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Acute Didecyl Dimethyl Ammonium Chloride Toxicity to Larval Lake Sturgeon, *Acipenser fulvescens* Rafinesque, Walleye *Sander vitreus* Mitchill, and Northern Pike, *Esox lucius* Linnaeus

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Didecyl dimethyl ammonium chloride (DDAC) is a cationic surfactant of the quaternary ammonium compound (QAC) family. DDAC is most commonly used as an antisapstain fungicide and is employed globally in the forest industry to prevent growth of fungi that can result in dark stains on softwood lumber products. Not surprisingly, the majority of aquatic toxicity studies evaluating the impact of DDAC (and other antisapstain chemicals) have been conducted on the west coast of Canada, where the province of British Columbia (BC) is the largest user of this class of compounds. BC was the first and only province in Canada to introduce legislation for lumber mill effluents, setting the limit at 700 µg/L based on available data (British Columbia 1990 in Juergensen et al. 2000). Following that, a series of studies found acute toxicity values ranging from 10 to 1100 µg/L for early life stages of several different freshwater species, most of which are native to BC (Henderson 1992; Wood et al. 1996; Bennett and Farrell 1998; Farrell et al. 1998; Bailey et al. 1999; Teh et al. 2003). In 2000, the interim CCME (Canadian Council for Ministers of the Environment) guideline for the protection of aquatic life was set at 1.5 µg/L for DDAC after applying a safety factor to an LC_{50} (48h) for *Daphnia magna* derived by Farrell et al. (1998) (Juergensen et al. 2000).

Despite the importance of DDAC to the lumber industry, there are actually over 250 registered formulations that contain the chemical as an active ingredient. These products are used in applications such as molluscicides for zebra mussel control, disinfectants, and recirculating cooling towers (see Juergensen et al. 2000). As it is possible that these formulations may be used in areas of North America other than the west coast, we conducted early life-stage exposures of DDAC on three-day old lake sturgeon (*Acipenser fulvescens*), walleye (*Sander vitreus*), and northern pike (*Esox lucius*) larvae to evaluate the acute toxicity of this compound to these fish species.

MATERIALS AND METHODS

Lake sturgeon larvae were raised from eggs and milt obtained from wild adult sturgeon captured by dip net in the Wolf River, Wisconsin, USA. Eggs from one

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female and milt from multiple males were hand-stripped, after which adult fish were released. Eggs were fertilized at the site of capture and transported to the Canadian Rivers Institute Manitoba Field Station in Pinawa, Manitoba, Canada in a sealed container within a cooler maintained (with ice) at a temperature similar to that in the Wolf River (~13°C). Upon arrival at the field station, eggs were gently rolled for several days in Winnipeg River water (at ambient temperatures: 13 to 18 °C) that was pumped through a MacDonald-type hatching jar. Larvae that emerged on the first day of hatching were removed from the jar and placed in an aquarium. Those that hatched on day two were kept in a separate aquarium, and this process was repeated until all viable eggs had hatched. All exposures began at 3 days post-hatch (dph).

Walleye and northern pike larvae were obtained from mature fish captured in a trap net set in Falcon Creek. Manitoba, Canada. Egg and milt collection, fertilization, and transportation, and larvae handling were performed in a manner similar to that for lake sturgeon described above.

Chemical additions were made to the exposure vessels such that the fish were exposed to the nominal concentration of DDAC, rather than the antisapstain formulation (Bardac 2280). Bardac 2280 was donated by Lonza (Fair Lawn, NJ, USA) and contains 80-82% DDAC as the active ingredient, 10% ethanol, 7-10% water, and < 1% amine chloride. A 1 L stock solution of 100 mg/L DDAC was obtained by adding Bardac 2280 to Winnipeg River water, taking into account that Bardac 2280 is 80% DDAC (e.g. 140.45 µL Bardac 2280 in 999.86 mL river water). The range of volumes added to the exposure vessels to obtain concentrations between 50 and 3000 µg/L was 0.5 to 30 ml; final volumes in all exposure vessels was 1 L. General water quality values for the Winnipeg River water used to prepare the stock solution and for the exposure vessels were: total dissolved solids 145 mg/L; total suspended solids 33 mg/L; hardness: 60 mg/L; conductivity 191 µmhos/cm; nitrite/nitrate 0.08 mg/L; total kjedahl nitrogen 1.01 mg/L; total phosphorus 0.084 mg/L; dissolved oxygen 7.9 mg/L; pH 7.85; and ammonia 0.03 mg/L.

Ten larvae (3 dph) were transferred to 1 L glass jars, with duplicate treatments for each concentration. Jars were partially submerged in a water bath, which was held at 18 °C to ensure that water temperatures in the exposure jars did not fluctuate appreciably during the study. Exposure conditions were static, with no water or chemical renewals over the 96 h period. Fish were not fed for the duration as they were all at the yolk sac stage. Lake sturgeon were exposed to 0, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 1.2, and 1.6 mg/L; walleye larvae were exposed to 0, 0.2, 0.4, 0.5, 0.6, 0.65, 0.7, 0.75, 0.8, and 1.0 mg/L; and northern pike larvae were exposed to 0, 0.2, 0.4, 0.8, 1.2, 1.6, 1.8, 2.0, 2.2, 2.5, and 3.0 mg/L. Exposure vessels were monitored for mortalities approximately every 2 hours (between 8 am and 11 pm), and any dead fish were immediately removed upon observation.

Quality assurance and quality control were performed on water samples of all

stock and selected experimental solutions to determine actual concentrations of DDAC in exposure vessels. Water samples were collected and preserved according to the procedures laid out by the Organic Chemistry Analytical Laboratory, Pacific Environmental Science Centre, Environment Canada, North Vancouver, BC, Canada (5 mL Rexonic N25-7 solution and 10 ml formaldehyde). Samples were stored in brown bottles covered in tin foil and maintained at 4°C.

DDAC water samples were shipped in coolers to Simon Fraser University, Vancouver, Canada, and analyzed by Envirochem Consultants (North Vancouver, BC) following methods outlined in the British Columbia Environmental Laboratory Manual (Horvath 2003). Briefly, the water samples were extracted using dichloromethane, and analyzed on a gas chromatograph (HP 5890 Series II GC), equipped with a nitrogen phosphorus detector. Didecyl dimethyl ammonium bromide (DDAB) was added to water samples as a surrogate, and was measured at a mean recovery of 70.1 ± 2.6% (SE; n=16), with a detection limit from this lab of 20 µg/L. Recovery of DDAB was assumed to be similar for DDAC, and so the actual concentration of DDAC was calculated using the formula:

$$\frac{\text{reported concentration DDAC}}{\text{recovery of DDAB}} \times 100$$

Actual concentrations were on average, 95.5 ± 2.6% (SE; n=10) of the nominal DDAC concentrations.

Mortality data from duplicate treatments were pooled and cumulative mortality was analyzed to calculate LC_{50}-96 h values and 95% confidence intervals for each fish species using the probit method (SoftTox™ software, WindowChem™, Fairfield, CA).

RESULTS AND DISCUSSION

No mortalities were observed for any species in any control treatment. The concentration-response relationships for DDAC exposure for all study species were very steep (Figure 1). Specifically, larval walleye, lake sturgeon, and northern pike displayed a range of 0% to 100% mortality between 0.4 to 0.8 mg/L, 0.4 to 0.8 mg/L, and 0.8 to 2.0 mg/L DDAC, respectively. This result is consistent with reports from other researchers, who have shown that 0 to 100% mortality occurs within substantially less than an order of magnitude for many fish (*Oncorhynchus mykiss,* Wood et al. 1996; *Oncorhynchus kisutch* (many ages), *Pimephales promelas,* and *Platichthys stellatus,* Farrell et al. 1998), and invertebrate species (*Mysidopsis bahia, Hyallela azteca,* and *Neomysis mercedis,* Farrell et al. 1998). Thus, it is clear that acute DDAC toxicity to fishes and invertebrates occurs over a very narrow concentration range.

The variability in the 96-h acute toxicity of DDAC among the three juvenile fish species was less than an order of magnitude (Table 1). In addition, the toxicity

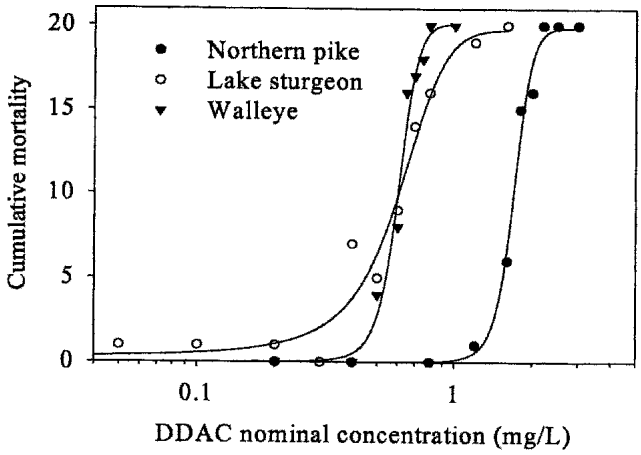

Figure 1. Concentration-response relationships for fish larvae exposed to didecyl dimethyl ammonium chloride (DDAC) for 96- h. Cumulative mortality is based on pooling duplicate trials for n = 10 fish each. There were no mortalities observed in any control treatments.

Table 1. Acute toxicity of didecyl dimethyl ammonium chloride (DDAC), for fish larvae exposed at 3 days post-hatch for 96-h using Winnipeg River water. Nominal concentrations are shown.

Fish species	LC_{50} 96-h (mg/L)	95% CI
Lake sturgeon	0.45	0.44 - 0.46
Walleye	0.59	0.56 - 0.62
Northern pike	1.04	1.03 - 1.05

values observed in the present study are higher than other reported values for larval fish exposed to DDAC. For example, Bennett and Farrell (1998) found that the 96-h LC50 was considerably lower (0.01 µg/L) for 8 d-old white sturgeon tested in filtered, dechlorinated Vancouver municipal water, which is known for being soft (hardness: 34 mg/L; Bennett and Farrell 1998), and having low levels of dissolved and suspended solids (20 to 30 mg/L combined; Gibson KA, Greater Vancouver Regional District, pers. comm.). However, Teh et al. (2003) studied white sturgeon in relatively hard (235 mg/L) well water and reported higher 96-h LC_{50} values (10-50, 58.4, 101.8, and 100-250 µg/L for 3, 8, 11, and 42 d-old white sturgeon, respectively). Our values for lake sturgeon in river water (dissolved and suspended solids: 145 and 33 mg/L, respectively) are higher still, which suggests that differences in acute toxicity among studies may be related to differences in water quality. DDAC has a strong affinity for adsorption to particulate matter, which tends to limit the bioavailability of the chemical (Szenasy et al. 1999) and likely reduces the effective toxicity in most environmental situations. Indeed,

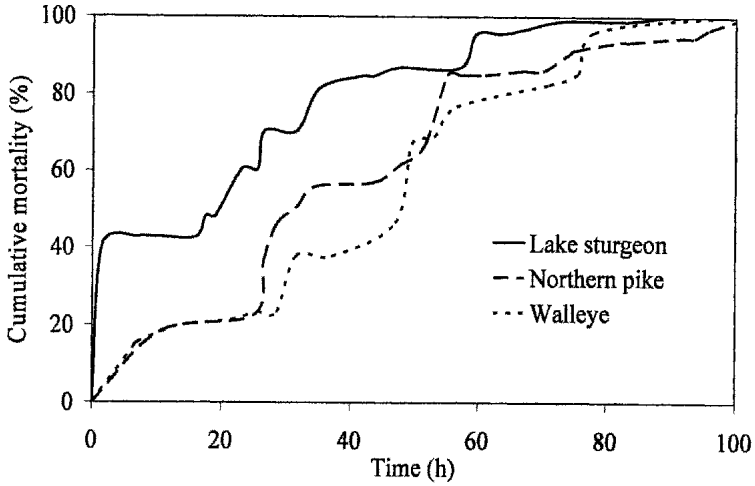

Figure 2. Time dependent cumulative mortality (%) for fish larvae exposed to didecyl dimethyl ammonium chloride (DDAC). Cumulative mortality represents all concentrations within a species combined.

there are reports of five-fold reductions in toxicity to fish when exposed using river water rather than laboratory water sources (AQUA-Science 1997). Consequently, application of these DDAC acute toxicity data must take local water quality into consideration.

While water quality appears to be an important consideration in establishing the acute toxicity of DDAC, results of the present study also show an important intra-specific species difference for fish tested at the same developmental stage and with the same water quality conditions. The timing of mortality may reflect some interspecific differences in the metabolism of the chemical or the mechanism of lethal action (Figure 2). Larval lake sturgeon suffered rapid early mortality (<2-h) compared to northern pike and walleye, which both experienced their most rapid mortality between 24- and 48-h. Although the cumulative mortality versus time shown here is directly related to the exposure concentrations of the chemical, the relationships reflect the overall response of the different species. In addition, the 2-fold higher 96-h LC_{50} value for northern pike as compared with both lake sturgeon and walleye larvae compares favourably with the interspecific range of previously reported 96-h LC_{50} values for various fishes. For example, in a review of available DDAC toxicity studies Juergensen et al. (2000) showed that across 7 fish species, and various life stages, the 96-h LC_{50} values were all within a five-fold range.

The current interim guideline for DDAC, for the protection of aquatic life, has been set at 1.5 µg/L after applying a safety factor to the 48h LC_{50} value for *Daphnia magna* produced by Farrell et al. (1998) (Juergensen et al. 2000).

Environmental concentrations of 449 μg/L have been measured at Fraser River storm-water discharge points; however, values fell to 11 μg/L at a distance of 5 m and became undetectable 10 m downstream of the discharge (Szensay et al. 1999). Given these environmental concentrations, and results shown here and elsewhere that natural waters can have a protective effect on DDAC toxicity to biota, current guidelines appear to be somewhat conservative, especially for waters with high levels of dissolved and/or suspended solids.

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