ELSEVIER

Aquatic Toxicology 71 (2005) 283–296

AQUATIC TOXICOLOGY

www.elsevier.com/locate/aquatox

Chronic toxicity of glutaraldehyde: differential sensitivity of three freshwater organisms

Larissa L. Sano[a], Ann M. Krueger[a,1], Peter F. Landrum[b,*]

[a] *Cooperative Institute for Limnology and Ecosystems Research, University of Michigan, 401 E. Liberty St., Suite 330, Ann Arbor, MI 48104, USA*

[b] *Great Lakes Environmental Research Laboratory, NOAA, 2205 Commonwealth Boulevard, Ann Arbor, MI 48105, USA*

Received 8 June 2004; accepted 1 December 2004

Abstract

The biocide, glutaraldehyde, is a potential environmental contaminant due to its widespread use in medical applications, off-shore oil extraction, and pulp mill processing. It has also been proposed as a candidate for treating the ballast water of vessels, which could result in a substantial increase in environmental release. To assess the potential for environmental impacts associated with glutaraldehyde, three standard chronic toxicity bioassays were performed: 96-h phytoplankton growth bioassays using *Pseudokirchneriella subcapitata* (formerly, *Selenastrum capricornutum*), three-brood reproduction bioassays using *Ceriodaphnia dubia*, and an embryo–larval bioassay using steelhead trout, *Oncorhynchus mykiss*. For the green alga, *P. subcapitata*, significant decreases in growth were observed at glutaraldehyde concentrations greater than or equal to 1.0 mg L^{-1}. Embryos of *O. mykiss* demonstrated a similar sensitivity with exposures of 2.5 mg L^{-1} resulting in a 97% reduction in hatch rate. In most cases, this failure to hatch was due to the inability of the embryo to leave the chorion and not to early embryo mortality. In contrast, reproduction and mortality rates in *C. dubia* were not as sensitive to glutaraldehyde: decreased reproduction was detected at 4.9 mg L^{-1} (the lowest observed effect concentration), and is similar to concentrations causing acute mortality in adults (4.7 mg L^{-1} for the estimated LC_{50}, or 50% lethal concentration). These data indicate that both algae and fish embryos may be particularly sensitive to long-term glutaraldehyde exposure; however, this is predicated on whether glutaraldehyde concentrations will achieve high enough environmental concentrations and for a sufficient period of time to elicit such effects.

Keywords: Glutaraldehyde; Chronic toxicity; *Pseudokirchneriella subcapitata*; *Ceriodaphnia dubia*; *Oncorhynchus mykiss*

1. Introduction

Glutaraldehyde (1,5-pentanedial: CAS 11-30-8) is a saturated five-carbon dialdehyde, characterized by strong antimicrobial (biocidal) properties. Like other compounds in the aldehyde family, glutaraldehyde possesses carbonyl groups that react readily with nucleic

* Corresponding author. Tel.: +1 734 741 2276; fax: +1 734 741 2055.
E-mail address: Peter.Landrum@noaa.gov (P.F. Landrum).
[1] Present address: Department of Molecular, Cellular, and Developmental Biology, University of Michigan, 830 North University, Ann Arbor, MI 48109, USA.

0166-445X/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquatox.2004.12.001

acids and proteins by alkylating sulfhydryl, hydroxyl, carboxyl, and amino groups (Miner et al., 1977; Rutala, 1990). This high degree of reactivity allows glutaraldehyde to cross-link amine groups on the outer cell walls and cell membranes of bacteria (and other microorganisms), and is believed to be responsible for its efficacy against Gram-negative bacteria, fungi, and viruses (Chambon et al., 1992; Simons et al., 2000).

Currently, the largest application of glutaraldehyde is in the medical and dental industries, where it is used primarily as a high-level disinfectant to clean heat-sensitive equipment (e.g., endoscopes, transducers, bronchoscopes, mirrors, etc.; Rutala, 1990). It is also employed, to a lesser degree, for oil drilling applications and gas pipelines to reduce populations of sulfate bacteria (Eagar et al., 1988) and in the pulp and paper-mill industry to control populations of microorganisms (Pereira et al., 2001). Most of these applications result in relatively small environmental releases of glutaraldehyde; however, even for medical applications, in which waste effluent is released through the sanitary sewer system, conditions can arise that result in releases of glutaraldehyhde that may be high enough to cause environmental impacts (Jolibois et al., 2002).

In addition to these current uses, glutaraldehyde has been proposed as a candidate biocide for treating the ballast water of vessels to decrease the number of non-indigenous species released into receiving waters (NRC, 1996). One of the more promising applications is for treating unballasted overseas vessels (i.e., NOBOBs or no ballast on board) that enter the Laurentian Great Lakes (Lubomudrov et al., 1997). These vessels represent the majority of ships that enter the Great Lakes from overseas ports (Colautti et al., 2003), and therefore present the highest risk for release of non-native species (MacIsaac et al., 2002). These vessels also undergo cross-transfer of lake water into ballast tanks, which would reduce the effective concentration of biocide in the tank prior to environmental release. The magnitude of this dilution can be substantial. A report by Farley (1996) indicated that the amount of lake water taken on by NOBOBs can range from 1000 to over 20,000 metric tonnes. Depending on the initial amount of residual sediments and water in the vessels and on the amount of water cross-transferred, dilution of glutaraldehyde could range from 10- to 100-fold. This dilution factor, coupled with the time it takes to transit between different ports, may promote biocide degradation prior to residuals being released into receiving waters.

Table 1
Physical properties and fate characteristics of glutaraldehyde

Characteristic	Value
Molecular formula	$C_5H_8O_2$
Molecular weight (g mol^{-1})	100.11
Density (kg m^{-3})	0.72
Melting point (°C)	−14
Boiling point (°C, at 1002 hPa)	188
Solubility	Miscible
log K_{ow}	−0.11
Vapor pressure (Torr, at 25 °C)	0.6
Henry's constant (atm m^{-3} mol)	1.1×10^{-7}
Aquatic biodegradation (aerobic) (h)	$t_{1/2} = 10.6$

Although the potential for ecological impacts will likely be limited due to the relatively rapid degradation rates of glutaraldehyde (Leung, 2001a; Landrum et al., 2003; Table 1), the possible magnitude and nature of effects on sensitive taxa in the vicinity of vessel discharges need to be evaluated. The overall viability of this application will partly depend on the degree of these environmental impacts.

The objective of this study is to determine the relative sensitivity of three different taxa of organisms to longer term glutaraldehyde exposure. This was assessed through standard chronic toxicity bioassays employing representative freshwater organisms: the green algal species, *Pseudokirchneriella subcapitata* (formerly, *Selenastrum capricornutum* and *Rhaphidocelis subcapitata*), the cladoceran, *Ceriodaphnia dubia*, and embryonic and larval stages of the steelhead trout, *Oncorhynchus mykiss*. This selection of organisms was designed not to be exhaustive but instead to include organisms that are widely used as standard test organisms, that inhabit the Great Lakes, and that encompass a range of taxa. The use of these species also permits assessment of glutaraldehyde effects on different endpoints including survival, growth, and reproduction.

2. Materials and methods

2.1. Chemicals and analytical methods

Glutaraldehyde was obtained as a 50% solution (w/w) from Fisher Scientific (Fairfield, NJ, USA).

Solutions of glutaraldehyde were analyzed using a spectrophotometric assay that employs 3-methyl-2-benzothiazonlinone hydrazone hydrochloride as the color-developing agent (Pakulski and Benner, 1992; Sawicki et al., 1961). Actual glutaraldehyde concentrations were estimated from a standard curve consisting of three concentrations (0.5, 1, and 8 mg L^{-1}), which was run during each analysis. The detection range of this method was 0.5–8.0 mg L^{-1} of glutaraldehyde. All reported glutaraldehyde concentrations are the measured values and are expressed as mg glutaraldehyde L^{-1}.

2.2. Algal growth bioassay

To assess the toxicity of glutaraldehyde to a representative algal population, 96-h growth bioassays were conducted using the unicellular Chlorophycea, *P. subcapitata* (Hindàk, 1990). The starter culture was obtained from Carolina Biological Supply (Burlington, NC, USA) and maintained in artificial culture media (including the addition of disodium (ethylenedinitrilo) tetraacetate (EDTA); ASTM, 1998a). The base for the media was Ann Arbor (MI, USA) city water that was passed through a carbon filtration system, and then filtered through a Ropure® ST reverse osmosis system (Barnstead, Dubuque, IA, USA) and a Nanopure® UV ultrapure water system (Barnstead, Dubuque, IA, USA). After the macro- and micronutrients were added to the purified water, the pH of the solution was adjusted to 7.5 (±0.1) using dilute solutions of either HCl or NaOH. The resulting culture water had an average hardness of 20 mg L^{-1} (as $CaCO_3$) and an average alkalinity of 30 mg L^{-1} (as $CaCO_3$), and the entire solution was then filter-sterilized through a sterile 0.20 μm Micronics filter (Redmond, WA, USA). The media were subsequently inoculated with algae and maintained in Erlenmeyer flasks in an incubator set at 25 °C with a 16 h light:8 h dark photoperiod.

The protocol for growth inhibition bioassays was based on standard procedures outlined in ASTM (1998a) and USEPA (2002). Experiments were conducted in 250 mL Erlenmeyer flasks containing 100 mL of sterilized culture media per replicate, with four replicates per concentration and six concentrations (including the control) per experiment. Each experiment was initiated using algae from a culture that was in an exponential growth phase and approximately 5–7 days old. Test flasks were inoculated with algal samples that had been concentrated to a density of 10,000 cells mL^{-1} (±1000). The appropriate volume of glutaraldehyde was added to each flask from a single stock solution, and glutaraldehyde concentrations were measured at the start of the experiment. Test flasks were placed on an orbital shaker operating at 100 rotations per minute (rpm). Flask position on the shaker was randomized daily by treatment. The flasks were kept at 25 °C with a 16 h light:8 h dark photoperiod. Full spectrum lights (American Environmental Products, Fort Collins, CO, USA) were used, which provided an approximate uniform illumination of 86 μE m^{-2} s^{-1}. At the end of the exposure period, algal biomass (number of cells) was determined through manual counts using a hemacytometer (Bright-Line counting chamber, Hausser Scientific, Horsham, PA, USA).

2.3. Zooplankton reproduction bioassay

The brood stock of *C. dubia* used in the bioassays was obtained from Wright State University (Dayton, OH, USA). Cultures were maintained at 24 ± 1 °C with a 16 h light:8 h dark photoperiod. The culturing medium was artificial moderately hard reconstituted water (USEPA, 2002), and the base water was the same used for the algal bioassays (described above). Culture water was changed approximately every 72 h, and organisms were fed a combination of yeast, Cerophyll®, trout chow mixture (YCT: 2 mL per beaker; USEPA, 2002), and *P. subcapitata* (3 mL per beaker) after each water change.

Neonates of cultured organisms were used to initiate the static renewal three-brood reproduction bioassay, which was conducted according to ASTM (1998b). Experiments began with 12-h neonates, which were randomly distributed across the different test solutions in a stratified manner (i.e., all of the neonates for each replicate for the different treatments originated from the same parthenogenic female). Six concentrations were tested (ranging from 0 to 7.9 mg L^{-1}) and consisted of 10 replicates each, with one 12-h neonate per replicate. Test solutions were renewed daily from fresh stock solutions, and glutaraldehyde concentrations were measured before and after solution renewal. Test organisms were fed YCT and *P. subcapitata* at a rate of 200 μL each per replicate per day. The toxicity test was terminated when at least 60% of the control organisms had

produced three broods, which occurred 8 days after initiation of the experiment. Observations were made daily on the status of the female daphnids and on the number of neonates. The DO and pH were also measured and recorded daily. At the end of the experiment, surviving females were gently blotted, dried for 48 h (at 60 °C), and then weighed.

2.4. Embryo–larval survival and growth bioassay

A static renewal embryo–larval bioassay was conducted using embryos of *O. mykiss*. The protocol for this experiment was adopted from Canaria et al. (1999) and ASTM (1998c). The experiment was initiated with newly fertilized embryos (approximately 12-h post-fertilization). These embryos were collected from wild steelhead trout (*O. mykiss*) and obtained from the Wolf Lake Hatchery (Kalamazoo, MI, USA). Fertilized embryos were sealed in plastic bags and placed in an ice chest for transit. Once the embryos arrived at the Great Lakes Environmental Research Laboratory (Ann Arbor, MI, USA), the embryos were removed from the container, placed into a crystallizing dish, and examined under the microscope. All damaged or infertile eggs were discarded. Viable embryos were randomly distributed into shallow plastic containers and then randomly assigned to treatments.

The experiment consisted of six treatments (including controls) with four replicates per treatment and 30 embryos per replicate. The test concentrations for the embryo period (up to sac-fry) ranged from 0 to 13.6 mg L^{-1}, while test concentrations for the larval period ranged from 0 to 1.0 mg L^{-1}. The experiments started with embryos being placed in 1000 mL borosilicate beakers containing 500 mL of test solution. Air was bubbled into the beakers at a rate of approximately 100 ± 10 bubbles/min. The water used for the experiments was hard reconstituted city water, which is similar to that described in USEPA (2002). The addition of $NaHCO_3$, KCl, Mg, and $CaSO_4$ yielded media with hardness and alkalinity comparable to Great Lakes water (140 and 100 mg L^{-1} as $CaCO_3$, respectively).

Experiments were conducted at 11 °C (±2 °C), and glutaraldehyde solutions and control water were changed approximately daily. During the water renewal process, 75–80% of the test solution was removed, and new solution was gently poured into the container using a baffle to minimize disturbance to the embryos. Concentrations of glutaraldehyde were measured both prior to and after renewing test solutions. Daily observations were made on embryo condition (alive/dead/deformed) and hatching time. The pH and DO were also checked with each water change.

Embryos were maintained in the dark until 1 week after 50% of the control group hatched from the chorion (i.e., became alevins or sac-fry). After the majority of embryos hatched in each beaker, they were transferred to 5 L aquaria and maintained under low-intensity light with a 16 h light:8 h dark photoperiod. When the alevins reached the swim-up stage, they were fed ground juvenile trout chow at a rate of 4% fish body weight per day (distributed over three feeding periods). The initial amount of food was approximately 125 mg/fry, which then increased to 250 mg/fry by the end of the second week. Water changes were continued on a 24 h basis, and pH, DO, and glutaraldehyde concentrations were measured daily. This experiment lasted for a total of 62 days.

Prior to the end of the experiment, food was withheld from larval fish for 24 h to allow for gut evacuation. At the end of the experiment, larvae were euthanized with MS-222. Individual fish were gently blotted and wet weights were collected. Larvae were then dried for 48 h at 60 °C, re-weighed, and measured for length.

2.5. Data analysis

Threshold concentrations including the no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC) were estimated using traditional hypothesis testing techniques. Normality of data was first assessed using Shapiro–Wilks test and homogeneity of variance was assessed with Bartlett's test. Depending on the data, the NOEC and LOEC were determined using either Dunnett's test, a parametric test for comparing individual treatment concentrations against controls (Winer et al., 1991), or Steel's Many–One Rank Test, a non-parametric test if either normality or homogeneity of variance assumptions was violated (USEPA, 2002). Point estimates of lethal concentrations (LC values) were estimated using either Probit analysis or the Spearman–Karber method (Finney, 1978). A linear interpolation method was used to estimate inhibition concentrations (IC) for algal growth, reproduction rates, and hatch-out rates,

using ICPIN software (Version 2.0 (USEPA, Duluth, MN, USA)). When fewer than seven replicates were used in an experiment, an expanded 95% confidence interval for the IC value is reported. One-way ANOVA was used to assess differences in dry weights for the *C. dubia* and embryo–larval experiments (using SYSTAT, Version 10 (SPSS, Inc., Chicago, IL, USA)).

3. Results

3.1. Algal growth bioassays

Two bioassays were conducted using *P. subcapitata*. Initial glutaraldehyde values were similar to nominal estimates, and concentrations for the first experiment decreased by an average of 27% over the 5-day experiment, with the lower concentrations ($\leq$2 mg L^{-1}) decreasing by a larger percentage (approximately 50%). The concentrations in the second experiment remained relatively unchanged over the 96-h test period. All reported concentrations are the measured 96-h average concentrations.

Violations of normality (first experiment) and variance (second experiment) required the use of the non-parametric test, Steel's Many–One Rank Test, to estimate endpoints. The NOEC and LOEC for the first experiment were 0.7 and 1.4 mg L^{-1}, respectively, and for the second experiment were 1.3 and 2.1 mg L^{-1}, respectively (Table 2). For the first experiment, the IC_{25} value was 0.6 mg L^{-1} (95% CI: 0.12–1.10 mg L^{-1}), and the IC_{50} value was 1.0 mg L^{-1} (95% CI: 0.44–1.29 mg L^{-1}). For the second experiment, the estimated IC_{25} was 1.3 mg L^{-1} (95% CI: 1.05–1.54 mg L^{-1}), and the estimated IC_{50} was 1.8 mg L^{-1} (95% CI: 1.59–1.90 mg L^{-1}), which are higher than the values estimated from the first experiment. For both experiments, the IC values were close together, indicating a steep concentration–response curve. At effect-level concentrations, the cells of *P. subcapitata* appeared enlarged and bloated compared to controls.

3.2. Zooplankton reproduction

Glutaraldehyde concentrations decreased over the 24-h exposure period between solution renewals. Although in most cases the initial (renewing) solution was close to the nominal concentration, by the end of the 24-h period, most concentrations had decreased by an average of 75%. There was no indication of differences in degradation by day or by treatment (one-way ANOVA, $p > 0.05$). The following results are based on a time-weighted average for the different treatments over the entire 8-day period.

Adult survival of *C. dubia* was adversely affected by glutaraldehyde exposure at the concentrations tested. The estimated LC_{50} for adults was 4.7 (95% CI: 3.92–5.17) (Table 2; Fig. 1). Based on the range of concentrations employed, the NOEC under these exposure conditions was 2.4 mg L^{-1}, and the LOEC was 4.9 mg L^{-1}.

Table 2
Estimated effect concentrations for the three test organisms exposed to glutaraldehyde

Species	Endpoint	IC_{25} and LC_{25} (95% CI)	IC_{50} and LC_{50} (95% CI)	NOEC	LOEC
P. subcapitata (experiment #1)	Cell growth	0.6 (0.12–1.10)	1.0 (0.44–1.29)	0.7	1.4
P. subcapitata (experiment #2)	Cell growth	1.3 (1.05–1.54)	1.8 (1.59–1.90)	1.3	2.1
C. dubia	Survival	NC	4.7 (3.92–5.17)	2.4	4.9
	Reproduction	3.5 (2.91–4.97)	4.7 (3.85–5.95)	2.4	4.9
	Growth	NC	NC	4.9	NC
O. mykiss	Survival (embryos)	NC	NC	13.6	NC
	Hatch-out rate	1.5 (1.32–1.55)	1.82 (1.73–1.89)	1.3	2.5
	Survival (larvae)	NC	NC	1	NC
	Growth (larvae)	NC	NC	1	NC

Endpoints represent exposures of 96 h for *Pseudokirchneriella subcapitata* and 8 days for *Ceriodaphnia dubia*. Embryo–larval exposures for *Onchorhynchus mykiss* lasted a total of 62 days, with the embryo period lasting for 35 days. All values are presented as mg glutaraldehyde L^{-1}. NC indicates that endpoints were not calculable based on these data (e.g., there was no statistically significant response for that endpoint at the concentrations tested).

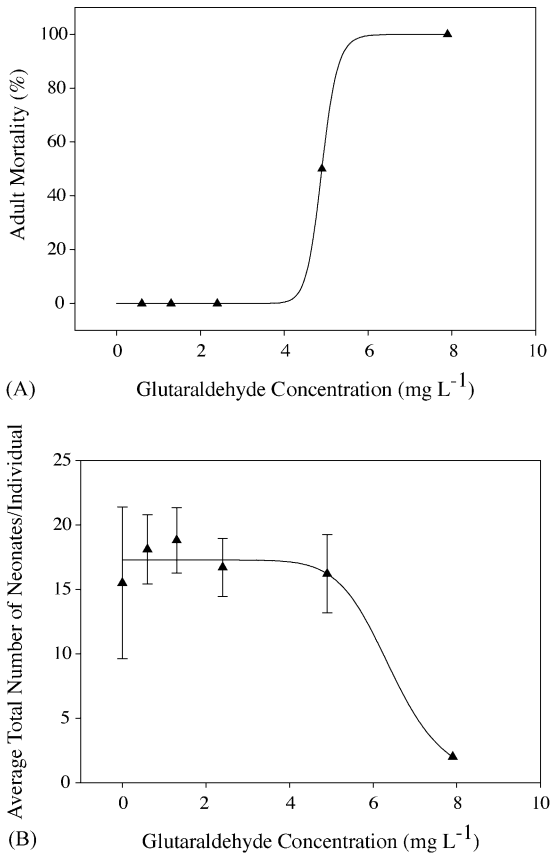

Fig. 1. Toxicity data from an 8-day experiment exposing 12 h neonates of *Ceriodaphnia dubia* to glutaraldehyde: (A) mortality data of initial neonates over the course of the experiment (endpoint at 8 days); (B) reproductive rate of exposed organisms based on the average of the total number of neonates produced per parthenogenic female over the course of the 8-day exposure period.

In terms of reproduction effects, the estimated IC values were close together, with an IC_{25} of 3.5 mg L^{-1} (95% CI: 2.91–4.97) and an IC_{50} of 4.7 mg L^{-1} (95% CI 3.85–5.95; Table 2). There were no significant differences between treatments up to, and including, 4.9 mg L^{-1} and the controls for several reproduction parameters including days to first brood, average number of young per brood, and average total number of young per adult (Fig. 1; Table 3). The estimated sensitivity (minimum significant difference) for the reproduction endpoint in this experiment was a 21% reduction compared to controls.

Dry weights of the *C. dubia* females indicate that glutaraldehyde exposure did not adversely affect growth rate (Table 3). However, a one-way ANOVA revealed statistically significant differences between the different treatments, with dry weights from the 1.3 and 2.4 mg L^{-1} treatments being higher than the dry weight of the controls ($p \ll 0.01$; two-sided *t*-test using the Bonferroni correction).

3.3. Embryo–larval growth

Glutaraldehyde concentrations fluctuated substantially both over the 24-h period between solution renewals and over the 62-day exposure period. For the embryonic period (through hatch-out, day 34), the initial (renewing) solution was close to the nominal concentration, but by the end of the 24-h period, most concentrations had decreased by an average of 20%. There was no indication of differences in degradation by day or by treatment (one-way ANOVA, $p > 0.05$). In contrast, the amount of degradation was much greater after the embryos reached the sac-fry stage (and the

Table 3
Results of 8-day chronic toxicity test for *Ceriodaphnia dubia* exposed to glutaraldehyde

24-h weighted average concentration (mg L^{-1})	Days to first brood (mean ± S.D.)	Adult survival (%)	No. of young/adult (mean ± S.D.)	No. of young/brood (mean ± S.D.)
0	4.1 ± 0.3	70	18.3 ± 4.2	6.3 ± 0.9
0.6	4.2 ± 0.4	100	18.1 ± 2.7	5.9 ± 1.1
1.3	4.0 ± 0.0	100	18.8 ± 2.5	6.3 ± 0.8
2.4	4.2 ± 0.4	100	16.7 ± 2.3	5.8 ± 0.7
4.9	4.4 ± 0.5	50*	16.2 ± 3.0	5.8 ± 0.3
7.9	5.0 ± 0.0*	0*	NA	NA

Experiments were initiated with 12 h neonates, and mortality rates of these organisms are expressed in adult survival rates. The remaining parameters are reproductive estimates of the exposed parthenogenic females. Organisms that died before the end of the experiment were excluded from estimates of reproduction parameters. NA: not available, since all original adults died before the end of the experiment.

* Indicates a significant difference compared to control ($p < 0.05$).

Table 4
Results of the chronic toxicity test using *Oncorhynchus mykiss* exposed to glutaraldehyde

24-h weighted average concentration (mg L^{-1})	Average embryo survival[a] (%)	Time to first hatch[b] (mean ± S.D., days)	Time to complete hatch[c] (mean ± S.D., days)	Hatching rates[d] (average % ± S.D.)
−0.1	83	25.3 ± 0.5	3.25 ± 1.26	89.8 ± 7.3
0.6	95	25.8 ± 0.5	3.5 ± 0.58	90.3 ± 7.5
1.3	98	26.8 ± 2.2	15.5 ± 3.87	74.9 ± 3.6
2.5	89	29.7 ± 1.5	NC	3.0 ± 2.0
5.1	93	NC	NC	0
13.6	77	NC	NC	0

The data listed in this table reflect parameters derived during the embryo period through hatch out into the sac-fry phase, up to 35 days post-fertilization. NC indicates that endpoints were not calculable.

[a] Embryo survival is derived from the number of embryos surviving up until the first day when embryos in the replicate began hatching.

[b] Time to first hatch is based on first day when embryos in a given replicate began to hatch.

[c] Time to complete hatch is based on the period from when embryos in a given replicate first started hatching to the day when all embryos had completely hatched into sac-fry.

[d] Hatching rates are based on the total number of viable embryos that successfully hatched during the hatch out period (less the number of embryos that died during that period).

experimental conditions changed due to light exposure and the addition of food): after day 35, test solutions declined on an average of 65% over the 24-h period between renewals. In addition, the concentration in the renewed solution was often much lower than the nominal value (usually about 50% less). The data presented below are separated out by the two different exposure periods, pre-hatch (embryonic) and post-hatch (sac-fry and larval).

Survival rates of *O. mykiss* embryos, up until the controls began to hatch on day 25, were comparable for all concentrations tested including the controls (Table 4). Although most of the embryos survived the initial embryonic period, the majority of organisms at the 2.5 mg L^{-1} treatment level and higher were not able to hatch from the embryo stage into the sac-fry stage (resulting in "imprisoned" larvae; Fig. 2). Hatch rates were assessed for up to 10 days after 50% of the control embryos successfully emerged from the chorion (ASTM, 1998c); however, because many of the embryos at 1.3 mg L^{-1} had partially hatched, these organisms were maintained throughout the remainder of the experiment. After the 10-day post-hatch period (up to day 35), only 3% of the surviving embryos treated at 2.5 mg L^{-1} had successfully emerged from the chorion (Table 4; Fig. 3) and none of the embryos at the higher concentrations had survived. In many cases, the embryos treated at 2.5 mg L^{-1} and higher clouded up and disintegrated over time (particularly towards the end of the hatching period, day 35). In other cases, the embryos appeared viable until the chorion first started to break open, after which the embryos quickly clouded up, with no viable sac-fry emerging.

Even at concentrations as low as 1.3 mg L^{-1}, embryos had difficulty emerging from the chorion (Table 4). This effect manifested itself primarily as an extended hatching period, during which sac-fry remained in a "partially hatched" condition (similar to the imprisoned larva in Fig. 2). The average time for all viable embryos to complete hatching ranged from 3.25

Fig. 2. Picture of an "imprisoned" larva of *O. mykiss*. This larva was exposed to a concentration of 2.5 mg L^{-1} and this picture was taken 10 days after 60% of the control organisms successfully hatched from the chorion.

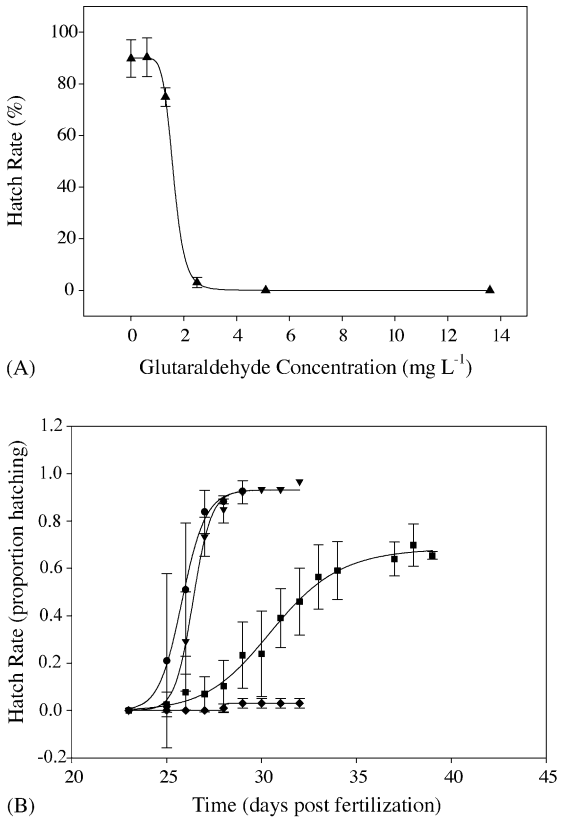

Fig. 3. Effects of glutaraldehyde on hatch rate success of *Oncorhynchus mykiss* embryos exposed to glutaraldehyde. (A) Percent successful hatch rate of embryos exposed at each treatment. Value was based on the number of embryos that fully hatched into viable sac-fry divided by the total number of viable embryos present once the embryos at that particular treatment level first began hatching; thus, it is based on both the number of successful hatches and embryos that became viable sac-fry (versus those embryos that died during the hatch-out period). (B) Hatching rate by day for different treatment groups. Again, these data reflect only those embryos that fully (not partially) hatched into viable sac-fry. Treatments from the 5.1 and 13.6 mg L^{-1} group were excluded from the graph, since they produced no viable sac-fry over the exposure period. (●) 0 mg L^{-1}, (▼) 0.6 mg L^{-1}, (■) 1.3 mg L^{-1}, (♦) 2.5 mg L^{-1}.

days (1 S.D.: 1.26) for the controls to 15.5 days (1 S.D.: 3.87) for the 1.3 mg L^{-1} treatment group. The overall hatching rate for this group was also lower than that for the controls (Table 4). Based on the experimental protocol, the estimated minimum significant difference for embryo survival rates was 22%.

The survival rates of larval fish were estimated separately due to the large effect of glutaraldehyde exposure on hatching success and due to differences in measured glutaraldehyde concentrations over the experimental period. Larval fish were followed for 27 days after hatching, through the alevin and the fry stages. At the end of this period, there was no significant difference between survival in the controls and the two remaining treatments (0.4 and 1.0 mg L^{-1}; Table 5). One of the replicates from the 0.4 mg L^{-1} exposure was excluded from analysis, because 75% of the larvae died during a 2-day period soon after hatching. This data point was considered an outlier, because it was significantly different than the three other replicates (in which mortality rates averaged 3%) and because it did not appear to be due solely to glutaraldehyde exposure, since acute mortality due to glutaraldehyde would not be expected at 0.4 mg L^{-1} concentrations. More likely, these mortalities were due to an undetected condition, such as some form of bacterial contamination, which may occur at lower, non-biocidal concentrations of glutaraldehyde.

Table 5
Survival and growth data for the larval period of *Oncorhynchus mykiss* exposed to glutaraldehyde

24-h weighted average concentration (mg L^{-1})	Average larvae survival (%)	Larval dry weight (mean ± S.D., mg)
−0.2	98	0.035 ± 0.006
0.4	97	0.029 ± 0.007
1.0	94	0.031 ± 0.008

The average larval survival is based on the number of viable sac-fry from the start of the larval period until the end of the experiment (day 62 post-fertilization).

Differences in larval fish growth were assessed using Bonferroni's adjustment, due to the unequal number of replicates. The data indicate no significant difference in growth (at $p < 0.05$), although the p-value for 1.0 mg L^{-1} was close to the critical value (2.14 versus 2.23, respectively). Given the experimental set-up, the estimated minimum significant difference is a 17% reduction in growth.

4. Discussion

Chronic toxicity bioassays, such as those detailed here, are regularly used to assess the effects of longer term exposure of chemicals on organism health. The purpose of employing these bioassays is to assess the potential sensitivity both of different classes and of dif-

ferent life stages of organisms in an effort to establish permissible environmental concentrations that should protect populations and communities of organisms (Ferson et al., 1996; USEPA, 2002). The results from these bioassays are often used to estimate effect-level concentrations in the environment; however, many issues have been raised regarding translating single species results into population- and community-level effects (Burmaster et al., 1991). Although the ecological relevance of these tests has been questioned, there are indications that effect-level concentrations derived from single species bioassays can estimate population- and ecosystem-effect levels (Versteeg et al., 1999). More importantly, perhaps, these types of data are essential for developing "weight of evidence" approaches for evaluating the potential environmental impacts of different chemicals (Hall and Giddings, 2000).

4.1. Summary of results

Chronic toxicity bioassays were employed to assess the potential for environmental effects associated with the release of the biocide, glutaraldehyde. Of the three organisms tested, the algal species, *P. subcapitata*, was most sensitive. The IC_{50} estimate of approximately 1.5 mg L^{-1} (ranging from 1.0 to 1.8 mg L^{-1}) indicates that low concentrations of glutaraldehyde can dramatically impact growth. In addition, the narrow interval between the IC_{25} and IC_{50} suggests a steep concentration–response curve; small increases in glutaraldehyde concentration would be expected to cause a disproportionate decrease in growth. In terms of relevance, *P. subcapitata* has been found to be more sensitive both to certain metals (Rojíčková-Padrotová and Maršálek, 1999; Versteeg et al., 1999) and to some organic compounds (Fairchild et al., 1998) compared to other algal species. This sensitivity should be expected with glutaraldehyde, given its mode of action: because glutaraldehyde interacts strongly with amine and sulfhydryl groups, it is likely to be more toxic to organisms with exposed cell walls. Since *P. subcapitata* possesses only a cellulosic cell wall as an external covering (Sheath and Wehr, 2003), this may make it more vulnerable to the cross-linking action of glutaraldehyde compared to other taxa that have a more substantial protective shield. It is thus likely that glutaraldehyde will also adversely affect similar algal species (especially those that lack protective coatings) at concentrations comparable to those reported here.

The survival rate of embryos of *O. mykiss* also proved sensitive to glutaraldehyde, and the exposure duration to elicit this effect was long (>20 days). This sensitivity manifested itself not in embryo survival rates, but instead in the successful hatching of embryos from the chorion. Embryo survival up to the time of hatch was not adversely impacted by exposures of up to 13.6 mg L^{-1}; yet, organisms exposed to concentrations greater than 2.5 mg L^{-1} were not able to emerge successfully from the chorion. While most other studies of toxicity effects on fish embryos have reported the hatching period to be relatively insensitive to chemical toxicity (Scudder et al., 1988; Görge and Nagel, 1990; Nguyen and Janssen, 2002), glutaraldehyde effects on embryo survival appeared to be particularly prominent. Glutaraldehyde exposure did not appear to adversely affect growth and survival of the remaining larvae up to a concentration of 1 mg L^{-1}, although this effect-level includes those embryos that had been exposed to 2.5 mg L^{-1} and that had delayed hatching (reflecting the entire history of glutaraldehyde exposure). Thus, the delay in hatch time did not appear to affect final growth of the larvae (up to 27 days post-hatch), although the ecological significance of this delay is difficult to determine from this experiment alone. The overall estimated NOEC for this experiment (embryo to fry stage) was 1.0 mg L^{-1}, and the LOEC was 2.5 mg L^{-1}, with the LOEC reflecting the substantial impact of glutaraldehyde exposure on embryo hatch rates. These concentrations are similar to the effect-levels for *P. subcapitata*, although the required exposure duration was much longer for *O. mykiss*.

Reproduction and mortality rates of *C. dubia* were the least sensitive endpoints tested. The LOEC for reproduction and adult survival was 4.9 mg L^{-1}, which is approximately twice as high as the LOEC values for the algal and embryo–larval experiments. In general, *C. dubia* has been found to be relatively sensitive to a range of compounds compared to other aquatic invertebrates (Von Der Ohe and Liess, 2004). Acute toxicity tests using glutaraldehyde have also found neonates and adult *C. dubia* to be the most sensitive freshwater invertebrate tested, with a 24 h LC_{50} estimate of 9 and 10 mg L^{-1}, respectively (Sano et al., 2003; Table 6). Growth of *C. dubia* proved relatively insensitive to glutaraldehyde exposure, with dry weights of organisms increasing

with higher concentration (up to 4.9 mg L^{-1}), indicating potential hormesis.

4.2. Comparative data

Although published toxicity data comparable to those reported here were not found, a review article by Leung (2001b) provides a variety of unpublished toxicity values. Results from 96-h growth experiments using *P. subcapitata*, for example, reported a NOEC of 0.50 mg L^{-1} and an EC_{50} of 0.81 mg L^{-1} based on biomass growth (WIL, 1997; Table 6). These statistical endpoints are roughly comparable to those reported here, and further indicate that this algal species is sensitive to glutaraldehyde. A 21-day reproduction experiment using *D. magna* estimated a NOEC for mortality of 4.25 mg L^{-1} (CCR, 1990: Table 3). Even at this concentration, however, there were effects on reproduction rates with a reported NOEC of 2.13 mg L^{-1}. This contrasts with the results from this study, since reproduction effects in *C. dubia* were observed at the same concentrations that caused mortality in adults. It is difficult to determine whether this is due to differences in experimental protocol or to differences in species sensitivity. Both the neonates and adults of *C. dubia* have been found to be more sensitive to glutaraldehyde than those of *D. magna* (Sano et al., 2003). Thus, it is surprising that reproduction rates in *D. magna* were affected at concentrations lower than those that caused effects in *C. dubia*.

Finally, results were reported from an early life-stage study using the fathead minnow, *Pimephales promelas* (WIL, 1999; Table 6). The results suggested no differences in time-to-hatch or in hatching success of embryos, which hatched between days 4 and 5, post-fertilization; however, embryo survival was reduced at 2.9 mg L^{-1}. For the larvae from the same exposure group, no growth differences were detected between the controls and the group exposed to 1.4 mg L^{-1}. These results differ from those reported in this study: for *O. mykiss*, embryo survival was not impacted by glutaraldehyde exposure; however, hatching success was markedly affected at concentrations higher than 1.3 mg L^{-1}. The discrepancy between these results may be due to differences in treatment temperatures, to differences in experimental design (flow-through versus static renewal), or to interspecific variability in sensitivity, particularly due to the shorter incubation time of *P. promelas* (5 days) compared to *O. mykiss* (25 days). In terms of larval growth, however, the data are comparable, as concentrations up to 1 or 2 mg L^{-1} do not appear to adversely impact growth rates.

Because glutaraldehyde is also employed as a disinfectant in aquaculture, there are additional data on glutaraldehyde toxicity to other fish species, primarily marine. These applications are more representative of

Table 6
Chronic toxicity values for glutaraldehyde as estimated from studies employing similar organisms

	Exposure	EC_{50} and LC_{50}	NOEC	LOEC	Reference
Acute data					
Oncorhynchus kisutch	96 h flow-through	3	–	–	SFU (1993)
Pimephales promelas	96 h static	5.4	2.6	–	UCC (1996)
Oncorhynchus mykiss	96 h static	11	8	–	UCC (1977)
Daphnia magna	48 h static	5	–	–	UCC (1981)
Ceriodaphnia dubia neonates	24 h static	9	–	–	Sano et al. (2003)
Ceriodaphnia dubia adults	24 h static	10	–	–	Sano et al. (2003)
Daphnia magna neonates	24 h static	14	–	–	Sano et al. (2003)
Daphnia magna adults	24 h static	56	–	–	Sano et al. (2003)
Chronic data					
Pseudokirchneriella subcapitata	5 days	0.81	0.5	–	WIL (1997)
Daphnia magna	21 days	–	2.13[a]	4.25[a]	CCR (1990)
Pimephales promelas	28 days	–	1.4[b]	2.9[b]	WIL (1999)

Most data are reported in Leung (2001b), with the exception of values reported by Sano et al. (2003). All toxicity endpoints are reported as mg glutaraldehyde L^{-1}.

[a] Indicates reproduction effect.

[b] Indicates survival endpoint.

acute exposures, since the prophylactic treatments employed in aquaculture involve exposures to high concentrations of glutaraldehyde (200–400 mg L^{-1}) for short periods of time in order to reduce bacterial loads (Harboe et al., 1994; Salvesen et al., 1997). Despite these differences, the data provide important information regarding sensitive developmental periods and interspecific differences in sensitivity. In terms of the former, Escaffre et al. (2001) reported that glutaraldehyde toxicity to gilthead sea bream (*Sparus aurata*) was influenced by timing of exposure: embryos exposed at the four to eight cell stage had low hatching rates, 49% compared to controls, while embryos exposed at the blastopore closure stage (end of epiboly) exhibited higher hatch rates (98%) than controls. They also found that longer glutaraldehyde exposures (>6 min) resulted in decreased hatching rates and a higher number of imprisoned larvae (at exposures >8 min). This increase in the number of imprisoned larvae was attributed to the effect of glutaraldehyde on the egg chorion (much like that seen in this experiment), which may be due to increased hardening of the egg chorion (Salvesen and Vadstein, 1995) and/or interference of glutaraldehyde with the hatching enzyme (Escaffre et al., 2001).

In terms of interspecific variations in sensitivity, Salvesen et al. (1997) reported glutaraldehyde to be more toxic to turbot (*Scophthalmus maximus*) embryos exposed at 12 °C than to Atlantic halibut (*Hippoglossus hippoglossus*) embryos exposed at 5 °C. This difference may reflect either true differences in species sensitivity or temperature effects, since glutaraldehyde efficacy is known to decrease with decreasing temperatures (Gardner and Peel, 1986; Sano et al., 2004). Thus, it is possible that different species of fish are differentially affected by glutaraldehyde, either because of true interspecific differences or because of temperature-related effects on toxicity.

4.3. Relevance of results

The data from this study provide an initial assessment of the toxicity of glutaraldehyde to representative aquatic organisms and give an indication of the types of species and endpoints that might be more susceptible to glutaraldehyde exposure. There are several important limitations in applying these data to predicting possible environmental impacts, including the small number of surrogate species tested, the difficulty in translating effects on single species into population-, community-, and ecosystem-level impacts, and the limited range of exposure conditions tested. In terms of the first issue, the data provided from these experiments may be best utilized for identifying the taxa and life history stages of freshwater organisms that are mostly likely to be sensitive to glutaraldehyde exposure. The general indication is that certain species of phytoplankton and the early life history stages (i.e., embryos) of fish will likely be most affected by environmental releases of glutaraldehyde. This information is helpful in addressing the second shortcoming, extrapolating single species experiments to higher level impacts. Any additional efforts at assessing potential population- and community-level impacts of glutaraldehyde should thus focus on indirect effects caused by fluctuations in phytoplankton populations and impacts on embryo survival, specifically hatching rate success, of representative fish species. These indirect effects can range from alterations in behavior to changes in competition and predation (see review by Fleeger et al., 2003) and may be particularly complex for fish populations, since decreases in embryo survival or increases in hatching time may trigger a range of either compensatory (negative) or depensatory (positive) feedback processes (Myers et al., 1995). The exact magnitude and nature of both direct and indirect effects will depend on the characteristics (timing and concentration) of glutaraldehyde released into the environment and on site-specific differences in the trophic community.

The third limitation (a narrow range of testing conditions) is equally difficult to accommodate. The exposure conditions tested in this study generally resulted in pulsed exposure of the toxicant, followed by relatively rapid degradation. The initial exposure concentrations for *C. dubia*, for example, were found to vary by up to 75% over a 24-h period. This is expected with a compound such as glutaraldehyde, which is relatively reactive and readily degraded by many microorganisms (Leung, 2001a). In addition, yeast has been reported to react with glutaraldehyde, which may have further reduced active concentrations (Navarro and Monsan, 1976). Although flow-through conditions would have reduced the variability in exposure concentrations, the fluctuations reported here are more likely to be representative of environmental exposures, due to the relatively rapid degradation rate of glutaraldehyde and the pulsed nature of glutaraldehyde releases. Current

environmental releases of glutaraldehyde are generally due either to overflow from the sanitary sewer system (Jolibois et al., 2002) or to periodic releases from oil drilling and gas applications. If glutaraldehyde were implemented as a ballast water treatment method, these environmental releases would also be periodic, due to the deballasting characteristics of vessels (see Colautti et al., 2003). The impact of this exposure variability, however, is difficult to predict. In some cases, continuous chemical exposure can augment toxicity (Kallander et al., 1997; Hosmer et al., 1998), while in other situations, pulsed exposures increase toxicity (Gustavson et al., 2003).

In addition, the toxicity data reported here were collected under a limited range of temperatures (25 °C for *P. subcapitata* and *C. dubia* and 11 °C for *O. mykiss*). There is strong evidence that glutaraldehyde toxicity will be temperature dependent (Sano et al., 2004), with lower environmental temperatures partially mitigating toxicity and higher temperature augmenting it. This is further complicated by the fact that glutaraldehyde is degraded by microorganisms, which will demonstrate the opposite effect with higher temperatures resulting in more rapid degradation rates (Landrum et al., 2003). The net effect will depend on the ambient temperatures where glutaraldehyde is released and the rates of degradation and dispersion. In terms of ballast water treatment applications for the Great Lakes, sea surface temperatures in most of the lakes are less than 25 °C for most of the year. Thus, for *P. subcapitata* and *C. dubia*, the estimated toxicity from these laboratory experiments may overestimate effects for most months, except during summer periods when sea surface temperatures can reach 25 °C. The relevance of any temperature-related effect is difficult to determine, but should be considered in any evaluation of potential environmental effects.

5. Conclusions

This study provides preliminary indication of the taxa and life history stages that are most vulnerable to glutaraldehyde releases into the environment. Largely because of its mechanism of action, chronic exposure to glutaraldehyde is likely to be most detrimental to small, unprotected organisms (i.e., those lacking protective covering). In addition, for certain sensitive taxa such as *P. subcapitata*, small increases in glutaraldehyde may have a disproportionately large effect on growth rates. For sensitive life history stages such as fish embryos, the impact of glutaraldehyde at environmental concentrations will likely be on the hatching stage, unless the species has substantially shorter incubation times. The translation of these impacts into actual effects on populations and communities is more difficult to assess. Although certain algal populations are likely to be affected by longer term exposure to glutaraldehyde, these taxa demonstrate rapid growth rates, and populations may be able to rebound quickly from periodic glutaraldehyde exposure. Decreases in the hatching rate of fish, however, may cause more of an impact at the population-level, depending on the influence of density-dependent responses. Thus, the indirect impacts at both the population- and community-level are important considerations for assessing ecological effects. In addition, reliable estimates of exposure concentrations of glutaraldehyde are critical for assessing potential impacts. Most current applications of glutaraldehyde result in relatively infrequent environmental releases of glutaraldehyde over limited spatial areas. The additional application of glutaraldehyde for treating ballast water of vessels would also result in episodic releases of glutaraldehyde. The net environmental impact of these releases will depend on how much is released and the periodicity of releases. When environmental concentrations approach 1 or 2 mg L^{-1}, it will likely cause local effects on growth rates of certain algal populations. Longer term releases at this concentration level may further affect hatching success of sensitive fish species. Rapid degradation and dispersion of glutaraldehyde are likely to mitigate longer term effects, although this will depend on the nature and location of releases.

Acknowledgements

This project was funded in part through the Cooperative Institute for Limnology and Ecosystems Research, University of Michigan, under cooperative agreement (NA67RJ0148) from the Office of Oceanic and Atmospheric Administration, U.S. Department of Commerce. We thank Duane Gossiaux and Rob Jennings for help with maintaining cultures and Martha Wolgamood for supplying the steelhead trout embryos. We

also thank Russell Moll for procuring funds and providing the initial vision of the project. This manuscript is GLERL contribution no. 1338.

References

American Society for Testing and Materials (ASTM), 1998a. Standard guide for conducting static 96-h toxicity tests with microalgae. E1218-97a. In: Annual Book of ASTM Standards, vol. 11.05, Biological Effects and Environmental Fate; Biotechnology; Pesticides. Section 11, Water and Environmental Technology. American Society for Testing and Materials, West Conshohocken, PA.

American Society for Testing and Materials (ASTM), 1998b. Standard guide for conducting three-brood, renewal toxicity tests with *Ceriodaphnia dubia*. E1295-89. In: Annual Book of ASTM Standards, vol. 11.05, Biological Effects and Environmental Fate; Biotechnology; Pesticides. Section 11, Water and Environmental Technology. American Society for Testing and Materials, West Conshohocken, PA.

American Society for Testing and Materials (ASTM), 1998c. Standard guide for conducting early life-stage toxicity tests with fishes. E1241-92. In: Annual Book of ASTM Standards, vol. 11.05, Biological Effects and Environmental Fate; Biotechnology; Pesticides. Section 11, Water and Environmental Technology. American Society for Testing and Materials, West Conshohocken, PA.

Burmaster, D.E., Menzie, C.A., Freshman, J.S., Burris, J.A., Maxwell, N.I., Drew, S.R., 1991. Assessment of methods for estimating aquatic hazards at superfund-type sites—a cautionary tale. Environ. Toxicol. Chem. 10, 827–842.

Canaria, E.C., Elphick, J.R., Bailey, H.C., 1999. A simplified procedure for conducting small scale short-term embryo toxicity tests with salmonids. Environ. Toxicol. 14, 301–307.

CCR, 1990. Influence of Piror 850 on the reproduction of *Daphnia magna*. Project 164002. Cytotest Cell Research GmbH & Co., Rossdorf, Germany.

Chambon, M., Bailly, J.-L., Peigue-Lafeuille, H., 1992. Activity of glutaraldehyde at low concentrations against capsid proteins of polio-virus type 1 and echoirus type 25. Appl. Environ. Microbiol. 58, 3517–3521.

Colautti, R., Niimi, A., van Overdijk, C.D.A., Mills, E.L., Holeck, K., MacIsaac, H.J., 2003. Spatial and temporal analysis of shipping vectors to the Great Lakes. In: Ruiz, G.M., Carlton, J.T. (Eds.), Invasive Species: Vectors and Management Strategies. Island Press, Washington, DC, pp. 227–246.

Eagar, R.G., Leder, J., Stanley, J.P., Theis, A.B., 1988. The use of glutaraldehyde for microbiological control in waterflood systems. Mater. Perform. 27, 40–45.

Escaffre, A.M., Bazin, D., Bergot, P., 2001. Disinfection of *Sparus aurata* eggs with glutaraldehyde. Aquacult. Int. 9, 451–458.

Fairchild, J.F., Ruessler, D.S., Carlson, A.R., 1998. Comparative sensitivity of five species of macrophytes and six species of algae to atrazine, metribuzin, alachlor, and metolachlor. Environ. Toxicol. Chem. 17, 1830–1834.

Farley, R.B., 1996. Analysis of Overseas Vessel Transits into the Great Lakes and Resultant Distribution of Ballast Water. Report no. 331. University of Michigan, Department of Naval Architecture and Marine Engineering, Ann Arbor, MI.

Ferson, S., Ginzburg, L.R., Goldstein, R.A., 1996. Inferring ecological risk from toxicity bioassays. Water Air Soil Pollut. 90, 71–82.

Fleeger, J.W., Carman, K.R., Nisbet, R.M., 2003. Indirect effects of contaminants in aquatic ecosystems. Sci. Total Environ. 317, 207–233.

Finney, D.J., 1978. Statistical Methods in Biological Assay, third ed. Charles Griffin & Co. Ltd., London, 508 pp.

Gardner, J.F., Peel, M.M., 1986. Introduction to Sterilization and Disinfection. Churchill Livingstone, Melbourne, 183 pp.

Görge, G., Nagel, R., 1990. Toxicity of lindane, atrazine and deltamethrin to early life stage of zebrafish (*Brachydania rerio*). Ecotoxicol. Environ. Saf. 20, 246–255.

Gustavson, K., Møhlenberg, F., Schlüter, L., 2003. Effects of exposure duration of herbicides on natural stream periphyton communities and recovery. Arch. Environ. Contam. Toxicol. 45, 48–58.

Hall, L.W., Giddings, J.M., 2000. The need for multiple lines of evidence for predicting site-specific ecological effects. Hum. Ecol. Risk Assess. 6, 679–710.

Harboe, T., Huse, I., Øie, G., 1994. Effects of egg disinfection on yolk sac and first feeding stages of halibut (*Hippoglossus hippoglossus* L.) larvae. Aquaculture 119, 157–165.

Hindàk, F., 1990. Biologicke prace (Slovenskej Akademie Vied) 36, 209.

Hosmer, A.J., Warren, L.W., Ward, T.J., 1998. Chronic toxicity of pulse-dosed fenoxycarb to *Daphnia magna* exposed to environmentally realistic concentrations. Environ. Contam. Toxicol. 17, 1860–1866.

Jolibois, B., Guerbet, M., Vassal, S., 2002. Glutaraldehyde in hospital wastewater. Arch. Environ. Contam. Toxicol. 42, 137–144.

Kallander, D.B., Fisher, S.W., Lydy, M.J., 1997. Recovery following pulsed exposure to organophosphorus and carbamate insecticides in the midge, *Chironomus riparius*. Arch. Environ. Contam. Toxicol. 233, 29–33.

Landrum, P.F., Sano, L.L., Mapili, M.A., Garcia, E., Krueger, A.M., Moll, R.A., 2003. Degradation of chemical biocides with application to ballast water treatment. NOAA Technical Memorandum, NOAA.

Leung, H.-W., 2001a. Aerobic and anaerobic metabolism of glutaraldehyde in a river water-sediment system. Arch. Environ. Contam. Toxicol. 41, 267–273.

Leung, H.-W., 2001b. Ecotoxicology of glutaraldehyde: review of environmental fate and effects studies. Ecotoxicol. Environ. Saf. 49, 26–39.

Lubomudrov, L.M., Moll, R.A., Parsons, M.G., 1997. An Evaluation of the Feasibility and Efficacy of Biocide Application in Controlling the Release of Nonindigenous Aquatic Species from Ballast Water. Report to the Michigan Department of Environmental Quality, Office of the Great Lakes, Lansing, MI.

MacIsaac, H.J., Robbins, T.C., Lewis, M.A., 2002. Modeling ships' ballast water as invasion threats to the Great Lakes. Can. J. Fish. Aquat. Sci. 59, 1245–1256.

Miner, N.A., McDowell, J.W., Wilcockson, G.W., Bruckner, N.I., Stark, R.L., Whitmore, E.J., 1977. Antimicrobial and other

properties of a new stabilized alkaline glutaraldehyde disinfectant/sterilizer. Am. J. Hosp. Pharm. 34, 376–382.

Myers, R.A., Barrowman, N.J., Hutchings, J.A., Rosenberg, A.A., 1995. Population dynamics of exploited fish stocks at low population levels. Science 269, 1106–1108.

National Research Council (NRC), 1996. Stemming the Tide: Controlling Introductions of Nonindigenous Species by Ships' Ballast Water. Committee on Ships' Ballast Operations and Marine Board Commission on Engineering and Technical Systems for the National Research Council. National Academy Press, Washington, DC.

Navarro, J.M., Monsan, P., 1976. Études du mecanismes d'interaction du glutaraldehyde avec les micro-organismes. Ann. Microbiol. (Paris) B127, 295–307.

Nguyen, L.T.H., Janssen, C.R., 2002. Embryo–larval toxicity tests with the African catfish (*Clarias gariepinus*): comparative sensitivity of endpoints. Arch. Environ. Contam. Toxicol. 42, 256–262.

Pakulski, J.D., Benner, R., 1992. An improved method for the hydrolysis and MBTH analysis of dissolved and particulate carbohydrates in seawater. Mar. Chem. 40, 143–160.

Pereira, M.O., Vieira, M.J., Beleza, V.M., Melo, L.F., 2001. Comparison of two biocides—carbamate and glutaraldehyde—in the control of fouling in pulp and paper industry. Environ. Technol. 22, 781–790.

Rojíčková-Padrotová, R., Maršálek, B., 1999. Selection and sensitivity comparisons of algal species for toxicity testing. Chemosphere 38, 3329–3338.

Rutala, W.A., 1990. APIC guideline for selection and use of disinfectants. Am. J. Infect. Control 18, 99–117.

Salvesen, I., Øie, G., Vadstein, O., 1997. Surface disinfection of Atlantic halibut and turbot eggs with glutaraldehyde: evaluation or concentrations and contact times. Aquacult. Int. 5, 249–258.

Salvesen, I., Vadstein, O., 1995. Surface disinfection of eggs from marine fish: evaluation of four chemicals. Aquacult. Int. 3, 155–171.

Sano, L.L., Mapili, M.A., Krueger, A., Garcia, E., Gossiaux, G., Phillips, K., Landrum, P.F., 2004. Comparative efficacy of potential chemical disinfectants for treating unballasted vessels. J. Great Lakes Res. 30, 201–216.

Sano, L.L., Moll, R.A., Krueger, A.M., Landrum, P.F., 2003. Assessing the potential efficacy of glutaraldehyde for biocide treatment of un-ballasted transoceanic vessels. J. Great Lakes Res. 29, 545–557.

Sawicki, E., Hauser, T.R., Stanley, T.W., Elbert, W., 1961. The 3-methyl-2-benzothiazolinone hydrazone test: sensitive new methods for the detection, rapid estimation, and determination of aliphatic aldehydes. Anal. Chem. 33, 93–96.

Scudder, B.C., Carter, J.L., Leland, H.V., 1988. Effects of copper on development of the fathead minnow, *Pimephales promelas*. Aquat. Toxicol. 12, 107–124.

Sheath, R.G., Wehr, J.D., 2003. Introduction to freshwater algae. In: Wehr, J.D., Sheath, R.G. (Eds.), Freshwater Algae of North America: Ecology and Classification. Elsevier Science, San Diego, CA, pp. 1–9.

Simons, C., Walsh, S.E., Mailar, J.-Y., 2000. A note: *ortho*-phthaldehyde: proposed mechanism of action of a new antimicrobial agent. Lett. Appl. Microbiol. 31, 299–302.

SFU, 1993. The acute toxicity of glutaraldehyde to juvenile Coho salmon, *Oncorhynchus kisutch*. Simon Fraser University, Burnaby, British Columbia, Canada.

UCC, 1977. Acute toxicity of 25% aqueous glutaraldehyde to the rainbow trout, *Salmo gairdneri*. Union Carbide Corporation Environmental services Project No. 11506-61-02, Tarrytown, NY.

UCC, 1981. Glutaraldehyde ecological fate and effects studies. Union Carbide Corporation File No. 29570, South Charleston, WV.

UCC, 1996. Glutaraldehyde–reduction of fish toxicity by reaction with sodium bisulfite. Union Carbide Corporation File No. 41843, South Charleston, WV.

United States Environmental Protection Agency (USEPA), 2002. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Water to Freshwater Organisms, fourth ed, EPA-821-T-02-013.

Versteeg, D.J., Belander, S.E., Carr, G.J., 1999. Understanding single-species and model ecosystem sensitivity: data-based comparison. Environ. Toxicol. Chem. 18, 1329–1346.

Von Der Ohe, P.C., Liess, M., 2004. Relative sensitivity distribution of aquatic invertebrates to organic and metal compounds. Environ. Toxicol. Chem. 23, 150–156.

WIL, 1997. Chemical deactivation products of glutaraldehyde: a 5-day toxicity test with the freshwater alga (*Selenastrum capricornutum*). Project 142A-105. Wildlife International Ltd., Easton, MD.

WIL, 1999. Ucarcide® 250 Antimicrobial: an early life-stage toxicity test with the fathead minnow (*Pimephales promelas*). Project no. 142A-105. Wildlife International Ltd., Easton, MD.

Winer, B.J., Brown, D.R., Michels, K.M., 1991. Statistical Principles in Experimental Design, third ed. McGraw-Hill, New York.