U.S. Army Center for Health Promotion and Preventive Medicine

USCHPPM

Wildlife Toxicity Assessment for Perchlorate

FINAL REPORT
FEBRUARY 2007

Prepared by
Health Effects Research Program
Environmental Health Risk Assessment Program

USACHPPM Document No: 87-MA02T6-05D

Readiness Thru Health

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Approved for Public Release; Distribution Unlimited

Acknowledgements

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When referencing this document use the following citation:
USACHPPM. 2007. Wildlife Toxicity Assessment for Perchlorate. U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM) Project Number 87-MA02T6-05D, Aberdeen Proving Ground, Maryland.

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Department of the Army
U.S. Army Center for Health Promotion and Preventive Medicine

Wildlife Toxicity Assessment for Perchlorate

CAS No. 7790-98-9, 7601-89-0, and 7778-74-7 **February 2007**

1. INTRODUCTION

This Wildlife Toxicity Assessment is based on a thorough review of the scientific literature regarding the toxicological characteristics of perchlorates that may pertain to the health of wildlife (mammals, birds, reptiles, and amphibians) exposed to these substances. Perchlorate salts are used as oxidizers in solid rocket propellants and munitions. They have been discovered in groundwater around military installations, fireworks and munitions manufacturing facilities, and in the groundwater. Although the military uses large quantities of perchlorate salts, their uses are not strictly related to munitions and solid rocket propellants. Perchlorate can be used as an etching and engraving agent; it can also be used in paper matches, fireworks, and automobile air bags. Until the 1970s, perchlorate was used to treat specific types of hyperthyroid conditions at very high dose levels in the United States and is still used for this purpose in Germany (Von Burg 1995).

This report assesses the current knowledge of the toxic effects of perchlorate ions (ClO_4^-). The protocol for the development of this assessment is documented in the U.S. Army Center for Health Promotion and Preventive Medicine Technical Guide 254, the Standard Practice for Wildlife Toxicity Reference Values (USACHPPM 2000).

2. TOXICITY PROFILE

2.1 Literature Review

Relevant biomedical, toxicological, and ecological databases were electronically searched in late November and early December 2003 to identify primary reports, studies, and reviews of perchlorate toxicology. These searches were focused on finding effects and exposure information relevant to terrestrial wildlife (vertebrate) species. The Defense Technical Information Center also was searched for relevant U.S. Department of Defense reports. Secondary sources reviewed included: Perchlorate Study Group's perchlorate literature review (ERM 1995); U.S. Air Force perchlorate literature review (Sterner and Mattie 1998); U.S. Air Force perchlorate ecological risk studies (Callahan and Sprenger 1998); and EPA's perchlorate toxicological review (USEPA 2002). In early 2004, additional perchlorate studies were located in birds (McNabb et al. 2004) and amphibians (Dean et al. 2004). Recommendations from external reviewers in early 2006 suggested that another, more recent search be conducted to capture the

more current, rapidly published material. Consequently, another literature review was conducted in June of 2006. Separate searches were carried using the keyword "perchlorate" and laboratory mammals, birds, reptiles and amphibians, or wild mammals. All available abstracts of those articles were evaluated for relevancy as being appropriate for Toxicity Reference Value (TRV) derivation. For perchlorate, 24 articles were selected for retrieval from the initial 99 hits during the 2006 search. Of those, twelve were not relevant in that they addressed exposure and not effects, fate and transport, dealt with aquatic organisms exclusively, were review articles, or were already cited. Additionally, one Chinese paper could not be obtained. In late 2006, two additional papers were found and incorporated. Since the incorporation of these data, the external reviewers were asked to review the document once again for accuracy. Details of the search strategy and the results of the search are documented in Appendix A.

2.2 Environmental Fate and Transport

A perchlorate anion consists of a chlorine (oxidation number of +7) surrounded by four oxygen (oxidation number of -2) atoms to form an oxychlorine anion (-1 charge). Perchlorate is a very stable anion that forms salts with cations such as sodium, potassium, and ammonium. Salts of perchlorates have high solubility in non-aqueous (e.g. soil) and aqueous environments (USEPA 2002; Table 1). Although no specific information on degradation rates (half-lives) was located, ionic perchlorate reportedly can persist in surface and groundwater for more than a decade (Callahan and Sprenger 1998). This is because of the high kinetic barrier for perchlorate to react with other constituents in water (Callahan and Sprenger 1998). Perchlorate salts are stable, powerful oxidants when concentrated. It is for this reason that ammonium and other perchlorate salts are used in solid rocket propellants, fireworks, and munitions.

A major pathway for perchlorates to enter the environment is during manufacturing and recharging of munitions and solid rocket motors. These activities represent the primary release mechanisms of perchlorate to the environment, and have resulted in perchlorate contamination of groundwater at many military installations and rocket manufacturing facilities (Callahan and Sprenger 1998, Sterner and Mattie 1998). Other anthropogenic sources of perchlorate include Chilean nitrate fertilizers, fireworks, safety flares, blasting explosives, and electrochemically-prepared (ECP) chlorine products (GeoSyntec Consultants 2005).

Perchlorates have been found in biological tissue at sites with concentrations found in the soil and surface water. Smith et al. (2004) found elevated concentrations in vegetation, but rarely in rodent tissue. Cows drinking from a perchlorate-contaminated stream were found to have very low levels in plasma, but exhibited no change in circulating thyroid hormone levels (Cheng et al. 2004). Results of a market basket survey in the Colorado River region found that traces of perchlorate in leafy vegetables, but at levels that

would result in < 10% of the reference dose recommended by the National Academy of Sciences (Sanchez et al. 2005a&b). A controlled laboratory study investigating food chain transfer found that perchlorate has a limited ability to bioaccumulate in aquatic systems (Park et al. 2005).

Key physicochemical properties of the three most common perchlorate salts, estimated using EPI-Suites 2000 Software (developed by the United States Environmental Protection Agency [USEPA]), and are provided in Table 1. Because the three perchlorate salts are estimated to have low vapor pressures (3.96×10^{-26} - 4.34×10^{-18} mm Hg at 25 °C; [USEPA 2000]), partitioning to air will be limited. Additionally, all are highly soluble in water (0.15×10^{5} to 21×10^{5} mg/L at 25 °C) and have been identified in both surface and groundwater.

Table 1. Summary of Physical-Chemical Properties of Perchlorate

Physical Property	Cations associated with Perchlorate anion		
	$NH_4(ClO_4)$	**$Na(ClO_4)$**	**$K(ClO_4)$**
CAS No.	7790-98-9	7601-89-0	7778-74-7
Percent Perchlorate Ion by Weight	84.67%	81.22%	71.8%
Molecular weight	117.49	122.44	138.54
Color	White	White	White
Physical state	Crystalline solid	Crystalline solid	Powder
Melting point[a]	266.8 °C	302.6 °C	302.6 °C
Boiling point[a]	616.0 °C	692.7 °C	692.7 °C
Odor	No data	Odorless	Odorless
Solubility in water at 25 °C (g/L)[b]	2.1×10^{2}	2.0×10^{2}	15×10^{1}
Partition coefficients [a]:			
Log K_{ow}	-5.84	-7.18	-7.18
Log K_{oc}	1.985	1.687	1.687
Henry's Law constant at 25 °C (atm-m^3/mole)[a]	2.8×10^{-11}	9.15×10^{-19}	2.17×10^{-19}
Vapor pressure at 25 °C (mm Hg)[a]	4.34×10^{-18}	1.47×10^{-25}	3.96×10^{-26}
Conversion factors	1 ppm = 4.80 mg/m^3 1 mg/m^3 = 0.208ppm	1 ppm = 5.0 mg/m^3 1 mg/m^3 = 0.20ppm	1 ppm = 5.66 mg/m^3 1 mg/m^3 = 0.177ppm
(% is ClO_4^- ion by weight)	(84.7%)	(81.2%)	(71.8%)

[a]Values estimated with EPA EPI Software (USEPA 2000)

[b]HSDB 2006

2.3 Summary of Mammalian Toxicity

Published toxicological studies on perchlorates have focused primarily on laboratory mammals such as rats and mice. This is partly due to the medical interest in perchlorate's ability to block iodine uptake and thus prevent hyperthyroidism (Grave's disease). Most toxicity evaluations of perchlorate focus on the levels of thyroid hormones, triiodothyronine (T3) and thyroxine (T4), because it is believed that deficiencies of these hormones affect growth and development, as well as metabolism, in animals. (Note: a glossary of terms specific to perchlorate effects is presented in Appendix B). As part of the homeostatic mechanism, thyroid-stimulating hormone (TSH) secreted by the pituitary would increase with decreased levels of T3 and T4 (T3, T4, and TSH are often measured concurrently). Virtually all vertebrates have thyroid glands (Callahan and Sprenger 1998). However, few studies were found that included testing of thyroid hormone levels in wildlife species. The evaluation of perchlorate toxicity relies mostly on studies in common laboratory animals (mice, rats, and rabbits).

Due to the high solubility of perchlorate salts and the ubiquitous environmental distribution of their associated cations (Na^+, K^+, NH_4^+), this toxicological review assumes that the contribution from the cations to overall toxicity is negligible. This may not be true for aquatic (or semi-aquatic, e.g. amphibian species). All observed effects are mostly assumed to be associated with the perchlorate anion (ClO_4^-). Additionally, all dose levels presented in the text and Tables 2 and 3 have been converted and are expressed in terms of perchlorate anion concentrations; calculations for the conversions are presented in Appendix C.

2.3.1 Mammalian Oral Toxicity

2.3.1.1 Mammalian Oral Toxicity - Acute/Subacute

In a brief review of perchlorate toxicity, Von Burg (1995) noted that acute mammalian toxicity data were limited. The oral LD_{50} of ammonium perchlorate in white rats was reported to be 3556 mg/kg perchlorate (Shigan 1963 cited in ERM (1995))[1]. It is important to note that these levels approach many limit test constraints (Table 2). In general, it appears that rabbits are more sensitive to the acute effects of perchlorate than rodents.

[1] Note that the study may report additional clinical symptoms. However, because the text is in Russian, additional interpretation was not possible.

Table 2. Summary of Median Lethal Dose (LD_{50}) Data for Perchlorate Salts [a]

Species	Route of Administration	Chemical Form	Perchlorate Ion Concentration[b] (mg/kg)
Rabbit	Oral	NH_4ClO_4	635 – 1610
Mouse	i.p.	$NaClO_4$	934
Mouse	Oral	NH_4ClO_4	1610 – 1690
Guinea Pig	Oral	NH_4ClO_4	2800
Rat	Oral	NH_4ClO_4	2960 – 3560

i.p. = intraperitoneal
[a] Source: Von Burg (1995)
[b] Perchlorate ion is determined by multiplying the concentration of perchlorate salt by percent perchlorate ion by weight (Table 1).

In addition to the derived LD_{50}, Mannisto et al. (1979) dosed Sprague-Dawley rats through drinking water for four days at 0, 7.6, 15.3, and 76.3 mg/kg-d perchlorate ion. At the end of the 4th day, T3 and T4 in blood serum were found to be depressed, while TSH was elevated at 15.3 mg/kg-d perchlorate ion (Mannisto et al, 1979). Nervous system effects were reported in rabbits exposed for 3 months at 190 mg/kg-d, yet no other details were provided (Von Burg 1995). No direct references could be found.

Khan et al (2005) investigated the effects of short-term repetitive exposures of perchlorate and chlorate in male Fischer 344 rats. One-hundred and sixty rats were evenly distributed into one of 16 treatments and were exposed to various combinations of ammonium perchlorate (AP) and/or sodium chlorate (SC) or a control via the drinking water for seven days. Analytical data regarding water concentrations and drinking rates were measured. Hypertrophy and colloid depletion of the thyroid was observed in the high dose AP treatment (8.7 ml/L, 1.2 mg/kg-d), most treatments containing sodium chlorate (> 0.5 mg/L or 0.69 mg/kg-d) and all groups containing mixtures of these compounds (Khan et al. 2005). Lower serum T4 concentrations were found in all mixtures having greater than 0.36 mg/L AP and 57 mg/L SC; however, T3 levels were not different across treatments. Of concern is that SC levels were detected in the control water of 0.5 mg/L. No changes in body weight, drinking rates, or any other clinical signs of toxicity were observed.

Keil et al. (1999) exposed female B6C3F1 mice to calculated doses of AP equivalent to receiving 0, 0.1, 1.0, 3.0, or 30 mg/kg-d through the drinking water for 14 or 90 days. Doses were within 10% of targets. Thyroid histology, systemic thyroid hormone levels, organ and body weights, and several immunological indices were endpoints of concern. Immunological indices included cellularity of lymphoid organs, CD4/CD8 thymic and splenic subpopulations, stem cell synthesis, natural killer cell activity, cytotoxic T-cell activity, nitrite production of peritoneal macrophages, phagocytosis, IgM and IgG antibody response to T-dependant antigen (sheep red blood cell), delayed type hypersensitivity,

melanoma tumor challenge model, and an antinuclear antibody screening assay. Changes in T4 levels, but not T3 or TSH were found respective to treatment (3 and 30 for 14-days, 1, 3, and 30 following 90-days). Effects in the immunological assays produced mixed results with few significant or dose-related outcomes. However, changes in delayed-type hypersensitivities were observed at the 30 mg/kg-d dose for mice at durations of both 14 and 90-days.

2.3.1.2 Mammalian Oral Toxicity - Subchronic

EPA (Crofton 1998) re-analyzed the subchronic 14-day and 90-day exposure data supplied by the Air Force Research Laboratory/Operational Toxicology Branch (AFRL/HEST) and originally analyzed by Springborn Laboratories (1998). In the re-analysis, Crofton (1998) compared the interaction between gender, time, and dose levels. Sprague-Dawley rats in groups of 9 to10 per treatment per sex were exposed to different doses of ammonium perchlorate via drinking water over a period of 90days followed by a 30-day recovery period (no perchlorate exposure). Thyroid hormone levels were measured at 14 and 90 days, and measured again after the 30-day recovery period. Using data for the 14-day subchronic ammonium perchlorate exposure (perchlorate ion levels of 0, 0.00847, 0.0423, 0.169, 0.847, and 8.47 mg/kg-d), Crofton (1998) reported that NOAELs for both males and females for T4 and TSH effects were 0.847 mg/kg-d and 0.00847 mg/kg-d perchlorate ions, respectively. The effect of perchlorate on T3 serum levels in males differed statistically from that in females. The lowest perchlorate ion dose (0.00847 mg/kg-d) reduced T3 levels in male rats. This dose was considered an unbounded LOAEL. In contrast, T3 levels in female rats were statistically unchanged at all dose levels; consequently, the 8.47 mg/kg-d dose (as perchlorate ion) was considered an unbounded NOAEL. Because the experiment continued to 90-days followed by a 30-day recovery period, additional discussion of the study is presented in Section 2.3.1.3.

The data from the 90-day exposure study were evaluated for interactions between gender, time, and perchlorate dose levels. Sprague-Dawley rats in groups of 9 to10 per treatment per sex were exposed to different doses of ammonium perchlorate in drinking water over a period of 90 days. Thyroid hormone levels were measured at 90 days, and measured again after a 30-day recovery period in which there was no perchlorate exposure. Crofton (1998) reported an unbounded LOAEL of 0.00847 mg/kg-d for both male and female rats based on decreases in T3 and T4. Although Crofton (1998) could not determine a NOAEL for T3 and T4, a LOAEL of 0.0423 mg/kg-d based on increased TSH levels in both sexes was calculated.

In another subchronic study, King (1995) reported significant gender differences in sensitivity to ammonium perchlorate. Ammonium perchlorate was administered to Spague-Dawley rats at eight

different doses from 0 to 18.8 mg/kg-d perchlorate ion for a period of 14 days. The following endpoints were measured at the end of the study: (1) thyroid/body weigh ratio, (2) TSH level, (3) water ingestion rate; (4) thyroglobulin (Tg) level, and (5) T3 level. The NOAEL and LOAEL levels are described below:

- Thyroid/body weight ratio - There were no statistical differences in thyroid/body weight ratios between male and female rats; thus, sexes were combined. The NOAEL, based on an increased thyroid/body weight ratio, occurred at 3.66 mg/kg-d; the corresponding LOAEL was 9.69 mg/kg-d.

- Water Ingestion rate - Water consumption was not influenced by the concentration of perchlorate administered; however, there was a difference in water consumption between males and females. Male Sprague-Dawley rats had higher water consumption rates (~40 ml/d) compared to females (~28 ml/d).

- Thyroglobulin (Tg), T3, and T4 Levels - Iodized Tg is required to synthesize monoiodo-L-thyronine (MIT) and 3,5-diiodo-L-thyronine (DIT), which are combined to produce T3 and T4. Although King (1995) measured the level of Tg in blood serum of Sprague-Dawley rats after exposure to ammonium perchlorate for 14 days, he did not perform further analysis on the threshold dose. This was because Capen (1997) demonstrated that "the lowest dose which lowers T3 and/or T4 and simultaneously increased TSH could be considered a threshold dose". Therefore, the statistical analyses focused on T3 and TSH levels. T4 hormone level, considered to be as important as T3 (Capen [1997] cited in King [1995]), did not exhibit a significant dose-response relationship, and therefore, was not discussed in further detail.

- T3 Level – Circulating T3 levels were measured in eight groups of 12 Sprague-Dawley rats (6 males, 6 females). Although T3 level decreased with increased perchlorate dose, T3 hormone level decreased in female rats at a lower dose than in males. The unbounded LOAEL for female rats was 0.105 mg/kg-d. In male rats, the NOAEL was 0.0931 mg/kg-d and the corresponding LOAEL was 0.375 mg/kg-d.

- TSH Level – Among female rats, the NOAEL and LOAEL for elevated TSH levels were 0.105 and 0.395 mg/kg-d, respectively. For male rats, the NOAEL and LOAEL for elevated TSH were 0.375 and 0.942 mg/kg-d perchlorate ion, respectively.

Isanhart et al (2005) exposed five prairie voles (*Microtus ochrogaster*) to target ammonium perchlorate (AP) exposures of 0, 1, and 10 mg/kg-d for 51 and 180 days (see Section 2.3.1.3). Voles were exposed to AP in the drinking water that resulted in average perchlorate ion exposures of 0, 1.13,

and 9.89 mg/kg-d. The purpose of this study was to measure changes in oxygen utilization (energy metabolism) by measuring oxygen consumption under normal resting and cold stress conditions (12°C for 20 minutes followed by a drop to 5°C until voles became hypothermic). Perchlorate exposure did not affect metabolism (oxygen consumption relative to subchronic AP treatments and controls). No differences were found in oxygen consumption (metabolic rates) between treatments and durations in voles during the resting phase (room temperature) or in voles cold challenged (Isanhart et al. 2005). No differences were found in plasma T4 or T3 concentrations between treatment groups; however, T4 concentrations per thyroid weight were different between controls and high dose treatments. Mean kidney weights were lower for the high dose compared with the low and controls; however, the clinical significance of that finding is uncertain. There were no changes in liver weights relative to controls, nor were any other clinical signs of toxicity observed.

2.3.1.3 Mammalian Oral Toxicity - Chronic

Chronic exposure to rats was studied by Kessler and Kruskemper (1966, original text in German and cited in ERM 1995). Groups of 40 rats were provided drinking water containing perchlorate at 1% weight per volume (w/v; approximately 513 mg/kg-d). Body and thyroid gland weights were the measurement endpoints. At the end of 0, 40, 120, 220, and 730 days, groups of 7 to 8 rats were sacrificed and body weight and thyroid gland measurements were made. Although no statistically significant differences were found in body weight for any of the exposure periods, statistically significant changes in thyroid histology were observed starting at day 40. Observed thyroid pathologies developed into fibroses and follicular adenomas as the experiment progressed.

In the second part of a two-part study, Isanhart et al. (2005) exposed five prairie voles (*Microtus ochrogaster*) to ammonium perchlorate in the drinking water that resulted in average perchlorate ion exposures of 0.79 mg/kg-d for 180 days and measured oxygen consumption under cold stress conditions (12°C for 20 minutes followed by a drop to 5°C until voles became hypothermic). Perchlorate exposure did not affect metabolism (oxygen consumption relative to subchronic AP treatments and controls). No differences were found in oxygen consumption (metabolic rates) between treatments and durations in voles during the resting phase (room temperature) or in voles cold challenged (Isanhart et al. 2005). No difference was found between in plasma T4 concentrations from voles exposed to 0.79 mg/kg-d for 180 days and those exposed to 1.12 mg/kg-d for 51 days. No changes were found in kidney or liver weights relative to controls, nor were any other clinical signs of toxicity observed.

2.3.1.4 Mammalian Oral Toxicity - Other

Another study observed thyroid hypertrophy in rabbits following perchlorate exposure (Lampe et al. 1967, in German and cited in ERM 1995). Rabbits were given a dose of 71.8 mg/kg-d perchlorate ion via diet from conception through day 21 or 28 days of gestation. Fetal thyroid weights in the experimental group were approximately four times higher than the control weights, while the maternal thyroid weights in the experimental group were three times higher than the controls (ERM 1995).

Perchlorate effects on thyroid weight and deciduoma formation (uterine deciduoma are formed in pseudopregnant dams, similar to decidua in normal pregnancy) were investigated by inducing false-pregnancy in female Wistar rats (Brown-Grant 1966). Female rats were administered 0.25% and 1.0% KClO4 in drinking water for 7 days (day 2 through 8 of gestation). Because exposure occurred during gestation these data were considered representative of a chronic exposure. At the end of the 7-day exposure period, thyroid weights were significantly increased at exposures of 1.0% w/v (599 mg/kg-d perchlorate ion); deciduoma formation was unaffected by either exposure level.

In a related study, Brown-Grant and Sherwood (1971) performed a two-generation study of potassium perchlorate in rats. The dams were dosed with 1740 mg/kg-d perchlorate ion at the termination of pregnancy with sacrifice of the pups on *post-partum* day 9. Relative thyroid weight for both the dams and pups were significantly different from that of the controls. Because of the single treatment group, only the unbounded LOAEL (1740 mg/kg-d perchlorate ion) was determined.

Postel (1957; cited in Sterner and Mattie 1998) administered potassium perchlorate to female guinea pigs at 528.5 mg/kg-d (converted from 1% w/v) during the final 21 to 48 days of gestation for a total of 27 days. This investigation is considered a chronic exposure because it was administered during a sensitive life stage of the species and fetal development was potentially affected. Female guinea pigs received perchlorate via drinking water and subcutaneous injections of 0, 8, 18 or 32 μg T3 supplement per day. The control groups received 257 mg/kg-d perchlorate ion in drinking water and saline solution in the subcutaneous injection. Although maternal thyroid weight and histology were not affected, fetal thyroid weight averaged fifteen times greater than the controls. The authors reported that the T3 supplements at all levels did not appear to have mitigated the effects of perchlorate and were suspected to have intensified observed effects.

York et al. (2001a) presented a toxicology evaluation of perchlorate based on a report to the Perchlorate Study Group by Argus Research Laboratories (Argus 1998a). In this study, Sprague-Dawley rats were administered ammonium perchlorate at levels of 0.254, 2.54, and 25.4 mg/kg-d perchlorate ion over two generations. P1 generation rats were exposed to perchlorate over 140 days for male and at least 126 days for the females. F1 generation rats were exposed to perchlorate for 124 days for male and at

least 90 days for females. Because of the extended duration of perchlorate exposure, the P1 and F1 generations qualify as chronic studies. York and coworkers (2001a) evaluated the effect on the male and female reproductive systems, growth and development of F1 offspring, neonatal morbidity, mortality and potential prenatal developmental toxicity. York et al. (2001a) considered 2.54 mg/kg-d perchlorate ion as the LOAEL, the lowest dose that resulted in a statistically significant increase in thyroid hypertrophy/hyperplasia in the male and female F2 generation, the female F1 generation pups, the male F1 generation adults, and the male and female P generation rats. Consequently, the next lowest level, 0.254 mg/kg-d perchlorate ion was considered the NOAEL**.**

The level at which a reproductive effect from perchlorate exposure becomes significant is equivocal. York et al (2001a) concluded that ammonium perchlorate ion is not a reproductive toxicant in rats at doses as high as 25.4 mg/kg-d; no deaths, abortions or premature deliveries could be attributed to this level of exposure to perchlorate in rats. However, Argus (2000) (a subset of these data were published in York 2001a) reports developmental effects including significantly reduced ossification sites per litter for sternal centers and forelimb phalanges at 25.4 mg/kg-d perchlorate ion. Although these are considered reversible developmental delays and not biologically significant, USEPA (2002) argues that "permanent or reversible" delays in development cannot be discounted as they may be a "potential indicator of developmental toxicity". USEPA suggests 25.4 mg/kg-d perchlorate ion as a LOAEL with a corresponding NOAEL of 2.54 mg/kg-d for developmental toxicity. USEPA (2002) acknowledges that a definitive assessment cannot be made on the available data, with the findings of Argus (2000) important considering the available data and the mode of action for perchlorate.

In a companion study on rabbits, York et al. (2001b) presented a toxicology evaluation of perchlorate to the Perchlorate Study Group as Argus Research Laboratory (Argus 1998b). York et al. (2001b) evaluated the maternal and embryo-fetal toxicity potential of ammonium perchlorate via drinking water. Female rabbits were exposed to ammonium perchlorate during gestation for a total of 23 days (gestation day 6 through 28). Considering the exposure occurred during a critical life stage of the species, this test is considered equivalent in value to a chronic study. Adult female rabbits were dosed at 0, 0.0847, 0.762, 8.81, 25.7, and 86.7 mg/kg-d perchlorate ion. For hypertrophy of the thyroid gland, the NOAEL level for adult female rabbits was 0.762 mg/kg-d perchlorate ion; the LOAEL level was 8.81 mg/kg-d perchlorate ion. York et al. (2001b) reported no adverse effects of ammonium perchlorate on fetal development.

Subsequent to the 2001 studies, York et al. (2004) investigated the neurodevelopmental effects of ammonium perchlorate in exposed adults and offspring of Sprague-Dawley rats. In this study, mated rats were exposed to 0, 0.1, 1.0, 3.0 and 10 mg ammonium perchlorate/kg-day (0, 0.0847, 0.847, 2.54, and 8.47 mg perchlorate ion/kg-d) in drinking water from gestation day 0 (mating) through lactation

(postpartum) day 10. Because this represents a critical life stage of the species and the affected endpoint is potentially relevant to fetal development, the data from this study is considered equal in weigh from data of chronic duration. Neurodevelopmental effects in offspring (evaluated by passive avoidance, watermaze, motor activity, and auditory startle testing) were not observed at any dose group. Also, there were no observed differences in brain weights, morphometry, neuropathology, body weights, feed consumption, clinical signs, or sexual maturation of pups. In dams, no effects were observed at parturition, clinical signs, body weights, or feed and water consumption rates.

As with previous studies, thyroid toxicity was observed in the offspring, although there were no observations of follicular hypertrophy or hyperplasia in dams (York et al., 2004). F1 generation pups culled at 5 days postpartum exhibited significantly different T3, T4, and TSH levels in the 0.847, 2.54, and 8.47 mg/kg-d perchlorate ion dose groups compared to controls. There was a non-significant increase in the incidence of follicular cell hypertrophy at all dose levels, with pronounced hypertrophy at the highest dose in male pups and the two highest doses in female pups. Other thyroid effects included a dose-related decrease in thyroid follicular lumen diameter that was significant at 0.847, 2.54, and 8.47 mg/kg-d perchlorate ion dose groups for males, and 2.54 and 8.47 mg/kg-d perchlorate ion for females. In addition, lumen area was decreased in the male 2.54 and 8.47 mg/kg-d perchlorate ion dose groups and female 8.47 mg/kg-d perchlorate ion dose group. Based on these thyroid effects, a NOAEL of 0.0847 for pups was identified by York et al. (2004). Thyroid hyperplasia in pups occurred in the two highest dose groups. As opposed to thyroid follicular hypertrophy (not considered a reliable biomarker for adverse effects of perchlorate), hyperplasia at a perchlorate ion dose of 2.54 mg/kg-d was considered the LOAEL for adverse thyroid effects, with a corresponding NOAEL of 0.847 mg/kg-d perchlorate ion. In addition to the thyroid effects, histological evaluation of the brain indicated increased thickness of the corpus callosum at the 8.47 mg/kg-d perchlorate ion exposure level in female pups culled 12 days postpartum (LOAEL). However, the method at which the corpus was measured may be considered subjective. A NOAEL for this endpoint in the pup at 2.54 mg/kg-d perchlorate ion was identified by the authors.

York et al. (2005a) conducted another developmental study in Sprague-Dawley rats where they were exposed for longer than the recommended guidelines. Female rats were exposed *ad libitum* to ammonium perchlorate via the drinking water two–weeks prior to cohabitation and continuing through pregnancy to postpartum day (PPD) 22. Sequential sacrifices of subgroup occurred throughout the exposure period. Water consumption was monitored during each stage for each of the animals. Rats were exposed to either 0, 0.01, 0.1, 1.0, or 30.0 mg ammonium perchlorate/kg-d (0, 0.0085, 0.085, 0.85, or 25.4 mg perchlorate ion/kg-d, respectively). There were no deaths, adverse clinical symptoms, or necropsy findings that were considered exposure-related during precohabitation, gestation, and/or lactation period. There were no

changes in a host of reproductive and developmental parameters (number of litters, corpora lutea, implantations, litter size, number of fetuses, resorptions, or post natal pup deaths). Pups were larger in the 0.01 mg/kg-d and higher exposures compared with controls at PPD 14, but only at 30.0 mg/kg-d at PPD 22. The authors consider this to be due to a decrease in weights for controls and not necessarily treatment related. Thyroid hormone levels (TSH and T4) were altered for dams at GD 21 at exposures of 0.01 and greater, but T3 levels were only affected at exposures of 30.0 mg/kg-d. Thyroid weight and colloid depletion was also affected at 30.0 mg/kg-d. These effects were less marked at lactational day (LD) 10. Male and female pups at PPD 22 had similar thyroid changes as dams at GD 21. TSH levels varied between intervals of exposure and were not consistent between life stages or sex.

In a companion study, York et al. (2005b) investigated brain morphometry, histology, and the motor behavior of pups from the previous experiment. Twenty linear morphometric measurements were made from each rat brain from pups at PPD 10 and 22. No treatment-related changes were found in any of the nine measurements of motor activity. Microscopic analysis of multiple sections of rat pup brains did not indicate any evidence of exposure-related neuropathologic changes. Although some differences were found between treatments in some of the brain measurements, they were inconsistent and not considered dose-related from a lack of a dose-response relationship.

Baldridge et al. (2004) exposed 24 pregnant Long-Evans rats to either 0, 5, or 50 mg/L ammonium perchlorate (corresponding to 0, 0.4 or 4.0 mg/kg-d or 0, 0.34, and 3.39 mg perchlorate ion/kg-d, respectively) from GD 7-21 and to post natal day (PND) 24-25 through maternal exposure and lactation. Some of these rats were also co-administered T4 supplements along with perchlorate in the drinking water to simulate levels suggested for normal development in humans. On PND 24-25, female pups were euthanized and ovaries were examined histologically. Number of ovarian (preantral and antral) follicles were reduced in the 4 mg/kg-d groups, but not the 0.4 mg/kg-d groups. Rats treated with 4 mg/kg-d and receiving T4 were not different from controls, suggesting that T4 ameliorated the effects of the perchlorate ion. Many of these follicles counted in the high dose group were also atretic (Baldridge et al. 2004). No change in mean ovarian area was observed between treatments.

Thuett et al. (2000, 2002a, and 2002b) and Roots et al. (2000) were the only investigations located that examined the effects of perchlorate in a wild mammal species. In these studies, captive-born, wild-type breeding pairs of deer mice (*Peromyscus maniculatus*) were exposed to 0, 1 nM, 1 µM, and 1 mM ammonium perchlorate (0, 1.59×10^{-5}, 1.60×10^{-2}, and 15.78 mg/kg-d perchlorate ion; 10 pairs per dose group) in drinking water. The 2000 studies represent preliminary data that were reported only in abstracts. As such this information is presented here, but not used from which to derive TRVs. These preliminary data suggest that ammonium perchlorate may adversely affect growth (i.e., significant

decrease in pup body weights at the highest dose, a), and development (i.e., significant differences in kidney, liver, and heart weights in pups) of deer mice when exposed from the time of cohabitation until post lactation; however, data were largely equivocal. Subsequent studies using the same dose groups extended the exposure time to the weaning of the third litter. Effects were evaluated in the second litter pups at postnatal day 21 and were reported in Thuett et al. (2002a and 2002b).

Reproductive and growth parameters were evaluated in Thuett et al. (2002a). Although there was a dose-related decrease in litter size, this decrease was not significant at any dose level. Additionally, there were no significant differences in body or organ weights when the data were analyzed using individual litters as experimental units. When individual pups were considered the experimental unit, there was a significant decrease in body weight at the 1 µM dose level for the 5-day and 20-day postpartum measurements. This effect, however, was not observed in the highest (1 mM) dose group. Therefore, this effect was not dose-dependent and its significance uncertain. The only statistically significant dose-related effect was in the 1 µM and 1 mM dose groups with a decrease in heart weights (using body weight as a covariate) in male pups on postnatal day 21. A similar trend was observed for female pups, but the decrease was not statistically significant. As described by the authors, effects on the heart have previously been related to improper functioning of the thyroid gland. Therefore, the decreased heart weights may have resulted from the effects of perchlorate on the thyroid observed during the study (Thuett et al. 2002b).

Concentrations of T4 were significantly greater than controls in the low and medium dose groups, but not in the high dose group. Concentrations of T3 did not differ among dose groups, and testosterone (except for one case in the high dose group) and estradiol were undetectable. Histological changes in the thyroid gland (i.e., decreased follicle number per unit area) were observed in the low and high dose groups, but not in the medium dose group. These studies indicate that exposure to ammonium perchlorate in deer mice resulted in altered thyroid hormone levels and histopathological changes to the thyroid gland that may be related to developmental effects observed in the heart. However, the results are equivocal and difficult to interpret given that the thyroid effects were not dose-dependent and the biological significance of the reduced heart weight is uncertain. Using decreased heart weight as the endpoint, the 1 nM dose level (1.59×10^{-5} mg/kg-d perchlorate ion) may be considered the NOAEL and the 1 µM dose level (1.60×10^{-2} mg/kg-d perchlorate ion) may be considered the LOAEL.

One component of the AFRL/HEST perchlorate study was an evaluation of recovery of thyroid hormones (T3/T4) and TSH production following the cessation of perchlorate exposure (Springborn 1998, Crofton 1998). Rats were provided clean water for 30 days following a 90-day perchlorate exposure. The result of the thyroid level measurement taken at the end of the 30 days revealed that males

and females respond differently during the recovery period. For males, T3 and TSH returned to the control level after 30 days of no perchlorate exposure, while T4 remained depressed at all dose levels. For female rats, no statistically significant levels were observed for T4 at any dose, while T3 remained depressed at 8.47 mg/kg-d and TSH increased at a dose of 0.042 mg/kg-d. Given the widely accepted belief that development of an adverse impact on the thyroid requires a simultaneous depressed T3/T4 and an increased TSH, none of the administered doses produced this combined effect. Therefore, the unbounded NOAEL for combined thyroid and pituitary hormone effects of perchlorate in rats in this study is 10 mg/kg-d. Because no significant depression in T4 levels were observed in females at any dose, a higher dose would need to be administered to rats before reliable chronic NOAEL and LOAEL levels can be established.

A recent study was conducted to test for potential endocrine disrupting effects on male reproductive development as a consequence of perchlorate exposure. Offspring from 23 Wistar rats were exposed to either 0, 62.5, 125, 250, or 500 mg perchlorate/kg-d in a corn oil vehicle from post natal day 23-53 (31-days) via gavage (Stoker et al. 2006). Onset of puberty was assessed through preputial separation of the penis of males (an indication of delay in male reproductive maturity), histology of testes, epididymides, prostate, seminal vesicles, and other relevant organs, organ and body weights along with serum testosterone, TSH, T3, and T4 levels. No significant effects were found related to dose in any of the reproductive related parameters; however, testosterone levels were found to increase to 250 mg/kg-d, and decrease to control levels at exposures of 500 mg/kg-d. Only the 250 mg/kg-d treatment was statistically higher relative to controls. Changes in thyroid histology (e.g. follicular height and colloid area) occurred at 62.5 mg/kg-d. Serum TSH and T4 were different at 125 mg/kg-d.

Smith et al. (2006) investigated effects of perchlorate-contaminated food versus water in prairie voles (*M. ochrogaster*) and deer mice (*Peromyscus maniculatus*) for 22 days, then evaluated reproductive success and subsequent exposure for non-mating individuals. Pups were necropsied and evaluated at post-natal day 21. Perchlorate-contaminated food prepared from incorporating soy bean plant matter from plants grown with perchlorate-irrigated water. During the 21-d exposure period, voles in the water-exposed and feed-exposed groups received 0.6 and 0.7 mg/kg-d, and mice received 0.9 and 1.1 mg/kg-d, respectively. During cohabitation (following the initial 21-d exposures) voles received 0.4 and 0.3, and mice received 1.0 and 1.1 mg/kg-d, respectively. Although some mortality occurred, it was not attributed to treatment by the authors. No statistical differences were reported in reproductive success between treatments for either species (e.g. number pups/litter, pup survival, duration to birth). Male voles in the perchlorate-food treatment had lower plasma levels of T4 than of the perchlorate-water groups or control; no other differences in either T3 or between sexes or treatments were found. Plasma T3 concentrations

were lower for mice in the perchlorate-food group compared with controls; no other differences in T3 or T4 levels were found. No differences in thyroid hormone content were found. Thyroid cell height and mean colloidal area was different in voles exposed to perchlorate-water than voles exposed to perchlorate-food or controls.

2.3.1.5 Studies Relevant for Mammalian TRV Development for Ingestion Exposures

The primary target organ for perchlorate toxicity is the thyroid gland, with histopathological changes and alterations in thyroid hormone production the most common effects of toxicant exposure (Table 3 and Figure 1). Changes in thyroid configuration and hormone levels were found in New Zealand white rabbits (York et al. 2001b), Sprague-Dawley and Fischer 344 rats (King 1995, Crofton 1998, York et al. 2001a, and York et al. 2004, Khan et al. 2005), and deer mice (Thuett et al. 2002b). However, given the reserve capacity of thyroid hormone in mammalian species, the competition of iodine and the perchlorate ion at the NIS, and the lack of marked adverse health effects, these data are of uncertain significance and are limited for use in determining a health-based threshold level.

In deer mice, thyroid hormone and organ effects did not show a clear dose-response relationship and reliable NOAEL and LOAEL values could not be identified. Reduced heart weights, possibly related to altered thyroid function, were also reported for deer mice (Thuett et al. 2002a), but were very small in magnitude. Although a NOAEL and LOAEL for this effect could be developed, the reliability of these values is uncertain given the discontinuous dose-response relationships, minimal reduction in weights, and lack of corroborative data for adverse effects observed for other study effects. Other contributing factors include three orders of magnitude difference between the NOAEL and LOAEL values from the studies of Thuett et al. (2002a) and the limited data that determined NOAELs, LOAELs, and LD_{50}s for rabbits, mice, guinea pigs, and rats (Von Burg 1995). Isanhart et al. (2005) conducted a study investigating oxygen consumption under cold stressed conditions in prairie voles for 51 and 180 days at target doses of 0, 1, 10 mg/kg-d (0, 0.85, and 8.5 mg perchlorate ion/kg-d, respectively). No changes were found in oxygen consumption or clinical toxicity; however, a trend was apparent for decreased T4 concentration relative to thyroid mass for 51d exposures only. No other effects were observed and as such may represent a LOAEL/NOAEL for another wildlife species. Care must be used in evaluating these data, as small animals numbers coupled with suspect statistical comparisons (e.g. comparing data from 51d controls with 180d treatment) were conducted.

Smith et al. (2006) compared perchlorate exposures in prairie voles and deer mice using either perchlorate-incorporated food or perchlorate-containing water. Although levels of thyroid hormone levels were variable between treatments, they were not necessarily corroborated with histology, which makes

interpretation difficult. However, this is the only study found that evaluated reproductive success in two wildlife species in a controlled laboratory environment using biologically-incorporated food. In this respect, no differences were found in measures of reproductive success between treatments including controls (i.e. time to birth, number of pups/litter, and pup survival rates).

Several studies were found that were current, well designed, and appropriate in the consideration of TRVs for mammals. The findings of York et al. (2001a, 2001b, and 2004) are of particular value because two different species were used. The studies by Thuett et al. (2002a and 2002b) are important because they represent the only available data for a wildlife species. Despite this, they are unable to be used in the derivation of mammalian TRVs because of inconsistent findings. Two orders and two families of *Mammalia* are represented in these studies and include *Rodentia* (*Muridae*) and *Lagomorpha* (*Leporidae*). Despite the variability with the deer mice studies, the effects from perchlorate exposure are consistent across the three species for which data are available. Although Crofton (1998) measured only hormone levels, York et al. (2001a, 2001b, and 2004) conducted a broader toxicological evaluation in which NOAELs and LOAELs were identified for various endpoints and included ecologically relevant organ systems (e.g., the reproductive system). None found adverse health effects. Although Baldridge et al (2004) reported changes in ovarian follicles in the female pups of exposed dams; more rigorous reproductive studies have found no adverse effects, suggesting these findings are of uncertain biological significance. The studies of York et al. (2001a, 2991b, 2004, 2005a&b, Bekkedal et al. 2000) are important in that no adverse health effects or changes in various measures of reproductive success to include behavior were found from several long-term reproductive studies. The work evaluating the male reproductive maturity respective to perchlorate exposure further finds no evidence of adverse effects (Stoker et al. 2006). Thyroid histology (hyperplasia, hypertrophy) and changes in systemic thyroid levels were found in all of these studies, but these changes are of uncertain biological significance and do not differentiate between adaptive mechanisms and adverse effects. Changes in thyroid cell number or structure may be important as a precursor for cancer; however, cancer generally occurs in senescent individuals, long after primary reproductive events and so is unlikely to have population-level consequences. Changes in T3 or T4 levels have not been parameterized, and therefore have no context from which to determine levels from which adverse effects occur.

Data from acute studies where gavage methods were employed were only considered when subchronic or chronic studies were not available for a species (e.g., mice and guinea pigs presented in Von Burg 1995). However, given the lack of marked adverse health effects, mortality is an important endpoint.

Table 3. Summary of Relevant Mammalian Data for TRV Derivation

Test Type	Study	Test Organism	Test Duration	Test Results		
				NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Effects Observed at the LOAEL
Chronic/other	Argus 2000	Rat (Sprague-Dawley)	>70 d	2.54	25.4	Reduced ossification sites per litter for sternal centers and forelimb phalanges (transient effect)
	York et al. 2001a	Rat (Sprague-Dawley)	>70 d	0.254 (♂+♀)	2.54 (♂+♀)	Histopathologic changes to the thyroid: hypertrophy and hyperplasia.
	York et al. 2001b	Rabbit (New Zealand white)	23 d (GD)	0.762 (♀)	8.81 (♀)	Hypertrophy of the thyroid gland. Authors noted no adverse effects on fetal development.
	York et al. 2004	Rat (Sprague-Dawley)	2 gen	0.847 (F1)	2.54 (F1)	Histopathological changes to the thyroid: hyperplasia.
				2.54 (F1)	8.47 (F1)	Increased thickness of the corpus callosum; potential indication of neurotoxicity.
				8.47 (P1)		No observed effects on reproduction or thyroid in adults. Unbounded NOAEL.
	Baldridge et al. 2004	Rat (Long-Evans)	GD 6-21, PND 1-25	0.34	3.4	Changes in ovarian follicular morphology and number.
	Isanhart et al. 2005	Prairie vole	180d	0.79	NA	No observed effects when compared oxygen metabolism with controls from 51d exposure; no effects in T4 or other signs.
	York et al. 2005a	Rat (Sprague-Dawley)	Precohabitation – 2 wks, GD 0-lactation D10 (PPD 22)	0.85	25.4	Dams GD21 - Hypertrophy of the thyroid, inc. mass, changes in plasma thyroid hormone levels.

Table 3. Summary of Relevant Mammalian Data for TRV Derivation

Test Type	Study	Test Organism	Test Duration	Test Results		
				NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Effects Observed at the LOAEL
	York et al. 2005a	Rat (Sprague-Dawley)	Precohabitation – 2 wks, GD 0-lactation D10 (PPD 22)	0.85	25.4	Dams LD10/22 – Changes in thyroid mass, hypertrophy, colloid depletion. Thyroid hormone changes at LD22. PPD22 male pup thyroid mass and colloid depletion; female pups thyroid mass, colloid, and T3.
				0.0085	0.085	Male pups PPD 22 - Changes in thyroid hormone levels.
				25.4	Not determined	No changes in found various reproductive indices measuring success. Pup weights larger at NOAEL compared with controls (not considered treatment-related).
	York et al. 2005b	Rat (Sprague-Dawley)	Precohabitation – 2 wks, GD 0-lactation D10 (PPD 22)	25.4	Not determined	No dose-related changes in brain morphometry, histology, or motor behavior of pups at various early growth stages.
	Smith et al. 2006	Prairie vole Deer mouse	21-d + mating to PND 21	0.7 (voles) 1.1 (mice)	NA	Data based on reproductive success (see text). Results in thyroid hormone levels variable between perchlorate-water and perchlorate-food treatments.
	Stoker et al. 2006	Rat (Wistar)	31d PND to puberty	500	NA	No changes in delay of puberty in males; no changes in reproductive organ histology, weights. Testosterone increased to 250 mg/kg-d; equal to controls at 500 mg/kg-d; thyroid and TSH/T4 changes at 62.5 and 125 mg/kg-d, respectively.

Table 3. Summary of Relevant Mammalian Data for TRV Derivation

Test Type	Study	Test Organism	Test Duration	Test Results		
				NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Effects Observed at the LOAEL
Sub- chronic	USEPA 2002	Rat (Sprague-Dawley)	14 d	0.847 (♂+♀)	8.47 (♂+♀)	Decrease in T4 thyroid hormone level. This study is a re-analysis of Springborn (1998) data by EPA analyzing raw data by gender, time, and treatment levels. The 30-d recovery observation after the 90-d exposure indicated an unbounded LOAEL at 0.05 mg/kg-d.
			15 d	0.00847 (♀)	0.0423 (♀)	Increase in TSH thyroid hormone level. This study is a re-analysis of Springborn (1998) data by EPA analyzing raw data by gender, time, and treatment levels.
				0.0423 (♂)	0.169 (♂)	The 30-d recovery observation after the 90-d exposure indicated an unbounded LOAEL at 0.05 mg/kg-d.
	Crofton 1998	Rat (Sprague-Dawley)	90 d	0.00847 (♂+♀)	0.0423 (♂+♀)	Decrease in T4 thyroid hormone level. This study is a re-analysis of Springborn (1998) data by EPA analyzing raw data by gender, time, and treatment levels.
				0.00847 (♂)	0.0423 (♂)	The 30-d recovery observation after the 90-d exposure indicated an unbounded LOAEL at 0.05 mg/kg-d.
			90 d	0.0423 (♂+♀)	0.169 (♂+♀)	Increase in TSH thyroid hormone level. This study is a re-analysis of Springborn (1998) data by EPA analyzing raw data by gender, time, and treatment levels. The 30-d recovery observation after the 90-d exposure indicated an unbounded LOAEL at 0.05 mg/kg-d.

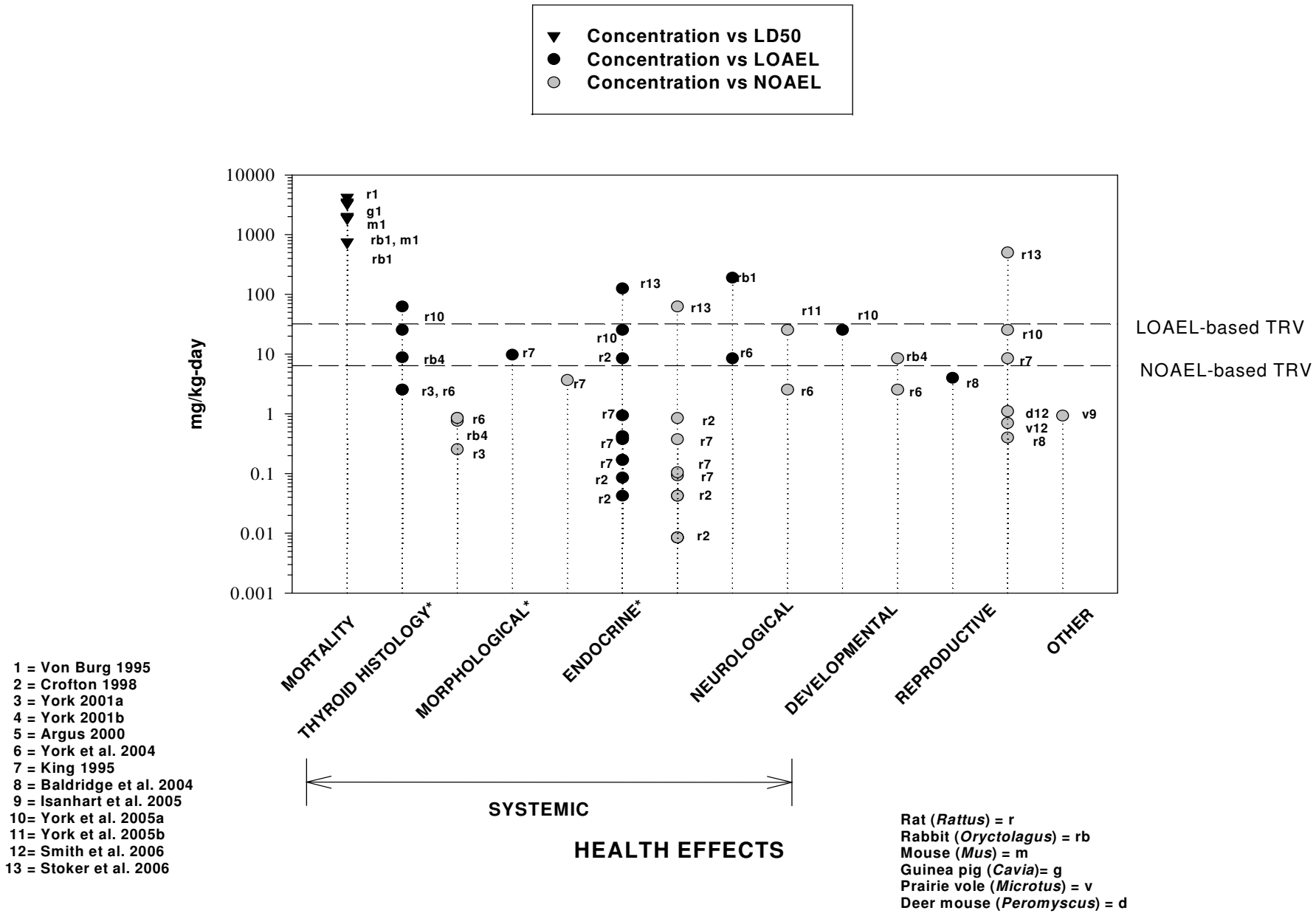
Table 3. Summary of Relevant Mammalian Data for TRV Derivation

Test Type	Study	Test Organism	Test Duration	Test Results		
				NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Effects Observed at the LOAEL
Sub-chronic	Isanhart et al. 2005	Prairie vole	51d	1.13	9.89	Lower T4 levels/thyroid mass, lower kidney mass
	Von Burg 1995	Rabbit	3 months	NA	190	Nervous system effects (not specified)
	King 1995[a]	Rat (Sprague-Dawley)	14 d	3.66 (♂+♀)	9.69 (♂+♀)	Increase in thyroid-to-body weight ratio; author observed that sex does not statistically affect treatment results.
			14 d	0.0931 (♂)	0.375 (♂)	Decrease in T3 thyroid hormone level; author concludes that sex is unrelated to treatment results.
			14 d	0.105 (♀) 0.375 (♂)	0.395 (♀) 0.942 (♂)	TSH increases with increase in dose; sex responds to dose differently.
Acute	Von Burg 1995	Rabbit	LD_{50}	635 - 1610		Mortality
			LD_{50}	1610 - 1690		Mortality

[a] Inferred from the statistical table; author did not explicitly indicate the LOAEL or NOAEL.

PERCHLORATE: HEALTH EFFECTS TO MAMMALS

Figure 1.



*Histologic, morphologic, and endorcrine changes relative to thyroid gland effects

2.3.2 Mammalian Inhalation Toxicity

Only one study was found that investigated the inhalation toxicity of perchlorate in mammals. However, no nominal or measured perchlorate concentration was reported, moreover, other gases (e.g. H2S, SO2, HCl) as vapor products of rocket propellants were measured (Feinsilver et al. 1955). Von Burg (1995) stated that because of the low vapor pressure of perchlorate salts and acids, exposure to fumes and vapors by wildlife would be negligible. Acute exposure to perchlorate fumes would be expected to affect the upper respiratory tract to produce signs and symptoms such as sneezing, coughing, chest pain, and pulmonary edema. No specific information on the dose required to produce these signs/symptoms were provided (Von Burg 1995).

2.3.3 Mammalian Dermal Toxicity

Mammalian toxicity data for the effects of perchlorate via dermal exposure were not located.

2.4 Summary of Avian Toxicology

2.4.1 Avian Oral Toxicity

2.4.1.1 Avian Oral Toxicity - Acute

No data were available for acute exposures of birds to perchlorate.

2.4.1.2 Avian Oral Toxicity - Subchronic

McNabb et al. (2004a) administered ammonium perchlorate to 3 to 4 day post-hatch northern bobwhite quail (*Colinus virginianus*) chicks via drinking water. One study group was exposed to ammonium perchlorate at concentrations of 0, 0.05, 0.5, 5, 50 and 250 mg/L for 2 weeks to measure thyroid hormone levels. A second group was exposed to ammonium perchlorate at concentrations of 0, 250, 500, 1000, 2000, and 4000 mg/L to measure thyroid hormone levels and growth parameters for an exposure period of 8 weeks. The authors reported that plasma T3 and T4 thyroid hormones and thyroid weight were less sensitive indicators of thyroid function as thyroidal T4 concentrations. Thyroidal T4 was significantly reduced in dose groups $\geq$ 0.5 mg/L for 2 weeks, whereas a significant increase in thyroid weight occurred at doses exceeding 1000 mg/L in the 8-week study. The NOAEL and LOAEL for decreased T4 hormone were determined using the 2-week exposure because doses in the 8-week exposure were too high to obtain a NOAEL. At the end of the 2-week exposure period, the 0.5 mg/L dose group (0.0326 mg/kg-d) was the LOAEL and the 0.05 mg/L dose group (0.00326 mg/kg-d) was the NOAEL for decreased T4 hormone levels. The NOAEL and LOAEL for increased thyroid weight were 33 and 65 mg/kg-d perchlorate ion, respectively, based on thyroid weights at 8 weeks. Tibia growth was

also significantly decreased at the end of the 8-week exposure period; NOAEL and LOAEL were 130 and 261 mg/kg-d perchlorate ion, respectively.

McNabb et al. (2004b) reports the results of a series of experiments (including those reported above in McNabb et al. 2004a) with varying exposures (range includes 0.013, 0.025, 0.05, 0.5, 5, 25, 50, 250, 500, 1000, 2000, and 4000 mg/L) of ammonium perchlorate and exposure durations (2, 4, or 8 weeks). Calculated doses as perchlorate ion were 0.00085, 0.0016, 0.0033, 0.033, 0.326, 1.6, 3.3, 16.3, 33, 65, 130, and 261 mg/kg-d. Significant reductions in T4 concentrations observed at 2 weeks in 0.5 and 5 mg/L dose groups were not apparent by 4 and 8 weeks (i.e., there was full compensation of T4 levels). Partial compensation was observed at 50 mg/L, but there was no evidence of compensation in the high dose group (i.e., T4 levels were reduced at 8 weeks to a degree similar to the 2 week measurements). Despite the observed compensation at 50 mg/L, T4 concentrations remained significantly decreased compared to controls. Thyroid weight at 2 weeks was significantly increased at ≥500 mg/L dose levels, whereas thyroid weight at 8 weeks was significantly increased at ≥1000 mg/L. This study suggests that developing young quail have a limited ability to compensate T4 concentrations during early exposure to ammonium perchlorate. Doses estimated in this WTA adopted the authors' conservative use of a daily water ingestion rate of 7.7% of adult quail body weight of 165 g. Using the results at 8 weeks, the NOAEL and LOAEL for T4 hormone levels were 0.326 and 3.3 mg/kg-d perchlorate ion, respectively (Note: the 5 mg/L dose level was the highest no-effect dose level for the T4 endpoint; data for T4 values were not reported for the 25 mg/L dose level). For increased thyroid weight, the NOAEL and LOAEL were 33 and 65 mg/kg-d perchlorate ion, respectively; McNabb et al. 2004a).

2.4.1.3 Avian Oral Toxicity - Chronic

No data are available for chronic exposures of birds to perchlorate.

2.4.1.4 Avian Oral Toxicity - Other

Another avian study was available, but the secondary literature that described the study did not indicate its duration of exposure (original study in German). Doses of 0, 14.4, 21.5, and 28.7 mg/kg-d perchlorate ion were administered to chickens (N = 3-dose). Thyroid and body weight were reduced beginning at 21.5 mg/kg-d. Other signs indicative of perchlorate toxicity were noted at all dose levels and included reduced feather exfoliation, sexual development, and failure of the bursa of Fabricius (organ responsible for B-lymphocyte maturation; ERM 1995). Although the doses and toxicological endpoints were obtained from the original paper, ERM (1995) noted that exposure duration prior to animal sacrifice was not evident. Due to the lack of exposure duration, a NOAEL was unable to be derived from the secondary literature.

Gentles et al. (2005) exposed twenty-three adult female northern bobwhite to one of four exposures 0.12, 0.012, 0.0012, or 0 mg/L ammomium perchlorate in the drinking water for 30 days (1, 0.1, 0.01, or 0 nM solutions, respectively). Liver, gizzard, heart, kidney, and thyroid were weighed at the end of the study, and eggs were collected daily during the study. Ammonium perchlorate did not affect body or organ weights. Birds exposed to the 1 nM group had a decrease in mean colloidal area and an increase in the height of the follicle cells of the thyroid. No change in number of eggs was found. Concentrations of perchlorate were found in the eggs that corresponded to exposure group.

2.4.1.5 Studies Relevant for Avian TRV Development for Ingestion Exposures

Of the four avian studies located, only the two quail studies (McNabb et al. 2004a and 2004b) were suitable for TRV derivation (ERM 1995 had insufficient information regarding exposure duration; the Gentles et al (2005) study did not measure dose (drinking rate) and reported no effects that were biologically relevant. The McNabb et al. studies (2004a and 2004b) represent a single species (northern bobwhite) with effects observed for three endpoints (thyroid hormone levels, thyroid weight, and tibia growth). NOAELs and LOAELs for these endpoints are outlined below (Table 4). Although these studies are useful in development of an avian TRV for perchlorate, confidence is reduced because only a single species is represented.

Table 4. Summary of Relevant Avian Data for TRV Derivation

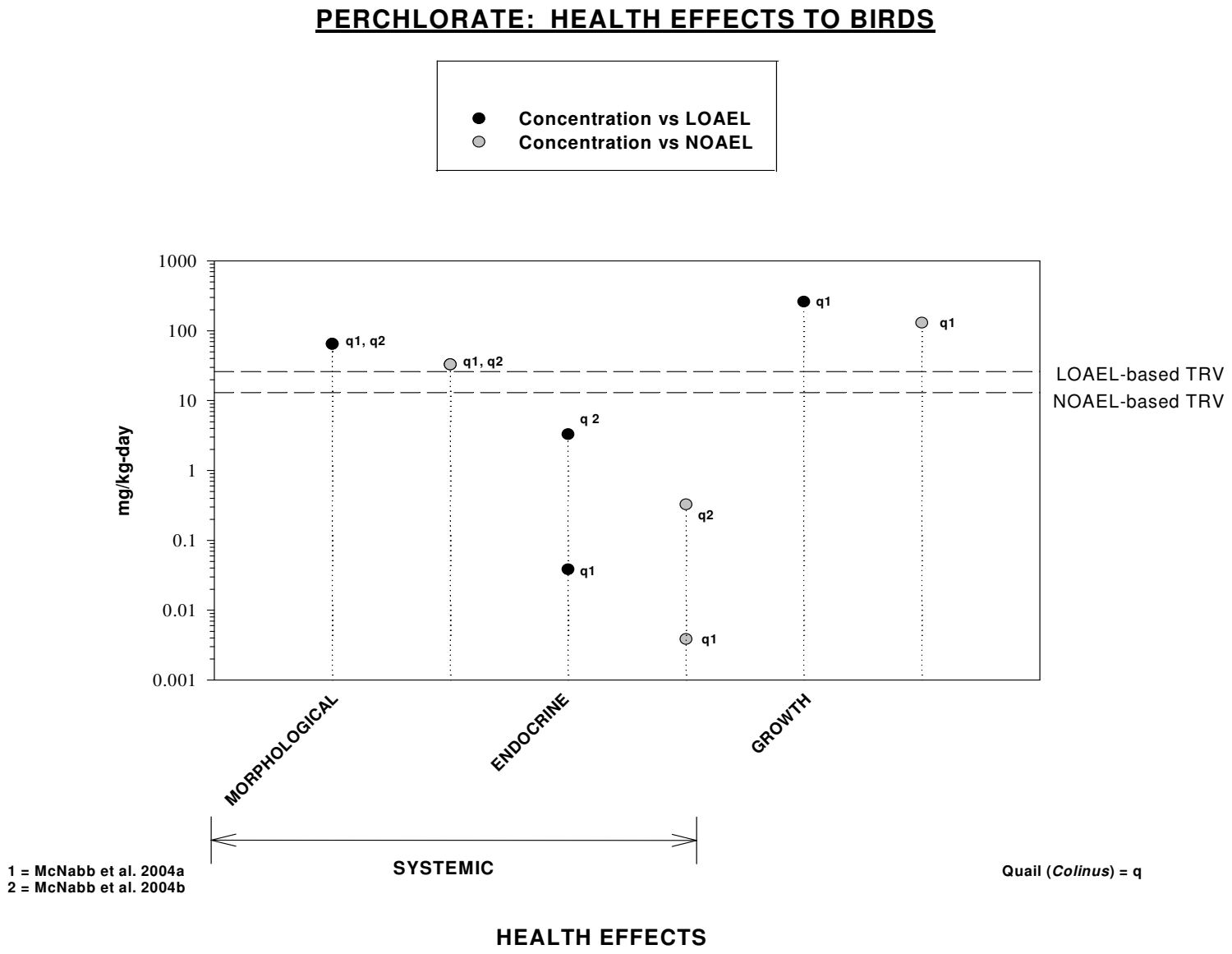
Test Type	Study	Test Organism	Test Duration	Test Results		
				NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Effects Observed at the LOAEL
Subchronic			2 weeks	0.00326	0.0326	Decrease in thyroidal T_4 hormone level.
	McNabb et al. 2004a	Bobwhite quail (*Colinus virginianus*)*	8 weeks	33	65	Increased thyroid weight.
			8 weeks	130	261	Decrease in tibia length. The authors note that tibia growth was decreased significantly relative to increased ammonium perchlorate concentration
	McNabb et al. 2004b	Bobwhite quail (*Colinus virginianus*)*	8 weeks	0.326	3.26	Decrease in thyroidal T_4 hormone level.

Table 4. Summary of Relevant Avian Data for TRV Derivation

Test Type	Study	Test Organism	Test Duration	Test Results		
				NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Effects Observed at the LOAEL
			8 weeks	33	65	Increased thyroid weight.

* = 3-4 week old chicks; sex unknown

Figure 2.


PERCHLORATE: HEALTH EFFECTS TO BIRDS
Concentration vs LOAEL
Concentration vs NOAEL
1000
100
10
1
0.1
0.01
0.001
mg/kg-day
q1, q2
q1, q2
q 2
q1
q2
q1
q1
q1
LOAEL-based TRV
NOAEL-based TRV
MORPHOLOGICAL
ENDOCRINE
GROWTH
SYSTEMIC
1 = McNabb et al. 2004a
2 = McNabb et al. 2004b
Quail (Colinus) = q
HEALTH EFFECTS

2.4.2 Avian Inhalation Toxicity

No data available.

2.4.3 Avian Dermal Toxicity

No data available.

2.5 Summary of Amphibian Toxicology

2.5.1 Amphibian Toxicity - Acute

Dean et al. (2004), exposed green frog tadpoles (*Rana clamitans*) to sodium perchlorate under measured flow-through test conditions for 96-hours (other shorter exposures were also conducted, but the 96-hour exposure is the standard exposure duration in water quality criteria development). Water hardness was 146 mg/L as CaCO3 at the beginning of the test and pH ranged from 7.89 to 8.20 in all solutions spanning the duration of the test. The LC_{50} was observed at 5,500 mg/L. The only sublethal effect observed was a loss of equilibrium in the test organism (i.e., the tadpole could not maintain proper orientation in the water). The EC_{50} for this behavioral effect was 5,100 mg/L.

Goleman and Carr (2006) exposed *Xenopus laevis* tadpoles to ammonium chloride (AC) or sodium perchlorate (SP) to help understand the relative toxicity of the ammonium cation. AC was slightly more lethal than AP or SP (LC_{50} = 83, 510, and 2780 mg/L for AC, AP and SP, respectively). Incidences of edema were increased in SP, though skeletal abnormalities were seen in high dose groups from both SC and AC treatments (> 159 mg/L).

Sparling and Harvey (2006) also investigated the relative toxicological influence of ammonium using various concentrations of ammonium perchlorate and ammonium bicarbonate. Northern leopard frog tadpoles (*Rana pipiens*) of Gosner stage 25 were exposed to concentrations of 0, 0.41, 1.13, 3.08, 8.4, and 61.3 mg/L perchlorate ion or at the relative same NH4 concentrations of ammonium bicarbonate for 7 days. Given inconsistent results from the 7-d exposures, LC_{50}s from the 96-hr data were developed. Seven-day LD_{50} values of 170 and 96-hr LC_{50} of 329 for perchlorate ion were calculated relative to 96-hr LC_{50} of 57.9 and 7-d 29.9 mg/L for ammonium ion, respectively. Based on similar values for other ammonium-containing compounds, and relationships between sublethal effects (e.g. growth measurements), the authors suggested that the NH_4-cation was most responsible the observed toxicity. They support this statement by providing LC_{50} values for NH_4-nitrate (25-32 mg/L) and from citing data from Sparling et al. (2003) where concentrations up to the maximum tested (50 mg/L) of potassium perchlorate caused no adverse effects on growth.

2.5.2 Amphibian Toxicity - Subchronic

In the first of a two part experiment, Tietge et al. (2005) exposed two developmental stages of *Xenopus laevis* larvae (NF stage 51 and 54) to five concentrations of sodium perchlorate (18, 62, 247, 972, and 4000 µg/L as perchlorate ion) and a control for 14 days. Tadpoles exposed to concentrations of 247 µg/L (analytical concentration) and higher exhibited inhibited metamorphic development. Colloid depletion and follicular cell hypertrophy (i.e. larger cell size) was observed in all treatments (>17 µg/L); the severity was concentration dependant. No treatment-related mortality occurred, and there was an interesting positive relationship between dose and body weight in both developmental groups of tadpoles.

In the second of a two-part experiment, Goleman et al. (2002a) conducted a 14-day Endocrine Disruptor Screening and Testing Committee (EDSTAC) Tier 1 frog metamorphosis assay with an exposure concentration of 14.04 mg/L ammonium perchlorate or 11.89 mg/L perchlorate ion. The percentage of tadpoles completing tail resorption was reduced and mean tail length was significantly greater in the treatment group (11.89 mg/L perchlorate ion) compared to controls. The authors note that tail length at the end of the experiment was less than at day 0; therefore, tail resorption was not completely inhibited by perchlorate ion at this exposure level. The authors also discuss the possibility that observed effects are due to the ammonium ion rather than perchlorate. Because perchlorate is known to cause thyroid toxicity and specifically affects thyroid hormone level sensitive aspects of growth and metamorphosis, it is unlikely the ammonium ion contributed to the observed effects.

2.5.3 Amphibian Toxicity - Chronic

Four chronic studies of the effects of perchlorate exposures to amphibians were located. In a study reported by Sterner and Mattie (1998), both male and female African clawed frogs (*Xenopus laevis*) were exposed to sodium perchlorate under static-renewal test conditions for five months following hatch to the end of the metamorphosis. The authors observed at a single exposure level (1% or 8,120 mg/L perchlorate ion) significant differences in blood chemistry measures (e.g., reduced number of circulating red blood cells) and retarded growth (e.g., less than half) relative to controls (Sterner and Mattie 1998). Additionally, maturation of the adult-specific immune system was affected at this exposure level as evidenced by reduced numbers of adult-specific T-cells and reduced splenic B-cells (15% of controls) Therefore, an unbounded LOAEL for this study is 8,120 mg/L. This study was considered chronic because of its duration (five months) and because it was conducted during a critical lifestage (i.e., metamorphosis).

Goleman et al. (2002a) conducted two ammonium perchlorate exposure experiments using *X. laevis.* The first experiment included a range finding study in which approximately 50 fertilized eggs were

exposed to one of seven concentrations of ammonium perchlorate (0.001175, 0.01175, 0.1175, 1.175, 11.75, 117.5, and 1175 mg/L) for 70 days and run in duplicate. Based on the results of the range finding study, two additional concentration groups were added. Measured exposure concentrations for these nine dose groups were 0.005, 0.018, 0.147, 1.412, 14.4, 133, 425, 585, and 1175 mg /L ammonium perchlorate (0.0042, 0.0152, 0.125, 1.196, 12.2, 113, 360, 495, and 995 mg/L perchlorate ion).

LC_{50} values of 510 mg/L (432 mg/L perchlorate ion) for 5-day exposures and 223 mg/L (189 mg/L perchlorate ion) for 70-day exposures were determined. Hatching success was significantly reduced above 1000 mg/L ammonium perchlorate, and survival of larvae was significantly reduced to only 6 to 7 percent in the 425 mg/L exposure group. Larvae that survived the 425 mg/L exposure group had reduced snout-vent length (SVL). Other effects observed in the first experiment included concentration - dependent reductions in hindlimb length, percent forelimb emergence (thyroid-hormone-dependent process that indicates the beginning of metamorphic climax), and percent completing tail resorption (process that indicates the completion of metamorphosis). Hindlimb length was significantly reduced at perchlorate ion exposure concentrations of 0.125 mg/L and greater. Forelimb emergence was the most sensitive endpoint with reductions starting at the lowest exposure level (0.0042 mg/L perchlorate ion), whereas reductions in percent completing tail resorption occurred at concentrations ≥ 0.0152 mg/L perchlorate ion.

In a second study, Goleman et al. (2002b) evaluated the effects of ammonium perchlorate on *X. laevis* using two environmentally relevant concentrations. Approximately 250 embryos were exposed to either 0.038 or 14.04 mg/L ammonium perchlorate (actual concentrations were 0.06 and 14.1 mg/L ammonium perchlorate or 0.06 and 11.9 mg/L perchlorate ion) for 70 days beginning < 24 hours after oviposition, followed by a 28-day non-treatment recovery period. Effects similar to those observed in Goldman et al. (2002a) were observed including significant reductions in hindlimb length, percent forelimb emergence, and percent completing tail resorption at both dose levels. Additionally, whole-body T4 concentrations were reduced at the highest exposure level and significant hypertrophy of the thyroid follicular epithelium occurred at both treatment concentrations. Moreover, the percentage of males at metamorphosis was decreased in both exposure groups compared to controls suggesting that ammonium perchlorate disrupts thyroid activity and impairs testes differentiation in developing *Xenopus*. The effects on metamorphosis and thyroid function were reversed after 28 days of non-treatment. Based on the results of this study, the low dose group (0.06 mg/L perchlorate ion) represents an unbounded LOAEL for endpoints relating to metamorphosis, thyroid function, and gonadal differentiation.

In the second part of a two-part study, Tietge et al. (2005) exposed *X. laevis* at the NF Stage 51 to target concentrations of sodium perchlorate at 0, 8, 16, 32, 63, or 125 µg/L (or analytical concentrations of 0, 9, 17, 34, 69, and 137 µg/L as perchlorate ion) for 44 days (until NF stage 66) and evaluated histologically for thyroid effects and other gross effects. The authors report that mean time to complete

metamorphic development was increased at exposure to 125 µg/L (137 µg/L analytical concentration). Thyroid hypertrophy was observable in frogs exposed to 125 µg/L (137 µg/L analytical concentration) and higher. Total thyroid area, as an indicator of size, was increased in frogs exposed to 60 µg/L (69 µg/L analytical concentration) and higher. Growth was not affected by perchlorate at these concentrations tested. These results suggest that the 14d exposures may have been more sensitive in the development of these measures; however, that longer exposures may be important in the development of tolerance.

In an effort to discriminate the effects of the ammonium ion component, Goleman and Carr (2006) conducted paired exposures to ammonium chloride (AC) or sodium perchlorate (SP) using *X. laevis*. Acute (5-day) and chronic (70-day) experiments were conducted. Ammonium perchlorate was used as an additional treatment for the chronic studies. In the chronic experiment, *X. laevis* embryos were to one of two concentrations (38 or 14,000 µg/L of AC, SP, or AP for 70 days through metamorphosis. Both concentrations of AP and the high concentration of SP inhibited hindlimb length and development. A similar relationship was observed in colloid depletion and hyperplasia of the thyroid. Both AP and SP affected sex ratios resulting in a greater percentage of females exposed to the high concentrations (10,645 and 10,672 µg/L perchlorate ion concentrations).

Sparling et al (2003) conducted two tests to investigate: 1) the concentration of perchlorate that would inhibit metamorphosis, and 2) determine if by adding iodide metamorphosis could be induced. Early larval *Hyla versicolor* tadpoles (Gosner stage 24 or 25) were exposed to either 0, 2.2, 4.8, 10.5, 22.9, 33.8 or 50 ppm potassium perchlorate in the water using bi-weekly static renewal design until metamorphosis (70-100 days). In addition, two other treatments were added: a 0 ppm perchlorate with 0.10 ppm iodide (as KI) and a 30 ppm perchlorate with 0.10 ppm iodide. Survival was high throughout all treatments (> 89%). No tadpoles completed metamorphosis in the 22.9 or the 33.8 ppm treatments, and only one completed metamorphosis in the 50 ppm treatment. There were no statistical differences between controls and the treatments that received iodide; however, the frequency of tadpoles that did complete metamorphosis within the first 70 days was different between these three treatments and all others that received perchlorate ($p < 0.0001$). There were few significant differences between treatments were found in the time to complete metamorphosis, but there was a significant difference in the time to complete metamorphosis once a fore limb emerged. Though there were no significant differences between treatments, on average, tadpoles exposed to perchlorate without iodide took 0.5-4 days longer to complete metamorphosis. An approximate EC_{50} of 3.63 was calculated; however, a 95% CL nor a LOAEL could be determined. When adding iodide at 0.01 ppm iodide, 90-100% of tadpoles entered metamorphic climax stage (regardless of perchlorate concentration) however, only 75% at 2 ppm perchlorate, 82% at 4.8 ppm perchlorate and 70 % at 22.9 ppm perchlorate completed metamorphosis. These data suggest that *H. versicolor* do not preferentially transport perchlorate over iodide, and in environments where iodide is present, effects from perchlorate exposure may be ameliorated (Sparling et al. 2003).

2.5.4 Amphibian Toxicity - Other

Theodorakis et al. (2006) collected and evaluated thyroid histology from cricket frogs (*Acris crepitans*) collected from several perchlorate-contaminated streams in central Texas. There was no evidence of colloid depletion or follicle cell hyperplasia in any of the frogs studied (N=86); however, moderate follicle cell hypertrophy was found in frogs collected from two sites with the highest perchlorate concentrations (~26 and 6 µg/L estimated from bar graph). It is of note that data from water collected for perchlorate analyses at these sites were variable.

2.5.5 Studies Relevant for Amphibian TRV Development

In the acute study of perchlorate exposure to amphibians, toxicant concentrations associated with effects include an LC_{50} of 5,500 mg/L and an EC_{50} for loss of equilibrium of 5,100 mg/L (Dean et al. 2004). Goleman and Carr (2006) report LC_{50}s of 83, 510, and 2780 mg/L for AC, AP, and SP, respectively. Sparling and Harvey (2006) attribute toxicity of acute ammonium perchlorate studies to the ammonium, rather than the perchlorate ion (96-hr LC_{50} = 329, 7-d LC_{50} = 170 for perchlorate ion). The other studies (Sterner and Mattie 1998 and Goleman et al. 2002a and 2002b, Goleman and Carr 2006) were conducted over an extended period (5 months or 70 days) and during a critical life stage (metamorphosis), hence are considered as relevant as chronic data. These studies represent multiple exposure concentrations as well as multiple endpoints, despite evaluating only a single amphibian species (*X. laevis*). In the Sterner and Mattie study (1998), an unbounded LOAEL for aquatic amphibians of 8120 mg/L perchlorate ion was derived. However, this exposure level exceeds the LC_{50} and EC_{50} values (Dean et al. 2004), results of the Goleman et al. studies (2002a&b), Tietge et al. (2005), and Goleman and Carr (2006) suggest that effects of ammonium perchlorate on aquatic amphibians occur at much lower levels. For endpoints related to metamorphosis, forelimb emergence was the most sensitive endpoint with an unbounded LOAEL of 0.004 mg/L perchlorate ion (Goleman et al. 2002a); however, this is not consistent with Tietge et al. (2005) with no adverse effects on development observed with exposures up to 0.069 mg/L during a comparable exposure duration in the same species, nor is it consistent with Goleman et al. (2002b) where forelimb emergence was delayed at 11.9, but not at 0.06 mg/L or Goleman and Carr (2006) where they report exposures of 10.6 mg/L perchlorate ion caused a depression in growth rates and in metamorphosis, but exposures of AP, not SP, at 23 µg/L (as perchlorate ion) caused a mild depression in growth rates and metamorphosis (Figure 3). Other discrepancies exist. After for controlling for perchlorate ion concentration, levels that were reported to cause altered metamorphosis and development (measured by hindlimb development and NF stage) occurred in sodium perchlorate treatments at perchlorate ion concentrations of 10.7 mg/L; however, changes were reported for frogs exposed to ammonium perchlorate at perchlorate ion concentrations of 0.024 mg/L. Endpoints evaluating thyroid function represented by significant hypertrophy of the thyroid follicular epithelium and gonadal

differentiation (reduced percentage of males) had an unbounded LOAEL of 0.06 mg/L perchlorate ion; yet these endpoints may be adaptive and their biological relevance to the health of amphibian populations are difficult to interpret. Data from Theodorakis et al. (2006) provide information from field exposures to another species; however, the importance of the presence of thyroid hypertrophy alone, combined with variable perchlorate concentrations measured in the streams, would not allow for a definitive interpretation of an adverse effect level. These data are instructional and do provide an additional line of evidence of a threshold whereby frogs are responding to environmental concentrations of perchlorate. Regardless, this body of evidence is sufficient from these chronic studies to develop a TRV.

Figure 3.

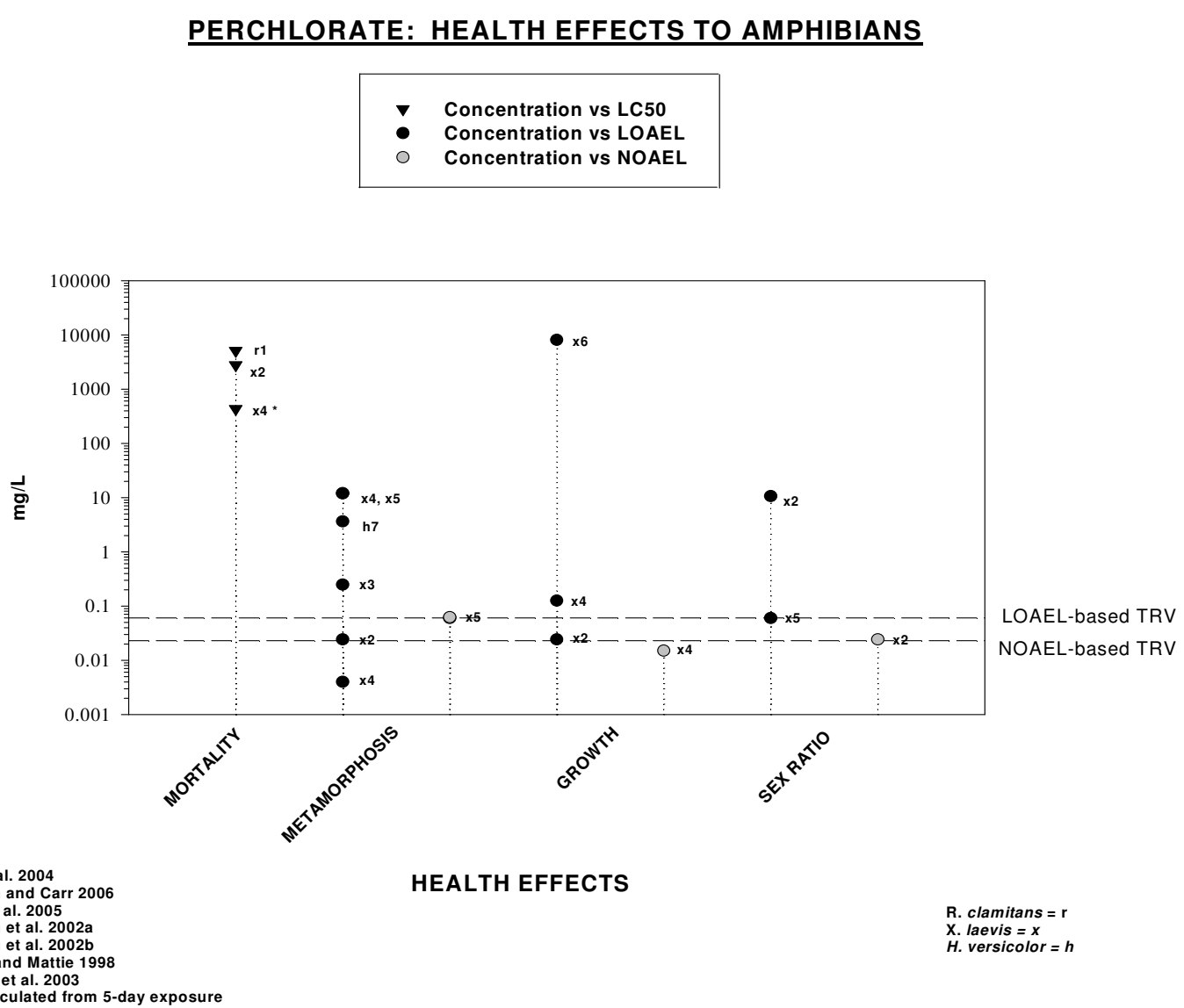

PERCHLORATE: HEALTH EFFECTS TO AMPHIBIANS
Concentration vs LC50
Concentration vs LOAEL
Concentration vs NOAEL
mg/L
100000
10000
1000
100
10
1
0.1
0.01
0.001
r1
x2
x4 *
x4, x5
h7
x3
x4
x5
x2
x6
x4
x2
x4
x5
x2
LOAEL-based TRV
NOAEL-based TRV
MORTALITY
METAMORPHOSIS
GROWTH
SEX RATIO
HEALTH EFFECTS
1 = Dean et al. 2004
2 = Goleman and Carr 2006
3 = Tietge et al. 2005
4 = Goleman et al. 2002a
5 = Goleman et al. 2002b
6 = Sterner and Mattie 1998
7 = Sparling et al. 2003
* = Value calculated from 5-day exposure
R. clamitans = r
X. laevis = x
H. versicolor = h

Table 5. Summary of Relevant Amphibian Data for TRV Derivation

Test Type	Study	Test Organism	Test Duration	Test Results		
				NOAEL (mg/L)	LOAEL (mg/L)	Effects Observed at the LOAEL
Acute	Dean et al. 2004	*R. clamitans*	LC_{50}	5100		Mortality (as sodium perchlorate)
	Goleman and Carr 2006	*X. laevis*	LC_{50}	2780		Mortality (as sodium perchlorate)
	Sparling and Harvey 2006	*R. pipiens*	96-hr	329		Mortality (as ammonium perchlorate)
Subacute	Tietge et al. 2005	*X. laevis*	14-d	0.069	0.137	Inhibited time to metamorphosis
	Goleman et al. 2002a	*X. laevis*	14-d	Not determined	11.9	Inhibited metamorphosis
	Goleman et al. 2002a	*X. laevis*	5-d	LC_{50} = 432		Mortality
	Sparling and Harvey 2006	*R. pipiens*	7-d	LC_{50} = 170		Mortality
Chronic	Sterner and Mattie 1998	*X. laevis*	5 months	Not determined	8120	Decrease in red blood cells, reduced growth, immune effects.
	Goleman et al. 2002a	*X. laevis*	70 days	Not determined	0.004	Delayed forelimb emergence
				0.015	0.125	Reduced hindlimb length
	Goleman et al. 2002b	*X. laevis*	70 days	0.06	11.9	Delayed forelimb emergence
				Not determined	0.06	Decreased male sex ratio
	Goleman and Carr 2006	*X. laevis*	70 days	0.024	10.6	Decreased male sex ratio
				Not determined	0.024	Decreased hindlimb length and NF stage

Table 5. Summary of Relevant Amphibian Data for TRV Derivation

Test Type	Study	Test Organism	Test Duration	Test Results		
				NOAEL (mg/L)	LOAEL (mg/L)	Effects Observed at the LOAEL
Chronic	Sparling et al. 2003	*H. versicolor*	70-100 days		EC50 3.63	Delays in metamorphosis* Effects ameliorated when adding iodide.

2.6 Summary of Reptilian Toxicology

Toxicological data for the effects of perchlorate on reptilian species were not located.

3. RECOMMENDED TOXICITY REFERENCE VALUES

3.1 Toxicity Reference Values for Mammals

3.1.1 TRVs for Ingestion Exposures for the Class Mammalia

The toxicological database for perchlorate is extensive, and includes chronic as well as reproductive and developmental data. More than three species of two orders are represented, as well as chronic NOAEL and LOAEL values. Interpretation of these data in an ecological context is challenging; few adverse effects have been identified, yet the database is comprehensive. There is a general lack of adverse effects. Changes in thyroid structure and function (systemic hormone levels) have been found; however, the relevance of these endpoints to wildlife at the individual or populational level of organization is questionable. Moreover, although changes in thyroid hormone levels and histology are consistent findings as a result of exposure, functional differences from these observations have not been observed. No changes in reproduction as a result of exposure have been observed, yet levels thyroid hormone levels may be decreased along with proportionate thyroid colloid depletion. Mortality is particularly relevant, but occurs only at relatively high exposures.

In the companion studies by York et al. (2001a and 2001b), the authors have stated the LOAEL for rats and rabbits based on structural histologic changes to the thyroid were 2.54 and 8.81 mg/kg-d, respectively; species comparable NOAELs were 0.254 and 0.762. York et al. (2004) used slightly different doses than the prior studies resulting in reported LOAEL and NOAEL for rats based on similar histologic changes to the thyroid of 2.54 and 0.847 mg/kg-d, respectively. Similar effects on the thyroid gland were reported in studies using deer mice (Thuett et al., 2002b). None of these histologic changes in themselves suggests organ failure, merely adaptation (e.g. changes in the size or number of follicular cells, changes in lumen shape, etc.) No evidence of cellular necrosis has been reported, and no other adverse effects (other than changes in TSH, T3 and T4 levels) have been linked to these findings.

Available studies on rats indicate that a change in thyroid and pituitary hormone levels (T4, T3, and TSH) is a more sensitive endpoint than observable histologic changes to the thyroid gland (i.e., the lowest LOAEL reported for the rat is 0.0423 mg/kg-d, where Crofton (1998) reported decreased T4 thyroid hormone levels). The results of Crofton (1998) and King (1995) reveal a significant and rapid response in thyroid hormone levels to low concentrations of perchlorate. However, at higher doses with longer exposure durations or during a critical life stage, adverse whole body responses (e.g., reproduction, fetal development, growth, and time to puberty) have not been observed (York et al. 2001a, 2001b; Stoker et al. 2006). Changes in ovarian follicle density and maturation was found (Baldridge et al. 2004); however, the biological significance of these findings is uncertain, particularly where greater than five reproductive studies have failed to find a relationship between perchlorate exposure and reproduction (York et al. 2001b, 2004, 2005a&b, Smith et al. 2006). It should be noted that USEPA (2002) considers a dose level of 25.4 mg/kg-d perchlorate ion to be a developmental LOAEL based on data reported in Argus 2000 (as cited in USEPA 2002), but was not considered biologically relevant by the study authors and therefore, not reported in the peer reviewed publication (i.e., York et al. 2001a).

The effects of pathologic changes in the thyroid gland to the health and ecology of the whole organism are not well understood. We could find no data that shows any detrimental health effects linked to a decrement to systemic thyroid levels or changes in thyroid histology as those mentioned in these studies. Moreover, changes in iodine levels or cessation of perchlorate values may ameliorate these endpoints, suggesting they are adaptive in nature. Due to the lack of definitive correlations between thyroid pathology and systemic thyroid hormone levels (indirect endpoints) and adverse health effects, we could not use these values in the derivation in a TRV value. Although the York et al. and Thuett et al. studies discussed in Section 2.3.1.5 meet the minimum data set requirement of the Standard Practice, Section 2.2 (USACHPPM 2000), the data for deer mice are equivocal and by themselves not considered sufficient for TRV derivation.

The only marked adverse effect from perchlorate exposure found in the literature is death. Von Burg (1995) reported the results of a 3 month rabbit study where nervous system effects were reported from exposures of 190 mg/kg-d; however, few details were provided and no original data could be found. If the approximation method is used to extrapolate a TRV from the most sensitive LD_{50} data (rabbit; using an UF of 100 for the NOAEL-based value and 20 for the LOAEL-based value), it results in a NOAEL-based TRV of 6.4 mg/kg-d and a LOAEL-based TRV of 32 mg/kg-d. These values are comparable, within an order of magnitude, to the NOAEL and LOAEL-based TRV values using the data from the most sensitive species (rat) in the York et al. studies (0.847 and 2.54 mg/kg-d, respectively) using histological changes in thyroid (i.e., thyroid hyperplasia) as an endpoint. Since these changes were reported as hyperplasia (increases in the number of normal cells), this finding is of uncertain biological significance for ecological receptors and not conducive for TRV derivation. These values are also

protective of the only other non-confirmed report of adverse effects (i.e. nervous system effects) in rabbits from exposures of 3 months in duration (Von Burg 1995). Together, these relationships provide a weight of evidence that shows the approximation approach is reasonable when applied to the acute mortality data. Therefore, the TRVs for the Class Mammalia were derived from the LD_{50} for the rabbit (most sensitive for lethality) using an UF of 100 for the NOAEL-based value and a UF of 20 for the LOAEL-based value.

Table 6 presents the selected TRVs. A moderate level of confidence has been given to these TRVs given the relative abundance of toxicity data for perchlorate and the numbers of species tested. However, though much data are available, there is considerable uncertainty regarding the relevance of thyroid structural and functional changes in wildlife. These changes may result in profound behavioral effects that could influence ecological interactions. More information is needed that focuses on neurodevelopmental endpoints that effect behavior important in maintaining populations and community structure. Presently, methods do not exist that measure these effects in controlled environments.

Table 6. Selected Ingestion TRVs for the Class Mammalia

TRV	Dose (mg/kg-d)	Confidence
NOAEL-based	6.4	Moderate
LOAEL-based	32	Moderate

3.1.2 TRVs for Ingestion Exposures for Mammalian Foraging Guilds

TRVs specific to particular guild associations (e.g., small herbivorous mammals) have not yet been derived. However, the class-specific TRVs shown in Table 5 may be considered to apply to herbivorous small mammals because both rats and rabbits are members of this guild. As with the class-specific TRVs, only two species are represented so confidence in the TRVs is medium. Data to derive TRVs for other guild associations (e.g., carnivorous mammals) is not available at this time.

3.1.3 TRVs for Inhalation Exposures for the Class Mammalia

Although one study (Von Burg 1995) discusses the inhalation toxicity of perchlorate to mammals, no information on the dose required to produce the symptoms listed in Section 2.3.2 was provided in the study. Therefore, no relevant data are available for derivation of an inhalation TRV for mammals.

3.1.4 TRVs for Dermal Exposures for the Class Mammalia

Not available at this time.

3.2 Toxicity Reference Values for Birds

3.2.1 TRVs for Ingestion Exposures for the Class Aves

Three avian studies were evaluated, with two (McNabb et al. 2004a and 2004b) important in TRV derivation. McNabb et al. (2004a) evaluated the effects of perchlorate on thyroid hormone levels (2- and 8-week study) and growth parameters (8-week study) in 3- to 4-day post-hatch bobwhite quail. In the 2-week study, there was a decrease in thyroidal T4 hormone levels in the 0.0326 mg/kg-d perchlorate ion dose group, while T4 levels were unchanged in the 0.00326 mg/kg-d perchlorate ion dose group. In the 8-week study, all dose groups experienced impacts to thyroid hormone levels. A NOAEL and LOAEL were determined in the 8-week study using thyroid weight and tibia length as endpoints. Thyroid weight was significantly increased at dose levels of ≥65 mg/kg-d perchlorate ion and similar to controls ≤at 33 mg/kg-d perchlorate ion. Tibia length was significantly decreased at 261 mg/kg-d, but similar to controls at 130 mg/kg-d. Changes in tibia length have been widely used as an indicator of growth rates in birds, though it is recognized that changes in thyroid weight are of uncertain significance.

In the second study (McNabb et al. 2004b), results from a series of experiments including those reported in McNabb et al. (2004a) are presented. It was found that reduced thyroidal T4 levels observed after 2 weeks exposure to 0.5 to 5 mg/L ammonium perchlorate were restored to control levels by week 4 and 8 of exposure. Exposure to concentrations of 50 mg/L ammonium perchlorate or greater continued to result in a significant decrease in thyroidal T4 levels. Therefore, the 8-week NOAEL and LOAEL for thyroidal T4 levels are 0.326 (5 mg/L dose group) and 3.26 mg/kg-d (50 mg/L dose group) perchlorate ion., respectively. As presented in McNabb et al. 2004a, the NOAEL and LOAEL for thyroid weight are 33 (500 mg/L dose group) and 65 mg/kg-d (1000 mg/L dose group) perchlorate ion, respectively.

As with mammals, a significant and rapid response in thyroid hormone levels was observed at low doses of perchlorate. Additionally, the McNabb et al. (2004a and 2004b) studies indicate that developing quail chicks appear to have limited ability to compensate for early thyroidal hormone effects resulting from exposure to low doses of ammonium perchlorate. Effects on growth parameters (thyroid weight and tibia length) were only observed at higher levels of exposure. Since birds, particularly nestlings, exhibit a relatively high rate of growth and are particularly vulnerable to predation, any reduction in growth has the potential to influence survival. Therefore, growth inhibition may be relevant to the health and ecology of the species within the class and thus constitutes data from which a TRV could be derived. Therefore, the data from McNabb et al (2004a) were used to derive the avian TRVs where growth rates (indicated by changes in tibia length) were used.

Because the avian toxicity database for perchlorate lack in meeting the minimum data set requirements of the Standard Practice, Section 2.2 (USACHPPM 2000), TRVs based on an approximation of the NOAEL and LOAEL were developed for Class Aves using UFs. Since these growth changes were

evaluated in a sensitive life stage in birds for growth parameters (chicks), these data are considered equivalent in value to chronic data (130 mg/kg-d and 261 mg/kg-d). Because only one species from a single taxonomic order are represented, an UF of 10 was applied to the NOAEL and LOAEL. Table 7 presents the selected TRVs. A Low-moderate level of confidence has been given to these TRVs because these studies were of high quality, but lacking in regards to data from other species and lack of other developmental and reproductive data.

Table 7. Selected Ingestion TRVs for the Class Aves

TRV	Dose (mg/kg-d)	Confidence
NOAEL-based	13	Low-Moderate
LOAEL-based	26	Low-Moderate

3.2.2 TRVs for Ingestion Exposures for Avian Foraging Guilds

TRVs specific to particular guild associations (e.g., herbivorous birds) have not yet been derived. However, the class-specific TRVs shown in Table 6 may be considered to apply to herbivorous birds, though the confidence in these TRVs is low. Data to derive TRVs for other guild associations (e.g., carnivorous birds) is not available at this time.

3.2.3 TRVs for Inhalation Exposures for the Class Aves

Not available at this time.

3.2.4 TRVs for Dermal Exposures for the Class Aves

Not available at this time.

3.3 Toxicity Reference Values for Amphibians

Although Sterner and Mattie (1998) is potentially relevant for TRV derivation, the information provided in this study is extremely limited. Only one species was evaluated at a single test exposure concentration and this concentration exceeded the LC_{50} and EC_{50} values reported in Dean et al. (2004) and the LC_{50} values from Goleman et al. (2002a). In contrast, the Goleman et al. studies provide multiple dose levels and an evaluation of multiple biologically relevant endpoints (e.g., metamorphosis and growth). Goleman et al. (2002a), in particular, demonstrates significant dose-response relationships for three relevant endpoints, while Goleman et al. (2002b) indicates that effects observed for these endpoints are reversible if perchlorate exposure is removed. Since thyroid changes can occur following relatively

brief exposures, and since the mechanism of delay metamorphosis and growth has not been fully elucidated in these species, a conservative approach is needed.

However, the data for delays in metamorphosis and in growth are inconsistent. Forelimb emergence was the most sensitive endpoint evaluated in Goleman et al. (2002a) at 0.004 mg/L perchlorate ion. However, Goleman et al. (2002b) using the same species, compound, and exposure duration, reported delayed forelimb emergence at much higher levels (11.9, but not at 0.06 mg/L), or Goleman and Carr (2006) where they report exposures of 10.6 mg/L perchlorate ion caused a depression in growth rates and in metamorphosis, but exposures of AP, not SP, at 23 μg/L (as perchlorate ion) caused a mild depression in growth rates and metamorphosis. Tietge et al. (2005) reported delays in metamorphosis at 137 μg/L; however, the biological relevance of these values in this model given the magnitude and variation is questionable (57.5 ± 3.6d relative to 54.1 ± 2.9d in controls).

Goleman and Carr (2006) investigated the relative influence of ammonium perchlorate, sodium perchlorate and sodium chloride in an effort to determine if the perchlorate ion was responsible for observed adverse effects. Although these acute data suggest the cationic portions of the molecule may help describe acute effects, depression in growth rates and changes in thyroid histology were relatively consistent between the two forms of perchlorate. Here, growth rate (and metamorphic stage) changes were reported from exposures to 10.6 mg/L perchlorate ion, and slight reduction were reported from exposures to only AP at 23 μg/L. Thyroid changes were scored at the 23 μg/L concentrations also, though these changes are uncertain in their biological significance. Greater ratios of females were found in the 10.6 mg/L exposures for both forms of perchlorate ion also. Tietge et al. (2005) found slightly differing results. Using the same species and compound as Goleman et al. (2002a&b) and Goleman and Carr (2006), they found no changes in development, but did find a delay in metamorphic stage at perchlorate ion concentrations at 137 μg/L from 44 day exposures. Sparling and Harvey (2006) provide data to suggest toxicity is due primarily to the ammonium ion and not perchlorate and in doing so add another species represented. Sparling and Harvey, however, did not provide long term data or data on sex ratio influence on perchlorate exposures. Sparling et al (2003) did evaluate and find delays in metamorphosis in gray treefrogs (*Hyla versicolor*) exposing tadpoles to concentrations of potassium perchlorate from 2.2 to 50 mg/L; however, no differences were observed in treatments where iodide was added at environmentally-relevant concentrations). These data suggest that the form of perchlorate as well as environmental iodide concentrations is important in understanding the potential for risk.

Because Goleman et al. (2002a and 2002b), Tietge et al. (2005), and Goleman and Carr (2006) conducted exposures from the embryo through the larval stage through metamorphosis, exposures through all life stages have been included. Tietge et al. (2005) did find histological changes in thyroid histology at these levels, consistent with earlier findings of Goleman et al. Therefore, considering the preponderance of these data, a fairly consistent NOAEL and LOAEL would be 0.023 and 0.06 mg/L for

perchlorate ion, respectively, the latter value based on changes in male sex ratios (Goleman et al. 2002b). These data satisfy the requirements for a chronic study. Data from two species of two different families are included, and are considered equivalent in terms of orders given the diversity in the Order Anura. Additionally, data from a field investigation of another species representing another family (*A. crepitans*) is used as an additional check on the value (see further). Two chronic LOAELs and one chronic NOAEL are available. Therefore, these data meet the minimum data set requirements of the Standard Practice, Section 2.2 (USACHPPM 2000), and the NOAEL/LOAEL approach was used for TRV derivation. Using this corroborative approach to the data, the NOAEL-based TRV is 0.023 and the LOAEL-based TRV is 0.06 mg/L, respectively. A "low" level of confidence was assigned given the variability in the data set.

The field data for Theodorakis et al. (2006) provide an additional line of evidence that even at low levels these values are protective of subtle responses of a native amphibian species in the field. It is important to note, however, that other compounds (e.g. nitrates) may be found in aquatic environments that also affect the thyroid in a similar way and may enhance the probability for the manifestation of effects.

Table 7. Selected Water TRVs for the Class Amphibia

TRV	Dose (mg/L)	Confidence
NOAEL-based	0.023	Low
LOAEL-based	0.06	Low

3.4 Toxicity Reference Values for Reptiles

Not available at this time.

4. IMPORTANT RESEARCH NEEDS

Mammalian TRVs derived for perchlorate have medium confidence; primary given the breadth of the toxicology studies provided to-date. However, the uncertainty regarding adaptive changes in the thyroid as a result of perchlorate exposure continues to be investigated and clear levels of change that are instructive in predicting adverse effects have yet to be elucidated. The present mammalian value was derived, albeit with a relative large uncertainty factor and consistent with other endpoints, from mortality data. It may very well be true that long term exposure to perchlorate may yield alterations in behavior or other effects that may have a profound influence on ecological interactions. Studies focused on

development and behavior relevant to wildlife in a natural setting g is needed as well as investigations using other species and taxonomic orders to provide a greater breadth of interspecific data. Moreover, the potential for adverse effects need to be considered respective to environmental iodide levels (which may ameliorate toxicity) as well as the impact of the reserve capacity of the thyroid in understanding likelihood of continuous exposure.

TRV derivation for birds, amphibians, and reptiles was even more uncertain due to the paucity of toxicity data for birds and amphibians and the absence of toxicity data for reptiles. Additional avian data are limited by the availability of useful models, however. The amphibian data set would benefit from additional studies with other native species conducted under high quality (GLP) conditions. Research studies should include experimental models of species genetically, biologically and behaviorally similar to wildlife exhibiting the greatest propensity for toxicant exposure. Experimental design should attempt to mimic both exposure type and duration, and include assessments of long-term effects.

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APPENDIX A
LITERATURE REVIEW

The following database were searched using the following keywords:

TOXNET/TOXLINE and PUBMED/MEDLINE (1966 to present)

Search Strategy 1: Perchlorate AND Wildlife	Number of hits: 3
Search Strategy 2: Perchlorate AND Toxicity	Number of hits: 93
Search Strategy 3: Perchlorate AND Mammal	Number of hits: 16
Search Strategy 4: Perchlorate AND Bird	Number of hits: 4
Search Strategy 5: Perchlorate AND Amphibian	Number of hits: 3
Search Strategy 6: Perchlorate AND Reptile	Number of hits: 0
Search Strategy 7: Perchlorate AND Snake	Number of hits: 0
Search Strategy 8: Perchlorate AND Toad	Number of hits: 1
Search Strategy 9: Perchlorate AND Salamander	Number of hits: 0

A majority of the search results are abstracts from conferences; they are not used because they are considered on-going studies and are limited in the presentation of methods and results; only peer-reviewed literature and gray literatures (reports, technical memoranda) were considered in wildlife toxicity assessments.

NTIS (1990 to present)

Search Strategy 1: Perchlorate AND Wildlife	Number of hits: 2
Search Strategy 2: Perchlorate AND Toxicity	Number of hits: 8
Search Strategy 3: Perchlorate AND Mammal	Number of hits: 0
Search Strategy 4: Perchlorate AND Bird	Number of hits: 3
Search Strategy 5: Perchlorate AND Amphibian	Number of hits: 0
Search Strategy 6: Perchlorate AND Reptile	Number of hits: 0
Search Strategy 7: Perchlorate AND Snake	Number of hits: 1
Search Strategy 8: Perchlorate AND Toad	Number of hits: 0
Search Strategy 9: Perchlorate AND Salamander	Number of hits: 0

Of the 14 total number of hits, 2 were duplicates, and others were considered irrelevant to our toxicity assessment purpose. Criteria that were considered irrelevant includes: exposure information only (concentration data), data pertinent to aquatic organisms (e.g. fish) or invertebrates (e.g. earthworms, copepods), fate and transport information, and review articles. Of these, 6 were retained for review.

Public Scientific and Technical Information Network (STINET) from the Department of Defense (1930 to present)

Search Strategy 1: Perchlorate AND Wildlife	Number of hits: 2
Search Strategy 2: Perchlorate AND Toxicity	Number of hits: 8
Search Strategy 3: Perchlorate AND Mammal	Number of hits: 0
Search Strategy 4: Perchlorate AND Bird	Number of hits: 3
Search Strategy 5: Perchlorate AND Amphibian	Number of hits: 0
Search Strategy 6: Perchlorate AND Reptile	Number of hits: 0
Search Strategy 7: Perchlorate AND Snake	Number of hits: 1
Search Strategy 8: Perchlorate AND Toad	Number of hits: 0
Search Strategy 9: Perchlorate AND Salamander	Number of hits: 0

After review of the titles, two were retained for review.

Based upon reviewers' recommendations, a subsequent review was review was conducted in June 2006 using the keyword "perchlorate" in the following databases: TOXNET/TOXLINE and PUBMED/MEDLINE going back to 2003. During this search, 99 new citations were found. Of these, abstracts were obtained for 24 since, based on the title, had promise of having relevant new toxicological information. Basis for rejection included parameters mentioned previously (e.g. contained fate and transport information exclusively, environmental concentration information without effects data, review articles, abstracts, invertebrate or aquatic toxicity studies, or were cited previously. Of those, 12 were considered relevant and included in this document. One possibly relevant paper could not be obtained:

Peng et al. 2003. Toxic effects of ammonium perchlorate on thyroid of rats. Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi. 21(6): 404-407 (in Chinese).

The abstract, however, was available in English and was reviewed. Since it is an abstract and details regarding the methods and translation could not be verified, a summary is presented here and not in the text. The study consisted of a 90-day drinking water study where rats of four treatment groups received either 0, 129, 257, or 514 mg/kg-d. Another study consisted on rats receiving either 0, 1.2, 46.5, or 465 mg/kg-d for 36 weeks. No differences in behavior or body weight were observed. Changes were evident in thyroid histology (suggesting follicle proliferation, colloid depletion) and in thyroid circulating hormone concentrations. It was determined that since no new information was presented, it would be infeasible to obtain the article and translation.

APPENDIX B
GLOSSARY

Diiodotyrosine (DIT)	A product of iodination of Tg with oxidized iodine. Combination of DIT with another DIT produces T4.
Hyperplasia	Increase in cell number. In this context, this typically refers to the growth of the thyroid gland as the lack of iodine prompts the thyroid gland to produce more thyroid hormones. The 1999 Peer Review Panel suggested the use of hyperplasia as a biomarker for adverse effects of perchlorate.
Hypertrophy	Increase in cell size. Usually refers to the enlargement of the thyroid gland due to preferential uptake of perchlorate over iodine. The 1999 Peer Review Panel concluded that thyroid hypertrophy is not a good biomarker for adverse effects of perchlorate.
Monoiodotyrosine (MIT)	A product of iodination of Tg with oxidized iodine. Combination of MIT with DIT produces T3.
Thyroglobulin (Tg)	A protein in the thyroid gland that contains iodine. It synthesizes T3 and T4 in the presence of iodine in the thyroid gland. Iodination of Tg and iodide produces monoiodotyrosine (MIT) and diiodotyrosine (DIT).
Thyroid hormones	The thyroid hormones are thought to promote developmental phase stages of life. The lack of thyroid hormones may negatively affect neurodevelopment, bone and central nervous system development.
Thyroxine (T4)	One of the two hormones produced by the thyroid gland. T4 contains four atoms of iodine and thus it is abbreviated as T4. T4 is synthesized in the thyroid gland by the combination of two DIT molecules. The anterior pituitary of the brain monitors the concentration of T3 and T4 in blood and regulates appropriate amounts of T3 and T4 by secreting thyroid-stimulating hormone (TSH).
Triiodothyronine (T3)	One of the two hormones produced by the thyroid gland. T3 contains three atoms of iodine and thus it is abbreviated as T3. T3 is synthesized in the thyroid gland by the combination of one DIT and one MIT molecules.
Thyroid-stimulating hormone (TSH)	Hormone produced by the anterior pituitary that promotes iodine uptake and iodination of Tg. The thyroid hormones (T3 and T4) control the rate of release of TSH. As part of the homeostatic regulatory mechanism in the body, decrease in T3 and T4 levels in blood would prompt an increase of TSH secretion in order to increase the production of T3 and T4.

APPENDIX C
CONVERSION FROM PERCHLORATE SALT TO PERCHLORATE ION

Compound: Ammonium perchlorate (NH_4ClO_4)
Form: NH_4CLO_4 (84.7 % ClO_4^-)
Reference: Crofton, 1998 (based on Springborn, 1998, data supplied by AFRL/HEST)
Test Species: Sprague-Dawley rats
Body weight: 0.35 kg (assumed; EPA 1988)
Life span: 2 years (assumed; EPA 1988)
Water Consumption: 41 g/animal-day (measured; Springborn, 1998)
Study Duration: 90-d (chronic)
Endpoint: Body weight and thyroid gland function (T_3, T_4, and TSH hormone levels)
Exposure Route: oral in water
Dosage: 0, 0.00847, 0.0423, 0.169, 0.847, 8.47 mg/kg-d as perchlorate ion (measured in NH_4ClO_4; Springborn, 1998)
Calculations:

Unbounded NOAEL (based on decreased T_3 and T_4):
(84.7%)*(0.01 mg/kg-d) = 0.00847 mg/kg-d CLO_4^-

NOAEL for increased TSH level
(84.7%)*(0.01 mg/kg-d) = 0.00847 mg/kg-d ClO_4^-

LOAEL for increased TSH level
(84.7%)*(0.20 mg/kg-d) = 0.169 mg/kg-d ClO_4^-

Comments: At the unbounded NOAEL dose of 0.00847 mg/kg-d (based on decreased T_3 and T_4), Sprague-Dawley rat responded with a T_3 decrease of 16% from 170 to 143 ng-dl, while it prompted a T_4 decrease of 17% from 4.75 to 3.94 ug-dl.

Final Unbounded LOAEL based on decreased T_3 and T_4: 0.00847 mg/kg-d
Final NOAEL based on increased TSH level: 0.00847 mg/kg-d
Final LOAEL based on increased TSH level: 0.169 mg/kg-d

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Compound: Potassium perchlorate ($KClO_4$)
Form: $KClO_4$ (71.8% ClO_4^-)
Reference: Kessler and Kruskemper, 1966
Test Species: Rat
Body weight: 0.35 kg (assumed; based on EPA 1988)
Life span: 2 years (assumed; based on EPA 1988)
Water Consumption: 0.025 L-d (estimated; calculated using allometric equation from EPA 1988)
Study Duration: 0, 40, 120, 220, and 730 d (chronic exposures were at 120, 220, and 730 days)
Endpoint: Body weight and thyroid gland structure (thyroid weight)
Exposure Route: oral in water
Dosage: Control and one dose level
1.0% w/v in water = 10,000 mg/L
Calculations:

Unbounded NOAEL (based on body weight) and unbounded LOAEL (based on thyroid structure); dose was adjusted accounting for perchlorate ion only:

(71.8%)*(10,000 mg/L)*(0.025 L-d)/0.35 kg = 513 mg/kg-d ClO_4^-

Comments: For thyroid structure and function, histological changes were observed starting at the 40-day exposure and continued to progress through fibrosis and on to follicular adenomas (goiters). Since there is no dose below the control dose of 513 mg/kg-d, this level was selected as the unbounded LOAEL for thyroid enlargement.

Final Unbounded NOAEL for body weight: 513 mg/kg-d
Final Unbounded LOAEL for increased thyroid weight: 513 mg/kg-d

==========

Compound: Potassium perchlorate ($KClO_4$)
Form: $KClO_4$ (71.8% ClO_4^-)
Référence: Lampe et al. 1967
Test Species: Rabbits
Body weight: 0.35 kg (assumed; EPA 1988)
Water Consumption: 41 g/animal-day (measured; Springborn, 1998)
Study Duration: 21-d during pregnancy (chronic)
Endpoint: Thyroid/body weight ratio
Exposure Route: oral in diet
Dosage: Control and one dose level
100 mg/kg-d as $KClO_4$ (nominal)

Calculations:
Unbounded LOAEL (based on increased thyroid/body weight ratio):
(71.8%)*(100 mg/kg-d) = 71.8 mg/kg-d ClO_4^-

Final Unbounded LOAEL based on thyroid/body weight ratio: 71.8 mg/kg-d

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Compound: Potassium perchlorate ($KClO_4$)
Form: $KClO_4$ (71.8% ClO_4^-)
Reference: Brown-Grant, 1966
Test Species: Adult female Wistar Rat
Body weight: 0.2974 kg (assumed; based on EPA 1988)
Water Ingestion: 246 mg/rat-day KClO4 (Brown-Grant, 1966)
Study Duration: 7-days (during gestation)
Endpoint: Thyroid hypertrophy
Exposure Route: Oral in water
Dosage: Control and one dose level
0.25% w/v in water (the result at this level was considered unsatisfactory, thus this data point was not used)
1.0% w/v in water = 10,000 mg/L
Calculations:
(71.8%)*(246 mg/rat-day)*(1 rat/0.2947 kg) = 599 mg/kg-d ClO_4^-

Final Unbounded LOAEL for thyroid hypertrophy: 599 mg/kg-d

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Compound: **Potassium perchlorate ($KClO_4$)**
Form: $KClO_4$ (71.8% ClO_4^-)
Reference: Postel, 1957 (Cited in Sterner and Mattie, 1998)
Test Species: Adult pregnant female guinea pig
Body weight: 0.72 kg (assumed; EPA, 1998)
Water Ingestion: 0.053 L-d KClO4 (allometrically estimated; EPA, 1998)
Study Duration: 27-days
Endpoint: Fetal thyroid weight
Exposure Route: Oral in water
Dosage: Control and one dose level
1.0% w/v in water = 10,000 mg/L
Calculations:
(71.8%)*(10,000 mg/L)*(0.053 L-d)/0.72 kg = 529 mg/kg-d ClO_4^-

Final Unbounded LOAEL for thyroid hypertrophy: 529 mg/kg-d

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Compound: Potassium perchlorate ($KClO_4$)
Form: $KClO_4$ (71.8% ClO_4^-)
Reference: ERM, 1995 [Primary study was from Pflungfelder (1959)]
Test Species: Adult chicken
Study Duration: Not available from ERM (1995)
Endpoint: Thyroid and body weight and other toxicity symptoms
Exposure Route: Not available from ERM (1995)
Dosage: Control and three dose level at 20, 30, and 40 mg/kg-d

Calculations:
(71.8%)*(20 mg/kg-d) = 14.4 mg/kg-d ClO_4^-
(71.8%)*(30 mg/kg-d) = 21.5 mg/kg-d ClO_4^-
(71.8%)*(40 mg/kg-d) = 28.7 mg/kg-d ClO_4^-

Final Unbounded NOAEL: NOAEL cannot be derived because the exposure duration is not available from the secondary literature.

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Compound: Ammonium perchlorate (NH_4ClO_4)
Form: NH_4ClO_4 (84.7% ClO_4^-)
Reference: York et al. (2001a)
Test Species: Sprague-Dawley rats
Study Duration: >90-days for P and F1 generations
Endpoint: Thyroid hyperplasia
Exposure Route: oral in water
Dosage: 0, 0.3, 3.0, and 30 mg/kg-d as ammonium perchlorate measured based on bodyweight and calculated water intake rate

Calculations:
NOAEL for hyperplasia of the thyroid gland
(84.7%)*(0.3 mg/kg-d) = 0.254 mg/kg-d ClO_4^-

LOAEL for hyperplasia of the thyroid gland
(84.7%)*(3.0 mg/kg-d) = 2.54 mg/kg-d ClO_4^-

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Compound: Ammonium perchlorate (NH_4ClO_4)
Form: NH_4ClO_4 (84.7% ClO_4^-)
Reference: York et al. (2001b)
Test Species: New Zealand Female Rabbits
Study Duration: 23-d (chronic because the rabbits were evaluated during a critical life stage)
Endpoint: body weight and thyroid gland function (T_3, T_4, and TSH hormone levels)
Exposure Route: oral in water
Dosage: 0, 0.1, 0.9, 10.4, 30.3, and 102.3 mg/kg-d as ammonium perchlorate measured based on bodyweight and calculated water intake rate

Calculations:

NOAEL for hypertrophy of the thyroid gland
(84.7%)*(0.9 mg/kg-d) = 0.762 mg/kg-d ClO_4^-

LOAEL for hypertrophy of the thyroid gland
(84.7%)*(10.4 mg/kg-d) = 8.81 mg/kg-d ClO_4^-

==========

Compound: Ammonium perchlorate (NH_4ClO_4)
Form: NH_4ClO_4 (84.7% ClO_4^-)
Reference: York et al. (2004)
Test Species: Sprague-Dawley rats (pups)
Study Duration: Day 0 of gestation until postpartum day 5
Endpoint: Thyroid hyperplasia, thickness of corpus callosum
Exposure Route: oral in water
Dosage: 0, 0.1, 1.0, 3.0, and 10 mg/kg-d as ammonium perchlorate measured based on bodyweight and calculated water intake rate

Calculations:

NOAEL for thyroid hyperplasia
(84.7%)*(1.0 mg/kg-d) = 0.847 mg/kg-d ClO_4^-

LOAEL for thyroid hyperplasia
(84.7%)*(3.0 mg/kg-d) = 2.54 mg/kg-d ClO_4^-

NOAEL for thickness of corpus callosum
(84.7%)*(3.0 mg/kg-d) = 2.54 mg/kg-d ClO_4^-

LOAEL for thickness of corpus callosum
(84.7%)*(10 mg/kg-d) = 8.47 mg/kg-d ClO_4^-

==========

Compound: Ammonium perchlorate (NH_4ClO_4)
Form: NH_4ClO_4 (84.7% ClO_4^-)
Reference: Thuett et al. 2002a
Test Species: Deer mice (pups)
Body weight: 8.73, 8.24, 7.68, and 8.95 g for control, 1 nM, 1µM, and 1 mM exposure groups, respectively
Water Consumption (L-d) = $0.099W^{0.90}$ (Assumed, EPA 1998)
Study Duration: From cohabitation until postnatal day 21
Endpoint: Heart weight
Exposure Route: Pup exposure to NH_4ClO_4 *en utero* and via lactation
Dosage: 0, 1.59×10^{-5}, 0.01602, and 15.78 mg/kg-d as perchlorate ion (estimated from nominal NH_4ClO_4 in drinking water, using the allometric equation for water consumption from EPA 1998)

Calculations:

NOAEL (based on decreased heart weight):
(84.7%)*(1.879×10^{-5} mg/kg-d) = 1.59×10^{-5} mg/kg-d ClO_4^-

LOAEL (based on decreased heart weight):
(84.7%)*(0.01893 mg/kg-d) = 0.0160 mg/kg-d ClO_4^-

Final NOAEL based on decreased heart weight: 0.0000159 mg/kg-d ClO_4^-
Final LOAEL based on decreased heart weight: 0.0160 mg/kg-d ClO_4^-

==========

Compound: Ammonium perchlorate (NH_4ClO_4)
Form: NH_4ClO_4 (84.7% ClO_4^-)
Reference: Thuett et al. 2002b
Test Species: Deer mice (pups)
Body weight: 8.73, 8.24, 7.68, and 8.95 g for control, 1 nM, 1µM, and 1 mM exposure groups, respectively
Water Consumption (L-d) = $0.099W^{0.90}$ (Assumed, EPA 1998)
Study Duration: From cohabitation until postnatal day 21
Endpoint: T_4 level
Exposure Route: Pup exposure to NH_4ClO_4 *en utero* and via lactation
Dosage: 0, 1.59×10^{-5}, 0.0160, and 15.78 mg/kg-d as perchlorate ion (estimated from nominal NH_4ClO_4 in drinking water, using the allometric equation for water consumption from EPA 1998)

Calculations:

Unbounded NOAEL (based on increased T_4):
(84.7%)*(1.879×10^{-5} mg/kg-d) = 1.59×10^{-5} mg/kg-d ClO_4^-

Final Unbounded NOAEL based on decreased heart weight: 1.59×10^{-5} mg/kg-d ClO_4^-

==========

Compound: Ammonium perchlorate (NH_4ClO_4)
Form: NH_4ClO_4 (84.7% ClO_4^-)
Reference: Thuett et al. 2002b
Test Species: Deer mice (pups)
Body weight: 8.73, 8.24, 7.68, and 8.95 g for control, 1 nM, 1µM, and 1 mM exposure groups, respectively
Water Consumption (L-d) = $0.099W^{0.90}$ (Assumed, EPA 1998)
Study Duration: From cohabitation until postnatal day 21
Endpoint: Thyroid follicle number/Unit Area
Exposure Route: Pup exposure to NH_4ClO_4 *en utero* and via lactation
Dosage: 0, 1.59×10^{-5}, 0.0160, and 15.78 mg/kg-d as perchlorate ion (estimated from nominal NH_4ClO_4 in drinking water, using the allometric equation for water consumption from EPA 1998)

Calculations:

Unbounded NOAEL (based on increased thyroid follicle number):
(84.7%)*(1.879×10^{-5} mg/kg-d) = 1.59×10^{-5} mg/kg-d ClO_4^-

Final Unbounded NOAEL based on decreased heart weight: 1.59×10^{-5} mg/kg-d ClO_4^-

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Compound: Ammonium perchlorate (NH_4ClO_4)
Form: NH_4ClO_4 (84.7% ClO_4^-)
Reference: McNabb et al. 2004a
Test Species: Bobwhite quail
Body weight: 165 g (assumed by the authors for dose calculation)
Life span: < 1 year (estimated)
Water Consumption: 0.0127 L-d (authors assumed water intake as 7.7% of adult quail body weight)
Study Duration: 2- and 8-weeks (subchronic)
Endpoint: body weight, limb growth, thyroid gland function (plasma and thyroidal T_4 hormone levels) and thyroid weight
Exposure Route: oral in water
Dosage: 0, 19.3, 32.6, 65.1, 130, and 261 mg/kg-d as perchlorate ion (estimated from nominal NH_4ClO_4 in drinking water, assuming a water ingestion rate of 7.7% in adult bobwhite quail weight of 165 g)

Calculations:

NOAEL for decreased thyroidal T4 hormone level at 2 weeks
(84.7%)*(0.05 mg/L)*(0.0127 L-d) / (0.165 kg) = 0.00326 mg/kg-d ClO_4^-

LOAEL for decreased thyroidal T4 hormone level at 2 weeks
(84.7%)*(0.5 mg/L)*(0.0127 L-d) / (0.165 kg) = 0.0326 mg/kg-d ClO_4^-

NOAEL for increased thyroid weight at 8 weeks
(84.7%)*(500 mg/L)*(0.0127 L-d) / (0.165 kg) = 33 mg/kg-d ClO_4^-

LOAEL for increased thyroid weight at 8 weeks
(84.7%)*(1000 mg/L)*(0.0127 L-d) / (0.165 kg) = 65 mg/kg-d ClO_4^-

NOAEL for decreased tibia length at 8 weeks
(84.7%)*(2000 mg/L)*(0.0127 L-d) / (0.165 kg) = 130 mg/kg-d ClO_4^-

LOAEL for decreased tibia length at 8 weeks
(84.7%)*(4000 mg/L)*(0.0127 L-d) / (0.165 kg) = 261 mg/kg-d ClO_4^-

Comments: Authors indicated that plasma thyroid hormones and thyroid weight were not as sensitive as an indicator of thyroid functional response as thyroidal T_4.

Final NOAEL based on decreased T_4: 0.0033 mg/kg-d
Final LOAEL based on decreased T_4: 0.033 mg/kg-d
Final NOAEL based on increased thyroid weight: 33 mg/kg-d
Final LOAEL based on increased thyroid weight: 65 mg/kg-d
Final NOAEL based on decreased tibia length: 130 mg/kg-d
Final LOAEL based on decreased tibia length: 261 mg/kg-d

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Compound: Ammonium perchlorate (NH_4ClO_4)
Form: NH_4ClO_4 (84.7% ClO_4^-)
Reference: McNabb et al. 2004b
Test Species: Bobwhite quail
Body weight: 165 g (assumed by the authors for dose calculation)
Life span: < 1 year (estimated)
Water Consumption: 0.0127 L-d (authors assumed water intake as 7.7% of adult quail body weight)
Study Duration: series of experiments of 2, 4, or 8 weeks (subchronic)
Endpoint: thyroid gland function (Plasma and thyroidal T_4 hormone levels) and thyroid weight
Exposure Route: oral in water
Dosage: 0, 0.0016, 0.0033, 0.033, 0.326, 1.6, 3.3. 16.3, 33, 65, 130, and 261 mg/kg-d as perchlorate ion (estimated from nominal NH_4ClO_4 in drinking water, assuming a water ingestion rate of 7.7% in adult bobwhite quail weight of 165 g)

Calculations:

NOAEL for decreased thyroidal T4 hormone level at 8 weeks
(84.7%)*(5 mg/L)*(0.0127 L-d) / (0.165 kg) = 0.326 mg/kg-d ClO_4^-
(Note: data for this endpoint were not reported for the 25 mg/L dose level, therefore, the next lower dose level [5 mg/L] was assumed to be the NOAEL)

LOAEL for decreased thyroidal T4 hormone level at 2 weeks
(84.7%)*(50 mg/L)*(0.0127 L-d) / (0.165 kg) = 3.3 mg/kg-d ClO_4^-

NOAEL for increased thyroid weight at 8 weeks
(84.7%)*(500 mg/L)*(0.0127 L-d) / (0.165 kg) = 33 mg/kg-d ClO_4^-

LOAEL for increased thyroid weight at 8 weeks
(84.7%)*(1000 mg/L)*(0.0127 L-d) / (0.165 kg) = 65 mg/kg-d ClO_4^-

Final NOAEL based on decreased T_4: 0.326 mg/kg-d
Final LOAEL based on decreased T_4: 3.3 mg/kg-d
Final NOAEL based on increased thyroid weight: 33 mg/kg-d

Final LOAEL based on increased thyroid weight: 65 mg/kg-d

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Compound: Ammonium perchlorate (NH_4ClO_4)
Form: NH_4ClO_4 (84.7% ClO_4^-)
Reference: Goleman et al. 2002a
Test Species: *Xenopus laevis*
Study Duration: 70-d egg through metamorphosis (chronic because the during critical life stage)
Endpoint: development (hatching success, hindlimb length, percent forelimb emergence, percent complete tail resorption
Exposure Route: in water (static-renewal)
Exposure: 0, 0.005, 0.018, 0.147, 1.412, 14.4, 133, 425, 585, and 1175 mg ammonium perchlorate/L

Calculations:

NOAEL for reduced hindlimb length
(84.7%)*(0.018 mg/kg) = 0.0152 mg ClO_4^-/L

LOAEL for reduced hindlimb length
(84.7%)*(0.147 mg/kg) = 0.125 mg ClO_4^-/L

Unbounded LOAEL for reduced percent forelimb emergence
(84.7%)*(0.005 mg/kg) = 0.0042 mg ClO_4^-/L

NOAEL for reduced percent complete to tail resorption
(84.7%)*(0.005 mg/kg) = 0.0042 mg ClO_4^-/L

LOAEL for reduced percent complete to tail resorption
(84.7%)*(0.018 mg/kg) = 0.0152 mg ClO_4^-/L

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Compound: Ammonium perchlorate (NH_4ClO_4)
Form: NH_4ClO_4 (84.7% ClO_4^-)
Reference: Goleman et al. 2002b
Test Species: *Xenopus laevis*
Study Duration: 70-d egg through metamorphosis (chronic because the during critical life stage)
Endpoint: development (hatching success, hindlimb length, percent forelimb emergence, percent complete tail resorption), endocrine (T4 concentrations), thyroid hypertrophy, gonadal differentiation (male to female ratio)
Exposure Route: in water (static-renewal)
Exposure: 0.059 and 14.1 mg ammonium perchlorate/L

Calculations:

Unbounded LOAEL for reduced hindlimb length, percent forelimb emergence, percent completing tail resorption, significant hypertrophy of the thyroid follicular epithelium, and increased percentage of males at metamorphosis
(84.7%)*(0.059 mg/kg-d) = 0.05 mg ClO_4^-/L

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