3B2v7.51c GML4.3.1 YEESA : 2334 Prod.Type:COM pp.1–7(col.fig.:NIL) ED:SG PAGN: NS/bmp SCAN: Nil

ARTICLE IN PRESS

Available online at www.sciencedirect.com

SCIENCE DIRECT®

ACADEMIC PRESS

Ecotoxicology and Environmental Safety ▮ (▮▮▮▮) ▮▮▮–▮▮▮

Ecotoxicology and Environmental Safety

www.academicpress.com

Chronic toxicity of arsenic to the Great Salt Lake brine shrimp, *Artemia franciscana*

Kevin V. Brix[a,*], Rick D. Cardwell[b], and William J. Adams[c]

[a] *EcoTox, 2001 NW Nye Street, Newport, OR 973659-8045, USA*
[b] *Parametrix, Inc., 5808 Lake Washington Blvd. NE, Suite 200, Kirkland, WA 98033, USA*
[c] *Kennecott Utah Copper, 8315 West 3595 South, P.O. Box 6001, Magna, UT 84044, USA*

Received 27 November 2001; received in revised form 26 August 2002; accepted 26 August 2002

Abstract

We determined the chronic toxicity of arsenic (sodium arsenate) to the Great Salt Lake brine shrimp, *Artemia franciscana*. Chronic toxicity was determined by measuring the adverse effects of arsenic on brine shrimp growth, survival, and reproduction under intermittent flow-through conditions. The study commenced with <24-h-old nauplii, continued through reproduction of the parental generation, and ended completed after 28 days of exposure. The concentrations tested were 4, 8, 15, 31, and 56 mg/L dissolved arsenic. The test was conducted using water from the Great Salt Lake, Utah as the dilution water. Adult survival was the most sensitive biological endpoint, with growth and reproduction somewhat less sensitive than survival. The no observed effect concentration (NOEC) for survival was 8 mg/L, and the lowest observed effect concentration (LOEC) was 15 mg/L dissolved arsenic. The LOEC for growth and reproduction was greater than the highest concentration tested, 56 mg/L. Based on survival, the final chronic value (geometric mean of the NOEC and LOEC) was 11 mg/L dissolved arsenic. The F_1 generation appeared to acclimate to the prior arsenic exposure of the parental generation and was significantly less sensitive than the parental generation. For example, survival for the F_1 generation through day 12 was 100% in 56 mg/L dissolved arsenic, compared to 26% for the parental generation. Growth of the F_1 generation was significantly less than that of the parental generation across all concentrations including the control, indicating a generational difference in brine shrimp growth rather than an arsenic effect. This study represents one of the few full life cycle toxicity tests conducted with brine shrimp.
© 2002 Elsevier Science (USA). All rights reserved.

Keywords: Arsenic; *Artemia franciscana*; Chronic toxicity; Great Salt Lake; Brine shrimp

UNCORRECTED PROOF

1. Introduction

The Great Salt Lake (GSL) in Utah is a unique aquatic habitat in the United States because of its water quality characteristics and extant biota. As a terminal lake with limited freshwater input, the lake has become hypersaline with a surface water salinity that ranges from approximately 75 to 150 g/L depending on annual rainfall. The lake is also characterized by relatively high dissolved organic carbon concentrations (15–55 mg/L) (Domagalski et al., 1990).

The hypersaline conditions severely limit the biological diversity of the GSL. No fish are capable of osmoregulating under these conditions and so are precluded from the lake. Only two invertebrates, brine shrimp (*Artemia franciscana*) and brine flies (*Ephydra* spp.), permanently occur throughout the lake. The brine shrimp have significant commercial value; approximately 10 million pounds of brine shrimp eggs are harvested each winter and sold as food for tropical fish, and the commercial shrimp industry generates over $10 million annually to the local economy and represents 90% of the world's commercial harvest. The brine fly larvae that occur in the lake provide, in combination with the brine shrimp, the main food supply for the millions of shorebirds that use the lake as a migratory stopover or breeding ground. Seven species of brine flies have been identified in the lake (Jorgenson, 1956). Water

*Corresponding author. Fax: +1-541-574-9490.
E-mail address: kevinbrix@actionnet.net (K.V. Brix).

0147-6513/02/$ - see front matter © 2002 Elsevier Science (USA). All rights reserved.
PII: S0147-6513(02)00054-4

boatmen (*Tricorixa* sp.) also periodically occur in the lake in some of the lower-salinity embayments, near significant freshwater inputs. The only other biota occurring in the lake are algae, diatoms, and bacteria. Approximately 23 species of algae and diatoms have been identified (Felix and Rushforth, 1979).

Given the unique habitat and biota, application of national water quality criteria to the lake is inappropriate, which USEPA explicitly acknowledges (Stephan et al., 1985). Rather, development of water quality standards for the lake should be based on site-specific toxicity studies on resident species. Further, these studies should be performed in water from the lake to account for how water quality characteristics influence chemical bioavailability.

There has been considerable methodological development for toxicity testing with brine shrimp over the past 20 years, although the vast majority of efforts have focused on acute testing regimes (Sorgeloos et al., 1978; Persoone and Castritsi-Catharios, 1989). Standard methods for performing chronic toxicity studies with brine shrimp have not been developed, although limited full chronic testing has been performed previously (Cunningham, 1976; Gebhardt, 1976). The objective of this study was to both develop a standardized chronic toxicity test method and use it to evaluate the effects of arsenic on brine shrimp. The potential for toxicity was evaluated through conduct of a full life-cycle test (Stephan et al., 1985). The approach used for this study was based on information gained from the literature and from preliminary testing that we performed. The study measured adverse effects on survival, growth, and reproduction after brine shrimp were exposed to a series of varying arsenic concentrations for 28 days. The objective of the study was to determine the no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC) for survival, growth, and reproduction for the parental generation and for survival and growth for the F_1 generation.

2. Materials and methods

2.1. Test substance

Arsenic, as reagent-grade sodium arsenate, $NaAsO_2$ (CAS No. 10048-95-0), was obtained from Sigma Chemical Co. (St. Louis, MO). A stock solution was prepared by adding 133 g of reagent-grade sodium arsenate to 2 L of deionized water and mixing for 2 h to achieve a 16 g/L stock solution. This solution was mixed for 2 h in a 2-L Pyrex volumetric flask using a Teflon-coated stir bar for mixing. After mixing, the stock solution was held in the dark at ambient temperature. Stock solutions were prepared in this manner three times during the study and all were analyzed for arsenic concentrations prior to use. All test concentrations in the remainder of this paper are reported as mg/L of arsenic.

2.2. Test organisms

Brine shrimp cysts were purchased from Argent Chemical Laboratories (Redmond, WA) and were certified to be *A. franciscana*, collected from the GSL. Cysts were stored in the dark at 5°C until used for testing. Cysts were hatched in seawater (salinity 28 g/L) at approximately 27°C under vigorous aeration. Nauplii < 24 h old were used to initiate the study.

2.3. Dilution water

Dilution water was collected from the GSL at a depth of 10–20 cm, on the north side of Antelope Island, approximately 60 miles north of Salt Lake City, Utah in the south arm. The dilution water was stored in two leached, 4000-L HDPE tanks for 20 days prior to use in testing. During storage, the dilution water was aerated by continuous circulation through the two storage tanks. The dilution water was analyzed for inorganic priority pollutants and conventional parameters prior to use in testing (Table 1). The total organic carbon, dissolved organic carbon, and total suspended solids concentrations measured in the dilution water are characteristic of the GSL (Domalgaski et al., 1990). Although these concentrations are higher than would normally be used in a laboratory toxicity tests (ASTM, 1996), they are appropriate for evaluating the site-specific conditions in the GSL.

Table 1
Dilution water quality

Analyte	Concentration (mg/L)
Antimony	<0.1
Arsenic	0.24
Beryllium	<0.05
Cadmium	<0.05
Chromium	<0.05
Copper	<0.15
Lead	<0.05
Mercury	<0.0004
Nickel	<0.05
Nitrogen, total ammonium	0.38
Nitrogen, nitrate	1
Nitrogen, nitrite	5
Organic carbon, dissolved	45
Organic carbon, total	46
Selenium	<0.1
Silver	<0.05
Thallium	<0.05
Total suspended solids	34
Zinc	<0.1

UNCORRECTED PROOF

2.4. Water quality parameters

Water quality parameters (pH, dissolved oxygen, and salinity) were measured in each test chamber and water temperature in the physical system at test initiation and every day thereafter. Water temperature was measured using a thermometer calibrated against a certified NBS thermometer. Test solution pH was measured using a Cole–Parmer Model 5398-00 digital pH meter. Dissolved oxygen was measured using a Hach Dissolved Oxygen Test Kit, Model OX-2P. Salinity was measured using a Reichert temperature-compensated refractometer.

2.5. Test methods

A proportional diluter system (USEPA, 1978) was used for the intermittent introduction of arsenic and dilution water. A calibrated laboratory pump (Fluid Metering Inc., Oyster Bay, NY) was used to inject the arsenic stock solution into the proportional diluter system. Prior to adding test organisms and initiating the study, the physical system was operated for 14 days to verify that measured arsenic concentrations were approximately equal to nominal concentrations.

The physical system was composed of an enclosed box in which the test chambers were housed and external light was controlled. Water temperature in the test chambers was maintained in a water bath. Test concentrations were mixed by the diluter and 1 L of test solution per replicate was delivered eight times per day. Each replicate underwent a 95% volume replacement every 50 h (Sprague, 1969). Gentle aeration (<100 bubbles per minute) was provided by a regenerative blower and delivered through glass bead airstones to each test chamber. The lighting for the test system consisted of fluorescent lights (100–150 foot candles) set on a 16-h light/8-h dark photoperiod.

The test design consisted of five concentrations and a dilution water control. A dilution water control and nominal concentrations of 4, 8, 16, 32, and 64 mg/L arsenic were used in the definitive study. These concentrations were based on a range-finding study that resulted in a 7-day LOEC of 64 mg/L arsenic for brine shrimp survival. Each concentration and control was represented by duplicate 7.8-L test chambers that were randomly located in relation to the others. Within each actual replicate were four acrylic cylinder pseudo-replicates (7 cm diameter by 14 cm deep) for a total of eight cylinders per test concentration. As discussed below, the pseudo-replicates within each test chamber were used to isolate breeding pairs of *Artemia* within each replicate so that nauplii production by the individual pairs could be accurately quantified. Each cylinder had four ports 2 cm wide by 10.5 cm high that allowed for exchange of test solution. The ports were covered by 120 μm Nitex screen to retain test organisms.

At test initiation, 10 nauplii <24 h old were introduced randomly into each cylinder for a total of 80 organisms per test concentration. Brine shrimp were observed daily; they were counted and recorded and dead specimens removed. Once sexual maturity was reached and adults began pairing (12 days after test initiation), three cylinders from each replicate were divided in half using an acrylic insert and one adult pair was placed in each half. This division of cylinders physically separated each pair, facilitating accurate quantification of nauplii production per pair. Each concentration contained a total of 12 pairs, with the exception of the highest concentration, which contained only five pairs, due to significant mortality during the first 12 days of exposure. One cylinder from each test chamber was not divided but instead used for evaluation of first-generation nauplii survival and growth. By not dividing this cylinder, the first-generation nauplii were exposed to the same conditions as those of parental generation prior to reproductive pairing. Of the remaining adults from each replicate concentration, 10 (five males and five females) were randomly selected for dry weight measurement. The brine shrimp were dried for 24 h at 60°C, after which they were weighed to the nearest 0.01 mg on an analytical balance. The brine shrimp were dried for 8 more hours and reweighed to ensure that constant weight was achieved.

The first release of live young occurred on day 13 and by day 15 all cylinders in all test concentrations had released live young. After pairing, any live nauplii or cysts released were counted and removed daily. Ten nauplii from each cylinder were selected randomly, placed in the fourth undivided cylinder, and allowed to grow for a duration equal to the number of days to thinning for the parental generation (12 days). F_1 generation exposure was terminated on days 25–27, depending on when the nauplii were first released (adult exposure continued through day 28). At this time, F_1-generation brine shrimp were counted and measured for dry weight.

At test initiation, the brine shrimp were fed marine green algae (*Platymonas* sp.) twice per day immediately after diluter cycling to provide adequate feeding time prior to flushing during the next diluter cycle. *Platymonas* sp. was used because preliminary studies demonstrated it to be a suitable food source. Each cylinder received 100,000 algal cells per animal twice daily. When the brine shrimp were thinned at day 12, each pair was fed 500,000 cells twice per day. The F_1 generation was fed 100,000 algal cells per animal twice daily.

UNCORRECTED PROOF

2.6. Analytical chemistry

Samples for arsenic analysis were collected at mid-depth from one replicate randomly selected from each test concentration at test initiation. Care was taken not to sample proximate to the chamber's sides or bottom and to avoid aspirating feces. For the remainder of the study, sampling of test concentrations alternated replicates. The time of sampling occurred at approximately the mid-point between dilutor cycles. Arsenic concentrations were measured at test initiation, each week thereafter, and at test termination for each concentration containing living test organisms. Duplicate and spiked samples were collected at a frequency of 5%. Samples were analyzed for both total and dissolved ($<0.45\,\mu m$) arsenic, using ICP-MS EPA Method 6020 (USEPA, 1995). The geometric mean of dissolved arsenic concentrations from the five sampling events was calculated and reported as the measured arsenic concentration for the test.

2.7. Analysis of biological data

Five test endpoints were evaluated in this study:

(1) survival of the parental generation prior to reproductive pairing on day 12 and survival on days 21 and 28;
(2) growth of the parental generation prior to reproductive pairing (day 12) and at day 28;
(3) reproduction in the parental generation on days 21 and 28;
(4) survival of the F_1 generation through day 12; and
(5) growth of the F_1 generation through day 12.

Data were analyzed a commercial software package, Statgraphics, version 5.0 (STSC, 1991). Data for the various test endpoints were first evaluated for normality and homogeneity of variance. The assumption of normality was tested by calculating the ANOVA model residuals and testing these residuals for normality by the normal probability plot method (Neter et al., 1990). The assumption of homogeneity of variances was evaluated by Bartlett's test using Statgraphics.

If a data set met the assumptions of normality and homogeneity of variances, an ANOVA was computed to determine whether any statistically significant differences existed among levels (concentrations or generations). If either of the assumptions could not be met, the nonparametric Kruskal–Wallis test was used to test for differences. Only survival of the F_1 generation was analyzed using nonparametric methods. Multiple-range evaluations for parametric data sets were computed by the least significant difference (LSD) test using Statgraphics. LSD tests for nonparametric data sets were computed with results from the Kruskal–Wallis tests using the multiple comparisons technique presented in Gibbons (1985).

3. Results

3.1. Water quality and test concentrations

The test temperature ranged from 25°C to 26°C, dissolved oxygen ranged from 0.6 to 3.2 mg/L, pH ranged from 7.8 to 8.2, and salinity ranged from 122 to 127 g/L over the course of the test.

Weekly arsenic concentrations are summarized as geometric means and coefficients of variation in Table 2. For the remainder of this paper, references to an arsenic concentration refers to the geometric mean of the measured dissolved arsenic concentrations. The dilution water had a mean concentration of 0.3 mg/L arsenic. The coefficient of variation for the dilution water was 60%; however, this was due to variability in relatively low (compared to test concentrations) arsenic concentrations (0.21 to 0.69 mg/L arsenic). Based on the analytical results, mean concentrations in the test were 0.3, 4, 8, 15, 31, and 56 mg/L arsenic as compared with nominal concentrations of 0, 4, 8, 16, 32, and 64 mg/L. Coefficients of variation for test concentrations ranged from 9% to 14%.

3.2. Biological results

Survival, growth, and reproduction were analyzed for the parental generation, and survival and growth were analyzed for the F_1 generation. Survival in the parental generation was the most sensitive endpoint through day 21 (Table 3). Between days 21 and 28, there was a general decline in survival across all concentrations, resulting in no statistically significant effects at any concentration compared to the control on day 28. After reproductive pairing on day 12, the dose–response relationship at the higher test concentrations became less distinct, with 31 mg/L arsenic exhibiting higher survival than 15 mg/L arsenic on days 21 and 28. None of the arsenic concentrations adversely affected survival

Table 2
Dissolved arsenic concentrations

Nominal As (mg/L)	Geometric mean dissolved As (mg/L)	Range in measurements	Coefficient of variation ($n = 6$) (%)
0	0.3	0.21–0.69	60
4	3.9	3.4–4.5	12
8	7.8	6.7–9.0	10
16	14.6	13.0–17.0	10
32	31.2	27.0–36.0	9
64	56.3	42.0–63.0	14

UNCORRECTED PROOF

Table 3
Parental and F_1 generation survival

Dissolved arsenic (mg/L)	Day 12		Day 21 Parental (SE)	Day 28 Parental (SE)
	Parental (SE)	F_1 (SE)		
0	93% (0%)	90% (0%)	75% (8%)	46% (4%)
4	86% (4%)	100% (0%)	58% (0%)	38% (4%)
8	89% (4%)	90% (10%)	58% (8%)	29% (13%)
15	90% (3%)	90% (10%)	42%* (8%)	33% (8%)
31	81%* (1%)	100% (0%)	79% (4%)	50% (8%)
56	26%* (1%)	100% (0%)	42%*[a](8%)	25% (17%)

[a] Percentage survival increased compared to that of day 12 due to thinning of test organisms at reproductive pairing.
*Statistically significant effect compared to the control.

Table 4
Parental and F_1 Generation Dry Weight (mg/Organism

Dissolved Arsenic (mg/L)	Day 12		Day 28 Parental (SE)
	Parental (SE)	F_1 (SE)	
0	0.37 (0.00)	0.24*(0.04)	0.45 (0.01)
4	0.43 (0.03)	0.31*(0.06)	0.72 (0.18)
8	0.40 (0.04)	0.23*(0.07)	0.51 (0.10)
15	0.43 (0.00)	0.25*(0.02)	0.52 (0.04)
32	0.47 (0.01)	0.28*(0.02)	0.49 (0.02)
56	0.45 (0.03)	0.25*(0.12)	0.57 (0.07)

*Statistically significant difference compared to the parental generation.

Table 5
Brine Shrimp Reproduction (Young Produced per Adult Reproduction Day

Dissolved arsenic (mg/L)	Young/Adult Reproduction Day (SE)	
	Day 21	Day 28
0	9.7 (0.3)	9.1 (0.5)
4	13.4 (2.0)	12.2 (1.1)
8	6.9 (2.5)	5.7 (1.5)
15	10.9 (1.7)	11.3 (2.0)
31	9.0 (0.5)	7.8 (1.0)
56	5.8 (1.8)	6.9 (3.0)

Table 6
Summary of Results for Biological Endpoints

	Evaluation	Measured dissolved arsenic (mg/L)
Parental Survival	NOEC	8
	LOEC	15
Parental growth	NOEC	56
	LOEC	>56
Parental reproduction	NOEC	56
	LOEC	>56
F_1 survival	NOEC	56
	LOEC	>56
F_1 growth	NOEC	56
	LOEC	>56
Final chronic value[a]		11

[a] The final chronic value is the geometric mean of the NOEC and the LOEC for the most sensitive endpoint measured. In this case, it constitutes parental generation survival, the geometric mean of 8 and 15 mg/L, or 11 mg/L.

for the F_1 generation over the exposure period. The F_1 generation was significantly less sensitive than the parental generation, with 100% survival at 56 mg/L arsenic compared to 26% survival at 56 mg/L for the parental generation over a 12-day period.

Arsenic did not affect growth in the parental or F_1 generation (Table 4). No statistically significant effects on growth occurred at any test concentration on days 12 or 28 for the parental generation. Although growth in the F_1 generation was significantly less than that in the parental generation through day 12, there was no significant difference between the control and the test concentrations (Table 4).

Brine shrimp reproduction was analyzed by the total number of young per adult reproductive day (YARD) per test chamber. This endpoint is calculated in a manner similar to the laboratory fish production index devised by Mount and Stephan (1967). Table 5 summarizes results for each test concentration for days 21 and 28. Although YARD was reduced at 56 mg/L (relative to the control) by 40% on day 21 and by 24% on day 28, this difference was not statistically significant, resulting in a reproductive NOEC of 56 mg/L.

4. Discussion

Of the biological endpoints measured for the adult generation, survival was the most sensitive, followed by reproduction and then growth (Table 6). After 21 days of exposure, survival was significantly affected at 15 mg/L arsenic (the survival LOEC), but not at 8 mg/L arsenic (the survival NOEC). Over the 21 days of exposure, neither growth nor reproduction was affected at the highest concentration tested (LOEC >56 mg/L arsenic), although reproduction at 56 mg/L was reduced by 40% relative to that of the control on day 21. Based on the

UNCORRECTED PROOF

day 21 survival results, the final chronic value is 11 mg/L dissolved arsenic, calculated as the geometric mean of the lowest NOECs and LOECs observed (8 and 15 mg/L).

The F_1 generation appeared to acclimate to the arsenic exposure and was significantly less sensitive than the parental generation in terms of survival. Why F_1 generation growth was significantly less than parental generation growth across all test concentrations, including the control, is unclear. One hypothesis is that brine shrimp produced oviparously and ovoviviparously either have different growth rates or, perhaps, initiate active feeding at different times.

There is a general lack of information concerning testing methodologies and performance of brine shrimp in chronic toxicity tests. The species has been used primarily for acute tests (Sorgeloos et al., 1978; Vanhaecke et al., 1981; Persoone and Castritsi-Catharios, 1989). Only three other chronic studies have been conducted with brine shrimp. The first (Gebhardt, 1976) evaluated the effects of cadmium, copper, and mercury on survival, growth and reproduction. It was conducted at 27°C using a static renewal test design (solution replacement every third day), GSL water was used as the dilution water, and brine shrimp were fed the hypersaline algae *Dunaliella viridis*. Under these conditions, the onset of reproduction was considerably later than that observed in our study with the control group beginning to reproduce on day 29. The number of nauplii produced was not quantified in that study.

The second study (Cunningham, 1976), although not a full life cycle study, investigated the effects of the insecticide Dimilin (TH 6040) on different life stages of brine shrimp under static renewal test conditions. In one experiment, brine shrimp reproduction was evaluated by exposing brine shrimp pairs and monitoring the number of nauplii produced. On day 21 of the experiment, adult survivorship in the control was approximately 90% and declined to approximately 70% on day 28. By day 40, survival had dropped below 50% and all shrimp were dead by day 80.

The only other data available for comparison to this study were from a preliminary study (dilution water only) that we conducted prior to initiating the definitive arsenic study. The primary difference between the preliminary study and the definitive study was that the preliminary study was conducted under static renewal conditions without constant aeration. The preliminary study had 78% and 75% survival on days 21 and 28 compared with 75% and 46% in this study. However, reproduction in the preliminary study was considerably different from that in the definitive study. An approximate 1:1 ratio of cysts and live young were produced in the preliminary study compared with only two cysts produced in any test concentration in the definitive study. Additionally, live young/surviving female on day 21 (156 in the control) in this study exceeded live young/surviving female on day 28 (113 in the control) in the preliminary study.

We hypothesize that under the conditions of this flow-through study, the brine shrimp completed their life cycle more quickly than those in the other studies. In other words, the mortality observed across all test concentrations between days 21 and 28 was caused by the shrimp reaching the end of their life span. This is supported by Gillespie and Stephens (1977), who suggested that the "generation time of *Artemia* may be less than three weeks under good conditions." Until additional studies are conducted though, this hypothesis cannot be verified.

5. Conclusions

Overall, we believe that this study demonstrates that chronic toxicity studies with brine shrimp can be performed successfully in the laboratory and that the results obtained are useful in assessing the potential of arsenic to limit survival, growth, and reproduction of brine shrimp in the Great Salt Lake. The difference in growth between parental and F_1 offspring and the decline in survival after day 21 indicates that additional research is needed to further define the life cycle of brine shrimp and appropriate testing and culturing needs of the organisms. However, given the available data, it appears unlikely that arsenic poses a significant risk to brine shrimp in the Great Salt Lake, given the relatively high effect levels determined in this study.

References

ASTM, 1996. Standard guide for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians. Standard E729-88. In: Annual Book of ASTM Standards: Biological Effects and Environmental Fate; Biotechnology; Pesticides, Vol. 11.05. American Society of Testing and Materials, Philadelphia, PA, pp. 249–268.

Cunningham, P.A., 1976. Effects of Dimilin (TH 6040) on reproduction in the brine shrimp, *Artemia salina*. Environ. Entomol. 5 (4), 701–706.

Domagalski, J.L., Eugster, H.P., Jones, B.F., 1990. Trace metal geochemistry of Walker, Mono and Great Salt lakes. In: Spencer, R. J., Chou, I. M. (Eds.), Fluid–Mineral Interactions: A Tribute to H. P. Eugster, The Geochemical Society.

Felix, E.A., Rushforth, S.R., 1979. The algal flora of the Great Salt Lake Utah USA. Nova Hedwigia 31, 163–195.

Gebhardt, K.A., 1976. Effects of Heavy Metals (Cadmium, Copper, and Mercury) on Reproduction, Growth, and Survival of Brine Shrimp (*Artemia salina*). Utah State University, Logan, UT.

Gibbons, J.D., 1985. Non-parametric Methods for Quantitative Analysis. American Science Press, Columbus, OH.

Gillespie, D.M., Stephens, D.W., 1977. Some aspects of plankton dynamics in the Great Salt Lake, Utah. International Conference on Desertic Terminal Lakes, Utah State University, Logan, UT.

UNCORRECTED PROOF

Jorgenson, E.C., 1956. Ephydra of Utah. University of Utah, Salt Lake City, UT.

Mount, D.I., Stephan, C.E., 1967. A method for establishing acceptable toxicant limits for fish—malathion and the butoxyethanol ester of 2,4-D. Trans. Am. Fish. Soc. 96, 185–193.

Neter, J., Wasserman, W., Kutner, M., 1990. Applied Statistical Linear Models. Irwin, New York.

Persoone, G., Castritsi-Catharios, J., 1989. A simple bioassay with *Artemia* larvae to determine the acute toxicity of antifouling paints. Water Res. 23, 893–897.

Sorgeloos, P., Remiche-Van Der Wielen, C., Persoone, G., 1978. The use of *Artemia nauplii* for toxicity tests—a critical analysis. Ecotoxicol. Environ. Saf. 2, 249–255.

Sprague, J.B., 1969. Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. Water Res. 3, 793–821.

Stephan, C.E., Mount, D.I., Hansen, D.J., Gentile, J.H., Chapman, G.A., Brungs, W.A., 1985. Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and their Uses. US Environmental Protection Agency, Environmental Research Laboratory, Duluth, MN.

STSC, I., 1991. Statgraphics. Rockville, MD.

USEPA, 1978. Manual for Construction and Operation of Toxicity-testing Proportional Diluters. US Environmental Protection Agency, Environmental Research Laboratory, Duluth, MN.

USEPA, 1995. Test methods for Evaluating Solid Waste (SW-846): Method 6020—Inductively Coupled Plasma–Mass Spectrometry, 3rd Edition, Revision 4. US Environmental Protection Agency, Office of Solid Waste, Washington DC.

Vanhaecke, P., Persoone, G., Claus, C., Sorgeloos, P., 1981. Proposal for a short-term toxicity test with Artemia nauplii. Ecotoxicol. Environ. Saf. 5, 382–387.

UNCORRECTED PROOF