Overview of Aquatic Toxicity Testing under the U.S. EPA Oil Research Program

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The U.S. EPA Office of Research and Development is developing baseline data on the ecotoxicity of selected petroleum products, chemical dispersants, and other spill mitigating substances as part of its Oil Research Program. Two diluted bitumens (dilbits) from the Alberta Tar Sands region are being tested for acute and chronic toxicity to standard freshwater and marine organisms given their spill potential during shipment within the United States. Separately, crude oils representing a range of characteristics and representative dispersants are being tested to evaluate acute and chronic toxicity to marine organisms in support of proposed regulatory amendments to Subpart J of the U.S. National Contingency Plan. Water accommodated fractions (WAF) of oil are prepared using traditional slow-stir methods and toxicity tests follow U.S. EPA standard effluent testing guidelines, modified for testing petroleum products. WAFs are characterized for petroleum hydrocarbons including alkyl PAH homologs. Future research plans include evaluating oil spill mitigating substances such as surface washing and bioremediation agents. The results of the research program will assist the U.S. EPA in assessing toxicity of unconventional oils (dilbits), and establish baseline toxicity data for selected crude oils and spill mitigating substances in support of planning and response activities.

INTRODUCTION

The National Oil and Hazardous Substances Pollution Contingency Plan (NCP) is the federal government's blueprint for responding to both oil spills and hazardous substance releases (*40 CFR 300*; www.epa.gov/emergency-response/national-oil-and-hazardous-substances-pollution-contingency-plan-ncp-overview). Subpart J of the NCP establishes a Product Schedule,

that includes dispersants and other spill mitigating substances that may be used in carrying out the NCP (*40 CFR 300*), and also testing requirements and authorization of use procedures for those substances. This research supports EPA's role under the NCP to provide "expertise on human health and ecological effects of oil discharges or releases of hazardous substances, pollutants, or contaminants; ecological and human health risk assessment methods; and environmental pollution control techniques."

EPA's Office of Research and Development established the Oil Research Program to support the Agency's role in planning and coordinating responses, providing guidance to Regional Response Teams, coordinating a national program of preparedness planning and response, and facilitating research to improve response activities. Currently three phases of research are being focused on specific aspects of the aquatic toxicology of petroleum products and spill mitigating substances. Phase 1 is determining the acute and chronic toxicity of two diluted bitumens (dilbits) from the Alberta Tar Sands region because there is only limited available toxicity data for dilbits, and the spill potential during shipment within the United States. Phase 2 of the research program is evaluating crude oils representing a range of oil physical properties to inform proposed amendments to Subpart J of the U.S. National Contingency Plan. Phase 2 is also evaluating the toxicity of representative dispersants following proposed revisions to the Subpart J regulation. A Phase 3 of the Oil Spills Research Program is planned to evaluate the aquatic toxicity of selected oil spill mitigating substances such as surface washing and bioremediation agents. This paper provides an overview of EPA's oil toxicology research program that will assist the Agency in assessing toxicity of unconventional oils (dilbits), and establish baseline toxicity data for selected crude oils and spill mitigating substances in support of planning and response activities.

PHASE 1 TOXICITY RESEARCH: DILBITS

Two diluted bitumens (dilbits) from the Alberta Tar Sands region have been selected for acute and chronic toxicity testing with four standard freshwater and marine organisms: Cold Lake Blend (CLB) and Western Canadian Select (WCS). These two dilbits represent a range of the diluted bitumens that are shipped extensively through pipelines in North America. Both fresh and artificially weathered CLB and WCS are being tested. Fresh Alaska North Slope crude oil is also being tested to allow comparison of dilbits to an oil of well-known toxicity. Water accommodated fractions (WAF) of oil were prepared using traditional slow-stir methods (Fig. 1A), and only the aqueous phase is used in toxicity tests. Test solutions are prepared using the variable dilution method (NRC, 2005).

Acute tests will be conducted with early life stages of four aquatic species (Table 1): a fresh water cladoceran zooplankton (*Ceriodaphnia dubia*) (Fig. 1B), a freshwater fish (fathead minnow, *Pimephales promelas*) (Fig. 1C), a marine invertebrate (mysid; *Americamysis bahia*) (Fig. 1D), and a marine fish (inland silverside; *Menidia beryllina*) (Fig. 1E). Acute and chronic toxicity tests follow U.S. EPA standard effluent testing guidelines for organism age and test methods, modified for use with petroleum mixtures (Table 1; Fig. 2). WAFs are characterized for a range of petroleum hydrocarbons including BTEX (benzene, toluene, ethylbenzene, xylenes), polycyclic aromatic hydrocarbons (PAHs) and their alkyl homologs, normal alkanes (C10-C35), and total petroleum hydrocarbons (TPH). This information will add to the limited available public information on the toxicity data of dilbits (e.g., Dew et al., 2015), and allow more informed hazard assessments in case of dilbit spills in fresh or marine waters of the United States.

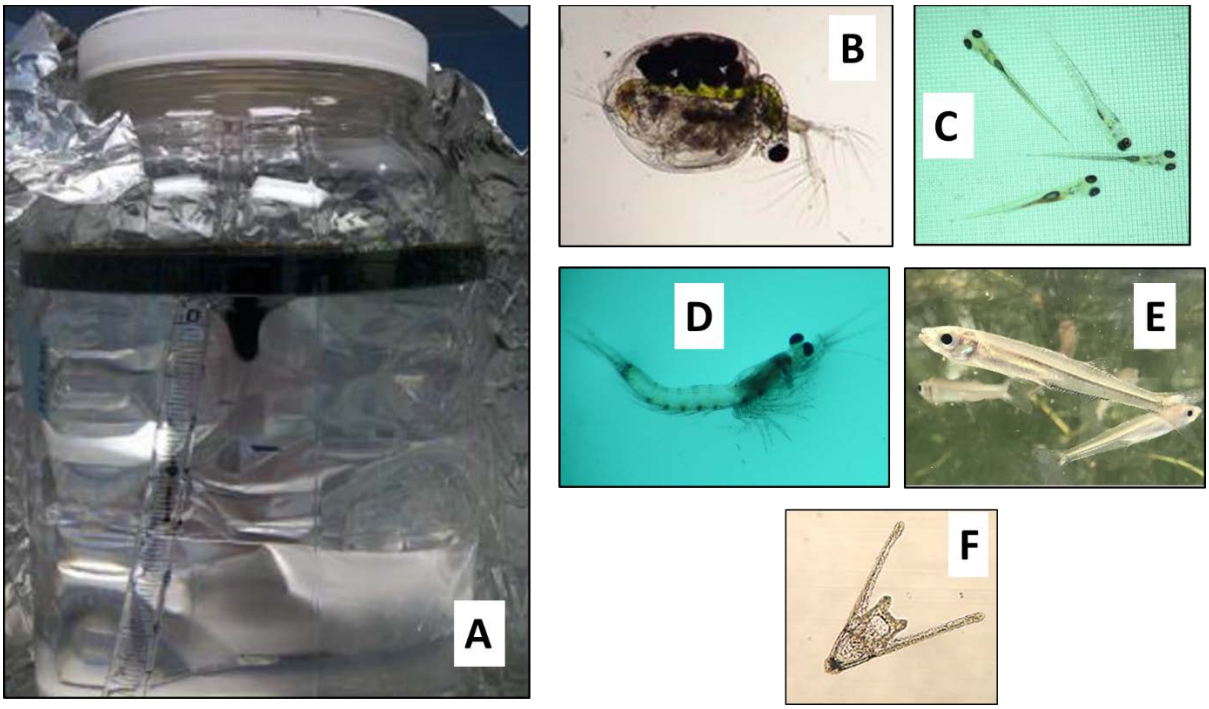

Figure 1. Photograph of a water accommodated fraction (WAF) prepared with dilbit (A), and life stages and species of aquatic test organisms: *Ceriodaphnia dubia* (B); fathead minnow, *P. promelas* (C); mysid, *A. bahia* (D); silverside, *M. beryllina* (E); and urchin, *A. punctulata* (F).

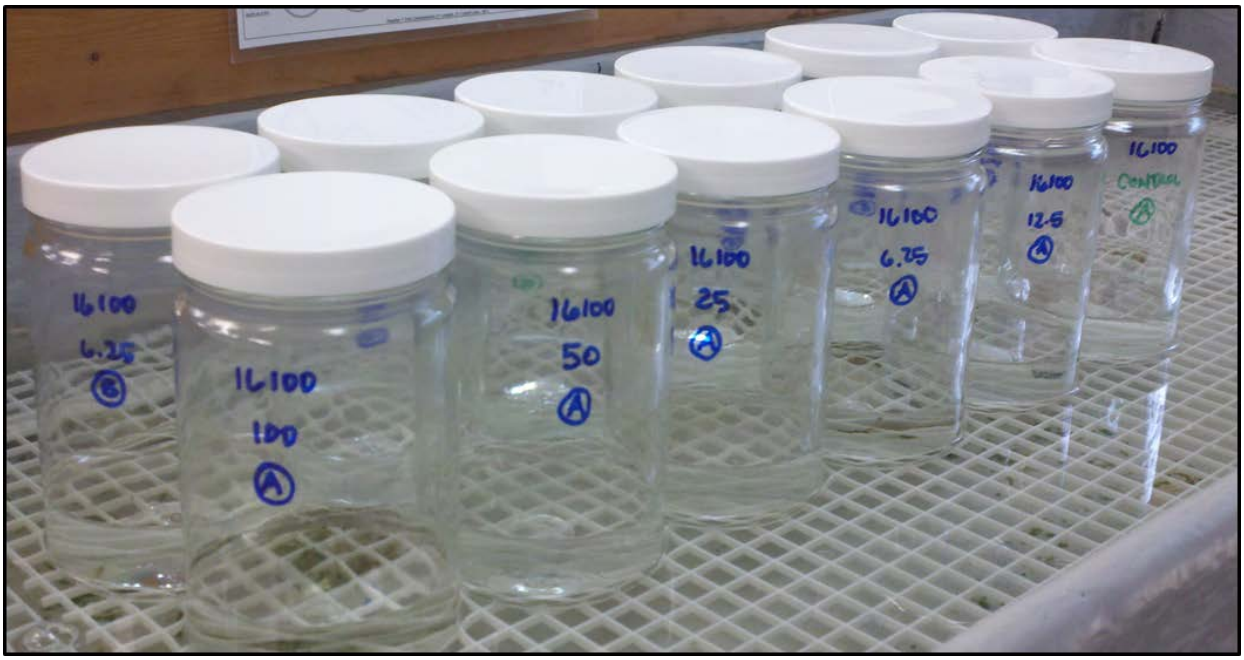

Figure 2. Photograph of example aquatic toxicity test chambers and temperature controlled water bath.

Table 1. Summary of acute and chronic toxicity test methods for four aquatic species.

Test species	Cladocern (*C. dubia)*	Mysid (*A. bahia)*	Fathead minnow *(P. promelas*	Inland silverside (*M. beryllina)*	Cladoceran (*C. dubia)*	Mysid (*A. bahia)*
Test method	EPA-821-R-02-012, Method 2002.0	EPA-821-R-02-012, Method 2007.0	EPA-821-R-02-012, Method 2000.0	EPA-821-R-02-012, Method 2006.0	EPA-821-R-02-013, Method 1002.0	EPA-821 R-02-014, Method 1007.0
Test type	Acute	Acute	Acute	Acute	Chronic	Chronic
Test renewal	Static	Static	Static	Static	Static renewal	Static renewal
Organism type	Fresh water invertebrate	Marine invertebrate	Fresh water fish	Marine fish	Freshwater invertebrate	Marine invertebrate
Organism Age	<24 hours	3-4 days	7-12 days	10-14 days	<24 hours	7 days
Test duration	48 hours	48 hours	96 hours	96 hours	7 days	7 days
Salinity	NA[1]	20 ± 2‰	NA	20 ± 2‰	NA	20 ± 2‰
Renewal	NA	NA	NA	NA	Daily	Daily
Temperature[2]	25 ± 1 °C. Test temperatures must not deviate by more than 3 °C during the test					
Light quality	Ambient laboratory illumination					
Light intensity	10–20 (E/m2/s)					
Photoperiod	16 h light, 8 h darkness, with phase in/out period recommended					
Test chamber size[3]	30 mL	500 mL	1 L	1 L	30 mL	500 mL
Test solution volume	20 mL	200 mL	200 ml	200 mL	20 mL	200 mL
organisms per test chamber	5	10	10	10	1	5
Replicate chambers	4	3	3	3	10	8
Feeding regime	Refer to specific feeding procedures provided in each test method					
Aeration	None, unless DO falls below 4.0 mg/L, then aerate all chambers. Rate: <100					
Physical / Chemical Measurement	Daily temperatures in one replicate of each test concentration. Exposure test solutions analyzed daily for pH, dissolved oxygen, and conductivity or salinity					
Test Levels	5 exposure concentrations and a control					

Test Acceptability	≥90% control survival	≥90% control survival	≥90% control survival	≥90% control survival	≥80% control survival; min 15 young per surviving female	≥80% control survival; min 0.20 mg mass
Endpoint	48 hr survival	48 hr survival	96 hr survival	96 hr survival	7 day survival and reproduction	7 day survival and growth

1. NA: not applicable.
2. Mysid temperature recommended at 26 ± 1 °C (see Table 2).
3. All test chambers covered.

PHASE 2 TOXICITY RESEARCH: CRUDE OILS AND DISPERSANTS

EPA is currently evaluating crude oils and testing example dispersants in support of proposed amendments to Subpart J of the NCP. Crude oils representing a range of physical properties are being evaluated in support of switching from Subpart J specified testing with fuel oil #2 to alternative crude oils. Crude oils will be considered that represent a range of physical, chemical and compositional characteristics. Candidate oils to be tested may include light and mid-range crude oils (e.g., Endicott, Dorado) or intermediate fuel oils. EPA is collecting baseline toxicity data with the two aquatic test species currently used for testing under Subpart J. Tests will use the acute methods of Table 1 with the mysid (*A. bahia*), a marine crustacean invertebrate, and the inland silverside (*M. beryllina*), a marine fish. Results from this effort are intended to support testing of spill mitigation substances for listing on the Product Schedule, pending any future amendments to the Subpart J regulation.

EPA is also testing four example dispersants that are currently listed on the NCP Product Schedule, as a component of Phase 2 of the Oil Research Program. In addition to the standard acute toxicity tests of dispersants with *A. bahia* and *M. beryllina*, chronic toxicity will also be assessed with these two species (Table 2). There is limited information concerning the sublethal toxicity of dispersants currently listed on the Product Schedule. This information would be of value

as guidance to responders on possible adverse effects on survival and growth of larval fish and invertebrates caused by longer-term exposure to dispersants such as those that may be encountered during atypical dispersant operations (NRT, 2013). Results of Phase 2 testing will be compared to exiting toxicity results on the Product Schedule and scientific literature. Chronic testing is also being conducted on a third species representative of an additional taxonomic group: the purple sea urchin, *Arbacia punctulata*, an echinoderm invertebrate (Table 3; Fig. 1F). Testing an additional species was deemed valuable because of the limited information on dispersant toxicity to species other than mysids and silversides, and the potential sensitivity of echinoderms. Dispersants being considered for testing to provide baseline comparative toxicity data include Corexit 9500A, Finasol, Accel, and other products.

Table 2. Summary of the chronic toxicity test methods with the myisd (*A. bahia*) and inland silverside (*M. beryllina*).

Test species	***Americamysis bahia***	***Menidia beryllina***
Test method	EPA-821-R-02-014, Method 1007.0	EPA-821-R-02-014, Method 1006.0
Test type	Static	
Test duration	7 days	
Salinity	20 ± 2‰	20 ± 2‰
Temperature	26 ± 1 °C. Test temperatures must not deviate (maximum minus minimum temperature) by more than 3 °C during test	
Light quality	Ambient laboratory illumination	
Light intensity	10–20 (E/m2/s)	
Photoperiod	16 h light, 8 h darkness, with phase in/out period recommended	
Test chamber size[1]	500 mL	1 L
Test solution volume	200 mL	500 mL
Age of test organism	7 days	7-11 days
Organisms per test chamber	5	10
Replicate chambers per concentration	8	4
Feeding regime	Refer to specific feeding procedures provided in each test method	
Aeration	None, unless DO falls below 4.0 mg/L, then aerate all chambers. Rate: <100 bubbles/minute	

Physical / Chemical Measurements	Daily temperatures measured in one replicate for each test concentration. Exposure test solutions analyzed daily for pH, dissolved oxygen, and salinity	
Test concentrations	5 exposure concentrations and a control	
Test acceptability	>80% survival and average dry weight > 0.20 mg in the controls	>80% survival and average dry weight > 0.50 mg in controls
Endpoints	Survival and growth	Survival and growth
1. Test chambers covered.		

Table 3. Summary of the 72 hour purple sea urchin development toxicity test.

Test species	***A. punctulata***
Test method	EPA-600-R-95-136, Method 1008.0
Test type	Static non-renewal
Temperature	20 ± 1oC
Light quality	Ambient laboratory illumination
Light intensity:	100 ft-c (ambient lab levels)
Replicates per concentration	4
Test chamber size	30-mL scintillation vials
Test solution volume	10-mL
Fertilized Egg Holding Time	< 1 hour
Fertilized Eggs #/vial	250
Dilution water	Synthetic or Natural Seawater
Salinity	30 ± 2‰
Effluent concentrations	5 plus controls
Dilution factor	>0.5 series
Test duration	72-hours
Effects measured	Normal development; mortality can be included.
Test Acceptability	≥80% normal shell development in the controls; must achieve a %MSD of <25%.
1. Test chambers sealed.	

PHASE 3 TOXICITY RESEARCH: OTHER OIL SPILL MITIGATION SUBSTANCES

Future research plans include evaluating the toxicity of other oil spill mitigating substances such as surface washing and bioremediation agents. Compared to oils and chemical dispersants, there is only very limited information on the toxicity of other types of spill mitigating substances

in the scientific literature. The objective of Phase 3 of the EPA Oil Research program will be to evaluate WAF preparation methods and behavior of these other spill mitigating substances under controlled laboratory conditions, and to collect baseline toxicity data using standardized test methods for both freshwater and marine species. Acute toxicity tests with four species (Table 1) are planned with one or two selected agents from each spill mitigation category (e.g., surface washing and bioremediation agents) that are listed on the NCP Product Schedule.

CONCLUSIONS

EPA's Office of Research and Development established the Oil Research Program to support the Agency's role in planning and coordinating responses, providing guidance to Regional Response Teams, coordinating a national program of preparedness planning and response, and facilitating research to improve response activities. Traditionally, the Research Program has focused on environmental degradation of oil, and dispersant efficacy. The addition of the toxicity assessment of oils and spill mitigating substances represents a significant research investment, and directly addresses EPA's responsibilities specified in the NCP regarding the ecological effects of oil discharges. The results of the three phases of aquatic toxicity research are anticipated to: (1) substantially add to the body of knowledge on toxicity of both fresh and weathered dilbits; (2) develop baseline toxicity data for new EPA reference oils; (3) determine the chronic toxicity of dispersants and other oil spill mitigation substances using standardized methods, and (4) establish the sea urchin as a third test species, in addition to the Subpart J requirements for testing a crustacean invertebrate (mysid) and fish (inland silverside).

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