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PERCHLORATE TROPHIC TRANSFER INCREASES TISSUE CONCENTRATIONS ABOVE AMBIENT WATER EXPOSURE ALONE IN A PREDATORY FISH

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Abstract

This study examined effects of varying concentrations of the environmental contaminant perchlorate in northern pike (*Esox lucius*) based on exposure in water and/or from prey (threespine stickleback, *Gasterosteus aculeatus*). Routes of exposure to pike were through contaminated water at 0, 10 or 100 mg/L perchlorate for 49 days and/or through feeding one stickleback per day over 14 days that were previously maintained in water at 0, 10 or 100 mg/L perchlorate. Both water and food significantly contributed to pike tissue concentrations of perchlorate as compared to controls, but, as expected for a water-soluble contaminant, perchlorate did not biomagnify from prey to predatory fish. Pike gastrointestinal tissue retained significantly more perchlorate than other tissues combined. Route of exposure and concentration of perchlorate in various media are important to consider in risk assessment when evaluating uptake and tissue concentration of perchlorate because significantly higher tissue concentrations may result from combined prey and water exposures than from prey or water exposures alone in a concentration dependent manner.

Keywords

Bioconcentration; *Esox lucius*; *Gasterosteus aculeatus*; Risk Assessment; Tissue Residue

Aquatic ecosystems contain xenobiotics that are persistent and/or accumulate in organisms (Lapworth et al. 2012; van der Oost et al. 2003). A fundamental understanding of the uptake, elimination, biotransformation and retention (disposition) of contaminants in tissues is essential when evaluating the impact of contaminants and their ability to concentrate between trophic levels. Bioaccumulation is the net increase of a contaminant concentration in an organism from all sources, including food, during its lifetime, while biomagnification is the increase of contaminant concentration from one trophic level (prey) to the next trophic level (predator) attributable to accumulation from food (Newman 2010). Some contaminants are well known to both bioaccumulate and biomagnify such as methyl-mercury, but the

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behavior of many contaminants is unknown, particularly for hydrophilic inorganic compounds.

The perchlorate anion is a persistent environmental contaminant found in many water sources throughout the United States (Brandhuber et al. 2009; EPA 2011). Perchlorate salts are both naturally occurring and synthesized for industrial uses such as ammonium perchlorate for solid rocket fuel (Trumpolt et al. 2005; Morrison et al. 2006). Anthropogenic sources are the main cause of contamination of most affected aquatic systems (Trumpolt et al. 2005; Morrison et al. 2006). Perchlorate salts are highly soluble and the perchlorate ion is stable once in solution, where it might reside for decades (Urbansky 1998; 2002; Morrison et al. 2006). Perchlorate is not expected to biomagnify in aquatic trophic webs because it is water soluble and likely easily eliminated from most organisms. The exception being plants, including important agricultural species such as spinach (*Spinacia oleracea*), which accumulates high concentrations of perchlorate and may be a significant source of dietary exposure (Ha et al. 2011; Jackson et al. 2005; Tan et al. 2004; Sanchez et al. 2005a; Voogt and Jackson 2010).

The known mechanism underlying adverse effects of perchlorate is competitive binding to the sodium/iodide symporter (NIS) in thyroid tissue (Wolff 1998). This effectively blocks the uptake of iodide and thereby disrupts production of thyroid hormone (TH) which is critical for the development and maintenance of many body systems (Wolff 1998). In addition, McDougal et al. (2011) showed that perchlorate exerts effects on thyroid unrelated to iodide deficiency. Perchlorate was found to interfere with development and reproduction in African clawed frogs (*Xenopus Laevis*) (Goleman et al. 2002a; 2002b), rats (*Rattus norvegicus*) (Baldridge et al. 2004; Gilbert and Sui 2008), mosquitofish (*Gambusia holbrooki*) (Park et al. 2006), zebrafish (*Danio rerio*) (Patiño et al. 2004; Mukhi et al. 2005; Patiño and Mukhi 2007; Mukhi and Patiño 2007), and threespine stickleback (*Gasterosteus aculeatus*) (Bernhardt et al. 2006; 2011; Bernhardt and von Hippel 2008). Exposure occurs through contaminated water and/or food (Murray et al. 2008; Huber et al. 2011).

Organisms in aquatic environments are susceptible to perchlorate because of its solubility and stability; and are exposed to perchlorate by way of ambient water (gills and integument) and/or gastrointestinal tract (GIT) (Theodorakis et al. 2006a). Exposure via water may be continuous or sporadic depending on the source of the contaminant, hydrological setting, and route of exposure. Exposure through diet might be important and has been implicated in higher than expected tissue residue concentrations in fish and other vertebrates at perchlorate contaminated sites (Smith et al. 2001; Theodorakis et al. 2006b). The importance of total diet intake for humans was previously investigated (Murray et al. 2008; Sanchez et al. 2009; Wang et al. 2009; Trumbo 2010; Valentin-Blasini et al. 2011), but few studies addressed routes of exposure for aquatic organisms (Park et al. 2005). Routes of perchlorate exposure may be more important for aquatic organisms because of presence in ambient water and physiological differences in exposed tissues such as gills, GIT and integument, species specific variation and the presence or absence of NIS (sodium-iodide symporter). This drives concern for a better understanding of the dynamics of perchlorate within aquatic food webs and of the relative merits of assessment techniques such as free ranging fish vs. aquatic animal models in lab settings.

Toxicokinetic studies of perchlorate in fish were conducted using zebrafish (Liu et al. 2006; Patiño et al. 2003), Bluegill sunfish (*Lepomis macrochirus*, (Dean et al. 2004)), mosquitofish (Bradford et al. 2006) and channel catfish (*Ictalurus punctatus*) (Park et al. 2007). General conclusions based on these studies include: 1) perchlorate is rapidly taken up from water, 2) tissue concentrations are well below the ambient water concentrations once a steady state is reached, 3) there are species differences in the concentrations of tissue residue

and tissue in which the chemical is found (head, fillet, GIT, etc.). Bioconcentration factors (BCF) ranged between 0.02 and 0.7 and were concentration-dependent. Two field studies of highly contaminated military sites found perchlorate was sporadically present in fish but were generally correlated with proximity to the point source (Smith et al. 2001; Theodorakis et al. 2006b). In contrast to lab studies, tissue concentrations in the field caught fish were often exceeded ambient water concentrations. Park et al. (2005) examined water and food routes of exposure in largemouth bass (*Micropterus salmonides*) and determined that perchlorate did not bioconcentrate but that food exposure was more than additive to body burden when fish were exposed via water and food.

The purpose of this study was to determine if varying concentrations of sodium perchlorate provided in prey only or prey plus water resulted in additive or greater than additive differences in predator tissue concentrations compared to water only exposure. Monitoring water concentrations alone are not sufficient to evaluate fish exposure if it is not the sole route by which fish are exposed. A 3×3 factorial study was used to evaluate predatory fish tissue concentrations and interactions between water and food routes of exposure. It was postulated that water and food routes of exposure might be additive to tissue concentrations. This investigation is expected to provide insights into the most appropriate aquatic animal models and experimental procedures for testing food web dynamics of perchlorate over a range of concentrations.

METHODS

Collection and husbandry

Threespine stickleback with a mean mass $\pm$ SD of 0.39 ± 0.2 g were collected with minnow traps from Bear Paw Lake (61° 36.830' N, 149° 45.413' W) in the Matanuska-Susitna Valley, Alaska, on 31 July, 2007. Juvenile northern pike (*Esox lucius*) with a mean mass $\pm$ SD of 14.5 ± 5.7 g and mean standard length $\pm$ SD (distance from the tip of the upper jaw to the posterior end of the hypural plate) of 139 ± 12.3 mm; (n = 43) were collected with a hand seine from Fire Lake (61° 20.978' N, 149° 32.837' W) in Eagle River, Alaska between 19 July and 7 August, 2007. All fish were transported in coolers with aerated lake water to the lab at the University of Alaska Anchorage, where they were housed in outdoor 1400 L polyethylene pools (diameter = 152 cm, height = 86 cm) filled with 1000 L of de-chlorinated city water. Instant Ocean® was added to maintain salinity at 1 g/L, which was previously found optimal for juvenile pike. Mean water temperature was 12°C during the experiment. Stickleback were fed frozen brine shrimp (*Artemia* spp.) twice a day to satiation. Pike were fed unexposed stickleback from Cheney Lake (61° 12.045' N, 149° 45.660' W) *ad lib* until the experiment began.

Experimental design

A 3×3 static renewal experiment with three replicates was used for pike exposures. There were 4 pools each with no perchlorate added (control), 10 or 100 mg/L perchlorate. These concentrations were selected to ensure detectable tissue concentrations in exposed fish. Environmental water concentrations > 10 mg/L were found by Smith et al (2001) to produce effects but most water sources contain < 4 µg/L (Brandhuber et al. 2009). The 4 pike in each pool were individually marked by fin clipping. Within every pool, each pike was randomly assigned a feeding treatment and fed individually in a large dip net. Stickleback used as prey for the feeding treatments were exposed to 10 or 100 mg/L perchlorate in two separate pools, and one control pool with no perchlorate. From day one of exposure, 5 stickleback from each treatment were collected every two days to generate a perchlorate uptake curve and determine steady state tissue concentrations.

Experimental procedures

Sodium perchlorate (> 98% purity, Sigma-Aldrich, St. Louis, MO, USA) was added to each tank by drying it in an oven at 90° C before weighing out 12.3 g or 123 g for 10 and 100 mg/L concentrations respectively. The salt was dissolved in water and added to each tank. Perchlorate concentrations were verified by ion chromatography (IC) over the exposure period and were within 1 to 10% of the target concentrations throughout (Figure S1). Slight contamination was detected in the control tanks due to the feeding treatments (Figure S1).

Stickleback and pike were exposed to perchlorate on the same day (August 1st) and allowed to reach a steady state (tissue concentration reached a plateau) prior to the feeding treatment. This was done to ensure perchlorate contributed from food was detectable in the interaction treatments. Following this period of water exposure, pike were fed according to their assigned feeding treatment for 14 days, beginning on September 1st. A single stickleback was fed to each pike per day. The mass of each stickleback was recorded before it was fed to the pike. Mean $\pm$ SD daily ration (wt/wt) over the duration of the feeding treatment for all pike was 3.07 ± 2.13 . Feeding treatments are referred to as the 10 and 100 mg/L exposure of the prey throughout the manuscript. At the end of the two week feeding period and approximately 24 hr after the last feeding, pike were killed instantly in liquid nitrogen and stored in a -80°C freezer until analysis. Steady state was presumed to be attained for the feeding treatments after two weeks. Pike mass and standard length were recorded at the beginning of the water exposure, at the beginning and after the two week feeding period. Throughout the manuscript, pike treatments are expressed as water:feeding (e.g., 0:100 = control water and prey exposed to 100 mg/L water).

Perchlorate Analysis of Tissue and Water

Fish tissue concentrations of perchlorate were determined using a modified method of Dodds et al. (2004). Stickleback were thawed and homogenized to produce a single homogenate for each sampling date for each treatment condition. The 5 fish collected from each pool every two days were treated as one sample to produce enough tissue to analyze. Pike were measured individually. Fish were thawed and GIT from esophagus to vent removed. The GIT contents, GIT and fish body were homogenized separately. Mean fractional weight of the GIT contents and GIT were: 5.65 and 3.04% respectively.

Perchlorate was extracted from homogenates using an Accelerated Solvent Extraction system (ASE 200; Dionex, Sunnyvale, CA, USA) with ultra-purified water (>18 mega ohm) as solvent. A 2 g sample was mixed with hydromatrix (diatomaceous earth) and placed in a 10 ml stainless steel ASE cell and extracted at a pressure of 689.5 kilopascal and 100°C. Two blanks and two spikes (3 ml of 1 µg/L perchlorate) were extracted and analyzed for quality control. After the extraction, 1 ml hydrogen peroxide was added to the extract, which was heated to 90°C for approximately one hr to degrade organic material. After organic material was removed, samples were reconstituted to 25 ml with ultra-purified water. For measurement, 2 ml of each sample was filtered at 0.45 µm and analyzed with a Dionex DX-500 ion chromatograph using a 4mm Dionex IonPac As16 column, 38mM KOH eluent and the suppresser method. Quantification was reached using linear regression of peak area (µS/cm *min) with 4 level calibration (10 to 500 ppb) prepared in 1:1 diluted Instant Ocean®. The perchlorate peak was readily observed at 9.22 ± 0.1 min. All reported measurements were within the range of the calibration curve. Water samples were diluted to match calibration concentration, filtered, and analyzed using the same IC method.

The detection limits (LOD) for perchlorate were 10 ng/ml in water and 30 ng/ml in tissue extracts. LOD was calculated from the regression line of the calibration curve as:

$$\text{LOD} = t * \frac{\text{intercept}}{m}$$

with t being the Student's t distribution, $\sigma_{\text{intercept}}$ the standard variation of the intercept and m the slope. Recovery was determined on 5 spiked pike tissue samples and was between 65 and 80%. Uncorrected, measured values are reported below. All analyses were performed in the Applied Science Engineering and Technology (ASET) Laboratory at the University of Alaska Anchorage.

Statistical Analysis

All statistical analyses were performed using R (version 3.0.1). Data were tested for normality using the Shapiro-Wilk test and homogeneity of residuals with the Modified Levenes test. Differences in tissue residue were analyzed using ANOVA. For multiple comparisons, a TukeyHSD contrast was used. Nonparametric tests (Kruskal-Wallis and Dunnett's Modified Tukey-Kramer Pairwise Multiple Comparison Test) were also used and qualitatively equal to the parametric tests. A paired Wilcoxon rank sum test was employed to determine if stomach contents and GIT perchlorate concentrations differed significantly. In addition, Response surface method (RSM) was used to evaluate interactions of effects between routes of exposure. A second order polynomial response function was employed to estimate $f(x)$:

$$E[y] = \mu_0 + \mu_1 X_1 + \mu_2 X_2 + \mu_{11} X_1^2 + \mu_{22} X_2^2 + \mu_{12} X_1 X_2,$$

where $X_1 = [\text{ClO}_4^-]$ in water, $X_2 = [\text{ClO}_4^-]$ in food, μ_1 , μ_{11} = unknown effects associated with $[\text{ClO}_4^-]$ in water, μ_2 , μ_{22} = unknown effects associated with $[\text{ClO}_4^-]$ in food, and μ_{12} = unknown effect from interaction of $[\text{ClO}_4^-]$ in water and $[\text{ClO}_4^-]$ in food. To equilibrate scaling factors, factorial data were transformed using the formula: $X_j = (\text{Actual level} - (\text{max level} + \text{min level})/2)/((\text{max level} - \text{min level})/2)$, where the level X_j is the j th factor (Kutner et al. 2005; Lenth 2009).

To determine if there was a significant effect of route of exposure, an expected value for each treatment (10:10, 10:100, 100:10 and 100:100) was calculated as: Expected value = water $[\text{ClO}_4^-]$ from tanks with food+water treatments * BCF from water only treatments + tissue $[\text{ClO}_4^-]$ from food only treatments. The expected value was then subtracted from actual tissue concentrations and a one sample t -test performed to detect differences from zero.

Pike body condition was calculated using a regression index to control for body size differences (Jakob et al. 1996). Body mass and length were transformed using the natural log before generating residuals by regression. The differences in residuals were then used to compare between treatments of three different time points during the perchlorate exposure: 1) 14 days after water exposure began, 2) 4 days into the feeding trial, and 3) the day of fish collection after the feeding trial.

RESULTS

Perchlorate Exposure from Food

The dose of total perchlorate from contaminated stickleback over the 14 day feeding period was 0.16 – 0.41 $\mu\text{g/day}$ for 10 mg/L exposed stickleback and 2.07 – 3.47 $\mu\text{g/day}$ for 100 mg/L exposed stickleback. The mean $\pm$ SD total doses to pike for the whole two week exposure were 3.37 ± 0.3 and 37.1 ± 1.6 μg , respectively and the mean body mass specific total dose was 0.21 mg/kg for pike eating 10 mg/L exposed prey and 2.2 mg/kg for pike eating 100 mg/L exposed prey.

Pike Body Condition

Pike sample sizes for each treatment were: 0:0 - five, 0:10- three, 0:100 - six, 10:0 - five, 10:10 - five, 10:100 - five, 100:0 - five, 100:10 - five, 100:100 - four. Pike body condition did not change significantly among treatments during the exposure period or during the feeding trial alone. Mean body condition increased over the whole exposure period.

Tissue

Stickleback tissue concentrations reached a steady state with contaminated water in 50–100 hr (Figure 1). Mean $\pm$ SD bioconcentration factors (BCF) were 0.047 ± 0.001 for 10 mg/L and 0.051 ± 0.003 for 100 mg/L perchlorate contaminated water. In contrast, the BCF of total tissue (body + GIT) of pike in 10 mg/L and 100 mg/L contaminated water were 0.132 ± 0.024 and 0.05 ± 0.004 , respectively. Tissue residue increased in concentration in a dose dependent relationship. Water only exposure resulted in greater tissue residues than food only exposures at 10 mg/L (0:10, 10:0) while there was no marked difference at the highest exposure level (0:100, 100:0; Figure 2). Food-borne exposure at the 100 mg/L level resulted in significant differences from water-borne only exposure (0:100, 10:100), with the exception 100 mg/L water in which food-borne exposure did not contribute to tissue residue (Figure 2). The GIT retained more perchlorate than the other tissues combined (Figure 2).

Total tissue concentrations of perchlorate in pike were significantly different between some of the food only, water only and food+water treatments and were concentration dependent (Figure 2). Pike exposed to contaminated water only (10:0 and 100:0) had total tissue concentrations significantly different from controls and each other. Pike from 0:100 had significantly higher perchlorate concentration in their tissues than the two other food only treatments 0:0, 0:10 and the 10:10 treatment. In the 0:100 and 10:100 treatments, diet-borne perchlorate from the 100 mg/L exposed prey resulted in significantly more tissue residue than controls (Figure 2). The pike in 100:0, 100:10 and 100:100 showed no significant effect of diet-borne perchlorate on tissue levels (Figure 2). The interaction term between water and food routes was significant. Smaller differences were detected in the 100 mg/L treatments compared to control and 10 mg/L (Figure S2). Tissue concentrations clearly rose with increasing exposure concentrations but there was a change in response with the highest concentrations. Gastrointestinal tract tissue did not contain significantly different perchlorate than associated stomach contents for pike in any of the treatments (Figure 2), although the 10:0 and 10:10 treatments displayed notable differences between these two tissues (Figure 2).

The response surface analysis resulted in a significant second order polynomial function (Figure 3). The regression coefficient and parameters showed that increasing concentrations of both water and food exposure resulted in rising pike tissue residues (Table 1). The negative interaction parameter indicates that there is not a greater than additive relationship between routes of exposure for all concentrations (Carter et al. 1988). The stationary points (maximum estimated response) for food and water perchlorate treatments were 65.1 and 66.2 mg/L, respectively (Figure 3).

Actual pike tissue concentrations differed significantly from calculated expected values in 2 of 4 combination treatments. The 10:10 treatment resulted in greater than expected tissue concentrations while the 100:100 treatment resulted in less than expected (Figure 4). The 10:100 and 100:10 treatments trended toward more than expected, but were not significantly different.

DISCUSSION

The perchlorate ion was retained in the tissue of both fish species at varying concentrations and related to water and prey contamination. Stickleback tissue reached steady state with contaminated water after approximately two days of exposure (Figure 1), which is similar to other studies for uptake of perchlorate in small fish (Patiño et al. 2003).

The water and food routes of exposure produced apparent concentration-dependent increases in pike tissue concentration of perchlorate. The 10:10 treatment resulted in significantly greater tissue residues than would be expected from simple addition (additivity) of the results from food only and water only treatments. Data suggest an interaction is occurring between these two routes at the lower concentration used in this study (Figure 3 and 4). This demonstrates that perchlorate does not act as a strict water soluble compound in some fish and that food might serve as a significant source of perchlorate for aquatic vertebrates. The RSM model resulted in a negative interaction coefficient, indicating an overall non additive relationship between routes, which is most likely due to the 100 mg/L water treatments where food contamination exerted no marked effect (Figure 2). Further, comparing the 100:0 and the 0:100 treatments, there was no marked difference in perchlorate levels in the GIT tissue concentration, but the body tissue concentration in the water only treatment was significantly greater (Figure 2) suggesting dispositional differences of the tissues involved. These results are consistent with a similar study by Park et al. (2005) who found higher than expected tissue concentrations in largemouth bass (*Micropterus salmoides*) that were exposed to both contaminated water and contaminated prey. Smith et al. (2001) and Theodorakis et al. (2006c) detected greater concentrations of perchlorate in some fish than in ambient water at highly contaminated sites in Texas, and consuming contaminated food may have contributed to the high tissue concentrations among other possible factors.

The higher than expected concentration of perchlorate in pike was in large part a result of the higher concentrations in the GIT (Figure 2). Some limitations of this study include the two week food exposure period and a small ration. Two weeks may not have been sufficient to reach a steady state for this route. Further research is required to determine accumulation curves via exposure from food. Pike eating more contaminated prey would potentially accumulate more perchlorate. The probable explanation for greater concentrations in the GIT is that GIT epithelium expresses NIS (Wolff 1998; Yu et al. 2002) and/or because this is the primary tissue exposed to ingested perchlorate. Perchlorate has a higher affinity than iodide for the NIS binding site and is 30-fold more potent than iodide at blocking $^{125}\text{I}^-$ transport across the cell membrane (De Groef et al. 2006; Tonacchera et al. 2004). Perchlorate may also be transported across plasma membranes (Tran et al. 2008; Attanasio et al. 2011; Cianchetta et al. 2010; Dohan et al. 2007). In pike, this would result in concentrating perchlorate in the gastric mucosa, which might account for the higher concentrations found in the GIT tissue. Indeed, three treatment combinations exhibited higher concentrations of perchlorate in the GIT than in the associated stomach contents (Figure 2). Feeding on contaminated food and/or drinking contaminated water results in greater GIT tissue residue of perchlorate. Iodide, which perchlorate is chemically similar, may be a rare anion in fresh water (Watanabe et al. 1997) and a mechanism to concentrate it from diet might likely increase fitness. Geven et al. (2007) found that Mozambique tilapia (*Oreochromis mossambicus*) accumulated ^{125}I in their intestinal tract and may therefore act as a reservoir for iodide, and therefore perchlorate, in fish.

There was no significant change in tissue residue for pike eating contaminated food in the 100 mg/L water treatment (i.e. exposure via food did not alter tissue concentrations, Figure 2) indicating there was a change in uptake and/or elimination at this water concentration. This is in agreement with the RSM analysis that resulted in the maximized perchlorate

exposure being approximately 65 mg/L water concentration. Uptake in the gut may have been limited by the density of NIS, or perhaps pike in the 100 mg/L water treatment reduced perchlorate and/or enhanced elimination of the ion. Perchlorate is known to be eliminated through urine (Wolff 1998). In fish, an additional site of ion exchange occurs at the gills and may play a role in the observed tissue concentrations

The ubiquitous presence of perchlorate in plants and animals at contaminated sites opens up the possibility that food webs contribute to the biotic distribution of this contaminant (Smith et al. 2001). Some plants bioaccumulate perchlorate to relatively high levels (Jackson et al. 2006). Herbivores may be exposed to high concentrations from contaminated plants and then become food for carnivores, effectively bridging the gap between bioaccumulating plants and non-bioaccumulating animals. Chronic exposure to high concentrations of perchlorate through diet in combination with exposure through water might result in body concentrations or burdens of perchlorate that exert detrimental health effects and would go undetected if models relied solely on water-borne exposure studies. Humans are exposed to perchlorate from green leafy vegetables grown with contaminated irrigation water (Sanchez et al. 2005b; Murray et al. 2008), drinks such as milk (Kirk et al. 2005) and water (Murray et al. 2008; Huber et al. 2011) and potentially through contaminated animals. Based on the outcome of the present study these multiple matrices and associated routes of exposure need to be considered in human as well as ecological risk assessments.

CONCLUSIONS

This study showed that perchlorate did not bioconcentrate (uptake from water) or bioaccumulate (food and water) in fish; which confirmed the expectation for a hydrophilic anion. Perchlorate did not biomagnify from fish prey to fish predator with the possible exception of the 10:10 treatment in which tissue concentration was greater than expected. Perchlorate tissue residue was correlated with exposure concentrations and varied by tissue compartment. These results demonstrate that risk assessment of perchlorate in aquatic organisms needs to take multiple routes of exposure into consideration, especially the contributions due to exposure to water and ingestion of food. The difference in ambient iodide concentrations in fresh water versus salt water, and adaptations of animals for efficient ion uptake or elimination, provide additional complications that need to be investigated to better understand health risks and selection of model organisms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Concentration (μg/g wet weight) of perchlorate in stickleback tissue in control, 10 and 100 mg/L contaminated water. Measurements are from the homogenate of 5 stickleback collected at each time point.

Figure 2.

Mean concentration (µg/g wet weight) of perchlorate in pike tissue. Black bar = whole body – gastrointestinal tract and GIT contents; White bar = only GIT; Diagonal cross bar = whole body + GIT; Diagonal bar = stomach contents only. C = control, 10 = 10 mg/L perchlorate, 100 = 100 mg/L perchlorate. Food concentrations on the X-axis are the levels of perchlorate stickleback were exposed to prior to feeding to the pike. Different letters indicate significant differences between treatments by tissue type for Body, GIT and Body+GIT (= 0.05; TukeyHSD contrast)..

Figure 3.

(A) Perspective plot of response surface of perchlorate tissue concentration in pike (body + gastrointestinal tract) for all water and food treatments. The stationary point (peak) for food is 65.1 mg/L and for water is 66.2 mg/L . Dose response curves are on the edge of the food and water axes. (B) Contour plot of response surface of perchlorate tissue concentration (centered on 0) in pike (body + gastrointestinal tract) for all water treatments. Concentration in food is given in mg/L because this is the concentration of perchlorate that stickleback were exposed to prior to feeding to pike.

Figure 4.

Pike tissue concentrations of perchlorate for the four combination (water and food) perchlorate exposure treatments. Black bars represent the predicted values of tissue residue calculated as: Expected $[\text{ClO}_4] = [\text{ClO}_4]$ in water from combination tanks * BCF from water only treatments + tissue $[\text{ClO}_4]$ from food only treatments. Asterisk indicates a significant ($\alpha = 0.05$) difference between expected and water and food treatments.

Table 1

Regression coefficients for pike tissue concentrations of perchlorate.

	Regression coefficient	Parameter estimate	p value
Intercept	0	9127.5	<0.001
[Water]	1	2255.4	<0.001
[Food]	2	1416.6	<0.001
[Water]^2	11	-3330.3	0.003
[Food]^2	22	-1843.4	0.088
Interaction	12	-742.6	<0.001