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Perchlorate Effects in Fish

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Introduction

Perchlorate anion is extremely water soluble and environmentally stable, resulting in rapid movement through ground and surface waters (Urbansky 2002). This fact, combined with the widespread use of perchlorate in industrial and military applications, indicates that the potential for perchlorate contamination in the aquatic environment, and thus the potential for exposure of fish to perchlorate, is significant. The number of known contaminated sites is probably limited by the small extent of monitoring for this contaminant. Furthermore, there is a paucity of knowledge concerning environmental fate, transport, toxicokinetics, bioavailability, and effects of this compound in aquatic systems.

Potential routes of entry of perchlorate into surface waters include

1) direct effluent discharge;
2) transfer from contaminated groundwater to the surface via seeps or springs, or use of contaminated groundwater for irrigation, with resultant runoff into surface waters;
3) percolation of water through contaminated soils; and
4) runoff from contaminated terrestrial ecosystems (Figure 7-1).

Furthermore, perchlorate may enter sediment pore water via surface water or ground water. Also, previous research has found that perchlorate accumulates in terrestrial plants (Smith et al. 2001) through uptake from soil, groundwater, or surface water, and most detritus in stream systems comes from fallen leaves of terrestrial plants in the riparian zone (i.e., streamside).

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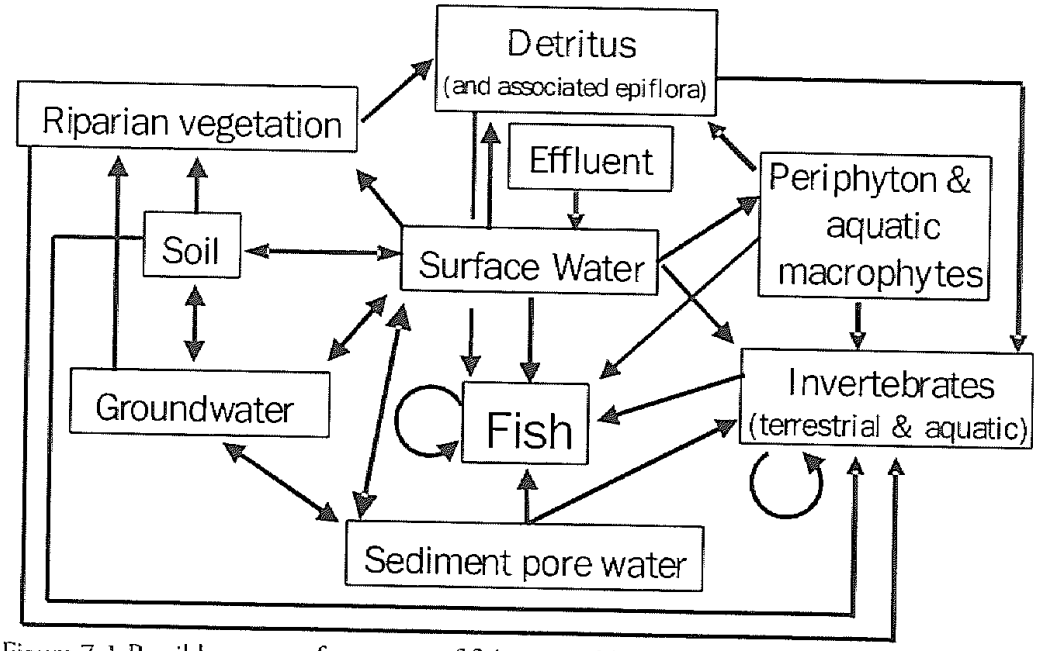

Figure 7-1 Possible routes of exposure of fish to perchlorate in aquatic ecosystems

(Such inputs of organic matter from terrestrial sources are known as "allochthonous inputs.") Thus, bioconcentration of perchlorate by terrestrial plants, followed by allochthonous inputs of detritus from such plants, is another possible pathway of entry of perchlorate into surface waters.

Fish may be exposed to perchlorate in contaminated surface waters via a number of different routes. A conceptual model of potential routes of uptake and transport through aquatic food webs is shown in Figure 7-1. First, because perchlorate is readily soluble, direct uptake from surface water through gills or permeable integument is probably a major route of entry. Another possible route of uptake in fish is through the food chain. Some fish feed on detritus, and so may be exposed to perchlorate in this fashion. The epiflora that lives upon detritus (saprophytic bacteria and fungi) could possibly take up perchlorate from the detritus or water. Perchlorate may also accumulate in periphyton, filamentous algae, and aquatic macrophytes (Anderson et al. 2004), representing a possible exposure pathway in herbivorous fish. Because many fish are invertebrivorous, any perchlorate taken up by aquatic invertebrates may be passed on to the fish. In addition, any fish feed upon terrestrial invertebrates that fall or are washed into the water, so this is an alternative pathway whereby terrestrial perchlorate in soil or plants may be transported to fish. Similar to fish, invertebrates may absorb perchlorate directly from contact with, or ingestion of, contaminated surface water, soil, sediment, or sediment pore water. Alternatively, invertebrates may be exposed to perchlorate from food

items such as periphyton, detritus (or associated epiflora), or other invertebrates. Piscivorous fish may also be exposed to perchlorate in their prey. Although, for larger predacious fish (and for some large, aggressive invertebrates), there is the possibility of food chain transfer via consumption of amphibians (larvae or adults) and other small aquatic or semi-aquatic vertebrates; this is probably not a major exposure route. Whatever the route of exposure, the primary concern of perchlorate exposure is that it is known to affect thyroid function, causing subsequent hormone disruption and potential perturbations of metabolic physiological and developmental activities.

Although there is not as much information on thyroid biology in fish as there is in other vertebrates, it is known that thyroid hormones (THs) influence many aspects of fish physiology and biochemistry. In at least some species of teleosts, THs may have been reported to influence mitochondrial function, carbohydrate and lipid metabolism, growth, gonad and gamete development, embryo or larval development, metamorphosis, immune function, behavior, melanocyte development and skin pigmentation, larval metamorphosis, osmoregulation, neurogenesis, temperature acclimation, and steroidogenesis (Prosser et al. 1991; Soyano et al. 1992; Eales and Brown 1993; Leatherland 1994; Mylonas et al. 1994; Brown and Kim 1995; Cyr and Eales 1996; Tyler and Sumpter 1996; Gomez et al. 1997; Castonguay and Cyr 1998; Iwata et al. 1999; Schreiber and Specker 1999; Kumar et al. 2000; Subash Peter et al. 2000; Power et al. 2001; Varghese et al. 2001; Aas-Hansen et al. 2003; Brown et al. 2004). Disruption of TH synthesis and signaling during zebrafish (*Danio rerio*) larval development may also inhibit yolk sac resorption and development of the fins, gastrointestinal system, swim bladder, and lower jaw (Brown 1997; Liu and Chan 2002). In addition, teleost TH signaling pathways may regulate or interact with other endocrine or paracrine signaling pathways, including growth hormone, sex steroids, retinoids, corticosteroids, and prostaglandins (Cyr and Eales 1996; Gomez et al. 1997; Kim and Brown 1997; Kuhn et al. 1998; Sternberg and Moav 1999; van Anholt et al. 2003). However, broad generalities cannot be made because there is considerable interspecific variation. For example, in some species, THs may influence growth hormone and embryo or larval development, whereas in other species they may not (Nishioka et al. 1985; Luo and McKeown 1991; Tagawa and Hirano 1991; Melamed et al. 1995; Brown 1997; Liu and Chan 2002; Rousseau et al. 2002). Also, in some species gondadotropin-releasing hormone and gonadal or sexual maturation may be stimulated by THs, while in other species they may be inhibited by THs (Sullivan et. al 1989; Parhar et al. 2000). Besides these differ-

ences between species, interspecific variation, there may be intraspecific variation in TH levels and their effects on fish physiology. TH levels may vary within species or individuals as a function of season, photoperiod, diurnal periodicity, nutritional status, sex, and reproductive condition (MacKenzie et al. 1989; Pavlidis et al. 1991; Gomez et al. 1997; Leiner et al. 2000; Gaylord et al. 2001; Fontainhas-Fernandes et al. 2002; Aas-Hansen et al. 2003; Reddy and Leatherland 2003). Thus, thyroid-disrupting chemicals have the potential to perturb many aspects of fish physiology, but the effects of these chemicals on fitness and performance parameters remain uncertain (Brown et al. 2004).

While many aspects of thyroid anatomy and physiology are conserved between fish and tetrapods, there are some differences. First, follicles in most fish are not encapsulated within a discrete gland, with some notable exceptions such as tuna (*Thunnus* spp.) and parrotfish (Scaridae; Eales and Brown 1993, and references therein). In most teleosts, thyroid follicles are scattered throughout the basibranchial region (in or near the lower mandible, near the base of the gills) and around the bulbus arteriosus, but may also be present in the gills, liver, thymus, spleen, or kidney (Eales and Brown 1993; Leatherland 1994). Second, there may be differences between teleosts and tetrapods in terms of regulation of the hypothalamus–pituitary–thyroid axis. For example, in fish, thyrotropin-releasing hormone plays a lesser role (if any) in regulating thyroid-stimulating hormone (TSH) secretion. Other hormones may be involved in this regard, such as somatostatin (inhibitory) or corticotropic-releasing factor (stimulatory). These hormones are also transported from the hypothalamus to the pitutuary via direct innervation, rather than a blood portal system (Brown et al. 2004). Third, the primary thyroid-binding plasma proteins in fish may include albumin and lipoproteins, as well as transthyretin, and a percentage of TH may be transported in red blood cells in some species (Brown et al. 2004). Fourth, in some species such as trout, some T_3 may be sequestered in a slowly equilibrating pool such as skeletal muscle (Brown et al. 2004). Finally, fish obtain iodine for use in TH synthesis not only from the diet but also (and primarily) through absorption from the water via the gills (Brown et al. 2004). However, despite these differences between fish and tetrapods, there is a high degree of conservation among vertebrates in terms of physiological responses to thyroid-disrupting chemicals: Chemicals that disrupt TH, homeostasis in mammals do so in fish as well, via similar mechanisms (Leatherland 1994; Brown et al. 2001; Fox 2001).

Thus, when teleost fish are exposed to perchlorate, the effects on their thyroids are similar to those found in other vertebrates. The thyroidal effects

of perchlorate are, in turn, affected by its uptake and toxicokinetics, and have the possibility to affect phyisiological and fitness parameters. The risk of perchlorate exposure to endemic fish populations may be assessing effects and toxicokinetics in the laboratory and the field, as well as by developing physiologically based models of the pharmacokinetics of perchlorate and TH kinetics in fish. This chapter summarizes current knowledge of perchlorate exposure and uptake in fish, lethal and sublethal effects, physiologically based pharmacokinetic (PBPK) models, and research needs.

Exposure

Uptake and toxicokinetics

There have been several studies on uptake and toxicokinetics of perchlorate in various fish species. These species include eastern mosquitofish (*Gambusia holbrooki*), zebrafish (*Danio rerio*), and bluegill sunfish (*Lepomis macrochirus*).

In one of these studies, female eastern mosquitofish were exposed to 0, 0.1, 1, 10, 100, and 1000 $mg \cdot L^{-1}$ sodium perchlorate for 2, 10, or 30 d, and then analyzed for perchlorate in whole-body homogenates (Bradford et al. 2006). No perchlorate was detected in mosquitofish exposed to the lowest concentrations of perchlorate (0, 0.1, and 1 $mg \cdot L^{-1}$ sodium perchlorate), for any exposure duration. Perchlorate was detected in mosquitofish exposed to 10, 100, and 1000 $mg \cdot L^{-1}$ sodium perchlorate, but the whole-body tissue concentrations were approximately 1/10 of the exposure concentration. Steady-state body burdens appeared to be reached relatively rapidly, within 2 d or less. The highest bioconcentration factors (BCFs) were in fish exposed to 10 and 100 $mg \cdot L^{-1}$ sodium perchlorate, with BCF, of 0.15 and 0.12, respectively. The BCF, for the 1000 $mg \cdot L^{-1}$ exposure was less than 0.01. An elimination experiment also was conducted in which fish were exposed to 100 $mg \cdot L^{-1}$ sodium perchlorate, and then allowed to depurate for 1, 2, 5, or 10 d. The uptake rate constant, elimination rate constant, and half-life of perchlorate in this species were 0.1 d^{-1}, 0.76 d^{-1}, and 0.91 d, respectively (Bradford et al. 2006).

Limited information on perchlorate uptake is also available for zebrafish. In a recent study, fish were exposed to ammonium perchlorate (AP)-derived perchlorate at measured concentrations of 677 $mg \cdot L^{-1}$ for 4 weeks or 18 $mg \cdot L^{-1}$ for 8 weeks (Patiño et al. 2003). The estimated BCFs in each case were 0.01 and 0.02, respectively.

In order to examine tissue distribution and tissue-specific uptake and elimination rates, Channel catfish (*Ictalurus punctatus*) were exposed to

100 mg·L^{-1} sodium perchlorate for 5 d, and allowed to depurate for 1, 2, 5, 10, and 20 d (Park 2003; Anderson et al. 2004). It was found that the BCFs were highest in the head and fillet. The fillet also had the lowest uptake rate constant and highest elimination rate constant, while the head and liver had the highest uptake rate constant and lowest elimination rate constant, respectively. These findings suggest that the head and fillet are the most important tissues to analyze when the toxicokinetics and uptake of perchlorate in fish are examined. Although perchlorate did accumulate in other tissues in the catfish (kidney, liver, gill, and gastrointestinal tract), these tissues had relatively low concentrations of perchlorate. Although perchlorate was rapidly eliminated from catfish tissues, it was detected in a few individuals even after 20 d of depuration. This finding suggests that depuration rates may vary greatly between individuals, and it has implications for interpretation of data from the field (see "Body burdens in field studies" below).

In another study, Dean et al. (2004) conducted bioconcentration tests on juvenile bluegill sunfish to 0, 1.5, and 15 mg·L^{-1} perchlorate anion (sodium salt) for 0, 1, 2, 3, 7, 14, 21, and 28 d. The BCF in this fish ranged from 0.73 L·kg^{-1} in the lowest treatment to 0.68 L·kg^{-1} in the highest treatment, with a geometric mean of 0.70 L·kg^{-1}.

In general, these results indicate that perchlorate is taken up by fish and reaches steady state relatively rapidly in some species, while it may take longer in others. Perchlorate was not detected in individuals exposed to environmentally relevant concentrations of perchlorate using ion chromatography (IC) with conductivity detection (mosquitofish, catfish, zebrafish), but for goldfish perchlorate detection was carried out using IC/MS/MS (ion chromatography/mass spectrometry/mass spectrometry). Differences between the catfish/mosquitofish vs. bluegill studies may represent species-specific differences or sensitivity of the techniques. Fish that were exposed to perchlorate in the laboratory did not bioaccumulate perchlorate because the tissue concentration of perchlorate was much lower than the exposure concentration of perchlorate. The uptake and elimination rate constants and BCFs may differ between species and between tissue types.

Body burdens in field samples

Environmental contamination of water sources occurs primarily near military and industrial installations where perchlorate is handled. To date, 14 of the United States, including Texas, have confirmed the presence of perchlorate in their ground and surface waters. In north-central and east Texas, perchlorate has been detected in water at 2 former military

manufacturing sites, the Longhorn Army Ammunition Plant (LHAAP) (TNRCC 1999; Smith et al. 2001) and the Texas Naval Weapons Industrial Reserve Plant (TNWIRP) (Motzer 2001; Theodorakis et al. 2006a). At both sites, perchlorate was also measured in tissues of ecological receptors including algae, aquatic insects, and fish (Smith et al. 2001; Anderson et al. 2004).

Long Horn Army Ammunition Plant, Texas

In 1999, several areas within LHAAP, Karnack, Texas, were analyzed for perchlorate (Smith et al. 2001). Two perchlorate-contaminated sites were sampled for small fish species: Harrison Bayou and Goose Prairie Creek. Until the fall of 2002, Harrison Bayou received an effluent of treated groundwater that was contaminated with perchlorate. Goose Prairie Creek flows past an area where perchlorate was ground and processed (Building 25C), and where soil concentrations were as high as 322 $\mu g \cdot kg^{-1}$ (Smith et al. 2001). Perchlorate was detected in water samples from both sites at concentrations less than 85 $ug \cdot L^{-1}$. Perchlorate was detected only sporadically and in small, insectivorous or omnivorous fish species in the families Centrarchidae, Cyprinidae, Fundilidae, and Poeliciidae. When perchlorate was detected, concentrations in fish tissues exceeded those in the water.

Texas Naval Weapons Industrial Reserve Plant and Lakes Waco and Belton, Texas

In 2001 and 2002, potential perchlorate contamination was investigated in the Bosque River and Leon River watersheds, Texas, because AP was discovered outside the TNWIRP (former Hercules Plant), which lies within those 2 watersheds. Water samples were collected from 6 streams in and around the TNWIRP. Harris Creek, North Fork South Bosque River, South Fork South Bosque River, and Station Creek all originate on the TNWIRP. Wasp Creek does not originate on the Plant, but a portion of the creek runs through it. Coryell Creek neither originates on nor runs through TNWIRP. Perchlorate was detected in water samples from the 4 streams that originate on the TNWIRP. Two reservoirs formed by impoundment of the Bosque and Leon Rivers, Lake Waco and Lake Belton, respectively, were also sampled. Samples of fish fillet (to assess possible human exposure in edible portions) and head (because perchlorate concentrations in laboratory fish were greatest in the head) were collected for perchlorate analysis.

Results for the streams were similar to those for LHAAP. Perchlorate was detected sporadically, and when it was found, it usually occurred in cyprinids, centrarchids, and mosquitofish, although it also was detected in

bullhead catfish (*Amerius spp.*). In particular, perchlorate was commonly found in green sunfish (*Lepomis cyanellus*). This finding is significant because the species is a popular sportfish and panfish in the area, colloquially known as "perch." Again, when perchlorate was detected, the concentration in biological tissues was much greater than in the water

A similar situation was observed for the fish sampled from Lakes Belton and Waco. Parts of the TNWIRP are located within the watersheds of both lakes, which receive inputs from streams originating on the TNWIRP. For these lakes, perchlorate was detected only sporadically in the fillets of several species of large fish, most commonly largemouth bass and channel catfish, although the concentration of perchlorate in the water was below the detection limit (1 $\mu g \cdot L^{-1}$) at the collection sites. These studies also found that the head had much higher concentrations of perchlorate than did the fillets, at least for black crappie.

Food chain transfer

In order to determine if perchlorate could be transferred through the food chain, Park et al. (2005) exposed bass to 500 $mg \cdot L^{-1}$ sodium perchlorate for 1, 10, or 30 d. There were 3 treatments for the bass: exposed to perchlorate via the food, via the water, or by a combination of food and water. The food consisted of fathead minnows exposed to 1000 $mg \cdot L^{-1}$ sodium perchlorate in the water for 2 d before they were fed to the bass. The body burden concentrations of perchlorate in largemouth bass indicated that food chain transfer of perchlorate occurred after 1 d of feeding. Moreover, fish exposed to perchlorate via food and water contained higher concentrations of perchlorate than did fish exposed to perchlorate in water only, although this was significant only at the 10-d exposure period. Two-way analysis of variance (ANOVA) indicated a significant interaction between the length of exposure and the mode of exposure ($P < 0.05$).

Effects

Acute toxicity

Several studies to date have found that perchlorate, either as sodium or ammonium salts, has low acute toxicity to fish. In one study, Patiño et al. (2003) found that the 5-d LC50 of AP to zebrafish embryos and larvae was 529 $mg \cdot L^{-1}$. Other studies using the sodium salt of perchlorate found 96-h LC50s of 404 and 1486 $mg \cdot L^{-1}$ for juvenile (<1 week old) eastern mosquitofish and 4-d old zebrafish, respectively (Liu et al. 2005; Park et al. 2006). Other species may be even less susceptible to perchlo-

rate: the 96-h LC50s for rainbow trout (*Oncorhynchus mykiss*), fathead minnow (*Pimephales promelas*), and bluegill sunfish (*Lepomis macrochirus*) exposed to sodium perchlorate were 2100, 1655, and 1500 $mg \cdot L^{-1}$, respectively (Engineering, Science, and Technology 1998; Parsons et al. 2002). Dean et al. (2004) also calculated the 96-h LC50s of perchlorate for rainbow trout and bluegill as 2010 and 1470 $mg \cdot L^{-1}$, respectively, in close agreement with results from rainbow trout, fathead minnow, and bluegill, above. Chronic toxicity (survival endpoint) LC50, no-observed-efffects concentration (NOEC), and lowest-observed-effects concentration (LOEC), for fathead minnows were reported as 614, 155, and 280 $mg \cdot L^{-1}$ by Callahan et al. (1998). In general, it seems that the LC50s are very much higher than are the water concentrations seen in the field (Motzer 2001; Smith et al. 2001).

Sublethal Toxicity

Thyroid effects

Laboratory studies. Adult zebrafish exposed to AP at measured water perchlorate concentrations of 18 $mg \cdot L^{-1}$ for 8 weeks showed signs of thyroid follicle cell (nuclear) hypertrophy, hyperplasia, colloid depletion, and increased vascularization (angiogenesis) relative to control fish (Patiño et al. 2003). The degree of thyroid follicle vascularization in this study was particularly remarkable (Patiño et al. 2003), suggesting its possible utility as new biomarker of perchlorate exposure. In a follow-up study (Mukhi et al. 2005), subadult (3-month-old) zebrafish were exposed to AP-derived perchlorate at measured concentrations of 0, 11, 90, 1131, and 11480 $\mu g \cdot L^{-1}$ for up to 12 weeks and allowed to recover in clean water for another 12 weeks. The LOEC of perchlorate that induced angiogenesis after 2 weeks of exposure was 90 $\mu g \cdot L^{-1}$, whereas hypertrophy was not detected at this time. By 12 weeks of exposure, the LOEC for angiogenesis remained at 90 $\mu g \cdot L^{-1}$, whereas the LOEC for hypertrophy was 1131 $\mu g \cdot L^{-1}$ (Mukhi et al. 2005). The content and distribution of thyroxine (T_4)-like immunoreactivity of the colloid in histological sections of thyroid follicles was also examined by immunocytochemistry. The thyroid follicles displayed a "colloidal T_4 ring" previously observed at the interphase between the follicular epithelium and the lumen, as reported in larval zebrafish (Wendl et al. 2002). The intensity of this colloidal T_4 ring was reduced by exposure to perchlorate, with a 2-week LOEC, of 1131 $\mu g \cdot L^{-1}$ and a 12-week LOEC of 11 $\mu g \cdot L^{-1}$ (Mukhi et al. 2005). Whole-body T_4 concentrations (measured by radioimmunoassay) were not affected by perchlorate in this study. Thus, a change in the intensity

of the colloidal T_4 ring following relatively extended periods of exposure (12 weeks) was the most sensitive of the parameters of thyroid toxicity condition examined. All perchlorate-induced changes in thyroid follicles were reversible, but residual effects on colloidal T_4 ring intensity as well as angiogenesis were still present after 12 weeks of recovery in the fish ptevi-ously exposed to the highest concentration of perchlorate (11480 $\mu g \cdot L^{-1}$). Thus, the sensitivity and longevity of changes in colloidal T_4 ring intensity and angiogenesis suggest their usefulness as novel markers of perchlorate exposure and toxicity thyroid effects in fish.

Effects of perchlorate on mosquitofish thyroid disruption also have been examined by Bradford et al. (2005). In this particular study, adult female eastern mosquitofish were exposed to 0, 0.1, 1, 10, 100, and 1000 $mg \cdot L^{-1}$ (nominal concentrations) sodium perchlorate for 2, 10, and 30 d in the laboratory. Thyroid follicle histopathology was examined in stained sections of the head, and whole-body T_4 concentrations were determined by radioimmunoassay. An increase in follicular epithelial height was noted, particularly in fish exposed to perchlorate for 30 d. In addition, an increase in the occurrence of hyperplasia, hypertrophy, and colloid depletion also was observed in a dose- and time-dependent fashion. There was a small decrease in T_4 concentrations in fish exposed to perchlorate for 30 d, but this effect was less pronounced than the effects on thyroid histopathology.

Additionally, adult male and female common goldfish (*Carassius auratus*) were exposed separately to environmentally relevant concentrations of sodium perchlorate (14 to 31 000 $\mu g \cdot L^{-1}$) in a laboratory flow-through exposure system (Crouch 2004). Treatments consisted of combinations of perchlorate concentration (measured anion concentration) and exposure duration as follows:

- control (<4 $\mu g \cdot L^{-1}$), 30 d;
- 14 $\mu g \cdot L^{-1}$, 30 d;
- 130 $\mu g \cdot L^{-1}$, 30 d;
- 31 000 $\mu g \cdot L^{-1}$-30 d;
- control (<4 $\mu g \cdot L^{-1}$), 60 d;
- and 31 000 $\mu g \cdot L^{-1}$, 60 d.

Both thyroid and reproductive endpoints were examined. A new, subjective, nonparametric rank-order assessment method was developed to assign activity scores to thyroid tissue in the subpharyngeal region (pharyngeal thyroid, lower jaw) and head kidney based on epithelial cell height, colloid depletion, number of follicles affected, and severity

of effects (Crouch 2004). A digital imaging system was used to measure thyroid follicle epithelial cell heights in the same tissues to allow a comparison between the new subjective assessment method and the more traditional and objective measurement method. Thyroid activity scores resulting from the assessment method were more sensitive to the effects of perchlorate than were epithelial cell height measurements. A significant increase in pharyngeal thyroid activity occurred in males exposed to 31 000 $\mu g \cdot L^{-1}$ for 60 d and in females exposed to 31 000 $\mu g \cdot L^{-1}$ for 30 d or 60 d. A, significant increase in head kidney thyroid activity occurred in males exposed to 31 000 $\mu g \cdot L^{-1}$ for 60 d and in females exposed to 1200 $\mu g \cdot L^{-1}$ for 30 d or to 31 000 $\mu g \cdot L^{-1}$ for 30 d or 60 d (Crouch 2004).

Field studies. Field studies on the effects of perchlorate are generally lacking. In studies by Theodorakis et al. (2006a) and Anderson et al. (2004), central stonerollers (*Campostoma anomalum*) and green sunfish (*Lepomis cyanellus*) were collected from the streams in and around TNWIRP. A reference site (Coryell Creek) was also chosen. Streams were sampled in October 2001 and March 2002. In October, Coryell, Wasp, Station, and Harris Creeks were sampled, while in March, the South Bosque River replaced Harris Creek as a sampling site. Histopathological indices, including the percentage of thyroid follicles that were hyperplastic or hypertrophic or that exhibited colloid depletion, were determined for stonerollers. The percentage of follicles that exhibited angiogenesis (extent of vascular tissue within the follicles), which has also been identified as an indicator of thyrotoxicity (Patiño et al. 2003), was measured (Anderson et al. 2004). The epithelial cell heights were determined for green sunfish collected in March (Anderson et al. 2004). In addition, the percent of individuals that exhibited evidence of angiogenesis in thyroidal tissue was also evaluated for fish collected in October.

Overall, the level of effect was associated with proximity to the TNWIRP. Fish collected from streams that originated on the TNWIRP grounds (Harris Creek and Station Creek) were impacted more than were fish collected from other streams. The Wasp Creek and South Bosque stonerollers seemed to be affected to a level intermediate between the level of effect observed in fish from the other streams that originate on TNWIRP and the level of effect in fish from Coryell Creek. This finding is consistent with the fact that Wasp Creek and the South Basque River are in closer proximity to TNWIRP than is Coryell Creek, and that Wasp Creek runs through TNWIRP while Coryell Creek does not. Furthermore, the trend in detectable perchlorate concentrations in fish among streams parallels the evidence of thyroid disruption. Wasp Creek was originally thought

to be a reference site, but perchlorate was found in some of the fish from Wasp Creek; however, the incidence of detectable perchlorate in fish was less in Wasp Creek than in the streams that originate on TNWIRP. Overall, researchers concluded that the data collected from this study suggested that perchlorate is negatively impacting thyroid homeostasis of the fish in streams that originate in or run through TNWIRP.

The data from the October 2001 sampling indicate the following:

- For thyroid follicle epithelial heights, the level of effect is in the order of Harris Creek > Wasp Creek > Station Creek > Coryell Creek.
- For percentage occurrence of hyperplasic follicles, the relative levels of effect were Station Creek > Wasp Creek = Harrison Creek > Coryell Creek.
- For percent occurrence of follicles with depleted colloid, the levels of effect were Harrison Creek = Station Creek > Wasp Creek = Coryell Creek.
- For angiogenesis, the levels of efffect were Station Creek > Wasp Creek > Harris Creek > Coryell Creek.

For the stonerollers collected in March, no statistically significant differences in the occurrence of hyperplasia were found, while the trends for occurrence of depleted colloid and epithelial cell height were Station = South Bosque = Wasp > Coryell and Coryell = South Bosque > Wasp = Station Creek. For occurrence of angiogenic responses, the level of effect was Wasp=Station > Coryell = Harris. Green sunfish from Coryell Creek had the smallest thyroid follicles, fish from the South Bosque River had the largest, and the other 2 sites had intermediate levels (Anderson et al. 2004; Theodorakis et al. 2006b). A comparison of thyroid epithelial cell heights between green sunfish and stonerollers suggests that the green sunfish in the South Bosque River are impacted more than stonerollers from the same river (Anderson et al. 2004).

Macrophage aggregates

Macrophage aggregates (MAs) or centers are generally considered to serve in the recycling and removal of cellular debris; the sequestering, destruction, and removal of cellular toxicants; antigen trapping and presentation to lymphocytes; and other functions (Tsujii and Seno 1990; Wolke 1992; Agius and Roberts 2003). In fish, exposure to environmental contaminants is known to increase the incidence of MAs in some tissues, and this response may indicate the occurrence of tissue injury or immunotoxic response (Weeks et al. 1992; Wolke 1992; Fournie et al. 2001; Agius and Roberts 2003). A recent study with zebrafish and eastern mosquitofish ex-

amined the histological effects of perchlorate on the trunk kidney, which in teleosts serves excretory and hemopoietic functions (Capps et al. 2005). Adult zebrafish of both sexes were exposed in the laboratory to waterborne, AP-derived perchlorate at measured concentrations of 18 $mg \cdot L^{-1}$ for 8 weeks. Adult male mosquitofish were exposed to waterborne sodium perchlorate at measured perchlorate concentrations of 1 to 92 $mg \cdot L^{-1}$ for 8 weeks. Control fish were kept in untreated water. Histological analyses showed that MAs were present in the hemopoietic region of the trunk kidney in both species (the head kidney was not examined). The estimated percent area of trunk kidney sections occupied by MAs was higher in zebrafish exposed to perchlorate at 18 $mg \cdot L^{-1}$ when compared to controls.

In male mosquitofish, the incidence of renal MAs increased proportionally with sodium perchlorate concentration and was significantly different from controls at 92 $mg \cdot L^{-1}$. These observations confirm that the kidney is affected by exposure to perchlorate in fish. The concentrations at which perchlorate had clear effects on the incidence of renal MAs (18 $mg \cdot L^{-1}$ in zebrafish and 92 $mg \cdot L^{-1}$ in mosquitofish) are at the high end of perchlorate concentrations previously reported in the environment; perchlorate concentrations of up to 33 $mg \cdot L^{-1}$ have been reported in some contaminated aquatic habitats (Smith et al. 2001). Thus, the environmental relevance of these findings is uncertain at the present time. However, it has been reported that the magnitude of the thyroidal effects of perchlorate is a function not only of exposure concentration but also of exposure duration (Fernandez Rodriguez et al. 1991; Mukhi et al. 2005). Thus, if the thyroid system were involved in the mechanism of perchlorate-induced kidney MAs, exposures to perchlorate at lower concentrations but for longer periods of time than those used in the present study may also affect the structure and function of the fish kidney.

Fitness parameters: growth, reproduction, development

A recent review of the effects of contaminants on the thyroid system concluded that xenobiotic-induced changes in thyroid function have yet to be clearly linked to impairments in the fitness or survival of fish (Brown et al. 2004). In agreement with this conclusion, the data presently available for fish have failed to demonstrate clear effects of perchlorate on parameters such as growth and reproduction at environmentally relevant concentrations.

Zebrafish. An 8-week exposure to AP-derived perchlorate at high environmentally relevant levels (18 $mg \cdot L^{-1}$) did not affect the reproductive performance (spawn volume and fertilization rate) of adult zebrafish, although it greatly disrupted their thyroid histological condition (Patiño et

al. 2003). Also, the growth and pubertal development of subadult zebrafish were not affected by exposure to AP-derived perchlorate (0 to 11 480 $\mu g \cdot L^{-1}$) during or after an experimental exposure period of 12 weeks (Mukhi et al. submitted). Results similar to those obtained with zebra-fish (Patiño et al. 2003; Mukhi et al. submitted) were reported in a study with rats, where a 2-generation exposure to AP did not affect reproductive performance or embryonic development (York et al. 2001).

Mosquitofish. Park et al. (2006) examined the effects of perchlorate on reproduction and growth of eastern mosquitofish exposed to sodium perchlorate. This species is commonly found at perchlorate-contaminated sites such as TNWIRP and LHAAP (Smith et al. 2001; Theodorakis et. al. 2006a). Adult and 7-d-old fry were exposed to sodium perchlorate at 0, 1, 10, and 100 $mg \cdot L^{-1}$ (nominal concentrations). Effects on growth and reproduction were determined after 14 and 56 d of exposure for fry and adults, respectively. Their results indicated that perchlorate exposure had a stimulatory effect on fecundity, gonadal somatic index (GSI = [gonad weight / body weight] *100), and average egg or embryo mass, at least for some treatments. Growth was also stimulated at 1 $mg \cdot L^{-1}$ but was inhibited at 10 $mg \cdot L^{-1}$. These results fail to provide evidence that perchlorate inhibits growth and reproduction in eastern mosquitofish at environmentally relevant concentrations.

Goldfish. Adult male and female common goldfish were exposed separately to environmentally relevant concentrations (14 to 31 000 $\mu g \cdot L^{-1}$) of sodium perchlorate for 30 to 60 d in a laboratory flow-through exposure system under conditions designed to stimulate spring gonadal recrudescence (Crouch 2004). Perchlorate exposure did not affect GSI in either sex, and there were no differences in gonad development, as assessed histologically, among treatment groups. However, the fish were not exposed during the period of gonadal recrudescence most sensitive to impairment of gonadal growth. Evaluations of plasma sex steroid concentrations 17ß-estradiol, testosterone, and 11-ketotestosterone) and TH concentrations (T_4 and T_3) are underway.

Fathead minnows. In chronic, 35-d early life-stage toxicity studies, no effects of perchlorate were found in fathead minnows at perchlorate ion concentrations up to 490 $mg \cdot L^{-1}$ perchlorate anion (Engineering, Science, and Technology 2000).

Lamprey. The effects of potassium perchlorate at very high concentrations (100 $mg \cdot L^{-1}$) have also been investigated in sea lamprey (*Petromyzon marinus*) from a landlocked population. Contrary to most teleosts, THs inhibit larval sea lamprey metamorphosis (Holmes and Youson 1993).

It was found that perchlorate treatment enhanced larval metamorphosis (Kao et al. 1999). Potassium perchlorate was also found to reduce serum TH concentrations and to affect lipid metabolism in this species (Youson et al. 1995; Kao et al. 1999). Similar effects were seen for American brook lamprey (*Lampetra appendix*; Holmes et al. 1999). Because of the high concentrations of perchlorate used in these studies, the environmental relevance of their findings remains uncertain.

Toxicity of perchlorate mixtures

Perchlorate and arsenate

Thyroid hormones have been found to influence levels of antioxidant enzymes and oxidative stress in fish (Chugh and Singh 1991; Varghese et al. 2001). Thus, thyroidal status may affect an organism's ability to respond to oxidative stress, or may determine the relative toxicity of pro-oxidant contaminants. In addition, perchlorate is a powerful oxidant, and so may itself induce oxidative stress in biological systems. Thus, there may be interaction between perchlorate and pro-oxidant compounds in inducing toxic responses. This hypothesis was tested by Liu et al. (2005), who exposed early-stage zebrafish larvae to sodium arsenate and sodium perchlorate. Arsenate was chosen because it is a model pro-oxidant that may co-occur with perchlorate in contaminated sites, and because several investigators (Lopez-Torrez et al. 2000, Das and Chainy 2001, Rahaman et al. 2001) have found that thyroid status modulates oxidative stress by arsenate in laboratory animals. Liu et al. (2005) found evidence that arsenate and perchlorate exhibited strictly additive toxicity, possibly because of similar, overlapping, or compensatory modes of action (e.g., they both may be pro-oxidants and thyroid-disrupting compounds).

Perchlorate and octylphenol

Recently, Cruz-Li (2004) completed a study examining the effects of perchlorate, octylphenol (a xenoestrogen), and their mixture on mortality and reproduction in zebrafish. For the mortality test, fish were exposed to 20 $\mu g \cdot L^{-1}$ AP and 3 $\mu g \cdot L^{-1}$ octylphenol (OP, nominal concentration), and a mixture of both during the embryo stage (0 to 3 d post fertilization [PF]), the larval stage (3 to 6 d PF), or the embryo–larval period (0 to 6 d PF). Mortality was noted during the exposure period and up to 30 d PF, (i.e., 24 to 27 d after exposure was ended). For all treatments (AP, OP, AP+OP), mortality was low (<20%) during the exposure period, but increased to approximately 60% to 80% after 20 d PF. Control mortality was approximately 50% to 60% at this time. For the fish that were exposed during the embryo and larval stages, no difference was found

among the treatments, but mortality was greater in treated fish than in control fish, especially at 20 d PF. This finding indicated that all treatments induced delayed mortality long after exposure ceased. For fish exposed during the embryo-larval period, the relative mortality increased in the order of control = AP < OP < AP+OP. Among the exposure regimes, the fish exposed during the entire embryo–larval period were least sensitive to AP, while fish exposed during the larval period were least sensitive to the mixture. For the reproduction tests, fish were exposed as above and allowed to reach sexual maturity in clean water (to test for latent effects). There were no differences among treatments for egg production, egg size, and fertilization success.

Predictive Physiologically Based Pharmacokinetic Models

Toxicokinetic models

A physiologically based toxicokinetic (PBTK) model was developed to simulate the movement of perchlorate within channel catfish (*Ictalurus punctatus*) organs (Albers 2004). Contaminant movement was governed by a series of mass-balanced differential equations programmed in Matlab. Model compartments and blood flow can be seen in Figure 7-2. General equations used in the model were taken from PBTKs developed by Nichols et al. for rainbow trout (1990, 1991) and channel catfish (1993). The rate of change for each of the 7 compartments (skin, kidney, muscle, GI tract, liver, gill, and thyroid) at individual time steps was obtained by solving the differential equations simultaneously using numerical integration. Fish gill physiology was kept biologically accurate by accounting for countercurrent chemical flux, including both flow and diffusion limitations (Erickson and McKim 1990b). Flow-limited distribution was assumed, that is, chemical equilibrium existed between the tissues and blood leaving the compartment. Additionally, portal blood flow was incorporated into the kidney and liver from poorly perfused tissue and richly perfused tissue, respectively. This represents the first and only PBTK model of perchlorate in fish.

Physiological parameters for blood flow and cardiac output in the channel catfish were obtained from existing literature (Hughes 1984; Erickson and McKim 1990a, 1990b; Nichols et al. 1990; McKim et al. 1994). Tissue blood flow was scaled by body weight to account for differences in literature and laboratory fish sizes. Tissue masses were measured in undosed juvenile channel catfish (Bradford 2002; Park 2003), with their

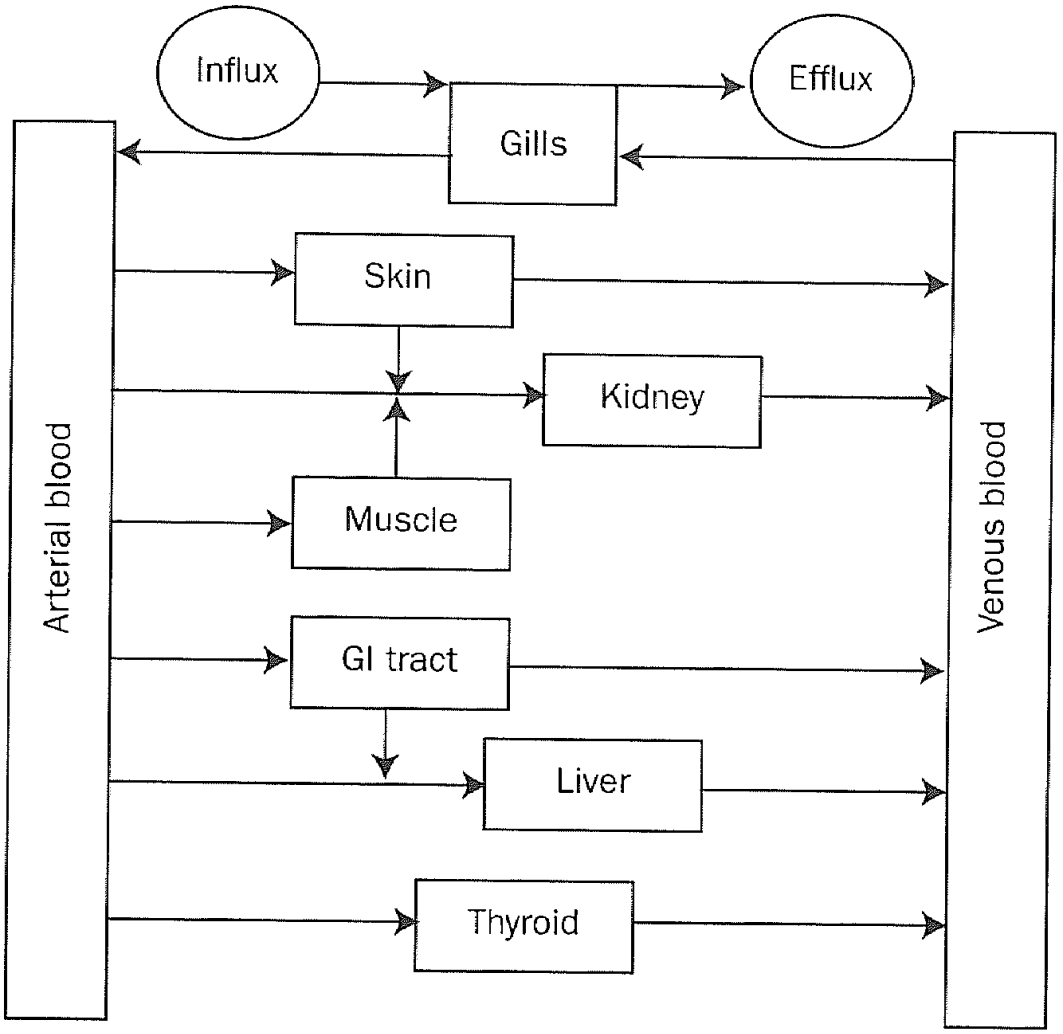

Figure 7-2 Flow diagram of the PBTK model for perchlorate inhalation in fish

averages used to determine their fractional mass. An equation for chemical flux at the gills (Erickson and McKim 1990b) was used to simulate uptake through inspired water. This method accounts for counter-current exchange with flows separated by a diffusion barrier made up of the gill epithelium and stagnant boundary layers in adjacent blood and water channels (Nichols et al. 1993). The PBTK model was calibrated using data from a 10-d elimination study in channel catfish (Park 2003) discussed previously (see "Uptake and toxicokinetics" above). By fitting the model to the elimination data, as well as to the uptake data, we were able to account for the lack of a urinary excretion term (Figure 7-3).

Thyroid hormone models

A 6-compartment model of combined T_3 and T_4 kinetics (Figure 7-4) originally developed for mammals (DiStefano 1986; Hershman et al. 1986; Bianchi et al. 1987; Pilo et al. 1990) and subsequently applied to rainbow trout (Sefkow et al. 1996) was used to simulate the secretion of

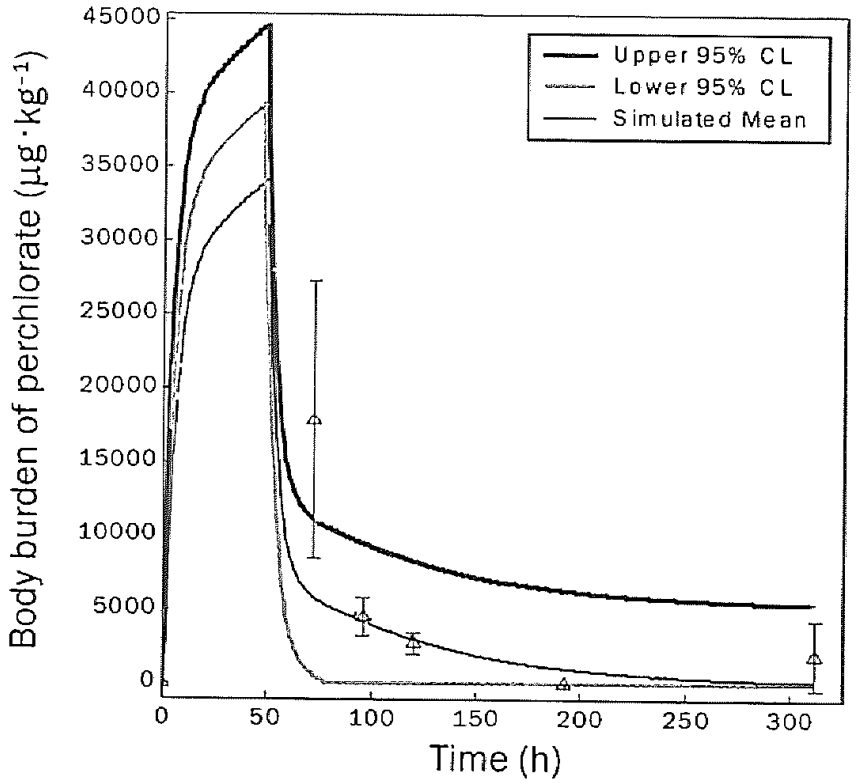

Figure 7-3 Calibration curve for thyroid compartment using elimination data

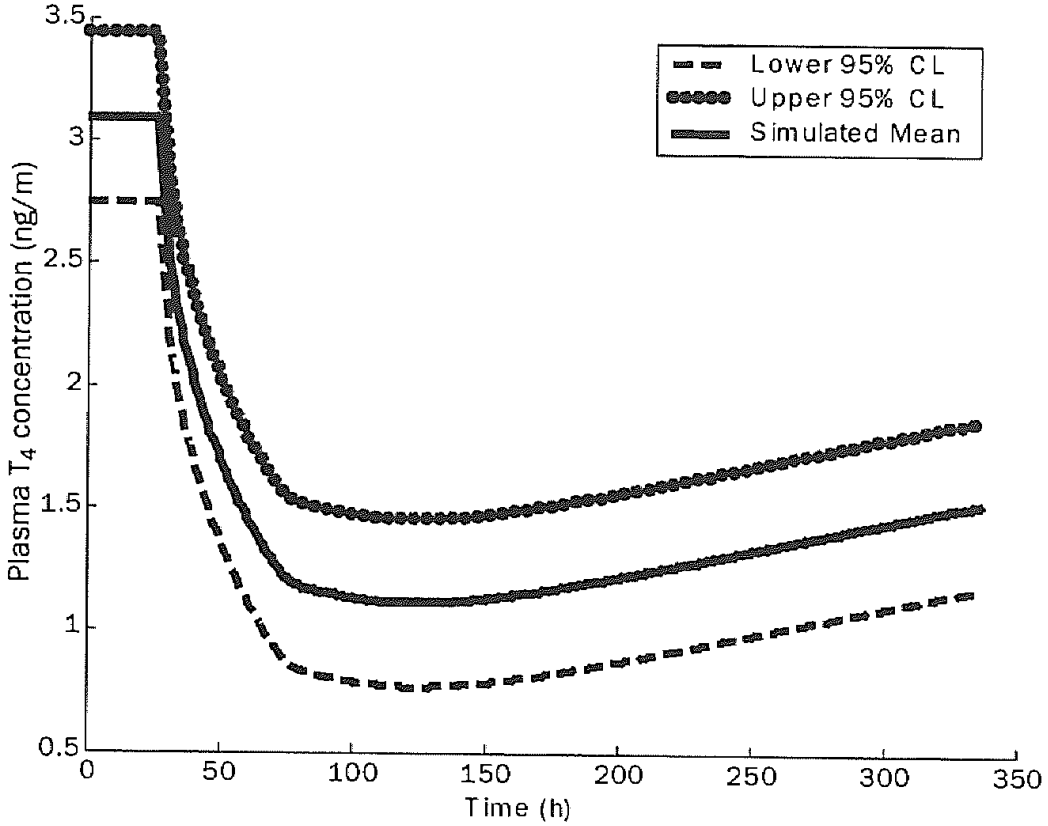

Figure 7-4 Simulated perchlorate-induced thyroid hormone inhibition in the T_4 plasma compartment based on thyroid tissue concentrations from Park (2003) 10-d elimination study

T_3 and T_4 in channel catfish, as well as the impact of perchlorate on the T_4 secretion rate (Albers 2004). The 6 compartments included

1) T_4 slow-exchange tissue pool,
2) T_4 rapid-exchange tissue pool,
3) T4 plasma pool,
4) T_3 slow-exchange tissue pool,
5) T_3 rapid-exchange tissue pool, and
6) T_3 plasma pool.

Skeletal muscle makes up the majority of the slow-exchange tissue pool. The rapid-exchange tissue pool is made up primarily of the liver but also of the kidney and gill, all of which possess deiodinating capacity (MacLatchy and Eales 1992; Eales and Brown 1993). Model parameters were reported by Sefkow et al. (1996) as either uniquely identifiable or determined within a range using the method of interval identifiability analysis (DiStefano 1983). Steady-state equations were developed by Sefkow et al. (1996) based on data from fasted rainbow trout injected with radiolabeled T_3 and T_4. Mass-balance ratios were used to calibrate the model, with plasma T_3 and T_4 compartments adjusted to meet levels reported by Gaylord et al. (2001) in channel catfish.

This model is a fairly simplistic representation of TH regulation in fish. It does not simulate the feedback loop and the related levels of TSH or corticotropin-releasing factor (CRF). The most notable drawback to this model is the lack of hormone pools. Fish, like mammals, possess stored TH pools that can be released even if iodide uptake is inhibited by perchlorate. As a result of these pools, there may be no observable effect of perchlorate exposure for some time, until the pool is depleted (Patiño et al. 2003). Until the nature of this pool is quantified, however, we are not able to incorporate that feature into the model.

T_4 hormone inhibition by perchlorate was calculated from data on mosquitofish dosed with sodium perchlorate for 2, 10, or 30 d at doses of 0, 0.1, 1, 10, 100, and 1000 $mg \cdot L^{-1}$ (Bradford et al. 2005). Whole-body T_4 concentrations were determined by radioimmunoassay for pooled groups of fish. The inhibition term was applied to the three T_4 compartments in the model (plasma, rapid exchange, and slow exchange) through adjustment of the T_4 secretion rate term.

The model was applied to the 10-d elimination study scenario to test its ability to rebound once perchlorate is removed from the system (Figure 7-4). The three T_4 compartments exhibited the greatest decrease in hormone levels (~51%) with a concomitant decrease in the T_3 compartments

(-16%) as expected. We saw a rapid decrease in hormone levels during exposure, followed by a gradual increase. Because the thyroid model is initiated with the simulated thyroid tissue concentrations from the PBTK model, hormone levels fluctuate with thyroid perchlorate concentrations. Evidence of this relationship can be seen in the plots of hormone concentrations that are the inverted shape of the plots of tissue data. While hormone recovery is evident once perchlorate exposure ceases, we do not know if the recovery rate is accurate.

In general, however, the modeled results do not coincide with actual results in other species to date. In the model, T_4 concentrations decrease by more than 3-fold within 3 d of exposure to perchlorate (Figure 7-4). In other fish species tested to date, much longer chronic exposures are needed to effect such reductions, and the effects are not this dramatic (Bradford et al. 2005). This result may be due to the lack of a model to accommodate TSH, feedback loops, or colloid storage of thyroglobulin. Until these issues are resolved, however, the utility of this model remains uncertain.

Data Gaps and Future Research Needs

Although there has been considerable research on the biological effects of perchlorate, there are still many gaps in the knowledge of its ecological risk. For example, more information is needed regarding the effects of perchlorate on fitness parameters. Although no adverse effects of environmentally relevant concentrations of perchlorate on growth or reproduction have been demonstrated in any fish species, the available data are insufficient to make generalizations, especially for the early life stages that are typically more sensitive to disruption by environmental contaminants. Accordingly, early life-stage and full life-cycle or partial life-cycle tests are necessary for adequate analyses of risk assessment. In mammals, TH disruption also has been implicated in male reproductive effects, including effects on sperm production, testicular function, and steroid synthesis (Buzzard et al. 2003; Krassas and Perros 2003; Maran 2003), but such effects have not been examined in fish.

Although it is known that perchlorate may affect the amount of THs produced by adult fish, the potential for perchlorate to cause trans-generational effects in fish has yet to be investigated. Embryonic fish require TH for development but generally do not synthesize the hormone themselves until after hatching. THs in embryos are supplied via maternal transfer to the eggs. If thyroid synthesis is disrupted in the mother, it may affect her ability to transfer THs to her eggs and thus affect embryonic develop-

ment. Although perchlorate may affect the amount of THs produced by adult fish, especially after prolonged exposures, the effects of perchlorate-induced thyroid endocrine disruption on maternal transfer of THs to eggs, and potential consequent effects on fish embryo development, are matters that have yet to be resolved.

Another potential area for future research is examination of alternative physiological fitness endpoints of perchlorate exposure. For example, embryonic neuronal development is controlled by THs (Denver et al. 1997); thus, it is possible that perchlorate-induced thyroid disruption could result in neurobehavioral effects, but this has not been investigated. In addition, perchlorate exposure and thyroid suppression have been found to suppress the immune system in amphibians (Rollins-Smith 1993; Weetman 1994), and THs have been found to affect immune function in fish as well (Harris and Bird 2000), but the potential effect of perchlorate on fish immune function has not been explored. Finally, possible effects of environmentally relevant concentrations of perchlorate on other thyroid-responsive physiological endpoints (e.g., lipid metabolism, temperature acclimation, osmoregulation, pigmentation, mitochondrial function, embryo or larval development) and expression of thyroid-responsive and fitness-related genes merit further attention. In particular, studies on thyroid-responsive gene expression could be used to provide

1) a method for distinguishing between pathological effects and compensatory responses and
2) tools for studying thyroid disruption in embryos, larvae, and early juveniles, which are too small for conventional TH assays.

In a related light, studies that examine interactions between THs and other endocrine-, exocrine-, and paracrine-signaling pathways are an important consideration. For example, because growth hormone gene expression may be controlled or influenced by TH status, does perchlorate affect growth hormone levels? Other potential effects could be less direct via cross talk between TH and sex hormone, corticosteroid-, and retinoid-signaling pathways.

An additional data gap that needs to be addressed is the interaction between perchlorate and naturally occurring and anthropogenic chemicals. The most relevant naturally occurring chemical is iodide or other oxidative states of iodine (e.g., iodate) because availability of iodine in the water could be critical for determining the magnitude of perchlorate-induced effects. In terms of anthropogenic chemicals, it is not known if perchlorate affects the toxicity, environmental chemistry and fate, toxicokinetics, or detoxification of other toxicants, and vice versa. Potential mechanisms for

such interactive effects could include redox reactions between chemicals, thyroid-mediated changes in metabolism of chemicals, or nonthyroidal effects on fish physiology. Finally, the toxicity and behavior of perchlorate in complex mixtures is unknown.

In terms of PBPK and TH models, more work needs to be done on the testing, validation, refinement, and sensitivity analysis of the models. In particular, inclusion of components for tissue- or compartment-specific peripheral deiodination, metabolism, excretion, and storage (especially storage in follicle colloids) would greatly enhance utility, realism, and accuracy of thyroid models. The models could also be expanded to include molecular, receptor- or ligand-based, toxicodynamic, and computational approaches.

Finally, more field studies need to be conducted to properly assess the ecotoxicological risk of perchlorate. These may include microcosm or mesocosm studies or field surveys to further characterize environmental fate of perchlorate and pathways of exposure for organisms in aquatic systems. Such studies would provide answers to questions that are critical for assessing ecological risk of perchlorate. For example, to what extent do each of the potential pathways and routes of exposure play in the exposure and effects of perchlorate in fish? Also, what are the possible indirect effects of perchlorate on fish populations? Examples of indirect effects include

1) perturbations of aquatic food webs resulting from toxic effects on plankton or macroinvertebrates,
2) differential sensitivities to perchlorate among species, and
3) interactions between perchlorate and natural variables or stressors (e.g., pathogens, salinity, pH, temperature, dissolved oxygen, suspended sediment, ultraviolet radiation, food availability).

Other manipulative or survey-type studies are needed to determine the extent of perchlorate exposure on reproduction and development in field situations, and the effects of perchlorate contamination on populations or communities of fish. Until these issues are resolved, it will be difficult to perform a complete ecological risk assessment of the effects of perchlorate in natural environments.

Conclusions

Research to date indicates that there is little risk to native fish populations due to acute toxic effects of perchlorate, but that chronic exposure to perchlorate in the laboratory and the field can result in structural and physiological perturbations to the fish thyroid gland tissue and its hor-

mones. Perchlorate does not bioconcentrate in fish tissues in laboratory settings, but measurement of water concentrations alone is insufficient to predict body burdens or toxicological effects in field situations. This discrepancy between field and laboratory studies may be due to complex multiple exposure pathways and/or highly variable perchlorate water concentrations in time and space. At the present time, there is no evidence of perchlorate-induced effects such as growth, survival, and reproduction at environmentally relevant concentrations. However, further studies are needed before sound risk assessments of perchlorate are possible. Full life-cycle and multigenerational studies will be necessary to fully understand the ecological effects of perchlorate on individuals and populations of fish. The models developed and under development have potential applications for calculating probability exposure or effects distributions for use in probabilistic risk assessments (see Chapter 10). To date, evidence for detrimental effects of perchlorate on ecologically relevant fitness parameters at environmentally relevant concentrations remains equivocal. However, because of the limited number of species tested, limited number and types of chronic tests, paucity of information on behavior and toxicity of perchlorate in complex mixtures, and the fact that THs play a part in so many critical aspects of fish physiology, further studies are needed before comprehensive risk assessments of perchlorate can be carried out.

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