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Toxicity Assessment of Individual Ingredients of Synthetic Based Drilling Muds (SBMs)

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ABSTRACT

Synthetic based drilling muds (SBMs) offer excellent technical characteristics while providing improved environmental performance over other drilling muds. The low acute toxicity and high biodegradability of SBMs suggest their discharge at sea would cause minimal impacts on marine ecosystems however, chronic toxicity testing has demonstrated adverse effects of SBMs on fish health. Sparse environmental monitoring data indicates effects of SBMs on bottom invertebrates however, no environmental toxicity assessment has been performed on fish attracted to the cutting piles. SBM formulations are mostly composed of synthetic base oils, weighting agents and drilling additives such as emulsifiers, fluid loss agents, wetting agents and brine. The present study aimed to evaluate the impact of exposure to individual ingredients of SBMs on the fish health. To do so, a suite of biomarkers [Ethoxyresorufin-*O*-deethylase (EROD) activity, biliary metabolites, sorbitol dehydrogenase (SDH) activity, DNA damage, heat shock protein] have been measured in pink snapper (*Pagrus auratus*) exposed for 21 days to individual ingredients of SBMs. The primary emulsifier (Emul S50) followed by the fluid loss agent (LSL 50) caused the strongest biochemical responses in fish. The synthetic base oil (Rheosyn) caused the least response in juvenile fish. The results suggest that the impact of Syndrill 80:20 on fish health might be reduced by replacement of the primary emulsifier Emul S50 with an alternative ingredient of less toxicity to aquatic biota. The research provides a basis for improving the environmental performance of SBMs by reducing the environmental risk of their discharge, and providing environmental managers with information regarding the potential toxicity of individual ingredients.

Keywords – Petroleum exploration, Environmental management, Synthetic based drilling mud (SBM), Synthetic base fluids (SBF), Chronic Toxicity, Biomarkers, *Pagrus auratus.*

INTRODUCTION

Drilling muds an essential part of drilling operations for oil and gas exploration. During the drilling process, limited quantities of drill cuttings coated with drilling mud are discharged to the marine environment, a disposal method that requires environmental management as fluid-coated cuttings act as attracting devices for fish. Cuttings generated by using SBMs are oil-wet and tend to clump together in large particles on their discharge in the ocean and settle rapidly in the bottom of sea. (Neff et al. 1987; Boethling et al. 2007: Neff, 2008).

The drill cuttings themselves are considered toxicologically inert; however, there is concern regarding adhering drilling mud, particularly if the cuttings are produced during drilling process with oil based muds (OBMs) or SBMs (Neff et al. 2000). Acute toxicity of SBMs is reported unlikely, as LC_{50} of > 1000 mg/kg has been commonly reported (Patin 1999).

However, data on chronic toxicity is limited. Few studies have demonstrated bioaccumulation of small amount of SBM in molluscs but it was uncertain that animal had actually assimilated some components of the SBM or if it retained chemicals under particulate form in the gills or gut (Neff et al, 2000).

Commercial formulations of SBM vary widely and may include a myriad of different ingredients. The main ingredient of SBM systems are; base oil [synthetic hydrocarbons such as Linear Alpha Olefin (LAO), Poly Alpha Olefin (PAO), Internal Olefin (IO), Linear Alkyl Benzene (LAB) and Paraffin (P) or synthetic chemicals such as ester, ether or acetals], emulsifiers, wetting agents, thinners, weighting agents (barite, clays, carbonates), gelling agents, and concentrated $CaCl_2$ brine. The relative proportions of the different components may vary depending on the type of SBM and the chemistry, geology, and depth of the formation being drilled (Friedheim and Conn 1996). The discharge of drill cuttings with

adhering drilling muds and the possible environmental impacts on marine ecosystems indicate the need for drilling mud systems to be formulated from environmentally friendly raw materials to protect the global environment (Amanullah 2006; Boethling et al. 2007).

Previous studies revealed that two main approaches have been applied so far for toxicological assessment of drilling mud ingredients, the first approach having tested short- term (acute) toxicity on marine organisms by measuring LC_{50} under relatively standardized conditions. The second approach has adapted the methodology for establishing the maximum permissible concentration of pollutants in the environment (Patin and Lesnikov 1988). Because of the variety of ingredients entering in the composition of drilling muds, the resulting toxicity varies greatly. The toxic properties of individual ingredients of drilling muds are mostly dependent on their molecular structures. Cyclic and high molecular weight compounds generally increase the toxicity of the ingredients (Patin 1999; Boethling et al. 2007). Almost 80 % of mud ingredients are considered practically non-toxic because their LC_{50} values exceeded 100 g/L (Neff 2005). However, acute toxicity results tested on algae and invertebrates under laboratory conditions are unable to predict the chronic impacts of these chemicals on higher vertebrates. Furthermore, some of the mud components that are considered non-toxic on the basis acute toxicity testing might demonstrate effects on aquatic organisms upon long-term exposure (Amanullah 2006).

In order to assess if synthetic drilling muds do impact the fish health, a suite of sub-lethal biomarkers can be measured in fish being chronically exposed to weathering drilling muds. Piles of drill cutting with adhering drilling muds create heterogeneity on the sea floor, attracting fish assemblages that adopt the stockpile and associated submerged structures as a permanent residence.

Recent research on two synthetic drilling muds, Syndrill 80:20 and Syndrill 90:10, have shown that Syndrill 80:10 causes less metabolic perturbations in fish chronically exposed to this mud, relative to the closely related Syndrill 90:10 (Bakhtyar and Gagnon 2009). In order to get better understandings of the induction of biomarker responses in fish chronically exposed to SBMs, and to assist in the design of drilling muds with lower impacts on fish health, additional investigations were required. The present study reports on fluorescence measurements of the individual ingredients entering in the composition of the muds, and relates fluorescence observations on individual ingredients to biomarker measurements made in exposed fish. In addition, fish were exposed to individual ingredients of the muds in order to identify the ingredients causing adverse impacts on fish health. This exploratory research does not attempt at simulating effects of drilling fluids in the marine environment but rather aims at informing on the toxic effects of individual ingredients entering in the composition if synthetic based drilling muds. It is expected that the information generated will assist in the design of drilling muds with excellent technical characteristics, high environmental performance and low toxicity to marine biota.

MATERIALS AND METHODS

The two previously tested mud systems Syndrill 80:20 and Syndrill 90:10 were composed of similar type of drilling base fluid, Rheosyn 1416 linear alpha olefin (LAO) which is a mixture of 1-dodecene ($C_{12}H_{24}$ 1-2%), 1-tetradecene ($C_{14}H_{24}$ 14%), 1-hexadecene ($C_{16}H_{32}$ 5%), and of 1-octadecene ($C_{18}H_{36}$ 2-3%). Both mud systems were constituted with different grades and proportions of ingredients including 30 – 40 % weighting agents [(barite ($BaSO_4$) , calcium carbonate ($CaCO_3$) omyacarb fine and omyacarb course], 10 % - 20 % brine ($CaCl_2$) and 0.5 – 5 % other mud additives such as emulsifier (Emul S50), wetting agent (Wetout), fluid loss agents (LSL 50R), fluid control (lubricant) agent (LSL 10), viscosifiers (Bentone 38,

Bentone 40) and lime (Table 1). The mud ingredients were supplied by Rheochem Ltd Henderson, Western Australia (WA) and these ingredients as well as the entire mud formulations are available worldwide. Chemical sources and their exact concentrations used in mud formulation are not described in this publication due to their commercial and confidential nature.

Chemical analysis of water soluble fractions of SBM Components

Water soluble fractions (WSFs) of each mud ingredients were prepared in order to investigate the possible contribution of each ingredient to trace metal concentration in later fish exposure. WSF were prepared in duplicate according to the method described in Tsvetnenko and Evans (2002). Each individual mud ingredient was added slowly to a 1 L Scott bottle filled with de-ionized (DI) water with continuous stirring. Instead of sea water, DI water was intentionally used in order to avoid any interference from seawater salts during the chemical analysis. Bottles were caped and samples were mixed for 24 hrs. Samples were kept standing for at least one hour for phase separation before gentle decanting through bottom tap of the amber Scott bottle to collect the aqueous portion representing the WSF of the mud. WSF samples were analyzed for total recovered hydrocarbons (TRH), polycyclic aromatic hydrocarbons (PAH) and trace metals content. TRH and PAH extractions were performed by using the liquid-liquid extraction according to the method USEPA 3510 (1986). TRH concentrations were determined by conventional gas chromatography - flame ionization detection (GC-FID) according to methods adopted from APHA 5520 F (2005). The acceptable spike recovery for TRH C_{10}-C_{36} was 50 – 150 %. The acceptable relative percentage difference (RPD) was 60 % and the limit of reporting was < 250 µg/L. TRH results are reported in µg/L. Specific PAHs (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene,

benzo(b+k)fluoranthene, benzo(a)pyrene, indeno(1,2,3,c,d)pyrene, dibenzo(a,h)anthracene, benzo(g,h,i)perylene) were extracted according to the method USEPA 3510 (1986) and PAHs concentrations were determined by the method USEPA 8270 C (2006) modified for Selected Ion Monitoring (SIM) by using gas chromatograph – mass spectrometry (GC-MS). The limit of reporting < 0.1 µg/L. and results are reported in µg/L.

Trace elements (metals) concentrations in WSF samples of LSL 10, LSL 50R, barite and bentone 38 (B 38) were determined using inductively coupled plasma atomic emission spectrometry (ICP-AES) and hydride generation technique (ICP-OES) according to USEPA methods 200.7 and 245.2 (2001) as detailed in Bakhtyar and Gagnon (2009). The measured limits of reporting (LOR) for trace elements silver (Ag), beryllium (Be), manganese (Mn), were < 0.001, for aluminium (Al), arsenic (As), boron (B), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), molybdenum (Mo), nickel (Ni), selenium (Se), strontium (Sr), titanium (Ti), vanadium (V), zinc (Zn) were < 0.005, for cadmium (Cd) was < 0.002 and for lead (Pb) and stannous (Sn) were < 0.010 mg/L. Trace metal results are reported in mg/L.

Fluorescence of individual ingredients

Past research has suggested that emulsifiers might be responsible for interference with PAH biliary metabolites in fish, a commonly measured biomarker of exposure to petroleum hydrocarbons (Bakhtyar and Gagnon 2009). Attempts were then made to investigate further which mud ingredients could interfere with biliary PAH fluorescence. For each mud ingredient, an initial solution was prepared by mixing the ingredient in a given volume of distilled water, with the mass of ingredient corresponding to its proportion in the drilling mud Syndrill 80:20. For example, if an ingredient represented 3 % of the weight of the mud

system, then this ingredient would have 30 g mixed in 1 liter of water. All mixtures were mixed with an ultrasonic homogenizer (Heidolph DIAX 900) and centrifuged at 3000 g for 10 minutes on a Jouan CR3i refrigerated centrifuge. The mixture of each chemical was further diluted in a series of concentrations (0.01 %, 0.1 %, 1 %, 10 %, 20 %, 50 % and 100 %) to obtain readable concentrations on spectrofluorimeter.

For the Emul S50, Rheosyn 1416 and Wetout the highest concentrations measured were 1.0 % of the total concentration present in the mud, as higher concentrations caused high turbidity and consequently, invalid readings. However, calcium carbonates [omyacarb fine (Carb 2) and omyacarb course (Carb 40)], barite, LSL 10, LSL 50R, Bentone 38 and lime were returning valid fluorescence measurements at naphthalene, pyrene and benzo-a-pyrene (BaP) wavelengths at high concentrations (10 %, 20 %, 50 % and 100 %).

The fluorescence of the water soluble fractions of each ingredient was determined on a Perkin Elmer LS-45 Luminescence spectrometer. The fluorescence of each ingredient was measured against standard curves using 1-naphthol and 1-hydroxypyrene as standards. These standards have been selected as they represent the wavelengths at which PAH biliary metabolites are measured in fish exposed to petroleum compounds, and the goal of the fluorescent reading was to assess if certain mud ingredients do cause interference with the reading of biliary metabolites. The fluorescence were read at excitation/emission wavelengths of 290/335 nm for 1-naphtol, at 340/380 nm for 1-hydroxypyrene and at 380/430 nm for BaP fluorescence equivalent (Aas et al. 1996). Therefore, the results expressed as 1-naphtol fluorescence equivalents, or 1-pyrenol fluorescence equivalents.

The emulsifier caused high turbidity therefore, only very low concentrations of emulsifier could be measured, that is, concentrations equivalent of less than 10.0 % of the original drilling mud concentrations were used for fluorescence measurements.

CHRONIC TOXICITY TESTING

Fish Exposure

In order to evaluate the relative toxicity of mud ingredients, the fish were exposed for 21 days to each individual ingredient of Syndrill 80:20 and Syndrill 90:10, as well as to the whole mud system Syndrill 80:20 pre-mixed for the exposure. The exposure concentration of each specific ingredient was set to be in similar concentration as for the fish exposed to the entire mud system. That is, the fish would be exposed to the same amount of that ingredient, but only to that ingredient, as it was in the entire mud system aquaria. For each ingredient, a typical concentration as would be present in a mud formulation was selected for fish exposures, and are listed in Table 1.

The juvenile pink snapper were purchased from a local hatchery (Fremantle TAFE, Perth WA). Juvenile fish were selected in order to avoid any reproductive hormones inference with biomarker determinations (Payne et al. 1987). Due to large size of the fish (mean weight ± SEM 333 ± 9.3 g, and mean length ± SEM, 25.7 ± 0.3 cm), only three fish were placed in each aquarium, and were acclimatized in 100 L aquaria (contain 80 L sea water) for two weeks prior to chemical exposures. Fish were exposed to each individual ingredient, and to the entire pre-mixed mud systems in duplicates, that is, six fish were exposed to each treatment in two separate aquaria (3 fish / aquarium). Fish were fed by commercially available fish pellets (Sketting, pellet type Nova ME, 45% protein. 20% lipid, 18% carbohydrates, 8% moisture) at a maintenance ratio of 1% body weight/day during the acclimation and exposure

periods (Palace et al. 1996). Fish handling and the daily 50% sea water renewal was done according to US Environmental Protection Agency document USEPA 821/R-02-013 (2002). Physicochemical characteristics such as pH, salinity and temperature were monitored on daily basis through out the exposures to maintain the water quality.

The fish were exposed in 80 L seawater containing individual drilling mud ingredients with the same proportion as it would be in 4 L complete mud system, that is, each ingredient was in similar concentration if fish were exposed to the entire mud system, or to an isolated mud ingredient. PVC pipes of 18 cm diameter were hanged in one corner of each aquarium and chemicals were placed in the pipes for direct contact of chemicals with water. This setting also helped to avoid the direct contact of animals with the chemicals and prevent air blockage from the surface due to immiscible floating chemical layers (drilling fluid base oil, high viscosity sticky emulsifiers, viscosifier and powdery fluid loss agents).

The floating chemical layers and settled mud components (barite, calcium carbonates) as well as the entire Syndrill 80:20 mud were left undisturbed during daily 50 % water change. Two control groups, negative control and positive control, were run parallel to experimental groups to allow comparison of fish biomarkers between exposed and non-exposed groups. Negative control groups received no chemical treatment whilst positive control groups were injected with BaP at a dose of 1.0 μg chemical/g fish after being anaesthetised with 3-aminobenzoic acid ethyl ester (70 mg/L water) to trigger biomarker responses. The positive control groups were killed four-days post-injection, a maximum period to trigger up responses to BaP (Gagnon and Holdway 1998).

Biomarker's Analysis

Fish exposure was stopped at the end of third week and blood samples were collected from

dorsal veins of live fish using vacutainer. A fresh subsample of blood was immediately sampled for DNA damage assessment. The remaining blood was allowed to clot on ice for 15 to 30 minutes, after which it was centrifuged at 5000 *g* for 10 minutes, and the serum isolated. The fish were then killed by *Iki jime* (spike through the brain) and total weight, and lengths (total, fork and standard) were measured. Gills were removed and immediately stored in liquid nitrogen. Liver samples were examined for any anomaly, weighed and also stored in liquid nitrogen. Similarly, bile was collected and placed immediately in liquid nitrogen. All samples were then transferred in freezer at - 80 ºC until biomarker's analysis.

The condition factor (CF), that is the measure of fattiness of fish (Heath 1995), was calculated according to the equations $CF = 100 \times (\text{gutted weight (g)} / \text{standard length (cm)})^3$. Liver somatic index (LSI), which measures the change, hyperplasia or hypertrophy or both, in liver cells due to chronic exposure to contaminants, was calculated according to equation $LSI = 100 \times [\text{gutted weight (g)} / \text{liver weight (g)}]$.

Ethoxyresorufin-O-deethylase (EROD) Activity

The ethoxyresorufin-*O*-deethylase (EROD) activity was determined according to Hodson *et al*. (1991). The enzyme activity measured on the S9 fraction was expressed as picomoles of resorufin produced per milligram of total protein per minute (pmol R. mg Pr^{-1} min^{-1}).

Biliary Metabolites

Biliary metabolites at three wavelengths; naphthalene, pyrene and BaP, were determined according to the methods Krahn et al. (1986) for pyrene metabolites and Lin et al. (1996) for naphthalene and BaP metabolites. The protein concentrations in the bile were determined

according to Lowry et al. (1951). Biliary metabolites were standardized to biliary proteins and reported as metabolite mg protein-^{1}.

Serum Sorbitol Dehydrogenase (SDH) Activity

Serum sorbitol dehydrogenase (SDH) activity was measured according to the method described in Webb and Gagnon (2007). The change in absorbance was measured on Pharmacia UV - Visible spectrophotometer at wavelength 340 nm. SDH levels of pink snapper are reported in milli-International Units (mU).

DNA damage (Comet Assay)

DNA damage was determined by comet assay as described in Singh et al. (1988). The DNA damage in blood cells was measured through computer software CASP (available at http://www.casp.com). The comet length was measured as the distance between the leading edge of the comet head and the end of the tail. The results are expressed as “tail moment” with a largest tail moments corresponding to an increased in DNA damage (Konca *et al.,* 2003).

Heat Shock Proteins (HSP 70)

The heat shock protein (HSP 70) was measured in the gill tissues of pink snapper as described in Webb and Gagnon (2009). Protein levels in gills were determined according to Lowry *et al.* (1951). The pixels densities obtained from the HSP 70 were quantified using the public domain NIH image program (available on site http://rsb.info.nih.gov/nih-image). HSP 70 in pink snapper were expressed as pixel density per µg of total protein (pixel µg pr $^{-1}$).

Statistical Analysis

Data for each biomarker was tested for normality of distribution and homodcedasticity of variances using SPSS statistical package (version 17) for Windows. All data required log transformation to achieve normality. Each treatment was run in duplicate and consequently, the results for duplicate tanks were compared using a t-test. In all cases, there were no differences ($p > 0.05$) between duplicate aquariums, and for each treatment data from both aquariums were pooled for subsequent statistical analysis. Data was compared using the one-way analysis of variance (ANOVA), and where differences were found between groups, Dunnett's post-hoc test ($p \leq 0.05$) was used to compare treatment groups to the negative control group. Data were presented as means ± standard error of the mean (SEM).

RESULTS

Chemical Analysis

TRH concentrations were measured in WSF samples of Emul S50, Rheosyn 1416 and Wetout. No significant PAHs were detected in any of the WSF samples. Most trace metals (Ag, As, Be, Cd, Co, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Sn, Se, Ti, V and Zn) analyses were below the limit of reporting in all WSF samples. However, low level of boron (B) was detected in the WSF of LSL 10, LSL 50R, Bentone 38 and barite. Whereas significant levels of Ba and Sr were determined in the WSFs of barite and Bentone 38, respectively (Table 2).

Fluorescence of drilling mud ingredients

The primary emulsifier Emul S50 had the highest fluorescence readings at three wavelengths relative to other mud ingredients, followed by barite and lime (Fig. 1). At 100% concentration

LSL 50R showed the highest reading at naphthalene wavelength only. Rheosyn 1416, Wetout, Bentone 38 and Carb 2 showed low fluorescence at the all wavelengths (Fig. 1 A, B & C).

Water Physicochemistry

The physiochemical measurements of salinity, temperature and pH remained stable through out the acclimation and exposure periods (Table 3). The good water quality resulted in the good physical condition of the fish, which were feeding well during the entire experiment.

Biomarker Analysis

Physiological Indices

No significant difference was detected between the CFs of exposed pink snappers ($p > 0.351$) relative to the negative control group. Fish exposed to individual mud ingredients had similar LSI ($p > 0.05$) relative to the negative control group, with the exception of LSI of barite exposed fish which was higher in the barite-exposed group than in the negative control group ($p > 0.009$; Table 4).

EROD Activity

Significant differences in EROD activity were noticed for fish exposed to Emul S50, LSL 10 and Bentone 38. Pink snapper exposed to Emul S50 had the highest EROD activity relative to negative control group (2.3 times; $p < 0.000$). EROD activity of LSL 10 and Bentone 38 exposed groups were 1.8 and 1.7 times higher than the negative control group ($p = 0.001$ and $p = 0.012$, respectively). Fish exposed to all other individual mud ingredients did not show significant ($p > 0.05$) EROD induction relative to the negative control group. However, the positive control group had a significantly higher ($p = 0.003$) EROD activity relative to the

negative control group, demonstrating the ability of juvenile pink snapper to exhibit EROD induction (Fig. 2).

Biliary Metabolites

At the naphthalene wavelength, significant fluorescence was detected only in the fish exposed to the Syndrill 80:20 ($p = 0.002$) and those exposed to the ingredients Emul S50 ($p = 0.001$), Wetout ($p = 0.030$) and LSL 10 ($p = 0.019$). Fish exposed to Emul S50 showed the highest fluorescence at naphthalene wavelength with 3.2 time higher fluorescence than the unexposed control group (Fig. 3A).

Fluorescence at the pyrene wavelength was also significantly higher for fish exposed to the mud Syndrill 80:20 ($p = 0.000$), as well as those fish exposed to the individual ingredients Emul S50 ($p = 0.000$), Wetout ($p = 0.002$) and LSL 10 ($p = 0.035$) relative to the negative control group. Fluorescence levels of these groups at pyrene wavelengths were 2.3 times, 4.2 times, 2.0 times and 1.7 times, respectively, higher than in the negative control group (Fig. 3B).

Relative to the negative control group, no significant fluorescence were detected at the BaP wavelength for any of the exposed groups. However, the positive control group had fluorescence levels 104 times higher than the negative control group ($p < 0.000$, Fig. 3C), demonstrating the biochemical responsiveness of juvenile pink snapper to a model contaminant. .

Serum Sorbitol Dehydrogenase (SDH) Activity

Fish exposed to the whole mud system Syndrill 80:20 as well as those exposed to the ingredient Emul S50 demonstrated significantly ($p = 0.043$ and $p = 0.006$) elevated SDH activity relative to the negative control group. The BaP injected positive control group also demonstrated high ($p = 0.008$) SDH activity relative to the negative control group. Fish exposed to all other individual ingredients were statistically similar to the unexposed negative control group ($p \geq 0.05$; Fig. 4).

DNA Damage

A significantly higher DNA damage was detected in the fish exposed to Syndrill 80:20, Emul S50, LSL 10, LSL 50R, as well as in the positive control group ($p \leq 0.001$), relative to negative control group. DNA damage in fish exposed to the other individual ingredients was not significantly higher than the negative control group ($p \geq 0.05$; Fig. 5).

Heat Shock Protein (HSP 70)

No significance differences were recorded in HSP 70 expressions between the exposed groups and the negative control group ($p = 0.117$; Fig. 6).

DISCUSSION

The discharge at sea of rock cuttings coated with drilling muds created heterogeneity of habitat, attracting fish assemblages which may be exposed chronically to the degrading drilling muds. It is not known if the health of the fish is affected by chronic exposure to drilling muds under field conditions however, other studies have demonstrated that fish health is affected by chronic exposure to drilling muds under laboratory conditions, and that biliary fluorescence is one of the obvious biomarker responses in drilling mud-exposed fish

(Bakhtyar and Gagnon 2009). Consequently, the present study proceeded with the initial screening of the fluorescence of individual ingredients. The assessment of exposure to individual ingredients on fish health, as conducted in the present study, aimed at the identification of the ill-causing ingredients in order to permute these ingredients with more environmentally acceptable constituents, resulting in ‘greener’ products with excellent technical characteristics, high environmental performance and low toxicity.

The CF and LSI are general and non-specific indices of fish health following long-term exposure under field conditions, but changes in their values are rarely observed under laboratory conditions (van der Oost et al. 2003). In the present study, no changes were observed in these parameters, except for the LSI of barite-exposed pink snapper which might be related to the barium content of the barite. No previous studies could corroborate that exposure to barium results in increased LSI in fish however, this sparingly soluble mineral has been identified as one of the least toxic ingredients of drilling muds (Neff 1987; Neff 2008), with the suspended particulates in the barite causing more detrimental effects to respiratory surfaces of aquatic organisms than the chemicals themselves (Carls and Rice 1984).

The activity of the liver detoxification enzymes, as measured by EROD activity, was increased in the groups exposed to Emul S50, LSL 10 and Bentone 38, although the maximum induction was at most of 2.3 times over the negative control group. EROD induction is mostly used as an early sign for potentially harmful effects of many organic pollutants (van der Oost et al, 2003). It is therefore not surprising that only weak induction have been observed in the present exposures. Under field situations where biomarker variability is higher than under laboratory conditions, it is unlikely that EROD activity could provide a good discriminatory power between drilling mud exposed and reference fish. When

drill cuttings are discharged on the seafloor, they are usually coated with drilling muds and crude oil, and the weak EROD induction caused by Emul S50, LSL 10 and Bentone 38 is likely to be masked by the stronger induction from exposure to the petroleum compounds.

It is possible that some water-soluble ingredients of the drilling muds are assimilated by the fish. Metabolisation of these compounds can lead to the presence of metabolites in the bile, which have the potential to interfere with the reading of PAH metabolites in field-collected animals. The presence of biliary chemicals fluorescing at the PAH wavelengths in SBM-exposed animals do not imply that these chemicals are PAH metabolites of petroleum origin. Rather, it simply points to the presence of unidentified chemicals fluorescing at the PAH wavelengths. Therefore, the presence of metabolized compounds in the biliary secretions has been included in the present study to assess if compounds originating from the drilling muds appeared in the bile, which could potentially interfere with the PAH determinations routinely done in field sampling. If the biliary fluorescence from fish exposed to the various drilling muds or to the ingredients shows no interference with PAH metabolites, then this biomarker will be able to discriminate exposure to drilling muds from exposure to drill cuttings containing petroleum compounds. The high fluorescence of Emul S50 translated in the high fluorescence of Emul S50 exposed fish. Although it cannot be ascertained from the present study design that there is a cause and effect link between Emul S50 exposure and high biliary fluorescence in pink snapper, the weight of evidence suggests that the primary emulsifier or its metabolites might have been eliminated via the bile. While the metabolised product might have a higher or lower fluorescence relative to its parent compound, the initial fluorescence measurements of the mud ingredients shows to be a useful screening tool to relate fluorescence of individual drilling mud ingredient to the likely consequences on fish biliary fluorescence measurements.

Serum-SDH activity is a biomarker of morphological hepatic changes with 75% better accuracy than other hepatic enzymes (Travlos et al. 1996). The measurement of sorbitol dehydrogenase activity in serum has been shown to be a precise and repeatable measure of sub-lethal liver damage in vertebrate species, which is organ-specific but not disease-specific (Levine et al. 1978). Significant increase in SDH activity in Emul S50 and Syndrill 80:20 exposed groups relative to other groups suggest liver damage. Normally, the cytoplasmic-enclosed SDH would remain in the cellular space however, upon rupture of the cellular membrane, the SDH enzyme is released and detectable in the blood stream. Disruptions of other cellular functions such as EROD activity can be associated to the loss of liver integrity, and it is recommended to measure SDH along other biomarkers that are related to liver metabolism (Holdway et al. 1994).

The single cell gel electrophoresis or comet assay method for DNA strand break detection in individual cells is increasingly becoming a popular biomonitoring tool in environmental contamination in both *in vivo* and *in vitro* studies (Fairbrain et al. 1994). Elevated DNA damage in pink snapper exposed to syndrill mud, Emul S50, LSL 10, LSL 50R or barite suggests that these chemicals might interact significantly with the DNA molecule. It has been commonly observed that many toxic chemicals cause strand breaks in DNA, either by direct interaction or by causing secondary concomitant types of DNA alterations (van der Oost et al. 2003). However, the observed increases in DNA damage levels were not elevated compared to other laboratory studies where DNA damage often increased by over 500 % of control groups (van der Oost et al. 2003). The modest increase in DNA damage measured in the present study might be related to the similar stress proteins levels in all groups. The stress proteins, which are involved in the protection and repair of the cell, were at similar levels in all exposed and negative control groups indicating that whilst there is some DNA damage

occurring in some exposed fish, the biological significance of this damage is not high enough to warrant induction of the HSP repair mechanism.

Previous studies have established that 20 h of mixing the mud ingredients at slow speed would achieve maximal level of dissolved hydrocarbons in the aqueous phase (Anderson et al. 1974; Singer et al. 2000). This approach was practiced in preparation of the WSFs. WSF analytical results demonstrated the extent of solubility of individual mud components in the water column and potential bioavailability of hydrocarbons and metals in the marine environment.

Barite is a prominent mineral component in the mud system (Starczak et al. 1992) and although it is considered one of the least toxic ingredients (Patin 1999), it may cause damage to benthic organisms by abrasion and clogging (Neff 1987). The detection of Al, B and high levels of Ba and Sr in WSF sample of barite, in addition to significant biological response in LSI and DNA damage in the barite exposed group indicate that the inclusion of barite as a drilling mud ingredient may contribute to the mud system overall toxic effect on marine organisms.

The solubility of barite in sea water has been reported to be about 80 µg/L (Neff 2005). Barium as barite is lethal to embryos of crab *Cancer anthonyi* at concentrations greater than 1000 mg/L (Macdonald et al. 1988). This concentration is 20,000 times higher than aqueous solubility of barium in sea water and therefore, any adverse effects probably associated with physical effects of fine-grained barite particles (Neff 2005; Neff 2008).

The health of Pink snapper exposed to Rheosyn 1416 base oil was not affected by the chronic exposure, as measured by the biomarkers including EROD activity, biliary metabolites, SDH activity, DNA damage and HSP 70. The results suggest that this particular base oil is not

biologically active, and consequently, has a very low toxicity. Olefins, used in SBMs are relatively large linear chain molecules and are highly hydrophobic chemicals therefore; they do not permeate membranes efficiently (Neff et al. 2000). The bioaccumulation of olefins occurs very slowly and their equilibration in marine animals may require more than a year of continuous exposure (Hawker and Connell 1985; 1986). Comprehensive studies (NRC 1983; Neff 1987; Swan et al. 1994) on environmental impacts of drilling muds reveal that marine organisms are more sensitive to the suspended particulate phase of drilling muds than to the water soluble phase. In accordance with these studies, the low levels of TRH in WSF samples support the low potential of Rheosyn 1416 to contribute significant hydrocarbons enrichment to the marine environment. Therefore, it is unlikely that bioaccumulation of Rheosyn 1416 base oil represent an environmentally important risk to marine organisms.

CONCLUSION

The study demonstrates that the various drilling mud ingredients induce a wide spectrum of biological reactions from absence of toxic effects to clear biomarker responses. Of all the mud ingredients, the emulsifier caused the strongest biomarker responses which support previous findings that emulsifiers represent the greatest environmental risk because of their potential toxicity (Rye et al. 1997). Rheosyn 1416 base oil was the most inert of the ingredients tested, and did not alter any of the biomarker measured in this study. The environmental performance of SBMs might be improved by the replacement of aggressive emulsifiers with alternative, less biologically active emulsifiers. The results from the present study might assist in the development of environmentally acceptable drilling mud formulations. The information generated by the present research represents a starting point on which the ecotoxicological and environmental management of drilling fluid discharges in the marine environment can be based.

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Figure 1. Fluorescence levels of selected drilling mud ingredients and whole mud Syndrill 80:20 and Syndrill 90:10 at naphthalene, pyrene and BaP wavelengths. **A**. Fluorescence at naphthalene wavelengths. **B**. Fluorescence at pyrene wavelengths. **C**. Fluorescence at BaP wavelengths.

Figure 2. Hepatic EROD activity in liver of juvenile pink snapper exposed to drilling mud ingredients and whole mud Syndrill 80:20 for 21 days. *indicates a significant difference ($p < 0.05$) from negative control fish.

Figure 3. Biliary metabolite levels in the bile of fish exposed to mud ingredients and whole mud Syndrill 80:20 for 21 days. * indicates a significant difference ($p < 0.05$) from negative control fish. **A**. Biliary metabolites at naphthalene wavelength. **B**. Biliary metabolites at pyrene wavelengths **C**. Biliary metabolites at BaP wavelengths, Positive control fish were injected with BaP in order to induce the presence of biliary metabolites at high levels.

Figure 4. Sorbitol dehydrogenase (SDH) levels in fish blood serum exposed to selected drilling mud ingredients and whole mud syndrill 80:20 for 21 days. * indicates a significant difference ($p < 0.05$) from negative control fish.

Figure 5. DNA Damage activity in blood of juvenile pink snapper exposed to selected drilling mud ingredients and whole mud Syndrill 80:20 for 21 days. *indicates a significant difference ($p < 0.05$) from negative control fish.

Figure 6. Heat Shock protein levels measured in the gills of juvenile pink snapper exposed to selected drilling mud ingredients and whole mud Syndrill 80:20 for 21 days. *indicates a significant difference ($p < 0.05$) from negative control fish.

1 **Table 1:** Ingredients used in formulation for drilling mud Syndrill 80:20 and Syndrill 90:10, and selected concentrations in

2 the exposure tanks.

Product's Function	Brand Name	Syndrill 80:20 composition	Exposed Conc. g/L	Syndrill 90:10 composition	Exposed Conc. g/L
Base Oil	Rheochem	35 % - 55 %.	18.20	35 % - 55 %.	26.25
Water	$CaCl_2$ Brine	10 - 20 %	_	10 - 20 %	_
Primary Emulsifier	Emul S50	< 5 %	1.05	< 5 %	1.30
Secondary Emulsifier	Wetout	_	_	< 5 %	0.40
Viscosifier	Bentone 38	< 5 %	1.05	< 5 %	1.30
Fluid Control Agent	LSL 10	< 5%	0.25	_	_
Fluid Loss Agent	LSL 50	_	_	< 5 %	0.35
Lime	$CaCl_2$	10 - 20 %	_	10 - 20 %	_
Barite	$BaSO_4$	30 - 40 %	21.00	_	_
Calcium Carbonate (Carb 2)	Omyacarb 2	_	_	7 - 10 %	3.65
Calcium Carbonate (Carb 40)	Omyacarb 40	_	_	20 - 30 %	11.00

3

4

5 **Table 2**. Chemical analysis (mean ± SEM) of drilling mud ingredients. (ND = peak not detected).

SBM Ingredients	pH	% TRH Recovery (µg/L)	PAHs (µg/L)	Trace Metals (mg/L)			
				Al	**B**	**Ba**	**Sr**
Rheosyn 1416	6.60	0.11 ± 0.08	ND	-	-	-	-
Emul S50	5.06	1.18± 0.32	ND	-	-	-	-
Wetout	5.91	1.74 ± 0.21	ND	-	-	-	-
LSL 10	7.13	ND	ND	< 0.005	0.038 ± 0.001	0.11 ± 0.002	< 0.005
LSL 50R	6.65	ND	ND	< 0.005	0.035 ± 0.003	0.021 ± 0.003	< 0.005
Barite	5.79	ND	ND	0.014 ± 0.002	0.041 ± 0.001	0.61 ± 0.002	1.4 ± 0.001
Bentone 38	6.74	ND	ND	< 0.005	0.032 ± 0.002	1.08 ± 0.004	0.23 ± 0.002

6

7 **Table 3.** Physicochemical parameters (mean ± SEM) of the seawater during the acclimation (N = 14 days) and experimental periods (N = 21

8 days).

Treatment	Salinity (ppt) during Acclimation	Salinity (ppt) during Exposure	pH during Acclimation	pH during Exposure	Temp (ºC) during Acclimation	Temp (ºC) during Exposure
Negative Control	33.4 ± 0.07	33.2 ± 0.31	6.97 ± 0.02	7.10 ± 0.07	21.5 ± 0.13	21.8 ± 0.43
Syndrill 80:20	33.3 ± 0.09	33.8 ± 0.12	7.11 ± 0.05	6.89 ± 0.27	22.8 ± 0.35	21.9 ± 0.45
Rheosyn 1416	33.8 ± 0.09	33.9 ± 0.02	7.23 ± 0.03	6.99 ± 0.21	21.6 ± 0.05	20.7 ± 0.58
Emul S50	34.1 ± 0.03	33.6 ± 0.07	6.88 ± 0.01	7.10 ± 0.04	21.7 ± 0.42	20.9 ± 0.35
Wetout	33.9 ± 0.17	34.2 ± 0.11	7.17 ± 0.04	7.12 ± 0.17	22.5 ± 0.03	22.3 ± 0.11
LSL 10	33.7 ± 0.09	33.0 ± 0.09	7.11 ± 0.11	7.19 ± 0.02	21.8 ± 0.03	21.9 ± 0.13
LSL50R	34.1 ± 0.03	34.4 ± 0.03	6.79 ± 0.23	7.15 ± 0.14	21.9 ± 0.08	22.1 ± 0.18
Barite	34.3 ± 0.04	34.6 ± 0.03	6.98 ± 0.11	7.10 ± 0.08	22.2 ± 0.07	21.9 ± 0.03
Bentone 38	33.1 ± 0.07	33.9 ± 0.06	7.15 ± 0.08	6.88 ± 0.11	21.8 ± 0.15	22.1 ± 0.07
Positive Control	33.6 ± 0.13	33.8 ± 0.08	7.08 ± 0.05	6.90 ± 0.05	21.7 ± 0.08	21.9 ± 0.12

9

10 **Table 4:** Physiological parameters (mean ± SEM) measured on juvenile pink snapper. ** indicates a statistical difference between this group and

11 the negative control group.

Fish Exposure	N	Total Weight (g)	Total Length (cm)	Condition Factor (CF)	Liver Somatic Index (LSI)
Negative Control	5	332 ± 41.2	26.1 ± 0.79	2.78 ± 0.07	0.80 ± 0.08
Syndrill 80:20	5	325 ± 22.2	24.8 ± 0.47	3.20 ± 0.23	1.19 ± 0.14
Rheosyn 1416	5	329 ± 25.0	26.2 ± 0.60	2.81 ± 0.08	1.15 ± 0.13
Emul S50	6	347 ± 18.5	26.0 ± 0.64	3.07 ± 0.18	1.12 ± 0.13
Wetout	5	391 ± 44.8	27.1 ± 0.92	2.89 ± 0.17	1.08 ± 0.12
LSL 10	5	324 ± 34.2	25.4 ± 1.31	2.93 ± 0.15	1.19 ± 0.09
LSL50R	6	324 ± 24.2	25.4 ± 0.61	3.10 ± 0.16	1.12 ± 0.14
Barite	5	300 ± 42.2	25.4 ± 1.07	2.83 ± 0.05	1.38 ± 0.17**
Bentone 38	6	325 ± 16.9	25.4 ± 0.36	3.13 ± 0.13	1.21 ± 0.11
Positive Control	6	338 ± 30.7	25.1 ± 0.89	3.20 ± 0.24	0.94 ± 0.10

12