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Report

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Toxicity testing of produced formation water (PFW) from Chinguetti FPSO in Mauritania

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Summary

An ecotoxicological study of produced formation water (PFW) from the Berge Helene FPSO at the Chinguetti oil field, operated by Woodside in Mauritania was performed. The study was requested by MRAG Ltd as a part of Woodside's Environmental Monitoring Plan. Toxicity was investigated at four trophic levels using Microtox® microbial (*Vibrio fisheri*) screen test, marine algae (*Skeletonema costatum*) growth inhibition test, copepod (*Acartia tonsa*) acute toxicity test, oyster (*Crassostrea gigas*) larval development toxicity test and limited fish (*Scophthalmus maximus*) acute toxicity test. In addition, dioxin-like toxicity and estrogen activity were assessed using DR CALUX assay and YES assay respectively. The oyster larval development test was the most sensitive to the PFW, with a 24 hour EC_{50} of 3.6%. By contrast, the Microtox® microbial toxicity test was the least sensitive, with a 5 min and 15 min EC50 of 27.9% and 24.2% respectively. The dioxin-like activity of PFW was 233 ± 21.2 pg TCDD TEQ/L and the estrogenic activity was 27 ng E2/L.

1. Introduction

MRAG Ltd has requested IMARES to design and organize a basic ecotoxicological study of produced formation water (PFW) from Berge Helene FPSO at the Chinguetti oil field operated by Woodside in Mauritania. This document reports the methods and results of the ecotoxicological tests conducted. Toxicity was investigated at four trophic levels using bacteria (Microtox® Basic test with *Vibrio fisheri*), marine algae (*Skeletonema costatum*), copepods (*Acartia tonsa*), oysters (*Crassostrea gigas*) and turbot (*Scophthalmus maximus*) as test organisms. In addition, dioxin-like toxicity and estrogen activity were measured using DR CALUX and YES assays to enable assessment of chronic toxicity of PFW. The ecotox tests were carried out by Norwegian Institute for Water Research (NIWA), Oslo, Norway (marine algae growth inhibition, copepod acute toxicity, dioxin-like toxicity and estrogen activity) and by Brixham Environmental Laboratory, Devon, UK (Microtox, oyster larval development and limited fish acute toxicity test).

2. Methods

2.1. Sampling, transport and storage of PFW

Sampling of PFW was performed on 24.8.2006 by IMARES. In total, 12 L of PFW was collected into twelve 1-L glass bottles. Samples were taken at the same time (filling bottles one by one) from the valve just after the on-line analyser and were collected into bottles directly, in order to avoid contamination. Samples were stored and shipped to IMARES under temperature controlled conditions (4 to 8˚C). The samples were received at IMARES on the 30th of August 2006 and were stored in darkness at 0–2˚C until the 6th of October 2006, when they were homogenized. Homogenization was performed by mixing the contents of the 12 bottles in one 20-L glass tank. The homogenized sample was subsequently distributed into twenty-four 1-L bottles, filling ca. 0.5L of the sample into each bottle. The samples were then frozen and stored at -20˚C until 10th of October, when samples (isolated in cool-boxes with icepacks) were dispatched to testing laboratories. Ten bottles (5 L of the sample) were sent to NIWA for marine algae growth inhibition, copepod acute toxicity, dioxin toxicity and estrogen activity tests and 14 bottles (7 L of the sample were sent to Brixham Environmental Laboratory, Devon, UK for Microtox, oyster larval development and fish acute toxicity tests. Samples were received by the test laboratories on the 12th of October 2006 and were stored at -20˚C until testing. The tests were performed between 1 and 15 November 2006.

2.2. Microtox® microbial toxicity screen

Heterotrophic bacteria are decomposers of organic matter and, as such, are important in nutrient cycles.

The test was performed using the Microtox® model 500, following the procedure, which utilises a suspension of the luminescent marine bacteria, *Vibrio fisheri*, as test organisms for measuring acute toxicity in aqueous samples. The Microtox® analyser measures the light emitted by the bacteria before and after the addition of the test substance. A reduction in light emission from the bacteria when a test substance is added signifies that the substance exhibits toxicity. Light emissions were recorded after five and fifteen minutes. The results were compared to a blank control and expressed as percentage effect (light inhibition). The nominal test concentration range deployed was 1.0, 3.2, 10, 32 and 100% v/v (v/v – dilution ratio based on volumes) together with the blank control).

2.3. Marine algae growth inhibition test

Algae are primary producers of organic matter upon which animals depend on either directly or indirectly through the food chain. As such, test procedures using algae are valuable for determining the primary productivity of a water sample and for testing the toxicity of chemicals present in the water.

The test measured the effect of the PFW on the growth of the marine algae *Skeletonema costatum* over a 48- and 72-hour exposure period. The test was performed according to ISO

10253: "Marine algal growth inhibition test with *Skeletonema costatum* and *Phaeodactylum tricornutum*". The test design included 8 concentrations (1.0, 1.8, 3.2, 5.6, 10, 18, 32 and 56%) of the test PFW sample and controls in pure test medium. Natural sea water was used for all dilutions and the control solution. Three replicate cultures of test algae at each concentration of the test sample and six replicate control cultures were incubated on a shaking table at 21±1˚C, under continuous illumination. Growth was monitored by daily counting of cell numbers using a Coulter Multisizer.

2.4. Copepod acute toxicity test

Copepods are primary consumers. The test measured the effect of the PFW on the mortality of the copepod *Acartia tonsa* over a 24- and 48-hour exposure period. The test was performed according to ISO 14669: "Determination of acute lethal toxicity to marine copepods (*Copepoda*, *Crustacea*)". The test was performed on the PFW sample diluted in natural sea water adjusted to a salinity of 32‰. The test design included 5 concentrations (5.6, 10, 18, 32 and 56%) and controls. The toxicity test was conducted with 4 replicates of each concentration and 12 control replicates. The number of animals added to each replicate vessel was 4-8. The test vessels were incubated in the dark at 20±1˚C. The number of surviving copepods in each test vessel was counted after 24 and 48 hours using a low power stereoscopic microscope. Animals showing no swimming or appendage movement within an observation period of 10 seconds were considered to be dead.

2.5. Oyster larval development toxicity test

This test provides a rapid and sensitive assessment of toxicity to a representative marine mollusc, *Crassostrea gigas*, and was carried out according to the methods detailed in BEL SOP BA270. Pre-conditioned oysters (*Crassostrea gigas*), obtained from a commercial facility, were induced to spawn, or, if unsuccessful, the gonads were physically excised to obtain the gametes. Egg suspensions were fertilised with sperm suspension and incubated at 24±2°C, for about 2 hours, with continual agitation, after which time the condition of the embryo suspensions were assessed for suitability. The density of the selected embryo suspension was determined using a Sedgewick-Rafter cell and was adjusted as appropriate. The test was then initiated by the addition of embryo suspension to each test solution (4 × 30 ml replicates per control and test concentration). The solutions were then incubated at 24±2°C, in the dark, until the end of the test (24 hours), at which point each test vessel was formalin fixed, pre- sampling. The numbers of normal and abnormal larvae were then recorded in sub-samples of each replicate using a microscope. The nominal test concentration range deployed was 1.0, 3.2, 10, 32 and 100% v/v, together with a dilution water control. Aged and filtered natural seawater (0.2 µm) was used for all dilutions and the control solution.

2.6. Fish acute toxicity - limit test

The test for acute toxicity to turbot, *Scophthalmus maximus*, was based on Brixham Environmental Laboratory Standard Operating Procedure (BEL SOP) BA465. The test was carried out on a static basis over 24 hours, at a temperature of 15±1°C, with a daily photoperiod of 16 hours light: 8 hours dark, including dusk and dawn transition periods. The single glass vessels each contained 5 fish in 20 litres of test solution. Full natural seawater (35‰) was used for the preparation of the test solutions. Based on range-finding data and the associated oyster larval test data, a single nominal concentration, at 10% v/v, was tested, together with a dilution water control. The 10% v/v test solution was observed as pale yellow and slightly cloudy at test start; the control was clear and colourless. The number of mortalities and any symptoms of toxicity observed were recorded after 3 and 24 hours.

2.7. Dioxin toxicity test

DR CALUX (Chemical-Activated Luciferase Gene Expression) assay was employed to evaluate 'dioxin-like' toxicity of the PFW. The test involved exposing genetically modified cell-line (rat-hepatoma H4IIE) to the test material for 24 h and measuring light production by activated luciferase which is related to the amount of dioxin-like compounds in the sample. The results are reported relatively to the toxicity of the most toxic 2,3,7,8-tetrachlorodibenzodioxin (TCDD) as total TCDD-TEQ/L.

2.8. Estrogenic activity test

Yeast estrogen screen (YES) assay was used for assessing the estrogenic activity of the test material. In this assay, the human estrogen receptor (hER) and estrogen response elements (hERE) are integrated into *Saccharomyces cerevisae* (yeast do not normally express the estrogen receptor). Since the ERE is linked to the ß-galactosidase reporter gene, a colorimetric change at 405 nm is observed when an ER ligand binds the receptor and is translocated to the ERE. The test involved exposing *S. cerevisiae* cell culture to the test mixture for 24 h, β-D-galactopyranoside-dependent colour development for additional 24 h and absorbance measuring. The results are reported relatively to the response of 17ß-estradiol (E2), *i.e.* in ng E2/L.

3. Results

3.1. Overview

Table 1 summarizes the ecotoxicological results for in vivo tests, showing the PFW concentrations for each test. Table 2 shows results for in vitro tests based on cell lines, showing response to 100% PFW. A discussion on the results is given below.

Table 1. Summary of test results for in vivo tests of PFW from Chinguetti field in Mauritania

Taxon	Test Species	Test Endpoint	Duration	EC_{50} (95% confidence interval) (%PFW)	NOEC[1] (%PFW)	LOEC[2] (%PFW)
Bacteria	*Vibrio fischeri*	Decrease in light output	5 min	27.9 (23.5 – 33.1)	1.0	3.2
			15 min	24.2 (16.7 – 35.1)	3.2	10
Algae	*Skeletonema costatum*	Growth/cell yield	48 h	15 (14.6 – 15.5)	8.8[3]	10
			72 h	14 (13.6 – 15)	8.0[3]	10
Copepod	*Acartia tonsa*	Survival	24 h	15 (12.8 – 17.7)	5.6	<10[4]
			48 h	6.9 (5.2 – 8.5)	–	<5.6[4]
Oyster	*Crassostrea gigas*	Larval development	24 h	3.6 (3.4 – 3.9)	1.0	<3.2[4]
Fish	*Scophthalmus maximus*	Survival	24 h	>10	10	–

[1] NOEC – no observed effect concentration estimated as the highest tested concentration showing less than 10% effect

[2] LOEC – lowest observed effect concentration estimated as the lowest tested concentration showing more than 10%, but less than 20% effect

[3] EC_{10} used instead of NOEC concentration

[4] values are set as below (<) values because more than 20% of population was affected at the lowest tested concentration, which is usually not accepted by administrations as LOEC for risk assessment.

Table 2. Results for in vitro tests of PFW from Chinguetti field in Mauritania

Test	Cell line	Response
DR-CALUX	Genetically modified rat-hepatoma H4IIE	233 ± 21.2 pg TCDD TEQ/L
YES	*Saccharomyces cerevisiae*	27 ± 7.8 E2/L

3.2. Microtox® microbial toxicity screen

The EC_{50} estimate for the produced formation water sample were 27.9% and 24.2% for the 5 and 15 minute exposure respectively. Significant inhibitions of light output were observed at low concentrations, with LOEC values of 3.2 and 10%, and NOEC values of 1.0 and 3.2% for the 5 and 15 minute exposures respectively. The 5 min exposure yields the most reliable results, as the response after 15 min exposure departs too much from linearity. As a result, NOEC and

LOEC values for longer exposure (15 min) are higher than those for the shorter exposure (5 min) time and the confidence interval around the EC_{50} is much broader.

3.3. Marine algae growth inhibition test

The PFW sample caused significant inhibition of the growth of the marine algae *Skeletonema costatum* at concentrations above 5.6% and consequently the NOEC and LOEC estimates for both exposure times were 5.6% and 10% PFW respectively. The 48-hour EC_{50} estimate was 15% (14.6% – 15.5%) PFW and 72-hour EC_{50} estimate was 14% (13.6 – 15%) PFW.

3.4. Copepod acute toxicity test

The PFW samples significantly affected survival of *Acartia tonsa* after 48-hour exposure. The lowest concentration evaluated was 5.6% PFW and at this concentration 58% of population survived. Therefore, the LOEC is much less than 5.6% PFW and a NOEC could not be assessed. The EC50 estimate was 6.9% (5.2% – 8.5%) PFW.
The test protocol ISO 14669 used prescribes reporting the concentration corresponding to 0% mortality, which was not possible from the results obtained. In spite of the non-optimal concentration range tested, the EC50-value obtained is reasonably well defined. The shape of the curve is supported by the data from 24 hours exposure, when partial lethality was obtained at 4 exposure concentrations and the EC50 value was 15% (5.6% – 10%) PFW.

3.5. Oyster larval development toxicity test

The oyster larvae proved to be the most sensitive species tested. Exposure to concentration of 3.2% PFW for 24 hours caused abnormal larval development to 28% of the test population. Consequently, the NOEC was set at 1.0% PFW, while the LOEC somewhat less than 3.2% PFW. From the results an EC_{50} of 3.6% (3.4% – 3.9%) PFW was estimated.

3.6. Fish larvae acute toxicity - limit test

Only limited test for acute toxicity to turbot was performed because of the restrictions on animal tests by animal welfare authorities. A single nominal concentration of 10% PFW was tested. All exposed fish survived the 24 hour test period. Consequently, the NOEC value in this test is 10% PFW and LC_{50} is indicated to be more than 10%. With this results turbot is proven to be less sensitive to PFW than all other species tested in this series.

3.7. Dioxin-like toxicity test

The measured dioxin-like activity of PFW is equal to 233 ± 21.2 pg/L of 2,3,7,8-tetrachlorodibenzodioxin.

3.8. Estrogenic activity test

The estrogenic activity of PFW was found to be equal to 27 ± 7.8 ng/L of 17ß-estradiol (E2).

4. Conclusions

Five marine ecotoxicity tests were performed on samples taken form the Berge Helene FPSO to assess the actual toxicity of the PFW produced.
The oyster larvae development test was the most sensitive to the PFW, with a 24 hour EC_{50} of 3.6% PFW. The marine copepod *Acartia tonsa* was only slightly less sensitive, with an EC_{50} of 6.9% PFW By contrast, the Microtox® test was the least sensitive, with a 5 min EC_{50} of 27.9%. However, the Microtox® test yielded a NOEC of only 1% PFW, the same as for oyster larvae. The results compare well to the results reported by Phillips (2004). Most tests yielded similar results, although the copepods seem to be more sensitive than prawns.

Ecotoxicity test are performed in order to account for mixture toxicity and chemicals not covered by chemical analyses. The causes of the observed effects can, however, not directly be deduced from the results. The bioassays applied have different sensitivities for different compound groups. The DR-CALUX response is rather high. This may indicate the presence of

dioxin or dioxin-like PCB. However, the DR-CALUX is also sensitive to high molecular PAH. These will disappear from the extract with extended exposure. High molecular PAH may indicate the presence of 'oil'. This may be responsible for the effects on the copepod *Acartia tonsa.* The Microtox test and oyster test are, however, relatively sensitive to inorganics, such as metals. To assess the causes of the observed toxicity the application of Toxicity Identification and Evaluation (TIE) techniques are required.

The results indicate that severe (EC_{50}-like) effects may be expected in the environment in the area where the discharged effluent is diluted less than approx. 25 times. No effects are expected when the effluent is diluted at least 100 times (NOEC level). To assess the actual volume around the FPSO that might be impacted by the PFW, dispersion modelling is needed, incorporating actually discharged volumes and local conditions.

5. References

Phillips R.R. (2004), Cossack Pioneer produced formation water assessment. Woodside ENV-REP-02-078-CP, 2004 update.

Ir. W. van der Galliën

Signature: _____

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