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Acute Toxicity of Methanol to *Mytilus edulis*

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Methanol is being promoted as an alternative fuel because of the clean air benefits of reduced carbon monoxide and other by-product emissions. In the event of an accidental spill or leakage from a storage tank, there is limited data available on the impact of alternative fuels on marine ecosystems. Before considering the impact of methanol on ecosystem processes, it is necessary to establish the acute toxicity. The blue mussel (*Mytilus edulis*) was selected for study because of its use as an indicator species of marine ecosystem health (Widdows and Donkin 1992). Our primary objective was to determine the LC-50 value of methanol to adult *Mytilus edulis.* We also note sublethal effects that were observed during the course of the 96-hr exposure.

MATERIALS AND METHODS

Mussels were collected from Narragansett Bay in early January of 1993 using a scallop dredge. They were brought to the United States Environmental Protection Agency-Atlantic Ecology Division Laboratory, Narragansett, Rhode Island (USEPA, formerly USEPA-ERLN) and placed in a 760 L flow-through seawater holding tank. Unfiltered seawater was pumped into the tank and was maintained at 3 °C, ambient field temperature. After being held in the tank for 1 wk, approximately 200 mussels were taken out of the flow-through tank and placed in a flow-through acclimation table. The water temperature was raised every 3 d by 2 °C until the mussels were acclimated to 15 °C. At this temperature, the organism is not stressed and is able to maintain normal feeding activity (Bayne et al. 1976, Gosling 1992).

The mussels were fed a unicellular algae, *T-Iso*, a strain of *Isochrysis galbana.* This was prepared under sterile conditions from a pure algae stock. Algal nutrient solution was prepared in deionized water and contained 6% $NaNO_3$; 0.4% NaH_2PO_4; 0.348% Na_2EDTA; 1 mL of sodium silicate solution (27% in 14% NaOH) and 0.260% $FeCl_3$ (added as an $FeCl_3$ solution); a 250-mL volume of nutrient solution was added to the seawater and stock.

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Algae was grown in a phycotron that was equipped with overhead fluorescent lights. The cultures were aerated and the temperature was maintained at 21 °C. Cells were allowed to mature for approximately 10 d and were fed to the mussels when cultures were assumed to be in the log phase of growth.

The toxicity study was conducted at the USEPA laboratory. Narragansett Bay seawater was obtained via the in-house sand filtration system and was re-filtered through a 10 µm Aqua Pure® filter (Cuno, Inc., Connecticut). HPLC grade methanol (Fisher Scientific, New Jersey) was used*.

After the mussels had been maintained in 15 °C water for 3 d, they were removed and placed in the dosing system (Figure 1). The system was contained in a temperature controlled water bath and consisted of a diluter, algae pump, solvent pump, 6 carboys containing the test solutions for the six treatments, 1 carboy containing algae solution, eighteen 1-L glass beakers, and a timer that controlled the pumps (Nelson 1990). Every hour, 750 mL of test solution (premixed) and 150 mL of algae were transferred to each respective chamber of the diluter. In each diluter chamber, the test solution was split three ways, delivering 250 mL of test solution and 50 mL of algae solution to each of three replicate beakers per treatment. This entire process occurred over a period of 3 min. Over a 24-hr period, the total volume of each beaker was replaced six times. The solutions were prepared daily; the temperature of the water bath was maintained at 15 ± 0.5 °C.

At the start of the exposure period, six acclimated mussels (5 - 7 cm length) were arbitrarily chosen, measured, and placed in the exposure beakers. The treatments consisted of 0%, 1%, 2%, 3%, 5% and 10% methanol/seawater (v/v) solutions. There were 3 replicates per treatment and 6 mussels per replicate, giving a total of 18 mussels per treatment. The delivery system was closed and the solution in each beaker was replaced approximately every 4 hr. The beakers were covered with plexiglass to minimize potential volatile losses of methanol. There was no evidence for volatile losses when MeOH concentrations in the beakers were verified with the corresponding calibration standard (below). Based on the dimensionless Henry's Law constant for methanol (on the order of 10^{-4}), less than 0.2% of the methanol was expected to volatilize Overestimating the potential volatile loss for illustrative purposes, the reported 2% methanol solution would decrease to 1.996%.

The experiment was monitored daily for pH (ORION 200 Series, ORION Research Inc., Cambridge, MA), dissolved oxygen (LG Nester oxygen meter, L.G. Nester Comp., Milville, NJ), temperature (ASTM ±0.1°C) and salinity (ATAGO S/Mill hand refractometer, ATAGO Co., LTD., Japan) of the influent seawater.

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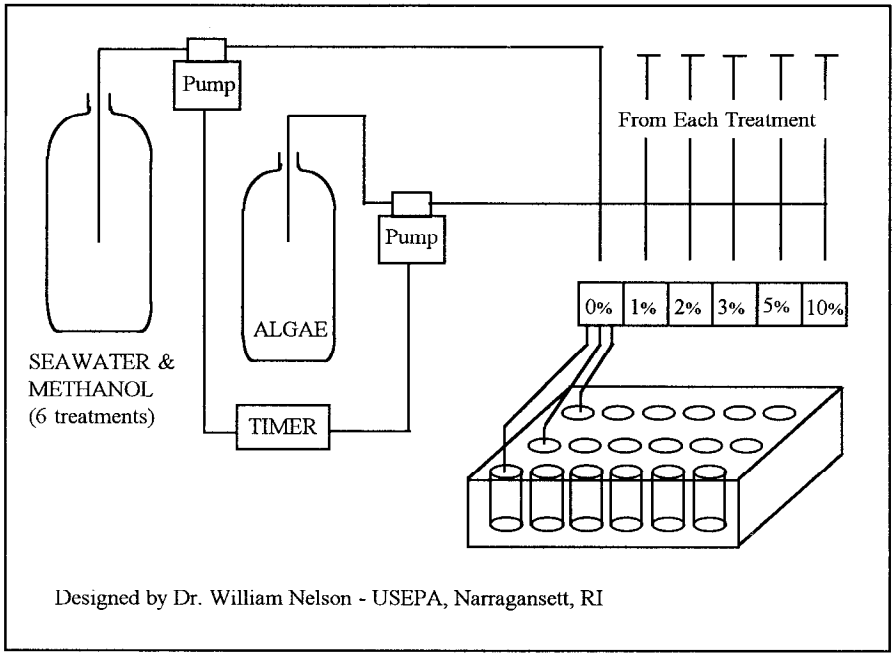

Figure 1. Dosing System

The methanol concentrations in the carboys and exposure beakers were verified daily using standard solutions and the density reading on the refractometer. Fresh standards for each methanol concentration were prepared daily. The density reading for each treatment was compared with the reading for the corresponding standard; the readings were always in agreement. With this technique we could verify the methanol concentration in each treatment and distinguish between treatments; we did not attempt to measure differences smaller than 1%.

Mussels were monitored daily for mortality which was evident by the bivalves being open and lack of movement when a stimulus was applied. Dead mussels were removed from the exposure chambers immediately upon discovery. During the test, physical parameters of the system and the behavior of the mussels were observed.

Two statistical methods were used to determine an LC50 for the binomial distribution. With the Trimmed Spearman-Karber method (TSK) (Hamilton et al. 1977) an a value of 0.05 is generally chosen as the amount of mortality data to be trimmed from the ends of the tolerance distribution. In this experiment, all mortality fell within 95% of the central mortality distribution and the data were not trimmed. In applying the TSK method, the mortality data from each respective treatment is pooled, thus variability between replicates is not considered. In contrast, the Inhibition Concentration Percentage method (ICp) (Norberg-King 1988) accounts for the variability between replicates when determining an LC-50.

RESULTS AND DISCUSSION

Mortality in each of the 3 replicate beakers was the same within each treatment, with the exception of the 2% treatment. Mortalities for each replicate of the 2% treatment are shown in Table 1. Response among replicates and within each treatment was very consistent and an approximate LC-50 of 2%, equivalent to 15,900 mg/L is obvious.

Table 1. Mortality of *Mytilus edulis* exposed to methanol.

Percent Methanol	0	1	2	2	2	3	5	10
Replicate	A-C	A-C	A	B	C	A-C	A-C	A-C
Number Exposed	18	18	6	6	6	18	18	18
Number Dead	0	0	2	3	3	18	18	18

Using the TSK method, an LC-50 of 1.92% (1.69 - 2.18) methanol for *Mytilus edulis* was calculated and is equivalent to 15,200 (13,400-17,300) mg/L. Similarly, an LC-50 of 2.10% (2.00 - 2.18) equivalent to 16,700 (15,900 - 17,300) mg/L was determined by the ICp method; the 95% confidence interval is provided in parentheses. Due to the lack of variability among replicates, the ranges calculated with both methods are quite similar.

A comparison of methanol toxicity for other aquatic species is provided in Table 2. All organisms were freshwater except for *Nitocra spinipes, Mytulis edulis, Alburnas alburnas,* which are all brackish/marine. The LC-50 of 2% (15,900 mg/L) determined in this study falls within the range of methanol toxicity reported for other species. It is important to note that Tarkpea and Svanberg (1982) performed their test for only 24 hr and did not verify the methanol concentration. In our study (below), mussels in the 3% treatment survived beyond 72 hours, but were dead at 96 hours.

All of the physical parameters of the test system were in normal range for acceptable criteria (Price et al., 1974) throughout the experiment (Table 3). The salinity was ambient for Narragansett Bay, 29 - 31 g/kg (Nelson, 1990). Although the temperature in the beakers varied (temporally) between 16 and 17 °C (16.3 ± 0.9), there were no differences among treatments. The dissolved oxygen (DO) in all exposures is reported in Table 3, and was always within 86%-90% saturation. Behavioral data for the 5% and 10% methanol treatments are not reported because the mussels died within 13.5 hr of initial exposure.

During the first 24 hr period, the control mussels exhibited normal behavior, constant filtering, and rapid closing of their shells if a stimulus was applied. All mussels in the 1%, 2% and 3% methanol were alive and feeding. The 5% and 10% methanol treatments were acutely toxic to the mussels within 13.5 hr of the initial exposure, which was

Table 2. Methanol toxicity to aquatic species.

Species		Time (hr)	LC-50 (mg/L)	Reference
Artemia salina	(brine shrimp)	24	10,000	Price et al. 1974
Lepomis macrochirus	(bluegill)	96	10,000	Poirier et al. 1986
Nitocra spinipes	(bleak)	96	12,539	Tarkpea and Svanberg 1982
Mytilus edulis	**(blue mussel)**	**96**	**15,900**	**Present Study**
Salmo gairdneri	(rainbow trout)	96	20,100	Poirier et al. 1986
Anabaena	(blue-green algae)	≥240	24,701	Stratton 1987
Alburnas alburnas	(bleak)	96	>28,000	Tarkpea and Svanberg 1982
Pimephales promela	(fathead minnow)	96	28,100	Veith et al. 1983
Chlorella pyrenoidosa	(green algae)	≥240	28,501	Stratton and Smith 1988

evident by the mussels' lack of movement and open shells (Maciorowski and Clarke, 1980). The mussels in the 5% and 10% treatments were dead when measurements were made at 13.5 hours.

After 48 hr, the control mussels and the mussels in the 1% and 2% methanol exhibited normal behavior. The mussels exposed to 3% methanol displayed various behavioral changes in comparison to the controls, presumably due to the effect of methanol on their central nervous system (Tephly, 1991). Slow movement and sporadic filter-feeding suggested narcosis, defined as a reversible state of arrested activity of protoplasmic structure due to the presence of an organic chemical (Veith et al., 1983).

Alcohol narcosis is physiologically reversible in fish (Veith et al., 1983). Reversibility was not determined in this experiment, but other work suggests that it may be possible for the filtration rate and reflexes to improve after methanol is removed from a system (Maciorowski and Clarke, 1980).

Table 3. Mean experimental conditions during 96-hr exposure to methanol.

Percent Methanol	0	1	2	3	5	10
D.O. (mg/L)	7.3 ± 0.2	7.0 ± 0.6	7.3 ± 0.2	7.3 ± 0.4	7.3 ± 0.01	7.3 ± 0.01
pH	7.5 ± 0.2	7.7 ± 0.1	7.6 ± 0.05	7.8 ± 0.1	8.1 ± 0.1	8.2 ± 0.01

Harris and Morgan (1984) reported that *Corophium volutator* (estuarine amphipod) that were placed in a 1% or higher solution of ethanol were dead within 24 hr, however, they were tolerant of greater concentrations when administered in short pulses.

By 72 hours, the mussels in the 3% methanol were sluggish, some had their siphons extended and exhibited slow reflexes when touched; these responses are characteristic of the narcotic effects of methanol (Mane et al., 1979). There was also a clear increase in fetal material in the 3% and 5% treatments and a slight increase in the 2% treatment. Similar observations were made by Mane et al. (1979), in their study with *Katelysia opima* (estuarine bivalve) and *Donax cuneatus* (marine bivalve) which were exposed to methanol. In the present study, filtration rate was not measured. We did note that the algae that was added as food remained visible in the water column of the 2% and 3% treatments longer than in the control and 1% treatments; this is indicative of a decrease in filtration rate. Mussels were observed at 89 hours to check for mortality; all of the mussels in the 3% treatment were dead.

At 96 hours, when the test was terminated, the mussels in the control and 1% treatments were still feeding and responsive to touch, thus the NOEC was 7960 mg/L. After continuous exposure for 96 hours, 8 of the 18 mussels in the 2% treatment were dead and the remaining mussels had slow reflexes.

In summary, an LC-50 of 15,900 mg L^{-1} of methanol, equivalent to 2% (v/v), was determined. Sublethal effects (e.g., narcosis) were observed for exposure concentrations of 2% and 3% methanol. Data from the literature suggest that some sublethal effects may be reversed if methanol is removed from the system. In marine ecosystems, exposure to extremely high concentrations (>5%) for 12 hours or more is lethal to *Mytilus edulis.* Mussels exposed to 3% methanol survived for greater than 72 hours, and those exposed to 1% exhibited no adverse effects during the entire 96 hr exposure period (NOEC = 7960 mg/L).

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