agonist[3]
77764-84-2	3',6-Dimethoxyaurone			✓	**60**	2.68	Sus AhR agonist[3]
574-77-6	Papaveroline			✓	83	2.5	Sus AhR agonist[3]
613-13-8	2-Anthracenamine			✓	**64**	**4.3**	Sus AhR agonist[2]
947-73-9	9-Phenanthrenamine			✓	**65**	3.6	Sus AhR agonist[2]
345-92-6	4,4-difluorobenzophenone			✓	**96**	3.6	Sus AhR agonist[3]
4177-16-6	Pyrazine, ethenyl-			✓	18	0.84	Sus AhR agonist[2]
86-74-8	Carbazole			✓	24	3.7	Sus AhR agonist[2]
EPA-138137	Carbazole, 1,3,4-trimethyl-			✓	**58**	**4.93**	Sus AhR agonist[3]
EPA-138124	Carbazole, 1,3-dimethyl-			✓	48	**4.39**	Sus AhR agonist[3]
EPA-138125	Carbazole, 1,4-dimethyl-			✓	48	**4.39**	Sus AhR agonist[3]
EPA-138126	Carbazole, 1,5-dimethyl-			✓	48	**4.39**	Sus AhR agonist[3]
EPA-138133	Carbazole, 3,4-dimethyl-			✓	48	**4.39**	Sus AhR agonist[3]
3770-48-7	4-Methylcarbazole			✓	44	3.84	Sus AhR agonist[3]
EPA-80557	N-Hydroxymethylcarbazole			✓	48	2.37	Sus AhR agonist[3]

[1] Where experimental data is not available, predictions are taken from PBT predict. [2] Confirmed by assay. [3]Predicted by QSAR.

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