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Chemosphere 65 (2006) 1679–1689

CHEMOSPHERE

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Uptake, accumulation and depuration of sodium perchlorate and sodium arsenate in zebrafish (*Danio rerio*)

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Received 9 September 2005; received in revised form 3 May 2006; accepted 17 May 2006
Available online 24 July 2006

Abstract

In toxicokinetics studies, interactions between chemicals in mixtures has been largely neglected. This study examines a mixture of perchlorate and arsenate because (1) they have the potential to co-occur in contaminated aquatic habitats, and (2) a previous study by the authors found possible toxicological interactive effects. In the present study, zebrafish (*Danio rerio*) were exposed to two concentrations of sodium perchlorate (10 and 100 mg l^{-1}), sodium arsenate (1 and 10 mg l^{-1}), and the mixture-sodium perchlorate + sodium arsenate (10 + 1 mg l^{-1} and 100 + 10 mg l^{-1} Na_2HAsO_4-high mixture) for 90 d. Their uptake and accumulation by zebrafish was evaluated at 10, 30, 60, and 90 d. In addition, depuration was examined at 1, 3, and 5 d after cessation of the exposure. The uptake of either chemical was concentration-dependent, with significantly higher uptake at high concentrations at either exposure interval. In contrast, there was no significant difference in whole body residue between single chemicals and the corresponding mixture except for 100 mg l^{-1} sodium arsenate at 90 d. However, there was increasing accumulation over time at the high concentration of either chemical alone and their mixture, and this increasing trend was more pronounced in the single chemical exposures than in the mixture. At the concentrations tested in the current study, both chemicals reduced the uptake but enhanced the depuration of the other chemical from the zebrafish. This study represents the first examination of the interaction of two anions-perchlorate and arsenate with respect to toxicokinetics.

Keywords: Zebrafish; Uptake; Depuration; Sodium arsenate; Sodium perchlorate

1. Introduction

In contaminated environments, chemicals very rarely occur singly, but usually occur as a part of a complex mixture. Unfortunately, however, toxicokinetics studies do not generally examine potential interactive effects of chemicals in mixtures. Thus, there is a need for more studies that expose organisms simultaneously to two or more chemicals, in order to provide more accurate input for ecological risk assessments of chemical mixtures.

With regards to this, the present study examines uptake and elimination of sodium perchlorate and sodium arsenate, alone and in mixtures. These two chemicals were chosen for the following reasons: (1) Both perchlorate and arsenic are contaminants in aquatic ecosystems (Urbansky and Shock, 1999; Pedlar and Klaverkamp, 2002), and have been found to co-occur in aquatic habitats (Pitten et al., 1999). These two anions may be taken up and accumulated in aquatic animals, and an interaction in uptake and accumulation between them would affect their toxicity to aquatic animals. (2) This research paper is part of a larger series of studies designed to examine the effects of xenobiotic-induced thyroid disruption (using perchlorate as a model

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0045-6535/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2006.05.030

thyroid disruptor) and oxidative stress (using sodium arsenate as a model pro-oxidant compound). One of our studies found an additive interaction between these two anions in terms of acute toxicity (96-h LC_{50}; Liu et al., 2005). However, no information is available with respect to interactive effects on the uptake, accumulation, and elimination of these two anions. More importantly, we have found an interaction between these two chemicals in terms of thyrotoxicity (Liu et al., 2006) and oxidative stress responses (Liu, 2006). Although a cellular mechanism may at least partially explain the patterns of interaction, it is also possible that toxicokinetic interactions also play a role.

Perchlorate was a focus of these studies not only because it is a model thyrotoxicant, but also because it is an environmental contaminant of concern. Perchlorate, is and has been, used in many applications, such as a therapeutic drug, a solid rocket propellant, and an ignitable source in munitions and fireworks (Strawson et al., 2004). In addition, perchlorate occurs naturally in nitrate-rich mineral deposits used in fertilizers, and thus may cause perchlorate contamination of agricultural crops (Susarla et al., 1999). Water contamination by perchlorate has been widely reported in many states in the United States, such as Arizona, California, Utah, Colorado, Texas, and Nevada (Strawson et al., 2004).

Perchlorate contamination is of concern not only because of its widespread occurrence—particularly in drinking water—but also its toxicology as a thyroid hormone disruptor. Perchlorate competitively inhibits iodide uptake through the NIS and therefore reduces thyroid hormone synthesis (Strawson et al., 2004). For these reasons it was added to the Contaminant Candidate List (CCL) for drinking water by EPA in 1998 (US EPA, 1998).

Perchlorate fate and effects in aquatic environments is also of concern because perchlorate salts are very water soluble and stable in water, and thus are persistent in aquatic environments (Urbansky and Shock, 1999). Perchlorate occurrence and effects has been examined in a variety of fish and frog species in Texas, USA (Theodorakis et al., 2005, 2006). Hence, there is possibility that humans are exposed to perchlorate by consumption of contaminated fish.

In addition, our previous research focused on arsenic not only because it is a model pro-oxidant, but it is also a contaminant of concern. In the environment, arsenic is found as a result of anthropogenic activities and natural processes (Bernstam and Nriagu, 2000). Arsenic toxicity depends on its chemical form (Aposhian et al., 2004). Arsenate (+5) is usually the dominant form in natural waters (Liu et al., 2005). Arsenic exerts its effect mainly through consumption of antioxidants in organisms via binding to sulfhydryl groups on proteins and production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Aposhian et al., 2004). Therefore, it is listed as a carcinogen (Huang et al., 2004).

Although there is the potential for perchlorate and arsenate to co-occur in the environment, and there is evidence that they may interact toxicologically (Liu, 2006; Liu et al., 2006), there is little information on the effects of one chemical on the uptake of another in such mixtures. Thus, the purpose of this study was to evaluate the uptake and depuration kinetics of arsenate and perchlorate anions. Zebrafish have been widely used in various fields, such as genomics, pharmacology, and toxicity studies (Patiño et al., 2003; Goldsmith, 2004). We used zebrafish as a model in the current study. In the current study, we intended to explore if there was interaction, which was indicated by thyrotoxicity in a similar study (Liu et al., 2006), between two anions at low and high concentrations in terms of toxicokinetics. The hypotheses tested in the current study are (1) these two anions are not accumulated significantly in the zebrafish due to their strong hydrophilicity, and (2) either chemical does not affect the accumulation and depuration of the other chemical.

2. Materials and methods

2.1. Experimental chemicals and animals

Anhydrous sodium perchlorate was purchased from EM Science (Gibbstown, NJ, USA), and sodium arsenate (dibasic 7-hydrate) from J.T. Baker (Phillipsburg, NJ, USA). Stock solutions (sodium arsenate: 50 g l^{-1}; sodium perchlorate: 100 g l^{-1}) were prepared by adding appropriate amounts of chemicals to 18.3-MΩ Milli-Q water to ensure concentrations of stock solutions below the solubility of the chemicals. The concentration of perchlorate was reported as $NaClO_4$, and that of arsenate as Na_2HAsO_4.

Male zebrafish, *Danio rerio*, were used and supplied commercially by Ekkwill Waterlife Resources (Gibsonton, FL). Fish were treated with antibiotics upon arrival to the lab for 5 d and then maintained in reconstituted water (60 mg l^{-1} Instant Ocean® sea salts in reverse osmosis water) free from antibiotics. Fish were allowed to acclimate to laboratory conditions for two weeks before initiation of the exposure. Fish were fed goldfish flake food twice per day *ad libitum*. Those fish free of any deformities, disease, or lesions were used in the experiment. Fish weight used in the experiment averaged 0.28 ± 0.03 g (wet weight ± SD).

2.2. Water quality

Water quality parameters tested included dissolved oxygen (DO), conductivity, pH, temperature, salinity, and unionized ammonia, DO, salinity, conductivity, and temperature were measured with an YSI Model 85 meter (Yellow Springs Instrument Co., Yellow Springs, OH, USA). The pH was measured using an Oakton® pH meter (Gresham, OR, USA). Unionized ammonia ion was determined with a Hach® spectrophotometer model DR/2000 (Loveland, CO, USA).

2.3. Experimental design

The experiment was conducted in 80-l indoor rectangular glass aquaria filled with 60 l test solutions. Zebrafish

were randomly assigned to each aquarium with triplicates of 7 concentrations, including control, 10, and 100 mg l^{-1} sodium perchlorate, 1 and 10 mg l^{-1} sodium arsenate, 10 mg l^{-1} sodium perchlorate + 1 mg l^{-1} sodium arsenate, or 100 mg l^{-1} sodium perchlorate + 10 mg l^{-1} sodium arsenate. Each experimental unit (tank) contained approximately 70 fish. Test solutions consisted of 60 mg l^{-1} Instant Ocean® sea salts with desired amount of stock solution of either chemical added to the test aquaria.

During the exposure, 1/3 of the test solutions were changed twice per week. The aquaria were refilled with reconstituted water, and the desired chemical stock solutions were added to maintain constant chemical concentrations. Water quality and water temperature were measured before water change. Water samples were taken from the center of each tank before water changes. Water samples for perchlorate analysis were stored at 4 °C and analyzed within 2 weeks. The sampling, storage, and analysis for arsenic in water samples were conducted following EPA Method 200.9 (1994). Briefly, a 10-ml water sample was acidified with 30 μl 35% nitric acid to reach a final pH of less than 2, and then stored at 4 °C until analysis. The water temperature in the aquaria was maintained around 24 °C using submerged heaters. During the experiment, the photoperiod was set at 14-h light: 10-h dark. The water level was checked routinely, and water was added as needed to maintain a constant water level.

The exposure lasted for 90 d. At 10, 30, 60, and 90 d, fish were sampled from each aquarium for chemical analysis. There were three replicate aquaria sampled per concentration at each sampling time point. Within each replicate, three fish were analyzed for arsenic and three for perchlorate. Due to the small size of zebrafish, the three fish from each replicate aquarium at each interval were pooled to get enough tissue for perchlorate analysis whereas arsenic analysis was conducted on individual fish. Following the uptake test, the remaining fish were rinsed four times in deionized water and then transferred to reconstituted water free from chemicals. At 1, 3, and 5 d after the transfer, three fish were sampled from each replicate aquarium for chemical analysis. At the time of sampling, fish were removed from the aquaria, rinsed with deionized water, euthanized in MS-222 (0.5 g l^{-1}), and weighed. The fish samples for arsenic analysis were placed in plastic bottles and stored at −80 °C until analysis. The fish samples for perchlorate analysis were desiccated in a fume hood until a constant weight was reached, and they were then processed for chemical analysis.

2.4. Characterization of uptake and depuration

Depuration constants were derived for either exposure scenario. Depuration characters were modeled using the first-order one-compartment bioconcentration model (Newman, 1995). Depuration data were fitted to a single-exponential elimination model, a power elimination model, and their linearized transformed models. The single-exponential model and its linearized form are as follows:

$$C_t = C_0 e^{-k_e t} \quad (1)$$

$$\ln C_t = \ln C_0 - k_e t \quad (2)$$

The following is the power elimination model and its linearized transformation:

$$C_t = C_0 t^{-p} \quad (3)$$

$$\ln C_t = \ln C_0 - p \ln t \quad (4)$$

where C_t is the concentration of chemical in whole fish (ng g^{-1} wet weight) at time t (hour), C_1 the concentration of chemical in whole fish (ng g^{-1} wet weight) at $t = 0$, and p a constant (h^{-1}).

The biological half-life ($t_{1/2}$) and the mean lifetime (τ) of chemicals in the zebrafish were calculated according to the following equations:

$$t_{1/2} = \frac{\ln 2}{k_e} \quad (5)$$

where $t_{1/2}$ is the biological half-life (h), and k_e the elimination rate constant (h^{-1})

$$\tau = \frac{1}{k_e} \quad (6)$$

where τ is the mean lifetime (h) and the k_e the elimination rate constant (h^{-1}).

The model selection was based on Akaike's information criterion (AIC) along with consideration of biological plausibility. The models chosen had the lowest AIC value. In toxicology, nonlinear models are routinely used to depict chemical depuration. In the current study, if a linearized model best fit the data, it was backtransformed to the corresponding nonlinear model with a provisional back transformation factor (Newman, 1995).

Uptake constants at day 10, 30, 60, and 90 were determined by the following first-order bioconcentration model (EPA, 1996) after the depuration constants were determined

$$C_t = C_w \times \frac{k_u}{k_e} \times (1 - e^{-k_e t}) \quad (7)$$

where C_t and C_w are the concentrations of chemical in whole fish (μg g^{-1} wet weight) and in test solution (mg l^{-1}), respectively, at time t (d), k_u(d^{-1}) and k_e(d^{-1}) the uptake constants and depuration constant, respectively, at time t.

2.5. Chemical analysis

A Thermo Model M series atomic absorption spectrometer (Thermo electron corporation, Cambridge, United Kingdom) equipped with a GF95 graphite furnace atomizer was used for arsenic analysis. Fish samples were first digested following EPA Method 200.3 (1991). In brief, the sample was digested at 95 °C with concentrated nitric acid (69%, tracemetal grade) followed by further oxidation with hydrogen peroxide (30%). The volume of the digestate

was adjusted to 5 ml. Acidified water samples (pH < 2) and fish digestates were analyzed following EPA Method 200.9 (1994). In addition to running laboratory reagent blanks (LRB), laboratory fortified blanks (LFB), and a laboratory fortified matrix (LFM), an additional analytical quality control for fish samples was achieved by digesting and analyzing standard reference material (Dog fish muscle, DORM-2, NRC-CNRC, Canada). Based on results from the LFM for fish samples, there were components in fish digestate which markedly depressed arsenic signal, thus seriously underestimating arsenic residue in fish. Therefore, the standard addition technique was applied for arsenic analysis in fish digestates (EPA, 1994). Namely, a standard calibration curve was prepared by using digestates of non-exposed fish instead of water.

Desiccated fish samples were extracted for perchlorate with a Dionex 200 Accelerated Solvent Extractor (ASE). The extracts were collected, and a portion was cleaned using silica and C18 solid-phase extraction (SPE) cartridges followed by filtration (0.45 μm). Water samples were filtered (0.45 μm) prior to analysis. All samples were analyzed following a method similar to EPA Method 314.0 (Hautman et al., 1999) using a Dionex DX-500 Ion Chromatography System equipped with a GP50 gradient pump, and a CD20 conductivity detector (Dionex Corp., Sunnyvale, CA, USA). A Dionex IonPac AS16 (250-mm × 4.0-mm) analytical column was used for ion separation. The quality controls used included blanks, matrix spikes, and check standards. Percent recoveries and detection limits were determined using spiked water samples, fish tissues, and aqueous fish extracts.

2.6. Statistical analysis

Statistical analysis was performed using SAS software (SAS®, version 9.1, Cary, NC, USA). Normality within groups was tested using Shapiro-Wilk test, and homogeneity of variance across groups using Levene's test. One-way ANOVA followed by a DUNCAN's multiple comparison test was used to test: (1) mean comparisons of whole body concentrations among treatments of either chemical, singly or in a mixture, for each exposure interval or (2) comparisons among exposure intervals for the same treatment and chemical concentration. The difference was considered significant at $p \leqslant 0.05$. The data were expressed as mean (±SD) or mean (±SE).

3. Results

3.1. Water quality and chemical concentrations in water

Water quality during exposure is presented in Table 1. The temperature over the course of the study was 23.4 ± 0.8 °C (Mean ± SD).

Actual concentrations for sodium perchlorate and sodium arsenate were 103.4 ± 11.7% and 104.0 ± 11.6% of the nominal concentrations, respectively (Table 2).

The percent recovery of perchlorate from fish tissues ranged from 81% to 96%. The detection limit of perchlorate in fish tissues was 2.5 μg kg^{-1} and 1.0 μg l^{-1} in water samples. The detection limit for arsenic in water samples was 0.65 μg l^{-1} and in fish samples 0.033 μg g^{-1} wet weight. For arsenic analysis, the recovery rate from the standard reference material was 95.6 ± 2.1%.

3.2. Uptake, accumulation, and depuration of chemicals by fish

A correction factor, 0.2, for conversion from wet weight to dry weight of fish has been widely cited in literature (Jjarvinen and Ankley, 1999; Pedlar and Klaverkamp, 2002). In the current study, when zebrafish were desiccated to a constant dry weight, the factor was found to be 0.33 ± 0.04. This is probably due to the different size among fish species. This factor was applied in the current study when the comparison of chemical residue in fish body was conducted between the current study and the literature.

Fig. 1 illustrates whole body residues of sodium perchlorate in zebrafish at the uptake and depuration phases. The uptake of perchlorate by zebrafish in 100 and 100 + 10 mg l^{-1} treatments was significantly higher than that in 10 and 10 + 1 mg l^{-1} treatments ($\alpha = 0.05$), resulting in much higher whole body residue of perchlorate in fish. In addition, the perchlorate residue in 100 mg l^{-1} perchlorate treatment was significantly higher than that in

Table 1
Water quality in different treatments over experiment time (Mean ± SE)[a]

Chemical	Concentration (mg l^{-1})	pH	Special conductivity (mS cm^{-1})	DO (mg l^{-1})	Salinity (mg l^{-1})	NH_3-N (mg l^{-1})
Control	0	6.8 ± 0.4	122.3 ± 11.7	7.1 ± 0.2	60.0 ± 4.0	0.65 ± 0.18
$NaClO_4$	10	6.7 ± 0.2	137.7 ± 10.1	7.2 ± 0.2	67.8 ± 5.1	0.68 ± 0.17
$NaClO_4$	100	6.7 ± 0.1	218.3 ± 10.8	7.2 ± 0.2	105.2 ± 5.8	0.73 ± 0.17
Na_2HAsO_4	1	6.7 ± 0.1	130.9 ± 10.7	7.3 ± 0.2	62.9 ± 4.6	0.67 ± 0.19
Na_2HAsO_4	10	6.8 ± 0.1	137.1 ± 10.7	7.2 ± 0.2	66.6 ± 5.5	0.66 ± 0.16
$NaClO_4 + Na_2HAsO_4$	10 + 1	6.8 ± 0.1	140.2 ± 10.1	7.2 ± 0.3	68.5 ± 4.5	0.71 ± 0.18
$NaClO_4 + Na_2HAsO_4$	100 + 10	6.8 ± 0.1	224.7 ± 13.1	7.1 ± 0.3	110.7 ± 4.7	0.73 ± 0.16

[a] Water quality was measured twice per week just prior to water change and in all treatments. Water samples were removed from the center of the aquarium and then measured for non-ion ammonia concentrations and pH values. The average and standard error were calculated using all data points over exposure time for each treatment.

Table 2
Chemical concentrations (mg l^{-1}) in test solutions during exposure (Mean ± SE)[a]

Chemical	Treatments (mg l^{-1})	$NaClO_4$ (mg l^{-1})		Na_2HAsO_4 (mg l^{-1})	
		Nominal	Actual	Nominal	Actual
Control	0	0	ND[b]	0	ND
$NaClO_4$	10	10	9.28 ± 0.56	0	ND
$NaClO_4$	100	100	113.99 ± 10.70	0	ND
Na_2HAsO_4	1	0	ND	1	0.92 ± 0.13
Na_2HAsO_4	10	0	ND	10	9.63 ± 1.45
$NaClO_4 + Na_2HAsO_4$	10 + 1	10	9.35 ± 0.61	1	0.93 ± 0.12
$NaClO_4 + Na_2HAsO_4$	100 + 10	100	114.58 ± 11.15	10	9.57 ± 1.24

[a] 20 ml and 10 ml water samples were removed twice per week prior to water change for the analysis of perchlorate and arsenic, respectively. The water samples for perchlorate analysis were remained at 4 °C and analyzed within two weeks, and those for arsenic analysis were acidified with nitric acid until pH was less than 2 and then kept at 4 °C until analysis.
[b] ND: not detected.

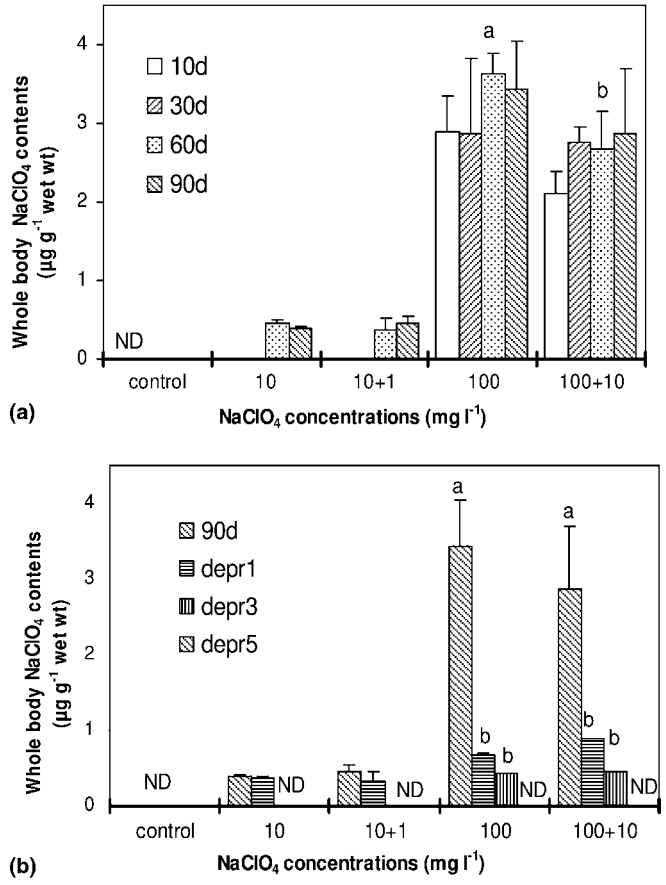

Fig. 1. The whole body residue of sodium perchlorate (Mean + SD, $n = 3$) in zebrafish during the uptake stage (A) and the depuration stage (B). Fish were exposed to chemicals for 90 d and then transferred to chemical-free reconstituted water for 5 d. Perchlorate residue in 100 and 100 + 10 mg l^{-1} treatments at depuration day 5 was not detected. ND = not detected and depr = depuration. Due to an accidental contamination of fish samples at day 10 and 30 from 10 and 10 + 1 mg l^{-1} treatments, no data were reported here for these two exposure scenarios. Significant difference was found between the high and low chemical concentrations at either exposure intervals, regardless of the single chemical or the mixture exposure. The bar bearing different letters indicated significant difference ($\alpha = 0.05$).

100 + 10 mg l^{-1} at day 60 interval ($\alpha = 0.05$). Because the fish samples from 10 and 10 + 1 mg l^{-1} treatments at day 10 and 30 were accidentally contaminated by perchlorate during pretreatment for perchlorate analysis by IC (i.e., during ASE extraction), the data were not presented. High tissue retention of perchlorate in the higher concentration treatments (100 and 100 + 10 mg l^{-1}) during uptake stage resulted in high body residue at elimination day 1 and 3, but by elimination day 5 no perchlorate was detected even in high perchlorate concentration treatments. At the lower perchlorate concentration treatments (10 and 10 + 1 mg l^{-1}), no significant difference was found among exposure intervals ($\alpha = 0.05$). As seen from Fig. 1, the elimination of perchlorate was very fast at the high concentration treatments during elimination day 1, and then the elimination rate apparently decreased. In contrast, the elimination during day 1 at the lower concentration treatments was slow, and no significant difference was observed between 90 d of exposure and elimination day 1 ($\alpha = 0.05$). The perchlorate residue in fish at lower concentration treatments was not detected at depuration day 3 in contrast to at depuration day 5 at the higher concentration treatments (Fig. 1).

The comparison of the whole body perchlorate residue among different perchlorate exposure scenarios and bioconcentration factors (BCFs) was demonstrated in Fig. 2. The ratio of sodium perchlorate residue in fish at high/low perchlorate treatments was lower than that of sodium perchlorate in test solutions (10:1), regardless of single chemical or the mixture exposure (Fig. 2a). Instead, lower ratios were observed at day 60 and 90. In particular, at elimination day 1, the ratio was just around 2 (Fig. 2a), which suggested that the depuration rate from fish in high perchlorate treatments was far faster than that in the lower perchlorate treatments during depuration day 1. The uptake and depuration of perchlorate was faster in 100 mg l^{-1} sodium perchlorate treatment than that in 100 + 10 mg l^{-1} treatment (i.e., the ratio was greater than one at the uptake stage and less than one at the depuration stage) but no constant trend was observed in the lower treatments (Fig. 2b). As seen from Fig. 2c, BCFs were at a range from 0.06 to 0.14, and thus, perchlorate is not accumulated in zebrafish significantly.

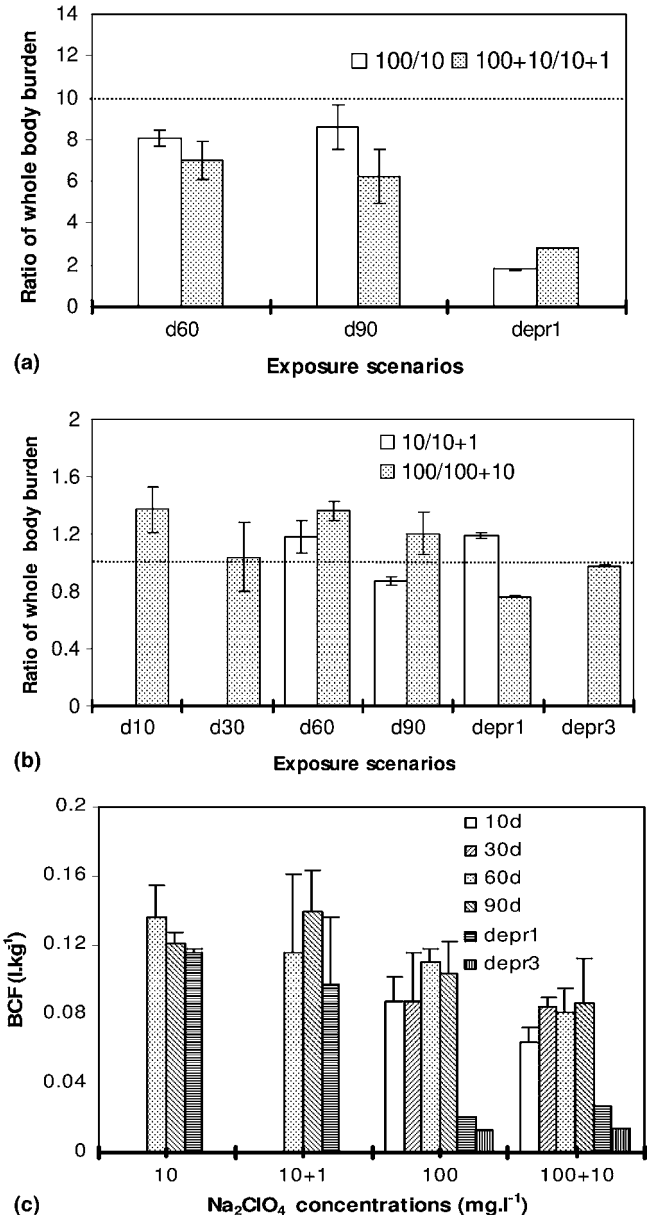

Fig. 2. Ratios of whole body residue (Mean + SE, $n = 3$) and bioconcentration factors of sodium perchlorate and bioconcentration factors of sodium arsenate (Mean ± SE, $n = 9$) among different exposure scenarios ($mg\,l^{-1}$). (a) displayed the ratios between high concentration and low concentration treatment for either perchlorate alone or the mixture, (b) the ratios between perchlorate alone and the mixture at the same sodium perchlorate concentration, and (c) the bioconcentration factors during the exposure course. Due to the accidental contamination for the samples in 10 and $10 + 1\,mg\,l^{-1}$ treatments at day 10 and 30, those ratios were not displayed. Perchlorate was not detected in 10 and $10 + 1\,mg\,l^{-1}$ at depuration day 3 and no ratios were displayed for this specific exposure.

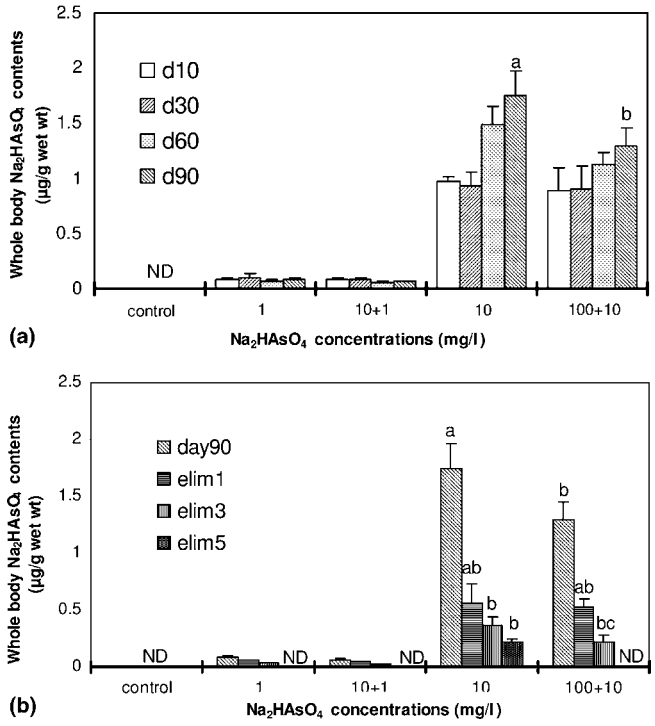

Fig. 3. The whole body residue of sodium arsenate (Mean + SE, $n = 9$) in zebrafish during the uptake stage (a) and the depuration stage (b). Fish were exposed to chemicals for 90 d and then transferred to chemical-free reconstituted water for 5 d. ND = not detected and depr = depuration. Significant difference was found between the high and low chemical concentrations at either exposure intervals, regardless of the single chemical or the mixture exposure. The bar bearing different letters indicated significant difference ($\alpha = 0.05$).

The whole body arsenic residue in fish at the uptake and depuration stages was plotted in Fig. 3. The arsenic residue at the high sodium arsenate treatments (10 and $100 + 10\,mg\,l^{-1}$) was significantly higher than that at the low sodium arsenate treatments (1 and $10 + 1\,mg\,l^{-1}$) at either interval. The highest residue occurred in $10\,mg\,l^{-1}$ sodium arsenate treatment at day 90 and was significantly higher than that in the mixture of 100 sodium perchlorate + $10\,mg\,l^{-1}$ sodium arsenate ($\alpha = 0.05$; Fig. 3). During the depuration stage, a similar pattern was found in that arsenic body residue at the high chemical treatments was much higher than that in the low treatments. Arsenic was not detected at depuration day 5 in $1\,mg\,l^{-1}$ treatment, at depuration day 3 in $10 + 1\,mg\,l^{-1}$ treatment, at depuration day 5 in $100 + 10\,mg\,l^{-1}$ treatment, respectively, but was detected at depuration day 5 in $10\,mg\,l^{-1}$ treatment. No statistically significant difference was observed during uptake course in either exposure scenario although an apparent increase over time occurred in the $10\,mg\,l^{-1}$ treatment ($\alpha = 0.05$; Fig. 3). As seen from Fig. 3, compared with the low arsenate concentration treatments, where the depuration rate was slow, the depuration was rapid at the high concentration treatments and followed by a slower depuration. The capability of fish to accumulate arsenic in the mixture was lower than that in the corresponding arsenic alone treatment, but depuration was faster in the mixture than the corresponding arsenate alone treatment.

The most pronounced accumulation of arsenic by zebrafish occurred at day 60 and 90, where approximately 20 times more arsenic was accumulated in high arsenic treatments compared to that in the low arsenic treatments (Fig. 4a). At other uptake intervals, arsenic was accumulated proportionally in low and high concentrations (i.e.,

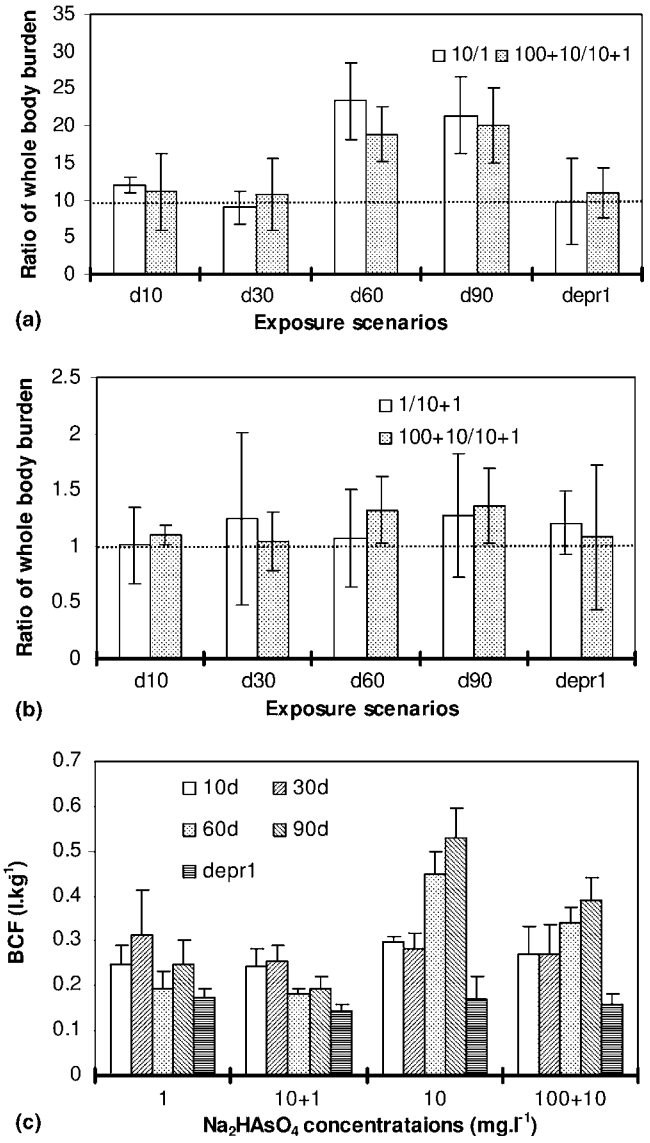

Fig. 4. Ratios of whole body residue (Mean ± 95% confidence limit) and bioconcentration factors of sodium arsenate (Mean + SE, $n = 9$) among different exposure scenarios (mg l^{-1}). (a) displayed the ratios between the high concentration and low concentration treatments for either perchlorate alone or the mixture, (b) the ratios between perchlorate alone and the mixture at the same sodium perchlorate concentration, and (c) the bioconcentration factors during the exposure course.

around 10 times the arsenic residue at high arsenic concentrations compared with the residue at low concentrations), regardless of chemical alone or the mixture. In addition, arsenic was depurated by zebrafish at a similar rate at depuration day 1 and 3 at different arsenic concentrations, indicated by the body arsenic ratio (close to 10:1) similar to the arsenic ratio in test solutions. This was a different pattern from perchlorate, which was depurated at a higher rate at high concentrations. A general trend for the difference between the arsenic alone and the mixture is that the arsenic uptake was faster but the arsenic depuration was slower in the chemical alone exposure than the mixture (Fig. 4b), though in most cases no statistical significant difference was observed (Fig. 3). Arsenic was not bioconcentrated in the fish, because the highest BCF for arsenic was 0.53 (Fig. 4c).

3.3. Mathematical characterization of uptake and depuration of chemicals by fish

Uptake constants were determined and used to evaluate the uptake rate over the course of exposure. The analysis results were listed in Table 3. As seen from Table 3, except for the 10 mg l^{-1} sodium arsenate treatment at day 10 and 30, in all single chemical treatments the uptake constants were higher than that in the corresponding mixture treatments at either exposure interval. This indicated that the uptake of one chemical was reduced by the other chemical at the concentrations of either chemical tested in the current study. Due to the accidental contamination of fish samples from 10 and 10 + 1 mg l^{-1} sodium perchlorate treatments at day 10 and 30, no uptake constants were determined for these two treatments. Depuration data of two chemicals were fitted to nonlinear models as well as their linearized models. The modeling results were showed in Table 4. Based on the AIC values, the linearized exponential models were best for 100 mg l^{-1} sodium perchlorate and 1 mg l^{-1} sodium arsenate. The linearized exponential models were backtransformed to the nonlinear exponential model, and a bias correction factor was applied for the backtransformation (Newman, 1995). Namely, a value for whole body residue of a chemical during depuration stage should be the

Table 3
Uptake constants over exposure time of day 10, 30, 60, and 90 at differential exposure scenarios

Treatment	Concentration (mg l^{-1})	Depuration constant (k_e) (d^{-1})	Uptake constants (k_u) over time course (d^{-1})			
			10	30	60	90
$NaClO_4$	100	0.64	0.018	0.018	0.023	0.025
$NaClO_4 + NaAsO_4$	100 + 10	0.56	0.012	0.016	0.015	0.016
$NaAsO_4$	1	0.25	0.025	0.031	0.019	0.024
$NaClO_4 + NaAsO_4$	10 + 1	0.26	0.022	0.021	0.015	0.016
$NaAsO_4$	10	0.4	0.042	0.040	0.064	0.075
$NaClO_4 + NaAsO_4$	10 + 1	0.56	0.050	0.050	0.063	0.072

The uptake and depuration constants were derived by using whole body chemical contents with unit of μg l^{-1} wet weight and were not conducted for 10 and 10 + 1 mg l^{-1} sodium perchlorate due to the accidental contamination of fish samples by perchlorate.

Table 4
Depuration modeling of chemicals in fish while they were transferred to a chemical-free reconstituted water after a 90-day exposure to the chemicals (Y: whole body residue of chemical; unit: ng/g wet wt)

Chemical	Concentration (mg l^{-1})	Models fitted	R^2	Bias correction factors	$t_{1/2}$ (h)	τ (h)
$NaClO_4$	100	$Y = 2421.14EXP(-0.027 \times hour)$	0.66	1.39	25.68	36.98
$NaAsO_4$	1	$Y = 85.55EXP(-0.01 \times hour)$	0.66	1.18	69.94	100.72
$NaAsO_4$	10	$Y = 1176.15EXP(-0.016 \times hour)$	0.66	1.32	42.99	61.92

one predicted by the model and then multiplied by the bias correction factor. A power elimination model, $Y = 1032.8 \times hour^{-0.273}$, was found to best depict the arsenic depuration pattern at the 10 mg l^{-1} sodium arsenate treatments based on the AIC value. However, the biological half-life was 2.54 h based on this model, which significantly underestimated the retention time of arsenic at 10 mg l^{-1} treatment. Hence, the linearized exponential model was chosen, which gave a reasonable biological half-life at this exposure scenario. Arsenic was depurated more rapidly in the 10 mg l^{-1} arsenic treatment ($k_e = 0.016\ h^{-1}$) than in the 1 mg l^{-1} arsenic treatment ($k_e = 0.01\ h^{-1}$), and thus the biological half-life of arsenic in zebrafish was reduced with increased exposure concentrations (Table 4). The depuration constants in the mixture were bigger than that in the corresponding single chemical exposure, regardless of the chemicals. Therefore, the existence of one chemical enhanced the depuration of the other chemical in the test solution.

4. Discussion

In general, the results indicated that these two chemicals were not significantly accumulated in zebrafish even chronically exposed to high concentrations of either chemical. However, the hypothesis of no interaction between perchlorate and arsenic occurs in terms of uptake, accumulation, and depuration, was not supported.

In regards to perchlorate toxicokinetics, the findings of the current study are similar to what has been found in other fish species. In a series of papers, Theodorakis and coworkers examined the perchlorate toxicokinetics (uptake, accumulation, and depuration) in fish. After adult mosquitofish *Gambusia holbrooki* were exposed to a variety of sodium perchlorate concentrations, including 0, 0.1, 1, 10, 100, and 1000 mg l^{-1}, for 2, 10, and 30 d, perchlorate uptake and accumulation were examined. Perchlorate was not detected at the concentrations of 0, 0.1, and 1 mg l^{-1}, regardless of exposure duration. Sodium perchlorate residue in whole body fish at day 2, 10, and 30 were 2.61, 0.75, and 1.25 μg g^{-1} dry weight (dw) at 10 mg l^{-1}, and 16.03, 10.09, and 9.72 μg g^{-1} dw at 100 mg l^{-1} (Bradford et al., 2006). In the current study, due to the accidental perchlorate contamination of fish samples from 10 and 10 + 1 mg l^{-1} perchlorate treatments at 10 and 30 d, the comparison of the capability and characteristics of perchlorate accumulation between these two species could not be conducted. However, the sodium perchlorate residues in 100 mg l^{-1} treatments were similar between these two species at two intervals (i.e., 10 and 30 d). The sodium perchlorate residues by dry weight in the current study were 8.76 and 8.73 μg g^{-1} dw at day 10 and 30. Therefore, the perchlorate residue in zebrafish exposed to 10 mg l^{-1} sodium perchlorate was probably roughly 1μg g^{-1} dw.

Rapid uptake of perchlorate has also been reported in rats (*Rattus rattus*), hens (*Gallus gallus*), and cattle (*Bos primigenius taurus*); Batjoens et al., 1993; Peña et al., 1976; Yu et al., 2002. This occurred in fish as well. In the study by Bradford et al. (2006), peak perchlorate concentration was observed at day 2 after initiation of the exposure at 10, 100, and 100 mg l^{-1} sodium perchlorate among all exposure intervals tested in the study. This indicated that a steady-state concentration of perchlorate was reached in fish relatively quickly. However, an increase of 29% and 16% of sodium perchlorate residue occurred at day 60 and 90, respectively, compared with 30 d at the 100 mg l^{-1} treatment although the increases were not statistically significant (Fig. 1). Perchlorate equilibrium is determined by uptake and excretion processes. In mammals, gastrointestinal tract and skin may be perchlorate uptake sites since they express the sodium-iodide symporters (NIS), which may competitively take up perchlorate (Yu et al., 2002). Perchlorate excretion occurs mainly in urine in mammals (Yu et al., 2002). However, in freshwater teleosts this may not be a major route of excretion due to their low urine production (Suhendrayatna et al., 2002). In teleosts, many water-soluble chemicals are excreted via the gills (Evans, 2002), and presumably this is the major route of perchlorate elimination as well. The rapid uptake, elimination, and achievement of steady state are similar to other water-soluble xenobiotics, taken up and excreted through the gills.

In the current study, perchlorate uptake and elimination rates were dependant on exposure concentrations. Similar results were also found in many other studies, in which a dose-dependent uptake and accumulation of perchlorate was observed (Batjoens et al., 1993; Peña et al., 1976; Yu et al., 2002). In the current study, significant differences existed between the high and the low perchlorate concentrations in terms of perchlorate residue, regardless of the chemical alone or the mixture exposure (Fig. 1). A time-dependent accumulation was also found for the high perchlorate concentrations whereas this time-dependent accumulation did not occurred at the low perchlorate concentrations (Fig. 1). This is similar to other studies

(Bradford et al., 2006). As seen in Fig. 2a, the ratio of the whole body perchlorate residue between the high and low perchlorate concentrations were less than 10, which was the ratio of high and low perchlorate concentrations in the test solutions, as was in agreement with the observation in the mosquitofish study (Bradford et al., 2006). Therefore, fish do not take up and accumulate perchlorate proportionally to the concentrations of waterborne perchlorate.

As with uptake, perchlorate depuration occurred rapidly. A previous study showed that most of perchlorate accumulated during a 2-day exposure to 100 mg l^{-1} sodium perchlorate was depurated from all tissues examined of catfish *Ictalurus punctatus* and whole body mosquitofish *Gambusia holbrooki* within 2 d (Park, 2003). In the current study, 80%, 88%, and 100% of perchlorate accumulated during the 90 d exposure were depurated at depuration day 1, 3, and 5 at 100 mg l^{-1}, and 5%, 100% at depuration day 1 and 3 at 10 mg l^{-1} perchlorate treatment. The depuration rate was higher at the high perchlorate concentration than that at the low concentration because the perchlorate residue ratio between two treatments at depuration day 1 was significantly lower than that during uptake period (Fig. 2a). The half-life of 100 mg l^{-1} perchlorate was 25.7 h. A similar half-life was obtained for mosquitofish (21.8 h) (Park et al., 2005). The slight difference with respect to the half-life of perchlorate in two species was due to the slight different depuration rate constants in zebrafish (0.64 d^{-1}) and mosquitofish (0.76 d^{-1}).

In contrast to perchlorate, there are no studies on the whole body accumulation of waterborne arsenic in fish in chronic exposure studies. In the current study, the arsenic accumulation in the zebrafish showed concentration-dependent fashion at all exposure intervals (Fig. 3). A time-dependent accumulation was observed at the high concentrations (10 and 100 + 10 mg l^{-1}) but not at the low concentrations (1 and 10 + 1 mg l^{-1}). As seen from Table 4, the arsenic residue levels in fish at the low-concentration exposure demonstrated a slight decrease over time (i.e., negative slopes) compared with the apparent increase at the high arsenic concentrations (i.e., positive slopes). Based on a series of studies, McGeachy and Dixon (1989) proposed that intoxication, or even death, would occur if a whole-body burden of arsenic is over 8 μg g^{-1} dw, while 4–6 μg g^{-1} dw is associated with chronic toxicity to fish. However, McGeachy and Dixon (1989, 1990, 1992) found that 2–3 μg arsenic g^{-1} dw resulted in no toxic effects in rainbow trout (*Oncorhynchus mykiss*). In the current study, the arsenic residue was in the range of 0.08 to 1.7 μg g^{-1} wet weight (Fig. 3) (i.e., 0.24–5.15 μg g^{-1} dw). However, most of the arsenic residues fell below 3 μg g^{-1} dw except for those in the high arsenic treatments at day 60 and 90. No mortality occurred in zebrafish for the current study, which is in agreement with McGeachy and Dixon (1989).

Similar to what has been found in the current chronic study, the concentration-dependent arsenic uptake in fish has been widely reported in other species in short-time exposure. When Tilapia (*Oreochromis mossambica*) were exposed to sodium arsenate at concentrations of 0.1, 5, and 10 mg l^{-1} for 7 d, the whole body arsenic residues were 3.4, 7.6, and 11.2 μg g^{-1} dw respectively. However, the arsenic concentrations in fish did not proportionally increase with the increase of the water borne arsenic (Suhendrayatna et al., 2002). In the current study, a significant concentration-related arsenic accumulation was observed (Fig. 3). The ratio of waterborne arsenic concentrations in the high:low treatments was 10:1. This was the same in fish whole body residues for high:low treatments at 10 and 30 d of exposure, but at 60 and 90 d the ratio of whole body residues at 10 mg l^{-1}: whole body residues at 1 mg l^{-1} is around 20:1 (Fig. 4a). The arsenic residues in the current study at the 10-day, 10 mg l^{-1} arsenic treatment was only 2.9 μg g^{-1} dw, which was far below that in the Tilapia exposure study (11.2 μg g^{-1} dw at day 7).

Different fish species may have differential capability to accumulate arsenic. The arsenic bioconcentration factors for short exposure (7 d) in Tilapia were around 1.0 (Suhendrayatna et al., 2002). However, in the current study, the highest bioconcentration factor was observed at 10 mg l^{-1} treatment at day 90 (0.53). Arsenic accumulation is a function of uptake and clearance rates of arsenic in tissues. It appears that either the uptake rates increase and/or the elimination rates decrease at the higher arsenic concentrations, and that this may have implications for risk assessments and toxicokinetic modeling. Further studies are needed to elucidate the possible mechanisms for these phenomena.

There are few studies that examine the depuration of arsenic from aquatic animals, but these few studies have indicated that arsenic depuration occurs rapidly in fish. For example, tilapia and Japanese medaka (*Oryzias latipes*) that were exposed to 1 mg l^{-1} sodium arsenite for 7 d depurated most (about 90%) of arsenic residue 1 d after being transferred to As-free water (Suhendrayatna and Maeda, 2001, 2002). In the current study, when fish were exposed to 1 mg l^{-1} arsenate, 30%, 55% and 100% of the original arsenic body burden (i.e., the whole body concentration after 90 d of exposure) had been eliminated by 1, 3, and 5 d, respectively. When fish were exposed to 10 mg l^{-1} arsenate, 58%, 83%, and 88% of the original arsenic body burden had been eliminated at 1, 3, and 5 d, respectively (Fig. 3). This indicates that the depuration of arsenate after high concentration exposures was much slower than that after lower exposures.

Studies have demonstrated that both perchlorate and arsenate were not bioconcentrated in animals (McGeachy and Dixon, 1992; Park, 2003; Bradford et al., 2006). In the current study, the whole body BCFs were 0.18–0.30 and 0.30–0.53 in fish exposed to 1 and 10 mg l^{-1} sodium arsenate, respectively, and 0.12–0.14 and 0.09–0.11 in fish exposed to 10 and 100 mg l^{-1} sodium perchlorate, respectively (Figs. 2c and 4c). However, based on the BCFs, we can surmise that BCFs of arsenate are greater than that of perchlorate in zebrafish.

In terms of whole body concentrations, in most cases no significant statistical differences were observed between the single chemical exposures and the corresponding mixture treatment at either concentration, regardless of arsenate or perchlorate (Figs. 1 and 3). Hence, it seemed that the steady state of either chemical accumulation was reached relatively quickly (i.e., within 10 d). The uptake constants indicated that there was a difference in the uptake capability between the single chemical and the corresponding mixtures at the concentrations of both chemicals in the chronic exposure (Table 3). This indicated that the uptake of either chemical was reduced by the co-existence of the other chemical in the environment when chemicals occurred at concentrations showed in Table 3. On the other hand, the opposite pattern was observed in the depuration scenario. Co-exposure of one chemical enhanced the elimination of another chemical when high concentrations of both chemicals occurred in the environment (Table 3). Arsenate is taken up by phosphate transporter (Aposhian et al., 2004), and perchlorate by sodium iodide symporter (NIS) (Strawson et al., 2004). Theoretically, it seems that they do not affect toxicokinetics of another anion in mixture. Potential contributing factors for alterations of uptake and depuration kinetics could include: (1) similar uptake and/or excretion routes in fish (e.g., competition for common uptake/elimination transporters), (2) alterations in bioavailability of one chemical by the other, due to chemical or physical processes in the water, (3) influence of one chemical on the cellular uptake and excretion mechanisms of the other. For example, excretion of arsenic may involve glutathione (Aposhian et al., 2004), and glutathione levels in zebrafish may be affected by thyroid hormone status and/or perchlorate exposure (Liu, 2006).

5. Conclusion

Uptake and depuration of both perchlorate and arsenate were rapid in zebrafish. The steady-state was reached by day 10 for both chemicals. At the concentrations tested in the current study, a slower uptake rate and a faster depuration of either chemical were found when the other chemical was present. Thus, any ecological assessments that assume no interactions between chemicals, in terms of toxicokinetics, may result in biased estimations of body burdens. More information is in need if such toxicokinetic interactions occur among other inorganic chemicals as well.

Acknowledgements

We thank Mike Wages and Hongmei Wu for their assistance in the experiment. This research was supported in part by the US Department of Defense contract CU1141, through the Strategic Environmental Research and Development Program (SERDP) under a Cooperative Agreement IERA-99-001 with the USAF, Institute for Environment, Safety and Occupational Health, Brooks AFB, TX. The views and conclusions contained herein are those of the authors and should not be interpreted as necessarily representing the official policies or endorsements, either expressed or implied, of the 311 HSW/AFIERA or the US Government.

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