Bull. Environ. Contam. Toxicol. (1998) 60:312-317

Bulletin of Environmental Contamination and Toxicology

Lithium Chloride: A Flow-Through Embryo-Larval Toxicity Test with the Fathead Minnow, *Pimephales promelas* Rafinesque

K. E. Long,[1] R. P. Brown, Jr.,[2] K. B. Woodburn[1]

[1] Health and Environmental Research Laboratories, The Dow Chemical Company, Midland, Michigan 48674, USA
[2] Exxon Biomedical Sciences, Mettler Road, CN 2350, East Millstone, New Jersey 08875-2350, USA

Received: 15 August 1997/Accepted: 10 November 1997

Lithium is a Group IA alkali metal on the periodic table with many industrial uses, ranging from medical applications to aerospace technology. It is the least reactive alkali metal, and its outermost electron is so heavily shielded from the attractive force of the nucleus that the ionization energy (to Li^+) is quite low; the element occurs as the ^6Li (7%) and ^7Li (93%) isotopes (Heslop and Jones 1976). Lithium comprises 0.0065% of the earth's lithosphere and may be found in aluminosilicates such as petalite ($LiNaAlSi_4O_{10}$) and spodumene ($LiAl(SiO_3)_2$). Lithium does not react readily with oxygen to form the oxide Li_2O below 100 °C but is highly reactive with nitrogen, even at room temperature, to yield Li_3N (Heslop and Jones 1976).

Lithium is used in high-energy batteries, as a strong reducing agent, is added to aluminum, magnesium, and zinc alloys to increase hardness, and is a useful catalyst in the polyolefin plastics industry (Seiler et al. 1988). ^6Li is used in regulator rods in thermonuclear reactors and lithium hydroxide (LiOH) and Li_2O are used to absorb carbon dioxide and regenerate atmospheres in submarines and spacecraft. Lithium also finds use in the medical industry because of its pharmaceutical applications (Rayner-Canham 1996).

In nature, lithium is normally found associated with sedimentary deposits such as clay and shale (Seiler et al. 1988). Aqueous-phase concentrations of lithium in the Great Lakes from 1980-1985 varied from 0.64 µg/L (ppb) in Lake Superior to 2.4 ppb in Lake Ontario (Rossmann and Barres 1988).

Lithium is considered slightly toxic to aquatic organisms, with a measured 96-hr LC_{50} in fathead minnows of 42 mg/L and a no-observed effect concentration (NOEC) of 13 mg/L (*Dow Report ES-2340, 1991*). The 48-hr EC_{50} for immobility with the water flea (*Daphnia magna*) was 24 mg/L and the NOEC was 11 mg/L(*Dow Report ES-2341, 1991).* High concentrations (10 mM) of lithium ion

Correspondence to: K. B. Woodburn

can inhibit the growth of bacterial cells; Inaba et al. (1993) have shown that such levels of Li^+ may affect pyruvate kinase enzyme activity in *Escherichia coli* bacteria.

In conjunction with production and use, lithium salts may be discharged with industrial effluent waste water. The objective of this study was to evaluate the chronic toxicity of lithium chloride to the early life stages of the fathead minnow, *Pimephales promelas* Rafinesque, following continuous exposure to water containing the test material. Data generated may then be used to evaluate and determine appropriate discharge levels for lithium chloride resulting from pharmaceutical or industrial processes.

MATERIALS AND METHODS

The study was conducted in accordance with the procedures formulated by the U.S. Environmental Protection Agency (EPA) and the American Society for Testing and Materials or ASTM (ASTM 1992; US EPA 1986). This study was designed and conducted in general accordance with U.S. EPA Good Laboratory Practices Standards (US EPA 1989). The testing was conducted at the Health and Environmental Research Laboratories of The Dow Chemical Company in Midland, Michigan. The sample of lithium chloride was obtained from Fisher Scientific Company (Pittsburgh, PA).

Laboratory water for the experiment was obtained from the upper Saginaw Bay of Lake Huron off Whitestone Point and was limed and flocculated with ferric chloride by the City of Midland to reduce its hardness. Prior to its use in the laboratory, the water was sand-filtered, pH-adjusted with CO_2, carbon-filtered, and UV irradiated. The laboratory water was monitored weekly for pH, alkalinity, conductivity, and hardness.

The test organism was the fathead minnow, *Pimephales promelas* Rafinesque. Fathead minnow embryos less than 24 hours old were obtained from a commercial supplier (Aquatic Biosystems, Inc., Fort Collins, Colorado). Prior to test initiation, the embryos were gently removed from the shipping container and pooled in a large glass dish. The embryos were inspected using a stereo-microscope and those that appeared abnormal or fungus-infected were discarded. Normal appearing embryos were used to set the test.

An intermittent-flow proportional diluter system designed to provide up to six test concentrations and a laboratory water control was used during the study. The diluter was calibrated so that the concentration of the test substance in each treatment was approximately 60 percent of that in the next higher treatment level. The temperature of the water in the trough was controlled by an electric temperature controller set to maintain a temperature of 25 ± 1 °C. Diluter and laboratory lighting provided a 16h light/8 h dark transitional photoperiod during

testing. The diluter was calibrated prior to the definitive study. During the test, the diluter provided at least six volume turnovers in each test vessel during a 24-h period.

Each glass test aquarium measured approximately 15 x 10 x 9 cm, had a 243 µm Nitex® screen-covered drain guard, and held a test volume of approximately 750 mL. Embryos were incubated in circular glass cups with 243 µm Nitex® plastic screen bottoms. The incubation cups were suspended in a cylindrical glass chamber which were supported by glass beads in the test vessel. Flow from the delivery tubes was directed into the cup to produce an intermittent flow of water around the embryos during the incubation period.

Nominal exposure concentrations were set at 1.2, 1.9, 3.2, 5.4, 9.0, and 15.0 mg/L, but chemical analysis of the test solutions was not conducted. Groups of fifty embryos were exposed to each treatment level and water control (25 embryos/replicate, 2 replicates per concentration). The test was initiated by distributing 25 embryos to each incubation cup. Once each cup contained 25 embryos, they were randomly distributed to the test vessels. Test concentrations were prepared by the proportional diluter system described earlier.

The embryos/larvae were observed and counted daily through day 5 of the study; dead or fungus-infected embryos/larvae were removed at each observation. On day 2, embryos were thinned down to 20 per replicate, as per guideline recommendation (ASTM 1992; US EPA 1986). Prior to thinning, the percent survival was calculated for each replicate. Dead or deformed larvae were subtracted from the total number of larvae to determine the number of normal larvae at hatch. The percent of embryos that hatched and the day-to-mean hatch for each replicate were recorded.

Larvae were observed every Monday, Wednesday, and Friday until test termination, with mortality and sublethal effects recorded; dead larvae were removed. The test continued for 22 days post day-to-mean hatch of the controls. At test termination, all surviving fish were sacrificed for weight and length measurements using tricaine methanesulfonate for euthanization. Before weighing the organisms, 5 larvae from each replicate were randomly measured; an average was then taken of both replicates. To determine the dry weight of the organisms, Nalgene™ Centrifuge Ware Organism Weight tubes were initially dried in a 60 °C oven for three days, then cooled and weighed. A maximum of five fish per dose level were placed in each tube, which was then placed in the 60 °C oven for 48 hours. The tubes were then removed, cooled, and weighed, and the average dried weight per fish in each tube was calculated.

Dissolved oxygen, pH and temperature data were recorded on days 0, 4, and weekly thereafter in each test and control vessel. Temperature was continuously monitored from one test vessel throughout the study using a chart recorder.

Loading did not exceed 0.5 g fish per liter of test solution in any 24-hour period and did not exceed 5 g/L at any time. Water quality parameters such as alkalinity, hardness, pH, and conductivity were measured on days 0, 4 and weekly thereafter from one control and one 15.0 mg/L exposure vessel.

Fish (post-hatch) were fed a diet of green algae, (*Ankistrodesmus convolutus* Corda) and newly hatched (less than 24-h old) brine shrimp, (*Artemia salina*), two to five times a day with at least 2 hours between feedings. The brine shrimp supplemented the green algae diet after approximately 1-2 days post-hatch.

The percent of embryos that hatched, normal larvae at hatch, day-to-mean hatch, percent survival to thinning, post-thinning survival, and overall survival were calculated. A computer program was used to calculate the EC_{50} and LC_{50} values and corresponding 95% confidence intervals. The program used two methods for this study: moving average angle analysis (Thompson 1982), and binomial probability/non-linear interpolation (Johnson 1969). The moving average methods calculates both the estimated EC_{50}/LC_{50} value and its confidence interval. The binomial method calculates only the confidence interval, while a point estimate of the EC_{50}/LC_{50} is obtained using non-linear interpolation, i.e., log transformation of the concentration and angle transformation of the number dead. The appropriateness of a given method is determined in the program by the concentration-response data, e.g., the number of concentrations resulting in adverse effects between 0 and 100 percent. The growth (length and weight) data was statistically evaluated by ANOVA at p=0.05, to determine the no-observed effect concentration (NOEC), lowest-observed effect concentration (LOEC), and maximum-acceptable toxicant concentration (MATC). The difference between groups was determined by the method of least significant differences.

RESULTS AND DISCUSSION

Over the course of the study, all water quality parameters were within the required limits. The dissolved oxygen ranged from 6.8 to 8.6 mg/L and remained >84% saturation during the entire exposure period. The temperature ranged from 24.4-25.0 °C, pH from 7.2-7.5, hardness from 56-70 mg/L as $CaCO_3$, and conductivity from 160-270 µmhos/cm.

The percent survival for the control larvae at the end of the study was 97.5%, within the range specified in the guidelines. The 26-day fathead minnow larvae LC_{50} for lithium chloride of 8.7 mg/L (95% confidence interval 5.4-15.0 mg/L) was calculated using the binomial method (Thompson 1982) and was based on percent survival post hatch at study termination (see Table 1). The moving average technique (Johnson 1969) was used to compute the EC_{50} value, based on the percent of normal larvae at study termination (see Table 1); the EC_{50} value was 6.4 mg/L (95% confidence interval 5.68-7.36 mg/L). Sublethal effects were noted in all concentrations greater than 5.4 mg/L of lithium chloride or 0.89 mg/L of

Table 1. Fathead minnow percent survival and day-to-mean hatch.

Concentration of LiCl (mg/L)	% Survival Pre-Thinning	Day-to-Mean Hatch	% Hatch	% Normal Hatch	% Survival Post Hatch	% Normal Survived Post Hatch
Water Control	98.0	Day 3	100	100	97.5	97.5
1.2	98.0	Day 3	100	100	92.5	92.5
1.9	98.0	Day 3	95.0	100	92.5	92.5
3.2	100	Day 3	100	100	87.5	87.5
5.4	98.0	Day 3	100	100	82.5	82.5
9.0	94.0	Day 4	100	75.0	47.5	27.5
15.0	100	Day 4	95.0	0	0	0

lithium ion (based on the 16.4% proportion of lithium's atomic weight of LiCl). The data concerning the percent survival pre-thinning, day-to-mean hatch, percent hatch, percent of normal hatch, percent survival post hatch, and percent normal survival post hatch results are presented in Table 1.

There was a minimal difference in the number of larvae that survived (%survival post hatch) to the end of the 26-day exposure period (22-23 days post-hatch) and those that were normal at the end of the study (%normal survived post hatch). This suggests that when exposed to lithium, the fathead minnow larvae were either lethally affected or not affected at all after crossing an exposure time threshold.

Effects on growth (length and dry weight) were statistically analyzed using ANOVA. The LOEC for length was 1.9 mg/L and for dry weight was 9.0 mg/L (see Table 2). Based on these results, the most sensitive endpoint was length. Using length, the NOEC for lithium chloride was 1.2 mg/L the LOEC was 1.9

Table 2. Summary of fathead minnow mean lengths and weights on Day 26.

Nominal Concentration of LiCl (mg/L)	Mean Length (mm)	Standard Deviation	Mean Weight (mg)	Standard Deviation
Water Control	13.1	0.9	3.7	0.4
1.2	12.8	0.8	3.9	0.7
1.9	12.1*	0.7	3.3	0.5
3.2	11.9*	1.5	3.3	0.6
5.4	11.0*	0.9	3.0	0.4
9.0	11.4*	1.2	2.2**	1.1

* Significantly different if mean <12.2 mm

**Significantly different if mean <2.4 mg

Table 3. Summary of lithium ion and lithium chloride effect concentrations,

Endpoint	Lithium Ion Concentration (mg/L)	Lithium Chloride Concentration (mg/L)
LC_{50}	1.4	8.7
EC_{50}	1.0	6.4
NOEC	0.20	1.2
LOEC	0.31	1.9
MATC	0.25	1.5

mg/L, and the MATC was 1.5 mg/L. Effect concentrations may be calculated for lithium ion (16.4% of total atomic weight): the EC_{50} value for lithium ion (Li^+) was 1.0 mg/L; the LC_{50} was 1.2 mg/L; the NOEC was 0.20 mg/L; the LOEC was 0.31 mg/L; and the MATC was 0.25 mg/L. The endpoint concentrations for either LiCl or Li^+ are presented in Table 3.

REFERENCES

American Society for Testing and Materials (1992) Standard guide for conducting earl life-stage tests with fishes. ASTM Standard E 1241-92, Philadelphia, PA

Heslop RB, Jones, K (1976) Inorganic chemistry, A guide to advanced study. Elsevier Scientific Publishing Company, New York

Inaba K, Kuroda T, Shimamoto T, Kayahara T, Tsuda M, Tsuchiya T (1993) Lithium toxicity and $Na^+(Li^+)/H^+$ antiporter in *Escherichia coli*. Biol Pharmacol Bull 17: 395-398

Johnson NL (1969) Discrete distributions. Houghton Mifflin, Boston, MA

Rayner-Canham G (1996) Descriptive inorganic chemistry. W.H. Freeman & Co., New York, New York

Rossmann R, Barres J (1988) Trace element concentrations in near-surface waters of the Great Lakes and methods of collection, storage, and analysis. J Great Lakes Res 14: 188-204

Seiler HG, Sigel H, Sigel A (1988) Handbook on toxicity of inorganic compounds. Marcel Dekker Inc., New York, New York

Thompson WR (1947) Use of moving averages and interpolation to estimate median-effective dose. Bacterial Rev 11: 115- 145

US Environmental Protection Agency (1986) Hazard evaluation division. Standard evaluation procedure: Fish early life-stage. EPA 540/86- 138

US Environmental Protection Agency Title (1989) 40 CFR part 792 - Toxic Substances Control Act (TSCA). Good laboratory practice standards - Final rule