ACUTE EFFECTS OF PETROLEUM HYDROCARBONS ON THE ARCTIC LITTORAL MYSID, *MYSIS OCULATA* (FABRICIUS)

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ON *MYSIS OCULATA* (FABRICIUS)

ABSTRACT

Acute lethal toxicity tests were conducted with young-of-the-year *Mysis oculata* exposed to oil-in-water dispersions (OWDs) and water-soluble fractions (WSFs) of Norman Wells crude oil. Median lethal concentrations (96-h LC50s) are among the lowest reported for arctic marine crustaceans, ranging from 0.49-0.62 mg/l for WSFs and 4.51-7.57 mg/l for OWDs. Sealed-jar tests with OWDs were more toxic to mysids than open-jar exposures. The retention of volatile hydrocarbons in test solutions caused behavioral abnormalities and latent toxic effects to occur at concentrations statistically lower than the LC50. Adverse effects were caused primarily by the chemical toxicity of water-soluble aromatic compounds since there were no apparent physical effects due to dispersed oil droplets. The water-soluble component of OWDs was more toxic than WSFs, probably due to the higher proportion of alkylated aromatics in the former (i.e. ethylbenzene to C_3-naphthalene). Vigorous shaking as opposed to gently stirring oil-in-water mixtures is recommended as a method for producing a saturated and more toxic WSF. Additional experiments and testing methods are suggested for arctic marine species, including the use of *M. oculata* as a standard test organism.

RESUME

Des éssais de toxicité létale ont été exécutés en exposant des jeunes mysidacés arctique (*Mysis oculata*) à des fractions dispersées et des fractions solubles de pétrole brut "Norman Wells" dans l'eau de mer. Les concentrations létales médianes (LC50 de 96 heures) sont parmi les plus basses reportées pour des crustacés marins arctiques (4.51-7.57 mg/l pour le pétrole dispersé et 0.49-0.62 mg/l pour les fractions solubles). Les tests de pétrole dispersé exécutés dans des bocaux scellés étaient plus toxiques que ceux exécutés avec des bocaux ouverts. Dû à la conservation en solution des hydrocarbures volatiles, des comportements anormaux ainsi que des effets toxiques retardés sont survenus à des concentrations statistiquement plus basses que le LC50. Les effets adverses ont été causés principalement par la toxicité chimique des fractions solubles aromatiques puisque les gouttellettes de pétrole dispersé n'avaient apparemment aucun effet physique sur les mysidacés. La fraction soluble des solutions dispersées, préparées par agitation vigoureuse, était plus toxique que la fraction soluble des solutions préparées par agitation lente. Ce phénomène est probablement dû à la proportion plus élevée d'hydrocarbures aromatiques alkyles (éthylbenzene à C_3-naphtaline) dans les solutions dispersées. L'agitation vigoureuse est la méthode conseillée pour produire une fraction soluble saturée et plus toxique. Des expériences complémentaires et des méthodes améliorées sont suggérées pour les espèces marines de l'Arctique, incluant l'utilisation de *Mysis oculata* comme organisme standard pour les tests de toxicité.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PREFACE

Oil exploration in Arctic waters over the past two decades has raised concerns about the effects of oil pollution on the arctic ecosystem. In the North American sector, offshore oil exploration and production has been limited mostly to the southern Beaufort Sea (Nelson-Smith 1982). However, in recent years, there have also been periodic shipments of crude oil from Cameron Island, in the high arctic, to southern ports, via Lancaster Sound and Davis Strait (Anonymous 1987). A further expansion of arctic oil development activities can be expected to follow an economic recovery in Canada and the U.S., increasing energy demands and competitive prices of arctic oil on the market (Conant 1982). Although such an expansion may not take place for several decades, it is expected because of the large amounts of recoverable petroleum resources believed to exist beneath arctic waters. Hence, there is a continuing threat of oil entering the arctic marine environment from oil-well blowouts, spills during transportation, and operational discharges. Although levels of petroleum hydrocarbons in arctic waters are now relatively low (Clark and Finley 1982; Levy 1984), it has been suggested that they could become comparable to those found in the "polluted" Mediterranean Sea (Mackay 1977).

The relative environmental impact of an oil spill in polar seas will likely be far greater than that of a comparable one in temperate seas. The reasons for this have been clearly described by Percy and Wells (1984):

> ... Clean-up efforts may be less effective in ice-covered waters given present technology, allowing a greater proportion of the oil to remain in the environment untreated. The low temperature, low nutrients and ice cover will slow the weathering and biodegradation of the oil, thus prolonging its toxic influence. Certain of the ice-associated habitats, such as sub-ice, ice edge and polynyas, that are ecologically important to many polar species are especially vulnerable to contamination by oil. ... Finally, the rate at which most polar marine populations are able to recover from a major population decline is much lower as a result of reduced fecundity, dispersal and growth.

According to Grainger (1975), the lower species diversity in arctic marine food webs may further increase the vulnerability of the ecosystem to pollutant stress.

In order to predict the ecological impacts of oil spilled in arctic waters, more research is needed in ecosystem dynamics (i.e. physical and biological oceanography) and toxicology. One of the first steps in oil toxicity research is to determine the sensitivities of key species to petroleum hydrocarbons by conducting laboratory studies. Although a number of arctic marine species have already been tested (see Percy and Wells 1984, for review), the coverage is sparse. This paper contributes to our knowledge by examining the acute effects of petroleum hydrocarbons on the littoral mysid, *M. oculata*.

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The scientific and technical assistance of the following people was essential in completing this project: M. Fingas and A. Bobra (Environment Canada, Ottawa) supplied the crude oils and gas chromatograms; D. Murray (Dept. of Fisheries and Oceans, Winnipeg) determined volatile hydrocarbon concentrations and identified peaks on the GC profiles of oil-in-water samples; C. Stephan (U.S.- E.P.A., Duluth, Minnesota) provided computer programs and useful information concerning statistics; D. Pike (Univ. of Manitoba) supplied data on the under-ice distribution of *M. oculata* in the high arctic. I gratefully acknowledge all these people for their valuable professional help.

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INTRODUCTION

Littoral mysids are useful experimental organisms for pollution impact studies (Jacobs and Grant 1974; Nimmo et al. 1977; Laughlin and Linden 1983; Smith and Hargreaves 1984; McLusky and Hagerman 1987). Their ease of culture and sensitivity to pollutants make them ideal subjects for laboratory toxicity tests (Nimmo and Hammaker 1982). To date, arctic littoral mysids have received little attention apart from a few studies dealing with effects of petroleum hydrocarbons on *Mysis litoralis* and *Mysis relicta* (Schneider 1980; Carls and Korn 1985). *Mysis oculata*, a closely related species, was chosen for study because of its ecological importance, its potential vulnerability to crude oil and its availability. A brief review of the ecology of this species will help in understanding its potential vulnerability to oil pollution.

Littoral mysids are important secondary producers in many estuarine and marine food webs (Mauchline 1980). In the arctic, *M. oculata* makes up a significant portion of the diets of marine birds, fish and mammals, including commercially important species such as arctic charr (*Salvelinus alpinus*) and ringed seal (*Phoca hispida*) (Dunbar 1941; Grainger 1953; McLaren 1958; Bradstreet 1980; Green 1983; Smith 1987).

M. oculata has a circumpolar distribution throughout the arctic and subarctic (Holmquist 1958). It inhabits nearshore shallow waters (i.e. less than 50 m deep) and is known to occur in sheltered embayments, such as lagoons, where bottom sediments are of the sandy mud type

(Crane and Cooney 1973; pers. observ.). During the open water season, large dense shoals of *M. oculata* are usually found associated with beds of macroalgae in the subtidal zone. In Frobisher Bay, young-of-the-year mysids migrate into the intertidal zone during flood tides. Like many mysid species, it is hyperbenthic during daylight hours and undergoes vertical migrations to surface waters at dusk and nighttime (Kaufman 1979). Young-of-the-year mysids appear to prefer shallow water (i.e. 1 to 5 m deep) whereas adults are commonly found in deeper water (i.e. 20 to 30 m) (Thomson et al. 1986). In the winter, *M. oculata* has been found associated with the under-ice surface in water depths of 12 to 18 m at 0.3 to 0.6 km from shore (D. Pike, pers. comm.).

The arctic nearshore and sub-ice environments are among the most vulnerable habitats to oil pollution (Percy and Wells 1984). Following a spill in open water, oil can become stranded on beaches and incorporated rapidly into nearshore sediments. The vulnerability of nearshore areas depends largely on the physical features of the intertidal zone, such as substrate type, wave action, erosion and sediment transport (Sanborn 1977; Mann and Clark 1978; Owens 1985). In certain sheltered areas, weathering can be slow and oil can remain buried in intertidal beaches for years, often retaining its toxic aromatic hydrocarbons (Clark and Finley 1982). An increase in retention time of stranded oil may have a longer-term impact on shallow subtidal fauna as a result of continuous leaching of dissolved hydrocarbons over extended periods. Additional contamination of subtidal sediments may result from dispersed oil adsorbing to particulate matter in the water column and

sinking to the bottom. This process may considerably increase the impact on the subtidal benthos, especially in waters containing high levels of sedimentary and organic particulate matter, such as the Mackenzie Delta. The sub-ice habitat may be even more vulnerable to spilled oil. In the event of a spill beneath the ice surface, such as a sub-sea blowout, oil accumulates rapidly at the ice-water interface where it can spread and remain in a relatively "fresh" state for extended periods. Ice-cover greatly reduces the weathering of crude oil because evaporative losses are completely inhibited (Payne and McNabb 1984). Toxic volatile fractions contained in the oil (i.e. aromatic hydrocarbons) are retained in the water, thus increasing the potential hazard to sub-ice communities. During fall and winter, oil can become encapsulated in the growing ice sheet and remain preserved until its release into the water again during spring, when biological activity is near its peak. A variety of spill scenarios are possible in ice-infested waters and these have been reviewed by Nelson-Smith (1982).

With increasing amounts of oil entering the arctic marine environment, the chance of oil exposure will be greatly increased for species inhabiting vulnerable areas. Dense shoals of *M. oculata* utilizing the intertidal, subtidal and sub-ice habitats could become exposed to oil in the particulate and dissolved states. In addition, behaviors such as shoaling and vertical migration may increase exposure. *M. oculata* populations are clearly vulnerable to spilled oil; their sensitivity however, has not yet been determined[1]. As a first step in understanding the effects of petroleum hydrocarbons on this species, acute toxicity

tests were conducted to determine sensitivity and behavioral changes of animals exposed to oil-in-water dispersions and water-soluble fractions of crude oil. This study also represents the first effort in holding and maintaining *M. oculata* for extended periods under laboratory conditions.

MATERIALS AND METHODS

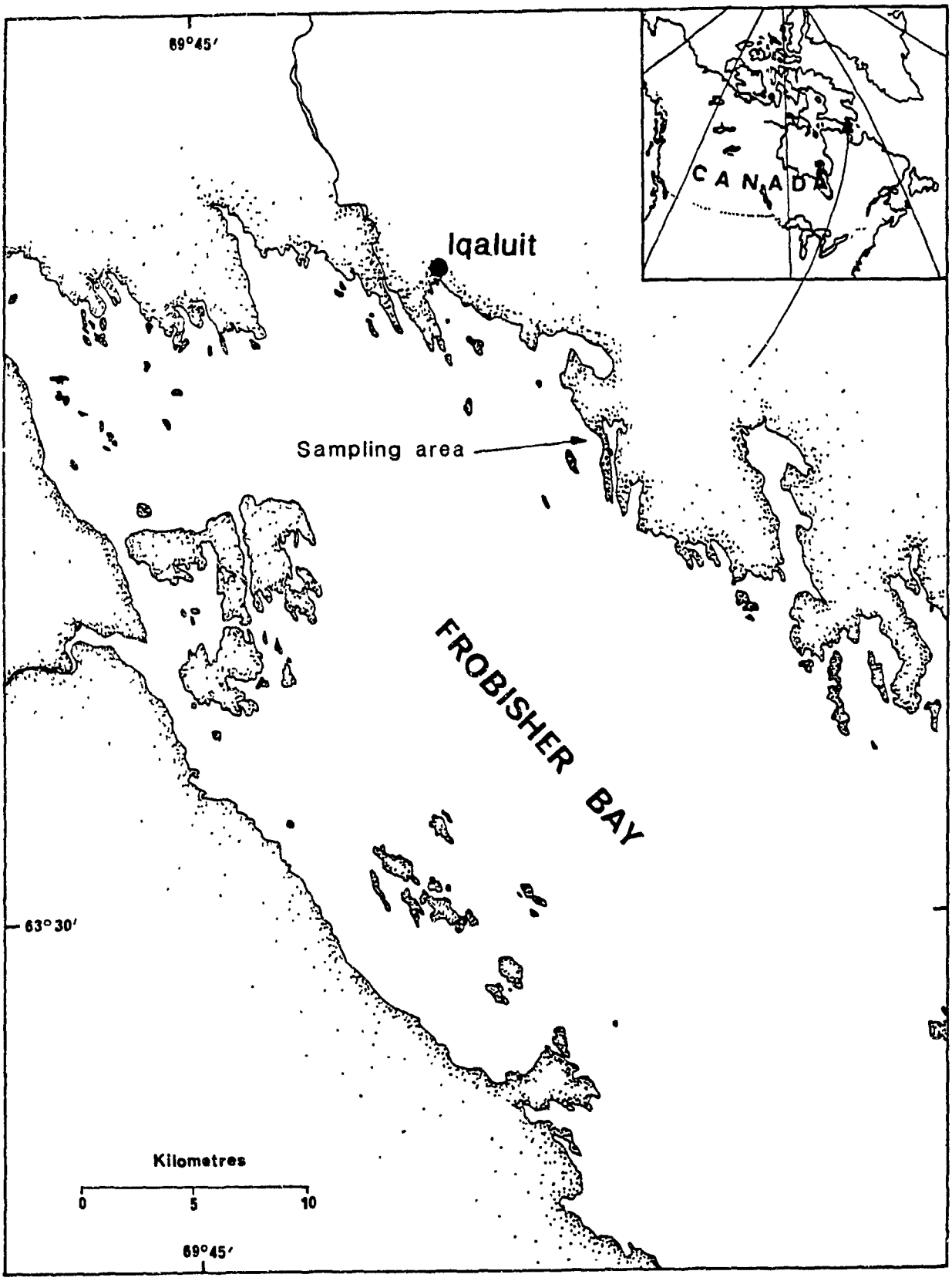
Collection and maintenance of animals

In August 1986 and 1987, young-of-the-year *M. oculata* were collected by dip-net from a sheltered embayment in upper Frobisher Bay, N.W.T., Canada (63° 44'N, 68° 31'W) (Fig. 1). During both years, large dense shoals of young mysids were present in the intertidal and shallow subtidal zones.

Animals were transported in cold seawater (T = 0-2°C) to the Iqaluit Research Laboratory. At the lab the animals were transferred to 9-L polystyrene containers of clean seawater (T = 0°C, S = 32 ppt) and kept in an incubator at 0°C until shipping. Live mysids for toxicity tests were flown to the Arctic Biological Station (ABS) in polyethylene bags of seawater packed in insulated boxes. Total transit took less than 7 hrs and no mortality occurred.

At the ABS, mysids were held in an artificial seawater (Instant Ocean(™)) re-circulating system (T = 1.5 ±1.0°C, S = 33.0 ±1.0 ppt, pH = 7.9). Approximately 1200 animals were held in three 65-L holding tanks in a cold room illuminated by incandescent lights. The light intensity above the tanks was 6 μeinsteins $m^{-2}s^{-1}$ (or 60 lux) and the photoperiod was similar to the light regime of Frobisher Bay (i.e. ranging from 6 to 15 daylight hours with a 30 min gradual brightening and dimming period at the beginning and end). The mysids were fed dried fish food (Cora Feed(™)) *ad libitum* every 2-3 d. Feeding was monitored

FIG. 1. Location of sampling area in upper Frobisher Bay, N.W.T.


69°45′
CANADA
Iqaluit
Sampling area
FROBISHER BAY
63°30′
Kilometres
0
5
10
69°45′

regularly and animals appeared healthy during the experimental periods. Some unused individuals lived for more than one year, indicating favorable conditions within the seawater system.

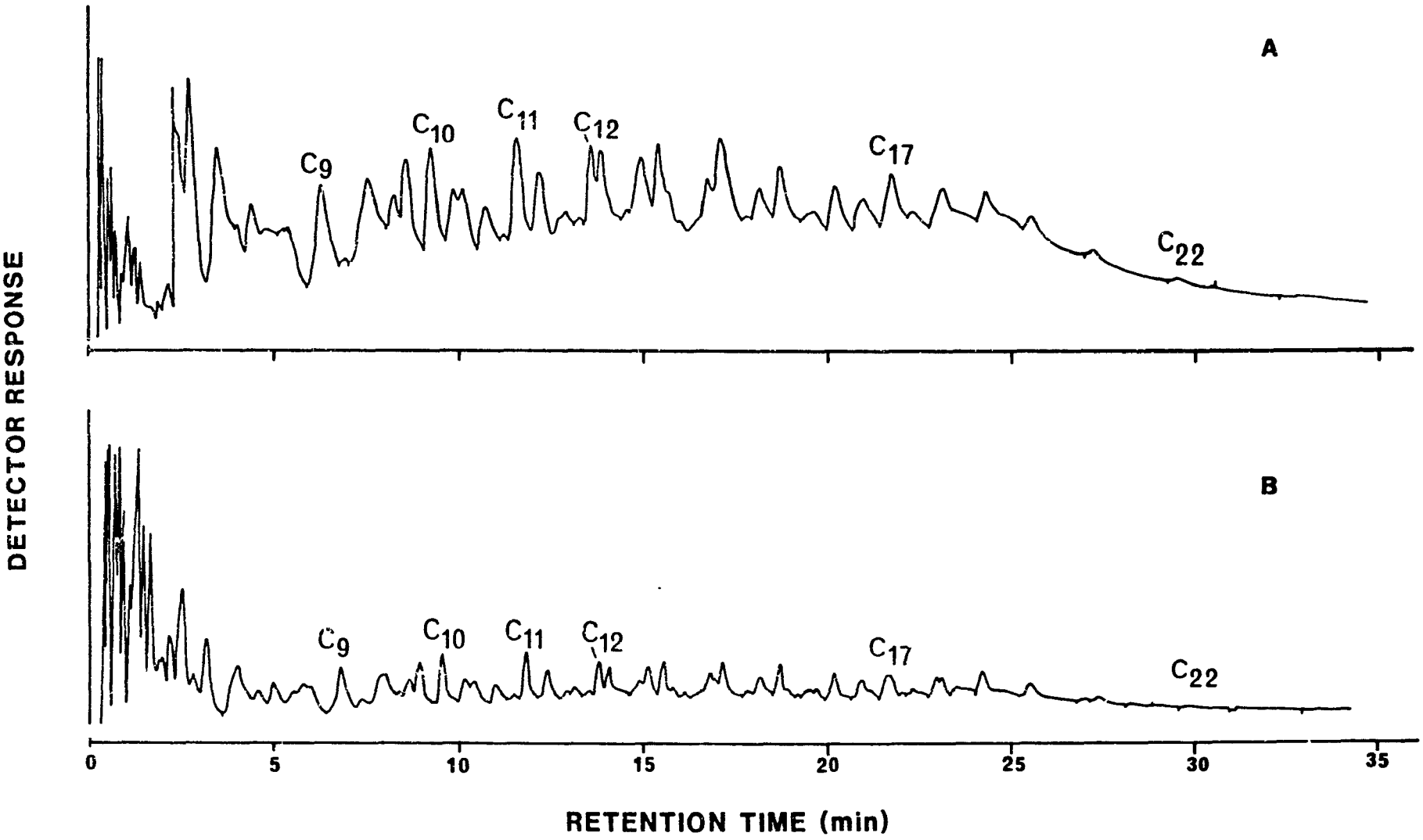
An acclimation period of at least 10 d preceded experimentation. Forty-eight hrs prior to testing, 80 to 100 animals were transferred to smaller holding tanks and starved.

Toxicants

Two different batches of Norman Wells crude oil (July 1983 and July 1986) and one reference toxicant (phenol) were used in toxicity experiments. The oil was received in tightly capped nalgene(TM) containers (1-L and 25-L) and aliquots were immediately transferred to several smaller containers to minimize the loss of volatile compounds during subsequent sample removal. Oil to be used for oil-in-water dispersions (OWD) was stored in tightly-capped 14-ml glass vials in a dark incubator at 0°C. Oil to be used for water-soluble fractions (WSF) was transferred to 500-ml glass erlenmeyer flasks and similarly stored.

Gas chromatograms (Fig. 2) show that Norman Wells crude is a light gravity oil with a relatively high percentage (i.e. 60-65%) of volatile hydrocarbons (below n-C_{12}). Typically, it has a pour point of -50°C, a specific gravity of 0.833 at 16°C, and a viscosity of 6.5 at 16°C centipoise (Keevil and Ramseier 1975). However, the properties and composition of crude oil recovered from the same field often vary according to the time of recovery and the location and depth of the oil well. For

FIG. 2. Gas chromatograph traces of Norman Wells crude oils. A: July 1983 oil; B: July 1986 oil.


A
B
C_9
C_{10}
C_{11}
C_{12}
C_{17}
C_{22}
DETECTOR RESPONSE
0
5
10
15
20
25
30
35
RETENTION TIME (min)

this reason, the Norman Wells oils used in this study differed in their appearance. The July '83 oil was darker, more viscous and less odorous than the July '86 oil. A comparison of GC profiles indicates that the former has almost double the proportion of high molecular weight alkanes (C_{17} - C_{22}) than the latter (i.e. 12.19% vs 6.71%), and slightly less volatile hydrocarbons (i.e. 60.81% vs 64.25%). Also, the higher number of peaks in Fig. 2B shows that the lighter '86 oil is a more diverse mixture of compounds. Additional quantities of each batch of oil are available for testing by interested investigators.

Reagent grade phenol was used as a reference toxicant[z] to compare the sensitivities of the two different collections of mysids used in this study (i.e. 1986 vs 1987). Preliminary tests with dodecyl sodium sulfate (DSS) showed that this reference toxicant was unsuitable because of its low solubility in cold seawater (also see Rice et al. 1976a; Foy 1978). Phenol was finally chosen because of its high water solubility and its toxicity to marine crustaceans (Tatem et al. 1978). It is commonly found in crude oils (Posthuma 1977), and has been recommended as one reference toxicant for bioassays (Klaverkamp et al. 1975; Fogels and Sprague 1977; P.G. Wells, pers. comm.).

Preparation of test mixtures

Toxicant and control solutions were prepared using filtered (Whatman(TM) in-line filter, 1 μm) Instant Ocean(TM) artificial seawater (S = 32 ppt) which was aerated and cooled to 0-2°C. During the

preparation of toxicant mixtures all solutions were maintained at a temperature of 0-2°C and kept sealed to minimize the evaporation of volatile compounds. Preliminary acute toxicity tests were conducted with *M. oculata* to determine concentration ranges to be used in final experiments.

OWDs were prepared with July '83 oil using methods similar to Percy and Mullin (1975) and Foy (1978). Depending on the test, a series of 3 to 6 concentrations were prepared by adding a given amount of oil (i.e. 5, 10, 15, 20, 30 or 40 µl) to 550 ml of filtered seawater in a 750-ml wide-mouth glass jar. The solutions were shaken vigorously on a reciprocating shaker at 280 oscillations/minute for 30 min and allowed to settle in 500-ml separatory funnels for 90 min. This method produces a "semi-stable dispersion" of Norman Wells crude oil in seawater (Percy and Mullin 1975). The lower 425 ml was slowly drained into a 2-L erlenmeyer flask, hand-stirred, subsampled for hydrocarbon analysis, and transferred to test jars which were sealed with aluminum foil until the animals were added.

WSFs were prepared by the method of Anderson et al. (1974a)[a]. A solution of 1 part oil to 9 parts seawater was stirred on a magnetic stirrer for 22 ±1 hrs. The stirring speed was adjusted so that the oil vortex did not break up and release oil droplets in solution. After mixing, the solution was left to settle for 1 to 3 hrs before the water phase was siphoned off from beneath the oil layer and utilized immediately in experiments. The stock WSF was diluted with filtered seawater to prepare a series of 6 concentrations (i.e. 5, 15, 25, 35, 45, and 60%

of the stock WSF).

Phenol mixtures were prepared by diluting a stock solution of 10 g/l of phenol in seawater. A series of 5 or 6 concentrations were prepared (i.e. in the range of 5 to 125 mg/l) by adding a given amount of stock solution to 600 ml of seawater in a 750-ml wide-mouth glass jar. The mixtures were stirred with a glass rod and the jars were sealed until the animals were added. All phenol concentrations are expressed as nominal amounts added to seawater, not values measured in test mixtures.

Each replicate toxicity test included 3 to 6 concentrations of toxicant and a control solution consisting of filtered seawater taken from the same lot that was used to prepare toxicant mixtures. All solutions were replaced daily with fresh mixtures.

Between each use, all glassware was washed with detergent, either in a dishwasher or by hand, and rinsed several times with hot de-ionized water. Aluminum foil used to seal jars was rinsed with hot tap water and de-ionized water.

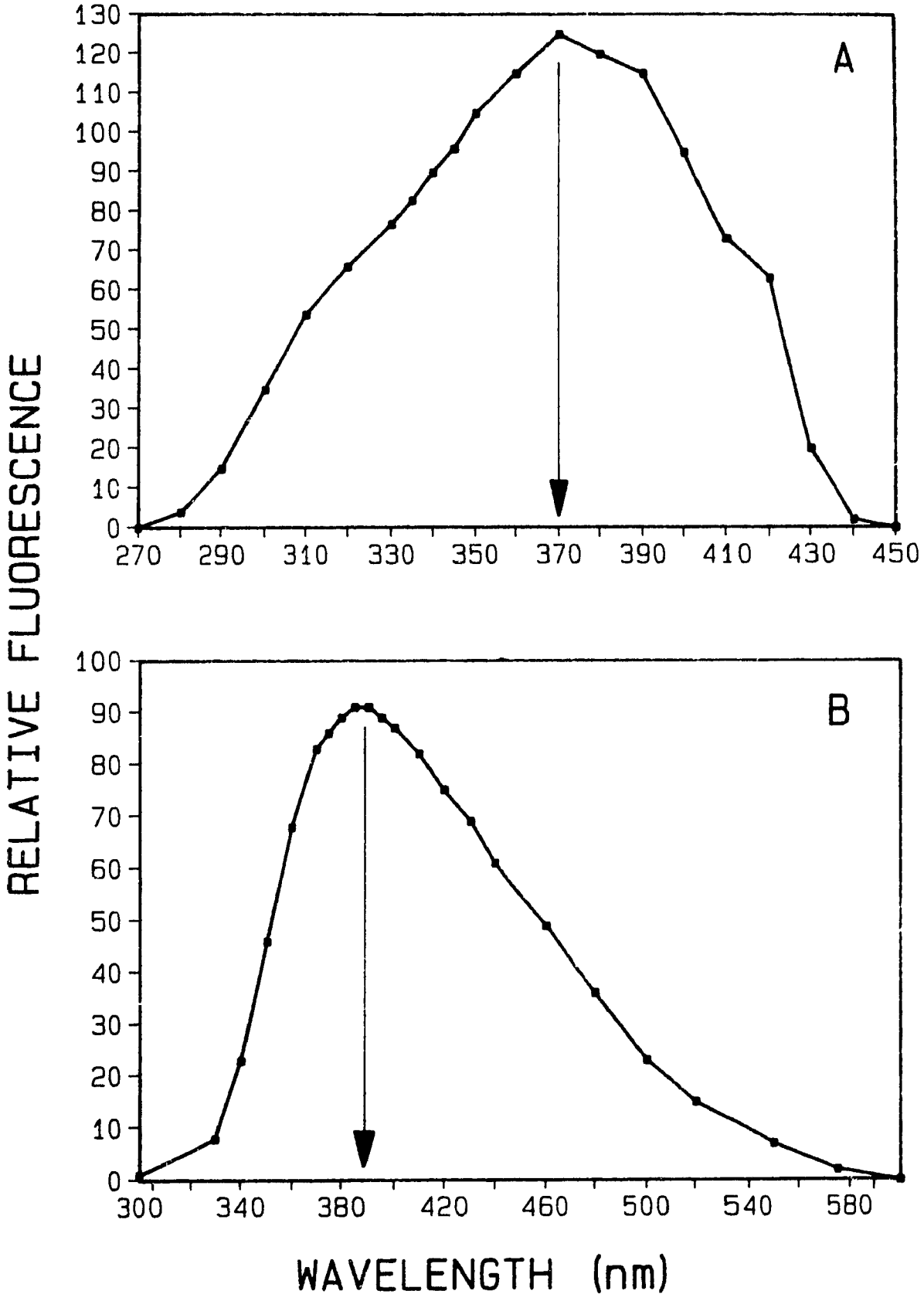
Hydrocarbon analyses

Petroleum hydrocarbons present in the aqueous phase of OWDs and WSFs were determined by fluorescence spectroscopy and gas chromatography. Preliminary analysis of WSF mixtures by fluorescence spectroscopy yielded results showing a high degree of variance among replicate samples. Overall precision was low, possibly due to losses of volatile

hydrocarbons during the solvent concentration step (Warner et al. 1980). Hence, a more accurate gas chromatographic technique was chosen to analyze WSFs.

For OWDs, the method of Keizer and Gordon (1973) was used to measure the concentration of total fluorescing hydrocarbons[4]. During toxicity tests, 115-ml subsamples of 0-h (fresh) and 24-h (old) mixtures were taken at each concentration and controls. Fresh oil-water mixtures were poured directly into sample bottles whereas 24-h solutions were carefully siphoned from 3-4 cm above the bottom of test jars, to avoid contamination from any surface oil film. Subsamples were kept in 125-ml glass serum bottles which were tightly sealed and stored at 0°C until the extraction step (i.e. 1 to 2 hrs). Before extraction with spectrophotometric grade dichloromethane, each subsample was vigorously hand-shaken to re-disperse oil which may have settled at the water surface. Subsamples were extracted twice with 10 ml of dichloromethane and the extracts were stored in tightly capped glass culture tubes until the evaporation step (i.e. 1-2 weeks). Samples were evaporated under vacuum at 31°C in a rotary evaporator. The residues were dissolved in 10 ml of spectrophotometric grade hexane and stored in glass culture tubes which were kept at -20°C until further analysis[5]. Fluorescence was measured in a Turner Model 430 Spectrofluorometer using a slit width of 15 nm. The optimum excitation (370 nm) and emission (385 nm) wavelengths used were determined from a fluorescence scan of a 60 µl/l standard of Norman Wells crude oil in hexane (Fig. 3). The oil concentration of each sample was estimated by a linear regression equation obtained from a

FIG. 3. Fluorescence scans of July '83 Norman Wells crude oil. A: excitation scan with emission wavelength set at 425 nm; B: emission scan with excitation wavelength set at 325 nm. The arrows indicate optimum excitation (A) and emission (B) wavelengths.


A
B
RELATIVE FLUORESCENCE
WAVELENGTH (nm)
130
120
110
100
90
80
70
60
50
40
30
20
10
0
270
290
310
330
350
370
390
410
430
450
100
90
80
70
60
50
40
30
20
10
0
300
340
380
420
460
500
540
580

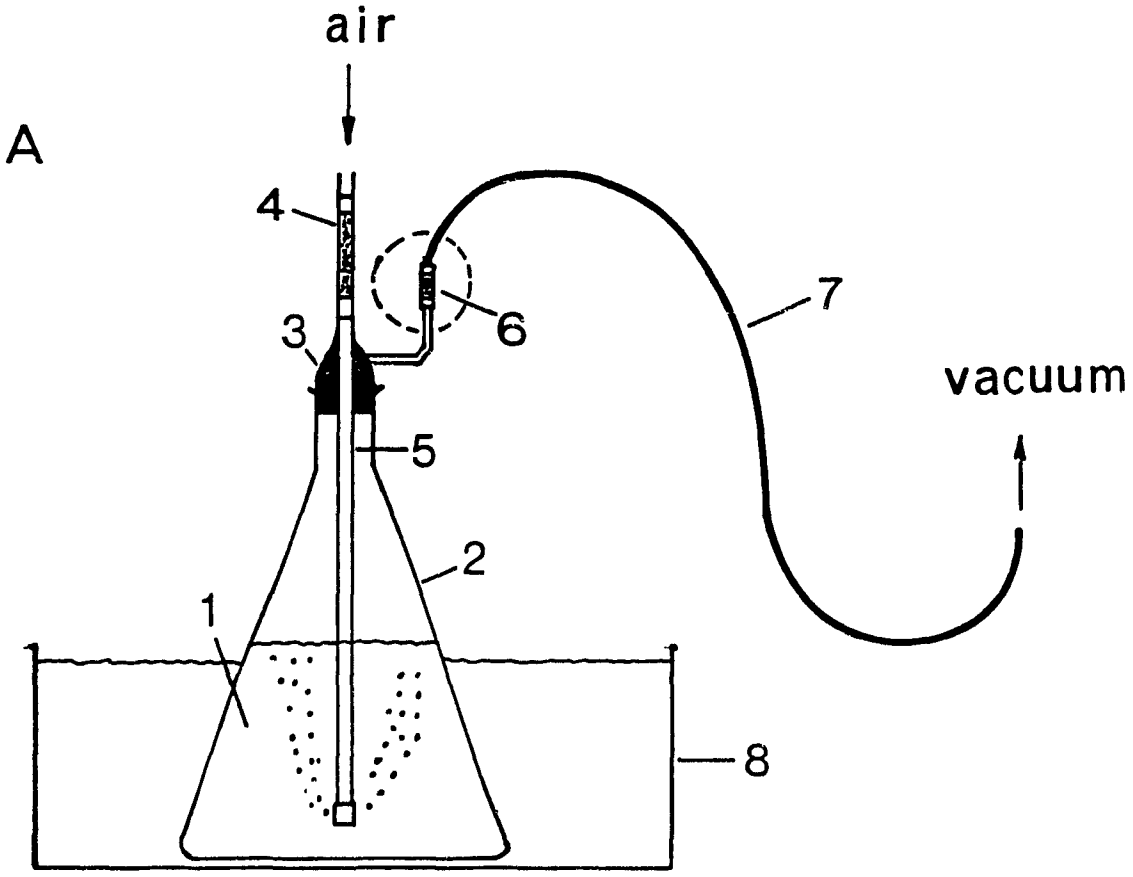
calibration curve ($r > 0.9$) prepared using 5 or 6 standards of known concentration. Since fluorescence quenching occurred at concentrations above 200 µl/l, samples which had a higher concentration were diluted with hexane and re-read. All readings were corrected for the fluorometric readings of the seawater used in the toxicity tests.

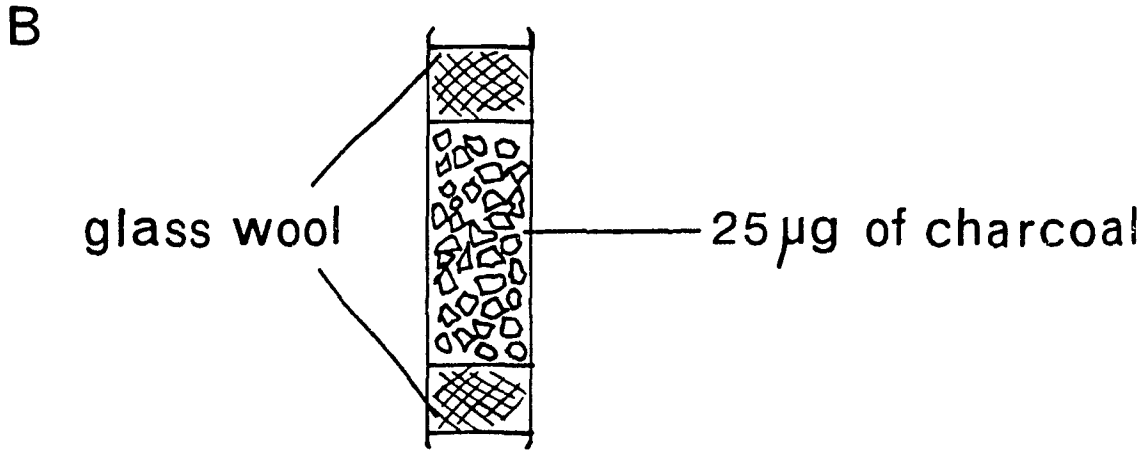
Oil concentrations were first determined as ppm (µl/l) and converted to mg/l. The weight of July '83 oil was determined using a Mettler(TM) AE163 dual range balance. Three volumes (i.e. 0.5, 1.0 and 2.0 ml) were weighed and three replicate readings were taken at four different times after the oil was added to the weighing pan (i.e. 0, 15, 25 and 60 seconds). At 0°C, the average weight of 1 µl of oil was 0.82 mg.

Gas chromatography was used to determine the concentrations of total volatile hydrocarbons (C_6 to C_{10}, or benzene to C_3-naphthalene) as well as the composition of WSF and OWD mixtures. A series of eight 1-L samples consisting of 0-h and 24-h-old OWDs and WSFs were prepared separately from toxicity tests and shipped to the Freshwater Institute (Dept. of Fisheries and Oceans, Winnipeg, Canada) for GC analysis. To test the effect of mysids on WSF concentration, 5 mysids were added to certain test jars and removed after 24 hours. Prior to shipping, samples of OWD were settled for 48 hrs to insure the separation from solution of most particulate hydrocarbons (i.e. C_{12} alkanes and up), thus producing a stable water-soluble fraction (Lockhart et al. 1984). A small amount of sodium azide was added to each sample to prevent bacterial degradation during shipping and storage. Samples were shipped

in tightly capped 1-L glass bottles which were wrapped in aluminum foil and kept refrigerated (at about 5°C) until analysis (i.e. 7 weeks later). GC analysis was done using a method originally designed to analyze volatile organic compounds in fish tissues (Murray and Lockhart, in press). The technique, which combines the principles of purge and trap, has now been successfully applied to water samples with recovery rates in the range of 80-98% for 8 common volatile hydrocarbons (D. Murray, pers. comm.). The method involves bubbling air through a 1-L water sample in order to purge volatile fractions from the solution and collect them using a charcoal trap (Fig. 4). A vacuum pump is used to draw air into the sample flask via a charcoal filter and a glass tube. The pump is connected to the charcoal trap via a capillary tube and a U-shaped glass tube in which a few drops of water are kept to monitor vacuum and to prevent water vapor from blocking the capillary tube. A series of 4-5 capillary tubes connected to the same vacuum pump allow for the simultaneous processing of several samples. Each sample is kept in a 60°C water bath and left to bubble for 3 hrs, after which the charcoal trap is removed and hydrocarbons are extracted using carbon disulfide, as described by Murray and Lockhart (in press). The identification of peaks on the GC traces were confirmed by gas chromatography/mass spectrometry applied to one oil-in-water sample.

FIG. 4. Schematic of purge and trap apparatus used to remove volatile hydrocarbons from water samples. A: 1 = 1-L water sample; 2 = 2-L glass erlenmeyer flask; 3 = ground glass stopper providing air-tight seal; 4 = air-purifying charcoal filter; 5 = glass tube; 6 = charcoal trap; 7 = U-shaped glass tube connected to vacuum pump via a capillary tube; 8 = water bath. B: Charcoal trap used to concentrate volatile hydrocarbons (i.e. as in Fig. 4A, item 6).


air
A
4
3
6
7
vacuum
5
2
1
8


B
glass wool
25 µg of charcoal

Test procedures

Standard 96-h acute lethal toxicity tests[6] were conducted to determine median lethal concentrations (LC50s) and median effective concentrations (EC50s). The basic methods used in this study are similar to those recommended by the American Public Health Association (i.e. APHA 1980).

Mysids were exposed to semi-static (i.e. replaced daily) toxicant mixtures and controls for 96 hrs and then transferred to clean seawater for 48 hrs to test for post-exposure recovery and latent effects (Hansen and Kawatski 1976; Wright 1976). Animals were placed in test jars randomly by drawing numbered slips of paper as recommended by Sprague (1969). The time elapsed between the addition of the first animal and the last one was always less than 1 h. Most tests were conducted using 750-ml wide-mouth glass jars containing 670 ml of solution and 5 mysids per jar (organism loading = 0.17 g/l). However, a series of OWD tests were conducted with 1-L glass beakers, each containing 10 animals per jar (loading = 0.23 g/l). In all replicate tests, 10 animals were exposed to each concentration and controls.

Table 1 summarizes the various experimental methods used in this study. For each different toxicant, sealed jar tests were conducted in which 750-ml jars were sealed with a screw cap lined with aluminum foil. In tests with 1-L beakers, these were sealed with foil only. In all cases there was an air space of 1 cm between the cap and the water surface. Furthermore, a series of open-jar tests was conducted with

TABLE 1. Summary of the toxicity tests conducted and the experimental methods used.

Test No.	Date	Mysid collection year	Toxicant	Open or sealed jars	No. of animals per jar	Size of jar (ml)	No. of concentrations tested	No. of tests conducted
1-2	09/86	1986	OWD83[1]	OPEN	5	750	5	2
3-7	10/86	1986	OWD83[1]	OPEN	10	1000	4	5
8-12	12/86	1986	PHENOL	SEALED	5	750	6	5
13-17	01/87	1986	OWD83[1]	SEALED	10	1000	2-3	5
18-21	09/87	1987	PHENOL	SEALED	5	750	5	4
22-23	10/87	1987	WSF83[2]	SEALED	5	750	6	2
24-27	10/87	1987	WSF86[3]	SEALED	5	750	6	4
28	11/87	1987	OWD83[1]	SEALED	5	750	6	1

1 - Oil-in-water dispersion prepared with oil from the July '83 batch.
2 - Water-soluble fraction prepared with oil from the July '83 batch.
3 - Water-soluble fraction prepared with oil from the July '86 batch.

OWDs using both 750-ml jars and 1-L beakers. All exposure jars were kept in a Hotpack(™) incubator (model 1231) at 0°C. A "cool white" fluorescent light illuminated the jars at an intensity of about 1 μeinstein $m^{-2}s^{-1}$ (10 lux) during 10 hrs per day.

On the basis of preliminary experiments, death and four behaviors of M. oculata exposed to oil-water mixtures were rigorously defined as follows:

A - Animal in normal upright position; walking or swimming; no loss of equilibrium.

B - Loss of equilibrium; animal lying on dorsal or lateral side; more than half of the thoracic appendages moving.

C - Animal lying on dorsal side; less than half of the thoracic appendages moving.

D - Visible motion only after gentle prodding with a glass rod.

DEATH - No visible motion after prodding with a glass rod. Only stages B, C and D are considered behavioral abnormalities.

During exposure to toxicants and controls, observations of behavior, death, molting and cannibalism were generally made after 2, 5, 10, 24, 30, 48, 54, 72, 78 and 96 hrs. Animals were then observed 24 and 48 hrs after their transfer to clean seawater. For observation, each jar was removed from the incubator, opened and the behaviors of the animals were assessed for 30 s. Molts and dead animals were removed as recommended by standard testing methods (Doudoroff et al. 1951;

Sprague 1973; APHA 1980). Dead mysids were examined under a dissecting microscope and the total length of each individual (i.e. tip of rostrum to tip of telson) was measured using a Wild(TM) MMS235 digital length-measuring device. The presence of parasites and external anomalies were noted and species identifications were verified according to Holmquist (1958) for *Mysis*, and Richardson (1905) for the epicarid isopod parasites.

Several parameters of the toxicant mixtures and control seawater were monitored regularly during experiments and are summarized in Table 2. According to APHA (1980) standards, the water quality remained favorable at all concentrations during toxicity tests.

All experiments were conducted at the Arctic Biological Station from Sept. '86 to Feb. '87 and from Sept. '87 to Dec. '87.

Statistical Methods

Exposure concentrations for OWD and WSF tests were determined by calculating the means of measured concentrations at 0 h (fresh) and 24 h (old). In the case of OWDs measured by fluorescence spectroscopy, geometric means were used since dispersed oil concentrations decrease exponentially over a 24-h period (Percy and Mullin 1975). In all other cases, arithmetic means were calculated to obtain average oil concentrations. To determine statistical differences between arithmetic means, a Student's t-test was used (SAS 1985).

TABLE 2. Summary of water parameter measurements for toxicity tests with *M. oculata*.

Parameter measured	Mean	S.D.	n	Measurement method
Temperature (°C)	0.6	0.7	320	Wahl(™) platinum 392M digital heat-prober
Salinity (ppt)	31.0	1.0	9	Goldberg(™) T/C refractometer (Model 10419)
pH	7.86	0.06	6	Radiometer(™) PHM63 digital pH-meter
Carbonate alkalinity (meq/l)	2.45	0.004	7	Parsons et al. (1984)
Ammonia (µg at NH_3-N)	5.8	3.4	14	Dal Pont et al. (1974)
Dissolved oxygen (ml/l)	5.7	0.5	8	Orbisphere(™) microprocessor oxygen indicator (Model 2609) + oxygen electrode
Dissolved oxygen (% saturation)	77.1	6.4	4	As above

Median lethal concentrations were calculated using only the percentage of animals which died during 96-h tests. Median effective concentrations were calculated for abnormal behavioral stages (i.e. B, C, and D) using the percentage of individuals exhibiting the behavior under study as well as behaviors indicative of progressive deterioration in health. For example, EC50s for stage B were calculated using the percent animals exhibiting stages B, C, D and DEATH. Hence, the EC50 value corresponds to the concentration which causes a stage B or "worse" in half of the mysid population tested under the conditions reported here.

Data obtained from post-exposure treatments in clean seawater were used to calculate EC50s for "moribundity" and "ecological death". Animals which died during the post-exposure period were considered to have been moribund and therefore counted as dead within the 96-h test, as suggested by Rice et al. (1977). "Ecologically dead" animals were defined as those which did not fully recover to stage "A" within the post-exposure period and, therefore, may die because of increased vulnerability to environmental stresses and/or predation (Rice et al. 1977).

LC50s, EC50s, their confidence limits[7] and slope functions were calculated using three different methods: probit analysis (Finney 1971; Sokal and Rohlf 1981), the moving average method (Bennet 1952), and the binomial test (Siegel 1956; Sokal and Rohlf 1981). Thus, the amount of statistically sound information derived from dose-response data was maximized. For example, the binomial test produced LC50s, EC50s and

confidence limits with data sets for which the probit method was not appropriate, such as those with less than two "partial kills" (Stephan 1977). However, since probit analysis is the most statistically powerful of the three methods, its results are believed to be more accurate than those of the other two methods. Moving average results are not reported because they are similar to probit results and hence, do not yield useful additional information. Binomial test results are reported as a supplement to probit results but they are not used in statistical comparisons. Dose-response data analyzed by the probit method were corrected for control mortality/behavioral changes by using Abbott's formula (Finney 1971). All calculations were performed using two BASIC computer programs supplied by C. Stephan.

For data sets analyzed by the probit method, a number of conditions outlined by Stephan (unpubl. MS) were respected in order to report statistically sound results:

- Chi square probability higher than 0.05.
- Concentration - effect relationship demonstrated over a reasonable range of percent dead (e.g. less than 37% to more than 63%).
- Highest confidence limit of the LC50 is less than 10 times the lowest confidence limit.
- If the calculated spontaneous mortality is greater than zero, at least two concentrations must have killed a fraction of exposed organisms that is greater than the spontaneous mortality and less than 1.0.

Statistical differences between "probit" LC50s (or EC50s) were tested at the 5% level of significance by the formula for Standard Error of the Difference, as used in Litchfield and Wilcoxon (1949).

RESULTS

Hydrocarbon analyses

Fluorescence spectrophotometry. Table 3 summarizes the results obtained by fluorescence spectroscopy on OWD mixtures. In the four series of tests conducted, the means of most 0-h concentrations increased significantly with the amount of oil added to test jars. Overall, the results of three concentrations were discarded because their initial means were not statistically different ($P > 0.05$) from those of bracketing concentrations. Overlap between concentrations probably occurred due to the unstable nature of oil in water and the narrow intervals between the volumes of oil added (i.e. 5 or 10 µl/l). Over a 24-h static period (i.e. between daily mixture replacement), the concentration of dispersed oil decreased significantly, mostly due to oil droplets coalescing and rising to the surface (Anderson et al. 1974a; Percy and Mullin 1975). The percentage of oil lost from the water ranged from 25.1 to 77.5%, with an overall mean of 58.4% (s.d. = 11.9%). Some of this loss may also be attributable to other factors such as volatilization, uptake by organisms, microbial degradation and photochemical oxidation (Foy 1978). In an attempt to decrease oil loss in 24 hrs, experimental methods in test no. 28 were slightly modified. Mixtures were settled in separatory funnels for 2.5 hrs instead of 1.5 hrs, to obtain a more stable OWD. Since more oil was lost due to coalescence during settling, overall means in test no. 28 were generally

TABLE 3. Results of spectrofluorometric analysis on oil-in-water dispersions.

Test No.		Oil added (mg/l)	Total fluorescing hydrocarbons (mg/l)								
			n	Range	Mean 0-h	S.D. 0-h	Mean 24-h	S.D. 24-h	Geom. mean 0-h + 24-h	Confidence limits Low	Confidence limits High	Average % oil loss
1-2		7.7	n=12	1.21 - 4.70	4.43	0.23	1.76	0.74	2.71	1.89	3.89	65.2
		15.5	n=10	1.94 - 8.29	6.72	1.19	2.23	0.20	3.84	2.51	5.86	66.9
	*	23.2	n=12	1.44 - 9.40	7.98	1.24	2.40	0.60	4.28	2.79	6.57	70.0
		29.8	n=16	4.14 - 16.69	15.14	0.93	7.41	1.99	10.40	8.24	13.12	51.1
		44.7	n=14	8.59 - 24.11	21.05	1.44	10.85	1.40	15.05	12.22	18.53	48.5
		59.6	n=18	5.89 - 26.52	25.01	1.13	13.10	3.42	17.74	14.42	21.82	47.6
3-7		7.5	n=22	1.78 - 9.91	7.79	1.40	4.41	1.22	5.68	4.74	6.81	45.7
		14.9	n=16	3.68 - 12.00	10.33	0.97	4.68	0.81	6.90	5.48	8.69	54.8
		22.4	n=22	2.72 - 15.55	13.58	1.55	5.43	1.84	8.34	6.53	10.65	60.0
	*	29.8	n=14	5.07 - 18.38	15.21	1.92	7.17	2.26	10.22	6.92	12.53	52.9
		44.7	n=14	3.73 - 21.20	16.89	3.17	6.65	2.51	10.22	7.31	14.27	60.6
13-17		3.0	n=24	2.02 - 5.06	3.43	1.02	2.57	0.69	2.87	2.52	3.26	25.1
		29.8	n=8	3.78 - 17.49	14.36	2.62	5.55	1.97	8.68	5.34	14.12	61.3
		59.6	n=12	4.01 - 36.33	29.57	5.65	6.64	1.60	13.71	8.15	23.05	77.5
28		1.5	n=6	0.33 - 1.89	1.25	0.82	1.09	0.13	1.03	0.54	1.96	75.0
		7.5	n=6	0.88 - 6.01	4.85	1.01	1.22	0.44	2.36	1.01	5.54	70.7
		14.9	n=8	1.24 - 8.35	7.33	1.07	2.15	0.96	3.81	2.04	7.15	56.8
	*	29.8	n=8	2.96 - 11.76	9.36	2.03	4.05	0.86	6.04	4.00	9.12	56.7
		44.7	n=6	4.76 - 12.48	12.06	0.38	5.34	0.64	8.00	4.96	12.91	55.8
		59.6	n=8	2.51 - 15.75	14.29	1.15	4.91	2.83	7.84	4.20	14.66	65.7

* - Concentrations not used in the calculations of LC50's and EC50's. The means of 0-hr measured concentrations were not statistically different ($P > 0.05$) than those of bracketing concentrations.

lower than in test no. 1-2 (Fig. 5). However, increasing the settling time by 1 h did not narrow confidence limits nor did it significantly change concentrations lower than about 23 mg/l of oil added. Although OWDs were unstable under the conditions used in this study, geometric means are believed to be a reliable estimate of exposure concentrations. For a more complete discussion on the behavior of particulate oil in seawater the reader should consult Anderson et al. (1974a) and Percy and Mullin (1975).

Gas chromatography. An examination of the GC traces of both WSF and OWD mixtures revealed that most of the major peaks of volatile hydrocarbons in the aqueous phase were aromatic compounds, primarily alkylated benzenes and naphthalenes (Fig. 6). All of these fractions are known for their toxicity to marine crustaceans, especially the naphthalenes (Anderson et al. 1974a; Tatem et al. 1978). Since concentrations of specific hydrocarbon compounds were not determined, only the obvious visual differences between chromatograms were noted. All mixtures had similar compositions but certain compounds occurred in different proportions depending on the sample. Overall, the most obvious difference occurred between fresh OWDs and WSFs; the former contained a higher proportion of volatile hydrocarbons from ethylbenzene to C_3-naphthalene (Fig. 6).

Total volatile hydrocarbon concentrations were determined for OWD and WSF mixtures (Table 4). Both WSFs prepared without animals had a similar composition, however, the WSF86 mixture had a slightly higher concentration of aromatic hydrocarbons as well as a higher percentage of

FIG. 5. Concentrations of crude oil-in-seawater dispersions measured during toxicity tests conducted using four different exposure methods. Geometric means and 95% confidence limits are indicated.

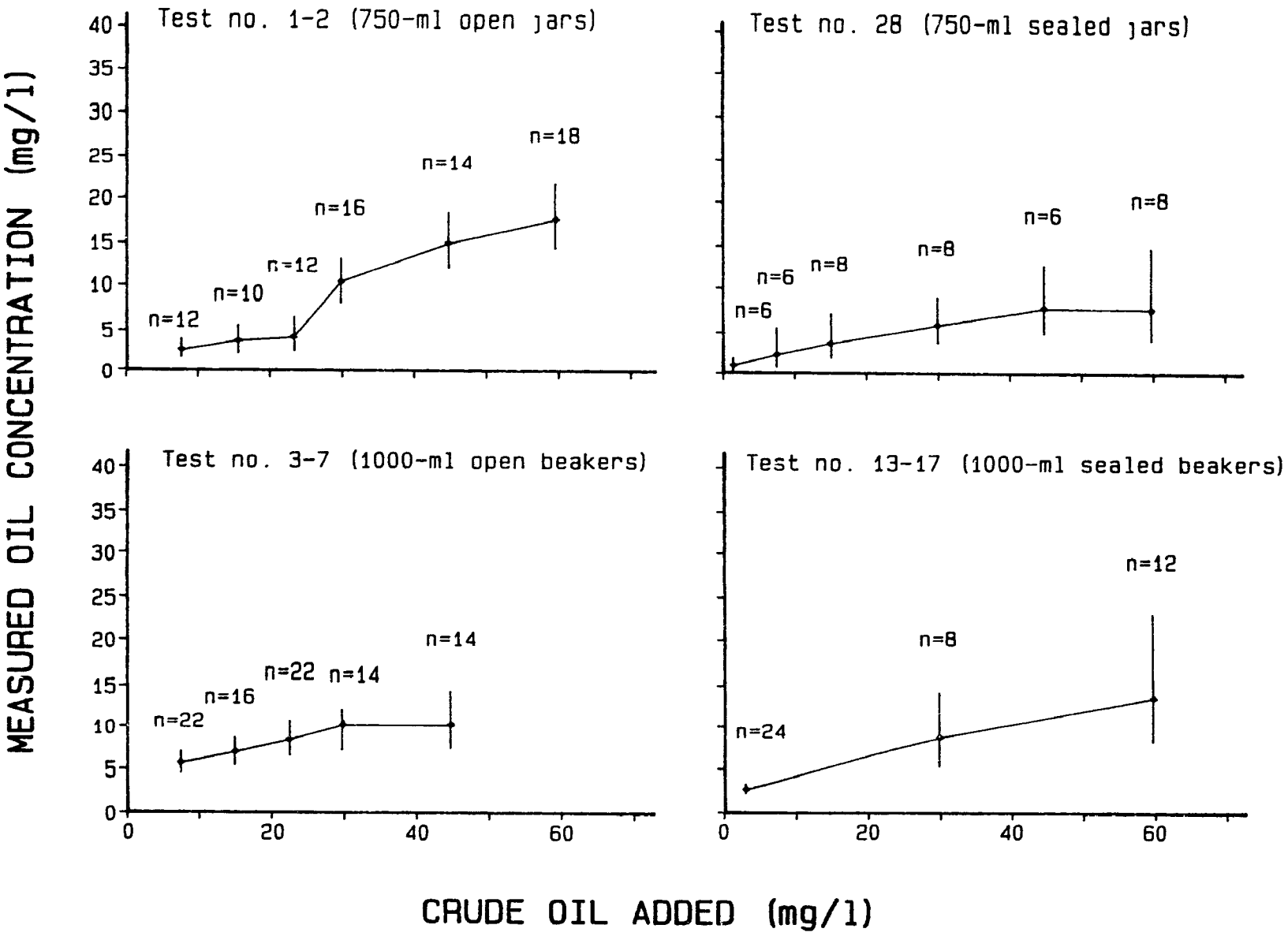

Test no. 1-2 (750-ml open jars)
Test no. 28 (750-ml sealed jars)
Test no. 3-7 (1000-ml open beakers)
Test no. 13-17 (1000-ml sealed beakers)
n=12
n=10
n=12
n=16
n=14
n=18
n=6
n=6
n=8
n=8
n=6
n=8
n=22
n=16
n=22
n=14
n=14
n=24
n=8
n=12
0
5
10
15
20
25
30
35
40
0
20
40
60
MEASURED OIL CONCENTRATION (mg/l)
CRUDE OIL ADDED (mg/l)

FIG. 6. GC traces showing volatile hydrocarbon compounds contained in Norman Wells crude oil-in-water mixtures. S = carbon disulfide solvent; 1 = benzene; 2 = toluene; 3 = ethylbenzene; 4 = *m*- + *p*-xylene; 5 = *o*-xylene; 6 = iso-propylbenzene; 7 = *n*-propylbenzene; 8 = C_3-alkylated benzenes; 9 = C_4-alkylated benzenes; 10 = naphthalene; 11 = 2-methyl-naphthalene; 12 = 1-methylnaphthalene; 13 = C_2-alkylated naphthalenes; 14 = internal standard (*n*-decyl-benzene).

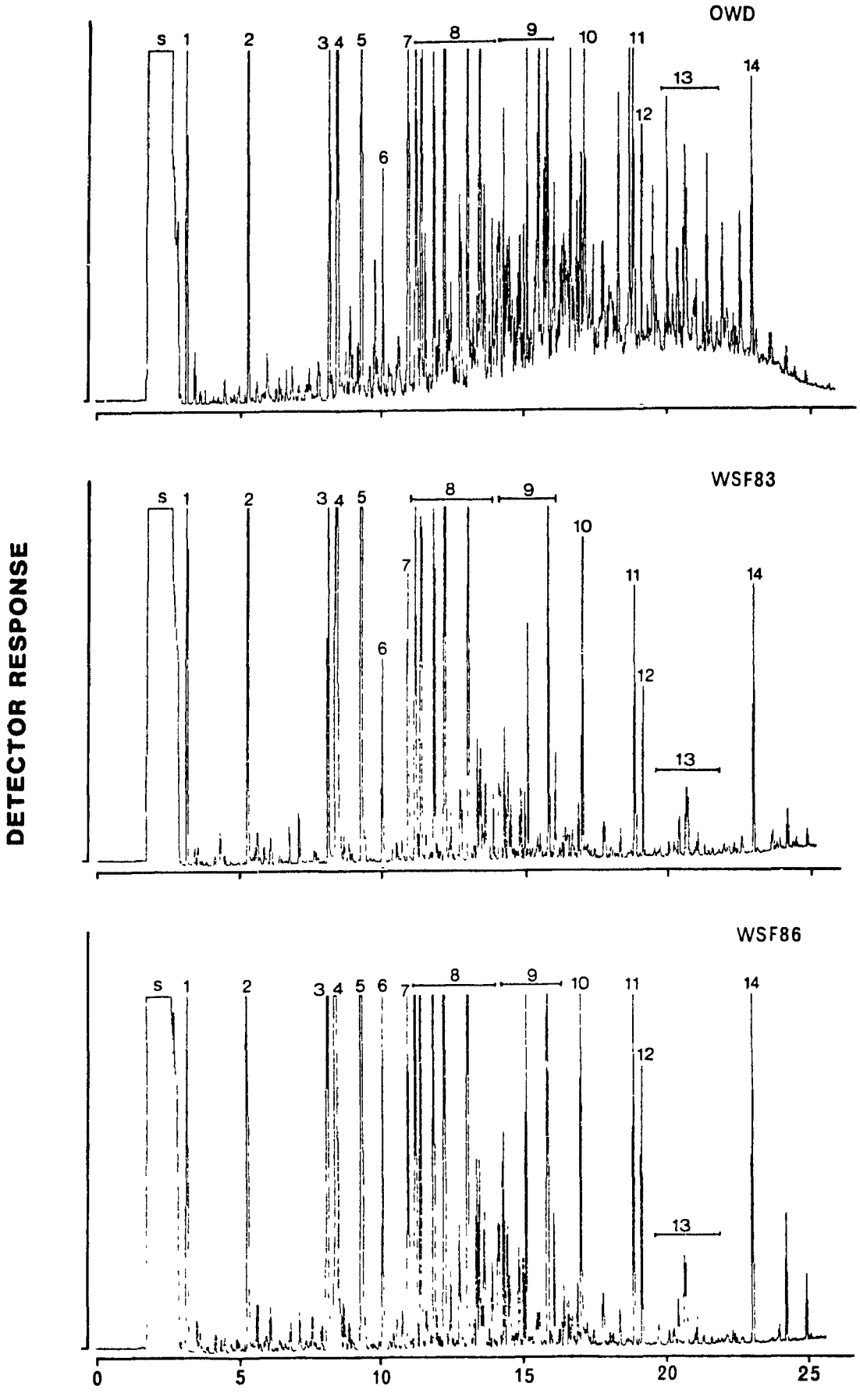

OWD
WSF83
WSF86
S
1
2
3
4
5
6
7
8
9
10
11
12
13
14
DETECTOR RESPONSE
0
5
10
15
20
25
RETENTION TIME (min)

TABLE 4. Concentrations of volatile hydrocarbons in oil-water mixtures as determined by gas chromatography.

Toxicant	Total volatile hydrocarbons (µg/l)				Average % oil loss
	0-h	24-h	Mean 0-h + 24-h	S.D.	
WSF83[1]	1110.7 1047.5	1009.7 974.4	1035.6	58.3	8.1
WSF86[1]	1293.8 1250.1	1131.1 1024.6	1174.9	121.5	15.3
WSF86[1] + 5 mysids	1293.8 1250.1	817.8 800.5	1040.6	800.5	36.4
OWD83[2]	1103.4 1033.4	578.2 577.7	823.2	284.6	45.9
OWD83[3]	1103.4 1033.4	766.0 749.0	913.0	181.9	29.1

1 - 60% of stock WSF; sealed jars.
2 - 60 mg/l of oil added; open jars.
3 - 60 mg/l of oil added; sealed jars.

oil lost in 24 hrs. These results may reflect the composition of each crude oil. When mysids were added to the test jars, the average percent oil loss was increased by about 20%, suggesting that *M. oculata* take up a significant portion of dissolved volatiles. Both OWDs and WSFs had a similar initial (0-h) concentration of volatiles, but OWDs had the lowest mean concentration due to relatively high losses during a 24-h period. The average percent loss for OWDs in open jars was about 17% higher than in sealed jars. Sealing test jars was an efficient method for retaining at least 70% of the volatile hydrocarbons contained in OWD mixtures and at least 85% of those in WSFs.

The concentrations of volatile hydrocarbons for all dilutions used in toxicity tests were calculated as percentages of the average concentration of each toxicant sample measured by gas chromatography. Only concentrations measured from samples without animals were used in the calculations.

Toxicity tests

Median lethal and effective concentrations for all tests are reported in the appendix (Tables A1 to A6). The levels of precision of LC50s and EC50s were calculated using data from dispersed oil tests. For data sets analyzed by the probit method, the upper and lower confidence limits were 39% (s.d. = 13%, n = 23) of the LC50 or EC50, on the average. In the case of the binomial test, much wider confidence limits[a] of 80% (s.d. = 51%, n = 16) of the LC50 were obtained. Ideally,

higher levels of precision are preferred and can be obtained by exposing more animals to each concentration (Jensen 1972; Hodson et al. 1977; APHA 1980). However, due to a limited supply of mysids, 10 individuals were exposed to each concentration and replicate tests were conducted, as suggested by Hodson et al. (1977). Most LC50s and EC50s of different exposure times (i.e. 2 to 96 hrs) were not statistically different because of overlapping confidence limits. Therefore, incipient LC50s and EC50s could not be calculated from toxicity curves and test results are presented as several 96-h LC50s and 96-h EC50s, or as a range.

Although control mortality/behavioral changes occurred in 2 out of 28 toxicity tests, corrected dose-response data were not used in statistical comparisons of LC50s and EC50s. The use of Abbott's formula is justified only if the cause of the spontaneous mortality (or effect) does not make the rest of the test organisms more or less susceptible to the toxicant (Sprague 1973; Stephan 1977; APHA 1980). This assumption is questionable in most acute mortality tests with aquatic organisms. Therefore, it is generally agreed that if control mortality (or effect) is inconsistent and below 10% (i.e. representing one weak organism in a group), the test is valid and correction of the LC50 (or EC50) for this mortality (or effect) would seem to be a meaningless exercise (Doudoroff et al. 1951; Sprague 1973; Stephan 1977). Table A3 shows that corrected LC50 and EC50 values and confidence limits are only slightly different (i.e. 0.2-0.3 mg/l higher) than non-corrected ones. Preliminary calculations showed that the use of corrected data did not significantly change the statistical results obtained with non-corrected data.

Mysid length and parasitism. Mysids from the two different collections differed in their mean lengths and percent parasitism (Table 5). Animals sampled in 1986 had a statistically higher mean total length ($P < 0.0001$) and twelve times more parasitized individuals than those of 1987. The epicarid isopod, *Dajus mysidis*, was the most visible parasite and was usually found attached to the last thoracic somite of the mysid, on the ventral surface (also see Tattersall and Tattersall 1951). In some cases, ciliates were seen swimming on the body surface of dead mysids.

Phenol. To compare the sensitivity of mysid collections the 96-h LC50s and 96-h EC50s for moribundity and "ecological death" were compared (Table 6). Although the two groups differed in their mean length and percent parasitism, they did not differ in their sensitivity to phenol. There was significant overlap in the ranges of LC50s, EC50s and their confidence limits. LC50s and EC50s for all toxicity tests were compared under the assumption that both stocks of mysids were equally sensitive to oil-in-water mixtures.

Oil-in-water dispersions. The 96-h LC50s of mysids exposed to OWDs ranged from 4.51 to 7.57 mg/l, using four different experimental methods. To show differences in LC50s and EC50s of sealed- and open-jar tests, data were grouped according to behavioral/mortality stage (Table 7). To eliminate bias due to the potential effect of jar size on animal sensitivity, only tests conducted with the same size jars were statistically compared. Mysids held in 1-L beakers as opposed to 750-ml jars, were more crowded and may have been more sensitive partly due to stress

TABLE 5. Total length and percent parasitism of young *Mysis oculata* from the 1986 and 1987 collections.

Collection year	Total length (mm)				Percentage of animals parasitized with *Dajus mysidis*
	n	Range	Mean	S.D.	
1986	572	10.0 - 17.3	14.1	1.2	11.0 %
1987	350	9.4 - 15.8	12.9	1.1	0.9 %

N.B. Mean total lengths are statistically different ($P < 0.0001$).

TABLE 5. Ranges of 96-h LC50s, 96-h EC50s and their confidence limits for two different collections *M. oculata* exposed to phenol.

Calculation method	Stage	Mysid collection year	No. of tests	Range of 96-h LC50s or EC50s[1] (mg/l)	Range of confidence limits
Probit	DEATH	1986	4	37.8 - 75.0	26.4 - 107.8
		1987	2	37.2 - 56.1	22.7 - 104.8
	MOR24[2]	1986	4	29.8 - 71.2	19.3 - 98.8
		1987	3	25.8 - 36.9	13.1 - 60.8
Binomial	DEATH	1986	5	50 - 89	5 - 125
		1987	4	35 - 74	5 - 125
	MOR24[2]	1986	5	25 - 69	5 - 125
		1987	4	21 - 40	5 - 125
	ECO24[3]	1986	5	11 - 23	5 - 125
		1987	4	8 - 10	5 - 15

1 - LC50s correspond to values for stage DEATH, all others are EC50s.
2 - Moribund animals which died during a 24-h post-exposure recovery period.
3 - "Ecologically dead" animals which did not fully recover within a 24-h post-exposure period.

TABLE 7. 96-h LC50s, 96-h EC50s and their confidence limits for *M. oculata* exposed to oil-in-water dispersions. Data are grouped by stage.

Stage	Method no.[1]	No. of tests	96-h LC50s[2] or EC50s (mg/l)		Range of confidence limits
B[3]	1	2	3.91 - 4.38		2.62 - 6.02
	4	1	1.90	*	1.30 - 2.53
C[3]	1	2	5.31 - 5.32		3.54 - 7.31
	4	1	2.27	*	1.50 - 3.16
D[3]	1	1	5.61		3.80 - 7.67
	4	1	2.41	*	1.63 - 3.36
DEATH	1	1	5.61		3.80 - 7.67
	4	1	4.51		3.09 - 8.20
	2	3	6.43 - 7.57		5.35 - 8.61
	3	1	4.93		3.35 - 6.89

1 - Method no. 1 : 750-ml open jars
Method no. 2 : 1000-ml open beakers
Method no. 3 : 1000-ml sealed beakers
Method no. 4 : 750-ml sealed jars

2 - When more than one test was conducted, a range of LC50s (or EC50's) is reported.

3 - Animals exhibit this behavior or one showing more health deterioration (e.g. B or 'worse').

* - LC50 (or EC50) is statistically different from other replicates at the 0.05 probability level.

rather than solely due to the sealing of test jars. In tests with 750-ml jars, LC50s and EC50s were consistently 1-3 mg/l lower when jars were sealed (Fig. 7). There were no statistical differences between the LC50s, but as a result of prolonged exposure to volatile hydrocarbons certain EC50s for sealed-jar test (i.e. method no. 4) were statistically lower than comparable EC50s obtained using the open-jar method (Table 7, Fig. 7). In tests with 1-L beakers there was no statistical difference between the LC50s of sealed- and open-jar tests. The similar toxicity of both mixtures may have been caused by losses of volatile hydrocarbons from sealed beakers due to their aluminum covers not providing an air-tight seal. To compare LC50s and EC50s of different stages, data were grouped by experimental method (Table 8). EC50s for all OWD tests were lower than LC50s, with stages B and "ecological death" producing the lowest values. Method no. 4 had the lowest EC50 values (i.e. 1.54 - 2.73 mg/l) and most of these were statistically different from the LC50 (Fig. 8). Differences in LC50s and EC50s due to jar size were small (i.e. 1-2 mg/l) and not statistically different, as demonstrated by a comparison of the values for open-jar tests in Table 8 (i.e. method no.1 vs method no.2).

Similar trends are shown by the binomial test results (Table A4). Approximate LC50 and EC50 values ranged from 2 to 9 mg/l and the lowest ones also occurred in method no. 4 (2 - 4 mg/l). EC50s for stages B, MOR24, MOR48, ECO24 and ECO48 were usually 1 - 3 mg/l lower than the 96-h LC50.

FIG. 7. 96-h LC50s and 96-h EC50s of *M. oculata* exposed to oil-in-water dispersions. Data are grouped by behavioral stage, moribundity, and "ecological death" to show differences between replicate EC50s (or LC50s) obtained using different experimental methods. Vertical lines correspond to 95% confidence limits. Numbers above the lines correspond to the experimental method used: 1 = 750-ml sealed jars; 2 = 1000-ml open beakers; 3 = 1000-ml sealed beakers; 4 = 750-ml sealed jars.

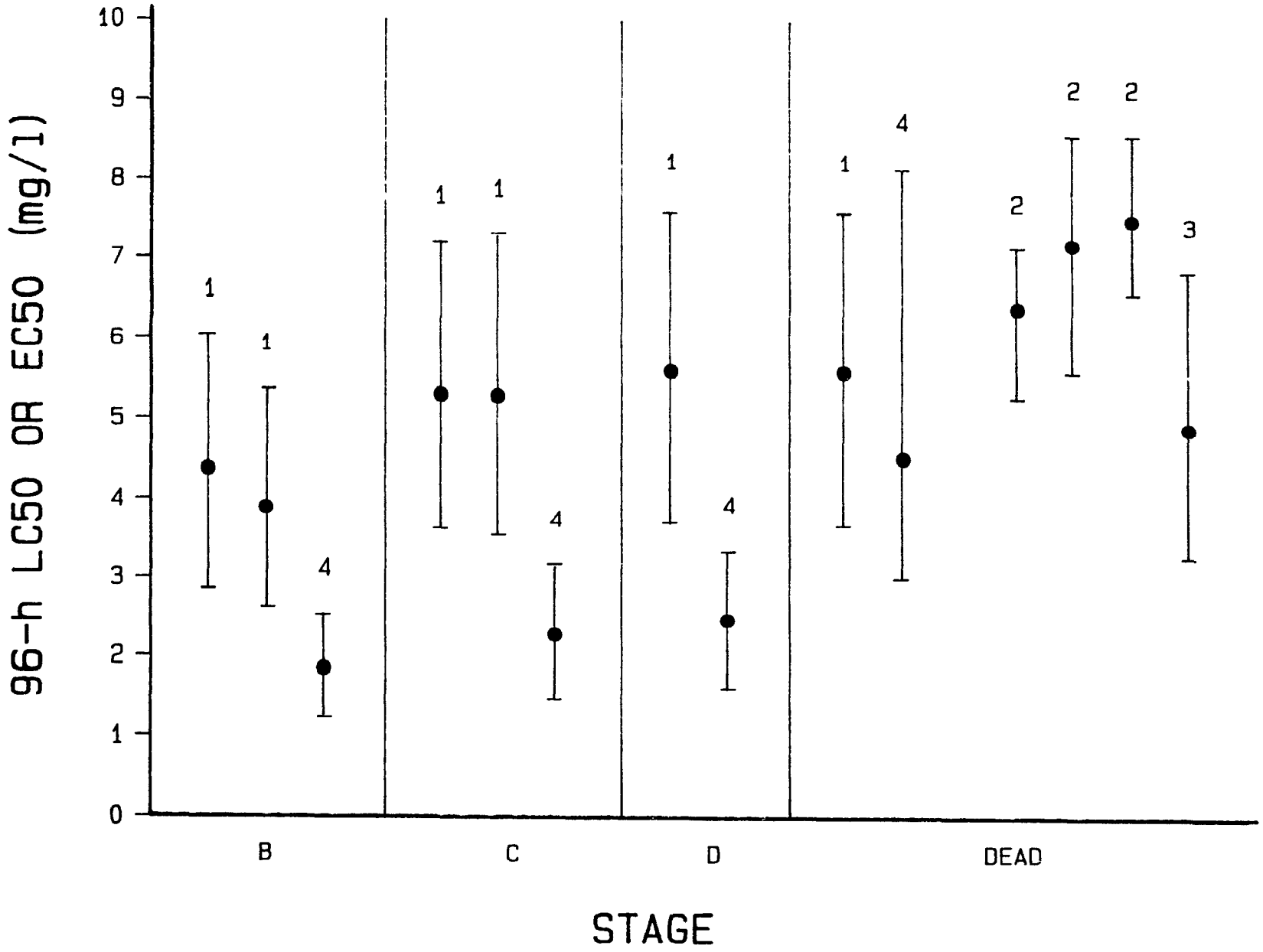

96-h LC50 OR EC50 (mg/l)
10
9
8
7
6
5
4
3
2
1
0
1
1
4
1
1
4
1
4
1
4
1
2
2
2
3
B
C
D
DEAD
STAGE

TABLE 8. 96-h LC50s, 96-h EC50s and their confidence limits for *M. oculata* exposed to oil-in-water dispersions. Data are grouped according to the experimental method used.

Method no.	Stage	No. of tests	96-h LC50s or EC50s[1] (mg/l)		Range of confidence limits
1	B	2	3.91 - 4.38		2.62 - 6.02
	C	2	5.31 - 5.32		3.54 - 7.31
	D	1	5.61		3.80 - 7.67
	DEATH	1	5.61		3.80 - 7.67
2	C	1	6.78		5.79 - 7.54
	D	2	6.85 - 7.12		5.02 - 8.02
	DEATH	3	6.43 - 7.57		5.35 - 8.61
	MOR24	1	6.44		5.65 - 7.07
	ECO24	1	6.45		5.65 - 7.10
3	DEATH	1	4.93		3.35 - 6.89
4	B	1	1.90	*	1.30 - 2.35
	C	1	2.27	*	1.50 - 3.16
	D	1	2.41	*	1.63 - 3.36
	DEATH	1	4.51		3.09 - 8.20
	MOR24	1	2.73		1.81 - 3.96
	MOR48[2]	1	2.41		1.57 - 3.42
	ECO24	1	1.81	*	1.07 - 2.56
	ECO48[3]	1	1.54	*	0.80 - 2.19

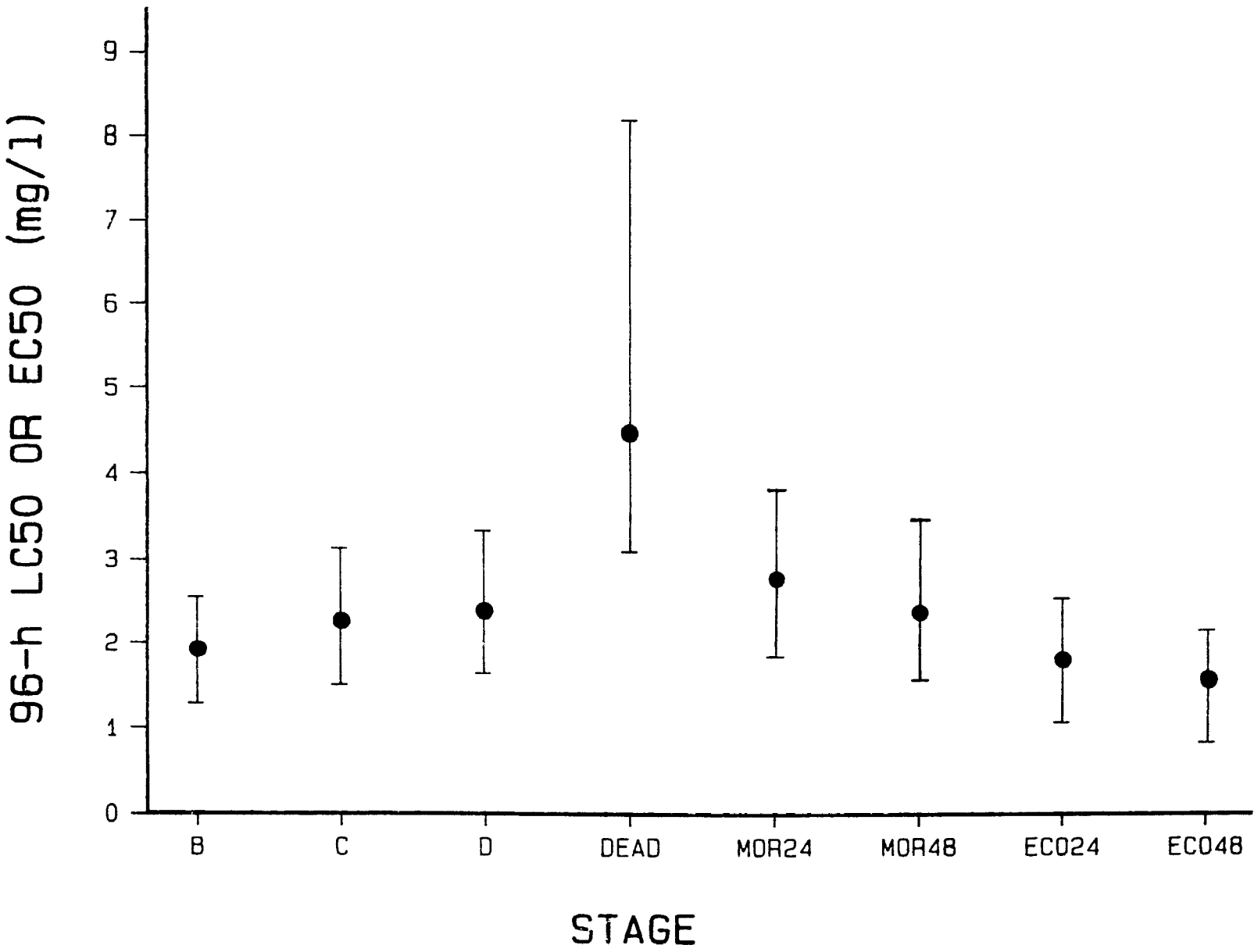
1 - When more than one test was conducted, LC50s (or EC50s) are reported as a range.

2 - Moribund animals which died within a 48-h post-exposure period in clean seawater.

3 - "Ecologically dead" animals which did not fully recover to stage A within a 48-h post-exposure period in clean seawater.

* - EC50s are statistically different from the LC50 at the 0.05 probability level.

FIG. 8. 96-h LC50, 96-h EC50s, and confidence limits of *M. oculata* exposed to oil-in-water dispersions using experimental method no. 4 (i.e. 750 ml sealed jars).


96-h LC50 OR EC50 (mg/1)
0
1
2
3
4
5
6
7
8
9
B
C
D
DEAD
MOR24
MOR48
EC024
EC048
STAGE

Water-soluble fractions. 96-h LC50s for WSF83 and WSF86 ranged from 486 to 624 µg/l. The two mixtures did not produce statistically different results (Fig. 9). For both mixtures, EC50s were the lowest for stage B, and values increased in the following order of stage: EC024 & 48, MOR24 & 48, C, D and DEATH (Fig. 10). In both cases, EC50s for stage B were statistically lower than the LC50 (Table 9). Also, tests conducted with WSF86 resulted in EC50s for "ecological death" which were statistically lower than the LC50.

Results from the binomial test show similar patterns in the data (Table A6).

Cannibalism and molting. The incidence of cannibalism during 96-h tests was highest in low concentrations of OWD (i.e. 1 - 4 mg/l) and low to intermediate concentrations of WSF (i.e. 259 - 685 µg/l) (Table 10). In most cases, cannibalism occurred because mysids which had lost equilibrium or died (i.e. stages B, C, D and DEATH) were eaten by more resistant individuals (i.e. stage A's). This did not occur at high concentrations because all mysids in test jars lost equilibrium rapidly, including the most resistant ones. Molting occurred in low and intermediate concentrations of toxicant as well as in control jars.

The mortality of marine crustaceans exposed to oil can increase during molting (Wells and Sprague 1976; Mecklenburg et al. 1977) and cannibalism (Capuzzo and Lancaster 1981; Derby and Capuzzo 1984). However, there were no detectable relationships between the percent cannibalism or molting and 96-h LC50s. The lowest LC50s indicating increased sensitivity, were rarely related to the highest incidence of

FIG. 9. 96-h LC50s, 96-h EC50s, and confidence limits of *M. oculata* exposed to water-soluble fractions. Data are grouped by behavioral stage (Fig. 9A), moribundity, and "ecological death" (Fig. 9B) to show differences between LC50s and EC50s due to different WSF mixtures. Numbers above the vertical lines correspond to the mixture used in toxicity tests: 1 = WSF83; 2 = WSF86.

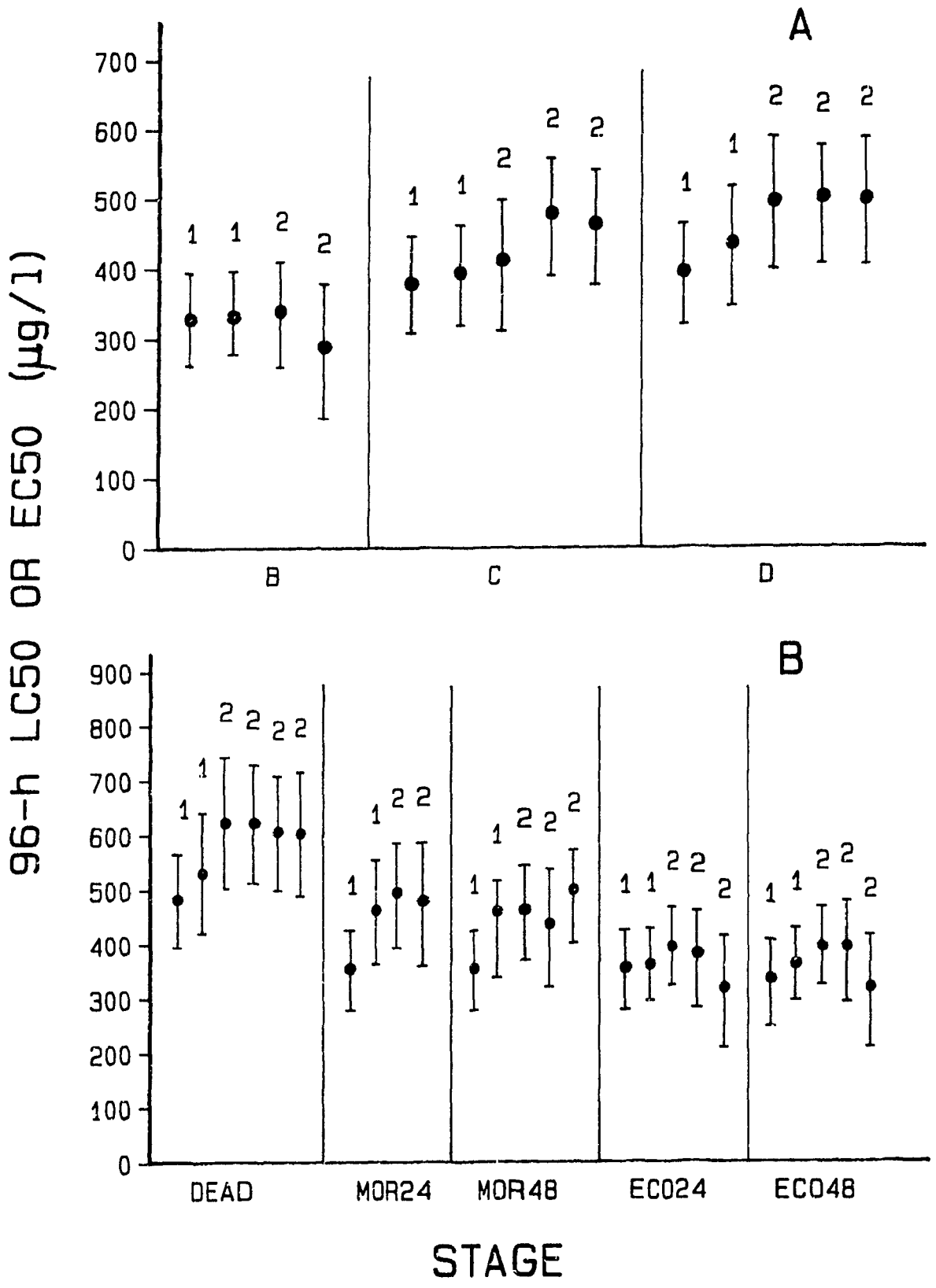

A
700
600
500
400
300
200
100
0
B
C
D
96-h LC50 OR EC50 (µg/1)
B
900
800
700
600
500
400
300
200
100
0
DEAD
MOR24
MOR48
EC024
EC048
STAGE

FIG. 10. 96-h LC50s, 96-h EC50s, and confidence limits of *M. oculata* exposed to two different WSF mixtures (i.e. WSF83 and WSF86). The chart shows differences in LC50s and EC50s according to behavioral stage, death, moribundity and "ecological death".

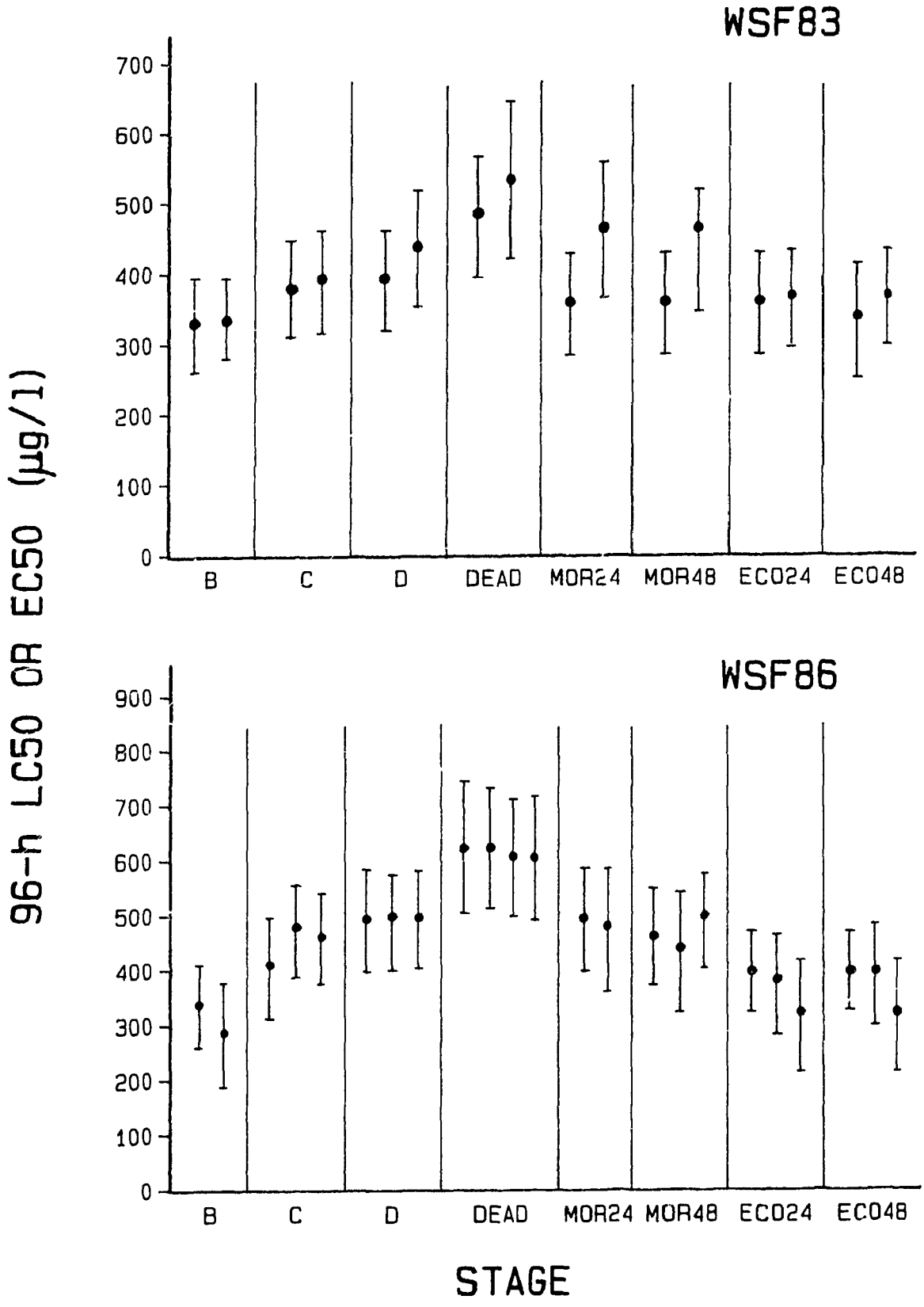

WSF83
700
600
500
400
300
200
100
0
B
C
D
DEAD
MOR24
MOR48
EC024
EC048
WSF86
900
800
700
600
500
400
300
200
100
0
B
C
D
DEAD
MOR24
MOR48
EC024
EC048
96-h LC50 OR EC50 (µg/l)
STAGE

TABLE 9. Ranges of 96-h LC50s, 96-h EC50s and their confidence limits for *M. oculata* exposed to water-soluble fractions. Data are grouped according to the WSF mixture used in toxicity tests (i.e. WSF83 or WSF86).

Toxicant	Stage	No. of tests	Range of 96-h LC50s or EC50s (µg/l)		Range of confidence limits
WSF83	B	2	331 - 334	*	262 - 396
	C	2	379 - 394		307 - 462
	D	2	394 - 437		319 - 517
	DEAD	2	486 - 533		396 - 644
	MOR24	2	358 - 463		280 - 556
	MOR48	2	358 - 435		280 - 519
	EC024	2	358 - 365		280 - 430
	EC048	2	337 - 365		249 - 430
WSF86	B	2	289 - 340	*	187 - 410
	C	3	413 - 481		310 - 556
	D	3	496 - 500		396 - 586
	DEAD	4	605 - 624		492 - 745
	MOR24	2	481 - 496		361 - 587
	MOR48	3	440 - 500		321 - 575
	EC024	3	321 - 397	*	211 - 469
	EC048	3	321 - 397	*	211 - 481

* - All replicate EC50s are statistically lower than the LC50 at the 0.05 probability level.

TABLE 10. Percent cannibalism and percent molting in toxicity test with *M. oculata*.

Toxicant	Concentration (mg/l)	Total no. of animals exposed	Percent cannibalism[1]	Percent molting[2]
OWD	Control	110	0.9	3.6
	1.03	10	10.0	0.0
	2.36	10	20.0	10.0
	2.71	20	10.0	5.0
	2.87	50	22.0	2.0
	3.81	10	20.0	0.0
	3.84	20	0.0	0.0
	5.68	50	4.0	10.0
	6.91	40	0.0	7.5
	8.00	10	0.0	0.0
	8.34	50	0.0	2.0
	8.68	10	0.0	0.0
	10.22	50	0.0	4.0
	10.40	20	0.0	0.0
	13.71	50	0.0	0.0
	15.05	20	0.0	0.0
	17.74	20	0.0	0.0
WSF	Control	60	0.0	13.3
	0.086	20	0.0	5.0
	0.098	40	0.0	20.0
	0.259	20	15.0	15.0
	0.294	40	7.5	10.0
	0.432	20	0.0	0.0
	0.490	40	32.5	2.5
	0.604	20	0.0	0.0
	0.685	40	12.5	0.0
	0.777	20	0.0	0.0
	0.881	40	0.0	0.0
	1.036	20	0.0	0.0
	1.175	40	0.0	0.0

1 - Percent animals partially eaten within 96-h tests.
2 - Percent full or partial molting during 96-h tests.

cannibalism and/or molting.

Post-exposure recovery. Table 11 shows the percent delayed mortality and "ecological death" for *M. oculata* during post-exposure periods in clean seawater. Latent effects were consistently higher for organisms exposed to OWDs as opposed to WSFs.

TABLE 11. Percent delayed mortality and "ecological death" of *M. oculata* during post-exposure to clean seawater.

Toxicant	Method no.	No. of animals transferred to clean seawater	Percent delayed mortality in 24 hrs	Percent delayed mortality in 48 hrs	Percent "ecological death" in 24 hrs	Percent "ecological death" in 48 hrs
OWD83	1	48	12.5	--	29.2	--
	2	83	19.3	38.6	57.8	63.9
	3	50	20.0	42.0	54.0	62.0
	4	26	26.9	34.6	50.0	57.7
WSP83		62	19.4	22.6	30.7	32.3
WSP86		131	15.3	24.4	26.0	26.0

DISCUSSION

Behavior of oil in water

The mixing method used in toxicity tests greatly influences the composition of oil-in-water solutions. It is generally agreed that vigorous shaking, as used here in the preparation of oil-in-water dispersions, creates a solution which resembles the parent oil in composition (NRC 1985). Gentle stirring on the other hand, produces a water-soluble fraction which is greatly enriched in aromatic hydrocarbons (Anderson et al. 1974a). Although similar patterns were found in this study, WSFs prepared by the method of Anderson et al. (1974a) were not saturated with aromatic hydrocarbons. Conversely, OWDs produced by shaking contained a higher proportion of several aromatic compounds from ethylbenzene to C_3-naphthalene. These results suggest that short-term vigorous shaking is a more efficient method of dissolving all volatile oil fractions and producing a solution saturated with aromatic hydrocarbons. Gently stirring oil-in-water solutions selectively dissolves a high proportion of soluble fractions (i.e. mono-nuclear aromatics) but a lesser proportion of those which require more mixing energy, such as the di- and tri-nuclear aromatics (Rice et al. 1977; Carls and Korn 1985; NRC 1985). In order to saturate WSF mixtures, certain authors (Higgins et al. 1982; Murray et al. 1984) have used short-term vigorous shaking of 10-50 ml of oil in 1 L of water followed by a 48-h settling period. Such a method may be useful in cold water toxicity testing because more

mixing energy is required to transport oil into the aqueous phase at lower temperatures.

Toxicity of test solutions

Out of the two types of oil-in-water mixtures tested, WSFs were more harmful to young *M. oculata* than OWDs. Although different analytical techniques were used to measure each mixture, comparisons are reliable because preliminary analysis of WSFs by fluorescence spectroscopy showed that estimated concentrations were similar to those determined by gas chromatography. Both EC50s and LC50s show that WSFs are at least an order of magnitude more toxic than OWDs. Similar results have been reported for other species of marine fish and crustaceans (Rice et al. 1977). The increased toxicity of WSFs is generally attributed to the high concentration of aromatic hydrocarbons in solution. The lesser toxic effect of OWDs may be due to the fact that a significant portion of hydrocarbons in the dispersion are present as small oil droplets and that these are less bioavailable than dissolved hydrocarbons, thus contributing less than the latter to the toxicity of the oil (Neff and Anderson 1981). *M. oculata* exposed to OWDs did not suffer any apparent adverse physical effects due to oil droplets in solution (e.g. adhesion, ingestion). Hence, it is suggested that oil toxicity is due to the chemical toxicity of soluble aromatics, rather than the physical effects of dispersed droplets, as is the case for several other marine species reviewed by Rice et al. (1977). The higher toxicity of dispersions in

sealed jars as opposed to open jars further suggests that oil toxicity is due primarily to volatile aromatic compounds. When an approximate 96-h LC50 was calculated using only the concentration of aromatics present in sealed-jar OWD83 (i.e. method no. 4), results showed that the soluble component of the OWD83 was more toxic than that of the WSF83 (i.e. 228 µg/l vs 526-604 µg/l). This increased toxicity may be due to the presence of a higher concentration of alkylated benzenes and naphthalenes which are generally absent in WSFs prepared by gentle stirring. Larger and less-soluble aromatic hydrocarbons are known to be more toxic to aquatic organisms than mono-nuclear aromatics (Anderson et al. 1974b; Neff and Anderson 1981).

The total volatile hydrocarbon content of the parent oil influenced the aromatic content of WSF mixtures since the most volatile oil (i.e. July '86 batch) produced the richest WSF (Table 4). However, this mixture was less potent than the WSF83 prepared using a crude oil with a lower proportion of total volatiles. A high concentration of total volatiles does not necessarily imply that an oil is toxic. Certain specific hydrocarbons may have been responsible for the increased toxicity of WSF83 but these were not identified.

Sensitivity of *M. oculata*

Median lethal concentrations of young mysids exposed to crude oil-in-seawater mixtures are among the lowest reported for arctic crustaceans (i.e. 5.5 - 9.2 ppm for OWDs and 0.6 - 0.8 ppm for WSFs).

Unfortunately, comparisons between studies are hampered by the lack of experimental standardization. In certain other studies, exposure tests were conducted with open jars and test solutions were aerated, promoting the loss of toxic volatile hydrocarbons. Resulting LC50s may be relatively higher than mortalities determined from sealed-jar tests. In the present study, sealed-jar tests represent a 'worst-case' scenario for *M. oculata* since toxic fractions were retained in test mixtures. This scenario is likely to be representative of the exposure conditions that would occur in the sea in the vicinity of oil trapped under an ice cover that prevents evaporation of volatile components. The sealed-jar testing method has been recommended by Lockhart et al. (1984) for static toxicity tests with crude oil. LC50 values reported here are consistently near the lower limits of lethal concentration ranges for arctic invertebrates and fish (see Percy and Wells 1984). For instance, Foy (1979, 1982) reports 96-h LC50s ranging from 32 to 68 ppm for 6 species of arctic amphipods exposed to OWDs of Prudhoe Bay crude oil. In another study, larval and adult decapods exposed to WSFs of various crude oils had 96-h LC50s ranging from 0.2 to 7.0 ppm (Rice et al. 1976a, 1976b). Although comparisons are difficult to make, *M. oculata* appear sensitive to oil-water mixtures, particularly water-soluble fractions. Others have also reported the sensitivity of littoral mysids exposed to petroleum hydrocarbons (Anderson et al. 1974a; Wells 1982; Laughlin and Linden 1983; Smith and Hargreaves 1984; Carls and Korn 1985).

Although median lethal concentrations are useful values for comparing the relative toxicities of oils, or the relative sensitivities of species, they can imply a level of tolerance that is erroneous. In most cases, ecologically-harmful levels of pollutants are over-estimated (Kimball and Levin 1985; Clark et al. 1986). Hence, any additional information concerning critically-toxic lower levels would enhance the usefulness of an acute toxicity test. In the present study, median effective concentrations causing gross behavioral disturbances such as loss of equilibrium were determined. These sublethal effects are clearly correlated with concentrations of hydrocarbons in the water and may be useful in predicting the onset of death. In *M. oculata*, acute toxicity followed a gradual pattern of deterioration in activity which corresponded to stages B, C and D, in that order. In tests with WSFs, only stage B (i.e. loss of equilibrium) occurred at concentrations statistically lower than the LC50. However, in sealed 750-ml jar tests with OWDs, EC50s of all behavioral stages were statistically lower than the LC50. These results indicate that the volatile component of OWD mixtures was significantly more toxic to *M. oculata* than WSFs, possibly due to the presence of more alkylated aromatic compounds in the former (i.e. as discussed previously). Such a comparison of EC50s and LC50s can yield more useful information concerning acute toxicity than if only LC50s were determined. In addition, post-exposure recovery data provide a more realistic estimate of acute toxicity (Hansen and Kawetski 1976). EC50s producing delayed mortality and "ecological death" show that latent toxic effects in *M. oculata* can occur at concentrations

than the LC50, although differences do not exceed 3 mg/l. The recovery of mysids during post-exposure periods was dependent on the behavioral stage of the animal and the toxicant mixture to which it was exposed. In most cases (i.e. over 90%) stage B animals recovered, but weaker ones (i.e. stages C and D) rarely recovered during a 48-h period in clean seawater (i.e. less than 5%). The ability of mysids to recover following oil exposure may be of some ecological pertinence and may be linked to their ability to metabolize petroleum hydrocarbons.

Marine crustaceans exposed to oil are known to accumulate certain aromatic compounds in their tissues (Anderson 1977; NRC 1985). The present study shows that *M. oculata* accumulates a significant portion (i.e. about 20%) of volatile hydrocarbons present in test solutions. Caldwell et al. (1977) have suggested that the relative toxicity of aromatic compounds is largely due to their ability to partition from seawater into the lipids of crustacean larvae. Hydrocarbon uptake in young *M. oculata* may be due to the fact that they contain a large number of lipid globules. Although it is not known which hydrocarbons are taken up, alkylated naphthalenes are thought to be particularly hazardous since they rapidly partition from the water into the lipid phase (Laughlin and Linden 1983). Also, these compounds are usually the ones which are retained in tissues longer than non-alkylated forms (Anderson 1977; NRC 1985). If this is true for *M. oculata*, the increase in latent effects due to OWD exposure (Table 11) may be explained by an increase in uptake of alkylated aromatics since these compounds were more concentrated in OWDs than in WSFs. More research is needed on uptake and

more concentrated in OWDs than in WSFs. More research is needed on uptake and depuration of petroleum hydrocarbons in lipid-rich arctic organisms such as *M. oculata*. At low temperatures, low metabolic rates of organisms and increased solubilities of toxic aromatics may render arctic marine species more vulnerable and sensitive to oil. Many arctic crustaceans rely on lipids as a source of energy (Percy and Fife 1981) and sublethal levels of petroleum are known to disrupt the normal pattern of lipid storage, utilization and synthesis (Capuzzo and Lancaster 1981). Laughlin and Linden (1983) discuss physiological changes which may occur in mysids as a result of acute and chronic oil exposure.

Acute mortality tests are a useful first step in determining the sensitivity of *M. oculata* and its suitability as a test organism for further laboratory experiments. In order to predict the ecological impact of spilled oil on this species, more information is needed on behavioral and physiological responses to sublethal levels of petroleum hydrocarbons. Whether or not these mobile organisms will avoid contaminated areas still needs to be determined. Oil has been reported to both attract (Atema et al. 1973) and repel (Percy 1976) marine crustaceans. Following the 'Sefir' oil spill in the Baltic Sea, large mortalities of littoral fauna including mysids, were observed (Laughlin and Linden 1983; Linden et al. 1983). Due to their shoaling behavior and vertical migrations into surface waters, mass mortalities of *M. oculata* in contaminated nearshore areas cannot be ruled out. Such events may have a significant disruptive effect on mysid populations and associated food webs in localized arctic regions.

Although in this study phenol was used primarily as a reference toxicant, it is also a potentially important marine pollutant. The importance of phenols in discharge water from offshore oil production platforms has recently been emphasized (Grahl-Nielsen 1987). It has been suggested that these compounds are more hazardous than aromatic hydrocarbons due to their higher water solubility and persistence in the aqueous phase. However, little is known about the fate of phenol in the marine environment and whether it can accumulate in certain areas. Thus the results of the toxicity tests carried out with this compound are themselves of considerable interest above and beyond their use as a physiological control. In discharge waters from North Sea production platforms, concentrations ranging from 2.4 to 7.3 mg/l have been detected (Grahl-Nielsen 1987). Results of acute toxicity tests suggest that prolonged exposure of marine crustaceans to such concentrations may cause lethal or sublethal effects. Tatem et al. (1978) have reported a 96-h LC50 of 5.8 ppm of phenol for grass shrimp (*Palaemonetes pugio*) and the present study shows that *M. oculata* suffers gross behavioral damage during 96-h exposures at 8-10 mg/l. To assess the environmental impact of phenol, more research is needed to determine concentrations in various marine habitats and their effects on vulnerable organisms.

Conclusions and recommendations

Lethal and effective concentrations reported in this study clearly show that *M. oculata* is more sensitive to crude oil, particularly the

water-soluble fractions, than other arctic crustaceans that have been tested. Furthermore, young-of-the-year mysids are very vulnerable to oil exposure because they inhabit areas where hydrocarbons tend to accumulate, i.e. the sub-tidal benthos and under-ice surface. The high sensitivity coupled with the high vulnerability suggests that oil spilled in nearshore arctic waters may have a disastrous effect on *M. oculata* populations. In order to predict more precisely the effects of spilled oil on arctic mysid populations more information is needed about their spatial and temporal distribution, feeding habits and reproductive biology. Physiological and behavioral toxicity experiments are also needed to determine preference/avoidance reactions and chronic effects of low levels of oil on *M. oculata*. This species has been shown to be an ideal subject for toxicity tests due to its sensitivity, its vulnerability, its availability in nearshore waters, and its ease of maintenance under laboratory conditions. Therefore, it is recommended that *M. oculata* be used as a standard test organism for arctic marine toxicity testing and environmental monitoring.

The present study demonstrates the need for further improvement and standardization of methods in toxicity testing with aquatic organisms. Firstly, due to the difficulty in obtaining comparable batches of oil, there is a definite need in Canada for an "oil bank" which would stock well-characterized batches of crude and refined oils for testing by interested researchers. Secondly, to simulate under-ice conditions in toxicity tests, efforts should be made to minimize the loss of toxic volatile fractions from test mixtures by tightly sealing

containers and refrigerating all solutions. Standard methods of preparing saturated oil-in-water mixtures, such as described by Murray et al. (1984), should also be more widely used. Finally, phenol should be considered as a routine reference toxicant in cold-water testing, primarily because of its high solubility and toxicity.

FOOTNOTES

1 - Habitat vulnerability and population sensitivity are two distinct considerations in the study of the environmental impacts of oil pollution (Percy and Wells 1984). The vulnerability of a population depends primarily on the vulnerability of its habitat to spilled oil. Organisms living in areas where hydrocarbons may persist, such as the intertidal zone, are more vulnerable than those inhabiting the open ocean, where oil is weathered more rapidly. The sensitivity of a population depends on the physiological effect of the pollutant on the organisms. For example, a population can be termed "sensitive" if most organisms die when exposed to low concentrations for short periods.

2 - Reference toxicants are used for periodic tests of animal stocks with the aim of revealing major changes in resistance due to disease or other causes, and providing a baseline for comparing results from different laboratories. The use of a single-compound chemical, such as phenol, is preferable since it yields a highly reproducible solution of uniform composition. Such reference toxicants are useful in toxicity tests with unstable oil-in-water mixtures because they can help determine whether changes in mortality are due to animal sensitivity or variability in the composition of oil-water mixtures.

3 - Although the mixtures prepared were believed to be single-phase solutions, they may have contained some oil particles of 100 μm diameter or less, which are not discernible to the human eye (NRC 1985).

4 - The limitations of fluorescence spectrophotometry are explained by Levy (1977).

> ...only molecules that contain multiple conjugated double bonds or possess a high degree of resonance stabilization are potentially capable of fluorescing. Included in these categories are compounds having aromatic nuclei, unsaturated heterocyclics, and long chain polyenes, although some simple ketones and aldehydes may be very weakly fluorescent. Among the most intensely fluorescent compounds are the polynuclear aromatic hydrocarbons.

5 - Preliminary experiments demonstrated that these storage methods did not affect the fluorometric readings of oil-in-hexane samples.

6 - There is a basic difference between toxicity tests and bioassays which has been emphasized by Stephan (1977):

> One difference between the two is that the effect used in a bioassay may be either a beneficial effect or an adverse one, whereas toxicity tests, by definition, are concerned with adverse effects. ... In bioassays as in many chemical assays, the results of the test on the unknown (chemical) are calculated on the basis of a standard curve obtained with known standards. The generally accepted best way to show that a bioassay is valid and is not subject to interference is to demonstrate that the slope of the regression for the unknown is the same as that of the regression curve for the known, that is, to demonstrate that the two regression curves are parallel. The slopes of the regression cannot be shown to be parallel unless at least two partial kills are obtained with both the known and the unknown. Thus, concern about the validity of bioassays results in a concern about parallelism and therefore a concern about partial kills.... When bioassays are conducted with aquatic organisms, the established principles of bioassays, such as the test of validity based

> on parallelism, should be followed..... If a test is conducted to compare the relative potencies or concentrations, then it ought to be called a bioassay, and it is important to obtain partial kills. If a test is conducted to obtain information about the adverse effects of an agent on organisms, then it ought to be called a toxicity test ..... With toxicity tests, there is no reason to be concerned about parallelism and so there is no reason to obtain partial kills.

7 - These limits are usually numerically equal to fiducial limits (Finney 1971), and therefore no distinction is made between the two.

8 - For data analyzed by the binomial test, the confidence level is above 99% when more than 6 organisms per treatment are used (Stephan 1977).

9 - In some cases data from several tests are pooled to obtain a combined sample estimate of the LC50 which usually appears to be an average LC50. However, this method does not detect patterns in the frequency distribution of individual LC50s and it gives no indication of repeatability between replicate tests (Hodson et al. 1977). For these reasons, LC50 and EC50 values are presented for each replicate test conducted. Furthermore, because of the variabilities of the individual test results, it is not statistically acceptable to calculate a mean LC50 (or EC50) by simply averaging the values from different tests. A procedure for combining probit lines from several toxicity tests was recently developed by Hong et al. (1988).

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APPENDIX

TABLE A1. Results of probit analysis for reference toxicity tests with two different collections of *M. oculata* exposed to phenol.

Stage	Collection year	Replicate test no.	96-h LC50 or EC50 (mg/l)	Confidence limits		Slope	Confidence limits		Chi square	Prob.
				Low	High		Low	High		
DEATH	1986	1	75.0	56.3	107.8	3.76	1.80	5.72	0.3837	0.9838
		2	71.5	48.0	98.0	4.35	1.93	6.77	4.4624	0.2157
		3	37.8	26.4	51.2	3.37	1.88	4.86	6.5042	0.1645
		5	58.7	46.0	73.6	5.76	3.02	8.50	2.9070	0.5735
	1987	1	56.1	33.5	104.8	2.00	0.94	3.06	7.5385	0.0566
		3	37.2	22.7	57.0	2.54	1.37	3.71	4.8248	0.1851
MOR24	1986	1	71.2	53.6	98.8	3.93	1.94	5.92	0.7965	0.9389
		3	28.9	19.6	39.3	3.38	1.89	4.88	3.2930	0.5100
		4	38.6	24.1	60.3	2.09	1.11	3.08	8.8339	0.0654
		5	33.3	19.3	54.2	1.83	0.95	2.72	7.6775	0.1041
	1987	1	25.8	13.1	44.6	1.77	0.90	2.64	5.0019	0.1717
		2	29.9	18.4	44.4	2.83	1.57	4.09	2.0513	0.5618
		4	36.9	21.2	60.8	2.05	1.08	3.02	6.9938	0.0721

TABLE A2. Binomial test results for reference toxicity tests.

Stage	Mysid collection year	Replicate test no.	Approx. 96-h LC50 (mg/l)	Confidence limits[1]	
				Low	High
DEATH	1986	1	75	30	---
		2	89	30	125
		3	53	5	85
		4	65	50	85
		5	50	30	125
	1987	1	74	5	125
		2	63	40	85
		3	54	5	125
		4	35	15	125
MOR24	1986	1	69	30	---
		2	25	5	125
		3	25	5	85
		4	56	5	85
		5	39	---	125
	1987	1	35	5	125
		2	40	5	85
		3	21	5	85
		4	25	5	125
ECO24	1986	1	23	5	85
		2	13	5	30
		3	11	5	30
		4	11	5	30
		5	12	---	125
	1987	1	9	5	15
		2	10	5	15
		3	9	5	15
		4	8	5	15

1 - These are not 95% confidence limits. When the binomial test is used and there are more than six organisms per treatment, the confidence level is above 99% (Stephan 1977).

TABLE A3. Probit analysis results for toxicity tests with oil-in-water dispersions of Norman Wells crude oil.

Method no.[1]	Stage	Replicate test no.	96-h LC50 or EC50 (mg/l)	Confidence limits		Slope	Confidence limits		Chi square	Prob.	% of animals affected in controls
				Low	High		Low	High			
1	B	1	4.38	2.89	6.02	3.56	1.89	5.22	3.9360	0.2685	0
		2	3.91	2.62	5.35	3.86	1.88	5.84	1.4407	0.6960	0
	C	1	5.32	3.66	7.22	3.60	2.06	5.14	3.2285	0.3577	0
		2	5.31	3.54	7.31	3.22	1.81	4.63	6.6046	0.0856	0
	D	1	5.61	3.80	7.67	3.37	1.91	4.82	5.0874	0.1655	0
	DEATH	1	5.61	3.80	7.67	3.37	1.91	4.82	5.0874	0.1655	0
2	C	4	6.78	5.79	7.54	10.26	4.51	16.01	1.4964	0.4732	0
	D	3	6.85	5.02	8.02	6.65	1.93	11.38	2.6958	0.2598	0
		4	7.12	6.45	7.80	14.19	7.01	21.37	0.2259	0.8932	0
	DEATH	2	6.43	5.35	7.17	10.17	4.17	16.18	0.9752	0.6141	0
		3	7.21	5.57	8.61	6.50	1.87	11.12	2.0510	0.3586	0
		4	7.57	6.64	8.61	9.29	4.11	14.46	2.2791	0.3200	0
	MOR24	4	6.44	5.65	7.07	13.99	5.86	22.12	2.2070	0.3317	0
	ECO24	4	6.45	5.65	7.10	13.75	5.61	21.89	2.0950	0.3508	0
3	DEATH	1	4.93	3.35	6.89	5.55	2.70	8.40	0.0943	0.7588	0

TABLE A3. (cont'd)

Method no.[1]	Stage	Replicate test no.	96-h LC50 or EC50 (mg/l)	Confidence limits		Slope	Confidence limits		Chi square	Prob.	% of animals affected in controls
				Low	High		Low	High			
4	B	1	1.90 (2.09)[2]	1.30 (0.59)	2.53 (2.81)	4.66 (5.41)	2.17 (0.98)	7.14 (9.83)	0.1228 (0.3576)	0.9404 (0.8363)	10
	C	1	2.27 (2.46)	1.50 (1.29)	3.16 (3.45)	3.64 (4.04)	1.71 (1.37)	5.57 (6.71)	1.0047 (1.1227)	0.6051 (0.5705)	6.67
	D	1	2.41 (2.66)	1.63 (1.34)	3.36 (3.69)	3.69 (4.34)	1.75 (1.27)	5.64 (7.42)	0.5728 (0.5383)	0.7510 (0.7641)	6.67
	DEATH	1	4.51 (4.85)	3.09 (3.28)	8.20 (7.37)	2.93 (4.64)	1.19 (1.55)	4.67 (7.72)	4.6056 (3.3178)	0.1000 (0.1903)	3.33
	MOR24	1	2.73 (3.15)	1.81 (1.52)	3.96 (4.59)	3.24 (4.10)	1.50 (1.08)	4.97 (7.13)	2.4916 (2.4779)	0.2877 (0.2897)	10
	MOR48	1	2.41 (2.71)	1.57 (1.24)	3.42 (3.91)	3.36 (3.90)	1.57 (1.12)	5.16 (6.69)	2.0950 (2.2976)	0.3508 (0.3170)	10
	EC024	1	1.81 (2.04)	1.07 (0.94)	2.56 (2.95)	3.36 (3.77)	1.48 (1.27)	5.24 (6.27)	0.5741 (0.6393)	0.7505 (0.7264)	10
	EC048	1	1.54 (1.74)	0.80 (0.77)	2.19 (2.53)	3.31 (3.67)	1.35 (1.27)	5.28 (6.08)	0.1547 (0.1030)	0.9256 (0.9498)	10

1 - Method no. 1: 750-ml open jars
Method no. 2: 1000-ml open beakers
Method no. 3: 1000-ml sealed beakers
Method no. 4: 750-ml sealed jars

2 - Numbers in parentheses correspond to values corrected for control mortality/behavioral changes using Abbott's formula.

TABLE A4. Binomial test results for toxicity tests with oil-in-water dispersions.

Method no.	Stage	Replicate test no.	Approx. 96-h EC50 or LC50 (mg/l)	Confidence limits		% control animals affected
				Low	High	
1	B	1	4	3	15	0
		2	6	--	10	0
	C	1	5	3	15	0
		2	4	3	15	0
	D	1	6	3	15	0
		2	6	3	15	0
	DEATH	1	6	3	15	0
		2	6	3	15	0
	MOR24	1	4	3	10	0
		2	3	--	10	0
	ECO24	1	4	3	10	0
	ECO48	2	3	--	10	0
2	B	4	6	--	8	0
	C	2	6	--	8	0
		4	7	--	8	0
		5	6	--	10	0
	D	2	6	--	8	0
		4	7	6	10	0
		5	9	--	10	0
	DEATH	2	6	--	10	0
		3	7	--	--	0
		4	7	6	--	0
		5	9	--	10	0
	MOR24	4	7	--	8	0
	MOR48	4	6	--	8	0
	ECO24	4	7	--	8	0
	ECO48	4	6	--	8	0

TABLE A4. (cont'd)

Method no.	Stage	Replicate test no.	Approx. 96-hr EC50 or LC50 (mg/l)	Confidence limits		% control animals affected
				Low	High	
3	B	1	3	--	9	0
	C	1	3	--	9	0
		4	4	--	14	0
	D	1	4	--	9	0
		2	3	--	14	0
		3	5	--	14	0
		4	4	--	14	0
		5	3	--	14	10
	DEATH	1	5	3	9	0
		2	3	--	14	0
		3	5	3	14	0
		4	5	3	14	0
		5	5	--	14	10
	MOR24	5	3	--	14	10
	ECO24	5	3	--	14	10
4	B	1	2	1	4	10
	C	1	2	1	8	6.67
	D	1	2	1	8	6.67
	DEATH	1	4	2	--	3.33
	MOR24	1	2	--	8	10
	MOR48	1	2	--	4	10
	ECO24	1	2	--	8	10
	ECO48	1	2	--	4	10

TABLE A5. Probit analysis results for toxicity tests with *M. oculata* exposed to water-soluble fractions of Norman Wells crude oil.

Toxicant	Stage	Replicate test no.	96-h LC50 or EC50 (µg/l)	Confidence limits		Slope	Confidence limits		Chi square	Prob.
				Low	High		Low	High		
WSF83	B	1	331	262	395	8.45	3.84	13.06	0.2542	0.9926
		2	334	278	396	11.69	5.24	18.14	0.0147	1.0000
	C	1	379	307	446	8.93	4.36	13.50	0.9374	0.9191
		2	394	319	462	8.71	4.31	13.10	1.7592	0.7799
	D	1	394	319	462	8.71	4.31	13.10	1.7592	0.7799
		2	437	350	517	6.92	3.69	10.16	1.1895	0.8798
	DEATH	1	486	396	566	7.70	3.99	11.42	7.2919	0.1212
		2	533	423	644	5.38	2.84	7.92	4.4179	0.3524
	MOR24	1	358	280	427	7.38	3.65	11.10	1.5505	0.8177
		2	463	363	556	5.69	3.06	8.32	4.7826	0.3103
	MOR48	1	358	280	427	7.38	3.65	11.10	1.5505	0.8177
		2	435	343	519	6.30	3.38	9.22	1.6428	0.8011
	EC024	1	358	280	427	7.38	3.65	11.10	1.5505	0.8177
		2	365	296	430	9.36	4.48	14.25	0.4111	0.9816
	EC048	1	337	249	408	6.37	3.06	9.67	2.1863	0.7015
		2	365	296	430	9.36	4.48	14.25	0.4111	0.9816
WSF86	B	2	340	259	410	8.54	3.24	13.85	0.0685	0.9994
		4	289	187	378	4.05	2.22	5.87	4.6757	0.3222
	C	2	413	310	499	6.04	3.07	9.01	5.5154	0.2384
		3	481	389	556	10.05	4.45	15.65	0.6610	0.9561
		4	463	375	542	8.62	4.29	12.95	2.9512	0.5660

TABLE A5. (cont'd)

Toxicant	Stage	Replicate test no.	96-h LC50 or EC50 (µg/l)	Confidence limits		Slope	Confidence limits		Chi square	Prob.
				Low	High		Low	High		
WSF86	D	2	496	396	586	6.92	3.69	10.16	1.1891	0.8799
		3	500	404	575	10.59	4.38	16.79	0.2832	0.9909
		4	497	402	583	7.70	4.00	11.40	2.7204	0.6056
	DEATH	1	623	504	745	5.93	3.14	8.73	7.7793	0.1000
		2	624	513	731	7.09	3.70	10.48	3.8488	0.4269
		3	608	499	710	7.27	3.79	10.74	5.3102	0.2569
		4	605	492	716	6.42	3.41	9.43	4.9834	0.2890
	MOR24	1	496	397	586	6.92	3.69	10.16	1.1891	0.8799
		2	481	361	587	5.04	2.66	7.42		
	MOR48	1	463	371	547	7.47	3.89	11.05	0.5495	0.9685
		2	440	321	540	5.01	2.61	7.41	3.2645	0.5146
		3	500	404	575	10.59	4.38	16.79	1.1194	0.8912
	ECO24	1	397	325	469	10.14	4.70	15.58	0.1246	0.9981
		2	382	283	463	6.37	3.06	9.67	2.1845	0.7019
		4	321	211	417	4.06	2.20	5.92	7.8599	0.0968
	ECO48	1	397	325	469	10.14	4.70	15.58	0.1246	0.9981
		2	397	296	481	6.16	3.06	9.26	3.6481	0.4557
		4	321	211	417	4.06	2.20	5.92	7.8599	0.0968

TABLE A6. Binomial test results for toxicity tests with water-soluble fractions.

Toxicant	Stage	Replicate test no.	Approx. 96-h LC50 or EC50 (µg/l)	Confidence limits	
				Low	High
WSF83	B	1	334	86	604
		2	334	259	432
	C	1	393	259	432
		2	432	259	604
	D	1	432	259	604
		2	468	259	777
	DEATH	1	526	432	777
		2	604	259	777
	MOR24	1	382	86	604
		2	511	259	777
	MOR48	1	382	86	604
		2	432	259	777
	ECO24	1	382	86	604
		2	368	259	604
	ECO48	1	365	86	604
		2	368	259	604
WSF86	B	1	417	294	685
		2	344	98	490
		3	379	294	490
		4	359	98	685
	C	1	417	294	685
		2	511	98	685
		3	459	294	685
		4	511	294	685
	D	1	436	294	685
		2	531	294	881
		3	490	294	685
		4	544	294	685
	DEATH	1	601	294	--
		2	579	294	1175
		3	601	294	881
		4	645	490	881

TABLE A6. (cont'd)

Toxicant	Stage	Replicate test no.	Approx. 96-h LC50 or EC50 (µg/l)	Confidence limits	
				Low	High
WSF86	MOR24	1	531	294	881
		2	531	98	881
		3	521	294	685
		4	601	294	881
	MOR48	1	490	294	685
		2	531	98	881
		3	490	294	685
		4	511	98	685
	ECO24	1	396	294	685
		2	414	98	685
		3	436	294	685
		4	401	98	685
	ECO48	1	396	294	685
		2	490	98	685
		3	436	294	685
		4	401	98	685