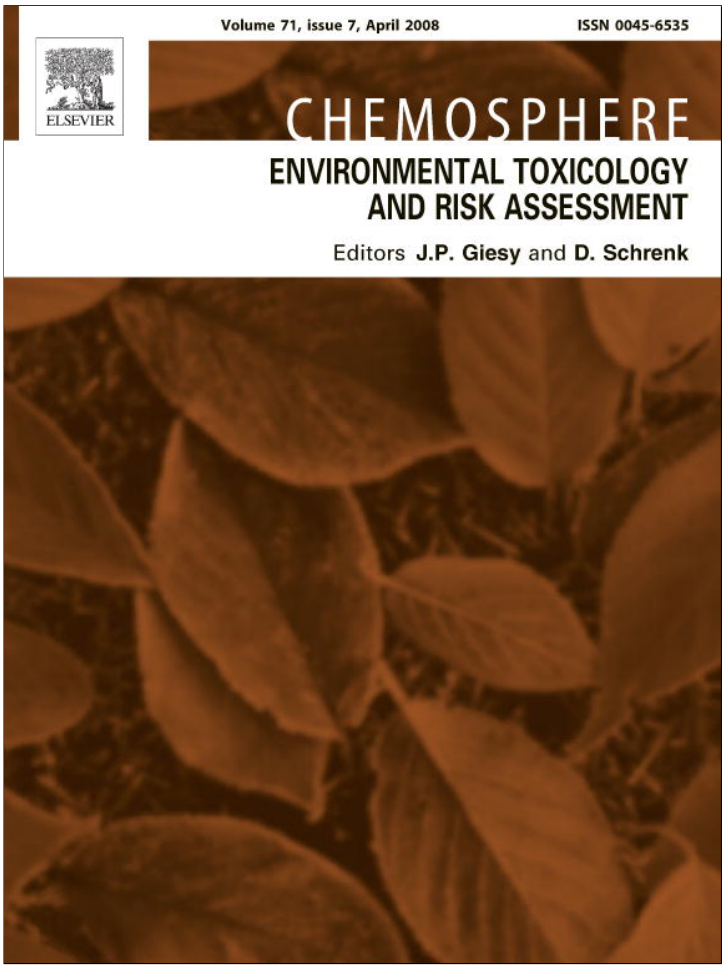

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Arsenate and perchlorate toxicity, growth effects, and thyroid histopathology in hypothyroid zebrafish *Danio rerio*

Fujun Liu [a], Angella Gentles [a], Christopher W. Theodorakis [b,*]

[a] *The Institute of Environmental and Human Health and Department of Environmental Toxicology, Texas Tech University, Lubbock, TX 79409-1163, USA*
[b] *Department of Biological Sciences and Environmental Sciences Program, Southern Illinois University Edwardsville, P.O. Box 1651, Edwardsville, IL 62026, USA*

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Abstract

Exposure to perchlorate or other thyrotoxic compounds can cause hypothyroidism in most vertebrates, and this may affect levels of endogenous antioxidants and cause oxidative stress. Arsenic also induces oxidative stress in animals by modifying the antioxidant capacity and may alter the thyroid homeostasis. Therefore, hypothyroidism may affect the toxicity of arsenate. In order to test this hypothesis, zebrafish (*Danio rerio*) were made hypothyroid by exposure to perchlorate, and toxicity of arsenate in hypothyroid and euthyroid fish was compared. The endpoints were LC50 and thyroid histopathology. Additionally, the recovery of thyroid histopathological indices after cessation of perchlorate exposure was determined. The current study showed that 96 h LC50 of perchlorate anion and arsenate ion to juveniles fish (37 day post-fertilization) were 2532 and 56 mg l^{-1}, respectively. In addition, hypothyroid fish were more sensitive to arsenate, with a 96 h LC50 of 43 mg l^{-1}. Growth rates were also significantly retarded by perchlorate exposure. After cessation of perchlorate exposure, there was recovery of thyroid histopathology in terms of epithelial cell height, but not colloid area or growth rate. In conclusion, perchlorate enhances arsenate toxicity to juvenile zebrafish, and the rate of thyroid recovery after cessation of perchlorate exposure depends on the endpoints examined.

Keywords: Perchlorate; Arsenate; Zebrafish; Acute toxicity; Thyroid histopathology

1. Introduction

The interactive effect of contaminant mixtures is an active area of research (Altenburger et al., 2003), because living organisms are commonly exposed to mixtures of contaminants in natural environments. One possible interaction is that some chemicals with low acute toxicity may enhance the toxicity of more toxic chemicals. For example, many chemicals may disrupt thyroid function (Brown et al., 2004), and it has been found that an altered thyroid state may induce oxidative stress or compromise endogenous antioxidant capacity in fish (Varghese et al., 2001). Thus, thyroid disruptors may affect the toxicity of pro-oxidant chemicals. One well-studied thyroid-disrupting chemical is perchlorate Smith et al., 2001; Theodorakis et al., 2006a, 2006c), and one well-studied pro-oxidant chemical is arsenate (Liu et al., 2001).

Arsenic is listed as a US EPA contaminant of concern because of risk of widescale environmental exposure, deleterious effects, and environmental persistence (Phillips, 1990). Aquatic habitats receive arsenic from many anthropogenic activities, such as mining, smelting, metal refinery, military activities, and agricultural application (ATSDR, 1998). Thus, there is a widespread risk of exposure and effects from arsenic to humans, fish, and wildlife. Thus, arsenate is the dominant form in surface water that contains sufficient oxygen to support fish populations.

* Corresponding author. Address: Department of Biological Sciences and Environmental Sciences Program, Southern Illinois University Edwardsville, P.O. Box 1651, Edwardsville, IL 62026, USA. Tel.: +1 618 650 5235; fax: +1 618 650 3174.
E-mail address: ctheodo@siue.edu (C.W. Theodorakis).

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Induction of oxidative stress is one of major mechanisms by which arsenic causes toxicity (Liu et al., 2001).

Thyroid hormones have been reported to regulate antioxidant levels in fish (Varghese et al., 2001), which suggests that disruption of thyroid homeostasis may affect the responses of organisms to oxidative stress. Thus, hypothyroidism could affect the toxicity of pro-oxidant compounds. Since the toxicity of arsenate is primarily mediated by oxidative stress (Liu et al., 2001), hypothyroidism caused by perchlorate exposure may change the picture of arsenate toxicity to animals. In addition, arsenic may disrupt thyroid homeostasis as well (Meltzer et al., 2002). Hence, interaction between these two chemicals may be additive in nature. This has been indicated in previous acute toxicity tests (Liu et al., 2005).

In zebrafish, thyroid hormones are necessary for embryonic to larval transition and larval to juvenile transition (Brown, 1997; Liu and Chan, 2002). Hence, if thyroid disruption occurs during either transition, growth retardation might occur (Brown, 1997). However, there were no reports on how thyroid follicles are disrupted in hypothyroid zebrafish during early developmental stages.

Thus, the objectives of the current study were to obtain basic acute toxicity data for these two anions to juvenile zebrafish, to evaluate the effect of these two chemicals at the thyroid histopathology level during early development stages, to determine the effect of perchlorate-induced hypothyroidism on arsenate toxicity, and to determine if the thyroid can recover from perchlorate exposure and how long the recovery will take if recovery occurs. These two chemicals were chosen (1) as prototypical thyroid-disrupting (perchlorate) and pro-oxidant (arsenate) chemicals, (2) because of their potential co-occurrence in surface waters due to military, agricultural, or industrial activities (Urbansky and Schock, 1999). The following hypotheses were tested, (1) hypothyroidism enhances the toxicity of arsenate to zebrafish, (2) arsenate is a thyroid disruptor in zebrafish, and (3) the thyroid recovers after cessation of perchlorate exposure.

2. Materials and methods

2.1. Chemicals

Perchlorate (anhydrous) was purchased from EM Science (Gibbstown, NJ, USA) and arsenate (dibasic 7-hydrate) from J.T. Baker (Phillipsburg, NJ, USA). Stock solutions were prepared by adding appropriate amounts of chemicals to 18.3-MΩ Milli-Q water to ensure concentrations of stock solution below the solubility limit of the chemicals. The concentration of perchlorate was reported as ClO_4^-, and that of arsenate as ASO_4^-.

2.2. Animals

Adult zebrafish (*Danio rerio*) were obtained commercially from Ekkwill Waterlife Resources (Gibsonton, Florida). Fish breeding was as described by Patiño et al. (2003) and Liu et al. (2005). Fish were raised and tested in 60 mg l^{-1} Instant Ocean® sea salts (Specrum Brands, Inc., Atlanta, GA, USA). After spawning, eggs were collected and washed. Viable eggs were selected and kept in an incubator thereafter. Larvae were fed Hikari®1st Bite (Hayward, CA, USA) during the early stage and later artemia cysts (Grantsville, UT, USA) and Bio-pure® brine shrimp (Hayward, CA, USA). The food was fed *ad libitum*. Juvenile fish were fed Aquatox® flake food (Zeigler Bros., Gardner PA). According to manufacturer's information on iodine content of their products, the iodide concentration in the test water was calculated as 0.4 μg/l, and in the food as 9 mg/kg. The iodide concentration of the brine shrimp is not known, but the iodide concentration in the water in which they were raised was calculated to be 0.2 mg l^{-1}.

2.3. Water chemistry

Dissolved oxygen and salinity were measured using an YSI® model 85 meter (Yellow Springs, OH, USA). The pH was measured using an Oakton® pH meter (Gresham, OR, USA). Unionized ammonia was determined with a Hach® spectrophotometer model DR/2000 (Loveland, CO, USA). Measurement of perchlorate and arsenate in water was conducted by ion chromatography and atomic absorption spectroscopy, respectively, as described in Liu et al. (2005, 2006a).

2.4. Juvenile acute toxicity test

A 96-h acute toxicity test of either arsenate or perchlorate was conducted following the standard procedure (ASTM, 2003). Thirty-seven day post-fertilization (dpf) juveniles were used in the test. A geometric series of five concentrations of either chemical plus one control group was used, with three replicates for each treatment (10 fish in each replicate). The exposure was conducted in an incubator, with temperature set at 28 °C and photoperiod at 14, 10-h light/dark. A static-renewal method was applied for the test. Every day, half of the test solution (250 ml) was changed. The mortality was checked twice daily, and the dead fish were removed. The toxicity was determined by converting mortality data to toxic units, as described previously (Liu et al., 2005). Perchlorate is stable and non-volatile under these conditions, and so it is expected that a static-renewal system is appropriate for this type of study. Arsenate is assumed to be stable under static-renewal conditions, because it is the oxidized form of the arsenic anion. Even so, in order to take into account changes in perchlorate due to biodegradation or other losses, or losses of arsenate due to sorption or volatilization, water samples for verification of doses were collected before water changes from each replicate beaker on day one and day four of the test.

2.5. Effect of perchlorate-induced hypothyroidism on arsenate toxicity

In addition to the LC50 test for either chemical, a third test was designed to evaluate the effect of hypothyroidism caused by perchlorate exposure on arsenate acute toxicity to zebrafish juveniles. Beginning from 6 dpf, larvae were exposed to perchlorate anion at a concentration of 120.6 ppm (0.1 times the 96 h LC50 of perchlorate to 6 dpf zebrafish larvae; Liu et al., 2005) until 37 dpf ("35-day exposure"). This concentration was chosen because preliminary study found that this concentration induced hypothyroidism in zebrafish yet was not overtly toxic to these fish. They were exposed to perchlorate from 6 to 37 dpf, because zebrafish experienced transition from larvae to juvenile during this stage and we wanted to examine the perchlorate exposure on thyroid status during this transition. After 37 dpf, these juveniles were additionally exposed to arsenate for another 96 h for a LC50 test. This was done to determine if a hypothyroid condition enhances arsenate toxicity. At the end of the exposure, the surviving fish from all tests were sacrificed for thyroid histopathology. There were 20 fish used for each treatment, divided into five replicate tanks.

2.6. Thyroid recovery test after cessation of perchlorate exposure

Larvae (6 dpf) were exposed to 120.6 mg l^{-1} perchlorate until 37 dpf (juvenile stage), and then were transferred to perchlorate-free water or the exposure was continued until 52 dpf ("46-day exposure, perchlorate + depuration"). An additional group was exposed to 120.6 mg l^{-1} perchlorate continuously from 6 dpf until 52 dpf ("52-day exposure"). Control groups were set up for either exposure scenario. All juveniles at the end of the exposure were sacrificed for thyroid histopathology. There were 24 fish used for each treatment, divided into five replicate tanks.

2.7. Effects of perchlorate on fish growth

In order to determine the effects of perchlorate-induced thyroid depletion on fish growth, fish from each replicate tank were pooled and weighed to determine average mass per fish. This was done for the 35-, 42-, and 52-day exposure treatments, as well as their respective controls. There were five replicate tanks in each treatment.

2.8. Thyroid histology

Fish heads were fixed in Bouin's fixative, embedded in paraffin, serially sectioned on a microtome, and stained as detailed in Liu et al. (2006b). Thyroid histopathology endpoints, including follicle area, colloid area, and epithelial cell height on the pictures were quantified using the SimplePCI imaging system (version 4.01.1605, Compix Inc., Sewickley, PA, USA). For epithelial cell height determination, five measurements along the follicle circumference were made.

2.9. Statistical analysis

SAS software was used in data analysis unless otherwise noted (SAS®, version 8.02, Cary, NC, USA). LC50 were determined using the Probit Unit procedure. To obtain concentration–response curves, a logistic model was used (Haanstra et al., 1985).

$$Y = c/(1 + e^{b(X-a)}) \quad (1)$$

where Y = survival (%), c = survival in control (set to 100%), a = logLC50 (mg l^{-1}), b = slope, and X = log concentration (mg l^{-1}). The difference among slopes of concentration–response curves and among treatments with respect to thyroid histopathology was analyzed using one-way ANOVA analysis followed by DUNCAN's multiple comparison. The difference is considered significant at $p = 0.05$. The data were expressed as mean (± S.D.) unless specifically stated.

3. Results

3.1. Water chemistry

The average water characteristics in the test solution were as follows, temperature, 27 ± 1 °C; dissolved oxygen, 5.61 ± 0.14 mg l^{-1}; pH, 6.81 ± 0.34; salinity, 100 mg l^{-1} in the arsenate toxicity test and 100 to 2700 mg l^{-1} in the perchlorate toxicity test; ammonia concentration, 0.07 ± 0.01 mg l^{-1}. Protocols for determining the water chemistry were as reported in Liu et al. (2006b). This concentration of ammonia exceeds the recommended limit of 0.035 mg l^{-1} (ASTM, 2003). Detection limits and% recovery rates are described in Liu et al. (2005, 2006a). Concentrations of chemicals are reported as actual concentrations, rather than nominal. Protocols for determining arsenic and perchlorate concentrations in water, as well as variability of actual measured concentrations, are reported in Liu et al. (2006a).

3.2. Acute toxicity

The 96 h LC50 of perchlorate and arsenate anions with or without pre-exposure to perchlorate (37 dpf) are listed in Table 1. As shown in Table 1, arsenate was much more toxic than perchlorate. Except for 24 h, there is significant difference between LC50 for arsenate with and without pre-exposure to perchlorate. Pre-exposure to perchlorate resulted in a statistically significant increase in juvenile zebrafish sensitivity to arsenate (i.e. no overlap of the 95% confidence intervals). This was also observed in the concentration–response curves (Fig. 1). With a pre-exposure to perchlorate, the curve significantly deviated from that without pre-exposure ($p < 0.05$). This indicates that perchlorate mediates arsenate toxicity. However, there was no

Table 1
Acute toxicity as LC50 (mg l^{-1}) of sodium perchlorate and sodium arsenate to 37 dpf zebrafish juveniles

Chemicals	LC50 (mg l^{-1}) (95% confidence limit)			
	24 h	48 h	72 h	96 h
ClO_4^-	2816^{a} (2586–3032)	2693^{ab} (2473–2899)	2640^{ab} (2528–2739)	2532^{b} (2392–2659)
$HAsO_4^{-2}$	63^{a}_{u} (45–81)	60^{a}_{u} (45–76)	59^{a}_{u} (54–62)	56^{a}_{u} (44–67)
$HAsO_4^{-2}$ [A]	67^{a}_{u} (53–80)	48^{b}_{v} (44–53)	47^{bc}_{v} (41–53)	43^{c}_{v} (42–43)

[A] With pre-exposure to sodium perchlorate (148.5 mg l^{-1}) from 6 to 37 dpf. Superscript denotes statistically significant differences among exposure periods, and subscript letters between exposures to sodium arsenate with and without pre-exposure to perchlorate; values bearing same letters denote no significant difference at 0.05 p level, otherwise significant difference exists.

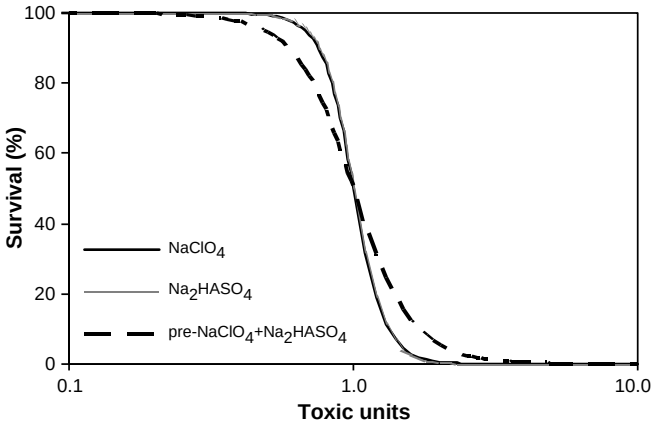

Fig. 1. Concentration–response curves for acute exposure to perchlorate, arsenate, and arsenate with pre-exposure to perchlorate. Data are plotted as a percent survival as a function of toxic units based on experimentally derived 96 h LC50. X-axis is based on the log scale. The curve shape for arsenate with pre-exposure to perchlorate is significantly different from that without pre-exposure.

significant difference between curves of perchlorate and arsenate (Fig. 1). There was no mortality in control fish in any experiment.

3.3. Thyroid histopathology

Exposure of zebrafish to 120.6 mg l^{-1} perchlorate from the larval to juvenile stages caused hypertrophy of the epithelial cells and altered the epithelial cells from squamous in control to columnar shape in perchlorate treatment, as well as reduction in the cross-sectional area of the colloid (Fig. 2). For the 96 h exposure to arsenate (no pre-exposure) and perchlorate, no significant difference was found between treatment and control for any endpoint, regardless of the chemical (data not shown). Thus, 96 h acute expo-

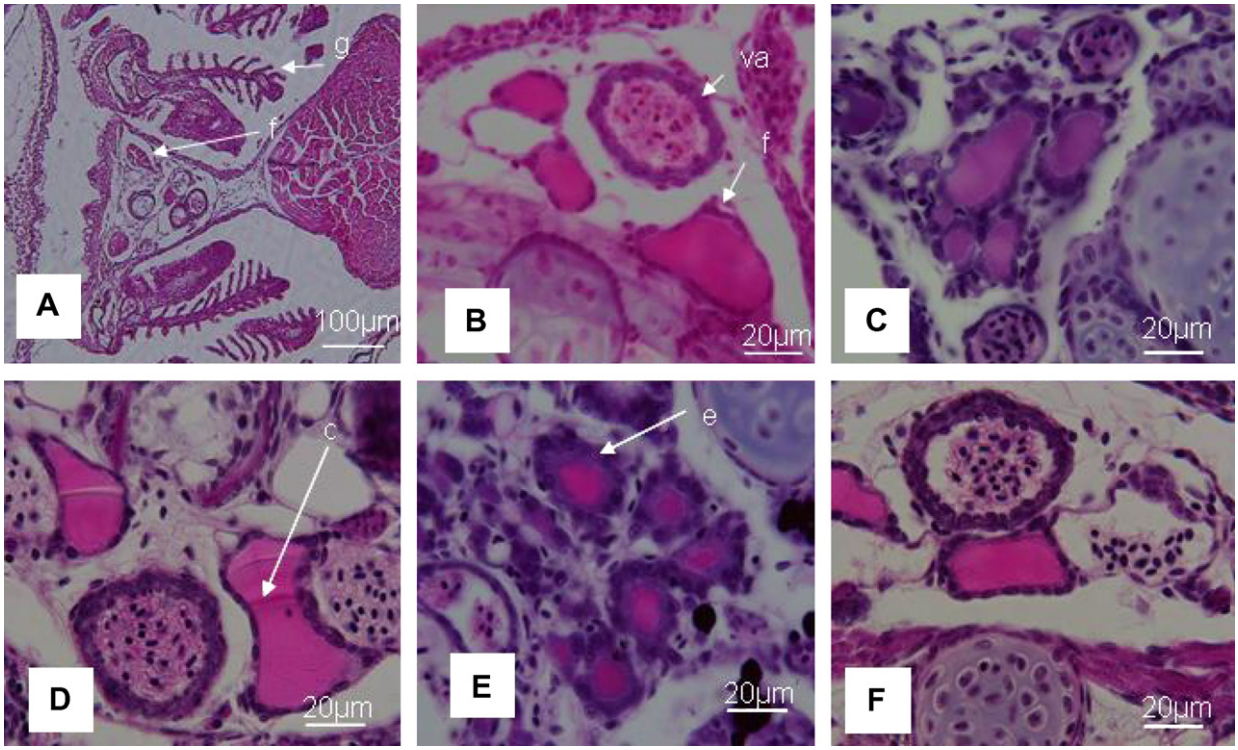

Fig. 2. Histological structure of thyroid follicles in zebrafish exposed to perchlorate (120.6 mg l^{-1}) from 6 dpf through 37 dpf (A–C) and 52 dpf (D–F). (A) the sagittal section of fish head area around the ventral aorta, (B) and (D), thyroid follicles in control group, (C) and (E), thyroid follicles in fish exposed to perchlorate, and (F), thyroid follicles in zebrafish exposed to perchlorate from 6 to 37 dpf followed by elimination for 15 days Compare the squamaous epithelial cells in (B) and (D) with the columnar epithelial cells in (C) and (E) (more pronounced hypertrophy). va = ventral aorta, f = thyroid follicle, c = colloid, g = gill, and e = thyroid follicle epithelial cell.

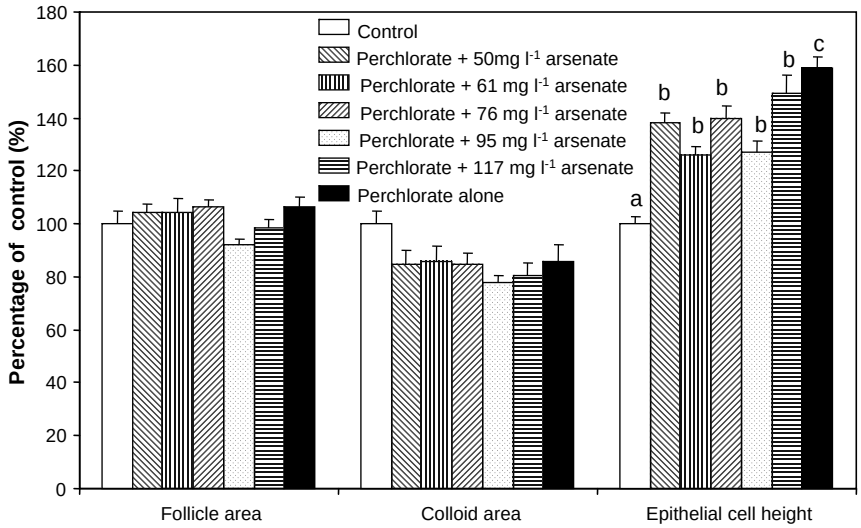

Fig. 3. Thyroid histopathology in 41 dpf zebrafish juveniles exposed to 120.6 mg l^{-1} perchlorate ("perchlorate alone") from 6 dpf to 41 dpf, or exposed to 148 g mg l^{-1} perchlorate from 6 dpf to 37 dpf and then exposed to arsenate for four days (perchlorate + arsenate). Mean ± S.E., $n = 24$ for each treatment.

sure of juveniles to either chemical did not affect the thyroid in terms of histopathology. This suggests that the histological change in thyroid tissue is a slow process and needs a longer period of time to be manifested.

The thyroid histopathology in the arsenate acute exposure with pre-exposure to perchlorate is illustrated in Fig. 3. Epithelial cell height in the perchlorate- and arsenate-exposed fish was significantly lower than that in the fish exposed to perchlorate alone, but significantly higher than that in the control. There were no statistically significant differences in follicle area and colloid area among treatments (Fig. 3).

The thyroid histopathology in the depuration test is illustrated in Fig. 4. Mean colloid area was statistically significantly less, and epithelial cell height was statistically significantly greater than controls after 31 days of exposure to perchlorate (Fig. 4A). Epithelial cell height after depuration was significantly reduced and showed no significant difference from that in the control (Fig. 4B). However, the colloid area after perchlorate exposure and elimination was not significantly different from that in the continuous perchlorate exposure (Fig. 4B). Therefore, the thyroid recovery occurred in terms of epithelial cell height, but not colloid area.

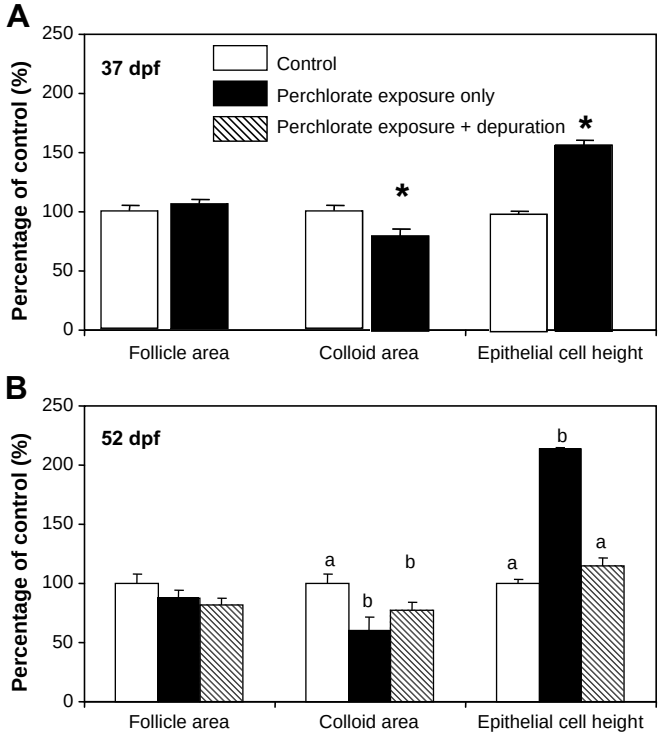

Fig. 4. Thyroid histopathology in zebrafish juveniles. (A) The perchlorate exposure (120.6 mg l^{-1}) was initiated from 6 dpf and finished at 37 dpf. Asterisks indicate significant difference from controls ($p < 0.05$, t-test). (B) The perchlorate exposure (120.6 mg l^{-1}) was initiated from 6 dpf and finished at 52 dpf. In the depuration scenario, the exposure finished at 37 dpf and then juveniles were transferred to clean water until 52 dpf. Bars labeled with difference letters are statistically significantly different ($p < 0.05$, Mean ± S.E., $n = 20$ for each treatment.

3.4. Fish growth

For the 35-day exposures, the mean mass of control fish was statistically significantly greater than that of fish exposed to 120.6 mg l^{-1} perchlorate from 6 to 37 dpf (Fig. 5). In the recovery test, the mass of control fish was statistically significantly greater than both the fish exposed to 120.6 mg l^{-1} perchlorate for 46 days (from 6 to 52 dpf) and fish exposed to perchlorate for 37 days and allowed to depurate for an additional 15 days (38–52 dpf; Fig. 5). The number of replicates for each treatment was five ($n = 5$).

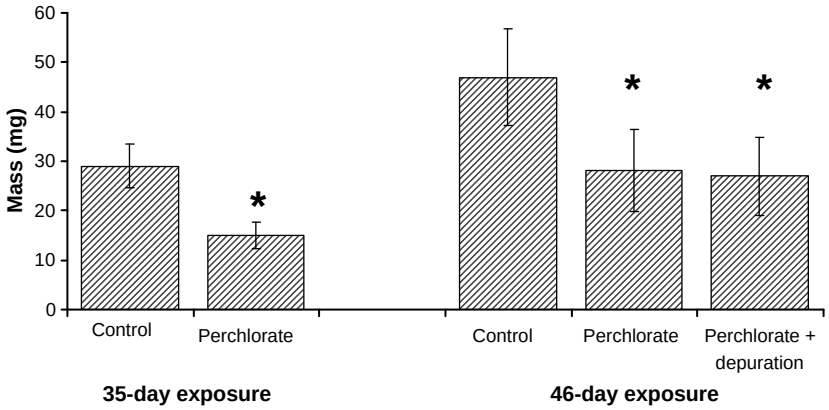

Fig. 5. Final mass (mg) of zebrafish fry exposed to perchlorate for various periods of time. The "35-day exposure" fish were exposed to 120.6 mg l^{-1} perchlorate from 6 days post-fertilization (dpf) until 41 dpf (larval to juvenile transition). The "46-day exposure" group was exposed to 120.6 mg l^{-1} perchlorate until 52 dpf. The "perchlorate + depuration" group was exposed to perchlorate until 37 dpf and then allowed to depurate in clean water until 52 dpf. Bars and error bars are means ± 95% confidence intervals. Asterisks represent treatments statistically significantly different from controls. Statistical significance was assessed by overlap of 95% CI. For the "35-day exposure" fish, $n = 24$; for the "46-day exposure" fish, $n = 20$ for each treatment.

4. Discussion

Thyroid hormones are necessary for zebrafish development and growth. For example, previous studies have found that exposure of zebrafish to goitrogens led to retarded growth, and was negated by simultaneous administration of thyroxine (Brown, 1997). In addition, development and metamorphosis were also delayed (Brown, 1997). Likewise, thyroid hormones are required for embryonic to larval transition, which is also retarded by exposure to goitrogens (Liu and Chan, 2002). Our results are in agreement with these studies. With an exposure extending from 6 dpf until 41 or 52 dpf, the zebrafish growth was severely retarded. Depuration for 15 day starting from 37 dpf (mean fish mass of 0.028 g) did not allow for an increase in growth rates above continuously perchlorate-exposed fish. Therefore, the recovery of growth rate from perchlorate-induced hypothyroidism is a slow process.

Although adults are typically more tolerant to toxic chemicals than larval stages, a single chemical can exert differential toxicity to different life stages of the same species. In the current study, the LC50 of perchlorate and arsenate for juveniles are 2532 and 56 mg l^{-1}, respectively. In the larval stage (6 dpf), the values are 1204 and 205 mg l^{-1}, respectively (Liu et al., 2005). This indicates that juveniles are more tolerant to perchlorate, but more sensitive to arsenate than larvae. The higher sensitivity of juveniles than post-hatch larvae to arsenate is unexpected. The fate of arsenate relative to an organism or a cell includes uptake (i.e. absorption), metabolism, and excretion. Further work is necessary to elucidate which processes lead to the differential sensitivity of zebrafish to arsenate at different developmental stages.

Perchlorate exposure enhances arsenate toxicity to juvenile zebrafish. Both LC50 and concentration–response relationship showed that sensitivity of juveniles to arsenate was enhanced by pre-exposure to perchlorate. This is consistent with the hypothesis that hypothyroidism enhances the toxicity of arsenate. There was no significant difference between the two chemicals regarding concentration–response curve (Fig. 1). Chemicals that have similar modes of action and similarly shaped concentration–response curves behave like dilutions of each other, and may have similar or at least compensating modes of action (Altenburger et al., 2003). Perchlorate causes a hypothyroid condition in fish (Theodorakis et al., 2006a,c), which was reported to antagonize the antioxidant defense system (Konukoğu et al., 1998). This may enhance arsenic toxicity because of oxidative stress caused by arsenic exposure (Liu et al., 2001). This may elucidate the observation in the current study that perchlorate exposure reduced the tolerance of zebrafish juveniles to arsenate. Preliminary studies have suggested that perchlorate exposure may modify arsenate-induced oxidative stress parameters in zebrafish (unpublished data).

Perchlorate salts are well documented as thyroid disruptors, and the effect can be manifested as thyroid histopathology as well as alteration of thyroid hormone status (Brown et al., 2004). For example, hypertrophy (i.e. increased epithelial cell height), hyperplasia, angiogenesis, and/or colloid depletion were observed when fish and amphibians were exposed to perchlorate in laboratory and field situations (Carr et al., 2003; Patiño et al., 2003; Capps et al., 2004; Crane et al., 2005; Mukhi et al., 2005, 2007; Bradford et al., 2006a; Burkhardt et al., 2006; Carr and Theodorakis, 2006; Liu et al., 2006b; Park et al., 2006; Theodorakis et al., 2006a,c; Mukhi and Patiño, 2007). In the current acute test described above, the thyroid tissues of juvenile zebrafish were only affected after prolonged exposure to perchlorate. An interesting finding was that acute arsenate exposure seemed to slightly ameliorate the effects of perchlorate However, more information was needed to make a definitive conclusion regarding the

effect of arsenic on thyroid status. It will be informative to conduct a chronic study to evaluate the effect of arsenate on thyroid status.

There is also evidence of a refractory effect of perchlorate after a 15-day depuration period in the perchlorate-free water. For example, the epithelial cell height in the "perchlorate + depuration" was not different from controls (Fig. 4), which may suggest recovery of thyroid tissue. However, no significant recovery in colloid area was observed. Additionally, perchlorate exposure caused significant growth retardation, and growth rates did not seem to recover after cessation of perchlorate exposure (Fig. 5). Thus, there seems to be refractory effects of perchlorate exposure on growth of zebrafish, even after cessation of exposure for 15 days. This is probably not due to residual perchlorate residues, because it is rapidly eliminated from fish tissues (Park et al., 2005; Bradford et al., 2006b; Liu et al., 2006a). The fact that thyroidal effects (i.e. colloid area) of perchlorate exposure were also persistent during the depuration phase is consistent with the hypothesis that the effects on growth were related to thyroidal perturbations.

The present study indicates that perchlorate exposure affects growth rate of zebrafish, and that growth rate does not seem to recover after the fish are moved to clean water. Because perchlorate is a known thyroid disruptor, it is possible that these affects may be directly attributed to effects of thyroid hormone on growth. However, growth may be affected by indirect factors as well. For instance, alterations of thyroid hormone levels or exposure to perchlorate itself could alter food intake rates, and this may indirectly affect growth rates. Additionally, there may be some toxic effects of perchlorate other than thyroid hormone alterations that affect growth rates. It could also be that the apparent effects on growth rate are due to a combination of direct and indirect effects. Additional research is needed to differentiate among these alternative hypotheses.

5. Conclusion

Based on LC50s, we concluded that arsenate is much more toxic than perchlorate to 37 dpf zebrafish juveniles, and that perchlorate pre-exposure augments the sensitivity of zebrafish juveniles to arsenate by reducing its LC50 and changing the concentration–response curve shape. Acute exposure of juvenile zebrafish to either chemical did not alter the thyroid status in terms of thyroid histopathology endpoints, including epithelial cell height, follicle area, and colloid area. Acute arsenate exposure (96 h) following the cessation of perchlorate exposure did not alter the acute toxicity of perchlorate exposure in terms of thyroid histopathology, and thyroid recovery was observed with respect to epithelial cell height. Alteration of thyroid status is a slow process and short-term exposure is not effective in this regard. Transferring of perchlorate-exposed juveniles to clean water for 15 day caused recovery of thyroid in terms of epithelial cell height but not colloid area. The concentrations of perchlorate used in this study are much higher than those typically found in surface waters (Smith et al., 2001; Mayer et al., 2006; Theodorakis et al., 2006b), although the arsenic concentrations in surface waters may exceed those used in this study (Kuwabara and Fuller, 2003). Given that Bradford et al. (2006a) and Mukhi et al. (2005, 2007) and Mukhi et al. (2005, 2007) have found effects of perchlorate on thyroid histopathology at environmentally relevant concentrations, more research is needed to determine if the trends seen in the present study are also seen when zebrafish are exposed to environmentally-relevant concentrations of perchlorate.

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