SPILL TECHNOLOGY

NEWSLETTER

ISSN 0381-4459

VOLUME 19 (2)
April-June 1994

THE DEVELOPMENT OF A STANDARD METHOD FOR PRODUCING WATER ACCOMMODATED FRACTIONS FROM PETROLEUM HYDROCARBONS FOR AQUATIC TOXICITY TESTING

by
Sandra Blenkinsopp,
Gary Sergy, Pierre Boileau, and
Dan Kyle
Western Office, Environmental
Technology Centre
Environment Canada, Edmonton

Don Aurand
Marine Spill Response
Corporation, Washington, D.C.

Petroleum hydrocarbons range greatly in physical and chemical properties, from light and volatile refined products to heavily weathered and persistent crude oil residues. Through spills and waste discharges, petroleum hydrocarbons, more conveniently termed 'oils', can contaminate fresh and seawater environments. In order to assess environmental impact and determine the appropriate cleanup response, it is important to have a reliable and consistent means of evaluating hydrocarbon toxicity. Aquatic toxicity test procedures are well standardized in Canada; however, the mode by which oil samples are prepared for exposure to the test organism is inconsistent. Generally, the oil and water are stirred for a defined time period, followed by a settling period after which the water is collected and used for aquatic toxicity testing. This type of approach is used to measure the effect of the oil in the water, and excludes the effects that are solely due to direct physical contact with the bulk oil. Most investigators use a method unique to their laboratory and the resulting variations in sample preparation can alter the chemical composition of the sample produced. This in turn

This issue includes two articles related to aquatic toxicity testing of spilled substances. The first article is a summary of work underway to develop a standard method of producing water-accommodated fractions for toxicity testing. The second is a summary of toxicity testing programs of the Emergency Science Division.

Canada

Environment Canada / Environnement Canada

affects the actual toxicity results, comparative toxicity assessments, and test reproducibility.

Over the past year, the Emergencies Science Division of Environment Canada has been developing a standard method for preparing water accommodated fractions (WAFs) from a range of different petroleum hydrocarbons. A working group of experts and interested parties was formed to help determine the most efficient way to approach the development of a standard method. It was apparent that although a variety of methods existed, the underlying principles on which the existing methods were based were similar. Methods from the literature were therefore broken down into their component parts and factors that remained relatively constant were identified. For factors which varied greatly, such as mixing duration, it was obvious that testing needed to be performed. The working group was provided with suggestions for fixed and experimental factors. These factors were modified where necessary to reflect the experience of the group.

The main variables being tested for a range of liquid oils are:

- mixing energy,
- mixing duration, and
- settling time.

The issue of "headspace" in the mixing vessel is also being addressed (no headspace versus minimal headspace).

For this work, our definition of a 'liquid' oil is an oil that spreads over the water surface after pouring at room temperature. Three liquid oils were eventually selected for use in the experiments:

- Alberta sweet mixed blend crude oil,
- No. 6 Fuel Oil (Bunker C), and
- Gasoline (Sunoco 'Ultra 94').

Experiments are also being performed to determine whether plastic carboys are more suitable than glass vessels for generating water accommodated fractions. The main disadvantage of glass is the cost of vessels large enough to prepare 20-L WAFs, the volume of most interest. Discussions with the expert committee revealed that because of the prohibitive cost of scientific glassware, a variety of glassware is used, sometimes of dubious safety (i.e., thin-walled wine carboys). Fluorinated Nalgene carboys are being investigated as an alternative, and have the advantage of being unbreakable, relatively inexpensive, lightweight and easy to sample through ports placed directly in the side of the vessel. All containers are tightly capped or sealed for the experiments.

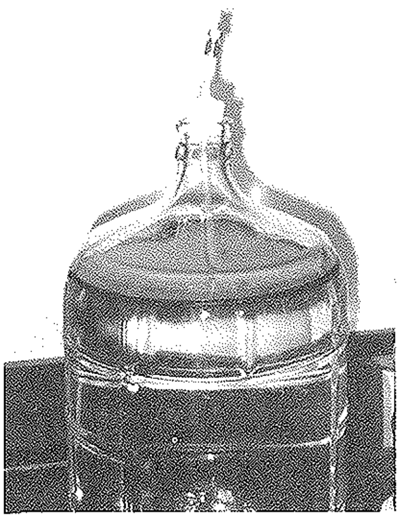
In addition, work is being performed on the best method for producing a water accommodated fraction from solid oils. In this case a 'solid' oil is defined as an oil that does not spread over the water surface after pouring at room temperature. Burn residue from the Newfoundland Offshore Oil Burn Experiment (NOBE) will be used to determine the best means of handling solid residue.

Experiments are nearing completion and the final report should be available in early 1995. To receive further information, contact Sandra Blenkinsopp at (403) 951-8705.

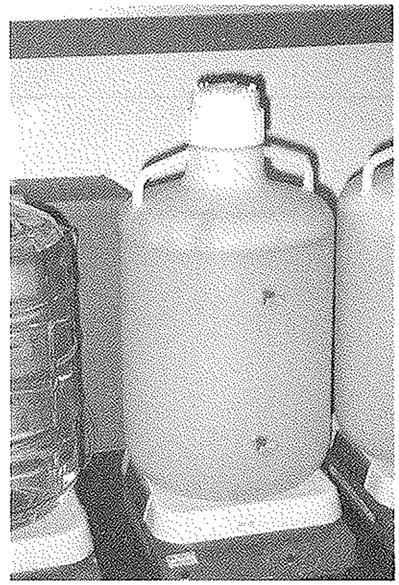
Acknowledgments

This work is funded by the Emergencies Science Division of Environment Canada and the Marine Spill Response Corporation. We thank Merv Fingas, Paula Jokuty, and Ken Li of the Emergencies Science Division for useful discussions.

Comparison of Glass and Nalgene Carboys



Glass Carboy with
10 000 mg/L of Alberta
Sweet Mixed Blend Crude
Oil on "Sea Water"



Nalgene Carboy at
10 000 mg/L Loading

Nalgene Carboys at Various Loadings

Glass Carboys at 100 and 10 mg/L
Loading

TOXICITY DETERMINATIONS OF SPILL SUBSTANCES AND SPILL COUNTERMEASURES

by
Sandra Blenkinsopp and
Gary Sergy
Western Office, Environmental Technology Centre
Environment Canada, Edmonton

The Emergencies Science Division of Environment Canada maintains a continuing program to ascertain the toxicity of a wide variety of spill-related substances and products. This includes substances that are spilled, or likely to be released into the environment by spills, or products that are used to combat spills. Information on the toxicity of such materials becomes essential both during an actual spill event, and in preparing for spills. It is used to help gauge the seriousness of a spill event, evaluate countermeasures, and decide on how to respond in the most appropriate manner.

Toxicity means the inherent potential or capacity of an material to cause adverse effects on living organisms. The effect should be *lethal* or *sublethal*, *acute*, or *chronic*. Information is gathered on toxicity in two ways. First, databases and the literature are searched for the many thousands of substances of interest, for example, chemicals on the "spill substance priority list" (Fingas *et al.* , 1992) for which manuals are being developed. Secondly, toxicity testing is conducted on materials for which further information is required, such as products used at spills.

Over the past 15 years, laboratory testing has been conducted on hundreds of samples. Almost all testing has been aquatic in nature and the primary test used has been the Rainbow Trout Acute Lethality Test (EC, 1990a). More recentlty, other types of assays have also been used.

Procedures recently developed and standardized by Environment Canada, as well as several others in common use in North America include:

- Acute Lethality Test using *Daphnia* spp. (EC, 1990b)
- Acute Lethality Test using Threespine Stickleback (EC, 1990c)
- Test of Reproduction and Survival using the Cladoceran *Ceriodaphnia dubia* (EC, 1992a)
- Test of Growth and Survival using Fathead Minnows (EC, 1992b)
- Toxicity Test using Luminescent Bacteria *Photobacterium phosphoreum* (EC, 1992c)
- Test of Growth and Inhibition using Green Algae *Selenastrum capricornutum* (EC, 1992d)
- Sediment Lethality Test using Marine or Estuarine Infaunal Amphipods (EC, 1992e)
- Test for Ferilization Success and Embryo Survival using Echinoids (EC, 1992f)
- Inland Silverside Test (U.S. EPA, 1991)
- Bivalve Larvae Test (ASTM, 1989)

Generally the types of samples tested are categorized as either spill-treating agents (STAs), petroleum hydrocarbons (commonly known as oils), or chemicals. Spill-treating agents are substances deliberately applied to spilled petroleum hydrocarbons, other spilled chemicals, and/or to the associated contaminated environment. They are applied with the intention of changing the fate and behaviour of the material spilled, improving the efficiency of other countermeasures, and/or reducing the environmental effects of the spill. The use of STAs is generally assessed together with other types of countermeasures during the spill response decision-making process. Various factors are considered during this process, including the environmental acceptability of a particular option. One frequently asked question pertains to the toxicity.

Because of the interest in STAs, most of the toxicity testing we have done has been on this type of sample. The Rainbow Trout Acute Lethality Test (EC, 1990a) has been the standard bioasssay performed on STAs over the years. Recently, is has been posssible to perform Microtox testing and all STA samples within the last 18 months have been tested using both trout and Microtox assays. More comprehensive toxicity testing (EC, 1990b; 1992f; U.S. EPA, 1991) is sometimes performed for projects or products of special concern.

For purposes of setting criteria and limits of acceptable product toxicity, the STA category has been broken down into 15 different classes after Fingas.

- Bioaugmentation Agent (microbes)
- Bioenhancement Agent (no microbes)
- Bird/Human Cleaning Agent
- Chemical Neutralizer
- Chemical Solidifier
- Oil Dispersant
- Oil Emulsion Breaker
- Oil Herder
- Oil Recovery Agent (Visco-elasticizers)
- Oil Sinking Agent (Banned)
- Oil Solidifier (Gelling Agent)
- Oil Surface Washing Agent (Beach Cleaner)
- Shoreline Pre-treatment Agent
- Sorbent
- Vapour Suppression Foam

Spill-treating agents which meet preset toxicity criteria (together with other requirements) are considered to have potential application for their intended use and are placed on a short-list of 'approved' products. The results from the testing are made available to various operational and spill repsonse personnel to assist them in assessing the conditions and acceptability for use of that product in response to an oil spill in the environment.

As a new project, a protocol for sorbent toxicity testing is being developed. Three tests are being used to assess the toxicity of differing sorbent materials to detemine the most appropriate organism(s) for a standard sorbent toxicity test. The tests under consideration are the Rainbow Trout Acute Lethality Test (EC, 1990a), the Echinoderm Ferilization Assay (EC, 1992f), and the Threespine Stickleback Acute Lethality Test (EC, 1990c).

In addition to STAs, various oils were also tested during the late 1980s and early 1990s by Maclean *et al.* (1989). These tests were performed using water soluble fractions of the oils against the test species *Artemia* spp. (brine shrimp) (22 oils and refined products) and *Daphnia magna* (water flea) (57 oils and refined products). Because sample preparation can influence the outcome of toxicity tests, further testing of oils has been halted pending the development of a standard protocol for the preparation of water soluble fractions (WSFs) from oil (Blenkinsopp *et al.*, 1994).

The results of the aquatic toxicity testing are public information and are provided in response to public inquiry and in the form of advice and recommendations to spill repsonse agencies. To facilitate the timely transfer of information, toxicity data are being entered into a toxicity database which will be placed on the ETC bulletion board (read-only mode). Completion is expected in eary 1995.

Glossary

Acute means within a short period in relation to the life span of the organism, and would be of the order of minutes for bacteria, usually ≤ 2 days for daphnids, and usually ≤ 4 days for fish.

Chronic means occurring during a realtively long-term period of exposure, usually a significant portion of the life span of the organism such as 10% or more. For tests using Cladocerans, chronic is typically defined as continuing until three broods are produced.

Lethal means causing death by direct action. Death is usually defined as the cessation of all visible signs of movement of other activity.

Sublethal means detrimental to a living organisms, but below the level that directly causes death within the test period.

Toxicity Test means a determination of the effect of a material on a group of selected organisms, under defined conditions. An aquatic toxicity test usually measures either the proportions of organisms affected (quantal), or the degree of effect shown (graded or quantitative), after exposure to specific concentrations of chemical, wastewater, receiving water, or liquid derived from sediment or similar solid material.

References

ASTM (American Society for Testing and Materials), "Standard Guide for Conducting Static Acute Toxicity Tests Starting with Embryos of Four Species of Saltwater Bivlave Molluscs", Method E724-89, in: *Annual Book of ASTM Standards, Water and Environmental Technology, Volume 11.04* (1989).

Blenkinsopp, S. G. Sergy, P. Boileau, and D. Kyle, "The Development of a Standard Method for Producing Water Soluble Fractions from Petroleum Hydrocarbons for Aquatic Toxicity Testing", *Spill Technology Newsletter, 19:* 2 (1994).

EC (Environment Canada), "Biological Test Method: Acute Lethality using Rainbow Trout", Environmental Protection Series Report, EPS 1/RM/9, Environment Canada, Ottawa, Ontario (1990a).

EC (Environment Canada), "Biological Test Method: Acute Lethality Test using *Daphnia* spp.", Environmental Protection Series Report, EPS 1/RM/11, Environment Canada, Ottawa, Ontario (1990b).

EC (Environment Canada), "Biological Test Method: Acute Lethality Test using Threespine Stickleback (*Gasterosteus aculeatus*)", Environmental Protection Series Report, EPS 1/RM/10, Environment Canada, Ottawa, Ontario (1990c).

EC (Environment Canada), "Biological Test Method: Test of Reproduction and Survival using the Cladoceran *Ceriodaphnia dubia*", Environmental Protection Series Report, EPS 1/RM/21, Environment Canada, Ottawa, Ontario (1992a).

EC (Environment Canada), "Biological Test Method: Test of Larval Growth and Survival using Fathead Minnows", Environmental Protection Series Report, EPS 1/RM/22, Environment Canada, Ottawa, Ontario (1992b).

EC (Environment Canada), "Biological Test Method: Toxicity Test using Luminescent Bacteria (*Photobacterium phosphoreum*)", Environmental Protection Series Report, EPS 1/RM/24, Environment Canada, Ottawa, Ontario (1992c).

EC (Environment Canada), "Biological Test Method: Growth Inhibition Test using the Freshwater Alga *Selenastrum capricornutum*", Environmental Protection Series Report EPS 1/RM/25, Environment Canada, Ottawa, Ontario (1992d).

EC (Environment Canada), "Biological Test Method: Acute Test for Sediment Toxicity using Marine or Estuarine Amphipods", Environmental Protection Series Report, EPS 1/RM/26, Environment Canada, Ottawa, Ontario (1992e).

EC (Environment Canada), "Biological Test Method: Fertilization Assay using Echinoids (Sea Urchins and Sand Dollars)", Environmental Protection Series Report, 1/RM/27, Environment Canada, Ottawa, Ontario (1992f).

Fingas, M. F., N. D. Laroche, G. Sergy, G. Cloutier, and P. Mazerolle, "Environment Canada's Chemical Spill Priority List", *Spill Technology Newsletter 16*(3):1-8 (1992).

Harris, G. "The Comparative Toxicity of Crude and Refined Oils to *Daphnia magna*", Report Series EE: in press, Environmental Technology Centre, Emergencies Science

Division, Environment Canada, Ottawa, Ontario (1994).

MacLean, M. Margo, and K.G. Doe, "The Comparative Toxicity of Crude and Refined Oils to *Daphnia magna* and *Artemia*", Report Series EE-111, Environmental Technology Centre, Emergencies Science Division, Environment Canada, Ottawa, Ontario (1989).

U.S. EPA (United States Environmental Protection Agency), *Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms*, (fourth edition), C.I. Weber (ed.), Environmental Monitoing Support Laboratory, U.S. EPA, Cincinnati, Ohio, EPA/600/4-90/027 (1991).

The Spill Technology Newsletter was started with modest intentions in 1976 to provide a forum for the exchange of information on spill countermeasures and other related matters. We now have more than 2000 subscribers in over 40 countries.

To broaden the scope of this newsletter, and to provide more information on industry and foreign activities in the field of spill control and prevention, readers are encouraged to submit articles on their work and views in this area.

Mr. M.F. Fingas
Technical Editor
Environmental Emergencies Science Division
Technology Development Directorate
Environmental Protection Service
Environment Canada
Ottawa, Ontario
K1A 0H3
Phone: (613) 990-7297

Environmental Protection Publications
Technology Development Directorate
Environmental Protection Service
Environment Canada
Ottawa, Ontario
K1A 0H3
Phone: (819) 953-5921 or (819) 953-5750