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Food Chain Transfer of Perchlorate in Largemouth Bass, *Micropterus salmoides*

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Uptake of contaminants by aquatic organisms is of concern due to the possibility of transfer to organisms at higher levels of the food chain. However, the potential for food chain transfer of many environmental contaminants are unknown. The various salts of perchlorate are among these contaminants (Urbansky 2002). Perchlorate (ClO_4^-) has been primarily used as an oxidizer in solid propellant systems for rockets and missiles (Urbansky 2002). Concern over perchlorate contamination is due to its stability and mobility in water and soils (Motzer 2001). In general, the behavior of perchlorate in the aquatic environment has been characterized by rapid mobility, stability, and non-reactivity. Thus, perchlorate in aquatic systems can persist and be mobile from years to decades (Motzer 2001). Recently, widespread perchlorate contamination was found in many US states where fireworks, solid fuel rockets, and illuminating munitions are manufactured and processed (Motzer 2001, Smith et al. 2001 and refs therein, Urbansky 2002). Naturally occurring perchlorate in nitrate-rich mineral deposits used as fertilizers can also contribute to environmental perchlorate contamination in biota, such as in plants (Ellington et al. 2001).

Perchlorate disrupts thyroid hormone synthesis via competitive inhibition of iodide uptake in thyroid follicles (Clark 2000). Because thyroid hormones have important roles for regulating growth, cell differentiation, metabolism, and neuro-development (Clark 2000, Brown et al. 2004), concerns about effects in aquatic organisms such as fish and amphibians have recently emerged (Goleman et al. 2002; Patiño et al. 2003). Fishes may be exposed to perchlorate through ambient water and food. Field studies have shown that perchlorate concentrations in fish from contaminated sites may exceed water concentrations (Smith et al. 2001), contrary to what has been found in the laboratory (unpublished results). Thus, routes of uptake other than via absorption from the water may contribute to this phenomenon in wild fish. Although the mechanism of perchlorate thyrotoxicity is well defined, there is little information on the contribution of perchlorate uptake through different routes of exposure. Thus, the purposes of this study were to assess perchlorate accumulation in fish through contaminated prey as well as ambient water, and whether contaminated prey can contribute to the body burden of fish, following long-term exposure. The hypothesis is that perchlorate will be taken up via water and food, with equal contribution from both.

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MATERIALS AND METHODS

Sodium perchlorate (99% purity, EM Science, Gibbstown, NJ) was used to make a stock solution at a concentration of 500 g/L, pH 7.4. Test water consisted of reverse osmosis water reconstituted with 60 g/L of Instant Ocean® sea salts. Largemouth bass, *Micropterus salmoides,* were selected in this study because they are common and widespread in the U.S. Mean (± SD) mass and standard length were 19.0 ± 5.2 g and 112.0 ± 10.5 mm, respectively. Fathead minnows, *Pimephales promelas,* were used as prey for the bass (average mass 1.3 ± 0.32 g per fish). Dissolved oxygen, conductivity, temperature, pH, and salinity were measured with a YSI® model 85 meter (Yellow Springs OH, USA), and the unionized ammonia was measured with a Hach® spectrophotometer model DR/2000 (Loveland, CO, USA) every other day.

Fifty minnows per exposure were placed in 80-L aquaria and exposed to 1000 mg/L sodium perchlorate for 2 days. These exposure conditions were based upon preliminary data (not shown), which indicated that the minnows would need to be exposed to this amount of perchlorate for there to be equal contributions from water and food to the bass' total body burden, assuming 100% assimilation efficiency. Minnows were fed to the bass daily in the amount of approximately 10% of the bass body weight. Prior to feeding the bass, minnows and water samples were sampled and analyzed for perchlorate. There was one bass per aquarium (30 L of water) and 5 replicates in each exposure period. Water samples were collected from each aquarium every other day and analyzed for perchlorate. Uneaten prey items were removed daily. After 1, 10, and 30 days, largemouth bass were collected, euthanized with MS-222 (1.5 g/L), and stored at -80℃ until perchlorate analysis. For the "food only" treatment, bass were kept in non-contaminated water and fed contaminated minnows. For the "water only" treatment, bass were exposed to 500 mg/L sodium perchlorate and fed non-contaminated minnows. This concentration of sodium perchlorate was chosen to ensure that perchlorate could be detected in tissues. For the "water plus food" treatment, bass were exposed to sodium perchlorate via both food and water routes. Minnows were pooled (about 5 g) for perchlorate body burden analysis.

Fish and water samples were analyzed for perchlorate following Anderson and Wu (2002). Briefly, samples were extracted with distilled Milli-Q water using a Dionex 200 Accelerated Solvent Extractor (ASE 200, Dionex Corp., Sunnyvale, CA, USA). Extracts were cleaned with silica solid phase extraction (SPE) cartridges and analyzed using a Dionex DX-500 Ion Chromatograph equipped with a CD 20 conductivity detector. A Dionex IonPac AS16 (4 mm) analytical column provided analyte separation with 100 mM sodium hydroxide as the eluent. An 8-point standard curve was constructed using a certified sodium perchlorate standard (Accustandard, Inc., New Haven, CT USA). Perchlorate was identified based on the retention time. The water samples were analyzed following similar procedures without an extraction step. Detection limits for water and tissue extracts were determined using a series of samples with known perchlorate concentrations. Percent recoveries for spiked extracts were determined by

adding 100 μg perchlorate to tissue extracts. Percent recoveries for fish tissues were determined by perfusing (via multiple syringe injections) wet tissues with 100 μg sodium perchlorate in DI, before dessication and extraction of the tissues.

Differences among treatments were tested using Kruskall-Wallis tests (multiple comparisons) or Wilcoxon's rank sum test, as appropriate. Regressions were tested using non-parametric Pearson's tests. Non-parametric tests were used because this was found to be more statistically powerful due to the skewed distribution of the data, non-homogeneous variances, and small sample sizes.

RESULTS AND DISCUSSION

Detection limits for perchlorate were 1 μg/L and 5 μg/L in water and tissue extracts, respectively. Percent recoveries from spiked water samples were 98.5 ± 0.42. Percent recoveries from spiked fish tissues were 93.8 ± 10.2 (mean ± standard deviation, n = 11). There were no differences in percent recoveries between species ($p > 0.05$, Student's t-test). Thus, assuming a minimum of 5 g extracted tissue and 20 ml extraction volume (according to the authors' standard procedures), and a conservative estimate of 80% extraction recovery, the detection limit in fish tissues was roughly 25 ng/g. The data for water chemistry parameters are presented in Table 1.

Table 1. Water quality[a] measured in aquaria in which largemouth bass exposed to perchlorate via different routes for 1, 10, or 30 days.

Route	Days	pH	Temp. (°C)	Dissolved O_2 (%)	Cond. (μS/cm)	NH_3 (mg/L)
Food	1	7.1 ± 0.1	20.2 ± 0.2	82.1 ± 3.8	160 ± 19	1.7 ± 0.5
	10	7.1 ± 0.1	20.5 ± 0.5	81.9 ± 2	148 ± 8	2 ± 0.6
	30	7.3 ± 0.1	20.1 ± 0.7	84.3 ± 2.8	148 ± 16	1.8 ± 0.3
Water	1	7.2 ± 0.1	19.8 ± 1.1	82.3 ± 2.4	159 ± 24	1.7 ± 0.3
	10	7.2 ± 0.1	20.4 ± 0.8	82 ± 2.4	158 ± 8	1.4 ± 0.4
	30	7.1 ± 0.1	20.7 ± 0.5	81.2 ± 2.8	148 ± 25	1.4 ± 0.4
Both	1	7.1 ± 0.1	20.9 ± 0.4	80.2 ± 3.7	142 ± 23	1.4 ± 0.4
	10	7.1 ± 0.2	20.7 ± 0.2	80.8 ± 3.3	151 ± 27	1.6 ± 0.6
	30	7.2 ± 0.2	20.5 ± 0.3	83.1 ± 2.8	148 ± 21	1.7 ± 0.7

[a]Water quality values are based upon cumulative means (± standard deviation) of samples taken every two days for the duration of the test. Dissolved O_2 is percent saturation. "Cond." is conductivity. "NH_3" is total ammonia.

The concentrations of perchlorate measured in the aquarium water at the time of tissue sampling are presented in Fig. 1. The low perchlorate concentrations reported in the "food only" aquarium are probably due to the elimination of perchlorate from the bass. Concentrations of perchlorate in the water were not

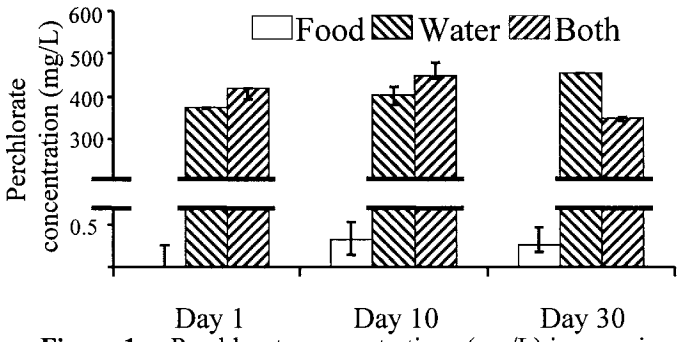

Figure 1. Perchlorate concentrations (mg/L) in aquaria water containing bass exposed to sodium perchlorate via food only, water only, or both food and water. Bars represent medians and error bars are first and third quartiles.

statistically significantly different among the "food only" treatments (1, 10, 30 days), but all other differences (among exposure routes within exposure periods, and among exposure periods within exposure routes) were statistically significant ($p < 0.05$, 2-way ANOVA, Fig. 1). Average (± standard deviation) concentrations of perchlorate in the minnows fed to the bass for 1, 10, and 30 day exposures were 35.2 ± 7.5, 45.9 ± 22.6, and 51.5 ± 16.0, respectively.

Fish can take up the contaminants through the diet or directly from water, and it is expected that the contributions of these two routes of uptake would contribute additively to the total body burden of the fish. This hypothesis was tested by comparing the "expected" body burden in the "water and food" treatment with the measured body burden in the "water and food" treatment. The expected body burden was calculated from the bioconcentration factor from the "water only", the water concentration in the "both water and food", and the body burden from the "food only" treatments. It was found that, for the 10 and 30 day exposures, the body burden was significantly greater than would be expected based upon additive contributions from water and food ($p < 0.05$, Wilcoxon rank-sum test; Fig 2A). Thus, there may be some interaction between absorption of perchlorate when both uptake routes are involved. Because the tissue concentration of perchlorate in the "food only" were much less than the "water only" fish, this suggests that the assimilation efficiency of perchlorate is much less than 100%.

In addition, the concentrations of perchlorate in the water at the time of sampling were significantly different among treatments and time periods. This may have affected the patterns of perchlorate in the tissues of the bass. In fact, the trends in bass body burdens relative to route and exposure duration are remarkably similar to the trends in water concentrations (Fig. 1 and Fig 2A). Thus, the body burdens were normalized by dividing them by the water concentration (note that for the "water only" treatment, the normalized body burden corresponds to the bioconcentration factor). For these data, the patterns of statistical significance vs. time were the same as those for the non-normalized data. This indicated that

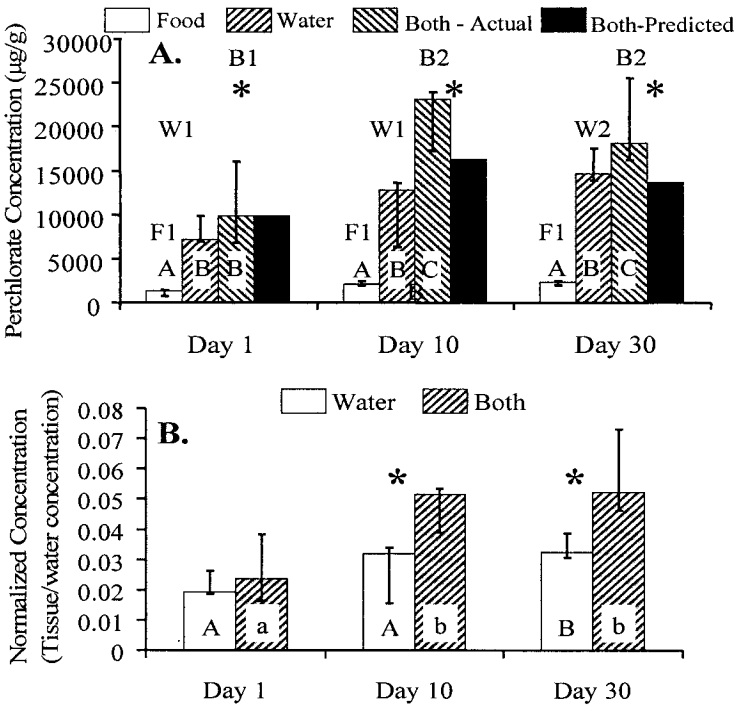

Figure 2. (A) Perchlorate body burdens (µg/g) in largemouth bass exposed to perchlorate via food only, water only or both food and water for 1-30 days. Bars represent medians and error bars are first/third quartiles. Bars labeled with different letters are not significantly different among treatments *within exposure durations* ($p > 0.05$, Kruskall-Wallis test). Alphanumeric labels (e.g., F1, W2, B2, etc.) denote significant differences between exposure durations for food only (F), water only (W), and both food and water (B) groups, respectively. Asterisks denote significant differences between observed and predicted values in the "both water and food" group. (B) Body burdens in bass normalized for water concentrations (body burden/water concentration). Bars represent medians and error bars are first/third quartiles. Bars labeled with different letters are significantly different ($p < 0.05$, Kruskall-Wallis test). Asterisks denote significant differences between "water" and "both" treatments.

differences in body burdens among time periods were not driven by differences in water concentrations. Furthermore, there were significant differences between the "water" and "both food and water" treatments, again suggesting that patterns in non-normalized data were not driven by differences in water concentrations. Analyses were also conducted using total mass of perchlorate rather than tissue concentrations (Fig. 3). There were differences between "water only" and "both water and food" treatments only for 10-d exposures ($p < 0.05$, Kruskall-Wallis test; Fig. 3). Also, the 1-day exposures were different from 10 and 30 d for "food" and "both food and water" treatments. For the "water only" group, the 30-d exposure perchlorate mass was greater than the other two test durations.

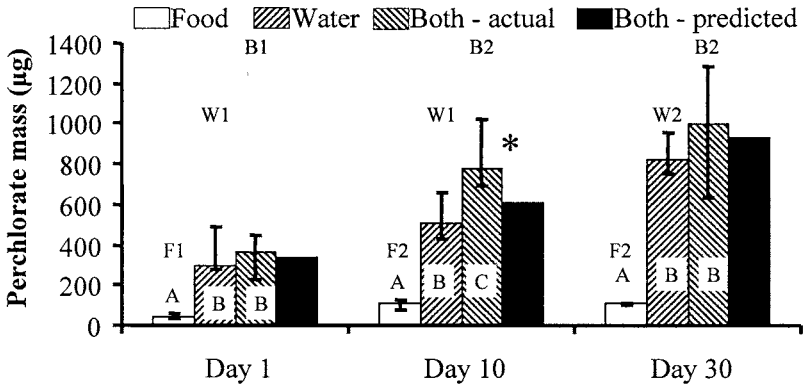

Figure 3. Perchlorate total mass (µg) in largemouth bass exposed to perchlorate via food only, water only or both food and water for 1- 30 days. Bars represent medians and error bars are first and third quartiles. Bars labeled with different letters are significantly different for comparisons among treatments *within exposure durations only* ($p > 0.05$, Kruskall-Wallis test). Alphanumeric labels (e.g., F1, W2, etc.) denote significant differences between exposure durations within each treatment: Food only (F), water only (W), and both food and water (B). Asterisks denote significant differences between observed and predicted values for body burdens in the "both water and food".

Predicted values for "both food and water" were calculated by summing median perchlorate mass in the "water only" and "food only" treatments, but there was only a difference between predicted and actual levels for the 10-day exposure ($p > 0.05$, Wilcoxon rank-sum test; Fig. 3). Overall, these trends tend to support the conclusions given above for perchlorate tissue concentrations.

Because there seemed to be a trend of increasing perchlorate in tissues over time, correlations were calculated between perchlorate metrics and time. There were statistically significant correlations for all time points and treatments (Table 2). These data indicate that perchlorate whole body concentrations in this species increase over time (not reaching steady state) until at least 30 d.

Non-parametric correlation analysis (simple linear models) indicated significant ($p < 0.05$) correlations between bass mass and perchlorate tissue concentrations after 1 d for "food only" and "both food and water", and after 10 d for "water only" exposures (Table 3). There were also marginally significant ($p < 0.10$) differences for "water only" and "both food and water" treatments at 30 d. The general trend was for negative correlations for tissue concentrations vs bass body mass. However, when the analyses were conducted using mass of perchlorate, there were some marginally significant negative correlations ($p = 0.10$), but also some significant positive correlations ($p < 0.05$), indicating increased perchlorate body burden with increasing body mass. Correlations were also run using "power models" of the form $\log(B) = \log(a) + b \log(W)$ (Newman 1995), where B

Table 2. Correlation between perchlorate tissue concentration or total mass and time of exposure in largemouth bass exposed to perchlorate for 1-30 days via food, water, or both.

		Treatment		
Perchlorate metric	Parameter[a]	Food only	Water only	Both
Concentration	r_s	0.441	0.777	0.646
	p	0.05	0.00	0.01
Mass	r_s	0.441	0.553	0.478
	p	0.05	0.02	0.04

[a]Pearson correlation coefficients (r_s) and significance (p-values).

is perchlorate concentration or mass and W is bass mass. Using this model, there were no significant correlations for perchlorate concentration, and the results for perchlorate mass were identical to those using simple linear models (not shown). These results imply that simple linear models are equivalent to, or better at, power models in describing allometric relationships for perchlorate body burdens, as is the case when there is little variation in body size (Newman 1995).

Table 3. Correlation between body mass and perchlorate (tissue concentration or mass of perchlorate in bass) in largemouth bass exposed to perchlorate for 1-30 days (D) via food, water, or both.

		Perchlorate concentration			Perchlorate mass		
Treatment	Parameter[a]	1 D	10 D	30 D	1 D	10 D	30 D
Food	r_s	-0.90	0.10	-0.70	-0.70	0.40	0.00
	p	0.04	0.53	0.12	0.10	0.26	0.53
Water	r_s	-0.40	-0.89	-0.83	0.90	-0.60	0.90
	p	0.26	0.04	0.08	0.042	0.18	0.04
Both	r_s	-0.90	-0.23	0.80	-0.70	0.90	-0.30
	p	0.04	0.71	0.07	0.10	0.04	0.3

[a]Pearson correlation coefficients (r_s) and statistical significance (p-values).

These results also indicated that perchlorate did not bioconcentrate in fish tissues, similar to other studies (Patiño et al. 2003). This is, however, contrary to field studies, in which perchlorate body burdens may be much higher than those in the ambient water (Smith et al. 2001). The present results do, however, indicate that perchlorate may be taken up from food items in field situations, and that complex interactions between food and water uptake may occur. The results also showed that higher concentrations of ClO_4^- were observed in "water only" and "water plus food" treatments after 1 day of exposure, verifying that perchlorate is taken up rapidly. The present results indicate that water is not the only route of

perchlorate uptake, but food chain transfer may occur and be responsible for the higher concentration of perchlorate found in fish collected from the field, compared to the ambient water concentrations. Taken together, these data indicate that sodium perchlorate can be taken up through both the water and the food. Although water is the predominant route of uptake, uptake from food can contribute to perchlorate body burdens when both routes of exposure are present, and there may be supra-additive interactions between the two. These results are essential for developing models of fate, effects, and transport of ClO_4^-, as well as to assess ecological risk in perchlorate-contaminated ecosystems.

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