# Toxicity of methyl-*tert*-butyl ether (MTBE) to California marine life

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## ABSTRACT

The sublethal effects of methyl-*tert*-butyl ether (MTBE) on three southern California marine species was examined by conducting the purple sea urchin (*Strongylocentrotus purpuratus*) 3-d embryo development test, the giant kelp (*Macrocystis pyrifera*) 2-d germination and growth test, and the 7-d mysid (*Holmesimysis costata*) growth test. In addition, the effects of MTBE on the survival of mysids and amphipods (*Grandidierella japonica*) were measured. These two species of crustaceans were found to be most sensitive, with a 50% rate of mortality at an MTBE concentration of approximately 150 mg/L. The threshold for toxic effects in the most sensitive species (amphipod) was 37 mg/L. The least sensitive species was the giant kelp, with a 50% reduction in growth at 2,236 mg/L. The highest concentration of MTBE measured in receiving water was less than 0.1% of the threshold effects level for the amphipod, the most sensitive California species tested.

## INTRODUCTION

Methyl-*tert*-butyl ether (MTBE) is used as a gasoline additive to reduce exhaust emissions and improve air quality; however, mounting concerns have been expressed over its potential to adversely impact human health and contaminate the aquatic environment. Gasoline leaks from underground storage tanks and emissions from motorized watercraft have resulted in the contamination of groundwater and lakes with MTBE (Oswalt 1997, Reuter *et al.* 1998).

MTBE also has been found in the marine environment (see *Concentrations of MTBE in inputs and receiving waters of southern California* in this annual report). Of the 21 largest National Pollutant Discharge Elimination System (NPDES) facilities discharging to southern California coastal waters, 48% detected MTBE in their effluents. MTBE was detected in 83% of samples taken at locations in Santa Monica Bay, Los Angeles Harbor, Mission Bay, and San Diego Bay. The highest levels of MTBE in receiving water were found in Mission Bay, with concentrations up to 34 µg/L. MTBE was also detected in Humboldt Bay and San Francisco Bay (Bay and Brown 2000). A likely source of MTBE discharge to marine waters is the operation of two-stroke boat engines. These engines are relatively inefficient, discharging up to 30% of their fuel unburned into the environment (ARB 1999).

Previous studies have indicated that aquatic organisms are relatively insensitive to MTBE (Drottar *et al.* 1998, Mancini *et al.* 1999). However, few marine species are represented by the available data, and no information exists on the effects of MTBE on marine algae. In addition, none of the previous studies examined the effects of MTBE on the species found in California. This article examines the short-term toxicity of MTBE to four California marine organisms. The species tested represent diverse taxonomic groups with commercial and recreational importance from habitats having the potential to expose marine life to MTBE.

## METHODS

### Toxicity Measurement

Three tests were conducted to examine the sublethal effects of MTBE, including the purple sea urchin (*Strongylocentrotus purpuratus*) 3-d embryo development test, the giant kelp (*Macrocystis pyrifera*) 2-d germination and growth test, and the 7-d mysid (*Holmesimysis costata*) growth test. In addition, the effects of MTBE on the survival of the mysid and a sediment-dwelling amphipod (*Grandidierella japonica*) were measured.

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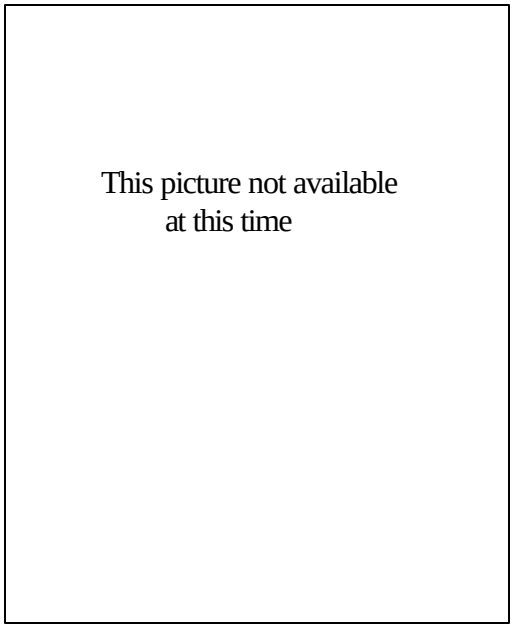
The exposures were conducted under static conditions, with solution renewals in the mysid and amphipod tests. Solutions were renewed after 48 h in the amphipod test, and after 48 and 96 h in the mysid test. All exposures were conducted in sealed containers to minimize the loss of MTBE. Glass 250 mL Boston bottles with screw tops were used for the sea urchin, amphipod, and mysid tests. Each Boston bottle had 25 mL headspace. The kelp test was conducted in 400 mL glass beakers covered with Saran Wrap and providing 50 mL headspace. The placement of each test chamber was random in each test. All tests were conducted at 15° C, with a 16 h light/8 h dark photoperiod.

Water quality parameters (pH, dissolved oxygen, total ammonia, and salinity) were measured at the beginning and end of each experiment. These parameters, excluding total ammonia, were also measured in the old mysid test solutions at the 48 and 96 h time points. Dissolved oxygen was also measured at the time of renewal for amphipods. Temperature was monitored continuously.

A reference toxicant exposure with copper (sea urchin and kelp), cadmium (amphipod), or zinc (mysid) was conducted concurrently with each MTBE experiment to document test organism health.




*Purple sea urchin embryo development test*

Purple sea urchins (*S. purpuratus*) were collected from a reference location at Point Dume, California. The sea urchins were maintained in a flow-through seawater culture system at 15° C and fed brown algae for more than one month before testing.

Sea urchins were spawned on the initial day of the MTBE experiment, and the resulting gametes were combined to produce embryos. The embryos were exposed to MTBE for 72 h following the methods of U.S. EPA (1995). There were 25 eggs/mL test solution for each treatment. The experiment was a static non-renewal test.

Nominal test concentrations were 0 (control), 200, 500, 1,000, 2,000, and 4,000 mg MTBE/L. Dilution seawater for all tests was obtained from a site 0.5 mile off Redondo Beach, California, and filtered to 0.4 µm. Four replicates of each concentration were tested. The test was terminated after 72 h by preserving the embryos with formalin. Embryo development was assessed by microscopic examination. At least 100 embryos from each container were examined. The test endpoint was the percentage of normally developed embryos.

*Giant kelp germination and germ tube growth test*

Giant kelp (*M. pyrifera*) sporophylls were collected from kelp beds off Point Loma, California, and transported to the laboratory on the initial day of the test.
The spores were released from the sporophylls following desiccation of the kelp blades. The spores were exposed to MTBE for 48 h following the methods of the U.S. EPA (1995). The experiment was a static non-renewal test.

Nominal test concentrations were 0 (control), 100, 200, 500, 1,000, 2,000, and 4,000 mg MTBE/L. Five replicate beakers were used for each treatment. The test was terminated after 48 h by preserving a subsample of the settled spores with glutaraldehyde. Germination and germ tube length were assessed by microscopic examination. The endpoints for the kelp test were the percentage of germinated spores and germ tube growth.

*Amphipod survival test*

Amphipods (*G. japonica*) were collected 4 d before testing from upper Newport Bay, California. Sediment from the site was partially screened in the field and returned to the laboratory. Holding bins were kept at 15° C and provided aeration and flowing seawater.

Test procedures followed methods recommended by ASTM (1996) for 96 h reference toxicant tests. On the day of MTBE test initiation, juvenile amphipods (2 to 4 mm in length) were screened out of the holding sediment and placed into large petri dishes. Ten animals were distributed randomly into each exposure container. The amphipods were exposed to MTBE for 96 h under static conditions with one renewal of solution at 48 h. Amphipods were not fed during the test.

Nominal test concentrations were 0 (control), 25, 50, 100, 200, and 400 mg MTBE/L. Five replicates of each concentration were tested. After 96 h, the test solutions were poured though a screen to remove the animals. Live animals were counted and the percent of survival was calculated.

*Mysid survival and growth test*

Adult mysids (*H. costata*) were collected off Point Loma, California, and transported to a nearby holding facility. Juvenile mysids released from adult females were collected and transported to the laboratory.

Juvenile mysids were exposed to MTBE for 7 d following the methods of the U.S. EPA (1995). The exposure was conducted under static conditions, with two solution renewals, after 48 and 96 h. Each container had five animals per replicate, with five replicates per treatment. Each container received 40 *Artemia* nauplii per mysid each day of the exposure to feed the test organisms.

Nominal test concentrations were 0 (control), 25, 50, 100, 200, and 400 mg MTBE/L. Survival was recorded at each water change, as well as at the end of the experiment. The surviving mysids were placed in tin weigh boats, desiccated at 60° C overnight, and then weighed. The endpoints for the mysid test were the percent of survival and the growth rate.

**MTBE Spiking Procedure**

All test solutions were prepared in gallon jars. The jars were filled with seawater to a volume that minimized headspace; then they were covered with Saran Wrap. A magnetic stirrer was used to mix the seawater vigorously while neat MTBE (99.99% purity) was added slowly, using a gas-tight syringe. The jars were then sealed with an additional layer of Saran Wrap and the contents were mixed for five minutes. The solutions were then transferred to Cubitainers for temporary storage (< 2 h) before their addition to the exposure containers.

**Chemical Analysis**

The concentration of MTBE was verified by gas chromatography/mass spectrometry (GC/MS) for each concentration tested. Samples were collected for analysis at the start and end of each test, as well as at each water change. Both the new and old solutions from the water changes were analyzed in order to document temporal changes in exposure concentration. Each sample prepared for analysis was a composite of equal volumes of solution from several replicate exposure containers. Water samples were placed in 40 mL volatile organic analysis (VOA) vials containing acid preservative and stored under refrigeration until analyzed. Samples were analyzed within 5 d of sampling, using EPA SW-846 Method 8260B (U.S. EPA 1996).

Quality control procedures for the MTBE measurements included the analysis of method blanks, recovery surrogates, and matrix spike/matrix spike duplicates for each set of samples analyzed. These measurements were made on samples prepared by the analytical laboratory. In addition, duplicate samples of water spiked to 5 or 500 mg/L at SCCWRP were analyzed in order to estimate variability associated with sampling, handling, and dilution of the samples.

The results of the analytical lab QC samples indicate that all method blanks were below detection limits, and high recovery (103%) of MTBE was obtained from spiked samples. Matrix spike duplicates had low variability, indicating that the instrumental analysis procedure had high precision. Measurements of duplicate samples spiked at SCCWRP showed slightly higher variability (7 to 21%), as expected. The variability between duplicates was similar to that measured between water changes during the toxicity tests.

**Data Analysis**

The data were analyzed using Toxstat statistical analysis software (West and Gulley 1996). The assumptions of data normality and homogeneity of variance were tested using the Shapiro-Wilk's test and Bartlett's test, respectively (U.S. EPA 1995). Both assumptions were met for all four species. For the kelp, amphipod, and mysid tests, Dunnett's test was used to identify treatments that were significantly different from the control ($\alpha = 0.05$) for determination of the LOEC lowest observed effect concentration (LOEC) and no observed effect concentration (NOEC). For the urchin test, t-tests were used with Bonferroni adjustment to compensate for the unequal replication in the data. The concentrations causing 50% mortality in amphipods or mysids ($LC_{50}$) were determined using probit analysis. The concentrations estimated to inhibit the development or growth by 25 and 50% ($IC_{25}$ and $IC_{50}$, respectively) of the control sea urchin, kelp, or mysid values were determined by linear interpolation. All calculations used the measured MTBE concentrations.

Measures such as the LOEC and NOEC, which indicate the threshold effects concentration for each species, can be misleading because they do not describe the magnitude of a response. Examination of the response over a concentration gradient for each species provides a more reliable measure of the overall sensitivity of each species (Figure 1). This dose-response relationship is often described using a measure of the median response for survival ($LC_{50}$) or sublethal ($IC_{50}$) effects (Table 1).

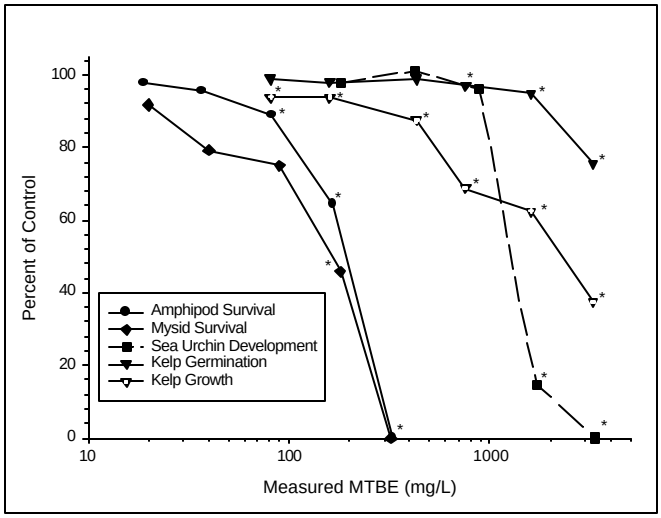
## RESULTS

**Toxicity Test Quality Control**

Results of the chemical verification measurements indicated that the MTBE dosing method produced accurate and stable exposure concentrations. The average measured con-

centrations of samples collected at the beginning and end of the exposure period were generally greater than 80% of the nominal values for all tests. The MTBE concentrations remained relatively constant during each experiment, with concentrations declining only 2.6 to 14.8% during the tests. Water quality parameters remained within acceptable limits throughout each test, indicating that the use of sealed exposure containers did not cause a significant decrease in oxygen content or a build-up of waste products during the test. All of the toxicity tests met the acceptability criteria for control performance and sensitivity to a standard reference toxicant.

**FIGURE 1. Response of marine species to MTBE exposure. Values are the mean of the treatment replicates, normalized to the control response. The asterisks (*) indicate concentrations that are significantly different from the control.**




## Response of California Species to MTBE

A wide range of responses to MTBE were found among the tests, as indicated by a greater than 10 times difference in toxicity summary statistics among species (Table 1). The kelp growth and amphipod survival tests had the most sensitive initial responses to MTBE, with significant reductions in growth or survival (LOEC) first occurring at approximately 80 mg/L. In contrast, the mysid survival LOEC was twice this amount (180 mg/L), and the sea urchin development LOEC was more than 20 times higher (1,700 mg/L).

Both crustacean species (amphipod and mysid) showed a similar dose-response pattern and had the greatest overall sensitivity to MTBE (Figure 1). The median effects on survival occurred at similar concentrations for the amphipod (155 mg/L) and mysid (141 mg/L) tests. No survival was observed for either species at a test concentration of 320 mg/L, while less than a 15% effect was recorded on kelp and sea urchins at a similar exposure concentration.

The mysid dose-response plot suggests that survival effects were occurring at 40 mg/L MTBE (24% reduction in survival), but the response was not statistically significant. Amphipods and mysids surviving at intermediate test concentrations often displayed significantly reduced swimming activity, typical of a narcotic mode of toxicity observed in other MTBE toxicity tests (Drottar *et al*. 1998).

Mysid growth was not affected by MTBE. The weights of mysids surviving in the MTBE exposures were not significantly different from the controls. Consequently, NOEC and LOEC values could not be calculated. The median response ($IC_{50}$) of 245 mg/L for mysid growth was derived by including data for the highest test concentration, which had no growth because there was no survival.

The high sensitivity of the kelp growth test to the initial effects of MTBE was a statistical effect of the high precision of the test. While statistically significant growth effects were measured at the two lowest MTBE concentrations, they were quite small in magnitude (6% reduction relative to the control). as shown in Figure 1. The overall sensitivity of the kelp test was lower than the overall sensitivity of the other tests, with a median inhibition concentration for germ tube growth of 2,236 mg/L, 14 times higher (less sensitive) than the amphipod $LC_{50}$ (155 mg/L). The kelp germination endpoint had the lowest overall sensitivity

**TABLE 1. Summary of MTBE toxicity results for California marine species. Kelp germ tube growth was significantly different from the control value at all MTBE concentrations tested. Concentrations greater than 90 mg/L were tested for the mysid growth endpoint, but the data were not used for analysis due to significantly reduced survival, as directed by U.S. EPA 1995. Abbreviations: NOEC = No effect concentration; LOEC = lowest effective concentration; $LC_{50}$ = concentration lethal to half of the organisms; IC = concentration that inhibits the growth or development by a proportion of the control.**

| | MTBE (mg/L) | | | | | |
|---|---|---|---|---|---|---|
| | Days | NOEC | LOEC | LC50 | IC25 | IC50 |
| Sea Urchin (*S. purpuratus*) | | | | | | |
| Development | 3 | 885 | 1,700 | | 1,093 | 1,341 |
| Kelp (*M. pyrifera*) | | | | | | |
| Percent Germination | 2 | 430 | 755 | | > 3,250 | > 3,250 |
| Growth | 2 | < 81 | 81 | | 616 | 2,236 |
| Amphipod (*G. japonica*) | | | | | | |
| Survival | 4 | 37 | 82 | 155 | | |
| Mysid (*H. costata*) | | | | | | |
| Survival | 7 | 90 | 180 | 141 | | |
| Growth | 7 | ≥ 90 | > 90 | | 206 | 245 |

to MTBE, with less than a 25% reduction in germination at the highest concentration tested (3,250 mg/L).

Sea urchin embryos were intermediate in sensitivity compared to kelp and crustaceans, with an $IC_{50}$ of 1,341 mg/L. The dose-response pattern for this species was very steep. No inhibition of development was seen at 885 mg/L MTBE, while the next higher exposure level (1,700 mg/L) produced 86% abnormal development.

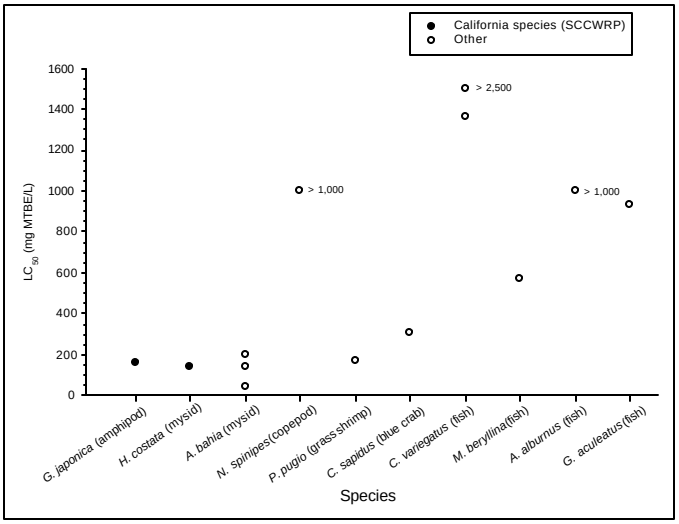
## DISCUSSION

### Comparison to Other Species

The effects of MTBE on the survival of California crustacean species were similar to those reported for other crustaceans (Figure 2). The $LC_{50}$ for mysids and amphipods in this study (155 and 141 mg/L, respectively) were similar (within a factor of two) to the values for the marine mysid *Americamysis (Mysidopsis) bahia* (mean = 127 mg/L), the grass shrimp *Palaemontes pugio* (166 mg/L), and the blue crab *Callinectes sapidus* (306 mg/L), but at least four times lower than the $LC_{50}$ for the marine copepod *Nitocra spinipes* (> 1,000 mg/L) (Drottar *et al.* 1998, Mancini *et al.* 1999). Fish survival appears to be less sensitive to MTBE than crustacean survival. The $LC_{50}$ values for California crustaceans were at least 4 to 16 times lower than values for four species of fish (*Menidia beryllina* $LC_{50}$ = 574 mg/L; *Gasterosteus aculeatus* $LC_{50}$ = 929 mg/L; *Alburnus alburnus* $LC_{50}$ > 1,000 mg/L; *Cyprinodon variegatus* $LC_{50}$s = 1,358, and > 2,500 mg/L).

Three of the four sublethal endpoints measured in this study were less sensitive to MTBE than previous studies (Figure 3). The sea urchin development $IC_{50}$ (1,341 mg/L) was 2 to 9 times higher than the results of short-term (≤96 h) tests using other marine species (*Crassostrea virginica* $EC_{50}$ = 150 mg/L; *A. bahia* $EC_{50}$ = 187 mg/L; *G. aculeatus* $EC_{50}$ = 297 mg/L; *C. variegatus* $EC_{50}$ = 663 mg/L) (Drottar *et al.* 1998, Mancini *et al.* 1999). The kelp growth $IC_{50}$ (2,236 mg/L) was 3 to 15 times higher than the values for these species. The mysid growth $IC_{50}$ in this study (245 mg/L), however, was similar to the responses obtained for other species.

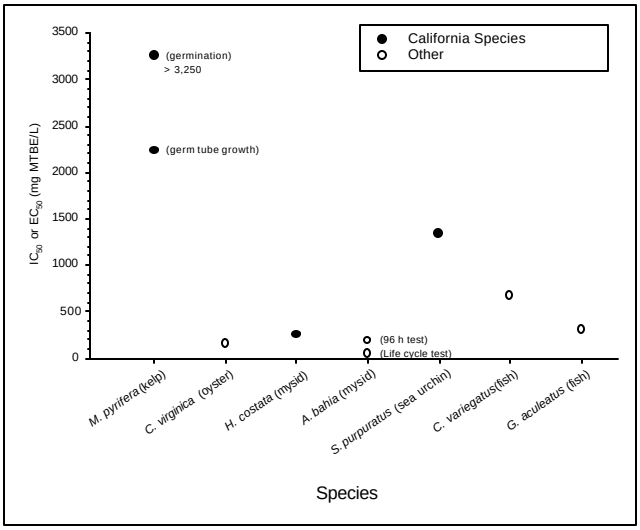
**FIGURE 2. Effects of MTBE on survival of marine species. Closed symbols indicate SCCWRP data.**




### Potential for MTBE Toxicity in Receiving Water

Comparison of the toxicity data with receiving water concentrations demonstrates that the concentrations of MTBE measured in the marine environment are not toxic to marine fish and invertebrates. The highest concentration measured in marine waters, 34 µg/L in Mission Bay (see *Concentrations of MTBE in inputs and receiving waters in southern California* in this annual report), is less than 0.1% of the threshold effect level (NOEC) for amphipod survival, the most sensitive California species tested (Figure 4).

**FIGURE 3. Sublethal effects of MTBE on marine species. All tests are short-term exposures (≤7d), except where noted.**



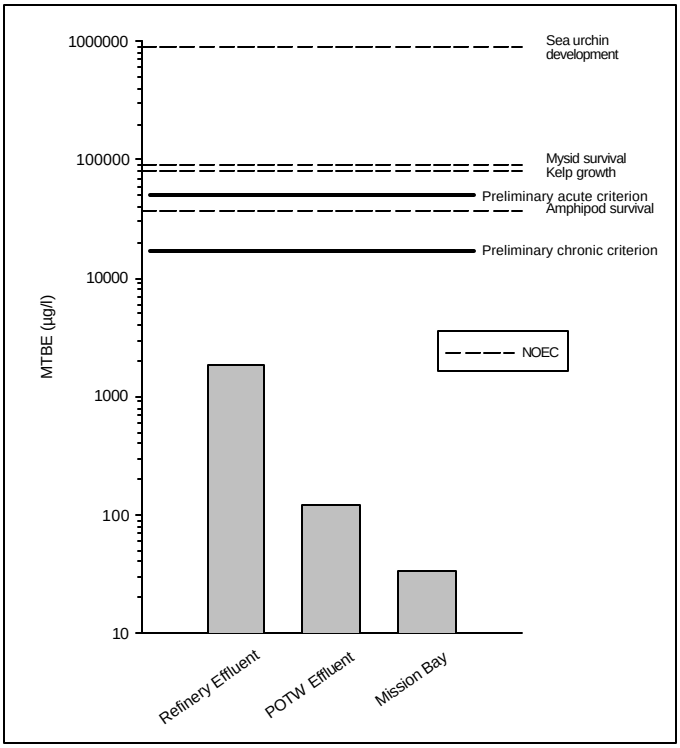

Data from this and other studies are being used by the MTBE Water Quality Criteria Work Group of the American Petroleum Institute to derive proposed acute and chronic water quality criteria for MTBE (Mancini *et al.* 1999). Development of the proposed criteria is still in progress, but preliminary calculations yield acute and chronic values for marine life of 53 mg/L and 18 mg/L, respectively. The proposed criteria will be submitted to the U.S. EPA for review.

Using the preliminary acute criterion as an estimate of the concentration protective of a diverse group of animals also indicates that MTBE concentrations in receiving water and undiluted effluent are below the levels that cause toxicity to marine life. Concentrations in Mission Bay were less than 0.05% of the acute criterion. Undiluted effluent from the Chevron El Segundo Refinery, which had the highest MTBE concentration of any NPDES facility in a recent statewide study (1,878 µg/L), contained 4% of the acute effects value (Figure 4). Undiluted publicly owned treatment work (POTW) effluents, with lower MTBE concentrations, contained 0.2% of the acute effects criterion.

While our experiments did not examine chronic exposure effects, other studies have found that concentrations in receiving waters are below levels that cause chronic toxicity. The only lifecycle test conducted with a marine species reported an $EC_{50}$ = 44 mg/L for effects on the survival and growth of *A.* bahia (Drottar *et al.* 1998). Tests of chronic MTBE effects on freshwater species yielded similar values (Mancini *et al.* 1999), with $IC_{20}$ values of 42 mg/L for the water flea (*Daphnia magna*) and 289 mg/L for the minnow (*Pimephales promelas*). The highest concentration of MTBE measured in Mission Bay (34 µg/L) is far below these effect levels and only 0.2% of the proposed chronic effects criterion of 18 mg/L. A large margin of safety is also present for MTBE discharges from point sources. For example, undiluted effluent from the Chevron El Segundo Refinery contained MTBE concentrations that were 11% of the chronic effects value (Figure 4). Concentrations in undiluted POTW effluents were less than 1% of the chronic effects criterion.

Impacts to the sediment-dwelling (benthic) organisms were indirectly investigated in the present study. One of the test species, the amphipod *G. japonica*, lives in the sediments of coastal bays and harbors, such as Los Angeles Harbor and San Diego Bay. This species was found to have a sensitivity to MTBE that was similar to other crustaceans (Figure 2). Contaminated sediments and prey organisms are common sources of pollutant exposure for many organisms; these sources are not expected to have a significant role in the exposure to MTBE as this compound does not have a tendency to bind onto sediments or bioaccumulate in tissues (Squillace *et al.* 1997). The available data indicate that the principal exposure source of benthic organisms to MTBE is water, which contains amounts of MTBE that are below the levels known to cause toxicity to marine life.

**FIGURE 4. Comparison of environmental MTBE concentration and effect levels. The highest concentrations for NPDES discharge categories and receiving water are shown. Dashed lines indicate NOEC for California species. Solid lines indicate preliminary water quality criteria from Mancini *et al.* (1999).**

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