Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Ecotoxicology and Environmental Safety 59 (2004) 300–308
Ecotoxicology and Environmental Safety
http://www.elsevier.com/locate/ecoenv

Oil dispersant increases PAH uptake by fish exposed to crude oil

Shahunthala D. Ramachandran,[a] Peter V. Hodson,[a,*] Colin W. Khan,[a] and Ken Lee[b]

[a] *School of Environmental Studies, Queen's University, Kingston, Ont., Canada K7L 3N6*
[b] *Department of Fisheries and Oceans, Bedford Institute of Oceanography, Centre for Offshore Oil and Gas Environmental Research, Halifax, NS, Canada B2Y 4A2*

Received 14 May 2003; received in revised form 12 August 2003; accepted 25 August 2003

Abstract

The use of oil dispersants is a controversial countermeasure in the effort to minimize the impact of oil spills. The risk of ecological effects will depend on whether oil dispersion increases or decreases the exposure of aquatic species to the toxic components of oil. To evaluate whether fish would be exposed to more polycyclic aromatic hydrocarbon (PAH) in dispersed oil relative to equivalent amounts of the water-accommodated fraction (WAF), measurements were made of CYP1A induction in trout exposed to the dispersant (Corexit 9500), WAFs, and the chemically enhanced WAF (dispersant; CEWAF) of three crude oils. The crude oils comprised the higher viscosity Mesa and Terra Nova and the less viscous Scotian Light. Total petroleum hydrocarbon and PAH concentrations in the test media were determined to relate the observed CYP1A induction in trout to dissolved fractions of the crude oil. CYP1A induction was 6- to 1100-fold higher in CEWAF treatments than in WAF treatments, with Terra Nova having the greatest increase, followed by Mesa and Scotian Light. Mesa had the highest induction potential with the lowest EC_{50} values for both WAF and CEWAF. The dispersant Corexit was not an inducer and it did not appear to affect the permeability of the gill surface to known inducers such as β-napthoflavone. These experiments suggest that the use of oil dispersants will increase the exposure of fish to hydrocarbons in crude oil.
© 2003 Published by Elsevier Inc.

Keywords: Crude oils; Dispersant Corexit EC9500; CYP1A; EROD activity; Water-accommodated fraction

1. Introduction

Dispersants have been in use in oil spill clean-up since the 1950s. Chemical dispersion of oil in spill contingency exercises was not favored due to the toxicity of the early dispersant formulations to aquatic organisms. Laboratory studies of their observed toxicity (Linden, 1974; Hartwick et al., 1982; Carr and Linden, 1984) were further validated by field reports (Smith, 1968) from the Torrey Canyon and more recently the Sea Empress oil spills for which dispersants were used (Lewis and Aurand, 1997). However, many effective and less toxic dispersants have since been developed.

Risks to aquatic organisms from chemical dispersion could arise from exposure to the dispersant as well as to the dispersed oil. Much research has been conducted on the toxicity of dispersants (Nelson-Smith, 1977; Singer et al., 1993; Law, 1995; Carr and Linden, 1984; Cotou et al., 2001), but in general less is known about the combined effects of oil and dispersants (Linden, 1975; Cohen et al., 2001; Gagnon and Holdway, 2000). Aquatic organisms are unlikely to be exposed to dispersant alone but instead to both dispersant and oil in combination, which may either exacerbate or mitigate toxic effects (Getter and Baca, 1984).

Dispersants are essentially surfactants comprising anionic and nonionic molecules in fixed ratios that render both hydrophilic and hydrophobic properties to the dispersant. Their purpose is to orient at the oil–water interface and lower interfacial tension, thus facilitating the formation of small ($<100\,\mu m$) mixed oil–surfactant micelles (Canevari, 1978). These oil emulsion droplets are driven into the water column forming a plume, thus breaking up the slick. The observed increased toxicity of dispersed (chemically or mechanically) oil has been attributed to particle size in dispersion (Bobra et al., 1989) and to aromatic hydrocarbon content (Anderson et al., 1974). The primary route of hydrocarbon uptake is via the gills

*Corresponding author. Fax: +1-613-533-6617.
E-mail address: hodsonp@biology.queensu.ca (P.V. Hodson).

0147-6513/$ - see front matter © 2003 Published by Elsevier Inc.
doi:10.1016/j.ecoenv.2003.08.018

(Thomas and Rice, 1981); thus, the dissolved fraction would be the most available form to animals.

The majority of studies of the toxicity of oil and oil–dispersant treatments concerns their impacts on invertebrate species, presumably because they are relatively sessile compared to fish. However, recent studies of the impacts of oil from the Exxon Valdez spill on embryonic and larval stages of salmon and herring demonstrated both exposure and chronic toxicity in situ. The coincidence of spawning and oil deposition in spawning shoals resulted in CYP1A induction, blue sac disease of larvae, and recruitment failure, and effects were associated with exposure to the polycyclic aromatic hydrocarbon (PAH) fractions (Carls et al., 1999). The risk to fish from oil and oil–dispersant treatments can be assessed in terms of exposure to PAH, and changes in exposure after the use of dispersants. PAH exposure can be estimated by a standardized laboratory bioassay of CYP1A induction in juvenile rainbow trout, *Oncorhynchus mykiss* (Hodson et al., 1996).

Dispersant effectiveness is usually influenced by a number of factors, which include sea energy, temperature, salinity, and the nature of the crude oil itself. Crude oils are a complex matrix of organic compounds, which, upon spilling, evaporate or dissolve and disperse into the water column. Light oils are known to have a higher proportion of volatile aromatics, some of which dissolve more readily (Volkmann et al., 1994). Dispersant effectiveness on these oils is less noticeable than in medium and heavy crude oils that comprise a higher proportion of longer and branched carbon chains with reduced solubility.

The objective of this study was to determine the effects of the dispersant Corexit 9500 on the exposure and toxicity to fish of hydrocarbons from three kinds of crude oil of varying viscosities. Fish were exposed to the water-accommodated fraction (WAF) and chemically enhanced (dispersed) WAF (CEWAF) of the oils. The hepatic CYP1A activity in juvenile rainbow trout exposed to WAF and CEWAF was used to measure the changes in exposure to dissolved and dispersed PAH created by the use of dispersant. Lethality was used as an index of toxicity. Total petroleum hydrocarbon (TPH) and PAH concentrations in the test solutions were also measured as an index of actual exposure concentrations.

2. Materials and methods

2.1. Experimental design

Tests (48 h) were conducted to assess the effects of Corexit on the extent of hydrocarbon uptake, which was indicated by CYP1A induction in fish exposed to mixtures of WAF and CEWAF. Test conditions were determined by preliminary assays to evaluate the lethality and CYP1A induction potential of Corexit alone, and the duration of the tests required to produce maximal CYP1A activity.

2.2. Crude oil and dispersant

Three kinds of weathered crude oil of varying viscosities were tested (1) Mesa sour crude (viscosity of 42.3 cP), (2) Terra Nova crude (viscosity of 50.1 cP), and (3) Scotian Light crude (viscosity of 3.76 cP) at 21°C. Mesa oil was weathered by evaporation (sparging with air for 130 h) to simulate the loss (approximately 14%) of volatile components at sea shortly after a spill (Hodson et al., 2002). The Terra Nova and Scotian Light crude oils were not weathered. Dispersant-type Corexit EC9500 was used for dispersing the oils. Corexit 9500 is a hydrocarbon-based reformulation of water-based Corexit 9527 and is meant to be used on higher viscosity oils and emulsions. The acute aquatic toxicity of Corexit 9500 was reported to be not much different from that of Corexit 9527and Corexit 9554 (Singer et al., 1996).

2.3. Fish stock

Juvenile rainbow trout (8–10 weeks of age) were obtained from a trout farm (Rainbow Springs, Thamesford, ON, Canada) and acclimatized for at least 1 week in dechlorinated water (12–15°C) prior to the bioassays. During acclimation, the trout were fed daily with a commercial fish food (Martins Feed Mills, ON, Canada) daily at a rate of 3% body weight per day. Feed was withheld 48 h prior to bioassay and throughout the exposure period.

2.4. Exposure experiments

2.4.1. Preparation of WAF and CEWAF

The WAF was prepared fresh daily by the stirring of crude oil with dechlorinated municipal water at a ratio of 1:9 in sealed containers with minimum head space for 18 h at 18°C. The vortex was adjusted to no more than a third of the height of the mixture from the oil–water interface (Singer et al., 2000). This particular ratio of oil to water has been reported as the optimum to maximize the TPH content of the water column (Gagnon and Holdway, 2000). The mixture was allowed to settle for 1 h for the separation of the water and oil phases, and the water phase was drained off for testing via a tap at the bottom of the tank.

CEWAF was made by mixing oil and water in the same ratio as that for WAF and by stirring for the same duration. The dispersant Corexit 9500 was added with a Pasteur pipette to the surface of the oil–water mixture at the recommended ratio of 1:20 dispersant:oil (Gilbert,

1996) and allowed to stir for an additional hour. The resulting solution contained droplets of dispersed oil, and was allowed to settle for another hour before the cloudy emulsion layer at the bottom was drained.

2.4.2. Exposure group

For each oil treatment, groups of five fish were exposed to a series of WAF and CEWAF concentrations in 10 L of water. Waterborne β-napthoflavone (10 μg/L in 10 L), a known CYP1A inducer, served as a positive control while Corexit EC9500, mineral oil, and dechlorinated municipal water were used as negative controls. All solutions were renewed every 24 h.

Exposure concentrations causing CYP1A induction and lethality were determined from range-finder tests. Solution concentrations ranged from 0.0001 to 0.56% of WAF or CEWAF (v/v) for Mesa, 0.0001–0.10% of WAF or CEWAF (v/v) for Terra Nova; and 0.001–1.0% of WAF or CEWAF (v/v) for Scotian Light. The exposure duration was fixed at 48 h because preliminary tests showed that 48 and 96 h of exposure caused comparable CYP1A induction. At the end of each 48 h test, exposed fish were sacrificed and their livers removed for the CYP1A assay. Physicochemical factors (temperature, pH, dissolved oxygen, and conductivity) were measured daily in each experiment. The concentration of ammonia was measured at the end of each test. Test conditions are presented in Table 1.

2.5. EROD assay

CYP1A activity (ethoxyresorufin-*o*-deethylase, or EROD, activity) was assessed by a fluorometric method that measures the rate of deethylation of ethoxyresorufin (Hodson et al., 1996, modified by Fragoso et al., 1998). Livers were homogenized in HEPES/KCl buffer at pH 7.4 and centrifuged at 9000*g* for 20 min at 4°C to isolate the microsomes (S9 fraction), which were quick-frozen in liquid nitrogen and stored at −86°C until assayed. The rate of resorufin production was measured using a microplate spectrofluorometer at excitation and emission wavelengths of 530 and 586 nm, respectively. The values were normalized against crude protein concentrations assayed by the Bio-Rad Protein Kit and measured on a microplate spectrophotometer at 600 nm. All assays were performed in triplicate. EROD activity was expressed as picomoles of resorufin produced per minute per milligram protein in the S9 fraction (pmol/min/mg Pr). EROD values were normalized to water controls. The mineral oil exposures provided a baseline induction.

2.6. Analysis of hydrocarbons

Water samples of 250 and 300 mL were taken during the daily renewal for TPH and PAH analysis, respectively. Samples for PAH extraction were spiked with 1.0 mL of surrogate standard comprising nine PAHs prior to solvent extraction. Samples for TPH analysis were extracted in three 50-mL lots of hexane, while those for PAH were extracted in three 20-mL lots of AR-grade dichloromethane and dried by filtration through sodium sulfate. The extract was concentrated by roto-evaporation and further reduced to 1.0 mL by drying with compressed nitrogen. TPHs were analyzed by gas chromatography, and concentrations in three boiling point fractions, C10–C16, C 16–C34, and C34–C50, were calculated from calibration curves derived for the C10, C16, and C34 hydrocarbons. The analytical method used was an adaptation of a Canadian Council of Ministers of the Environment Tier 1 Method (CCME, 2000).

Dichloromethane extracts for PAH analysis were sent to the Centre for Offshore Oil and Gas Environmental Research (COOGER–CREPGE), Bedford Institute of Oceanography (Nova Scotia, Canada), for analysis by gas chromatography/mass spectrometry (Garcia-Blanco et al., 2001).

2.7. Statistical analysis and LC_{50} calculations

Statistical analyses were performed using SigmaStat software. For each oil, EROD activity values were log transformed to achieve a normal distribution, as demonstrated by the Kolmogorov–Smirnov normality test and the Levene median equal variance test. One-way analysis of variance with treatment as a factor was applied to detect differences among treatments (control, WAF, and CEWAF). The *post hoc* Bonferroni multiple comparison test was used to identify treatment concentrations that were significantly different from each another. Median effects concentrations (EC_{50} as percentage of maximum EROD activity) for the WAF and CEWAF exposures of each oil were calculated from induction curves using Graph Pad–Prism software to fit a linear regression.

Table 1
Test conditions for juvenile trout 48-h static bioassays with daily renewal

Fish weight (g)	pH	Temperature (°C)	Conductivity (μS/cm)	Dissolved oxygen (% saturated)	Total ammonia (ppm)
2.62 ± 1.34	7.98 ± 0.31	13.7 ± 1.2	265 ± 41	94.6 ± 13.7	0.55 ± 0.18

$n = 2$ days × three oils × 14 buckets; mean ± SD.

3. Results

3.1. Toxicity of Corexit EC9500, WAF, and CEWAF

The 96-h LC_{50} of Corexit EC9500 was estimated to be between 100 and 1000 mg/L. There was no increase in EROD activity at dispersant concentrations ranging from 1.0 to 10,000 mg/L even though there was lethality at the higher concentrations. In terms of toxicity, Scotian Light was the most toxic, as 1% (v/v) CEWAF was lethal. CEWAF treatments for both Mesa and Terra Nova were lethal at 10% (v/v). There was no mortality in all WAF treatments up to 100% (v/v).

3.2. EROD activity in fish exposed to WAF and CEWAF

EROD activity in trout exposed to all three crude oils increased in response to CEWAF exposure at concentrations of 0.0001 (v/v) to 0.32% (v/v) (Fig. 1). Scotian Light concentrations above 0.32% were lethal. Fish in both WAF and CEWAF treatments showed up to a 60-fold higher EROD activity than fish exposed to mineral oil and water controls ($P<0.05$, Bonferroni pair-wise comparison). In both the WAF and CEWAF treatments for all three oils, EROD activity increased in response to increasing exposure concentrations up to 0.1% (v/v). Further increases in concentration (>0.1%) for CEWAF treatments resulted in decreased activity.

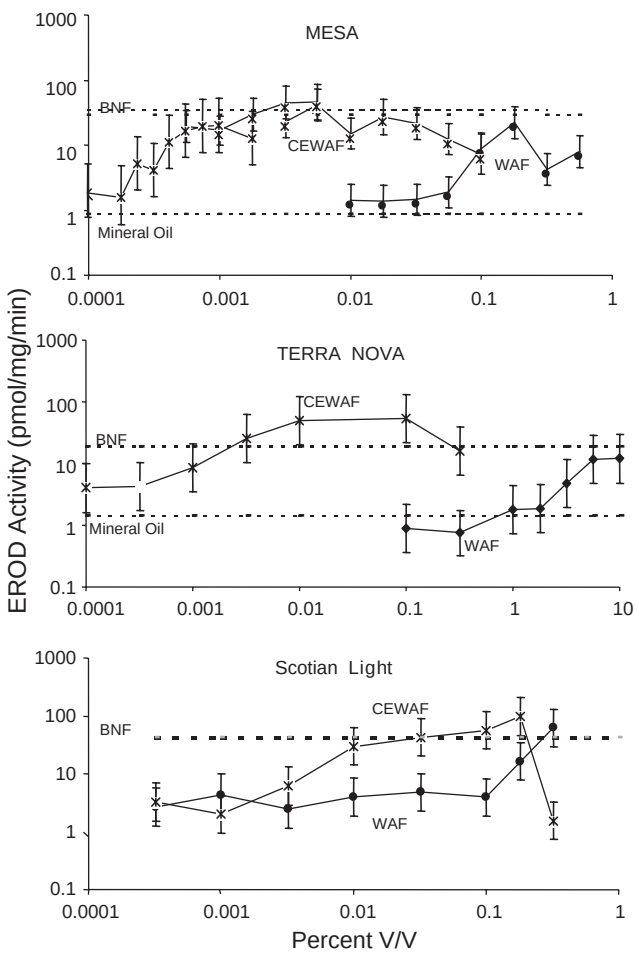

Fig. 1. EROD activity (pmol/min/mg protein) in trout exposed to a range of WAF or CEWAF concentrations from crude oils or to one concentration of mineral oil or 10 μg/L β-napthoflavone (BNF). Error bars, 95% confidence limits.

The exposure–response curve for Mesa crude oil was derived from three separate tests, with the second and third added at successively lower exposure concentrations to define the EC_{50}, as indicated in Fig. 1. There was very good concordance in EROD activity for overlapping concentrations among the three tests.

The consistent shift of the CEWAF induction curves to the left of that of WAF for Mesa and Terra Nova reflects exposure to higher hydrocarbon concentrations for CEWAF concentrations for a given percentage v/v. In the case of Scotian Light, the curves overlapped, but EROD activity values were significantly higher for CEWAF treatments than for WAF treatments.

EC_{50} values (Table 2) for both WAF and CEWAF of the three oils showed the following rank order of induction potency: Mesa CEWAF > Terra Nova CEWAF > Scotian Light CEWAF > Mesa WAF > Scotian Light WAF > Terra Nova WAF. PAH concentrations (estimated from the PAH data in Table 3) corresponding to the EC_{50} were between 0.60 and 2.00 μg/L for both treatments from all three oils. The difference in induction potential calculated from the ratio of EC_{50} WAF: EC_{50} CEWAF was greatest for Terra Nova (1090 ×), followed by Mesa (110 ×) and Scotian Light (6 ×). Scotian Light was more easily dissolved and dispersed than the other two oils and was also the most toxic (0.32% v/v CEWAF; Fig. 1).

3.3. Polycyclic aromatic hydrocarbons

The PAHs shown in Table 3 were detectable in WAF or CEWAF of Mesa, Terra Nova, and Scotian Light. However, only three, methylfluorene, dimethyldibenzothiophene, and methylphenanthrene, were common and detectable among most of the exposure solutions. More PAH compounds were detected in the higher CEWAF concentrations, such as 0.32 and 0.56% (v/v) of Mesa and Scotian Light crude oils, but the concentrations were all below 2.5 μg/L (Table 3; instrument detection limit of 50 ng/L).

PAH measurements of both WAF and CEWAF test solutions for the three kinds of oil are shown in Fig. 2. Increasing concentrations of some PAHs (methylphenanthrene, dimethyldibenzothiophene, and methylfluorene) were observed at the higher concentrations (0.032, 0.056, and 0.10%) (v/v) in CEWAF from Mesa crude oil. Concentrations of methylphenanthrene were 2–4 times higher in CEWAF compared to WAF, while dimethyldibenzothiophene was 5–10 times higher.

Table 2
EC_{50} values for CYP1A induction (EROD activity) in trout exposed to WAF or CEWAF of three oils

Types of crude oil	EC_{50} WAF		EC_{50} CEWAF		Induction potential
	% (v/v)	PAH concentration	% (v/v)	PAH concentration	$\frac{EC_{50}\ WAF}{EC_{50}\ CEWAF}$
Terra Nova	3.350	1.80	0.003	1.50	1116
Mesa	0.106	0.72	0.001	0.60	106
Scotian Light	0.390	1.56	0.066	2.00	5.91

EC_{50} values were calculated from a Graph–Pad Prism using percentage EROD activity values (maximum EROD activity of 100%). PAH concentrations (μg/L) were the sum of estimated PAH concentrations (see Table 3) corresponding to the EC_{50}s for WAF or CEWAF (% v/v).

In Terra Nova crude oil exposures, concentrations of acenapthylene increased in CEWAF and WAF treatments of 0.1% and 1.0% (v/v), respectively, with concentrations in CEWAF being double those of WAF at 0.1% (v/v). The concentrations of other detected PAHs in CEWAF treatments of $<0.1\%$ were equal to those in the more concentrated WAF treatments (Fig. 2). For Scotian Light, the concentrations for dimethyldibenzothiophene were 10 times higher in the CEWAF than in the WAF treatments. The PAHs that were most concentrated in oil were most concentrated in CEWAF.

3.4. Total petroleum hydrocarbons

Concentrations of TPH for the less concentrated CEWAF and for all WAF treatments were below a detection limit of 10 μg/L. Scotian Light CEWAF treatments of 0.18, 0.32, and 0.56 (v/v) had TPH concentrations of 30, 83, and 266 μg/L, respectively, while Terra Nova CEWAF mixtures of 0.10% and 0.32% (v/v) had TPH concentrations of 34 and 53 μg/L TPH.

4. Discussion

4.1. EROD activity in fish exposed to WAF and CEWAF

EROD activity was a reliable and repeatable measure of exposure to hydrocarbons from crude oil. The exposure response curves for all three oils clearly denoted a greater EROD response to CEWAF than to WAF. PAHs responsible for CYP1A induction were presumably at higher concentrations in CEWAF treatments than in WAF treatments. CYP1A induction was observed at very low concentrations of dispersed oil (as little as 0.0001% (v/v)), up to 1100× less than in nondispersed oil, suggesting that fish exposed to dispersed oil are at greater risk of hydrocarbon toxicity. Similar observations of higher EROD activity in fish exposed to CEWAF versus WAF have been reported (Gagnon and Holdway, 2000; Cohen et al., 2001).

EROD activity decreased at the highest concentrations of CEWAF for all three oils, presumably arising from liver damage (Gagnon and Holdway, 2000). The EC_{50} values confirmed the higher induction potential of CEWAF compared to WAF. Mesa oil had the lowest EC_{50} for both WAF and CEWAF, suggesting the presence of either high-potency CYP1A inducers or inducers at higher concentrations. This was further apparent in the higher number of PAHs detected in Mesa oil and the correspondingly higher total PAH concentration.

The ratio of WAF EC_{50} to CEWAF EC_{50} (for each oil) indicates the extent of dissolution of PAH with dispersion. Terra Nova, being the most viscous, had the highest ratio, followed by Mesa and Scotian Light. Thus, Corexit 9500 seems effective for its intended use on higher viscosity oils.

PAH concentrations at the EC_{50} estimated for all three oils show comparable values for WAF and CEWAF over a narrow range (Table 2), thus lending weight to the reliability and accuracy of EC_{50} values. The finding is consistent with the assumption that PAHs are primarily responsible for CYP1A induction in fish. Mesa had the lowest EC_{50} (i.e., greatest toxicity), confirming the presence of strong inducers.

There were no confounding factors from hydrocarbons present in Corexit EC9500, as it caused no discernable increase in the EROD activity of exposed trout. There was, however, some toxic response at higher concentrations. LC_{50} values for Corexit EC9500 were similar to reported LC_{50} values for Corexit 9527 (Gagnon and Holdway, 2000).

EROD activity in the negative controls (water and mineral oil) was minimal and equivalent to background levels previously observed for trout (Fragoso et al., 1998). Corexit EC9500 had no effect on the permeability of gill surfaces, because EROD activity did not increase when fish were exposed to β-napthoflavone in the presence of varying concentrations of dispersant (data not reported). The robustness of the EROD assay for measuring exposure was further illustrated by the tight overlap of three separate induction curves derived from three tests of Mesa crude oil (Fig. 1).

Table 3
PAH concentrations (μg/L) detected in WAF and CEWAF treatments for three crude oils

Mesa WAF % (v/v)			0.010	0.018	0.032	0.056	0.100	0.18	0.32	0.56	
Methylfluorene			0.13	0.28	0.16	0.03	0.24		0.18	0.02	
Dimethyldibenzothiophene			bdl	bdl	bdl	bdl	bdl		bdl	bdl	
Trimethyldibenzothiophene				0.18							
Methylphenanthrene			0.47	0.51	0.38	0.38	0.48		0.43	0.42	
Mesa CEWAF % (v/v)	0.0032	0.0056	0.010	0.018	0.032	0.056	0.100				
Fluorene											
Methylfluorene	0.18		0.13	0.29		0.37	0.66				
Dibenzothiophene							0.33				
Methyldibenzothiophene						0.87	1.45				
Dimethyldibenzothiophene	bdl	bdl	bdl	0.15		0.55	1.06				
Trimethyldibenzothiophene				0.26		1.13	1.39				
Tetramethyldibenzothiophene						0.74	1.10				
Phenanthrene							0.54				
Methylphenanthrene	0.43		0.46	0.45		1.07	1.89				
Dimethylphenanthrene						1.35	2.31				
Trimethylphenanthrene						1.29	1.95				
Tetramethylphenanthrene						0.55	0.74				
Methylpyrene							0.30				
Trimethylpyrene						0.29	0.42				
Tetramethylpyrene						0.23	0.36				
Dimethylpyrene						0.33	0.44				
Methylnapthobenzothiophene						0.28	0.39				
Dimethylnapthobenzothiophene						0.67	1.02				
Dimethylchrysene						0.29	0.39				
Trimethylchrysene							0.29				
Scotian Light WAF % (v/v)		0.0032	0.01	0.032	0.10		0.32		1.0		
Acenaphthylene		1.65	1.72	1.76	1.37		1.40		1.56		
Methylfluorene		bdl*	0.008	0.04	0.056		0.07		bdl		
Methyldibenzothiphene									0.30		
Dimethyldibenzothiphene		0.02	bdl	0.04	0.03		0.09		0.05		
Scotian Light CEWAF %(v/v)	0.001	0.0032	0.010		0.10	0.18	0.32	0.56			
Naphthalene							0.20				
Dimethylnaphthalene							0.29				
Trimethylnaphthalene							0.41				
Tetramethylnaphthalene							1.14				
Acenaphthylene	1.68	1.69	1.57		1.80	1.92	2.02	1.77			
Methylfluorene	bdl	bdl	0.19		0.07	bdl	0.30	0.03			
Methyldibenzothiphene						0.23	0.98				
Dimethyldibenzothiophene	bdl	bdl	0.36		0.13	0.30	0.87	0.17			
Anthracene			0.29				0.18				
Dimethylphenanthrene							0.41				
Trimethylphenanthrene						0.31	0.43				
Methylpyrene							0.19				
Dimethylpyrene							0.17				
Terra Nova WAF % (v/v)						0.10	0.32	1.0	1.8	5.6	10.0
Acenaphthylene						1.06	1.35	1.47	1.63	1.42	1.58
Methylfluorene						0.09	0.14	0.05	bdl	0.02	0.06
Dimethyldibenzothiophene						0.13	0.17	0.07	0.06	0.10	0.05
Trimethyldibenzothiophene						0.056	0.076	0.17	0.17	0.18	0.17
Methylphenanthrene						0.14	0.21	0.04	0.02	0.10	0.12
Terra Nova CEWAF % (v/v)	0.0001	0.0003	0.0010	0.0032	0.01	0.10	0.32				
Acenaphthylene		0.98		1.48	1.39	2.21	1.68				
Methylfluorene		0.06		bdl	0.04	0.09	0.04				
Dimethyldibenzothiophene	bdl	0.12		0.02	0.09	0.30	0.22				
Trimethyldibenzothiophene		0.18				0.18	0.15				
Methylphenanthrene	bdl	0.08		bdl	0.07	0.16	0.42				
Dimethylphenanthrene							0.39				
Trimethylphenanthrene							0.22				

bdl, below detection limit.

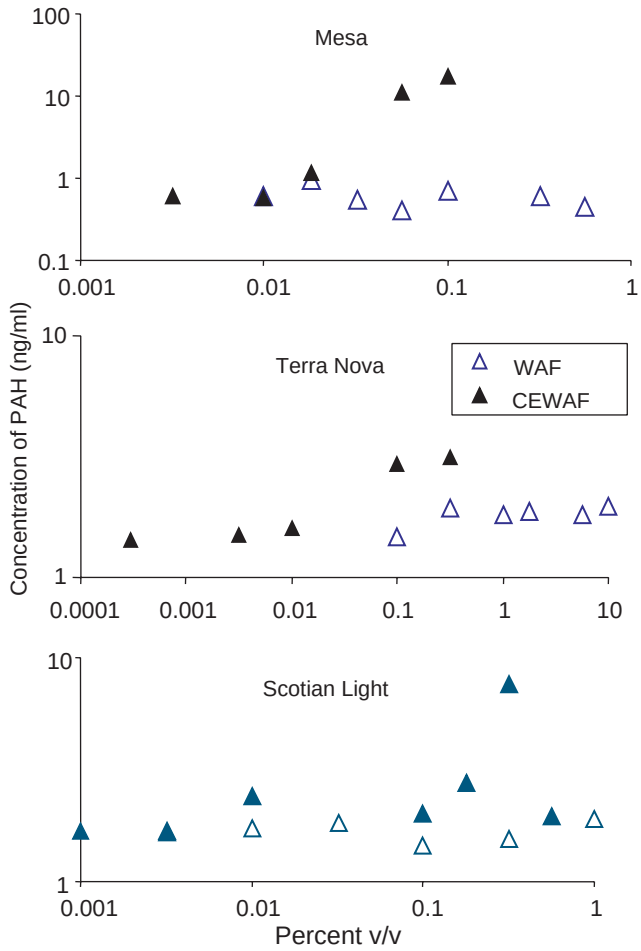

Fig. 2. Sum of polycyclic hydrocarbon concentrations detected in the WAF and CEWAF test solutions.

4.2. Polycyclic aromatic hydrocarbons

Dispersing oil markedly increased hydrocarbon concentrations in test solutions based on measured concentrations of PAH. More napthalenes were detected in CEWAF than in WAF from Mesa crude oil. Fucik (1994) found hydrocarbon concentrations in dispersed Central Gulf oil four to five times higher than WAF with napthalenes as the dominant fraction. Cohen and Nugegoda (2000) also reported that dispersed oil WAF (CEWAF) had the highest concentration of TPHs compared to crude oil WAF. The increase in hydrocarbons could be due to the presence of oil droplets in emulsion or increased dissolution of hydrocarbons from the surfaces of the numerous droplets (surface area effects). One must realized that in WAF, hydrocarbon concentrations are determined by the solubilities of the various compounds. Conversely, CEWAF concentrations are primarily influenced by the presence of bulk oil droplets. This is largely due to the emulsifying action of the dispersant that drives the oil–surfactant micelles into solution. Therefore, the amount of PAH in solution is determined by the solubility of each compound, the rate of partitioning into solution across the oil–water interface, and the size of the surface area available for partitioning, as determined by droplet size.

4.3. Total petroleum hydrocarbon

For TPH, most of the concentrations were below detection limits (3 μg/L). The more concentrated solutions of CEWAF from Scotian Light crude oil (0.32 and 0.56% v/v) had TPH concentrations of 60 and 266 μg/L, respectively, which are comparable to observations of 200 μg/L for the 0.5% (v/v) CEWAF of Bass Strait Crude Oil (Cohen et al., 2001).

Most TPH concentrations were above the detection limit, but because of higher blank values, they were not quantified. Despite this, an exposure–response relationship of EROD activity with hydrocarbon concentration was observed for C16 in WAF of Terra Nova and C10 and C16 in CEWAF of Scotian Light. A correlation coefficient (Pearson r value) of 0.952 was calculated between EROD activity and the C10 TPH fraction of the CEWAF treatment of Scotian Light crude. Dispersant effectiveness is a major controlling factor in the bioavailability of hydrocarbons. The higher ratio of hydrocarbons in CEWAF versus WAF for Scotian Light compared to Terra Nova is an indication that lighter oils would disperse more effectively as bulk oil droplets than heavier ones (Fingas, 1995). However, the EROD response of fish in this experiment suggests that Corexit EC9500 solubilizes PAH more effectively from heavier oils than from light oils.

While the preparation of WAF and CEWAF was carried out as prescribed in the literature, there may have been some conditions that were overlooked. Factors relevant to this study include the manner and order in which the dispersant was added. When dispersant was added to the oil layer surface and then vortexed, the likelihood of sufficient dispersant contact was reduced; oil can be stranded on the vessel walls because of herding at initial dispersion (Singer et al., 2000). This would have been especially significant in the case of the less viscous Scotian Light. A second factor of relevance is the preparation of the test concentrations. Test treatments should be derived from a range of oil loadings and not serial dilutions, because partitioning of compounds into the aqueous phase is not directly correlated to the oil:water ratio (Girling et al., 1994).

The following conclusions can be drawn from this study. Dispersing crude oil increased the bioavailability of PAHs to fish, as reflected by greater CYP1A induction in the livers of fish exposed to CEWAF versus WAF treatments. Second, the action of the dispersant was influenced by the viscosity of the crude oils. Terra Nova, which had the highest viscosity value, had the biggest difference in induction for CEWAF and

WAF treatments. Scotian Light had the lowest viscosity value and showed the smallest difference in induction values, while Mesa was intermediate.

While it was difficult to detect most of the PAHs and petroleum hydrocarbons at the lower doses of WAF and CEWAF by sensitive analytical techniques such as gas chromatography/mass spectrometry, the EROD assay proved to be much more reliable and sensitive for detecting effects at low concentrations ($<0.0032\%$ v/v).

In an ecological context, this study revealed an increase in exposure of fish to hydrocarbons with dispersion for all three oils. Consequently, the risk of PAH toxicity to pelagic species of fish, especially to sensitive life stages such as eggs and larvae, is enhanced by chemical dispersion. The effectiveness of Corexit EC9500 was most pronounced on Terra Nova oil, while Mesa oil had the greatest induction potential. Dispersing these oils would cause sublethal exposures to PAH, with Mesa potentially having a greater sublethal effect. Scotian Light was the most toxic by virtue of its higher solubility and reached a lethal concentration limit during dispersion.

Acknowledgments

The authors thank the staff at Analytical Services Unit, Queens University, and COOGER–CREPGE at the Bedford Institute of Oceanography for their technical support. We also thank Barbara Sobota, Heather Orr, and Vivien Dayeh for their assistance and contribution. We wish to extend our gratitude to Gary Wohlgeschaffen for his prompt response to queries and requests. The research was funded by a contract from the Department of Fisheries and Oceans, Halifax, Nova Scotia, Canada.

References

Anderson, J.W., Neff, J.M., Cox, B.A., Tatem, H.E., Hightower, G.M., 1974. Characteristics of dispersions and water-soluble extracts of crude and refined oils and their toxicity to estuarine crustaceans and fish. Mar. Biol. 27, 75–88.

Bobra, A.M., Shiu, W.Y., MacKay, D., Goodman, R.H., 1989. Acute toxicity of dispersed fresh and weathered crude oil and dispersants to *Daphnia magna*. Chemosphere 19, 1199–1222.

Canevari, G.P., 1978. Some observations on the mechanism and chemistry aspects of chemical dispersion. In: McCarthy, L.T.J., Lindblom, G.P., Walter, H.F. (Eds.), Chemical Dispersants for the Control of Oil Spills. Am. Soc. Testing and Materials, Philadelphia, pp. 2–5.

Carls, M.G., Rice, S.D., Hose, J.E., 1999. Sensitivity of fish embryos to weathered crude oil: Part 1. Low-level exposure during incubation causes malformations, genetic damage, and mortality in larval pacific herring (*Clupea pallasi*). Environ. Toxicol. Chem. 18, 481–493.

Carr, R.S., Linden, O., 1984. Bioenergetic responses of *Gammarus salinus* and *Mytilus edulis* to oil and oil dispersants in a model ecosystem. Mar. Ecol. Progr. Ser. 19 (3), 285–292.

CCME, 2000. Reference Method for the Canada Wide Standard for Petroleum Hydrocarbons in Soil. Draft Copy of CCME—Tier 1 Method, Canadian Council of Ministers of the Environment, Winnipeg, Man., Canada.

Cohen, A.M., Nugegoda, D., 2000. Toxicity of three oil spill remediation techniques to the Australian bass *Macquaria novemaculeata*. Ecotoxicol. Environ. Saf. 47, 178–185.

Cohen, A.M., Nugegoda, D., Gagnon, M.M., 2001. The effect of different oil spill remediation techniques on petroleum hydrocarbon elimination in Australian bass *Macquaria novemaculeata*. Arch. Environ. Contam. Toxicol. 40, 264–270.

Cotou, E., Castritsi-Catharios, I., Moraitou-Apostolopoulou, M., 2001. Surfactant-based oil dispersant toxicity to developing nauplii of *Artemia*: effects on atpase enzymatic system. Chemosphere 42, 959–964.

Fingas, M., 1995. Oil spills and their cleanup. Chem. Ind. 24, 1005–1008.

Fragoso, N., Parrott, J.L., Hahn, M.E., Hodson, P.V., 1998. Chronic retene exposure causes sustained induction of CYP1A activity and protein in rainbow trout (*Oncorhynchus mykiss*). Environ. Toxicol. Chem. 17, 2347–2353.

Fucik, K., 1994. Dispersed oil toxicity tests with species indigenous to the Gulf of Mexico. Technical Summary: Minerals Management Service Publication 94-0021, Gulf of Mexico Region, Environmental Information, US Department of the Interior, New Orleans, LA, USA.

Gagnon, M.M., Holdway, D.A., 2000. EROD induction and biliary metabolite excretion following exposure to the water accomodated fraction of crude oil and to chemically dispersed crude oil. Arch. Environ. Contam. Toxicol. 38, 70–77.

Garcia-Blanco, S., Motelab, M., Venosa, A.D., Suidan, M.T., Lee, K., King, D.W., 2001. Restoration of the oil contaminated St. Lawrence River shoreline: bioremediation and phytoremediation. In: Proceedings of the 2001 Oil Spill Conference. Am. Petroleum Inst., Washington, DC, pp. 303–308.

Getter, C.D., Baca, B.J., 1984. A laboratory approach for determining the effects of oils and dispersants on mangroves. In: Allen, T.E. (Ed.), Oil Spill Chemical Dispersants: Research, Experience, and Recommendations, STP 840. Am. Soc. Testing and Materials, Philadelphia, pp. 5–13.

Gilbert, T., 1996. Dispersant use/oil/sea temperature suitability matrix and oil chemistry and the impact on the effectiveness of chemical dispersants. Sixth Scientific Support Coordinators Workshop, Launceston, Tasmania, Australia. Australian Maritime Safety Authority, Canberra, ACT 2601, Australia, URL: http://www.amsa.gov.au/me/netplan/disper.htm

Girling, A.E., Whale, G.F., Adema, D.M.M., 1994. A guideline supplement for determining the aquatic toxicity of poorly water-soluble complex mixtures using water-accommodated fractions. Chemosphere 29, 2645–2649.

Hartwick, E.B., Wu, R.S.S., Parker, D.B., 1982. Effects of a crude oil and a oil dispersant (Corexit 9527) on populations of the littleneck clam (*Protothaca staminea*). Mar. Environ. Res. 6, 291–306.

Hodson, P.V., Efler, S., Wilson, J.Y., El-Shaarawi, A., Maj, M., Williams, T.G., 1996. Measuring the potency of pulp mill effluents for induction of hepatic mixed function oxygenase activity in fish. J. Toxicol. Environ. Health 49, 101–128.

Hodson, P.V., Ibrahim, I., Zambon, S., Ewert, A., Lee, K., 2002. Bioavailability to fish of sediment PAH as an indicator of the success of in situ remediation treatments at an experimental oil spill. Bioremediat. J. 6 (3), 297–313.

Law, A.T., 1995. Toxicity study of the oil dispersant Corexit 9527 on *Macrobrachium rosenbergii* (de Man) egg hatchability by using a flow-through bioassay technique. Environ. Pollut. 88, 341–343.

Lewis, A., Aurand, D., 1997. Putting dispersants to work: overcoming obstacles. 1997 International Oil Spill Conference Issue Paper,

Technical Report No. IOSC-004.23. Am. Petroleum Inst., Washington, DC.

Linden, O., 1974. Effects of oil dispersants on the early development of Baltic Herring. Ann. Zool. Fennici 11, 141–148.

Linden, O., 1975. Acute effects of oil and oil/dispersant mixture on larvae of Baltic herring. Ambio 4, 130–133.

Nelson-Smith, A., 1977. Effects of dispersant use on shore life. In: McCarthy Jr., L.T., Lindblom, G.P., Walter, H.F. (Eds.), Chemical Dispersants for the Control of Oil Spills, ASTM STP 659. Am. Soc. Testing and Materials, Philadelphia, pp. 18–34.

Singer, M.M., Aurand, D., Bragins, G.E., Clarks, J.R., Coelho, G.M., Sowby, M.L., Tjeerdema, R.S., 2000. Standardization of the preparation and quantitation of water-accommodated fractions of petroleum for toxicity testing. Mar. Pollut. Bull. 40 (11), 1007–1016.

Singer, M.M., George, S., Benner, D., Jacobson, S., Tjeerdema, R.S., Sowby, M.L., 1993. Comparative toxicity of two oil dispersants to the early life stages of two marine species. Environ. Toxicol. Chem. 12, 1855–1863.

Singer, M.M., George, S., Jacobson, S., Lee, I., Weetman, L.L., Tjeerdema, R.S., Sowby, M.L., 1996. Comparison of acute aquatic effects of the oil dispersant Corexit 9500 with those of other Corexit series dispersants. Ecotoxicol. Environ. Saf. 35, 183–189.

Smith, J.E., 1968. "Torrey Canyon" Pollution and Marine Life. Cambridge University Press, Cambridge House, Cambridge, UK, pp. 23–25.

Thomas, R.E., Rice, S.D., 1981. Excretion of aromatic hydrocarbons and their metabolites by freshwater and seawater Dolly Varden char. In: Vernberg, F.J., Calabrese, A., Thurberg, F., Vernberg, W. (Eds.), Biological Monitoring of Marine Pollutants. Academic Press, New York, pp. 425–448.

Volkmann, J.K., Miller, G.J., Revill, A.T., Connell, D.W., 1994. Oil spills. In: Swan, J.M., Neff, J.M., Young, P.C. (Eds.), Environmental Implications of Offshore Oil and Gas Development in Australia—The Findings of an Independent Review. Australian Petroleum Exploration Assoc., Sydney, Australia, pp. 509–695.