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Chronic Uranium Toxicity to White Sucker Fry (*Catostomus commersoni*)

K. Liber,[1] S. Stoughton,[1] A. Rosaasen[2]

[1] Toxicology Center, University of Saskatchewan, 44 Campus Drive, Saskatoon, SK, S7N 5B3, Canada
[2] Environment, Health and Safety Department, COGEMA Resources Inc., 817–825 45th Street West, Saskatoon, SK, S7K 3X5, Canada

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Large-scale uranium mining has taken place in northern Saskatchewan, Canada, for over 40 years. Despite this and the fact that surface water contamination from mine and mill effluents has occurred to varying extent over this entire period, relatively little is known about uranium toxicity to aquatic species inhabiting the associated environments. One of the more common fish species in these areas, especially those directly receiving effluent discharge, is white sucker (*Catostomus commersoni*).

A prior study designed to evaluate the hatching and development success of white sucker eggs collected from an impacted lake near the Cluff Lake uranium mine in northwestern Saskatchewan (de Rosemond and Liber 2002) provided the opportunity to evaluate the sensitivity of those fish to chronic uranium exposure. Excess eggs obtained for the hatching and development study were reared separately in the laboratory to the fry stage at which time they were used in the experiment described here. Ideally, fish would also have been obtained from an uncontaminated reference lake for comparison, but this could not be accomplished due to poor capture success of adults.

This study therefore provided an assessment of how white sucker fry that would be born downstream of uranium mining operations would respond to chronic uranium exposure. Since the adult fish used to obtain embryos came from such an environment, this experiment provides insight into how their offspring would tolerate chronic uranium exposure during the fry phase of their development.

MATERIALS AND METHODS

White sucker fry were reared at the Toxicology Centre, University of Saskatchewan. The original eggs were obtained from mature adult fish captured in the southern section of Island Lake at the Cluff Lake uranium mine in northern Saskatchewan, Canada, on June 2, 2002. The eggs were fertilized in the field using a dry fertilization method and the embryos transported to the laboratory that same day

Correspondence to: K. Liber

(arrived ~12 h post-fertilization). Upon arrival at the laboratory, eggs were placed in a 0.000075% provone-iodine solution (Betadine Surgical Scrub, Purdue Pharma, Pickering, ON, Canada) for 10-20 min to discourage fungal growth on the developing embryos. The embryos were hatched in the laboratory and reared in uncontaminated water collected from Cluff Lake (an uncontaminated reference lake at the Cluff Lake mine site) at a temperature of 14 ± 2 °C until used in the present experiment. Prior to use, the water was vacuum filtered through #4 Whatman® filter papers, 20-25 µm (Whatman International Ltd., Maidstone, England), to remove most suspended solids and biota, acclimated to test temperature (14 ± 1°C), and aerated overnight. Once they reached the fry stage, fish were fed daily with a combination of brine shrimp (*Artemia franciscana*) and Tetramin® aquarium fish food (Tetra, Melle, Germany). There was negligible mortality in the culture vessels and fry appeared to be in healthy condition.

On day 52 post-fertilization, healthy fry were arbitrarily removed from the holding vessels and transferred to test vessels for the present study. Test vessels (modified from the general design provided in Environment Canada 1998), consisted of specially designed 1-L polypropylene incubation chambers with screened-sides (1.5-mm mesh) situated inside 2-L high density polyethylene containers. All test vessels were individually aerated using 0.7 mm dia. Tygon® tubing and housed in a controlled environment chamber at 14 ± 1 °C with a 16:8 L:D photoperiod. Separate timer-operated lights created low intensity lighting for 30 min before and after the main lights turned on/off thus creating short dawn and dusk periods.

The experimental design consisted of five uranium treatments and an untreated control, each with four replicates. There were 10 fish per replicate. Every 24 to 48 h, fry were monitored and observations recorded on general behaviour, swimming behaviour, respiration, integument condition, and pigmentation, and dead fry were removed. Fish were fed a diet of newly hatched (~36 h old) brine shrimp (*Artemia franciscana*) and ground up Tetramin® fish food. The brine shrimp were hatched in Cluff Lake water with added aquarium salt (Nutrafin® freshwater aquarium salt, Rolf C. Hagen, Montreal, QC, Canada; final concentration: 30 g/L). Each test container received 2 ml of brine shrimp and approximately 0.25 g of Tetramin® twice daily.

Fish were exposed to one of five uranium concentrations spiked into the dilution water as $UO_2(NO_3)_2 \cdot 6H_2O$ (99+ % purity; Strem Chemicals Inc., Newburyport, MA, USA), or to untreated dilution water (control). The nominal uranium concentrations were 0.1, 0.4, 1.6, 6.4, and 25.6 mg U/L. The water volume in each exposure vessel was 1 L and the fish loading density never exceeded the Environment Canada (1998) threshold of 0.125 g/L/d. Control and test solutions were changed every 2 d over the 30-d test period by carefully transferring the screened inner container containing the

fish and a small volume of exposure solution to a clean 2-L container with 1 L of new test solution. Fish were never touched or completely removed from water during the 30-d test period.

At the time of test initiation on day 0, 40 randomly selected fish from the culture vessels were anaesthetized using MS-222 (tricaine methanesulphonate, Argent Chemical Laboratories, Redmond, WA, USA) and initial mean total length and dry weight determined (dried at 60 °C for 24 h). At test termination on d 30, all surviving fish were anaesthetized and their total length and dry weight determined as described above. Test endpoints consisted of survival, growth and behavioural observations. Growth data were compared using conventional one-way ANOVA followed by a Dunnett's test for pair-wise multiple comparisons to the control (α = 0.05).

Dissolved oxygen and temperature were measured in all test chambers before and after each water change with an Orion Dissolved Oxygen Meter Model 370 (Orion Research, Beverly, MA, USA). Samples for other routine water quality analyses were collected every 6 d, before and after water changes. Two replicates from each treatment were sampled and pH, alkalinity, hardness, and ammonia measured. pH was measured with an Orion PerpHecT LogR Meter Model 370 (Orion Research, Beverly, MA, USA), and ammonia was measured on a Beckman Coulter™ DU™ 640 spectrophotometer (Beckman Instruments Inc., Fullerton, CA, USA). Water hardness and alkalinity were measured with a Hach Digital Titrator Model 16900 (Hach Company, Loveland, Co, USA).

Water samples for uranium analysis were collected at the beginning (0 h) and end (48 h) of water changes on days 0-2, 8-10, 16-18, and 24-26, and then filtered through Nalgene® 0.45µm surfactant-free cellulose acetate membrane syringe filters into 8-mL HDPE bottles. Distilled nitric acid was added to all bottles to preserve samples which were stored at 4 °C until the time of analysis by ICP-MS in the Department of Geological Sciences, University of Saskatchewan. Samples were collected from three replicates per treatment at each sampling time, but only two of the four sets were analyzed for the lowest three treatments. Separate ICP-MS analysis of Cluff Lake water revealed no background contamination by any of the trace elements comprising the 56 element scan.

RESULTS AND DISCUSSION

General water quality throughout the 30-d experimental period remained within limits deemed acceptable for white sucker (Table 1). Total water hardness, alkalinity and pH increased marginally over the 2-d water renewal periods, but never by

consequential amounts. Total ammonia increased from below detection (<0.050 mg/L) to a mean of 0.84 mg/L over the 2-d water renewal periods. Measured uranium exposure concentrations were very close to nominal levels (Table 2). There were generally slight increases in uranium concentrations over the 2-d water renewal periods, likely the result of minor evaporation of dilution water.

Table 1. Summary of water quality (mean ± SE) measured at the beginning (new water) and end (old water) of 2-d water renewal periods.

Variable	New water	Old water	Overall mean [1]
Temperature (°C)	14.4 ± 0.1	14.1 ± 0.1	14.3 ± 0.2
Dissolved oxygen (mg/L)	8.9 ± 0.1	8.9 ± 0.1	8.9 ± 0.1
Alkalinity (mg/L as $CaCO_3$)	67 ± 2	70 ± 2	68 ± 3
Hardness (mg/L as $CaCO_3$)	72 ± 4	73 ± 2	72 ± 3
pH	7.8 ± 0.2	8.0 ± 0.0	7.9 ± 0.2
Ammonia (mg/L) [2]	BDL	0.84 ± 0.13	n.a.

[1] Mean ± SE calculated across all treatments and sampling periods (n = 360 for temperature and DO, and 60 for other variables).

[2] BDL = below detection limit (<0.050 mg/L). n.a. = not applicable.

Table 2. Uranium concentrations (mean ± SD) in water samples collected from the control and the five treatment groups at the beginning and end of selected 2-d water renewal periods. All data are in mg/L. [1]

Nominal concentration	New water	Old water	Overall mean
Control	0.001 ± 0.000	0.001 ± 0.000	0.001 ± 0.000
0.1	0.110 ± 0.016	0.125 ± 0.002	0.117 ± 0.015
0.4	0.445 ± 0.067	0.506 ± 0.016	0.476 ± 0.066
1.6	1.718 ± 0.145	1.886 ± 0.055	1.802 ± 0.159
6.4	7.146 ± 0.371	7.514 ± 0.129	7.330 ± 0.550
25.6	27.48 ± 0.83	28.25 ± 0.46	27.86 ± 1.31

[1] Sample size (n) = 6 for control to 1.6 mg/L treatments and 12 for 6.4 and 25.6 mg/L treatments. The detection limit for uranium was 0.00001 mg/L.

There were no significant effects on survival of white sucker fry, but the mean lengths and weights of fish in the highest test concentration were significantly lower than for controls (Table 3). The greatest difference was for weight which was

reduced by 25.6 %. Most of the fish in the highest treatment also exhibited general hyperexcitability and on one occasion erratic swimming behaviour. Unconsumed food was observed on the bottom of all replicate test containers in both the highest and second highest uranium treatments, but not in lower treatments. In addition, all fish in the two highest treatments were lighter in colour and pigmentation than control fish.

Overall, white sucker fry were relatively tolerant of chronic uranium exposure; even the highest test concentration (27.86 mg/L) failed to cause significant mortality over a 30-d exposure period. Significant sublethal effects were, however, observed at this concentration, but not at 7.33 mg/L (the no observed effect concentration).

Table 3. Final survival, total length and dry weight (mean ± SE) of white sucker (*C. commersoni*) fry exposed to different uranium concentrations for 30 d ($n = 4$).

Treatment (mg/L)	Survival (%)	Total length (mm)	Dry weight (mg)
Control	100 ± 0	24.8 ± 0.5	16.3 ± 1.0
0.1	100 ± 0	25.3 ± 0.4	16.8 ± 0.8
0.4	97.5 ± 5.0	25.5 ± 1.0	17.9 ± 1.3
1.6	100 ± 0	25.3 ± 0.8	16.6 ± 1.8
6.4	100 ± 0	25.4 ± 0.7	17.2 ± 1.7
25.6	90.0 ± 8.2	23.2 ± 0.5*	12.1 ± 0.6*

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

These observations are in reasonable agreement with other reported findings on uranium toxicity to fish, allowing for important differences in test duration and water hardness, the latter of which averaged 72 mg/L as $CaCO_3$ in the present study. For example, Hamilton (1995) reported 96-h LC50 values for swim-up fry and juveniles of three small fish species (Colorado squawfish, *Ptychocheilus lucius*, razorback sucker, *Xyrauchen texanus*, and bonytail, *Gila elegans*) from the Green River, Utah, USA, to all be 46 mg/L at a mean water hardness of 196 ± 5 mg/L as $CaCO_3$. Poston et al. (1984) observed only 16.7 % mortality of juvenile fathead minnow, *Pimephales promelas*, at 100 mg/L uranium in a 96-h static test with a water hardness of 66-73 mg/L $CaCO_3$ equivalent. Tarzwell and Henderson (1960) showed the importance of water hardness in the expression of uranium toxicity by reporting 96-h LC50s for fathead minnow of 2.8 mg/L in soft water (20 mg/L) and 135 mg/L in hard water (400 mg/L). Similarly, Parkhurst et al. (1984) reported 96-h LC50s for juvenile brook trout, *Salvelinus fontinalis*, of 5.5 and 23 mg/L in soft (35 mg/L) and hard (208 mg/L) water, respectively. Somewhat lower toxicity values (LC50s ranging from 0.7 to 3.5 mg/L) have been reported for tropical freshwater fishes from northern

Australia in very soft water (Bywater et al. 1991; Holdway 1992). None of these published studies evaluated effects on growth after a longer exposure duration (e.g., 30 d).

It should be noted that the fry used in this study came from adult fish that had been collected from a lake with known contamination (including uranium levels over the previous five years as high as 0.2-0.4 mg/L) from mining operations at the Cluff Lake uranium mine. Therefore, it is conceivable that the adult fish had over the years developed some tolerance to uranium contamination and that this tolerance was passed on to the fry (although these were reared in clean water from the moment of fertilization). It is, however, unlikely that this would have substantially altered their susceptibility relative to fry obtained from previously unexposed adults. This hypothesis is based on the observation that both lake trout (*S. namaycush*) and northern pike (*Exos lucius*) fry obtained from adults from uncontaminated lakes displayed very comparable toxicity responses over much longer (141 d and 65 d, respectively) exposure periods (Liber et al. 2004a,b). Both the lake trout and northern pike studies also revealed that the hatching process of the fish early life cycle was the most sensitive stage to uranium toxicity, so it is possible that hatching of white sucker embryos could have been affected at a concentration somewhat lower than the one observed here to impact growth of fry.

Considering that the highest uranium concentrations routinely measured immediately downstream of discharges from the Cluff Lake and other northern Saskatchewan uranium mines are generally substantially below 1 mg/L (and much lower outside of the immediate effluent dilution zones), it is unlikely that exposure to such uranium concentrations would adversely affect white sucker fry survival and growth in the field, even over longer exposure periods.

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