ACUTE TOXICITY OF THE OIL DISPERSANT COREXIT 9500, AND FRESH AND WEATHERED ALASKA NORTH SLOPE CRUDE OIL TO THE ALASKAN TANNER CRAB (*C. bairdi*), TWO STANDARD TEST SPECIES, AND *V. fischeri* (MICROTOX® ASSAY)

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A

THESIS

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ABSTRACT

Toxicity assays of the oil dispersant Corexit 9500, and fresh and weathered Alaska North Slope (ANS) crude oil were conducted on Alaskan tanner crab larvae (*Chionoecetes bairdi*) under cold-region conditions, the reference species, *Mysidopsis bahia* and *Menidia beryllina,* and *Vibrio fischeri* (Microtox® bioassay). Acute 96-hour toxicity data for *C. bairdi* were calculated using the response "affected" (decreased phototactic response and ability to swim). *C. bairdi* were most sensitive to non-dispersed weathered oil (EC_{50} = 0.4 mg/L), least to dispersant-only solutions (EC_{50} = 1,267 mg/L), and were typically more sensitive than the reference species. Dispersant-only solutions were consistently least toxic for all species tested. Dispersed fresh oil was frequently more toxic than non-dispersed oil. Weathered oil data are greatly influenced by aqueous solubilities, indicating non-dispersed weathered oil was most toxic, although those solutions required the highest oil loading (25 g/L). Interpretations of toxicity data are dependent upon expression of solution concentrations.

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ACRONYMS

LC_{50}	Lethal Concentration to 50 percent of the population
EC_{50}	Effective Concentration to 50 percent of the population
LL_{50}	Lethal Loading to 50 percent of the population
EL_{50}	Effective Concentration to 50 percent of the population
MEC	Median Effective Concentration (LC_{50}, EC_{50}, LL_{50}, or EL_{50})
VOA	Volatile Organic Analyte (C_6-C_9)
TPH	Total Petroleum Hydrocarbons (C_{10}-C_{36})
THC	Total Hydrocarbon Content (C_6-C_{36})
WAF	Water-Accommodated Fraction
CE-WAF	Chemically Enhanced Water-Accommodated Fraction
CROSERF	Chemical Response to Oil Spills: Ecological research Forum

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INTRODUCTION

AND

BACKGROUND

INTRODUCTION

Chemical dispersants are an important oil spill response option (Pace and Clark, 1993). Although dispersants are applied in order to mitigate oil spills, they continue to draw the concern of environmental regulators and decision-makers. In part, this is due to uncertainties surrounding the exposure tolerances of local marine organisms to potentially toxic substances (Pace and Clark, 1993), and the possibility that dispersing oil leads to increased toxicity (NRC, 1989). The Oil Pollution Act of 1990 called for the reevaluation of dispersant use as a response option and required the consideration of pre-authorization plans where dispersants may or may not be used (Coelho *et al.,* 1995). When evaluating dispersant use to respond to an oil spill, dispersant effectiveness must be considered first followed by an evaluation of environmental acceptability. Many regulatory agencies that have a pre-approval process for dispersant use must have a basis to assess the potential impact to the local marine environment (Pace and Clark, 1993). Results from aquatic toxicity tests are an integral part of the information needed to assess those potential environmental impacts.

Standard toxicity testing in the United States involves determining the LC_{50} (lethal concentration to 50% of the population) of a test material to particular species under continuous exposure (Bragin *et al.,* 1994). However, data from field experiments where dispersants have been applied to an oil slick indicate that within several hours, initial concentrations of hydrocarbons decrease by an order of magnitude in the water column (Bragin *et al.,* 1994; Singer *et al.,* 1996a). Additionally, dispersant application to mitigate an oil spill is recommended only under certain conditions in which high energy states exist (*i.e.,* wind and wave intensity, sea swell), favoring rapid dilution (Pace and Clark, 1993; Singer *et al.,* 1996a). Thus, toxicity results based upon a continuous exposure may not be representative of actual exposures that may occur in the field (Bragin *et al.,* 1994; Singer *et al.,* 1996a). In light of this, a spiked exposure (declining concentration), flow-through experimental system was designed by Singer and others (1993). This method of toxicity testing was adopted by the State of California for dispersant approval using native marine species (Pace and Clark, 1993). However, continuous exposure tests are a more commonly used laboratory method nation-wide (Singer *et al.,* 1990; 1991; Bragin *et al.,* 1994) for which a larger toxicity database has been established. Thus, use of both exposure regimes allows comparisons of the data to both past and future work of similar nature.

Toxicity tests have been conducted using oil dispersants and dispersed oil on various species (Wilson, 1977; Lonning & Falk-Peterson, 1978; Singer *et al.*, 1991, 1993, and 1996a). Most tests focus on warm-water species under more temperate conditions than are found in Alaska. Among the standard test species used by the U.S. Environmental Protection Agency (EPA) are *Mysidopsis bahia* and *Menidia beryllina*, for which a large database of information regarding toxicity of oil and dispersants exists. However, little or no data are available describing the exposure response of cold-region, Alaskan species to oils and dispersants.

Both spiked and continuous exposure assays were used to in this study to evaluate the toxicity of crude oil and the oil dispersant, Corexit 9500, to an Alaskan marine species, *Chionocetes bairdi.* Methods used in this study followed protocols established by the

Chemical Response to Oil Spills: Ecological Research Forum (CROSERF) group. CROSERF is a "group of individuals from State and Federal government, academia, and industry dedicated to improving laboratory and mesocosm research on the ecological effects of chemical agents used in oil spill response" (Coelho and Aurand, 1997). The main emphasis of the group's work concerns the toxicity and effectiveness of petroleum dispersants, with a major focus on developing standardized laboratory testing procedures for toxicity assays, a research need identified by the National Research Council to improve the comparability of data sets (National Research Council, 1989).

The purpose of this study was to evaluate the acute toxic effects of an oil dispersant (Corexit 9500) and fresh and weathered ANS crude oil (dispersed and non-dispersed) on early life stages of an Alaskan marine organism, *C. bairdi*, and two EPA standard reference species, *M. bahia* and *M. beryllina*. Use of the EPA reference species in this study facilitated the cross-comparison of results between laboratories. Additionally, by associating the results obtained for *C. bairdi* to those of the standard EPA species tested under similar conditions, the toxicity database for cold-regions species could effectively be augmented. Also to facilitate inter-laboratory comparisons of toxicity data, the reference crude oil, Prudhoe Bay Crude Oil, was evaluated using the reference species, *M. beryllina*. In addition, Microtox® analyses were run concurrently on samples collected from the aquatic toxicity tests. Microtox® is a commercially available bioassay system that is based on inhibition of luminescence of the bacterium, *Vibrio fischeri*. Because Microtox® analysis is a relatively rapid and inexpensive bioassay, its ability to predict possible environmental impact in a "real-time" fashion is of particular interest to regulators and spill response teams. Split samples were collected from aquatic toxicity tests on *C. bairdi* larvae and the two standard test species, *M. bahia* and *M. beryllina* and analyzed using the Microtox® test system.

This study represents the first effort to evaluate a cold-regions marine species under both spiked and continuous exposures to crude oil and oil dispersants. The results of this study have been provided to the Alaska Department of Environmental Conservation for use in determining the relative risk of dispersant use in response to oil spill events occurring in Alaska.

Project Objectives

The objectives of this study were to evaluate the toxicity of the dispersant, Corexit 9500, and dispersed and non-dispersed fresh and weathered Alaska North Slope crude oil to a cold-region, sensitive life-stage marine organism. Results from this study provide insight into the potential environmental impacts of using dispersants in response to an oil spill at high latitude.

BACKGROUND

Oil Spill Response Options

The decision of how best to respond to a spill is often considered one of "environmental trade-offs," choosing options which impart a net-environmental benefit greater than environmental losses (Trudel, 1998). In the event of an oil spill, major response options include: 1) mechanical containment and recovery, 2) use of chemical dispersants, 3) *in-situ* burning; 4) shoreline cleanup, and 5) natural attenuation (no-response option) (NRC, 1989). Viability of each response option depends upon such factors as local geography, energy states (*i.e.,* current, wave, and wind action), mixing depth, and environmental sensitivity. Most oil spills spread rapidly, forming a slick ranging from 1 μm to 1 mm in thickness (NRC, 1989), thus further encumbering response efforts. Ideally, when oil is spilled, it all would be contained and removed from the sea surface. Unfortunately, mechanical recovery of oil can collect only a small amount, "leaving the rest to cause environmental damage" (Trudel, 1998). The recovery rate of skimmers (mechanical recovery) is "negligible at thicknesses of less than about 1 mm," and for large spills has been as low as 10 percent (NRC, 1989). There may be other logistical limitations to mechanical containment and recovery. For example, if the slick is large, the number of vessels required to contain it may not be practical, the time to deploy equipment for response may be slower than other measures, or the cost may be prohibitive (White *et al.*, 1999).

Use of chemical dispersants may be the only option available where mechanical equipment physically cannot fit into a spill location, or when wave height exceeds the oil containment capacity of booms (NRC, 1989). Dispersants are applied to an oil spill by being sprayed from either aircraft or sea vessels. The time to respond using dispersant application can be much more rapid than mechanical removal. However, dispersant application can be hindered by low visibility due to darkness or fog, or high winds preventing accurate targeting for dispersant application to the oil slick (NRC, 1989).

Use of dispersants is recommended for offshore areas in order to protect shorelines (Gulec and Holdway, 1997; Wells, 1984), and to reduce the threat to surface inhabitants such as seabirds and marine mammals (Wolfe *et al.,* 1998). Chemical dispersants are not recommended for use in shallow or restricted waters where dilution rates are low or where sensitive habitats, such as near-shore benthic communities, may be affected (Coelho *et al.,* 1995). Field studies have shown that non-dispersed oil that reached the shoreline was less biodegraded than dispersed oil that was collected near-shore (Lunel, 1998). This suggests that non-dispersed oil stranded on shorelines may be more persistent than dispersed oil, and may thus have greater potential to cause prolonged exposures to local organisms. Furthermore, dispersed oil has a reduced ability to adhere to solid surfaces than non-dispersed oil, effectively reducing the exposure time to organisms living near-shore. Pink salmon embryos, for example, were found to be adversely effected under both short-term exposure (reduced survival) and long-term exposure (slowed growth) when exposed to oiled gravel at levels consistent with samples of contaminated stream sediments collected from Prince William Sound (PWS) (Heintz *et al.,* 1995). Incidentally, oil released from the grounding of the *Exxon Valdez* in PWS in 1989 was not treated with dispersants (Coelho *et al.,* 1995).

Physicochemical Characteristics of Crude Oils

Crude oils are complex and variable chemical mixtures (Bobra *et al.,* 1983). The physicochemical characteristics of the individual compounds in oil contribute to its ability to form water-soluble fractions, enabling contact with aquatic biota through which a toxic effect may occur. A chemical must be able to interact with water in order to establish concentrations in the aqueous media (Lipnick, 1995). The extent to which oil will go into solution depends upon the parent oil composition, temperature, salinity, mixing energy and duration, and oil to water ratio (*i.e.,* oil loading) (Shiu *et al.,* 1990; Caldwell *et al.,* 1977; Rice *et al.,* 1977).

The physicochemical characteristics of the oil that contribute to formation of water-soluble fractions include molecular size, polarity, and partitioning preference between aqueous and lipid or gaseous phases (*i.e.,* hydrophobicity and volatility). Lower molecular weight hydrocarbons are more soluble than heavier ones (Shaw, 1977; Abernathy *et al.,* 1986). In fresh crude oils, monoaromatics are the most soluble (Bobra *et al.,* 1983). The solubility of aromatic hydrocarbons decreases with increasing degree of alkyl substitution, and number of aromatic rings (Rice *et al.,* 1977). Aliphatic hydrocarbons are among the least soluble with solubility decreasing with increasing carbon number (Rice *et al.,* 1977).

Hydrocarbons that are soluble in water often are also volatile. The rates of dissolution can be much slower than the rates of evaporation (Peterson *et al.,* 1993), often making dissolution a minor process (McAuliffe, 1977). In addition, once in solution, the more water-soluble hydrocarbons can rapidly volatilize out of solution (Peterson *et al.,* 1993). Volatility can be described using Henry's law constant (ratio of the chemical's vapor pressure to solubility). The partitioning preferences (*e.g.,* hydrophobicity, or affinity for lipids in biological membranes) of a chemical are estimated using the octanol-water partitioning coefficient (K_{ow}) (LaGreaga *et al.,* 1994; Lyman, 1995; Lipnick, 1995).

The characteristics above contribute to a chemical's toxicity as well. Once in solution, a chemical can make contact with an aquatic organism, making a toxic effect possible. If that chemical has a large octanol-water partitioning coefficient, it may preferentially partition out of solution into the biological lipids of the organism. Molecular size and structure can influence a chemical's ability to interact with biological lipids. The diffusion capacity of larger molecules may be less than those that are smaller (Abernathy *et al.*, 1986; Bobra *et al.*, 1993). Molecular structure can play a role in toxicity, but the octanol-water partitioning coefficient is a more important factor (Abernathy *et al.*, 1986).

Fate of Oil in the Environment

Immediately following a spill, the nature of crude oil begins to alter through weathering processes. The first compositional changes to occur are competing processes of evaporation and solution of volatile compounds (McAuliffe, 1977). Rates of evaporation are generally much greater than those of dissolution (Peterson *et al.*, 1993; NRC, 1985; McAuliffe, 1977), depending upon the vapor pressure and solubility of individual compounds. Evaporation may result in the loss of up to one third of the oil by mass (Mackay *et al.*, 1982), leaving behind persistent components of lower solubility, and increased viscosity (Bobra *et al.*, 1983; McAuliffe, 1977; Shiu *et al.*, 1990). The most immediately toxic and sub-toxic fractions of crude oil are those soluble in water (benzene to naphthalenes) (McDonald *et al.*, 1984; Bobra *et al.*, 1983), with the chronic toxicity of oil being related to the non-volatile, persistent aromatic hydrocarbons (Maher, 1986). Since the more soluble light aromatics are also more volatile, their removal through weathering processes would imply a reduction in toxicity (Bobra *et al.*, 1983). Moreover, since toxicity is related to a substance's ability to interact with aqueous media (Bobra *et al.*, 1983; Abernathy *et al.*, 1986), increased viscosity of weathered oil further inhibits the oil's ability to form water-soluble fractions. Due to response logistics, oil spills typically are not treated until one or more days have passed since release. During this time, substantial weathering and loss of the volatile fraction can occur (Singer *et al.*, 1998; Mackay *et al.*, 1982). Therefore, concern for toxicity due to volatile fractions is more relevant to subsurface releases or surface spill events in which treatment occurs shortly after release (Singer *et al.*, 1998). To best understand the consequences of dispersing oil under either treatment scenarios (*i.e.*, rapid vs. delayed response post-release), toxicological data from both fresh and weathered oils must be compared.

Dispersants: History and Function

Dispersants are complex mixtures of surface-active agents (surfactants), solvents, and additives (Clayton *et al.*, 1993). Their design purpose is to reduce interfacial tension between the oil-water interface so as to promote the dispersion of oil into the water column, effectively increasing the surface area of the oil slick (NRC, 1989). Surfactants are the primary agent in reducing interfacial tension (Clayton *et al.*, 1993). Containing both hydrophobic (*i.e.*, oil-compatible) and hydrophilic (*i.e.*, water-compatible) components, a surfactant molecule reduces the interfacial tension by "residing" half in the oil phase and half in the water phase (Clayton *et al.*, 1993; NRC, 1989). As the concentration of the surfactants increase, the interfacial tension decreases until a critical micelle concentration (CMC) is reached (NRC, 1989). Micelles are droplets of oil surrounded by surfactants that disperse into the water column with the aid of wave action, currents, and wind. Solvents in the dispersant mixture are present to maintain homogeneity in the dispersant mixture of surfactants and additives (Payne *et al.,* 1993), and to reduce the oil's viscosity and facilitate dispersal (NRC, 1989). The acute toxicity of dispersants alone is often attributed to the surface-active components in the dispersant mixture, interacting equally with biological lipids as with other lipids (Singer *et al.,* 1996a; 1990). The additives in a dispersant are intended to aid in biodegradation (Payne *et al.,* 1993).

Dispersants have been used worldwide for the more than 30 years in response to oil spills, and have received more focus and research than any other response option (Hillman, 1998). This is in part due to the huge public outcry over use of dispersants in the *Torrey Canyon* spill in 1967. Since then, there has been a long history of apparent successes that does not receive much notice from opponents of dispersant use (Lewis and Aurand, 1997). So-called first-generation dispersants, as were used in the *Torrey Canyon* spill, were derived from engine room degreasers and were as toxic as the oil being treated (NRC, 1989; Singer *et al,* 1990). Second and third generation dispersants have been reformulated to contain surfactants that are less toxic than those of their predecessors. Dispersants currently considered for use in the United States and Canada are of low toxicity compared to crude oil and refined petroleum products (NRC, 1989).

Dispersant effectiveness depends on the length of time crude oil is allowed to weather, the contact time between the dispersants and crude oil, and the dispersant to oil ratio

(White *et al.*, 1999). Factors that affect dispersant effectiveness include: 1) temperature – decreased temperature results in increased viscosity, requiring more mixing energy; 2) salinity – increased salinity results in the reduced solubility of dispersant in saltwater, thereby making the dispersant's surfactant more available for interaction with oil; 3) mixing energy – sufficient mixing energy is required for both oil-dispersant contact and to facilitate breaking the oil into micelles; and 4) local conditions, for example, high winds may prohibit the use of aerial dispersant spraying, or excessive energy states may reduce the contact time between the dispersant and oil (White *et al.*, 1999). Oil properties such as viscosity, pour point, boiling point, and surface tension also factor into the effectiveness of dispersants (White *et al.*, 1999).

Corexit 9500, the dispersant used in this study, is a newer oil dispersant that was designed to treat higher viscosity oils than its predecessor, Corexit 9527 (Singer *et al.*, 1996a). Although Corexit 9527 is currently stockpiled for response in the areas of Prince William Sound and the Gulf of Alaska, and has been shown effective (30-80%) in field tests conducted in south Alaskan seas (Hillman, 1998), this product is no longer manufactured. Consequently, the focus of more recent research has been directed toward Corexit 9500 (White *et al.*, 1999; Lindstrom *et al.*, 1999; Singer *et al.*, 1996a).

Fate of Dispersed Oil in the Environment

Initial concentrations of dispersants alone (*i.e.*, not in the presence of oil or other chemicals) applied to water might range from 0.1 to 13 mg/L at various depths (5 to 10 m) (Wells, 1984; Singer *et al.*, 1991; Trudel, 1998). Chemical dispersion of oil results in formation of micelles (stabilized oil droplets surrounded by surfactant molecules) that may range in size from 1 to 70 μm (Mackay *et al.*, 1982; Lunel, 1998). Dispersion is believed to be rapid within the first 5 to 20 minutes (Mackay *et al.*, 1982). Under an untreated slick, 0.1 to 0.2 percent of the oil released can be detected; however, following treatment with chemical dispersants, this amount increases to 1.8 to 3.5 percent (Pace *et al.*, 1995). Concentrations under a treated slick are greatest initially at shallower depths (*e.g.*, 40 ppm at 1 m vs. 0.1 ppm at 9 m at 0.25 h following treatment) (Mackay *et al.*, 1982; Wright *et al.*, 1994; Trudel, 1998). However, over time (approximately 28 hours) concentrations normalize throughout a depth of approximately 10 m where a "diffusion floor" apparently exists, as little oil penetrates to greater depths (Mackay *et al.*, 1982). The decrease in concentration is due to diffusion in both the vertical and horizontal directions, with horizontal diffusion being greater (Mackay *et al.*, 1982). Once treated, oil droplets are sufficiently small and have neutral buoyancy, thus remaining dispersed in the water column (Mackay *et al.*, 1982). Dispersed oil will not sink unless associated with sediment or as feces after being ingested by organisms. In most spills, association with sediment is not a significant transport pathway for the fate of the oil, unless sediment is re-suspended by storm action or other disturbances (Lunel, 1998).

By increasing the aqueous concentrations of oil through enhanced solubilization or emulsification, dispersant use is advantageous based on the belief that degradation is enhanced (Wolfe *et al,* 1998). Recent studies however, suggest that microorganisms may preferentially degrade hydrocarbons originating from the dispersant and not from the dispersed oil (Lindstrom, *et al.*, 1999). This may potentially result in selective enhancement of a certain hydrocarbon group, actually increasing its persistence in the environment (Lindstrom, *et al.,* 1999). Through the action of dispersants, the bioavailable fraction of oil is increased by the presence of more hydrocarbons in the water column and altered interactions between oil, dispersants, and biological membranes (Wolfe *et al.,* 1998). This can lead to a concomitant increase in bioaccumulation, direct dermal contact, or ingestion (Middaugh *et al.,* 1996; Wolfe *et al.,* 1998). These are all factors surrounding dispersant use and the ultimate fate of oil that must be considered when dispersants are used in response to an oil spill.

Dispersant Policy in Alaska

Alaska has oil spill response zones classified as "Zone 1" in Prince William Sound (PWS) and Cook Inlet, where use of dispersants has been pre-approved (Morris, 1998). This means that a Coast Guard Federal On-Scene Coordinator (FOSC) can consider use of dispersants in response to an oil spill (after first considering mechanical means) without being required to receive approval from the EPA or the State of Alaska (Morris, 1998). Zone 1 regions are characterized by bathymetry and currents that are conducive to dispersant use. Zones 2 and 3 are more sensitive areas. Zone 2 is characterized as having biological parameters that must be considered such as sensitive habitats or biota (Morris, 1998). Zone 3 typically is adjacent to shorelines where impacts to human activities are a concern. Zones 2 and 3 require more collaboration between response teams and agencies during a spill event in order to make spill-response decisions (Morris, 1998). Pre-spill response approvals have the benefit of determining beforehand where and when dispersant use could be used effectively to respond to a spill event (Morris, 1998). This eliminates the need to review extensive scientific and technical information during a spill event in order to support sound decision-making (Morris, 1998). The pre-approval process also allows assignment of more stringent seasonal zone status to regions that are more biologically sensitive during certain times in the year. For instance, the PWS tanker lane is classified as Zone 1 except during the period of March 1 through October 15 when its classification becomes Zone 2 to protect important fisheries resources and commercial fishing activities. Such designations are made in part based upon information gained from toxicological assays.

Toxicity Tests

Toxicity tests are designed to identify the concentration of a chemical at which a percentage, usually 50 percent, of the population responds with a specified effect (*e.g.*, reduced ability to swim, or death). Typically, the effect specified is death, since death is often more easily discernable in an organism than other sub-lethal responses. Toxicity tests thus provide information about what response an organism may have when exposed to specific concentrations of chemicals under conditions similar to those used in the laboratory analysis. In addition, when compared to other species tested with the same chemicals under similar conditions, toxicity tests can provide some indication of relative species sensitivities to the test chemicals.

To identify the concentration that elicits a 50 percent response from the organism, a series of solutions with increasing concentration of the chemical are prepared. Ideally, organisms in the control group will exhibit no response (*e.g.*, no mortality), followed by either no or a very low response to the lowest concentration, then two or more partial responses in the mid-range concentrations (*i.e.*, 20,40, or 60% mortality), and finally, a complete response by the test organisms in the highest concentration (*i.e.*, 100% mortality). Data collected from this design defines a curve illustrating the relationship between exposure to the chemical at increasing concentrations and the organisms' response, referred to as a "dose-response" curve. The result from each test concentration is plotted along the ordinate against the proportion responding along the abscissa. Typically, lines are drawn between these data points to aid the eye and suggest the trend in toxicity with respect to increasing concentration. However, these lines are not meant to imply what the actual relationship between effect and test concentration is between those data points. Statistical methods are used to estimate the mid-point of the slope on the curve where the greatest change in response to concentration occurs; this point is defined as the concentration at which 50 percent of the population responds. In order to estimate that concentration, at least a 50 percent response by the organisms must be observed in the toxicity test.

An important aspect of toxicity testing is species selection. The selection of a species requires identifying one that is: 1) sensitive (so as to provide a conservative estimate of the toxic effect on local biota); 2) of local ecological and economic importance; and 3) amenable to laboratory testing (Rand *et al.*, 1995). *Chionocetes bairdi* was used in this study as it met these criteria. Marketed and sold as "Tanner Crab" (Williams *et al.*, 1988), *C. bairdi* is both economically and ecologically significant to the State of Alaska.

Newborn tanner crab larvae move into the upper 30 meters of the water column where they feed on phytoplankton. Since animals in early life stages are generally more sensitive than adults (Broderson *et al.,* 1977; Karinen and Rice, 1974), and dispersants are generally found in the upper water column (Mackay *et al.,* 1982), tanner crab larvae may experience comparatively greater risk of exposure during an oil spill than other species. This species typically is found in waters with an ambient salinity of approximately 32 parts per thousand (‰) and temperature of about 7°C. Information obtained from the toxicity assays on *C. bairdi* from this study can be used in the decision-making process for spill response actions and plans. Also, this species was evaluated to determine its laboratory suitability (*e.g.,* ability to survive laboratory procedures, and availability) and its suitability as a cold-regions reference species.

The EPA reference species, *M. bahia,* is an estuarine shrimp found in the waters of the Atlantic and Gulf of Mexico, and is among the most sensitive of standard test species (Pace *et al.,* 1995). *M. beryllina* are fish found in estuaries along the coasts of the Atlantic ocean and the Gulf of Mexico (Middaugh *et al.,* 1996). This species serves as a forage fish for larger species of economic importance (Webber, 1993). Both *M. bahia* and *M. beryllina* are commonly used in toxicity assays, and are being considered for dispersant-testing protocols (Pace and Clark, 1993). *M. beryllina* was recently selected by CROSERF as the organism of choice for laboratory inter-calibration of standard dispersants, and dispersed and non-dispersed oil solutions (Coelho and Aurand, 1998). Hence, *M.beryllina* was tested in this study to allow comparisons with other CROSERF laboratories. Toxicity assays of *M. bahia* also were conducted to allow comparisons to other studies that used this species, prior to selection of *M. beryllina* as the CROSERF standard.

In addition to the test chemical itself and species sensitivity, factors that influence the results of toxicity assays include: 1) the methods used to prepare solutions; and 2) the manner in which organisms are exposed to the test solution. Two exposure regimes (spiked and continuous) were used in this study to evaluate acute toxicity (96 hour) of three types of solutions: 1) dispersant in saltwater; 2) water accommodated fraction (WAF) of crude oil in saltwater; and 3) the chemically enhanced water accommodated fraction (CE-WAF) of crude oil mixed with dispersant in saltwater. WAF solutions were not filtered or placed into a centrifuge to remove all traces of bulk particulate oil. Thus, the term WAF is preferred to water soluble fraction (WSF) in this case since WSF indicates that particulate oil (*i.e.*, oil droplets) has been removed from the solution (Singer *et al.*, 1996b). Although the spiked exposure regime is a better model for actual exposure conditions (Pace and Clark, 1993), continuous toxicity assays are common standardized tests that facilitate comparison of toxicity data between local and non-local species (Singer *et al.*, 1990; 1991).

Field Extrapolations

When using toxicity data as a tool to assess potential environmental impact, a basic understanding of how the data were generated is important to properly interpret the results as they apply to the natural environment. Laboratory methods used to prepare test solutions for organism exposure are designed to mimic conditions likely to occur in nature. However, because a variety of conditions can exist in the environment (*e.g.*, high-energy storms or, especially, calm waters), these methods are intended to re-create only one realistic concentration profile. Thus, an understanding of the test procedures (both solution preparation and exposure type) is necessary to properly extrapolate toxicity data to field conditions.

An equally, if not more, important consideration when extrapolating laboratory data to field conditions is how the concentrations of the test solutions were characterized in order to calculate a toxicity value. That is, are the toxicity values reported in a study calculated based upon analytically determined concentrations or nominal concentrations (the amount of chemical added to a known volume of aqueous media), or only certain fractions of the measured concentration? In the case of a test material that is composed of a mixture of chemicals, if the toxicity values are calculated based only on a chemical subset of the material, those data may not represent of the actual material concentration that caused the toxic effect. This is an especially important consideration when test materials contain chemicals with varying abilities to interact with aqueous media. In such cases, reporting toxicity values based on chemical subset groups may erroneously omit other chemicals or groups of chemicals that may be more influential on the toxic response of the organism.

Finally, seasonal variations in biological sensitivity must be considered. For example, *C. bairdi* larvae are present in the upper reaches of the water column during the spring and early summer months. If a spill event were to occur in November in the same location where these zooplankton bloom in the spring, these animals would not experience any exposure to oil or dispersed oil. Therefore, concern for a given species in a particular life-stage may not always be relevant.

CHAPTER 1

FRESH OIL AND DISPERSANT STUDY

MATERIALS AND METHODS

Materials

All toxicity assays in this study were conducted using solutions made from oil, dispersant, or dispersed oil using the following materials: 1) Alaska North Slope crude oil (ANS) (Williams Alaska Petroleum, Inc., North Pole, Alaska); 2) Prudhoe Bay crude oil (PBCO), a reference oil (R.T. Corporation, Laramie, Wyoming); and 3) Corexit 9500 (Nalco/Exxon Energy Chemicals, L.P., Sugar Land, Texas). Sub-samples of the dispersant and crude oil were collected and dispensed separately, with no headspace, into certified organic-free 20-mL or 40-mL septum vials and stored at 4°C until use.

Corexit 9500 is a dispersant with both anionic and nonionic properties, and contains an oleophilic solvent carrier designed to treat higher viscosity oils and emulsions (Singer *et al.*, 1996a). This dispersant is described as a blend of oxyalkylate polymers, organic sulfonic acid salt, substituted fatty ester, glycol ether, and aliphatic hydrocarbon (Nalco/Exxon Energy Chemicals, L.P., 1997). ANS used in this study was collected in October 1997 from the Trans-Alaska Pipeline. Approximately one-third (w/w) of ANS is composed of volatiles, compounds with a boiling point of 400 to 525°F (204 to 274°C) or less (pers. comm., Mead, 1997). PBCO is a U.S. Environmental Protection Agency (EPA) standard, and is described as a "medium light crude" (Wolfe *et al.*, 1998) with 23.2 percent (by weight) of its components having a boiling point of 205°C or less (NRC, 1985).

All toxicity assays of *Chionocetes bairdi* were conducted using natural, 0.5-μm filtered seawater (20-μm pleated cellulose paper, 10-μm granular activated carbon, 0.5-μm block-activated carbon; Ametek, Sheboygan, Wisconsin) taken from an 80-m depth from Resurrection Bay, Seward, Alaska, at ambient temperature and salinity (typically 7°C and 31.5‰, respectively). For toxicity assays of *M. bahia* and *M. beryllina*, re-constituted saltwater made from de-ionized water (≥ 18 MΩ–cm) and Crystal Sea Marinemix (formerly Forty Fathoms Seasalt, Marine Enterprises International, Inc., Baltimore, Maryland) was used at a temperature of 25°C and salinity of 20‰ (Webber, 1993; Pace and Clark, 1993; Bragin *et al.,* 1994). Saltwater used in the toxicity assays for all species was also used for all saltwater needs including animal holding, test solution preparation, and dilution water in the spiked exposure (declining concentration) tests.

Test Solutions

Each species was evaluated for acute toxic effects using three solution types prepared with saltwater: 1) dispersant only (Corexit 9500), 2) water-accommodated fractions (WAF) of crude oil (no dispersant added), and 3) chemically-enhanced water-accommodated fractions (CE-WAF) of crude oil (dispersant added). *C. bairdi* and *M. bahia* were evaluated for acute toxic effects using ANS only, whereas tests for *M. beryllina* included both ANS and PBCO. Both oils were tested on *M. beryllina*, because this species is a recognized standard test species. Thus results from this species will further facilitate the cross-comparison of results with other laboratories.

Selection of the nominal concentrations tested in the toxicity assays was based on the results of range-finding tests (Webber, 1993) for both dispersant and oil solutions. Results from these assays helped identify the concentrations that bracketed a 50 percent response by the animals when exposed to the test solutions, thus enabling calculation of an estimated median-effect concentration (*i.e.,* EC_{50} or LC_{50}).

Dispersant solutions were prepared separately for each concentration (*i.e.*, not serially diluted) by dispensing a known mass of Corexit 9500, determined by the difference between initial and final masses, weighed in a 1 mL gas-tight syringe (Hamilton, Reno, NV), into a 2-L volumetric flask partially filled with saltwater. Due to the limited solubility of Corexit 9500 in saltwater (Wells, 1984; Singer *et al.*, 1996a), the flask was inverted three times to ensure complete mixing, brought up to the proper volume with saltwater, then inverted three more times before samples were collected for chemical analysis and the test solution was dispensed into test chambers for the toxicity tests.

Water-accommodated fractions (WAF) of crude oil were prepared using a standardized method of low-energy mixing adopted by researchers in both Canada and the United States (Blenkinsopp *et al.*, 1996; Coelho and Aurand, 1997). This method, adopted and outlined by CROSERF (Chemical Response to Oil Spills - Ecological Effects Research Forum), was followed in this study (Coelho and Aurand, 1997). WAF preparation involved adding a known mass of crude oil, determined from initial and final masses weighed either in a 5 mL gas-tight syringe (Hamilton, Reno, NV) or a 50 mL beaker, into a low-mixing energy (0% water depth vortex; ca. 180-240 rpm; Blenkinsopp *et. al.*, 1996) 4-L aspirator bottle filled with 3.5 L of saltwater, resulting in a standardized headspace of 25% by volume (Singer *et al.*, 1996b; Coelho and Aurand, 1997; Blenkinsopp *et al.*, 1996). Mixing energy was provided to the aspirator bottles by magnetic stir plates (Model No. 948050, Troemner Inc., Philadelphia, Pennsylvania) and 2-inch teflon-coated stir bars. Bottles were kept in a water bath to maintain a constant temperature during mixing (7°C for *C. bairdi*; 25°C for *M. bahia* and *M. beryllina*), and were covered with aluminum foil to reduce evaporative losses and volatilization. The entire water bath was kept dark in order to avoid photooxidation of the test solutions mixing in the bath. Following a 24-hour mixing period and a 5-minute settling period, the WAF solution was collected for chemical analysis and immediate delivery into the test chambers. WAF solution was collected from the bottom 90 percent of the water depth through the aspirator bottle's sampling port fitted with silicon tubing. Each WAF was individually prepared (*i.e.*, not serially diluted), because components of the oil with varying solubilities may not be transferred in equal proportions during serial dilutions (Girling *et al.*, 1992).

In preliminary investigations during development of this WAF preparation method, other researchers found that using high mixing energies tended to entrain oil droplets or form emulsions, thereby contributing to greater variability in the solution profiles (Singer *et al.*, 1998; Blenkinsopp *et al.*, 1996). The method of WAF preparation used in this study used low-mixing energy, yielding solutions free of oil droplets greater than 1 μm in diameter as verified by epifluorescence microscopy (Blenkinsopp *et al.*, 1996). Because the WAF solutions are relatively free of oil droplets, a settling time of only five minutes was needed to allow for organic/aqueous phase separation.

The term "WAF" is used instead of "water-soluble fraction" (WSF) to describe untreated crude oil solutions to indicate that the test media did not undergo separation procedures such as filtration or centrifugation to remove undissolved, dispersed components of crude oil (Girling *et al.*, 1992; 1994; Singer *et al.*, 1996b; 1998; Maher, 1986). Separation procedures such as these would require physical handling of the test solution, potentially altering its chemical composition (Singer *et al.*, 1996b; 1998). Because solutions may contain oil droplets of 1 μm in diameter or less, the word "solution" is used here recognizing that test solutions, including those made with dispersant only, may be more accurately described as suspensions.

Chemically-enhanced water-accommodated fractions (CE-WAF) of crude oil (chemically dispersed oil) were prepared in a manner similar to WAF solutions, with some exceptions. These included the addition of dispersant in a 10:1 (w/w) ratio of oil-to-dispersant, increasing the mixing energy to achieve a 20-25% water depth vortex (ca. 360-680 rpm; Singer *et al.*, 1996b; 1998), and altering the mixing-to-settling time ratios. Increasing mixing energy compared to that used for WAF solutions was necessary to ensure good contact between oil and dispersant and to promote effective dispersion (Singer *et al.*, 1998). CE-WAF solutions were mixed for a period of 18 to 24 hours, followed by a settling period of 3 to 6 hours (Coelho and Aurand, 1997). The settling period facilitated separation of large oil droplets from solution, generally leaving behind a soluble fraction of oil. Following the settling period, CE-WAF solutions were collected from the bottom 90 percent of the water column, sampled for hydrocarbon analyses, and immediately dispensed into the test chambers for the toxicity assays. Any remaining suspension of crude oil on the water's surface inside the aspirator bottle was avoided during collection. As with the WAF preparation method, recommendations for CE-WAF preparation are set forth by CROSERF and were followed in this study (Coelho and Aurand, 1997).

This method of CE-WAF preparation is similar to those described elsewhere (Singer *et al.,* 1998). These authors report that, despite differences in mixing duration (*i.e.,* WAF for 24 hours, CE-WAF for 18 to 24 hours), CE-WAF solutions were essentially equivalent to WAFs in number of whole oil droplets provided that the CE-WAF mixing-to-settling time ratios remained within (9 h to 24 h): 6 h (pers. comm., Singer, 1999). Additionally, CE-WAF solutions allowed to settle for 3 to 6 hours were not found to be statistically different from one another with respect to the number of oil particulates present in solution (pers. comm., Singer, 1999), leading to the 3- to 6-hour settling period set forth by CROSERF (Coelho and Aurand, 1997) and followed in this study. The mixing-to-setting ratios employed for preparation of CE-WAF in this study were selected to produce solutions with similar profiles (with respect to number of oil particulates) to those of WAF solutions.

Toxicity Test Procedures

Short-term tests (96 h) were conducted to evaluate the responses of early life-stages of Alaskan Tanner crab (*Chionocetes bairdi*), a mysid (*Mysidopsis bahia*), and the inland silverside (*Menidia beryllina*) when exposed to the test solutions. These species were tested under two exposure regimes in this study, spiked and continuous exposure. Spiked exposure tests were used to evaluate acute toxic effects under declining concentration conditions. Continuous exposure tests model exposure to a constant concentration. Animals were exposed in triplicate to a saltwater control and five test solutions of increasing concentration. The test temperature for *C. bairdi* assays was an ambient temperature of 7°C ± 1°C, while for *M. bahia* and *M. beryllina*, the temperature was 25°C ± 1°C (Webber, 1993). Tests for *C. bairdi* were conducted in a temperature-controlled room or in water baths. Other environmental parameters were controlled in the tests, including salinity (20‰ ± 2‰ for *M. bahia* and *M. beryllina*, and 31.5‰ ± 3‰, ambient salinity, for *C. bairdi*), dissolved oxygen (DO, ≥ 60% saturation), and pH (range of 6 to 9; Webber, 1993; ASTM, 1996). Temperature, pH, DO, and conductivity (an index of salinity) were monitored daily.

In the continuous exposure tests, five to twelve animals were placed in a 400-mL beaker covered with a watch glass to minimize evaporative losses and keep contaminants out (ASTM, 1996). The beakers were supplied filtered air (granular activated carbon filter) via a 4-mm ID glass tube at a rate of 50 to 100 bubbles per minute (1.68 to 3.35 cm^3/min) (Webber, 1993). A low aeration rate was used to avoid production of turbulence in the beakers that could be a source of stress to the animals (ASTM E 729-96). This method was employed to assure that sufficient dissolved oxygen concentrations were maintained throughout the duration of the test (ASTM, 1996; Webber, 1993); it was not intended to minimize volatile losses from the test solution. Every 24 hours, test solutions in the beakers were gently decanted off the top 90 percent and then slowly re-filled with fresh solution. Any dead animals or detritus present in the beakers were removed along with the day-old test solution. Post-24-hour samples of test solution were collected at random from mid- to high-concentrations to observe changes in concentration of the more volatile hydrocarbons.

Spiked exposure tests (declining concentrations) were conducted in 280-mL, airtight, borosilicate glass flow-through chambers (Singer *et al.*, 1990; 1996a) (Figure 1-1). These chambers have a top and bottom portion, each fitted with a grooved flange. An airtight seal is created when the top and bottom parts of the chamber are assembled with a silicone O-ring seated in the groove of the two flanges. A U-clamp, tightened with spring-loaded screws, is attached firmly to the flange to hold the two parts together. The tops of the chambers are equipped with two threaded ports, one, to accept influent diluent (fresh, aerated saltwater), and the other to provide food for the animals. The bottoms contain only one threaded port to carry outflow, or chamber effluent, and are fitted with a 40 to 60-µm mesh fritted glass filter for animal containment. All tubing used in this system was made of inert materials (silicon, glass, or platinum-cured silicon).

In order to ensure that all flow-through chambers received equal treatment, each chamber was prepared for the toxicity assay one-at-a-time and in the same manner as the others. Particular care was taken to standardize the amount of time between loading the chambers with test solution and animals, and the addition of diluent. This prevented having test animals in one or more of the triplicate chambers at each test concentration experience a longer period of exposure than others.

The process to prepare each flow-through chamber for the test began by partially filling the chamber with test solution. Five to twelve animals randomly selected from a

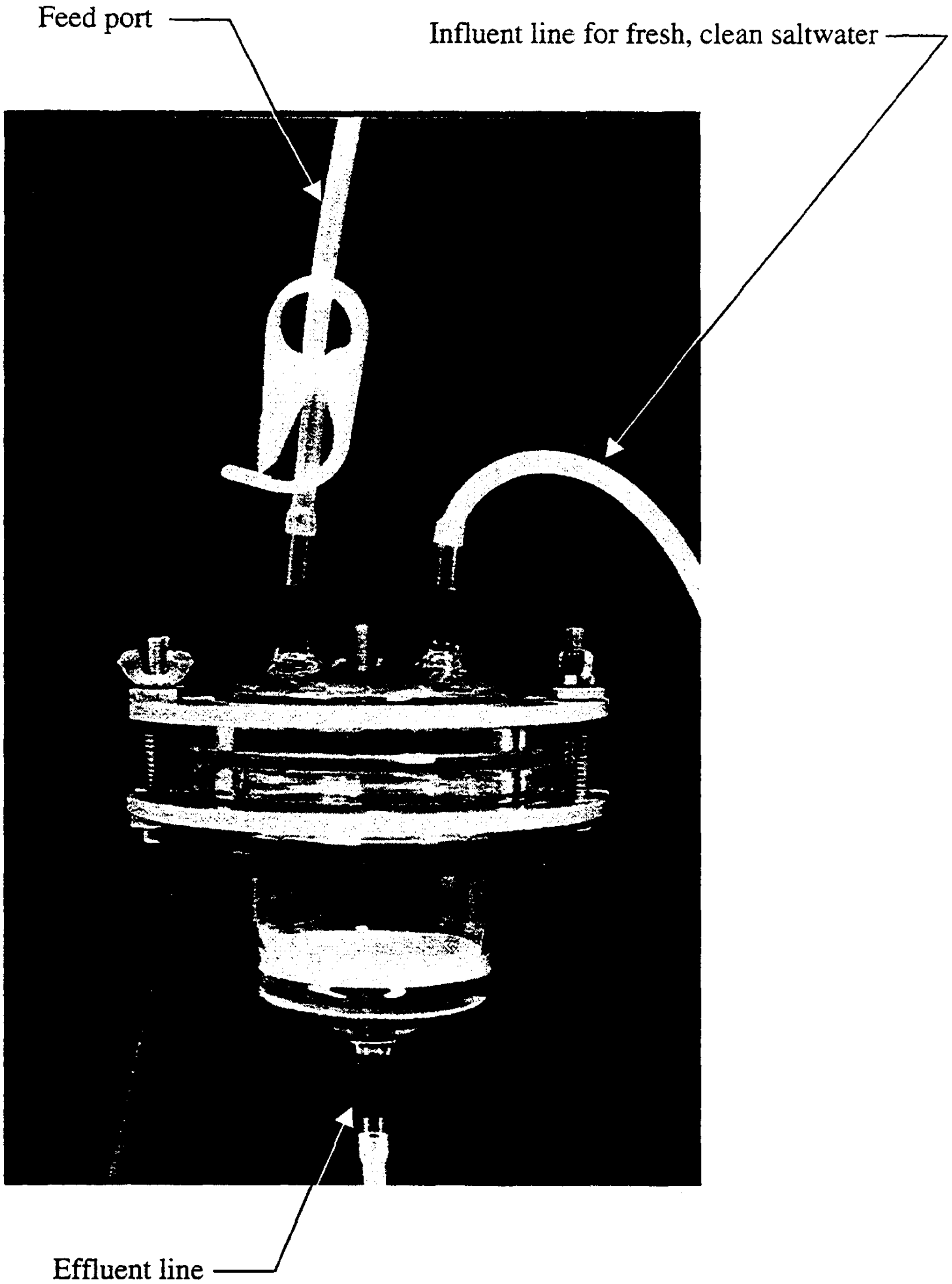

Fig. 1-1. Flow-through chamber used in spiked exposure tests

group of several hundred were then placed in the chamber, which was immediately sealed and clamped shut. The remainder of the chamber was filled with test solution just to the base of the influent and feed ports in order to prevent animal escape through influent lines. Once filled, the chamber was immediately connected to an influent line that supplied the chamber with the saltwater diluent. The time the influent line was connected was recorded, marking the beginning of the 96 hour test (*i.e.,* t = 0 hours) for that chamber. That recorded time was then followed accordingly for the time of disassembly (for that specific chamber) at the end of the 96-hour test. This process of loading the flow-through chambers was repeated for each chamber in the assay, until all 18 were loaded.

The saltwater diluent was supplied to all flow-through chambers over the duration of the test using a peristaltic pump (Model No. 7332-00, Cole-Parmer, Vernon Hills, Illinois) at a rate of 1 to 2 mL per minute from a 100-L covered reservoir, and was oxygenated with air filtered through granular activated carbon. Hourly composite samples of test solution from triplicate chambers were collected, typically at hours 2, 4, 7, and 12 from each of the six concentrations (a saltwater control and five test solutions). A minimum of a middle and high concentration was sampled to verify that concentrations in the test chambers were declining. This also served as a periodic maintenance check on the diluent delivery system to ensure that all chambers were receiving an adequate supply of fresh, aerated saltwater.

Test Species

Tanner crab larvae (*Chionocetes bairdi*) were obtained from gravid females collected in January 1998 from Kachemak Bay, Alaska, USA. The gravid females and larvae were kept in natural saltwater at ambient temperature and salinity. The larvae tested were less than 24 hours old. Prior to and during the tests, the tanner crab larvae were fed once daily with 5 to 10 mL of a solution containing a mixture of diatoms (*Chaetocerus calcitrans*, *Chaetocerus gacile, and Thalassiosira pseudonana*; Qutekcak Shellfish Hatchery, Seward, Alaska). Although *C. bairdi* larvae are known to be phototacticly responsive, it is not known whether the larvae require light for survival (pers. comm., McDonald, 1998). Therefore when other on-going research projects sharing the same laboratory facilities could not support long periods of illumination, the decision was made not to use a regimented photoperiod prior to or during *C. bairdi* tests.

The standard reference species *Mysidopsis bahia* and *Menidia beryllina* were both obtained from Aquatic Bio Systems, Inc., Ft. Collins, Colorado. These animals were fed 1 mL of a suspension of saltwater-rinsed, concentrated, newly hatched (≤ 24 hour old) brine shrimp nauplii (*Artemia*; approximately 100 *Artemia* per animal) once or twice daily prior to and during a test (Webber, 1993). *M. bahia* were found to be highly cannibalistic, requiring careful attention to the feeding needs of the test animals. Six day-old *M. bahia* and 12 day-old *M. beryllina* were tested in each chamber. Each reference species was acclimated to the test salinity and temperature for two days prior to initiation of the test with changes in temperature and salinity not more than 3°C or 3 ppt in any 12 hour period, respectively (Webber, 1993; ASTM, 1996). A photoperiod of 8 hours of dark and 16 hours of light was maintained for *M. bahia* and *M. beryllina* throughout both the acclimation period and toxicity test using ambient laboratory lighting (approximately 10 to 20 $\mu E/m^2/s$; Webber, 1993). Water quality parameters monitored during the acclimation period included temperature, pH, dissolved oxygen (DO) concentration, conductivity (salinity), and ammonia concentration.

Microtox® Assay

Microtox® is a commercially available bioassay system based on inhibition of luminescence of the bacterium, *Vibrio fischeri*. Split samples were collected from test solutions from the aquatic toxicity tests on tanner crab (*C. bardi*) larvae and the two standard test species, *M. bahia* and *M. beryllina,* and were analyzed using the Microtox® test system. Samples were collected in 40 mL VOA vials (no headspace) and stored at 4°C until analysis could be performed (within two weeks of sample collection). All samples were run using the acute toxicity basic test protocol (Azur Environmental, 1995) for the Microtox® system (Azur Environmental, Carlsbad, CA). All reagents were obtained from Azur Environmental and were stored and used as indicated in the test protocol. Phenol (a well-characterized toxicant) standards were run periodically for quality assurance that the test system was set up optimally. In every case, the results obtained fell within the range published for phenol (Azur Environmental, 1995).

Chemical Analysis: Dispersant Solutions

Dispersant test solutions were characterized using ultra-violet (UV) spectrophotometery on a Milton Roy Spectronic 1201 (Ivyland, Pennsylvania) for *C. bairdi* tests and a Gilford Response UV Spectrophotometer (Westchester, Pennsylvania) for *M. bahia* and *M. beryllina* tests. The nominal concentrations of dispersant solutions were also determined by gravimetric means (known mass into known volume). Although the UV-measured concentrations showed good linearity with respect to nominal concentrations, for concentrations below 120 mg/L ($r^2 = 0.99$ for *C. bairdi*, $r^2 = 0.93$ for *M. bahia* and *M. beryllina*), these measured values were not necessarily concordant with nominal concentrations. Additionally, those Corexit 9500 solutions prepared with higher dispersant loadings (typically ≥ 1000 ppm for solutions at 25°C, and ≥ 500 ppm for solutions at 7°C) were observed to exhibit a biphasic nature, suggesting limited solubility of the dispersant mixture and its oleophilic components. Because the manner in which dispersant-only test solutions were prepared (completely mixed immediately before being decanted into the test chambers), animals in the toxicity tests were exposed to all components of the dispersant. As a result, good agreement (*i.e.*, ± 10% of loading) between measured and nominal concentrations of dispersant test solutions was considered important to accurately portray the toxicity of dispersant solutions to which the animals were exposed. Therefore, when a comparison of the UV-measured concentrations to their respective nominal concentrations indicated that some test solutions contained as little as 8 percent of the initial dispersant added, the ability of this analytical technique to accurately depict the solutions' concentrations was questioned. As a result, dispersant test solution concentrations in this study are presented as nominal concentrations determined gravimetrically. Exceptions include the measured declining concentrations of dispersant-only solutions in spiked exposure tests. These hourly samples from *C. bairdi* tests were determined using UV spectrophotometry, and from *M. bahia* and *M. beryllina* tests by total organic carbon (TOC) analysis. TOC analyses were done using a Shimadzu TOC-5000A (Columbia, Maryland).

Chemical Analysis: Oil Solutions

Both WAF and CE-WAF solutions were analyzed using Gas Chromatography/ Flame Ionization Detection (GC/FID). Solutions were analyzed for total volatile organic analytes (VOA; range defined as C_6-C_9) and total petroleum hydrocarbons (TPH; range defined as C_{10}-C_{36}; Coelho and Aurand, 1997). The summation of these analytes is the total hydrocarbon content (THC; C_6-C_{36}) (Coelho and Aurand, 1997). Guidelines used in the development of this analytical method are outlined in the Proceedings of the Sixth Meeting of CROSERF (Coelho and Aurand, 1997), U.S. EPA SW-846 methods 5030, 8000B, and 8021B (U.S. EPA, 1986), and ADEC method AK101 and AK102 Appendix D, Revision 3.0 (ADEC, 1996).

Chromatographic measurements of THC were made using a Hewlett Packard 5890 GC/FID with nitrogen as the carrier-gas. Two columns in series were used to facilitate the separation of organic compounds for VOA and TPH analytes. The first column was a 30 m x 0.53 mm (ID) Rtx®-1 fused-silica capillary column with a film thickness of 0.25 μm (Restek Corp., Bellefonte, PA); the second column was a 30 m x 0.53 mm (ID) HP-1 flexible fused silica capillary column with a film thickness of 1.5 μm (Hewlett Packard, San Fernando, California). A purge and trap condenser equipped with a 16-port Autosampler (Model 7695, Hewlett Packard, San Fernando, California) was used to analyze samples for VOA content using nitrogen as the carrier gas. Following the analysis of samples collected from the *C. bairdi* tests and prior to commencement of the *M. bahia* and *M. beryllina* tests, the gas chromatograph required recalibration, allowing for more target analytes to be included in the calibration (Table 1-1).

Samples analyzed for TPH were serially extracted using a three aliquots of 75 mL of dicholormethane (DCM) as extraction solvent (U.S. EPA SW-846 Method 5030). The extraction volumes for WAF and CE-WAF samples were 1000 and 500 mL, respectively. A surrogate standard (*o*-terphenyl) was added to all samples prior to extraction to monitor the extraction efficiency.

The GC was calibrated using a suite of neat or pre-made hydrocarbon solutions purchased from chemical suppliers (Chem Service, Inc, West Chester, Pennsylvania; Restek Corporation, Bellefonte, Pennsylvania; Alltech Associates, Inc., Deerfield, Illinois). A calibration curve was prepared for each of those hydrocarbons and used to calculate a response factor (RF). An average RF was then determined from the RF for each analyte (U.S. EPA, 1992; ADEC, 1996). Samples were measured by summing the peaks of both resolved (*i.e.*, those hydrocarbons for which an RF was determined from individual, known standards) and unresolved compounds (Coelho and Aurand, 1997),

Table 1-1. Minimum target analytes for chemical analysis of fresh oil test solutions

Minimum target analyte list for VOA analysis (*C. bairdi*)

Saturates	Unsaturates
hexane	benzene
nonane	toluene
	ethylbenzene
	m-xylene
	p-xylene
	o-xylene
	n-propylbenzene

Minimum target analyte list for VOA analysis (*M. bahia* and *M. beryllina*)

Saturates	Unsaturates
2-methylpentane	benzene
hexane	toluene
cyclopentane	ethylbenzene
heptane	m-xylene
2,4 dimethylpentane	p-xylene
cyclohexane	o-xylene
octane	n-propylbenzene
nonane	1,2,4-trimethyl-benzene
	1,3,5-trimethyl-benzene

Minimum target analyte list for TPH analysis (*C. bairdi, M. bahia,* and *M. beryllina*)

n-Alkanes:			
Decane	C10	Tetracosane	C24
Undacane	C11	Hexacosane	C26
Dodecane	C12	Octacosane	C28
Tetradecane	C14	Triacontane	C30
Hexadecane	C16	Dotriacontane	C32
Octadecane	C18	Tetratriacontane	C34
Eicosane	C20	Hexatriacontane	C36
Docosane	C22		

without subtracting the Corexit 9500 peak, and were then quantified using the average RF.

Test solutions in the continuous exposure tests were renewed every 24 hours, yet had to be reported as one concentration for the full 96-hour test. As a result, concentrations for continuous exposure tests were determined in a variety of ways depending upon the hydrocarbon group being analyzed. For *C. bairdi* tests, values reported for VOA and TPH concentrations are the mean of values measured from samples collected on each of four days. For *M. bahia* and *M. beryllina* tests, measured values reported for VOA for both WAF and CE-WAF test solutions are of the composite of samples collected from days one through four. TPH values from WAF tests for *M. bahia* and *M. beryllina* are the values from samples collected on day one only. This approach to characterizing TPH content in WAF test solutions was adopted after verifying that TPH content in WAF solutions was consistently low regardless of increased oil loading due to the limited solubility of hydrocarbons in the range of C_{10} to C_{36}. For CE-WAF solutions from *M. bahia* and *M. beryllina* tests, measured TPH values are from the composite of samples collected from days one through four. TPH samples were composited using an equal volume from each sample collected. Hourly-samples collected from spiked exposure tests of WAF and CE-WAF were analyzed for VOA content to verify that concentrations were declining within the flow-through chambers.

All samples collected for analysis (including those for dispersant-only solutions) were preserved with an 18% HCl (hydrochloric acid) solution (0.25, 0.5, and 2.5 mL of 18% HCl for vials with volumes of 20, 40, and 1000 mL, respectively). Only Microtox®

sample vials were not preserved with acid, as acid will kill the bacteria used to conduct the bioassay (Azur Environmental, 1995).

Toxicity Analysis

Median-effect concentration (MEC) values were determined for each species. For *M. bahia* and *M. beryllina*, LC_{50} (lethal concentration to 50 percent of the population) values of test solutions were determined. For *C. bairdi,* these values were calculated as the effective concentration to 50 percent of the population (EC_{50}), since lethal effects were rarely observed. Each individual larva (*C. bairdi*) was observed under a microscope (30x magnification) and assigned a health status of alive, affected, mortally affected, or dead. The effect used to calculate the EC_{50} was the status of at least "affected, " which also included those animals categorized as "mortally affected" and "dead." Definitions of the four health categories used are shown in Table 1-2.

Table 1-2. *C. bairdi* health evaluation categories

Alive	Vigorously swimming; tail bent under in a normal position; active internal organ movement; good phototactic response (successfully swimming to water's surface to obtain phytoplankton); swims away when touched
Affected	Passively swimming; phototactic response diminished; tail cocked or flipped backwards; organ movement detectable; reduced response to being touched
Mortally affected	Not swimming, but twitching; slight organ movement; no phototactic response; no response to being touched
Dead	No internal organ movement; opaque beige in color

This method is similar to earlier studies of *C. bairdi* larvae as reported in Brodersen and others (1977), where the reported responses to oil solution exposure were similar to those observed in this study. Both Brodersen and others (1977) and Buchanan and others (1970) used moribundity (death imminent) as the lethal indicator for crab larvae to calculate median lethal concentration, and defined moribundity in larvae as "the cessation of swimming."

Median-effect concentrations for dispersant-only solutions are based on nominal concentrations. Median-effect concentrations for oil solutions were calculated based upon the combined measured concentrations of total volatile organic analytes (VOA range; C_6-C_9) and total petroleum hydrocarbons (TPH range; C_{10}-C_{36}), referred to as total hydrocarbon content (THC). Microtox® toxicity values are presented as EC_{50} values and are calculated based upon both single and combined fractions (*i.e.*, VOA, TPH, and THC), as well as oil loading rates. This was done to evaluate the differences in results of comparing the toxicities of two or more solutions with toxicity values standardized to different descriptors of solution content and concentration (*e.g.*, VOA and TPH).

Statistical Analysis

Three replicate exposure chambers were used in the tests to assess the variation within and among test species. The estimated median-effect concentrations (LC_{50} and EC_{50}) were calculated using probit analysis where possible (Finney, 1971), and Trimmed Spearman-Karber (TSK) or Spearman-Karber (TSK with 0% trim) when conditions for probit analysis were not met (Hamilton *et al.*, 1977). When conditions for the analysis methods previously described were not met or when no partial mortalities were observed data were analyzed by the graphical method (Webber, 1993). Probit and TSK estimation are preferred to graphical methods, because confidence intervals can be calculated. Probit analyses were made using Probit Program Version 1.5, and Trimmed Spearman-Karber with Trimmed Spearman-Karber (TSK) Program Version 1.5 (Environmental Monitoring Systems Laboratory, Cincinnati, Ohio). Both programs automatically adjust for any mortality observed in the controls using Abbott's formula, and smooth the data when response proportions were not non-monotonically increasing (Webber, 1993). These programs prompt the user to enter toxicity data in the form of total number of animals responding out of the total number exposed at each concentration, effectively pooling the data from the triplicate chambers at each test concentration. The percent trim with Trimmed Spearman-Karber analysis is automatically calculated when the program is run. Percent trim values are reported with median-effect concentration values (see Table 1-5). For clarification, the median-effect concentrations are often qualified as "estimated," since an LC_{50} or EC_{50} is the median response of a given test population that is "an estimate of the 'true' median lethal [or sub-lethal] concentration of that test material for the entire species" (Greenberg *et. al.,* 1992).

For those tests where a minimum response of 50 percent needed to calculate an LC_{50} or EC_{50} was not observed, median-effect concentrations are reported as values greater than the highest concentration tested. Tests with 20% effect or less in the controls were considered acceptable (Singer *et al.*, 1998; Markarian *et al.*, 1995; Ward, 1995). There can be variability in the percent responses observed in each of the triplicate chambers for a single test concentration (*e.g.*, for the same concentration, observations in chambers a, b, and c may be 20%, 40% and 10%, respectively). Therefore, use of some sort of descriptive statistic (*e.g.*, mean, median, range, etc.) is used to report on the distribution of the data. Variations observed in each test concentration are shown graphically on the dose-response curves as the mean (data point) plus or minus the standard error (error bars, n = 3) of the percent responses.

RESULTS

General Test Conditions

Temperature, salinity, pH, and dissolved oxygen (DO) remained within acceptable limits during the *C. bairdi* tests (Table 1-3). Oxygen concentrations in test solutions were above 60% saturation at all times in all tests, and pH remained in the range of 6 to 9. Temperatures for *M. bahia* and *M. beryllina* tests were maintained close to test protocols 25°C ± 1°C and 20‰ ± 10%, respectively (Table 1-3).

Dispersant Solutions

UV-measured concentrations of dispersant solutions often were not in agreement with their respective nominal concentrations. Although they showed good linearity between measured versus nominal concentrations for dispersant loadings less than 120 ppm, only 54.1 percent of all measured values were within ± 20% of the nominal concentration, with the mean at 85.7% agreement (SD = 31.2, n = 61) ranging from 8 to 138%.

Table 1-3. Summary of water quality parameters measured for dispersant and fresh oil toxicity tests

Test Species		pH	Salinity (ppt)	Temp. (°C)	D.O. (mg/L)
C. bairdi	Mean	8.04	31.42	6.91	9.27
	Std. Dev.	0.25	0.16	0.89	0.42
	n	87	87	35	34
	Maximum	8.44	31.97	8.10	10.18
	Minimum	7.65	30.77	4.60	8.63
M. bahia	Mean	8.24	20.42	25.15	6.79
	Std. Dev.	0.31	1.11	1.16	0.65
	n	72	72	72	72
	Maximum	8.65	23.51	29.00	8.60
	Minimum	7.50	17.67	23.00	4.80
M. beryllina	Mean	8.07	20.99	25.47	6.71
	Std. Dev.	0.29	1.13	1.65	0.36
	n	126	127	129	128
	Maximum	8.70	24.24	28.50	8.30
	Minimum	7.44	19.54	22.00	5.80

Dispersant solutions made in 7°C seawater were found to be soluble up to 500 ppm, half of what was reported by Singer and others (1996a) for similar solutions made at 15°C. A phase-separation was observed in dispersant solutions of higher concentrations (approximately ≥ 800 ppm at 7°C; ≥ 1100 ppm at 25°C) that were left to sit overnight, suggesting the solubility of Corexit 9500 in saltwater is limited, though the product literature states it is completely soluble in water (Nalco/Exxon Energy Chemicals, L.P, 1997). Analytical problems associated with UV-spectrophotometry are likely a result of the limited solubility of dispersant in saltwater.

Oil Solutions

The total hydrocarbon content (THC) in the fresh oil test solutions generally increased with increasing oil loading (Figures 1-2a, 1-2b, and 1-2c). In both WAFs and CE-WAFs the concentration of the lighter, more volatile fraction of crude oil (VOA; C_6-C_9) was observed to increase with increased oil loading, with this increase being significantly greater (t-test; $P < 0.05$) for CE-WAFs than WAFs. The concentrations of total petroleum hydrocarbons (TPH; C_{10}-C_{36}) in WAFs were consistently low despite increased oil loading, characteristic of their low-solubilities and Henry's law constants. In contrast, TPH concentrations in dispersed oil solutions (CE-WAF) increased with increased oil loading, and at a rate significantly higher (t-test; $P < 0.05$) than that of the VOA components.

Mean measured hydrocarbon concentrations of VOA, TPH, and combined as THC and their relative proportions in WAF and CE-WAF solutions are presented in Table 1-4.

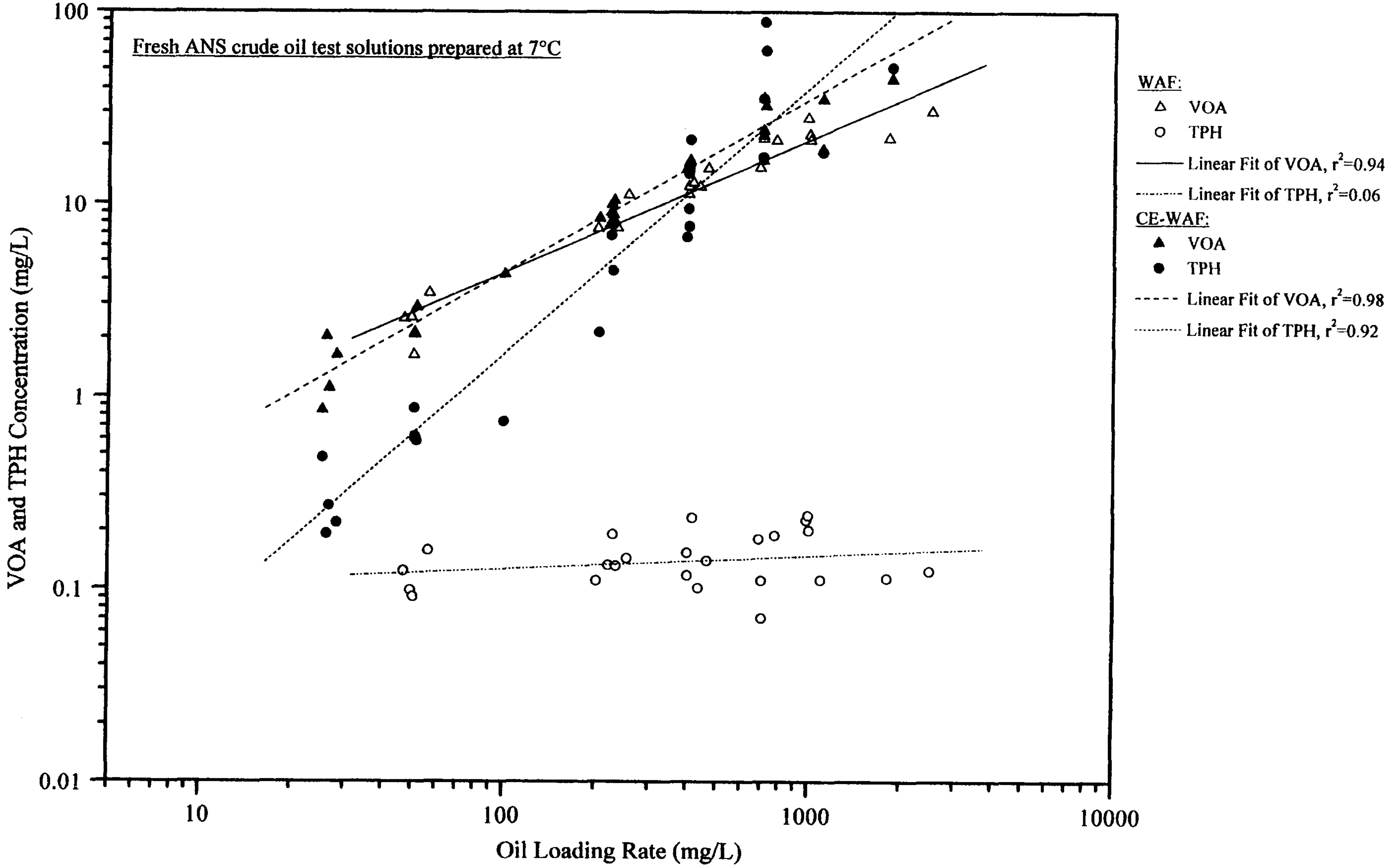

Fig 1-2a. Relationship of measured concentrations of volatile organic analytes (VOA) and total petroleum hydrocarbons (TPH) to oil loading rates in fresh ANS WAF and CE-WAF test solutions for *C. bairdi* in 7°C saltwater

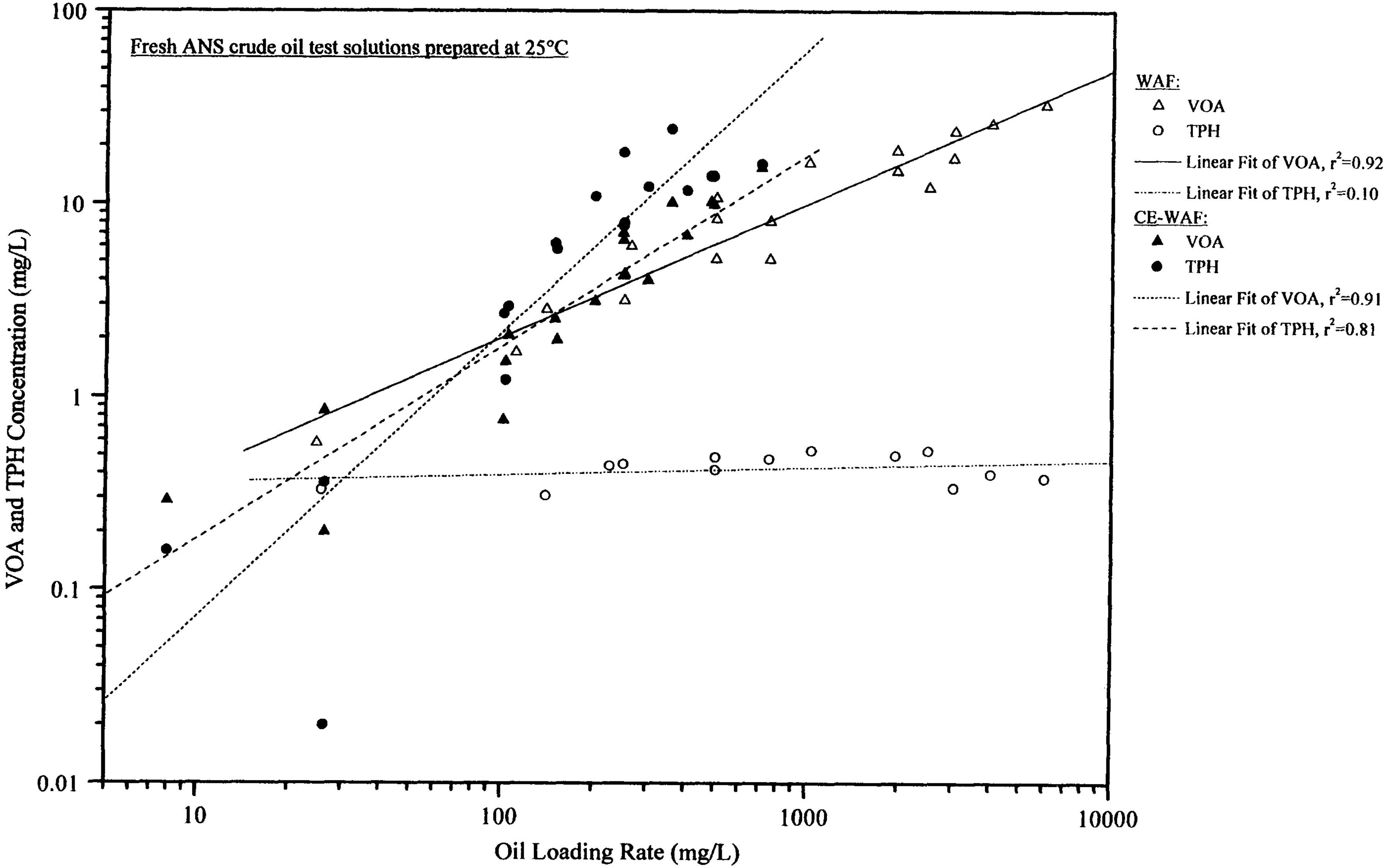

Fig 1-2b. Relationship of measured concentrations of volatile organic analytes (VOA) and total petroleum hydrocarbons (TPH) to oil loading rates in fresh ANS WAF and CE-WAF test solutions for *M. bahia* and *M. beryllina* in 25°C saltwater

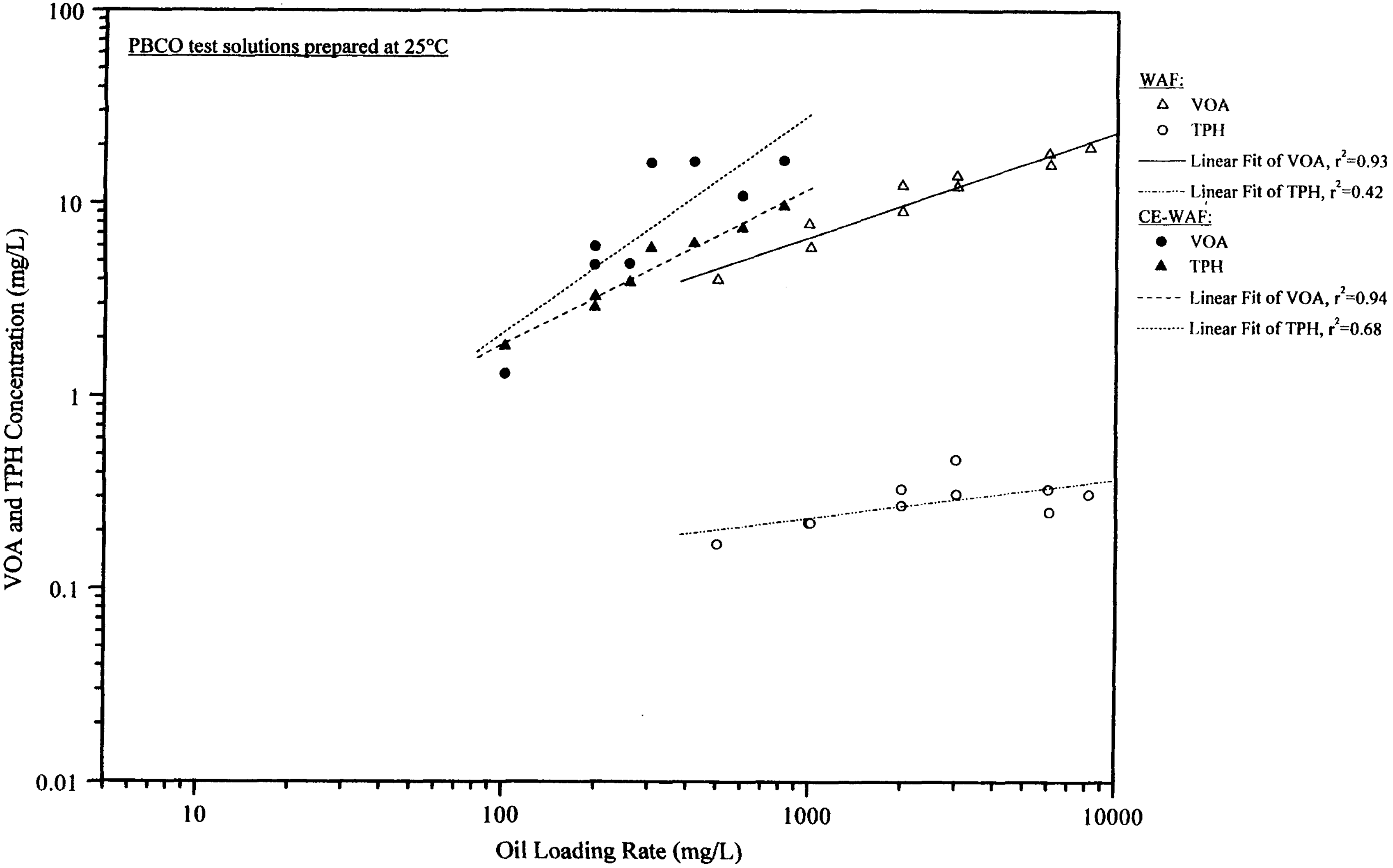

Fig 1-2c. Relationship of measured concentrations of volatile organic analytes (VOA) and total petroleum hydrocarbons (TPH) to oil loading rates in fresh PBCO WAF and CE-WAF test solutions for *M. beryllina* in 25°C saltwater

Table 1-4. Mean concentrations of hydrocarbons measured in WAF and CE-WAF solutions

Hydrocarbon Fraction	Oil Type	Mean +/- Std. Error (SE) Hydrocarbon Conc. (mg/L)							
		WAF	SE	n	% THC	CE-WAF	SE	n	% THC
VOA	Fresh ANS	17	1.2	43	98.7	11	1.7	39	40.2
	PBCO	12	1.1	20	99.1	5.3	0.64	15	34.3
TPH	Fresh ANS	0.23	0.02	28	1.3	17	3.3	28	59.8
	PBCO	0.30	0.02	18	2.5	10	1.5	15	65.7
THC	Fresh ANS	17	1.2	43	-	28	3.8	41	-
	PBCO	12	1.1	20	-	16	1.7	19	-

n = number of samples

On average, WAF solutions of ANS contained of 17 mg/L of VOA and 0.23 mg/L TPH. WAF solutions of PBCO contained of 12 mg/L of VOA and 0.30 mg/L TPH. With the addition of dispersants, the concentrations of hydrocarbons, especially TPH, changed noticeably. CE-WAF solutions of ANS contained of 11 mg/L VOA and 17 mg/L of TPH on average. Approximately ten times more TPH were in CE-WAF solutions than were in WAF solutions. As proportions, VOA in fresh ANS and PBCO WAF solutions comprised 98.7 and 97.5 percent of the total hydrocarbon content for, respectively, with very little contributions from TPH. CE-WAF solutions contained more TPH as a proportion of THC than WAF with 59.8 and 65.7 percent of THC for ANS and PBCO, respectively.

TPH concentrations were typically greater in CE-WAF solutions than in WAF solutions at any given oil loading rate. However, the results of some CE-WAF TPH concentrations may have been influenced by oil droplets in sample solutions that would have been included in the solvent extract (*e.g.*, see Figures 1-2a, b, c). This would cause the TPH measurement to be biased high.

Concentrations of VOA and TPH components measured in solutions made from PBCO were less than those made from fresh ANS crude oil for any given oil loading rate (Figures 1-2b and 1-2c, and Table 1-4). Temperature had a significant effect (t-test; $P < 0.05$) on the concentrations of VOA and TPH in both WAF and CE-WAF solutions made with ANS, causing VOA concentrations to be significantly greater at 7°C than at 25°C. The reverse was observed for TPH concentrations, which were significantly greater at 25°C than at 7°C (Figures 1-2a and 1-2b). Concentrations of VOA in both WAF and CE-

WAF solutions were linearly related to the oil loading rate ($r^2 \geq 0.91$). This relationship was not seen for TPH in WAF solutions ($r^2 < 0.42$), though TPH in CE-WAF solutions showed fair linearity with oil loading ($0.68 < r^2 < 0.92$). Analysis of concentration decline in spiked exposure tests indicated that solution concentrations generally followed a trend of first order exponential decay, stabilizing between the sixth and ninth hour of the 96-hour test (Figures 1-3a, 1-3b, and 1-3c). Similar observations were made by Singer and others (1996a), where stabilization in spiked exposure tests occurred between the sixth and eighth hour. In samples from WAF and CE-WAF tests analyzed for VOA content, concentrations measured for the twelfth hour were no greater than 1.7 mg/L, with concentrations typically less than 1.0 mg/L.

Chionocetes bairdi Tests

In both spiked and continuous exposure tests, dose-response relationships were typically sigmoidal for all test solution types (dispersant only, WAF, and CE-WAF). Data for dispersant only solutions were standardized to the nominal concentrations, and to the measured total hydrocarbon content (THC) for oiled solutions (Figures 1-4a and 1-4b). The loading rates used to prepare dispersant and fresh oil test solutions and their respective measured concentrations are summarized in Table 1-5. In general, solutions for spiked exposure tests were prepared using 2.5 to 20 times more test material than those for continuous exposure tests. Similarly, WAFs required 1.4 to 2.0 times more test material (fresh ANS crude oil) than CE-WAFs.

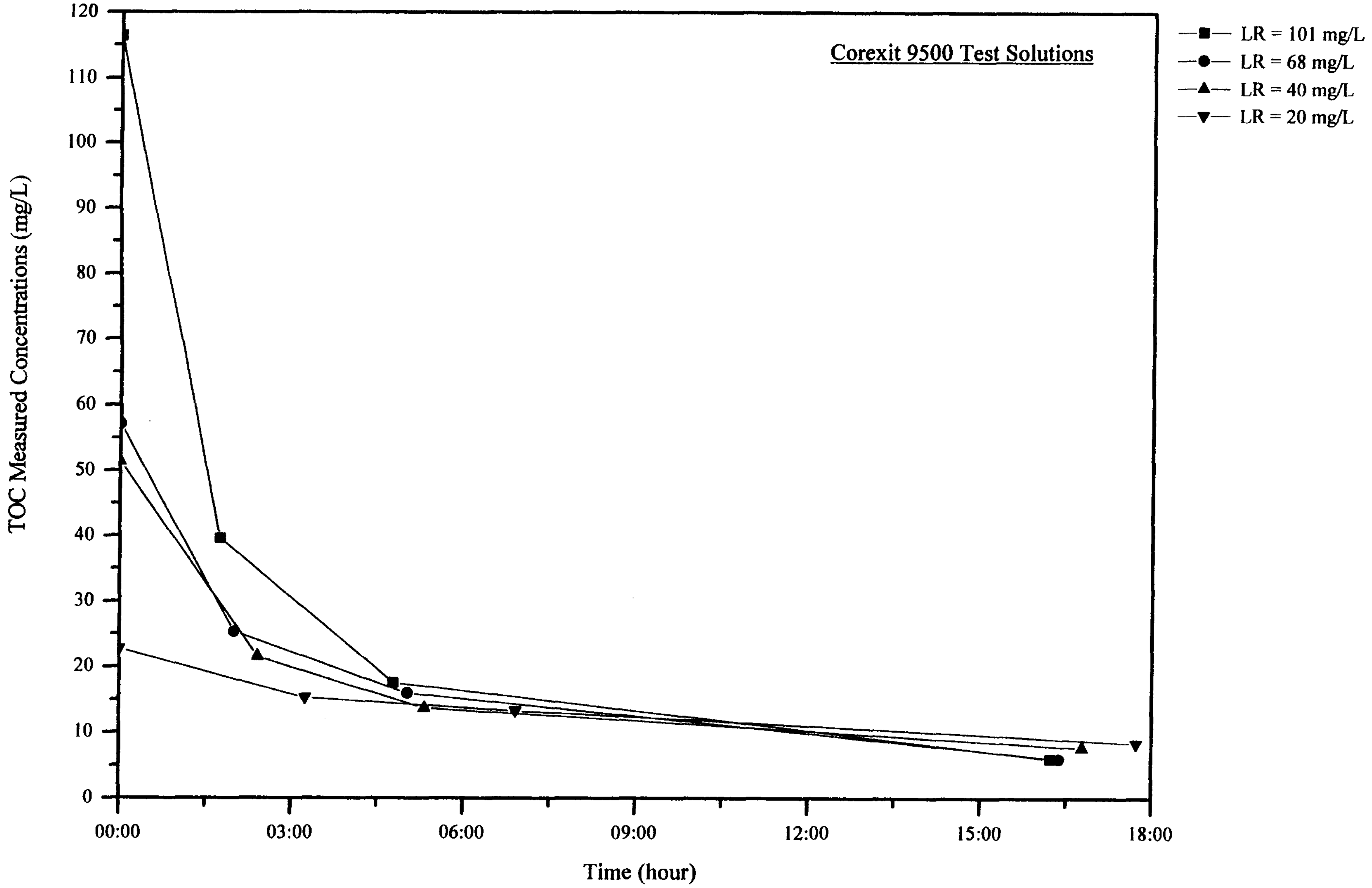

Fig. 1-3a. Concentration decline in spiked exposure tests of Corexit 9500 test solutions of different loading rates (LR)

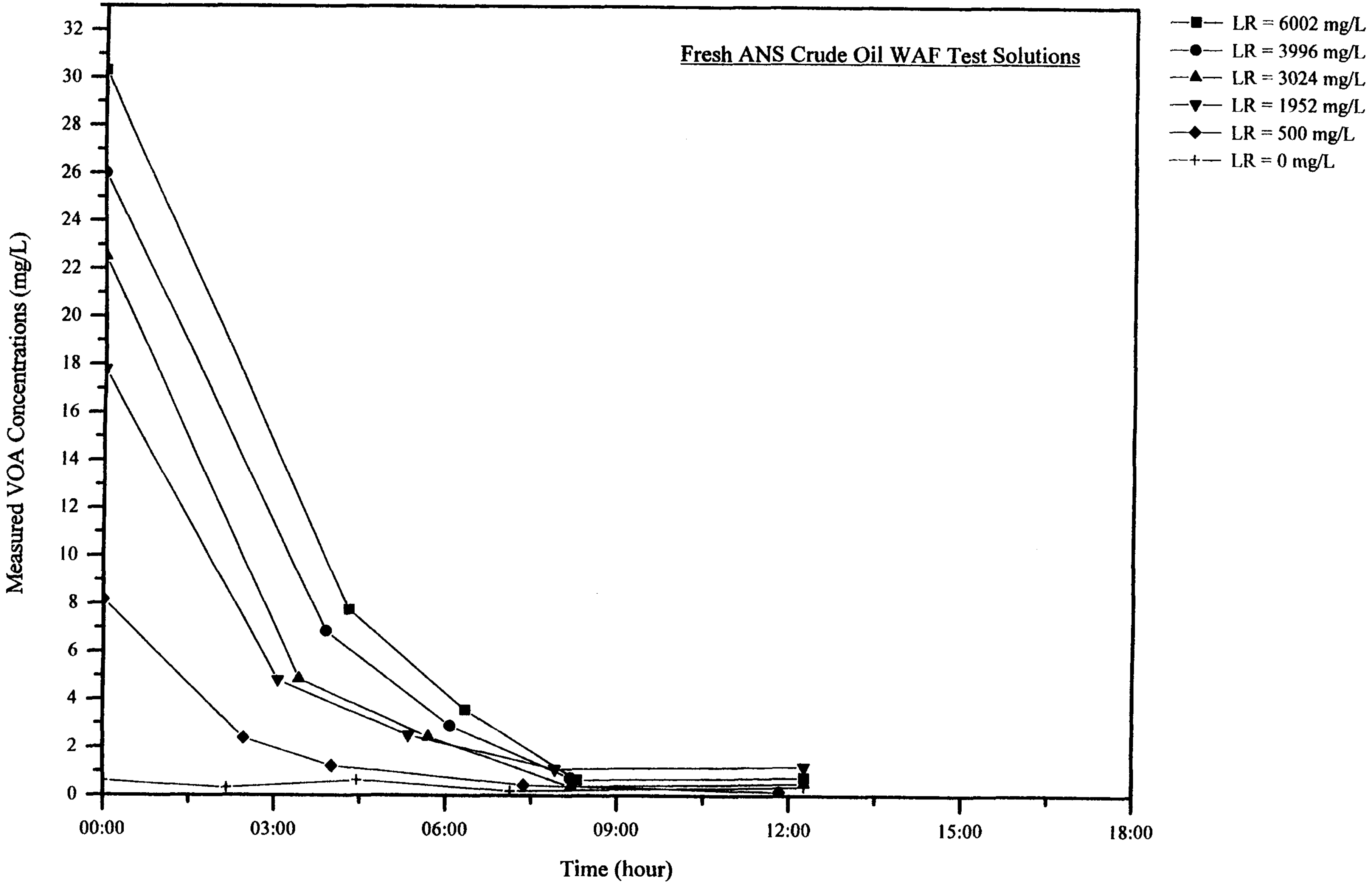

Fig. 1-3b. Concentration decline in spiked exposure tests of fresh ANS WAF test solutions of different loading rates (LR)

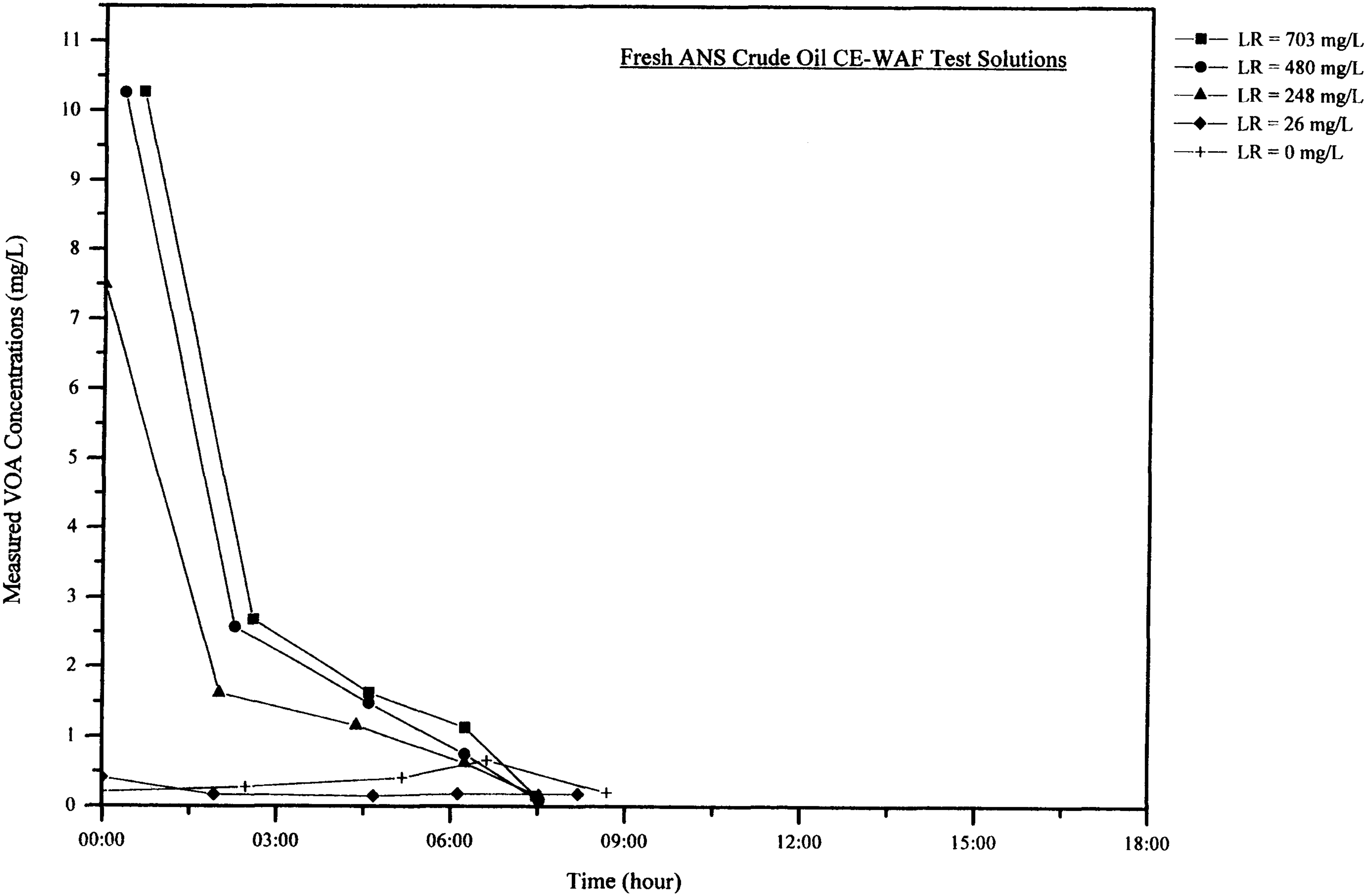

Fig. 1-3c. Concentration decline in spiked exposure tests of fresh ANS CE-WAF test solutions of different loading rates (LR)

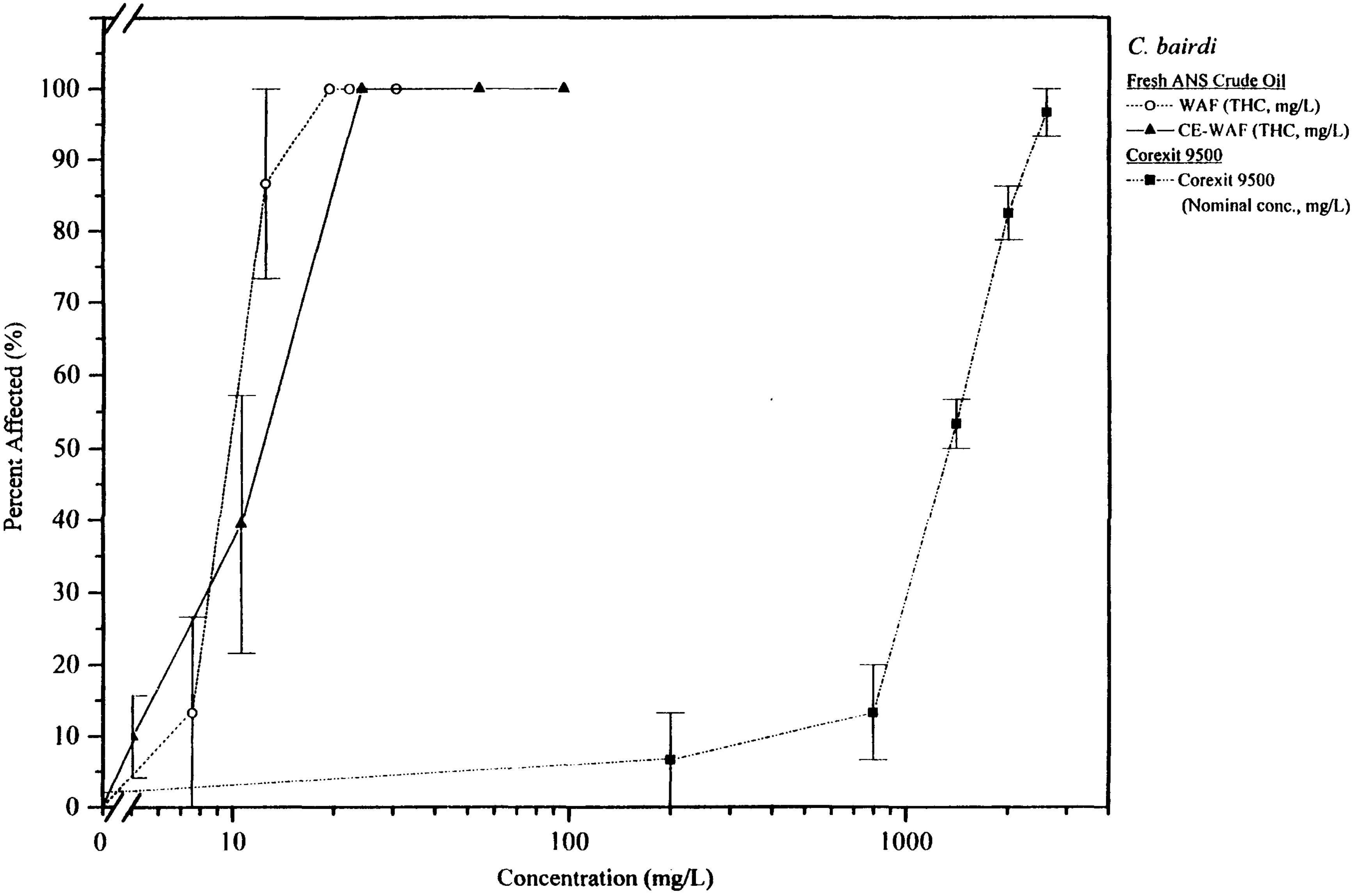

Fig. 1-4a. Dose-response relationships for *C. bairdi* to dispersant and fresh ANS spiked exposure tests. Symbols are mean ± SE for each concentration (n = 3)

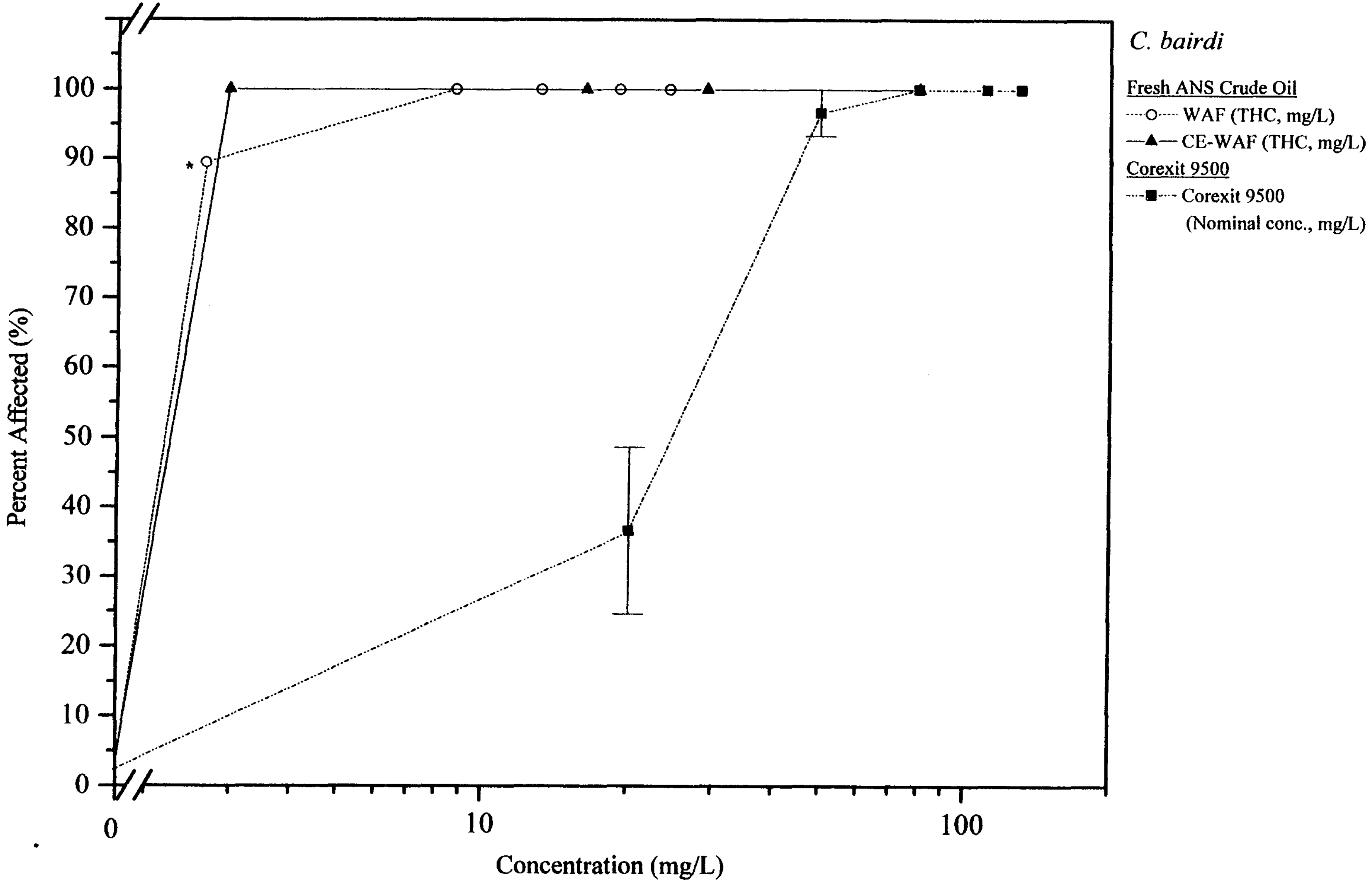

Fig. 1-4b. Dose-response relationships for *C. bairdi* to dispersant and fresh ANS continuous exposure tests. Symbols are mean ± SE for each concentration (n = 3, except where noted by "*" where SE could not be calculated)

Table 1-5. Summary of the ranges of dispersant and fresh oil loading rates (mg/L) and respective measured THC (C_6-C_{36}) concentrations (mg/L) for oil solutions used in spiked and continuous exposure tests

			C. bairdi		*M. bahia*		*M. beryllina*	
Test Solution	Exposure Regime	Range	Loading Rate	Measured THC	Loading Rate	Measured THC	Loading Rate	Measured THC
Corexit 9500	Spiked	Low	200	-	300	-	20	-
		High	2600	-	1900	-	120	-
	Continuous	Low	20	-	15	-	20	-
		High	130	-	80	-	120	-
ANS WAF	Spiked	Low	200	7.56	140	3.14	500	11.2
		High	2500	30.6	2500	12.7	6000	33.1
	Continuous	Low	50	2.47	25	0.91	260	6.44
		High	1000	24.3	750	5.61	4000	26.4
ANS CE-WAF	Spiked	Low	100	5.02	26	0.22	148	8.72
		High	1850	96.2	700	31.6	400	18.6
	Continuous	Low	30	1.70	8	0.45	100	3.45
		High	700	80.2	490	23.9	300	16.3
PBCO WAF	Spiked	Low	-	-	-	-	990	8.03
		High	-	-	-	-	8150	19.9
	Continuous	Low	-	-	-	-	500	4.17
		High	-	-	-	-	6050	16.1
PBCO CE-WAF	Spiked	Low	-	-	-	-	200	7.68
		High	-	-	-	-	820	26.3
	Continuous	Low	-	-	-	-	100	3.10
		High	-	-	-	-	420	22.6

Median-effect concentrations are presented in Table 1-6. EC_{50} estimates for *C. bairdi* Corexit 9500 tests under spiked and continuous exposures are 1266.84 and 23.76 mg/L, respectively. Having non-overlapping fiducial limits (*i.e.*, confidence intervals) suggests that values are significantly different, meaning that spiked and continuous exposure concentrations are significantly different. For water-accommodated fractions of fresh ANS crude oil, EC_{50} estimates were 9.73 and 2.54 mg/L for spiked and continuous exposures, respectively. The continuous exposure WAF test yielded results that did not meet the assumptions necessary to calculate the estimated EC_{50} using probit or TSK analyses; therefore, the value was determined using the graphical method, where fiducial limits are not available (Webber, 1993). For chemically-enhanced water-accommodated fractions of fresh ANS crude oil, the estimated EC_{50} for spiked exposure was 10.72 mg/L and for continuous exposure was 1.30 mg/L. No partial effect (*i.e.*, values for percent affected between, but not equal to 0 and 100%) was observed in the continuous exposure CE-WAF test, making use of the graphical method necessary to estimate the median-effect concentration. Confidence limits cannot be calculated when the graphical method is used.

Toxicity values were also calculated using the loading rates required to produce effective solutions (*i.e.*, those that produce a response by the organisms). When presented in this manner, these values are referred to as EL_{50} (effective loading to 50 percent of the population). These values are presented in Table 1-7 alongside the EC_{50} values. Estimates for EL_{50} values for *C. bairdi* exposed to WAF solutions are 285 mg/L and 12.48 mg/L for spiked and continuous exposures, respectively. For CE-WAF

Table 1-6. Acute 96-hour median lethal and effect concentration (mg/L) estimates (95% confidence limits) for Corexit 9500, WAF, and CE-WAF fresh ANS and PBCO tests

Test Solution	*C. bairdi* EC_{50} Values Spiked Exposure	*C. bairdi* EC_{50} Values Continous Exposure	*M. bahia* LC_{50} Values Spiked Exposure	*M. bahia* LC_{50} Values Continous Exposure	*M. beryllina* LC_{50} Values Spiked Exposure	*M. beryllina* LC_{50} Values Continous Exposure	*M. beryllina* LC_{50} Values Spiked Exposure	*M. beryllina* LC_{50} Values Continous Exposure
Corexit 9500[*]	1266.84[‡] (1030.88, 1556.82) a = 8.33%	23.76[†] (19.26, 28.40)	330.72[‡‡]	29.06[‡] (24.85, 33.99) a = 3.57%	115.18[‡] (105.75, 125.46) a = 40%	54.67[†] (46.70, 62.94)		
	ANS						PBCO	
WAF[**]	9.73[†] (8.83, 10.68)	2.54[‡‡]	8.21[†] (7.05, 9.27)	2.61[†] (1.40, 3.24)	26.36[‡] (25.54, 27.22) a = 0%	15.59[‡] (13.98, 17.38) a = 0%	>19.86[***]	14.81[†] (9.79, 68.75)
CE-WAF[**]	10.72[†] (9.08, 12.72)	1.3[‡‡]	5.08[‡] (3.13, 8.26) a = 0%	1.40[‡] (1.04, 1.88) a = 0%	12.22[‡] (7.79, 19.17) a=40%	12.42[‡] (11.40, 13.54) a=0%	12.29[‡] (10.90, 13.86) a = 6.67%	4.57[‡] (4.16, 5.02) a = 20%

Notes:

[*] Corexit 9500 values based on loading rate in mg/L

[**] WAF and CE-WAF values based on total hydrocarbon content (THC) in mg/L

[***] Highest concentration tested had a 8,152 mg/L loading rate

Statistical Methods Used:

[†] Probit analysis

[‡] Trimmed Spearman-Karber analysis, a = % trim

[‡‡] Graphical method, 95% confidence limits not available (Webber, 1993)

Table 1-7. Acute 96-hour median lethal and effect concentration estimates (mg/L) based on measured concentrations and oil loading rates (95% confidence limits)

		ANS				PBCO			
		WAF		CE-WAF		WAF		CE-WAF	
Species	Exosure type	Measured THC Conc. LC_{50}	Loading Rate LL_{50}	Measured THC Conc. LC_{50}	Loading Rate LL_{50}	Measured THC Conc. LC_{50}	Loading Rate LL_{50}	Measured THC Conc. LC_{50}	Loading Rate LL_{50}
C. bairdi †	Spiked	9.73 (8.83, 10.68)	285 (249, 325)	10.72 (9.08, 12.72)	203 (174, 236)	*	*	*	*
	Continous	2.54 (N/A)	12.48 (N/A)	1.30 (N/A)	5.16 (N/A)	*	*	*	*
M. bahia	Spiked	8.21 (7.05, 9.27)	654 (488, 875)	5.08 (3.13, 8.26)	127 (101, 161)	*	*	*	*
	Continous	2.61 (1.40, 3.24)	160 (63, 217)	1.40 (1.04, 1.88)	30 (22, 41)	*	*	*	*
M. beryllina	Spiked	26.36 (25.54, 27.22)	3520 (3326, 3725)	12.22 (7.79, 19.17)	272 (171, 425)	>19.86 (N/A)	>8152 (N/A)	12.29 (10.90, 13.86)	272 (230, 312)
	Continous	15.59 (13.98, 17.38)	1641 (1317, 2044)	12.42 (11.40, 13.54)	227 (212, 244)	14.81 (9.79, 68.75)	4965 (2293, 117423)	4.57 (4.16, 5.02)	130 (115, 149)
Vibrio fischeri †	N/A	4.2 +/- 0.25	310 +/- 41	2.0 +/- 0.17	29 +/-2.6	3.7 +/- 0.29	960 +/-160	1.9 +/- 0.09	46 +/- 4.0

† Measured Conc. as EC_{50}, Loading Rate as EL_{50}, both in mg/L

* Not tested

N/A = not available; confidence limits could not be calculated solutions, these values are slightly smaller at 203 mg/L and 5.16 mg/L for spiked and continuous exposures, respectively. Because dispersant-only solutions are calculated using nominal concentrations, those presented in Table 1-6 could also be considered as EL_{50} values. Thus, toxicity values for dispersant-only solutions are not presented in Table 1-7.

Temporal responses (*i.e.*, EL_{50} values for hours 24, 48, and 72) to test solution exposure are not available for *C. bairdi*, since evaluation of their health status required careful (microscopic) observations that could not be made during a test. These data are available for *M. bahia* and *M. beryllina* and are presented in the next section.

Mysidopsis bahia Tests

Dose-response relationships for *M. bahia* tended to be sigmoidal with most tests showing an increase in mortality with increasing concentration, with one exception in the spiked exposure test of Corexit 9500 solutions (Figures 1-5a and 1-5b). The loading rates used to produce a lethal effect in at least 50 percent of the animals in the spiked exposure dispersant-only tests were about 20 times greater than those used for continuous exposure tests (Table 1-5). The difference between loading rates used for oiled solutions ranged from 1.5 to 5.5 times greater for spiked exposures than for continuous exposures. WAFs were prepared using loading rates 1.5 to 5.0 times greater than those for CE-WAFs.

Estimated median-lethal concentrations listed in Table 1-6 also include the respective 95% confidence limits where available, which for *M. bahia* tests are fairly narrow. Estimated LC_{50} values for Corexit 9500 tests under spiked and continuous

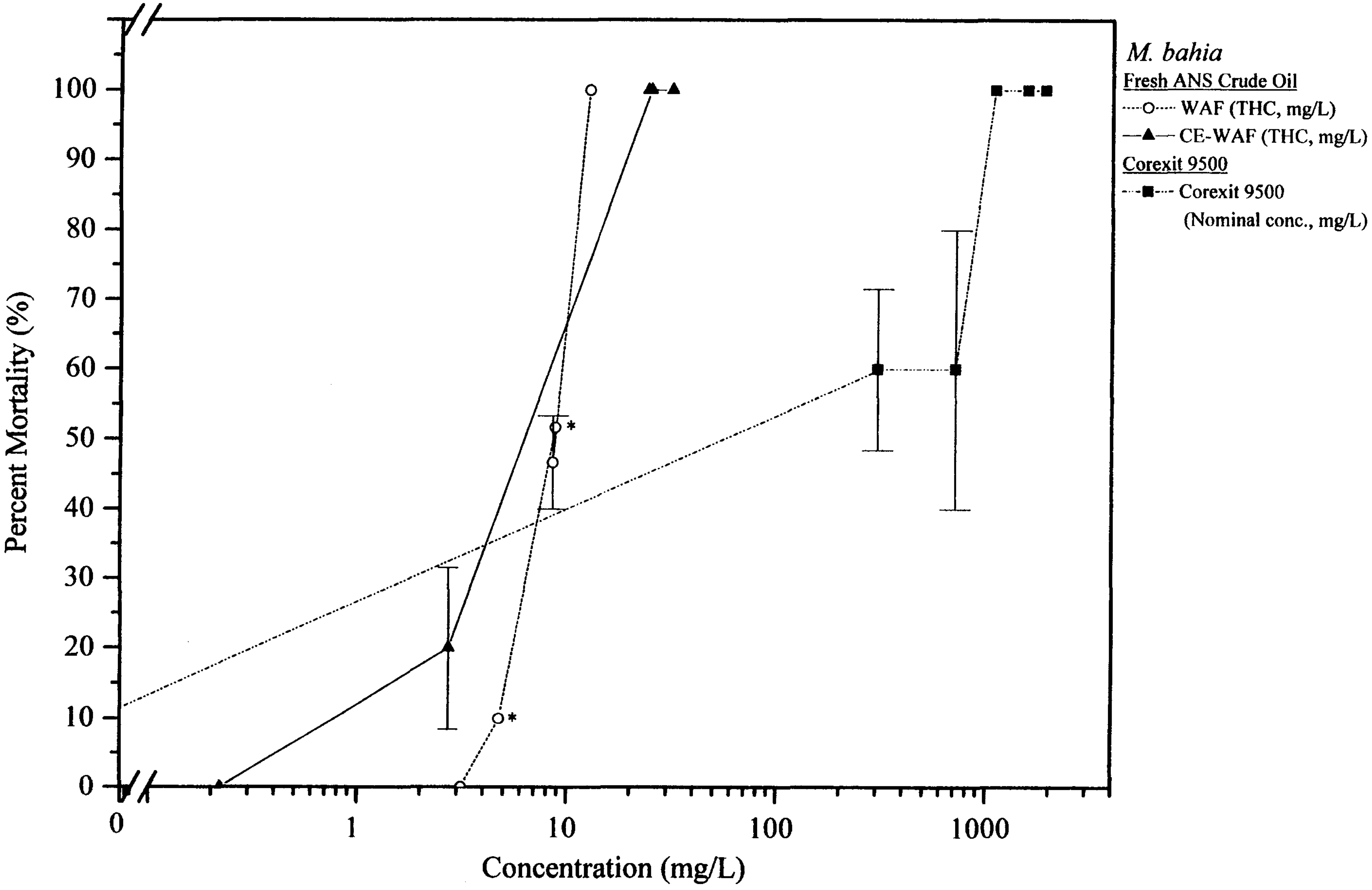

Fig. 1-5a. Dose-response relationships for *M. bahia* to dispersant and fresh ANS spiked exposure tests. Symbols are mean ± SE for each concentration (n = 3, except where noted by "*" where SE could not be calculated)

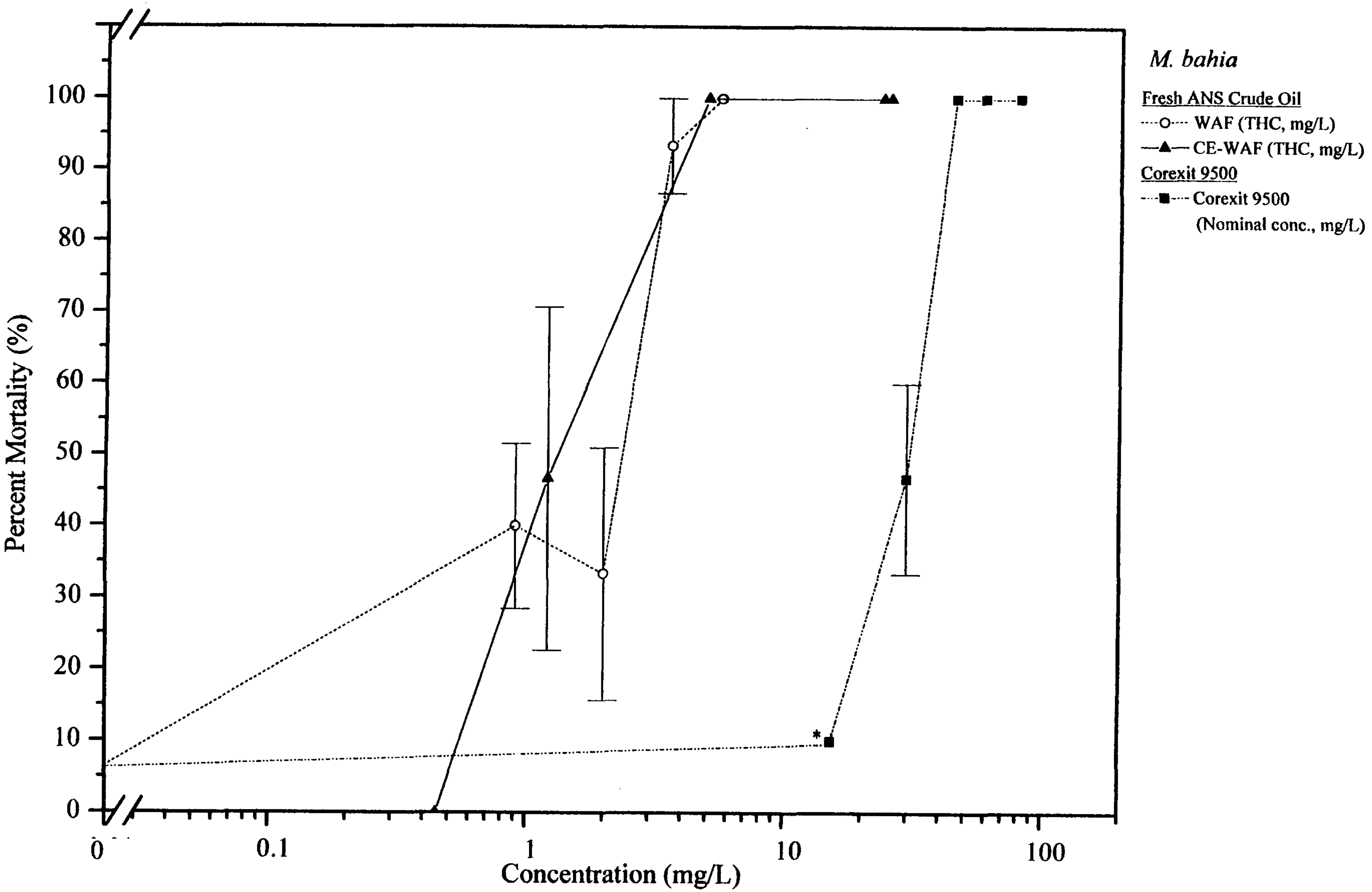

Fig. 1-5b. Dose-response relationships for *M. bahia* to dispersant and fresh ANS continuous exposure tests. Symbols are mean ± SE for each concentration (n = 3, except where noted by "*" where SE could not be calculated)

exposures were 330.72 and 29.06 mg/L, respectively. Two partial mortalities were observed in the Corexit 9500 spiked exposure test; however, both were of equal value. This caused estimates of an LC_{50} by the statistical methods of probit and TSK to be unreliable. As a result, the LC_{50} reported was estimated using graphical analysis of mortality data up to and including the first 100 percent mortality. WAF tests resulted in estimates of 8.21 and 2.61 mg/L for LC_{50} values under spiked and continuous exposures, respectively. Estimates for CE-WAFs were slightly less than those for WAFs at 5.08 and 1.40 mg/L for the respective spiked and continuous exposures. Non-overlapping confidence limits between spiked and continuous exposure tests suggest a significant difference between acute toxic response of *M. bahia* under the two exposure regimes. However, comparison between WAFs and CE-WAFs via LC_{50} values from either spiked or continuous exposure tests reveal a slight overlap, indicating no significant difference between the toxicity of these solution types for *M. bahia.*

Qualitative estimates of temporal median-lethal concentrations at hours 24, 48, 72, and 96 of the 96-hour tests based upon the dispersant and oil loading rates (nominal concentrations) are presented in Tables 1-8 and 1-9. The values for days one through three are "qualitative," because these data are based on observations made by peering into the flow-through chambers where a clear viewing of the animals is somewhat obscured. All tests indicate that *M. bahia* experience an increase in mortality over time to test solution exposure, except in the CE-WAF spiked exposure test. For the CE-WAF spiked exposure test, the response of *M. bahia* occurred during the first 24 hours of the test, and remained stable throughout. However for the WAF spiked exposure test, *M. bahia*

Table 1-8. Daily median-lethal loading (LL_{50},mg/L) estimates (95% confidence limits) for Corexit 9500, WAF, and CE-WAF fresh oil spiked exposure tests

	M. bahia				*M. beryllina*				*M. beryllina*			
	Observation time (hr)				Observation time (hr)				Observation time (hr)			
Test Solution	24	48	72	96	24	48	72	96	24	48	72	96
Corexit 9500	545 (263, 1130)	544 (265, 1117)	331*	331*	115 (106, 125)	115 (106, 125)	115 (106, 125)	115 (106, 125)				
	Fresh ANS Crude Oil				Fresh ANS Crude Oil				Fresh PBCO			
WAF	717 (549, 937)	654 (488, 875)	654 (488, 875)	654 (488, 875)	3520 (3326, 3725)	3520 (3326, 3725)	3520 (3326, 3725)	3520 (3326, 3725)	>8152	>8152	>8152	>8152
CE-WAF	127 (101, 161)	127 (101, 161)	127 (101, 161)	127 (101, 161)	272 (171, 425)	272 (171, 425)	272 (171, 425)	272 (171, 425)	272 (230, 312)	272 (230, 312)	272 (230, 312)	272 (230, 312)

* Graphical method, 95% confidence limits not available (Webber, 1993)

Table 1-9. Daily median-lethal loading (LL_{50},mg/L) estimates (95% confidence limits) for Corexit 9500, WAF, and CE-WAF fresh oil continuous exposure tests

	M. bahia				*M. beryllina*				*M. beryllina*			
	Observation time (hr)				Observation time (hr)				Observation time (hr)			
Test Solution	24	48	72	96	24	48	72	96	24	48	72	96
Corexit 9500	39 (33, 45)	31 (27, 37)	29 (25, 34)	29 (25, 34)	63 (54, 71)	59 (49, 68)	56 (47, 65)	55 (47, 63)				
	Fresh ANS Crude Oil				Fresh ANS Crude Oil				Fresh PBCO			
WAF	209 (77, 320)	209 (77, 320)	179 (93, 248)	160 (63, 217)	3180 (2204, 4587)	1970 (1620, 2395)	1935 (1593, 2349)	1641 (1317, 2044)	>6054	>6054	>6054	4965 (2293, 117423)
CE-WAF	110 (80, 150)	35 (26, 47)	35 (26, 47)	30 (22, 41)	255 (243, 268)	249 (236, 262)	227 (212, 244)	227 (212, 244)	177 (157, 199)	177 (157, 199)	146 (138, 156)	130 (115, 149)

response stabilized at the observation time of 48 hours. For all continuous exposure tests, mortality steadily increased over the course of the 96-hour assay. Similar to spiked exposure tests, CE-WAF tests experienced the greatest change in toxicity approximately 24 hours earlier than WAF tests (between 24-48 hours vs. 48-72 hours). These 96-hour values (based upon loading rates) are also presented alongside those calculated using measured THC concentrations (mg/L) in Table 1-7.

Menidia beryllina Tests

The same concentrations of Corexit 9500 were tested in both the spiked and continuous exposure tests (Table 1-5). Oil loading rates for spiked exposures of both fresh ANS and PBCO ranged from 1.3 to 2.0 times greater than those used in continuous exposure tests. For fresh ANS, WAFs were prepared using 2.6 to 5.0 times more crude oil than in CE-WAFs, and for PBCO, WAFs used 4.5 to 10.0 times more oil than CE-WAFs. Oil loading rates were 1.4 to 2.0 times more PBCO than ANS for WAFs, and 1.0- to 2.0-fold more PBCO than ANS for CE-WAFs.

Dose-response relationships for *M. beryllina* tests using fresh ANS crude oil and some with PBCO were often not monotonically increasing with increasing concentration (Figures 1-6a, 1-6b, 1-7a, and 1-7b). Results from chemical analyses (VOA and TPH) of solutions occasionally indicated that measured concentrations of total hydrocarbons in solution were lower than those measured in solutions prepared with less initial crude oil. This is likely due to the presence of oil droplets in samples of lower concentrations, causing those measurements to be higher. This, in addition to the variability of the

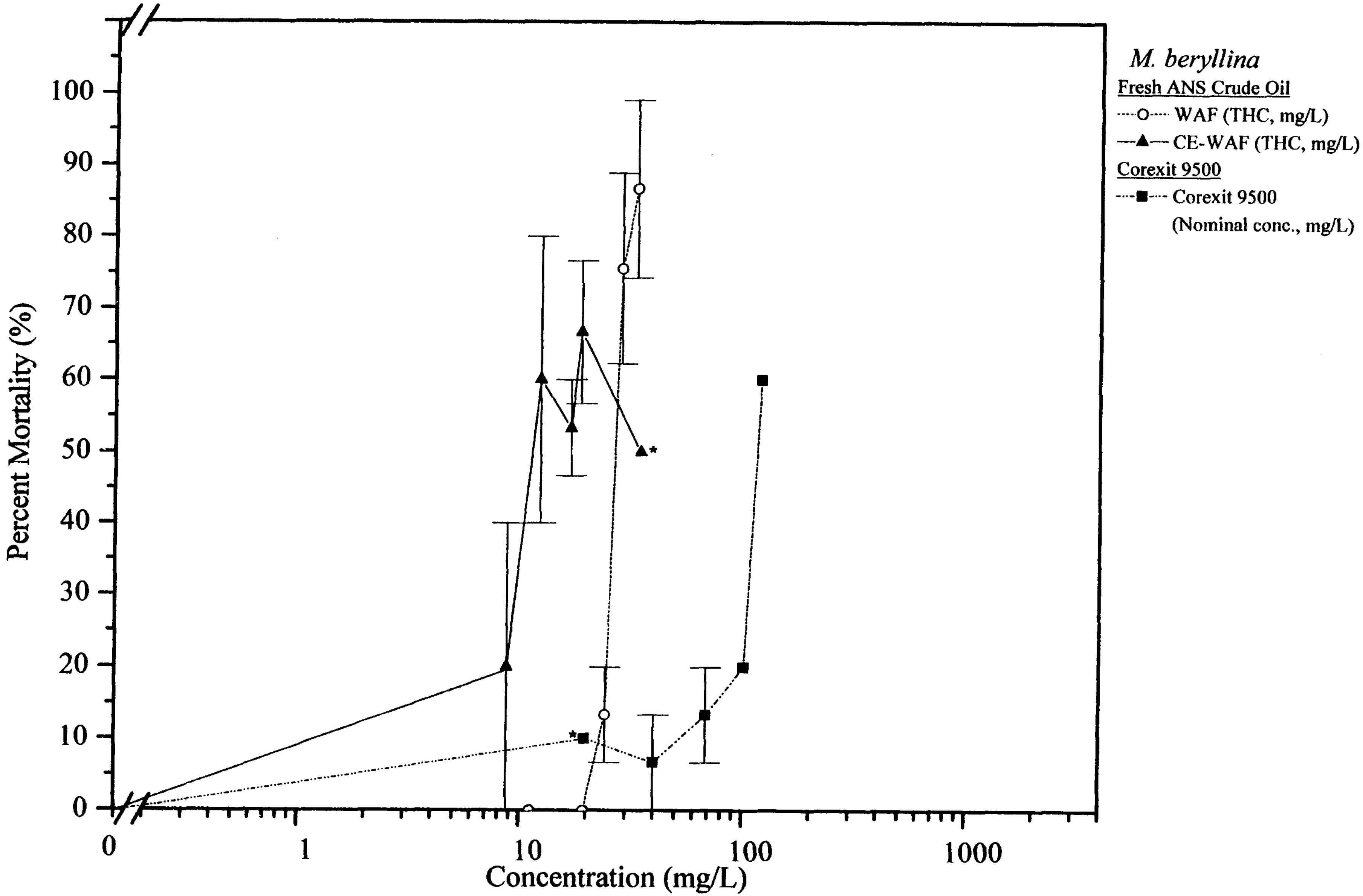

Fig. 1-6a. Dose-response relationships for *M. beryllina* to dispersant and fresh ANS spiked exposure tests. Symbols are mean ± SE for each concentration (n = 3, except where noted by "*" where SE could not be calculated)

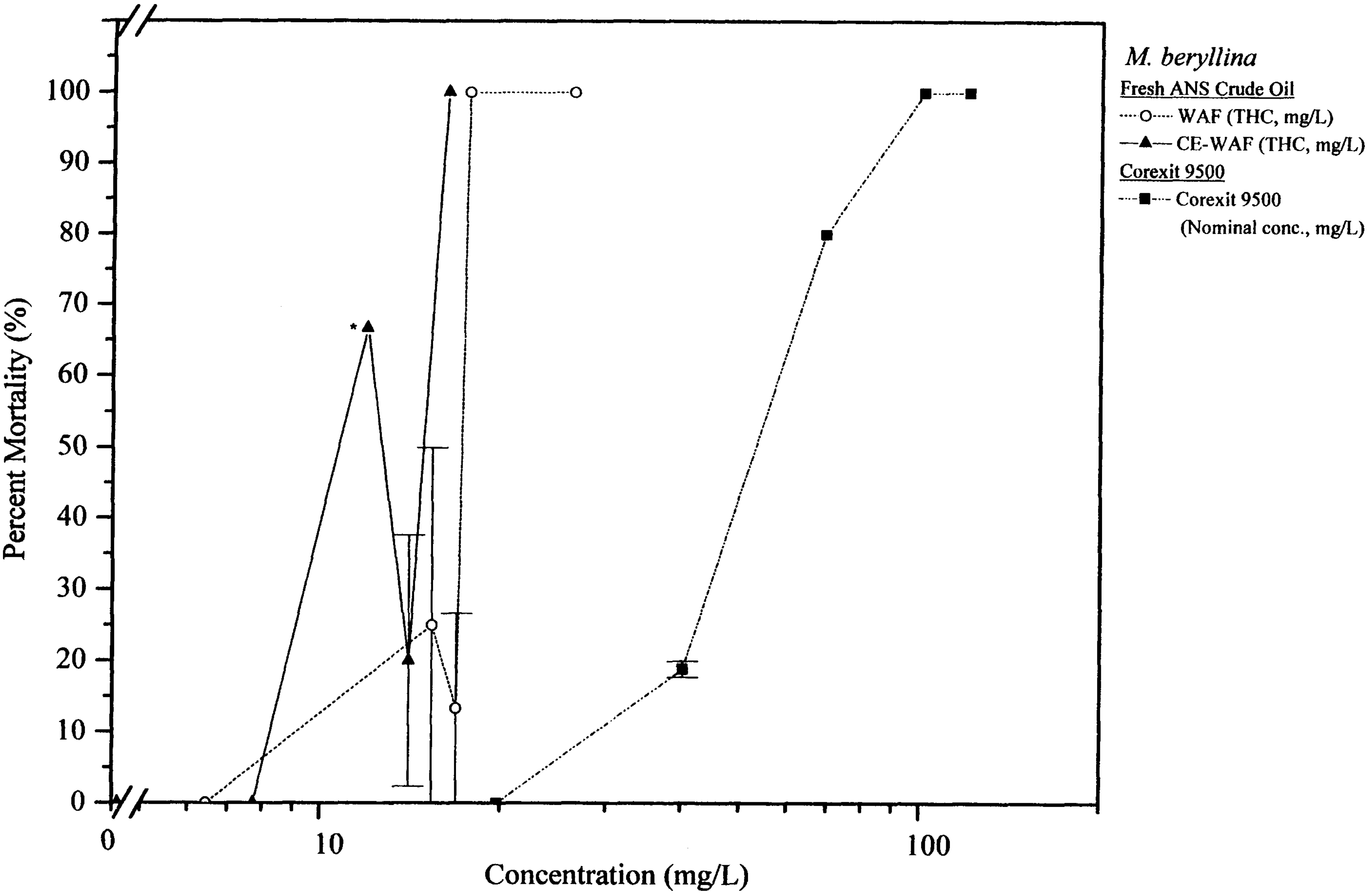

Fig. 1-6b. Dose-response relationships for *M. beryllina* to dispersant and fresh ANS continuous exposure tests. Symbols are mean ± SE for each concentration (n = 3, except where noted by "*" where SE could not be calculated)

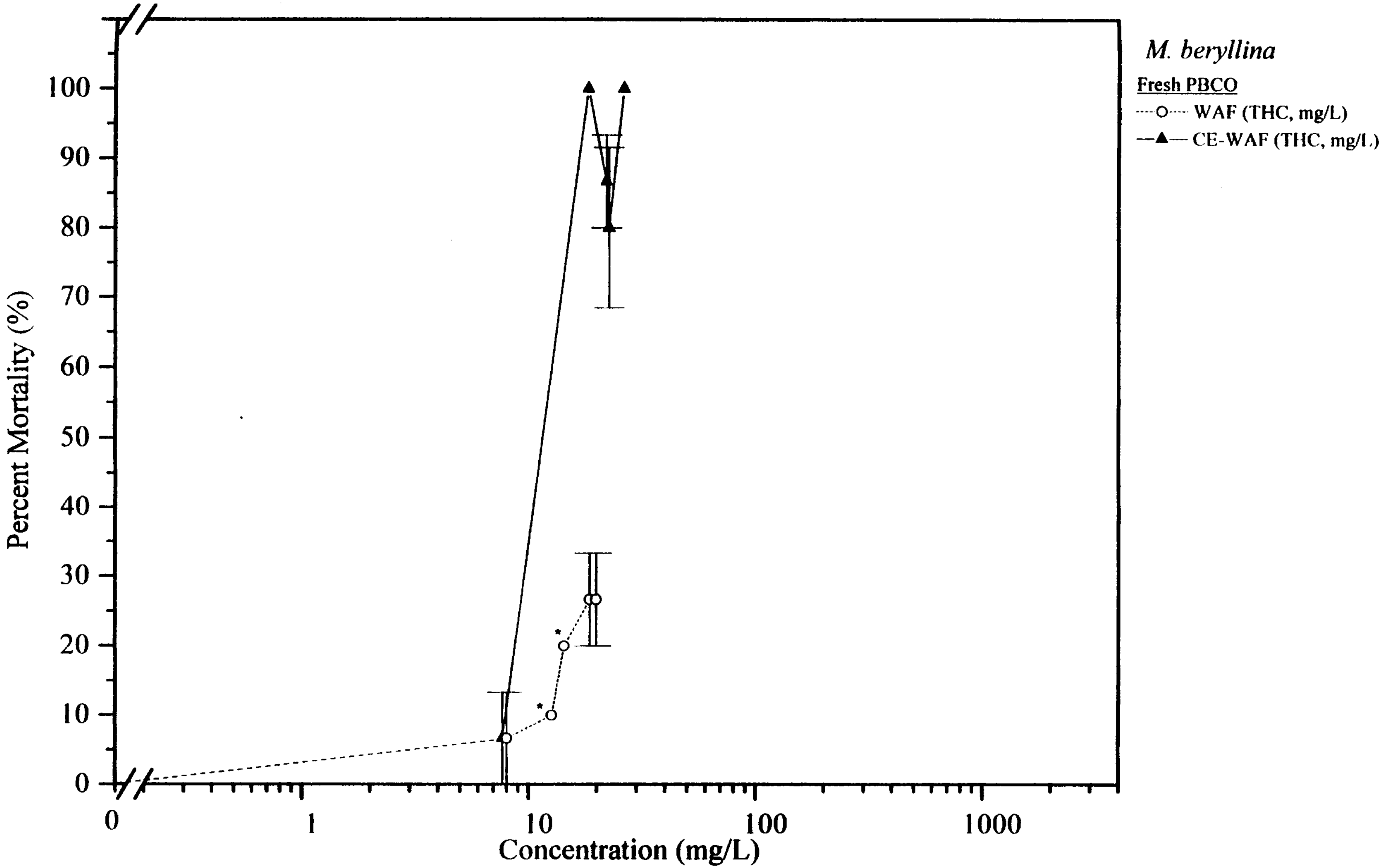

Fig. 1-7a. Dose-response relationships for *M. beryllina* to fresh PBCO spiked exposure tests.
Symbols are mean ± SE for each concentration (n = 3, except where noted by "*" where SE could not be calculated)

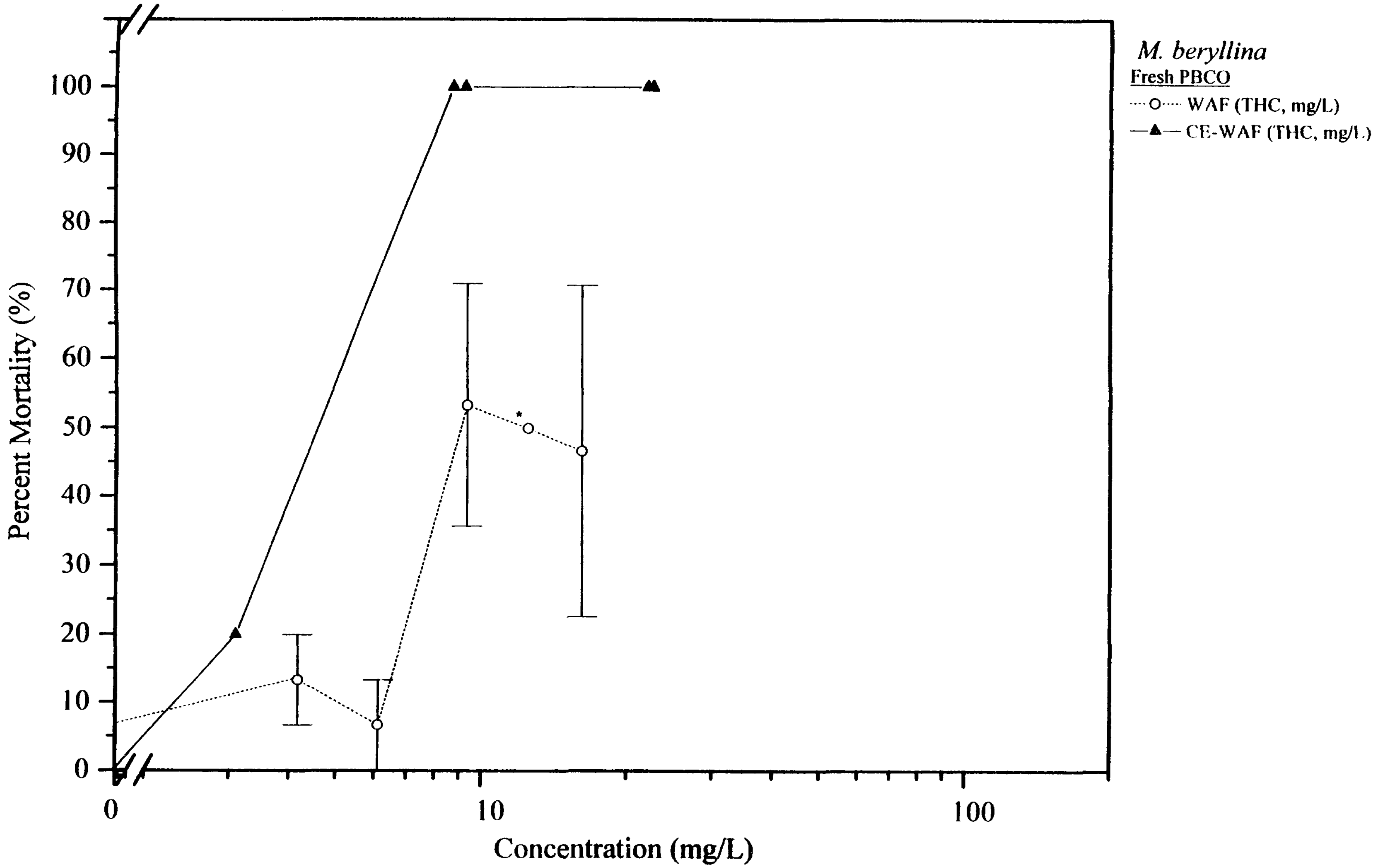

Fig. 1-7b. Dose-response relationships for *M. beryllina* to fresh PBCO continuous exposure tests.
Symbols are mean ± SE for each concentration (n = 3, except where noted by "*" where SE could not be calculated)

response of *M. beryllina* to the test solutions, contributes to the shape of these curves and non-monotonicity (Figures 1-6a and 1-6b). Dose-response relationships for Corexit 9500 tests under both spiked and continuous exposures and CE-WAFs of PBCO under continuous exposure were typically sigmoidal.

Despite the variations from a typical sigmoidal curve, assumptions necessary to estimate median-lethal concentrations using probit or TSK analyses were satisfied. Estimated LC_{50} values for both fresh ANS and PBCO are listed in Table 1-5. For Corexit 9500 tests, the estimated LC_{50} for *M. beryllina* was 115.18 mg/L for spiked exposure, and 54.67 mg/L for continuous exposure. Estimated LC_{50} values for WAFs of fresh ANS were 26.36 and 15.59 mg/L for spiked and continuous exposures, respectively. For CE-WAF spiked exposure the estimated LC_{50} was 12.22 mg/L, and 12.42 mg/L for continuous exposure. An estimated LC_{50} was not calculable for the WAF PBCO test since the highest percent mortality observed in the test was 27% at an oil loading rate of 8151 mg/L. As a result, this LC_{50} is reported as an inequality. WAFs of PBCO resulted in estimated median-lethal concentrations of >19.86 mg/L and 14.81 mg/L for spiked and continuous exposures, respectively. The estimated LC_{50} values for CE-WAFs of PBCO were 12.29 and 4.57 mg/L for spiked and continuous exposures, respectively. Non-overlapping fiducial limits for median-lethal concentrations of dispersant-only and fresh ANS WAF spiked and continuous exposure tests suggest that the LC_{50} values for these two types of regimes are significantly different. The converse is true for spiked and continuous exposures of CE-WAF solutions made with fresh ANS crude oil – there is no significant difference between LC_{50} values of the two exposures. Comparison of 95%

confidence limits between the solution types of dispersant-only, and WAFs and CE-WAFs of fresh ANS under either spiked or continuous exposure reveal non-overlapping limits, suggesting the LC_{50} values from these tests are significantly different from one another. Unfortunately, comparisons to PBCO WAFs for significance of LC_{50} values are not possible since the LC_{50} is reported as a greater-than value. However, LC_{50} values for PBCO CE-WAF solutions were significantly different under spiked and continuous exposures. CE-WAF solutions of PBCO were more toxic than those of WAF, although this comparison for WAFs under spiked exposure is somewhat extrapolated since this is a greater-than value. Comparisons between solutions made from the different oil types (ANS and PBCO) suggest no significant difference in the toxicity, with one exception. That exception is with PBCO CE-WAF solutions under continuous exposure where a smaller LC_{50} value suggests this solution was more toxic than the ANS CE-WAF (also under continuous exposure).

Temporal responses by *M. beryllina* under spiked exposure tests to all solution types (dispersant, fresh ANS, and PBCO) stabilized within the first 24 hours of the 96-hour test (Table 1-8). The estimated LL_{50} values for CE-WAF solutions made with fresh ANS and PBCO were identical, differing only in associated fiducial limits. Under continuous exposure to all solutions, *M. beryllina* exhibited a steady increase in mortality over the course of the 96-hour test (Table 1-9). Exceptions include responses to CE-WAFs of fresh ANS where estimated LL_{50} values stabilized at the observation time of 72 hours, and the WAFs of PBCO where data did not satisfy the assumptions necessary to calculate an estimated median-lethal concentration, resulting in this value to be reported as an inequality.

Microtox® Assay

Mean 5-minute EC_{50} values obtained by the Microtox® system were calculated by pooling all data available (samples collected from *C. bairdi, M. bahia,* and *M. beryllina* tests) for a particular test solution from both spiked and continuous exposure tests (Table 1-10). The data from all individual tests used to calculate the mean EC_{50} values are found in Appendix J. Mean EC_{50} values (Table 1-10) were calculated based on all possible representations of the test material's concentration in solution: 1) measured concentrations of volatile organic analytes (VOA); 2) total petroleum hydrocarbons (TPH); 3) total hydrocarbon content (THC; defined as VOA + TPH); and 4) loading rates (nominal concentrations). No matter what fraction was used to calculate the toxicity data, for any given oil type, EC_{50} values for WAF and CE-WAF were always significantly different (t-test; $P < 0.05$). When the data were standardized to VOA or to loading rates, CE-WAF solutions were more toxic than WAF solutions. When standardized to TPH, the opposite trend was seen. When standardized to THC, CE-WAF solutions were more toxic than WAF for fresh oil. Dispersant-only solutions were relatively low in toxicity (mean $EC_{50} = 220 \pm 26$ mg/L). Toxicity appeared to be strongly related to the solubilities of the hydrocarbon fractions measured.

Table 1-10. Mean 5-minute EC_{50} values obtained by the Microtox Toxicity Assay. Values were calculated based on measured hydrocarbon fractions and on total oil added (loading rates)

Hydrocarbon Fraction	Oil Type	Mean +/- Std. Error (SE) EC_{50} (mg/L)*					
		WAF	SE	n	CE-WAF	SE	n
VOA	Fresh ANS	4.2	0.25	43	0.86	0.09	39
	PBCO	3.6	0.29	20	0.69	0.04	15
TPH	Fresh ANS	0.06	0.01	28	1.0	0.13	28
	PBCO	0.10	0.01	18	1.2	0.10	15
THC	Fresh ANS	4.2	0.25	43	2.0	0.17	41
	PBCO	3.7	0.29	20	1.9	0.09	19
Loading Rates	Fresh ANS	310	41	34	29	2.6	33
	PBCO	960	160	13	46	4.0	13

Dispersant only: EC_{50} (mg/L) = 220 +/- 26

n = number of tests

* For each oil type and a given hydrocarbon fraction used to standardize the data, the EC_{50} value for WAF was significantly different ($P < 0.05$) from that for CE-WAF

Toxicity Value Comparisons: Test Solutions

Overall, for comparisons made using toxicity values calculated based on both measured concentrations (LC_{50} and EC_{50} values) and loading rates (LL_{50} and EL_{50} values), for all species and exposure regimes, dispersant-only solutions were the least toxic followed by WAF and CE-WAF solutions of fresh oil (n = 12 out of N = 20; only 1 of the 12 was not significantly different, and 2 of the 12 were without fiducial limits for significance testing). WAF solutions were more often less toxic than CE-WAF solutions (n = 17 out of N = 20; where 3 of the 17 were not significantly different, and 2 of 17 were without fiducial limits for significance testing).

For test solution comparisons made using toxicity values based on measured concentrations only (LC_{50} and EC_{50} values; denoted as LC_{50}/EC_{50}), dispersant-only solutions were least toxic in all cases (n = 10 out of N = 10; where only 1 was not significant, and 2 did not have fiducial limits for significance testing). WAF solutions were less toxic than CE-WAF solutions in most cases (n = 9 out of N = 10; where 3 of the 9 were not significantly different, and 2 of the 9 were without fiducial limits for significance testing). These trends are consistent with those above for the combined data sets of LC_{50} and EC_{50} values and LL_{50} and EL_{50} values.

Comparisons made using toxicity values based upon the loading rates only (LL_{50} and EL_{50} values; denoted as LL_{50}/EL_{50}), yielded three different scenarios: 1) dispersant was least toxic and CE-WAF most toxic (n = 2 out of N = 10 for *C. bairdi*); 2) WAF was least toxic and CE-WAF most toxic (n = 4 out of N = 10 for *M. bahia* and *V. fischeri* for ANS and PBCO); and 3) WAF was least toxic and dispersant most toxic (n = 4 out of N = 10 for *M. beryllina*). The most consistent trend is that WAF concentrations were least toxic for all species (n = 8 out of N = 10; where only 1 of the 8 was not significantly different), except for *C. bairdi* where dispersant-only solutions were least toxic.

Toxicity Value Comparisons: Species Sensitivities

C. bairdi were least sensitive to the dispersant solutions and most sensitive to the CE-WAF concentrations when compared using both EC_{50} and EL_{50} values (n = 3 out of N = 4; where 1 of the 3 did not have fiducial limits to test significance between the WAF and CE-WAF values). In only one case were *C. bairdi* more sensitive to a WAF solution than a CE-WAF solution, although this relationship was not significant. *M. bahia* and *V. fischeri* shared the same pattern of sensitivity. When compared using toxicity values by measured concentrations only (LC_{50}/EC_{50}), these species were again least sensitive to dispersant-only solutions and most to CE-WAF solutions (n = 4 out of N = 4; where 2 WAF and CE-WAF values were not significantly different, and 1 dispersant test did not have fiducial limits to test significance). *M. bahia* and *V. fischeri,* when compared using toxicity values by loading rates only (LL_{50}/ EL_{50}), were least sensitive to WAF solutions and most sensitive to CE-WAF solutions (n = 4 out of N = 4; where only 1 did not have fiducial limits for significance testing). According to LC_{50} values, *M. beryllina* was most sensitive to CE-WAF solutions and least to dispersant-only solutions (n = 4 out of N = 4; where only 1 did not have fiducial limits for significance testing). According to LL_{50} values, however, *M. beryllina* was most sensitive to dispersant-only solutions (although just over twice that of CE-WAF concentrations) and least sensitive to WAF solutions (n = 4 out of N = 4; all were significant).

When comparing sensitivities between species using toxicity values calculated from both measured concentrations (LC_{50}/ EC_{50}) and loading rates (LL_{50}/ EL_{50}), *M. beryllina* was most resistant overall (n = 8 out of N = 10; where 1 of the 8 was not significant), and most resistant to oil solutions (n = 8 out of N = 8; where 1 of the 8 was not significant). In most tests for all solution types and exposure regimes, *C. bairdi* was the most sensitive of all species (n = 6 out of N = 10; where 1 of the 6 was not significant, and 4 of the 6 did not have fiducial limits for significance testing). *C. bairdi* and *M. bahia* often were more sensitive than both *M. beryllina* and *V. fischeri* (n = 4 out of N = 10; all were significant). Relative to the other species evaluated, *V. fischeri* showed no clear trend to suggest that it was either more or less sensitive to the test solutions evaluated. However, results from the Microtox® Assay were consistent in predicting when and to a similar degree how much of a biological impact could be expected from these solutions. This suggests that *V. fischeri* in the Microtox® Assay may be a useful, rapid screening tool to obtain information about a material's toxicity.

DISCUSSION

Data Evaluation

The toxicity tests were designed to provide information about the relative acute toxicity of the test solutions and the sensitivities of the species evaluated. There are some aspects of the methods that differ between tests and should be considered to properly interpret the data. *Chionocetes bairdi* tests were evaluated using a behavioral endpoint of "affected," defined as diminished phototactic response, reduced ability to swim, and reduced response to touching. Tests of *Mysidopsis bahia* and *Menidia beryllina* were evaluated using death as an endpoint. A sub-lethal response (effect of reduced luminescence) of the bacterium, *Vibro fischeri*, was used in Microtox® Assays. Thus, comparisons between EC_{50} and LC_{50} values encompass responses by organisms that experience sub-lethal and lethal effects of exposure to the test solutions. For example, identical values for EC_{50} and LC_{50} for two different organisms tested with the same potential toxicant indicate that the organism reporting an LC_{50} is more sensitive, having exhibited a lethal rather than a sub-lethal response.

EC_{50} values were considered to be more appropriate than LC_{50} values for *C. bairdi* since death as an endpoint was not typically observed. Internal organ movement was observable even when the animals were obviously adversely affected and unlikely to survive. Consequently, four health categories (alive, affected, mortally affected, and dead) were developed to reflect the observations made during the tests. The most important effect observed was the diminished phototactic response and ability to successfully swim to the surface, because this indicated a reduced ability to obtain food and a potentially increased vulnerability to predation. Similar health stages of *C. bairdi* were observed by Brodersen and others (1977) in which larvae first experienced changes in their ability to swim, ranging from successfully lifting from the bottom of the chamber to merely twitching their appendages, then failure to move, and finally death.

Test solution preparation methods differed slightly among tests in loading rates and mixing energy used. All test solutions were prepared with a range of concentrations that would either bracket or cause, at minimum, a response by 50 percent of the test species. This enables calculation of median-effect concentrations, allowing comparison of the test solution's toxicity values and relative sensitivities among different species. Because the test materials differ in chemical composition, their ability to interact with saltwater and form soluble fractions to which the organisms were exposed varied. This, in addition to the influence of the preparation methods on the resulting test solutions (Girling, 1989; Maher, 1986), resulted in the requirement to use loading rates of Corexit 9500 and oil that usually were not equivalent between tests (Table 1-6).

In keeping with CROSERF protocols, mixing energies used for CE-WAF solutions were different than those used for WAF solutions (Coelho and Aurand, 1997). This is based on the necessity to provide adequate energy for good oil-dispersant contact in order to effectively disperse the oil, yet produce solutions relatively free of bulk oil droplets following a specified settling period (Singer *et al.*, 1998). Having solutions relatively free of oil droplets was important since the purpose of the toxicity tests was to evaluate the toxicity of predominately water-soluble components of non-dispersed and dispersed oil. Matching mixing energy for the solution preparation of WAF to CE-WAF solutions resulted in either formation of an emulsion in WAF concentrations, or failure to disperse the oil due to insufficient oil-dispersant contact in CE-WAF (Singer *et al.*, 1998).

Dispersant Solutions

Use of dispersant loadings in excess of the detected solubility range (>500 ppm at 7°C; >1000 ppm at 15°C; Singer *et al.*, 1996a) was necessary to produce an effect greater than 50 percent in *C. bairdi* and *M. bahia* spiked exposure tests. Because the purpose of these tests was not to evaluate the toxicity of a water soluble fraction of the dispersant chemical mixture, but rather the dispersant as a whole, these solutions were completely mixed without use of a settling period. Some solutions, made at concentrations in excess of the detected solubility range that were allowed to settle after mixing, were observed to develop a phase separation. This is likely due to coalescence of the lipophilic portion of the surfactant in Corexit 9500. Such a tendency towards a bi-phasic nature at higher concentrations complicates analysis of solutions by spectrophotometric methods. The presence of large particulates (*i.e.*, droplets) can interfere with light transmittance and alter the results in an inconsistent, unpredictable manner. Calibration curves prepared for UV-spectrophotometric analysis of dispersant solutions showed good linearity within detectable limits (*C. bairdi* maximum detect was 380 ppm, $r^2 = 0.991$, $\lambda_{max} = 236$ nm; *M. bahia* and *M. beryllina* maximum detect was 250 ppm, $r^2 = 0.995$, $\lambda_{max} = 238$ nm). For samples that were more concentrated than the maximum detectable limits, dilution into the linear range was necessary. However, this procedure was observed to be problematic, producing questionable results. This is most likely due to the limited solubility of dispersant in saltwater at higher concentrations causing non-uniform sample dilutions that may not have been representative. Dispersant-only solutions tested in this study were completely mixed prior to use in the toxicity tests producing a solution that was characteristically homogeneous. As a result, test species in these tests were initially exposed to all chemicals in the mixture which constitute the dispersant. Therefore, good agreement between nominal concentrations and measured concentrations was considered important. When results were not observed to have good agreement, an effort was made to select an alternate analytical technique to UV-spectrophotometry with which to measure these solutions. That alternate technique was total organic carbon (TOC) analysis.

The TOC equipment available had been only recently acquired, and instrument calibration was on-going during toxicity assays. Initial results from TOC analysis indicated difficulties similar to UV spectrophotometery in agreement between measured and nominal concentrations. Once calibrated, however, results showed both good linearity and concordance. TOC analysis measures the amount of carbon dioxide (CO_2) evolved from total oxidation of dissolved organic material in one of two ways. One is via gas chromatography, the other is by measurement of the change in conductivity of CO_2 absorbed in ultra-pure water, correcting first for CO_2 contributions from inorganic carbon sources (carbonate and bi-carbonate) by acid digestion (Manahan, 1994). With TOC analysis, potential error and variability in measured concentrations due to the presence of particulates in solution is less of a concern than with spectrophotometric analyses. It is therefore recommended that TOC analysis be considered for future measurements of dispersant solutions, particularly if non-soluble droplets are suspected to be present in the solution matrix.

The bi-phasic nature of dispersant solutions at higher concentrations may also affect toxicity assays. It is likely that in more concentrated, dispersant-only test solutions the solution profiles to which animals were exposed were subject to change over time due to separation of the dispersant's hydrophobic components from the bulk solution. Phase separation was observed to occur in the three highest concentrations (1100, 1500, and 1900 ppm) in the flow-through chambers of the spiked exposure test of *M. bahia* at 25°C, and also in the higher concentrations (> 800 ppm) of the spiked exposure test of *C. bairdi* at 7°C. Occurrence of quiescent sea states conducive to phase separation of dispersant components is unlikely. Therefore, toxicity analyses of dispersant-only solutions prepared at concentrations greater than their solubility limits may require alterations to the exposure system design to allow for maintenance of a completely mixed solution throughout the duration of the assay, while minimizing stress to the test organisms. Alternatively, these solutions may need to be considered essentially non-toxic given the unrealistically high loadings required to produce an effect, especially when compared to reported dispersant concentrations of less than 1 to 13 ppm measured at various depths during a sea trials (Singer *et al.,* 1991). Also, if a solution must be prepared in excess of its detected saturation concentrations, the test material may need to be considered essentially non-toxic at normal application concentrations.

Oil Solutions

Oil solutions (WAF and CE-WAF) were prepared with different oil loadings in order to produce results that either bracketed or caused at minimum a 50 percent effect by the test species. Solutions prepared with dispersant added (CE-WAF) required much less oil (1.4 to 10.0 times less) than those without (WAF) to produce solutions with similarly effective hydrocarbon concentrations (Figures 1-2a, b, c). These differences in oil loadings directly reflect the design purpose of dispersants, that is to enhance the entry of oil droplets into the water column (Singer *et al.*, 1998; Clayton *et al.*, 1993). For instance, a CE-WAF prepared with equivalent oil loadings as a WAF produced a substantially more concentrated solution (*e.g.*, see Figure 1-2a, oil loading rate of 1000 mg/L), resulting in a higher level of exposure to test organisms. Additionally, the solution profiles of WAF and CE-WAF were substantially different with WAF being essentially devoid of lower-solubility TPH (C_{10} - C_{36}) components, and CE-WAF showing enhanced aqueous solubility of both TPH and VOA (C_6 -C_9) components.

Concentrations of VOA were higher in CE-WAF than WAF for equivalent oil loadings starting at loading rates greater than approximately 100 mg/L (Figures 1-2a, b, c). On average, however, VOA was more concentrated in WAF than in CE-WAF (*e.g.*, 17 mg/L vs. 11 mg/L, Table 1-4). VOA was also greater in proportion to TPH in WAF than in CE-WAF solutions (*e.g.*, 98% for WAF vs. 42% for CE-WAF, Table 1-4). TPH was larger in proportion than VOA in CE-WAF for solutions with loading rates also of approximately 100 ppm or greater. However, the rate of inclusion (*i.e.*, increase in measured concentration in solution per increase in loading rate indicated by the slope of the lines shown in Figures 1-2a, b, and c) for TPH was always greater than that for VOA in CE-WAF. These two observations may indicate that at lower oil loading rates (approximately <100 ppm), the inherent solubilities of VOA components influenced dissolved hydrocarbon concentrations more than the dispersant's action of enhancing solubilities of TPH components. Additionally, dispersant application has a greater effect on the rates of incorporation of low-solubility chemicals than those of inherently higher solubility.

Oil solutions of PBCO were observed to have significantly lower concentrations of VOA in solution than those of ANS (t-test; $P < 0.05$). This is likely because the parent oils have different compositions, with ANS containing approximately 33 percent volatiles (boiling points < 204°C) (Mead, pers. comm., 1997), and PBCO containing approximately 26 percent volatiles (boiling point < 200°C) (NRC, 1985). Not surprisingly, the loading rates required to obtain hydrocarbon concentrations similarly effective to *M. beryllina* were higher for PBCO than ANS. Even with higher loading rates for PBCO, the TPH fraction in WAF solutions from the two oil types was quite small (Figure 1-2b, 1-2c). Without dispersant addition, the heavier fractions of these oils retained their characteristic of having low aqueous solubility, irrespective of their relative proportion to VOA in the parent oils.

Temperature had a significant effect on the rates of inclusion. For VOA, those rates were greater in colder solutions (7°C); but for TPH, they were greater in warmer solutions (25°C). This is consistent with the understanding that hydrocarbons evaporate more slowly from cold than warmer waters (Neff, 1990). A possible explanation for a higher rate of inclusion of heavier fractions (TPH) in warmer solutions is perhaps that viscosities are reduced at warmer temperatures, allowing for their enhanced solubility (McDonald *et al.,* 1977). Salinity can also effect solubility with increases in salinity resulting in decreased solubility (Shaw, 1977). However, the concentrations of VOA were greater in colder waters of higher salinity than in warmer waters of lower salinity for solutions prepared with similar oil loading rates (*e.g.*, see VOA Figures 1-2a and 1-2b). Therefore, salinity apparently had less of an effect on solubility than temperature.

Overall, the relationships between oil loading rates and the resulting hydrocarbon concentrations in solution depended upon three things. The most basic parameter was the composition and chemical and physical characteristics of the parent oil. Also important were the conditions under which solutions were prepared (*e.g.*, temperature, salinity, dispersant-to-oil ratio, mixing energy and duration). Finally, treatment with dispersant strongly affected hydrocarbon concentrations.

Toxicity Basis: Measured Concentrations or Loadings

Toxicity values calculated based upon measured concentrations may not illustrate the large differences in loadings required to obtain effective concentrations of WAF or CE-WAF (see Table 1-7). For example, when considering toxicity values from Table 1-7 (using values where fiducial limits are available), the average ratio of toxicity values for WAF to CE-WAF (*e.g.*, WAF/CE-WAF) is 2.02 by measured concentrations (LC_{50} values) and 8.25 by loading rates (LL_{50} values). Presumably, the toxic effect of each solution to the test organisms was the same irrespective of how the toxicant concentration was expressed (*i.e.*, by measured concentrations or by loading rates). Therefore, if the ratios of toxicity values (WAF-to-CE-WAF) by both LC_{50} and LL_{50} values were equal, one could deduce that these chemicals interact with saltwater equally. However, since the ratio of WAF-to-CE-WAF determined by loading rates (LL_{50} values) was larger than that by measured concentrations (LC_{50} values), this indicates that some physical or chemical parameter varies among the test materials, affecting their interactions with saltwater. Some of these conditions may be known for a test material, such as with crude oils, which are known to contain poorly soluble constituents whose solubilities are enhanced with the addition of dispersants or changes in temperature, or with chemical changes due to weathering. What may not be apparent when comparing the LC_{50} and LL_{50} values of test materials is that a solution may be much more toxic in terms of the smaller amount of material required to generate a toxic effect than LC_{50} values would suggest (*e.g.*, Figure 1-8 compares Loading Rate vs. VOA, TPH, or THC). For example, CE-WAF solutions are 2.02 more toxic than WAF according to measured concentrations, but 8.25 times more toxic according to loading rates. This is one reason why several authors suggest the use of an LL_{50} or EL_{50} (lethal loading or effective loading to 50% of the population) to express the results of tests for materials containing poorly soluble constituents (Girling *et al.*, 1992; Markarian *et al.*, 1995; Peterson, 1994). Use of an LL_{50} is more demonstrative of a material's ability to produce toxic concentrations in aqueous media. This type of information may be more useful for product comparisons or for quick hazard assessments in the field (Girling, 1992). However, since test solutions are strongly dependent upon their method of preparation (Girling *et. al.*, 1989), toxicity values in terms of loading are of limited value unless identical preparation methods are used (Singer *et. al.*, 1998; Rice *et. al.*, 1977). Additionally, an LL_{50} could not be used to

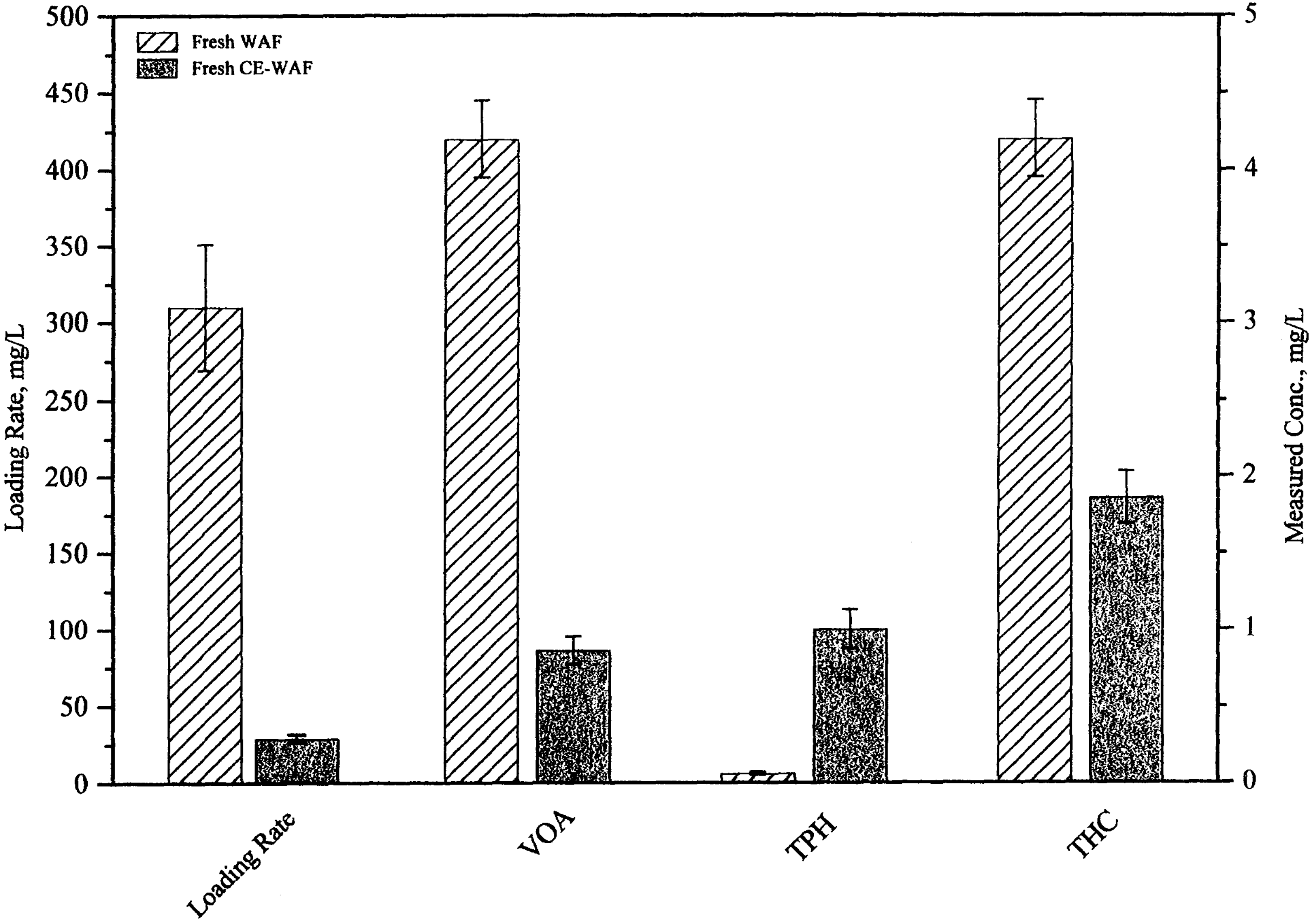

Fig. 1-8. EC_{50} values for *V. fischeri* calculated based on measured hydrocarbon fractions (VOA, TPH, and THC) and total oil added (Loading Rate)

evaluate the toxic effect a particular hydrocarbon or group of hydrocarbons in solution has as analytically determined hydrocarbon concentrations could. Therefore, the question being asked of the data (*e.g.*, which substance is more toxic; or what is the dominant toxicant) will dictate in which form the toxicity values are most useful. However, concurrent use of the two forms is preferred since together they provide the most complete information concerning toxicity, incorporating both physical and chemical conditions influencing the test solutions' formation and the solutions themselves. This is particularly important when the comparison of LC_{50} and LL_{50} values of two test solutions provides opposite conclusions as to which solution or material is more toxic. Such an occurrence suggests that other factors, such as solubility, in addition to the test material's concentrations measured in solution should be considered when the overall toxicity of a test material is evaluated.

Toxicity Basis: Fractional or Total Measured Concentrations

Differences in solution profiles of WAF and CE-WAF can greatly influence the interpretation of the toxicity of these solutions depending upon which solution component (VOA, TPH, or combined as THC) is used to calculate the toxicity value (Figure 1-8). For example, in the *M. bahia* spiked exposure tests, when the toxicity value (LC_{50}) is based solely on the TPH fraction, the LC_{50} of WAF is 0.48 mg/L, and that of CE-WAF is 2.15 mg/L. Because a smaller toxicity value denotes a more toxic solution (*i.e.*, less test material in solution was required to produce a response of 50 percent by the test species), the interpretation of the example given above would lead to the conclusion that WAF solutions are more toxic than CE-WAF solutions. However, if the same exercise is applied to the VOA components also from *M. bahia* spiked exposure tests, the toxicity value for WAF is 7.32 mg/L and 2.22 mg/L for CE-WAF, leading to the opposite conclusion that CE-WAF concentrations are more toxic than WAF concentrations. In fact, when comparing these toxicity values calculated by VOA, THC, and loading rates using these data for WAF solutions versus CE-WAF solutions, in all cases except for those values standardized to TPH, CE-WAF is more toxic than WAF. Similar trends were observed in the other species as well as *V. fischeri* in the Microtox® Assays (see Table 1-10 and Figure 1-8).

The fact that such dichotomous conclusions can be drawn from the same data set is an artifact of two related conditions. First, test solutions must be characterized as a single toxicant, even if the test material is composed of many chemicals as with crude oil or dispersants – all with varying aqueous solubilities, K_{ow} values, Henry's law constants, and presumably toxicities. Second, toxicity values are influenced by the manner in which the concentration of test material in solution is characterized as a single toxicant. All statistical methods estimate a toxicity value in the same general manner by estimating the location of the inflection point (the point which corresponds to the estimated 50 percent response by the test species) on a dose-response curve with respect to the concentration of a single toxicant plotted along the abscissa. Because "there is no such thing as an 'oil molecule'" (Singer *et al.*, 1998) from which to calculate a single toxicant concentration, all measured components of oil must be combined in some fashion to estimate a toxicity value. In so doing, the toxicity of each individual component or group of components of oil is not easily identifiable, nor is the "driver" of toxicity (*i.e.*, that chemical or group of chemicals which is most responsible for the toxic effect). To base conclusions about the relative toxicity of solutions standardized solely to one fraction (*e.g.*, TPH) would be to ignore possible synergistic effects of the combined fractions. Moreover, to do so may erroneously overlook important toxic effects of other fractions that may be present in larger proportion in the test solution but were omitted from the solution's concentration characterization. This is the case with the *M. bahia* spiked exposures discussed earlier; the TPH fraction contributed only 1.3 percent of the total hydrocarbon content measured in WAF solutions. If toxicity values were based only on the TPH fraction, 98.7 percent of the total hydrocarbon content (THC) in solution would not be accounted for in that toxicity value. To omit such a large portion of the solution's hydrocarbons, especially the fraction which is often attributed to being most responsible for acute toxic effects (Maher, 1986; Rice *et al.*, 1984; Bobra *et al.*, 1983; McDonald *et al.*, 1984), could result in gross inaccuracies in the portrayal of the toxicity of a test solution.

Consequently, it is advocated here that the total measured hydrocarbons in solution (*e.g.*, THC) be used to calculate toxicity values. Additionally, the manner used to characterize solution concentration should be reported along with the calculated toxicity values. All toxicity comparisons in this study are made using the combined fractions of VOA and TPH, referred to as THC (LC_{50}) accompanied with consideration of results determined using loading rates (LL_{50}).

Toxicity: Spiked versus Continuous Exposure

Responses by test species to test solutions were always greater under continuous exposure than under spiked, declining exposures. This is consistent with observations by Pace and others (1995) and Bragin and others (1994), in studies that used similar exposure regimes as this study. Since the continuous exposure regime is a more widely accepted standard (Singer *et. al.*, 1990; 1991) that has been and is still commonly used in toxicity tests (Broderson *et. al.*, 1977; Wright *et. al.*, 1994; Webber, 1993), responses under continuous exposure were evaluated in this study to facilitate comparison with results from other studies. However, this type of exposure for an equivalent duration (96 hours) may not be representative of what organisms might encounter in the field, and may in fact overestimate the toxicity of a solution (Pace *et al.*, 1995; Bragin *et. al.*, 1994). Additionally, problems associated with continuous exposure tests arise with a potential decline in concentrations in the test chambers due to aeration, temperature, and other factors; yet, the exposure is modeled as a constant exposure. This may cause the toxicity of the solution under continuous exposure (modeled as constant exposure) to be underestimated (Rice *et. al.*, 1977).

The methods employed in this study for the continuous exposure tests held the potential for loss of the volatile fraction from oil solutions. To assess the degree of underestimation of the toxicity values, a qualitative analysis of the change in volatile compounds (VOA) in continuous exposure test solutions was made. A series of samples were collected during the first 24 hours of a simulated continuous exposure test from beakers containing a low- and high-concentration WAF solution, and a mid-concentration solution for a CE-WAF. These samples were analyzed for VOA content, since that is the fraction most likely to be affected by aeration. The measured concentrations were plotted against time in order to determine the change in VOA concentration over time and the area under the curve (AUC). The AUC calculated was compared to the that of the theoretical constant exposure. VOA from WAF concentrations were observed to decline near to detection limits in approximately 12 hours, causing the AUC to be 90 percent less than the theoretical exposure. Declines in CE-WAF concentration were much slower with some VOA remaining at the end of 24 hours. The AUC for the CE-WAF was 83 percent less than the theoretical exposure. With these factors taken into account, results from continuous exposure tests are considered in the following discussion. However, it must be understood that the toxicity reported for the continuous exposure tests are likely underestimated for an actual "constant" exposure (*i.e.,* toxicity values would be smaller, indicating greater toxicity, if generated under an absolute "constant" exposure). Greater emphasis is placed upon results from spiked exposure tests, as concentrations in those tests more closely resemble concentration profiles observed in the field (Pace and Clark, 1993; Singer *et al.,* 1993).

Toxicity: Test Solution Toxicity Comparisons

The toxicity of test solutions are compared here using toxicity values calculated using both measured concentrations (LC_{50} and EC_{50} values) and loading rates (LL_{50} and EL_{50} values). Evaluations in trends are made using the combined results from measured concentrations (LC_{50} and EC_{50} values) and loading rates (LL_{50} and EL_{50} values) first, then considering each method individually.

Overall, the trend was that dispersant-only solutions were least toxic, followed by WAF and CE-WAF solutions of fresh oil, with CE-WAF being more frequently the most toxic. This implies that dispersed hydrocarbon compounds of CE-WAF solutions are more bioreactive than the non-dispersed hydrocarbons of WAF. This observation is likely related to the amount of hydrocarbons found in solution for dispersed versus non-dispersed solutions. For example, measured TPH concentrations were always much higher for CE-WAF than for WAF solutions, and were more concentrated (THC) than the WAF solutions of similar oil loading.

Trends in the data according to values based upon measured concentrations (LC_{50} and EC_{50} values; denoted as: LC_{50}/EC_{50}) were quite consistent. In general, dispersant-only solutions were least toxic in all cases, followed by WAF solutions, then CE-WAF solutions as most toxic – similar to the trend above using all of the data. Not all of these relationships, however, were significantly different or had fiducial limits with which to test significance. For instance, for *C. bairdi* and *M. bahia,* there was no significant difference in toxicity of WAF and CE-WAF solutions; however, the toxicity values for WAF solutions were generally larger than those for CE-WAF solutions. These trends are also most likely related to the hydrocarbon content (*i.e.,* less toxic) as was presented previously.

Trends in the data when compared using toxicity values calculated by loading rates (LL_{50} and EL_{50} values; denoted as: LL_{50}/EL_{50}) were less consistent than those made by

LC_{50} and EC_{50} values. Two prominent trends were observed. One, WAF solutions were least toxic for all species except for *C. bairdi*, where dispersant-only solutions were least toxic. And two, relative to CE-WAF, WAF solutions were generally less toxic (*i.e.*, had the largest loading rate). The former is not surprising since WAF solutions required the largest amount of test material to be added to saltwater in order to form effective solutions thus influencing a higher calculated toxicity value. For two equally effective solutions produced with different product loading rates, the more concentrated (in terms of loading) solution will also have the largest toxicity value (*i.e.*, lowest toxicity) when calculated using loading rates. As a result, toxicity values calculated using loading rates were largest for WAF solutions, indicating that these solutions were least toxic. That latter trend (that WAF solutions were less toxic than CE-WAF solutions) in comparisons of LL_{50} and EL_{50} values is also related to the amount of hydrocarbons in solution.

According to these data, the best response to the question of which is more toxic, dispersed or non-dispersed oil, is that it depends upon the species and endpoint tested, and how the data is presented. Singer and others (1998) report similar results when considering the relative toxicity of WAF and CE-WAF solutions, reporting the differences in toxicity of these solutions is dependent upon "species, time, and endpoint." Wells (1984) indicates that some studies report dispersed oil solutions as more toxic, while others studies show no difference in toxicity of dispersed and non-dispersed oil solutions.

Toxicity: Intra-Species Sensitivities

Comparisons made here are by the combined data sets of LC_{50} and EC_{50} values and LL_{50} and EL_{50} values, unless stated otherwise. *C. bairdi* were least sensitive to the dispersant solutions, and were more or less sensitive to the CE-WAF solutions depending upon the exposure type. Although, under spiked exposure, there was no significant difference in toxicity of these solutions to *C. bairdi*. *M. bahia* and *V. fischeri* shared the same pattern of sensitivity. These species tended to be most sensitive to the CE-WAF solutions. The least toxic solutions for *M. bahia* and *V. fischeri* were dependant upon how the toxicity value was calculated (measured or loading). According to LC_{50}/ EC_{50}, these species were least sensitive to dispersant-only solutions. According to LL_{50}/ EL_{50}, they were least sensitive to WAF solutions. Although, the difference in toxicity between WAF and CE-WAF solutions for *M. bahia* was not significant. *M. beryllina* was most sensitive to CE-WAF solutions and least to dispersant-only solutions according to LC_{50} values. The trend was slightly different according to LL_{50} values, where *M. beryllina* tended to be most sensitive to dispersant-only solutions and least sensitive to WAF solutions. That *M. beryllina* were most sensitive to dispersant-only solutions may be because fish may be more susceptible to some types of waterborne toxicants (Singer *et al.*, 1998) — perhaps the surfactants in the dispersant mixture. Surfactants are intended to reduce the interfacial tension between the aqueous and lipid phases, and do so non-selectively for biogenic or non-biogenic lipids. It is possible that the decreased interfacial tension between gill epithelial cells and the surrounding medium reduced the amount of oxygen exchanged, causing hypoxia and eventually asphyxia (Singer *et al.*, 1994).

Toxicity: Inter-Species Sensitivities

M. beryllina was most resistant overall. Similar observations were made by other researchers working with *M. beryllina,* where the authors state that *M. beryllina* was one of the least sensitive species tested (compared to *M. bahia,* another mysid, and oyster larvae) under continuous exposure (Bragin and Clark, 1996). However, for dispersant-only solutions, *M. beryllina* was either most or least sensitive depending upon the exposure regime. Under continuous exposure, *M. beryllina* were most resistant to dispersant-only solutions, but least under spiked exposure. This indicates that both *M. bahia* and *C. bairdi* were substantially more sensitive to dispersant-only solutions under a continuous exposure. This may imply that under longer exposures, dispersant surfactants have more time to act upon and damage to the membranes of these species. Surfactants are known to have a number of effects on aquatic organisms, such as disrupting normal cell function by altering membrane permeability, interrupting cellular respiration, and causing membrane lysis (Singer *et al.,* 1998). It is possible that crustaceans are more susceptible to this type of damage when dispersants and biological membranes are in contact for periods longer than six to nine hours (the detected concentration decline in spiked exposure tests), potentially approaching equilibrium.

M. beryllina were also most resistant to oil solutions. C. *bairdi* and *M. bahia* often were more sensitive than of both *M. beryllina* and *V. fischeri.* It has been suggested that crustacean larvae may be more sensitive to oil and oil-components than fish (Rice *et al.,* 1977). *C. bairdi* showed greatest sensitivity to oil solutions under continuous exposure; however, under spiked exposure, *C. bairdi* tended to be more resistant than both *M. bahia* and *V. fischeri*. Of all tests, *C. bairdi* was most frequently the most sensitive of all species. It has been suggested that because of lower temperatures, the persistence of toxic aromatic hydrocarbons is increased (Rice *et al.,* 1977), potentially extending the exposure period for cold-region species. Also, cold-region crustacean species may be more sensitive to oil pollution than those of warmer regions because they develop more slowly therefore existing in the more sensitive larval state longer (Brodersen *et al.,* 1977). No clear trend for *V. fischeri* was observed in terms of relative sensitivity to suggest that this bacterium was consistently more or less sensitive than *C. bairdi* or *M. bahia* (after *M. beryllina* as least sensitive).

Toxicity: Temporal Responses

Qualitative, temporal assessments of lethal responses by *M. bahia* and *M. beryllina* were made for all test solutions. These are considered "qualitative" because these assessments were based upon observations made by viewing through the flow-through chambers that are somewhat obscured. Similar assessments are not available for *C. bairdi* because evaluation of this species' response to test solution exposure required close (microscopic) observations, not possible during the toxicity test.

The type of exposure, spiked or continuous, had a noticeable effect on the response by the test species to test solution exposure over time. Not surprisingly, the toxic effect in spiked exposure tests generally stabilized within the first 24 hours of the 96-hour test (Table 1-8). However, continuous exposure tests generally caused a steady increase in mortality over the duration of the test. Providing renewed toxicant every 24 hours resulted in further increasing the mortality of the test species. Under spiked exposure, *M. bahia* tended to exhibit more of a delay in lethal effect than *M. beryllina*. As was seen previously for comparisons made both by LC_{50} and LL_{50} values, *M. beryllina* was most resistant to oil solutions, and least to dispersant-only solutions with one exception, where under continuous exposure, *M. beryllina* was more resistant to dispersant-only solutions than *M. bahia* and *C. bairdi*.

Inter-laboratory Comparisons

Test protocols used in this study followed those set forth by CROSERF in order to facilitate comparison of toxicity data determined by other laboratories following similar protocols. Other research groups employing CROSERF protocols (which generally includes oil solution preparation protocols, the spiked exposure regime, and guidelines for chemistry analysis of test oil solutions) have evaluated the toxicity of dispersants and oils of local interest to local species, much in the way toxicity tests were designed in this study. Inter-laboratory comparisons are possible here by use of the national standard species *M. bahia* and *M. beryllina,* the more recently accepted CROSERF standard, and the reference oil, PBCO. Comparisons of toxicity values from other laboratories were made here where data are available and directly comparable (*i.e.,* same species and test solution evaluated). Because Corexit 9500 is a newer dispersant than Corexit 9527, more toxicity data exists for 9527 than 9500, thus few direct comparisons are available. One value that is directly comparable to the Corexit 9500 *M. bahia* continuous exposure test was reported in Coelho and Aurand (1996). The median-lethal concentration value reported was 35.9 ppm, which agrees well with the value obtained in this study of 29.1 ppm (approximately 20% difference). Other toxicity values are available for comparison and are made here; however, it should be noted that these tests were evaluated using different species and/or test materials. Considering only spiked exposure tests for *M. bahia* and *M. beryllina*, values reported in Bragin and Clark (1996) found for WAF solutions of Kuwait crude oil, the toxicity was greater than 2.93 ppm and 2.0 ppm, for *M. bahia* and *M. beryllina,* respectively. When compared to the values obtained in this study of 8.21 and 26.36 ppm, respectively, these values appear very different. However, it is important to note that the values reported in Bragin and Clark (1996) for both WAF concentrations and CE-WAF concentrations were standardized to the TPH fraction only, quite possibly causing these values to be low. CE-WAF concentrations, also from Kuwait crude oil, but with the addition of the dispersant Corexit 9527 instead of Corexit 9500, resulted in values of 6.6 ppm and 16.8 ppm for *M. bahia* and *M. beryllina*, respectively (Bragin and Clark, 1996). Values obtained in this study for the same species, but using fresh ANS and Corexit 9500, were 5.08 and 12.22 ppm for *M. bahia* and *M. beryllina*, respectively. These values differ slightly, but are within the same order of magnitude of those reported in Bragin and Clark (1996). To date, there have been no other laboratories that have followed protocols set forth by CROSERF and have evaluated responses of *C. bairdi* larvae. However, several toxicity tests on the same (*C. bairdi*) or similar cold-regions species have been conducted (Rice *et al.,* 1977; Broderson *et al.,* 1977). Broserson and others (1977) exposed Tanner crab larvae (*C. bairdi*) to a static (constant concentration) water soluble fraction of Cook Inlet crude oil and determined the median-lethal concentration to be 1.7 ppm, analyzed using freon extracts and infrared spectrophotometry. In their study, the researchers defined the lethal indicator as "moribundity (death imminent)," which was identified as "the cessation of swimming" – not unlike the definition used in this study for "affected" (Broderson *et al.*, 1977). Rice and others (1977) report an LC_{50} of 2.0 ppm for King crab larvae exposed to static water soluble fractions of crude oil. In results from this study for the continuous exposure to water-accommodated fractions of ANS, the median-effect concentration for *C. bairdi* was determined to be 2.54 ppm. Given the differences in testing protocols and crude oils evaluated, these values are in good agreement.

Toxicity Driver

The general trend of increased toxicity of dispersed oil solutions over that of non-dispersed oil solutions may be due to the increased TPH fraction. Since TPH is nearly absent from the solution profile in WAF concentrations, it presumably contributes little to the toxic effect. For example, *M. beryllina* was significantly more sensitive to CE-WAF concentrations than WAF concentrations (WAF LC_{50} = 26.36 mg/L; CE-WAF LC_{50} = 12.22 mg/L). A WAF from that test produced with a loading rate of 500 mg/L resulted in a solution with a measured concentration of 24.21 mg/L THC, which is similar to the estimated LC_{50}. Of that total hydrocarbon content (24.21 mg/L), 23.87 mg/L were VOA and 0.34 mg/L were TPH. In comparison, a CE-WAF from that test, produced with half of the loading of the WAF at 250 mg/L, resulted in a measured THC concentration of 12.26 mg/L, also similar to the estimated LC_{50}. Of that 12.26 mg/L THC, 4.29 mg/L were VOA and 7.97 mg/L were TPH. The VOA concentration in the CE-WAF solution is approximately 6 times less than that in the WAF; however, the TPH concentration is near 27 times greater. The largest difference between these solutions is the increase of TPH measured in CE-WAF solutions. Therefore a possible explanation for the increase in toxicity of CE-WAF solutions is due to the increase in the TPH fraction or some other unmeasured parameter.

Another influence on the toxic effect of a solution may be from the individual hydrocarbons themselves. When considering the toxicity of water soluble fractions of untreated oil, Bobra and others (1983) suggested that the "potency" (defined as the ratio of the individual substance's solubility to the overall LC_{50} of the hydrocarbon mixture) for a single hydrocarbon decreases as molecular weight increases. Larger molecules may have slower diffusivities in both the aqueous and lipid phases (Abernathy *et al.,* 1986). A larger size may also affect the molecule's ability to partition through the membranes of an organism to access sites of toxic action (Bobra *et al.,* 1983). These factors may contribute to the decrease in potency for larger molecules (Bobra *et al.,* 1983). Moreover, larger molecules are less soluble than their smaller counterparts (Shaw, 1977; Rice *et al.,* 1977), making them less able to establish concentrations in "aqueous media through which transport must occur" in order to produce a toxic effect (Abernathy *et al.,* 1986). These factors potentially lead to a lesser degree of contribution to the overall toxic effect of TPH in WAF concentrations. Testing the toxicity of individual hydrocarbons is a research endeavor that has been explored and promoted for predicting the toxicity of a mixture of hydrocarbons (Rice *et al.*, 1984; Peterson, 1994). This may be the only possible way in which to identify which component of a mixture contributes most to the toxic effect. However, Rice and others (1984) tested the toxicity of a water soluble fraction (WSF) that modeled those prepared from Cook Inlet crude oil. The synthetic Cook Inlet WSF was prepared from a mixture of the ten aromatic hydrocarbons that were predominant in the whole oil WSF. Rice and others (1984) found that the synthetic mixture had a toxic effect that was only 20 to 30 percent of that the whole oil WSF, "even though proportions of individual hydrocarbons were the same that that of the whole crude oil." This suggests that synergistic effects of a whole product may not be re-produceable by a synthetic mixture or summation of toxic effects from single hydrocarbons.

However, altering the solution profile by the action of dispersants tends to increase the toxicity of the oil solution. Once dispersed, oil is in the form of micelles presumably containing compounds that are most hydrophobic at the center surrounded by a zone of lower-soluble fractions with enhanced solubility. Those compounds that were initially of low-solubility are introduced into the aqueous media by the action of the dispersants, where they can more easily make initial contact with an organism. Once in the aqueous media, these molecules may preferentially partition out of the water phase in a non-specific manner into the lipid phase, having equal affinity for biological lipids as other lipids present in the system.

The addition of chemical dispersants enhances the dissolution of inherently low-soluble compounds that normally would not go into "solution." Through this action, it is possible that larger hydrocarbons of low-solubility and slightly larger octanol-water partitioning coefficients (K_{ow}) than those naturally soluble would be incorporated into the water column for exposure to organisms. Since the octanol-water partitioning coefficients (K_{ow}) of these chemicals are believed to be indicative of a chemical's ability to partition between biological lipid and water phases (Lipnick, 1995), a higher K_{ow} would indicate a greater propensity to partition into biological membranes rather than water. For example, the log K_{ow} for n-hexane is 4.11, and slightly higher for n-decane at 6.69. Since smaller hydrocarbons solubilize in water easier than larger hydrocarbons (Shaw, 1977), the addition of dispersants would increase the concentration of these larger molecules that may also have a larger K_{ow}. However, this may only be true for certain mid-range hydrocarbons (*e.g.*, 10 to 15 carbons), since according to Abernathy and others (1986), larger molecules have a tendency to be less soluble in octanol.

Alternatively, the K_{ow} coefficients of molecules may be altered in some way by the addition of dispersants. If it is assumed that the VOA fraction is responsible for the toxic effect, then WAF solutions would have been found to be more toxic than CE-WAF solutions since they were more concentrated with VOA. It is possible then, that the K_{ow} coefficients of hydrocarbons enhanced into solution, were altered such that their original values were increased. In such an event, these chemicals would have increased in their biological reactivity.

The observation that WAF solutions were generally less toxic than CE-WAF solutions reflects both the chemical and the physical effects of the dispersed oil solutions. CE-WAF solutions were measured to have higher hydrocarbon concentrations than WAF solutions, presumably contributing more to chemical toxicity of dispersed oil solutions.

In addition, CE-WAF solutions were likely to have more oil particulates in solution than WAF solutions through the action of the dispersant. The solution preparation methods for WAFs and CE-WAFs were intended to produce solutions that are essentially equivalent to one another with respect to the number of oil particulates (≤ 1 μm in diameter) in solutions (Singer *et al.*, 1998; pers. comm., Singer, 1999). However, CE-WAF solutions were noticeably more concentrated than WAF solutions by increased opacity. From this observation and given the understanding of dispersant action, it is reasonable to assume that CE-WAF solutions may actually have slightly more oil particulates in solution that WAF solutions. Thus, the mere presence of micro-oil droplets approximately 1 μm in diameter in CE-WAF solutions, if brought into contact with an organism of approximate 4 mm in size, could conceivably increase toxicity due to physical effects rather than chemical ones (Singer *et al.*, 1998; Karinen and Rice, 1974; Wells, 1985).

The increase in toxicity of CE-WAF solutions in some cases may due to the following factors: 1) incorporating additional hydrocarbons that may be of higher octanol-water partitioning coefficients into the aqueous media that might not normally go into "solution" under mixing conditions similar to those use to prepare WAF solutions; 2) altering the partitioning ratios (*e.g.*, K_{ow}) of a chemical once enhanced into solution by dispersant addition; and/or 3) introducing micro-droplets of oil into solution/suspension via formation of micelles possibly contributing to toxicity by physical means (*e.g.*, coating).

Field Extrapolation

An important question when considering toxicity values is how and when they are relevant to actual field conditions. To be able to answer that, additional variables must be considered to appropriately apply information obtained in laboratory toxicity tests to local field conditions. Wells (1985) states that in addition to physicochemical properties of oil and dispersed oil, the quantity and location of an oil spill, and population sensitivity, variables such as species, life stages, season, physiology, biochemistry, behavior, and habitat vulnerability must be considered.

Ideally, toxicity tests should be evaluated using a species that is sufficiently sensitive so results will be representative of potential toxic effects to other organisms in the biological system. The most sensitive life-stage of an ecologically and economically important species, *C. bairdi*, was selected for evaluation in this study. However, the possibility exists that another Alaskan species, not yet tested and/or reported, possess a greater sensitivity to oil pollution than *C. bairdi.* Thus, other cold-region species may need to be evaluated to augment the database of toxic responses to oil pollution in order to make more-informed oil spill response decisions.

In addition to species and life-stage considerations of when to apply toxicity data to field conditions, are the methods used to prepare the test solutions. Because numerous different mixing regimes (*i.e.,* sea stages) can occur in the environment, an effort to simulate all of the possible solutions resulting from various different sea states is impractical (Rice *et al.,* 1977). In fact, Rice and others (1977) state that acute toxicity tests can only provide an approximate idea of what is likely to occur in the environment.

However, toxicity assays should resemble the natural environment as realistically as possible. As a result, spiked exposure tests that more closely resemble exposures likely to occur in the environment (Pace and Clark, 1993; Singer *et al.*, 1996a) were evaluated in this study. Rapid dispersion of chemically treated oil on the order of 5 to 20 minutes is expected to occur in the field (Mackay *et al.*, 1982). Also, high dilution rates (*e.g.* sea swell, wind and wave intensity) are a pre-requisite for dispersant application to an oil slick (Pace and Clark, 1993).

When applying toxicity data derived in the laboratory to field conditions, it should be reiterated that toxicity values are the product of the values that characterize the chemical concentrations in solution. Singer and others (1998) suggest that toxicity values based on loading rates alone (LL_{50}) are of little practical value, since often concentration data available during response to an oil spill is determined using fluorometric or chromatographic analytical methods. In such a case, use of toxicity values based upon measured concentrations (LC_{50} values) would be more appropriate to enable direct comparison with analytically determined concentrations. However, when analytically determined concentrations are not available, but the initial volume of oil spilled per approximate mixing volume are known (*e.g.*, size of local mixing depth, length, and width), toxicity values based upon loading rates may be more applicable.

Likewise, a toxicity value based solely on one chemical fraction (*e.g.*, TPH) may be of little value. For example, suppose a crude oil containing a high proportion of lighter, more soluble fractions is spilled into the environment, and application of chemical dispersants within short succession of the spill is being considered for a mitigation response. In such a case, the lighter fractions would be enhanced into solution preferentially over evaporation by the action of the dispersant. They would exist in a dissolved form where their contribution to the toxic effect is more likely. Consequently, a toxicity value based solely on the heavier fractions (TPH) could overlook the effects of these lighter hydrocarbons, possibly leading to a greater environmental impact than would be expected from the toxicity values reported. Therefore, it is important for researchers to report which fractions were used to obtain the toxicity values; and conversely, for users of this information to consider from where these values were calculated.

Another important consideration when extrapolating laboratory-derived toxicity data to field conditions is whether or not material loadings used in the test to generate a response by 50 percent of the population is realistic or unrealistically high. According to Shiu and others (1990) an excess of oil implies a water-to-oil ratio of 20:1 or less. Similarly, Singer and others (1998) suggest that a water-to-oil ratio of 40:1 (25 g/L) is "unrealistically high." Dispersant-to-oil ratios used in the field are typically 1:20 or less (NRC, 1989); a smaller ratio than that was used in this study (1:10) to match that used by other researchers (Singer *et al.,* 1998; Bragin *et al.,* 1994) for purposes of comparison. Based on the above information, an excessive dispersant loading may be expected to be from 2.5 g/L to 5.0 g/L (water to dispersant ratio of 400:1 or 200:1). Therefore, the loading rates used in the dispersant-only spiked exposure tests of *C. bairdi* and *M. bahia* would be considered excessive (see Table 1-6); however, all other tests were not. This implies that for those species tested with solutions that were not considered excessive, under similar conditions in the field, a toxic effect could occur from dispersant alone. However, all other tests (oil solutions, continuous exposure dispersant-only tests for *C. bairdi* and *M. bahia,* and the *M. beryllina* dispersant-only) were evaluated with loading rates that are not considered excessive by the above standards. Therefore, it is possible that these concentrations could occur in the environment and under similar conditions, a toxic effect may be observed. The next consideration is that of the actual toxicity values determined for each species and how those relate to concentrations observed in the field.

In order to speculate if a toxic effect could be expected under field conditions, the estimated median-effect concentrations (LC_{50} and EC_{50} values) must be compared to concentrations that have been measured under field conditions, or are expected to occur in the field. For dispersants alone, initial concentrations might range from 0.1 to 13 mg/L at depths of 5 to 10 m (Wells, 1984; Singer *et al.,* 1991; Trudel, 1998). Since all median-effect concentrations calculated in this study are greater than 13 ppm, this would suggest that no toxic effect would occur in the field as a result of dispersant addition alone.

In a field investigation of an oil spill that was treated with Corexit 9527 soon after release, concentrations were measured at depths of 1, 3, and 9 meters at 0.25, 0.6, and 3 hours following dispersant application; those concentrations were as follows: 1) 0.25 h: 40, 9, and 0.1 ppm at 1, 3, and 9 m, respectively; 2) 0.6 h: 12, 14, and 2 ppm at 1, 3, and 9 m, respectively; and 3) 3 h: 1, 2, and 0.5 ppm at 1, 3, and 9 m, respectively (Trudel, 1998). Similar values based upon data published in Lewis and Aurand (1997) are shown in Figure 1-9. Over time (approximately 28 hours) these concentrations normalize throughout a depth of approximately 10 m (Mackay *et al.,* 1982), as can be seen from the

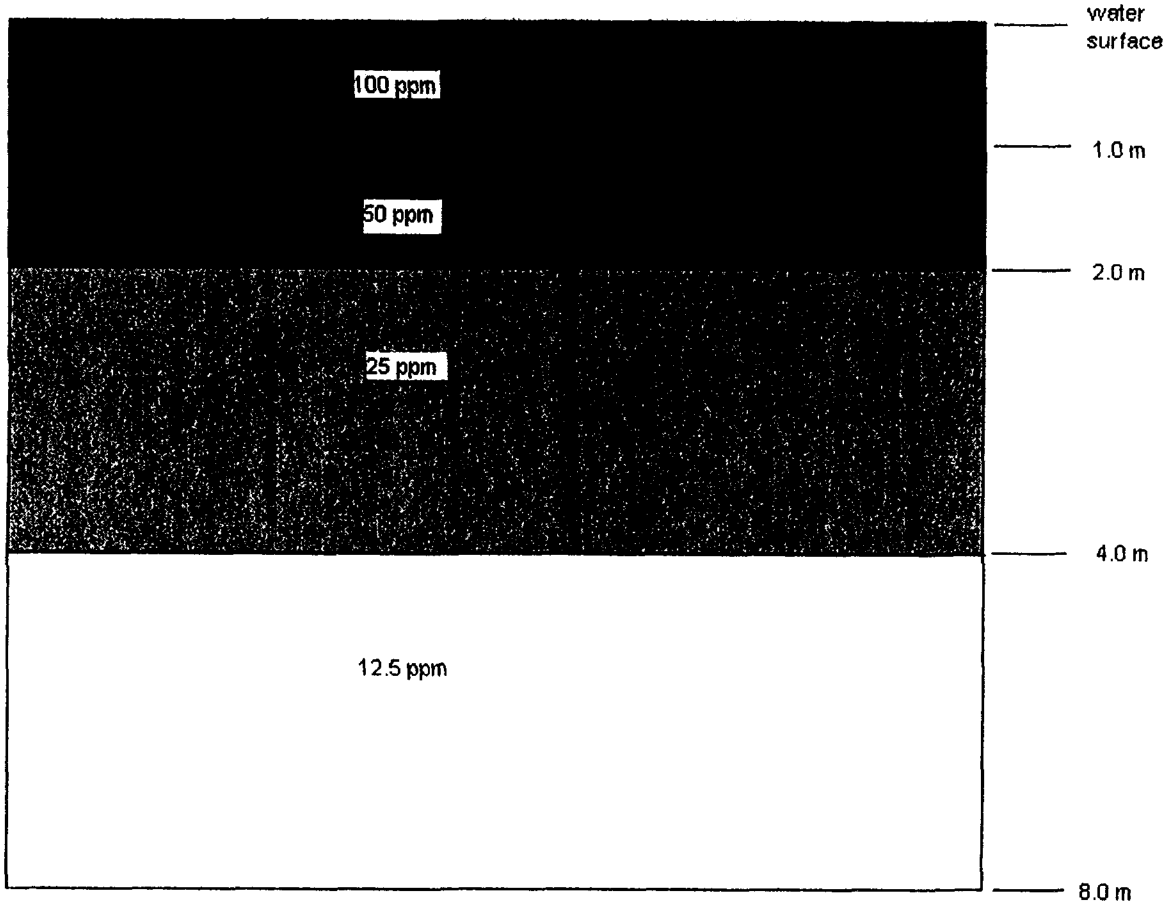

Fig. 1-9. Concentrations of oil in the water column following dispersal of a 0.1 mm thick slick of fresh oil treated with a chemical dispersant (after Lewis and Aurand, 1997)

values above. This information supports the use of the spiked exposure tests, with dispersed oil concentrations declining and stabilizing at an average concentrations of 0.65 ppm (less than all toxic thresholds calculated for the species tested in this study) at the seventh hour of CE-WAF tests. Comparing these values to the toxicity values calculated for CE-WAF solutions tested under spiked exposure reveals that a toxic effect could be expected for *C. bairdi, M. bahia,* and *M. beryllina* at a 1 m depth 0.25 h following dispersal of a spill, and would continue to 0.6 h at depths of 1 and 3 m. Three hours after dispersing, however, no toxic effect would be expected. Therefore, based upon these data and the concentrations provided above (Trudel, 1999), only those organisms in the immediate vicinity at the time of dispersal would experience an acute toxic effect due to dispersed oil.

Season and habitat are also two very important variables that must be considered when using toxicity data as a decision-making tool for oil spill response actions. For instance, chemical dispersion in shallow waters where the dilution volume is very small may adversely impact benthic communities (Coelho *et al.,* 1995). Also, the season in which an oil spill occurs is important to consider, since a sensitive species or life-stage of a species may only be present in the upper reaches of the water column during certain periods of the year. A case in point, *C. bairdi* larvae evaluated in this study are only present in the upper 30 meters of the water column beginning in the spring months, until they enter into the megalops larval stage of development and seek habitat at greater depths. As a result, if an oil spill were to occur any time other than during the spring planktonic bloom, the expected acute toxic effects to larval *C. bairdi* from exposure to chemically dispersed oil could be little to none.

CHAPTER 2

WEATHERED OIL STUDY

MATERIALS AND METHODS

The toxicity study of weathered oil is a continuation of the fresh oil study covered in Chapter 1. The methods and materials employed for weathered oil tests are the same as for fresh oil tests with the exception of weathering of the crude oil. Consequently, much of this section refers to the Materials and Methods section of Chapter 1; any differences are identified in the following sections.

Materials

Toxicity assays in this study were conducted using solutions made from the dispersant Corexit 9500 (Nalco/Exxon Energy Chemicals, L.P., Sugar Land, Texas) and weathered Alaska North Slope crude oil (ANS) (Williams Alaska Petroleum, Inc., North Pole, Alaska). Corexit 9500 used in the weathered oil study is from the same supply that was used in the fresh oil study. Chemical characteristics of Corexit 9500 and fresh ANS are described in Chapter 1. Fresh ANS is approximately one-third by weight volatiles (components with boiling points 204 to 274°C or less; pers. comm., Mead, 1997). ANS was collected from the Trans-Alaskan Pipeline in December 1998 and sent to Battelle Ocean Sciences (Duxbury, Massachusetts), where it was artificially weathered using a modified method of ASTM D86/82, resulting in losses of components with boiling points below 200°C (pers. comm., Macomber, 1998).

All toxicity assays of *Chionocetes bairdi* were conducted using natural, 0.5-µm filtered seawater (20-µm paper-pleated polypropylene, 5-µm carbon-wrapped, 0.5-µm block-activated carbon; OMNIFilter, Hammond, Indiana) taken from an 80-m depth from Resurrection Bay, Seward, Alaska, at ambient temperature (typically 7°C) and salinity (≈ 31.5‰). Saltwater used in toxicity assays of *Menidia beryllina* was identical to that used in the fresh oil study – reconstituted saltwater made from de-ionized water (≥ 18 MΩ–cm) and Crystal Sea® Marinemix (formerly Forty Fathoms® Seasalt, Marine Interprises International, Inc., Baltimore, Maryland) at a temperature of 25°C and salinity of 20‰ (Webber, 1993).

Test Solutions

Each species was evaluated for acute toxic effects using water-accommodated fractions (WAF) of weathered ANS (no dispersant added) and chemically-enhanced water-accommodated fractions (CE-WAF) of weathered ANS (dispersant added). Toxicity assays for dispersant-only solutions were conducted as part of the fresh oil study and were not duplicated here. WAF and CE-WAF solutions of weathered ANS were prepared according to procedures described in Chapter 1, Materials and Methods, Test Solutions.

Toxicity Test Procedures

Short-term toxicity tests (96 h) were conducted to evaluate the sub-lethal and lethal responses to weathered ANS of the early life-stages of Alaskan Tanner crab (*Chionocetes* *bairdi*) and the inland silverside (*Menidia beryllina*), respectively. Responses of the test species to test solutions when exposed under spiked and continuous concentrations were observed. Microtox® Assays were also run on spilt samples collected from test solutions from the *C. bairdi* and *M. beryllina* tests. Experimental design, methods, and materials employed in this study were identical to those described in Chapter 1, Materials and Methods, Toxicity Test Procedures, with one exception. Temperature control during the *C. bairdi* tests were maintained using water baths, instead of in a temperature-controlled room.

Test Species

Tanner crab larvae (*Chionocetes bairdi*) were obtained from gravid females collected from Kachemak Bay, Alaska, in January 1998. Larvae were from the same females that were used in the fresh oil study. This was possible due to the reproductive characteristics of this species. Multiparous ("females producing second and subsequent egg clutches;" Paul and Paul, 1992) female *C. bairdi* store sperm in their spermathecae that remains viable for up to two years. This allows the females to re-inseminate themselves if no males are present during the mating season (Paul, 1984). This may suggest that the genetic material of progeny used in the fresh oil study was identical to that in the weathered oil study. However, because it is possible for females to copulate with more than one male, there may be variations in the genetics of the larvae born from one female from year-to-year (Paul and Paul, 1992). However, since progeny from the same females were used in both the fresh and weathered oil studies these variations are likely to be less than if larvae from a different set of females were used.

Between the times of the fresh and weathered oil studies, the adult females and hatching larvae were kept in ambient saltwater at the Alaska SeaLife Center in Seward, Alaska. The larvae tested were less than 24 hours old. Prior to and during testing, the tanner crab larvae were fed twice daily with 5 to 10 mL of a solution containing chain-forming diatoms (*Tetraselmis striata, Chaetocerus calcitrans, Chaetocerus gacile, and Thalassiosira pseudonana*) (Qutekcak Shellfish Hatchery, Seward, Alaska).

Larvae of the standard reference species, *Menidia beryllina*, used in this study were obtained from Aquatic Bio Systems, Inc., Ft. Collins, Colorado, the same source as those tested in the fresh oil study. These larvae were handled and cared for using the same protocols employed in the fresh oil tests. Water quality parameters monitored during acclimation periods included the following: temperature, pH, dissolved oxygen (DO), conductivity (salinity), and ammonia.

Microtox® Assay

Split samples of weathered oil test solutions were collected from toxicity tests of both species (*C. bairdi* and *M. beryllina*) under both exposure regimes (spiked and continuous), and were analyzed using the Microtox® test system, which is based upon the response (defined as luminescence inhibition) of the bacterium, *Vibrio fischeri*. The same procedures used for the Microtox® Assays used in the fresh oil study were used here in the weathered oil study.

Chemical Analysis

Test solutions were analyzed using Gas Chromatography/ Flame Ionization Detection (GC/FID). Solutions were analyzed for total volatile organic analytes (VOA range defined as C_6-C_9) and total petroleum hydrocarbons (TPH range defined as C_{10}-C_{36}) (Coelho and Aurand, 1997). The summation of these analytes is the total hydrocarbon content (THC: C_6-C_{36}). A list of the minimum target analytes can be found in Table 2-1. Chromatographic measurements of THC were conducted in identical manner as described in Chapter 1, Materials and Methods, Chemical Analysis: Oil Solutions.

Values reported for VOA for both WAF and CE-WAF test solutions are the composite of samples collected from days one through four. TPH values from WAF tests are the measured values from samples collected on day one only. This approach to analyzing WAF solutions for TPH content was employed after verifying that due to the limited solubility of hydrocarbons in the range of C_{10} to C_{36}, TPH content in WAF solutions was consistently low regardless of increased oil loading. For CE-WAF solutions, measured TPH values are from the composite of samples collected from days one through four. TPH samples were composited using an equal volume from each sample collected. For spiked exposure tests, hourly samples were collected to verify that VOA concentrations were declining within the flow-through chambers. Generally, samples from a mid- and high-concentration test solution were collected over the first 24 hours of the test, typically at hours 2, 4, 7, and 12 and were analyzed for VOA content.

Table 2-1. Minimum target analytes for chemical analysis of weathered oil test solutions

Minimum target analyte list for VOA analysis (*C. bairdi* and *M. beryllina*)

Saturates	Unsaturates
2-methylpentane	benzene
hexane	toluene
cyclopentane	ethylbenzene
heptane	m-xylene
2,4 dimethylpentane	p-xylene
cyclohexane	o-xylene
octane	n-propylbenzene
nonane	1,2,4-trimethyl-benzene
	1,3,5-trimethyl-benzene

Minimum target analyte list for TPH analysis (*C. bairdi* and *M. beryllina*)

n-Alkanes:		Aromatic Hydrocarbons:
Decane	C10	Naphthalene
Dodecane	C12	2-methylnaphthalene
Tetradecane	C14	Acenaphthylene
Hexadecane	C16	Acenaphthene
Octadecane	C18	Fluorene
Nonadecane	C19	Fluoranthene
Eicosane	C20	Pyrene
Docosane	C22	
Tetracosane	C24	
Hexacosane	C26	
Octacosane	C28	
Triacontane	C30	
Hexatriacontane	C36	

Toxicity Analysis

Median-effect concentrations of weathered oil solutions were determined for each species. For *M. beryllina*, LC_{50} (lethal concentration to 50 percent of the population) values of the test solutions were determined. For *C. bairdi*, these values were calculated as the effective concentration to 50 percent of the population (EC_{50}) since lethal effects were rarely observed. Toxicity test protocols (*e.g.*, spiked and continuous exposure; animal handling) and criteria (*e.g.*, sub-lethal or lethal) used to determine toxic effects in this study are identical to those used in the fresh oil study.

Statistical Analysis

In the same manner as was done in the fresh oil study, three replicate exposure chambers were used in each test to assess the variation within and among test species. The median-effect concentrations (LC_{50} and EC_{50}) were calculated in the same manner as the fresh oil study described in Chapter 1. As with the fresh oil study, tests with 20% effect or less in the controls were considered acceptable (Singer *et al.*, 1998; Markarian *et al.*, 1995).

RESULTS

General Test Conditions

Temperature, salinity, pH, and dissolved oxygen (DO) remained within acceptable limits during the tests (Table 2-2). Oxygen concentrations in test solutions were above

Table 2-2. Summary of water quality parameters measured for weathered oil toxicity tests

Test Species		pH	Salinity (ppt)	Temp. (°C)	D.O. (mg/L)
C. bairdi	Mean	8.35	31.48	6.91	8.90
	Std. Dev.	0.09	0.17	0.77	0.21
	n	60	60	60	60
	Maximum	8.46	31.90	9.70	9.54
	Minimum	8.01	31.10	5.90	8.31
M. beryllina	Mean	7.86	20.32	25.15	6.13
	Std. Dev.	0.10	0.41	0.45	0.66
	n	59	59	59	59
	Maximum	8.01	21.69	26.00	7.20
	Minimum	7.63	19.11	24.00	4.70

60% saturation at all times; pH remained within a range of 6 to 9. Temperatures and salinity for all tests were maintained according to test protocols (25°C ± 1°C and 20‰ ± 10% for *M. beryllina*; ambient conditions: 7°C ± 1°C and 31.5‰ ± 10% for *C. bairdi*) with little variability.

Oil Solutions

In the weathered oil test solutions (WAF and CE-WAF), the total hydrocarbon content (THC, C_6-C_{36}) was measured and observed to generally increase with increased oil loading (Figures 2-1a and 2-1b). In water-accommodated fractions (WAF), however, the volatile organic analyte group (VOA, C_6-C_9) increased some and total petroleum hydrocarbons (TPH, C_{10}-C_{36}) increased only slightly, if at all, despite the wide range of oil loading rates (500 to 10,000 mg/L) used to prepare these solutions. In chemically-enhanced water-accommodated fractions (CE-WAFs), TPH concentrations were observed to increase at a rate significantly (t-test; $P < 0.05$) higher than VOA concentrations with increased oil loadings. In CE-WAF solutions, concentrations of TPH were greater than VOA for all oil loadings used. By the addition of dispersants, TPH concentrations were greater in CE-WAF solutions than in WAF solutions for any given oil-loading rate.

A linear relationship of oil loading rates to resultant VOA and TPH concentrations showed good correlation for all CE-WAF solutions ($r^2 \geq 0.93$), and less so for WAF VOA components ($r^2 \geq 0.68$). TPH components of WAF solutions were poorly correlated linearly ($0.01 < r^2 < 0.16$). A closer inspection of WAF VOA and TPH

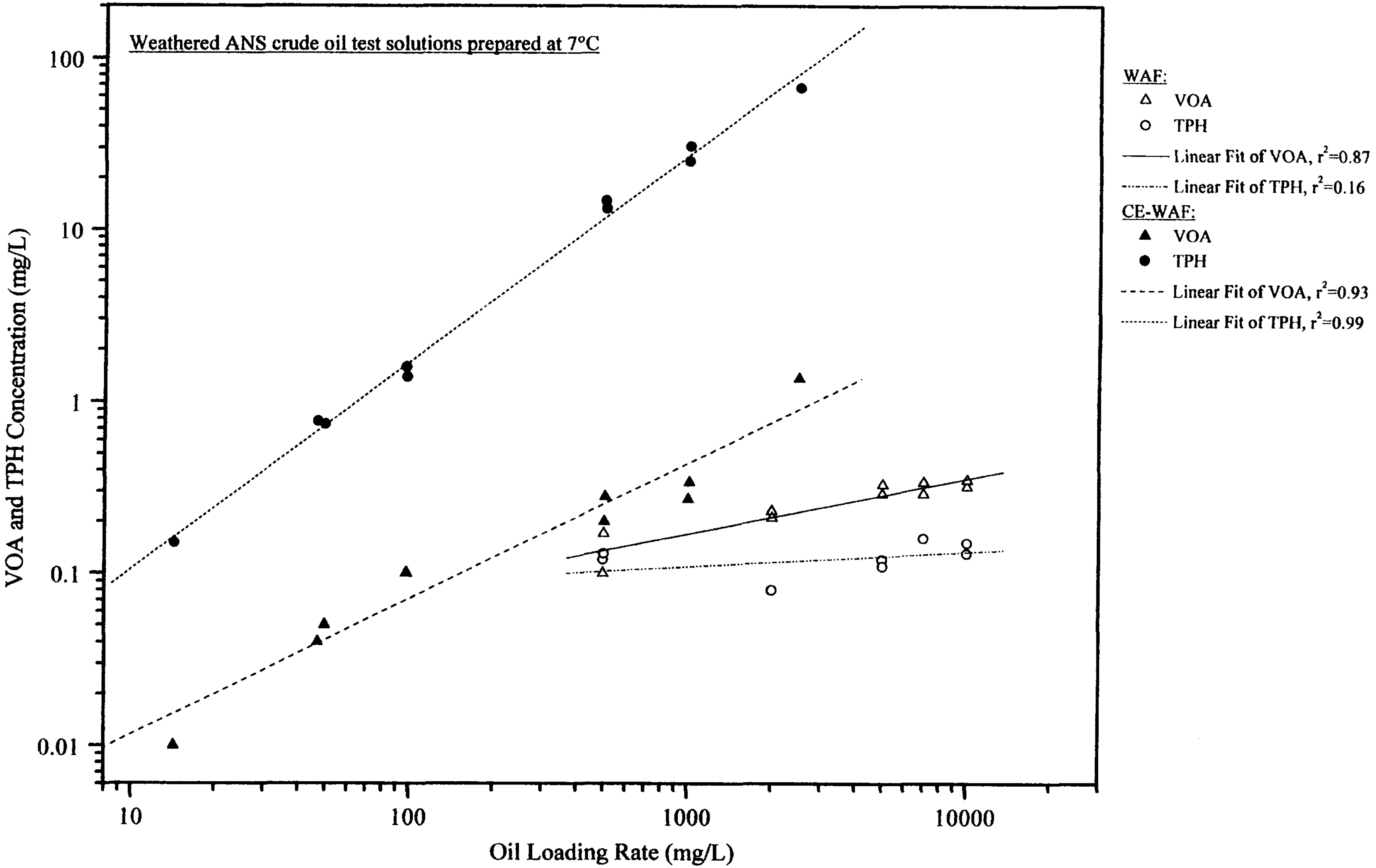

Fig 2-1a. Relationship of measured concentrations of volatile organic analytes (VOA) and total petroleum hydrocarbons (TPH) to oil loading rates in weathered ANS WAF and CE-WAF test solutions for *C. bairdi* in 7°C saltwater

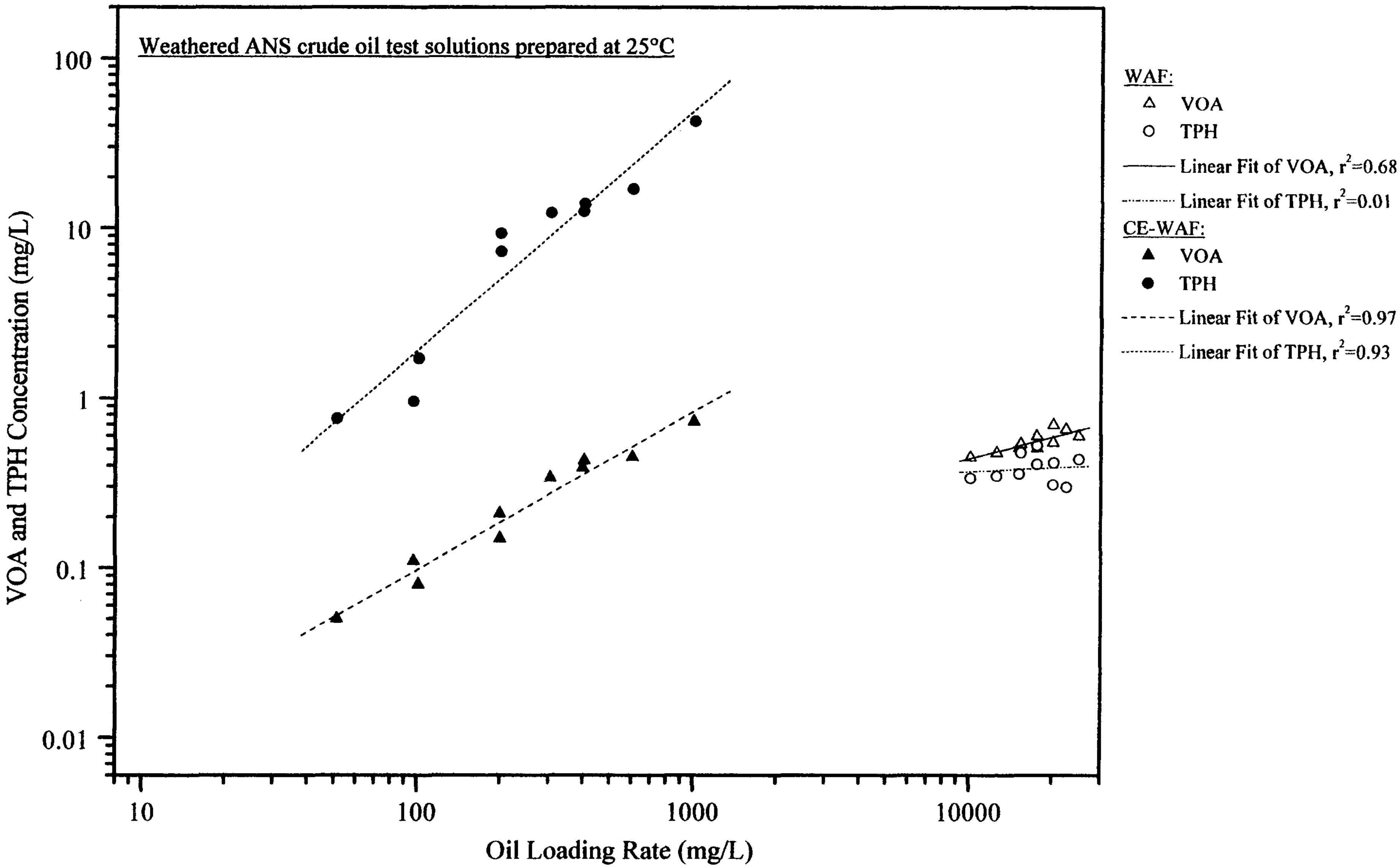

Fig 2-1b. Relationship of measured concentrations of volatile organic analytes (VOA) and total petroleum hydrocarbons (TPH) to oil loading rates in weathered ANS WAF and CE-WAF test solutions for *M. beryllina* in 25°C saltwater

concentrations suggests these solutions reach a quasi-saturation state. For solutions prepared for *M. beryllina* (25°C and 20‰), this occurred at an oil loading rate of 20,000 mg/L for VOA, and 10,000 for TPH. Similarly, for *C. bairdi* (6°C and 31.5‰), a quasi-saturation of solutions was observed at a loading rate of 5,000 mg/L for VOA components and less than 1,250 mg/L for TPH components (Figures 2-2a and 2-2b). At loading rates higher that those mentioned above, concentrations of THC increase very slowly if at all.

Following the artificial weathering process, with temperatures topped off at 200°C, the weight of residual oil was on average 70.4 percent (SD = 0.009, N = 5) of the un-weathered (*i.e.,* fresh) ANS. This suggests that 29.6 percent by weight of the fresh oil is comprised of compounds with a boiling point of 200°C or less. These results are consistent with the understanding that fresh ANS is approximately one-third by weight volatiles (components with boiling points 204 to 274°C or less; pers. comm., Mead, 1997).

Analysis of concentration-decline in spiked exposure tests indicated that solution VOA concentrations generally follow a trend of first order exponential decay, stabilizing between the sixth and ninth hour of the 96-hour test (Figures 2-3a and 2-3b) similar to observations in the fresh oil study. In samples from WAF and CE-WAF tests analyzed for VOA content, concentrations measured for the twelfth hour were no greater than 0.02 mg/L.

The average resulting concentrations of weathered WAF and CE-WAF solutions are presented in Table 2-3. As expected, the VOA content in weathered oil solutions was

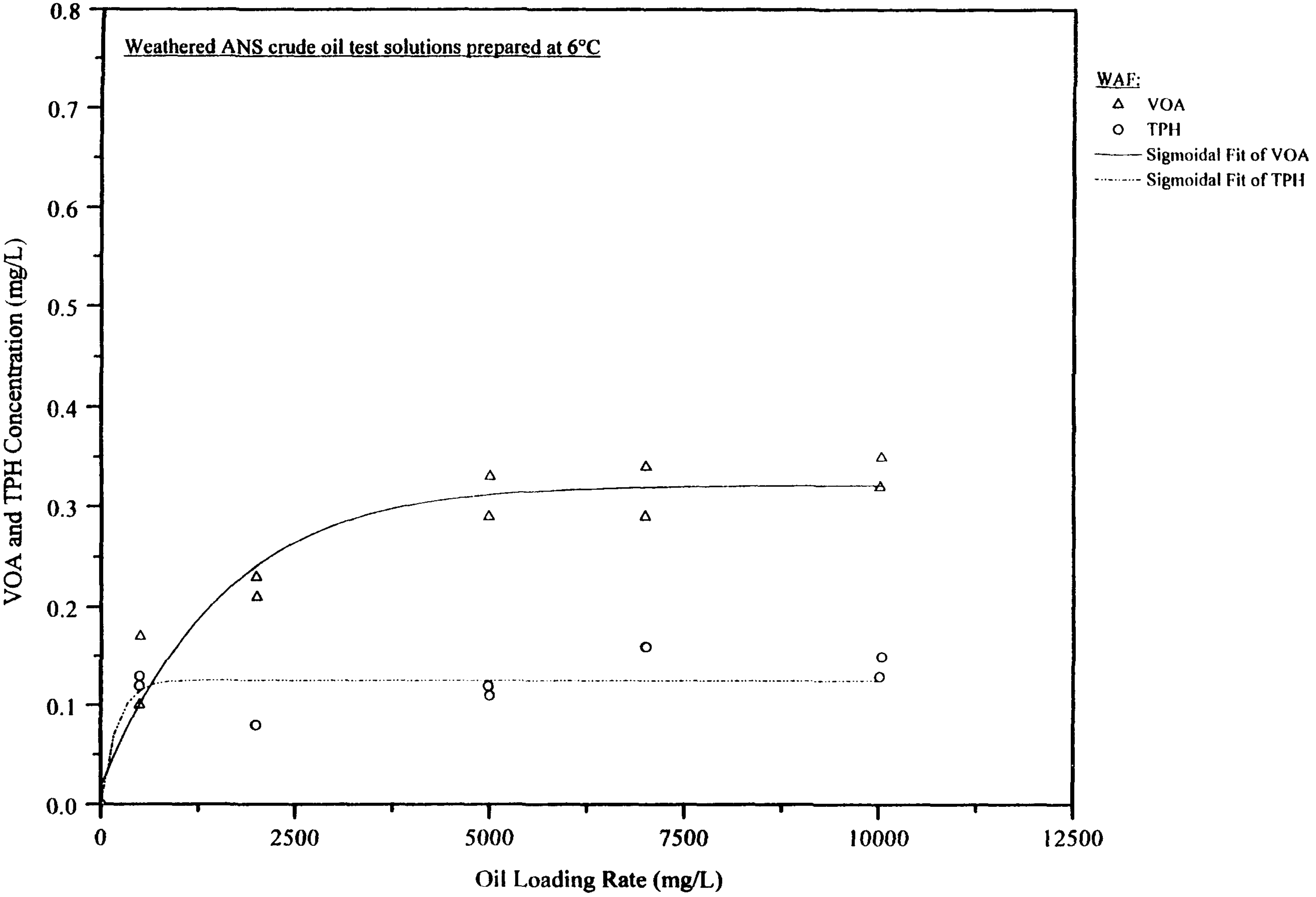

Fig. 2-2a. Relationship of measured concentrations of volatile organic analytes (VOA) and total petroleum hydrocarbons (TPH) to oil loading rates in weathered ANS test solutions for *C. bairdi* in 7°C saltwater – linear scale

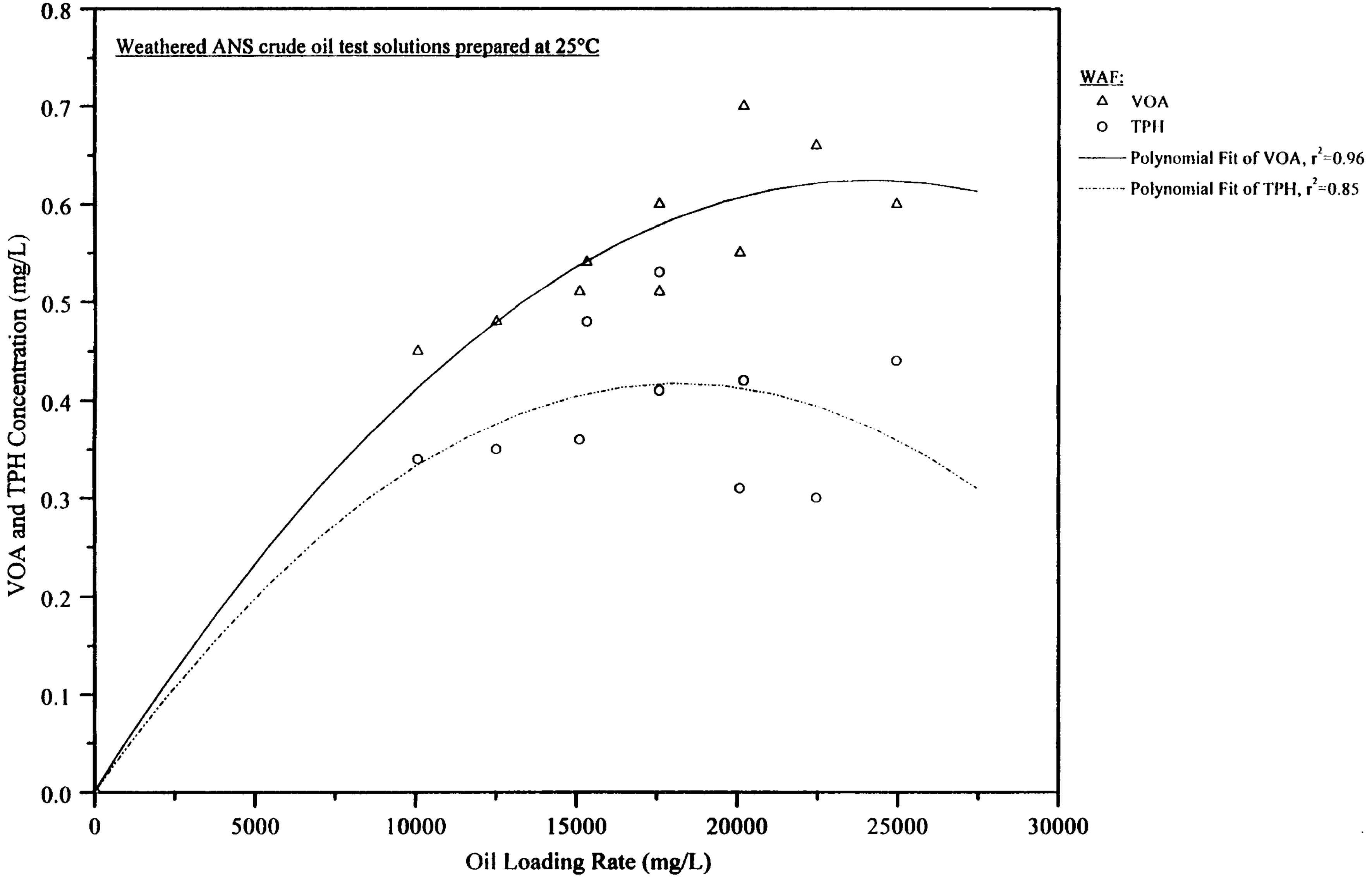

Fig. 2-2b. Relationship of measured concentrations of volatile organic analytes (VOA) and total petroleum hydrocarbons (TPH) to oil loading rates in weathered ANS test solutions for *M. beryllina* in 25°C saltwater – linear scale

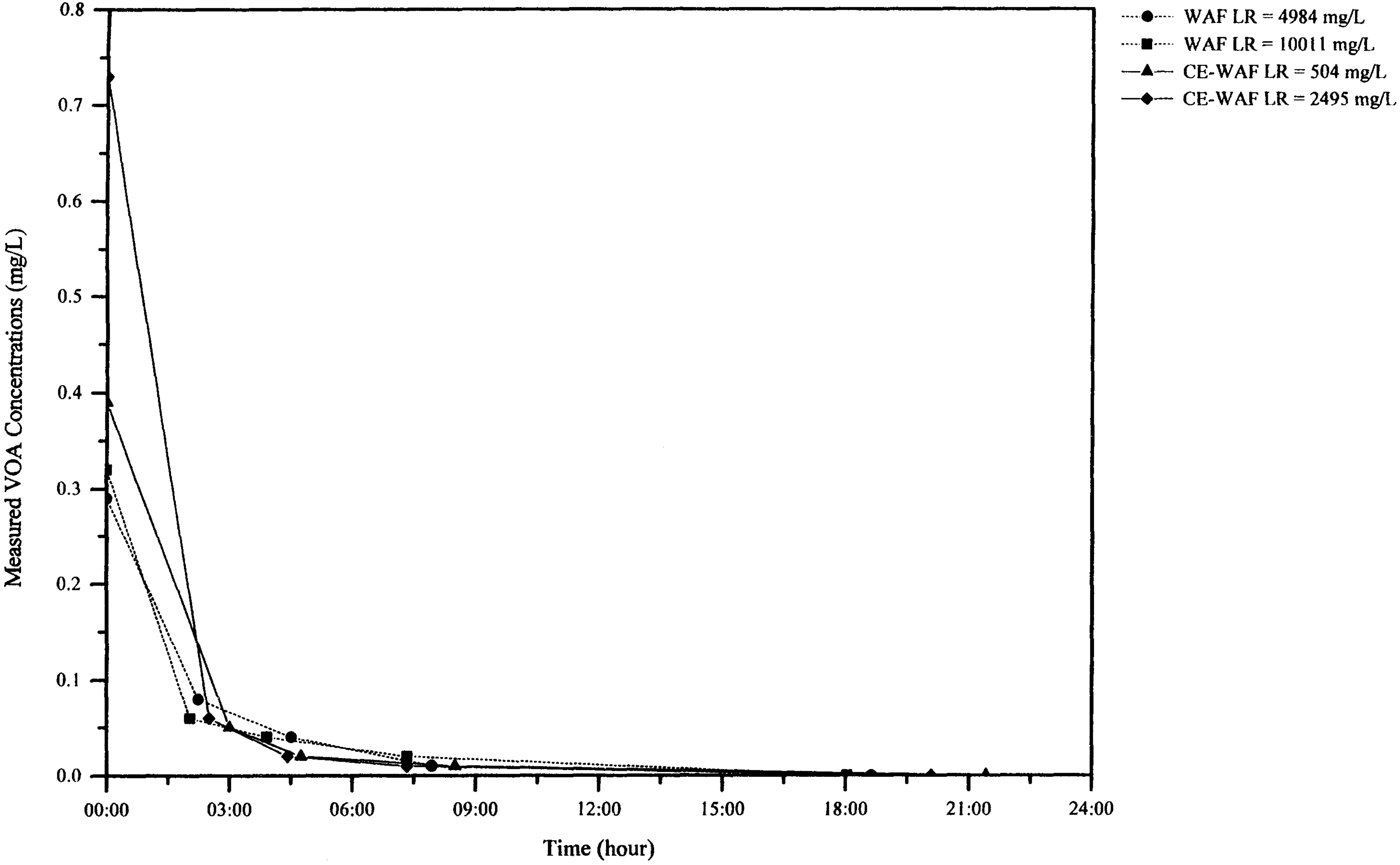

Fig. 2-3a. Concentration decline in spiked exposure tests of weathered ANS WAF and CE-WAF test solutions of different loading rates (LR) for *C. bairdi*

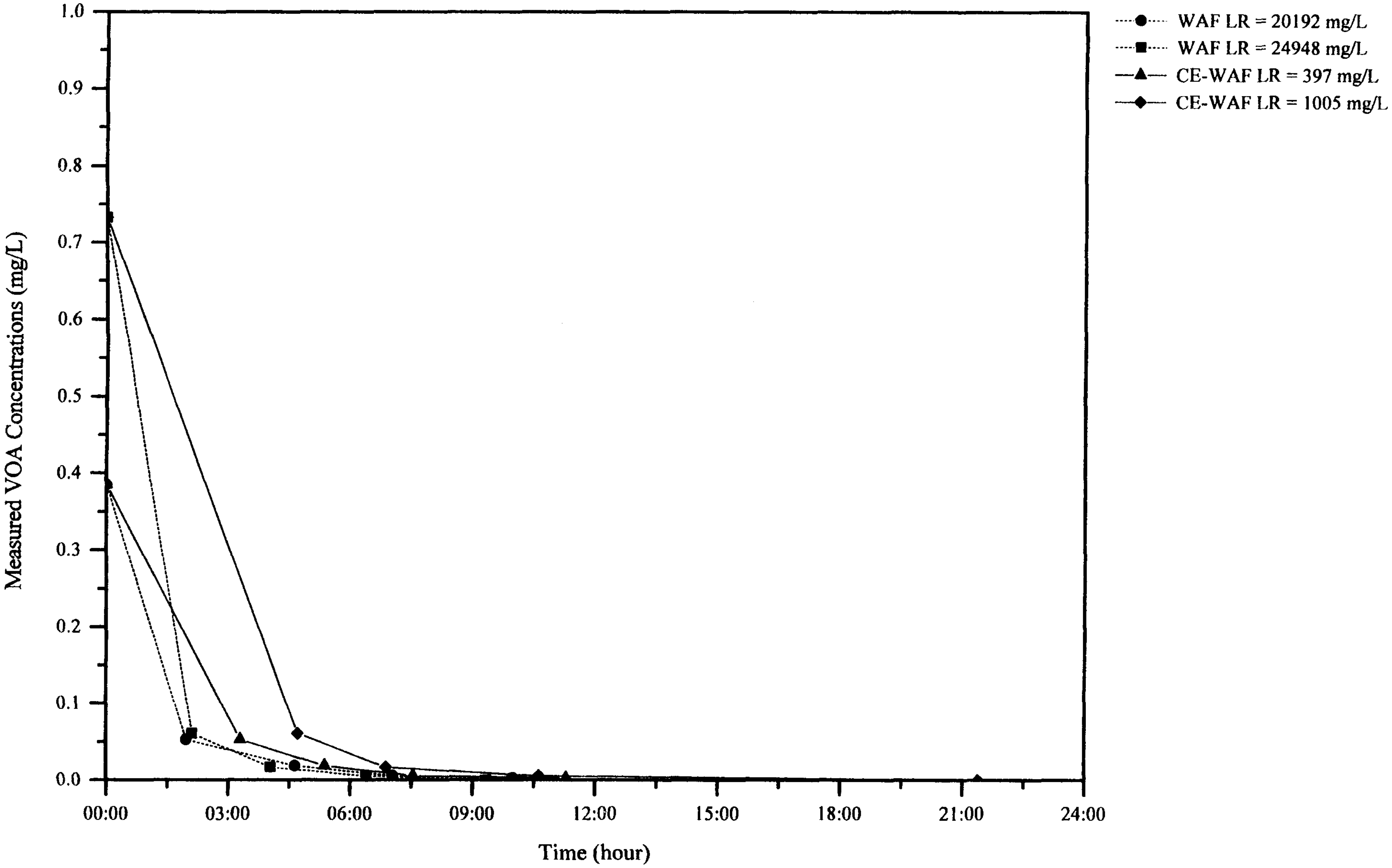

Fig. 2-3b. Concentration decline in spiked exposure tests of weathered ANS WAF and CE-WAF test solutions of different loading rates (LR) for *M. beryllina*

Table 2-3. Mean concentrations of hydrocarbons measured in WAF and CE-WAF solutions

Hydrocarbon Fraction	Oil Type	Mean +/- Std. Error (SE) Hydrocarbon Conc. (mg/L)							
		WAF	SE	n	% THC	CE-WAF	SE	n	% THC
VOA	Weathered ANS	0.53	0.04	14	60.2	0.55	0.11	13	1.9
TPH	Weathered ANS	0.35	0.04	14	39.8	28	5.7	13	98.1
THC	Weathered ANS	0.88	0.08	14	-	28	5.8	13	-

n = number of samples

considerably less than those made from fresh, with average concentrations of 0.53 and 0.55 mg/L for WAF and CE-WAF, respectively. Similar to fresh oil solutions, with the addition of dispersant, weathered CE-WAF solutions were considerable more concentrated with TPH than WAF with an average of 28 versus 0.35 mg/L. Proportionally, weathered WAF solutions were comprised of 60.2 percent VOA and 39.8 percent TPH, and for weathered CE-WAF, 1.9 percent VOA and 98.1 percent TPH solutions.

Chionocetes bairdi Tests

In both spiked and continuous exposure tests, dose-response relationships were approximately sigmoidal for both WAF and CE-WAF test solutions (Figures 2-4a and 2-4b). The range of loading rates for dispersant and fresh oil tests and their respective measured concentrations for spiked and continuous exposure regimes are summarized in Table 2-4. Solutions for continuous exposure CE-WAF tests were generally prepared using 2.5 to 3.4 times less test material (weathered ANS crude oil) than solutions prepared for spiked exposure tests; whereas for WAF solutions, identical oil loading rates were used in each exposure regime. WAF solutions were prepared using 10 to 36 times more weathered crude oil than CE-WAF solutions tested. The resulting THC concentrations from WAF and CE-WAF solutions were very different. A high WAF oil loading rate of 10,030 mg/L resulted in a concentration of only 0.51 mg/L THC. But a high CE-WAF oil loading rate of 1011, approximately 10 times less initial material than

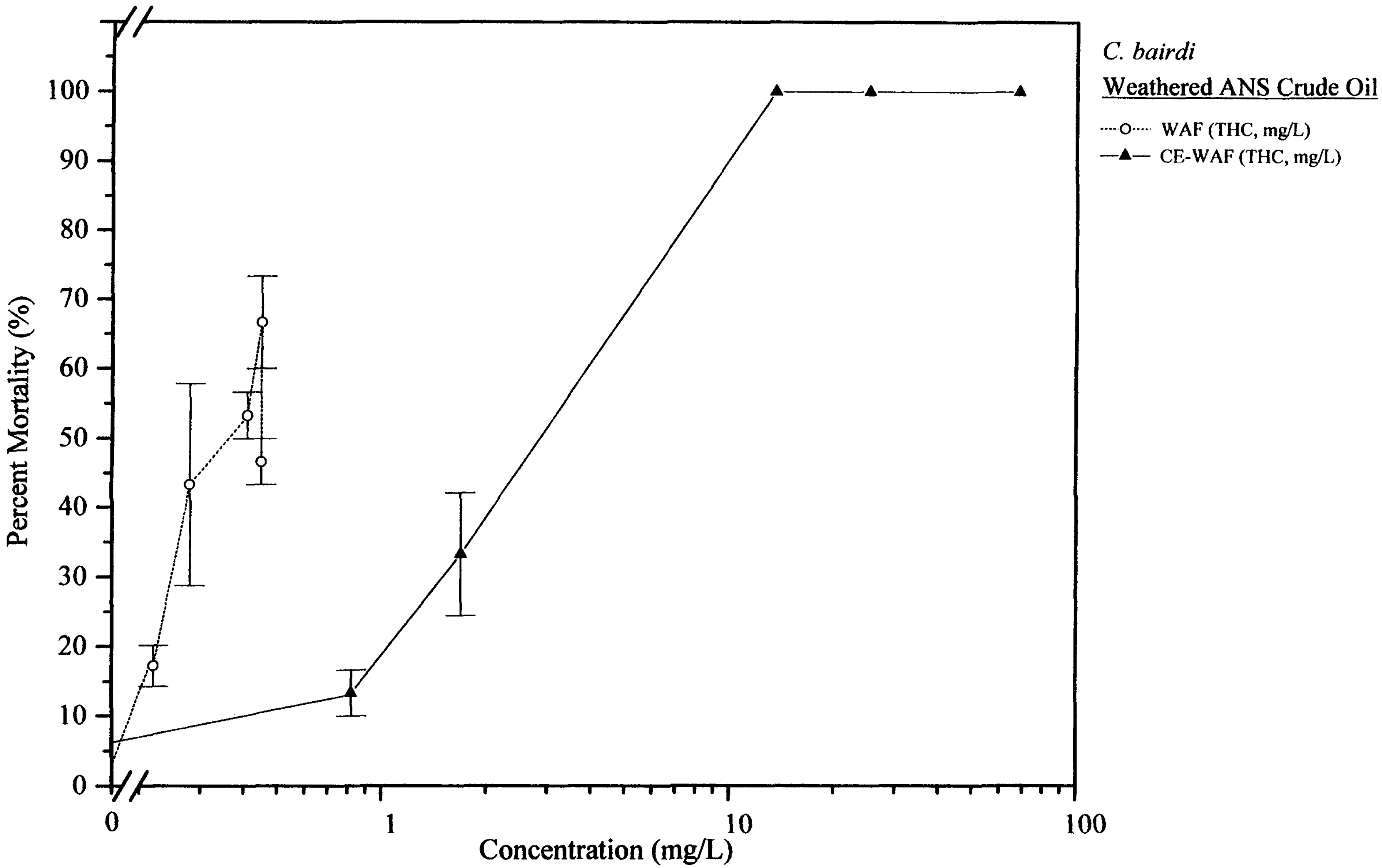

Fig. 2-4a. Dose-response relationships for *C. bairdi* to weathered ANS spiked exposure tests. Symbols are mean ± SE for each concentration (n = 3)

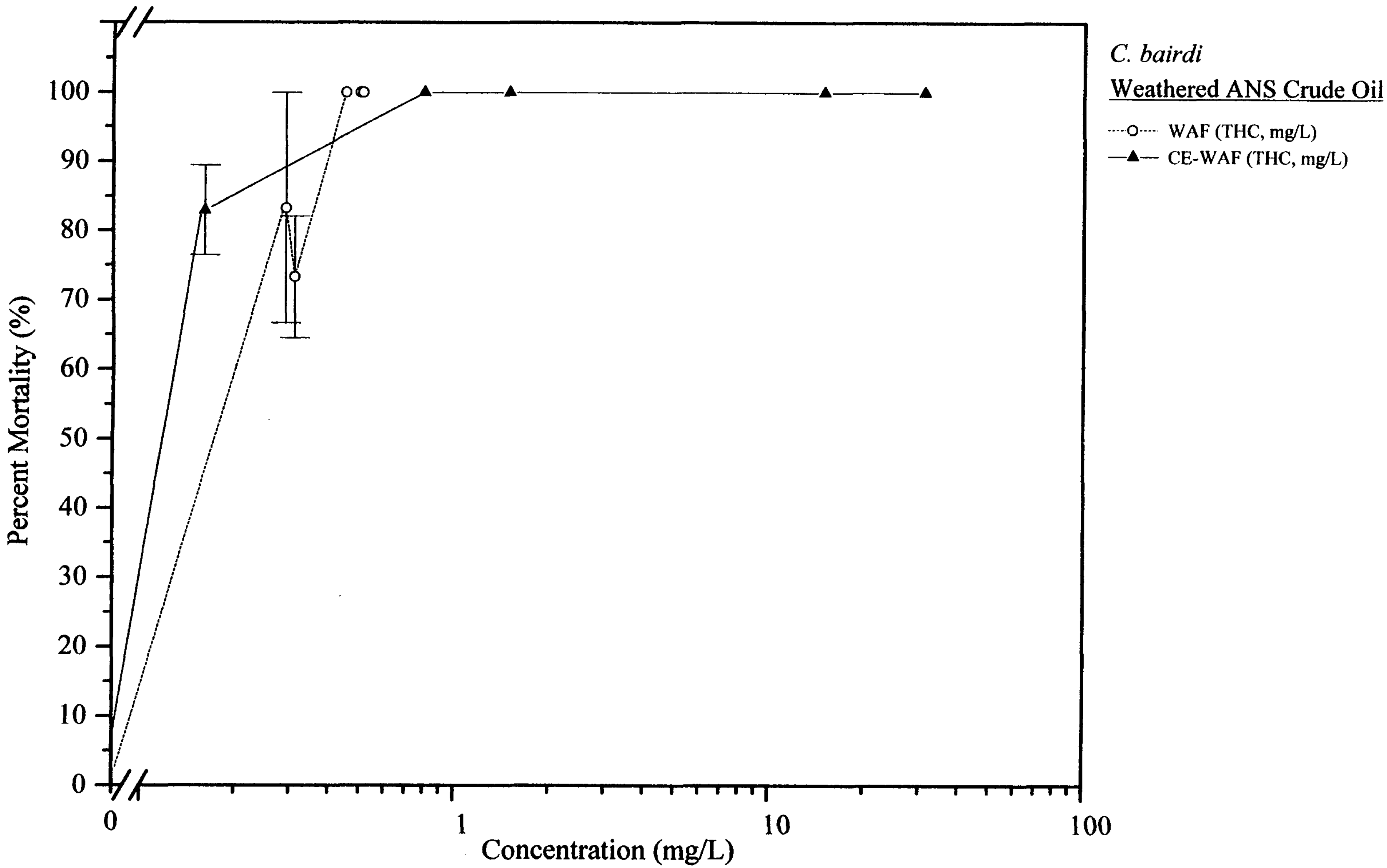

Fig. 2-4b. Dose-response relationships for *C. bairdi* to weathered ANS continuous exposure tests. Symbols are mean ± SE for each concentration (n = 3)

Table 2-4. Summary of the ranges of weathered ANS crude oil loading rates (mg/L) and respective measured THC (C_6-C_{36}) concentrations (mg/L) used in spiked and continuous exposure tests

			C. bairdi		*M. beryllina*	
Test Solution	Exposure Regime	Range	Loading Rate	Measured THC	Loading Rate	Measured THC
WAF	Spiked	Low	500	0.22	15300	1.02
		High	10010	0.45	25000	1.04
	Continuous	Low	500	0.29	10100	0.79
		High	10030	0.51	20100	0.86
CE-WAF	Spiked	Low	50	0.82	100	1.07
		High	2500	68.1	1000	43.2
	Continuous	Low	10	0.16	50	0.81
		High	1010	31.1	400	14.5

was used in the WAF solution, resulted in a concentration of 31.06 mg/L THC, over 60 times the concentration in the WAF solution (Table 2-4).

Median-effect concentrations are presented in Table 2-5. EC_{50} estimates for WAFs of weathered ANS crude oil were 0.40 and 0.27 mg/L for spiked and continuous exposures, respectively. For CE-WAFs of weathered ANS crude oil, the estimated EC_{50} for spiked exposure was 2.36 mg/L and was 0.36 mg/L for continuous exposure. Only one partial effect at a high percentage (83%) was observed in the continuous exposure CE-WAF test; this prevented assumptions necessary for use of Probit and Trimmed Spearman-Karber analyses to be satisfied. Consequently, the median-effect concentration for the CE-WAF continuous exposure test was estimated using graphical analysis.

Qualitative, temporal observations are not available for *C. bairdi*, since evaluating the response of this species required close observations that could not be made during the assay.

Menidia beryllina Tests

Oil loading rates for spiked exposures of weathered ANS crude oil ranged from 1.2 to 2.5 times greater than those used in continuous exposure tests (Table 2-4). WAFs of weathered ANS crude oil were prepared using 158 to 238 times more test crude oil than in CE-WAFs for spiked exposure tests, and 50 to 197 times more for continuous exposure tests. Similar to weathered oil solutions prepared for *C. bairdi* tests, the resulting THC concentrations are much greater for chemically treated oil solutions than

Table 2-5. Acute 96-hour median lethal and effect concentration (mg/L) estimates (95% confidence limits) for WAF and CE-WAF weathered oil tests

	Weathered ANS Crude Oil			
	C. bairdi EC_{50} Values		*M. beryllina* LC_{50} Values	
Test Solution	Spiked Exposure	Continous Exposure	Spiked Exposure	Continous Exposure
WAF[*]	0.40[†] (0.33, 0.51)	0.27[†] (0.24, 0.28)	>1.13[**]	0.79[†] (0.32, 0.83)
CE-WAF[*]	2.36[†] (1.66, 6.66)	0.36[‡‡]	18.89[†] (15.78, 24.71)	0.65[†] (0.10, 1.25)

Notes:

[*] WAF and CE-WAF values based on total hydrocarbon content (THC) in mg/L

[**] Highest concentration tested had a 24,948 mg/L loading rate

Statistical Methods Used:

[†] Probit analysis

[‡‡] Graphical method, 95% confidence limits not available (Webber, 1993)

untreated solutions. For an oil loading rate of 10,058 mg/L in a WAF solution, the resulting THC concentration was 0.79 mg/L. Comparatively, a 1005 mg/L oil loading rate in a CE-WAF solution, approximately 10 times less initial oil, resulted in a solution over 40 times more concentrated than the WAF at 43.23 mg/L (Table 2-4).

Dose-response relationships for *M. beryllina* tests using of weathered ANS WAF solution were often not monotonically increasing with increasing concentration. This is likely due to the limited ability of weathered crude oil to form soluble (or accommodated) fractions. Also, chemical analysis results occasionally indicated that measured concentrations of total hydrocarbons in solution were lower than those measured in solutions prepared with less initial crude oil. This, in addition to the variability of the response of *M. beryllina* to the test solutions, contributes to the shape of these curves (Figures 2-5a and 2-5b). The dose-response relationships for both spiked and continuous exposures to CE-WAFs of weathered ANS crude oil are approximately sigmoidal.

Assumptions necessary to estimate median-lethal concentrations using Probit analysis were satisfied in all but one of the four tests. Under spiked exposure for WAF test solutions, the estimated LC_{50} for *M. beryllina* was >1.13, and 0.79 mg/L for continuous exposure (Table 2-5). An estimated LC_{50} could not calculated for the WAF test since the highest percent mortality observed in the test was 20% at a measured THC of 1.13 mg/L from the highest oil loading of 24,948 mg/L. Estimated LC_{50} values for CE-WAF spiked exposure was 18.89 mg/L, and 0.65 mg/L for continuous exposure. Non-overlapping fiducial limits for median-lethal concentrations of weathered ANS CE-WAF spiked and continuous exposures tests suggest that these values are significantly different. Because

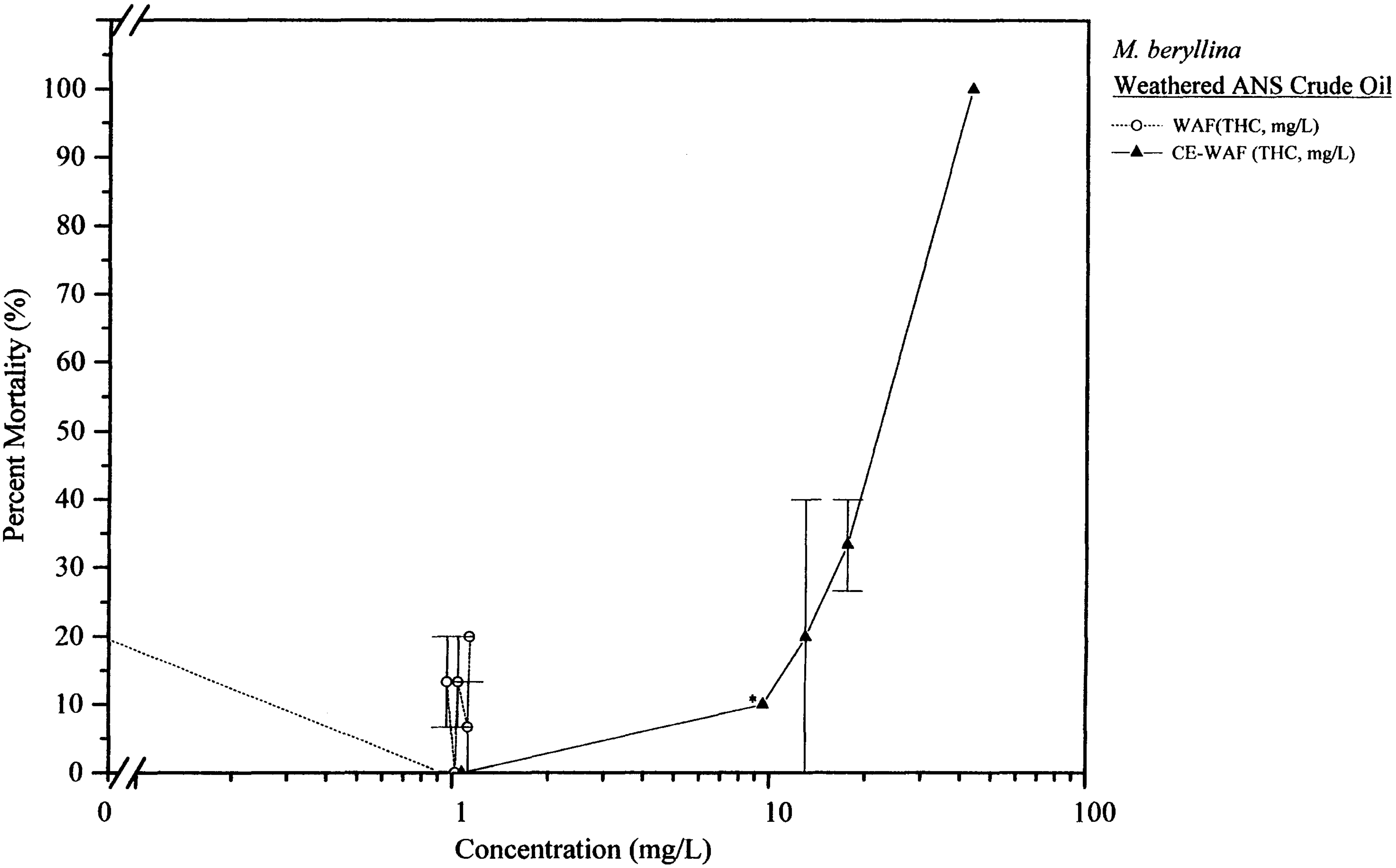

Fig. 2-5a. Dose-response relationships for *M. beryllina* to weathered ANS spiked exposure tests. Symbols are mean ± SE for each concentration (n = 3, except where noted by "*" where SE could not be calculated)

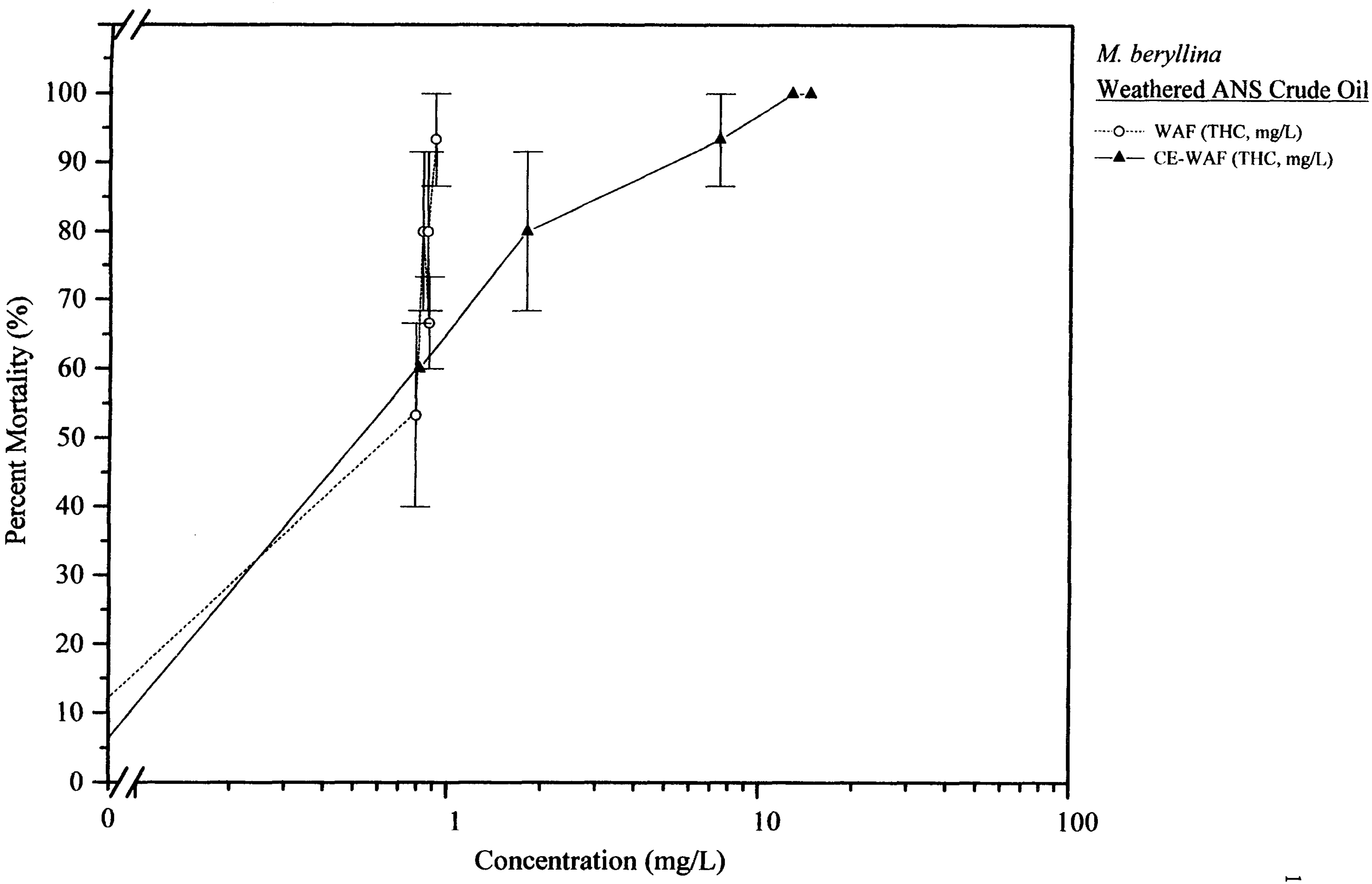

Fig. 2-5b. Dose-response relationships for *M. beryllina* to weathered ANS continuous exposure tests. Symbols are mean ± SE for each concentration (n = 3)

fiducial limits were not available for the WAF spiked exposure test, comparisons of LC_{50} values for spiked and continuous exposures of WAF test solutions, and between spiked exposures of WAF and CE-WAF tests are not possible. However, overlapping limits of LC_{50} values for continuous exposure WAF and CE-WAF tests suggest these values are not significantly different.

Qualitative estimates of temporal median-lethal concentrations at hours 24, 48, 72, and 96 of the 96-hour tests based upon the weathered oil loading rates used for solution preparation are presented in Table 2-6. The response of *M. beryllina* to spiked exposures of WAF and CE-WAF solutions stabilized within the first 24 hours of the 96-hour test. However under continuous exposure, *M. beryllina* experience an increase in mortality over the duration of the test. Where assumptions necessary to calculate an estimated median-lethal concentration were not satisfied, these values are reported as a greater-than number.

Microtox® Assay

Mean 5-minute EC_{50} values obtained by the Microtox® system were calculated by pooling all data available (from analysis samples collected from tests using *C. bairdi* and *M. beryllina* from both static and flow through experiments) for a particular oil (Table 2-7). The data from all individual tests used to calculate the mean EC_{50} values are found in the Appendix J. Mean EC_{50} values (Table 2-7) were standardized to all manners of concentration characterization (measured volatile organic analysis (VOA), total

Table 2-6. Daily median-lethal loading (LL_{50},mg/L) estimates (95% confidence limits) for *M. beryllina* weathered ANS crude oil WAF and CE-WAF spiked and continuous exposure tests

	Weathered ANS Crude Oil Spiked Exposure				Weathered ANS Crude Oil Continuous Exposure			
	Observation time (hr)				Observation time (hr)			
Test Solution	24	48	72	96	24	48	72	96
WAF*	>24948	>24948	>24948	>24948	>20077	>20077	13366 (N/A)**	9512 (N/A)**
CE-WAF*	555.15 (450, 684)	555.15 (450, 684)	555.15 (450, 684)	555.15 (450, 684)	239.49 (198, 289)	165 (129, 204)	78 (40, 112)	47 (14, 72)

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* WAF and CE-WAF values based on oil loading rate in mg/L

**Not available

Table 2-7. Mean 5-minute EC_{50} values obtained by the Microtox Toxicity Assay. Values were calculated based on measured hydrocarbon fractions and on total oil added (loading rates)

Hydrocarbon Fraction	Oil Type	Mean +/- Std. Error (SE) EC_{50} (mg/L)*					
		WAF	SE	n	CE-WAF	SE	n
VOA	Weathered ANS	0.22	0.01	14	0.12	0.02	13
TPH	Weathered ANS	0.15	0.02	14	5.9	1.0	13
THC	Weathered ANS	0.37	0.03	14	6.0	1.1	13
Loading Rates	Weathered ANS	6400	570	18	180	39	15

n = number of tests

* For each oil type and a given hydrocarbon fraction used to standardize the data, the EC_{50} value for WAF was significantly different ($P < 0.05$) from that for CE-WAF

petroleum hydrocarbons (TPH), total hydrocarbon content (THC; defined as VOA + TPH), or loading rates). No matter what fraction was used to standardize the data, for any given oil type, EC_{50} values for WAF and CE-WAF were always significantly (t-test; $P < 0.05$). When the data were standardized to VOA or to loading rates, CE-WAF solutions were calculated to be more toxic than WAF solutions. When standardized to TPH or THC the opposite trend was seen; WAF was more toxic.

Toxicity Value Comparisons: Test Solutions

When the toxicity data were standardized to loading rates (LL_{50} and EL_{50} values; denoted as: LL_{50}/EL_{50}), CE-WAF solutions were more toxic than WAF solutions in all cases (n = 5 out of N = 5; where 1 of the 5 did not have fiducial limits to test significance). In contrast, when the toxicity data were standardized to measured concentrations of THC (LC_{50} or EC_{50} values; denoted as: LC_{50}/EC_{50}), WAF solutions were more toxic than CE-WAF (n = 4 out of N = 5; note; 1 of the 4 cases cannot be tested for significant difference due to absence of fiducial limits for the greater-than toxicity value). The fact that two conflicting results can be drawn from the same data set as a result of the method of calculation for the toxicity values is confounding. Two possible interpretations exist for these data: 1) WAF is more toxic than CE-WAF according to LC_{50}/EC_{50} values; or 2) CE-WAF is more toxic than WAF according to the LL_{50}/EL_{50}.

For toxicity values calculated using only fractional groups of hydrocarbons in solution (*e.g.*, TPH or VOA), similar contradictory results concerning which test solutions is more toxic can be observed. To illustrate this, toxicity values were calculated for *V. fischeri* based upon the hydrocarbons groups of VOA, TPH, and THC (Table 2-7). From the Microtox® Assays for *V. fischeri*, weathered CE-WAF solutions were most toxic when standardized either VOA or THC fractions. However, when standardized to TPH, weathered WAF solutions were most toxic. This same observation was made for solutions made from fresh oil when the toxicity data was standardized to individual hydrocarbon groups; WAF solutions appeared more toxic when comparisons were made using the TPH fraction only. The results appear to be directly related to the solubility of the test material and the manner in which the data are presented (*e.g.*, TPH or THC; THC or loading rate).

Dispersant-only solutions, determined in the fresh oil study, were less toxic than the weathered oil solutions (WAF and CE-WAF) in all but two cases ($n = 6$ out of $N = 8$; with all 4 relationships being significant). Those cases were for *V. fischeri* and *M. beryllina* spiked exposure, where according to EL_{50}, dispersant-only solutions were more toxic than weathered WAF (both relationships were significant).

Toxicity Value Comparisons: Species Sensitivities

All three species (*C. bairdi, M. beryllina,* and *V. fischeri*) were either more or less sensitive to dispersed weathered oil solutions depending upon the manner in which the solution concentrations were portrayed, as measured concentrations or loading rates. For every species tested, when comparisons are made using toxicity values based upon LC_{50}/EC_{50} values, weathered WAF is more toxic than the CE-WAF. Conversely, according to LL_{50}/EL_{50} values, weathered CE-WAF is more toxic than WAF to all species.

According to both LC_{50}/EC_{50} and LL_{50}/EL_{50} values, *M. beryllina* was always the least sensitive species of those tested (n = 8 out of N = 8; where 3 of the 8 did not have fiducial limits to test significance). *C. bairdi* was consistently the most sensitive species (n = 7 out of N = 8; where 2 of the 8 were without fiducial limits). *V. fischeri*, therefore, was moderately sensitive compared to *M. beryllina* and *C. bairdi*. Similar trends were observed in the fresh oil study, in which *M. beryllina* was least sensitive and *C. bairdi* the most.

DISCUSSION

Data Evaluation

To properly interpret the toxicity data, differences in end-points (*i.e.*, lethal vs. sub-lethal) and test solution preparation methods (*i.e.*, CE-WAF and WAF) should be considered. Discussion of these topics in Chapter 1 of the fresh oil study also apply here to the weathered oil study.

Oil Solutions

As in the fresh oil study, oil solutions (WAF and CE-WAF) were prepared with different oil loadings in order to produce results that either bracketed or caused at minimum a 50 percent effect by the test species. Solutions prepared with dispersant added (CE-WAF) required substantially less oil (4 to 170 times less) than those without

(WAF) to produce solutions with similarly effective hydrocarbon concentrations (Figures 2-1a and 2-1b). However, for equivalent oil loading rates, CE-WAF solutions were substantially more concentrated in both TPH and VOA components (*e.g.*, see Figure 2-1a oil loading rate 500 mg/L). For example, from the *C. bairdi* tests, a WAF solution prepared at 496 mg/L oil loading resulted in a total hydrocarbon content (THC, C_6-C_{36}) concentration of 0.22 mg/L; whereas, at a similar oil loading of 504 mg/L for a CE-WAF, the resulting THC concentration was 13.5 mg/L (see Appendix G). On average, over 60 times the hydrocarbons went into solution in the CE-WAF than WAF as a result of dispersant addition (*e.g.*, see data in Table 2-3; 0.35 mg/L vs. 28 mg/L).

Weathering of ANS crude oil resulted in a reduction of approximately 30 percent by weight through loss of volatiles, components with boiling points 204 to 274°C or less, which constitute approximately one-third by weight of the crude oil (pers. comm., Mead, 1997). Compounds that make up the VOA fraction possess a greater propensity to dissolve in water than TPH, and have boiling points generally less than 200°C. Therefore, that portion of crude oil which was most likely to form soluble fractions with aqueous media (VOA) has now been removed from the system through the weathering process. As expected, measured concentrations of VOA in weathered WAF were considerably lower compared to fresh WAF (*e.g.*, VOA from an approximate loading rate of 500 was 0.10 mg/L for weathered and approximately 15 mg/L for fresh). Because TPH compounds are of inherently low-solubility, this hydrocarbon fraction has limited interaction with aqueous media. As a result, the concentration of TPH in WAF solutions is relatively low. Additionally, TPH exhibits an apparent saturation occurring at low oil loading rates (1250 ppm), and is typically unaffected by increases in oil loadings. Similar trends in TPH were observed in fresh WAF solutions, suggesting that the weathering process had little effect on the nature of TPH interactions with saltwater. Weathered WAF solutions are therefore low in THC (VOA + TPH) concentration for two reasons: 1) the more soluble fraction of crude oil (*i.e.,* VOA) has been removed from the system in the weathering process and is no longer available to form water soluble fractions; and 2) the inherent low solubility of TPH compounds limits the ability of this fraction to interact with aqueous media to form water soluble fractions.

In all solutions, temperature had a significant (t-test; $P < 0.05$) effect on the rates of inclusion (*i.e.,* the degree of increase in measured concentration in solution per increase in loading rate; slope of the line). These rates were significantly greater in warmer saltwater for all solution components (*i.e.,* WAF-VOA, CE-WAF-VOA, and CE-WAF-TPH) except for one. That exception being the TPH components in WAF solutions, where these were greater in solutions at colder temperatures (7°C). Although the rate of TPH solution was greater in WAF solutions of cold waters than in warm, inspection of Figures 2-1a through 2-2b reveal warm waters were slightly more concentrated with TPH, even so, TPH in both WAF solutions were low. Additionally, the range of loading rates tested for weathered WAF solutions were very different for 25°C and 7°C. Had these loading rates overlapped, results of concentrations with respect to temperature and salinity may be different, altering interpretations of these results. By inspection of Figures 2-1a and 2-1b, the linear relationships for concentrations of all components were generally greater in the warmer solutions than in colder. This in addition to significantly greater rates of inclusion in warmer solutions may be a result of reduced viscosity of the compounds remaining in the weathered ANS crude oil, including those in the VOA range enhancing their solubility to some degree (McDonald *et al.,* 1977).

Mean measured hydrocarbon concentrations for WAF and CE-WAF are presented in Table 2-3. In WAF solutions, VOA compounds remaining after the weathering process contributed on average 60.2 percent of the total hydrocarbon content, reflecting the greater solubility than that of TPH. As expected with the addition of dispersant, the proportion of TPH in solution increased substantially. CE-WAF solutions contained 98 percent TPH, compared to the 39.8 percent in WAF. This implies that the dispersant acted as designed, enhancing solubility of inherently low-soluble hydrocarbons (Singer *et al.,* 1998). Corexit 9500 is designed to treat more viscous oils (Nalco/Exxon Energy Chemicals, L.P, 1997). The removal of VOA by the weathering process can be seen in its low presence in CE-WAF solution (2%). TPH was always more concentrated than VOA in CE-WAF solutions. These observations may indicate the following: 1) by weathering crude oil – removing the inherently soluble fraction, resulting WAF solutions are of low THC concentration; 2) dispersant addition has a greater effect on the rates of incorporation of low-solubility chemicals than those naturally of higher solubility; and 3) since VOA are primarily removed from the parent weathered crude oil, dispersant addition results in solutions more concentrated in TPH than VOA for all oil loadings.

Overall, as was with fresh oil solutions, the relationships between oil loading rates and the resulting hydrocarbon concentrations in solution for weathered oil were dependent upon: 1) the composition and chemical and physical characteristics of the parent oil, 2) conditions under which solutions were prepared (*e.g.*, mixing energy, temperature, etc.), and 3) whether the oil was treated with dispersant or not.

Toxicity Basis

The same discussion covered in Chapter 1 for fresh oils concerning the form in which toxicity values are presented also applies here, and is perhaps more relevant. To understand how the hydrocarbon fraction that is selected to calculate the toxicity data affects interpretation of the data, mean hydrocarbon chemistry data collected for the solutions used in the toxicity tests (Table 2-3) is summarized. As expected, weathered oil is depleted in VOA. Measured TPH values are low for all WAF solutions, but the addition of dispersant in the CE-WAF solutions substantially increases the concentrations of measured TPH. THC concentrations are dominated by whichever fraction is higher (VOA or TPH). When concentrations of a specific fraction are much less for WAF than CE-WAF solutions (*e.g.*, see TPH data), then WAF solutions appear to be significantly more toxic.

Since the median-effect concentration (MEC) values are calculated based upon the value used to characterize the solution concentration, a small number for concentration will result in calculation of a small MEC value. A small toxicity value indicates high toxicity. However, as was seen in the fresh oil study, omission of a hydrocarbon fraction may erroneously overlook an important, even dominant, contributor to toxicity – unless the toxicity is attributed to some unmeasured parameter. For example, when the data are based upon the TPH fraction only, WAF solutions appear to be quite toxic. Preferably, these values should be calculated based on the total hydrocarbon content (THC) so as to avoid erroneously omitting a fraction of the hydrocarbons that may be the dominant group contributing to the toxic effect. Therefore, along with the toxicity value, the fraction to which it was standardized should be reported as well.

When the toxicity data are based upon the loading rate required to produce a response by 50 percent of the population, WAF solutions are least toxic (Table 2-8). The values in this table are based upon the measured total hydrocarbon content (LC_{50} by THC) and the loading rate (LL_{50}). Interpretation of these data lead to conclusions that are exactly opposite. By LC_{50}, weathered WAF solutions are more toxic than CE-WAF, but by LL_{50}, CE-WAF solutions are more toxic. Similar observations were made by Bobra and others (1982) in which weathering of crude oil produces aqueous WAF solutions that are more toxic in the sense of having lower LC_{50} values, but the weathered WAF solutions are apparently saturated at very low hydrocarbon concentrations compared to CE-WAF solutions. Therefore, under these solution preparation conditions, these saturated solutions are essentially non-toxic, since above the level of saturation no additional material goes into solution.

However, 50 percent of the organisms tested elicited a response to weathered WAF solutions, with the exception of *M. beryllina*, suggesting that factors other than those measured may contribute to the organisms' response. For example, weathering crude oil may alter some physical parameters (*e.g.*, increasing viscosity) of the accommodated fractions that were not measured. Alternatively, at the higher oil loadings required to

Table 2-8. Acute 96-hour median lethal and effect concentration estimates (mg/L) based on measured concentrations and oil loading rates (95% confidence limits)

		Weathered ANS			
		WAF		CE-WAF	
Species	Exosure type	Measured THC Conc. LC_{50}	Loading Rate LL_{50}	Measured THC Conc. LC_{50}	Loading Rate LL_{50}
C. bairdi[†]	Spiked	0.40 (0.33, 0.51)	4485 (2216, 10248)	2.36 (1.66, 6.66)	128 (96, 426)
	Continous	0.27 (0.24, 0.28)	149 (N/A)	0.37 (N/A)	6.44 (N/A)
M. beryllina	Spiked	> 1.13 (N/A)	> 24948 (N/A)	18.89 (15.78, 24.71)	555 (450, 684)
	Continous	0.79 (0.32, 0.83)	9512 (N/A)	0.65 (0.10, 1.25)	47 (14, 72)
Vibrio fischeri[†]	N/A	0.37 +/- 0.03	6400 +/- 570	6.00 +/- 1.1	180 +/- 39

[†] Measured Conc. as EC_{50}, Loading Rate as EL_{50}, both in mg/L

* Not tested

produce an effect by 50 percent of the population, a greater number of oil particulates may be present in solution, causing the response to be more of a physical nature rather than a chemical nature.

Because opposite or conflicting conclusions can be drawn from toxicity data based upon either measured concentrations or oil loadings, concurrent use of the two forms, LC_{50} and LL_{50}, is preferred. Together they can provide more comprehensive information concerning toxicity of these solutions, incorporating both physical and chemical characteristics influencing the test solutions' formation and the solutions themselves. Opposite conclusions from the LC_{50} and LL_{50} about which solution is more toxic (or which organism more sensitive) may be indicative that other factors about the test solution besides measured concentrations (*e.g.*, solubility) should be considered when the toxicity of a material is evaluated.

Toxicity

As was observed in the fresh oil study, responses to test solutions were always greater under continuous exposure than under spiked, declining exposures. Discussion covering the subject of spiked versus continuous exposures for the fresh oil study in Chapter 1 is applicable here, but is not re-stated.

Toxicity Value Comparisons: Test Solution Toxicities

Comparisons are made here using toxicity values calculated based on measured concentrations of test solutions (LC_{50} and EC_{50} values) and loading rates used to prepare the solutions (LL_{50} and EL_{50}). When results from such comparisons agree, then arriving at a conclusion about which solution is more toxic is simpler, as was demonstrated with results from the fresh oil study. Results from this study of weathered oil toxicity; however, are not as straightforward. Two possibilities exist for weathered oil: 1) WAF is more toxic than CE-WAF according to measured THC concentrations in solution; 2) CE-WAF is more toxic than WAF according to the amount of product required to produce effective solutions (*i.e.,* those which result in a minimum of 50 percent response by the test organisms).

The same situation exists when comparisons are made between measured concentrations of fractional hydrocarbon groups in solution. For toxicity values calculated using only fractional groups of hydrocarbons in solution (*e.g.,* TPH or VOA), similar contradictory results concerning which test solutions is more toxic can also be observed here. When standardized to VOA, non-dispersed weathered oil (WAF) is less toxic than dispersed weathered oil as CE-WAF. The converse is true when standardized to TPH, dispersed weathered oil is less toxic. From the Microtox® Assays for *V. fischeri*, weathered CE-WAF solutions were most toxic when standardized either to VOA or THC fractions. However, when standardized to TPH, weathered WAF solutions were most toxic. This same observation was made for solutions made from fresh oil when the toxicity data was standardized to individual hydrocarbon groups; WAF solutions appeared more toxic when compared using the TPH fraction only. The results appear to be directly related to the solubility of the test material and the manner in which the data are presented (*e.g.,* TPH or THC; THC or loading rate).

Toxicity Value Comparisons: Species Sensitivities

The same conflicting interpretations as were seen above for which test solution is more toxic (weathered WAF or CE-WAF solutions) are observed when making comparisons of species sensitivity using the toxicity data. All three species tested (*C. bairdi, M. beryllina,* and *V. fischeri*) were either more or less sensitive to dispersed weathered oil solutions depending upon the manner in which the solution concentrations were portrayed, as measured concentrations or loading rates. When comparisons are made using toxicity values based upon measured concentrations, weathered WAF is more toxic than the CE-WAF; conversely, according to toxicity values calculated using loading rates, weathered CE-WAF is more toxic than WAF.

According to both LC_{50}/EC_{50} and LL_{50}/EL_{50} values, *M. beryllina* was always the least sensitive species of those tested (n = 8 out of N = 8; where 3 of the 8 did not have fiducial limits to test significance). *C. bairdi* was consistently the most sensitive species (n = 7 out of N = 8; where 2 of the 8 were without fiducial limits). *V. fischeri*, therefore, was moderately sensitive compared to *M. beryllina* and *C. bairdi*. Similar trends were observed in the fresh oil study, in which *M. beryllina* was least sensitive and *C. bairdi* the most. Bragin and Clark (1996) noted in their study that of the species tested (*M. bahia*, and *Crassostrea gigas*, oyster larvae), that *M. beryllina* was the least sensitive. Some researchers suggest that crustacean larvae are more sensitive than fish (Rice *et al.*, 1977; Singer *et al.*, 1998).

CHAPTER 3

FRESH AND WEATHERED OIL SOLUTIONS

RESULTS

The results of the fresh and weathered oil studies are combined and presented here to more directly compare the differences between these two states of oil. Comparisons are based on the chemical characteristics of fresh and weathered, dispersed and non-dispersed oil solutions, and their toxicity to *C. bairdi*, *M. beryllina*, and *V. fischeri* from Microtox® Assays.

Oil Solutions

Graphical representations of the dispersed and non-dispersed fresh and weathered oil solutions are shown in Figures 3-1a through 3-2b. Test solutions are compared based upon the temperature and salinity in which they were prepared. All WAF solutions have low TPH concentration, as expected, and have a distinct reduction in VOA from fresh to weathered WAF solutions. Both CE-WAF solutions had comparable amounts of TPH in solution, but the weathered CE-WAF solutions showed a marked reduction in VOA components, as expected. Mean hydrocarbon contents and relative proportions for fresh and weathered oils are shown in Table 3-1.

Toxicity Value Comparisons

The comparisons made in the sections below, further demonstrate how the aqueous solubility of the test material and the manner in which the test solution concentrations are characterized (*i.e.*, in terms of loading rate, TPH, THC, or VOA) can have an effect on

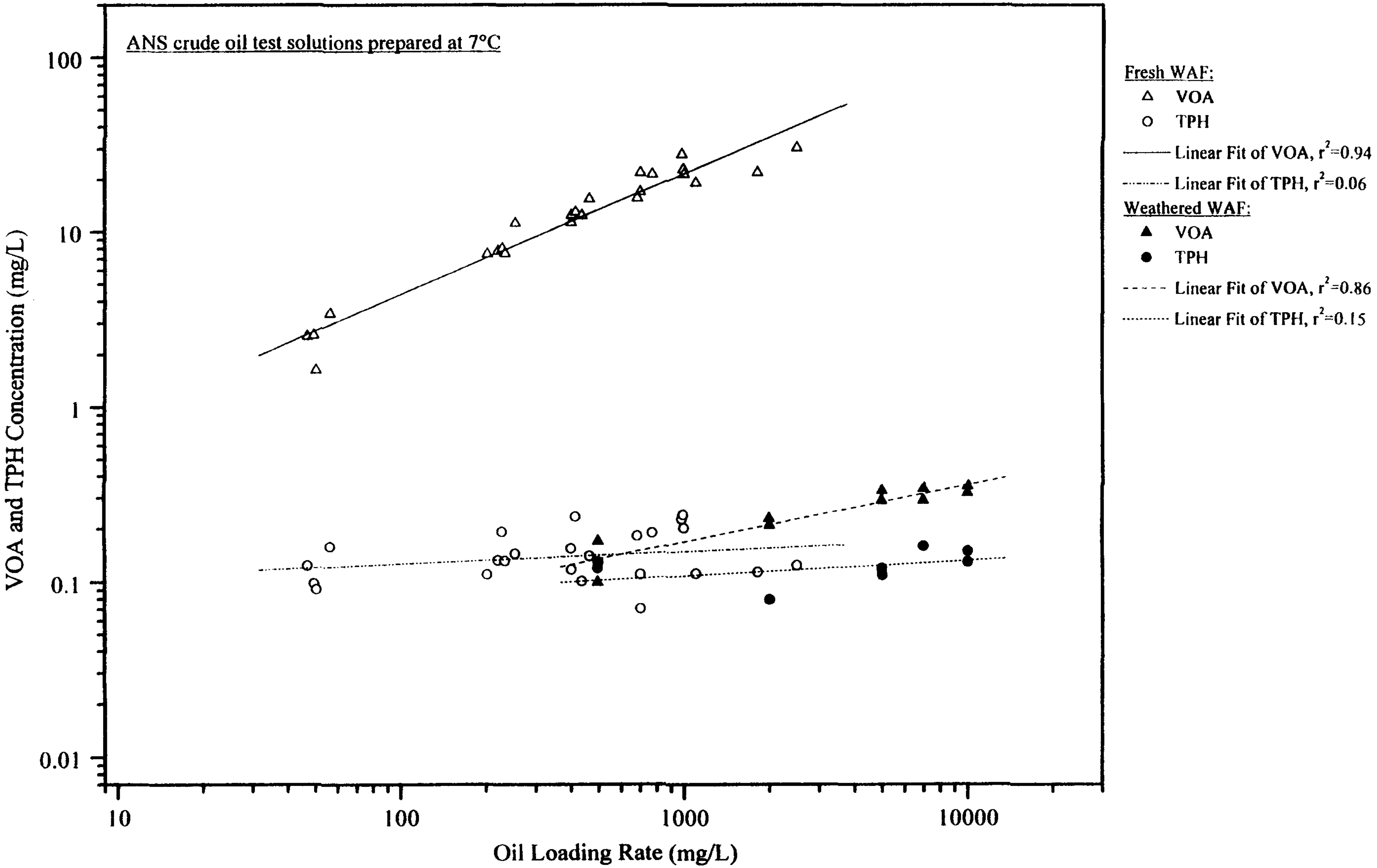

Fig. 3-1a. Relationship of measured concentrations of volatile organic analytes (VOA) and total petroleum hydrocarbons (TPH) to oil loading rates in fresh and weathered ANS WAF test solutions for *C. bairdi* in 7°C saltwater

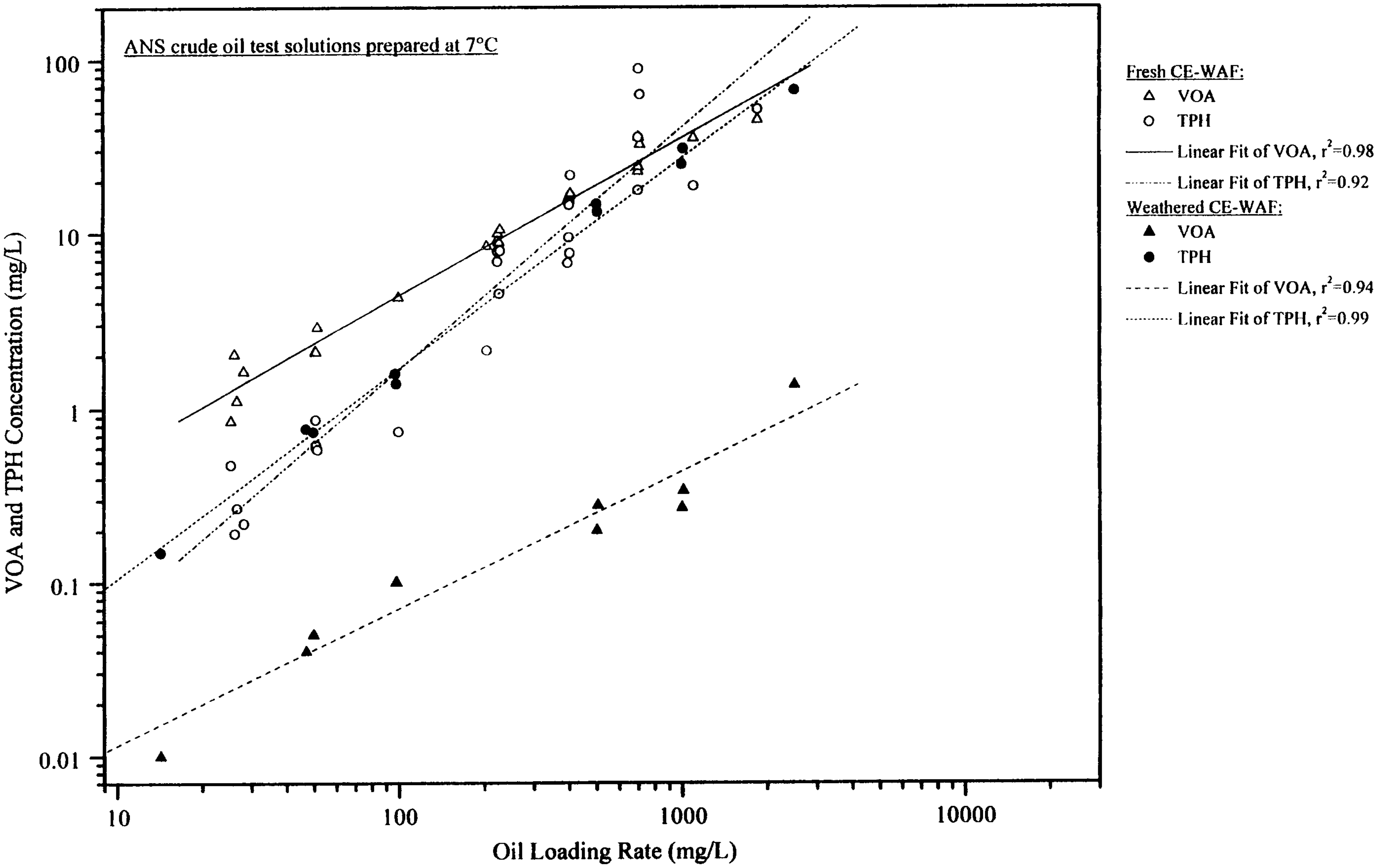

Fig. 3-1b. Relationship of measured concentrations of volatile organic analytes (VOA) and total petroleum hydrocarbons (TPH) to oil loading rates in fresh and weathered ANS CE-WAF test solutions for *C. bairdi* in 7°C saltwater

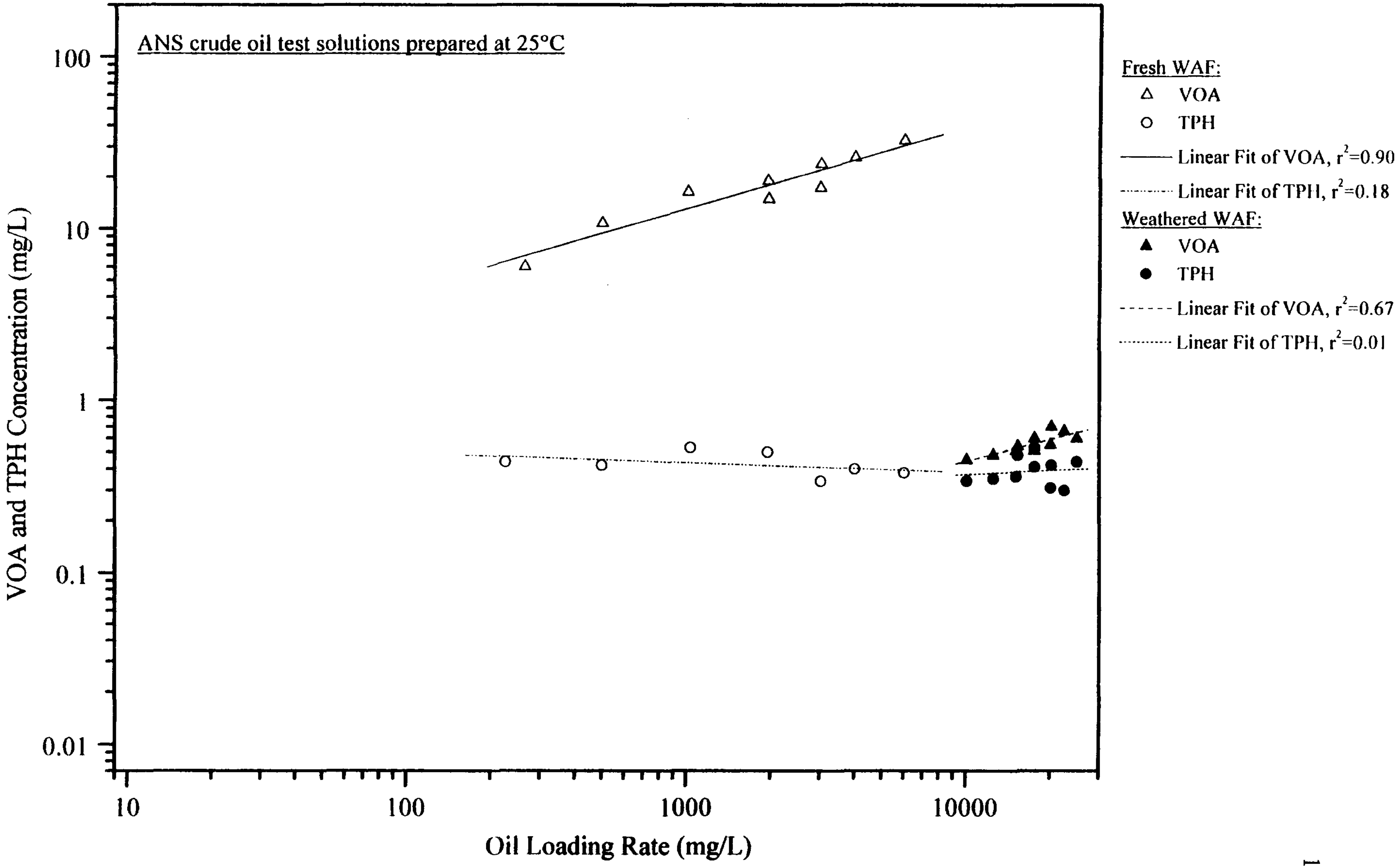

Fig. 3-2a. Relationship of measured concentrations of volatile organic analytes (VOA) and total petroleum hydrocarbons (TPH) to oil loading rates in fresh and weathered ANS WAF test solutions for *M. beryllina* in 25°C saltwater

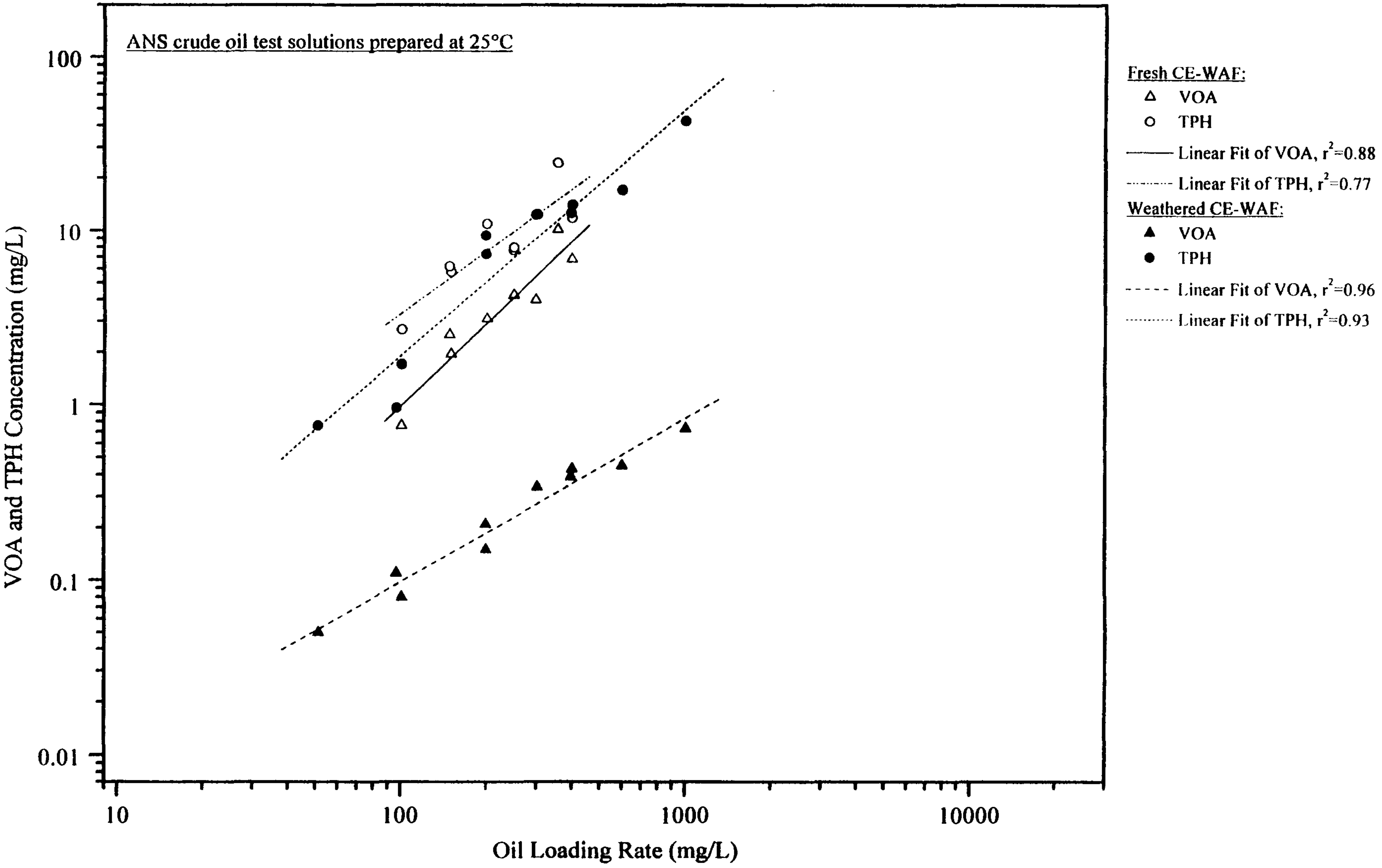

Fig. 3-2b. Relationship of measured concentrations of volatile organic analytes (VOA) and total petroleum hydrocarbons (TPH) to oil loading rates in fresh and weathered ANS CE-WAF test solutions for *M. beryllina* in 25°C saltwater

Table 3-1. Mean concentrations of hydrocarbons measured in WAF and CE-WAF solutions

Hydrocarbon Fraction	Oil Type	Mean +/- Std. Error (SE) Hydrocarbon Conc. (mg/L)							
		WAF	SE	n	% THC	CE-WAF	SE	n	% THC
VOA	Fresh ANS	17	1.2	43	98.7	11	1.7	39	40.2
	Weathered ANS	0.53	0.04	14	60.2	0.55	0.11	13	1.9
	Fresh PBCO	12	1.1	20	99.1	5.3	0.64	15	34.3
TPH	Fresh ANS	0.23	0.02	28	1.3	17	3.3	28	59.8
	Weathered ANS	0.35	0.04	14	39.8	28	5.7	13	98.1
	Fresh PBCO	0.30	0.02	18	2.5	10	1.5	15	65.7
THC	Fresh ANS	17	1.2	43	-	28	3.8	41	-
	Weathered ANS	0.88	0.08	14	-	28	5.8	13	-
	Fresh PBCO	12	1.1	20	-	16	1.7	19	-

n = number of samples

toxicity results and conclusions drawn from those data. These data are presented in Tables 3-2, 3-3, and 3-4.

Toxicity Value Comparisons: Test Solution Toxicities

When the toxicity data were standardized to measured (THC) concentrations (LC_{50} and EC_{50} values; denoted as: LC_{50}/EC_{50}), weathered oil solutions (WAF and CE-WAF) were more toxic than fresh oil solutions in all cases (Figure 3-3; n = 8 out of N = 10; where 3 of the 8 did not have fiducial limits to test significance). The two cases that were contrary to this (*i.e.*, fresh more toxic than weathered, according to LC_{50}/EC_{50}) were: 1) *M. beryllina* under spiked exposure, in which weathered CE-WAF was less toxic than fresh CE-WAF, however, not significantly; and 2) weathered CE-WAF was significantly less toxic than fresh CE-WAF for *V. fischeri.*

Fresh and weathered oils differ in the total amount of hydrocarbons in the parent oil (*i.e.*, fresh oil has ~100% its components and the weight of weathered oil is reduced by 30%). When the toxicity data were standardized to loading rates (LL_{50} and EL_{50} values; denoted as: LL_{50}/EL_{50}), weathered oil solutions were less toxic than fresh (n = 8 out of N = 10 where 3 of the 8 were without fiducial limits). The two cases in which fresh oil solutions were more toxic than weathered according to LL_{50}/EL_{50} were as follows: 1) *C. bairdi* to CE-WAF under spiked exposure, but this relationship was not significant; and 2) *M. beryllina* to CE-WAF under continuous exposure, where this relationship was significant.

Table 3-2. Acute 96-hour median lethal and effect concentration (mg/L) estimates (95% confidence limits) for Corexit 9500, and fresh and weathered oil WAF and CE-WAF tests

Test Solution	*C. bairdi* EC$_{50}$ Values		*M. beryllina* LC$_{50}$ Values	
	Spiked Exposure	Continous Exposure	Spiked Exposure	Continous Exposure
Corexit 9500[*]	1266.84[‡] (1030.88, 1556.82) a = 8.33%	23.76[†] (19.26, 28.40)	115.18[‡] (105.75, 125.46) a = 40%	54.67[†] (46.70, 62.94)
Fresh ANS				
WAF[**]	9.73[†] (8.83, 10.68)	2.54[‡‡]	26.36[‡] (25.54, 27.22) a = 0%	15.59[‡] (13.98, 17.38) a = 0%
CE-WAF[**]	10.72[†] (9.08, 12.72)	1.3[‡‡]	12.22[‡] (7.79, 19.17) a = 40%	12.42[‡] (11.40, 13.54) a = 0%
Weathered ANS				
WAF[**]	0.40[†] (0.33, 0.51)	0.27[†] (0.24, 0.28)	>1.13[***]	0.79[†] (0.32, 0.83)
CE-WAF[**]	2.36[†] (1.66, 6.66)	0.37[‡‡]	18.89[†] (15.78, 24.71)	0.65[†] (0.10, 1.25)

Notes:

[*] Corexit 9500 values based on loading rate in mg/L

[**] WAF and CE-WAF values based on total hydrocarbon content (THC) in mg/L

[***] Highest concentration tested had a loading rate of 24,948 mg/L

Statistical Methods Used:

[†] Probit analysis

[‡] Trimmed Spearman-Karber analysis, a = % trim

[‡‡] Graphical method, 95% confidence limits not available (Webber, 1993)

Table 3-3. Mean 5-minute EC_{50} values obtained by the Microtox Toxicity Assay. Values were calculated based on measured hydrocarbon fractions and on total oil added (loading rates)

Hydrocarbon Fraction	Oil Type	Mean +/- Std. Error (SE) EC_{50} (mg/L)*					
		WAF	SE	n	CE-WAF	SE	n
VOA	Fresh ANS	4.2	0.25	43	0.86	0.09	39
	Weathered ANS	0.22	0.01	14	0.12	0.02	13
	Fresh PBCO	3.6	0.29	20	0.69	0.04	15
TPH	Fresh ANS	0.06	0.01	28	1.0	0.13	28
	Weathered ANS	0.15	0.02	14	5.9	1.0	13
	Fresh PBCO	0.10	0.01	18	1.2	0.10	15
THC	Fresh ANS	4.2	0.25	43	2.0	0.17	41
	Weathered ANS	0.37	0.03	14	6.0	1.1	13
	Fresh PBCO	3.7	0.29	20	1.9	0.09	19
Loading Rates	Fresh ANS	310	41	34	29	2.6	33
	Weathered ANS	6400	570	18	180	39	15
	Fresh PBCO	960	160	13	46	4.0	13

Dispersant only: EC_{50} (mg/L) = 220 +/- 26

n = number of samples

* For each oil type and a given hydrocarbon fraction used to standardize the data, the EC_{50} value for WAF was significantly different ($P < 0.05$) from that for CE-WAF

Table 3-4. Median lethal and effect concentration estimates (mg/L) based on measured concentrations and oil loading rates

		Fresh ANS				Weathered ANS			
		WAF		CE-WAF		WAF		CE-WAF	
Species	Exosure type	Measured THC Conc. LC$_{50}$	Loading Rate LL$_{50}$	Measured THC Conc. LC$_{50}$	Loading Rate LL$_{50}$	Measured THC Conc. LC$_{50}$	Loading Rate LL$_{50}$	Measured THC Conc. LC$_{50}$	Loading Rate LL$_{50}$
C. bairdi†	Spiked	9.73 (8.83, 10.68)	285 (249, 325)	10.72 (9.08, 12.72)	203 (174, 236)	0.40 (0.33, 0.51)	4485 (2216, 10248)	2.36 (1.66, 6.66)	128 (96, 426)
	Continous	2.54 (N/A)	12.48 (N/A)	1.30 (N/A)	5.16 (N/A)	0.27 (0.24, 0.28)	149 (N/A)	0.37 (N/A)	6.28 (N/A)
M. beryllina	Spiked	26.36 (25.54, 27.22)	3520 (3326, 3725)	12.22 (7.79, 19.17)	272 (171, 425)	> 1.13 (N/A)	> 24948 (N/A)	18.89 (15.78, 24.71)	555 (450, 684)
	Continous	15.59 (13.98, 17.38)	1641 (1317, 2044)	12.42 (11.40, 13.54)	227 (212, 244)	0.79 (0.32, 0.83)	9512 (N/A)	0.65 (0.10, 1.25)	47 (14, 72)
Vibrio fischeri†	N/A	4.2 +/- 0.25	310 +/- 41	2.0 +/- 0.17	29 +/-2.6	0.37 +/- 0.03	6400 +/- 570	6.00 +/- 1.1	180 +/- 39

† Measured Conc. as EC$_{50}$, Loading Rate as EL$_{50}$, both in mg/L

N/A = not available; confidence limits could not be calculated

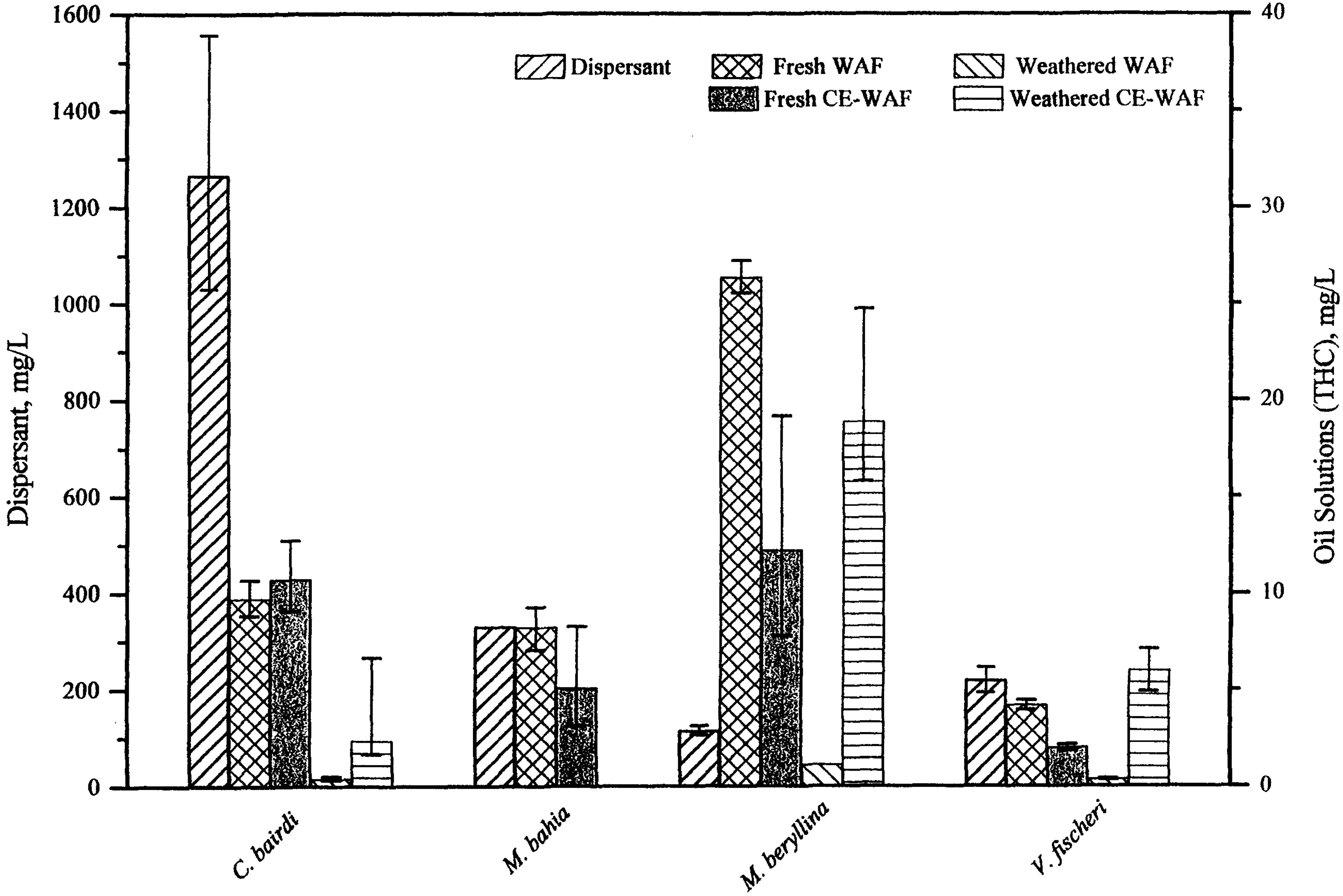

Fig. 3-3. Acute 96-hour median lethal and effect concentrations for spiked exposure tests based on measured concentrations (LC_{50} and EC_{50}) values) grouped by species

Toxicity Value Comparisons: Species Sensitivities

All three species (*C. bairdi, M. beryllina,* and *V. fischeri*) were either more or less sensitive to dispersed weathered oil solutions depending upon the manner in which the solution concentrations were portrayed. For every species tested, when comparisons are made using toxicity values based upon LC_{50}/EC_{50} values, weathered WAF was nearly always most toxic (Figure 3-4a). Conversely, according to LL_{50}/EL_{50} values, weathered WAF was generally least toxic to all species (Figure 3-4b). Exceptions are mentioned in the section above.

DISCUSSION

Oil Solutions

The resulting fresh and weathered oil solutions agreed well with the information provided concerning the weight reduction of crude oil. Weathered oil solutions contained approximately 30 percent less VOA than the fresh oil solutions, which corresponds with the amount reported lost during the weathering process. There were little changes in concentration of hydrocarbons with boiling points greater than 200°C between the fresh and weathered oil solutions. The solubility of weathered oil was apparently decreased based on the observation that more weathered oil was required to produce effective solutions and were generally less concentrated than those prepared with fresh oil.

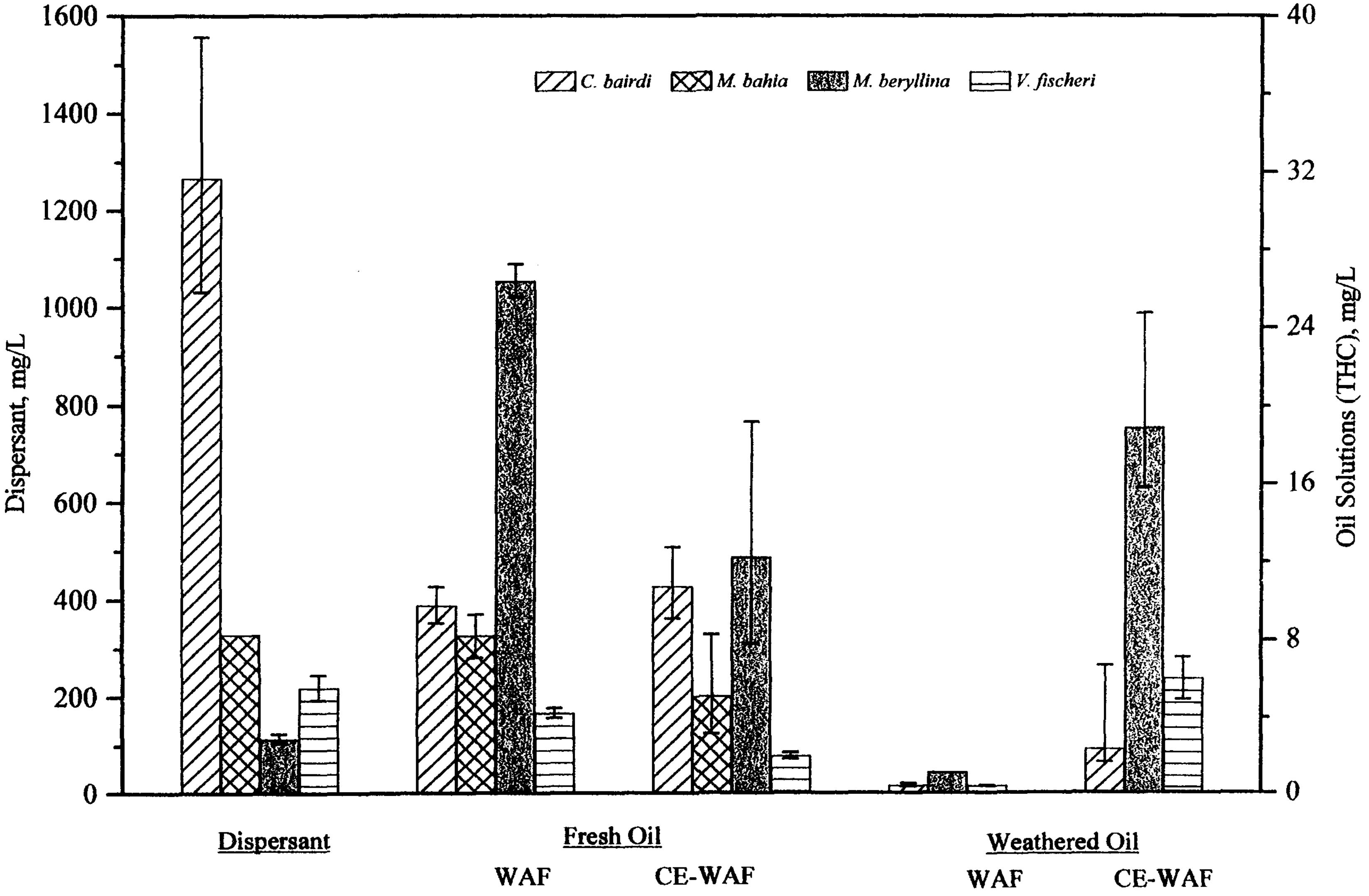

Fig. 3-4a. Acute 96-hour median lethal concentrations for spiked exposure tests based on measured concentrations (LC_{50} and EC_{50} values) grouped by solution type

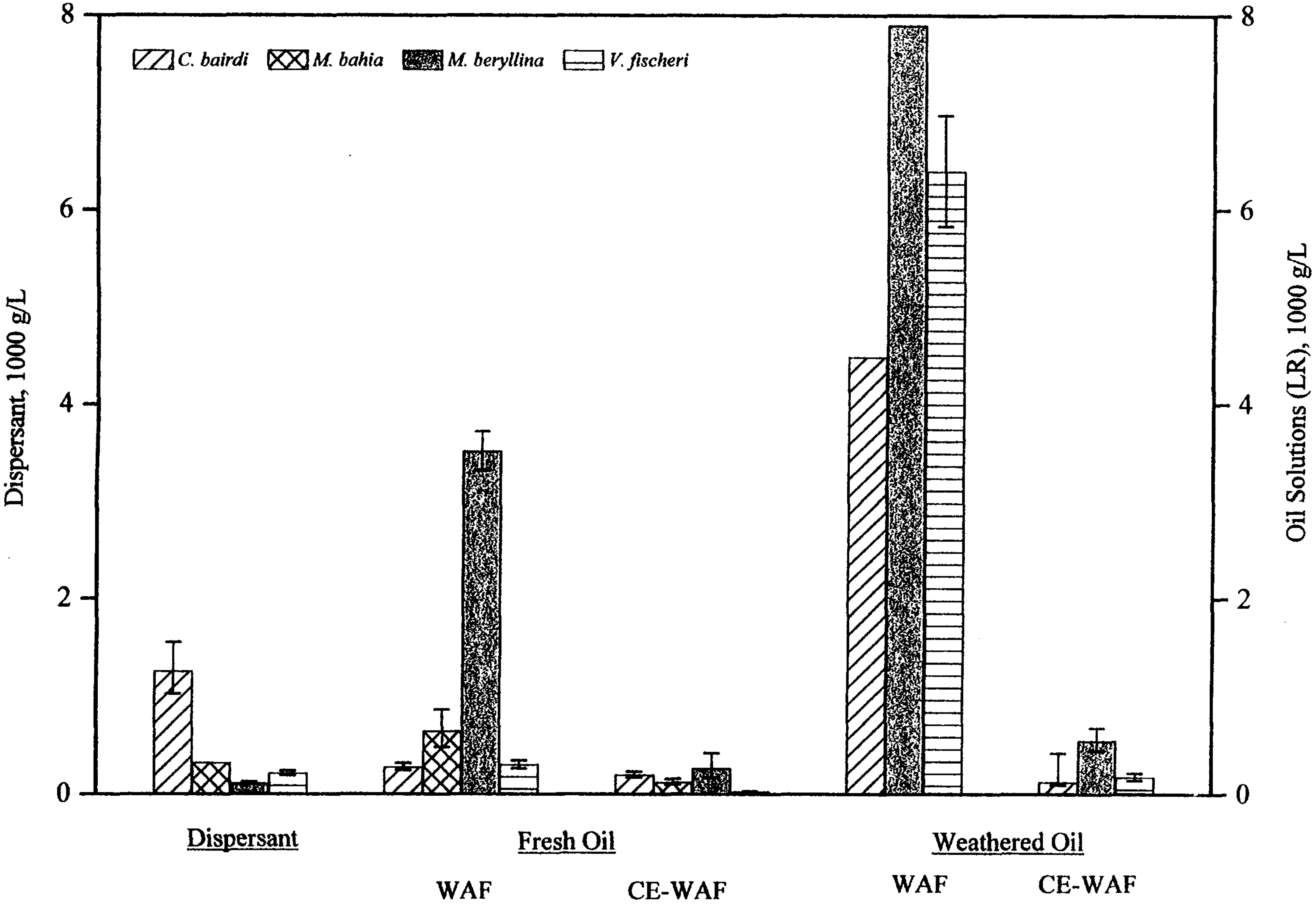

Fig. 3-4b. Acute 96-hour median lethal concentrations for spiked exposure tests based on loading rates (LL_{50} and EL_{50} values) grouped by solution type

Toxicity

Toxicity appeared to be strongly related to the solubilities of the hydrocarbon fractions measured. This was initially observed in the fresh oil study with median-effect concentrations calculated based upon fractional groups of hydrocarbons possessing distinct differences in solubility. The notion that solubilities greatly influence resulting calculated toxicity values was further elucidated with results from the weathered oil study. Similar results were observed by Bobra and others (1983) when the authors evaluated the toxicity of fresh and weathered water soluble fractions to *Daphnia magna*. Weathered oil caused a reduction in both solubility and LC_{50} values, but caused a marked increase in the oil loadings required to form effective solutions. The loadings used for some of the weathered WAF solutions approached levels that were unrealistically high and impractical from the consideration that these solutions behaved as saturated solutions. Bobra and others (1983) noted that with non-dispersed weathered crude oil, it becomes nearly impossible to form a lethal aqueous solution. If solutions are at near-saturation, yet barely produce effective solutions, these solutions may in fact be essentially non-toxic, even though the LC_{50} and EC_{50} values would suggest they are very toxic due to these low values. Bobra and others (1983) propose correlating toxicity directly to a chemical's aqueous solubility. In light of the observations made from the fresh and weathered studies, such an exercise would be a worthy endeavor. However, in addition to that, the octanol-water partitioning coefficients of the hydrocarbons in solution should be correlated to the toxicity. This is useful because not all hydrocarbons may be equally potent.

Toxicity Commentary

At their most basic, toxicity tests provide information about how much is too much of a test material to cause a defined response (*e.g.*, death) by 50 percent of the population. Intuitively, a chemical that requires only minimal quantities to elicit a response in a test species is much more (acutely) toxic than another chemical that requires large quantities to generate the same response in the same species. Aquatic toxicity tests differ somewhat from other toxicity tests (*e.g.*, direct injection in mice provide an LD_{50}, lethal "dose" rather than lethal "concentration") in that exposure to the test material must occur via the media in which the test organisms reside, in this case saltwater (Hodgson and Levi, 1987). In order to generate a response by the test organism, first the test material must be able to interact with the aqueous media. Second, the concentration of the chemical in the aqueous media must be analyzed in some manner to estimate the actual exposure concentration experienced by the organism. And a third, more advanced procedure, might involve analyzing the concentration of the test chemical in the organism following the assay to determine with more accuracy what the actual exposure concentration to that organism was (*e.g.*, via tissue dosimetry; Rand *et al.*, 1995). Such an exercise would provide information about the chemical's propensity to partition out of the aqueous phase into the lipid phases of biological membranes, which is often estimated by the octanol-water partitioning coefficient (K_{ow}) of that chemical (LaGreaga *et al.*, 1994). Therefore, through these transformations (*e.g.*, dissolution, concentration characterization, partitioning into an organism), the results from an aquatic toxicity assay reflect the following: 1) the ability of the test material (chemical) to interact with aqueous media; 2) the analytical capabilities used to characterize the aquatic concentrations; 3) the test material's ability to partition into an organism (K_{ow}) to contact a toxic site of action; and finally 4) the test organisms' response to exposure to the test chemical, which can be chemical and/or physical, or a combination of chemical and physical effects of that chemical.

Given these considerations, concurrent use of the LC_{50} and LL_{50} (or EC_{50} and EL_{50}) values from a toxicity assay, provides a relatively quick and inexpensive way to detect that some other factors besides what is characterized as the test solution concentrations may have an influence on these results and should be considered. For instance, had the interpretations of the LC_{50} and LL_{50} for weathered WAF and CE-WAF solutions been in agreement, then the conclusion that one solution type is more toxic than the other would be straightforward. In such a case, the use of these two forms of toxicity values would simply provide additional information about the test material's ability to interact with saltwater by illustrating the breadth of loadings required to form effective solutions; this was demonstrated in Chapter 1 with the fresh oil study. This may be of value to the end user, who may only consider the final values. If that final value is based upon measured concentrations alone (or only a fraction of the measured concentration as with TPH or VOA vs. THC), the end user will not be fully informed of the physicochemical nature also at play with respect to this material's toxicity.

In the case of weathered oil, however, where dichotomous conclusions are drawn from LC_{50} and LL_{50} values concerning which is more toxic, weathered WAF or CE-WAF solutions, (note: the same applies to "fractional" toxicity for toxicity values standardized to VOA and TPH fractions in both fresh and weathered crude oil), clearly, there is more to be considered than the solution's measured concentration to properly interpret the relative toxicity of two or more solutions or species' sensitivities. In the case of weathered WAF solutions, several factors contribute to the resulting toxicity values: 1) decreased VOA content in the parent oil, 2) increased viscosity from the weathering process (in part due to removal of VOA), 3) reduced solubility by increased viscosity and remaining hydrocarbons of lower solubility, 4) all previously listed factors (1 through 3) require that the loading rates be increased substantially to form effective solutions; and/or 5) another unknown parameter that was not measured in this study.

Test Solution Toxicities

Which then is more toxic: 1) weathered WAF or weathered CE-WAF; or 2) fresh or weathered WAF? If the concern is only for how much product (test material) is required to produce an effect to 50 percent of the organisms, then clearly, dispersed weathered oil (CE-WAF) is more toxic. However, if the focus is more on the actual accommodated fractions, then non-dispersed weathered oil (WAF) is more toxic. Because the measured values for weathered WAF concentrations were very low, their resulting LC_{50} values were also very low. On one hand, WAF is clearly less toxic due to excessive loadings (Figure 3-4b); on the other hand, WAF is more toxic due to the low concentrations of hydrocarbons in solution (Figure 3-4a). Yet the fact remains that weathered WAF solutions still managed to produce a response by 50 percent of the population in all test species except *M. beryllina* under spiked exposure. Therefore, something about these solutions causes an effect to these species. As was previously suggested, perhaps this is indicative of 1) another parameter not measured in this study that is responsible for the species' response (*e.g.*, viscosity), 2) oil particulates may be more numerous in solution due to the high oil loadings ("unrealistically" high loadings are considered to be 25 g/L, Singer *et al,* (1998), which coincides with those used for *M. beryllina*) needed to produce effective WAF solutions causing a physical toxicological impact, or less likely, 3) the weathering process causes some alteration of the residual crude oil's compounds, leaving behind chemicals that are more toxic than they were in their fresh oil state.

CONCLUSIONS

In general, the toxicity data suggest that of the solutions tested, dispersant solutions alone were least toxic, water-accommodated fractions were moderately toxic, and dispersed oil (chemically-enhanced water-accommodated fractions) were most toxic. However, these relationships varied depending upon the species and end-point tested. Dispersant addition to oil solutions (CE-WAF solutions) indicated increased toxicity, as reflected by lower median-effect concentration (toxicity) values. However, broader scale decisions on whether or not to use dispersants must rely on other factors as well. These factors include the short-term effectiveness of the dispersant product and the effects of the product on the long-term persistence of oil residues in the environment.

Toxicity values obtained from this study suggest that the cold-water species, *C. bairdi,* is more sensitive to oil solutions than the warmer standard test species *M. bahia* and *M. beryllina*. This is consistent with findings from other researchers who have evaluated the toxicity of oil solutions to *C. bairdi* and speculate that their greater sensitivity can be attributed to effects of colder temperatures. Cold temperatures lead to slower development times for larvae and increased persistence of aromatic hydrocarbons in solution (Brodersen *et al.,* 1977; Rice *et al.,* 1977). Overall, *M. beryllina* was least sensitive to oil, but most sensitive to dispersant-only solutions.

The response of the species *V. fischeri* evaluated using the Microtox® Assay, although possibly not directly correlative to the toxic response of the zooplankton tested in this study, were indicative of whether or not a biological impact could be expected from exposure to dispersants, oil, or dispersed oil. Additionally, the toxicity values for *V. fischeri* for oil solutions were within the same order of magnitude as those determined for the other test species.

Toxicity results that were directly comparable (*i.e.,* same species and test material) to other laboratories employing protocols put forth by CROSERF were in agreement with those obtained in this study, suggesting that laboratory methods employed in this study were reliable. The spiked exposure model is more representative of an exposure likely to occur in the environment, and consequently provides toxicity values that do not overestimate toxicity as some constant exposure tests may (Bragin *et al.,* 1994). Use of continuous exposure tests to estimate the toxicity of a solution is problematic in that a constant exposure is difficult to preserve given factors such as volatilization and biodegradation (Rice *et al.,* 1977). In the future, to more accurately determine the toxic effect due to continuous exposure, aeration of the test solution should be avoided when possible, or sub-samples of the test solutions should be collected over time from the test chambers to better estimate the actual concentration profile of exposure to the organisms.

Presentation of the toxicity data is of utmost importance when considering the information contained therein as it applies to field conditions. Values based solely on one hydrocarbon fraction are subject to either under or over-estimating the toxicity of a solution. Also, use of either an LC_{50} or an LL_{50} may be more accessible in a field situation depending upon what analytical instruments are available to estimate the concentration of dispersants, oil, or dispersed oil that may be introduced in the water column by an oil spill mitigation action. When comparing the toxicity of two solutions (*e.g.*, dispersed or not), use of an LL_{50} (or EL_{50}) should be considered and reported in conjunction with the more standard LC_{50} (or EC_{50}). Together, the these two are of particular value when presenting toxicity data as they may reveal a test material's inherent ability (or lack of) to form water accommodated fractions in aqueous media through which exposure can occur. Concurrent reporting of these two forms of toxicity data may provide the end-user of this data some information about the volume of material required to produce a toxic effect to 50 percent of the population.

In summary, the factors that should be considered when using toxicity data include: 1) composition and physicochemical characteristics of the parent oil or dispersant; 2) the form of and to which chemical fractions the toxicity values are based (*e.g.*, LL_{50} or LC_{50}; based only on TPH or THC); 3) laboratory protocols for test solution preparation and how they relate to actual field condition; and 4) species and life stage from which the toxicity data was derived. For field extrapolations, the following should also be considered in additions to those factors listed above: 1) local mixing energy conditions (*e.g.*, high dilution via sea swell, intense wind/wave action); 2) local habitat sensitivities or vulnerabilities; 3) season (*e.g.*, are sensitive species currently present in the water column).

Decision to Disperse

The consideration of whether a dispersed oil solution is more or less toxic than a non-dispersed oil solution is an important one when deciding to use dispersants in response to an oil spill. However results from toxicity tests as to which is more toxic can be confounding, as was observed in this study. In either event, if the question being posed is whether or not to disperse weathered oil, the answer is still one of environmental trade-offs and seasonal considerations. First, there should be reasonable assurance that application of dispersants will be effective in mitigating the potential damage caused by an oil spill. Once, this has been confirmed, then the environmental impacts must be considered of all response options in order to determine which one causes the least net environmental damage. Potential effects to Alaskan Tanner crab larvae may occur if hydrocarbon concentrations in the field resemble those shown in Figures 3-5a through 3-6 b expressed as oil loadings and measured concentrations. These figures illustrate the range of concentrations for both dispersed and non-dispersed oil solutions that cased an effect to Tanner crab larvae.

Chemically dispersed weathered oils appear to be more toxic with respect to oil loadings; however, if the test species is not present in the water column at the time of dispersal (*i.e.,* out of season), no effect should be expected. Even if the test species is present in the water column, the decision to disperse weathered oil should be based upon

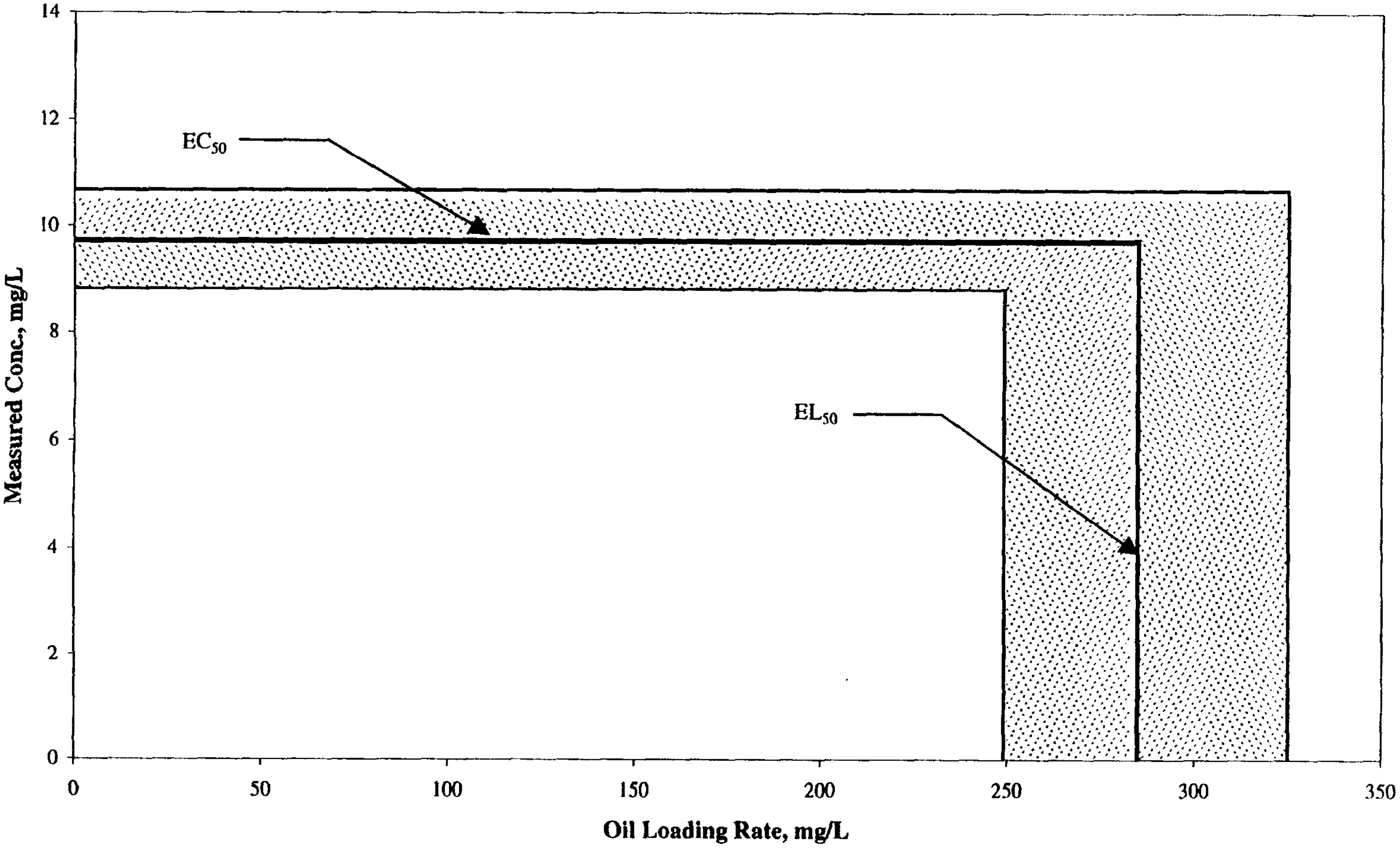

Figure 3-5a. Relationship of median effective concentrations by measured concentrations (EC$_{50}$) and oil loading rates (EL$_{50}$) of fresh ANS WAF test solutions for *C. bairdi* under spiked exposure

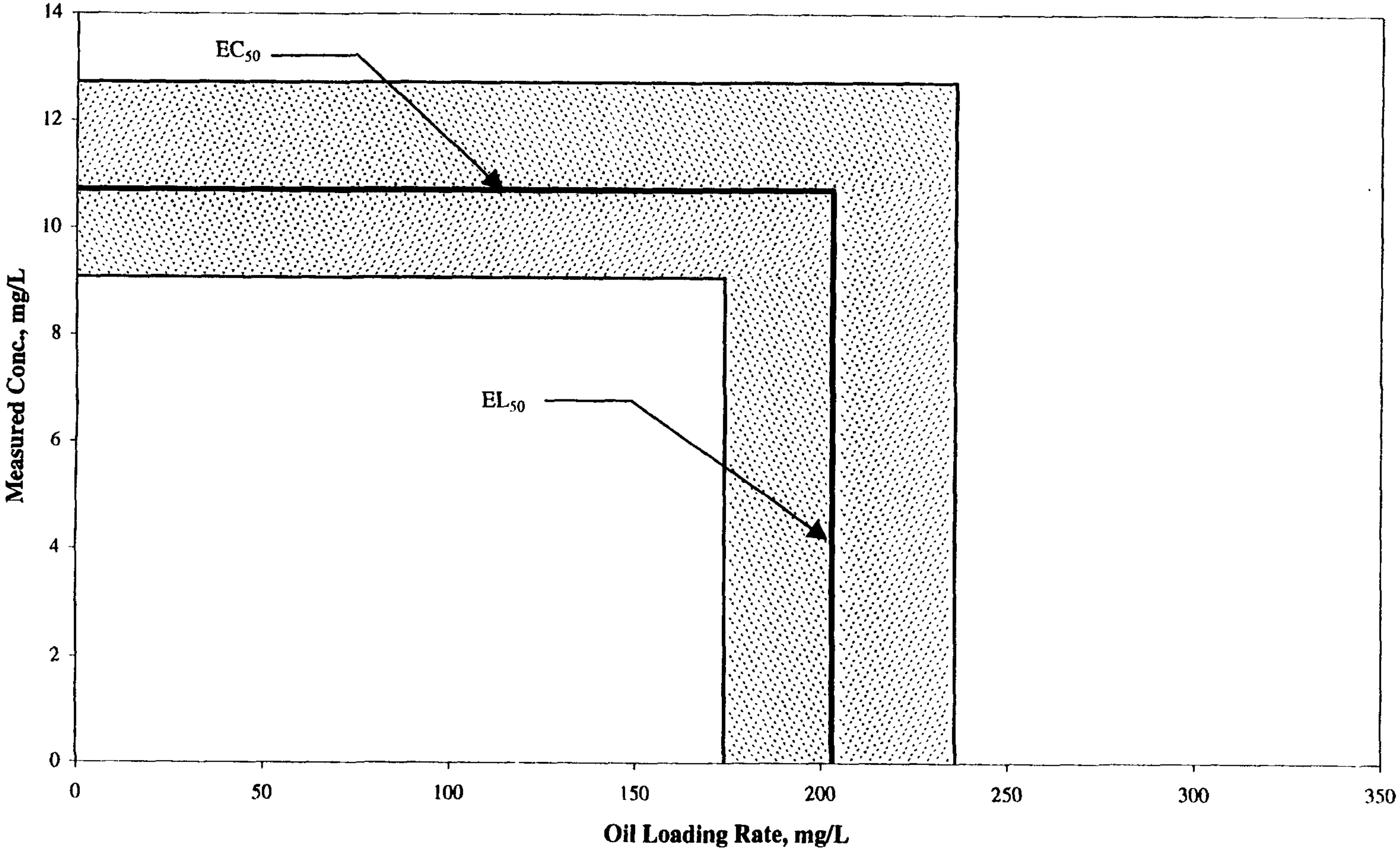

Figure 3-5b. Relationship of median effective concentrations by measured concentrations (EC_{50}) and oil loading rates (EL_{50}) of fresh ANS CE-WAF test solutions for *C. bairdi* under spiked exposure

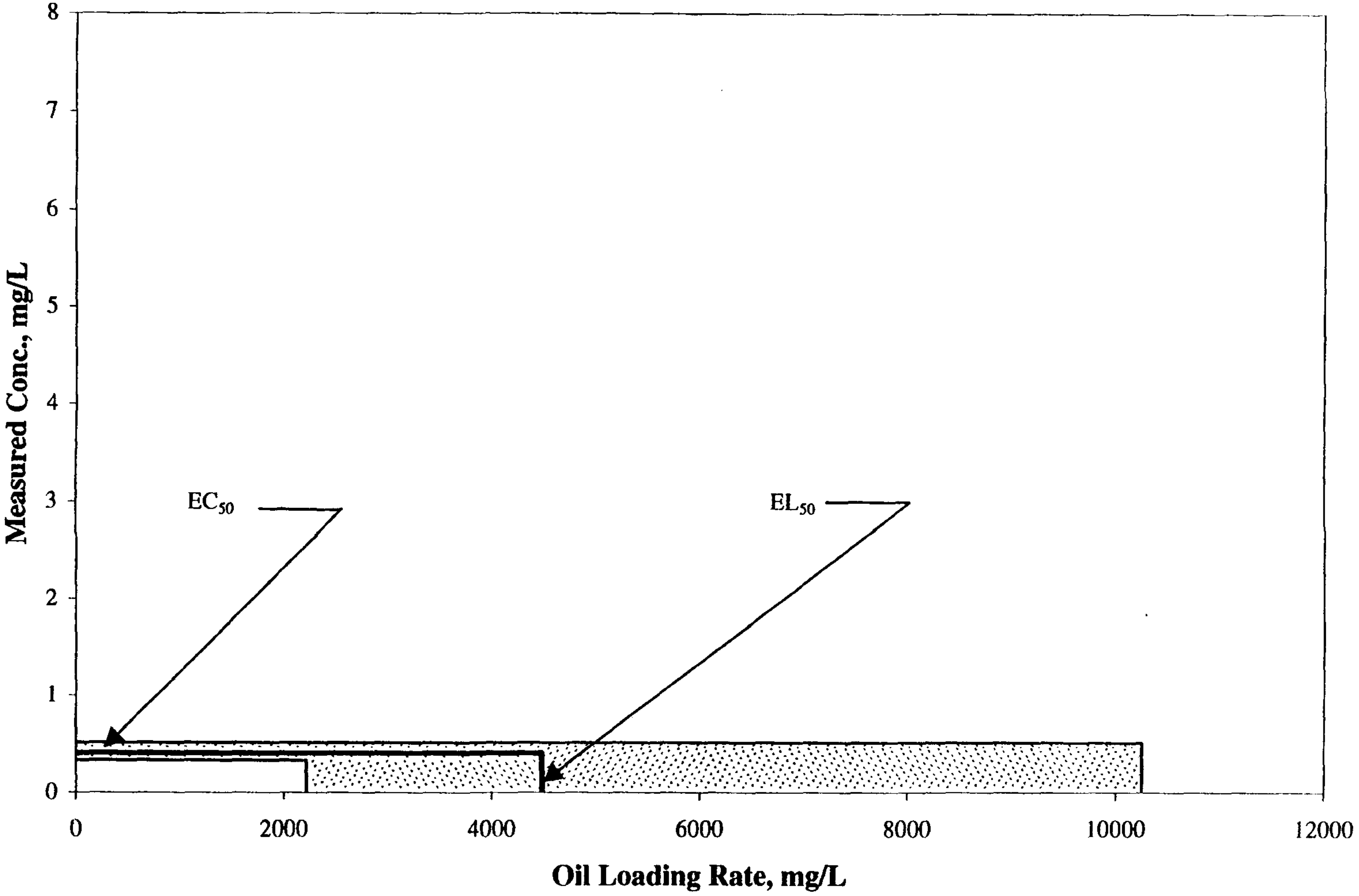

Figure 3-6a. Relationship of median effective concentrations by measured concentrations (EC_{50}) and oil loading rates (EL_{50}) of weathered ANS WAF test solutions for *C. bairdi* under spiked exposure

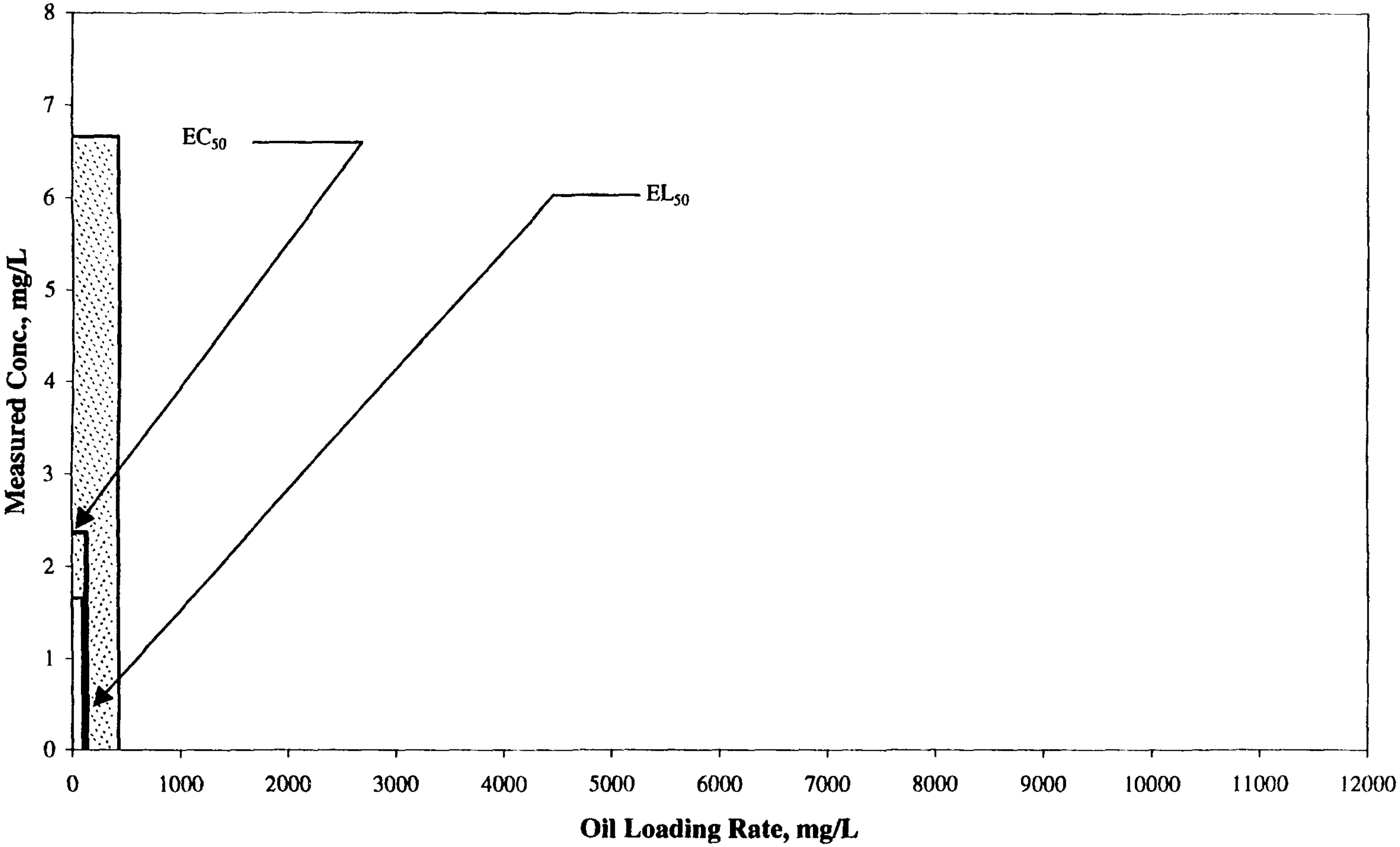

Figure 3-6b. Relationship of median effective concentrations by measured concentrations (EC_{50}) and oil loading rates (EL_{50}) of weathered ANS CE-WAF test solutions for *C. bairdi* under spiked exposure

a net environmental benefit analysis (Lewis and Aurand, 1997). For example, what are the effects of a "one-time hit" – to zooplankton, for instance, an important yet numerous food-web species – by dispersing oil versus not dispersing? Not dispersing could possibly result in oiled gravel beaches that may affect an important ecological and economical species (*e.g.*, pink salmon embryos; Heintz *et al.*, 1995) or larger mammalian species that may be more sensitive than the most-sensitive life-stages of zooplankton with respect to the length (and number of progeny) of their reproductive cycle.

Finally, long-term effects should be considered. Dispersing oil is generally believed to enhance biodegradation through increasing the surface area of the oil and observation of increased biodegradation rates (NRC, 1989). However, the actual fate of dispersed oil may not be one of complete mineralization, as a recent study suggests that dispersant addition may cause selective enrichment of more persistent hydrocarbons (Lindstrom *et al.*, 1999). Additionally, if dispersed oil becomes associated with sediment, the bioavailability of some hydrocarbons can decrease, limiting biodegradation (Braddock and Richter, 1997).

Future Investigations

Toxicity tests of crude oil, both weathered or fresh, conducted in the future should consider the following: 1) the contributions of physical toxicity factors due to the presence of particulate oil in dispersed oil solutions; 2) the role of enhanced concentrations of soluble compounds that may possess a greater potency as estimated by their octanol-water partitioning coefficients; and 3) the possibility that octanol-water partitioning coefficients may be altered by the addition of dispersants.

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APPENDIX

APPENDIX A

FRESH OIL AND DISPERSANT STUDY TOXICITY DATA

Tanner Crab (*Chionoecetes bairdi*)
Dispersant Spiked Exposure
Start Date of Test: 27-Apr-99

Test Chamber #	Design Conc. (mg/L)	Nominal Conc. (mg/L)	EC_{50} Test				
			Number of Animals Alive/Total	Individual Percent Affected	Mean Percent Affected	Standard Error	EC_{50} (mg/L) 95% CI (Analysis mthd.)
1a	0	0.00	9/9	0.00	0.00	0.00	**1266.84** LL=1030.88 UL=1556.82 (TSK a=8.33%)
1b			10/10	0.00			
1c			9/9	0.00			
2a	200	199.40	9/9	0.00	6.67	6.67	
2b			5/5	0.00			
2c			8/10	20.00			
3a	800	796.00	8/10	20.00	13.33	6.67	
3b			8/10	20.00			
3c			10/10	0.00			
4a	1400	1401.85	4/10	60.00	53.33	3.33	
4b			5/10	50.00			
4c			5/10	50.00			
5a	2000	2002.80	2/9	77.78	82.59	3.76	
5b			2/10	80.00			
5c			1/10	90.00			
6a	2600	2599.60	1/10	90.00	96.67	3.33	
6b			0/10	100.00			
6c			0/10	100.00			

Defined responses: Alive, Affected, Mortally Affected, Dead

EC_{50} response: Affected

Tanner Crab (*Chionoecetes bairdi*)
Dispersant Continuous Exposure
Start Date of Test: 27-Apr-99

Test Chamber #	Design Conc. (mg/L)	Mean Nominal Conc. (mg/L)	EC_{50} Test				
			Number of Animals Alive/Total	Individual Percent Affected	Mean Percent Affected	Standard Error	EC_{50} (mg/L) 95% CI (Analysis Mthd.)
1a	0	0.00	9/10	10.00	3.33	3.33	**23.76** LL=19.26 UL=28.40 (Probit)
1b			10/10	0.00			
1c			10/10	0.00			
2a	20	20.21	7/10	30.00	36.67	12.02	
2b			4/10	60.00			
2c			8/10	20.00			
3a	50	50.00	0/10	100.00	96.67	3.33	
3b			1/10	90.00			
3c			0/10	100.00			
4a	80	79.93	0/10	100.00	100.00	0.00	
4b			0/10	100.00			
4c			0/10	100.00			
5a	110	110.53	0/10	100.00	100.00	0.00	
5b			0/10	100.00			
5c			0/10	100.00			
6a	130	130.21	0/10	100.00	100.00	0.00	
6b			0/10	100.00			
6c			0/10	100.00			

Defined responses: Alive, Affected, Mortally Affected, Dead
EC_{50} response: Affected

Tanner Crab Larvae (*Chionocetes bairdi*)
WAF Spiked Exposure; ANS Fresh Oil Study
Start Date of Test: 15-Apr-98

Test Chamber #	Design Conc. (mg/L)	Loading Rate (mg/L)	Measured Concentrations			EC_{50} Test				
			VOA (mg/L)	TPH (mg/L)	VOA + TPH (mg/L)	Number of Animals Alive/Total	Individual Percent Affected	Mean Percent Affected	Standard Error	EC_{50} (mg/L) 95% CI (Analysis mthd.)
1a	0	0.00	0.00	0.00	0.00	11/11	0.00	0.00	0.00	**9.73**
1b						LOST[1]	LOST[1]			LL=8.83
1c						11/11	0.00			UL=10.68 (Probit)
2a	200	201.86	7.45	0.11	7.56	10/10	0.00	13.33	13.33	
2b						10/10	0.00			
2c						6/10	40.00			
3a	400	401.09	12.43	0.12	12.54	0/10	100.00	86.67	13.33	
3b						4/10	60.00			
3c						0/10	100.00			
4a	1100	1099.66	19.24	0.11	19.35	0/10	100.00	100.00	0.00	
4b						0/10	100.00			
4c						0/10	100.00			
5a	1800	1814.86	22.10	0.11	22.21	0/10	100.00	100.00	0.00	
5b						0/10	100.00			
5c						0/11	100.00			
6a	2500	2499.23	30.45	0.12	30.58	0/10	100.00	100.00	0.00	
6b						0/10	100.00			
6c						0/10	100.00			

[1] Broken Chamber

Defined responses: Alive, Affected, Mortally Affected, Dead
EC_{50} response: Affected

Tanner Crab Larvae (*Chionocetes bairdi*)
WAF Continuous Exposure; ANS Fresh Oil Study
Start Date of Test: 8-Apr-98

Test Chamber #	Design Conc. (mg/L)	Mean Loading Rate (mg/L)	Measured Concentrations			EC_{50} Test				
			Mean VOA (mg/L)	Mean TPH (mg/L)	VOA + TPH (mg/L)	Number of Animals Alive/Total	Individual Percent Affected	Mean Percent Affected	Standard Error	EC_{50} (mg/L) 95% CI (Analysis mthd.)
1a	0	0.00	0.000	0.000	0.000	8/9	11.11	3.70	3.70	**2.54** (CNC)[2] (Graphical)
1b						9/9	0.00			
1c						10/10	0.00			
2a	50	50.89	2.535	0.118	2.653	1/10	90.00	89.44	0.56	
2b						LOST[1]	LOST[1]			
2c						1/9	88.89			
3a	225	234.14	8.631	0.151	8.782	0/10	100.00	100.00	0.00	
3b						0/9	100.00			
3c						0/10	100.00			
4a	400	428.58	13.018	0.158	13.176	0/10	100.00	100.00	0.00	
4b						0/10	100.00			
4c						0/10	100.00			
5a	700	716.01	19.018	0.139	19.157	0/9	100.00	100.00	0.00	
5b						0/10	100.00			
5c						0/10	100.00			
6a	1000	998.79	24.108	0.224	24.331	0/9	100.00	100.00	0.00	
6b						0/9	100.00			
6c						0/10	100.00			

[1] Animals accidentally lost on solution change.

[2] CNC = Could not calculate; Confidence intervals cannot be calculated for graphical analysis.

Defined responses: Alive, Affected, Mortally Affected, Dead

EC_{50} response: Affected

Tanner Crab Larvae (*Chionocetes bairdi*)
CE-WAF Spiked Exposure; ANS Fresh Oil Study
Start Date of Test: 20-Apr-98

Test Chamber #	Design Conc. (mg/L)	Loading Rate (mg/L)	Measured Concentrations			EC$_{50}$ Test				
			VOA (mg/L)	TPH (mg/L)	VOA + TPH (mg/L)	Number of Animals Alive/Total	Individual Percent Affected	Mean Percent Affected	Standard Error	EC$_{50}$ (mg/L) 95% CI (Analysis mthd.)
1a	0	0.00	0.00	0.00	0.00	11/11	0.00	0.00	0.00	**10.72**
1b						LOST[1]	LOST[1]			LL=9.08
1c						10/10	0.00			UL=12.72 (Probit)
2a	100	99.83	4.28	0.74	5.02	8/10	20.00	10.00	5.77	
2b						9/10	10.00			
2c						10/10	0.00			
3a	200	204.36	8.42	2.15	10.57	11/12	8.33	39.44	17.80	
3b						6/10	40.00			
3c						3/10	70.00			
4a	400	402.32	16.48	7.67	24.14	0/10	100.00	100.00	0.00	
4b						0/9	100.00			
4c						0/10	100.00			
5a	1100	1100.75	35.19	18.74	53.93	0/9	100.00	100.00	0.00	
5b						0/9	100.00			
5c						0/9	100.00			
6a	1800	1853.79	44.64	51.60	96.23	LOST[2]	LOST[2]	100.00	#DIV/0!	
6b						LOST[2]	LOST[2]			
6c						0/10	100.00			

[1] Broken chamber.
[2] Drained chamber.

Defined responses: Alive, Affected, Mortally Affected, Dead
EC$_{50}$ response: Affected

Tanner Crab Larvae (*Chionocetes bairdi*)
CE-WAF Continuous Exposure; ANS Fresh Oil Study
Start Date of Test: 17-Apr-98

			Measured Concentrations			EC_{50} Test				
Test Chamber #	Design Conc. (mg/L)	Mean Loading Rate (mg/L)	Mean VOA (mg/L)	Mean TPH (mg/L)	VOA + TPH (mg/L)	Number of Animals Alive/Total	Individual Percent Affected	Mean Percent Affected	Standard Error	EC_{50} (mg/L) 95% CI (Analysis mthd.)
1a	0	0.00	0.000	0.000	0.000	10/11	9.09	3.03	3.03	**1.30** (CNC)[1] (Graphical)
1b						10/10	0.00			
1c						10/10	0.00			
2a	25	26.62	1.408	0.291	1.700	0/10	100.00	100.00	0.00	
2b						0/10	100.00			
2c						0/10	100.00			
3a	50	51.06	2.306	0.671	2.976	0/11	100.00	100.00	0.00	
3b						0/10	100.00			
3c						0/10	100.00			
4a	225	225.31	9.581	6.785	16.367	0/12	100.00	100.00	0.00	
4b						0/11	100.00			
4c						0/10	100.00			
5a	400	400.04	15.953	13.149	29.102	0/11	100.00	100.00	0.00	
5b						0/10	100.00			
5c						0/11	100.00			
6a	700	704.84	28.739	51.454	80.193	0/12	100.00	100.00	0.00	
6b						0/10	100.00			
6c						0/11	100.00			

[1] CNC = Could not calculate; Confidence intervals cannot be calculated for graphical analysis.

Defined responses: Alive, Affected, Mortally Affected, Dead
EC_{50} response: Affected

Mysidopsis bahia
Dispersant Spiked Exposure
Start Date of Test: 23-Jun-98

Test Chamber #	Design Conc. (mg/L)	Nominal Conc. (mg/L)	LC_{50} Test				
			Number of Animals Alive/Total	Individual Percent Mortality	Mean Percent Mortality	Standard Error	LC_{50} (mg/L) 95% CI (Analysis mthd.)
1a	0	0	4/5	20.00	13.33	6.67	**330.72**
1b			4/5	20.00			(CNC)[1]
1c			5/5	0.00			(Graphical)
2a	300	303.9	3/5	40.00	60.00	11.55	
2b			1/5	80.00			
2c			2/5	60.00			
3a	700	714.35	1/5	80.00	60.00	20.00	
3b			1/5	80.00			
3c			4/5	20.00			
4a	1100	1093.2	0/5	100.00	100.00	0.00	
4b			0/5	100.00			
4c			0/5	100.00			
5a	1500	1561.9	0/5	100.00	100.00	0.00	
5b			0/5	100.00			
5c			0/5	100.00			
6a	1900	1899.3	0/5	100.00	100.00	0.00	
6b			0/5	100.00			
6c			0/5	100.00			

[1] CNC = Could not calculate; Confidence intervals cannot be calculated for graphical analysis.

Mysidopsis bahia
Dispersant Continuous Exposure
Start Date of Test: 23-Jun-98

			LC_{50} Test				
Test Chamber #	Design Conc. (mg/L)	Mean Nominal Conc. (mg/L)	Number of Animals Alive/Total	Individual Percent Mortality	Mean Percent Mortality	Standard Error	LC_{50} (mg/L) 95% CI (Analysis mthd.)
1a	0	0.00	4/5	20.00	6.67	6.67	**29.06** LL=24.85 UL=33.99 (TSK a=3.57%)
1b			5/5	0.00			
1c			5/5	0.00			
2a	15	15.26	LOST[1]	LOST[1]	10.00	10.00	
2b			4/5	20.00			
2c			5/5	0.00			
3a	30	29.85	2/5	60.00	46.67	13.33	
3b			4/5	20.00			
3c			2/5	60.00			
4a	45	45.70	0/5	100.00	100.00	0.00	
4b			0/5	100.00			
4c			0/5	100.00			
5a	60	59.30	0/5	100.00	100.00	0.00	
5b			0/5	100.00			
5c			0/5	100.00			
6a	80	80.85	0/5	100.00	100.00	0.00	
6b			0/5	100.00			
6c			0/5	100.00			

[1] Animal fatality likely resulting from mysterious contaminant on glassware.

Mysidopsis bahia
WAF Spiked Exposure; ANSC Fresh Oil Study
Start Date of Test: 3-Aug-98

			Measured Concentrations			LC_{50} Test				
Test Chamber #	Design Conc. (mg/L)	Loading Rate (mg/L)	VOA (mg/L)	TPH (mg/L)	VOA + TPH (mg/L)	Number of Animals Alive/Total	Individual Percent Mortality	Mean Percent Mortality	Standard Error	LC_{50} (mg/L) 95% CI (Analysis mthd.)
1a	0	0.00	0.00	0.00	0.00	5/5	0.00	0.00	0.00	**8.21**
1b						5/5	0.00			LL=7.05
1c						5/5	0.00			UL=9.27 (Probit)
2a	100	139.26	2.83	0.31	3.14	5/5	0.00	0.00	0.00	
2b						5/5	0.00			
2c						5/5	0.00			
3a	250	250.51	4.32	0.45	4.77	4/5	20.00	10.00	10.00	
3b						5/5	0.00			
3c						LOST[1]	LOST[1]			
4a	500	500.34	8.34	0.49	8.83	1/6	83.33	51.67	31.67	
4b						4/5	20.00			
4c						LOST[1]	LOST[1]			
5a	750	752.51	8.11	0.48	8.59	3/5	40.00	46.67	6.67	
5b						2/5	60.00			
5c						3/5	40.00			
6a	2500	2497.86	12.20	0.53	12.73	0/5	100.00	100.00	0.00	
6b						0/5	100.00			
6c						0/5	100.00			

[1] Chambers' flow lapsed.

Mysidopsis bahia
WAF Continuous Exposure; ANSC Fresh Oil Study
Start Date of Test: 3-Aug-98

Test Chamber #	Design Conc. (mg/L)	Mean Loading Rate (mg/L)	Measured Concentration			LC_{50} Test				
			Mean VOA (mg/L)	Mean TPH (mg/L)	VOA + TPH (mg/L)	Number of Animals Alive/Total	Individual Percent Mortality	Mean Percent Mortality	Standard Error	LC_{50} (mg/L) 95% CI (Analysis mthd.)
1a	0	0.00	0.00	0.00	0.00	5/5	0.00	6.67	6.67	2.61
1b						5/5	0.00			LL=1.40
1c						4/5	20.00			UL=3.24 (Probit)
2a	25	24.67	0.58	0.33	0.91	4/5	20.00	40.00	11.55	
2b						2/5	60.00			
2c						3/5	40.00			
3a	100	110.90	1.69	0.31	2.00	5/5	0.00	33.33	17.64	
3b						2/5	60.00			
3c						3/5	40.00			
4a	250	250.69	3.15	0.45	3.60	0/5	100.00	93.33	6.67	
4b						1/5	80.00			
4c						0/5	100.00			
5a	500	499.89	5.15	0.49	5.64	0/6	100.00	100.00	0.00	
5b						0/5	100.00			
5c						0/5	100.00			
6a	750	751.27	5.13	0.48	5.61	0/5	100.00	100.00	0.00	
6b						0/5	100.00			
6c						0/5	100.00			

Mysidopsis bahia
CE-WAF Spiked Exposure; ANSC Fresh Oil Study
Start Date of Test: 31-Aug-98

			Measured Concentrations			LC_{50} Test				
Test Chamber #	Design Conc. (mg/L)	Loading Rate (mg/L)	VOA (mg/L)	TPH (mg/L)	VOA + TPH (mg/L)	Number of Animals Alive/Total	Individual Percent Mortality	Mean Percent Mortality	Standard Error	LC_{50} (mg/L) 95% CI (Analysis mthd.)
1a	0	0.00	0.00	0.00	0.00	5/5	0.00	0.00	0.00	**5.08**
1b						LOST[1]	LOST[1]			LL=3.13
1c						5/5	0.00			UL=8.26 (TSK a=0%)
2a	25	26.26	0.20	0.02	0.22	5/5	0.00	0.00	0.00	
2b						5/5	0.00			
2c						5/5	0.00			
3a	100	102.46	1.51	1.22	2.73	5/5	0.00	20.00	11.55	
3b						4/5	20.00			
3c						3/5	40.00			
4a	250	248.29	6.54	18.51	25.05	0/5	100.00	100.00	0.00	
4b						0/5	100.00			
4c						0/5	100.00			
5a	500	479.66	10.24	14.00	24.24	0/5	100.00	100.00	0.00	
5b						0/5	100.00			
5c						0/5	100.00			
6a	750	702.80	15.41	16.21	31.62	0/5	100.00	100.00	0.00	
6b						0/5	100.00			
6c						0/5	100.00			

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[1] Broken chamber.

Mysidopsis bahia
CE-WAF Continuous Exposure; ANSC Fresh Oil Study
Start Date of Test: 31-Aug-98

			Measured Concentration			LC_{50} Test				
Test Chamber #	Design Conc. (mg/L)	Mean Loading Rate (mg/L)	Mean VOA (mg/L)	Mean TPH (mg/L)	VOA + TPH (mg/L)	Number of Animals Alive/Total	Individual Percent Mortality	Mean Percent Mortality	Standard Error	LC_{50} (mg/L) 95% CI (Analysis mthd.)
1a	0	0.00	0.00	0.00	0.00	5/5	0.00	0.00	0.00	**1.40**
1b						6/6	0.00			LL=1.04
1c						5/5	0.00			UL=1.88 (TSK a=0%)
2a	10	7.97	0.29	0.16	0.45	6/6	0.00	0.00	0.00	
2b						5/5	0.00			
2c						5/5	0.00			
3a	25	26.19	0.85	0.36	1.21	6/6	0.00	46.67	24.04	
3b						2/5	60.00			
3c						1/5	80.00			
4a	100	104.17	2.07	2.93	5.00	0/5	100.00	100.00	0.00	
4b						0/5	100.00			
4c						0/5	100.00			
5a	250	249.71	7.04	18.51	25.55	0/5	100.00	100.00	0.00	
5b						0/5	100.00			
5c						0/5	100.00			
6a	500	489.35	9.89	14.00	23.89	0/5	100.00	100.00	0.00	
6b						0/5	100.00			
6c						0/5	100.00			

Menidia beryllina
Dispersant Spiked Exposure
Start Date of Test: 6-Jul-98

Test Chamber #	Design Conc. (mg/L)	Nominal Conc. (mg/L)	LC_{50}				
			Number of Animals Alive/Total	Individual Percent Mortality	Mean Percent Mortality	Standard Error	LC_{50} (mg/L) 95% CI (Analysis mthd.)
1a	0	0.00	5/5	0.00	0.00	0.00	**115.18** LL=105.75 UL=125.46 (TSK a=40%)
1b			5/5	0.00			
1c			5/5	0.00			
2a	20	19.60	5/5	0.00	10.00	10.00	
2b			LOST[1]	LOST[1]			
2c			4/5	20.00			
3a	40	40.00	5/5	0.00	6.67	6.67	
3b			5/5	0.00			
3c			4/5	20.00			
4a	70	68.45	5/5	0.00	13.33	6.67	
4b			4/5	20.00			
4c			4/5	20.00			
5a	100	101.10	4/5	20.00	20.00	0.00	
5b			4/5	20.00			
5c			4/5	20.00			
6a	120	120.30	2/5	60.00	60.00	0.00	
6b			2/5	60.00			
6c			2/5	60.00			

[1] Drained.

Menidia beryllina
Dispersant Continuous Exposure
Start Date of Test: 6-Jul-98

			LC_{50} Test				
Test Chamber #	Design Conc. (mg/L)	Mean Nominal Conc. (mg/L)	Number of Animals Alive/Total	Individual Percent Mortality	Mean Percent Mortality	Standard Error	LC_{50} (mg/L) 95% CI (Analysis mthd.)
1a	0	0.00	5/5	0.00	0.00	0.00	**54.67**
1b			5/5	0.00			LL=46.70
1c			5/5	0.00			UL=62.94 (Probit)
2a	20	19.74	5/5	0.00	0.00	0.00	
2b			5/5	0.00			
2c			5/5	0.00			
3a	40	40.29	5/6	16.67	18.89	1.11	
3b			4/5	20.00			
3c			4/5	20.00			
4a	70	69.41	1/5	80.00	80.00	0.00	
4b			1/5	80.00			
4c			1/5	80.00			
5a	100	101.10	0/5	100.00	100.00	0.00	
5b			0/5	100.00			
5c			0/5	100.00			
6a	120	120.30	0/5	100.00	100.00	0.00	
6b			0/5	100.00			
6c			0/6	100.00			

Menidia beryllina
WAF Spiked Exposure; ANSC Fresh Oil Study
Start Date of Test: 17-Aug-98

			Measured Concentrations			LC$_{50}$ Test				
Test Chamber #	Design Conc. (mg/L)	Loading Rate (mg/L)	VOA (mg/L)	TPH (mg/L)	VOA + TPH (mg/L)	Number of Animals Alive/Total	Individual Percent Mortality	Mean Percent Mortality	Standard Error	LC$_{50}$ (mg/L) 95% CI (Analysis mthd.)
1a	0	0.00	0.00	0.00	0.00	5/5	0.00	0.00	0.00	**26.36** LL=25.54 UL=27.22 (SK)
1b						5/5	0.00			
1c						5/5	0.00			
2a	500	499.51	10.73	0.42	11.15	5/5	0.00	0.00	0.00	
2b						5/5	0.00			
2c						5/5	0.00			
3a	2000	1952.14	18.97	0.50	19.47	5/5	0.00	0.00	0.00	
3b						6/6	0.00			
3c						LOST [1]	LOST [1]			
4a	3000	3023.80	23.87	0.34	24.21	4/5	20.00	13.33	6.67	
4b						4/5	20.00			
4c						5/5	0.00			
5a	4000	3996.23	27.91	0.40	28.31	0/5	100.00	86.67	13.33	
5b						0/5	100.00			
5c						2/5	60.00			
6a	6000	6001.71	32.67	0.38	33.05	2/5	60.00	75.56	12.37	
6b						0/5	100.00			
6c						2/6	66.67			

[1] Chambers' flow lapsed.

Menidia beryllina
WAF Continuous Exposure; ANSC Fresh Oil Study
Start Date of Test: 17-Aug-98

Test Chamber #	Design Conc. (mg/L)	Mean Loading Rate (mg/L)	Measured Concentrations			LC$_{50}$ Test				
			Mean VOA (mg/L)	Mean TPH (mg/L)	VOA + TPH (mg/L)	Number of Animals Alive/Total	Individual Percent Mortality	Mean Percent Mortality	Standard Error	LC$_{50}$ (mg/L) EC$_{50}$ (Analysis mthd.)
1a	0	0.00	0.00	0.00	0.00	5/5	0.00	0.00	0.00	**15.59** LL=13.98 UL=17.38 (TSK a=0%)
1b						5/5	0.00			
1c						5/5	0.00			
2a	250	263.78	6.00	0.44	6.44	5/5	0.00	0.00	0.00	
2b						5/5	0.00			
2c						5/5	0.00			
3a	1000	1008.39	16.35	0.53	16.88	5/5	0.00	13.33	13.33	
3b						5/5	0.00			
3c						3/5	40.00			
4a	2000	1959.29	14.86	0.50	15.36	LOST[1]	LOST[1]	25.00	25.00	
4b						3/6	50.00			
4c						6/6	0.00			
5a	3000	VOA: 2989.27 TPH: 2997.18	17.31	0.34	17.65	0/5	100.00	100.00	0.00	
5b						0/5	100.00			
5c						0/5	100.00			
6a	4000	4004.72	26.00	0.40	26.40	0/5	100.00	100.00	0.00	
6b						0/5	100.00			
6c						0/5	100.00			

[2] N/A = VOA sample for Day 3 was destroyed.

Menidia beryllina
CE-WAF Spiked Exposure; ANSC Fresh Oil Study
Start Date of Test: 12-Oct-98

Test Chamber #	Design Conc. (mg/L)	Loading Rate (mg/L)	Measured Concentrations			LC_{50} Test				
			VOA (mg/L)	TPH (mg/L)	VOA + TPH (mg/L)	Number of Animals Alive/Total	Individual Percent Mortality	Mean Percent Mortality	Standard Error	LC_{50} (mg/L) 95% CI (Analysis mthd.)
1a	0	0.00	0.00	0.00	0.00	5/5	0.00	0.00	0.00	**12.22** LL=7.79 UL=19.17 (TSK, a=40%)
1b						5/5	0.00			
1c						5/5	0.00			
2a	150	148.43	2.51	6.21	8.72	5/5	0.00	20.00	20.00	
2b						5/5	0.00			
2c						2/5	60.00			
3a	250	250.06	4.29	7.97	12.26	4/5	20.00	60.00	20.00	
3b						1/5	80.00			
3c						1/5	80.00			
4a	300	298.83	3.99	12.82	16.81	2/5	60.00	53.33	6.67	
4b						2/5	60.00			
4c						3/5	40.00			
5a	350	356.06	10.14	24.48	34.62	3/5	40.00	50.00	10.00	
5b						2/5	60.00			
5c						LOST [1]	LOST [1]			
6a	400	400.14	6.83	11.79	18.62	1/5	80.00	66.67	6.67	
6b						2/5	60.00			
6c						2/5	60.00			

[1] Drained.

Menidia beryllina
CE-WAF Continuous Exposure; ANSC Fresh Oil Study
Start Date of Test: 12-Oct-98

			Measured Concentrations			LC_{50} Test				
Test Chamber #	Design Conc. (mg/L)	Mean Loading Rate (mg/L)	Mean VOA (mg/L)	Mean TPH (mg/L)	VOA + TPH (mg/L)	Number of Animals Alive/Total	Individual Percent Mortality	Mean Percent Mortality	Standard Error	LC_{50} (mg/L) 95% CI (Analysis mthd.)
1a	0	0.00	0.00	0.00	0.00	5/5	0.00	0.00	0.00	**12.42** LL=11.40 UL=13.54 (TSK a=0%)
1b						5/5	0.00			
1c						5/5	0.00			
2a	100	100.97	0.76	2.69	3.45	5/5	0.00	0.00	0.00	
2b						5/5	0.00			
2c						5/5	0.00			
3a	150	150.48	1.95	5.78	7.73	5/5	0.00	0.00	0.00	
3b						5/5	0.00			
3c						5/5	0.00			
4a	200	200.65	3.11	10.95	14.06	4/5	20.00	20.00	0.00	
4b						4/5	20.00			
4c						4/5	20.00			
5a	250	248.96	4.24	7.70	11.94	2/5	60.00	66.67	17.64	
5b						3/5	40.00			
5c						0/5	100.00			
6a	300	298.83	3.99	12.28	16.27	0/5	100.00	100.00	0.00	
6b						0/5	100.00			
6c						0/5	100.00			

Menidia beryllina
WAF Spiked Exposure; PBCO Fresh Oil Study
Start Date of Test: 26-Oct-98

			Measured Concentrations			LC_{50} Test				
Test Chamber #	Design Conc. (mg/L)	Loading Rate (mg/L)	VOA (mg/L)	TPH (mg/L)	VOA + TPH (mg/L)	Number of Animals Alive/Total	Individual Percent Mortality	Mean Percent Mortality	Standard Error	LC_{50} (mg/L) 95% CI (Analysis mthd.)
1a	0	0.00	0.00	0.00	0.00	5/5	0.00	0.00	0.00	**>19.86**
1b						5/5	0.00			
1c						5/5	0.00			
2a	1000	990.03	7.81	0.22	8.03	5/5	0.00	6.67	6.67	
2b						4/5	20.00			
2c						5/5	0.00			
3a	2000	1994.26	12.36	0.33	12.69	4/5	20.00	10.00	10.00	
3b						5/5	0.00			
3c						LOST[1]	LOST[1]			
4a	3000	2997.70	13.88	0.47	14.35	5/5	0.00	20.00	20.00	
4b						3/5	40.00			
4c						LOST[2]	LOST[2]			
5a	6000	5994.89	18.25	0.33	18.58	4/5	20.00	26.67	6.67	
5b						4/5	20.00			
5c						3/5	40.00			
6a	8000	8151.74	19.55	0.31	19.86	4/5	20.00	26.67	6.67	
6b						4/5	20.00			
6c						3/5	40.00			

[1] Chamber drained rapidly; animals not exposed to normal spiked exposure regime.

[2] Chambers' flow lapsed.

Menidia beryllina
WAF Continuous Exposure; PBCO Fresh Oil Study
Start Date of Test: 26-Oct-98

			Measured Concentrations			LC$_{50}$ Test				
Test Chamber #	Design Conc. (mg/L)	Mean Loading Rate (mg/L)	Mean VOA (mg/L)	Mean TPH (mg/L)	VOA + TPH (mg/L)	Number of Animals Alive/Total	Individual Percent Mortality	Mean Percent Mortality	Standard Error	LC$_{50}$ (mg/L) 95% CI (Analysis mthd.)
1a	0	0.00	0.00	0.00	0.00	5/5	0.00	6.67	6.67	**14.81** LL=9.79 UL=68.75 (Probit)
1b						4/5	20.00			
1c						5/5	0.00			
2a	500	498.67	4.00	0.17	4.17	4/5	20.00	13.33	6.67	
2b						4/5	20.00			
2c						5/5	0.00			
3a	1000	1004.16	5.90	0.22	6.12	4/5	20.00	6.67	6.67	
3b						5/5	0.00			
3c						5/5	0.00			
4a	2000	1996.80	9.06	0.27	9.33	2/5	60.00	53.33	17.64	
4b						4/5	20.00			
4c						1/5	80.00			
5a	3000	3011.05	12.18	0.31	12.49	1/5	80.00	50.00	30.00	
5b						LOST[1]	LOST[1]			
5c						4/5	20.00			
6a	6000	6053.85	15.89	0.25	16.14	5/5	0.00	46.67	24.04	
6b						2/5	60.00			
6c						1/5	80.00			

[1] Beaker found without air supply.

Menidia beryllina
CE-WAF Spiked Exposure; PBCO Fresh Oil Study
Start Date of Test: 19-Oct-98

			Measured Concentrations			LC_{50} Test				
Test Chamber #	Design Conc. (mg/L)	Loading Rate (mg/L)	VOA (mg/L)	TPH (mg/L)	VOA + TPH (mg/L)	Number of Animals Alive/Total	Individual Percent Mortality	Mean Percent Mortality	Standard Error	LC_{50} (mg/L) 95% CI (Analysis mthd.)
1a	0	0.00	0.00	0.00	0.00	5/5	0.00	0.00	0.00	**12.29**
1b						5/5	0.00			LL=10.90
1c						5/5	0.00			UL=13.86
										(TSK a=6.67%)
2a	200	198.43	2.89	4.79	7.69	5/5	0.00	6.67	6.67	
2b						5/5	0.00			
2c						4/5	20.00			
3a	300	302.66	5.84	16.22	22.06	0/5	100.00	86.67	6.67	
3b						1/5	80.00			
3c						1/5	80.00			
4a	400	417.54	6.18	16.44	22.63	2/5	60.00	80.00	11.55	
4b						1/5	80.00			
4c						0/5	100.00			
5a	600	599.91	7.42	10.92	18.34	0/5	100.00	100.00	0.00	
5b						0/5	100.00			
5c						0/5	100.00			
6a	800	817.60	9.64	16.70	26.34	0/5	100.00	100.00	0.00	
6b						0/5	100.00			
6c						0/5	100.00			

* Note: EC50 is greater than highest concentration.

Menidia beryllina
CE-WAF Continuous Exposure; PBCO Fresh Oil Study
Start Date of Test: 19-Oct-98

			Measured Concentrations			LC_{50} Test				
Test Chamber #	Design Conc. (mg/L)	Mean Loading Rate (mg/L)	Mean VOA (mg/L)	Mean TPH (mg/L)	VOA + TPH (mg/L)	Number of Animals Alive/Total	Individual Percent Mortality	Mean Percent Mortality	Standard Error	LC_{50} (mg/L) 95% CI (Analysis mthd.)
1a	0	0.00	0.00	0.00	0.00	5/5	0.00	0.00	0.00	**4.57** LL = 4.16 UL = 5.02 (TSK, a=20%)
1b						5/5	0.00			
1c						5/5	0.00			
2a	100	101.38	1.80	1.30	3.10	4/5	20.00	20.00	0.00	
2b						4/5	20.00			
2c						4/5	20.00			
3a	200	198.76	3.28	5.97	9.25	0/5	100.00	100.00	0.00	
3b						0/5	100.00			
3c						0/5	100.00			
4a	250	257.94	3.87	4.86	8.73	0/5	100.00	100.00	0.00	
4b						0/5	100.00			
4c						0/5	100.00			
5a	300	302.66	5.84	16.22	22.06	0/5	100.00	100.00	0.00	
5b						0/5	100.00			
5c						0/5	100.00			
6a	400	417.54	6.18	16.44	22.62	0/5	100.00	100.00	0.00	
6b						0/5	100.00			
6c						0/5	100.00			

[1] CNC = Could not calculate; 95% Confidence Intervals not reliable.

APPENDIX B

FRESH OIL AND DISPERSANT STUDY CHEMISTRY DATA

Measured concentrations for Corexit 9500 test solutions.

C. bairdi spiked exposure

Loading Rate (mg/L)	UV (mg/L)	TOC (mg/L)
0.00	0.00	N/S
199.40	233.13	N/S
796.00	714.38	N/S
1401.85	762.97	N/S
2002.80	890.47	N/S
2599.60	1032.19	N/S

M. bahia spiked exposure

Loading Rate (mg/L)	UV (mg/L)	TOC (mg/L)
0.00	9.13	2.36
303.90	136.86	288.20
714.35	123.78	378.70
1093.20	123.37	573.70
1561.90	123.22	857.20
1899.30	283.11	952.20

M. beryllina spiked exposure

Loading Rate (mg/L)	UV (mg/L)	TOC (mg/L)
0.00	8.51	7.03
19.60	18.54	22.70
40.00	34.65	51.37
68.45	57.07	57.17
101.10	84.37	116.30
117.2**	90.24	100.20
120.3**	62.03	68.03

C. bairdi continuous exposure

Day 1	0.00	0.00	N/S
	20.05	12.29	N/S
	49.50	49.11	N/S
	79.95	89.01	N/S
	109.70	126.77	N/S
	130.30	148.96	N/S
Day 2	0.00	0.00	N/S
	19.90	8.54	N/S
	50.30	35.16	N/S
	79.80	71.88	N/S
	111.70	124.69	N/S
	130.45	140.73	N/S
Day 3	0.00	0.00	N/S
	20.95	11.46	N/S
	50.05	46.09	N/S
	79.90	80.10	N/S
	110.25	118.96	N/S
	129.90	146.51	N/S
Day 4	0.00	0.00	N/S
	19.95	8.70	N/S
	50.15	40.57	N/S
	80.05	83.80	N/S
	110.45	117.97	N/S
	130.20	142.97	N/S

M. bahia continuous exposure

Day 1	0.00	9.24	4.50
	13.95	16.04	16.30
	29.40	25.95	27.60
	44.90	36.60	37.10
	59.75	42.58	239.27
	80.60	50.66	187.83
Day 2	0.00	N/S	5.13
	13.30	N/S	51.30
	29.45	N/S	63.00
	46.10	N/S	44.80
	55.85	N/S	51.33
	81.10	N/S	74.37
Day 3	0.00	N/S	4.07
	18.00	N/S	19.13
	31.05	N/S	27.47
	46.10	N/S	39.07
	*	*	*
	*	*	*
Day 4	0.00	N/S	3.97
	18.00	N/S	17.03
	31.05	N/S	33.87
	46.10	N/S	41.86
	*	*	*
	*	*	*

M. beryllina continuous exposure

Day 1	0.00	8.51	7.03
	19.60	18.54	22.70
	40.00	34.65	51.37
	68.45	57.07	57.17
	101.10	84.37	116.30
	117.2**	90.24	100.20
	120.3**	62.03	68.03
Day 2	0.00	N/S	10.53
	19.90	N/S	29.10
	39.70	N/S	40.17
	69.75	N/S	71.00
	*	*	*
	*	*	*
Day 3	0.00	N/S	5.37
	19.30	N/S	20.87
	40.10	N/S	39.90
	70.10	N/S	70.27
	*	*	*
	*	*	*
Day 4	0.00	N/S	3.83
	20.15	N/S	24.07
	41.35	N/S	40.43
	69.35	N/S	68.03
	*	*	*
	*	*	*

* Test solution concentrations were not prepared or analyzed due to complete test orgainism die off for that concentration.

** Additional test solution was mixed to complete the test at the 120 mg/L target concentration.

Measured concentrations of Alaska North Slope crude oil spiked exposure test solutions.

Species	Test Solution[a]	Loading Rate (mg/L)	BTEX (mg/L)	VOA C_6-C_9 (mg/L)	TPH C_{10}-C_{36} (mg/L)	THC[b] C_6-C_{36} (mg/L)
C. bairdi	WAF	0.00	0.00	0.17	0.00	0.17
		201.86	6.72	7.45	0.11	7.56
		401.09	11.52	12.43	0.12	12.54
		1099.66	18.29	19.24	0.11	19.35
		1814.86	21.24	22.10	0.11	22.21
		2499.23	29.17	30.45	0.12	30.58
	CE-WAF	0.00	0.00	0.15	1.52	1.67
		99.83	3.77	4.28	0.74	5.02
		204.36	7.28	8.42	2.15	10.57
		402.32	12.31	16.48	7.67	24.14
		1100.75	24.25	35.19	18.74	53.93
		1853.79	29.35	44.64	51.60	96.23
M. bahia	WAF	0.00	0.05	0.97	0.07	1.04
		139.26	2.15	2.83	0.31	3.14
		250.51	3.60	4.32	0.45	4.77
		500.34	7.20	8.34	0.49	8.83
		752.51	6.76	8.11	0.48	8.59
		2497.86	11.27	12.20	0.53	12.73
	CE-WAF	0.00	0.10	0.14	0.01	0.15
		26.26	0.14	0.20	0.02	0.22
		102.46	1.17	1.51	1.22	2.73
		248.29	3.92	6.54	18.51	25.05
		479.66	6.83	10.24	14.00	24.24
		702.80	13.05	15.41	16.21	31.62
M. beryllina	WAF	0.00	0.05	0.07	0.00	0.07
		499.51	9.40	10.73	0.42	11.15
		1952.14	17.31	18.97	0.50	19.47
		3023.80	21.87	23.87	0.34	24.21
		3996.23	25.51	27.91	0.40	28.31
		6001.71	30.67	32.67	0.38	33.05
	CE-WAF	0.00	0.00	0.01	0.00	0.01
		148.43	2.12	2.51	6.21	8.72
		250.06	3.44	4.29	7.97	12.26
		298.83	3.27	3.99	12.82	16.81
		356.06	6.34	10.14	24.48	34.62
		400.14	5.13	6.83	11.79	18.62

[a] WAF = water-accommodated fraction; CE-WAF = chemically enhanced water-accommodated fraction.

[b] THC concentrations are the sum of VOA and TPH values.

Measured concentrations of Alaska North Slope crude oil continuous exposure test solutions.

Species	Test Solution[a]	Mean Loading Rate (mg/L)	BTEX (mg/L)	VOA C_6-C_9 (mg/L)	TPH C_{10}-C_{36} (mg/L)	THC[b] C_6-C_{36} (mg/L)
C. bairdi[c]	WAF	0.00	0.00	0.00	0.00	0.00
		50.89	1.64	2.35	0.12	2.47
		234.14	7.75	8.63	0.15	8.78
		428.58	12.11	13.02	0.16	13.18
		716.01	17.44	19.02	0.14	19.16
		998.79	22.17	24.11	0.22	24.33
	CE-WAF	0.00	0.00	0.00	0.00	0.00
		26.62	1.10	1.41	0.29	1.70
		51.06	2.04	2.31	0.67	2.98
		225.31	7.98	9.58	6.79	16.37
		400.04	12.52	15.95	13.15	29.10
		704.84	18.17	28.74	51.45	80.19
M. bahia[d]	WAF	0.00	0.00	0.28	0.07	0.35
		24.67	0.34	0.58	0.33	0.91
		110.90	1.45	1.69	0.31	2.00
		250.69	2.70	3.15	0.45	3.60
		499.89	4.61	5.15	0.49	5.64
		751.27	4.68	5.13	0.48	5.61
	CE-WAF	0.00	0.13	0.54	0.08	0.62
		7.97	0.15	0.29	0.16	0.45
		26.19	0.55	0.85	0.36	1.21
		104.17	1.69	2.07	2.93	5.00
		249.71	4.16	7.04	18.51	25.55
		489.35	2.08	9.89	14.00	23.89
M. beryllina[d]	WAF	0.00	0.01	0.43	0.04	0.47
		263.78	5.04	6.00	0.44	6.44
		1008.39	10.86	16.35	0.53	16.88
		1959.29	13.00	14.86	0.50	15.36
		2989.27	15.86	17.31	0.34	17.65
		4004.72	22.85	26.00	0.40	26.40
	CE-WAF	0.00	0.40	0.50	0.04	0.54
		100.97	0.56	0.76	2.69	3.45
		150.48	1.56	1.95	5.78	7.73
		200.65	2.45	3.11	10.95	14.06
		248.96	3.21	4.24	7.70	11.94
		298.83	3.27	3.99	12.28	16.27

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[a] WAF = water-accommodated fraction; CE-WAF = chemically enhanced water-accommodated fraction.

[b] THC concentrations are the sum of VOA and TPH values.

[c] BTEX, VOA, and TPH concentrations for *C. bairdi* are the mean values for solutions from days 1-4.

[d] BTEX and VOA concentrations for *M. bahia* and *M. beryllina* are the composite of solutions from days 1-4; TPH concentrations for WAF solutions are values from day 1 solutions; TPH concentrations for CE-WAF solutions are the composite of solutions from days 1-4.

Measured concentrations of Prudhoe Bay crude oil test solutions for *M. beryllina*.

Exposure Regime	Test Solution[a]	Loading Rate (mg/L)	BTEX (mg/L)	VOA C_6-C_9 (mg/L)	TPH C_{10}-C_{36} (mg/L)	THC[b] C_6-C_{36} (mg/L)
Spiked	WAF	0.00	0.24	0.25	0.00	0.25
		990.03	7.13	7.81	0.22	8.03
		1994.26	11.08	12.36	0.33	12.69
		2997.70	12.71	13.88	0.47	14.35
		5994.89	16.84	18.25	0.33	18.58
		8151.74	18.11	19.55	0.31	19.86
	CE-WAF	0.00	0.06	1.41	0.00	1.41
		198.43	1.91	2.89	4.79	7.68
		302.66	3.41	5.84	16.22	22.06
		417.54	4.33	6.18	16.44	22.62
		599.91	5.40	7.42	10.92	18.34
		817.60	6.51	9.64	16.70	26.34
Continuous[c,d]	WAF	0.00	0.00	0.00	0.00	0.00
		498.67	3.59	4.00	0.17	4.17
		1004.16	5.39	5.90	0.22	6.12
		1996.80	8.40	9.06	0.27	9.33
		3011.05	11.27	12.18	0.31	12.49
		6053.85	14.80	15.89	0.25	16.14
	CE-WAF	0.00	0.06	0.54	0.00	0.54
		101.38	1.00	1.80	1.30	3.10
		198.76	2.29	3.28	5.97	9.25
		257.94	2.74	3.87	4.86	8.73
		302.66	3.41	5.84	16.22	22.06
		417.54	4.33	6.18	16.44	22.62

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[a] WAF = water-accommodated fraction; CE-WAF = chemically enhanced water-accommodated fraction.

[b] THC concentrations are the sum of VOA and TPH values.

[c] BTEX and VOA concentrations for *M. beryllina* are the composite of solutions from days 1-4.

[d] TPH concentrations for WAF solutions are values from day 1 solutions; TPH concentrations for CE-WAF solutions are the composite of solutions from days 1-4.

Declining, spiked concentrations of Corexit 9500 test solutions.

C. bairdi

Loading Rate (mg/L)	Hour	UV (mg/L)
1401.85	0	762.97
	2	400.78
	4	219.79
	7	100.16
	12	130.73
2599.60	0	1032.19
	2	707.97
	4	425.52
	7	231.41
	12	178.75

M. bahia

Loading Rate (mg/L)	Hour	TOC (mg/L)
303.90	0	288.20
	2	93.27
	4	24.73
	7	18.20
	12	10.46
714.35	0	378.70
	2	190.43
	4	84.02
	7	68.13
	12	15.89
1093.20	0	573.70
	2	245.60
	4	141.27
	7	107.27
	12	26.36
1561.90	0	857.20
	2	405.80
	4	131.60
	7	119.37
	12	50.24
1899.30	0	952.20
	2	399.47
	4	121.00
	7	109.27
	12	43.02

M. beryllina

Loading Rate (mg/L)	Hour	TOC (mg/L)
19.60	0	22.70
	2	15.30
	4	13.30
	18	8.40
40.00	0	51.37
	2	21.50
	4	13.70
	18	7.70
68.45	0	57.17
	2	25.30
	4	16.00
	18	6.00
101.10	0	116.30
	2	39.60
	4	17.60
	18	6.00

Declining, spiked concentrations of Alaska North Slope crude oil (ANS) and Prudhoe Bay crude oil (PBCO) test solutions.

ANSC

WAF[1]

M. beryllina Spiked exposure

Loading Rate (mg/L)	Hour	BTEX (mg/L)	VOA C_6-C_9 (mg/L)
0	0	0.00	0.61
	2	0.03	0.33
	4	0.02	0.65
	7	0.00	0.19
	12	0.27	0.38
499.51	0	7.33	8.17
	2	1.97	2.38
	4	1.02	1.23
	7	0.35	0.45
	12	0.05	0.17
1952.14	0	16.33	17.78
	2	4.41	4.81
	4	2.28	2.50
	7	0.95	1.11
	12	0.30	1.20
3023.80	0	20.64	22.50
	2	4.55	4.87
	4	2.23	2.43
	7	0.27	0.38
	12	0.33	0.52
3996.23	0	24.10	26.01
	2	6.24	6.86
	4	2.49	2.89
	7	0.58	0.74
	12	nt	nt
6001.71	0	24.83	30.30
	2	7.13	7.76
	4	3.30	3.56
	7	0.48	0.66
	12	0.54	0.76

CE-WAF[1]

M. bahia Spiked exposure

Loading Rate (mg/L)	Hour	BTEX (mg/L)	VOA C_6-C_9 (mg/L)
0	0	0.07	0.21
	2	0.07	0.28
	4	0.13	0.41
	7	0.31	0.66
	12	0.16	0.20
26.26	0	0.33	0.41
	2	0.08	0.17
	4	0.05	0.15
	7	0.08	0.18
	12	0.10	0.18
102.46	0	1.21	1.47
	2	0.5	0.56
	4	0.31	0.41
	7	0.99	1.13
	12	1.53	1.70
248.29	0	4.97	7.49
	2	1.23	1.62
	4	0.77	1.16
	7	0.33	0.62
	12	0.04	0.15
479.66	0	6.75	10.26
	2	2.19	2.57
	4	1.26	1.48
	7	0.62	0.75
	12	0.01	0.09
702.8	0	8.06	10.27
	2	2.37	2.68
	4	1.42	1.63
	7	0.96	1.13
	12	0.03	0.15

PBCO

WAF[1]

M. beryllina Spiked exposure

Loading Rate (mg/L)	Hour	BTEX (mg/L)	VOA C_6-C_9 (mg/L)
1994.26	0.0	9.98	10.96
	2.5	2.25	4.23
	6.0	1.73	1.85
	20.5	0.07	0.08
8151.74	0.0	14.60	15.55
	5.0	2.31	2.39
	19.5	0.22	0.23

CE-WAF[1]

M. beryllina Spiked exposure

Loading Rate (mg/L)	Hour	BTEX (mg/L)	VOA C_6-C_9 (mg/L)
198.43	0	1.52	1.82
	2	0.59	0.64
	4	0.43	0.47
	7	0.10	0.13
	10	0.00	0.01
817.6	0	5.66	6.76
	2	1.77	2.02
	5	0.56	0.62
	18	0.07	0.10

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[1] WAF = water-accommodated fraction; CE-WAF = chemically enhanced water-accommodated fraction.

Post 24hr concentrations of Alaska North Slope crude oil test solutions.

WAF[1]			
M. beryllina continuous exposure			
Loading Rate (mg/L)	End of day	BTEX (mg/L)	VOA C_6-C_9 (mg/L)
1034.11	24	0.00	0.16
986.26	72	0.02	0.29
1980.30	48	0.04	0.53
4058.74	48	0.04	0.29
3948.20	72	0.07	0.28

CE-WAF[2]			
M. bahia continuous exposure			
Loading Rate (mg/L)	End of day	BTEX (mg/L)	VOA C_6-C_9 (mg/L)
26.26	24	0.02	0.08
26.86	48	0.03	0.06
28.09	72	0.22	0.57
102.46	24	0.02	0.07
248.29	24	0.10	0.19
479.66	24	0.07	1.00

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[1] WAF = water-accommodated fraction.

[2] CE-WAF = chemically enhanced water-accommodated fraction.

APPENDIX C

FRESH OIL AND DISPERSANT STUDY WATER QUALITY DATA

Water quality parameters for spiked exposure to test solutions of Alaska North Slope crude oil used on *C. bairdi*.

Test Solution[a]	Loading Rate (mg/L)	pH	Conductivity Reading (mS)	Salinity (ppt)	Temp.[†] (°C)	D.O. (mg/L)
Corexit 9500[b]	0.00	8.37	48.52	31.3	6.2	9.15
	199.40	8.42	48.40	31.4	8.1	8.74
	796.00	8.41	48.41	31.3	7.6	8.82
	1401.85	8.41	48.20	31.2	7.7	8.78
	2002.80	8.38	48.06	31.1	7.6	8.77
	2599.60	8.36	47.97	31.0	7.8	8.84
WAF	0.00	7.86	46.80	31.83	5.30	10.16
	201.86	7.95	46.20	31.41	N/A	N/A
	401.09	7.92	46.10	31.34	N/A	N/A
	1099.66	7.95	46.20	31.41	N/A	N/A
	1814.86	7.89	46.20	31.41	N/A	N/A
	2499.23	7.91	46.40	31.55	N/A	N/A
CE-WAF	0.00	N/S	N/S	N/S	N/A	10.18
	99.83	7.65	47.00	31.97	N/A	N/A
	204.36	7.72	46.10	31.34	N/A	N/A
	402.32	7.77	46.20	31.41	N/A	N/A
	1100.75	7.77	46.30	31.48	N/A	N/A
	1853.79	7.81	46.20	31.41	N/A	N/A
Mean	-	8.03	47.02	31.40	6.92	9.18
Std. Dev.	-	0.29	0.98	0.23	1.04	0.62
n	-	17	17	17	9	8
Maximum	-	8.42	48.52	31.97	8.10	10.18
Minimum	-	7.65	46.10	31.00	5.30	8.74

[a] WAF = water-accommodated fraction; CE-WAF = chemically enhanced water-accommodated fraction.

[b] Measurments from Corexit 9500 test repeated in 1999.

[†] IMS Ressurection Bay average seawater temperatures for WAF and CE-WAF test solutions for the months of March-April 1998 plus individual measurments.

N/S = Not Sampled.

Water quality parameters for continuous exposure dispersant only solutions of Corexit 9500 used on *C. bairdi.*

Test Solution		Loading Rate (mg/L)	pH	Conductivity Reading (mS)	Salinity (ppt)	Temp. (°C)	D.O. (mg/L)
Corexit 9500[a]	Day 1	0.00	8.37	48.52	31.30	6.20	9.15
		20.05	8.42	48.39	31.30	7.90	8.63
		49.50	8.43	48.73	31.40	7.60	8.82
		79.95	8.44	48.70	31.50	7.70	8.87
		109.70	8.43	48.80	31.50	7.70	8.87
		130.30	8.41	48.72	31.40	7.70	8.84
	Day 2	0.00	8.33	48.32	31.20	5.10	9.86
		19.90	8.37	48.63	31.40	7.10	9.34
		50.30	8.37	48.66	31.40	6.70	9.49
		79.80	8.40	48.65	31.40	6.50	9.48
		111.70	8.42	48.63	31.40	6.60	9.56
		130.45	8.40	48.69	31.40	6.50	9.51
	Day 3	0.00	8.34	49.02	31.40	5.30	10.03
		20.95	8.32	48.66	31.40	7.20	9.16
		50.05	8.35	48.60	31.40	7.20	9.19
		79.90	8.35	48.53	31.30	7.50	9.14
		110.25	8.37	48.59	31.30	7.20	9.14
		129.90	8.39	48.57	31.40	7.00	9.09
	Day 4	0.00	8.36	49.19	31.40	4.60	9.96
		19.95	8.33	48.70	31.50	7.30	9.30
		50.15	8.37	48.61	31.40	7.20	9.28
		80.05	8.37	48.58	31.40	7.00	9.18
		110.45	8.40	48.66	31.40	6.80	9.29
		130.20	8.39	48.68	31.40	6.50	9.36
	Mean	-	8.38	48.66	31.39	6.84	9.27
	Std. Dev.	-	0.03	0.17	0.07	0.85	0.35
	n	-	24	24	24	24	24
	Maximum	-	8.44	49.19	31.50	7.90	10.03
	Minimum	-	8.32	48.32	31.20	4.60	8.63

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[a] Measurments from Corexit 9500 test repeated in 1999.

Water quality parameters for continuous exposure WAF test solutions of Alaska North Slope crude oil used on *C. bairdi*.

Test Solution		Loading Rate (mg/L)	pH	Conductivity Reading (mS)	Salinity (ppt)	Temp.[†] (°C)	D.O. (mg/L)
WAF[a]	Day 1	0.00	7.78	46.30	31.48	N/A	N/A
		47.03	7.80	46.30	31.48	N/A	N/A
		254.00	7.80	46.30	31.48	N/A	N/A
		463.94	7.78	46.30	31.48	N/A	N/A
		773.94	7.85	46.60	31.69	N/A	N/A
		1017.37	7.83	46.30	31.48	N/A	N/A
	Day 2	0.00	7.74	46.50	31.62	N/A	N/A
		56.54	7.93	46.40	31.55	N/A	N/A
		233.80	7.94	46.50	31.62	N/A	N/A
		414.74	7.84	46.70	31.76	N/A	N/A
		702.11	7.84	46.50	31.62	N/A	N/A
		994.71	7.90	46.50	31.62	N/A	N/A
	Day 3	0.00	7.79	46.50	31.62	N/A	N/A
		49.49	7.92	46.40	31.55	N/A	N/A
		228.17	7.93	46.30	31.48	N/A	N/A
		399.83	7.89	45.30	30.77	N/A	N/A
		685.63	7.87	46.00	31.27	N/A	N/A
		1001.14	7.88	45.90	31.20	N/A	N/A
	Day 4	0.00	7.78	46.70	31.76	N/A	N/A
		50.49	7.80	46.20	31.41	N/A	N/A
		220.57	7.90	46.30	31.48	N/A	N/A
		435.80	7.90	46.20	31.41	N/A	N/A
		702.34	7.90	46.30	31.48	N/A	N/A
		981.94	7.97	46.30	31.48	N/A	N/A
	Mean	-	7.86	46.32	31.49	6.00	-
	Std. Dev.	-	0.06	0.29	0.20	0.28	-
	n	-	24	24	24	2	-
	Maximum	-	7.97	46.70	31.76	6.20	-
	Minimum	-	7.74	45.30	30.77	5.80	-

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[a] WAF = water-accommodated fraction.

[†] IMS Ressurection Bay average seawater temperatures for the months of March-April 1998.

Water quality parameters for continuous exposure CE-WAF solutions of Alaska North Slope crude oil used on *C. bairdi*.

Test Solution		Loading Rate (mg/L)	pH	Conductivity Reading (mS)	Salinity (ppt)	Temp.† (°C)	D.O. (mg/L)
CE-WAF[a]	Day 1	0.00	7.94	46.00	31.27	7.70	9.66
		25.39	7.86	46.30	31.48	N/A	N/A
		50.95	7.91	46.00	31.27	N/A	N/A
		223.67	7.90	46.10	31.34	N/A	N/A
		395.99	7.89	46.20	31.41	N/A	N/A
		701.26	7.91	46.00	31.27	N/A	N/A
	Day 2	0.00	N/S	N/S	N/S	7.90	9.63
		26.17	7.88	46.40	31.55	N/A	N/A
		50.89	7.89	46.10	31.34	N/A	N/A
		228.09	7.88	46.20	31.41	N/A	N/A
		399.41	7.89	46.20	31.41	N/A	N/A
		704.06	7.92	46.20	31.41	N/A	N/A
	Day 3	0.00	7.88	46.30	31.48	N/A	N/A
		26.69	7.90	46.20	31.41	N/A	N/A
		50.80	7.91	46.30	31.48	N/A	N/A
		223.28	7.92	46.30	31.48	N/A	N/A
		400.40	7.92	46.30	31.48	N/A	N/A
		702.19	7.94	46.20	31.41	N/A	N/A
	Day 4	0.00	N/S	N/S	N/S	N/A	N/A
		28.22	7.87	46.20	31.41	N/A	N/A
		51.58	7.85	46.10	31.34	N/A	N/A
		226.19	7.85	46.10	31.34	N/A	N/A
		404.37	7.81	46.10	31.34	N/A	N/A
		711.86	7.89	46.30	31.48	N/A	N/A
	Mean	-	7.89	46.19	31.40	6.90	9.65
	Std. Dev.	-	0.03	0.11	0.08	1.06	0.02
	n	-	22	22	22	4	2
	Maximum	-	7.94	46.40	31.55	7.90	9.66
	Minimum	-	7.81	46.00	31.55	5.80	9.63

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[a] CE-WAF = chemically enhanced water-accommodated fraction.

† IMS Ressurection Bay average seawater temperatures for the months of March-April 1998 plus individual measurments.

N/S = Not Sampled.

Water quality parameters for spiked exposure to test solutions of Alaska North Slope crude oil used on *M. bahia*.

Test Solution[a]	Loading Rate (mg/L)	pH	Conductivity Reading (mS)	Salinity (ppt)	Temp. (°C)	D.O. (mg/L)
Corexit 9500	0.00	8.11	28.50	20.67	23.00	8.60
	303.90	N/A	N/A	N/A	N/A	N/A
	714.35	N/A	N/A	N/A	N/A	N/A
	1093.20	7.99	28.50	20.67	25.00	7.60
	1561.90	7.86	28.30	20.51	25.00	7.50
	1899.30	7.84	28.30	20.51	25.00	7.40
WAF	0.00	8.12	32.00	23.51	25.00	6.80
	139.26	7.68	30.10	21.97	24.00	6.60
	250.51	7.75	29.40	21.40	24.00	6.60
	500.34	7.61	28.80	20.91	24.00	6.60
	752.51	7.50	28.10	20.35	24.00	6.60
	2497.86	7.65	28.80	20.91	25.00	6.40
CE-WAF	0.00	8.52	27.40	19.78	27.00	6.40
	26.26	8.43	26.90	19.37	25.00	6.00
	102.46	8.48	27.40	19.78	26.00	5.80
	248.29	8.40	27.00	19.46	26.00	5.60
	479.66	8.42	27.00	19.46	25.00	5.00
	702.80	8.35	27.10	19.54	26.00	4.80
Mean	-	8.08	28.33	20.54	25.08	6.10
Std. Dev.	-	0.40	1.57	1.27	1.00	0.67

[a] WAF = water-accommodated fraction; CE-WAF = chemically enhanced water-accommodated fraction.

Water quality parameters for continuous exposure dispersant only solutions of Corexit 9500 used on *M. bahia*.

Test Solution		Loading Rate (mg/L)	pH	Conductivity Reading (mS)	Salinity (ppt)	Temp. (°C)	D.O. (mg/L)
Corexit 9500	Day 1	0.00	8.37	29.10	21.16	25.00	7.50
		13.95	8.29	28.90	21.00	25.00	7.60
		29.40	8.35	28.80	20.91	25.00	7.60
		44.90	8.36	28.90	21.00	25.00	7.40
		59.75	8.35	28.40	20.59	25.00	7.40
		80.60	8.34	29.00	21.08	25.00	7.40
	Day 2	0.00	8.50	29.30	21.32	25.00	7.20
		13.30	8.55	29.10	21.16	25.00	7.20
		29.45	8.58	29.40	21.40	25.00	7.30
		46.10	8.58	29.40	21.40	25.00	7.20
		58.85	8.55	29.10	21.16	25.00	7.20
		81.10	8.54	29.00	21.08	25.00	7.20
	Day 3	0.00	8.46	29.50	21.48	25.00	7.20
		18.00	8.54	29.10	21.16	25.00	7.20
		31.05	8.51	29.10	21.16	25.00	7.20
		46.10	8.52	29.20	21.24	24.00	7.20
		**	**	**	**	**	**
		**	**	**	**	**	**
	Day 4	0.00	8.49	28.30	20.51	26.00	6.90
		15.80	8.50	28.00	20.27	26.00	7.00
		29.50	8.49	28.40	20.59	25.00	7.10
		44.55	8.48	28.70	20.83	25.00	7.40
		**	**	**	**	**	**
		**	**	**	**	**	**
	Mean	-	8.47	28.94	21.02	25.05	7.27
	Std. Dev.	-	0.09	0.40	0.32	0.39	0.18

** Not sampled because of complete die off in this concentration.

Water quality parameters for continuous exposure WAF test solutions of Alaska North Slope crude oil used on *M. bahia*.

Test Solution		Loading Rate (mg/L)	pH	Conductivity Reading (mS)	Salinity (ppt)	Temp. (°C)	D.O. (mg/L)
WAF[a]	Day 1	0.00	8.12	32.00	23.51	25.00	6.80
		25.66	7.78	29.60	21.56	24.00	6.60
		139.26	7.68	30.10	21.97	24.00	6.60
		250.51	7.75	29.40	21.40	24.00	6.60
		500.34	7.61	28.80	20.91	24.00	6.60
		752.51	7.50	28.10	20.35	24.00	6.60
	Day 2	0.00	8.13	27.60	19.94	24.00	6.80
		25.43	8.29	29.50	21.48	24.00	6.90
		99.94	8.21	29.60	21.56	24.00	6.80
		251.89	8.24	29.80	21.73	23.00	7.00
		499.23	8.07	28.10	20.35	23.00	7.00
		750.03	8.64	26.20	18.81	24.00	7.00
	Day 3	0.00	8.15	27.90	20.18	26.00	7.00
		21.77	8.07	24.80	17.67	24.00	7.20
		104.97	8.09	25.10	17.91	24.00	7.20
		250.54	8.05	24.80	17.67	24.00	7.20
		500.09	8.03	25.10	17.91	24.00	
		**	**	**	**	**	**
	Day 4	0.00	8.02	28.90	21.00	29.00	6.60
		25.80	7.97	27.40	19.78	26.00	7.00
		99.43	8.08	26.50	19.05	26.00	7.20
		249.83	8.02	26.90	19.37	26.00	7.20
		**	**	**	**	**	**
		**	**	**	**	**	**
	Mean	-	8.02	27.91	20.20	24.57	6.90
	Std. Dev.	-	0.25	1.99	1.61	1.36	0.24

[a] WAF = water-accommodated fraction.

** Not sampled because of complete die off in this concentration.

Water quality parameters for continuous exposure CE-WAF solutions of Alaska North Slope crude oil used on *M. bahia*.

Test Solution		Loading Rate (mg/L)	pH	Conductivity Reading (mS)	Salinity (ppt)	Temp. (°C)	D.O. (mg/L)
CE-WAF[a]	Day 1	0.00	8.52	27.40	19.78	27.00	6.40
		10.09	8.65	26.80	19.29	26.00	6.00
		26.26	8.43	26.90	19.37	25.00	6.00
		102.46	8.48	27.40	19.78	26.00	5.80
		248.29	8.40	27.00	19.46	26.00	5.60
		479.66	8.42	27.00	19.46	25.00	5.00
	Day 2	0.00	8.30	27.20	19.62	27.00	7.00
		5.89	8.48	27.50	19.86	26.00	6.80
		26.86	8.59	27.20	19.62	26.00	6.30
		99.34	8.54	28.00	20.27	26.00	6.40
		**	**	**	**	**	**
		**	**	**	**	**	**
	Day 3	0.00	8.28	27.70	20.02	28.50	6.40
		10.00	8.29	26.90	19.37	26.00	6.80
		**	**	**	**	**	**
		**	**	**	**	**	**
		**	**	**	**	**	**
		**	**	**	**	**	**
	Day 4	0.00	8.31	27.90	20.18	28.00	6.50
		5.91	8.39	28.10	20.35	26.00	6.70
		23.54	8.40	28.00	20.27	26.00	6.70
		**	**	**	**	**	**
		**	**	**	**	**	**
		**	**	**	**	**	**
	Mean	-	8.43	27.40	19.78	26.30	6.29
	Std. Dev.	-	0.11	0.45	0.36	0.96	0.53

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[a] CE-WAF = chemically enhanced water-accommodated fraction.

** Not sampled because of complete die off in this concentration.

Water quality parameters for spiked exposure to test solutions of Alaska North Slope crude oil on *M. beryllina*.

Test Solution[a]	Loading Rate (mg/L)	pH	Conductivity Reading (mS)	Salinity (ppt)	Temp. (°C)	D.O. (mg/L)
Corexit 9500	0.00	8.53	28.80	20.91	26.00	6.90
	19.60	8.16	29.90	21.81	27.00	6.60
	40.00	8.12	29.40	21.40	27.00	6.60
	68.45	N/A	N/A	N/A	27.00	6.60
	101.10	8.10	28.90	21.00	27.00	6.60
	120.30	N/A	N/A	N/A	N/A	N/A
WAF	0.00	8.12	28.00	20.27	26.00	6.90
	499.51	8.19	27.10	19.54	24.00	6.60
	1952.14	7.88	29.40	21.40	22.00	6.20
	3023.80	8.25	27.30	19.70	22.00	6.70
	3996.23	8.23	27.40	19.78	22.00	6.30
	6001.71	7.88	27.40	19.78	22.00	6.50
CE-WAF	0.00	8.34	31.70	23.26	24.00	6.80
	148.40	8.34	32.80	24.16	24.00	7.10
	250.06	8.40	32.90	24.24	22.00	7.00
	298.83	8.38	32.70	24.08	23.00	7.10
	356.06	8.36	32.90	24.24	22.00	6.90
	400.14	8.34	32.80	24.16	23.00	6.80
Mean	-	8.23	30.20	22.05	23.00	6.74
Std. Dev.	-	0.18	2.62	2.13	1.28	0.29

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[a] WAF = water-accommodated fraction; CE-WAF = chemically enhanced water-accommodated fraction.

Water quality parameters for continuous exposure dispersant only solutions of Corexit 9500 used on *M. beryllina*.

Test Solution		Loading Rate (mg/L)	pH	Conductivity Reading (mS)	Salinity (ppt)	Temp. (°C)	D.O. (mg/L)
Corexit 9500	Day 1	0.00	8.53	28.80	20.91	26.00	6.90
		19.60	8.16	29.90	21.81	27.00	6.60
		40.00	8.12	29.40	21.40	27.00	6.60
		68.45	N/A	N/A	N/A	27.00	6.60
		101.10	8.10	28.90	21.00	27.00	6.60
		117.20	8.30	28.60	20.75	27.00	6.60
	Day 2	0.00	8.64	29.80	21.73	24.00	7.20
		19.90	8.60	29.80	21.73	24.00	6.40
		39.70	8.56	29.80	21.73	24.00	6.20
		69.75	8.61	29.70	21.64	24.50	6.40
		**	**	**	**	**	**
		**	**	**	**	**	**
	Day 3	0.00	8.70	28.20	20.43	27.50	6.60
		19.30	8.66	29.20	21.24	27.00	6.60
		40.10	8.66	29.30	21.32	27.00	6.60
		70.10	8.68	29.20	21.24	27.00	6.50
		**	**	**	**	**	**
		**	**	**	**	**	**
	Day 4	0.00	8.57	30.20	22.05	26.00	7.70
		20.15	8.53	29.70	21.64	26.00	7.30
		41.35	8.58	29.80	21.73	26.00	7.30
		69.35	8.54	29.60	21.56	26.00	7.30
		**	**	**	**	**	**
		**	**	**	**	**	**
	Mean	-	8.50	29.41	21.41	26.11	6.78
	Std. Dev.	-	0.20	0.53	0.43	1.20	0.41

** Not sampled because of complete die off in this concentration.

Water quality parameters for continuous exposure WAF test solutions of Alaska North Slope crude oil used on *M. beryllina*.

Test Solution		Loading Rate (mg/L)	pH	Conductivity Reading (mS)	Salinity (ppt)	Temp. (°C)	D.O. (mg/L)
WAF[a]	Day 1	0.00	8.12	28.00	20.27	26.00	6.90
		255.83	8.25	27.30	19.70	24.00	6.80
		1034.11	8.25	27.40	19.78	23.00	6.60
		1952.14	7.88	29.40	21.40	22.00	6.20
		3023.80	8.25	27.30	19.70	22.00	6.70
		3996.23	8.23	27.40	19.78	22.00	6.30
	Day 2	0.00	8.12	29.10	21.16	25.50	6.70
		269.34	8.04	27.50	19.86	26.00	6.60
		1004.86	8.12	27.80	20.10	26.00	6.60
		1980.80	8.19	28.30	20.51	27.00	6.40
		2954.74	8.16	28.40	20.59	27.00	6.40
		4058.74	8.24	28.60	20.75	26.00	6.20
	Day 3	0.00	8.12	28.50	20.67	27.00	6.40
		279.51	8.04	27.90	20.18	27.00	6.20
		986.26	8.01	28.60	20.75	26.00	6.60
		1953.86	8.06	28.50	20.67	26.00	6.60
		**	**	**	**	**	**
		3948.20	8.12	28.60	20.75	27.00	6.60
	Day 4	0.00	8.10	28.20	20.43	27.00	6.70
		250.43	8.23	28.20	20.43	27.00	6.40
		1008.31	8.26	29.00	21.08	27.00	6.10
		1950.34	8.24	27.90	20.18	27.00	6.20
		**	**	**	**	**	**
		4015.71	8.21	28.20	20.43	27.00	6.00
	Mean	-	8.15	28.19	20.42	25.66	6.46
	Std. Dev.	-	0.10	0.59	0.48	1.81	0.24

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[a] WAF = water-accommodated fraction.

** Not sampled because of complete die off in this concentration.

Water quality parameters for continuous exposure CE-WAF solutions of Alaska North Slope crude oil used on *M. beryllina*.

Test Solution		Loading Rate (mg/L)	pH	Conductivity Reading (mS)	Salinity (ppt)	Temp. (°C)	D.O. (mg/L)
CE-WAF[a]	Day 1	0.00	8.34	31.70	23.26	24.00	6.80
		98.31	8.37	32.60	24.00	24.00	7.40
		148.40	8.34	32.80	24.16	24.00	7.10
		199.26	8.31	32.90	24.24	24.00	7.20
		250.06	8.40	32.90	24.24	22.00	7.00
		298.83	8.38	32.70	24.08	23.00	7.10
	Day 2	0.00	7.94	28.80	20.91	27.00	6.80
		97.89	8.17	29.80	21.73	28.00	6.20
		149.23	8.28	29.80	21.73	27.00	6.20
		201.89	8.35	29.80	21.73	28.00	6.00
		248.06	8.34	29.70	21.64	28.00	8.30
		**	**	**	**	**	**
	Day 3	0.00	7.99	28.70	20.83	26.00	7.20
		100.57	8.01	28.90	21.00	27.00	7.10
		151.46	8.10	29.40	21.40	27.00	6.80
		198.54	8.15	29.60	21.56	25.00	6.70
		250.20	8.08	29.40	21.40	27.00	6.60
		**	**	**	**	**	**
	Day 4	0.00	7.86	27.60	19.94	28.50	6.70
		107.09	7.95	28.60	20.75	27.00	6.70
		152.83	8.00	28.80	20.91	26.50	6.60
		202.89	8.04	28.50	20.67	26.00	6.50
		247.51	7.88	28.80	20.91	26.00	6.60
		**	**	**	**	**	**
	Mean	-	8.16	30.09	21.96	25.95	6.84
	Std. Dev.	-	0.18	1.73	1.40	1.82	0.49

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[a] CE-WAF = chemically enhanced water-accommodated fraction.

** Not sampled because of complete die off in this concentration.

Water quality parameters for spiked exposure to test solutions of Prudhoe Bay crude oil on *M. beryllina*.

Test Solution[a]	Loading Rate (mg/L)	pH	Conductivity Reading (mS)	Salinity (ppt)	Temp. (°C)	D.O. (mg/L)
WAF	0.00	7.77	28.50	20.67	25.00	6.90
	990.03	7.73	27.70	20.02	25.00	6.80
	1994.26	7.76	27.70	20.02	25.00	6.70
	2997.70	7.81	27.70	20.02	25.00	6.60
	5994.89	7.76	27.70	20.02	25.00	6.60
	8151.74	7.77	27.70	20.02	25.00	6.20
CE-WAF	0.00	8.13	28.20	20.43	27.00	7.50
	198.43	8.00	28.40	20.59	24.00	6.90
	302.66	7.92	28.20	20.43	27.00	6.60
	417.54	7.58	28.00	20.27	22.00	5.80
	599.91	7.80	27.90	20.18	26.00	6.60
	817.60	7.44	28.30	20.51	26.00	6.30
Mean	-	7.79	28.00	20.26	25.17	6.63
Std. Dev.	-	0.18	0.31	0.25	1.34	0.42
n	-	12	12	12	12	12
Maximum	-	8.13	28.50	20.67	27.00	7.50
Minimum	-	7.44	27.70	20.02	22.00	5.80

[a] WAF = water-accommodated fraction; CE-WAF = chemically enhanced water-accommodated fraction.

Water quality parameters for continuous exposure WAF solutions of Prudhoe Bay crude oil on *M. beryllina*.

Test Solution		Loading Rate (mg/L)	pH	Conductivity Reading (mS)	Salinity (ppt)	Temp. (°C)	D.O. (mg/L)
WAF[a]	Day 1	0.00	7.77	28.50	20.67	25.00	6.90
		496.60	7.80	27.70	20.02	25.00	6.90
		990.03	7.73	27.70	20.02	25.00	6.80
		1994.26	7.76	27.70	20.02	25.00	6.70
		2997.70	7.81	27.70	20.02	25.00	6.60
		5994.89	7.76	27.70	20.02	25.00	6.60
	Day 2	0.00	7.83	28.10	20.35	26.00	7.00
		503.31	7.83	28.10	20.35	25.00	6.90
		1009.37	7.83	28.10	20.35	25.00	6.60
		1990.34	7.81	28.20	20.43	25.00	6.60
		2997.97	7.80	28.10	20.35	25.00	6.90
		5986.40	7.90	28.10	20.35	25.00	6.60
	Day 3	0.00	7.67	28.10	20.35	25.00	6.80
		495.80	7.66	28.10	20.35	25.00	6.80
		991.51	7.71	28.00	20.27	25.00	6.80
		2004.60	7.72	28.10	20.35	25.00	7.00
		3013.68	7.71	28.00	20.27	25.00	6.80
		6078.97	7.74	28.10	20.35	25.00	6.80
	Day 4	0.00	7.62	28.80	20.91	26.00	6.90
		498.97	7.80	28.40	20.59	25.00	6.80
		1025.74	7.75	28.40	20.59	25.00	7.10
		1998.00	7.79	28.40	20.59	25.00	6.80
		3034.83	7.79	28.40	20.59	25.00	6.70
		6155.14	7.77	28.40	20.59	25.00	6.60
	Mean	-	7.77	28.12	20.36	25.08	6.79
	Std. Dev.	-	0.06	0.29	0.23	0.28	0.14
	n	-	24	24	24	24	24
	Maximum	-	7.90	28.80	20.91	26.00	7.10
	Minimum	-	7.62	27.70	20.02	25.00	6.60

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[a] WAF = water-accommodated fraction..

Water quality parameters for continuous exposure CE-WAF solutions of Prudhoe Bay crude oil on *M. beryllina*.

Test Solution		Loading Rate (mg/L)	pH	Conductivity Reading (mS)	Salinity (ppt)	Temp. (°C)	D.O. (mg/L)
CE-WAF[a]	Day 1	0.00	8.13	28.20	20.43	27.00	7.50
		99.74	8.01	28.40	20.59	24.00	7.00
		198.43	8.00	28.40	20.59	24.00	6.90
		257.94	7.95	28.30	20.51	24.00	6.60
		302.66	7.92	28.20	20.43	27.00	6.60
		417.54	7.58	28.00	20.27	22.00	5.80
	Day 2	0.00	N/A	28.50	20.67	27.00	N/A
		103.09	7.83	28.50	20.67	27.00	6.90
		200.91	7.84	28.50	20.67	27.00	7.20
		**	**	**	**	**	**
		**	**	**	**	**	**
		**	**	**	**	**	**
	Day 3	0.00	7.94	28.20	20.43	28.00	6.60
		102.71	7.76	28.90	21.00	27.00	6.60
		197.77	7.59	28.50	20.67	26.00	6.60
		**	**	**	**	**	**
		**	**	**	**	**	**
		**	**	**	**	**	**
	Day 4	0.00	7.60	29.30	21.32	28.00	6.90
		99.97	7.75	29.20	21.24	27.00	6.80
		197.94	7.93	29.00	21.08	26.50	6.80
		**	**	**	**	**	**
		**	**	**	**	**	**
		**	**	**	**	**	**
	Mean	-	7.85	28.54	20.70	26.10	6.77
	Std. Dev.	-	0.17	0.39	0.31	1.75	0.38
	n	-	14	15	15	15	14
	Maximum	-	8.13	29.30	21.32	28.00	7.50
	Minimum	-	7.58	28.00	20.27	22.00	5.80

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[a] CE-WAF = chemically enhanced water-accommodated fraction.

** Not sampled because of complete die off in this concentration.

APPENDIX D

FRESH OIL AND DISPERSANT STUDY TOXICITY DAILY OBSERVATION DATA

Menidia beryllina
Dispersant Spiked Exposure
Start Date of Test: 6-Jul-98

Test Chamber	Design Concentration (mg/L)	Nominal Conc. (N.C.) (mg/L)	End of Day 1 Number of Animals Alive/Total	End of Day 2 Number of Animals Alive/Total	End of Day 3 Number of Animals Alive/Total	End of Day 4 Number of Animals Alive/Total
1a	0	0.00	5/5	5/5	5/5	5/5
1b			5/5	5/5	5/5	5/5
1c			5/5	5/5	5/5	5/5
2a	20	19.60	5/5	5/5	5/5	5/5
2b			5/5	LOST[1]	LOST[1]	LOST[1]
2c			5/5	5/5	5/5	5/5
3a	40	40.00	5/5	5/5	5/5	5/5
3b			5/5	5/5	5/5	5/5
3c			4/5	4/5	4/5	4/5
4a	70	68.45	5/5	5/5	5/5	5/5
4b			4/5	4/5	4/5	4/5
4c			4/5	4/5	4/5	4/5
5a	100	101.10	4/5	4/5	4/5	4/5
5b			4/5	4/5	4/5	4/5
5c			4/5	4/5	4/5	4/5
6a	120	120.30	2/5	2/5	2/5	2/5
6b			2/5	2/5	2/5	2/5
6c			2/5	2/5	2/5	2/5
	LC_{50} (mg/L) based on N.C.		**129.11**	**129.38**	**129.38**	**129.38**
	95% CI		LL=100.35 UL=291.06	LL=100.38 UL=309.61	LL=100.38 UL=309.61	LL=100.38 UL=309.61
	(Analysis Method)		(Probit)	(Probit)	(Probit)	(Probit)

[1] Drained.
LL = lower limit
UL = upper limit

Menidia beryllina
Dispersant Continuous Exposure
Start Date of Test: 6-Jul-98

Test Chamber	Design Concentration (mg/L)	Mean Nominal Conc. (N.C.) (mg/L)	End of Day 1 Number of Animals Alive/Total	End of Day 2 Number of Animals Alive/Total	End of Day 3 Number of Animals Alive/Total	End of Day 4 Number of Animals Alive/Total
1a	0	0.00	5/5	5/5	5/5	5/5
1b			5/5	5/5	5/5	5/5
1c			5/5	5/5	5/5	5/5
2a	20	19.74	5/5	5/5	5/5	5/5
2b			5/5	5/5	5/5	5/5
2c			5/5	5/5	5/5	5/5
3a	40	40.29	5/6	5/6	5/6	5/6
3b			5/5	4/5	4/5	4/5
3c			5/5	4/5	4/5	4/5
4a	70	69.41	3/5	3/5	2/5	1/5
4b			2/5	2/5	2/5	1/5
4c			2/5	2/5	1/5	1/5
5a	100	101.10	0/5	0/5	0/5	0/5
5b			0/5	0/5	0/5	0/5
5c			0/5	0/5	0/5	0/5
6a	120	120.30	0/5	0/5	0/5	0/5
6b			0/5	0/5	0/5	0/5
6c			0/6	0/6	0/6	0/6
		LC_{50} (mg/L) based on N.C.	**62.62**	**58.69**	**55.69**	**52.67**
		95% CI	LL=53.77 UL=71.28	LL=49.02 UL=67.98	LL=46.63 UL=64.52	LL=44.44 UL=61.00
		(Analysis Method)	(Probit)	(Probit)	(Probit)	(Probit)

LL = lower limit
UL = upper limit

Menidia beryllina
WAF Spiked Exposure; ANSC Fresh Oil Study
Start Date of Test: 17-Aug-98

Test Chamber #	Design Concentration (mg/L)	Loading Rate (L.R.) (mg/L)	Measured VOA + TPH (mg/L)	End of Day 1 Number of Animals Alive/Total	End of Day 2 Number of Animals Alive/Total	End of Day 3 Number of Animals Alive/Total	End of Day 4 Number of Animals Alive/Total
1a	0	0.00	0.00	5/5	5/5	5/5	5/5
1b				5/5	5/5	5/5	5/5
1c				5/5	5/5	5/5	5/5
2a	500	499.51	11.15	5/5	5/5	5/5	5/5
2b				5/5	5/5	5/5	5/5
2c				5/5	5/5	5/5	5/5
3a	2000	1952.14	19.47	5/5	5/5	5/5	5/5
3b				6/6	6/6	6/6	6/6
3c				LOST[1]	LOST[1]	LOST[1]	LOST[1]
4a	3000	3023.80	24.21	4/5	4/5	4/5	4/5
4b				4/5	4/5	4/5	4/5
4c				5/5	5/5	5/5	5/5
5a	4000	3996.23	28.31	0/5	0/5	0/5	0/5
5b				0/5	0/5	0/5	0/5
5c				2/5	2/5	2/5	2/5
6a	6000	6001.71	33.05	2/5	2/5	2/5	2/5
6b				0/5	0/5	0/5	0/5
6c				2/6	2/6	2/6	2/6
		LC_{50} (mg/L) based on L.R.		**3519.80**	**3519.80**	**3519.80**	**3519.80**
		95% CI		LL=3325.77 UL=3725.16	LL=3325.77 UL=3725.16	LL=3325.77 UL=3725.16	LL=3325.77 UL=3725.16
		(Analysis Method)		(TSK, a=19.35%)	(TSK, a=19.35%)	(TSK, a=19.35%)	(TSK, a=19.35%)

[1] Chambers' flow lapsed.
LL = lower limit
UL = upper limit

Menidia beryllina
WAF Continuous Exposure; ANSC Fresh Oil Study
Start Date of Test: 17-Aug-98

Test Chamber #	Design Concentration (mg/L)	Mean Loading Rate (L.R.) (mg/L)	Measured VOA + TPH (mg/L)	End of Day 1 Number of Animals Alive/Total	End of Day 2 Number of Animals Alive/Total	End of Day 3 Number of Animals Alive/Total	End of Day 4 Number of Animals Alive/Total
1a	0	0.00	0.00	5/5	5/5	5/5	5/5
1b				5/5	5/5	5/5	5/5
1c				5/5	5/5	5/5	5/5
2a	250	263.78	6.44	5/5	5/5	5/5	5/5
2b				5/5	5/5	5/5	5/5
2c				5/5	5/5	5/5	5/5
3a	1000	1008.39	16.88	5/5	5/5	5/5	5/5
3b				5/5	5/5	5/5	5/5
3c				5/5	5/5	4/5	3/5
4a	2000	1959.29	15.36	4/5	0/5	0/5	0/5
4b				6/6	3/6	3/6	3/6
4c				6/6	6/6	6/6	6/6
5a	3000	VOA: 2989.27 TPH: 2997.18	17.65	2/5	0/5	0/5	0/5
5b				4/5	0/5	0/5	0/5
5c				2/5	0/5	0/5	0/5
6a	4000	4004.72	26.40	0/5	0/5	0/5	0/5
6b				0/5	0/5	0/5	0/5
6c				5/5	4/5	4/5	0/5
		LC$_{50}$ (mg/L) based on L.R.		**3179.80**	**1969.77**	**1934.50**	**1640.72**
		95% CI		LL=2204.36 UL=4586.88	LL=1620.20 UL=2394.76	LL=1593.26 UL=2348.83	LL=1316.81 UL=2044.30
		(Analysis Method)		(TSK, a=33.33%)	(TSK, a=13.33%)	(TSK, a=13.33%)	(TSK, a=0%)

LL = lower limit
UL = upper limit

Menidia beryllina
CE-WAF Spiked Exposure; ANSC Fresh Oil Study
Start Date of Test: 12-Oct-98

Test Chamber #	Design Concentration (mg/L)	Loading Rate (L.R.) (mg/L)	Measured VOA + TPH (mg/L)	End of Day 1 Number of Animals Alive/Total	End of Day 2 Number of Animals Alive/Total	End of Day 3 Number of Animals Alive/Total	End of Day 4 Number of Animals Alive/Total
1a	0	0.00	0.00	5/5	5/5	5/5	5/5
1b				5/5	5/5	5/5	5/5
1c				5/5	5/5	5/5	5/5
2a	150	148.43	8.72	5/5	5/5	5/5	5/5
2b				5/5	5/5	5/5	5/5
2c				2/5	2/5	2/5	2/5
3a	250	250.06	12.26	4/5	4/5	4/5	4/5
3b				1/5	1/5	1/5	1/5
3c				1/5	1/5	1/5	1/5
4a	300	298.83	16.81	2/5	2/5	2/5	2/5
4b				2/5	2/5	2/5	2/5
4c				3/5	3/5	3/5	3/5
5a	350	356.06	34.62	3/5	3/5	3/5	3/5
5b				3/5	3/5	3/5	2/5
5c				LOST [1]	LOST [1]	LOST [1]	LOST [1]
6a	400	400.14	18.62	1/5	1/5	1/5	1/5
6b				3/5	2/5	2/5	2/5
6c				2/5	2/5	2/5	2/5
		LC$_{50}$ (mg/L) based on L.R.		**272.43**	**272.43**	**272.43**	**272.43**
		95% CI		LL=171.08 UL=425.15	LL=171.08 UL=425.15	LL=171.08 UL=425.15	LL=171.08 UL=425.15
		(Analysis Method)		(Probit)	(Probit)	(Probit)	(Probit)

[1] Drained.
LL = lower limit
UL = upper limit

Menidia beryllina
CE-WAF Continuous Exposure; ANSC Fresh Oil Study
Start Date of Test: 12-Oct-98

Test Chamber #	Design Concentration (mg/L)	Mean Loading Rate (L.R.) (mg/L)	Measured VOA + TPH (mg/L)	End of Day 1 Number of Animals Alive/Total	End of Day 2 Number of Animals Alive/Total	End of Day 3 Number of Animals Alive/Total	End of Day 4 Number of Animals Alive/Total
1a	0	0.00	0.00	5/5	5/5	5/5	5/5
1b				5/5	5/5	5/5	5/5
1c				5/5	5/5	5/5	5/5
2a	100	100.97	3.45	5/5	5/5	5/5	5/5
2b				5/5	5/5	5/5	5/5
2c				5/5	5/5	5/5	5/5
3a	150	150.48	7.73	5/5	5/5	5/5	5/5
3b				5/5	5/5	5/5	5/5
3c				5/5	5/5	5/5	5/5
4a	200	200.65	14.06	5/5	5/5	4/5	4/5
4b				5/5	5/5	4/5	4/5
4c				5/5	5/5	4/5	4/5
5a	250	248.96	11.94	4/5	4/5	2/5	2/5
5b				3/5	3/5	3/5	3/5
5c				3/5	1/5	0/5	0/5
6a	300	298.83	16.27	0/5	0/5	0/5	0/5
6b				0/5	0/5	0/5	0/5
6c				0/5	0/5	0/5	0/5
		LC$_{50}$ (mg/L) based on L.R.		**255.24**	**248.55**	**227.12**	**227.12**
			95% CI	LL=243.16	LL=236.12	LL=211.53	LL=211.53
				UL=267.92	UL=261.64	UL=243.85	UL=243.85
			(Analysis Method)	(TSK, a=0%)	(TSK, a=0%)	(TSK, a=0%)	(TSK, a=0%)

LL = lower limit
UL = upper limit

Mysidopsis bahia
Dispersant Spiked Exposure
Start Date of Test: 23-Jun-98

Test Chamber	Design Concentration (mg/L)	Nominal Conc. (N.C.) (mg/L)	End of Day 1 Number of Animals Alive/Total	End of Day 2 Number of Animals Alive/Total	End of Day 3 Number of Animals Alive/Total	End of Day 4 Number of Animals Alive/Total
1a	0	0	5/5	4/5	4/5	4/5
1b			4/5	4/5	4/5	4/5
1c			5/5	5/5	5/5	5/5
2a	300	303.9	3/5	3/5	3/5	3/5
2b			3/5	3/5	1/5	1/5
2c			2/5	2/5	2/5	2/5
3a	700	714.35	1/5	1/5	1/5	1/5
3b			1/5	1/5	1/5	1/5
3c			4/5	4/5	4/5	4/5
4a	1100	1093.2	1/5	0/5	0/5	0/5
4b			0/5	0/5	0/5	0/5
4c			0/5	0/5	0/5	0/5
5a	1500	1561.9	0/5	0/5	0/5	0/5
5b			0/5	0/5	0/5	0/5
5c			0/5	0/5	0/5	0/5
6a	1900	1899.3	0/5	0/5	0/5	0/5
6b			0/5	0/5	0/5	0/5
6c			0/5	0/5	0/5	0/5
		LC_{50} (mg/L) based on N.C.	**465.93**	**543.65**	**98.00**	**98.00**
		95% CI	LL=157.06 UL=1373.51	LL=264.68 UL=1116.65	(N/A)	(N/A)
		(Analysis Method)	(TSK, a=42.86%)	(TSK, a=38.46%)	(Graphical)	(Graphical)

LL = lower limit
UL = upper limit

Mysidopsis bahia
Dispersant Continuous Exposure
Start Date of Test: 23-Jun-98

Test Chamber	Design Concentration (mg/L)	Mean Nominal Conc. (N.C.) (mg/L)	End of Day 1 Number of Animals Alive/Total	End of Day 2 Number of Animals Alive/Total	End of Day 3 Number of Animals Alive/Total	End of Day 4 Number of Animals Alive/Total
1a	0	0.00	4/5	4/5	4/5	4/5
1b			5/5	5/5	5/5	5/5
1c			5/5	5/5	5/5	5/5
2a	15	15.26	5/5	5/5	LOST[1]	LOST[1]
2b			5/5	4/5	4/5	4/5
2c			5/5	5/5	5/5	5/5
3a	30	29.85	3/5	2/5	2/5	2/5
3b			4/5	4/5	4/5	4/5
3c			3/5	2/5	2/5	2/5
4a	45	45.70	2/5	1/5	0/5	0/5
4b			2/5	0/5	0/5	0/5
4c			5/5	2/5	0/5	0/5
5a	60	59.30	0/5	0/5	0/5	0/5
5b			0/5	0/5	0/5	0/5
5c			0/5	0/5	0/5	0/5
6a	80	80.85	0/5	0/5	0/5	0/5
6b			0/5	0/5	0/5	0/5
6c			0/5	0/5	0/5	0/5
		LC_{50} (mg/L) based on N.C.	**38.55**	**31.43**	**29.06**	**29.06**
		95% CI	LL=32.96 UL=45.09	LL=26.84 UL=36.80	LL=24.85 UL=33.99	LL=24.85 UL=33.99
		(Analysis Method)	(TSK, a=0%)	(TSK, a=0%)	(TSK, a=3.57%)	(TSK, a=3.57%)

[1] Animal fatality likely resulting from mysterious contaminant on glassware.
LL = lower limit
UL = upper limit

Mysidopsis bahia
WAF Spiked Exposure; ANSC Fresh Oil Study
Start Date of Test: 3-Aug-98

Test Chamber #	Design Concentration (mg/L)	Loading Rate (L.R.) (mg/L)	Measured VOA + TPH (mg/L)	End of Day 1 Number of Animals Alive/Total	End of Day 2 Number of Animals Alive/Total	End of Day 3 Number of Animals Alive/Total	End of Day 4 Number of Animals Alive/Total
1a	0	0.00	0.00	5/5	5/5	5/5	5/5
1b				5/5	5/5	5/5	5/5
1c				5/5	5/5	5/5	5/5
2a	100	139.26	3.14	5/5	5/5	5/5	5/5
2b				5/5	5/5	5/5	5/5
2c				5/5	5/5	5/5	5/5
3a	250	250.51	4.77	4/5	4/5	4/5	4/5
3b				5/5	5/5	5/5	5/5
3c				5/6	LOST[1]	LOST[1]	LOST[1]
4a	500	500.34	8.83	2/6	1/6	1/6	1/6
4b				4/5	4/5	4/5	4/5
4c				3/5	LOST[1]	LOST[1]	LOST[1]
5a	750	752.51	8.59	4/5	3/5	3/5	3/5
5b				2/5	2/5	2/5	2/5
5c				3/5	3/5	3/5	3/5
6a	2500	2497.86	12.73	0/5	0/5	0/5	0/5
6b				0/5	0/5	0/5	0/5
6c				0/5	0/5	0/5	0/5
		LC_{50} (mg/L) based on L.R.		**717.38**	**653.54**	**653.54**	**653.54**
		95% CI		LL=549.00 UL=937.40	LL=488.01 UL=875.22	LL=488.01 UL=875.22	LL=488.01 UL=875.22
		(Analysis Method)		(TSK, a=0%)	(TSK, a=0%)	(TSK, a=0%)	(TSK, a=0%)

[1] Chambers' flow lapsed.
LL = lower limit
UL = upper limit

Mysidopsis bahia
WAF Continuous Exposure; ANSC Fresh Oil Study
Start Date of Test: 3-Aug-98

Test Chamber #	Design Concentration (mg/L)	Mean Loading Rate (L.R.) (mg/L)	Measured VOA + TPH (mg/L)	End of Day 1 Number of Animals Alive/Total	End of Day 2 Number of Animals Alive/Total	End of Day 3 Number of Animals Alive/Total	End of Day 4 Number of Animals Alive/Total
1a	0	0.00	0.00	5/5	5/5	5/5	5/5
1b				5/5	5/5	5/5	5/5
1c				5/5	5/5	4/5	4/5
2a	25	24.67	0.91	5/5	5/5	4/5	4/5
2b				3/5	3/5	3/5	2/5
2c				3/5	3/5	3/5	3/5
3a	100	110.90	2.00	5/5	5/5	5/5	5/5
3b				3/5	3/5	2/5	2/5
3c				4/5	4/5	4/5	3/5
4a	250	250.69	3.60	1/5	1/5	0/5	0/5
4b				2/5	2/5	2/5	1/5
4c				0/5	0/5	0/5	0/5
5a	500	499.89	5.64	2/6	2/6	1/6	0/6
5b				0/5	0/5	0/5	0/5
5c				0/5	0/5	0/5	0/5
6a	750	751.27	5.61	1/5	1/5	0/5	0/5
6b				0/5	0/5	0/5	0/5
6c				1/5	1/5	0/5	0/5
		LC$_{50}$ (mg/L) based on L.R.		**209***	**209***	**178.50**	**160.48**
		95% CI		LL=77 UL=320	LL=77 UL=320	LL=92.84 UL=248.10	LL=62.77 UL=216.66
		(Analysis Method)		(Probit)	(Probit)	(Probit)	(Probit)

*Note: Calculation made using control mortality from Days 3 and 4.
LL = lower limit
UL = upper limit

Mysidopsis bahia
CE-WAF Spiked Exposure; ANSC Fresh Oil Study
Start Date of Test: 31-Aug-98

Test Chamber #	Design Concentration (mg/L)	Loading Rate (L.R.) (mg/L)	Measured VOA + TPH (mg/L)	End of Day 1 Number of Animals Alive/Total	End of Day 2 Number of Animals Alive/Total	End of Day 3 Number of Animals Alive/Total	End of Day 4 Number of Animals Alive/Total
1a	0	0.00	0.00	5/5	5/5	5/5	5/5
1b				LOST[1]	LOST[1]	LOST[1]	LOST[1]
1c				5/5	5/5	5/5	5/5
2a	25	26.26	0.22	5/5	5/5	5/5	5/5
2b				5/5	5/5	5/5	5/5
2c				5/5	5/5	5/5	5/5
3a	100	102.46	2.73	5/5	5/5	5/5	5/5
3b				4/5	4/5	4/5	4/5
3c				3/5	3/5	3/5	3/5
4a	250	248.29	25.05	0/5	0/5	0/5	0/5
4b				0/5	0/5	0/5	0/5
4c				0/5	0/5	0/5	0/5
5a	500	479.66	24.24	0/5	0/5	0/5	0/5
5b				0/5	0/5	0/5	0/5
5c				0/5	0/5	0/5	0/5
6a	750	702.80	31.62	0/5	0/5	0/5	0/5
6b				0/5	0/5	0/5	0/5
6c				0/5	0/5	0/5	0/5
		LC$_{50}$ (mg/L) based on L.R.		**127.41**	**127.41**	**127.41**	**127.41**
		95% CI		LL=101.02	LL=101.02	LL=101.02	LL=101.02
				UL=160.68	UL=160.68	UL=160.68	UL=160.68
		(Analysis Method)		(TSK, a=0%)	(TSK, a=0%)	(TSK, a=0%)	(TSK, a=0%)

[1] Broken chamber.
LL = lower limit
UL = upper limit

Mysidopsis bahia
CE-WAF Continuous Exposure; ANSC Fresh Oil Study
Start Date of Test: 31-Aug-98

Test Chamber #	Design Concentration (mg/L)	Mean Loading Rate (L.R.) (mg/L)	Measured VOA + TPH (mg/L)	End of Day 1 Number of Animals Alive/Total	End of Day 2 Number of Animals Alive/Total	End of Day 3 Number of Animals Alive/Total	End of Day 4 Number of Animals Alive/Total
1a	0	0.00	0.00	5/5	5/5	5/5	5/5
1b				6/6	6/6	6/6	6/6
1c				5/5	5/5	5/5	5/5
2a	10	7.97	0.45	6/6	6/6	6/6	6/6
2b				5/5	5/5	5/5	5/5
2c				5/5	5/5	5/5	5/5
3a	25	26.19	1.21	6/6	6/6	6/6	6/6
3b				4/5	2/5	2/5	2/5
3c				4/5	3/5	3/5	1/5
4a	100	104.17	5.00	5/5	0/5	0/5	0/5
4b				2/5	0/5	0/5	0/5
4c				5/5	0/5	0/5	0/5
5a	250	249.71	25.55	0/5	0/5	0/5	0/5
5b				0/5	0/5	0/5	0/5
5c				0/5	0/5	0/5	0/5
6a	500	489.35	23.89	0/5	0/5	0/5	0/5
6b				0/5	0/5	0/5	0/5
6c				0/5	0/5	0/5	0/5
		LC$_{50}$ (mg/L) based on L.R.		**109.62**	**34.96**	**34.96**	**29.77**
		95% CI		LL=79.98 UL=150.25	LL=25.95 UL=47.08	LL=25.95 UL=47.08	LL=21.64 UL=40.94
		(Analysis Method)		(TSK, a=0%)	(TSK, a=0%)	(TSK, a=0%)	(TSK, a=0%)

LL = lower limit
UL = upper limit

APPENDIX E

1998 *C. bairdi* DISPERSANT ONLY DISCARDED TESTS

Tanner Crab Larvae (*Chionocetes bairdi*)
Dispersant Spiked Exposure
Start Date of Test: 26-Mar-98

			EC$_{50}$ Test				
Test Chamber #	Design Conc. (mg/L)	Nominal Conc. (mg/L)	Number of Animals Alive/Total	Individual Percent Mortality	Combined Percent Mortality	Standard Error	EC$_{50}$ (mg/L) 95% CI (Analysis mthd.)
1a 1b 1c	0	0.00	12/12 8/12 8/14	0.00 33.33 42.86	25.40	12.99	**1042.33** LL=604.46 UL=1797.41 (TSK, a=32.57)
2a 2b 2c	200	200.03	9/9 8/9 0/10	0.00 11.11 100.00	37.04	31.64	
3a 3b 3c	800	803.07	0/11 7/8 0/12	100.00 12.50 100.00	70.83	29.17	
4a 4b 4c	1400	1440.22	12/12 0/5 LOST [2]	0.00 100.00 LOST [2]	50.00	50.00	
5a 5b 5c	2000	2005.18	0/10 0/10 3/10	100.00 100.00 70.00	90.00	10.00	
6a 6b 6c	2600	2600.81	9/10 0/10 LOST [2]	10.00 100.00 LOST [2]	55.00	45.00	

[1] Sample bottle broke.

[2] Drained.

* EC50 is greater than highest concentration.

<u>Defined responses:</u> <u>Alive, Affected, Mortally Affected, Dead</u>
EC$_{50}$ response: Affected

LL = lower limit
UL = upper limit

Tanner Crab Larvae (*Chionocetes bairdi*)
Dispersant Continuous Exposure
Start Date of Test: 27-Mar-98

Test Chamber #	Design Conc. (mg/L)	Mean Nominal Conc. (mg/L)	EC_{50} Test Number of Animals Alive/Total	Individual Percent Mortality	Combined Percent Mortality	Standard Error	EC_{50} (mg/L) 95% CI (Analysis mthd.)
1a	0	0.00	7/10	30.00	30.20	1.75	**15.24**
1b			6/9	33.33			LL=4.42
1c			8/11	27.27			UL=23.95
							(Probit)
2a	20	21.39	2/10	80.00	78.18	13.15	
2b			5/11	54.55			
2c			0/12	100.00			
3a	50	53.44	0/11	100.00	90.00	10.00	
3b			3/10	70.00			
3c			0/12	100.00			
4a	80	83.55	0/10	100.00	100.00	0.00	
4b			0/12	100.00			
4c			0/11	100.00			
5a	110	112.63	0/10	100.00	100.00	0.00	
5b			0/10	100.00			
5c			0/10	100.00			
6a	130	130.97	0/10	100.00	100.00	0.00	
6b			0/10	100.00			
6c			0/10	100.00			

* EC50 is greater than highest concentration.

Defined responses: Alive, Affected, Mortally Affected, Dead
EC_{50} response: Affected

LL = lower limit
UL = upper limit

Measured concentrations for 1998 Corexit 9500 test solutions.

C. bairdi spiked exposure

Loading Rate (mg/L)	UV (mg/L)	TOC (mg/L)
0.00	0.00	N/S
200.03	222.00	N/S
803.07	769.00	N/S
1440.22	N/S	N/S
2005.18	1986.00	N/S
2600.81	2689.00	N/S

C. bairdi continuous exposure

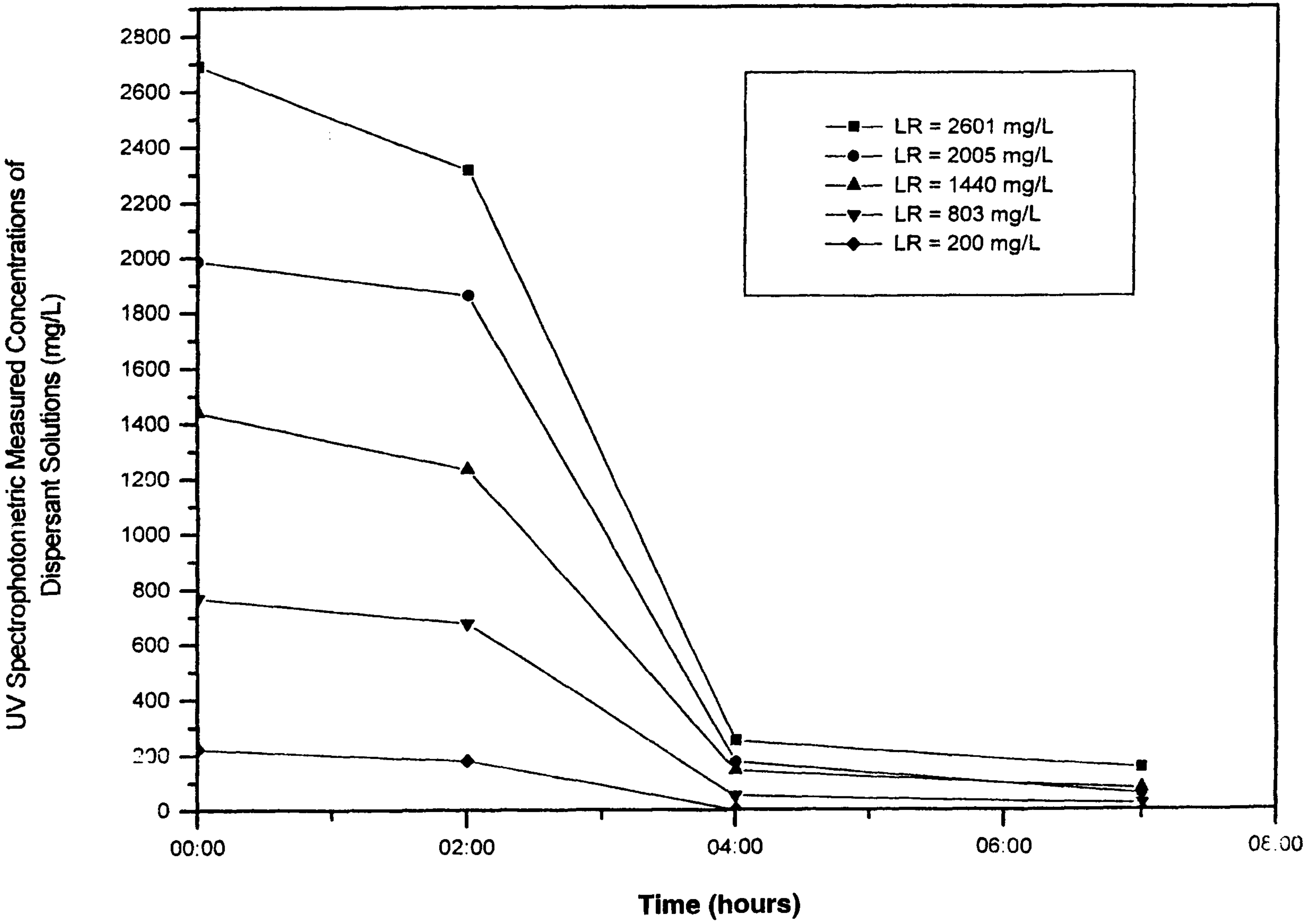
Day 1	0.00	0.00	N/S
	21.36	21.00	N/S
	52.83	54.70	N/S
	82.04	98.20	N/S
	111.16	136.20	N/S
	130.96	150.90	N/S
Day 2	0.00	N/S	N/S
	21.47	N/S	N/S
	55.38	N/S	N/S
	84.57	N/S	N/S
	114.31	N/S	N/S
	131.92	181.80	N/S
Day 3	0.00	0.00	N/S
	21.66	9.60	N/S
	52.72	31.80	N/S
	84.29	66.90	N/S
	114.69	106.10	N/S
	130.62	131.40	N/S
Day 4	0.00	0.00	N/S
	21.07	20.50	N/S
	52.84	62.40	N/S
	83.29	87.80	N/S
	110.35	133.60	N/S
	130.37	162.70	N/S

Declining, spiked concentrations of Corexit 9500 test solutions.

C. bairdi

Loading Rate (mg/L)	Hour	UV (mg/L)
200.00	0	222.00
	2	181.00
	4	3.20
	7	1.10
800.00	0	769.00
	2	676.00
	4	52.90
	7	24.80
1440.00	0	N/A
	2	1232.00
	4	144.30
	7	77.80
2000.00	0	1986.00
	2	1860.00
	4	176.00
	7	61.00
2600.00	0	2689.00
	2	2314.00
	4	252.00
	7	154.30

Concentration Decline of Corexit 9500 Test Solutions
for *Chionocetes bairdi* Spiked Exposure Test



Water quality parameters for continuous exposure dispersant only solutions of Corexit 9500 used on *C. bairdi.*

Test Solution		Loading Rate (mg/L)	pH	Conductivity Reading (mS)	Salinity (ppt)	Temp. (°C)	D.O. (mg/L)
Corexit 9500[a] Spiked Exposure		0.00	8.88	46.00	31.27	N/A	N/A
		200.03	8.85	44.80	30.42	N/A	N/A
		803.07	8.92	44.50	30.21	N/A	N/A
		1440.22	8.91	44.90	30.49	N/A	N/A
		2005.18	8.91	44.50	30.21	N/A	N/A
		2600.81	8.85	44.90	30.49	N/A	N/A
Continuous Exposure	Day 1	0.00	7.77	47.60	32.40	N/A	N/A
		21.36	7.91	45.40	30.84	N/A	N/A
		52.83	7.91	45.90	31.20	N/A	N/A
		82.04	7.93	44.20	30.00	N/A	N/A
		111.16	7.92	45.60	30.99	N/A	N/A
		130.96	7.89	45.30	30.77	N/A	N/A
	Day 2	0.00	8.23	45.10	30.63	N/A	N/A
		21.47	8.34	46.00	31.27	N/A	N/A
		55.38	8.40	45.40	30.84	N/A	N/A
		84.57	8.39	45.40	30.84	N/A	N/A
		114.31	8.41	46.00	31.27	N/A	N/A
		131.92	8.42	46.70	31.76	N/A	N/A
	Day 3	0.00	7.78	46.60	31.69	N/A	N/A
		21.66	7.82	46.20	31.41	N/A	N/A
		52.72	7.88	46.60	31.69	N/A	N/A
		84.29	7.90	46.60	31.69	N/A	N/A
		114.69	7.89	46.80	31.83	N/A	N/A
		130.62	7.91	46.80	31.83	N/A	N/A
	Day 4	0.00	7.78	45.90	31.20	N/A	N/A
		21.07	7.85	45.40	30.84	N/A	N/A
		52.84	7.91	45.30	30.77	N/A	N/A
		83.29	7.93	45.40	30.84	N/A	N/A
		110.35	7.95	45.10	30.63	N/A	N/A
		130.37	7.96	46.10	31.34	N/A	N/A
	Mean	-	8.25	45.74	31.09	N/A	N/A
	Std. Dev.	-	0.43	0.88	0.62	N/A	N/A
	n	-	24	24	24	N/A	N/A
	Maximum	-	8.92	47.60	32.40	N/A	N/A
	Minimum	-	7.77	44.20	30.00	N/A	N/A

[a] Measurments from Corexit 9500 test conducted in 1998

APPENDIX F

WEATHERED OIL STUDY TOXICITY DATA

Tanner Crab (*Chionoecetes bairdi*)
WAF Weathered ANS Crude Oil Spiked Exposure
Start Date of Test: 8-Apr-99

			Measured Concentrations			EC_{50} Test				
Test Chamber #	Design Conc. (mg/L)	Loading Rate (mg/L)	VOA (mg/L)	TPH (mg/L)	VOA + TPH (mg/L)	Number of Animals Alive/Total	Individual Percent Affected	Mean Percent Affected	Standard Error	EC_{50} (mg/L) 95% CI (Analysis mthd.)
1a	0	0.00	0.00	0.00	0.00	8/9	11.11	3.45	3.70	**0.40**
1b						10/10	0.00			LL=0.33
1c						10/10	0.00			UL=0.51 (Probit)
2a	500	496.26	0.10	0.12	0.22	8/10	20.00	17.24	2.96	
2b						8/9	11.11			
2c						8/10	20.00			
3a	2000	2002.83	0.21	0.08	0.28	8/10	20.00	43.33	14.53	
3b						6/10	40.00			
3c						3/10	70.00			
4a	5000	4983.86	0.29	0.12	0.41	5/10	50.00	53.33	3.33	
4b						5/10	50.00			
4c						4/10	60.00			
5a	7000	6993.25	0.29	0.16	0.45	4/10	60.00	66.67	6.67	
5b						4/10	60.00			
5c						2/10	80.00			
6a	10000	10011.26	0.32	0.13	0.45	5/10	50.00	46.67	3.33	
6b						6/10	40.00			
6c						5/10	50.00			

Defined responses: Alive, Affected, Mortally Affected, Dead
EC_{50} response: Affected

LL = lower limit
UL = upper limit

Tanner Crab (*Chionoecetes bairdi*)
WAF Weathered ANS Crude Oil Continuous Exposure
Start Date of Test: 8-Apr-99

			Measured Concentrations			EC_{50} Test				
Test Chamber #	Design Conc. (mg/L)	Mean Loading Rate (mg/L)	Mean VOA (mg/L)	Mean TPH (mg/L)	VOA + TPH (mg/L)	Number of Animals Alive/Total	Individual Percent Affected	Mean Percent Affected	Standard Error	EC_{50} (mg/L) 95% CI (Analysis Mthd.)
1a	0	0.00	0.00	0.00	0.00	10/10	0.00	3.33	3.33	**0.27**
1b						9/10	10.00			LL=0.24
1c						10/10	0.00			UL=0.28 (Probit)
2a	500	498.57	0.17	0.13	0.29	5/10	50.00	83.33	16.67	
2b						0/10	100.00			
2c						0/10	100.00			
3a	2000	1996.45	0.23	0.08	0.31	1/10	90.00	73.33	8.82	
3b						3/10	70.00			
3c						4/10	60.00			
4a	5000	4999.62	0.33	0.11	0.45	0/10	100.00	100.00	0.00	
4b						0/10	100.00			
4c						0/10	100.00			
5a	7000	7004.21	0.34	0.16	0.50	0/10	100.00	100.00	0.00	
5b						0/10	100.00			
5c						0/10	100.00			
6a	10000	10030.13	0.35	0.15	0.51	0/10	100.00	100.00	0.00	
6b						0/10	100.00			
6c						0/10	100.00			

Defined responses: Alive, Affected, Mortally Affected, Dead
EC_{50} response: Affected

LL = lower limit
UL = upper limit

Tanner Crab (*Chionoecetes bairdi*)
CE-WAF Weathered ANS Crude Oil Spiked Exposure
Start Date of Test: 15-Apr-99

Test Chamber #	Design Conc. (mg/L)	Loading Rate (mg/L)	Measured Concentrations			EC_{50} Test				
			VOA (mg/L)	TPH (mg/L)	VOA + TPH (mg/L)	Number of Animals Alive/Total	Individual Percent Affected	Mean Percent Affected	Standard Error	EC_{50} (mg/L) 95% CI (Analysis mthd.)
1a	0	0.00	0.00	0.00	0.00	10/10	0.00	7.14	3.82	**2.36**
1b						9/10	10.00			LL=1.66
1c						7/8	12.50			UL=6.66 (Probit)
2a	50	46.86	0.04	0.77	0.82	9/10	10.00	13.33	3.33	
2b						9/10	10.00			
2c						8/10	20.00			
3a	100	97.14	0.10	1.59	1.69	7/10	30.00	33.33	8.82	
3b						8/10	20.00			
3c						5/10	50.00			
4a	500	504.40	0.28	13.25	13.53	0/10	100.00	100.00	0.00	
4b						0/10	100.00			
4c						0/6	100.00			
5a	1000	999.51	0.27	25.00	25.27	0/10	100.00	100.00	0.00	
5b						0/10	100.00			
5c						0/10	100.00			
6a	2500	2494.68	1.36	66.73	68.09	0/10	100.00	100.00	0.00	
6b						0/10	100.00			
6c						0/10	100.00			

LL = lower limit
UL = upper limit

Tanner Crab (*Chionoecetes bairdi*)
CE-WAF Weathered ANS Crude Oil Continuous Exposure
Start Date of Test: 15-Apr-99

			Measured Concentrations			EC_{50} Test				
Test Chamber #	Design Conc. (mg/L)	Mean Loading Rate (mg/L)	Mean VOA (mg/L)	Mean TPH (mg/L)	VOA + TPH (mg/L)	Number of Animals Alive/Total	Individual Percent Affected	Mean Percent Affected	Standard Error	EC_{50} (mg/L) 95% CI (Analysis Mthd.)
1a	0	0.00	0.00	0.00	0.00	9/10	10.00	6.67	3.33	**0.37** (CNC)[1] (Graphical)
1b						10/10	0.00			
1c						9/10	10.00			
2a	10	14.26	0.01	0.15	0.16	1/9	88.89	82.96	6.49	
2b						1/10	90.00			
2c						3/10	70.00			
3a	50	49.82	0.05	0.74	0.80	0/10	100.00	100.00	0.00	
3b						0/10	100.00			
3c						0/10	100.00			
4a	100	97.85	0.10	1.39	1.49	0/10	100.00	100.00	0.00	
4b						0/10	100.00			
4c						0/10	100.00			
5a	500	500.37	0.20	14.73	14.93	0/10	100.00	100.00	0.00	
5b						0/9	100.00			
5c						0/10	100.00			
6a	1000	1010.54	0.34	30.73	31.06	0/10	100.00	100.00	0.00	
6b						0/10	100.00			
6c						0/10	100.00			

[1] CNC = Could not calculate; Confidence intervals cannot be calculated for graphical analysis.

Defined responses: Alive, Affected, Mortally Affected, Dead
EC_{50} response: Affected

LL = lower limit
UL = upper limit

Menidia beryllina
WAF Weathered ANS Crude Oil Spiked Exposure
Start Date of Test: 25-Jan-99

			Measured Concentrations			LC_{50} Test				
Test Chamber #	Design Conc. (mg/L)	Loading Rate (mg/L)	VOA (mg/L)	TPH (mg/L)	VOA + TPH (mg/L)	Number of Animals Alive/Total	Individual Percent Mortality	Mean Percent Mortality	Standard Error	LC_{50} (mg/L) 95% CI (Analysis mthd.)
1a	0	0.00	0.00	0.00	0.00	4/5	20.00	20.00	11.55	**>1.13** (CNC)[1]
1b						5/5	0.00			
1c						3/5	40.00			
2a	15000	15315.94	0.54	0.48	1.02	5/5	0.00	0.00	0.00	
2b						5/5	0.00			
2c						5/5	0.00			
3a	17500	17569.68	0.60	0.53	1.13	4/5	20.00	20.00	0.00	
3b						4/5	20.00			
3c						4/5	20.00			
4a	20000	20192.11	0.70	0.42	1.12	5/5	0.00	6.67	6.67	
4b						4/5	20.00			
4c						5/5	0.00			
5a	22500	22457.11	0.66	0.30	0.96	4/5	20.00	13.33	6.67	
5b						5/5	0.00			
5c						4/5	20.00			
6a	25000	24948.00	0.60	0.44	1.04	4/5	20.00	13.33	6.67	
6b						4/5	20.00			
6c						5/5	0.00			

[1] CNC = Could not calculate; Confidence limits cannot be calculated since 50 percent mortality was not observed.

LL = lower limit
UL = upper limit

Menidia beryllina
WAF Weathered ANS Crude Oil Continuous Exposure
Start Date of Test: 25-Jan-99

			Measured Concentrations			LC_{50} Test				
Test Chamber #	Design Conc. (mg/L)	Mean Loading Rate (mg/L)	Mean VOA (mg/L)	Mean TPH (mg/L)	VOA + TPH (mg/L)	Number of Animals Alive/Total	Individual Percent Mortality	Mean Percent Mortality	Standard Error	LC_{50} (mg/L) 95% CI (Analysis mthd.)
1a	0	0.00	0.00	0.00	0.00	4/5	20.00	13.33	6.67	0.79
1b						5/5	0.00			LL=0.32
1c						4/5	20.00			UL=0.83 (Probit)
2a	10000	10057.79	0.45	0.34	0.79	3/5	40.00	53.33	13.33	
2b						3/5	40.00			
2c						1/5	80.00			
3a	12500	12504.42	0.48	0.35	0.83	0/5	100.00	80.00	11.55	
3b						1/5	80.00			
3c						2/5	60.00			
4a	15000	15091.74	0.51	0.36	0.87	2/5	60.00	66.67	6.67	
4b						2/5	60.00			
4c						1/5	80.00			
5a	17500	17572.26	0.51	0.41	0.91	1/5	80.00	93.33	6.67	
5b						0/5	100.00			
5c						0/5	100.00			
6a	20000	20077.06	0.55	0.31	0.86	0/5	100.00	80.00	11.55	
6b						2/5	60.00			
6c						1/5	80.00			

LL = lower limit
UL = upper limit

Menidia beryllina
CE-WAF Weathered ANS Crude Oil Spiked Exposure
Start Date of Test: 8-Feb-99

			Measured Concentrations			LC_{50} Test				
Test Chamber #	Design Conc. (mg/L)	Loading Rate (mg/L)	VOA (mg/L)	TPH (mg/L)	VOA + TPH (mg/L)	Number of Animals Alive/Total	Individual Percent Mortality	Mean Percent Mortality	Standard Error	LC_{50} (mg/L) 95% CI (Analysis mthd.)
1a	0	0.00	0.00	0.00	0.00	5/5	0.00	0.00	0.00	**18.89**
1b						5/5	0.00			LL=15.78
1c						5/5	0.00			UL=24.71 (Probit)
2a	100	96.80	0.11	0.96	1.07	5/5	0.00	0.00	0.00	
2b						5/5	0.00			
2c						5/5	0.00			
3a	200	199.11	0.21	9.33	9.54	4/5	20.00	10.00	10.00	
3b						LOST[1]				
3c						5/5	0.00			
4a	400	396.77	0.39	12.58	12.97	2/5	60.00	20.00	20.00	
4b						5/5	0.00			
4c						5/5	0.00			
5a	600	600.25	0.45	17.11	17.56	4/5	20.00	33.33	6.67	
5b						3/5	40.00			
5c						3/5	40.00			
6a	1000	1004.91	0.73	42.50	43.23	0/5	100.00	100.00	0.00	
6b						0/5	100.00			
6c						0/5	100.00			

[1] Chambers' flow lapsed.

LL = lower limit
UL = upper limit

Menidia beryllina
CE-WAF Weathered ANS Crude Oil Continuous Exposure
Start Date of Test: 8-Feb-99

			Measured Concentrations			LC_{50} Test				
Test Chamber #	Design Conc. (mg/L)	Mean Loading Rate (mg/L)	Mean VOA (mg/L)	Mean TPH (mg/L)	VOA + TPH (mg/L)	Number of Animals Alive/Total	Individual Percent Mortality	Mean Percent Mortality	Standard Error	LC_{50} (mg/L) 95% CI (Analysis mthd.)
1a	0	0.00	0.00	0.00	0.00	5/5	0.00	6.67	6.67	**0.65** LL=0.10 UL=1.25 (Probit)
1b						4/5	20.00			
1c						5/5	0.00			
2a	50	51.25	0.05	0.76	0.81	2/5	60.00	60.00	0.00	
2b						2/5	60.00			
2c						2/5	60.00			
3a	100	100.88	0.08	1.71	1.79	0/5	100.00	80.00	11.55	
3b						1/5	80.00			
3c						2/5	60.00			
4a	200	199.58	0.15	7.29	7.44	0/5	100.00	93.33	6.67	
4b						1/5	80.00			
4c						0/5	100.00			
5a	300	302.10	0.34	12.33	12.67	0/5	100.00	100.00	0.00	
5b						0/5	100.00			
5c						0/5	100.00			
6a	400	401.05	0.43	14.05	14.48	0/5	100.00	100.00	0.00	
6b						0/5	100.00			
6c						0/5	100.00			

LL = lower limit
UL = upper limit

APPENDIX G

WEATHERED OIL STUDY CHEMISTRY DATA

Measured concentrations of weathered Alaska North Slope crude oil spiked exposure test solutions.

Species	Test Solution[a]	Loading Rate (mg/L)	BTEX (mg/L)	VOA C_6-C_9 (mg/L)	TPH C_{10}-C_{36} (mg/L)	THC[b] C_6-C_{36} (mg/L)
C. bairdi	WAF	0.00	0.00	0.00	0.00	0.00
		496.26	0.08	0.10	0.12	0.22
		2002.83	0.17	0.21	0.08	0.28
		4983.86	0.22	0.29	0.12	0.41
		6993.25	0.22	0.29	0.16	0.45
		10011.26	0.24	0.32	0.13	0.45
	CE-WAF	0.00	0.00	0.00	0.00	0.00
		46.86	0.02	0.04	0.77	0.82
		97.14	0.04	0.10	1.59	1.69
		504.40	0.13	0.28	13.25	13.53
		999.51	0.15	0.27	25.00	25.27
		2494.68	0.36	1.36	66.73	68.09
M. beryllina	WAF	0.00	0.00	0.00	0.00	0.00
		15315.94	0.37	0.54	0.48	1.02
		17569.68	0.41	0.60	0.53	1.13
		20192.11	0.46	0.70	0.42	1.12
		22457.11	0.45	0.66	0.30	0.96
		24948.00	0.41	0.60	0.44	1.04
	CE-WAF	0.00	0.00	0.00	0.00	0.00
		96.80	0.04	0.11	0.96	1.07
		199.11	0.08	0.21	9.33	9.54
		396.77	0.15	0.39	12.58	12.97
		600.25	0.11	0.45	17.11	17.56
		1004.91	0.27	0.73	42.50	43.23

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[a] WAF = water-accommodated fraction; CE-WAF = chemically enhanced water-accommodated fraction.

[b] THC concentrations are the sum of VOA and TPH values.

Measured concentrations of weathered Alaska North Slope crude oil continuous exposure test solutions.

Species	Test Solution[a]	Loading Rate (mg/L)	BTEX (mg/L)	VOA C_6-C_9 (mg/L)	TPH C_{10}-C_{36} (mg/L)	THC[b] C_6-C_{36} (mg/L)
C. bairdi[d]	WAF	0.00	0.00	0.00	0.00	0.00
		498.57	0.11	0.17	0.13	0.29
		1996.45	0.18	0.23	0.08	0.31
		4999.62	0.22	0.33	0.11	0.45
		7004.21	0.25	0.34	0.16	0.50
		10030.13	0.25	0.35	0.15	0.51
	CE-WAF	0.00	0.00	0.00	0.00	0.00
		14.26	0.00	0.01	0.15	0.16
		49.82	0.02	0.05	0.74	0.80
		97.85	0.04	0.10	1.39	1.49
		500.37	0.10	0.20	14.73	14.93
		1010.54	0.16	0.34	30.73	31.06
M. beryllina[c,d]	WAF	0.00	0.00	0.00	0.00	0.00
		10057.79	0.31	0.45	0.34	0.79
		12504.42	0.33	0.48	0.35	0.83
		15091.74	0.35	0.51	0.36	0.87
		17572.26	0.35	0.51	0.41	0.91
		20077.06	0.37	0.55	0.31	0.86
	CE-WAF	0.00	0.00	0.01	0.00	0.00
		51.25	0.02	0.05	0.76	0.81
		100.88	0.04	0.08	1.71	1.79
		199.58	0.07	0.15	7.29	7.44
		302.10	0.13	0.34	12.33	12.67
		401.05	0.16	0.43	14.05	14.48

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[a] WAF = water-accommodated fraction; CE-WAF = chemically enhanced water-accommodated fraction.

[b] THC concentrations are the sum of VOA and TPH values.

[c] BTEX and VOA concentrations for *M. beryllina* are the composite of solutions from days 1-4.

[d] TPH concentrations for WAF solutions are values from day 1 solutions;
TPH concentrations for CE-WAF solutions are the composite of solutions from days 1-4.

Declining, spiked-exposure and Post-24-Hour, continuous-exposure concentrations of weathered Alaska North Slope crude oil (ANS) test solutions for *C. bairdi*.

Spiked exposure

WAF[1]				CE-WAF[1]			
Loading Rate (mg/L)	Hour	BTEX (mg/L)	VOA C_6-C_9 (mg/L)	Loading Rate (mg/L)	Hour	BTEX (mg/L)	VOA C_6-C_9 (mg/L)
4983.86	0	0.22	0.29	504.40	0	0.15	0.39
	2	0.07	0.08		2	0.02	0.05
	4	0.03	0.04		4	0.01	0.02
	7	0.01	0.01		7	0.00	0.01
	12	0.00	0.00		12	0.00	0.00
10011.26	0	0.24	0.32	2494.68	0	0.27	0.73
	2	0.04	0.06		4	0.02	0.06
	4	0.03	0.04		7	0.01	0.02
	7	0.01	0.02		12	0.00	0.01
	12	0.00	0.00		24	0.00	0.00

Continuous exposure

WAF[1]				CE-WAF[1]			
Loading Rate (mg/L)	End of Day	BTEX (mg/L)	VOA C_6-C_9 (mg/L)	Loading Rate (mg/L)	End of Day	BTEX (mg/L)	VOA C_6-C_9 (mg/L)
4983.86	1	0.02	0.03	97.14	1	0.00	0.00
5004.77	2	0.00	0.01	96.14	2	0.00	0.00
4995.77	3	0.00	0.02	97.57	3	0.00	0.00
				100.54	4	0.00	0.01
7032.37	4	0.00	0.02				
				999.51	1	0.01	0.14
10011.26	1	0.00	0.01	1035.46	2	0.01	0.13
9996.57	2	0.00	0.01	999.66	3	0.00	0.07
				1007.51	4	0.01	0.13

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[1] WAF = water-accommodated fraction; CE-WAF = chemically enhanced water-accommodated fraction.

Declining, spiked-exposure and Post-24-Hour, continuous-exposure concentrations of weathered Alaska North Slope crude oil (ANS) test solutions for *M. beryllina*.

Spiked exposure

WAF[1]				CE-WAF[1]			
Loading Rate (mg/L)	Hour	BTEX (mg/L)	VOA C_6-C_9 (mg/L)	Loading Rate (mg/L)	Hour	BTEX (mg/L)	VOA C_6-C_9 (mg/L)
20192.11	0	0.46	0.70	396.77	0	0.15	0.39
	2	0.10	0.13		2	0.02	0.05
	4	0.04	0.05		4	0.01	0.02
	7	0.03	0.04		7	0.00	0.01
	12	0.01	0.01		12	0.00	0.00
24948.00	0	0.41	0.60	1004.91	0	0.27	0.73
	2	0.06	0.07		4	0.02	0.06
	4	0.04	0.05		7	0.01	0.02
	7	0.03	0.04		12	0.00	0.01
	12	0.01	0.02		24	0.00	0.00

Continuous exposure

WAF[1]				CE-WAF[1]			
Loading Rate (mg/L)	End of Day	BTEX (mg/L)	VOA C_6-C_9 (mg/L)	Loading Rate (mg/L)	End of Day	BTEX (mg/L)	VOA C_6-C_9 (mg/L)
12349.63	1	0.00	0.01	48.08	3	0.00	0.01
12601.45	2	0.00	0.00	204.60	2	0.00	0.01
12559.08	3	0.00	0.00	196.48	3	0.00	0.01
17564.05	2	0.00	0.00	300.85	1	0.00	0.02
17577.20	3	0.00	0.00	396.77	1	0.00	0.01
20192.11	1	0.28	0.40	407.57	2	0.00	0.03

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[1] WAF = water-accommodated fraction; CE-WAF = chemically enhanced water-accommodated fraction.

APPENDIX H

WEATHERED OIL STUDY WATER QUALITY DATA

Water quality parameters for spiked exposure to test solutions of weathered Alaska North Slope crude oil used on *on C. bairdi*.

Test Solution[a]	Loading Rate (mg/L)	pH	Conductivity Reading (mS)	Salinity (ppt)	Temp. (°C)	D.O. (mg/L)	D.O (%)
WAF	0.00	8.01	49.33	31.9	8.0	8.95	94.3
	496.26	8.38	48.51	31.5	9.7	8.87	96.1
	2002.83	8.25	48.76	31.5	7.1	8.81	95.0
	4983.86	8.27	48.82	31.6	7.5	8.80	92.5
	6993.25	8.41	48.81	31.5	7.6	8.82	92.3
	10011.26	8.36	48.82	31.5	7.0	8.89	92.9
CE-WAF	0.00	8.38	48.78	31.3	6.1	9.45	92.4
	46.86	8.27	48.72	31.4	5.9	8.74	88.4
	97.14	8.38	48.71	31.4	6.7	8.82	89.7
	504.40	8.35	48.64	31.4	6.6	8.81	89.9
	999.51	8.36	48.68	31.4	6.4	8.81	89.7
	2494.68	8.32	48.52	31.3	6.5	8.60	90.0
Mean	-	8.31	48.76	31.48	7.09	8.86	91.93
Std. Dev.	-	0.11	0.21	0.16	1.03	0.20	2.42
n	-	12	12	12	12	12	12
Maximum	-	8.41	49.33	31.90	9.70	9.45	96.10
Minimum	-	8.01	48.51	31.30	5.90	8.60	88.40

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[a] WAF = water-accommodated fraction; CE-WAF = chemically enhanced water-accommodated fraction.

Water quality parameters for continuous exposure WAF test solutions of weathered Alaska North Slope crude oil used on *C. bairdi*.

Test Solution		Loading Rate (mg/L)	pH	Conductivity Reading (mS)	Salinity (ppt)	Temp. (°C)	D.O. (mg/L)	D.O (%)
WAF[a]	Day 1	0.00	8.01	49.33	31.9	8.0	8.95	94.3
		496.26	8.38	48.51	31.5	9.7	8.87	96.1
		2002.83	8.25	48.76	31.5	7.1	8.81	95.0
		4983.86	8.27	48.82	31.6	7.5	8.80	92.5
		6993.25	8.41	48.81	31.5	7.6	8.82	92.3
		10011.26	8.36	48.82	31.5	7.0	8.89	92.9
	Day 2	0.00	8.34	48.82	31.7	8.0	8.96	99.2
		500.08	8.34	48.99	31.7	7.5	9.04	95.8
		1993.11	8.33	49.14	31.8	6.8	9.13	96.1
		5004.77	8.35	49.15	31.8	7.1	8.94	93.6
		6992.82	8.37	49.15	31.8	7.1	8.90	92.8
		9996.57	8.34	49.07	31.7	7.4	8.66	92.2
	Day 3	0.00	8.34	48.28	31.3	7.8	9.07	99.8
		497.43	8.33	48.88	31.6	6.9	9.20	96.2
		1991.37	8.35	48.91	31.6	6.5	9.17	94.1
		4995.77	8.34	49.09	31.7	7.2	8.98	93.0
		6998.40	8.33	49.15	31.7	6.5	9.08	93.5
		10104.34	8.32	49.20	31.8	6.4	8.77	90.8
	Day 4	0.00	8.40	47.94	31.1	8.4	8.69	95.5
		500.51	8.39	48.63	31.4	6.5	8.95	93.7
		1998.48	8.37	48.44	31.2	6.5	8.94	92.2
		5014.08	8.39	48.90	31.5	6.6	8.85	90.5
		7032.37	8.40	48.93	31.5	6.5	8.70	89.1
		10008.34	8.37	48.91	31.5	6.5	8.65	89.1
	Mean	-	8.34	48.86	31.58	7.21	8.91	93.76
	Std. Dev.	-	0.08	0.32	0.20	0.78	0.16	2.69
	n	-	24	24	24	24	24	24
	Maximum	-	8.41	49.33	31.90	9.70	9.20	99.80
	Minimum	-	8.01	47.94	31.10	6.40	8.65	89.10

[a] WAF = water-accommodated fraction.

Water quality parameters for continuous exposure CE-WAF solutions of weathered Alaska North Slope crude oil used on *C. bairdi*.

Test Solution		Loading Rate (mg/L)	pH	Conductivity Reading (mS)	Salinity (ppt)	Temp. (°C)	D.O. (mg/L)	D.O (%)
CE-WAF[a]	Day 1	0.00	8.38	48.78	31.3	6.1	9.45	92.4
		13.65	8.13	48.65	31.3	6.3	8.92	92.4
		46.86	8.27	48.72	31.4	5.9	8.74	88.4
		97.14	8.38	48.71	31.4	6.7	8.82	89.7
		504.40	8.35	48.64	31.4	6.6	8.81	89.9
		999.51	8.36	48.68	31.4	6.4	8.81	89.7
	Day 2	0.00	8.40	49.08	31.5	6.2	9.11	94.2
		14.31	8.37	48.65	31.4	6.3	9.09	93.4
		50.23	8.40	48.69	31.4	6.3	8.84	92.9
		96.14	8.40	48.58	31.3	6.2	8.79	92.3
		499.37	8.42	48.73	31.4	6.3	8.91	91.5
		1035.46	8.42	48.59	31.3	6.6	8.95	91.5
	Day 3	0.00	8.37	48.81	31.4	6.7	9.54	96.2
		13.74	8.42	48.75	31.5	6.7	8.81	92.4
		49.71	8.42	48.55	31.3	6.3	8.93	90.7
		97.57	8.46	48.61	31.4	6.3	8.93	91.3
		499.89	8.42	48.72	31.4	6.3	8.95	91.8
		999.66	8.44	48.63	31.4	6.2	8.94	92.9
	Day 4	0.00	8.39	48.80	31.4	6.7	9.40	94.6
		15.34	8.37	48.62	31.5	7.1	8.75	92.5
		52.49	8.42	48.45	31.4	6.8	8.79	92.8
		100.54	8.44	48.53	31.4	7.2	8.73	92.1
		497.80	8.45	48.63	31.4	7.1	8.31	91.2
		1007.51	8.45	48.44	31.3	7.0	8.63	90.5
	Mean	-	8.39	48.67	31.39	6.51	8.91	91.97
	Std. Dev.	-	0.07	0.13	0.06	0.35	0.26	1.72
	n	-	24	24	24	24	24	24
	Maximum	-	8.46	49.08	31.50	7.20	9.54	96.20
	Minimum	-	8.13	48.44	31.30	5.90	8.31	88.40

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[a] CE-WAF = chemically enhanced water-accommodated fraction.

Water quality parameters for spiked exposure to test solutions of weathered Alaska North Slope crude oil on *M. beryllina*.

Test Solution[a]	Loading Rate (mg/L)	pH	Conductivity Reading (mS)	Salinity (ppt)	Temp. (°C)	D.O. (mg/L)
WAF	0.00	7.90	29.40	21.69	25.00	7.10
	15315.94	7.81	27.40	19.91	25.00	5.50
	17569.68	7.87	27.50	20.00	25.00	5.20
	20192.11	7.81	28.20	20.62	25.00	5.20
	22457.11	7.82	28.10	20.53	25.00	4.90
	24948.00	7.86	28.00	20.44	25.00	4.70
CE-WAF	0.00	7.80	27.70	20.18	25.00	7.00
	96.80	7.70	27.70	20.18	25.00	6.60
	199.11	7.68	28.00	20.44	25.00	6.40
	396.77	7.67	28.00	20.44	25.00	6.00
	600.25	7.75	27.90	20.36	25.00	6.00
	1004.91	7.63	28.00	20.44	25.00	5.70
Mean	-	7.78	27.86	20.44	25.00	5.86
Std. Dev.	-	0.09	0.25	0.45	0.00	0.80
n	-	12	12	12	12	12
Maximum	-	7.90	29.40	21.69	25.00	7.10
Minimum	-	7.63	27.40	19.91	25.00	4.70

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[a] WAF = water-accommodated fraction; CE-WAF = chemically enhanced water-accommodated fraction.

Water quality parameters for continuous exposure WAF test solutions of weathered Alaska North Slope crude oil on *M. beryllina*.

Test Solution		Loading Rate (mg/L)	pH	Conductivity Reading (mS)	Salinity (ppt)	Temp. (°C)	D.O. (mg/L)
WAF[a]	Day 1	0.00	7.90	29.40	21.69	25.00	7.10
		9874.63	7.81	27.50	20.00	25.00	6.10
		12349.63	7.84	27.80	20.27	25.00	6.00
		15315.94	7.81	27.40	19.91	25.00	5.50
		17569.68	7.87	27.50	20.00	25.00	5.20
		20192.11	7.81	28.20	20.62	25.00	5.20
	Day 2	0.00	7.96	28.00	20.44	25.00	7.20
		10202.02	7.94	28.50	20.89	25.00	6.20
		12601.45	7.92	28.10	20.53	25.00	6.00
		15035.11	7.96	28.50	20.89	25.00	5.80
		17564.05	7.96	28.60	20.98	26.00	5.40
		20006.45	7.96	28.50	20.89	25.00	5.20
	Day 3	0.00	7.74	27.50	20.00	24.00	7.20
		10125.60	7.70	28.20	20.62	25.00	6.00
		12559.08	7.86	28.10	20.53	25.00	5.80
		15008.62	7.86	28.20	20.62	25.00	5.60
		17577.20	7.89	28.10	20.53	25.00	5.20
		20010.48	7.88	28.20	20.62	25.00	5.20
	Day 4	0.00	7.96	27.40	19.91	24.00	6.80
		10028.91	7.82	27.40	19.91	26.00	6.20
		12507.51	7.91	27.80	20.27	25.00	5.80
		15007.28	7.92	27.60	20.09	25.00	5.60
		17578.11	7.93	27.70	20.18	25.00	5.60
		20099.20	7.94	27.60	20.09	25.00	5.10
	Mean	-	7.88	27.99	20.44	25.00	5.88
	Std. Dev.	-	0.07	0.49	0.44	0.42	0.65
	n	-	24	24	24	24	24
	Maximum	-	7.96	29.40	21.69	26.00	7.20
	Minimum	-	7.70	27.40	19.91	24.00	5.10

---

[a] WAF = water-accommodated fraction.

Water quality parameters for continuous exposure CE-WAF solutions of weathered Alaska North Slope crude oil used on *M. beryllina*.

Test Solution		Loading Rate (mg/L)	pH	Conductivity Reading (mS)	Salinity (ppt)	Temp. (°C)	D.O. (mg/L)
CE-WAF[a]	Day 1	0.00	7.80	27.70	20.18	25.00	7.00
		52.20	7.80	27.60	20.09	25.00	6.50
		96.80	7.70	27.70	20.18	25.00	6.60
		199.11	7.68	28.00	20.44	25.00	6.40
		300.85	7.66	28.00	20.44	25.00	6.20
		396.77	7.67	28.00	20.44	25.00	6.00
	Day 2	0.00	7.96	27.60	20.09	25.00	7.00
		53.88	7.99	27.90	20.36	25.00	6.70
		104.11	7.97	28.00	20.44	25.00	6.50
		204.60	7.94	27.90	20.36	25.00	6.20
		312.02	7.88	27.80	20.27	25.00	6.20
		407.57	7.80	27.70	20.18	25.00	6.10
	Day 3	0.00	7.74	26.50	19.11	26.00	7.00
		48.08	7.94	27.80	20.27	25.00	6.70
		101.05	7.94	27.50	20.00	26.00	6.60
		196.48	7.95	27.60	20.09	26.00	6.50
		294.54	7.96	27.60	20.09	25.00	6.40
		398.82	8.01	27.90	20.36	26.00	6.20
	Day 4	0.00	7.79	27.30	19.82	26.00	7.00
		50.82	7.96	27.20	19.73	26.00	6.60
		101.54	7.96	27.40	19.91	26.00	6.80
		198.11	7.97	27.50	20.00	26.00	6.80
		299.00	7.95	27.60	20.09	26.00	6.60
		**	**	**	**	**	**
	Mean	-	7.87	27.64	20.13	25.39	6.55
	Std. Dev.	-	0.12	0.34	0.30	0.50	0.30
	n	-	23	23	23	23	23
	Maximum	-	8.01	28.00	20.44	26.00	7.00
	Minimum	-	7.66	26.50	19.11	25.00	6.00

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[a] CE-WAF = chemically enhanced water-accommodated fraction.

** Not sampled because of complete die off in this concentration.

APPENDIX I

WEATHERED OIL STUDY TOXICITY

DAILY OBSERVATION DATA

Menidia beryllina
WAF Weathered ANS Crude Oil Spiked Exposure
Start Date of Test: 25-Jan-99

Test Chamber #	Design Concentration (mg/L)	Loading Rate (L.R.) (mg/L)	VOA + TPH (mg/L)	End of Day 1 Number of Animals Alive/Total	End of Day 2 Number of Animals Alive/Total	End of Day 3 Number of Animals Alive/Total	End of Day 4 Number of Animals Alive/Total
1a	0	0.00	0.00	5/5	4/5	4/5	4/5
1b				5/5	5/5	5/5	5/5
1c				4/5	3/5	3/5	3/5
2a	15000	15315.94	1.02	5/5	5/5	5/5	5/5
2b				5/5	5/5	5/5	5/5
2c				5/5	5/5	5/5	5/5
3a	17500	17569.68	1.13	5/5	5/5	5/5	4/5
3b				5/5	4/5	4/5	4/5
3c				5/5	4/5	4/5	4/5
4a	20000	20192.11	1.12	5/5	5/5	5/5	5/5
4b				4/5	4/5	4/5	4/5
4c				5/5	5/5	5/5	5/5
5a	22500	22457.11	0.96	5/5	5/5	4/5	4/5
5b				5/5	5/5	5/5	5/5
5c				5/5	4/5	4/5	4/5
6a	25000	24948.00	1.04	5/5	5/5	5/5	4/5
6b				5/5	5/5	4/5	4/5
6c				5/5	5/5	5/5	5/5
			LC_{50} (mg/L) based on L.R.	**>24948**	**>24948**	**>24948**	**>24948**
			95% CI	CNC[1]	CNC[1]	CNC[1]	CNC[1]
			(Analysis method)				

[1] CNC = Could not calculate due to no observation of 50-percent mortality or greater.

Menidia beryllina
WAF Weathered ANS Crude Oil Continuous Exposure
Start Date of Test: 25-Jan-99

Test Chamber #	Design Concentration (mg/L)	Mean Loading Rate (L.R.) (mg/L)	Measured VOA + TPH (mg/L)	End of Day 1 Number of Animals Alive/Total	End of Day 2 Number of Animals Alive/Total	End of Day 3 Number of Animals Alive/Total	End of Day 4 Number of Animals Alive/Total
1a	0	0.00	0.00	4/5	4/5	4/5	4/5
1b				5/5	5/5	5/5	5/5
1c				5/5	4/5	4/5	4/5
2a	10000	10057.79	0.79	5/5	5/5	3/5	3/5
2b				5/5	5/5	4/5	3/5
2c				5/5	5/5	1/5	1/5
3a	12500	12504.42	0.83	5/5	4/5	1/5	0/5
3b				5/5	4/5	2/5	1/5
3c				5/5	5/5	3/5	2/5
4a	15000	15091.74	0.87	4/5	4/5	2/5	2/5
4b				5/5	5/5	2/5	2/5
4c				4/5	4/5	2/5	1/5
5a	17500	17572.26	0.91	4/5	3/5	1/5	1/5
5b				3/5	3/5	2/5	0/5
5c				2/5	2/5	0/5	0/5
6a	20000	20077.06	0.86	3/5	1/5	1/5	0/5
6b				5/5	5/5	4/5	2/5
6c				4/5	4/5	4/5	1/5
		LC$_{50}$ (mg/L) based on L.R.		**>20077.06**	**>20077.06**	**13365.98**	**9512.23**
		95% CI		CNC[1]	CNC[1]	N/A[2]	N/A[2]
		(Analysis method)				(Probit)	(Probit)

[1] CNC = Could not calculate due to no observation of 50-percent mortality or greater.

[2] Confidence limits not reliably calculable; calculated chi-squared value very similar to the tabulted chi-squared value.

Menidia beryllina
CE-WAF Weathered ANS Crude Oil Spiked Exposure
Start Date of Test: 8-Feb-99

Test Chamber #	Design Concentration (mg/L)	Loading Rate (L.R.) (mg/L)	VOA + TPH (mg/L)	End of Day 1 Number of Animals Alive/Total	End of Day 2 Number of Animals Alive/Total	End of Day 3 Number of Animals Alive/Total	End of Day 4 Number of Animals Alive/Total
1a	0	0.00	0.00	5/5	5/5	5/5	5/5
1b				5/5	5/5	5/5	5/5
1c				5/5	5/5	5/5	5/5
2a	100	96.80	1.07	5/5	5/5	5/5	5/5
2b				5/5	5/5	5/5	5/5
2c				5/5	5/5	5/5	5/5
3a	200	199.11	9.54	4/5	4/5	4/5	4/5
3b				4/5	LOST[1]	LOST[1]	LOST[1]
3c				5/5	5/5	5/5	5/5
4a	400	396.77	12.97	2/5	2/5	2/5	2/5
4b				5/5	5/5	5/5	5/5
4c				5/5	5/5	5/5	5/5
5a	600	600.25	17.56	4/5	4/5	4/5	4/5
5b				3/5	3/5	3/5	3/5
5c				3/5	3/5	3/5	3/5
6a	1000	1004.91	43.23	0/5	0/5	0/5	0/5
6b				0/5	0/5	0/5	0/5
6c				0/5	0/5	0/5	0/5
		LC_{50} (mg/L) based on L.R.		**555.15**	**555.15**	**555.15**	**555.15**
		95% CI		450.43 684.21	450.43 684.21	450.43 684.21	450.43 684.21
		(Analysis method)		(TSK, a=0%)	(TSK, a=0%)	(TSK, a=0%)	(TSK, a=0%)

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[1] Chambers' flow lapsed.

Menidia beryllina
CE-WAF Weathered ANS Crude Oil Continuous Exposure
Start Date of Test: 8-Feb-99

Test Chamber #	Design Concentration (mg/L)	Mean Loading Rate (L.R.) (mg/L)	Measured VOA + TPH (mg/L)	End of Day 1 Number of Animals Alive/Total	End of Day 2 Number of Animals Alive/Total	End of Day 3 Number of Animals Alive/Total	End of Day 4 Number of Animals Alive/Total
1a	0	0.00	0.00	5/5	5/5	5/5	5/5
1b				5/5	5/5	4/5	4/5
1c				5/5	5/5	5/5	5/5
2a	50	51.25	0.81	5/5	5/5	3/5	2/5
2b				5/5	5/5	3/5	2/5
2c				4/5	4/5	2/5	2/5
3a	100	100.88	1.79	5/5	4/5	0/5	0/5
3b				5/5	4/5	2/5	1/5
3c				5/5	5/5	5/5	2/5
4a	200	199.58	7.44	2/5	2/5	1/5	0/5
4b				4/5	4/5	1/5	1/5
4c				4/5	1/5	1/5	0/5
5a	300	302.10	12.67	3/5	2/5	0/5	0/5
5b				2/5	0/5	0/5	0/5
5c				1/5	0/5	0/5	0/5
6a	400	401.05	14.48	1/5	0/5	0/5	0/5
6b				0/5	0/5	0/5	0/5
6c				0/5	0/5	0/5	0/5
		LC$_{50}$ (mg/L) based on L.R.		**239.49**	**165.31**	**78.24**	**46.89**
			95% CI	198.13	129.19	40.09	13.95
				289.47	204.32	111.87	71.90
			(Analysis method)	(TSK, a=6.67%)	(Probit)	(Probit)	(Probit)

APPENDIX J

DISPERSANT, FRESH OIL, AND WEATHERED OIL STUDY

MICROTOX DATA

C. bardi; Fresh ANSC; WAF; THC

C. bardi Flowthrough exposure

Loading Rate (mg/L)	THC Conc. (mg/L)	Rep. 1 5min exposure EC$_{50}$%	Rep. 1 5min exposure EC$_{50}$ (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 1 15min exposure EC$_{50}$%	Rep. 1 15min exposure EC$_{50}$ (mg/L)	Rep. 1 15min exposure CR	Rep. 1 15min exposure SE
0.00	0.17	*	*	-	-	*	*	-	-
201.86	7.56	36.79	2.78	0.85	0.22	46.77	3.54	2.27	0.58
401.09	12.54	25.36	3.18	0.48	0.12	35.62	4.47	2.12	0.54
1099.66	19.35	25.55	4.94	1.47	0.38	35.49	6.87	2.46	0.63
1814.86	22.21	29.10	6.46	1.77	0.45	37.66	8.36	3.21	0.82
2499.23	30.58	23.80	7.28	1.57	0.40	31.90	9.76	3.03	0.77

C. bardi Static exposure

Day 1	0.00	0.00	*	*	-	-	*	-	-	-
	47.03	2.47	*	*	-	-	*	-	-	-
	254.00	8.78	38.35	3.37	1.11	0.28	45.34	3.98	2.18	0.56
	463.94	13.18	31.18	4.11	0.30	0.08	39.79	5.24	0.87	0.22
	773.94	19.16	22.23	4.26	0.80	0.20	28.20	5.40	1.58	0.40
	1017.37	24.33	28.91	7.03	3.06	0.78	38.84	9.45	4.80	1.22
Day 2	0.00	0.00	-	-	-	-	-	-	-	-
	56.54	2.47	-	-	-	-	-	-	-	-
	233.80	8.78	*	*	-	-	*	*	-	-
	414.74	13.18	32.49	4.28	0.89	0.23	43.44	5.73	2.95	0.75
	702.11	19.16	22.94	4.40	0.73	0.19	29.02	5.56	1.21	0.31
	994.71	24.33	14.43	3.51	0.53	0.13	18.16	4.42	0.93	0.24
Day 3	0.00	0.00	-	-	-	-	-	-	-	-
	49.49	2.47	-	-	-	-	-	-	-	-
	228.17	8.78	40.82	3.58	1.89	0.48	49.56	4.35	3.48	0.89
	399.83	13.18	40.18	5.30	-1.41	-0.36	*	*	-	-
	685.63	19.16	42.74	8.19	2.64	0.67	*	*	-	-
	1001.14	24.33	31.01	7.54	0.32	0.08	40.65	9.89	3.98	1.02
Day 4	0.00	0.00	-	-	-	-	-	-	-	-
	50.49	2.47	*	*	-	-	*	*	-	-
	220.57	8.78	35.48	3.12	0.85	0.22	41.85	3.67	1.30	0.33
	435.80	13.18	43.02	5.67	1.90	0.48	*	*	-	-
	702.34	19.16	37.82	7.25	6.24	1.59	*	*	-	-
	981.94	24.33	22.69	5.52	0.76	0.19	29.34	7.14	1.04	0.27

* = EC50% was greater than the highest concentration tested.
\- = not tested.
CR = control ratio correction factor.
SE = standard error.

C. bardi; Fresh ANSC; CE-WAF; THC

C. bardi Flowthrough exposure

Loading Rate (mg/L)	THC Conc. (mg/L)	Rep. 1 5min exposure EC$_{50}$%	Rep. 1 5min exposure EC$_{50}$ (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 1 15min exposure EC$_{50}$%	Rep. 1 15min exposure EC$_{50}$ (mg/L)	Rep. 1 15min exposure CR	Rep. 1 15min exposure SE
0.00	1.67	*	*	-	-	*	*	-	-
99.83	5.02	23.70	1.19	0.21	0.05	31.81	1.60	0.16	0.04
204.36	10.57	14.28	1.51	0.05	0.01	18.66	1.97	0.30	0.08
402.32	24.14	3.56	0.86	0.20	0.05	5.43	1.31	0.21	0.05
1100.75	53.93	1.72	0.93	0.03	0.01	2.22	1.20	0.33	0.09
1853.79	96.23	1.02	0.98	0.19	0.05	1.26	1.22	0.22	0.06

C. bardi Static exposure

Day 1	0.00	0.00	*	*	-	-	*	*	-	-
	25.39	1.70	-	-	-	-	-	-	-	-
	50.95	2.98	55.02	1.64	0.85	0.22	*	*	-	-
	223.67	16.37	10.51	1.72	0.06	0.01	14.16	2.32	0.16	0.04
	395.99	29.10	7.61	2.21	0.31	0.08	11.18	3.25	0.46	0.12
	701.26	80.19	3.20	2.57	0.15	0.04	4.11	3.29	0.09	0.02
Day 2	0.00	0.00	-	-	-	-	-	-	-	-
	26.17	1.70	-	-	-	-	-	-	-	-
	50.89	2.98	*	*	-	-	*	*	-	-
	228.09	16.37	12.99	2.13	0.05	0.01	20.84	3.41	0.39	0.10
	399.41	29.10	7.68	2.24	0.05	0.01	9.90	2.88	0.32	0.08
	704.06	80.19	1.58	1.27	0.10	0.03	2.58	2.07	0.48	0.12
Day 3	0.00	0.00	-	-	-	-	-	-	-	-
	26.69	1.70	-	-	-	-	-	-	-	-
	50.80	2.98	48.34	1.44	1.42	0.36	*	*	-	-
	223.28	16.37	13.79	2.26	0.27	0.07	22.80	3.73	0.86	0.22
	400.40	29.10	8.66	2.52	0.32	0.08	11.71	3.41	0.33	0.08
	702.19	80.19	2.87	2.30	0.17	0.04	3.77	3.02	0.17	0.04
Day 4	0.00	0.00	-	-	-	-	-	-	-	-
	28.22	1.70	-	-	-	-	-	-	-	-
	51.58	2.98	*	*	-	-	*	*	-	-
	226.19	16.37	36.95	6.05	0.65	0.17	47.12	7.71	2.09	0.53
	404.37	29.10	7.79	2.27	0.30	0.08	11.67	3.40	0.59	0.15
	711.86	80.19	2.69	2.16	0.26	0.07	3.54	2.84	0.55	0.14

* = EC50% was greater than the highest concentration tested.
- = not tested.
CR = control ratio correction factor.
SE = standard error.

C. bardi; Weathered ANSC; WAF; THC

C. bardi Flowthrough/Static exposure

Loading Rate (mg/L)	THC Conc. (mg/L)	Rep. 1 5min exposure EC_{50}%	Rep. 1 5min exposure EC_{50} (mg/L)	Rep. 1 5min exposure CR.	Rep. 1 5min exposure SE	Rep. 1 15min exposure EC_{50}%	Rep. 1 15min exposure EC_{50} (mg/L)	Rep. 1 15min exposure CR.	Rep. 1 15min exposure SE
0.00	0.00	*	*	-	-	*	*	-	-
496.26	0.22	*	*	-	-	*	*	-	-
2002.83	0.28	*	*	-	-	*	*	-	-
4983.86	0.41	*	*	-	-	*	*	-	-
6993.25	0.45	49.48	0.22	0.12	0.03	*	*	-	-
10011.26	0.45	48.89	0.22	0.03	0.01	*	*	-	-

Loading Rate (mg/L)	Loading Rate (mg/L)	Rep. 2 5min exposure EC_{50}%	Rep. 2 5min exposure EC_{50} (mg/L)	Rep. 2 5min exposure CR.	Rep. 2 5min exposure SE	Rep. 2 15min exposure EC_{50}%	Rep. 2 15min exposure EC_{50} (mg/L)	Rep. 2 15min exposure CR.	Rep. 2 15min exposure SE
0.00	0.00	*	*	-	-	*	*	-	-
496.26	0.22	*	*	-	-	*	*	-	-
2002.83	0.28	*	*	-	-	*	*	-	-
4983.86	0.41	*	*	-	-	*	*	-	-
6993.25	0.45	54.15	0.24	0.04	0.01	*	*	-	-
10011.26	0.45	45.27	0.20	0.02	0.01	*	*	-	-

* = EC50% was greater than the highest concentration tested.
\- = not tested.
CR = control ratio correction factor.
SE = standard error.

C. bardi; Weathered ANSC; CE-WAF; THC

C. bardi Flowthrough/Static exposure

Loading Rate (mg/L)	THC Conc. (mg/L)	Rep. 1 5min exposure EC$_{50}$%	Rep. 1 5min exposure EC$_{50}$ (mg/L)	Rep. 1 5min exposure CR.	Rep. 1 5min exposure SE	Rep. 1 15min exposure EC$_{50}$%	Rep. 1 15min exposure EC$_{50}$ (mg/L)	Rep. 1 15min exposure CR.	Rep. 1 15min exposure SE
0.00	0.00	*	*	-	-	*	*	-	-
46.86	0.82	*	*	-	-	*	*	-	-
97.14	1.69	*	*	-	-	*	*	-	-
504.40	13.53	45.11	6.10	0.90	0.23	*	*	-	-
999.51	25.27	21.63	5.47	1.08	0.28	30.69	7.76	0.89	0.23
2494.68	68.09	21.73	14.80	1.61	0.41	27.54	18.75	1.83	0.47

Loading Rate (mg/L)	Loading Rate (mg/L)	Rep. 2 5min exposure EC$_{50}$%	Rep. 2 5min exposure EC$_{50}$ (mg/L)	Rep. 2 5min exposure CR.	Rep. 2 5min exposure SE	Rep. 2 15min exposure EC$_{50}$%	Rep. 2 15min exposure EC$_{50}$ (mg/L)	Rep. 2 15min exposure CR.	Rep. 2 15min exposure SE
0.00	0.00	*	*	-	-	*	*	-	-
46.86	0.82	*	*	-	-	*	*	-	-
97.14	1.69	*	*	-	-	*	*	-	-
504.40	13.53	*	*	-	-	*	*	-	-
999.51	25.27	24.25	6.13	1.26	0.32	36.47	9.22	3.42	0.87
2494.68	68.09	20.52	13.97	1.63	0.42	28.84	19.64	4.69	1.20

* = EC50% was greater than the highest concentration tested.
\- = not tested.
CR = control ratio correction factor.
SE = standard error.

M. bahia; Fresh ANSC; WAF; THC

M. bahia Flowthrough exposure

Loading Rate (mg/L)	THC Conc. (mg/L)	Rep. 1 5min exposure EC_{50} %	Rep. 1 5min exposure EC_{50} (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 2 5min exposure EC_{50} %	Rep. 2 5min exposure EC_{50} (mg/L)	Rep. 2 5min exposure CR	Rep. 2 5min exposure SE
0.00	1.04	*	*	-	-	*	*	-	-
139.26	3.14	37.08	1.16	0.52	0.13	*	*	-	-
250.51	4.77	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-
500.34	8.83	30.75	2.72	0.41	0.11	35.51	3.14	0.55	0.14
752.51	8.59	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-
2497.86	12.73	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-

M. bahia Static exposure

0.00	0.35	*	*	-	-	*	*	-	-
25.66	0.91	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-
139.26	2.00	37.08	0.74	0.33	0.08	*	*	-	-
250.51	3.60	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-
500.34	5.64	30.75	1.73	0.26	0.07	35.51	2.00	0.35	0.09
752.51	5.61	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-

* = EC50% was greater than the highest concentration tested.
- = not tested.
CR = control ratio correction factor.
SE = standard error.

M. bahia; Fresh ANSC; CE-WAF; THC

M. bahia Flowthrough exposure

Loading Rate (mg/L)	THC Conc. (mg/L)	Rep. 1 5min exposure EC_{50} %	Rep. 1 5min exposure EC_{50} (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 2 5min exposure EC_{50} %	Rep. 2 5min exposure EC_{50} (mg/L)	Rep. 2 5min exposure CR	Rep. 2 5min exposure SE
0.00	0.15	*	*	-	-	-	-	-	-
26.26	0.22	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-
102.46	2.73	33.53	0.92	0.07	0.02	38.60	1.05	0.58	0.15
248.29	25.05	4.47	1.12	0.26	0.07	5.06	1.27	0.35	0.09
479.66	24.24	9.06	2.20	0.41	0.10	10.26	2.49	0.38	0.10
702.80	31.62	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-

M. bahia Static exposure

Loading Rate (mg/L)	THC Conc. (mg/L)	Rep. 1 5min exposure EC_{50} %	Rep. 1 5min exposure EC_{50} (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 2 5min exposure EC_{50} %	Rep. 2 5min exposure EC_{50} (mg/L)	Rep. 2 5min exposure CR	Rep. 2 5min exposure SE
0.00	0.62	*	*	-	-	-	-	-	-
10.09	0.45	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-
26.26	1.21	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-
102.46	5.00	33.53	1.68	0.14	0.03	38.60	1.93	1.07	0.27
248.29	25.55	4.47	1.14	0.26	0.07	5.06	1.29	0.36	0.09
479.66	23.89	9.06	2.16	0.40	0.10	10.26	2.45	0.38	0.10

* = EC50% was greater than the highest concentration tested.
\- = not tested.
CR = control ratio correction factor.
SE = standard error.

M. beryllina; Fresh ANSC; WAF; THC

M. beryllina Flowthrough exposure

Loading Rate (mg/L)	THC Conc. (mg/L)	Rep. 1 5min exposure EC_{50} %	Rep. 1 5min exposure EC_{50} (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 2 5min exposure EC_{50} %	Rep. 2 5min exposure EC_{50} (mg/L)	Rep. 2 5min exposure CR	Rep. 2 5min exposure SE
0.00	0.07	*	*	-	-	-	-	-	-
499.51	11.15	26.13	2.91	1.47	0.37	27.57	3.07	0.68	0.17
1952.14	19.47	20.92	4.07	1.28	0.33	23.71	4.62	1.04	0.27
3023.80	24.21	18.83	4.56	1.23	0.31	22.56	5.46	1.15	0.29
3996.23	28.31	14.44	4.09	0.47	0.12	16.71	4.73	0.91	0.23
6001.71	33.05	15.33	5.07	0.50	0.13	15.46	5.11	0.93	0.24

M. beryllina Static exposure

Day 1	0.00	0.47	*	*	-	-	-	-	-	-
	255.83	6.44	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-
	1034.11	16.88	24.55	4.14	1.25	0.32	nt	nt	-	-
	1952.14	15.36	20.92	3.21	1.01	0.26	23.71	3.64	0.82	0.21
	3023.80	17.65	18.83	3.32	0.90	0.23	22.56	3.98	0.84	0.21
	3996.23	26.40	14.44	3.81	0.44	0.11	16.71	4.41	0.84	0.22

= EC50% was greater than the highest concentration tested.
= not tested.
R = control ratio correction factor.
E = standard error.

M. beryllina; Fresh ANSC; CE-WAF

M. beryllina Flowthrough exposure

	Loading Rate (mg/L)	THC Conc. (mg/L)	Rep. 1 5min exposure EC_{50} %	Rep. 1 5min exposure EC_{50} (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 2 5min exposure EC_{50} %	Rep. 2 5min exposure EC_{50} (mg/L)	Rep. 2 5min exposure CR	Rep. 2 5min exposure SE
	0.00	0.01	*	*	-	-	*	*	-	-
m	152.91	8.72	23.21	2.02	0.01	0.00	23.24	2.03	0.40	0.10
	250.06	12.26	M. bahia	M. bahia	-	-	M. bahia	M. bahia	-	-
m	299.88	16.81	8.85	1.49	0.09	0.02	9.35	1.57	0.24	0.06
m	355.02	34.62	13.56	4.69	1.01	0.26	15.08	5.22	0.34	0.09
	400.14	18.62	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-

M. beryllina Static exposure

Day 1	0.00	0.54	*	*	-	-	*	*	-	-
	98.31	3.45	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-
m	152.91	7.73	23.21	1.79	0.01	0.00	23.24	1.80	0.36	0.09
	199.26	14.06	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-
	250.06	11.94	M. bahia	M. bahia	-	-	M. bahia	M. bahia	-	-
m	299.88	16.27	8.85	1.44	0.09	0.02	9.35	1.52	0.23	0.06

M. beryllina Flowthrough exposure

	Loading Rate (mg/L)	THC Conc. (mg/L)	Rep. 1 15min exposure EC_{50} %	Rep. 1 15min exposure EC_{50} (mg/L)	Rep. 1 15min exposure CR	Rep. 1 15min exposure SE	Rep. 2 15min exposure EC_{50} %	Rep. 2 15min exposure EC_{50} (mg/L)	Rep. 2 15min exposure CR	Rep. 2 15min exposure SE
	0.00	0.01	*	*	-	-	*	*	-	-
m	152.91	8.72	28.15	2.45	0.23	0.06	26.74	2.33	0.54	0.14
	250.06	12.26	M. bahia	M. bahia	-	-	M. bahia	M. bahia	-	-
m	299.88	16.81	10.64	1.79	0.13	0.03	11.33	1.90	0.23	0.06
m	355.02	34.62	18.44	6.38	1.57	0.40	18.20	6.30	0.35	0.09
	400.14	18.62	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-

M. beryllina Static exposure

Day 1	0.00	0.54	*	*	-	-	*	*	-	-
	98.31	3.45	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-
m	152.91	7.73	28.15	2.18	0.20	0.05	26.74	2.07	0.48	0.12
	199.26	14.06	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-
	250.06	11.94	M. bahia	M. bahia	-	-	M. bahia	M. bahia	-	-
m	299.88	16.27	10.64	1.73	0.13	0.03	11.33	1.84	0.22	0.06

* = EC50% was greater than the highest concentration tested.
\- = not tested.
CR = control ratio correction factor.
SE = standard error.

M. beryllina; Fresh PBCO; WAF; THC

M. beryllina Flowthrough exposure

Loading Rate (mg/L)	THC Conc. (mg/L)	Rep. 1 5min exposure EC_{50} %	Rep. 1 5min exposure EC_{50} (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 2 5min exposure EC_{50} %	Rep. 2 5min exposure EC_{50} (mg/L)	Rep. 2 5min exposure CR	Rep. 2 5min exposure SE
0.00	0.25	*	*	-	-	*	*	-	-
990.03	8.03	24.76	1.99	0.22	0.06	39.08	3.14	0.17	0.04
1994.26	12.69	38.07	4.83	0.61	0.16	34.72	4.41	1.04	0.27
2997.70	14.35	40.98	5.88	0.16	0.04	39.33	5.64	2.02	0.52
5994.89	18.58	24.24	4.50	0.60	0.15	21.24	3.95	2.03	0.52
8151.74	19.86	22.03	4.38	0.61	0.16	22.68	4.50	1.54	0.39

M. beryllina Static exposure

Day 1	0.00	0.00	*	*	-	-	*	*	-	-
	496.60	4.17	46.28	1.93	2.03	0.52	38.64	1.61	6.96	1.78
	990.03	6.12	24.76	1.52	0.17	0.04	39.08	2.39	0.13	0.03
	1994.26	9.33	38.07	3.55	0.45	0.11	34.72	3.24	0.76	0.19
	2997.70	12.49	40.06	5.00	0.48	0.12	39.33	4.91	1.76	0.45
	5994.89	16.14	24.24	3.91	0.52	0.13	21.24	3.43	1.76	0.45

* = EC50% was greater than the highest concentration tested.
- = not tested.
CR = control ratio correction factor.
SE = standard error.

M. beryllina; Fresh PBCO; CE-WAF; THC

M. beryllina Flowthrough exposure

Loading Rate (mg/L)	THC Conc. (mg/L)	Rep. 1 5min exposure EC_{50} %	Rep. 1 5min exposure EC_{50} (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 2 5min exposure EC_{50} %	Rep. 2 5min exposure EC_{50} (mg/L)	Rep. 2 5min exposure CR	Rep. 2 5min exposure SE
0.00	1.41	*	*	-	-	*	*	-	-
198.43	7.68	25.57	1.96	0.27	0.07	27.29	2.10	0.332544	0.08
302.66	22.06	8.55	1.89	0.59	0.15	7.62	1.68	0.6212096	0.16
417.54	22.62	9.57	2.17	0.34	0.09	11.18	2.53	0.558714	0.14
599.91	18.34	9.51	1.74	0.48	0.12	10.55	1.93	0.574042	0.15
817.60	26.34	8.30	2.19	0.49	0.13	7.88	2.08	0.351639	0.09

M. beryllina Static exposure

	Loading Rate (mg/L)	THC Conc. (mg/L)	Rep. 1 5min exposure EC_{50} %	Rep. 1 5min exposure EC_{50} (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 2 5min exposure EC_{50} %	Rep. 2 5min exposure EC_{50} (mg/L)	Rep. 2 5min exposure CR	Rep. 2 5min exposure SE
Day 1	0.00	0.54	*	*	-	-	*	*	-	-
	99.74	3.10	*	*	-	-	43.87	1.36	0.40269	0.10
	198.43	9.25	25.57	2.37	0.32	0.08	27.29	2.52	0.400525	0.10
	257.94	8.73	14.34	1.25	0.29	0.07	12.77	1.11	0.261027	0.07
	302.66	22.06	8.55	1.89	0.59	0.15	7.62	1.68	0.6212096	0.16
	417.54	22.62	9.57	2.17	0.34	0.09	11.18	2.53	0.558714	0.14

* = EC50% was greater than the highest concentration tested.
- = not tested.
CR = control ratio correction factor.
SE = standard error.

***M. beryllina*; Weathered ANSC; WAF; THC**

M. beryllina Flowthrough and Static exposures

Loading Rate (mg/L)	THC Conc. (mg/L)	Rep. 1 5min exposure EC_{50} %	Rep. 1 5min exposure EC_{50} (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 2 5min exposure EC_{50} %	Rep. 2 5min exposure EC_{50} (mg/L)	Rep. 2 5min exposure CR	Rep. 2 5min exposure SE
0.00	0.00	*	*	-	-	-	-	-	-
15315.90	1.02	50.14	0.51	0.10	0.03	46.00	0.47	0.124848	0.03
17569.68	1.13	43.51	0.49	0.03	0.01	46.36	0.52	0.055031	0.01
20192.11	1.12	26.45	0.30	0.02	0.00	33.76	0.38	0.100688	0.03
22457.11	0.96	41.01	0.39	0.06	0.02	44.41	0.43	0.032064	0.01
24948.00	1.04	39.06	0.41	0.06	0.01	42.20	0.44	0.125944	0.03

M. beryllina Flowthrough and Static exposure

Loading Rate (mg/L)	THC Conc. (mg/L)	Rep. 1 15min exposure EC_{50} %	Rep. 1 15min exposure EC_{50} (mg/L)	Rep. 1 15min exposure CR	Rep. 1 15min exposure SE	Rep. 2 15min exposure EC_{50} %	Rep. 2 15min exposure EC_{50} (mg/L)	Rep. 2 15min exposure CR	Rep. 2 15min exposure SE
0.00	0.00	-	-	-	-	-	-	-	-
15315.90	1.02	58.44	0.60	0.19	0.05	51.97	0.53	0.203388	0.05
17569.68	1.13	49.88	0.56	0.15	0.04	53.67	0.61	0.280918	0.07
20192.11	1.12	43.60	0.49	0.27	0.07	29.85	0.33	0.03304	0.01
22457.11	0.96	44.82	0.43	0.18	0.05	50.96	0.49	0.1537248	0.04
24948.00	1.04	-	-	-	-	46.46	0.48	0.100776	0.03

* = EC50% was greater than the highest concentration tested.
\- = not tested.
CR = control ratio correction factor.
SE = standard error.

M. beryllina; Weathered ANSC; CE-WAF; THC

M. beryllina Flowthrough and Static exposure

Loading Rate (mg/L)	THC Conc. (mg/L)	Rep. 1 5min exposure EC_{50} %	Rep. 1 5min exposure EC_{50} (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 2 5min exposure EC_{50} %	Rep. 2 5min exposure EC_{50} (mg/L)	Rep. 2 5min exposure CR	Rep. 2 5min exposure SE
0.00	0.00	-	-	-	-	*	-	-	-
96.80	1.07	*	*	-	-	*	*	-	-
199.11	9.54	44.45	4.24	0.80	0.20	40.63	3.88	0.440748	0.11
396.77	12.97	25.62	3.32	0.37	0.09	23.95	3.11	0.164719	0.04
600.25	17.56	25.09	4.41	1.69	0.43	31.55	5.54	0.9039888	0.23
1004.91	43.23	9.11	3.94	0.67	0.17	8.01	3.46	0.8658969	0.22

M. beryllina Flowthrough and Static exposure

Loading Rate (mg/L)	THC Conc. (mg/L)	Rep. 1 15min exposure EC_{50} %	Rep. 1 15min exposure EC_{50} (mg/L)	Rep. 1 15min exposure CR	Rep. 1 15min exposure SE	Rep. 2 15min exposure EC_{50} %	Rep. 2 15min exposure EC_{50} (mg/L)	Rep. 2 15min exposure CR	Rep. 2 15min exposure SE
0.00	0.00	*	*	-	-	*	*	-	-
96.80	1.07	*	*	-	-	*	*	-	-
199.11	9.54	*	*	-	-	*	*	-	-
396.77	12.97	34.09	4.42	0.30	0.08	36.82	4.78	0.929949	0.24
600.25	17.56	32.57	5.72	3.24	0.83	40.67	7.14	1.0327036	0.26
1004.91	43.23	12.51	5.41	0.86	0.22	10.02	4.33	1.2432948	0.32

* = EC50% was greater than the highest concentration tested.
\- = not tested.
CR = control ratio correction factor.
SE = standard error.

C. bardi; Fresh ANSC; WAF; VOA

C. bardi Flowthrough exposure

Loading Rate (mg/L)	VOA Conc. (mg/L)	Rep. 1 5min exposure EC_{50}%	Rep. 1 5min exposure EC_{50} (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 1 15min exposure EC_{50}%	Rep. 1 15min exposure EC_{50} (mg/L)	Rep. 1 15min exposure CR	Rep. 1 15min exposure SE
0.00	0.17	*	*	-	-	*	*	-	-
201.86	7.45	36.79	2.74	0.84	0.21	46.77	3.48	2.24	0.57
401.09	12.43	25.36	3.15	0.47	0.12	35.62	4.43	2.11	0.54
1099.66	19.24	25.55	4.92	1.46	0.37	35.49	6.83	2.44	0.62
1814.86	22.10	29.10	6.43	1.77	0.45	37.66	8.32	3.20	0.82
2499.23	30.45	23.80	7.25	1.57	0.40	31.90	9.71	3.02	0.77

C. bardi Static exposure

Day 1	0.00	0.00	*	*	-	-	*	-	-	-
	47.03	2.35	*	*	-	-	*	-	-	-
	254.00	8.63	38.35	3.31	1.09	0.28	45.34	3.91	2.15	0.55
	463.94	13.02	31.18	4.06	0.30	0.08	39.79	5.18	0.86	0.22
	773.94	19.02	22.23	4.23	0.79	0.20	28.20	5.36	1.57	0.40
	1017.37	24.11	28.91	6.97	3.04	0.77	38.84	9.36	4.75	1.21
Day 2	0.00	0.00	-	-	-	-	-	-	-	-
	56.54	2.35	-	-	-	-	-	-	-	-
	233.80	8.63	*	*	-	-	*	*	-	-
	414.74	13.02	32.49	4.23	0.88	0.23	43.44	5.66	2.91	0.74
	702.11	19.02	22.94	4.36	0.73	0.19	29.02	5.52	1.20	0.31
	994.71	24.11	14.43	3.48	0.52	0.13	18.16	4.38	0.92	0.23
Day 3	0.00	0.00	-	-	-	-	-	-	-	-
	49.49	2.35	-	-	-	-	-	-	-	-
	228.17	8.63	40.82	3.52	1.85	0.47	49.56	4.28	3.42	0.87
	399.83	13.02	40.18	5.23	-1.39	-0.35	*	*	-	-
	685.63	19.02	42.74	8.13	2.62	0.67	*	*	-	-
	1001.14	24.11	31.01	7.48	0.32	0.08	40.65	9.80	3.95	1.01
Day 4	0.00	0.00	-	-	-	-	-	-	-	-
	50.49	2.35	*	*	-	-	*	*	-	-
	220.57	8.63	35.48	3.06	0.83	0.21	41.85	3.61	1.28	0.33
	435.80	13.02	43.02	5.60	1.88	0.48	*	*	-	-
	702.34	19.02	37.82	7.19	6.20	1.58	*	*	-	-
	981.94	24.11	22.69	5.47	0.75	0.19	29.34	7.07	1.03	0.26

* = EC50% was greater than the highest concentration tested.
- = not tested.
CR = control ratio correction factor.
SE = standard error.

C. bardi; Fresh ANSC; CE-WAF; VOA

C. bardi Flowthrough exposure

Loading Rate (mg/L)	VOA Conc. (mg/L)	Rep. 1 5min exposure EC_{50}%	Rep. 1 5min exposure EC_{50} (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 1 15min exposure EC_{50}%	Rep. 1 15min exposure EC_{50} (mg/L)	Rep. 1 15min exposure CR	Rep. 1 15min exposure SE
0.00	0.15	*	*	-	-	*	*	-	-
99.83	4.28	23.70	1.01	0.18	0.05	31.81	1.36	0.13	0.03
204.36	8.42	14.28	1.20	0.04	0.01	18.66	1.57	0.24	0.06
402.32	16.48	3.56	0.59	0.14	0.03	5.43	0.89	0.14	0.04
1100.75	35.19	1.72	0.60	0.02	0.01	2.22	0.78	0.22	0.06
1853.79	44.64	1.02	0.46	0.09	0.02	1.26	0.56	0.10	0.03

C. bardi Static exposure

Day 1	0.00	0.00	*	*	-	-	*	*	-	-
	25.39	1.41	-	-	-	-	-	-	-	-
	50.95	2.31	55.02	1.27	0.66	0.17	*	*	-	-
	223.67	9.58	10.51	1.01	0.03	0.01	14.16	1.36	0.09	0.02
	395.99	15.95	7.61	1.21	0.17	0.04	11.18	1.78	0.25	0.06
	701.26	28.74	3.20	0.92	0.05	0.01	4.11	1.18	0.03	0.01
Day 2	0.00	0.00	-	-	-	-	-	-	-	-
	26.17	1.41	-	-	-	-	-	-	-	-
	50.89	2.31	*	*	-	-	*	*	-	-
	228.09	9.58	12.99	1.24	0.03	0.01	20.84	2.00	0.23	0.06
	399.41	15.95	7.68	1.23	0.03	0.01	9.90	1.58	0.17	0.04
	704.06	28.74	1.58	0.45	0.04	0.01	2.58	0.74	0.17	0.04
Day 3	0.00	0.00	-	-	-	-	-	-	-	-
	26.69	1.41	-	-	-	-	-	-	-	-
	50.80	2.31	48.34	1.12	1.10	0.28	*	*	-	-
	223.28	9.58	13.79	1.32	0.16	0.04	22.80	2.18	0.50	0.13
	400.40	15.95	8.66	1.38	0.17	0.04	11.71	1.87	0.18	0.05
	702.19	28.74	2.87	0.82	0.06	0.02	3.77	1.08	0.06	0.02
Day 4	0.00	0.00	-	-	-	-	-	-	-	-
	28.22	1.41	-	-	-	-	-	-	-	-
	51.58	2.31	*	*	-	-	*	*	-	-
	226.19	9.58	36.95	3.54	0.38	0.10	47.12	4.51	1.22	0.31
	404.37	15.95	7.79	1.24	0.16	0.04	11.67	1.86	0.32	0.08
	711.86	28.74	2.69	0.77	0.09	0.02	3.54	1.02	0.20	0.05

* = EC50% was greater than the highest concentration tested.
- = not tested.
CR = control ratio correction factor.
SE = standard error.

C. bardi; Weathered ANSC; WAF; VOA

C. bardi Flowthrough/Static exposure

Loading Rate (mg/L)	VOA Conc. (mg/L)	Rep. 1 5min exposure EC$_{50}$%	Rep. 1 5min exposure EC$_{50}$ (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 1 15min exposure EC$_{50}$%	Rep. 1 15min exposure EC$_{50}$ (mg/L)	Rep. 1 15min exposure CR	Rep. 1 15min exposure SE
0.00	0.00	*	*	-	-	*	*	-	-
496.26	0.10	*	*	-	-	*	*	-	-
2002.83	0.21	*	*	-	-	*	*	-	-
4983.86	0.29	*	*	-	-	*	*	-	-
6993.25	0.29	49.48	0.14	0.08	0.02	*	*	-	-
10011.26	0.32	48.89	0.16	0.02	0.00	*	*	-	-

Loading Rate (mg/L)	VOA Conc. (mg/L)	Rep. 2 5min exposure EC$_{50}$%	Rep. 2 5min exposure EC$_{50}$ (mg/L)	Rep. 2 5min exposure CR	Rep. 2 5min exposure SE	Rep. 2 15min exposure EC$_{50}$%	Rep. 2 15min exposure EC$_{50}$ (mg/L)	Rep. 2 15min exposure CR	Rep. 2 15min exposure SE
0.00	0.00	*	*	-	-	*	*	-	-
496.26	0.10	*	*	-	-	*	*	-	-
2002.83	0.21	*	*	-	-	*	*	-	-
4983.86	0.29	*	*	-	-	*	*	-	-
6993.25	0.29	54.15	0.16	0.02	0.01	*	*	-	-
10011.26	0.32	45.27	0.14	0.02	0.00	*	*	-	-

* = EC50% was greater than the highest concentration tested.

\- = not tested.

CR = control ratio correction factor.

SE = standard error.

C. bardi; Weathered ANSC; CE-WAF; VOA

C. bardi Flowthrough/Static exposure

Loading Rate (mg/L)	VOA Conc. (mg/L)	Rep. 1 5min exposure EC_{50}%	Rep. 1 5min exposure EC_{50} (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 1 15min exposure EC_{50}%	Rep. 1 15min exposure EC_{50} (mg/L)	Rep. 1 15min exposure CR	Rep. 1 15min exposure SE
0.00	0.00	*	*	-	-	*	*	-	-
46.86	0.04	*	*	-	-	*	*	-	-
97.14	0.10	*	*	-	-	*	*	-	-
504.40	0.28	45.11	0.13	0.02	0.00	*	*	-	-
999.51	0.27	21.63	0.06	0.01	0.00	30.69	0.08	0.01	0.00
2494.68	1.36	21.73	0.30	0.03	0.01	27.54	0.37	0.04	0.01

Loading Rate (mg/L)	VOA Conc. (mg/L)	Rep. 2 5min exposure EC_{50}%	Rep. 2 5min exposure EC_{50} (mg/L)	Rep. 2 5min exposure CR	Rep. 2 5min exposure SE	Rep. 2 15min exposure EC_{50}%	Rep. 2 15min exposure EC_{50} (mg/L)	Rep. 2 15min exposure CR	Rep. 2 15min exposure SE
0.00	0.00	*	*	-	-	*	*	-	-
46.86	0.04	*	*	-	-	*	*	-	-
97.14	0.10	*	*	-	-	*	*	-	-
504.40	0.28	*	*	-	-	*	*	-	-
999.51	0.27	24.25	0.07	0.01	0.00	36.47	0.10	0.04	0.01
2494.68	1.36	20.52	0.28	0.03	0.01	28.84	0.39	0.09	0.02

* = EC50% was greater than the highest concentration tested.

\- = not tested.

CR = control ratio correction factor.

SE = standard error.

M. bahia; Fresh ANSC; WAF; VOA

M. bahia Flowthrough exposure

Loading Rate (mg/L)	VOA Conc. (mg/L)	Rep. 1 5min exposure EC_{50} %	Rep. 1 5min exposure EC_{50} (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 2 5min exposure EC_{50} %	Rep. 2 5min exposure EC_{50} (mg/L)	Rep. 2 5min exposure CR	Rep. 2 5min exposure SE
0.00	0.97	*	*	-	-	*	*	-	-
139.26	2.83	37.08	1.05	0.47	0.12	*	*	-	-
250.51	4.32	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-
500.34	8.34	30.75	2.56	0.39	0.10	35.51	2.96	0.52	0.13
752.51	8.11	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-
2497.86	12.20	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-

M. bahia Static exposure

0.00	0.28	*	*	-	-	*	*	-	-
25.66	0.58	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-
139.26	1.69	37.08	0.63	0.28	0.07	*	*	-	-
250.51	3.15	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-
500.34	5.15	30.75	1.58	0.24	0.06	35.51	1.83	0.32	0.08
752.51	5.13	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-

* = EC50% was greater than the highest concentration tested.
\- = not tested.
CR = control ratio correction factor.
SE = standard error.

M. bahia; Fresh ANSC; CE-WAF; VOA

M. bahia Flowthrough exposure

Loading Rate (mg/L)	VOA Conc. (mg/L)	Rep. 1 5min exposure EC_{50} %	Rep. 1 5min exposure EC_{50} (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 2 5min exposure EC_{50} %	Rep. 2 5min exposure EC_{50} (mg/L)	Rep. 2 5min exposure CR	Rep. 2 5min exposure SE
0.00	0.14	*	*	-	-	-	-	-	-
26.26	0.20	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-
102.46	1.51	33.53	0.51	0.04	0.01	38.60	0.58	0.32	0.08
248.29	6.54	4.47	0.29	0.07	0.02	5.06	0.33	0.09	0.02
479.66	10.24	9.06	0.93	0.17	0.04	10.26	1.05	0.16	0.04
702.80	15.41	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-

M. bahia Static exposure

Loading Rate (mg/L)	VOA Conc. (mg/L)	Rep. 1 5min exposure EC_{50} %	Rep. 1 5min exposure EC_{50} (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 2 5min exposure EC_{50} %	Rep. 2 5min exposure EC_{50} (mg/L)	Rep. 2 5min exposure CR	Rep. 2 5min exposure SE
0.00	0.54	*	*	-	-	-	-	-	-
10.09	0.29	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-
26.26	0.85	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-
102.46	2.07	33.53	0.69	0.06	0.01	38.60	0.80	0.44	0.11
248.29	7.04	4.47	0.31	0.07	0.02	5.06	0.36	0.10	0.03
479.66	9.89	9.06	0.90	0.17	0.04	10.26	1.01	0.16	0.04

* = EC50% was greater than the highest concentration tested.
\- = not tested.
CR = control ratio correction factor.
SE = standard error.

M. beryllina; Fresh ANSC; WAF; VOA

M. beryllina Flowthrough exposure

Loading Rate (mg/L)	VOA Conc. (mg/L)	Rep. 1 5min exposure EC$_{50}$ %	Rep. 1 5min exposure EC$_{50}$ (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 2 5min exposure EC$_{50}$ %	Rep. 2 5min exposure EC$_{50}$ (mg/L)	Rep. 2 5min exposure CR	Rep. 2 5min exposure SE
0.00	0.07	*	*	-	-	-	-	-	-
499.51	10.73	26.13	2.80	1.41	0.36	27.57	2.96	0.65	0.17
1952.14	18.97	20.92	3.97	1.25	0.32	23.71	4.50	1.01	0.26
3023.80	23.87	18.83	4.49	1.21	0.31	22.56	5.39	1.14	0.29
3996.23	27.91	14.44	4.03	0.47	0.12	16.71	4.66	0.89	0.23
6001.71	32.67	15.33	5.01	0.50	0.13	15.46	5.05	0.92	0.23

M. beryllina Static exposure

Day 1	0.00	0.43	*	*	-	-	-	-	-	-
	255.83	6.00	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-
	1034.11	16.35	24.55	4.01	1.21	0.31	nt	nt	-	-
	1952.14	14.86	20.92	3.11	0.98	0.25	23.71	3.52	0.79	0.20
	3023.80	17.31	18.83	3.26	0.88	0.22	22.56	3.91	0.83	0.21
	3996.23	26.00	14.44	3.75	0.43	0.11	16.71	4.34	0.83	0.21

* = EC50% was greater than the highest concentration tested.

- = not tested.

CR = control ratio correction factor.

SE = standard error.

***M. beryllina*; Fresh ANSC; CE-WAF; VOA**

M. beryllina Flowthrough exposure

	Loading Rate (mg/L)	VOA Conc. (mg/L)	Rep. 1 5min exposure EC_{50} %	Rep. 1 5min exposure EC_{50} (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 2 5min exposure EC_{50} %	Rep. 2 5min exposure EC_{50} (mg/L)	Rep. 2 5min exposure CR	Rep. 2 5min exposure SE
	0.00	0.01	*	*	-	-	*	*	-	-
m	152.91	2.51	23.21	0.58	0.00	0.00	23.24	0.58	0.12	0.03
	250.06	4.29	M. bahia	M. bahia	-	-	M. bahia	M. bahia	-	-
m	299.88	3.99	8.85	0.35	0.02	0.01	9.35	0.37	0.06	0.01
m	355.02	10.14	13.56	1.37	0.30	0.08	15.08	1.53	0.10	0.03
	400.14	6.83	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-

M. beryllina Static exposure

Day 1	0.00	0.50	*	*	-	-	*	*	-	-
	98.31	0.76	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-
m	152.91	1.95	23.21	0.45	0.00	0.00	23.24	0.45	0.09	0.02
	199.26	3.11	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-
	250.06	4.24	M. bahia	M. bahia	-	-	M. bahia	M. bahia	-	-
m	299.88	3.99	8.85	0.35	0.02	0.01	9.35	0.37	0.06	0.01

M. beryllina Flowthrough exposure

	Loading Rate (mg/L)	VOA Conc. (mg/L)	Rep. 1 15min exposure EC_{50} %	Rep. 1 15min exposure EC_{50} (mg/L)	Rep. 1 15min exposure CR	Rep. 1 15min exposure SE	Rep. 2 15min exposure EC_{50} %	Rep. 2 15min exposure EC_{50} (mg/L)	Rep. 2 15min exposure CR	Rep. 2 15min exposure SE
	0.00	0.01	*	*	-	-	*	*	-	-
m	152.91	2.51	28.15	0.71	0.07	0.02	26.74	0.67	0.15	0.04
	250.06	4.29	M. bahia	M. bahia	-	-	M. bahia	M. bahia	-	-
m	299.88	3.99	10.64	0.42	0.03	0.01	11.33	0.45	0.05	0.01
m	355.02	10.14	18.44	1.87	0.46	0.12	18.20	1.85	0.10	0.03
	400.14	6.83	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-

M. beryllina Static exposure

Day 1	0.00	0.50	*	*	-	-	*	*	-	-
	98.31	0.76	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-
m	152.91	1.95	28.15	0.55	0.05	0.01	26.74	0.52	0.12	0.03
	199.26	3.11	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-
	250.06	4.24	M. bahia	M. bahia	-	-	M. bahia	M. bahia	-	-
m	299.88	3.99	10.64	0.42	0.03	0.01	11.33	0.45	0.05	0.01

* = EC50% was greater than the highest concentration tested.
\- = not tested.
CR = control ratio correction factor.
SE = standard error.

M. beryllina; Fresh PBCO; WAF; VOA

M. beryllina Flowthrough exposure

Loading Rate (mg/L)	VOA Conc. (mg/L)	Rep. 1 5min exposure EC_{50} %	Rep. 1 5min exposure EC_{50} (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 2 5min exposure EC_{50} %	Rep. 2 5min exposure EC_{50} (mg/L)	Rep. 2 5min exposure CR	Rep. 2 5min exposure SE
0.00	0.25	*	*	-	-	*	*	-	-
990.03	7.81	24.76	1.93	0.21	0.05	39.08	3.05	0.16	0.04
1994.26	12.36	38.07	4.71	0.60	0.15	34.72	4.29	1.01	0.26
2997.70	13.88	40.98	5.69	0.16	0.04	39.33	5.46	1.95	0.50
5994.89	18.25	24.24	4.42	0.59	0.15	21.24	3.88	1.99	0.51
8151.74	19.55	22.03	4.31	0.60	0.15	22.68	4.43	1.51	0.39

M. beryllina Static exposure

Day 1	0.00	0.00	*	*	-	-	*	*	-	-
	496.60	4.00	46.28	1.85	1.95	0.50	38.64	1.55	6.68	1.70
	990.03	5.90	24.76	1.46	0.16	0.04	39.08	2.31	0.12	0.03
	1994.26	9.06	38.07	3.45	0.44	0.11	34.72	3.15	0.74	0.19
	2997.70	12.18	40.06	4.88	0.47	0.12	39.33	4.79	1.71	0.44
	5994.89	15.89	24.24	3.85	0.51	0.13	21.24	3.38	1.73	0.44

* = EC50% was greater than the highest concentration tested.
\- = not tested.
CR = control ratio correction factor.
SE = standard error.

M. beryllina; Fresh PBCO; CE-WAF; VOA

M. beryllina Flowthrough exposure

Loading Rate (mg/L)	VOA Conc. (mg/L)	Rep. 1 5min exposure EC_{50} %	Rep. 1 5min exposure EC_{50} (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 2 5min exposure EC_{50} %	Rep. 2 5min exposure EC_{50} (mg/L)	Rep. 2 5min exposure CR	Rep. 2 5min exposure SE
0.00	1.41	*	*	-	-	*	*	-	-
198.43	2.89	25.57	0.74	0.10	0.03	27.29	0.79	0.125137	0.03
302.66	5.84	8.55	0.50	0.16	0.04	7.62	0.45	0.1644544	0.04
417.54	6.18	9.57	0.59	0.09	0.02	11.18	0.69	0.152646	0.04
599.91	7.42	9.51	0.71	0.19	0.05	10.55	0.78	0.232246	0.06
817.60	9.64	8.30	0.80	0.18	0.05	7.88	0.76	0.128694	0.03

M. beryllina Static exposure

Day 1	0.00	0.54	*	*	-	-	*	*	-	-
	99.74	1.80	*	*	-	-	43.87	0.79	0.23382	0.06
	198.43	3.28	25.57	0.84	0.11	0.03	27.29	0.90	0.142024	0.04
	257.94	3.87	14.34	0.55	0.13	0.03	12.77	0.49	0.115713	0.03
	302.66	5.84	8.55	0.50	0.16	0.04	7.62	0.45	0.1644544	0.04
	417.54	6.18	9.57	0.59	0.09	0.02	11.18	0.69	0.152646	0.04

* = EC50% was greater than the highest concentration tested.
- = not tested.
CR = control ratio correction factor.
SE = standard error.

M. beryllina; Weathered ANSC; WAF; VOA

M. beryllina Flowthrough and Static exposures

Loading Rate (mg/L)	VOA Conc. (mg/L)	Rep. 1 5min exposure EC_{50} %	Rep. 1 5min exposure EC_{50} (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 2 5min exposure EC_{50} %	Rep. 2 5min exposure EC_{50} (mg/L)	Rep. 2 5min exposure CR	Rep. 2 5min exposure SE
0.00	0.00	*	*	-	-	-	-	-	-
15315.90	0.54	50.14	0.27	0.05	0.01	46.00	0.25	0.066096	0.02
17569.68	0.60	43.51	0.26	0.01	0.00	46.36	0.28	0.02922	0.01
20192.11	0.70	26.45	0.19	0.01	0.00	33.76	0.24	0.06293	0.02
22457.11	0.66	41.01	0.27	0.04	0.01	44.41	0.29	0.022044	0.01
24948.00	0.60	39.06	0.23	0.03	0.01	42.20	0.25	0.016896	0.00

M. beryllina Flowthrough and Static exposure

Loading Rate (mg/L)	VOA Conc. (mg/L)	Rep. 1 15min exposure EC_{50} %	Rep. 1 15min exposure EC_{50} (mg/L)	Rep. 1 15min exposure CR	Rep. 1 15min exposure SE	Rep. 2 15min exposure EC_{50} %	Rep. 2 15min exposure EC_{50} (mg/L)	Rep. 2 15min exposure CR	Rep. 2 15min exposure SE
0.00	0.00	-	-	-	-	-	-	-	-
15315.90	0.54	58.44	0.32	0.10	0.03	51.97	0.28	0.107676	0.03
17569.68	0.60	49.88	0.30	0.08	0.02	53.67	0.32	0.14916	0.04
20192.11	0.70	43.60	0.31	0.17	0.04	29.85	0.21	0.02065	0.01
22457.11	0.66	44.82	0.30	0.12	0.03	50.96	0.34	0.1056858	0.03
24948.00	0.60	-	-	-	-	46.46	0.28	0.05814	0.01

* = EC50% was greater than the highest concentration tested.
- = not tested.
CR = control ratio correction factor.
SE = standard error.

M. beryllina; Weathered ANSC; CE-WAF; VOA

M. beryllina Flowthrough and Static exposure

Loading Rate (mg/L)	VOA Conc. (mg/L)	Rep. 1 5min exposure EC$_{50}$ %	Rep. 1 5min exposure EC$_{50}$ (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 2 5min exposure EC$_{50}$ %	Rep. 2 5min exposure EC$_{50}$ (mg/L)	Rep. 2 5min exposure CR	Rep. 2 5min exposure SE
0.00	0.00	-	-	-	-	*	-	-	-
96.80	0.11	*	*	-	-	*	*	-	-
199.11	0.21	44.45	0.09	0.02	0.00	40.63	0.09	0.009702	0.00
396.77	0.39	25.62	0.10	0.01	0.00	23.95	0.09	0.004953	0.00
600.25	0.45	25.09	0.11	0.04	0.01	31.55	0.14	0.023166	0.01
1004.91	0.73	9.11	0.07	0.01	0.00	8.01	0.06	0.0146219	0.00

M. beryllina Flowthrough and Static exposure

Loading Rate (mg/L)	VOA Conc. (mg/L)	Rep. 1 15min exposure EC$_{50}$ %	Rep. 1 15min exposure EC$_{50}$ (mg/L)	Rep. 1 15min exposure CR	Rep. 1 15min exposure SE	Rep. 2 15min exposure EC$_{50}$ %	Rep. 2 15min exposure EC$_{50}$ (mg/L)	Rep. 2 15min exposure CR	Rep. 2 15min exposure SE
0.00	0.00	*	*	-	-	*	*	-	-
96.80	0.11	*	*	-	-	*	*	-	-
199.11	0.21	*	*	-	-	*	*	-	-
396.77	0.39	34.09	0.13	0.01	0.00	36.82	0.14	0.027963	0.01
600.25	0.45	32.57	0.15	0.08	0.02	40.67	0.18	0.0264645	0.01
1004.91	0.73	12.51	0.09	0.01	0.00	10.02	0.07	0.0209948	0.01

* = EC50% was greater than the highest concentration tested.

\- = not tested.

CR = control ratio correction factor.

SE = standard error.

C. bardi; Fresh ANSC; WAF; TPH

C. bardi Flowthrough exposure

Loading Rate (mg/L)	TPH Conc. (mg/L)	Rep. 1 5min exposure EC_{50}%	Rep. 1 5min exposure EC_{50} (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 1 15min exposure EC_{50}%	Rep. 1 15min exposure EC_{50} (mg/L)	Rep. 1 15min exposure CR	Rep. 1 15min exposure SE
0.00	0.00	*	*	-	-	*	*	-	-
201.86	0.11	36.79	0.04	0.01	0.00	46.77	0.05	0.03	0.01
401.09	0.12	25.36	0.03	0.00	0.00	35.62	0.04	0.02	0.01
1099.66	0.11	25.55	0.03	0.01	0.00	35.49	0.04	0.01	0.00
1814.86	0.11	29.10	0.03	0.01	0.00	37.66	0.04	0.02	0.00
2499.23	0.12	23.80	0.03	0.01	0.00	31.90	0.04	0.01	0.00

C. bardi Static exposure

Day 1	0.00	0.00	*	*	-	-	*	-	-	-
	47.03	0.12	*	*	-	-	*	-	-	-
	254.00	0.15	38.35	0.06	0.02	0.00	45.34	0.07	0.04	0.01
	463.94	0.16	31.18	0.05	0.00	0.00	39.79	0.06	0.01	0.00
	773.94	0.14	22.23	0.03	0.01	0.00	28.20	0.04	0.01	0.00
	1017.37	0.22	28.91	0.06	0.03	0.01	38.84	0.09	0.04	0.01
Day 2	0.00	0.00	-	-	-	-	-	-	-	-
	56.54	0.12	-	-	-	-	-	-	-	-
	233.80	0.15	*	*	-	-	*	*	-	-
	414.74	0.16	32.49	0.05	0.01	0.00	43.44	0.07	0.04	0.01
	702.11	0.14	22.94	0.03	0.01	0.00	29.02	0.04	0.01	0.00
	994.71	0.22	14.43	0.03	0.00	0.00	18.16	0.04	0.01	0.00
Day 3	0.00	0.00	-	-	-	-	-	-	-	-
	49.49	0.12	-	-	-	-	-	-	-	-
	228.17	0.15	40.82	0.06	0.03	0.01	49.56	0.07	0.06	0.02
	399.83	0.16	40.18	0.06	-0.02	0.00	*	*	-	-
	685.63	0.14	42.74	0.06	0.02	0.00	*	*	-	-
	1001.14	0.22	31.01	0.07	0.00	0.00	40.65	0.09	0.04	0.01
Day 4	0.00	0.00	-	-	-	-	-	-	-	-
	50.49	0.12	*	*	-	-	*	*	-	-
	220.57	0.15	35.48	0.05	0.01	0.00	41.85	0.06	0.02	0.01
	435.80	0.16	43.02	0.07	0.02	0.01	*	*	-	-
	702.34	0.14	37.82	0.05	0.05	0.01	*	*	-	-
	981.94	0.22	22.69	0.05	0.01	0.00	29.34	0.06	0.01	0.00

* = EC50% was greater than the highest concentration tested.
\- = not tested.
CR = control ratio correction factor.
SE = standard error.

C. bardi; Fresh ANSC; CE-WAF; TPH

C. bardi Flowthrough exposure

Loading Rate (mg/L)	TPH Conc. (mg/L)	Rep. 1 5min exposure EC_{50}%	Rep. 1 5min exposure EC_{50} (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 1 15min exposure EC_{50}%	Rep. 1 15min exposure EC_{50} (mg/L)	Rep. 1 15min exposure CR	Rep. 1 15min exposure SE
0.00	1.52	*	*	-	-	*	*	-	-
99.83	0.74	23.70	0.18	0.03	0.01	31.81	0.24	0.02	0.01
204.36	2.15	14.28	0.31	0.01	0.00	18.66	0.40	0.06	0.02
402.32	7.67	3.56	0.27	0.06	0.02	5.43	0.42	0.07	0.02
1100.75	18.74	1.72	0.32	0.01	0.00	2.22	0.42	0.12	0.03
1853.79	51.60	1.02	0.53	0.10	0.03	1.26	0.65	0.12	0.03

C. bardi Static exposure

	Loading Rate (mg/L)	TPH Conc. (mg/L)	Rep. 1 5min exposure EC_{50}%	Rep. 1 5min exposure EC_{50} (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 1 15min exposure EC_{50}%	Rep. 1 15min exposure EC_{50} (mg/L)	Rep. 1 15min exposure CR	Rep. 1 15min exposure SE
Day 1	0.00	0.00	*	*	-	-	*	*	-	-
	25.39	0.29	-	-	-	-	-	-	-	-
	50.95	0.67	55.02	0.37	0.19	0.05	*	*	-	-
	223.67	6.79	10.51	0.71	0.02	0.01	14.16	0.96	0.07	0.02
	395.99	13.15	7.61	1.00	0.14	0.04	11.18	1.47	0.21	0.05
	701.26	51.45	3.20	1.65	0.10	0.02	4.11	2.11	0.05	0.01
Day 2	0.00	0.00	-	-	-	-	-	-	-	-
	26.17	0.29	-	-	-	-	-	-	-	-
	50.89	0.67	*	*	-	-	*	*	-	-
	228.09	6.79	12.99	0.88	0.02	0.01	20.84	1.42	0.16	0.04
	399.41	13.15	7.68	1.01	0.02	0.01	9.90	1.30	0.14	0.04
	704.06	51.45	1.58	0.81	0.06	0.02	2.58	1.33	0.31	0.08
Day 3	0.00	0.00	-	-	-	-	-	-	-	-
	26.69	0.29	-	-	-	-	-	-	-	-
	50.80	0.67	48.34	0.32	0.32	0.08	*	*	-	-
	223.28	6.79	13.79	0.94	0.11	0.03	22.80	1.55	0.36	0.09
	400.40	13.15	8.66	1.14	0.14	0.04	11.71	1.54	0.15	0.04
	702.19	51.45	2.87	1.48	0.11	0.03	3.77	1.94	0.11	0.03
Day 4	0.00	0.00	-	-	-	-	-	-	-	-
	28.22	0.29	-	-	-	-	-	-	-	-
	51.58	0.67	*	*	-	-	*	*	-	-
	226.19	6.79	36.95	2.51	0.27	0.07	47.12	3.20	0.87	0.22
	404.37	13.15	7.79	1.02	0.13	0.03	11.67	1.53	0.27	0.07
	711.86	51.45	2.69	1.38	0.17	0.04	3.54	1.82	0.35	0.09

* = EC50% was greater than the highest concentration tested.
\- = not tested.
CR = control ratio correction factor.
SE = standard error.

C. bardi; Weathered ANSC; WAF; TPH

C. bardi Flowthrough/Static exposure

Loading Rate (mg/L)	TPH Conc. (mg/L)	Rep. 1 5min exposure EC$_{50}$%	Rep. 1 5min exposure EC$_{50}$ (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 1 15min exposure EC$_{50}$%	Rep. 1 15min exposure EC$_{50}$ (mg/L)	Rep. 1 15min exposure CR	Rep. 1 15min exposure SE
0.00	0.00	*	*	-	-	*	*	-	-
496.26	0.12	*	*	-	-	*	*	-	-
2002.83	0.08	*	*	-	-	*	*	-	-
4983.86	0.12	*	*	-	-	*	*	-	-
6993.25	0.16	49.48	0.08	0.04	0.01	*	*	-	-
10011.26	0.13	48.89	0.06	0.01	0.00	*	*	-	-

Loading Rate (mg/L)	Loading Rate (mg/L)	Rep. 2 5min exposure EC$_{50}$%	Rep. 2 5min exposure EC$_{50}$ (mg/L)	Rep. 2 5min exposure CR	Rep. 2 5min exposure SE	Rep. 2 15min exposure EC$_{50}$%	Rep. 2 15min exposure EC$_{50}$ (mg/L)	Rep. 2 15min exposure CR	Rep. 2 15min exposure SE
0.00	0.00	*	*	-	-	*	*	-	-
496.26	0.12	*	*	-	-	*	*	-	-
2002.83	0.08	*	*	-	-	*	*	-	-
4983.86	0.12	*	*	-	-	*	*	-	-
6993.25	0.16	54.15	0.09	0.01	0.00	*	*	-	-
10011.26	0.13	45.27	0.06	0.01	0.00	*	*	-	-

* = EC50% was greater than the highest concentration tested.

\- = not tested.

CR = control ratio correction factor.

SE = standard error.

C. bardi; Weathered ANSC; CE-WAF; TPH

C. bardi Flowthrough/Static exposure

Loading Rate (mg/L)	TPH Conc. (mg/L)	Rep. 1 5min exposure EC$_{50}$%	Rep. 1 5min exposure EC$_{50}$ (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 1 15min exposure EC$_{50}$%	Rep. 1 15min exposure EC$_{50}$ (mg/L)	Rep. 1 15min exposure CR	Rep. 1 15min exposure SE
0.00	0.00	*	*	-	-	*	*	-	-
46.86	0.77	*	*	-	-	*	*	-	-
97.14	1.59	*	*	-	-	*	*	-	-
504.40	13.25	45.11	5.98	0.88	0.22	*	*	-	-
999.51	25.00	21.63	5.41	1.07	0.27	30.69	7.67	0.88	0.22
2494.68	66.73	21.73	14.50	1.57	0.40	27.54	18.38	1.80	0.46

Loading Rate (mg/L)	Loading Rate (mg/L)	Rep. 2 5min exposure EC$_{50}$%	Rep. 2 5min exposure EC$_{50}$ (mg/L)	Rep. 2 5min exposure CR	Rep. 2 5min exposure SE	Rep. 2 15min exposure EC$_{50}$%	Rep. 2 15min exposure EC$_{50}$ (mg/L)	Rep. 2 15min exposure CR	Rep. 2 15min exposure SE
0.00	0.00	*	*	-	-	*	*	-	-
46.86	0.77	*	*	-	-	*	*	-	-
97.14	1.59	*	*	-	-	*	*	-	-
504.40	13.25	*	*	-	-	*	*	-	-
999.51	25.00	24.25	6.06	1.25	0.32	36.47	9.12	3.39	0.86
2494.68	66.73	20.52	13.69	1.60	0.41	28.84	19.24	4.60	1.17

* = EC50% was greater than the highest concentration tested.

\- = not tested.

CR = control ratio correction factor.

SE = standard error.

M. bahia; Fresh ANSC; WAF; TPH

M. bahia Flowthrough exposure

Loading Rate (mg/L)	TPH Conc. (mg/L)	Rep. 1 5min exposure EC$_{50}$ %	Rep. 1 5min exposure EC$_{50}$ (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 2 5min exposure EC$_{50}$ %	Rep. 2 5min exposure EC$_{50}$ (mg/L)	Rep. 2 5min exposure CR	Rep. 2 5min exposure SE
0.00	0.07	*	*	-	-	*	*	-	-
139.26	0.31	37.08	0.11	0.05	0.01	*	*	-	-
250.51	0.45	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-
500.34	0.49	30.75	0.15	0.02	0.01	35.51	0.17	0.03	0.01
752.51	0.48	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-
2497.86	0.53	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-

M. bahia Static exposure

0.00	0.07	*	*	-	-	*	*	-	-
25.66	0.33	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-
139.26	0.31	37.08	0.11	0.05	0.01	*	*	-	-
250.51	0.45	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-
500.34	0.49	30.75	0.15	0.02	0.01	35.51	0.17	0.03	0.01
752.51	0.48	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-

* = EC50% was greater than the highest concentration tested.
\- = not tested.
CR = control ratio correction factor.
SE = standard error.

M. bahia; Fresh ANSC; CE-WAF; TPH

M. bahia Flowthrough exposure

Loading Rate (mg/L)	TPH Conc. (mg/L)	Rep. 1 5min exposure EC_{50} %	Rep. 1 5min exposure EC_{50} (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 2 5min exposure EC_{50} %	Rep. 2 5min exposure EC_{50} (mg/L)	Rep. 2 5min exposure CR	Rep. 2 5min exposure SE
0.00	0.01	*	*	-	-	-	-	-	-
26.26	0.02	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-
102.46	1.22	33.53	0.41	0.03	0.01	38.60	0.47	0.26	0.07
248.29	18.51	4.47	0.83	0.19	0.05	5.06	0.94	0.26	0.07
479.66	14.00	9.06	1.27	0.23	0.06	10.26	1.44	0.22	0.06
702.80	16.21	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-

M. bahia Static exposure

0.00	0.08	*	*	-	-	-	-	-	-
10.09	0.16	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-
26.26	0.36	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-
102.46	2.93	33.53	0.98	0.08	0.02	38.60	1.13	0.62	0.16
248.29	18.51	4.47	0.83	0.19	0.05	5.06	0.94	0.26	0.07
479.66	14.00	9.06	1.27	0.23	0.06	10.26	1.44	0.22	0.06

* = EC50% was greater than the highest concentration tested.
- = not tested.
CR = control ratio correction factor.
SE = standard error.

M. beryllina; Fresh ANSC; WAF; TPH

M. beryllina Flowthrough exposure

Loading Rate (mg/L)	TPH Conc. (mg/L)	Rep. 1 5min exposure EC$_{50}$ %	Rep. 1 5min exposure EC$_{50}$ (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 2 5min exposure EC$_{50}$ %	Rep. 2 5min exposure EC$_{50}$ (mg/L)	Rep. 2 5min exposure CR	Rep. 2 5min exposure SE
0.00	0.00	*	*	-	-	-	-	-	-
499.51	0.42	26.13	0.11	0.06	0.01	27.57	0.12	0.03	0.01
1952.14	0.50	20.92	0.10	0.03	0.01	23.71	0.12	0.03	0.01
3023.80	0.34	18.83	0.06	0.02	0.00	22.56	0.08	0.02	0.00
3996.23	0.40	14.44	0.06	0.01	0.00	16.71	0.07	0.01	0.00
6001.71	0.38	15.33	0.06	0.01	0.00	15.46	0.06	0.01	0.00

M. beryllina Static exposure

Day 1	0.00	0.04	*	*	-	-	-	-	-	-
	255.83	0.44	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-
	1034.11	0.53	24.55	0.13	0.04	0.01	nt	nt	-	-
	1952.14	0.50	20.92	0.10	0.03	0.01	23.71	0.12	0.03	0.01
	3023.80	0.34	18.83	0.06	0.02	0.00	22.56	0.08	0.02	0.00
	3996.23	0.40	14.44	0.06	0.01	0.00	16.71	0.07	0.01	0.00

* = EC50% was greater than the highest concentration tested.
\- = not tested.
CR = control ratio correction factor.
SE = standard error.

M. beryllina; Fresh ANSC; CE-WAF; TPH

M. beryllina Flowthrough exposure

	Loading Rate (mg/L)	TPH Conc. (mg/L)	Rep. 1 5min exposure EC_{50} %	Rep. 1 5min exposure EC_{50} (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 2 5min exposure EC_{50} %	Rep. 2 5min exposure EC_{50} (mg/L)	Rep. 2 5min exposure CR	Rep. 2 5min exposure SE
	0.00	0.00	*	*	-	-	*	*	-	-
m	152.91	6.21	23.21	1.44	0.01	0.00	23.24	1.44	0.29	0.07
	250.06	7.97	M. bahia	M. bahia	-	-	M. bahia	M. bahia	-	-
m	299.88	12.82	8.85	1.13	0.07	0.02	9.35	1.20	0.18	0.05
m	355.02	24.48	13.56	3.32	0.72	0.18	15.08	3.69	0.24	0.06
	400.14	11.79	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-

M. beryllina Static exposure

Day 1	0.00	0.04	*	*	-	-	*	*	-	-
	98.31	2.69	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-
m	152.91	5.78	23.21	1.34	0.01	0.00	23.24	1.34	0.27	0.07
	199.26	10.95	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-
	250.06	7.70	M. bahia	M. bahia	-	-	M. bahia	M. bahia	-	-
m	299.88	12.28	8.85	1.09	0.07	0.02	9.35	1.15	0.18	0.04

M. beryllina Flowthrough exposure

	Loading Rate (mg/L)	TPH Conc. (mg/L)	Rep. 1 15min exposure EC_{50} %	Rep. 1 15min exposure EC_{50} (mg/L)	Rep. 1 15min exposure CR	Rep. 1 15min exposure SE	Rep. 2 15min exposure EC_{50} %	Rep. 2 15min exposure EC_{50} (mg/L)	Rep. 2 15min exposure CR	Rep. 2 15min exposure SE
	0.00	0.00	*	*	-	-	*	*	-	-
m	152.91	6.21	28.15	1.75	0.16	0.04	26.74	1.66	0.38	0.10
	250.06	7.97	M. bahia	M. bahia	-	-	M. bahia	M. bahia	-	-
m	299.88	12.82	10.64	1.36	0.10	0.03	11.33	1.45	0.17	0.04
m	355.02	24.48	18.44	4.51	1.11	0.28	18.20	4.46	0.24	0.06
	400.14	11.79	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-

M. beryllina Static exposure

Day 1	0.00	0.04	*	*	-	-	*	*	-	-
	98.31	2.69	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-
m	152.91	5.78	28.15	1.63	0.15	0.04	26.74	1.55	0.36	0.09
	199.26	10.95	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-
	250.06	7.70	M. bahia	M. bahia	-	-	M. bahia	M. bahia	-	-
m	299.88	12.28	10.64	1.31	0.10	0.02	11.33	1.39	0.16	0.04

* = EC50% was greater than the highest concentration tested.
\- = not tested.
CR = control ratio correction factor.
SE = standard error.

M. beryllina; Fresh PBCO; WAF; TPH

M. beryllina Flowthrough exposure

Loading Rate (mg/L)	TPH Conc. (mg/L)	Rep. 1 5min exposure EC_{50} %	Rep. 1 5min exposure EC_{50} (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 2 5min exposure EC_{50} %	Rep. 2 5min exposure EC_{50} (mg/L)	Rep. 2 5min exposure CR	Rep. 2 5min exposure SE
0.00	0.00	*	*	-	-	*	*	-	-
990.03	0.22	24.76	0.05	0.01	0.00	39.08	0.09	0.00	0.00
1994.26	0.33	38.07	0.13	0.02	0.00	34.72	0.11	0.03	0.01
2997.70	0.47	40.98	0.19	0.01	0.00	39.33	0.18	0.07	0.02
5994.89	0.33	24.24	0.08	0.01	0.00	21.24	0.07	0.04	0.01
8151.74	0.31	22.03	0.07	0.01	0.00	22.68	0.07	0.02	0.01

M. beryllina Static exposure

Day 1	0.00	0.00	*	*	-	-	*	*	-	-
	496.60	0.17	46.28	0.08	0.08	0.02	38.64	0.07	0.28	0.07
	990.03	0.22	24.76	0.05	0.01	0.00	39.08	0.09	0.00	0.00
	1994.26	0.27	38.07	0.10	0.01	0.00	34.72	0.09	0.02	0.01
	2997.70	0.31	40.06	0.12	0.01	0.00	39.33	0.12	0.04	0.01
	5994.89	0.25	24.24	0.06	0.01	0.00	21.24	0.05	0.03	0.01

* = EC50% was greater than the highest concentration tested.
- = not tested.
CR = control ratio correction factor.
SE = standard error.

M. beryllina; Fresh PBCO; CE-WAF; TPH

M. beryllina Flowthrough exposure

Loading Rate (mg/L)	TPH Conc. (mg/L)	Rep. 1 5min exposure EC$_{50}$ %	Rep. 1 5min exposure EC$_{50}$ (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 2 5min exposure EC$_{50}$ %	Rep. 2 5min exposure EC$_{50}$ (mg/L)	Rep. 2 5min exposure CR	Rep. 2 5min exposure SE
0.00	0.00	*	*	-	-	*	*	-	-
198.43	4.79	25.57	1.22	0.17	0.04	27.29	1.31	0.207407	0.05
302.66	16.22	8.55	1.39	0.43	0.11	7.62	1.24	0.4567552	0.12
417.54	16.44	9.57	1.57	0.24	0.06	11.18	1.84	0.406068	0.10
599.91	10.92	9.51	1.04	0.28	0.07	10.55	1.15	0.341796	0.09
817.60	16.70	8.30	1.39	0.31	0.08	7.88	1.32	0.222945	0.06

M. beryllina Static exposure

Day 1	0.00	0.00	*	*	-	-	*	*	-	-
	99.74	1.30	*	*	-	-	43.87	0.57	0.16887	0.04
	198.43	5.97	25.57	1.53	0.21	0.05	27.29	1.63	0.258501	0.07
	257.94	4.86	14.34	0.70	0.16	0.04	12.77	0.62	0.145314	0.04
	302.66	16.22	8.55	1.39	0.43	0.11	7.62	1.24	0.4567552	0.12
	417.54	16.44	9.57	1.57	0.24	0.06	11.18	1.84	0.406068	0.10

* = EC50% was greater than the highest concentration tested.
\- = not tested.
CR = control ratio correction factor.
SE = standard error.

M. beryllina; Weathered ANSC; WAF; TPH

M. beryllina Flowthrough and Static exposures

Loading Rate (mg/L)	TPH Conc. (mg/L)	Rep. 1 5min exposure EC_{50} %	Rep. 1 5min exposure EC_{50} (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 2 5min exposure EC_{50} %	Rep. 2 5min exposure EC_{50} (mg/L)	Rep. 2 5min exposure CR	Rep. 2 5min exposure SE
0.00	0.00	*	*	-	-	-	-	-	-
15315.90	0.48	50.14	0.24	0.05	0.01	46.00	0.22	0.058752	0.01
17569.68	0.53	43.51	0.23	0.01	0.00	46.36	0.25	0.025811	0.01
20192.11	0.42	26.45	0.11	0.01	0.00	33.76	0.14	0.037758	0.01
22457.11	0.30	41.01	0.12	0.02	0.00	44.41	0.13	0.01002	0.00
24948.00	0.44	39.06	0.17	0.02	0.01	42.20	0.19	0.0123904	0.00

M. beryllina Flowthrough and Static exposure

Loading Rate (mg/L)	TPH Conc. (mg/L)	Rep. 1 15min exposure EC_{50} %	Rep. 1 15min exposure EC_{50} (mg/L)	Rep. 1 15min exposure CR	Rep. 1 15min exposure SE	Rep. 2 15min exposure EC_{50} %	Rep. 2 15min exposure EC_{50} (mg/L)	Rep. 2 15min exposure CR	Rep. 2 15min exposure SE
0.00	0.00	-	-	-	-	-	-	-	-
15315.90	0.48	58.44	0.28	0.09	0.02	51.97	0.25	0.095712	0.02
17569.68	0.53	49.88	0.26	0.07	0.02	53.67	0.28	0.131758	0.03
20192.11	0.42	43.60	0.18	0.10	0.03	29.85	0.13	0.01239	0.00
22457.11	0.30	44.82	0.13	0.06	0.01	50.96	0.15	0.048039	0.01
24948.00	0.44	-	-	-	-	46.46	0.20	0.042636	0.01

* = EC50% was greater than the highest concentration tested.
\- = not tested.
CR = control ratio correction factor.
SE = standard error.

M. beryllina; Weathered ANSC; CE-WAF; TPH

M. beryllina Flowthrough and Static exposure

Loading Rate (mg/L)	TPH Conc. (mg/L)	Rep. 1 5min exposure EC_{50} %	Rep. 1 5min exposure EC_{50} (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 2 5min exposure EC_{50} %	Rep. 2 5min exposure EC_{50} (mg/L)	Rep. 2 5min exposure CR	Rep. 2 5min exposure SE
0.00	0.00	-	-	-	-	*	-	-	-
96.80	0.96	*	*	-	-	*	*	-	-
199.11	9.33	44.45	4.15	0.78	0.20	40.63	3.79	0.431046	0.11
396.77	12.58	25.62	3.22	0.36	0.09	23.95	3.01	0.159766	0.04
600.25	17.11	25.09	4.29	1.64	0.42	31.55	5.40	0.8808228	0.22
1004.91	42.50	9.11	3.87	0.66	0.17	8.01	3.40	0.851275	0.22

M. beryllina Flowthrough and Static exposure

Loading Rate (mg/L)	TPH Conc. (mg/L)	Rep. 1 15min exposure EC_{50} %	Rep. 1 15min exposure EC_{50} (mg/L)	Rep. 1 15min exposure CR	Rep. 1 15min exposure SE	Rep. 2 15min exposure EC_{50} %	Rep. 2 15min exposure EC_{50} (mg/L)	Rep. 2 15min exposure CR	Rep. 2 15min exposure SE
0.00	0.00	*	*	-	-	*	*	-	-
96.80	0.96	*	*	-	-	*	*	-	-
199.11	9.33	*	*	-	-	*	*	-	-
396.77	12.58	34.09	4.29	0.29	0.07	36.82	4.63	0.901986	0.23
600.25	17.11	32.57	5.57	3.16	0.81	40.67	6.96	1.0062391	0.26
1004.91	42.50	12.51	5.32	0.85	0.22	10.02	4.26	1.2223	0.31

* = EC50% was greater than the highest concentration tested.
\- = not tested.
CR = control ratio correction factor.
SE = standard error.

C. bardi; Dispersant Only; Loading Rates

Corexit 9500

C. bardi Flowthrough exposure

Loading Rate (mg/L)	Rep. 1 5min exposure EC$_{50}$%	Rep. 1 5min exposure EC$_{50}$ (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 1 15min exposure EC$_{50}$%	Rep. 1 15min exposure EC$_{50}$ (mg/L)	Rep. 1 15min exposure CR	Rep. 1 15min exposure SE
0.00	**	**	-	-	**	**	-	-
200.03	**	**	-	-	**	**	-	-
803.07	**	**	-	-	**	**	-	-
1440.22	**	**	-	-	**	**	-	-
2005.18	**	**	-	-	**	**	-	-
2600.81	11.35	295.19	147.73	37.69	13.91	361.77	130.82	33.37

C. bardi Static exposure

	Loading Rate (mg/L)	Rep. 1 5min exposure EC$_{50}$%	Rep. 1 5min exposure EC$_{50}$ (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 1 15min exposure EC$_{50}$%	Rep. 1 15min exposure EC$_{50}$ (mg/L)	Rep. 1 15min exposure CR	Rep. 1 15min exposure SE
Day 1	0.00	*	*	-	-	*	*	-	-
	21.36	*	*	-	-	*	*	-	-
	52.83	*	*	-	-	*	*	-	-
	82.04	*	*	-	-	*	*	-	-
	111.16	*	*	-	-	*	*	-	-
	130.96	*	*	-	-	*	*	-	-
Day 2	0.00	*	*	-	-	*	*	-	-
	21.47	*	*	-	-	*	*	-	-
	55.38	*	*	-	-	*	*	-	-
	84.57	*	*	-	-	*	*	-	-
	114.31	*	*	-	-	*	*	-	-
	131.92	*	*	-	-	*	*	-	-
Day 3	0.00	*	*	-	-	*	*	-	-
	21.66	*	*	-	-	*	*	-	-
	52.72	*	*	-	-	*	*	-	-
	84.29	*	*	-	-	*	*	-	-
	114.69	*	*	-	-	*	*	-	-
	130.62	*	*	-	-	*	*	-	-
Day 4	0.00	*	*	-	-	*	*	-	-
	21.07	*	*	-	-	*	*	-	-
	52.84	*	*	-	-	*	*	-	-
	83.29	*	*	-	-	*	*	-	-
	110.35	*	*	-	-	*	*	-	-
	130.37	*	*	-	-	*	*	-	-

* EC50 concentration was to high to calculate or the EC50 was an extrapolated value.
** See C. bairdi make up test next page.

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* = EC50% was greater than the highest concentration tested.
- = not tested.
CR = control ratio correction factor.
SE = standard error.

Corexit 9500

C. bardi make up test

Loading Rate (mg/L)	Rep. 1 5min exposure EC_{50}%	Rep. 1 5min exposure EC_{50} (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 2 5min exposure EC_{50}%	Rep. 2 5min exposure EC_{50} (mg/L)	Rep. 2 5min exposure CR	Rep. 2 5min exposure SE
0.00	*	*	-	-	*	*	-	-
208.80	*	*	-	-	*	*	-	-
864.00	28.88	249.52	50.29	12.83	*	*	-	-
1475.60	25.92	382.48	34.68	8.85	36.68	541.25	105.65	26.95
2050.80	18.16	372.43	53.32	13.60	20.79	426.36	174.52	44.52
2604.40	11.00	286.48	65.11	16.61	4.75	123.71	29.77	7.59

* = EC50% was greater than the highest concentration tested.
\- = not tested.
CR = control ratio correction factor.
SE = standard error.

C. bardi; Fresh ANSC; WAF; Loading Rates

	Loading Rate (mg/L)	Rep. 1 5min exposure EC$_{50}$%	Rep. 1 5min exposure EC$_{50}$ (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 1 15min exposure EC$_{50}$%	Rep. 1 15min exposure EC$_{50}$ (mg/L)	Rep. 1 15min exposure CR	Rep. 1 15min exposure SE
C. bardi Flowthrough exposure									
	0.00	*	*	-	-	*	*	-	-
	201.86	36.79	74.26	22.81	5.82	46.77	94.41	60.72	15.49
	401.09	25.36	101.72	15.24	3.89	35.62	142.87	67.94	17.33
	1099.66	25.55	280.96	83.68	21.35	35.49	390.27	139.55	35.60
	1814.86	29.10	528.12	145.01	36.99	37.66	683.48	262.61	66.99
	2499.23	23.80	594.82	128.46	32.77	31.90	797.25	247.92	63.24
C. bardi Static exposure									
Day 1	0.00	*	*	-	-	*	-	-	-
	47.03	*	*	-	-	*	-	-	-
	254.00	39.23	99.64	43.99	11.22	45.80	116.33	72.32	18.45
	463.94	31.18	144.66	10.67	2.72	39.79	184.60	30.72	7.84
	773.94	22.23	172.05	32.12	8.19	28.20	218.25	63.93	16.31
	1017.37	28.91	294.12	88.92	22.68	38.84	395.15	200.62	51.18
Day 2	0.00	-	-	-	-	-	-	-	-
	56.54	-	-	-	-	-	-	-	-
	233.80	*	*	-	-	*	*	-	-
	414.74	32.49	134.75	28.17	7.19	43.44	180.16	92.74	23.66
	702.11	22.94	161.06	26.82	6.84	29.02	203.75	44.16	11.27
	994.71	14.43	143.54	21.49	5.48	18.16	180.64	37.89	9.67
Day 3	0.00	-	-	-	-	-	-	-	-
	49.49	-	-	-	-	-	-	-	-
	228.17	40.82	93.14	49.01	12.50	49.56	113.08	90.45	23.07
	399.83	40.18	160.65	42.70	10.89	*	*	-	-
	685.63	42.74	293.04	94.34	24.07	*	*	-	-
	1001.14	30.30	303.35	115.23	29.40	33.73	337.68	200.23	51.08
Day 4	0.00	-	-	-	-	-	-	-	-
	50.49	*	*	-	-	*	*	-	-
	220.57	35.48	78.26	21.31	5.44	41.85	92.31	32.75	8.35
	435.80	43.02	187.48	62.80	16.02	*	*	-	-
	702.34	37.82	265.62	228.82	58.37	*	*	-	-
	981.94	22.69	222.80	30.64	7.82	29.34	288.10	42.13	10.75

* = EC50% was greater than the highest concentration tested.
- = not tested.
CR = control ratio correction factor.
SE = standard error.

C. bardi; Fresh ANSC; CE-WAF; Loading Rates

C. bardi Flowthrough exposure

Loading Rate (mg/L)	Rep. 1 5min exposure EC$_{50}$%	Rep. 1 5min exposure EC$_{50}$ (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 1 15min exposure EC$_{50}$%	Rep. 1 15min exposure EC$_{50}$ (mg/L)	Rep. 1 15min exposure CR	Rep. 1 15min exposure SE
0.00	*	*	-	-	*	*	-	-
99.83	23.70	23.66	4.20	1.07	31.81	31.76	3.14	0.80
204.36	14.28	29.18	1.06	0.27	18.66	38.13	5.79	1.48
402.32	3.56	14.32	3.33	0.85	5.43	21.83	3.47	0.89
1100.75	1.72	18.92	3.75	0.96	2.22	24.39	6.00	1.53
1853.79	1.02	18.93	3.74	0.95	1.26	23.43	4.17	1.06

C. bardi Static exposure

	Loading Rate (mg/L)	Rep. 1 5min exposure EC$_{50}$%	Rep. 1 5min exposure EC$_{50}$ (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 1 15min exposure EC$_{50}$%	Rep. 1 15min exposure EC$_{50}$ (mg/L)	Rep. 1 15min exposure CR	Rep. 1 15min exposure SE
Day 1	0.00	*	*	-	-	*	*	-	-
	25.39	-	-	-	-	-	-	-	-
	50.95	43.79	22.31	10.56	2.69	*	*	-	-
	223.67	10.51	23.51	0.78	0.20	14.16	31.67	2.21	0.56
	395.99	7.61	30.14	4.23	1.08	11.18	44.27	6.22	1.59
	701.26	3.20	22.47	1.31	0.33	4.11	28.80	0.74	0.19
Day 2	0.00	-	-	-	-	-	-	-	-
	26.17	-	-	-	-	-	-	-	-
	50.89	*	*	-	-	*	*	-	-
	228.09	14.64	33.39	5.17	1.32	23.40	53.37	18.27	4.66
	399.41	7.68	30.68	0.62	0.16	9.90	39.55	4.37	1.11
	704.06	1.58	11.12	0.88	0.22	2.58	18.18	4.17	1.06
Day 3	0.00	-	-	-	-	-	-	-	-
	26.69	-	-	-	-	-	-	-	-
	50.80	48.34	24.56	24.27	6.19	*	*	-	-
	223.28	13.79	30.79	3.67	0.94	22.80	50.91	11.70	2.98
	400.40	8.66	34.67	4.37	1.11	11.71	46.89	4.57	1.17
	702.19	2.87	20.14	1.49	0.38	3.77	26.44	1.47	0.38
Day 4	0.00	-	-	-	-	-	-	-	-
	28.22	-	-	-	-	-	-	-	-
	51.58	*	*	-	-	*	*	-	-
	226.19	36.95	83.58	9.02	2.30	47.12	106.58	28.89	7.37
	404.37	7.79	31.48	4.12	1.05	11.67	47.19	8.21	2.09
	711.86	2.19	15.61	5.04	1.29	2.82	20.07	1.53	0.39

* = EC50% was greater than the highest concentration tested.
\- = not tested.
CR = control ratio correction factor.
SE = standard error.

C. bardi; Weathered ANSC; WAF; Loading Rates

C. bardi Flowthrough/Static exposure

Loading Rate (mg/L)	Rep. 1 5min exposure EC_{50}%	Rep. 1 5min exposure EC_{50} (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 1 15min exposure EC_{50}%	Rep. 1 15min exposure EC_{50} (mg/L)	Rep. 1 15min exposure CR	Rep. 1 15min exposure SE
0.00	*	*	-	-	*	*	-	-
496.26	*	*	-	-	*	*	-	-
2002.83	*	*	-	-	*	*	-	-
4983.86	*	*	-	-	*	*	-	-
6993.25	49.48	3460.26	1918.95	489.53	*	*	-	-
10011.26	48.89	4894.51	592.67	151.19	*	*	-	-

Loading Rate (mg/L)	Rep. 2 5min exposure EC_{50}%	Rep. 2 5min exposure EC_{50} (mg/L)	Rep. 2 5min exposure CR	Rep. 2 5min exposure SE	Rep. 2 15min exposure EC_{50}%	Rep. 2 15min exposure EC_{50} (mg/L)	Rep. 2 15min exposure CR	Rep. 2 15min exposure SE
0.00	*	*	-	-	*	*	-	-
496.26	*	*	-	-	*	*	-	-
2002.83	*	*	-	-	*	*	-	-
4983.86	*	*	-	-	*	*	-	-
6993.25	54.15	3786.84	600.72	153.24	*	*	-	-
10011.26	45.27	4532.10	538.61	137.40	*	*	-	-

* = EC50% was greater than the highest concentration tested.

\- = not tested.

CR = control ratio correction factor.

SE = standard error.

C. bardi; Weathered ANSC; CE-WAF; Loading Rates

C. bardi Flowthrough/Static exposure

Loading Rate (mg/L)	Rep. 1 5min exposure EC_{50}%	Rep. 1 5min exposure EC_{50} (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 1 15min exposure EC_{50}%	Rep. 1 15min exposure EC_{50} (mg/L)	Rep. 1 15min exposure CR	Rep. 1 15min exposure SE
0.00	*	*	-	-	*	*	-	-
13.65	*	*	-	-	*	*	-	-
46.86	*	*	-	-	*	*	-	-
97.14	*	*	-	-	*	*	-	-
504.40	45.11	227.53	33.54	8.56	*	*	-	-
999.51	21.63	216.19	42.88	10.94	30.69	306.75	35.08	8.95
2494.68	21.73	542.09	58.87	15.02	27.54	687.03	67.11	17.12

Loading Rate (mg/L)	Rep. 2 5min exposure EC_{50}%	Rep. 2 5min exposure EC_{50} (mg/L)	Rep. 2 5min exposure CR	Rep. 2 5min exposure SE	Rep. 2 15min exposure EC_{50}%	Rep. 2 15min exposure EC_{50} (mg/L)	Rep. 2 15min exposure CR	Rep. 2 15min exposure SE
0.00	*	*	-	-	*	*	-	-
13.65	*	*	-	-	*	*	-	-
46.86	*	*	-	-	*	*	-	-
97.14	*	*	-	-	*	*	-	-
504.40	*	*	-	-	*	*	-	-
999.51	24.25	242.38	49.88	12.72	36.47	364.52	135.43	34.55
2494.68	20.52	511.91	59.87	15.27	28.84	719.47	171.88	43.85

* = EC50% was greater than the highest concentration tested.
- = not tested.
CR = control ratio correction factor.
SE = standard error.

M. bahia; Dispersant Only; Loading Rates

Corexit 9500

M. bahia Flowthrough exposure

Loading Rate (mg/L)	Rep. 1 5min exposure EC_{50} %	Rep. 1 5min exposure EC_{50} (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 2 5min exposure EC_{50} %	Rep. 2 5min exposure EC_{50} (mg/L)	Rep. 2 5min exposure CR	Rep. 2 5min exposure SE	Rep. 3 5min exposure EC_{50} %	Rep. 3 5min exposure EC_{50} (mg/L)	Rep. 3 5min exposure CR
0.00	*	*	-	-	*	*	-	-	nt	nt	-
303.90	33.35	101.35	117.91	30.08	nt	nt	-	-	nt	nt	-
714.35	*	*	-	-	2.01	14.34	11.48	2.93	27.02	193.02	119.37
1093.20	21.29	232.74	187.48	47.83	28.31	309.48	103.20	26.33	nt	nt	-
1561.90	22.55	352.21	298.79	76.22	1.63	25.38	11.96	3.05	12.34	192.74	81.06
1899.30	8.49	161.31	62.70	15.99	3.85	73.20	50.37	12.85	13.75	261.15	172.84

Loading Rate (mg/L)	Rep. 4 5min exposure EC_{50} %	Rep. 4 5min exposure EC_{50} (mg/L)	Rep. 4 5min exposure CR	Rep. 4 5min exposure SE	Rep. 5 5min exposure EC_{50} %	Rep. 5 5min exposure EC_{50} (mg/L)	Rep. 5 5min exposure CR	Rep. 5 5min exposure SE
0.00	nt	nt	-	-	nt	nt	-	-
303.90	nt	nt	-	-	nt	nt	-	-
714.35	nt	nt	-	-	nt	nt	-	-
1093.20	nt	nt	-	-	nt	nt	-	-
1561.90	15.81	246.94	136.98	34.94	nt	nt	-	-
1899.30	11.54	219.18	109.59	27.96	4.97	94.34	37.95	9.68

We ran so many replicates here because of the large variability in the EC50 concentration value.
This was caused by the small slope on the dose response curve

Notes:

* EC_{50} greater than solution concentration.

** Test organisms died solutions were not prepared.

nt Sample was not collected or not tested.

* = EC50% was greater than the highest concentration tested.

\- = not tested.

CR = control ratio correction factor.

SE = standard error.

M. bahia; Fresh ANSC; WAF; Loading Rates

M. bahia Flowthrough exposure

Loading Rate (mg/L)	Rep. 1 5min exposure EC_{50} %	Rep. 1 5min exposure EC_{50} (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 2 5min exposure EC_{50} %	Rep. 2 5min exposure EC_{50} (mg/L)	Rep. 2 5min exposure CR	Rep. 2 5min exposure SE
0.00	*	*	-	-	*	*	-	-
139.26	37.08	51.64	23.01	5.87	*	*	-	-
250.51	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-
500.34	30.75	153.85	23.46	5.98	35.51	177.67	31.02	7.91
752.51	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-
2497.86	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-

M. bahia Static exposure

0.00	*	*	-	-	*	*	-	-
25.66	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-
139.26	37.08	51.64	22.81	5.82	*	*	-	-
250.51	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-
500.34	30.75	153.85	23.46	5.98	35.51	177.67	31.02	7.91
752.51	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-

* = EC50% was greater than the highest concentration tested.
- = not tested.
CR = control ratio correction factor.
SE = standard error.

M. bahia; Fresh ANSC; CE-WAF; Loading Rates

M. bahia Flowthrough exposure

Loading Rate (mg/L)	Rep. 1 5min exposure EC_{50} %	Rep. 1 5min exposure EC_{50} (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 2 5min exposure EC_{50} %	Rep. 2 5min exposure EC_{50} (mg/L)	Rep. 2 5min exposure CR	Rep. 2 5min exposure SE
0.00	*	*	-	-	-	-	-	-
26.26	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-
102.46	33.53	34.35	2.78	0.71	38.60	39.55	21.84	5.57
248.29	4.47	11.09	2.58	0.66	5.06	12.57	3.47	0.89
479.66	9.06	43.46	8.05	2.05	10.26	49.21	7.53	1.92
702.80	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-

M. bahia Static exposure

0.00	*	*	-	-	-	-	-	-
10.09	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-
26.26	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-
102.46	33.53	34.35	2.78	0.71	38.60	39.55	21.84	5.57
248.29	4.47	11.10	2.58	0.66	5.06	12.56	3.47	0.89
479.66	9.06	43.46	8.05	2.05	10.26	49.21	7.53	1.92

* = EC50% was greater than the highest concentration tested.
- = not tested.
CR = control ratio correction factor.
SE = standard error.

M. beryllina; Dispersant Only; Loading Rates

Corexit 9500

M. beryllina Flowthrough exposure

Loading Rate (mg/L)	5min exposure EC_{50} %	5min exposure EC_{50} (mg/L)	5min exposure CR	5min exposure SE
0.00	*	*	-	-
19.60	-	-	-	-
40.00	-	-	-	-
68.45	*	*	-	-
101.10	57.27	57.90	44.47	11.34
117.20	79.80	93.53	167.75	42.79
120.30	57.41	69.06	51.46	13.13

M. beryllina Static exposure

	Loading Rate (mg/L)	5min exposure EC_{50} %	5min exposure EC_{50} (mg/L)	5min exposure CR	5min exposure SE
Day 1	0.00	*	*	-	-
	19.60	-	-	-	-
	40.00	-	-	-	-
	68.45	*	*	-	-
	101.10	57.27	57.90	44.47	11.34
	117.20	79.80	93.53	167.75	42.79
	120.30	57.41	69.06	51.46	13.13
Day 2	0.00	-	-	-	-
	19.90	-	-	-	-
	39.70	-	-	-	-
	69.75	-	-	-	-
	**				
	**				
Day 3	0.00	-	-	-	-
	19.30	-	-	-	-
	40.10	-	-	-	-
	70.10	-	-	-	-
	**				
	**				
Day 4	0.00	-	-	-	-
	20.15	-	-	-	-
	41.35	-	-	-	-
	69.35	-	-	-	-
	**				
	**				

Notes;

* EC_{50} greater than solution concentration.

** Test organisms died solutions were not prepared.

m Make-up Test

* = EC50% was greater than the highest concentration tested.

\- = not tested.

CR = control ratio correction factor.

SE = standard error.

M. beryllina; Fresh ANSC; WAF; Loading Rates

M. beryllina Flowthrough exposure

Loading Rate (mg/L)	Rep. 1 5min exposure EC_{50} %	Rep. 1 5min exposure EC_{50} (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 2 5min exposure EC_{50} %	Rep. 2 5min exposure EC_{50} (mg/L)	Rep. 2 5min exposure CR	Rep. 2 5min exposure SE
0.00	*	*	-	-	-	-	-	-
499.51	26.13	130.52	65.62	16.74	27.57	137.71	30.32	7.73
1952.14	20.92	408.39	128.25	32.72	23.71	462.85	104.24	26.59
3023.80	18.83	569.38	153.61	39.19	22.56	682.17	144.24	36.80
3996.23	14.44	577.06	66.73	17.02	16.71	667.77	127.88	32.62
6001.71	15.33	920.06	91.23	23.27	15.46	927.86	168.65	43.02

M. beryllina Static exposure

Day 1

0.00	*	*	-	-	-	-	-	-
255.83	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-
1034.11	24.55	253.87	76.63	19.55	nt	nt	-	-
1952.14	20.92	408.39	128.25	32.72	23.71	462.85	104.24	26.59
3023.80	18.83	569.38	153.61	39.19	22.56	682.17	144.24	36.80
3996.23	14.44	577.06	66.73	17.02	16.71	667.77	127.88	32.62

* = EC50% was greater than the highest concentration tested.
\- = not tested.
CR = control ratio correction factor.
SE = standard error.

M. beryllina; Fresh ANSC; CE-WAF; Loading Rates

M. beryllina Flowthrough exposure

	Loading Rate (mg/L)	Rep. 1 5min exposure EC_{50} %	Rep. 1 5min exposure EC_{50} (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 2 5min exposure EC_{50} %	Rep. 2 5min exposure EC_{50} (mg/L)	Rep. 2 5min exposure CR	Rep. 2 5min exposure SE
	0.00	*	*	-	-	*	*	-	-
m	152.91	23.21	35.49	0.24	0.06	23.24	35.54	7.06	1.80
	250.06	M. bahia	M. bahia	-	-	M. bahia	M. bahia	-	-
m	299.88	8.85	26.52	1.61	0.41	9.35	28.04	4.28	1.09
m	355.02	13.56	48.14	10.40	2.65	15.08	53.54	3.45	0.88
	400.14	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-

M. beryllina Static exposure

Day 1	0.00	*	*	-	-	*	*	-	-
	98.31	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-
m	152.91	23.21	35.49	0.24	0.06	23.24	35.54	7.06	1.80
	199.26	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-
	250.06	M. bahia	M. bahia	-	-	M. bahia	M. bahia	-	-
m	299.88	8.85	26.54	1.61	0.41	9.35	28.04	4.28	1.09

M. beryllina Flowthrough exposure

	Loading Rate (mg/L)	Rep. 1 15min exposure EC_{50} %	Rep. 1 15min exposure EC_{50} (mg/L)	Rep. 1 15min exposure CR	Rep. 1 15min exposure SE	Rep. 2 15min exposure EC_{50} %	Rep. 2 15min exposure EC_{50} (mg/L)	Rep. 2 15min exposure CR	Rep. 2 15min exposure SE
	0.00	*	*	-	-	*	*	-	-
m	152.91	28.15	43.04	4.05	1.03	26.74	40.89	9.41	2.40
	250.06	M. bahia	M. bahia	-	-	M. bahia	M. bahia	-	-
m	299.88	10.64	31.91	2.37	0.60	11.33	33.98	4.02	1.03
m	355.02	18.44	65.47	16.12	4.11	18.20	64.61	3.55	0.91
	400.14	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-

M. beryllina Static exposure

Day 1	0.00	*	*	-	-	*	*	-	-
	98.31	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-
m	152.91	28.15	43.04	4.05	1.03	26.74	40.89	9.41	2.40
	199.26	C. bairdi	C. bairdi	-	-	C. bairdi	C. bairdi	-	-
	250.06	M. bahia	M. bahia	-	-	M. bahia	M. bahia	-	-
m	299.88	10.64	31.91	2.37	0.60	11.33	33.98	4.02	1.03

Notes:
m make up test

* = EC50% was greater than the highest concentration tested.
- = not tested.
CR = control ratio correctior. factor.
SE = standard error.

M. beryllina; Fresh PBCO; WAF; Loading Rates

M. beryllina Flowthrough exposure

Loading Rate (mg/L)	Rep. 1 5min exposure EC_{50} %	Rep. 1 5min exposure EC_{50} (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 2 5min exposure EC_{50} %	Rep. 2 5min exposure EC_{50} (mg/L)	Rep. 2 5min exposure CR	Rep. 2 5min exposure SE
0.00	*	*	-	-	*	*	-	-
990.03	24.76	245.13	27.13	6.92	39.08	386.90	20.40	5.20
1994.26	38.07	759.21	96.32	24.57	34.72	692.41	163.33	41.67
2997.70	40.98	1228.46	33.57	8.56	39.33	1179.00	422.08	107.67
5994.89	24.24	1453.16	194.24	49.55	21.24	1273.31	654.04	166.85
8151.74	22.03	1795.83	249.44	63.63	22.68	1848.81	630.95	160.96

M. beryllina Static exposure

Day 1	0.00	*	*	-	-	*	*	-	-
	496.60	46.28	229.83	241.74	61.67	38.64	191.89	828.93	211.46
	990.03	24.76	245.13	27.13	6.92	39.08	386.90	20.40	5.20
	1994.26	38.07	759.21	96.32	24.57	34.72	692.41	163.33	41.67
	2997.70	40.06	1200.88	114.51	29.21	39.33	1179.00	422.08	107.67
	5994.89	24.24	1453.16	194.24	49.55	21.24	1273.31	654.40	166.94

* = EC50% was greater than the highest concentration tested.
- = not tested.
CR = control ratio correction factor.
SE = standard error.

M. beryllina; Fresh PBCO; CE-WAF; Loading Rates

M. beryllina Flowthrough exposure

Loading Rate (mg/L)	Rep. 1 5min exposure EC$_{50}$ %	Rep. 1 5min exposure EC$_{50}$ (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 2 5min exposure EC$_{50}$ %	Rep. 2 5min exposure EC$_{50}$ (mg/L)	Rep. 2 5min exposure CR	Rep. 2 5min exposure SE
0.00	*	*	-	-	*	*	-	-
198.43	25.57	50.74	6.93	1.77	27.29	54.15	8.6	2.19
302.66	8.55	25.89	8.05	2.05	7.62	23.06	8.52	2.17
417.54	9.57	39.97	6.19	1.58	11.18	46.68	10.31	2.63
599.91	9.51	57.06	15.54	3.96	10.55	63.29	18.78	4.79
817.60	8.30	67.83	15.23	3.89	7.88	64.41	10.91	2.78

M. beryllina Static exposure

	Loading Rate (mg/L)	Rep. 1 5min exposure EC$_{50}$ %	Rep. 1 5min exposure EC$_{50}$ (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 2 5min exposure EC$_{50}$ %	Rep. 2 5min exposure EC$_{50}$ (mg/L)	Rep. 2 5min exposure CR	Rep. 2 5min exposure SE
Day 1	0.00	*	*	-	-	*	*	-	-
	99.74	*	*	-	-	43.87	43.76	12.96	3.31
	198.43	25.57	50.74	6.93	1.77	27.29	54.15	8.6	2.19
	257.94	14.34	36.99	8.54	2.18	12.77	32.94	7.71	1.97
	302.66	8.55	25.89	8.05	2.05	7.62	23.06	8.52	2.17
	417.54	9.57	39.97	6.19	1.58	11.18	46.68	10.31	2.63

* = EC50% was greater than the highest concentration tested.

\- = not tested.

CR = control ratio correction factor.

SE = standard error.

M. beryllina; Weathered ANSC; WAF; Loading Rates

M. beryllina Flowthrough and Static exposures

Loading Rate (mg/L)	Rep. 1 5min exposure EC$_{50}$ %	Rep. 1 5min exposure EC$_{50}$ (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 2 5min exposure EC$_{50}$ %	Rep. 2 5min exposure EC$_{50}$ (mg/L)	Rep. 2 5min exposure CR	Rep. 2 5min exposure SE
0.00	*	*	-	-	-	-	-	-
9874.63	46.86	4627.25	1096.00	279.59	39.31	3881.72	412.00	105.10
12349.63	31.21	3854.32	718.71	183.34	32.46	4008.69	455.68	116.24
15315.90	50.14	7679.39	1478.00	377.04	46.00	7046.00	1875.00	478.32
17569.68	43.51	7643.99	430.00	109.69	46.36	8144.99	855.00	218.11
20192.11	26.45	5340.81	325.09	82.93	33.76	6816.00	1816.00	463.27
22457.11	41.01	9209.00	1395.00	355.87	44.41	9973.20	75.00	19.13
24948.00	39.06	9743.99	1348.00	343.88	42.20	10527.98	3023.00	771.17

M. beryllina Flowthrough and Static exposure

Loading Rate (mg/L)	Rep. 1 15min exposure EC$_{50}$ %	Rep. 1 15min exposure EC$_{50}$ (mg/L)	Rep. 1 15min exposure CR	Rep. 1 15min exposure SE	Rep. 2 15min exposure EC$_{50}$ %	Rep. 2 15min exposure EC$_{50}$ (mg/L)	Rep. 2 15min exposure CR	Rep. 2 15min exposure SE
0.00	-	-	-	-	-	-	-	-
9874.63	55.33	5463.63	1000.00	255.10	47.51	4691.44	744.00	189.80
12349.63	32.93	4066.73	789.10	201.30	33.99	4197.64	397.64	101.44
15315.90	58.44	8950.00	2879.00	734.44	51.97	7959.00	3053.00	778.83
17569.68	49.88	8763.98	2384.00	608.16	53.67	9428.94	4368.00	1114.29
20192.11	43.60	8803.76	4926.00	1256.63	29.85	6027.34	595.67	151.96
22457.11	44.82	10065.28	2727.00	695.66	50.96	11444.14	3596.00	917.35
24948.00	-	-	-	-	46.46	11591.99	2418.00	616.84

* = EC50% was greater than the highest concentration tested.
\- = not tested.
CR = control ratio correction factor.
SE = standard error.

M. beryllina; Weathered ANSC; CE-WAF; Loading Rates

M. beryllina Flowthrough and Static exposure

Loading Rate (mg/L)	Rep. 1 5min exposure EC_{50} %	Rep. 1 5min exposure EC_{50} (mg/L)	Rep. 1 5min exposure CR	Rep. 1 5min exposure SE	Rep. 2 5min exposure EC_{50} %	Rep. 2 5min exposure EC_{50} (mg/L)	Rep. 2 5min exposure CR	Rep. 2 5min exposure SE
0.00	-	-	-	-	*	-	-	-
52.20	*	-	-	-	*	-	-	-
96.80	*	*	-	-	*	*	-	-
199.11	44.45	88.50	16.67	4.25	40.63	80.90	9.2	2.35
300.85	26.28	79.06	12.69	3.24	25.71	77.35	13.27	3.39
396.77	25.62	101.65	11.23	2.86	23.95	95.03	5.04	1.29
600.25	25.09	150.60	57.70	14.72	31.55	189.40	30.9	7.88
1004.91	9.11	91.57	15.66	3.99	8.01	80.45	20.13	5.14

Loading Rate (mg/L)	Rep. 1 15min exposure EC_{50} %	Rep. 1 15min exposure EC_{50} (mg/L)	Rep. 1 15min exposure CR	Rep. 1 15min exposure SE	Rep. 2 15min exposure EC_{50} %	Rep. 2 15min exposure EC_{50} (mg/L)	Rep. 2 15min exposure CR	Rep. 2 15min exposure SE
0.00	*	*	-	-	*	*	-	-
52.20	*	*	-	-	*	*	-	-
96.80	*	*	-	-	*	*	-	-
199.11	*	*	-	-	*	*	-	-
300.85	36.15	108.76	11.49	2.93	34.99	105.27	4.3	1.10
396.77	34.09	135.26	9.08	2.32	36.82	146.09	28.45	7.26
600.25	32.57	195.50	110.9	28.29	40.67	244.10	59.1	15.08
1004.91	12.51	125.70	20.00	5.10	10.02	100.70	28.9	7.37

* = EC50% was greater than the highest concentration tested.
- = not tested.
CR = control ratio correction factor.
SE = standard error.

APPENDIX K

TOC AND UV ANALYSIS DATA

Measured concentrations for *Mysidopsis bahia*, *Menidia beryllina*, and *Chionocetes bairdi* Corexit 9500 test solutions using Total Organic Carbon (TOC), and UV-Vis (UV) Spectrophotometer analysis.

C. bairdi Spiked Exsposure

Loading Rate (mg/L)	UV (mg/L)	UV=X%(LR)	TOC (mg/L)	TOC=X%(LR)
0.00	0.00		-	-
200.03	222.00	110.98	-	-
803.07	769.00	95.76	-	-
1440.22	N/S		-	-
2005.18	1986.00	99.04	-	-
2600.81	2689.00	103.39	-	-

M. bahia Spiked Exsposure

Loading Rate (mg/L)	UV (mg/L)	UV=X%(LR)	TOC (mg/L)	TOC=X%(LR)
0.00	9.13	-	2.36	-
303.90	136.86	45.03	288.20	94.83
714.35	123.78	17.33	378.70	53.01
1093.20	123.37	11.29	573.70	52.48
1561.90	123.22	7.89	857.20	54.88
1899.30	283.11	14.91	952.20	50.13

M. beryllina Spiked Exsposure

Loading Rate (mg/L)	UV (mg/L)	UV=X%(LR)	TOC (mg/L)	TOC=X%(LR)
0.00	8.51	-	7.03	-
19.60	18.54	94.59	22.70	115.82
40.00	34.65	86.63	51.37	128.43
68.45	57.07	83.37	57.17	83.52
101.10	84.37	83.45	116.30	115.03
117.20	90.24	77.00	100.20	85.49
120.30	62.03	51.56	68.03	56.55

C. bairdi Continuous Exsposure

	Loading Rate (mg/L)	UV (mg/L)	UV=X%(LR)	TOC (mg/L)	TOC=X%(LR)
Day 1	0.00	0.00		-	-
	21.36	21.00	98.31	-	-
	52.83	54.70	103.54	-	-
	82.04	98.20	119.70	-	-
	111.16	136.20	122.53	-	-
	130.96	150.90	115.23	-	-
				-	
Day 2	0.00	-		-	-
	21.47	-		-	-
	55.38	-		-	-
	84.57	-		-	-
	114.31	-		-	-
	131.92	181.80	137.81	-	-
Day 3	0.00	0.00		-	-
	21.66	9.60	44.32	-	-
	52.72	31.80	60.32	-	-
	84.29	66.90	79.37	-	-
	114.69	106.10	92.51	-	-
	130.62	131.40	100.60	-	-
Day 4	0.00	0.00		-	-
	21.07	20.50	97.29	-	-
	52.84	62.40	118.09	-	-
	83.29	87.80	105.41	-	-
	110.35	133.60	121.07	-	-
	130.37	162.70	124.80	-	-

M. bahia Continuous Exsposure

	Loading Rate (mg/L)	UV (mg/L)	UV=X%(LR)	TOC (mg/L)	TOC=X%(LR)
Day 1	0.00	9.24	-	4.50	-
	13.95	16.04	114.98	16.30	116.85
	29.40	25.95	88.27	27.60	93.88
	44.90	36.60	81.51	37.10	82.63
	59.75	42.58	71.26	239.27	400.45
	80.60	50.66	62.85	187.83	233.04
Day 2	0.00	-	-	5.13	-
	13.30	-	-	51.30	385.71
	29.45	-	-	63.00	213.92
	46.10	-	-	44.80	97.18
	55.85	-	-	51.33	91.91
	81.10	-	-	74.37	91.70
Day 3	0.00	-	-	4.07	-
	18.00	-	-	19.13	106.28
	31.05	-	-	27.47	88.47
	46.10	-	-	39.07	84.75
	*	-	-	*	-
	*	-	-	*	-
Day 4	0.00	-	-	3.97	-
	18.00	-	-	17.03	94.61
	31.05	-	-	33.87	109.08
	46.10	-	-	41.86	90.80
	*	-	-	*	-
	*	-	-	*	-

M. beryllina Continuous Exsposure

Day 1 This data set is identical to the one above for Spiked Exposure, since the same test concentrations were used for both spiked and continuous exposure tests.

	Loading Rate (mg/L)	UV (mg/L)	UV=X%(LR)	TOC (mg/L)	TOC=X%(LR)
Day 2	0.00	-	-	10.53	-
	19.90	-	-	29.10	146.23
	39.70	-	-	40.17	101.18
	69.75	-	-	71.00	101.79
	*	-	-	*	-
	*	-	-	*	-
Day 3	0.00	-	-	5.37	-
	19.30	-	-	20.87	108.13
	40.10	-	-	39.90	99.50
	70.10	-	-	70.27	100.24
	*	-	-	*	-
	*	-	-	*	-
Day 4	0.00	-	-	3.83	-
	20.15	-	-	24.07	119.45
	41.35	-	-	40.43	97.78
	69.35	-	-	68.03	98.10
	*	-	-	*	-
	*	-	-	*	-

Total Count	20	% of total	0	% of total		10	% of total	21	% of total		6	% of total	15
Count w/in +/-10%	6	30.00	0	-		0	0	9.00	42.86		1	16.67	7
Count w/in +/-20%	9	45.00	0	-		3	30	13.00	61.90		4	66.67	12

* Absent test solution concentrations were not prepared or analyzed due to complete test orgainism die off for that concentration.

** Additional test solution was mixed to complete the test at the 120 mg/L target concentration.

Measured concentrations of Corexit 9500 test solutions
using UV-Vis (UV) Spectrophotometric analysis.

C. bairdi 1999 Spiked Exposure

Loading Rate (mg/L)	UV (mg/L)	UV=X%(LR)
0.00	0.00	-
199.40	233.13	116.91
796.00	714.38	89.75
1401.85	762.97	54.43
2002.80	890.47	44.46
2599.60	1032.19	39.71

C. bairdi 1999 Continuous Exposure

Day 1	0.00	0.00	-
	20.05	12.29	61.31
	49.50	49.11	99.22
	79.95	89.01	111.33
	109.70	126.77	115.56
	130.30	148.96	114.32
Day 2	0.00	0.00	-
	19.90	8.54	42.92
	50.30	35.16	69.89
	79.80	71.88	90.07
	111.70	124.69	111.63
	130.45	140.73	107.88
Day 3	0.00	0.00	-
	20.95	11.46	54.69
	50.05	46.09	92.10
	79.90	80.10	100.26
	110.25	118.96	107.90
	129.90	146.51	112.79
Day 4	0.00	0.00	-
	19.95	8.70	43.60
	50.15	40.57	80.90
	80.05	83.80	104.69
	110.45	117.97	106.81
	130.20	142.97	109.81

Mean Loading Rate (mg/L)	Mean UV-Measured Conc. (mg/L)
0.00	0.00
20.21	10.25
50.00	42.73
79.93	81.20
110.53	122.10
130.21	144.79

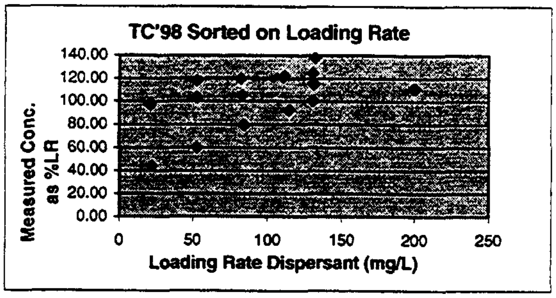
Total Count	25	% of total
Count w/in +/-10%	9	36
Count w/in +/-20%	17	68

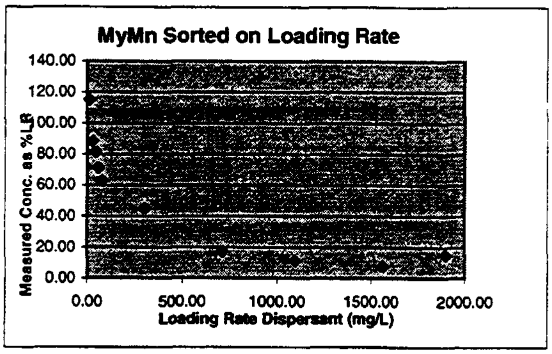
Pooled UV Data Sored on Loading Rates

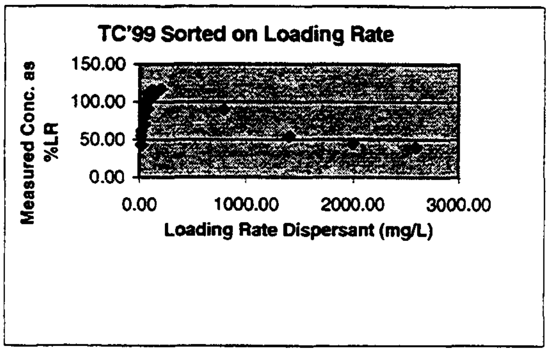
	Loading R	UV=X%(LR)
TC'98	21.07	97.29
	21.36	98.31
	21.66	44.32
	52.72	60.32
	52.83	103.54
	52.84	118.09
	82.04	119.70
	83.29	105.41
	84.29	79.37
	110.35	121.07
	111.16	122.53
	114.69	92.51
	130.37	124.80
	130.62	100.60
	130.96	115.23
	131.92	137.81
	200.03	110.98
	803.07	95.76
	2005.18	99.04
	2600.81	103.39

	Loading R	UV=X%(LR)
MyMn	13.95	114.98
	13.95	114.98
	29.40	88.27
	29.40	88.27
	44.90	81.51
	44.90	81.51
	59.75	71.26
	59.75	71.26
	80.60	62.85
	80.60	62.85
	303.90	45.03
	303.90	45.03
	714.35	17.33
	714.35	17.33
	1093.20	11.29
	1093.20	11.29
	1561.90	7.89
	1561.90	7.89
	1899.30	14.91
	1899.30	14.91

	Loading Rat	UV=X%(LR)
TC'99	19.90	42.92
	19.95	43.60
	20.05	61.31
	20.95	54.69
	49.50	99.22
	50.05	92.10
	50.15	80.90
	50.30	69.89
	79.80	90.07
	79.90	100.26
	79.95	111.33
	80.05	104.69
	109.70	115.56
	110.25	107.90
	110.45	106.81
	111.70	111.63
	129.90	112.79
	130.20	109.81
	130.30	114.32
	130.45	107.88
	199.40	116.91
	796.00	89.75
	1401.85	54.43
	2002.80	44.46
	2599.60	39.71


TC'98 Sorted on Loading Rate

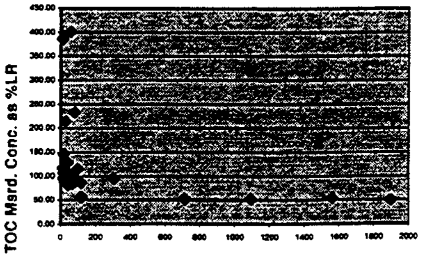

MyMn Sorted on Loading Rate


TC'99 Sorted on Loading Rate

Pooled TOC Data Sored on Loading Rates

	LR	TOC=X%(LR)
MyMn	13.3	385.71
	13.95	116.85
	18	106.28
	18	94.61
	19.3	108.13
	19.6	115.82
	19.9	146.23
	20.15	119.45
	29.4	93.88
	29.45	213.92
	31.05	88.47
	31.05	109.08
	39.7	101.18
	40	128.43
	40.1	99.50
	41.35	97.78
	44.9	82.63
	46.1	97.18
	46.1	84.75
	46.1	90.80
	55.85	91.91
	59.75	400.45
	68.45	83.52
	69.35	98.10
	69.75	101.79
	70.1	100.24
	80.6	233.04
	81.1	91.70
	101.1	115.03
	117.2	85.49
	120.3	56.55
	303.9	94.83
	714.35	53.01
	1093.2	52.48
	1561.9	54.88
	1899.3	50.13
	Average:	117.88
	Max:	400.45
	Min:	50.13

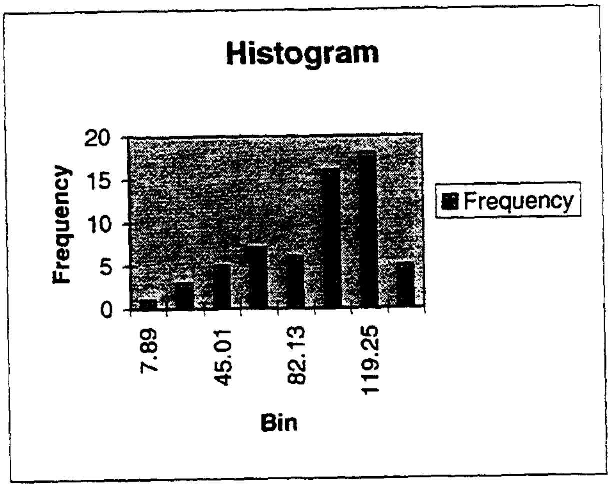
MyMn TOC Sorted on Loading Rate



CONCORDANCE ANALYSIS

	UV=X%(LR)
TC'98	110.98
	95.76
	99.04
	103.39
	98.31
	103.54
	119.70
	122.53
	115.23
	137.81
	44.32
	60.32
	79.37
	92.51
	100.60
	97.29
	118.09
	105.41
	121.07
	124.80
MyMn	45.03
	17.33
	11.29
	7.89
	14.91
	114.98
	88.27
	81.51
	71.26
	62.85
	94.59
	86.63
	83.37
	83.45
	77.00
	51.56
TC'99	116.91
	89.75
	54.43
	44.46
	39.71
	61.31
	99.22
	111.33
	115.56
	114.32
	42.92
	69.89
	90.07
	111.63
	107.88
	54.69
	92.10
	100.26
	107.90
	112.79
	43.60
	80.90
	104.69
	106.81
	109.81
Count, N=	61
Mean	85.65
SD	31.23
Max	137.81
Min	7.89

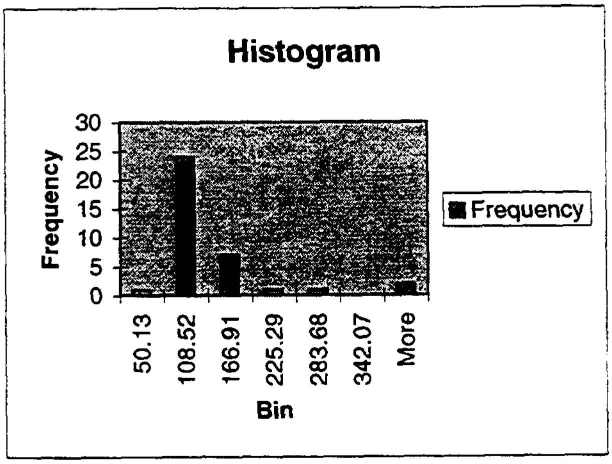
Bin	*Frequency*
7.89	1
26.45	3
45.01	5
63.57	7
82.13	6
100.69	16
119.25	18
More	5



CONCORDANCE ANALYSIS

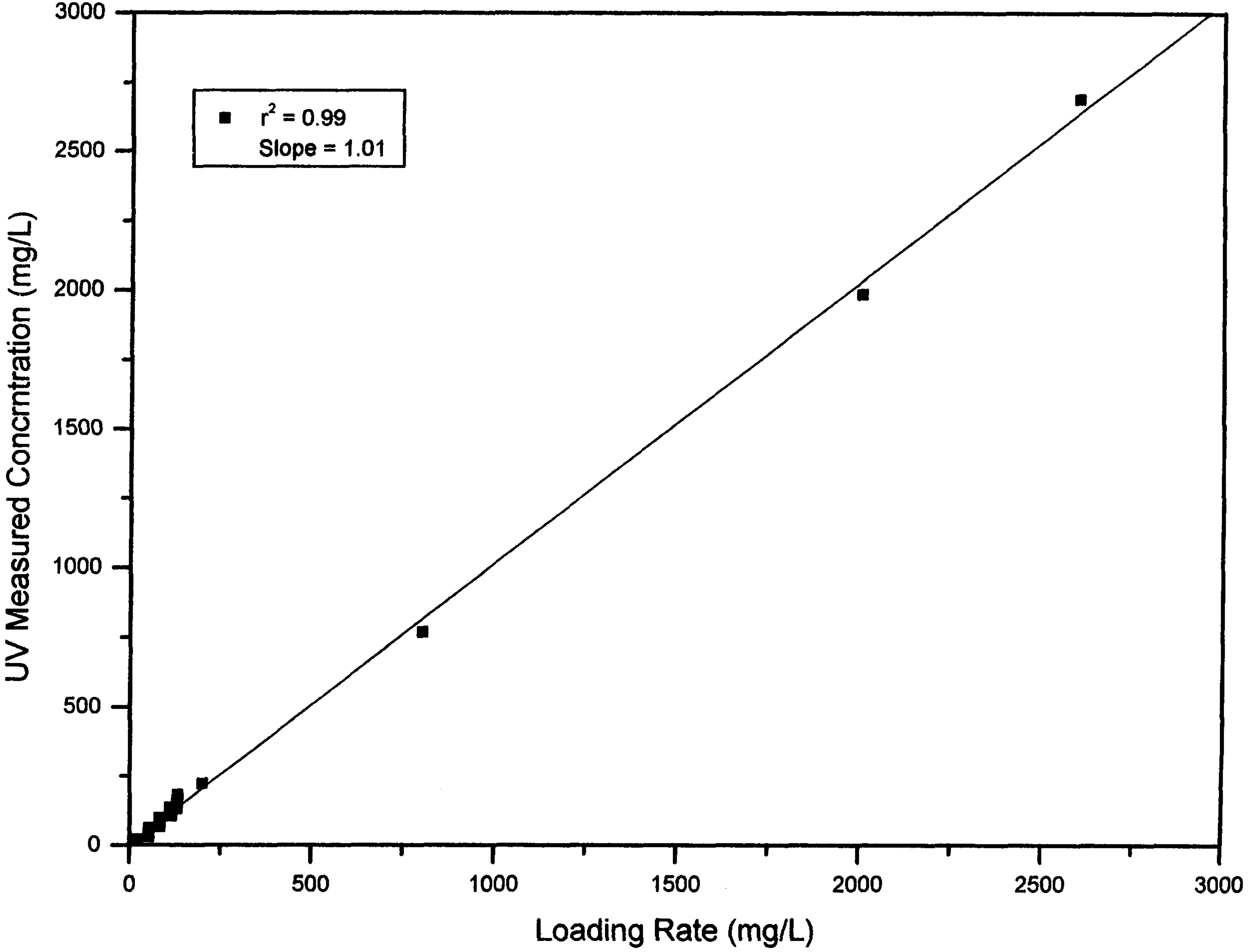
	TOC=X%(LR)
MyMn	94.83
	53.01
	52.48
	54.88
	50.13
	116.85
	93.88
	82.63
	400.45
	233.04
	385.71
	213.92
	97.18
	91.91
	91.70
	106.28
	88.47
	84.75
	94.61
	109.08
	90.80
	115.82
	128.43
	83.52
	115.03
	85.49
	56.55
	146.23
	101.18
	101.79
	108.13
	99.50
	100.24
	119.45
	97.78
	98.10
Count, N=	36
Mean	117.88
SD	77.03
Max	400.45
Min	50.13

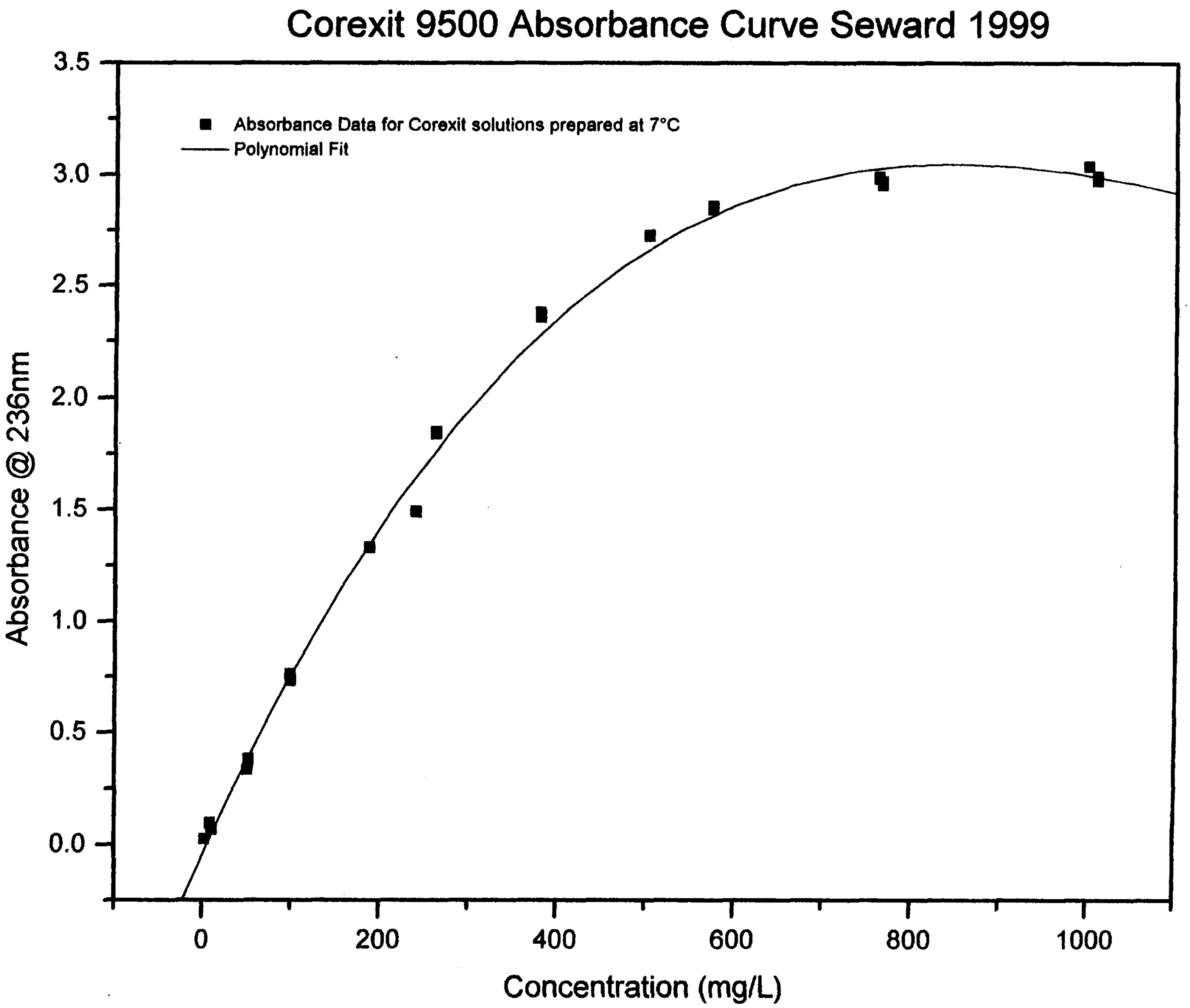
Bin	*Frequency*
50.13	1
108.52	24
166.91	7
225.29	1
283.68	1
342.07	0
More	2



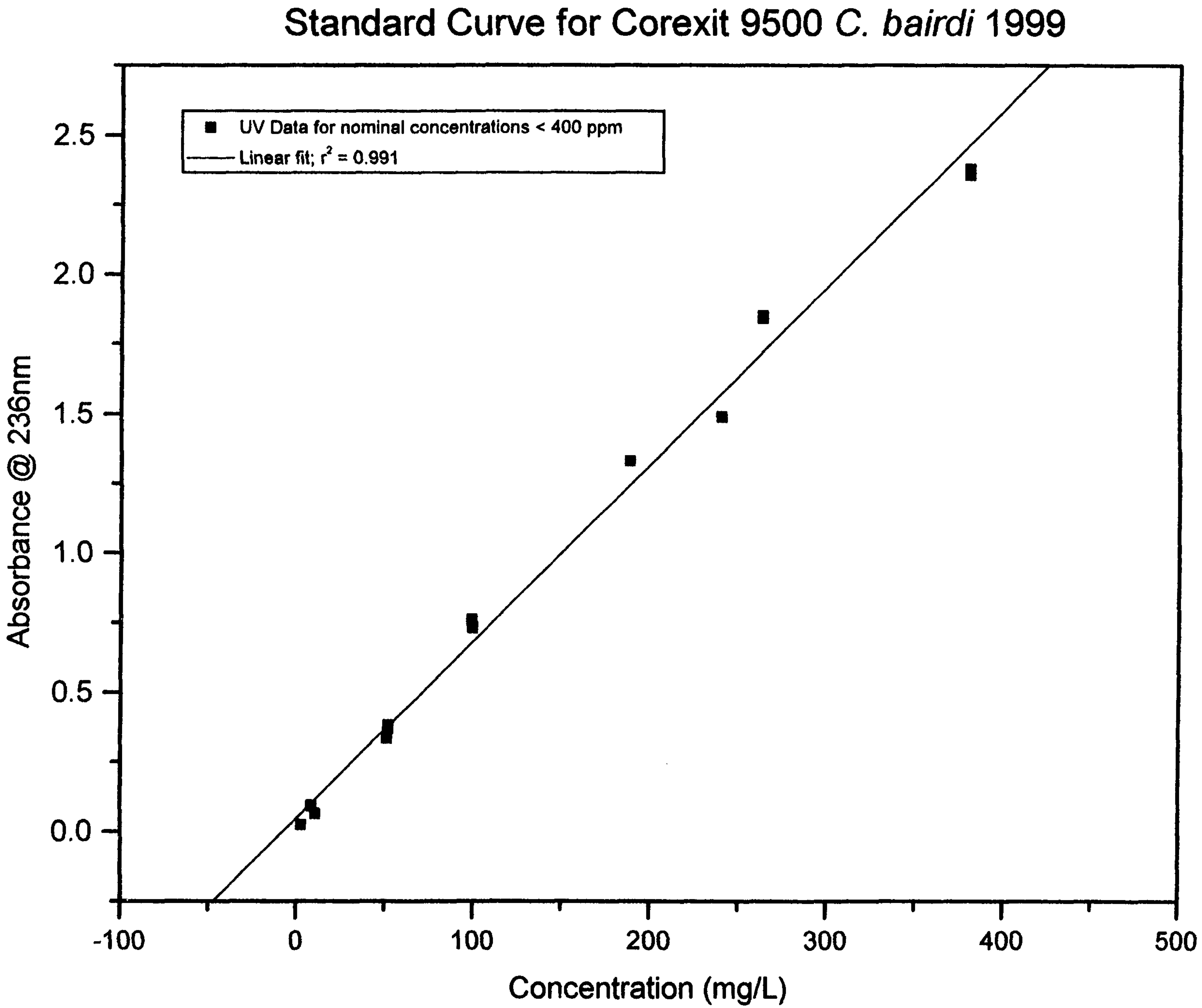
C. *bairdi* 1998 Corexit 9500

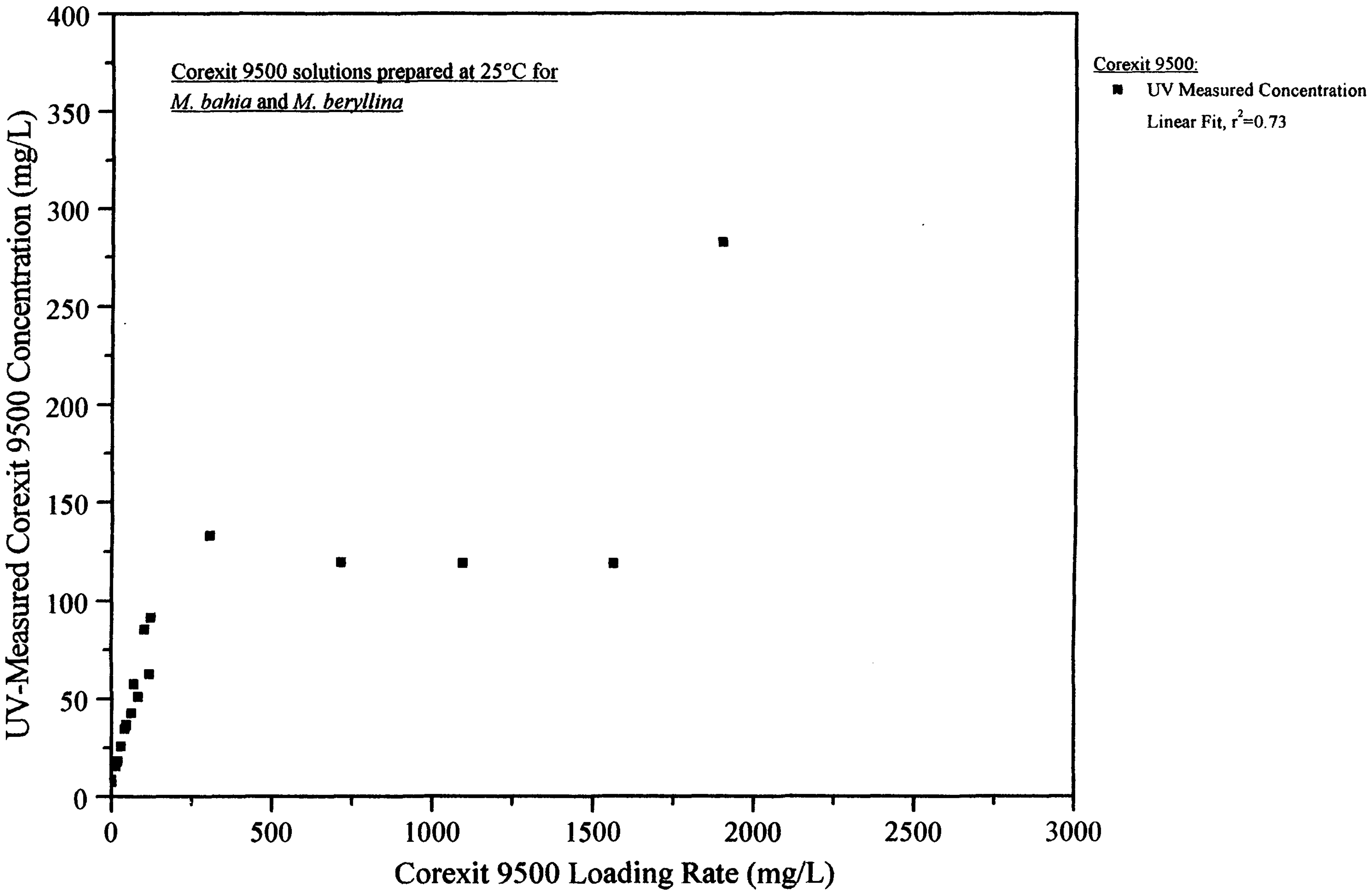
UV Measured Concentrations vs. Loading Rate

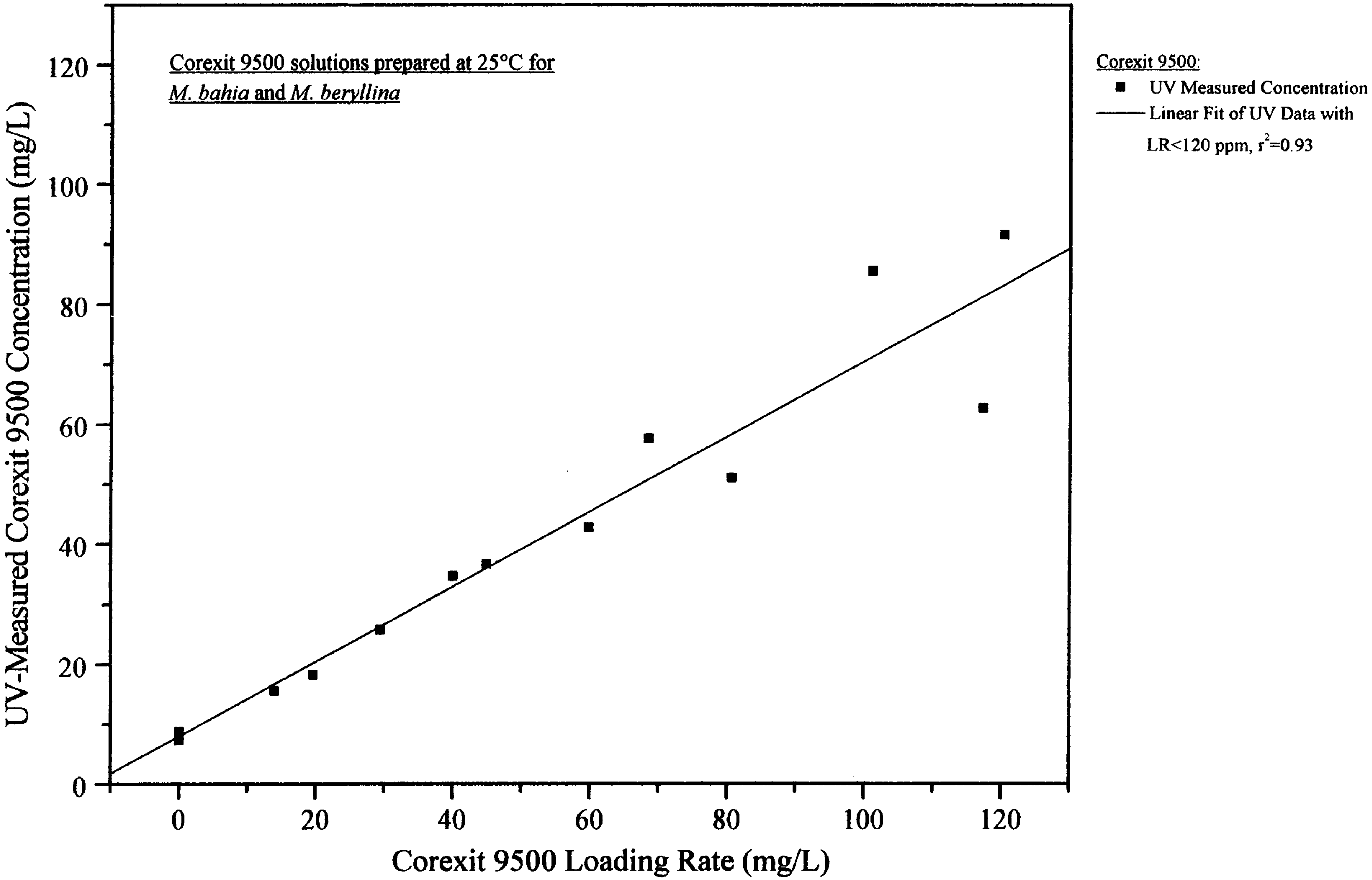


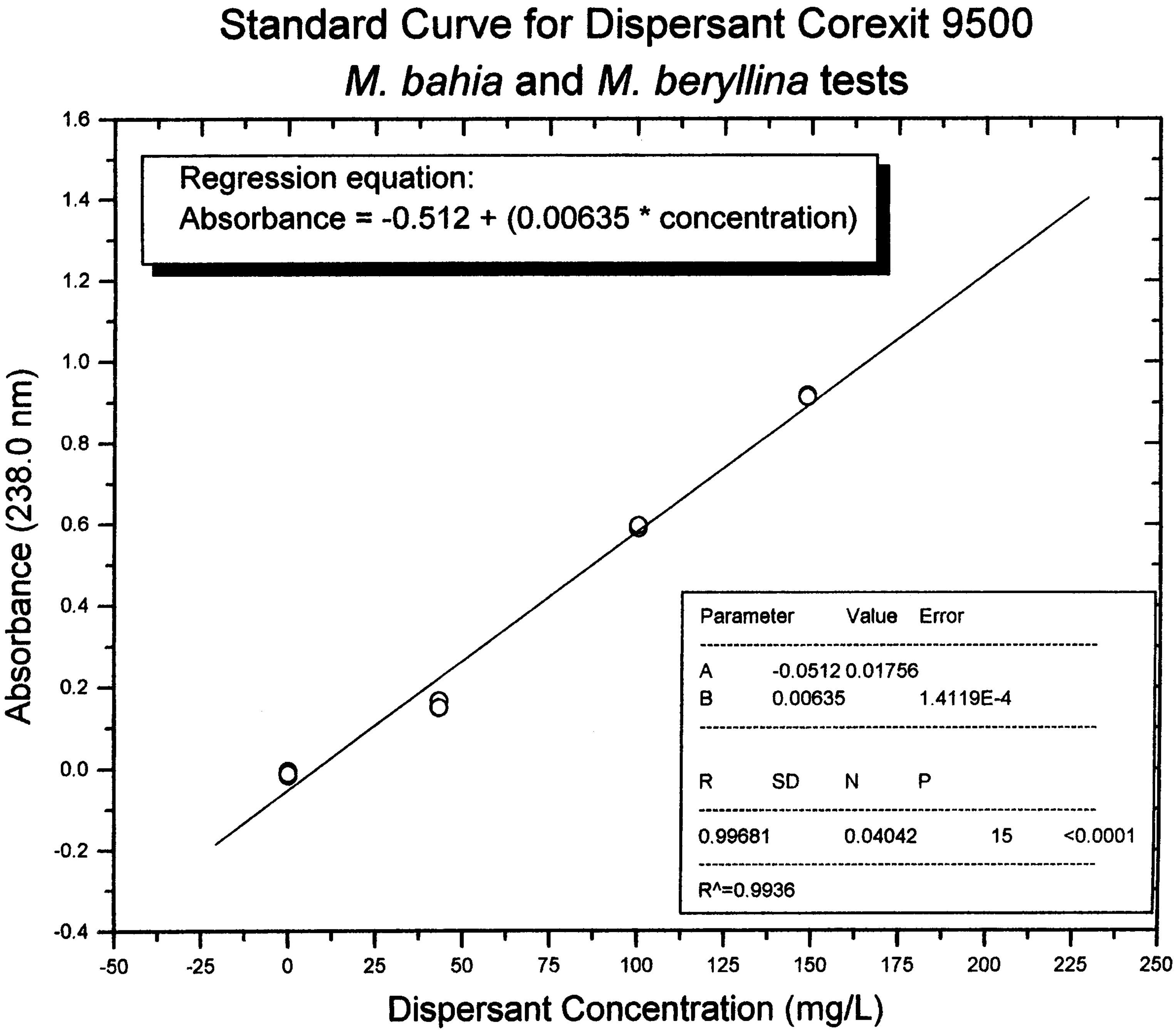


Corexit 9500 Absorbance Curve Seward 1999


Standard Curve for Corexit 9500 *C. bairdi* 1999
UV Data for nominal concentrations < 400 ppm
Linear fit; r^2 = 0.991
Absorbance @ 236nm
0.0
0.5
1.0
1.5
2.0
2.5
-100
0
100
200
300
400
500
Concentration (mg/L)


Corexit 9500 solutions prepared at 25°C for
M. bahia and *M. beryllina*
Corexit 9500:
■ UV Measured Concentration
Linear Fit, r²=0.73
UV-Measured Corexit 9500 Concentration (mg/L)
0
50
100
150
200
250
300
350
400
0
500
1000
1500
2000
2500
3000
Corexit 9500 Loading Rate (mg/L)


Corexit 9500 solutions prepared at 25°C for
M. bahia and *M. beryllina*
Corexit 9500:
■ UV Measured Concentration
Linear Fit of UV Data with LR<120 ppm, r²=0.93
UV-Measured Corexit 9500 Concentration (mg/L)
Corexit 9500 Loading Rate (mg/L)
0
20
40
60
80
100
120



Standard Curve for Dispersant Corexit 9500

M. bahia and *M. beryllina* tests

Regression equation:

Absorbance = -0.512 + (0.00635 * concentration)

Parameter	Value	Error
A	-0.0512	0.01756
B	0.00635	1.4119E-4

R	SD	N	P
0.99681	0.04042	15	<0.0001

R^=0.9936

APPENDIX L

OIL WEATHERING DATA

Battelle Duxbury Operations
Topping Statistics
N003652-0001
Oil Type: MAPCO Crude

Column:	0	1	2	3	4	5	6
	Topping Replicate	Mass Fresh Oil (g)	Mass Residual Oil (g)	Density Fresh Oil (g/mL)	Density Residual Oil (g/mL)	Volume Fresh Oil (mL) col1/col3	Volume Residual Oil (mL) col2/col4
	Replicate 1	1387.00	960.20	0.8773	0.9403	1580.99	1021.16
	Replicate 2	1392.00	981.90	0.8773	0.9391	1586.69	1045.58
	Replicate 3	1418.10	998.70	0.8773	0.9398	1616.44	1062.67
	Replicate 4	1447.10	1032.80	0.8773	0.9382	1649.49	1100.83
	Replicate 5	1436.55	1021.25	0.8773	0.9366	1637.47	1090.38
	Average				0.9388		
	Standard Deviation				0.0015		
	Percent Coefficient of Variation				0.1556		

col = column

7 Volume Distillate (mL)	8 Volume % Residual Oil (%) col6/col5 X100	9 Volume % Distillate Measured (%) col7/col5 X100	10 Volume % Distillate Calculated (%) 100-col8	11 Volume % Lost (%) col10-col9	12 Weight % Residual Oil (%) col2/col1
530	64.59	33.52	35.41	1.89	0.69
510	65.90	32.14	34.10	1.96	0.71
505	65.74	31.24	34.26	3.02	0.70
525	66.74	31.83	33.26	1.43	0.71
520	66.59	31.76	33.41	1.65	0.71
		32.0983	34.0889		
		0.8598	0.8539		
		2.6785	2.5050		

Battelle Duxbury Operations
Topping Data
N003652-0001
Oil Type: MAPCO Crude Rep 1

Running Time	Actual Time	Temperature Residual Oil (°C)
t_0	13:15	23
t_1	13:25	33
t_2	13:35	46
t_3	13:45	59
t_4	14:00	78
t_5	14:10	91
t_6	14:20	102
t_7	14:30	116
t_8	14:40	129
t_9	14:50	146
t_{10}	15:00	159
t_{11}	15:10	172
t_{12}	15:20	187
t_{13}	15:35	204
t_{14}	15:45	218
t_{15}	15:55	234
t_{16}	16:10	252
t_{17}	16:20	266
t_{18}	16:25	275
t_{19}	16:30	284
t_{20}	16:36	294

Temperature Vapor (°C)	Volume Distillate (mL)
Not Available	Not Present
Not Available	Not Present
Not Available	Not Present
Not Available	Not Present
48	<25
62	25
64	50
62	70
68	90
80	130
90	165
102	205
108	245
118	295
132	335
142	375
158	425
173	465
183	490
190	>500
204	530

Battelle Duxbury Operations
Topping Data
N003652-0001
Oil Type: MAPCO Crude Rep 2

Running Time	Actual Time	Temperature Residual Oil (°C)
t_0	13:15	23
t_1	13:25	33
t_2	13:35	45
t_3	13:45	57
t_4	14:00	77
t_5	14:10	89
t_6	14:20	100
t_7	14:30	113
t_8	14:40	125
t_9	14:50	140
t_{10}	15:00	152
t_{11}	15:10	164
t_{12}	15:20	179
t_{13}	15:35	195
t_{14}	15:45	209
t_{15}	15:55	225
t_{16}	16:10	245
t_{17}	16:20	261
t_{18}	16:25	270
t_{19}	16:30	279
t_{20}	16:35	287

Temperature Vapor (°C)	Volume Distillate (mL)
Not Available	Not Present
Not Available	Not Present
Not Available	Not Present
Not Available	Not Present
42	<25
60	<25
68	35
63	55
62	70
76	100
86	125
97	165
108	210
116	255
129	300
142	345
156	400
174	440
184	465
194	490
200	510

Battelle Duxbury Operations
Topping Data
N003652-0001
Oil Type: MAPCO Crude Rep 3

Running Time	Actual Time	Temperature Residual Oil (°C)
t_0	9:15	23
t_1	9:30	43
t_2	9:40	56
t_3	9:50	70
t_4	10:00	81
t_5	10:10	93
t_6	10:20	106
t_7	10:40	131
t_8	10:50	144
t_9	11:00	158
t_{10}	11:10	172
t_{11}	11:25	195
t_{12}	11:35	210
t_{13}	11:45	226
t_{14}	11:55	244
t_{15}	12:05	260
t_{16}	12:15	279
t_{17}	12:20	287

Temperature Vapor (°C)	Volume Distillate (mL)
Not Available	Not Present
Not Available	Not Present
Not Available	Not Present
Not Available	Not Present
58	<25
57	25
54	35
67	65
80	95
92	135
102	175
120	240
133	285
143	335
159	380
174	425
190	475
202	505

Battelle Duxbury Operations
Topping Data
N003652-0001
Oil Type: MAPCO Crude Rep 4

Running Time	Actual Time	Temperature Residual Oil (°C)
t_0	9:15	23
t_1	9:30	39
t_2	9:40	54
t_3	9:50	67
t_4	10:00	78
t_5	10:10	89
t_6	10:20	101
t_7	10:40	128
t_8	10:50	135
t_9	11:00	149
t_{10}	11:10	163
t_{11}	11:25	184
t_{12}	11:35	198
t_{13}	11:45	214
t_{14}	11:55	232
t_{15}	12:05	250
t_{16}	12:15	270
t_{17}	12:20	280

Temperature Vapor (°C)	Volume Distillate (mL)
Not Available	Not Present
Not Available	Not Present
Not Available	Not Present
Not Available	Not Present
50	<25
58	<25
62	40
66	80
76	105
88	140
98	180
116	250
128	290
136	335
152	385
173	435
190	495
200	525

Battelle Duxbury Operations
Topping Data
N003652-0001
Oil Type: MAPCO Crude Rep 5

Running Time	Actual Time	Temperature Residual Oil (°C)
t_0	13:35	24
t_1	13:55	46
t_2	14:10	66
t_3	14:20	81
t_4	14:30	93
t_5	14:40	108
t_6	14:50	122
t_7	15:00	138
t_8	15:10	153
t_9	15:20	170
t_{10}	15:35	193
t_{11}	15:50	222
t_{12}	16:00	238
t_{13}	16:10	256
t_{14}	16:20	275
t_{15}	16:25	284

Temperature Vapor (°C)	Volume Distillate (mL)
Not Available	Not Present
Not Available	Not Present
Not Available	Not Present
52	<25
59	30
62	50
66	75
79	110
88	150
106	205
118	275
142	355
154	400
173	450
190	500
200	520