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Acute Toxicity of Sodium Selenite to Juvenile Walleye

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Selenium is a trace element normally found in surface waters at concentrations of approximately 0.1-0.3 µg/L (Lemly 1985). At slightly elevated concentrations (1-5 µg/L), it can bioaccumulate in the aquatic food chain, becoming highly toxic to fish and wildlife (Lemly and Smith 1987). Sandholm et al. (1973) found that fish accumulate selenium primarily through the food chain, although uptake across gills is very efficient at low waterborne concentrations (Hodson and Hilton 1983). Selenium can also be passed to offspring from maternal transfer to the eggs where it is possible to cause complete reproductive failure (Gillespie and Baumann 1986; Coyle et al. 1993; Lemly 1993).

Selenium can enter aquatic environments by many processes, including natural weathering processes, combustion of fossil fuels, agricultural irrigation, metal mining processes and other industrial activities. In South Dakota, selenium concentrations are highest in soils associated with coal deposits and marine shales (Ruelle 1993). Normal background selenium concentrations in shale are about 0.6 µg/g (Eisler 1985). In South Dakota along the Missouri River, maximum selenium concentrations in marine shale are more than 20 times higher than normal background concentrations (Eisler 1985). The Cheyenne River drains areas that are known to have marine shales containing high selenium concentrations that ultimately deposit in lower Lake Oahe.

Historically, the Cheyenne River has exceeded the 4-day average chronic limit of 5 µg/L established for the protection of aquatic organisms (USEPA 1987). USGS (1983-1995) water quality data has documented during 1983-1995 a dissolved selenium range from <1.0 to 13 µg/L ($\overline{x}$ = 3.50 µg/L; n = 72) at the confluence of Cherry Creek and the Cheyenne River. Waterborne concentrations of selenium from 10-25 µg/L have been associated with apparent reproductive failure and disappearance of certain freshwater fish populations (e.g., Lemly 1985; Baumann and Gillespie 1986). A decline in the walleye (*Stizostedion vitreum*) population in lower Lake Oahe was first documented in the early 1980's (Riis 1983) and has since been attributed to poor natural reproduction and/or recruitment (Fielder 1992). Also, the percent egg hatch from walleye broodstock collected for hatchery purposes in the Cheyenne River area is frequently lower than hatches obtained from broodstock collected from the middle and upper regions of Lake Oahe. This study was conducted to assess the sodium selenite 96-h LC50 for juvenile walleye to determine if selenium concentrations in the Cheyenne River might be of concern to possible juvenile walleye survival.

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MATERIALS AND METHODS

Walleyes were obtained from the Blue Dog State Fish Hatchery, Webster County, South Dakota, and transported to South Dakota State University where they were placed into a recirculating system (21 °C) consisting of 24 110-L aquaria, a solids settling chamber, a biofilter, and a sand filter. Walleyes were trained to feed on a prepared diet (BioKyowa C-1000) and reared to a mean length of 66.7 mm (SE 0.8; n = 96) before being placed in test chambers.

Test chambers consisted of six 37-L aquaria with a glass partition to create two chambers of equal volume per aquaria, or a total of 12 test chambers for the experiment. This configuration allowed the testing of four treatments with three replicates. Constant toxicant volume, delivered from a serial diluter, was maintained by overflowing the treated water through a standpipe fixed to the base of the chamber. All test aquaria were submersed in a water bath to maintain constant temperature of 21° C during the experiment. Continuous aeration was supplied to all test chambers with an oil-less compressor and monitored for dissolved oxygen levels (Model 54, YSI Inc., Yellow Springs, CO).

Toxicant was delivered using a six-concentration intermittent flow-through proportional serial diluter (Lemke et al. 1978) with a dilution factor of 0.50 between concentrations and a control. The diluter delivered 2 L of each concentration of the toxicant every 17 minutes. The flow of each concentration was split three ways for each of the three replicates. The four selenite treatment target concentrations used to determine the 96-h LC50 for walleyes were 25 mg/L, 12.5 mg/L, 6.25 mg/L, and a control. Reagent grade sodium selenite (Na_2SeO_3; Sigma Chemical Corporation) was used in the experiment. Sodium selenite was chosen because most of the published data on acute waterborne selenium toxicity is for the selenite form.

Walleyes were starved for 24 h before stocking in test chambers to reduce metabolic wastes (USEPA 1993). The toxicity test was begun by randomly distributing two walleye per test chamber, then repeating until all test chambers held eight walleyes. Walleyes were placed into the test chambers within 1 h of the chambers being filled with the toxicant. To minimize organic wastes, walleyes were not fed for the duration of the LC50 trial. Total lengths and weights of the walleyes were recorded at the conclusion of the experiment to assure that no size differences existed between treatments and to determine loading densities (total tank weight/tank volume). Mortality and behavior were observed every 24 h after initiation of the experiment and mortality was recorded and dead walleyes were removed at each observation period.

Water samples were collected in 500 ml polypropylene bottles and acidified with 5 ml concentrated nitric acid until pH was below 2 pending verification of selenium concentrations. Water samples were analyzed for selenium concentrations by a modified fluorometric method described by Olson et al. (1975) and Koh and Benson (1983). Other water quality parameters monitored were alkalinity, and total hardness (Model 16900, Hach, Loveland, CO), pH (Model EC10, Hach), conductivity (Model CO150, Hach), and temperature. Statistical analysis of the data to calculate the sodium selenite LC50 and 95% confidence limits for walleyes was done using probit analysis (SAS 1990).

RESULTS AND DISCUSSION

Water temperature was maintained at 21 °C, which is near the optimum water temperature of 22°C for juvenile walleye growth. Dissolved oxygen levels remained near saturation. Total alkalinity ($CaCO_3$) was 86 mg/L, total hardness ($CaCO_3$) was 380 mg/L, conductivity was 770 µmhosl/m, and pH was 7.8 for the duration of the experiment. Mean selenium concentrations were 1.6 µg/L (SE = 0.8; range = 0.7-3.2 µg/L) for the control, and 7.6 mg/L (SE = 0.2; range = 7.3-8.1 mg/L), 12.9 mg/L (SE = 0.4; range = 12.1-13.4 mg/L), and 25.7 mg/L(SE = 0.1; range = 25.6-25.9 mg/L) for the three supplemented treatments.

Loading densities for the test chambers were 1.2 g/L. Mortalities occurred after 9 h in the test chambers containing the highest selenium concentration; seven dead walleyes were observed in each of the three replicates. By 24 h, 100% mortality was achieved at the highest concentration. It was observed that before mortality took place, a slight discoloration occurred in the walleye, they would swim erratically, often spiraling, and would be found near the surface.

Using probit analysis in SAS (1990), it was determined that the LC50 occurred at 11.7 mg/L with 95% confidence intervals of 10.2-13.1 mg/L (Figure 1). Based on these data, juvenile walleye mortality during the 96-h experiment would be expected to reach 1% mortality at a concentration of 6.5 mg/L (95% confidence intervals, 3.1 to 8.2 mg/L). Ninety-nine percent of the mortality for 96 h would be expected at a concentration of 21.1 mg/L (95% confidence intervals, 17.0 to 40.6 mg/L).

Basic to assessment of the potential hazards of toxicants to aquatic biota is the principle that an evaluation of the hazard can be obtained by comparing the exposure concentration to the effect concentration (Kimerle et al. 1979). Maki (1979) determined that of thirteen hazard evaluation schemes, the ratio of LC50 to the estimated environmental concentration (EEC) was the most useful and objective decision criteria for assessing hazards.

The difference between LC50 and EEC is the margin of safety or the margin of uncertainty (Hamilton 1995). The LC50 will depend upon the species and the life stage tested, health of organism, genetic constitution, exposure route, and water quality parameters (Hamilton 1995). The EEC is dependent upon the test conditions such as water quality parameters, degradation, and mixing characteristics (Hamilton 1995). There are other factors which will influence the LC50 and the EEC which are not listed above. All of these factors will create variability in both the LC50 and EEC concentration used to determine the margin of safety.

Margins of safety less than 100 based on 96-h LC50 studies indicate a high potential for an environmental hazard, whereas margins greater than 1000 usually indicate low hazard potential (Hamilton 1995). In this study, the safety margin was found to be 1,113 for fingerling walleye when compared to the selenium EEC of 13 µg/L for the Cheyenne River at the Cherry Creek confluence. This low hazard ratio would suggest that it is safe to stock fingerling walleyes into the Cheyenne River. However, the stocking of walleye fry would need to be examined because it is expected that the LC50 for walleye fry would show that swim-up fry would be more sensitive to selenium than the more advanced

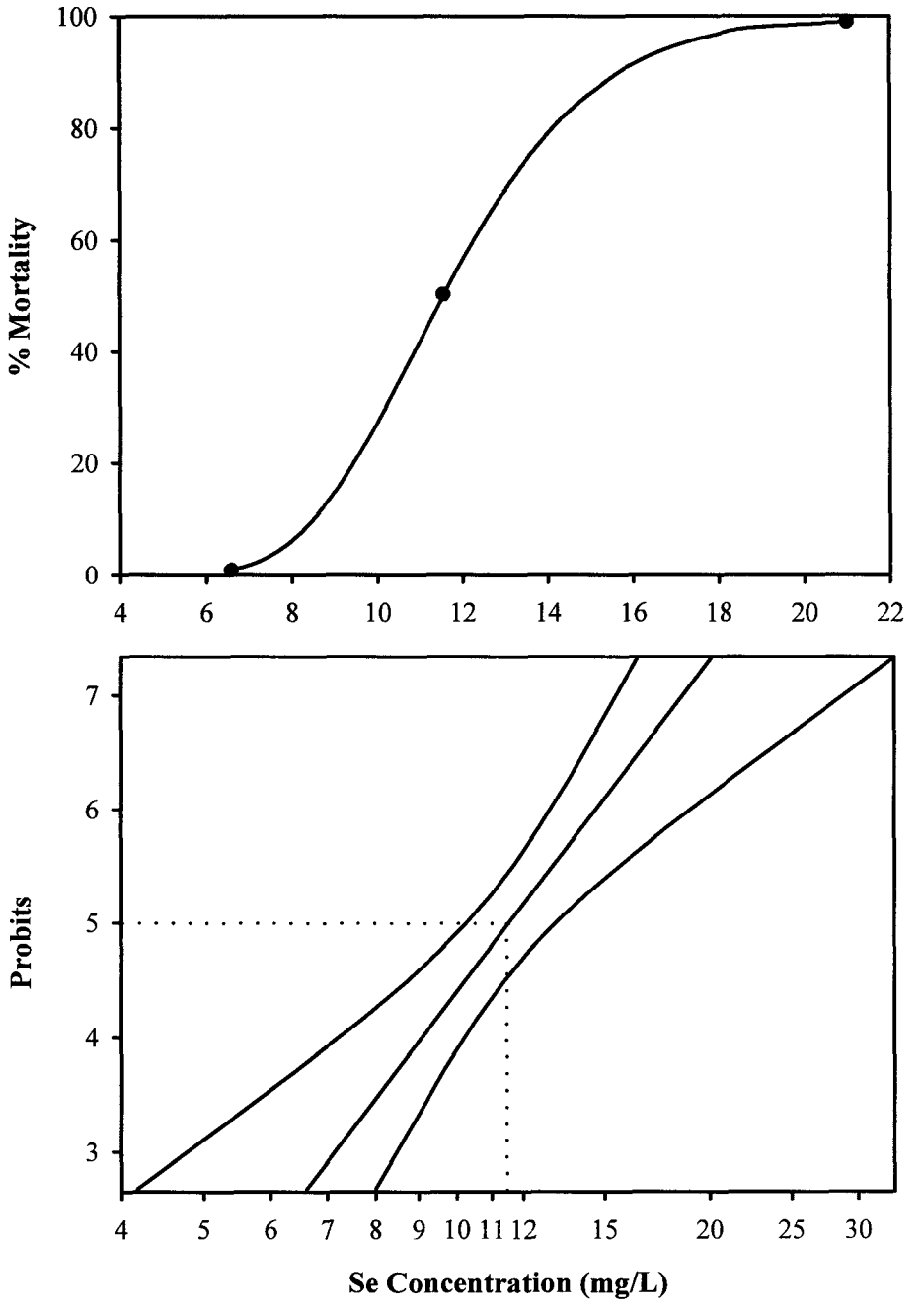

Figure 1. LC50 (96 h) for juvenile walleye as influenced by selenite. Top graph shows unaltered data whereas bottom graph shows same data analyzed by probit analysis with 95% confidence intervals. Data points in the top graph represent 1, 50 and 99% mortality at 6.5, 11.7 and 21.1 mg/L, respectively.

juvenile stage examined in this study. This difference in sensitivity is because both embryos and juveniles are typically less sensitive than are swim-up fry to selenium toxicity. Also, testing should be done over a range of temperatures for fingerling walleye, because the selenium effects are most pronounced at elevated temperatures (Klaverkamp et al. 1983). Lemly (1993) found combined dietary and waterborne selenium in conjunction with decreased water temperature (4°C) caused significant mortality in juvenile bluegills (*Lepomis macrochirus).* Lemly (1993) stated that seasonal metabolic changes in test organisms should be considered when assessing toxicity hazard potential.

Consideration should also be given to the fact that although waterborne selenium concentrations may seem to be within prescribed safety standards, selenium can be biologically magnified in food chains even at low water concentrations. The margin of safety for selenium between normal waterborne concentrations and those that lead to substantial bioaccumulation is extremely small (Lemly 1993). Biomagnification can affect growth, behavior, or reproduction leading to the elimination of organisms at the top of the trophic web (Lemly 1985). Also of concern when investigating toxic effects of selenium is that it will come in contact with other chemicals in the environment, possibly producing an antagonistic or synergistic effect.

Lemly (1985) stated that the dynamics of waterborne selenium uptake may mask the true toxic potential of selenium during the first few days of exposure such as a 96-h LC50 study. The kinetics of selenium in fish involves uptake and metabolism from the water and from the diet. The toxic responses from these two sources can be quite different, with each source contributing differently to the concentrations observed in different tissue samples (Hodson and Hilton 1983). Selenium uptake from water is very rapid for about 20 d, especially when present in sublethal concentrations (Lemly 1982). However, equilibrium concentrations in tissues are not likely reached for at least 60 d (Adams 1976; Lemly 1982). It has been hypothesized that during this time frame, selenium is being incorporated into selenoproteins (Lunde 1972). The time periods for waterborne selenium uptake and deposition to various tissues differs, thus acute toxicity tests may be the result of variable peripheral involvement of factors (i.e., superficial gill tissue, differential levels of immediate metabolic stress resulting from rapid physiological or chemical changes) and not reflect true interspecific sensitivity differences (Lemly 1985).

In freshwater organisms, approximately 36% of total selenium is present as selenate, the rest as selenite and selenide (Eisler 1985). Selenium can occur as its elemental state, although the most probable form is as an inorganic selenite because of its low redox potential. Selenite is generally more toxic to early life stages (Niimi and LaHam 1976) and at higher temperatures (Adams 1976). Most of the published data on acute selenium toxicity to fish is for the selenite form. Studies have determined that 96-h LC50 values range from 1 to 35 mg/L for fish species (e.g., Klaverkamp et al. 1983; Niimi and LaHam 1976; Sato et al. 1980). For coolwater fish species similar to walleye, Klaverkamp et al. (1983) report a 75.5-h LC50 of 11.1 mg/L for northern pike (*Esox lucius*) and a 10-d LC50 of 4.8 mg/L for yellow perch (*Perca flavescens*). Those published LC50s are similar to the walleye LC50 determined in this study, considering differences in the study protocols. The chronic effects of selenium might prove to be more important than the acute effects. For example, Crane et al. (1992) reported that approximately 50% of a yellow perch population disappeared during a 550-d pond experiments with selenium concentrations of 25 µg/L. This concentration (25 µg/L) also reduced yellow perch reproduction, but no clear effects were found at a selenium concentration of 10 µg/L.

From this study, the margin of safety for juvenile walleyes in the Cheyenne River is such that the selenium hazard is not critical. However, more research is warranted on the chronic and acute effects of elevated waterborne selenium on walleye. Minimally, different life stages and water temperatures need to be examined.

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