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Short Communication

JOINT TOXICITY OF SODIUM ARSENATE AND SODIUM PERCHLORATE TO ZEBRAFISH *DANIO RERIO* LARVAE

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Abstract—Joint toxicity of arsenate and perchlorate was tested in larvae of *Danio rerio*. Results indicated that the 96-h median lethal concentrations of sodium arsenate and sodium perchlorate were 258.8 and 1,401.2 mg/L, respectively, and that arsenate and perchlorate generally showed a concentration-additive effect.

Keywords—Zebrafish Toxicity Arsenate Perchlorate Mixtures

INTRODUCTION

Aquatic ecosystems can be contaminated by mixtures of compounds that induce interactions distinct from a single-chemical toxicity [1]. Arsenate and perchlorate are two such contaminants that may be encountered in aquatic ecosystems [2–4] (http://www.atsdr.cdc.gov/HAC/PHA/nsw/nsw_p1.html). Arsenate is commonly found in surface waters because of anthropogenic activities, such as mining, metal refining, and agriculture [5]. Production of oxidative stress is one major mechanism by which arsenic causes toxicity [6]. Perchlorate contamination is found where perchlorate-containing materials, such as solid-fuel rocket propellants, fireworks, matches, and munitions, have been manufactured, processed, or used [7]. Perchlorate also has been found in certain fertilizers, irrigation water, or agricultural products [8]. The main health concern regarding perchlorate is that it inhibits thyroid function by perturbing thyroidal iodide uptake and, in turn, the synthesis of thyroid hormones [9].

Some data regarding acute toxicity of perchlorate salts and arsenate salts in fish are available [10–13], but to our knowledge, no information is available regarding their interaction. Therefore, a series of acute toxicity tests using zebrafish larvae (*Danio rerio*) were conducted to evaluate the interactive effects of perchlorate and arsenate exposure on mortality. We hypothesized that the joint toxicity of arsenate and perchlorate would differ from additivity because of the different modes of toxic action of arsenate and perchlorate.

MATERIALS AND METHODS

Adult zebrafish were reared in reconstituted water following the method described by Patiño et al. [11] and were fed goldfish flake food and frozen adult brine shrimp ad libitum. Reconstituted water consisted of reverse-osmosis water containing 60 mg/L of Instant Ocean® sea salts (Aquarium Systems, Mentor, OH, USA). Fish were induced to reproduce by maintaining the water temperature at approximately 28°C, with a photoperiod of 14:10-h light:dark and lights-on at 0800 h Central Standard Time. Eggs were collected and hatched, and larvae (age, 4 d posthatch) were used for the toxicity test.

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Sodium perchlorate (purity, 99%; CAS 7790-89) was purchased from EM Science (Gibbstown, NJ, USA) and sodium arsenate (dibasic 7-hydrate; purity, 99%; CAS 7778-43-0) from J.T. Baker (Phillipsburg, NJ, USA). The static-renewal toxicity test followed standard procedures [14] using individual glass Petri dishes (60 × 15 mm) as replicates. Tests with sodium arsenate, sodium perchlorate, and the mixtures consisted of a geometric series of five concentrations plus one control group, with 10 fish per replicate and five replicates per treatment. Exposure was conducted at a constant 28°C and a photoperiod of 14:10-h light:dark. Two individual toxicity tests were first conducted to determine 96-h median lethal concentrations (LC50s) of individual chemicals (arsenate and perchlorate). The design of the mixture-toxicity tests followed that described by Berenbaum [15] based on 96-h LC50s of the individual chemicals. Briefly, five concentrations of these two chemicals were used in a fixed ratio (sodium arsenate:sodium perchlorate, 1:6.7) at approximately 12.5, 25, 50, 100, and 200% of their respective 96-h LC50s.

Water samples were taken 24 h after the start and at the end of the experiment. Samples then were analyzed for perchlorate by ion chromatography following the method described by Smith et al. [16] and for total arsenic by atomic absorption spectrophotometer following U.S. Environmental Protection Agency Method 200.9 [17]. Measured water-quality parameters included dissolved oxygen, salinity (measured using a YSI® model 85, Yellow Springs, OH, USA), and pH (measured using an Oakton® pH meter, Gresham, OR, USA).

A logistic response model was used to obtain the shape of the concentration–response curves [18]. The LC50s and their 95% confidence limits were determined using the Probit procedure in SAS® statistical software (version 8.02; SAS, Cary, NC, USA). Joint toxicity between sodium arsenate and sodium perchlorate in the mixture was evaluated using the toxic unit (TU) concept according to the method described by Sprague and Ramsey [19]. Survival rates were plotted against TUs to obtain TU-based concentration–survival curves ($LC50_{mix}$), and the following criterion was used to evaluate the type of joint action by arsenate and perchlorate (TU_{mix}): Concentration additive ($LC50_{mix} = 1\ TU_{mix}$), greater than additive ($LC50_{mix} < 1\ TU_{mix}$), or less than additive ($LC50_{mix} > 1\ TU_{mix}$) [20]. To

evaluate the nature of the joint toxicity between these two chemicals at different exposure concentrations, the estimated lethal effect concentrations (LC*i*s) were calculated at intervals of 10%, ranging from LC10 to LC90 (i.e., i = 10, 20, 30, 40, 50, 60, 70, 80, or 90). Toxic units were then calculated for each LC*i* level (e.g., TU10, TU20, . . . , TU90). The TU*i*s were plotted against the respective LC*i*s to evaluate the nature of the joint action (additive, greater than additive, or less than additive) of these two chemicals at different effect levels.

RESULTS

The survival rate in the control group was greater than 95%, which satisfies the American Society for Testing and Materials standard [14]. Water characteristics in the reconstituted water were as follows (mean ± SD): Dissolved oxygen, 6.68 ± 0.5 mg/L; pH 6.38 ± 0.10; salinity, 100–1,100 mg/L. Goodness-of-fit tests indicated that the data were accurately predicted by the logistic model ($p < 0.05$). The 96-h LC50s (and 95% confidence intervals) for sodium perchlorate and arsenate were 1,365 (1,219–1,531) and 272 (248–296) mg/L, respectively (based on measured rather than nominal concentrations). The LC50 of the combined exposure of arsenate and perchlorate was equal to 1.0 TU, indicating an additive toxicity between these chemicals (Fig. 1). In addition, an overall strict concentration addition exists between sodium salts of arsenate and perchlorate at all LC*i*s, as indicated by the inclusion of 1 TU within the 95% confidence limits (Fig. 1C).

DISCUSSION

The present results provide two important contributions to the field of environmental toxicology. First is the investigation of the pattern of joint toxicity of these two contaminants of concern. Overall, the hypothesis that toxicity would differ from strict additivity was not supported. Second is the additional background information regarding the toxicity of arsenate and perchlorate to fish.

The acute toxicity of arsenate has been determined only for a few fish. For example, the 96-h LC50 for flannelmouth suckers (*Catostomus latipinnis*), fathead minnows (*Pimephales promelas*), mosquitofish (*Gambusia affinis*), rainbow trout (*Oncorhynchus mykiss*), razorback sucker (*Xyrauchen texanus*), and Colorado squawfish (*Ptychocheilus lucius*) were 82.1, 25.6, 49.0, 10.8, 17.8, and 105 mg/L, respectively [10,12,13]. In the present experiments, the 96-h LC50 of arsenate anion was 203.8 mg/L, and according to these results, zebrafish seem to be much less sensitive to arsenate compared with other fish species studied to date.

The acute toxicity of perchlorate been studied in even fewer fish species than that of arsenate. In one such study, the LC50 of ammonium perchlorate to early stage zebrafish (embryo to larvae) was 529 mg/L [11]. However, ammonium may have contributed to the toxicity of perchlorate. Rainbow trout, fathead minnow, and bluegill sunfish (*Lepomis macrochirus*) were more tolerant to perchlorate, with 96-h LC50s of 2,100, 1,655, and 1,470 mg/L, respectively [21,22].

In terms of toxicity of mixtures, chemicals with similarly shaped concentration–response curves behave like dilutions of each other and may have similar, or at least compensating, modes of action [1]. This might have been the case in the present study. Initially, it was predicted that the two chemicals would have different concentration–response shapes, because they were assumed to have different modes of action [6,9]. This prediction was not supported by the results. Perchlorate has been well documented as a definitive thyroidal disruptor in vertebrates, causing hypothyroidism [11,23], and hypothyroid fish also may have increased oxidative stress [24]. In addition, arsenate has been found to alter thyroid homeostasis in fishes and amphibians as well as in other vertebrates [25] (unpublished data). Therefore, it could be hypothesized that these two anions share similar, or at least compensating, modes of action (i.e., thyroid disruption and/or oxidative stress). Additional studies will be needed to test this hypothesis.

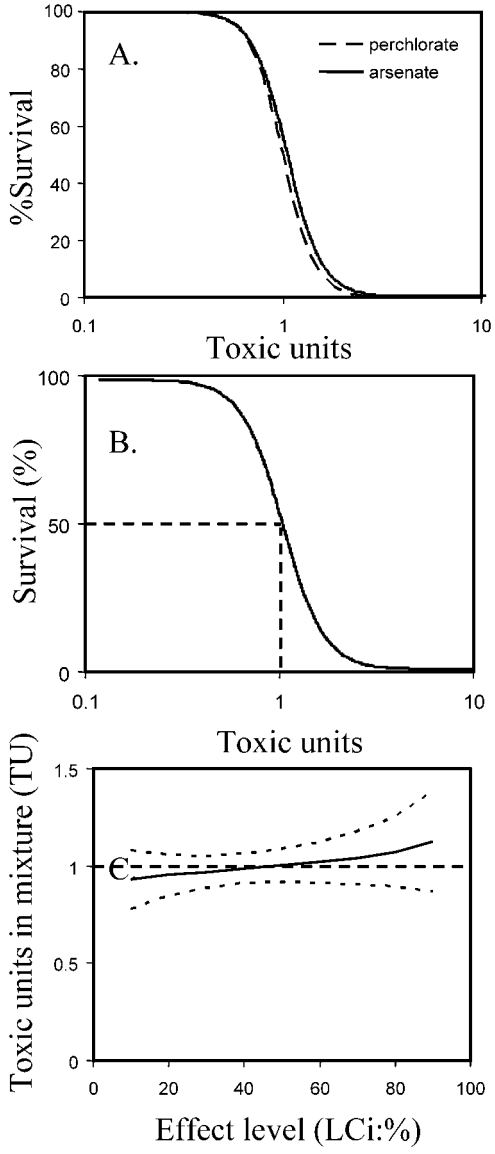

Fig. 1. Concentration–response curves for sodium perchlorate, sodium arsenate (**A**), or a mixture of two (**B**) for zebrafish larvae. Toxic units (TUs) of the mixture equals the TUs of sodium perchlorate plus the TUs of sodium arsenate. Data are plotted as the percentage survival as a function of the TUs based on experimentally derived, 96-h median lethal concentration (LC50). (**C**) The joint action between sodium arsenate and sodium perchlorate at different effect levels (LC_i = concentration that causes i [%] mortality) for zebrafish larva exposed to a mixture of sodium arsenate and sodium perchlorate. Toxic units in the mixture were plotted against the effect levels on which the TUs in the mixture were calculated. Dashed lines indicate the corresponding 95% confidence interval.

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