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Environmental Toxicology and Chemistry, Vol. 28, No. 6, pp. 1159–1167, 2009

Printed in the USA
0730-7268/09 $12.00 + .00

IDENTIFICATION OF NONREGULATED POLLUTANTS IN NORTH SEA–PRODUCED WATER DISCHARGES

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(*Received* 30 *September* 2008; *Accepted* 9 *December* 2008)

Abstract—Produced water collected from oil and gas platforms on the United Kingdom Continental Shelf was characterized for nonregulated pollutants through an effects-directed analysis procedure. Produced water samples were characterized for the presence of aryl hydrocarbon receptor (AhR) and estrogen receptor (ER) agonists using the dioxin-responsive, chemical-activated luciferase gene expression assay (DR-CALUX®) and yeast estrogen screen (YES) bioassays. The AhR and ER agonists were then isolated by normal-phase, high-performance liquid chromatography and identified using gas chromatography coupled to mass spectrometry in a number of formats. The identified compounds were cross-referenced with those compounds routinely analyzed and regarded by the Oslo and Paris (OSPAR) Commission for the North East Atlantic as priority hazardous substances. The occurrence in produced water of a number of nonregulated compounds with demonstrable potential environmental effects is presented, to our knowledge for the first time. These include persistent organic contaminants, such as hexachlorobenzene, decachlorobiphenyl, and octachlorodibenzofuran.

Keywords—Produced water Effects-directed analysis Toxicity testing Characterization

INTRODUCTION

Produced water (PW) is the largest offshore discharge associated with oil and gas production. The total North Sea discharge of PW has been estimated at more than 240×10^6 m^3/year (Department of Trade and Industry; http://www.ug.dti.gov.uk/). Produced water is complex in composition and typically contains small amounts of dispersed oil, dissolved organics (including hydrocarbons), organic acids, phenols, production chemicals, and inorganic compounds [1]. Numerous environmental protection strategies (e.g., Oslo and Paris [OSPAR] Commission for Protection of the North East Atlantic) require that monitoring data are available to establish the environmental impact of offshore oil- and gas-related activities.

On the United Kingdom continental shelf, platform operators are required to have their discharges analyzed biannually for total aliphatics, total aromatics, total hydrocarbons, BTEX (benzene, toluene, ethylbenzene, and xylene), NPD (naphthalene, phenanthrene, and dibenzothiophene, including C1–C3 alkyl homologues), polycyclic aromatic hydrocarbons (PAHs; U.S. Environmental Protection Agency [EPA] 16 priority pollutants, excluding naphthalene and phenanthrene), organic acids (total organic acids, *n*-C1 to *n*-C6 organic acids), phenols (total phenols. sum C0–C3 phenols, and C1–C9 alkylphenols), and metals (arsenic, cadmium, chromium, copper, lead, mercury, nickel, and zinc).

Similar requirements also are placed on operators in other sectors of the North Sea (e.g., Norway). Discharges of PW, however, do not exclusively contain the above compounds. Other compounds and homologues of those listed above are present in crude oil as well as in oil production chemicals added by the operators. These compounds also can partition into PW [2]. Furthermore, novel compounds may be produced during use (e.g., degradation of drilling fluids downhole under the conditions of high temperature and pressure), and domestic waste (along with the disinfection agents it contains) may be added to PW before discharge [3]. To complicate the issue further, the composition of PW changes during the production life of an oil field, because water is reinjected into the reservoir to maintain pressure. The composition also differs considerably between oil and gas production and between different production fields.

The complex and variable composition of PW makes it difficult to assess which components have the potential to cause the most environmental harm. A conventional approach would be to determine the environmental risk by correlating toxicity and known contaminant concentration within PW to suggest causes of the observed effects. This is a suitable approach when a group of substances have been identified as having a detrimental effect. An example is the recent risk assessment performed for C3–C6 alkylphenols, which are known estrogen receptor (ER) agonists [4] (http://www.olf.no/miljoerapporter/risk-assessment-of-reproductive-effects-of-alkyl-phenols-in-produced-water-on-fish-stocks-in-the-north-sea-article1933-247.html), in PW discharges. In addition, the biannual monitoring data and the operators' knowledge of which oil production chemicals have been used allow the environmental impact of the individual discharges to be estimated. Typically this is performed through the use of risk quotients, which are calculated using predicted environmental concentrations obtained from computer models (i.e., DREAM [5]) and the known acute effects (no-observed-effect concentrations) of the substances assessed. Although these methods are universally applied, there is room for improvement. For instance, compounds causing toxicity may not be included in the biannual surveys of chemicals if they are reaction products formed from added treatment chemicals. In addition, a number

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Published on the Web 1/30/2009.

of studies recently have demonstrated that PW effluent contains compounds that can exert sublethal toxic effects [6]. Effluents from certain oil and gas production platforms are known to contain in vitro ER [7] as well as aryl hydrocarbon receptor (AhR) agonists and androgen receptor antagonists [8]. Are the compounds being analyzed biannually, however, the cause of these responses?

The present study examined the ER and AhR agonist potency of 22 PW effluents, representing 17 oil and gas installations from the United Kingdom continental shelf. Two in vitro bioassays were employed to screen quantitatively extracts for ER and AhR agonists: The yeast estrogen screen (YES), and the dioxin-responsive, chemical-activated luciferase gene expression assay (DR-CALUX®). A combination of mass balance and effects-directed analysis was then used to establish which substances or classes of compounds were responsible for the measured ER agonist potency and AhR agonist potency. A combination of a recently developed fractionation procedure [9], two-dimensional gas chromatography (GC)–mass spectrometry (MS), and quantitative structure–activity relationships (QSARs) was used to identify ER and AhR agonists that are not included in current PW monitoring schemes.

MATERIALS AND METHODS

Sample collection

Clean, stainless-steel barrels (30 L) and a standard operating procedure on sample collection were provided to each operator. Briefly, samples were to be collected at a sampling point immediately downstream of the last conventional water-treating vessel or other designated sampling location. These sampling points are taps from the main PW effluent discharge. The taps were to be opened for several minutes before sampling to flush through PW in the pipes, and the container was rinsed with sample, filled to the brim, and labeled. Samples were then to be dispatched to the Cefas Burnham Laboratory (Burnham on Crouch, Essex, UK) within 5 d. Samples were collected from a total of 22 discharge points on 17 installations. These installations were a mixture of oil and gas condensate platforms, with a variety of oil/water separation methods. Installations 1, 7, 15, 16, and 17 each had two points of PW discharge. In these cases, both were collected and then labeled as a and b.

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 a 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. 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 dichloromethane (5 ml) and methanol (5 ml). These extracts were combined and reduced to 5 ml using a Turbovap (Zymark) at 35°C. Samples were stored in methanol and solvent-exchanged when necessary for assay or chemical analysis. Solvent exchange was carried out by reducing to near dryness under a stream on N_2 at 30°C (Turbovap) and reconstituting with the required solvent (methanol for YES and dimethyl sulfoxide for DR-CALUX).

Assay for ER agonist potency

Samples were tested for estrogenic activity using a yeast-based estrogen screen (YES) that has been fully validated and used in similar effects-directed investigations [6,10,11]. The bioassay detects all known estrogens (e.g., 17β-estradiol [E2] and estrone) and xenoestrogens (e.g., alkylphenols and bisphenol A) and was carried out using methods described previously [12].

Samples (50 μl in methanol, corresponding to 300 ml of PW) 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, consisting of the chromogenic substrate and a growth medium that had been inoculated with yeast cells containing the human ER, was added to the plate. The plate was then incubated for 3 days at 32°C. On the third day, any change in the color of the chromogenic substrate was read colorimetrically using a ultraviolet–visible light plate reader (Bio-Tek Instruments) at an absorbance of 540 nm for color and 620 nm for turbidity. A dilution series of E2 (as a positive control and estrogenic standard) together with a solvent blank were assayed alongside each batch of samples. The estrogenic activity of each sample was determined by comparing responses with that of the E2 standard and are expressed as E2 equivalents per liter.

Assay for AhR agonist potency

The DR-CALUX assay was performed as described previously [13], without modification. Cells for assay were seeded into the central 60 wells of a 96-well culture plate 24 h before exposure. Samples (8 μl) in dimethyl sulfoxide were diluted in α-minimum essential medium, and 100 μl were added to each well. Samples were assayed in triplicate and compared to a 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) dose–response curve generated on the same plate. Dimethyl sulfoxide was used as a negative control. Samples were analyzed before cleanup for a total aryl hydrocarbon response and after acid-activated silica cleanup to remove PAHs, leaving just dioxins and furans. Toxic equivalents (TEQs) are expressed as TCDD equivalents per liter.

Sample fractionation

Selected samples were fractionated to separate active compounds from interferences. Normal-phase, high-performance liquid chromatography (HPLC) was performed using a Partisil PAC semipreparative HPLC column (length, 25 cm; inner diameter, 10 mm; film thickness, 10 μm; Phenomenex) fitted with a guard column (length, 5 cm; inner diameter, 10 mm; film thickness, 10 μm; Phenosphere; Phenomenex) at a flow rate of 5 ml/min using HPLC-grade hexane, dichloromethane, and *iso*-propylalcohol as a mobile phase. One milliliter of the original 5-ml samples was loaded onto the column. 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% dichloromethane, and ending with a 100% *iso*-propylalcohol flush for 5 min. Thirty 5-ml fine fractions were collected at 5-min intervals. Fractions were solvent-exchanged by reducing to near dryness under a stream on N_2 at 30°C (Turbovap) and then reconstituting with hexane. Fractions were stored at −20°C before analysis.

GC with electron-impact MS

Fractions giving a positive response during bioassay testing were analyzed by GC with electron-impact (EI) MS, which

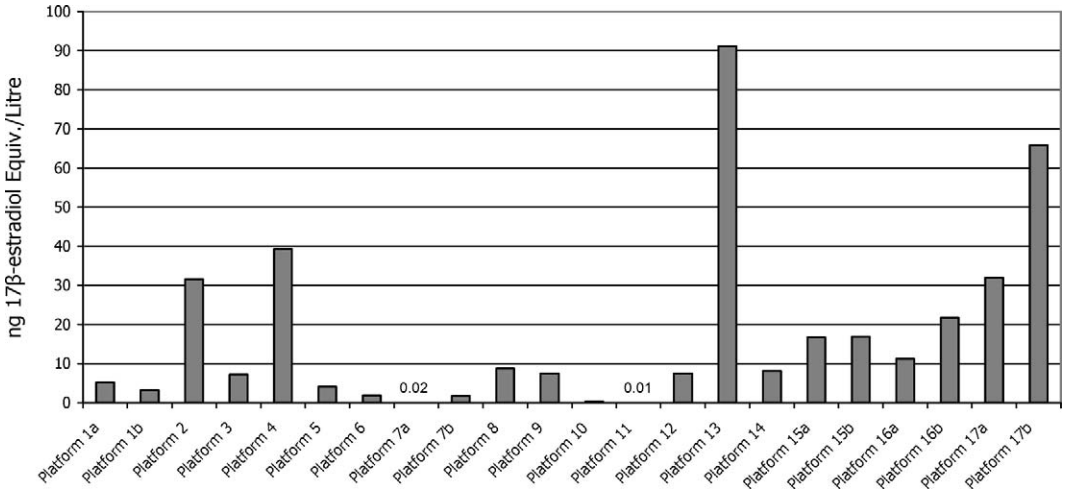

Fig. 1. Estrogen receptor agonist potency of the tested United Kingdom produced water effluents.

was performed using a ThermoFinnigan PolarisQ ion-trap mass spectrometer (Thermo Fisher Scientific) coupled to a Trace GC 2000. Separations were carried out using a DB5 column (length, 30 m; inner diameter, 0.25 mm; film thickness, 0.25 μm; J&W Scientific) with a temperature gradient of 40 to 280°C at 5°C/min and then 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 Da). Mass spectra of major peaks (>0.01% above baseline) were compared to reference spectra in the National Institute of Standards and Technology mass spectral database using Automated Mass Spectral Deconvolution and Identification System software for tentative identification.

GC-MS using negative chemical ionization

Gas chromatography–mass spectrometry using negative chemical ionization (NCI) was carried out on hexane extracts using a Hewlett-Packard 5973 GC with mass-selective detector. Chromatographic separations were carried out using a DB5 column (length, 50 m; inner diameter, 0.25 mm; film thickness; J&W Scientific) with a temperature gradient of 60 to 300°C at 5°C/min and then held isothermally at 300°C for 20 min. Splitless injection was used (2 μl; injector temperature, 270°C). The GC column was directly coupled to the source of the mass spectrometer via a transfer line heated to 295°C and using methane as the reagent gas. The mass spectrometer was operated in full-scan mode (50–650 Da). The acquired mass spectra were then compared manually with reference spectra [14] for tentative identification.

Comprehensive GC with time-of-flight MS

The comprehensive GC (GC×GC) with time-of-flight (TOF) MS system was built from a Trace 2D (ThermoElectron) gas chromatograph coupled to a TEMPUS TOF mass spectrometer (ThermoElectron). Separations were carried out using a DB1 column (length, 30 m; inner diameter, 0.25 mm; film thickness, 0.25 μm; J&W Scientific) as the first-dimension column and a HT8 column (length, 1 m; inner diameter, 0.1 mm; film thickness, 0.1 μm; SGE International) as the second-dimension column. One end of the second-dimension column was coupled directly to the first-dimension column, and the other end was coupled to the 30-cm × 0.1-mm retention gap mounted in the GC-MS interface. Modulation was performed at the beginning of the second column, with a modulation period of 6 s. Helium gas (Hoek Loos) was used as carrier gas at a constant flow of 1.2 ml/min. Samples were injected manually (1 μl) into a Programmed Temperature Vaporization injector inlet port operated in constant-temperature splitless mode at 280°C, with the split opening 2 min after injection. The mass range of 50 to 750 Da was acquired at a data acquisition rate of 40 Hz.

QSAR analysis

Quantitative structure–activity relationship analysis was performed using the COREPA model for human ER binding affinity [15] and the COREPA-M model for AhR binding affinity [16]. A list of suggested compounds was analyzed in each QSAR, resulting in a probability of receptor binding for each compound. This was expressed as a relative binding affinity for estrogenic activity and as log(1/median effective concentration [EC50]) for AhR binding affinity.

RESULTS AND DISCUSSION

Estrogen-like activity of PW extracts

All the PW extracts contained in vitro ER agonists (Fig. 1). The E2 equivalent concentration ranged from 0.01 to 91 ng/L. These data compare with E2 equivalent concentrations of between <1 and 91 ng/L that have been reported previously for PW extracts collected from the North Sea [6,7]. Dose–response curves for the most potent extracts are shown (Fig. 2); a linear relationship with that of E2 can be seen clearly and confirms the presence of ER agonists in PW effluents. The sample extract collected from platform 13 contained the highest concentration of ER agonists and was selected for further characterization.

Dioxin-like activity of PW extracts

Extracts of all 22 effluents were screened for the presence of AhR agonists before and after multilayer acid silica column fractionation (Fig. 3). Before cleanup, all 22 extracts elicited a positive response of between 4.8 and 417 ng TCDD TEQ_{CALUX}/L, compared with concentrations of between 1 and 430 ng TCDD TEQ_{CALUX}/L reported previously for PW [13]. Only 13 of the 22 extracts elicited a response following the removal of PAHs by the acid silica column (Fig. 3). Aryl

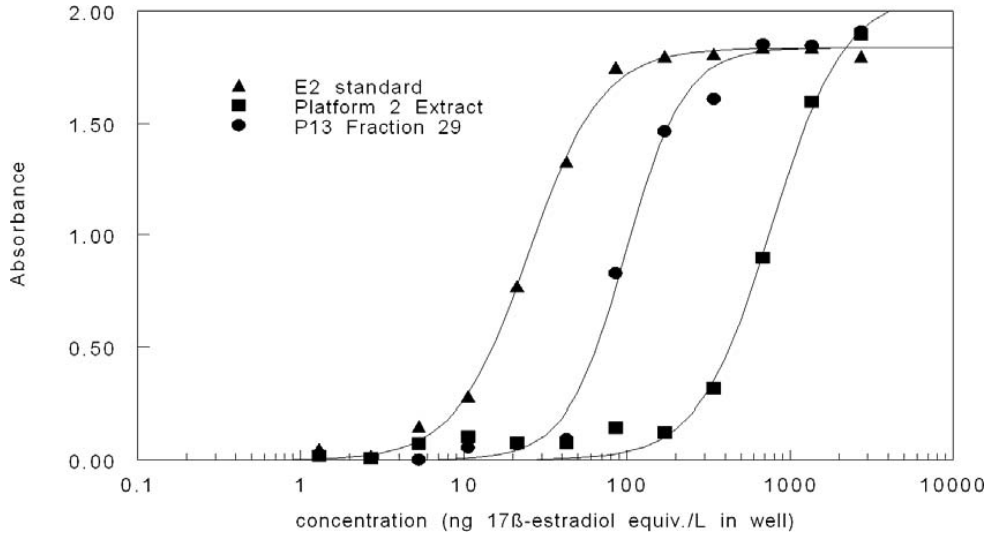

Fig. 2. Relative absorbance of 17β-estradiol (▲), a produced water extract (■), and a produced water fraction (●).

hydrocarbon receptor agonist potencies of between 1.2 and 1,004 pg TCDD TEQ_{CALUX}/L support the hypothesis that AhR active substances other than PAH can occur in PW effluents, although these only account for less than 1% of the total activity in most cases. The extract isolated from platform 7b contained the highest concentration of total AhR agonists, whereas the extract obtained from platform 11 contained the highest concentration of isolated dioxin-like substances. Platform 11 is the only gas condensate facility investigated in the present study; therefore, the extract obtained from the oil production facility at platform 14, which also contains high concentrations of dioxin-like compounds (Fig. 3), was chosen along with the extract from platform 11 for further characterization.

Characterization of ER active compounds

Normal-phase HPLC fractionation of the extract from solid-phase extraction obtained from platform 13 provided eight estrogenic fractions (fractions 22–29). The ER agonist potency of these fractions was between 0.03 and 1.62 ng/L, which accounted for 5% of the activity in the total extract. The dose–response curves in Figure 2 show a linear relationship between the E2 standard curve and that of the PW fraction. Major losses were in this fractionation procedure, which may result from one or more of the following: The unknown nature of the compounds involved means that some compounds may be lost during fractionation; synergistic effects may have occurred in the mixture that are lost once fractionated; and antagonistic effects in the fractions may exist that are not found in the presence of more potent interferences in the mixture.

A combination of GC-EI-MS, GC-TOF-MS, and GC×GC-TOF-MS identified 63 compounds of interest. Numerous alkylphenols, which have been identified previously as ER agonists in PW [7] and are now routinely monitored, were identified in these PW samples. Also, a number of alkylated meth-

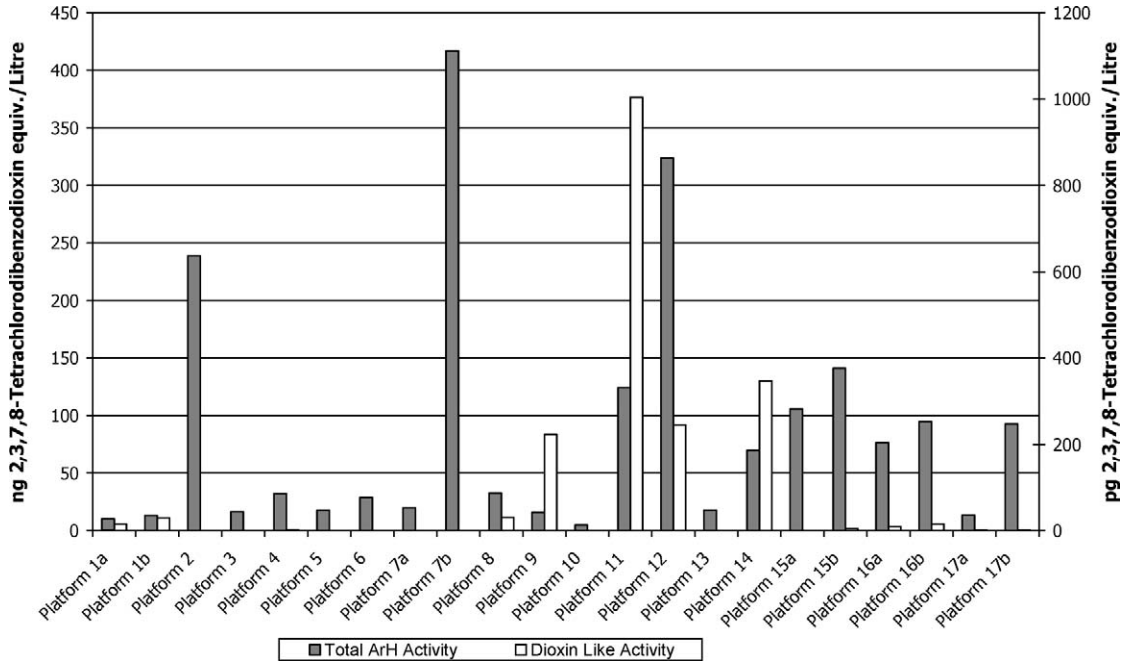

Fig. 3. Aryl hydrocarbon receptor agonist potency of the tested United Kingdom produced water fractions.

Table 1. Estrogen receptor (ER) agonist potency of potential ER agonists identified in the extract of produced water from platform 13 assessed by quantitative structure–activity relationships (QSARs) and the yeast estrogen screen (YES) assay

Chemical Abstracts Service no.	Chemical name	Fraction	QSAR result[a]	YES activity[b]
526-75-0	2,3-Dimethylphenol	26, 28	$0 < RBA < 0.1\%$	1.53×10^{-5}
3520-52-3	2-Methyl-6-propylphenol	26	$0 < RBA < 0.1\%$	9.82×10^{-6}
89-72-5	2-sec-Butylphenol	25, 28	$0 < RBA < 0.1\%$	8.46×10^{-6}
88-18-6	2-*tert*-Butylphenol	26	Not active	1.08×10^{-5}
108-68-9	3,5-Dimethylphenol	24, 27–29	$0 < RBA < 0.1\%$	—
620-17-7	3-Ethylphenol	28	$0 < RBA < 0.1\%$	8.96×10^{-6}
618-45-1	3-Isopropylphenol	26, 28	$0 < RBA < 0.1\%$	6.61×10^{-6}
31143-55-2	3-Methyl-6-propylphenol	26	$0 < RBA < 0.1\%$	—
1988-89-2	4-(1-Phenylethyl) phenol	24, 29	$0 < RBA < 0.1\%$	—
1515-95-3	4-Ethylanisole	23	Not active	9.43×10^{-6}
123-07-9	4-Ethylphenol	26, 28, 29	$0 < RBA < 0.1\%$	1.71×10^{-5}
4074-46-8	4-Methyl-2-propylphenol	26	$0 < RBA < 0.1\%$	—
14938-35-3	4-Pentylphenol	28	$0 < RBA < 0.1\%$	1.19×10^{-3}
645-56-7	4-Propylphenol	29	$0 < RBA < 0.1\%$	3.32×10^{-5}
99-71-8	4-sec-Butylphenol	28	$0 < RBA < 0.1\%$	5.3×10^{-5}
20294-32-0	6-Methyl-4-indanol	29	$0 < RBA < 0.1\%$	—
120-44-5	Desoxyanisoin	23	$0.1 < RBA < 10\%$	Not active

[a] RBA = relative binding affinity as determined by QSAR.
[b] 17β-Estradiol has a potency of one. Where no results are shown, standards were unavailable.

oxybenzenes and other multiring petrogenic compounds with structures similar to those known to have estrogenic activity were identified. Analysis of the 63 compounds using the COREPA QSAR model showed that 34 of the compounds identified were potential ER agonists (Table 1). The in vitro ER agonist potency of the compounds was determined when it was possible to obtain an authentic standard. Many were inactive in the YES assay at the concentration tested, whereas two compounds that were predicted to have no activity using QSAR showed activity in the YES assay (Table 1). Of the 63 compounds initially investigated, only 17, which were either positive using the YES assay or had a QSAR relative binding affinity of greater than 0.1%, are shown in Table 1. Targeted chemical analysis of those identified ER agonists (when authentic reference compounds were available) identified only 35% of the total activity observed in those fractions (Table 2) and only approximately 5% of total activity of the sample.

High concentrations of weakly potent alkylphenols, when combined, cause measurable effects [17]. For this reason, the compounds identified as estrogenically active in fractions 27, 28, and 29 were combined in the same proportions as found in the PW extract. In fraction 29, the inactive compounds also were included to see if this addition enhanced the activity of the fraction. All fractions gave the same response as the sum of their active parts, indicating that in this case, the weakly acting alyklphenols did not combine to give enhanced effects. This indicates that the identity of approximately 95% of the ER agonists in these PW is unknown. Existing environmental risk assessments therefore may underestimate the risk posed by PW to the offshore environment, because not all active chemicals are being measured. It is possible that the compounds responsible are not amenable to gas chromatography, because they are either too polar or insufficiently volatile, or that they are present in such complex mixtures we are unable to resolve them by GC or GC×GC. Alternatively, the compounds may not have been eluted from the normal-phase HPLC column or may not be present in chromatographic libraries (because they are not widely used or known). Research has shown that estrogenic compounds can be chlorinated in wastewater treatment works, altering their estrogenic potency [18,19]. At installations where domestic waste, which is treated with chlorine, is mixed with PW before discharge, this also

Table 2. Quantification of estrogenic compounds in platform 13 fractions that contained estrogen receptor (ER) agonists[a]

Compound	Platform 13 fraction (ng/L)								17β-Estradiol equivalent binding affinity	Contribution (17β-estradiol ng/L)
	22	23	24	25	26	27	28	29		
2,3-Dimethylphenol	4,327	9,091	4,575	99	90	ND	ND	ND	1.53 E−05	0.28
3-Ethylphenol	ND	ND	ND	ND	ND	3,953	14,730	ND	8.96 E−06	0.17
3-Isopropylphenol	ND	ND	ND	3	3,407	3,777	ND	ND	6.61 E−06	0.05
4-Ethylphenol	ND	ND	ND	ND	ND	ND	ND	1,487	1.71 E−05	0.03
4-Pentylphenol	ND	ND	ND	ND	ND	277	116	45	1.19 E−03	0.52
4-Propylphenol	ND	ND	ND	ND	ND	ND	442	753	3.32 E−05	0.04
4-sec-Butylphenol	ND	ND	240	257	1,880	2,198	2,678	2,163	5.30 E−05	0.50
Total										1.58
17β-Estradiol equivalents accounted for	0.07	0.14	0.08	0.02	0.12	0.51	0.43	0.22	1.58	
17β-Estradiol equivalents from yeast estrogen screen	0.93	0.03	0.08	0.18	0.38	0.10	1.62	1.21	4.51	
Percentage	7.1	413	109	8.6	32	505	26	18	35	

[a] ND = not detected.

Table 3. Compounds identified as potential aryl hydrocarbon receptor agonists using quantitative structure–activity relationship (QSAR) and dioxin-responsive, chemical-activated luciferase gene expression (DR-CALUX®)[a]

Chemical Abstracts Service no.	Compound	Fraction			log(1/EC50) (QSAR)	Toxicity equivalency factor (DR-CALUX)
		Platform 7a	Platform 11	Platform 14		
613-13-8	2-Anthracenamine	13			>10	2.61×10^{-3}
4630-20-0	3-Methylcarbazole	19			5.75	Not active
345-92-6	4,4-Difluorobenzophenone	19, 20			2.77	—
3770-48-7	4-Methylcarbazole	18			4.29	—
947-73-9	9-Phenanthrenamine	13			6.59	7.22×10^{-3}
120-12-7	Anthracene		6		8.76	7.38×10^{-5}
246-02-6	Benz[*a*]azulene	8	7		4.13	—
86-74-8	Carbazole	19			4.13	1.56×10^{-4}
EPA-138137	Carbazole, 1,3,4-trimethyl-	16			6.20	—
EPA-138124	Carbazole, 1,3-dimethyl-	18			5.93	—
EPA-138125	Carbazole, 1,4-dimethyl-	18			4.78	—
EPA-138126	Carbazole, 1,5-dimethyl-	18			4.50	—
EPA-138133	Carbazole, 3,4-dimethyl-	18			5.97	—
2051-24-3	Decachlorobiphenyl			5	7.85	5.54×10^{-5}
132-64-9	Dibenzofuran			7	3.15	—
132-65-0	Dibenzothiophene	7			6.15	Not active
86-73-7	Fluorene	7	6		3.50	Not active
118-74-1	Hexachlorobenzene			5	5.08	Not active
91-20-3	Naphthalene		6	7	0.68	Not active
86-52-2	Naphthalene, 1-(chloromethyl)-			7	2.20	—
573-98-8	Naphthalene, 1,2-dimethyl-			6	2.97	—
69-41-5	Naphthalene, 1,8-dimethyl-			6	2.07	1.14×10^{-3}
1127-76-0	Naphthalene, 1-ethyl-			6	2.94	Not active
90-12-0	Naphthalene, 1-methyl-			7	1.29	5.19×10^{-5}
827-54-3	Naphthalene, 2-ethenyl-			6	5.50	Not active
91-57-6	Naphthalene, 2-methyl-			6	2.58	Not active
EPA-80557	*N*-hydroxymethylcarbazole	20			4.30	—
39001-02-0	Octachlorodibenzofuran			5	7.48	—
1321-64-8	Pentachloronaphthalene			5	4.21	Not active
85-01-8	Phenanthrene		6, 7		4.92	Not active
4177-16-6	Pyrazine, ethenyl-	9			<0	3.92×10^{-5}

[a] EC50 = median effective concentration; EPA = U.S. Environmental Protection Agency.

may occur, leading to estrogenic compounds that are neither in chromatographic libraries nor available as analytical standards.

Characterization of AhR active compounds

Fractions 7 to 9, 11 to 14 ,and 16 to 28 of the PW extract obtained from platform 7b contained measurable amounts of AhR agonists, representing the complex mixture of AhR agonists found in PW effluents. Dioxin-like compounds were isolated in fractions 6 to 12 of the extracts from gas condensate platform 11 and in fractions 7 to 9 of the extract from oil platform 14. The total AhR agonist potency of all platform 7b fractions was 10% of the total extract, indicating poor recovery of potent AhR agonists through the fractionation system. The recovery of dioxin-like substances from the platform 11 and 14 extracts was 168 and 270%, respectively. This indicated the presence of many antagonists in the mixture, which when removed by fractionation increase the potency of the individual fractions. Antagonists bind to a receptor but do not activate it and stop processes normally carried out by that receptor.

A combination of GC-EI-MS, GC-NCI-MS, GC-TOF-MS, and GC×GC-TOF-MS of the extracts from the three samples identified a broad range of potential AhR agonists. Many of the compounds that were identified as having the potential to bind to the AhRs are PAHs, which would be present in formation water and are routinely monitored in PW. Other compounds with similar structures are present but have not been documented previously as appearing in PW; these include 2-aminoanthracene and clopidol (3,5-dichloro-2,6-diemthyl-4-pyridinol). Analysis using the COREPA-M QSAR model for AhR binding affinity showed that only 5 of the 41 potential AhR agonists had a high affinity for the AhR (defined as log(1/EC50) > 7). Seventeen were indicated as having medium affinity (defined as 4 < log(1/EC50) < 7), and 19 were indicated to have low affinity (defined as log(1/EC50) < 4). Comparison of the QSAR and DR-CALUX data for aryl hydrocarbon activity shows that many more positives are assigned using QSAR analysis compared to the DR-CALUX assay (Table 3). In addition, there seems to be very little correlation between the results for COREPA-M model and the DR-CALUX assay.

In the sample from platform 7b (Table 4), which was analyzed for total aryl hydrocarbon activity, four compounds from the initial investigations are present and active in the DR-CALUX assay: Anthracene, 1-methyl naphthalene, dimethylnaphthalene, and carbazole. These account for approximately 2.8% of the observed activity. In the platform 11 and 14 fractions (Table 5), the only compounds that are present and also active are 1-methyl naphthalene and dimethylnaphthalene. These two compounds account for 3.8 and 1.9% of the observed activity for platform 11 and platform 14, respectively. As with the ER agonists, several compounds were not readily available for purchase, and two of these showed a relatively high activity in the QSAR analysis and may contribute to the overall activity of the sample. It is unlikely, however, that they would represent 95% of the activity. Furthermore, the compounds causing this activity may not be represented in chromatographic libraries, and some compounds may occur only

Table 4. Quantification of aryl hydrocarbon receptor agonists in platform 7b fractions[a]

Compound	Platform 7b fraction (ng/L)												Toxicity equivalency factor	Contribution (TCDD ng/L)
	7	8	9	11	12	13	16	18	19	20	21	23		
Anthracene	11					1							7.38×10^{-5}	0.0008
Dimethylnaphthalene	28												1.14×10^{-3}	0.03
1-Methylnaphthalene	139		2			2							5.19×10^{-5}	0.007
Carbazole			212						4,130	2,288			1.6×10^{-4}	1.035
TEQs accounted for	0.04	0.00	0.03	0.00	0.00	0.0001	0.00	0.00	0.64	0.36	0.00	0.00		1.08
TEQs 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
Percentage	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

[a] DR-CALUX® = dioxin-responsive, chemical-activated luciferase gene expression; TCDD = 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TEQ = toxic equivalents.

Table 5. Quantification of aryl hydrocarbon receptor in platform 11 and 14 fractions[a]

Compound	Platform 11 fraction (ng/L)						TEF	Contribution (TCDD ng/L)	Platform 14 fractions (ng/L)					TEF	Contribution (TCDD ng/L)
	6	7	8	9	11	12			5	6	7	8	9		
Dimethylnaphthalene		10					1.14 E−03	0.01			14			1.14 E−03	0.015
1-Methylnaphthalene		210					5.19 E−05	0.01			468	20	7	5.19 E−05	0.025
TEQs calculated	0	0.02	0	0	0	0		0.02	0	0	0.04	0.001	0.0004		0.04
TEQs in DR-CALUX® assay		0.28	0.20	0.10				0.58		0.26	0.91	0.54	0.43		2.14
Percentage		8.07	0	0				3.8		0	4.35	0.19	0.09		1.9

[a] DR-CALUX® = dioxin-responsive, chemical-activated luciferase gene expression; TCDD = 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TEF = toxic equivalency factor; TEQ = toxic equivalent.

Table 6. Summary of substances identified, including persistence (P), bioaccumulation (B), and toxicity (T) parameters[a]

Chemical Abstracts Service no.	Substance	OSPAR listing category: Priority action	Possible concern	No listing	P (half-life; d)[b]	B (log K_{OW})[b]	T
88-18-6	Phenol, 2-(1,1-dimethylethyl)-			√	55	3.3	ER agonist[b]
89-72-5	Phenol, 2-(1-methylpropyl)-			√	22	3.3	ER agonist[b]
526-75-0	Phenol, 2,3-dimethyl-			√	21	2.5	ER agonist[b]
1687-61-2	Phenol, 2-ethyl-5-methyl-			√	22	3.1	ER agonist[c]
618-45-1	Phenol, 3-(1-methylethyl)-			√	22	3	ER agonist[b]
1197-34-8	Phenol, 3,5-diethyl-			√	22	3.6	ER agonist[c]
620-17-7	Phenol, 3-ethyl-			√	21	2.4	ER agonist[b]
31143-55-2	Phenol, 3-methyl-6-propyl-			√	22	3.6	ER agonist[b]
99-71-8	Phenol, 4-(1-methylpropyl)-			√	22	3.1	ER agonist[b]
123-07-9	Phenol, 4-ethyl-			√	22	2.6	ER agonist[b]
14938-35-3	Phenol, 4-pentyl-			√	20	4.02	ER agonist[b]
645-56-7	Phenol, 4-propyl-			√	22	3.2	ER agonist[b]
4074-46-8	4-Methyl-2-propylphenol			√	20	3.59	ER agonist[c]
3520-52-3	2-Methyl-6-propylphenol			√	20	3.59	ER agonist[c]
1515-95-3	Benzene, 1-ethyl-4-methoxy-			√	17	3.1	Suspected ER agonist[c]
20294-32-0	6-Methyl-4-indanol			√	56	3.5	Suspected ER agonist[c]
2051-24-3	Decachlorobiphenyl		√		579	8.27	AhR agonist[b]
39001-02-0	Octachlorodibenzofuran	√			1,200	8.6	Suspected AhR agonist[c]
120-12-7	Anthracene		√		73	4.45	AhR agonist[b]
573-98-8	Naphthalene, 1,2-dimethyl-			√	41	4.31	AhR agonist[c]
569-41-5	Naphthalene, 1,8-dimethyl-			√	42	4.38	AhR agonist[c]
90-12-0	Naphthalene, 1-methyl-			√	10	3.87	AhR agonist[c]
86-52-2	Naphthalene, 1-(chloromethyl)-			√	44	3.97	Suspected AhR agonist[d]
132-64-9	Dibenzofuran			√	19	4.12	Suspected AhR agonist[d]
246-02-6	Benz[*a*]azulene			√	18	4.38	Suspected AhR agonist[d]
77764-84-2	3′,6-Dimethoxyaurone			√	60	2.68	Suspected AhR agonist[d]
574-77-6	Papaveroline			√	83	2.5	Suspected AhR agonist[d]
613-13-8	2-Anthracenamine			√	64	4.3	AhR agonist[c]
947-73-9	9-Phenanthrenamine			√	65	3.6	AhR agonist[c]
345-92-6	4,4-Difluorobenzophenone			√	96	3.6	AhR agonist[c]
4177-16-6	Pyrazine, ethenyl-			√	18	0.84	AhR agonist[c]
86-74-8	Carbazole			√	24	3.7	AhR agonist[c]
EPA-138137	Carbazole, 1,3,4-trimethyl-			√	58	4.93	Suspected AhR agonist[d]
EPA-138124	Carbazole, 1,3-dimethyl-			√	48	4.39	Suspected AhR agonist[d]
EPA-138125	Carbazole, 1,4-dimethyl-			√	48	4.39	Suspected AhR agonist[d]
EPA-138126	Carbazole, 1,5-dimethyl-			√	48	4.39	Suspected AhR agonist[d]
EPA-138133	Carbazole, 3,4-dimethyl-			√	48	4.39	Suspected AhR agonist[d]
3770-48-7	4-Methylcarbazole			√	44	3.84	Suspected AhR agonist[d]
EPA-80557	*N*-hydroxymethylcarbazole			√	48	2.37	Suspected AhR agonist[d]

[a] AhR = aryl hydrocarbon receptor; EPA = U.S. Environmental Protection Agency; K_{OW} = octanol–water partition coefficient; ER = estrogen receptor; OSPAR = Oslo and Paris.
[b] When experimental data were not available, predictions were taken from the EPI (Estimation Programs Interface) Suite™ (U.S. Environmemtal Protection Agency).
[c] Confirmed by assay.
[d] Predicted by quantitative structure–activity relationships.

under the specific conditions associated with the formation of PW (i.e., high temperatures and pressures). Dioxins, which are particularly potent in the DR-CALUX assay, are chlorinated planar aromatic compounds. If other planar aromatic compounds are chlorinated in the PW, they may form compounds similar to dioxins, which also may have a high affinity for the AhR but not appear in chromatographic libraries.

Assessment of substances identified

A full summary of the substances identified in PW collected from United Kingdom offshore installations is presented in Table 6. All the substances identified have been cross-referenced with the OSPAR substances for priority action and substances of possible concern lists (http://www.ospar.org/content/content.asp?menu=00940304440000_000000_000000). Under the OSPAR convention (agreement 2005/9), a compound is initially considered to be of increased concern when it meets the following criteria: Toxic, persistent (half-life, >50 d), and bioaccumulative (log octanol–water partition coefficient >4 or bioconcentration factor >500). These criteria were applied to the compounds identified in the present study that were shown to have toxic action in a particular assay by experiment or by QSAR (when 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 therefore are estimated using EPIWIN (U.S. Environmental Protection Agency Exposure Assessment Tools and Models; http://www.epa.gov/opptintr/exposure/pubs/episuitedl.htm), whereas the toxicity data are from the experimental data in the current project. Five compounds are of particular interest: Decachlorobiphenyl, anthracene, octachlorodibenzofuran, 1,3,4-trimethylcarbazole, and 2-anthraceneamine. Of these, anthracene, octachlorodibenzofuran,

and decachlorobiphenyl already appear on the OSPAR possible concern list, whereas the other two are persistent, bioaccumulative, and toxic but are not listed by OSPAR.

The compounds in Table 6 belong to a number of function or use categories: Drugs, organohalogens, phenols, and personal care products. Many are used in the food, cosmetics, and drug industries. A comparison with available data regarding chemicals used in the offshore industry did not give any positive results. The libraries used in the present study for the analysis of spectra, however, may not contain the spectra of chemicals used in the offshore industry. Therefore, certain offshore chemicals may not be identified in the samples when being compared to the library. Of the five compounds identified as both bioaccumulative and toxic, all are AhR agonists (octachlorodibenzofuran, 2-anthraceneamine, 1,3,4-trimethylcarbazole, anthracene, and decachlorobiphenyl). Substances that contribute to any of the measured biological activity in PW, that are persistent, and that can bioaccumulate in organisms or become associated with sediments are of concern, because they may increase in concentration over time. Contaminated sediments can act as a source of exposure for sediment-dwelling organisms or for the remobilization of contaminants into the water column. This may be particularly significant for those compounds, such as octachlorodibenzofuran, that have a long half-life (1,200 d) and a relatively high sediment partition value (50%).

Acknowledgement—This work was supported by contributions by Defra, Dti, Shell, United Kingdom Offshore Operators Association, and the European Union (contract 511237 [GOCE]). The authors would also like to thank participating offshore installations for their assistance in sample collection.

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