Bull Environ Contam Toxicol (2012) 89:296–301
DOI 10.1007/s00128-012-0673-0

Toxicity of Chloride Under Winter Low-Flow Conditions in an Urban Watershed in Central Missouri, USA

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Received: 23 February 2012 / Accepted: 3 May 2012 / Published online: 23 May 2012

Abstract Deicers such as sodium chloride and calcium chloride are used to treat snow and ice on road surfaces and have been identified as potential stressors on aquatic life. Hinkson Creek is an urban stream on the Missouri 303(d) list of impaired waters and is classified as impaired due to urban non-point source pollution. A 7-day toxicity test using *Ceriodaphnia dubia* was conducted to assess the toxicity of stream water during snowmelt at seven sites within the Hinkson Creek watershed. Chloride concentrations at two sites (Site 6, 1252 mg Cl/L; Site 4, 301 mg Cl/L) exceeded the U.S. Environmental Protection Agency chronic criterion (230 mg Cl/L). Survival (30 %) and total reproduction (6.9 young/adult) of *C. dubia* at Site 6 was significantly lower than survival (100 %) and total reproduction (30.4 young/adult) at Site 1 (reference site). Results indicate that chloride concentrations are elevated above water-quality criteria and that chloride may be a significant chemical stressor for macroinvertebrate communities during winter low-flow conditions in the Hinkson Creek watershed.

Keywords Urban streams · Snowmelt · Chloride toxicity · *Ceriodaphnia dubia*

Deicers such as sodium chloride (NaCl) and calcium chloride ($CaCl_2$) are used to treat snow and ice on impervious surfaces in northern temperate zones for road safety (Corsi et al. 2010; Daley et al. 2009; USEPA 1988). Chloride concentrations in urban streams have been found to increase steadily through winter months, typically spiking during snowmelt and low-flow conditions (Corsi et al. 2010; Kelly et al. 2010). Long-term exposure to elevated chloride concentrations can cause a range of effects such as mortality, growth abnormalities, and reproductive failure in fishes and invertebrates, with possible changes in community structure (Benbow and Merrit 2004; Corsi et al. 2010 and references therein; Health Canada 1999; Hecnar and Sanzo 2006).

In 1996, the Missouri Department of Natural Resources (MDNR) placed a 10-km segment of Hinkson Creek on the Missouri 303(d) list of impaired waters due to measured impacts on macroinvertebrates related to "unknown pollutants" associated with non-point source stormwater under peak-flow conditions (USEPA 2011). The Hinkson Creek Total Maximum Daily Loads document calls for a 40 % reduction in stormwater runoff to minimize the effects of possible stressors including metals, pesticides, polycyclic aromatic hydrocarbons, and chlorides (MDNR 2002–2004, 2004–2005, 2005–2006; USEPA 2011). However, we hypothesize that the maximum exposure to chemical stressors occurs under low-flow conditions. This study was conducted to assess the toxicity of stream water to the

All procedures conformed to the USGS guidelines for the humane treatment of test organisms during culture and experimentation. Use of trade names does not constitute USGS or U.S. Government endorsement.

Electronic supplementary material The online version of this article (doi:10.1007/s00128-012-0673-0) contains supplementary material, which is available to authorized users.

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survival and reproduction of *Ceriodaphnia dubia* during low-flow snowmelt conditions in the Hinkson Creek watershed.

Methods and Materials

Hinkson Creek flows about 42 km in a southwesterly direction through the City of Columbia in Boone County, Missouri, USA. Seven sites were selected within a 22-km segment of watershed (Fig. 1). Site 1 was selected as a reference site and was located upstream of the urbanized and impaired section of Hinkson Creek. Site 4 was located in the Grindstone Creek tributary, where rapid urban development is occurring. Site 6 was located in the Flat Branch tributary, which is heavily urbanized and receives the greatest volume of stormwater runoff from the downtown business district. Sites 2, 3, 5, and 7 were located on the mainstem of Hinkson Creek. A 7-day *C. dubia* survival and reproduction toxicity test was conducted according to standard protocols (USEPA 2002) at the U.S. Geological Survey Columbia Environmental Research Center (CERC). Test water was collected daily at seven stream sites after a major (>42 cm) snow event from January 24–February 1, 2011. Daily discharge (0.14–0.71 cubic meter per sec) in Hinkson Creek over the 7-day test period was approximately the same as the 24-year medium daily discharge (USGS 2011). In situ water quality (e.g., temperature, pH, specific conductance, dissolved oxygen, and turbidity) was measured daily during the 7-day toxicity test with a Hydrolab® Quanta meter.

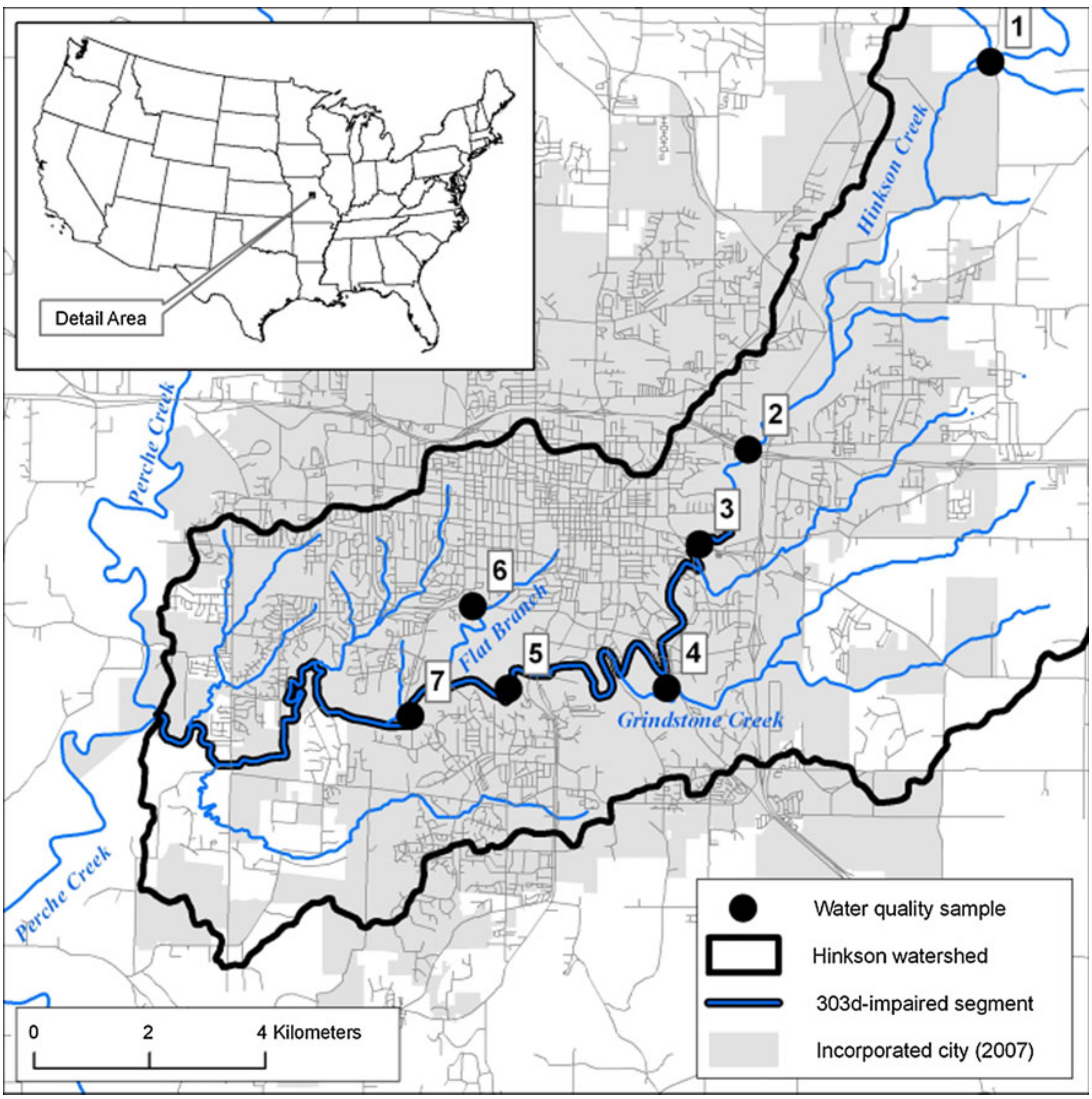

Fig. 1 Map of study area with sites in Hinkson Creek watershed, Missouri, USA

Prior to testing, *C. dubia* were individually maintained in 25-ml polycarbonate beakers containing 15-ml CERC well water (pH = 7.7; alkalinity = 254 $CaCO_3$ mg/L; hardness = 286 $CaCO_3$ mg/L; chloride = 23 mg Cl/L) at 25 ± 1°C for approximately 6 weeks to document reproductive fitness of brood females (USEPA 2002). We tested water from seven stream sites; three reference concentrations of NaCl mixed into CERC well water (0.63 g NaCl/L; 1.25 g NaCl/L; 2.5 g NaCl/L), and a control water (CERC well water). Test waters were placed in 30-ml borosilicate glass test beakers in a waterbath held at the nominal test temperature (25 ± 1°C) for 24 h prior to being used. At the start of the test, each test beaker contained 15-ml of test water; one < 12-h old neonate; and 0.1 ml of a mixture of yeast-trout chow-cerophyll (YTC; 1.7–1.9 g solids/ml; USEPA 2002) and 0.1 ml of *Selenastrum capricornutum* (1×10^6 cells/ml; Aquatic Biosystems, Fort Collins, Colorado, USA). Ten replicates were tested for each stream water, NaCl solution, and CERC well water. After daily observations were made, *C. dubia* were transferred to beakers containing fresh test water with YTC and *S. capricornutum*. Endpoints measured daily were lethality (absence of movement) and reproduction (number of neonates or young produced per adult). Dissolved oxygen and pH were measured daily in discarded test-chamber water (i.e., final water quality) and dissolved oxygen, pH, and specific conductance were measured daily in renewal water (i.e., initial water quality; USEPA 2002). Alkalinity and hardness were measured by titration daily during the study (APHA 2005). Chloride concentrations were determined in at least one sample of water collected from stream sites during the 7-day test using a Hach® DR2000 spectrophotometer (Method 70) and verified with a Dionex® integrated ion chromatography machine (Model 1100). Detection and recovery of all reference standards were within 20 % of nominal concentrations; therefore, none of the sample results were corrected for recovery.

Data was analyzed using the Statistical Analysis System (SAS) to determine if data were normally distributed using the Shapiro–Wilk's Statistic (SAS 2008). The data were not normally distributed. Survival and reproduction data were evaluated using analysis-of-variance based on rank-transformed data (USEPA 2002). In the analyses, mean survival and mean total reproduction for *C. dubia* over the 7-day test (including dead individuals) were the dependent variables. Experiment-wise error rates for all pairwise comparisons were controlled at $\alpha = 0.05$ with Tukey's method (Hochberg and Tamhane 1987). Specific conductance is widely used for estimating the total dissolved solids content of water which includes ions such as sodium, chloride, sulfate, calcium, and magnesium. Correlation (Spearman) analysis was conducted to confirm the association of specific conductance and chloride concentration in field-collected and laboratory reference water.

Results and Discussion

Mean chloride concentrations at Sites 3, 5, 6, and 7 were significantly greater than at the reference site (Table 1).

Chloride concentrations at Site 4 (301 mg Cl/L) and Site 6 (1,252 mg Cl/L) were 1.4- to 4-times greater than the U.S. Environmental Protection Agency (USEPA) chronic criterion for chloride (230 mg Cl/L; USEPA 1988) and 2- to 8-times greater than the recommended Canadian 30-day average concentration (150 mg Cl/L; Nagpal et al. 2003). Mean specific conductance at Sites 2–7 (Table 1) and NaCl solutions (Supplemental Table S1) were significantly greater than Site 1 or CERC well water. Specific conductance and chloride concentrations varied the most at

Table 1 Mean values ± standard deviation of water-quality variables for stream sites during 7-day toxicity test

Site	N[a]	In situ					N[a]			N[a]	
		Temperature (°C)	pH	Specific conductance (mS/cm)	Dissolved oxygen (mg/L)	Turbidity (NTU)		Alkalinity (mg $CaCO_3$/L)	Hardness (mg $CaCO_3$/L)		Chloride (mg/L)
1	8	0.1 ± 0.1	8.1 ± 0.5	0.5 ± 0.0	16.4 ± 1.4	8.0 ± 2.0	10	143 ± 5	229 ± 12	4	21 ± 2
2	8	0.1 ± 0.1	8.3 ± 0.3	1.0* ± 0.1	16.9 ± 0.6	6.8 ± 1.9	9	163 ± 10	312* ± 15	5	90 ± 59
3	8	0.1 ± 0.1	8.3 ± 0.4	1.2* ± 0.2	17.5 ± 0.7	7.2 ± 2.0	13	163 ± 12	333* ± 20	4	139* ± 98
4	7	0.2 ± 0.2	8.4 ± 0.2	1.2* ± 0.1	17.4 ± 0.9	8.1 ± 2.7	8	201* ± 11	337* ± 16	1	301 ± –
5	8	0.2 ± 0.1	8.4 ± 0.2	1.2* ± 0.2	16.6 ± 0.6	7.1 ± 1.0	9	178* ± 14	332* ± 14	4	119* ± 79
6	7	0.8* ± 0.7	8.1 ± 0.3	3.7* ± 1.5	16.0 ± 0.9	15 ± 10	7	173* ± 28	362* ± 70	2	1252* ± 981
7	8	0.2 ± 0.2	8.4 ± 0.6	1.4* ± 0.1	16.6 ± 0.5	7.6 ± 1.0	11	179* ± 14	330*[b] ± 16	4	158* ± 121

* $p < 0.05$ compared to Site 1

[a] N = Number of samples

[b] N = 10

Table 2 Mean ± standard deviation for survival and number of young per adult *Ceriodaphnia dubia* during 7-day toxicity test

Site/test water	No. young per adult replicate										No. live adults	Mean ± standard deviation young per adult
	1	2	3	4	5	6	7	8	9	10		
1	35	29	27	31	29	33	30	35	26	29	10	30.4 ± 3.1
2	27	30	36	37	38	36	29	40	29	31	10	33.3 ± 4.6
3	36	33	43	–	42	37	46	34	–	39	8[a]	38.5 ± 4.8*
4	35	35	37	32	36	39	33	43	0	40	9	33.3 ± 6.7
5	39	22	38	48	0	21	42	38	38	0	10	28.6 ± 17.2
6	0	0	0	0	0	0	14	6	0	2	3**	2.20 ± 4.57**
7	31	18	35	36	35	38	33	29	37	34	10	33.3 ± 12.1
0.63[b]	10	26	12	20	29	28	0	0	9	19	8	15.3 ± 10.8
1.25[b]	0	24	0	22	0	20	0	0	0	3	6	6.90 ± 10.50**
2.50[b]	0	0	0	0	0	0	0	0	0	0	0**	0.00 ± 0.00**
Well[c]	27	29	17	8	41	28	28	0	30	26	10	26.0 ± 9.1

* $p < 0.05$ compared to Columbia Environmental Research Center (CERC) well water

** $p < 0.05$ compared to Site 1 and CERC well water

[a] Two individuals were killed during transferred between beakers; mean young per adult calculated with n = 8

[b] mg NaCl/L mixed into CERC well water

[c] Well = CERC well water

Site 6 (Table 1). Mean hardness of Sites 3–7 were significantly greater than Site 1; however, all test waters were classified as moderately hard water (Table 1 and Supplemental Table S2). Mean specific conductance in stream waters was significantly correlated with mean chloride concentrations (r = 0.89) and mean hardness (r = 0.75), but not mean alkalinity (r = 0.43), indicating specific conductance was a good surrogate measure for chloride. Mean values of initial or final pH and initial or final dissolved oxygen of all test waters during the 7-day toxicity test were not significantly different from Site 1 or CERC well water (Supplemental Table S1).

Survival of *C. dubia* was significantly lower at Site 6 (30 %) and the 2.50 mg Cl/L solution (0 %) than the reference site (100 %) or CERC well water (100 %; Table 2). Mean total reproduction of *C. dubia* in water from Site 6 (2.20 young/adult), 1.25 mg NaCl/L solution (6.90 young/adult), and 2.5 mg NaCl/L solution (0 young/adult) were significantly lower than Site 1 (30.4 young/adult) and CERC well water (26.0 young/adult; Table 2).

Comparison of the response distributions of *C. dubia* exposed to field-collected stream samples and the NaCl solutions gave similar results, indicating that chloride was the likely toxicant of concern (Fig. 2). Mean survival and total reproduction of *C. dubia* decreased sharply above the USEPA chronic chloride criterion despite moderately high hardness in test waters (Elphick et al. 2011; Iowa DNR 2009; USEPA 1988).

Measured chloride concentrations in the Hinkson Creek watershed are approximately 20- to 100-times greater than

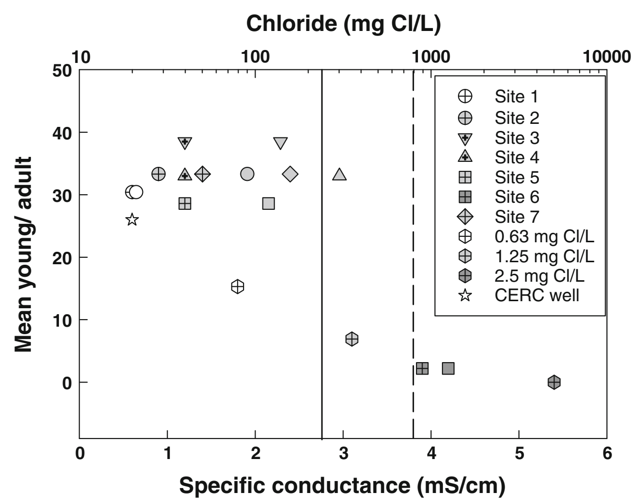

Fig. 2 Mean number of young per adult in relationship to specific conductance (*hatched symbols*) or chloride concentrations (*open symbols*). Lines represent U.S. Environmental Protection Agency chronic (*solid*) and acute (*dashed*) chloride criteria

reference streams within the same Ecological Drainage Unit during low flow despite the removal of a Missouri Department of Transportation salt facility (Fig. 3; MDNR 2004–2005). Chloride concentrations at three sites in this and previous studies were greater than the Canadian 30-day average and the USEPA chronic chloride criterion (Fig. 3; MDNR 2002–2004, 2004–2005, 2005–2006; Nagpal et al. 2003; USEPA 1988). This study is the first to report toxicity and reproductive impairment of *C. dubia* exposed to water from Flat Branch (Site 6). Chloride concentration at

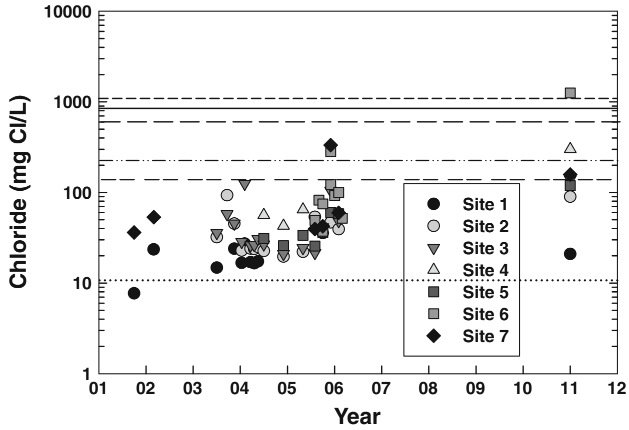

Fig. 3 Chloride concentrations within Hinkson Creek watershed from 2001–2011; data from this study and MDNR (2002–2004, 2004–2005, 2005–2006). Lines represent (1) mean chloride concentration of reference streams within the Ecological Drainage Unit (*dotted*; MDNR 2002–2004, 2004–2005, 2005–2006); (2) recommended Canadian 30-day average criteria (medium–medium dashes; Nagpal et al. 2003); (3) U.S. Environmental Protection Agency (USEPA) chronic criteria (*dash dot* dot; USEPA 1988); (4) recommended Canadian maximum (long dash; Nagpal et al. 2003); (5) USEPA acute criteria (*solid*; USEPA 1988); and (6) acute 48-h median lethal concentration (LC_{50}) toxicity value for NaCl (short dash; Mount et al. 1997)

Site 6 (1252 mg Cl/L) was 5-times greater than previously reported for the watershed and were greater than the USEPA acute chloride criterion (860 mg Cl/L), the recommended Canadian maximum chloride concentration (600 mg Cl/L), and the acute 48-h median lethal concentration (LC_{50}) toxicity value for chloride (1189 mg Cl/L; MDNR 2002–2004, 2004–2005, 2005–2006; Mount et al. 1997; USEPA 1988). The percentage of sites exceeding the USEPA chronic (43 %) and acute (14 %) criteria in winter months is comparable to other northern states (Corsi et al. 2010).

Previous data indicated that Sites 1, 3, 5, and 7 were only partially sustaining of macroinvertebrate communities for one or more sampling periods (MDNR 2002–2004, 2004–2005, 2005–2006). We did not see any effects on survival or reproduction of *C. dubia* in water from these sites during our toxicity test; however, chloride concentrations at Sites 3, 5, and 7 exceeded several chloride criteria. These data indicate that current deicing practices within the Hinkson Creek watershed are likely contributing to chloride concentrations in the watershed and that chloride may be a significant chemical stressor during winter low-flow conditions in the Hinkson Creek watershed. Additional monitoring of chloride concentrations under variable stream flows is needed to investigate whether or not chloride is having adverse effects on macroinvertebrate communities in the Hinkson Creek watershed.

Acknowledgments L. Johnson and S. Olson assisted with the field collections, laboratory testing, and manuscript. M. Stuckhoff developed Fig. 1. This manuscript has been reviewed in accordance with U.S. Geological Survey (USGS) policy.

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