

## Studies of produced water toxicity using luminescent marine bacteria

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### Abstract

The main aqueous discharge from oil production platforms is produced water (PW). Produced water is contaminated with a range of pollutants including crude oil, inorganic salts, trace metals, dissolved gases, produced solids and oilfield chemical residues. Concern has been expressed on the impact these discharges, and particularly the dissolved oil component, may be having on the marine environment. In this investigation the toxicity of synthetic produced waters contaminated with petroleum hydrocarbons was compared to PW samples received from the field using the luminescent marine bacterium *Vibrio fischeri*. The objective was to correlate toxicity to specific PW components. Initial studies of individual oil components showed that both aromatic and aliphatic compounds exhibited toxicity. Naphthalene was the most toxic aromatic compound measured and cycloheptane the most toxic aliphatic. For benzenes, toxicity increased with alkyl substitution. Synthetic PW samples, based on the composition of those obtained offshore, had lower toxicities than the field PW samples. The addition of oilfield chemicals at dosage levels used offshore increased the toxicity of the synthetic PW mixtures, but not to the original values. Removal of the oil components by solid-phase extraction reduced PW toxicity in both synthetic and real samples. The results suggest that a range of hydrocarbons, both aliphatic and aromatic, along with heavy metals and oilfield chemical residues, contribute to the toxicity of produced water. Removal of petroleum hydrocarbons significantly reduces the acute toxicity of produced water. However, differences in toxicity between real and synthetic PW samples suggest that components other than hydrocarbons, heavy metals and oilfield chemical residues, are also influencing the toxicity of the effluent.

*Keywords:* toxicity testing, luminescent marine bacteria, produced water, hydrocarbons, oilfield chemicals.



## 1 Introduction

Produced water is the main aqueous discharge from offshore oil production. The composition of produced water varies greatly between production fields. It consists mainly of water, with minor amounts of inorganic and organic constituents derived from the source geologic formation, associated hydrocarbon resource, and chemicals added during processing CAPP [1].

Detailed studies on the precise chemical composition of produced water have been carried out in the North Sea (Stephenson [2]; Utvik, [3]), Gulf of Mexico (Neff and Sauer [4]), and Canadian waters [1]. The organic compounds in produced water include mainly hydrocarbons, in the form of dissolved oil, phenols, organic acids, and naphthenic acids (Frost et al. [5]). The dissolved oil component contains mainly lower molecular weight aliphatic compounds (C5 and C6 normal, branched and cycloalkanes) and 1-3 ring aromatic hydrocarbons. The organic acids, the major organic component, are dominated by C1-C6 acids and phenols, alkylated up to C9. The inorganic component consists of salts (the salinity of produced water can range from a few ppt to 300 ppt [1]), heavy metals, for example zinc, lead, copper, cadmium, chromium and nickel (Garland et al. [6]), and in some produced waters, radionuclides [1]. In addition, produced water contains residues of oilfield chemicals used on the platform to prevent corrosion and scale formation, in the separation of oil and water (demulsifiers), and to mitigate a range of other operational problems.

### 1.1 Produced water toxicity studies

A wide range of species have been used to investigate the toxicity of produced water (Somerville et al. [7]; Stromgren et al. [8]; Flynn et al. [9]) including the marine bacterium *Vibrio fisheri* ([7, 9], Johnsen et al. [10]; Whale [11]). The objectives have been to elucidate those components posing the greatest threat to the marine environment through an assessment of their toxicity and to compare the sensitivities of different test species. In these studies whole produced waters were utilised for toxicity testing and attempts made to correlate toxicity to chemical composition.

In this investigation the toxicity of individual produced water components were assessed. Synthetic produced waters were then made up based on the composition of real produced waters obtained from the field and the toxicity of the two compared. Finally produced waters (both real and synthetic) were subjected to chromatography to remove the dissolved oil component and the subsequent toxicity measured. The objective was to obtain a better understanding of the toxicity contribution of individual components and to see whether synthetic produced waters could account for all of the toxicity of the field samples upon which they were based.

## 2 Methods

Aliphatic and aromatic solvents for toxicity testing were obtained from Rathburn Chemicals Limited, Walkerburn, Scotland. All solvents were HPLC Grade. All



other chemicals were purchased from Sigma-Aldrich Co. Ltd, Gillingham, England. Toxicity testing was performed on a Microtox Model 500 analyser. Materials and reagents for Microtox testing were purchased from SDI Europe Limited, Hampshire, England.

Chemicals for testing were dissolved in brine (35 g/l sodium chloride in distilled water) or in 1% ethanol in brine. Poorly water soluble chemicals and synthetic produced water samples were made up in ethanol prior to dilution. Produced water samples were received from 14 North Sea production platforms. On arrival at the laboratory they were stored in the dark at 4°C until required for analysis. Prior to testing, the salinity and pH of each sample was checked to ensure it was in the optimal range for the bacteria. Toxicity testing was carried out using standard test protocols: Basic Test eight dilutions in duplicate or four dilutions, duplicate samples (Microbics Corporation 1992).

Solid phase extraction was performed using Strata C<sub>18</sub>-E 6 ml 1 g solid-phase extraction (SPE) cartridges (Phenomenex UK, Cheshire, England). The presence of oil components was determined using a Thermoquest Trace gas chromatograph fitted with a 30 m, 0.25 mm i.d. HP-5MS capillary column (Agilent Technologies, UK) and flame ionisation detector.

## 2.1 Toxicity of individual produced water components

Toxicity values for a range of components typically found in produced water are shown in Figure 1. The components have been selected as those representing the most abundant members of their type reported in the literature. The values given are the mean of duplicate analyses. The toxicity of different components varies with exposure time. The exposure times reported represent maximum toxicity values. For the aliphatic and aromatic hydrocarbons this was 5 minutes and the organic acids 15 minutes. For the heavy metals maximum toxicity occurred after an exposure time of 30 minutes. The quality of the data was based on an assessment of the confidence range calculated for each EC<sub>50</sub> value and the coefficient of determination ( $R^2$ ), an expression of the quality of the estimating equation from which the EC<sub>50</sub> is obtained. Ideally 95% confidence range values for each replicate should not exceed 30% of the EC<sub>50</sub> value. Greater confidence range values were observed for some of the replicates, in particular the less water soluble components. This is detailed in the description of their toxicity.

### 2.1.1 The aliphatic hydrocarbons

Crude oil derived aliphatic hydrocarbons are present in produced water. They tend to be poorly water soluble and a solvent (1% ethanol) was used to aid solubility. The EC<sub>50</sub> values of the normal alkanes (pentane C<sub>5</sub>, hexane C<sub>6</sub> and heptane C<sub>7</sub>) were significantly higher than those of the other components tested (i.e. these compounds were less toxic to the bacteria). Mean EC<sub>50</sub> values were 3668, 1403 and 231.3 mg/l respectively. The EC<sub>50</sub> of trimethylpentane (TMP) C<sub>8</sub> was 124.3 mg/l. There appears to be a trend of increasing toxicity with increasing carbon number (correlation coefficient  $r = -0.9785$ ,  $p = 0.05$ ).



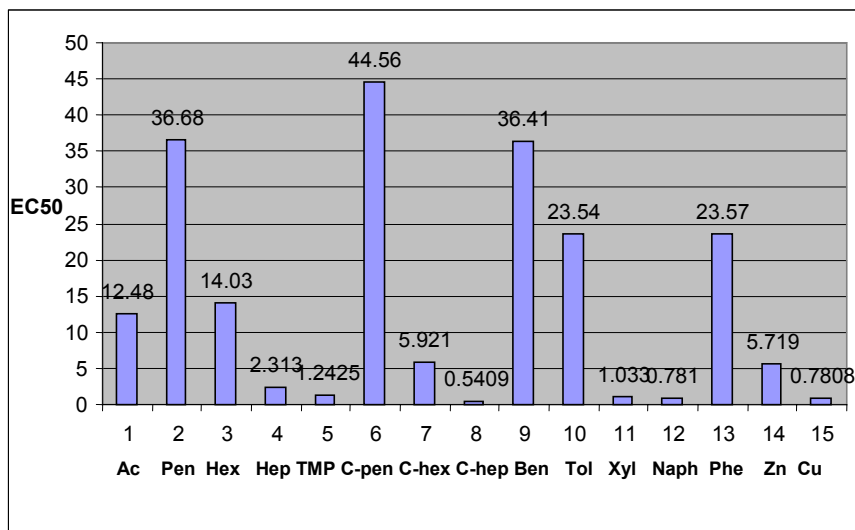


Figure 1: EC<sub>50</sub> values (mg/l) for produced water components. The EC<sub>50</sub> values for Components 1-5 have been divided by 100 to enable easier comparison of data.

Caution must be observed as the 95% confidence range values exceeded 30% of the EC<sub>50</sub> for some of the replicates. However, for all of the alkanes, either the two EC<sub>50</sub> values were within 20% of one another or the 95% confidence range values of at least one of the replicates were less than 30%. Further, the coefficient of determination ( $R^2$ ) for all replicates was  $>0.91$ , suggesting the estimating equation for calculating the EC<sub>50</sub> was of reasonable quality for all replicates.

The C5 – C7 cycloalkanes were significantly more toxic than their linear counterparts (range 44.56 – 0.5409 mg/l). As with the normal alkanes, there appears to be a trend of increasing toxicity with increasing carbon number. For the cycloalkanes, EC<sub>50</sub> values fell by an order of magnitude for each additional carbon atom in the ring.

### 2.1.2 The aromatic hydrocarbons

Benzenes (single ring aromatics) are relatively water soluble and are the most abundant aromatic hydrocarbons present in produced water. As with the aliphatic hydrocarbons, they are predominantly derived from crude oil. The toxicity of the benzene ring was observed to increase with increasing alkylation ( $r = -0.9657$ ). This was particularly noticeable for xylene (dimethylbenzene), which was significantly more toxic than either benzene itself or toluene (methylbenzene). Naphthalene (a two ring aromatic hydrocarbon) had a similar toxicity to xylene. Significant levels of phenols have been reported in produced water. The toxicity of phenol was similar to toluene.

As expected, the aromatics were more toxic than the linear and branched alkanes. Interestingly, the cycloalkanes were of similar toxicity to the aromatics.

### 2.1.3 The organic acids and heavy metals

Organic acids are the most abundant organic material present in produced water. The most abundant acid reported, acetic, was relatively non-toxic (EC<sub>50</sub> 1123 mg/l). The toxicity of zinc (the most abundant heavy metal reported in a majority of the field samples) was 5.719 mg/l. The EC<sub>50</sub> for copper (also present in some of the produced waters tested) was 0.7808 mg/l.

## 2.2 Toxicity of real versus synthetic produced waters

The toxicity of 17 produced water samples from 14 different North Sea oil platforms was measured. The values ranged from 3.74 – 37.34%, the majority (14) having values between 3 and 10%. Synthetic produced waters were made up based on the chemical compositions of six of the field samples. Their compositions are shown in Table 1. Their EC<sub>50</sub> values compared to the field samples upon which the compositions were based are shown in Figure 2.

Table 1: Synthetic produced water compositions.

Chemical Group	Component	Platform					
		A	B	C	D	E	F
Oil in water (OIW) content (mg/l)		39	36	25	12	22	16
Oil in water aliphatics (mg/l)	Pentane	5.5	5.5	1.5	2	5.5	5
	Cyclopentane	5.5	5.5	1.5	2	5.5	5
BTEX (mg/l)	Benzene	1.9	8.2	7.2	3	5	2.9
	Toluene	1.3	3	3.2	1.8	3.2	3.2
	Xylene	0.7	0.7	0.9	0.5	1	1.1
NPD (ug/l)	Naphthalene	861	352	328	473	880	795
	Phenanthrene	74	39	6	24	98	21
	Dibenzothiophene	50	17	3	19	27	19
PAH (ug/l)	Fluorene	2	5	2	4	4	8
	Chrysene	0	0	0	0	2	0
Organic acids (mg/l)	Acetic Acid	260	86	231.7	116.2	377.4	566.9
	Formic Acid	0.3	0	1	7	0.4	3.3
Heavy metals (mg/l)	Copper	0	0	0	0	0.42	0
	Zinc	0.36	0.1	0.05	0	0.24	0

The toxicity of the synthetic produced waters was significantly less than the field samples upon which they were based. One reason may be the presence of oilfield chemical residues in the samples collected from the field. In order to investigate this, the synthetic produced waters were dosed with production chemicals at concentrations used at the respective platforms. A list of the chemical types and dosage levels is given in Table 2, the resultant toxicity values are shown in Figure 2.



Table 2: Types and concentrations of oilfield production chemicals dosed at the different platforms.

PLATFORM	CHEMICAL AND DOSING CONCENTRATION (MG/L)
A	demulsifier (3) corrosion inhibitor/scale inhibitor (10)
B	demulsifier (4) scale inhibitor (6) antifoamer (2)
C	demulsifier (4) corrosion inhibitor (10) scale inhibitor (6) antifoamer (2)
D	demulsifier (7) corrosion inhibitor/scale inhibitor (30) antifoamer (10)
E	demulsifier (10)
F	demulsifier (2) scale inhibitor (30) water clarifier (15)

The addition of oilfield chemicals significantly increased the synthetic produced water toxicity values for all samples. However, with the exception of platforms A and D, the values were less than the field samples. This suggests that toxicity may be a function of compounds other than those measured. For platform D the toxicity of the synthetic PW + oilfield chemicals was significantly greater than the field sample. It should be noted that the addition of oilfield chemicals in the laboratory does not take into account partitioning in the topside process stream nor degradation/loss of components following dosing offshore. The toxicity measured may therefore be an overestimate of the field situation.

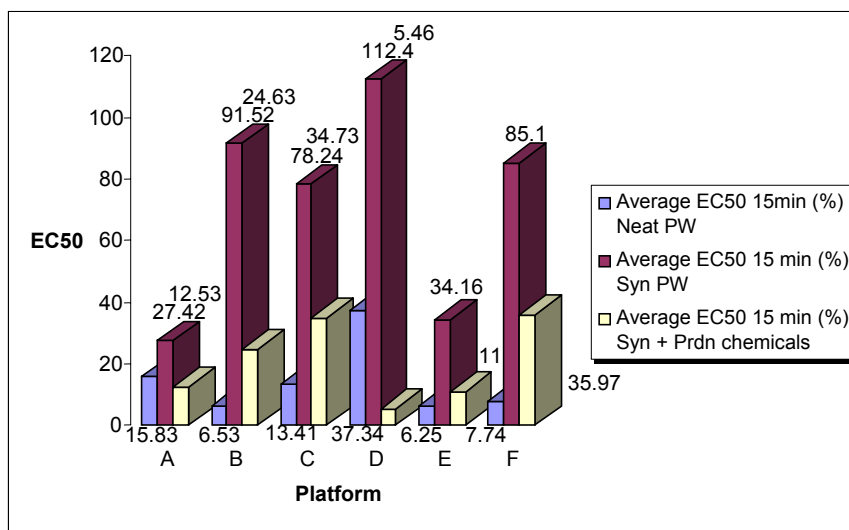


Figure 2: Microtox EC50 15 minute determinations of the field PW samples, synthetic PW and synthetic PW + production chemicals.

### 2.2.1 Statistical analysis of produced water sample data

PCA analysis was performed on the compositional data from the 17 field PW samples. Components not common to all samples were removed from the

analysis. PCA suggested that toxicity did not appear to have a strong dependence on a single variable or group of variables – all had an effect to a certain extent.

### 2.3 The effect of dissolved oil on PW toxicity

It has been suggested that produced water toxicity is primarily dependent on dissolved oil components [12]. This was examined in this study. Brent blend crude oil (50 ml) was partitioned with brine (200 ml, 35 g/l NaCl) by shaking in a separating funnel and allowing to equilibrate for 24hrs. The oil concentration measured by infrared analysis in the brine following equilibration was 19.89 mg/l. The 15 minute Microtox EC50 value for the oily brine was 9.14% (confidence range 8.58 – 9.72%) indicating that dissolved oil may contribute significant toxicity to produced water. In order to assess the impact of removing these components, the 17 field produced water samples were eluted through C18-E Strata SPE cartridges. An initial study had shown that these cartridges removed 40 mg/l crude oil in brine (as determined by gas chromatography). Elution through SPE cartridges reduced the toxicity of the field PW samples significantly. In seven of the samples hormesis (stimulation of the bacteria) was observed. In the remaining samples toxicity was reduced by a factor ranging from 2 to 8 times. This data suggests that although dissolved oil is a major contributor to acute toxicity at some fields other components also influence the toxicity of the produced water.

## 3 Discussion

The Microtox EC50 values for the produced waters tested lay within the range of North Sea production platforms reported in the literature [7, 9, 10]. There is a general assumption that aromatic hydrocarbons contribute significantly to produced water toxicity [1]. However, studies have implicated a range of different components that contribute toxicity to produced water. These include: zinc and hydrocarbons [8]; the aromatic and phenolic fraction [9, 10]; and naphthenic acids [5]. Oilfield chemicals have also been reported to increase the “risk” (Roe et al. [13]). Little attention has been paid to the “non-toxic” aliphatic component of crude oil.

This study has shown that aliphatic hydrocarbons do indeed contribute toxicity to produced water. Their toxicity contribution appears to be related to their structure, molecular weight and their octanol-water partition coefficient ( $K_{ow}$ ). These structure activity relationships (SARs) have been used for many years to predict the impact of chemicals on the environment (Clements [14]). QSARS (Quantitative Structure Activity Relationships) have been used by Sverdup and Kelley [15] to investigate the toxicity of n-pentane, n-hexane, benzene and toluene to *Daphnia magna*. Their results showed good agreement between measured toxicity and theoretical predictions based on  $K_{ow}$ . Indeed, the normal alkanes, cycloalkanes and benzenes all gave increasing toxicity with increasing molecular weight and  $K_{ow}$ . However, our studies indicate that toxicity of these compounds to *Vibrio fischeri* depends on both  $K_{ow}$  and chemical structure, with



toxicity varying between each hydrocarbon class. Both the cycloalkanes and benzenes were significantly more toxic than the normal alkanes, with the cycloalkanes producing similar EC50s to the benzenes. This may be significant when assessing the toxicity of produced waters as cycloalkanes are the most common molecular structures in crude oil (Hunt [16]). Further, they are significantly more water soluble, and hence higher produced water concentrations would be expected, than for their linear counterparts. They have also been reported as “not readily biodegradable” (ARKEMA [17]).

Principal component analysis suggested the levels of soluble, oil-derived organics strongly influence the produced water toxicity. Removal of these components with SPE significantly reduced PW toxicity. However, at some platforms the reduction was only a factor of two. This suggests that at these platforms other components were having a significant influence. Frost [5] has suggested that naphthenic acids may contribute significant toxicity to produced water. These compounds have not been previously studied and are not routinely measured in produced water. Phenols have also been reported to contribute significant toxicity to produced water [9, 10] although the toxicity of phenol itself is less than a number of other components measured, including cyclohexane. Phenols were not included in the analytical reports received for the field samples and were therefore not included in the synthetic PW samples.

The toxicity of synthetic produced waters was also significantly less than the field samples upon which they were based. One possible reason was the omission of oilfield chemicals from these samples. The addition of oilfield chemicals to the synthetic produced waters significantly increased their toxicity, in one case to close to and, in another case, exceeding that of the field samples. The effect of oilfield chemicals on the acute toxicity of produced waters depends on their structure, oil:water partitioning characteristics ( $K_{ow}$ ) and their dose rate. Johnsen et al. [10] concluded that, at normal dosing concentrations, process chemicals did not alter the toxicity of produced water significantly. At high concentrations there was evidence that a minor contribution to measured toxicity was observed. Studies in our own laboratory have also indicated that, for the majority of chemicals tested, the acute toxicity of the aqueous phase (following oil partitioning) was not significantly altered by process chemicals at normal dosing levels, although there was some evidence that certain chemicals may increase the partitioning of oil components into the aqueous phase by an order of magnitude when applied at high dosage rates [12]. The significant increases in toxicity observed in the synthetic produced water samples probably reflects a lack of partitioning of the more oil-soluble, and often toxic, components. Oilfield chemicals almost certainly increase the environmental risk associated with produced water discharges. Roe et al. [13] used modelling to show that this was the case with produced water from the Statfjord and Gullfaks North Sea oil fields. Frost [5] calculated that oilfield chemicals contributed 41% of the Environmental Impact Factor (EIF) at an unnamed Statoil operated field.

Microtox data for whole effluents (produced water) from oil platforms in the North Sea have been reported to be similar to toxicity data from a number of other marine toxicity tests [11]. However, differences can be observed for





individual components. The EC50 values for the normal and branched alkanes measured in this study were all >100 mg/l, which would place them in the least toxic categories (D/E) of the Revised OCNS for offshore chemicals (CEFAS [18]). However, wide variation in EC50 values for these compounds is reported in the literature. This may reflect difficulties in maintaining these compounds in aqueous solution over the duration of the test or differences in species sensitivity. Sverdup and Kelley [15] reported significantly lower pentane and hexane EC50 values for *Daphnia magna* than were measured in this study using *Vibrio fischeri*. Indeed the *Daphnia* EC50 values would place these compounds in OCNS category B (>1 – 10mg/l), the second most toxic category. The mechanism of action of the alkanes is thought to be non-polar narcosis. The endpoint of the *Daphnia* test is immobilisation and the different sensitivity may reflect different modes of action in the two test species.

The reported sensitivity of different species to other produced water components can also vary greatly. In the case of cyclopentane, EC50 values ranging from 11.1 µg/l for brown shrimp to 116 mg/l for algae, have been reported [17]. By contrast, the toxicity to *Vibrio* of more water soluble compounds, such as benzene, was similar to that reported for *Daphnia*, as was the EC50 value of naphthalene.

The results of this study suggest that a number of components, including those not normally regarded as toxic, contribute to the acute toxicity of produced water. However, reducing the levels of oil-in-water may not have a concomitant effect on PW toxicity as other components may also be making a contribution.

Once produced water has been discharged, dilution will have the most immediate effect in mitigating potential environmental impacts of produced water [7]. Volatilisation and biodegradation are also important mechanisms. For example, benzene is a known carcinogen but is relatively water soluble and will disperse in the water column very rapidly. It is also reported to be readily biodegradable [9].

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