OCS Study
MMS 89-0042

Oil and Dispersant Toxicity Testing

Proceedings of a Workshop on Technical Specifications Held in New Orleans

January 17-19, 1989

MMS U.S. Department of the Interior
Minerals Management Service
Gulf of Mexico OCS Region

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Editors

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ABSTRACT

The Minerals Management Service (MMS) conducted a workshop entitled "Technical Specifications for Oil and Dispersant Toxicity Testing" in New Orleans, Louisiana, from January 17-19, 1989. The purpose of this workshop was to discuss the latest information available on testing the effects of dispersants, oils, and chemically dispersed oil on marine organisms, and to recommend to MMS the oils, dispersants, and marine organisms to be tested, as well as the methods for testing. Participants at the workshop were experts in the areas of aquatic toxicology, marine biology, chemistry, and the formulation and application of dispersants. The workshop consisted of two parts: individual presentations on several specific subjects (including selection of oils and dispersants for testing; toxicity of oils and dispersants to marine plants, invertebrates, and vertebrates; principles of bioaccumulation; critical life stages for testing; and modeling) and separation of participants into working groups dealing with methods for testing vertebrates, invertebrates, and plants, and selection of dispersants and oil for testing.

Participants developed a flow diagram illustrating the level of testing (using a hierarchical approach) to evaluate test materials; a summary table matching level of testing, test organism, and test method; and named four oils and six dispersants that should be tested.

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LIST OF ACRONYMS AND ABBREVIATIONS

API	American Petroleum Institute
ASTM	American Society for Testing Materials
cm	centimeters
EC50	Median effective concentration that produces a sublethal response in 50% of a test population
EPA	Environmental Protection Agency
l	liters
LC50	Median lethal concentration that kills 50% of a test population
mm	millimeters
MMS	Minerals Management Service
NCP	National Contingency Plan
NRC	National Research Council
OWD	oil-in-water dispersion
PAH	polyaromatic hydrocarbons
PBC	Prudhoe Bay Crude
ppb	parts per billion
ppm	parts per million
RRT	Regional Response Team
RTWG	Regional Technical Working Group
TRI	Technical Resources, Inc.
WAF	water-accommodated fraction
WSF	water-soluble fraction

ACKNOWLEDGEMENTS

The contributions of Workshop participants to this Proceedings through their informed and lively discussions of ideas and issues are gratefully acknowledged. Special thanks go to the speakers whose timely presentations stimulated discussions and exchange of technical information. The discussion leaders, Drs. Jack Anderson, Mike Flaherty, Jerry Neff, and Howard Teas, deserve special recognition for channeling the energies of the four working groups to the tasks at hand and, with Dr. Peter Wells, for improving this document with their editorial skills.

EXECUTIVE SUMMARY

Test methodologies for determining the effects of oil, dispersed oil, and dispersants on marine organisms were addressed at a Workshop sponsored by the Minerals Management Service (MMS) and held in New Orleans, Louisiana, January 17-19, 1989. The purpose of the Workshop, entitled "Technical Specifications for Oil and Dispersant Toxicity Testing," was to discuss the latest information available on testing the effects of dispersants, oil, and chemically dispersed oil on marine organisms and to recommend to MMS the oils, dispersants, and marine organisms that should be tested, as well as the methods for testing them.

The Workshop involved a group of experts in the fields of aquatic toxicology, marine biology and chemistry, biochemistry, transportation of oil and oil products, and modeling. Criteria for their selection were based primarily on scientific expertise as demonstrated in scientific publications and presentations at professional meetings, knowledge of organisms in the Gulf of Mexico, and knowledge of oil and dispersants. Thirteen of those invited were chosen to speak on subjects of special interest to MMS and served as an Advisory Panel through their presentations and comments during the Workshop. Others participated in discussions of papers and in formal work groups.

The Workshop consisted of two parts: an initial presentation of topical papers on reports by the experts, and organization of participants into four working groups that considered 1) selection of oils and dispersants for testing, and toxicity testing with 2) invertebrates, 3) vertebrates, and 4) plants. In order to report the results of the Workshop as accurately as possible, the Proceedings document is organized as follows: Introduction, Recent and Ongoing Studies, Perspectives on Testing, Working Group Recommendations, Summary and Findings, Abstracts, and Appendices.

Recent and Ongoing Studies

The organizers and participants involved in the Workshop were aware that some effort already had been directed to understanding the impacts of dispersants and dispersed oil on the marine environment. Because of their relevance to the Workshop, two recent developments in particular were addressed--deliberations of the National Research Council (NRC) Committee on Effectiveness of Oil Spill Dispersants and a recently developed Gulf of Mexico spill assessment model. At the time of the Workshop, only the Executive Summary and Conclusions and Recommendations of the NRC Report were available.

The NRC study was initiated due to concern about possible "aesthetic, ecological, and economic impacts of oil spills in the ocean, and the adequacy of technologies for controlling them." The study addressed two basic questions about the use of dispersants: "Do they do any good?" and

"Do they do any harm?" Some documented field tests and laboratory experiments have shown dispersants to effectively remove a major part of the oil from the water surface. Other studies have reported low effectiveness, which could be due to inadequate application techniques, poor formulations, and other poor techniques. The Executive Summary of the NRC Report suggests that recent application techniques have improved and experience has shown that the viscosity of oil increases rapidly with weathering. Because the difficulty of dispersing increases with increasing viscosity, it is important to add dispersants within a few hours in order to obtain maximum efficiency. With respect to harm to marine life, toxicity tests conducted in the laboratory indicate that the acute lethal toxicities of dispersants currently in use are usually lower than crude oils and their refinery products. Other toxicity tests have shown that the acute toxicity of dispersed oil resides in the more toxic fractions of the oil as opposed to the dispersant.

The other development discussed at the Workshop concerned a computerized system (spill impact assessment model) to advise decision-makers on using dispersants under a given set of circumstances in the Gulf of Mexico. This system can predict the impacts of oil spills by showing the results of treating oil with a dispersant compared with not treating the oil. Briefly, the model functions as follows: a computerized map of the untreated oil spill is generated based on an oil fate model, which predicts the location and concentration of oil with time. This map is then compared with a series of computerized maps of 70 important Gulf resources that could be affected by the spill. A special geographical information system is then employed to calculate that portion of each resource affected at specific concentrations of oil. These mapped areas-of-effect are compared with mapped distributions of species' appropriate life stages that lie within the area-of-effect. After adjustments for the effects of other variables, an estimate is made of the proportions of populations of each resource at risk from the spill. The same procedure is applied for chemically dispersed oil. The results are lists of resource-specific effects of treated and untreated oil.

Perspectives on Toxicity Testing

A general testing scheme was developed by the participants that reflected their experiences in testing oils and dispersants, which include collecting and maintaining test organisms and dosing of test systems to obtain desired concentrations of test materials. The participants developed a flow diagram to illustrate the level of testing recommended to evaluate the toxicity of dispersants, oil, and dispersed oil. The testing scheme entails aspects of both fate and effects and contains basic ingredients of an aquatic risk assessment.

The Workshop participants agreed that several tiers of testing are required in order to determine the toxicity of dispersants and dispersed oil. The first stage of the proposed testing scheme consists of screening tests. These tests can be conducted in a short period of time (6 to 96

hr), and may or may not result in determination of a concentration that kills 50% of the test population (LC50). Definitive tests, the next step, usually result in determination of an LC50 or an EC50, an effective concentration that reduces some physiological indices by half. Screening and definitive toxicity tests involve reasonably uniform mixtures and exposures and the tests are applicable to both oils and dispersants.

Modifications of standard toxicity methods to accommodate shorter testing duration and application of test materials to test systems will provide viable testing methodology for the water phase. The modifications suggested include using the water-accommodated fraction of 1:9 oil/sea water mixture. When testing dispersed oils, use 10% dispersant in sea water, mixed together first then added to diluted water. Toxicity endpoints for water-phase tests include mortality and various sublethal effects such as reproduction and growth. It was pointed out that the NRC Report on dispersants will also provide information on the preparation of test solutions and other aspects of toxicity testing.

Time did not permit much consideration of microcosms or mesocosms, which were included in the flow diagram, but there was general consensus on their usefulness in estimating the toxicity of the materials in question, especially where sediments are concerned. Micro/mesocosms can vary in size from small aquaria to large tanks holding hundreds of liters of water. They can contain communities of organisms with their natural sediment, or clean sediment placed in the system, with natural sea water flowing through the system, and the effects of the test material on larvae settling on the clean sediment can be observed. The need for ecosystem-level testing was noted and there were some discussions of field studies conducted in the past.

Working Group Recommendations

The participants were organized into four working groups: 1) selection of oils and dispersants for testing, and toxicity testing of 2) invertebrates, 3) vertebrates, and 4) plants. MMS charged the working groups with developing a hierarchical testing system to evaluate the potential impact of dispersants, dispersed oil, and crude oil on the coastal environment. This system will involve a progression of more complicated tests, from 96-hour LC50 (lethality) tests and sublethal chronic tests with sensitive life stages of selected organisms, to measurements of structural and functional aspects of ecosystems. Because of the limited time available at the Workshop, the working groups were also given priorities in the following order: identify protocols for testing, comment on availability of test organisms, match species from a prepared list with testing protocol where appropriate, and address ecological implications of test results when possible.

Working Group on Selection of Oils and Dispersants for Testing

The participants at the Workshop discussed the need for careful selection (for toxicity testing purposes) of oils produced or transported within the Gulf of Mexico and chemicals applied to disperse them in case of a spill. Because it is not feasible to test all of the oils and dispersants, criteria were developed for selecting those appropriate for a reasonable testing program.

The kinds, amounts, and some properties of oil transported or produced in the Gulf of Mexico can be found in Appendices D-1, D-1a, and D-2 of the U.S. Environmental Protection Agency's (EPA) Proposed National Oil and Hazardous Substance Pollution Contingency Plan. The volume of any given oil or product that is transported may vary from year to year, but the oil properties do not vary substantially. For consideration of dispersant use (and thus toxicity testing), the oil properties may be more important than volumes. Approximately 95 different kinds of crude oil are transported through the Gulf. The amount of drilling in the Gulf is down while the number of tankers is up.

The working group developed criteria for testing oil and dispersants and based on these criteria and personal experiences, recommended four oils and six dispersants that should be tested. The criteria for testing oil included availability of enough amounts to complete an entire hierarchical testing procedure, sufficient characterization of physical and chemical properties, and sufficient volumes transported through the Gulf. The criteria for testing dispersants included availability of sufficient quantities for spill control, existence of a database on toxicity, and availability of chemical composition.

Working Group on Testing Invertebrates

Invertebrates are an especially important group of test organisms because of their abundance in the marine environment and their sensitivity to oil and oil-related compounds. A table was developed to match the level of test shown in the flow diagram with materials, the kind of organism, and the stage in the life cycle most appropriate for the test.

The following summarizes the suggestions from the working groups concerning specific invertebrates and their life stages that are most appropriate for testing the effects of dispersants, oil, and dispersed oil: the American oyster is a valuable bivalve test species and could be tested as an adult for reproductive and growth effects; postlarvae and/or juvenile stages of penaeid shrimp should be tested; mud crabs could be used in first-level testing; however, stone crabs, fiddler crabs, or the spiny lobster could be used, and mysids and sea urchins should be included as reference test organisms. Certain species of polycheate worms and corals were also designated for testing.

Working Group on Testing Vertebrates

Several groups of marine vertebrates were suggested for testing purposes including fish, turtles, and birds. The species recommended for testing in each group, the life stage of each that is most appropriate for testing, the criteria for effects, and an indication of the availability of test species was documented and displayed in a table. Fish suggested for testing included planktonic spawners such as menhaden, croaker, and spot. Benthic spawners included a redfish and speckled trout. Discussions also included marine cetaceans, birds, and turtles.

Working Group on Testing Plants

The participants developed information on testing seagrasses, mangroves, marsh grasses, algae, and phytoplankton. Seagrass species were selected based on their Gulfwide distribution, ease of maintenance in the laboratory, occupation of separate ecological niches, sensitivity to oil and dispersants, and contribution to the total biomass of seagrasses in the Gulf of Mexico. The red and black mangrove were selected as test species to represent mangroves in general and criteria included importance to coastal ecosystems, sensitivity of seedlings to the chemicals in question, and occupation of a unique ecological niche. Because it is possible that oil and dispersed oil could reach marsh grass through wave and current action and dispersants could inadvertently reach marsh grass through aerial spraying action, it was recommended that all three materials be tested with needlerush and smoothcord grass. Testing unicellular plants such as phytoplankton should be required when water column information is needed.

Speakers' Abstracts

Scientists representing many scientific disciplines including toxicology, biology, chemistry, ecology, and biochemistry presented specific information regarding methods for evaluating the toxicity of oils and dispersants on marine organisms. Each speaker prepared an extended abstract, and the talks and abstracts were the basis for many productive discussions at the Workshop. A discussion of the abstracts is presented here to indicate the technical basis for many of the discussions and conclusions of the Workshop.

The first two abstracts give information on a recent report by the NRC on the use of dispersants in marine waters and on development of a model relevant to the fate and effect of oil and dispersants in the Gulf of Mexico. Dr. P.G. Wells relates the findings of the NRC Report, which were especially pertinent to deliberations at the Workshop. Dr. K. Trudel speaks of the development of an oil spill impact assessment model that can predict the impacts of oil spills by showing results of treating oil with dispersants compared with not treating the oil.

The next four abstracts set the tone for the status of toxicity testing in a general sense. Dr. R.F. Lee gives information on the pharmacodynamics of bioaccumulation that is of special interest to those conducting toxicity tests. Drs. J.D. Costlow and A.S. Clare discuss the need for testing sensitive life stages of organisms in order to properly determine the vast array of effects (such as reproduction, larval release/brooding, larval development, and others) that can be exerted at different stages of development. Dr. J.M. Neff discusses the manner in which organisms can be exposed to oil and suggests that ideally, laboratory exposures should simulate as closely as possible the types of exposures in the field. He also identifies a variety of acute and chronic biological effects that can result from such exposures. Dr. J.W. Anderson presents additional information on exposure regimes and toxicity of dispersed and undispersed oil as well as data on toxicity of dispersants to marine organisms.

The remainder of the abstracts are directed to topics addressed by the subgroups: selection of oil and dispersants for testing, and the toxicity of oil, dispersed oil, and dispersants to invertebrates, vertebrates, and plants. Dr. M.L. Flaherty discusses data required for registration of dispersants. He points out that Regional Response Teams are encouraged to "preapprove" certain dispersants or chemical agents in their area of geographical responsibility. Dr. J.P. Fraser comments on considerations in selecting dispersants for testing, which include the presence of the dispersant on the EPA product schedule, whether the dispersant contains certain chemical ingredients, and whether it is designed for aerial or boat spray application. Dr. E.N. Powell discusses approaches to testing the toxicity of materials to invertebrates, particularly oysters and coral. His approaches include both field and laboratory tests. Dr. P.L. Lutz addresses methods for determining the toxicity of oil and dispersants to sea turtles and suggests that these animals are surprisingly sensitive to oil contamination. He proposes a conceptual model of the potential effects of oils and dispersants on sea turtles.

The last two abstracts are concerned with the effects of the compounds and chemicals of interest on seagrasses and mangroves. These particular plant communities were included because of their potential exposure to oils and dispersants and because of the sensitivity of the communities of organisms that share these habitats. Dr. T.W. Duke describes a microcosm system containing cores of seagrasses and attendant sediment that could be used to evaluate the effects of oil and dispersants on seagrass communities. The criteria for effects on these communities include changes in growth rate of the seagrass and diversity and richness of macroinvertebrate species. Dr. H.J. Teas discusses methods for determining the toxicity of oil and dispersants to mangroves and lists plant stages or parts that have been tested with these compounds.

Summary and Findings

Based on their expertise and experience in the fields of aquatic toxicology, ecology, marine biology, and chemistry, the participants developed a flow diagram to illustrate the level of testing recommended to evaluate the toxicity of dispersants, oil, and dispersed oil to marine organisms. The organisms for testing were selected on the basis of general sensitivity to the test chemicals, adaptability to laboratory conditions, availability, and other considerations. The methods for testing were discussed in some detail and the necessity for chemical measurements of oil or other test materials and the need to know the chemical composition of dispersants to be tested were emphasized. The following findings are a result of comments and discussions during the meeting:

Certain toxicological tests need to be conducted with dispersants, oil, and dispersed oil but they should be conducted with the knowledge that there is already an extensive toxicity database available.

1. There are few "standard" toxicity tests designed specifically for testing dispersants, oil, and dispersed oil. However, many standard tests found in American Society for Testing and Materials standard practices documents and other similar documents can be used if the exposure techniques are modified to accommodate oil and dispersed oil. Furthermore, some methods for testing these chemicals specifically can be found in various scientific articles and reports. The relationships among recommended test species, test methods, and level of testing with reference to the flow diagram are shown in Table 6.

2. The manner in which organisms are exposed to test chemicals should be consistent, when possible, with the manner in which organisms are exposed in the natural environment. For example, contaminated sediment can be layered over clean sediment to test the impact on benthic organisms because this simulates contaminated suspended particulates settling to the bottom in the natural environment.

3. The concentration of dispersants, oil, and dispersed oil used in laboratory experiments should include concentrations found in the environment or predicted to be there, and those inducing and not inducing effects. Chemical analysis should be conducted to determine the actual concentrations and, if possible, the composition of the chemicals in the test systems.

4. Prudhoe Bay, South Louisiana, No. 2 Fuel Oil, and Saudi Arabian Light were recommended as the oils to be tested.

5. The following dispersants were recommended for testing: Corexit 9527, Chemlink D609, Finasol OSR7, Cold Clean 500, Slickgone NS, and Gold Crew.

6. Models, such as the MIRG/SLR spill impact assessment model described by Trudel, should be peer reviewed, "validated," and used as another tool for predicting the impact of oil, dispersed oil, and related chemicals on the environment.

7. There is a need for micro/mesocosm studies on the fate and effects of oil and dispersants in marine ecosystems. There is also a need for testing the impact of oil and dispersants on the structure and function of ecosystem in the laboratory and in the field. However, the consensus of the Workshop was that the subject of ecosystem-level testing should be addressed in another forum.

8. Test results produced from toxicity tests such as those described at the Workshop should be integrated into a risk assessment process in order to obtain a broader view of the fate and effects of the materials involved. One approach is to integrate the results of the proposed hierarchical testing scheme with an Ecological Risk Assessment consisting of hazard identification, effects assessment (water column and sediment), exposure assessment, and risk characterization.

Appendices

Four appendices are included in the Proceedings:

A. Workshop Agenda and Charge to Working Groups

B. List of Attendees and Speakers

C. Sources for Test Species Recommended by the Workshop

D. Existing Toxicity Testing and/or Dispersant Registration Requirements used by Federal and State Agencies.

NARRATIVE REPORT

Introduction

The Minerals Management Service (MMS) conducted a Workshop entitled "Technical Specifications for Oil and Dispersant Toxicity Testing" in New Orleans, Louisiana, January 17-19, 1989. The purpose of the Workshop was to discuss the latest information available on testing the effects of dispersants, oil, and dispersed oil on marine organisms and to recommend to MMS the oil, dispersants, and marine organisms that should be tested, as well as the methods for testing them. Because of present uncertainties regarding the effects of dispersants and dispersed oil on the marine environment, the information from this Workshop could be useful in planning any study proposed to synthesize existing data on the toxicity of dispersants and dispersed oil to potentially sensitive organisms in the Gulf of Mexico.

The MMS began developing information on the potential impact of these oil products on the flora and fauna of the Gulf of Mexico by working with the Regional Technical Working Group (RTWG) of the MMS Gulf of Mexico OCS Region. The members of the RTWG, who represent the States bordering the Gulf, were asked to consult with appropriate State agencies and interested scientists to identify those animals and plants and their life stages that should be tested. After consultation with the States and others, MMS provided a list of species and life stages and asked the States to rank them in priority for which testing is required and to add to the list as necessary.

Technical Resources, Inc. (TRI) was asked by the MMS to assist in organizing and presenting a workshop to develop technical specifications for determining the toxicity of oil, chemically dispersed oil, and dispersants on marine organisms. Specifically, TRI was asked to:

1. Review and summarize the information provided by the States on appropriate test organisms and their life stages,

2. Convene a group of experts (Advisory Panel) for the purpose of recommending toxicity tests and studies for commercially available dispersants,

3. Assist in selection of test organisms that represent commercial fishery species, recreational species, sea turtles, corals, seagrasses, and mangroves, and

4. Consider the life stages of the organisms; embryonic (seed) through larval to adult as appropriate. Testing shall be with dispersant alone, oil alone, and chemically dispersed oil.

Therefore, the emphasis of the Workshop was on hierarchical testing with representative organisms (or their surrogates) with the oil products. Several related topics were considered important but were not examined in the context of the Workshop and have been studied by others. These include the long-term effects of oil on marine organisms (Boesch and Rabalais 1987), ecological testing in the marine environment (Persoone et al. 1984), and the physical and chemical properties of oil products in sea water (NRC 1988).

The MMS asked that a group of experts be convened in the areas of aquatic toxicology, marine biology and chemistry, biochemistry, transportation of oil and oil products, and modeling. Such a group was invited to attend the Workshop. The criteria for selection were based primarily on scientific expertise as demonstrated in scientific publications and presentations at professional meetings, knowledge of organisms in the Gulf of Mexico, and knowledge of oils and dispersants. Thirteen participants were invited to speak on specific subjects of interest to MMS and served as an Advisory Panel through their presentations and comments during the Workshop. Others participated in discussions of the papers and formal working groups.

The Workshop consisted of two parts: (1) presentation of topical papers or reports, including the recent study on oil dispersants by the National Research Council, and studies on the selection of oils and dispersants for testing, on bioaccumulation, on sensitive life stages for testing, and on methods for testing marine organisms; and (2) organization of participants into four working groups discussing vertebrates, invertebrates, plants, and materials for toxicity testing (see Workshop agenda, Appendix A, and List of Attendees and Speakers, Appendix B).

The MMS directed the working groups to develop a hierarchical testing system to evaluate the potential impact of dispersants, dispersed oil, and crude oil on the coastal environment. This system would involve a progression of more complicated tests, from 96-hour LC50 (lethality) tests and sublethal chronic tests with sensitive life stages of selected organisms, to measurements of structural and functional aspects of ecosystems. Because of the limited time available at the Workshop, the working groups were given the following priorities:

1. Identify protocols for testing that are:

   a. Currently available,
   b. Under development, and
   c. Those that should be developed.

2. Comment on availability of test organisms.

3. Match species from "A Preliminary Listing of Species and Life Stages for Testing Effects of Oil Dispersants" (Duke and Shuba 1988) to testing protocol where appropriate. Indicate species priority.

4. Address ecological implications of test results where possible.

The working groups prepared written summaries of their deliberations, which were distributed to the participants and served as the basis for the summary discussion of the Workshop. The participants were asked to submit comments on the Workshop in general and the working group summaries in particular within 10 days of the close of the Workshop.

This report was derived from the abstracts provided by the speakers, reports from the working groups, discussions held throughout the Workshop, and comments furnished by the participants after the Workshop. The summary discussion held at the last session was especially beneficial in this regard.

In order to report the results of the Workshop as clearly as possible, the Proceedings document is organized as follows: Introduction, Recent and Ongoing Studies, Perspectives on Testing, Working Group Recommendations, Summary and Findings, Abstracts, and Appendices.

Recent and Ongoing Studies

The organizers and participants involved in the Workshop were aware that some effort already had been directed to understanding the impacts of dispersants and dispersed oil on the marine environment. The purpose of this section was not to review the literature, but to address two studies that were particularly relevant to the Workshop--deliberations of the National Research Council (NRC) Committee on Effectiveness of Oil Spill Dispersants (NRC 1989) and a recently developed Gulf of Mexico spill assessment model (see abstract, Trudel). At the time of the Workshop, only the Executive Summary and Conclusions and Recommendations of the NRC Report were available. Dr. Peter Wells (see abstract) addressed the highlights on biological effects from the Executive Summary.

NRC Study

The following discussion is based on the NRC Summary and Dr. Wells's presentation.

The NRC study was initiated due to concern about possible "aesthetic, ecological, and economic impacts of oil spills in the ocean, and the adequacy of technologies for controlling them." The study addressed two basic questions about the use of dispersants: "Do they do any good?" and "Do they do any harm?" Some documented field tests and laboratory experiments have shown dispersants to effectively remove a major part of the oil

from the water surface. Other studies have reported low effectiveness, which could be due to inadequate application techniques, poor formulations, and other poor techniques. The NRC Executive Summary suggests that recent application techniques have improved and experience has shown that the viscosity of oil increases rapidly with weathering. Because the difficulty of dispersing increases with increasing viscosity, it is important to add dispersants within a few hours of an oil spill in order to obtain maximum efficiency. With respect to harm to marine life, toxicity tests conducted in the laboratory indicate that the acute lethal toxicities of dispersants currently in use are usually lower than crude oils and their refinery products. Other toxicity tests have shown that the acute toxicity of dispersed oil resides in the more toxic fractions of the oil as opposed to the dispersant.

The NRC Committee recommended several research initiatives including:

- Assessment of ecological effects of dispersed oil on marine life in shallow water environments, and other habitats having restricted water exchange to better define conditions under which dispersant use can be environmentally safe.

- Research on the interaction of oil and dispersed oil with suspended particulates, sediments, plankton, and benthic organisms to establish a better quantitative basis for comparing the adhesion properties of untreated and dispersant-treated oil.

- Additional investigations of the toxic effects of both untreated oil and dispersant-treated oil on sea surface-dwelling organisms that could be affected by oil slicks.

One of the conclusions and recommendations stated that the biological benefits or objectives of chemically dispersing oil effectively are to

- Prevent stranding of oil in intertidal zones,

- Reduce hazards of discharged oil to marine birds and mammals,

- Enhance degradation of oil components, and

- Reduce chronic impacts on some habitats, such as mangroves, because of the shorter persistence of dispersed oil.

Further, acute biological effects are expected to be slight in most open-sea applications because the dispersed oil mixes into a relatively large volume of water, resulting in concentrations and times of exposure that are low compared with those showing effects in laboratory studies. However, it is pointed out that in shallow water with poor circulation, and in protected bays and inlets, the acute biological effect at high

concentrations of dispersed oil on some organisms and habitats may be greater than the effects of untreated oil.

Dr. Wells points out the relevance of the results reported in the NRC Summary Report to the goals and objectives of the Workshop. For example, the Workshop was charged with developing a hierarchical testing system and the NRC Summary introduced a number of topics crucial to the design of such a system: choice of exposure conditions; rationale and choice of species and life stages; comparative toxicology of marine species to the materials; and predictability of field effects from laboratory experiments. The necessity for using environmentally relevant concentrations and composition of dispersants and dispersed oil and for working with measured versus nominal concentrations was discussed by Dr. Wells and emphasized in group discussions. The difficulties in determining what component of dispersed oil should be analyzed in order to determine the concentration of the mixture in sea water was also discussed. There was no consensus on the most efficient analysis for this purpose. Dr. Wells strongly recommended that an investigation be made of the effect of dispersing surface oil slicks on marine (particularly diving) birds. The "stickiness" of dispersed oil on the plumage of birds and fur of mammals was also discussed.

Perhaps the best fractions of dispersed or nondispersed oil to monitor in the water columns are the low-molecular-weight polycyclic aromatic hydrocarbons (PAH's). Lighter aromatics such as benzene, toluene, and xylene are too volatile and may be lost by evaporation in hours. There is a great need for the application of standard analytical methods for analysis of petroleum hydrocarbons.

Oil Spill Assessment Model (Marine Industry Research Group/S.L. Ross; MIRG/SLR)

Another recent development discussed at the Workshop concerned a computerized system to advise decision-makers on using dispersants under a given set of circumstances in the Gulf of Mexico (see Dr. Ken Trudel's abstract). This system can reputedly predict the impacts of oil spills by showing the results of treating oil with a dispersant (effects on marine organisms) compared with not treating the oil. Briefly, a computerized map of the untreated oil spill is generated based on an oil fate model predicting the location and concentration of oil with time. This map is then compared with a series of computerized maps of 70 important Gulf resources that could be affected by the spill. A geographical information system is then employed to calculate that portion of each resource affected at specific concentrations of oil. These mapped areas-of-effect are compared with mapped distributions of species' appropriate life stages that lie within the area-of-effect. After adjustments for the effects of other variables, a calculation is made of the proportions of populations of each resource at risk from the spill. The same procedure is applied for chemically dispersed oil. The results are lists of resource-specific effects of treated and untreated oil.

That portion of the model pertaining to toxicology and resource sensitivity were of particular interest to the participants because these factors are determined in part by toxicity tests with dispersed and nondispersed oil. There is a large body of data available on the effects of oil on marine organisms and these effects vary from reversible physiological effects to irreversible effects such as larval deformity and mortality. In this model, estimates of spill impact on resources are computed by a series of resource-specific impact assessment algorithms that present and interpret data on the fate of oil and the sensitivity and vulnerability of the resources. Separate algorithms were developed for each resource because of the many interactions between oil and different resources. An example of an algorithm for a shrimp fishery is presented in Dr. Trudel's abstract. The algorithm considers both the damage to the shrimp population itself and the losses to the fishery from tainting and possible interference of fishing activity. With respect to future toxicity tests that would be useful for modeling purposes, Dr. Trudel cites the need to employ realistic exposure conditions, including episodic or short exposure times. He also notes gaps in information on population-level effects, economics, and real-world exposure.

Discussions at the Workshop indicated the participants' concern about the capacity of oil spill models in general to accurately predict the dose-effect relationships for oil and marine organisms, and the manner in which the oil weathering processes are modeled. Some believe that the dose-effect relationship (within a factor of 10) does not affect the model significantly but that weathering rates may have a tremendous effect on the time component of dose. An associated concern was the effects of dispersants on different weathering processes. There appears to be little information on this subject.

Perspectives on Testing

Introduction

Oil and dispersants introduced into the marine environment can remain in the water and undergo microbiological and chemical degradation, move into the atmosphere through the sea/air interface, be sorbed onto particles and sediments and be accumulated by plants and animals. Discussions of the interactions of oil in sea water are cited in NRC, 1985, and interaction of dispersants in the sea are discussed in NRC, 1989. The Workshop emphasized the accumulation of oil, dispersed oil (in this Proceedings, dispersed oil refers to chemically dispersed oil), and dispersants by marine organisms, the potential effects of those oil products on them, and the methods for measuring the effects.

Bioaccumulation and Critical Life Stages

According to Dr. Richard F. Lee (see abstract), the bioaccumulation of oils and dispersants by marine organisms is a reflection of the relative importance of uptake, metabolism, and elimination. Filtering

bivalves, such as oysters, mussels, and some clams, are used in monitoring studies because they filter large volumes of water and have a limited capacity to metabolize many organics (thus, they are bioaccumulated). The extent of bioaccumulation by marine organisms is related to the hydrophobic properties of the compound; therefore, high-molecular-weight polycyclic aromatic hydrocarbons (hydrophobic) are concentrated in some animals by several orders of magnitude over their concentration in the water. Compounds or formulations with high water solubility (hydrophilic), such as dispersants, are accumulated in animals only to a limited extent since elimination is so rapid. The responses of detoxifying systems such as cytochrome P-450 systems and glutathione S-transferases to organic pollutants are the result of bioaccumulation and these responses appear to be useful in environmental pollution studies to indicate exposure has occurred.

The need for testing sensitive life stages of marine organisms was introduced by Dr. John Costlow and Dr. A.S. Clare (see abstract), who elaborated on the desirability of testing all life stages and thus the entire life cycle of test organisms. However, practicality in testing dictates another approach. Because each of the life stages is an integral part of the whole, any modification of a single stage (possibly a toxic effect) will impact the entire life cycle. Several points in the life cycle were identified as critical links: reproduction, which includes the maturation of gametes and the production and release of pheromones; larval development, including dispersal to desired habitats; and growth and maturation of juveniles to sexual maturity. Thus, information gained by testing one of the critical links would provide insight into the entire life cycle effects. Dr. Costlow points out that physiological processes are affected by combinations of environmental factors, for example, extremes of salinity coupled with extremes of temperature, light, and diet. Additional stresses are imposed upon organisms when anthropogenic factors are added.

Dr. Costlow identifies four organisms found in different temperate and west Atlantic habitats as providing excellent characteristics to be used as testing organisms: blue crab, Callinectes sapidus, mud crab, Rhithropanopeus harrisii, barnacle, Balanus amphitrite, and horseshoe crab, Limulus polyphemus. The adults of these species may be affected by pollutants affecting intertidal or benthic surfaces and each has a larval stage that could be affected in the water column. The larvae of Callinectes sapidus are difficult to culture and use in tests but the larvae of Rhithropanopeus are an excellent surrogate for Callinectes.

A General Aquatic Testing Scheme

A general testing scheme was developed by participants that reflected their experiences in testing and experimenting with oils and dispersants, which include collecting and maintaining test organisms and dosing of test systems to obtain the desired concentrations of test materials. The

testing scheme includes aspects of both fate and effects and contains basic ingredients of an aquatic hazard assessment (see Figure 1).

The Workshop participants agreed that several tiers of testing are required in order to determine the toxicity of dispersants and dispersed oil and the first stage of the proposed testing scheme consists of screening tests. These tests can be conducted in a short period of time (6 to 96 hr), and may or may not result in determination of a lethal concentration that kills 50% of the test population within a defined time (LC50). Definitive tests, the next step, usually result in determination of an LC50 or an effective concentration (EC50), a concentration that reduces some physiological indices by half. Screening and definitive toxicity tests involve reasonably uniform mixtures and exposures and the tests are applicable to both oils and dispersants. The methodology for screening and definitive tests can be found in publications of the American Public Health Association, American Water Works Association, Water Pollution Control Federation (1981), and the American Society for Testing and Materials (1980), and EPA (Federal Register, 1984), supplemented by Rand and Petrocelli (1985).

Water-phase testing is designed for oils and chemically dispersed oils and should simulate exposure of the test organisms to these materials in the water column under controlled conditions. For this reason, the participants recommended that test organisms be exposed in a flowing water system in covered containers with no air space for 24 hr, removed and placed in clean water for weeks to months, depending upon the organism being tested. They also recommended an increase in the number of chemical analyses normally made during toxicity tests and a reduction in the number of test species (relative to the RTWG species list). Several discussions were held concerning the amounts of oil and dispersed oil that should be used in these toxicity tests. The group agreed that concentrations measured in the field after application of dispersants should be employed where possible. However, concentrations resulting from aerial spraying are difficult to predict and may be on the low side for toxicity testing purposes. It is important that concentrations bracket effect and no-effect levels. There was some concern expressed about the amount of suspended particulates in test water because nondispersed oil adheres to particles and dispersed oil may not. If comparisons of toxicity results are made among different laboratories, this could be one of several complicating factors.

Modifications of standard toxicity methods to accommodate shorter testing duration and application of test materials to test systems will provide viable testing methodology for the water phase (see abstracts by Anderson and Neff, and Wells et al. 1984). The modifications suggested include using the water-accommodated fraction of 1:9 oil/sea water mixture as in Anderson et al. (1974). When testing dispersed oils, use 10% dispersant in oil, mixed together first then added to diluent sea water. Neff presents a discussion of the different kinds of water-phase exposure

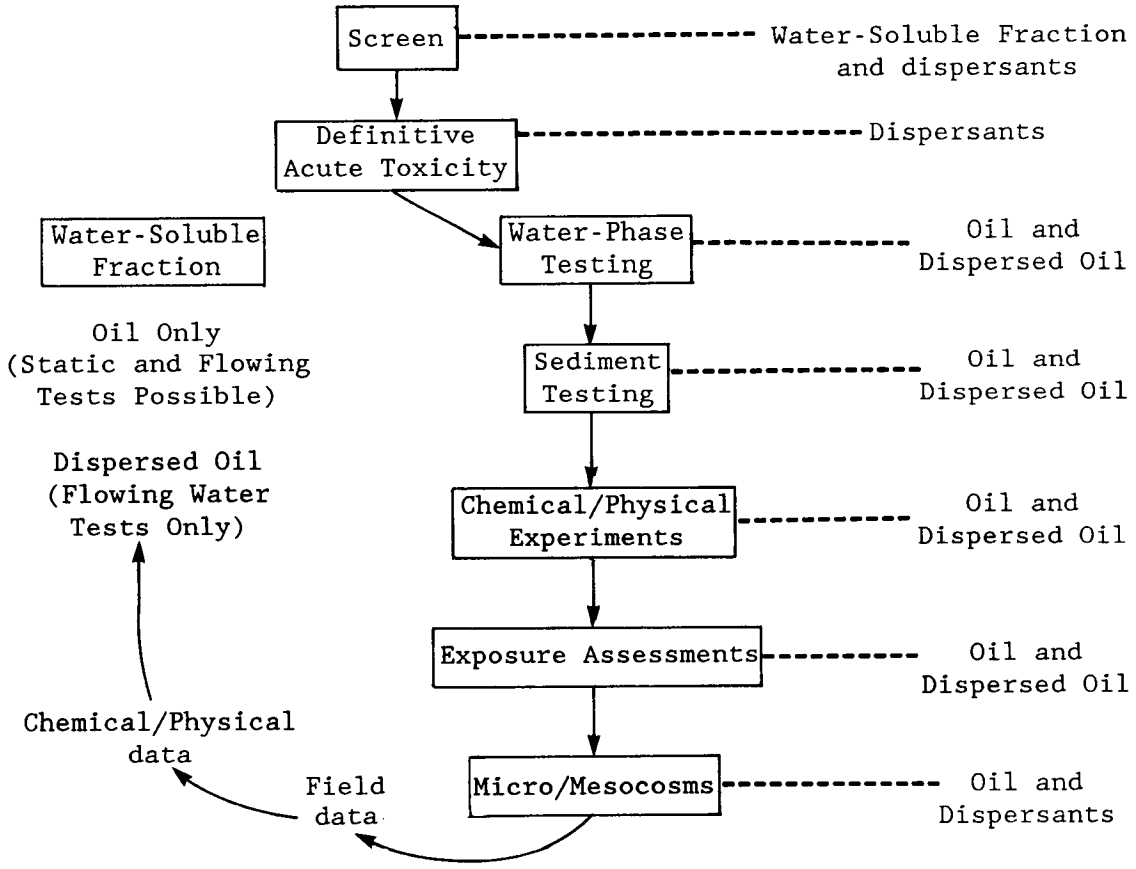

Figure 1. A flow diagram for testing dispersants, oil, and dispersed oil.

that can be used in laboratory toxicity experiments including water-soluble phase, water-accommodated phase, and oil/water mixture. Since the kind of exposure can affect toxicity, the method of exposure should be noted in all toxicity tests. Toxicity endpoints for water-phase tests include mortality and various sublethal effects such as reproduction, development, and growth. It was pointed out that the NRC Report (1989) on dispersants also provides information on the preparation of test solutions and other considerations for toxicity testing. An earlier NRC Report (1985) also has an extensive methods section.

Other discussions on the subject of toxicity testing included suggestions that results of toxicity tests should be expressed as 24-, 48-, and 96-hour LC50s or EC50s and as parts per millon (ppm)-hour of total hydrocarbon or total aromatics (see Anderson's abstract). One of the problems of interpreting toxicity data is that in most instances only a 96-hr LC50 is reported. It would be most helpful to have the toxicity curve (mortality with time at given concentrations of the test material) that was used to calculate the LC50 value so that effects at times less than 96 hours could be estimated. This is because most water column organisms are exposed to oil and dispersants for less than 96 hours; indeed, some are exposed for only a few hours. Several papers concerned with determining the effects of dispersants and oil on marine organisms and on physical/chemical characteristics of these chemicals provide information on techniques for adding the chemicals to the test system. These include Anderson (1984), Anderson et al. (1974 and 1987), Wells et al. (1982 and 1984), Wells (1984), and Shuba and Heikamp (1989). In sediment exposures, chemical and physical experiments should be conducted to determine how much oil or dispersed oil reaches the sediment, how much is attached to suspended particulates, and how much is bioaccumulated. It may be possible to determine rates of exchange of the oil constituents and dispersed oil among sediment, water, and biota.

There are no standard test methods for measuring the effects of oils and dispersants on organisms associated with sediments, but testing of these materials differs only from other protocols in the manner of dosing and sometimes in selection of response parameters as indicated by Swartz et al. (1985), Anderson et al. (1984), and Tagatz et al. (1978 and 1980). Also, the ASTM Sediment Toxicology Subcommittee is developing guides for testing the impact of pollution on benthic organisms. The Workshop participants recommended that contaminated sediments be generated in the following manner: add sediment to well-mixed water; while still mixing slurry, add oil to slurry; stir slurry. Dosing can be varied by either varying the time the slurry is withdrawn or by varying the amount of sediment:oil ratio. Contaminated sediments can be layered on top of clean sediment or can be mixed with it. Several participants stated the importance of knowing and reporting the particle size of the sediment and matching test organisms with particle size. Test organisms should be exposed to contaminated sediment for 10 days or longer as appropriate. Chemical analyses should be conducted on sediment, but on overlying water only if a static sediment test is being conducted.

Time did not permit much consideration of the microcosms/mesocosms portion of the flow diagram but there was general consensus on their usefulness in estimating the toxicity of the materials in question, especially where sediments are concerned. Micro/mesocosms can vary in size from small aquaria to large tanks holding thousands of liters of water. They can contain communities of organisms with their natural sediment, or clean sediment placed in the system, with natural sea water flowed through the system, and the effects of the test material on settling larvae can be observed.

With the chemical/physical data obtained in laboratory studies, information developed from experimental releases of oil and dispersants in the natural environment, as well as from accidental spills, realistic concentrations can be used in the laboratory to determine the effects of the water-soluble phase of dispersed oil.

Dr. Jerry Neff and Dr. Jack Anderson (see abstracts) presented general information on determining the toxicity of dispersants, oils, and dispersed oil on marine organisms, as well as data on the impact of these materials on specific organisms. Various methods for introducing oil and dispersants into test systems and some biological effects of these chemicals are presented. Dr. Neff mentions the need for a tiered or hierarchical testing system (this subject was also addressed briefly by the participants and was reflected in their flow diagram for testing). The first tier consists of standard acute lethal toxicity tests, the second includes a variety of early life stage tests and abbreviated chronic tests. Tier 3 tests measure a variety of physiological and biochemical responses, including oil-mediated pathology. Field and mesocosm studies constitute the fourth tier. An overall assessment of the relative merits of 14 chemical dispersants is discussed by Anderson. He also presented information indicating the water-soluble or water-accommodated fraction (WSF or WAF) of oils and dispersant alone can be tested in static systems, but dispersed oil exposure will require flowing systems. Chemical characterization of exposure concentrations of WSF, WAF, and dispersed oil will be required.

Testing of sediments with oil and dispersed oil is important and the procedure used must allow differences in sorption characteristics of the two materials to be exhibited. In other words, oil in combination with effective dispersants is less likely to bind to sediment particles under natural conditions. Mixing systems should not be so artificial that this difference is masked.

WORKING GROUP RECOMMENDATIONS

Group A. Selection of Oils and Dispersants for Toxicity Testing. Dr. M.L. Flaherty, Chairman

Group B. Methods for Testing Invertebrates. Dr. J. Anderson, Chairman

Group C. Methods for Testing Vertebrates. Dr. J. Neff, Chairman

Group D. Methods for Testing Plants. Dr. H. Teas, Chairman

Working Group Recommendations

Introduction

Four working groups of small numbers (7-20) of participants were formed to discuss in more detail the points of interest raised by the speakers and others. The working groups were formed to discuss A) selection of oils and dispersants for toxicity testing, and methods for testing toxicity of oils and dispersants to B) invertebrates, C) vertebrates, and D) plants. The working groups were given priorities in the following order: identify protocols for testing; comment on the availability of test organisms; match species from list provided to proposed toxicity tests; and address ecological implications of tests where possible. The groups were asked to consider a hierarchical testing system that involves a progression of more complicated tests, from 96-hour LC50 tests, sublethal chronic tests with sensitive life stages of selected organisms, to measurements of structural and functional aspects of the ecosystem.

Time did not permit the working groups to complete all aspects of their charge from MMS. For example, the sense of the groups was that ecosystem-level testing in the field and in the laboratory was important, but there was not time (and in some instances not the required expertise) to discuss this subject in any detail during the Workshop deliberations.

The following summaries consist mainly of the recommendations of each of the working groups.

Selection of Oils and Dispersants for Testing

Selection of Oils

Because it is not feasible to test all of the oils and dispersants, the participants at the Workshop discussed the need for careful selection (for toxicity testing purposes) of oils produced or transported within the Gulf of Mexico and chemicals applied to disperse them. Criteria were developed for selecting those appropriate for a reasonable testing program.

The kinds, amounts, and some properties of oil transported or produced in the Gulf can be found in Appendices D-1, D-1a, and D-2 of EPA's Proposed National Oil and Hazardous Substance Pollution Contingency Plan. Lt. Edward Stanton of the U.S. Coast Guard emphasized that the volume of any given oil or product that is transported may vary from year to year, but that oil properties do not vary substantially. For consideration of dispersant use (and thus toxicity testing), the oil properties may be as important as volumes. Lt. Stanton noted that 95 different kinds of crude oil are transported through the Gulf and that Mexican and Alaskan oils make up the bulk of the volumes transported. However, data presented in the Petroleum Supply Annual 1986 indicate that imports from the Organiza-

tion of Petroleum-Exporting Countries (OPEC), chiefly Arabian Crude, make up the bulk of products entering Gulf ports (DOE 1986). The amount of drilling in the Gulf is down while the number of tankers has increased.

The following criteria were developed by the participants for the selection of oils to be tested:

1. **Oils should be available in large enough batches to complete an entire set of hierarchical testing procedures.** It is important, for comparative purposes, to test the same oil throughout the testing procedure.

2. **Oils tested should be sufficiently characterized before testing.** Many oils already have been carefully analyzed and their chemical and physical properties noted. Detailed analyses are needed here. (However, it was noted in discussions that crude and refined products are not "standard" products with uniform and consistent compositions. Unless they are from stocks maintained by EPA and the American Petroleum Institute (API), the oils should be carefully characterized.)

3. **Oils tested should be those produced or transported in major quantities in the Gulf of Mexico.** Furthermore, the oils used for testing should be selected from oil stocks from the EPA/API reference oils at the Ohmsett Laboratory in Leonardo, New Jersey. The following oils are stored at Ohmsett:

   A. North Slope (essentially the same as Prudhoe Bay)
   B. Prudhoe Bay
   C. Alberta Sweet
   D. No. 6 Fuel Oil
   E. South Louisiana
   F. Saudi Arabian Light
   G. Bunker C Heating Oil
   H. High Aromatic Heating Oil
   I. Canadian Crude
   J. Typical Heating Oil

Based on the criteria mentioned above and the availability of oil at Ohmsett, the following oils are recommended for testing in order of preference:

   A. Prudhoe Bay
   B. South Louisiana
   C. No. 2 Fuel Oil
   D. Saudi Arabian Light

Selection of Dispersants

The participants next discussed the selection of dispersants. Dispersants are primarily mixtures of solvents and surface active agents for reducing surface tension and are used to remove oil slicks from surface waters. The treated oil enters the water column as fine droplets, where it is dispersed by currents and undergoes degradation from natural microbiological and physical processes. Thus, if the application is timely and the action of the dispersant and natural processes proceed as planned, an oil slick may be prevented from entering a sensitive biological area such as a bay or estuary (NRC 1989).

The participants also reviewed the numbers and kinds of dispersants procurable for testing and agreed to consider primarily those dispersants that are realistically available for use (see Appendix C to Part 300 of EPA's Proposed National Oil and Hazardous Substance Pollution Contingency Plan for a product list that displays dispersants by product name, manufacturer, and the date listed).

The chemical and physical properties of dispersants in general were discussed. According to Dr. John Fraser (see abstract), the job of choosing which dispersants to consider for testing is simplified because only a limited number of chemicals are currently used in dispersant formulations; these include anionic surfactants, nonionic surfactants, and solvents.

Based on this information and the experiences of several participants, the following criteria were developed for selecting dispersants for testing:

1. Sufficient quantities should be available for spill control.

2. The chemical composition and variability should be known (water base, solvent base, or other base).

3. There should be an existing database of toxicity studies.

4. The chemical composition should be available.

5. The dispersant should have been subjected to EPA's 96-hour toxicity test with No. 2 Fuel Oil according to procedures in the Proposed National Oil and Hazardous Substance Pollution Contingency Plan, Appendix C to Part 300.

Dr. Michael Flaherty presented a listing of dispersants by product name, manufacturer, and the date listed, as found in the Product Schedule, Part 300 of the National Contingency Plan. As a result of considerations of this listing and the criteria developed by the group, Table 1 was constructed to show the recommended priorities for testing dispersants and to display some relevant information about the dispersants. Based on new

Table 1. Dispersants selected for testing and some characteristics of each.

Dispersant	Sufficient Quantities	Variability of Chemical Composition	Existing Toxicity Test Database	Knowledge of Chemical Composition	96-hr LC50 with 1:10 Mixture (one part dispersant) of No. 2 Fuel Oil*	
					Fundulus (*Fundulus heteroclitus*)	Brine Shrimp (*Artemia salina*)
Corexit 9527	Most widely stocked (1100 drums)	Solvent-Based	Excellent	Yes	4	40
Chemlink D609	100+ drums available in Gulf of Mexico	Very similar to Corexit 9527	Considerable available	Yes	1230	470
Finasol OSR7	None	Water-based product	Full data available	Yes	2000	35
Cold Clean 500	100+ drums available in Gulf of Mexico	Unknown**	Minor at best	Yes	240	12
Slickgone NS	None***	Water-based, very different to above 5 dispersants	Data available from Warren Springs, England and from EPA	Yes	498	101
Gold Crew	Small stock in Atlanta, S.F., and San Diego	Water-based product	Complete test data available	EPA aware	71	14,000

* Source: Technical Product Bulletins - Emergency Response Division, U.S. Environmental Protection Agency

** To the knowledge of the participants in the working group

*** Soon to be available in Gulf Coast (5 mfg. plants planned)

information obtained after the Workshop, the Working Group Chairman recommended the following order of priority testing:

1. Chemlink D609	Solvent base
2. Gold Crew	Water base
3. Slickgone N.S.	Low solvent/water base
4. Finasol OSR7	Water base
5. Corexit 9527	Solvent base
6. Cold Clean 500	Water base

An example of the manner in which toxicity data on dispersants can be used is illustrated in Table 2 (based on Anderson et al. 1985), where toxicity values are combined with measurements of effectiveness and cost of the dispersants to produce a number by which various dispersants can be compared. This number is one of several criteria that can be employed by environmental managers and on-scene coordinators to determine whether or not a dispersant should be applied. It was noted that Corexit 9527 and 7664 are among those stockpiled for use in the Gulf of Mexico.

Toxicologists in the group strongly expressed their opinion that in order to properly test and evaluate dispersants, it is necessary to know the formulations involved. This is necessary not only for toxicological interpretations but also for the safety of laboratory personnel involved in testing. The need for this information often conflicts with that of the manufacturer to maintain confidentiality of the formulation for marketing purposes. It was pointed out that the manufacturer often furnishes a list of ingredients without revealing the quantities or ratios involved and this may be sufficient to test the dispersant safely. The general composition of some formulations can be obtained from patent literature, as well.

Testing Invertebrates

Invertebrates are a particularly important group of test organisms because of their diversity and abundance in the marine environment, their commercial importance, and their sensitivity to oil and oil-related compounds. Table 3 was developed to match the level of test shown in the flow diagram with materials, the kind of organism, and the stage in the life cycle most appropriate for the test.

The participants selected certain species found in the Gulf of Mexico as especially appropriate for testing purposes. This selection was based on the availability of the species at certain life stages, its sensitivity to oil products, its being hardy enough to be maintained under laboratory testing conditions, the availability of background information on its physiology, and other criteria (Table 3). This is not to say that other scientists could not produce other appropriate test organisms or that other test organisms should not be developed. Rather, the Working Group suggested these species based on their testing experience and knowledge of the literature.

Table 2. Relative effectiveness, toxicity, and cost of chemical dispersants.*

Dispersant	(15°C) Dispersant:Oil Ratio (DOR_{90})[1]	(25°C) 96-hr LC50 (ppm)	RET[2]	REC[3]	REC/T[4]
Finasol OSR7 (C)	0.038	204.0	2	0.28	1
Arcochem O-609 (D)	0.007	29.0	2	0.08	3
Corexit 9527	0.009	31.9	3	0.11	3
Corexit 7664 (F)	0.500	515.0	10	4.40	8
Petrocon N/T #4	0.018	15.0	12	0.16	11
Magnus Maritec (N)	0.012	8.0	15	0.09	11
Petromend (P)	0.008	3.7	22	0.08	22
BP1100X (L)	0.150	17.0	88	0.56	33
BP1100WD (B)	0.009	1.4	68	0.07	50
Slick-A-Way (J)	0.240	16.0	150	1.30	81
Corexit 8667 (G)	0.028	2.0	140	0.17	85
Conco K (K)	0.580	3.5	170	0.59	169
Ameroid OSD/LT (I)	0.110	6.7	170	1.20	179
Atlantol AT-7 (A)	0.130	8.6	197	1.20	182

* After Anderson et al. 1985, as presented by Mike Flaherty

[1] DOR_{90} is the ratio of dispersant to oil required to disperse 90% of the oil.

[2] RET is the relative effective toxicity or the combination of the 96-hr LC50 value and the ratio (DOR_{90}) of oil to dispersant. The formula used is

$$\frac{DOR_{90} \times 10^4}{\text{96-hr LC50}} = \text{RET}.$$

[3] REC is the relative effective cost of sufficient dispersant to disperse 90% of 1 gal. of oil under the conditions of the Mackay apparatus.

[4] REC/T[4] represents the combination of effectiveness, cost, and toxicity of the products. The formula used is

$$\frac{\text{REC} \times 10^3}{\text{96-hr LC50}}$$

Table 3. Testing invertebrates: recommended species and life stages to be tested.

SPECIES	LIFE-STAGE
Bacteria (*Photobacterium phosphoreum*[R*])	Population
Crustaceans	
Mysids (*Mysidopsis bahia*)	Juvenile (5-8 days)
Brown shrimp (*Penaeus aztecus*)	Juvenile, post larvae, Adult
White shrimp (*Penaeus setiferus*)	Megalops, juvenile
Pink shrimp (*Penaeus duorarum*)	Adult
Mud crab (*Rhithropanopeus harrisii*)	
Fiddler crab (*Uca* spp.)	Adult, sperm
Stone crab (*Menippe* spp.)	Adult
Spiny lobster (*Panulirus argas*)	
Sea urchin (*Arbacia punctulata*)	
Amphipod (*Ampelisca* spp.)	
Molluscs	
Oyster (*Crassostrea virginica*)	Post larvae, adult
Coral (*Acropora cervicornis*)	___
Gorgonian, *Briareum* spp.	___
Polychaete worm (*Neanthes arenaceodentata*)	Adult

*R = Microtox

The participants also made the following suggestions concerning specific invertebrates and their life stages that are most appropriate within a Gulf of Mexico context for testing the effects of dispersants, oil, and dispersed oil:

1. The American oyster, Crassostrea virginica, could be used as the bivalve test species and should be tested as an adult for reproductive and growth effect.

2. Any of the three decapod shrimp, Penaeus aztecus, Penaeus setiferus, or Penaeus duorarum could be used; the selection of species would depend upon the geographical location of interest. Postlarvae and\or juvenile stages could be tested.

3. The mud crab, Rhithropanopeus harrisii, could be used as a first-level test species for decapod crustaceans. However, the stone crab (Menippe mercenaria), the spiny lobster (Panulirus argas), and the fiddler crab (Uca spp.) also could be used.

4. The mysid, Mysidopsis bahia, the sea urchin (Arbacia), and the bacterium (Photobacterium phosphoreum[R*]) could be included as reference test organisms.

5. A specific polychaete worm was not named but an infaunal worm must be tested. The amphipod, Ampelisca spp., could be used as an infaunal crustacean.

6. The coral, Acropora cervicornis, and the gorgonian, Briareum, could be used as test organisms.

Since corals are often considered to be "sensitive biological areas" in relation to pollution, Dr. Eric Powell was asked to describe toxicity tests conducted in the field with these organisms (see abstract). He pointed out that most coral biomass accretes yearly by growth, not reproduction. Hence, measurements of sublethal effects should include growth and the primary mediator of growth, zooxanthellae health. Good assays are available for both of these based on pH/alkalinity measurements. Dr. Powell and others expressed the need for conducting tests in both turbid and nonturbid water and in conducting recovery studies where exposed corals are placed in clean water for several weeks or, in some instances, months.

Testing Vertebrates

Several groups of marine vertebrates were suggested for testing purposes including finfish (planktonic and benthic spawners), turtles, and

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*R = Microtox

birds. The species recommended for testing in each group, the life stage of each that is the most appropriate for testing, the criteria for effects, and an indication of availability of the test organisms are shown in Table 4. Of the planktonic spawners, menhaden (Brevoortia patronus), croaker (Micropogonias undulatus), and spot (Leiostomus xanthurus) were considered to be of the highest testing value and the most easily maintained. Also, it is possible to work with the embryos and larvae of these species. Redfish (Sciaenops ocellatus) and speckled trout (Cynoscion nebulosus) are more difficult to maintain in the early life stages and should be tested as adults. There is ongoing research on the endocrinology of the latter two species. The participants encouraged the use of micro/mesocosms containing mixtures of plants and animals to evaluate the primary and secondary effects of the test materials. Secondary effects on such critical links in the community as predation can destabilize the community but could not be detected with single species tests.

Marine cetaceans were discussed but it was noted that they were not considered especially vulnerable or sensitive to oil spills, although it was noted that little is known about avoidance effects on eye tissues, etc. Furthermore, they are not conducive to toxicity testing.

Marine birds are both vulnerable and sensitive and it is estimated that 90% of the birds that contact oil in a spill situation die. For purposes of toxicity testing, the participants recommended diving birds as prime candidates for testing and skimmers as representative of another vulnerable group. Some test species of birds may be available from the U.S. Fish and Wildlife Service Laboratory at Patuxent, Maryland.

The possible effects of dispersants, oil, and dispersed oil on marine turtles received special attention at the meeting. Turtles can be exposed to these materials in many ways. For example, adults could experience prolonged exposure by continually resurfacing through an oil slick. Juveniles could be exposed through association of flotsam contaminated with oil, hatchlings could be exposed to oiled beach sand as well as oil at the water/beach interface, and eggs could be exposed when buried in oiled sand. With reference to exposure, it is necessary to think in terms of oil concentrations on a square meter of water surface or in liters per square meter and in thickness of the oil on the water or beach. The black terrapin was suggested as a surrogate test organism (for certain tests) for marine turtles because all species of marine turtles found in the Gulf of Mexico are considered threatened or endangered. According to Dr. Peter Lutz (see abstract), general studies for turtles exposed to oil (or dispersed oil) should include observations on the effects of surface contact, alterations in respiration, effects of ingested materials, and absorption of toxic products across the gut wall.

Table 4. Testing vertebrates: showing organisms and life stages most appropriate for testing and availability of test organisms.

Species	Life Stage	Effects Criteria	Availability
FISH			
Planktonic Spawners	Planktonic embryo and larvae	Hatch/survival Growth Buoyancy Larval-juvenile Development	Available 6 months of year
1. Menhaden (*Brevoortia patronus*) 2. Croaker (*Micropogonias undulatus*) 3. Spot (*Leiostomus xanthurus*) 4. Redfish (*Sciaenops ocellatus*) 5. Trout (spotted)(*Cynoscion nebulosus*)	Adults	Reproduction	
Benthic Spawners	All life stages	Hatch/survival Growth Survival Reproduction	Available 12 months of year
1. Sheepshead minnow (*Cyprinodon variegatus*) 2. Silverside minnow (*Menidia menidia*) 3. Mummichog (*Fundulus heteroclitus*)			
Micro-Mesocosms Sheepshead Minnow plus other	Community studies	Survival Growth Species interaction (2nd-degree effects)	Available 12 months of year
TURTLES			
Loggerhead (*Caretta caretta*) Green Turtle (*Chelonia mydas*)	Egg Juveniles	Survival/hatch Development Histopathology plus clinical	Available 6 months of year Juveniles 12 months However, threatened/ endangered
BIRDS			
Cormorants Pelicans Ducks Terns (bull)	Literature search of all material Eggs (embryos) Juveniles Adults	Survival/hatch Development	Literature search for maximum dose

Testing Plants

The participants provided information on testing seagrasses, mangroves, marsh grasses, algae, and phytoplankton (see Table 5). Manatee grass (Syringodium filiforme), shoal grass (Halodule wrightii), and turtle grass (Thalassia testudinum) were selected as test seagrass species based on their Gulfwide distribution, ease of maintenance in the laboratory, occupation of separate ecological niches, demonstrated sensitivity to oil and dispersants, and the facts that some relevant toxicity data are available on these grasses and they compose a large portion of seagrass biomass in the Gulf of Mexico. As with the mangroves and marsh grasses, there are no standard methods for testing with oil and dispersants. While some methods are found in various scientific papers, most existing methods must be modified to accommodate oil and dispersants as test materials. In some cases, tests will have to be developed for specific toxicological studies.

The red mangrove (Rhizophora mangle) and black mangrove (Avicennia germinans) were selected as test species to represent mangroves in general. The criteria for selection included importance to the coastal ecosystem, sensitivity of seedlings to the chemicals in question, composition of a large biomass, and occupation of a unique ecological niche. The criteria for effects include measurement of lenticel functions and membrane permeability. A screening test should be developed that links effects on seedlings to adult plants.

Marsh grasses play an important role in the ecology of coastal zones and should be considered in a testing program. Because it is possible that oil and dispersed oil could reach marsh grass through wave and current action and dispersants could be sprayed inadvertently on the grasses, it is recommended that all three materials be tested with recommended species. The black needlerush (Juncus roemerianus) and smooth cordgrass (Spartina alterniflora) were recommended as test marsh grass species. These species compose a large biomass and are widely distributed in the Gulf of Mexico. Unfortunately, the plants are slow to recover after a severe impact of any kind. Because there are no standard or other testing protocols for these plants and the materials in question, the group recommended that relevant tests be developed with ecologically and physiologically relevant endpoints. This includes a reasonable exposure regime and endpoints such as O_2 production, growth, maturation, and mortality. The tests should involve both seedlings and adults because of potential exposure in the field.

The need for testing unicellular plants also was discussed and the group recommended red macroalgae (Champia parvula) and red algae (Caloglossa lepre); the phytoplankton (Minutocellus polymorphus) was added later. The red algae are amenable to standard test methods developed by the EPA and the phytoplankton has been used by Walsh et al. (1988) to test other contaminants. Reproduction and mortality are the endpoints for previous studies with these plants. It should be noted that oil may

either inhibit or stimulate phytoplankton growth, and this combined with the rapid recovery time of phytoplankton may make it difficult to interpret the results of these tests.

Table 5. Testing plants: recommended species and life stages to be tested.

TEST SPECIES	LIFE-STAGE
Seagrasses	
Manatee grass (*Syringodium filiforme*)	Adult and new growth
Shoal grass (*Halodule wrightii*)	
Turtle grass (*Thalassia testudinum*)	
Mangroves	
Black mangrove (*Avicennia germinans*)	Seedlings and adult
Red mangrove (*Rhizophora mangle*)	
Marsh grasses	
Black needlerush (*Juncus roemerianus*)	Seedlings and adult
Smooth cordgrass (*Spartina alterniflora*)	
Algae	Immature and mature
Red macroalgae (*Champia parvula*)	
Red algae (*Caloglossa lepre*)	
Phytoplankton	
Diatom (*Minutocellus polymorphus*)	Population
Diatom (*Skeletonema costatum*)	Population

Several recommendations of general concern were highlighted during group discussions:

1. Chemical analyses must be performed in order to properly determine exposure and effects.

2. A special need exists to develop testing procedures for sensitive life stages (seedlings) of plants.

3. Concentrations of dispersants, oil, and dispersed oil used in toxicity tests should be consistent with expected field concentrations.

4. Tests should be developed to measure the recovery of affected plants.

Dr. Tom Duke and Dr. Howard Teas (see abstracts) presented additional information on sea grasses and mangroves. Dr. Duke introduced a method produced by Morton et al. (1986) for testing seagrasses that involves microcosms in which cores of seagrass with intact sediment are removed from the environment, placed in microcosms, and exposed to contaminants in flowing sea water. These systems have not yet been used with oil or dispersants alone, but have been used by others to study the effects of drilling fluids containing diesel oil. The endpoints for effects include changes in numbers and composition of the invertebrate community as well as changes in chlorophyll a content of epiphytes and grasses.

Dr. Teas (see abstract) pointed out that mangroves are important because of the many forms of life they support and their stabilizing effect on the shoreline. A reason for special concern over mangroves is the long time required for them to regrow after they are killed. He presented data on the plant stages or parts that have been used in testing oil and dispersants and indicated that petroleum hydrocarbons have been shown to disrupt mangrove root membranes.

Summary and Findings

Based on their expertise and experience in the fields of aquatic toxicology, ecology, marine biology, and chemistry, the participants developed a flow diagram to illustrate the level of testing recommended to evaluate the toxicity of dispersants, oil, and dispersed oil to marine organisms. The organisms for testing were selected on the basis of general sensitivity to the test chemicals, adaptability to laboratory conditions, availability, and other considerations. The methods for testing were discussed in some detail and the necessity for chemical measurements of oil or other test materials was emphasized. The following findings are a result of comments and discussions during the meeting:

Certain toxicology tests need to be conducted with dispersants, oil, and dispersed oil but they should be conducted with the knowledge that there is already an extensive toxicity database available.

1. There are few (NRC 1989) "standard" toxicity tests designed specifically for testing dispersants, oil, and dispersed oil. However, many standard tests found in American Society for Testing and Materials standard practices documents and other similar documents can be used if the exposure techniques are modified to accommodate oil and dispersed oil. Furthermore, some methods for testing these chemicals specifically can be found in various scientific articles and reports. The relationships among recommended test species, test methods, and level of testing with reference to the flow diagram are shown in Table 6.

Table 6. A summary table showing level of testing, suggested test species, and appropriate test methods.*

LEVEL OF TESTING	INVERTEBRATES	VERTEBRATES	PLANTS
Screen (Water-soluble phase)	Bacteria (Photobacterium phosphoreum R**)[1,2] Sea urchin (Arbacia)[3]		
Definitive Acute Toxicity (Dispersants)	Mysids (Mysidopsis bahia)[4,5,6] Brown shrimp (Penaeus aztecus)[4,5,6] White shrimp (Penaeus setiferus)[4,5,6] Oyster (Crassostrea virginica) Sea urchin[3,4,5,6]	Menhaden (Brevoortia patronus)[4,5,6] Croaker (Micropognias undulatus)[4,5,6] Spot (Leiostomus xanthurus)[4,5,6] Redfish (Sciaenops ocellatus)[4,5,6] Trout (speckeled) (Cynoscion nebulosus)[4,5,6] Sheepshead minnow (Cyprinodon variegatus)[4,5,6] Silverside (Menidia beryllina)[4,5,6] Mummichog (Fundulus heteroclitus)[4,5,6]	Phytoplankton (Minutocellus polymorphus)[8,9] Phytoplankton (Skeletonema costatum)[9,7] Red macroalgae (Champia parvula)[3,7] Red algae (Caloglossa lepre)[7,9]

* Methods cited are not necessarily designed for testing dispersants, oil, or dispersed oil, or for using the specific organism, but are applicable with some modification. The purpose of the citation is to provide examples of applicable methods and the cited methods are not intended to be inclusive.

** R = Microtox

Table 6. A summary table showing level of testing, suggested test species, and appropriate test methods (continued).*

LEVEL OF TESTING	INVERTEBRATES	VERTEBRATES	PLANTS
Water Phase (Oil only) and Static and flowing possible (Dispersants and Oil) Only flowing water possible	Coral (Acropora cervicornis)10,11 Oyster 4,5,6,10,12 Crab (Rhithropanopeus harrisii)4,5,6 Brown shrimp 4,5,6,13,14 White shrimp 4,5,6,13,14 Pink shrimp 4,5,6,13,14 Sea urchin 4,5,6,13,14 Mysids 4,5,6,13,14	Menhaden 4,5,6,12,13 Croaker 4,5,6,12,13 Spot 4,5,6,12,13 Redfish 4,5,6,12,13 Trout 4,5,6,12,13 Sheepshead minnow 4,5,6,12,13 Silverside 4,5,6,12,13 Loggerhead (Caretta caretta)**15 Green turtle (Chelonia mydas)**15 Cormorants 22 Pelicans 22 Ducks 22 Terns 22	Manatee grass (Syringodium filiforne)10,16,17,18,19,20 Shoal grass (Halodule wrightii))10,16,17,18,19,20 Turtle grass (Thalassia testudinum)10,16,17,18,19,20 Black mangrove (Avicennia germinans)21 Red mangrove (Rhizophora mangle)21 Black Needlerush (Juncus roemerianus)30 Smooth cordgrass (Spartina alterniflora)30
Sediment (Oil only and dispersed oil)	Clam, 29 Crab, Uca spp. 23,24,25,26,27 Crab, Menippe spp. 23,24,25,26,27 White shrimp 23,24,25,26,27 Pink shrimp 23,24,25,26,27 Amphipod, Ampelisca spp. 23,24,25,26,27 Polychaete worm (Neanthes arenaceoclentata) 23,24,25,26,27 Gorgonian, Briareum spp. 10,11	Sheepshead minnow 28 Silverside 28 Mummichog 28 Ducks 22	Manatee grass 10,16,17,18,19,20 Shoal grass 10,16,17,18,19,20 Turtle grass10,16,17,18,19,20 Black mangrove 22 Red mangrove 22 Black needlerush 30 Smooth cordgrass 30

* Methods cited are not necessarily designed for testing dispersants, oil, or dispersed oil, or for using the specific organism, but are applicable with some modification. The purpose of the citation is to provide examples of applicable methods and the cited methods are not intended to be inclusive.

** Endangered or rare species.

Table 6. A summary table showing level of testing, suggested test species, and appropriate test methods (continued).*

LEVEL OF TESTING	INVERTEBRATES	VERTEBRATES	PLANTS
Micro/mesocosm	Multispecies test including some or all of species listed for sediment	Sheepshead minnow plus others	One or all species of seagrasses and marsh grasses listed for sediment. Mangrove seedlings could be included.

* Methods cited are not necessarily designed for testing dispersants, oil, or dispersed oil, or for using the specific organism, but are applicable with some modification. The purpose of the citation is to provide examples of applicable methods and the cited methods are not intended to be inclusive.

** Endangered or rare species.

1. Hagan and Halm, 1985
2. Jones, 1989
3. EPA, 1988a
4. APHA, AWWA, WPCF, 1981
5. ASTM, 1980
6. EPA, 1985
7. ASTM, 1981
8. Walsh et al., 1988
9. EPA, 1988b
10. Thorhaug et al., 1989
11. Kendall and Powell, 1988
12. Neff and Haensly, 1982
13. Anderson et al., 1974
14. Wells et al., 1982
15. Lutz, abstract in this Proceedings
16. Morton et al., 1986
17. Kelly et al., 1987
18. Hatcher and Larkum, 1982
19. Thorhaug and Marcus, 1987
20. Thorhaug et al., 1986
21. Teas, abstract in this Proceedings
22. To be developed
23. Army Corps of Engineers, 1977
24. Tagatz et al., 1978
25. Tagatz and Deans, 1983
26. Tagatz et al., 1980
27. Santschi, 1985
28. Rubenstein et al., 1984
29. Anderson et al., 1984
30. Lane et al., 1987

2. The manner in which organisms are exposed to test chemicals should be consistent, when possible, with the manner in which organisms are exposed in the natural environment. For example, contaminated sediment can be layered over clean sediment to test the impact on benthic organisms because this simulates contaminated suspended particulates settling to the bottom in the natural environment.

3. The concentrations of dispersants, oil, and dispersed oil used in laboratory experiments should include concentrations found in the environment or predicted to be there, and those inducing and not inducing effects. Chemical analysis should be conducted to determine the actual concentrations and, if possible, the composition of the chemicals in the test systems.

4. Prudhoe Bay, South Louisiana, No. 2 Fuel Oil, and Saudi Arabian Light were recommended as the oils to be tested.

5. The following dispersants were recommended for testing: Corexit 9527, Chemlink D609, Finasol OSR7, Cold Clean 500, Slickgone NS, and Gold Crew.

6. Models, such as the MIRG/SLR spill impact assessment model described by Trudell, should be peer reviewed, "validated," and used as another tool for predicting the impact of oil, dispersed oil, and related chemicals on the environment.

7. There is a need for micro/mesocosm studies on the fate and effects of oils and dispersants in marine ecosystems. There is also a need for testing the impact of oils and dispersants on the structure and function of ecosystems in the laboratory and in the field. However, the consensus of the Workshop was that the subject of ecosystem-level testing should be addressed in another forum.

8. Test results produced from toxicity tests such as those described at the Workshop should be integrated into a risk assessment process in order to obtain a broader view of the fate and effects of the materials involved. One approach is to integrate the results of the proposed hierarchical testing scheme with an Ecological Risk Assessment consisting of hazard identification, effects assessment (water column and sediment), exposure assessment, and risk characterization.

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ABSTRACTS OF

SPEAKERS' PRESENTATIONS

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Introduction

Scientists representing many scientific disciplines including toxicology, biology, chemistry, ecology, and biochemistry presented specific information regarding methods for evaluating the toxicity of oils and dispersants on marine organisms. Each speaker prepared an extended abstract, and the talks and abstracts were the basis for many productive discussions at the Workshop. The abstracts are presented here to indicate the technical basis for many of the discussions and conclusions of the Workshop.

The first two abstracts give information on a recent report by the NRC on the use of dispersants in marine waters and on development of a model relevant to the fate and effect of oil and dispersants in the Gulf of Mexico. Dr. P.G. Wells relates the findings of the NRC Report, which were especially pertinent to deliberations at the Workshop. Dr. K. Trudel speaks of the development of an oil spill impact assessment model that can predict the impacts of oil spills by showing results of treating oil with dispersants compared with not treating the oil.

The next four abstracts set the tone for the status of toxicity testing in a general sense. Dr. R.F. Lee gives information on the pharmacodynamics of bioaccumulation that is of special interest to those conducting toxicity tests. Drs. J.D. Costlow and A.S. Clare discuss the need for testing sensitive life stages of organisms in order to properly determine the vast array of effects (such as reproduction, larval release/brooding, larval development, and others) that can be exerted at different stages of development. Dr. J.M. Neff discusses the manner in which organisms can be exposed to oil and suggests that ideally, laboratory exposures should simulate as closely as possible the types of exposures in the field. He also identifies a variety of acute and chronic biological effects that can result from such exposures. Dr. J.W. Anderson presents additional information on exposure regimes and toxicity of dispersed and undispersed oil as well as data on toxicity of dispersants to marine organisms.

The remainder of the abstracts are directed to topics addressed by the subgroups: selection of oil and dispersants for testing, and the toxicity of oil, dispersed oil, and dispersants to invertebrates, vertebrates, and plants. Dr. M.L. Flaherty discusses data required for registration of dispersants. He points out that Regional Response Teams are encouraged to "preapprove" certain dispersants or chemical agents in their area of geographical responsibility. Dr. J.P. Fraser comments on considerations in selecting dispersants for testing, which include the presence of the dispersant on the EPA product schedule, whether the dispersant contains certain chemical ingredients, and whether it is designed for aerial or boat spray application. Dr. E.N. Powell discusses approaches to testing the toxicity of materials to invertebrates, particularly oysters and

coral. His approaches include both field and laboratory tests. Dr. P.L. Lutz addresses methods for determining the toxicity of oil and dispersants to sea turtles and suggests that these animals are surprisingly sensitive to oil contamination. He proposes a conceptual model of potential effects of oils and dispersants on sea turtles.

The last two abstracts are concerned with the effects of the compounds and chemicals of interest on seagrasses and mangroves. These particular plant communities were included because of their potential exposure to oils and dispersants and because of the sensitivity of the communities of organisms that share these habitats. Dr. T.W. Duke describes a microcosm system containing cores of seagrasses and attendant sediment that could be used to evaluate the effects of oil and dispersants on seagrass communities. The criteria for effects on these communities include changes in growth rate of the seagrass and diversity and richness of macroinvertebrate species. Dr. H.J. Teas discusses methods for determining the toxicity of oil and dispersants to mangroves and lists plant stages or parts that have been tested with these compounds.

USING OIL SPILL DISPERSANTS ON THE SEA--ISSUES AND ANSWERS

Peter G. Wells

The National Research Council (NRC) Committee on Effectiveness of Oil Spill Dispersants has recently completed a critical detailed literature review and summary of experience of workers in the field of oil spill dispersants. Twelve committee members,[1] the Marine Board staff, and numerous others contributed to the review (NRC 1989).

The review was stimulated by the concern about the aesthetic, ecological, and economic impacts of oil spills in the ocean and the adequacy of technologies for controlling them. The study addressed two basic questions about the use of dispersants: Do they do any good (i.e., do dispersants effectively disperse oils), and do they do any harm (i.e., how do dispersants interact with oil and affect marine biota and ecosystems)?

This presentation, based directly on the NRC review, addressed *only* the highlights on biological effects (toxicity of dispersants, dispersed oils), biodegradation, ecological effects, and effects on birds and mammals. The assumption was made for this presentation that only effective dispersants would be considered for use. Biological issues of particular importance to this Workshop are summarized after the review synopsis.

General Conclusions

The principal biological benefits of chemically dispersing oil effectively are to (a) prevent stranding of oil in intertidal zones, (b) reduce hazards of discharged oil to marine birds and mammals, (c) enhance degradation of oil components, and (d) reduce chronic impact on some habitats due to the shorter persistence of oil. Biological concerns include the effects of expanded oil slicks, the effects of dispersed oil on marine life in the upper water column, and the effect of oil dispersed offshore that may reach coastal marine habitats and communities. Acute biological effects are expected to be slight in most open-sea applications. In shallow water with poor circulation, and in protected bays and inlets, the acute biological effects on some organisms and habitats from high concentrations of dispersed oil may be greater than the effect of untreated oil.

Recommendation: Additional ecological assessments of dispersed versus untreated oil at sites where water is shallow and circulation is well defined are needed.

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[1] J.N. Butler (Chairman), L.P. Atkinson, J.P. Fraser, M.J. Herz, C.M. Jones, J.P. Marum, C.D. McAuliffe, R.J. Meyers, L.A. "Skip" Onstad, J.R. Payne, J.M. Teal, and P.G. Wells.

Biological Effects

Toxicity of Dispersants

The acute lethal toxicity of most dispersants currently considered for use in the United States and Canada is low compared with the constituents and fractions of crude oils and refined products, based on literature to 1987. Toxicities are higher at higher temperatures. A wide range of sublethal responses, usually at high exposure concentrations, has been observed, but the sublethal effects of dispersants at realistic concentrations are only partially understood. It is considered unlikely, at recommended application rates, that dispersants would contribute significantly to lethal or sublethal toxicities of dispersed oils. Direct application of dispersants to seabirds or marine mammals is to be avoided. Knowledge of the chemical composition of formulations is necessary for making responsible decisions about dispersant use.

> Recommendations: Biological research and toxicity screening in the laboratory should use exposure conditions that more closely reflect dispersant use and probable dilution in the water column. New products should be screened for short-term toxicity using standard methods that will consider the physicochemical characteristics of the dispersant solutions and both lethal and sublethal responses of test organisms. Some members of the NRC Committee felt that information on dispersant chemical structures and formulations should be made readily available to researchers.

Toxicity of Dispersed Oils

Many earlier laboratory studies of the joint toxicity of oil and dispersants erroneously concluded that dispersed oils were more toxic than oil alone, because only nominal concentrations were used. Proper comparisons must be made using only the measured oil fraction in the water. For most species tested to date, the acute lethal toxicity of chemically dispersed oils resides in the low-molecular-weight and dissolved, aromatic, and aliphatic fractions of the oil. Different species and life stages show sensitivity to chemically dispersed oils at exposures varying by three to four orders of magnitude. Laboratory tests of the toxicology of dispersed oil should cover the ranges of exposures that would be expected in the field. Several studies have shown that chemically dispersed oil does not adhere as much as untreated oil to some organisms and habitats.

> Recommendations: Better methods for comparing laboratory and field exposures should be developed, and additional research should compare the effects of untreated and treated oil adhesion to organisms.

Biodegradation of Dispersants and Dispersed Oils

Dispersant components biodegrade, based on laboratory and mesocosm experiments. Some laboratory studies and all mesocosm studies to date have shown that the rate of biodegradation of dispersed oil is equal to or greater than that of nondispersed oil.

Recommendation: Further field studies of biodegradation rates of dispersed oils and hydrocarbon components should be undertaken.

Ecological Effects

Exposure of communities of organisms and their habitats to oil, dispersants, and dispersed oil depends on many factors. Dispersing oil before it reaches sensitive inshore habitats may keep them from becoming oiled or may reduce the persistence of oil that comes in contact with them. Acute effects of chemically dispersed oils on organisms and habitats vary. Organisms in the water column, particularly in upper layers, will experience greater short-term exposure to oil components if the oil is dispersed. Long-term harmful effects to benthic organisms may be reduced by chemically dispersing oil rather than by not treating the spill. In a habitat with restricted water exchange, the acute effects of dispersed oil on some organisms or marine plants may be greater than that of oil alone; however, mangroves and other intertidal habitats such as mudflats are less damaged by dispersed oil that is treated before entering the habitat, and can recover faster. Intertidal areas do not benefit from dispersant application after the oil reaches the shore. Reducing chronic exposure is a key to reducing biological damage. No measurable effects of dispersed or untreated oil on commercial fisheries and their supporting food webs have yet been found.

Recommendations: Additional ecological studies under controlled or established water circulation in shallow environments should be conducted. Long-term studies of the recovery of selected ecosystems exposed to oil are desirable, including continued studies of those sites where the impact of oil and dispersed oil has been compared already.

Birds and Mammals

Adverse effects of oil, dispersants, and dispersed oil have been shown in laboratory tests and from limited field tests and observations on seabirds and some mammals. These effects include reduced water repellency of fur and feathers, reduced hatchability of eggs, and physiological and biochemical effects. These laboratory results are consistent with field observations for untreated oil but may not appropriately represent exposures to dispersants and dispersed oil in the field. Over a short period, residual sheen from dispersed oil slicks may cover a greater area than untreated oil, and more birds rather than fewer may be oiled. Theoretical considerations indicate that the exposure of birds and mammals

to dispersed oil in the water column may be less damaging than exposure to untreated floating oil. Concern about the effects of dispersants on birds and marine mammals centers on the question of the extent of exposure rather than on enhanced toxicity of the oil.

> Recommendations: Laboratory studies on water repellency of fur and feathers and on hatchability of eggs need to be conducted under more realistic exposure conditions. Field observations of the effects of ingested oils should be increased.

Applications to the Workshop Objectives

The Workshop was charged with developing a hierarchical testing system to evaluate the potential impact of dispersants, dispersed oil, and crude oil on the coastal environment. The NRC review introduces a number of topics crucial to the design of such a testing system: identification of specific test objectives; choice of tested materials, including reference materials; rationale and choice of species and life stages; choice of exposure conditions and their characterization; comparative toxicology of marine species to the materials; and predictability of field effects from laboratory experiments. It is clear from the NRC review, and from previously published works, that composition and properties of dispersants and oils, temperature, phylogeny, species and life stages of the organisms, and exposure regime and duration must be considered. It is the author's opinion that the simplest screening system for dispersants should be strived for, one that takes into account the above factors and our current knowledge in marine ecotoxicology and hydrocarbon toxicology. This system should also acknowledge the complexity and responses of marine ecosystems under chemical stress not accounted for by laboratory or mesocosm testing systems of any kind to date.

Acknowledgments

This abstract is adapted directly from the prepublication version of the conclusions and Recommendations of the NRC (1989) dispersant review. I acknowledge all contributors to that review and the Marine Board of the National Research Council for permission to use the material.

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THE SPILL IMPACT ASSESSMENT MODEL FROM A USER'S PERSPECTIVE

Ken Trudel

This submission describes the recently developed Gulf of Mexico spill impact assessment model and considers the problem of toxicity testing programs and protocols from the perspective of the dispersant decision-maker and the modeler.

Before dispersants can be approved for use during a spill, a decision-maker from a regulatory agency may be called upon to decide whether or not chemical dispersants might reduce the overall environmental impact of the spill. This decision-making problem is complicated because the environmental impact of oil spills is influenced by a large number of variables. The approach we have taken in the Gulf of Mexico has been to estimate the potential environmental impact of the spill if left untreated, and compare this with the potential impact if dispersants are used. The decision then becomes a trade-off. On the one hand, the untreated spill poses certain risks to a variety of resources; on the other hand, the use of dispersants offers some protection to certain resources while increasing the risks to others. The trade-off is made by the user, based on the relative effects of the dispersed and untreated spills and the relative importance or value of the resources threatened in each case.

The impact assessment model computes spill impact on a wide variety of resources and expresses impact in terms of the proportions of identifiable stocks or populations of resources (and the fisheries that are supported by these resources) that are at risk from the spill. Estimates of impact take into account the following:

- Oil fate and movement
- Resource sensitivity to oil or chemically dispersed oil
- Resource recovery potential
- Resource vulnerability (distribution/aggregation, habits and habitat use, and seasonality of life-history events).

The impact assessment model functions as follows. The oil fate model accepts user-specified input concerning spill conditions (spill location, spill volume, oil type, ocean currents, winds) and computes the fate and movements of oil slicks and clouds of chemically or physically dispersed oil. The output of the oil fate subroutine is used to estimate the concentrations or amounts of oil to which resources may be exposed. This information is combined with data concerning oil exposure-response relationships of the various resources to estimate the size and location of areas within which exposure conditions are lethal or have an effect on different types of resources (called areas-of-effect). The mapped areas-of-effect are compared with mapped distributions of the appropriate life stages of populations of the various resources to estimate the

proportion of each life stage that lies within the area-of-effect. These estimates are then adjusted for the effect of other variables that influence resource vulnerability, such as vertical distribution in the water column and age-structure of the species populations. The final result of the calculation is output as the proportions of populations of each resource that are at risk from the spill. Unique impact algorithms have been developed for each resource and an example is given in Figure 2. Databases, impact assessment algorithms, and toxicity criteria have been developed for more than 70 resources that were selected by the regulatory agencies of the five Gulf of Mexico States. These resources are listed in Table 7.

For each scenario the system produces, as output, maps of predicted oil fate and a summary of predicted spill impact on all resources that have been specified by the user. An example of this output summary appears in Table 8.

During discussions with potential end-users of this system and during the development of the model itself, certain problems routinely arise concerning the use of laboratory data to predict the impact of natural resources. These problems fall into certain basic categories: response criteria, accuracy or applicability of laboratory test results, and precision of laboratory test results.

Response Criteria

Each biological resource suffers a variety of effects from exposure to oil. These effects are related to the level of oil exposure and range in severity from reversible physiological responses to more severe irreversible effects, such as larval deformity, histopathology, and mortality. In oil spill situations where exposures to toxicants are brief, reversible physiological effects may be of little long-term consequence to the population, while more severe effects like mortality are clearly significant at the population level. Ideally, it would be useful to understand the exposure-response relationships for all responses of all life stages of all species, but the number of species is large, and time and funds are limited. In selecting the response criteria to be used in testing, it is important that they have long-term significance to the population. It is also important that the same response criteria be used for all resources because dispersant decisions require a comparison of population-level effects on a variety of resource types.

Accuracy or Applicability of Laboratory Test Results

The problem of predicting real-world responses of organisms to hydrocarbon exposures from laboratory tests is complicated by the fact that exposure protocols used in many laboratory tests do not accurately reflect exposure experienced by resources under natural conditions. The available data show that toxicity threshold values in laboratory tests vary widely (several orders of magnitude) with exposure parameters such as exposure

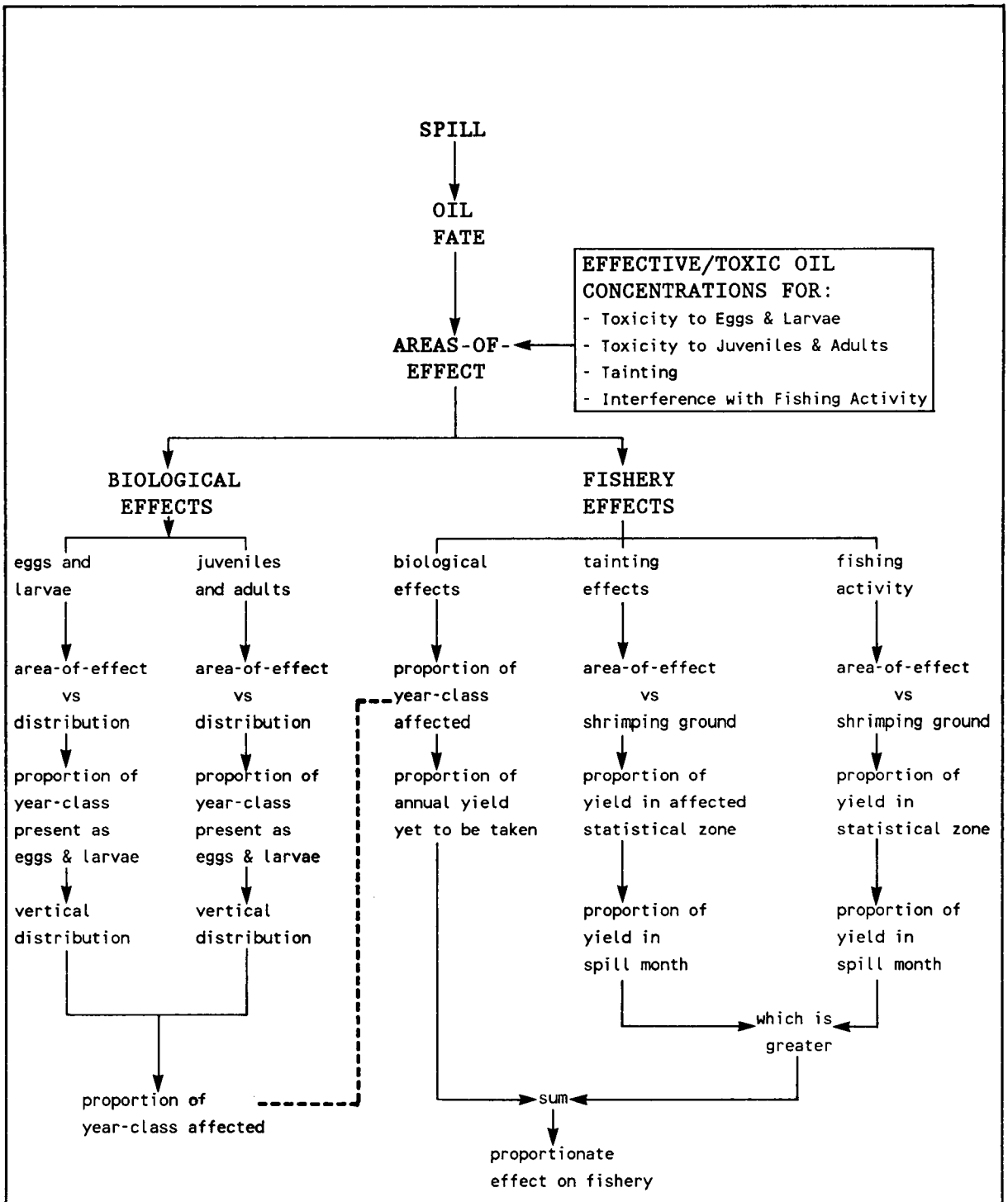


Figure 2. Algorithm for computing effects of dispersed or untreated oil on the brown shrimp stock and its associated fishery.

Table 7. Biological resources included in the Gulf of Mexico dispersant study.

HABITATS

salt marsh
mangroves
seagrass
coral reefs
oyster reef

REPTILES

green seaturtle
loggerhead seaturtle
Kemp's ridley seaturtle
leatherback seaturtle
American crocodile
gulf saltmarsh snake

MAMMALS

manatee
right whale

INVERTEBRATES

brown shrimp
pink shrimp
white shrimp
blue crab
stone crab
spiny lobster
eastern oyster
mercenaria clam
bay scallop
queen conch

BIRDS

lesser scaup
mottled duck
redhead duck
common loon
laughing gull
royal tern
sooty tern
black skimmer
reddish egret
snowy egret
great white heron
sanderling
piping plover
snowy plover
oystercatcher
northern gannet
magnificent frigatebird
brown pelican
whooping crane
roseate spoonbill
bald eagle
osprey

FINFISH

bay anchovy
gulf menhaden
spotted seatrout
black drum
red drum
southern kingfish
striped mullet
sheepshead
tarpon
snook
southern flounder
spanish mackerel
king mackerel
cobia
crevalle jack
Florida pompano
Atlantic croaker
red snapper
mangrove snapper
scamp grouper
sailfish
sand seatrout

Table 8. Summary of effects of dispersed and untreated spills.

Spill Conditions:
i) Spill: 25,000 m^3, light crude oil
ii) Winds: constant from the west at 12km/h
iii) Location/Month: 82°30′, 26°30′; June

Resources (Stocks)[1]	Impact by treatment %	
	Untreated	Dispersed (100%)
Pink shrimp (East Gulf)	0.1 (0.5)[2]	0.4 (1)
Blue crab (East Gulf)	0.2 (0.2)	0.0 (0)
Spotted seatrout (Charlotte)	0.8 (3) 0.5 (3)	
Red drum (East Gulf)	0.0 (2) 0.2 (0.1)	
Reddish egret (Florida)	2	0
Least tern (West Florida)	5	0
Brown pelican (East Gulf)	3	0
Mangrove (Charlotte)	10	0
Marinas	8[3]	0
Shorelines[4]		
Beach, nonamenity	8[5]	0
Mangrove	39	0

1. Parentheses show location of stock of resource.
2. Values are percentage of stock at risk, values in parentheses are percentage of reduction in annual yield to fishery. In this case, the target fishery is the total fishery for all five Gulf States.
3. Numbers of marinas at risk regardless of size.
4. Mean level of shoreline oiling = 28 l/m.
5. Length of shoreline oiled in km.

duration. In addition, the degree of variability differs between different types of resources. It is important, therefore, that testing protocols used for predictive purposes simulate as closely as possible the exposure experienced by organisms in the field.

Precision

The available toxicity database shows that for certain species, the toxicity threshold values for episodic (declining concentrations) exposure to chemically dispersed oil (which simulate real-world exposures) differ by several orders of magnitude from threshold values that might have been predicted based on experiments with continuous (> 96 hour) or short-term exposures (24 hours) to the water-soluble fraction (WSF) or oil-water dispersion. These observations, combined with the wide variability of threshold values reported for certain exposure types (e.g., 96-hour LC50 values for WSF for adult fish), suggest that it is important to evaluate reproducibility and natural variability in determining threshold values. Wide variations in threshold values can be accommodated in the model, but in situations where only a limited number of tests can be devoted to evaluating threshold values for each of many resources, it is important to be able to clearly distinguish between real differences in resource sensitivity and natural experimental variability.

It is important to point out that the level of precision required in estimating toxicity threshold values for many resources is not high. In the model system, the significance of variability in toxicity threshold values for any resource is determined by the degree of uncertainty that this error causes in estimating impact on resources and in formulating the final dispersant decision. For many resources, vulnerability is low, and hence, impact estimates remain low regardless of the toxicity threshold used (within limits).

PRINCIPLES OF BIOACCUMULATION OF OILS AND DISPERSANTS BY MARINE ANIMALS

Richard F. Lee

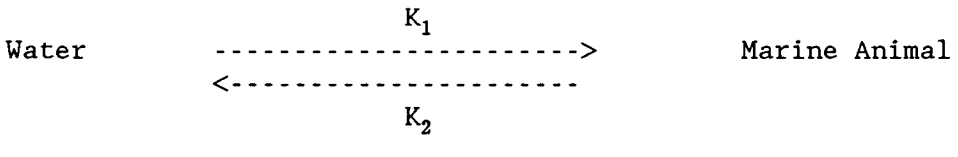
The accumulation of organic pollutants by marine organisms depends on various pharmacokinetic processes including uptake, distribution in tissues, metabolism, and elimination. Each of these processes has its own rate constant. The type of compound and the animal used will be important factors in any bioaccumulation study. Animals such as filtering bivalves, which have a limited ability to metabolize xenobiotics, bioaccumulate such compounds to a greater extent than fish or crustaceans, which rapidly metabolize and eliminate many xenobiotics. Compounds that bioaccumulate to the greatest extent, i.e., persistent pollutants, are poorly metabolized, and have low water solubility and high lipid solubility.

Bioaccumulation often correlates with the octanol/water partition coefficient (P). Those compounds with high P values are more likely to be bioaccumulated than those with low P values. Under discussion here are two very different groups of chemicals. One group is compounds associated with petroleum, many of which have high P values, while dispersants with high water solubility have low P values. Most of the work on the accumulation of petroleum by marine animals deals with hydrocarbons, both aromatics and alkanes, which are highly lipophilic. Dispersants comprise a large number of different chemicals that work by decreasing the interfacial tension between the oil and water, causing the oil slick to break up. The action of waves and currents causes the oil to break up into small droplets, which enter the sub-surface water. The active ingredient in the dispersant is the surfactant, which has two ends with differing solubility, one hydrophobic and one hydrophilic. The hydrophilic end can be ionic (+ or -) or nonionic, such as in the polyethanoxys or polyglycols.

This paper will address in a general manner the factors affecting the bioaccumulation of both petroleum and dispersants from water, food, and sediments by various marine animals.

Accumulation from Water

In its simplest term, accumulation reflects the relative importance of uptake and elimination:



K_1 = uptake rate constant
K_2 = elimination rate constant

In a simple model, hydrocarbon uptake is a first-order process with respect to hydrocarbon concentration in water and elimination rate is a first-order process with respect to hydrocarbon concentration in the animal.

$\frac{dc}{dr}$ = uptake-elimination = $k\mu c_w - k_e c$

c_w = concentration of hydrocarbon in water
c = concentration of hydrocarbon in animal
t = time
$k\mu$ = uptake rate constant
k_e = elimination rate constant

If the rate of uptake is constant and the rate of elimination is exponential, the concentration c will increase until a steady state is reached where elimination is equal to uptake. The k_e for a particular hydrocarbon can be influenced by such factors as temperature, salinity, and reproductive status. Clams, oysters, and mussels differ in their rates of hydrocarbon uptake, possibly because of differences in filtering rates and amounts of body lipids (Clark and Finely 1987; Neff et al. 1976). Oysters with high lipid content have been found to take up more fuel oil (314 $\mu g\ g^{-1}$) from the water than low lipid oysters (162 $\mu g\ g^{-1}$) (Stegeman and Teal 1973).

The steady-state bioaccumulation can be defined:

$$\text{Bioconcentration factor} = \frac{k\mu}{k_e}$$

For nondegradable, lipophilic, nonionized organic chemicals, the following relationship has been found for fish (Mackay 1982):

$$KB = 0.048\ P$$

P is the octanol/water partition coefficient and K_B is the bioconcentration factor, which is the ratio of the concentration of the chemical in the water to the concentration of the surrounding water. Since fish metabolize hydrocarbons, this relationship is not applicable for them. However, for daphnids and some mollusks, which metabolize polycyclic aromatic hydrocarbons very slowly, the relationship of K_B to P applies to their accumulation of polycyclic aromatic hydrocarbons (Hawker and Connell 1986).

In most marine animals, entry of pollutants from the water is via the gill. The gill tissues of bivalves have a micellar layer on their surfaces which absorbs hydrophobic compounds. Most of the compounds found in petroleum, e.g., hydrocarbons, are taken into the body by passive diffusion across lipid-rich membranes. For hydrophilic dispersants, entry will likely be through hydrophilic channels in the membrane. The form of the compound, i.e., dissolved or adsorbed to particle, significantly

influences the bioavailability of the pollutant compound. Numerous studies have shown that marine animals can rapidly take up petroleum hydrocarbons from an oil-in-water dispersion and that transfer to clean water results in rapid elimination of most of the hydrocarbons (Neff et al. 1976; Anderson et al. 1974). It is likely that most of the alkanes and heavier aromatics associated with petroleum are adsorbed to particulates. As particles deliver pollutants to membrane vesicles, the rate-limiting step is the desorption from the particle (Lakowicz et al. 1980; Bevan et al. 1981). Free dispersants would be dissolved but when mixed with oil much of the dispersant will be associated with small droplets containing both oil and dispersant. Copepods and protozoans can take up such small oil droplets. The aromatics associated with oil droplets or adsorbed to particulates are not taken up to the same extent by most marine animals as aromatics dissolved in water (Neff et al. 1976; Lyes 1979).

As noted above, the elimination of a compound is often first order and directly proportional to the concentration in the animal. The elimination of hydrocarbons often is in two phases, with a rapid elimination in the first phase and a much slower second phase. Mussels from the oil-polluted Lagoon of Venice, Italy, which initially contained 250 μg/g of petroleum hydrocarbon, retained 30 μg/g after 36 days of depuration (Fossato 1975). The relatively polar dispersants with their high water solubility are not tightly bound to tissues and there should be rapid, one-phase elimination of dispersants from animals.

Elimination occurs by several routes. One often suggested for fish is direct partitioning through the gills into water. Thomas and Rice (1981) present evidence that substantial proportions of naphthalene and toluene could be excreted directly through the gills. Biliary, renal, and epidermal routes may be used for disposition of xenobiotics. For example, substantial amounts of naphthalene were seen in the bile of fish exposed to the compound (Roubal et al. 1977; Varanasi et al. 1979).

A factor affecting the release of pollutants by bivalves and other invertebrates is the peaks in lipid reserves prior to spawning. The pollutants accumulated in the lipid-rich gametes will be discharged during gamete release. Thus, the seasonal reproductive cycle is an important factor in pollutant accumulation and discharge. Maxima for benzo(a)pyrene and perylene concentrations in Mytilus edulis from the Lagoon of Venice occurred in January and minima in May (Fossato et al. 1979). Spawning took place from March to April.

Accumulation from Food

A study of fish uptake of hydrophobic organic chemicals from food showed that uptake efficiency is related in octanol-water partition coefficient (Gobas et al. 1988). The relative importance of food uptake will depend on the compound and the animal. In copepods, dietary uptake of naphthalene was more important than uptake from the water (Corner et

al. 1976). In blue crabs (Callinectes sapidus), hydrocarbons in the food were only slowly taken up and, in fact, most were voided in the feces (Lee et al. 1976). It is often assumed that uptake of a hydrocarbon from food and water are additive. However, the metabolism of hydrocarbon and dispersants by the gastrointestinal tract of fish may complicate this assumption. It is also assumed that elimination rates of food and water-derived hydrocarbons and dispersants are first order and identical. More work needs to be carried out on this latter assumption.

Accumulation from Sediment

Highly lipophilic compounds will tend to adsorb to sediments resulting in very low concentrations in the water. When a variety of benthic marine animals have been exposed to sediments containing petroleum compounds, uptake of the compounds take place (Anderson et al. 1977; Varanasi and Gmur 1981; Lyes 1979; Foster et al. 1987). Generally, it appears that bioaccumulation from sediment by marine animals is of limited importance and, in fact, some benthic animals do not accumulate sediment-sorbed hydrocarbons, e.g., clams, Mya arenaria (Foster et al. 1987). When accumulation from sediment occurs, it is assumed that hydrocarbons desorb from the sediment particles into the interstitial water where it is more bioavailable to a marine animal.

A first-order rate equation has been used to estimate uptake transfer coefficients and elimination rate constants using the following equation (Foster et al. 1987):

$$\frac{dC_A}{dt} = TC_S - k_d C_A$$

where T is uptake transfer coefficient, C_S is concentration of hydrocarbon in sediment, k_d is elimination rate constant, C_A is concentration of hydrocarbon in animal, and t is time.

Dispersants with their higher water solubility do not readily absorb to sediment.

Metabolism

The compounds found in petroleum and dispersants are readily metabolized by many groups of marine animals. Aromatic hydrocarbons are metabolized by most groups of animals, but transformation rates differ greatly between groups, being extremely slow in bivalves and relatively fast in fish. The final equilibrium concentration within tissue depends on the ability of the animal to metabolize the hydrocarbon and the physical-chemical properties of the compounds, which affects the animal's rate of uptake and elimination.

In many cases, transformation rates may balance uptake rates with low apparent bioaccumulation. Lipophilic foreign compounds, often referred

to as xenobiotics, are converted by reduction, oxidation, hydrolysis, or conjugation to more soluble metabolites, which facilitates their elimination from the animal. Cytochrome P-450-mediated mixed-function oxygenase systems (MFO) oxidize foreign compounds by hydroxylation, O-dealkylation, N-dealkylation, or epoxidation reactions. The polar metabolites formed can be conjugated with sugars, sulfates, or peptides and disposed of in the urine and feces. Such enzyme systems could act on a variety of dispersants. For example, hydroxyl groups on the dispersant could be sulfated since active sulfotransferases are found in many marine invertebrates and vertebrates. The hydrophobic end of the dispersant could be oxidized by the P-450 system. For many dispersants the high water solubility would allow elimination from the animal without any modification of the compound.

The characteristics, functions, and presence of P-450 systems in aquatic, principally marine, species have been reviewed in detail (Bend and James 1978; Stegeman 1981; Lee 1981). The liver is the primary site of pollutant metabolism in fish; the hepatopancreas serves this function in invertebrates. The rate of pollutant metabolism and elimination can be influenced by a variety of environmental and physiological fates that might affect the catalytic function of the P-450 system, alter the pharmacokinetics of parent or product, or affect the response of an animal to inducers of P-450. One of the most important factors is the induction of cytochrome P-450 by environmental pollutants such as aromatic hydrocarbons. Induction in numerous marine and freshwater fish has been described (e.g., Payne and Penrose 1975; Bend et al. 1977) including induction in embryonic and larval forms (Binder and Stegemen 1980). Induction has also been noted in polychaetes and crabs after exposure to xenobiotics (Lee 1981; Lee et al. 1982). Induction can result in increased rates of pollutant metabolism in the liver and some extrahepatic tissues of fish and some invertebrates.

There is evidence that induction occurs in fish after exposure to petroleum in the environment (Payne 1976; Kurelec et al. 1977; Stegemen 1978). There is also increasing evidence of widespread induction of P-450 in fish by chemicals of unknown origin in the environment (Bend 1980; Stegeman et al. 1981). The causes of such induction have not been established, although there have been correlations with polycyclic aromatic hydrocarbons in the sediment. In the polychaete Capitella capitata exposed to crude oil, the third generation had much higher mixed-function oxygenase activity than the first or second generations (Lee et al. 1981). Grassle and Grassle (1976, 1977) showed that on the basis of electrophoretic patterns, C. capitata is a complex of at least six sibling species. Thus, exposure to oil may result in selection for species for strains that are resistant to oil because of high mixed-function oxygenase activity.

Summary and Conclusions

The extent of bioaccumulation of petroleum and dispersants is a reflection of the relative importance of uptake, metabolism, and elimination. Because they filter large volumes of water and have a limited ability to metabolize many organic pollutants, the filtering bivalves, e.g., oysters and mussels, have been used for monitoring studies. However, the consequences of elevated concentrations of petroleum and/or dispersants in bivalves and other marine animals is not well understood. For carcinogenic hydrocarbon some of the metabolites, e.g., diol epoxides, are the active carcinogen, not the parent compound. The extent of bioaccumulation by a marine animal is related to the hydrophobic properties of the compound; therefore, high-weight polycyclic aromatic hydrocarbons are concentrated in some animals by several orders of magnitude more than their concentration in the water. Compounds with high water solubility, such as dispersants, are accumulated in animals only to a very limited extent because elimination is so rapid. The response of detoxifying systems such as cytochrome P-450 systems and glutathione S-transferases to organic pollutants are responses resulting from bioaccumulation that appear to be useful for environmental pollution studies.

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TESTING FOR TOXICITY WITH VARIOUS LIFE STAGES OF MARINE ORGANISMS

John D. Costlow and A.S. Clare

Marine and estuarine organisms, like their terrestrial counterparts, exhibit life cycles whose stages and complexity are governed by various factors, among the more important of which are phylum, habitat, size, and feeding habits. It is a formidable task to develop a protocol for toxicity testing that would encompass an organism's entire life cycle. However, because each of the life cycle stages is an integral part of the whole, any factor that modifies a single stage, such that the potential for survival is reduced for that stage (a toxic effect), will have repercussions for the entire cycle and the propagation of the species.

The points in a life cycle that may be modified by external factors are by definition almost infinite, but for convenience critical links in the chain may be identified as follows: (1) reproduction, which includes maturation of gametes, production and release of pheromones used to both recognize and attract potential mates, behavior associated with egg fertilization, cleavage and embryonic development of the fertilized eggs, and release/brooding of larval stages within the water column; (2) larval development, metamorphosis, and settlement, which together result in dispersal of the species to desirable habitats; and (3) growth and maturation of the juvenile forms to sexual maturity when the life cycle is complete.

In any consideration of toxicity, be it related to petroleum and its products, oil dispersants, heavy metals, pesticides, or other anthropogenic or natural compounds, one must consider a vast array of effects that may be exerted at the different stages detailed above. It is also necessary to understand how natural and anthropogenic factors may act synergistically and to determine possible accumulative effects, which, while not necessarily lethal, may contribute to sublethal changes in one or more of the essential life processes. There is now reasonable evidence that most biochemical and physiological processes are affected by combinations of several factors, for example, extremes of salinity coupled with extremes of temperature, light, and diet. If anthropogenic factors are added to these natural environmental factors, then further stresses are imposed upon the organism.

Selection of Species

Over and above any consideration of pollutant effects and the identification of appropriate points in the life cycles where these effects may be studied, is the selection of species to be tested. The literature contains numerous references to the impact of anthropogenic compounds, including petroleum and to a lesser extent dispersants, on representatives of all of the major phyla found within the continental shelf and estuarine environments around the world. A few organisms appear

to have received the most attention for one or more reasons, including their habitat, economic importance, sensitivity, the degree to which they can be maintained within the laboratory, and their ease of collection. Species with wide geographic distribution offer more opportunity to compare and apply results and it is useful to consider the various habitats occupied by organisms prior to any decision as to how appropriate they are for study. Our own work, which largely comprises studies on Brachyura (true crabs) and Cirripedia (barnacles), has tended to concentrate on those species that are representative of the temperate waters and marshes found within the southeastern area of the United States. Examples have been drawn from the water column, intertidal areas, the benthos and tidal marshes, and they include both adult and larval forms. Of great importance is the degree to which the organisms can provide reliable and consistent results such that clear conclusions can be derived from statistical analyses of the data.

Laboratory Experiments and Field Observations

In considering the entire realm of toxicity, one must also grapple with the problem of laboratory experiments and the degree to which results derived from them can aid in predicting events in the field. Although it is generally agreed that field observations offer a far more realistic approach to understanding the impact of toxic compounds, it is frequently difficult or impractical to carry out such studies, owing to the nature of the organism, the complexity of the natural environment, and our inability to clearly delineate cause and effect. Some studies involving adults can be performed under natural conditions within the marine and estuarine environments. The literature contains numerous references to the impact of a variety of pollutants, including petroleum and dispersants, on survival and growth of adult organisms, viability of the eggs and sperm, and susceptibility to lesions, infections, and parasites.

There have been general studies on the degree to which fluctuations in planktonic organisms can occur under normal and polluted field conditions, and mesocosm studies have identified zooplankton as the most severely affected component of the simulated ecosystem. However, it is virtually impossible to conduct experiments on specific impacts of pollutants of these organisms under conditions other than those within the laboratory. A number of laboratory studies on planktonic organisms have contributed to an understanding of the way in which extremes of one or more factors contribute to survival during specific stages. For example, these studies have helped to show how these factors may result in normal versus abnormal morphological development, the way in which their physiology, as expressed by oxygen consumption, can be altered by pollutants, the identification of biochemical/molecular changes at specific stages under the influence of anthropogenic compounds, and how long-term genetic modifications by contaminants may affect the capacity for survival in subsequent generations.

Unfortunately, in many of the laboratory experiments the primary consideration has been "survival," i.e., to what concentration of a particular compound is an organism unable to adapt. Although this information is useful in ranking components according to their toxicity, it provides little insight into the effect of lower concentrations of pollutants and chronic exposures. Also, it does not contribute to our understanding of accumulative impacts over long periods of time.

Progress in Laboratory Experiments

Over the past two decades, considerable progress has been made in developing techniques for maintaining some planktonic organisms under controlled conditions in the laboratory. Although there continues to be criticism that these controlled conditions do not duplicate those found in the natural environment, it has been possible to identify specific criteria by which the normality of the observations in a simulated environment can be judged. Thus, our own studies on larval development of a number of crustaceans evolved from an interest in the impact of single environmental factors to more complex studies to provide a better understanding of the synergistic relationship between two or more natural factors. Our more recent studies have combined, in classical matrix form, natural environmental factors (salinity, temperature, light, and diet) with anthropogenic factors, including various concentrations of drilling muds, pesticides, heavy metals, and insect growth regulators.

Initially these studies were focused largely on how one or more factors contributed to survival at specific stages of development, coupled with observations on the degree to which molting and the duration of the intermolt stage was modified. Acknowledging that the understanding of lethal levels left much to be desired, we have more recently identified a technique using regeneration of larval appendages that gives an extremely sensitive "bioassay" from which to determine sublethal levels. These studies, in addition to giving us indications of sublethal levels and their effects, also provide for detailed observations on the mode of action of specific compounds.

One can design experiments to provide for multifactorial analysis and identification of synergism involving a variety of natural and anthropogenic factors, the degree to which the concept of toxicity should be extended to include mixtures of anthropogenic pollutants at sublethal levels, and the usefulness of employing indices of sublethal effects of toxic compounds introduced into the marine and estuarine environments.

Four species of marine organisms found commonly along the Gulf and Atlantic coasts of the United States provide advantages for study: Callinectes sapidus, Rhithropanopeus harrisii, Balanus amphitrite, and Limulus polyphemus. In each case, the adult organisms occupy different habitats that may be subject to the impact of pollutants, e.g., intertidal surfaces for barnacles, the benthos for Limulus, and the Spartina marsh for Rhithropanopeus. Similarly, each of these species has larvae that are

subject to those pollutants that might be found in the water column. Although Callinectes sapidus is recognized as a major commercial species, the larval stages are extremely difficult to raise under controlled conditions in the laboratory. Consistency in survival is low when dealing with eggs and larvae from different females. A surrogate species, Rhithropanopeus harrisii, has proven to be extremely useful because of its consistency, reliability, wide geographical distribution, and the body of information accumulated on it over the past two decades. Thus, these four species enable one to address the issues of pollutants in the water column, in the benthos, and within the tidal marshes. Because of the extreme sensitivity of the larval stages, as compared to the adult stage, they offer a broad spectrum of sensitivity for comparative studies.

METHODS FOR TESTING THE TOXICITY OF OIL TO MARINE ORGANISMS

Jerry M. Neff

A variety of laboratory and field methods have been used to determine the effects of oil on marine organisms. Laboratory investigations usually are designed to gain a better understanding of the toxicity and other biological effects of crude and refined petroleum products on marine organisms. This information may be used to predict the impacts of spills or other short- or long-term inputs of petroleum on the marine environment. Results of laboratory studies may also be useful in designing better, less damaging approaches to combatting oil spills and cleaning up after them when they occur.

Field investigations are used to validate the results of laboratory studies and to assess how impacts measured in the laboratory at the level of individual or small groups of organisms are expressed at the population and community levels. Field studies of chronic inputs of petroleum to the ocean (e.g., produced water discharges and refinery effluents) or of accidental spill events are performed to assess the nature and extent of the damage to different habitats, to determine the effectiveness of different types of spill countermeasures, and to predict and document the rate of recovery of damaged ecosystems following a spill. In cases where a CERCLA Type B damage assessment is required, the field investigation is designed to assess financial damages to be assessed against the company responsible for the spill.

Laboratory Test Methods

Exposure Methods

Petroleum is an extremely complex and variable mixture of thousands of organic and inorganic compounds. Hydrocarbons are the major ingredient of crude oil, usually representing more than 75% of its volume. The remainder consists primarily of various sulfur-, nitrogen-, and oxygen-containing organic compounds. The relative proportions of hydrocarbons and other organic components in oil differ substantially in different crude oils and even in oil from different levels in one well.

Due to this compositional complexity and variability, crude oils vary substantially in solubility, dispersibility, and persistence in sea water. In addition, weathering of spilled oil in the ocean will markedly alter its aqueous solubility and dispersibility, as well as the composition of the fraction of the oil that is accommodated in the water column.

The compositional complexity and physical behavior of petroleum make it very difficult to design laboratory studies of its effects on marine animals. Ideally, the laboratory exposures should simulate as closely as

possible the type of exposure an organism might encounter in its natural environment. Several approaches have been used to introduce oil into exposure tanks:

- As a water-soluble fraction (WSF)
- As an oil-in-water dispersion (OWD)
- As a surface slick
- Dispersed in the water column with chemical dispersant
- In oil-contaminated food
- In oil-contaminated sediments.

Each type of exposure has advantages and disadvantages in predicting the effects of oil in the marine environment (Neff and Anderson 1981; Capuzzo 1987).

Petroleum is highly hydrophobic, and most components of oil are, at best, only sparingly soluble in sea water. When oil and sea water are mixed, small amounts of the lower molecular weight hydrocarbons, particularly aromatic hydrocarbons, go into solution in the water. Depending on the mixing energy, variable amounts of large and small oil droplets are dispersed in the aqueous phase. The large droplets return quickly to the surface oil slick. Droplets smaller than about 0.1 mm may remain in the water column indefinitely.

The WSF, sometimes called the water-accommodated fraction, is thought to resemble the type of exposure marine organisms might encounter in the water column down current from a spill or a point source of chronic oil input, such as a produced water or tanker ballast water discharge. The WSF is prepared by gently stirring set ratios of oil in water (usually one part oil in nine parts sea water) for a set period of time (usually about 1 day)(Anderson et al. 1974). Stirring is followed by an equilibration period to allow separation of particulate and water-accommodated (soluble and colloidal fractions) oil. The aqueous phase is then diluted to the desired concentrations for the exposures. If preparation conditions are carefully standardized, a reproducible WSF can be produced. Several systems have been produced to continuously prepare the WSF, dilute it to different concentrations, and introduce it into exposure aquaria (e.g., Benville et al. 1981; Ostgaard and Jensen 1983).

The oil-in-water dispersion is prepared by very energetic mixing of set ratios of oil and water for short periods of time (Anderson et al. 1974). The energetic mixing generates a high concentration of microdroplets of oil in the water column, as might occur during an oil spill at sea under stormy conditions. After a short settling period to allow the larger oil droplets to return to the surface, the aqueous phase, which constitutes the OWD, is recovered and diluted to different concentrations for introduction into exposure aquaria. Automated systems for continuously producing the OWD and dosing it into aquaria have been developed (e.g., Vanderhorst et al. 1977).

The composition of the WSF is quite different from that of the OWD. The WSF is enriched in the low-molecular-weight, more soluble hydrocarbons (particularly the toxic, low-molecular-weight, one- and two-ring aromatic hydrocarbons), compared with the oil from which it was prepared. The composition of the OWD, on the other hand, closely resembles that of the oil from which it was prepared. Because most of the petroleum hydrocarbons in the OWD are present in the form of oil droplets, the OWD is not very stable in sea water, and much of the oil returns quickly to the surface slick.

Oil slicks are prepared by gently layering oil on the surface of sea water in exposure aquaria with little or no mixing. They do not simulate the type of exposure expected in the marine environment and therefore have not been used frequently.

The toxic effects of ingestion of oil-contaminated food are evaluated by incorporating oil into prepared diets such as pelleted feeds, by exposing living food organisms to oil-water mixtures, or by feeding contaminated detritus to detritus-feeding animals. Such experiments can be used to evaluate the physiological effects of oil ingestion, the transfer of petroleum hydrocarbons and hydrocarbon metabolites through marine food chains, or the potential for hydrocarbon biomagnification in marine food webs.

Most hydrocarbons, because of their hydrophobic nature, rapidly become adsorbed to suspended particles in the water column and settle with them to the bottom. The oil associated with marine sediments is quite persistent and may continue to cause damage to marine ecosystems for years after a spill. Therefore, test systems have been designed to evaluate the effects of oil-contaminated sediments on marine organisms.

Artificially contaminated sediments or sediments from the vicinity of an oil spill or site of a chronic point source of petroleum hydrocarbons are introduced into experimental aquaria, preferably supplied with a continuous supply of clean natural sea water. Experimental organisms are introduced into the aquaria and allowed to swim in the water over or burrow into the contaminated sediments. Mortality or sublethal biological responses of the test organisms are recorded at different times. Artificially oiled sediments may be prepared by introducing oil into the water column over the sediments and allowing the phases to mix for a period of time before replacing the contaminated sea water with clean sea water and introducing the test organisms. Alternatively, they may be prepared by energetic mixing of an oil/water/ sediment slurry. The three phases are allowed to separate, and the sediment phase is removed, rinsed with clean sea water, and introduced into test aquaria.

Biological Effects

These oil exposure media can be used to determine a variety of acute and chronic biological effects. The standard acute lethal toxicity test,

in which the end point is the median mortality at 96 hr (96-hr LC50), is useful for comparing the relative toxicities of different oils. It can be used as a Tier I screening test but is not useful for assessing the potential impacts of an oil spill on a particular species or ecosystem. There are few acute toxicity tests available for determining the toxicity of chemicals associated with sediments to benthic marine animals. One sediment bioassay protocol, utilizing the infaunal amphipod Rhepoxinius abronius, has been validated with a variety of chemical contaminants in sediments (Swartz et al. 1985). It may be useful for determining the toxicity of oil-contaminated sediments to benthic marine animals.

A variety of sublethal responses can also be measured. They should be selected carefully to reflect the most sensitive life processes or life stages of the organism and to represent responses that can be interpreted readily in terms of long-term impacts at the population and community levels. Examples of such responses are effects on reproduction and early growth and development.

Tier II tests might include a variety of early life stage tests and abbreviated chronic tests designed to determine effects of oil on early life stages and sensitive biological processes. The U.S. Environmental Protection Agency has developed a series of rapid chronic tests for the routine evaluation of the toxicity of complex effluents permitted by NPDES permits for discharge to the ocean (U.S. EPA 1988). These tests include an algal (Champia parvula) growth test, a mysid (Mysidopsis bahia) early life stage test, a sheepshead minnow (Cyprinodon variegatus) early life stage test, and a sea urchin (Arbacia punctulata) sperm cell test. The tests have not been evaluated extensively with petroleum, but they have been evaluated with a variety of other complex mixtures. Therefore, they should be useful for evaluating sublethal responses of representative sensitive marine organisms to oil.

Tier III tests might measure a variety of physiological and biochemical responses to oil. The focus of such studies should be to elucidate the mechanisms of oil-mediated pathology and to predict long-term effects at the population level (Capuzzo et al. 1988). Reproduction in fish and crustaceans is under complex endocrine control. Oil may affect reproduction in fish in several ways. These can be examined by, for example, evaluating ovarian growth in sexually mature female fish, investigating circulating titers of gonadotropins and sex steroids, and monitoring the production of vitellogenin and the viability of eggs (Thomas 1988). Responses found to be sensitive to oil exposure can be used in field assessments of oil spills.

Fish and many marine invertebrates are able to metabolize the aromatic hydrocarbons in oil and produce a variety of more polar metabolites. Some of the metabolites are more readily excreted, but some are highly reactive and covalently bind to tissue macromolecules, including DNA, causing a variety of pathological responses, including cancer (Neff 1979; Stegeman 1981). Laboratory studies of these processes are useful for determining

the mechanisms of oil-mediated enzyme induction (in fish) and the effects of natural environmental factors on it, the nature of the metabolites produced and their reactions in conjugating systems and with tissue macromolecules, and the mechanisms of the toxic responses to aromatic hydrocarbon metabolism. This information can then be used to develop monitoring tools for assessing the effects of oil in the marine environment.

Field and Mesocosm Studies

Field studies following an oil spill or in the vicinity of a chronic input of petroleum hydrocarbons can use many of the biological responses characterized in laboratory studies as indices of oil-mediated damage (Spies 1987). A variety of physiological and biochemical responses have been recommended as indices of pollutant stress in marine animals (Neff 1985; Capuzzo et al. 1988). Some, such as induction of the cytochrome P-450 mixed-function oxygenase system in fish liver (Payne et al. 1987) and accumulation of aromatic hydrocarbon metabolites in fish liver and gallbladder (Krahn et al. 1982), are fairly specific indices of previous exposure to petroleum hydrocarbons. However, these indices are indicative of an adaptive response to pollution and are not in themselves an indication of current or impending pathology.

The marine ecosystems experiencing the most damage and requiring the longest period for recovery from an oil spill are benthic environments, salt marshes, coral reefs, and mangroves. Assessments of the impacts on benthic environments should focus on alterations of infaunal community structure and the processes of ecosystem recovery following the spill event (e.g., Glemarec and Hussenot 1982). Studies of effects on commercially valuable populations of demersal fish and crustaceans that depend on the benthic infauna may also be useful (e.g., Neff and Haensly 1982).

Oil spills are not always available for study. Therefore, marine mesocosms are useful for studying various aspects of oil spill impacts at the community level. Settlement of benthic fauna can be studied in flow-through sea water aquaria containing oiled substrates (Tagatz and Deans 1983). Alternatively, various types of shallow water marine ecosystems can be simulated in mesocosm tanks. Spills of various types of oil can be simulated and community responses measured (e.g., Santschi 1985; Linden et al. 1987). Another alternative is to perform a controlled oil spill in a marine ecosystem. This approach tends to be very expensive and the conditions of exposure are hard to control, as was the case in the BIOS experimental oil spill on the north shore of Baffin Island, Canada (Sergy and Blackall 1987).

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TOXICITY OF DISPERSANTS AND DISPERSED OIL FOR MARINE ORGANISMS

Jack W. Anderson

Introduction

Because there are several aspects of this topic, which have been the subjects of four separate papers published previously with several coauthors, this expanded abstract is a collection of these abstracts.

Diluting Concentration Method for Testing Dispersed Oil

The first study developed a diluting concentration method for testing the acute toxicity of chemically dispersed oil (Anderson 1984). Using a gradient pump, dispersed oil was delivered at a predetermined rate to produce diluting concentrations reaching zero in either 8 or 24 hr. Both dispersants, t9527 (E) and BP1100WD (B), and the two test oils (Prudhoe Bay crude--PBC--and light Arabian crude) were used in this system to produce acute toxicity for shrimp (Pandalus danae) and fish (Ammodytes hexapterus). The toxicity index (in ppm-days) was used to compare different exposure conditions and species.

In earlier tests on shrimp with water-soluble fractions (WSF) of PBC and in those studies with dispersed PBC, shrimp were shown to be significantly more sensitive ($P < 0.01$) in the spring and summer than during fall or winter. Dispersions produced by the two chemicals (E and B) applied at a 1:20 ratio with PBC did not result in significantly ($P > 0.05$) different toxicity indices in summer, but PBC+E was more toxic (lower index, $P < 0.01$) than PBC+B in the winter. Comparison of PBC+E and light Arabian crude+E in summer demonstrated that the Arabian oil was more toxic ($P < 0.01$).

Toxicity indices for shrimp were used to compare spring and summer exposures of water-soluble PBC at constant concentrations with those of dispersed PBC at both constant and diluting concentrations. All dispersed oil conditions were shown to be somewhat less toxic for shrimp (in ppm-days of total hydrocarbons) than exposures to WSF. There was very close agreement between the toxicity indices of shrimp exposed to constant concentrations of dispersed PBC and those exposed to diluting concentrations (8- and 24-hr tests).

These findings lead to the conclusion that it is not essential to conduct diluting exposures to assess the sensitivity of an organism to a specific oil or dispersant. It is, however, necessary to consider the natural dilution of dispersed oil in sea water under realistic conditions. By utilizing appropriate dilutions in the calculation of an estimated total exposure (time x concentration), it is possible to determine the likelihood of significant impact on species similar in sensitivity to Pandalus.

The sand lance (Ammodytes) was found to be more resistant to dispersed oil than shrimp and mortality occurred 3 to 5 days after the 24-hr exposures were terminated. Latent mortality was considered the result of the total exposure (ppm-days) provided during a 24-hr period. The values were about 3 times greater than toxicity indices for shrimp during the same summer period (16 vs. 5 ppm-days). While not directly comparable to shrimp data, the incorporation of latent mortality of sand lance data into the calculation of a toxicity index may be a very valuable and realistic approach.

Because the stability of a chemical dispersion and perhaps the potential for bioaccumulation and toxicity may be a function of the sizes of oil droplets produced, it was necessary to characterize droplets produced by the two products. Using PBC oil, the oil droplet size distributions were:

Product E = 1.2 to 3.8 microns diameter
Product B = 2.4 to 5.2 microns diameter

The major portion of the oil volume in dispersant E mixtures was in 2-micron particles, while most oil dispersed with product B was in 4-micron droplets. As noted above, the toxicity of the two dispersions was very similar except in winter. We do not know if the lower toxicity of PBC+B in winter was the result of differences in the behavior of the two dispersants at colder temperature (8-9°C) or some other factor associated with shrimp sensitivity.

Shrimp (Pandalus danae) exposed to PBC crude oil dispersed with both products in flowing systems accumulated approximately the same concentrations (10-13 ppm) of naphthalenes and phenanthrenes. These were the same compounds as those accumulated from oil WSF's. Saturates that are dominant in the oil droplets were apparently not accumulated by either ingestion or adsorption on the exoskeleton. It can be concluded that droplet sizes within the range examined may reflect the stability of the dispersion, but do not have an effect on the rate or extent of hydrocarbon accumulation by shrimp.

Reports in the literature indicate that water column organisms such as fish and shrimp might be exposed to as high as 0.5 ppm soluble components for 1 day (0.5 ppm-days) or 1 ppm-day of dispersed oil. These would not appear to be capable of producing significant toxicity for the species studied unless longer exposure times resulted.

Testing with Constant of Decreasing Concentrations of Oil

The next study involved toxicity testing with constant or decreasing concentrations of chemically dispersed oil (Anderson et al. 1984). An exposure system and method of quantitating toxicity were developed to provide an estimate of the effects of dispersed oil on marine organisms under a variety of exposure conditions. The results of constant con-

centration exposures (4 hr or days) can be compared to those of diluting exposures (decreasing to zero in 8 or 24 hr) on a basis of the "toxicity index." This index is equal to the total exposure when time in hours or days is multiplied by the concentration at each hour (ppm-hr or ppm-days).

Tests were conducted with shrimp (Pandalus danae), two oils (PBC and a light Arabian crude), and two dispersants. There was a seasonal pattern to the tolerance of the shrimp. Tests in the colder months (fall/winter) produced toxicity indices approximately three times higher than summer/spring values. Testing shrimp with PBC crude oil and a chemical dispersant during the fall/winter season, we found constant and 24-hr dilution exposures produced toxicity indices of 11 (+ 1.1 standard error) and 10 (+ 0.6 standard error) ppm-days, respectively. During the fall/winter season (greatest tolerance), tests with PBC and two different chemical dispersants produced toxicity indices for P. danae of 10 (+ 0.6) and 12 (+ 1.1) ppm-days. During tests in summer, there was also little difference observed when the toxicity of the light Arabian oil was compared to that of PBC (2.3 and 3.4 ppm-days, respectively). The usefulness of these methods is that, in addition to the comparisons already noted, it is possible to predict the outcome of dispersant applications under varying environmental conditions.

Toxicity of Dispersed and Undispersed PBC Oil

Many previous studies of oil toxicity used high oil concentrations and WSFs. The aim of this study was to approximate field conditions, in which weathering and chemical dispersions reduce the volatile fractions of spilled crude oil (Anderson et al. 1987). The objective was to determine the extent of toxicity reduction produced by decreased concentrations of monoaromatics and diaromatics.

The study measured the relative toxicity of fresh PBC oil and two distillation fractions (Stage I and Stage II) and their chemical dispersions to the shrimp Pandalus danae and the fish Ammodytes hexapterus (sand lance). The hydrocarbon composition of the three oils, the WSF of the oils, and the chemical dispersions were measured.

Distillation of fresh PBC oil produced a Stage I oil containing very low amounts of monoaromatics (benzene and alkylbenzenes) but with the diaromatics relatively unchanged. Further distillation produced a Stage II oil that contained only higher molecular-weight aromatics of three rings (phenanthrenes) and greater. Saturated hydrocarbons with corresponding boiling points also were removed.

Bioassays on shrimp with dispersed oils showed that the removal of monoaromatics (Stage I) reduced toxicity about sevenfold. The WSF of Stage I oil and both the WSF and dispersions of Stage II oil were not toxic to shrimp. Toxicity from fresh PBC oil WSF and dispersions was likely the result of the combination of monoaromatic and diaromatic compounds.

Sand lance (Ammodytes hexapterus) mortality did not correlate with the aromatic content of the oils, but appeared to be affected by dispersed oil droplets of all three oils to about the same degree. The fish were more resistant to dispersed oil than the shrimp (higher toxicity index). However, when latent mortality is considered, the data show that the fish may be more sensitive than shrimp to dispersed oil.

Evaluation of Relative Merits of Chemical Dispersants

The last study was a laboratory evaluation of chemical dispersants for use on oil spills at sea (Anderson et al. 1985). Data on the toxicity and effectiveness of 14 chemical dispersants were combined in a straightforward equation to provide an overall assessment of the relative merits of the oil spill chemicals. When a decision is made by regional response authorities to mitigate the damage of spilled oil to the shoreline, these findings should aid in the selection of an effective low-toxicity product. The products were evaluated by using standard toxicity tests with a mysid shrimp (Mysidopsis bahia) and a standard effectiveness test using the Mackay-Nadeau-Steelman (MNS) apparatus. The ratios of dispersant to oil required to maintain 90% dispersions of oil in sea water (15°C and 30°/oo) with a standard mixing energy (1.0 in of water pressure) of air flow were derived for each chemical by using PBC oil. Toxicity tests with M. bahia were conducted at 25°C and 25°/oo by using freshly hatched juveniles (15 per concentration x 5 concentrations) in small dishes in an incubator.

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CURRENT TESTING REQUIREMENTS

Michael L. Flaherty

A new Subpart J (final draft) to the National Contingency Plan (NCP) will replace the current Subpart H to the NCP. The main change is an official listing of the new category of miscellaneous spill control agents and the data requirements for this category, proposed in Subsection 300.915.

The language in Subsection 300.910 has been modified slightly to emphasize the importance of obtaining concurrence for the use of dispersants and other chemicals from the appropriate Regional Response Team (RRT), State representatives, and DOC/DOI contingency planning efforts. RRT's are further encouraged to make "pre-approval determinations" with respect to the use of certain dispersants or chemical agents in their area of geographical responsibility. Sinking agents are still prohibited for application to oil discharges.

The U.S. Environmental Protection Agency (EPA) has a step-by-step procedure for getting a new product tested and listed on the NCP Product Schedule. A description of this procedure is available from John Cunningham, Office of Solid Waste and Emergency Response, U.S. EPA, Washington, D.C., 20460. The full, detailed product notebook is also available from EPA. This contains special information on each listed product, i.e., use, toxicity data, effectiveness data, safety precautions, storage limitations, shelf life, application recommendations, and other important information.

An outline of the ASTM Dispersant Use Guidelines for Salt Water and the pending Guidelines for Freshwater, as well as the Computerized Decision Tree, are also available from EPA at the above address.

CONSIDERATIONS IN SELECTING DISPERSANTS FOR TESTING

John P. Fraser

The overall objective of the proposed study by MMS on oil and dispersant toxicity is to provide guidance to regulatory authorities in determining whether it is appropriate to use dispersants as one means of mitigating an oil spill. This guidance should consider primarily those dispersants that are realistically available for use. Thus, we need not consider surfactant chemicals that are not presently in use. Our job of choosing which dispersants to consider for inclusion in the test program is simplified by the fact that only a limited number of chemicals are currently used in dispersant formulations. These include:

- Anionic surfactants such as
  - Sodium dioctyl sulfosuccinate
  - Sodium ditridecyl sulfosuccinate
- Nonionic surfactants such as
  - Sorbitan monooleate
  - Ethoxylated sorbitan monooleate
  - Polyethylene glycol esters of oleic acid
- Solvents such as
  - Water
  - Kerosene
  - Monobutyl glycol ether.

A large majority of the dispersants on the EPA Product Schedule contain the above ingredients in varying proportions, and most can be associated into about three or four general groups. Thus, it appears that dispersants for the proposed test program can be chosen from a limited number of commercially available dispersants, rather than having to test all dispersants that are marketed. Information on dispersant compositions is available to a limited extent from the manufacturers and from the patent literature.

Further, consideration in selecting dispersants for testing should be given to those that are available in fairly large quantities. The chemical dispersion of an oil spill will most likely be appropriate when there is a large spill. This means that the dispersants will need to be available in large quantities. Therefore, the primary emphasis in testing should preferably be on those materials of which there are large stocks, i.e., on the order of at least 25 drums or more.

With the above considerations in mind, the following criteria and variables might be included in a broad program to study the toxicity of dispersants:

- Dispersant must be on the EPA Product Schedule
- Dispersant properties to include
  - Water based
  - Hydrocarbon based
- Major chemical systems (active ingredients) to include
  - Polyethylene glycol esters of C18 unsaturated fatty acids
  - Ethoxylated/propoxylated fatty alcohol
  - Mixtures of nonionic and anionic surfactants
  - Ethoxylated octylphenol
- Dispersant to be designed for
  - Aerial application
  - Boat spray application
- Effects of the oil type to include
  - Low, medium, or high viscosity
  - High- or low-sulfur content (or other oil compositional variable).

In a study of the toxicity of oils and dispersed oils, a question that should be addressed by toxicologists is whether they can predict potential toxicological concerns from composition, either of the dispersant or of the oil.

METHODS FOR DETERMINING THE TOXICITY OF OIL AND DISPERSANTS TO CORALS

Eric N. Powell

Laboratory experiments on acute and chronic toxicity are handicapped in two important ways. First, some important effects may only become obvious subsequent to the normally short exposure periods, and some pollutants induce effects with long-term recovery periods. Neither can be adequately documented or investigated except under field conditions. In corals and oysters, where important symbiotic or pathogenic associations can be facilitated or inhibited by even brief changes in animal health, even short-term acute exposures can exert a substantial long-term impact on the population by changing growth and reproductive capacity. In oysters, for example, one disease organism, Perkinsus marinus, accounts for about 50% of the reproductive capacity in Texas populations. Chronic hydrocarbon pollution facilitates this disease, as do many short-term acute stresses. In corals, zooxanthellae loss occurs during or after exposure to many stresses. In both cases, delayed responses and extended recovery periods may be associated with acute exposures of hours to days. In both oysters and corals, these recovery periods last at least 6 months. Consequently, successful toxicity testing may require extended population monitoring, which cannot be conducted except in the field.

The second handicap is that laboratory experiments of any kind impose a certain degree of stress on the experimental organism. In toxicity tests, where one ultimate goal should be the elucidation of potential effects on field populations, laboratory animals typically do not adequately mimic animals under field conditions. The reduction or elimination of the experimental stress response should be one aspect of the development of toxicity protocols. Some animals are more sensitive than others to experimental stress--for example, corals. However, even less sensitive animals, such as oysters, can be considerably affected.

Field Experimental Approaches

The use of field-experimental approaches that might mitigate or eliminate one or both of these problems has been a concern. In research on corals, a field protocol has been developed that reduced the experimental stress response and allowed short-term monitoring of recovery after acute exposure. The same protocol has been used successfully in oysters for experimental periods of up to 2 months. The protocol was based on the use of a STACH (short-term aerated coral habitat) unit consisting of a series of plexiglass domes and bases with inflow and outflow ports on a PVC holding frame erected in the animal's habitat for extended periods. The experimental protocol is described in detail in Kendall and Powell (1988).

In experiments with Acropora cervicornis, coral branches about 10 to 12 cm long were inserted into egg-crate holding trays suspended by plexiglass rods above the base of each dome. Suspending the holding trays eliminated any buildup around the corals of materials that settled out of solution during the exposure period. Each dome was held in place by bolts and nuts running through a flange around the dome's lower edge. The seal between the dome and base was a silicone-coated gasket permanently attached to the dome's flange.

During exposure periods, flow-through conditions were obtained by gravity feed from a set of holding tanks elevated about 3 m above the water line (6 m above the domes). To monitor the calcification rate, toward the end of a 1- to 4-day exposure period, the flow-through system was stopped, Ca^{45} added, and mixing and aeration within the dome achieved by slow steady streams of air injected via the base ports from a set of twin 80-ft^3 scuba tanks. To monitor recovery after exposure, the solid dome was replaced with one that had a series of holes, which allowed free movement of sea water but excluded large organisms.

Although a certain degree of experimental stress remained in these experiments, produced by the relocation of the experimental organisms, the experimental stress response was reduced considerably in both corals and oysters. Consequently, the results of toxicity assays were more nearly what might be expected in actual field populations. A consistent response to drilling muds was observed in corals under these conditions. Both growth rates and biochemical composition were altered. During recovery, two separate responses were observed:

- Delayed effects of exposure occurred during recovery in some tests, indicating that exposure time and changes in the measured parameter need not occur simultaneously. Obviously, certain serious effects--zooxanthellae loss, for example--can have a latent period prior to expression.

- Recovery did not proceed at the same rate in all measured parameters. Growth rate recovered much more rapidly than certain indices of biochemical composition.

Analogous experiments on oysters yielded similar results. Growth rate typically recovered more rapidly than most other parameters, and delayed responses frequently occurred. In the latter case, pathogens rather than symbionts mediated the delayed response. Most important, had either series of experiments, on corals or oysters, been ended after the exposure period, an altogether inadequate result would have been obtained.

Consequently, the choice of the parameters to monitor health must include those that might be expressed immediately and those that might be delayed. In both cases, monitoring only growth rate would have indicated complete recovery soon after exposure, whereas in both organisms, delayed responses (weeks to months) eventually would have resulted in substantial

mortality in both populations. Furthermore, in both cases, the effects included substantial impacts on symbionts or pathogens. Although symbionts are not present in most species, many well-studied species are known to have substantial prevalences of disease-causing and parasitic organisms, which may, as in the case of oysters, exert a primary effect on population health. Hence, the use of populations of diseased rather than uninfected organisms may substantially affect the results of toxicity evaluation in the field.

Long-term Experiments

Longer term experiments and a further reduction of experimental stress require more or less continuous monitoring of the field population. Among the problems associated with such experiments are (1) the inadequacy of frequently used normalizing parameters in experiments of as short as 2 days (e.g., in corals, protein content changes significantly within 1 day, and in oysters, total weight may change considerably upon spawning and during disease intensification), (2) the need to establish and monitor equivalency between control and experimental populations (e.g., flow regime and food availability), (3) the need to monitor some selected stress responses nondestructively, and (4) the need to choose parameters for monitoring that manifest themselves in the primary effects of decreased net productivity and reproductive capacity (lifetime fecundity or the number of daughters that live to reproduce).

In longer term experiments in field populations, the microflow regime around animals must be well known. Variations in food availability in suspension-feeding populations, for example, are well described and may produce significant variations in health between populations. A micro-current meter has been developed that is capable of measuring current speed within 1 cm of animals such as corals and oysters at current speeds of 0 to 50 cm sec^{-1} (Murphy et al. 1988). The array consists of up to 20 thermistors connected to an underwater data logger so that continual long-term measurements can be made. Each meter uses a simple bridge with a heated thermistor and a constant supply voltage to determine the power dissipated across the thermistor for a given flow speed and ambient temperature. Temperature compensation is obtained mathematically through the calculation of a temperature-independent power dissipation coefficient (K)

$$K = P_b(T_b - T_a)$$

where

$$P_b = V^2_t / R_t$$

(P_b is the power supplied to the sensor bead; T_b, the bead temperature; T_a, the ambient temperature; V_t, the voltage drop across the thermistor; R_t, the thermistor resistance), which can be directly related to current speed at any ambient temperature. Adequate temperature compensation has been obtained over a 25°C range in temperature.

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METHODS FOR DETERMINING THE TOXICITY OF OIL AND DISPERSANTS TO SEA TURTLES

Peter L. Lutz

Introduction

Although direct lethal effects of oil pollution on sea turtles have been demonstrated (e.g., hatchlings apparently starved to death as a result of their beaks and esophagus being blocked with tar balls), sublethal encounters are likely to be much more common and, if harmful, will have a considerably greater deleterious impact on sea turtle populations. Oil pollution, therefore, poses a potential threat to sea turtles that must be taken seriously.

Sea turtles are vulnerable to the harmful effects of oil pollution in many ways. By continuously resurfacing through an oil slick, adult sea turtles experience prolonged physical contact with the floating oil. Ocean convergent zones and drift lines, where early juveniles are found, also are regions where floating tar accumulates. The sea turtle's mode of respiration, a rapid inspiration of surface layer air before diving, introduces petroleum vapor into the lungs; eating contaminated food or tar balls brings petroleum materials into the intestine. On nesting beaches, oil deposits can interfere with normal development of the embryos in the egg clutch as well as present a lethal hazard to newly emerged hatchlings.

It has been found that despite the high tolerance of marine turtles to physical damage, sea turtles are surprisingly sensitive to oil. Experiments on the physiological and clinicopathological effects of oil carried out on loggerhead sea turtles (approximately 15 to 18 months old) showed that the major physiological systems in these turtles were adversely affected by both chronic (0.05 cm oil layer for 96 hr) and acute (0.5 cm oil layer for 48 hr) exposures to weathered South Louisiana crude oil. The skin of exposed turtles, particularly the soft pliable areas of the neck, showed consistent gross histologic changes with a proliferation of acute inflammatory cells, cell death, and abnormal cell development (dysplasia). Statistically significant changes occurred in some respiratory parameters of acutely oiled turtles, in particular, breathing rates and oxygen uptake were affected. Oil was found in the feces, possibly interfering with normal gut function. Internal effects such as changes in blood chemistry, a substantial increase in white blood cell number, and the cessation of salt gland function all indicated that some toxic oil products were being absorbed.

Although all of these conditions were reversed after the turtles were removed from the oil and cleaned up, the question remained as to how the turtles' viability would have been diminished in the wild after an oil exposure.

The potentially harmful effects of an oil spill on sea turtles must clearly be taken seriously (see Figure 3), and any strategy to prevent turtles from encountering the oil must be regarded as a preferred front-line defense. One attractive approach is to use chemical dispersants to break up oil slicks at sea or to clean the beach; chemical dispersants also could be delivered parenterally to oiled turtles to accelerate the passage of hydrocarbons out of the digestive system. However, although more benign dispersants are becoming available, some dispersants have proved as toxic to marine life as spilled crude oil. In addition, on the beach, dispersants may actually increase oil penetration. Unfortunately, nothing is known about the effect of dispersants on sea turtles, but in view of the sea turtle's susceptibility to oil, it is essential that only those dispersants proved to be relatively harmless to sea turtles are used with an approved protocol.

Methods

Studies on sea turtles must take fully into account that all species are at risk and have either threatened or endangered species status. Investigation must be confined to sublethal effects that are fully reversible once the treatment is halted. This restricts the scope of toxicity studies that can be carried out, especially the study of internal effects, and investigations of natural defense mechanisms, such as the liver mixed-function oxygenase system, would be very difficult. Nevertheless, physiological and clinical studies are most valuable because they can be used to detect harmful changes and suggest therapies.

Based on our studies and on a synthesis of the effects of oil and dispersants on sea birds and marine mammals, we suggest that studies on the effects of oil and dispersant exposure concentrate on the primary routes of impact. More subtle long-term effects, such as a decrease in reproductive fitness, which are nonetheless serious, are not considered here.

Protocol

For general studies, sea turtles should be held individually for 3 to 5 days in tanks that have a minimal surface layer of oil (0.05 cm). To study skin lesions in particular, sections of turtle skin should be subject to oil application for periods of time up to 1 month. Similar protocols should be performed with dispersant and oil/dispersant mixes. All studies must have proper controls with untreated animals.

1. Surface contact--Direct visual observation for inflamed and irritated regions in the skin, eyes, nares, and buccal region should be made. Histopathologic effects should be evaluated via serial incisional tissue biopsies, paying special attention to the occurrence and development of epidermal cellular dysplasia.

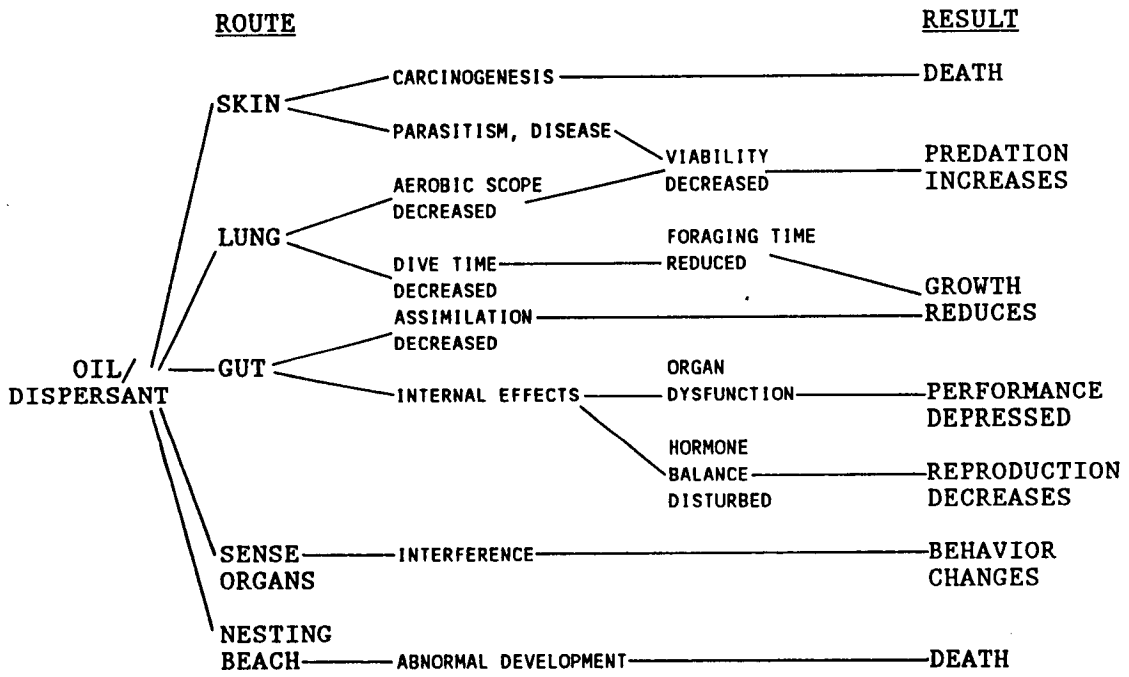

Figure 3. Conceptual model of potential effects of oil or dispersants on sea turtles.

2. Respiration--Mammals inhaling noxious substances are known to have decreased lung mucus transport and increased potential for pneumonitis. In the sea turtle, inspiration of petroleum vapor could cause an irritation of the pulmonary mucosa, and aspiration of dispersant could interfere with lung function through its surfactant effect. At the behavioral level, a decrease in respiratory efficiency could be seen as a change in the dive time, rate of breathing, and time spent at the surface, and an alteration in the ratio of total breathing time/time spent diving + time spent breathing. Diagnostic tests should measure lung tidal volume, pulmonary diffusing capacity, and the efficiency of lung oxygen extraction.

3. Ingestion--Swallowed oil or dispersant could interfere with normal gut function. Feces should be examined for the presence of oil and dispersant products and gastrointestinal hemorrhage identified by testing for occult blood. Previous studies suggest that changes in absorption efficiency and, in particular, the role of the gut in salt and water balance should be monitored.

4. Absorption--The transfer of toxic products across the gut wall could have widespread harmful effects compromising organ functions.

   (a) Hematology--Our earlier study showed that oil exposure resulted in changes in the numbers of circulating blood cells. It is important to measure red blood cell indices, hematocrit, red blood cell number, and cell size frequency to identify anemia and a reduced blood oxygen-carrying capacity. Total leukocyte count and a differential leukocyte count will give information on stress and the degree of evoked immunoresponses.

   (b) Blood chemistry--Changes in some blood constituents are useful indicators of metabolic disorders. The rate of recovery from hypoglycemia after exposure to oil has been used as an indicator of energy metabolism imbalance. An increase in plasma alanine transferase (ALT/SGPT) can indicate liver dysfunction, and increases in serum alkaline phosphatase (SAP) may indicate cell damage from a variety of tissues. Increased plasma cortisol has been used as a rapid and effective indicator of stress in many vertebrates.

(c) Organ dysfunction--As mentioned above, invasive studies on organ performance are precluded, and for the liver, for example, indirect blood enzyme changes will have to be relied on. The salt gland is an exception. The orbital salt gland is a key organ for salt and water balance in the sea turtle. It has been shown that the active salt gland receives a blood flow equivalent to that of the mammalian kidney and, as in marine birds, its activity appears to be compromised in oil-exposed turtles. Salt gland dysfunction can be tested by giving a saline challenge to the sea turtle and monitoring the resultant salt gland response.

5. Development--Oil can adversely affect the egg clutch by interfering with gas transport through the surrounding sand, causing a reduction in oxygen transport and a buildup of CO_2. Changes in oxygen and CO_2 levels should be monitored in experimental nests, the percentage hatched recorded, and the condition of the embryos from unhatched eggs noted. Through contact with the egg shell and by crossing the egg shell wall, oil and dispersant can have direct effects on development. In a properly constructed study, these effects can be tested for by applying small amounts of oil, dispersant, and oil/dispersant to the shells of a sample of turtle eggs and measuring the mortality and viability of the hatchlings as well as recording any malformations.

A PROPOSED MICROCOSM SYSTEM FOR EVALUATING THE IMPACTS OF CRUDE OIL AND DISPERSANTS ON SEAGRASS COMMUNITIES

Thomas W. Duke

Introduction

Seagrass microcosms have been used successfully to measure the impact of drilling fluids (mixtures of chemicals and clay) and tributyltin on experimental seagrass communities (Morton et al. 1986; J.R. Kelly, personal communication[2]). A modification of the system has also been proposed for evaluating the effects of microbiological pest control agents (EPA internal document). The purpose of this paper is to propose the use of the microcosms or modifications of them for testing the impact of oil and dispersants on seagrass communities.

Seagrasses play an important ecological role in the dynamics of the coastal zone. The roots bind the sediment and resist erosion; although few animals feed directly on the grass, many feed on associated epiphytes and detritus; and grasses produce from 114 to 228 g/C/m/yr, a relatively high production rate. The health of seagrass beds may be indicative of the health of the coastal zones in which they occur. In fact, seagrasses and their attendant communities may be considered "sensitive biological areas" from a regulatory perspective.

The use of microcosms to study the effects of oil, dispersed oil, and dispersants is not new. Hatcher and Larkum in 1982 used microcosms of sublittoral seagrass meadow from Botany Bay to evaluate the effects of a crude oil and dispersant. Thorhaug and Marcus (1987) and Thorhaug et al. (1986) studied the impacts of oil, dispersed oil, and dispersants on subtropical and tropical seagrasses in large laboratory tanks.

The proposed test method involves construction and utilization of seagrass microcosms in which cores of seagrass are removed from the environment, placed in test cylinders, and exposed to pollutants in a flowing water system. At a specified time, the seagrass communities are harvested, and various measurements are made.

This test procedure originally was developed to evaluate the effects of complex mixtures of chemicals on a rooted aquatic plant (seagrass) community (Morton et al. 1986). Crude oil and dispersants have not yet been tested in the system. Also, larger predators were excluded from the microcosms and this could result in some difficulties when extrapolating results to field situations. Nevertheless, with slight modifications the microcosm test system described by Morton et al. (1986) appears to be a

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[2] J.R. Kelly. Ecosystems Research Center, Cornell University, Ithaca, New York.

reasonable tool for determining the potential impact of these pollutants on the near-shore environment.

Microcosm and Support Facilities

The microcosm is designed to simulate desirable physical, chemical, and biological conditions existing in the natural environment where seagrass samples are taken. The support facilities (room, light fixtures, air system) ensure proper environment for testing. The experimental microcosms provide a community testing chamber that reflects conditions in the environment as much as possible.

The microcosms are plexiglass cylinders, 16.0 cm inside diameter and 50 cm high, that are attached to a plate on the bottom. The cores collected from the seagrass beds are slipped intact from the core cylinder into the test cylinder.

Samples of the seagrass community are taken by divers with plexiglass cylinders 14.0 cm inside diameter and 50 cm high. The cores penetrate 10 cm deep into the grass beds and upon withdrawal, contain sediment with attendant macroinvertebrates and grasses.

In the laboratory, microcosms can be operated with a continuous flow of sea water or by replenishing the water in the microcosms daily.

Flowing Water System

Unfiltered sea water of known quality is pumped into a settling reservoir above the test apparatus (see Morton et al. 1986). A 1-mm Nytex^{R} filter is fitted over the delivery standpipe in the settling reservoir to exclude larger material from reaching and clogging the delivery tubes to the microcosms. Sea water flows from the settling reservoir to a baffled 20-liter plexiglass primary head-box suspended over the microcosms. Twelve glass standpipes, six at each end of the head-box, are fitted with silicone stoppers and calibrated to deliver approximately 200 ml/min. A larger diameter glass overflow standpipe at each end of the primary head-box maintains the water at a constant level. From the 12 standpipes, the water enters glass mixing tubes that deliver the sea water to 12 secondary head-boxes. From each secondary head-box, sea water is delivered separately to 4 microcosms at a rate of about 40 ml/min. A glass cover is placed over the open end of the microcosm cylinder.

Static (Renewal) System

If flowing sea water is not available,it is possible to conduct the test in a static system. In this system, 8 liters of sea water are placed in the microcosms. The water is drained (syphoned) from each microcosm every 24 hours and replaced by fresh sea water of known salinity, pH, temperature, and dissolved oxygen.

Leaf litter or detritus bags can be added to each microcosm in order to determine the impact of the microbiological pest control agents on

decomposers. (The weight loss of bags in the treated microcosm is compared with that of control-weight loss is equated to degradation.) The bags are constructed of 2-mm mesh Nytex nylon netting and are 10-cm square. Glass rods anchor the bags in the microcosms just above the sediment.

Lighting for the test is provided by four 400-WATT multivapor lamps and four high-intensity fluorescent bulbs rated at 250 watts. Suspended 46.0 cm above the microcosms, the bulbs provide an average light energy of 217 $u_H E/m^2/S$. The lights are connected to a 24-hr timer (12 hr light and 12 hr dark).

Definitive Test

The flow chart for conducting the test is found in Table 9 (modification of Morton et al. 1986). There should be at least 12 replicates of exposed microcosm in each test. The test is conducted for 6 weeks with flowing sea water and for 2 weeks with replenished water.

Harvest and Treatment of Samples

The 48-microcosm test system is harvested randomly at the end of the test period. Fifteen seagrass leaves with associated epiphytes are taken from each treatment for analysis of biomass and chlorophyll content. The remaining contents are sieved to separate plant and sediment portions and placed into 0.5-mm nylon bags. All portions should be immersed in propylene phenoxetol in sea water to relax organisms. The plant materials are fixed in 5% formalin until the animals are picked from them and grass weights are determined. The sediment portions are placed in 10% formalin in sea water with 100 mg/liter rose bengal stain. The sediments are rinsed with freshwater, then sieved through 1.0- and 0.5-mm sieves. The sieved material is stored in 60% 2-propanol until the macroinvertebrates can be sorted and identified. All macrofauna are identified to the lowest taxonomic level possible.

The litter bags can be removed at intervals for measurements of rates of decomposition or can be removed at the end of the test. The bags are removed from the microcosms, rinsed in freshwater, dried at 100°C for 24 hr, weighed, and the weight change (loss) is calculated.

Measurements of the Thalassia leaf samples include chlorophyll a per g tissue or per cm^2 of blade area. The effects on epiphytic communities are observed by measuring differences (between "controls" and treatment) in epiphytic biomass (ash-free dry weight--AFDW) per cm^2 of Thalassia leaf as a biomass indicator, chlorophyll a per g AFDW epiphytic tissue as a community health indicator, and chlorophyll a per cm^2 leaf area as an indicator of photosynthetic potential. The details of these analyses to determine the health and productivity of the epiphytes can be found in Price et al. (1986).

A summary of selected criteria for effects on seagrass microcosms is presented in Table 10.

Table 9. Flow chart for microcosm test.

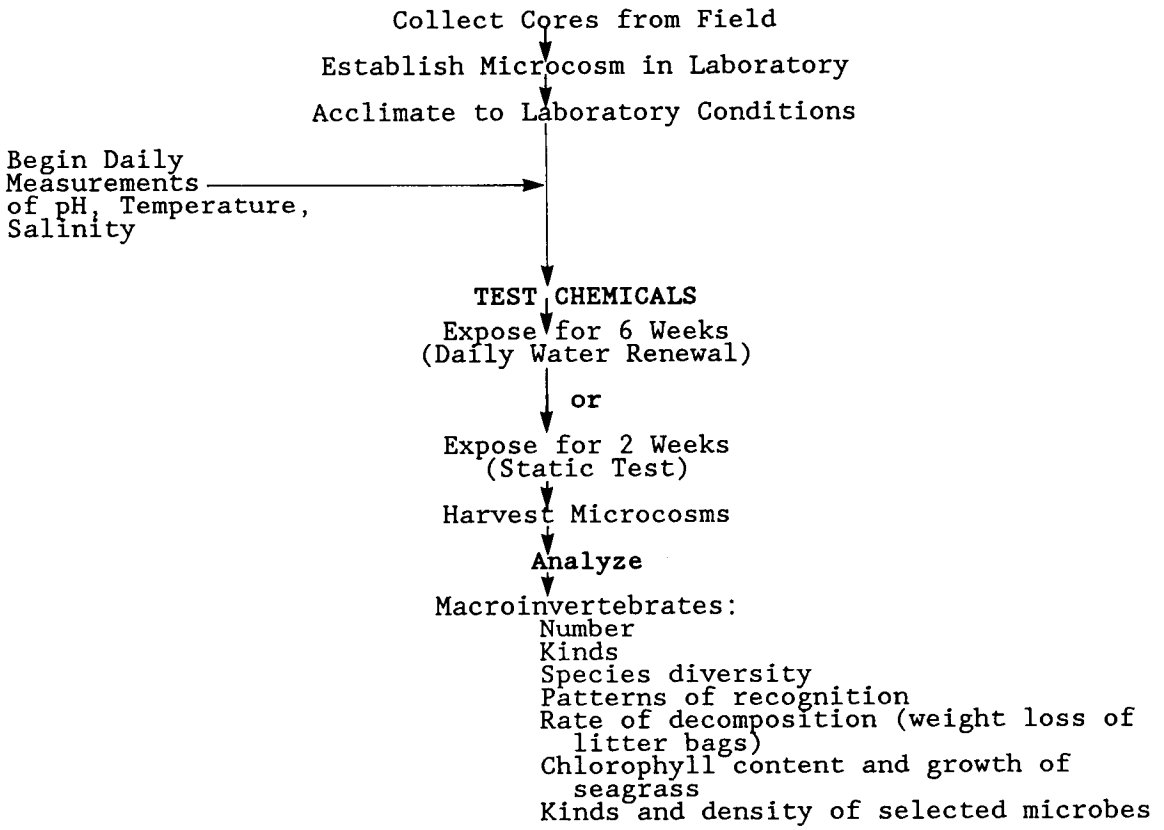

Table 10. Criteria for effects on seagrass microcosms.

COMPONENT	CRITERIA	METHOD	REFERENCE
Seagrass	Chlorophyll *a* per g blade area	Acetone extraction Spectrophotometer analysis	Strickland and Parsons (1972)
	Growth rate	Movement of aluminum tape during experiment	Modification of Zieman (1974)
Macroinvertebrates	Number of individuals per species Species richness Total number of individuals Species diversity Cluster or discriminate analysis or other methods of pattern recognition.	Microcosms are harvested at the end of desired time. Macroinvertebrates are sieved (1.0 and 5.0 mm), identified, and enumerated. Ten numerically dominant species can be analyzed.	Morton et al. (1986)

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METHODS FOR DETERMINING THE TOXICITY OF OIL AND DISPERSANTS TO MANGROVES

Howard J. Teas

Mangroves dominate the shorelines of low-energy tropical and subtropical marine waters of the world. Mangroves are valuable because they are primary photosynthetic producers that support a variety of animals, they provide habitat for many organisms, and they stabilize shorelines. Three mangrove species occur along the U.S. Gulf Coast: the red mangrove, Rhizophora mangle; the black mangrove, Avicennia germinans; and the white mangrove, Laguncularia racemosa. All three species grow along southern peninsular Florida. Rhizophora and Laguncularia are more cold sensitive than Avicennia: their northern ranges extend a short distance north of Tampa, Florida. The more cold-tolerant Avicennia is found as shrubby plants growing discontinuously from the Florida coast westward to Texas and Mexico. Cordgrass, Spartina, tends to replace mangrove in colder areas (Teas 1977).

A reason for special concern over mangroves, beyond their ecological role, is the time it takes for them to regrow after they are killed. It has been estimated that more than 20 years are required for new mangrove forests to develop (Odum and Johannes 1975). For reasons that are not clearly understood, mangrove forests that have been killed by clear-cutting or other means are often not replaced by mangroves (Macnae 1968).

The Gulf of Mexico mangrove species reproduce by seeds or propagules that float and are distributed by tidal waters. Avicennia and Laguncularia produce seeds that are ordinarily viable for a few weeks. Seeds of Laguncularia are approximately 1.5 cm long by 1 cm wide. The seeds of Avicennia are flat and lima bean shaped; they may be up to 2.5 by 3.5 cm and are viable for a longer period than are those of Laguncularia. Rhizophora mangroves are viviparous--that is, they produce seeds that germinate within the fruit on the parent tree. The propagules that develop from the germinated seeds are pencil shaped, about 1.5 to 2 cm in diameter and 15 to 25 cm long. When the propagules mature, they fall from the parent tree. Rhizophora propagules often survive for weeks or months.

Mangroves grow in areas of anaerobic soils and therefore must cope with the problem of root aeration (Scholander et al. 1955). They do this by means of snorkel-like connections between the subsurface roots and the atmosphere. Rhizophora trees have arching prop roots that contain large amounts of aerenchyma, which is air-conducting tissue that extends to the subsurface roots (Tomlinson 1986). The openings of the aerenchyma to the atmosphere are through small structures called lenticels, which are found on the prop roots. Lenticels consist of openings that are covered with loose packets of microscopic waxy particles. The spaces between the particles allow air to pass into the plant at low tide but the high surface tension of the waxy particles keeps water from entering at high tide. Avicennia do not have prop roots. Their subsurface roots are

aerated by pneumatophores, which are narrow aerial roots that project vertically above the soil so that they are above the water at low tides. Avicennia pneumatophores are typically 0.5 to 1 cm in diameter and 5 to 15 cm high. Like the prop roots of Rhizophora, the surface of the pneumatophores contain lenticels that allow air to enter at low tides and to keep water out at high tides (Tomlinson 1986). Laguncularia lack prop roots; however, they have lenticels on their lower trunks and often have pneumatophores with lenticels. Laguncularia pneumatophores are short: they may be 2 cm in diameter but are not usually more than 8 to 10 cm high.

In addition to root aeration problems, mangroves have special features that enable them to grow in brackish or saline waters. The adaptations that enable mangroves to grow in anaerobic soils and to survive in salt water are associated with features that could make mangroves especially sensitive to oil and dispersants.

The mangrove is able to grow in salt water because its root membranes restrict salt entry into the plant (Scholander 1968). In Avicennia and Laguncularia, the root membranes pass small amounts of salt, much of which is excreted through salt glands on the leaves. Rhizophora mangroves have more efficient salt-excluding systems than the other two species but have no salt-secreting glands.

Petroleum hydrocarbons have been shown to disrupt mangrove root membranes in a way that allows lethal amounts of salt to enter the trees (Page et al. 1985). In addition, it has been found that heavy or weathered oils can seal over the lenticels on prop roots or pneumatophores, which cuts off the supply of air to the roots (Scholander et al. 1955). Laboratory studies have shown that dispersants can reduce the surface tension of the waxy lenticel packets so that sea water penetrates into the aerenchyma, presumably with serious consequences for the tree.

Government agency approvals for field application of oils and dispersants to mangrove forests are difficult to obtain. The availability of enough mature trees for testing a series of oils and dispersants is unlikely anywhere.

The seedlings of propagules are the most readily available form of mangroves for large-scale tests. (Propagules or seeds of the three Florida mangrove species are available in large numbers only in the fall.) However, the specific anatomical or morphological aspects of oil or dispersant effects may limit the transfer value of seedling tests to mature trees. For example, if mature Avicennia trees are killed by oil and/or dispersant through a mechanism that involves the pneumatophores, results from tests on seedlings might have little value in protecting mature forests. Similarly, if killing or survival of Rhizophora were based even partially on the aeration system in prop roots, seedlings might not be a suitable model.

It is presumably the mature mangrove trees that one wants to protect because of the time necessary for replacements to grow. Seedlings appear to be more sensitive to oiling than mature trees. The killing of all of a year's crop of mangrove seedlings probably would not be nearly as significant ecologically as killing a fraction of the mature trees.

Table 11 identifies the plant stages or parts that have been used for tests with oil and dispersant.

Table 11. Plant stages or parts that have been used for tests with oil and dispersant.

Mangrove	Stage or Part	Where	Oil	O + D	D
Rhizophora	Propagule	Florida	*	*	*
	Seedling	Florida	*		
	Seedling	South Carolina	*	*	*
	Seedling	India	*		
	Sapling	Malaysia	*	*	
	Mature trees	Florida	*	*	
	Mature trees	Panama	*	*	
	Prop roots	Florida			*
Avicennia	Seedling	South Carolina	*	*	*
	Saplings	Malaysia	*	*	
	Mature trees	Australia	*	*	*
	Pneumatophores	Florida			*
Laguncularia	Seedlings	Florida			*
	Pneumatophores	Florida			*

* Indicates plant stage or part has been tested.

References

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Getter and Baca (1984)
Jagtap and Untawale (1980)
Lai and Feng (1985)
Wardrop et al. (1987)

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Getter, C.D. and B.J. Baca. 1984. A laboratory approach for determining the effect of oil and dispersants on mangroves, pp. 5-13. In T.E. Allen, ed. Oil spill chemical dispersants: research, experience and recommendations. STP 840. Amer. Soc. for Test. Mat., Philadelphia, PA.

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APPENDIX A

WORKSHOP AGENDA AND CHARGE TO WORKING GROUPS

APPENDIX A

Minerals Management Service

Workshop on Technical Specifications for Oil and Dispersant Toxicity Testing

Tuesday, January 17, 1989

Day 1

Time	Speaker	Presentation
8:30 am	Gary Petrazzuolo Technical Resources, Inc. (TRI) and Robert M. Avent Minerals Management Service	Welcome, Introductions, and Purpose of the Workshop
9:00 am	Peter Wells Environment Canada Dartmouth, Nova Scotia	Summary of the National Academy of Sciences Dispersants Report
9:30 am	Mike Flaherty Environmental Consultant	Current Testing Requirements
10:00 am	Break	
10:30 am	Edward Stanton U.S. Coast Guard	Considerations in Selecting Crude Oil for Testing
11:00 am	John Fraser Shell Oil Company	Considerations in Selecting Dispersants for Testing
11:30 am	Ken Trudel S.L. Ross Environmental Research Ltd.	The Spill Impact Assessment Model from a User's Perspective
12:00 noon	Lunch	
1:30 pm	Richard Lee Skidaway Institute of Oceanography	Principles of Bioaccumulation of Oil and Dispersants
2:00 pm	John Costlow Duke University Marine Laboratory	Testing for Toxicity with Various Life Stages of Marine Organisms
2:30 pm	Jerry Neff Battelle, New England	Methods for Determining Toxicity of Oil to Marine Organisms

Tuesday January 17, 1989 (continued)

3:00 pm	Jack Anderson Southern California Coastal Water Research Project	Methods for Determining Toxicity of Dispersants to Marine Organisms
3:30 pm	Break	
4:00 pm	Peter Lutz University of Miami	Methods for Determining Toxicity of Oil and Dispersants to Turtles
4:30 pm	Thomas Duke Technical Resources, Inc.	Methods for Determining Toxicity of Oil and Dispersants to Seagrasses
5:00 pm	Howard Teas University of Miami	Methods for Determining Toxicity of Oil and Dispersants to Mangroves
5:30 pm	Eric Powell Texas A & M University	Methods for Determining Toxicity of Oil and Dispersants to Corals

Wednesday, January 18, 1989

Day 2

Time	Speaker	Presentation
8:30 am	Gary Petrazzuolo Technical Resources, Inc.	Summary of Speakers' Reports
9:00 am	Formation of Working Groups on Toxicity of Oil and Dispersants	

Group	Topic
A.	Invertebrates
B.	Vertebrates
C.	Plants
D.	Materials for Toxicity Testing

9:30 am Working Group Deliberations

7:00 pm Deadline for submission of working group reports for word processing

(Breaks at the discretion of individual groups)

Thursday, January 19, 1989

Day 3

Time	Speaker	Presentation
8:30 am	Presentations and Discussions of Working Group Results	
	Invertebrates (Group A) Vertebrates (Group B) Plants (Group C) Materials for Toxicity Testing (Group D)	
10:30 am	Gary Petrazzuolo Technical Resources, Inc.	Synthesis and Final Remarks
12:00 noon	Adjourn	

CHARGE TO WORKING GROUPS

The Minerals Management Service (MMS) is interested in developing a hierarchical testing system to evaluate the potential impact of dispersants, dispersed oil, and crude oil on the coastal environment. This system will involve a progression of more complicated tests, from 96-hour LC50 (lethality) tests, sublethal chronic tests with sensitive life stages of selected organisms, to measurements of structural and functional aspects of ecosystems. The purpose of Working Groups A, B, and C is to recommend specific toxicity tests that encompass the nominated species where possible. The working groups are asked to consider where recommended toxicity tests fit into a hierarchical testing system.

The specific questions to be addressed by each group include:

- Should species or life stages be added to or deleted from the MMS survey list provided?
- What species, life stages, and toxicity endpoints are recommended?
- What is the feasibility, either under laboratory or natural conditions, of these recommended tests?
- What are the prospects for culturing organisms that are difficult to obtain or maintain? Are any of these critical to the program? Can surrogate species be used?
- What experimental protocols are available for the species of concern? Which are most appropriate? What is the state of development of these protocols?
- Which species and laboratory facilities are currently available for this work?
- What are the limits of applicability of results from recommended toxicity tests in predicting environmental effects?
- Are there specific instances where ecosystem-level tests should be conducted in addition to (or instead of) tests with individual species?

APPENDIX B

LIST OF ATTENDEES AND SPEAKERS

APPENDIX B

Minerals Management Service

Workshop on Technical Specifications for Oil and Dispersants Toxicity Testing

Attendees

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APPENDIX C

SOURCES FOR TEST SPECIES RECOMMENDED BY WORKSHOP ON TECHNICAL SPECIFICATIONS FOR OIL AND DISPERSANT TOXICITY TESTING

APPENDIX C

Sources for Test Species Recommended by Workshop on Technical Specifications for Oil and Dispersant Toxicity Testing

The Minerals Management Service asked Technical Resources, Inc. to poll Federal, State, academic, and commercial facilities/institutions to determine the availability of test species that were nominated for consideration by the Workshop on Technical Specifications for Oil and Dispersants Toxicity Testing that was held in New Orleans, LA, in January, 1989. The poll was taken and the results are recorded in the following table. Contacts were made at various Federal, State, academic, and commercial facilities as requested to determine where the nominated species could be obtained by those interested in conducting the tests. Those contacted quickly revealed that those species required for NPDES and other permit testing activities were readily available and often cultured while other species would have to be collected from the natural environment as needed. In other words, it may be necessary for those conducting toxicity tests to collect some species or have them collected from the environment at the time the tests are conducted. Several commercial suppliers mentioned that species other than those presently used in permit testing activities could be cultured or maintained if the demand were great enough. Although it was impossible to contact all agencies, academic institutions, and commercial facilities, the following information represents a reasonable profile of contacts to those conducting toxicity tests to determine their suppliers and to the suppliers to make certain that the species are available. This compilation does not include data on the quality of the organisms provided, shipping or transient time, etc., and suppliers are listed alphabetically, not in any particular order of preference.

Test Species	Availability Season[1]	Source
INVERTEBRATES		
Bacteria:		
Photobacterium phosphoreum (Microtox)	All year	Commercial supplier[2] 6
Sea urchin:		
Arbacia punctulata	All year	Commercial supplier 10, 13, 19, 33
Polychaete:		
Neanthes arenaceodentata	All year	Field collection[3]
(Nereis virens)[4]	All year	Commercial supplier 32
Oyster:		
Crassostrea virginica	All year	Commercial supplier 13, 27
Coral:[5]		
Acropora cervicornis	All year	Field collection
Gorgonian:[5]		
Briareum spp.	All year	Field collection
Amphipod:		
Ampelisca spp.		Field collection
Mysid:		
Mysidopsis bahia	All year	Commercial supplier 2 ,3, 4, 5, 8, 9, 11, 12, 13, 15, 16, 17, 20, 21, 22, 24, 25, 31, 32
Shrimp:		
Penaeus aztecus (Brown shrimp)	May-Sept	Field collection
Penaeus setiferus (White shrimp)	Mar-June	Field collection
Penaeus duorarum (Pink shrimp)	May-Sept	Field collection
(P. vannamei and P. monodon)[4]	All year	Commercial supplier 1, 18, 27
Crabs:		
Rhithropanopeus harrisii	Mar-Sept	Field collection
Uca spp.	All year	Field collection
Menippe spp.	All year	Field collection

Test Species	Availability Season[1]	Source
VERTEBRATES		
Fishes[6]		
Sheepshead minnow: *Cyprinodon variegatus*	All year	Commercial supplier 2, 3, 4, 5, 8, 9, 11, 12, 13, 14, 19, 20, 21, 22, 24, 25, 31, 32
Silversides: *Menidia beryllina*	All year	Commercial supplier 3, 4, 8, 13, 19, 22, 24, 31, 32
Mummichog: *Fundulus heteroclitus*	May-Sept	Commercial supplier 13, 32
Redfish: *Sciaenops ocellatus*	Mar-Sept	Commercial supplier 23, 25, 27, 28, 29, 30, 34
Croaker: *Micropogonia undulatus*	Jan-Mar	Field collection
Menhaden: *Brevoortia patronus*	Jan-Mar	Field collection
Spot: *Leiostomus xanthurus*	Mar-Sept	Field collection
Speckled trout: *Cynoscion nebulosis*	Mar-May	Field collection
Loggerhead turtles:[5] *Caretta caretta*		
Green turtle:[5] *Chloria mydas*		
PLANTS		
Phytoplankton: *Minutocellus polymorphus*	All year	Commercial supplier 7

Test Species	Availability Season[1]	Source
Skeletonema costatum	All year	Commercial supplier 7
Red macroalgae: Champia parvula	All year	Commercial supplier
Red algae: Caloglossa lepre	Growing season	Field collection
GRASSES AND MANGROVES[5]		
Manatee seagrass: Syringodium filiforme	Growing season	Field collection
Shoal grass: Halodule wrightii	Growing season	Field collection
Turtle grass: Thalassia testudinum	Growing season	Field collection
Black mangrove: Avicennia germinans	Growing season	Field collection
Red mangrove: Rhizophora mangle	Growing season	Field collection
Black needlebrush: Juncus roemerianus	Growing season	Field collection
Smooth cordgrass: Spartina alternaflora	Growing season	Field collection

1. Time of year that species is available from either field collection or commercial suppliers.
2. See the list of suppliers and their corresponding numbers that follows this appendix.
3. Field collection indicated that the species is not maintained in culture and must be collected from the environment when required for testing. Commercial suppliers will often provide this service.
4. Supplies of cultured surrogate species are available.
5. Special permission from proper State and Federal offcials may be/will be needed to collect these species.
6. Embryo, larval, and juveniles were the suggested test ages.

List of Commercial Suppliers of Test Organisms and Corresponding Number

1. Amorient Aquaculture International
   P.O. Box 131
   Kahuku, HI 96731
   (808) 293-8531

2. Aquatic Bioassay Labs
   8142 Grenwell Springs Road
   Baton Rouge, LA 70816
   (504) 924-2618

3. Aquatic Biosystems, Inc.
   375 E. Horsetooth Road
   Shores 4, Suite 103
   Ft. Collins, CO 80525
   (800) 331-5916

4. Aquatic Indicators
   P.O. Box 632
   St. Augustine, FL 32085-0632
   (904) 825-2320

5. Aquatic Research Organism
   P.O. Box 1271
   Hampton, NH 03842

6. Beckman Instrument, Inc.

7. Bigelow Laboratory for Ocean Sciences
   Mckown Point
   West Boothbay Harbor, MA 04575
   (207) 633-2173

8. Biomonitoring Services Laboratory
   6601 East Bay Boulevard
   Gulf Breeze, FL 32561-9432
   (904) 932-2717

9. Biota
   3925 Terry Lane
   Mobile, AL
   (205) 633-6100

10. Carolina Biological Supply
    2700 York Road
    Burlington, NC 27215
    (919) 584-0381

11. Chesapeake Cultures
    P.O. Box 507
    Hayes, VA 23072
    (804) 693-4046

12. Cosper Environmental Services, Inc.
    Northport Environmental Research Center
    P.O. Box 525
    Northport, NY 11768
    (516) 754-4455

13. Cultures Aquatics
    Eatons Neck Road
    Northport, NY 11768
    (516) 575-8182

14. Danbury Fish Farms
    P.O. Box 528
    Danbury, TX 77534
    (409) 922-8414

15. Environmental Research Lab
    2601 E. Airport Drive
    Tuscon, AZ 85706
    (602) 741-1990

16. Environmental Sciences & Engineering
    P.O. Box ESE
    Gainesville, FL 32602
    (904) 332-3318

17. Florida Biological Consultants
    University of Tampa
    Tampa, FL 33603
    (813) 253-3333

18. Granada Corporation
100 Research Parkway
College Station, TX 77840
(409) 268-7000

19. Gulf Specimen Company
P.O. Box 237
Panacea, FL 32346
(904) 984-5297

20. IT Corporation
165 Field Crest Avenue
Edison, NJ 08818
(201) 225-2000

21. Mar, Inc.
101 N.W. Interchange
Bay St. Louis, MS 39520
(601) 255-1461

22. Marinco
7524 Castle Drive
Sarasota, FL 34240
(813) 377-5219

23. Matagorda Bay Aquaculture
414 Elizabeth
Palacios, TX 77465
(512) 972-2801

24. Multi-Aquaculture Systems
Box 679, Cranberry Hole Road
Amagansett, NY 11903
(516) 267-3341

25. Mysitech
2521 Avenue O 1/2
Galveston, TX 77550
(409) 765-9792

26. Red Ewald, Inc.
P.O. Box 519
Karnes City, TX 78119-0529
(800) 242-3524

27. The Redfish Hatchery
Rt. 4, Box 130
Pass Christian, MS 39571
(601) 452-2034

28. Redfish Ranch
P.O. Box 70
Port O'Conner, TX 77982
(512) 983-4330

29. Redfish Unlimited
5 Post Oak Park, Suite 1820
Houston, TX 77027
(713) 622-1946

30. Rowland Fiberglass, Inc.
P.O. Box 971
Ingleside, TX 78362
(512) 776-7753

31. Shealey Environmental Services
400 Graymont Avenue
Columbia, SC 29205
(803) 254-9915

32. SP Engineering Technology
29 Congress Street
Salem, MA 01970
(617) 745-4569

33. Westover Farms, Inc.
P.O. Box 778
Jeanerette, LA 70544
(318) 276-4716

34. Woods Hole Marine Biology Laboratory
Dept. of Marine Resources
Woods Hole, ME 02543
(617) 548-3705

APPENDIX D

EXISTING TOXICITY TESTING AND/OR DISPERSANT REGISTRATION REQUIREMENTS USED BY FEDERAL OR STATE AGENCIES

APPENDIX D

Existing Toxicity Testing and/or Dispersant Registration Requirements Used by Federal or State Agencies

Telephone interviews were conducted with personnel from regulatory agencies in states that have marine coastlines. The objective of the interviews was to ascertain information on existing toxicity testing and registration requirements for chemical dispersants used in oil spill clean-ups. The interviews were conducted during December 1988.

California is the only state contacted with specific and separate requirements for testing the effects of dispersants on marine organisms. Most states utilize National Pollutant Discharge Elimination System (NPDES) testing requirements, those found in Standard Methods, tests developed specifically for detergents by the U.S. Environmental Protection Agency (EPA) or equivalent tests. Presently, the California State Water Resources Control Board requires tests with two fish and the brine shrimp to generate LC50's for each dispersant tested, and a minimum of two tests with two species of fish in sea water containing 1:5 mixture of dispersant to No. 6 Fuel Oil. According to Kim McCleneghan, California State Department of Fisheries, a project is underway to develop new tests with more sensitive species and life stages than those now being tested.

Respondents from several states indicated that the EPA protocol for testing dispersants would be used should testing be required. This protocol is contained in the Federal Register, Volume 49, No. 139, pages 29204-29207 (attachment 3). The test for toxicity of dispersants involves exposing two species (Fundulus heteroclitus and Artemia salina) to five concentrations of the test dispersant and No. 2 Fuel Oil alone and in a 1:10 mixture of dispersant to oil. To aid in comparing results from assays performed by different workers, reference toxicity tests are conducted using dodecyl sodium sulfate as a reference toxicant. The LC50's are calculated based on mortality data at the end of the exposure period. According to discussions with several respondents, the advantage of this test is that it is "generic" and results can be compared nationwide, i.e., one test is used and results can be compared. The disadvantages include the sensitivity of the suggested test species -- neither Artemia nor Fundulus are considered "sensitive" species and would not be considered endemic species for most spills.

- Mr. Lee Dunbar, Connecticut Department of Environmental Protection, stated that Connecticut has no specific legislation or testing program for dispersants. The Department does regulate effluent dischargers under the NPDES System and requires 48- to 96-hour tests using

sheepshead minnows (Cyprinodon variegatus), Atlantic silversides (Menidia menidia), and the mysid shrimp (Mysidopsis bahia). The methods described by the EPA for effluent testing are required by the Department. If a request were received to use a dispersant, the Department would request data generated by the EPA effluent methods.

- Mr. Richard Greene, Delaware Department of Natural Resources and Environmental Control, stated there are no specific testing requirements for dispersants in Delaware. They do regulate point source dischargers under NPDES, but he is not aware of any plan to use NPDES test procedures for any materials other than effluents. He also indicated that the Department is generally against the use of dispersants in State waters because of the sensitivity of local estuaries.

- Mr. Norm Marcotte, Maine Department of Environmental Protection, stated there are no testing or registration requirements for dispersants in Maine. In 1982, Boudin College conducted an experimental oil spill in Maine waters. The project created a tremendous public outcry and the Maine Legislature passed a law forbidding the issue of research permits for similar projects in the future.

- Mr. Ed Gertler, Maryland Department of the Environment, stated there are no specific testing requirements for chemical dispersants in Maryland. However, the Department has recently been reorganized and now has an Oil Spill Control Group. The use of toxicity tests for approval of dispersant use has been discussed within the group, but a definitive plan has not been formulated.

- Mr. Tom Quinn, New York State Department of Environmental Conservation (Oil Spill Group), stated there are no specific testing requirements for chemical dispersants in New York and that the Department depends on EPA to provide any needed information. The State has a Memorandum of Understanding with the U.S. Coast Guard and EPA which defines the very limited use of dispersants based in part on the geographic area where the spill occurs.

- Mr. Kent Wiggins, North Carolina Department of Natural Resources and Community Development (Emergency Response Group), stated there are no specific testing

requirements for chemical dispersants in North Carolina. The state has an Environmental Management Commission which must approve any request for dispersant use. Approval is based on toxicity and efficacy data submitted by the applicant. No dispersants have been approved by the Commission, but they have reviewed data submitted by applicants. He also stated that the Department is generally against the use of dispersants in State waters because of the possibility of the dispersed oil entering their estuaries. The Department favors mechanical methods of clean-up.

- Mr. Steve Williams, Virginia Water Control Board, stated there are no specific testing requirements for chemical dispersants in Virginia. Virginia is a member of the Regional Response Team. The Board has the responsibility of approving any request for dispersant use and has reviewed toxicity data on a small number of dispersants. The Board is generally against the use of chemical dispersants because of fishery resources in Virginia estuaries. It would consider permitting dispersant use for offshore areas.

The Department of the Interior Mission

As the Nation's principal conservation agency, the Department of the Interior has responsibility for most of our nationally owned public lands and natural resources. This includes fostering sound use of our land and water resources; protecting our fish, wildlife, and biological diversity; preserving the environmental and cultural values of our national parks and historical places; and providing for the enjoyment of life through outdoor recreation. The Department assesses our energy and mineral resources and works to ensure that their development is in the best interests of all our people by encouraging stewardship and citizen participation in their care. The Department also has a major responsibility for American Indian reservation communities and for people who live in island territories under U.S. administration.

The Minerals Management Service Mission

As a bureau of the Department of the Interior, the Minerals Management Service's (MMS) primary responsibilities are to manage the mineral resources located on the Nation's Outer Continental Shelf (OCS), collect revenue from the Federal OCS and onshore Federal and Indian lands, and distribute those revenues.

Moreover, in working to meet its responsibilities, the **Offshore Minerals Management Program** administers the OCS competitive leasing program and oversees the safe and environmentally sound exploration and production of our Nation's offshore natural gas, oil and other mineral resources. The MMS **Minerals Revenue Management** meets its responsibilities by ensuring the efficient, timely and accurate collection and disbursement of revenue from mineral leasing and production due to Indian tribes and allottees, States and the U.S. Treasury.

The MMS strives to fulfill its responsibilities through the general guiding principles of: (1) being responsive to the public's concerns and interests by maintaining a dialogue with all potentially affected parties and (2) carrying out its programs with an emphasis on working to enhance the quality of life for all Americans by lending MMS assistance and expertise to economic development and environmental protection.