Toxicity of Mexel 432 to Three Species of Freshwater Organisms

For

RTK Technologies, Inc
Baton Rouge, LA

By

Lake Superior Research Institute
University of Wisconsin-Superior
Superior, WI 54880

Larry Brooke, Project Leader

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Introduction

Certain species of life are incompatible with mechanisms associated with industries using natural waters for cooling or manufacturing. Many industries have increasing concerns with the interference of water flows due to aquatic organism accumulations ultimately causing flows to decrease and interfering with the mechanical processes. One species of particular concern in the United States and Europe is the zebra mussel (*Dreissena polymorpha*). Many control methods for this species have been attempted with limited success. A new chemical product developed in France, Mexel 432, shows promise as a control agent. The Lake Superior Research Institute (LSRI) has conducted tests with this mixture with sensitive non-target species to determine the acute toxicity of Mexel.

The three animal species tested were a cladoceran, *Daphnia magna*, fathead minnow, *Pimephales promelas*, and the rainbow trout, *Oncorhynchus mykiss*. *Daphnia magna* was chosen because it represents sensitive invertebrate species that may inhabit lakes and large rivers where Mexel could potentially be discharged if used as control agent for the zebra mussel. Organic matter was varied between acute tests with the cladoceran because it has been shown in studies conducted in Europe that water quality has a strong influence on the toxicity of Mexel to aquatic organisms. The cladoceran was tested in both short-term acute tests and a long-term chronic test. The fathead minnow was chosen because it represents the warmwater group of fish. Rainbow trout represent the coldwater fish species. Both fish species were tested as juveniles (very sensitive life stage) in seven-day exposures.

Methods

Test Organisms--The cladoceran was cultured at UW-Superior for many generations. The original stock was acquired from the U.S. Environmental Protection Agency, Mid-Continent Ecology Division Laboratory located at Duluth, MN. The culture was maintained with adults grown separately to monitor their reproductive health. The culture procedures met the requirements recommended by ASTM (1993a). The parents of the test organisms had the culture water renewed three times each week. The renewal water contained food which consisted of a solution containing 14.4 mg/L dry weight of yeast-trout chow-cereal leaf mixture per liter and 2.5 mL of $1x10^8$ cells/mL of the green algae *Selenastrum capricornutum* per liter. During the first week, the parental organisms were cultured in moderately hard reconstituted water (ASTM 1993b). After seven days, the culture water was changed to laboratory water and the $\leq$24-h-old neonates used in the toxicity tests were collected from the laboratory water.

The fathead minnow was cultured at UW-Superior for many generations. The original stock was acquired from the U.S. Environmental Protection, Agency Mid-Continent Ecology Division Laboratory located at Duluth, MN. The culture was maintained with adults kept separate to monitor their health and reproductive capacity. The procedures used to culture met the requirements recommended by ASTM (1993b). Adult fish were fed a diet of adult brine shrimp (*Artemia salina*).

The rainbow trout were acquired from the Wisconsin Department of Natural Resources fish hatchery located at Bayfield, WI. They were transported to the UW-Superior culture facility as sac fry before swim-up. They were cultured in 12 °C laboratory water for two weeks before use in the toxicity test. The trout larvae were nourished by the remnants of their yolk sac and were not fed during the Mexel exposure.

Test Water--Water used for culturing the cladoceran test organisms for the first seven days was reconstituted moderately hard water (ASTM 1993b). During the second and third weeks when the broods are produced for testing, the culturing water was changed to laboratory water. Water for culturing the fish species was UW-Superior laboratory water. The laboratory water is municipal water from the City of Superior, WI water supply which takes its raw water from Lake Superior. In the laboratory, the water is partially dechlorinated by contact with active bone charcoal. Then it is passed through an ion exchange resin that removes metallic cations that may be present. Prior to temperature adjustment of the water, a sodium sulfite solution is injected into the water line. The sulfite reduces the residual chlorine to chloride. Chlorine residues were not measured above the detection limit of 3 μg/L for the ion specific electrode method.

Water used for conducting the toxicity tests was slightly modified by the addition of humic acid in most cases. One acute test with the cladoceran was conducted with the usual amount of organic carbon present (<1 mg/L) and the remainder of the tests had the sodium salt of humic acid (Aldrich Incorporated, Milwaukee, WI; Lot No. 061197) added to the water at two concentrations (4.5 and 9.5 mg/L). Humic acid was weighed (0.001g) and mixed into a common batch of water used to prepare solutions for renewal of the test solutions.

Test Procedures--The acute tests with the cladoceran were conducted in 100 mL glass beakers containing approximately 80 mL of test solution. Test solutions were prepared with laboratory water with humic acid added in two of the tests at 4.5 and 9.5 mg/L; the third test did not have humic acid added. Five neonate organisms ≤24-h old were used to start the 48-h exposures. The organisms were selected from a common pool of organisms and added to each test beaker until five organisms were present. Four replicate beakers were used for each Mexel concentration and control. The organisms were from the fifth, sixth or seventh broods and were not fed during the acute tests. Test solutions were prepared each day from a fresh stock of water and Mexel. Test beakers were conditioned by adding test solutions to the beakers for a minimum of one hour before the start of the test. At the start of the test, the conditioning solutions were discarded and new solutions were added immediately before the test organisms were added. Test containers were randomly placed on a tray, covered with another tray, and placed in a temperature and photoperiod (16 h light : 8 h dark) controlled chamber. Test solutions were renewed at 24 h and test organisms were observed for affected behavior or death before transferring the organisms with a large-diameter glass tube to a second set of test chambers containing fresh test solutions. Death was defined as lack of mobility and no heart movement.

The chronic (14-d) cladoceran test was conducted in 250 mL glass beakers with approximately 150 mL of test solution. The test solutions were prepared using laboratory water with humic acid added at 4.5 mg/L. Prior to beginning the test, two sets of test containers were conditioned with the appropriate concentrations of test solutions. Test solutions were renewed daily with freshly prepared Mexel concentrations that contained food. Food consisted of the same ration fed during culturing . Test chambers were placed in random order on trays, covered and placed in a temperature and photoperiod (16 h light: 8 h dark) controlled chamber. Each day when the test solutions were renewed, test organisms were observed for survival and the production of young before transferring them with a glass tube to fresh solutions in a second set of exposure chambers. When observed, neonates were counted and discarded.

The fathead minnow toxicity test was conducted in glass chambers that were subdivided into four replicate test chambers with a fifth chamber in common with the other four. The fifth chamber had a side that was constructed of stainless steel mesh that was in common with each of the test chambers. The mesh insured that each replicate chamber received a portion of the same test solution. The total volume of test solution in the four replicate test chambers was 2.0 liter. The chambers were conditioned with the appropriate test solutions prior to beginning the test. Ten larval fish were placed in each replicate chamber in a random order at the start of the test. Each day the old test solution was removed with a siphon to approximately ten percent of the original level and a fresh solution added. During siphoning, uneaten food and feces were removed. Larval fish used in the toxicity test were fed a diet of freshly hatched brine shrimp that was washed free of salt brine. Each replicate test chamber was fed two or three times daily equal volumes in excess of what the fish could eat. The test chambers (five containing Mexel solutions and another with control solution) were placed in a temperature controlled water bath. Photoperiod was controlled at 16 h light and 8 h dark. Each day the test organisms were observed for behavior changes and death. Death was defined as unable to respond to prodding and no observed movement of the heart. At the termination of the test, the surviving fish were dried at 60 °C for 24 h and weighed.

Rainbow trout sac fry were tested in one liter beakers containing approximately 800 mL of test solution. Five concentrations of Mexel and a control were tested with four replicates each containing five test organisms (20 fish total for each Mexel concentration and control). The fish were randomly selected from a common pool and randomly placed into the test chambers. The chambers were conditioned with the appropriate test solutions prior to beginning the test. Each day the test solutions were siphoned to less than 100 mL and replaced with freshly made solutions. The test beakers were covered with aluminum foil to prevent the fish from leaping from the beaker. Temperature of the test solutions was maintained by placing the beakers, in random positions, in a water bath. Each day the fry were observed for behavioral changes or death. Death was defined as lack of response to prodding when found lying motionless at the bottom of the test beaker. Photoperiod was controlled at 16 h light and 8 h dark. At the termination of the test, the surviving fish were dried at 60 °C for 24 h and weighed.

Chemical Analysis--Concentrations of Mexel in water were measured using the method recommended by Dr. Michel Khalanski of Electricite de France (EDF), Paris, France. The method is referred to as the methyl orange colorimetric method.

The procedure for analysis is described in detail.

1. Prepare calibration standards and transfer 50 mL to 125 mL amber glass bottles. Collect 50 mL samples of water to be analyzed and place in amber bottles.
2. Add 2.0 mL of buffer solution (125 g KCl, 70 g NaAcetate, 300 mL glacial acetic acid and dilute to 1.0 liter with deionized water; pH = 3.75) to 50 mL of water sample in a 125 mL brown bottle.
3. Add 1.25 mL 0.1 % (w/v) methyl orange solution.
4. Add 3.5 g of KCl to the sample to aid in the separation of the aqueous and organic layer.
5. Add 10.0 mL of 1,2-dichloroethane.
6. Shake for 15 minutes on a mechanical shaker (100 oscillations per minute).
7. While samples are shaking, prepare spectrophotometer for analysis. Set wavelength to 414 nm and 100% transmittance (0.000 absorbance) using a 2 cm cuvette filled with 1,2-dichloroethane.
8. Immediately transfer the sample mixture to a 125 mL separatory funnel to allow for separation of the organic and aqueous phases.
9. Transfer the 1,2-dichloroethane extract to a test tube and then into a 2 cm cuvette. This step removes any water drops that may be present.
10. Absorbance is measured 10 minutes after being removed from the mechanical shaker. The absorbance is measured and recorded at 414 nm and 750 nm.
11. Regress the absorbance (414 nm absorbance minus the absorbance at 750 nm) against the Mexel concentration to compute a prediction equation.
12. Using the linear equation, sample concentrations can be determined from the sample absorbance measurements (414 nm measurement minus the 750 nm measurement).
13. A detection limit of 0.200 mg/L can be obtained with this method. High linearity is obtained with the standards (i.e., $R^2 = 0.98$).

Modifications:

1. To reduce the detection limit, use 100 mL samples, 4.0 mL of buffer solution and 7.0 g KCl in the analytical procedure. Do not increase the volume of 1,2-dichloroethane.
2. In the Mexel concentration range of 5 to 15 mg/L, a linear regression slope of approximately 18.4 was measured. For lower concentrations, a slope of lower values (approximately 11.8 to 12.2) was measured. Therefore, it may be desirable to prepare two sets of standards of different ranges if a large concentration range is expected in samples.

Glassware Cleaning:

1. Two rinses with acetone.
2. One rinse with 10 % nitric acid.
3. Three rinses with deionized water.

Statistical Analysis

Median lethal concentrations (LC50) were computed using the Trimmed Spearman-Karber analysis (Hamilton et al. 1977). LC50s were computed using the nominal Mexel concentrations.

Significant differences in fish weight at test termination were tested using hypothesis test methods. One-way Analysis of Variance (ANOVA) and Dunnett's Procedure were used to determine significant ($p < 0.05$) differences from control fish weights.

Results

Analytical Determinations of Mexel--Analytical determinations of Mexel were not made in tests conducted with *Daphnia magna*. The acute tests were conducted below the analytical detection limit of 200 μg/L and the chronic test was omitted due to a laboratory error. The fathead minnow and rainbow trout tests were measured and showed reasonable agreement with the nominal concentrations that were determined from carefully weighed and diluted stock concentrations. Freshly prepared test solutions in the fish tests were an average of 89.6 ± 22.6% of the nominal concentrations. When the test solutions were 24-h old, they were 49.0 ± 13.5% of the fresh solution measured concentrations in the fathead minnow test. The concentrations at 24 h were 87.0 ± 27.5% of the fresh solution measurements in the rainbow test. The much lower (11.5 vs 22.5 °C) temperature in the rainbow trout test may have reduced the degradation rate of the Mexel solutions.

Duplicate analyses of samples were measured during the rainbow trout test and resulted in percent agreement of 71.7 and 98.0%. An extraction recovery of a Mexel spiked standard was determined for the same test and resulted in 130% recovery. Comparison of measured concentrations of Mexel from two consecutive days of fresh test solutions showed an average agreement of 90.4%. Because of the variability in analytical determinations and the generally good agreement with nominal concentrations, nominal concentrations are used to describe effect concentrations for biological responses.

Analytical Measurements of Test Water Characteristics--Measurements of six water characterists were made at various times during most of the toxicity tests (Table 1). Water was measured with a digital thermometer (Fisher Scientific, Inc.) that was calibrated using a National Bureau of Standards thermometer. The hydrogen ion concentration (pH) was measured using Orion Model 290A meter that was calibrated daily with three standards. Dissolved oxygen was measured using a YSI (Yellow Springs Instrument, Inc.) Model 58 digital dissolved oxygen meter that was calibrated each week by the Winkler Titration Method. Total hardness (as $CaCO_3$) was determined by titration with EDTA using the ManVer 2 powder pillows of the Hach, Co (Loveland, CO). Total alkalinity (as $CaCO_3$) was determined by titration with sulfuric acid and the bromocresol green-methyl red powder pillow color indicator of Hach, Co. Conductivity was measured using a digital conductivity meter (Fisher Scientific, Co.) that was calibrated with a KCl standard solution. All measured water characteristics (except temperature that was controlled) agreed well between tests and was similar to the values measured in Lake Superior water.

Biological Effects--Cladoceran neonates <24-h old were exposed to Mexel in tests with various concentrations of organic matter. In the test solutions containing only the usual amount of organic matter available from laboratory water ($\leq$1.0 mg/L), the lowest EC50s were determined (Table 2). Test concentrations of Mexel ranged from 0.58 to 9.2 μg/L and all mortalities were observed during the first two hours of exposure (Table 3). An EC50 of 6.51 μg/L was measured at two hours of exposure and did not change during the remainder of the test. In the test with 4.5 mg/L of humic acid added to laboratory water, exposure concentrations ranged from 12.9 to 206 μg/L (Table 4). An EC50 of 30.7 μg/L was measured at two hours of exposure and the EC50 continued to decrease with time to 18.2 μg/L at 24 h (Table 2). At a third level of organic carbon (9.5 mg/L), the toxicity of Mexel was further reduced. Exposure concentrations ranged from 1,960 to 31,400 μg/L. Dead organisms were not observed until 24 h of exposure (Table 5). At 24 h of exposure, an EC50 of 18,000 μg/L was estimated and it was further reduced at 48 h to 2,770 μg/L. The organisms became slowed in their swimming prior to their deaths.

A chronic exposure with the cladoceran to Mexel with 4.5 mg/L of added organic matter was attempted for the recommended 21 days (ASTM 1993a). The test was terminated on Day 14 of exposure due to the loss of 30 % of the control organisms. An obvious reason for the deaths of two control organisms on this day is not apparent, but handling the organism seven rather than three times during a week (three is the usual procedure for conducting a twenty-one day chronic test) could have contributed to the mortalities. Increased rates of mortality occurred in all test concentrations at Day 14.

Although Mexel concentrations were not measured, test organisms appeared slowed in growth prior to death at Mexel concentrations $\geq$6.0 mg/L. At the lower test concentrations ($\leq$3.0 mg/L) growth did not appear to differ from the control organisms. Organisms survived <24 h at the highest test concentration (24 mg/L), <48 h at 12 mg/L, and some organisms survived to the end of the test at lower test concentrations $\leq$6.0 mg/L (Table 6). LC50s of 17.0, 8.5, 8.5, and 3.4 mg/L were calculated for days 1, 2, 4, and 14, respectively. The LC50s are approximately 1,000 times less toxic than those measured for the acute tests (Table 2). The acute tests did not have food present whereas the chronic test was fed. The extra organic matter contributed by the food can further reduce the toxicity of Mexel. At Day 14 of exposure, survival was 10% at the 6.0 mg/L and 60 to 70% at lower exposure concentrations and the control. Time to produce the first brood was slower at 6.0 mg/L (8.8 days) than at lower exposure concentrations (7.6 days) and controls (7.9 days). Mean number of young produced per surviving adult was approximately one third the young production of the controls at Mexel concentrations $\geq$3.0 mg/L (Table 6). Reproduction was not reduced at the Mexel concentration of 1.5 mg/L when compared to the control organism reproduction. Based upon reproduction from a fourteen-day exposure to Mexel with daily renewal of test solutions the Lowest Observed Effect Concentration (LOEC) is 3.0 mg/L and the No Observed Effect Concentration (NOEC) is 1.5 mg/L. The chronic value is 2.121 mg/L for the 14-day exposure.

Fathead minnow larvae <24-h old were exposed to Mexel for seven days. Two tests were attempted, the first was conducted at a range of Mexel concentrations of 0.025 to 0.400 mg/L. Survival ranged from 92.5 to 100%. The fish were dried and weighed to determine if sublethal effects on growth occurred due to exposure to Mexel. Individual fish weights averaged from 0.85 to 1.00 mg dry weight. No significant ($p<0.05$) effects were observed in this test.

The second seven-day exposure of the fathead minnow to Mexel was conducted at higher Mexel concentrations. Nominal Mexel concentrations ranged from 0.75 to 12.0 mg/L (Table 7). The concentrations were analytically verified in fresh and 24-h old solutions. The fresh solutions agreed well (89.6%) with the nominal concentrations and the 24-h old solutions were approximately one half (49.0±13.5%) of the fresh solution concentrations. Survival of control organisms was 95% and ranged from 92.5 to 100% for Mexel treatments of ≤6.0 mg/L. None of the fish survived the 12.0 mg/L treatment. LC50s were computed for 2, 4, and 7 days of exposure and were 8.34, 8.06, and 7.87 mg/L, respectively. At the end of the test, surviving fish were dried, weighed, and compared to the control fish weight to determine if growth was impacted by the Mexel exposures. Fish exposed to Mexel concentrations ≥1.5 mg/L had significant ($p<0.05$) reductions in growth (Table 8). Fish exposed to Mexel concentrations of 1.5 and 3.0 mg/L grew 14% less than the controls and fish exposed to 6.0 mg/L grew 30% less.

Rainbow trout were exposed as late sac fry juveniles for seven days to Mexel concentrations ranging from 2.0 to 32.0 mg/L. Mexel concentrations were analytically determined for fresh and 24-h old test solutions (Table 7). Freshly prepared test solutions agreed well (89.6%) between the nominal values and the measured values. Solutions that were 24-h old had only a 13% decrease of concentrations when compared to the measured fresh solution concentrations. This is considerably less than the fathead minnow solutions that decreased an average of 49%. The difference between the two tests in the rate of the disappearance of Mexel is likely due to the temperature differences between the two tests. (The fathead minnow test was conducted at approximately 23 °C and the rainbow trout test at approximately 12 °C.) Survival of the fish during the test ranged from 100% in the controls to 0% in the treatment concentrations ≥16.0 mg/L. LC50s were computed for 12 hours, 1, 4, and 7 days and were 22.6, 12.6, 11.3, and 7.0 mg/L, respectively. Because the fish were living on stored yolk sac energy and not fed during the test, growth was not expected to be a useful endpoint in this test. However, the surviving fish were dried and weighed at the termination of the test and compared to the control fish weights (Table 8). No significant ($p<0.05$) differences were observed in Mexel exposure concentrations ≤4.0 mg/L. The fish exposed to the Mexel concentration of 8.0 mg/L showed a significant increase in weight of 46%. This may be due to only a few fish surviving (25%) and they may have been the largest of the group.

Table 1. Measured Characteristics of Test Solutions for Mexel 432 Exposures of Various Durations with Three Species of Freshwater Organisms. Measurements are the Mean, ± Standard Deviation and (Number of Observations).

	Daphnia magna Acute Tests					
Characteristic	<1 mg/L Humic Acid	4.5 mg/L Humic Acid	9.5 mg/L Humic Acid	*Daphnia magna* Chronic Test	Fathead Minnow Seven-Day Test	Rainbow Trout Seven-Day Test
Water Temperature (°C)	20.3±0.4 (3)	20.9±0.4 (2)	20.4±0.3 (5)	20.2±0.4 (252)	24.2±0.6 (58)	11.5±0.1 (33)
pH	7.77±0.01 (3)	-	7.62±0.03[1/] (2)	7.76±0.15 (27)	8.00±0.29 (28)	8.03±0.42 (7)
Dissolved Oxygen (mg/L)	9.0±0.0 (3)	-	-	8.3±1.0 (27)	7.3±0.7 (30)	8.4±0.2 (19)
Total Hardness (mg/L as $CaCO_3$)	48.0±0.0 (3)	-	51.8±0.6[1/] (2)	49.5±0.7 (27)	47.9±3.2 (9)	51.5±0.9 (3)
Total Alkalinity (mg/L as $CaCO_3$)	47.3±1.5 (3)	-	51.6±0.0[1/] (2)	52.1±2.5 (27)	48.0±3.0 (9)	51.3±1.2 (3)
Conductivity (μmhos/cm)	153.7±4.9 (3)	-	158.0±0.0[1/] (2)	161.3±3.4 (27)	151.9±3.4 (11)	167.0±2.0 (3)

[1/] Measured in previous test that was unsuccessful due to no observed effects.

Table 2. EC50 Values ($\mu g/L$) for *Daphnia magna* <24-Hour Old Neonates Exposed to Mexel 432 for Various Time Intervals in Three Types of Water.

	Time (Hour)			
Test Water	2	4	24	48
Lake Superior Water[1/]	6.51 (NR)[2/]	6.51 (NR)	6.51 (NR)	6.51 (NR)
Lake Superior Water with 4.5 mg/L Humic Acid Added	30.7 (25.0 - 37.5)	25.8 (21.6 - 30.8)	18.2 (NR)	18.2 (NR)
Lake Superior Water with 9.5 mg/L Humic Acid Added	>31,400	>31,400	18,000 (15,500 - 21,000)	2,770 (NR)

[1/] Lake Superior water usually contains between 0.5 to 1.0 mg/L of organic carbon.
[2/] Confidence limits (95%) of the EC50 estimate. Not reliable (NR) when an all or nothing response was seen instead of a gradual response with the concentration of Mexel 432.

Table 3. Mortalities Observed at various Exposure Times for *Daphnia magna* Neonates Exposed to Mexel 432 in Lake Superior Water without Additional Organic Carbon Added. Twenty Test Organisms were Exposed at All Test Concentrations and Controls.

	Nominal Mexel Concentration (μg/L)					
Time (Hr)	0	0.58	1.15	2.30	4.60	9.20
2	0	0	0	0	0	20
24	0	0	0	0	0	20
48	0	0	0	0	0	20

Table 4. Mortalities Observed at various Exposure Times for *Daphnia magna* Neonates Exposed to Mexel 432 in Lake Superior Water with 4.5 mg/L of Humic Acid Added. Twenty Test Organisms were Exposed at All Test Concentrations and Controls.

	Nominal Mexel Concentrations (μg/L)					
Time (Hr)	0	12.9	25.8	51.5	103	206
2	0	0	9	19	17	20
4	0	0	12	20	18	20
24	0	0	20	20	20	20
48	0	0	20	20	20	20

Table 5. Mortalities Observed at various Exposure Times for *Daphnia magna* Neonates Exposed to Mexel 432 in Lake Superior Water with 9.5 mg/L of Humic Acid Added. Twenty Test Organisms were Exposed at All Test Concentrations and Controls.

	Nominal Mexel Concentrations (μg/L)					
Time (Hr)	0	1,960	3,920	7,850	15,700	31,400
2	0	0	0	0	0	0
4	0	0	0	0	0	0
24	0	0	1	5	20	20
48	0	0	20	20	20	20

Table 6. Survival , Time to First Brood, Number of Young Produced, and the Mean Number of Young Produced per Surviving Adult During the Fourteen-Day Exposure of *Daphnia magna* To Mexel 432.

Concentration of Mexel (mg/L) and Time of Observation	Percent Survival	Day of First Brood	Number of Young Produced	Mean Number of Young per Surviving Adult
Control				
Day 2	100		0	0
Day 7	100	7.9	11	1.1
Day 14	70		752	83.6
1.5				
Day 2	100	7.6	0	0
Day 7	100		34	3.4
Day 14	60		741	82.3
3.0				
Day 2	100	7.6	0	0
Day 7	100		13	1.3
Day 14	60		198	24.8
6.0				
Day 2	100	8.8	0	0
Day 7	100		11	1.1
Day 14	10		0	0
12.0				
Day 2	0	-	0	0
24.0				
Day 2	0	-	0	0

Table 7. Nominal and Measured Concentrations of Mexel 432 from Toxicity Tests with the Fathead Minnow and Rainbow Trout.

	Fathead Minnow Test[2/]						Rainbow Trout Test[3/]					
Nominal Mexel Concentrations (mg/L)	0.0	0.75	1.5	3.0	6.0	12.0	0.0	2.0	4.0	8.0	16.0	32.0
Fresh Soln.	<DL[1/]	0.88	1.3	3.2	5.8	-	<DL	1.2	3.9/2.8	8.7	-	-
Fresh Soln.	<DL	0.92	1.2	2.3	5.4	12.5						
24-hour old	<DL	0.46	0.6	1.8	1.8	-	<DL	1.4	2.7	5.5/5.4	-	-
Fresh Soln.	<DL	0.42	1.0	2.4	7.6	-						
24-hour old	-	-	-	-	1.4	-						

[1/] Less than detection limit.
[2/] Spike recovery = 80.0%.
[3/] Spike recovery =130%; Duplicate analysis = 71.5 and 98.0% agreement.

Table 8. Percent Survival and Mean Weight, at End of Test, of the Surviving Fish in Seven-Day Exposures to Mexel 432 with Juvenile Fathead Minnow and Rainbow Trout.

Test Species	Nominal Test Concentration (mg/L)	Percent Survival	Mean Weight (mg)
	0	95	0.695±0.040
	0.75	97.5	0.672±0.028
Fathead Minnow	1.5	100	0.598±0.040*
	3.0	95	0.598±0.036*
	6.0	92.5	0.488±0.043*
	12.0	0	-
	0	100	11.62±1.33
	2.0	100	12.84±0.96
Rainbow Trout	4.0	100	12.65±2.33
	8.0	25	16.98±1.54*
	16.0	0	-
	32.0	0	-

* Significantly different from the control organism average weight ($p<0.05$).

References

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