ELSEVIER

Chemosphere 63 (2006) 1591–1597

CHEMOSPHERE

www.elsevier.com/locate/chemosphere

Uptake and elimination of perchlorate in eastern mosquitofish

Carrie M. Bradford, June-Woo Park [1], Jacques Rinchard [2], Todd A. Anderson, Fujun Liu, Christopher W. Theodorakis [*,3]

The Institute of Environmental and Human Health, Department of Environmental Toxicology, Texas Tech University, Box 41163, Lubbock, TX 79409-1163, United States

Received 27 April 2005; received in revised form 18 August 2005; accepted 30 August 2005
Available online 21 November 2005

Abstract

The purpose of this study was to investigate the uptake and elimination of perchlorate in eastern mosquitofish (*Gambusia holbrooki*). Fish were exposed to 0.1–1000 mg/l sodium perchlorate for 12 h, 1, 2, 5, 10, and 30 days, and perchlorate was determined in whole body extracts. Perchlorate was not detected in mosquitofish exposed to the low concentrations of perchlorate (0, 0.1, and 1 mg/l sodium perchlorate), regardless of the exposure time, whereas it was detected when fish were exposed to 10, 100, and 1000 mg/l. The tissue concentrations were approximately 10 times less than that in the water. There was no difference in the uptake of perchlorate depending upon the exposure time, however, a difference in perchlorate uptake depending upon the concentration of the exposure dose ($P < 0.001$) was observed. Uptake (K_u) and elimination (K_e) rate constants were 0.09 l/mg day and 0.70 day^{-1}, respectively. The half-life ($T_{1/2}$) of perchlorate was 0.99 day. Thus, it appears that perchlorate is rapidly taken up and eliminated in eastern mosquitofish. These results are critical and may be used to develop models of fate, effects, and transport of perchlorate in natural systems, as well as to assess ecological risk in affected ecosystems.

Keywords: Fish; Toxicokinetics; Thyroid disruptor; Body burden

1. Introduction

Ammonium perchlorate is used as an oxidizer in rocket propellants and explosives and is present in commercially available products such as road flares, fireworks, airbags, and some fertilizers (Urbansky et al., 2001; Urbansky, 2002). Because it has the potential to disrupt thyroid function in humans as well as in other vertebrates (Brechner et al., 2000; Soldin et al., 2001; Goleman et al., 2002; Greer et al., 2002; Yu et al., 2002; Carr et al., 2003; Patiño et al., 2003), there has been growing concern over the effects of

* Corresponding author. Tel.: +1 618 650 5235; fax: +1 618 650 3174.
E-mail address: ctheodo@siue.edu (C.W. Theodorakis).
[1] Present address: Department of Zoology, 203 Natural Science Building, Michigan State University, East Lansing, MI 48824-1115, United States.
[2] Present address: School of Natural Resources, The Ohio State University, 2021 Coffey Road, Columbus, OH 43210, United States.
[3] Present address: Environmental Sciences Program and Department of Biology, Southern Illinois University Edwardsville, P.O. Box 1651, Edwardsville, IL 62026, United States.

0045-6535/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2005.08.073

environmental perchlorate contamination. Perchlorate has been found in ground and surface waters near military installations and perchlorate manufacturing plants (Smith et al., 2001; Urbansky, 2002). There have been several studies that examined environmental fate, transport, and degradation of perchlorate (Yoon et al., 2002; Tipton et al., 2003) and others which have explored its remediation in environmental media (Urbansky, 1998; Hare, 2000). Because perchlorate is water soluble and persistent in the environment, it is probable that organisms such as fish are exposed to perchlorate at contaminated sites (Smith et al., 2001). In fact, Smith et al. (2001) found that perchlorate was present in fish at concentrations greater than in the ambient water. However, based on its physical properties, namely the ionic nature and high water solubility, it is not expected to bioconcentrate. Therefore, more information on bioconcentration factors and toxicokinetics is needed to test this hypothesis. Although toxicokinetics of perchlorate have been examined in plants (Urbansky et al., 2000; Susarla et al., 2000; Yu et al., 2004), humans, and other mammals (Batjoens et al., 1993; Fisher et al., 2000; Clewell et al., 2001; Yu et al., 2002), little is known about its toxicokinetics and tissue distribution in fish.

The purpose of this study was to determine the uptake and the elimination of perchlorate into whole body eastern mosquitofish (*Gambusia holbrooki*). This species was chosen because: (1) it is widely distributed in the United States, (2) it is of ecological importance, (3) there is a large amount of literature on the biology, ecology, and toxicology of this species, and (4) field studies have shown that this species take up perchlorate from environmental media (Smith et al., 2001).

Because perchlorate is a water soluble chemical, we hypothesized that it will be rapidly taken up into the fish and reach steady state. In order to determine bioconcentration factors at various water concentrations and exposure periods, mosquitofish were exposed to perchlorate at various concentrations ranging from 0.1 to 1000 mg/l for 2, 5, 10, and 30 days. In subsequent experiments, uptake and elimination rate constants and mosquitofish were determined for fish exposed to 100 mg/l perchlorate at for 12 h to 30 days (uptake) and 12 h – 20 days (elimination).

2. Materials and methods

2.1. Test chemical

Sodium perchlorate (99% purity) was purchased from EM Science (Gibbstown, NJ). Sodium perchlorate was chosen over the ammonium salt to examine the effects of perchlorate ion alone, without confounding effects of ammonium toxicity.

2.2. Animals

Adult mosquitofish (0.86 ± 0.34 g) were purchased from a commercial hatchery (Ken's Hatchery and Fish Farm, Alapaha, GA). Upon arrival, fish were transferred into a 400-l tank supplied with filtered, recirculated reconstituted water (60 mg/l Instant Ocean® sea salts in deionized water). They were allowed to acclimate to laboratory conditions for at least 5 days prior to the start of the experiments. A 12-h photoperiod was maintained. During that period, fish were fed ad libitum with Aquatox® flake food (Zeigler Bros. Inc., Gardners, PA, USA). The concentration of iodine in the food was 6 mg/kg (data provided by the manufacturer).

2.3. Uptake experiments

Bioconcentration factor vs. exposure time and concentration: These experiments were carried out in a static renewal system. Four hundred and fifty female mosquitofish were randomly assigned to thirty 15-l aquaria (15 fish per aquarium) and were exposed to 0, 0.1, 1, 10, 100, and 1000 mg/l (nominal concentration) sodium perchlorate for 2, 5, 10, and 30 days with 5 replicates for each exposure. The low concentrations were chosen based upon perchlorate concentrations found in surface waters (Smith et al., 2001), and the higher to ensure perchlorate detection. Fish were fed Aquatox® flake food twice daily ad libitum. Each aquarium was supplied with aeration and 12-h photoperiod maintained. Feeding time and daily observations, including behavior and number of sick and dying fish, were recorded.

Aquaria water consisted of reconstituted water (60 mg/l Instant Ocean® sea salts in deionized water) with the appropriate amount of sodium perchlorate stock solution added to each test aquarium. 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. Water quality tests were conducted to determine dissolved oxygen, salinity, pH, ammonia, and conductivity on days between water changes. Water samples from each aquarium were collected on the first day and last day of each exposure and once a week for the longer exposures of the experiment.

After each exposure, mosquitofish were euthanized in 3-aminobenzoic acid ethyl ester (MS-222, 0.5 g/l), rinsed with deionized water, individually weighed and measured. Eight fish were then pooled and frozen in liquid nitrogen for perchlorate analysis.

Uptake: This experiment was carried out as previously described. Adult mosquitofish were exposed to sodium perchlorate at 100 mg/l for 12 h, 24 h, 48 h, 10, and 30 days. Fish were fed and sampled as previously described. This concentration was chosen to maximize the potential for detecting perchlorate in fish tissues.

2.4. Elimination experiments

A renewal static system was also used to evaluate perchlorate elimination in mosquitofish. Two hundred and sixty mosquitofish were exposed to 100 mg/l sodium perchlorate for five days in an 80-l aquarium. This concentration was chosen because it was high enough to ensure detectable perchlorate in the fish tissues, but not high enough to induce stress-related behaviors, overt mortality, or other signs of stress in the fish. Fish were then transferred to 15 l aquaria containing clean reconstituted water. Seven fish were collected from each aquarium at 0, 1, 2, 5, 10, and 20 days, euthanized with MS-222, rinsed with deionized water and individually weighed and measured. There were five aquaria for each sampling period. Fish were pooled and frozen in liquid nitrogen for perchlorate analysis. Every other day, 1/3 of the water in the elimination aquaria was replaced. Fish were fed as previously described. Water samples were taken from each aquarium everyday during the exposure period and at the fish collection during the elimination phase.

2.5. Tissue extraction and extract clean-up

Perchlorate was extracted from fish tissue according to the procedure previously described by Anderson and Wu (2002). Briefly, fish samples rinsed three times with deionized water were desiccated in a fume hood for at least 48 h and extracted in 11-ml cells using Milli-Q water (18 MΩ) with a Dionex Accelerated Solvent Extractor (ASE, 200, Dionex, Sunnyvale, CA). Cells were heated for 5 min at 100 °C, filled with Milli-Q water, and pressurized to 1500 psi. Total extraction time was 15 min. At the completion of the extraction, extract volume was recorded. Extracts (1 ml) were then cleaned using silica solid-phase extraction (SPE) cartridges and then diluted to 5 ml with Milli-Q water. The diluted extracts were then filtered through a 0.45 μm Acrodisc® filter into 5-ml vial IC vials.

2.6. Water sample clean-up

Water samples (5 ml) were filtered through a 0.45 μm Acrodisc® filter into 5-ml vial IC vials and either analyzed directly for perchlorate ion or diluted with Milli-Q water prior to analysis.

2.7. Ion chromatography analysis

Samples were analyzed on a Dionex DX-500 Ion Chromatography System equipped with a GP50 pump, a CD20 conductivity detector, and an AS40 automated sampler (Yu et al., 2004). Ion separation was made with a Dionex IonPac AS16 (4 mm) analytical column. An eight-point standard curve consisting of 2.5, 5, 10, 20, 50, 100, 200, and 500 μg/l perchlorate was constructed using a 100 μg/ml certified sodium perchlorate standard (Accustandard, Inc.). Sodium hydroxide (100 mM) was used as eluent at a flow rate of 1 ml/min. Resulting chromatograms were optimized and the perchlorate peak (when present) was identified based on the retention time of the standards. Perchlorate concentrations in the tissues were determined based upon the area under the perchlorate peak, the wet weight of the tissue, and the volume extracted from each sample. Percent recovery and detection limits were determined from spiked perchlorate tissues (perchlorate solutions injected into fish prior to extraction) and adding known amounts of perchlorate to aqueous tissue extracts, respectively.

2.8. Data analysis

Results are presented as means ± standard error. All statistical tests were performed using SAS (version 8.0) with significance level α set at 0.05. Uptake and elimination of perchlorate data were analyzed using non-parametric Kruskall–Wallis H test. This test was applied because normalization of the data and homogeneity of the variances were not satisfied.

The bioconcentration factor (ratio between the perchlorate concentration in the fish to the measured concentration in the water) was calculated. BCF among concentrations were compared using non-parametric Kruskall–Wallis test.

The uptake rate constants (K_u) was calculated from $K_u = K_e \cdot C_{ss}/C_w$; C_{ss} is the concentration in tissues at steady state and C_w is the concentration in water (Newman, 1995). When the fish were transferred to clean water, the elimination rate constant (K_e) was determined by fitting a non-linear regression to the model $C_t = C_0 e^{-K_e t}$, where C_0 is the concentration of perchlorate in the whole body at the beginning of the experiment, C_t the whole body concentration of perchlorate at time t, and t is time (days). The half-life ($t_{1/2}$) of perchlorate in whole body was estimated according to the equation $t_{1/2} = -\ln 0.5/K_2$ (Spacie and Hamelink, 1995; Sancho et al., 1998). The 95% confidence interval was estimated for K_e via regression analysis. The 95% confidence interval for the K_u was obtained by calculating a K_u for each fish i at steady state (i.e, for all fish exposed to perchlorate for 2 or more days), calculating the standard error and multiplying the standard error by 1.96.

3. Results

3.1. Uptake of perchlorate

Uptake depending upon exposure time and concentration: Water quality was within the acceptable parameters (Table 1; ASTM, 2003), and perchlorate concentrations

Table 1
Time-weighted averages (±SD) of water quality parameters during perchlorate uptake and elimination experiments[a]

Exposure concentration (mg/l)	Water quality parameters				
	pH	Temperature (°C)	Dissolved oxygen (%)	Conductivity (μS/cm)	NH_3 (ppm)
Uptake 0	6.85 ± 0.15	19.77 ± 0.69	98.69 ± 2.47	92.77 ± 2.61	0.51 ± 0.06
Uptake 0.1	6.82 ± 0.17	19.99 ± 1.1	89.16 ± 3.02	91.18 ± 2.24	0.68 ± 0.06
Uptake 1	6.86 ± 0.19	20.26 ± 0.74	98. 15 ± 2.89	86.74 ± 1.83	0.51 ± 0.06
Uptake 10	7.04 ± 0.2	20.6 ± 0.67	93.14 ± 2.81	87.92 ± 3.18	0.63 ± 0.07
Uptake 100	6.95 ± 0.19	19.47 ± 1.03	88.3 ± 1.91	149.71 ± 10.96	0.64 ± 0.07
Uptake 1000	7.13 ± 0.19	19.83 ± 0.95	90.28 ± 1.75	486.62 ± 40.59	0.52 ± 0.07
Elimination	6.85 ± 0.15	19.77 ± 0.69	98.69 ± 2.47	92.77 ± 2.61	0.51 ± 0.06

[a] Average of 5 aquaria sampled every other day.

Table 2
Mean (±SD) measured (“actual”) concentrations (mg/l)[a] of perchlorate in aquarium water in which eastern mosquitofish (*Gambusia holbrooki*) were exposed to various nominal concentrations of perchlorate for 12 h to 30 day

Nominal concentration	Actual concentration				
	12 h	24 h	2 day	10 day	30 day
0	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]
0.1	0.09 ± 0.01	0.08 ± 0.02	0.08 ± 0.01	0.10 ± 0.04	0.18 ± 0.10
1	0.89 ± 0.12	0.91 ± 0.05	0.80 ± 0.05	0.75 ± 0.05	0.90 ± 0.36
10	7.8 ± 0.20	7.6 ± 0.18	7.5 ± 0.29	7.0 ± 0.32	7.10 ± 0.39
100	87.5 ± 1.53	95.6 ± 0.33	96.0 ± 18.0	92.0 ± 15.0	88.0 ± 4.0
1000	954 ± 56.8	993 ± 113	906.0 ± 149.0	1100.0 ± 318.0	867.0 ± 49.0

[a] Perchlorate quantified by ion chromatography. $N = 5$ water samples for each data point. Perchlorate concentrations are reported on the basis of perchlorate ion, rather than sodium perchlorate.
[b] Not detected (detection limit = 0.001 mg/l).

within each aquarium were close to the nominal concentration (Table 2). Two 10 g samples of the food were analyzed for perchlorate, as above, and none was detected. Percent recoveries of perchlorate from fish tissues ranged from 81% to 92%. The detection limit for perchlorate in water was 1 μg/l, and in aqueous tissue extracts was 2.5 μg/l. Given that the extract volume used ranged from 0.020 to 0.022 l, and the mass of fish used ranged from 3.7 to 8.0 g, this would give tissue detection limits of 0.006–0.015 μg/g.

Perchlorate was not detected in mosquitofish exposed to the lowest concentrations of perchlorate (0, 0.1, and 1 mg/l sodium perchlorate), regardless of the exposure time (Table 3). Perchlorate was detected in mosquitofish exposed to 10, 100, and 1000 mg/l sodium perchlorate, but the whole body tissue concentrations were approximately 10 times less than the exposure concentration (Table 3). There was no difference in the BCF values depending upon the exposure time ($P = 1.000$). However, a difference in BCF values was observed depending upon the exposure concentration ($P < 0.001$).

Bioconcentration factors (BCF) for the uptake of perchlorate into whole mosquitofish were determined (Table 3). The highest BCF was found in fish exposed to 10 mg/l sodium perchlorate for 2 days. The Kruskall–Wallis H test revealed a significant difference among the treatment groups for each exposure period ($P = 0.001$).

Uptake depending upon short exposure times: The results of this experiment indicated a rapid uptake of perchlorate in eastern mosquitofish. The concentration of perchlorate in whole body increased ($P < 0.01$) up to 24 after which the concentration remained stable (Fig. 1A). This was assumed to be steady state.

3.2. Elimination of perchlorate

The concentration of perchlorate in water measured after five days of exposure (73.72 ± 2.00 mg/l) was close to the nominal concentration. The concentration of perchlorate in the water before the first water change (day 2) was 0.016 ± 0.001 mg/l.

The kinetic of perchlorate elimination in whole body mosquitofish is presented in Fig. 1B. The uptake rate constant (K_u) of perchlorate was estimated to be 0.09 l/mg day, the elimination rate constant (K_e) 0.70 per day, and the half-life ($T_{1/2}$) 0.99 day.

Table 3
Mean(±SD) bioconcentration[a] factor of perchlorate in eastern mosquitofish (*Gambusia holbrooki*) exposed to various concentrations of sodium perchlorate (nominal concentration) for 2, 10, or 30 days

Nominal concentration (mg/l)[c]	Bioconcentration factor (mean ± SD mass)[b]		
	2 day	10 day	30 day
0	ND (4.5 ± 1.4)[d]	ND (5.5 ± 2.6)[d]	ND (5.2 ± 1.8)[d]
0.1	ND (6.5 ± 2.4)[d]	ND (4.3 ± 1.4)[d]	ND (5.5 ± 1.7)[d]
1	ND (6.3 ± 1.4)[d]	0.27 ± 0.61 (4.6 ± 1.1)	0.06 ± 0.12 (4.5 ± 1.9)
10	0.35 ± 0.33 (6.3 ± 2.2)	0.11 ± 0.15 (5.2 ± 1.4)	0.18 ± 0.09(5.6 ± 2.1)
100	0.17 ± 0.07 (6.5 ± 1.8)	0.12 ± 0.02 (5.3 ± 1.7)	0.14 ± 0.02 (4.8 ± 1.4)
1000	0.09 ± 0.03 (4.9 ± 2.2)	0.06 ± 0.02 (5.5 ± 1.9)	0.13 ± 0.03 (6.1 ± 2.1)

[a] Concentration in the whole body tissues/measured concentration in the water, $n = 5$. Perchlorate ion concentrations measured by ion chromatography.
[b] Mass of the composite fish sampled analyzed.
[c] Concentrations based upon perchlorate ion rather than sodium perchlorate.
[d] Perchlorate not detected in tissues. Tissue detection limit = 0.006–0.015 mg/kg.

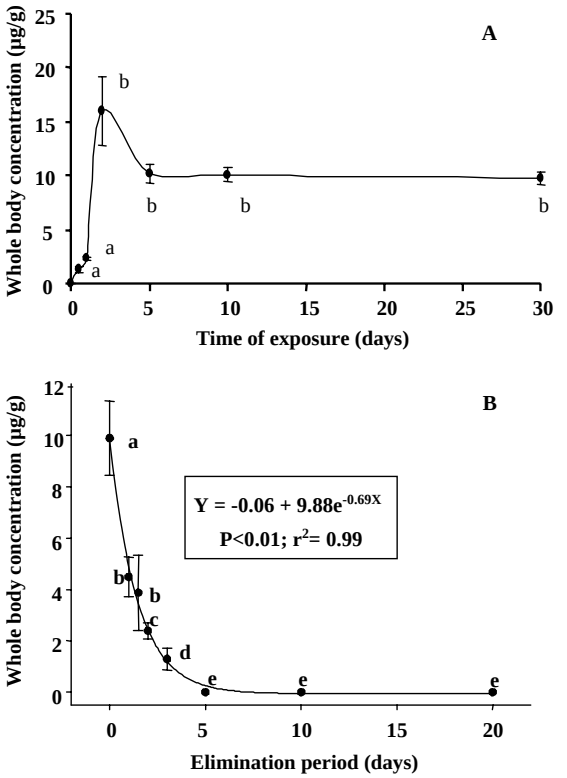

Fig. 1. (A) Perchlorate uptake in eastern mosquitofish (*Gambusia holbrooki*) exposed to 100 mg/l sodium perchlorate for 12, 24, 48, 240, and 720 h (means ± standard error, $n = 4$ for 12 and 24 h and $n = 5$ for 48, 240 and 720 h). (B) Perchlorate elimination in eastern mosquitofish after a 5-day exposure at 100 mg/l (means ± standard error, $n = 5$). Perchlorate concentrations are based upon the wet weight of the sample. Data points with different letters are significantly different ($P < 0.05$, Kruskal–Wallis test).

4. Discussion

The results of this study clearly indicate that perchlorate is rapidly taken up, reaches steady state, and is rapidly eliminated in eastern mosquitofish. This is in accordance with several studies rats (*Rattus rattus*), which indicated that perchlorate was rapidly absorbed when administrated orally in drinking water (Von Burg, 1995; Wolff, 1998; Clark, 2000).

The concentration of perchlorate in whole body of mosquitofish when detected was always lower than the exposure concentration. The highest BCF was found at an exposure of 10 mg/l perchlorate. Although concentrations at this level have been found in field analyses (Smith et al., 2001), in field situations, the measured concentrations are typically at most an order of magnitude lower – most commonly in the range of 0.010–0.080 mg/l (Smith et al., 2001; Anderson et al., 2004; Theodorakis et al., in press). A low BCF was also found in tissues of channel catfish exposed to 100 mg/l perchlorate for 5 days (Park, 2003). Thus, it appears that perchlorate does not bioaccumulate in fish.

This information is in apparent contrast to field studies, in which perchlorate was detected in fish captured in and around the Longhorn Army Ammunition Plant at Karnack, Texas (Smith et al., 2001) and the Naval Weapons Reserve Plant at Waco, Texas (Anderson et al., 2004; Theodorakis et al., in press), with tissue concentrations greater than those in the water. In fact, in some cases perchlorate was detected in the fish when it was not detected in the water (Theodorakis et al., in press). The exact same methodologies were used in Smith et al. (2001), Theodorakis et al. (in press), and in the current study. All field and laboratory samples from the current study and the studies cited above were conducted under the same Good Laboratory Practice

guidelines, using the same set of Standard Operating Procedures, and in all cases the data and analysis procedures were audited by and internal, but independent, Quality Assurance/Quality Control team to insure data quality. Also, the samples from Smith et al. (2001) were analyzed prior to, and those of Theodorakis et al. (in press) were analyzed subsequent to, the samples in this study. Finally, perchlorate has been frequently detected in mosquitofish from field sites cited in the above studies (Smith et al., 2001; Theodorakis et al., in press), which would argue against the hypothesis that the field and lab studies differed because of differences in false positive rate or detection limit due to species differences. These facts would argue against the hypothesis that the findings of perchlorate concentrations in fish tissues greater than water concentrations—as reported in Smith et al. (2001) and Theodorakis et al. (in press)—were due to false positives or other artifacts of analysis.

Therefore, there two possible explanations for the discrepancy between field and laboratory studies may be that: (1) there may be some other pathway of uptake, e.g., through the food chain, that explains the higher concentration in fish than in water in field situations, or (2) the perchlorate water concentrations in the field may be highly temporally variable, with large spikes in perchlorate concentrations or short durations followed by rapid declines in water concentrations. Evidence for such dramatic fluctuations in perchlorate water concentrations has been found in previous field studies (Anderson et al., 2004). Also, the elimination rate can be highly variable from fish to fish: at some points during the depuration experiments perchlorate was detected in some fish and not in others (Park, 2003, and the current study). Thus, large fluctuations in perchlorate water concentrations, coupled with variation in elimination, could, at least partially, explain the findings of Smith et al. (2001) and Theodorakis et al. (in press), where perchlorate was detected in fish when it was not detected in water, but only in some individual fish in some species.

In addition, the uptake and accumulation of perchlorate in fish may be affected by the concentrations to which they are exposed. This has been found for a wide variety of other contaminants. For example, in aquatic organisms exposed to metals, there is an inverse relationship between the exposure concentration and BCF, with high values occurring with low exposure concentrations (indicating hazard) and low values occurring with high exposure concentrations (indicating no hazard) (McGeer et al., 2003). Findings in this study show that, with the exception of the highest dose group, BCF was influenced by exposure concentration. This is an important consideration since BCF is one of the criteria used in determining toxicity in aquatic environments.

Perchlorate was rapidly eliminated when the mosquitofish were transferred to clean water. Perchlorate in mosquitofish had a lower elimination rate and higher half-life than any tissue in the catfish (Park, 2003). This fact could be considered as the effect of body size on elimination of perchlorate between both species because it has been reported that elimination of water-soluble chemicals in fish is affected by allometric (size) relationship (Newman, 1995). The half-life of perchlorate was very short, approximately 1 day. In rats the perchlorate half-life ranged from 8 to 20 h (Clark, 2000).

The results obtained in the present study are critical and may be used to develop models of fate, effects, and transport of perchlorate in natural systems, as well as to assess ecological risk in affected ecosystems.

Acknowledgements

We thank E. Cruz-Li, M. Wainscott, E. Galo, J. Canas, S. Williams, M. Landrum, L. Yu, and B. Adair for technical assistance. This research was supported in part by the US Department of Defense contract GUI 141, through the Strategic Environmental Research and Development Program (SERDP) under a Cooperative Agreement IERA-99-001 with the USAF, Institute for Environment, Safety, and Occupational Health, Brooks AFB, TX. The views and conclusions contained herein are those of the authors and should not be interpreted as necessarily representing the official policies or endorsements, either expressed or implied, of the 311 HSW/AFIERA or the US Government. Portions of this work were presented at the 24th annual meeting of the Society of Environmental Toxicology and Chemistry, 2003, Salt Lake City, UT.

References

American Society for Testing and Materials, 2003. Standard guide for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians. E 729-96. Annual Book of ASTM Standards, Vol. 11.04. American Society for Testing and Materials, Philadelphia, PA.

Anderson, T.A., Wu, T.H., 2002. Extraction, cleanup, and analysis of the perchlorate anion in tissue samples. Bull. Environ. Contam. Toxicol. 68, 684–691.

Anderson, T.A., Smith, P.N., McMurry, S.T., Carr, J.A., Theodorakis, C.W., Jackson, W.A., Dixon, K.R. 2004. Ecological risk assessment of ammonium perchlorate on fish, amphibian, and mammals in the Lake Belton and Lake Waco watersheds, An integrated laboratory and field investigation. In: U.S. Army Corps of Engineers, Bosque and Leon River Watershed Study, Final Report. U.S. Army Corps of Engineers, Fort Worth District, Fort Worth, TX.

Batjoens, P., DeBrabander, H.F., Tkindt, L., 1993. Ion chromatographic determination of perchlorate in cattle urine. Anal. Chim. Acta. 275, 335–340.

Brechner, R.J., Parkhurst, G.D., Humble, W.O., Brown, M.B., Herman, W.H., 2000. Ammonium perchlorate contamination of Colorado River drinking water is associated with

abnormal thyroid function in newborns in Arizona. J. Occup. Environ. Med. 42, 777–782.

Carr, J.A., Urquidi, L.J., Goleman, W.L., Hu, F., Smith, P.N., Theodorakis, C.W., 2003. Ammonium perchlorate disruption of thyroid function in natural amphibian populations, assessment and potential impact. In: Linder, G., Krest, S., Sparling, D., Little, E.E. (Eds.), Multiple Stressor Effects in Relation to Declining Amphibian Populations, ASTM STP 1443. ASTM International, West Conshohocken, PA, pp. 130–142.

Clark, J.J., 2000. Toxicology of perchlorate. In: Urbansky, E.T. (Ed.), Perchlorate in the Environment. Kluwer Academic/Plenum Publishers, New York, pp. 15–29.

Clewell, R.A., Merrill, E.A., Robinson, P.J., 2001. The use of physiologically based models to integrate diverse data sets and reduce uncertainty in the prediction of perchlorate and iodide kinetics across life stages and species. Toxicol. Ind. Health. 17, 210–222.

Fisher, J., Todd, P., Mattie, D., Godfrey, D., Narayanan, L., Yu, K., 2000. Preliminary development of a physiological model for perchlorate in the adult male rat, a framework for further studies. Drug. Chem. Toxicol. 23, 243–258.

Goleman, W.L., Carr, J.A., Anderson, T.A., 2002. Environmentally relevant concentrations of ammonium perchlorate inhibit thyroid function and alter sex ratios in developing *Xenopus laevis*. Environ. Toxicol. Chem. 21, 590–597.

Greer, M.A., Goodman, G., Pleus, R.C., Greer, S.E., 2002. Health effects assessment for environmental perchlorate contamination, the dose response for inhibition of thyroidal radioiodine uptake in humans. Environ. Health. Perspect. 110, 927–937.

Hare, J.P., 2000. Remediation of perchlorate contamination at NWIRP McGregor, Texas. Fed. Facil. Environ. J. 10, 33–38.

McGeer, J.C., Brix, K.V., Skeaff, J.M., DeForest, D.K., Brigham, S.I., Adams, W.J., Green, A., 2003. Inverse relationship between bioconcentration factor and exposure concentration for metals, implications for hazard assessment of metals in the aquatic environment. Environ. Toxicol. Chem. 22, 1017–1037.

Newman, M.C., 1995. Bioaccumulation. Quantitative Methods in Aquatic Ecotoxicology. CRC Press, Inc., FL, USA, pp. 59–111.

Park, J.-W., 2003. Toxicological effects and kinetics of perchlorate on fishes. Master's Thesis. Texas Tech University, Lubbock, TX.

Patiño, R., Wainscott, M.R., Cruz-Li, E.I., Balakrishman, S., McMurry, C., Blazer, V.S., Anderson, T.A., 2003. Effects of ammonium perchlorate on the reproductive performance and thyroid follicle histology of zebrafish. Environ. Toxicol. Chem. 22, 1115–1121.

Sancho, E., Ferrando, M.D., Lleo, C., Andreu-Moliner, E., 1998. Pesticide toxicokinetics in fish, Accumulation and elimination. Ecotoxicol. Environ. Safe. 41, 245–250.

Smith, P.N., Theodorakis, C.W., Anderson, T.A., Kendall, R.J., 2001. Preliminary assessment of perchlorate in ecological receptors at the Longhorn Army Ammunition Plant. LHAAP., Karnack, Texas. Ecotoxicology 10, 305–313.

Soldin, O.P., Braverman, L.E., Lamm, S.H., 2001. Perchlorate clinical pharmacology and human health, a review. Ther. Drug. Monit. 23, 316–331.

Spacie, A., Hamelink, L., 1995. Bioaccumulation. In: Rand, G.M. (Ed.), Fundamentals of Aquatic Toxicology, second ed. Taylor & Francis, Philadelphia, pp. 1052–1082.

Susarla, S., Bacchus, S.T., Harvey, G., McCutcheon, S.C., 2000. Phytotransformations of perchlorate contaminated waters. Environ. Technol. 21, 1055–1065.

Theodorakis, C.W., Rinchard, J., Anderson, T., Liu, F., Park, J.-W., Costa, F., McDaniel, L., Kendall, R., Waters, A., in press. Perchlorate in fish from a contaminated site in east-central Texas. Environ. Pollu.

Tipton, D.K., Rolston, D.E., Scow, K.M., 2003. Transport and biodegradation of perchlorate in soils. J. Environ. Qual. 32, 40–46.

Urbansky, E.T., 1998. Perchlorate chemistry, implications for analysis and remediation. Biorem. J. 2, 81–95.

Urbansky, E.T., 2002. Perchlorate as an environmental contaminant. Environ. Sci. Pollut. Res. Int 9, 187–192.

Urbansky, E.T., Magnuson, M.L., Kelty, C.A., Brown, S.K., 2000. Perchlorate uptake by salt cedar. *Tamarix ramosissima* in the Las Vegas wash riparian ecosystem. Sci. Total. Environ. 256, 227–232.

Urbansky, E.T., Brown, S.K., Magnuson, M.L., Kelty, C.A., 2001. Perchlorate levels in samples of sodium nitrate fertilizer derived from Chilean caliche. Environ. Pollut. 112, 299–302.

Von Burg, R., 1995. Toxicology update. Perchlorates J. Appl. Toxicol. 15, 237–241.

Wolff, J., 1998. Perchlorate and the thyroid gland. Pharmacol. Rev. 50, 89–105.

Yoon, Y., Amy, G., Cho, J., Her, N., Pellegrino, J., 2002. Transport of perchlorate. ClO_4^- through NF and UF membranes. Proceedings, Volume 4, Desalination 147, 11–17.

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., 2002. The pharmacokinetics of perchlorate and its effect on the hypothalamus–pituitary–thyroid axis in the male rat. Toxicol. Appl .Pharmacol. 182, 148–159.

Yu, L., Canas, J.E., Cobb, G.P., Jackson, W.A., Anderson, T.A., 2004. Uptake of perchlorate in terrestrial plants. Ecotox. Environ. Safe. 58, 44–49.