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Laboratory Exposures of Invertebrate and Vertebrate Species to Concentrations of IA- 35 (Petro-Canada) Drill Mud Fluid, Production Water, and Hibernia Drill Mud Cuttings

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April 2001

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Canadian Manuscript Report of Fisheries and Aquatic Sciences No. 2560

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Canadian Manuscript Report of
Fisheries and Aquatic Sciences No. 2560

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Laboratory Exposures of Invertebrate and Vertebrate Species to Concentrations of IA- 35 (Petro-Canada) Drill Mud Fluid, Production Water, and Hibernia Drill Mud Cuttings

by

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Cat. No. Fs 97-04/2560E ISSN 0706-6473

Correct citation for this publication:

Payne, J., L. Fancey, C. Andrews, J. Meade, F. Power, K. Lee, G. Veinott and A. Cook. 2001. Laboratory exposures of invertebrate and vertebrate species to concentrations of IA-35 (Petro-Canada) drill mud fluid, production water and Hibernia drill mud cuttings. Can. Manuscr. Rep. Fish. Aquat. Sci. No.2560: iv + 27 p.

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ABSTRACT

Payne, J., L. Fancey, C. Andrews, J. Meade, F. Power, K. Lee, G. Veinott and A. Cook. 2001. Laboratory exposures of invertebrate and vertebrate species to concentrations of IA-35 (Petro-Canada) drill mud fluid, production water and Hibernia drill mud cuttings. Can. Manuscr. Rep. Fish. Aquat. Sci. No. 2560: iv + 27 p.

Data are presented on the short term effect of synthetic drill mud fluid, produced water and drill mud cuttings on brine shrimp nauplii (Artemia franciscana), capelin larvae (Mallotus villosus), marine copepods (Calanus finmarchicus), juvenile yellowtail flounder (Limanda ferruginea) and ctenophores (Pleurobrachius pileus). Included are data on the water solubility of Petro Canada drill mud fluid IA-35 and metal analysis of production water from the Sable Island Offshore Exploration Project. The studies indicated a very low acute toxicity potential for drill mud fluid, production water and Hibernia drill cuttings for the species and life stages tested. The results support the hypothesis that the wastes pose little or no risk of an acute toxic nature to the marine environment.

RÉSUMÉ

Payne, J., L. Fancey, C. Andrews, J. Meade, F. Power, K. Lee, G. Veinott and A. Cook. 2001. Laboratory exposures of invertebrate and vertebrate species to concentrations of IA-35 (Petro-Canada) drill mud fluid, production water and Hibernia drill mud cuttings. Can. Manuscr. Rep. Fish. Aquat. Sci. No. 2560: iv + 27 p.

Des données concernant la toxicité aigue des liquides et déblais de forage à hydrocarbures et de l'eau produite sont présentées dans ce rapport. Ces données concernent des larves de crevettes (*Artemia franciscana*), des larves de capelans (*Mallotus villosus*), des copépodes marins (*Calanus finmarchicus*), des plies juvéniles (*Limanda ferruginea*) et des cténophores (*Pleurobrachia pileus*). Sont incluses également, des données sur la solubilité du liquides de forage IA-35 de Petro Canada et l'analyse des métaux des eaux produites de la plate-forme Offshore de lîle des Sables. Ces études indiquent une très légère toxicité aigue des liquides de forage, de l'eau produite et des déblais de forage et ce pour toutes les espèces et stades de développement considérés. Les résultats renforcent l'hypothèse que les déchets des sites d'exploitation pétrolière posent peu de risques pour l'environnement marin.

INTRODUCTION

Offshore exploration for oil and gas is rapidly developing on the Grand Banks of Newfoundland, Canada. The Hibernia, Terra Nova, and White Rose oil fields are being developed in the North East area of the Banks where, traditionally, important commercial fishing activities have been carried out. The co-existence of the oil industry activities with fish, fish habitat and commercial fishing activities leads to potential concern for harmful cumulative effects or other negative environmental impacts on marine organisms at these sites.

The potential for oil industry discharges to cause environmental impact is of concern. Potential negative impacts may be associated with the types of discharges that accompany offshore exploration and production including drilling muds or cuttings, production waters and displacement waters. Review and discussion on the environmental impacts of production water may be found in Ray and Englehardt (1992). Potential for impact of oil industry discharges has been a topic of review by the Joint Group of Experts on the Scientific Aspects of Marine Pollution (GESAMP 1993).

The body of knowledge obtained in recent years from field studies and supporting laboratory studies conducted in the North Sea, Gulf of Mexico and California have indicated that offshore impacts will be minimal due to the rapid dilution effect in the offshore (e.g. GESAMP 1993). However there still remains a level of uncertainty especially with regard to the potential for chronic effects. This is particularly important in the Grand Banks where there is potential to effect a large and rich fishing ground. There is also the need to provide assurance though monitoring activities, on predicted effects of oil industry activities particularly on localized effects on larval and planktonic communities.

In this study, a series of test trials were performed using brine shrimp nauplii (*Artemia franciscana*), capelin larvae (*Mallotus villosus*), marine copepods (*Calanus finmarchicus*), juvenile yellowtail flounder (*Limanda ferruginea*) and ctenophores (*Pleurobrachia pileus*) to investigate the short term effect of test solutions of oil industry discharges such as synthetic drill mud fluid, production waters and drill mud cuttings.

MATERIALS AND METHODS

Production water was obtained from Dr. Ken Lee, Institute Maurice Lamontagne, Fisheries and Oceans and was stored in PETE (polyethylene teraphthalate) bottles at 4°C until used. This production water was taken from the Sable Island Offshore Exploration Project, June 21, 1999. Drill mud cuttings were obtained from Mr. David Taylor, Hibernia Management and Development Company, and were stored in a 20 L bucket at 4°C until used. The synthetic drill mud fluid, IA -35, was obtained from Mr. Urban Williams, Petro-Canada, and held at room temperature (approximately 20°C) in HPDE (high-density-polyethylene) bottles until used. *Artemia franciscana* cysts were obtained from Pet City,

St. John's, NF, Canada, (San Francisco Bay Brand, 8239 Enterprise Dr., Newark, CA 94560). Capelin larvae (*Mallotus villosus*) were collected from the surf at Middle Cove Beach, NF on July 31, 2000. Marine copepods were collected by plankton net tow: 333 micron Nitex mesh; 2 kn towing speed; off St. John's Harbour, NF; N 47°34.4' latitude and W52°40.4' longitude; January 26, 2000. Copepods used for test exposures were identified as species, *Calanus finmarchicus* (Marshall and Orr 1955). Ctenophores were collected by the same method as the copepods off St. Phillip's, Conception Bay, NF; N 47°35.2' latitude and W52°52.8' longitude; February 2, 2000. Ctenophores used for test exposures were identified as species, *Pleurobrachia pileus* (Smith 1964). On November 23, 1998, 100 juvenile yellowtail flounder (*Limanda ferruginea*) were obtained from Danny Boyce at the Ocean Science Centre, where they were raised at 10°C. Experimental protocols are detailed in each experiment section below.

Experiment 1: IA-35 Synthetic Drill Mud Fluid

1.1 Water Solubility: (IA-35 - Synthetic Drill Mud Fluid)

The water solubility of synthetic drilling mud fluid was determined by adding 410 mg of IA-35 to 1 L of seawater at room temperature in a 1 L separatory funnel and shaken vigorously for 5 min. After 48 h the bottom, middle and upper 300 mL was decanted into 3 secondary 1 L separatory funnels and the top 100 mL discarded (to remove surface drilling fluid). All separatory funnels were cleaned with Fisherbrand Versaclean then rinsed 3 times with tap water, acetone, methylene chloride and seawater before use.

Each 300 mL aliquot was then extracted 3 times with 40 mL methylene chloride, which was run though 10 g of sodium sulphate (cleaned with methylene chloride and baked) into 250 mL round bottom flasks. The extracts were evaporated to ~1 mL by rotoevaporation then to dryness under nitrogen gas. The residues were redissolved in 5 mL of ultra violet grade hexane using 5 mL volumetric flasks. The samples were transferred to autosampler vials and analyzed using a Hewlett Packard 5890 gas chomatograph with a flame ionization detector. Concentrations of soluble IA-35 were determined using a standard curve of 1, 2, 3, 4 and 5 ppt IA-35 and naphthalene as an internal standard. The detection limit for IA-35 analysis is 10µg/mL. All glassware used for the extractions were cleaned with Fisherbrand Versaclean then rinsed well with tap water and rinsed 3 times with acetone, hexane and methylene chloride before use.

The procedure was done in duplicate. The upper 300 mL portion of Replicate B was accidentally wasted. The results are recorded in Table 1:

Table 1. Water Solubility of IA-35 after 48 h.

Replicate A	IA-35 (mg/mL) in solvent extract	IA-35 (mg/L) in seawater	% Soluble IA-35 in seawater
Bottom (300 mL)	0.13	2	0.5
Middle (300 mL)	0.18	3	0.7
Upper (300 mL)	0.18	3	0.7
Replicate B		IA-35 (mg/L)	% Soluble IA-35
Bottom (300 mL)	0.13	2	0.5
Middle (300 mL)	0.22	4	1
Upper (300 mL)	Lost	lost	Lost
Average	**0.17**	**3**	**0.7**

1.2 Exposure Trials with IA-35 Drill Mud Fluid

1.2.1 *Artemia* nauplii Exposure to Synthetic Drill Mud Fluid

Trial 1: *Artemia* desiccated cysts were sprinkled on the surface of 1 liter of seawater at room temperature (~20°C) and aerated using an airstone and supplied air. Aeration continued for 72 h at which time the hatched nauplii were then used in the exposure trial. Exposures were conducted in 300 mL clear-sided drinking glasses containing the appropriate IA-35 exposure concentration. All exposure glasses were washed with Fisherbrand Versaclean and were rinsed several times with tap water and were allowed to dry. Each glass was again rinsed with seawater before use in an exposure trial. All exposures trials were performed in duplicate per concentration.

Concentrations of IA-35 were prepared by shaking (a consistent number of times) 0, 1 mL and 5 mL of IA-35 in a total of 500 mL of room temperature aerated seawater in a separatory funnel. Two separatory funnels were used, one to prepare the seawater control test solutions and the other to prepare the IA-35 test solutions starting with mixing of the least concentrated solution. Half of the shaken solution (250 mL) was poured into one of the replicate glasses (A) and the other half was poured into the second replicate glass (B). This provided a total of 6 exposures per trial consisting of 3 solutions (seawater only as control; 1 mL IA-35 in 500 mL seawater or 0.2%; and 5 mL IA-35 in 500 mL seawater or 1.0%) in duplicate exposure glasses.

Approximately twenty *Artemia* nauplii were counted out and transferred, using a glass Pasteur pipette, to each glass exposure concentration A and its replicate B.

The replicate exposure concentrations were incubated at room temperature for 24 h and the number of dead nauplii were counted. The transparent exposure glasses allowed good determination of numbers of dead and live nauplii. Nauplii were considered dead if no movement could be discerned. After the nauplii were counted at the 24 h stage the exposure was allowed to continue and surviving nauplii were counted again at 48 h. Number of nauplii dead and alive in each exposure replicate, as well as survival (%), are recorded in Table 2.

Table 2. Survival Data -Trial 1 with IA –35.

Test: Solution/Concentration	24 h Exposure results		48 h Exposure results	
	Replicate A	Replicate B	Replicate A	Replicate B
Seawater only (control)	all alive 100% survival	all alive 100% survival	all alive 100% survival	all alive 100% survival
1 mL IA-35/500 mL seawater (0.2%)	19 alive 2 dead 90.5% survival	all alive 100% survival	4 alive 17 dead 19% survival	3 alive 17 dead 15% survival
5 mL IA-35/500 mL seawater (1%)	all alive 100% survival	all alive 100% survival	2 alive 19 dead 9.5% survival	2 alive 18 dead 10% survival

Trial 2: A second exposure trial was conducted with IA-35 synthetic drill mud fluid. Conditions of exposure were the same as in the IA-35 Trial 1 except that aeration continued for 48 h (instead of 72 h) and one more concentration, 0.5 mL IA-35/500 mL seawater (0.1%) was used. This provided a total of 8 exposures per trial consisting of 4 solutions (seawater only as control; 0.5 mL IA-35 in 500 mL seawater, 1 mL IA-35 in 500 mL seawater; and 5 mL IA-35 in 500 mL seawater) in duplicate exposure glasses. Number of nauplii dead and alive in each exposure replicate are recorded in Table 3.

Table 3. Survival Data -Trial 2 with IA-35.

Test: Solution/Concentration	24 h Exposure results		48 h Exposure results	
	Replicate A	Replicate B	Replicate A	Replicate B
Seawater only (control)	all alive 100% survival	all alive 100% survival	all alive 100% survival	all alive 100% survival
0.5 mL IA-35/500 mL seawater (0.1%)	all alive 100% survival	all alive 100% survival	15 alive 5 dead 75% survival	14 alive 6 dead 70% survival
1mL IA-35/500 mL seawater (0.2%)	all alive 100% survival	all alive 100% survival	14 alive 6 dead 70% survival	14 alive 6 dead 70% survival
5mL IA-35/500 mL seawater (1%)	all alive 100% survival	all alive 100% survival	16 alive 3 dead 84% survival	12 alive 8 dead 60% survival

1.2.2 Capelin Larvae Exposure to Synthetic Drill Mud Fluid

Trial 3: A third exposure trial was done using IA-35 synthetic drill mud fluid. Capelin larvae were transported to the aquarium at the Northwest Atlantic Fisheries Centre, and held at 12°C in a 20 L container with a flow though system for 24 h, at which time they were used in the exposure trial. The exposure was conducted in 1.5 L glass mason jars that were washed in Fisherbrand Versaclean and rinsed well with tap water.

Concentrations of IA-35 were prepared by shaking vigorously for 5 min, 0 mL and 0.5 mL of IA-35 in a total of 1 L aerated seawater. Twenty-five capelin larvae were counted and transferred, using a 7 in. glass Pasteur pipette, to each exposure. The exposures were held at 12°C for 72 h and then the number of surviving larvae were counted. The larvae were considered to be alive if activity was present. The number of larvae alive and dead in each exposure, as well as percent survival, are recorded in Table 4.

Table 4. Survival Data -Trial 3 with IA-35.

Test: Solution/Concentration	72 h Exposure results
Seawater only (control)	22 alive 3 dead 88% survival
0.5 mL IA-35/1 L seawater (0.05%)	22 alive 3 dead 88% survival

1.2.3 Marine Copepods Exposure to Synthetic Drill Mud Fluid

Trial 4: Marine copepods were held in clean seawater in five gallon plastic buckets and were transported the same day to the laboratory. Stock copepods were held in clean aerated seawater under static conditions in plastic buckets until used in exposure trials. Buckets were placed within 240 L aquaria which had running ambient temperature seawater to maintain a constant holding temperature (~5°C).

For each test exposure, copepods were transferred from the holding buckets to the test jars with a 50 mL glass beaker. Each transfer contained approximately 20-30 copepods in 20 mL seawater. All test exposures were done in duplicate (A and B) in glass 250 mL Mason jars. Each concentration of test solution was prepared to give a final volume of 100 mL. First, test solutions were prepared by adding a volume of IA-35 to seawater to make 80 mL volume. Jars were then capped and shaken very vigorously to ensure mixing of test solution before addition of test organisms. Twenty milliliters of copepods (containing approximately 20-30 individuals) in seawater were then added to bring final volume of test solution to 100 mL. Four concentrations of IA-35 (0%, 0.5%, 1% and 2%) were tested (see Table 5).

After addition of the test organisms, the test jars were placed in an aquarium and held static surrounded by running ambient temperature seawater to maintain constant holding temperature. No aeration was supplied to the jars. After 24 h incubation, each jar was examined for dead copepods, the number of dead individuals was recorded and the jars were then allowed to continue incubating until exposure time was 48 h. After 48 h, each jar was re-examined for dead copepods. Contents of each jar were then poured into a 250 μm sieve, and copepods were preserved in a glass vial in 70% ethanol to confirm both the total number of copepods in each jar, and the species identification.

Table 5 indicates the number of alive and dead copepods in each exposure, as well as the percent survival.

Table 5. Survival Data -Trial 4 with IA-35.

Test: Concentration	IA-35 volume	Seawater volume	Copepods in seawater volume	24 h survival	48 h survival
A: Control	0 mL	80 mL	20 mL	22 alive 0 dead 100% survival	22 alive 0 dead 100% survival
B: Control	0 mL	80 mL	20 mL	19 alive 0 dead 100% survival	19 alive 0 dead 100% survival
A: 0.5%(0.5 mL IA-35/100 mL seawater)	0.5 mL	79.5 mL	20 mL	16 alive 0 dead 100% survival	16 alive 0 dead 100% survival
B: 0.5%(0.5 mL IA-35/100 mL seawater)	0.5 mL	79.5 mL	20 mL	30 alive 0 dead 100% survival	30 alive 0 dead 100% survival
A: 1% (1 mL IA-35/100 mL seawater)	1.0 mL	79 mL	20 mL	35 alive 0 dead 100% survival	35 alive 0 dead 100% survival
B: 1% (1 mL IA-35/100 mL seawater	1.0 mL	79 mL	20 mL	25 alive 0 dead 100% survival	25 alive 0 dead 100% survival
A: 2% (2 mL IA-35/100 mL seawater	2.0 mL	78 mL	20 mL	26 alive 0 dead 100% survival	26 alive 0 dead 100% survival
B: 2% (2 mL IA-35/100 mL seawater	2.0 mL	78 mL	20 mL	18 alive 0 dead 100% survival	18 alive 0 dead 100% survival

Trial 5: Exposure of marine copepods to IA-35 was repeated using identical methods and the same stock of copepods as in Trial 4. Results are recorded in Table 6.

Table 6. Survival Data -Trial 5 with IA-35.

Test Concentration	IA-35 volume	Seawater volume	Copepods in seawater volume	24 h survival	48 h survival
A: Control	0 mL	80 mL	20 mL	27 alive 0 dead 100% survival	27 alive 0 dead 100% survival
B: Control	0 mL	80 mL	20 mL	23 alive 0 dead 100% survival	23 alive 0 dead 100% survival
A: 0.5%(0.5 mL IA-35/100 mL seawater)	0.5 mL	79.5 mL	20 mL	31 alive 0 dead 100% survival	31 alive 0 dead 100% survival
B: 0.5%(0.5 mL IA-35/100 mL seawater)	0.5 mL	79.5 mL	20 mL	18 alive 0 dead 100% survival	18 alive 0 dead 100% survival
A: 1% (1 mL IA-35/100 mL seawater)	1.0 mL	79 mL	20 mL	24 alive 0 dead 100% survival	23 alive 1 dead 96% survival
B: 1% (1 mL IA-35/100 mL seawater	1.0 mL	79 mL	20 mL	21 alive 0 dead 100% survival	21 alive 0 dead 100% survival
A: 2% (2 mL IA-35/100 mL seawater	2.0 mL	78 mL	20 mL	19 alive 0 dead 100% survival	19 alive 0 dead 100% survival
B: 2% (2 mL IA-35/100 mL seawater	2.0 mL	78 mL	20 mL	33 alive 0 dead 100% survival	33 alive 0 dead 100% survival

1.2.4 Juvenile Yellowtail Flounder Exposure to Synthetic Drill Mud Fluid

Trial 6: Juvenile yellowtail flounder for this trial came from a 30 L tank of 100 flounder obtained from the Ocean Science Centre. These flounder were acclimated to ambient temperature (2°C) in an aerated, flowthough system.

For this exposure, two 5 L tanks were set-up. Tank A contained 4 L seawater and Tank B contained 4 L seawater that had been shaken with 10 mL IA-35. The IA-35 was added to a 1 L separatory funnel containing 1 L seawater and shaken vigorously for 5 min. All but the top 100 mL (which contained drilling fluid that had settled out of solution) was added to Tank B. Another 900 mL seawater was added to the separatory funnel, shaken as before and again all but the top 100 mL added to Tank B. Once again 900mL was added to the separatory funnel, shaken and 1 L added to Tank B. The volume of Tank B was brought up to 4 L with seawater. Twenty fish were transferred to Tank A and Tank B at 2°C, 10 fish per tank. After 30 days the fish were counted to determine the percent survival. Results are recorded in Table 7.

Table 7. Survival Data -Trial 6 with IA-35.

Test: Solution/Concentration	30 day exposure results
Seawater only (control)	10 alive 100% survival
10 mL IA-35/4 L seawater (0.25%)	10 alive 100% survival

1.2.5 Ctenophore Exposure to Synthetic Drill Mud Fluid

Trial 7: Ctenophores were exposed to six concentrations of IA-35 (0 mL/L; 0.25 mL/L; 0.50 mL/L; 1.0 mL/L; 1.5mL/L and 2.0 mL/L). Each test solution was made up in duplicate (A and B) to a final volume of 500 mL of clean seawater in 1L Mason jars and the jars shaken vigorously to mix. Duplicate control exposures using only clean seawater were also done. Ten ctenophores were added to each test solution in the jars. Jars were placed in an aquarium containing running ambient temperature seawater (~5°C). Each jar was gently aerated using an air stone and supplied air.

Ctenophores in each test exposure were examined at intervals from 12 hr to 120 hr to determine the number remaining alive. Results are reported in Table 8.

Table 8. Trial 7 - Survival data after exposure of ctenophores to IA-35.

Concentration IA-35 Replicates: A, B	Exposure Time					
	12 h	24 h	48 h	72 h	96 h	120 h
	% Survival					
A: 0 mL/L (Seawater only-control)	100	100	100	100	100	100
B: 0 mL/L (Seawater only-control)	100	100	100	100	100	100
A: 0.25 mL/L	100	100	100	100	100	100
B: 0.25 mL/L	100	100	100	100	100	100
A: 0.50 mL/L	100	100	100	100	100	100
B: 0.50 mL/L	100	100	100	100	100	100
A: 1.0 mL/L	100	100	100	100	100	100
B: 1.0 mL/L	100	100	100	100	100	100
A: 1.5 mL/L	100	100	100	100	100	100
B: 1.5 mL/L	100	100	100	100	100	100
A: 2.0 mL/L	100	100	100	100	100	100
B: 2.0 mL/L	100	100	100	100	100	100

Trial 8: The exposure setup was repeated as in Trial 7 except only six ctenophores were used in each test exposure (only enough ctenophores left from collected stock to do six per exposure). Results are recorded in Table 9.

Table 9. Trial 8 - Survival Data after exposure of ctenophores to IA-35.

Concentration IA-35 Replicates: A, B	Exposure time					
	12 h	24 h	48 h	72 h	96 h	120 h
	% Survival					
A: 0 mL/L (Seawater only-control)	100	100	100	100	100	100
B: 0 mL/L (Seawater only-control)	100	100	100	100	100	100
A: 0.25 mL/L	100	100	100	100	100	100
B: 0.25 mL/L	100	100	100	100	83.3	83.3
A: 0.50 mL/L	100	100	100	100	100	100
B: 0.50 mL/L	100	100	100	100	100	100
A: 1.0 mL/L	100	100	100	100	83.3	83.3
B: 1.0 mL/L	100	100	100	100	100	100
A: 1.5 mL/L	100	100	100	100	100	100
B: 1.5 mL/L	100	100	100	100	100	100
A: 2.0 mL/L	100	100	100	100	100	100
B: 2.0 mL/L	100	100	100	100	100	100

Experiment 2: Production Water

2.1 Metal Analysis

Production water was analyzed for 19 elements using inductively coupled plasma mass spectrometry (ICPMS). Production water was analyzed in two parts:

1. 20 mL production water was taken from the middle of a 1 L stock bottle. A 2 mL sub-sample was taken, diluted with nanopure water (1:9) and acidified with 2% ultra pure nitric acid. A blank was prepared with nanopure water and 2% nitric acid.

2. The precipitate in the bottom of the 1L stock bottle of production water was removed

and dried at 60°C. After 48 h the dried precipitate was weighed (0.3381 g), transferred to a polypropylene tube and 6 mL acid mixture added (2:1 $HN0_3$:HF). The sample was left to stand overnight then heated to ~80°C. After 24 h., 4 mL of a second acid mixture (1:1 $HN0_3$:HF) was added and the sample remained heated at ~80°C for another 24 h. The sample was then slowly heated to dryness on a hotplate, cooled, and then 3 drops of HCl and 4 mL $HN0_3$ were added. After gentle warming for a few minutes 20 mL of nanopure water was added and the sample heated for an additional 20 min. The sample was then diluted with nanopure water (1:9) to give a final acid concentration of 2%. The sample was analyzed directly using ICPMS and the results recorded in Table 10.

The method used for digestion was slightly modified from Trace Metals in Soils and Sediments MDS SOP #3010/#4079. A reagent blank and Standard Reference Material (SRM) Iron Ore were prepared as outlined above. The reagent blank was analyzed along with the sample; however, the NIST SRM 692 Iron Ore would not digest so could not be analyzed.

In both A and B, NIST SRM1640 Trace Elements in Natural Water was run as an external standard. Greater than 90% of analyzed values were within 17% of the certified value. Matrix errors were corrected using internal standards scandium, germanium, indium and terbium. Detection limit was determined by blank count rate +3 standard deviations of blank count rate divided by sensitivity (counts/sec/ppm).

Table 10. Trace elements in production water and production water precipitate.

Element	Production water Analyzed value (ppm)	Precipitate Analyzed value (ppm)	Detection limit (ppm)
Li	1.11	11.2	<0.01
Al	ND	33	0.010
Si	17.8	2090	0.05
Ca	1390	14000	0.5
V	0.197	0.084	<0.001
Cr	0.027	6.86	0.002
Mn	1.52	23.5	<0.001
Fe	5.08	14000	0.05
Co	0.00277	0.0627	<0.001
Ni	0.0634	1.13	<0.001
Cu	0.596	2.28	<0.001
Zn	0.00267	0.42	0.001
As	0.231	0.406	<0.001
Se	0.456	0.351	0.002
Sr	314	2310	<0.001
Mo	0.00573	0.651	<0.001
Cd	0.000412	0.00503	<0.001
Ba	58.5	615	<0.001
Pb	0.00264	0.402	<0.001

2.2 Exposure Trials with Production Water

2.2.1 *Artemia* nauplii exposure to Production Water

Trial 1: In this experimental trial, *Artemia* nauplii were exposed to different concentrations of production water to give approximate determinations of the concentration lethal to 50 percent of the test organisms after 24 h exposure (24 h LC50). The *Artemia* exposure method was adapted from Vanhaecke and Persoone, 1984. *Artemia* desiccated cysts (~100 mg) were sprinkled on the surface of 1L of seawater in a beaker held at room temperature (~20°C). The incubation beaker was aerated using an airstone with air supplied by a small electric aquarium pump. The cysts were aerated in the beaker and incubated for 48 h under constant room illumination. At 48 h, the nauplii were used in the dosing experiments.

Several hundred nauplii were transferred, by Pasteur pipette, from the incubation beaker to a 9 cm glass petri dish containing aerated seawater at room temperature. This first transfer facilitated the picking of 10 vigorously swimming individual larvae which were then transferred to each of the smaller (5 cm diameter) test petri dishes. Ten nauplii were transferred manually to each small test petri dish (5 cm diameter) using a glass Pasteur pipette in a volume of no more than 50 µl (total) of seawater so as not to upset the final concentration of each dose test solution. The test solutions were prepared in new 20 mL glass scintillation vials. After the 10 nauplii were transferred to the test petri dish, the test solution was immediately poured carefully from the scintillation vial over the nauplii in the petri dish. Each concentration was tested in duplicate with 10 nauplii each. Petri dishes containing nauplii in exposure solutions were covered lightly with a small sheet of polyethylene film to protect from dust and to slow evaporation. Nauplii were exposed to the 1% and 5% test concentrations for 24 h and for the 25% test concentration for 48 h.

Each test concentration of either production water, "1/3 production water", or sodium chloride was made up by adding the appropriate volume of test solution to aerated seawater at room temperature (20°C). Production water was shaken vigorously before use to resuspend particulate sediment. Each solution was prepared in 20 mL glass scintillation vials (in duplicate) and shaken vigorously to mix before pouring carefully into a labeled petri dish containing 10 *Artemia* nauplii. Exposure trials were conducted at room temperature using production water or appropriate control solutions. Counts of nauplii remaining alive were done at 24 h for all test solutions and again at 48 h for the most concentrated test solutions. Nauplii were determined to be alive if movement was detected. The exposure trial protocols are outlined in the following tables. Experiments A to F were run concurrently.

Setup A: Production Water

Three concentrations (1%, 5% and 25%) of production water were tested in duplicate (A and B) in 5 cm glass petri dishes. Seawater was used as a diluent to mix test solutions.

Results are reported in Table 11.

Table 11. Survival data - production water.

Test Concentration of Production Water Duplicate A or B	Production water	Seawater	*Artemia* nauplii per dish	# Alive 24 h	# Alive 48 h
A:1%(0.1 mL/10 mL)	100 µL	9.9 mL	10	10 alive 0 dead	
B:1%(0.1 mL/10 mL)	100 µL	9.9 mL	10	10 alive 0 dead	
A:5%(0.5 mL/10 mL)	500 µL	9.5 mL	10	10 alive 0 dead	
B:5%(0.5 mL/10 mL)	500 µL	9.5 mL	10	10 alive 0 dead	
A:25%(2.5 mL/10 mL)	2500 µL	7.5 mL	10	10 alive 0 dead	8 alive 2 dead
B:25%(2.5 mL/10 mL)	2500 µL	7.5 mL	10	10 alive 0 dead	7 alive 3 dead

Setup B - 1/3 Dilution of Production Water

Production water was first diluted (1 volume of production water to 2 volumes of distilled water) to give a "1/3 production water" test solution. This "1/3 production water solution" was then further diluted with seawater as indicated in the table in a similar manner to Setup A. Production water (salinity 10.3%) was first diluted to 1/3 to approximate the salinity of seawater (3.2%) and served as a control for salinity. Each concentration tested was conducted in duplicate (A and B) in 5 cm glass petri dishes. Results are reported in Table 12.

Table 12. Survival data - "1/3 production water".

Test Concentration of 1/3 production water Duplicate A or B	1/3 Production water	Seawater	*Artemia* nauplii per dish	# Alive 24 h	# Alive 48 h
A:1%(0.1 mL/10 mL)	100 μL	9.9 mL	10	10 alive 0 dead	
B:1%(0.1 mL/10 mL)	100 μL	9.9 mL	10	10 alive 0 dead	
A:5%(0.5 mL/10 mL)	500 μL	9.5 mL	10	10 alive 0 dead	
B:5%(0.5 mL/10 mL)	500 μL	9.5 mL	10	10 alive 0 dead	
A:25%(2.5 mL/10 mL)	2500 μL	7.5 mL	10	10 alive 0 dead	9 alive 1 dead
B:25%(2.5 mL/10 mL)	2500 μL	7.5 mL	10	10 alive 0 dead	10 alive 0 dead

Setup C - Control with 10.3% Sodium chloride solution

The sodium chloride exposure controlled for effects of salinity as production water had been previously determined to be 10.3% salinity. Each concentration tested was conducted in duplicate (A and B) in 5 cm glass petri dishes. Results are reported in Table 13.

Table 13. Survival data - 10.3% sodium chloride solution.

Test Concentration Duplicate A or B	10.3% NaCl	Seawater	*Artemia* nauplii per dish	# Alive 24 h	# Alive 48 h
A:1%(0.1 mL/10 mL)	100 µL	9.9 mL	10	10 alive 0 dead	
B:1%(0.1 mL/10 mL)	100 µL	9.9 mL	10	10 alive 0 dead	
A:5%(0.5 mL/10 mL)	500 µL	9.5 mL	10	10 alive 0 dead	
B:5%(0.5 mL/10 mL)	500 µL	9.5 mL	10	9 alive 1 dead	
A:25%(2.5 mL/10 mL)	2500 µL	7.5 mL	10	10 alive 0 dead	7 alive 3 dead
B:25%(2.5 mL/10 mL)	2500 µL	7.5 mL	10	10 alive 0 dead	5 alive 5 dead

Setup D - Control with Seawater

Exposures were conducted in duplicate (A and B) in 5 cm glass petri dishes using aerated seawater as control. Results are reported in Table 14.

Table 14. Survival data - seawater control.

Test solution Duplicate A or B	Seawater	*Artemia* nauplii per dish	# Alive 24 h	# Alive 48 h
A: Straight Seawater	10 mL	10	10 alive 0 dead	5 alive 5 dead
B: Straight Seawater	10 mL	10	8 alive 2 dead	6 alive 4 dead

Setup E: 1/3 Production water

Production water diluted 1 volume of production water to 2 volumes of distilled water, was used (without further dilution with seawater) as the test solution. Number of nauplii alive were also checked after 72 h. Duplicate exposures were not done. Results are reported in Table 15.

Table 15. Survival data - "1/3 production water".

Test solution (No replicate)	1/3 Production water	*Artemia* nauplii per dish	# Alive 24 h	# Alive 48 h	# Alive 72 h
1/3 Production water	10 mL	10	10 alive 0 dead	10 alive 0 dead	9 alive 1 dead

Setup F: Straight Production water

Undiluted production water was used. Duplicate exposures were not done. Results are reported in Table 16.

Table 16. Survival data - production water.

Test solution (No replicate)	Production water	*Artemia* nauplii per dish	# Alive 24 h	# Alive 48 h	# Alive 72 h
Straight Production water	10 mL	10	10 alive 0 dead	10 alive 0 dead	0 alive 10 dead

Trial 2: Repeat of Trial 1 using only Highest Test Concentrations for Exposures

In Trial 2 some of the experimental exposures done in Trial 1 were repeated. For each test solution, exposures were performed in triplicate (A, B and C) in 5 cm glass petri dishes using the same methodology as for Trial 1 and using only the highest concentration (25%) for each of the test solutions (straight production water, "1/3 production water" dilution, sodium chloride solution, and seawater control) as used in Trial 1. Results are reported in Tables 17, 18, 19 and 20, respectively.

Table 17. Production water.

Test Concentration Replicate: A, B or C	Production water	Seawater	*Artemia* nauplii per dish	# Alive 24 h
A:25%(2.5 mL/10 mL)	2500 µL	7.5 mL	10	10 alive 0 dead
B:25%(2.5 mL/10mL)	2500 µL	7.5 mL	10	10 alive 0 dead
C:25%(2.5 mL/10 mL)	2500 µL	7.5 mL	10	10 alive 0 dead

Table 18. "1/3 Production water" dilution.

Test Concentration Replicate A, B or C	"1/3 Production water" dilution	Seawater	*Artemia* nauplii per dish	# Alive 24 h
A:25%(2.5 mL/10 mL)	2500 µL	7.5 mL	10	10 alive 0 dead
B:25%(2.5 mL/10 mL)	2500 µL	7.5 mL	10	10 alive 0 dead
C:25%(2.5 mL/10 mL)	2500 µL	7.5 mL	10	10 alive 0 dead

Table 19. 10% NaCl control.

Test Concentration Replicate A, B or C	10.3 % NaCl	Seawater	*Artemia* nauplii per dish	# Alive 24 h
A:25%(2.5 mL of 10.3% NaCl/10 mL)	2500 µL	7.5 mL	10	10 alive 0 dead
B:25%(2.5mL of 10.3% NaCl /10 mL)	2500 µL	7.5 mL	10	10 alive 0 dead
C:25%(2.5mL of 10.3% NaCl /10 mL)	2500 µL	7.5 mL	10	10 alive 0 dead

Table 20. Seawater control.

Control Replicate A, B or C	Seawater	*Artemia* nauplii per dish	# Alive 24 h
A: Seawater only	10 mL	10	10 alive 0 dead
B: Seawater only	10 mL	10	10 alive 0 dead
C: Seawater only	10 mL	10	10 alive 0 dead

2.2.2 Marine Copepod Exposure to Production Water

Trial 3: The same stock of copepods collected for use in Experiment 1, Trials 4 and 5, was used to test the effects of production water in this experiment. All exposures were done in 250 mL Mason jars. Three concentrations of production water (1%, 5%, 25%) were tested in this set of exposures. Seawater was used as a diluent to prepare the production water test concentrations.

The salinity of the production water used was determined to be 103 ppt or 10.3%, (Dr. Philip Yeats, Bedford Institute of Oceanography, pers. comm.). To control for the effects of the high salinity of production water, a set of control exposures using 10.3% NaCl as the test solution was run concurrently with the production water test concentrations.

Seawater was also used as a diluent to prepare the NaCl test solutions.

All test solutions were prepared in duplicate. After the seawater control and either production water or NaCl solutions were prepared, the jars were capped and shaken very vigorously to ensure mixing. Twenty milliliters of seawater containing approximately 20-30 copepods was added to each jar to bring the total volume per jar to 100 mL. The jars were then placed in an aquarium which contained running seawater at ambient temperature. The jars received no aeration. After the test exposures had been run for 24 h, the number of dead copepods was recorded. Dead copepods were removed, and preserved in 70% ethanol in a glass vial. Exposures were continued and after 48 h the number of dead copepods was again recorded. Any dead copepods were removed, and preserved in 70% ethanol in a glass vial. The preserved copepods were used to confirm species identification. Table 21 outlines the composition of each replicate test solution and the survival data for each exposure.

Table 21 -Survival data for test concentrations of production water.

Test Concentration	Production water volume	10.3 % NaCl aqueous solution	Seawater volume	Copepods in seawater volume	24 h survival	48 h survival
A: 1% Control (NaCl)	0 mL	1 mL	79 mL	20 mL	24 alive 0 dead 100% survival	24 alive 0 dead 100% survival
B: 1% Control (NaCl)	0 mL	1 mL	79 mL	20 mL	18 alive 0 dead 100% survival	18 alive 0 dead 100% survival
A: 1% Production water	1 mL	0 mL	79 mL	20 mL	20 alive 0 dead 100% survival	20 alive 0 dead 100% survival
B: 1% Production water	1 mL	0 mL	79 mL	20 mL	39 alive 0 dead 100% survival	39 alive 0 dead 100% survival
A: 5% Control (NaCl)	0 mL	5 mL	75 mL	20 mL	19 alive 0 dead 100% survival	18 alive 1 dead 94.7% survival
B: 5% Control (NaCl)	0 mL	5 mL	75 mL	20 mL	24 alive 0 dead 100% survival	23 alive 1 dead 95.8% survival
A: 5% Production water	5 mL	0 mL	75 mL	20 mL	29 alive 0 dead 100% survival	29 alive 0 dead 100% survival
B: 5% Production water	5 mL	0 mL	75 mL	20 mL	36 alive 0 dead 100% survival	35 alive 1 dead 97.2% survival
A: 25% Control (NaCl)	0 mL	25 mL	55 mL	20 mL	0 alive 16 dead 0% survival	0% survival at 24 h
B: 25% Control (NaCl)	0 mL	25 mL	55 mL	20 mL	0 alive 19 dead 0% survival	0% survival at 24 h
A: 25% Production water	25 mL	0 mL	55 mL	20 mL	8 alive 19 dead 29.6% survival	0 alive 8 dead 0% survival
B: 25% Production water	25 mL	0 mL	55 mL	20 mL	5 alive 12 dead 29.4% survival	0 alive 5 dead 0% survival

Experiment 3: Hibernia Drill Mud Cuttings

3.1 Exposure Trials with Hibernia Drill Mud Cuttings

3.1.1 *Artemia* nauplii Exposure to Seawater Extraction of Hibernia Drill Mud Cuttings

Trial 1: One gram of wet Hibernia drill mud cuttings was weighed into each of thee disposable polypropylene test tubes. Ten milliliters of aerated seawater at room temperature was added to each test tube. The suspensions in the three tubes were mixed using a Vortex mixer for 5 min. on high speed. The tubes were centrifuged at approximately 1200 x g for 5 min. The supernatant seawater was carefully removed with a glass Pasteur pipette leaving the mud pellet and approximately 1 mL of supernatant undisturbed at the bottom, and was transferred to a second polypropylene test tube and re-centrifuged at approximately 1200 x g for 5 min. The supernatant liquid was again removed and transferred to a third tube.

Artemia nauplii were prepared by incubating desiccated cysts in seawater for 48 h, as in previous trials conducted with production water. After 48 h incubation, 10 *Artemia* nauplii were transferred to each of thee small 5 cm glass petri dishes. Each test solution prepared from the three drill mud extractions above, was slowly poured into each one of the three petri dish replicate exposures A, B and C. Thee control replicate dishes were also set up with 10 *Artemia* nauplii each in 10 mL of aerated seawater. Each test petri dish was held at room temperature for 24 h under constant illumination. After 24 h exposure, the number of nauplii remaining alive were counted. Results are reported in Table 22.

Table 22. Survival data -Trial 1 Hibernia drill mud cuttings seawater extraction.

Exposure	# Alive/total after 24 h exposure		
	Replicate A	Replicate B	Replicate C
Control (Seawater)	10/10	10/10	10/10
Hibernia drill cuttings seawater extract	10/10	10/10	10/10

Trial 2: One gram of wet Hibernia drill mud cuttings was weighed into each of three disposable polypropylene test tubes. Ten milliliters of aerated seawater at room temperature was added to each test tube. The three tubes were mixed with a Vortex mixer for 5 min. Ten milliliters of the over laying seawater with suspended flocculent material was pipetted into a second polypropylene tube. (The mud that remained settled on the bottom of the test tube was discarded). Three replicate 5 cm petri dishes were prepared by adding 10 *Artemia* nauplii (48 h old) to each as in previous trials. The mixture in each test tube was first re-suspended with a Pasteur pipette and then carefully poured into one of the replicate petri dishes. This process was repeated for the second and third test tubes

before pouring into the other two replicate dishes. Three replicate petri dishes with 10 nauplii each were setup with 10 mL of seawater each to serve as controls. Nauplii were exposed to the test solutions for 24 h at room temperature as in Trial 1. Results are reported in Table 23.

Table 23 - Survival Data -Trial 2 Hibernia Drill Mud Cuttings seawater extraction.

Exposure	# Alive/total after 24 h exposure		
	Replicate A	Replicate B	Replicate C
Control (Seawater)	10/10	10/10	10/10
Hibernia drill cuttings seawater extract with flocculent material	10/10	10/10	10/10

3.1.2 Exposure of Juvenile Yellowtail Flounder to seawater extraction of Hibernia Drill Mud Cuttings

Trial 3:

Replicate A:

Two tanks were set up with a continuous air flow at 7°C: a control tank contained 4.5 L seawater only and an exposure tank contained 50 g of wet Hibernia drill mud cuttings that had been shaken vigorously 3 times with 1 L seawater in a 1 L mason jar. The 3 L of extract along with the Hibernia drill cuttings were transferred to the exposure tank and the volume brought up to 4.5 L.

Twenty six of the 100 juvenile yellowtail flounder obtained from the Ocean Science Centre (where they were raised at 10°C) were transferred to the control and exposure tank the same day, 13 fish per tank at 7°C. After 4 h the fish in both tanks were alive but not very active (they did not respond when gently touched with a net). After 24 h, all fish were still not responding well and two were dead in the exposed tank. The two dead fish were removed and the tanks were placed in a larger 260 L tank that was regulated at 10°C to act as a water bath. The temperature of our exposure and control tanks was slowly raised to 9°C and the remaining fish became livelier. The exposure was checked a couple times per week and left for 51 days.

Replicate B:

Six days following Set Up A, 20 more juvenile yellowtail flounder were obtained from the Ocean Science Centre. The fish were left in 2 bags of water from the Ocean Science

Centre and placed in two 5 L tanks set up at 9°C as above; one control and one with Hibernia drill mud cuttings. After 30 min, the fish were transferred to the tanks, 10 in each. The exposure was checked 2-3 times per week and left for 45 days.

The results of Replicates A and B are reported in Table 24.

Table 24. Survival data -Trial 3 Hibernia drill mud cuttings.

Exposure	Replicate A (45 days)	Replicate B (39 days)	Replicate A (51 days)	Replicate B (45 days)
Control 4.5 L seawater	0 dead 13 alive 100% survival	0 dead 10 alive 100% survival	0 dead 13 alive 100% survival	0 dead 10 alive 100% survival
50g drill cuttings per 4.5 L seawater	0 dead 11 alive 100% survival	0 dead 10 alive 100% survival	2 dead 11 alive 85% survival	9 dead 1 alive 10% survival

Note: Exposure trial was checked January 8, 1999 (45 and 39 day exposures), all fish were living. Exposure trial was checked again on January 14, 1999 (51 and 45 day exposure) when 2 deaths from Replicate A and 9 deaths from Replicate B were reported. We do not expect the deaths to be a result of the exposure to drill cuttings since the replicates did not give the same results. An explanation for the deaths could be bacterial build-up from fish left under static water conditions.

3.1.3 Exposure of Juvenile Yellowtail Flounder to Hibernia Drill Mud Cuttings in Sediment

Trial 4: The Ocean Science Centre collected sand from the beach at Lower Pond, Witless Bay, NF. Concentrations of drill mud cuttings were prepared by mixing 0, 3, 9, 27, 81 and 243 g of drill mud cuttings per liter of dry sand. Homogeneous mixtures were ensured by gradual addition and thorough mixing of small quantities of sand into the drill cuttings. Each concentration was transferred to six 5 L tanks supplied with aerated seawater in a flow though system and left overnight. Juvenile yellowtail flounder were obtained from the Ocean Science Centre and were acclimated to ambient temperature (2°C) in an aerated, flowthough system. Each tank received 19 juvenile yellowtail flounder and was held under ambient conditions (~2-5°C) for 95 days, in a darkened room. The fish were checked 2-3 times per week for survival status. Results are reported in Table 25.

Table 25. Survival data - Trial 4 Hibernia drill mud cuttings in sand.

Concentrations of drill mud Cuttings per liter of sand	# Surviving/total (95 days exposure)
0g/L	19/19
3g/L	19/19
9g/L	19/19
27g/L	19/19
81g/L	18/19
243g/L	19/19

Note: Death in 81g/L exposure occurred after 21 days exposure.

CONCLUSIONS

A number of studies have been carried out on plankton, fish larvae and juvenile fish to investigate the acute toxicity potential of synthetic base cuttings and production water. Acute toxicity potentials were demonstrated to be very low indicating that the wastes pose little or no risk of an acute toxic nature to the marine environment.

ACKNOWLEDGMENTS

We thank the Program on Energy Research and Development (PERD) for providing support for these studies, Hibernia for supplying drill cuttings, Terra Nova for drilling fluids and SOEP for production water.

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