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Perchlorate Affects Thyroid Function in Eastern Mosquitofish (*Gambusia holbrooki*) at Environmentally Relevant Concentrations

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The purpose of this study was to determine the effects of perchlorate on thyroid function in mosquitofish. Adult mosquitofish were exposed to 0, 0.1, 1, 10, 100, and 1000 mg/L sodium perchlorate for 2, 10, and 30 d. Whole body thyroxin (T_4) content and histological assessment of thyroid follicles (e.g., follicular epithelial height, hyperplasia, hypertrophy, and colloid depletion) were used to gauge alterations in thyroid function. Follicular epithelial cell height, hyperplasia, and hypertrophy increased with increasing perchlorate concentration, especially in fish exposed for 30 d, and these effects were statistically significantly different from control at concentrations as low as 0.1 mg/L (nominal concentration). The percent occurrence of follicles with depleted colloid decreased with increasing perchlorate concentration, which is contrary to what is expected with thyroid inhibition. There also was a decrease in whole body T_4 concentration in fish exposed to perchlorate for 30 d, but clear dose−response relationships were less evident for whole body T_4 than for histopathological endpoints. In conclusion, thyroid histopathology provides a sensitive biomarker for thyroid endocrine disruption at environmentally relevant concentrations of sodium perchlorate, and whole body T_4 is a less sensitive indicator of perchlorate exposure than is histopathology.

Introduction

Perchlorate salts are used as oxidizers in solid-fuel rockets and missiles, illuminating munitions, fireworks, automobile airbags, and flares (*1*). The perchlorate anion is also a contaminant found in some nitrate-based fertilizers (*1*). Environmental contamination of soil and surface or groundwater has been found at facilities where these materials are, or have been, manufactured, used, or processed (*1*−*3*). Concern over environmental perchlorate contamination stems from the fact that it competitively inhibits thyroidal iodide uptake by reversibly binding to the sodium/iodide symporter, thus hindering synthesis of thyroid hormones (TH) (*4*).

Perturbation of TH homeostasis may be manifested as hypertrophy and hyperplasia of thyroid follicle epithelial cells as well as modulation of thyroid follicle colloid volume (*5*). This is because a reduction in the amount of circulating TH results in an increase in the secretion of thyroid stimulating hormone (TSH) via a negative feedback loop. Elevated blood concentrations of TSH cause hypertrophy and hyperplasia of thyroid follicle cells and depletion of colloid. Alteration of the thyroid follicular colloid volume may occur when there is a perturbation of TH synthesis, for example as affected by reduced iodine availability (*6*).

Although perchlorate has been known to inhibit thyroid production in mammals for some time (*7*−*10*), its effects in other vertebrates, such as fish and amphibians, have only recently been recognized (*11*−*14*). In teleost fish, thyroid hormones regulate such ecologically relevant processes as growth, embryo/larval development, metamorphosis, reproduction, and behavior (*15*−*18*). Although perchlorate-induced thyroid disruption has been studied in zebrafish (*Danio rerio*) and sea lamprey (*Petromizon marinus*) (*14*, *19*), effects in native North American teleosts have not been determined. This information is necessary for developing biomarkers of exposure and effects of thyroid disrupting chemicals and for assessing risk to ecological receptors such as fish. Thus, the purpose of this research is to determine the effects of perchlorate on thyroid histopathology and T_4 concentrations in the eastern mosquitofish (*Gambusia holbrooki*). Mosquitofish were chosen because they are often present at perchlorate-contaminated sites, and perchlorate has been detected in mosquitofish from these sites (*3*, *20*). It was hypothesized that T_4 levels would be decreased in a dose−response manner and that changes in thyroid follicle morphologies such as hyperplasia and hypertrophy would also be apparent.

Experimental Section

Chemicals. Sodium perchlorate (99% purity) was purchased from Sigma-Aldrich (St. Louis, MO). Sodium perchlorate was chosen over the ammonium salt to eliminate the confounding effects of ammonium toxicity. In addition, although the ammonium salt is most frequently used in manufacturing processes and is the form most frequently released to the environment, in field conditions ammonium perchlorate is rapidly converted to the sodium salt (*21*). Thus, sodium perchlorate is most commonly encountered in the environment (*21*) and would be the perchlorate salt that is most environmentally significant.

Animals. Adult female mosquitofish were used for all experiments below. They were purchased from commercial hatcheries (Ken's Hatchery and Fish Farm, Alapaha, GA). Females only were used in order to control for effects of sex on thyroid endpoints. Male were not used because, due to the fact that they are much smaller in size than the females, it would have been more difficult to obtain adequate tissues for analysis from them. Also, males were very rare in the fish shipments received from the hatchery (probably due to the fact that they are much smaller than females, and so they could more easily slip through the mesh of the netting used to capture them from stock ponds). Fish were allowed to acclimate to laboratory conditions for at least 5 d prior to the start of the experiments. A 12-h photoperiod and constant water temperature were maintained throughout the experiment. Fish were fed ad libitum with commercial flake food daily (Aquatox Flake, Zeigler Bros. Corp., Gardners, PA). This

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food contains 9 mg/kg of iodine, as determined by the manufacturer. The test and acclimation water consisted of 60 mg/L of Instant Ocean sea salts in deionized (DI) water, containing 0.4125 μg/L of iodide anion (reconstituted water) following ref *14*. This level of iodine is within the range of iodine concentrations typically found in surface waters in the U.S. and elsewhere: surface water total iodine concentrations have been found to vary between 0.3 and 5 μg/L (*22*−*25*). Iodine concentration in the water is significant, because even low concentrations of iodine in the water can negate the effects of perchlorate, at least in amphibians (*26*).

Experimental Design

Mosquitofish were exposed to 0, 0.1, 1, 10, 100, and 1000 mg/L sodium perchlorate in reconstituted water for 2, 10, and 30 d. There was 15 L water per aquarium. Fifteen mosquitofish were randomly assigned to each aquarium. There were 5 replicate aquaria for each of the treatment concentration and duration combination. There were six concentrations used (control plus five concentrations of perchlorate). There were 3 exposure time periods (i.e., there were a total of 18 treatments − 6 concentrations × 3 exposure periods, and a total of 90 aquaria (18 × 5 replicates per treatment). The aquaria were arranged in a randomized block design, with 1 replicate per treatment assigned to each block (laboratory shelf), minimizing possible effects due to environmental variation in the laboratory. Fish were fed Aquatox Flake food daily, ad libutium, and all feedings were at the same time of the day.

Every other day, 1/3 of the water was changed, and the aquaria were refilled with reconstituted water and the appropriate amount of sodium perchlorate stock solution. Dissolved oxygen, salinity, pH, ammonia, and conductivity were determined on days between water changes. Water samples were also taken on the first day and last day of each exposure, and once weekly for the longer exposures, and analyzed for perchlorate as described in Anderson and Wu (*27*). Fish were fed ad libitum and were fed at the same time of the day each feeding period.

Following exposure, the fish were euthanized in 1 g/L of MS-222 (methanesulfonate salt, 3-aminobenzoic acid ethyl ester), and the standard length and mass of each fish were recorded. Two fish were randomly selected from each aquarium and were preserved in Bouin's fixative (75% picric acid-saturated water, 20% formalin, and 5% glacial acetic acid) for histological processing, and the remainder of the fish were frozen whole in liquid nitrogen and stored at −80 °C for determination of whole body T_4 content.

Histopathology

The fish heads severed from whole fish fixed in Bouin's fixative (as described above). The heads were were immersed in Bouin's fixative for 2 d to decalcify and fix tissues, after which the fixative was removed by rinsing in running DI water (24 h) and soaking in 70% ethanol. The ethanol was replaced every 24 h until there was no longer any yellow discoloration (due to Bouin's fixative) in the ethanol. Tissues were processed with the Tissue-Tek V.I.P. 2000 Processor (Miles Laboratories, Elkhart, IN) and then embedded in paraffin. Transverse cross-sections were cut with a microtome (5 μm) and mounted on microscope slides. The slides were then stained with hematoxylin and Eosin Y for light microscopic observation following ref *28*.

The number of follicles per section and the height of the follicular epithelium were measured in 10 fish randomly selected from each exposure group. In each fish, the height of 100 epithelial cells from a total of 10 follicles (10 cells per follicle) was determined with a light microscope. Five follicles were chosen from a section from the rostral end (the first section to have at least five follicles), and five were chosen from a section from the caudal end of the basibranchial region. Follicles were chosen on the basis of lack of histological artifacts (tears, folding of the sections, distortions due to cutting, cutting through the wall of the follicle). The slides were also examined to score the percent occurrence of hyperplasic, hypertrophic, and colloid-depleted follicles; in this case, all follicles (without discernible artifacts of preparation, see above) were scored. When a particular section was chosen for scoring, the preceding and subsequent sections were also examined qualitatively to ensure that the follicle was not cut through the follicle wall. Because artifacts of histological processing may cause conditions that may appear similar to colloid depletion, follicles were scored as colloid-depletion only if there were indicators of colloid depletion other than reduced colloid size (i.e, pale, "lacy", or eosinic colloid, increase number of vacuoles, follicular wall infolding) (*29*, *30*). All slides were coded and scored blind: one investigator performed the exposures, while another performed the histology.

The severity of thyroid damage was also assessed based upon the guidelines established by the EPA Pathology Working Group (*29*, *30*). Based upon these guidelines, the degree of colloid depletion, hyperplasia, and hypertrophy was given a score of 0, 1, or 2 in order of increasing severity. The overall score was then determined by calculating the mean of the colloid depletion, cell hypertrophy, and hyperplasia scores for all sections and summing to determine the mean histological grade for the fish (*30*). Scoring was done on all follicles located in three section per fish. The sections were from the rostral, caudal, and midpoint of the follicle-containing region of the basibranchial apparatus. Sections were spaced far enough apart as to avoid scoring the same follicle in more than one section. "Hypertrophy" was defined as follicles with cuboidal-high columnar epithelium (*30*), i.e., cells where the width (dimension parallel to the axis of the basement membrane) ≤ height (dimension perpendicular to the axis of the basement membrane).

Determination of T_4 Concentration

Methods for determination of whole body T_4 followed Goleman et al. (*12*) and Denver (*31*). Approximately 2.5 g of fish tissue (about 5 fish) were pooled into 1 sample from each test aquarium (90 samples total) and frozen in liquid nitrogen for determination of whole body T_4 concentration by radioimmunoassay (RIA). Fish were thawed, minced, homogenized, and sonicated in 3 volumes of MeOH/PTU (methanol containing 1 mM propylthiouracil). An aliquot was removed for spectrophotometric protein determination. $^{125}I-T_4$ (New England Nuclear, Boston, MA, 1000−1500 μCi/μg) tracer was added to each sample to achieve a specific activity of 1000 counts per minute (CPM). Samples were then extracted with chloroform (2:1, chloroform:MeOH/PTU) and then back-extracted with 0.1 volumes of 2 N ammonium hydroxide (NH_4OH). The aqueous supernatants were pooled and dried overnight in a rotary evaporator.

Dried extracts were reconstituted in 1 mL of 2 N NH_4OH and again extracted with chloroform. The aqueous phase was added to a AG 1 × 2 resin (200−400 mesh, chloride form) chromatography column and sequentially washed with acetate buffer (AB, 16.4 g of Na-acetate/L deionized water, pH 7), absolute ethanol, AB (pH 4), AB (pH 3), 1% acetic acid, 35% acetic acid, and 70% acetic acid. The samples were then eluted in 70% acetic acid, dried in a vacuum evaporator, and reconstituted in 125 μL of RIA buffer (bovine gamma globulin-EDTA-thimerosal-barbital buffer, 15.46 g of barbital, 0.5 g of EDTA, 0.1 g of thimerosal, 1.0 g of bovine gamma globulins/L deionized water, pH adjusted to 8.6 with 1 N HCl) with 1.5 mg of ANS (8-anilino-1-naphthalenesulfonic acid)/mL RIA buffer (RIA buffer/ANS). The T_4 content of reconstituted

TABLE 1. Mean (±SD)[a] Perchlorate Concentrations (mg/L) Measured in Water during Exposures

nominal concn, mg/L	exposure period		
	2 day	10 day	30 day
0.1	0.08 ± 0.008	0.10 ± 0.039	0.18 ± 0.103
1	0.80 ± 0.05	0.75 ± 0.046	0.90 ± 0.360
10	7.5 ± 0.29	7.0 ± 0.32	7.1 ± 0.39
100	96 ± 18	82 ± 15	68 ± 4
1000	906 ± 149	1100 ± 318	667 ± 49

[a] Mean value for each exposure period for samples taken at least once per week.

extract samples was determined by radioimmunoassay following ref *32* using T_4 antiserum (rabbit anti-T_4, ICN Pharmaceuticals, Inc., Irvine, CA; 1:320) and $^{125}I-T_4$ and measured in a Cobra 5005 Gamma-Counter (Packard, Downers Grove, IL). Intraassay variation was determined by comparing two sets of standards, one at the beginning of the assay and one at the end of the assay. The T_4 concentrations were then converted to ng/g based upon the original wet weight of the sample and the reconstituted volume of 125 μL. Validation of the assay was achieved by comparing the displacement of $^{125}I-T_4$ in serially diluted mosquitofish extracts with that produced by authentic T_4 standards.

Statistical Analysis of Histopathology and T_4 Concentrations. Nonparametric statistics were used because of the small number of samples, heterogeneity of variances, and non-normality of the data. Nested ANOVA on the ranked data indicated no differences among blocks or aquaria within treatments, so for histopathological analysis all fish were pooled within each treatment. Differences among treatments were tested with the Kruskal-Wallis test with Dunn's post-hoc comparisons of all treatments vs control.

Because different parameters may respond in different directions or with different magnitudes, a more integrative approach would include multivariate analysis of multiple endpoints in order to determine *overall* differences in oxidative responses among thyroidal states. Also, multivariate analyses may be more sensitive indicators of responses than univariate analyses (*33*). Thus, multivariate ANOVA was also performed on the ranked histopathological data, and multivariate *t*-tests with Bonferoni adjustments were used for multiple comparisons among treatments within each exposure period. To visualize relative multivariate differences among treatments, discriminant analysis was performed on the rank-transformed data, and the group centroids were plotted as a function of the first and second discriminant functions.

Results and Discussion

Mortality for the 2-day exposure was 0%. Mortality for the 10-day exposure ranged from 0 to 0.4%. Mortality for the 30-day exposure ranged from 0 to 0.9%. Mortality did not vary in response to exposure concentration. There were no statistically significant differences in size of the fish before or after the treatments ($P>0.05$, ANOVA). Measured perchlorate concentrations within each aquarium are presented in Table 1, and water quality parameters were within acceptable ranges for fish chronic toxicity tests (*34*) (Table 2).

The types of histolopathological abnormalities observed are illustrated in Figure 1. There was an increase in the height of the follicular epithelium, especially after the 30 d exposure (Figure 2A). Apparent differences in epithelial cell height in fish exposed for 2 and 10 d were minimal, but after 30 d large differences were apparent. The presence of hyperplasia and hypertrophy were also apparent (Figures 2B and 3A). Although these effects were statistically significantly different from control as soon as 2 days after exposure, there was not a discernible pattern of increasing response with increasing dose until after 30 d of exposure (Figures 2 and 3). A notable finding is that perchlorate exposure induced effects that were statistically significantly different from controls at environmentally relevant concentrations (0.1, 1, and 10 mg/L). Other studies have found that these concentrations are within the ranges of perchlorate surface water concentrations found in the field (*3*, *20*, *35*).

The results for colloid depletion were somewhat unexpected. Instead of increasing with perchlorate exposure, the levels of colloid depletion actually decreased. At 30 d, there was a definite tendency toward decreasing occurrence of colloid depletion with increasing dose (Figure 3B). This suggests that there is a perturbation in the balance between thyroid hormone secretion into the colloid and pinocytosis from the colloid.

Histopathology score increased significantly with increasing concentration in mosquitofish exposed to sodium perchlorate for 30 d (Figure 3C). A definite dose−response pattern was less evident in mosquitofish exposed to sodium perchlorate for 10 d (Figure 3C). At 2 days, there were no follicles evident with epithelial hypertrophy (cell height > width) or depleted colloid, so the scores for day 2 could not be reported.

Multivariate analysis of variance indicated that, when all variables are used in the analysis, there were highly significant differences between all treatments and control ($p<0.001$) after 10 and 30 d of exposure. The plots of the group centroids in discriminant space indicated that for environmentally relevant concentrations (0.1−10 mg/L, nominal concentrations), there was in increase in distance (in discriminant space) between control and treated centroids with increasing dose concentrations (Figure 4). This was more apparent at 30 d than at 10 d (Figure 4). For higher concentrations (100 and 1000 mg/L), this was not the case.

Except for the colloid depletion, results from the histological analysis are consistent with pathologies seen in amphibians, zebrafish, and rats exposed to perchlorate (*12*, *14*, *36*−*40*) and in other species of fish exposed to various

TABLE 2. Time-Weighted Average of Water Quality Parameters during Experiments in Which Mosquitofish Were Exposed to Sodium Perchlorate at the Concentrations Indicated

nominal concn, mg/L	water quality parameters					
	pH	temperature (°C)	dissolved oxygen (%)	conductivity (μS/cm)	salinity (ppt)	NH_3 (μg/L)
0	6.03 ± 0.13	18.4 ± 1.6	87.0 ± 4.5	145 ± 5.3	0.1	0.77 ± 0.12
0.1	6.81 ± 0.26	18.3 ± 0.09	89.8 ± 3.2	178 ± 8.9	0.1	0.86 ± 0.16
1	6.07 ± 0.16	18.3 ± 1.2	92.7 ± 2.0	165 ±15.8	0.1	0.77 ± 0.41
10	6.23 ± 0.023	18.4 ± 1.8	93.6 ± 3.1	178 ± 15.3	0.1	0.97 ± 0.14
100	6.34 ± 0.18	18.4 ± 0.1	84.1 ± 2.9	186 ± 18.3	0.1	0.86 ± 0.12
1000	6.56 ± 0.12	18.9 ± 1.8	83.9 ± 1.3	1026 ± 42	0.6	0.88 ± 0.16

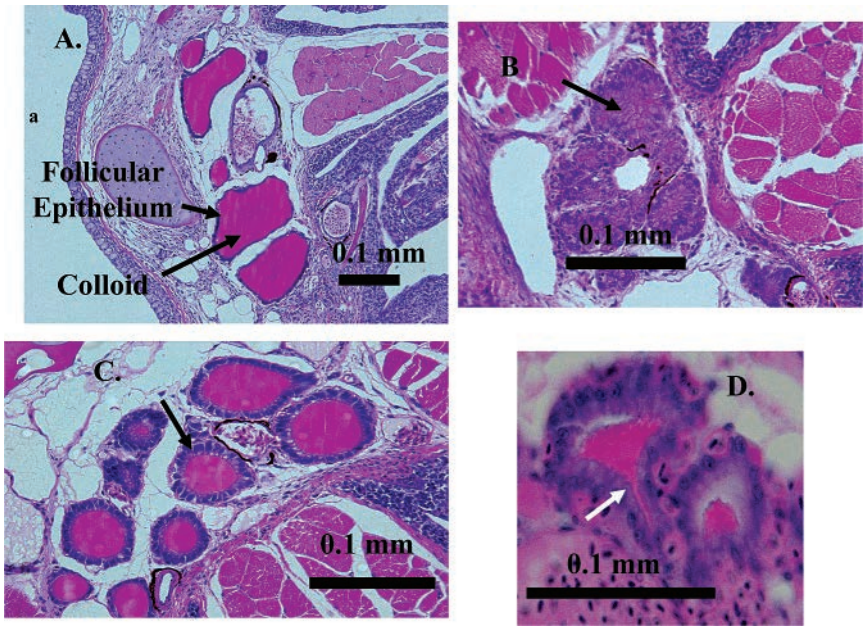

FIGURE 1. Photomicrographs of hematoxylin/eosin stained sections of thyroids. (A) Control mosquitofish follicle with simple squamous epithelium. (B–D) Thyroid alterations due to exposure to sodium perchlorate: (B) total colloid depletion with follicular collapse and hyperplasia; (C) moderate hypertrophy (follicle with low columnar epithelium); and (D1) colloid depletion with severe hypertrophy (high columnar epithelium).

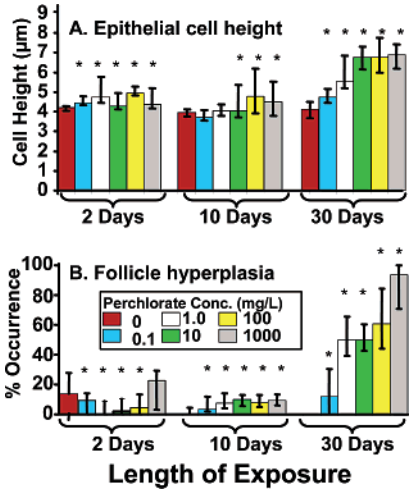

FIGURE 2. Hyperplasia (A) and epithelial cell height (B) in mosquitofish exposed to 0, 0.1, 1, 10, 100, and 1000 mg/L sodium perchlorate for 2, 10, and 30 d. Bars labeled with an asterisk are significantly different from control ($p<0.05$, Kruskal-Wallis test, Dunn's post hoc test of all treatments vs controls). Bars are medians and error bars are first and third quartiles ($n=5$).

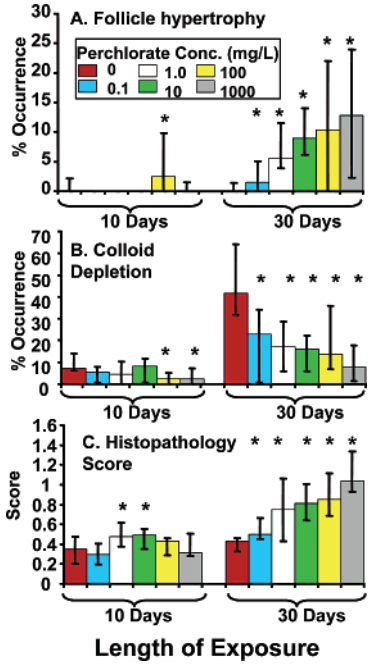

FIGURE 3. Hypertrophy (A), colloid depletion (B), and histopathology score (C) in mosquitofish exposed to 0, 0.1, 1, 10, 100, and 1000 mg/L sodium perchlorate for 10 and 30 d. Bars labeled with an asterisk are significantly different from control ($p<0.05$, Kruskal-Wallis test, Dunn's post hoc test of all treatments vs controls). Bars are medians and error bars are first and third quartiles ($n=5$).

thyroid disrupting compounds (*18*, *41*–*44*). The colloid depletion data are in contrast to the effects seen by Patiño et al. (*14*), in which increasing levels of perchlorate exposure led to increasing occurrence of depleted colloid. In the mosquitofish, hyperplasia, hypertrophy, and colloid depletion were evident in fish chronically exposed to perchlorate. In a previous study, histological examination of the thyroid from amphibians exposed to ammonium perchlorate also indicated an increase in the epithelial cell height compared to the controls (*12*). Since differences were seen in the epithelial cell height between the control group and the group exposed to the lowest concentration, Goleman et al. suggest that epithelial cell height is the most sensitive indicator of ammonium perchlorate exposure (*12*). Other histological studies of amphibians also indicate the presence of hypertrophy and hyperplasia after exposure to perchlorate (*11*–*13*, *36*–*38*), similar to what was found in mosquitofish from the present study.

In terms of whole body T_4 concentration, there was a decrease in exposed fish for at least some treatments for all exposure periods, but this decrease did not follow a definite dose–response pattern (Figure 5). The results of this study indicate that whole body T_4 is a less sensitive or less predictive (in terms of dose–response relationships) indicator of perchlorate exposure than thyroid histology, as was indicated by Goleman et al. (*12*). Interassay variation was less than a

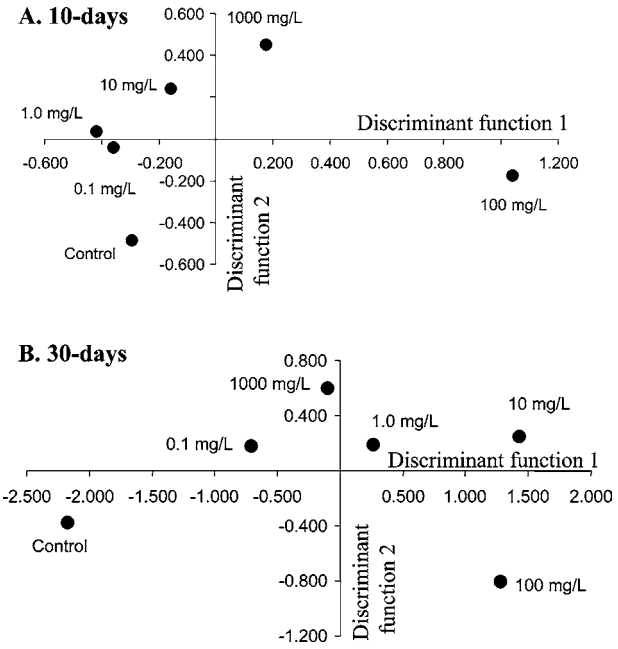

FIGURE 4. Group centroids using the first two discriminant functions generated by discriminant analysis using the thyroid follicle histopathological variates percent hyperplastic follicles, percent hypertrophic follicles, and percent follicles with colloid depletion for eastern mosquitofish exposed to 0−1000 mg/L sodium perchlorate for (A) 10 d and (B) 30 d.

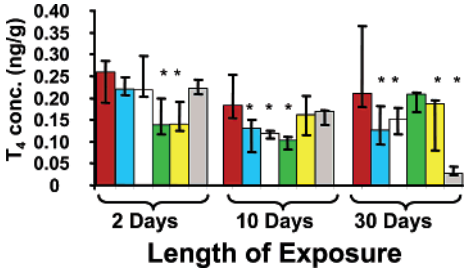

FIGURE 5. Whole body T4 content in mosquitofish exposed to 0, 0.1, 1, 10, 100, and 1000 mg/L sodium perchlorate for 2, 10, and 30 d. Bars labeled with an asterisk are significantly different from control (p<0.05, Kruskal-Wallis test, Dunn's post hoc test of all treatments vs controls). Bars are medians and error bars are first and third quartiles (n=5).

10% difference between any set of standards, in terms of amount of $^{125}I-T_4$ displaced by authentic T_4 standards. There was a strong correlation between amount of displacement of $^{125}I-T_4$ in serially diluted mosquitofish extracts and that produced by authentic T_4 standards ($P<0.01$, $r^2=0.91$).

Although only a slight decrease in whole body T_4 concentration was indicated in the mosquitofish exposed to perchlorate ($0.05<P<0.1$), other studies have indicated large decreases in serum T_4 concentrations. Sea lamprey that were exposed to potassium perchlorate for 4, 8, and 16 wk had lower serum T_4 levels than the control group (*19*), and in a following study with a 23 wk exposure, serum T_4 levels were more than 60% lower than in the control. In amphibians that were exposed for 42, 70, and 98 d, whole body T_4 concentrations were significantly lower than in the control in the amphibians exposed for the 2 longest time periods (*12*). A 2-generation study in the rat (*41*) and a 90-d exposure of rats to perchlorate in drinking water (*40*) both gave evidence of significant decreases in serum T_4 concentrations. These significant findings may be due to the fact that previous studies examined T_4 in the plasma rather than in the whole body. Because of the small size of the fish used to determine T_4 levels in the current study, the T_4 had to be extracted from the whole fish. Thus, there was no differentiation among T_4 in blood, thyroid, and extrathyroidal tissues, which may have complicated the analysis. If it had been possible to determine plasma T_4 and triiodothyronine (T_3) levels, thyroid hormones may have been a more sensitive indicator of perchlorate exposure than was evident in the current study.

Whole body T_4 levels decreased with increasing concentration of perchlorate, as expected. However, occurrence of depleted colloid decreased with increasing perchlorate dose, and this is opposite of what would be expected. T_4 levels are expected to drop as colloidal stores of iodinated thyroglobulin (a protein used to make thyroid hormones) fall. Further studies focusing on these alternative endpoints are needed to clarify these results.

In conclusion, results were consistent with the hypotheses in that thyroid homeostasis was affected by exposure of fish to perchlorate for long periods of time, at least for thyroid histopathological endpoints was observed in fish exposed to perchlorate. Definitive concentration−response relationships were only seen for the longest exposure scenario. Whether these results are indicative of compensatory responses or pathological conditions remains to be determined. Also, it is not known if such effects would be translated into effects on fitness parameters such as reproduction, development, growth, survival, and lifespan. Such endpoints must be the focus of future research.

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Literature Cited

(1) Urbansky, E. T. Perchlorate as an environmental contaminant. *Environ. Sci. Pollut. Res. Int.* **2002**, *9*, 187−192.
(2) Urbansky, E. T. *Perchlorate in the environment*; Kluwer Academic/Plenum Publishers: New York, 2000.
(3) Smith, P. N.; Theodorakis, C. W.; Anderson, T. A.; Kendall, R. J. Preliminary assessment of perchlorate in ecological receptors at the Longhorn Army Ammunition Plant (LHAAP), Karnack, Texas. *Ecotoxicology* **2001**, *10*, 305−13.
(4) Norris, D. O. *Vertebrate endocrinology*; Academic Press: San Diego, CA, 1997.
(5) Hadley, M. E. *Endocrinology*; Prentice Hall: Upper Saddle River, NY, 2000.
(6) Leatherland J. F. Reflections on the thyroidology of fishes, from molecules to humankind. *Guelph Ichthyol. Rev.* **1994**, 3−67.
(7) Yu, K. O.; Narayanan, L.; Mattie, D. R.; Godfrey, R. J.; Todd, P. N.; Sterner, T. R.; Mahle, D. A.; Lumpkin, M. H.; Fisher, J. W. The pharmacokinetics of perchlorate and its effect on the hypothalamus-pituitary-thyroid axis in the male rat. *Toxicol. Appl. Pharmacol.* **2002**, *182*, 148−59.
(8) Greer, M. A.; Goodman, G.; Pleus, R. C.; Greer, S. E. Health effects assessment for environmental perchlorate contamination, the dose response for inhibition of thyroidal radioiodine uptake in humans. *Environ. Health. Perspect.* **2002**, *110*, 927−37.
(9) Lawrence, J.; Lamm, S.; Braverman, L. E. Low dose perchlorate (3 mg daily) and thyroid function. *Thyroid* **2001**, *11*, 295.

(10) Fisher, J.; Todd, P.; Mattie, D.; Godfrey, D.; Narayanan, L.; Yu, K. Preliminary development of a physiological model for perchlorate in the adult male rat, a framework for further studies. *Drug Chem. Toxicol.* **2000**, *23*, 243−58.
(11) Carr, J. A.; Urquidi, L. J.; Goleman, W. L.; Hu, F.; Smith, P. N.; Theodorakis, C. W. Ammonium perchlorate disruption of thyroid function in natural amphibian populations, assessment and potential impact. In *Multiple Stressor Effects in Relation to Declining Amphibian Populations*; Linder, G., Krest, S., Sparling, D., Little, E. E., Eds.; ASTM International: West Conshohocken, PA, 2003; Vol. ASTM STP1443.
(12) Goleman, W. L.; Carr, J. A.; Anderson, T. A. Environmentally relevant concentrations of ammonium perchlorate inhibit thyroid function and alter sex ratios in developing *Xenopus laevis*. *Environ. Toxicol. Chem.* **2002a**, *21*, 590−597.
(13) Goleman, W. L.; Urquidi, L. J.; Anderson, T. A.; Smith, E. E.; Kendall, R. J.; Carr, J. A. Environmentally relevant concentrations of ammonium perchlorate inhibit development and metamorphosis in *Xenopus laevis*. *Environ. Toxicol. Chem.* **2002b**, *21*, 424−430.
(14) Patiño, R.; Wainscott, M. R.; Cruz-Li, E. I.; Balakrishman, S.; McMurry, C.; Blazer, V. S.; Anderson, T. A. Effects of ammonium perchlorate on the reproductive performance and thyroid follicle histology of zebrafish. *Environ. Toxicol. Chem.* **2003**, *22*, 1115−1121.
(15) Power, D. M.; Llewellyn, L.; Faustino, M.; Nowell, M. A.; Bjornsson, B. T.; Einarsdottir, I. E.; Canario, A. V.; Sweeney, G. E. Thyroid hormones in growth and development of fish. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* **2001**, *130*, 447−459.
(16) Brown, D. D. The role of thyroid hormone in zebrafish and axolotl development. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 13011−13016.
(17) Plohman, J. C.; Dick, T. A.; Eales, J. G. Thyroid of lake sturgeon, *Acipenser fulvescens*. II. Deiodination properties, distribution, and effects of diet, growth, and a T3 challenge. *Gen. Comput. Endocrinol.* **2002**, *125*, 56−66.
(18) Rolland, R. M. A review of chemically induced alterations in thyroid and vitamin A status from field studies of wildlife and fish. *J. Wildl. Dis.* **2000**, *36*, 615−635.
(19) Manzon, R. G.; Eales, J. G.; Youson, J. H. Blocking of $KC1O_4$-induced metamorphosis in premetamorphic sea lampreys by exogenous thyroid hormones (TH); effects of $KC1O_4$ and TH on serum TH concentrations and intestinal thyroxine outer-ring deiodination. *Gen. Comput. Endocrinol.* **1998**, *112*, 54−62.
(20) Theodorakis, C. W.; Rinchard, J.; Anderson, T.; Liu, F.; Park, J.-W, Costa, F.; McDaniel, L.; Kendall, R.; Waters, A. Perchlorate in fish from a contaminated site in east-central Texas. *Environ. Pollut.* **2005**, in press.
(21) Urbansky, E. T. Perchlorate Chemistry, Implications for analysis and remediation. *Biorem. J.* **1998**, *2*, 81−95.
(22) Kamavisdar, A.; Patel, R. M. Flow injection spectrophotometric determination of iodide in environmental samples. *Microchim. Acta* **2002**, *140* (1−2), 119−124.
(23) Moran, J. E.; Oktay, S. D.; Santschi, P. H. Sources of iodine and iodine 129 in rivers. *Water Resour. Res.* **2002**, *38* (8), Art. No. 1149.
(24) Ghose, N. C.; Das, K.; Saha, D. Distribution of iodine in soil−water system in the Gandak basin, Bihar. *J. Geol. Soc. India* **2003**, *62* (1), 91−98.
(25) Schwehr, K. A.; Santschi, P. H. Sensitive determination of iodine species, including organo-iodine, for freshwater and seawater samples using high performance liquid chromatography and spectrophotometric detection. *Anal. Chim. Acta* **2003**, *482* (1), 59−71.
(26) Renner, R. Iodine counteracts perchlorate effects in frogs. *Environ. Sci. Technol.* **2003**, *37* (3), 52A.
(27) Anderson, T. A.; Wu, T. H. Extraction, cleanup, and analysis of the perchlorate anion in tissue samples. *Bull. Environ. Contam. Toxicol.* **2002**, *68*, 684−91.
(28) Humason, G. E. *Animal Tissue Techniques*; Freeman and Company: San Francisco, 1967.
(29) Mann, P. C. *The effects of ammonium perchlorate on thyroids, Pathology working group report;* Experimental Pathology Laboratories, Inc.: Research Triangle Park. 2000. http://oaspub.epa.gov/eims/eimscomm.getfile?p_download_id=4605.
(30) Hooth, M. J.; Deangelo, A. B.; George, M. H.; Gaillard, E. T.; Travlos, G. S.; Boorman, G. A.; Wolf, D. C. Subchronic sodium chlorate exposure in drinking water results in a concentration-dependent increase in rat thyroid follicular cell hyperplasia. *Toxicol. Pathol.* **2001**, *29*, 250−259.
(31) Denver, R. J. Acceleration of anuran amphibian metamorphosis by corticotropin-releasing hormone-like peptides. *Gen. Comput. Endocrinol.* **1993**, *91*, 38−51.
(32) MacKenzie, D. S.; Licht, P.; Papkoff, H. Thyrotropin from amphibian (*Rana catesbeiana*) pituitaries and evidence for heterothyrotropic activity of bullfrog luteinizing hormone in reptiles. *Gen. Comput. Endocrinol.* **1978**, *36*, 566−574.
(33) Anderson, T. W. *An introduction to multivariate statistical analysis;* Wiley: New York, 1984.
(34) ASTM. *Standard Practice For Conducting Chronic Toxicity Tests with Early Life Stages of Fish;* ASTM E-1241-98; American Society for Testing and Materials: Philadelphia, PA, 1999.
(35) Smith, P. N.; Yu, L.; McMurry, S. T.; Anderson, T. A. Perchlorate in water, soil, vegetation, and rodents collected from the Las Vegas Wash, Nevada, U.S.A. *Environ. Pollut.* **2004**, *132* (1), 121−127.
(36) Miranda, L. A.; Paz, D. A.; Dezi, R. E.; Pisano, A. Immunocytochemical and morphometric study of TSH, PRL, GH, and ACTH cells in *Bufo arenarum* larvae with inhibited thyroid function. *Gen. Comput. Endocrinol.* **1995**, *98*, 166−176.
(37) Miranda, L. A.; Pisano, A.; Paz, D. A. Effect of potassium perchlorate on thyroid activity of *Bufo arenarum* larvae. *Comun. Biol.* **1992**, *10*, 125−135.
(38) Sparling, D. W.; Harvey, G.; Nzengung, V. Interaction between perchlorates and iodine in the metamorphosis of *Hyla versicolor*. In *Multiple Stressor Effects in Relation to Declining Amphibian Populations*; Linder, G., Krest, S., Sparling, D., Little, E. E., Eds.; ASTM International: West Conshohocken, PA, 2003; Vol ASTM STP1443.
(39) Rodriguez, A. F.; Davidson, H. G.; Villadiego, M. S.; Fernandez, A. M.; Lacave, I. M.; Sanz, J. F. Induction of thyroid proliferative changes in rats treated with antithyroid compound. *J. Vet. Med. C* **1991**, *20*, 289−298.
(40) Siglin, J. C.; Mattie, D. R.; Dodd, D. E.; Hildebrandt, P. K.; Baker, W. H. A 90-day drinking water toxicity study in rats of the environmental contaminant ammonium perchlorate. *Toxicol. Sci.* **2000**, *57*, 61−74.
(41) York, R. G.; Brown, W. R.; Girard, M. F.; Dollarhide, J. S. Two-generation reproduction study of ammonium perchlorate in drinking water in rats evaluates thyroid toxicity. *Int. J. Toxicol.* **2001**, *20*, 183−97.
(42) McMaster, M. E. A review of the evidence for endocrine disruption in Canadian aquatic ecosystems. *Water Qual. Res. J. Can.* **2001**, *36*, 215−231.
(43) Brown, S. B.; Adams, B. A.; Cyr, D. G.; Eales, J. G. Contaminant effects on the teleost fish thyroid. *Environ. Toxicol. Chem.* **2004**, *23*, 1680−1701.
(44) Leatherland, J. F. Contaminant-altered thyroid function in wildlife. In *Environmental Endocrine Disrupters, An Evolutionary Perspective*; Guillette, L. J., Jr., Crain, D. A., Eds.; Taylor and Francis: New York, 1999.

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