



# Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization

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National Center for Environmental Assessment  
Office of Research and Development  
U.S. Environmental Protection Agency  
Washington, DC

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## Preface

The purpose of this review is to provide scientific support and rationale for hazard identification and dose-response assessments based on the emerging data for both human health and ecological effects caused by exposures to perchlorate. It is not intended to be a comprehensive treatise on the chemical or the toxicological nature of perchlorate.

In Chapter 10, the U.S. Environmental Protection Agency (EPA) has characterized its overall confidence in the quantitative and qualitative aspects of hazard and dose-response (U.S. Environmental Protection Agency, 1995) for both the human health and ecotoxicological effects of perchlorate. Matters considered in this characterization include knowledge gaps, uncertainties, quality of data, and scientific controversies. This characterization is presented in an effort to make apparent the limitations of the individual assessments and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

Development of these hazard identifications and dose-response assessments for perchlorate have followed the general guidelines for risk assessments set forth by the National Research Council (1983). Other EPA guidelines that were used in the development of this health risk assessment include the Assessment of Thyroid Follicular Cell Tumors (U.S. Environmental Protection Agency, 1998a), Guidelines for Neurotoxicity Risk Assessment (U.S. Environmental Protection Agency, 1998b), 1996 Proposed Guidelines for Carcinogen Risk Assessment (Federal Register, 1996), Guidelines for Reproductive Toxicity Assessment (U.S. Environmental Protection Agency, 1996a), Use of the Benchmark Dose Approach in Health Risk Assessment (Crump et al., 1995), Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry (U.S. Environmental Protection Agency, 1994), Proposed Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicology Studies (Whalan and Redden, 1994), Guidelines for Developmental Toxicity Risk Assessment (Federal Register, 1991), Recommendations for and Documentation of Biological Values for Use in Risk Assessment (U.S. Environmental Protection Agency, 1988), The Risk Assessment Guidelines of 1986 (U.S. Environmental Protection Agency, 1987), and the Guidelines for Ecological Risk Assessment (U.S. Environmental Protection Agency, 1998c).

The document presents the hazard identification or dose-response assessment for noncancer toxicity for each route of exposure, either the oral reference dose (RfD) or the inhalation

reference concentration (RfC). The RfD and RfC are meant to provide information on long-term effects other than carcinogenicity, although more recently, the value of mode-of-action information to inform the potential for a continuum from noncancer toxicity as precursor lesions to carcinogenicity presented as tumors has been recognized (Federal Register, 1996; Wiltse and Dellarco, 1996). Consideration of this continuum is especially pertinent to the evaluation of the potential toxicity of perchlorate. When such a continuum can be characterized, the dichotomous approaches to “noncancer” versus “cancer” toxicity can be harmonized into one route-specific estimate. The objective is to select a prominent toxic effect or key event that is pertinent to the chemical’s key mode of action, defined as a chemical’s influence on molecular, cellular, and physiological functions (Wiltse and Dellarco, 1996). A harmonized approach to the neurodevelopmental and neoplastic effects of perchlorate is proposed herein.

In a default characterization without mode-of-action information, the RfD typically is based, in part, on the assumption that a threshold exists for certain toxic effects, both for the individual and the population; whereas, a threshold may not exist for other carcinogenic effects. Thus, if the critical toxic effect is prevented, then all toxic effects are prevented. In general, the RfD or RfC is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure or continuous inhalation exposure to the human population (including sensitive subpopulations) that is likely to be without deleterious noncancer effects during a lifetime. The oral RfD is expressed in units of milligrams per kilogram per day. The inhalation RfC considers toxic effects for both the respiratory tract as the portal of entry, as well as for effects remote to the respiratory tract (extra-respiratory or systemic effects). The RfC is expressed in units of milligrams per cubic meter.

The carcinogenicity assessment is meant to provide information on three aspects of the carcinogenic risk assessment for perchlorate: the EPA classification and quantitative estimates of risk from both oral and inhalation exposure. The classification reflects a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed.

The screening-level ecological risk assessment of environmental contamination by perchlorate follows the Guidelines for Ecological Risk Assessment (U.S. Environmental Protection Agency, 1998c).

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# EXECUTIVE SUMMARY

The purposes of this document is to present an assessment that updates previous provisional values issued by the U.S. Environmental Protection Agency (EPA) for an oral reference dose (RfD) for perchlorate and revises the assessment previously released as a draft external review document (U.S. Environmental Protection Agency, 1998d). The objective of this assessment is to derive a human health risk estimate, based on an evaluation of its potential to cause toxicity or cancer, and to provide a screening-level ecological risk assessment for perchlorate based on all toxicity data that recently have become available to the Agency as of fall 2001. Another important objective was to evaluate the evidence for indirect exposures, i.e., those exposures not by direct ingestion of contaminated water. This revised assessment incorporates data from new studies and analyses in response-level to recommendations made at a previous peer review of the 1998 draft (Research Triangle Institute, 1999). Most of these data were obtained as results of a testing strategy that was designed with knowledge of the mode of action for perchlorate toxicity that identified major data gaps in the data available prior to 1997. This executive summary concisely presents key findings from the present assessment.

## SUMMARY FINDINGS

### Sources of Perchlorate Contamination and Occurrence

- Perchlorate is an oxidizing anion that originates as a contaminant in ground and surface waters from the dissolution of ammonium, potassium, magnesium, or sodium salts. Perchlorate is exceedingly mobile in aqueous systems and can persist for many decades under typical ground and surface water conditions.
- Ammonium perchlorate is manufactured for use as the oxidizer component and primary ingredient in solid propellant for rockets, missiles, and fireworks. Because it is a reducing agent, it can undergo a variety of intramolecular redox reactions that lead to the release of gaseous products. Through such reactions, it acts as a thrust booster.
- Perchlorate salts are also used on a large scale as a component of air bag inflators. Perchlorate salts are also used in nuclear reactors and electronic tubes, as additives in lubricating oils, in

1 tanning and finishing leather, as a mordant for fabrics and dyes, in electroplating, in aluminum  
2 refining, and in rubber manufacture, as a mordant for fabrics and dyes, and in the production of  
3 paints and enamels. Chemical fertilizer had been reported to be a potential source of  
4 perchlorate contamination, but new investigations by the Agency have determined that this is  
5 not an issue for agricultural applications.

- 6 • Large-scale production of perchlorate-containing chemicals in the United States began in the  
7 mid-1940s. Because of its shelf life, perchlorate must be washed out of the United States'  
8 missile and rocket inventory to be replaced with a fresh supply. Thus, large volumes have been  
9 disposed of in various states since the 1950s.
- 10 • Perchlorate began to be discovered at various manufacturing sites and in well water and  
11 drinking water supplies within the months following the April 1997 development of an ion  
12 chromatography analytical method that achieved a method detection limit (MDL) of  
13 approximately 1 ppb and a minimum reporting limit (MRL) of 4 ppb. There are 20 states with  
14 confirmed releases in ground or surface water. There are 40 states that have confirmed  
15 perchlorate manufacturers or users based on EPA Information Request responses.  
16 In California, most of the locations where perchlorate has been detected are associated with  
17 facilities that have manufactured or tested solid rocket fuels for the Department of Defense or  
18 the National Aeronautics and Space Administration.
- 19 • To date, there has not been a systematic national survey of perchlorate occurrence and a  
20 National Primary Drinking Water Regulation for perchlorate does not currently exist.  
21 Perchlorate was placed on the Contaminant Candidate List (CCL) in March 1998. The CCL  
22 lists priority contaminants (defined as either known or anticipated to occur in public water  
23 systems) in need of research, guidance development, regulatory determinations, or monitoring  
24 by the states. Perchlorate was listed as a contaminant that required additional research and  
25 occurrence information before regulatory determinations could be considered.
- 26 • Perchlorate was placed on the Unregulated Contaminants Monitoring Rule (UCMR) in March  
27 1999 (Federal Register, 1999) to gather needed exposure information. Under the UCMR, all  
28 large public water systems and a representative sample of small public water systems were  
29 required to monitor for perchlorate beginning in January 2001. This effort does not extend to  
30 investigating potential sources in ground and surface water that have not migrated into public  
31 water supplies. Identification of the magnitude and extent of perchlorate occurrence in the

1 environment is important in assessing the routes of exposure to humans and for determining the  
2 different types of organisms and ecosystems that may be affected.

- 3 • In early 2000, an analytical method to detect perchlorate in drinking water (EPA Method 314.0)  
4 using ion chromatography was published as a direct final rule (Federal Register, 2000). The  
5 EPA Method 314.0 was also approved as a monitoring method for the UCMR (Federal  
6 Resister, 2000). The MDL for the method is 0.53 ppb and the MRL is 4 ppb. Improvements  
7 developed commercially in the analytical capabilities may lower the MRL to the sub-part per  
8 billion level in the near future.
- 9 • Adequate exposure characteristics of transport and transformation in the environment are also  
10 absent. Preliminary biotransport studies at six contaminated sites indicate a potential for  
11 uptake into plant and animal tissues in ecosystems. Extension of analytical methods to detect  
12 perchlorate in plant and animal tissues awaits validation before a conclusive determination can  
13 be made.

#### 14 15 **An Integrated Approach to Comprehensive Risk Characterization**

- 16 • Perchlorate is of concern for several reasons. First, there were uncertainties in the toxicological  
17 database available that could be used to evaluate the potential for perchlorate to produce human  
18 health effects when present at low levels in drinking water. The purpose of the targeted  
19 toxicity testing strategy was to develop a database to address key data gaps. Secondly, the  
20 actual extent of the occurrence of perchlorate in ground and surface waters is not known at this  
21 time. Additionally, the efficacy of different treatment technologies for various water uses (such  
22 as drinking water or agricultural applications) and different scales (i.e., large or small volumes)  
23 is still being determined. Finally, the extent and nature of ecological impact or transport and  
24 transformation phenomena in various environmental media have only, as yet, been studied  
25 superficially.
- 26 • To adequately and comprehensively characterize the risks posed by perchlorate contamination  
27 and to develop scientifically-based management strategies that effectively mitigate the potential  
28 risks posed by perchlorate contamination, several advances are essential. The analytical  
29 methods used to characterize various exposures must be accurate and precise. The exposure  
30 estimates cannot be gauged with respect to their risk unless robust health and ecological risk  
31 estimates are available. Treatment technologies should be targeted to levels of concern and

1 tailored to the intended water use. Technology transfer is necessary so that all affected parties  
2 and concerned citizens are apprised of accurate and reliable information that is up to date with  
3 the evolving state of the science.

- 4 • The toxicity testing strategy was expedited through a unique partnership between the  
5 Department of Defense and EPA, together with members of an Interagency Perchlorate  
6 Steering Committee (IPSC), which includes other governmental representatives from the  
7 National Institute for Environmental Health Sciences (NIEHS) and affected state, tribal, and  
8 local governments.
- 9 • The charge of the IPSC is to facilitate and coordinate accurate accounts of related technological  
10 issues (occurrence surveys, health assessment, ecotoxicology assessment, treatability, waste  
11 stream handling, and analytical detection). This assessment is intended to address the need for  
12 evaluation of perchlorate's potential to cause human health effects or impact on ecological  
13 systems, based on currently available data.

#### 14 15 **Physicochemical Characteristics**

- 16 • As an oxidant, perchlorate is kinetically nonlabile. This means the reduction of the central  
17 chlorine atom from an oxidation state of +7 (perchlorate) to -1 (chloride ion) occurs extremely  
18 slowly. Sorption is not expected to attenuate perchlorate because it absorbs weakly to most soil  
19 minerals. Natural chemical reduction in the environment is not expected to be significant.  
20 These two factors account for perchlorate being both very mobile in aqueous systems and  
21 persistent for many decades under typical ground and surface water conditions.
- 22 • The activation energy to perchlorate reduction is so high that it cannot be expected to act as an  
23 oxidant under human physiological conditions (i.e., dilute solution, unelevated temperatures,  
24 neutral pH). This is supported by absorption, distribution, metabolism, and elimination studies  
25 that show perchlorate is excreted virtually unchanged in the urine after absorption.

#### 26 27 **Hazard Identification and Mode of Action Testing Strategy**

- 28 • The health effects and toxicity database available in the spring of 1997 was determined to be  
29 inadequate for quantitative risk assessment by an independent (non-EPA) peer review. A  
30 testing strategy was developed based on a hazard identification using the available data and the  
31 suspected mode of action for perchlorate to target testing on potential effects of perchlorate.

1 Data from this effort was used to support the previous EPA draft assessment and this revised  
2 assessment in 2002.

- 3 • To design a testing strategy based on the mode of action for a chemical, it is necessary to  
4 understand its toxicokinetics and toxicodynamics. Perchlorate is readily absorbed from the  
5 intestinal tract, and oral uptake is considered to be the major route of exposure. Because of its  
6 high charge, perchlorate does not pass readily through the skin. Exposure via inhalation is  
7 expected to be negligible because the vapor pressure of perchlorate salts and acids is expected  
8 to be low at room temperatures. Droplet size during showering likely would preclude  
9 inhalation of perchlorate-contaminated water as an aerosol. Perchlorate is known to inhibit the  
10 uptake of iodide in the thyroid at the sodium (Na<sup>+</sup>)–iodide (I<sup>-</sup>) symporter, or NIS, thereby  
11 causing a reduction in the hormones thyroxine (T4) and triiodothyronine (T3). When these  
12 hormones enter the blood circulation, they are bound to plasma proteins. There may be other  
13 locations of inhibition of iodide transport in the gland. Perchlorate itself is not metabolized in  
14 the thyroid or peripheral tissues.
- 15 • Control of circulating concentrations of these hormones is regulated primarily by a negative  
16 feedback known as the hypothalamic-pituitary-thyroid axis or feedback system involving three  
17 organs: (1) the thyroid, which produces T4 and T3; (2) the pituitary gland which produces  
18 TSH; and (3) the hypothalamus, which also responds to and helps to maintain optimal T4 and  
19 T3 levels. The hypothalamus stimulates the pituitary gland through thyrotrophic-releasing  
20 hormone (TRH) to produce thyroid stimulating hormone (TSH), which then prompts the  
21 thyroid to produce T4 and T3. Cells in the hypothalamus and pituitary gland respond to the  
22 levels of circulating T4 and T3, such that when thyroid production levels are low, there is a  
23 signal to increase the output of TRH and TSH. Circulating hormone levels (T4, T3, and TSH)  
24 can be monitored readily to serve as biomarkers of exposure and effect of agents that disrupt  
25 the status of this negative feedback system.
- 26 • The hypothalamic-pituitary-thyroid feedback system for regulation of thyroid hormones is  
27 conserved across species. Differences in plasma protein binding between rats and humans  
28 account for differences in the circulating half-life of the hormones and in thyroid response to  
29 TSH between the species. New studies since 1999 have confirmed that the inhibition of iodide  
30 uptake by perchlorate at the NIS is essentially the same sensitivity across species. This is

1 important when considering decrements in T4 as important to neurodevelopmental effects  
2 versus neoplasia that results in the gland due to stimulation by TSH.

- 3 • Given its mode of action as an inhibitor of iodide uptake that results in disturbances of the  
4 hypothalamic-pituitary-thyroid axis, concerns arose about the potential for perchlorate to cause  
5 carcinogenic, neurodevelopmental, developmental, reproductive, and immunotoxic effects.  
6 Further, there is concern for ecotoxicology effects on various aquatic and terrestrial plants and  
7 animals.
- 8 • The human health testing strategy for perchlorate developed in 1997 originally included eight  
9 different recommended studies to address data gaps and enhance the mechanistic information  
10 on the mode of action. The goal of these studies was to provide a comprehensive database on  
11 which to arrive at a revised human health risk assessment with greater confidence than previous  
12 recommended provisional values. These studies are described briefly below.
  - 13 (1) A 90-day oral bioassay to identify other target tissues in young adult rats; to provide data  
14 on the effects of repeated exposures to perchlorate on T3, T4, and TSH levels; to  
15 evaluate recovery of effects after 30 days; and to screen for some reproductive  
16 parameters. A genotoxicity assay also was performed on rats from the terminal sacrifice.
  - 17 (2) A neurodevelopmental study in rats to evaluate the potential for functional and  
18 morphological effects in offspring from the mother exposed during pregnancy and  
19 lactation.
  - 20 (3) A Segment II developmental study in rabbits to evaluate the potential for perchlorate to  
21 cause birth defects and to provide data on thyroid hormone effects in a second species  
22 other than the rat.
  - 23 (4) A two-generation reproductive toxicity study to evaluate the potential for perchlorate to  
24 cause deficits in reproductive performance in adult rats and for toxicity in the young  
25 offspring.
  - 26 (5) Absorption, distribution, metabolism, and elimination (ADME) studies to characterize  
27 the pharmacokinetics of perchlorate in laboratory animals and humans and to provide  
28 data necessary to allow construction of models for quantitative description of different  
29 internal dose metrics and interspecies extrapolation.

- 1 (6) Mechanistic studies that characterize the effects of perchlorate on the iodide uptake  
2 mechanism across species as a link with the ADME studies to aid in the quantitative  
3 extrapolation of dose across species.
- 4 (7) A battery of genotoxicity assays to evaluate the potential for carcinogenicity by  
5 evaluating the potential for direct effects on deoxyribonucleic acid (DNA).
- 6 (8) Immunotoxicity studies to evaluate the potential for perchlorate to disrupt immune  
7 function, including cell-mediated and humoral toxicity.
- 8 • After the External Peer Review in 1999, additional studies were performed to replicate the  
9 neurodevelopmental study (i.e., changes in brain morphometry and motor activity); determine  
10 the developmental toxicity potential in rats versus rabbits; investigate additional aspects of  
11 immunotoxicity; and develop a consistent nomenclature and scoring system for the  
12 histopathological lesions in the thyroid gland. Additional pharmacokinetic data was also  
13 developed into physiologically-based pharmacokinetic (PBPK) models of perchlorate and  
14 iodide distribution.
  - 15 • A battery of ecological screening tests as part of the 1997 testing strategy was conducted as  
16 part of the 1997 testing strategy in laboratory organisms representative of ecological receptors  
17 across soil, sediment, and water to evaluate dose-response relationships. These were  
18 considered to be a tier of tests to give an idea of gross toxicity that would determine the need  
19 and types of tests to be performed in the next tier. The tests did not measure the amount of  
20 perchlorate in the tissues of the species being tested. Based on stakeholder input and the need  
21 for a more focused battery of tests, lettuce was substituted for duckweed because of Tribal  
22 concerns regarding the sizable lettuce crop along the Colorado river. The following species  
23 were selected for the first round of testing:
    - 24 (1) *Daphnia magna* (water flea) to represent an aquatic invertebrate
    - 25 (2) *Ceriodaphnia magna* (water flea) to represent an aquatic invertebrate
    - 26 (3) *Lactuca sativa* (lettuce) to represent a vascular plant
    - 27 (4) *Pimephales promelas* (fathead minnow) to represent an aquatic invertebrate
    - 28 (5) *Eisenia foetida* (earthworm) to represent a soil invertebrate
    - 29 (6) *Microtus pennsylvanicus* (meadow vole) to represent an herbivore
  - 30 • Other studies in the set of tests included the Frog Embryo Teratogenesis Assay: *Xenopus*  
31 (FETAX) and a phytoremediation study to examine uptake, distribution, and degradation in

1 experimental systems with rooted cuttings of woody plants, including willow, Eastern  
2 Cottonwood, and eucalyptus.

- 3 • Additional studies, some of chronic duration, on effect levels in aquatic animals, an aquatic  
4 plant, a terrestrial plant, and a soil invertebrate have been performed since 1999. A study of  
5 perchlorate occurrence in six selected sites with known or suspected contamination also  
6 examined perchlorate concentrations in site media and in various ecological receptors.

### 7 8 **Human Health Assessment**

- 9 • The testing strategy confirmed that the target tissue for perchlorate toxicity was the thyroid  
10 gland. Anti-thyroid effects included iodide uptake inhibition, perturbations of T3, T4, and TSH  
11 hormones, and thyroid histopathology in adult, fetal, and postnatal rats across studies with a  
12 range of experimental design. Thyroid weight in these studies was also effected. Other than  
13 effects in the thyroid, no effects were observed in rabbits of the developmental study, but the  
14 developmental study in rats identified 30 mg/kg-day as the lowest observed adverse effect level  
15 (LOAEL).
- 16 • Competitive inhibition of iodide uptake at the NIS by perchlorate is the key event leading to  
17 both potential neurodevelopmental and neoplastic sequelae. The decrement in iodide uptake  
18 leads to subsequent drops in T4 and T3 that can lead to permanent neurodevelopmental  
19 deficits. Because of strong correlations between changes in iodide uptake inhibition with  
20 decrements in T3 and T4; between T3 and T4 with changes in TSH; and between changes in  
21 T3, T4, or TSH with thyroid histopathology, an assessment model was proposed that used the  
22 changes in T3, T4, and TSH as the precursor lesions to subsequent effects that potentially could  
23 lead to thyroid tumors or to altered neurodevelopment. This assessment approach essentially  
24 harmonizes noncancer and cancer approaches because it is presumed that the no-observed-  
25 adverse-effect-level (NOAEL) for the precursor lesions will preclude any subsequent sequelae  
26 at higher doses.
- 27 • Thyroid tumors were observed in previous studies in rats exposed in long-term bioassays at  
28 high doses. Thyroid tumors were more recently also diagnosed in the first-generation (F1)  
29 adults (second parental generation [P2]) at 19 weeks in a two-generation reproductive study.  
30 Both the latency and incidence of these tumors were significant relative to the entirety of the  
31 National Toxicology Program data base for this type of tumor and in this strain of rat. These

1 effects and the demonstration of a progression with duration of effects on hormones and thyroid  
2 histopathology in the 90-day study raised the concern that extended exposures to perchlorate  
3 may change the hypothalamic-pituitary-feedback system or the cellular sensitivity and demand  
4 for thyroid hormones.

- 5 • The rat model is considered relevant yet conservative for human health risk assessment of  
6 potential thyroid neoplasia because of the differences in thyroid structure and hormone  
7 half-lives. Perchlorate was demonstrated to be nongenotoxic in the testing battery employed,  
8 suggesting the antithyroid effects are an indirect mode of action for thyroid tumor formation.
- 9 • Due to the age- and time-dependent nature of the key event of perchlorate toxicity and its  
10 anti-thyroid effects, the revised RfD was based on weight-of-the-evidence approach to the  
11 entire data base. The RfD is proposed to be protective of both neurodevelopmental and  
12 neoplastic sequelae. An administered dose of 0.01 mg/kg-day was supported as a lowest-  
13 observed-adverse-effect level (LOAEL) based on effects on brain morphometry in pups from a  
14 PND21 sacrifice in a neurodevelopmental study that repeated similar observations made in a  
15 similar 1998 study, hormonal effects indicative of hypothyroidism (decreased T4 and increased  
16 TSH) in the dams of those same pups on GD21, thyroid histopathology and hormone changes  
17 in these same pups at various developmental stages (GD21, PND4, PND9, and PND21),  
18 thyroid histopathology and hormone changes at the 14- and 90-day sacrifice dates in a  
19 subchronic study, and indications of immunotoxicity (dermal contact hypersensitivity).
- 20 • A human equivalent exposure (HEE) was calculated using physiologically-based  
21 pharmacokinetic (PBPK) models for interspecies adjustment based on the area under the curve  
22 (AUC) of perchlorate in the serum as the dose metric. The HEE for the maternal dams was  
23 chosen for operational derivation because brain morphometry effects may have been  
24 programmed *in utero* and because the dams of effected pups were hypothyroid.
- 25 • A composite uncertainty factor of 300 was used to address uncertainties in the extrapolations  
26 required for the RfD derivation. A three-fold factor for intraspecies variability was retained  
27 due to the variability observed in the data and PBPK modeling for the adult humans and  
28 because the subjects used to develop the models did not provide kinetic data for the potentially  
29 susceptible population. There was also uncertainty in the parallelogram approach to extending  
30 the adult structures to predict doses for different life stages in the human. A full factor of ten  
31 was applied to extrapolate the LOAEL for the adverse effects (brain morphometry, colloid

1 depletion and hormone changes) observed in various studies at the 0.01 mg/kg-day dosage  
2 level. A three-fold factor for duration was applied due to the concern for the biological  
3 importance of the statistically significant increase in tumors observed in the F1-generation pups  
4 (second parental, P2 generation) at 19 weeks and the evidence for progression of effects with  
5 extended exposure in the 90-day study. The finding of tumors at 19 weeks raised concern for  
6 *in utero* programming, i.e., that disruption of thyroid hormones in the developing fetus may  
7 predispose the developing neonate and adult to future insults to the thyroid gland. This factor  
8 can also be viewed as part of a data base deficiency since there are no adequate long-term  
9 bioassays of perchlorate. Finally, a three-fold factor was applied for inaccurate characterization  
10 of immunotoxicity since recent studies reinforced concern for this potential endpoint. Because  
11 the test article was ammonium perchlorate, an adjustment factor of 0.85 was made for the  
12 percent of molecular weight of the salt from ammonium (15.35%), so that the RfD is expressed  
13 for perchlorate as the anion alone. This was done to be compatible with the analytical methods  
14 that measure the anion in environmental samples and because most perchlorate salts readily  
15 dissolve in water. The resultant revised RfD value for perchlorate is 0.00003 mg/kg-day.  
16 Confidence in the principal study, the data base and the RfD were all designated as medium.

### 17 18 **Screening Ecological Risk Assessment**

- 19 • A secondary acute value of 5 mg/L (as perchlorate) was derived to be protective of 95% of  
20 aquatic organisms during short-term exposures with 80% confidence. The secondary chronic  
21 value of 0.6 (as perchlorate) likewise was derived to be protective of 95% of aquatic organisms  
22 during short-term exposures with 80% confidence. These values were derived based on  
23 sodium perchlorate and are probably protective even if ammonium perchlorate is the  
24 contaminant released. Calculated ammonia-nitrogen concentrations corresponding to those  
25 values are below the acute and chronic ambient water quality criteria for ammonia, regardless  
26 of pH.
- 27 • For terrestrial plants, the quartile inhibitory concentrations for growth in soil and sand were  
28 78 mg/kg (293 mg/L) and 41 mg/kg (160 mg/L), respectively. A factor of 10 was applied to  
29 account for interspecies variance to obtain a screening benchmark of 4 mg/kg.

- 1 • Because of limited data on effects for soil invertebrates, a conservative estimate of a threshold  
2 for soil community effects was derived at 1 mg/kg. The equivalent aqueous phase benchmark  
3 is 2.8 mg/L.
- 4 • A factor of 10 for interspecies variance and LOAEL to NOAEL extrapolation was applied to  
5 the human health risk LOAEL estimate based on rat data (0.01 mg/kg-day) to obtain a  
6 screening benchmark of 0.001 mg/kg-day for the representative herbivore (meadow vole)  
7 because it also is a rodent. The population-level implications of this effect are unknown, but it  
8 seems likely that such effects on the thyroid could diminish survivorship and fecundity, which  
9 would diminish population production.
- 10 • Data are available showing that perchlorate accumulates in the tissues of exposed fish,  
11 amphibians, and invertebrates. However, data are insufficient to determine whether perchlorate  
12 is concentrated in those tissues to levels exceeding the levels of exposure. By contrast, several  
13 studies have shown that perchlorate is taken up and concentrated in aerial plant parts, especially  
14 leaves, although studies designed for the purpose of quantifying plant concentration factors  
15 have not yet been conducted.

#### 16 17 **Uncertainties and Assessment Research Needs**

- 18 • Accurate exposure information is a requisite for risk characterization for both human and  
19 ecological assessments. These data should include transport and transformation processes,  
20 notably the fate of perchlorate in irrigated soils because of the potential for evaporative  
21 concentration.
- 22 • Research concerning the human health risks of perchlorate needs to better characterize the  
23 dose-response for perchlorate inhibition of iodide uptake in adults, fetuses, and neonates. More  
24 definitive studies linking iodide uptake inhibition and the degree of perturbation of the  
25 hypothalamic-pituitary-thyroid axis (i.e., changes in T3, T4, and TSH levels) and association  
26 with neurobehavioral problems, thyroid changes, and neoplastic sequelae may continue to  
27 improve the confidence in the assessment. Understanding the relative sensitivity of laboratory  
28 animal assays of neurodevelopmental effects versus epidemiological studies of  
29 neuropsychological development also needs to be advanced. Research on potential factors  
30 influencing sensitivity is critically requisite. Animal models of thyroid impairment such as  
31 iodide deficiency and “womb to tomb” exposure designs should be explored.

- 1 • Because only a screening tier of tests has been performed, the major uncertainty derives from  
2 data gaps. Data on bioaccumulation in aquatic biota would allow evaluation of exposure of  
3 organisms that feed on fish and other aquatic organisms. Effects of perchlorate on algae and  
4 aquatic macrophytes are required to estimate risks to aquatic primary producers. Data on  
5 bioaccumulation in aquatic plants are necessary to assess direct impact to primary consumers  
6 (i.e., planktonic and benthic invertebrate communities). Data on accumulation in terrestrial  
7 vascular plants also should be investigated further. The factor applied for the use of subchronic  
8 data in fish could be addressed by chronic effect testing. Effects also should be determined in  
9 nondaphnid invertebrates and of dietary exposure in birds and herbivorous or litter-feeding  
10 invertebrates.

### 11 **Risk Characterization**

- 13 • As noted above, the lack of exposure information precludes comparison with the human health  
14 and ecological toxicity assessment for accurate characterization of risk. Indirect human  
15 exposure pathways can be addressed best by a new EPA document, Methodology for Assessing  
16 Health Risks Associated with Multiple Pathway of Exposure to Combustor Emissions, which is  
17 scheduled for final release in January 2002.
- 18 • Noncancer neurobehavioral effects have been shown at lower doses. The estimate for  
19 perchlorate has been based on precursor effects considered protective for both the thyroid  
20 neoplasia and neurodevelopmental effects. It is appropriate for comparison against direct oral  
21 exposures. The frequency and magnitude of exposure are key attributes for characterization  
22 compared with those assumptions of continuous lifetime exposure assumed in the derivation.  
23 The degree to which the particular suspected population at risk fits with the assumptions used  
24 in the RfD derivation should be kept in mind when performing any risk characterization.  
25 Further, RfD estimates are not intended to serve as a “bright line” because, by definition, there  
26 is an order-of-magnitude uncertainty around the estimate. This typically translates into a range  
27 of threefold below to threefold above the RfD.
- 28 • Ecological risk could not be precluded nor accurately characterized because of the significant  
29 data gaps described above.

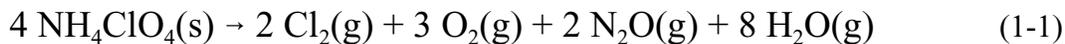
# 1. INTRODUCTION

The purpose of this document is to revise the previous human health and ecological risk assessment external review draft (ERD) document (U.S. Environmental Protection Agency, 1998d). This revision is based on recommendations made at the 1999 external peer review (Research Triangle Institute, 1999). The peer review panel recommended some alternative analyses and several additional studies. This revised assessment addresses these recommendations and is based on all data made available to the Agency as of Fall 2001; including new studies from the perchlorate testing strategy. The purpose of this chapter is to provide background information on the current status of perchlorate ( $\text{ClO}_4^-$ ) contamination in the United States and an historical perspective on how certain issues of concern have evolved to prominence. The role of this risk assessment will be placed in context with respect to the overall integrated approach to addressing the perchlorate contamination and regulatory readiness.

## 1.1 PRODUCTION USES AND SOURCES OF PERCHLORATE CONTAMINATION

Perchlorate is an oxidizing anion that originates as a contaminant in ground and surface waters from the dissolution of perchloric acid and of the salts including ammonium, potassium, magnesium, or sodium. With the exception of potassium perchlorate, each of these compounds is extremely soluble. Potassium perchlorate is regarded as sparingly soluble; however, it may dissolve completely under the appropriate environmental conditions.

Ammonium perchlorate is the oxidizer and primary ingredient (by mass) in solid propellant for rocket motors. For example, ammonium perchlorate ( $\text{NH}_4\text{ClO}_4$ ) makes up 69.7% of the propellant for the space shuttle rocket motors and 65 to 75% of the Stage I motors of the Minuteman III and 68% of the Titan missile motors (Rogers, 1998). Because the ammonium ion is a reducing agent, ammonium perchlorate can undergo a variety of intramolecular redox reactions that lead to the release of gaseous products. The explosive decomposition shown in Equation 1-1 is induced thermally and occurs at temperatures below 300 °C (Schilt, 1979a).



2  
3 Through such reactions, ammonium perchlorate also acts as a thrust booster. Even after such  
4 decomposition, the dichlorine and dioxygen thus produced remain capable of engaging in  
5 subsequent redox reactions with fuels.

6 Specific uses of various perchlorate salts include: solid rocket fuel oxidizer, flares, and  
7 pyrotechnics (potassium); solid rocket fuel oxidizer, explosives, chemical processes and  
8 pyrotechnics (ammonium); precursor to potassium and ammonium perchlorate and in explosives  
9 (sodium); and military batteries (magnesium) (Rogers, 1998). Perchlorate salts also are used on a  
10 large scale as a component of air bag inflators. Other industrial or commercial applications of  
11 perchlorate salts include their use in nuclear reactors and electronic tubes; as additives in  
12 lubricating oils; in tanning and finishing leathers; as a mordant for fabrics and dyes;  
13 in electroplating, aluminum refining, and rubber manufacture; and in the production of paints and  
14 enamels (Siddiqui et al., 1998). A 1998 report raised the concern that chemical fertilizer is  
15 a potential source of perchlorate contamination (TRC Environmental Corporation, 1998). More  
16 recent studies limit concern to certain types of fertilizer containing Chilean caliche (Urbansky,  
17 2000; U.S. Environmental Protection Agency, 2001a,b; Urbansky and Collette, 2001); however,  
18 production practices have been changed to address that issue. Besides their large-scale  
19 commercial uses, perchlorate salts often are employed on a small scale in laboratory chemical  
20 studies as ionic strength adjustors or as noncomplexing counterions. Some still are used in  
21 medical diagnostics in thyroid function tests. Perchloric acid is used for various laboratory  
22 applications requiring a strong acid. Wet ashing organic matter with perchloric acid still is  
23 performed today as a means of preparation for certain samples. Anhydrous magnesium  
24 perchlorate ( $\text{Mg}(\text{ClO}_4)_2$ ) is a strong desiccant; however, historically, Anhydrone<sup>®</sup>, a slightly  
25 hydrated form of  $\text{Mg}(\text{ClO}_4)_2$ , has been used to collect the water formed in combustion analyses.

26 The large-scale production of perchlorate-containing chemicals in the United States began  
27 in the mid-1940s. The approximate percentages sold for specific end uses are 92% as an  
28 oxidizer, 7% as an explosive, and 1% other uses (American Pacific Corporation, 1998). The  
29 typical volume of production ranged from 1 to 15 million lb per year (Rogers, 1998) although  
30 production in the 1980's was generally 20 to 30 million pounds per year (Kerr-McGee Chemical  
31 LLC, 1998; American Pacific Corporation, 1998). Solid rocket fuel inventories are growing at a

1 significant rate as systems reach the end of their service life and as treaties mandate motor  
2 disposal. The current disposal method for these motors is open burning or open detonation, both  
3 of which are becoming increasingly difficult to perform under intense public and regulatory  
4 pressure based, in part, on concern over incomplete destruction via these methods. Currently, the  
5 large solid rocket motor disposal inventory shows 55 million lb of propellant awaits disposal, and  
6 this number is expected to be over 164 million lb by the year 2005 (Siddiqui et al., 1998).  
7 A significant portion of this inventory contains ammonium perchlorate, which now can be  
8 reclaimed and recycled into new motor propellants. The accepted method for removal and  
9 recovery of solid rocket propellant from rocket motors is high-pressure water washout. This  
10 method generates large amounts of aqueous solution containing low concentrations of  
11 ammonium perchlorate. Although ammonium perchlorate can be recovered from these aqueous  
12 solutions, it is cost-prohibitive to remove it entirely. Most of the locations where perchlorate has  
13 been detected in ground or surface waters are in areas associated with development, testing, or  
14 manufacture of aerospace materials. Explosives and fireworks manufacturing and disposal  
15 operations have also been implicated in a number of environmental releases. Laboratory  
16 activities and fertilizer operations are potential sources of contamination in relatively few known  
17 instances. Perchlorate contamination also may occur where mining activities use explosives  
18 extensively (Siddiqui et al., 1998).

19 When ammonium perchlorate is released to water, the salt is highly soluble and dissociates  
20 completely releasing ammonium (NH<sub>4</sub>) and perchlorate (ClO<sub>4</sub><sup>-</sup>):



23 Its high solubility is not affected by pH or temperature. It is likely that most of the ammonium  
24 has been biodegraded, and the cation in the environment is best viewed as mostly sodium (Na<sup>+</sup>)  
25 or possibly hydrogen (H<sup>+</sup>), especially where contamination levels are below 100 ppb;  
26 nevertheless, those regions with high concentrations of perchlorate ion probably retain a small  
27 fraction of ammonium ion (Urbansky, 1998a). At those sites where contamination has occurred  
28 for decades, very little (if any) ammonium ion has been found. To date, there has been no  
29 quantitative determination of the cations responsible for the charge balance.  
30

1 As an oxidant, perchlorate is kinetically nonlabile. This means that reduction of the central  
2 chlorine atom from an oxidation state of +7 (perchlorate) to -1 (chloride ion) occurs extremely  
3 slowly. This will be elaborated on in Chapter 2 in the discussion of physicochemical  
4 characteristics. Sorption is not expected to attenuate perchlorate concentrations because it  
5 absorbs weakly to most soil minerals. Natural chemical reduction in the environment is also not  
6 expected to be significant. Together, these two factors account for perchlorate's high mobility  
7 and persistence for many decades under typical groundwater and surface water conditions.  
8 Figure 1-1 summarizes the various pathways through which perchlorate can reach ground and  
9 surface water sources.

## 12 **1.2 EVOLUTION OF ANALYTICAL DETECTION METHODS AND** 13 **EMERGING OCCURRENCE DATA**

14 The Region 9 Office of the U.S. Environmental Protection Agency (EPA) first became  
15 aware of the potential contamination issues with perchlorate in 1985 when samples measured  
16 with a colorimetric method reported contamination in 14 wells ranging from 0.11 to 2.6 ppm  
17 (Takata, 1985). The Region 9 office requested assistance from the Centers for Disease Control  
18 and Prevention (CDC) to evaluate the potential health effects of these levels of perchlorate  
19 (Takata, 1985). In response the CDC recommended validation of the colorimetric measures but  
20 could not address the potential for toxicity of the chemical because of toxicity data  
21 insufficiencies (Margolis, 1986). The CDC also recommended additional testing to determine  
22 potential target tissues and the effects from long-term, low-level exposures. The absence of a  
23 valid analytical method to measure low concentrations of perchlorate and the lack of data with  
24 which to characterize the risk of toxicity led Region 9 of EPA to focus on chemicals other than  
25 perchlorate at these sites. By the early 1990s, however, perchlorate at detectable levels  
26 (>1 mg/L) was found in monitoring wells at a California Superfund site, and EPA Region 9  
27 increased its effort to establish a human-health-based reference dose (RfD) in order to help gauge  
28 the risk of the contamination that was beginning to be characterized. In 1997, after perchlorate  
29 was discovered in a number of California water supplies, the California Department of Health  
30 Services (CA DHS) adopted 18 ppb as its provisional action level.

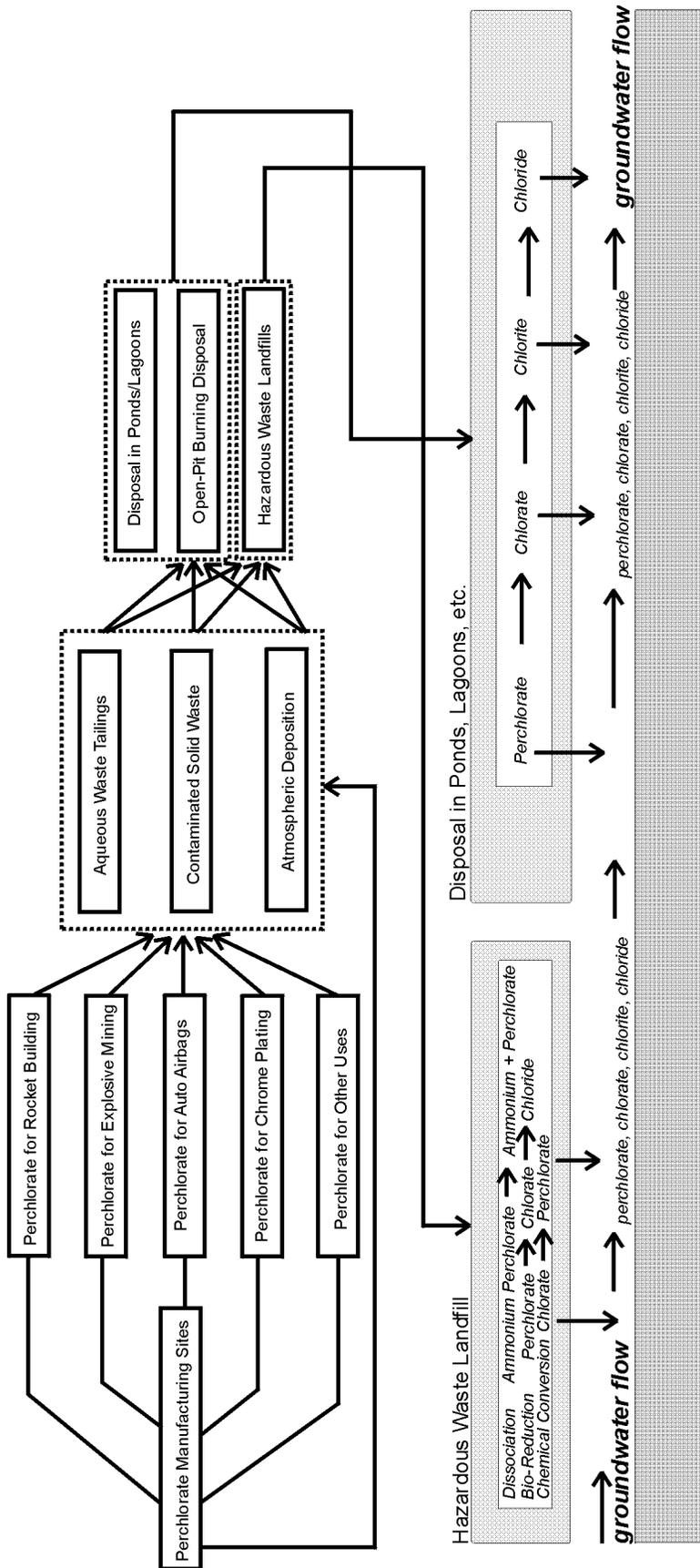


Figure 1-1. Sources and pathways of groundwater contamination for perchlorate. (Modified from Siddiqui et al., 1998.)

1           In January 1997, the California Department of Health Services' Division of Drinking Water  
2 and Environmental Management requested the Sanitation and Radiation Laboratory Branch  
3 (SRLB) test for perchlorate in drinking water wells potentially affected by groundwater migrating  
4 from the Aerojet facility near Sacramento. Based on its provisional action level, Region 9 of  
5 EPA indicated that a reporting limit of at least 4 ppb would be necessary. However, procedures  
6 to measure perchlorate at such low levels were not available. An ion chromatographic (IC)  
7 method was capable of detecting 400 ppb; and, during the previous year, Aerojet had improved  
8 the method to detect 100 ppb. By March 1997, SRLB and an analytical equipment manufacturer  
9 had developed an IC method that achieved a method detection limit of approximately 1 ppb and a  
10 reporting limit of 4 ppb. Within several months following the March 1997 development of the  
11 low-level (4 ppb) IC detection method, perchlorate was discovered at various manufacturing sites  
12 and in well water and drinking water supplies in California, Nevada, and Utah.

13           Efforts in several additional laboratories helped improve the IC method (Eldridge et al.,  
14 1999; Urbansky, 2000). Although IC is the dominant analytical method used at this time, a  
15 variety of additional techniques are being refined for perchlorate analysis, including: mass  
16 spectrometry, Raman spectrometry, capillary electrophoresis, and others (Urbansky, 2000).  
17 Recent publications have reported detection of perchlorate in tap water at levels as low as 0.1 ppb  
18 (Handy et al., 2000; Koester et al., 2000).

19           In March 1999, EPA included perchlorate in the Unregulated Contaminant Monitoring  
20 Rule (UCMR) (Federal Register, 1999). Under the UCMR, all large public water systems and a  
21 representative sample of small public water systems were required to monitor for perchlorate  
22 beginning in January 2001. The EPA Method 314.0 for the analysis of perchlorate in drinking  
23 water using IC methods was published in early 2000 as a direct final rule (Federal Register,  
24 2000). The EPA Method 314.0 was also approved as a monitoring method for the UCMR  
25 (Federal Register, 2000). However, this effort does not extend to investigating potential sources  
26 in groundwater and surface water that have not migrated into public water supplies. Additional  
27 information about the UCMR is available at the web site [http://www.epa.gov/safewater/  
28 ucmr.html](http://www.epa.gov/safewater/ucmr.html).

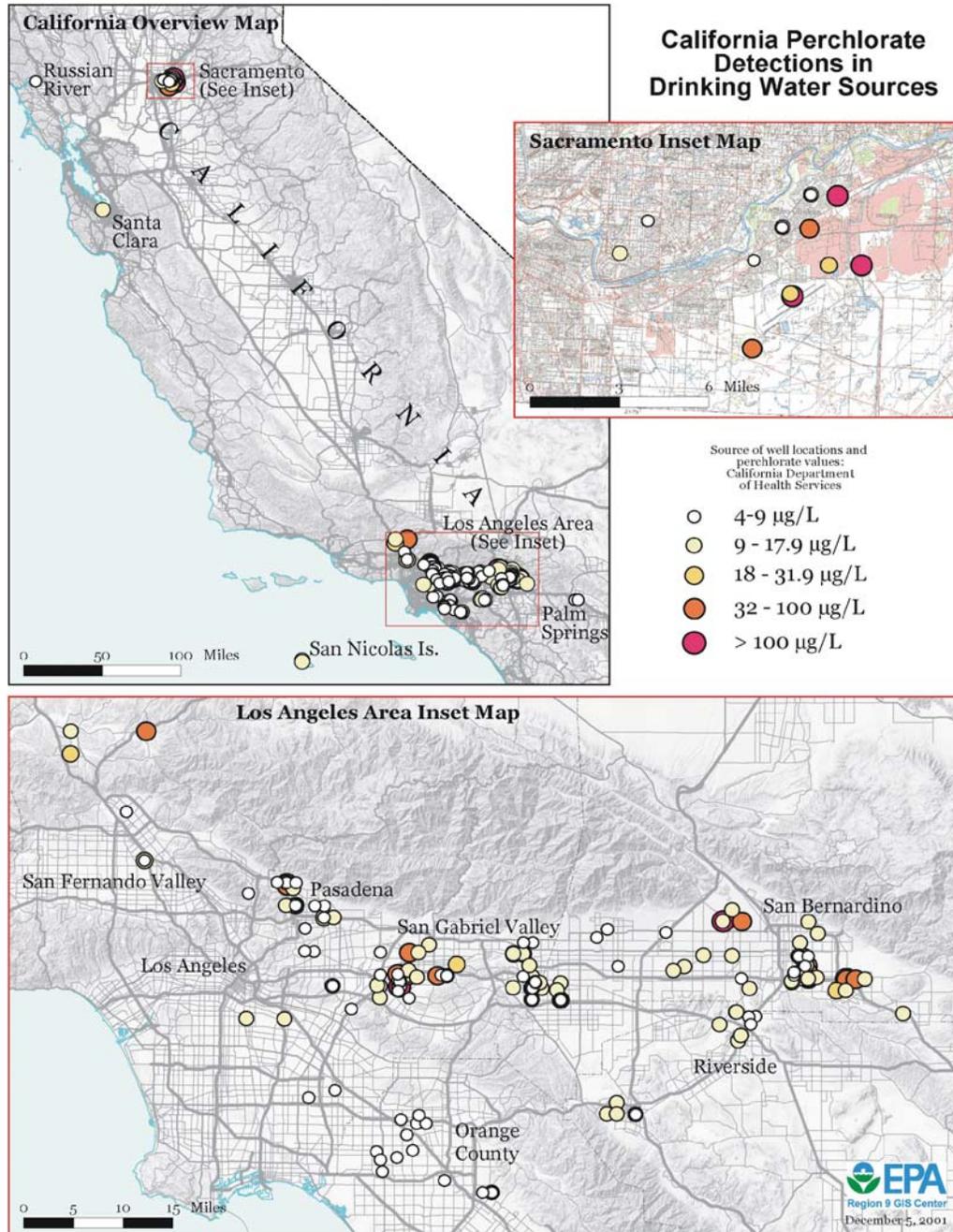
29           The CA DHS adopted 18 ppb as its provisional action level in 1997 after perchlorate was  
30 discovered in a number of California water supplies. The CA DHS also added perchlorate to the  
31 list of unregulated chemicals for which monitoring is required in 1999 (Title 22, California Code

1 of Regulations §64450). By September 2001, over 2,800 sources of public water supply had  
2 been sampled in California by water supply agencies responding to CA DHS requirements. Most  
3 of these sources represent water supply wells. Of the sources sampled, 206 (over 7 percent) had  
4 perchlorate concentrations greater than 5 ppb in at least two samples (Figure 1-2). Most of these  
5 wells have as their source groundwater plumes that have spread as far as nine miles from the site  
6 of original release.

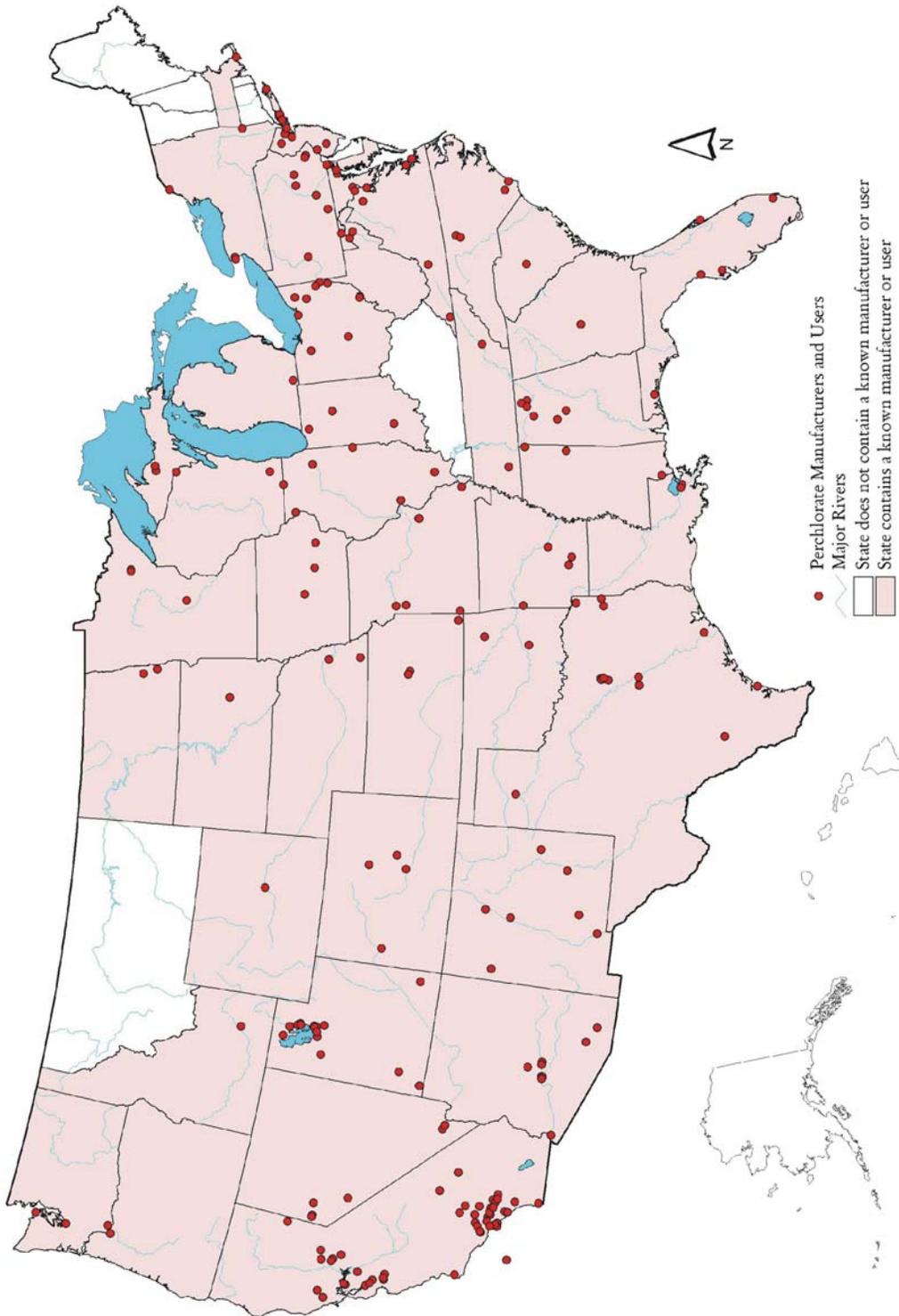
7 At this time, there has not been a systematic national survey of perchlorate occurrence.  
8 Several states and EPA regions are taking significant steps to test water supplies for perchlorate,  
9 notably the states of Arizona, Utah, and Texas, EPA Regions 6 and 7, and Suffolk County,  
10 New York. Figure 1-3 indicates states with confirmed perchlorate manufacturers or users, and  
11 Figure 1-4 indicates those states in which facilities have, in response to reported releases, directly  
12 measured perchlorate in groundwater or surface water. Table 1-1 describes these locations. The  
13 data published in Siddiqui et al., 1998 (drinking water systems in New Mexico, Indiana,  
14 Pennsylvania, and Iowa) are displayed in Figure 1-3 and in Table 1-1, but they have not been  
15 independently confirmed.

16 Information on other potential sites across the country is being gathered from the  
17 Department of Defense (DoD) and National Aeronautics and Space Administration (NASA)  
18 searches and from EPA information requests made to perchlorate manufacturers. The EPA has  
19 notified state, tribal, and local governments when it has evidence of perchlorate manufacture and  
20 use in these governmental jurisdictions. The American Water Works Association Research  
21 Foundation is coordinating a survey to characterize possible perchlorate contamination of  
22 drinking water sources in areas of high risk. The EPA will build on these survey data and other  
23 information to discover potential sources and evaluate threats to water resources.

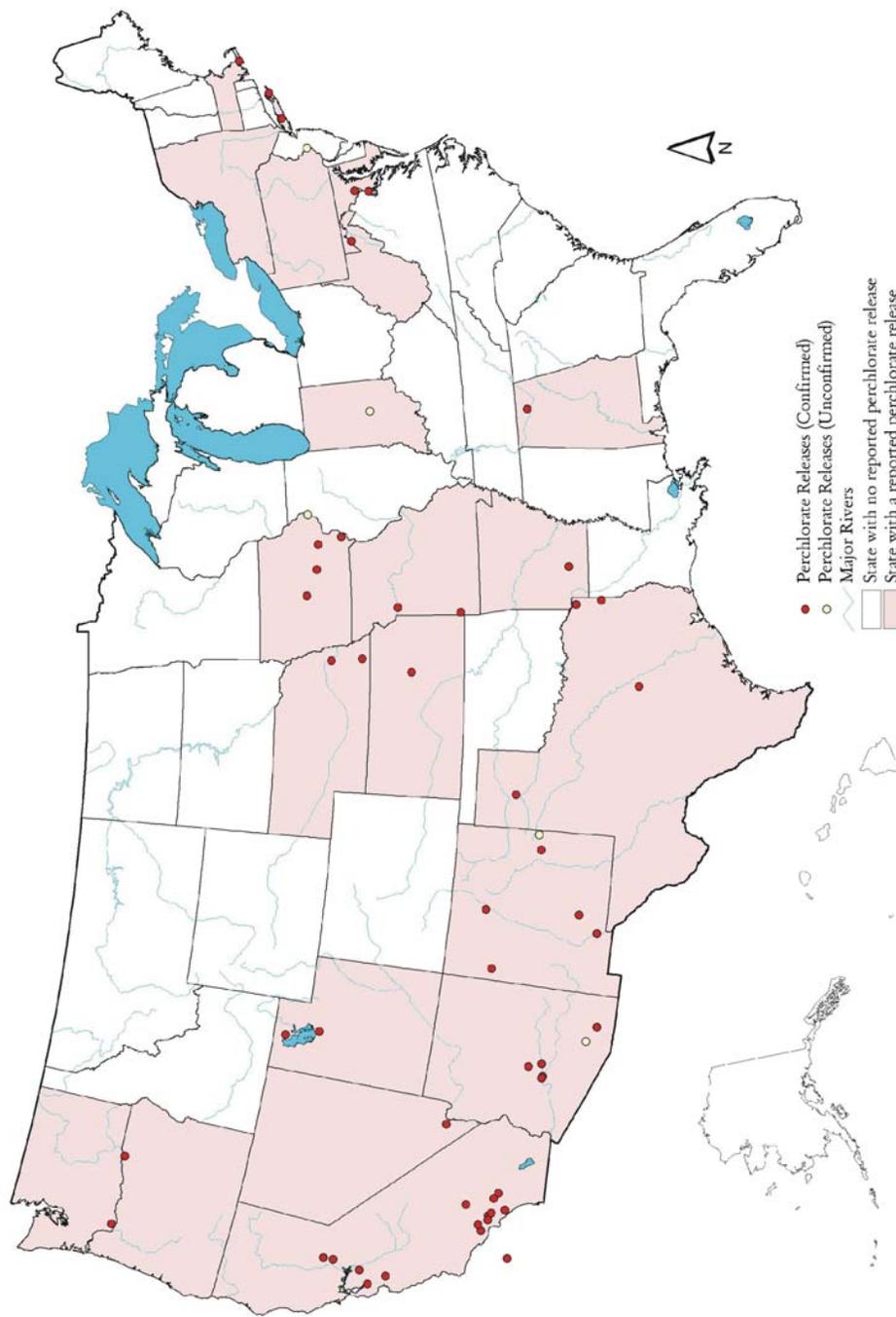
24 Region 9 officials have collected information concerning detected perchlorate releases in  
25 20 different states (Table 1-1). For two of these states, Pennsylvania and Indiana, the only  
26 reported releases have not been confirmed by a state or federal agency and should be considered  
27 questionable until the detections can be independently validated. In Washington State, propellant  
28 was observed scattered around open burn/open detonation sites although results of solid rocket  
29 chemical analyses of groundwater samples are not yet available. In California, most areas where  
30 perchlorate has been detected are associated with facilities that have manufactured, tested, or  
31 disposed of solid rocket fuels and propellants for DoD or NASA. Two facilities that



**Figure 1-2. Distribution of perchlorate detected in public water supply sources in California. Also noted are several large sites of groundwater contamination that include perchlorate releases (Mayer, 2001).**



**Figure 1-3. Locations of specific perchlorate manufacturers or users identified through responses to EPA Information Requests from current manufacturers (identifying shipments of at least 500 pounds in any year) and through investigations by state and local authorities (Mayer, 2001).**



**Figure 1-4. Locations of reported environmental releases of perchlorate to groundwater, surface water, or soil. Perchlorate measured in four water supplies in New Mexico, Iowa, Indiana, and Pennsylvania has been published in Siddiqui et al., 1998, but has not been confirmed independently by EPA or state authorities. Monitoring for perchlorate releases in most states is very limited or nonexistent (Mayer, 2001).**

**TABLE 1-1. OCCURRENCE AND POTENTIAL SOURCES OF PERCHLORATE  
RELEASES TO THE ENVIRONMENT AS OF NOVEMBER, 2001<sup>a</sup> (Mayer, 2001)**

<b>State</b>	<b>Location</b>	<b>Suspected Source</b>	<b>Type of Contamination</b>	<b>Max. Conc. ppb</b>
AL	Redstone Army Arsenal - NASA Marshall Space Flight Center Huntsville, AL	Propellant Manufacturing, Testing, Research, Disposal	Monitoring Well Springs/Seeps	19,000 37
AZ	Apache Nitrogen Products Benson, AZ	Explosives Manufacturing	Monitoring Well	670
AZ	Aerodyne Gila River Ind. Res., Chandler, AZ	Propellant Testing	Monitoring Well	18
AZ	Davis Monthan AFB Tucson, AZ	Explosives/Propellant Disposal	Soil	Not confirmed
AZ	Unidynamics Phoenix Inc. Phoenix Goodyear Airport, Goodyear, AZ	Explosives/Ordnance Manufacturing	Monitoring Well	80
AZ	Universal Propulsion Phoenix, AZ	Rocket Manufacturing	Soil	—
AZ	Unidynamics Phoenix Inc. Whiter Tanks Disposal Area Maricopa County, AZ	Explosives/Ordnance Disposal	Public Water Supply Well (Unconfirmed Report) Soil	(4) —
AR	Atlantic Research East Camden, AR	Rocket Manufacturing Disposal - Open Burn/Open Detonation	Monitoring Well Surface Water Soil	1,500 480,000 —
CA	Aerojet General also affects Mather AFB Rancho Cordova, CA	Rocket Manufacturing	Public Water Supply Well Monitoring Well	260 640,000
CA	Alpha Explosives Lincoln, CA	Explosives Manufacturing	Monitoring Well Reported in Surface Water	67,000
CA	Boeing/Rocketdyne, NASA at Santa Susana Field Lab U.S. DOE Santa Susana, CA	Rocket Research, Testing and Production	Monitoring Well	750
CA	Edwards AFB Jet Propulsion Lab, North Base Edwards, CA	Rocket Research	Monitoring Well	300
CA	El Toro Marine Corps Air Station Orange Co., CA	Explosives Disposal	Monitoring Well	380
CA	Lawrence Livermore National Laboratory Site 300 Tracy, CA	U.S. DOE Explosives Research	Monitoring Well	84

**TABLE 1-1 (cont'd). OCCURRENCE AND POTENTIAL SOURCES OF  
PERCHLORATE RELEASES TO THE ENVIRONMENT AS OF NOVEMBER, 2001<sup>a</sup>  
(Mayer, 2001)**

<b>State</b>	<b>Location</b>	<b>Suspected Source</b>	<b>Type of Contamination</b>	<b>Max. Conc. ppb</b>
CA	Lockheed Propulsion Upper Santa Ana Valley Redlands, CA	Rocket Manufacturing	Public Water Supply Well	87
CA	NASA - Jet Propulsion Lab Raymond Basin Pasadena, CA	Rocket Research	Public Water Supply Well	54
CA	Rialto, CA	Fireworks Facility (?) B.F. Goodrich (?) Rocket Research and Manufacturing	Public Water Supply Well (inactive)	811
CA	San Fernando Valley Glendale, CA	Grand Central Rocket (?) Rocket Manufacturing	Monitoring Well	84
CA	San Gabriel Valley Baldwin Park, CA	Aerojet Rocket Manufacturing	Public Water Supply Well Monitoring Well	159 2,180
CA	San Nicholas Island Ventura Co., CA	U.S. Navy Firing Range	Public Water Supply (Springs)	12
CA	Stringfellow Superfund Site Glen Avon, CA	Hazardous Waste Disposal Facility	Monitoring Well Private Well	682,000 37
CA	UTC (United Technologies) San Jose, CA	Rocket Testing	Monitoring Well	180,000
CA	Whittaker-Bermite Ordnance Santa Clarita, CA	Ordnance Manufacturing	Public Water Supply Well	47
CA	Whittaker Ordnance Hollister, CA	Ordnance Manufacturing	Private Well Monitoring Well	810 88
IN	American Water Works Service Greenwood, IN	Unknown Source	Public Water Supply Well (Unconfirmed Report)	(4)
IA	American Water Works Service Clinton, IA	Unknown Source	Public Water Supply Well (Unconfirmed Report)	(6)
IA	Ewart, IA	Unknown Source	Livestock Well	29
IA	Hills, IA	Unknown Source	Private Well	30
IA	Napier, IA	Agriculture (?)	Private Well	10
KS	Herington, KS	Ammunition Facility	Monitoring Well	9
MA	Massachusetts Military Res. Barnstable Co., MA	Disposal - Open Burn/ Open Detonation	Monitoring Well	300
MD	Naval Surface Warfare Center Indian Head, MD	Propellant Handling	Waste Discharge to Surface Water	>1,000

**TABLE 1-1 (cont'd). OCCURRENCE AND POTENTIAL SOURCES OF  
PERCHLORATE RELEASES TO THE ENVIRONMENT AS OF NOVEMBER, 2001<sup>a</sup>  
(Mayer, 2001)**

<b>State</b>	<b>Location</b>	<b>Suspected Source</b>	<b>Type of Contamination</b>	<b>Max. Conc. ppb</b>
MD	White Oak Fed. Research Center (Naval Surface Warfare Center) White Oak, MD	Propellant Handling	Monitoring Well	72
MO	ICI Explosives Joplin, MO	Explosives Facility	Monitoring Well	107,000
MO	Lake City Army Amm. Plant Independence, MO	Propellant Handling	Monitoring Well	70
NE	Lewiston, NE	Agricultural Chemical Facility	Shallow Private Well	5
NE	Mead, NE	Fireworks Facility	Monitoring Well	24
NV	Kerr-McGee/BMI Henderson, NV	Chemical Manufacturing	Public Water Supply Monitoring Well Surface Water	24 3,700,000 120,000
NV	PEPCON Henderson, NV	Chemical Manufacturing	Monitoring Well	600,000
NM	American Water Works Service Clovis, NM	Unknown	Public Water Supply Well (Unconfirmed Report)	(4)
NM	Ft. Wingate Depot Activity Gallup, NM	Explosives Disposal	Monitoring Well	2,860
NM	Holloman AFB Alamogordo, NM	Rocket Testing	Monitoring Well Seasonal Surface Water Soil	40 16,000 —
NM	Los Alamos National Lab Los Alamos, NM	U.S. Dept. of Energy Lab Chemicals	Public Water Supply Well Monitoring Well Deep Borehold Water	3 220 1,662
NM	Melrose Air Force Range Melrose, NM	Explosives	Public Water Supply Well	25
NM	White Sands Missile Range White Sands, NM	Rocket Testing	Monitoring Well Soil	21,000 —
NY	West Hampton Suffolk County, NY	Unknown Source(s)	Public Water Supply Well Monitoring Well	16 3,370
NY	Yaphank Suffolk County, NY	Fireworks	Private Well Monitoring Well	26 122
PA	American Water Works Service Yardley, PA	Unknown	Public Water Supply Well (Unconfirmed Report)	(5)

**TABLE 1-1 (cont'd). OCCURRENCE AND POTENTIAL SOURCES OF PERCHLORATE RELEASES TO THE ENVIRONMENT AS OF NOVEMBER, 2001<sup>a</sup> (Mayer, 2001)**

State	Location	Suspected Source	Type of Contamination	Max. Conc. ppb
TX	Longhorn Army Ammunition Depot Karnak, TX	Propellant Handling	Monitoring Well Reported in Surface Water Soil	169,000 — —
TX	McGregor Naval Weapons Plant McGregor, TX	Propellant Handling	Monitoring Well Reported in Surface Water Soil	91,000 — —
TX	PANTEX Plant (USDOE) Amarillo, TX	Explosives	Monitoring Well	5
TX	Red River Army Depot Texarkana, TX	Propellant Handling	Monitoring Well	80
UT	Alliant Tech Systems Magna, UT	Rocket Manufacturing	Public Water Supply Well	16
UT	Thiokol Promontory, UT	Rocket Manufacturing	Well Supply Well (Inactive)	42
WA	Camp Bonneville near Vancouver, WA	Explosives/Propellant Disposal	Soil	—
WV	Allegheny Ballistics Lab Rocket Center, WV	Rocket Research, Production, Open Burn/Open Detonation	Surface Discharge of Groundwater Extraction	400

<sup>a</sup>Data reported to EPA Region 9 as of November 2001. All reports have been confirmed by federal, state, or county agencies except where noted. Soil concentrations are not listed.

1 manufactured ammonium perchlorate in Nevada were found to have released perchlorate to  
 2 groundwater resulting in low levels (4 to 24 ppb) in Lake Mead and the Colorado River. This  
 3 water is used for drinking, irrigation, and recreation for millions of people in Nevada, California,  
 4 Arizona, and by Native American tribes.

5 The concentrations reported in wells and surface water vary widely. At one facility near  
 6 Henderson, NV, perchlorate in groundwater monitoring wells was measured as high as 0.37%  
 7 (3.7 million ppb). The highest level of perchlorate reported in any public water supply well was  
 8 800 ppb in an inactive well in California. Few active public water supply wells have perchlorate  
 9 greater than 100 ppb, and none are reported at this level outside of California.

1 Perchlorate was found in a number of water supply wells on Long Island, NY, including  
2 several downgradient from a fireworks facility. It has been speculated that the wide distribution  
3 pattern of the New York contamination could be a result of low levels of perchlorate contained in  
4 fertilizer imported from Chile (TRC Environmental Corporation, 1998; Urbansky, 2000; Suffolk  
5 County Department of Health Services, 2001a,b). Agricultural chemicals also have been  
6 implicated as a potential source of perchlorate contamination in Nebraska at a shallow well near  
7 a speciality fertilizer facility (Williams, 2000). After state and federal officials in Region 7  
8 added perchlorate analyses in their program testing hundreds of rural wells for fertilizers and  
9 agricultural chemicals. Their results showed that fertilizer application to farmlands is an unlikely  
10 source of perchlorate in Midwestern states.

11 In addition to discoveries at facilities involved with rocket propellants, explosives, and  
12 fireworks, a number of perchlorate detections have been made at current or former military  
13 facilities where propellants and explosives were disposed of by detonation and burning.  
14 Cooperation from Department of Defense (DoD) and Department of Energy (DoE) officials will  
15 continue to be important for examining these types of potential sources.

16 In the past three years, the increasing interest in investigating the environment has resulted  
17 in increasing detections. It is likely that regional positive efforts at detection may largely explain  
18 the distribution of known areas of release to the environment (Figure 1-4) when compared to the  
19 potential distribution suggested in Figure 1-3. As the efforts for detection become more uniform  
20 nationwide, the occurrence of perchlorate in the environment may more closely resemble the  
21 pattern of perchlorate usage.

22 It is important to distinguish between minimum detection limit (MDL) and the minimum  
23 reporting limit (MRL), which is also called the practical quantitation limit (PQL). MDLs are  
24 calculated from the precision of replicate low level measurements and are assumed to reflect  
25 99% confidence that a trace concentration above zero can be detected. MRLs are higher values  
26 that reflect actual quantifiable concentrations. The EPA calculated and published an MDL for  
27 Method 314 (Ion Chromatography) at  $0.53 \mu\text{g/L}$  (Federal Register, 2000). This was derived  
28 through the analysis of 7 replicate samples fortified at  $2.0 \mu\text{g/L}$ . Based upon this result, an MRL  
29 for perchlorate was established at  $4.0 \mu\text{g/L}$ . Dionex, the manufacturer of the ion chromatography  
30 column, published an MDL of  $0.2 \mu\text{g/L}$  and MRL of  $2.0 \mu\text{g/L}$  (Dionex, 2000).

1 Method 314 does not represent the lowest possible MRL or MDL. Unpublished  
2 improvements in the ion chromatography method may lower the MRL to the sub-part per billion  
3 level (Yates, 2001). Several research and commercial laboratories have been developing mass  
4 spectrometry methods to detect sub-ppb levels of perchlorate (Urbansky et al., 1999; Magnuson  
5 et al., 2000 a,b; Urbansky, 2000; Handy et al., 2000; Koester et al., 2000; Winkler, 2001). It is  
6 reasonable to expect that a reliable sub-ppb MRL for perchlorate will be commercially available  
7 in the very near future. The Agency encourages development of these emerging methods (e.g.,  
8 LC/MS/MS) to eliminate interferences that can be encountered by extending IC methods for  
9 low-level analysis in a variety of matrices (e.g., soil or plants and animal tissues). The market  
10 demand for this capability may determine the commercial availability and expense of this  
11 method. Regulatory pressure to ensure protection of water supplies and to maintain treatment  
12 process control is also a factor driving the development of lower reporting limits for perchlorate.  
13 Thorough method validation and quality assurance information must be compiled to establish a  
14 standard analytical method in the sub-ppb range for various media.

### 17 **1.3 HEALTH AND ECOTOXICOLOGY RISK ASSESSMENTS—** 18 **HISTORICAL OVERVIEW**

19 This section briefly summarizes how the assessments for the health and ecotoxicology risks  
20 of perchlorate contamination have evolved. This document represents the revised assessment  
21 that incorporates additional data and analyses recommended at the external peer review convened  
22 by the Agency in February, 1999 (Research Triangle Institute, 1999).

#### 24 **1.3.1 Overview of Perchlorate Health Risk Assessment**

25 The EPA Region 9 office requested evaluation of the toxicology data from the EPA  
26 Superfund Technical Support Center (Stralka, 1992). The EPA Superfund Technical Support  
27 Center issued a provisional RfD in 1992 (Dollarhide, 1992) and a revised provisional RfD in  
28 1995 (Dollarhide, 1995) based on a literature review (Environmental Resources Management,  
29 Inc., 1995) submitted by the Perchlorate Study Group (PSG). Ideally, an RfD is based on a  
30 database that evaluates an array of endpoints that address potential toxicity during various critical  
31 life stages, from developing fetus through adult and reproductive stages. The provisional RfD

1 values (1992 and 1995) were based on an acute study in which single doses of potassium  
2 perchlorate caused the release of iodide (I<sup>-</sup>) from the thyroids of patients with Graves' disease, an  
3 autoimmune condition that results in hyperthyroidism. It was difficult to establish a  
4 dose-response for the effects on thyroid function from daily or repeated exposures in normal  
5 humans from the data on patients with Graves' disease because of a variety of confounding  
6 factors, including that the disease itself has effects; that often only a single exposure, rather than  
7 repeated exposures was tested; that only one or two doses were employed; and that often the only  
8 effect monitored was iodide release from the thyroid or control of the hyperthyroid state.  
9 Nevertheless, a no-observed-adverse-effect-level (NOAEL) was determined to be  
10 0.14 mg/kg-day based on release of iodide in the thyroid, followed by incomplete inhibition of  
11 iodide uptake. Uncertainty factors that ranged from 300 to 1,000 were applied to account for  
12 data missing on additional endpoints and extrapolations required to calculate a lifetime human  
13 exposure level. The provisional RfD values issued are listed as such by EPA because they did  
14 not undergo the internal EPA and external peer review required of estimates available on the  
15 EPA's Integrated Risk Information System (IRIS). Standard assumptions for ingestion rate and  
16 body weight were applied to the RfD to calculate the reported range in the groundwater cleanup  
17 guidance levels of 4 to 18 ppb.

18 In recognition of the potential influence of the reduced analytical detection limit, a  
19 reevaluation of the provisional 1992 and 1995 RfDs that serve as the basis of the provisional  
20 action level was warranted. An external non-EPA peer review convened in March 1997 to assess  
21 an analogous RfD derivation by an independent organization (Toxicology Excellence for Risk  
22 Assessment, 1997) determined that the health effects and toxicity data were insufficient for a  
23 credible quantitative risk analysis (Toxicology Excellence for Risk Assessment, 1998a). The  
24 external peer review panel concluded that the limited database was insufficient to rule out effects  
25 of perchlorate on other organs, so it could not be determined unequivocally that the effect on the  
26 thyroid was the critical effect. In particular, the reviewers were concerned that developmental  
27 toxicity, notably neurological development affected by hypothyroidism during pregnancy, could  
28 be another critical effect of perchlorate that had not been examined adequately in studies to date.  
29 In response to the March 1997 external peer review of the provisional RfD value, a subsequent  
30 external peer review of experts was convened in May 1997 to recommend and prioritize a set of  
31 studies to address the key data gaps and to reduce uncertainties in various extrapolations

1 (Toxicology Excellence for Risk Assessment, 1998b). The objective of the new studies is to  
2 provide a comprehensive database that will support development of a robust RfD estimate that  
3 reduces the uncertainties inherent in the provisional values. The strategical basis of the new  
4 battery of toxicity studies is discussed in Chapter 3. These data featured prominently in the  
5 external peer review draft of the assessment issued by the EPA in December 1998. At the  
6 subsequent external peer review convened by the Agency in February 1999, recommendations  
7 were made for additional studies and analyses, including completion of those on studies that were  
8 only available as preliminary data at that time (Research Triangle Institute, 1999). The EPA  
9 committed to a second external peer review and a revised risk assessment in order to benefit from  
10 the additional insights that these data might bring to bear. The purpose of this current revised  
11 document is to incorporate all of the data from new studies and to respond to recommendations  
12 made at the previous external peer review.

13 Because the Agency is committed to utilizing the latest available science to support its  
14 human and ecotoxicological risk estimates, the Office of Research and Development (ORD)  
15 issued interim guidance in 1999 to its risk assessors and risk managers to be followed until this  
16 revised assessment became publicly available (Noonan, 1999). The recommendation was to  
17 continue using the standing provisional RfD range of 0.0001 to 0.0005 mg/kg-day for  
18 perchlorate-related risk assessment activities because of the significant concerns and  
19 uncertainties that remained to be addressed. This recommendation was based on the  
20 determination that important new analyses on emerging data would likely have an impact on the  
21 previously proposed health risk benchmark in the 1998 external review draft (U.S.  
22 Environmental Protection Agency, 1998d) and that, while the new estimates would reflect greater  
23 accuracy, the resultant revised risk estimate could be either higher or lower.

24 This recommendation helped to ensure that the Agency bases its risk management  
25 decisions on the best available peer reviewed science and was in keeping with the full and open  
26 participatory process embodied by the proposed series of external peer review workshops.  
27 It should be noted that, due to the uncertainty of whether the final proposed revised oral human  
28 health risk benchmark would increase or decrease based on the new data and analyses, the  
29 standing provisional RfD range was the more conservative of the estimates available at the time  
30 of the interim guidance and, therefore, more likely to be protective of public health. The  
31 recommendation was also consistent with Agency practice that existing toxicity estimates remain

1 in effect until the review process to revise them is completed. The steps necessary to complete  
2 this assessment are outlined in Section 1.4. Once completed, this assessment will be included on  
3 the Agency's Integrated Risk Information System (IRIS).  
4

### 5 **1.3.2 Overview of Ecotoxicology Screening Level Assessment**

6 The mobility and persistence of perchlorate discussed in the beginning of this chapter also  
7 may pose a threat to ecological receptors and whole ecosystems either by direct harm to  
8 organisms or by indirectly affecting their ability to survive and reproduce. There were very  
9 limited data in 1997 with which to evaluate the effects of perchlorate on ecological systems; nor  
10 were there data about the possible uptake of perchlorate into agricultural products irrigated by  
11 contaminated water. Analytical tests had been derived to detect perchlorate in water, but little  
12 work had been done to extend these methods to testing plant and animal tissues or food crops for  
13 perchlorate.

14 Searches of available databases revealed minimal information on the ecological effects of  
15 ammonium perchlorate or any of perchlorate's other salts. Little data exist to describe  
16 perchlorate's ecological effects on various soil, sediment, or aquatic receptors, including aquatic  
17 vertebrates, aquatic or sediment invertebrates, and bacteria or plants. The data that were  
18 available suggested effects on thyroid-hormone-mediated development in the South African  
19 clawed frog, *Xenopus laevis*, in the range of 50 to 100 ppm, and 1,000 ppm had been shown to  
20 completely block the metamorphosis of tadpoles. Effects on development and population growth  
21 also had been indicated in the freshwater lamprey at 100 ppm and the freshwater hydra at  
22 350 ppm. Mortality was observed in cold-water trout (6,000 to 7,000 ppm) and *Daphnia magna*  
23 (670 ppm). Effects on seed germination and growth of agricultural plants were reported at  
24 10 ppm.

25 Under the auspices of the Ecological/Transport and Transformation Subcommittee of the  
26 Interagency Perchlorate Steering Committee (IPSC, see Section 1.5), the U.S. Air Force (USAF)  
27 Detachment 1, Human Systems Center, Brooks Air Force Base (AFB), in conjunction with EPA,  
28 developed a proposal for a battery of screening-level bioassays in laboratory-reared organisms  
29 representative of soil, sediment, and water column receptors, to evaluate dose-response  
30 relationships. The identified tests focus on identifying gross (direct) toxicity tests whose  
31 endpoints can include mortality, growth, and reproductive success. Bioassays with standard

1 protocols and general regulatory acceptance were chosen. Although these were screening-level  
2 tests and provided only an indication of gross toxicity, they provided the dose-response  
3 information required to make decisions about the need for a next tier of tests to be completed  
4 (e.g., bioavailability, bioaccumulation, histopathology).

5 Additional new studies were recommended at the 1999 external peer review in the  
6 ecotoxicology arena as well, and some additional data has become available that improves the  
7 information base somewhat. Most significantly, additional data are available on effect levels in  
8 aquatic animals, an aquatic plant, a terrestrial plant, and a soil invertebrate; and some of these  
9 data are for chronic durations. In addition, surveys have been conducted at several sites of  
10 known or suspected perchlorate contamination with analysis of environmental and biological  
11 materials for perchlorate levels. While these new data have been incorporated in the current  
12 revision and are described in Chapter 8, the knowledge in this arena requires that the ecological  
13 assessment must still be characterized as a screening level rather than definitive. The number of  
14 species is still quite low and the site surveys aimed only to describe the range of exposures at the  
15 sites. The ecotoxicological review will undergo the same peer review process as the health risk  
16 assessment that is described in Section 1.4.

## 17 18 19 **1.4 RISK CHARACTERIZATION AND REGULATORY AGENDA**

20 This section briefly describes pending regulatory activities that this evaluation and  
21 characterization will likely influence. Particular emphasis is placed on the revised health risk  
22 assessment and ecotoxicology assessments.

### 23 24 **1.4.1 U.S. Environmental Protection Agency Regulatory Plans**

25 The Safe Drinking Water Act (SDWA), enacted by Congress in 1974 and amended in 1986  
26 and again in 1996 (U.S. Code, 1996), provides the basis for safeguarding public drinking water  
27 systems from contaminants that pose a threat to public health. The purpose of the SDWA is to  
28 protect public health by ensuring that public drinking water systems provide tap water that is safe  
29 for drinking and bathing. Within EPA, the Office of Ground Water and Drinking Water

1 develops National Primary Drinking Water Regulations (NPDWR) to control the levels of  
2 contaminants that may occur in public drinking water systems.

3 The 1996 amendments to the SDWA require EPA to publish a list of contaminants that are  
4 not currently subject to a NPDWR and are known or anticipated to occur in public water systems.  
5 This list, known as the Contaminant Candidate List (CCL), is the source of priority contaminants  
6 for research, guidance development, regulatory determinations, and monitoring by the states.  
7 The SDWA requires EPA to determine whether or not to regulate at least five contaminants from  
8 the CCL by 2001. The CCL also must be reviewed and updated every 5 years; the next review is  
9 scheduled for 2003.

10 With broad public input and consultation with the scientific community, a draft CCL was  
11 published on October 6, 1997. The draft CCL specifically requested comment on whether to  
12 include perchlorate on the CCL based on the limited information EPA had received on  
13 perchlorate's occurrence in drinking water supplies at the time of publication. As a result of the  
14 public comments and the obtainment of additional occurrence information, EPA determined that  
15 sufficient information exists to raise concern over perchlorate's potential public health impact  
16 and added perchlorate to the final CCL published on March 2, 1998.

17 The CCL consists of 50 chemical and 10 microbiological contaminants and is divided into  
18 two categories: (1) contaminants for which sufficient information exists to begin to make  
19 regulatory determinations in 2001, and (2) contaminants for which additional research and  
20 occurrence information is necessary before regulatory determinations can be made. Perchlorate  
21 falls into the latter category because of the need for additional research in the areas of health  
22 effects, treatment technologies, analytical methods, and extent of occurrence.

#### 24 **1.4.2 State Regulatory Plans**

25 The CA DHS and the California EPA Office of Environmental Health Hazard Assessment  
26 (CA EPA OEHHA) reviewed the EPA risk assessment reports for perchlorate and established its  
27 action level at 18 ppb based on the provisional RfD values from the EPA Superfund Technical  
28 Support Center. The CA DHS advises water utilities to remove drinking water supplies from  
29 service if they exceed the 18-ppb action level. If the contaminated source is not removed from  
30 service because of system demands, and if drinking water provided by the utility exceeds the  
31 action level, the CA DHS advises the utility to notify its customers. On August 1, 1997, the CA

1 DHS informed drinking water utilities of its intention to develop a regulation requiring  
2 monitoring of perchlorate as an unregulated chemical. Legislative action to establish a state  
3 drinking water standard for perchlorate by January 2000 (California Senate Bill 1033 [California  
4 State Senate, 1998]) was vetoed by the governor after passage by both houses. The governor  
5 supported prioritizing the regulation of perchlorate in drinking water but objected to the strict  
6 time schedule required.

7 In July 2001, the CA EPA OEHHA posted a notice on its web site indicating that it was  
8 initiating a risk assessment for perchlorate in connection with the development of a public health  
9 goal (PHG) for a number of chemicals in drinking water ([www.oehha.ca.gov/public\\_info/public/  
10 phgannounc.html](http://www.oehha.ca.gov/public_info/public/phgannounc.html)). PHGs are concentrations of chemicals in drinking water that are not  
11 anticipated to produce adverse health effects following long-term exposures. These goals are  
12 non-regulatory in nature but are to be used as the health basis with which to update the state  
13 primary drinking water standards established by CA DHS for chemicals in drinking water subject  
14 to regulation. A 45-day public comment period will be provided after posting, followed by a  
15 public workshop. Scientific peer reviews are arranged through the University of California. The  
16 overall process will include time for revisions, further public comment, and responses to  
17 comments. The new PHGs are scheduled for publication in 2003.

18 New York, Arizona, and Texas also initially adopted the level of 18 ppb as their version of  
19 advisory levels for water supply systems. Texas and Arizona health departments revised their  
20 perchlorate advisory levels based on research presented in EPA's December 1998 External  
21 Review Draft Toxicity Assessment. In July 1999, Texas arrived at a value of 22 ppb in drinking  
22 water by calculating the exposure of a 15 kg child drinking 0.64 liter per day and using the  
23 reference dose proposed in the 1998 EPA ERD document. Texas revised this value to 4 ppb in  
24 October 2001 based in part on the interim ORD guidance (Noonan, 1999). Arizona derived a  
25 14 ppb level in March 2000, based on a 15 kg child drinking 1 liter per day and using the  
26 proposed RfD in the 1998 EPA ERD document. New York State has continued to use 18 ppb as  
27 the advisory level for perchlorate in drinking water.

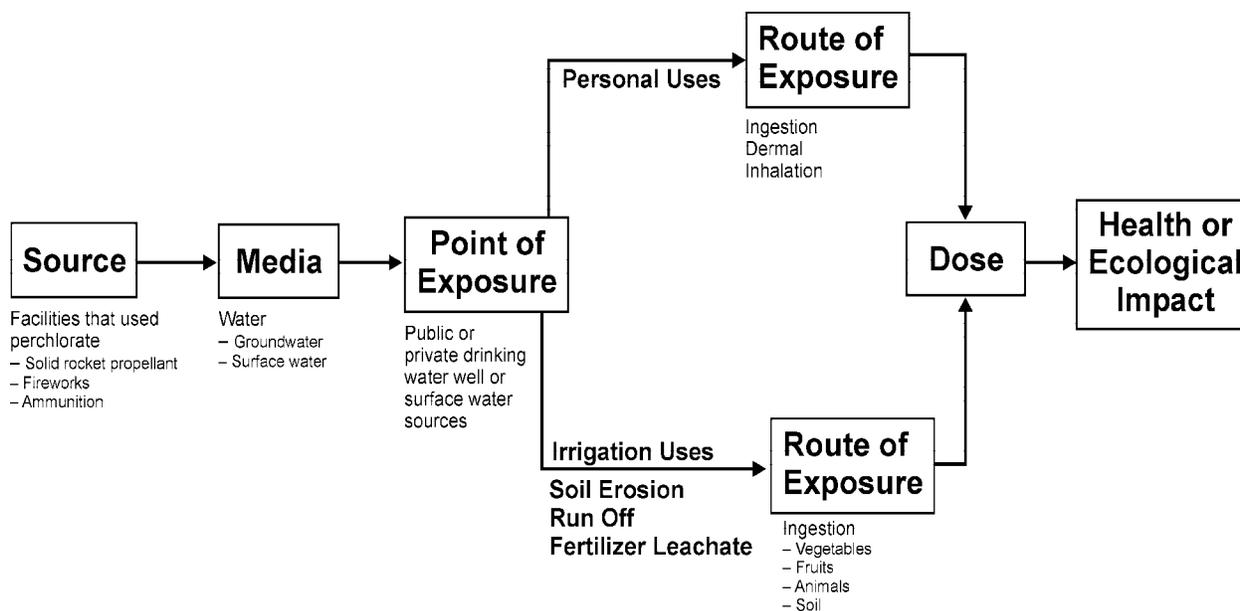
28 The Nevada Division of Environmental Protection (NDEP) has authority under Nevada  
29 Water Pollution Control Regulations to address pollutants in soil or groundwater. The state's  
30 Corrective Action Regulations direct NDEP to establish action levels for hazardous substances,  
31 pollutants, or contaminants, using drinking water standards such as a maximum contaminant

1 level (MCL), health advisories, or background or protective levels (determined by IRIS or the  
2 equivalent). In August 1997, Nevada determined that the action level of 18 ppb, as established  
3 by EPA, would be the recommended action level for cleanup, pending a more current risk  
4 assessment.

## 7 **1.5 SUMMARY**

8 Perchlorate contamination is a concern for several reasons. First, there are uncertainties in  
9 the toxicological database that is used to address the potential of perchlorate to produce human  
10 health effects when present at low levels in drinking water. Additionally, the actual extent of  
11 perchlorate occurrence in ground and surface waters and other media (soils or plant and animal  
12 tissues) is unknown—a problem compounded by limits to the analytical detection method. The  
13 efficacy of different treatment technologies for various water uses, including drinking and  
14 irrigation, is also not well established. Finally, the nature and extent of ecological effects and  
15 details about transport and transformation phenomenon in various environmental media have  
16 been studied only superficially. EPA aims to more comprehensively characterize the risks to  
17 human and ecological health from perchlorate contamination through the integrative approach  
18 presented in Figure 1-5.

19 Thus, a number of key pieces of information and scientific advances are essential to  
20 adequately characterize the risks of perchlorate contamination and to develop scientifically-based  
21 management strategies that effectively mitigate the potential risks posed by perchlorate  
22 contamination. Accurate characterization of exposures relies on reliable analytical detection  
23 methods. The exposure estimates cannot be gauged with respect to their risk unless a robust  
24 health risk estimate is available. Treatment technologies should be targeted to levels of concern  
25 and tailored to the intended water use. Technology transfer is necessary so that all affected  
26 parties and concerned citizens are apprised of accurate and reliable information that is  
27 up-to-date with the evolving state-of-the-science. The purpose of the revised risk  
28 characterizations presented in this document is to serve in this integrative approach by providing  
29 more robust risk estimates than those that currently exist provisionally in order to better gauge  
30 the potential human health and ecological impacts.



**Figure 1-5. Considerations for comprehensive characterization of perchlorate contamination. (Modified from Underwood, 1998.)**

1 The National Center for Environmental Assessment (NCEA) in the Office of Research and  
 2 Development (ORD) of EPA first evaluated the emerging information and new human  
 3 health/toxicity and ecotoxicity data from the testing strategy (see Chapter 3) and issued an  
 4 external peer review draft in December 1998. In February 1999, an external peer review  
 5 workshop was convened. The peer review panel endorsed the conceptual approach proposed by  
 6 NCEA and recommended additional studies and analyses. This revised risk characterization  
 7 document represents a response to those recommendations and includes data made available to  
 8 the EPA as of Fall 2001.

9 As with any risk assessment, incorporation of new data is an iterative process. Because of  
 10 regulatory schedule constraints, this assessment has gone forward with the recognition that new  
 11 data may warrant further revision at a future date. Data in additional analyses that are warranted  
 12 and which will be arriving in the period between the issuance of the external peer review draft  
 13 and the external peer review workshop are identified herein and may be presented at that  
 14 workshop.

1 Independent, external peer review of the study protocols, toxicity studies, and revised risk  
2 assessment for perchlorate will be critical to ensuring that future decisions will be protective of  
3 human health and that the potential for ecotoxicology is characterized appropriately. The IRIS  
4 program will oversee the external peer review and has tasked a qualified contractor to manage  
5 the peer review of technical issues related to the development of the human health and  
6 ecotoxicology assessments, including system design, conduct of toxicity studies, statistical  
7 analysis of data, designation of effect levels, selection of critical effect and uncertainty factors,  
8 and risk characterization. The peer review will be conducted by a panel of technical experts  
9 selected by contractors in ecotoxicology; neurotoxicology; developmental, reproductive, genetic,  
10 and general toxicology; endocrinology; pathology; biostatistics; dose-response modeling; and  
11 risk assessment.

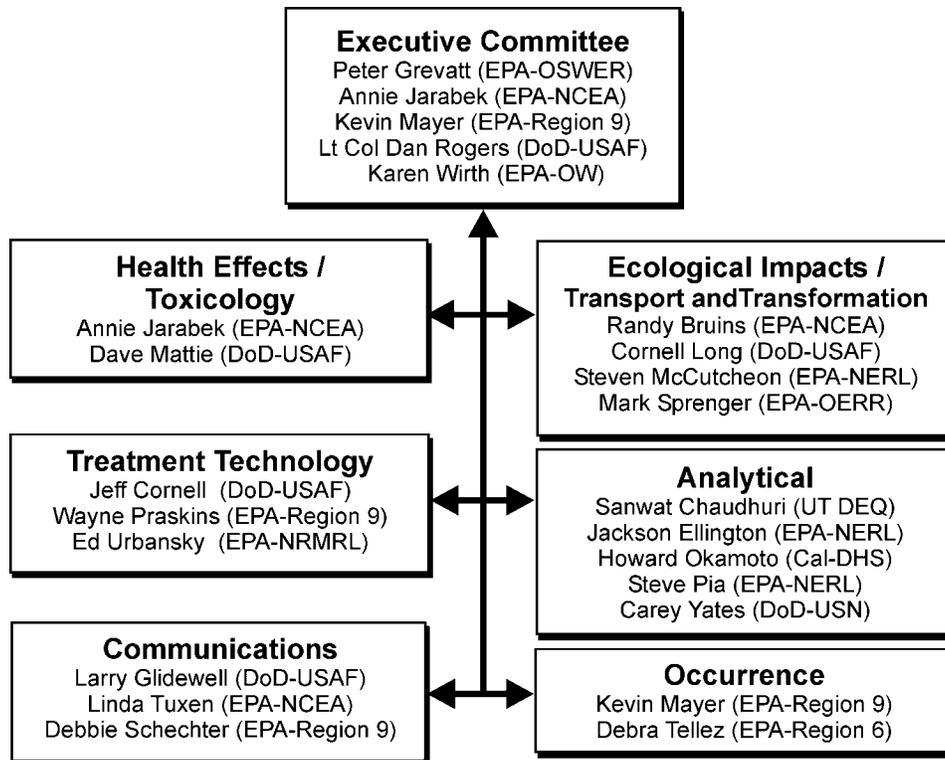
12 The risk characterization assessment package, supporting studies, and study protocols for  
13 the new data will be distributed to the peer review panel in advance of the peer review meeting.  
14 The peer reviewers are charged with evaluating experimental protocols, performance, and results  
15 for any new studies since 1999 in addition to how the data are used in this risk assessment. Peer  
16 reviewers will independently review the risk assessment package and supporting studies and will  
17 submit their written comments to the IRIS contractor prior to the peer review meeting. The IRIS  
18 contractor will compile the peer reviewers comments and distribute them to all of the reviewers  
19 prior to the meeting which will be held on March 5 and 6, 2002. Sacramento was selected as a  
20 location for its accessibility to stakeholders and peer reviewers. The public will be invited to  
21 attend and observe the peer review meeting and may obtain pre-meeting comments at that time.  
22 Following the peer review meeting, the peer review panel will generate a report detailing their  
23 comments on the reference dose package and supporting studies. NCEA then will generate a  
24 responsiveness summary report that will discuss how comments made by the peer reviewers have  
25 been addressed. The revised risk characterization will be issued subsequently by EPA as a final  
26 IRIS assessment after Agency consensus review across offices and laboratories and a final IRIS  
27 program clearance.

28 It should be noted that this assessment effort was accomplished in an expedited time frame  
29 through the partnership and cooperation of a number of governmental entities. The IPSC was  
30 formed in January 1998 to bring together government representatives from EPA; DoD; the  
31 National Institute for Environmental Health Sciences (NIEHS); and affected state, tribal, and

1 local governments. Participation in IPSC also has been solicited from other governmental  
2 entities. The purpose of the IPSC is to facilitate and coordinate accurate accounts of related  
3 technological issues (occurrence, health effects, treatability, waste stream handling, analytical  
4 detection, and ecological impacts) and to create information-transfer links for interagency and  
5 intergovernmental activities regarding these areas of concern.

6 Figure 1-6 shows the structure of the IPSC, members of its executive committee, and  
7 co-chairs of the subcommittees. Note that a subcommittee exists for each of the outstanding  
8 controversial issues regarding perchlorate contamination. These are identified in the  
9 comprehensive characterization framework presented in Figure 1-5. Research to obtain  
10 additional data and the development of new methods and applications is underway in these  
11 human health and ecotoxicology areas, as well as in most of the others, to ensure that the state-  
12 of-the-science is brought to bear in addressing the unique issues of perchlorate contamination.

13 The IPSC collaborated in 1998 with EPA ORD NCEA on a draft report to a Congressional  
14 committee that assesses the state-of-the-science on the health effects of perchlorate on humans  
15 and the environment and the extent of perchlorate contamination. The report also contained  
16 recommendations for future research to address emerging issues (U.S. Environmental Protection  
17 Agency, 1998e). This report will be finalized and sent to Congress after the IRIS file is  
18 completed. Updates on activities of IPSC can be found on the EPA Office of Water (OW) web  
19 site at the following address: <http://www.epa.gov/ogwdw/ccl/perchlor/perchlo.html>. Discussion  
20 papers presented by the IPSC present additional information on the areas (e.g., analytical and  
21 treatment technology) that have not been discussed in detail herein.

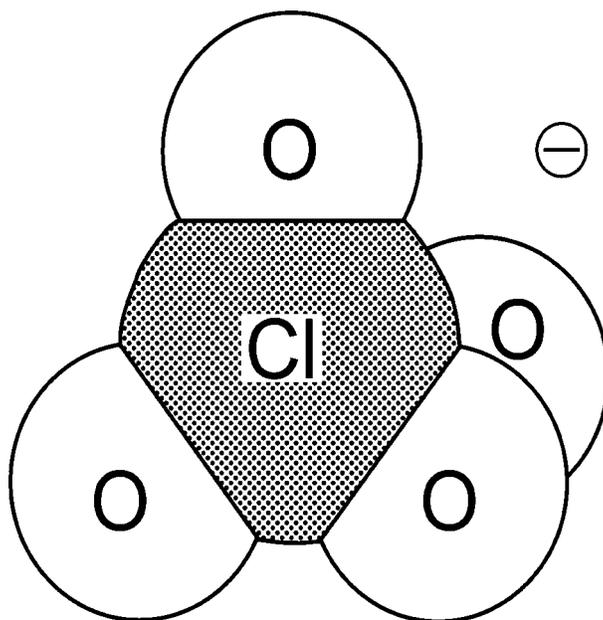


**Figure 1-6. Structure and membership of the executive committee, subcommittees areas, and co-chairs of IPSC. The IPSC is designed to ensure an integrated approach to addressing the perchlorate contamination challenge and to provide accurate accounts of technical issues to stakeholders. (OSWER = Office of Solid Waste and Emergency Response, NCEA = National Center for Environmental Assessment, DoD = Department of Defense, USAF = U.S. Air Force, OW = Office of Water, NERL = National Exposure Research Laboratory, OERR = Office of Emergency Response and Remediation, NRMRL = National Risk Management Research Laboratory, Cal DHS = California Department of Health Services, USN = U.S. Navy, UT DEQ = Utah Department of Environmental Quality).**

## 2. PHYSICOCHEMICAL CHARACTERISTICS

This chapter provides an overview of the physicochemical properties of perchlorate. These are important to understanding the persistence of perchlorate in the environment and to understanding how perchlorate is processed in various plants and animals. Additional toxicokinetic and toxicodynamic information can be found in Chapters 3 and 6; additional data on transport and transformation, including biotransport, are discussed in Chapters 8 and 9.

In the solid state, the perchlorate anion has been determined by X-ray diffraction to have a nearly perfect tetrahedral geometry with the four oxygen atoms at the vertices and the chlorine atom at the center as shown in Figure 2-1. In aqueous solution, the geometry is probably perfectly tetrahedral. The average chlorine-to-oxygen bond distance is 1.42 pm (Schilt, 1979b), and the oxygen-to-oxygen distance is 2.43 pm. The partial molar ionic volume is 44.5 cm<sup>3</sup>/mol at 25 °C, compared with 36.7 for iodide.



**Figure 2-1. Chemical structure of perchlorate.**

1 Perchlorate is widely known to be a very poor complexing agent and is used extensively as  
 2 a counter anion in studies of metal cation chemistry, especially in nonaqueous solution (Urbansky,  
 3 1998). In this application, it is comparable with other noncomplexing or weakly ligating anions,  
 4 such as trifluoromethanesulfonate (triflate [CF<sub>3</sub>SO<sub>3</sub><sup>-</sup>]), tetrafluoroborate (BF<sub>4</sub><sup>-</sup>), and, to a lesser  
 5 extent, nitrate (NO<sub>3</sub><sup>-</sup>). Some exceptions are known, but are rare, such as some copper  
 6 compounds (Burke et al., 1982). All of these anions have a highly delocalized (CF<sub>3</sub>SO<sub>3</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup>,  
 7 ClO<sub>4</sub><sup>-</sup>) or sterically blocked (BF<sub>4</sub><sup>-</sup>) monovalent anionic charge and large volume. The low charge  
 8 density reduces their affinity for cations and their extent of aquation (see Table 2-1).

**TABLE 2-1. GIBBS FREE ENERGIES OF FORMATION FOR  
 SELECTED ANIONS IN AQUEOUS SOLUTION (Urbansky, 1998)**

Anion	$\Delta G_f^\circ$ , kJ Mol <sup>-1</sup>
BF <sub>4</sub> <sup>-</sup>	-1,490
PO <sub>4</sub> <sup>3-</sup>	-1,019
SO <sub>4</sub> <sup>2-</sup>	-744
HCO <sub>3</sub> <sup>-</sup>	-587
OH <sup>-</sup>	-157
Cl <sup>-</sup>	-131
NO <sub>3</sub> <sup>-</sup>	-109
Br <sup>-</sup>	-104
ClO <sub>4</sub> <sup>-</sup>	-8.5
ClO <sub>3</sub> <sup>-</sup>	-8.0

1 This low association with cations is responsible for the extremely high solubilities of perchlorate  
 2 salts in aqueous and nonaqueous media. As noted, the ammonium and the alkali metal salts of  
 3 perchlorate generally are readily soluble in water. Salts of the smaller univalent cations (i.e.,  
 4 ammonium [NH<sub>4</sub><sup>+</sup>], lithium [Li<sup>+</sup>], and sodium [Na<sup>+</sup>]) are very soluble; whereas, those of the  
 5 larger univalent cations are less so (i.e., potassium [K<sup>+</sup>], rubidium [Rb<sup>+</sup>], and cesium [Cs<sup>+</sup>]).  
 6 Quaternary ammonium salts are less soluble still. The outstanding example is sodium

perchlorate, which is extremely soluble ( $>8 \text{ mol dm}^{-3}$ ). Table 2-2 lists these solubilities as well as other key physicochemical properties.

**TABLE 2-2. PHYSICOCHEMICAL PROPERTIES OF AMMONIUM AND ALKALI METAL PERCHLORATES AT 25 °C (Schilt, 1979).**

Physical Property	Magnitude of Physicochemical Property of Perchlorate					
	NH <sub>4</sub>	Li	Na	K	Rb	Cs
Molecular Weight (g mol <sup>-1</sup> )	117.49	106.40	122.44	138.55		
Density	1.952	2.429	2.499	2.5298	2.9	3.327
Solubility (w/w %)						
Water	24.922	59.71	209.6	2.062	1.338	2.000
Methanol	6.862	182.25	51.36	0.105	0.000	0.093
Ethanol	1.907	151.76	14.71	0.012	0.009	0.011
<i>n</i> -Propanol	0.387	105.00	4.888	0.010	0.006	0.006
Acetone	2.260	136.52	51.745	0.155	0.095	0.150
Ethyl Acetate	0.032	95.12	9.649	0.001	0.016	0.000
Ethyl Ether	0.000	113.72	0.000	0.000	0.000	0.000
Thermochemical data						
$\Delta H_f^\circ$ , kJ mol <sup>-1</sup>	-290.4	-384.0	-385.7	-435.5	-434.7	-434.7
$\Delta G_f^\circ$ , kJ mol <sup>-1</sup>	-88.9 <sup>b</sup>	-254 <sup>c</sup>	-255 <sup>b</sup>	-304	-306	-307
$\Delta S_f^\circ$ , kJ mol <sup>-1</sup>	186 <sup>b</sup>	130 <sup>c</sup>	142 <sup>b</sup>	151	161	175
$\Delta H_{\text{soln}}^\circ$ , kJ mol <sup>-1</sup>	-26.6	26.1	14.7	50.6	56.8	55.6
Magnetic susceptibility ( $\times 10^6$ )	46.3	32.8	37.6	47.4	—	69.9
Molar refraction	17.22	—	13.58	15.27	—	—

<sup>a</sup>Thermochemical data converted from kcal/mol using 1,000 cal = 4.184 J.

<sup>b</sup>Weast (1989).

<sup>c</sup>Dean (1985).

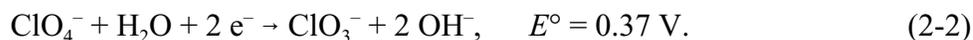
Because of their large solubilities, the health risk assessment for perchlorate anion (ClO<sub>4</sub><sup>-</sup>) is appropriate for perchlorate salts, including ammonium perchlorate [CASRN 7790-98-9], sodium perchlorate [CASRN 7601-89-0], potassium perchlorate [CASRN 7778-74-7], and

1 lithium perchlorate [CASRN 7791-03-9]. The estimate is not appropriate to characterize the risk  
2 of effects of perchloric acid (HClO<sub>4</sub>) [CASRN 7601-90-3] because it is a strong acid, and the  
3 dominant mode of toxicity is the irritating action of the hydrogen ion on skin and mucous  
4 membranes.

5 Perchlorate can be a strong oxidizing agent under certain conditions as indicated by its high  
6 reduction potential; therefore, the question has arisen as to whether or not it has the potential to  
7 behave as an oxidant in biological systems. The thermodynamics of the halogen oxoanions and  
8 oxoacids to participate in redox reactions are well understood. Under standard conditions in 1 M  
9 acid, where the species is reduced to chloride, the oxidizing strength and standard reduction  
10 potential ( $E^\circ$ ) increase as follows: Cl<sub>2</sub> < HOCl < HClO<sub>2</sub> < ClO<sub>3</sub><sup>-</sup> < ClO<sub>4</sub><sup>-</sup>. The reduction  
11 potentials for the oxoanions increase with increasing acidity or decreasing pH (i.e., they are  
12 stronger oxidizing agents in acidic solution). Consider, for example, the reduction of  
13 chlorine(VII) to chlorine(V) under both acidic and alkaline conditions. In 1.0 M H<sup>+</sup>(aq) solution  
14 (pH = 0),



17  
18 In 1.0 M OH<sup>-</sup>(aq) solution (pH = 14),



21  
22 The effect of pH can be explained in terms of Le Châtelier's principle. In Reaction 2-1,  
23 hydrogen ion is plentiful and acts a reactant; this drives the reaction forwards. In Reaction 2-2,  
24 hydroxide ion is a product of the reaction and is already present at 1 M. This reduces the driving  
25 force for this reaction to take place. The reaction is still spontaneous, as shown by the positive  
26 value of  $E^\circ$ ; nonetheless, the driving force is considerably smaller for this case.

27 Thermodynamically, perchlorate is a stronger oxidant in the chlorine oxoanion series at the  
28 extremes of the pH scale; however, such extremes are difficult to achieve in vivo (Tsui, 1998).

29 In Chapter 1, perchlorate anion was described as a nonlabile oxidant. Although the driving  
30 force for reduction is very high, the activation energy required to start the process is also very  
31 high. With the chlorine oxoanions, kinetic lability runs counter to the thermodynamic stability.

1 That is, the most stable species, hypochlorite ( $\text{ClO}^-$ ), reacts fastest; whereas, the least stable  
2 species, perchlorate ( $\text{ClO}_4^-$ ), reacts the slowest. It is important to note that the activation energy  
3 required for the reduction of perchlorate to take place is a function not only of the perchlorate,  
4 but also of the chemical nature of the reductant. With common reducing agents (e.g., thiosulfate,  
5 sulfite, or ferrous ions), the activation energy is too high for any reaction to be observed. In fact,  
6 this property (lack of lability) is exploited routinely in chemical studies where perchlorate salts  
7 are used to control the ionic medium and strength, but do not themselves react.

8 An alternative way of expressing the thermodynamic driving force for a reaction is the  
9 Gibbs free energy function. Although the driving force for redox reactions is often conveniently  
10 expressed in terms of the potential, there are practical limitations to this approach. For example,  
11 in the decomposition reaction of ammonium perchlorate in Equation 1-1, an electric potential  
12 cannot be measured. The Gibbs free energy of reaction,  $\Delta G_{\text{rxn}}^\circ$ , is a measure of the energy  
13 available to do work when a reaction is performed under constant pressure at standard state  
14 conditions.<sup>1</sup> When ammonium perchlorate explodes, the gaseous products push against the  
15 surrounding air and thereby perform expansion work on the atmosphere.<sup>2</sup>  $\Delta G_{\text{rxn}}^\circ$  specifies the  
16 maximal nonexpansion mechanical work that can be obtained from a chemical reaction carried  
17 out at constant temperature and pressure.<sup>3</sup> If the nonexpansion work is the electrical work of a  
18 redox process, then an additional relationship applies (Equation 2-3), where  $n$  is the number of  
19 electrons transferred;  $F$  is the Faraday constant,  $96,485 \text{ C (mol e)}^{-1}$ ; and  $E^\circ$  is the electric  
20 potential for the reaction under standard state conditions.

---

<sup>1</sup>This is the case with reactions occurring exposed to the open air, rather than inside a sealed container. In a sealed container, where volume is constant and pressure changes, a different thermodynamic quantity, the Helmholtz free energy  $\Delta A_{\text{rxn}}^\circ$ , is used instead. The superscript circle indicates standard state conditions (i.e., solution concentrations of  $1 \text{ mol dm}^{-3}$  and gas pressures of 1 bar). All thermodynamic data are for a temperature of 298 K. All of the thermodynamic relationships herein apply at other conditions, and reference tables exist only for standard conditions. For other conditions, appropriate corrections must be made.

<sup>2</sup>Expansion work ( $W_{\text{exp}}$ ) is significant only when a reaction has a net change in the number of gas molecules and can be calculated from the equation of state for a perfect gas:  $W_{\text{exp}} = -P\Delta V = \Delta nRT$  (where  $P$  = pressure (atm),  $V$  = volume (L),  $n$  = number of moles,  $R$  = ideal gas constant ( $\text{L atm K}^{-1}$ ), and  $T$  = temperature (K) and  $T$  and  $P$  are constant). For reactions occurring in the condensed phases,  $W_{\text{exp}} \approx 0$ .

<sup>3</sup>To obtain the maximal nonexpansion work, it is assumed that the process occurs reversibly so the loss of energy as heat is minimized. Although this is approximately true for an electrochemical cell, most chemical reactions do not take place under conditions that approach reversibility. For example, explosions are so irreversible because so much internal energy is lost as heat that the nonexpansion work is much smaller than  $\Delta G_{\text{rxn}}^\circ$ .

1 
$$\Delta G_{\text{rxn}}^{\circ} = -w_{\text{max}} = -nFE^{\circ} \quad (T, P \text{ constant}) \quad (2-3)$$

2  
3 The negative sign is necessary because the work done on the environment represents a loss of  
4 free energy from the chemical system. Nonexpansion work includes, but is not limited to,  
5 causing an electric current to flow or lifting an object against gravity. Whenever a chemical  
6 reaction has the ability to do work on the surroundings, it will take place spontaneously.<sup>4</sup>  $\Delta G_{\text{rxn}}^{\circ}$   
7 is calculated as follows using Hess's law:

8  
9 
$$\Delta G_{\text{rxn}}^{\circ} = \Sigma \Delta G_{\text{f}}^{\circ} (\text{all products}) - \Sigma \Delta G_{\text{f}}^{\circ} (\text{all reactants}). \quad (2-4)$$

10  
11 The Gibbs free energy of formation,  $\Delta G_{\text{f}}^{\circ}$ , is calculated for the formation of a compound from its  
12 standard state as an element; consequently,  $\Delta G_{\text{f}}^{\circ} = 0$  for  $\text{Cl}_2(\text{g})$  and  $\text{O}_2(\text{g})$ . For Reaction 1-1,

13  
14 
$$\begin{aligned} \Delta G_{\text{rxn}}^{\circ} &= 2\Delta G_{\text{f}}^{\circ} [\text{N}_2\text{O}(\text{g})] + 8\Delta G_{\text{f}}^{\circ} [\text{H}_2\text{O}(\text{g})] - 4\Delta G_{\text{f}}^{\circ} [\text{NH}_4\text{ClO}_4(\text{s})] \\ &= 2(104) + 8(-229) - 4(-89) \text{ kJ} = -1,268 \text{ kJ}. \end{aligned} \quad (2-5)$$

15  
16  
17 This large negative value for  $\Delta G_{\text{rxn}}^{\circ}$  suggests that the decomposition of ammonium perchlorate is  
18 spontaneous and has a large quantity of energy available to do work. When 4 moles (468 g) of  
19 ammonium perchlorate decompose, enough energy is released to lift a 1 kg mass 130 km, heat  
20 and completely boil 0.5 kg of water (starting from 25 °C), or power a 100-W light bulb for 3.5 h.  
21 Each molecule contains a large amount of potential chemical energy; however, a handful of  
22 ammonium perchlorate will not spontaneously explode. The free energy is not released because  
23 the reaction kinetics are too slow at room temperature—only an infinitesimal fraction of the  
24 molecules possesses enough energy to reach the activation energy of the transition state at any  
25 point. The activation energy for the reaction between an ammonium cation and a perchlorate  
26 anion also is too great for a reaction to occur.

---

<sup>4</sup>Readers who have studied thermodynamics will recall that the determining factor for the spontaneity of a chemical process is a net increase in the entropy of the universe (i.e.,  $\Delta S_{\text{univ}}^{\circ} > 0$ ). It can be shown that  $\Delta G_{\text{rxn}}^{\circ} = -T\Delta S_{\text{univ}}^{\circ}$ ; therefore,  $\Delta S_{\text{univ}}^{\circ} > 0$  means  $\Delta G_{\text{rxn}}^{\circ} < 0$ , and  $\Delta S_{\text{univ}}^{\circ} < 0$  means  $\Delta G_{\text{rxn}}^{\circ} > 0$  (because  $T > 0$ ). As a consequence of these relationships, it can be stated definitively that negative free energy available to do positive nonexpansion work is a measure of the thermodynamic spontaneity of a chemical reaction. This implies that any chemical reaction capable of performing positive nonexpansion work will occur spontaneously. Conversely, positive free energy suggests that the reverse reaction is spontaneous.

1 The distinction between thermodynamic spontaneity and kinetic lability must be  
 2 emphasized. A reaction with  $\Delta G_{\text{rxn}}^{\circ} \ll 0$  and  $E^{\circ} \gg 0$  is thermodynamically favored, but may be  
 3 so slow as to take virtually an infinite amount of time to occur (as is the case with most  
 4 perchlorate reductions). On the other hand, a reaction that occurs very quickly may have a very  
 5 small driving force. Reaction rates are fast when the combined internal energies of the reactants  
 6 closely approach the activation energy required to form the transition state. In a similar case, the  
 7 kinetic barrier (activation energy) explains why an open gas jet does not burst into flame until the  
 8 heat of a match is applied.

9 It is well established that, in aqueous solution, chlorine(I), chlorine(III), and chlorine(V)  
 10 species undergo their most facile reductions via nucleophilic attack at the chlorine atom rather  
 11 than at the oxygen atom. When oxoanions are dissolved in water, the rate of net oxygen atom  
 12 exchange (Equation 2-6) can be used to understand how reactions proceed:



15  
 16 Reaction 2-6 proceeds through an associative mechanism in which the incoming water molecule  
 17 attacks the central chlorine atom. Consider the simplest example, hypochlorous acid, for which  
 18 the following mechanism is the accepted explanation (where O is again a labeled oxygen atom):



20  
 21  
 22  
 23 The aquated species  $[\text{HO}\cdots\text{Cl}\cdots\text{OH}_2]^\ddagger$  represents the activated complex and is the transition state  
 24 of Reaction 2-7; the proton is not directly transferred from the labeled water molecule to the  
 25 hydroxide that is part of the HOCl molecule. Rather, a proton is lost to the bulk water of the  
 26 solution from the activated complex, and another proton is gained. This activated complex may  
 27 revert back to reactants or proceed to products.<sup>5</sup> As the number of oxygen atoms increases, the  
 28 water has greater difficulty accessing the reaction site. The oxidation state of the chlorine  
 29 increases by 2 with each additional oxygen atom; accordingly, the chlorine becomes more and

---

<sup>5</sup>Note that  $\Delta G_{\text{rxn}}^{\circ} = 0$  because the reactants and products are chemically identical. This suggests a process at equilibrium in which the forward and reverse rates are balanced.

1 more electron-poor and holds the oxygen atoms closer to share their electrons. (This factor will  
2 be expanded on further when perchlorate is examined specifically.)

3 In perchlorate, which contains chlorine(VII), the central chlorine atom is sterically blocked  
4 from the attack of an incoming reducing agent by the tetrahedrally oriented oxygen atoms.  
5 As the oxidation state of the central chlorine atom increases, the strength of the chlorine-oxygen  
6 bonds also increases. The electron-deficient chlorine(VII) draws electron density from the  
7 oxygen ligands resulting in increased  $O(p\pi)\rightarrow Cl(d\pi)$  back donation despite the high  
8 electronegativity of the oxygen atoms. Increased O-Cl bond strength thus further complicates  
9 oxoanion reduction by making oxygen-atom abstraction even more difficult.

10 Perchloric acid normally exhibits oxidizing behavior when heated and concentrated. When  
11 cold and dilute,  $HClO_4$  acts only as a strong Brønsted-Lowry acid with no more oxidizing  
12 character than other mineral acids, such as sulfuric or hydrochloric acids. In the absence of free  
13  $H^+$ , as in vivo, a reducer or a catalyst with a lot of free potential energy would be requisite to  
14 increase the rate (Tsui, 1998).

15 All observable perchlorate reductions reported in the literature are initiated via oxygen  
16 atom abstraction by air-sensitive transition metal species (Urbansky, 1998). The metal cations  
17 that react with perchlorate are all sensitive to atmospheric oxygen because they are strong  
18 (thermodynamically) and labile (kinetically facile) reductants. None of these metal ions would  
19 survive under human physiologic conditions. Certainly, any reductant capable of reacting with  
20 perchlorate, such as  $Ti^{III}(aq)$  (Earley et al., 2000),  $Ch_3ReO_2$  (Abu-Omar et al., 1996), or certain  
21  $Re^V$  complexes (Abu-Omar et al., 2000) would not survive in a physiologic environment. Thus,  
22 the activation energy to perchlorate reduction is so high that perchlorate cannot be expected to  
23 act as an oxidant under human physiological conditions (i.e., dilute solution, moderate  
24 temperatures, and nearly neutral pH). This is supported by absorption, distribution, metabolism,  
25 and elimination studies that show perchlorate is excreted virtually unchanged after absorption  
26 (see Chapters 3 and 6).

27 A catalyst increases the rate of chemical reactions by reducing the activation energy,  
28 increasing the number of collisions, or properly orienting chemical reactants. Many catalysts  
29 reduce the activation energy, but some have multiple effects. When a perchlorate ion collides  
30 with a reducing agent, the two entities can recoil unaffected or they can interact. If they interact,  
31 the entity they form is called an activated complex and is a transition state from which they can

1 separate or react. If they have sufficient internal energy (enough to overcome the activation  
2 energy), the species will react. For perchlorate, this means an oxygen atom is transferred to the  
3 reductant. If a catalyst is involved, it can act as an intermediate, removing oxygen atoms from  
4 the perchlorate and transferring them to the reductant. In the case of the rhenium (V) catalysts,  
5 the coordinated rhenium center accepts oxygen atoms from (and is therefore oxidized by) the  
6 perchlorate. This oxidized species (now containing  $\text{Re}^{\text{VII}}$ ) then transfers an oxygen atom to (and  
7 is therefore reduced by) any reducing agent; however, the authors used thioethers and mercaptans  
8 for this purpose (Abu-Omar et al., 2000). Of particular interest in this work was that the  
9 conditions were not nearly so forcing as what is normally required for perchlorate reduction. The  
10 reaction took place at roughly neutral pHs and ambient temperatures.

11 Some bacteria have catalysts (i.e, enzymes known as reductases) that allow the microbes to  
12 use perchlorate as an oxidant in anaerobic metabolic pathways. Although oxygen is a stronger  
13 oxidant than perchlorate, bacteria will utilize perchlorate under low-oxygen conditions. For  
14 example, perchlorate-reducing monera use perchlorate reductases under conditions where  
15 conventional inorganic chemistry suggests that perchlorate reduction should be imperceptibly  
16 slow (Urbansky, 1998; Logan, 1998). Over the past few years, there has been a profusion of  
17 work in this area, mostly slanted towards bioremediation (Coates et al., 1999, 2000; Logan, 2001;  
18 Nzungu and Wang, 2000).

19 This chapter provides a brief summary of some physiochemical properties of the  
20 perchlorate anion, especially the salient features that might bear on its environmental and  
21 toxicological chemistry. Additional chemical issues are explored in some depth in Chapter 9 as  
22 related to analysis of environmental samples. Additional chemical-specific issues as related to  
23 the pharmacokinetics of perchlorate in organisms are discussed in Chapters 3 and 6.  
24

### 3. TOXICOKINETICS/TOXICODYNAMICS AND MODE-OF-ACTION TESTING STRATEGY

This chapter explains the rationale that was the basis of the testing strategy which was designed to evaluate the potential critical targets for perchlorate and to establish a database robust enough to support a quantitative risk assessment. Aspects of the toxicokinetics and toxicodynamics of perchlorate and its interaction with the thyroid are discussed as the basis for the development of a testing strategy based on the mode of action of perchlorate. *Mode of action* is defined as a chemical's influence on molecular, cellular, and physiological functions (Federal Register, 1996; Wiltse and Dellarco, 1996). Understanding the mode of action helps to interpret the relevancy of the laboratory animal and human data to inform the most appropriate dose-response procedure (see Chapter 7).

As discussed in Chapter 2, perchlorate salts dissolve readily in water. The resultant anion is easily absorbed from the gastrointestinal tract. However, because of its high charge, neither perchlorate, nor other electrolytes applied from aqueous solution or aqueous media penetrate the skin readily (Scheuplein and Bronaugh, 1983). Uptake of inorganic ions such as perchlorate through the skin is typically less than 10% and frequently less than 1%. Exposure via inhalation of fumes or vapors is considered negligible because the vapor pressure of perchlorate salts and acids is low at room temperatures. The risk from exposure to particles would depend on the particle size distribution. Thus, the ingestion route is the major concern for the risk posed by the perchlorate contamination and is the focus of this characterization.

#### 3.1 ABSORPTION, DISTRIBUTION, METABOLISM, AND ELIMINATION OF PERCHLORATE

Limited absorption, distribution, metabolism, and elimination (ADME) studies were in existence prior to the testing strategy discussed in Section 3.5. Although experimental studies in laboratory species and humans had been performed using radiolabeling techniques, most were at high concentrations, and the published data were expressed simply as thyroid: blood ratios of

1 radioactivity counts that provided no information on internal dose to biological tissues. Oral  
2 drinking water administration, the most relevant to the contamination issue, was not the norm.  
3 Time-course studies were very limited and essentially nonexistent for repeated administration.  
4 More importantly, no data existed on the co-administration of iodide (I<sup>-</sup>) and perchlorate even  
5 though data were necessary to develop a physiologically based pharmacokinetic model (Fisher,  
6 1998a). The following section describes the limited pharmacokinetic information that was  
7 considered when the data gap was highlighted during the development of protocols for the testing  
8 strategy. The development of physiologically-based pharmacokinetic models that describe  
9 ADME for perchlorate with data from the testing strategy will be discussed in Chapter 6.

10 Perchlorate appears to be eliminated rapidly, primarily in the urine (>90%), and virtually  
11 unchanged from both rats (Eichler and Hackenthal, 1962) and humans (Anbar et al., 1959).  
12 Durand (1938) measured urinary elimination from two human subjects who ingested 794 mg of  
13 sodium perchlorate in 100 g of water. Urinary elimination accounted for 50% of the  
14 administered dose within 5 hr and 95% within 48 hr. Half-lives have been reported for the rat  
15 ranging from <8 hr (95% in 60 hr) to ≈20 hr (Wolff, 1998). Stanbury and Wyngaarden (1952)  
16 reported that perchlorate appears in the urine within 10 to 15 min of oral dosing and that peak  
17 plasma levels occur within 3 hr. Perchlorate was reported to undergo a two-phased urinary  
18 elimination process in rats and calves. In rats, the first phase accounted for approximately 96%  
19 of the administered dose and had a half-life of 1 to 2 hr. The second phase accounted for 4% and  
20 had a half-life that ranged from 72 to 80 h. In calves, the first-phase half-life was reported to be  
21 2 to 2.5 hr, and the second 23 to 27 hr (Selivanova et al., 1986, as cited in Allred, 1998). The  
22 kinetics of long-term administration of perchlorate have not been characterized. The distribution  
23 and metabolism of perchlorate and its relevance to potential toxicity in the thyroid will be  
24 discussed in greater detail in Section 3.3 following discussions of iodine metabolism and thyroid  
25 physiology in Section 3.2.

### 26 27 **3.1.1 Human Studies**

28 The majority of the human data on perchlorate ADME prior to the strategy was comprised  
29 of the therapeutic case and clinical studies of Graves' disease patients described in Section 4.2.2.  
30 These studies established the effect of perchlorate on the sodium (Na<sup>+</sup>)-iodide (I<sup>-</sup>) symporter  
31 (NIS) but were of limited use in establishing quantitative dose-response relationships.

1 Anbar et al. (1959) demonstrated that perchlorate was not metabolized in humans. Four  
2 patients were administered 200 mg (approximately 2.9 mg/kg using a default body weight of  
3 70 kg) double-labeled  $K^{36}Cl^{18}O_4$ , and urine was collected 3 h after dosing. Perchlorate was found  
4 to be excreted at approximately 200  $\mu\text{g}/\text{min}$  in the urine. Total urine radioactivity was  
5 distributed between  $^{36}\text{Cl}$ ,  $^{36}\text{Cl}^{18}\text{O}_4^-$ ,  $^{36}\text{ClO}_4^-$  and  $^{36}\text{Cl}^-$  and indicated that perchlorate was excreted  
6 unchanged in the urine. No human data existed with which to adequately characterize the  
7 pharmacokinetics of perchlorate during steady-state, low-dose, repeated administration.

### 9 **3.1.2 Laboratory Animal Studies**

10 Although the perchlorate discharge test has been performed in rats (Atterwill et al., 1987),  
11 the procedure is very different than that used in humans and does not readily allow for  
12 comparison or extrapolation. Rats are dosed intraperitoneally (ip) with 100  $\mu\text{L}$  (1  $\mu\text{Ci}$ )  $^{125}\text{I}$ , then  
13 dosed ip with potassium perchlorate at 5, 10, 25, or 50 mg/kg body weight from 1 to 6 h  
14 afterwards. Results are expressed as thyroid: blood ratios, which differ from how most human  
15 data are expressed. Additionally, the time points at which uptake is measured are highly  
16 dissimilar to those used in human studies.

17 Anbar et al. (1959) also attempted to confirm the lack of perchlorate accumulation and lack  
18 of metabolism in the thyroid in rats. White rats were injected ip with  $^{36}\text{KClO}_4$ , and the specific  
19 activity per gram of tissue was measured at 30 min, 4 hr, and 12 hr. The activity was greatest in  
20 the thyroid and peaked at 4 h. The salivary and adrenal glands also had high activity levels.  
21 Rabbits also were tested; the thyroid activity levels were again the highest of any tissue and  
22 peaked at 2 h. Rabbit testes had the next highest specific activities.

23 In one of the only co-administration studies, Anbar et al. (1959) simultaneously  
24 administered  $^{131}\text{I}^-$  and  $^{36}\text{ClO}_4^-$  in equimolar concentrations. The thyroid: blood specific activity for  
25 iodide was slightly higher than the ratio for perchlorate (1.80 and 1.69, respectively).

26 Halmi et al. (1956) examined iodide uptake in male Sprague-Dawley rats when active  
27 transport was completely blocked via sodium perchlorate. The rats were first administered 6 mg  
28 of propylthiouracil (PTU) subcutaneously to prevent iodide organification. Iodide uptake was  
29 prevented by administration of 100, 200, or 400 mg sodium perchlorate with half of each dose  
30 administered along with the PTU and the other half administered 45 min later with 5 to 50  $\mu\text{Ci}$   
31  $^{131}\text{I}^-$ . The rats were sacrificed 1.0 to 1.5 h after the iodide administration. Perchlorate reduced the

1 thyroid: blood ratio from 22.7 to 0.45; radioiodide was found to account for 30% of the thyroid  
2 gland volume when it entered the gland by diffusion alone. Rats sacrificed 4.0 to 4.5 h after  
3 iodide administration produced similar results, indicating that equilibrium is reached prior to  
4 1.0 to 1.5 h. The distribution of radioiodide in other tissues also was examined. Perchlorate did  
5 not affect the organ: serum iodide ratios in the following organs: submaxillary salivary gland,  
6 parotid salivary gland, pituitary gland, adrenal glands, testes, spleen, kidneys, lung, skin, or  
7 diaphragm. However, perchlorate administration did affect the stomach wall: serum and gastric  
8 juice: serum iodide ratios (0.36 and 0.75, respectively) compared with the ratios for controls  
9 administered sodium chloride (1.45 and 15.8, respectively). This suggested a gastric iodide  
10 pump subject to inhibition by perchlorate and, as will be discussed in Chapter 6, the  
11 gastrointestinal tract is another tissue with NIS.

12 Goldman and Stanbury (1973) administered 0.1  $\mu\text{Ci}$  of the potassium salt of  $^{36}\text{Cl}$ -labeled  
13 perchlorate ( $\text{K}^{36}\text{ClO}_4$ ) by ip injection to male Sprague-Dawley rats that had been maintained on a  
14 low-iodine diet for 4.5 to 5.0 weeks prior to dosing (approximately 40  $\mu\text{g}$  stable perchlorate per  
15 injection). The radionuclide retention in the thyroid, expressed as percent of dose per gram of  
16 tissue, was recorded at 2 h (6 rats), 4 h (5 rats), 8 h (6 rats), 24 h (6 rats), 48 h (6 rats), and 96 h  
17 (5 rats). The peak was reported to appear around 4 h and then to fall to approximately 5% of this  
18 peak value after approximately 96 h. An exponential function was used to estimate a half-life of  
19 20 h. Urinary excretion data indicated that the disappearance rate from the plasma and thyroid  
20 and the appearance rate in the urine corresponded closely although the question was raised as to  
21 whether there is some curvilinearity to the urinary excretion, which may suggest limited  
22 saturation. The retained dose and its standard deviation in tissues at 96 h were reported as  
23  $0.142 \pm 0.1$ ,  $0.125 \pm 0.09$ ,  $0.098 \pm 0.03$ ,  $0.048 \pm 0.04$ , and background for the thyroid, kidney,  
24 spleen, liver, and brain, respectively.

25 Chow et al. (1969) compared the uptake of radiolabeled perchlorate and iodide ions with  
26 stable ions in normal and thyroid-impaired rodents. Intact male Sprague-Dawley rats were  
27 injected ip with 0.1, 0.2, or 5.0 meq/kg stable potassium perchlorate (14, 28, or 690 mg/kg,  
28 respectively) 2 h prior to sacrifice. The specific activity of the chlorine label ( $^{36}\text{Cl}$ ) was  
29  $25.2 \mu\text{Ci}/\text{mmol}$ . Thyroid impairment was affected by pretreatment with thyroid-stimulating  
30 hormone (TSH) (1 international unit [IU] TSH in 0.9% saline solution ip 18 h prior to  
31 perchlorate administration), hypophysectomization (removal of the pituitary), TSH and

1 hypophysectomization, or PTU (0.1% PTU in drinking water for 2 weeks prior to perchlorate  
2 administration). Perchlorate at the 0.1- and 0.2-meq/kg dose levels was found to preferentially  
3 concentrate in the rat thyroid as compared to the plasma, and the concentration was related  
4 inversely to dose. The high dose level did not result in the concentration of radiolabeled  
5 perchlorate in the thyroid. Rats pretreated with TSH or PTU also concentrated perchlorate at the  
6 lower dose levels. At the two lower levels, hypophysectomized rats were not able to concentrate  
7 perchlorate compared with intact rats, but the thyroid perchlorate concentration at the high dose  
8 level did not differ between intact and altered rats. In a second subset of the same study, rats  
9 were exposed to 0.005, 0.01, 0.02, 0.05, or 0.10 meq/kg perchlorate (0.69, 1.4, 2.8, 6.9, or  
10 14 mg/kg, respectively) under the same general conditions. The concentration of radiolabeled  
11 perchlorate in the thyroid again was related inversely to perchlorate dose. Male albino guinea  
12 pigs also were exposed to the same doses. The guinea pigs displayed the same relationships as  
13 the rats, but concentrated more perchlorate in the thyroid compared to plasma levels.

14 Chow and Woodbury (1970) demonstrated that perchlorate is actively sequestered by the  
15 thyroid gland at low doses but that the capacity of the symporter to actively sequester perchlorate  
16 is exceeded at higher doses. Male Sprague-Dawley rats were functionally nephrectomized by  
17 ligating the renal pedicle of both kidneys 24 h before the rats were sacrificed. Perchlorate was  
18 administered as the radiolabeled potassium salt ( $K^{36}ClO_4$ ) in solution by ip injection at 0.005,  
19 0.1, or 2.0 mmol/kg stable potassium perchlorate (0.69, 14, and 280 mg/kg body weight,  
20 respectively, assuming 0.266 kg body weight; actual weight  $226 \pm 4$  g) 2 to 240 min before  
21 sacrifice. A group of control rats received [ $^{14}C$ ]-insulin,  $^{35}SO_4^{-2}$  or  $^{36}Cl^-$  2 h prior to sacrifice to  
22 determine thyroid follicle volume and intrafollicular membrane potential. Concentrations of  
23 perchlorate in the thyroid and plasma were measured at 0.033, 0.067, 0.13, 0.2, 0.50, 1.0, 2.0,  
24 and 4.0 h after sacrifice. Again, perchlorate was actively sequestered by the thyroid gland at the  
25 low dose, but the capacity of the symporter to actively sequester perchlorate was exceeded at the  
26 higher doses (e.g., the thyroid:plasma [milligrams per gram:milligrams per liter] ratios at 15 min  
27 or 4 h post-dosing were 6.4, 0.69, and 0.36 or 13.8, 0.93, and 0.44 at the 0.5, 14.0, or  
28 280.0 mg/kg doses, respectively). These data suggest that maximal inhibition by perchlorate of  
29 active uptake of iodide probably occurs below 14 mg/kg potassium perchlorate (10.0 mg/kg as  
30 perchlorate). If perchlorate-induced inhibition of active iodide uptake is substantial, iodide still  
31 may enter the thyroid by diffusion, but in a smaller amount. Likewise, if inhibition of iodide

1 uptake by perchlorate is incomplete, then iodide still may be actively sequestered into the thyroid,  
2 again in a smaller amount. Thus, perchlorate-induced thyroid hormone perturbations may  
3 plateau in adult rats dosed with perchlorate greater than approximately 5 to 10 mg/kg of  
4 perchlorate (Fisher, 1998a).

5 Wolff and Maurey (1962) demonstrated the competitive nature of the perchlorate inhibition  
6 in sheep thyroid tissue slices incubated at 37 °C for 100 min. This study showed that the  
7  $K_m$  constants for anion accumulation and the  $K_i$  constants for inhibition of accumulation were  
8 identical within the error of the method.

9 Eichler and Hackenthal (1962) presented perchlorate elimination data for male and female  
10 Wistar rats dosed subcutaneously with 0.2, 1.0, or 6.0 of the  $^{36}\text{Cl}^-$  sodium perchlorate salt  
11 ( $\text{Na}^{36}\text{ClO}_4$ ) per 100 g body weight (2, 10, or 60 mg/kg). The elimination curves showed nearly  
12 linear, rapid excretion of perchlorate until 6 hr, at which time the curve slope started to decrease.  
13 The rate of excretion increased with dose. The elimination rates of the different doses prior to  
14 24 h were significantly different from each other but were similar after 24 h. Over 60 hr, 93.4 to  
15 97.4% of the administered dose was recovered, again suggesting that perchlorate was not  
16 metabolized.

17 In a recent review (Von Burg, 1995), perchlorate elimination curves in rats and calves were  
18 described as biphasic in both species. For rats, 96% of administered perchlorate is eliminated  
19 with a half-life of 1 to 2 hr. The second portion of the curve accounts for 4% of the dose, with  
20 half-life of 72 to 80 hr. Calves have a faster overall rate of elimination, but the initial elimination  
21 is slower. The first-phase half-life was 2.0 to 2.5 hr, and the second-phase half-life ranged from  
22 23 to 27 hr.

23 An intravenous (iv) study performed at AFRL/HEST in Sprague-Dawley rats with  
24 perchlorate to characterize its inhibition of iodide uptake supports the conclusion that there is  
25 inhibition at low concentrations and there is a gradual plateau at higher concentrations (Meyer,  
26 1998). Rats were dosed once by iv tail-vein injection with either 0.01, 0.1, 1.0, or 3.0 mg/kg of  
27 cold (i.e., not radiolabeled) ammonium perchlorate in saline. Perchlorate was administered as  
28 ammonium perchlorate, and the data are presented as milligrams perchlorate per kilogram body  
29 weight. Two hours after dosing with perchlorate, the rats were dosed again by iv tail-vein  
30 injection with 33  $\mu\text{g}/\text{kg}$   $^{125}\text{I}$  dissolved in saline. Rats were sacrificed at selected times (n = 6 per  
31 time point) up to 24 h. Total and free  $^{125}\text{I}$  were measured in serum, thyroid, and urine.

1 Perchlorate serum, thyroid, tissue, and urine analyses began in January 1999 and are reported in  
2 Chapter 6. For control comparison, rats were dosed once by iv tail-vein injection with 33  $\mu\text{g}/\text{kg}$   
3 nonradiolabeled iodide and  $^{125}\text{I}$  mixed in physiologic saline. Rats ( $n = 6$ ) were sacrificed at the  
4 same selected time points up to 24 hr.

5 Table 3-1 shows the percent of inhibition of  $^{125}\text{I}$  uptake as measured by bound  $^{125}\text{I}$  in the  
6 thyroid. Inhibition of  $^{125}\text{I}$  uptake into the thyroid by perchlorate was measured by bound or free  
7  $^{125}\text{I}$  in the thyroid at various time points after the single-dose of perchlorate. Because the  $^{125}\text{I}$  was  
8 administered 2 hr after dosing with ammonium perchlorate, these time points correspond to 4, 8,  
9 and 11 h after dosing. The most profound inhibitory effects were found at the 1.0- and 3.0-mg  
10 perchlorate/kg dose group; however, the trend for  $^{125}\text{I}$  inhibition is evident at the 0.01- and  
11 0.1-mg/kg levels (Meyer, 1998). By 24 h (26 h after dosing with perchlorate), inhibitory effects  
12 on  $^{125}\text{I}$  uptake were still observed at the 1.0- and 3.0-mg/kg dose groups.

13 Recovery of  $^{125}\text{I}$  in urine 24 hr after dosing with  $^{125}\text{I}$  (26 h after ammonium perchlorate) was  
14 between 79 and 88% for control  $^{125}\text{I}$ -dosed rats and perchlorate-dosed rats. The control  $^{125}\text{I}$ -dosed  
15 rats excreted 79.5% (SD  $\pm$  5.50) of the  $^{125}\text{I}$  dose over the 24-hr period; whereas, the perchlorate-  
16 dosed rats excreted 87% (SD  $\pm$  7.84), 86% (SD  $\pm$  4.47), 87.8 (SD  $\pm$  20.20) and 79.3 (SD  $\pm$  10.58)  
17 of the  $^{125}\text{I}$  dose in urine at the 0.01-, 0.1-, 1.0-, and 3.0-mg/kg dose levels, respectively. The  
18 amount of  $^{125}\text{I}$  in serum was elevated in the perchlorate-dosed animals compared to the control  
19  $^{125}\text{I}$ -dosed rats for up to 6 hr in all dose groups, suggesting that thyroid function was altered by  
20 perchlorate and that a transient “discharge” of organified  $^{125}\text{I}$  occurred as reported in studies  
21 summarized in Chapter 3. Free  $^{125}\text{I}$  levels in serum were similar between perchlorate-dosed and  
22 control  $^{125}\text{I}$ -dosed rats (Meyer, 1998). These results are consistent with those of Chow et al.  
23 (1969) and Chow and Woodbury (1970). The pattern for the inhibition of iodide uptake, albeit  
24 only after a single dose, is strikingly similar to the patterns shown for the thyroid hormone  
25 decreases. Consequently, data on the species differences (i.e., rat versus human in particular) in  
26 perchlorate inhibition of the symporter will provide a basis for evaluating the degree of  
27 uncertainty that should be applied when utilizing laboratory animal data as the model for humans  
28 (see Chapter 7).

29 Repeated dose studies in rats (Fisher, 1998a) and in humans (Channel, 1998a) to establish  
30 the kinetics of perchlorate at steady-state performed by AFRL/HEST to further characterize the  
31 inhibition of iodide uptake by perchlorate are discussed in Chapter 6.

**TABLE 3-1. PERCENT INHIBITION OF IODIDE UPTAKE IN THE THYROID GLAND OF SD RATS DOSED WITH PERCHLORATE (Meyer, 1998)**

Time Points <sup>a</sup>	Dose (mg perchlorate/kg)	[Iodide] ( $\mu\text{g/g}$ )	Percentage of Inhibition
2 hr	Control <sup>b</sup>	24.4	—
	0.01	21.3	13
	0.1	18.6	24
	1	7.4	70
	3	2.99	88
6 hr	Control <sup>b</sup>	46.5	—
	0.01	36.7	21
	0.1	32.0	31
	1	19.2	59
	3	9.13	80
9 hr	Control <sup>b</sup>	55	—
	0.01	49.2	11
	0.1	39.2	29
	1	24.7	55
	3	10.0	82

<sup>a</sup>Time points correspond to dosing with <sup>125</sup>I and to 4, 6, and 11 hr after dosing with ammonium perchlorate.

<sup>b</sup>Dosed with only iodide (33  $\mu\text{g/kg}$ ).

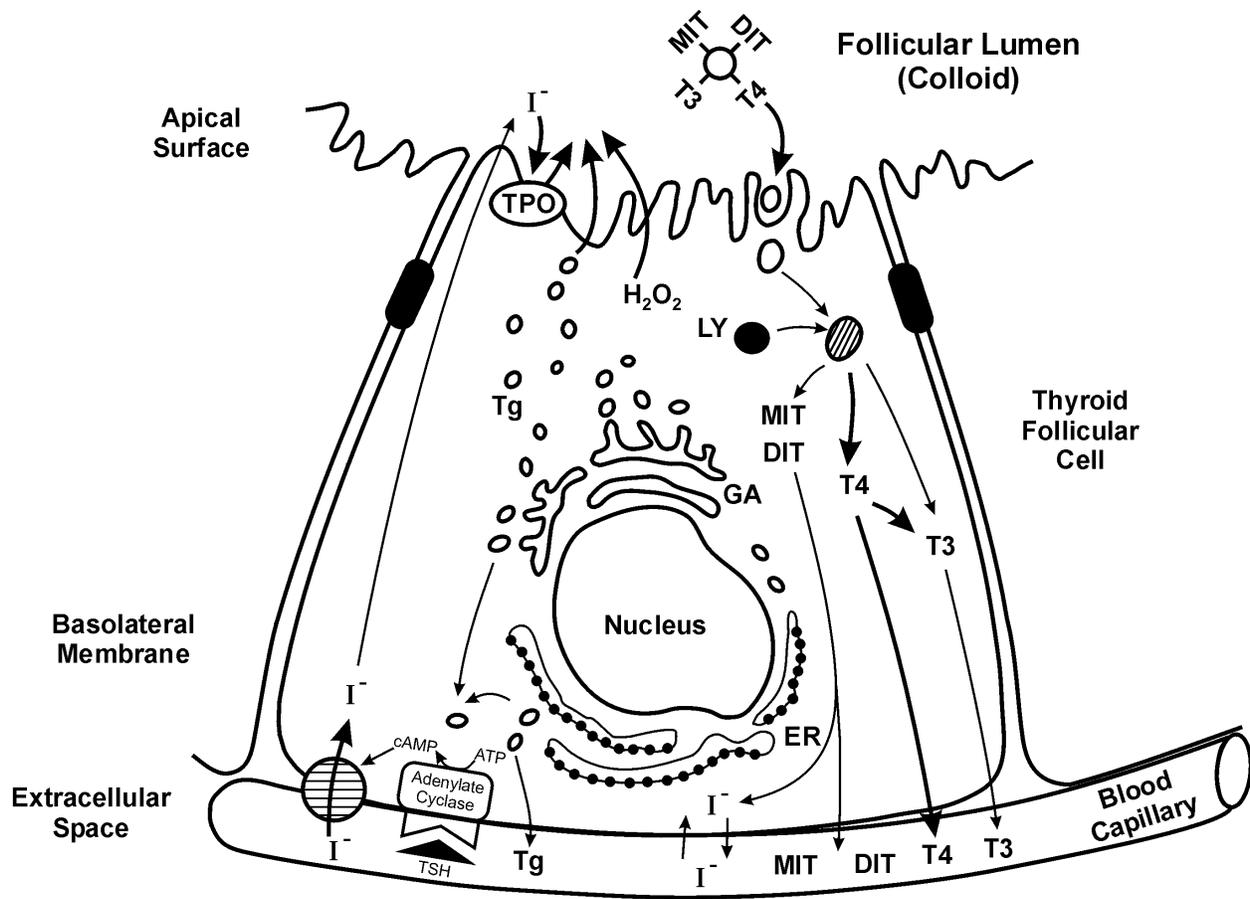
### 1    **3.2 IODINE METABOLISM AND THYROID PHYSIOLOGY**

2            Iodine plays a central role in thyroid physiology as both a constituent of thyroid hormones  
3 and a regulator of thyroid gland function. Like perchlorate, iodine is absorbed efficiently from  
4 the gastrointestinal tract. Iodine in organic form is converted mostly to iodide before absorption  
5 (Cavalieri, 1997). The kidneys account for about two-thirds of the iodide cleared from plasma  
6 and more than 90% of the iodide cleared from the body. Sweat and breast milk account for  
7 various fractions of iodide loss, and fecal elimination constitutes approximately 1% of total body  
8 iodide clearance.

1           The thyroid gland concentrates iodide against an electrochemical gradient by a carrier-  
2 mediated mechanism driven by adenosine triphosphate (ATP). The activation energy required  
3 for perchlorate reduction is so high that it cannot act as an oxidant under physiological conditions  
4 (i.e., dilute solution, moderate temperatures, and neutral pH). Plasma membrane experiments  
5 indicate that the sodium cation ( $\text{Na}^+$ ) and iodide cotransport are electrogenic, with a  
6 thermodynamically downhill transport of approximately two  $\text{Na}^+$  ions driving one iodide ion  
7 against its electrochemical gradient into the cell. The transport is sensitive to ouabain, an  
8 inhibitor of ATPase. The molecule responsible for the transport of iodide has been named the  
9 *sodium (Nat)/iodide (I) symporter or NIS*. The thyroid thus has a specialized ability to  
10 concentrate iodide selectively from the surroundings where the concentration is very low ( $10^{-8}$  to  
11  $10^{-7}$  M) and where the concentration of chloride ions is in the order of 0.01 to 0.1 M. The  
12 transport is “active,” not only by electrochemical criteria, but also by metabolic ones: it does not  
13 occur in the cold, it requires oxygen, and, as mentioned, it is a function of the ATP level.  
14 In addition to the thyroid, other organs that can concentrate iodide include the salivary glands,  
15 gastric mucosa, choroid plexus, mammary glands, and the placenta. Iodide secreted into the  
16 saliva and gastric juice is reabsorbed in the small intestine (Cavalieri, 1997).

17           Nevertheless, it is essentially only in the thyroid that the newly concentrated iodide can be  
18 metabolized further to form thyroid hormone; and, only in the thyroid, does TSH regulate the  
19 process. Thyroid hormones play numerous and profound roles in regulating metabolism, growth,  
20 development, and maintenance of homeostasis. It is generally thought that these actions result  
21 from the effects of the thyroid hormones on protein synthesis (Hill et al., 1989).

22           Figure 3-1 shows a schematic representation of thyroid hormone biosynthesis and secretion  
23 in a single thyroid follicular cell. The thyroid hormones are stored as amino acid residues in  
24 thyroglobulin (Tg), a protein constituting most of the colloid in the thyroid follicles. In situ, the  
25 follicular cell displays functional and structural polarity: the vascular space is at the bottom, and  
26 the lumen of the follicle is at the top. The striated circle straddling the basolateral membrane  
27 represents the iodide transporter. The process of thyroid hormone biosynthesis is first stimulated  
28 by TSH binding to the follicular cell TSH receptor and cyclic adenosine monophosphate (cAMP)  
29 activation (Hard, 1998). The protein portion of Tg is synthesized on rough endoplasmic  
30 reticulum (ER), and carbohydrate moieties are added by the Golgi apparatus (GA).  
31 Thyroglobulin proceeds to the apical surface in secretory vesicles (small open circles) that



**Figure 3-1. Schematic representation of thyroid hormone biosynthesis and secretion in a single thyroid follicular cell. (Modified from Hill et al., 1989; Cavaliere, 1997; and Fisher, 1996.)**

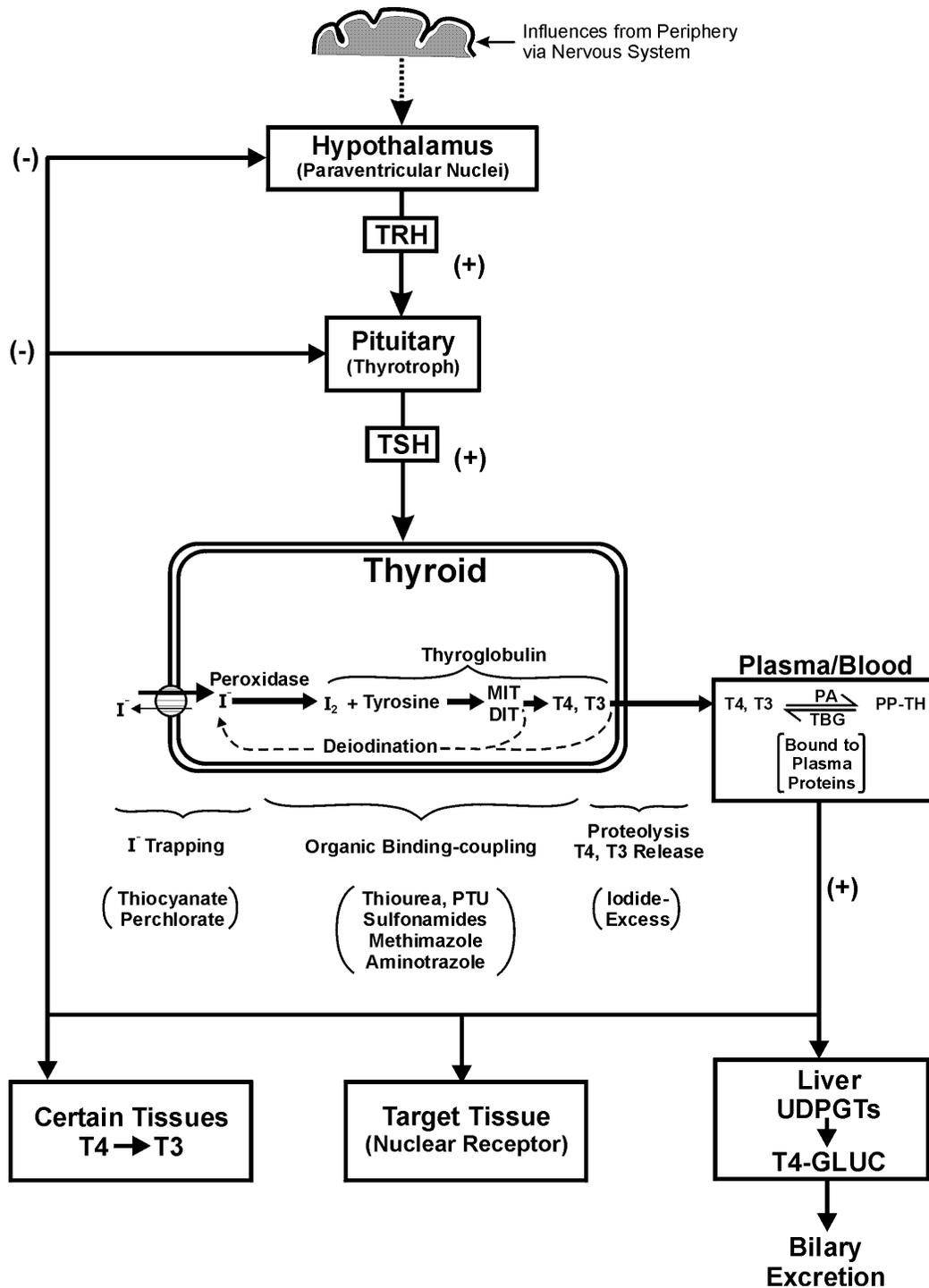
- 1 fuse with the cell membrane and discharge their contents into the follicular lumen. Iodide enters
- 2 the cell by active transport, and then, at the apical surface, is oxidized by thyroid peroxidase
- 3 (TPO). The hydrogen-peroxide-generating system is represented by hydrogen peroxide ( $H_2O_2$ ).
- 4 Organification occurs at or near this apical cell-colloid interface; the oxidized iodide is
- 5 incorporated into tyrosine residues in peptide linkage in Tg. Two iodinated tyrosyl groups couple
- 6 in ether linkage to form tetraiodothyronine (T<sub>4</sub>), which initially remains trapped in Tg. Hormone
- 7 secretion first involves pinocytosis of colloid-containing iodinated Tg (large open circle) at the
- 8 apical border of the follicular lumen and resolution into vesicles that fuse with lysosomes (LY,
- 9 dark circle). Lysosome proteolysis (striated circle) then converts Tg to amino acids, T<sub>4</sub>,

1 triiodothyronine (T3), diiodotyrosine (DIT) and monoiodotyrosine (MIT). Iodotryosine  
2 dehalogenase regenerates iodide from MIT and DIT for reuse within the thyroid or release into  
3 the blood, accounting for the iodide leak in the chronic state of iodine excess in certain thyroid  
4 disorders. Type I iodothyronine deiodinase converts a fraction of the free T4 to T3. Both  
5 hormones (T4 and T3) are released into the blood circulation by a process that is not well  
6 understood. The thyroid also releases Tg, of which some is iodinated and some uniodinated as  
7 newly synthesized protein.

8         Although T4 is by far the major hormone secreted by the thyroid (typically at 8 to 10 times  
9 the rate of T3), T4 is considered a prohormone because about 33% of the T4 secreted undergoes  
10 5'-deiodination to T3 in the peripheral tissues and T3 is about fourfold more potent than T4.  
11 Another 40% undergoes deiodination of the inner ring to yield the inactive material, reverse  
12 triiodothyronine (rT3), which recently has been postulated to play an inhibitory role on the  
13 conversion of T4 to T3. T3 is regarded as the active hormone because it is the form that appears  
14 to activate a response by nuclear DNA. Upon entering the circulation, both T4 and T3 are bound  
15 and transported in strong, but not covalent, association with plasma proteins.

16         The major plasma-protein carrier in humans is thyroxine-binding globulin, a glycoprotein  
17 with a very high affinity for T4 and a lower affinity for T3. In rats, the T4 and T3 are bound to  
18 prealbumin (PA) or albumin with a weaker attachment. Control of the circulating concentrations  
19 of these hormones is regulated primarily by a negative feedback involving three organs: (1) the  
20 thyroid, which produces thyroid hormone, and (2) the pituitary gland and (3) hypothalamus,  
21 which respond to and help maintain optimal T3 and T4 levels (Hill et al., 1998). Figure 3-2  
22 shows the schematic for this hypothalamic-pituitary-axis and the feedback mechanisms.

23         The hypothalamus stimulates the pituitary gland through thyrotropin-releasing hormone  
24 (TRH) to produce TSH, which prompts the thyroid to produce T4 and T3. Once secreted into the  
25 blood, T4 and T3 are bound to plasma proteins (thyroid-binding globulin [TBG] in humans or  
26 prealbumin [PA] and albumin in rats). In addition to the aforementioned conversion of T4 to T3  
27 in peripheral tissues, thyroid hormone also is metabolized irreversibly in the liver by uridine  
28 diphosphyl glucuronosyl transferases (UDPGTs) to either glucuronic (T4) or sulfate (mainly T3)  
29 conjugates that are excreted in bile. A portion of the conjugated material is hydrolyzed in the  
30 intestine, and the free hormones thus released are reabsorbed into the blood via enterohepatic  
31 circulation. The remaining portion of the conjugated material is excreted in the feces.



**Figure 3-2. Schematic of the hypothalamic-pituitary-thyroid axis and feedback mechanisms (PP-TH = plasma protein-thyroid hormone, PTU = propylthiouracil, UDPGT = uridine diphosphyl glucuronosyl transferase, T4 GLUC = T4-glucuronide conjugate). (Modified from U.S. Environmental Protection Agency, 1998a; Hill et al., 1998; and Capen, 1997).**

1 Cells in the hypothalamus and pituitary gland respond to levels of circulating T4 and T3  
2 such that when thyroid production levels are high, there is a signal to reduce the output of (TRH)  
3 and TSH. Similarly, when thyroid hormone levels are low, the pituitary is prompted to deliver  
4 more TSH to the thyroid in order to increase the output of T4 and T3. This negative feedback  
5 loop helps the body respond to varying demands for thyroid hormone and to maintain hormone  
6 homeostasis. Thus circulating T4, T3, and TSH are monitored readily in experimental animals  
7 and humans and so may serve as biomarkers of exposure to and indicators of the effects from  
8 agents that disrupt the status of the hypothalamic-pituitary-thyroid axis (U.S. Environmental  
9 Protection Agency, 1998a).

10 In the absence of thyroid-binding globulin, as in the rat and mouse, a greater fraction of  
11 thyroid hormone is free of protein binding and subject to metabolism and removal from the body.  
12 As a consequence, the half-life of T4 in the rat is only about 1 to 24 hr, in contrast to the 6 to  
13 7 day half-life in humans. Rats compensate for the increased turnover rate by secreting more  
14 TSH from the pituitary gland. Table 3-2 provides the interspecies and intraspecies differences in  
15 both thyroid hormone and gland structure between rats and humans. The consequences of  
16 disrupting the status of the hypothalamic-pituitary-axis will be discussed in Section 3.4.

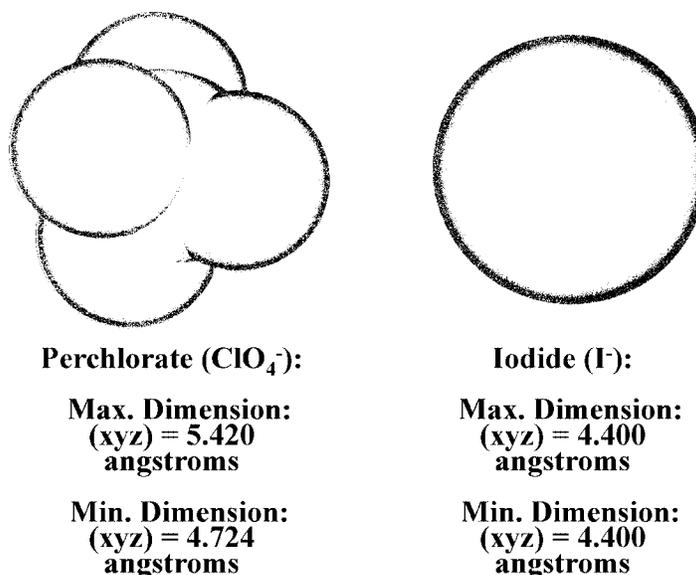
### 19 **3.3 TOXICOKINETICS OF PERCHLORATE**

20 Because of the complex anatomy of the thyroid follicle, all of the locations where  
21 perchlorate inhibition is exerted remain to be established (Wolff, 1998). Perchlorate has been  
22 established as a competitive inhibitor of iodide uptake across the basolateral membrane (i.e., acts  
23 by the inhibition at NIS). Figure 3-3 shows a comparison of the molecular dimensions of  
24 perchlorate and iodide. The following potency series was constructed for monovalent anion-  
25 based inhibition of iodide transport in thyroid slices:  $\text{TcO}_4^- \geq \text{ClO}_4^- > \text{ReO}_4^- > \text{SCN}^- > \text{BF}_4^- > \text{I}^- >$   
26  $\text{NO}_3^- > \text{Br}^- > \text{Cl}^-$  (Wolff, 1998). However, it is not clear whether this anion sequence, measured  
27 at very high concentrations, has any mechanistic relation to what occurs at low concentrations in  
28 the thyroid. It is important to determine which solution properties of the anions determine this  
29 sequence (e.g., crystal radius, hydrated radius, hydration enthalpy, charge density). Strong base  
30 anion-exchange resins (usually a large cation with a weak field) exhibit a marked preference for  
31  $\text{ClO}_4^-$  (e.g., compared to  $\text{Cl}^-$ ); thus, it seems likely that selectivity for iodide or perchlorate in the

**TABLE 3-2. INTERSPECIES AND INTRASPECIES DIFFERENCES IN  
THYROID STRUCTURE AND T<sub>3</sub>, T<sub>4</sub>, AND TSH HORMONES  
(U.S. Environmental Protection Agency, 1998a)**

Parameter	Human	Rat
Thyroxine-binding globulin	Present	Essentially absent
T <sub>4</sub> Half-life	5 to 6 Days	0.5 to 1 Day
T <sub>3</sub> Half-life	1 Day	0.25 Day
T <sub>4</sub> Production rate/kg body weight	1 ×	10 × that in humans
TSH	1 ×	6 to 60 × that in humans
Follicular cell morphology	Low cuboidal	Cuboidal
Sex differences		
Serum TSH	M <sup>a</sup> = F <sup>a</sup>	M ≤ 2 × F
Cancer sensitivity	F = 2.5 × M	M > F

<sup>a</sup>M = male, F = female.



**Figure 3-3. Comparison of the molecular dimensions for the perchlorate (left) and iodide (right) anions.**

1 thyroid may be based on an anion-exchange mechanism using a large cation such as a quaternary  
2 amine (e.g., arginine) (Wolff, 1989).

3 Perchlorate also has been used to stimulate the efflux of iodide already stored in the  
4 follicular lumen of the gland (Atterwill et al., 1987). The exact nature of the mechanism for this  
5 effect has not been established, however. Transport of iodide out of the cell is downhill  
6 electrically, but this could be accounted for by the high concentration gradient that is established  
7 from follicular lumen (iodide stored in the colloid) to the basolateral and extracellular space.  
8 This may be the rate-limiting aspect for perchlorate efflux effect. Perchlorate added to the apical  
9 side of a polarized thyroid cell monolayer is substantially less effective than when added to the  
10 basolateral side (Wolff, 1998). Moreover, perchlorate rapidly increases the secretory response to  
11 TSH, and TSH increases iodide efflux before it increases iodide influx, suggesting that additional  
12 control points may exist.

13 Thus, perchlorate appears to have no effect on the iodination process itself but, rather,  
14 displaces iodide by competitive uptake at the NIS. Perchlorate is concentrated by thyroid tissue  
15 in a manner similar to iodide, but it is not significantly metabolized in the gland nor peripherally,  
16 as mentioned previously. It is not unequivocally established whether there are additional effects  
17 of perchlorate on iodide transport within the thyroid. Pharmacokinetic studies with perchlorate,  
18 both acute and particularly once steady state has been achieved, have provided some useful data  
19 with which to gain insight on this issue. The potential impacts as health endpoints of interest for  
20 human health risk assessment of this perturbation in the hypothalamic-pituitary-thyroid axis and  
21 hormone economy will be discussed in Section 3.4.

## 22 23 24 **3.4 TOXICODYNAMICS OF THYROID HORMONE PERTURBATIONS**

25 Given the established mode of action for perchlorate as the inhibition of iodide uptake at  
26 the NIS, it is important to distinguish the temporal aspects with respect to potential adverse tissue  
27 response.

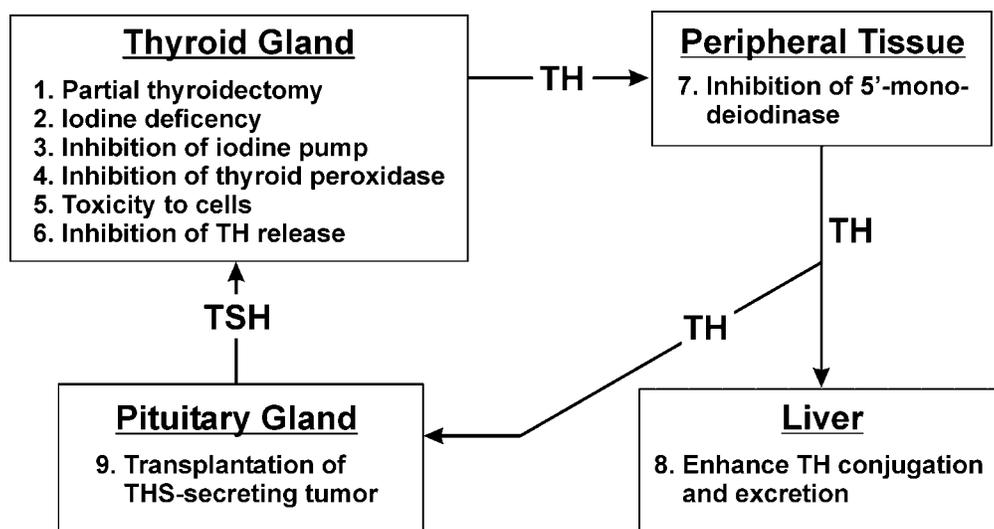
### 28 29 **3.4.1 Carcinogenic Effects**

30 In higher organisms, when demands for more thyroid hormone are small, existing thyroid  
31 follicular cells can meet the demand. With increased need, as a result of certain chemical

1 exposures or iodine deficiency, the thyroid responds by increasing the size (hypertrophy) and  
2 number (hyperplasia) of thyroid follicular cells to enhance hormone output. With continued TSH  
3 stimulation, there is actual enlargement of the thyroid (goiter) and, at least in rodents, eventual  
4 neoplasia of the thyroid follicular cells. Because TSH-producing pituitary cells also are  
5 stimulated, they too sometimes undergo hyperplasia and neoplasia (U.S. Environmental  
6 Protection Agency, 1998a; Hill et al., 1998). The EPA Assessment of Thyroid Follicular Cell  
7 Tumors (U.S. Environmental Protection Agency, 1998a), as well as reviews recommended  
8 therein, provides details about thyroid follicular cell carcinogenesis. Figure 3-4 shows  
9 schematically the possible antithyroid effects that could influence carcinogenesis. Note that  
10 effects, not only in the thyroid but also in peripheral tissues and the liver, may cause demand on  
11 thyroid hormone production such that the TSH stimulation of the thyroid to produce more  
12 hormone is enlisted. Table 3-3 lists mechanisms of antithyroid-mediated neoplasia in rodents.  
13 The potential for an indirect effect of perchlorate has been established, but genotoxicity  
14 information was required to evaluate its potential for direct effects. As will be discussed in  
15 Section 3.5, a battery of genotoxicity assays was included in the testing strategy.

16 Long-term perturbations in the hypothalamic-pituitary-thyroid axis by the various  
17 influences listed in Table 3-3 are more likely to predispose the laboratory rat to a higher  
18 incidence of proliferative lesions (Capen, 1997). One factor that may play a role in this  
19 interspecies quantitative difference in sensitivity to thyroid stimulation is the influence of protein  
20 carriers of thyroid hormones in the blood (Table 3-2). Both humans and rodents have  
21 nonspecific, low-affinity protein carriers of thyroid hormones (e.g., albumin). However, in  
22 humans, other primates, and dogs, there is a high-affinity binding protein, thyroxine-binding  
23 globulin, which binds T4 (and T3 to a lesser degree). This protein is missing in rodents and  
24 lower vertebrates. As previously indicated, T4 is bound to proteins with lower affinity in the  
25 rodent and is more susceptible to removal from the blood, by metabolism, and through excretion  
26 than in dogs and primates.

27 In keeping with this finding, the serum half-life of T4 is much shorter in rats (less than  
28 1 day) than it is in humans (5 to 9 days); this difference in T4 half-life results in a 10-fold greater  
29 requirement for exogenous T4 in the rat with a nonfunctioning thyroid than in the adult human.  
30 Serum T3 levels also show a species difference: the half-life in the rat is about 6 hr; whereas, it is  
31 about 24 hr in humans. High thyroid hormone synthetic activity is demonstrated in thyroid



**Figure 3-4. Schematic of antithyroid effects that influence thyroid carcinogenesis. (U. S. Environmental Protection Agency, 1998a; and Hill et al., 1998).**

**TABLE 3-3. MECHANISMS OF ANTITHYROID-MEDIATED NEOPLASIA IN RODENTS (U.S. Environmental Protection Agency, 1998a).**

- 
- **DNA Directed**
    - X rays
    - $^{131}\text{I}$
    - Genotoxic chemicals
  
  - **Indirect**
    - Partial thyroidectomy
    - Transplantation of TSH-secreting pituitary tumors
    - Iodide deficiency
    - Chemicals inhibiting iodide uptake
    - Chemicals inhibiting thyroid peroxidase
    - Chemicals inhibiting TH
    - Chemicals inhibiting conversion of T3 and T4
    - Chemical inhibiting hepatic thyroid hormone metabolism and excretion
-

1 follicles in rodents, where the follicles are relatively small and are surrounded by cuboidal  
2 epithelium. Follicles in primates demonstrate less activity and are large with abundant colloid,  
3 and follicular cells are relatively flattened (low cuboidal) (McClain, 1992).

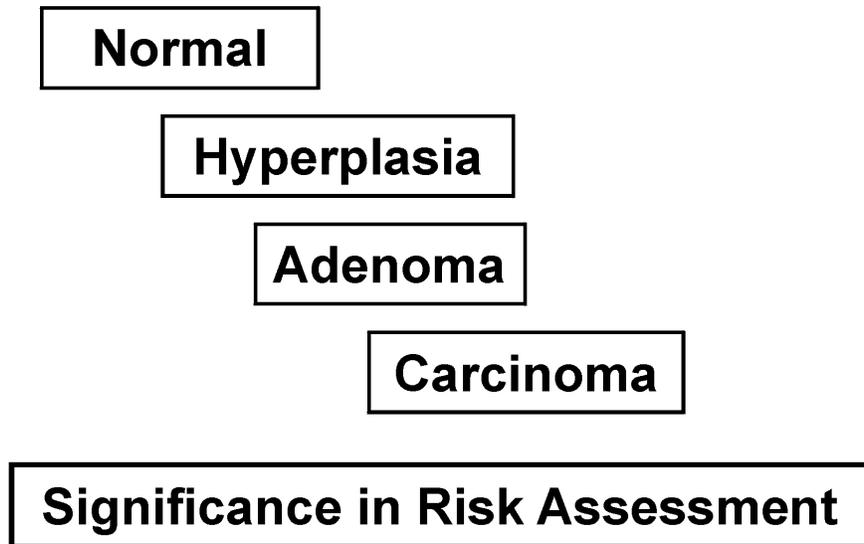
4 The accelerated production of thyroid hormones in the rat is driven by serum TSH levels  
5 that are probably about 6- to 60-fold higher than in humans. This assumes a basal TSH level in  
6 rats and humans of 200 ng/mL and 5  $\mu$ U/mL, respectively, and a potency of human TSH of 1.5 to  
7 15 IU/mg of hormone (U.S. Environmental Protection Agency, 1998a). Thus, it appears that the  
8 rodent thyroid gland is chronically stimulated by TSH levels to compensate for the increased  
9 turnover of thyroid hormones. It follows that increases in TSH levels above basal levels in rats  
10 could more readily move the gland towards increased growth and potential neoplastic change  
11 than in humans. In addition to considerations about the influence of serum thyroid hormone  
12 carrier proteins, there are differences between humans and laboratory animals in size and life  
13 span and in the pharmacokinetics and pharmacodynamics of endogenous and exogenous  
14 chemicals. Any comparison of thyroid carcinogenic responses across species should be  
15 cognizant of all these factors.

16 A number of goitrogenic compounds, those that either interfere with thyroid hormone  
17 synthesis or secretion, have been demonstrated to result in thyroid follicular cell adenomas in  
18 rats. Excessive secretion of TSH alone has been reported to produce a high incidence of thyroid  
19 follicular cell adenomas. The pathogenic mechanism of thyroid follicular cell tumor  
20 development in rodents involves a sustained excessive stimulation of the thyroid by TSH. In the  
21 multistage model of this pathogenesis, the proliferative lesions often begin as hyperplasia, may  
22 proceed to the development of benign tumor (adenomas), and infrequently develop into  
23 malignant tumors (Figure 3-5).

24 The precise molecular steps in the carcinogenic process leading to thyroid follicular cell  
25 cancer have not been elucidated totally although significant insights into the problem have been  
26 described (Farid et al., 1994; Said et al., 1994). Normal cell division in the thyroid seems to be  
27 affected by an interplay among several mitogenic factors, namely TSH, insulin-like growth  
28 factor-1 (IGF-1), insulin, epidermal growth factor (EGF), and possibly fibroblast growth factor  
29 (FGF). Additionally, other factors, such as transforming growth factor  $\beta$ , certain interferons, and  
30 interleukin 1, may inhibit growth.

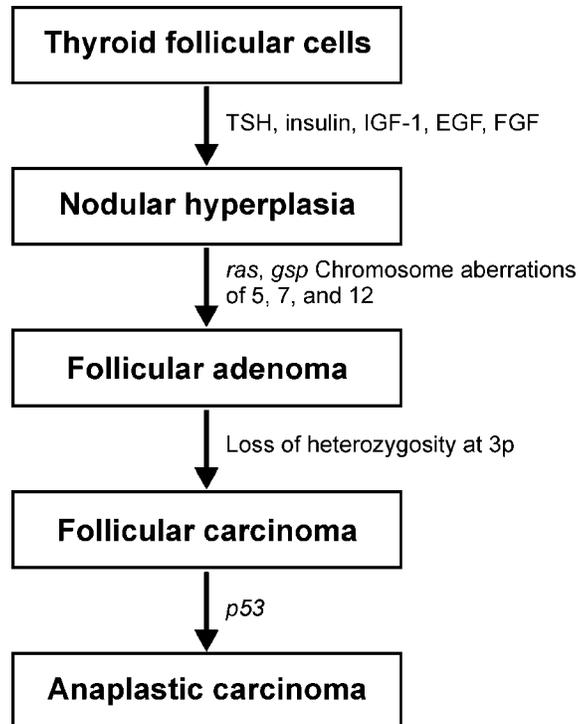
31

# Morphologic Continuum



**Figure 3-5. Proliferative changes involved in the multistage characterization of thyroid follicular cell neoplasia in rodents represent a morphologic continuum. Although these lesions typically are classified as discrete entities, the overlap in morphologic features should be emphasized because only imprecise criteria to separate borderline proliferative lesions exist. Thyroid neoplasia in rodents is considered relevant to human risk assessment (U.S. Environmental Protection Agency, 1998a) but thought to be protective (Capen, 1997).**

1            Figure 3-6 shows the possible molecular events in human thyroid follicular carcinogenesis.  
2 In spite of the potential qualitative similarities, there is evidence that humans may not be as  
3 sensitive quantitatively to thyroid cancer development from thyroid-pituitary disruption as are  
4 rodents. Rodents readily respond to reduced iodide intake with the development of cancer;  
5 whereas, humans develop profound hyperplasia with “adenomatous” changes with only  
6 suggestive evidence of malignancy. Even with congenital goiters from inherited blocks in  
7 thyroid hormone production, only a few malignancies have been found in humans. Thus, despite  
8 a common physiology in regard to the thyroid-pituitary feedback system, the role of disruption of  
9 this axis in human cancer development is much less convincing. EPA has adopted the following  
10 science



**Figure 3-6. Possible molecular events in human thyroid follicular carcinogenesis (*ras* = *ras* protooncogene, *gsp* = GTP-binding protein mutation, *p53* = *p53* tumor suppressor gene) ( U.S. Environmental Protection Agency, 1998a and Hill et al., 1998).**

- 1 policy that recognizes the role of mode-of-action information regarding thyroid-pituitary  
 2 disruption and mutagenesis to potential thyroid carcinogenesis (U.S. Environmental Protection  
 3 Agency, 1998a).
- 4 • It is presumed that chemicals that produce rodent thyroid tumors may pose a carcinogenic  
 5 hazard for the human thyroid.
  - 6 • In the absence of chemical-specific data, humans and rodents are presumed to be equally  
 7 sensitive to thyroid cancer caused by thyroid-pituitary disruption. This is a conservative  
 8 position when thyroid-pituitary disruption is the sole mode of action because rodents appear to  
 9 be more sensitive to this carcinogenic mode-of-action than are humans. When the thyroid  
 10 carcinogen is a mutagenic chemical, the possibility that children may be more sensitive than  
 11 adults needs to be evaluated on a case-by-case basis.

- 1 • Adverse rodent noncancer thyroid effects (e.g., thyroid enlargements) following short- and  
2 long-term reductions in thyroid hormone levels are presumed to pose human noncancer health  
3 hazards.

4 The new data on the antithyroid activity of perchlorate that has resulted from the testing  
5 strategy will be evaluated in Chapter 7 according to criteria provided in the guidance (U.S.  
6 Environmental Protection Agency, 1998a) to determine the likelihood that the chemical would  
7 act indirectly, via disruption of the thyroid-pituitary axis, or directly on DNA.

### 8

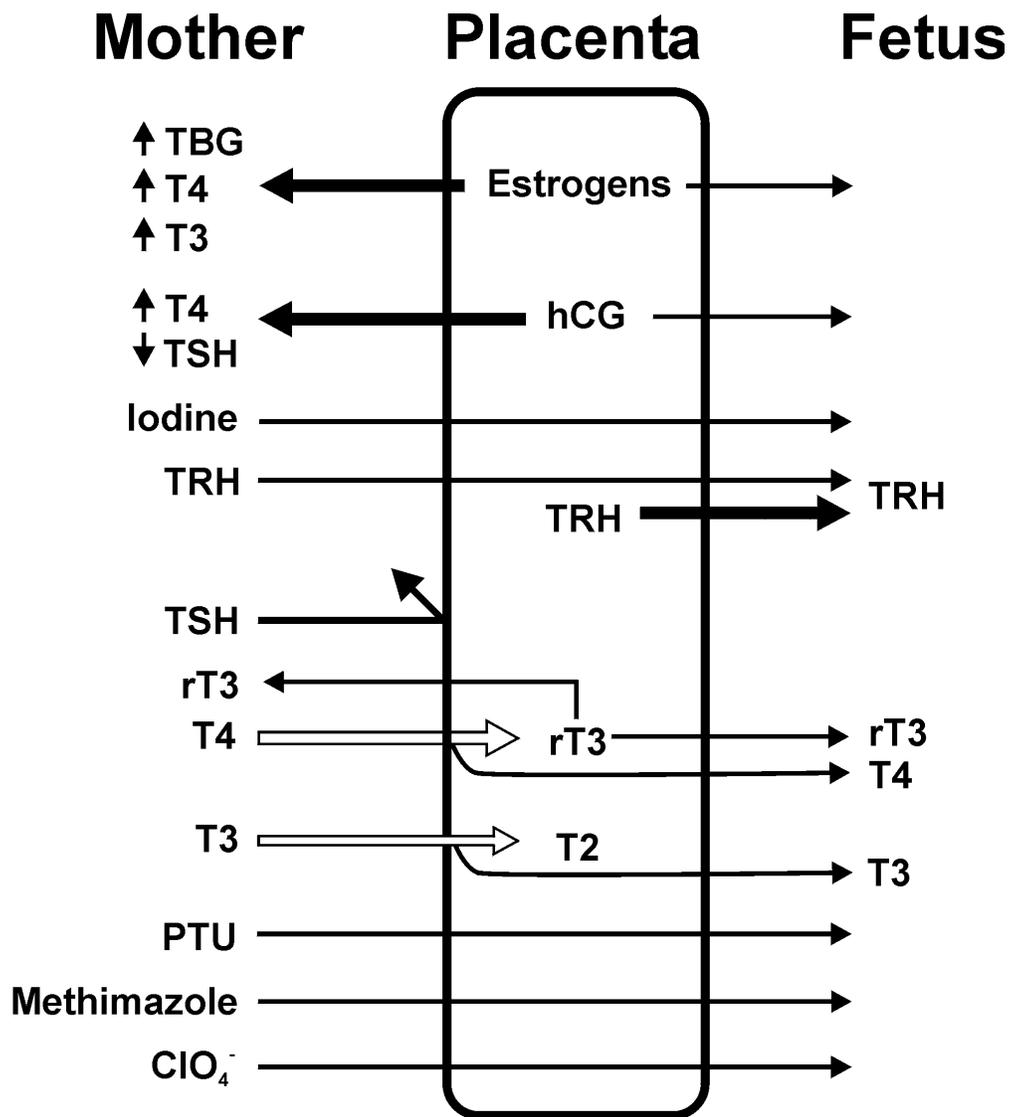
### 9 **3.4.2 Neurodevelopmental Deficits and Other Potential Adverse Effects**

### 10 **Resulting from Thyroid Hormone Disruption**

11 As expressed by the external review panel convened by Toxicology Excellence for Risk  
12 Assessment (TERA) in 1997, there was concern about other potential adverse effects of  
13 perchlorate-induced hypothyroidism. Humans respond as do experimental animals in regard to  
14 short- and mid-term disturbances in thyroid functioning from various anti-thyroid stimuli such as  
15 iodide deficiency, partial thyroidectomy (surgically or <sup>131</sup>I induced), and goitrogenic chemicals  
16 such as thionamides (U.S. Environmental Protection Agency, 1998a). For instance, thyroid  
17 hormone is critical to normal brain and physical development. This dependency begins in the  
18 uterus and extends to 3 years of age in humans. Thus, there was concern that hypothyroidism  
19 during pregnancy could result in neurodevelopmental effects.

20 The role of the placenta in thyroid hormone metabolism is shown in Figure 3-7. Although  
21 the fetus is initially dependent on maternal thyroid hormone levels, the potential for disruption of  
22 fetal hormone production remains once the fetal thyroid assumes this function because  
23 perchlorate can cross the placenta. Disruption of circulating thyroid hormones can have  
24 drastically different effects on fetuses and infants than on adults, depending on the developmental  
25 stage at exposure (Table 3-4). It is important to emphasize that even transient disruption may  
26 lead to permanent effects in the developing organism.

27 Chemical-induced alterations in thyroid hormone homeostasis are known to adversely  
28 affect the development of many organ systems, including the nervous and reproductive systems  
29 (Porterfield, 1994; Jannini et al., 1995). Severe developmental hypothyroidism caused by iodine  
30 deficiencies or a congenital condition has devastating effects on fetal and postnatal development,  
31 including mental deficiencies and hearing, speech, and motor deficits (Porterfield, 1994; Sher



**Figure 3-7.** Schematic representation of the role of the placenta in thyroid hormone metabolism during human pregnancy. The placenta produces estrogens and hCG that increase maternal TBG levels and stimulate maternal thyroid hormone production, respectively. Both activities tend to increase maternal T4 and T3 concentrations and to inhibit maternal TSH secretion. Iodide and TRH readily cross the placenta, and the placenta itself synthesizes TRH. The placenta is impermeable to TSH and only partially permeable to T4 and T3. Placental Type III iodothyronine monodeiodinase enzymes degrade T4 to rT3 and T3 to 3,3'-diiodothyronine (T2). Propylthiouracil and methimazole readily cross the placenta. Given its physicochemical characteristics and similarity to iodide, perchlorate also is anticipated to cross readily. (Modified from Fisher, 1996 and Underwood, 1998).

**TABLE 3-4. MAIN SYMPTOMS AND EFFECTS OF HYPOTHYROIDISM**

<b>Developmental</b> <i>(Transient disruption leads to permanent effects.)</i>	<b>Adult</b> <i>(Transient disruption leads to transient effects.)</i>
<ul style="list-style-type: none"><li>• Delayed reflex ontogeny</li><li>• Impaired fine motor skills</li><li>• Deaf-mutism, spasticity</li><li>• Gait disturbances</li><li>• Mental retardation</li><li>• Speech impairments</li></ul>	<ul style="list-style-type: none"><li>• Run down, slow, depressed</li><li>• Sluggish, cold, tired</li><li>• Dryness and brittleness of hair</li><li>• Dry and itchy skin, constipation</li><li>• Muscle cramps</li><li>• Increased menstrual flow</li><li>• Thyroid tumors in rodents</li></ul>

1 et al., 1998). It is important to emphasize that these effects are caused by a lack of thyroid  
2 hormones alone, rather than by tumor development or thyroid hypertrophy/hyperplasia due to  
3 increases in TSH. Thus, the important species comparison may be perchlorate's action of iodide  
4 uptake inhibition at the NIS. In fact, data discussed in Chapters 5 and 6 show that the sensitivity  
5 of the NIS is quite similar across species.

6 During development, thyroid hormones regulate cell proliferation, migration, and  
7 differentiation. Intracellularly, THs bind to thyroid hormone receptors that interact with thyroid  
8 response elements to alter expression of messenger ribonucleic acids (mRNAs) and subsequent  
9 protein synthesis. The pituitary-thyroid TSH feedback loop may or may not be activated during  
10 development, depending on the mechanism of action of the chemical. The adversity of  
11 congenital hypothyroidism, usually less severe than endemic cretinism, can be ameliorated via  
12 early postnatal thyroxine therapy. In contrast, the effects of developmental iodine deficiency can  
13 not be corrected with only postnatal therapy, indicating that iodine deficiency during pregnancy  
14 is the causative action (Cao et al., 1994). Clearly, xenobiotics that contribute to fetal or maternal  
15 hypothyroidism or hypothyroxenemia are of concern.

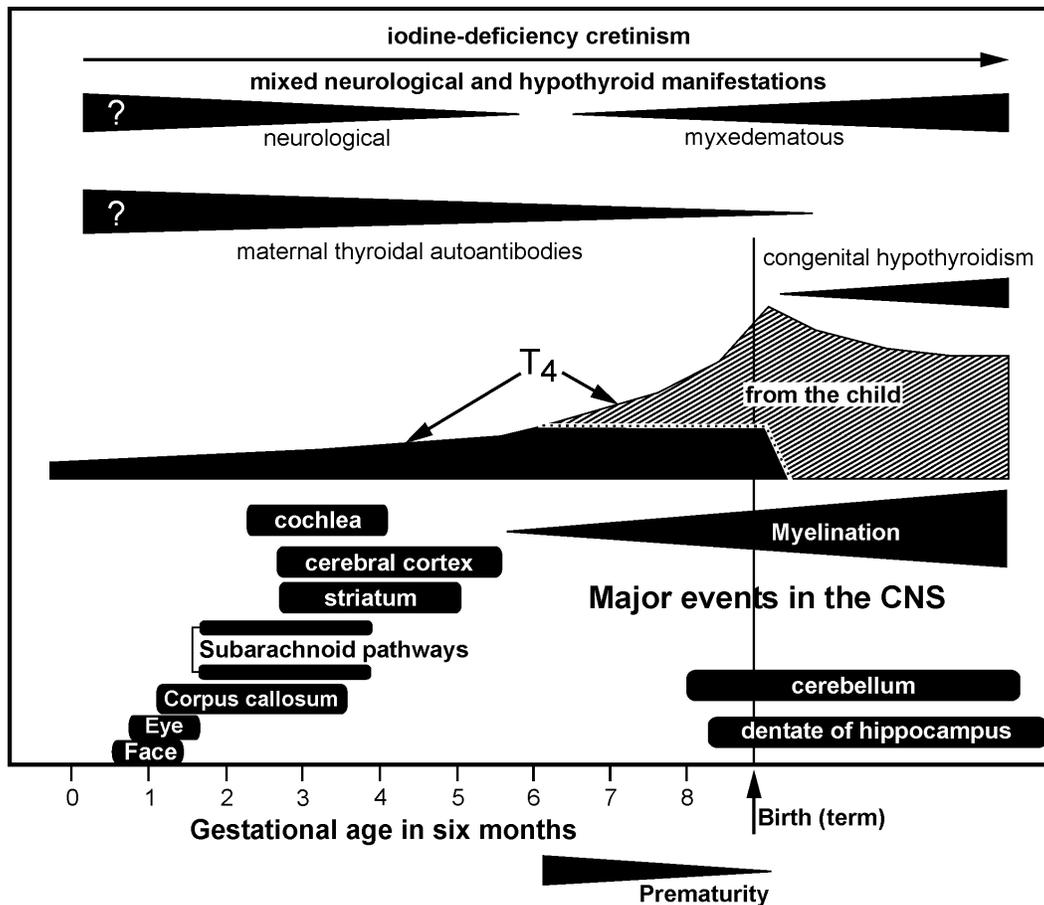
16 Since the previous external peer review, studies reported in the clinical and epidemiological  
17 literature have reinforced concerns for deficits in neuropsychological development related to  
18 maternal thyroid deficiency. Haddow et al. (1999) showed an effect on IQ scores in children  
19 (ages seven to nine) who had normal thyroid function at birth but were born to women with  
20 abnormal thyrotropin levels versus children born to a matched cohort of women with normal  
21 thyrotropin levels as controls. Haddow et al. (1999) concluded that even mild and probably

1 asymptomatic hypothyroidism in pregnant women can adversely affect their children's  
2 subsequent performance on neuropsychological tests.

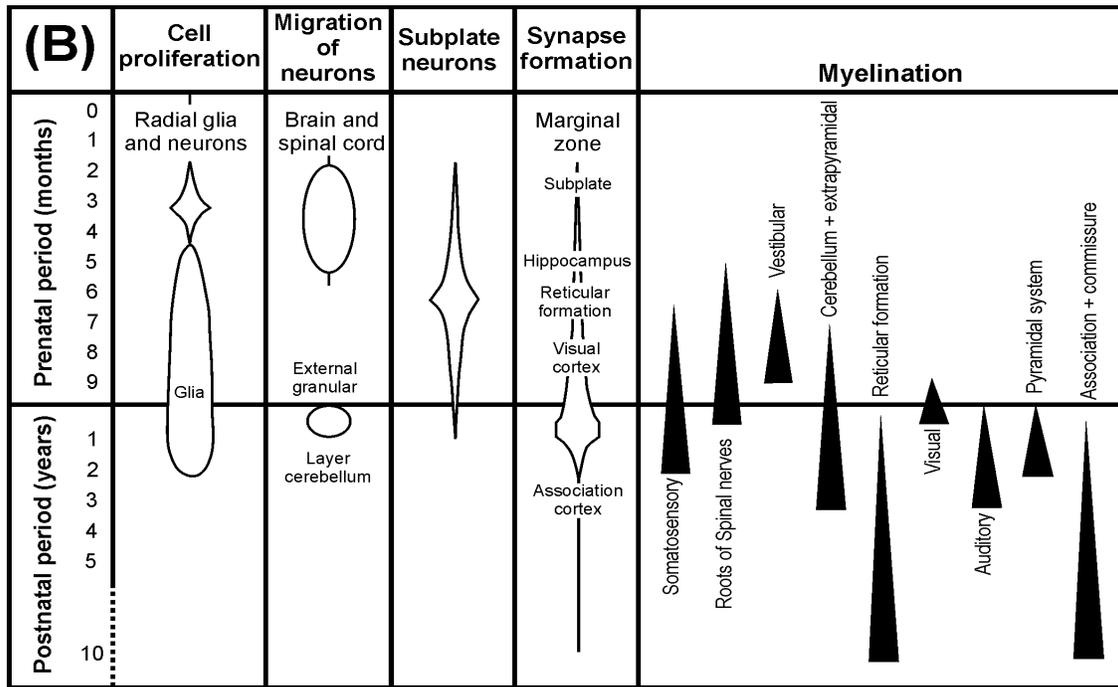
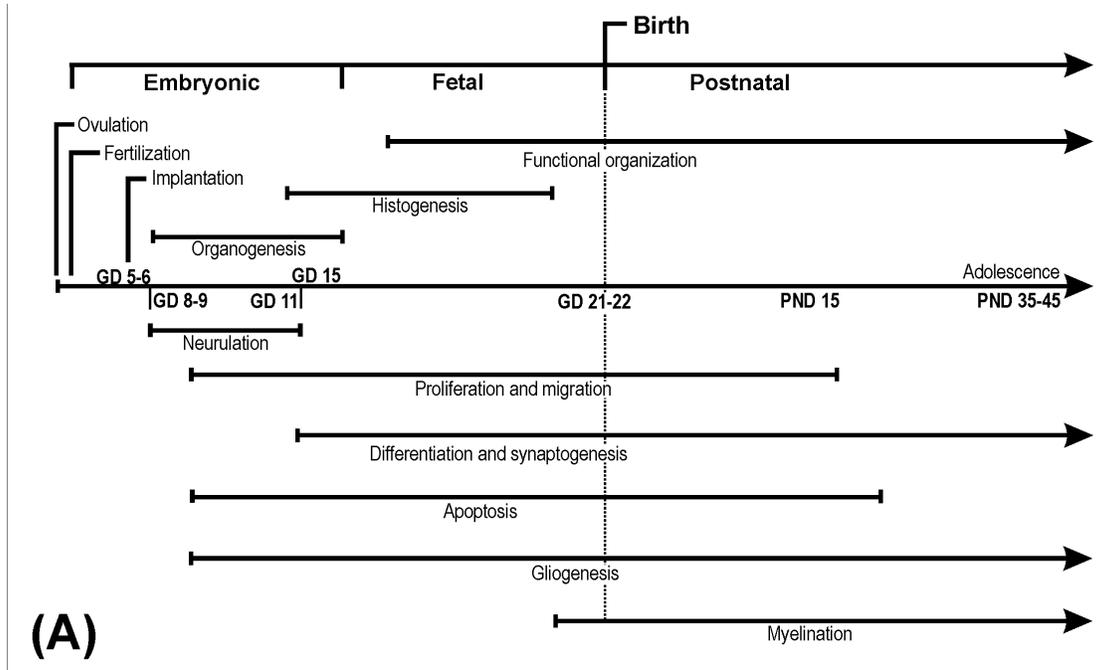
3 Pop et al. (1995) noted an average impairment of 10.5 IQ points in the offspring of mothers  
4 with high thyroid peroxidase antibody (TPO-Ab) titers during pregnancy. In a later prospective  
5 study these same researchers evaluated developmental indices at 3 weeks, 10 months, 1 and  
6 2 years of age and demonstrated that a maternal free T4 blood level that was less than the 10<sup>th</sup>  
7 percentile of first trimester values (10.4 pmol/L in their study series) was associated with  
8 distinctly impaired psychomotor development whether or not TSH and TPO-Abs were elevated  
9 (Pop, et al., 1999). Smit et al. (2000) reported a similar relationship between free T4 and early  
10 neurodevelopment of children born from treated hypothyroid women.

11 Morreale de Escobar et al. (2000) evaluated epidemiological, clinical, and basic research  
12 data to ascertain if the principal factor leading to neurodevelopmental deficits in children was  
13 related to maternal hypothyroidism, whether clinical or subclinical (as defined by TSH higher  
14 than the 98<sup>th</sup> percentile of the normal population); or if they were instead related to maternal  
15 hypothyroxinemia per se (decrement in T4 without concomitant increase in TSH). These  
16 researchers concluded that conditions resulting in hypothyroxinemia alone (a low for gestational  
17 age circulating maternal free T4 level whether or not TSH was increased) poses an increased risk  
18 for poor neuropsychological development of the fetus. T4 is the required substrate for the  
19 ontogenically-regulated generation of T3 in the amounts needed for optimal brain development,  
20 both temporally and spatially. Normal maternal T3 concentrations did not seem to prevent the  
21 potential damage of a low T4 supply (Morreale de Escobar et al., 2000). Hypothyroxinemia  
22 seems to be much more frequent in pregnant women than either clinical or subclinical  
23 hypothyroidism and autoimmune thyroid disease (AITD), especially in regions where the iodine  
24 intake of the pregnant woman is inadequate to meet her increased needs for T4 (Morreale de  
25 Escobar et al., 2000).

26 Figure 3-8 illustrates the windows of susceptibility for insults to the brain resulting from  
27 hypothyroxinemia. A similar map has been developed for rats, and time lines have begun to be  
28 compared and correlated (Rice and Barone, 2000), as shown in Figure 3-9. Morreale de Escobar  
29 et al. (2000) reported findings that altered early migration of cortical cells can be observed in rats  
30 with severe iodine deficiency. Porterfield (2000) has also discussed the potential for



**Figure 3-8.** Approximate timing of major insults to the brain resulting from hypothyroxinemia, superimposed on major neurodevelopmental events in humans. Conditions resulting in early maternal hypothyroxinemia, combined to later impairment of the fetal thyroid, are the most damaging, with central nervous system (CNS) damage that is irreversible at birth. The most frequent cause is maternal iodine deficiency (ID) and the presence of maternal autoimmune thyroid disease (AITD). Unless ID is also present, the CNS damage in congenital hypothyroidism is preventable by early postnatal treatment because the normal maternal thyroxinemia has avoided damage to the brain until birth. If maternal hypothyroxinemia persists, normal maternal concentrations of  $T_3$  do not protect the fetal brain because of its dependence on intracerebral regulation of local  $T_3$  availability by deiodinating pathways using  $T_4$  as a substrate. Interruption of the contribution of maternal  $T_4$  in premature infants with an immature thyroid may also underlie their increased risk of neurodevelopmental problems, the more severe the earlier their birth. The *question mark* indicates that it is unknown whether very early CNS development, corresponding to a period when the general morphogenesis of the pros encephalon (neurolation and segmentation) is being determined, is thyroid hormone sensitive or not (Morreale de Escobar et al., 2000).



**Figure 3-9. Timelines of developmental processes in the nervous system of rats (a) and humans (b). Rat timeline is compared to timing of fertilization, organogenesis, and histogenesis. Human perinatal period is scaled in months and the postnatal development is scaled in years (Rice and Barone, 2000).**

1 neurotoxicity and altered brain development that may result from exposure to environmental  
2 chemicals that disrupt thyroid function even on a transient basis.

3       These concerns for the potential adverse effects of perchlorate on T4 and T3, especially  
4 during pregnancy, are compounded by the growing appreciation that women of childbearing age  
5 have relatively low iodide intake. A January 2001 report by the National Academy of Sciences  
6 (NAS) concerning the dietary reference intake of trace-mineral nutrients, including iodine,  
7 indicated that less than 25% of the total population was below the estimated average requirement  
8 for iodide and stressed a need to look at levels of adequacy for susceptible age groups and status  
9 during pregnancy and lactation. The higher requirements during this time indicate a potential  
10 susceptibility as shown in Table 3-5. The NAS also cautions against using urinary iodine as a  
11 biomarker for iodine status unless the data are from 24-hour collections or are normalized against  
12 creatinine. Other reports suggest that the level of iodide intake is less than a third of the range  
13 recommended for pregnant women by the World Health Organization (WHO) (Caron et al.,  
14 1997).

15  
16  
**TABLE 3-5. DIETARY REFERENCE INTAKES (DRI) FOR IODIDE**  
**(National Academy of Sciences, 2001)**

<b>Age or Status</b>	<b>Adequate Intake (AI)</b> <b>µg/day</b>	<b>Estimated Average Requirement (EAR)</b> <b>µg/day</b>	<b>Recommended Dietary Allowance (RDA)</b> <b>µg/day</b>
0-6 months	110		
6-12 months	130		
1-3 years		65	90
4-8 years		65	90
9-13 years		73	120
14-18 years		95	150
19-15 years		95	150
51 + years		95	150
Pregnancy		160	220
Lactation		209	290

1           The prevalence of abnormal thyroid function continues to be debated and this is  
2           confounded by the variable definitions of the disease state as well as the different measures of  
3           thyroid function (Canaris et al., 2000). Most reports are still defined by TSH levels rather than  
4           for hypothyroxinemia per se, but recent presentations suggest that TSH is a poor test to assess the  
5           severity of tissue hypothyroidism (Meier et al., 2001), and recommendations in the epidemiologic  
6           literature are proposing that screening of pregnant women should include the determination of  
7           free T4 (Morreale de Escobar et al., 2000). Age, sex and dietary iodine levels are confounding  
8           factors, although virtually all studies report higher prevalence rates for hypothyroidism  
9           (as defined by increased TSH) in women with age (Canaris et al., 2000). Rates as high as 24%  
10          among women older than 60 years have been reported. Suppressed TSH levels have been  
11          associated with decreased bone density, increased risk of atrial fibrillation, premature atrial beats,  
12          and effects on serum lipids notably elevated serum cholesterol levels.

13          Together these findings strongly suggest that a susceptible population of particular concern  
14          for perchlorate exposure is pregnant women with hypothyroxinemia and that the iodine  
15          deficiency represents an additional potential insult that could exacerbate the effects of perchlorate  
16          toxicity. The elderly, especially women, represent another potentially susceptible population, as  
17          well as people with cardiac dysfunction or risk factors such as elevated serum cholesterol.

18          As mentioned above, reproductive toxicity was also a concern as a potential effect of  
19          perchlorate's mode of action. In females, thyroid hormones appear to have a role in stimulating  
20          the onset of human chorionic gonadotropin (hCG) production by the placenta early in pregnancy.  
21          Human chorionic gonadotropin is essential for the maintenance of pregnancy. Therefore, a  
22          hypothyroid condition has potential to interfere with normal placental function and fetal  
23          survival, as well as the potential to interfere with lactation. Suppression of thyroid hormone  
24          secretion with radioactive iodine or goitrogens reduces milk yield in lactating animals. This  
25          effect may be caused by suppression of placental lactogen production. Thyroid-releasing  
26          hormone is known to play a role in prolactin release during the estrous cycle. Additionally, the  
27          thyroid is necessary for the transition to the anestrus state in seasonally breeding species.  
28          In summary, effects on thyroid hormone levels have roles in estrous cycle regulation, pregnancy  
29          maintenance, fetal growth, and lactation.

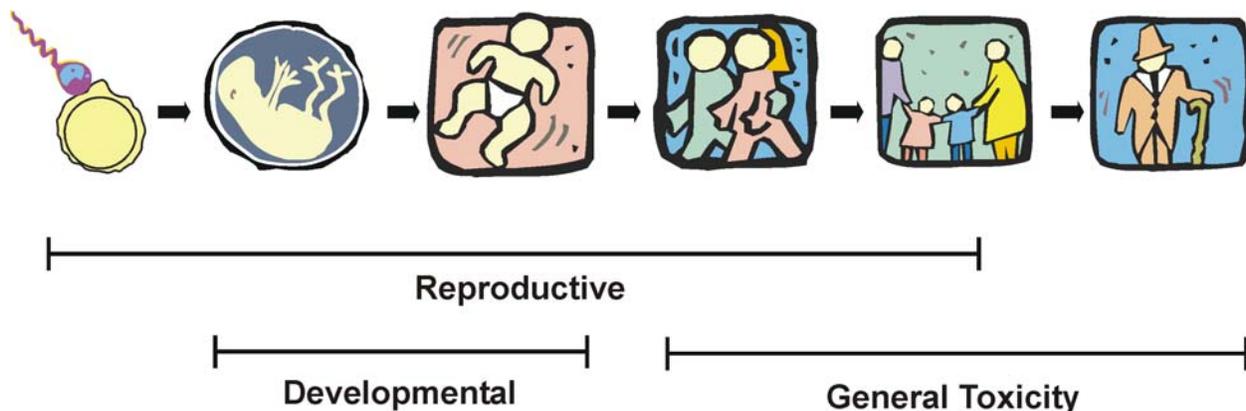
30          In males, the primary effects of hypothyroidism appear to occur during testicular  
31          development. The testis is responsive to thyroid hormones only during a limited time during the

1 perinatal and prepubertal periods. Thyroid hormone is a major regulator of seminiferous  
2 epithelium development by inducing the normal differentiation of Sertoli cells, gonocytes, and  
3 Leydig cells, and by limiting the proliferation of those cell types. In the hypothyroid condition,  
4 those cells proliferate beyond the norm, and the steroidogenic function of the Leydig cells, on a  
5 per-cell basis (but not necessarily in total), is impaired. Secretory activity of the Sertoli cells also  
6 appears to be impaired. In boys, untreated hypothyroidism is associated with marked and  
7 precocious testis enlargement, but low androgen activity. In a small study, hypothyroid men had  
8 complaints of reduced libido that was probably related to a defective leutenizing hormone  
9 response to gonadotropin-releasing hormone.

10 The inclusion of an immunological evaluation of mice exposed to perchlorate was  
11 warranted because of evidence from earlier clinical studies that indicated a link between the  
12 treatment of Graves' disease with perchlorates and serious hematological effects that may be  
13 linked to immune mechanisms. A small number of patients undergoing perchlorate therapy have  
14 been reported to develop aplastic anemia, agranulocytosis, lymphadenopathy, leukopenia, or skin  
15 rashes. The antithyroid drugs propylthiouracil and methimazoles are reported to exert their  
16 effects on the hematopoietic system through immune mechanisms. Because the use of these  
17 antithyroid drugs by a small number of patients also resulted in sequelae similar to that of some  
18 patients under perchlorate treatment, it has been postulated that perchlorate also may act via the  
19 immune system.

### 22 **3.5 DEVELOPMENT OF A TOXICITY TESTING STRATEGY BASED** 23 **ON MODE OF ACTION**

24 Because the RfD is intended to be a lifetime dose-response estimate, the typical objective  
25 of a database to support such a quantitative assessment is to evaluate a comprehensive array of  
26 testing endpoints that represent various life stages during which potential effects could occur  
27 (e.g., the developing fetus through adult) and for effects on reproductive capability (shown  
28 schematically in Figure 3-10). As discussed in the previous sections, thyroid hormone  
29 deficiencies, such as those induced by perchlorate, can affect normal metabolism, growth, and  
30 development. No robust data existed prior to this time to evaluate other potential target tissues or  
31 effects. There were limited data on effects caused by long-term exposures and no data with



**Figure 3-10. Schematic illustrating that a high confidence RfD is based on data that address all potentially critical stages over a lifetime.**

1 which to evaluate the effects of perchlorate in potentially susceptible populations such as in  
 2 developing fetuses, nor were there data on the effects of perchlorate on the reproductive capacity  
 3 of male or female laboratory animals. Table 3-6 shows the minimum database for derivation of  
 4 an RfD with low confidence (a 90-day bioassay) and the rationale for other tests typically  
 5 included to bolster the confidence in the derivation—the same suite of tests that has been  
 6 discussed for perchlorate. These data typically also reduce the uncertainty for which uncertainty  
 7 factors are applied (see Table 3-7), either because the absence of data on a suspected endpoint  
 8 (e.g., developmental toxicity) has been addressed or because mechanistic data provide insight on  
 9 the relevance of the laboratory animal model, including the magnitude of interspecies and  
 10 intrahuman variability in toxicokinetics and toxicodynamics. Any individual chemical database  
 11 may fall in between this range of high and low certainty, depending on the quality of the  
 12 individual studies and whether the dose response for suspected endpoints is characterized well.

13 The objective of the testing strategy was to provide a comprehensive database that  
 14 described the mode-of-action-based pathogenesis in quantitative terms so that the resultant  
 15 estimate could be more predictive and ultimately support the development of a robust RfD  
 16 estimate that reduced the uncertainties inherent in the provisional, presumably protective values  
 17 (see Figure 3-11).

**TABLE 3-6. MINIMUM DATABASE FOR DERIVATION OF AN ORAL REFERENCE DOSE**

Mammalian Database <sup>a</sup>	Confidence	Comments
Two chronic oral bioassays in different species One two-generation reproductive study Two developmental toxicity studies in different species	High <sup>b</sup>	Minimum database for high confidence
One subchronic oral bioassay	Low	Minimum database for estimation of an RfD

<sup>a</sup>Rationale is to use different species to evaluate variability in species sensitivity unless a particular laboratory animal model is more appropriate.

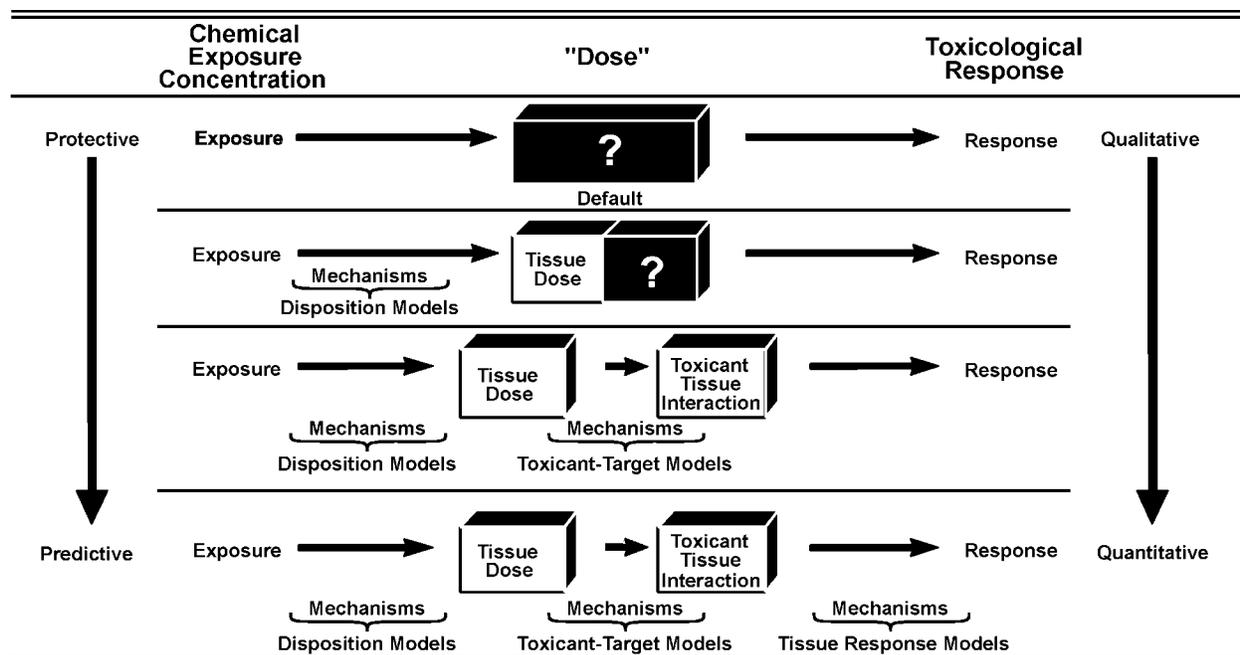
<sup>b</sup>Rationale is to address all potentially critical life stages.

**TABLE 3-7. FACTORS FOR UNCERTAINTIES IN APPLIED EXTRAPOLATIONS USED TO DERIVE REFERENCE DOSES<sup>a</sup>**

10 <sub>H</sub>	– Human to sensitive human
10 <sub>A</sub>	– Experimental animal to human
10 <sub>S</sub>	– Subchronic to chronic duration
10 <sub>L</sub>	– LOAEL(HEE) <sup>a</sup> to NOAEL(HEE) <sup>a</sup>
10 <sub>D</sub>	– Incomplete to complete database
MF	– Modifying factor. Professional assessment of scientific uncertainties of the study and database not explicitly addressed above. Default for the MF is 1.0 (e.g., applied for small sample size or poor exposure characterization).

<sup>a</sup>HEE = human equivalent exposure.

1 As illustrated in Figure 3-11, it is ultimately desirable to have a comprehensive  
2 biologically-based dose-response model that incorporates the mechanistic determinants of  
3 chemical disposition, toxicant-target interactions, and tissue responses integrated into an overall  
4 quantitative model of the pathogenesis (Jarabek, 1995a). Because the internal tissue dose of the  
5 chemical or its toxic moiety in a target tissue is not always proportional to the applied dose of a  
6 compound, emphasis has been placed on the need to distinguish clearly between the exposure  
7 concentration and the dose to critical target tissues. Consequently, the term “exposure-dose-  
8 response” has been recommended as more accurate and comprehensive (Andersen et al., 1992).  
9 This expression refers, not only to the determination of the quantitative relationship between  
10 exposure concentrations and target tissue dose, but also to the relationship between tissue dose  
11 and the observed or expected responses in laboratory animals and humans. The process of



**Figure 3-11. Schematic characterization of comprehensive exposure-dose-response continuum and the evolution of protective to predictive dose-response estimates (U.S. Environmental Protection Agency, 1994 and Jarabek 1995b).**

1 determining the exposure-dose-response continuum is achieved by linking the mechanisms or  
 2 critical biological factors that regulate the occurrence of a particular process and the nature of the  
 3 interrelationships among these factors. This can be especially important for interspecies  
 4 extrapolation and to understanding intrahuman variability.

5 Dose-response estimates based on characterization of the exposure-dose-response  
 6 continuum at the rudimentary (“black box”) level necessarily incorporate large uncertainty  
 7 factors to ensure that the estimates are protective in the presence of substantial data gaps. With  
 8 each progressive level, incorporation and integration of mechanistic determinants allow  
 9 elucidation of the exposure-dose-response continuum and, depending on the knowledge of model  
 10 parameters and fidelity to the biological system, a more accurate characterization of the  
 11 pathogenesis process (Jarabek, 1995a). Because of the increase in accuracy of the  
 12 characterization with each progressive level, dose-response estimates also progress from more  
 13 protective to factually-based (predictive).

1 Eight new studies were recommended as part of the original testing strategy after the May  
 2 1997 external peer review to provide such a comprehensive array of endpoints. These studies are  
 3 described below along with the role they were anticipated to play in informing the revised health  
 4 risk assessment (see Table 3-8).

5  
 6  
**TABLE 3-8. PERCHLORATE PEER REVIEW RECOMMENDED STUDIES SUMMARY**

Study	Description	Potential Use in Assessment
90-Day subchronic bioassay + TH <sup>a</sup> + reproductivity + genotoxicity + recovery	Tests for other target tissues; evaluates effect on TH in young adult rats; reproductive parameters added; mouse micronuclei and a recovery group	Minimum database for RfD dose-response for TH in young adult rats; additional information on others; may allow decrease in uncertainty factor (UF) for database deficiencies
Developmental neurotoxicity + TH	Evaluates nervous system in fetal and postnatal rats; TH in does (P0-generation) and pups (F1-generation)	Potentially critical effect; comparison of developmental versus adult effects on TH
Developmental study + TH	Evaluates birth defects in rabbits; TH in does at end of gestation	Potentially critical effect; data in second species for TH effects; may reduce UF for database deficiencies
Two-Generation reproductive toxicity + TH	Evaluates fertility of adult rats and toxicity in offspring over two generations; TH in parents (F0-generation) and offspring (F1- and F2-generations)	Potentially critical effect; may reduce UF for database deficiencies
ADME studies	Characterize absorption, distribution, metabolism, and elimination in rats and humans; iodine inhibition and perchlorate kinetics and hormone homeostasis	Interspecies extrapolation
Mechanistic studies	Evaluate mechanism of TH response and sensitivity in rats and humans	Interspecies extrapolation; determine susceptible subpopulation
Genotoxicity assays	Test for toxicity to DNA	Mode-of-action information for thyroid neoplasia; may reduce UF for database deficiencies
Immunotoxicity	Evaluates immune system structure and function	Potentially critical effect; may reduce UF for database deficiencies

<sup>a</sup>Thyroid hormones (T4 and T3); Thyroid stimulating hormone (TSH), a pituitary hormone, was also assayed in those studies.

1     **(1) 90-Day Subchronic Oral Bioassay Study.** This study was considered the minimum data  
2     requirement for derivation of an oral RfD. The study aimed to identify other target tissues,  
3     to test young adult rats, and to provide data on the effect of repeated exposure to perchlorate  
4     on thyroid hormone levels. The 30-day recovery phase, i.e., evaluation of the thyroid status  
5     30 days after perchlorate was stopped, would provide data necessary to characterize its  
6     anti-thyroid effects with respect to carcinogenicity (U.S. Environmental Protection Agency,  
7     1998a). These data were collected to allow reduction of the uncertainty factor applied for  
8     database deficiencies.

9  
10    **(2) Developmental Neurotoxicity Study.** This study was designed to evaluate the potential for  
11    developmental neurotoxicity of perchlorate by assessing functional and morphological  
12    endpoints in offspring from the mother exposed during pregnancy and lactation.  
13    Neurotoxicity endpoints were likely to be a critical effect, and the developing organism a  
14    sensitive subpopulation. It was hoped that these data would allow reduction of the  
15    uncertainty factors applied for intrahuman variability and database deficiencies.

16  
17    **(3) Segment II Developmental Study.** This study was conducted to evaluate the potential for  
18    perchlorate to cause birth defects in rabbits and to evaluate a potentially critical effect and  
19    subpopulation. This study also was conducted to provide data on the thyroid hormone  
20    effects in a second species (in addition to rats). These data might allow reduction of the  
21    uncertainty factor applied for database deficiencies.

22  
23    **(4) Two-Generation Reproductive Toxicity Study.** This study was designed to evaluate the  
24    potential for perchlorate to cause deficits in reproductive performance in adult rats and for  
25    toxicity in the young offspring. The primary goal of this study was to identify a potentially  
26    critical effect and to allow for reduction of the uncertainty factor applied for database  
27    deficiencies.

28  
29    **(5) Absorption, Distribution, Metabolism, and Elimination Studies.** These ADME studies  
30    aimed to understand the pharmacokinetics (i.e., how perchlorate is absorbed, distributed,  
31    metabolized, and excreted) of perchlorate in test animals and humans. These data were to

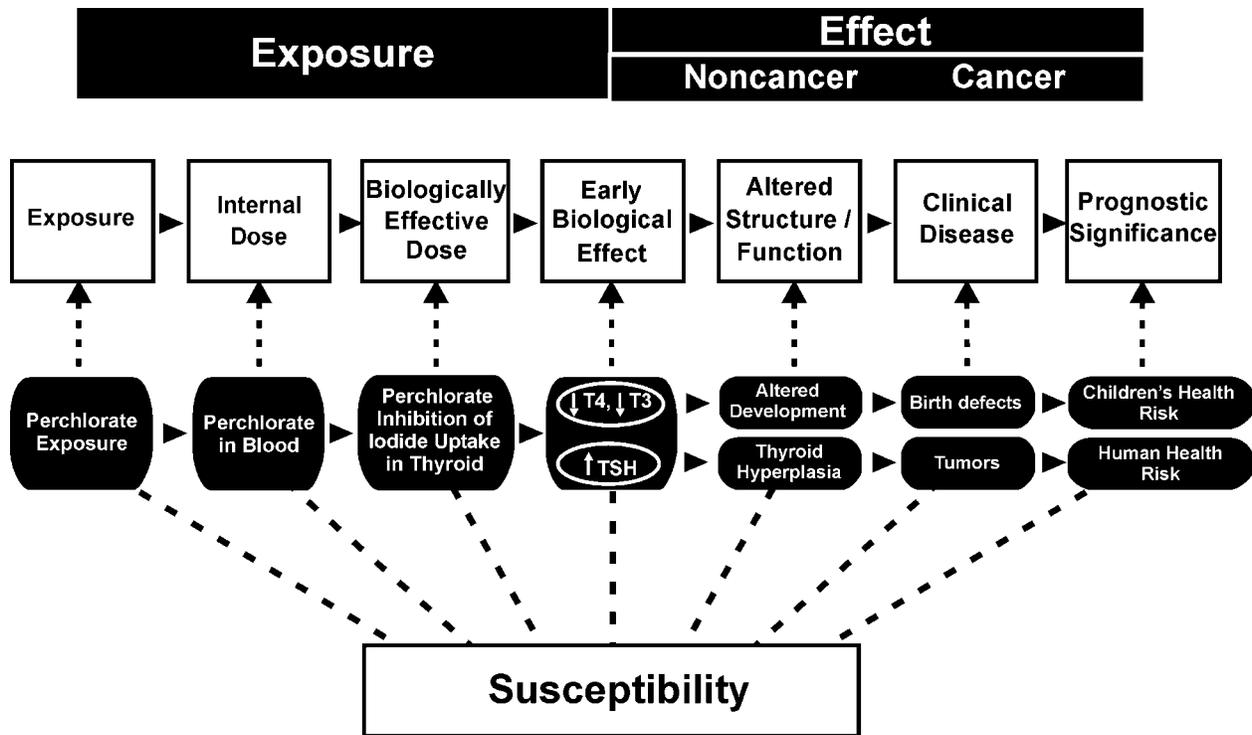
1 provide information to support construction of quantitative extrapolation of dose across  
2 species (e.g., rat to human).

3  
4 **(6) Perchlorate Mechanism Studies.** These studies provided a link to the pharmacokinetic  
5 studies and were conducted via a comparison of existing literature and of new *in vitro* and  
6 *in vivo* data that evaluated the effects of perchlorate on the iodide uptake mechanism across  
7 species to aid in the quantitative extrapolation of dose.

8  
9 **(7) Genotoxicity Assays.** These studies evaluated the potential for carcinogenicity by  
10 evaluating mutations and toxic effects on DNA. These data were useful to determining  
11 whether the benign thyroid tumors were likely to be a result of the proposed threshold  
12 pathogenesis process.

13  
14 **(8) Immunotoxicity Studies.** These studies were planned to evaluate the potential for  
15 perchlorate to disrupt immune function and identify a potentially critical effect. These data  
16 would help to reduce the uncertainty factor applied for database deficiencies. Because  
17 concern was raised for these potential adverse effects based on the previous clinical  
18 experience with treatment of Graves' disease patients, these studies were considered  
19 necessary to a comprehensive database for perchlorate.

20  
21 In the 1998 external review draft (U.S. Environmental Protection Agency, 1998d), a model  
22 based on mapping the events of the mode of action for perchlorate was proposed as shown in  
23 Figure 3-12. The key event was identified as the inhibition of iodide uptake at the NIS, followed  
24 by decreases in thyroid hormones and increases in TSH. Both the potential neurodevelopmental  
25 and neoplastic sequelae of this perturbation in thyroid hormone economy were proposed as  
26 downstream adverse health outcomes. The conceptual model was endorsed by the external peer  
27 review panel in 1999 (Research Triangle Institute, 1999), and additional studies were  
28 recommended to reevaluate indications of developmental and neurodevelopmental in rats for  
29 effects observed in the 1998 database. Delineating the continuum of histopathological changes  
30 in the thyroid was also recommended. The results of all the studies in the testing strategy (both  
31



**Figure 3-12. Mode-of-action model for perchlorate toxicity proposed by the U.S. EPA (U.S. Environmental Protection Agency, 1998d). Schematic shows the exposure-dose-response continuum considered in the context of biomarkers (classified as measures of exposure, effect, and susceptibility) and level of organization at which toxicity is observed (U.S. Environmental Protection Agency, 1994; Schulte, 1989). The model maps the toxicity of perchlorate on this basis by establishing casual linkage or prognostic correlations of precursor lesions.**

1 “old ” 1998 and “new” 2001), as well as additional studies now available in the literature, will be  
 2 reported together with EPA’s interpretation and evaluation in Chapter 5.

## 4. HUMAN HEALTH EFFECTS DATA

The available data on the human health effects of perchlorate exposures are limited. Until the emerging concern regarding environmental contamination, the majority of the studies were clinical reports on patients treated with potassium perchlorate for Graves' disease. The non-EPA, independent peer review held in March 1997 (Toxicology Excellence for Risk Assessment, 1998a) concluded that the experimental design limitations of the studies prior to that time precluded their use in quantitative dose-response assessment. The CA DHS also determined in 1997 that there were major limitations on the human studies. Nevertheless, the studies were useful in hazard identification and supported the conceptual model for the mode of action of perchlorate available at the time as described in Chapter 3.

Since the external peer review of the previous 1998 external review draft held in 1999 by the U.S. Environmental Protection Agency (Research Triangle Institute, 1999), some ecological studies have been performed that have addressed the limitations in the human data with some success. Two occupational populations with inhalation exposure to perchlorate were also studied, and some additional clinical studies in healthy adults performed. On December 14, 2001, after internal peer review of this document, the Agency articulated its interim policy on the use of third-party studies submitted by regulated entities (U.S. Environmental Protection Agency, 2001c). For these purposes, EPA is considering "third party studies" as studies that have not been conducted or funded by a federal agency pursuant to regulations that protect human subjects. Under the interim policy, the Agency will not consider or rely on any such human studies (third-party studies involving deliberate exposure of human subjects when used to identify or quantify toxic endpoints such as those submitted to establish a NOAEL or NOEL for systemic toxicity of pesticides) in its regulatory decision making, whether previously or newly submitted. Some of the clinical studies contained in this database fall in this category of studies not to be considered. However, the scientific and technical strengths and weaknesses of these studies were described before this Agency policy was articulated. Therefore, because of the scientific shortcomings of these studies, they will not be used as "principal studies" in the derivation of a RfD. The ethical issues surrounding the conduct of these studies or their use for

1 regulatory purposes in light of the Agency's interim policy will not be discussed in this  
2 document. The Agency is requesting that the National Academy of Sciences conduct an  
3 expeditious review of the complex scientific and ethical issues posed by EPA's possible use of  
4 third-party studies which intentionally dose human subjects with toxicants to identify or quantify  
5 their effects.

#### 8 **4.1 EPIDEMIOLOGICAL DATA**

9 To be informative to quantitative dose-response analysis for risk assessment applications,  
10 epidemiological studies must pose research questions that are based on appropriate physiological  
11 issues relevant to the mode of action for the chemical and its toxic effect. In some contexts, a  
12 sufficient specification may take relatively simple form. For example, with occupational cancer,  
13 the generally assumed underlying mechanisms lead to a simple test: does exposure to a substance  
14 or mixture specified as a dependent parameter,  $X$ , at time  $t_1$  increase the incidence of specific  
15 cancers at time  $t_2 > t_1 + a$ , where  $a > 0$  is some lag time. The relation of risk at  $t_2$  to the history of  
16 prior exposure may be a complex one, but almost always, risk is an increasing function of  
17 exposure at various time intervals,  $X_{(it)}$ . This test may require controlling for confounding  
18 factors, which is usually not difficult when relevant detailed information is available.

19 In contrast, determining the effect of an environmental exposure on a regulated system  
20 could be more of a challenge. Thus, cancers whose risk depends on endocrine status introduce  
21 increased complexity. Environmental perturbations of physiological systems that have inherent  
22 variability over time and are imbedded in control networks that function to minimize disruption  
23 make it a challenge to determine which endpoints to measure. Cross-sectional assessments  
24 during chronic exposures may capture variability in some regulated biological parameters while  
25 other parameters will tend to stabilize at "normal" levels despite substantial environmental  
26 impact on production and function. In such instances it can be difficult to distinguish alterations  
27 due to the xenobiotic from the variation that occurs in response to other environmental factors.  
28 Short-term fluctuations in exposure often have no effect independent of cumulative dose for  
29 chronic diseases such as lung cancer or other respiratory diseases but may be important for  
30 endocrine system functions that affect neurodevelopmental, hyperplastic, neoplastic, immune, or  
31 autoimmune events (Park, 2001).

1           The effect of the perchlorate anion on the hypothalamic-pituitary-thyroid feedback system  
2 is an example of a regulated system that is potentially difficult to characterize. Important effects  
3 may be evident as shifts in average levels of measurable factors, but more important effects may  
4 involve alterations in transient responses to demands on the regulated system (Park, 2001).  
5 Multiple covariates that may influence potential perchlorate health effects include iodine  
6 availability, age, gender, ethnicity, health status, diet, and possibly social class. For neonates, the  
7 birth process itself stimulates an endocrine cascade with the amplitudes of endpoint variation  
8 depending on birth weight, gestational age, age at sampling (in hours), and possibly  
9 environmental temperature. Post-partum developmental risk factors for the neonate and growing  
10 child include perchlorate exposure via lactation or consumption of contaminated water.

11           Individual perchlorate exposures are difficult to measure or even estimate in population-  
12 based studies. This makes their usefulness to quantitative dose-response analysis limited,  
13 particularly if confounding variables are not controlled and small population sizes are evaluated.  
14 The few population-based studies from geographic areas that have experienced perchlorate  
15 contamination offer little help beyond indicating that clinical thyroid disease is not greatly  
16 increased in populations with sustained drinking water contamination as high as 15  $\mu\text{g/L}$  in the  
17 past. However, most of the studies have principally evaluated thyroid function or hormone status  
18 and have not evaluated neurodevelopmental or other deficits in children or adults resulting from  
19 perturbed thyroid function over sustained periods of exposure.

#### 21 **4.1.1 Ecological Studies**

22           Rockette and Arena (1983) reviewed death certificates for workers known to have been  
23 exposed to perchloric acid, magnesium perchlorate, and other chemicals in a U.S. chemical plant.  
24 Because the workers had received multiple chemical exposures, the authors could not associate  
25 an elevated death rate for a particular time period or work area and a specific chemical.

26           The Environmental Health Investigations Branch within the CA DHS, under a cooperative  
27 agreement with ATSDR, conducted health assessment activities and consultations on the  
28 Aerojet-General Corporation Superfund site in Sacramento County, CA (California Department  
29 of Health Services, 1997; 1998a,b,c,d,e). A preliminary health review (California Department of  
30 Health Services, 1997) analyzed several statewide databases for possible perchlorate-related  
31 outcomes during the suspected years of contamination within the zip codes most likely exposed.

1 In California, thyroid hormone levels in newborns are measured and kept on file by the Genetic  
2 Disease Branch of the Centers for Disease Control and Prevention. Data for the period 1985  
3 through 1996 from relevant zip codes was assessed for a total of 11,814 thyroid hormone screens.  
4 Although an extrapolation of the statewide rate predicted there would be 3.76 cases of  
5 hypothyroidism, four cases were observed. In the non-exposed areas, six cases of  
6 hypothyroidism were found although 6.41 cases were predicted. These data suggested no  
7 association between residence in the potentially-exposed zip codes and neonatal hypothyroidism.  
8 The TSH levels (ascertained only in neonates with initially low T4 levels) in the potentially-  
9 exposed areas were statistically significantly lower than those in the nonexposed areas. The  
10 database also was evaluated for diagnosis of goiter among the first five reported hospitalized  
11 individuals residing in the zip code of most likely contamination from the years 1991 to 1995.  
12 Because there are so many diseases or conditions that can produce a goiter other than perchlorate  
13 ingestion, and because the database can not differentiate this aspect, it was concluded that these  
14 data would not be useful in determining the prevalence of thyroid enlargement due to perchlorate  
15 in the affected water district. The same zip code also was evaluated for agranulocytosis or  
16 aplastic anemia as one of the top five diagnoses for the years 1991 to 1995. There were a total of  
17 76 cases in 5 years, less than the statewide rate of 41.6 per year. The rate for aplastic anemia was  
18 3.8 hospitalizations per 100,000 individuals per year, a rate higher than the statewide rate of 2.2.  
19 However, all but one of the hospitalizations also had an additional diagnosis of cancer with  
20 chemotherapy or radiation treatment; these treatments are likely explanations for this  
21 observation; acquired immunodeficiency syndrome (AIDS) may be another. The registry also  
22 was searched for cases of childhood leukemia (either acute lymphocytic leukemia or acute  
23 myelogenous leukemia). Again, the rate for the potentially exposed zip code was less than the  
24 corresponding rate for California.

25 The CA DHS concluded that the data on goiter, agranulocytosis, and aplastic anemia did  
26 not indicate an increase in incidence; however, these data do not provide definitive causative  
27 information because other likely causes for these conditions existed. Increases in the incidence  
28 of decreased neonatal thyroid hormone levels, hypothyroidism, or childhood leukemia rates were  
29 not observed. The CA DHS noted that the major limitation on studies of this nature is that  
30 imposed by the absence of good exposure estimates and the absence of data on transport and  
31 transformation models which would provide dose reconstruction for the affected population. It is

1 unclear when the contaminated plume entered the drinking water supply; consequently, the time  
2 period analyzed may have been too broad. Improving this exposure information was one of the  
3 recommendations made in the report to Congress regarding perchlorate (U.S. Environmental  
4 Protection Agency, 1998e). Finally, that perchlorate is not specific for producing thyroid  
5 dysfunction or hematological abnormalities makes assessing these outcome surveys difficult.  
6 Table 4-1 shows the approximate prevalence of these disorders in the neonatal period  
7 (1:30,000 to 1:100,000), and suggests that studies with large numbers of subjects may be  
8 necessary to detect subtle effects.

9 Based on these results, the CA DHS investigated several other water service areas for  
10 exposure (California Department of Health Services, 1998a,b,c,d,e) and ascertained that  
11 complete exposure pathways to perchlorate contaminated water existed in several areas. These  
12 studies reinforced the need for this document which attempts to properly characterize the risk  
13 posed by perchlorate contamination by providing better exposure estimates and a revised health  
14 risk estimate.

15 Since the 1999 external peer review, eight new population studies have been performed.  
16 One of these studies has examined effects in the general population (Li et al., 2001), another in  
17 school-age children (Crump et al., 2000), and six have been devoted to evaluating neonatal  
18 endocrine status in areas with contaminated drinking water (Crump et al., 2000; Lamm et al.,  
19 1999; Li et al., 2000a,b; Brechner et al., 2000; Schwartz, 2001). In each study, the critical  
20 covariates were captured with varying degrees of success and only one study (Schwartz, 2001)  
21 offers a convincing description of neonatal perchlorate effects (Park, 2001).

22 In a study of the general population, Li et al. (2001) investigated physician-generated  
23 medical insurance claims for thyroid problems in a Medicaid insured population in Nevada,  
24 comparing all counties that were known not to have perchlorate contaminated drinking water  
25 with the one county that had contamination at approximately 10  $\mu\text{g}/\text{L}$ . This was a study of  
26 period-prevalence, i.e., the proportion of the population that had claims for thyroid-related  
27 disorders anytime during a two-year period. Incident cases could not be identified within this  
28 database. Thyroid patients were defined as having one or more of the following diagnoses of  
29 thyroid disease according to the International Classification of Diseases, 9<sup>th</sup> Revision (ICD-9):  
30 (1) simple and unspecified goiter (ICD-9 Code 240); (2) non-toxic nodular goiter (ICD-9 Code  
31 241); (3) thyrotoxicosis with or without goiter (ICD-9 Code 242); (4) congenital hypothyroidism

**TABLE 4-1. THYROID DISORDERS AND THEIR APPROXIMATE PREVALENCES IN THE HUMAN NEONATAL PERIOD (Fisher, 1996).**

<i>Thyroid Dysgenesis</i>	1:4000
Agenesis	
Hypogenesis	
Ectopia	
<i>Thyroid Dyshormonogenesis</i>	1:30,000
TSH unresponsiveness	
Iodide trapping defect	
Organification defect	
Defect in thyroglobulin	
Iodotyrosine deiodinase deficiency	
<i>Hypothalamic-Pituitary Hypothyroidism</i>	1:100,000
Hypothalamic-pituitary anomaly	
Panhypopituitarism	
Isolated TSH deficiency	
Thyroid hormone resistance	
<i>Transient Hypothyroidism</i>	1:40,000
Drug induced	
Maternal antibody induced	
Idiopathic	

1 (ICD-9 Code 243); (5) acquired hypothyroidism (ICD-9 Code 244); (6) thyroiditis (ICD-9 Code  
 2 245); (7) other disorders of the thyroid (ICD-9 Code 246) and (8) malignant neoplasms of the  
 3 thyroid gland (ICD-9 Code 193). Two of these disorders have very low prevalence: congenital  
 4 hypothyroidism (0.01%) and thyroid cancer (0.02%).

5       Comparisons were made between the exposed county, which includes Las Vegas, and  
 6 (a) an unexposed county with a similar large city (Reno), and (b) all other counties (unexposed).  
 7 There were no statistically significant period-prevalence rate differences between the exposed  
 8 county and the two categories of comparison counties; however, the differences between the  
 9 comparison county groups themselves were quite large, indicating that either important  
 10 confounding risk factors were not controlled or estimates were unstable due to the small numbers  
 11 of cases in the comparison counties. For acquired hypothyroidism, prevalences (%) in the two  
 12 categories of unexposed counties were significantly different (Reno: 1.17 [95% CI = 1.05 to  
 13 1.30, using a normal approximation to the Poisson distribution for number of cases] and other

1 counties: 1.44 [95% CI = 1.29 to 1.59]). Age, gender, ethnicity, iodine intake, and other  
2 important risk factors were unavailable in this database and there could have been differential  
3 under- or over-diagnosis in this Medicaid population. Interestingly, when comparing the two  
4 counties with large urban centers and restricting focus to the 6 (out of 8) more prevalent  
5 outcomes (total n=3069), all 6 showed elevated (but not individually significant) rate ratios for  
6 the exposed county, ranging from 1.01 to 1.89. While these findings appear to rule out a large  
7 perchlorate-related excess (i.e., greater than two-fold) for some thyroid disorders such as  
8 acquired hypothyroidism (appearing as routine medical insurance claims), the study had a  
9 statistical power of less than 0.5 to detect a 50% excess for several specific thyroid disorders  
10 (i.e., the observed relative rises exceeded 1.50 but were not statistically significant).  
11 Unfortunately, owing to potentially overwhelming confounding (e.g., related to age, gender,  
12 ethnicity, or iodine intake) or because of small numbers of cases in the comparison counties, little  
13 else can be concluded from this study.

14 The Crump et al. (2000) study of school children (mean age 7.3 years) in three Chilean  
15 cities permitted comparisons on effects of drinking water with widely varying perchlorate  
16 content: 0, 5, and 100 ppb. A total of 162 school-age children were studied, 127 of whom had  
17 lifelong residence in their respective cities. Controlling for age, gender, and urinary iodine,  
18 a highly significant trend of increasing T4 levels—the opposite to the expected direction for  
19 effects on T4 from perchlorate—was observed with increasing perchlorate content in the water.  
20 The city with the highest concentrations (100 ppb) had a significant five-fold excess in family  
21 history of thyroid-related problems. Children in all three cities had elevated goiter prevalence,  
22 but it was highest in the city with intermediate concentrations (5 ppb) which was believed to also  
23 have iodine deficiencies. A variable introduction of iodized salt in earlier years may have  
24 affected these observations. It is not known what role boiling drinking water may have played or  
25 how the microbiological quality of drinking water varied across the cities studied. Ethnic and  
26 socioeconomic attributes were thought to be similar across the three groups of children but were  
27 not controlled for in the analysis. Whether ambient indoor and outdoor temperatures may have  
28 played a role in thyroid functional status was not investigated. It would appear that uncontrolled  
29 confounding effects, particularly from environmental or other factors, make it difficult to  
30 interpret the observed effects of drinking water contaminated with perchlorate at levels as low as  
31 5 ppb on thyroid function in this study. Controlling for urinary iodine in the analyses would

1 better address whether iodine deficiency differences across the three cities studied may have  
2 distorted the association of T4 changes with perchlorate exposure. The paradoxical trend  
3 observed in this study remains unexplained.

4 Crump et al. (2000) also studied newborns screened for hypothyroidism by a heel-stick  
5 blood sample between February 1996 and January 1999 in the same three Chilean cities.  
6 A systematic laboratory error gamma counter contamination occurred between December 1, 1997  
7 and June 30, 1998 which caused TSH to be reported very low ( $0.1 \mu\text{U}/\text{mL}$ ) for a high proportion  
8 (29.1%) of the blood samples analyzed. The error was reported to be limited to this 7-month  
9 period and to have affected a similar proportion of samples from each of the three cities. All data  
10 obtained during the 7-month period in question were excluded, leaving 9,784 neonatal records  
11 for analysis. Analysis revealed a statistically significant decline in TSH (log-transformed) with  
12 increasing city-perchlorate levels, a trend opposite to that hypothesized. The analysis was  
13 adjusted for gender and age at screening as categorical variables in days but covariates lacking  
14 included iodine intake (known to be low in one city), ethnicity, and birth weight. The ages at  
15 screening differed across the three cities studied; the median ages were 3, 4, and 6 for the  
16 unexposed, low, and high perchlorate studies, respectively. Other important environmental  
17 factors may have played a role such as ambient temperatures, caloric intake, and social class.  
18 This paradoxical finding parallels the similar result in school age children in the same Chilean  
19 population discussed above, and remains unexplained.

20 Lamm et al. (1999) examined rates of congenital hypothyroidism in 7 counties of California  
21 and Nevada with perchlorate contaminated drinking water. This outcome is defined as a result of  
22 a mandatory screening program at birth that involves a preliminary T4 determination followed by  
23 a TSH assay in a prescribed subset with low T4. Age at screen is not considered in this  
24 procedure for selecting candidates for TSH determination and screening age distributions by  
25 county were not reported. County-specific levels of perchlorate contamination were unavailable.  
26 Rates for the California births were adjusted only for Hispanic ethnicity, observed to be a risk  
27 factor in this and other studies (Brechner et al., 2000; Schwartz, 2001). The county rate ratios for  
28 congenital hypothyroidism ranged from 0.6 to 1.1 relative to the statewide expected rates and  
29 were not statistically significant for all exposed counties combined, the rate ratio was 1.03 (95%  
30 CI = 0.90 to 1.16). Expected rates based on the non-exposed counties of the two states were not  
31 used. Had only non-exposed counties been used for comparison (given that the exposed counties

1 comprise a substantial fraction but assuming it is less than half of the state's population) the  
2 resulting rate ratios for the exposed counties would have been 1% or higher. Most critically  
3 lacking in the analysis was classification on age at time of blood sample for the screening test.  
4 Birth weight and further detail on ethnicity and other risk factors were also unavailable.  
5 Therefore, it is likely that uncontrolled confounding has played a role in this study, making it  
6 difficult to interpret and allows for some role of perchlorate in the almost two-fold observed  
7 variation in risk of neonatal hypothyroidism across counties.

8 Li et al. (2000a) compared the mean monthly T4 levels derived from mandatory screening  
9 of all newborns in Las Vegas (exposed) and Reno (unexposed), controlling for birth weight  
10 (within the restricted range 2.5-4.5 kg) and for age at sample (days 1, 2 or 3 versus 4), for the  
11 period April 1998 through June 1999. Statistical differences in the mean birth weight and mean  
12 age at time of sample were noted for the Las Vegas (n = 17,308) and Reno (n = 5,882) newborns.  
13 The exposure variable was based on monthly measurements made on Las Vegas finished water  
14 by the Southern Nevada Water Authority using IC with a detection limit of 4 ppb. The source of  
15 the Las Vegas water supply, Lake Mead, is known to have thermal stratification that causes  
16 seasonal variation in drinking water perchlorate content. The water supply in Reno comes from  
17 the mountains via Lake Tahoe, the Truckee River, and local wells. Tests of these water sources  
18 for Reno were reported to detect no perchlorate (data not shown nor was it specified if these  
19 measurements were made monthly). A highly significant period or seasonal effect was observed  
20 for both cities (perhaps suggesting an ambient temperature effect), but no difference was  
21 observed between cities during the period of exposure (7 out of the 15 months of observation  
22 when perchlorate content was high in Las Vegas drinking water). Highly significant age effects  
23 were observed, but the dependence of these age effects on exposure (i.e., an exposure interaction)  
24 was not examined. For reasons that are obscure, T4 levels reported in this study were  
25 considerably higher than those reported by others (17 versus 7-10  $\mu\text{g/dL}$ ). The restriction on  
26 birth weight would be inappropriate if birth weight were an intervening variable (i.e., itself  
27 affected by thyroid changes resulting from perchlorate exposure). Regressions on first trimester  
28 and 9-month cumulative exposures using monthly perchlorate levels and grouping birth  
29 outcomes by month in Las Vegas and Reno revealed no trends for T4 differences between the  
30 two cities although more powerful analyses could have been performed using individual  
31 observations. This study suggests that clinically significant individual neonatal T4 differences

1 have not resulted from current perchlorate exposures although the possibility of important  
2 variation with exposure conditional on neonatal age was not examined.

3 In a parallel study design, Li et al. (2000b) studied TSH levels in Las Vegas and Reno  
4 newborns over an eleven-month period from December 1998 to October 1999. Las Vegas water  
5 had measurable perchlorate levels in 8 of the 11 months. The perchlorate exposure measures and  
6 assumptions were the same as in Li et al. (2000a). TSH levels were determined on screening  
7 samples that were below the 10th percentile on T4 in each daily batch of samples collected  
8 throughout the state, selected without regard to age at screening. TSH levels from the two cities  
9 for birth weights restricted to 2.5-4.5 kg were compared adjusting for gender and age at screen  
10 (days 2-7 versus 8-30). Births whose screening sample was taken on the first day were excluded  
11 because those TSH levels were considered unstable. The study did not report whether the age at  
12 screen distribution differed between the two cities. Ethnicity and other risk factors were not  
13 available. Using a log-transformed TSH level to facilitate statistical testing, they found no  
14 difference in TSH levels between the two populations (a very small negative effect was estimated  
15 for TSH with exposure), however, the log transformation may have suppressed important  
16 differences at the high end of the TSH distribution and the analysis was not restricted to the  
17 8 months when exposure differed between the two cities. Examination of an exposure with age  
18 interaction was not reported. Excluding births screened on the first day may have further  
19 obscured differences arising from perchlorate exposure, differences that pertain to thyroid  
20 responsiveness. This study suggests that TSH levels in newborns after the first day did not differ  
21 substantially between two cities with and without perchlorate contamination of drinking water as  
22 estimated by monthly measurements.

23 Brechner et al. (2000) studied TSH levels in Arizona newborns assayed over a three-year  
24 period between October 1994 and the end of December 1997 in the Arizona Newborn Screening  
25 Program. In this program, total T4 is assayed in daily batches of specimens received from all  
26 over the state. TSH is measured in selected samples, representing approximately 10% of the  
27 samples with the lowest T4 levels from each batch. TSH levels were compared between two  
28 cites, Flagstaff and Yuma, representing areas of nonexposure and exposure to perchlorate. Zip  
29 codes were used to determine that Yuma was the only area with essentially all of its drinking  
30 water supplied by the Colorado River below Lake Mead. Exposure data were not available for  
31 the period between 1994 and 1997. Measurements made by the U.S. Environmental Protection

1 Agency Region 9 laboratory in August 1999 reported perchlorate levels at 6 ppb in both raw and  
2 finished water for Yuma and not detectable in Flagstaff water. Because the water processing  
3 facilities have not changed in either city and perchlorate is known to persist for long periods,  
4 Brechner and colleagues presumed that comparable differences between the perchlorate levels in  
5 the two cities existed during the period of analysis. Controlling for age at screen (days 0, 1-4,  
6 5+) and Hispanic ethnicity, these investigators found a statistically significant elevation in TSH  
7 for the exposed population in Yuma (crude TSH: 19.9 versus 13.4 mU/L; adjusted TSH effect  
8 not reported). However, the age-at-screening distributions differed considerably between these  
9 two cities presenting a possibility for some residual confounding on age. In Yuma (exposed)  
10 5.9% of newborns were screened in the first 24 hours when TSH levels peak (mean TSH =  
11 30 mU/L), compared with 2.4% of Flagstaff newborns (mean TSH = 23 mU/L). Thirty-one  
12 percent of Yuma births were screened at day 6 compared with 46% of Flagstaff births.  
13 Additionally, because of this negative association between age and exposure, the analysis of  
14 variance procedure employed had the potential for bias arising from colinearity. The age and  
15 exposure effect estimates would be jointly affected: overestimating exposure and  
16 underestimating age effects, or visa versa. Other factors not controlled included gender and birth  
17 weight. This study offers positive support for an association of increased neonatal TSH with  
18 perchlorate exposures; however, similar to other studies on this question, it has some unresolved  
19 methodological issues, most notably the strong association between age at screen and perchlorate  
20 exposure.

21 There is a subtle form of bias in the Brechner and other studies where TSH was determined  
22 on a low - T4 percentile subset of all births that mixes on a daily basis ages at screen for samples  
23 from all over the state. Bloods with low T4 are selected, but the T4 distribution depends on age.  
24 Births with screen ages that usually have higher T4 (typically after 24 hr) are less likely to be  
25 selected for TSH determination; conversely, at ages under 24 hr, births are more likely to be  
26 selected. Both summary and age-specific TSH comparisons would be unbiased with respect to  
27 exposure effects only if the same age at screen distributions were obtained in both the exposed  
28 and unexposed populations. The effect of this bias on estimation of overall perchlorate exposure  
29 effects is difficult to predict, depending in part on how perchlorate exposure affects T4 as well as  
30 on its effects on TSH, and on how sampling age varied with exposure status. It is conceivable  
31 that this bias could explain some of the elevated TSH in perchlorate-exposed neonates of the

1 Brechner et al. (2000) study, but the same sampling bias was potentially present in the Li et al.  
2 (2000b) study that found no effect. The latter study, however, excluded neonatal blood samples  
3 taken during the first 24 hours. That is the period when the strongest perchlorate-related  
4 differences were observed in the Brechner et al. (2000) study.

5 Schwartz (2001) analyzed both T4 and TSH levels for all California newborns screened in  
6 1996, making use of detailed covariate information on age, birth weight, ethnicity and birth  
7 multiplicity. Perchlorate exposure was assigned using the mothers' postal zip codes that were  
8 linked to state water testing data on all drinking water sources. These estimates of perchlorate  
9 levels were ultimately collapsed into four exposure categories: 0, 1-2, 3-12, 13+ ppb. This level  
10 of exposure detail far exceeded that of any other studies, very likely resulting in the least  
11 exposure misclassifications.

12 An analysis of covariance (ANCOVA) model was used in this analysis. The ANCOVA  
13 model is a multiple linear regression model that can simultaneously estimate effects for levels  
14 categorical variables like gender as well as for continuous variables like age or birth weight.  
15 Controlling for age at screening (6-hour increments up to 48 hours), gender, single versus  
16 multiple birth, birth weight (in 5 levels), and ethnicity (20 categories), a highly statistically  
17 significant declining trend was observed for T4 with the four perchlorate exposure levels (0,  
18 -9.7, -11.2, -18.2). T4 levels in this model declined with age (relative to its final level after  
19 48 hours) until about 18 hrs (-50 mg/dL below final level) and then increased over the next 30  
20 hours (to 36 mg/dL above final level) before assuming its final level after 48 hours. For TSH  
21 (log-transformed), there was a significant increasing trend with perchlorate exposure (0, 0.029,  
22 0.03, 0.128), and the TSH level followed a more rapid time course increasing immediately after  
23 birth, then declining to a final level by 24 hours. Substantial birth weight, gender, ethnicity and  
24 birth multiplicity effects were observed for T4, and smaller effects were observed for TSH.

25 The models specified in this study tested for uniform additive exposure effects for T4 and  
26 TSH across all covariate categories, including baseline shifts. Another issue of considerable  
27 physiological interest would have been whether the amplitudes of the T4 and TSH surges  
28 depended on perchlorate exposure with baseline levels relatively unaffected, which could be  
29 tested by evaluating an interaction between age and exposure. An examination of interaction was  
30 not reported. The bias in TSH measurements introduced by the T4-triggered sample selection  
31 described above for other effects studies would also affect the Schwartz study. This bias would

1 not affect inferences on exposure effects if the age at screen distribution were similar across the  
2 four exposure levels. These distributions were not reported in the Schwartz study.

3 The Schwartz study also modeled the effect of two screening performance criteria on the  
4 same set of predictors: (a) “presumptive positive criterion” and (b) a positive finding of  
5 congenital hypothyroidism. Not surprisingly, these models did not predict the standard screening  
6 outcomes well because the screening algorithm does not take into account the several very  
7 important predictors identified in this study. Rather, finding a presumptive positive is based  
8 entirely on T4 without regard to age at screen, birth weight, etc. Similarly, identifying a case of  
9 congenital hypothyroidism is based only on T4-triggered sample selection and subsequent TSH  
10 determination ( $>25 \mu\text{U/ml}$ ).

11 The Schwartz study is by far the most convincing of the neonatal studies, being based on  
12 the most elaborate exposure assignment and the most detailed collection of covariate information  
13 pertaining to neonatal thyroid function. It is unlikely that bias arising from the TSH sampling  
14 could produce such a consistent TSH exposure response and would play no role in the stronger  
15 (based on narrower confidence intervals for the parameter estimates) exposure response observed  
16 for T4.

#### 18 **4.1.2 Occupational Studies**

19 There are two publications investigating workers in ammonium perchlorate production  
20 (Gibbs et al., 1998; Lamm et al., 1999). The route of exposure for each was by inhalation to  
21 perchlorate dust, introducing a considerable uncertainty in dose-response analysis especially due  
22 to poor characterization of particle size distribution. Both studies were also cross-sectional in  
23 design and, therefore, subject to survivor bias in that workers experiencing adverse effects could  
24 have left employment. This issue was not addressed in either study. It would have been  
25 particularly noteworthy had any former employee no longer in the cohort experienced thyroid  
26 disorders, aplastic anemia, or related hematological disorders, each of which have been reported  
27 in settings where perchlorate is used for short periods at higher doses in the treatment of disease  
28 (Lawrence et al., 1999). The airborne exposures that were characterized corresponded to daily  
29 doses on the order of 20 to 50 mg and possibly higher as the air-sampling methods excluded  
30 large particulate ( $> 50 \mu\text{m}$ ) that could add considerable mass to the daily inhaled or ingested

1 dose. In the study that investigated this (Lamm et al., 1999), the daily absorbed dose based on  
2 urinary perchlorate actually exceeded the inhaled dose.

3 There was no clear evidence for any perchlorate effect on thyroid function, as defined by  
4 the investigators, in these two cross-sectional occupational studies. However, historical exposure  
5 classification was limited in one study and absent in the other. Former employees were lost to  
6 follow-up, and neither study controlled for potential confounding arising from body mass,  
7 environmental temperatures, or socioeconomic status. There was no measurement of thyroid  
8 iodine status or of any index thyroid dynamic responsiveness that conceivably could be altered  
9 even though steady-state TSH and T4 levels appear to be in the normal range. Because of the  
10 cross-sectional design and measured endpoints, the studies did not evaluate the dynamics of  
11 hypothalamic-pituitary-thyroid feedback that are likely important in target populations such as  
12 hypothyroxinemic pregnant women and their fetuses.

13 Gibbs et al. (1998) performed a case control occupational epidemiology study to evaluate  
14 thyroid function and standard clinical blood test parameters of liver, kidney, and bone marrow  
15 function in employees exposed to ammonium perchlorate airborne dust at a production facility  
16 and an associated cross-blending facility. Exposure estimates were based on multiple samples  
17 (average sample number = 17) for eight homogenous exposure groups defined by similar job  
18 activities: control, maintenance/foreman, and six discrete operator job categories. Personal  
19 breathing zone samples (n = 119) were used for the work categories and full-shift area samples  
20 were used for the control group (n = 19). The control exposure was not zero but was several  
21 orders of magnitude below any exposure category. In their 1997 analyses, when ammonium was  
22 quantified using National Institute for Occupational Safety and Health Method 6016 which had a  
23 minimum reporting limit of 0.017 mg/m<sup>3</sup>, concentrations in a large number of the samples were  
24 reported as undetectable. The 1998 analyses were performed using the modified EPA 300.0  
25 methodology that determines perchlorate using ion chromatography and has a reporting limit of  
26 approximately 0.00004 mg/m<sup>3</sup>.

27 Effects were examined in either a single-shift design (pre- and post-shift parameter  
28 measurements) or working lifetime design based on medical surveillance data that included  
29 thyroid examination since 1996 (blood tests, physical exam, and history since 1994). Dose was  
30 reconstructed based on personnel records for job type and area samples.

1 Despite the lack of particle size distribution data, an inhaled “dose” was calculated for a  
2 single shift as (Gibbs et al., 1998):

$$\left( \begin{array}{c} \text{respiratory} \\ \text{rate} \end{array} \right) \times \left( \begin{array}{c} \text{inhalation} \\ \text{concentration} \end{array} \right) \times \left( \begin{array}{c} \text{exposure} \\ \text{duration} \end{array} \right) \times \left( \begin{array}{c} \text{fraction} \\ \text{absorbed} \end{array} \right). \quad (4-1)$$

3  
4 Working lifetime exposure estimates were calculated as:

$$\sum (\text{mean group exposure}) \times (\text{years in exposure group}) \times 2,000, \quad (4-2)$$

6  
7 where the 2,000 was an average of the number of hours worked yearly based on typical overtime  
8 rates at the facilities.

9 Daily respiratory rates of 0.0165 m<sup>3</sup>/kg-hr and 0.0068 m<sup>3</sup>/kg-hr were estimated for “active”  
10 and “sedentary” workers, respectively, based on Beals et al. (1996). These estimates are slightly  
11 lower than the default EPA respiratory rates and are moderately lower than those recommended  
12 by the International Commission on Radiological Protection in its recent human respiratory tract  
13 model (International Commission on Radiological Protection, 1994). Average body weights of  
14 the workers were larger than the typical default body weights. Because current practice usually  
15 scales ventilation rate based on body weight, higher ventilation rates were expected.

16 The absence of particle size diameter and distribution data is a significant limitation of the  
17 study because this data is required to assess the potential inhalability of the ammonium  
18 perchlorate aerosol. Data from another production facility indicate the majority of particles are  
19 200 μm (Hancock, 1998). Particles larger than 30 μm are typically not inhalable by humans  
20 (U.S. Environmental Protection Agency, 1996b). Further, there was no mention of face volume  
21 performance of the personal samplers using 5-μm filters although this is an important  
22 consideration in dusty environments when the particles under investigation have a large diameter.  
23 This consideration is especially important here because the filter cassettes were changed when  
24 respirators were used. Even if a 5-μm particle diameter could be assumed, the inhaled “dose”  
25 calculation should have included an adjustment for inhalability and deposition efficiency to

1 calculate the deposition fraction, approximately 0.3 at 5  $\mu\text{m}$  (U.S. Environmental Protection  
2 Agency, 1996b).

3 The assumption about the solubility of the inhaled particles is also problematic because this  
4 parameter is particle-diameter dependent. The particle diameter dictates the location  
5 (extrathoracic, tracheobronchial, pulmonary) where the particle deposits and the local milieu and  
6 clearance vary with location also influence solubility (U.S. Environmental Protection Agency,  
7 1996b; Snipes et al., 1997). The solubility of cesium chloride (CsCl) in beagles was used to  
8 estimate a fraction absorbed. Although CsCl and  $\text{NH}_4\text{ClO}_4$  may have similar solubilities,  
9 additional uncertainty is introduced because the CsCl particle diameter or inhalability function  
10 for the beagles was not taken into account; and the hygroscopicity, which influences the initial  
11 deposition site, may not be the same. The assumptions about dose could have been validated  
12 with a mass balance approach. For example, perchlorate could have been measured in the blood  
13 when samples were taken for thyroid hormone analyses. Additionally, urine samples could have  
14 been monitored for perchlorate because it is excreted in the urine. These additional  
15 measurements would have afforded some confidence that the inhaled dose estimates were  
16 reasonable.

17 Standard clinical thyroid profiles included a total serum T4, triiodothyronine resin uptake,  
18 and TSH. Bone marrow function was evaluated during medical surveillance examinations with  
19 standard tests from the complete blood count which included hemoglobin, hematocrit, red blood  
20 cell count, mean corpuscular volume, white blood cell count, and platelet count. Standard serum  
21 chemistries were used to assess kidney (serum creatinine level and blood urea nitrogen) and liver  
22 (serum glutamyl pyruvic transaminase [SGPT], serum glutamyl oxaloacetic transaminase  
23 [SGOT], g-glutamyl transpeptidase [GGTP], and alkaline phosphatase) functions.

24 Dependent variables for the single-shift study were the cross-shift change in measures of  
25 thyroid function. Explanatory variables included race, gender, age, hours awake prior to the  
26 pre-shift test, number of hours slept during the most recent period prior to the test, time of day,  
27 and shift length. Dependent variables for the working lifetime included measures of thyroid,  
28 bone marrow, liver, and kidney functions. For the thyroid tests, an additional explanatory  
29 variable was used to indicate whether the measurement was from a routine physical in 1996 or  
30 from a pre-shift or a post-shift examination in 1997 or 1998. The dose variables were group  
31 (control, low dose, or high dose) and estimated cumulative exposure. The dose group

1 designation was an arbitrary stratification of <8 mg/kg-day and >8 mg/kg-day. Multiple  
2 regression was used to analyze the relationship between effect measures and explanatory  
3 variables. A sequential approach was used to determine whether a dependent variable should be  
4 log-transformed and whether any outliers (defined as a value corresponding to a residual larger in  
5 absolute value than three standard deviations) should be eliminated from an analysis.

6 Estimated doses for the single shift-study ranged from 0.0002 to 0.436 mg/kg-day with a  
7 mean of 0.036 mg/kg-day and median of 0.013 mg/kg-day. The dose estimate was not a  
8 significant predictor of thyroid function parameters measured in 83 control (65 male, 18 female)  
9 or 18 exposed (15 male, 3 female) individuals. Working lifetime exposure estimates ranged from  
10 0.5 to 7.0 (mean 3.5) mg/kg for the low-dose group and from 8.0 to 88.0 (mean 38.0) mg/kg for  
11 the high-dose group. The duration of exposure ranged from 1 to 27 years (mean 8.3).  
12 No significant correlations were detected in any measures of thyroid, bone marrow, liver, or  
13 kidney function; however, significant gender and race differences were apparent in the clinical  
14 tests of bone marrow, liver, and kidney functions. Females were slightly lower in hemoglobin,  
15 hematocrit, SGPT, GGTP, and creatinine than males; black workers were slightly lower than  
16 whites in hemoglobin and hematocrit and slightly higher in creatinine.

17 The only significant finding ( $p = 0.01$ ) was that cross-shift TSH changes were greater for  
18 those who worked a 12-h shift than for those who worked 8-h shifts, accounting for a  
19 0.45 urinary IU/mL increase across the shift. This was attributed to the influence of circadian  
20 changes in serum TSH. However, the TSH increase (10%) across a single work shift in an  
21 exposed group ( $n = 18$ ) compared to an unexposed group ( $n=83$ ) was observed in groups that  
22 together comprised less than half of employees eligible for study. Comparison of workers in  
23 three groups (unexposed, low and high cumulative exposure) resulted in consistent patterns for  
24 all thyroid parameters in which the unexposed group had values intermediate between those of  
25 the low and the high cumulative dose groups. This suggests that important confounding was  
26 present (i.e., that the comparison group, which apparently included office workers, differed from  
27 the exposed groups on other important risk factors) as well. For thyroid (TSH) and liver  
28 outcomes (SGOT, GGPT, SGPT), there were subtle indications of exposure effects: the standard  
29 deviation increased substantially in the high dose group, as did the average values but not the  
30 percentiles up to the 75<sup>th</sup>, suggesting that a small subgroup had undergone a considerable upward

1 excursion. Statistical tests (regression analysis) of these effects were severely limited by the  
2 apparent confounding that affected baseline levels.

3 In the second study of ammonium perchlorate workers, Lamm et al. (1999) assembled a  
4 comparison group at the same facility from an unrelated process thought to have low exposure to  
5 inhaled perchlorate. Workers were classified using presumptive exposure based on visible dust  
6 generated. Pre- and post-shift urine samples were collected to measure urinary perchlorate,  
7 iodine and creatinine levels. Post-shift blood samples were analyzed for complete blood count  
8 (CBC), hemoglobin, hematocrit and additional red cell parameters (mean corpuscular volume,  
9 mean corpuscular hemoglobin, and mean corpuscular concentration). A clinical chemistry panel  
10 was also run on post-shift serum samples. Thyroid parameters included TSH, free T4, T4, T3,  
11 thyroid hormone binding ratio, thyroid peroxidase antibodies, and clinical examination. Urinary  
12 perchlorate measurements were used to calculate a post-shift level of perchlorate (mg) per g of  
13 creatinine as an excretion dose, D:

$$D = k[E_i - 0.354 E_0]/0.646. \quad (4-3)$$

15 The right hand term in brackets is the post-shift adjusted level in mg perchlorate per gram of  
16 creatinine. Perchlorate absorption was calculated as a time-weighted average exposure using an  
17 assumption that the percent absorbed which is excreted is 95%. The human adult creatinine  
18 excretion rate was then used to link perchlorate excretion rate in terms of creatinine to rates in  
19 terms of time, so that the exposure dose was then calculated as:

$$12 \text{ hours} \times 60 \text{ minutes} / \text{hour} \times 0.001 \text{ g/mg} \times 1 \text{ mg creatinine/min} \times [\text{post-shift}]/0.646. \quad (4-4)$$

21  
22  
23 While particle size distribution data were collected, this information was not utilized in the  
24 analyses. Inhalation exposure was instead categorized into either “total” or “respirable”. While  
25 these categories correlated with each other to a good degree ( $r = 0.82$ ), perchlorate absorption  
26 (mg/shift) did not correlate as well to respirable ( $r = 0.45$ ) as it did to total particles ( $r = 0.54$ ).  
27 The comparison group had current absorbed doses equal to 20% of the low perchlorate-exposed  
28 group and 3% of the high exposed group even though the inhaled dose of the comparison group  
29 was 4% of that of the low dose and 0.02% of the high dose group. This suggests that there was  
30

1 considerable exposure misclassification, arising perhaps from general environmental  
2 contamination at the work site or in clothing. In one subject, urinary perchlorate increased over a  
3 12 hr period during which there was thought to be no exposure. No significant associations were  
4 observed between perchlorate exposure and thyroid parameters; however, measures of  
5 cumulative exposure were not considered. Suggestions of increasing trends for T3, T4, and  
6 maximum-T3 were not statistically significant but were based on small numbers (numbers of  
7 workers in exposure groups: 21 for the unexposed versus 14, 8, and 15 in the low, medium, and  
8 high exposure groups).

## 9 10 11 **4.2 CLINICAL STUDIES**

12 The historical clinical data on perchlorate have been predominantly case reports of patients  
13 whose results would be confounded either with thyroid disease or other pharmaceutical agents.  
14 A few more recent studies have begun to evaluate thyroid function in healthy volunteers. This  
15 section will discuss the available data on thyroid function from several clinical studies. A more  
16 formal development of the pharmacokinetic data in humans is presented in Chapter 6.

### 17 18 **4.2.1 Studies in Healthy Human Subjects**

19 Few data are available to demonstrate the effects of perchlorate on healthy individuals and  
20 issues of ethics are likely to preclude clinical evaluation in sensitive populations such as pregnant  
21 women. Exposure duration to perchlorate is typically short, from a few days to 4 weeks.

22 Burgi et al. (1974) examined the effects of perchlorate on the secretion of endogenous  
23 iodine by the normal human thyroid gland. Five healthy volunteers (3 males, 2 females;  
24 ages 24 to 27 years) received tracers of <sup>125</sup>I-iodide and <sup>131</sup>I-thyroxine for 17 days, followed by  
25 600 mg/day perchlorate (9.7 mg/kg-day, based on actual reported average body weight of  
26 61.8 kg) for 8 days. Urine and serum were analyzed for <sup>125</sup>I and <sup>131</sup>I to determine if perchlorate  
27 can cause the discharge of endogenous, as well as exogenous iodide, from the thyroid. Results  
28 show that this dose of perchlorate also was sufficient to completely block iodide uptake by the  
29 thyroid. In addition, perchlorate caused a 65% increase in excretion of nonthyroxine iodide over  
30 background. The authors attributed this increase to additional secretion of endogenous iodide

1 from the thyroid. Treatment with carbimazole plus perchlorate caused an additional increase in  
2 the secretion of nonthyroxine iodide, suggesting that perchlorate causes only a partial release of  
3 endogenous iodide. This study suggests a Lowest-Observed-Adverse-Effect-Level (LOAEL) of  
4 9.7 mg/kg-day for thyroid effects in healthy patients.

5 Brabant et al. (1992) administered potassium perchlorate to five healthy male volunteers  
6 (age 25 to 28 years) to study changes in TSH concentration and release in response to a decrease  
7 in iodine supply to the thyroid. During the first 4 weeks of the study, the volunteers were given  
8 200  $\mu\text{g}/\text{day}$  iodine. After iodine supplementation was discontinued, the volunteers were  
9 administered 900 mg/day of potassium perchlorate orally for 4 weeks to induce a state of iodine  
10 depletion. At the end of the 4-week perchlorate treatment, levels of thyroid hormones were  
11 measured. Although perchlorate treatment had no effect on thyroid volume or levels of  
12 triiodothyronine (T3) and thyroxine (T4), intrathyroidal iodide concentration and serum levels of  
13 TSH were decreased significantly, and serum levels of thyroglobulin were nearly doubled. The  
14 authors speculate that the decrease of TSH, which is the opposite of the expected response, may  
15 be an early adaptive mechanism to the iodine deficiency induced by perchlorate. They suggest  
16 that, early in iodide deficiency, the thyroid becomes more sensitive to TSH creating a feedback  
17 mechanism that decreases TSH levels. Only as iodide deficiency becomes more prolonged do  
18 TSH levels increase. This study defined a LOAEL of 13 mg/kg-day for thyroid effects. In a  
19 follow-up study, Brabant (1994) repeated the earlier studies with perchlorate treatment lasting  
20 longer than 4 weeks. As a result of the longer treatment, thyroid volumes increased in all  
21 subjects although TSH levels did not increase.

22 Lawrence et al. (2000) performed a 14-day clinical study with nine euthyroid volunteers  
23 (ages 22 to 30 years). Each subject was enrolled after a normal complete physical exam that  
24 included a thyroid exam. Blood was obtained for baseline measurement of thyroid function tests,  
25 TPO antibodies, CBC, and routine blood chemistries. A spot urine was obtained for routine  
26 urinalysis. All baseline tests were normal.

27 Ten mg of perchlorate as potassium perchlorate was dissolved in 1-L bottles of spring  
28 water. Each subject was instructed to consume the 1-L bottle intermittently during waking hours.  
29 Assuming a body weight of 70 kg, this dosage is equivalent to 0.14 mg/kg-day. Blood specimens  
30 were drawn between 8:00 and 9:00 a.m and 24-hour urine samples were obtained on days 7 and  
31 14 during exposure and then again after another 14 days after perchlorate was discontinued.

1 Thyroid function was assessed by assays for TSH, free thyroxine index (FTI), total T3, (TT3) and  
 2 T4. Blood chemistries and CBC were also measured. Baseline thyroid radioactive iodine uptake  
 3 (RAIU) was measured using  $^{123}\text{I}$  at 4, 8, and 24 hours after the ingestion of  $150 \mu\text{Ci } ^{123}\text{I}$ .

4 As reported by the authors, statistical analysis for the thyroid RAIU was carried out by  
 5 analysis of variance (ANOVA) with post hoc pairwise comparisons using Tukey's method. The  
 6 outcome measure variable was log-transformed to achieve greater homoscedasticity and a more  
 7 Gaussian distribution. Serial analyses were done: a three-factor ANOVA with factors as patient,  
 8 treatment, and time and a set of two-factor ANOVAs, one for each of the three times. The  
 9 analogous mixed-model ANOVAs were also run with subject as a random effect to confirm that  
 10 repeated measures among the subjects did not affect the results. Statistical analyses of the  
 11 thyroid function tests and urine and serum perchlorate and iodine values were carried out by  
 12 ANOVA and Student Newman Keuls (SNK).

13 Urine and serum perchlorate levels at baseline and during and after ingestion of the daily  
 14 10 mg perchlorate dose are presented in Table 4-2. Perchlorate levels returned to baseline after  
 15 the two week recovery period. There was also no significant changes in urinary iodine excretion  
 16 during, or 2 weeks after stopping the perchlorate administration as shown in Table 4-3. The  
 17 authors note that the iodide ingestion of the volunteers was not controlled in the diet and were  
 18 variable. It may also be worthwhile to note that the urinary iodine values are relatively high (see  
 19 Chapter 3), indicating a potential protective status in these subjects for the inhibition of the NIS  
 20 by perchlorate.

**TABLE 4-2. URINE AND SERUM PERCHLORATE ( $\text{ClO}_4^-$ ) VALUES BEFORE, DURING, AND AFTER THE INGESTION OF 10 mg OF  $\text{ClO}_4^-$  DAILY FOR 14 DAYS (Lawrence et al., 2000)**

Time	Urine Perchlorate <sup>a</sup> (mg/24 hr)	Serum Perchlorate <sup>a</sup> ( $\mu\text{g/mL}$ )
Baseline	< 0.5	0
7 Days $\text{ClO}_4^-$	$7.7 \pm 0.8^a$	$0.61 \pm 0.02$
14 Days $\text{ClO}_4^-$	$7.5 \pm 1.0$	$0.59 \pm 0.02$
14 Days After $\text{ClO}_4^-$	< 0.5	0

<sup>a</sup>Mean  $\pm$  SE.

**TABLE 4-3. URINE AND SERUM IODINE VALUES BEFORE, DURING, AND AFTER THE INGESTION OF 10 mg OF ClO<sub>4</sub><sup>-</sup> DAILY FOR 14 DAYS (Lawrence et al., 2000)**

Time	Urine Iodine <sup>a</sup> (μg/24 hr)	Serum Iodine <sup>a</sup> (μg/dL)
Baseline	254 ± 69	6.5 ± 0.42 <sup>a</sup>
7 Days ClO <sub>4</sub> <sup>-</sup>	233 ± 49	6.2 ± 0.34
14 Days ClO <sub>4</sub> <sup>-</sup>	385 ± 123	6.4 ± 0.37
14 Days After ClO <sub>4</sub> <sup>-</sup>	208 ± 42	6.3 ± 0.57

<sup>a</sup>Mean ± SE.

1           A highly significant decrease in the <sup>123</sup>I thyroid RAIU with respect to baseline  
2 measurements at all three time points was noted (Table 4-4), 34%, 39%, and 41% at 4, 8, and  
3 24 hours. The decrease averaged over all three time points was 38%. Two weeks after  
4 perchlorate was discontinued, the thyroid RAIU values were significantly higher at all three time  
5 points (average increase of 25%), indicating a rebound that may represent upregulation of the  
6 NIS. The time course of the iodine inhibition could not be calculated since the subjects drank the  
7 dose ad libitum over the day and there was evidence that the full 10 mg/day dose was not  
8 achieved for at least some subjects because the average daily urinary excretion of perchlorate was  
9 7.6 for the 2-week course of perchlorate administrations. There was a corresponding increase in  
10 urinary iodide excretion during dosing followed by a drop below baseline during rebound. T3  
11 levels were observed to rise throughout the 28-day trial (trend not tested).

12           In a subsequent study reported as a letter to the editor by these same investigators,  
13 Lawrence et al. (2001) used nine healthy male volunteers and a dose of 3 mg/day (.04 mg/kg-day  
14 assuming 70 kg body weight) and again observed decreased RAIU. The mean 8-hour decrease  
15 from baseline was reported to be at 10% and at 24-hours to be 10.3%. Neither were significant  
16 based on Tukey paired t-test (data not shown). The RAIU after stopping the perchlorate  
17 ingestion for 14 days rebounded as in the first study and was reported to be an increase of 22% at  
18 8 hours and 18% at 24 hours (p < 0.02). It is worthwhile to note when evaluating these results  
19 that these data (Lawrence et al., 2000; 2001) were evaluated for use in the physiologically-based  
20 pharmacokinetic (PBPK) models described in Chapter 6, but the data were excluded due to the

**TABLE 4-4. THYROID <sup>123</sup>I UPTAKES BEFORE, DURING, AND AFTER THE INGESTION OF 10 mg ClO<sub>4</sub><sup>-</sup> DAILY FOR 14 DAYS (Lawrence et al., 2000)**

Time	Thyroid <sup>123</sup> I Uptake <sup>a</sup> (% Dose)		
	Baseline	14 days on ClO <sub>4</sub> <sup>-</sup>	14 days after ClO <sub>4</sub> <sup>-</sup>
4 Hours	12.5 ± 1.3	8.2 ± 0.7 <sup>b</sup>	16.6 ± 2.4 <sup>c</sup>
8 Hours	17.3 ± 1.9	10.6 ± 1.0 <sup>b</sup>	21.9 ± 2.8 <sup>c</sup>
24 Hours	23.6 ± 2.6	14.0 ± 1.6 <sup>b</sup>	27.1 ± 3.3 <sup>d</sup>

<sup>a</sup>mean ± S.E.

<sup>b</sup>*p* < 0.01 vs. baseline and after ClO<sub>4</sub><sup>-</sup>.

<sup>c</sup>*p* < 0.01 vs. baseline.

<sup>d</sup>*p* < 0.05 vs. baseline.

1 lack of availability of all records to the QA/QC process and unresolved issues regarding sample  
 2 sequences. Variability of serum and urine perchlorate results, potentially due to the unstructured  
 3 drinking water regimen (Merrill, 2001a,b) was noted. Serum levels from the 0.04 mg/kg-day  
 4 dose group ranged from non-detect to 495 mg/L on days when the subjects were supposed to  
 5 have consumed perchlorate. Given this variability and the unknown consequence of a 10%  
 6 change in thyroid RAIU of a small sample of healthy euthyroid individuals to potentially  
 7 hypothyroid or hypothyroxinemic pregnant women, it would be difficult to designate this effect  
 8 as a No-Observed-Adverse-Effect-Level (NOAEL) with any confidence.

9 Greer et al. (2000) described a third study of RAIU in healthy euthyroid subjects in an  
 10 abstract. Perchlorate was dissolved in 400 ml of drinking water at one of three doses to twenty-  
 11 four euthyroid volunteers (4 males and 4 non-pregnant females per dose; 18 to 57 years old).  
 12 The subjects were instructed to drink 100 ml at 4 set times throughout the day for 14 days.  
 13 Measurement of 8- and 24-hour RAIU was performed prior to perchlorate ingestion (baseline),  
 14 on exposures days 2 and 14, and on post-exposure Day 15. Expressed as a percentage of baseline  
 15 (mean ± S.E.), the abstract reports 24-hour RAIU values for the 0.02, 0.1 and 0.5 mg/kg-day dose  
 16 groups as: 83 ± 5.6, 59 ± 3.5 and 31 ± 2.6 on exposure day 2; 85 ± 5.6, 57 ± 4.7, and 34 ± 4.5  
 17 on exposure day 14; and 111 ± 5.1, 96 ± 12, and 108 ± 12 on post-exposure Day 15. These  
 18 correspond to RAIU inhibition values expressed as % of baseline (where “-” indicates inhibition  
 19 relative to baseline) for the 0.02, 0.1 and 0.5 mg/kg-day dose groups of -17, -41, and -69 on

1 exposure Day 2; -15, -43, and -66 on exposure Day 14; and +11, -4, and +8 on post-exposure  
2 Day 15. The authors report no difference between males and females and that a linear log-dose  
3 relationship was observed with the regression slopes indistinguishable between the 8- and  
4 24-hour measurements (data not shown).

5 In other unpublished data provided in Merrill (2001a; Attachment #7) these same  
6 investigators tested seven euthyroid subjects (six non-pregnant females and one male) at a dose  
7 of 0.007 mg/kg-day. Expressed as a percent of baseline, the average 8- and 24-hour RAIU  
8 inhibition values measured on exposure Day 14 were -6.2 and -1.8%. The inhibition values  
9 ranged from -38.6% to +27.9% of baseline at the 8-hour time point and -26.7 to +39% of  
10 baseline at the 24-hour time point. The range for the post-exposure Day 14 RAIU inhibition  
11 values was -19.3 to +45% of baseline. No measurements were made on Day 2 when the RAIU  
12 inhibition would have been greater. There was no RAIU inhibition measured on post-exposure  
13 Day 15. In the Greer et al. (2000) abstract, the authors estimate the no effect level at  
14 0.007 mg/kg-day.

15 In order to evaluate whether the 0.007 mg/kg-day dose had a sufficient sample size to  
16 detect a difference of the observed magnitude as in the other doses tested, the EPA calculated the  
17 power of the usual t-test for the 14-day exposure data. A log transform of the ratio of the  
18 individual values at Day 14 to their baseline values was based on the non-central t distribution.  
19 The power at the 0.007 mg/kg-day dose was low (0.1) compared to the other doses: 0.95, 0.998,  
20 and 0.999 at 0.02, 0.1, and 0.5 mg/kg-day.

21 The EPA has also been made aware of another human clinical study being performed at  
22 Loma Linda and funded by Lockheed Martin (Beck, 2001). The study is not yet completed  
23 because the objective sample size for each dose group has not yet been attained. Human  
24 euthyroid volunteers (male and non-pregnant females) have been dosed with perchlorate in gel  
25 caps at 0.007, 0.014, and 0.04 mg/kg-day. Measurements were made at baseline, 3-months,  
26 6-months, and after recovery from exposure for RAIU, T3, T4, and TSH levels. These dosages  
27 are the same as already tested so the added value to the human database, especially with respect  
28 to the now prominent concern for neurodevelopmental effects secondary to hypothyroxinemia or  
29 even transient decrements in T4, is not readily apparent. The additional data may potentially  
30 reduce the variability and low power due to the small sample sizes of the previous studies if  
31 sufficiently comparable in design.

## 4.2.2 Studies in Patients with Graves' Disease

Potassium perchlorate had been used to treat Graves' disease in humans; consequently, most of the prior data on perchlorate effects on humans are in patients with this disease. Graves' disease is an autoimmune disorder which causes patients to carry immunoglobulins in their blood that bind to TSH receptors on thyroid cells and act like TSH to stimulate DNA synthesis and cell divisions, leading to a hyperthyroid state. Symptoms of the disease include increased synthesis and secretion of iodide-containing hormones into the blood by the thyroid gland, thyroid gland enlargement, increased basal metabolism, and weight loss. Perchlorate inhibits the excessive synthesis and secretion of thyroid hormones by inhibiting the uptake of iodide into the thyroid and causes an efflux (discharge) of accumulated iodide in the gland.

Stanbury and Wyngaarden (1952) evaluated therapeutic perchlorate use in patients ( $n = 8$ , although reporting of exact numbers for various aspects [e.g., different dose levels] of the study is sketchy) with Graves' disease and found that perchlorate caused the discharge of iodide accumulated in the thyroid and blocked the uptake of iodide into the thyroid. Within 30 min of administration, a single dose of 100 mg potassium perchlorate caused the nearly complete release ( $\approx 80\%$ ) of  $^{131}\text{I}$  from the thyroids of Graves' disease patients previously treated with tracer amounts of  $^{131}\text{I}$  and 1-methyl-2-mercaptoimidazole (MMIA). MMIA was given to cause accumulation of  $^{131}\text{I}$  in the thyroid because MMIA prevents the oxidation of iodide ion to iodine and its attachment to tyrosyl groups (see Chapter 3). A single dose of 10 mg perchlorate appeared to cause a  $\sim 50\%$  release of accumulated iodine. The authors reported that perchlorate doses as low as 3 mg caused detectable, but incomplete, release of iodide from the thyroid (although quantitative data for doses less than 10 mg were not presented). In addition, Stanbury and Wyngaarden (1952) reported that the uptake of tracer levels of  $^{131}\text{I}$  into the thyroid glands of two patients with Graves' disease was markedly inhibited for as long as 6 hr when 100 mg of potassium perchlorate was given orally 1 h prior to administration of the tracer. Beyond 6 h, uptake of  $^{131}\text{I}$  recommenced. Inhibition of iodide uptake also occurred in three patients without MMIA treatment. The authors stated that no toxic effects were encountered in any patients who were given, in more than three doses, a total not exceeding 600 mg potassium perchlorate. This

1 study was used to identify a LOAEL of 1.4 mg/kg-day<sup>1</sup> for complete release of iodine from the  
2 thyroid for the RfD reviewed in March 1997 (Toxicology Excellence for Risk Assessment,  
3 1997). Because it was not clear what degree of iodide efflux constitutes an adverse effect, a  
4 NOAEL was not designated for this study. An expert peer review panel later determined this  
5 study was inadequate for RfD derivation (Toxicology Excellence for Risk Assessment, 1998b).

6 Godley and Standbury (1954) report using potassium perchlorate to treat 24 patients with  
7 Graves' disease. Patients were treated with 600 to 1,200 mg/day (typically 200 mg every 8 h)  
8 for at least 11 weeks with a few patients treated as long as 45 to 52 weeks. A decrease in iodide  
9 uptake was observed. Five patients became euthyroid after continuous administration for  
10 28 weeks. Two patients developed gastrointestinal problems that were assumed to result from  
11 perchlorate treatment. In one of these patients, these effects occurred at 600 mg/day, but the dose  
12 that the other patient received is not specified. Other side effects of antithyroid agents, such  
13 hematological changes, liver damage, and skin rash, were not observed. This study suggested a  
14 LOAEL of 9 mg/kg-day in humans for short-term exposures.

15 Crooks and Wayne (1960) observed one case of skin rash and three cases of nausea (12%)  
16 among 35 patients treated with 600 mg/day (9 mg/kg-day) and 165 patients given 1,000 mg/day  
17 (14 mg/kg-day). All patients had diffuse goiters and exophthalmos, classic signs of Graves'  
18 disease. In another group of 10 patients given 1,500 mg/day (21 mg/kg-day) and 40 patients  
19 given 2,000 mg/day (29 mg/kg-day), five cases of skin rash, two cases of nausea, and one case of  
20 agranulocytosis occurred (16%). Leukocyte counts returned to normal in the patient with the  
21 agranulocytosis when perchlorate treatment was stopped. The length of treatment was unclear  
22 but generally appears to have been less than 8 weeks although it appears that one patient was  
23 monitored for 22 weeks. The authors report that the "time to cure" Graves' disease using  
24 perchlorate is approximately 9 weeks. The authors also report that 1 of 12 infants born of  
25 mothers given 600 to 1,000 mg/day was born with a very slightly enlarged thyroid that returned  
26 to normal size in 6 weeks; no other abnormalities were noted. This study suggested a LOAEL  
27 between 9 and 14 mg/kg-day.

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<sup>1</sup>Unless otherwise indicated, for human studies in which the actual body weight of the subjects was not reported, the dose in milligrams per kilogram per day was calculated assuming a body weight of 70 kg. Thus, a dose of 100 mg/day ÷ 70 kg is 1.4 mg/kg-day.

1 Morgans and Trotter (1960) reported that 3% of 180 patients treated with 400 to  
2 1,000 mg/day (6 to 14 mg/kg-day) potassium perchlorate and 18% of 67 patients treated with  
3 1,200 to 2,000 mg/day (17 to 29 mg/kg-day) displayed a variety of adverse reactions that  
4 included skin rash, sore throat, gastrointestinal irritation, and lymphadenopathy. Reactions  
5 occurred within 2 to 3 weeks of drug administration. This study suggested a LOAEL between  
6 6 and 14 mg/kg-day.

7 Connell (1981) reported a case study of a single 72-year-old female Graves' disease patient  
8 who was treated with 200 mg/day (3 mg/kg-day) potassium perchlorate for 22 years without any  
9 indication of adverse side effects. Thyrotoxicosis recurred 4 weeks after stopping potassium  
10 perchlorate administration, suggesting that this dose level provided sufficient clinical control of  
11 the hyperthyroidism. The study also suggested that the adverse reactions seen at higher doses  
12 may not occur at lower doses, even after long-term treatment.

#### 14 **4.2.2.1 Hematological Effects**

15 Between 1961 and 1966, the occurrence of severe hematological side effects in patients  
16 receiving long-term potassium perchlorate treatment for Graves' disease led to a decreased use of  
17 potassium perchlorate as a therapeutic agent. Several authors (Hobson, 1961; Johnson and  
18 Moore, 1961; Fawcett and Clarke, 1961; Krevans et al., 1962; Gjerdal, 1963) report case studies  
19 in which a single patient suffered fatal aplastic anemia after treatment doses ranging from 6 to  
20 14 mg/kg-day. The duration of treatment ranged from 3 mo (Johnson and Moore, 1961) to 8 mo  
21 (Hobson, 1961). In all cases, patients were started at the high end of the treatment range for a  
22 period of time and then were reduced to the lower end of the treatment range after the appearance  
23 of side effects. In two cases (Hobson, 1961; Gjerdal, 1963), patients had co-exposures to other  
24 drugs. Other case reports are available that report nonfatal agranulocytosis in patients treated  
25 with 14mg/kg-day for 12 days (Southwell and Randall, 1960) or 3 mo (Sunar, 1963). Barzilai  
26 and Sheinfeld (1966) report that 11% of 76 patients developed leukopenia or other unspecified  
27 side effects after treatment with 1,000 mg/day (14 mg/kg-day) for a little as 2 mo. Within this  
28 group, there was one case of fatal aplastic anemia and one case of fatal agranulocytosis.

29 These studies suggest that doses in the range of 6 to 14 mg/kg-day may represent a frank  
30 effect level in patients with Graves' disease although there were questions as to whether these  
31 effects were caused by the disease itself, whether there was some contamination, or whether the

1 effects occurred only at high doses. A review by Wenzel and Lente (1984) concluded that the  
2 “severe adverse reactions, such as agranulocytosis, were likely to occur only when large doses of  
3 more than 1,000 mg potassium perchlorate were administered.” There is no information to  
4 suggest that humans without Graves’ disease would have a similar reaction to perchlorate.

5 Antithyroid drugs appear to exert their effects on the hematopoietic system through an  
6 immune mechanism. Wing and Fantus (1987) reviewed the adverse effects of two antithyroid  
7 drugs, propylthiouracil and methimazole, and concluded that most reactions were related to the  
8 immunologic effects of these drugs. They noted that skin rash and granulocytopenia were among  
9 the most commonly reported adverse effects of these drugs. Less commonly reported effects  
10 include aplastic anemia, leukopenia, and antibodies to insulin and glucagon. In fact, Wing and  
11 Fantus (1987) recommend that patients be instructed to report skin rash immediately, as this may  
12 be an early sign of adverse immune reaction caused by the antithyroid drugs. Although these  
13 authors did not include perchlorate in their investigation, the similarity of the effects seen after  
14 perchlorate treatment—including rash, leukopenia, agranulocytosis, and aplastic anemia—  
15 suggest that perchlorate also may act in a similar fashion to induce an immune effect.

16 There is a tight functional connectivity between the immune and endocrine systems which  
17 is mediated, at least in part, by shared receptors and mediators among the systems (Kammuller,  
18 1995). Thus, although the mechanism of perchlorate action on the hematopoietic system is not  
19 known, it is likely to be an immune reaction. Although it is possible that perchlorate may cause  
20 hematological effects in healthy humans, it appears that Graves’ disease patients are likely to be  
21 more sensitive to this type of immune-induced adverse effect than are healthy people. The  
22 increased sensitivity to immunologic function in Graves’ disease patients arises because of the  
23 underlying abnormal immunologic function in Graves’ disease. Immunoreactivity to antithyroid  
24 drugs is another expression of the compromised immune system in these patients (Wall et al.,  
25 1984; Wing and Fantus, 1987).

### 26 27 28 **4.3 SUMMARY OF CONCLUSIONS REGARDING HUMAN HEALTH** 29 **EFFECTS STUDIES**

30 The recent human studies support the established effect of perchlorate at the NIS. Using  
31 these data as the basis for quantitative dose-response assessment is more difficult. Of the five

1 population studies investigating the effects of perchlorate exposures on TSH levels in newborns  
2 (Lamm et al., 1999; Li et al. 2000b; Crump et al., 2000; Brechner et al., 2000; Schwartz, 2001),  
3 the Brechner et al. (2000) study had a somewhat better exposure classification owing to a more  
4 narrow, but still ecological, geographical focus (two small cities) and Schwartz had a relatively  
5 detailed exposure classification down to the level of zip codes. Only these two studies had  
6 positive findings in newborns. The restriction of birth weight in Li et al. (2000b) could have  
7 reduced study sensitivity if thyroid endpoints in non-normal birth weights are especially effected  
8 by perchlorate. The strong dependence of thyroid endpoints on birth weight observed in several  
9 studies raises the possibility that birth weight itself could be an intervening variable in  
10 perchlorate effects. That is, perchlorate exposure may affect birth weight. This would be a  
11 testable hypothesis in several of the studies. If birth weight were an intervening variable, birth  
12 weight restriction in the Li et al. (2000a,b) studies or controlling for birth weight as a confounder  
13 in the Li et al. (2000a,b), Brechner et al. (2000) and Schwartz (20001) studies may have resulted  
14 in an underestimation of perchlorate exposure effects.

15 In the one study that reported age-specific perchlorate exposure effects on TSH (Brechner  
16 et al., 2000), the largest effect was in the first 24 hours after birth. This observed exposure-age  
17 interaction was not statistically evaluated. The study with the strongest findings (Schwartz,  
18 2001) actually focused only on the first 2 days after birth. Therefore, excluding day-one screened  
19 births as in the Li et al. (2000b) study may severely reduce or eliminate the ability to detect a  
20 perchlorate effect.

21 The well-known TSH surge at birth is thought to represent a response to temperature  
22 change (Schwartz, 2001). This suggests that ambient temperatures – prenatal and perinatal –  
23 might be important determinants of thyroid endpoints. The strong period/seasonal effect  
24 observed in the Li et al. (2000b) study supports this temperature conjecture and the unexpected  
25 trends across Chilean cities in the Crump et al. (2000) and variations across U.S. counties in the  
26 Lamm et al. (1999) and Schwartz (2001) investigations could also be related to temperature.

27 It should also be noted that all of the studies in this review examined endpoints that may be  
28 insensitive to the consequences of altered thyroid function. No detailed models of thyroid  
29 dynamic response were postulated with subsequent analysis of relevant endpoints that would  
30 reliably detect the specific perchlorate- or environmentally-induced defects. Nonetheless, one  
31 study examining neonatal thyroid status in the first five days found a perchlorate effect that was

1 greatest in the first 24 hours and that rapidly declined over the next two days, suggesting  
2 alteration of thyroid response to the birth event. The issue of iodine depletion in exposed  
3 populations was not directly evaluated although experimental evidence of short-term depletion in  
4 adults at high doses was observed.

5 All of the observational field studies utilized “ecological” exposure rather than individual-  
6 specific dose measurements; the relative specificity of the dose metric varied widely from  
7 “exposed/not exposed”, to an average concentration in drinking water for a given zip code. The  
8 occupational studies used air sampling to estimate homogeneous exposure groups. Nevertheless,  
9 there was evidence of perchlorate effects on neonatal thyroid status, with the studies by Brechner  
10 et al. (2000) and Schwartz (2001) contributing the most compelling observations, and iodine  
11 depletion was observed experimentally. The presence of exposure misclassification and  
12 potentially serious confounding in many of the studies makes interpretation difficult and allows  
13 for the possibility of missed effects even at the level of current thyroid function (e.g., steady state  
14 levels of TSH or T4). The full implications of these findings are unclear; however, they should  
15 be taken seriously, especially in populations already at risk for thyroid deficiency. These  
16 considerations are summarized in Table 4-5.

17 The present review differs from a recent summary co-authored by two major participants in  
18 industry-funded perchlorate research (Soldin et al., 2001). That review argues that there is now  
19 sufficient evidence to recommend safe levels for regulatory purposes. The authors see no  
20 immediate need for refinement of the physiological issues underlying the existing epidemiologic  
21 study designs or for new initiatives in evaluating such issues in human populations. Potentially  
22 important aspects of the mode-of-action for perchlorate not well addressed in the available  
23 human studies include: (1) short-term effects of variable exposure during pregnancy, for  
24 example, on critical neurodevelopmental effects; (2) the effects of iodine depletion on the T4 or  
25 TSH surge response at birth, i.e., whether the effect of perchlorate on fetal thyroid status depends  
26 additionally on prior cumulative exposure; (3) the equilibration of this regulated system under  
27 chronic exposure and the masking of potential deficiency states such that steady-state T4 or TSH  
28 levels appear normal despite substantial impact on production and function; and (4) the special  
29 situation of populations or individuals with inadequate iodine intake where thyroid  
30 responsiveness may be compromised.

1           The recent clinical data (Lawrence et al., 2000; 2001; Greer et al., 2000) may be more  
2 useful in helping to characterize the potential effects on thyroid function if the mode of action  
3 framework is superimposed on the interpretation of the data (i.e., that prevention of significant  
4 iodide inhibition would preclude adverse neurodevelopmental and neoplastic sequelae).  
5 However, given the current controversy in evaluating thyroid status, particularly in pregnant  
6 women, it is difficult to ascertain the degree of iodide inhibition to designate as adverse. Further,  
7 there is considerable uncertainty associated with using small sample sizes of euthyroid  
8 individuals as the basis of such a determination, so that the use of a factor to account for this in a  
9 risk derivation would be warranted, particularly when the variability as noted is considered and  
10 the range of inhibition of iodide uptake at levels suggested to be “No-Observed-Adverse-Effect-  
11 Levels” include values as great as 38.6% below baseline. A discussion considering these human  
12 clinical data in comparison to the laboratory animal toxicological study results can be found in  
13 Section 7.1.5.1.

TABLE 4-5. SUMMARY OF HUMAN POPULATION STUDIES (Park, 2001)

Publication	Study Population	ClO <sub>4</sub> Source and Levels	Duration	Outcomes studied	Findings	Problems/Comment
1 Gibbs JP, Ahmad R, Crump KS, et al  JOEM 1998; 40:1072-1082. <i>Evaluation of a population with occupational exposure to airborne ammonium perchlorate for possible acute or chronic effects on thyroid function.</i>	Kerr-McGee workers in voluntary medical surveillance 1994-98; 170 out of 254 did survey; 130 did single shift evaluation	Airborne exposure to AP in 8 homogenous exposure groups: 0.04-627 $\mu\text{m}/\text{m}^3$ using closed face cassettes	1 day 1-27 yr.	T3U, T4, FTI, TSH, liver, kidney and hematol fcn  T4: 7.5 $\mu\text{g}/\text{dL}$ TSH: 2.0 $\mu\text{IU}/\text{ml}$	Indication of increase in TSH over work shift: 2.2 -> 2.5. In workforce, T4 declines and TSH increases from low to high exposure but also from low exposure to unexposed; see inconsistent TSH trends using two lab groups; for both thy and liv outc, SDs increased in high dose group: for thy and liv fcn, averages for low vs high AP very different but %iles up to 75th are not. Implies big excursion at high exposure end.	Possibly half of eligibles did not participate in shift study; possibly confounded by shift duration. Did not evaluate ITR.  Suggestion of inappropriate unexposed comparison group. In this steady state and cross-sectional population, difficult to assess thyroid regulatory status. SDs suggest heterogeneity of effect. <b>Indications of chronic effects.</b>
2 Lamm SH, Braverman LE, Li FX, et al. JOEM 1999; 41:248-260. <i>Thyroid health status of ammonium perchlorate workers: a Cross-sectional occupational health study.</i>	American Pacific workers: 37 AP and 21 azide workers: full feasible participation; all from same site with same other work attributes	Airborne exposure in 3 AP groups based on visible dust level; total and respirable AP by individual closed-face samplers 10-11 hrs on subset from each exp group; levels: total dust (mg/day): .01, .34, 6.57, 59.4; resp fraction (mg/day): .02, .09, .60, 8.6	1 day n=58; 6 days n=2	Urine AP, T3, T4, FTI, TSH, THBR, and hematologic fcn  T4: 7.0 pg/dL TSH: 2.6 JLrU/InI	18% of total airborne Mb is respirable (range 8-25); urinary excretion of P shows much higher absorbed dose in unexposed workers than expected from air samples: (mg): .88, 4.0, 10.9, 33.6 (assuming 8 hr halflife). Thy, hematol by current exp group: no association (T3, T4); absorb dose greatly exceeds resp total inhaled dose [F51. See aberrant clearance in 1 of 2 6-day subjects FF2]. Authors conclude no AP health effects.	Some misclassification apparent among exposure groups based on absorbed dose; non-inhalable contribution may constitute important deficit in air sampling results. Steady-state, cross-sectional population difficult to interpret. Thy, hematol results based on current, non-cumulative AP exposure are uninterpretable for chronic effects. Possible increasing trend for max(T3) with exposure group.
3 Lawrence JE, Lamm SH, Braverman LE.  J Endocrinol Invest 1999; 22:405-407. <i>The use of perchlorate for the prevention of thyrotoxicosis in patients given iodine rich contrast agents.</i>	Radiocontrast patient series	Therapeutic high oral doses (1000 mg) in day prior to contrast agent	1 day	Misc. thyroid parameters  —	Recommend in high risk patients (low iodide areas and elderly) a combination of perchlorate and contrast agent.	Not relevant to and uninformative on chronic exposure effects in adults and acute effects in infants.

TABLE 4-5 (cont'd). SUMMARY OF HUMAN POPULATION STUDIES (Park, 2001)

Publication	Study Population	ClO <sub>4</sub> <sup>-</sup> Source and Levels	Duration	Outcomes studied	Findings	Problems/Comment
4 Li FX, Squartsoff L, Lamm SH. JOEM 2001; 43:630-634. <i>Prevalence of thyroid diseases in Nevada counties with respect to perchlorate in drinking water.</i>	Medicaid population at risk for thyroid disease in Nevada in 1997-98.	Perchlorate in drinking water in one county (P= 8.9-11.6 µg/L) versus all others	Lifetime	ICD 240-246; ICD 193: thy cancer	Exposed county (Clark) with Las Vegas compared to another county with a city (Reno/Washoe) as well as with all other counties. No significant excesses found for exposed county for the 8 outcomes studied. Actually, the comparison counties (one with a city, and all others) for all important outcomes differed more between them than with the exposed county. For the 6 more prevalent outcomes (n=3069) the exposed county had higher rates than the unexposed (Washoe) county.	Based on period-prevalence rates. Two outcomes with small numbers are not informative: congenital hypothyroidism (n=22) and thyroid cancer (n=44). <b>The difference in the comparison counties suggests that uncontrolled confounders or uncertain estimates are affecting this analysis and that the study is uninterpretable for all but large effects.</b> Confounders might include age, gender, body mass, diet, iodine intake, ethnicity, occupational exposures.
5 Crump C, Michaud P, Tellez R et al. and Crump KS, Gibbs JP. JOEM 2000; 42:603-612. <i>Does perchlorate in drinking water affect thyroid function in newborns or school-age children?</i>	School children from 1 or 2 schools in three cities in Chile (n=53,49,60 in 0, low and high P cities); all newborns 2/96-1/99 in same cities (n=8888,468,428)	Geological Na-P in drinking water (0, 5.5, 111.6 µg/L)	Recent and lifetime for 6-8 yr-olds; gestation	T3, T4, free T4, FTI, TSH, hematomol, liver, kidney, prev:goiter, prev:family H <sub>x</sub> thy disease  T4:10.0 µg/dL TSH: 3.0 µIU/mL	Did comparisons across cities. Urinary I/creatinine low in city-2 lifetime residents: (1,092, 862, 963); goiter high in city-2 recent residents: (17.7, 26.5, 23.3%) and high in city-3 lifetime residents: (22.2, 19.5, 26.0 based on 8, 8, 13 cases); family H <sub>x</sub> of the disease high in city-3: OR=4.9 (11.1, 9.8, 30.0); highly significant increase in T4 with increased P (1.25, 1.34, 1.50). Highly significant decrease in log (TSH+1) in newborns in city-3-high P (.91, .91, .66) [T9], which is in the unexpected direction. There was a diverse age-at-screen distribution across cities.	Dietary, ethnic, birthwt, SES confounders of thy fcn uncontrolled; observe trends in unexpected directions; suggesting confounding. Unknown if some Chileans boil drinking water. <b>Significant paradoxical effects indicate uncontrolled confounding and inappropriate thy fcn model in relation to P in this population.</b> Possible role of ambient temperatures.
6 Lawrence JE, Lamm SH, Pino S, Richman K, Braverman LE. Thyroid 2000; 10:659-663. <i>The effect of short-term low-dose perchlorate on various aspects of thyroid function.</i>	9 healthy, male volunteers K-perchlorate – 10mg/day	Potassium perchlorate 10 mg/day	14 days	T3, T4, FTI, TSH, THBR, RAIU, liver, hematology  T4: 7.0 µg/dL TSH: 1.0 µIU/mL	Assumed identical P doses. Upward trend for T3 at BL, 7-, 14-, and 28-days (136, 140, 151, 157; trend not tested). See depressed I-uptake at 14 days (40%) with rebound at 28 days; non-24 hour urinary- and serum-I was unchanged throughout. Authors conclude: no thyroid impact because of large I-storage.	Hematomol, liver test results clinically “normal” but no data presented. <b>Inappropriate assessment: clinical rather than epidemiological.</b> T3 effect not addressed; dietary I not controlled or reported. <b>Suggests long term iodine depletion.</b>

TABLE 4-5 (cont'd). SUMMARY OF HUMAN POPULATION STUDIES (Park, 2001)

Publication	Study Population	ClO <sub>4</sub> <sup>-</sup> Source and Levels	Duration	Outcomes studied	Findings	Problems/Comment
7 Lawrence JE, Lamm S, Braverman LE. Thyroid 2001. 11:295 (letter) <i>Low dose perchlorate (3 mg daily) and thyroid function.</i>	8 healthy volunteers	Potassium perchlorate 3 mg/day	14 days	T3, T4, FTI, TSH, THBR, RAIU, liver, hematoI	No signif changes (data not presented) except for depressed I-uptake at 14 days (10%) with significant rebound (22%) at 28 days;	<b>Implies some I depletion over 2 weeks at 3 mg/day</b> (seen by other investigators at 1.4 mg/day).
8 Lamm SH, Doemland M. JOEM 1999; 41:409-411. <i>Has perchlorate in drinking water increased the rate of congenital hypothyroidism?</i>	Newborns in CA and NV in 1996-97 in 7 counties	Perchlorate in drinking water: 4-16 µg/L	Gestation	Congenital hypothyroid-ism based on neonatal screen  (expected= 35/10 <sup>5</sup> )  —	Compared counties. Hispanic-adjusted prevalence ratios by county: 0.6 (n=8) to 1.1 (n=136); none statistically significant.	No county-specific levels of P; no individual consumption. Should have used other CA and NV counties for expected rates. <b>Identification of cases is limited by screening procedure that does not consider age at screen, ethnicity and birthweight.</b> Unable to address transient developmental sequelae.
9 Li Z, Li FX, Byrd D, et al. and Lamm. JOEM 2000; 42:200-205. <i>Neonatal thyroxine level and perchlorate in drinking water.</i>	Newborns in Reno (n=5,882)and Las Vegas (n=17,308) NV 4/98 – 6/99 with birthwt 2.5-4.5kg and age at screen < 5 days and non ICU	Perchlorate in drinking water of Las Vegas: 0 up to 15 µg/L, measured monthly	Gestation	T4  T4:17.0 µg/dL	Compared cities. Significant period effect (seasonal) (ΔT4=.60) when adj for birthweight (.85/kg), age at screen (day 1,2,3 vs. 4: -1.275, .408, .758) and gender (.727). No city * period interaction implies no P effect. Age * exposure interaction not investigated. Did regressions on monthly means (T4, cum.P); also, used 10 percentile T4 as an outcome—no effect. See jump in T4 at newborn return visits in days 2-4.	<b>These T4 levels are much higher than in other neonate studies (7-10).</b> Birthweight may be intervening variable: P causing reduced birthwt via impaired thy fcn. Loss of power in regressions using monthly means instead of individual obs. Early return visits have selection bias: reason for early return.
10 Li FX, Byrd DM, Deyhle GM et al. and Lamm. Teratology 2000; 62:429-431. <i>Neonatal thyroid-stimulating hormone level and perchlorate in drinking water.</i>	Newborns in Reno and Las Vegas NV 12/98 – 10/99 with birthwt 2.5-4.5 kg	Perchlorate in drinking water of Las Vegas: 0 up to 15 µg/L, measured monthly	Gestation	TSH  TSH: 10.0 µIU/mL	Compared cities. TSH levels, adjusted for gender and age at screen (2-7 vs. 8-30): no difference for LV vs. Reno.	TSH log transformation for variance stabilization could suppress TSH differences in the high range; inadequate control for age at screen (LV vs Reno), ethnicity and birthwt (2.5-4.5 kg); birthwt may be intervening variable. TSH levels may not be relevant vs T4. <b>Insensitive to developmental issues and short-term time variability of P exposure.</b>

TABLE 4-5 (cont'd). SUMMARY OF HUMAN POPULATION STUDIES (Park, 2001)

Publication	Study Population	ClO <sub>4</sub> Source and Levels	Duration	Outcomes studied	Findings	Problems/Comment
11 Brechner RJ, Parkhurst GD, Humble WO et al. JOEM 2000; 42:777-782. <i>Ammonium perchlorate contamination of Colorado River drinking water is associated with abnormal thyroid function in newborns in Arizona.</i>	Newborns 10/94-12/97 in two Arizona cities whose T4 screen was below state-wide daily 10%ile	Perchlorate in drinking water <16 µg/L	Gestation	TSH  TSH: 13.4 µIU/mL	Compared cities. TSH higher in newborns from exposed city (median: 19.9 vs 13.4); age at screen distribution very different between two cities: exposed screened sooner. Stratifying on age at screen (0, 1-4, 5+ days) and Hispanicity, see signif increase (p=.017); adj effect not reported.	TSH levels (13-20) higher than reported for other newborns (7-10).] Selection on T4 level is problematic due to strong age dependence of T4 surge at birth thus causing variable percentile discrimination with age (8-40% were screened depending on age). This effect could increase TSH of the exposed city relative to unexposed city but the effect of the bias is difficult to predict. Uncontrolled other confounding e.g., birthwt, gest. age, iodine intake, SES.
12 Schwartz J. Dissertation, UC Berkeley, 2001. <i>Gestational exposure to perchlorate is associated with measures of decreased thyroid function in a population of California neonates.</i>	99% of California newborns screened for thy disease in 1996	Perchlorate in drinking water classified in 3 levels and assigned by zip code: 1-2,3-12, 13+ µg/L	Gestation	T4, TSH, presumptive positive; congenital hypothyroidm  T4: 160 mg/dL TSH: 7.6 µIU/mL	Compared across four levels of estimated exposure. Has detailed covariates: birthweight, age at screen in hours, ethnicity in 20 groups; birth multiplicity; ANCOVA model with extensive control of most confounders finds highly significant decrease in T4 (mean=166) with P level (0, -9.7, -11.2, -18.2) and large effects for birthweight (-72 for birthweight 1500-2500), age (-50 for hours 7-18) and ethnic groups (-10 to -30); see initial T4 fall followed by surge by 12 hours and stays elevated until 36 hours; initial onset of TSH surge unresolvable in time; stays elevated till 18 hours. Significant P effect on TSH (0, .029, .03, .128) but birthweight effects models (-.09 for <1.5 kg). Model for presumptive positives shows strong age at screen and ethnicity effects; for congenital hypothyroidism, insignificant effect.	[T4 is reported at levels 10,000-fold higher than in other studies.] presumptive positive criterion not clear (all at or below 9 mg/dl plus lowest 5% immediately above 9 mg/dl?). NO P-ITR reported, e.g., P * age (especially on surge amplitude), P * birthweight; possible selection bias in identification of TSH subjects. Age at screen was not included in logistic regression model of congenital hypothyroidemia. <b>This study presents strong evidence of perchlorate health effects in neonates from drinking water contamination with perchlorate.</b>

**TABLE 4-5 (cont'd). SUMMARY OF HUMAN POPULATION STUDIES (Park, 2001)**

Publication	Study Population	ClO <sub>4</sub> <sup>-</sup> Source and Levels	Duration	Outcomes studied	Findings	Problems/Comment
13 Soldin OP, Braverman LE, Lamm SH. Therapeutic Drug Monitoring 2001; 23:316-331. <i>Perchlorate clinical pharmacology and human health: a review.</i>	Review of animal and human evidence				This review, co-authored by two major participants in industry funded perchlorate research, argues that there is now sufficient evidence to recommend safe levels for regulatory purposes, i.e., at this time there is no need for further refinement of the physiological issues underlying the existing epidemiologic study designs or for new initiatives in evaluating such issues in human populations.	Not considered in this review are issues such as (1) short term effects of variable exposure during pregnancy, (2) the effects of maternal iodine depletion on T4 or TSH surge response at birth, (3) the equilibration of this system under chronic exposure and the masking of potential deficiency states, and (4) the special situation of populations with inadequate iodine intake.

I = iodine; P = perchlorate; AP = ammonium perchlorate; exp = exposure; thy = thyroid; liv = liver; hematol = hematologic; ITR = interaction; outc = outcomes; SD = standard deviation; H<sub>x</sub> = history; [Tn] = table in paper; [Fn] = figure in paper.

## 5. TOXICOLOGICAL EFFECTS IN LABORATORY ANIMAL STUDIES

This chapter provides a review of the relevant laboratory animal toxicity data for quantitative dose-response analysis of the toxic effects of perchlorate exposure. Evidence that both the neoplastic and non-neoplastic effects of perchlorate derive from its anti-thyroid effects at the sodium (Na<sup>+</sup>)-iodine (I) symporter (NIS) should be appreciated. Studies completed before the initiation of the perchlorate testing strategy described in Chapter 3 are included here, but the major emphasis is on these newer studies given their contemporary design and integrated approach to evaluating perchlorate's mode of action. This introduction provides a brief review of the status of issues after the previous external peer review and a summary of studies recommended and performed since that time. In response to the 1999 external peer review, the EPA committed to a second external peer review to address these recommendations and to evaluate the data from new analyses and studies (Noonan, 1999).

At the external peer review in February 1999, it was noted by the EPA that the thyroid histopathology that had made a significant contribution to the risk assessment had never undergone an independent peer review by a second pathologist in any of the studies. In addition, these studies had been performed at several different laboratories with several different study pathologists using different lesion grading systems. The external peer review panel agreed that these inconsistencies between study reports made it difficult to compare studies and could contribute to variability in the resultant dose-response estimate (Research Triangle Institute, 1999).

In response, the National Center for Environmental Assessment (NCEA) committed to a Pathology Working Group (PWG) process in collaboration with the NIEHS. The purpose of the independent peer review and PWG was to decrease variability in response across the studies by providing a common nomenclature for lesions and a consistent pathology review. Determination of No-Observed-Adverse-Effect-Levels (NOAELs) or designation of adversity was not the objective of this review. NCEA asked Dr. Douglas C. Wolf in the EPA's National Health and Environmental Effects Research Laboratory (NHEERL) to conduct the requisite independent

1 peer review (second pathology review) using one consistent lesion grading system on the  
2 materials. Dr. Wolf was chosen because he had not been involved in any of the work performed  
3 with ammonium perchlorate and because he had developed a thyroid grading scheme (Hooth  
4 et al., 2001) to analyze a similar thyroid response in rodents exposed to sodium chlorate that  
5 would be useful to the perchlorate review.

6 After the initial pathology review of 100% of the thyroid slides by Dr. Wolf, Dr. Peter  
7 Mann of Experimental Pathology Laboratories, Inc. (EPL), reviewed 100% of the slides for  
8 quality assurance/quality control (QA/QC) and consistency. Subsequent to this QA/QC review  
9 of the independent peer review, a NIEHS-sponsored PWG of 5 experienced veterinary  
10 pathologists was conducted on a subset of the slides. Recommendations of that PWG  
11 (Experimental Pathology Laboratories, 2000) were then incorporated into the final report on the  
12 independent review of 100% of the slides conducted and reported by Dr. Wolf (Wolf, 2000).  
13 Both of these reports were made available almost immediately to the public on the NCEA  
14 website. During subsequent analyses it was appreciated that the slides provided for the  
15 two-generation study (Argus Research Laboratories, Inc. 1999) were from animals not on test  
16 and some of the mean severity scores were miscalculated. These minor changes are provided in  
17 Wolf (2001).

18 The thyroid slides that underwent the PWG review included materials from the following  
19 studies: Argus Research Laboratories, Inc. (1998a,b,c); Caldwell, et al. (1995); Keil et al.  
20 (1998); and Springborn Laboratories, Inc. (1998). It should be noted that the two-generation  
21 reproduction study performed by Argus Research Laboratories (1999) was completed at the time  
22 of the PWG, and the review included all final thyroid tissue slides despite its listing in the PWG  
23 and Wolf (2000; 2001) reports as 1998c. The newest study, that of Argus Research Laboratories,  
24 Inc. (2001) described below in Section 5.3.3, was also performed with the new nomenclature and  
25 grading system. The study pathologist had been a member of the PWG; therefore, the pathology  
26 results can be considered consistent with the results of Wolf (2000, 2001). However, a second  
27 independent review of the pathology in that study has not been performed.

28 All analyses performed on thyroid histopathology in this revised risk assessment rely on  
29 either the PWG data (Wolf, 2000; 2001) or the new 2001 study (Argus Research Laboratories,  
30 Inc., 2001). The revised benchmark dose (BMD) analyses for thyroid colloid depletion,  
31 hypertrophy, and hyperplasia diagnosed in the studies reviewed by the PWG are presented in

1 Table 5-1 (Geller, 2001a). Figures 5-1 and 5-2 present these estimates and their distributions  
2 graphically in comparison to the previous 1998 assessment values. It is worthwhile to note that  
3 while hyperplasia occurs at slightly higher concentrations in the analysis of the overall data array,  
4 there is considerable overlap with the distributions of the other two thyroid histopathology  
5 indices (colloid depletion and hypertrophy). This overlap is especially evident when evaluating  
6 BMD or benchmark dose lower confidence level (BMDL) values within individual studies.

7 The potential for variability due to inconsistent handling of the radioimmunoassay (RIA)  
8 kits used for serum thyroid and pituitary hormone levels was also noted at the external peer  
9 review (Research Triangle Institute, 1999). In response, the Air Force Research Laboratory  
10 (AFRL) conducted a study to compare serum thyroid hormone and TSH data obtained by RAI  
11 procedures for three different research laboratories that participated in perchlorate toxicity  
12 studies involving hormone analysis (Narayanan, 2000). The purpose was to statistically  
13 investigate the reproducibility (i.e., variability across laboratories) and the repeatability (i.e.,  
14 variability within a laboratory) of the hormone measurements expressed as counts per minute  
15 (CPM). RIA kits from the same batch number and with the same expiration date were used for  
16 all the hormone measurements for all the standard and unknown samples. For unknown samples,  
17 six rat serum samples plus six samples obtained from different species (dog, guinea pig, rabbit  
18 and mouse) were used. Assays were performed using the RIA kits according to the  
19 manufacturers' recommended procedures and each laboratories' standard operating procedures.

20 Reproducibility limits (RL) for each sample and for each hormone were determined. The  
21 RL was defined as approximately 95% of all pairs of means from the same hormone and same  
22 sample; different laboratories should differ in absolute value by less than the RL. The difference  
23 in means between any two laboratories is a normally distributed random variable with a mean of  
24 zero. The range  $\pm$  RL is then the middle 95% for this distribution (i.e., 2.5% in each tail). The  
25 reproducibility varied for each hormone with T3 showing the best reproducibility and TSH the  
26 least. Three replicates ensured a more reproducible sample even when repeatability was not as  
27 consistent. The results suggest that the variability in the RIA determination should be considered  
28 when determining effect levels.

29 It was also recommended at the external peer review, by the biostatistician Dr. Joseph  
30 Haseman, that different approaches to the thyroid and pituitary hormone analyses be explored  
31 (Research Triangle Institute, 1999). EPA complied with this request and developed two new

**TABLE 5-1. BENCHMARK DOSE (BMD)<sup>a</sup> AND BENCHMARK DOSE LOWER CONFIDENCE LIMIT (BMDL)<sup>a</sup> ESTIMATES CALCULATED FROM THE WOLF (2000, 2001) THYROID HISTOPATHOLOGY DATA (Geller, 2001a)**

Study Name, Time Point Wolf (2000; 2001) Table Number	Ammonium perchlorate dose levels test (mg/kg-day)	Colloid Depletion				Hypertrophy				Hyperplasia			
		BMD	BMDL	$\chi^2$ <sup>b</sup>	Exp <sup>c</sup>	BMD	BMDL	$\chi^2$ <sup>b</sup>	Exp <sup>c</sup>	BMD	BMDL	$\chi^2$ <sup>b</sup>	Exp <sup>c</sup>
1. Caldwell Tbbs. 1 and 2	0, 1.25, 5, 12.5, 25, 50, 125, 250	13.29	0.72	0.97	4.37	Not done <sup>d</sup>				35.29	0.78	0.20	0.88
2. Subchronic, 14-day Tbbs. 3 and 6	0, 0.01, 0.05, 0.2, 1.0, 10.0	2.55	0.28	0.20	0.74	0.75	0.017	0.54	0.78	NOE <sup>e</sup>			
3. Subchronic, 90-day Tbbs. 4 and 7	0, 0.01, 0.05, 0.2, 1.0, 10.0	0.13	0.03	0.70	0.50	0.21	0.008	0.74	0.55	8.36	2.09	1.00	7.87
4. Subchronic, 120-day Tbbs. 5 and 8	0, 0.05, 1.0, 10.0	NOE				NOE				NOE			
5. Neurobehav., F0 Fem Tbl. 9	0, 0.1, 1, 3, 10	NOE				NOE				NOE			
6. Neurobehav., PND5 Tbbs. 10 and 11	0, 0.1, 1, 3, 10	0.45 0.53	0.009 0.33	0.46 0.67 <sup>f</sup>	0.94 1.0	0.92 1.27	0.24 0.88	.024 0.26 <sup>f</sup>	0.81 1.0	15.18 11.02	1.86 3.62	0.70 0.32 <sup>f</sup>	0.36 1.0
7. Neurobehav., adult Tbbs. 12 and 13	0, 0.1, 1, 3, 10	0.72	0.029	0.23	0.89	3.48	NC	0.72	0.29	NOE			
8. 2-gen., P1 Tbbs. 14 and 15	0, 0.3, 3, 30	1.97	0.11	0.68	3.84	Poor fit <sup>g</sup>				7.89	2.44	0.41	0.72
9. 2-gen., P2 Tbbs. 16 and 17	0, 0.3, 3, 30	2.16	0.90	0.06	1.16	0.99	0.15	0.67	0.70	4.62	0.0004	0.14	0.31
10. 2-gen., F1-weanling Tbbs. 18 and 19	0, 0.3, 3, 30	2.51	0.80	0.17	1.2	0.21	0.057	0.40	0.79	2.74	0.66	0.85	0.52
11. 2-gen., F2-weanling Tbbs. 20 and 21	0, 0.3, 3, 30	Poor fit				1.19	0.32	0.25	0.52	NOE			
<b>BMDL Range: Rat Studies</b>		<b>0.009 - 0.90</b>				<b>0.008 - 0.74</b>				<b>0.0004 - 3.62</b>			
12. Dev tox., rabbit dams Tbl. 22	0, 0.1, 1, 10, 30, 100	0.12	0.008	0.19	0.36	Poor fit				1.53	0.42	0.13	0.61
13. Immunotox. Mice, combined studies Tbl. 23	0, 0.1, 1, 3, 30	26.07	5.15	1.00	7.88	1.62	0.97	0.58	0.84	24.92	4.48	1.00	7.86

<sup>a</sup> Units of mg/kg-day.

<sup>b</sup>  $\chi^2$  p-value.

<sup>c</sup> Exponent in Weibull model fit not restrained to  $\geq 1.0$  unless indicated.

<sup>d</sup> Not done: Because of non-routine staining, cytological characteristics were not adequate to make determination of hypertrophy on these samples (Wolf, personal communication).

<sup>e</sup> No observed effect (NOE): Either no incidence of endpoint noted in animals tested or no notable difference between dosed and controls.

<sup>f</sup> Exponent in Weibull model fit restrained to  $\geq 1$ .

<sup>g</sup> Poor fit:  $p < 0.05$  for  $\chi^2$  test.

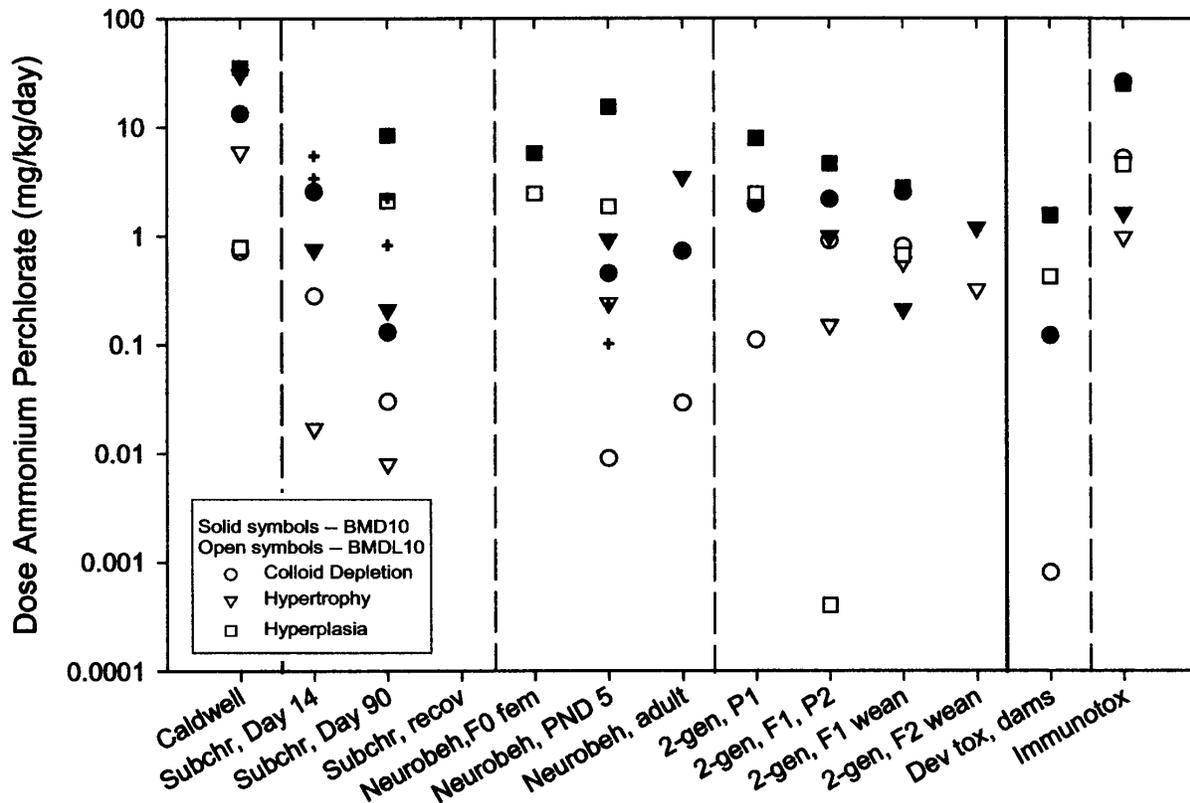
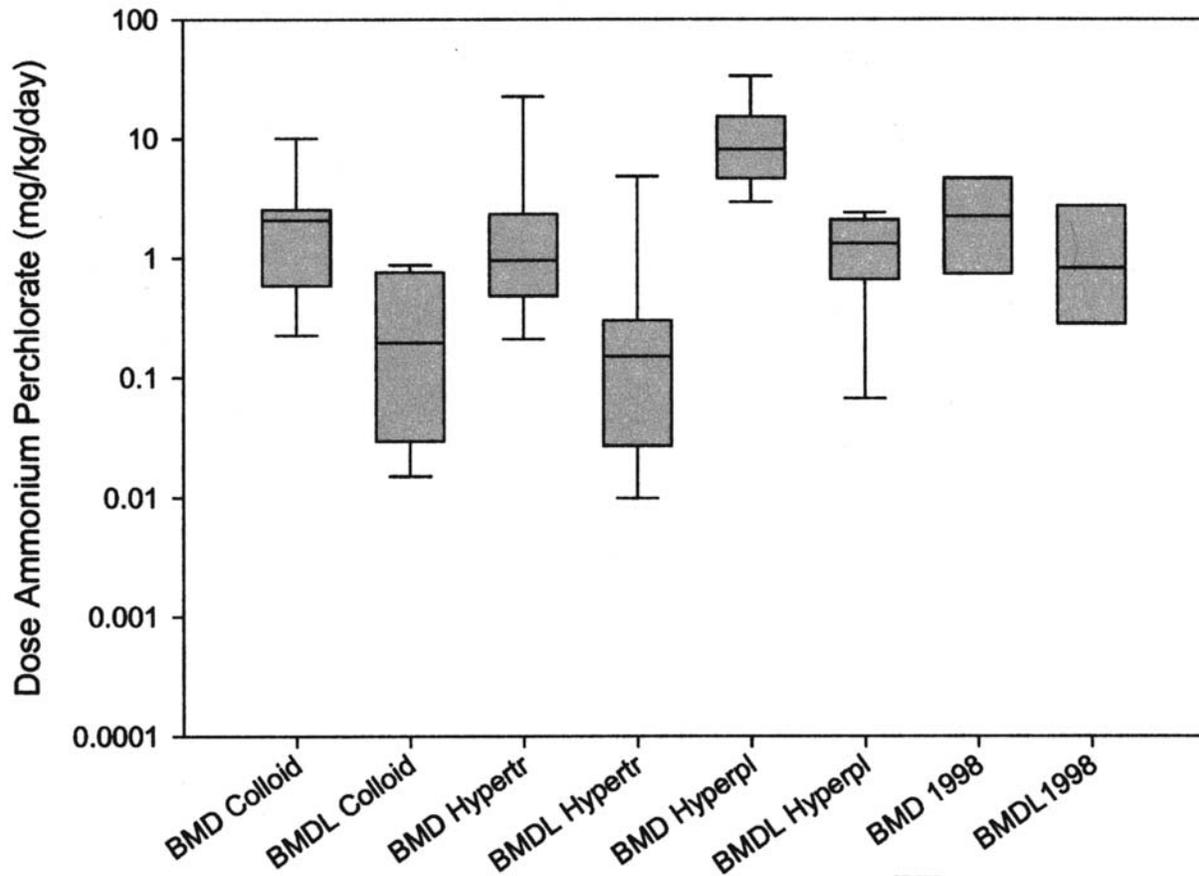


Figure 5-1. Benchmark dose (BMD) and benchmark dose lower limit (BMDL) estimates recalculated for thyroid histopathology based on 2000 Pathology Working Group review (Wolf, 2000; 2001). Data on incidence of colloid depletion, thyroid hypertrophy and thyroid hyperplasia were submitted to the EPA for the perchlorate risk characterization. Values used are presented in Table 5-1 (Geller, 2001a). Greater value represents the BMD and lesser value represents the BMDL. The + denotes BMD and BMDL from previous EPA risk characterization (U.S. Environmental Protection Agency, 1998d; Geller, 1998a). Values to the left of the vertical solid line are from the rat studies. Values to the right are from the developmental study in rabbits (Argus Research Laboratories, 1998c) and the mouse immunotoxicity studies (Keil et al., 1998). Study denoted by “Caldwell” refers to Caldwell et al. (1995); “Subchronic” to Springborn Laboratories, Inc. (1998); “Neurobeh” to the 1998 developmental neurobehavioral study (Argus Research Laboratories, 1998a); and “2-gen” to the completed 2-generation reproductive toxicity study in rats (Argus Research Laboratories, 1999).



**Figure 5-2. Distribution of BMD and BMDL estimates shown by “box and whisker” plots of colloid depletion (colloid), hypertrophy (hyptry), and hyperplasia (hyppls) from rat studies recalculated for thyroid histopathology based on 2000 Pathology Working Group review (Wolf, 2000; 2001). Values are presented in Table 5-1. Study #4 was excluded since it was a 30-day recovery experiment and #5 was excluded due to lack of monotonicity. The boundary of the box closest to zero indicates the 25<sup>th</sup> percentile, a line within the box denotes the median, and the boundary of the box farthest from zero indicates the 75<sup>th</sup> percentile. Whiskers above and below the box indicate the 90<sup>th</sup> and 10<sup>th</sup> percentiles. The two rightmost boxes plot values from the combined rat studies from the 1998 EPA risk characterization (U.S. Environmental Protection Agency, 1998d; Geller, 1998a).**

1 approaches to the analyses that address these comments (Crofton and Marcus, 2001; Marcus,  
2 2001; Crofton, 2001a). All thyroid and pituitary hormone analyses presented will utilize these  
3 new approaches. The reanalyses of the hormone data for the previous set of studies can be found  
4 in Table 5-2.

5 Finally, a number of additional new toxicology studies were recommended by the EPA and  
6 the external review panel in 1999. These included a study of the developmental effects of  
7 perchlorate (Section 5.4.3); a re-evaluation of the effects of perchlorate on neurodevelopmental  
8 motor activity (Section 5.3.2); refinement of the evaluation of immunotoxicity concerns with a  
9 repeat of the sheep red blood cell (SRBC) response using the established plaque-forming cell  
10 (PFC) assay for humoral effects and an additional test for contact hypersensitivity (Section 5.6);  
11 and what has become known as the “Effects Study” (Section 5.3.3). The objective of the  
12 “Effects Study” (Argus Research Laboratories, Inc., 2001) was to reevaluate brain morphometry  
13 effects and to evaluate thyroid histopathology and thyroid and pituitary hormones at various  
14 stages of development, including during gestation and post-natal days 5, 10 and 22.

## 17 **5.1 CHRONIC STUDIES AND GENOTOXICITY ASSAYS**

18 This section discusses the data establishing perchlorate as a carcinogen. A few long-term  
19 studies at comparatively high doses performed before the 1997 perchlorate testing strategy  
20 showed that perchlorate causes thyroid tumors. These studies are discussed in Section 5.1.1. In  
21 order to invoke the conceptual mode-of-action framework for the anti-thyroid effects of  
22 perchlorate causing thyroid neoplasia via a non-genotoxic mechanism, the testing strategy had to  
23 determine whether or not perchlorate acts directly with DNA. This evidence is discussed in  
24 Section 5.2.2. The completed genotoxicity data were presented at the 1999 external peer review  
25 as Attachment A to the February 1, 1999 submission provided by NCEA to the peer review panel  
26 (Zeiger, 1999a,b; Dellarco, 1999; BioReliance, 1999; Moore, 1999). Dr. David Brusick, the  
27 genetic toxicologist on the previous external peer review panel, agreed with the EPA conclusions  
28 (Research Triangle Institute, 1999) that perchlorate’s ability to cause thyroid tumors was not  
29 likely to be via a directly genotoxic mechanism.

30 It should be noted that perchlorate exposure also caused a statistically-significant increase  
31 in tumors at the 30 mg/kg-day dose in the F1-generation pups of the two-generation rat

**TABLE 5-2. A COMPARISON OF NOAELs AND LOAELs FROM THE ORIGINAL 1998 ANALYSES AND THE 2001 RE-ANALYSES FOR HORMONE AND MORPHOMETRY ON THYROID FOLLICULAR LUMEN SIZE (Crofton and Marcus, 2001; Marcus, 2001; Crofton, 2001a)**

Species/Study	Time Point, Age (Doses, mg/kg-day)	Endpoint	Sex	Original Analyses		Re-Analyses <sup>a,b</sup>	
				NOAEL	LOAEL	NOAEL	LOAEL
<b>Rat 14-Day (Caldwell et al., 1995)</b>	14-Day (males - 0.0, 0.11, 0.44, 1.11, 2.26, 4.32, 11.44, 22.16) (females - 0.0, 0.12, 0.47, 1.23, 3.06, 4.91, 11.47, 24.86)	T3	M	0.11	0.44	0.11	0.44
			F	—	0.11	—	0.12
		T4	M	—	0.11	—	0.11
			F	—	0.12	—	0.12
		TSH	M	0.44	1.11	0.44	1.11
			F	0.12	0.47	—	<b>0.12</b>
		hTg	M	—	0.11	—	0.11
			F	—	0.12	—	0.12
		rT3	M	0.44	1.11	<b>0.11</b>	<b>0.44</b>
			F	0.47	1.23	<b>0.12</b>	<b>0.47</b>
<b>Rat Subchronic Study (Springborn, 1998)</b>	14-Day (0, 0.01, 0.05, 0.2, 1.0, 10.0)	T3	M	—	0.01	—	0.01
			F	10.0	—	10.0	—
		T4	M	1.0	10.0	—	<b>0.05</b>
			F	—	—	—	—
		TSH	M	0.05	0.2	<b>0.01</b>	<b>0.05</b>
			F	0.01	0.05	—	<b>0.01</b>

**TABLE 5-2 (cont'd). A COMPARISON OF NOAELs AND LOAELs FROM THE ORIGINAL 1998 ANALYSES AND THE 2001 RE-ANALYSES FOR HORMONE AND MORPHOMETRY ON THYROID FOLLICULAR LUMEN SIZE (Crofton and Marcus, 2001; Marcus, 2001; Crofton, 2001a)**

Species/Study	Time Point, Age (Doses, mg/kg-day)	Endpoint	Sex	Original Analyses		Re-Analyses <sup>a,b</sup>	
				NOAEL	LOAEL	NOAEL	LOAEL
<b>Rat Subchronic Study (Springborn, 1998) (cont'd)</b>	90-Day (0, 0.01, 0.05, 0.2, 1.0, 10.0)	T3	M	—	0.01	—	0.01
			F	—	0.01	—	0.01
		T4	M	—	0.01	—	0.01
			F	—	0.01	—	0.01
		TSH	M	0.05	0.2	0.05	0.2
			F	0.05	0.2	0.05	0.2
<b>Rat Subchronic Study (Springborn, 1998)</b>	120-Day (0, 0.05, 1.0, 10.0)	T3	M	1.0	10.0	1.0	10.0
			F	1.0	10.0	1.0	10.0
		T4	M	—	0.05	—	<b>0.05</b>
			F	—	0.05	<b>1.0</b>	<b>10.0</b>
		TSH	M	10.0	—	—	<b>0.05</b>
			F	10.0	—	—	<b>0.05</b>
<b>Rat Developmental Neurotoxicity Study (Argus, 1998a)</b>	PND5 (0, 0.1, 1.0, 3.0, 10.0)	Lumen size	M	1	3	<b>0.3</b>	<b>3</b>
			F	1	3	<b>0.3</b>	<b>3</b>
	PND90 (0, 0.1, 1.0, 3.0, 10.0)	Lumen size	M	Data not available for original analyses		<b>10</b>	—
			F	Data not available for original analyses		<b>10</b>	—
	PND5 (0, 0.1, 1.0, 3.0, 10.0)	T4		1.0	3.0	<b>0.1</b>	<b>1.0</b>
		T3		0.1	1.0	0.1	1.0
		TSH		3.0	10.0	3.0	10.0
PND90	T4, T3, and TSH		No data available				

**TABLE 5-2 (cont'd). A COMPARISON OF NOAELs AND LOAELs FROM THE ORIGINAL 1998 ANALYSES AND THE 2001 RE-ANALYSES FOR HORMONE AND MORPHOMETRY ON THYROID FOLLICULAR LUMEN SIZE (Crofton and Marcus, 2001; Marcus, 2001; Crofton, 2001a)**

Species/Study	Time Point, Age (Doses, mg/kg-day)	Endpoint	Sex	Original Analyses		Re-Analyses <sup>a,b</sup>	
				NOAEL	LOAEL	NOAEL	LOAEL
<b>Mouse Hormone and Immunotoxicity (Keil et al., 1998)</b>	14-Day (0.0, 0.1, 1.0, 3.0, 30)	T4	M	3.0	30.0	—	<b>0.1</b>
		T3	M	Data not available at time of 1998 analysis		—	<b>0.1<sup>c</sup></b>
		TSH	M	No data			
	90-Day (0.0, 0.1, 1.0, 3.0, 30)	T4	M	0.1	3.0	—	0.1 <sup>c</sup>
		T3	M	Data not available at time of 1998 analysis		—	<b>0.1<sup>d</sup></b>
		TSH	M	30.0	—	—	<b>0.1<sup>d</sup></b>
	120-Day (0.0, 0.1, 1.0, 3.0, 30)	T4	M	30.0	—	30.0	—
		T3	M	Data not available at time of 1998 analysis		30.0	—
		TSH	M	30.0	—	—	—
<b>Rabbit Developmental Toxicity (Argus, 1998b)</b>	Gestation Day 28 (0.0, 0.1, 1.0, 10.0, 30.0, 100.0)	T4	F	0.1	1.0	0.1	1.0
		T3	F	100	—	100	—
		TSH	F	100	—	100	—

<sup>a</sup>Bold indicates where 2001 analyses is different than 1998 analyses.

<sup>b</sup>Results from the liberal and conservative statistical approaches were the same.

<sup>c</sup>No dose response - 0.1 and 1.0 differ from control; 0.3 and 30.0 do not differ from control.

<sup>d</sup>No dose response - 0.1 and 1.0 differ from control; 0.3 and 30.0 do not differ from control.

1 reproductive study (Argus Research Laboratories, 1999). These pups were used as the parents of  
2 the second generation (F2) pups in the study. When these F1 animals were sacrificed after only  
3 19-weeks, tumors were observed (Wolf, 2000). The type was the expected benign thyroid  
4 adenoma consistent with the anti-thyroid effect at the NIS (iodine uptake inhibition) with thyroid  
5 hormone disruption followed by TSH upregulation. The early onset at 19 weeks is remarkable  
6 and suggests the potential for in utero imprinting, a phenomenon beginning to be appreciated  
7 with other endocrine-disrupting compounds (Prins et al., 2001; Phillips et al., 1998; Seckl, 1997).  
8 These tumor results will be discussed in Section 5.5.

### 10 **5.1.1 Cancer Studies**

11 Kessler and Krüskemper (1966) provided potassium perchlorate in drinking water at a  
12 concentration of 0 or 1% to male Wistar rats for 2 years. Body weights and thyroid weights were  
13 reported for groups of 6 to 8 rats sacrificed after 0, 40, 120, 220, and 730 days of treatment, and  
14 thyroid glands from the animals were examined histologically. Using body weight data provided  
15 in the report to calculate a time-weighted average body weight of 0.336 kg and using an  
16 estimated water consumption of 0.045 L/day (calculated with the allometric equation  
17 recommended by U.S. Environmental Protection Agency [1987]), a dose of 1,339 mg/kg-day can  
18 be derived. Body weights of control and treated animals were comparable throughout the  
19 experiment. In contrast, thyroid weights, both relative and absolute, were increased markedly in  
20 treated rats compared to controls at each examination interval. Histological examination of  
21 thyroids from treated rats at 40 days revealed follicular cell hyperplasia. The authors  
22 characterized these changes as typical for a thyroid gland stimulated by TSH during a relatively  
23 short period of time. After 200 days of perchlorate treatment, diffusely degenerative changes  
24 with fibrosis and increased colloid were observed. The authors commented that the course of the  
25 histological changes in the thyroid was similar to that produced by long-term administration of  
26 thiouracil, another antithyroid agent. The authors further reported that 4 of 11 rats treated with  
27 potassium perchlorate for 2 years developed benign tumors of the thyroid gland and that  
28 20 untreated Wistar control rats displayed no thyroid gland tumors. The 1,339 mg/kg-day dose  
29 suggested a free-standing LOAEL because no other doses were tested.

30 Pajer and Kališnik (1991) administered 0 or 1.2% sodium perchlorate in drinking water to  
31 groups of 36 female BALB/c mice (12/group) for up to 46 weeks. Eight or 12 weeks after the

1 beginning of the experiment, one group of treated and control mice were totally irradiated with  
2 0.8 Gy on 5 consecutive days at a dose rate of 1.45 Gy/min so that each mouse received a total of  
3 4 Gy. Assuming a body weight of 0.0353 kg and a water consumption rate of 0.0063 L/day (U.S.  
4 Environmental Protection Agency, 1987), a dose of 2,147 mg/kg-day can be calculated. Thirty  
5 animals died during the experimental period; however, details about the cause of death were not  
6 provided. Forty-two animals were sacrificed at 46 weeks for histological examination of the  
7 thyroid and pituitary gland. No other tissues were examined. Obvious treatment-related  
8 histological changes were observed in the thyroid and pituitary gland, including thyroid follicular  
9 cell carcinoma. Immunoperoxidase staining of pituitary thyrotropic cells and antihuman TSH  
10 serum provided qualitative evidence of increased TSH production in the pituitary gland.  
11 Perchlorate treatment was associated with an increased total volume of the thyroid and of the  
12 distal parts of the anterior pituitary gland (adenohypophysis). In addition, increased average  
13 volume and numbers of epithelial, thyrotropic, and parafollicular cells were observed. Irradiation  
14 appeared to enhance the effects of perchlorate treatment. This study suggested a free-standing  
15 LOAEL of 2,147 mg/kg-day for thyroid effects.

### 17 **5.1.2 Genotoxicity Assays**

18 ManTech Environmental Technology, Inc. (1998) performed a battery of three genotoxicity  
19 assays (*Salmonella typhimurium*/microsome mutagenesis assay [Ames assay], the mouse  
20 lymphoma cell mutagenesis assay [L5178Y-TK test], and the *in vivo* mouse bone marrow  
21 micronucleus induction assay) with ammonium perchlorate to help determine its potential for  
22 various interactions with DNA and to gain insight into its possible carcinogenicity. To confirm  
23 the findings of ManTech Environmental Technology, Inc., the EPA requested that the National  
24 Toxicology Program (NTP) also evaluate ammonium perchlorate in the Ames assay and the  
25 mouse bone marrow micronucleus test (Zeiger, 1999a). The sponsor (PSG) also had the mouse  
26 lymphoma assay repeated (BioReliance, 1999).

27 Ammonium perchlorate was evaluated in the Ames assay (*Salmonella typhimurium*/  
28 microsome mutagenesis assay), which is a well-defined assay for detection of mutagens.  
29 It measures the reversion from a histidine-independent state ( $his^-$ ) induced by chemicals that  
30 cause base-pair changes or frameshift mutations in the genome of the organism (i.e., it measures  
31 for point mutations [e.g., substitution, addition, or deletion of one or a few DNA base pairs

1 within a gene]). In this assay, bacteria are exposed to the test chemical with and without a  
2 metabolic activation system (Arochlor 1254-induced rat liver S9 with co-factors).  
3 The mutagenicity is evaluated by the increase in the number of revertant colonies. The L5178Y  
4 mouse-lymphoma assay is another short-term *in vitro* assay to detect both point mutations and  
5 structural chromosomal changes. The *in vivo* mammalian micronucleus test detects the damage  
6 of chromosomes or of the mitotic apparatus caused by a clastogenic chemical in bone marrow  
7 cells (polychromatic erythrocyte [PCE] stem cells) of treated animals. Micronuclei are believed  
8 to be formed from chromosomes or chromosome fragments left behind during anaphase of  
9 mitosis. The induction of micronuclei indicates changes in either chromosome structure or  
10 number in bone marrow cells. ManTech Environmental Technology, Inc. (1998) performed this  
11 assay in Swiss-CD-1 mice and the NTP used B6C3F1 mice (Zeiger, 1999a). The micronucleus  
12 assay also was performed as part of the 90-day bioassay in Sprague-Dawley rats (Springborn  
13 Laboratories, Inc., 1998). This is considered an adequate series of tests to determine the  
14 mutagenic and clastogenic (chromosomal breaking) potential of an agent. It should be noted that  
15 perchlorate is not likely to be mutagenic, given its physical and chemical properties (i.e., it is  
16 simply an anion). Although perchlorate is an oxidizing agent, it is not expected to produce  
17 oxidative DNA damage because of the kinetic considerations discussed in Chapter 2.

#### 18 19 **5.1.2.1 *In Vitro* Assays**

20 Ammonium perchlorate was not found to be mutagenic in the *Salmonella typhimurium*  
21 (Ames assay) with and without Arochlor 1254-induced rat liver S9 activation by two separate  
22 laboratories (ManTech Environmental Technology, Inc., 1998; Zeiger, 1999a). In the ManTech  
23 study, ammonium perchlorate was dissolved in distilled water and tested at five concentrations  
24 (5,000, 2,500, 1,250, 625, and 312.5  $\mu\text{g}/\text{plate}$ ) in tester strains TA98, TA100, TA1535, and  
25 TA1537, with and without Arochlor 1254-induced rat liver S9 using the plate incorporation  
26 assay. Although this study was regarded as adequate, the EPA requested that the Ames assay be  
27 repeated by the NTP to confirm the negative findings and to include additional tester strains (i.e.,  
28 TA102, and TA104) that are able to detect a variety of oxidative mutagens. Therefore, the NTP  
29 evaluated ammonium perchlorate in the Salmonella/Ames assay in tester strains TA98, TA100,  
30 TA1535, TA97, TA102, and TA104 (Zeiger, 1999b). Ammonium perchlorate was dissolved in  
31 distilled water and tested using the preincubation procedure at doses of 10,000, 3,333, 1,000,

1 333, and 100  $\mu\text{g}/\text{plate}$ , with and without metabolic activation from Arochlor-induced rat and  
2 hamster livers. Ammonium perchlorate was neither toxic nor mutagenic under the conditions of  
3 the NTP assay.

4 The L5178Y/*tk*<sup>+/−</sup> mouse lymphoma assay also was used to evaluate the mutagenic and  
5 chromosomal breaking potential of ammonium perchlorate in vitro. Ammonium perchlorate was  
6 reported to be negative both in the absence and presence of rat Arochlor-induced S9 liver  
7 activation (ManTech Environmental Technology, Inc., 1998). Ammonium perchlorate was  
8 evaluated at 5.0, 2.5, 0.5, 0.25, 0.05, and 0.025 mg/mL without S9 activation, and at 2.5, 0.5,  
9 0.25, 0.05, and 0.025 mg/mL with S9 activation. Although a small increase in mutation  
10 frequency was found in the absence of S9 activation at 2.5 mg/mL, which appeared to be  
11 statistically significant ( $p < 0.05$ ) by the two-tail Student's t-test, a repeat assay found no increase  
12 in mutation frequency at this concentration compared with controls. Therefore, ammonium  
13 perchlorate is considered to be negative in the absence of S9 activation. Confidence in the  
14 negative findings without S9 activation is reinforced by the wide range of ammonium perchlorate  
15 concentrations evaluated. Although ammonium perchlorate also was reported as negative in the  
16 presence of S9 activation, the response of the positive control, 3-methyl cholanthrene (MCA), in  
17 the actual experiment was too low ( $182.6 \times 10^{-6}$ ) to be acceptable. The highest dose of  
18 ammonium perchlorate produced a mutation frequency of  $194 \times 10^{-6}$ . The MCA at 2.5  $\mu\text{g}/\text{mL}$   
19 should induce a mutation frequency of 300 to  $350 \times 10^{-6}$  or higher. Such a low positive control  
20 response weakens the confidence for the negative finding with S9 activation. In addition, the  
21 cloning efficiencies for the S9 test appear to be too high (143%), further reducing the confidence  
22 in a negative finding. Therefore, only the assays on ammonium perchlorate without S9 are  
23 considered unequivocally to be negative. Although perchlorate is not expected to be metabolized  
24 to a mutagenic intermediate, these S9 data were not of sufficient quality to support a  
25 negative-response conclusion.

26 Because of the problems described above, the sponsor (PSG) had the mouse lymphoma  
27 assay repeated. In this recent mouse lymphoma assay, ammonium perchlorate was evaluated at  
28 concentrations of 1000, 2000, 3000, 4000, and 5000  $\mu\text{m}/\text{ml}$  without and with Arochlor  
29 1254-induced rat liver S9 activation (BioReliance, 1999). No increase in mutant frequencies  
30 were found after treatment with perchlorate. The data were judged to be of sufficient quality to  
31 determine perchlorate to be nonmutagenic both with and without S9 activation. Although the

1 background mutant frequency was low, particularly in the S9 experiment, the data set still is  
2 considered to be very good overall, as well as internally consistent. The problems that were  
3 observed in the data generated by the first laboratory (ManTech Environmental Technology, Inc.,  
4 1998) were not present in the data form the BioReliance (1999) study.

#### 5 6 **5.1.2.2 *In Vivo* Assays**

7 The potential for ammonium perchlorate to induce micronuclei was evaluated in mice and  
8 rats. Ammonium perchlorate was administered by drinking water gavage for 3 consecutive days  
9 to Swiss CD-1 mice (5 females and 5 males per dose group) at 1,000, 500, 250, 125, and  
10 62.5 mg/kg-day (ManTech Environmental Technology, Inc., 1998). Twenty-four hours after the  
11 last dose, the mice were sacrificed, and the frequency of micronucleated cells were evaluated by  
12 counting 1,000 PCEs per animal. The assay was conducted in accordance with existing EPA  
13 Federal Insecticide, Fungicide, and Rodenticide Act/Toxic Substances Control Act  
14 (FIFRA/TSCA) testing guidelines. No increase in the frequency of micronuclei were found for  
15 any dose group. There is some uncertainty whether a maximum tolerated dose (MTD) was  
16 reached in this study. The study authors reported that at 2,000 mg/kg, 4 out of 6 animals died  
17 after one dosing of ammonium perchlorate. Typically, the assay is performed at 85% of the  
18 MTD, and the 1,000 mg/kg-day represents approximately 50% of the LD<sub>50</sub>. There was no  
19 indication of toxicity to the bone marrow cells because the polychromatic erythrocyte to  
20 normochromatic erythrocyte (PCE/NCE) ratio was not different from negative controls.  
21 Furthermore, the study authors did not report any indication of clinical signs of toxicity in the  
22 highest dose group. Despite a rebuttal submitted by Dourson (1998) on behalf of the sponsor  
23 (PSG), EPA remained concerned because of the importance of this test in the overall  
24 determination of the approach to be taken for the carcinogenicity assessment (i.e., to rule out  
25 direct genotoxicity).

26 The NTP agreed to expedite and repeat this test in response to an EPA request. The assay  
27 was performed by ip injection to ensure the greatest delivery to the bone marrow. Male B6C3F1  
28 mice were treated with 125, 250, 500, 1,000, 1,500, and 2,000 mg/kg ammonium perchlorate in  
29 buffered saline, plus solvent and positive (cyclophosphamide) controls. Note that this study uses  
30 two dose groups higher than those used in the previous study (i.e., 1,500 and 2,000 mg/kg).  
31 Furthermore, the use of ip injection as the route of administration would result in a direct

1 delivery of the compound to the bone marrow cells versus delivery from drinking water gavage.  
2 Five mice per group were injected daily for 3 consecutive days and were sacrificed 24 h after the  
3 last injection; 2,000 PCEs were scored per animal for micronuclei. All animals in the 1,500- and  
4 2,000-mg/kg groups died after the first ip injection, and 4/5 animals died in the 1,000-mg/kg  
5 group after the second ip injection. No increases in percent PCE were observed in any of the  
6 remaining test groups (125, 250, and 500 mg/kg). No bone marrow toxicity was seen as  
7 indicated by the percent of PCE (Zeiger, 1999a,b). These results are interpreted to be consistent  
8 with those of the ManTech Environmental Technology, Inc. (1998) study that used gavage  
9 drinking water administration, and confirm that perchlorate does not induce micronuclei in  
10 rodents.

11 The 90-day subchronic bioassay using Spraque-Dawley rats also evaluated micronuclei  
12 induction (Springborn Laboratories, Inc., 1998). The frequency of micronuclei induction was  
13 examined in both the males and females after the 90-day sacrifice in the 10-mg/kg-day dose  
14 group of ammonium perchlorate administered by drinking water. Although there was no  
15 induction of micronuclei at this dose, 10 mg/kg-day does not appear to reach a MTD because  
16 there were no overt signs of toxicity. However, the definition of MTD may be somewhat moot,  
17 given the changes in thyroid hormone economy and histopathology seen in the thyroids at that  
18 dose. There was significant reduction in the PCE/NCE ratio (i.e., an indicator of toxicity to the  
19 bone marrow cells).

### 21 **5.1.2.3 Summary of Genotoxicity Battery Results**

22 Negative results were reported in all genotoxicity assays conducted on ammonium  
23 perchlorate when evaluated by two independent laboratories. Ammonium perchlorate was not  
24 mutagenic in the Ames assay (with or without S9 activation). Negative results were also found  
25 in the mouse lymphoma gene mutation assay without and with S9 activation. Ammonium  
26 perchlorate did not induce chromosomal anomalies when evaluated for micronuclei induction in  
27 the bone marrow of mice when administered via drinking water gavage or ip injection.  
28 No increases in micronuclei were found in Spraque-Dawley rats when evaluated from the 90-day  
29 study at the highest dose, which produced both thyroid hormone perturbations and follicular cell  
30 hyperplasia.

1 In conclusion, ammonium perchlorate does not have the potential to be mutagenic or  
2 clastogenic. The *in vitro* and *in vivo* studies discussed above provide support for that conclusion.  
3 Therefore, mutagenicity is not considered a possible mode of carcinogenic action for this  
4 chemical.

## 7 **5.2 GENERAL TOXICITY: SHORT-TERM AND SUBCHRONIC** 8 **TESTING**

9 The majority of the data on perchlorate toxicity available from previous studies or as a  
10 result of the current perchlorate testing strategy involved either short-term or subchronic  
11 exposures and are presented in this section. As discussed in Chapter 3, the testing strategy  
12 included targeted studies to evaluate different endpoints, e.g., developmental neurotoxicity  
13 (Section 5.3), developmental studies (Section 5.4) reproductive studies (Section 5.5) and  
14 immunotoxicity assays (Section 5.6). The rationale behind the 90-day study (Section 5.2) with  
15 satellite examination of thyroid and pituitary hormones and a 30-day recovery period was to  
16 evaluate anti-thyroid effects as possible precursor lesions. If a NOAEL could be established for  
17 these precursor lesions, it was thought that a two-year bioassay would not be required. This  
18 assumption is now more tenuous due to the tumors observed in the F1-generation at 19 weeks.  
19 The integration of these results with the available human data to arrive at a risk assessment will  
20 be discussed in Chapter 7.

### 22 **5.2.1 Historical Data**

23 Mannisto et al. (1979) measured serum levels of TSH, T3, and T4 by RIA in groups of 5 to  
24 6 male Sprague-Dawley rats weighing 180 to 220 g that were exposed to potassium perchlorate  
25 in their drinking water at concentrations of 0, 10, 50, 100, or 500 mg/L for 4 days. Potassium  
26 perchlorate doses of 0, 1.5, 7.6, 15.3, or 76.3 mg/kg-day, respectively, were calculated assuming  
27 a body weight of 0.2 kg and a water consumption rate of 0.0305 L/day (U.S. Environmental  
28 Protection Agency, 1987). Perchlorate produced statistically significant increases in serum TSH  
29 levels and decreases in serum T3 and T4 levels. Significant changes in all three parameters were  
30 measured in the 100 and 500 mg/L (15.3 and 76.3 mg/kg-day, respectively) dose groups. In the  
31 50 mg/L (7.6 mg/kg-day) dose group, levels of T3 and T4 were decreased significantly; TSH

1 levels were increased slightly, but the increase was not significant. At the low dose, T3, T4, and  
2 TSH levels were unchanged from controls. This study suggested a NOAEL of 1.5 mg/kg-day  
3 and a LOAEL of 7.6 mg/kg-day for short-term exposures to potassium perchlorate.

4 Shigan (1963) administered 190 mg/kg-day of potassium perchlorate in water to rabbits and  
5 white rats (number, sex, and strain not identified) for 3 mo. The author did not indicate whether  
6 the compound was administered in drinking water or by gavage with water. The animals were  
7 examined for cardiac function; liver function, based on changes in serum proteins; immune  
8 function, based on leukocyte phagocytosis; and adrenal function. Perchlorate at the dose tested  
9 caused a change in the electrocardiogram and a decrease in serum proteins, indicating a  
10 disruption of the glycogen-forming function of the liver. Shigan (1963) did not indicate whether  
11 these changes were observed in both rabbits and rats. Perchlorate had no effect in the remaining  
12 tests. This study suggested a LOAEL of 190 mg/kg-day although the study translation is reported  
13 incompletely, limiting its usefulness for risk assessment.

14 In a second set of experiments, Shigan (1963) also treated rabbits and white rats (number,  
15 sex, and strain not identified) with 0, 0.25, 2.0, and 40 mg/kg-day of potassium perchlorate for  
16 9 mo. The medium for dosing was not reported. The animals were examined for cardiac and  
17 liver function, for conditioned reflexes, and for uptake and discharge of iodide by the thyroid. In  
18 the two highest dose groups, there was a statistically significant increase in the amount of iodide  
19 excreted from the thyroid; this increase was not observed in the 0.25-mg/kg-day dose group. The  
20 study does not indicate if the effect was seen in one or both species tested. This study suggested  
21 a NOAEL of 0.25 mg/kg-day and a LOAEL of 2 mg/kg-day for thyroid effects.

22 Hiasa et al. (1987) measured serum levels of T3, T4, and TSH by radioimmunoassay in  
23 groups of 20 male Wistar rats administered 0 or 1,000 ppm potassium perchlorate in the diet for  
24 20 weeks. Assuming a body weight of 0.34 kg (the average final body weight of rats treated with  
25 perchlorate) and a food consumption rate of 27.4 g/day (U.S. Environmental Protection Agency,  
26 1987), an estimated dose of 80.7 mg/kg-day was calculated. Absolute and relative thyroid  
27 weights were significantly increased compared to controls in perchlorate-treated rats. No effects  
28 were seen on liver weights. The T4 levels decreased slightly, but the decrease was  
29 not statistically significant. The T3 levels were unchanged compared to controls. The TSH  
30 levels were increased statistically significantly compared to controls. Histological examination

1 of the thyroid revealed diffused small follicles in perchlorate-treated rats and one case of  
2 follicular hyperplasia. Thus, the 80.7-mg/kg-day dose could be considered a LOAEL.

3 Gauss (1972) fed female NMRI mice a diet containing 0 or 1% potassium perchlorate for  
4 up to 160 days. Mice were between 50 and 60 days old at the beginning of treatment and  
5 weighed between 19 and 28 g (average, 23.23 g). During the first 2 mo of treatment, body  
6 weights increased about 12%; body weight data for longer treatment periods were not reported.  
7 Assuming a body weight of 23 g and a food consumption value of 4.625 g/day (U.S.  
8 Environmental Protection Agency, 1987), a dose of 2,011 mg/kg-day was calculated. Thyroid  
9 glands were examined histologically at 10- to 20-day intervals throughout the 160-day study  
10 period. Thyroid and nuclei volumes and height of epithelial follicles were increased in treated  
11 mice throughout the treatment period compared to controls. The histological examinations  
12 showed a progressive change in the histological appearance of the thyroid of treated mice,  
13 beginning with colloid loss, nuclei volume expansion, and rising epithelium height, followed by  
14 the appearance of hypertrophy and hyperplasia of the thyroid parenchyma. At later stages of the  
15 treatment period, hyperplastic follicles, areas of adenomatic tissue, adenoma complexes, and  
16 secreting cystadenomas were observed; however, no progression to malignancy was apparent.  
17 The 2,011 mg/kg-day dose suggested a free-standing LOAEL because no other doses were tested.

### 19 **5.2.2 Caldwell et al. (1995) 14-Day Study**

20 Caldwell et al. (1995) administered ammonium perchlorate in drinking water at  
21 concentrations of 0, 1.25, 5.0, 12.5, 25, 50, 125, or 250 mg/L to Sprague-Dawley rats  
22 (6/sex/group) for 14 days. The actual dose administered to each animal was calculated by  
23 multiplying the concentration of ammonium perchlorate administered in the drinking water by  
24 each rat's average water consumption over the 14-day period and dividing this number by each  
25 animal's average body weight for the same period, resulting in doses (male/female) of 0,  
26 0.11/0.12, 0.44/0.47, 1.11/1.23, 2.26/3.06, 4.32/4.91, 11.44/11.47, and 22.16/24.86 mg/kg-day,  
27 respectively (Caldwell et al., 1995). Caution must be used when interpreting these reports  
28 because the conversion is sometimes not included (e.g., the Channel [1998b] consultative letter  
29 reports results in units of the test concentrations rather than the dose converted to milligrams per  
30 kilogram per day). Thyroids were weighed, histopathology and morphometry performed, and  
31 thyroid hormone levels were measured with a radioimmune assay technique.

1 The consultative letter of Channel (1998b) provides results and comments on a  
2 histopathological analysis of the rat thyroids from the Caldwell et al. (1995) 14-day study that  
3 was performed by the Air Force Research Laboratory/Human Effectiveness Directorate  
4 (AFRL/HEST) and never officially published (Eggers, 1996, as cited in Channel, 1998b).  
5 As part of the previous assessment, EPA requested from the AFRL/HEST the previously  
6 unpublished histopathology data from the 14-day oral dosing study performed by Caldwell et al.  
7 (1995). The histopathology was discussed in the paper on the study design (Caldwell and Mattie,  
8 1995) but had not been published in either Caldwell et al. (1995) or King (1995). The  
9 histopathology data discussed herein were provided in a consultative letter from the AFRL/HEST  
10 (Channel, 1998b). The EPA also performed a reanalysis of the thyroid hormone data (T4, T3,  
11 rT3, TSH, and thyroglobulin [hTg]) found in the Caldwell et al. (1995) and King (1995) reports  
12 (Crofton, 1998a). Because these individual data were supplied only electronically on Microsoft  
13 Excel<sup>®</sup> spreadsheets and not submitted formally to EPA, Crofton, (1998a) represents the official  
14 publication of these data. These histopathology data and reanalyses of effect levels using the  
15 PWG results and new hormone analyses are found in the following sections.

#### 16 17 **5.2.2.1 Thyroid Histology Data**

18 Channel (1998a) submitted that the incidence of thyroid follicular cell hypertrophy  
19 determined by standard histology was significantly different from control at a lower dose  
20 (0.44 0.47 mg/kg-day) than for the incidence of decrease in follicular lumen size (2.26  
21 3.06 mg/kg-day), but the statistics indicate a NOAEL at 0.11 0.12 mg/kg-day. However, the  
22 documentation of the statistics was not provided, and Eggers (1996) apparently combined both  
23 sexes for the analyses. It is recommended in the report (Channel, 1998a), and EPA concurred,  
24 that a re-analysis was warranted for a number of reasons. First, there was a gender-by-treatment  
25 interaction observed in the thyroid hormone analyses (see Section 5.2.2.2). Secondly, there was  
26 an apparent dose trend, despite the limited sample size, in the incidence of response: male and  
27 female combined was 7/12, 6/11, 11/12, 10/12, 12/12, 12/12, 12/12, and 12/12; male only was  
28 3/6, 4/6, 5/6, 5/6, 6/6, 6/6, 6/6, and 6/6; and female only was 4/6, 2/5, 6/6, 5/6, 6/6, 6/6, 6/6, and  
29 6/6 for the 0, 0.1, 1.0, 5.0, 10, 20, 50, and 100 mg/kg-day groups, respectively. Finally, the  
30 analysis did not combine severity and incidence data for the decrease in lumen size, but the mean  
31 severity scores alone were statistically significant from control above the 0.44/0.47 mg/kg-day

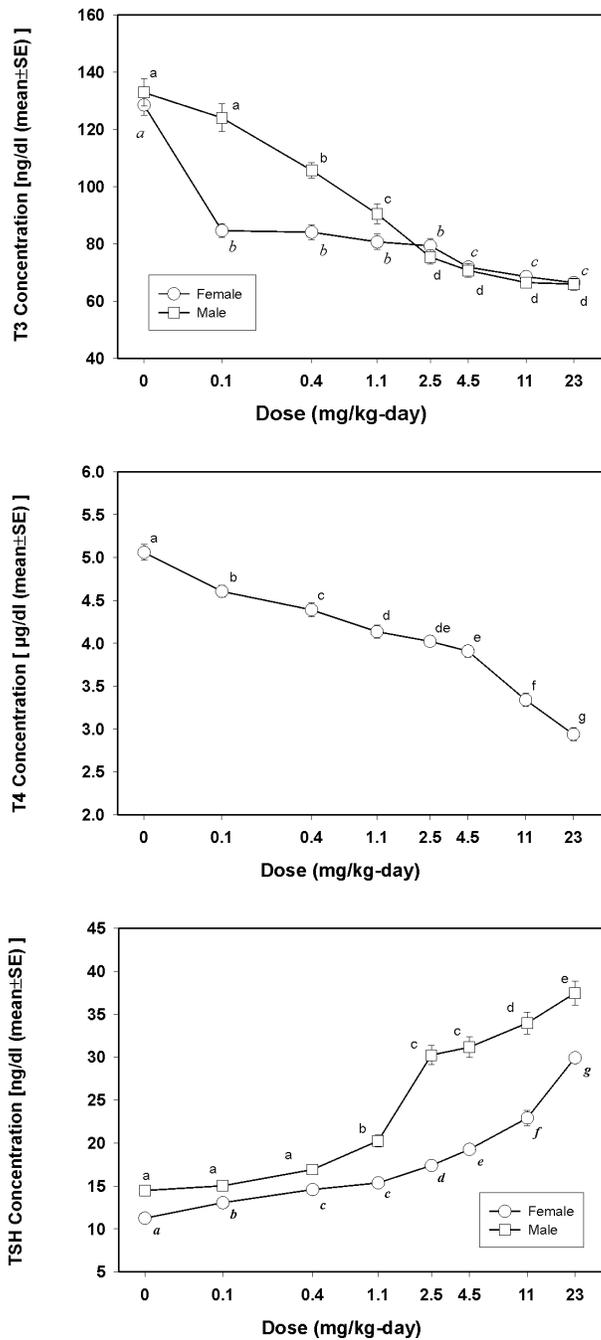
1 group. A separate computerized morphometric analysis of follicular lumen size was performed  
2 by AFRL/HEST for the 0, 0.11/0.12, 1.11/1.23, 4.32/4.91, and 22.16/24.86 mg/kg-day groups,  
3 and a statistically significant difference in the incidence of decrease in lumen size was evident in  
4 the males at the 1.11 mg/kg-day dose and, in females, at the 4.91 mg/kg-day dose; however, the  
5 gender-by-treatment effect was not taken into account. Relative thyroid weights were  
6 significantly increased in 11.44/11.47 and the 22.16/24.86 mg/kg-day dose groups compared to  
7 controls.

8 Results of the PWG analysis can be found in Wolf (2000; 2001; Tables 1 and 2). Female  
9 rats appeared to be slightly more sensitive in this study with a NOAEL designated at 1.23 mg/kg-  
10 day; whereas, in males it was somewhat difficult to ascertain. This may be due to the difficulty  
11 that the PWG had in reading the slides from this study due to the non-routine staining method  
12 (periodic acid shift [PAS] reaction with a green counterstain) as noted in Wolf (2000). BMD  
13 analysis (Table 5-1) for the combined female and male data results in BMDL values for a 10%  
14 increase in incidence at 0.72 mg/kg-day for colloid depletion and 0.78 mg/kg-day for hyperplasia.  
15 The difficulty noted above with the staining for this study was most prominent in evaluating  
16 hypertrophy (Wolf, personal communication), so that these estimates were not calculated.  
17 Re-analysis of the morphometry on thyroid follicular lumen size identified a NOAEL at the  
18 0.44/0.47 mg/kg-day dose.

#### 20 **5.2.2.2 Thyroid and Pituitary Hormone Analyses**

21 The thyroid and pituitary hormone data were reanalyzed using five two-way analysis of  
22 variance (ANOVA) tests, one each for all of the hormones (Crofton, 1998a). Data from  
23 dependent measures (T3, T4, rT3, TSH, and hTg) were subjected to separate two-way ANOVAs,  
24 with gender (male and female), and treatment (dose) as independent, between-subject variables.  
25 Step-down ANOVA tests were conducted as indicated by significant interactions and discussed  
26 in Crofton and Marcus (2001) and Marcus (2001). Mean contrasts were performed using  
27 Duncan's Multiple Range Test. Results of these reanalyses are similar to those stated in the  
28 Caldwell et al. (1995) and King (1995) reports with some notable exceptions. Figure 5-3 shows  
29 the dose-dependent effects on T3, T4, and TSH.

30 There was a significant gender-by-treatment interaction on total serum T3, and subsequent  
31 step-down ANOVA tests showed significant treatment effects for both genders. Figure 5-3(A)



**Figure 5-3. Effects in the Caldwell et al. (1995) study of 14-day drinking water administration of ammonium perchlorate to SD rats on serum total T3 (A), T4 (B), and TSH (C) concentrations (ng/mL; mean ± SE) as recalculated in Table 5-2 (Crofton and Marcus, 2001). Means with different letters were significantly different ( $p < 0.05$ ). Data of Channel (1998b) and Crofton (1998a). Daily dose was estimated from water consumption data.**

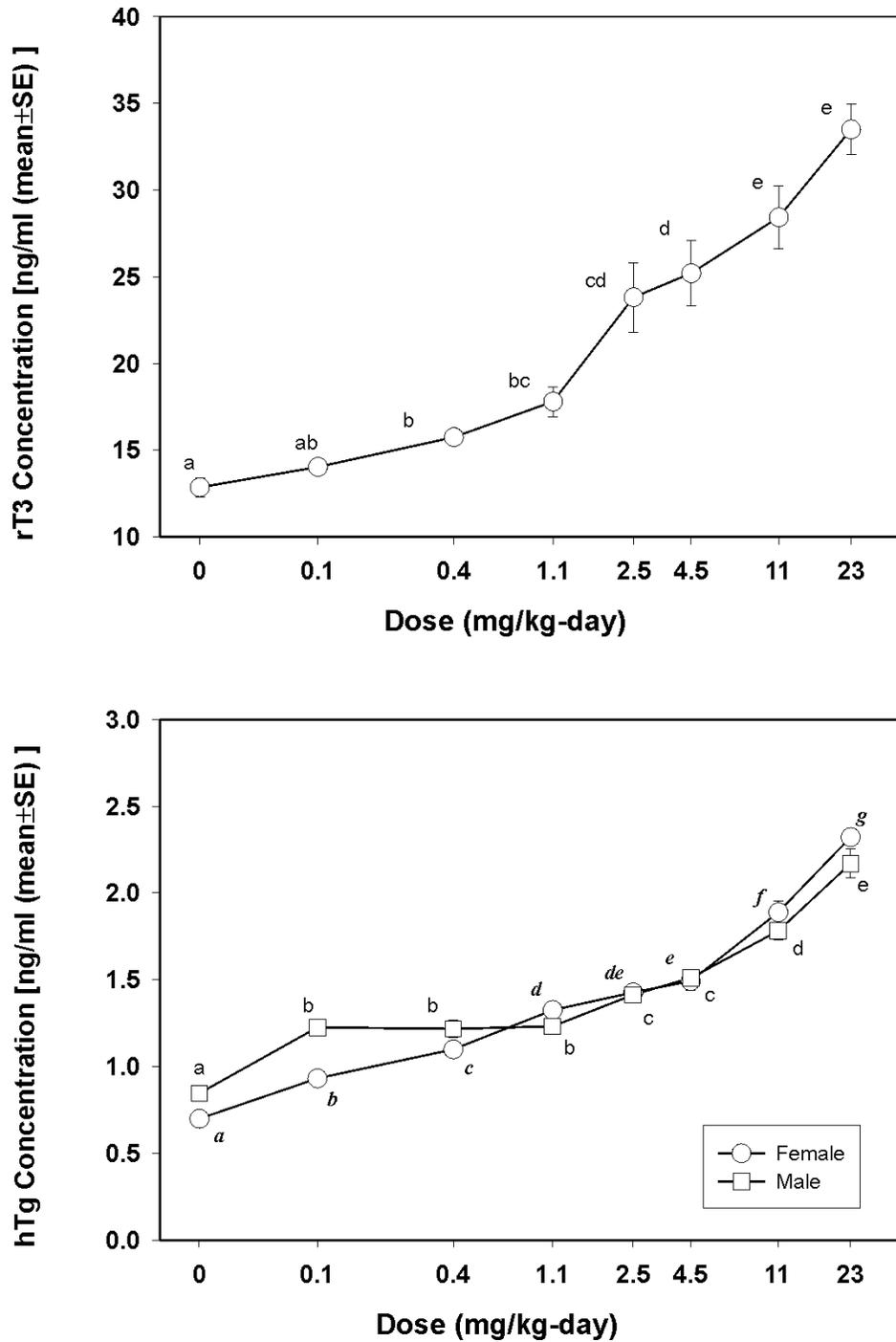
1 illustrates dose-dependent decreases in T3 for both genders while females were slightly more  
2 sensitive compared to males. The overall gender-by-treatment interaction was not significant for  
3 T4, but there was a significant main effect of treatment (Figure 5-3(B)). Perchlorate also  
4 decreased T4 in a dose-dependent manner. There was a significant gender-by-treatment  
5 interaction on total serum TSH, and subsequent step-down ANOVA tests showed significant  
6 treatment effects for both genders. Dose-dependent increases in TSH were observed for both  
7 genders; however, females were slightly more sensitive compared to males.

8 The Caldwell et al. (1995) study is the only one in which an additional thyroid hormone,  
9 rT3, and hTg were assayed (Tg in rats was assayed with a human RIA kit, thus the notation “h”).  
10 There was no significant gender-by-treatment interaction for rT3. Figure 5-4(A) clearly indicates  
11 that perchlorate increases rT3 in a dose-dependent manner. There was a significant gender-by-  
12 treatment interaction on hTg, and subsequent step-down ANOVA tests showed significant  
13 treatment effects for both genders. Figure 5-4(B) illustrates the dose-dependent increases in hTg  
14 for both genders. Both genders were equally sensitive, with males exhibiting a slightly greater  
15 response to the lowest dosage.

16 Perchlorate exposure decreased circulating T3 and T4 and increased TSH. This report also  
17 provides evidence that rT3, formed mostly in extrathyroidal tissues, was increased by this  
18 exposure. Thyroglobulin also was increased. The NOAELs and LOAELs are summarized in  
19 Table 5-2. A NOAEL for TSH was observed in males only at 0.44 mg/kg-day and at 0.11/0.12  
20 for rT3. Note that free-standing LOAELs (i.e., effects at the lowest dosage tested) were found at  
21 0.11/0.12 mg/kg-day for T3 in females, for T4 and hTg in both sexes, and for TSH in females.  
22

### 23 **5.2.3 The 90-Day Testing Strategy Bioassay in Rats**

24 The 90-day study that was part of the testing strategy consisted of oral administration of  
25 ammonium perchlorate via drinking water to male and female Sprague-Dawley rats at doses of  
26 0, 0.01, 0.05, 0.2, 1.0, and 10 mg/kg-day (Springborn Laboratories, Inc., 1998). This study has  
27 also been reported in the literature (Siglin et al., 2000), but because that manuscript did not use  
28 the thyroid histopathology as reported by the PWG (Wolf, 2000) it will not be discussed further  
29 in this document. A 14-day sacrifice also was included in the study for comparison with the  
30 Caldwell et al. (1995) study of that same duration. Ten rats/sex/dose were used, and an  
31 additional 10 rats/sex/dose were sacrificed after the 30-day recovery period following cessation



**Figure 5-4. Effects in the Caldwell et al. (1995) study of 14-day drinking water administration of ammonium perchlorate to SD rats on serum rT3 (A) and hTg (B) concentrations (ng/mL; mean ± SE) as recalculated in Table 5-2 (Crofton and Marcus, 2001). Data of Channel (1998b) and Crofton (1998a). Means with different letters were significantly different ( $p < 0.05$ ). Daily dose was estimated from water consumption data.**

1 of the 90-day exposure at doses of 0, 0.05, 1.0, and 10 mg/kg-day to evaluate reversibility of any  
2 observed lesions.

3 The stock solution of the test article was diluted with reverse osmosis (RO) water and  
4 prepared fresh five times during the study (at least once every 5 weeks). Stability analyses were  
5 performed by the sponsor (AFRL/HEST) and showed that ammonium perchlorate solutions were  
6 stable for 109 days (Tsui et al., 1998). The sponsor also confirmed that the stock and dosing  
7 solutions were within an acceptable concentration range (Springborn Laboratories, Inc., 1998;  
8 Appendix B). Control drinking water solutions were analyzed by the sponsor to confirm no  
9 contamination of detectable nitrate, an ion that could cause possible interference to estimating the  
10 dose of test article. Dosing solutions were prepared fresh for each week, and the administered  
11 concentrations were adjusted based on measured body weights and water intake.

12 The parameters evaluated included clinical observations, body and organ weights, food and  
13 water consumption, hematology, clinical chemistry, ophthalmology, and gross necropsy.  
14 Histopathology was performed on all tissues from the control and high-dose groups. The liver,  
15 kidneys, lungs, thyroid/parathyroid, and gross lesions from all intermediate dose groups and for  
16 the recovery groups also were examined microscopically. Evaluation of additional reproductive  
17 parameters, i.e., estrous cyclicity in females and sperm motility and morphology in males, also  
18 was performed. Thyroid hormone analyses were performed at the 14-, 90-, and 120-day  
19 sacrifices. Only the 0, 0.05, 1.0 and 10.0 mg/kg-day groups were continued until the 120-day  
20 time point. All hormone and tissue collection was balanced over time-of-day to control for the  
21 circadian rhythms of hormones.

### 22 23 **5.2.3.1 General Toxicity, Thyroid Histopathology Results, and Satellite** 24 **Reproductive Assay**

25 There were no clinical signs of toxicity observed during the treatment or recovery periods.  
26 All rats survived to scheduled sacrifice except one female rat in the 0.05 mg/kg-day group that  
27 was found dead during the recovery period. However, this death was considered unrelated to  
28 treatment because no deaths occurred in any of the higher dose groups, and the histopathologic  
29 evaluation for cause of death was inconclusive. No statistically significant or remarkable  
30 findings were observed among the groups with respect to clinical observations, body weights,  
31 food or water consumption, ophthalmology, hematology, or clinical chemistry. Miscellaneous

1 lesions that occurred with equal incidence and severity in all dose groups and controls included  
2 extramedullary hematopoiesis in the livers, inflammation in the lungs, minimal nephropathy in  
3 the kidneys and inflammation of the heart. Because none of these lesions demonstrated a dose  
4 response, and some are commonly seen in young rats, they were not considered treatment-related.  
5 The only treatment-related lesions observed at gross necropsy were reddened thyroids, attributed  
6 to minimal congestion of the blood vessels.

7 Absolute thyroid weight and thyroid weight relative to both final body weight and brain  
8 weight were increased significantly in males of the 10 mg/kg-day dose group after 14 and  
9 90 days of treatment and in females at the 10 mg/kg-day dose group after 90 days indicating  
10 LOAEL at 10 and a NOAEL at 1 mg/kg-day. These thyroid weight measures were comparable to  
11 control values in both males and females of the 10 mg/kg-day group at the end of the 30-day  
12 recovery period. Histopathology was evaluated on Days 14, 90, and 30 postexposure (120 days).  
13 The corresponding PWG review results can be found in Wolf (2000; 2001, Tables 3 through 8).  
14 Male rats appeared to be slightly more sensitive, exhibiting follicular cell hyperplasia by Day 14  
15 and not recovering fully for any of the thyroid histopathological indices by 30 days post  
16 exposure. On Day 14, females showed decreased colloid and follicular cell hypertrophy at  
17 10 mg/kg-day. Males also showed a significant increase in these two thyroid response measures  
18 at this dose but also exhibited changes at lower doses and in addition showed hyperplasia.  
19 By 90 days, all three response measures (colloid depletion, follicular cell hypertrophy, and  
20 follicular cell hyperplasia) in both sexes were significant at 10.0 mg/kg-day, again indicating a  
21 LOAEL at 10 and a NOAEL at 1 mg/kg-day. Recovery of the thyroid histopathological changes  
22 was essentially complete by 30 days post-exposure although the males did have some indication  
23 of residual toxicity.

24 The BMD analyses for these data are found in Table 5-1 and Figures 5-1 and 5-2. Data for  
25 females and males were combined. The BMDL for colloid depletion and hypertrophy at 14 days  
26 were 0.28 and 0.017 mg/kg-day, with no estimate for hyperplasia. By 90-days, the BMDL values  
27 decreased for colloid depletion and hypertrophy to 0.03 and 0.008 mg/kg-day. The BMDL value  
28 for hyperplasia was 2.09 mg/kg-day. No observed effect was estimated for the 120 day value.

29 Estrous cyclicity was evaluated for 3 weeks prior to sacrifice in all females of the 90- and  
30 120-day termination groups by examining daily vaginal smears. The number and percentage of  
31 females cycling and the mean cycle length were determined for each group. There is an apparent

1 dose-related response for the absolute number and proportion of females with an abnormal  
2 estrous cycle (defined as less than 3 or more than 5 days). The number and percentage of  
3 females with at least one abnormal cycle in those females cycling was 1/10 (10%), 1/10 (10%),  
4 5/9 (56%), 6/9 (67%), 0/8 (0%), and 0/10 (0%) at the 0, 0.01, 0.05, 0.2, 1.0, and 10-mg/kg-day  
5 doses. The proportion began to increase at the 0.05 mg/kg-day dose level, peaked at the  
6 0.2 mg/kg-day dose level, and then declined at the two higher doses. This suggests the  
7 possibility of an inverted U-shaped dose-response pattern. Examination of the 120-day data  
8 (after 30-day recovery) also revealed changes in cyclicity with 1/5 (20%), 1/7 (14%), 1/6 (16%),  
9 and 4/6 (67%) females not cycling in the 0.0, 0.05, 1.0, and 10-mg/kg-day groups, respectively.  
10 Because the number of rats in the add-on groups (n = 10) did not provide the level of statistical  
11 power that would be desired, this indication of an effect in a study with limited power was of  
12 concern in 1998, but the results of the two-generation reproductive study completed in 1999 did  
13 not indicate any effects on this endpoint (Section 5.5.1).

14 Sperm samples were obtained from all male rats terminated after 90 or 120 days for  
15 evaluation of sperm count, concentration, motility, and morphology. The mean percentage of  
16 normal sperm was calculated for each group. There were no treatment-related effects on sperm  
17 parameters noted although again the number tested is small. The effects on the percentage of  
18 normal sperm appear to be artifacts because of a single outlier in each of the two groups with  
19 lower means. These occurred at different dose levels in the exposure versus recovery phases.  
20

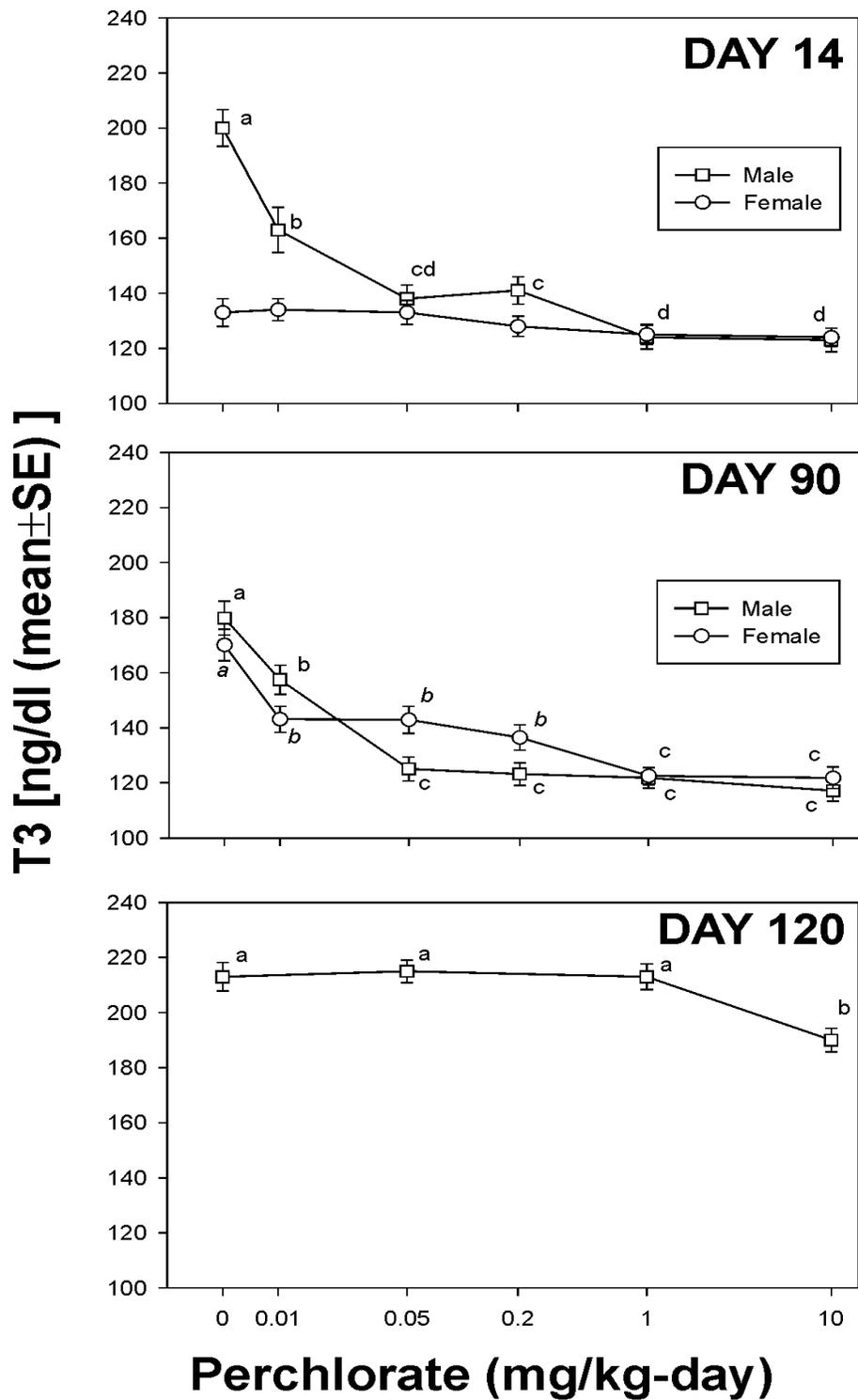
### 21 **5.2.3.2 Thyroid and Pituitary Hormone Analyses**

22 The assays for T4, T3, and TSH were performed using RIA kits according to the  
23 manufacturer's standard procedures. Assay kits from the same batch number and with the same  
24 expiration date were used for each animal termination period (Study Days 14, 90, or 120).  
25 Samples and standards were run in triplicate. The Springborn Laboratories report included an  
26 appendix (Springborn Laboratories, Inc., 1998; Appendix I) containing the results of these  
27 thyroid hormone assays. The Springborn report used a series of individual ANOVA tests to  
28 determine main effects of treatment for all three hormones in both genders and at three time  
29 points during the study (Day 14, Day 90, and Day 120 a [30-day recovery time]). As part of its  
30 1998 assessment, EPA reanalyzed these thyroid hormone data using three-way ANOVA tests,  
31 one for each of the three hormones, to allow for a statistical comparison of the interaction

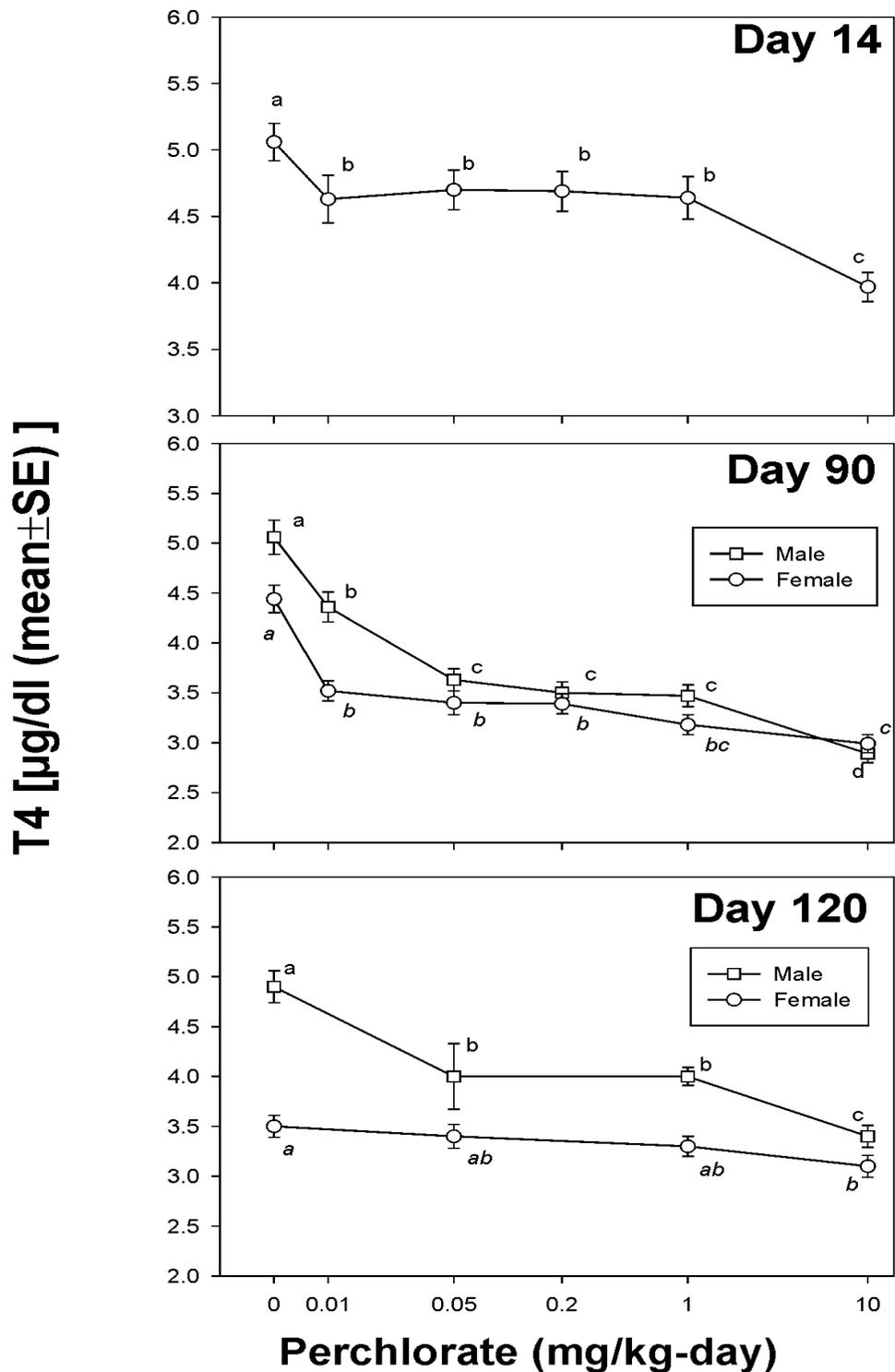
1 between gender, time, and treatment (Crofton, 1998b). The Crofton (1998b) analysis also  
2 contains a printout of all of the individual animal data, an omission from Springborn  
3 Laboratories, Inc. (1998). As suggested in the external peer review (Research Triangle Institute,  
4 1999), EPA reanalyzed these data from each hormone at each time point (Day 14, Day 90, and  
5 Day 120) with two-way ANOVA tests. Gender and treatment (dose) were used as independent  
6 between-subject variables. Dependent variables were T3, T4, and TSH. Step-down ANOVA  
7 tests were conducted as indicated by significant interactions (Crofton and Marcus, 2001; Marcus,  
8 2001). Mean contrasts were performed using Duncan's Multiple Range Test.

9 Results of the EPA reanalyses, shown in Table 5-2 and illustrated in Figures 5-5 through  
10 5-7, are similar to those stated in the contract report (Springborn Laboratories, Inc., 1998) with a  
11 few notable exceptions. First, there is only a marginal interaction between gender and treatment,  
12 resulting from a slight difference in magnitude of effects between genders. However, no  
13 differences in LOAELs between genders were observed (with minor exceptions likely caused by  
14 small changes in variance between groups, which are probably not biologically significant [see  
15 below]). Results of the analyses for each thyroid hormone and TSH are discussed individually.

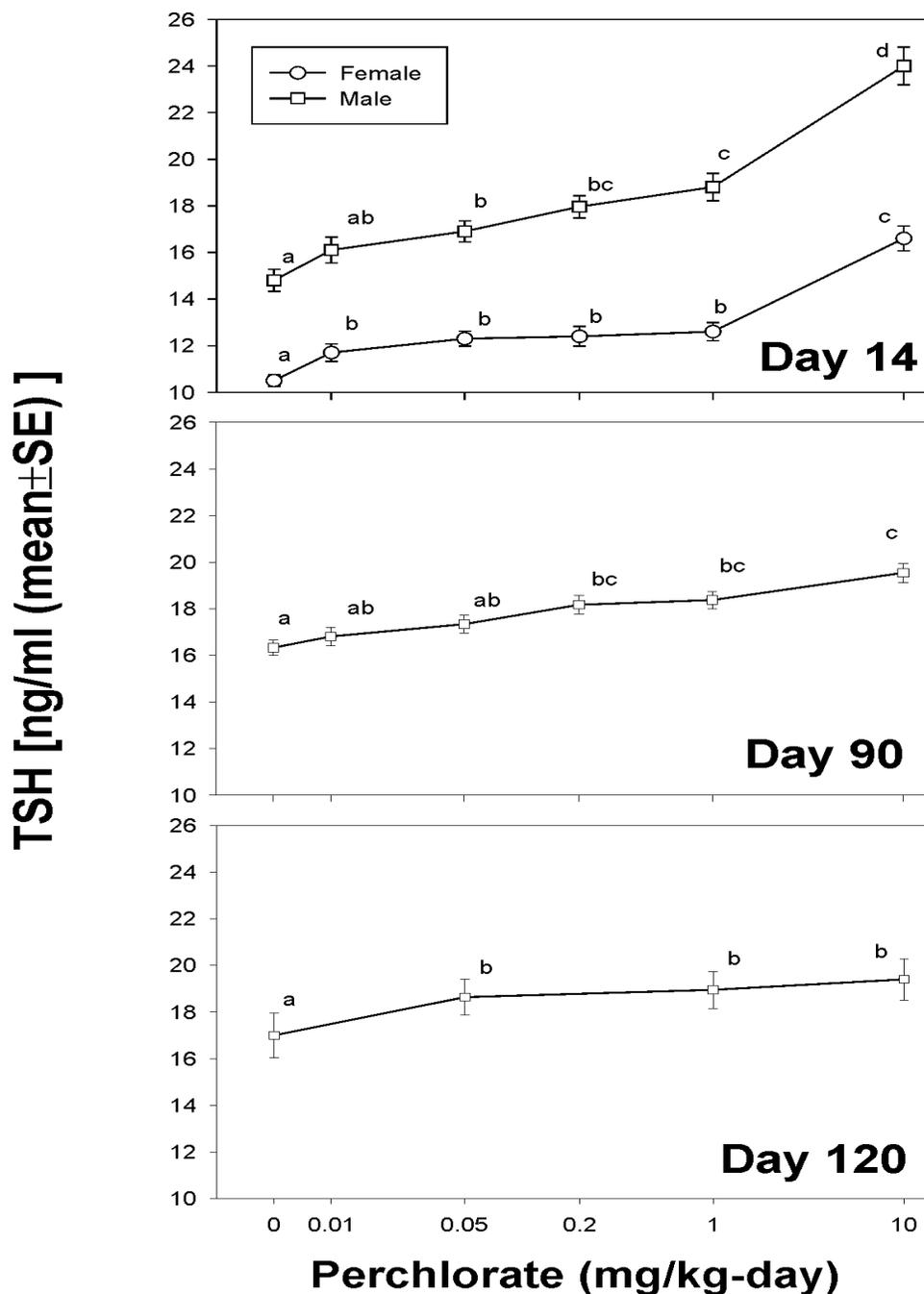
16 There were significant day-by-gender-by-treatment interactions for T3 on Day 14 and  
17 Day 90. Therefore, separate ANOVA tests were conducted on each gender to test for a main  
18 effect of treatment. Lack of a significant gender-by-treatment interaction on the 120-day data led  
19 to one subsequent ANOVA to test for a main effect of treatment. Data from Day 14 revealed a  
20 LOAEL of 0.01 mg/kg-day for males (see Figure 5-5). There was a NOAEL of 10 mg/kg-day for  
21 T3 in females. The low potency of perchlorate on T3 in females at the 14-day time point may be  
22 artifactual. Not plotted on the figure for Day 14 are all the available data from control female  
23 rats from this laboratory, including the Day 90 and Day 120 time points, and the data from two  
24 other studies. These historical data show that the group mean for females in Figure 5-5 for the  
25 14-day time point may be artificially low relative to some of the other data from the AFRL/HEST  
26 laboratory. Thus, the biological significance of this gender-dependent effect of perchlorate after  
27 14-days of exposure is suspect. Consistent with this conclusion is the significant dose-dependent  
28 decrease in T3 concentrations in female rats exposed to 0.125 to 250 mg/kg-day perchlorate in a  
29 previous 14-day exposure study by this same laboratory (Caldwell et al., 1995). The LOAEL for  
30 effects on T3 for both males and females was 0.01 on Day 90. The NOAEL for effects on T3 at



**Figure 5-5. Effects from 90-day drinking water administration of ammonium perchlorate to SD rats on serum total T3 concentrations as recalculated in Table 5-2 (Crofton and Marcus, 2001). Means with different letters were statistically different ( $p < 0.05$ ). The 120-day time point is 30 days after cessation of exposure.**



**Figure 5-6. Effects from 90-day drinking water administration of ammonium perchlorate to SD rats on serum total T4 concentrations as recalculated in Table 5-2 (Crofton and Marcus, 2001). Means with different letters were significantly different ( $p < 0.05$ ). The 120-day time point is 30 days after cessation of exposure.**



**Figure 5-7. Effects from 90-day drinking water administration of ammonium perchlorate to SD rats on serum total TSH as recalculated in Table 5-2 (Crofton and Marcus, 2001). Data of Springborn Laboratories, Inc. (1998). A main gender-by-treatment interaction was observed for Day 14, but not Days 90 and 120; therefore, data are presented separately for males and females on Day 14 and collapsed across gender for Days 90 and 120. Means with different letters were significantly different ( $p < 0.05$ ). The 120-day time point is 30 days after cessation of exposure.**

1 Day 120 was 10 mg/kg-day, indicative of a recovery of T3 concentrations after cessation of  
2 treatment.

3 There were significant day-by-treatment interactions for effects on T4 at the 90- and  
4 120-day time points but not at the 14-day time point. Mean contrast tests for Day 14 data  
5 revealed a free-standing LOAEL of 0.01 mg/kg-day for effects on T4 in both sexes. The  
6 0.01 mg/kg-day dosage was also a free-standing LOAEL on Day 90 for effects on T4 in both  
7 sexes. Analysis of the data from the 30-day recovery period (the Day 120 time point) revealed a  
8 free-standing LOAEL of 0.05 mg/kg-day in males and a NOAEL of 1.0 mg/kg-day in females for  
9 effects on T4.

10 There was a significant day-by-gender-by-treatment interaction for TSH only on Day 14.  
11 Therefore, separate ANOVA tests were conducted on each gender to test for a main effect of  
12 treatment for the Day 14 time point. Lack of a significant gender-by-treatment interaction for the  
13 90- and 120-day data led to subsequent one-way ANOVA tests at each time point to test for a  
14 main effect of treatment. Perchlorate caused a dose-dependent increase in TSH that was apparent  
15 at the Day 14 and Day 90 time points (see Figure 5-7). The NOAEL for effects on TSH at  
16 Day 14 data was 0.01 mg/kg-day in the males. The 0.01 mg/kg-day dose was a free-standing  
17 LOAEL in the females. This small difference between males and females likely is caused by  
18 small changes in variance between groups rather than by a biologically significant difference (the  
19 absolute increase relative to the control mean in the 0.05-mg/kg-day female group is actually  
20 smaller than the same comparison in the males). The TSH concentrations did not recover to  
21 control values 30 days after cessation of treatment with a free-standing LOAEL at 0.05 mg/kg-  
22 day in both sexes.

23 The data demonstrate a dose- and time-dependent effect of perchlorate on thyroid hormones  
24 and TSH. There was no LOAEL established in this data set due to multiple effects at the lowest  
25 dose of 0.01 mg/kg-day. There was some evidence of recovery at the Day 120-evaluation  
26 (30 days after cessation of treatment). The NOAEL for effects on T3 increased to 1.0 mg/kg-day.  
27 However, the omission of the 0.01 mg/kg-day dose group at the 120-day time point make it  
28 difficult to conclude about a recovery of effects on T4 and TSH.

## 5.3 DEVELOPMENTAL NEUROTOXICITY STUDIES

Concern for potential neurodevelopmental sequelae was warranted given the established mode of action for perchlorate, and the original 1997 testing strategy included a developmental neurotoxicity study (Argus Research Laboratories, Inc., 1998a). Results of that study raised additional issues and concerns so that the external peer review convened in 1999 recommended additional testing. This section describes results of the available studies that tested neurodevelopmental indices per se. The 1998 neurodevelopmental study is reviewed in Section 5.3.1. Results of the new study on motor activity are reviewed in Section 5.3.2. The “Effects Study” repeated the study of brain morphometry as a measure of neurodevelopmental toxicity and is reviewed in Section 5.3.3.

### 5.3.1 The 1998 Developmental Neurotoxicity Study

The neurobehavioral developmental study of ammonium perchlorate that was part of the original 1997 testing strategy was performed by drinking water administration in Sprague-Dawley rats (Argus Research Laboratories, Inc., 1998a). A schematic of this study design is provided as Figure A-1 (Appendix A) of this document to aid understanding of terminology and the protocol. It should be noted that Argus Laboratories identifies the day of birth as PND1; therefore, the age of PND10 and PND22 actually correspond to PND9 and PND21 in this study. The description of the study design will use the Argus nomenclature in order to readily compare with the contract report. Subsequent supplemental data submittals and additional analyses pertaining to this were requested by EPA and provided by Argus Laboratories study (York, 1998a,b,c,d,e).

Female rats (25/dosage group) were administered target doses of 0, 0.1, 1.0, 3.0, and 10 mg/kg-day by continual access to ammonium perchlorate in nonchlorinated RO deionized water beginning on gestation day zero (GD0) and ending at scheduled sacrifice. Test substance concentrations were evaluated weekly, based on actual water consumption levels recorded the previous week and adjusted as necessary to more closely achieve the target dose levels. Test solutions were prepared weekly. The stability of the stock solution and that concentrations agreed well with nominal concentrations were determined by AFRL/HEST (Argus Research

1 Laboratories, Inc., 1998a; Appendix J). Feed and water consumption were recorded daily during  
2 exposure.

3 After acclimation for 14 days, virgin female rats were cohabited with breeder male rats  
4 (one male rat per female rat) for a maximum of 7 days. Female rats with spermatozoa observed  
5 in a vaginal smear or a copulatory plug observed in situ were considered to be at GD0. The  
6 F0-generation dams were examined at approximately the same time each day during the exposure  
7 period for signs of maternal behavior, autonomic dysfunction, abnormal postures, abnormal  
8 movements or behavior patterns, and unusual appearance. Pregnancy outcome measures  
9 evaluated at birth included pregnancy rate, duration of gestation, number of implantation sites,  
10 gestation index (number with live pups/number pregnant), number of pups/litter, sex ratio of  
11 pups, and viability and lactation indices. Maternal body weight was recorded on GD0, daily  
12 during the exposure period, weekly during the post-weaning period, and at sacrifice. The same  
13 set of signs as examined during exposure were evaluated on a weekly basis during post-weaning.  
14 Thyroids from all F0-generation rats were weighed and evaluated histologically. Five dams per  
15 group were selected for sacrifice and blood collection on post-natal day 10 (PND10) from those  
16 with no surviving pups or with litters of less than eight pups. Thyroid and pituitary hormone  
17 analyses (T3, T4, and TSH) were done on the blood (see Section 5.3.1.3). All dams not selected  
18 for continued observation were sacrificed on PND22.

19 Pups (F1-generation) were counted and clinical signs were recorded once daily during  
20 pre-and post-weaning. Body weight was recorded on PNDs 1, 5, 8, 12, 14, 18, and 22 and then  
21 weekly during post-weaning. Feed consumption values were recorded weekly during  
22 post-weaning. Pups that appeared stillborn and those that died before initial examination on  
23 PND1 were examined for vital status, and the gross lesions were preserved. Pups that were not  
24 selected for continued observation were sacrificed and necropsied on PND5. Blood was sampled  
25 for thyroid and pituitary hormone analysis, and the thyroids were examined histologically. The  
26 F1-generation pups not selected for continued observation on PND10 (n = 102) were sacrificed  
27 and examined for gross lesions. Post-weaning pups that were selected for continued observation  
28 were given ammonium perchlorate in RO deionized water with chlorine (added at a maximum of  
29 1.2 ppm as a bacteriostat).

30 Other pups (F1-generation) were assigned to four different subsets for additional  
31 evaluations. The first male and female pup (1/sex/dose; total of 97 male and 100 female pups)

1 were assigned randomly to Subset 1 for brain weight and neurohistological examination  
2 (including morphometric measurements). All pups were selected for fixed brain weights on  
3 PND12; 6/sex/dose (total of 30 male and 30 female pups) were selected for neurohistological  
4 examination. The second male and female pup (1/sex/dose; total of 100 male and 100 female  
5 pups) were assigned randomly to Subset 2 for passive avoidance testing on PNDs 23 to 25 and  
6 PNDs 30 to 32; water maze testing on PNDs 59 to 63 and PNDs 66 to 70; and scheduled sacrifice  
7 at PNDs 90 to 92, with blood collection for thyroid and pituitary hormone analysis. The third  
8 male and female pup (1/sex/dose; total of 100 male and 100 female pups) were assigned  
9 randomly to Subset 3 for motor activity evaluation on PNDs 14, 18, 22, and 59; auditory startle  
10 habituation on PNDs 23 and 60; and scheduled sacrifice on PNDs 67 to 69. The fourth male and  
11 female pup (1/sex/dose; total of 100 male and 100 female pups) were assigned randomly to  
12 Subset 4 for regional brain weight evaluation on PNDs 81 to 86 (6/sex/dose; total of 30 male and  
13 30 female rats) and neurohistological examination on PNDs 82 to 85 (6/sex/dose; total of  
14 30 male and 30 female rats). Female pups also were evaluated for the age of vaginal patency  
15 beginning on PND28, and male pups were evaluated for the age of preputial separation beginning  
16 on PND39. A few of these measurements inadvertently went unrecorded, but the laboratory  
17 asserted that this did not affect the results because a sufficient amount of data on other rats was  
18 recorded.

### 20 **5.3.1.1 Results of General Toxicity Measures, Neurohistology, and Morphology**

21 Results in the dams (F0-Generation) revealed no treatment-related effects on food or water  
22 consumption (Argus Research Laboratories, Inc., 1998a; Appendix B, Tables B7 through B14),  
23 mortality (Appendix B, Tables B2 and B18), clinical signs (Appendix B, Table B2), necropsy  
24 (Appendix B, Table B18), body weight (Appendixes A and B, Figure A1 and Tables B3 through  
25 B6), or pregnancy outcome measures (Appendix B, Tables B15 through B16). Effects on thyroid  
26 weight, histopathology, and thyroid and pituitary hormone analyses will be discussed below in  
27 Sections 5.3.1.2 and 5.3.1.3.

28 Results in the pups (F1-generation) revealed no treatment-related effects on feed  
29 consumption (Argus Research Laboratories, Inc., 1998a; Appendix C, Tables C18 and C19),  
30 mortality (Appendix C, Tables C1 and C2), clinical signs (Appendix C, Tables C1 and C2), body  
31 weight (Appendixes A and C, Figures A2 and A3 and Tables C3 through C6), or sexual

1 development landmarks (Appendix C, Table C11). No treatment-related effects were observed  
2 on mortality, brain weight, or body weight in the pups of Subset 1 at PND12 (Argus Research  
3 Laboratories, Inc., 1998a; Tables D1 and D2), Subset 2 at PNDs 90 to 92 (Tables E3 and E4), or  
4 Subset 3 at PNDs 67 to 69 (Tables F5 and F6). Results of the neurobehavioral tests from  
5 Subsets 2 and 3 will be discussed in Section 5.3.1.4.

6 In the Subset 1 subgroup subjected to neurohistological examination (the F1 pups sacrificed  
7 on PND12), morphometric analyses revealed a 23.4% increase in the size of the corpus callosum  
8 in females and a 30.2% increase in males (not significant) at the high dose (10 mg/kg-day).  
9 Slight decreases in brain weight also were noted at the highest dose in females. In Subset 4 (the  
10 F1 pups sacrificed on PND82), there was a continued effect on the size of the corpus callosum  
11 (20.9% increase) in males, but no effect in females at the highest dose. There was also a 3.4%  
12 increase in the brain weight in males and increases in the size of the frontal cortex (9.2%) and the  
13 caudate putamen (10.2%). The EPA concluded that the effects may be significant and that  
14 analyses of the next lower dose (or, at least, historical control data for the affected endpoints)  
15 were warranted and requested additional analyses from the sponsor (PSG). York (1998d)  
16 responded with morphometry analyses of the next lower dose (3.0 mg/kg-day) of the Subset 1  
17 F1 pups at PND12. The new analysis noted, in addition to previous findings, a statistically  
18 significant increase in the anterior/posterior cerebellum size, a statistically significant decrease in  
19 the caudate putamen for the F1 PND12 female pups, and a statistical significant decrease in the  
20 hippocampal gyrus size for the F1 PND12 male pups. These effects were not considered  
21 treatment-related by the Primedica/Argus pathologist because they were not dose dependent.

22 A preliminary reanalysis by EPA (Crofton, 1998c) of the control, 3- and 10-mg/kg-day  
23 groups (York 1998d) was restricted to the corpus callosum because this was the area with the  
24 largest effect. The analysis revealed no interaction of gender and treatment; however, there was a  
25 significant effect of treatment ( $F[2,30] = 7.65, p < 0.0021$ ). There was a significant increase in  
26 the size of the corpus callosum only in the 10-mg/kg-day group. Group means were 288, 278,  
27 and 366  $\mu\text{m}$  for the controls and 3- and 10-mg/kg-day groups, respectively. Incorporation of  
28 historical control data from both PND10 and PND12 (mean for controls = 264  $\mu\text{m}$  for PNDs 10  
29 and 265  $\mu\text{m}$  for PND12; York, 1998a) supports the conclusion that the control values for corpus  
30 callosum size in the data set are within the “normal” range (York 1998a; see also Argus Research  
31 Laboratories, Inc., 1998a).

1 EPA did not agree with the argument put forth by Argus Research Laboratories, Inc.  
2 (1998a) that these effects were “not suggestive of a neurotoxic effect” because of “an unknown  
3 biological significance.” EPA considers a 27% increase in the size of any brain region to be a  
4 potentially adverse effect (U.S. Environmental Protection Agency, 1998e), and designated  
5 10 mg/kg-day as the LOAEL and the NOAEL at 3 mg/kg-day for these changes in brain  
6 histology. No additional evaluation of the brains from the neurohistological examination of  
7 Subset 4 pups (PND82 to PND85) were ever submitted to EPA although it was suggested again  
8 that the next lower dose group be analyzed because of the significant increases in brain weights  
9 and in the frontal cortex and corpus callosum measurements for the males in the high-dose group.

10 Additional analyses of the brain morphometry were provided by the EPA at the 1999  
11 external peer review (Geller, 1999a) that corroborated the preliminary finding of Crofton  
12 (1998c). The data were analyzed using a 2-way ANOVA, with dose and sex as independent  
13 variables. To correct for multiple comparisons, the acceptable alpha for significance (for all  
14 interaction main effects) was corrected to 0.016 (alpha of 0.05 divided by the square root of the  
15 number of ANOVA tests).

16 Significant effects of dose were found in corpus collosum, hippoacampal gyrus, anterior  
17 and posterior cerebellum, and caudate putamen. An effect of sex was also found in caudate  
18 putamen. The effect on corpus callosum was confirmed and showed an increase in size at the  
19 10 mg/kg-day dose. Hippocampal gyrus (12% less than control) and caudate putamen (7.3% less  
20 than control) showed a decrease in size at the 3 mg/kg-day dose, with no significant difference  
21 between control and high dose, yielding a U-shaped dose response. The anterior and posterior  
22 cerebellum showed a significant increase in size at the 3 mg/kg-day group (13%).

23 Because of concern for this effect voiced at the 1999 external peer review, the blocks of  
24 brain tissue were evaluated to determine if they could be refaced and additional sections  
25 evaluated. It was determined that the remaining materials were of insufficient quality for  
26 additional sectioning and histological evaluation (Harry, 2001). As an alternative, brain  
27 morphometry measurements were included in the “Effects Study”, described below in  
28 Section 5.3.3, to determine if the alteration in brain morphometry could be repeated.

### 1 **5.3.1.2 Evaluation of Thyroid Histopathology**

2 Appendix O of the Argus Research Laboratories, Inc. (1998a) neurodevelopmental study  
3 presents thyroid histopathology data provided by the sponsor (AFRL/HEST). Note that the data  
4 analyzed by EPA in the 1998 document for PND5 F1-generation rat pups are from the final  
5 report for the PND5 time point (Channel, 1998c). Channel (1998c) reported that the decrease in  
6 follicular lumen area in these pups at PND90 to PND92 showed no significant differences  
7 between dose groups and controls for either females or males based on t-test or Mann-Whitney  
8 Rank Sum Test (M-W RST). These data suggest a recovery from the effects observed in the  
9 thyroids of the pups at PND5.

10 The report also contained measurements, performed by Dr. William Baker of AFRL/HEST,  
11 of both follicular epithelial cell height and the follicular lumen diameter. These data were  
12 subsequently formally transmitted to EPA by consultative letter (Channel, 1998c) in Microsoft  
13 Excel<sup>®</sup> spreadsheets. For the final morphometric study (Channel, 1998c), the arbitrary decision  
14 based on ease of detection of this region in digitized images was made by Dr. William Baker to  
15 focus on only a lumen area measurement because of time constraints (Jarabek, 1998). The mean  
16 follicular lumen area represents the mean area of all follicular lumens measured from the three  
17 histological sections sampled from each rat and is expressed in microns. In the opinion of  
18 Dr. Charles Capen of Ohio State University (Crofton, 1998d), the measurement of follicular  
19 height is usually more sensitive than those of follicle diameter and lumen area. In support of this  
20 opinion, data collected by Dr. Baker (Argus Research Laboratories, Inc., 1998a; Appendix O)  
21 demonstrated significant increases in males rats in the incidence of follicular epithelial cell  
22 hypertrophy at doses much lower than those doses that increased the incidence of decreased  
23 lumen area. The difference observed between standard histopathology as originally reported by  
24 Argus Research Laboratories, Inc. (1998a) and the thyroid morphometry performed by Dr. Baker  
25 was analyzed extensively by the EPA in its 1998 assessment. The results indicated that the  
26 morphometry performed on lumen size was a less sensitive measure of thyroid histopathology.  
27 The analyses of the thyroid morphometry are retained in this reassessment; whereas, the PWG  
28 review results will be presented below for the histopathology.

29 Data from the dependent measure (follicle lumen size) based on the morphometric analyses  
30 (Channel, 1998c) were available for pups sacrificed at ages PND5 and PND90. These data were  
31 reanalyzed by EPA (Crofton and Marcus, 2001). Because there was only one block of animals at

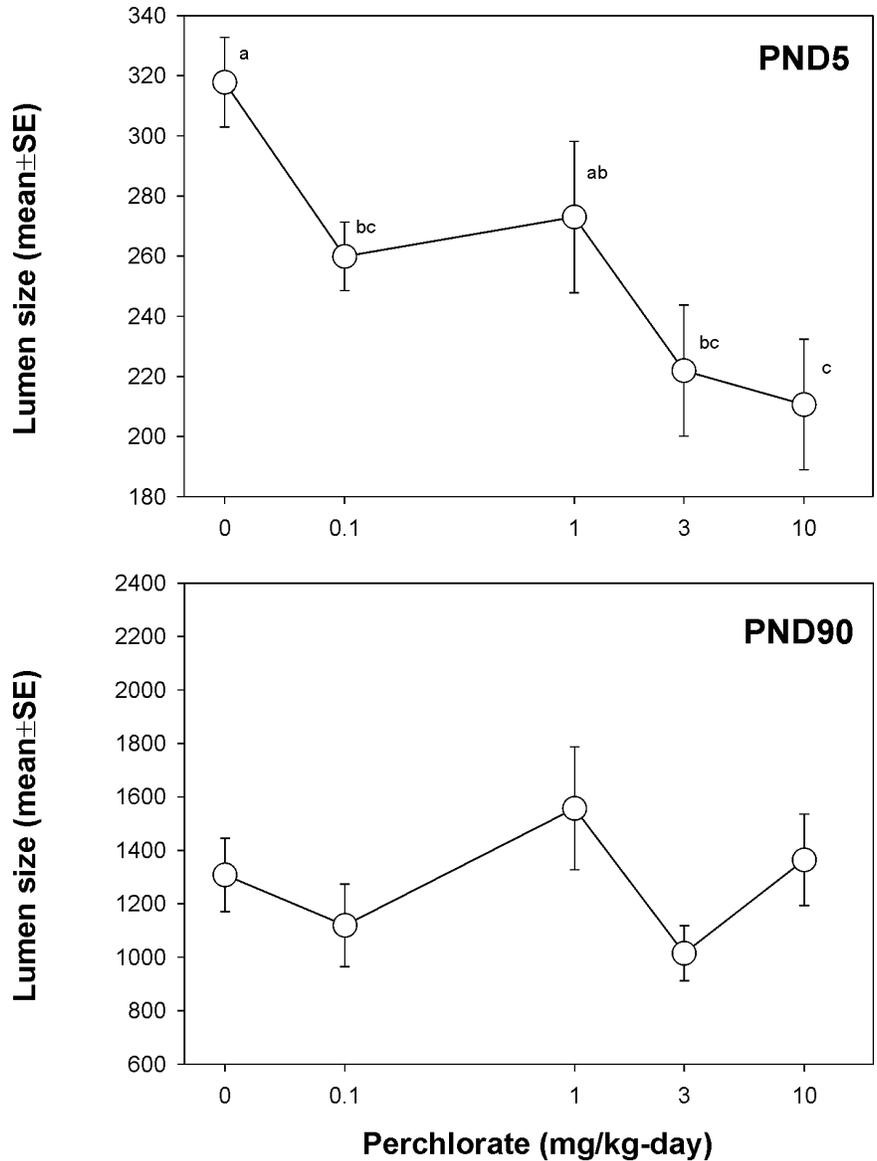
1 PND90 compared to two blocks of data at PND5, and because the slides for PND90 were  
2 processed at a much later time, the data for the two ages were analyzed separately. Data from  
3 PND5 pups were subjected to three-way ANOVA tests with gender, treatment (dose), and block  
4 (two separate analyses of separate blocks of data) as independent between-subjects variables.  
5 Data from PND90 were subjects to a two-way ANOVA with gender and treatment (dose) as  
6 independent between-subjects variables. Step-down ANOVA tests were conducted as indicated  
7 by significant interactions and recalculated by Crofton and Marcus (2001) and Marcus (2001).  
8 Mean contrasts were performed using Duncan's Multiple Range Test. Note that in the Crofton  
9 and Marcus (2001) memorandum the 0.1 mg/kg-day dose is incorrectly labeled as 0.3 mg/kg-day.  
10 There was a significant main effect of treatment on lumen size for all doses at PND5, resulting in  
11 a free-standing LOAEL of 0.1 mg/kg-day. The data are plotted in Figure 5-8. There was no  
12 significant effect of perchlorate on lumen size at PND90.

13 The thyroid histopathology as reviewed and reported by the PWG can be found in Wolf  
14 (2001; Tables 9 through 13). This report includes corrections for slides sent to EPA that  
15 contained animals with autolysis and those necropsied at different times than indicated for the  
16 study protocol or to exclude dams that did not have litters.

17 The F0 generation dams (Wolf, 2001: Table 9) exhibited decreased colloid and increases  
18 in both hypertrophy and hyperplasia. A clear dose-response was not evident, however, with the  
19 possible exception of colloid depletion at levels above 0.1 mg/kg-day.

20 Thyroid histopathology in the pups on PND4 (Wolf, 2001: Tables 10 and 11) was more  
21 pronounced, with colloid depletion and increases in hypertrophy at 0.1 and 3 mg/kg-day.  
22 Hyperplasia appeared to be effected at 3 mg/kg-day. The BMD analyses presented in Table 5-1  
23 support these levels with BMDL estimates for colloid depletion at 0.33, increased hypertrophy at  
24 0.88, and increased hyperplasia at 3.62 mg/kg-day. These results were obtained with a  
25 constrained model, but an adequate fit is obtained by fitting the model without restricting the  
26 exponent on dose to be  $\geq 1$  and results in a BMDL for pups on PND4 in this study at 0.009 for  
27 colloid depletion (Geller, 2001a).

28 The argument for the lack of biological plausibility of unrestricted functions is based on  
29 cancer modeling theory from the early 1960s (Mantel and Bryan, 1961) that attempted to derive a  
30 default procedure for modeling tumor data at the time when cancer was thought to be a one-stage  
31 process and many bioassays used only 1 dose and control. Given the increased sophistication of



**Figure 5-8. Effects from maternal drinking water administration of ammonium perchlorate to SD rats on thyroid gland follicular lumen size in F1-generation offspring on PND5 as recalculated in Crofton and Marcus (2001). Data of Channel (1998c) and Argus Research Laboratories, Inc. (1998a). Means with different letters were significantly different ( $p < 0.05$ ). Daily dose was estimated from water consumption data.**

1 contemporary bioassays and the level of organization at which effects are now being identified  
2 (i.e., precursor events at the cellular and molecular levels), Hasselblad et al. (1995) have argued  
3 that restricting the slopes of fits to the data prioritizes mathematical convenience over fitting the  
4 data. The thyroid hormone data show exquisite sensitivity to very low doses of perchlorate. This  
5 suggests that models fit with nonsupralinear slopes and lower doses need to be tested. It is  
6 interesting to note that PWG results for colloid depletion are very similar to the 1998 EPA  
7 analysis on the previous histopathological read by Argus Laboratories, Inc. (1998a) for  
8 hypertrophy/hyperplasia that resulted in a BMDL of 0.1 mg/kg-day.

9 Histopathology in the animals from PND90 and PND92 (Wolf, 2001: Tables 12 and 13)  
10 indicated variable effects on colloid depletion, hypertrophy, and hyperplasia. As indicated in  
11 Table 5-1, a BMDL was only calculated with confidence for colloid depletion with a resultant  
12 estimate of 0.03 mg/kg-day.

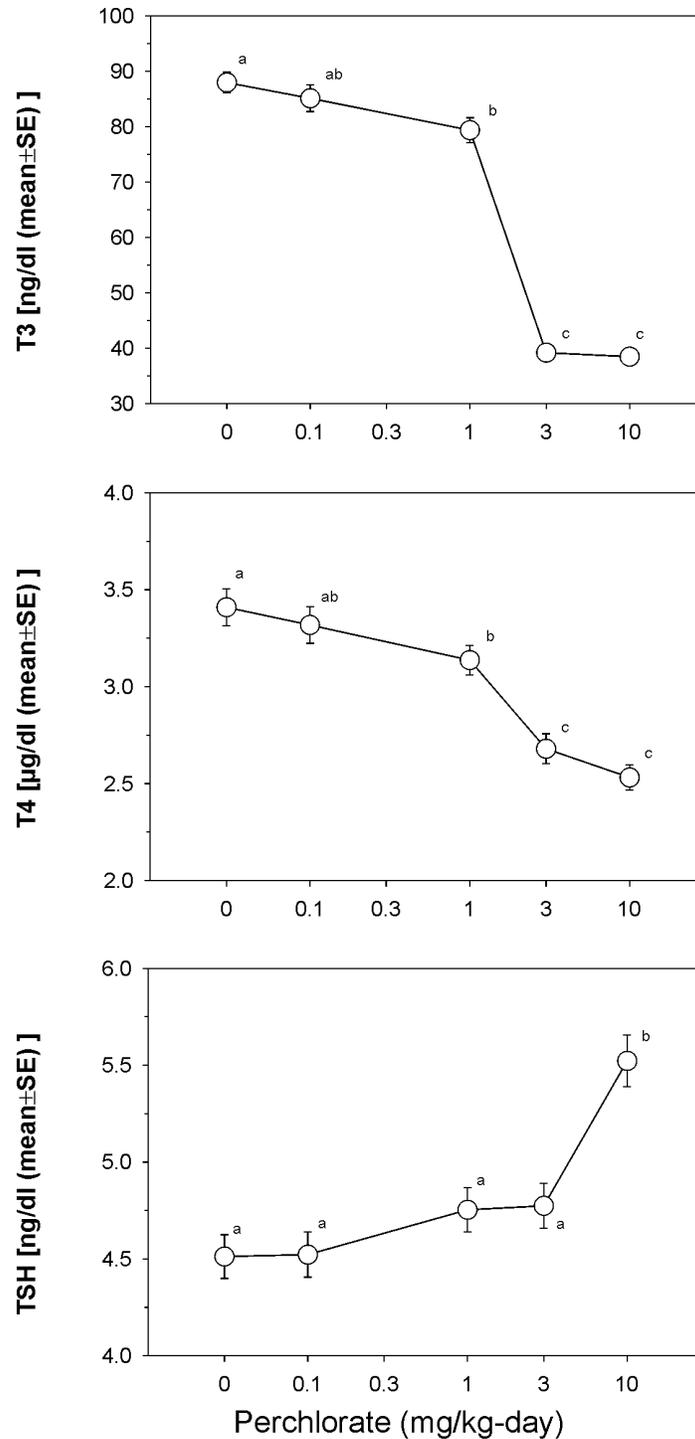
13 Evaluation of the histopathology in this study indicate that the pups are the most sensitive  
14 with a BMDL between 0.009 and 0.33 mg/kg-day.

### 16 **5.3.1.3 Thyroid and Pituitary Hormone Analyses**

17 Serum was collected and thyroid hormone analyses performed as part of the  
18 neurodevelopmental study (Argus Research Laboratories, Inc., 1998a; Crofton, 1998f)). The  
19 following is a statistical analysis of the thyroid and pituitary hormone data (T4, T3, and TSH)  
20 found in that report (Crofton and Marcus, 2001). At the time of this assessment, individual  
21 animal data were available from both the F1-generation pups (male and female samples were  
22 pooled for each litter) on PND5 and the F0 generation (parents) on post-partum Day 10 (PP10).  
23 Only the F1 data were reanalyzed because of the very limited (n = 2 to 5/group) data for the  
24 parental F0 PP10 group.

25 All data were supplied in Microsoft Excel<sup>®</sup> spreadsheets via E-mail by Dr. David Mattie  
26 (AFRL/HEST). Data for dependent measures (T4, T3, and TSH) were subjected to separate one-  
27 way ANOVA tests. Treatment (dose) was used as the independent, between-subjects variable.  
28 Mean contrasts were performed using Duncan's Multiple Range Test.

29 There were significant main effects of treatment for all the hormones. The data are plotted  
30 in Figure 5-9. Results of these reanalyses are similar to those stated in the report (Argus  
31 Research Laboratories, Inc., 1998a). There was a significant decrease in both T3 and T4, as well



**Figure 5-9. Effects from maternal drinking water administration of ammonium perchlorate to SD rat F1-generation pups on serum total T3 (A), T4 (B) and TSH (C) concentrations (ng/dL; mean ± SE) as recalculated in Table 5-2 (Crofton and Marcus, 2001). Data of Argus Research Laboratories, Inc. (1998a). Means with different letters were significantly different (p<0.05). Daily dose was estimated from water consumption data.**

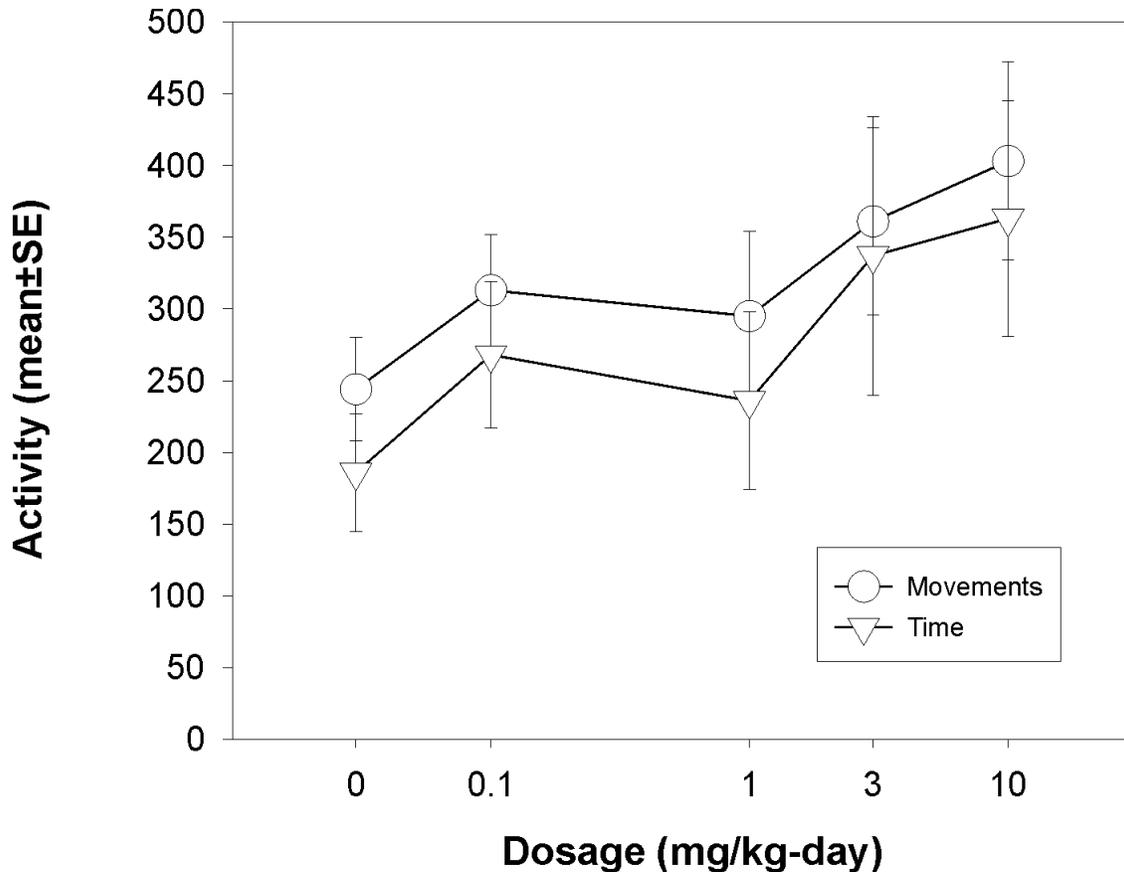
1 as the expected increase in TSH. The NOAEL for the effects of perchlorate on T3, T4, and TSH  
2 are 0.1, 0.1, and 3.0 mg/kg-day, respectively. These results are consistent with the known  
3 mechanism-of-action of perchlorate (inhibition of thyroid hormones). The increased TSH is  
4 likely a result of the activation of the pituitary-thyroid feedback mechanism.

#### 5 6 **5.3.1.4 Behavioral Evaluations**

7 The 1998 EPA review of the behavioral evaluations performed on Subset 3 pups agreed  
8 with the Argus Research Laboratories, Inc. (1998a) report with one exception regarding an  
9 increase in motor activity in male rats on PND14 that no perchlorate-induced changes were  
10 detected in any of the other behavioral indices (i.e., passive avoidance, water maze, auditory  
11 startle). The EPA disagreed with the Argus Research Laboratories, Inc. (1998a) report and  
12 subsequent submissions (York, 1998a,b,c,d,e) with regard to the significance of the motor  
13 activity changes.

14 The data originally were analyzed using two separate three-way ANOVA tests (age,  
15 treatment, and habituation block), one for each gender (Argus Research Laboratories, Inc.,  
16 1998a). This analysis demonstrated a significant decrease in the amount of habituation in the  
17 two highest dose groups on PND14 in the male pups. There were no changes detected at any  
18 other ages (i.e., PND18, PND22, PND59). On initial review by EPA, it was recommended to the  
19 sponsor (PSG) that an additional analysis of the data be conducted using gender as a  
20 within-subject variable, or alternatively, to use a nested design with gender nested under litter  
21 (see Holson and Pearce [1992] and Cox [1994], for a review of statistical methods used in  
22 developmental studies and the importance of using litter as the unit of measure). The EPA also  
23 questioned why the method or statistics did not detect significance for the dose-dependent  
24 increase in total session counts that amounted to a 95% increase over controls in the highest  
25 dosage group (see Figure 5-10). The response from Argus Laboratory (York, 1998b) included a  
26 new analysis in which gender was used as a between-subjects variable. No interactions with, or  
27 main effects of, treatment were found in this analysis.

28 EPA remained concerned that Argus Research Laboratory and the sponsor (PSG) failed to  
29 respond adequately to the request for an explanation of why the analysis failed to detect  
30 significance in the PND14 motor activity for the male rats. Figure 5-10 illustrates the clear  
31 dose-dependent increase in two different measurements of motor activity: (1) time-spent-in-



**Figure 5-10. The effects of developmental exposure to perchlorate on motor activity in male rats on PND14. Data of Argus Research Laboratories, Inc. (1998a). The dose-dependent increases in both number of movements and time spent in movement were not statistically different, even though the increases were substantial at the higher dosages.**

1 movement (“time”) and (2) total number of movements (“movements”). The time variable  
 2 increased over 95% at the highest dose relative to controls (group means of 363 and 186,  
 3 respectively). The number-of-movements variable increased approximately 65% relative to  
 4 controls. Expert opinion of EPA neurotoxicologists was sought, and it was their opinion that  
 5 increases in motor activity over 50%, especially in developing animals, were clearly of concern  
 6 from a biological perspective (Crofton et. al., 1998). The critical issue for evaluation of these  
 7 motor activity data was how to resolve the difference between what is a clearly a biologically  
 8 significant alteration in behavior with a lack of statistical significance. In an attempt to resolve  
 9 the issue, EPA also requested positive control data from the testing laboratory for this device that

1 was not provided in the original report, as well as any available historical control data. York  
2 (1998a) replied with a number of positive control studies and a limited amount of historical  
3 control data from PND14 pups.

4 The positive control data were requested to help understand the sensitivity of the device in  
5 detecting increases in motor activity (i.e., what is the smallest increase in motor activity that has  
6 been detected by this device). Unfortunately, the positive control data were of limited use in  
7 interpreting the sensitivity of the device. The submission (York, 1998a) contained data from  
8 experiments with amphetamine and triadimefon in adult rats. The smallest increase in activity  
9 that was induced by either chemical was a 109% increase relative to controls. Although these  
10 effects were statistically significant, they are greater than the effects produced by the highest  
11 dosage of perchlorate in the PND14 pups. There were also positive control data from  
12 chlorpromazine-treated animals that showed significant decreases ( $\geq 32\%$ ) in activity. However,  
13 ability to detect decreases does not necessarily translate to the detection of increases.

14 The historical control data from PND14 rats were requested to help understand the  
15 variability normally found in control animals. Unfortunately, the historical control data  
16 submitted were only useful in that the data raised more suspicion that the degree of experimental  
17 control over this behavior by the testing facility was inadequate. For the time data, the control  
18 mean for the perchlorate data set was 186 sec. For the three relevant historical control data sets,  
19 the means were 1026, 965, and 458 sec. Either the lab had very little control over the behavior,  
20 or the data were from a different test apparatus or from a different usage of the same apparatus.  
21 In any case, the data were of no use in helping EPA determine the historical profile of control  
22 animal behavior in this test apparatus.

23 In lieu of the absence of useful positive control and historical control data, EPA was left  
24 with the issue of ascertaining statistical versus biological significance. There were a number of  
25 reasons for the lack of statistical significance. The first reason was the extremely large within-  
26 group variability exemplified by coefficients of variation (CV) greater than 100%. It was the  
27 opinion of Crofton et al. (1998) that this was likely caused by the inability of the testing  
28 laboratory to gain adequate control over the behavior being tested. This large variability results  
29 in very little statistical power and increases the potential for Type II errors. Normally, an  
30 increase in sample size (by additional testing) allows for adequate power to refute or support the  
31 conclusion of an effect. Given the CVs of about 100%, simple power calculations (see Cohen,

1 1987) for detecting a 40% change in one group out of five results in needed group sizes of about  
2 70 to 90 animals per group. The second reason was that the effect, a 95% increase, while rather  
3 large from a biological perspective, occurs in only one gender on only 1 day out of 4 test days.  
4 The large variability coupled with the complicated design (treatment, age, gender, and block)  
5 would tend to mask anything other than extremely large effects. This conclusion is consistent  
6 with the content of a phone conversation (Crofton, 1998g) with Dr. Simon Mats. Dr. Mats was  
7 the statistician from the contract laboratory (Primedica/Argus) who conducted the revised  
8 statistical analysis of these data. Lastly, the effect seen in the males on PND14 may indeed be a  
9 Type I error and may not be found again if this experiment was repeated.

10 The assignment of biological significance to the effect seen was supported by both the  
11 underlying mode of action of perchlorate and the effects of other chemical and physical insults on  
12 the motor activity of post-natal rats. The hypothesis that a thyrotoxic chemical would induce a  
13 delay in any aspect of nervous system development is highly plausible. A delay in the onset of  
14 habituation would be evidenced by an increase in overall counts, as well as a decrease in the rate  
15 of a habituation (Ruppert et al., 1985a,b). This delay could be quite transient. Other agents that  
16 interfere with thyroid hormones during development are known to induce delays of a few days  
17 magnitude in developmental landmarks such as eye opening (Goldey et al., 1995a,b). This is the  
18 type of effect seen on PND14 in the Argus Research Laboratories, Inc. (1998a) report.

19 Developmental exposure to numerous hypothyroid-inducing agents (e.g., propylthiouracil,  
20 methimazole) are known to result in delays in the ontogeny in many behaviors (cf., Comer and  
21 Norton, 1982; Goldey et al., 1995a,b; Schneider and Golden, 1986; Tamasy et al., 1986),  
22 including the development of habituation. However, effects of these chemicals on total motor  
23 activity counts vary from increased to decreased, depending on the chemical and age of testing.  
24 Rice (2000) has noted parallels between the features of attention deficit hyperactivity disorder  
25 (ADHD) and the behavior of monkeys exposed to polychlorinated biphenyls (PCBs). The  
26 mechanism for the gender-dependent nature of the effect of perchlorate also remains to be  
27 determined. In addition, there are numerous reports from the literature that support the biological  
28 significance of a 40 to 50% increase in motor activity in postnatal rats (cf., Campbell et al., 1969;  
29 Ruppert et al., 1985a,b).

30 In summary, EPA maintained that the increase in activity should be considered biologically  
31 significant until additional data could be marshaled to suggest or prove otherwise. The

1 inadequacy of standard parametric statistics to detect a significant difference suggested that  
2 alternative analyses should be used on these data, such as the benchmark approach. This type of  
3 statistical approach may be useful because of the inverse relationship between the data variability  
4 and the benchmark dose (BMD). The BMDL estimates were calculated for data on the  
5 movement (number of movements) and time (time spent moving) measures from the motor  
6 activity test from PND14 pups. These data were fit by a linear function with fairly shallow slope,  
7 yielding BMD estimates for movement and time of 1.94 and 1.33 mg/kg-day and BMDL  
8 estimates of 1.04 and 0.66 mg/kg-day, respectively. These BMD and BMDL estimates could  
9 serve as estimates of LOAEL and NOAEL for this data set. The estimates are in accord with  
10 doses with activity values that may have emerged as significantly different from control had the  
11 data set not had its unusually high variability. These BMD analyses bring the motor activity  
12 NOAEL more within the range of the T3 and T4 NOAEL and below that for TSH.

### 14 **5.3.2 Motor Activity Study (Bekkedal et al., 2000)**

15 In response to recommendations at the 1999 peer review for an additional study, the United  
16 States Navy (USN) performed a study that included evaluation of motor activity in Sprague  
17 Dawley rats of both sexes (Bekkedal et al., 2000). Female Sprague-Dawley rats were dosed with  
18 ammonium perchlorate for two weeks at 0, 0.1, 1.0, 3.0 or 10.0 mg/kg-day prior to mating with  
19 the breeder males and through PND10. PND1 was counted as the day when the first pup was  
20 observed in the cage. All pups within a litter were weighed on PND5 when the litters were  
21 culled to eight pups of 4 males and 4 females or as close as possible to that combination. Pups  
22 and dams from any litters with less than 8 pups were eliminated. On PND14, one male and one  
23 female were randomly selected from each litter to be used in the motor activity testing. These  
24 same animals were tested on PND14, PND18 and PND22. Nine different measures of motor  
25 activity were automatically recorded using Opto-Varimex activity meters at ten minute intervals.  
26 The measures included: frequency and time of ambulatory movements, frequency and time of  
27 stereotypic movements, frequency of movements in the horizontal plane, distance traveled in the  
28 horizontal plane, frequency of rears, total number of horizontal movements made while in the  
29 rearing position (vertical plane movements), and time spent resting.

30 Bekkedal et al. (2000) analyzed each of the nine measures of motor activity separately  
31 using a univariate repeated-measures ANOVA. The between subjects variable was perchlorate

1 dose, with 5 levels. The three within-subject variable were sex (2 levels), age (3 levels), and time  
2 block (9 levels). Due to violation of the sphericity assumption, the Greenhouse-Geisser test was  
3 employed with a fiducial limit set at  $p < 0.05$ . No statistically significant differences were found  
4 for the main effect of perchlorate exposure for any of the 9 measures nor any reliable interactions  
5 related to dose. The authors do note, however, a general pattern of dose-dependent changes in  
6 the later sessions (90-minute). They also note that this pattern, as in the previous Argus  
7 Laboratories, Inc. (1998a), suggest that exposed pups have a slightly slower rate of habituation  
8 and thus maintain a higher level of activity as compared to untreated pups. Additional follow-up  
9 tests were suggested.

### 11 **5.3.2.1 EPA and NIEHS Statistical Analyses of Motor Activity Effects**

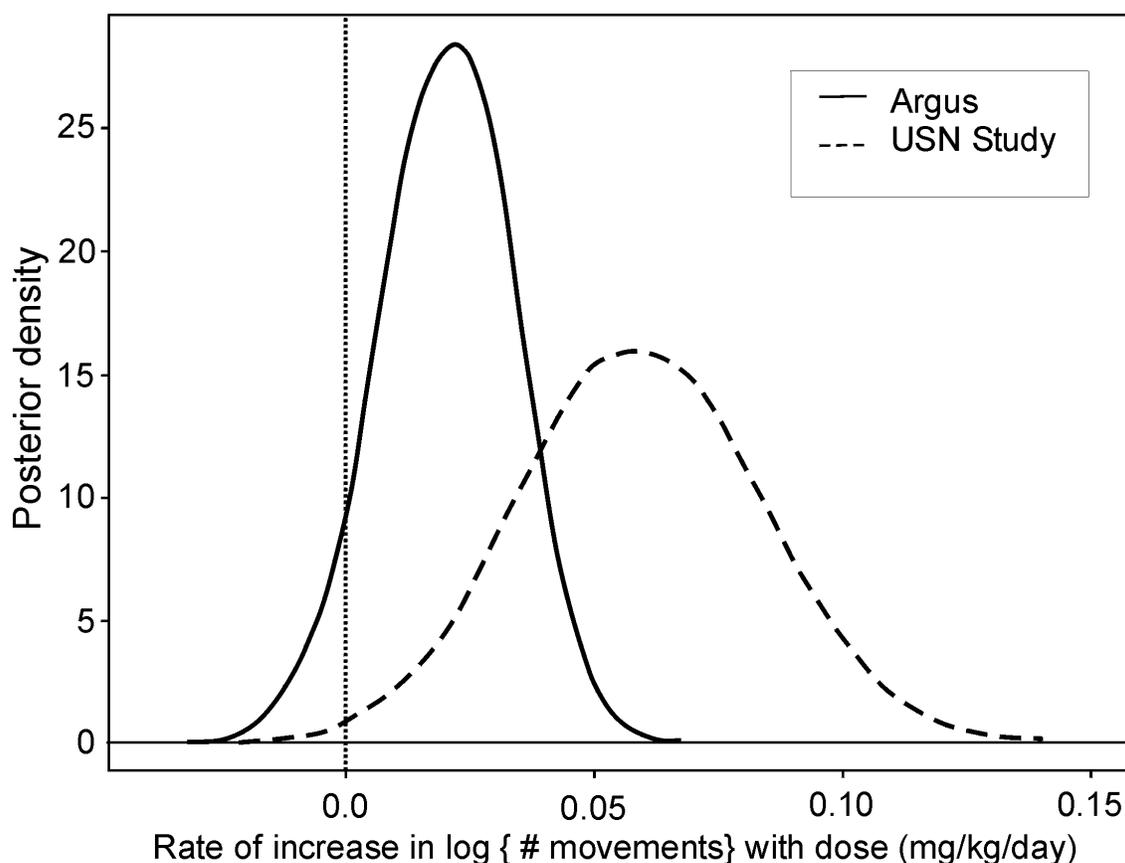
12 Because EPA was concerned about effect on motor activity in the original study and it  
13 appeared that a similar pattern of effects was again emerging in the study repeated by Bekkedal  
14 et al. (2000), EPA requested that NIEHS perform a statistical evaluation that could formally  
15 integrate the various measures together as well as statistically compare the two studies with each  
16 other (Dunson, 2001a). A Bayesian hierarchical model (Gelfand et al., 1990) was chosen to  
17 assess the weight of evidence of a dose-response trend in motor activity. A linear mixed-effects  
18 regression model (Laird and Ware, 1982) related dose, sex, age, habituation time and a  
19 habituation time x dose interaction term to the expected number of ambulatory movements, with  
20 an animal-specific intercept included to account for within-animal dependency. To complete a  
21 Bayesian specification of the model, a vague (or uninformative) but proper prior distributions for  
22 each of the unknown parameters was chosen. In particular, the prior for the parameters that  
23 related dose to motor activity was centered on a value corresponding to the null hypothesis of no  
24 effect of perchlorate. The model was fit using BUGS, a widely-used software package for  
25 Bayesian analyses (Gilks et al., 1994).

26 The analyses were conducted under a variety of different choices of prior variance for the  
27 dose parameters and prior means and variances for the other parameters in the model. The dose  
28 level associated with a 10% increase in the number of ambulatory movements by inverse  
29 estimation (refer to Appendix A in Dunson, 2001a). The choice of 10% as the benchmark level  
30 is consistent with standard practice for dichotomous outcomes. The 5% level often used for

1 continuous outcomes was judged to be too low for measuring a biologically significant increase  
2 in motor activity. Conclusions were consistent across the analyses.

3 As noted by Bekkedal et al. (2000), the effect of ammonium perchlorate on the number of  
4 ambulatory movements was found to increase significantly with habituation time (posterior  
5 probability = 0.98). In the first habituation interval there was modest evidence of an increase in  
6 motor activity with dose (posterior probability = 0.79), while in the final interval there was clear  
7 evidence of an increase in motor activity with dose (posterior probability > 0.99). The posterior  
8 density for the expected increase in the logarithm of the number of ambulatory movements at the  
9 final habituation time per unit (mg/kg-day) increase in dose of ammonium perchlorate is plotted  
10 in Figure 5-11 for the USN study (Bekkedal et al., 2000). The posterior density is centered on a  
11 positive slope and assigns low probability to a negative slope, suggesting a clear increase in  
12 motor activity with dose. The dose estimated to increase the mean number of ambulatory  
13 movements at the final habituation time by 10% is 1.62 with a 95% credible interval of (0.90,  
14 7.87). There was no evidence of an interaction between age and dose, nor of any effect of  
15 gender.

16 The previous study of Argus Laboratories, Inc. (1998a) was also analyzed in this fashion  
17 and results were very similar (Figure 5-11). In contrast to the Bekkedal et al. (2000) study,  
18 dosage began at the first day of gestation and continued through parturition and up to lactation  
19 day 10 (PND10). Dams were dosed at 0, 0.1, 1.0, 3.0 and 10.0 mg/kg-day. Movements of each  
20 pup were monitored by a passive infrared sensor. Each test session was 90 minutes in duration.  
21 The number and time spent in movement was tabulated at each five-minute interval. In order to  
22 be comparable with the USN analysis, every two of the five-minute intervals were combined into  
23 a ten-minute interval. However, the Bekkedal et al. (2000) study did not have data for PND59,  
24 so the results are not entirely comparable. Again, there was evidence of an increase in the effect  
25 of ammonium perchlorate on motor activity at the later habituation times (posterior probability =  
26 0.93). In the first habituation interval there was no evidence of an increase in motor activity with  
27 dose (posterior probability = 0.58), while in the final interval there was moderate evidence  
28 (posterior probability = 0.94). The dose estimated to increase the average of ambulatory  
29 movements in the final habituation time by 10% is 4.60 with a credible interval of (2.18,  
30 infinity). This interval was wider than the interval observed in the Bekkedal et al. (2000) study;



**Figure 5-11. Bayesian estimates of the posterior densities for the expected increase in the logarithm of the number of ambulatory movements at the final habituation time per unit dose (mg/kg-day) increase of ammonium perchlorate (Dunson, 2001a). A separate analysis for the Argus Research Laboratories, Inc. (1998a) and United States Navy (Bekkedal et al., 2000) was performed.**

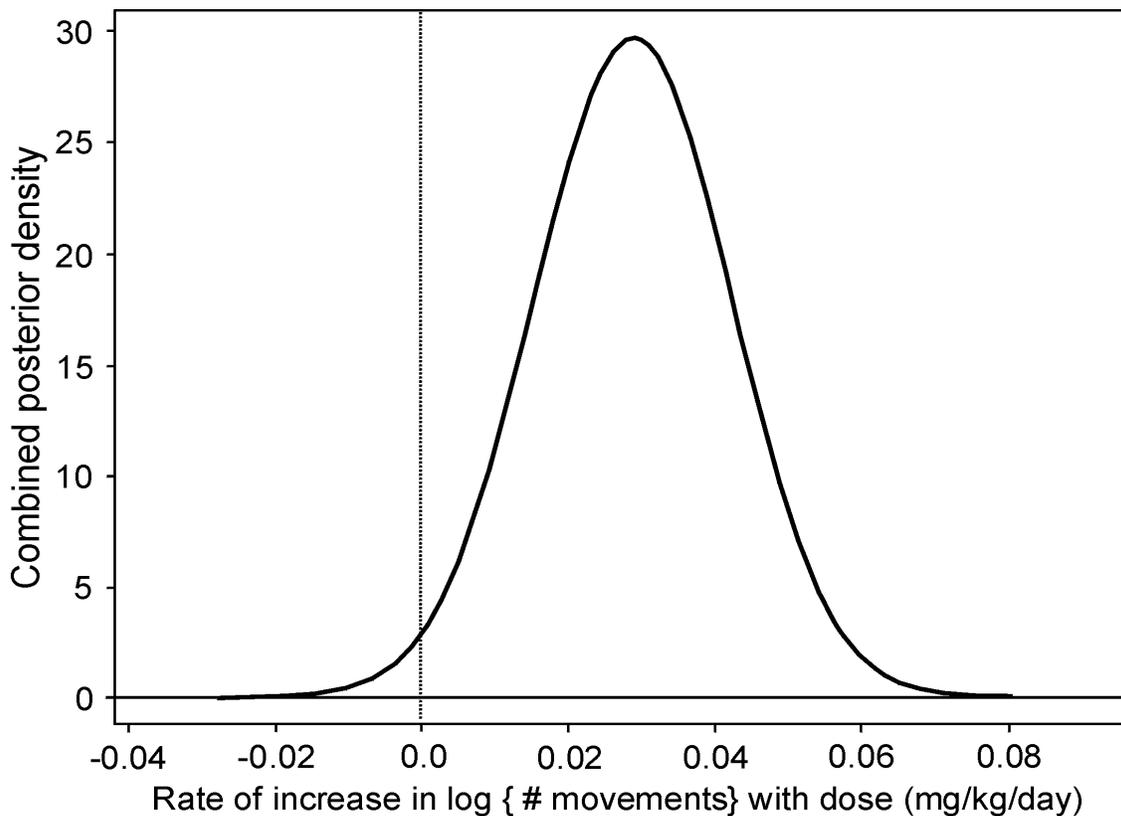
1 possibly due to greater variability in the Argus data as noted in 1998 by EPA. This result is  
 2 slightly higher than the BMD analysis (Section 5.3.1.4) estimate of 1.04 mg/kg-day.

3 One of the advantages of Bayesian analysis is that it provides for formal combination of  
 4 data from different studies. To perform a combined analysis of data from the USN Study  
 5 (Bekkedal et al., 2000) and the Argus (1998) study, a modification of the model described above  
 6 was used (Dunson, 2001a). The number of ambulatory movements was first standardized by  
 7 subtracting the overall mean and dividing by the standard deviation. A linear mixed-effects  
 8 regression model that incorporated distinct baseline parameters (i.e., intercept, age-effects,

1 habituation time effects, error variances) for the two studies was then fit, assuming common  
2 slope parameters. This approach allowed the different studies to have distinct baseline  
3 parameters, including aging effects.

4 Figure 5-12 shows the posterior density from the combined analysis of the Argus Research  
5 Laboratories, Inc. (1998a) study and the Bekkedal et al. (2000) study. In this combined analysis,  
6 the posterior probability of an increase in motor activity with dose was 0.99. For rats that  
7 averages 34.09 ambulatory movements at the final habituation time in the absence of exposure  
8 (the average value in the Argus study), the estimated dose needed to increase this average by  
9 10% is 3.33 [95% credible interval = (1.91,12.78)].

10



**Figure 5-12. Bayesian estimate of the posterior density for the expected increase in the logarithm of the number of ambulatory movements at the final habituation time per unit dose (mg/kg-day) increase of ammonium perchlorate for the combined data from the two studies of motor activity effects shown in Figure 5-12 (Dunson, 2001a).**

1           There was evidence of an increasing dose-response trend in motor activity in both the  
2 Argus Research Laboratories, Inc. (1998a) and Bekkedal et al. (2000) studies, although the effect  
3 in the Argus study was less pronounced, likely due to the variability in the data previously noted.  
4 Given this, it is remarkable that the two studies showed such similar results. The Bayesian  
5 analysis can be applied to risk assessment in an analogous fashion to the benchmark dose  
6 analysis (Hasselblad and Jarabek, 1996). The lower limit on the estimated dose corresponding to  
7 a 10% increase in motor activity relative to control can be used as a surrogate for the NOAEL for  
8 the point of departure for reference dose derivation. For the Argus Research study, the lower  
9 limit of the 95% credible interval for the dose was 2.18, while for the Bekkedal et al. (2000)  
10 study the corresponding estimate was 0.90. In the combined analysis, the lower limit was 1.91.  
11 Because of the variability in the Argus Research Laboratories, Inc. (1998a) study, a NOAEL that  
12 relied on the Bekkedal et al. (2000) was chosen at 1.0 mg/kg-day to represent effects on motor  
13 activity from these combined data.

### 14 15 **5.3.3 The 2001 “Effects Study”**

16           The Argus Research Laboratories, Inc. (2001) study was performed in response to  
17 recommendations made at the 1999 external peer review (Research Triangle Institute, 1999) for  
18 additional analyses of the thyroid and brain effects during gestation and post-natal days. Because  
19 Argus Laboratories identified the day of birth as PND1, the age nomenclature of PND5, PND10,  
20 and PND22 (Argus, 2001) is off by one day as referenced by EPA definition. These ages are  
21 therefore referred to as PND4, PND9, and PND21.

22           It should be noted that exposure in this study started two weeks prior to the start of  
23 cohabitation. The rationale was to ensure a hypothyroid state, but given the response of the rat  
24 system to perturbation, it is more likely that this resulted in the dams already compensating for  
25 the effect of perchlorate prior to pregnancy by upregulation of the NIS, making comparison with  
26 the 1998 developmental neurotoxicity study (Section 5.3.1) more difficult.

27           The thyroid and brain from one male and one female pup per litter were selected for  
28 histological and morphometric evaluation, with one set evaluated on PND4, PND9, and PND21.

1 **5.3.3.1 Results of General Toxicity Measures**

2 There were no remarkable clinical or necropsy observations. Average body weights and  
3 body weight changes for female rats were comparable among the five exposure groups through  
4 the pre-cohabitation and gestation periods. Body weight gains for female rats in the 1.0 and  
5 30.0 mg/kg-day target dosage groups were significantly increased on PND12 to PND15  
6 compared to the carrier group. These increases were not considered treatment-related because  
7 they were a singular occurrence and were transient.

8  
9 **5.3.3.2 Evaluation of Thyroid Histopathology**

10 The thyroid histopathology in this study was evaluated using the same scoring system as  
11 developed for the PWG review and was performed by one of the pathologists who served on the  
12 PWG. A second read of these slides has not occurred. The data will be discussed individually  
13 for each of the time points. Benchmark dose analyses conducted by EPA will be presented in  
14 Section 5.3.3.2.1.

15 Absolute thyroid weights were increased significantly in the 30.0 mg/kg-day group in the  
16 dams on GD21 and decreased colloid; increased hypertrophy and increased hyperplasia were also  
17 noted at this dose. Thyroid weights were not collected for fetuses on GD21, but colloid depletion  
18 was noted in both male and female fetuses at both the 1.0 and 30.0 mg/kg-day doses.

19 Thyroid weight in pups was measured on PND4, and the absolute weight was significantly  
20 effected at 30 mg/kg-day, suggesting a NOAEL at 1.0 mg/kg-day. Histopathology was evident at  
21 lower doses, suggesting a NOAEL at 0.1 for colloid depletion; however, no real dose-related  
22 trend in either hypertrophy or hyperplasia was evident.

23 Thyroid weight in dams on PND9 continued to be effected significantly at 30 mg/kg-day,  
24 with histopathology noted at lower doses. The pups on PND9 were more sensitive than the  
25 dams, exhibiting statistically increased absolute thyroid weights at 0.1 mg/kg-day and higher  
26 doses and suggesting a NOAEL at 0.01 mg/kg-day. A dose-related trend in histopathology in  
27 this same range of doses was noted in the pups, especially for colloid depletion.

28 Thyroid weight in dams on PND21 remained significantly effected at 30 mg/kg-day, with a  
29 clear dose-related trend in colloid depletion, hypertrophy and hyperplasia. All three  
30 histopathological indices were increased at 30 mg/kg-day, and hyperplasia was also significantly  
31 increased at the 1 mg/kg-day dose. It is interesting to note that hyperplasia was more sensitive

1 than both hypertrophy and colloid depletion in the dams at this time point, perhaps indicating a  
2 system coming into the chronic phase of compensation described in Chapter 6.

3 Pups on PND21 also continued to exhibit increased thyroid weights at both 1 and  
4 30 mg/kg-day (females only at 1.0 mg/kg-day). Colloid depletion was clearly significant at  
5 30 mg/kg-day, and hyperplasia was noted although not designated as significant. Despite the  
6 assertion by Argus Research Laboratories, Inc. (2001) that there was no dose-related trend in  
7 hyperplasia, a BMD analysis indicated otherwise (see below). Hypertrophy was not noted, again  
8 indicating an overlap among the three diagnostic indices of thyroid effects used by the PWG.

9 Benchmark dose analyses performed by EPA are presented in Table 5-3 (Geller, 2001b).  
10 A benchmark response level of a 10% increase in incidence over controls, i.e., BMD10 and  
11 BMDL10, was adopted for all studies. Data were fit with a log-logistic function constrained such  
12 that the slope was  $\geq 1$ .

#### 14 **5.3.3.2.1 Benchmark Dose Analyses of Thyroid Histopathology**

15 BMDL values in the dams on GD21 were 1.01, 1.19, and 8.51 mg/kg-day for colloid  
16 depletion, hypertrophy, and hyperplasia. By PND9, these values decreased to 0.13, 1.01, and  
17 0.92 mg/kg-day. Similar values for dams on PND21 were 0.62, 1.24, and 0.99 mg/kg-day for  
18 colloid depletion, hypertrophy, and hyperplasia. Of note is the overlap between the estimates for  
19 hypertrophy and hyperplasia.

20 The effects of ammonium perchlorate on the pups' thyroid glands are largely limited to  
21 colloid depletion. The dams show additional dose-related effects on thyroid histopathology that  
22 were evaluated as thyroid hypertrophy and hyperplasia. The low incidence of these latter two  
23 endpoints in pups may be related to the duration of exposure compared to the dams and the adult  
24 rats examined in earlier studies (Geller, 2001a). Alternatively, hyperplasia and hypertrophy may  
25 be have been difficult to detect in the smaller thyroid glands from the young pups.

26 The BMDL10 is lowest in the GD21 pups and is estimated at 0.12 mg/kg-day for the male  
27 and female pups combined, or for male pups alone, and for female pups alone at 0.04 mg/kg-day.  
28 The BMDL10 increases with age (Figure 5-13), suggesting that the thyroid gland may be most  
29 susceptible to the effects of perchlorate during gestation or at the time of parturition (Geller,  
30 2001b). This is likely due to the double effects of perchlorate inhibition of thyroid function in

**TABLE 5-3. BENCHMARK DOSE (BMD)<sup>a</sup> AND BENCHMARK DOSE LOWER CONFIDENCE LIMIT (BMDL)<sup>a</sup> ESTIMATES FROM THYROID HISTOPATHOLOGY IN THE “EFFECTS STUDY” (Argus Laboratories, Inc., 2001; Geller, 2001b)**

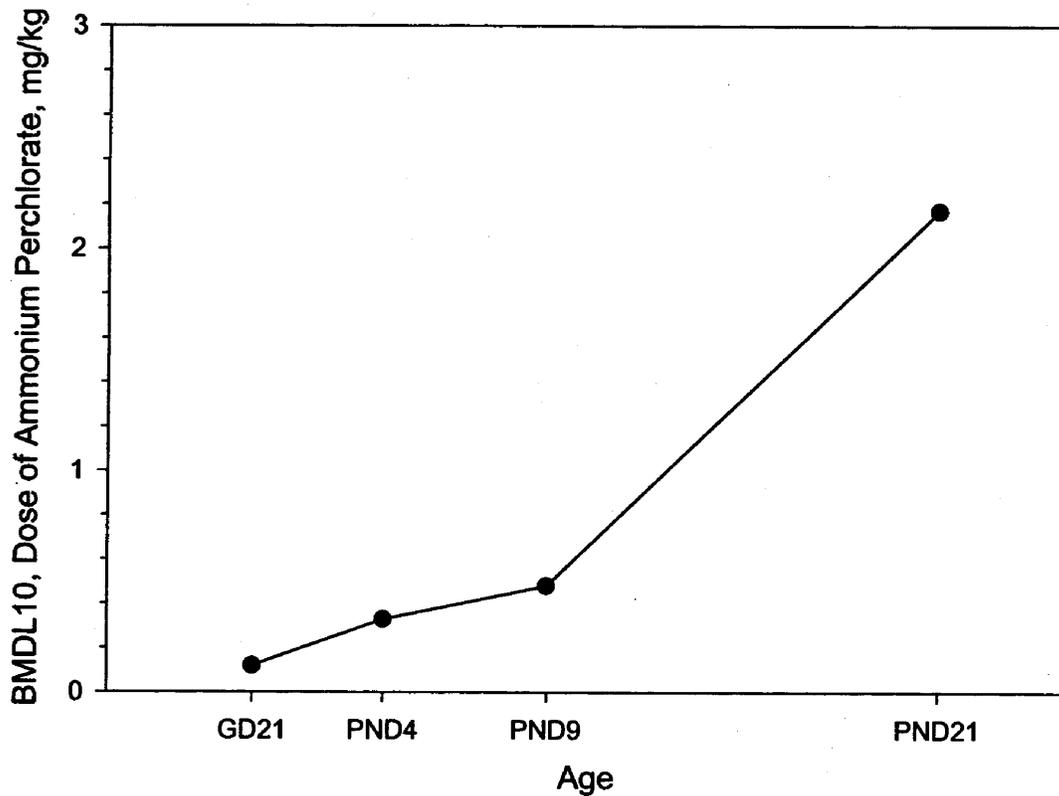
Study Population “Effects” Study (Argus, 2001)	Colloid Depletion				Hypertrophy				Hyperplasia			
	BMD	BMDL	$\chi^2$ <sup>b</sup>	Exp <sup>c</sup>	BMD	BMDL	$\chi^2$ <sup>b</sup>	Exp <sup>c</sup>	BMD	BMDL	$\chi^2$ <sup>b</sup>	Exp <sup>c</sup>
GD 21 Dams	5.10	1.01	1.00	17.90	15.46	1.19	1.00	6.25	28.54	8.51	1.0	5.03
GD 21 Male pups	0.69	0.12	1.00	8.82	NOE <sup>d</sup>	NOE	NOE	NOE	NOE	NOE	NOE	NOE
GD 21 Female pups	0.18	0.04	0.60	2.08	NOE	NOE	NOE	NOE	NOE	NOE	NOE	NOE
GD 21 M + F pups	0.65	0.12	0.16	7.80	NOE	NOE	NOE	NOE	NOE	NOE	NOE	NOE
PND4 Male pups	0.88	0.29	0.12	7.37	NOE	NOE	NOE	NOE	NOE	NOE	NOE	NOE
PND4 Female pups	0.82	0.18	0.12	7.78	NOE	NOE	NOE	NOE	NOE	NOE	NOE	NOE
PND4 M + F pups	0.84	0.33	0.02	7.50	NOE	NOE	NOE	NOE	NOE	NOE	NOE	NOE
PND9 Dams	0.62	0.13	0.59	2.65	2.65	1.01	0.22	17.86	2.24	0.92	0.49	1.0
PND9 Male pups	1.29	0.71	0.59	6.40	NOE	NOE	NOE	NOE	NOE	NOE	NOE	NOE
PND9 Female pups	0.33	0.13	0.61	1.30	NOE	NOE	NOE	NOE	NOE	NOE	NOE	NOE
PND9 M + F pups	0.93	0.48	0.36	3.77	NOE	NOE	NOE	NOE	NOE	NOE	NOE	NOE
PND21 Dams	1.21	0.62	0.34	4.90	15.60	1.24	1.0	6.34	3.59	0.99	0.66	1.0
PND21 Male pups	17.33	1.36	1.0	5.85	NOE	NOE	NOE	NOE	26.97	5.45	0.58	5.06
PND21 Female pups	16.42	1.24	1.00	5.94	NOE	NOE	NOE	NOE	NOE	NOE	NOE	NOE
PND21 M + F pups	17.32	2.17	1.0	5.92	NOE	NOE	NOE	NOE	54.17	13.70	0.24	1.0

<sup>a</sup> Units of mg/kg-day.

<sup>b</sup>  $\chi^2$  goodness of fit criterion

<sup>c</sup> Exponent in log-logistic function restricted to be  $\geq 1.0$ .

<sup>d</sup> NOE = No observed effect.



**Figure 5-13. Lower confidence limit on the dose of ammonium perchlorate in drinking water that produced a 10% increase in the incidence of colloid depletion in the thyroid gland as a function of post-natal age of rat pups. Data of Argus Laboratories, Inc. (2001). Male and female data combined (Geller, 2001b).**

1 the pup and the lack of protection of the pup by the dam because of her own compromised  
 2 thyroid function. After 21 days of post-natal exposure, the male pups also show follicular cell  
 3 hyperplasia.

4 The BMD and BMDL estimates of 0.84 and 0.33 mg/kg-day for the PND4 male and female  
 5 pups in this study (Table 5-3) do corroborate the BMD and BMDL for colloid depletion for the  
 6 PND4 pups from the 1998 Neurobehavioral Developmental study of 0.53 and 0.33 mg/kg-day  
 7 (Table 5-1). However, it should be noted that an unrestricted model also fits those data  
 8 adequately and results in a BMD and BMDL estimate of 0.45 and 0.009 mg/kg-day, suggesting  
 9 variability in those analyses (Geller, 2001b). Again, the lower estimates based on the 1998 data  
 10 at this time point (PND4) may be due to differences in the dosing of the dams between the two  
 11 studies.

1 The BMD and BMDL estimates of 17.32 and 2.17 mg/kg-day for the PND21 male and  
2 female pups in this “Effects Study” (Table 5-3) are somewhat higher than the previous 1999  
3 two-generation reproductive toxicity study estimates of 2.51 and 0.80 mg/kg-day (Table 5-1).  
4 However, comparison of the results of the two-generation reproductive toxicity study to the  
5 current results may be difficult because of differences in the spacing of doses tested.  
6

### 7 **5.3.3.3 Thyroid and Pituitary Hormone Analyses**

8 Thyroid (T3 and T4) and pituitary (TSH) hormones were also analyzed in the “Effects  
9 Study” at various time points. Thyroid hormones and TSH were evaluated in the dams and fetus  
10 on GD21, in the dams on PND10 and PND22, and in neonates on PND5, PND10, and PND22  
11 (corresponding to PND4, PND9, and PND21 according to EPA nomenclature as explained  
12 earlier). Table 5-4 presents the results of ANOVA analyses performed by EPA (Crofton, 2001b).  
13 Maternal serum measures of the hormones were subjected to separate two-way ANOVA.  
14 Treatment (dose) and age (GD21 or PND5, PND10 or PND22) were the independent between-  
15 subjects variables. Two separate approaches were used to address the offspring data due to  
16 differences in experimental design. The data from GD21, PND5 and PND10 were obtained from  
17 litter-pooled samples due to the small volumes of blood and no gender analyses were possible.  
18 These data were subjected to separate two-way ANOVA with age (GD21, PND5, or PND10) and  
19 treatment (dose) as between-subjects variables. Blood samples from PND22 were not pooled so  
20 that the data from this age were subjected to separate two-way ANOVA with gender and  
21 treatment (dose) as independent variables. Mean contrasts were performed using Duncan’s  
22 Multiple range test. Significant two-way ANOVA were followed by step-down one-way  
23 ANOVA to determine the main effects of treatment. If the interaction term was not significant,  
24 then the model was refit if main effects were found. A reduced model was then fitted to the data  
25 retaining only the main effects found significant previously, described as the “liberal” approach  
26 in Crofton and Marcus (2001) and Marcus (2001).

27 EPA benchmark dose analyses (Geller, 2001c) of these results will also be discussed. The  
28 benchmark estimates were generated using the Bench Mark Dose Software version 1.30, and fit a  
29 Hill equation constrained such that the exponent on dose was  $\geq 1.0$  (Geller, 2001c). The BMDL  
30 estimates indicate that the thyroid and pituitary hormones are exquisitely sensitive to the effects  
31 of perchlorate.

**TABLE 5-4. NOAELs AND LOAELs FOR EFFECTS ON THYROID AND PITUITARY HORMONES FROM THE ARGUS 2001 “EFFECTS STUDY” (Crofton, 2001b)**

Generation	Hormone	Age	Sex	Effect Level Designation		
				NOAEL	LOAEL	
Dams	T3	GD21	F	1.0	30.0	
		PND10	F			
		PND22	F			
	T4	GD21	F	—	0.01	
		PND10	F	0.1	1	
		PND22	F	1.0	30.0	
	TSH	GD21	F	—	0.01	
		PND10	F	—	0.01	
		PND22	F	0.01	0.1	
Fetus and Offspring	T3	GD21	Pooled	—	0.01	
		PND5	Pooled			
		PND10	Pooled			
		PND22	F			0.01
	T4	GD21	Pooled	0.01	0.1	
		PND5	Pooled			
		PND10	Pooled			
		PND22	F			no significant effects
			M			—
	TSH	GD21	Pooled	0.1	1.0	
		PND5	Pooled	no significant effects		
		PND10	Pooled	—	0.01	
PND22		F	0.01	0.1		
		M	—	0.01		

<sup>a</sup>Dosages of 0, 0.01, 0.1, 1.0, and 30 mg/kg-day.

#### 1 **5.3.3.3.1 Maternal Hormone Analyses**

2 Exposure to perchlorate produced significant decreases in thyroid hormones and an  
3 increase in TSH in the dams at the various ages tested. For effects on maternal T3, there was no  
4 age-by-treatment interaction and the NOAEL at all time points was 1.0 mg/kg-day. There was a  
5 significant age-by-treatment interaction for effects on maternal T4. Step-down analyses resulted  
6 in a LOAEL at 0.01, 1.0 and 30.0 mg/kg-day at GD21, PND9 and PND21. The 0.01 mg/kg-day  
7 level is a LOAEL for the dams at GD21. There was also a significant age-by-treatment  
8 interaction for the effects on maternal TSH. Step-down analyses resulted in a LOAEL at 0.01,  
9 0.01 and 0.1 mg/kg-day at GD21, PND9 and PND21. As for the effects on T4, there was no  
10 NOAEL at GD21 for the effects on TSH. There was no NOAEL for the effects on TSH at PND9  
11 as well. These effects on T4 and TSH at GD21 are consistent with the Argus Laboratories Inc.  
12 (2001) analyses. Benchmark dose analyses resulted in BMD estimates of 1.63, 0.006 and  
13 2.38 mg/kg-day for the effects on T3, T4, and TSH at GD21. BMDL estimates were only  
14 calculable for T4 in the dams and resulted in an estimate of 0.004 mg/kg-day. Benchmark dose  
15 calculations were not performed for the dams on PND9. At PND21, a BMDL estimate was  
16 calculable only for TSH in the dams with a resultant estimate of 0.53 mg/kg-day.

#### 17 18 **5.3.3.3.2 Fetal and Neonatal Hormone Analyses**

19 Maternal exposure to perchlorate resulted in hypothyroidism in the offspring. There were  
20 significant dose-related decreases in thyroid hormones and increases in TSH at all time points  
21 evaluated.

22 There were no age-by-treatment interactions for the effects on T3 at any age tested. The  
23 LOAEL for GD21, and post-natal days 4 and 9 was 0.01 mg/kg-day. This value is lower than  
24 that reported in the Argus Laboratories, Inc. (2001) analyses. The specified benchmark dose  
25 analysis were not computable for T3 at PND4 or PND21. There was no significant gender-by-  
26 treatment interaction for the effects on T3. The NOAEL for effects on T3 at PND21 was  
27 0.1 mg/kg-day. A BMDL was calculable only for the male pups and resulted in an estimate of  
28 0.13 mg/kg-day.

29 There were also no age-by-treatment for the effects on T4. The LOAEL was 0.1 mg/kg-day  
30 and the NOAEL was 0.01 mg/kg-day for GD21 and PND4 and PND9. At PND21, there was a  
31 significant gender-by-treatment interaction for the effects on T4. There was no NOAEL

1 established for the male pups and 0.01 mg/kg-day was a LOAEL, whereas 0.01 was suggested as  
2 a NOAEL in the Argus Laboratories, Inc. (2001) analyses. The females did not show significant  
3 effects in either the EPA or Argus Laboratories, Inc. (2001) analyses. BMDL estimates were  
4 extremely sensitive for changes in T4 at PND21 in the males with a BMD and BMDL at  
5 0.001 and  $2.86 \times 10^{-7}$  mg/kg-day. Benchmark analyses did not converge for the data from the  
6 female pups alone or for the combined data.

7 There was a significant age-by-treatment interaction for the effects on TSH. Step-down  
8 analyses revealed a NOAEL at 0.1 mg/kg-day for GD21. There was no significant effect on TSH  
9 at PND5, but then no NOAEL on PND9 with a LOAEL at 0.01 mg/kg-day. The LOAEL was  
10 also 0.01 mg/kg-day in male pups at PND21. The females were slightly less sensitive as  
11 suggested by the significant gender-by-treatment interaction. The NOAEL in female pups on  
12 PND21 was 0.01 mg/kg-day. Benchmark analyses on the combined data resulted in a BMD and  
13 BMDL of 0.06 and 0.02 mg/kg-day for the effects on TSH.

#### 14 15 **5.3.3.4 Brain Morphometry Effects**

16 Due to the deficiencies of the remaining tissue blocks from the previous developmental  
17 neurotoxicity study (Argus Research Laboratories, Inc. 1998a), it was determined that the  
18 recommendation of the external peer review panel to evaluate more sections could not be  
19 accomplished unless a new study was performed (Harry, 2001). Thus, one major objective of the  
20 Argus Laboratories, Inc. (2001) “Effects Study” was replication of brain morphometric  
21 measurements in order to address concerns raised by the US EPA, the NIEHS, and the external  
22 peer review panel regarding results observed in the 1998 developmental neurotoxicity study  
23 (Argus, Protocol Number 1613-002, 1998a; U.S. Environmental Protection Agency, 1998d). The  
24 purpose was to evaluate, under more rigorous experimental conditions and according to the EPA  
25 developmental neurotoxicity guidelines (U.S. Environmental Protection Agency, 1998b),  
26 whether the effect in the corpus callosum identified by the EPA in the previous assessment  
27 (Section 5.3.1) would be replicated.

28 In addition, another objective was to identify effects that may occur in other brain regions.  
29 Details with respect to the rationale motivating the experimental design can be found in Harry  
30 (2001). A brief summary of important points will be provided here, but the reader is referred to  
31 Harry (2001) for specifics on this protocol and to other review articles (Garman et al., 2001;

1 Adams et al., 2000; Rice and Barone, 2000; U. S. Environmental Protection Agency, 1998b,g,h)  
2 for a fuller appreciation of the state-of-the-science supporting the use of these measures as  
3 developmental neurotoxicity indices in risk assessment. The use of the rodent and not a non-  
4 human primate was based on the degree of difficulty and the ethical issues involved with  
5 conducting such screening studies in addition to the need to replicate previous findings. The  
6 work, to document the process of normal development and alterations in the rat cited in these  
7 reviews, supports the use of rodent models for determining potential adverse effects on the  
8 developing brain.

9 It should be noted that Argus Laboratories identifies the day of birth as PND1; therefore,  
10 the age nomenclature as recommended in the EPA guidelines for PND10 and PND22 actually  
11 corresponds to PND9 and PND21 in this study. Likewise, in the previous 1998 Argus Research  
12 Laboratories, Inc. Study (Section 5.3.1), the morphometry performed on PND12 was actually  
13 done on PND11. While the actual ages were slightly different between the two studies, the  
14 concept of capturing an active process of development with brain morphometry remains in effect  
15 (Harry, 2001).

16 The motivation for evaluation of brain morphometry was based on the fact that the  
17 formation and maturation of the nervous system is critically dependent upon both a temporal and  
18 spatial organization pattern (U.S. EPA, 1998b; Harry, 2001). Within this framework, an  
19 interdependency between the various cell types in the brain and a precise spatial relationship of  
20 one cell type to one cell type another has been demonstrated. During this time, the developing  
21 system is undergoing rapid maturation of organizational and regulatory processes. Thus, the  
22 disruption of the developmental profile of one cell type may significantly influence critical events  
23 in later development, resulting in an alteration of the normal formation of the brain and its  
24 functional connections. Many toxic agents have been shown to interfere with one or more of the  
25 developmental processes of the brain (i.e., cell division of neuronal and glia precursor cells, cell  
26 interaction with the immediate environment through surface receptors or cell adhesion  
27 molecules, regulation of cytoskeletal processes that control proliferation and migration, cell-cell  
28 interactions that underlie synaptogenesis, development of the cerebral circulation and the blood-  
29 brain barrier, myelination, and programmed cell death). Such perturbations may not be evident  
30 by standard histological assessments as often there is little, if any, evidence of cell death. Rather

1 what is seen is a delay or disruption in the normal development and maturation of specific neural  
2 regions (Harry, 2001).

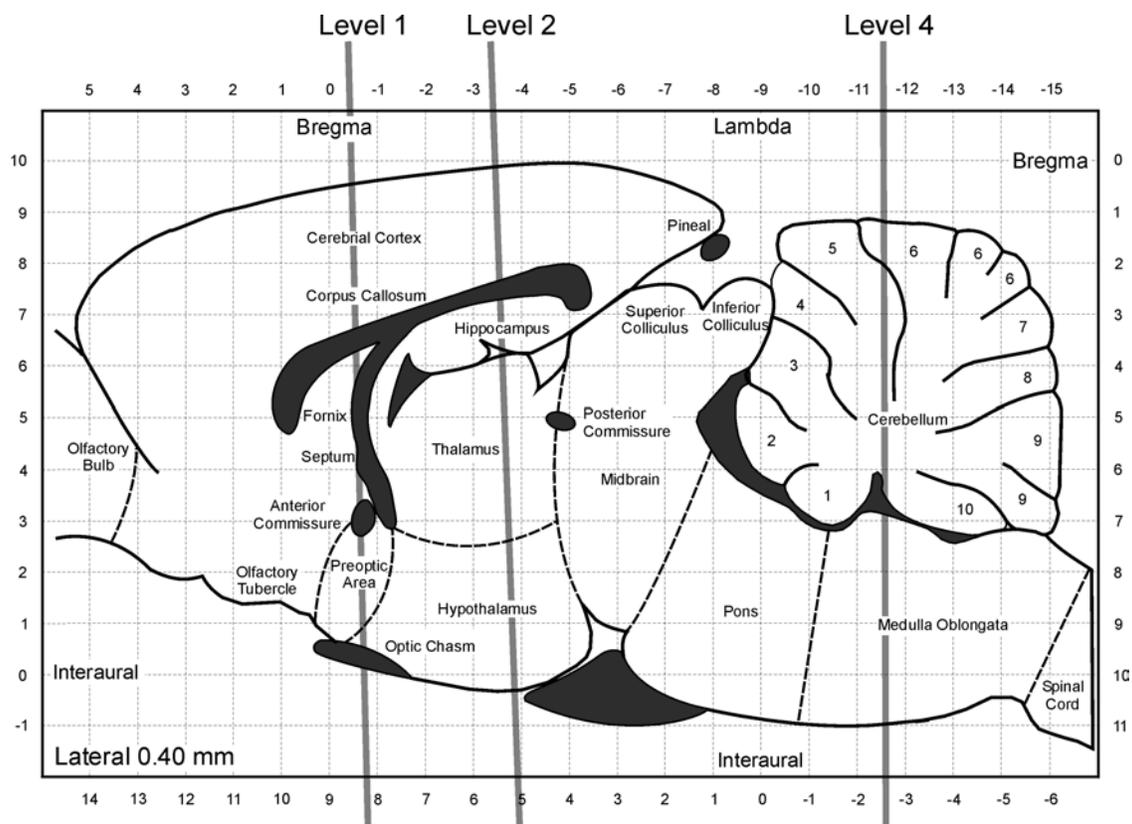
3 Immersion fixation was the tissue processing method of choice and was both recommended  
4 and agreed upon by both the EPA and the PSG for the study. While the tissue fixation method of  
5 choice in adult rodents is via cardiac perfusion, even this procedure is not without problems that  
6 can compromise tissue integrity. It has been documented that immersion fixation artifacts can  
7 influence histological and morphometric evaluations of adult brains; however, a less than optimal  
8 cardiac perfusion can also result in morphological artifacts. For the younger animal, there is less  
9 of a consensus on the proper manner of fixation. With the decreasing size and blood volume of  
10 the younger animal (PND4 and PND9) used in the protocol, the difficulty of ensuring a good  
11 fixation via cardiac perfusion is significantly increased over that in the adult. Further, because  
12 comparisons were to be made between the 1998 and the 2001 study, consistency in method of  
13 fixation was considered to be a critically important variable to maintain as constant across  
14 studies.

15 Following the review of the previous developmental neurotoxicity study (Argus Research  
16 Laboratories, Inc., 1998a), and in considering design considerations for the subsequent study, the  
17 plane of cut for the brain was discussed (Garman, 2001a,b). While sagittal sections for analysis  
18 were recommended for some aspects of morphometric analysis, coronal sections were ultimately  
19 adopted since comparisons were to be made between the 1998 and the 2001 study. This final  
20 design of the study also adhered to the EPA developmental neurotoxicity testing guidelines that  
21 call for coronal sections (U.S. Environmental Protection Agency, 1998g, h). It was originally  
22 recommended by the NIEHS that measurements of the corpus callosum in coronal sections  
23 should not be conducted at the midline due to possible edema artifacts that can occur from the  
24 close proximity of the ventricle. Three sites were recommended for measurement that would  
25 have been consistent with the evaluation conducted by NIEHS on the sections from the Argus  
26 Research Laboratories, Inc. (1998a) study (Section 5.3.1). It was agreed upon in the final design  
27 meeting with PSG contractors that, given the time constraints and need for comparison to the  
28 1998 study, one measurement per hemisphere would be recorded at the same site as used in this  
29 previous study (Garman, 2001a,b). This was a site just off of the midline of the two  
30 hemispheres.

1 Finally, a question raised in the PSG-contracted review (Toxicology Excellence for Risk  
2 Assessment, 2001) with regard to age of sampling as it relates to myelin formation should be  
3 addressed. The process of myelination is a “developmental landmark” for the maturation of the  
4 brain, that is initiated upon the presence of the axon and continues over an extended period of  
5 time. It is a structure that matures over time with the accumulation of protein and structural  
6 lamella. One major period of myelin protein and lipid synthesis occurs approximately between  
7 PND19 and PND35. Thus, while examination at PND21 would not capture the final  
8 accumulation of myelin, it would capture events occurring at a time during which myelin  
9 processing and lamella wrapping of the axon is actively occurring. Therefore, this may represent  
10 a period of critical development of the myelin sheath. Examination of animals with a mature  
11 myelin sheath (e.g., ages greater than PND40) may offer information regarding whether any of  
12 the changes seen at earlier time points represent a permanent structural alteration. The majority  
13 of studies that have examined myelin development and/or alterations in this developmental  
14 process have employed biochemical, molecular, as well as, morphological evaluations to make  
15 such determinations regarding delay or hypomyelination. From such studies, the time most  
16 appropriate for examination appears to be between the ages of PND15 and PND35. Thus,  
17 examination of the corpus callosum at PND9 is probably at the limit of early development for an  
18 evaluation of the myelin sheath. However, it should be noted again that this study was intended  
19 to determine if the effects seen previously (Argus Research Laboratories, Inc., 1998a) could be  
20 repeated. Effects in the corpus callosum in that previous study occurred at the early (PND11)  
21 and remained at the late (PND82) time points. Brain weight and the size of the frontal cortex and  
22 caudate putamen also were effected at the PND82 sacrifice (Section 5.3.1.1).

23 In addition, the development of the axonal pathways connecting the two hemispheres via  
24 the corpus callosum also continues to develop during this time period. While the study design  
25 allowed for the collection of tissue at PND4, it is felt that any measurements recorded at such age  
26 would be very limited in their contribution to the interpretation of the currently available data set.  
27 In addition, given the variability of the plane of cut and the difficulty in examining brains of  
28 young animals, EPA and NIEHS agree that examination of the corpus callosum in younger  
29 animals (the remaining materials available for PND4) would present an even greater problem.

30 Figure 5-14 illustrates where the section levels were taken for the brain morphometry  
31 measurements and shows the anatomical landmarks on the ventral and dorsal surfaces of the



**Figure 5-14. Topograph of the approximate anatomical landmarks on the ventral and dorsal surfaces of the brain used for making the morphometry measurements (Garman, 2001c). The topograph provided is for an adult brain, but the same landmarks are used for PND9 and PND21 brains although the sections at these two other ages would differ due to the rapid growth during this period.**

1 brain. The veterinary pathologist who performed the work has noted that while the landmarks  
 2 were the same for both the PND9 and PND21 brains, it must be appreciated that the sections  
 3 from one age versus the other would not look precisely similar (Garman, 2001c) due to the fact  
 4 that the brain is rapidly growing at this time.

5 Overall, the images of the brain sections from the PND9 and PND21 time points  
 6 demonstrated that the processing of the brain was adequate for conducting limited morphometric  
 7 measurements as outlined in the protocol. As mentioned by the PSG-contracted reviewers  
 8 (Toxicology Excellence for Risk Assessment, 2001) and stated in the study and additional reports  
 9 (Argus Research Laboratories, Inc., 2001; Consultants in Veterinary Pathology, 2001; Garman,

1 2001d), there was a greater degree of variation in the PND9 sections than in the PND21 brain  
2 sections (Harry, 2001). Many sections in the PND9 brains also showed signs of disruption or  
3 damage that may have compromised the measurements. For these reasons the EPA relied upon  
4 the PND21 measurements, despite corroborating effects from the materials at PND9.

5 There were no significant effects of treatment or sex on brain weight, anterior-posterior  
6 cerebrum length, or anterior-posterior cerebellar size at either age tested. As discussed in the  
7 Argus Research Laboratories, Inc. (2001) report, statistical analyses consisted of Students' t-test  
8 comparisons between the control and the corresponding group of each sex at each separate dose  
9 level. For example, PND9 male control striatum measurements were compared to measurements  
10 for the PND9 male 30 mg/kg-day dose group, then PND9 male control striatum measurements  
11 were compared to the PND9 1 mg/kg-day-dose group. These analyses were run separately for  
12 both sexes and ages and all brain areas, right and left sides. The Argus Laboratories, Inc. (2001)  
13 analyses found a large number of significant effects on brain morphometry at doses of 0.1 and  
14 0.01 mg/kg-day ammonium perchlorate in drinking water.

15 Guidelines on the assessment of neurotoxicity (U.S. Environmental Protection Agency,  
16 1998b) specify that alterations in brain structure should be considered adverse and relevant to  
17 human health risk assessment. Alterations in brain structure are consistent with the mode-of-  
18 action for perchlorate, i.e., transient decrements in T4 and T3 during development can result in  
19 neurodevelopmental effects. The significant findings reported in the Argus Laboratories, Inc.  
20 (2001) report strongly argue, therefore, that adverse effects of ammonium perchlorate are present  
21 at the lowest dose tested and that this data set contains only LOAELs, no NOAELs.

22 While the analysis in the Argus report was provocative, the number of t-tests run increases  
23 the risk of introducing Type I error into this analysis. To address this, a more conservative  
24 multivariate analysis, profile analysis (Johnson and Wichern, 1988; Tabachnick and Fidell,  
25 2001), was run by the EPA (Geller, 2001d). Profile analysis is more conservative than the  
26 analysis described above because a multiple analysis of variance (MANOVA) takes into account  
27 any correlations between the independent variables; whereas, the multiple t-tests assume  
28 complete independence. This analysis also reduced the number of main effects tests by nesting  
29 gender within litter and by constructing a vector composed of all of the morphometric data from  
30 each animal, then comparing these vectors. The approach is explained in more detail below.

#### 1 **5.3.3.4.1 Description of EPA Profile Analysis of Brain Morphometry Effects**

2 When a series of measurements are made from a single animal, i.e., within-subjects  
3 measurements, they can be used to build a profile or vector of scores across the measurement  
4 variables. Profile analysis makes between-groups comparisons using a vector composed of all of  
5 the (within-subject) measurements taken from each animal. Its primary test, for parallelism of  
6 the vectors, establishes whether the pattern of results between treatment groups is the same or  
7 different. It is a much more rigorous and conservative test, requiring that all of the measurements  
8 (i.e., all brain regions) show a dependence on dose with the same pattern. This determination  
9 also allowed examination of the entire set of data without an *a priori* expectation of effect in one  
10 brain region or another or the direction of the effect (i.e., decrease or increase). While there is  
11 indication that certain areas of the brain are likely susceptible to the effects on thyroid hormones  
12 of perchlorate (e.g., Madeira et al., 1991, 1992, 1993), and the previous study performed by  
13 Argus indicated that the corpus callosum was affected (U.S. Environmental Protection Agency,  
14 1998d; Crofton, 1998c), definitive gestational windows for specific brain areas are unknown.  
15 Profile analysis determines whether there were dose-related changes in the pattern of brain  
16 growth, i.e., brain growth in one region relative to another while precluding prior expectations  
17 about specific areas of the brain or the direction and magnitude of these changes.

18 The profile analysis was run on the data from the PND9 and PND21 animals separately  
19 with gender nested within litter (PROC GLM, SAS Institute, Inc, Cary, NC). The data were  
20 provided in electronic form from Argus Research Laboratories, Inc. (2001) and in an additional  
21 report (Garman, 2001d). Profile analysis requires data from each endpoint for each animal. Data  
22 from individual brain regions, both right and left sides, were missing from 8 animals in the PND9  
23 cohort and 3 animals in the PND21 cohort, eliminating these animals from the analysis (Geller,  
24 2001d: Table 1). If a sex by treatment interaction was found, separate analyses were run on  
25 males and females. Treatment effects within a brain region were examined with univariate  
26 analyses of variance with gender nested within litter. Dunnett's two-tailed t-test was used to  
27 compare each dose group to controls at  $\alpha = 0.05$  for step-down tests of treatment effects within a  
28 brain region as guided by the overall (univariate) treatment or sex by treatment effects.

29 Right and left side measures of the same brain structures were examined with profile  
30 analyses (whole set of data) and repeated measures analyses of variance (univariate analysis on  
31 each brain region). While there was no *a priori* reason to expect other than a bilateral effect, the

1 presence of this kind of bias could reflect either anisometries in brain regions (i.e., lateralization)  
2 or sectioning that was not perfectly perpendicular to the anterior-posterior axis of the brain and  
3 that would have resulted in sampling brain regions at different depths on right and left side.  
4 These analyses, together with examination of the images of the brain sections (Harry, 2001)  
5 demonstrated some systematic variability in the sectioning resulting in differences in right versus  
6 left measurements in different brain regions. The magnitude of the variability was small and not  
7 always in the same direction, even within a brain region (varying with the dose group sampled).  
8 The small magnitude of difference relative to the dose-related changes found in this study, the  
9 fact that different brain regions varied in their laterality bias in different directions, and that  
10 different dose groups varied in different directions all argue for simply averaging the right and  
11 left brain region measurements for each animal rather than tailoring different analyses for  
12 different brain regions. In addition, averaging could help to reduce variability in the data due to  
13 sampling only one histological section/brain region/animal. Therefore, data from right and left  
14 sides of the brain were averaged before the analysis of dose effects. Where data were missing  
15 from only one side of the brain, the existing measurement was used for the analysis.

16 Two additional analyses were run with adjustments to the raw morphometry data in  
17 response to suggestions made by reviewers hired by the PSG (Toxicology Excellence for Risk  
18 Assessment, 2001) designed to subtract variability due to variation in brain size and focus on  
19 changes in the sizes of brain areas relative to one another. As suggested by the PSG review, one  
20 analysis was run dividing all of the linear dimensions through by the post-fixation brain weight  
21 from each brain. However, EPA and NIEHS note that there are little historical data for  
22 normalizing data with post-fixation brain weight (Harry, 2001) and that fixation results in the  
23 loss of any evidence of hydration-related changes such as edema or other swelling.

24 The second additional analysis was suggested by the NIEHS and also adjusted for brain  
25 size using the anterior-posterior (a-p) measurements of cerebrum and cerebellum and the full  
26 width measure of hippocampus to adjust the linear dimensions. In this analysis, frontal, parietal,  
27 and corpus collosum dimensions were divided by a-p cerebrum size; dentate, CA1, and CA3  
28 were divided by hippocampal width; and the cerebellar linear measurement was divided by the  
29 a-p cerebellum measurement. Hippocampus, a-p cerebrum, and a-p cerebellum were not  
30 included in the analysis as separate measures. The striatum and external germinal layer  
31 measurements were not adjusted by these other linear dimensions.

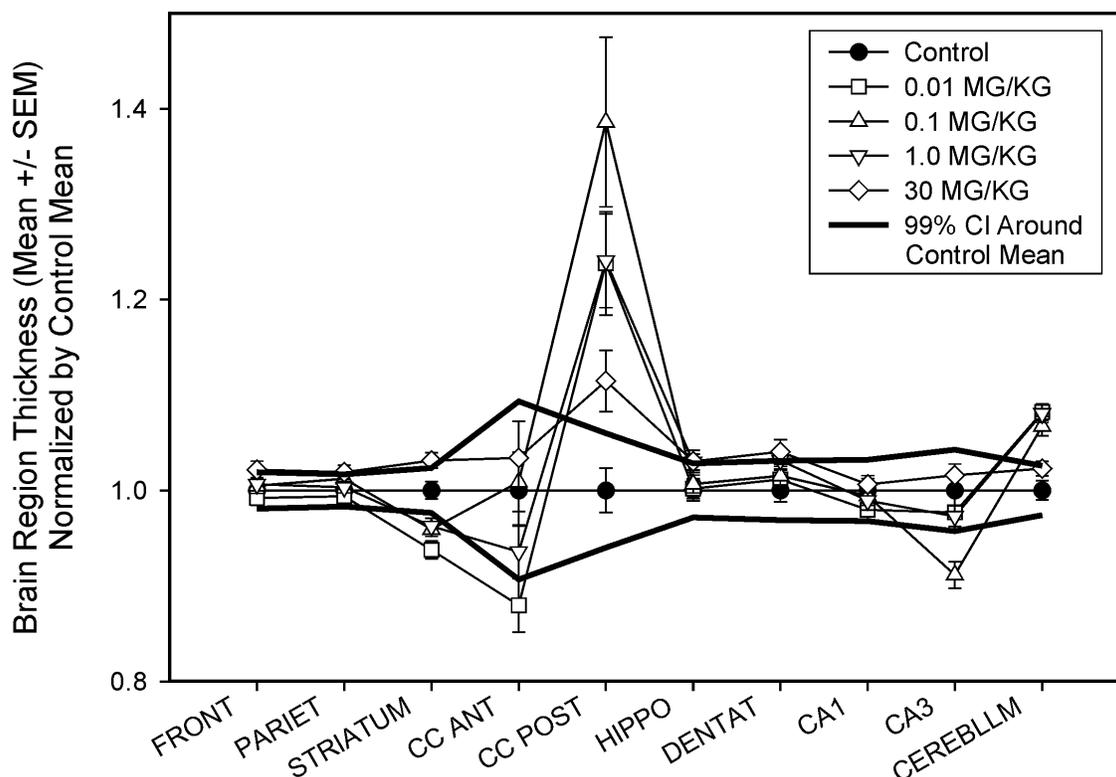
1 An additional two analyses were run on the PND21 data. These analyses omitted (1) the  
2 posterior corpus callosum measurement, or (2) the posterior corpus callosum and all  
3 hippocampal measures; i.e., all measures that came from the Level II section since there was  
4 some indication that there may have been a systematic difference in the plane of sectioning with  
5 dose (Harry, 2001).

#### 7 **5.3.3.4.2 Results of EPA Profile Analysis of Brain Morphometry Effects**

8 The brain morphometry profiles were not parallel across treatment groups for PND9 pups  
9 (Geller, 2001d: Table 2). The absence of parallel profiles obviates further analysis for equal  
10 profiles. This means that the effects of developmental dosing with ammonium perchlorate were  
11 different on different brain regions. Planned contrasts show that the 0.01 and 1.0 mg/kg-day  
12 doses were significantly different than controls (Geller, 2001d: Table 2A). Adjusting for brain  
13 weight had little effect on these results (Geller, 2001d: Table 2B), though the adjustment for the  
14 linear size of the different brain regions made the effect at the highest dose (30 mg/kg-day) also  
15 significantly different from control (Geller, 2001d: Table 2C).

16 The brain morphometry profiles were also not parallel across treatment groups for the  
17 PND21 pups (Geller, 2001d: Table 2A). Contrasts between each of the dose groups and controls  
18 showed that the controls differed from all other dose groups at better than  $p < 0.0001$ , including  
19 at the lowest dose used, 0.01 mg/kg-day ammonium perchlorate in drinking water. The absence  
20 of parallel profiles obviates further analysis for equal profiles. The analysis adjusting for brain  
21 weight or regional size yielded similar, highly significant effects (Geller, 2001d: Tables 2B, 2C).  
22 Sex by dose interactions were significant in the parallel profiles analysis of the raw data and with  
23 the data adjusted by brain region size. The parallel profile MANOVA remained significant at  
24  $p < 0.0001$  in the overall and contrast tests with the posterior corpus callosum or posterior corpus  
25 callosum and all hippocampal measurements (i.e., all measurements taken at section Level II  
26 removed from the analysis) decreasing concern for confounding introduced by potential bias in  
27 sectioning at this level suggested for the males (Harry, 2001).

28 The profile analysis was done using the raw (right-left averaged) data values. Because the  
29 brain structures measured yield a range of measurements varying 10-fold, it is difficult to plot the  
30 raw data vector in a meaningful way in order to see the differences driving the findings of  
31 significant differences between dose groups. Figure 5-15 plots the (unadjusted) region-by-region



**Figure 5-15. Profile analysis of brain morphometry measurements for PND21 rat pup brain regions. The male and female data on linear thickness measurements were combined and normalized by the control mean of each region. The control data are represented by the horizontal line at 1.0. Profile analysis determines whether the vectors of measurements from each treatment group differ from each other and control in a dose-dependent fashion. The heavy line represents the  $\pm 99\%$  confidence interval around the mean control values. Note that while this plot uses the normalized data to more easily illustrate the data vectors, the actual analysis was performed using raw data values (Geller, 2001d). A similar analysis showed effects in PND9 brains (data not shown).**

1 size of each brain structure normalized by the mean size of that brain structure in the controls,  
 2 male, and female combined for the PND21 pup data. The control group is therefore represented  
 3 by a horizontal line at 1.0 with associated variability. The other dose groups differ from this  
 4 horizontal line to different extents, and the parallel profiles analysis tests, in essence, whether  
 5 these departures make the other dose groups significantly “non-horizontal”. Note that the

1 analysis was not done on the normalized data; the control values were divided through to aid in  
2 visualizing the data vectors used in this analysis. The 99% confidence intervals around the  
3 control means represent an envelope inside of which comparable values  $\pm$  standard error of the  
4 mean (SEM) are not significantly different from controls.

#### 6 **5.3.3.4.2.1 Univariate analyses of brain morphometry**

7 While the main reason to use profile analysis was to benefit from the power it brings to an  
8 analysis by its conservative constraint that requires the entire vector of measurements depend on  
9 dose with a consistent pattern, univariate analyses also were evaluated to gain insights into  
10 effects on specific regions.

11 **PND9 brains.** Univariate tests yielded significant effects of treatment with ammonium  
12 perchlorate in the frontal and parietal regions of the cerebral cortex, the striatum, region CA1 of  
13 the hippocampus, the corpus callosum, and the external germinal layer of PND9 pup brains  
14 (Geller, 2001d: Table 3A). There is an increase in size at the 1.0 mg/kg-day dose in the frontal,  
15 parietal, and striatum measurements, and decreases in size in CA1 and the external germinal  
16 layer. There were also treatment-by-sex interactions in the corpus callosum and CA1 regions  
17 (Geller, 2001d: Table 3A). Both of these brain regions showed a treatment-related decrease in  
18 linear extent in females while showing an increase in size in males. While most of the changes  
19 in linear extent measured in the sampled brain regions were  $\pm 5$  to 11%, the male corpus callosum  
20 was increased 23% at both the 0.1 and 1.0 mg/kg-day doses.

21 The adjustment for brain size reduced the significance of treatment effects in the striatum,  
22 CA1, and external germinal layer (Geller, 2001d: Table 3A, center). The analysis using  
23 adjustment for regional size (Geller, 2001d: Table 3A, right) was nearly identical to the raw data  
24 analysis, with the addition of significant effects being noted on cerebellum.

25 A comparison of the profile analysis and the analysis presented in Argus Research  
26 Laboratories, Inc. (2001) shows similar results were obtained on the PND9 brain morphometry  
27 with one exception. Both analyses found an increase in linear extent of frontal, parietal, and  
28 striatum at 1.0 mg/kg-day ammonium perchlorate and in the corpus callosum at the 0.1 and  
29 1.0 mg/kg-day dose, with the corpus callosum increase limited to males. There was a decrease in  
30 the linear extent of the striatum at 0.1 mg/kg-day dose and decreases in the size of region CA1 of

1 females at the 0.01, 0.1, and 1.0 mg/kg-day doses. The Argus Laboratories, Inc. (2001) analysis  
2 did not detect a significant difference in female CA1 at the 0.01 mg/kg-day dose.

3 A post-hoc analysis of the plane of cut of the PND9 brain sections suggested that the  
4 0.1 and 1.0 mg/kg-day dose groups were sectioned at a different depth than were the other dose  
5 groups (Harry, 2001). This likely contributed to the small but significant increase in size of the  
6 frontal, parietal, and striatum sections in the 1.0 mg/kg-day dose groups and may have  
7 contributed to the large increase in size of the anterior corpus callosum seen in the PND9 males.

8 **PND21 brains.** The striatum, cerebellum, and corpus callosum II (posterior sample) all  
9 showed significant changes with the lowest administered dose of ammonium perchlorate, 0.01  
10 mg/kg-day (Geller, 2001d: Table 3B, left). The striatum was significantly reduced in size at all  
11 but the highest dose. Region CA3 of the hippocampus similarly showed a U-shaped dose  
12 response. The cerebellum and the posterior corpus callosum increased in size with dose in an  
13 inverted U-shape. There were sex-by-treatment interactions in striatum and frontal cortex such  
14 that the female rats showed a stronger dose-related decrease in linear measurement than males.  
15 Both males and females show a complex dose response in the anterior corpus callosum  
16 measurement. As in the PND9 animals, the changes in linear extent were generally in the  $\pm 5$  to  
17 11% range with the exception of the posterior portion of the corpus callosum, which showed an  
18 increase in size of 24% in the 0.01 and 1.0 mg/kg dose groups, and a 39% increase in the  
19 0.1 mg/kg dose group.

20 The adjustments for brain size had little effect on the region by region results at PND21  
21 (Geller, 2001d: Table 3B, center, right). Dividing through by the a-p or hippocampal  
22 measurements resulted in additional significant dose effects noted on CA1 and a sex by dose  
23 effect on cerebellum.

24 The Argus Research Laboratories Inc. (2001) and current EPA analyses agreed. Both  
25 analyses found a significant decrease in size of the striatum at 0.01, 0.1, and 1.0 mg/kg doses and  
26 increases in size of the corpus callosum II (posterior) and cerebellum at the same doses. Both  
27 analyses noted the decrease in size of CA3 at the 0.1 mg/kg dose, the decreased anterior corpus  
28 callosum in females at 0.01 mg/kg, and the increased size of the frontal region in males at 0.1 and  
29 30 mg/kg.

#### 1 **5.3.3.4.3 Conclusions of EPA Brain Morphometry Analyses of Brain Morphometry Effects**

2 There were significant differences in brain morphometry due to treatment with ammonium  
3 perchlorate at both PND9 and PND21 in this study. Tables 2 and 3 in Geller (2001d) enumerate  
4 strong effects of developmental exposure to ammonium perchlorate on brain morphometry  
5 considered across all regions tested and in the analysis of individual brain regions. These effects  
6 were present at PND9 and PND21, with the latter age group showing stronger effects. Many of  
7 these effects represent an increase or decrease of  $\pm 10\%$  in the size of a brain region, similar to  
8 the range of morphometric alteration noted in a recent study of fetal alcohol syndrome  
9 (Bookstein, et al., 2001). The corpus callosum showed a notable increase of 24% or more in  
10 linear extent at PND21 in the 0.01, 0.1, and 1.0 mg/kg ammonium perchlorate dosing groups.  
11 Adjusting the raw morphometric determinations by either brain weight or measurements of larger  
12 brain areas (i.e., cerebrum, cerebellum, and hippocampus) had no strong effect on the results of  
13 the analysis.

14 The significant differences in the parallel-profiles test demonstrate exposure-related  
15 changes in relative growth of different brain areas even at the lowest administered dose (Geller,  
16 2001d: Table 2). Univariate analyses to further investigate these effects showed effects on a  
17 number of different brain regions at both ages tested. The most sensitive endpoints were the  
18 linear dimensions of the striatum, corpus callosum, and cerebellum at the 0.01 mg/kg-day dose  
19 when males and females were considered together at PND21. Thus, these analyses ultimately  
20 agree with those submitted in Argus Laboratories, Inc. (2001): exposure to 0.01 mg/kg-day  
21 ammonium perchlorate during gestational and post-partum (weanling) development resulted in  
22 measurable changes in brain structures.

23 The increase in the size of the corpus callosum in this study replicates that seen in the  
24 previous morphometric analysis of rats developmentally exposed to ammonium perchlorate (U.S.  
25 Environmental Protection Agency, 1998d, Crofton, 1998c). This is notable given the differences  
26 between the two studies. The previous data were obtained from tissues from rats aged PND11  
27 rather than PND9 and PND21, and dose spacing included high doses of 3 and 10 mg/kg rather  
28 than 1 and 30 mg/kg as in this study. Fewer animals were used in the previous study (6/dose/sex)  
29 than in the current study (approximately 15/dose/sex), and litter identity was considered in the  
30 current analysis. It also has been noted by Garman (2001c), a principal investigator with

1 established experience in performing brain morphometry on a substantial number of studies, that  
2 such a treatment-related pattern has not been observed in other studies.

3 It should be noted that changes in thyroid hormone levels effect different brain regions  
4 differently during development. For example, developmental hypothyroidism prolongs the  
5 expansion of the external granular layer and increases fissure formation in the cerebellum  
6 (Lauder, et. al., 1974). Different brain regions show an inverted U or U-shape dose response;  
7 this is not uncommon in biological systems as compensatory or other mechanisms may be  
8 triggered at high doses.

9 Fixation artifacts are not a concern in the study because all brains were fixed and embedded  
10 at the same time. In addition, dose-related effects were seen as both increases and decreases in  
11 brain region size. EPA concludes from this that whatever artifacts may be present were not large  
12 enough to obviate alterations of the magnitude observed. There is some concern over sectioning  
13 artifacts because the brains from the different dose groups were sectioned at different intervals  
14 after sacrifice (Argus Research Laboratories, Inc., 2001) and post-hoc analysis of the brain  
15 sections did reveal some systematic differences in the PND9 animals and in a limited sample of  
16 sections examined from the PND21 animals (Harry, 2001). Additional sectioning is being  
17 performed by EPA to address whether the anterior to posterior bias selection suggested in the  
18 males (Harry, 2001) is a true confounder because normative data for brain measurements at these  
19 ages are not available. These new data will be made available to the external peer review panel  
20 as soon as possible. Because the analyses conducted without sections from this level still  
21 resulted in a significant effect at the 0.01 mg/kg-day dose and the dose-related changes noted in  
22 this study have not been noted in other studies with tissue sampler treated similarly (Garman,  
23 2001c), this concern is somewhat mitigated. Certainly to be protective of public health, these  
24 effects should be viewed as adverse until additional data either confirm or contradict that  
25 conclusion.

26 In summary, two different analyses of the brain morphometry data from the 2001 “Effects  
27 Study” (Argus Research Laboratories, Inc., 2001) yielded significant effects (i.e., alteration of  
28 brain structures) of developmental exposure to ammonium perchlorate in drinking water at doses  
29 of 0.01 mg/kg-day and higher in a mammalian (rat) model of neurodevelopment. These  
30 alterations included a 23-39% increase in the size of the corpus callosum over controls in the  
31 progeny of dams dosed with 0.01 to 1.0 mg/kg of ammonium perchlorate in drinking water.

1 Alteration of brain structures in a laboratory animal model is considered to be an adverse  
2 neurotoxic effect (U.S. Environmental Protection Agency, 1998b). One of the analyses used a  
3 series of t-tests; the other a more conservative multivariate analysis employing a nested model  
4 profile analysis followed by univariate analysis of specific brain regions. The latter method is  
5 more likely to be considered a valid analytic method because it better incorporates the design  
6 elements of the study and reduces the likelihood of Type I statistical error. These effects on brain  
7 morphometry dictate a designation of 0.01 mg/kg-day as a LOAEL.

## 10 **5.4 DEVELOPMENTAL STUDIES**

11 The 1997 testing strategy included a developmental study in rabbits to evaluate both a  
12 potential critical effect and to characterize the toxic effects of perchlorate in a species other than  
13 rats. Testing guidance for developmental toxicity typically requires data in two different species.  
14 A new study of developmental toxicity in rats was recommended at the 1999 external peer  
15 review. This section reviews the historical data on the developmental effects of perchlorate  
16 (5.4.1), the 1998 study in rabbits (5.4.2), and the new 2000 study in rats (5.4.3).

### 18 **5.4.1 Historical Studies**

19 Brown-Grant (1966) examined the effects of perchlorate on implantation and pregnancy  
20 outcome in Wistar rats. Potassium perchlorate or potassium chloride (control) was administered  
21 at 1.0% (w/v) in drinking water from GD2 through GD8. The daily calculated intake rates were  
22 237 and 371 mg/rat for potassium perchlorate and potassium chloride, respectively. Rats were  
23 administered methylothiouracil 45 min before injection of 5  $\mu$ Ci sodium radioiodide ( $^{131}$ I) and  
24 sacrificed 2 h later. Rats clearly not pregnant were sacrificed on Day 20; whereas, pregnant rats  
25 were allowed to deliver prior to sacrifice. Pregnancy was successful in 7/11 control rats and  
26 8/11 perchlorate-treated rats. Among nonpregnant animals, implantation sites were not found.  
27 Litter size, number of pups, and pregnancy were not affected.

28 In the same study, false pregnancy was induced by mating females with vasectomized  
29 males. Females were dosed as before on GD2 through GD8 to 0.25 or 1.0% potassium  
30 perchlorate or potassium chloride (control). These doses correspond to 63 and 246 mg potassium

1 perchlorate/rat and 82 and 308 mg potassium chloride per rat, respectively. Deciduoma  
2 formation was induced through traumatizing one uterine horn while under anesthesia. Rats  
3 exposed to the 0.25% dose were traumatized on GD3 and sacrificed on GD7. Trauma and  
4 sacrifice occurred on GD4 and GD8, respectively, in the 1.0%-dose group. Methylthiouracil and  
5 sodium radioiodide (<sup>131</sup>I) were administered prior to sacrifice as before. Deciduoma formation  
6 was not different between dosed and control rats. Thyroid weights were increased significantly  
7 in the rats of the 1.0% potassium perchlorate-dose group.

8 A related study was performed by Brown-Grant and Sherwood (1971). Wistar rats were  
9 mated shortly post-partum, and the present litter was culled to nine. The dams were then  
10 administered 0.1% potassium iodide or 1.0% potassium chloride, potassium perchlorate, or  
11 potassium iodide in the drinking water until sacrifice. The average daily intake of potassium  
12 perchlorate and potassium chloride was 615 and 655 mg/rat, respectively; calculated daily doses  
13 were approximately 2,440 and 2,660 mg/kg body weight. The litters were sacrificed on GD9 or  
14 GD10. The dams then were sacrificed on GD12 or GD13, allowing time for the new blastocysts  
15 to implant. Potassium perchlorate again did not affect blastocyst ability to survive prior to  
16 implantation or implantation rate after lactation ceased. Relative thyroid weights of the dams and  
17 litters were increased significantly compared with potassium-chloride-dosed controls. The high  
18 dose of potassium iodide (average daily intake of 234 mg/rat [approximately 1,150 mg/kg]) was  
19 maternally toxic.

20 All dams were sacrificed on Day 12 or 13 and examined for the number of implantation  
21 sites. There was 100% incidence of dams with implantation sites for all groups except the  
22 perchlorate-treated group in which only 70% of the dams had implantation sites. The number of  
23 implantation sites per dam was comparable for all groups. Thyroid weights in the perchlorate-  
24 treated dams appeared to be increased compared with the chloride- or iodide-treated dams. Also,  
25 thyroid weights of the offspring of perchlorate-treated dams were increased compared with  
26 offspring from iodide-treated dams. The authors concluded that treatment with potassium  
27 perchlorate had no significant effect on blastocyst survival or the ability to implant under  
28 conditions delaying implantation (i.e., concurrent lactation).

29 Postel (1957) reported administration of 1% potassium perchlorate in drinking water to  
30 pregnant guinea pigs (n=16) and a control group (n = 3) receiving a diet of 0.48 µg iodine per  
31 gram. Dosing with perchlorate during GD21 through GD48 produced enlarged thyroids in the

1 fetuses compared to the thyroids of control fetuses. In contrast, perchlorate treatment did not  
2 have any effect on the thyroids in dams. Enlarged fetal thyroids also occurred when perchlorate  
3 treatment was accompanied by daily subcutaneous treatment with T3 doses as high as  
4 32  $\mu\text{g}/\text{kg}/\text{day}$ . From water intake and body weight data, the author calculated an average daily  
5 dose to the dams of 740 mg/kg-day. The fetuses were not examined for other developmental  
6 effects. This study suggested a free-standing LOAEL of 740 mg/kg-day for fetal thyroid  
7 enlargement because no other doses were tested. In a separate experiment to test effects on adult  
8 guinea pigs, 0 or 1% potassium perchlorate was administered to nonpregnant female guinea pigs  
9 for 30, 60, or 90 days. Thyroid enlargement and hyperplasia were apparent in treated animals  
10 after 60 or 90 days of treatment.

11 Similar results in rabbits were described by Lampe et al. (1967). Dams were dosed with  
12 100 mg potassium perchlorate/kg by weight daily, mixed with feed. Dosing occurred from  
13 conception through GD21 or GD28. Maternal thyroid weights in treated animals were three  
14 times higher than control thyroids; fetal thyroids were nearly four times the control weights. The  
15 number of epithelial cells were increased, and the amount of colloid decreased in treated animals.  
16 The relative volume of the stroma, the supporting matrix, was increased because of the reduced  
17 follicle sizes. Likewise, maternal thyroids showed decreased luminal size and increased  
18 epithelial cells. The authors asserted that these results demonstrated that the placenta is  
19 permeable to perchlorate. Because fetal thyroids were more enlarged relative to maternal thyroid  
20 glands, the fetal thyroid system is independent of the maternal regulatory system and more  
21 sensitive to changes in iodine availability.

#### 22

#### 23 **5.4.2 Segment II Developmental Toxicity Study in Rabbits**

24 A developmental toxicity study was performed in New Zealand White (Hra:[NZW]SPF)  
25 rabbits as part of the overall perchlorate testing strategy (Argus Research Laboratories, Inc.,  
26 1998c). This study has also appeared in the literature (York et al., 2001a); however, because that  
27 manuscript did not use the PWG review of thyroid histopathology and its conclusions on other  
28 endpoints are the same as the contract report, the manuscript will not be discussed further in this  
29 document. To aid understanding of terminology and the protocol, a schematic of the study  
30 design is provided in Figure A-3 of Appendix A to this document. The study design meets the  
31 requirements of the 1998 EPA Office of Pollution Prevention and Toxic Substances (OPPTS)

1 870.3700 guideline. A deviation from the use of double staining was noted in Appendix D of the  
2 Argus report, but EPA determined that this should not have had an effect on the overall outcome  
3 of this study.

4 The dose groups tested were 0, 0.1, 1.0, 10, 30, and 100 mg/kg-day of ammonium  
5 perchlorate in RO water provided by continual access on presumed GD6 to GD28. Each group  
6 was comprised of 25 time-mated does assigned on a randomized basis stratified by weight.  
7 Doses were selected on the basis of a dose range-finding study (Argus Research Laboratories,  
8 Inc., 1998d) in which thyroid histopathology was evident in the does at 20, 50, and 100 mg/kg-  
9 day; thyroid hormone levels (T3, T4, and TSH) in the does were reduced at all doses; and three  
10 malformed fetuses from three litters in the 20-mg/kg-day group were observed upon gross  
11 external examination. EPA was concerned about these pilot study results, particularly because  
12 the original target doses of 0.1 and 10 mg/kg-day were changed on GD13 to 50 and 100 mg/kg-  
13 day based on the lack of clinical toxicity at these doses. The fact that these were the doses at  
14 which effects were observed, together with the fact that a low number of animals (n = 5) was  
15 used in this range-finding study caused EPA to counsel the sponsor (PSG) to examine an  
16 expanded range of doses in the definitive study. The dose groups chosen for the definitive  
17 developmental study were thus aimed to bracket the dose levels in the range-finding study and to  
18 go below the doses causing thyroid hormone perturbations and above those associated with the  
19 fetal malformations.

20 Dosing solutions of ammonium perchlorate were prepared at least weekly from stock  
21 solution, and the results of the concentration analyses were within acceptable ranges. Stability of  
22 solutions was assumed based on determinations by AFRL/HEST for the 90-day bioassay as  
23 discussed in Section 5.2.3. Rabbits were observed for viability at least twice daily, and body  
24 weight, food and water consumption, clinical observations, deaths, abortions, and premature  
25 deliveries were evaluated daily. On GD29, rabbits were terminated and cesarean sections were  
26 performed. Blood samples from the does were taken for evaluation of thyroid and pituitary  
27 hormones (T3, T4, and TSH). Gross necropsy was performed on the thoracic, abdominal, and  
28 pelvic viscera of each doe. Parameters evaluated in the does included pregnancy status, gravid  
29 uterine weight, number of corpora lutea in each ovary, number and distribution of implantations,  
30 early and late resorptions, and live and dead fetuses. The thyroids/parathyroids were evaluated  
31 histologically. Weight, gross external alterations, sex, in situ brain status (in one-half of the

1 fetuses in each litter), brain histology (in the other one-half of all fetuses in each litter), cavitated  
2 organs, and skeletal and cartilaginous alterations were examined in the fetuses. No  
3 measurements of thyroid structure or function were made in the fetuses.  
4

#### 5 **5.4.2.1 Results of Maternal Examinations and Thyroid Histopathology**

6 Two does in the 1.0-mg/kg-day group aborted either dead pups or had late resorptions on  
7 GD28. Both of these abortions were considered unrelated to treatment because the incidences  
8 were not dose-dependent and were consistent with historical control data for rabbits in that  
9 laboratory (Argus Research Laboratories, Inc., 1998c; Appendix J). One doe in the 100-mg/kg-  
10 day group delivered prematurely on GD27 (normal delivery in rabbits occurs on GD31), but it  
11 was assumed that this rabbit had been identified and shipped incorrectly by the supplier because  
12 the pups appeared to be full-term (i.e., they had fur and were nursing). There were no treatment-  
13 related effects on maternal clinical signs, body weight, body weight change, gravid uterine  
14 weight, or food and water consumption. It is interesting to note that there were decreases (not  
15 statistically significant) in several of these endpoints, at the 1.0-mg/kg-day group—the same at  
16 which the abortions occurred—as did one adverse necropsy observation of a mottled liver.  
17 However, none of these responses showed a dose-response with the current treatment regimen,  
18 and none were out of the range of normal occurrence.

19 The only remarkable histopathology in the does was observed in the thyroids. There was  
20 an apparent dose-related but not statistically significant decrease in thyroid weight). The  
21 histopathology in the dams as reviewed by the PWG can be found in Wolf (2000; 2001,  
22 Table 22). There was a clear dose-response for colloid depletion, hypertrophy, and hyperplasia,  
23 indicating that another species has conserved the hypothalamic-pituitary-thyroid feedback  
24 regulation. All three indices appeared to be significantly increased at 1.0 mg/kg-day and above.  
25 Benchmark dose analyses resulted in BMDL estimates of 0.008 for colloid depletion and 0.42 for  
26 hyperplasia. A poor fit prevented BMDL estimation for hypertrophy.  
27

#### 28 **5.4.2.2 Developmental Endpoints**

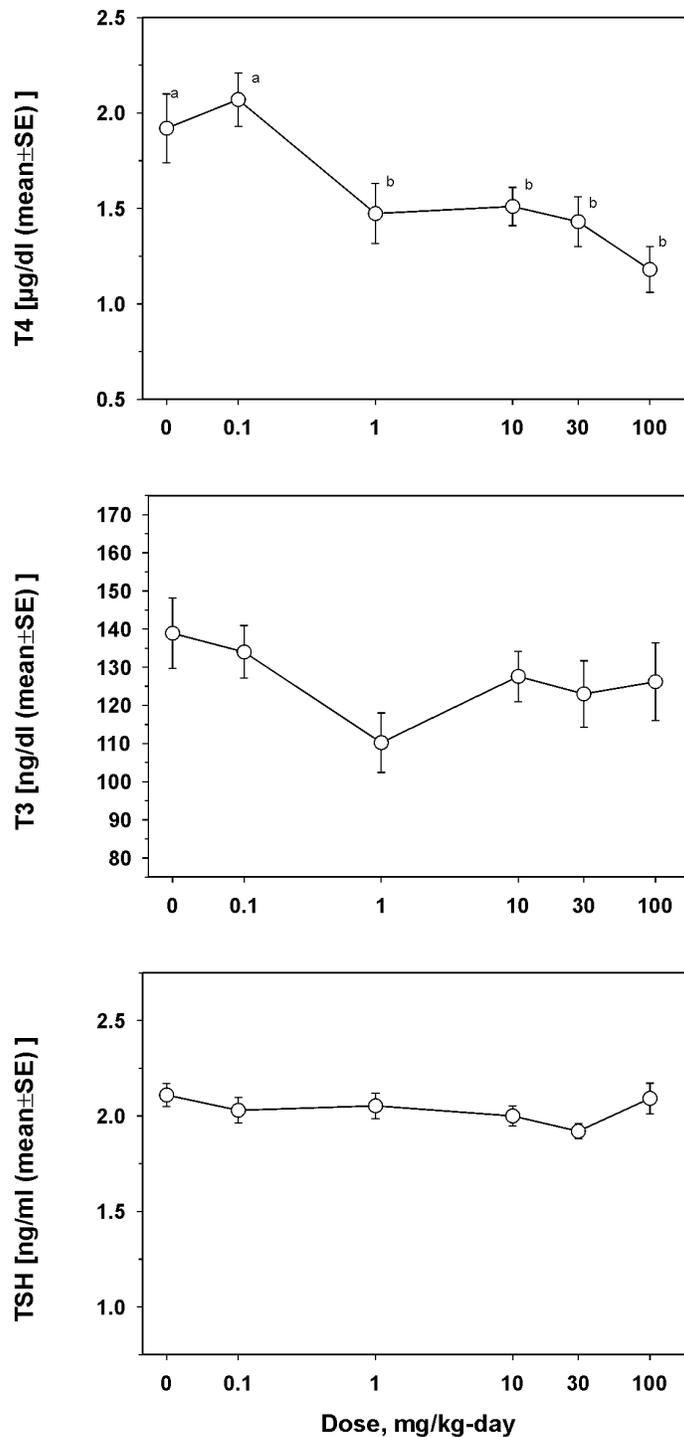
29 There were no treatment-related effects on gross external endpoints (Argus Research  
30 Laboratories, Inc., 1998c, Table 16). With regard to soft tissue anomalies (Argus Research  
31 Laboratories, Inc., 1998c, Table 17), there were several occurrences of lung lobe and gallbladder

1 absence, but their incidence was not treatment related. The statistically significant decrease in  
2 folded retina was attributed to an artifact of tissue processing. There were no treatment-related  
3 effects in skeletal or ossification alterations (Argus Research Laboratories, Inc.,1998c, Tables 18  
4 and 19), and no indication of an increased incidence of the more apical endpoint (i.e., any  
5 skeletal change). The fetal NOAEL thus is identified as greater than 100 mg/kg-day for embryo-  
6 fetal developmental toxicity, other than that which may have occurred in the thyroid.

### 7 8 **5.4.2.3 Maternal Thyroid and Pituitary Hormone Analyses**

9 The thyroid and pituitary hormone (T3, T4, and TSH) analyses were performed by  
10 AniLytics, Inc., for the does in the developmental rabbit study (Argus Research Laboratories,  
11 Inc.,1998c). Assays for T3 and T4 were performed using RIA kits according to manufacturer's  
12 standard procedures. Assay kits from the same batch number and with the same expiration date  
13 were used for the T3 and T4 measurements for each rabbit. The TSH assay was a  
14 double-antibody, RIA procedure developed for rabbits and performed by AniLytics, Inc. The  
15 analyses discussed in the Argus Research Laboratories, Inc. (1998c) report contain data from  
16 both pregnant and nonpregnant rabbits, with both groups combined in the analyses. Because of  
17 the known effects of pregnancy on thyroid hormones, EPA decided to reanalyze separately the  
18 data from the pregnant and nonpregnant animals. However, EPA determined that the analyses  
19 for nonpregnant animals were not useful because of the very limited number of subjects per  
20 group (final number of does: n = 3, 1, 0, 1, 1, and 1 nonpregnant does/group, and n = 22, 24, 25,  
21 24, and 23 pregnant does/group for the 0.0, 0.1, 1.0, 10, 30, and 100 mg/kg-day groups,  
22 respectively). Therefore, EPA conducted reanalyses for these two groups separately (Crofton,  
23 1998h). All data were taken from Appendix I of the report (Argus Research Laboratories,  
24 Inc.,1998c). The analyses used the pregnancy status data subsequently submitted (York, 1998e).  
25 Data from dependent measures (T3, T4, and TSH) were subjected to separate one-way ANOVA  
26 tests with treatment (dose) as the independent between-subjects variable as calculated in Crofton  
27 and Marcus (2001) and Marcus (2001). Mean contrasts were performed using Duncan's  
28 Multiple Range Test.

29 The main effect of treatment was not significant for T3. The T3 data are plotted in  
30 Figure 5-16A. There was a main effect of treatment and a significant difference between group  
31 means for the control versus 1.0, 10, 30, and 100 mg/kg-day groups on T4. These data are



**Figure 5-16. Effects from ammonium perchlorate in drinking water administration in pregnant New Zealand rabbits during GD6 to GD28 on T3 (A), T4 (B) and TSH (C) concentrations (ng/dL; mean ± SE) as recalculated in Table 5-2 (Crofton and Marcus, 2001). Data of Argus Research Laboratories, Inc. (1998c). Means with different letters were significantly different (p<0.05). Daily dose was estimated from water consumption data.**

1 plotted in Figure 5-16B. The main effect of treatment was not significant for TSH  
2 (Figure 5-16C). Results of these EPA reanalyses are different from those stated in the report.  
3 The report (Argus Research Laboratories, Inc., 1998c) states that the NOAEL for T4 was  
4 10 mg/kg-day. The current EPA analyses excluding nonpregnant animals, demonstrate a  
5 NOAEL at 0.1 mg/kg-day for T4. There was no statistical significance of any dose on T3 or  
6 TSH.

7 The lack of effect of any dose of perchlorate on T3 and TSH is difficult to explain. One  
8 must note that these data are from rabbits (the majority of other data are from rats) and that the  
9 data were collected 1 day prior to birth from the maternal compartment (whereas, all other data  
10 were collected in adults or from postnatal day time points). In a previous study in guinea pigs  
11 (Postel, 1957), enlarged thyroids were found in fetuses; whereas, there was no change in maternal  
12 weight or histology. Lampe et al. (1967) demonstrated a larger effect on fetal thyroid weight  
13 compared to maternal thyroid weights during late gestational exposure to perchlorate in rabbits.  
14 These data warrant caution when comparing effects of perchlorate in the maternal with the  
15 fetal/post-natal compartments.

### 16 17 **5.4.3 Segment II Developmental Study in Rats**

18 As recommended at the 1999 external peer review, a developmental study in addition to the  
19 one in rabbits was performed in rats (Argus Research Laboratories, Inc., 2000). The EPA review  
20 (Kimmel, 2000) was first performed on the audited final report (June 2000) and then on  
21 clarifications provided by the principal investigator (York, 2000) that do not appear in the final  
22 report.

23 Rats were given continuous access to target dosages of 0.01, 0.1, 1.0, and 30 mg/kg-day  
24 ammonium perchlorate in deionized drinking water beginning at least 15 days before  
25 cohabitation and continuing through the day of sacrifice. Each dosage group was comprised of  
26 24 females, assigned on a random basis, stratified by weight. There were no maternal deaths.  
27 Of these females, 20 were selected for evaluation; of these, 19, 19, 17, 20, and 20 were pregnant  
28 in the 0, 0.01, 0.1, 1.0, and 30 mg/kg-day groups. The EPA OPPTS 870.3700 testing guidelines  
29 recommend 20 pregnant animals per group at necropsy so that the power of the study to detect an  
30 exposure-related response was somewhat lower.

1 All rats were sacrificed on day 21 of presumed gestation (GD21), and a gross necropsy of  
2 the thoracic, abdominal, and pelvic viscera was performed. Gravid uterine weights were recorded,  
3 and the uterus then excised and examined for pregnancy, number and distribution of  
4 implantations, live and dead fetuses, and early and late resorptions. The number of corpora lutea  
5 in each ovary was recorded. Placentae were examined for abnormalities (size, color or shape).  
6 Each fetus was identified, weighed and examined for sex and gross external alterations.  
7 Approximately one-half of the fetuses in each litter were examined for soft tissue alterations.  
8 The heads of these fetuses were examined by free-hand sectioning. The remaining fetuses in  
9 each litter were examined for skeletal alterations and cartilage development.

#### 11 **5.4.3.1 Results of Maternal Examinations**

12 Three dams in the 30 mg/kg-day group showed an increase in localized alopecia that was  
13 statistically significant and was observed over 9-11 days during mid-late gestation. EPA feels  
14 that this should be considered biologically significant and exposure-related despite the claim by  
15 Argus Research Laboratories, Inc. (2000) and the study director (York, 2000) that such incidence  
16 is within the range observed historically at their testing facility.

17 There were no other maternal parameters that were clearly supportive of exposure-related  
18 effects. There was a statistically significant increase in corrected maternal body weight gain over  
19 gestation in the 0.1 and 30.0 mg/kg-day groups, and an increase (not statistically significant) in  
20 the 1.0 mg/kg-day group. There was also a reduction, again not statistically significant, in gravid  
21 uterine weight in three of the four exposure groups. These latter changes may be associated with  
22 reduced number of implants in the exposed groups (see below).

#### 24 **5.4.3.2 Developmental Endpoints**

25 The Argus Research Laboratories, Inc. (2000) report (Table B17) did not record  
26 preimplantation loss as an endpoint. EPA notes that there is an increase in this parameter over  
27 control (12%) at each dose level: 0.01 (18%), 0.1 (20%), 1.0 (16%), and 30.0 (25%) mg/kg-day.  
28 Whether this is statistically significant or biologically significant is unclear; although a decrease  
29 in live fetuses in three of the four exposure groups that was significant at the highest dosage was  
30 reported. Given the reduced power of this study to detect an effect, consideration was paid to  
31 this finding. The lack of an effect on live fetuses at the 1.0 mg/kg-day level is not clear, and

1 these results by themselves are insufficient to establish an effect level below 30 mg/kg-day. EPA  
2 recommends that preimplantation loss and embryo/fetal viability should be evaluated in any other  
3 study reports on this chemical.

4 Ossification sites per litter for sternal centers and forelimb phalanges were significantly  
5 reduced at 30 mg/kg-day, but Argus Laboratories, Inc. (2000) dismissed them as “reversible  
6 developmental delays.” EPA disagrees and contends that developmental delays, be they  
7 permanent or reversible, are not to be discounted as potential indicators of developmental  
8 toxicity. EPA additionally had some concern over the staining technique used for cartilage  
9 (Kimmel, 2000) which was not accepted by Argus Research Laboratories, Inc. (York, 2000) as an  
10 issue.

#### 11 **5.4.3.3 Conclusions Regarding Developmental Toxicity in Rats**

12 Based on the review of the maternal and fetal data, EPA concludes that there are signs of  
13 maternal and developmental toxicity at the 30.0 mg/kg-day level suggesting it as a LOAEL with  
14 a NOAEL then at 3.0 mg/kg-day. While none of the results were so clear that a definitive  
15 assessment can be made, the suggestive results are important to consider in light of the overall  
16 data base and mode of action for the toxicity of perchlorate.

### 17 **5.5 TWO-GENERATION REPRODUCTIVE TOXICITY STUDY**

18  
19  
20 The 1997 recommendation to characterize the potential perchlorate toxicity on reproductive  
21 parameters in a two-generation study was completed in 1999 (Argus Research Laboratories, Inc.,  
22 1999). This study has also been reported in the literature (York et al., 2001b), but since that  
23 manuscript did not use the PWG review of thyroid histopathology and its conclusions on other  
24 endpoints are the same as in the contract report, the manuscript will not be discussed further in  
25 this document. A schematic of the study design is provided as Figure A-2 of this document  
26 (Appendix A) to aid understanding of terminology and the protocol.

27  
28 The target doses (30 rats/sex/group) were 0, 0.3, 3.0, and 30 mg/kg-day of ammonium  
29 perchlorate in RO water provided by continual access. Concentrations were adjusted based upon  
30 actual water consumption and body weights recorded the previous week. Dosing solutions of  
31 ammonium perchlorate were prepared weekly, and the results of concentration analyses were

1 within acceptable ranges ( $\pm 10\%$ ) with one exception in the 3.0-mg/kg-day target group on May 5,  
2 1998 (15.8%). The stock solution was prepared at least once, but the exact number of times was  
3 not reported. Stability of solutions was assumed based on determinations by AFRL/HEST for  
4 the 90-day bioassay, as discussed in Section 5.2.3.

5 On arrival, Sprague-Dawley rats were assigned randomly to individual housing, and  
6 consecutive order was used to assign the P1 generation rats to cohabitation (one male rat per  
7 female rat). The cohabitation period lasted a maximum of 14 days. Females with spermatozoa  
8 observed in a vaginal smear or with a copulatory plug observed in situ were considered to be at  
9 GD0 and assigned to individual housing. Estrous cycling was evaluated daily by examination of  
10 vaginal cytology beginning 21 days before the scheduled cohabitation period and continuing until  
11 GD0. The rats were observed for viability at least twice each day of the study and daily for  
12 clinical signs. Body weights were recorded weekly during acclimation, on the first day of  
13 dosage, weekly thereafter, and at scheduled sacrifice. Feed consumption and water consumption  
14 values were recorded at least three times per week. Females were evaluated for duration of  
15 gestation (GD0 to the day the first pup was delivered). Day 1 of lactation (LD1, post-partum)  
16 was defined as the day of birth and was the first day on which all pups in a litter were weighed  
17 individually. Maternal behavior was observed on LD1, 4, 7, 14, and 21. Rats that did not deliver  
18 a litter were sacrificed on GD25 and examined for pregnancy status. Each litter was evaluated  
19 for litter size (live and dead pups versus live pups only) and pup viability at least twice each day  
20 of the 21-day post-partum period, and pups were counted daily. Deviations from expected  
21 nursing behavior also were recorded. All F1-generation rats were weaned at the same age based  
22 on observed growth and viability at LD21, unless required to be extended to LD28.

23 At the end of the 21-day post-partum period, all surviving P1 rats were sacrificed. Gross  
24 necropsy was performed on all animals, and all gross lesions were examined histologically.  
25 Organ weights were obtained for the thyroid, adrenal glands, brain, epididymides, heart, kidneys,  
26 liver, ovaries, pituitary, prostate, seminal vesicles, spleen, and testes. The thyroids and  
27 parathyroids were submitted for histopathological examination. Histopathology of other organs  
28 was performed for the control and high-dose groups. Blood was collected for determination of  
29 hormone levels (T3, T4, and TSH). Portions of the epididymides were used either for evaluation  
30 of sperm count or motility. The left testis was homogenized after weighing for analysis of  
31 spermatid concentration (spermatids per gram of tissue).

1 Pups not selected for continued evaluation in the study also were sacrificed on LD21.  
2 Blood was pooled by sex per litter for analysis of T3, T4, and TSH. At least 3 pups/sex/litter  
3 were necropsied and examined for gross lesions, including a single cross-section of the head at  
4 the level of the frontal-parietal suture and examination of the head for apparent hydrocephaly.  
5 Brain, thymus, spleen, and thyroid/parathyroid organ weights were obtained prior to fixation.  
6 The adrenal glands, thyroid/parathyroid, kidneys, and liver were retained in formalin.  
7

### 8 **5.5.1 General Toxicity Results and Evaluation of Reproductive Parameters**

9 There was a statistically significant decrease in water consumption by males, but not by  
10 females. The decrease with males and a smaller decrease with females were sufficiently small  
11 that they are not considered to be biologically significant (Argus Research Laboratories, Inc.,  
12 1999; Tables B5 and B6). There was a significant increase in ovarian weight at the 0.3-mg/kg-  
13 day dose level only (Argus Research Laboratories, Inc., 1999; Table C26). There also was  
14 slightly increased (not statistically significant) pituitary weight in females at the 0.3- and  
15 3.0-mg/kg-day dose levels.

16 The fertility results are potentially of concern, but the statistical analyses did not show any  
17 significant differences between groups for any of the tested parameters (Argus Research  
18 Laboratories, Inc., 1999; Table C21 through C23). However, at 0.3 mg/kg-day, there were four  
19 pairs that did not mate compared with one or two pairs in the other groups. Also at  
20 0.3 mg/kg-day, there were three females that showed at least one signal of persistent diestrus and  
21 one with persistent estrus (Argus Research Laboratories, Inc., 1999; Table C40). Incidences  
22 were lower in all other groups. Only one of those females did not have evidence of mating, but  
23 there were also four females that did not have evidence of mating in the 0.3 mg/kg-day group.  
24 When mating and conception failures are combined, pregnancy rates were 28/30, 22/30, 26/30,  
25 and 24/30 for the 0-, 0.3-, 3.0-, and 30-mg/kg-day groups, respectively. Of the females that were  
26 pregnant, litter size was slightly lower at the 3.0- and 30-mg/kg-day dose levels, with values of  
27 15.0, 14.9, 14.1, and 14.0 with increasing dose level. A similar trend was seen in the number of  
28 implantation sites (15.8, 15.8, 15.0, and 15.0). None of these results were statistically significant  
29 for the P1 generation, and the effect was not seen in the F1 generation. Consequently, this was  
30 not considered a significant finding (Clegg, 1999; Rogers, 2000). Note should be made that  
31 female intake of perchlorate during the last week of gestation was higher (Argus Research

1 Laboratories, Inc., 1999; Table C1). Additionally, in many of the perchlorate intake and feed  
2 consumption summary data, observations were reported for a low numbers of rats, apparently  
3 because of spillage.

4 In the F1 matings, all three perchlorate-dosed groups had a slightly higher fertility index  
5 than did the vehicle controls, but this appears to be due to a control value that was low (Clegg,  
6 1999; Rogers, 2000). These findings, the high dosage level of 30 mg/kg-day is designated as a  
7 NOAEL for reproductive parameters (Rogers, 2000), a finding that is consistent with the  
8 preliminary evaluation presented by EPA in 1999 (Clegg, 1999).

### 9 10 **5.5.2 Evaluation of Thyroid Histology**

11 The histopathology from the completed Argus Laboratories, Inc. (1999) two-generation  
12 reproductive study was limited to the thyroid gland and can be found in Wolf (2001; Tables 14  
13 through 21). In addition to the precursor lesion data (colloid depletion, hypertrophy, hyperplasia)  
14 discussed in Section 5.5.2.1, Wolf (2000) noted that two animals from the high dose group (30  
15 mg/kg-day) in the F1 generation (second parental generation, P2) in the study had adenomas and  
16 one of these animals had two adenomas for a total of three. Although statistically significant  
17 decreases in colloid were reported at both the 3.0 and 30.0 mg/kg-day dose levels (Argus, 1999),  
18 none of the rats in the other groups (0, 0.3, 3.0 mg/kg-day) developed thyroid follicular cell  
19 adenomas (0/30, 0/30, 0/30, respectively). These animals were dosed from conception to 19  
20 weeks of age (adult male F1 rats). The tumors were considered to be treatment related (Wolf,  
21 2000). Compared to the background incidence of thyroid follicular cell adenomas in male F344  
22 rats after 2 years on study at 38/3419 from 67 NTP studies or 1.1% incidence at the 2-year end  
23 sacrifice date, this study showed an incidence of 2/30 or 6.7% at 19 weeks. The tumors that  
24 occurred in the F1 generation male rat pups at 19 weeks were considered particularly remarkable  
25 (Wolf, 2000), and the EPA asked the NIEHS to review this incidence in context with the data  
26 from the National Testing Program (NTP). The finding is especially of concern since three of the  
27 F1 males in this high dosage group died of unknown causes (Rogers, 2000). This NIEHS  
28 analysis of the tumor incidence is described below (Dunson, 2001b) in Section 5.5.2.2.

### 5.5.2.1 Thyroid Weight, Colloid Depletion, Hypertrophy, and Hyperplasia

Absolute thyroid weight was increased significantly in the P1 males at the 3.0- and 30-mg/kg-day dose levels. An increase was significant in females at 30 mg/kg-day. A significant increase in thyroid weight relative to both body weight and brain weight also occurred at 30 mg in both sexes (Argus Research Laboratories, Inc., 1999; Tables B11 through B13 and C26 through C28). The histopathology for the P1 generation as reported by the PWG can be found in Wolf (2000; 2001, Tables 14 and 15). All three indices (colloid depletion, hypertrophy, and hyperplasia) were present with a clear suggestion of an increase in females for colloid depletion and hypertrophy at 3 and 30 mg/kg-day that supported the thyroid weight changes. Hyperplasia was more prominent at 30 mg/kg-day. Benchmark dose analyses using the male and female data for the P1 generation combined (Table 5-1; Geller, 2001a) result in BMDL estimates of 0.11 mg/kg-day for colloid depletion and 2.44 mg/kg-day for hyperplasia. The data for hypertrophy resulted in inadequate model fit.

The F1-generation (second parental, P2 generation) rats also exhibited all three thyroid histopathological indices in a dose-related fashion with 3 and 30 mg/kg-day as effect levels (Wolf, 2000; 2001, Tables 16 and 17). Benchmark dose analyses (Table 5-1; Geller, 2001a) using the male and female data combined for the P2 generation estimate 0.90, 0.15, and 0.0004 mg/kg-day as the BMDL for colloid depletion, hypertrophy, and hyperplasia. Of note is the dramatic overlap between colloid depletion and hypertrophy in this generation. It was the males in these rats, exposed in utero and then sacrificed at 19 weeks, that showed the 3 adenomas.

The F1-generation weanling rat data are presented in Tables 18 and 19 (Wolf, 2000; 2001) and also exhibit the three thyroid histopathology indices increased at 3 and 30 mg/kg-day. Benchmark dose analyses (Table 5-1; Geller, 2001a) using the male and female data combined result in BMDL estimates of 0.80, 0.057, and 0.66 mg/kg-day for colloid depletion, hypertrophy, and hyperplasia. Again, the overlap among indices is present.

Data for the second weanling generation (F2) rats are presented in Wolf (2000, 2001; Tables 20 and 21). Decreased colloid and hypertrophy remain increased at 3 and 30 mg/kg-day, but hyperplasia was not remarkable. Benchmark dose analyses (Table 5-1; Geller, 2001a) only provided adequate fit to the hypertrophy data and resulted in a BMDL of 0.32 mg/kg-day.

1 Across the generations, this study results in a range of BMDL estimates (mg/kg-day) for  
2 colloid depletion of 0.11 to 0.90, for hypertrophy of 0.057 to 0.32, and for hyperplasia of  
3 0.0004 to 2.44. Of note is the low BMDL value for hyperplasia (0.0004 mg/kg-day) in the P2  
4 generation, the same animals that exhibited tumors.

#### 6 **5.5.2.2 Bayesian Analysis of Tumor Incidence**

7 In order to properly interpret the results from a given toxicological study, it is often  
8 necessary to consider the data in light of additional information from outside of the study such as  
9 the variability and average level of response for positive and negative controls in past studies that  
10 are similar to the current study. It is also necessary to account for confounding effects that an  
11 exposure may have on variables that are associated with the outcome of interest. For example, it  
12 is important to adjust for animal survival to avoid bias in analyses of animal tumorigenicity  
13 (McKnight and Crowley, 1984) and reproductive toxicity (Dunson and Perreault, 2001).  
14 Typically, expert knowledge and information from related studies are accounted for only  
15 informally in the interpretation of a statistically significant or non-significant result. However,  
16 there are clear advantages to formally incorporating such extra information into the statistical  
17 analysis because it can be very difficult to interpret statistical significance when some aspect of  
18 the data is inconsistent with outside information (e.g., the control response is higher or lower  
19 than typically seen in related studies). In addition, the formal incorporation of outside  
20 information can improve sensitivity and limit bias when assessing toxicological effects. The  
21 advantages of including historical control data, in particular, has been well documented in the  
22 toxicological and statistical literature (Dunson and Dinse, 2001; Haseman, Huff, and Boorman,  
23 1984; Ibrahim, Ryan and Chen, 1998; Tarone, 1982).

24 Although frequentist (i.e., non-Bayesian) hypothesis tests can sometimes incorporate  
25 historical control data (see, for example, Tarone, 1982), outside information can be incorporated  
26 more naturally and flexibly within a Bayesian analysis. In Bayesian analyses, the unknown  
27 parameters in a statistical model are assigned prior probability distributions quantifying  
28 uncertainty prior to observing data from a current study. For example, based on experience with  
29 an assay system, a toxicologist may be 95% certain that the average level of response among  
30 vehicle control animals is between bounds *A* and *B* with *C* being the most likely value. This  
31 information can be formally incorporated into a Bayesian analysis through a prior distribution,

1 for a parameter measuring expected control response, which is centered on  $C$  and assigns 95%  
2 probability to values between  $A$  and  $B$ . Alternatively, the prior distribution can be estimated  
3 using data or summary statistics for control animals in historical studies if such information is  
4 available (Ibrahim, Ryan and Chen, 1998; Dunson and Dinse, 2001). For parameters about  
5 which little is known, noninformative or vague prior distributions that assign equal prior  
6 probability to a wide range of plausible values can be chosen.

7 Bayesian inferences about toxicological effects can be based on the posterior distribution  
8 for the parameters in the statistical model. The posterior distribution, which quantifies the  
9 current state of knowledge about the unknown quantities in the statistical model, is obtained by  
10 updating the prior distribution with the information in the data from the current study using  
11 Bayes theorem (refer to Gelman et al., 1995 for an overview). One can use the posterior  
12 distribution as a basis for conclusions about effects of interest by using posterior means, 95%  
13 credible intervals, and posterior probabilities as Bayesian alternatives to the maximum likelihood  
14 estimates, 95% confidence intervals, and p-values used in frequentist analyses. For example, as  
15 an alternative to a p-value, one could calculate the posterior probability of an increase in the  
16 proportion of animals with an adverse response in a treated group relative to the control.  
17 Bayesian approaches have been developed for a wide variety of toxicological applications,  
18 including risk assessment (e.g., Hill, 1996; Hasselblad and Jarabek, 1996), toxicokinetic  
19 modeling (e.g., Bernillon and Bois, 2000), and analysis of skin papilloma data (Dunson et al.,  
20 2000).

21 Without incorporating historical data on spontaneous neoplasms in Sprague-Dawley rats,  
22 the difference between 0/30 in the vehicle control and 2/30 in the 30 mg/kg-day group is  
23 non-significant by standard tests (e.g., Fisher's exact). However, the reported historical control  
24 incidence of thyroid follicular adenomas for male Sprague-Dawley rats in two-year studies is  
25 approximately 3-4% (Chandra et al., 1992; McMartin et al., 1992), suggesting that these tumors  
26 should be extremely rare among 19-week old animals in the absence of a treatment effect.  
27 Without formally incorporating this historical information into the statistical analysis through a  
28 prior distribution, it is very difficult to assess the weight of evidence in favor of a treatment-  
29 related increase in thyroid follicular adenoma incidence. A Bayesian approach was used to  
30 assess the effect of ammonium perchlorate in drinking water on thyroid follicular cell adenoma

1 incidence in male Sprague-Dawley rats from the two-generation study (Argus Research  
2 Laboratories, Inc., 1999).

#### 3 4 **5.5.2.2.1 Choosing prior distributions based on historical controls**

5 The proportion of control male Sprague-Dawley rats developing thyroid follicular cell  
6 adenomas in two-year carcinogenicity studies has been reported in the literature. Chandra et al.  
7 (1992) reported a rate of 48/1340 (3.6%), and McMartin et al. (1992) reported a rate of 23/583  
8 (3.9%). In order to incorporate this historical control data into our analysis of the effect of  
9 ammonium perchlorate on thyroid incidence at 19 weeks of age, we follow a Bayesian approach.  
10 The historical data can be summarized using a Beta (71,1852) prior distribution for the  
11 probability of a male Sprague-Dawley rat developing a thyroid follicular cell adenoma (in the  
12 absence of treatment with a test agent) by the time of natural death or sacrifice at two years. The  
13 Beta prior is the standard choice for a prior distribution on a probability (c.f., Dunson and  
14 Tindall, 2000 and Gelman et al., 1996 for further discussion of the Beta prior). The values  
15 71 and 1923 are simply the numbers of control male Sprague-Dawley that did and that did not  
16 develop thyroid follicular cell adenomas, respectively, from the Chandra et al. (1992) and  
17 McMartin et al. (1992) articles.

18 To account for the fact that the Argus (1999) study recorded thyroid incidence at 19 weeks  
19 and not at the time of natural death or at sacrifice at two years, a prior distribution for the ratio of  
20 the probability of thyroid follicular cell adenomas at 19 weeks to the lifetime probability in a  
21 two-year study was chosen. Portier, Hedges, and Hoel (1986) suggest that the probability of a  
22 control male Fischer 344/N rat developing a thyroid follicular cell adenoma increases  
23 approximately in proportion to age<sup>4.78</sup>. Based on this estimate and on the average survival time  
24 for male Fischer 344/N rats in the NTP historical control database (95.2 weeks), the prior  
25 expectation for the ratio is  $(19/95.2)^{4.78} = 5e-04$ . Allowing for a high degree of uncertainty in this  
26 prior expectation due to uncertainty in the Portier, Hedges, and Hoel (1986) estimate and in  
27 extrapolation from Fischer 344/N rats to Sprague-Dawley rats, a Beta (0.11, 2.6) for the ratio was  
28 chosen. This prior has median  $5e-04$  and 95% interval (0,0.379).

#### 1 **5.5.2.2.2 Results of NIEHS analysis**

2 Using the prior described in the previous subsection and “updating” the prior with control  
3 data from the Argus study (i.e., 0 tumors out of 30 control male rats), the NIEHS analysis  
4 estimated that a control rat has a 0.15% chance of developing a thyroid follicular cell adenoma by  
5 19 weeks (Dunson, 2001b). In addition, had perchlorate had no effect on the incidence of thyroid  
6 follicular cell adenomas, the probability of observing two or more rats with these tumors out of  
7 30 would be approximately 0.005. Thus, the data strongly support the hypothesis that  
8 ammonium perchlorate in the drinking water at 30 mg/kg-day causes an increase in the incidence  
9 of thyroid follicular cell adenomas.

#### 11 **5.5.2.2.3 Summary of NIEHS analysis of tumor data**

12 Incorporating historical control data in a Bayesian analysis, a significant increase in thyroid  
13 follicular cell adenoma incidence at 19 weeks in male Sprague-Dawley rats exposed to 30 mg/kg-  
14 day relative to controls was found (Dunson, 2001b). There was no evidence of an increase at low  
15 dose levels. This finding raises concern for in utero imprinting (i.e., that pups exposed in utero  
16 are subsequently more susceptible to thyroid hormone perturbation during post-natal  
17 development and adulthood), a phenomenon that is now appreciated in the endocrine disruption  
18 arena (Prins et al., 2001; Phillips et al., 1998; Seckl, 1997).

### 20 **5.5.3 Thyroid and Pituitary Hormone Analyses**

21 Thyroid and pituitary hormones were assayed in the P1-generation (both sexes), the  
22 F1-generation adults, the F1-generation pups (PND21) and the F2-generation pups.

23 In the P1-generation, there was an unexpected and unexplained increase in T3 levels.  
24 Effects on T4 and TSH were as expected, with a significant decrease in T4 and increase in TSH  
25 at the 30 mg/kg-day level.

26 An anomalous increase in T3 was also reported in the F1-generation adults. Significant  
27 ( $p \leq 0.01$ ) decreases in T4 of the F1-generation adult males occurred at the high dosage but  
28 increases ( $p \leq 0.05$ ) at the mid-doses are unexplained; TSH in the adult males was significantly  
29 increased ( $p \leq 0.01$ ) at the 30 mg/kg-day level. Similar results were reported for the  
30 F1-generation adult females.

1 In the F1-generation pups, the only statistically significant effects was an unexpected  
2 decrease in TSH at the mid doses in the males and an increase in the females at the lowest.  
3 Similarly seemingly spurious results were observed for the F2-generation pups.

4 Geller (1999b) presented the EPA analysis of thyroid hormones for this study for the  
5 P1-and F1-generation using separate repeated-measures ANOVAs with treatment as the  
6 independent between-subjects variable and sex as a within-litter repeated-measures variable.  
7 Mean contrasts were performed using Tukey's Studentized Range (HSD) test. In order to correct  
8 for multiple comparisons, the alpha for significance (for all interaction main effect tests) was  
9 adjusted to 0.029 (alpha of 0.05 divided by the square root of the number of ANOVA tests).

10 In the P1-generation rats, there was a significant dose effect and dose by sex interaction for  
11 both T4 and TSH. A NOAEL was identified for males only for T4 and TSH at a dose of  
12 3.0 mg/kg-day.

13 In the F1-generation (weanling pups) on PND21, the contract laboratories reported a  
14 decrease in TSH and an increase in T4. This effect was discounted by Argus Research  
15 Laboratories, Inc. (1999) because the decrease was not dosage-dependent and because TSH  
16 would be expected to increase and T4 to decrease. EPA found similar results with its analyses,  
17 noting that the significant dose effect on female T4 data was due to an elevated level in the  
18 0.3 mg/kg-day group relative to the high dose and also noting that the results were inconsistent  
19 with the mode of action for perchlorate (Geller, 1999b).

20 A significant increase in TSH was found in the adult F1 (P2 generation) rats at 30 mg/kg-  
21 day; a finding consistent with the tumors observed at this dosage, but T4 and T3 appeared to  
22 have increased in a dose-dependent fashion. Again the reason for this disparity is not clear.  
23  
24

## 25 **5.6 IMMUNOTOXICITY STUDIES**

26 As discussed in Chapter 3, immunotoxicity studies were included in the perchlorate testing  
27 strategy due to indications in humans and laboratory animals that perchlorate may affect immune  
28 and hematological function. For example, a study by Weetman et al. (1984) that appeared as a  
29 Letter to the Editor in *The Lancet*, investigated the effect of potassium perchlorate on human  
30 T- and B-cell responses to mitogens *in vitro*. Perchlorate at concentrations of 0, 0.01, 0.1, and  
31 10 mmol/L (1.17 g/L) were tested in cultures of human peripheral blood lymphocytes. IgG and

1 IgM in culture supernatants were measured by ELISA after culture for 10 days with pokeweed  
2 mitogen (PWM). Perchlorate at 0.1 to 10 mmol/L resulted in inhibition of PWM-induced IgG  
3 production at 10 mmol perchlorate/L inhibited IgM production. Lymphocyte proliferation as  
4 measured by <sup>3</sup>H-thymidine incorporation was reduced by 33 to 35% in cultures from five of six  
5 individuals in the presence of the T-lymphocyte mitogen phytohemagglutinin (PHA). Weetman  
6 et al. (1984) concluded that perchlorate had significant immunosuppressive activity on  
7 lymphocytes at pharmacologically-relevant concentrations in the absence of cytotoxicity, the  
8 latter of which was assessed by ethidium bromide/acridine orange fluorescence. Unfortunately,  
9 no details were provided as to when viability was determined during the 10 days of lymphocyte  
10 culture with perchlorate and PWM. While these and other results were not sufficient to infer that  
11 perchlorate was immunosuppressive or had other immunotoxic effects, there was uncertainty  
12 with respect to its potential to do so. It was not known whether this could be a direct effect of  
13 perchlorate but could plausibly also be due to its anti-thyroid effects.

14 An array of 14- and 90-day experiments, to evaluate the effects of drinking water  
15 administration of ammonium perchlorate on immunotoxicological and hematological parameters  
16 were performed using female B6C3F1 (Keil et al., 1998; Kiel et al., 1999; BRT-Burleson  
17 Research Technologies, Inc., 2000a,b,c,) or CBA/J Hsd mice (BRT-Burleson Research  
18 Technologies, Inc., 2000a,b,c). Parameters also were evaluated 30 days after one 90-day study to  
19 assess the reversibility on several observed effects. The mouse was chosen for these studies  
20 because it is the typical experimental species for immunotoxicological studies. In addition, data  
21 were collected on thyroid and pituitary hormones and thyroid histology to provide additional  
22 insight on interspecies variability by comparison with results of the rabbit and rat studies  
23 included in the testing strategy. The mice (8 to 10 weeks of age) were acclimated for 1 week  
24 prior to initiation of any study. Ammonium perchlorate was obtained from the sponsor  
25 (AFRL/HEST), and different lots were used for each of the major study groups (i.e., Keil, et al.,  
26 1998; Keil et al., 1999; BRT-Burleson Research Technologies, Inc., 2000a,b,c.). Primary stock  
27 solutions were prepared approximately every 1 to 2 months, and dosing solutions were prepared  
28 weekly. In the Keil et al. (1998) studies, there was an indication of a trend that mice exposed at  
29 the 30 mg/kg-day level consumed slightly less water on a weekly basis ( $\approx$  3 mL/week less than  
30 control). Consequently, differences were noted in the actual exposure for the high-dose group in  
31 the 14-day studies. This difference was not as marked in the 90-day studies. Concentration of

1 dosing solutions was verified by the sponsor (AFRL/HEST; data not shown). The one apparent  
2 disparity in dose level (0.1 mg/kg-day; experiment not specified) was rectified after  
3 reexamination of calculations (data not shown) (Keil et al., 1998). The mice were exposed to  
4 levels of 0, 0.1, 1.0, 3.0, or 30 mg/kg-day in the Keil et al., (1998, 1999) studies; while in the  
5 BRT-Burleson Research Technologies, Inc. (2000a,b,c) studies, the mice were exposed to levels  
6 of 0, 0.02, 0.06, 0.2, 2.0 or 50 mg/kg-day. The doses were established based on the mean body  
7 weight for each treatment group per week. Each dose group generally consisted of 6 to 10 mice,  
8 with the exception that some control groups in the BRT-Burleson Research Technologies, Inc.  
9 (2000a,b,c) studies had a group size of 20.

10 A number of 14-day experiments were conducted. In Experiments “C”, “G”, “I”, “J”, “T”,  
11 and “K” (Keil et al., 1998), the mice were sacrificed at Day 14; and body weight, organ weight  
12 and cellularity (thymus, spleen, liver, and kidney), a number of immunotoxicology and  
13 hematological parameters, thyroid histology, and thyroid and pituitary hormone levels were  
14 measured. These data are summarized in Tables 3, 6, 9, 12, 14, 16, 18, and 21 of the “Final  
15 Report” (Keil et al., 1999). In Experiments “U” and “V” (Keil et al., 1998), mice were  
16 challenged with sublethal amounts (2,300 or 2,700 colony-forming units [CFU]) of *Listeria*  
17 *monocytogenes* on Day 7 and then sacrificed on Day 14. The spleens were removed for a  
18 delayed-type hypersensitivity (DTH) assay (Keil et al. 1999: Table 31). In experiments “H”,  
19 “F”, and “M” (Keil et al., 1998), mice were challenged with P815 tumor cells by ip injection.  
20 At the 14-day terminal sacrifice, spleens were removed for the cytotoxic T lymphocyte (CTL)  
21 activity assay (Keil et al., 1999: Table 23).

22 A series of 90-day experiments also were conducted. In Experiments “A”, “D”, and “N”  
23 (Keil et al., 1998), mice were sacrificed after 90 days; and body weight, organ weight and  
24 cellularity (bone marrow, thymus, spleen, liver, and kidney), a number of immunotoxicology and  
25 hematological parameters, thyroid histology, and thyroid and pituitary hormone levels were  
26 measured (Keil et al., 1999: Tables 4, 7, 10, 13, 15, 17, 19, 20, and 22). In Experiments “B” and  
27 “E” (Keil et al., 1998), these same endpoints were measured after a 30-day recovery period (Keil  
28 et al., 1999: Tables 5, 8, 11, and 22.). In Experiment “P” (Keil et al., 1998), mice were  
29 challenged with P815 tumor cells by ip injection on Day 76. Spleens were removed at terminal  
30 sacrifice for the CTL activity assay (Keil et al., 1999: Table 24).

1 Two host resistance models, one a bacteria and the other a tumor, were used in 90-day  
2 experiments. Mice in Experiment “L” (Keil et al., 1998) were challenged with *Listeria*  
3 *monocytogenes* by iv injection. At terminal (90-day) sacrifice, spleens and livers were removed  
4 and cultured for *L. monocytogenes* growth. Unfortunately, the challenge concentration (i.e.,  
5 5360 CFU) of bacteria used was excessive, thereby prohibiting enumeration of the bacteria in the  
6 spleens of these mice. A second 90-day *L. monocytogenes*-challenge experiment (Keil et al.,  
7 1999) was subsequently undertaken using a lower (i.e., 2700 CFU) challenge concentration (see  
8 Keil et al., 1999: Table 34). For the tumor model, in Experiments “Q” and “O” (Keil et al.,  
9 1998), mice were challenged with B16F10 tumor cells by iv injection on Day 76. At the 90-day  
10 sacrifice, the lungs were removed, and the number of tumor nodules in both lungs were  
11 enumerated (Keil et al. 1999: Table 33).

12 The IgM and IgG antibody responses to sheep red blood cells (SRBCs) of mice exposed to  
13 ammonium perchlorate for 90 days and the IgM anti-SRBC response of mice exposed for 14 days  
14 was determined using an enzyme linked immunosorbent assay (ELISA) (figures on page 59, Keil  
15 et al., 1999). Based on EPA comments in 1998 and external peer review recommendation  
16 (Research Triangle Institute, 1999), a second contract was let to determine the antibody response  
17 to SRBCs using the more traditional antibody plaque-forming cell (PFC) assay (BRT-Burleson  
18 Research Technologies, Inc., 2000a,b,c). Unlike the ELISA, which measures SRBC-specific  
19 IgM antibody in serum, the PFC assay quantifies the number of plasma cells in the spleen which  
20 produce SRBC-specific IgM. The potent immunosuppressant cyclophosphamide (CP) was used  
21 as a positive control in these latter studies. In both the 14- and 90-day studies, mice were  
22 immunized iv with SRBCs 4 days prior to assay. The positive control mice were injected ip with  
23 15 mg/kg-day CP on the last 4 days of dosing prior to assay.

24 Concern about potential effects of ammonium perchlorate on contact hypersensitivity, also  
25 raised at the 1999 external peer review, were addressed in studies performed by Burleson et al.  
26 (2000). Eight-week-old female CBA/J Hsd mice that had been acclimated one week prior to  
27 dosing were exposed to 0, 0.02, 0.06, 0.2, 2.0, or 50.0 mg/kg-day for 14 or 90 days. The contact  
28 sensitizer, 2,4-dinitrochlorobenzene (DNCB), was applied to the surface of both ears on days 9,  
29 10 and 11 in the 14-day study, and on days 92, 93, and 94 in the 90-day study. Mice were  
30 assayed using the local lymph node assay (LLNA) on day 14 and 97 for the 14-day and 90-day

1 studies respectively. A CP-positive control group was included in each study, with  
2 administration of 15 mg/kg-day CP ip for 5 consecutive days prior to assay.

3 Data from the Keil et al. (1998, 1999) studies were analyzed as follows. Initially, analysis  
4 of variance was performed using Tukey's multicomparison ( $p < 0.05$ ) for the various parameters  
5 measured. A Fisher's multicomparison test was used in previous interim reports but not in the  
6 final one. The previous analyses reported effects. Consequent to criticisms of the analyses  
7 performed, as stated in the previous external review Draft Toxicological Review Document on  
8 Perchlorate (U.S. Environmental Protection Agency, 1998d), and reinforced by the comments of  
9 Dr. Kimber White at the previous external peer review (Research Triangle Institute, 1999), these  
10 and new data (i.e., the 14-day antibody response to SRBCs and the 90-day host resistance to  
11 *L. monocytogenes*) were analyzed as indicated in the "Final Report" (Keil et al., 1999). That is,  
12 data were combined from two or three experiments and evaluated by the Kolmogorv-Smirnov  
13 test for normality and Bartlett's test for homogeneity of variance. If data displayed a normal  
14 distribution and equal variance, two-way ANOVA, with experiments and treatments as factors,  
15 was performed. Tukey's pairwise comparison was performed to determine differences ( $p < 0.05$ )  
16 between control and treatments if no interaction was identified due to combining multiple  
17 experiments. If a significant interaction was identified in the ANOVA, data from each  
18 experiment were analyzed using one-way ANOVA and Tukey's pair wise analysis. The Kruskal-  
19 Wallis test was used if data were not normally distributed or variances were not equal; and if  
20 significant, the Mann-Whitney test was employed to determine differences ( $p < 0.05$ ) between  
21 control and treatments.

22 The results of the BRT-Burleson Research Technologies, Inc. (2000a,b,c) studies were  
23 analyzed as follows. Data from each treatment group were compared by first performing a  
24 Bartlett's Chi-Square test for variance of homogeneity. If found to be non-significant, ANOVA  
25 was employed using dose. If significant, then Dunnett's *t*-test was performed, with  $p < 0.05$  being  
26 significant. On the other hand, if Bartlett's Chi-square was significant, the non-parametric  
27 Kruskal-Wallis test was performed, which if significant was followed by a Jonckheere's-Terpera  
28 test for dose-dependent trends. The parametric ANOVA and the non-parametric extended  
29 Cochran-Mantel-Haenszel test were performed to determine whether the data could be pooled.

30 Results for the general toxicity and organ weight measures will be discussed in  
31 Section 5.6.1. Thyroid histopathology evaluations will be reported in Section 5.6.2, and analyses

1 of T3, T4, and TSH in Section 5.6.3. Results for the immunotoxicological and hematological  
2 parameters are discussed in Sections 5.6.4 and 5.6.5. A summary of the results and their  
3 potential significance is presented in Section 5.6.6.  
4

### 5 **5.6.1 Results for General Toxicity, Organ Weight, and Cellularity Measures**

6 There were no effects observed on body, thymus, spleen, liver, or kidney weights in the  
7 14-, 90-, or 120-day studies (Keil et al., 1999: Tables 6-8). Earlier interim reports indicated  
8 considerable variability in the splenic and thymic cellularity of ammonium perchlorate-exposed  
9 mice. This variability was due, in large part, to technical errors. Recognizing this, the contractor  
10 performed additional studies (i.e., “on at least two or more occasions”) in which “no significant  
11 changes in cellularity were observed.” (Keil et al., 1999). As such, in the “Final Report” no  
12 consistent alteration in splenic or thymic cellularity was observed in the 14-, 90-, or 120-day  
13 studies (Keil et al., 1999: Tables 9-11), nor in splenic lymphocyte CD4/CD8 subsets (Keil et al.,  
14 1999: Tables 14 and 15). With the exception that CD4-CD8+ thymic lymphocytes were  
15 increased in mice exposed to 0.1- and 1.0-mg/kg-day doses in the 14-day experiment, there were  
16 no other alterations in thymocyte subsets observed in the 14- or 90-day studies (Keil et al., 1999:  
17 Table 12). Furthermore, there were no alterations in the number of peritoneal macrophages  
18 obtained from mice exposed to any doses of ammonium perchlorate in the 14-, 90-, and 120-day  
19 studies (Keil et al., 1999: Tables 9-11), nor in bone marrow cellularity in the 14- and 90-day  
20 studies (Keil et al., 1999: Tables 9 and 10). Due to the absence of effects in the latter studies, no  
21 120-day study was performed.  
22

### 23 **5.6.2 Evaluation of Thyroid Histology**

24 Thyroid histopathology evaluation was performed for two experiments (A and D) in the  
25 Keil et al. (1998) study and eventually published in the final report (Keil et al., 1999). These data  
26 were transmitted by Warren (1999), and a preliminary review by EPA was presented at the 1999  
27 external peer review (Jarabek, 1999). The materials were provided to the PWG review, and the  
28 results are found in Wolf (2000, 2001; Table 23). These results corroborate the preliminary  
29 analyses that showed decreased colloid, follicular hypertrophy and hyperplasia to occur at the  
30 30 mg/kg-day dose. Congestion in the intrafollicular capillaries and the nuclear to cytoplasmic

1 ratio of the follicular cells were not recorded by the PWG but were both noted in the Warren  
2 (1999) report at 30 mg/kg-day (Jarabek, 1999). Hypertrophy was additionally observed in the  
3 lower doses of experiment “A”, and the reason for the disparity between the two studies is  
4 unclear. These results support the assertion that the hypothalamic-pituitary-thyroid feedback  
5 regulatory mechanism is conserved across species (rats, rabbits, mice and humans) and suggest a  
6 NOAEL of 3 mg/kg-day in this strain of mouse.

### 8 **5.6.3 Thyroid and Pituitary Hormone Analyses**

9 The report (Keil et al., 1998) contains thyroid hormone and thyrotrophin (TSH) data from  
10 14- and 90-day exposures to ammonium perchlorate in B6C3F1 mice. The following is a  
11 statistical analysis of the thyroid and pituitary hormone data (T4 and TSH) found in that report.  
12 There were no data for T3 reported in the original study submitted to EPA (Keil et al., 1998).  
13 The EPA reanalyzed the data that were supplied in Excel<sup>®</sup> spreadsheets to EPA by Dr. Deborah  
14 Keil, and the data are published therein (Crofton, 1998i). Subsequent submission of additional  
15 data files also containing data for T3 were included in reanalyses (Crofton, 2001a). Data for  
16 dependent measures (T4 and TSH) were subjected to separate analyses. The T4 and TSH data  
17 were analyzed with a two-way ANOVA, with duration (14, 90, and 120 days) and treatment  
18 (dose) as the independent between-subjects variables as reanalyzed by Crofton and Marcus  
19 (2001) and Crofton (2001a) as reported in Table 5-2. Mean contrasts were performed using  
20 Duncan’s Multiple Range Test.

21 Results of these EPA reanalyses are different from those stated in the Keil et al. (1998)  
22 report. The EPA reanalysis of the T3 data (Crofton, 2001a) found main effects of time and  
23 treatment, but no time-by-treatment interaction. Mean contrast testing showed a LOAEL of  
24 0.1 mg/kg-day; however, the dose-related decrease was not linear. The 0.1 and 3.0 mg/kg-day  
25 doses differed from controls but the 1.0 and 30.0 mg/kg-day doses did not. There was a  
26 significant time-by-treatment interaction for T4. After 14 days of exposure there was no effect  
27 with a NOAEL at 30 mg/kg-day; whereas, after 90 days of exposure the LOAEL was 0.1 mg/kg-  
28 day. T4 recovered after 30 days postexposure. There was no effect of perchlorate on TSH  
29 concentration contrary to the changes in histopathology discussed in Section 5.6.2.

1           These effects are of interest in that they demonstrate effects in mice comparable in nature to  
2 that in rats and indicate that the hypothalamic-pituitary-thyroid feedback system is conserved  
3 across species.  
4

#### 5 **5.6.4 Results of Immune Function Assays**

6           No consistent alteration in CTL activity was observed in three 14-day studies (“M”, “H”,  
7 and “F”, Keil et al., 1998). No effects were observed on CTL activity in Experiments “M” and  
8 “H”. However, in Experiment “F”, increases in CTL activity were observed at the 0.1-mg/kg-day  
9 ammonium perchlorate dose for effector to target cell (E:T) ratios of 100:1, 30:1, and 10:1, and,  
10 at the 1- and 3-mg/kg-day doses, for an E:T ratio of 10:1. In a 90-day study (“P”, Keil et al.,  
11 1998) there were no alterations in CTL activity at any dosages or E:T ratios. The variability and  
12 inconsistencies observed in the early interim reports were ascribed to “technical issues” that were  
13 consequently “corrected”. In fact, the data presented in Tables 23 and 24 (Keil et al., 1999)  
14 which includes data for dexamethasone, a potent immunosuppressant and positive control,  
15 indicates that there were no effects of ammonium perchlorate AP exposure on CTL activity.

16           There was also no consistent alteration in the DTH response, as measured by the  
17 lymphoproliferation (LP) of splenic lymphocytes from *L. monocytogenes*-challenged mice  
18 incubated with soluble *Listeria* antigen (SLA) in two 14-day studies (“U” and “V”, Keil et al.,  
19 1998). The LP response was increased only in cultured splenic lymphocytes from mice in the  
20 30-mg/kg-day group stimulated with 0.1  $\mu\text{g/mL}$  SLA in Experiment “U” and in cultures of  
21 splenic lymphocytes from mice in the 3-mg/kg-day group stimulated with 8  $\mu\text{g/mL}$  SLA in  
22 Experiment “V” (Keil et al., 1998). The “Results Summary and Status” page of Keil et al. (1998)  
23 indicates that a 90-day DTH study was planned. These 90-day data and a summary of the 14-day  
24 data are presented in Tables 32 and 31 respectively, of the “Final Report” (Keil et al., 1999). The  
25 data indicated an enhanced LP response in mice dosed at 30-mg/kg-day in both the 14-day and  
26 90-day studies.

27           No alteration in splenic natural killer (NK) cell activity was observed in two 14-day studies  
28 (“G” and “T”, Keil et al., 1998). The 14-day Experiment “T” data are presented in a table;  
29 however, the raw data and statistics for this study were not found in the submission. Inconsistent  
30 results were obtained in two 90-day studies (“D” and “N”, Keil et al., 1998) in which NK cell  
31 activity was increased at the 30-mg/kg-day ammonium perchlorate in Experiment “N”; however,

1 no effects were observed at any doses in Experiment “D”. A similar increase in NK cell activity  
2 at the 30-mg/kg-day dose was observed in the 120-day Experiment “E” (see also the data in  
3 Tables 21-22, Keil et al., 1999, in which the positive control dexamethasone was employed).  
4 The lack of any change in the number of B16F10 tumor nodules in the lungs of mice from the  
5 90-day “Q” study (Keil et al., 1998; see also Table 33, Keil et al., 1999), particularly at  
6 30 mg/kg-day, suggests that the increased NK-activity does not reflect a significant biological  
7 effect (see below). The EPA notes that there is a good deal of variation in NK activity data for  
8 the controls in the 14-day “G” study, the 90-day “D” and “N” studies, and the 120-day “E” study,  
9 which were 34, 6.4, 13.6, and 18.4 lytic units/10<sup>7</sup> splenic lymphocytes, respectively. Also, the  
10 14-day “G” study was not included in Table 33 (Keil et al., 1999).

11 Decreased *in vitro* phagocytosis of *L. monocytogenes* was observed at 3 and 30 mg/kg-day  
12 of ammonium perchlorate in the 14-day “C” and 90-day “A” studies (Keil et al., 1998). In the  
13 90-day “N” study, macrophage phagocytosis was decreased in all dose groups. However, in the  
14 14-day “G” and 90-day “D” studies and in two 120-day studies (“B” and “E”), no effect on  
15 macrophage phagocytosis was observed (Keil et al., 1998). In the “Final Report” (Keil et al.,  
16 1999), these alterations were confirmed (i.e., decreased phagocytosis at 1.0 and 30.0 mg/kg-day  
17 in the 14-day study (Keil et al., 1999: Table 27) and decreased phagocytosis at 0.1, 1.0, 3.0, and  
18 30.0 mg/kg-day in the 90-day study (Keil et al., 1999: Table 28). However, after a 30 day  
19 recovery period (i.e., 120-day study, Keil et al., 1999: Table 29) phagocytic function was  
20 comparable across control and treated mice. These data suggest that ammonium perchlorate  
21 suppresses the phagocytic capacity of peritoneal macrophages *in vitro*, but that this suppression  
22 may be reversed after a 30-day recovery period. Criticism of the use of an *in vitro* rather than an  
23 *in vivo* assessment of macrophage function was raised in the 1998 EPA ERD document and at  
24 the 1999 external peer review by Dr. Kimber White (Research Triangle Institute, 1999).

25 No consistent alteration in peritoneal macrophage nitrite production was observed in 14-,  
26 90-, and 120-day studies. Increased nitrite production from macrophages cultured with interferon  
27 (IFN) occurred at doses of 3 and 30 mg/kg-day and from macrophages cultured with IFN and  
28 lipopolysaccharide for the 30-mg/kg-day dose in the 90-day “D” study (Keil et al., 1998). Also,  
29 increased nitrite production from macrophages cultured with IFN was observed at 3 mg/kg-day in  
30 the 90-day “N” study (Keil et al., 1998). An increase in nitrite production for macrophages  
31 cultured with IFN or LPS alone also occurred for the 30-mg/kg-day group in the 120-day “B”

1 study (Keil et al., 1998). These data suggest a “trend” toward increased nitrite production at the  
2 higher doses of ammonium perchlorate.

3 A subsequent analysis of these data, as presented in Tables 25 and 26 (Keil et al., 1999),  
4 demonstrates “no significant difference in nitrite production of peritoneal macrophages” (Keil  
5 et al., 1999).

6 A 90-day study (“L”, Keil et al., 1998) was performed to determine if exposure of mice to  
7 ammonium perchlorate results in alterations in resistance to infection with *L. monocytogenes*.  
8 A trend toward increased resistance was suggested by the data; however, technical difficulties  
9 were encountered. For example, there was variability in the number of *L. monocytogenes* CFU/g  
10 liver recovered from control mice. It was not possible to enumerate the number of CFU/g spleen  
11 in mice due to the high concentration of bacteria injected and also to an inadequate dilution of  
12 spleen cell suspensions. In a subsequent 90-day study, mice were challenged with a lower  
13 concentration of bacteria such that both the CFU/g liver and spleen could be determined. These  
14 results, presented in Table 34 (Keil et. al., 1999), indicate that ammonium perchlorate exposure  
15 does not alter resistance to infectious challenge to *L. monocytogenes*.

16 No effects were observed in an initial 90-day B6F10 tumor challenge host-resistance model  
17 experiment (“Q”, Keil et al., 1998). Another 90-day B6F10 tumor challenge experiment (i.e.,  
18 “O”) was performed, and the combined results of these two experiments are presented in  
19 Table 33 (Keil et al., 1999). These data indicate that there were no differences in the number of  
20 tumors present in the lungs of ammonium perchlorate-exposed mice compared with control mice.

21 Two separate groups of studies examining the effect that ammonium perchlorate has on the  
22 antibody response to SRBCs were performed by independent contractors (Keil et al, 1999;  
23 BRT-Burleson Research Technologies, Inc, 2000a,b,c). Initial studies were performed by Keil  
24 et al. (1999), in which the IgM and IgG antibody responses were determined using ELISAs.  
25 As indicated in the figures on page 59 (Keil et al., 1999), no change in the IgM levels in a 14-day  
26 study, nor in the IgM and IgG levels in a 90-day study, was observed between control and any  
27 ammonium perchlorate treated mice .

28 In the second set of studies, the anti-IgM SRBC PFC assay was employed (BRT-Burleson  
29 Research Technologies, Inc, 2000a,b,c), using CP as a positive immunosuppressant control.  
30 In the 14-day study there were no differences in the PFC response between control and treated  
31 mice when expressed either as the number of PFC/spleen or PFC/10<sup>6</sup> spleen cells (BRT-Burleson

1 Research Technologies, Inc, 2000a,b,c: Figures 3 and 4). On the other hand, in the 90-day study  
2 the PFC response was increased in the 2.0 and 50.0 mg/kg-day groups when expressed as the  
3 PFC/spleen and increased only in the 50.0 mg/kg-day group expressed as PFC/10<sup>6</sup> spleen cells  
4 (BRT-Burleson Research Technologies, Inc., 2000a,b,c: Figures 5 and 6). This disparity was not  
5 due to any difference in splenic cellularity between the control and treated mice. In both the  
6 14- and 90-day studies, CP significantly inhibited the PFC response, expressed either as  
7 PFC/spleen or PFC/10<sup>6</sup> spleen cells compared to the controls.

8 The results of the effect that 14- and 90-day exposure to ammonium perchlorate has on the  
9 development of a contact hypersensitivity response to DNCB, as determined by the LLNA,  
10 indicate that an ammonium perchlorate dose as low as 0.06 mg/kg-day enhances this response.  
11 In the 14-day study, the LLNA was increased at doses of 0.06, 0.2, and 50.0 mg/kg-day, but not  
12 2.0 mg/kg-day (BRT-Research Technologies, Inc., 2000a,b,c: Figure 8). The results of the  
13 90-day study were somewhat different in that, while the LLNA was enhanced at 0.06 and  
14 0.2 mg/kg-day, it was suppressed at 50 mg/kg-day (BRT-Research Technologies, Inc., 2000a,b,c:  
15 Figure 9). Another disparity between these two studies was that while CP suppressed the LLNA  
16 in the 14-day study, it did not suppress this response in the 90-day study.

### 18 **5.6.5 Results for Evaluations of Hematological Parameters**

19 There were no differences observed between control and dosed mice in 14- or 90-day  
20 experiments for erythrocyte cell count, hemoglobin, hematocrit, mean corpuscular volume, mean  
21 corpuscular hemoglobin, and mean corpuscular hemoglobin concentration; nor in leukocyte  
22 differential counts of neutrophils, monocytes, and lymphocytes. Because of the absence of  
23 effects in these studies, no 120-day study was performed. No effects were observed in a single  
24 14-day study (Experiment "T", Keil et al., 1998) on platelet counts. An increase in the  
25 percentage of reticulocytes was observed in the peripheral blood of mice exposed to 3 mg/kg-day  
26 of ammonium perchlorate in a 90-day study ("N", Keil et al., 1998). No other reticulocyte data  
27 are available because of "the minimal availability of blood obtained from each mouse" in other  
28 studies (Keil et al., 1998). In a subsequent 14-day study, there were no changes in the  
29 hematological parameters examined between control and ammonium-perchlorate-treated mice  
30 (Keil, et al., 1999: Table 16).

1 No consistent alteration in the bone marrow stem cell assay was observed. An increase in  
2 the number of colony-forming units was observed in bone marrow cell cultures from mice dosed  
3 at 30 mg/kg-day in a 14-day study (“K”, Keil et al., 1998). However, there was no effect of  
4 ammonium perchlorate exposure on the stem cell assay in a 90-day study (“D”, Keil et al., 1998).  
5 In a subsequent 90-day study, while no alteration in the stem cell assay was observed between  
6 control and ammonium perchlorate-treated mice, exposure to the positive control dexamethasone  
7 resulted in suppression of the stem cell assay ( Keil, et al., 1999: Table 20).

### 9 **5.6.6 Results Summary**

10 The results of the various studies of immune function are summarized in Table 5-5.  
11 Although innate (i.e., macrophage and NK cell function) and cell-mediated (i.e., cytotoxic  
12 T lymphocytes [CTL], CD4, and CD8) immune functions were evaluated in the initial studies by  
13 Keil et al, (1998), EPA noted that humoral immunity (i.e., B cells and antibody response) was not  
14 (Smialowicz, 1999). The EPA suggested strongly that the antibody response to SRBCs is one of  
15 the most commonly effected functional parameters in animals exposed to chemical  
16 immunosuppressants (Luster et al., 1988). In fact, it is one of the assays required by EPA for test  
17 rules. The EPA also requested that an additional 90-day *L. monocytogenes* host-resistance study  
18 be undertaken consequent to technical problems associated with the initial 90-day study (Keil  
19 et al., 1998). As such, the EPA felt that these data would provide a more comprehensive  
20 evaluation of the potential for immunosuppression by ammonium perchlorate. In addition, the  
21 EPA requested that thyroid histology and thyroid and pituitary hormone data be obtained in order  
22 to provide additional insights on interspecies variability for this effect.

23 Consequently, the sponsor and investigators, Keil et al. (1998), agreed to perform these  
24 assays, the results of which are presented in the “Final Report” (Keil et al., 1999).

25 Subsequent to receipt of the results of the antibody response to SRBCs (Keil et al., 1999),  
26 in which antibody titers were expressed as the O.D. 50 or midpoint titer, rather than the more  
27 conventional titer to achieve a 0.5 O.D., a second request to determine the potential effects of  
28 ammonium perchlorate on the response to SRBCs was issued. In this same solicitation, the EPA  
29 also requested that a sensitization test be performed. The results of these studies are found in  
30 BRT-Burleson Research Technologies, Inc. (2000a, b, c).

**TABLE 5-5. SUMMARY OF IMMUNOTOXICITY TEST RESULTS**

Series/Strain/Sex (Study)	Exposures Period and Doses (mg/kg/d)	Endpoint	NOAEL/LOAEL Designations
<b>Mouse/B6C3F1/Female (Keil et al., 1998;1999)</b>	14-days 0, 0.1, 1.0, 3.0, or 30	Weights: body, thymus, spleen, liver, kidney	None
		Cellularity: spleen, thymus, bone marrow	None
		Splenic CD4CD8 cells	None
		NK cell activity/B16F10 tumor challenge	None/Not Done
		CTL to P815 cells ( <i>in vitro</i> )	Increased at 0.1, 1.0 and 3.0; no effect in subsequent “corrected” study.
		<i>L. monocytogenes</i> challenge	Not Done
		DTH to <i>L. monocytogenes</i> antigen	Increased at 30. NOAEL = 3.0, LOAEL = 30
	90-days 0, 0.1, 1.0, 3.0, or 30	Macrophage phagocytosis ( <i>in vitro</i> )	Decreased at 1.0 and 30. NOAEL = 0.1, LOAEL = 1.0
		Macrophage nitrate ( <i>in vitro</i> + IFN or LPS)	None
		IgM ELISA to SRBCs	None
		Weights: body, thymus, spleen, liver, kidney	None
		Cellularity: spleen, thymus, bone marrow	None
		Splenic CD4CD8 cells	None
		<b>Mouse/B6C3F1/Female (Kiel et al., 1998; 1999)</b>	90-days 0, 0.1, 1.0, 3.0, or 30
CTL to P815 cells ( <i>in vitro</i> )	None		
<i>L. monocytogenes</i> challenge	None		
DTH to <i>L. monocytogenes</i> antigen	Increase at 30. NOAEL = 3.0, LOAEL = 30		
Macrophage phagocytosis ( <i>in vitro</i> )	Decreased at 0.1, 1.0, 3.0 and 30, LOAEL = 0.1		

**TABLE 5-5 (cont'd). SUMMARY OF IMMUNOTOXICITY TEST RESULTS**

Series/Strain/Sex (Study)	Exposures Period and Doses (mg/kg/d)	Endpoint	NOAEL/LOAEL Designations
<b>Mouse/CBA/JHsd/Female (BRT-Burleson Research Technologies, Inc., 2000a,b,c)</b>	14-days 0, 0.02, 0.06, 0.2, 2.0, or 50	Macrophage nitrate ( <i>in vitro</i> + IFN or LPS)	None
		IgM ELISA to SRBCs	None
		anti-SRBC PFC/10 <sup>6</sup> cells	None
		anti-PFC/spleen	None
	90-days 0, 0.02, 0.06, 0.2, 2.0, or 50	LLNA to DNCB	Increased at 0.06, 0.2, and 50, but not at 2.0. NOAEL = .02, LOAEL = 0.06
		anti-SRBC PFC/10 <sup>6</sup> cells	Increased at 50. NOAEL = 2.0, LOAEL = 50
		anti-PFC/spleen	Increased at 2.0 and 50. NOAEL = 0.2, LOAEL = 2.0
		LLNA to DNCB	Increased at 0.06 and 0.2, but not at 2.0; decreased at 50. NOAEL = 0.02, LOAEL = 0.06

NK = natural killer; CTL = cytotoxic lymphocyte; DTH = delayed type hypersensitivity; IFN = interferon; SRBC = sheep red blood cell; PFC = plaque forming colony; LLNA = local lymph node assay; DNCB = 2,4-Dinitrochlorobenzene.

1           The three immune function parameters altered by ammonium perchlorate exposure were  
2 the following: suppression of *in vitro* peritoneal macrophage phagocytosis of *L. monocytogenes*,  
3 enhancement of the PFC response to SRBCs, and enhancement of the LLNA to DNCB. These  
4 results are summarized and discussed below.

5           Decreased *in vitro* phagocytosis of *L. monocytogenes* by peritoneal macrophages obtained  
6 from mice dosed for 14 days at 1- or 3- and 30-mg ammonium perchlorate/kg-day was observed  
7 (Keil et al, 1998, 1999). In mice exposed for 90-days, phagocytosis was decreased in all dosage  
8 groups (Keil, 1998, 1999). However, in the 120-day (i.e., 90-day ammonium perchlorate  
9 exposure followed by 30-day recovery) studies, no effect on macrophage phagocytosis of  
10 *L. monocytogenes* was observed (Keil et al., 1998, 1999). Taken together, these data suggest that

1 ammonium perchlorate suppresses the *in vitro* phagocytic capacity of peritoneal macrophages,  
2 but that this suppression is reversed after a 30 day recovery period.

3 This decrease in macrophage phagocytic activity could be expected to be reflected in the  
4 results of the *L. monocytogenes* infectivity data because, along with other immune system  
5 components, macrophages play a pivotal role in resistance to infection by this bacterium.  
6 For example, the pathogenesis of *L. monocytogenes* is associated with its ability to grow within  
7 mononuclear phagocytes. Complement (C') plays an important role in *L. monocytogenes*  
8 infections, as demonstrated by the fact that C'-deficient mice have impaired host resistance to  
9 this bacterium. This impairment in C'-deficient mice is caused by the absence of macrophage-  
10 associated C'. The T-lymphocytes also play a major role in defense against *L. monocytogenes*  
11 because complete elimination of bacteria from infected tissue is accomplished by macrophages  
12 activated by T-cell dependent mechanisms.

13 However, the *L. monocytogenes* host-resistance studies indicate that ammonium  
14 perchlorate exposure of mice does not alter the ability to combat this bacterial infection. With  
15 the exception that clearance of *L. monocytogenes* from the liver of mice given a 5360 CFU  
16 challenge following dosing at 3.0 mg AP/kg/day for 90 days was reduced, no other effect was  
17 observed (Keil et al., 1999: Table 43). These data imply that while *in vitro* phagocytosis by  
18 peritoneal macrophages of this bacterium was reduced following ammonium perchlorate  
19 exposure, the ability of macrophages from other *in situ* sites (e.g., spleen, liver) to clear  
20 *L. monocytogenes* was not altered.

21 Exposure of mice to 2.0 or 50.0 mg ammonium perchlorate/kg/day for 90, but not 14, days  
22 resulted in enhancement of the antibody response to SRBCs as determined by the PFC assay  
23 (BRI-Burleson Research Technologies, Inc., 2000a,b,c). In both the 14- and 90-day studies, the  
24 PFC response was suppressed by dosing mice with the immunosuppressive positive control CP.  
25 The PFC assay is routinely used for identifying chemicals that are immunosuppressive. The  
26 reason why the highest dose(s) of ammonium perchlorate, given over 90 days, enhanced this  
27 response is not known. It is possible that under these dosing conditions ammonium perchlorate  
28 may have an adjuvant-like or enhancing effect on the antibody response to SRBCs. The ELISA  
29 data for mice exposed to up to 30.0 mg ammonium perchlorate/kg/day, for 14 or 90 days (Keil  
30 et al., 1999), do not corroborate this enhanced response to SRBCs as determined by the PFC

1 assay. However, taken together, the PFC and ELISA data indicate that ammonium perchlorate  
2 does not suppress the immune response to SRBCs.

3 The LLNA is an accepted approach for identifying chemicals with the potential of causing  
4 dermal contact hypersensitivity (CHS) reactions in humans. In this assay the test substance,  
5 2,4-dinitrochlorobenzene (DNCB) was topically applied on three consecutive days to both ears of  
6 the mouse. Two days later the mice were injected iv with radioactive uridine (e.g., <sup>125</sup>IUDR).  
7 Five hours later, the lymph nodes draining the ears, referred to as the “auricular” lymph nodes,  
8 were removed and <sup>125</sup>IUDR incorporation by the lymph node cells determined. Since the nodes  
9 draining the ear (i.e., “auricular” nodes) have no standard anatomical nomenclature, experience  
10 in identifying these nodes as well as appropriate and consistent excision of these nodes from  
11 control and test mice is critical. The LLNA evaluates the induction phase of the CHS reaction by  
12 assessing the influx of lymphoid cells and the differential argumentation of lymphocyte  
13 proliferation elicited by exposure to the test chemical relative to that of a vehicle control.

14 The data from BRT-Burleson Research Technologies, Inc. (2000a,b,c) report that exposure  
15 to ammonium perchlorate enhances/exacerbates the LLNA response to DNCB at doses of 0.06,  
16 and 0.2 mg/kg/d in both the 14- and 90-day. While a dose of 50.0 mg/kg-day for 14 days also  
17 enhanced this response, a dose of 2.0 mg/kg-day did not. Similarly, a dose of 2.0 mg/kg-day in  
18 the 90-day study did not enhance the LLNA response to DNCB. In contrast to the 14-day study,  
19 exposure of mice to 50.0 mg ammonium perchlorate/kg/day in the 90-day study resulted in  
20 suppression of the LLNA response. In the 90-day study, the positive control CP did not suppress  
21 the LLNA response to DNCB. The failure of CP to suppress this response in the 90-day vs.  
22 14-day study is disquieting because CP was administered similarly (i.e., 15 mg/kg-day for  
23 5 consecutive days prior to the LLNA) in both studies. The only difference between these two  
24 studies was that the mice in the 90-day study were 11 weeks older. This difference in age,  
25 however, should not influence the ability of CP to suppress this response. The fact that CP did  
26 not suppress the LLNA response in the 90-day study calls into question the performance of this  
27 and perhaps the 14-day study.

28 Application of the LLNA for identification of chemicals that are contact sensitizers  
29 routinely involves a demonstration of a dose-related increase in the LLNA using, at a minimum,  
30 three increasing concentrations of the test agent. Neither the 14- nor 90-day ammonium  
31 perchlorate LLNA data demonstrate a dose-response relationship, which would be expected if

1 ammonium perchlorate was acting additively or synergistically with DNCB to increase the  
2 LLNA response. While higher concentrations of a contact sensitizing agent will increase the  
3 LLNA response, there is no information in the literature that indicates such an increase results in  
4 a more serious or potentially detrimental effect on the host. Consequently, the physiologic  
5 significance of the observed increase in the LLNA response to DNCB in ammonium perchlorate-  
6 exposed mice is unknown. This is unlike the situation with immunosuppressive agents where  
7 suppression of specific immune function(s) can be linked to a biological detrimental effect (i.e.,  
8 decreased host resistance to an infectious agent or tumor).

9 It is interesting to note that there are published reports in which non-sensitizing agents have  
10 been employed to improve the sensitivity of the LLNA to detect sensitizers. For example,  
11 Vitamin A acetate dietary supplementation enhances the detection of weak sensitizers, and at low  
12 concentrations of moderate sensitizers, assessed by the LLNA. The mechanism(s) for this  
13 increased detection of contact sensitizers is not known. However, topically applied retinol causes  
14 epidermal hyperplasia which may lead to increased numbers of antigen-presenting cells in the  
15 epidermis. Retinoids also up regulate the sensitization phase of DTH induction through direct or  
16 indirect stimulation of T cells. Non-sensitized mice, fed a diet supplemented with retinol,  
17 display somewhat higher LLNA responses compared to control mice on a normal diet. This  
18 suggests that dietary retinol itself causes cellular infiltration and/or proliferation in the absence of  
19 a contact sensitizer as measured by the LLNA. It may be that ammonium perchlorate, in the  
20 absence of DNCB, has the capability of raising the baseline LLNA response compared to water  
21 control mice. Unfortunately, there were no negative controls in the Burleson et al. (2000)  
22 studies. Appropriate negative controls would have included the following: (1) ammonium  
23 perchlorate-dosed non-sensitized mice; (2) ammonium perchlorate-dosed and ammonium  
24 perchlorate-challenged mice; and (3) water control mice dermally exposed to ammonium  
25 perchlorate on the ear pinna. Another group of appropriate and informative studies would  
26 involve ammonium perchlorate-dosed mice that would be challenged with a series of low to  
27 moderate concentrations of DNCB, for comparison with the current LLNA “optimal DNCB”  
28 response concentration data.

29 Enhancement of the LLNA to DNCB in mice exposed to 0.06 mg ammonium  
30 perchlorate/kg-day for 14 or 90 days represents the Lowest Observed Effect Level (LOEL) for all  
31 of the immune function tests performed. While this is the LOEL it is unknown if this is the

1 Lowest-Observed-Adverse-Effect- Level (LOAEL) because it is not clear that enhancement of  
2 the LLNA is a physiologically relevant adverse effect. Studies are needed to determine if  
3 ammonium perchlorate itself is a contact sensitizer as determined by the LLNA, as described  
4 above, and whether the degree of the LLNA response to ammonium perchlorate itself or to a  
5 known contact sensitizer can be linked to a quantifiable adverse outcome.

6 It is important to note that clinical studies in the 1960s reported that some patients suffering  
7 from Graves' disease and treated with potassium perchlorate presented with agranulocytosis  
8 and/or skin rashes. While the studies reported by Keil et al. (1998, 1999) indicated that there  
9 were no alterations in the proportion of peripheral blood leukocytes of mice dosed with  
10 ammonium perchlorate for 14- or 90-days, the work of BRI-Burleson Research Technologies,  
11 Inc. (2000a,b,c) suggests that ammonium perchlorate appears to exacerbate the contact  
12 sensitizing potential of the known skin sensitizer DNCB. However, due to the uncertainties  
13 associated with any attempt to extrapolate from the incomplete database of the mouse LLNA  
14 performed by BRI-Burleson Research Technologies, Inc. (2000a,b,c) to the clinical observations  
15 of skin rash and agranulocytosis in Graves' disease patients treated with potassium perchlorate,  
16 an uncertainty factor based on deficiencies in the database is recommended to be applied to this  
17 risk assessment.

## CHAPTER 6. CONSTRUCTION OF PBPK MODELS TO ADDRESS PERCHLORATE'S MODE-OF-ACTION

The purpose of this chapter is to describe the progress that has been made in developing physiologically-based pharmacokinetic (PBPK) models to aid interspecies extrapolation of effects observed in the toxicity studies. The models describe perchlorate and iodide kinetics in rats and humans. Because of the complex challenge posed in arriving at a representation of the regulation system for hypothalamic-pituitary-thyroid feedback, the modeling effort was not able to satisfactorily develop models that linked the observed effects of perchlorate inhibition of iodine uptake at the NIS with the resultant hormone perturbations and available toxicological information in the proposed mode-of-action framework.

Because of their potential role in the risk assessment and regulatory applications, the EPA required that all human clinical data utilized in this modeling effort undergo a quality assurance/quality check (QA/QC). The QA/QC report is presented in Merrill (2001a,b). These QA/QC data represent the most contemporary, comprehensive, and consistent set of human pharmacokinetic data available for perchlorate.

The PBPK models discussed herein (Merrill, 2001c,d; Clewell, 2001a,b) were developed by the AFRL/HEST to provide more accurate descriptions of the kinetics of iodide and perchlorate with respect to perchlorate's inhibition of iodide uptake at the NIS and their serum and tissue time courses as well as to aid evaluation of subsequent perturbations in thyroid hormones and TSH. A general discussion of the model development for the various PBPK model structures of perchlorate distribution will be provided in this chapter to aid appreciation of their attributes and applications. Because of the mode of action for perchlorate, an accurate description of iodide kinetics is critical to the description of perchlorate effects on iodide uptake at the NIS so that each of these models also includes iodide-specific parameters and accounts for iodide disposition.

A similar model was developed for each of the various life stages of importance to interspecies extrapolation of the laboratory animal data: adult, pregnant rat and fetus, and the lactating rat and neonate. The adult male rat model was developed using data from the ADME

1 studies in the perchlorate testing strategy, together with experimental data and parameter values  
2 available in the existing literature. The subsequent model structures for the human and various  
3 life stages of the rat were similarly developed based largely on the adult male rat structure  
4 through scaling and optimization of parameters to available data.

5 It should be noted that the original motivation for including human studies (as discussed in  
6 Chapter 3) in the perchlorate testing strategy was to support such interspecies extrapolation and  
7 not to derive NOAEL estimates for thyroid effects in the human population. As discussed in  
8 Chapter 4, the EPA feels that both the observational epidemiological and the human clinical  
9 studies have significant scientific and technical limitations that preclude their use as the basis for  
10 a quantitative dose-response assessment. In addition, some of the clinical studies contained in  
11 this database fall in the category of studies not to be considered under EPA's Dec. 14, 2001  
12 interim policy on the use of third-party human studies (U.S. Environmental Protection Agency,  
13 2001c). However, the scientific and technical strengths and weaknesses of these studies were  
14 described before this Agency policy was articulated. Therefore, because of the scientific  
15 shortcomings of these studies, they will not be used as "principal studies" in the derivation of  
16 an RfD. The clinical study subject attributes (euthyroid adults) and study design issues (sample  
17 size, RAIU time points, etc.) made these data less reliable than the laboratory animal toxicological  
18 data to ascertain effect levels for the basis of an RfD derivation. Models of perchlorate distribution  
19 for human pregnancy and lactation have not been developed.

20 More detailed discussion can be found for each model structure in the accompanying  
21 references provided for each in the sections that follow. The adult male rat and human model  
22 (Merrill, 2001c,d) will be discussed in Section 6.2. Section 6.3 discusses the pregnant dam and  
23 fetal rat PBPK model (Clewell, 2001a), and the lactating dam and neonate model (Clewell,  
24 2001b) is discussed in Section 6.4. The purpose of providing these model descriptions and a  
25 discussion of the data used to develop and validate their structures is to provide the external peer  
26 reviewers an opportunity to critically evaluate the model structures, the use of the data in model  
27 development or validation exercises, and the model applications.

28 The simultaneous ordinary differential equations used in the proposed PBPK models to  
29 simulate radioiodide and perchlorate distribution were written and solved using advanced  
30 continuous simulation language (ACSL) software (AEqis Technologies, Austin, TX).

1 **6.1 MODE-OF-ACTION FRAMEWORK AND UNDERLYING**  
 2 **MODELING APPROACH**

3 The mode-of-action model proposal by the EPA for the previous perchlorate assessment  
 4 and discussed in Chapter 3 served as the conceptual construct for the development of the PBPK  
 5 models. Shown again in Figure 6-1, the model lays out the biomarkers of exposure and effect in  
 6 a continuum from ingestion of perchlorate in drinking water and uptake into the blood, the key  
 7 event of iodide uptake inhibition at the NIS in the thyroid gland, and subsequent effects on  
 8 thyroid hormone economy leading to neurodevelopmental and neoplastic sequelae.  
 9

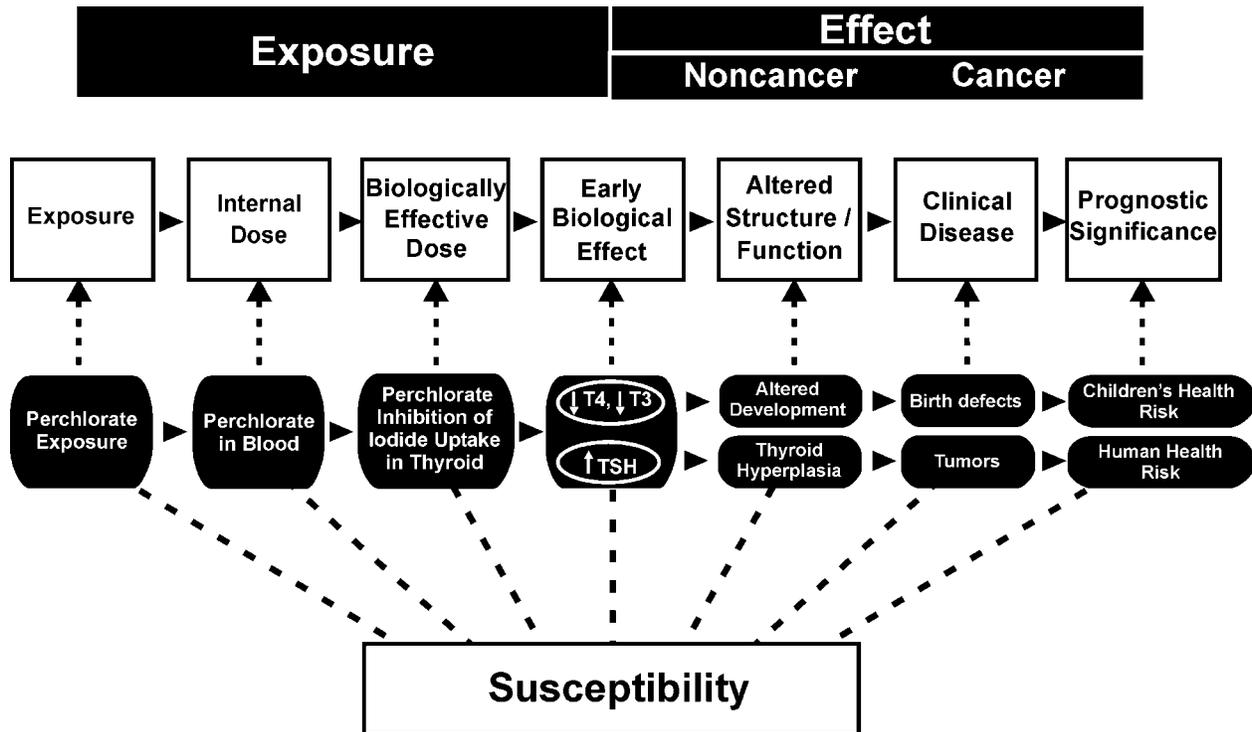


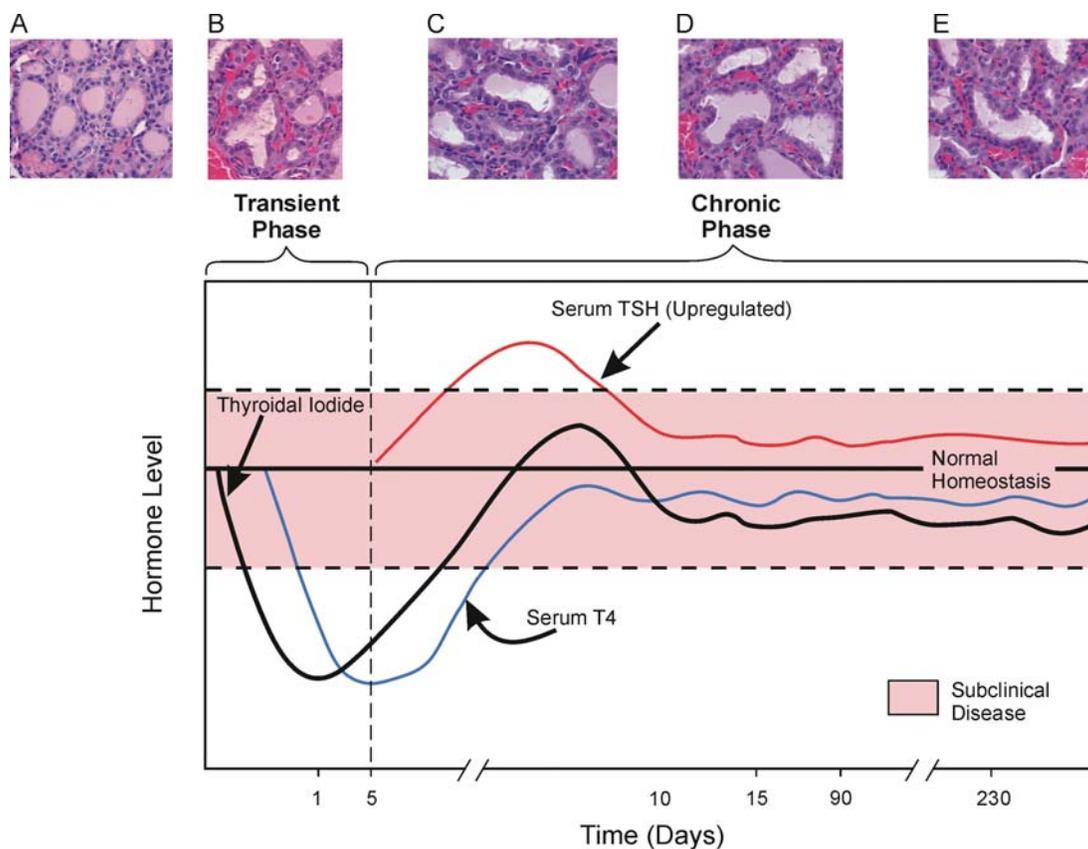
Figure 6-1. Mode-of-action model for perchlorate toxicity proposed by the U.S. EPA (U.S. Environmental Protection Agency, 1998d). Schematic shows the exposure-dose-response continuum considered in the context of biomarkers (classified as measures of exposure, effect, and susceptibility) and level of organization at which toxicity is observed (U.S. Environmental Protection Agency, 1994; Schulte, 1989). The model maps the toxicity of perchlorate on this basis by establishing causal linkage or prognostic correlations of precursor lesions.

1           The temporal pathological and serum hormone changes that accompany exposure to  
2 perchlorate corresponding to this continuum are represented in Figure 6-2. The inhibition of  
3 iodide uptake at the NIS results in a transient decrease in serum T4 and T3. This transient phase  
4 of thyroid hormone deficit is of concern during pregnancy and development due to the critical  
5 role that these hormones play in preventing adverse neurodevelopmental sequelae as described in  
6 Chapters 3 and 5. The hypothalamic-pituitary-thyroid feedback system is designed to regulate  
7 the circulating levels of thyroid hormone and will respond to the thyroid hormone decreases by  
8 upregulating TSH production in order to stimulate the thyroid to increase its production of  
9 thyroid hormones to compensate. Represented as the “chronic phase” in Figure 6-2, the  
10 upregulation of TSH would bring the system back into apparent homeostasis. As depicted in the  
11 figure, however, this apparent homeostasis may actually represent subclinical disease in that the  
12 system is only maintaining homeostasis by upregulation and can be considered a stressed system  
13 with respect to its ability to compensate for additional insults caused by other chemicals or  
14 diseases that might impact the thyroid. Further, it should be emphasized that recent  
15 epidemiological investigations have indicated concern about decrements in T4, i.e., thyroxinemia  
16 without concomitant upregulation of TSH that would constitute hypothyroidism (Morreal de  
17 Escobar, 2000; Haddow et al., 1999; Pop et al., 1999).

18           In order to adequately characterize the transient phase of events, evaluation of the initial  
19 effect of perchlorate at the NIS is necessary. This can be accomplished by determining  
20 perchlorate inhibition with radioactive iodide uptake (RAIU) studies. The timing and route of  
21 administration are important considerations in evaluating these types of studies. Studies of  
22 RAIU that occur during the chronic phase, such as longer-term studies of hormones, offer little  
23 insight to the critical decrements in T4 that may occur during the transient phase due to iodine  
24 inhibition. Likewise, longer-term studies of hormones often represent the upregulated system  
25 and may not be especially informative.

### 26 27 **6.1.1 Parallelogram Approach to Interspecies Extrapolation**

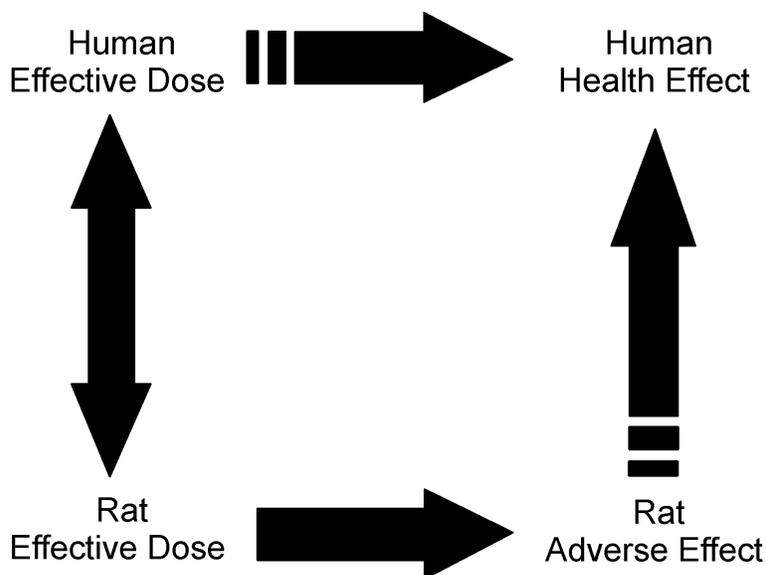
28           PBPK models have proven to be very useful tools for performing interspecies extrapolation  
29 of dose for applications in risk analysis. Interspecies extrapolation is often necessary because, as  
30 in this case of perchlorate, critical effects at levels of organization below that of the population  
31 (e.g., thyroid histopathology or brain morphometry) can not be evaluated easily or ethically in



**Figure 6-2. Schematic of thyroid and pituitary hormone levels with associated pathology after acute versus chronic dosing with perchlorate. The transient phase is represented by decreases in thyroidal iodide due to the inhibition by perchlorate at the NIS with subsequent drop in T4. The transient drops in T4 can lead to permanent neurodevelopmental sequelae. Once TSH is upregulated via the hypothalamic-pituitary-thyroid feedback, T4 appears to be in normal homeostasis but actually can represent subclinical or undiagnosed disease (hypothyroxinemia). The upregulation of TSH can result in neoplasia. Normal thyroid tissue is represented in Panel A. Panel B shows lace-like colloid depletion which is more pronounced in subsequent panels C, D and E. Panels D and E represent hypertrophy and hyperplasia.**

1 humans. A basic tenet of molecular epidemiology is that these precursor lesions are often more  
 2 closely related to the exposure than are the traditional outcome measures of morbidity and  
 3 mortality (U.S. Environmental Protection Agency, 1994).

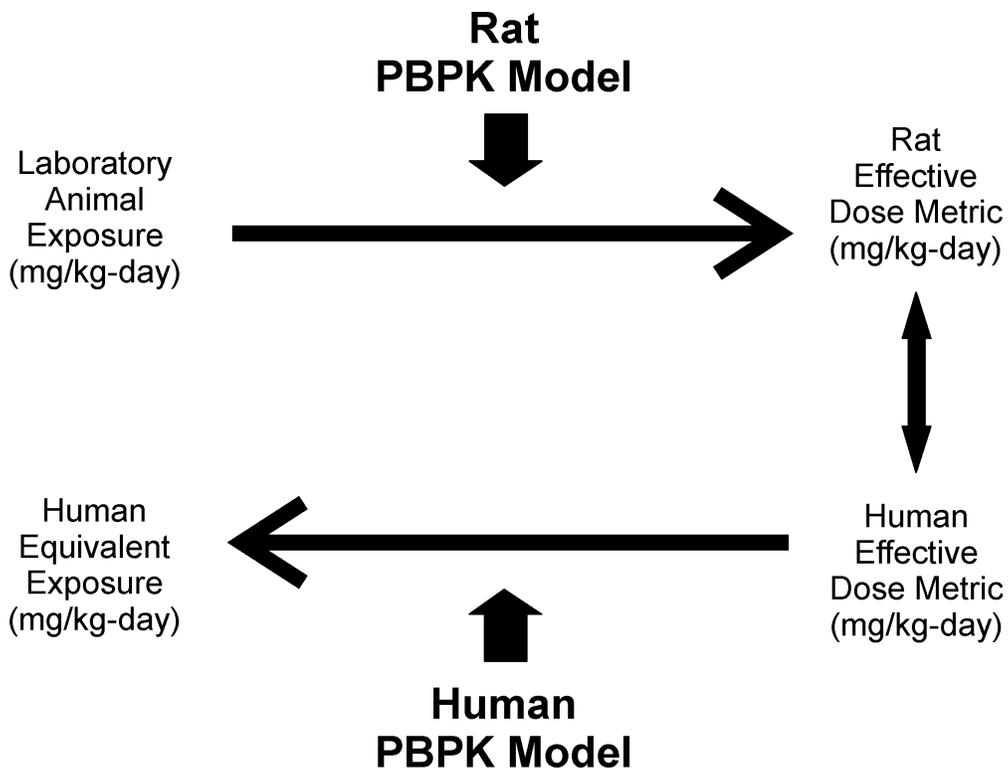
4 A parallelogram approach as shown in Figure 6-3 is used to predict the dose-response  
 5 relationship for humans based on the dose-response in laboratory animals. Because these critical



**Figure 6-3. Schematic of parallelogram approach used for interspecies extrapolation (U.S. Environmental Protection Agency, 1994). Dose and adverse effect in rat can be used to predict human effective dose and response.**

1 effects cannot be accurately measured in humans, the dose associated with an observed critical  
 2 effect in the laboratory animal is scaled to the human by adjusting the PBPK model with human  
 3 physiological parameters and variables. The human model is typically constructed by  
 4 allometrically scaling some parameters in the laboratory animal model based on body weight, and  
 5 some parameters such as partition coefficients can be measured *in vitro*. An administered dose  
 6 associated with the critical effect is determined based on an appropriate internal dose metric.  
 7 The internal dose is scaled to an equivalent exposure (HEE) in humans by exercising the human  
 8 model with human parameters and exposure assumptions. Thus, the HEE represents the human  
 9 exposure that would result in the same amount of internal dose metric in a human as that which  
 10 caused the effect in the laboratory animal.

11 The dose-response relationship is considered to be the same as that in the laboratory animal  
 12 as the default or more biologically-based models may contain additional parameters that also  
 13 account for species-specific determinants of toxicant-target interaction. Figure 6-4 illustrates the  
 14 use of the laboratory animal and human PBPK models to arrive at the HEE. Simulations used to



**Figure 6-4. Illustration of how human equivalent exposure (HEE) is calculated using PBPK models. An effective internal dose associated with a critical health effect at an administered dose (mg/kg-day) is calculated by simulating the experimental exposure regimen (e.g., 5 days/week) for a relevant metric (e.g., area under the curve in blood, [AUCB]). The human PBPK model is then used to simulate an exposure that achieves the same effective internal dose metric level using human parameters.**

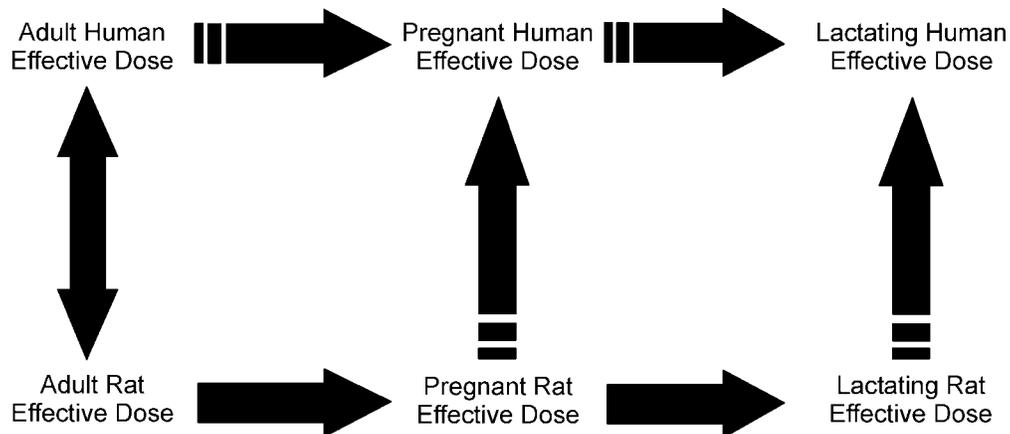
1 arrive at HEE for different internal dose metrics and a sensitivity analysis of the adult model  
 2 structure will be discussed in Section 6.5.

3 The parallelogram approach has also been used to predict effective doses for structurally  
 4 related chemicals (Jarabek et al., 1994). Disposition of one chemical associated with an effect  
 5 can be predicted for another after appropriate adjustments for chemical structure and activity are  
 6 made. In the case of these models, it should be appreciated that the accurate modeling of iodide  
 7 in addition to that of perchlorate represents such a validation.

8  
 9

1 **6.1.2 Extending the Parallelogram Approach to Various Experimental**  
2 **Life Stages**

3 Because effects at various life stages (adult, pregnant dam, fetus, lactating dam, and  
4 neonate) were evaluated in the perchlorate laboratory animal studies, the parallelogram approach  
5 had to be extended as shown in Figure 6-5. There are no human models of perchlorate  
6 disposition for pregnant women, lactating women, fetuses, or children, so the relationships to the  
7 adult human HEE had to rely on the relationships determined in the laboratory animal species.  
8 This approach assumes that the relationships, expressed as ratios between one life stage and  
9 another, will be comparable in humans.



10  
11  
**Figure 6-5. Schematic of extended parallelogram approach used for perchlorate due to effects at different life stages. Doses in the pregnant rat and fetus are related back to the adult male rat, likewise, the effects in lactating rats and neonates.**

1 The various PBPK models are used to predict equivalent effective doses at the various  
2 administered doses used in the experiments; e.g., 1.0 mg/kg-day ammonium perchlorate given in  
3 drinking water to both the adult male rat and the pregnant dam. Each PBPK model is exercised  
4 (adult rat and pregnant rat) to predict the amount of internal dose metric achieved at each life  
5 stage. The ratio of the effective internal dose metrics of the life stage in question is then used to

1 adjust the HEE based on the adult male rat. For example, the HEE for the pregnant dam would  
2 be found by adjusting the HEE for the adult male rat by the ratio of the male rat:pregnant rat as:

$$\text{Pregnant HEE (mg/kg-day)} = \text{Adult male rat HEE (mg/kg - day)} \times \frac{\text{Adult male rat internal effective dose metric}}{\text{Pregnant rat internal effective dose metric}} \quad (6-1)$$

5 This ratio is unitless and accounts for the differences between the two life stages in  
6 question in an analogous fashion to the dosimetric adjustment factor (DAF) used in the EPA's  
7 inhalation reference concentration methods to extrapolate respiratory tract doses in different  
8 regions of the laboratory animal to human equivalent concentrations (U.S. Environmental  
9 Protection Agency, 1994). The same ratio approach is used to extend the model predictions to  
10 HEE estimates for the fetus, lactating dam, and neonate. Development of the ratios for two  
11 internal effective dose metrics, perchlorate area-under-curve (AUC) concentrations in serum and  
12 iodide uptake inhibition, will be discussed in Section 6.5.

## 15 **6.2 ADULT RAT AND HUMAN MODEL STRUCTURES**

16 Because the same model structure is used to describe perchlorate and iodide disposition  
17 (absorption, distribution, and elimination) for both the adult male rat and human, this section will  
18 describe the development of both of these models together. Data supporting development and  
19 validation of the structures will be summarized in this section while additional detail, including  
20 some of the governing equations, can be found in the consultative letters from the AFRL/HEST  
21 (Merrill, 2001c,d).

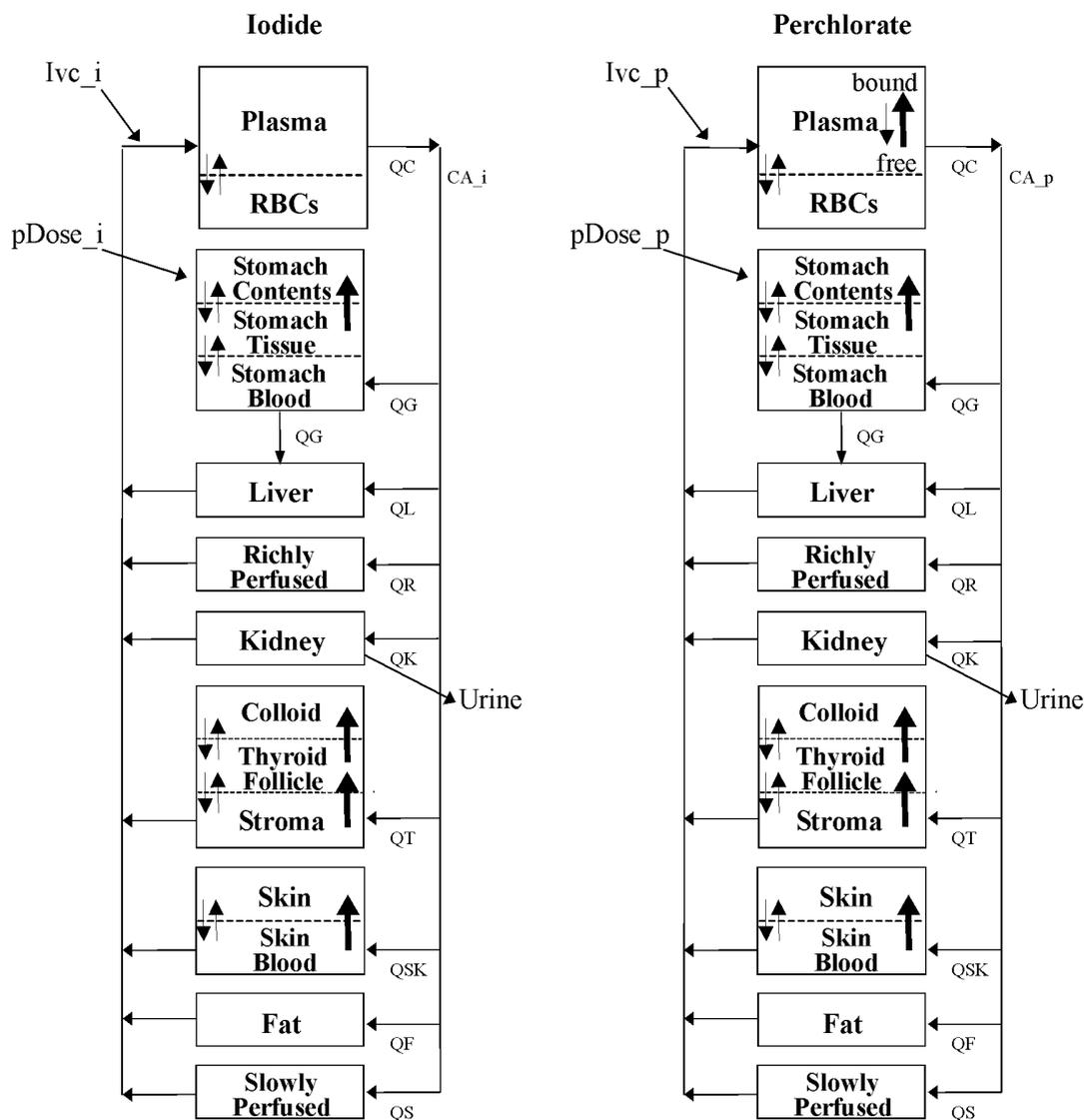
22 As discussed in Chapter 2, the perchlorate anion ( $\text{ClO}_4^-$ ) is very similar in ionic size, shape,  
23 and charge to that of iodide ( $\text{I}^-$ ). These shared properties allow perchlorate to interfere with the  
24 first stage of thyroid hormone synthesis by competitively inhibiting the active transfer of iodide  
25 into the thyroid by the sodium ( $\text{Na}^+$ )-iodide ( $\text{I}^-$ ) symporter or NIS. The NIS is a protein that  
26 resides in the basolateral membrane of thyroid epithelial cells (Spitweg et al., 2000). NIS  
27 simultaneously transports both sodium and iodide ions from extracellular plasma into the thyroid  
28 epithelial cell via an active process. Energy is provided by the electrochemical gradient across

1 the cell membrane. The low intracellular concentration of sodium is maintained by sodium-  
2 potassium pumps (Ajjan et al., 1998). The kinetics of perchlorate and iodide anions differ  
3 mainly in that iodide is organified in the thyroid (thyroid hormone production); whereas,  
4 perchlorate is thought to be unreactive and eventually diffuses from the thyroid into systemic  
5 circulation.

6 The proposed PBPK model structure for the adult male rat (Merrill, 2001c) and human  
7 (Merrill, 2001d) describes active uptake of iodide and perchlorate in gastric juice, thyroid, and  
8 skin, and competitive inhibition of iodide uptake by perchlorate in NIS-containing tissues, as  
9 well as venous equilibration with slowly and richly perfused tissues as shown in Figure 6-6.  
10 Tissues that exhibited evidence of sodium iodide symporter and were found to concentrate either  
11 anion were depicted as compartments with nonlinear uptake (Merrill, 2001c,d). Tissues with  
12 active uptake include the thyroid, skin, and gastric mucosa (Wolff, 1998; Chow et al., 1969;  
13 Kotani et al., 1998). Although other tissues have been known to sequester iodide and similar  
14 anions (e.g., salivary glands, choroid plexus, ovaries, mammary glands, placenta) (Brown-Grant,  
15 1961, Honour et al., 1952; Spitzweg et al., 1998), the iodide and perchlorate pools of these  
16 tissues was expected to be too small to significantly affect plasma levels. These tissues were  
17 lumped with slowly and richly perfused tissues.

18 The model also includes separate compartments for plasma, kidney, liver, and fat. These  
19 compartments do not maintain concentrations greater than the plasma at steady state, and  
20 therefore, were not described with terms for active uptake. The rapid urinary clearance of  
21 perchlorate (Yu, 2000) mandated the inclusion of a kidney compartment in the model. A liver  
22 compartment was also utilized due to its significant impact on iodide homeostasis. The majority  
23 of extrathyroidal deiodination takes place within the liver. Fat was primarily added as an  
24 exclusionary compartment. Due to its significant percentage of body weight, the skin represents  
25 an important pool for slow iodide turnover.

26 The modelers at AFRL/HEST found that a separate skin compartment was necessary.  
27 Experiments performed with radioiodide in rats resulted in skin:serum iodide ratios of close to  
28 one (Yu, 2000). Other researchers have reported higher ratios in rats, but results have not been  
29 consistent. Similar observations during dialysis with pertechnate of slow uptake and retention in  
30 human skin was observed by Hays and Green (1973) and the skin was therefore maintained as a  
31 separate compartment in the model. The skin contains two sub-compartments representing the



**Figure 6-6. Schematic for the adult male rat and human PBPK models of perchlorate and iodide distribution (Merrill, 2001c,d). Bold arrows indicate active uptake (except for plasma binding) at NIS sites in thyroid, gut, and skin. Plasma binding was also described with Michaelis-Menten terms for the association of perchlorate anion to binding sites with first-order clearance rates for dissociation. Small arrows indicate passive diffusion. Boxes represent specific compartments in the model structure. The thyroid consists of the stroma, the follicle, and the colloid; and the stomach consists of the capillary bed, stomach wall, and stomach contents. The skin contains two subcompartments: the capillary bed and skin tissue. Permeability area cross products and partition coefficients were used to describe the first-order movement of the perchlorate ( $\text{ClO}_4^-$ ) and iodide ( $\text{I}^-$ ) anions into deeper subcompartments.**

1 capillary bed and the skin tissue. The thyroid and stomach consist of three sub-compartments:  
2 the stroma, the follicle, and the colloid in the thyroid and the capillary bed, stomach wall, and  
3 contents in the case of the stomach.

4 Active uptake into the thyroid colloid, stomach contents, and skin were described using  
5 Michaelis-Menten kinetics for nonlinear processes (Figure 6-6, bold arrows). Permeability area  
6 cross products and partition coefficients were used to describe the first order movement of the  
7 anions ( $\text{ClO}_4^-$  and  $\text{I}^-$ ) between the capillary bed, tissue, and inner (deep) compartments  
8 (Figure 6-6, small arrows) that results from the inherent electrochemical gradient within the  
9 tissues. Passive diffusion through the kidney, liver, and fat compartments were described with  
10 partitions and blood flows. Plasma binding of perchlorate was described with Michaelis-Menten  
11 terms for the association of the perchlorate anions to plasma binding sites and a first order  
12 clearance rate for the dissociation. First-order clearance rates from the kidney were also used to  
13 describe urinary clearance of the anions.

14 The blood compartment differs between the perchlorate and iodide models. The  
15 perchlorate blood compartment is composed of plasma and plasma proteins to simulate binding.  
16 Plasma binding was required to simulate serum perchlorate concentrations at lower doses.  
17 Iodinated hormones bind to plasma proteins, but free iodide apparently does not. Therefore, a  
18 single compartment for plasma iodide was used. The free anions in plasma are available for  
19 diffusion and active uptake into tissues.

20 The presence of NIS is an indicator of active uptake for iodide. NIS is highly expressed in  
21 thyroid epithelial cells. Lower levels of expression have been detected in the mammary gland,  
22 salivary gland, skin, stomach, and colon (Ajjan et al., 1998; Spitzweg et al., 1998). However,  
23 only the thyroid has been found to organify iodide (Ajjan et al., 1998). The most important  
24 regulator of symporter gene and protein expression is thyroid-stimulating hormone (TSH). This  
25 is also the case for other important thyroid proteins such as thyroglobulin and thyroid peroxidase  
26 (Spitzweg et al., 1998).

27 The parameters used in the adult male rat and human model for the various compartments  
28 are provided in Table 6-1. The parameters were based on literature values or fitted to data using  
29 the model as described in the table. It is important to note that the model structure for both  
30 species is the same. The difference, per typical for PBPK models, is that there are species- and  
31 chemical-specific parameters for each. For example, the volume of the thyroid (as percent of

**TABLE 6-1. PHYSIOLOGICAL PARAMETERS FOR THE ADULT MALE RAT AND HUMAN PBPK MODELS  
(Merrill, 2001c,d)**

Physiological Parameters		Male Rat	Source	Human	Source
Tissue Volumes					
Body Weight	BW (kg)	0.3	Measured (rat specific)	~70.0	Subject-specific
Slowly Perfused	VSc (%BW)	74.6	Brown et al., 1997	65.1	Brown et al., 1997
Richly Perfused	VRc (%BW)	11.0	Brown et al., 1997	12.4	Brown et al., 1997
Fat	VFc (%BW)	7.4	Brown et al., 1997	♂ 21.0 ♀ 2.7	Brown et al., 1997
Kidney	VKc (%BW)	1.7	Brown et al., 1997	0.44	Brown et al., 1997
Liver	VLc (%BW)	5.5	Brown et al., 1997	2.6	Brown et al., 1997
Stomach Tissue	VGc (%BW)	0.54	In house male rat ClO <sub>4</sub> <sup>-</sup> kinetics (Yu et al., 2000)	1.7	Brown et al., 1997
Gastric Juice	VGJc (%BW)	1.68	In house male rat ClO <sub>4</sub> <sup>-</sup> kinetics (Yu et al., 2000)	0.071	Licht and Deen, 1988
Stomach Blood	VGBc (%VG)	4.1	Altman & Dittmer, 1971b	4.1	Altman & Dittmer, 1971a
Skin Tissue	VSkc (%BW)	19.0	Brown et al., 1997	3.7	Brown et al., 1997
Skin Blood	VSkBc (%VSk)	2.0	Brown et al., 1997	8.0	Brown et al., 1997
Thyroid	Vtrotc (%BW)	0.0077	Malendowicz, 1977	0.03	Yokoyama et al., 1986
Thyroid Follicle	VTc (%Vtrot)	59.9	Malendowicz, 1977	57.3	Brown et al., 1986
Thyroid Colloid	VDTc (%VTtot)	24.4	Malendowicz, 1977	15.0	Brown et al., 1986
Thyroid Blood	VTBc (%VTtot)	15.7	Malendowicz, 1977	27.6	Brown et al., 1986
Plasma	Vplasc (%BW)	4.1	Brown et al., 1997, Altman & Dittmer, 1971a	4.4	Marieb, 1992; Altman & Dittmer, 1971b
Red Blood Cells	VRBCc (%BW)	3.3	Brown et al., 1997, Altman & Dittmer, 1971a	3.5	Marieb, 1992; Altman & Dittmer, 1971b
Adjusted Slowly Perfused	VS (L)	0.138	Calculated from model	28.0	Calculated from model
Adjusted Richly Perfused	VR (L)	0.01	Calculated from model	5.34	Calculated from model

**TABLE 6-1 (cont'd). PHYSIOLOGICAL PARAMETERS FOR THE ADULT MALE RAT AND HUMAN PBPK MODELS (Merrill, 2001c,d)**

Physiological Parameters		Male Rat	Human	Source
Tissue Volumes		Male Rat	Human	Source
<b>Blood Flows</b>				
Cardiac Output	QCc (L/hr-kg)	14.0	16.5	Brown et al., 1997; Hanwell & Linzell, 1973
Slowly Perfused	QSc (%QC)	24.0	5.2	Brown et al., 1997
Richly Perfused	QRc (%QC)	76.0	17.5	Brown et al., 1997
Fat	QFc (%QC)	6.9	22.0	Brown et al., 1997
Kidney	QKc (%QC)	14.0	1.0	Leggett & Williams, 1995; Malik et al., 1976
Liver	QLc (%QC)	17.0	1.6	Brown et al., 1997
Stomach	QGc (%QC)	1.61	13.0	Calculated, using 24% QC as flow to all slowly perfused tissues (Brown et al., 1997)
Skin	QSkc (%QC)	5.8	33.0	Calculated, using 76% QC as flow to all richly perfused tissues (Brown et al., 1997)
Thyroid	QTc (%QC)	1.6		Brown et al., 1997
Adjusted Slowly Perfused	QS (%QC)	11.3		Calculated from model
Adjusted Richly Perfused	QR (%QC)	41.8		Calculated from model

1 body weight), the maximum capacity of thyroid iodide or perchlorate uptake, and plasma binding  
2 of perchlorate. The chemical-specific parameter for each model for both perchlorate and iodide  
3 are provided in Table 6-2.

4 In order to simulate the daily dosing regimen of the drinking water experiment, the rats  
5 were assumed to drink at constant rate for 12 of the 24 hours per day (1800 to 0600 hours).  
6 A pulse function in ACSL was used to introduce drinking water to the gastrointestinal (GI)  
7 compartment of the rat for the first 12 hours of each 24-hour period and to stop dosing while the  
8 rat was presumably sleeping. Intravenous (iv) dosing was introduced into the venous blood  
9 compartment of the model. Intraperitoneal (ip) injection was introduced into the model in the  
10 same manner as the iv dosing.

## 11 **6.2.1 Data and Methods**

13 This section summarizes the AFRL/HEST data and data available in the literature that were  
14 used for model development. Details on experimental methods, including protocol design,  
15 exposure regimen, chemical source and purity, animals (housing, feeding, surgical procedures,  
16 etc), and the analytical methods for measurement of RAIU; of perchlorate in plasma, urine and  
17 tissues; and of thyroid hormones and TSH can be found in the associated consultative letters  
18 from AFRL/HEST (Merrill, 2001c,d; Yu, 2000, 2001, 2002; Yu et al., 2000).

### 19 **6.2.1.1 Studies in Laboratory Rats**

21 The studies performed at AFRL/HEST included both “acute” iv experiments to measure  
22 radiolabeled iodide or perchlorate as well as measurements of the same after drinking water  
23 administration. These two different regimens provided a better characterization of the transient  
24 (“acute”) and chronic behavior necessary for an accurate description of the disposition of the  
25 anions. Adult male Sprague-Dawley rats ( $330 \pm 35$  g;  $n = 6$  rats per group) that were purchased  
26 from Charles River Laboratory (Raleigh, NC) were used in the experiments.

27 In these experiments, the term total iodine includes bound iodine plus free inorganic iodide.  
28 Carrier doses included tracer doses of carrier free radiolabeled iodide ( $^{125}\text{I}$ ) along with non-  
29 radiolabeled iodide. Free  $^{125}\text{I}$  radioactivity was determined by subtracting the bound from total  
30 measurements (Merrill, 2001c; Yu, 2000, 2001, 2002; Yu et al., 2000).

**TABLE 6-2. CHEMICAL-SPECIFIC PARAMETERS FOR THE ADULT MALE RAT AND HUMAN PBPK MODELS**  
(Merrill, 2001c,d)<sup>a</sup>

Partition Coefficients (unitless)	Male Rat			Human		
	Perchlorate	Iodide	Source	Perchlorate	Iodide	Source
Slowly Perfused/Plasma PS_	0.31	0.21	Yu et al., 2000; Halimi et al., 1956	0.31	0.21	Halimi et al., 1956; Yu et al., 2000
Richly Perfused/Plasma PR_	0.56	0.40	Yu <i>et al.</i> , 2000; Halimi <i>et al.</i> , 1956	0.56	0.40	Halimi <i>et al.</i> , 1956; Yu <i>et al.</i> , 2000
Fat/ Plasma PF_	0.05	0.05	Pena et al., 1976	0.05	0.05	Pena et al., 1976
Kidney/Plasma PK_	0.99	1.09	Perlman et al., 1941	0.99	1.09	Perlman et al., 1941; Yu et al., 2000
Liver/Plasma PL_	0.56	0.44	Perlman et al., 1941	0.56	0.44	Perlman et al., 1941; Yu et al., 2000
Gastric Tissue/Gastric Blood PG_	1.80	1.40	Yu et al., 2000; Yu, 2000	1.80	0.50	Yu et al., 2000; Yu, 2000
Gastric Juice/Gastric Tissue PGJ_	2.30	3.00	Yu et al., 2000; Yu, 2000	2.30	3.50	Yu et al., 2000; Yu, 2000
Skin Tissue/Skin Blood PSk_	1.15	0.70	Yu, 2000; Perlman et al., 1941	1.15	0.70	Perlman et al., 1941; Yu, 2000
Thyroid Tissue/Thyroid Blood PT_	0.13	0.15	Chow & Woodbury (1970)	0.13	0.15	Chow & Woodbury (1970)
Thyroid Lumen/Thyroid Tissue PDT_	7.00	7.00	Chow & Woodbury (1970)	7.00	7.00	Chow & Woodbury (1970)
Red Blood Cells/Plasma	0.80	1.00	Yu et al., 2000; Rall et al., 1950	0.80	1.00	Rall et al., 1950; Yu et al., 2000
<b>Max Capacity, Vmaxc (ng/hr-kg)</b>						
Thyroid Colloid Vmaxc_DT	1.0E+04	4.0E+07	Fitted	2.5E+5	1.0E+8	Fitted
Thyroid Follicle Vmaxc_T	2.2E+03	5.5E+04	Fitted	5.0E+4	~1.5E+5	Fitted
Skin Vmaxc_S	6.2E+05	5.0E+05	Fitted	1.0E+6	7.0E+5	Fitted
Gut Vmaxc_G	3.0E+05	1.0E+06	Fitted	1.0E+5	9.0E+5	Fitted
Plasma Binding Vmaxc_Bp	9.5E+03	—	Fitted	5.0E+2	—	Fitted

**TABLE 6-2 (cont'd). CHEMICAL-SPECIFIC PARAMETERS FOR THE ADULT MALE RAT AND HUMAN PBPK MODELS (Merrill, 2001c,d)<sup>a</sup>**

Partition Coefficients (unitless)	Male Rat			Human		
	Perchlorate	Iodide	Source	Perchlorate	Iodide	Source
<b>Affinity Constants, Km (ng/L)</b>						
Thyroid Lumen Km_DT	1.0E+08	1.0E+09	Golstein et al., 1992	1.0E+8	1.0E+9	Golstein et al., 1992
Thyroid Km_T	2.5E+05	4.0E+06	Gluzman & Niepomniszcze, 1983; Wolff, 1998	1.8E+5	4.0E+6	Gluzman & Niepomniszcze, 1983; Wolff, 1998
Skin Km_S	2.0E+05	4.0E+06	Gluzman & Niepomniszcze, 1983; Wolff, 1998	2.0E+5	4.0E+6	Gluzman & Niepomniszcze, 1983; Wolff 1998
Gut Km_G	2.0E+05	4.0E+06	Gluzman & Niepomniszcze, 1983; Wolff, 1998	2.0E+5	4.0E+6	Gluzman & Niepomniszcze, 1983; Wolff, 1998
Plasma binding Km_B	1.1E+04	—	Fitted	1.8E+4	—	Fitted
<b>Permeability Area Cross Products (L/hr-kg)</b>						
Gastric Blood to Gastric Tissue PAGc_	0.80	0.10	Fitted	0.6	0.2	Fitted
Gastric Tissue to Gastric Juice PAGJc_	0.80	0.10	Fitted	0.8	2.0	Fitted
Skin Blood to Skin Tissue PASKc_	1.0	0.10	Fitted	1.0	0.06	Fitted
Plasma to Red Blood Cells PARBCc_	0.10	1.00	Fitted	1.0	1.0	Fitted
Follicle to thyroid blood PATc_	4.0E-05	1.0E-04	Fitted	1.0E-4	1.0E-4	Fitted
Lumen to Thyroid Follicle PADTc_	0.01	1.0E-04	Fitted	0.01	1.0E-4	Fitted
<b>Clearance Values (L/hr-kg)</b>						
Urinary excretion CLUc_	0.07	0.05	Fitted	0.126	0.1	Fitted
Plasma unbinding CLumbc_	0.1	—	Fitted	0.025	—	Fitted

<sup>a</sup>All parameters listed are notated in the model by either an *i* (for iodide) or *p* (for perchlorate) following an underscore in the parameter name (e.g., PR\_*i*, PR\_*p*, Vmax\_*Ti*, Vmax\_*Tp*, etc.).

### 6.2.1.1.1 Acute iv Experiments in Rats

**Radiolabeled iodide ( $^{125}\text{I}^-$ ) kinetics.** Male rats were administered a single iv tail-vein injection with physiological saline (control group) or 33 mg/kg  $^{125}\text{I}^-$  (with carrier) in physiological saline. Rats were euthanized by  $\text{CO}_2$  asphyxiation at 5, 15, and 30 minutes (min), 1, 2, 6, 9, 24, 32, 48, and 96 hours (hr) post dosing to collect thyroid and blood from the vena cava. Rats for the 24 hour time point were placed individually in metabolism cages to collect urine.

In an additional study, male rats were intravenously dosed with 33 mg/kg  $^{125}\text{I}^-$  (with carrier) and euthanized at 0.5, 2 and 6 hours post dosing. Total, bound, and free  $^{125}\text{I}^-$  were analyzed in thyroid and serum, and total  $^{125}\text{I}^-$  was measured in skin and gastric contents (Yu, 2001).

**Radiolabeled  $^{36}\text{ClO}_4^-$  kinetics.** Naïve adult male rats ( $300 \pm 20$  g) were dosed once by iv tail-vein injection with 3.3 mg/kg radiolabeled perchlorate. Due to the low specific activity, a smaller dosing level could not be achieved. Each rat received less than 6  $\mu\text{Ci}$ . Rats were euthanized by  $\text{CO}_2$  asphyxiation at 0.5, 6, 12, 24, 32, and 48 hours after dosing. The thyroid, intestinal tract, intestinal tract contents, muscle, skin, liver, kidney, spleen, bladder, plasma, and red blood cells were harvested from the rats and stored at  $-20^\circ\text{C}$  until analysis of  $^{36}\text{ClO}_4^-$ . Rats for 12, 24, 32, and 48 hours time points were placed individually in metabolism cages for urine collection. Metabolism cages were washed with 500 mL de-ionized water. Urine and cage wash samples were stored under the same conditions until analysis.

**$^{125}\text{I}^-$  Kinetics and Inhibition from Acute iv Dosing with  $\text{ClO}_4^-$ .** Rats were injected with one of five doses of perchlorate (0.0, 0.01, 0.1, 1.0, and 3.0 mg/kg). At 2 hours post dosing, they were challenged with  $^{125}\text{I}^-$  with carrier (33 mg/kg) by intravenous injection and euthanized at 5, 15, and 30 min, 1, 2, 6, 9, and 24 hours post dosing of iodide. This corresponds to 2.08, 2.25, 2.5, 3, 4, 8, 11, and 26 hours, respectively, after dosing with perchlorate. Blood and thyroid were harvested from all time point groups; urine was collected from rats in the 24 hours dose group. Perchlorate and iodide levels were determined in the thyroid, serum and urine.

In an additional study, three rats were intravenously dosed with 0.0, 0.1, and 1.0 mg/kg perchlorate and challenged two hours later with 33 mg/kg  $^{125}\text{I}^-$ . Rats were euthanized at 15 min, 1, 2, and 4 hours after they were dosed with iodide. Levels of perchlorate and  $^{125}\text{I}^-$  were determined in thyroid, serum, skin and gastric contents (Yu, 2001).

### 6.2.1.1.2 Drinking Water Studies in Rats

Three drinking water studies (1, 5, and 14 days) were performed with target perchlorate concentrations of 0.0, 1.0, 3.0, 10.0, and 30.0 mg/kg-day with adult male rats continually exposed via drinking water. At the end of day 1, 5, or 14, rats (n=6 per group) were challenged once with 33 mg/kg  $^{125}\text{I}^-$  with carrier and euthanized at 2 hours post iodide dosing. Blood and thyroid gland were collected for  $\text{ClO}_4^-$  and  $^{125}\text{I}^-$  analyses in serum. For the 10 and 30 mg/kg dose groups, perchlorate was measured in serum and thyroid on day 5; however, the iodide inhibition study for these dose groups was conducted on Day 14.

### 6.2.1.2 Human Studies

The data used in development of the Merrill (2001d) human model were obtained from Hays and Solomon (1965) or recent data, both published and unpublished, that underwent the QA/QC check described in the introduction of this chapter (Merrill, 2001a,b). These data included the published and unpublished data from a human study of drinking water exposure to perchlorate that measured RAIU in the thyroid (Greer et al., 2000).

Data supporting model validation were obtained from another unpublished drinking water study conducted under contract to AFRL/HEST by Drs. Holger Leitolf and Georg Brabant of the Medizinische Hochschule, Hanover, Germany. Urinary perchlorate clearance data by Eichler (1929), Kamm and Drescher (1973), and Durand (1938) were also used to validate model predictions.

#### 6.2.1.2.1 Human Iodide Kinetic Data (Hays and Solomon, 1965)

A comprehensive human kinetic study on early iodide distribution was reported in 1965 by Hays and Solomon. The authors studied the effect of gastrointestinal cycling on iodide kinetics in nine healthy males after an iv dose of 10  $\mu\text{Ci}$  radiolabeled iodide ( $^{131}\text{I}^-$ ), approximately  $3.44 \times 10^{-3}$  ng  $^{131}\text{I}^-/\text{kg}$  body weight. Frequent measurements of radioiodide uptake in the thyroid, gastric secretions, plasma, and cumulative urine samples were taken during the three hours following injection. Gastric secretions were collected using a nasogastric tube with constant suction while the subjects remained in a resting position (only standing to urinate). Saliva was not collected separately and therefore pooled, to some extent, with gastric juices. To account for the removal of gastric iodide from circulation and to determine its impact on free iodide

1 distribution, the authors ran a control session on the same subjects without aspirating gastric  
2 secretions. Aspirated gastric secretions accounted for 23% of the  $^{131}\text{I}$  administered.

#### 3 4 **6.2.1.2.2 Perchlorate Kinetics and Inhibition of Thyroid Iodide Uptake (Greer et al., 2000)**

5 **Perchlorate data.** As described in Chapter 4, Greer et al. (2000) recently studied the  
6 effects of repeated low level exposure to perchlorate on humans. Subjects received 0.5, 0.1,  
7 0.02, or 0.007 mg/kg-day perchlorate in drinking water over a two week period. Each dose group  
8 consisted of eight healthy volunteers (four males and four females) with no signs or symptoms of  
9 thyroid disorders (euthyroid). The daily dose was dissolved in 400 mL water and divided into  
10 four 100 mL servings that were ingested at approximately 0800, 1200, 1600, and 2000 hours.

11 Baseline serum and urine samples were collected before the first perchlorate treatment.  
12 During perchlorate exposure, serum samples were collected at the following approximate times:  
13 day 1 at 1200 and 1600, day 2 at 0800, 1200, and 1700, day 3 at 0900, day 4 at 0800 and 1200,  
14 day 8 between 0800 and 0900 and day 14 at 0800 and 1700. Serum samples were also collected  
15 on post-exposure days 1, 2, 3, and 14. Twenty-four hour urine collections were taken on  
16 exposure days 1, 2, 14 and post-exposure days 1 through 3. Serum and 24-hour urine samples  
17 from the study were provided to AFRL/HEST compliments of Dr. Monte Greer of Oregon  
18 Health Science University (OHSU), Portland, OR, and Dr. Gay Goodman of Intertox, Seattle,  
19 WA. The samples were analyzed for perchlorate at the Operational Toxicology Branch, Human  
20 Effectiveness Directorate at the Air Force Research Laboratory (AFRL/HEST), Wright Patterson  
21 Air Force Base (WPAFB), OH, using the analytical methods described in Merrill (2001d).

22 **Iodide Inhibition Data.** Eight and 24 hour thyroid  $^{123}\text{I}$  uptakes (radioiodine uptake or  
23 RAIU) were measured one to two days prior to perchlorate treatment (baseline) on days 2 and  
24 14 of perchlorate exposure and 14 days after perchlorate exposure was discontinued. A gelatin  
25 capsule containing 100 mCi of  $^{123}\text{I}$  was administered orally at 0800, before the first perchlorate  
26 solution for that day was drunk. Thyroid scans were then taken 8 and 24 hours later.

27 **Thyroid and Pituitary Hormone Data.** The serum samples were also analyzed for TSH,  
28 T4, T3, and free T4 at OHSU. However, these hormone data were not used in the PBPK model  
29 described below. Statistical analysis of the data is described in Attachment 2 of Merrill (2001d).

30 In summary, there was little effect of perchlorate on levels of T4, free T4, or T3. TSH  
31 decreased significantly from baseline by Exposure Day 3. On Post-Exposure Day 1, the TSH

1 levels of the subjects in the 0.5 mg/kg-day group had decreased by an average of 35% from  
2 baseline (ranging from 17% to 52%). Therefore, it appears that TSH was dropping while  
3 inhibition remained the same. It is possible that there is an increase in thyroid sensitivity to TSH  
4 as an early response to inhibition (Brabant et al., 1992). This increased sensitivity (possibly an  
5 increase affinity of the TSH receptor) could possibly decrease circulating TSH levels while T4  
6 has not decreased sufficiently yet to stimulate the hypothalamus to increased TRH secretions.  
7 After perchlorate was discontinued, between Post-Exposure Days 1 and 15, the mean TSH level  
8 increased significantly over baseline (23% greater than baseline), with TSH of one subject  
9 remaining below baseline. The drop in TSH during perchlorate exposure and the rise above  
10 baseline measurements after perchlorate seem counter-intuitive to the TSH regulation expected  
11 but may be part of a rebound phenomenon as the NIS begins to upregulate.

12 In addition, the data by Greer et al. (2000) showed an increase in radioiodide uptake in  
13 excess of baseline measurements 14 days after perchlorate exposure. An increase in radioiodide  
14 uptake is expected due to the rise in TSH mentioned above. This rebound effect has been noted  
15 in other human inhibition studies (using both iodide and perchlorate as inhibitors). Saxena et al.  
16 (1962) evaluated the prophylactic doses of iodide required to suppress thyroid uptake of  $^{131}\text{I}$  in  
17 euthyroid mentally defective children. They found a minimal effective oral dose of 1500 to  
18 2000  $\mu\text{g}$  iodide per square meter of body surface per day was required to completely suppress  
19  $^{131}\text{I}$  uptake. Within a week after iodide administration was stopped, a rebound of uptake was  
20 noted. In some instances these uptakes were even higher in subsequent weeks.

### 21 22 **6.2.1.2.3 Supporting Kinetic Studies**

23 Both urine and serum perchlorate concentrations for a validation exercise were provided  
24 from a recent unpublished study by Drs. Brabant and Leitolf of Medizinische Hochschule,  
25 Hanover, Germany. In their study, seven healthy males ingested 12.0 mg/kg perchlorate  
26 dissolved in 1 liter of drinking water every day for two weeks. The daily perchlorate dose was  
27 divided equally in three portions and ingested three times per day (approximately between 0600  
28 and 0800, 1100 and 1300 and 1800, and 2000 hours). Blood specimens were collected on days 1,  
29 7, and 14 of perchlorate treatment and on the two mornings after perchlorate administration was  
30 discontinued. Samples were analyzed for perchlorate at AFRL/HEST.

1 Three published studies reported cumulative urine concentrations collected from healthy  
2 males after receiving a high oral dose of perchlorate (Durand, 1938; Kamm and Drescher, 1973;  
3 Eichler, 1929). Oral doses administered in these studies were 784 mg NaClO<sub>4</sub> (635 mg ClO<sub>4</sub><sup>-</sup>;  
4 Durand, 1938); 1000 mg NaClO<sub>4</sub> (765 mg ClO<sub>4</sub><sup>-</sup>; Kamm and Drescher, 1973), and 2000 mg  
5 KClO<sub>4</sub> (1400 mg ClO<sub>4</sub><sup>-</sup>; Eichler, 1929). The studies did not report serum perchlorate levels but  
6 could be used to validate the model.

7 Stanbury and Wyngaarden (1952) measured radioiodide uptake in a patient with Grave's  
8 disease. The patient received a tracer dose of <sup>131</sup>I<sup>-</sup> as a control before perchlorate dosing and  
9 again one hour after administration of 100 mg KClO<sub>4</sub>. Thyroid scans of radioiodide uptake were  
10 performed both after the control and perchlorate sessions to determine the level of inhibition.

## 11 12 **6.2.2 Adult Male Rat Model Development**

13 This section summarizes some key features necessary to the development of the adult male  
14 rat model structure and shows results of predictions made with simulations against experimental  
15 data used to parameterize and validate the model.

### 16 17 **6.2.2.1 Physiologic Parameters and Tissue Partition Coefficients**

18 The adult male rat volumes and blood flows were obtained from the literature or the  
19 AFRL/HEST studies as described in Table 6-1. Allometric scaling was used to account for  
20 parameter differences due differences in body weights between rats and humans. Because no  
21 steady-state values from infusion studies were available, the partition coefficients for iodide and  
22 perchlorate were estimated from the various studies listed in Table 6-2. The liver:serum and  
23 muscle:serum ratios of 0.56 and 0.31 were obtained in the AFRL/HEST radiolabeled perchlorate  
24 (<sup>36</sup>ClO<sub>4</sub><sup>-</sup>) iv study described above. The liver:serum partition value was used to represent  
25 partitioning to the liver and richly perfused compartments and the muscle:serum value to  
26 represent the slowly perfused compartment.

27 For compartments with nonlinear uptake of the anions, effective partition coefficients were  
28 used that represented either approximate tissue:serum concentration ratios or electrical potential  
29 gradients. Chow and Woodbury (1970) measured electrochemical potentials within the thyroid  
30 stroma, follicular membrane, and colloid at three different doses of perchlorate. The measured  
31 difference in electrical potential between the thyroid stroma and follicle was interpreted by

1 Merrill (2001c) as an effective partition coefficient for the perchlorate and iodide anions,  
2 hindering the entry of negatively charged ions into the follicle. The equal and opposite potential  
3 from the follicle to the colloid enhances passage of negatively charged species into the colloid  
4 and indicates an effective partition coefficient of greater than one. The equivalence between  
5 electrical potential differences  $\phi_i - \phi_f$  and effective partition coefficients for the thyroid  
6 subcompartments (stroma:follicle and follicle:colloid) were estimated in the manner of Kotyk  
7 and Janacek (1977) based on the Chow and Woodbury (1970) data as described in Merrill  
8 (2001c).

### 9 10 **6.2.2.2 Chemical-Specific Parameters**

11 The various active transport processes, tissue permeabilities, and clearance rates (excretion)  
12 are described in PBPK models for each species on a chemical-specific basis. This section  
13 outlines how the values for perchlorate and iodide used in the adult male rat model were derived.  
14 The values can be found in Table 6-2 and details on derivation in Merrill (2001c).

#### 15 16 **6.2.2.2.1 Affinity Constants and Maximum Velocities for Active Transport Processes**

17 Kinetic values for the saturable (Michaelis-Menten) active uptake process of perchlorate,  
18 the affinity constant and maximum velocity capacity ( $K_{m_p}$  and  $V_{maxc_p}$ ), were not available  
19 in the literature nor were they determined experimentally at AFRL/HEST. Only the affinity of  
20 iodide for NIS was available in the literature. The Merrill (2001c) adult rat model uses a  
21 Michaelis-Menten affinity constant ( $K_m$ ) value of  $4.0 \times 10^6$  ng/L to describe the affinity of iodide  
22 ( $K_{m_i}$ ) across compartments involving active transport by NIS (e.g., in the thyroid and gastric  
23 juices). This was based on the mean value of  $3.96 \times 10^6$  ng/L for iodide derived by Gluzman and  
24 Niepomnische (1983) from thyroid slices of 5 normal individuals. The thyroid slices were  
25 incubated with several medium iodide concentrations. The experimentally determined  $K_m$   
26 values for iodide are similar across species (Gluzman and Niepomnische, 1983) and across  
27 different tissues (Wolff, 1998). This average literature value was therefore used for iodide in  
28 tissues described with active uptake.

29 The values for perchlorate affinity were originally assumed to be the same as those for the  
30  $K_m$  of iodide, due to the similar mechanism in which the two anions are transported into the  
31 tissues. Thus, the iodide values were adjusted for the difference in mass to give an estimated

1 value for the affinity of perchlorate. The molar equivalent of iodide's  $K_m$  for perchlorate is  
2  $3.1 \times 10^6$  ng/L. However, these values were not adequate for use in the models. Several  
3 literature sources suggest that perchlorate may have a significantly higher affinity for NIS than  
4 iodide. In his 1963 paper (Wolff and Maurey, 1963) and his 1998 review, Wolff concluded that  
5 perchlorate has a greater affinity than iodide for the NIS. This assumption was based upon his  
6 work with iodide, perchlorate, and several other anions actively sequestered in the thyroid.  
7 Wolff measured the  $K_m$  of a few of the anions and inhibition constants ( $K_i$ 's) for several ions,  
8 including perchlorate. As noted in Chapter 2, Wolff found that the relative potency of inhibition  
9 by the various anions could be described with the following series:  $\text{TcO}_4 > \text{ClO}_4 > \text{ReO}_4 > \text{SCN}^-$   
10  $> \text{BF}_4 > \text{I} > \text{NO}_3 > \text{Br} > \text{Cl}^-$ . Wolff reported that the measured  $K_m$  values for several of these  
11 inhibiting anions were not the same as those measured for iodide. In fact, measured values for  
12  $K_m$  and  $K_i$  for several of the inhibiting anions revealed that affinity increased with increased  
13 inhibitory potency.

14 Several studies suggest perchlorate is a more potent inhibitor than iodide. In the rat  
15 thyroid, Wyngaarden et al. (1952) have shown that perchlorate was a more powerful inhibitor of  
16 the iodide trap than thiocyanate. Halmi and Stuelke (1959) showed that perchlorate was ten  
17 times as effective as iodide in depressing tissue to blood ratios in the rat thyroid and gut.  
18 Similarly, Harden et al. (1968) compared human saliva to plasma radioiodide concentration  
19 ratios after equimolar doses of perchlorate and iodide. The saliva:plasma iodide ratios during  
20 resting conditions were approximately seven times lower after a molar equivalent dose of  
21 perchlorate versus iodide. Lazarus et al. (1974) also demonstrated that perchlorate was taken up  
22 to greater extent in mice salivary glands than iodide. These studies, in addition to the work of  
23 Chow et al. (1969), support the use of a lower  $K_m$  for perchlorate uptake in the tissues with  
24 sodium iodide symporter. Based on this information, a value of  $2.5 \times 10^5$  ng/L for the thyroid  
25 ( $K_m_{Tp}$ ) and  $2.0 \times 10^5$  ng/L for skin ( $K_m_{Sp}$ ) or gut ( $K_m_{Gp}$ ), approximately 10 times lower  
26 than that of iodide, was estimated by Merrill (2001c,d) to represent perchlorate's affinity for  
27 transport by the NIS.

28 The apical follicular membrane (between the thyroid follicle and colloid) also exhibits a  
29 selective iodide uptake mechanism. Golstein et al. (1992) measured a  $K_m$  value of  
30 approximately  $4.0 \times 10^9$  ng/L for the transport of iodide between the thyroid follicle and colloid  
31 ( $K_m_{DTp}$ ) in bovine thyroid. This iodide channel also appears to be very sensitive to

1 perchlorate inhibition and shares a similar permeability to perchlorate as to iodide. The ability of  
2 perchlorate to inhibit iodide uptake at the apical follicular membrane suggests that the  $K_m$  of  
3 perchlorate at the apical follicular membrane ( $K_m_{Dtp}$ ) is also lower than that of iodide. Model  
4 simulations of thyroid inhibition supported a value of  $1.0 \times 10^8$  ng/L, approximately ten times  
5 less than that of iodide.

6 Whereas the  $K_m$  is similar across tissues containing NIS, the maximum velocity term  
7 ( $V_{maxc}$ ) does vary between tissues and species (Wolff, 1998), being lower in humans than other  
8 species (Gluzman and Niepomnische, 1983; Wolff and Maurey, 1961). Maximum velocities or  
9 capacities ( $V_{maxc}$ ) were not found in the literature and were estimated for a given compartment  
10 by fitting the simulation to the data at varying doses.

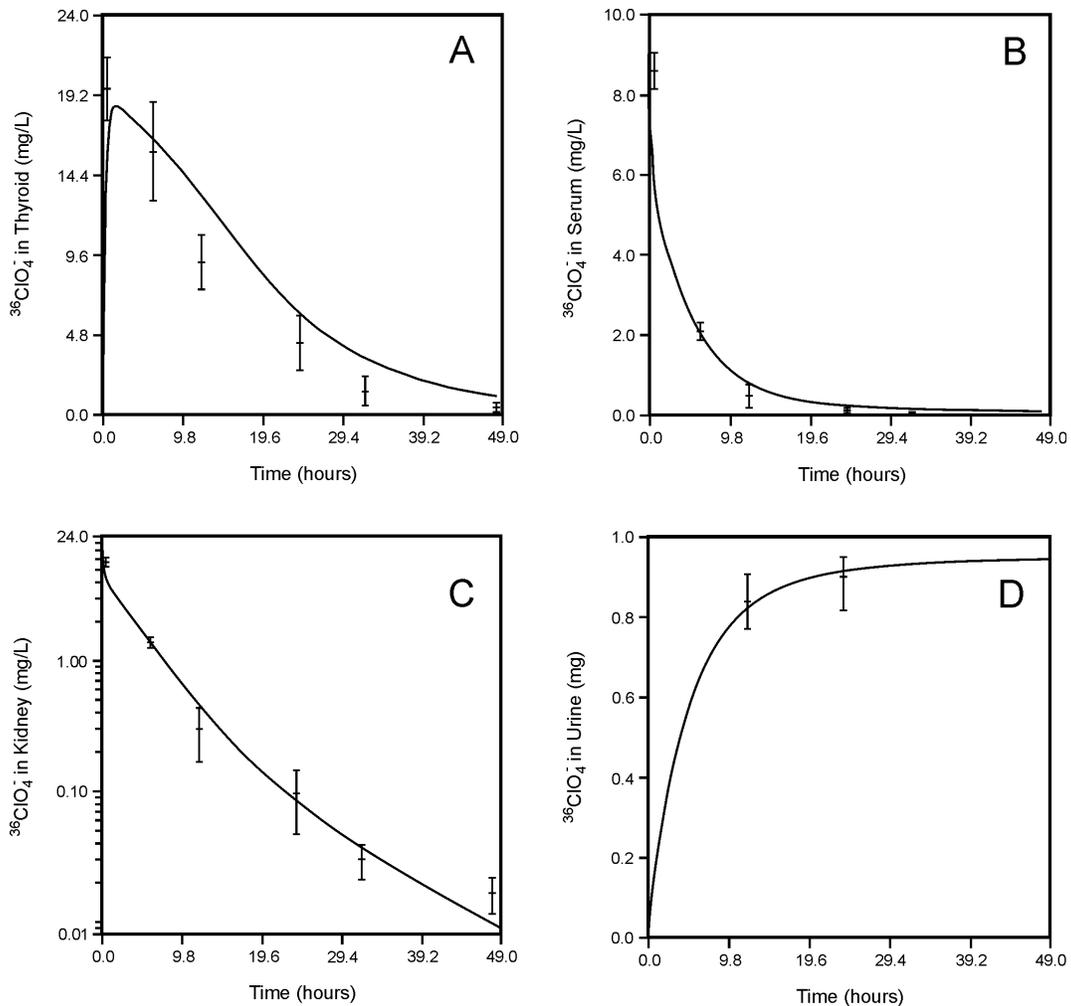
#### 11 **6.2.2.2.1 Effective Partitions, Permeability Area Cross Products and Clearance Values**

12 Permeability area cross products and partition coefficients were used to describe diffusion  
13 limited uptake in tissues requiring subcompartments. The permeability area values in the Merrill  
14 (2001c) model were fitted by setting the partition coefficients to the literature values in  
15 Table 6-2. Fitted clearance values were used to describe first-order urinary excretion rates and  
16 reversible plasma binding to serum. Equations for these representations are provided in Merrill  
17 (2001c).

#### 18 **6.2.2.3 Adult Male Rat Model Simulation Results and Validation**

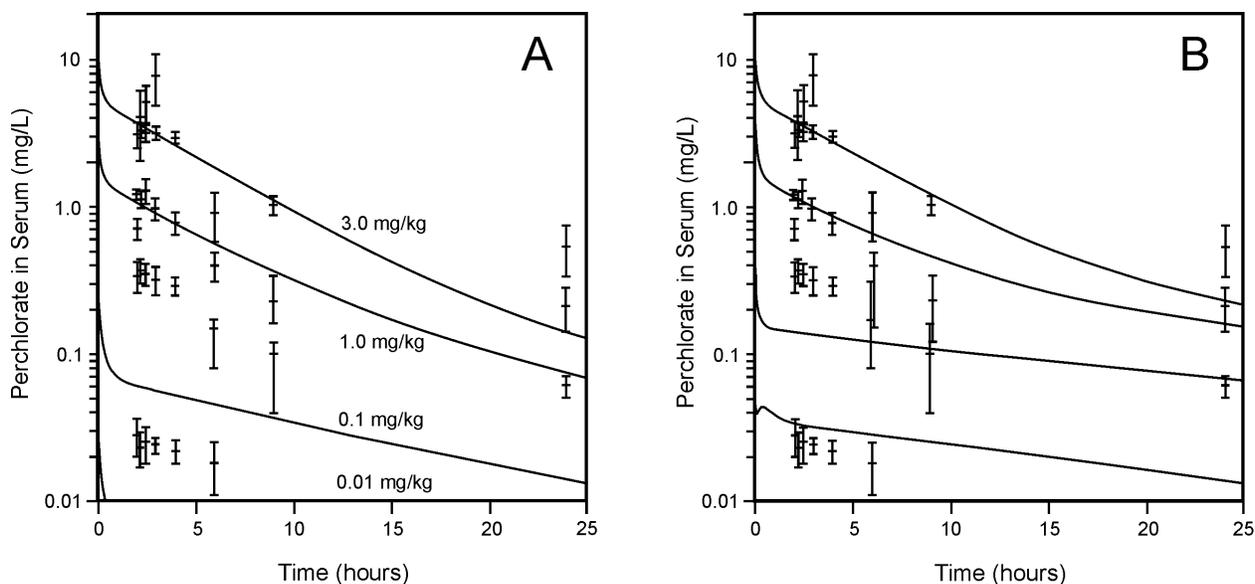
19 The simulations shown in this section result from exercising the model with the  
20 physiological and chemical-specific parameters provided in Tables 6-1 and 6-2. Figure 6-7  
21 illustrates the model predictions versus data time course for the iv radiolabeled perchlorate study  
22 described in Section 6.2.1.1.1. The model produced good simulations for the trend of the data  
23 but slightly over predicts the thyroid concentrations at later time points (Panel A). Model  
24 predictions fit the data well for perchlorate concentrations in the serum (Panel B) and kidney  
25 (Panel C), as well as the amount excreted in the urine (Panel D). Other tissue concentrations not  
26 shown herein also were predicted well by the model (Merrill, 2001c).

27 Figure 6-8 shows that plasma binding of perchlorate was necessary to provide adequate  
28 model predictions. Thyroid, serum, and urine were collected from the iv studies described in  
29 Section 6.2.1.1.1 using cold (i.e., not radiolabeled) perchlorate at 0.01, 0.1, 1.0, and 3.0 mg/kg.  
30  
31



**Figure 6-7. Adult male rat PBPK model predictions after an acute iv dosing with radiolabeled perchlorate ( $^{36}\text{ClO}_4^-$ ). Panels A and B show model predictions (lines) versus data time course (mean  $\pm$  SD) of labeled perchlorate (mg/L) in the thyroid and serum. Panel C shows model predictions versus data time course of labeled perchlorate (mg/L) in the kidney. Panel D shows cumulative excretion (mg) of labeled perchlorate in the urine (Merrill, 2001c).**

1 Model predictions without plasma binding (Panel A, left) resulted in an underestimation of  
 2 serum perchlorate concentrations at the 1 mg/kg-day dosage level and below. Low serum  
 3 predictions suggested either greater uptake into other tissues or protein binding. To provide  
 4 better estimates of perchlorate serum concentrations at the 0.01 and 0.1 mg/kg doses, Merrill  
 5 (2001c) added protein binding to the venous blood compartment of the model. An affinity



**Figure 6-8. Simulations illustrating the necessity of including plasma binding in the adult male rat PBPK model structure (Merrill, 2001c). Model predictions (lines) versus data time course (mean  $\pm$  SD) of perchlorate concentration (mg/L) in serum after doses of 3.0, 1.0, 0.1 and 0.01 mg/kg-day are shown in Panel A without and in Panel B with plasma binding. Only part of the simulation for the 0.01 dose in Panel A can be seen in the lower left corner. Data of Yu (2000).**

1 constant for this binding of perchlorate in the blood ( $Km_{Bp}$ ) of  $1.1E6$  ng/L and a maximum  
 2 velocity capacity for this blood binding ( $Vmaxc_{Bp}$ ) of  $9.3E3$  ng/h/kg was fitted to serum levels  
 3 from doses ranging 0.01 to 3.0 mg/kg (Panel B, right). The model underpredicts serum  
 4 perchlorate from the 0.1 mg/kg dose group; but it fits serum at 0.01 mg/kg and cumulative urine  
 5 across the doses. Interestingly, the urinary excretion at 0.01 mg/kg was lower than the other  
 6 doses, accounting for elevated serum concentrations. Mean 24 hour urinary excretions ( $\pm$  SD) of  
 7 perchlorate were approximately 97% ( $\pm$  2), 72% ( $\pm$  1), 87% ( $\pm$  17), and 91% ( $\pm$  13) of the  
 8 administered iv dose for the 0.01, 0.1, 1.0, and 3.0 mg/kg dose groups, respectively.

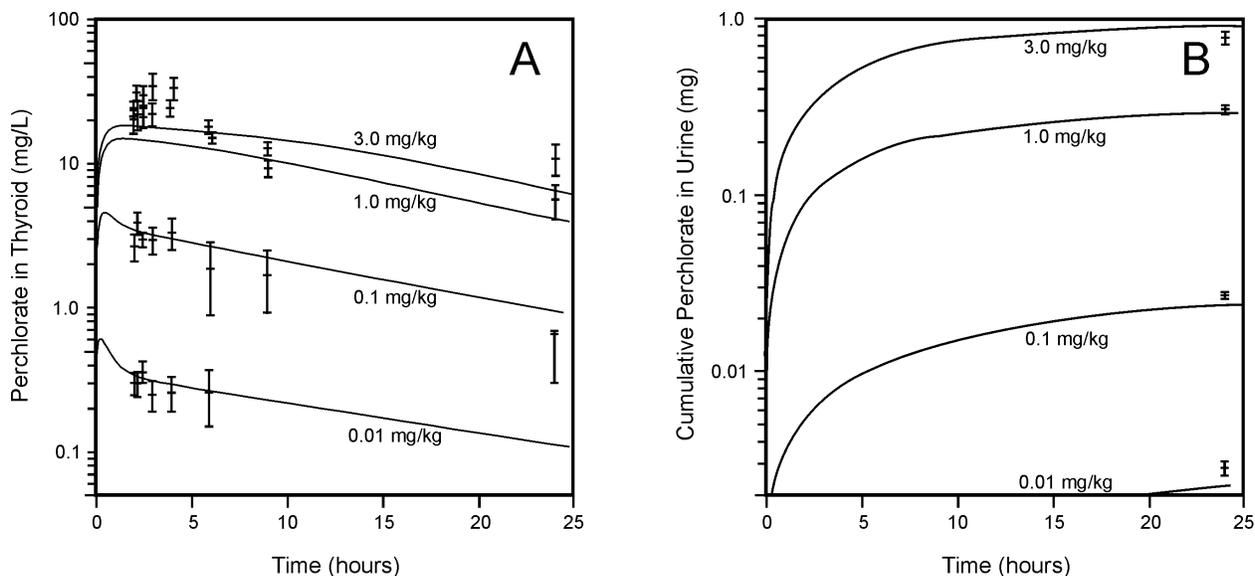
9 The literature discussed in Chapter 3 and in Merrill (2001c) suggests that serum albumin is  
 10 the major binding protein; however, it does not confirm that albumin is the only binding site.  
 11 Merrill (2001c) notes that no studies were found that evaluated whether perchlorate or similar  
 12 anions bind to thyroglobulin. However, Yamada (1967) studied the effects of perchlorate and

1 other anions on T4 metabolism and noted a significant decrease in serum protein-bound iodide  
2 (PBI) in thyroidectomized T4-maintained perchlorate-fed rats. In a 1968 in vitro study, Yamada  
3 and Jones reported that T4 was displaced from plasma protein as indicated by an uptake of T4 by  
4 muscle in the presence of plasma taken from perchlorate-fed rats. This suggested, but did not  
5 demonstrate directly, that perchlorate interferes with T4 binding with plasma proteins.

6       Pertechnetate is known to bind to plasma proteins. Hays and Green (1973) studied the  
7 blocking of pertechnetate binding with human serum proteins by other anions. Perchlorate was  
8 found to be one of the most effective, while iodide was ineffective. In dialysis studies, inorganic  
9 iodide did not bind to plasma proteins. The pertechnetate binding appeared to be reversible in  
10 serum.

11       Simulations of thyroid perchlorate concentrations and of the amount of perchlorate excreted  
12 in the urine from the four dose groups are shown in Figure 6-9. It was noted that the thyroid  
13 concentrations resulting from the 3.0 mg/kg cold perchlorate study were slightly higher than  
14 those from the radiolabeled perchlorate ( $^{36}\text{ClO}_4^-$ ) study at 3.3 mg/kg (Figures 6-9A and 6-7A,  
15 respectively). This may reflect the analytical differences in measuring cold versus radiolabeled  
16 perchlorate. The model slightly underpredicts the thyroid concentrations at 3.0 mg/kg, based on  
17 the cold perchlorate data (Figure 6-9A), and slightly overpredicts the  $^{36}\text{ClO}_4^-$  thyroid  
18 concentration at 3.3 mg/kg (Figure 6-7A).

19       The model is able to adequately predict data from studies that were not used in the  
20 development process. Figure 6-10 shows the model predictions versus the data of Chow and  
21 Woodbury (1970) and Eichler (1929). Model predictions fit the data well for radiolabeled  
22 perchlorate concentration in the thyroid (A); whereas, the serum (B) is underpredicted. Merrill  
23 (2001c) notes the difference and provides some plausible explanations. The rats in the Chow and  
24 Woodbury (1970) study were functionally nephrectomized by ligating the renal pedicle of both  
25 kidneys and given the radiolabeled perchlorate ip. Analytical differences between AFRL/HEST  
26 and Chow and Woodbury could exist, and it is also possible that the nephrectomization creates  
27 physiological changes that can not be accounted for sufficiently by “turning off” urinary  
28 excretion in the model simulations. One hypothesis is that saturation in NIS-containing tissues  
29 occurs to a lesser extent as a result of increased extracellular sodium cation ( $\text{Na}^+$ ) and possibly  
30 other competitive anions when renal clearance is blocked, thereby increasing the arterial  
31 radiolabeled perchlorate. While the underprediction in serum would suggest the need for an

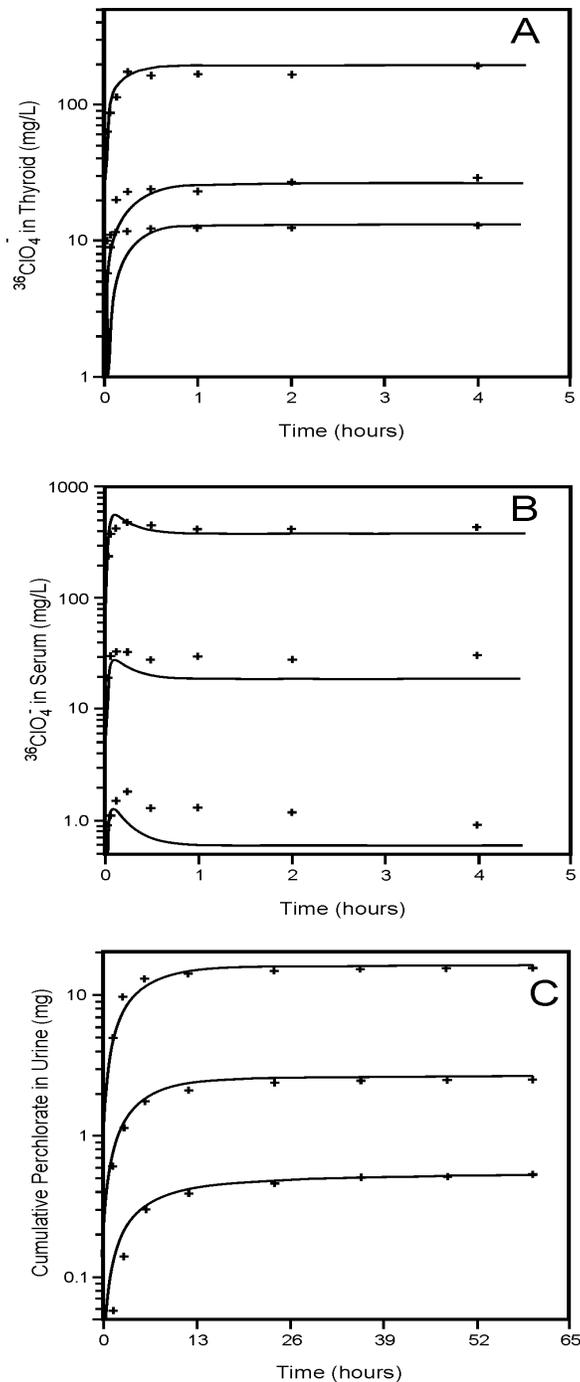


**Figure 6-9. Adult male rat PBPK model predictions (lines) versus data time course (mean  $\pm$  SD) of perchlorate concentrations in the thyroid (mg/L) in Panel A or cumulative excreted perchlorate in the urine (mg) in Panel B (Merrill, 2001c). Male rats were dosed iv with 3.0, 1.0, 0.1 or 0.01 mg/kg-day perchlorate (Yu, 2000).**

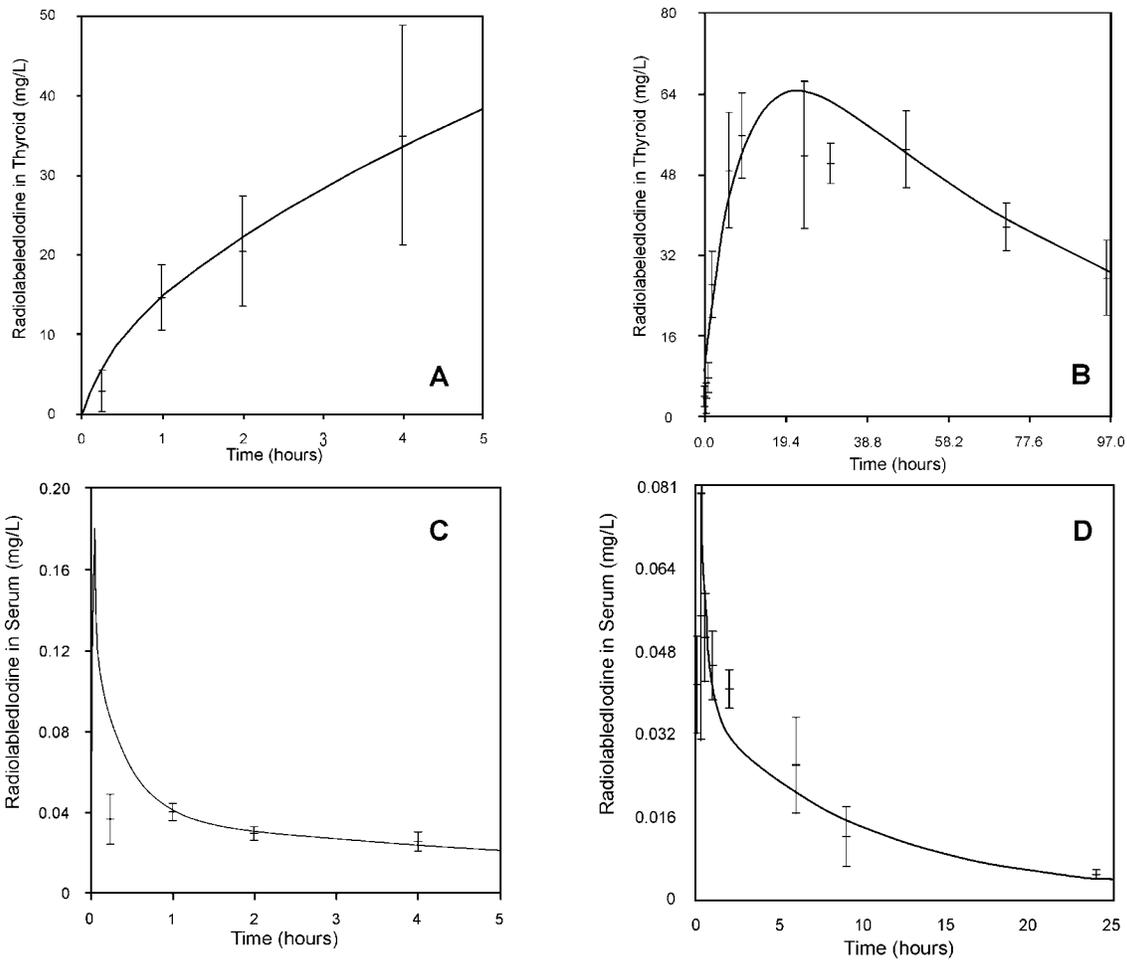
1 increased binding constant for perchlorate, this was not consistent with the data from  
 2 AFRL/HEST for studies at lower doses (Merrill, 2001c). Panel C in Figure 6-10 shows the  
 3 model predictions versus the data of Eichler (1929) for cumulative perchlorate excreted in the  
 4 urine. These rats were given perchlorate subcutaneously (sc) at doses of 1.6, 8.0, and 49 mg/kg.

5 The adult male rat model (Merrill, 2001c) is also able to predict iodide distribution.  
 6 Figure 6-11 shows the model predictions versus a time course for radiolabeled iodide data from  
 7 the AFRL/HEST experiments outlined in Section 6.2.1.1.1. Adequate fit is demonstrated for  
 8 both the thyroid and serum concentrations at doses of radiolabeled iodide differing by an order of  
 9 magnitude (0.033 and 0.33 mg/kg).

10 Figure 6-12 demonstrates the fit of the model simulations of perchlorate thyroid  
 11 concentration (mg/L) after drinking water exposures to perchlorate. The model was coded to  
 12 simulate oral dosing for 12 hours per day, assuming that rats drink fairly continuously during  
 13 their waking hours. The same perchlorate parameters used to describe the “acute” (iv) kinetics  
 14 also adequately described serum concentrations from these “chronic” drinking water exposures

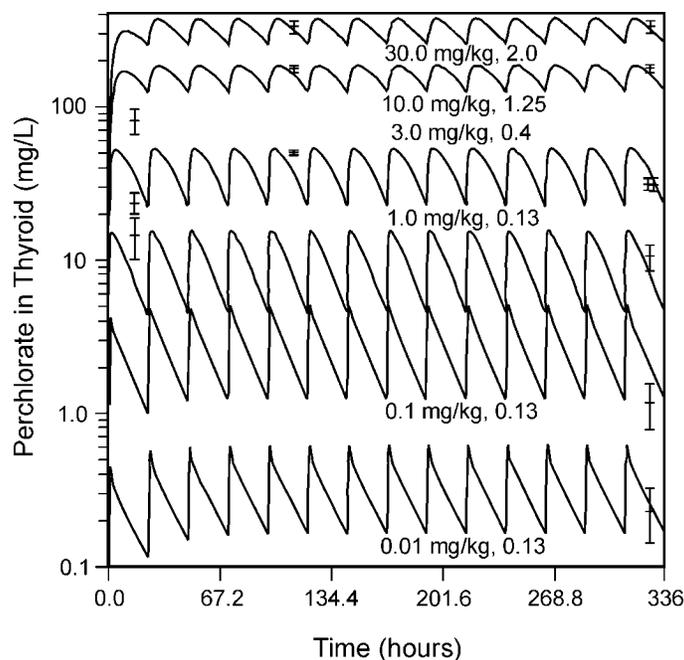


**Figure 6-10. Validation for male rat PBPK model of perchlorate disposition (Merrill, 2001c). Model predictions (lines) versus data time course for concentrations (mg/L) in the thyroid (A) and serum (B) for ip administration in rats of 200, 10, and 0.5 mg/kg  $^{36}\text{ClO}_4^-$  (data of Chow and Woodbury, 1970). Panel C shows model predictions (lines) and data time course for cumulative perchlorate in the urine (mg) of male rats after subcutaneous doses of 1.6, 8.0, and 49 mg/kg (data of Eichler, 1929).**



**Figure 6-11. Male rat PBPk model (Merrill, 2001c) predictions (lines) versus data time course (mean  $\pm$  SD) of iodide concentrations (mg/L) at two doses of  $^{125}\text{I}$  with carrier, 0.033 mg/kg or 0.33 mg/kg, in the thyroid (A) or (B) and in the serum (C) or (D). Data of Yu (2001).**

1 (data shown in Merrill, 2001c) but failed to predict thyroid concentrations from the 3.0 mg/kg-  
 2 day dose and higher. TSH in these same studies was increased during drinking water exposure  
 3 across all doses so that Merrill (2001c) accounted for the TSH-induced upregulation in the NIS  
 4 by fitting an increased effective thyroid follicle:stroma partition coefficient ( $PT_p$ ) at these  
 5 higher doses. Merrill (2001c) noted that TSH is not expected to increase NIS in tissues other than  
 6 the thyroid (Brown-Grant, 1961) and that these simulations agree. Given the small size of the  
 7 thyroid, its upregulation would not decrease serum concentrations significantly. This explains

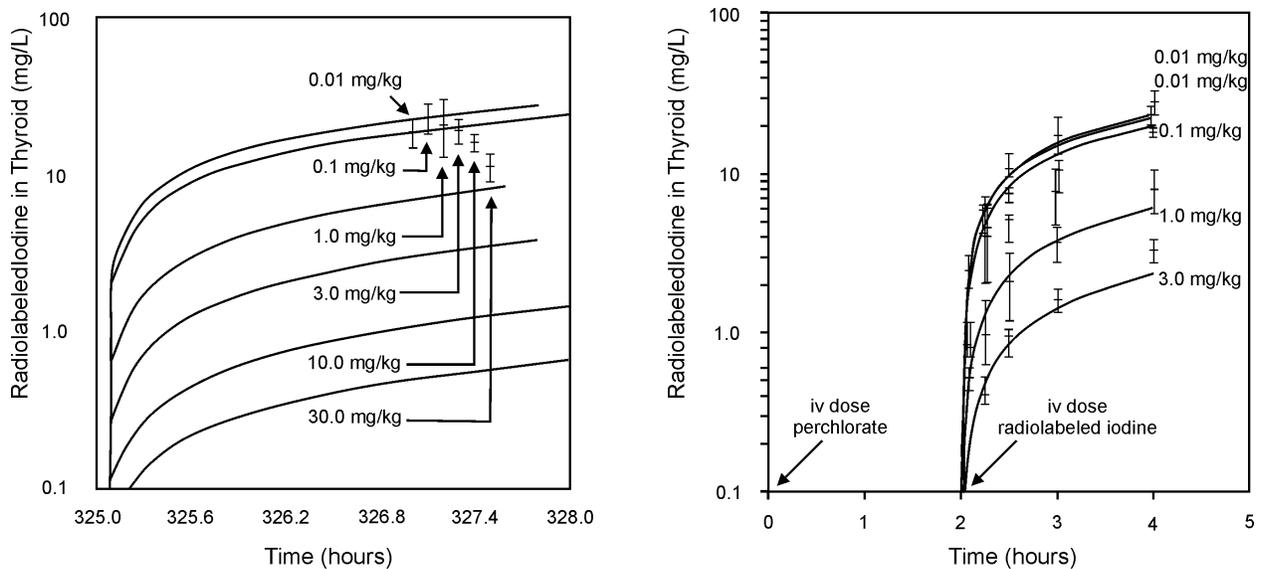


**Figure 6-12. Male rat PBPK model predictions (lines) versus data time course (mean  $\pm$  SD) of thyroid perchlorate concentrations (mg/L) in male rats during ingestion of 30, 10, 3.0, 1.0, 0.1, or 0.01 mg/kg-day in drinking water for 14 days (Merrill, 2001c). Data across the doses were fit by increasing the thyroid follicle:stroma effective partitioning for perchlorate ( $PT_p$ ) from 0.13 to 0.4, 1.25, and 2.0 at the 3, 10, and 30 mg/kg-day doses.**

1 why the model successfully predicted serum perchlorate concentrations across drinking water  
 2 doses with the same parameters used to describe acute exposures and why it could not predict  
 3 thyroid concentrations above 3 mg/kg-day.

4 It could be expected that other parameters (e.g., follicle size and follicular  $V_{maxc}$ ) would  
 5 also increase with TSH stimulation. There is an increase in percent of thyroid volume attributed  
 6 to the follicle cells (Conde et al., 1991; Ginda et al., 2000), total protein, RNA and DNA content,  
 7 and the incorporation of labeled amino acids into protein (Pisarev and Kleiman de Pisarev,  
 8 1980). However, Merrill (2001c) notes that adequate predictions could be achieved by adjusting  
 9 additional parameters; although, without incorporation of regulation by the hypothalamic-  
 10 pituitary-thyroid axis, such adjustments provide little additional insight.

11 The ability of the adult male rat model to predict iodide uptake inhibition in the thyroid is  
 12 demonstrated in Figure 6-13 for a single iv dose of perchlorate (right) or for a 14-day drinking



**Figure 6-13. Male rat PBPK model predictions (lines) versus data time course (mean  $\pm$  SD) of iodide uptake inhibition in male rats administered perchlorate either by a single iv dose (right) or in drinking water for 14 days (left), followed by an iv dose of 33  $\mu\text{g}/\text{kg}$   $^{125}\text{I}$  with carrier (Merrill, 2001c). Perchlorate doses were 3.0, 1.0, 0.1, and 0.01 mg/kg-day. Inhibition at the 0.01 and 0.1 mg/kg-day doses overlaps for the iv dose (right).**

1 water exposure (left). Perchlorate-induced inhibition of  $^{125}\text{I}$  uptake in the thyroid was 13, 24, 70,  
 2 and 88% at 2 hours and 11, 29, 55, and 82% at 9 hours after iv dosing with  $^{125}\text{I}$  with carrier for  
 3 the 0.01, 0.1, 1.0, and 3.0 mg/kg dose groups. Good simulations were achieved across doses.  
 4 However, at 3.0 mg/kg, the model slightly overpredicts inhibition 6 hrs after the perchlorate dose  
 5 (4 hours after  $^{125}\text{I}$  administration). TSH was measured from the highest dose level (3.0 mg/kg)  
 6 between 8 and 48 hours post dosing and was found to increase between 8 and 12 hrs. It is  
 7 possible that TSH was already elevated at 6 hrs, allowing upregulation of the thyroid to  
 8 compensate for inhibition at that time point, which the model would not predict. Yu (2000)  
 9 provides greater details on hormone fluctuations resulting from the AFRL/HEST experiments.

10 With respect to iodide inhibition after 14 days of drinking water exposure to perchlorate at  
 11 0.01, 0.1, 1.0, 3.0, 10.0, and 30.0 mg/kg-day (Figure 6-13, left), the model overpredicts inhibition  
 12 at the 1.0 mg/kg-day dosage and greater. TSH-induced upregulation of the thyroid compensates  
 13 for competitive inhibition, resulting in little or no inhibition of radioiodide uptake on Day 14 of

1 exposure in all dose groups except 30 mg/kg-day. In all treated groups, TSH levels were already  
2 increased after the first day. Serum T4 initially decreased in all dose groups except the  
3 0.01 mg/kg-day group. By day 14, T4 levels had increased to control values in the 0.1 and  
4 1.0 mg/kg-day dose groups. FT4 increased in all dose groups on day 1, returned to normal values  
5 by day 5, and were significantly elevated across all dose groups by day 14 (except the 0.1 mg/kg-  
6 day group).

### 7 8 **6.2.3 Human Model Development**

9 The adult human PBPK model (Merrill, 2001d) was developed concurrently with that for  
10 the adult male rat (Merrill, 2001c) and updates the preliminary structure provided to EPA  
11 (Merrill, 2000). Much of the early development was based upon generalizations from previous  
12 AFRL/HEST work on perchlorate (Fisher, 1998a; 2000) and the work of Hays and Wegner  
13 (1965) describing iodide kinetics. As discussed above and shown in Figure 6-1, a nearly  
14 identical model structure to that of the adult male rat was used for the adult human. The human  
15 physiological parameters will of course be different as these should be species-specific. This  
16 section will only highlight notable differences in parameter sources between the two models.

#### 17 18 **6.2.3.1 Physiologic Parameters and Tissue Partition Coefficients**

19 Human tissue volumes and blood flows were obtained from the literature as shown in  
20 Table 6-1. Merrill (2001d) notes that considerable variability was reported for some parameters.  
21 For example, blood flow to the gastrointestinal (GI) tract can increase ten-fold in response to  
22 enhanced functional activity (secretion and digestion) (Granger et al., 1985). Blood flows used  
23 in the model represent estimates of resting values. Human data on the volume of the gut  
24 capillary bed (VGBc) were not found in the published literature. Therefore, Merrill (2001d) used  
25 a value derived from rat stomach data (Altman and Dittmer, 1971a) for the volume of the  
26 gastrointestinal blood (VGBc) in the human model.

27 Thyroid volume was obtained from ultrasound measurements on 57 healthy volunteers with  
28 no thyroid disorders (37 to 74 years of age) in a study conducted by Yokoyama et al. (1986). The  
29 mean thyroid volume was  $13.4 \pm 4.1$  mL and mean thyroid volume to body weight ratio was  
30  $0.251 \pm 0.074$  mL/kg (mean  $\pm$  SD), approximately 0.03% of body weight. Yokoyama et al.  
31 (1986) found a positive correlation between thyroid volume and both body weight and age, with

1 weight having the most pronounced influence. The percent of total thyroid volume attributed to  
2 the thyroid follicular epithelium, colloid, and stroma were estimated from histometric  
3 measurements of patients at necropsy by Brown et al. (1986). Their findings on the histological  
4 features of thyroids of men and women showed overlapping distributions without evidence of a  
5 significant difference between sexes. However, a significant sex difference in total fat mass is  
6 reported in humans, with women having approximately 10% more fat than men (Brown et al.,  
7 1997). Based on these data, Merrill (2001d) used a gender-specific value for this parameter.  
8

### 9 **6.2.3.2 Chemical-Specific Parameters**

10 The various active transport processes, tissue permeabilities, and clearance rates (excretion)  
11 are described in PBPK models for each species on a chemical-specific basis. This section  
12 outlines how the values for perchlorate and iodide used in the human model were derived. The  
13 values can be found in Table 6-2, and the details on derivation are in Merrill (2001d).  
14

#### 15 **6.2.3.2.1 Affinity Constants and Maximum Velocities**

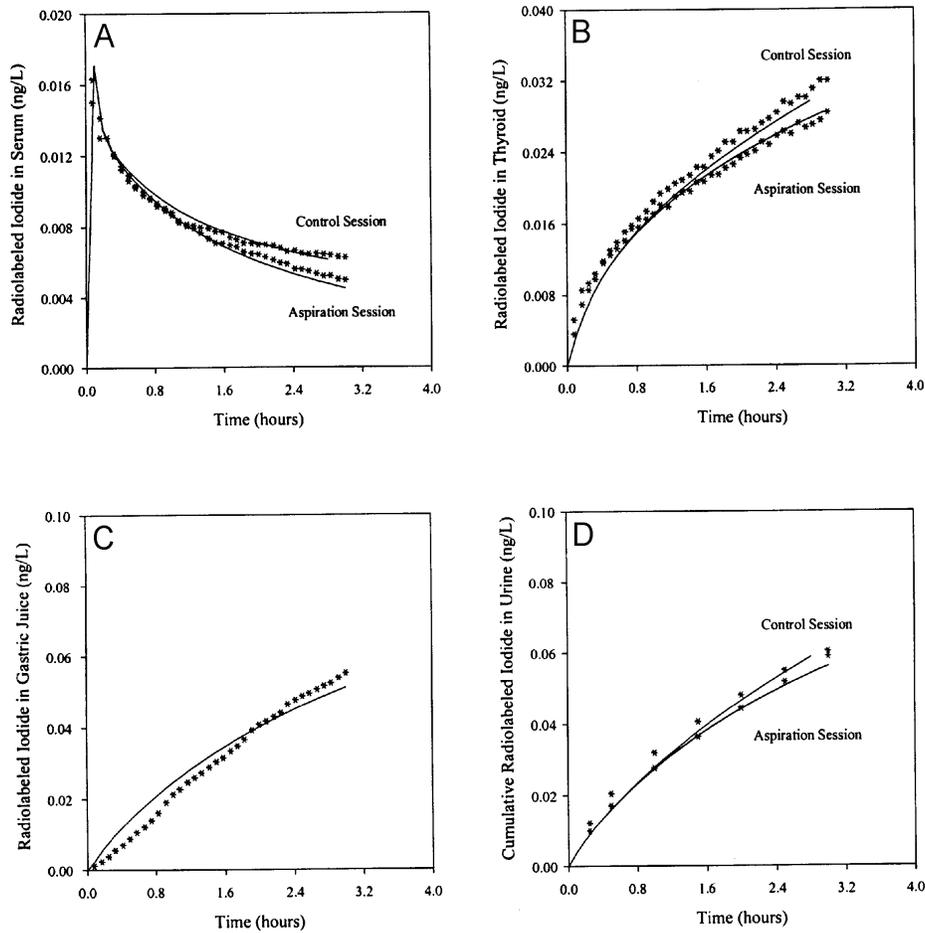
16 The Michaelis-Menten affinity constant ( $K_m$ ) estimates for perchlorate and iodide in the  
17 various tissues with active transport were developed in the human in an analogous fashion to that  
18 in the rat, as described above in Section 6.2.2.2., based on Golstein et al. (1992), Gluzman and  
19 Niepomnische (1983), and Wolff (1998). The maximum velocity capacity ( $V_{maxc}$ ) values were  
20 estimated for the various compartments by fitting the simulations to available data at various  
21 doses (Merrill, 2001d).  
22

#### 23 **6.2.3.2.2 Effective Partitions, Permeability Area Cross Products, and Clearance Values**

24 Permeability area cross products and clearance values for perchlorate and iodide were  
25 developed by fitting to literature values in an analogous fashion to that for the rat described in  
26 Section 6.2.2.3 (Merrill, 2001d).  
27

#### 28 **6.2.3.3 Adult Human Model Parameterization and Validation**

29 The human PBPK model for iodide was developed based on the data of Hays and Solomon  
30 (1965) described in Section 6.2.1.2.1. Model predictions versus the data are shown in  
31 Figure 6-14 for iodide concentrations (ng/L) in the serum (A), thyroid (B), and gastric juice (C);

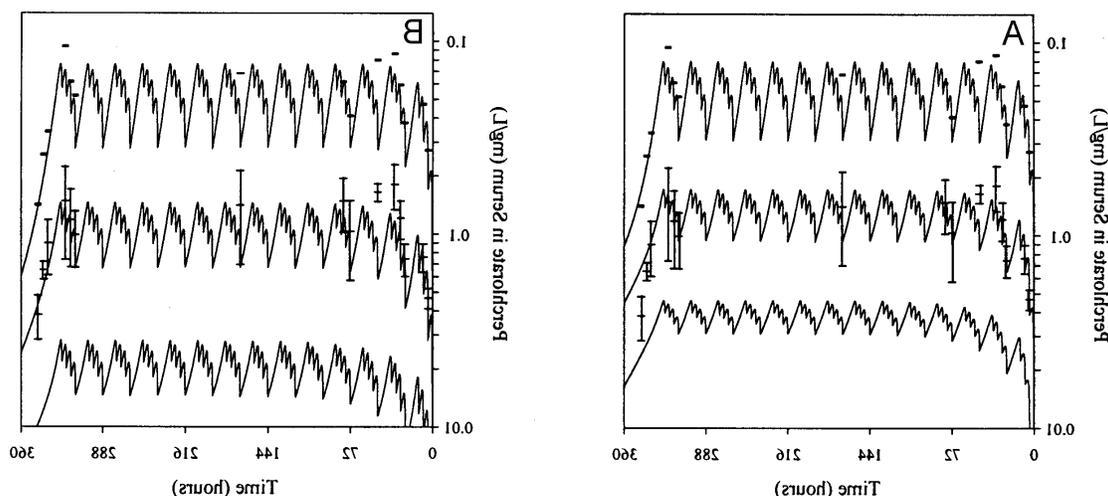


**Figure 6-14. Human PBPK model (Merrill, 2001d) predictions (lines) versus mean <sup>131</sup>I concentration (mg/L) time course (asterisks) in serum (A), thyroid (B), gastric juice (C), and urine (D). Data of Hays and Solomon (1965) are for nine healthy males dosed with 10  $\mu$ Ci <sup>131</sup>I (approximately 3.44 ng/kg).**

1 cumulative iodide excreted in the urine (ng) is shown in D. In this study, aspirated gastric juice  
 2 accounted for an average of 23% of the iv dose within 3 hours after iv injection with radiolabeled  
 3 iodide (<sup>131</sup>I) (Merrill, 2001d). Simulation of the gastric juice removed during the aspiration  
 4 session (Figure 6-14, C) required mathematically removing the amount of <sup>131</sup>I reabsorbed by the  
 5 stomach wall. This was accomplished by adjusting the rate of reabsorption of <sup>131</sup>I from gastric  
 6 juice to gastric tissue during the aspiration session as described in Merrill (2001d). The Vmaxc  
 7 values for the gut and thyroid were then obtained by fitting values of <sup>131</sup>I uptake into gastric juice

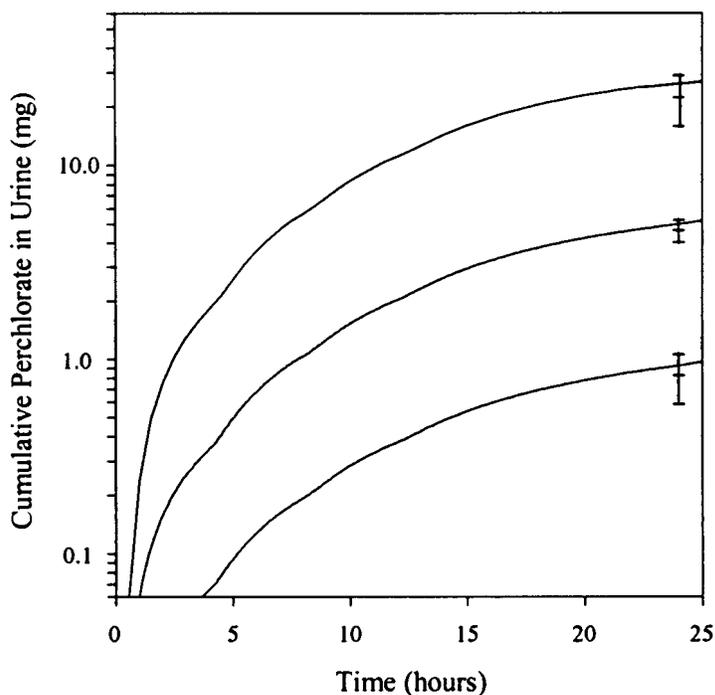
1 from the aspiration session (lower lines in Figures 6-14; B and C). The urinary clearance value  
 2 was fitted to simulate both cumulative urine content and serum iodide concentration from the  
 3 aspiration session data (lower lines in Figures 6-14; A and D). Once parameters were established  
 4 using the aspiration session, the rate of change in the gastric juice and partitioning back into the  
 5 gastric juice from the systemic circulation was fitted to predict the corresponding increase in  
 6  $^{131}\text{I}$  in plasma, thyroid, and urine seen in the control session versus the aspiration session (upper  
 7 lines in Figures 6-14; A, B and D).

8 Figure 6-15 illustrates that, as for the adult male rat model, plasma binding of perchlorate  
 9 was necessary to fit the serum concentration data of the 14-day study by Greer et al. (2000). The  
 10 model indicates that humans have a lower binding capacity for perchlorate than rats.  
 11 For example, the  $V_{maxc}$  value for perchlorate is  $9.3 \times 10^3$  ng/hr-kg in the male rat versus  $5.0 \times 10^2$   
 12 ng/hr-kg in the human. Merrill (2001d) noted that while the effect of the plasma binding is  
 13 subtle at 0.5 mg/kg-day dose, including the plasma binding improved the fit for uptake and  
 14 clearance at the 0.1 and 0.02 mg/kg-day dosage levels.



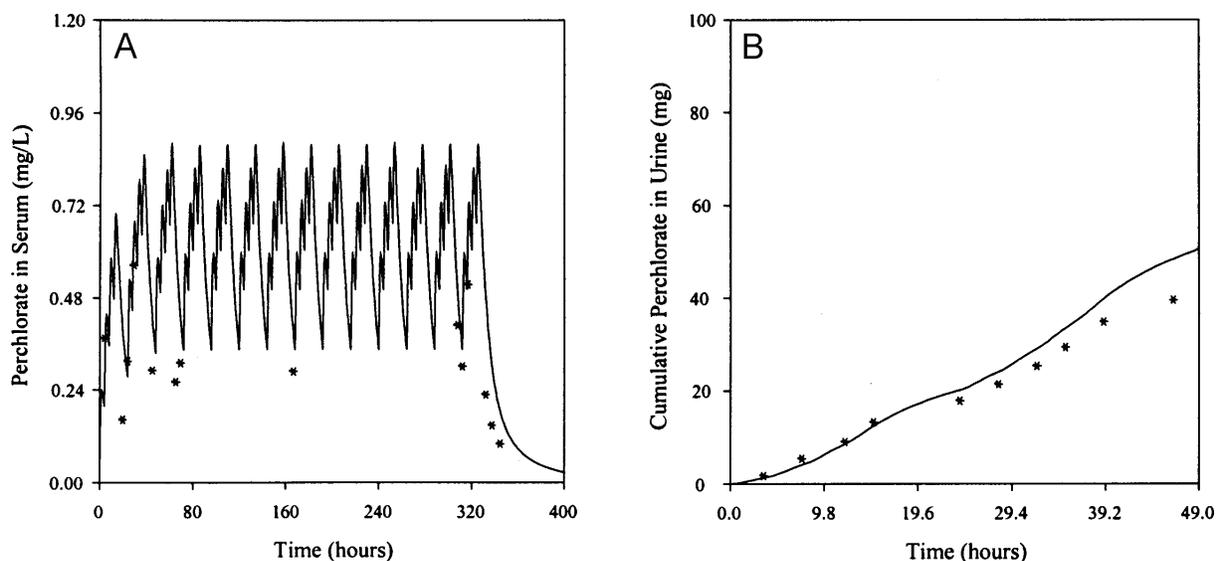
**Figure 6-15. Simulations illustrating the necessity of including plasma binding in the human PBPK model structure (Merrill, 2001d). Model predictions (lines) versus data time course (mean  $\pm$  SD) are shown with (A) and without (B) plasma binding for serum concentrations (mg/L) from 4 male subjects dosed with perchlorate at 0.5, 0.1, or 0.02 mg/kg-day for 14 days (data of Greer et al., 2000).**

1 Cumulative urinary perchlorate excretion (mg) predictions versus the data (mean  $\pm$  SD) at  
2 each dosage level are shown in Figure 6-16. Merrill (2001d) also simulated serum concentration  
3 (mg/L) and cumulative urinary perchlorate levels (mg) for each individual in the 0.5, 0.1, and  
4 0.02 mg/kg-day dose groups of the Greer et al. (2000) study. An average value for urinary  
5 clearance of perchlorate ( $Cl_{Uc_p}$ ) of 0.126 L/hr-kg ( $\pm$  0.050) was calculated from the  
6 individually fitted values. Figures 6-17 and 6-18 show a representative plot of model prediction  
7 versus individual subject data at the 0.5 and 0.1 mg/kg-day dosage. Additional plots provided in  
8 Merrill (2001d) provide an appreciation for the high degree of variability in the data.

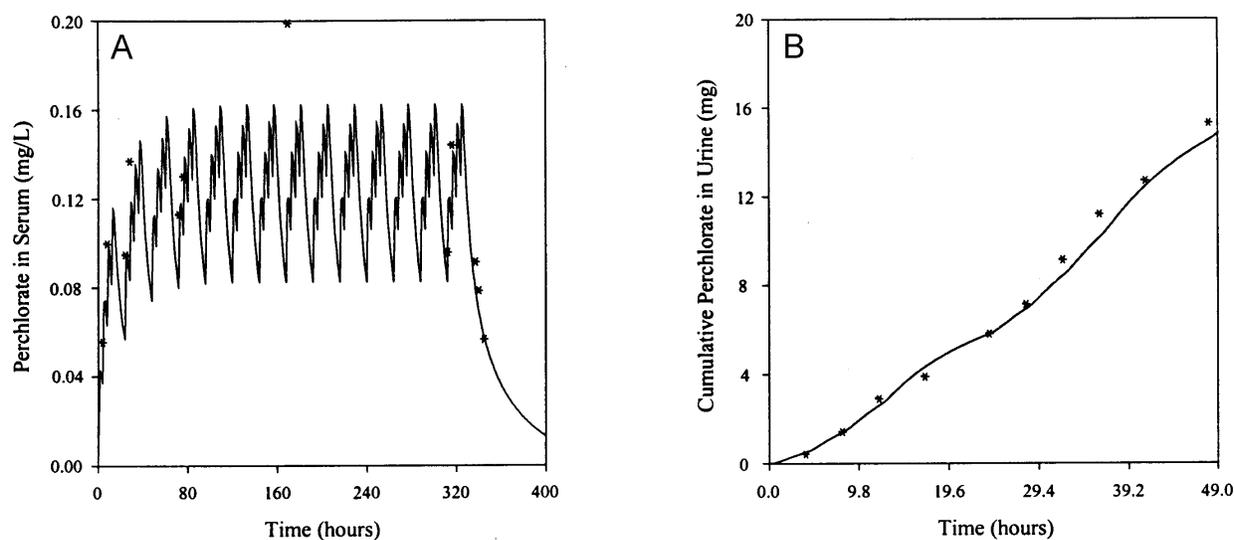


**Figure 6-16. Human PBPK model predictions (lines) versus data (mean  $\pm$  SD) of the observed cumulative urine excretion (mg) in male subjects dosed with perchlorate 0.5, 0.1, or 0.02 mg/kg-day for 14 days. Model of Merrill (2001d) and data of Greer et al. (2000).**

1 Serum perchlorate levels were not available for the 0.02 mg/kg-day dose group, but  
2 cumulative urinary excretion amounts (mg) for this group were fitted using the average

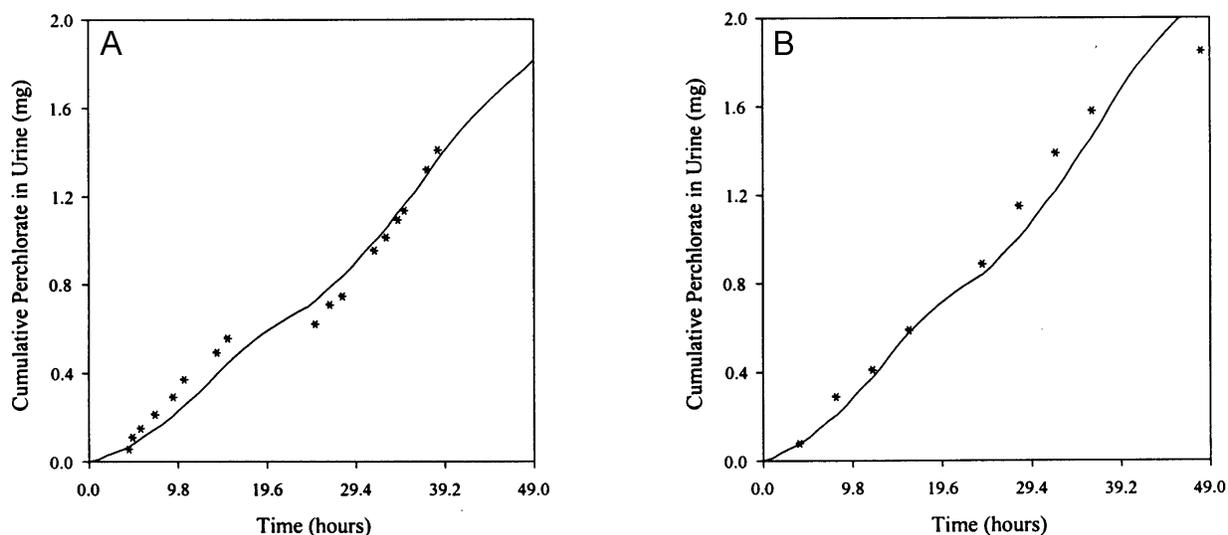


**Figure 6-17. Human PBPK model predictions (lines) versus data of one subject's serum perchlorate concentration (mg/L) shown in (A) and corresponding 48-hour cumulative urine perchlorate (mg) shown in (B). Subject consumed 0.5 mg/kg-day perchlorate in drinking water, 4 times per day, for 14 days. Model predictions for the individual obtained by using study average value of all subjects for urinary clearance of perchlorate (CIUc<sub>p</sub>). Model of Merrill (2001d) and data of Greer et al. (2000).**



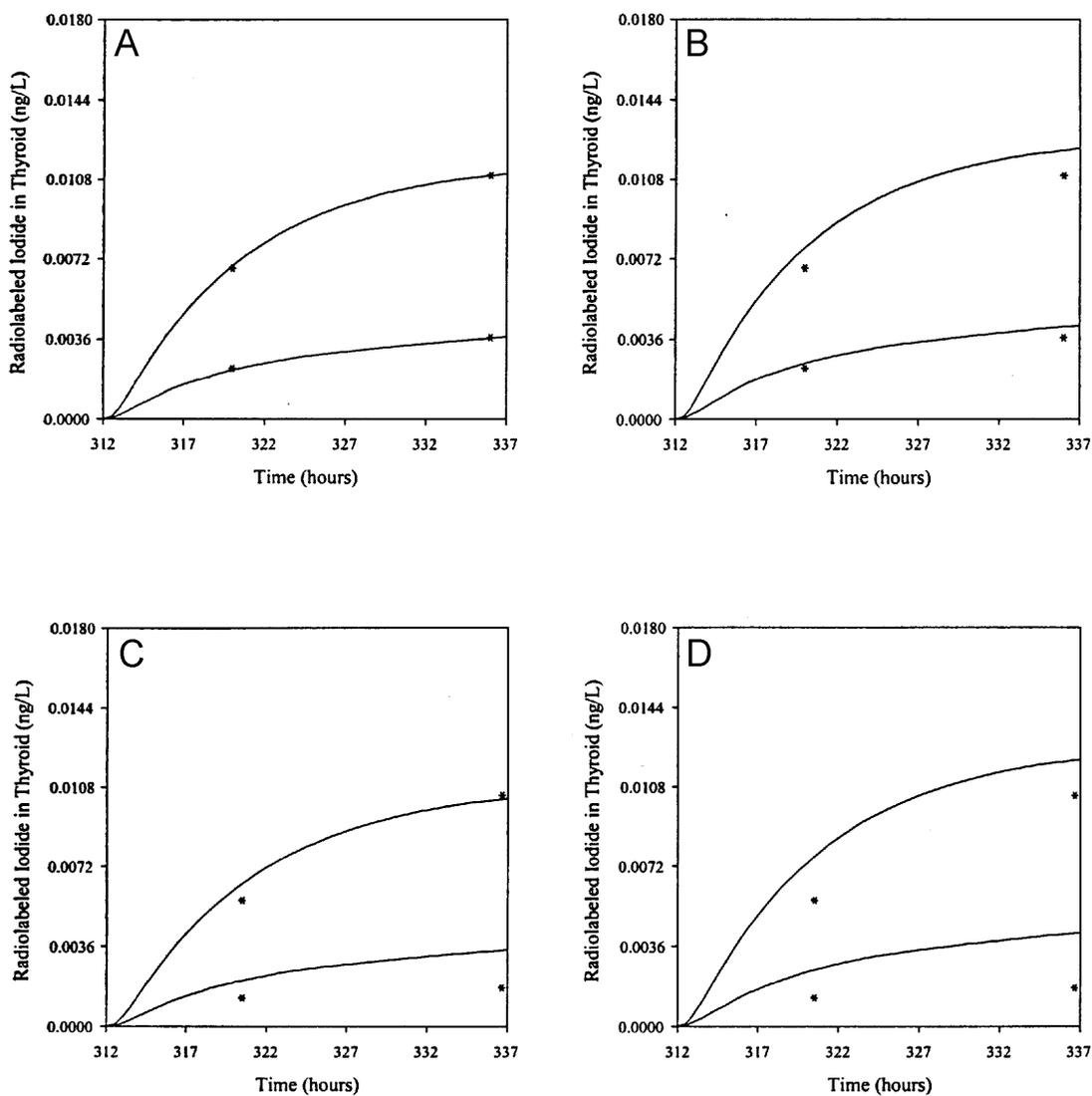
**Figure 6-18. Human PBPK model predictions (lines) versus data of one subject's serum perchlorate concentration (mg/L) shown in (A) and corresponding 48-hour cumulative urine perchlorate (mg) shown in (B). Subject consumed 0.1 mg/kg-day perchlorate in drinking water, 4 times per day, for 14 days. Model predictions for the individual obtained by using study average value of all subjects for urinary clearance of perchlorate (CIUc<sub>p</sub>). Model of Merrill (2001d) and data of Greer et al. (2000).**

1 perchlorate urinary clearance ( $CIUC_p$ ) value of 0.126 L/hr-kg calculated from the individual fits  
2 for the 0.1 and 0.5 mg/kg-day groups. Figure 6-19 shows the model predictions versus 48-hour  
3 cumulative urine perchlorate (mg) for two different subjects.  
4  
5

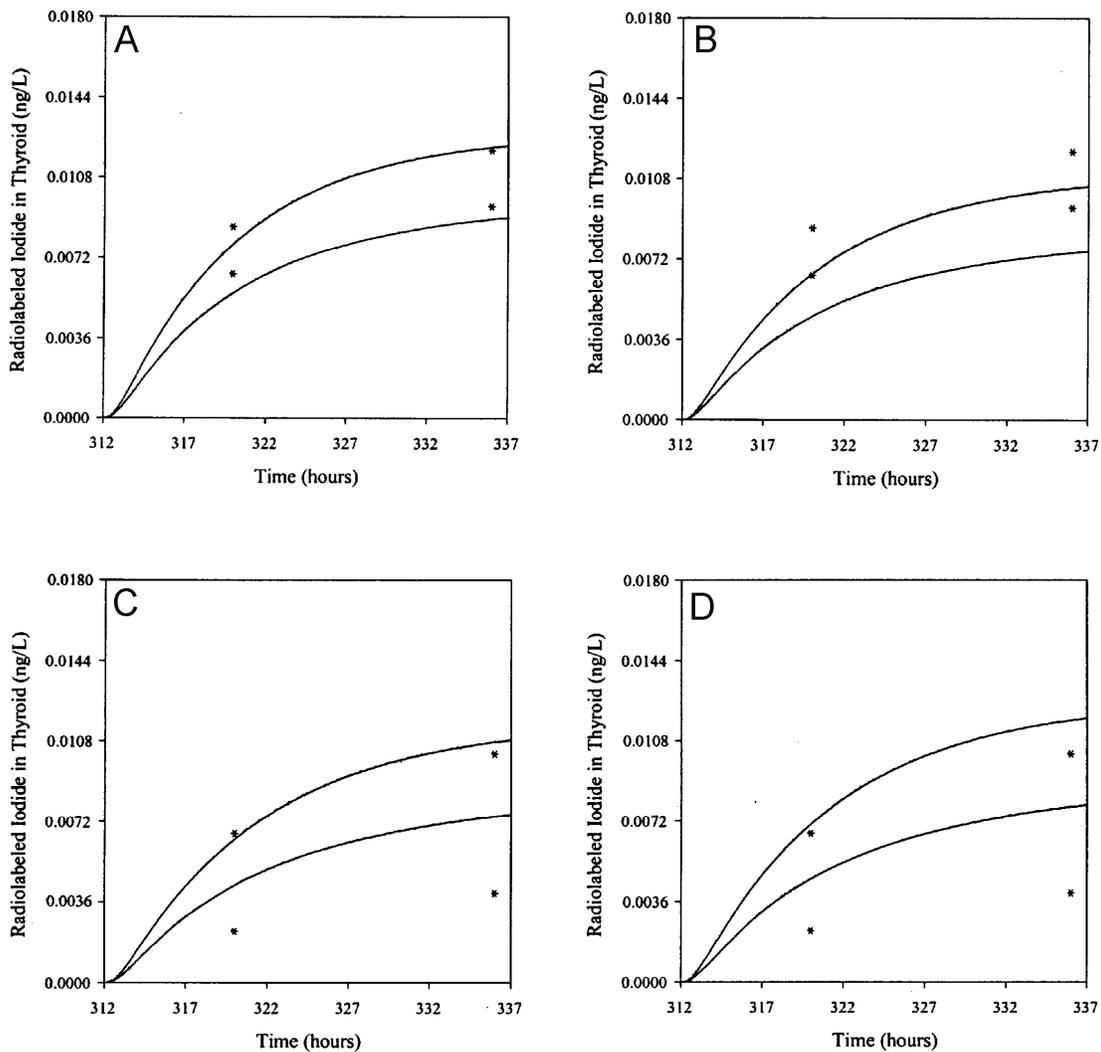


**Figure 6-19. Human PBPK model predictions (lines) versus data of 48-hour cumulative urine perchlorate (mg) shown for two different subjects. Subject consumed 0.02 mg/kg-day perchlorate in drinking water, 4 times per day, for 14 days. Model predictions for the individual obtained by using study average value of all subjects for urinary clearance of perchlorate ( $CIUC_p$ ). Model of Merrill (2001d) and data of Greer et al. (2000).**

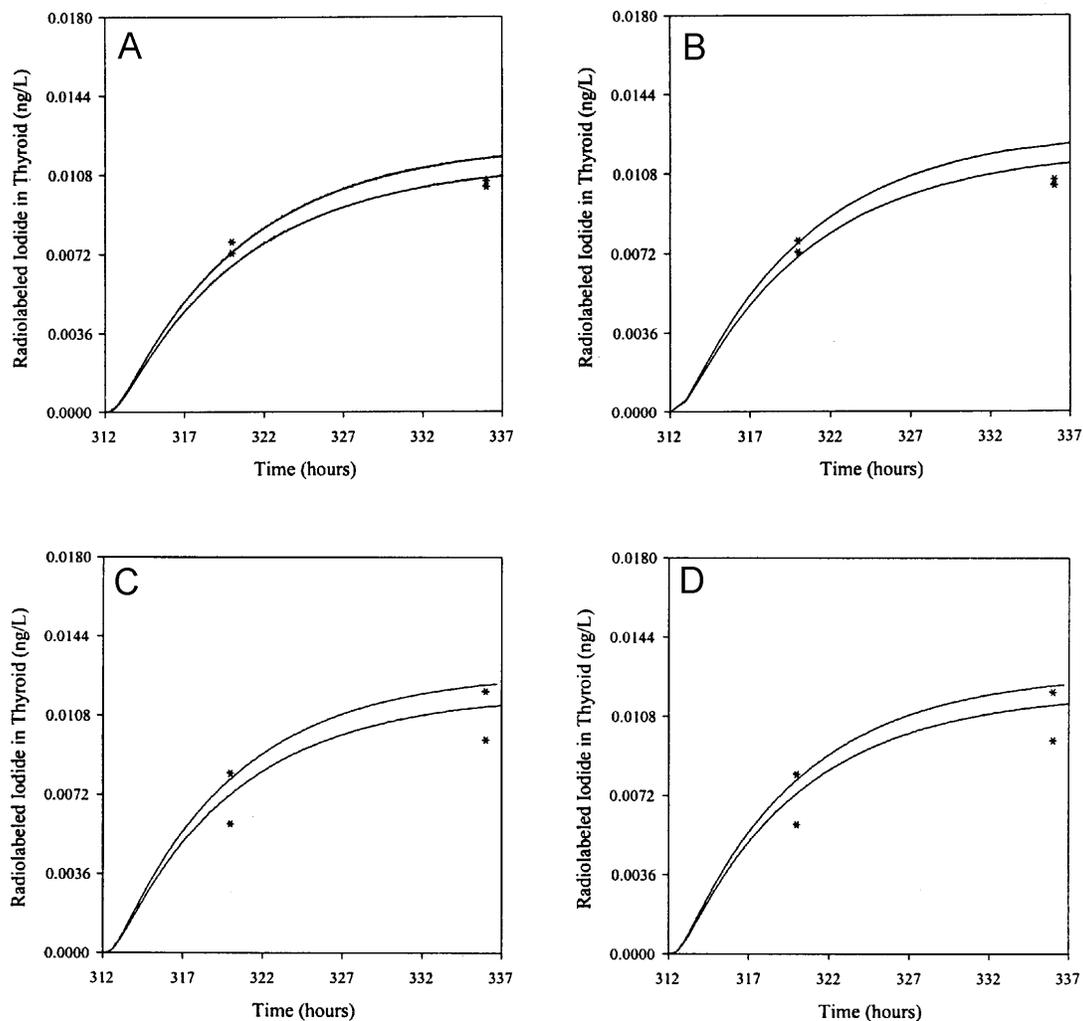
1 Due to its small size, variations in the thyroid parameters have little effect on serum  
2 concentrations of both iodide and perchlorate. As described for Figure 6-14, Merrill (2001d)  
3 estimated parameters for iodide disposition, including those of the thyroid, from fits to the data  
4 of Hays and Solomon (1965). Using these same iodide parameters, baseline thyroid RAIU  
5 measurements performed by Greer et al. (2000) were fit with the model by adjusting the  $V_{maxc}$   
6 for the thyroid follicular epithelium ( $V_{maxc\_Ti}$ ). Figures 6-20, 6-21, 6-22, and 6-23 illustrate  
7 the model predictions of thyroid RAIU versus data for subjects in the 0.5, 0.1, 0.02, and  
8 0.007 mg/kg-day dosage groups, using either the individual's  $V_{maxc\_Ti}$  (left) or an average  
9 value (right). The average  $V_{maxc\_Ti}$  ( $1.5 \times 10^5$  ng/hr-kg) was obtained from fitting baseline



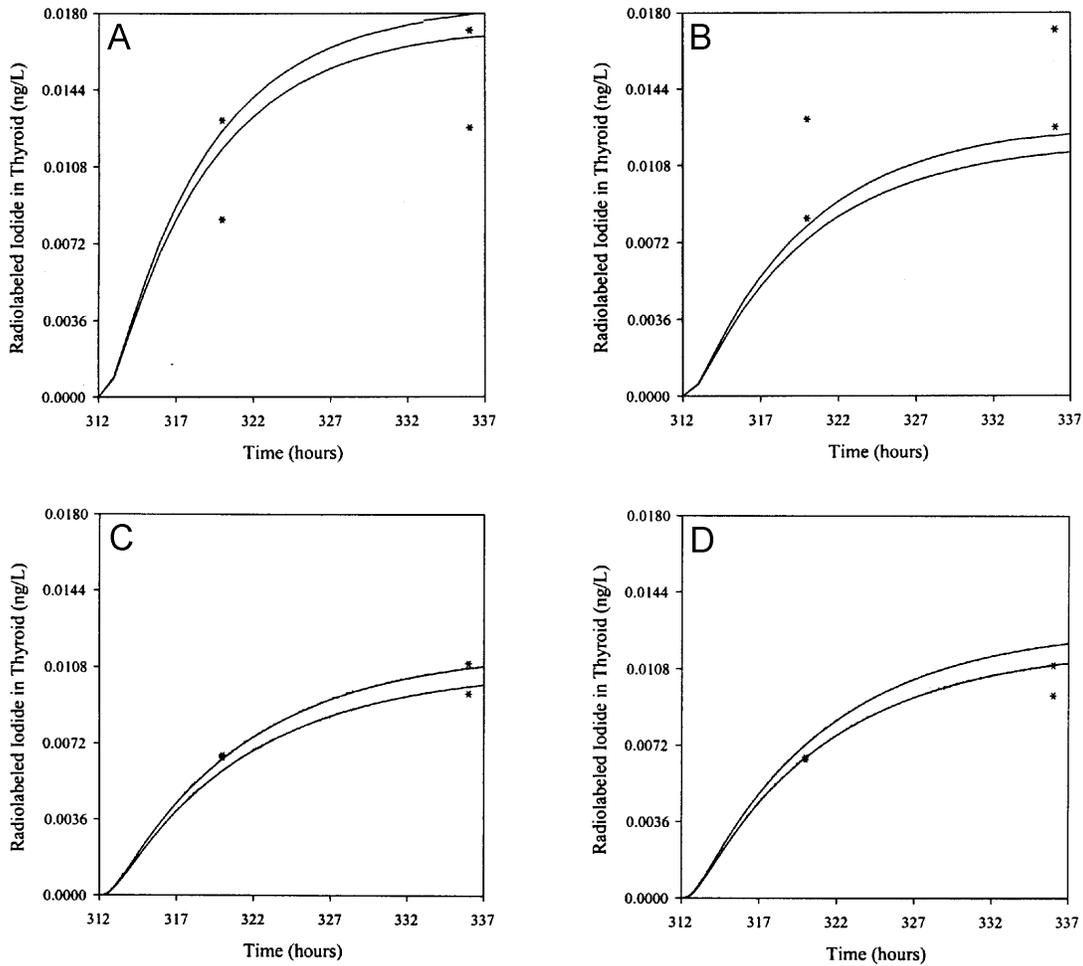
**Figure 6-20. Human PBPK model predictions (lines) versus data (asterisks) for thyroid RAIU (ng/L) on day 14 of perchlorate exposure at 0.5 mg/kg-day for a healthy female (top panel) and male (bottom panel). Prediction on left for female (A) obtained by using individually fitted maximum capacity (ng/hr-kg) for active transport of iodide into the thyroid follicular epithelium ( $V_{maxc\_Ti}$ ) of  $1.3 \times 10^5$  and on right (B) by using an average  $V_{maxc\_Ti}$ . Prediction on left for male (C) obtained by using individually fitted  $V_{maxc\_Ti}$  of  $1.24 \times 10^5$  and on right (D) by using an average  $V_{maxc\_Ti}$  of  $1.5 \times 10^5$ . Model of Merrill (2001d) and data of Greer et al. (2000).**



**Figure 6-21. Human PBPK model predictions (lines) versus data (asterisks) for thyroid RAIU (ng/L) on day 14 of perchlorate exposure at 0.1 mg/kg-day for a healthy female (top panel) and male (bottom panel). Prediction on left for female (A) obtained by using individually fitted maximum capacity (ng/hr-kg) for active transport of iodide into the thyroid follicular epithelium ( $V_{maxc\_Ti}$ ) of  $1.65 \times 10^5$  and on right (B) by using an average  $V_{maxc\_Ti}$ . Prediction on left for male (C) obtained by using individually fitted  $V_{maxc\_Ti}$  of  $1.2 \times 10^5$  and on right (D) by using an average  $V_{maxc\_Ti}$  of  $1.5 \times 10^5$ . Model of Merrill (2001d) and data of Greer et al. (2000).**



**Figure 6-22. Human PBPK model predictions (lines) versus data (asterisks) for thyroid RAIU (ng/L) on day 14 of perchlorate exposure at 0.02 mg/kg-day for a healthy female (top panel) and male (bottom panel). Prediction on left for female (A) obtained by using individually fitted maximum capacity (ng/hr-kg) for active transport of iodide into the thyroid follicular epithelium ( $V_{maxc\_Ti}$ ) of  $1.4 \times 10^5$  and on right (B) by using an average  $V_{maxc\_Ti}$ . Prediction on left for male (C) obtained by using individually fitted  $V_{maxc\_Ti}$  of  $1.5 \times 10^5$  and on right (D) by using an average  $V_{maxc\_Ti}$  of  $1.5 \times 10^5$ . Model of Merrill (2001d) and data of Greer et al. (2000).**



**Figure 6-23. Human PBPK model predictions (lines) versus data (asterisks) for thyroid RAIU (ng/L) on day 14 of perchlorate exposure at 0.007 mg/kg-day for a healthy female (top panel) and male (bottom panel). Prediction on left for female (A) obtained by using individually fitted maximum capacity (ng/hr-kg) for active transport of iodide into the thyroid follicular epithelium ( $V_{maxc\_Ti}$ ) of  $2.8 \times 10^5$  and on right (B) by using an average  $V_{maxc\_Ti}$ . Prediction on left for male (C) obtained by using individually fitted  $V_{maxc\_Ti}$  of  $1.24 \times 10^5$  and on right (D) by using an average  $V_{maxc\_Ti}$  of  $1.35 \times 10^5$ . Model of Merrill (2001d) and data of Greer et al. (2000).**

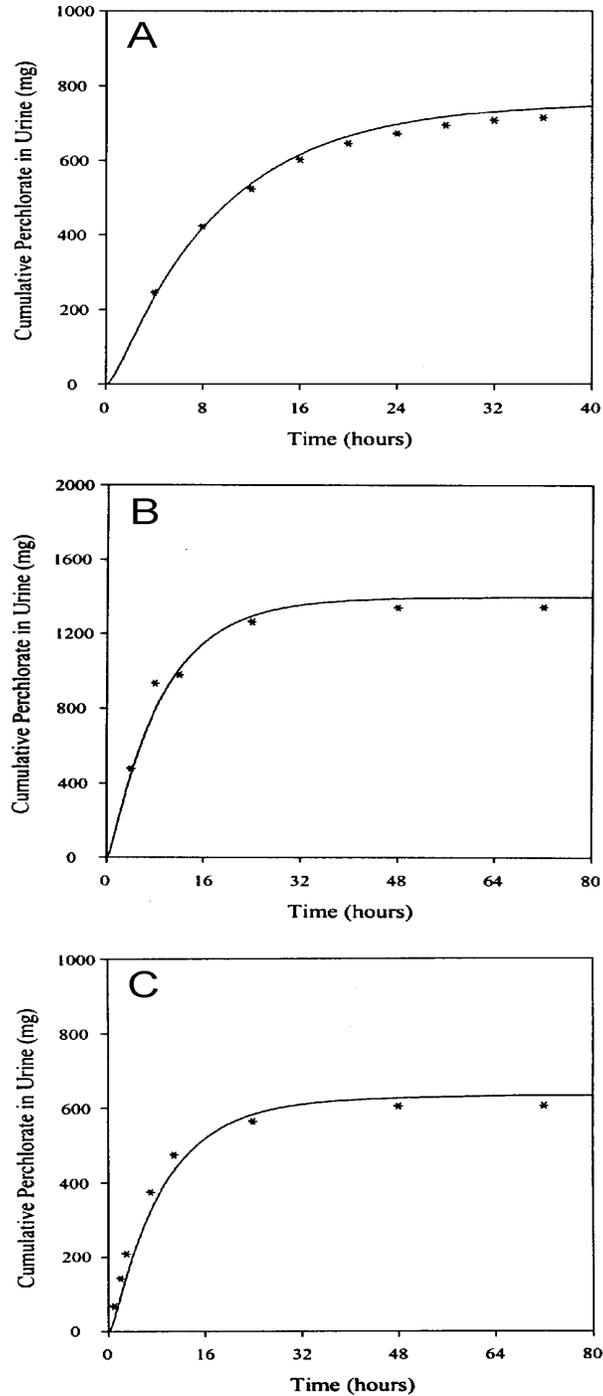
1 radioiodide uptake measurements provided by Greer et al. (2000) across doses (see Merrill,  
2 2001d; Table 3). Merrill (2001d) hypothesized that the large variability in  $V_{maxc\_Ti}$ , ranging  
3 from  $5.0 \times 10^4$  to  $5.0 \times 10^5$  ng/hr-kg, may be attributed to variability in endogenous iodide levels,  
4 as dietary iodide was not controlled. Merrill (2001d) estimated these values from best visual fits  
5 of baseline 8- and 24-hour thyroid RAIU data. Inhibition data restricted to each time point (i.e.,  
6 8- versus 24-hour time points) and from higher dose groups would be useful to test the  
7 robustness of the model to predict inhibition of uptake of iodide in the thyroid.

8 The ability of the human model to predict data from other independent experiments not  
9 used to develop the model is illustrated in Figure 6-24. The model adequately predicts  
10 cumulative perchlorate in urine (mg) reported in three published studies using therapeutic  
11 perchlorate dose levels (Merrill, 2001d). Oral doses administered in these studies were  
12 approximately 9.07 mg/kg (Durand, 1938), 9.56 mg/kg (Kamm and Drescher, 1973), and  
13 20 mg/kg (Eichler, 1929). It is worth noting that the previously determined urinary clearance  
14 value ( $Cl_{Uc\_p}$ ) of 0.126 L/hr-kg was used with all validation data and that an adequate fit was  
15 observed.

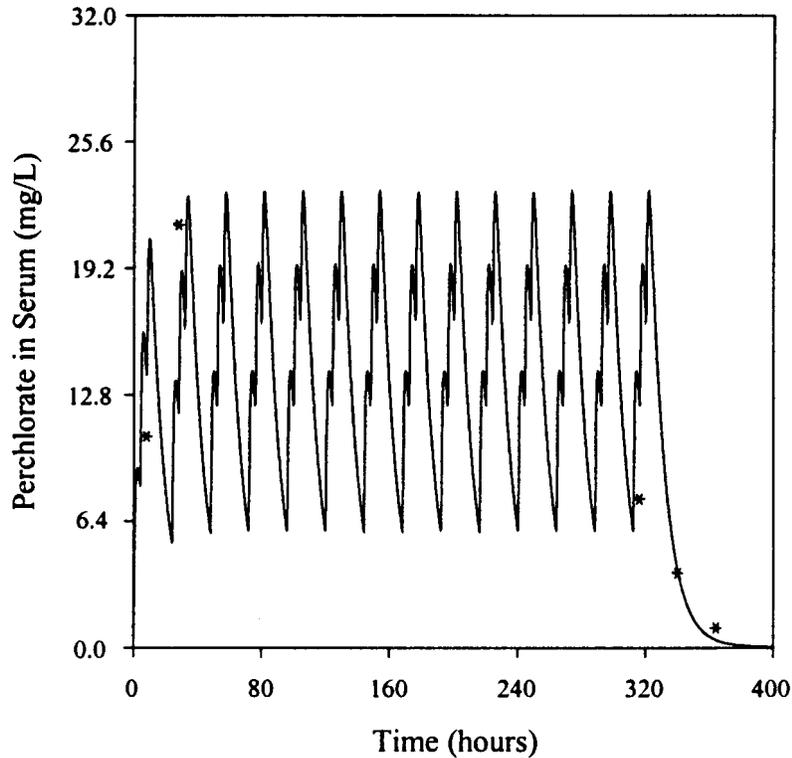
16 The ability of the model to predict cumulative perchlorate in urine from three different  
17 studies at three different doses with the same set of parameters, established from the studies by  
18 Hays and Solomon (1965) and Greer et al. (2000), demonstrates the usefulness of the model and  
19 provides validation for the model structure and the physiological and chemical parameters used.

20 The model also predicts serum perchlorate concentrations at 12 mg/kg-day from an  
21 unpublished study performed by Dr. Georg Brabant at the Medizinische Hochschule, Hanover,  
22 Germany (Figure 6-25). Subjects received 12 mg/kg-day perchlorate in drinking water near meal  
23 times. Variability in the observed serum measurements is believed to reflect variability in the  
24 dosing regimen, as the experimental protocol was less fixed than that used in Greer et al. (2000).  
25 Again the usefulness of the model is demonstrated by its ability to successfully predict serum  
26 concentrations from a dose 24 times higher than the high dose used to establish perchlorate  
27 parameters (0.5 mg/kg-day).

28 The model is also able to successfully predict the thyroidal iodide uptake in a subject from  
29 the Stanbury and Wyngaarden (1952) study with patients with Grave's disease. The maximum  
30 velocity capacity in the follicular epithelium ( $V_{maxc\_Ti}$ ) had to be increased to  $5.0E6$  ng/hr-kg,  
31 a factor of ten times higher than in normal subjects, in order to achieve this fit (upper line in

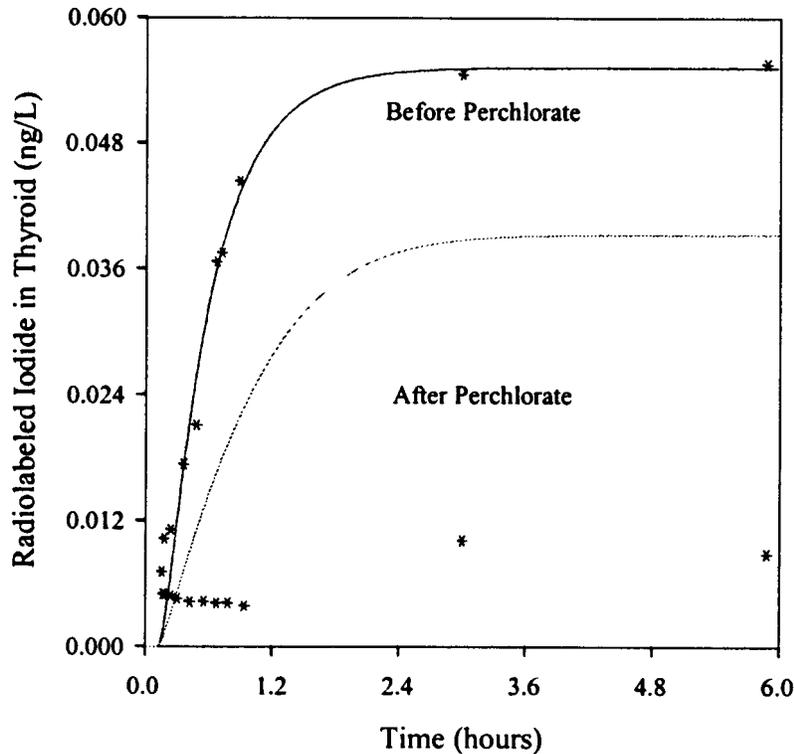


**Figure 6-24. Validation for human PBPK model (Merrill, 2001d). Model predictions (lines) versus data (asterisks) for cumulative perchlorate excretion in urine (mg) in a healthy male after an oral dose of 9.56 mg (A), 20 mg (B) or 9.07 mg (C). Data are from three different studies. Data of Kamm and Drescher (1973) for (A), Eichler (1929) for (B) and Durand (1938) for (C).**



**Figure 6-25. Validation for human PBPK model (Merrill, 2001d). Model predictions (lines) versus data (asterisks) for serum perchlorate concentrations (mg/L) in 5 subjects received 12 mg/kg-day in drinking water (data of Brabant and Letiolf, 2000 as cited in Merrill, 2001d). Subjects were instructed to ingest the solution 3 times/day for 14 days. Serum samples were collected 2 hours after the first dose, after 12 pm on day two, the morning of day 14 and post exposure days 1 and 2. Usefulness of the model is demonstrated by its ability to successfully predict serum concentrations at a dose 24 times higher than the dose used to develop parameters in the model.**

1 Figure 6-26). This increase in  $V_{maxc\_Ti}$  is supported in the literature, as Gluzman and  
 2 Niepomnische (1983) measured elevated  $V_{maxc(s)}$  in thyroid specimens from subjects with  
 3 Grave's disease. However, the model underpredicts the degree of inhibition caused by  
 4 perchlorate in this subject (Figure 6-26, lower line). It would appear that the increased inhibition  
 5 could be attributed to a lower  $K_m$  value. However, Gluzman and Niepomnische (1983) noted  
 6 that the  $K_m$  did not differ greatly between thyroid specimens from hyperthyroid subjects and



**Figure 6-26. Validation for human PBPK model (Merrill, 2001d). Model predictions (lines) versus data (asterisks) for RAIU in the thyroid ( $^{131}\text{I}$  ng/L) of a male with Graves' disease after an iv dose of  $10 \mu\text{Ci } ^{131}\text{I}$  before and after a 100 mg dose of potassium perchlorate. Data of Stanbury and Wyngaarden (1952).**

1 normal subject. This suggests that the increased inhibition by perchlorate seen in Grave's disease  
 2 may be attributed to a mechanism other than NIS affinity (Merrill, 2001d).

#### 4 **6.2.4 Summary**

5 The proposed model structures for the adult male rat (Merrill, 2001c) and adult human  
 6 (Merrill, 2001d) have been shown to adequately describe both perchlorate and iodide disposition  
 7 by demonstrating good correspondence between predicted tissue compartment concentrations  
 8 and measured values in the thyroid, serum, red blood cells, urine, liver, muscle, skin, and  
 9 stomach in the rat and by adequately predicting serum concentrations and cumulative urine after  
 10 drinking water exposure to perchlorate spanning four orders of magnitude (0.02 to 12.0 mg/kg-  
 11 day) in the human. Serum perchlorate levels for human subjects were not available at

1 0.02 mg/kg-day; however, the model did predict the cumulative urine from that dose group  
2 (Figure 6-19).

3 The model structure of the thyroid requires three compartments (stroma, follicle, and  
4 colloid) to quantify rapid organification in the gland. Differences in model parameters between  
5 iodide and perchlorate indicate that iodide kinetics are very similar to perchlorate kinetics, but  
6 cannot be applied directly. The main differences involve the saturable kinetics of the thyroid,  
7 skin, and stomach, with perchlorate exhibiting higher  $V_{max}$ 's except in the skin. Because  
8 organification of iodide occurs in both the thyroid follicle and colloid, their respective  $V_{max}$ 's  
9 are over 1,000 and 10 times higher than those for perchlorate, which is discharged unchanged.  
10 Perchlorate affinity for the symporters into the follicle and colloid were approximately an order  
11 of magnitude greater (lower  $K_m$ ) than those of iodide.

12 The thyroid perchlorate concentrations from high drinking water exposures in the rat were  
13 fitted by increasing the effective follicle:stroma partition coefficient ( $PT_p$ ) to account for TSH  
14 stimulation and upregulation of NIS. Since these values were not supported by additional data,  
15 thyroid concentrations may not be as reliable. Further, the toxic effects of perchlorate are most  
16 likely due to secondary effects on thyroid hormones due to its action at the NIS.

17 The model, however, could simulate serum concentrations from drinking water exposures  
18 using parameters established from the acute data. The thyroid, given its small size, would not be  
19 expected to significantly alter serum concentrations, even during hyperstimulation. Although  
20 TSH has not been shown to increase the NIS in other tissues, NIS-containing tissues were not  
21 obtained from the AFRL/HEST studies to support this.

22 The models support plasma protein binding of perchlorate in both species; a saturable term  
23 is required to simulate serum concentrations at lower doses. It is possible that perchlorate  
24 competes with thyroxine for the same binding sites of plasma proteins, as the work of Yamada  
25 and Jones (1968) suggests. Urinary clearance values of 0.05 L/hr for iodide and 0.07 L/hr for  
26 perchlorate were used across data sets in the rats, and average urinary clearance values were  
27 found to be 0.1 L/hr-kg for iodide and 0.126 L/hr-kg in humans. Excretion constants were  
28 highest among the 0.1 mg/kg-day group. With the urinary excretion rates fitted to cumulative  
29 urine data, the model tends to slightly underestimate serum perchlorate levels at repeated low  
30 doses. Elevated serum concentrations may indicate plasma binding of perchlorate. Yamada and  
31 Jones (1967) studied effects of different anions on plasma binding to thyroxine and noted that

1 some of the thyroxine had been displaced after perchlorate was introduced. Thus, it is possible  
2 that perchlorate competes with thyroxine for the same binding sites of plasma proteins (Merrill,  
3 2001c,d; Clewell, 2001a).

4 While there are limited data suggesting iodide and perchlorate uptake through the skin, the  
5 models and the kinetic studies required this assumption in the models for both rats and humans.  
6 Without the skin compartment, the models overestimated circulating plasma inorganic iodide and  
7 perchlorate in both species. Due to its large size, skin appears to be an important pool for slow  
8 turnover of these anions. Brown-Grant (1961) noted that the uptake of iodide was higher in the  
9 male rat and pup than in the female. The findings of Merrill (2001c) agree, with the rat model  
10 requiring a higher  $V_{max}$  in the skin for the male rat than that reported for the pregnant rat  
11 (Clewell, 2001a) discussed in the next section. Cutaneous uptake of iodide and perchlorate in  
12 mice and rats has been reported (Brown-Grant and Pethes, 1959; Zeghal et al., 1995). The lack  
13 of reported iodide in human skin from clinical radioiodide scans may be due to the difficulty in  
14 differentiating it from background radioactivity.

15 Merrill (2001d) notes that GI clearance of iodide is rapid and plays an important role in  
16 radioiodide conservation. Further, Merrill (2001d) suggests that the appearance of time-course  
17 radioiodine in stomach contents of any species is complicated by the fact that it reflects more  
18 than sequestration of radioiodide by NIS. Its appearance also reflects radioiodide contributed  
19 through the gradual accumulation of iodide in saliva that is swallowed involuntarily throughout  
20 the study. Several studies that examined sequestration of these anions in digestive juices have all  
21 shown high variability in the concentrations measured over time (Honour et al., 1952; Hays and  
22 Solomon, 1965; Merrill, 2001d). There is a tendency for the gastric juice to plasma ratio to be  
23 low when the rate of secretion of juice is high (Honour et al., 1952). Fluctuations in the secretion  
24 rate are probably the most important factor in determining the pattern of the concentration ratios  
25 in individuals. Therefore, variability in stomach or GI tract parameters between models is  
26 expected. However, the early rise in the gastric juice:plasma ratio mentioned earlier is a constant  
27 feature across these data sets, whether or not an attempt was made to eliminate contamination of  
28 gastric juices by dietary contents or saliva. The human model successfully predicted this same  
29 trend.

30 Merrill (2001d) also noted dietary iodine and endogenous inorganic iodide levels to be  
31 clearly important in modeling iodide and perchlorate kinetics, because excessive iodide levels

1 cause the ion to inhibit its own uptake. Plasma inorganic iodide (PII) is rarely reported in the  
2 literature due to analytical difficulties, and it was not available in any of the studies presented in  
3 this paper. While measurements of tracer radioiodide can be fitted to predict transfer rates, its  
4 use is limited when attempting to predict the saturation of nonlinear compartments, such as the  
5 thyroid that are dependent upon the existing amount of iodide already present. Subsequent  
6 modeling efforts on predicting subsequent effects of iodide inhibition on thyroid hormone  
7 synthesis and regulation in humans will require the capability of the model to predict PII.

### 10 **6.3 PREGNANT RAT AND FETAL MODEL STRUCTURE**

11 This section describes the model developed by AFRL/HEST in response to concerns about  
12 interspecies extrapolation of effects due to perchlorate exposure during gestation (Clewell,  
13 2001a). The model predicts the distribution of perchlorate within the pregnant and fetal rat  
14 through gestation and at birth and predicts the short-term effect of acute perchlorate exposure on  
15 iodide kinetics, including iodide uptake into the maternal thyroid. The general model structure  
16 relied on the adult male rat model (Merrill, 2001c) described in Section 6.2 and approaches to  
17 gestational growth of the dam and fetus were based on the work of O’Flaherty et al. (1992) and  
18 Fisher et al. (1989) with weak acids.

19 The model structure is shown in Figure 6-27. Table 6-3 provides the physiological  
20 parameters for the pregnant rat and fetus PBPK models. Table 6-4 provides the perchlorate-  
21 specific parameters, and Table 6-5 provides the iodide-specific parameters for each.

22 The compartments shared with the adult male rat were developed as described in  
23 Section 6.2. The pregnant rat model also includes a mammary gland and placenta compartment.  
24 The mammary gland consists of two subcompartments that represent the capillary bed and the  
25 tissue. The mammary gland has been shown to concentrate both perchlorate and iodide during  
26 lactation. However, the mammary NIS is regulated by hormones produced during lactation and  
27 has been found to increase at the onset of lactation (Tazebay et al., 2000). This concentrating  
28 mechanism does not appear to be as established during pregnancy. Studies reported by Yu  
29 (2000) showed mammary gland:plasma ratios of less than one for perchlorate. However,  
30 mammary gland perchlorate levels are slowly built up and remain high well into the clearance  
31 phase of the serum. This behavior suggested a very slow diffusion between the mammary gland

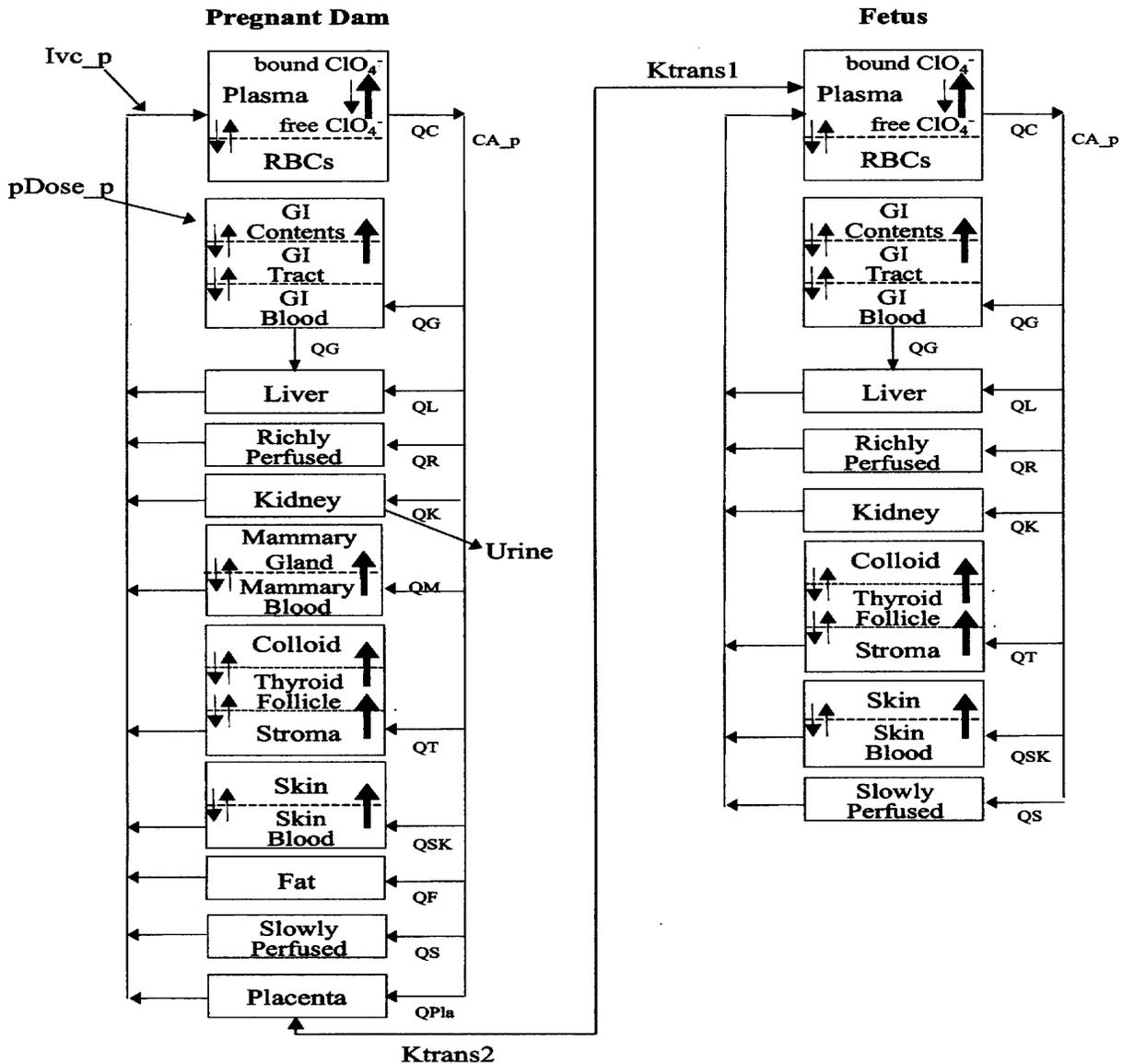


Figure 6-27. Schematic for the pregnant dam and fetal rat PBPK model of perchlorate and iodide distribution (Clewell, 2001a). Bold arrows indicate (except for plasma binding) active uptake at NIS sites into the thyroid, GI contents, and skin. Plasma binding was also described with Michaelis-Menten terms for the association of perchlorate anion to binding sites with first-order clearance rates for dissociation. Small arrows indicate passive diffusion. Boxes represent specific compartments in the model structure. The thyroid consists of the stroma, the follicle, and the colloid; and the stomach consists of the capillary bed, GI wall, and contents. The skin and mammary gland each contain two subcompartments representing the capillary bed and tissue. Permeability area cross products and partition coefficients were used to describe the first-order movement of the perchlorate ( $\text{ClO}_4^-$ ) and iodide ( $\text{I}^-$ ) anions into deeper subcompartments. Placental-fetal transfer and urinary clearance were represented by first order clearance rates.

**TABLE 6-3. PHYSIOLOGICAL PARAMETERS FOR THE PREGNANT RAT AND FETUS PBPK MODEL (Clewell, 2001a)**

Physiological Parameters	Pregnancy		Source
	Dam	Fetus	
<b>Tissue Volumes (%BW)</b>			
Body Weight <i>BW</i> and <i>VIfet</i> (kg)	0.280 - 0.361	0.0 - .0045	O'Flaherty et al., 1992
Slowly Perfused <i>VSc</i> (%BW)	74.6	74.6	Brown et al., 1997
Richly Perfused <i>VRc</i> (%BW)	11	11	Brown et al., 1997
Fat <i>VFc</i> (%BW)	10.0 - 11.0	0.0	Naismith et al., 1982
Kidney <i>VKc</i> (%BW)	1.7	1.7	Brown et al., 1997
Liver <i>VLc</i> (%BW)	3.4	3.4	Brown et al., 1997
GI Tract <i>VGc</i> (%BW)	3.60	3.60	Brown et al., 1997
GI Contents <i>VGJc</i> (%BW)	7.20	7.20	Yu et al., 2000
GI Blood <i>VGBC</i> (%VG)	2.9	2.9	Altman and Dittmer, 1971
Skin Tissue <i>VSkc</i> (%BW)	19.0	19.0	Brown et al., 1997
Skin Blood <i>VSkBc</i> (%VSk)	2.0	2.0	Brown et al., 1997
Thyroid Total <i>VTtote</i> (%BW)	0.0105	0.0234	Malendowicz and Bednarek, 1986; Florsheim et al., 1966
Thyroid Follicle <i>VTc</i> (%BW)	45.9	61.4	Malendowicz and Bednarek, 1986; Conde et al., 1991
Thyroid Colloid <i>VDTc</i> (%BW)	45	18.3	Malendowicz and Bednarek, 1986; Conde et al., 1991
Thyroid Blood <i>VTBc</i> (%VT)	9.1	20.3	Malendowicz and Bednarek, 1986; Conde et al., 1991
Plasma <i>VPlasc</i> (%BW)	4.7	4.7	Brown et al., 1997; Altman and Dittmer, 1971
Red Blood Cells <i>VRBCc</i> (%BW)	2.74	2.74	Brown et al., 1997; Altman and Dittmer, 1971
Placenta <i>VPlac</i> (%BW)	0.0 - 2.57	—	O'Flaherty et al., 1992
Mammary Tissue <i>VMc</i> (%BW)	1.0 - 5.5	—	Knight et al., 1984; O'Flaherty et al., 1992
<b>Blood Flows (%QC)</b>			
Cardiac Output <i>QCc</i> (L/hr-kg)	14	14.0	Buelke-Sam, 1982a & b; O'Flaherty et al., 1992
Slowly Perfused <i>QSc</i> (%QC)	24.0	24.0	Brown et al., 1997
Richly Perfused <i>QRc</i> (%QC)	76.0	76.0	Brown et al., 1997
Fat <i>QFc</i> (%QC)	7 - 8.1	0.0	Brown et al., 1997
Kidney <i>QKc</i> (%QC)	14.0	14.0	Brown et al., 1997
Liver <i>QLc</i> (%QC)	18.0	18.0	Brown et al., 1997
GI <i>QGc</i> (%QC)	13.60	13.60	Brown et al., 1997
Thyroid <i>QTc</i> (%QC)	1.6	1.6	Brown et al., 1997
Mammary <i>QMc</i> (%QC)	0.2 - 1.2	—	Hanwell and Linzell, 1973
Placenta <i>QPlc</i> (%QC)	0.0 - 12.3	—	O'Flaherty et al., 1992

**TABLE 6-4. PERCHLORATE-SPECIFIC PARAMETERS FOR THE PREGNANT RAT AND FETUS PBPK MODEL (Clewell, 2001a)<sup>a</sup>**

Pregnancy Parameters	Perchlorate Values			
	Partition Coefficients (unitless)	Dam	Fetus	Source
Slowly Perfused/Plasma PS_	0.31	0.31	0.31	Yu et al., 2000
Rapidly Perfused/Plasma PR_	0.56	0.56	0.56	Yu et al., 2000
Fat/Plasma PF_	0.05	—	—	Pena et al., 1976
Kidney/Plasma PK_	0.99	0.99	0.99	Yu et al., 2000
Liver/Plasma PL_	0.56	0.56	0.56	Yu et al., 2000
Gastric Tissue/Gastric Blood PG_	0.50	1.80	1.80	Yu et al., 2000
GI Contents/GI Tissue PGJ_	1.30	2.30	2.30	Yu, 2000
Skin Tissue/Skin Blood PSk_	1.15	1.15	1.15	Yu, 2000
Thyroid Tissue/Thyroid Blood PT_	0.13 / 2.25	0.13 / 2.25	0.13 / 2.25	Chow and Woodbury, 1970 <sup>b</sup>
Thyroid Lumen/Thyroid Tissue PDT_	7.00	7.00	7.00	Chow and Woodbury, 1970
Red Blood Cells/Plasma	0.73	0.73	0.73	Yu et al., 2000
Placenta/Plasma PPL_	0.56	—	—	Assume same as richly perfused
Mammary/Plasma PMam_p	0.66	—	—	Anbar et al., 1959
<b>Max Capacity, Vmaxc (ng/hr-kg)</b>				
Thyroid Follicle Vmaxc_T	1.80E+03	1.80E+03	1.80E+03	Fitted <sup>c</sup>
Thyroid Colloid Vmaxc_DT	1.00E+04	1.00E+04	1.00E+04	Fitted <sup>c</sup>
Skin Vmaxc_S	6.00E+05	4.00E+05	4.00E+05	Fitted
Gut Vmaxc_G	8.00E+05	8.00E+05	8.00E+05	Fitted
Mammary Gland Vmaxc_M	3.90E+04	---	---	Molar equivalent to Vmaxc_Mi
Plasma Binding Vmaxc_Bp	5.00E+03	1.50E+03	1.50E+03	Fitted
<b>Affinity Constants, Km (mg/L)</b>				
Thyroid Follicle Km_T	1.00E+05	1.00E+05	1.00E+05	Wolff, 1998
Thyroid Colloid Km_DT	1.00E+08	1.00E+08	1.00E+08	Golstein et al., 1992; Wolff, 1998
Skin Km_S	1.00E+05	1.00E+05	1.00E+05	Wolff, 1998
Gut Km_G	1.00E+05	1.00E+05	1.00E+05	Wolff, 1998
Mammary Gland	1.00E+5	—	—	Wolff, 1998
Plasma Binding Km_Bp	1.00E+05	1.00E+05	1.00E+05	Fitted
<b>Permeability Area Cross Products, (L/hr-kg)</b>				
GI Blood to GI Tissue PAGc_	1.00	1.00	1.00	Fitted
GI Tissue to GI Contents PAGJc_	1.00	1.00	1.00	Fitted
Thyroid Blood to Thyroid Tissue PATc_	4.0E-5 / 6.0E-4	4.0E-5 / 6.0E-4	4.0E-5 / 6.0E-4	Fitted <sup>b</sup>
Thyroid Tissue to Thyroid Lumen PADTc_	0.01	0.01	0.01	Fitted
Skin Blood to Skin Tissue PASKc_	1.00	1.00	1.00	Fitted
Plasma to Red Blood Cells PRBCc_	1.00	1.00	1.00	Fitted
<b>Clearance Values, (L/hr-kg)</b>				
Urinary Excretion CLUc_	0.07	—	—	Yu et al., 2000
Transfer from Placenta to Fetus Cltrans1c_	0.10	0.10	0.10	Yu, 2000
Transfer from Fetus to Placenta Cltrans2c_	0.19	0.19	0.19	Yu, 2000
Dissociation from Plasma Binding Sites Clunbc_p	0.034	0.010	0.010	Yu, 2000

<sup>a</sup>All parameters listed are notated in the model by either an *i* (for iodide) or *p* (for perchlorate) following an underscore in the parameter name (e.g., PR\_*i*, PR\_*p*, Vmaxc\_*Ti*, etc.)

<sup>b</sup>Parameters with two values indicate acute and drinking water parameters, respectively.

<sup>c</sup>Fetus was given maternal values for Vmax (scaled by fetal body weight) in the absence of data.

**TABLE 6-5. IODIDE-SPECIFIC PARAMETERS FOR THE PREGNANT RAT AND FETUS PBPK MODEL (Clewell, 2001a)<sup>a</sup>**

<b>Pregnancy Parameters</b>	<b>Iodide Values</b>			
	<b>Partition Coefficients (unitless)</b>	<b>Dam</b>	<b>Fetus</b>	<b>Iodide Source</b>
Slowly Perfused/Plasma PS_	0.21	0.21		Halmi et al., 1956
Rapidly Perfused/Plasma PR_	0.40	0.40		Halmi et al., 1956
Fat/Plasma PF_	0.05	—		Pena et al., 1976
Kidney/Plasma PK_	1.09	1.09		Yu et al., 2000
Liver/Plasma PL_	0.44	0.44		Yu et al., 2000
GI Tissue/GI Blood PG_	1.0	1.0		Yu, 2000
GI Contents/GI Tissue PGJ_	2.0	2.0		Yu, 2000
Skin Tissue/Skin Blood PSk_	0.70	0.70		Perlman et al., 1941
Thyroid Tissue/Thyroid Blood PT_	0.15	0.15		Chow and Woodbury, 1970
Thyroid Lumen/Thyroid Tissue PDT_	7.00	7.00		Chow and Woodbury, 1970
Red Blood Cells/Plasma	1.00	1.00		Yu et al., 2000
Placenta/Plasma PPL_	0.99	—		Unpublished GD20 data
Mammary/Plasma PMam_p	0.66	—		Anbar et al., 1959 (for ClO <sub>4</sub> <sup>-</sup> )
<b>Max Capacity, Vmaxc (ng/hr-kg)</b>				
Thyroid Follicle Vmaxc_T	4.00E+04	0.0 – 7.5E+04	Fitted	
Thyroid Colloid Vmaxc_DT	6.00E+07	6.00E+07	Fitted	
Skin Vmaxc_S	6.00E+04	3.00E+05	Fitted	
Gut Vmaxc_G	1.00E+06	2.00E+05	Fitted	
Mammary Gland Vmaxc_M	5.00E+04	—	Fitted	
<b>Affinity Constants, Km (mg/L)</b>				
Thyroid Follicle Km_T	4.00E+06	4.00E+06		Gluzman and Niepomniscze, 1983
Thyroid Colloid Km_DT	1.00E+09	1.00E+09		Golstein et al., 1992
Skin Km_S	4.00E+06	4.00E+06		Gluzman and Niepomniscze, 1983
Gut Km_G	4.00E+06	4.00E+06		Gluzman and Niepomniscze, 1983
Mammary Gland Km_M	4.00E+06	—		Gluzman and Niepomniscze, 1983
<b>Permeability Area Cross Products, (L/hr-kg)</b>				
GI Blood to GI Tissue PAGc_	0.80	0.10	Fitted	
GI Tissue to GI Contents PAGJc_	0.60	0.30	Fitted	
Thyroid Blood to Thyroid Tissue PATc_	1.000E-04	1.000E-04	Fitted	
Thyroid Tissue to Thyroid Lumen PADTc_	1.00E-04	1.00E -04	Fitted	
Skin Blood to Skin Tissue PASkc_	0.10	0.02	Fitted	
Plasma to Red Blood Cells PRBCc_	1.00	1.00	Fitted	
<b>Clearance Values, (L/hr-kg)</b>				
Urinary excretion CLUc_	0.03	—	Fitted	
Transfer from Placenta to Fetus Cltrans1c_	0.06	0.06		Unpublished GD 20 Iodide iv Data
Transfer from Fetus to Placenta Cltrans2c_	0.12	0.12		Unpublished GD 20 Iodide iv Data

<sup>a</sup>All parameters listed are notated in the model by either an *i* (for iodide) or *p* (for perchlorate) following an underscore in the parameter name (e.g., PR<sub>*i*</sub>, PR<sub>*p*</sub>, Vmaxc<sub>*Ti*</sub>, etc.)

1 and blood, so that Clewell (2001a) described the two-subpartment mammary gland with both  
2 diffusion of iodide and active uptake by the NIS.

3 Although it has been suggested that the placenta may contain the capability for active  
4 uptake in the rat, AFRL/HEST data did not indicate placenta:plasma levels greater than one for  
5 perchlorate or iodide (Yu, 2000), and unpublished iodide time course data indicate that the  
6 behavior of iodide in the placenta closely mirrors that of the plasma (Clewell, 2001a). Thus, the  
7 placenta was simulated with a single, flow-limited compartment.

8 Partitioning into the mammary, placenta, and other diffusion-limited compartments was  
9 based on effective partitioning. This effective partitioning is probably very similar to that in the  
10 thyroid where an electrochemical gradient is responsible for allowing the  $\text{ClO}_4^-$  anion to move  
11 between the serum and the tissue (Chow and Woodbury, 1970). Urinary clearance and placental-  
12 fetal transfer of the anions were represented by first order clearance rates.

13 The structure of the fetal perchlorate model is similar to that of the pregnant rat, with the  
14 exception of the mammary gland and placenta compartments. In order to simplify the model, all  
15 of the fetuses from a single litter were combined in the structure of the model, essentially  
16 viewing the individual fetuses as one entity, or one large fetus. The dose to the fetus is based on  
17 the transfer of perchlorate from the maternal placenta to the serum of the fetus, rather than  
18 through direct exposure to the drinking water. Though a kidney is included in the fetal model,  
19 urinary excretion is not used to identify the loss of perchlorate for the fetus. Since the ability to  
20 produce urine is not well developed until after parturition, the loss from the fetus is described as  
21 first order clearance from the fetal arterial blood to the placenta (Clewell, 2001a).

22 The pregnancy model attempts to describe perchlorate distribution in a highly dynamic  
23 system. In addition to total body weight changes in the dam and fetus, maternal mammary tissue  
24 and blood flow, cardiac output, fractional body fat, placenta and fetus body weight, and fractional  
25 body fat are also changing with respect to time. Growth equations, based on O'Flaherty et al.  
26 (1992) were used to account for these changes (Clewell, 2001a). All tissue volume and blood  
27 flow values were adjusted with respect to the changing parameters.

### 28 29 **6.3.1 Data and Methods**

30 This section summarizes the data that Clewell (2001a) used for development and validation  
31 of the pregnant and fetal rat model structures. Details on experimental methods, including:

1 protocol design, exposure regimen, chemical source and purity, animals (housing, feeding,  
2 surgical procedures, etc.), and the analytical methods can be found in the consultative letter and  
3 associated reports from AFRL/HEST or cited papers therein.

#### 4 5 **6.3.1.1 AFRL/HEST Experiments in Laboratory Rats**

6 These studies are described in the consultative letters and reports of Clewell (2001a),  
7 Yu (2000, 2001, 2002) and Yu et al. (2000).

##### 8 9 **6.3.1.1.1 Drinking Water Study**

10 Perchlorate drinking water experiments used in model development were performed at  
11 AFRL/HEST and described in detail in the report Yu (2000). Pregnant dams of the Sprague-  
12 Dawley strain were exposed to drinking water treated with perchlorate from gestational day (GD)  
13 2 through 20, at perchlorate doses of 0.0, 0.01, 0.1, 1.0 and 10.0 mg/kg-day. GD0 was  
14 determined by the presence of a vaginal plug. Both dams and fetuses were sacrificed on GD20  
15 and maternal and fetal serum analyzed for free and total thyroxine (fT4 and tT4), triiodothyronine  
16 (T3), and TSH. Maternal serum, thyroid, skin, GI contents, placenta, and amniotic fluid were  
17 analyzed for perchlorate at all of the above doses. Fetal serum, skin and GI tract were also  
18 analyzed for perchlorate at all of the doses. Two hours before sacrifice, the dams were given iv  
19 doses of 33 mg/kg radiolabeled iodide ( $^{125}\text{I}$ ) with carrier. Tissue concentrations of iodide were  
20 measured in order to determine the inhibition in the various tissues after long-term exposure to  
21 perchlorate.

##### 22 23 **6.3.1.1.2 Preliminary Iodide Kinetics Study**

24 A preliminary study of radiolabeled ( $^{125}\text{I}$ ) kinetics was performed by AFRL/HEST in which  
25 timed-pregnant dams of the Sprague-Dawley strain were exposed via tail-vein injection to a  
26 tracer dose (average dose = 2.19 ng/kg body weight) of the radiolabeled anion on GD20. Dams  
27 (n=6) were sacrificed at 0.5, 2, 4, and 8 hours post-dosing. Maternal serum, thyroids, skin, GI  
28 contents, placenta and mammary gland tissue, as well as fetal serum, skin, and GI tract were  
29 collected and analyzed for iodide content at each time point. Serum was pooled for all fetuses  
30 within a litter, due to limited sample volume. Fetal skin and GI tract were analyzed individually.

### 6.3.1.1.3 Iodide Inhibition Kinetics Study

A more in-depth study was performed by AFRL/HEST, in which Sprague-Dawley timed-pregnant dams were given 1.0 mg/kg body weight perchlorate via tail-vein injection on GD20; control rats were given saline. The perchlorate or saline dose was followed two hours post dosing with a tail-vein injection of carrier free  $^{125}\text{I}$  at an average dose of 1.87 ng/kg BW. Dams (n=6) were sacrificed after 0.5, 1, 2, 4, 8, 12, and 24 hours. Maternal serum, thyroids, skin, GI contents, placenta, mammary gland tissue, and fetal serum, skin, and GI tract were collected and analyzed for iodide content at each time point. Serum was again pooled for all fetuses within a litter. Fetal skin and GI tract were analyzed individually. At this time, only the maternal serum, maternal thyroids and fetal serum from this study were available for use with the model. Clewell (2001a) states that further validation of the model structure will be performed at a later time with the remaining data, but no further work has been provided to the EPA. Additional data were provided by Yu (2002).

### 6.3.1.2 Data Published in the Literature

Data available in the literature used in a validation exercise of the model are described briefly in this section.

#### 6.3.1.2.1 Versloot et al., 1997

Versloot and coauthors measured  $^{125}\text{I}$  as percent of dose in maternal and fetal thyroid, mammary gland, placenta, and fetal carcass without the thyroid. Pregnant Wistar rats (body weight [BW] =  $300 \pm 5$  g) were given an injection of 10  $\mu\text{Ci}$  carrier free  $^{125}\text{I}$  into the right vena jugularis on GD19. Measurements of the maternal thyroid were taken at 4 and 24 hours post dosing. Mammary gland, placenta, fetal thyroid, and fetal carcass minus the thyroid were taken only 24 hours post dosing.

#### 6.3.1.2.2 Sztanyik and Turai 1988

Sztanyik and Turai measured the uptake of iodide into the placenta and fetal whole body 24 hours post dosing. Five groups of CFY albino rats (BW = 200 to 250 g) were dosed ip with 370 kBq (0.081 ng) carrier free radiolabeled iodide ( $^{131}\text{I}$ ) on GDs 17, 18, 19, 20, and 22. Although this is a different strain of rat, the GD20 fetal weights (average BW = 4.088 g) compare

1 favorably with those seen on GD20 in the Sprague-Dawley fetus. As a result, Clewell (2001a)  
2 used the GD20 time point as a means of validating GD20 parameters for iodide across different  
3 data sets and doses. Placental and whole body fetal  $^{131}\text{I}$  were measured in a well-type  
4 scintillation detector.

#### 6.3.1.2.3 Feldman et al., 1961

7 Feldman and coauthors measured the uptake of iodide into the fetal thyroid and rest of body  
8 carcass on GDs 16, 17, 18, and 19 in pregnant female Holtzman rats. A single subcutaneous  
9 injection was given to the dam, containing 50  $\mu\text{Ci}$  of  $^{131}\text{I}$  on each of the days mentioned above.  
10 Fetal thyroid and carcasses were measured at 24 hours post dosing.

### 6.3.2 Pregnant Rat and Fetus Model Development

13 This section summarizes only the key features that were different than the adult male rat  
14 model previously described in Section 6.2.

#### 6.3.2.1 Physiological Parameters and Tissue Partition Coefficients

17 Maternal parameters were scaled allometrically based on body weight as previously  
18 described for the male rat. Fetal values were scaled in the same manner as the maternal  
19 parameters. However, since the model actually represents several fetuses, it was necessary to  
20 first scale the values for the individual fetus and then adjust for the total number of fetuses in the  
21 litter (Clewell, 2001a).

22 Clewell (2001a) based the physiological description of the maternal and fetal rat during  
23 gestation on O'Flaherty et al. (1992). However, growth descriptions, body weights, and organ  
24 descriptions were optimized for use within this particular model structure. The model is able to  
25 account for differences in gestation time, pup birth weight, and litter size between experiments  
26 and strains of rats. Growth equations and parameters that change over time were described with  
27 mathematical descriptions of available literature and experimental data. Details and equations  
28 are provided in the consultative letter (Clewell, 2001a).

### 6.3.2.1.1 Maternal Tissues

The body weight of the dam is known to change significantly throughout the relatively short gestation time in the rat (21 days). However, the traditional approach utilizing allometric scaling to describe tissue growth in relation to the change in body weight is not a sufficient description for the changes taking place during pregnancy. As opposed to the typical growth scenario, organs and tissues cannot be assumed to increase at the same rate in this dynamic system (Clewell, 2001a). The placenta, fetal volume, and mammary tissue grow at an accelerated rate in comparison to the other organs. These require additional descriptions for their growth beyond the previously described allometric scaling by body weight.

Since the growth of the other tissues is negligible in comparison to the change in the placenta, mammary gland, fat and fetal volume, Clewell (2001a) described the total change in the maternal body weight as simply the change in these four volumes added to the initial (pre-pregnancy) body weight ( $BW_{ini}$ ). All other maternal organs were assumed to remain constant and were scaled allometrically relative to the initial body weight (see Table 6-5).

Mammary tissue growth during gestation was described by Knight and Peaker (1982). Based on this work, Clewell (2001a) described mammary tissue growth as a linear process during which the mammary gland reaches a maximum volume for gestation on GD21 of 4.6% of the maternal body weight.

Clewell (2001a) also described the growth of maternal fat as a linear process throughout gestation based on the work of Naismith et al. (1982). Naismith reported a 40% increase in body fat throughout gestation. Thus, in the model a linear equation was employed to describe a 40% increase in body fat during the length of gestation with an initial (non-pregnant) value of 7.0% body weight for Sprague-Dawley rats (Brown et al., 1997).

Placental volume was described in the model as a sum of three stages of growth, based on the data of Buelke-Sam et al. (1982a), Sikov and Thomas (1970), and the mathematical description of data provided in O'Flaherty et al. (1992). The placenta volume is negligible during gestational days 1 through 5. Individual yolk sac placenta enter a stage of rapid growth between days 6 and 10 of gestation, and was described by an equation that accounted for yolk sac placenta, the total volume of the placenta during this time period, and the number of fetuses present. Placental growth during gestational days 6 through 10 is defined solely by this equation. Total placenta volume changes during gestational days 10 through 21 (parturition) were defined

1 by two separate processes: the exponential decline in yolk sac volume and the increase in  
2 chorioallantoic placenta (Clewell, 2001a).

3 O’Flaherty et al. (1992) also described the growth of the uterus and liver during gestation.  
4 However, Clewell (2001a) did not include a specific description of growth in these organs  
5 because the liver is not believed to have a major role in perchlorate kinetics. Further, because the  
6 iodide model does not describe deiodination, the description of liver growth was deemed  
7 unnecessary. The use of a uterine compartment was also not included in the Clewell (2001a)  
8 model due to the lack of available perchlorate and iodide data. The uterus was considered to be  
9 part of the lumped richly perfused tissue. EPA agrees that adding a description of liver growth  
10 would only bring additional complexity to the model structure without providing a real benefit to  
11 the description of perchlorate and total iodide kinetics and that the uterine compartment would be  
12 purely hypothetical and could not be validated without pertinent data.

#### 14 **6.3.2.1.2 Maternal Blood Flow**

15 Clewell (2001a) described temporal changes in maternal cardiac output during gestation as  
16 the sum of the initial cardiac output, given in Brown et al. (1997) for a non-pregnant rat, and the  
17 change in blood flow to the placenta, mammary, and fat tissues. The approach of O’Flaherty  
18 et al. (1992) to changing blood flows was utilized in placental, mammary, and fat blood flows.  
19 The fraction of cardiac output to the mammary gland and fat tissues are described as proportional  
20 to the change in volume of the tissue. The change in blood flow to the yolk sac placenta is  
21 approximately proportional to the change in volume of the yolk sac. However, the blood flow to  
22 the chorioallantoic placenta increases at a faster rate than the change in volume, so three different  
23 equations were used to describe the blood flow for each different stage of placental growth (GD1  
24 to GD6, GD7 to GD10, GD11 to GD12, and GD13 to GD21).

#### 26 **6.3.2.1.3 Fetal Tissues**

27 A three stage description of fetal growth was also described in O’Flaherty et al. (1992) in  
28 order to mathematically reproduce data obtained from Beaton et al. (1954), Sikov and Thomas  
29 (1970), Goedbloed (1972), and Buelke-Sam et al. (1982a). Because data are not available for  
30 fetal volume between gestational days 1 through 11, an exponential growth curve was used as a  
31 reasonable approximation of fetal growth and was fit to the first available data for fetal volume

1 (Clewell, 2001a). The second stage of growth describes a slower increase in fetal volume,  
2 beginning on GD11, based on the same data. Clewell (2001a) described the third stage of fetal  
3 growth as a linear increase between days 18 and the day of parturition. The equation is  
4 dependent on the weight of the pup at the time of birth so that the model can account for the  
5 differences in birth weight encountered when simulating different data sets.

6 Individual fetal organ weights were assumed to increase linearly with respect to change in  
7 fetal body weight and were therefore scaled allometrically to account for changes in tissue  
8 volumes. Values for tissue volumes were taken from the literature and from experimental data  
9 for the fetus when available. However, most volumes were taken from adult rat data and scaled  
10 allometrically for the fetus due to the lack of tissue data in fetuses.

11 Florsheim et al. (1966) measured thyroid and body weight of the rat fetus and pup from  
12 GD18 through PND22 and reported a linear relationship between the thyroid weight and body  
13 weight throughout the time period. The value given for the thyroid of the fetus in %fetal body  
14 weight for GD19 was used in the Clewell (2001a) model. On the other hand, the physiology of  
15 the developing thyroid was found by Conde et al. (1991) to change significantly between birth  
16 and PND120. Conde reported a decrease in follicle volume from 61.4% to 37.2% of the total  
17 volume of the thyroid from birth to 120 days. An increase in colloid volume from 18.3% of the  
18 total thyroid volume at birth to 32.5% at 120 days was also reported. In the absence of  
19 histometric data in the fetal thyroid, the follicle, colloid, and stroma volumes for the fetus were  
20 described using the thyroid fractions measured at birth. The value for thyroid stroma was  
21 calculated within the model by subtracting the colloid and follicle volumes from the total thyroid  
22 volume.

23 The fetal body fat content was assumed to be zero in the Clewell (2001a) model. This  
24 assumption is reasonable in light of the data given in Naismith et al. (1982). Naismith et al.  
25 (1982) measured values for the body fat of PND2 and 16 rat pups, corresponding to 0.16% and  
26 3.7% of the body weight. Given that body fat quickly increases in the neonatal period, it is not  
27 unreasonable to assume that body fat in the fetus is negligible. The volume is certainly not large  
28 enough to interfere with iodide or perchlorate kinetics. All other parameters were scaled  
29 allometrically by fetal weight from the adult male rat. The male rat physiological parameters  
30 were used rather than female parameters for several reasons. First, the male rat pups have been  
31 shown to be more sensitive to perturbation of hormone homeostasis by perchlorate, and therefore

1 are considered the sensitive endpoint (Yu, 2000). Additionally, Clewell (2001a) asserts that  
2 sufficient evidence was not found to indicate that physiological parameters between male and  
3 female rats were present in the fetus.

#### 4 5 **6.3.2.1.4 Fetal Blood Flow**

6 Fetal blood flow was assumed to operate independently from the mother. The transfer of  
7 the chemical was accomplished via diffusion between the placenta and fetal blood. Therefore,  
8 the fetal cardiac output and blood flow to organs (as % cardiac output) were scaled allometrically  
9 from the male rat values relative to the fetal volume.

#### 10 11 **6.2.2.2 Chemical-Specific Parameters**

12 The various active transport processes, tissue permeabilities, and clearance rates (excretion)  
13 are described in PBPK models for each species on a chemical-specific basis. This section  
14 outlines how the values for perchlorate and iodide used in the pregnant and fetal rat model were  
15 derived. The values can be found in Tables 6-4 and 6-5; details on the derivation can be found in  
16 Clewell (2001a).

#### 17 18 **6.3.2.2.1 Affinity Constants and Maximum Velocities for Active Uptake Processes**

19 These were developed as described previously for the adult male rat model (Merrill, 2001c)  
20 in Section 6.2. The chemical specific parameters were kept the same in male, female, neonatal  
21 and fetal rats, and humans whenever possible. However, it was necessary to change a few of the  
22 parameters, including the maximum velocities ( $V_{maxc}$ 's) in the Clewell (2001a) model for  
23 pregnant rat and fetus. The  $K_m$  values were similar between tissues and between female and  
24 male rat and human models. However, the maximum velocity or capacity differs between tissues  
25 (Wolff and Maurey, 1961). Since  $V_{maxc}$  values for perchlorate were not given in literature, the  
26 values were estimated with the model. In order to determine  $V_{maxc}$  using the model, the  
27 simulation for the tissue of interest was compared to various data sets with several different  
28 perchlorate dose levels. The value for  $V_{maxc}$  within a given compartment was then determined  
29 by the best fit of the simulation to the data.

#### 6.3.2.2.2 Effective Partitioning Permeability Area Cross Products and Clearance Values

These were developed as described previously for the adult male rat model (Merrill, 2001c) in Section 6.2. The value of 0.05 was used to represent the partitioning of perchlorate into the fat for the pregnant rat and fetus (Clewel, 2001a). This value was based on the data of Pena et al. (1976) who measured tissue:blood ratios in the laying hen after intra-muscular dosing with either a single injection of 10  $\mu$ Ci or 3 sequential doses of 10  $\mu$ Ci radiolabeled perchlorate. Although the hen is a very different species, several other tissues were reported to have values comparable to those found by Yu (2000) and Yu et al. (2000) in the male and female rat (0.3 vs. 0.31 in muscle, 0.1 vs. 0.1 in brain, 0.8 vs. 0.99 in the kidney). Clewel (2001a) noted that the use of this value is supported by the fact that the polarity of the perchlorate anion would severely limit the movement of perchlorate into fatty lipophilic tissue. Anbar et al. (1959) measured the mammary gland:blood ratios in the rat four hours after ip injection of radiolabeled perchlorate (100 mg  $\text{KClO}_4$ ), and they reported an effective partition of 0.66 for the rat mammary gland. This value is in general agreement with that chosen by Clewel (2001a).

Maternal and fetal skin were described using the value Perlman et al. (1941) determined after a sc tracer dose of iodide for the partition coefficient in this compartment. Iodide partition coefficients were calculated from the tissue:blood ratios measured during the clearance phase of iodide data in the tissue of interest. The preliminary iodide kinetics study described in the supporting experiments was utilized for the determination of the placenta partition coefficients. For example, values for the GI tract and its contents were determined from the clearance portion of the iodide kinetic study in the adult male rat (Yu et al., 2000).

For all tissues in which a clearance was described (urinary clearance, transfer between placenta and fetal serum, and dissociation of perchlorate from the binding sites), a clearance value was determined. Since perchlorate is quickly excreted in urine and binding has little effect on serum levels at high doses, the simulation for the 10 mg/kg-day dose group was primarily dependent on the urinary clearance value ( $\text{CIUc}_p$ ). The urinary clearance value for perchlorate was therefore based on the fit of the model to the serum data at the high dose. Iodide is incorporated into many of the constituents in plasma. However, it is not bound to the plasma proteins (i.e., albumin) in the same manner as perchlorate. Additionally, the iodide model is currently simplified to account for the distribution of total iodine. Therefore, the urinary clearance value ( $\text{CIUc}_i$ ) was determined primarily by fitting the model simulation to the iodide

1 serum data, as blood levels were more dependent on excretion than on the amount of iodide in  
2 other tissues. The clearance of both iodide and perchlorate between the fetal serum and maternal  
3 placenta were based on the fit of the model simulation to the fetal and maternal blood levels and  
4 to the placenta concentration.

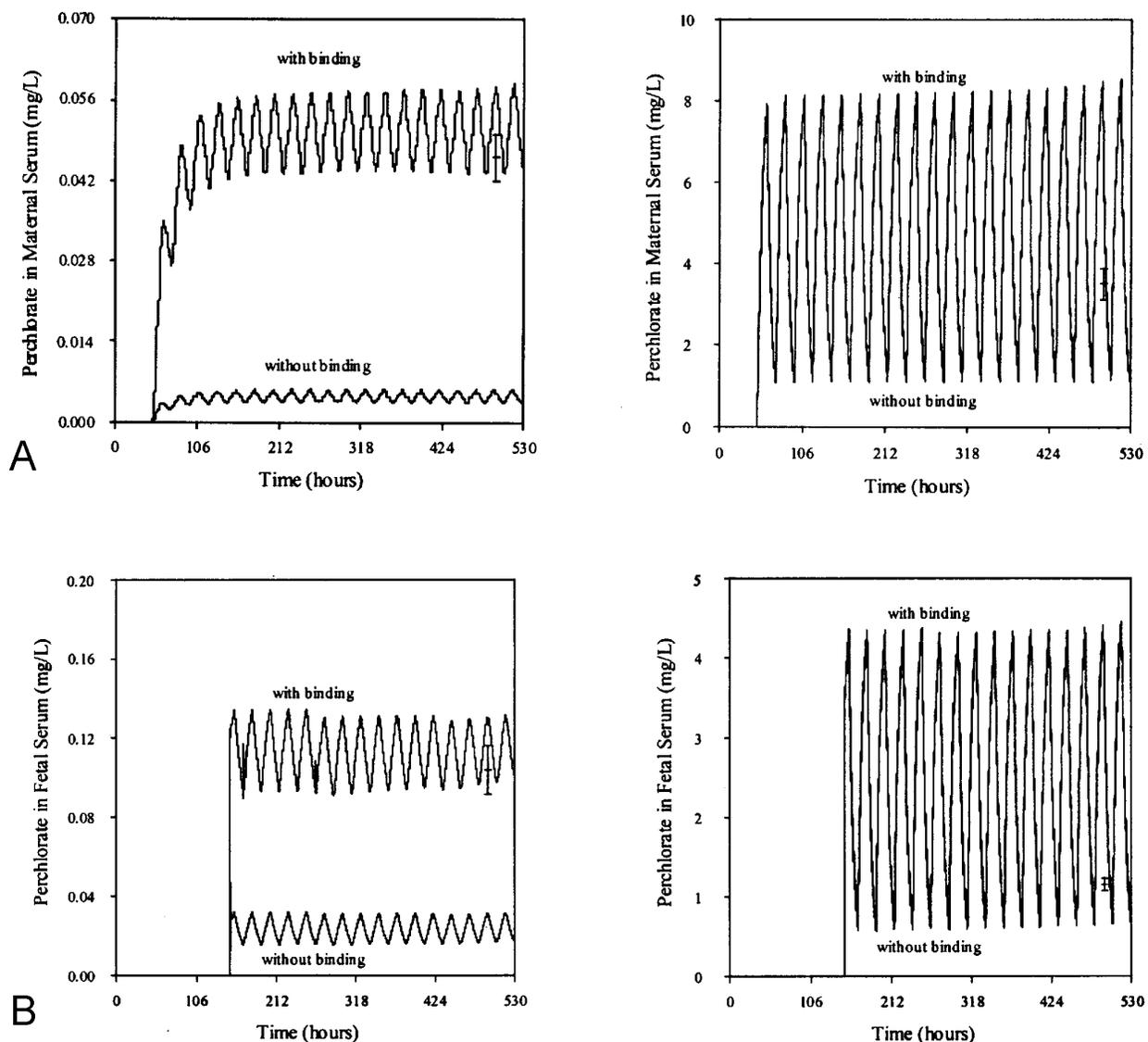
### 6.3.2.3 Pregnant Rat and Fetus Model Parameterization and Validation

7 This section summarizes how Clewell (2001a) used the various data sets to parameterize  
8 the model and the validation exercises performed.

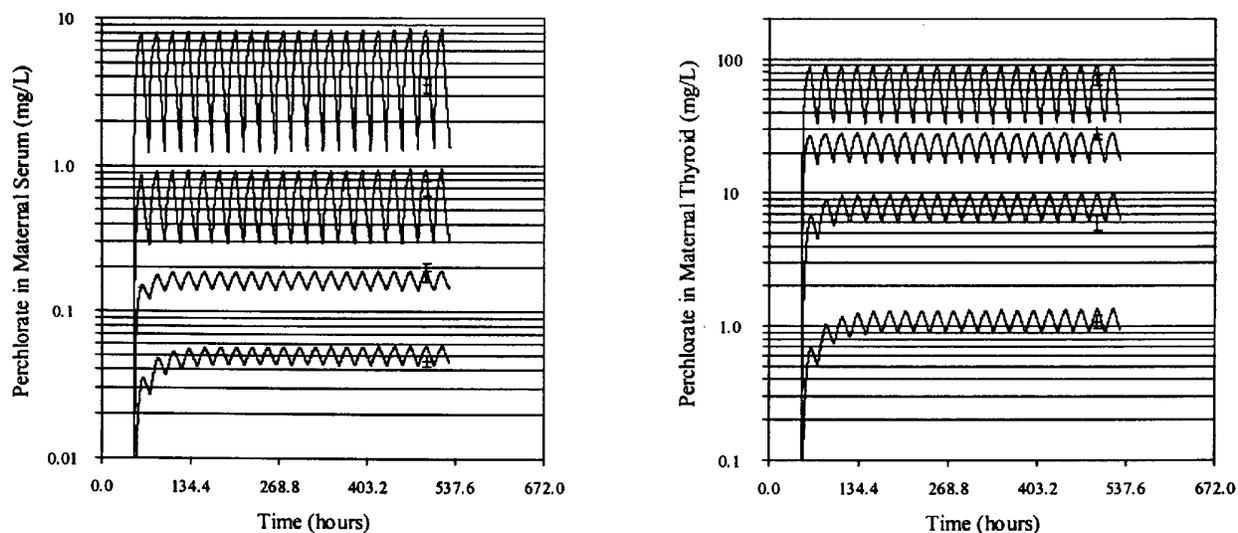
#### 6.3.2.3.1 Perchlorate Model Parameterization

11 Clewell (2001a) performed model parameterization for perchlorate using the data obtained  
12 from the AFRL/HEST drinking water studies on GD20. Optimized kinetic parameters ( $V_{max}$   
13 and permeability area values) were determined by fitting the model simulation to the  
14 experimental data. As for the adult male rat and human, it was necessary to account for the  
15 serum binding of perchlorate in order to adequately describe the blood perchlorate concentrations  
16 at the lower doses (0.01 and 0.1 mg/kg-day). Figure 6-28 illustrates the importance of binding in  
17 the model simulations of both maternal (A) and fetal (B) serum at 0.01 (left) versus the 10.0  
18 (right) mg/kg-day dose. Binding does not have a noticeable effect on the plasma concentrations  
19 in the highest dose. However, as the perchlorate dose decreases, the effect of binding is more  
20 pronounced. Therefore, at lower levels, a larger percent of the injected dose will be bound.  
21 As the amount consumed is increased, the binding process is saturated and eventually the amount  
22 of perchlorate that is bound is negligible in contrast to the large amount of free perchlorate in the  
23 plasma. This is to be expected because the number of binding sites is limited.

24 Figure 6-29 shows the fit of the model to the maternal serum (left) and thyroid (right)  
25 perchlorate concentration (mg/L) in the dam on GD20. Since saturation of the symporter occurs  
26 between the 1.0 and 10.0 mg/kg-day dose groups, the influence of  $V_{maxc}$  in the tissues was  
27 primarily in the 0.01 to 1.0 mg/kg-day doses. Thus, the fit of the model simulation to the data in  
28 the lower three doses was used to determine the values for  $V_{maxc}$  in the tissues. On the other  
29 hand, the  $V_{maxc}$  did not have a significant effect on the highest dose. The model fits to the  
30 10 mg/kg-day dose group were primarily affected by the partition coefficients and permeability  
31 area values. Clewell (2001a) obtained the permeability area values in the tissues by fitting the



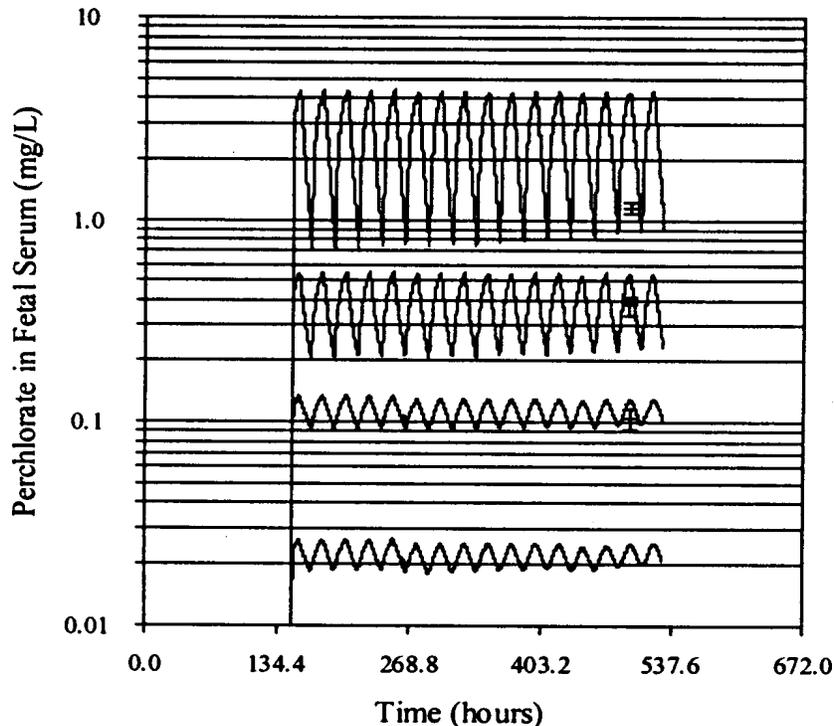
**Figure 6-28. Simulations illustrating the necessity of including plasma binding in the pregnant dam and fetal rat PBPK model structure (Clewell, 2001a). Model predictions (lines) versus data time course (mean  $\pm$  SD) are shown with and without plasma binding for maternal (A) and fetal (B) serum concentrations (mg/L) at two different doses, 0.01 mg/kg-day (left) and 10.0 mg/kg-day (right).**



**Figure 6-29. Pregnant dam and fetal rat PBPK model predictions (lines) versus data time course (mean  $\pm$  SD) of perchlorate concentrations (mg/L) in maternal serum (left) and thyroid (right) on GD20 (Clewel, 2001a). Pregnant rats were dosed in drinking water with 10.0, 1.0, 0.1, or 0.01 mg/kg-day perchlorate. Data of Yu (2000).**

1 highest dose to the 10 mg/kg-day data in the tissues. Maternal placenta, mammary gland, and GI  
 2 tract concentrations were available at the 10 mg/kg dose only. These tissues were used to verify  
 3 the applicability of the assigned partition coefficients to the model. Since mammary glands were  
 4 not available for the 0.01 through 1.0 mg/kg-day dose groups, it was not possible to fit the  
 5  $V_{maxc}$  value to data at which the symporter has a significant effect. Therefore, the  $V_{maxc}$  in the  
 6 mammary gland was assigned the molar equivalent of the iodide  $V_{maxc}$ . This is probably a  
 7 reasonable value in the non-lactating gland. Clewel (2001a) provides additional figures that  
 8 demonstrate the fit of the model to the GI tract, mammary glands, and placenta in the pregnant  
 9 dam.

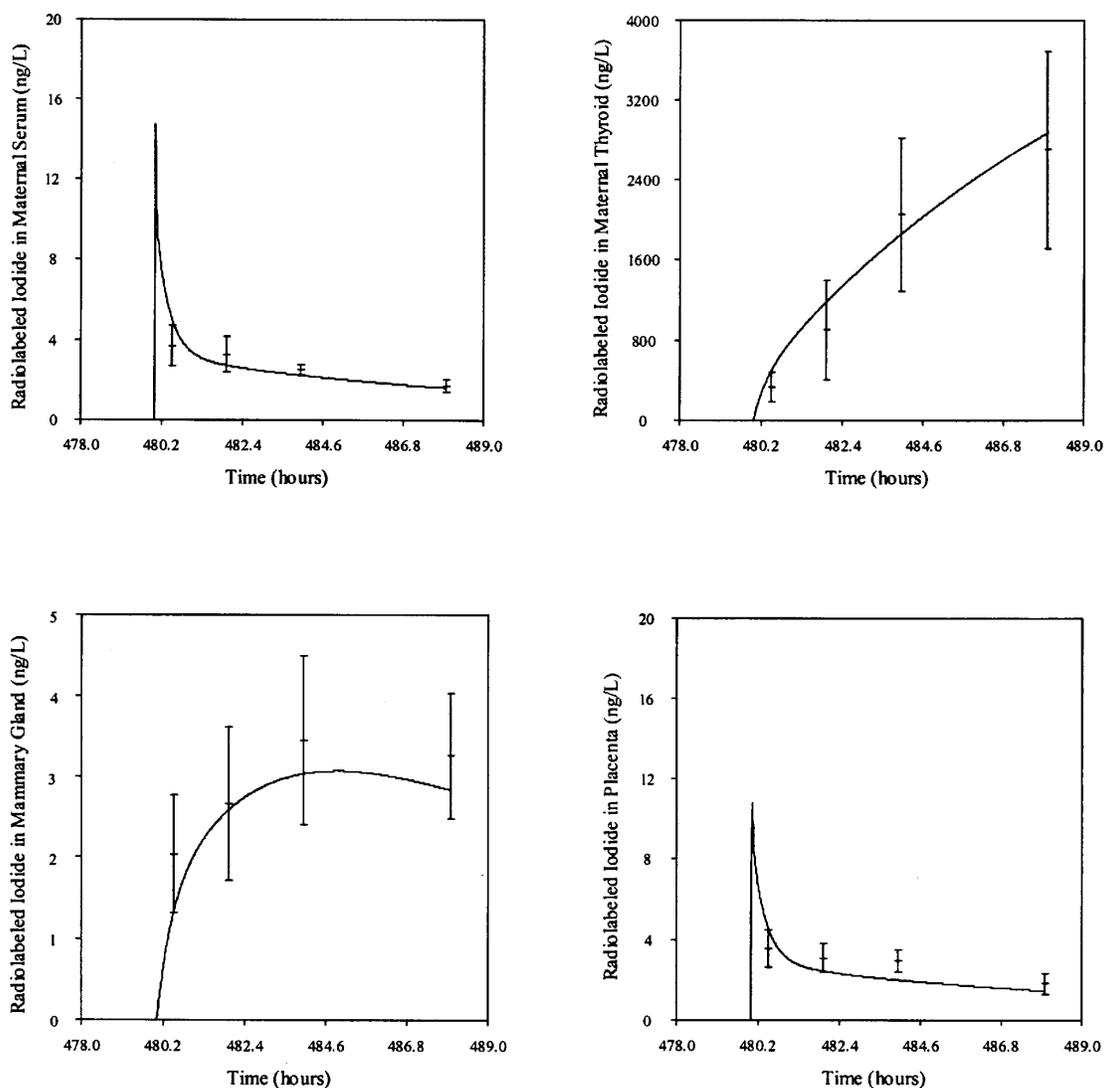
10 Fewer data were available for perchlorate distribution in the fetus than in the dam due to  
 11 the experimental difficulty involved in sampling the small fetal tissues. Figure 6-30 depicts the  
 12 model simulation of the fetal serum concentration (mg/L) compared to the data obtained in the  
 13 drinking water study. Fetal serum and skin were pooled by litter. Fits to additional  
 14 compartments are provided in Clewel (2001a).



**Figure 6-30. Pregnant dam and fetal rat PBPK model predictions (lines) versus data time course (mean  $\pm$  SD) of perchlorate concentrations (mg/L) in pooled fetal serum on GD20 (Clewell, 2001a). Pregnant rats were dosed in drinking water with 10.0, 1.0, 0.1, or 0.01 mg/kg-day perchlorate. Data of Yu (2000).**

### 6.3.2.3.2 Iodide Model Parameterization

Development of the iodide model was performed by fitting the model to the kinetic data in the tissues of the dam and fetus from the preliminary iodide study. Only the values for  $V_{maxc}$  and permeability area needed to be fit with the model. The clearance value for urinary excretion was determined by fitting the maternal serum prediction to the above data while keeping good fits in the other tissues, such as the maternal skin and gut and the fetal skin. Permeability area values were adjusted to describe the behavior of the iodide data, where varying the permeability area values toward 1.0 L/hr-kg generally increased the rate at which uptake and clearance in a particular tissue occurred; and decreasing permeability area slowed the uptake and clearance. Figure 6-31 shows the model simulation of the iv injection of 2.19 ng/kg  $^{125}\text{I}$  on GD20 versus the experimental data for the maternal iodide concentrations in serum (top left), thyroid (top right), mammary gland (bottom left) and placenta (bottom right). The data are described well by the



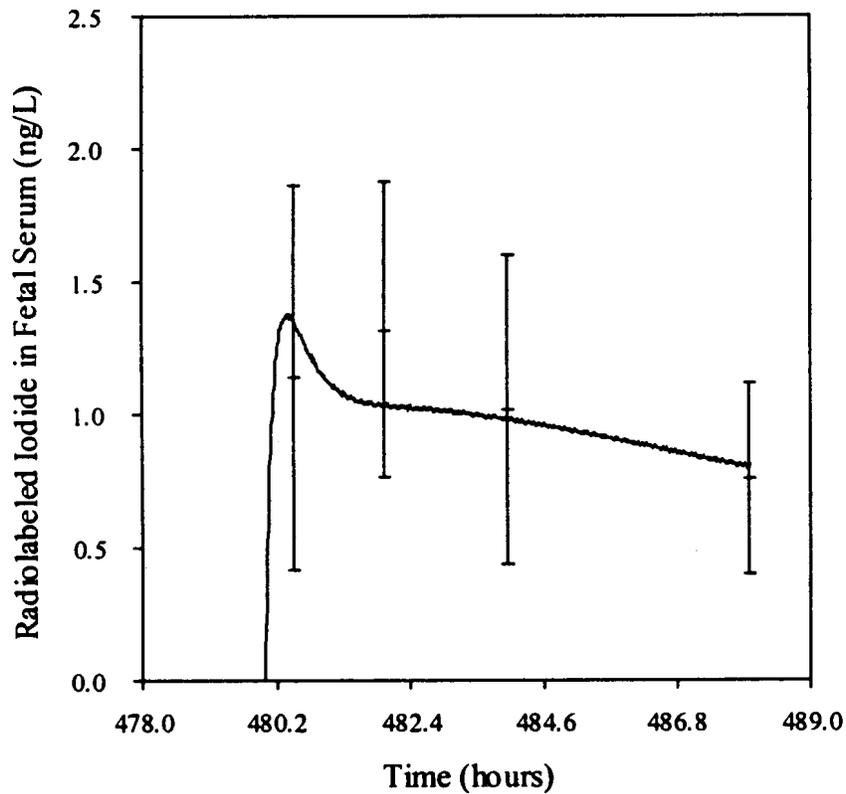
**Figure 6-31. Pregnant dam and fetal rat PBPK model predictions (lines) versus data time course (mean  $\pm$  SD) of  $^{125}\text{I}$  radiolabeled iodide concentrations (ng/L) in maternal serum (top left), thyroid (top right), mammary gland (bottom left), and placenta (bottom right) on GD20 (Clewell, 2001a) Pregnant rats were dosed by iv injection with 2.19 ng/kg  $^{125}\text{I}^-$  on GD20. Data of Yu (2002).**

1 model. The behavior of these mammary tissue data indicate that iodide is maintained in the  
 2 mammary gland well into the clearance phase of the serum. In order to simulate this behavior, it  
 3 was necessary to use a low permeability area value (0.02 L/hr-kg) in the mammary gland

1 (Clewell, 2001a). The mammary:plasma ratios of greater than one were fit with the Vmaxc for  
2 mammary NIS.

3 Clearance values for the transfer of iodide between the placenta and fetal blood were  
4 determined by optimizing the fit of the fetal serum to the data points while maintaining the fit of  
5 the simulations of the maternal blood and fetal tissue data. Figure 6-32 shows the model  
6 simulation versus the fetal data in the preliminary iodide time course study for radiolabeled  
7 iodide in fetal serum (ng/L). Clewell (2001a) shows additional simulations for fetal skin and  
8 fetal GI tract.

9  
10



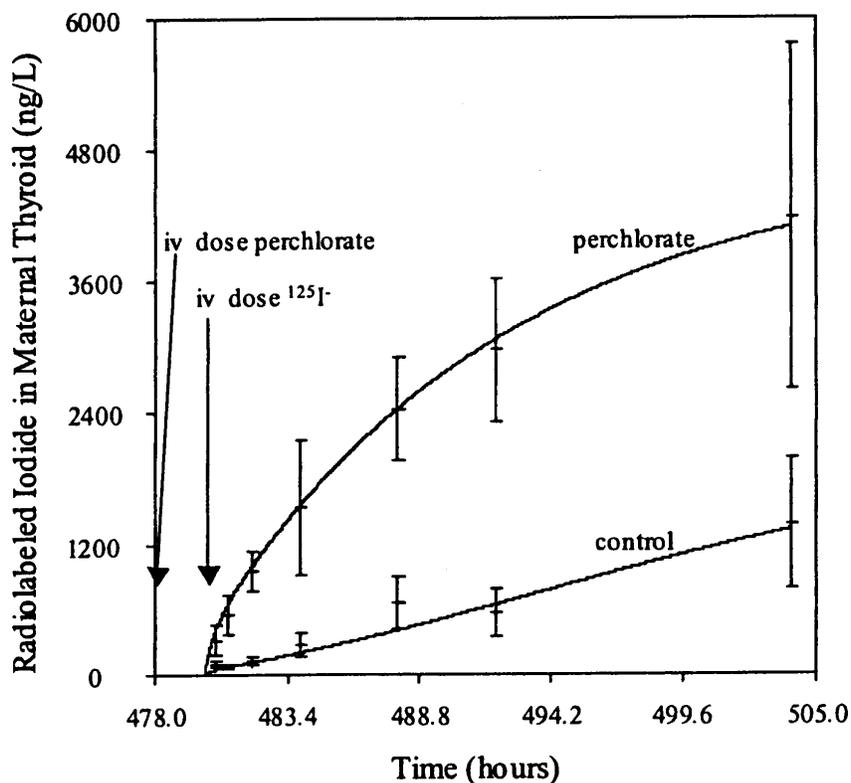
**Figure 6-32. Pregnant dam and fetal rat PBPK model predictions (lines) versus data time course (mean  $\pm$  SD) of  $^{125}\text{I}^-$  radiolabeled iodide concentrations (ng/L) in fetal serum on GD20 after an iv injection to the dam with 2.19 ng/kg  $^{125}\text{I}^-$  (Clewell, 2001a). Data of Yu (2002).**

1 The data of Feldman et al. (1961) were used by Clewell (2001a) to determine the values for  
2 maximum velocity of iodide uptake in the fetal thyroid. An exponential function was fit to the  
3 experimental values and time points where time was gestation in hours. This equation was then  
4 used in the model to account for the increasing ability of the fetal thyroid to incorporate iodide.  
5 Iodide levels were negligible on GD16 but increased dramatically from GD17 to GD19 (see  
6 Clewell, 2001a; Figure 25).

### 7 8 **6.3.3 Model Validation**

9 The Clewell (2001a) model predictions for the inhibition of iodide uptake into the thyroid  
10 and the resulting effect on the maternal and fetal serum was validated against the data collected  
11 by AFRL/HEST during the inhibition study on GD20. The kinetic parameters derived from the  
12 perchlorate drinking water and preliminary iodide data sets were used in the model. Because the  
13 inhibition study was performed with an acute perchlorate dose, it was necessary to make some  
14 slight changes in the parameters describing thyroid perchlorate kinetics. The long-term exposure  
15 to perchlorate in the drinking water studies (18 days) that were used to determine the perchlorate  
16 parameters is sufficient to induce up-regulation in the thyroid (Yu, 2000). Thus, it was  
17 determined that the thyroid parameters in the dam at this point would be different from those  
18 seen in an acute situation. The only parameters altered in order to model the acute perchlorate  
19 were the partition coefficient (from 2.25 to 0.13) and permeability area value (from 6.0E-4 to  
20 4.0E-5) into the thyroid at the basolateral membrane (thyroid follicle). The value for the  
21 partitioning into the follicle in a naive thyroid was calculated as described previously from Chow  
22 and Woodbury (1970). The permeability area value in the naive thyroid follicle was determined  
23 with the lactation model, which is described in another consultative letter describing model  
24 development for the lactating rat (Clewell, 2001b).

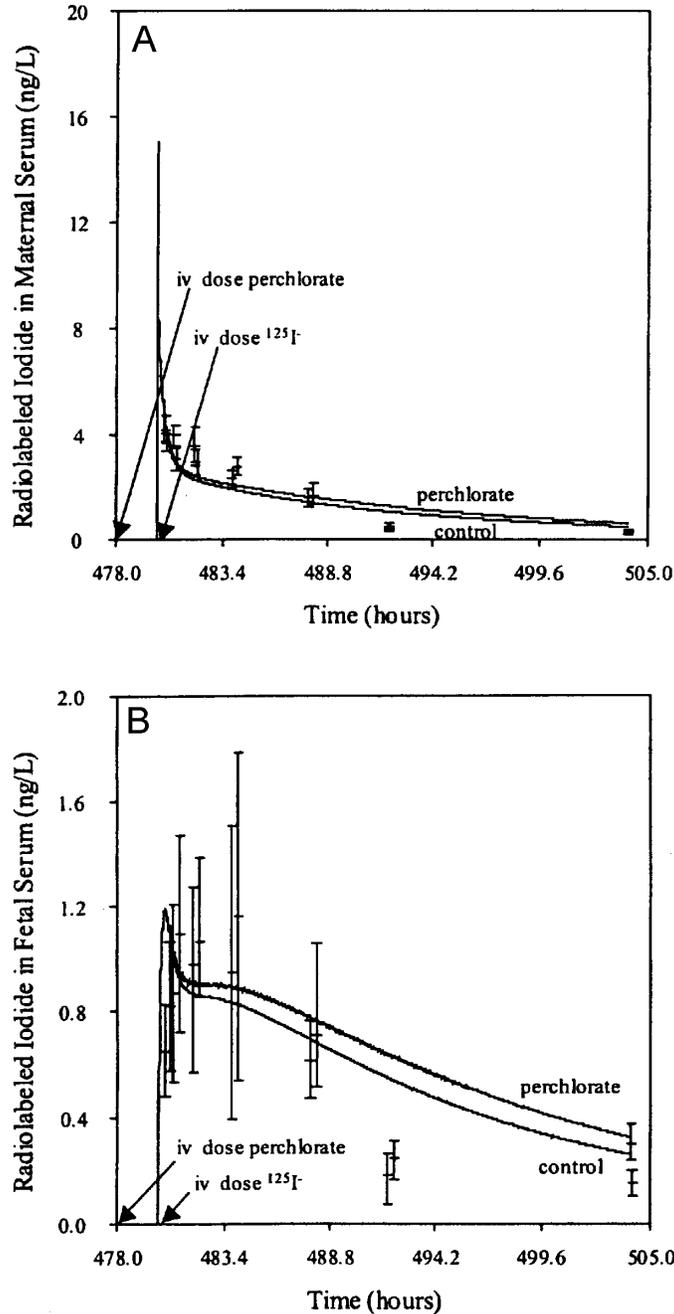
25 The model simulation was fit to the available kinetic data in the thyroid while keeping all  
26 other thyroid parameters identical to those in the pregnancy model. Figure 6-33 illustrates the  
27 model prediction of thyroidal iodide uptake with and without perchlorate inhibition, utilizing  
28 these pre-set parameters. The model prediction of inhibition in the thyroid gland at 0.5, 1., 2, 4,  
29 8, 12, and 24 hours after dosing with iodine shows an excellent fit to the data. The use of  
30 parameters derived from the drinking water perchlorate data for acute iodide uptake kinetics is  
31 well supported by the inhibition of iodide because inhibition is highly dependent on the



**Figure 6-33. Validation for pregnant dam and fetal rat PBPK model (Clewell, 2001a). Model predictions (lines) versus data time course (mean  $\pm$  SD) of  $^{125}\text{I}^-$  radiolabeled iodide concentrations (ng/L) in maternal thyroid with and without 1.0 mg/kg perchlorate administered by iv injection to the dam 2 hours prior to an iv injection with 1.87 ng/kg  $^{125}\text{I}^-$  (Clewell, 2001a). The top simulation represents the control thyroid and the lower indicates the inhibited thyroid. Data of Yu (2000, 2002).**

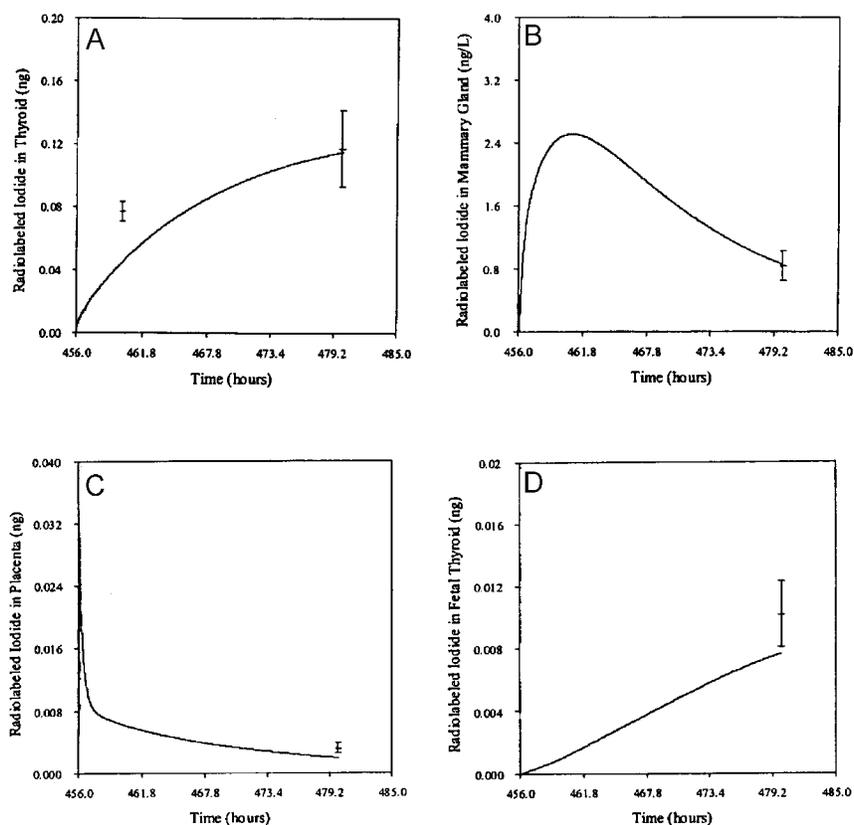
1 perchlorate concentration in the thyroid and the perchlorate affinity constants in the apical and  
 2 basolateral membranes of the thyroid. Figure 6-34 illustrates the effect of perchlorate thyroid  
 3 inhibition on the maternal (top) and fetal (bottom) blood iodide levels. Significant differences  
 4 were found in the maternal serum iodide concentrations collected at the 1, 4, and 24 hour time  
 5 points. Fetal serum, however, did not show any significant differences in the total serum iodide  
 6 between the control and inhibited groups. Additional statistical analysis of these data are  
 7 provided as Attachment #2 in Clewell (2001a).

8 Clewell (2001a) performed a model simulation of data presented by Versloot et al. (1997)  
 9 in order to test the ability of the model to predict diverse data sets collected under different



**Figure 6-34. Validation for pregnant dam and fetal rat PBPK model (Clewell, 2001a). Model predictions (lines) versus data time course (mean  $\pm$  SD) of <sup>125</sup>I radiolabeled iodide concentrations (ng/L) in maternal (A) and fetal (B) serum with and without a 1.0 mg/kg perchlorate dose administered by iv injection to the dam 2 hours prior to an iv injection with 1.87 ng/kg <sup>125</sup>I (Clewell, 2001a). The top simulations in each represents the serum during thyroid inhibition and the lower represents the control serum. Data of Yu (2000, 2002).**

1 conditions. This data set provided an additional time point for the iodide model validation  
2 (GD19). Dams were exposed by iv injection to 10  $\mu\text{Ci}$  (1.74 ng/kg) carrier-free radiolabeled  
3 iodide ( $^{125}\text{I}$ ) on GD19. Figure 6-35 shows the model predictions versus data (mean  $\pm$  SD) for the  
4 amount (ng) of iodide taken up in maternal thyroid (A), mammary gland (B), and placenta (C), or  
5 fetal thyroid (D). The model is able to accurately describe these tissues of interest and fits other  
6 compartments (data shown in Clewell, 2001a) within a two-fold factor without changing any  
7 parameters. This illustrates its predictive power and usefulness to the extrapolations required.



**Figure 6-35. Validation for pregnant dam and fetal rat PBPK model (Clewell, 2001a). Model predictions (lines) versus data time course (mean  $\pm$  SD) of total  $^{125}\text{I}$  radiolabeled iodide in the maternal thyroid (A), mammary gland (B), placenta (C), or fetal thyroid (D) at 24 hours after exposure to the dam by iv injection of 10  $\mu\text{Ci}$  (1.74 ng/kg carrier-free)  $^{125}\text{I}$  in GD19 dams. Data of Versloot et al. (1997).**

1 Model predictions were also shown to be in good agreement with another unrelated data  
2 set, that of Sztanyik and Turai (1988), who measured carrier-free radiolabeled iodide ( $^{131}\text{I}$ ) in  
3 GD20 dams and in the total (whole body) fetuses after an iv injection (Clewell, 2001a). This  
4 validation illustrated adequate model fit to another time point and radioactive species of iodide.  
5 The model was additionally validated against AFRL/HEST data for dams and fetuses after  
6 administration of radiolabeled iodide ( $^{125}\text{I}$ ) with carrier at doses four orders of magnitude greater  
7 than that used to parameterize the model (33000 ng/kg versus 2.19 ng/kg). These validation  
8 simulations are shown in Clewell (2001a).

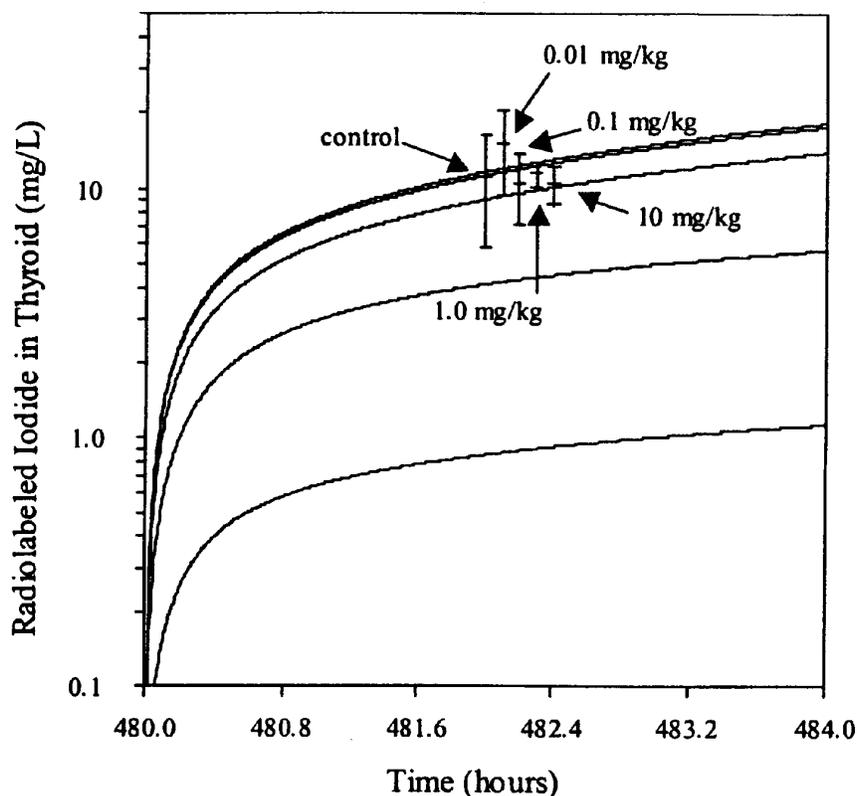
9 As a final validation exercise, the model was used to predict radiolabeled iodide uptake  
10 inhibition after perchlorate exposures in drinking water for 18 days at 0.0, 0.01, 1.0, and 10  
11 mg/kg-day (Yu, 2000). It was apparent that even at the lowest dose, the hormonal system had  
12 experienced a perturbation and was attempting to compensate for the interruption caused by the  
13 perchlorate exposure (Clewell, 2001a). Maternal T4 decreased in a dose-dependent manner,  
14 while TSH increased. The maternal total T4 and TSH changes were statistically significant at all  
15 doses. Free T4 was significantly increased at the 0.1, 1.0, and 1.0 mg/kg-day doses and total T3  
16 was significantly decreased at the 1.0 and 10.0 mg/kg-day doses. The fetus appeared to follow  
17 the same trends as those seen in the dam. However, only the 1.0 and 10.0 mg/kg-day dose  
18 groups show significant decreases in total T4 and the 0.01, 1.0, and 10.0 mg/kg-day doses  
19 resulted in significant increases in fetal free T4 and TSH. No significant decrease was seen in  
20 fetal T3. The statistical analysis of the hormone data is provided as Attachment #3 in Clewell  
21 (2001a).

22 From the perspective of iodide kinetics, these hormone changes are important indicators of  
23 thyroid up-regulation. When TSH is increased, the thyroid is stimulated to increase iodide  
24 uptake. It is evident, then, that after exposure to perchlorate in drinking water for 18 days, the  
25 thyroid of the pregnant dam has experienced both inhibition and up-regulation and has  
26 successfully compensated for the competition of perchlorate for binding sites of NIS. Therefore,  
27 it is not surprising that no inhibition was reported on GD20. It is not that the inhibition is not  
28 taking place, but rather that the system has compensated for the effect.

29 None of the models is currently equipped with the capability to account for up-regulation of  
30 the thyroid. Therefore, when a simulation of the inhibition is performed with the model, the  
31 concentration of iodide is under-predicted in a perchlorate-dose dependent manner (Clewell,

1 2001a). Figure 6-36 shows the model prediction of iodide in the thyroid of the dam at drinking  
2 water doses of 0.0, 0.1, 1.0, and 10.0 mg/kg-day. The  $V_{max}$  for iodide was decreased to  
3  $2.5 \times 10^4$  to fit the mean from the control data with the control simulation in order to make the  
4 comparison of the inhibition data and simulations clearer. All experimental data were actually  
5 taken two hours post dosing. However, the data points were separated slightly by time on the  
6 plot in order to make them more visible. The prediction of thyroid perchlorate levels from this  
7 same study can be seen in Figure 6-29 (right).

8  
9



**Figure 6-36. Validation for pregnant dam and fetal rat PBPK model (Clewell, 2001a). Model predictions (lines) versus data time course (mean  $\pm$  SD) of radiolabeled iodide in the maternal thyroid of the dam at doses of perchlorate in drinking water at 0.0, 0.01, 1.0, and 10.0 mg/kg-day for 18 days. Data of Yu (2000).**

#### 1     **6.3.4 Summary**

2           The proposed model for the pregnant rat and fetus developed by Clewell (2001a) appears to  
3 adequately describe perchlorate and iodide distribution in a highly dynamic, changing system, by  
4 accounting for growth with age-specific functions. The model predicts the transfer of perchlorate  
5 to the fetus and is also able to describe the uptake into fetal tissues of interest, such as the serum  
6 and thyroid. Fetal and dam tissues were predicted well by fitting data that spans three orders of  
7 magnitude (i.e., 0.01 to 10.0 mg/kg-day).

8           In addition to the requisite compartments for pregnancy (mammary gland, placenta, fetus),  
9 some differences exist that affect the kinetics of both perchlorate and iodide. The thyroidal  
10 maximum capacities are lower in the pregnant dam than in the male rat. Model parameterization  
11 in the male rat indicated the need for  $V_{max}$  values for uptake into the follicle of the thyroid of  
12  $2.2 \times 10^3$  L/hr-kgr for perchlorate and  $5.5 \times 10^4$  L/hr-kg for iodide, while the gestation model  
13 required values of  $1.8 \times 10^3$  L/hr-kg and  $4.0 \times 10^4$  L/hr-kg for the same parameters. This  
14 difference is supported in the literature. Versloot et al. (1997) suggest that the pregnant rat may  
15 have a lowered reserve of iodide in the thyroid toward the end of pregnancy, causing increased  
16 activity in the thyroid. The increased response of the pregnant rat was also seen in the studies  
17 performed by Yu (2000) and Yu et al. (2000) that reported a greater than average inhibition in the  
18 thyroid of the pregnant dam than in the male rat at the same perchlorate dose (78% vs. 70% over  
19 8 hours). The skin of the pregnant dam also required a smaller value for  $V_{maxc}$  than the male  
20 rat. This is supported by the work of Brown-Grant and Petes (1959), which reported higher  
21 levels of iodide in the skin male rats than in female rats. Skin, therefore, appears to be a more  
22 important iodide reserve in the male rat than the female. It is reassuring that the model is able to  
23 account for the majority of differences in the uptake, distribution, and excretion between the  
24 male and pregnant female by incorporating known differences in physiology.

25           Clewell (2001a) notes that at this time the amount of data concerning perchlorate kinetics  
26 in the pregnant rat is very limited. Although perchlorate has been used extensively in literature  
27 to study the thyroidal uptake of iodide, it has not been commonly used in rat gestation studies.  
28 As such, the perchlorate model was limited to utilizing the drinking water studies for  
29 parameterization. However, acute kinetic data were available for perchlorate in the lactating dam  
30 and were utilized in the development of the rat lactation model (Clewell, 2001b; see Section 6.4).  
31 This system is similar to that of the pregnant dam. Consequently, it was possible to simulate the

1 perchlorate kinetics of the dam with the same general model structure, changing only the  
2 physiological parameters. Therefore, it seemed reasonable to use the acute perchlorate  
3 parameters from the lactation model. The use of the described parameters for acute perchlorate  
4 kinetics is also supported by the ability of the model to predict inhibition in the pregnant dam.  
5 Clewell (2001a) discusses that acute perchlorate kinetic data to further verify the model are  
6 currently being analyzed by AFRL/HEST, and these were provided to the EPA too late for  
7 evaluation (Yu, 2002). In these studies, tissues were collected from pregnant dams and fetuses at  
8 various time points after iv injections of perchlorate. The use of these data in the modeling effort  
9 may be described in draft manuscripts provided to the external peer review.

10 The kinetic behavior of iodide was also accurately simulated with a range of doses that  
11 spans nearly five orders of magnitude (0.36 to 33,000 ng/kg). The active sequestration of iodide  
12 in maternal and fetal tissues and the transfer of iodide between mother and fetus was described  
13 kinetically with the model, and data have been simulated at a variety of doses and at various time  
14 points up to 24 hours post exposure. The fact that the model was able to simulate data from  
15 other laboratories under a variety of different conditions attests to the validity of the model  
16 structure and its applicability to other studies. The ability of the model to predict iodide was  
17 indicative of the usefulness of the model for predictive purposes. It was possible to predict  
18 inhibition out to 24 hours while simulating the serum and thyroid perchlorate and iodide levels  
19 with satisfactory accuracy. This provides support for the chosen model structure, as well as  
20 validation for the physiological and chemical descriptions used.

21 Clewell (2001a) notes that the inability of the model to respond to this auto-regulation  
22 presents a considerable need for further model development since drinking water scenarios  
23 would allow time for the hypothalamic-pituitary-feedback system to upregulate. Given that the  
24 temporal windows of developmental susceptibility are not well established across species, this  
25 issue may have to wait for further fundamental neurodevelopmental research.

26 The EPA has also become aware of a recent human biokinetic model for iodine and  
27 radionuclides at various ages (fetus, children, mothers) that may provide some additional  
28 information with which to validate the iodide kinetic components of the proposed models from  
29 AFRL/HEST scientists (International Commission on Radiological Protection, 2001, 1989).

## 6.4 LACTATING AND NEONATAL RAT MODEL STRUCTURE

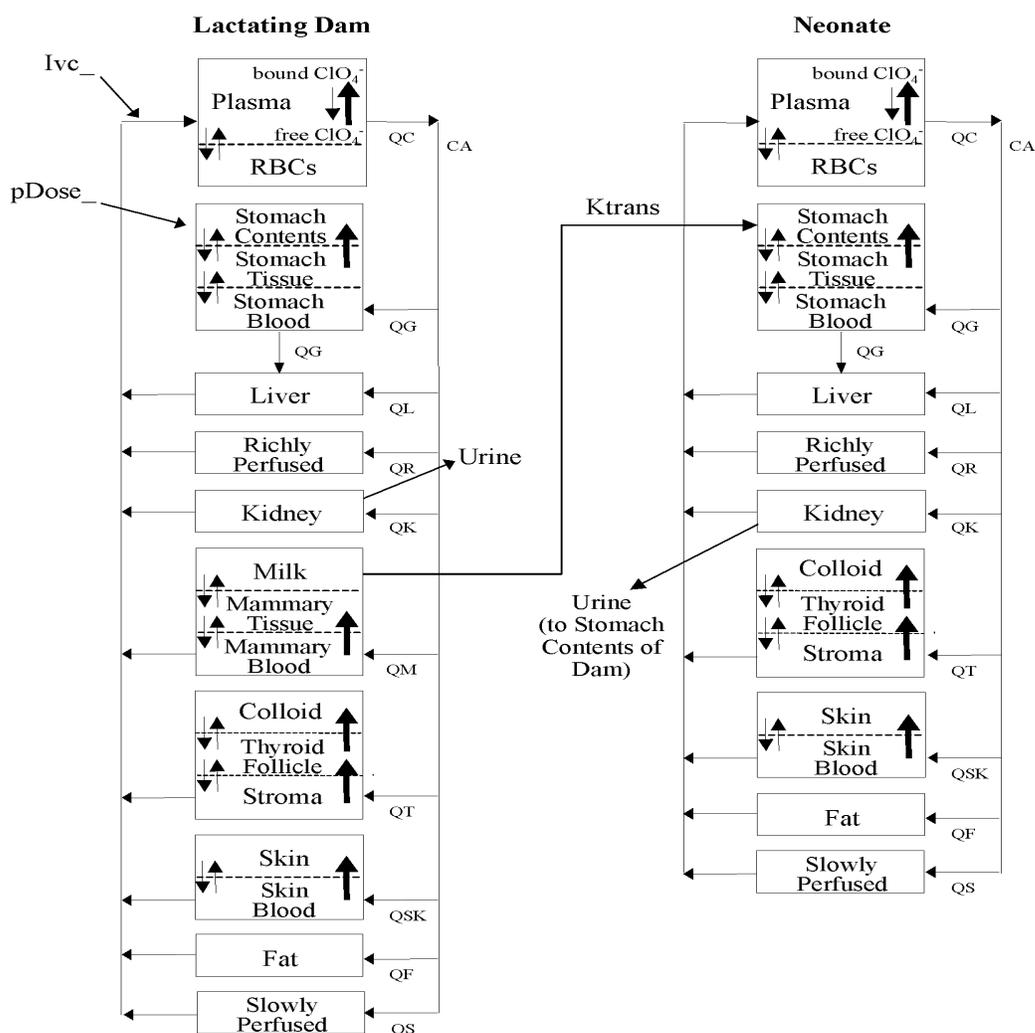
This section describes the model developed by AFRL/HEST in response to concerns about interspecies extrapolation of effects observed in laboratory rats immediately after parturition up to about PND22 (Clewell, 2001b) and updates the preliminary structure provided to EPA (Clewell, 2000). The model predicts the distribution of perchlorate within the lactating dam and neonatal rat during these first few weeks of life, and also predicts the short-term effect of acute perchlorate exposure on iodide kinetics, including iodide uptake in the maternal thyroid.

Concern regarding the kinetics of perchlorate in lactating dams and neonates was motivated by the knowledge that the mammary gland is another tissue with active transport via the NIS, as described in Section 6.3. Perchlorate can thus competitively inhibit the uptake of iodide into the mammary gland in a manner reminiscent of the thyroid, and reduce the amount of available iodide to the infant. Studies utilizing radiolabeled iodide in lactating rats have shown perchlorate to be an effective inhibitor of iodide secretion of into breast milk (Potter et al., 1959, Brown-Grant, 1961). The fact that perchlorate not only inhibits the uptake of iodide, but is also taken up itself into the mammary tissue by way of the NIS, results in an additional risk to the neonate. The perchlorate is then concentrated in the milk and transferred to the litter through suckling.

Although early papers suggest that perchlorate is not transferred in milk (Zeghal et al., 1992), newer technology with better analytical sensitivity has detected perchlorate in the milk of rats dosed with as little as 0.01 mg/kg-day perchlorate in drinking water at the AFRL/HEST. The perchlorate levels in 5- and 10-day old neonate serum are comparable to those of the mother (Yu et al., 2000), indicating that the pups are in fact exposed to significant levels of perchlorate through the maternal milk. This information highlighted the need for more information regarding the effect of perchlorate exposure on the neonate.

The model structure is shown in Figure 6-37. Table 6-6 provides the physiological parameters used in the lactating and neonatal rat PBPK models. Table 6-7 provides the perchlorate-specific parameters, and Table 6-8 provides the iodide-specific parameters for each.

The model structure was developed to be consistent with the previously discussed structures for the adult male rat, pregnant rat, and fetus. In fact, an important linking to the pregnancy model was required. Since the experimental data used to develop the lactation model were taken from drinking water studies in which the dosing began on GD2, it was necessary to include initial perchlorate concentrations in the tissues at the time of birth (0 hours). In order to



**Figure 6-37. Schematic for the lactating dam and neonatal rat PBPK model of perchlorate and iodide distribution (Clewell, 2001b). Boxes represent specific compartments in the model structure. The thyroid consists of the stroma, the follicle, and the colloid, and the stomach consists of the capillary bed, stomach wall, and contents. The skin contains two subcompartments representing the capillary bed and skin tissue. Bold arrows indicate active uptake at NIS sites in the thyroid, skin, mammary gland and GI tract. Plasma binding was also described with Michaelis-Menten terms for the association of perchlorate anion to binding sites with first-order clearance rates for dissociation. Sequestration of the perchlorate ( $\text{ClO}_4^-$ ) and iodide ( $\text{I}^-$ ) anions into milk was also described with Michaelis-Menten kinetics. Permeability area cross products and partition coefficients were used to describe the first-order movement of the perchlorate ( $\text{ClO}_4^-$ ) anion into deeper subcompartments which results from the inherent electrochemical gradient within the tissues. Urinary clearance and transfer of the anions through suckling were represented by first order clearance rates.**

**TABLE 6-6. PHYSIOLOGICAL PARAMETERS FOR LACTATING DAM AND NEONATE PBPK MODEL (Clewell, 2001b)**

Physiological Parameters	Lactation		Source
	Dam	Neonate	
<b>Tissue Volumes<sup>a</sup></b>			
Body Weight <i>BW</i> (kg)	0.277 - 0.310	0.0075 - 0.1985	Yu, 2000
Slowly Perfused <i>VSc</i> (%BW)	37.07-40.42	53.92-49.31	Brown et al., 1997
Richly Perfused <i>VRc</i> (%BW)	5.35	5.36	Brown et al., 1997
Fat <i>VFc</i> (%BW)	12.45 - 6.9	0.0 - 4.61	Naismith et al., 1982
Kidney <i>VKc</i> (%BW)	1.7	1.7	Brown et al., 1997
Liver <i>VLc</i> (%BW)	3.4	3.4	Brown et al., 1997
Stomach Tissue <i>VGc</i> (%BW)	0.54	0.54	male rat ClO <sub>4</sub> <sup>-</sup> kinetics
Gastric Juice <i>VGJc</i> (%BW)	1.68	1.68	Yu, 2000
Stomach Blood <i>VBc</i> (%VG)	2.9	2.9	Altman & Dittmer, 1971
Skin Tissue <i>VSkc</i> (%BW)	19.0	19.0	Brown et al., 1997
Skin Blood <i>VSkBc</i> (%VSkc)	2.0	2.0	Brown et al., 1997
Thyroid Total <i>VTtotc</i> (%BW)	0.0105	0.0125	Malendowicz & Bednarek, 1986; Florsheim et al., 1966
Thyroid Follicle <i>VTc</i> (%VTtot)	45.89	37.2	Malendowicz & Bednarek, 1986; Conde et al.,1991
Thyroid Colloid <i>VDTc</i> (%VTtot)	45	13.8	Malendowicz & Bednarek, 1986; Conde et al.,1991
Thyroid Blood <i>VTBc</i> (%VTtot)	9.1	49.0	Malendowicz & Bednarek, 1986; Conde et al.,1991
Plasma <i>VPlasc</i> (%BW)	4.7	4.7	Brown et al., 1997, Altman & Dittmer, 1971
Red Blood Cells <i>VRBCc</i> (%BW)	2.74	2.74	Brown et al., 1997, Altman & Dittmer, 1971
Mammary Tissue <i>VMc</i> (%BW)	4.4 - 6.6	—	Knight et al., 1984
Mammary Blood <i>VMBc</i> (%VM)	18.1	—	Assume same % as Thyroid Blood
Milk <i>VMk</i> (L)	0.002	—	Fisher et al., 1990
<b>Blood Flows</b>			
Cardiac Output <i>QCc</i> (L/hr-kg)	14.0 - 21.0	14.0	Hanwell & Linzell, 1973; Brown et al., 1997
Slowly Perfused <i>QSc</i> (%QC)	7.9-1.9	16.9	Brown et al., 1997
Richly Perfused <i>QRc</i> (%QC)	40.8	40.8	Brown et al., 1997
Fat <i>QFc</i> (%QC)	7.0	7.0	Brown et al., 1997
Kidney <i>QKc</i> (%QC)	14.0	14.0	Brown et al., 1997
Liver <i>QLc</i> (%QC)	18.0	18.0	Brown et al., 1997
GI <i>QGc</i> (%QC)	1.61	1.61	Brown et al., 1997
Skin <i>QSkc</i> (%QC)	0.058	0.058	Brown et al., 1997
Thyroid <i>QTc</i> (%QC)	1.6	1.6	Brown et al., 1997
Mammary <i>QMc</i> (%QC)	9.0 - 15.0	—	Hanwell & Linzell, 1973

<sup>a</sup>For calculation of volumes from body weight, a density of 1.0 g/mL was assumed.

**TABLE 6-7. PERCHLORATE-SPECIFIC PARAMETERS FOR LACTATING DAM AND NEONATE PBPK MODEL (Clewell, 2001b)<sup>a</sup>**

Perchlorate Parameters	Lactation Values		Source
	Dam	Neonate	
<b>Partition Coefficients (unitless)</b>			
Slowly Perfused/Plasma PS_	0.31	0.31	Yu et al., 2000
Rapidly Perfused/Plasma PR_	0.56	0.56	Yu et al., 2000
Fat/ Plasma PF_	0.05	0.05	Pena et al., 1976
Kidney/ Plasma PK_	0.99	0.99	Yu et al., 2000
Liver/Plasma PL_	0.56	0.56	Yu et al., 2000
Gastric Tissue/Gastric Blood PG_	1.80	3.21	Yu, 2000; Yu et al., 2000
Gastric Juice/Gastric Tissue PGJ_	2.30	5.64	Yu, 2000; Yu et al., 2000
Skin Tissue/Skin Blood PSk_	1.15	1.15	Yu et al., 2000
Thyroid Tissue/Thyroid Blood PT_	0.13/2.0	0.13/2.0	Chow and Woodbury, 1970; Yu, 2000 <sup>b</sup>
Thyroid Lumen/Thyroid Tissue PDT_	7.0	7.0	Chow and Woodbury, 1970; Yu, 2000
Red Blood Cells/Plasma PRBC_	0.73	0.73	Yu et al., 2000
Mammary Tissue/Mammary Blood PM_	0.66	—	Anbar et al., 1959
Milk/Mammary Tissue PMk_	2.39	—	Yu, 2000
<b>Max Capacity, Vmaxc (ng/hr-kg BW)</b>			
Thyroid Follicle Vmaxc_T	1.50E+03	1.50E+03	Fitted <sup>c</sup>
Thyroid Colloid Vmaxc_DT	1.00E+04	1.00E+04	Fitted <sup>c</sup>
Skin Vmaxc_S	8.00E+05	8.00E+05	Fitted
Gut Vmaxc_G	1.00E+06	1.00E+06	Fitted
Mammary Tissue Vmaxc_M	2.0E+5/2.0E+4	—	Fitted <sup>bc</sup>
Milk Vmaxc_Mk	2.00E+04	—	Fitted
Plasma Binding Vmaxc_B	9.00E+03	1.00E+03	Fitted
<b>Affinity Constants, Km (ng/L)</b>			
Thyroid Follicle Km_T	1.00E+05	1.00E+05	Gluzman & Niepomniszcze, 1983; Wolff, 1998
Thyroid Colloid Km_DT	1.0E+09	1.0E+09	Golstein et al., 1992; Wolff, 1998
Skin Km_S	1.00E+05	1.00E+05	Gluzman & Niepomniszcze, 1983; Wolff, 1998
Gut Km_G	1.00E+05	1.00E+05	Gluzman & Niepomniszcze, 1983; Wolff, 1998
Mammary Km_M	1.0E+05	—	Gluzman & Niepomniszcze, 1983; Wolff, 1998
Milk Km_Mk	1.00E+06	—	Fitted
Plasma Binding Km_B	1.00E+04	1.00E+04	Fitted

**TABLE 6-7 (cont'd). PERCHLORATE-SPECIFIC PARAMETERS FOR LACTATING DAM AND NEONATE PBPK MODEL (Clewell, 2001b)<sup>a</sup>**

Perchlorate Parameters	Lactation Values		Source
	Dam	Neonate	
<b>Permeability Area Cross Products, (L/hr-kg)</b>			
Gastric Blood to Tissue PAGc_	1.00	1.00	Fitted
Gastric Tissue to Juice PAGJc_	1.00	1.00	Fitted
Thyroid Blood to Tissue PATc_	4.0E-05/6.0E-04	4.0E-05/6.0E-05	Fitted <sup>b,c</sup>
Thyroid Tissue to Colloid PADTc_	0.01	0.01	Fitted
Skin Blood to Tissue PASKc_	0.50	1.00	Fitted
Mammary Blood to Tissue PAMc_	0.01	—	Fitted
Mammary Tissue to Milk PAMkc_	0.001/1.0		Fitted
Plasma to Red Blood Cells PRBCc_	1.00	1.00	Fitted
<b>Clearance Values, (L/hr-kg)</b>			
Urinary excretion CLUc_	0.07	0.005	Fitted
Dissociation from Binding Sites Clunbc_	0.034	0.034	Fitted
Transfer from Milk to Pup KtranSc	6.4E-04/1.04E-03	6.4E-04/1.04E-03	Sampson & Jansen, 1984

<sup>a</sup>All parameters listed are notated in the model either by an *i* (for iodide) or *p* (for perchlorate) following an underscore in the parameter name (e.g., PR\_*i*, PR\_*p*, Vmaxc\_*Ti*, Vmaxc\_*Tp*, etc.).

<sup>b</sup>Neonate was given maternal values for Vmax (scaled by body weight) in the absence of data.

<sup>c</sup>Parameters with two values indicate acute and drinking water parameters, respectively.

1 obtain these initial values for tissue loading at birth, the pregnancy model had to include all of  
 2 the compartments contained in the lactation model (Clewell, 2001a). The pregnancy model was  
 3 then allowed to run until the day of birth (GD22), and the average tissue concentrations of  
 4 perchlorate or iodide for the final day of gestation were used as the starting values for the  
 5 respective tissues in the lactation model (Clewell, 2001b).

6 As discussed, the mammary tissue has been shown to concentrate both perchlorate and  
 7 iodide during lactation via the NIS symporter. Additionally, hormones produced during lactation  
 8 such as prolactin which stimulates milk production, have been shown to regulate the mammary  
 9 NIS. Suckling of the neonatal rats has also been shown to stimulate mammary NIS activity  
 10 (Tazebay et al., 2000). An additional symporter has been identified in the experiments of  
 11 Shennan (2001). *In vitro* studies of iodide transport into the mammary gland and the resulting  
 12 efflux of sulfate from the cells in the absence of sodium cation (Na<sup>+</sup>), indicates that another form  
 13 of transport exists for iodide in the mammary gland in addition to the NIS. Shennan suggests

**TABLE 6-8. IODIDE-SPECIFIC PARAMETERS FOR LACTATING DAM AND NEONATE PBPK MODEL (Clewell, 2001b)<sup>a</sup>**

Iodide Parameters	Lactation Values		Source
	Partition Coefficients (unitless)	Dam	
Slowly Perfused/Plasma PS_	0.21	0.21	Halmi et al., 1956
Rapidly Perfused/Plasma PR_	0.40	0.40	Halmi et al., 1956
Fat/Plasma PF_	0.05	0.05	Pena et al., 1976
Kidney/Plasma PK_	1.09	1.09	Perlman et al., 1941
Liver/Plasma PL_	0.44	0.44	Perlman et al., 1941
Gastric Tissue/Gastric Blood PG_	1.00	1.00	Unpublished Lactation Inhibition Study
Gastric Juice/Gastric Tissue PGJ_	1.00	3.50	Unpublished Lactation Inhibition Study
Skin Tissue/Skin Blood PSk_	0.70	0.70	Perlman et al., 1941
Thyroid Tissue/Thyroid Blood PT_	0.15	0.15	Chow and Woodbury, 1970
Thyroid Lumen/Thyroid Tissue PDT_	7.00	7.00	Chow and Woodbury, 1970
Red Blood Cells/Plasma	1.00	1.00	Rall et al., 1950
Mammary Tissue/Mammary Blood PM_	0.66	—	Anbar et al., 1959
Milk/Mammary Tissue PMk_	4.00	—	Yu, 2000
<b>Max Capacity, Vmaxc (ng/hr-kg BW)</b>			
Thyroid Follicle Vmaxc_T	4.00E+04	4.00E+04	Fitted <sup>b</sup>
Thyroid Colloid Vmaxc_DT	6.00E+07	6.00E+07	Fitted <sup>b</sup>
Skin Vmaxc_S	6.00E+04	2.50E+05	Fitted
Gut Vmaxc_G	1.00E+06	2.00E+05	Fitted
Mammary Tissue Vmaxc_M	8.00E+05	—	Fitted
Milk Vmaxc_Mk	5.00E+06	—	Fitted
<b>Affinity Constants, Km (ng/L)</b>			
Thyroid Follicle Km_T	4.00E+06	4.00E+06	Gluzman and Niepomniszcze, 1983
Thyroid Colloid Km_DT	1.00E+09	1.00E+09	Golstein et al., 1992
Skin Km_S	4.00E+06	4.00E+06	Gluzman and Niepomniszcze, 1983
Gut Km_G	4.00E+06	4.00E+06	Gluzman and Niepomniszcze, 1983
Mammary Km_M	4.00E+06	—	Gluzman and Niepomniszcze, 1983
Milk Km_Mk	1.00E+06	—	Fitted
<b>Permeability Area Cross Products, (L/hr-kg)</b>			
Gastric Blood to Gastric Tissue PAGc_	0.80	0.05	Fitted
Gastric Tissue to Gastric Juice PAGJc_	0.60	0.06	Fitted
Thyroid Blood to Thyroid Tissue PATc_	1.00E-04	1.00E-04	Fitted <sup>b</sup>
Thyroid Tissue to Thyroid Colloid PADTc_	1.00E-04	1.00E-04	Fitted <sup>b</sup>
Skin Blood to Skin Tissue PASKc_	0.50	0.02	Fitted
Mammary Blood to Tissue PAMc_	0.02	—	Fitted
Mammary Tissue to Milk PAMkc_	1.00	—	Fitted
Plasma to Red Blood Cells PRBCc_	1.00	1.00	Fitted
<b>Clearance Values, (L/hr-kg)</b>			
Urinary excretion CLUc_	0.03	0.02	Fitted
Transfer from Milk to Pup Ktransc	6.4E-04 - 1.04E-03		Sampson & Jansen, 1984

<sup>a</sup>All parameters listed are notated in the model either by an *i* (for iodide) or *p* (for perchlorate) following an underscore in the parameter name (e.g., PR<sub>*i*</sub>, PR<sub>*p*</sub>, Vmaxc<sub>*Ti*</sub>, Vmaxc<sub>*Tp*</sub>, etc.).

<sup>b</sup>Neonate was given maternal values for Vmax (scaled by body weight) in the absence of data.

1 that this anion transport mechanism is able to transfer perchlorate and iodide into the secretory  
2 cells against a concentration gradient. Since the secretory cells are responsible for secreting their  
3 contents into the milk, the anion transport mechanism was included in the milk compartment of  
4 the Clewell (2001b) model.

5 The structure of the Clewell (2001b) neonatal model is similar to that of the pregnant and  
6 fetal rat model, with the exception of the mammary gland compartment as will be described in  
7 6.4.2.1.1. In order to simplify the model, all neonates from a single litter were combined in the  
8 structure of the model, essentially viewing the entire litter as one entity, or one large neonate.  
9 The dose to the neonate is based on the transfer of perchlorate from the maternal milk to the GI  
10 contents of the neonate rather than through direct exposure to the drinking water. The 60% of  
11 urinary excretion of the neonate is then entered back into the GI contents of the dam in order to  
12 account for maternal ingestion of the pup's urine during cleaning, based on the work of Samuel  
13 and Caputa (1965).

14 The same challenge posed by the pregnancy model (i.e., to describe perchlorate and iodide  
15 distribution in a highly dynamic system) was the objective of the lactating and neonatal rat model  
16 (Clewell, 2001b). In addition to total body weight changes in the dam and neonate, maternal  
17 mammary tissue and blood flow, cardiac output, fractional body fat and neonatal body weight,  
18 and fractional body fat change with respect to time. All tissue volume and blood flow values  
19 were adjusted with respect to the changing parameters.

20 Clewell (2001b) assumed the neonate to be nursing at a constant rate, 24 hours a day. This  
21 assumption is based on the fact that young nursing rats are unable to go for long periods of time  
22 without suckling. The loss through suckling was then described with a first order clearance rate  
23 from the mother's milk to the gastric juice of the neonate, based on the experiments of Sampson  
24 and Jansen (1984). The milk production rate was assumed to be equal to the amount of milk  
25 ingested by the litter.

## 26 27 **6.4.1 Data and Methods**

28 This section summarizes the data that Clewell (2001b) used for development and validation  
29 of the lactating and neonatal rat model structures. Details on experimental methods, including:  
30 protocol design, exposure regimen, chemical source and purity, animals (housing, feeding,

1 surgical procedures, etc.), and the analytical methods can be found in the consultative letter and  
2 associated reports from AFRL/HEST or papers cited therein.

### 3 4 **6.4.1.1 AFRL/HEST Experiments in Laboratory Rats**

5 These studies are described in the consultative letter and reports of Clewell (2001b), Yu  
6 (2000, 2002), Yu et al. (2000), and Mahle (2000; 2001).

#### 7 8 **6.4.1.1.1 Drinking Water Study**

9 Perchlorate drinking water experiments used in development of the Clewell (2001b) model  
10 included this study in which pregnant Sprague-Dawley dams were exposed to drinking water  
11 treated with perchlorate from GD 2 through PND5 or PND10 at perchlorate doses of 0.0, 0.01,  
12 0.1, 1.0, and 10.0 mg/kg-day. GD0 was determined by the presence of a vaginal plug. Litters  
13 were standardized to eight pups (four male and four female, when possible) on PND2. Dams and  
14 their litters were euthanized on either PND5 or PND10; maternal and neonatal serum was  
15 analyzed for fT4, tT4, T3, and TSH. Maternal serum, thyroid, skin, and gastric contents were  
16 analyzed for perchlorate at all doses. Neonatal serum, skin, and GI contents were also analyzed  
17 for perchlorate at all doses. Milk was analyzed only on PND10 at all doses. Perchlorate analysis  
18 was performed only on maternal gastric tract, mammary tissue, and neonatal gastric tract samples  
19 from the PND5 study at the 10.0 mg/kg-day dose. Two hours before euthanization, the dams  
20 were given iv doses of 33 mg/kg radiolabeled iodide ( $^{125}\text{I}$ ) with carrier. Tissue concentrations of  
21 iodide were measured in order to determine the inhibition in the various tissues after long-term  
22 exposure to perchlorate. This study is described in detail in the consultative letter (Yu, 2000).

#### 23 24 **6.4.1.1.2 Cross-fostering Study**

25 The cross-fostering study involved four groups of rats with varied experimental conditions:  
26 true control, control, exposed, and true exposed. True control rats were never dosed with  
27 perchlorate. Neonates remained with the dam after birth. In the control group, dams were never  
28 exposed to perchlorate in drinking water. However, at the time of birth, the neonates were  
29 replaced with pups (less than 24 hours old) that had been exposed to perchlorate throughout  
30 gestation (1.0 mg/kg-day to mother through drinking water). In the exposed group, the dams  
31 were dosed with 1.0 mg/kg-day perchlorate in drinking water from GD2 to PND10. At the time

1 of birth, the neonates were replaced with pups (less than 24 hours old) that had never been  
2 exposed to perchlorate. The true exposed dams were dosed with 1.0 mg/kg-day perchlorate from  
3 GD2 to PND10. Neonates remained with their mother after birth. All dams and pups were  
4 euthanized on PND10. The skin, GI contents, and serum from the neonates and dam were  
5 analyzed for perchlorate. Results indicated that both true control and control (exposed neonates  
6 with control dams) showed no perchlorate present on PND10. True exposed and exposed  
7 (exposed dams with control litters) showed comparable perchlorate levels on PND10. This study  
8 is described in detail in the consultative letters (Mahle, 2000; 2001).

#### 9 10 **6.4.1.1.3 Perchlorate Kinetics Study**

11 In order to evaluate the acute kinetics of perchlorate in the lactating dam and neonate,  
12 AFRL/HEST performed a study of the kinetic behavior of perchlorate after the administration of  
13 an acute dose. PND10 Sprague-Dawley dams were given 0.1 mg/kg perchlorate by tail-vein  
14 injection. The dams were left with their neonates until the time of euthanization at 0.5, 1, 2, 4, 8,  
15 or 12 hours post-dosing. Maternal serum, thyroid, stomach contents, skin, and mammary gland  
16 were collected and analyzed for perchlorate content at all time points. Neonate serum, stomach  
17 contents, and skin were also collected for perchlorate analysis at all time points. Fat, liver,  
18 kidney and bladder tissues were also collected from the dam at the eight hour time point.  
19 Perchlorate analysis was performed on the serum of the dam and neonates and the maternal  
20 thyroid, mammary gland, GI contents, and skin.

#### 21 22 **6.4.1.1.4 Iodide Inhibition Kinetics Study**

23 A study of iodide time course and inhibition kinetics was performed by AFRL/HEST in  
24 which Sprague-Dawley timed-pregnant dams were given 1.0 mg/kg body weight perchlorate via  
25 tail-vein injection on PND10. The perchlorate dose was followed at two hours post-dosing with  
26 a tail-vein injection of carrier free radiolabeled iodide ( $^{125}\text{I}$ ) at an average dose of 2.10 ng/kg.  
27 Dams (n=6) were euthanized after 0.5, 1, 2, 4, 8, and 24 hours. Maternal and neonatal serum,  
28 skin, GI contents and tract, as well as the maternal thyroid and mammary gland tissue, were  
29 collected and analyzed for total iodide content at each time point. Neonatal serum was pooled by  
30 sex in each litter. Neonatal skin and GI contents and tract were analyzed individually.

## 6.4.1.2 Data Published in the Literature

Data available in the literature and used in development and validation of the model are described briefly in this section.

### 6.4.1.2.1 Sztanyik and Turai, 1988

Five groups of CFY albino rats (BW = 200 to 250 g) were dosed ip with either 370 kBq (0.081 ng) or 740 kBq (1.61 ng) carrier-free radiolabeled iodide ( $^{131}\text{I}$ ) on PND1 (after 24 hours). Sztanyik and Turai measured the total iodide burden of each litter at 29 hours and on PNDs 2, 5, 7, 9, and 14. Since the litters were not standardized, the number of pups in each litter varied.

### 6.4.1.2.2 Potter et al., 1959

Four dams of the Long-Evans strain (PND 17-18) were dosed ip with 500  $\mu\text{Ci}$  of carrier-free radiolabeled iodide ( $^{131}\text{I}$ ). Iodide uptake was measured in the milk and plasma of the dam 3, 6, and 24 hours postdosing and in the maternal thyroids 24 hours postdosing.

## 6.4.2 Lactating and Neonatal Rat Model Development

This section summarizes only the key features that were different than the preceding model structures described in Sections 6.2 and 6.3.

### 6.4.2.1 Physiological Parameters and Partition Coefficients

Maternal parameters were scaled allometrically based on body weight as previously described for the male rat. Neonatal values were scaled in the same manner as the maternal parameters. However, since the model actually represents several neonates, it was necessary to scale the values for the individual pup first, then to adjust for the total number of pups in the litter as was done in an analogous fashion as for the fetuses in the pregnant rat model (Clewell, 2001a,b).

#### 6.4.2.1.1 Maternal Tissues

During lactation, the mammary gland grows in response to the increased need for milk production by the growing neonates. Knight et al. (1984) measured the mammary gland on several days during lactation. They found the mammary tissue to be 4.4, 5.6, 6.3, and 6.6% of

1 the maternal body weight on days 2, 7, 14, and 21, respectively. The residual milk was assumed  
2 to be 0.002 L based on the model of Fisher et al. (1990). Naismith et al. (1982) examined the  
3 change in body fat content of the lactating rat. They reported values for the volume of maternal  
4 body fat of 15.2 and 6.9% of the body weight on PND 2 and 16, respectively. The body fat  
5 composition of the dam on PND1 was calculated to be 12.4% from the PBPK model for  
6 perchlorate and iodide kinetics in the pregnant rat model described in Section 6.3 (Clewell,  
7 2001a).

8 In order to describe the changes in the physiology of the lactating rat, it was not sufficient  
9 to simply scale some of the parameters allometrically. As opposed to the typical growth  
10 scenario, some of the tissues in the lactating rat cannot be assumed to increase at the same rate in  
11 this dynamic system. Rather, a few tissues, such as the mammary gland and fat, are changing at  
12 an accelerated rate in comparison to the other organs. These parameters required additional  
13 descriptions for their growth beyond the previously described allometric scaling by body weight.  
14 Clewell (2001b) based the approach to modeling these changing parameters on the work of  
15 Fisher et al. (1990) with trichloroethylene.

16 Additionally, the thyroid of the female rat was found by investigators to be significantly  
17 larger than that of the male rat (Malendowicz and Bednarek, 1986). Clewell (2001b) assigned  
18 values to these parameters based on these data and relevant to the gender and condition (i.e.,  
19 lactation) of the animal. A value of 1.05% of the maternal body weight was used for the thyroid  
20 in the lactation model. The volume fractions of the colloid, follicle, and stroma were given  
21 values of 45, 46, and 9% of the thyroid volume. These are significantly different from the values  
22 given for the male rat. The volume of the colloid in particular is much greater in the female than  
23 the male rat (46 vs. 24% of the thyroid volume). Parameters that were not available specifically  
24 for the female were described by adjusting the values for the male rat by body weight.

25 In the PND10 drinking water study performed by AFRL/HEST (see Attachment #2;  
26 Clewell, 2001b), the body weight of the dam showed an average increase of 12% between PND1  
27 and PND10, but did not show a significant difference in weight between dose groups. As a  
28 result, Clewell (2001b) calculated the average body weight of the dams for all dose groups for  
29 each day of the study and then programmed this changing body weight into the model as a table  
30 function.

#### 6.4.2.1.2 Neonatal Tissues

As for the lactating rats, Clewell (2001b) programmed the overall average body weights of the neonates measured on PNDs 3, 5, 7, 9 and 10 into the model as a table function, in order to estimate growth. Naismith et al. (1982) reported the body fat in the pup at PND2 and PND16 to be 0.167 and 3.65% of the neonatal body weight. The amount of body fat in a 41-day old rat was given in Brown et al. (1997) as 4.61% of the body weight.

The volume of the thyroid was studied by Florsheim et al. (1966). The volume of the thyroid was found to increase in a fairly linear relationship with body weight between PND1 and PND22. These investigators reported thyroid volumes of 0.0125, 0.0146, 0.0120, 0.0137, 0.0130, 0.0130, and 0.0131% body weight for neonates on PND1 through 5, 7, and 11. These values were used in a table function in the model to describe the growth of the neonatal thyroid (Clewell, 2001b). The histometry of the thyroid in the neonate was examined by Conde et al. (1991). The authors found a significant difference between the volume fractions of the colloid, follicle and stroma in the neonatal rat versus those in the adult. The reported values of 18.3, 61.4, and 20.3% thyroid volume were used to describe the colloid, follicle, and stroma fractions in the neonatal rat (Clewell, 2001b).

The suckling rate of the neonatal rat has been examined in more than one literature study and has been shown to change over time in response to the growth of the neonatal rats. As the pups grow, they require larger amounts of milk. Sampson and Jansen (1984) measured the amount of milk suckled in rats by removing neonates from the dams for two hours and then allowing the pups to suckle for two hours. This process was repeated throughout the day on several days of lactation. By assuming that the weight gained by the neonates during the suckling period was due to the milk intake and the weight lost while separated from the dam was through excretion, Sampson and Jansen were able to develop an equation that describes the suckling rate of the neonatal rat. Since this equation is dependent on the body weight and growth rate of the neonates, it is able to account for the change over time and the difference between strains and studies. The equation was used in the Clewell (2001b) model which assumed the milk yield of the dam was equal to the suckling rate of the neonate.

### 1 **6.4.2.1.3 Blood Flows**

2 All maternal and neonatal blood flows that were not directly affected by the changes  
3 induced by lactation were scaled by weight from the adult male rat parameters. For those blood  
4 flow parameters that change in response to lactation, some additional description was required  
5 (Clewell, 2001b). Cardiac output has been shown to increase during lactation (Hanwell and  
6 Linzell, 1973). The values given by Hanwell and Linzell (1973) of 14.0, 18.6, 19.0, and  
7 21.0 L/hr-kg for days 3, 8, 13, and 23 of lactation were used in the model as a table function to  
8 describe the change in cardiac output over time (Clewell, 2001b). Additionally, the blood flow  
9 to the mammary tissue was also found to increase during lactation. Reported fractional blood  
10 flows to the mammary tissue of 9, 10, 11, 14, 14, and 15% of the cardiac output on PNDs 1, 5,  
11 10, 15, 17, and 21, again from Hanwell and Linzell (1973), were used.

### 13 **6.4.2.2 Chemical-Specific Parameters**

14 The various active transport processes, tissue permeabilities and clearance rate (excretion)  
15 are described in PBPK models for each species on a chemical-specific basis. This section  
16 outlines how the values for perchlorate and iodide used in the lactating and neonatal rat model  
17 were derived. The values can be found in Tables 6-7 and 6-8. Details on the derivation can be  
18 found in Clewell (2001b).

#### 20 **6.4.2.2.1 Affinity Constants and Maximum Velocities for Active Uptake Processes**

21 Whenever possible, chemical specific parameters were kept the same in human and in  
22 male, female, neonatal, and fetal rats. However, it was necessary to change a few of the  
23 parameters, including the maximum velocity capacity ( $V_{maxc}$ ). The  $K_m$  values were similar  
24 between tissues and between female and male rat and human models. However, the maximum  
25 velocity capacity differs between tissues (Wolff and Maurey, 1961). Since values for the tissue  
26 maximum velocity capacity for perchlorate ( $V_{maxc-p}$ ) were not given in literature, the values  
27 were estimated with the model. In order to determine  $V_{max}$  with the model, the simulation for  
28 the tissue of interest was compared to various data sets with several different perchlorate dose  
29 levels. The value for  $V_{maxc}$  within a given compartment was then determined by the best fit of  
30 the simulation to the data.

#### 6.4.2.2.2 Effective Partitions, Permeability Area Cross Products and Clearance Values

Anbar et al. (1959) measured the mammary gland:blood ratios in the rat four hours after an intra-peritoneal injection of 100 mg radiolabeled perchlorate ( $^{36}\text{ClO}_4^-$ ) as potassium perchlorate. They reported an effective partition of 0.66 for the rat mammary gland. Clewell (2001b) used this value in the model. Since the partition for iodide into the mammary gland was not available in the literature, Clewell (2001b) assigned the same effective partition coefficient as used for perchlorate.

When available, iodide partition coefficients were calculated from the tissue:blood ratios measured during the clearance phase of iodide data in the tissue of interest. For example, GI tract and contents were determined from the clearance portion of the data from the iodide kinetic study in the lactating rat.

For tissues in which a clearance was described (urinary clearance and dissociation of perchlorate from the binding sites), a clearance value was determined by fitting the model simulation to the appropriate tissue data. Since perchlorate is quickly excreted in urine and binding has little effect on serum levels at high doses, the simulation for the 10 mg/kg-day dose group was primarily dependent on the urinary clearance value ( $\text{ClUc}_p$ ). The urinary clearance value for perchlorate was therefore based on the fit of the model to the serum data at the high dose. The value obtained in this manner was similar to that determined by fitting the male rat PBPK simulation to urinary perchlorate at several doses (Merrill, 2001a) and to the high dose in the pregnant rat (Clewell, 2001a). The rate of dissociation of perchlorate from the binding sites was fit to the serum data across doses.

#### 6.4.2.3 Lactating Rat and Neonate Model Parameterization and Validation

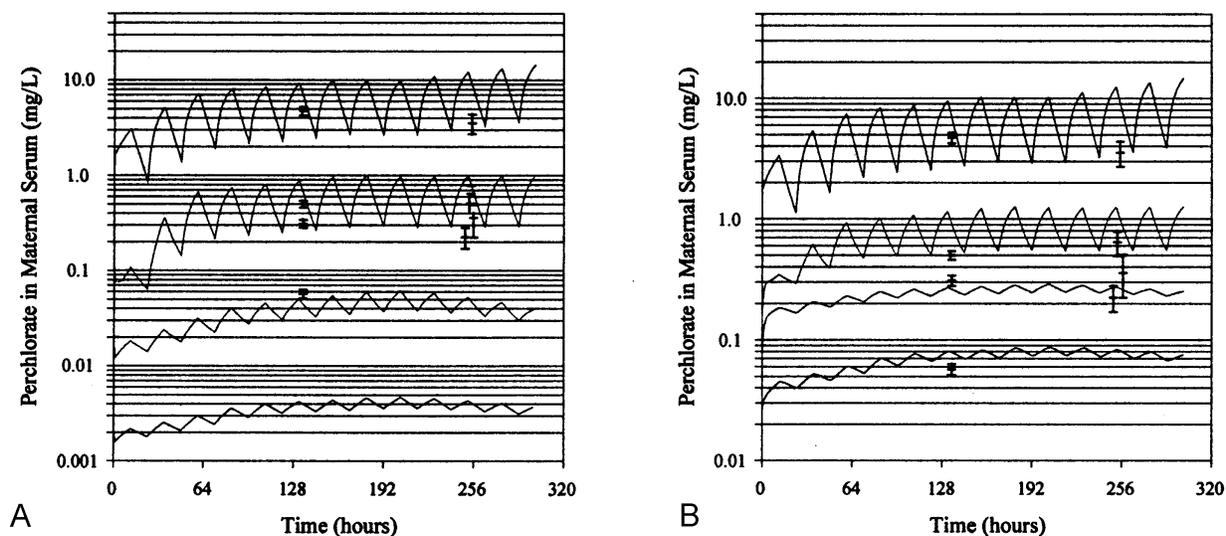
This section summarizes how Clewell (2001b) used the various data sets to parameterize the model and how the validation exercises were performed.

##### 6.4.2.3.1 Perchlorate Model Parameterization

Clewell (2001b) performed model parameterization for perchlorate using the data obtained for the tissues from the AFRL/HEST drinking water studies on PND5 and PND10. Optimized kinetic parameters ( $V_{\text{maxc}}$  and permeability area) were determined by visually fitting the model simulation to the experimental data. As for the previous model structures (adult male rat, human,

1 pregnant rat and fetus), it was necessary to account for the serum binding of perchlorate in order  
2 to adequately describe the serum perchlorate concentrations at the lower doses (0.01 and  
3 0.1 mg/kg-day). Figure 6-38 illustrates the importance of binding in the model simulations in the  
4 dam on these days.

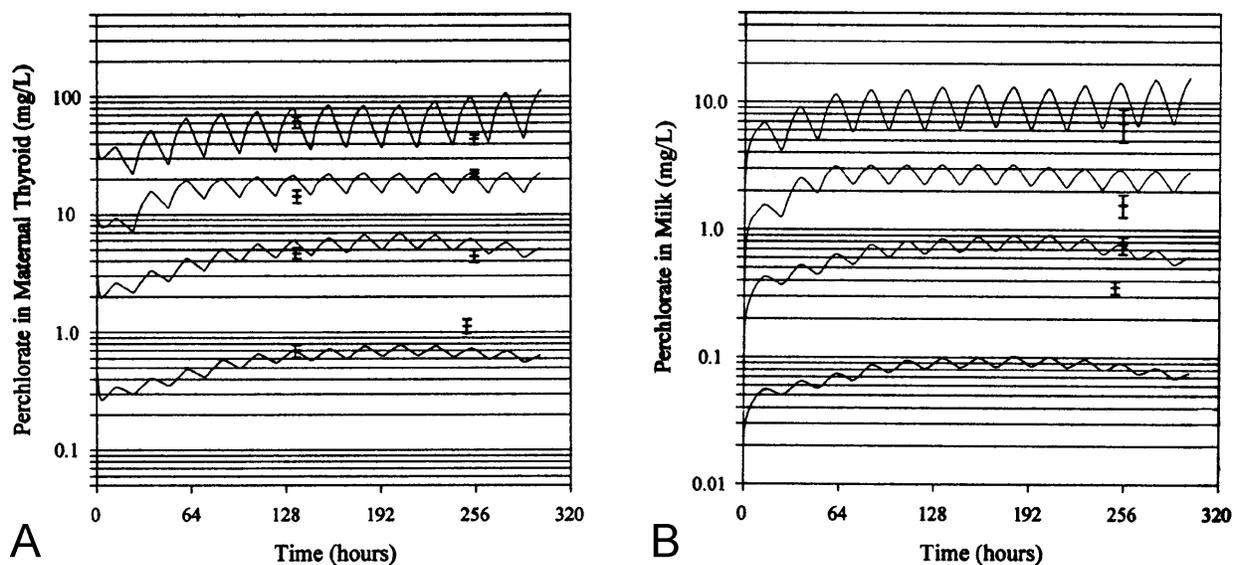
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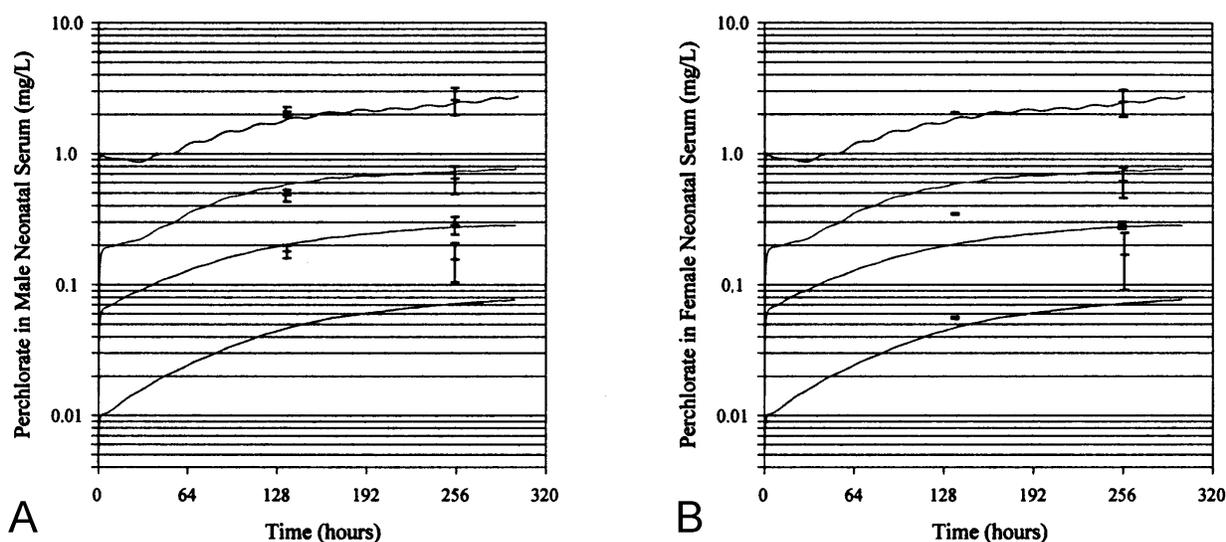
**Figure 6-38. Simulations illustrating the necessity of including plasma binding in the lactating dam and neonatal rat PBPK model structure (Clewell, 2001b). Model predictions (lines) versus data time course (mean  $\pm$  SD) for maternal serum perchlorate concentrations (mg/L) on PND5 and PND10 at doses to the dam of 10.0, 1.0, 0.1, and 0.01 mg/kg-day are shown with (A) and without (B) plasma binding.**

1 Figure 6-39 shows the perchlorate tissue concentrations (mg/L) in the lactating dam thyroid  
2 (A) and in maternal milk (B) at PND5 and PND10 for the 0.01, 0.1, 1.0 and 10.0 mg/kg-day  
3 doses. It was noticed that during the drinking water studies, the daily dose to the dams varied  
4 somewhat due to their changing water intake. Therefore, all of the model simulations of the  
5 drinking water studies reflect the actual daily dose to the dam, which Clewell (2001b) calculated  
6 from the daily water consumption and body weight measurements.

7 Figure 6-40 shows the model simulations of the male and female neonate plasma levels  
8 compared to the data obtained in the AFRL/HEST drinking water study. Plasma concentrations



**Figure 6-39.** Lactating dam and neonatal rat PBPK model predictions (lines) versus data time course (mean  $\pm$  SD) of perchlorate concentrations (mg/L) in the maternal thyroid (A) and milk (B) on PND5 and PND10 at doses in drinking water to the dam of 10.0, 1.0, 0.1, or 0.01 mg/kg-day perchlorate (Clewell, 2001b).



**Figure 6-40.** Lactating dam and neonatal rat PBPK model predictions (lines) versus data time course (mean  $\pm$  SD) of perchlorate concentrations (mg/L) in the serum of male (A) and female (B) neonates on PND5 and PND10 at doses in drinking water to the dam of 10.0, 1.0, 0.1, or 0.01 mg/kg-day perchlorate (Clewell, 2001b).

1 varied significantly between the male and female neonates, and Clewell (2001b) noted that the  
2 difference appears to be a function of age. At PND5, the male neonatal plasma concentrations  
3 were nearly 4 times higher than those of the female neonates in the 0.1 mg/kg-day dose group.  
4 By PND10, however, no significant sex difference was found in the plasma perchlorate  
5 concentrations at the same dose.

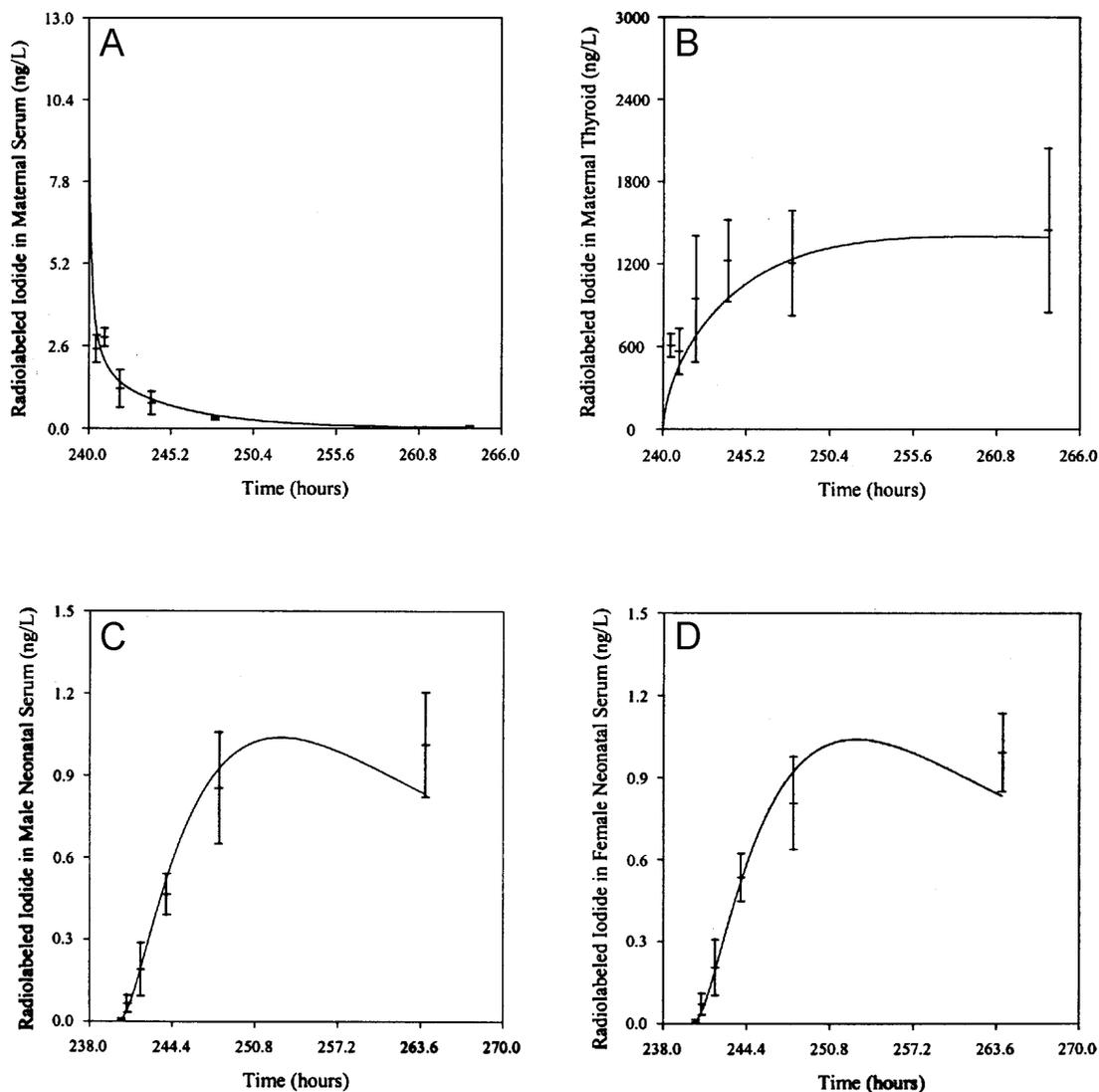
6 Clewell (2001b) fit the male neonatal serum data because the male pups showed higher  
7 perchlorate concentrations in the serum than the female pups (Yu, 2000). The neonatal serum  
8 was under-predicted by the model in the 0.01 mg/kg-day dose group. Clewell (2001b) strongly  
9 asserts that this was due to the fact that the milk concentration was also under-predicted in that  
10 same dose group. The three higher doses are well described in the male neonate. The female  
11 pups also show acceptable fits at PND10. However, since the PND5 data were much lower in  
12 the female than male neonates, the model over-predicts the PND5 time-points in the 0.1 and  
13 1.0 mg/kg-day doses. Fits of the model to neonatal skin and GI tract are discussed in Clewell  
14 (2001b).

15 As in the maternal model, the clearance value for urinary excretion was determined by the  
16 fit of the model to the serum from the 10 mg/kg-day dose, while the lower doses were used to  
17 determine the kinetic parameters for the binding in the neonate. Both binding and urinary  
18 clearance were considerably lower in the pup than in the dam (Table 6-7).

#### 20 **6.4.2.3.2 Iodide Model Parameterization**

21 Clewell (2001b) developed the iodide aspect of the model by visually fitting the model to  
22 measured tissue concentrations in the dam and neonate from the control group of the inhibition  
23 kinetic study. Only the values for  $V_{max}$  and permeability area needed to be fit with the model.  
24 As shown in Figure 6-41, the model simulations of iodide concentrations (ng/L) after an iv  
25 injection of 2.10 ng/kg radio-labeled iodide ( $^{125}\text{I}$ ) on PND10 versus the experimental data in the  
26 lactating dam are shown in the dam serum (A) and thyroid (B) and in male (C) and fetal (D)  
27 neonatal serum.

28 The model simulations describe the data well with the exception of the longest time point  
29 in the neonates. The clearance value for urinary excretion was determined by fitting the maternal  
30 serum prediction to the above data while keeping good fits in the other tissues, such as maternal  
31 skin, GI, and mammary gland (Clewell, 2001b). Permeability area values were adjusted to



**Figure 6-41. Lactating dam and neonatal rat PBPK model predictions (lines) versus data time course (mean  $\pm$  SD) of iodide concentrations (mg/L) in the maternal serum (A) or thyroid (B) and in male (C) or female (D) neonatal pups on PND10 after an iv dose to the lactating dams of 2.10 ng/kg  $^{125}\text{I}^-$  (Clewell, 2001b). Data of Yu (2000, 2002).**

- 1 describe the behavior of the iodide data; varying the permeability area values toward 1.0 L/hr-kg
- 2 generally increased the rate at which uptake and clearance in a particular tissue occurred;
- 3 decreasing permeability area slowed the uptake and clearance.

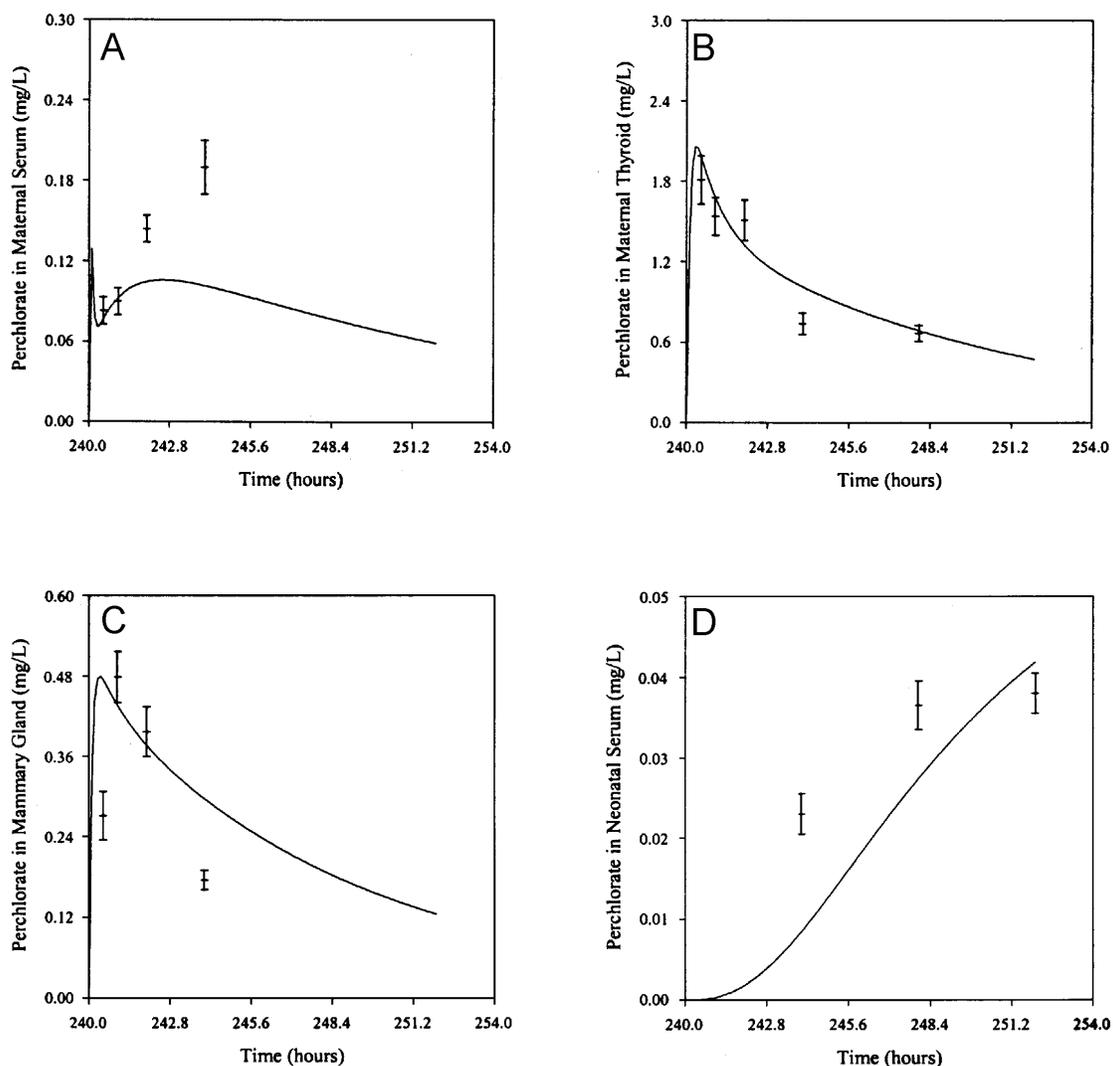
1 The behavior of the iodide in the neonatal skin and GI tract and contents appeared to be  
2 different from the dam. The iodide tended to stay in the tissue of the neonate longer, requiring a  
3 slower clearance in the fetal tissues than was used in the corresponding maternal tissue. As a  
4 result, permeability area values used for the GI and skin in the neonate were lower than those  
5 used in the dam (Table 6-8). For example, the permeability area value in the skin was  
6 determined to be 0.5 L/hr-kg in the dam, but was decreased to 0.02 L/hr-kg in the neonate.  
7 However, these values correspond well to the values used for the fetus in the pregnancy model  
8 (Clewell, 2001a).

9 The neonatal urinary clearance value was determined to be 0.02 L/hr-kg in the neonate,  
10 which is very similar to the maternal value (0.03 L/hr-kg of the dam). This was a surprise,  
11 because the neonate was expected to have a much lower rate of excretion than the more mature  
12 dam; however, Clewell (2001b) notes that this trend is supported in the literature. Capek and  
13 Jelinek (1956) measured the amount excreted by pups at various ages. The neonates required  
14 external stimulation by the mother in order to release the urine from their bladders. However,  
15 when that stimulation was supplied, the neonates were able to excrete urine at the same rate as an  
16 adult rat. Therefore, it is reasonable that the urinary excretion rate is similar between the pup and  
17 adult. The amount of iodide lost to urine is then dependent on both the urinary clearance value  
18 and the concentration of the ion in the kidney (Clewell, 2001b).

### 20 **6.4.3 Model Validation**

21 The ability of the model to simulate the kinetics of perchlorate in the lactating dam and  
22 neonate was tested against the perchlorate time course data collected *in vivo* by AFRL/HEST.  
23 Since the study was performed with an acute perchlorate dose, it was necessary to make minor  
24 changes in the thyroid perchlorate parameters. The long-term exposure to perchlorate in the  
25 drinking water studies that were used to determine the perchlorate parameters is sufficient to  
26 induce up-regulation in the thyroid (Yu, 2000). Therefore, the thyroid parameters in the dam at  
27 this point would be different from those seen in an acute situation. Clewell (2001b) achieved the  
28 model fits to the acute data by altering the partition coefficient (from 2.25 in the drinking water  
29 to 0.13 in the acute exposure) and permeability area value (from 6.0E-4 to 4.0E-5) into the  
30 thyroid at the basolateral membrane (thyroid follicle). The value for the partitioning into the  
31 follicle in a naïve thyroid was calculated as described previously from Chow and Woodbury

1 (1970). The permeability area value in the naïve thyroid follicle was determined by fitting the  
2 model prediction to the thyroid data, while keeping good fits in the serum and other tissues.  
3 Figure 6-42 shows the model predictions versus the data time course of perchlorate  
4 concentrations in maternal serum (A), thyroid (B), or mammary gland (C) and in neonatal serum.  
5  
6

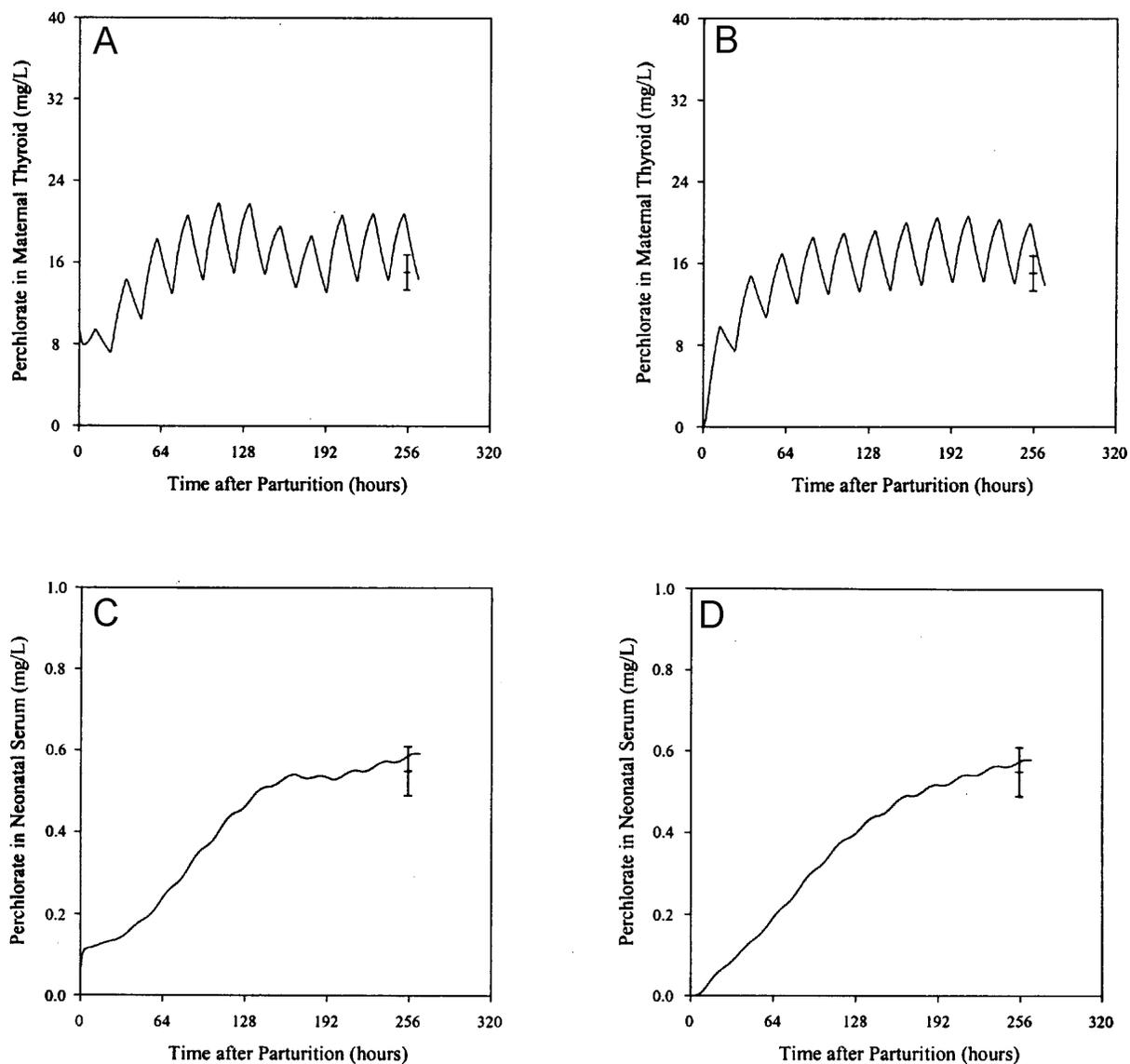


**Figure 6-42. Validation for lactating dam and neonatal rat PBPK model (Clewell, 2001b). Model predictions (lines) versus data time course (mean  $\pm$  SD) of perchlorate in the maternal serum (A), thyroid (B), or mammary gland (C) and in neonatal serum (D) after an iv dose of  $1.0 \times 10^6$  mg/kg perchlorate on PND10. Data of Yu (2000, 2002) and Yu et al. (2000).**

1           The maternal serum is not fit particularly well and the neonatal serum fit could also be  
2 improved. Clewell (2001b) notes the difficulties may be due to the use of the iv kinetic data as  
3 well as some additional challenges not yet met by the model with respect to the mammary gland.  
4 Clewell (2001b) increased the transfer of perchlorate through the milk in the acute studies in  
5 order to fit the model derived on drinking water studies to these acute (iv) data. That is, the  
6 value for the  $V_{max}$  into the mammary tissue was increased in order to allow more perchlorate  
7 into the mammary compartment, and the permeability area into the milk was decreased in order  
8 to minimize the back flow of perchlorate into the mammary from the milk. This essentially  
9 forced the perchlorate in the milk to be passed to the neonate rather than return to the mammary  
10 tissue of the mother. The  $V_{maxc}$  for the binding in the neonate was decreased slightly from the  
11 value used in the drinking water simulations. This may have been due to increased transfer of  
12 iodide in the acute simulations. When the same parameters were used in the mammary  
13 compartment that were determined with the drinking water studies, the amount in the mammary  
14 tissue was low and the clearance of the mammary was too slow. As a result, acute neonatal  
15 serum levels were under-predicted. By adjusting the  $V_{maxc}$ , the model was able to achieve  
16 reasonable fits to the available data in the maternal and neonatal tissues. Clewell (2001b)  
17 suggests that different fractions of the dose are transferred through the milk during an acute (iv)  
18 exposure versus a drinking water scenario.

19           Figure 6-43 shows the model predictions against the data obtained in the AFRL/HEST  
20 cross-fostering study described in Section 6.4.1.1.2. Perchlorate concentrations (mg/L) in the  
21 maternal thyroid of dams exposed during gestation (A) or only during lactation (B) show similar  
22 results. Perchlorate concentrations (mg/L) in neonatal serum exposed only during gestation (C)  
23 or only during lactation (D) also contained similar levels. Because the data were taken on  
24 PND10, the sex difference seen at the earlier time points was not present and the simulation is  
25 shown for the average of all pups.

26           The model is able to predict the data from the cross-fostering study very well. It is apparent  
27 from the data and from the model prediction of the cross-fostering data that the gestational  
28 exposure to perchlorate does not affect the perchlorate concentrations of the maternal serum and  
29 thyroid or the neonatal serum. This is in agreement with other studies that indicate the rapid  
30 clearance of perchlorate in the urine (Yu et al., 2000), but not in agreement with the toxicological  
31



**Figure 6-43. Validation for lactating dam and neonatal rat PBPK model (Clewell, 2001b). Model predictions (lines) versus data time course (mean  $\pm$  SD) of perchlorate in the maternal thyroid during gestation (A) or during lactation only (B) and in the neonatal serum during gestation (C) or during lactation only (D) after an iv dose of  $1.0 \times 10^6$  mg/kg perchlorate on PND10. Data of Mahle (2001).**

1 observations between the 1998 and 2001 developmental neurotoxicological studies performed by  
 2 Argus Research Laboratories, Inc. (1998; 2001). Differences in the hormone data are discussed  
 3 in Clewell (2001c) and other differences may be due to strain differences (Fail et al., 1999).  
 4 From the model, even though the neonatal urinary excretion is much lower than that of the dam

1 (0.005 vs. 0.07 L/hr-kg), the prenatal exposure does not affect the serum levels of the neonate  
2 past PND2. This is in accord with the observations made of the BMDL estimates for the post-  
3 natal thyroid discussed in Chapter 5.

4 Additional validation exercises were performed by Clewell (2001b), showing reasonably  
5 adequate model fits to the data of Potter et al. (1959) and that of Sztanyik and Turai (1988) as  
6 shown in Clewell (2001b). Maternal radiolabeled iodide concentrations were overpredicted in  
7 the thyroid on PND18. The maternal milk concentrations were also overpredicted for the earlier  
8 time point, but were within the range at the later. The model predicted the radiolabeled iodide  
9 data obtained in the litters of Sztanyik and Turai (1988) quite well. This indicates that the  
10 lactation and neonatal kinetics are characterized accurately.

11 Figure 6-44 shows that the Clewell (2001b) model is able to predict the radiolabeled iodide  
12 ( $^{125}\text{I}$ ) uptake-inhibition data in maternal thyroids on PND10 from the AFRL/HEST “acute” (iv)  
13 studies with perchlorate. The inhibition was described well by the model across the range of  
14 time points from 0.5 to 24 hours postdosing. The top line indicates the prediction for the control  
15 thyroid, and the bottom line shows the effect of perchlorate. The model is able to describe the  
16 kinetics of iodide under both conditions.

17 The Clewell (2001b) model is also able to predict the radiolabeled iodide uptake inhibition  
18 data from AFRL/HEST obtained after “chronic” drinking water exposures. Figure 6-45 shows  
19 the radiolabeled iodide ( $^{125}\text{I}$ ) concentrations (mg/L) in the maternal thyroids at PND5 after  
20 23 days of dosing with perchlorate at 0.0, 0.01, 1.0, and 10.0 mg/kg-day.

#### 22 **6.4.4 Summary**

23 Clewell (2001b) highlights some important differences in the lactating dam and neonatal rat  
24 model structure that were necessary in order to adequately describe the distribution kinetics of  
25 perchlorate and iodide. The loss of iodide and perchlorate in the milk results in much faster  
26 clearance rates of the anions from the dam. Studies also suggest that the loss of iodide to the  
27 mammary gland and milk decreases the iodide available for the maternal thyroid (Brown-Grant,  
28 1961; Yu, 2000; Yu et al., 2000). The thyroidal maximum capacities are lower in the lactating  
29 and pregnant dam than in the male rat. Model parameterization in the male rat indicated the need  
30 for  $V_{\text{maxc}}$  values for uptake into the follicle of the thyroid of  $2.2 \times 10^3$  L/hr-kg for perchlorate  
31 and  $5.5 \times 10^4$  L/hr-kg for iodide while the gestation model required values of  $1.5 \times 10^3$  L/hr-kg

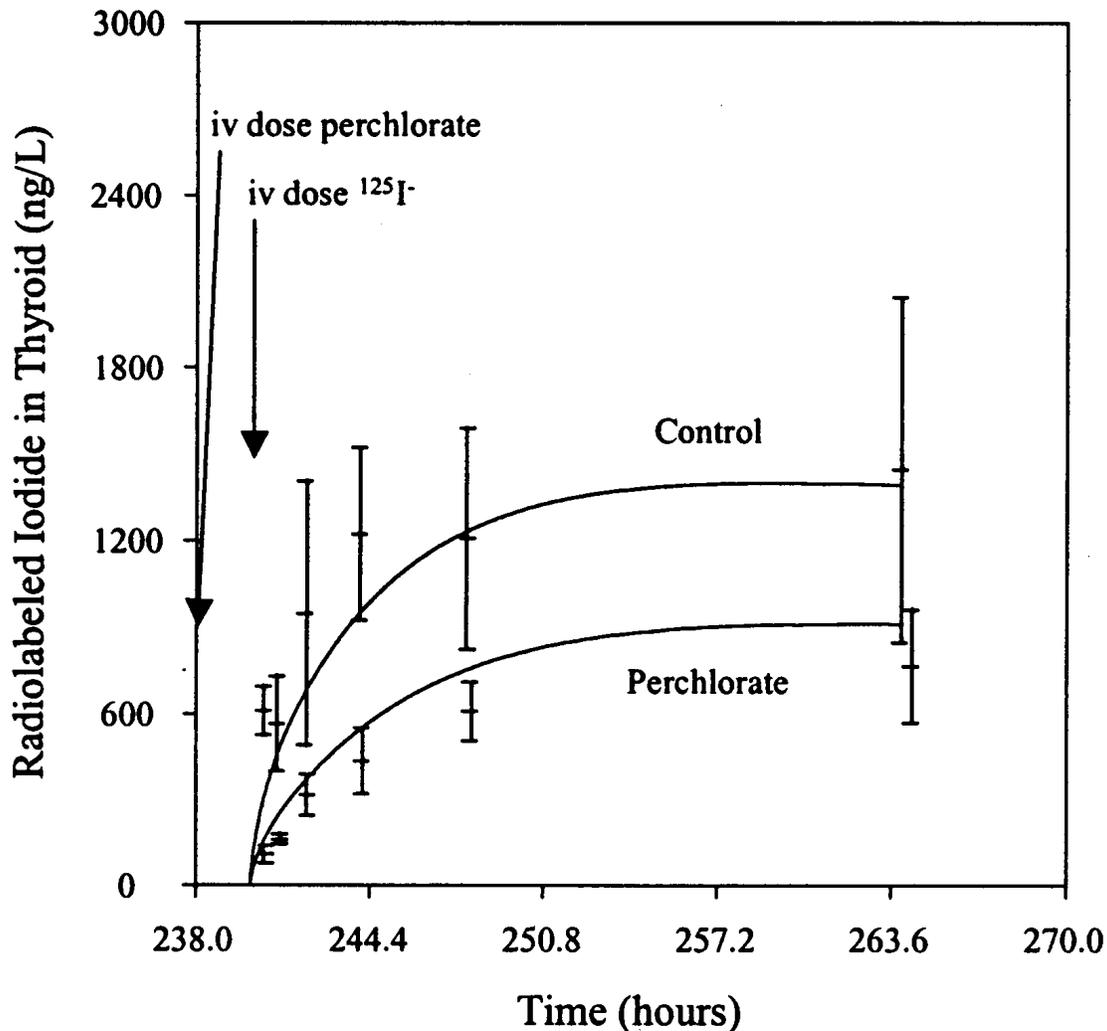
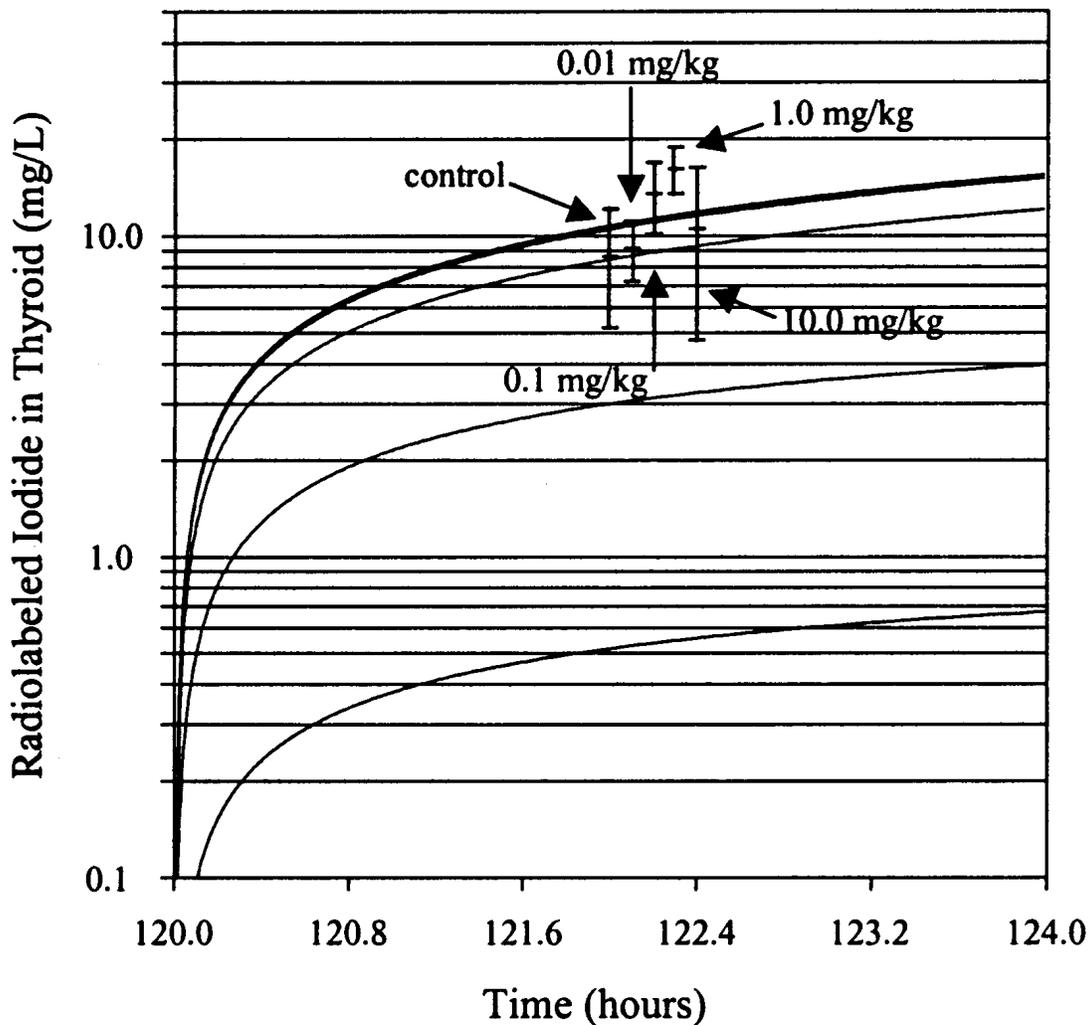


Figure 6-44. Validation for lactating dam and neonatal rat PBPK model (Clewel, 2001b). Model predictions (lines) versus data time course (mean  $\pm$  SD) of <sup>125</sup>I- radiolabeled iodide in the maternal thyroid with and without an iv dose of perchlorate at 1.0 mg/kg perchlorate 2 hours prior to an iv dose of 2.10 ng/kg <sup>125</sup>I- to the dam on PND10. The top simulation indicates the control thyroid and the lower indicates the inhibited thyroid. Data of Yu (2000) and Yu et al. (2000).

- 1 and  $4.0 \times 10^4$  L/hr-kg for the same parameters. This difference is supported in the literature.
- 2 Versloot et al. (1997) suggest that the pregnant rat may have a lowered reserve of iodide in the
- 3 thyroid toward the end of pregnancy, causing increased activity in the thyroid. This may also be
- 4 true in the lactating rat. The skin of the lactating dam also required a smaller value for  $V_{maxc}$



**Figure 6-45. Validation for lactating dam and neonatal rat PBPK model (Clewley, 2001b). Model predictions (lines) versus data time course (mean  $\pm$  SD) of  $^{125}\text{I}^-$  radiolabeled iodide in the maternal thyroid on PND5 after 23 days dosing with perchlorate in drinking water at 0.0, 0.1, 1.0, and 10.0 mg/kg-day. All experimental data were taken two hours post-dosing. Data of Yu et al. (2000).**

1 than the male rat. This is supported by the work of Brown-Grant and Pethes (1959), who  
 2 reported higher levels of iodide in the skin of male rats than in female rats. Skin, therefore,  
 3 appears to be a more important iodide reserve in the male rat than the female.

4 The described PBPK lactation model is able to predict the distribution of perchlorate in the  
 5 tissues of active uptake and serum of the lactating dam and neonate on PND5 and PND10 after  
 6 exposure to perchlorate in drinking water. Perchlorate distribution in this dynamic system is

1 described utilizing a pharmacokinetic approach to the modeling and accounting mathematically  
2 or physiological changes, such as changing tissue volumes and maternal and neonatal growth.  
3 The model predicts the transfer of perchlorate to the neonate and is also able to describe the  
4 uptake into tissues of interest in the neonate, such as the GI contents and skin; however, the EPA  
5 believes that both the maternal and neonatal serum fits could be improved. This may already be  
6 accomplished with the additional data to which Clewell (2001b) alludes or, as noted previously,  
7 the radionuclide modeling efforts of the ICRP (2001, 1989) may be informative.

8 The kinetic behavior of iodide is well described with the existing model, in spite of the  
9 physiological complexity of the described system. The dam and neonate were accurately  
10 simulated at a range of doses that spans four orders of magnitude (2.10 to 33,000 ng/kg) between  
11 days 1 and 18 of lactation. The active sequestration of iodide in maternal and neonatal tissues  
12 and the transfer of iodide between mother and neonate was described kinetically with the model;  
13 data have been simulated at a variety of doses and at various time points up to 14 days after  
14 exposure. The fact that the model was able to simulate data from other laboratories under a  
15 variety of different conditions attests to the validity of the model structure and its applicability to  
16 other studies. This also provides greater confidence in the model structure.

17 The clear differences between the perchlorate data from iv and drinking water studies draw  
18 attention to unresolved issues in the transfer kinetics of perchlorate. Although lactational transfer  
19 has long been studied, the transport mechanisms of this ion have yet to be elucidated in the  
20 literature. A second transporter has been identified in the mammary gland, which actively  
21 transports anions against the chemical gradient. However, the relationship of this transporter and  
22 the anion concentration resulting from prolonged exposure to the high doses of perchlorate used  
23 in these studies is not known. Clewell (2001b) suggests that it is possible that the high anion  
24 load resulting from the long-term exposure to perchlorate may have resulted in decreased  
25 transport of the ion. It is feasible that the movement of iodide may be regulated in the mammary  
26 tissue, because the ion is vital to the development of the newborn. The data obtained between  
27 the acute and drinking water studies suggest that a feedback mechanism is in place, because the  
28 model over-predicts the milk transfer in the drinking water data when the acute parameters are  
29 used. Clewell (2001b) notes in-house experiments that may help resolve these issues are  
30 currently underway. Additional data were provided by Yu (2002), but is not clear that all these  
31 data have been provided to the Agency or how these will be used to improve the modeling effort.

## 6.5 APPLICATION OF PBPK MODEL STRUCTURES TO INTERSPECIES EXTRAPOLATION

As discussed in the introduction to this chapter, the purpose of developing the proposed PBPK model structures was to aid interspecies extrapolation. All of the proposed model structures adequately describe both perchlorate and iodide distributions as evidenced by the fit of the model predictions against the experimental data shown in the preceding sections of this chapter. The degree of confidence in the model descriptions differed for the acute (iv) versus chronic (drinking water) data to some degree in the laboratory animals. A rather large degree of intersubject variability was evident among the human subjects, but in general the structures are accepted as quite sound and informative to the task.

The models do not link the perchlorate and iodide kinetics to perturbations in thyroid hormone. The existing data and current structures were not designed to address the complex issues involved with hormone homeostasis of the hypothalamic-pituitary-thyroid feedback axis as described in Chapter 3 or illustrated in the beginning of this chapter. Such a model would need to incorporate the hormone levels in tissues and serum and processes such as hormone production, storage, and secretion in the thyroid; conversion of T4 to T3 in the tissues; deiodination of T4 and T3 to less active forms and a feedback mechanism between the hormone levels, TSH, and the thyroid NIS. Kohn et al. (1996) developed a PBPK model that attempts to describe the effect of dioxin on thyroid hormones. Although perchlorate and dioxin act on the endocrine system through different modes of action, it is likely that a similar approach to that of Kohn et al. (1996) would be required to begin to address the hormone feedback system in the case of perchlorate. Parameterization and validation of such a model system would take a significant number of additional studies.

Nevertheless, the model structures as they exist currently are useful, particularly when employed in the conceptual framework proposed in Section 6.1. Because the models predict perchlorate and iodide kinetics, two relevant dose metrics to the mode of action can be evaluated: (1) the area under the curve (AUC) of perchlorate in the serum and (2) the degree (expressed as a % of baseline) of iodide uptake inhibition in the thyroid.

Because developmental effects are of concern, an argument could be made that peak and not AUC is the appropriate dose metric—the rationale being that any transient dose could be responsible for permanent deficits. However, the AUC values, as opposed to peak

1 concentrations, were used based on the assumption that these dose metrics would represent an  
2 averaging of the serum and thyroid perchlorate concentrations and would be better correlated  
3 with the inhibition effect on iodide uptake. The correlation was shown to be good between the  
4 AUC and the degree of inhibition (see Section 6.5.2). Further, due to the rapid phase of  
5 distribution after an iv dose, measurements of concentrations are very difficult to attain  
6 experimentally and are more variable. Using simulated peak concentrations after iv injections is  
7 potentially problematic due to the inexact modeling of the actual distribution of dose in the tail-  
8 vein volume and the exact time of mixing in the whole blood compartment (Merrill, 2001e).  
9 It was also observed by EPA that the ratios for peak perchlorate serum values (Merrill, 2001e;  
10 Table 6) were in good agreement with those for the perchlorate serum AUC and that the serum  
11 AUC were slightly more conservative if really different at all at the lower doses of concern to the  
12 risk assessment.

13 The perchlorate AUC concentration in the thyroid was also considered, but the EPA and  
14 AFRL/HEST agreed that this was a less satisfactory dose metric based on a number of  
15 considerations. These included the following: that the thyroid  $V_{maxc}$  estimates had to be  
16 adjusted to account for upregulation of the NIS, but that this adjustment was more an empirical  
17 exercise than a true biological model (since the hormone changes discussed above regulate the  
18 NIS); that the thyroid concentrations were not actually measured in the fetus and neonate so that  
19 verification of the parameters was not possible; and that the effects of perchlorate are related to  
20 its effects on the NIS and secondary impact on thyroid hormone economy rather than to the  
21 concentrations in the gland itself. Results of a sensitivity analysis on the adult male rat model  
22 structure supported these conclusions (Merrill, 2001e). The results of the sensitivity analysis will  
23 be discussed in Section 6.5.1. Thus, the models were exercised to develop human equivalent  
24 exposure (HEE) estimates based on internal perchlorate concentration and iodide uptake  
25 inhibition, both components of exposure in the proposed EPA model (Merrill, 2001e). The  
26 purpose of Section 6.5.2 is to describe the modeling exercises underlying the HEE estimates that  
27 are used in Chapter 7.

### 28 29 **6.5.1 Sensitivity Analysis of Proposed Adult Male Rat Model**

30 A sensitivity analysis was performed on the adult male rat model of Merrill (2001c) in  
31 order to determine which parameters had the most significant impact on serum and thyroid AUC

1 perchlorate concentrations. All chemical specific kinetic parameters were increased individually  
2 by 1% from the original, optimized values. The model-predicted dosimetrics were recalculated  
3 after each change to determine the effect on the AUC estimates. This exercise was performed at  
4 the four-hour time point after iv dosing for the 0.1 and 1.0 mg/kg-day doses. The equation  
5 describing the calculation of the Sensitivity Coefficient value for each PBPK perchlorate  
6 parameter tested is (Merrill, 2001e):

$$\text{Sensitivity Coefficient} = \frac{(A - B)/B}{(C - D)/D} \quad (6-2)$$

7  
8  
9  
10  
11 where:

12  
13 A = AUC for either serum or thyroid perchlorate with 1% increased parameter value,

14 B = AUC for either serum or thyroid perchlorate at initial parameter value,

15 C = Parameter value increase 1% over initial parameter value, and

16 D = Original initial starting parameter value.  
17

18 Results are presented for the physiological parameters and chemical specific parameters  
19 separately. Tables 6-9 and 6-10 provide the results for the 0.1 mg/kg-day dose, and  
20 Tables 6-11 and 6-12 provide the results for the 1.0 mg/kg-day dose. The sensitivity coefficients  
21 for the AUC estimates in both the thyroid and serum are provided and the changes in predicted  
22 AUC estimates for the thyroid and serum are presented in the final two columns (Merrill, 2001e).

23 The sensitivity of serum and thyroid concentrations to model parameters is not linear.  
24 At an iv dose level of 1.0 mg/kg, the model prediction of the AUC for serum  $\text{ClO}_4^-$  concentration  
25 is most sensitive to urinary clearance ( $\text{ClUc}_p$ ). A one percent increase in this value, from  
26  $0.07$  to  $0.0707$  ng/hr-kg, causes a decrease in AUC serum  $\text{ClO}_4^-$  concentration from  $4.69 \times 10^5$  to  
27  $4.63 \times 10^5$  ng, with a sensitivity coefficient of  $-1.271$  (Table 6-12). Serum concentration is next  
28 most sensitive to the rate  $\text{ClO}_4^-$  unbinds from plasma proteins ( $\text{Clunbc}_p$ ), with a sensitivity  
29 coefficient of  $-0.869$  (Table 6-12).  
30

**TABLE 6-9. SENSITIVITY ANALYSIS FOR PHYSIOLOGICAL PARAMETERS IN THE ADULT MALE RAT MODEL AT 0.1 mg/kg PERCHLORATE (ClO<sub>4</sub><sup>-</sup>) DOSE (Merrill, 2001e)**

Parameter <sup>a</sup>	Original Parameter Value	1% Increase in Parameter Value	AUC Thyroid Sensitivity Coefficient	AUC Serum Sensitivity Coefficient	Increase in AUC Thyroid ClO <sub>4</sub> <sup>-</sup> (ng) <sup>b</sup>	Increase in AUC Serum ClO <sub>4</sub> <sup>-</sup> (ng) <sup>c</sup>
BW	3.00E-01	3.03E-01	0.315	0.182	1.88E+06	9.95E+04
<b>Blood Flows (fraction of cardiac output, QCc [L/hr])</b>						
QCc	1.40E+01	1.41E+01	-0.005	-0.006	1.88E+06	9.94E+04
QTc	1.60E-02	1.62E-02	NS <sup>d</sup>	NS	1.88E+06	9.94E+04
QSKc	5.80E-02	5.86E-02	NS	-0.003	1.88E+06	9.94E+04
QGc	1.60E-02	1.62E-02	0.011	0.008	1.88E+06	9.94E+04
QLc	1.70E-01	1.72E-01	NS	NS	1.88E+06	9.94E+04
QKc	1.40E-01	1.41E-01	-0.016	-0.010	1.88E+06	9.93E+04
QFc	6.90E-02	6.97E-02	NS	NS	1.88E+06	9.94E+04
<b>Tissue Volumes (fraction of body weight)</b>						
Vplasc	4.10E-02	4.14E-02	0.155	0.079	1.88E+06	9.94E+04
VRBCc	3.30E-02	3.33E-02	0.192	0.109	1.88E+06	9.95E+04
Vttotc	7.70E-05	7.78E-05	0.187	0.113	1.88E+06	9.95E+04
VDTc	2.44E-01	2.46E-01	0.928	0.114	1.89E+06	9.95E+04
VTBc	1.57E-01	1.58E-01	0.203	0.114	1.88E+06	9.95E+04
VTc	6.00E-01	6.05E-01	0.453	0.114	1.88E+06	9.95E+04
VGc	5.40E-03	5.45E-03	0.197	0.112	1.88E+06	9.95E+04
VGJc	1.68E-02	1.70E-02	0.165	0.091	1.88E+06	9.94E+04
VGBc	4.10E-02	4.14E-02	0.197	0.114	1.88E+06	9.95E+04
VSkc	1.90E-01	1.92E-01	-0.053	-0.023	1.87E+06	9.93E+04
VSkBc	2.00E-02	2.02E-02	0.203	0.117	1.88E+06	9.95E+04
VLc	5.50E-02	5.56E-02	0.197	0.114	1.88E+06	9.95E+04
VKc	1.70E-02	1.72E-02	0.197	0.113	1.88E+06	9.95E+04
VFc	7.40E-02	7.47E-02	0.208	0.118	1.88E+06	9.95E+04

<sup>a</sup>Parameters as defined in Tables 6-1 and 6-2.

<sup>b</sup>AUC Thyroid Concentration using original parameters = 1.88E+06 ng ClO<sub>4</sub><sup>-</sup>.

<sup>c</sup>AUC Serum Concentration using original parameters = 9.94E+04 ng ClO.

<sup>d</sup>NS = sensitivity coefficient less than 0.001.

**TABLE 6-10. SENSITIVITY ANALYSIS FOR CHEMICAL SPECIFIC PARAMETERS IN THE ADULT MALE RAT MODEL AT 0.1 mg/kg PERCHLORATE (ClO<sub>4</sub><sup>-</sup>) DOSE (Merrill, 2001e)**

Parameter <sup>a</sup>	Original Parameter Value	1% Increase in Parameter Value	AUC Thyroid Sensitivity Coefficient	AUC Serum Sensitivity Coefficient	Increase in AUC Thyroid ClO <sub>4</sub> <sup>-</sup> (ng) <sup>b</sup>	Increase in AUC Serum ClO <sub>4</sub> <sup>-</sup> (ng) <sup>c</sup>
<b>Iodide Tissue/Blood Partition Coefficients</b>						
PS <sub>p</sub>	3.10E-01	3.13E-01	0.149	0.085	1.88E+06	9.94E+04
PR <sub>p</sub>	5.60E-01	5.66E-01	0.192	0.111	1.88E+06	9.95E+04
PK <sub>P</sub>	9.90E-01	1.00E+00	0.192	0.111	1.88E+06	9.95E+04
PL <sub>p</sub>	5.60E-01	5.66E-01	0.187	0.108	1.88E+06	9.95E+04
PG <sub>p</sub>	1.80E+00	1.82E+00	0.160	0.088	1.88E+06	9.94E+04
PGJ <sub>p</sub>	2.30E+00	2.32E+00	0.165	0.090	1.88E+06	9.94E+04
PT <sub>p</sub>	1.30E-01	1.31E-01	1.184	0.113	1.90E+06	9.95E+04
PDT <sub>p</sub>	7.00E+00	7.07E+00	0.928	0.114	1.89E+06	9.95E+04
PF <sub>p</sub>	5.00E-02	5.05E-02	0.197	0.114	1.88E+06	9.95E+04
PSk <sub>p</sub>	7.00E-01	7.07E-01	11.154	6.024	2.08E+06	1.05E+05
PRBC <sub>p</sub>	8.00E-01	8.08E-01	11.324	6.112	2.09E+06	1.05E+05
PS <sub>p</sub>	3.10E-01	3.13E-01	0.149	0.085	1.88E+06	9.94E+04
PR <sub>p</sub>	5.60E-01	5.66E-01	0.192	0.111	1.88E+06	9.95E+04
PK <sub>P</sub>	9.90E-01	1.00E+00	0.192	0.111	1.88E+06	9.95E+04
PL <sub>p</sub>	5.60E-01	5.66E-01	0.187	0.108	1.88E+06	9.95E+04
PG <sub>p</sub>	1.80E+00	1.82E+00	0.160	0.088	1.88E+06	9.94E+04
PGJ <sub>p</sub>	2.30E+00	2.32E+00	0.165	0.090	1.88E+06	9.94E+04
PT <sub>p</sub>	1.30E-01	1.31E-01	1.184	0.113	1.90E+06	9.95E+04
PDT <sub>p</sub>	7.00E+00	7.07E+00	0.928	0.114	1.89E+06	9.95E+04
PF <sub>p</sub>	5.00E-02	5.05E-02	0.197	0.114	1.88E+06	9.95E+04
PSk <sub>p</sub>	7.00E-01	7.07E-01	11.154	6.024	2.08E+06	1.05E+05
PRBC <sub>p</sub>	8.00E-01	8.08E-01	11.324	6.112	2.09E+06	1.05E+05
<b>Perchlorate Active Uptake Parameters - Vmaxc (ng/hr-kg BW) Km (ng/L)</b>						
Vmaxc <sub>Tp</sub>	2.90E+03	2.93E+03	47.830	6.088	2.77E+06	1.05E+05
Km <sub>Tp</sub>	2.50E+05	2.53E+05	45.154	6.090	2.72E+06	1.05E+05
Vmaxc <sub>DTp</sub>	1.00E+05	1.01E+05	55.875	6.081	2.92E+06	1.05E+05
Km <sub>DTp</sub>	1.00E+08	1.01E+08	55.673	6.081	2.92E+06	1.05E+05
Vmaxc <sub>Gp</sub>	1.00E+04	1.01E+04	55.769	6.080	2.92E+06	1.05E+05
Km <sub>Gp</sub>	2.00E+05	2.02E+05	55.774	6.081	2.92E+06	1.05E+05
Vmaxc <sub>Sp</sub>	6.50E+05	6.57E+05	54.713	5.678	2.90E+06	1.05E+05
Km <sub>Sp</sub>	2.00E+05	2.02E+05	55.060	5.811	2.91E+06	1.05E+05

**TABLE 6-10 (cont'd). SENSITIVITY ANALYSIS FOR CHEMICAL SPECIFIC PARAMETERS IN THE ADULT MALE RAT MODEL AT 0.1 mg/kg PERCHLORATE (ClO<sub>4</sub><sup>-</sup>) DOSE (Merrill, 2001e)**

Parameter <sup>a</sup>	Original Parameter Value	1% Increase in Parameter Value	AUC Thyroid Sensitivity Coefficient	AUC Serum Sensitivity Coefficient	Increase in AUC Thyroid ClO <sub>4</sub> <sup>-</sup> (ng) <sup>b</sup>	Increase in AUC Serum ClO <sub>4</sub> <sup>-</sup> (ng) <sup>c</sup>
<b>Perchlorate Plasma Binding Parameters</b>						
Vmaxc_Bp	9.50E+03	9.60E+03	54.857	6.417	2.90E+06	1.06E+05
km_Bp	1.10E+04	1.11E+04	54.916	5.590	2.91E+06	1.05E+05
Kunbc_p	1.00E-01	1.01E-01	54.948	5.096	2.91E+06	1.04E+05
<b>Perchlorate Urinary Clearance and Permeability Area Cross Products (L/hr-kg)</b>						
ClUc_p	7.00E-02	7.07E-02	54.047	5.399	2.89E+06	1.05E+05
PAGc_p	8.00E-01	8.08E-01	54.905	5.752	2.91E+06	1.05E+05
PAGJc_p	8.00E-01	8.08E-01	54.905	5.752	2.91E+06	1.05E+05
PATc_p	5.00E-05	5.05E-05	23.273	5.776	2.31E+06	1.05E+05
PADTc_p	1.00E-02	1.01E-02	24.398	5.775	2.33E+06	1.05E+05
PASKc_p	4.00E-01	4.04E-01	3.759	-4.354	1.95E+06	9.50E+04
PARBCc_p	1.00E-01	1.01E-01	3.455	-4.508	1.94E+06	9.49E+04

<sup>a</sup>Parameters as defined in Tables 6-1 and 6-2.

<sup>b</sup>AUC Thyroid concentration using original parameters = 1.88E+06 ng ClO<sub>4</sub><sup>-</sup>.

<sup>c</sup>AUC Serum concentration using original parameters = 9.94E+04 ng ClO<sub>4</sub><sup>-</sup>.

1 The predicted AUC for total thyroid concentration at a dose level of 1.0 mg/kg-day is most  
2 sensitive to changes in the maximum capacity of the thyroid colloid (Vmaxc\_DTp). A one  
3 percent increase in this value from  $1.00 \times 10^5$  to  $1.0110^5$  ng/hr-kg results in an increase in AUC  
4 thyroid concentration from  $9.84 \times 10^6$  to  $1.04 \times 10^7$  ng (Table 6-12). However, the AUC thyroid  
5 concentration is almost equally sensitive to other parameters of saturable processes, including  
6 Vmaxc, Km, and the permeability area cross product values of other saturable tissues.

7 With a lower iv dose of 0.1 mg/kg, the blood serum concentration remains sensitive to  
8 changes in urinary clearance, but demonstrates increased sensitivity to the parameters of  
9 saturable compartments and effective partitioning with skin (PSk\_p) and red blood cells  
10 (PRBC\_p). Serum concentration is most sensitive to the maximum capacity for plasma binding  
11 (Vmaxc\_Bp) at this dose level (Table 6-10).

12 At the lower dose level of 0.1 mg/kg, thyroid concentrations show a similar sensitivity to  
13 parameters of saturable processes, including plasma binding, permeability area cross products,

**TABLE 6-11. SENSITIVITY ANALYSIS FOR PHYSIOLOGICAL  
PARAMETERS IN THE ADULT MALE RAT MODEL  
AT 1.0 mg/kg PERCHLORATE (ClO<sub>4</sub><sup>-</sup>) DOSE (Merrill, 2001e)**

<b>Parameter<sup>a</sup></b>	<b>Original Parameter Value</b>	<b>1% Increase in Parameter Value</b>	<b>AUC Thyroid Sensitivity Coefficient</b>	<b>AUC Serum Sensitivity Coefficient</b>	<b>Increase in AUC Thyroid ClO<sub>4</sub><sup>-</sup> (ng)<sup>b</sup></b>	<b>Increase in AUC Serum ClO<sub>4</sub><sup>-</sup> (ng)<sup>c</sup></b>
BW	3.00E-01	3.03E-01	-5.944	-0.534	9.81E+06	4.67E+05
<b>Blood Flows [fraction of cardiac output, QCc (L/hr)]</b>						
QCc	1.40E+01	1.41E+01	-0.192	-0.014	9.84E+06	4.69E+05
QTc	1.60E-02	1.62E-02	0.021	NS <sup>b</sup>	9.84E+06	4.69E+05
QSKc	5.80E-02	5.86E-02	-0.085	0.001	9.84E+06	4.69E+05
QGc	1.60E-02	1.62E-02	0.128	0.005	9.84E+06	4.69E+05
QLc	1.70E-01	1.72E-01	0.021	NS	9.84E+06	4.69E+05
QKc	1.40E-01	1.41E-01	-0.234	-0.021	9.84E+06	4.69E+05
QFc	6.90E-02	6.97E-02	0.021	NS	9.84E+06	4.69E+05
<b>Tissue Volumes (fraction of bodyweight)</b>						
Vplasc	4.10E-02	4.14E-02	-7.734	-0.701	9.80E+06	4.66E+05
VRBCc	3.30E-02	3.33E-02	-7.649	-0.691	9.80E+06	4.66E+05
VTtotc	7.70E-05	7.78E-05	-7.841	-0.683	9.80E+06	4.66E+05
VDTc	2.44E-01	2.46E-01	7.606	-0.683	9.87E+06	4.66E+05
VTBc	1.57E-01	1.58E-01	-7.500	-0.682	9.80E+06	4.66E+05
VTc	6.00E-01	6.05E-01	-2.322	-0.683	9.83E+06	4.66E+05
VGc	5.40E-03	5.45E-03	-7.649	-0.685	9.80E+06	4.66E+05
VGJc	1.68E-02	1.70E-02	-7.883	-0.710	9.80E+06	4.66E+05
VGBc	4.10E-02	4.14E-02	-7.628	-0.682	9.80E+06	4.66E+05
VSkc	1.90E-01	1.92E-01	-8.799	-0.829	9.80E+06	4.65E+05
VSkBc	2.00E-02	2.02E-02	-7.606	-0.680	9.80E+06	4.66E+05
VLc	5.50E-02	5.56E-02	-7.628	-0.683	9.80E+06	4.66E+05
VKc	1.70E-02	1.72E-02	-7.628	-0.685	9.80E+06	4.66E+05
VFc	7.40E-02	7.47E-02	-7.585	-0.676	9.80E+06	4.66E+05

<sup>a</sup>Parameters as defined in Tables 6-1 and 6-2.

<sup>b</sup>Original AUC Thyroid concentration = 9.84E+06 ng ClO<sub>4</sub><sup>-</sup>.

<sup>c</sup>Original AUC Serum concentration = 4.69E+05 ng ClO<sub>4</sub><sup>-</sup>.

<sup>d</sup>NS = sensitivity coefficient less than 0.001.

**TABLE 6-12. SENSITIVITY ANALYSIS FOR CHEMICAL-SPECIFIC  
PARAMETERS IN THE MALE RAT MODEL  
AT 1.0 mg/kg PERCHLORATE (ClO<sub>4</sub><sup>-</sup>) DOSE (Merrill, 2001e)**

<b>Parameter<sup>a</sup></b>	<b>Original Parameter Value</b>	<b>1% Increase in Parameter Value</b>	<b>AUC Thyroid Sensitivity Coefficient</b>	<b>AUC Serum Sensitivity Coefficient</b>	<b>Increase in AUC Thyroid ClO<sub>4</sub><sup>-</sup> (ng)<sup>b</sup></b>	<b>Increase in AUC Serum ClO<sub>4</sub><sup>-</sup> (ng)<sup>c</sup></b>
<b>Perchlorate Tissue/Blood Partition Coefficients</b>						
PS <sub>p</sub>	3.10E-01	3.13E-01	-7.862	-0.728	9.80E+06	4.66E+05
PR <sub>p</sub>	5.60E-01	5.66E-01	-7.649	-0.688	9.80E+06	4.66E+05
PK <sub>P</sub>	9.90E-01	1.00E+00	-7.649	-0.688	9.80E+06	4.66E+05
PL <sub>p</sub>	5.60E-01	5.66E-01	-7.670	-0.692	9.80E+06	4.66E+05
PG <sub>p</sub>	1.80E+00	1.82E+00	-7.905	-0.714	9.80E+06	4.66E+05
PGJ <sub>p</sub>	2.30E+00	2.32E+00	-7.883	-0.711	9.80E+06	4.66E+05
PT <sub>p</sub>	1.30E-01	1.31E-01	12.911	-0.683	9.90E+06	4.66E+05
PDT <sub>p</sub>	7.00E+00	7.07E+00	7.606	-0.683	9.87E+06	4.66E+05
PF <sub>p</sub>	5.00E-02	5.05E-02	-7.628	-0.684	9.80E+06	4.66E+05
PSk <sub>p</sub>	7.00E-01	7.07E-01	-8.885	-0.846	9.80E+06	4.65E+05
PRBC <sub>p</sub>	8.00E-01	8.08E-01	-7.649	-0.691	9.80E+06	4.66E+05
<b>Perchlorate Active Uptake Parameters - Vmaxc (ng/hr-kg BW), Km (ng/L)</b>						
Vmaxc <sub>Tp</sub>	2.90E+03	2.93E+03	12.890	-0.683	9.90E+06	4.66E+05
Km <sub>Tp</sub>	2.50E+05	2.53E+05	-15.745	-0.682	9.76E+06	4.66E+05
Vmaxc <sub>DTP</sub>	1.00E+05	1.01E+05	123.554	-0.687	1.04E+07	4.66E+05
Km <sub>DTP</sub>	1.00E+08	1.01E+08	120.784	-0.687	1.04E+07	4.66E+05
Vmaxc <sub>Gp</sub>	1.00E+04	1.01E+04	122.062	-0.687	1.04E+07	4.66E+05
Km <sub>Gp</sub>	2.00E+05	2.02E+05	122.062	-0.687	1.04E+07	4.66E+05
Vmaxc <sub>Sp</sub>	6.50E+05	6.57E+05	120.997	-0.806	1.04E+07	4.66E+05
Km <sub>Sp</sub>	2.00E+05	2.02E+05	122.914	-0.641	1.04E+07	4.66E+05
<b>Perchlorate Plasma Binding Parameters - Vmaxc (ng/hr-kg BW), Km (ng/L)</b>						
Vmaxc <sub>Bp</sub>	9.50E+03	9.60E+03	122.062	-0.500	1.04E+07	4.67E+05
km <sub>Bp</sub>	1.10E+04	1.11E+04	122.062	-0.694	1.04E+07	4.66E+05
Kunbc <sub>p</sub>	1.00E-01	1.01E-01	122.275	-0.869	1.04E+07	4.65E+05
<b>Perchlorate Urinary Clearance and Permeability Area Cross Products (L/hr-kg)</b>						
CIUc <sub>p</sub>	7.00E-02	7.07E-02	115.031	-1.271	1.04E+07	4.63E+05
PAGc <sub>p</sub>	8.00E-01	8.08E-01	122.275	-0.685	1.04E+07	4.66E+05
PAGJc <sub>p</sub>	8.00E-01	8.08E-01	122.275	-0.686	1.04E+07	4.66E+05
PATc <sub>p</sub>	5.00E-05	5.05E-05	100.969	-0.686	1.03E+07	4.66E+05
PADTc <sub>p</sub>	1.00E-02	1.01E-02	120.784	-0.687	1.04E+07	4.66E+05
PASKc <sub>p</sub>	4.00E-01	4.04E-01	123.341	-0.567	1.04E+07	4.67E+05
PARBCc <sub>p</sub>	1.00E-01	1.01E-01	122.062	-0.687	1.04E+07	4.66E+05

<sup>a</sup>Parameters as defined in Tables 6-1 and 6-2.

<sup>b</sup>Original AUC Thyroid concentration = 9.84E+06 ng ClO<sub>4</sub><sup>-</sup>.

<sup>c</sup>Original AUC Serum concentration = 4.69E+05 ng ClO<sub>4</sub><sup>-</sup>.

1 and urinary clearance. However, the predicted thyroid concentrations at both dose levels (1.0 and  
2 0.1 mg/kg) are most sensitive to a change in  $V_{max\_DTp}$ . The  $V_{max}$  values of the thyroid were  
3 established by empirically fitting thyroid radioiodide and perchlorate uptake from several data  
4 sets ranging in three orders of magnitude

## 6 6.5.2 Derivation of Human Equivalent Exposure Estimates

7 As discussed, the following internal dosimetrics were chosen to represent output from each  
8 of the PBPK models: area under the curve (AUC) perchlorate concentrations in serum and  
9 thyroid; peak serum and thyroid perchlorate concentrations; the total amount of perchlorate  
10 excreted in the urine; the AUC for the lactational and placental transfer of perchlorate; and the  
11 percent inhibition of iodide uptake into the thyroid. In order to explore the dose-response  
12 relationship of these values, the target dosimetrics were evaluated across several doses in both  
13 acute and sub-chronic exposure scenarios using previously developed PBPK models at the  
14 AFRL/HEST; i.e., the models for the adult male rat (Merrill, 2001c) and human (Merrill, 2001d)  
15 described in Section 6.2, the pregnant and fetal rat model (Clewell, 2001a) and the lactating and  
16 neonatal rat model (Clewell, 2001b).

17 Acute (iv) pharmacokinetic studies in the adult male rat were used as the basis for this  
18 dose-response analysis because iodide uptake inhibition could be correlated to perchlorate levels.  
19 Further, as discussed in Section 6.1, the initial inhibition of iodide is viewed in the conceptual  
20 model as the important step in the transient phase (Figure 6-2). Transient decrements in T4 can  
21 result in permanent neurodevelopmental sequelae. In drinking water studies, upregulation of NIS  
22 in the rat is so rapid that it resulted in no measurable thyroid iodide inhibition, so the iv doses  
23 were used to estimate this initial insult. The target internal dosimetrics were first calculated in  
24 each of the rat models for acute exposure to perchlorate (single iv administration) at doses of  
25 0.01, 0.1, 1.0, 3.0, 5.0, 10.0, 30.0, and 100.0 mg/kg. In order to correlate perchlorate parameters  
26 to data-validated inhibition, the 2 to 4 hr time-frame was used for all acute calculations. The  
27 AUC for thyroid and serum were calculated by integrating predicted tissue concentrations from  
28 2 to 4 hrs post dosing.

29 These same dosimetrics calculated for acute exposures were also determined for subchronic  
30 (drinking water) perchlorate exposures at doses of 0.01, 0.1, 1.0, 3.0, 5.0, 10.0, 30.0, and  
31 100.0 mg/kg-day. In order to achieve steady state concentrations, the models were run until the

1 predicted peak and trough heights did not change from one day to the next (Merrill, 2001e).  
2 Serum and thyroid perchlorate AUC concentrations were then determined over a 24 hr period  
3 (240-264 hrs in male, lactating, and neonatal rats; 480-504 hrs in pregnant and fetal rats).  
4 Although the tissues reach steady state perchlorate concentrations within one week, the above  
5 time-points were chosen in the lactation and gestation models for their ability to be verified with  
6 data (Clewell, 2001a,b). The male rat model was run at the same time as lactation for the sake of  
7 consistency with the other models. The total perchlorate AUC in the serum and thyroid were  
8 determined from each the models at 240 and 264 hrs (or 480 and 504 hrs). The difference in the  
9 two values was then divided by 24 hrs to give the AUC in units of ng/L-hr.

10 The AFRL/HEST experiments (Yu, 2000; Yu et al., 2000) have shown upregulation of the  
11 NIS to be both time and dose-dependent. Thus, at lower doses, the rat thyroid was completely  
12 upregulated after only a few days of drinking water exposure. Iodide uptake in the thyroid at  
13 higher perchlorate doses ( $\geq 10$  mg/kg-day) was completely restored by the 18<sup>th</sup> day of exposure,  
14 the time of data collection in the pregnant and fetal rats (Clewell, 2001).

15 Drinking water studies in the adult male rats showed elevated perchlorate uptake in the  
16 thyroid at drinking water doses of 3.0 mg/kg-day and higher (Yu et al., 2000; Merrill et al.,  
17 2001c). Increased perchlorate uptake also results from upregulation of NIS. Since perchlorate is  
18 transferred into the thyroid via NIS, the inhibiting anion is “upregulated” along with iodide.  
19 In order to simulate increased perchlorate concentrations in thyroids of the 3.0, 10.0, and  
20 30.0 mg/kg-day dose groups, the original value for follicular  $V_{maxc}$  ( $V_{maxc\_Tp}$ ) was adjusted  
21 to obtain the best fit of the model simulation to experimental data (Table 6-13). Since there were  
22 no pharmacokinetic data available for the 5.0 and 100.0 mg/kg-day dose groups, values for  
23  $V_{maxc\_Tp}$  were estimated from a Michaelis-Menten fit to the adjusted  $V_{maxc}$ 's at 3.0, 10.0, and  
24 30.0 mg/kg-day doses (Figure 6-46). Target dosimetrics in the male rat were calculated for both  
25 originally optimized parameters and these adjusted (“upregulated”) parameters.

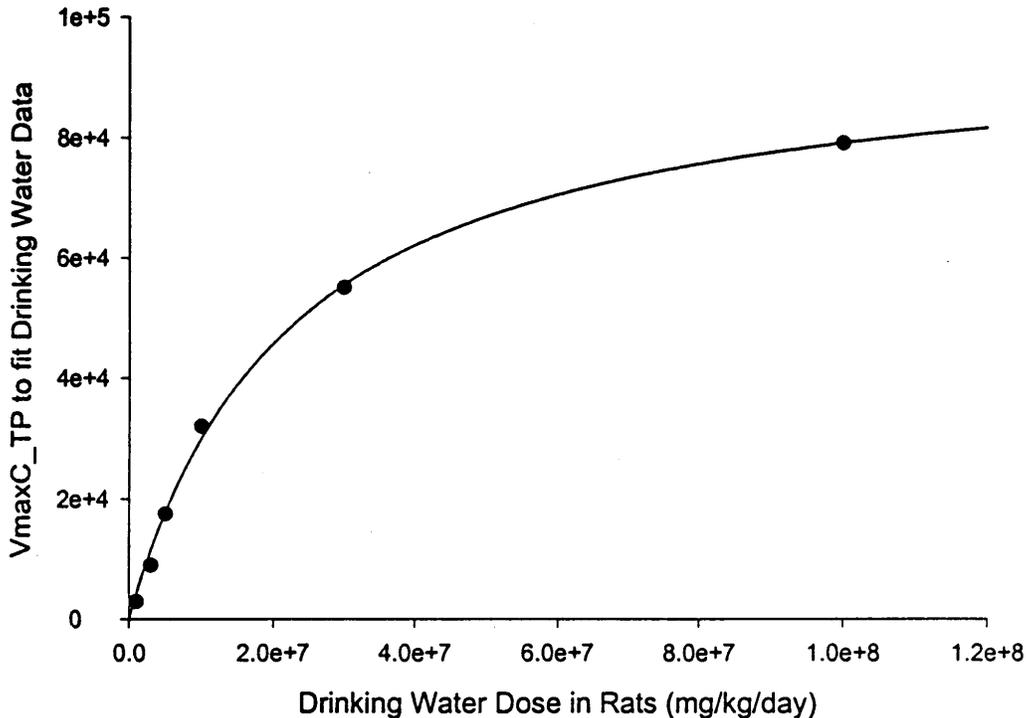
26 This process of adjusting (“upregulating”) the  $V_{maxc\_Tp}$  values was not necessary in the  
27 gestation, lactation, or human models, as they were able to successfully describe perchlorate  
28 concentrations in serum and thyroid at all measured doses (0.01 – 10.0 mg/kg-day in gestation  
29 and lactation; 0.02 – 12 mg/kg-day in human) using one set of model parameters (Clewell,  
30 2001a,b; Merrill, 2000). Merrill (2001e) posits that it was not necessary because it is likely that a  
31 loss of maternal iodide to the fetus and neonate causes dams to exist in a chronic state of

**TABLE 6-13. “UP-REGULATED” VALUES OF VMAXC\_Tp<sup>a</sup> AFTER PERCHLORATE DRINKING WATER EXPOSURE IN THE ADULT MALE RAT MODEL (Merrill, 2001e)**

Drinking Water Dose (mg/kg-day)	Adjusted Vmaxc_Tp (ng/hr-kg)
0.01	2900
0.1	2900
1	2900
3	9000
5	17500 <sup>b</sup>
10	32000
30	55000
100	79000 <sup>b</sup>

<sup>a</sup>Maximum velocity capacity of active transport in the thyroid follicle.

<sup>b</sup>Data not available for these dose levels.



**Figure 6-46. Upregulation of maximal capacity (ng/kg-hr) of active transport into the thyroid follicle for perchlorate (Vmaxc\_Tp) optimized by fitting to drinking water data in the rat. Upregulation is first noted in the 3.0 mg/kg-day dose group.**

1 thyroidal up-regulation. As a result, the effect of perchlorate on the thyroid was less dramatic  
2 than in the male rat where a completely naïve system is perturbed by an inhibiting chemical.  
3 Thus, the PBPK models for gestation and lactation were able to describe thyroid perchlorate  
4 levels at drinking water doses from 0.01 to 10.0 mg/kg-day without adjusting the follicular  
5  $V_{maxc}$  ( $V_{maxc\_Tp}$ ) values with dose.

6 Increased follicular  $V_{maxc}$  values were not needed to fit the human data likely due to the  
7 larger size of the human thyroid colloid versus that of the rat and to the differences in plasma  
8 protein binding discussed in Chapter 3.

9 The human PBPK model (Merrill, 2001d) was used to calculate all target dose metrics in  
10 both acute and two-week drinking water perchlorate exposures in a 70 kg adult at doses of 0.01,  
11 0.1, 1.0, 3.0, 5.0, 10.0, 30.0, and 100.0 mg/kg-day. Acute serum and thyroid perchlorate AUC  
12 concentration estimates were calculated with the model over an eight hr time period (from 24 to  
13 32 hrs post-exposure) in order to correlate perchlorate parameters to data-validated iodide  
14 inhibition. For two-week drinking water exposures, the thyroid and serum perchlorate AUC  
15 concentration estimates were calculated over a 24 hr period after serum and thyroid  
16 concentrations reached steady state. The 240 to 264 hr time period was chosen for consistency  
17 with the male rat model (Merrill, 2001c).

18 The adult human model (Merrill, 2001d) was also used to predict dosimetry in a 15 kg  
19 child. The same dosimetrics were run in the model for the child and adult. However, since an  
20 average child drinks less water than an adult (approximately 1 L/d as opposed to 2 L/d in the  
21 adult), the actual exposures of a child and adult from the same water source would be different.  
22 For example, a 15kg child consuming 1 L of contaminated water would receive a daily dose (per  
23 kg bodyweight) that was 2.3 times that of a 70 kg adult consuming 2 L of water. Table 2 shows  
24 the concentration of the drinking water required to deliver the same dose to a 15 kg child and a  
25 70 kg adult. For the purpose of this paper, dosimetric comparisons were calculated using the  
26 same dose (mg/kg-day) in the adult and child.

27 Figure 6-47 shows the curve generated from plotting the experimentally-determined percent  
28 inhibition versus the corresponding PBPK-derived serum (A) and thyroid (B) perchlorate AUC  
29 concentration estimates after acute (iv) exposure in rats. Thyroidal radiolabeled iodide ( $^{125}\text{I}$ )  
30 uptake measurements were taken two hours after iv administration of perchlorate. The solid line

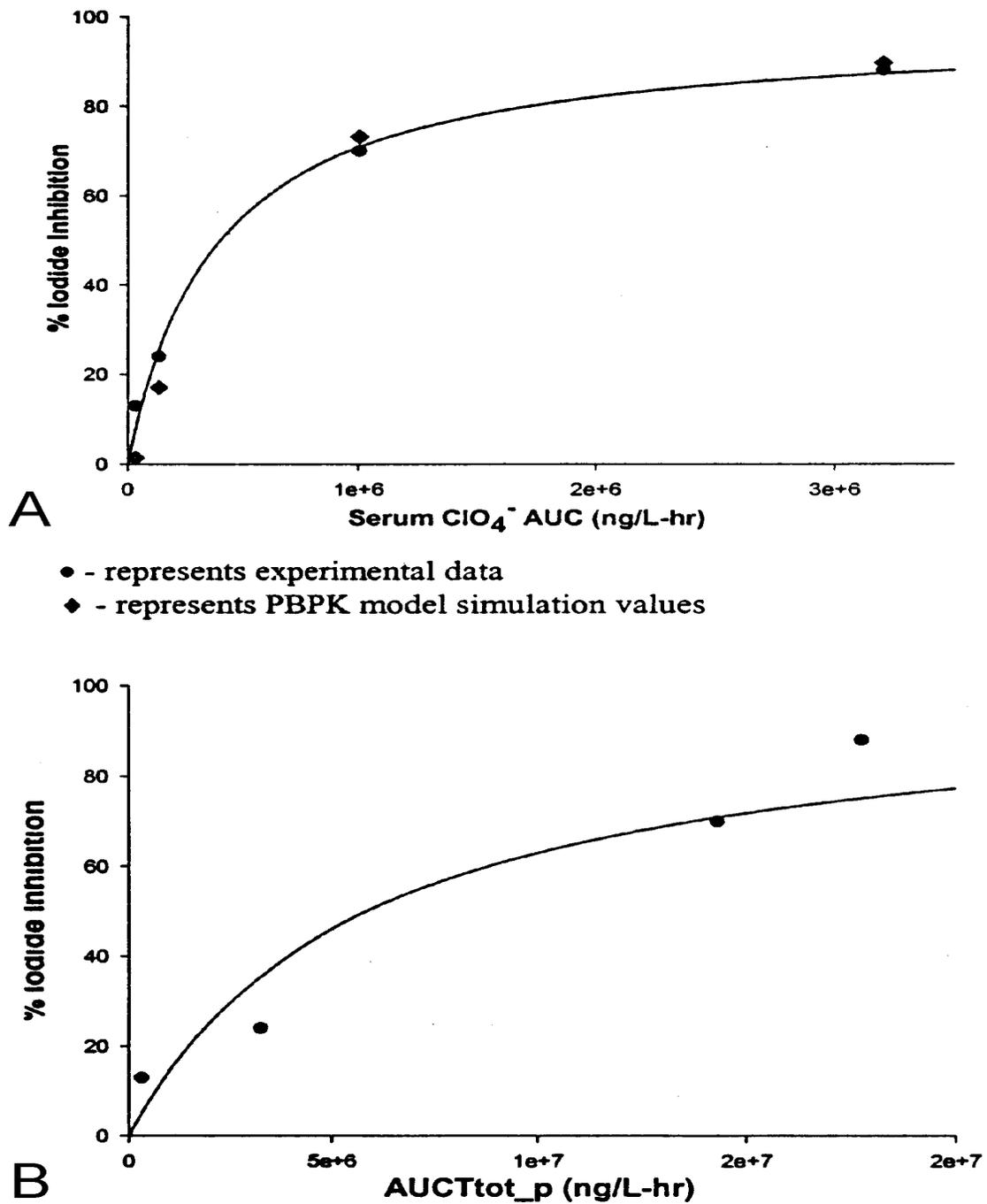


Figure 6-47. Michaelis-Menten fit of the “acute” male rat area under the curve (AUC) for serum (A) and thyroidal perchlorate (AUCTtot\_p) in ng/L-hr. Model predictions and actual data shown for percent radiolabeled iodide uptake inhibition after iv injection of perchlorate.

1 represents a fit (not a PBPK model simulation) using the Michaelis-Menten type equation given  
2 below:

$$Y = (A \times AUC_{\text{dose}})/(AUC_{\text{dose}} + B) \quad (6-3)$$

3  
4  
5  
6 Where 'Y' represents the predicted percent inhibition of radioiodide uptake, 'A' represents the  
7 maximal percent inhibition of radioiodide uptake, 'B' is related to the affinity of iodide uptake  
8 based on serum concentration, and  $AUC_{\text{dose}}$  represents the AUC at each specific dose of  
9 perchlorate. The above equation was also used to derive the dose-response relationship in  
10 subsequent figures. The correlation coefficient ( $r^2$ ) greater than 0.91 in all cases indicated  
11 excellent fit for all (see Merrill, 2001e; Table 3).

12 Figure 6-48 shows the PBPK-derived AUC perchlorate concentration estimates for  
13 drinking water exposure to the adult male rat versus the calculated percent inhibition of  
14 radioiodide in the serum (A) and thyroid (B). The values for AUC of perchlorate concentration  
15 in the serum were determined by running the adult male rat model (Merrill, 2001c) across doses.  
16 Corresponding percent inhibitions were calculated by putting serum AUC perchlorate  
17 concentration values into the equation from Figure 6-47. Human response (thyroid inhibition) to  
18 subchronic exposure is similar to that of an acute exposure in the rat. This approach allows the  
19 sub-chronic serum levels in the rat be related to iodide uptake in the native thyroid. The values  
20 for AUC of thyroid perchlorate concentration (B) were determined by running the male rat model  
21 (Merrill, 2001c) at steady state (between 240 and 264 hours of drinking water exposure) across  
22 the doses shown. Corresponding percent inhibitions were calculated by putting thyroid AUC  
23 values in the equation from Figure 6-47.

24 The actual human iodide inhibition data (Greer et al., 2000) were plotted as a function of  
25 the perchlorate AUC concentration estimates for serum and thyroid calculated with the PBPK  
26 model in Figure 6-49. The measured percent inhibition of radiolabeled iodide uptake in the  
27 serum and thyroid on Day 2 of drinking water exposure to perchlorate is shown versus the  
28 PBPK-derived estimates for human volunteers (both male and female). Inhibition data from time  
29 points earlier than Day 2 of perchlorate in the human drinking water (Greer et al., 2000) and  
30 inhibition data from acute perchlorate dosing in humans were not available. Therefore, the  
31 inhibition measurements on Day 2 of perchlorate drinking water exposure were the closest-

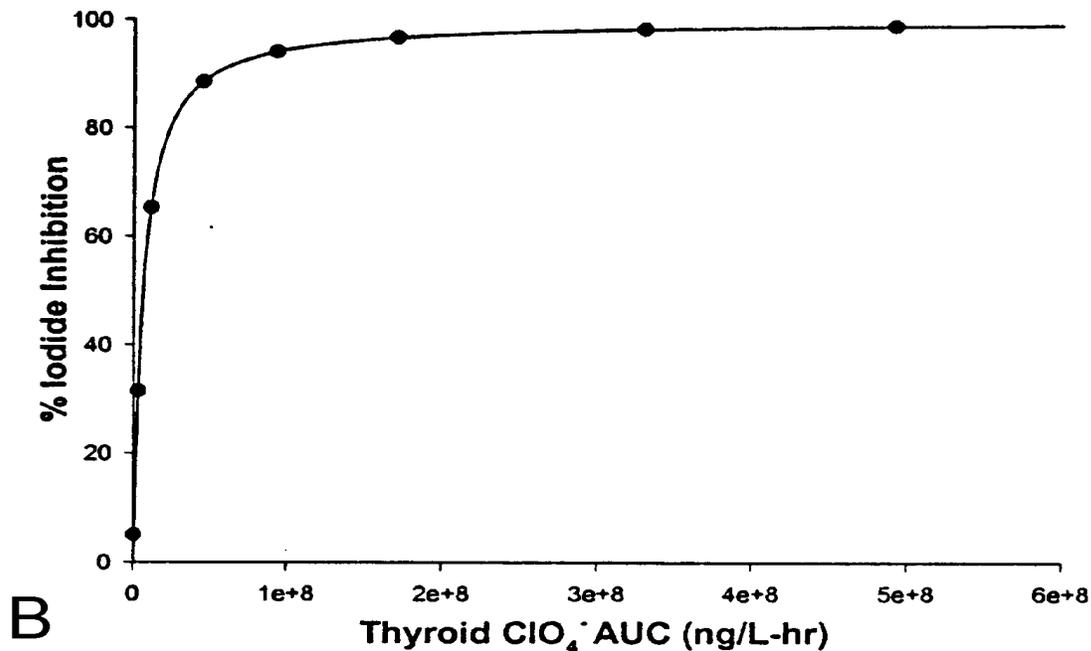
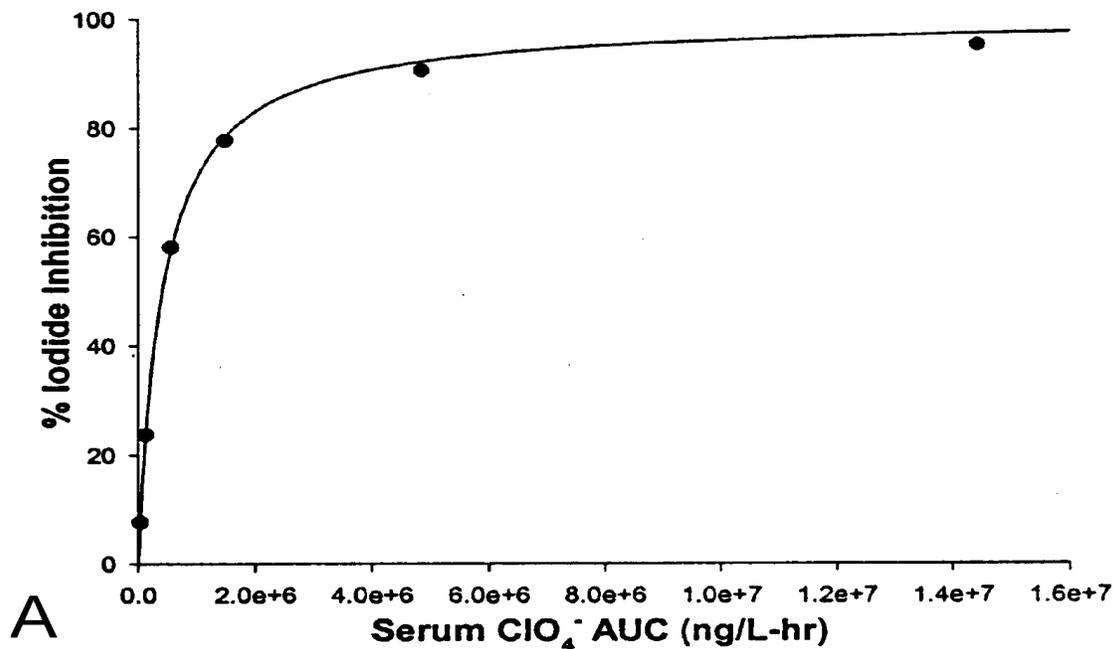
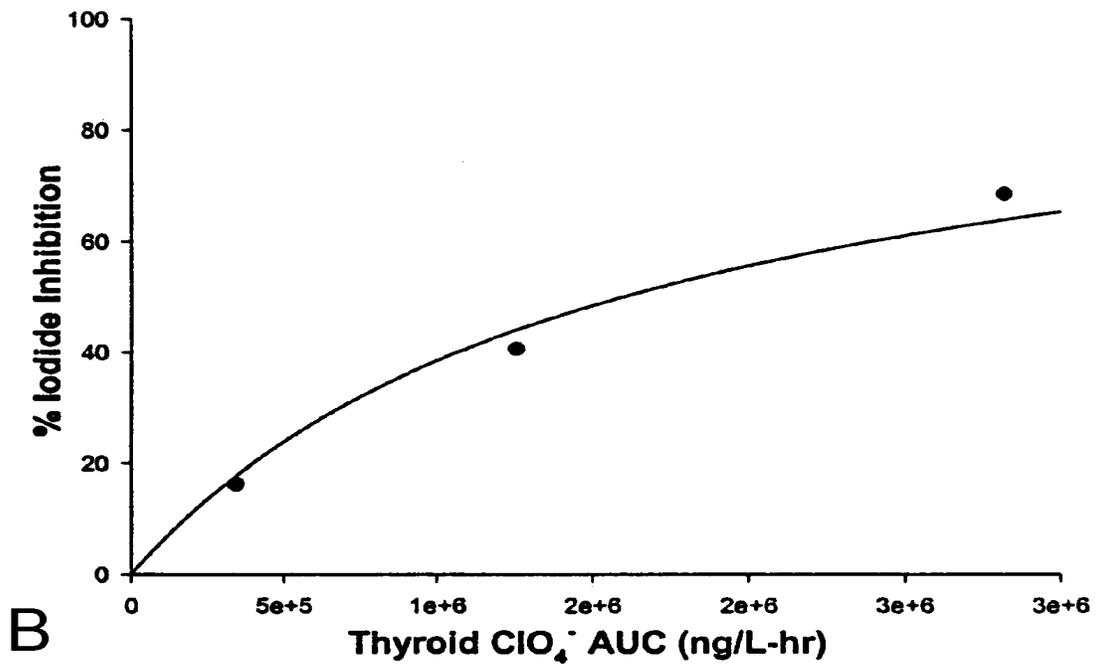
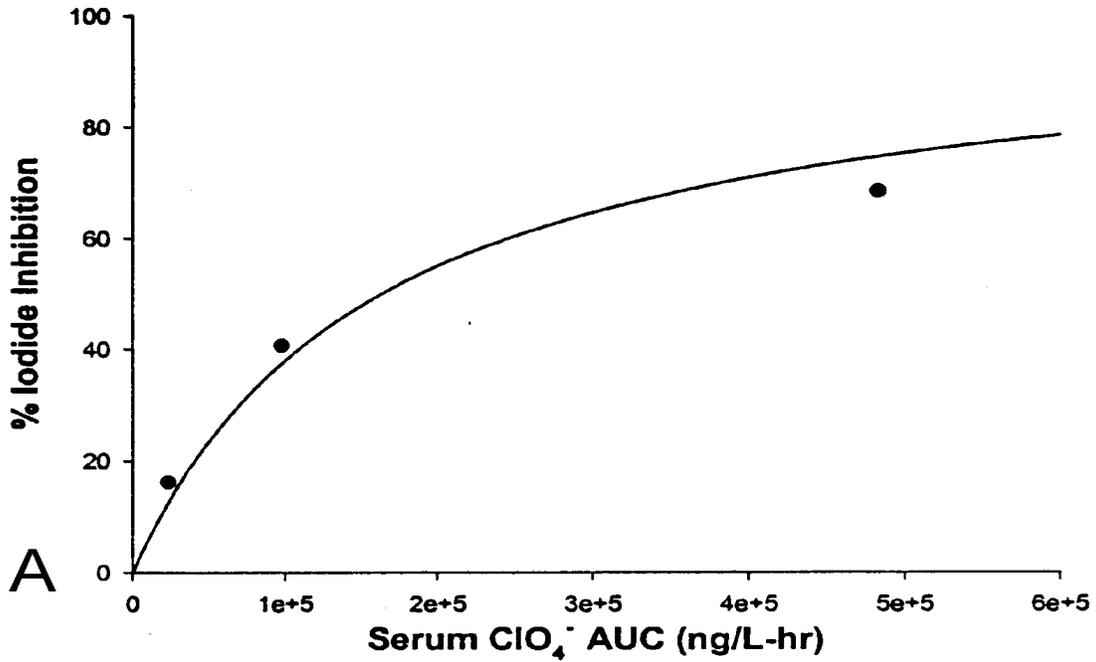


Figure 6-48. Michaelis-Menten fit of the “chronic” male rat area under the curve (AUC) for serum (A) and thyroidal (B) perchlorate (ng/L-hr). Model predictions and actual data shown for percent radiolabeled iodide uptake inhibition after drinking water exposure of perchlorate. Fit for serum calculated percent inhibition of radioiodide uptake calculated from equation used in Figure 6-47 (A) and for thyroid from Figure 6-47(B).



**Figure 6-49. Michaelis-Menten fit of the human area under the curve (AUC) for serum (A) and thyroidal (B) perchlorate (ng/L-hr) on exposure Day 2. Model predictions and actual data shown for percent radiolabeled iodide uptake inhibition after drinking water exposure of perchlorate.**

1 available representation of an acute human dose. Measured serum TSH and thyroid hormones  
2 indicated that thyroids were in normal homeostatic state in human volunteers during the entire  
3 two week study (Merrill, 2001d).

4 The HEE estimates were calculated using the models as described in Section 6.1  
5 (Figure 6-4). The HEE that would result in the same perchlorate AUC concentration estimates  
6 for serum (A) and thyroid (B) in the human and rat and the corresponding percent inhibition of  
7 iodide uptake is presented in Figure 6-50. Values for percent inhibition were determined from  
8 the rat serum AUC during drinking water exposures to perchlorate using the Michaelis-Menten  
9 equations from Figure 6-47. The correlation coefficient for both the serum and thyroid AUC  
10 versus percent iodide uptake inhibition relationship was 0.99.

### 12 **6.5.3 Summary**

13 The correlation coefficients for the dose-response relationships using the PBPK-model  
14 generated HEE estimates between serum and thyroid perchlorate AUC concentration versus  
15 iodide inhibition indicated good fits. Tables of the actual estimates and their ratios can be found  
16 in Merrill (2001e).

17 The rat serum ratios (AUC and peak concentrations) change significantly between 0.1 and  
18 3.0 mg/kg-day due to binding of perchlorate by plasma proteins. Plasma binding is saturated at  
19 doses greater than 1.0 mg/kg-day. Male rat to human ratios are notably lower than those ratios  
20 between rats, as plasma binding of perchlorate occurs to a much lesser extent in humans.

21 HEE estimates were calculated for both a 15 and 70 kg human. The differences between  
22 the 15 and 70 kg human HEE estimates were never greater than 75%, indicating that body weight  
23 doesn't significantly affect the target dose metrics. Interestingly, the HEE estimates were greater  
24 in the 15 kg child. One might expect the adult and child HEE estimates to be nearly equal, given  
25 no parameters were changed in the human model except body weight. However, physiological  
26 parameters within the model are linearly scaled by body weight; whereas, chemical-specific  
27 parameters are scaled nonlinearly (e.g., as a multiple of body weight to a power of  $3/4$ ).

28 As indicated later in the sensitivity analysis, the internal dose metrics presented are more  
29 sensitive to chemical-specific parameters, especially those describing saturable kinetics.

30 Therefore, the chemical-specific parameter values for the 15 kg child are proportionally greater

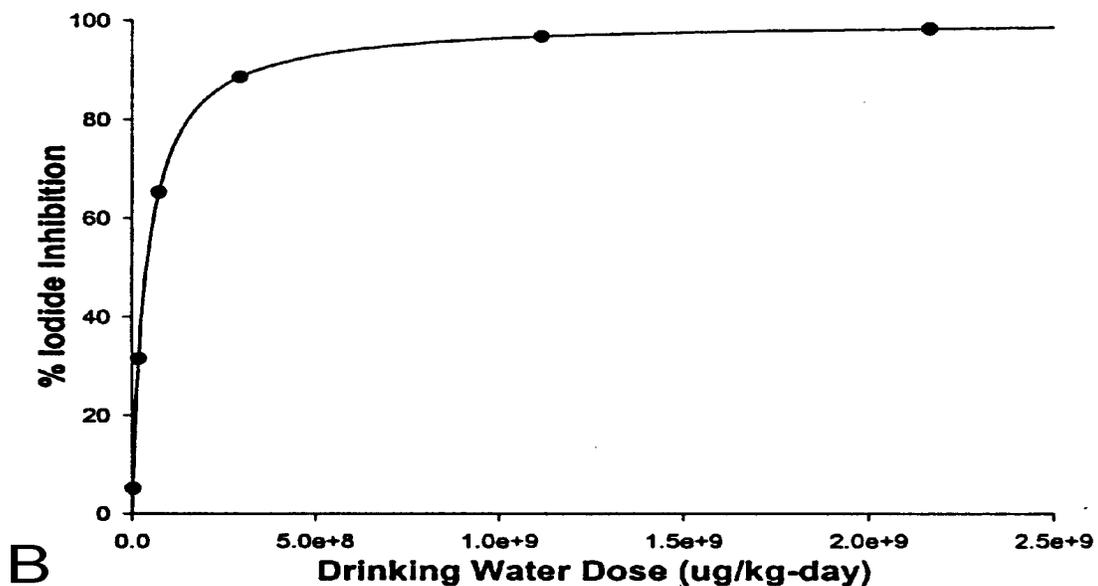
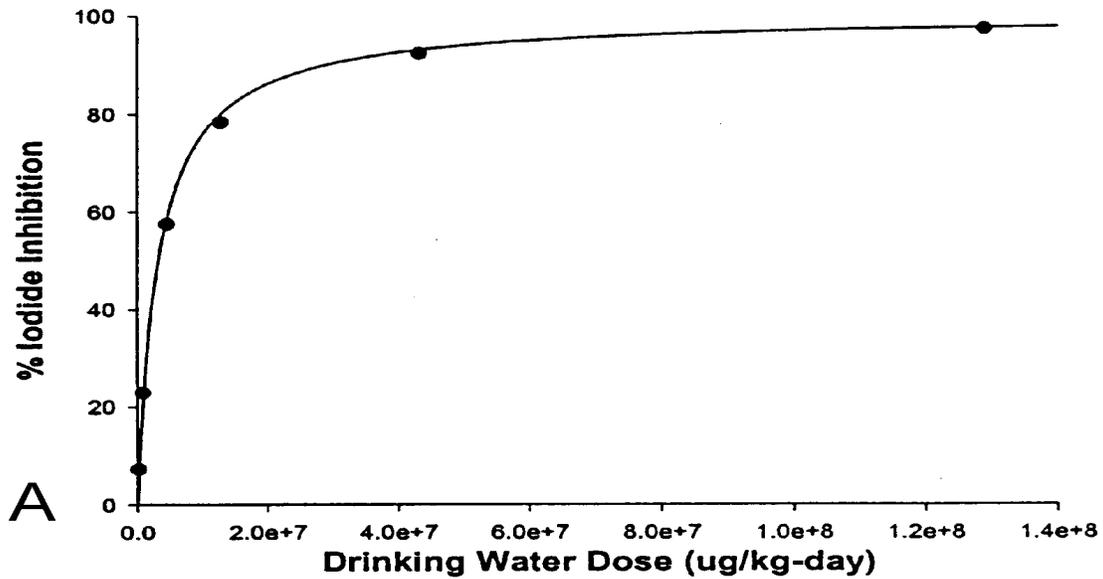


Figure 6-50. Michaelis-Menten fit of the human equivalent exposure (HEE) of perchlorate in drinking water derived from the area under the curve (AUC) for serum (A) or thyroid (B) versus percent predicted inhibition in the rat after an “acute” (iv) dose.

1 (in terms of body weight) than those of the adult. As a result, a slightly higher dose is required to  
 2 saturate these tissues in a child.

1           When comparing the dose metrics for serum versus thyroid, the HEE estimates calculated  
2 from the thyroid were less than the HEE estimates calculated from the serum by a factor of 100 at  
3 a 0.01 mg/kg-day dose level. This difference became a factor of 10 starting at the 5.0 mg/kg-day  
4 concentration for the 15 kg child and at 10.0 mg/kg-day for the adult.

5           These considerations will be explored in Chapter 7 to develop dosimetric adjustment  
6 factors for the observed effect levels.

7

## 7. DOSE-RESPONSE ASSESSMENTS FOR HUMAN HEALTH

The available database prior to initiation of the perchlorate testing strategy in 1997 (see Chapter 3) on the health effects and toxicology of perchlorate or its salts was very limited. The majority of human data were clinical reports of patients treated with potassium perchlorate for hyperthyroidism resulting from an autoimmune condition known as Graves' disease. Potassium perchlorate still is used diagnostically to test TSH, T3, and T4 production in some clinical settings. The primary effect of perchlorate is to decrease the production of thyroid hormones by competitively inhibiting iodide anion uptake into the thyroid at the *sodium (Na<sup>+</sup>)-iodide (I) symporter (NIS)* and by causing a discharge of stored iodide from the thyroid gland.

It was difficult to establish a dose-response for the effects on thyroid function from daily or repeated exposures in healthy humans based on the data in patients with Graves' disease because of a variety of confounding factors, including that the disease itself has effects; that often only a single exposure and not repeated exposures were tested; that only one or two doses were employed; and that often the only effect monitored was iodide release from the thyroid or control of the hyperthyroid state. There were limited data in normal human subjects and laboratory animals that support the effect of perchlorate on thyroid hormones, but the majority of these studies suffer from the same limitations as those with the Graves' disease patients, with respect to the number of doses and exposures. These limitations prevent establishment of a quantitative dose-response estimate for the effects on thyroid hormones after long-term repeated exposures to perchlorate in healthy human subjects.

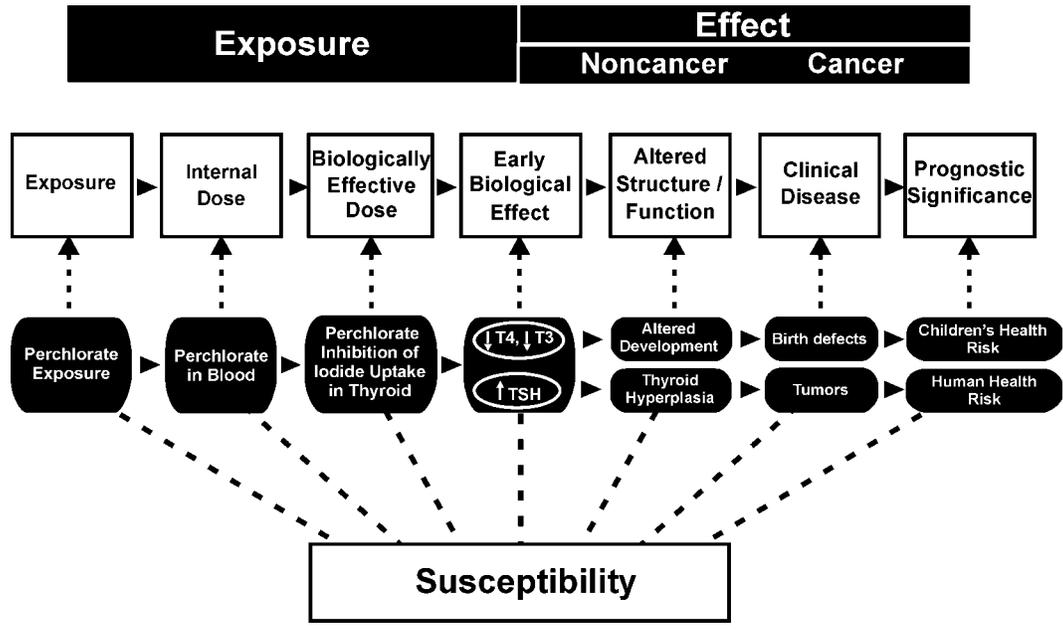
In addition, on December 14, 2001, after internal peer review of this document, the Agency articulated its interim policy on the use of third-party studies submitted by regulated entities (U.S. Environmental Protection Agency, 2001c). For these purposes, EPA is considering "third party studies" as studies that have not been conducted or funded by a federal agency pursuant to regulations that protect human subjects. Under the interim policy, the Agency will not consider or rely on any such human studies (third-party studies involving deliberate exposure of human subjects when used to identify or quantify toxic endpoints such as those submitted to establish a

1 NOAEL or NOEL for systemic toxicity of pesticides) in its regulatory decision making, whether  
2 previously or newly submitted. Some of the clinical studies contained in this database fall in this  
3 category of studies not to be considered. However, the scientific and technical strengths and  
4 weaknesses of these studies were described before this Agency policy was articulated.  
5 Therefore, because of the scientific shortcomings of these studies, they will not be used as  
6 “principal studies” in the derivation of an RfD. The ethical issues surrounding the conduct of  
7 these studies or their use for regulatory purposes in light of the Agency’s interim policy will not  
8 be discussed in this document. The Agency is requesting that the National Academy of Sciences  
9 conduct an expeditious review of the complex scientific and ethical issues posed by EPA’s  
10 possible use of third-party studies which intentionally dose human subjects with toxicants to  
11 identify or quantify their effects.

12 Thyroid hormone deficiencies, such as those induced by perchlorate, can affect normal  
13 metabolism, growth, and development. However, no robust data existed previously with which  
14 to evaluate potential target tissues or effects other than those in the thyroid. The data on the  
15 thyroid effects were also insufficient for quantitative dose-response assessment. Additionally,  
16 there were no data with which to evaluate the effects of perchlorate in potentially susceptible  
17 populations, such as developing fetuses; nor were there data on the effects of perchlorate on the  
18 reproductive capacity of male or female laboratory animals.

19 Benign tumors had been reported in the thyroids of male Wistar rats and female BALB/c  
20 mice treated with repeated, high-dose exposures (2 years at 1,339 mg/kg-day and 46 weeks at  
21 2,147 mg/kg-day, respectively) of potassium perchlorate in drinking water, establishing  
22 perchlorate as a carcinogen. Benign tumors in the thyroid have been established to be the result  
23 of a series of progressive changes that occur in the thyroid in response to interference with  
24 thyroid-pituitary homeostasis (i.e., perturbation of the normal stable state of the hormones and  
25 functions shared between these two related glands). This progression is similar regardless of the  
26 cause of the thyroid hormone interference (Hill et al., 1989; Capen, 1997; Hurley et al., 1998).  
27 EPA has adopted the policy that for the dose-response of chemicals that cause disruption in the  
28 thyroid but that do not have genotoxic activity (i.e., cause damage to DNA or show other genetic  
29 disruption) a threshold for carcinogenicity is to be based on precursor lesions (U.S.  
30 Environmental Protection Agency, 1998e).

1 In the case of perchlorate, an overall model based on its mode of action has been developed  
 2 as shown in Figure 7-1. The model supports iodide inhibition as the key event that precedes the  
 3 hormone and thyroid changes with subsequent neurodevelopmental and neoplastic sequelae.  
 4 Focusing on the key event of iodide uptake inhibition allows a harmonized approach to both the  
 5 “noncancer” and “cancer” toxicity that occurs downstream along the continuum. Thus, one  
 6 harmonized risk estimate is derived for both sequelae based on their common mode of action.  
 7  
 8



**Figure 7-1. Mode-of-action model for perchlorate toxicity proposed by the U.S. EPA (U.S. Environmental Protection Agency, 1998d). Schematic shows the exposure-dose-response continuum considered in the context of biomarkers (classified as measures of exposure, effect, and susceptibility) and level of organization at which toxicity is observed (U. S. Environmental Protection Agency, 1994a; Schulte, 1989). The model maps the toxicity of perchlorate on this basis by establishing casual linkage or prognostic correlations of precursor lesions.**

1 This chapter presents the synthesis of the most relevant data for deriving a revised  
 2 quantitative assessment of human health risk for perchlorate. The new data were consistent with  
 3 the limited historical characterization and the 1998 EPA assessment in that the anti-thyroid

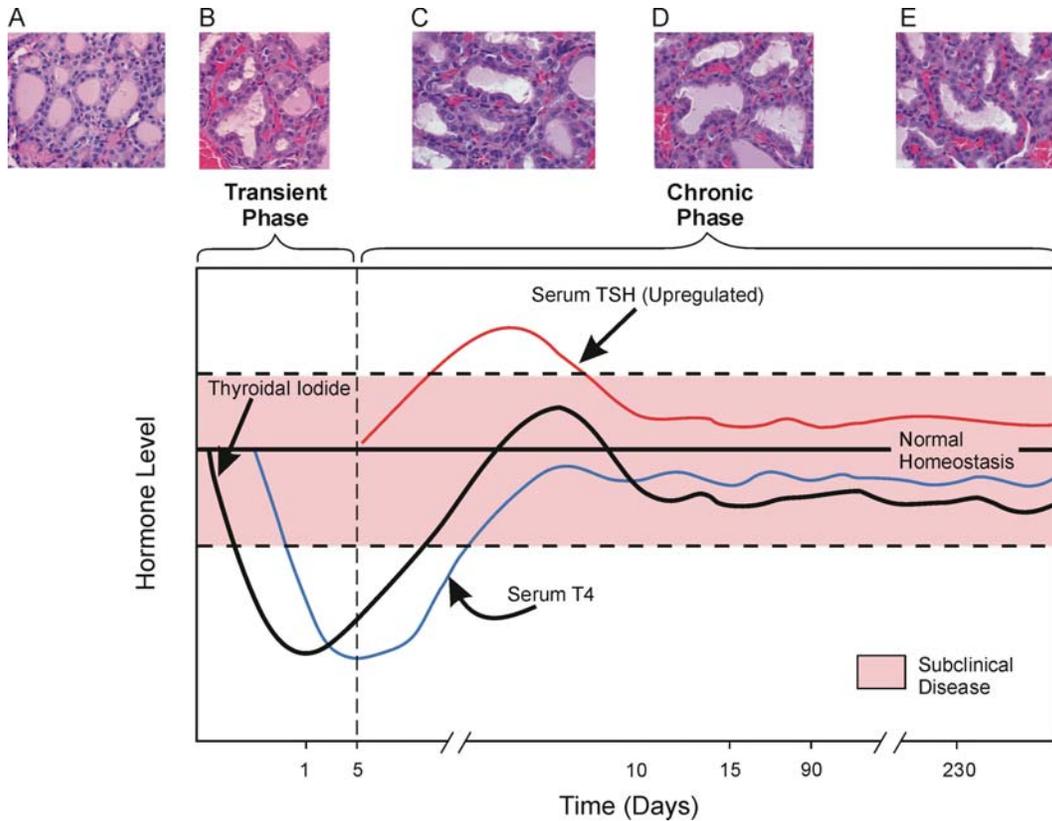
1 effects remain the focus of concern and the key event of its mode of action remained identified as  
2 the inhibition of iodide uptake at the NIS. However, data from the testing strategy allowed a  
3 more comprehensive evaluation of the possible sequelae of the iodide uptake inhibition and its  
4 thyroid-pituitary axis perturbations with respect to other endpoints, notably effects in dams and  
5 their offspring and on neurodevelopmental, reproductive, and immunotoxicity parameters.

6 The key event is defined as an empirically observable precursor step that is a necessary  
7 element of the mode of action or is a marker for such an element. This will be discussed in  
8 Section 7.1.1. Section 7.1.2 discusses dosimetric adjustment of effect levels observed in the  
9 laboratory animals to human equivalent exposures (HEE). Choice of the point of departure for  
10 the assessment based on a quantitative consideration of the key event, observed effects, and  
11 weight of the evidence is discussed in Section 7.1.3. Application of factors to account for  
12 uncertainty and variability in the extrapolations required to use the data is discussed in Section  
13 7.1.4. The overall operational derivation is presented in Section 7.1.5, and the assignment of  
14 confidence levels is discussed in Section 7.1.6. Section 7.1.5 also presents a discussion of the  
15 cancer assessment in the context of the RfD. Section 7.2 discusses the inhalation reference  
16 concentration. Susceptible population considerations are discussed in Section 7.1.5.3. Section  
17 7.3 presents a brief summary of the findings.

### 19 **7.1.1 Key Events and Weight of the Evidence**

20 Results of the testing strategy have established that the critical target tissue for perchlorate  
21 is the thyroid gland, with some remaining concern for adequate characterization of its potential  
22 for immunotoxicity, notably contact hypersensitivity. Changes in thyroid weights, three response  
23 indices of thyroid histopathology (colloid depletion, hypertrophy and hyperplasia), and thyroid  
24 and pituitary hormones were consistently altered across the array of experimental designs  
25 represented by the data base. The developmental and reproductive NOAEL and LOAEL values  
26 were higher than those associated with thyroid toxicity per se.

27 Figure 7-2 highlights the temporal considerations that have to be superimposed on  
28 evaluation of the data from the various studies in laboratory animals and humans in order to  
29 characterize the anti-thyroid effects from perchlorate exposure. Conceptually, competitive  
30 inhibition of iodide uptake at the NIS by perchlorate is the key event leading to both potential  
31 neurodevelopmental and neoplastic sequelae. The decrement in iodide uptake leads to



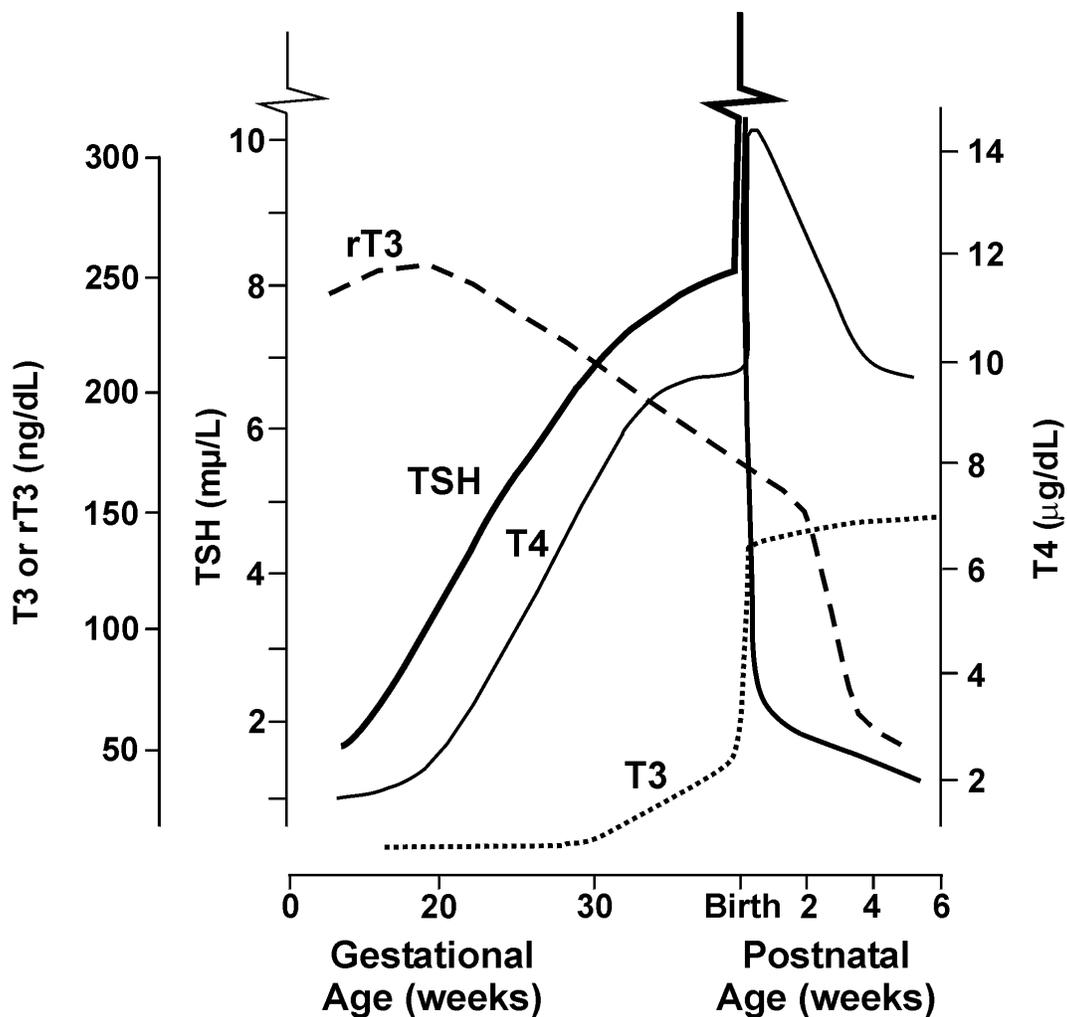
**Figure 7-2. Schematic of thyroid and pituitary hormone levels with associated pathology after acute versus chronic dosing with perchlorate. The transient phase is represented by decreases in thyroidal iodide due to the inhibition by perchlorate at the NIS with subsequent drop in T4. The transient drops in T4 can lead to permanent neurodevelopmental sequelae. Once TSH is upregulated via the hypothalamic-pituitary-thyroid feedback, T4 appears to be in normal homeostasis but actually can represent subclinical or undiagnosed disease (hypothyroxinemia). The upregulation of TSH can result in neoplasia. Normal thyroid tissue is represented in Panel A. Panel B shows lace-like colloid depletion which is more pronounced in subsequent panels C, D and E. Panels D and E represent hypertrophy and hyperplasia.**

- 1 subsequent drops in T4 (and T3) that can lead to permanent neurodevelopmental deficits.
- 2 Corroborating evidence for this likely outcome given the mode of action of perchlorate comes
- 3 from the iodide deficiency literature and recent studies showing that maternal hypothyroxinemia
- 4 (i.e., decrements in T4 with or without concomitant increases in TSH) is linked to poor
- 5 developmental, neuropsychological and cognitive outcomes (Haddow, et al., 1999; Pop et al.,

1 1999; Morreale de Escobar, et al., 2000). It should be noted that medical concern for  
2 hypothyroxinemia remains in the “chronic phase”; i.e., once TSH upregulates to attempt to  
3 regulate the hypothalamic-pituitary-thyroid feedback system back to an apparent homeostasis,  
4 because this stress on the system essentially represents a “subclinical” disease state. Indeed,  
5 adverse outcome in women with hypothyroxinemia per se has been demonstrated because  
6 adversity includes the inability of an organism to respond to additional stressors. The system in  
7 this case, particularly when considered on a population level, would present a diminished  
8 capacity to compensate for other anti-thyroid insults. Since a large percentage of women are  
9 believed to already be hypothyroid, the importance of this effect to women in general, pregnant  
10 women, and fetuses on a population level can not be discounted. Weiss (2000) has noted that  
11 even if the magnitude of effect may be relatively small for most environmental levels, such  
12 neurotoxicity is extremely significant for public health.

13 Of notable concern, as previously discussed in Chapter 3, is that the developing fetus is  
14 dependent on the mother for its T4 and T3 through parturition, as illustrated in Figure 7-3 for  
15 humans with a similar pattern in rats. During the period illustrated in Figure 7-3, a number of  
16 critical stages in neural development take place, some of which depend on thyroid hormones.  
17 The cell precursors of the brain and spinal cord which compose the central nervous system  
18 (CNS) begin to develop early in embryogenesis through the process called neurulation.  
19 Beginning early in the second week of gestation in rodents (GD9.5 in rats) and the first month of  
20 gestation in humans, specific areas of the CNS begin to form with the neurogenesis and  
21 migration of cells in the forebrain, midbrain, and hindbrain. This sequence of developmental  
22 processes includes proliferation, migration, differentiation, synaptogenesis, apoptosis, and  
23 myelination (Rice and Barone, 2000). As discussed in Chapter 3, thyroid hormones play a role  
24 throughout this process, regulating proliferation, migration, and differentiation. Alterations in  
25 these processes can result in abnormalities of the brain and developmental delays.

26 The upregulation in TSH in the “chronic phase” (see Figure 6-2) also presents an increased  
27 potential for neoplasia because stimulation of the thyroid to produce more T4 and T3 can result  
28 in hyperplasia. Both the decrement in T4 and T3 and increase in TSH is shown in Figure 7-1 at  
29 the same step along the continuum. Which of these thyroid responses is the most sensitive to  
30 hormone changes has not specifically been studied in the perchlorate testing strategy. As noted  
31 in the analyses of the studies in Chapter 5, there is a considerable degree of overlap among the



**Figure 7-3. Pattern of change in fetal and neonatal thyroid function parameters during pregnancy and extrauterine adaptation in the human (from Fisher, 1996). A similar pattern is thought to exist in the rat (see text for further details).**

1 three different diagnoses of thyroid histopathology: colloid depletion, hypertrophy, and  
 2 hyperplasia.

3 Colloid depletion does appear to be slightly more sensitive across the perchlorate studies.  
 4 The fact that thyroid follicular colloid depletion is a consistent finding not only across this study,  
 5 but in rodents in general, would suggest that it is a good indicator of sufficient exposure to inhibit  
 6 thyroid hormone synthesis. From a physiologic point of view this may be logical and supports  
 7 the mode-of-action model. If there is any reserve thyroid hormone in the colloid, it is depleted

1 before serum hormones are altered. Once serum levels are altered, TSH is upregulated and  
2 hypertrophy and hyperplasia are initiated in an attempt by the gland to restore circulating levels  
3 of T4 and T3. The diagnosis of colloid depletion has been reported with a similar compound,  
4 sodium chlorate, in the rat (Hooth et al., 2001), with many other chemicals in the rat, and with  
5 numerous goitrogens and pharmaceutical agents in the mouse. Colloid depletion in association  
6 with hypertrophy and hyperplasia suggests sufficient dose of the compound to inhibit colloid  
7 synthesis and decreases of circulating serum thyroid hormone levels sufficient to stimulate TSH.

8 Colloid depletion as the most sensitive indicator is most notable in the pups of the 2001  
9 “Effects Study” on GD21 and then immediately post parturition on PND4. Alternatively, as  
10 discussed in Chapter 5, it may have been harder to diagnose hypertrophy and hyperplasia in the  
11 younger (smaller) and growing glands. The BMDL for colloid depletion increased with post-  
12 natal age and by PND21, hyperplasia was also present. In contrast, all three thyroid indices were  
13 present in the PND4 pups of the previous Argus Laboratories, Inc. (1998a) study. This may be  
14 due to the difference in dosing of the dams. The dams in the 1998 study were only dosed during  
15 gestation and, therefore, likely had a greater decrement in thyroid hormones. The dams in the  
16 2001 study were dosed for two weeks during cohabitation, sufficient time as evidenced in the  
17 data described in Chapter 6, for upregulation of NIS to compensate.

18 Other studies indicate that whichever index is most sensitive could be dependent on dose  
19 spacing in the study, age of animals on test, and sacrifice time point. For example, hyperplasia  
20 was the most sensitive of the three in the P2-generation adults (19 week F1-generation pups) and  
21 these same pups developed thyroid adenomas.

22 The proposed mode of action mapped in Figure 7-1 is supported by correlations between  
23 thyroid hormones and TSH and between thyroid hormones or TSH and an objective measure of  
24 lumen size from laboratory animals exposed to ammonium perchlorate. There were positive  
25 correlations between T3 and T4, and negative correlations between either T3 and T4 and TSH, as  
26 expected based on the mode of action model (Appendix 7A). The positive correlation between  
27 TSH and decreased follicular lumen size and negative correlation between T4 or T3 and  
28 decreased follicular lumen size similarly support the proposed model (Appendix 7A). Some of  
29 the correlations used in the 1998 assessment were precluded due to the limited severity scoring  
30 system used by the PWG.

1 Additional support for the mode of action comes from data that now allow the linkage of  
2 both neurodevelopmental and neoplastic sequelae into the model. These definitive data were not  
3 available prior to the 1997 perchlorate testing strategy and especially not before the most recent  
4 studies recommended by the 1999 external peer review. The repeat of observed effects on the  
5 motor activity and brain morphometry results by new studies allowed definitive determination  
6 that perchlorate exposure poses a neurodevelopmental hazard.

7 Repeatability and variability in statistics, sometimes a concern for evaluation of behavioral  
8 assays (Cory-Slechta et al., 2001) were addressed by the Bayesian approach employed for the  
9 motor activity analysis (Dunson, 2001a) that showed remarkable reproducibility between the two  
10 studies despite the deficits previously noted for the Argus Research Laboratories, Inc. (1998a)  
11 study. The effects on the size of the corpus callosum measurements were also repeated, and  
12 effects on additional brain regions identified. The new data were subject to a more rigorous  
13 statistical analysis than in 1998. The profile analysis described in Chapter 5 required that all  
14 areas of the brain measured were altered in a dose-dependent fashion and effects were again  
15 demonstrated not only in the corpus callosum but other brain regions as well (Geller, 2001d).

16 Likewise the neoplastic potential for perchlorate that had been demonstrated only at high  
17 doses in historical studies was confirmed at lower doses by the thyroid adenomas reported by the  
18 PWG (Wolf, 2000; 2001) for the F1-generation pups at 19 weeks (P2 parents) from the  
19 two-generation reproductive study (Argus Laboratories, Inc., 1999). Consistent with the  
20 proposed mode-of-action model, the anti-thyroid effects leading to neoplasia are likely to be via  
21 the non-linear mechanism described above. The genotoxicity battery established that perchlorate  
22 is not directly damaging to DNA.

23 Thus, the key event for the anti-thyroid effects of perchlorate is its perturbation of the  
24 hypothalamic-pituitary-thyroid axis by competitive inhibition of iodide uptake at the NIS. The  
25 evidence for this effect is built upon the observation of consistent changes across a range of  
26 experimental designs, including various species. These changes demonstrate effects on thyroid  
27 and pituitary hormones, increases in thyroid weight, and increases in three different diagnoses of  
28 thyroid histopathology (colloid depletion, hypertrophy, and hyperplasia). In addition,  
29 corresponding neurodevelopmental (motor activity and brain morphometry) and neoplastic  
30 outcomes were observed in special assays; these outcomes are also consistent with the proposed

1 mode of action and provide further evidence to confirm that the perturbation of the thyroid  
2 hormone economy should be viewed as adverse.

3 Due to the age and time-dependent nature of the critical effect, no one principal study is  
4 being chosen for this derivation. Instead, a weight-of-the-evidence approach will be taken to  
5 arrive at a point of departure in Section 7.1.3.

### 7 **7.1.2 Dosimetric Adjustment of Exposures Associated with Effect Levels**

8 Adjustments for interspecies differences in the internal dose delivered to target tissues  
9 should be made before an evaluation of the data array for valid comparisons across endpoints  
10 (U.S. Environmental Protection Agency, 1994). Based on the mode of action and the available  
11 PBPK model structures, two dose metrics were considered to describe the biologically effective  
12 dose for perchlorate: (1) the area under the curve (AUC) for perchlorate in the serum associated  
13 with drinking water exposures and (2) the percent of iodide uptake inhibition in the thyroid.  
14 These correspond to the different exposure components along the exposure-dose-response  
15 continuum in the mode-of-action model (Figure 7-1).

16 As described in Chapter 6, the serum perchlorate AUC was developed as the first dose  
17 metric based on data in rats and humans after drinking water exposures. To predict the  
18 “transient” phase of initial iodide inhibition in the rat, i.e., before upregulation of the NIS or  
19 increases in TSH, the second dose metric was based on RAIU measurements made in adult male  
20 rats dosed with perchlorate by iv two hours prior to an iv dose of radiolabeled iodide. Table 7-1  
21 presents the human equivalent exposures (HEE) estimates calculated using the PBPK models for  
22 serum perchlorate AUC as the dose metric. Table 7-2 shows the ratios for this same dose metric  
23 that can be applied in the parallelogram approach to arrive at estimates for different life stages  
24 used to observe effects in the different experimental endpoints. Fetal rat predictions were based  
25 on data developed for GD21. Neonatal rat predictions were based on data for PND10. This  
26 approach was taken since PBPK models for human pregnancy and lactation do not exist for  
27 perchlorate distribution. The calculation using the ratios approach is described in Chapter 6.  
28 The resultant adult HEE values for the different life stages of the rat experiments are shown in  
29 Table 7-3.

30 It can be observed in the tables in Merrill (2001e) that the pregnant and lactating rats have  
31 significantly higher average serum perchlorate concentrations at the lowest drinking water dose

**TABLE 7-1. PBPK-MODEL CALCULATED HUMAN EQUIVALENT EXPOSURES (HEE) TO VARIOUS EXPERIMENTAL DOSES IN THE MALE RAT FOR 15 AND 70 KG HUMAN BASED ON PERCHLORATE AREA UNDER THE CURVE (AUC) IN SERUM OR THYROID AS THE DOSE METRIC (Merrill, 2001e)**

<b>Adult Male Rat DW<sup>a</sup> Dose (mg/kg-day)</b>	<b>Human 15 kg HEE (mg/kg-day) based on serum<sup>b</sup> AUC</b>	<b>Human 70 kg HEE (mg/kg-day) based on serum<sup>b</sup> AUC</b>	<b>Human 15 kg HEE (mg/kg-day) based thyroid<sup>b</sup> AUC</b>	<b>Human 70 kg HEE (mg/kg-day) based on thyroid<sup>b</sup> AUC</b>
0.010	0.030	0.021	0.0002	0.0001
0.1	0.145	0.100	0.002	0.001
1.0	0.745	0.505	0.008	0.006
3.0	2.05	1.35	0.052	0.035
5.0	3.35	2.25	0.145	0.098
10.0	6.75	4.45	0.725	0.460
30.0	20.3	13.2	163.0	110.0
100.0	65.0	43.8	490.0	330.0

<sup>a</sup>DW = drinking water.

<sup>b</sup>Calculated from PBPK-derived rat AUC(s) at steady state between 240 and 264 hrs during DW exposure, using upregulated V<sub>maxv\_T<sub>p</sub></sub> values from (Merrill, 2001e: Table 1).

**TABLE 7-2. RATIO OF PBPK-DERIVED PERCHLORATE AREA UNDER THE CURVE (AUC) SERUM CONCENTRATIONS IN DRINKING WATER FOR VARIOUS EXPERIMENTAL LIFE STAGES (Merrill, 2001e)**

<b>Rat DW<sup>a</sup> Dose (mg/kg-day)</b>	<b>Male Rat: Pregnant Rat</b>	<b>Male Rat: Lactating Rat</b>	<b>Male Rat: Fetal Rat</b>	<b>Male Rat: Neonate Rat</b>	<b>Pregnant Rat: Fetal Rat</b>	<b>Lactating Rat: Neonate Rat</b>
0.01	0.63	0.58	1.44	1.16	2.28	1.99
0.1	0.73	0.54	1.06	0.85	1.46	1.56
1.0	0.90	0.84	1.44	1.01	1.61	1.20
3.0	0.94	0.95	1.67	1.71	1.77	1.80
5.0	0.95	0.98	1.74	2.14	1.82	2.18
10.0	0.96	1.01	1.80	2.70	1.87	2.69
30.0	0.97	1.02	1.84	3.33	1.90	3.26
100.0	0.97	1.03	1.85	3.65	1.92	3.55

<sup>a</sup>DW = drinking water.

**TABLE 7-3. PBPK-MODEL CALCULATED HUMAN EQUIVALENT EXPOSURES (HEE) TO VARIOUS EXPERIMENTAL LIFE STAGES IN THE RAT USING SERUM PERCHLORATE AREA UNDER THE CURVE (AUC) AS THE DOSE METRIC**

Dose (mg/kg-day)	Human Equivalent Exposure <sup>a</sup> (mg/kg-day)				
	Adult Male Rat	Pregnant Rat	Fetal Rat	Lactating Rat	Neonate Rat
0.01	0.02	0.01	0.03	0.01	0.02
0.1	0.10	0.07	0.10	0.05	0.08
1.0	0.51	0.46	0.73	0.43	0.52
3.0	1.35	1.3	2.3	1.3	2.4
5.0	2.25	2.14	3.92	2.20	4.82
10.0	4.4	4.22	7.9	4.4	11.9
30.0	13.2	12.8	24.3	13.5	43.95
100.0	43.8	42.5	81.0	45.11	160.0

<sup>a</sup>Based on predicting the area under the curve in the blood (AUCB) using the human PBPK model that achieves an equivalent degree to that simulated for the rat experimental regimen associated at different life stages. See Tables 7-1 and 7-2 and Chapter 6 for explanation of calculation.

(0.01 mg/kg-day). This is likely due to increased binding in the serum (Merrill, 2001e). It has been shown that the estrus cycle affects the concentration of binding proteins within the blood. Thyroxine, which is displaced from plasma proteins by perchlorate, is bound to a greater extent in the pregnant rat (Iino and Greer, 1960). It follows then that perchlorate would also be bound to a greater extent during pregnancy and possibly lactation. Since serum binding affects only the low doses, it is reasonable that the higher doses (1.0 through 100 mg/kg-day) would be similar across the male, pregnant and lactating rats (Merrill, 2001e).

Tables 7-4 through 7-7 are a comparable set of tables but are based on using thyroid uptake inhibition as the dose metric. Table 7-5 shows the percent of iodide uptake inhibition predicted at each dose for the various life stages used in the various laboratory rat experiments.

#### 7.1.2.1 Choice of Dose Metric

Because developmental effects are of concern, an argument could be made that peak—and not AUC—is the appropriate dose metric with the rationale that any transient dose could be responsible for permanent deficits. However, the AUC values, as opposed to peak

**TABLE 7-4. PBPK-MODEL CALCULATED HUMAN EQUIVALENT EXPOSURES (HEE) TO VARIOUS EXPERIMENTAL DOSES IN THE ADULT MALE RAT FOR 15 AND 70 KG HUMAN BASED ON % IODIDE UPTAKE INHIBITION IN THE THYROID**

<b>Rat iv Dose (mg/kg)</b>	<b>Adult male rat inhibition at 2-hr post iv dose</b>	<b>Human 15 kg HEE (mg/kg-day)</b>	<b>Human 70 kg HEE (mg/kg-day)</b>
0.01	1.5%	0.006	0.004
0.1	16.3%	0.075	0.048
1.0	74.5%	1.5	0.9
3.0	90.0%	4.8	2.7
5.0	93.5%	8.0	4.9
10.0	96.2%	16.0	9.0
30.0	98.1%	35.0	19.3
100.0	98.7%	50.0	33.0

**TABLE 7-5. PBPK-MODEL PREDICTED % INHIBITION OF IODIDE UPTAKE IN THE THYROID<sup>a</sup>**

<b>Rat DW<sup>b</sup> Dose (mg/kg-day)</b>	<b>Adult Male Rat</b>	<b>Pregnant Rat</b>	<b>Fetal Rat<sup>c</sup></b>	<b>Lactating Rat<sup>d</sup></b>	<b>Neonate Rat<sup>c,d</sup></b>	<b>70 kg Human</b>
0.01	1.5%	3.2%	-129.1%	0.5%	0.4%	2.8%
0.1	16.3%	30.1%	27.9%	5.3%	1.3%	23.7%
1.0	74.5%	88.7%	81.2%	62.9%	3.0%	80.2%
3.0	90.0%	93.8%	90.3%	92.8%	3.3%	92.3%
5.0	93.5%	97.0%	90.4%	95.8%	3.1%	95.2%
10.0	96.2%	97.9%	97.9%	97.6%	3.8%	97.4%
30.0	98.1%	98.6%	98.9%	98.5%	6.1%	98.9%
100.0	98.7%	98.8%	99.2%	98.8%	13.4%	99.4%

<sup>a</sup>Based on iv administration to rat and drinking water in human.

<sup>b</sup>DW = drinking water

<sup>c</sup>Values for these tissues not validated versus data.

<sup>d</sup>All calculations are for PND10 in lactating and neonatal rat.

**TABLE 7-6. RATIOS OF PBPK-DERIVED % IODIDE UPTAKE INHIBITION IN DRINKING WATER FOR VARIOUS EXPERIMENTAL LIFE STAGES<sup>a</sup>**

Rat DW <sup>b</sup> Dose (mg/kg-day)	Male Rat: Pregnant Rat	Male Rat: Lactating Rat	Male Rat: Fetal Rat <sup>c</sup>	Male Rat: Neonate Rat <sup>c</sup>	Pregnant Rat: Fetal Rat	Lactating Rat: Neonate Rat <sup>c,d</sup>
0.01	0.48	3.24	-0.01	4.02	-0.02	1.2
0.1	0.54	3.06	0.59	12.75	1.08	4.2
1.0	0.84	1.18	0.92	24.53	1.09	20.7
3.0	0.96	0.97	1.00	27.49	1.04	28.4
5.0	0.96	0.98	1.03	30.45	1.07	31.2
10.0	0.98	0.99	0.98	25.61	1.00	26.0
30.0	0.99	1.00	0.99	16.06	1.00	16.1
100.0	1.00	1.00	1.00	1.37	1.00	7.4

<sup>a</sup>Inhibition in human was PBPK-derived from 2 wks ClO<sub>4</sub><sup>-</sup>-exposure in drinking water (DW); all rat values simulated from an iv dose.

<sup>b</sup>DW = drinking water

<sup>c</sup>Model predicted in fetal and neonate rats not validated with data.

<sup>d</sup>All calculations are for PND10 in lactating and neonatal rat.

**TABLE 7-7. PBPK-MODEL CALCULATED HUMAN EQUIVALENT EXPOSURES (HEE) TO VARIOUS EXPERIMENTAL LIFE STAGES IN THE RAT USING % IODIDE UPTAKE INHIBITION IN THE THYROID AS THE DOSE METRIC**

Dose (mg/kg-day)	Human Equivalent Exposure <sup>a</sup> (mg/kg-day)				
	Adult Male Rat	Pregnant Rat	Fetal Rat	Lactating Rat	Neonate Rat
0.01	0.004	0.002	—	0.01	0.02
0.1	0.048	0.026	0.03	0.15	0.61
1.0	0.90	0.756	0.83	1.06	22.05
3.0	2.7	0.259	2.70	2.62	74.2
5.0	4.9	4.70	5.05	4.80	149.2
10.0	9.0	8.82	8.82	8.91	230.5
30.0	19.3	19.1	19.1	19.3	309.96
100.0	33.0	33.0	33.0	33.0	33.0

<sup>a</sup>Based on predicting the % iodide uptake in the thyroid using the human PBPK model that achieves an equivalent degree to that simulated for the rat experimental regimen associated at different life stages. See Tables 7-4 and 7-6 and text for explanation of calculation.

1 concentrations, were used based on the assumption that these dose metrics would represent an  
2 averaging of the serum and thyroid perchlorate concentrations and would be better correlated  
3 with the inhibition effect on iodide uptake. The correlation was shown to be good between the  
4 AUC and the degree of inhibition (Figures 6-47 through 6-50). Further, due to the rapid phase of  
5 distribution after an iv dose, measurement of concentrations are very difficult to attain  
6 experimentally and are more variable. Using simulated peak concentrations after iv injections is  
7 potentially problematic due to the inexact modeling of the actual distribution of dose in the  
8 tail-vein volume and the exact time of mixing in the whole blood compartment (Merrill, 2001e).  
9 It was also observed by EPA that the ratios for peak perchlorate serum values (Merrill, 2001e:  
10 Table 6) were in good agreement with those for the perchlorate serum AUC and that the serum  
11 AUC were slightly more conservative if different at all.

12 Merrill (2001e) expressed concern regarding the thyroid values in neonates and fetuses  
13 because these values were not validated against experimental data. Fetal and neonatal thyroid  
14 were never actually analyzed for perchlorate concentration. In the case of the fetus, kinetic  
15 parameters were determined by fitting model simulations of fetal thyroid concentration to  
16 available iodide data and assuming that the perchlorate:iodide ratio would be similar to that of  
17 the mother. In the case of the neonatal rat, no data were available for thyroid concentrations for  
18 either perchlorate or iodide. Thus, model predictions were based on allometrically scaling  
19 maternal parameters for thyroid uptake. It was the opinion of the AFRL/HEST authors that while  
20 the thyroid parameters in the fetus and neonatal rat were highly informative, they should not be  
21 used in the formal risk assessment (Merrill, 2001e). EPA concurs with these considerations and  
22 recommendation.

23 In general, the models were believed to provide a good description of perchlorate and  
24 iodide disposition in the blood. Using the models to describe dose metrics in the thyroid was  
25 viewed as less reliable due to assumptions regarding parameters and the lack of experimental  
26 data for validation. The models were able to successfully describe serum perchlorate and iodide  
27 concentrations for both acute (based on iv doses) and chronic drinking water in the adult male,  
28 pregnant, neonatal and fetal rat, and greater confidence can be afforded these predictions  
29 (Merrill, 2001e).

30 Tables 7-3 and 7-7 demonstrate good correspondence in the HEE estimates predicted for  
31 both dose metrics at the lower doses for the lactating and neonatal rats, but not for the male adult,

1 pregnant or fetal rats where there is an order of magnitude difference. The iodide inhibition  
2 metric predicts a 10-fold lower HEE in both the adult male and pregnant dam when compared to  
3 the HEE estimated based on the serum AUC. The fetal rat value for iodide inhibition was  
4 viewed as unreliable for the reasons stated above. All of the factors influencing this disparity are  
5 not fully appreciated at this time but can reasonably be ascribed to uncertainty in the thyroid  
6 descriptions that were not validated with experimental data, and will require additional studies to  
7 characterize accurately. For these reasons, the adjustment factor to arrive at an HEE estimate  
8 was based on perchlorate serum AUC as the dose metric.

### 9 10 **7.1.3 Point-of-Departure Analysis**

11 Various statistical procedures were used for each of the different outcome measures for the  
12 various endpoints described in Chapter 5. The weight-of-evidence approach herein relies on the  
13 results, and the details on the statistical analyses are provided in Chapter 5 and associated  
14 memoranda from EPA and NIEHS scientists. In general, benchmark dose analysis was used for  
15 the thyroid histopathology because the EPA advocates the use of quantitative dose-response  
16 modeling to diminish the influence of dose-spacing, sample size, and variability on the NOAEL  
17 designation (Crump et al., 1995). Likewise, ANOVA was used to evaluate the thyroid and  
18 pituitary hormone data (Crofton and Marcus, 2001) although benchmark analyses were also  
19 performed as a comparison (Geller, 2001c). The 1998 benchmark analyses for the hormone data  
20 from the previous set of studies (Geller, 1998a) is provided in Appendix 7B.

21 Specific Bayesian statistical analyses were employed for the motor activity data and for  
22 evaluating the significance of the tumors in the 19-week old F1-generation adult rats (Dunson,  
23 2001a,b). Another specific statistical approach, profile analysis, was used to evaluate the brain  
24 morphometry effects (Geller, 2001d).

25 Several studies suggest 0.01 mg/kg-day as the exposure dose that is a level of concern for  
26 the adverse effects of perchlorate. The first is the profile analysis on brain morphometry effects  
27 in PND21 pups in the “Effects Study” (Argus Research Laboratories, Inc., 2001) which  
28 demonstrated a dose-dependent and significant effect on the size of the corpus callosum and  
29 other brain regions. Statistically significant changes were also demonstrated in the PND9 pups.  
30 This effect repeated effects on brain morphometry observed in the previous neurodevelopmental  
31 study (Argus Research Laboratories, Inc. 1998a) that were a noted concern to the EPA in the

1 1998 risk assessment. Changes in the corpus callosum at a later time point on PND82 were also  
2 observed in that previous study.

3 An increase in the corpus callosum plausibly represents a delay in developing brain  
4 structures since this area is known to increase in size and then decrease later during development.  
5 Neurodevelopmental toxicity suggestive of delays was also demonstrated by effects on motor  
6 activity in both the Argus Research Laboratories, Inc. (1998a) and repeated in the Bekkedal et al.  
7 (2000) study. The increases in motor activity represent activity that should have subsided by  
8 these test dates. A type of hyperactivity has been noted in monkeys exposed to PCBs (Rice,  
9 2000).

10 These effects on brain morphometry and motor activity are of particular concern because  
11 the relative sensitivity of laboratory animal assays to adequately characterize the types of deficits  
12 related to maternal hypothyroxinemia in large population studies is unknown (Morreale de  
13 Escobar, 2000; Haddow et al., 1999; Pop, 1999). Screening neurodevelopmental studies may not  
14 have the power to ascertain neurological effects that might result from small changes in the  
15 thyroid-pituitary hormone economy. As pointed out by Crofton (1998j), the sensitivity of animal  
16 models used to explore the role of thyroid hormones in neural development is currently  
17 equivocal. Most of the data collected and published to date were with high doses of thyrotoxic  
18 chemicals (e.a., methimazole, propylthiouracil) or with thyroidectomy. It is not known whether  
19 the available tests are capable of detecting more subtle changes in nervous system development.  
20 An analysis presented by Crofton (1998j) suggested that measurements of nervous system  
21 development are less sensitive than measurements of T4. Two reasons for this relationship were  
22 presented. First, the brain may be protected from perturbations in circulating concentrations of  
23 T4, as demonstrated by upregulation of deiodinases in brain tissue that compensate for very large  
24 decreases in circulating T4. The second reason, and one for concern in the context development  
25 of this model, is that currently available testing methods, particularly screening methods, may not  
26 be sufficiently sensitive. Recent data suggest that the battery is insensitive to alterations in  
27 thyroid hormones during development (Goldey, 1995a,b).

28 The 0.01 mg/kg-day dosage as a level for concern was also supported by thyroid  
29 histopathology in the database. Changes in colloid depletion observed on PND4 in both the 1998  
30 neurodevelopmental study (Argus Research Laboratories, Inc., 1998a) and the newer 2001  
31 “Effects Study” (Argus Research Laboratories, Inc. 2001) were demonstrated. The BMDL

1 estimated for those studies on PND4 was 0.33 mg/kg-day, but an estimate of 0.009 mg/kg-day is  
2 also obtained with a model demonstrating adequate fit to the data. The BMDL for colloid  
3 depletion in pups on GD21 was 0.12 mg/kg-day, but for female pups alone on GD21 was 0.04  
4 mg/kg-day. The BMDL estimated for thyroid hypertrophy in weanling pups from the two-  
5 generation study (Argus Research Laboratories, Inc., 1999) was 0.06 mg/kg-day. Of notable  
6 concern to this consideration was that the BMDL estimates decreased with duration in the 90-day  
7 study. The BMDL estimates for colloid depletion were 0.28 and 0.03 mg/kg-day at the 14-day  
8 and 90-day time points in the Springborn Laboratories, Inc. (1998) study. The BMDL estimates  
9 for hypertrophy were 0.017 and 0.008 mg/kg-day at the 14-day and 90-day time points. This  
10 effect of duration was of concern as it was also evident by the observation of tumors in the  
11 F1-generation adults at 19 weeks. Both observations suggest concern that duration may  
12 recalibrate either the homeostatic interactions of the hypothalamic-pituitary-feedback system or  
13 the cellular sensitivity and demand for the thyroid hormones.

14 The thyroid hormone data in a number of studies also designated 0.01 mg/kg-day as a  
15 LOAEL. Levels of T4 were significantly decreased and TSH levels statistically increased at this  
16 dosage in the dams on GD21 in the same study as the significant brain morphometry  
17 measurements in the PND21 pups (Argus Research Laboratories, Inc. 2001), revealing no  
18 NOAEL for hypothyroidism in the dams. The pups in that study were also affected at  
19 0.01 mg/kg-day. Effects on T3 occurred at GD21, PND5, and PND9 at this dosage. The  
20 0.01 mg/kg-day dose was the LOAEL for effects on T4 and TSH at PND21 in the male pups and  
21 for TSH in both sexes at PND9 as well. This same dose (0.01 mg/kg-day) was also the LOAEL  
22 for decreases in T4 and increases in TSH at the 14-day and 90-day time points in the 90-day  
23 study (Springborn Laboratories, Inc., 1998).

24 The ANOVA estimates for hormone data were used to characterize this effect after serious  
25 consideration. While in clinical studies a normal range typically is defined by a control healthy  
26 population, the ANOVA approach is an equally valid approach in that a statistically significant  
27 value represents a shift in the mean for the population. The control group defines the range for  
28 the unexposed, presumably healthy population, and statistically significant differences indicate  
29 that the mean for an exposed group is outside of that normal range. Circadian fluctuations are  
30 addressed because the same fluctuations in the control population occur as in the exposed  
31 population. A small shift in the mean of a population can have significant consequences to

1 individuals in the tails of the distributions of those populations. Indeed, such an evaluation  
2 underlies the basis for the blood lead level used in the National Ambient Air Quality Standard  
3 (Davis and Elias, 1996) and has been noted as an important consideration for neurotoxicity  
4 (Weiss, 2000).

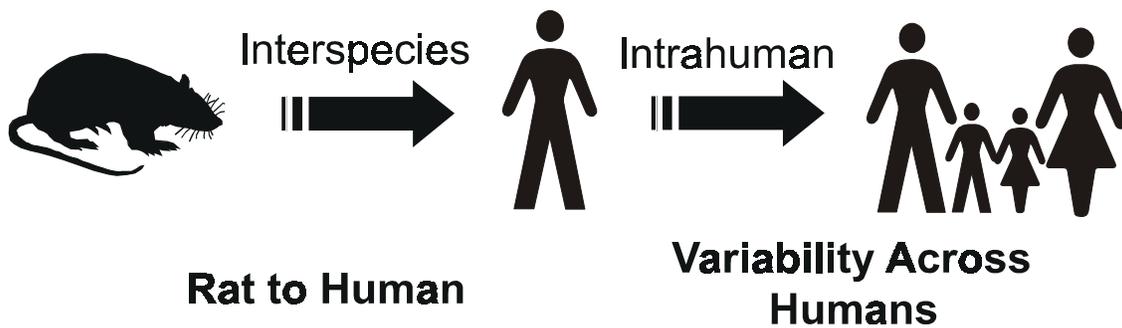
5 The notion that continuous data should be considered in the context of the specific dose-  
6 response rather than to *a priori* categories defined outside of the data under analysis is supported  
7 in the benchmark dose literature. Murrell et al. (1998) point out that a continuous quantity  
8 measurement such as the hormone data should be scaled by the range from background response  
9 level to maximum response level (for increasing response functions). The authors go on to note  
10 that it is a biological reality that, whatever the mechanism of effect of the toxicant, there is some  
11 dose level beyond which no further change in response is seen or is theoretically feasible.  
12 In general, there is some type of limitation or saturation phenomenon that occurs at high enough  
13 doses (e.g., in the saturation of the symporter capacity, as suggested by the modeling effort in  
14 Chapter 6 and the data of Chow and Woodbury [1970] and of Meyer [1998]).

15 An analogy to the case of quantal data for which an effect is defined as a probability metric  
16 in which the response reaches a maximum at one, is, that for continuous measures, the extra  
17 effect can be defined as the change in effect from background standardized by the total range of  
18 response (Murrell et al., 1998). The total response range is not necessarily the response range of  
19 the observed responses in a study; rather, it is defined by a determination of the minimum and  
20 maximum possible responses according to, for example, a model equation fitted to the data as in  
21 the case of benchmark analyses. In all BMD analyses, however, the hormone BMDL estimates  
22 were shown to be extremely low (Geller, 1998a; Geller, 2001c). This may not necessarily be  
23 surprising given that hormones are operative at low doses by definition, but corresponding  
24 changes in thyroid histopathology were more consistent with the ANOVA estimates.

25 Finally, the NOAEL for immunotoxicity suggested by the dermal contact hypersensitivity  
26 assay at 0.02 mg/kg-day can be viewed as supportive, especially since deficiencies in this study  
27 raise concern for the characterization and because a LOAEL for the effect was demonstrated at  
28 0.06 mg/kg-day.

#### 7.1.4 Application of Uncertainty Factors

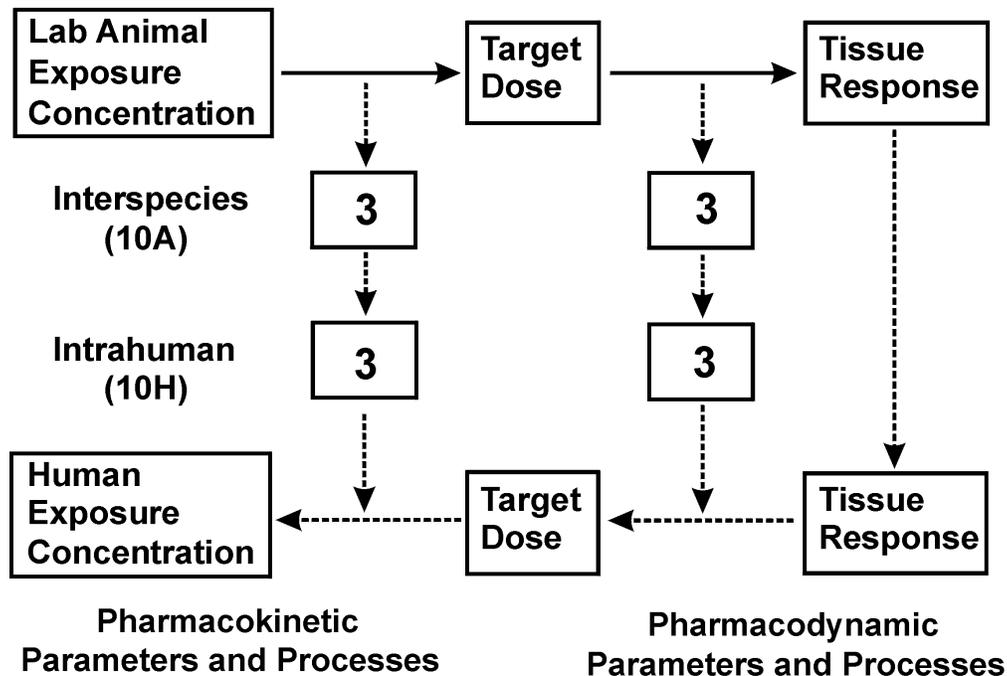
The types of uncertainty factors (UF) applied for various extrapolations required to arrive at a reference dose were discussed in Chapter 3. Figure 7-4 illustrates schematically that the interspecies and intraspecies UFs embody attributes of both uncertainty and variability. A factor for variability across humans typically is applied to account for potentially susceptible portions of the population. As shown in Figure 7-5 (Jarabek, 1995b), both of these factors typically are broken into components of approximately three each for pharmacokinetics (toxicokinetics) and pharmacodynamic (toxicodynamic) processes. This scheme is consistent with that used by the World Health Organization (WHO) (Jarabek, 1995b).



**Figure 7-4. Consideration of uncertainty and variability influence interspecies and intrahuman extrapolation.**

There were a total of four (4) uncertainty factors applied in this derivation, resulting in a composite factor of 300. The partial factors of 3 represent “halving” of each UF that is believed to be an upper bound on a lognormal distribution; i.e.,  $10^{0.5}$ , so that multiplication of the various partial factors results in a composite of 100 (U.S. Environmental Protection Agency, 1994).

A 3-fold factor for intraspecies variability was retained due to the variability observed in the data and PBPK modeling for the adult humans and because these subjects do not represent kinetic data for the potentially susceptible populations of the hypothyroid or hypothyroxinemic pregnant women and their fetuses. There was also uncertainty in the parallelogram approach to extending the adult structure to address different life stages. These uncertainties might be



**Figure 7-5. Schematic of uncertainty factor components incorporated into exposure-dose-response characterization for interspecies and intrahuman extrapolations (Jarabek, 1995b).**

1 mitigated by further development of pregnancy and lactation models or the models might be  
 2 further validated with radionuclide data using a parallelogram approach between perchlorate  
 3 and iodide as described in Chapter 6. This reduced factor was a point of considerable debate,  
 4 especially given the concern over the animal neurodevelopmental assays for adequately  
 5 characterizing neuropsychological development deficits in susceptible populations. However, it  
 6 was also discussed that the UF values are not entirely independent; e.g., aspects underlying the  
 7 duration extrapolation also might underlay the intrahuman UF (Jarabek, 1995b).

8 The interspecies factor was omitted due to general confidence that the extrapolation based  
 9 on perchlorate distribution (and on iodide inhibition by perchlorate at lower doses) was  
 10 accurately characterized by the PBPK modeling effort described in Chapter 6. Concern for  
 11 eliminating this factor was again considered in the context of the lack of independence with other  
 12 applied UF. The concern that the HEE was not based on iodide inhibition but rather the serum

1 perchlorate AUC was assuaged somewhat by the correlations that demonstrated a close  
2 relationship between these two measures.

3 A full 10-fold factor was applied to extrapolate the LOAEL for the brain morphometry,  
4 thyroid histopathology, and hormone changes observed at the 0.01 mg/kg-day level. Designating  
5 these changes to be adverse is consistent with the proposed mode of action and existing Agency  
6 guidance and procedures. The neurotoxicity assessment guidelines (U.S. EPA, 1998a) specify  
7 changes in brain structure as adverse. The OPPTS has used thyroid hormone changes to  
8 designate effect levels. Finally, the shallow slope of the response curve at these lower levels  
9 suggested that a full factor should be applied.

10 A 3-fold factor for duration was applied due to the concern for the biological importance of  
11 the statistically significant increase in tumors in the F1-generation pups at 19 weeks (P2, second  
12 parental generation). The occurrence of these tumors with a dramatically reduced latency and  
13 with a significance in incidence greater than the NTP historical data (Dunson, 2001b) for thyroid  
14 tumors in this strain of rat was reason for concern. As discussed earlier, the concerns were that  
15 this observation represented the potential for *in utero* programming; and that the decrease in the  
16 NOAEL/LOAEL estimates for hormone perturbations and histopathology between the 14-day  
17 and 90-day time points represented a recalibration of the regulatory feedback system or changes  
18 in cellular sensitivity and demand for thyroid hormones with extended exposures. This factor  
19 can also be viewed as part of a data base deficiency because there are no long-term bioassays of  
20 perchlorate with contemporary design and data quality. While the original strategy aimed at  
21 determining a NOAEL for thyroid histopathology as a precursor lesion to tumors in the 90-day  
22 study, this finding in the F1-generation cannot be ignored, especially in light of an emerging  
23 appreciation of findings suggesting a phenomenon known as *in utero* imprinting with endocrine  
24 disruption (Prins et al., 2001; Phillips et al., 1998; Seckl, 1997). Thus, *in utero* disruption of  
25 thyroid hormones in the developing fetus may predispose the developing neonate and adult to  
26 future environmental insults to the thyroid gland by making the fetus more sensitive. Weiss  
27 (2000) has noted that changes in brain functions occur throughout life and some consequences of  
28 early damage may not even emerge until advanced age. This could be exacerbated if  
29 environmental insults to the thyroid were to be continued throughout life.

30 The potential for perchlorate to cause immunotoxicity remains a concern so that a 3-fold  
31 factor was applied for the database insufficiency. New studies based on recommendations at the

1 1999 external peer review had some deficiencies and reinforced concern about the lack of an  
2 accurate characterization of this endpoint.

### 3 4 **7.1.5 Operational Derivation of the Reference Dose**

5 The HEE for the neonatal rat corresponding to brain morphometry and hormone changes  
6 observed in the PND21 pups (also the PND9 pups) at the 0.01 mg/kg-day dosage would be 0.02  
7 mg/kg-day (Table 7-3). However, because the dams on GD21 were shown to be hypothyroid  
8 (with statistically-significant decreases in T4 and increases in TSH) at this same dosage, and  
9 because the temporal windows underlying the neonatal brain morphometry effects are unknown,  
10 and because the brain morphometry effects may have occurred *in utero* due to the dams'  
11 hormone deficiency, the HEE estimate for dams of 0.01 mg/kg-day was chosen as the operational  
12 derivation. It was noted that this choice was not as conservative as using the HEE for iodide  
13 inhibition in the dams (0.002 mg/kg-day), but it was viewed as more accurate given the concerns  
14 for the reliability of the thyroid estimates.

15 According to Dollarhide (1998), who spoke with Argus laboratory on behalf of the sponsor  
16 (PSG), the reported doses were of ammonium perchlorate and not the anion itself. Thus, an  
17 adjustment for percent of the molecular weight of the salt from ammonium (15.35%) must also  
18 be made. Further, because the analytical methods measure the anion concentration in  
19 environmental samples, this is the appropriate expression for the RfD to use while making valid  
20 comparisons for risk characterization. Thus, the derivation for an RfD for the perchlorate anion  
21 as itself is as follows:

$$22 \qquad 0.01 \text{ mg/kg-day} \times 0.85 / 300 = 0.00003 \text{ mg/kg-day.} \qquad (7-1)$$

23  
24  
25 Note that the appropriate adjustment for any salt of perchlorate (e.g., adjustment by a factor of  
26 0.72 for potassium perchlorate) should be made when evaluating toxicity data for similar  
27 assessment activities.

28 It is critically important to distinguish the proposed RfD from any guidance value that may  
29 result. An RfD would be only one step in the future regulatory process of determining, based on  
30 a variety of elements, whether a drinking water standard for perchlorate is appropriate. As with  
31 any draft EPA assessment containing a quantitative risk value, that risk estimate is also draft and

1 should be construed at this stage to represent Agency policy. The units for an RfD are mg/kg-  
2 day. Conversion of an RfD to a drinking water equivalent level (DWEL) is based on adjusting  
3 by body weight (kg) and drinking water consumption (L) to arrive at a level expressed in units of  
4 mg/L (ppb). Derivation of a maximum contaminant level goal (MCLG) from a DWEL by the  
5 OW typically involves the use of a relative source contribution (RSC) factor to account for non-  
6 water sources of exposures such as those discussed in Chapters 8 and 9.

7 Because the effect is viewed to be the result of neurodevelopmental deficits resulting from  
8 the hypothyroid or hypothyroxinemic state induced by the mother's exposure, and because  
9 developmental neurotoxicity may emerge later in the life or be exacerbated later in life,  
10 conversion factors for the adult of 70 kg body weight and 2 L of water per day are considered  
11 appropriate. Recent guidance from the OW in its Methodology for Deriving Ambient Water  
12 Quality Criteria for the Protection of Human Health (U.S. Environmental Protection Agency,  
13 2000) provides a decision flow chart for derivation of the RSC and recommends 80% as a ceiling  
14 and 20% as the floor for this factor when data are adequate to estimate sources of exposure.  
15 When data are not adequate to estimate other anticipated exposures, OW recommends a default  
16 RSC of 20%. (U.S. Environmental Protection Agency, 2000: Chapter 4, Section 4.2.2.4 on  
17 apportionment decisions). EPA does not recommend that high-end intakes be assumed for every  
18 exposure source since the combination may not be representative of any actually exposed  
19 population or individual.

20 A hypothetical adjustment of the 0.00003 mg/kg-day RfD by 70 kg and 2 L would thereby  
21 result in a DWEL of 1 ug/L (ppb) and application of an RSC between 0.2 to 0.8 would thereby  
22 result in an MCLG in the range of 0.2 to 0.8 ug/L (ppb). These values are in the range of current  
23 analytical capabilities. As discussed in Chapter 1, improvements to the analytical methods on the  
24 near horizon or expected to be published this spring could result in minimum reporting limits in  
25 this range and lower (Yates, 2001).

26 Concern is often expressed in the regulatory arena for the potential added susceptibility of  
27 children in developing DWEL estimates based on different conversion factors (15 kg and 1 L).  
28 Consequently, the EPA asked for additional PBPK simulations to help inform this dialogue.  
29 As shown in Table 7-1, the HEE estimates for a 15 kg human for serum perchlorate AUC can be  
30 as great as two-fold higher than those predicted for the 70 kg human due to differences in  
31 distribution volumes and excretion. Thus, if the 15 kg and 1 L values are used to convert this 2-

1 fold higher HEE value in an analogous derivation to the adult RfD derivation and DWEL  
2 calculation above, an estimate of 1 ppb that is equivalent to the adult conversion results.

### 3 4 **7.1.5.1 Comparison with Derivation Considering Human Data**

5 It is important to evaluate this derivation in context with the evidence from the available  
6 and relevant human data. As described in Chapter 4, the EPA felt that both the observational  
7 epidemiological and the human clinical studies have significant scientific and technical  
8 limitations that preclude their use as the basis for a quantitative dose-response assessment. The  
9 clinical study subject attributes (euthyroid adults) and study design issues (sample size, RAIU  
10 time points, etc.) made these data less reliable than the laboratory animal toxicological data to  
11 ascertain effect levels for the basis of an RfD derivation. In addition, on December 14, 2001,  
12 after internal peer review of this document, the Agency articulated its interim policy on the use of  
13 third-party studies submitted by regulated entities (U.S. Environmental Protection Agency,  
14 2001c). For these purposes, EPA is considering "third party studies" as studies that have not  
15 been conducted or funded by a federal agency pursuant to regulations that protect human  
16 subjects. Under the interim policy, the Agency will not consider or rely on any such human  
17 studies (third-party studies involving deliberate exposure of human subjects when used to  
18 identify or quantify toxic endpoints such as those submitted to establish a NOAEL or NOEL for  
19 systemic toxicity of pesticides) in its regulatory decision making, whether previously or newly  
20 submitted. Some of the clinical studies contained in this database fall in this category of studies  
21 not to be considered. However, the scientific and technical strengths and weaknesses of these  
22 studies were described before this Agency policy was articulated. Therefore, because of the  
23 scientific shortcomings of these studies, they will not be used as "principal studies" in the  
24 derivation of an RfD. The ethical issues surrounding the conduct of these studies or their use for  
25 regulatory purposes in light of the Agency's interim policy will not be discussed in this  
26 document. The Agency is requesting that the National Academy of Sciences conduct an  
27 expeditious review of the complex scientific and ethical issues posed by EPA's possible use of  
28 third-party studies which intentionally dose human subjects with toxicants to identify or quantify  
29 their effects.

30 These issues notwithstanding, a dose of 0.007 mg/kg-day has been suggested by some  
31 authors in an abstract (Greer et al., 2000) to be a NOAEL estimate. This was based on an

1 average 6.2 % decrease relative to baseline of RAIU measured on Day 14 of exposure to seven  
2 subjects at the 8-hour time point (unpublished data presented in Merrill, 2001a; Attachment #7).  
3 The values for RAIU ranged from a 38.6% decrease in a 34-year old female to a 27.9% increase  
4 in a 49-year old female at that dosage.

5 Prior to the articulation of the Agency's interim policy, the Agency had conducted a  
6 comparison of its reference dose derivation considering the results of the study described above,  
7 which falls within the category of a "third-party study" described by the authors as demonstrating  
8 a NOAEL in humans. If this study were to be considered in lieu of the laboratory animal data  
9 and PBPK modeling, the following would need to be considered. The seven subjects (six  
10 females and one male) were euthyroid and ranged in age from 18 to 49. Because this is a limited  
11 data set (small sample size), with noted variability and because of relevance to the elderly  
12 woman, cardiac risk patient, hypothyroid or hypothyroxinemic pregnant woman, or fetus as the  
13 susceptible population is difficult to ascertain, an uncertainty factor of 3-fold for this iodide  
14 uptake inhibition level as a minimal LOAEL as well as a 3-fold factor for intrahuman variability  
15 would be warranted. This is particularly relevant if this value is viewed in context with the  
16 neurodevelopmental effects in laboratory animal data. At a minimum each factor should be  
17 3-fold, and discussion with respect to the meaning of these factors with respect to population  
18 effects again entertained. None of the human studies of perchlorate reviewed in Chapter 4 have  
19 adequately investigated neurodevelopmental outcomes. The concern for duration of exposure  
20 was at least a 3-fold factor per the above laboratory animal data discussion and should also be  
21 applied, as well as the 3-fold factor for database deficiencies because these considerations and  
22 deficiencies are not obviated by the use of human data.

23 Thus, a derivation based on the available human data would estimate the RfD at a  
24 maximum of 0.00007 mg/kg-day, an estimate in rather good agreement with that proposed based  
25 on the laboratory animal data (0.00003 mg/kg-day). If a larger UF were to be applied to the  
26 human data, as could be justified for the intrahuman factor, the resultant estimate would be  
27 essentially equivalent to that proposed using the laboratory animal data.

28 The consistency between the estimates based on the laboratory animal versus the human  
29 data is likely due, at least in part, to the use of AFRL/HEST PBPK modeling (Merrill, 2001c,d;  
30 Clewell, 2001a,b) to perform the interspecies extrapolation rather than the use of default factors.  
31 It should be noted that the original motivation for performing these human studies (as discussed

1 in Chapter 3) in the perchlorate testing strategy was to support such interspecies pharmacokinetic  
2 extrapolation and not to derive NOAEL estimates for thyroid effects in the human population. In  
3 addition, as noted in Chapter 4, the EPA felt that both the observational epidemiological and the  
4 human clinical studies have significant scientific and technical limitations that precluded their  
5 use as the basis for a quantitative dose-response assessment. As mentioned previously, under the  
6 interim policy articulated on December 14, the Agency will not consider or rely on any such  
7 human studies (third-party studies involving deliberate exposure of human subjects when used to  
8 identify or quantify toxic endpoints such as those submitted to establish a NOAEL or NOEL for  
9 systemic toxicity of pesticides) in its regulatory decision making, whether previously or newly  
10 submitted. Nonetheless, the use of both previously published and newly-derived human data by  
11 the Air Force in its modeling efforts was important. The AFRL/HEST PBPK model approach  
12 allowed EPA to confirm that humans were as sensitive as rats to the iodide uptake inhibition  
13 effects of perchlorate at the NIS, the key event for the proposed mode-of action of perchlorate on  
14 the thyroid. In addition, the PBPK models increased the accuracy of interspecies extrapolation  
15 by allowing the incorporation and integration of ADME data to describe perchlorate and iodide  
16 disposition relative to the key event. These two outcomes from the integration of human and  
17 animal data in the AFRL/HEST models provide greater confidence than would the laboratory  
18 animal data alone that the reference dose that is derived will be protective of human health.

#### 20 **7.1.5.2 Comparison with Derivation Based on Tumor Data**

21 To address neoplasia as the other potential adverse endpoint, this section will discuss how  
22 an estimate could be derived based on the recently acquired tumor data.

##### 24 **7.1.5.2.1 Choice of Dose-Response Procedure**

25 As discussed in Chapter 5, the genotoxicity assays included in the testing strategy  
26 determined that perchlorate was not likely to be mutagenic. This was one of the critical  
27 determinants in deciding on a dose-response approach for a cancer derivation. The EPA  
28 guidance on assessment of thyroid follicular cell tumors (U.S. Environmental Protection Agency,  
29 1998a) sets forth data needs to establish the default dose-response procedure that should be used  
30 to establish that a chemical has antithyroid activity (i.e., that it is disrupting the thyroid-pituitary  
31 hormone status). Table 7-8 lists the default procedures for thyroid carcinogens that would be

**TABLE 7-8. DEFAULT DOSE-RESPONSE PROCEDURES FOR  
THYROID CARCINOGENS (U.S. Environmental Protection Agency, 1998a)**

Example	Array of Effects		Dose-Response Methodology
	Mutagenic	Antithyroid	
1	Either or both unknown		Linear
2	Yes	No	Linear
3	No	Yes	Margin of exposure
4	Yes	Yes	Linear and margin of exposure

1 used. The thyroid lesions observed (colloid depletion, hypertrophy, and hyperplasia) are among  
 2 the required lesions to demonstrate antithyroid activity. Table 7-9 shows the types of data  
 3 required.

**TABLE 7-9. DATA DEMONSTRATING ANTITHYROID ACTIVITY  
(U.S. Environmental Protection Agency (1998a))**

Required	Desirable
1. Increases in cellular growth	6. Lesion progression
2. Hormone changes	7. Structure-activity relationships
3. Site of action	8. Other studies
4. Dose correlations	
5. Reversibility	

1 What has been proposed in this assessment is the harmonization of the “noncancer” and  
 2 “cancer” assessment approaches because the target tissue is the thyroid and the mode of action is  
 3 the same for both the neurodevelopmental and neoplastic sequelae. The proposed RfD based on  
 4 precursor lesions is analogous to a nonlinear approach and viewed as a protective for thyroid  
 5 tumors.

6 Perchlorate has clearly demonstrated an effect in both adult, fetal, and neonatal stages in  
 7 thyroid histopathology, as well as a decrease in lumen size in a dose-dependent fashion. Thyroid  
 8 and pituitary hormone changes and expected correlations all have been demonstrated for T3, T4,

1 and TSH across an array of studies at different time points. The site of action has been  
2 established as competitive inhibition of the iodide symporter although there remains some  
3 uncertainty as to whether that is the only locus for the effect (e.g., evidence for intrathyroidal  
4 activity) because of the efflux (discharge) phenomenon. Dose-correlations in this case were not  
5 with tumors, but rather for precursor lesions (colloid depletion, hypertrophy, hyperplasia, and  
6 decreased follicular lumen size). Reversibility has been demonstrated in thyroid weight, colloid  
7 depletion, hypertrophy, hyperplasia, and thyroid and pituitary hormones in the 30-day recovery  
8 period after the 90-day study in rats and in T4 levels of the various immunotoxicity experiments  
9 in mice.

10 Lesion progression was difficult to determine because of dose-spacing and differences in  
11 sample size and histological methods among the studies. However, there was a progression  
12 within the 90-day study between the 14- and 90-day time points.

13 Analyses of other anions have fairly well established that the mode of action of perchlorate  
14 arises from it being an anion that is recognized by the NIS (see Chapter 3).

15 Thus, the appropriate dose-response procedure for perchlorate would be a nonlinear  
16 margin-of exposure approach based on demonstration that it is not genotoxic and that its  
17 anti-thyroid effects are consistent with a mode of action leading from inhibition of iodide uptake  
18 at the NIS through precursor lesions of perturbation of thyroid hormone economy and resultant  
19 histopathological changes in the thyroid gland.

#### 21 **7.1.5.2.2 Dose-response Assessment for Thyroid Neoplasia**

22 Thyroid adenomas were statistically increased in the high dose (30 mg/kg-day) group of  
23 F1-generation animals sacrificed as adults (P2-generation) at 19 weeks in the Argus Research  
24 Laboratories, Inc. (1999) two-generation reproductive study. Both the latency and incidence of  
25 these tumors were remarkable relative to the entirety of the NTP data base for this type of tumor  
26 in this strain of rat (Dunson, 2001b). Colloid depletion, hypertrophy, and hyperplasia were all  
27 observed at dosages of 0.3 mg/kg-day and above with BMDL estimates of 0.9, 0.15, and  
28 0.0004 mg/kg-day. This last estimate is outside the range of possible dosimetric adjustment so it  
29 will not be carried forward, but consideration of the overlap among colloid depletion,  
30 hypertrophy, and hyperplasia should be superimposed on the derivation. The HEE values for  
31 adult versus neonatal rats are comparable at these dosages. Using the adult male rat dosimetric

1 adjustment factor to correspond to sacrifice date results in HEE estimates of 0.45 and 0.02 for  
2 colloid depletion and hypertrophy.

3 Using the nonlinear approach and applying a composite factor of 100 to the HEE estimates  
4 to account as above for uncertainty in intrahuman variability, duration, and database deficiencies;  
5 and with factor for a minimal LOAEL of 3 to account for the fact that hyperplasia occurred at  
6 over an order of magnitude lower than these two thyroid histopathology estimates, results in an  
7 RfD derivation in the range of 0.005 to 0.0002 mg/kg-day. Applying a larger uncertainty factor  
8 for intrahuman variability would result in a range of 0.002 to 0.00007 mg/kg-day. Thus, the  
9 derivation based on tumor outcome data supports the mode-of-action concept and corroborates  
10 that the proposed RfD that as derived would be protective of both neurodevelopmental and  
11 neoplastic sequelae.

### 13 **7.1.5.3 Possible Susceptibility**

14 Based on the mode-of-action for perchlorate, the competitive inhibition of iodide uptake,  
15 and the subsequent perturbation of thyroid hormone homeostasis, a number of factors potentially  
16 could cause an increase in susceptibility of a population to perchlorate toxicity. As already  
17 indicated by the choice of critical effect, the fetus, and perhaps the developing child, may  
18 represent susceptible populations. However, critical data on the steady-state pharmacokinetics  
19 and placental dosimetry are lacking to definitively state whether or not there is an inherent  
20 pharmacodynamic component to the apparent sensitivity of pups versus dams in the laboratory  
21 animal models. Individuals that are iodine deficient may be another susceptible population. The  
22 elderly, especially women, and hypothyroid and hypothyroxinemic individuals or those treated  
23 with anti-thyroid drugs, may be others more susceptible than the general population to the effects  
24 of perchlorate. Patients with cardiac dysfunction or elevated levels of cholesterol may also be at  
25 increased risk.

### 27 **7.1.6 Designation of Confidence Levels**

28 Confidence in the principal study is medium. The dose level of 0.01 mg/kg-day was the  
29 lowest tested, and it was determined to be a LOAEL (not NOAEL). The small sample size for  
30 the critical effect also reduces confidence in the study. Despite the new data, the confidence in  
31 the database at this time remains medium because the sensitivity of these animal assays versus

1 evaluation of neuropsychological development in human population studies is not known, and  
2 because a concern for potential immunotoxicity remains. Based on confidence in the study and  
3 on the database together in setting the overall confidence in the RfD, the confidence in the RfD  
4 currently is also medium.

## 7.2 INHALATION REFERENCE CONCENTRATION

8 Derivation of an inhalation reference concentration is precluded because there are no  
9 inhalation data available with which to characterize dose-response or the portal-of-entry  
10 modulation of internal dose. However, the EPA has been questioned as to whether the potential  
11 for inhalation exposure of perchlorate from showering with contaminated water poses a health  
12 risk. Given the low vapor pressure of perchlorate, it is not likely that it would come out of  
13 solution. Further, Giardino et al. (1992) characterized shower particle droplet size as ranging  
14 from 200 to 3,000  $\mu\text{m}$ . Thus, there is minimal chance for inhalation or deposition of perchlorate-  
15 laden droplets in the respiratory tract.

## 7.3 SUMMARY

19 The model based on mode of action for perchlorate served as a useful construct for the  
20 integration of a diverse set of data. Results of studies in the testing strategy confirmed that the  
21 target tissue for perchlorate is the thyroid and that the key event for its antithyroid effects is the  
22 inhibition of iodide uptake at the NIS with corresponding perturbations of thyroid hormone  
23 economy. Disturbances in thyroid hormone economy were confirmed to result in thyroid  
24 histology as diagnosed by decreases in colloid depletion or follicular lumen size and increases in  
25 hypertrophy and hyperplasia. Effects on both neurodevelopmental indices (brain morphometry  
26 and motor activity) and neoplasia that could be expected based on the mode of action were also  
27 demonstrated. Other developmental and reproductive effects were not observed to be as  
28 sensitive as the neurodevelopmental and thyroid histopathological changes. Accurate  
29 characterization of the immunotoxicity of perchlorate, notably its potential to cause contact

1 hypersensitivity, either secondarily to these hormone effects or possibly via a direct effect of the  
2 anion itself, remains a concern.

1 **APPENDIX 7A**

2 **CORRELATION ANALYSES**

3  
4 The correlation analyses were of two types. Hormone levels are continuous, ratio-scaled  
5 values, so correlations were computed using the conventional Pearson's r statistic. Correlations  
6 between ratio-scaled hormone levels and ordinaly-scaled standard histology ratings must be  
7 computed using nonparametric correlations. To compare variables from the different scales, it is  
8 simplest to recode the data by converting the variable values into rank scores. Spearman's rank  
9 order ( $r_s$ ) was used to compute the correlation between the rankings of two variables. When there  
10 were ties in the ranks, as there were in this data set, each value was assigned the mean of the  
11 ranks that they would otherwise occupy. A correlation coefficient was then computed for the  
12 rankings of the variables of interest.

13 An alternative statistic used for comparing the data sets was Kendall's tau, best thought of  
14 as a measure of agreement or concordance between two sets of ranked data. It searches for the  
15 number of inversions in two sets of ranked data (i.e., observations are ranked according to the  
16 first variable, then reranked according to the second, and the number of interchanges that occur is  
17 used to compute the statistic). The Spearman and Kendall statistics produced nearly identical  
18 results. Statistics were computed using SAS<sup>®</sup> software (PROC RANK and PROC CORR,  
19 SAS Institute, Cary, NC). All statistics corresponding to Figures 7A-1 through 7A-7 can be  
20 found in Tables 7A-1 through 7A-6.

21  
22 **TABLE 7A-1. PEARSON'S r CORRELATIONS (n = 96) BETWEEN THYROID HORMONES AND TSH IN RATS OF THE CALDWELL et al. (1995) 14-DAY STUDY**

	T3	T4	TSH
T3	1.00 p = 0.00	0.81 p = 0.0001	-0.65 p = 0.0001
T4		1.00 p = 0.00	-0.67 p = 0.0001
TSH			1.00 p = 0.00

**TABLE 7A-2. SPEARMAN'S  $r_s$  CORRELATIONS (n = 95) BETWEEN THE RANK ORDER OF HORMONE LEVELS AND HISTOLOGICAL SEVERITY RATING DECREASE IN FOLLICULAR LUMEN SIZE (LS) IN RATS OF THE CALDWELL et al. (1995) 14-DAY STUDY**

	LS
T3	-0.74 p = 0.0001
T4	-0.70 p = 0.0001
TSH	0.79 p = 0.0001
FH	0.75 p = 0.0001

**TABLE 7A-3. PEARSON'S r CORRELATIONS (n = 223) BETWEEN THYROID HORMONES AND TSH IN RATS FOR THE COMBINED 14- AND 90-DAY DATA OF THE SPRINGBORN LABORATORIES, INC. (1998) SUBCHRONIC RAT STUDY**

	T3	T4	TSH
T3	1.00 p = 0.00	0.42 p = 0.0001	-0.18 p = 0.007
T4		1.00 p = 0.00	-0.20 p = 0.0027
TSH			1.00 p = 0.00

**TABLE 7A-4. PEARSON'S r CORRELATIONS (n = 104) BETWEEN THYROID HORMONES AND TSH FOR THE 14-DAY DATA OF THE SPRINGBORN LABORATORIES, INC. (1998) SUBCHRONIC RAT STUDY**

	T3	T4	TSH
T3	1.00 p = 0.00	0.36 p = 0.0001	-0.11 p = 0.27
T4		1.00 p = 0.00	0.20 p = 0.04
TSH			1.00 p = 0.00

**TABLE 7A-5. PEARSON'S r CORRELATIONS (n = 119) BETWEEN THYROID HORMONES AND TSH OF THE 90-DAY DATA OF THE SPRINGBORN LABORATORIES, INC. (1998) SUBCHRONIC RAT STUDY**

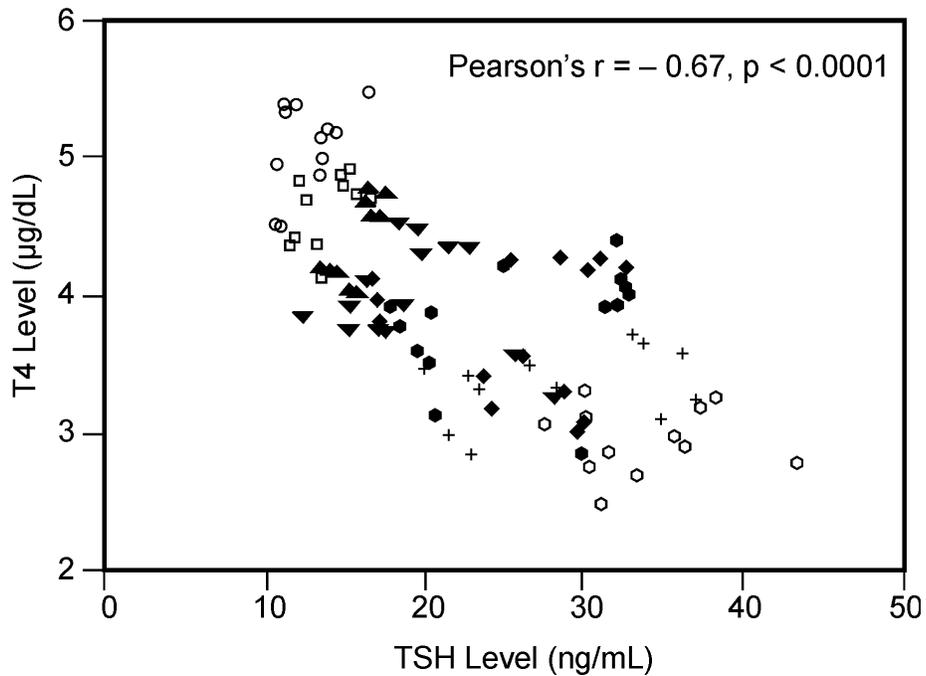
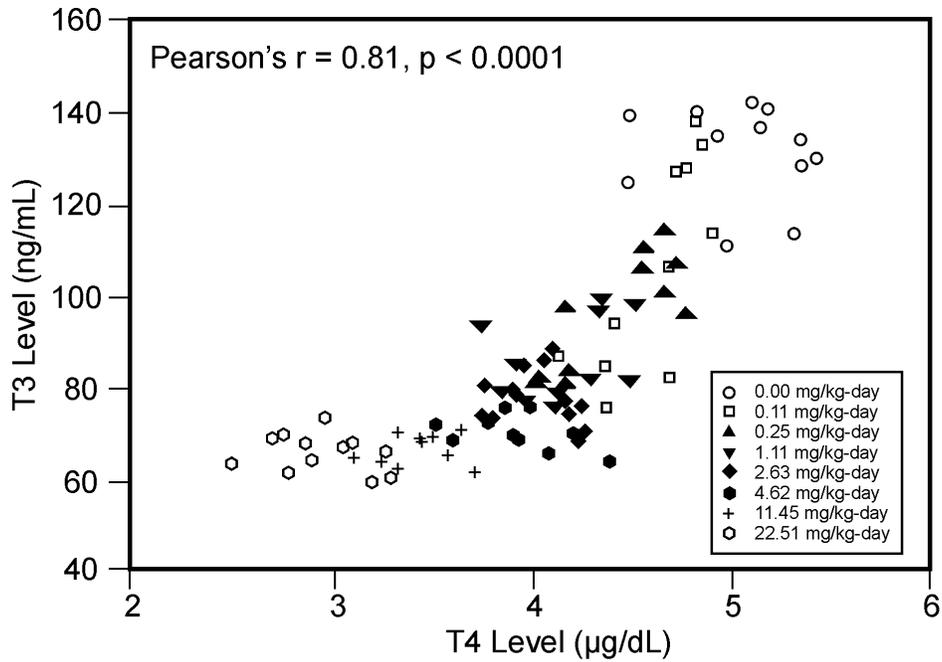
	T3	T4	TSH
T3	1.00 p = 0.00	0.66 p = 0.0001	-0.40 p = 0.0001
T4		1.00 p = 0.00	-0.38 p = 0.0001
TSH			1.00 p = 0.00

**TABLE 7A-6. PEARSON'S r CORRELATIONS (n = 22 to 27) BETWEEN THYROID HORMONES AND TSH FOR THE F1 RAT PUPS ON PND5 IN THE DEVELOPMENTAL NEUROTOXICITY STUDY (Argus Research Laboratories, Inc., 1998a)**

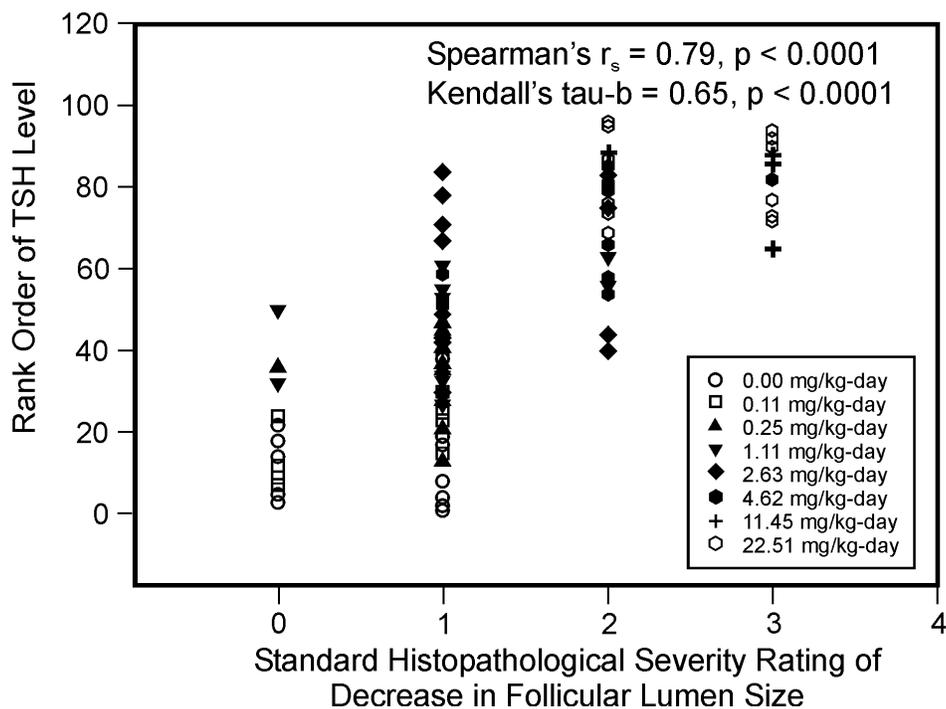
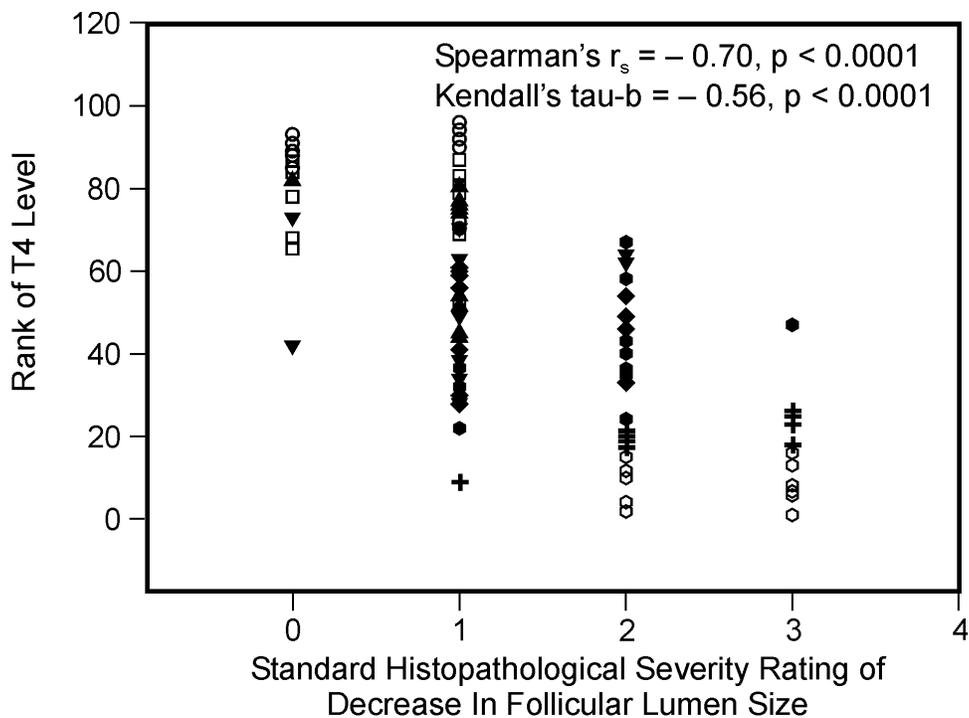
	T3	T4	TSH
T3	1.00 p = 0.00	0.87 p = 0.0001	-0.43 p = 0.03
T4		1.00 p = 0.00	-0.57 p = 0.0046
TSH			1.00 p = 0.00

1 In general, positive correlations were expected between T3 and T4 and between TSH and  
 2 the histopathology rating. Negative correlations were expected between T4 and TSH and  
 3 between T4 and histopathology.

4 Figure 7A-1 shows the correlations between T3 and T4 and between T4 and TSH levels  
 5 from the 14-day Caldwell et al. (1995) study in rats. Robust relationships are illustrated:  
 6 a positive correlation is shown between T3 and T4; whereas, the T4 and TSH varied inversely.  
 7 Hormone levels also correlated highly with decrease in follicular lumen size. Figure 7A-2 shows  
 8 the rank of T4 level and TSH level versus the severity rating for follicular lumen size to be highly  
 9 correlated inversely. Figure 7A-3 shows the correlations for the combined 14-day and 90-day  
 10 time points (male and female) from the subchronic study performed in rats (Springborn



**Figure 7A-1. Correlations between T3 versus T4 (top panel) and T4 versus TSH (bottom panel) in rats of the 14-day Caldwell et al. (1995) study (Geller, 1998a). Data of Channel (1998a) and Crofton (1998a).**



**Figure 7A-2. Correlations between the rank order of T4 (top panel) and TSH (bottom panel) versus decrease in follicular lumen size in rats of the 14-day Caldwell et al. (1995) study (Geller, 1998a). Data of Channel (1998a) and Crofton (1998a).**

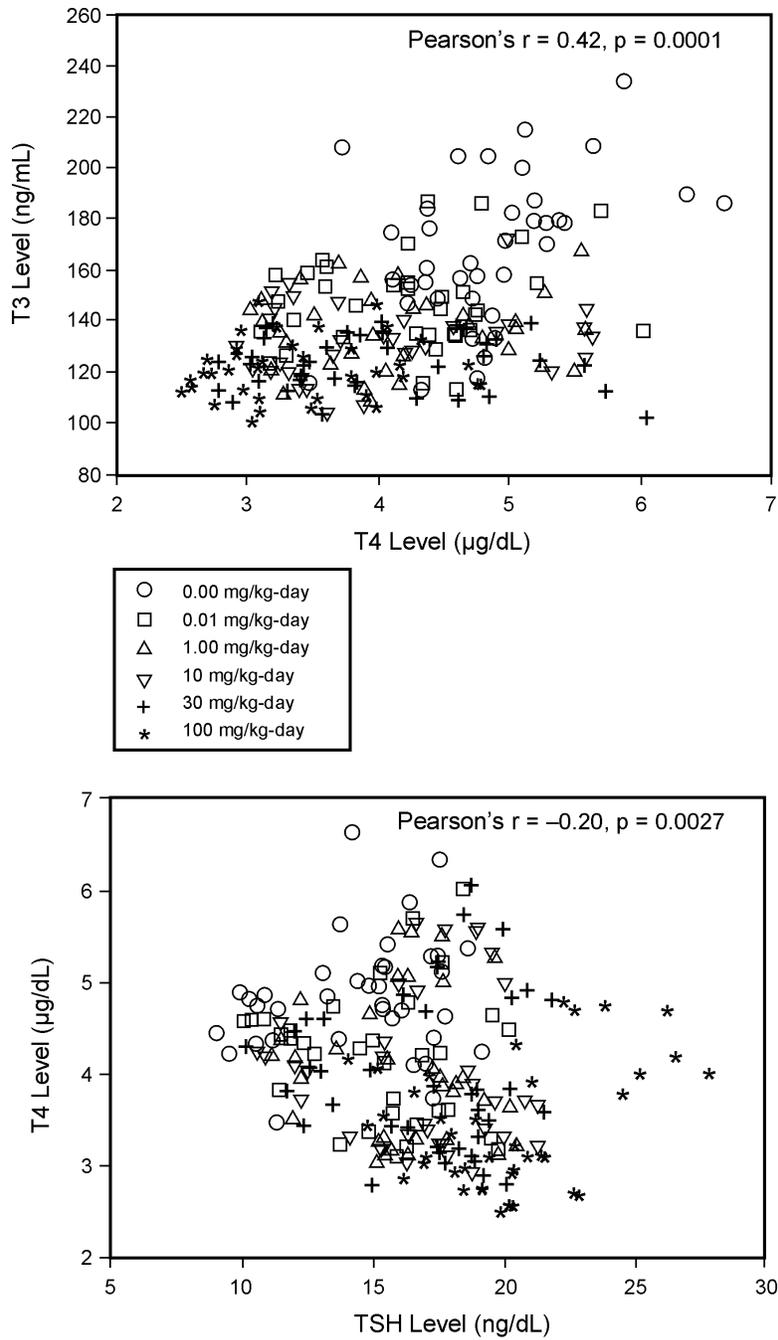
1 Laboratories, Inc., 1998). As shown in Figure 7A-3 (top panel), T3 and T4 were highly  
2 significantly correlated, with low levels of T3 and T4 associated with high doses. Both T4 and  
3 TSH were significantly negatively correlated (bottom panel). After 14-days of dosing  
4 (Figure 7A-4), T3 and T4 are highly associated (top panel), but there is an unexpected positive  
5 relation between T4 and TSH (bottom panel). At the 90-day time point, there are the expected  
6 strong correlations between T3 and T4 (Figure 7A-5, top panel) and between T4 and TSH  
7 (bottom panel).

8 Correlations also were performed on the data from the neurodevelopmental study for the  
9 PND5 pups (Argus Research Laboratories, Inc., 1998a). T3 and T4 were strongly positively  
10 correlated, and T4 and TSH were negatively correlated (Figure 7A-6). Figure 7A-7 (top panel)  
11 shows that T4 is negatively associated with a significant decrease in lumen area. Figure 7A-7  
12 (bottom panel) also shows that TSH is positively correlated with a decrease in lumen size.

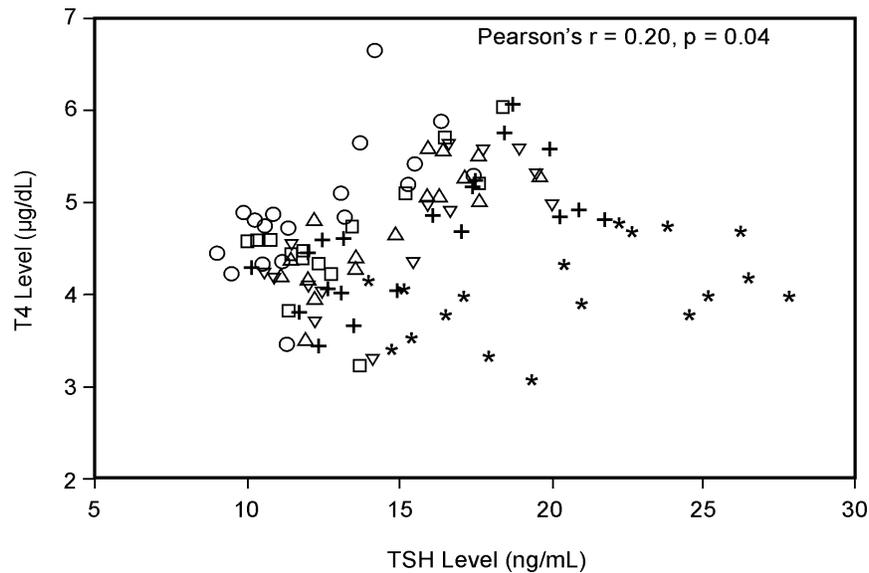
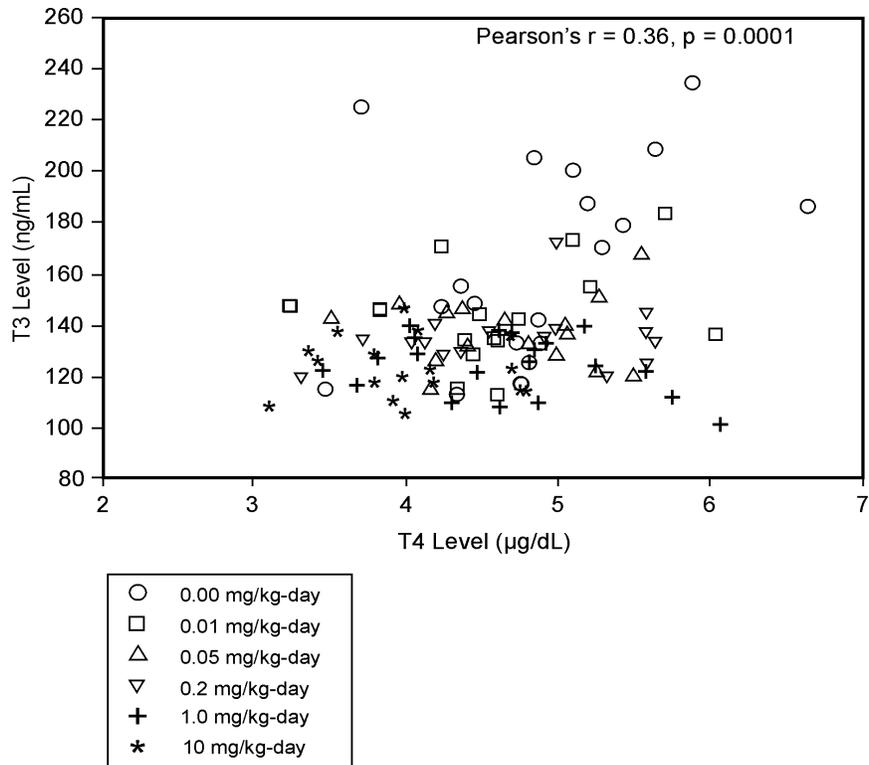
13 In total, these correlations lent strong support to the mapping model proposed. Strong  
14 correlations were observed between T3 and T4 levels, T3 or T4, and TSH levels, and hormone  
15 levels and a decrease in thyroid lumen size. These relationships were most definitive in the  
16 Caldwell et al. (1995) study, in which strong correlations existed between the elements of the  
17 thyroid hormone homeostasis feedback loop and between hormone levels and severity ratings for  
18 lumen size decrease as a measure of thyroid histopathology. In the subchronic (Springborn  
19 Laboratories, Inc., 1998) study, correlations were established between hormone levels across  
20 both the 14- and 90-day dosing points and for each time point individually. At 14 days of dosing,  
21 the expected inverse relationship between T4 and TSH was not found. At the 90-day dosing  
22 point, the inverse relationships between T3 or T4 and TSH were found.

23 Similar relationships were observed in pups on PND5 of the developmental neurotoxicity  
24 study (Argus Research Laboratories, Inc., 1998a; York, 1998c). The T4 and TSH were  
25 significantly correlated negatively, as expected. The T3, T4, and TSH were all significantly  
26 correlated with decrease in lumen size. The correlations in the rat studies support the model that  
27 manipulations resulting in decreased levels of circulating thyroid hormone are linked to thyroid  
28 histopathological changes that are thought to result directly from elevation of TSH.

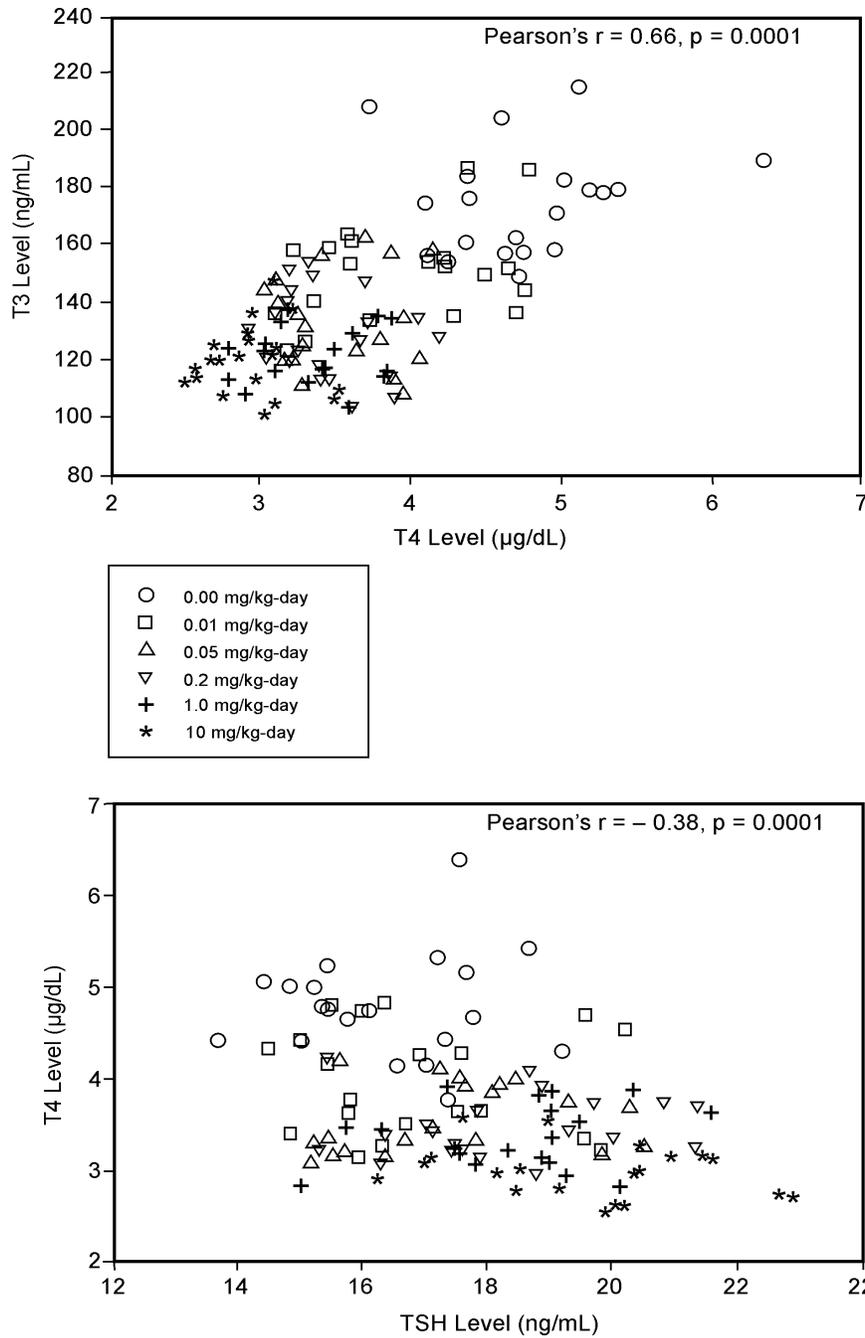
29



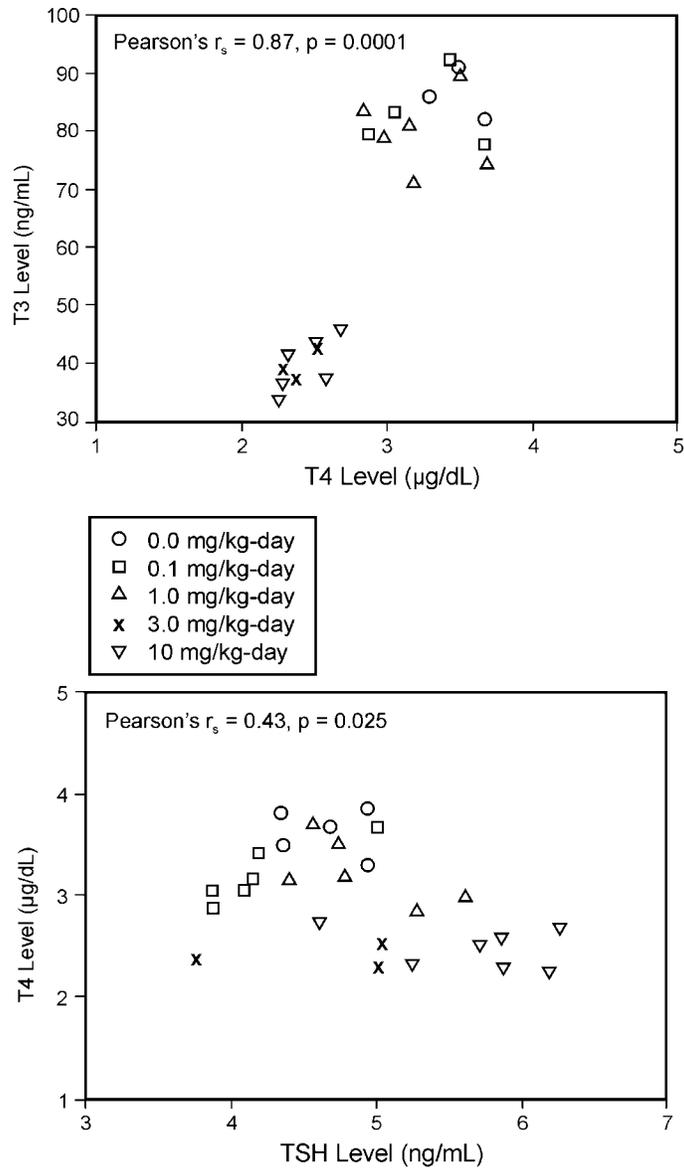
**Figure 7A-3. Correlations between T3 versus T4 (top panel) and T4 versus TSH (bottom panel) for the combined male and female data of the 14-day and 90-day time points from the Springborn Laboratories Inc. (1998) subchronic study (Geller, 1998a).**



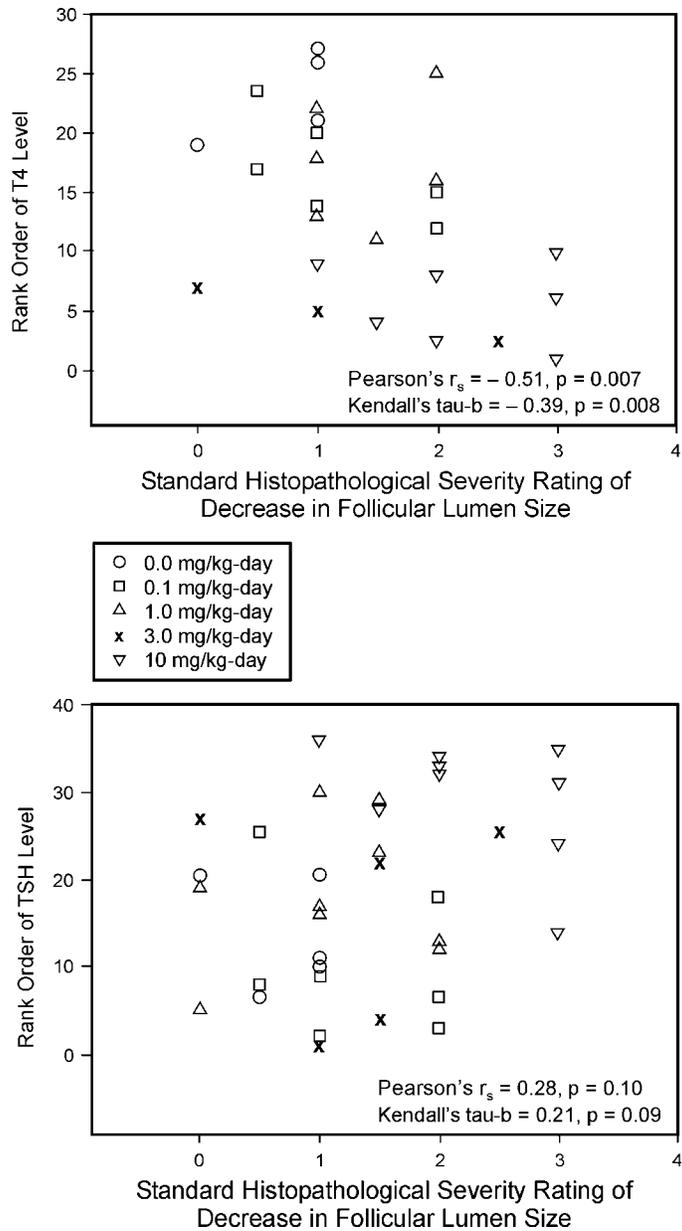
**Figure 7A-4. Correlations between T3 versus T4 (top panel) and T4 versus TSH (bottom panel) for the combined male and female data of the 14-day time point from the Springborn Laboratories Inc. (1998) subchronic study in rats (Geller, 1998b).**



**Figure 7A-5. Correlations between T3 versus T4 (top panel) and T4 versus TSH (bottom panel) for the combined male and female data of the 90-day time point from the Springborn Laboratories Inc. (1998) subchronic study in rats (Geller, 1998b).**



**Figure 7A-6. Correlations between T3 versus T4 (top panel) and T4 versus TSH (bottom panel) for the F1-generation rat pups on PND5 in the developmental neurotoxicity study (Geller, 1998b). Data of Argus Research Laboratories, Inc. (1998a), York (1998c), Channel (1998c), and Crofton (1998f).**



**Figure 7A-7. Correlations between the rank order of T4 (top panel) and TSH (bottom panel) versus histopathology severity rating of the decrease in follicular lumen size for the postnatal day 5 (PND5) pups in the 1998 neurodevelopmental study (Geller, 1998b). Data of Argus Research Laboratories, Inc. (1998b), Channel (1998c), and Crofton (1998e, f).**

# Appendix 7B

## Benchmark Dose Statistics for Hormone Analyses

As mentioned in Chapter 5, benchmark dose analyses were performed in addition to the ANOVA for all hormone data. Benchmark analysis of the 2001 “Effects Study” is presented in Geller (2001c). This appendix presents analyses performed on the other data sets provided in the 1998 assessment.

For the continuous hormone data, the BMD and BMDL estimates were calculated using a variety of benchmark response (BMR) values. Generally, the BMR was equal to a response 10% less than the control mean (i.e., 10% of the actual control response was subtracted from the estimate of the control value generated by the fit to the data). This is a less rigorous standard than the (control minus 5% of control) BMR that provided a close match to NOAELs in the evaluation of BMD for developmental toxicity by Kavlock et al. (1995) although this may be warranted because other endpoints (thyroid hormone and histopathology) are being evaluated. For the natural log (ln) transformed data, this means subtracting the constant 0.1053 from the control value, which is equivalent to multiplying the control value by 0.90. The BMD and BMDLs at 20 and 30% less than control and control standard deviations also are provided as a yardstick for evaluating how other clinical criteria may affect the estimates. Hormone data were fit with polynomial (linear or quadratic) or power functions (Table 7B-1).

**TABLE 7B-1. CONTINUOUS FUNCTIONS USED IN BENCHMARK DOSE (BMD) MODELING**

Power function	$f(\text{dose}) = \text{control} + \text{slope} * \text{dose}^{\text{power}}$
Polynomial function (includes linear and quadratic)	$f(\text{dose}) = \beta_0 + \beta_1 * \text{dose} + \beta_2 * \text{dose}^2 + \dots$

Adequacy of fit for continuous data was evaluated by the statistical goodness-of-fit ( $-2 \times \log$  likelihood ratio) test provided by the EPA BMD program output, visual comparison,

1 and whether the fit was biologically plausible. The latter criterion in most cases,  
2 non-monotonocities in the function fit to the data, precluded a fit from consideration. In general,  
3 the second order quadratic fits suffered from minima or maxima between the data points from the  
4 two highest data points in a given experiment. This consideration also precluded the use of  
5 polynomials of higher than second order because these higher order polynomials generally had a  
6 local maxima or minima between data points (dose levels) and did not model the data plausibly.  
7 It should be noted that the interpretation of the test for constant variance included in the output of  
8 the version of the BMD software (version 0.96) was not reliable.

### 9 10 **7B.1 Benchmark Dose Estimates Submitted to U.S. Environmental** 11 **Protection Agency**

12 Two sets of BMD calculations were derived from the Caldwell et al. (1995) 14-day study  
13 and submitted to the EPA (Dollarhide and Dourson, 1997). One set was calculated for TSH and  
14 T4 levels for males and females separately using the THC (polynomial fit) module of the Crump  
15 software, and the model coefficients were restricted to be nonnegative to prevent  
16 non-monotonicity. This resulted in linear fits to curvilinear data, and the fits were judged to be  
17 poor by both visual inspection and statistical goodness-of-fit criteria (Geller, 1998a).

18 An alternative approach to calculating BMD estimates based on additional risk also was  
19 derived using the Kodell-West algorithm (Kodell-West, 1993). This model generates a quadratic  
20 fit to the dose-response data using a maximum likelihood estimator, defines an adverse effect  
21 level based on the variability present in the data, and then calculates additional risk. The EPA  
22 recalculated these fits using Kodell's SAS<sup>®</sup> program (Geller, 1998a). The EPA estimates  
23 correspond to those previously reported, as shown in Table 7B-2 of Appendix 7B. The  
24 coefficients of the fits are provided in Table 7B-3. None of the fits to the data reached statistical  
25 significance, and all contain minima (T3 and T4) or maxima (TSH) within the dose range tested.  
26 Again, the lack of fit raises difficulties with interpretation and suggests that these estimates  
27 should not be used as the basis for risk assessment. The EPA also calculated BMD estimates on  
28 ln-transformed data because the Kodell-West algorithm assumes constant variance, and the  
29 transformed data is more likely to fit this assumption. The BMD estimates calculated with the ln  
30 transform, however, were virtually identical to those of the previous estimates.

**TABLE 7B-2. BENCHMARK DOSE (BMD) ESTIMATES FOR MALE HORMONE  
DATA OF CALDWELL et al. (1995) 14-DAY RAT STUDY, USING  
KODELL-WEST ALGORITHM**

Responders	BMD Associated with 1% Additional Risk (mg/kg-day)		BMD Associated with 10% Additional Risk (mg/kg-day)		BMD:N(L)OAEL 1%; 10%
	EPA <sup>a</sup>	D&D, 1997 <sup>b</sup>	EPA <sup>a</sup>	D&D, 1997 <sup>b</sup>	
<b>TSH</b>	EPA <sup>a</sup>	D&D, 1997 <sup>b</sup>	EPA <sup>a</sup>	D&D, 1997 <sup>b</sup>	1.11
k = 3	0.832	0.823	2.078	2.074	0.75; 1.87
k = 2	0.176	0.172	0.972	0.970	0.16; 0.88
<b>ln TSH</b>					1.11
k = 3		0.845		2.115	0.76; 1.91
k = 2		0.181		0.987	0.16; 0.89
<b>T3</b>	EPA <sup>a</sup>	D&D, 1997 <sup>b</sup>	EPA <sup>a</sup>	D&D, 1997 <sup>b</sup>	0.11 <sup>c,d</sup>
k = 3	0.980	0.983	2.485	2.495	8.1; 22.59
k = 2	0.209	0.207	1.146	1.151	1.9; 10.42
<b>lnT3</b>					0.11 <sup>c,d</sup>
k = 3		0.891		2.244	8.1; 20.4
k = 2		0.190		1.042	1.73; 9.47
<b>T4</b>	EPA <sup>a</sup>	D&D, 1997 <sup>b</sup>	EPA <sup>a</sup>	D&D, 1997 <sup>b</sup>	0.11 <sup>c,d</sup>
k = 3	0.797	0.658	1.969	1.639	7.25; 17.9
k = 2	0.172	0.136	0.927	0.774	1.56; 8.43
<b>ln (T4)</b>					0.11 <sup>c,d</sup>
k = 3		1.002		2.490	9.11; 22.64
k = 2		0.215		1.169	1.95; 10.63

<sup>a</sup>EPA refers to BMD estimates calculated using SAS<sup>®</sup> software received from Dr. Ralph Kodell for Kodell-West calculations (Geller, 1998a).

<sup>b</sup>D&D refers to BMDs included in Dollarhide and Dourson (1997).

<sup>c</sup>LOAEL; otherwise, value indicates NOAEL.

<sup>d</sup>LOAEL from combined male and female.

**TABLE 7B-3. COEFFICIENTS AND GOODNESS-OF-FIT STATISTICS OF KODELL-WEST (QUADRATIC POLYNOMIAL) MODEL FITS TO MALE HORMONE DATA OF CALDWELL et al. (1995) 14-DAY RAT STUDY<sup>a</sup>**

Responders	B0	B1	B2	Dose (mg/kg-day) of Global Max/Min	p of Fit <sup>2b</sup>
TSH	17.182	2.895	-0.0914	max: 15.84	<0.00001
ln TSH	2.825	0.1269	-0.004202	max: 15.11	<0.00001
T3	112.871	-8.987	0.3169	min: 14.18	<0.00001
lnT3	4.7114	-0.09702	0.0034	min: 14.27	<0.00001
T4	4.7712	-0.1791	0.00445	min: 20.11	<0.00001
ln (T4)	1.563	-0.0414	0.0009	min: 23.00	0.00012

<sup>a</sup>Coefficients generated by using SAS software received from Dr. Ralph Kodell (Geller, 1998a). Identical coefficients were generated by using EPA BMD software.

<sup>b</sup>p > 0.05 denotes significant fit. Goodness-of-fit derived using -2 log (likelihood ratio) test from EPA BMD software (see Geller, 1998a).

## 7B.2 U.S. Environmental Protection Agency Benchmark Dose Estimates for Thyroid and Pituitary Hormones

The hormone data from the Caldwell et al. (1995) subchronic (Springborn Laboratories, Inc., 1998) and rabbit developmental studies (Argus Research Laboratories, Inc., 1998c) were best fit by unrestricted power functions. The hormone data from the developmental neurotoxicity study (Argus Research Laboratories, Inc., 1998a; York, a,b,c,d,e) and mouse immunotoxicity study (Keil et al., 1998) were fit by either unrestricted power or polynomial functions. It is noted that the unrestricted power function fits generally have an extremely high slope as dose approaches zero. Tables 7B-4 through 7B-14 provide the statistics for each study.

Many of the BMDL estimates derived from these studies were lower than the NOAEL or LOAEL values derived by ANOVA, particularly those derived from power function fits. Murrell et al. (1998) suggested that this occurs when sampling statistics (i.e., small group sample sizes and few dose groups) play a large role in inflating NOAELs while depressing BMDL estimates. This may be the case for some of the data examined herein. Murrell et al. (1995) suggested that under such conditions using the BMD point estimate, rather than the lower confidence limit, would be a more accurate representation of the dose-response behavior.

**TABLE 7B-4. BENCHMARK DOSE (BMD) ESTIMATES USING POWER FUNCTION FIT TO COMBINED MALE AND FEMALE HORMONE DATA OF CALDWELL et al. (1995) 14-DAY RAT STUDY  
(Benchmark response based on 10% change from control value.)**

Endpoint	p of Fit	BMD	BMDL	NOAEL/ LOAEL	BMD: N(L)OAEL	BMDL: N(L)OAEL	BMR: 10% control SD
TSH <sup>a</sup>	0.272	0.014	0.0002	0.44	0.032	4.55e-4	1.29 1.88
ln TSH <sup>a</sup>	0.099	0.017	0.002	0.44	0.039	4.55e-3	-0.1053
Female TSH <sup>b</sup>	0.077	0.19	0.032	0.1	1.90	0.32	1.125 0.48
Female ln(TSH) <sup>a</sup>	0.50	0.078	0.035	0.1	0.78	0.35	-0.1053
Male TSH	No significant fits to male TSH or male ln(TSH) data						
T3 <sup>a</sup>	0.107	0.00035	0.00	0.1 <sup>c</sup>	0.0035	NA	13.07 10.21
lnT3 <sup>a</sup>	0.091	0.0004	2e-6	0.1 <sup>c</sup>	0.004	2.00e-5	-0.1053
T4 <sup>a</sup>	0.303	0.243	0.096	0.1 <sup>c</sup>	2.43	0.96 <sup>c</sup>	0.506 0.321
ln (T4) <sup>d</sup>	0.172	0.340	0.0997	0.1 <sup>c</sup>	3.40	1.00 <sup>c</sup>	-0.1053

<sup>a</sup>Unrestricted quadratic: fit nonmonotonic, not significant. Restricted polynomial (linear): fit not significant.

<sup>b</sup>Unrestricted quadratic: fit monotonic but not significant. Restricted polynomial (linear): fit not significant.

<sup>c</sup>LOAEL; otherwise, value is NOAEL.

<sup>d</sup>Unrestricted quadratic: fit not significant, global minimum at approximate high dose. Restricted polynomial (linear): fit not significant.

1           The BMD estimates calculated with a benchmark response of 10% less than control on the  
2 TSH hormone dose-response data are spread over 2.5 orders of magnitude, a similar range to that  
3 seen in the distribution of NOAELs calculated for TSH. The BMDL estimates are distributed  
4 more widely, over 5 orders of magnitude. These reflect the steepness of the confidence limits on  
5 the slope at low doses.

6           The T3 BMD estimates are spread over approximately two orders of magnitude, similar to  
7 the variability seen across studies in the LOAEL and NOAEL estimates. The T3 BMD estimates  
8 are 100-fold lower than the NOAEL/LOAEL estimates, however. A BMDL could be calculated

**TABLE 7B-5. BENCHMARK DOSE (BMD) AND BMD 95% LOWER LIMIT (BMDL) ESTIMATES USING POWER FUNCTION FIT TO COMBINED MALE AND FEMALE HORMONE DATA OF CALDWELL et al. (1995) 14-DAY RAT STUDY (Benchmark response based on 10, 20, and 40% changes from control value.)**

Endpoint	p of Fit	BMD BMDL (10%)	BMD BMDL (20%)	BMD BMDL (40%)	Mean	NOAEL
TSH	0.272	0.014 0.0002	0.083 0.0038	0.507 0.0604	12.861	0.44
ln(TSH) <sup>a</sup>	0.099	0.002	0.043	1.11		0.44
T3	0.0108	0.00035 0.00	0.0338 0.000036	3.27 0.042 <sup>c</sup>	130.69	0.10 <sup>b</sup>
ln(T3) <sup>a</sup>	0.091	0.000002	0.000642	0.478		0.10 <sup>b</sup>
T4	0.303	0.243 0.096	2.28 1.299	21.44 16.78	5.06	0.10 <sup>b</sup>
ln(T4) <sup>a</sup>	0.172	0.100	1.213	16.89		0.10 <sup>b</sup>

<sup>a</sup>For ln-transformed data, only BMDL estimates are displayed.

<sup>b</sup>LOAEL, not NOAEL.

<sup>c</sup>BMDL calculation failed at some values. This means BMDL value may not be accurate.

1 for only one of the data sets, and this value was approximately 10,000 times lower than the  
2 LOAEL. The BMD estimates comprising the 25th to 75th percentiles for T4 cover the same  
3 2.5 orders of magnitude as those covered by the NOAEL and LOAEL estimates for T4. The  
4 BMDL estimates for this same percentile range are distributed a little more widely, but do  
5 include the range of T4 NOAEL and LOAEL estimates.

6

### 7 **7B.3 Summary of U.S. Environmental Protection Agency Benchmark** 8 **Dose Analyses**

9 The BMD analyses of previously reported estimates for the hormone data of Caldwell et al.,  
10 (1995)14-day study in rats (Dollarhide and Dourson, 1997) were shown to be based on  
11 inadequate model fits. The EPA was able to successfully model the hormone data. However,  
12 these estimates raised a number of issues with respect to approaches for these types of data.  
13 An alternative may be to pursue a model form of the Hill equation which recently has been used  
14 for endocrine disruption data (Barton et al., 1998).

**TABLE 7B-6. BENCHMARK DOSE (BMD) AND BMD 95% LOWER LIMIT (BMDL) ESTIMATES FOR COMBINED MALE AND FEMALE HORMONE DATA OF 14-DAY TIME POINT IN THE SPRINGBORN LABORATORIES, INC. (1998) SUBCHRONIC STUDY (Benchmark response based on 10% change from control value.)**

Endpoint	Model	p of Fit	BMD	BMDL	NOAEL/ LOAEL	BMDL: N(L)OAEL	BMD: N(L)OAEL	BMR: 10% control SD
TSH	Power	0.45	0.037	0.000075	0.01	0.0075	3.7	1.26
	Quadratic	0.069	Fit significant, but not monotonic		0.01			2.52
ln TSH	Power	0.43	0.043	Could not calculate	0.01	NA	4.3	-0.1053
	Quadratic	Fit not significant, nonmonotonic			0.01			
T3	Power	0.41	0.000033	Lower limit includes 0	0.01 <sup>a</sup>	NA	0.0033	16.65 38.51
	Quadratic	Fit not significant, nonmonotonic			0.01 <sup>a</sup>			
lnT3	Power	0.35	0.000168	Lower limit includes 0	0.01 <sup>a</sup>	NA	0.0168	-0.1053
	Quadratic	Fit not significant, nonmonotonic			0.01 <sup>a</sup>			
T4	Power	0.203	1.16	0.0035	1.0	0.0035	1.16	0.506
	Quadratic <sup>b</sup>	0.12	3.27	1.09	1.0	1.09	3.27	0.603
ln (T4)	Power	0.22	1.64	0.04	1.0	0.04	1.64	-0.1053
	Quadratic <sup>b</sup>	0.16	3.25	1.06	1.0	1.06	3.25	

<sup>a</sup>LOAEL; otherwise, value is NOAEL.

<sup>b</sup>Global minimum of quadratic function is at dose ≈9.50 mg/kg-day.

**TABLE 7B-7. BENCHMARK DOSE (BMD) AND BMD 95% LOWER LIMIT (BMDL) ESTIMATES FOR COMBINED MALE AND FEMALE HORMONE DATA OF 14-DAY TIME POINT IN THE SPRINGBORN LABORATORIES, INC. (1998) SUBCHRONIC STUDY (Benchmark response based on 10, 20, and 40% changes from control value.)**

Endpoint	Model	p of Fit	BMD BMDL (10%)	BMD BMDL (20%)	BMD BMDL (40%)	Mean	NOAEL
T4	Power	0.203	1.16 0.0035	12.73 1.21	138.94 38.33	5.066	1.0
ln(T4)	Power	0.22	0.037	3.899	36.48		1.0
T3	Power	0.41	0.000033 —	0.207 —	129.39 0.129 <sup>a</sup>	166.5	0.01 <sup>b</sup>
ln(T3)	Power	0.35	Lower limit includes 0	0.000054 <sup>a</sup>	43.16 <sup>a</sup>		0.01 <sup>b</sup>
TSH	Power	0.45	0.037 0.000076	0.326 0.005	2.89 0.36	12.616	0.01
ln(TSH)	Power	0.43	0.0015	0.098	6.587		0.01

<sup>a</sup>BMDL calculation failed at a number of values. This means BMDL value may not be accurate.

<sup>b</sup>LOAEL, not NOAEL.

**TABLE 7B-8. BENCHMARK DOSE (BMD) ESTIMATES FOR COMBINED MALE AND FEMALE HORMONE DATA OF 90-DAY TIME POINT IN THE SPRINGBORN LABORATORIES, INC. (1998) SUBCHRONIC STUDY (Benchmark response based on 10% change from control value.)**

Endpoint	p of Fit	BMD	BMDL	NOAEL/ LOAEL	BMD: N(L)OAE	BMDL: N(L)OAE	BMR: 10% Control SD
TSH <sup>a</sup>	0.42	0.269	0.018	0.05	5.38	0.36	1.633 1.464
ln TSH <sup>a</sup>	0.40	0.492	0.0796	0.05	9.84	1.6	-0.1053
T3 <sup>a</sup>	0.01	No fit	No fit	0.01 <sup>b</sup>	NA	NA	17.50 18.924
lnT3 <sup>a</sup>	0.01	No fit	No fit	0.01 <sup>b</sup>	NA	NA	NA
T4 <sup>a</sup>	0.14	6e-6	Lower limit includes 0	0.01 <sup>b</sup>	6e-4	NA	0.475 0.576
ln (T4) <sup>a</sup>	0.17	1.10e-5	0.00	0.01 <sup>b</sup>	1.1e-3	∞	-0.1053

<sup>a</sup>Unrestricted quadratic: fit nonmonotonic, not significant. Restricted polynomial (linear): fit not significant.

<sup>b</sup>LOAEL; otherwise, value is NOAEL.

**TABLE 7B-9. BENCHMARK DOSE (BMD) AND BMD 95% LOWER LIMIT (BMDL) ESTIMATES FOR COMBINED MALE AND FEMALE HORMONE DATA OF 90-DAY TIME POINT IN THE SPRINGBORN LABORATORIES, INC. (1998) SUBCHRONIC STUDY (Benchmark response based on 10, 20, and 40% changes from control value.)**

	Model	p of Fit	BMD BMDL (10%)	BMD BMDL (20%)	BMD BMDL (40%)	Mean	NOAEL
T4	Power	0.14	0.000006 —	0.01 0.000001	15.09 0.52 <sup>a</sup>	4.75	0.01 <sup>b</sup>
ln(T4)	Power	0.165	0.00	0.004	4.87		0.01 <sup>b</sup>
T3	Power	0.01		No significant fit		174.96	0.01 <sup>b</sup>
ln(T3)	Power	0.01		No significant fit			0.01 <sup>b</sup>
TSH	Power	0.43	0.272 0.019	8.808 2.404	285.52 73.80	16.33	0.05
ln(TSH)	Power	0.40	0.082	7.94	405.14		0.05

<sup>a</sup>BMDL calculation failed at a number of values. This means BMDL value may not be accurate.

<sup>b</sup>LOAEL not NOAEL.

**TABLE 7B-10. BENCHMARK DOSE (BMD) AND BMD 95% LOWER LIMIT (BMDL) ESTIMATES FOR HORMONE AND THYROID MORPHOMETRY DATA OF F1-GENERATION PUPS AT PND5 IN THE DEVELOPMENTAL NEUROTOXICITY STUDY  
(Argus Research Laboratories, Inc., 1998a, and Channel, 1998c)<sup>a</sup>  
(Benchmark response based on 10% change from control value.)**

Endpoint	Model	p of Fit	BMD	BMDL	NOAEL or LOAEL	BMD: N(L)OAEL	BMDL: N(L)OAEL	BMR: 10% Control SD
TSH	Linear	0.50	4.64	3.77	3.0	1.55	1.26	0.45 0.465
	Power	0.31	4.48	1.43	3.0	1.49	0.48	
ln TSH	Linear	0.48	5.51	4.43	3.0	1.84	0.54	-0.1054
	Power	0.30	5.03	2.11	3.0	1.68	0.70	
T3	Neither linear, quadratic, or power FCNS fit data	<0.00001 for all	No fit	No fit	0.1	NA	NA	
lnT3	Neither linear, quadratic, or power FCNS fit data	<0.00001 for all	No fit	No fit	0.1	NA	NA	
T4	Nonmonotonic quadratic significant fit	0.50 min = 7.45 mg/kg	<i>1.26</i>	<i>0.98</i>	<i>1.0</i>	<i>1.26</i>	<i>0.98</i>	0.341 0.370
ln (T4)	Nonmonotonic quadratic significant fit	0.50 min = 7.14 mg/kg	<i>1.18</i>	<i>0.92</i>	<i>1.0</i>	<i>1.18</i>	<i>0.92</i>	
Morphometry	Control-10% Control (=31.78); SD = 0.37 Nonmonotonic quadratic significant fit Power FCN BMDL interval includes 0.00	0.19 global min = 6.81 mg/kg	<i>1.053</i>	<i>0.644</i>	<i>1.00</i>	<i>1.053</i>	<i>0.644</i>	
ln (morph)	Control-10% Control (= 0.341); SD = 0.37 Nonmonotonic quadratic significant fit Power FCN BMDL computational failures	0.19 global min = 7.01 mg/kg	<i>0.822</i>	<i>0.538</i>	<i>1.00</i>	<i>0.822</i>	<i>0.538</i>	

<sup>a</sup>Italics denote estimates derived from nonmonotonic fits to data. FCN = function and SD = standard deviation.

**TABLE 7B-11. BENCHMARK DOSE (BMD) AND BMD 95% LOWER LIMIT (BMDL) ESTIMATES FOR HORMONE DATA OF F1-GENERATION PUPS AT PND5 IN THE DEVELOPMENTAL NEUROTOXICITY STUDY (Argus Research Laboratories, Inc., 1998a, and Channel, 1998c) (Benchmark response based on 10, 20, and 40% changes from control value.)**

	p of Fit	BMD BMDL (10%)	BMD BMDL (20%)	BMD BMDL (40%)	Mean	NOAEL
T4	<u>0.50</u> <sup>a</sup>	<u>1.26</u> <sup>a</sup> <u>0.973</u> <sup>a</sup>	<u>2.89</u> <sup>a</sup> <u>2.16</u> <sup>a</sup>	<u>BMD set to</u> <sup>a</sup> <u>1,000</u> <sup>a</sup>	3.41	1.0
ln(T4)	<u>0.50</u> <sup>a</sup>	<u>0.92</u> <sup>a</sup>	<u>NC</u> <sup>a</sup>	<u>NC</u> <sup>a</sup>		1.0
T3	<0.00001	NC	NC	NC	87.97	0.1
ln(T3)	<0.00001		NC	NC		0.1
TSH	0.50	4.64 3.77	9.30 7.55	18.61 15.10	4.51	3.0
ln(TSH)	0.48	NC	NC	NC		3.0

<sup>a</sup>Underlined values from nonmonotonic fits to data. (NC = not computed.) The BMDL calculation failed at a number of values. This means BMDL value may not be accurate.

**TABLE 7B-12. BENCHMARK DOSE (BMD) AND BMD 95% LOWER LIMIT (BMDL) ESTIMATES USING THE LINEAR MODEL FIT TO THE MOTOR ACTIVITY DATA OF F1-GENERATION PUPS AT PND14 IN THE DEVELOPMENTAL NEUROTOXICITY STUDY (Argus Research Laboratories, Inc., 1998a) (Benchmark response based on 10% change from control value.)**

Endpoint	p of Fit	BMD	BMDL	NOAEL/ LOAEL	BMD: N(L)OAEL	BMDL: N(L)OAEL	BMR: 10% control SD
Movement <sup>a</sup>	0.72	1.94	1.04	None	NA	NA	24.45 162.75
Time <sup>b</sup>	0.69	1.33	0.66	None	NA	NA	18.60 184.78

<sup>a</sup>Number of movements.

<sup>b</sup>Time spent in activity.

**TABLE 7B-13. BENCHMARK DOSE (BMD) AND BMD 95% LOWER LIMIT (BMDL) ESTIMATES USING THE POWER MODEL FIT TO THE HORMONE DATA OF FEMALE RABBITS ON GESTATION DAY 29 IN THE DEVELOPMENTAL STUDY (Argus Research Laboratories, Inc., 1998c)  
(Benchmark response based on 10% change from control value.)**

Endpoint	p of Fit	BMD	BMDL	NOAEL/ LOAEL	BMD: N(L)OAEL	BMDL: N(L)OAEL	BMR
TSH, ln TSH						NA	No effect of dose
T3, ln T3						NA	No effect of dose
T4	0.06	0.54	Lower limit includes 0	0.1	5.4	NA	0.187
ln (T4)	0.0503	1.69	0.002	0.1	16.9	0.02	0.1053

**TABLE 7B-14. BENCHMARK DOSE (BMD) AND BMD 95% LOWER LIMIT (BMDL) ESTIMATES USING THE POWER MODEL FIT TO THE HORMONE DATA OF FEMALE RABBITS ON GESTATION DAY 29 IN THE DEVELOPMENTAL STUDY (Argus Research Laboratories, Inc., 1998c)  
(Benchmark response based on 10, 20, and 40% changes from control value.)**

	p of Fit	(10%)	(20%)	(40%)	Mean	NOAEL
T4	0.06	0.54 —	7.05 —	91.76 0.63	1.874	0.1
ln(T4)	0.05	1.69 0.0018	10.97 0.033	86.19 7.278		0.1
T3						No effect
ln(T3)						No effect
TSH						No effect
ln(TSH)						No effect

# 8. SCREENING ECOLOGICAL RISK ASSESSMENT FOR PERCHLORATE

## 8.1 INTRODUCTION

As discussed in Section 1.1, perchlorate salts including ammonium, potassium, sodium, and magnesium perchlorate, are manufactured as oxidizer components for propellants and explosives. The manufacture or use of perchlorate salts has been reported in most of the states of the continental United States (Figure 1-3). In some areas involved with the manufacture, use, or disposal of perchlorate salts, perchlorate, as the anion dissociated from these salts, has contaminated soils or ground or surface waters (Figure 1-4). These releases of perchlorate into the environment have been confirmed to have occurred in 20 states, clustered primarily in the southwestern United States where most sampling has occurred (Figures 1-3 and 1-4). Currently, there is a research need to determine whether perchlorate ion is causing any potential effects on ecosystems or ecosystem components. This chapter presents a screening-level ecological risk assessment of environmental contamination by perchlorate. In organization, it follows the Guidelines for Ecological Risk Assessment (U.S. Environmental Protection Agency, 1998c).

### 8.1.1 Management Goals and Decisions

The discovery that perchlorate release in some sites has contaminated ground and surface waters in certain locations has raised public and regulatory agency concerns. Much concern has focused on potential public exposures through drinking water and on the possible needs to improve analytical and treatment methods and to develop drinking water regulations (Section 1.4). Consequently, an extensive scientific assessment effort is underway to address those concerns (Section 1.5). A balanced approach requires assessing ecological effects as well. The goal of this screening-level ecological risk assessment is, therefore, to indicate the likelihood that adverse ecological effects (i.e., toxicity to specific organisms or effects on aquatic or terrestrial ecosystems) will result from observed levels of environmental contamination by perchlorate. The results of this assessment may be used to address the following questions:

- 1 • Are ecological risks best characterized as *de minimis* (exposures clearly are below levels of  
2 concern), *de manifestis* (risks are clearly significant and require management action to reduce  
3 exposures); or somewhere in between and requiring further characterization?
- 4 • Are analytical detection methods for determining levels of perchlorate in the environment  
5 sufficient, or is it likely that adverse ecological effects occur at levels below current detection  
6 limits?
- 7 • Is the available ecotoxicological information on perchlorate sufficient, or are additional studies  
8 needed?

### 9 10 **8.1.2 Scope, Complexity, and Focus**

11 In the previous ERD version of this document (U.S. EPA, 1998d), the available  
12 information for this ecological risk assessment was characterized as “very limited” and the  
13 assessment was characterized as “screening-level.” Information about the environmental levels  
14 of perchlorate to which organisms were exposed and about its effects on diverse taxonomic  
15 groups was practically nonexistent. Since then, additional information has become available that  
16 improves the database in some respects. Most significantly, additional data are available on  
17 effect levels in aquatic animals, an aquatic plant, a terrestrial plant, and a soil invertebrate; some  
18 of these data are for chronic exposures. Effect levels in rodents have been reevaluated as part of  
19 the human health risk assessment for perchlorate, and the ecological implications of those  
20 changes are reflected herein. In addition, surveys have been conducted at several sites of known  
21 or suspected perchlorate contamination, and environmental and biological materials have been  
22 analyzed for perchlorate. Nonetheless, the level of knowledge on this issue must still be  
23 characterized as limited because the number of species tested is still quite minimal, and the site  
24 surveys focused only on the range of exposures at those sites. This ecological risk assessment is  
25 therefore still a screening-level, rather than definitive, assessment. The materials used in the  
26 1998 ERD and those that are new to this present draft, are described in this section.

27 **Interagency Perchlorate Steering Committee Report.** Perchlorate Ecological Risk  
28 Studies is a report of the IPSC’s Ecological Risk/Transport and Transformation Subcommittee,  
29 dated November 13, 1998 (Interagency Perchlorate Steering Committee, 1998). This report  
30 presents a literature review on perchlorate toxicity to nonmammalian organisms, recognizing that  
31 few published studies exist, and a rationale for the selection of a battery of ecotoxicology tests

1 conducted for the USAF Armstrong Laboratory by EA Engineering, Science and Technology,  
2 Inc. It then summarizes those test results, discusses the findings in the context of observed  
3 exposures, discusses uncertainties, and makes recommendations for further study. The present  
4 report constitutes a reevaluation of much of the same information from EPA's perspective,  
5 except that EPA did not examine the open literature studies reviewed by the IPSC subcommittee.

6 **Test Battery Reports.** The EA Engineering, Science and Technology, Inc. (1998) final  
7 report, *Results of Acute and Chronic Toxicity Testing with Sodium Perchlorate*, dated November  
8 1998, details the test methods and results of the ecotoxicology battery. A follow-up report (EA  
9 Engineering, Science and Technology, Inc., 2000) details the test methods and results from  
10 additional chronic toxicity testing with the freshwater amphipod *Hyaella azteca* and the fathead  
11 minnow *Pimephales promelas*.

12 **Block Environmental Services, Inc., Report.** The report, *LC<sub>50</sub> Aquatic Toxicity Test*  
13 *Results for Ammonium Perchlorate—A Two-Species Chronic Definitive Bioassay* (Block  
14 Environmental Services, Inc., 1998) presents additional bioassay results that were not included in  
15 the IPSC report.

16 **Algal Toxicity Testing.** The EA Engineering, Science and Technology, Inc. (1999) final  
17 report, *Results of Algal Toxicity Testing with Sodium Perchlorate*, dated September 1999, details  
18 the test methods and results of the ecotoxicological testing with the algae, *Selenastrum*  
19 *capricornutum*.

20 **Frog Embryo Teratogenesis Assay: *Xenopus* (FETAX) Study.** The report, FETAX  
21 Analysis of Ammonium Perchlorate (Dumont and Bantle, 1998), prepared by the Department of  
22 Zoology, Oklahoma State University, and dated May 22, 1998, presents results of the Frog  
23 Embryo Teratogenesis Assay: *Xenopus* (FETAX) conducted with ammonium perchlorate.  
24 Recent data received by the EPA that the Agency has not yet fully reviewed indicate effects on  
25 thyroid function, metamorphosis and sex ratio in developing *Xenopus laevis* (Goleman et al.,  
26 2002). These data are made available with this document to the external peers for their review.

27 **Phytotransformation Study.** Two sets of studies report on the accumulation and potential  
28 degradation of perchlorate by plants. The study, *Laboratory Characterization of*  
29 *Phyto-transformation Products of Perchloroethylene (PCE), Trichloroethylene (TCE) and*  
30 *Perchlorate* (Nzengung, n.d.; Nzengung et al., 1999), examined perchlorate distribution and  
31 degradation in experimental systems containing sand, aqueous perchlorate solution, and rooted

1 cuttings of woody plant species. This study also examined systems containing chopped leaves or  
2 microbial mats and aqueous perchlorate solution. A second study, *Potential Species for*  
3 *Phytoremediation of Perchlorate* (Susarla et al., 1999a; Susarla et al., 2000a), reported  
4 perchlorate depletion from test media over a ten day period by 13 vascular plant species and their  
5 potential for phytoremediation of perchlorate contaminated sites.

6 **Biotransport Investigation Studies.** These studies assess the potential for  
7 bioaccumulation of perchlorate in food webs by answering the question of whether perchlorate is  
8 present in biological receptors. The report *Scientific and Technical Report for Perchlorate*  
9 *Biotransport Investigation: A Study of Perchlorate Occurrence in Selected Ecosystems* (Parsons,  
10 2001) examined perchlorate concentrations in site media and in various ecological receptors at  
11 six sites with known or suspected perchlorate contamination: (1) sites associated with withdraw  
12 of irrigation water from the Colorado River in the vicinity of Yuma, Arizona; (2) Las Vegas  
13 Wash and Lake Mead near Las Vegas, Nevada; (3) Allegany Ballistics Laboratory, Rocket  
14 Center, West Virginia; (4) Holloman Air Force Base in Otero County, New Mexico; (5) Naval  
15 Surface Warfare Center, Indian Head, Maryland; and (6) Longhorn Army Ammunition Plant,  
16 Karnack, Texas. Additional data are available for one of these sites, Longhorn Army  
17 Ammunition Plant (LHAAP), Texas, in a paper published by Smith et al. (2001). In both studies,  
18 ion chromatography with an AS-16 analytical column was used to measure for perchlorate  
19 concentrations. Analyses with this analytical column have been shown to be superior than other  
20 columns for detecting and quantifying perchlorate (Ellington and Evans, 2000; Susarla et al.,  
21 2000b).

22 All these sites, except for those in the vicinity of Yuma, are associated with localized  
23 contamination related to the manufacture, handling, or use of perchlorate in solid propellants.  
24 The Yuma sites are approximately 250 miles downstream along the Colorado River from the Las  
25 Vegas Wash and Lake Mead sites; the report suggests that there is no localized source of the  
26 perchlorate; therefore, the most likely potential source of any perchlorate contamination in these  
27 soils is believed to be Colorado River irrigation water. However, portions of the Yuma Proving  
28 Grounds are drained by washes that pass near some of the agricultural locations sampled, and the  
29 information provided in the report was not sufficient for ruling out the possibility of  
30 contamination from the Yuma Proving Grounds.

1 **8.2 PROBLEM FORMULATION**

2 The characteristics of perchlorate and its sources are described earlier in this document  
3 (Chapters 1 and 2). Because this assessment is site independent, this problem formulation  
4 focuses on the selection of assessment endpoints, derivation of the conceptual model, and the  
5 analysis plan.

6  
7 **8.2.1 Assessment Endpoints**

8 In ecological risk assessment, assessment endpoints are operational definitions of the  
9 environmental values to be protected. They are chosen based on policy goals and societal values,  
10 their ecological relevance, and their susceptibility to the stressor and are defined in terms of an  
11 entity and a property of that entity. The assessment endpoints for this ecological risk assessment  
12 are described in the following five subsections.

13  
14 **8.2.1.1 Fish Community Richness and Productivity**

15 Fish communities are valued societally and are ecologically important. The productivity of  
16 these communities is important in terms of the support of fisheries. Species richness is important  
17 in terms of maintaining biodiversity. This importance is reflected by the use of species  
18 sensitivity distributions in the derivation of national ambient water quality criteria and the use of  
19 fish species richness as an important component of bioassessment procedures for enforcement of  
20 the Clean Water Act.

21  
22 **8.2.1.2 Aquatic Invertebrate Community Richness and Productivity**

23 Aquatic invertebrate communities have little direct societal value but are important to  
24 energy and nutrient dynamics in aquatic ecosystems. The productivity of these communities is  
25 important in terms of trophic support of fisheries, of other groups of aquatic species, and of some  
26 terrestrial insectivores. Species richness is important in terms of maintaining biodiversity. This  
27 importance is reflected by the use of species sensitivity distributions in the derivation of national  
28 ambient water quality criteria and the use of invertebrate species richness as an important  
29 component of bioassessment procedures for enforcement of the Clean Water Act.

1 **8.2.1.3 Aquatic Plant Richness and Productivity**

2 Algae and other aquatic plants have little direct societal value but are important to energy  
3 and nutrient dynamics in aquatic ecosystems. Species richness is important in terms of  
4 maintaining biodiversity. Because of their importance to the trophic support of fisheries and  
5 other aquatic consumers, productivity is an important endpoint for this assemblage.  
6

7 **8.2.1.4 Soil Invertebrate Community Richness and Productivity**

8 Soil invertebrate communities have little direct societal value, but, in nearly all terrestrial  
9 ecosystems, they are important to energy and nutrient dynamics and to maintenance of soil  
10 structure. The productivity of these communities is also important in terms of trophic support of  
11 some terrestrial insectivores. Species richness is important in terms of the policy of maintaining  
12 biodiversity.  
13

14 **8.2.1.5 Terrestrial Plant Richness and Productivity**

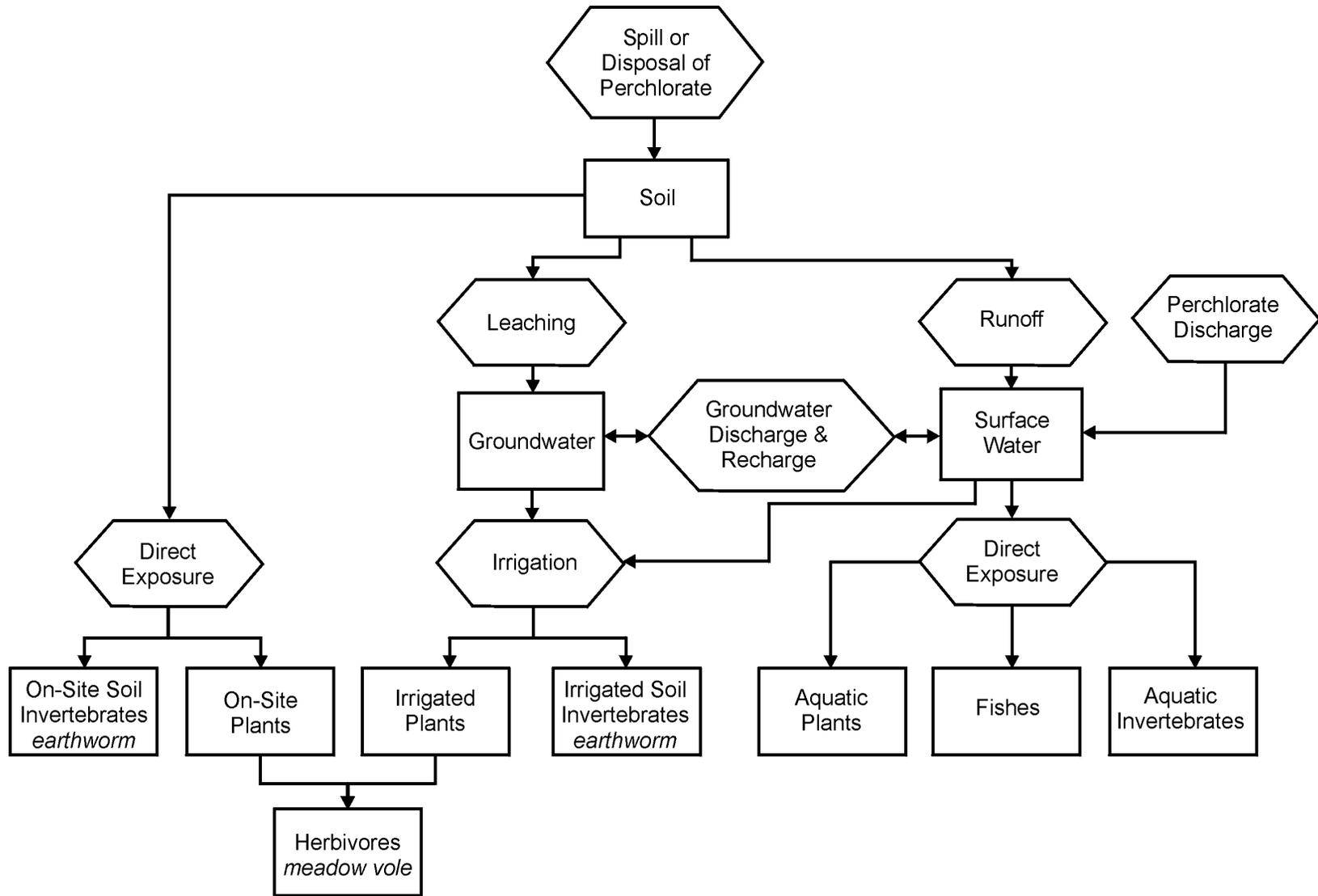
15 Terrestrial plants are valued highly by society for production of food, fiber, and timber, as  
16 well as their aesthetic value. The primary valued property of terrestrial plants is their  
17 productivity. As autotrophs, plants are the basis of energy and nutrient dynamics in most  
18 terrestrial or aquatic food webs. Moreover, species richness is important in terms of the policy of  
19 maintaining biodiversity.  
20

21 **8.2.1.6 Population Productivity of Herbivorous Wildlife**

22 Herbivorous wildlife are included as an endpoint entity because of the apparent  
23 bioconcentration of perchlorate in plant foliage. The meadow vole (*Microtus pennsylvanicus*) is  
24 used as a representative species for this group. Population productivity is used as the endpoint  
25 property because growth and reproduction are generally sensitive properties and because  
26 herbivores are valued for their production of food for human and nonhuman carnivores.  
27

28 **8.2.2 Conceptual Models**

29 The conceptual model describes the relationships between sources of perchlorate and the  
30 endpoint receptors (Figure 8-1). Sources include spills during the flushing of rockets; the  
31 combustion of rocket fuel; the improper disposal of rocket fuel, open burn or open detonation



**Figure 8-1. A conceptual model of exposure of ecological endpoint receptors to perchlorate. Specific endpoint taxa are identified in italics; all other endpoints are defined at the community level. Processes are designated by hexagonal boxes, compartments by rectangular boxes.**

1 operations, explosives, or manufacturing wastes; and the aqueous discharge of waste water from  
2 manufacturing of perchlorate. The most recent information on perchlorate content in fertilizers  
3 demonstrates that fertilizer use is unlikely to constitute an environmentally significant source of  
4 perchlorate contamination, and ecological risks from this source are not considered further (see  
5 Chapter 9). Spills contaminate the soil at the site and, through leaching and run-off, contaminate  
6 the surface water and groundwater. The discharge of groundwater to surface water may result in  
7 locally high levels of perchlorate in surface waters. Aquatic communities are exposed directly to  
8 contaminated surface water; soil invertebrate and plant communities are exposed to perchlorate  
9 in soil at the spill site and through irrigation with either surface or groundwater; and herbivorous  
10 terrestrial wildlife are exposed through their consumption of plants that have bioconcentrated  
11 perchlorate. However, the potential for transfer of perchlorate further up the terrestrial food web  
12 is currently unknown.

13 This conceptual model is relatively simple because it excludes some potential routes and  
14 receptors. Dietary exposures are excluded from aquatic systems because, as of this writing,  
15 available data have not shown perchlorate to bioconcentrate to any significant extent.  
16 Information newly received from the U.S. Army Corps of Engineers (Condike, 2001) report on  
17 the analysis of environmental samples from perchlorate-contaminated water bodies near  
18 McGregor Naval Weapons Industrial Reserve Plant (NWIRP), TX, and purports to show fish  
19 tissue concentrations that exceed comparable water concentrations. These data suggest that  
20 perchlorate not only accumulates but is bioconcentrated. This information, which has not yet  
21 been fully reviewed by the U.S. EPA, is herewith made available with this document to external  
22 peers for their review.

23 Wildlife are assumed to have negligible exposure from air or from direct exposure to soil.  
24 Exposures of wetlands to groundwater or surface water are not included explicitly because their  
25 exposures and effects are assumed to be equivalent to irrigation exposures. That is, plants and  
26 invertebrates are assumed to be exposed to pore-water concentrations equal to surface or  
27 groundwater concentrations. Exposures to contaminated sediments also are not included  
28 explicitly because they are believed to be equivalent to surface water exposures. Perchlorate salts  
29 are highly water soluble and the anion is unlikely to adsorb to anionic particles, such as soils or  
30 humic substances, to a significant extent. Therefore, sediment exposures are expected to be  
31 dominated by exposure to pore water, which is assumed to be equal to surface water.

1 **8.2.3 Analysis Plan**

2 This screening assessment uses existing information to determine whether the existing  
3 environmental contamination by perchlorate poses a clearly significant risk, insignificant risk, or  
4 an ambiguous risk. The analysis of effects will consist of the derivation of screening benchmarks  
5 through the application of conservative extrapolation models. The analysis of exposure for  
6 ecological endpoints consists of measured concentrations reported in Chapter 1 or derived from  
7 Parsons (2001) or Smith et al. (2001). Soil exposure estimates are based on exposure to  
8 perchlorate in irrigation water.  
9

10  
11 **8.3 ANALYSIS**

12 **8.3.1 Characterization of Exposure**

13 **8.3.1.1 Water Concentrations**

14 As previously described, fishes, aquatic invertebrates, and aquatic plants may be exposed  
15 directly to concentrations of perchlorate in surface waters. These concentrations may result from  
16 surface run-off from perchlorate-contaminated soil, from leaching of perchlorate from  
17 contaminated soil via shallow groundwater, or from direct discharge of aqueous wastes. Surface  
18 or groundwater may be used for irrigation, resulting in direct exposure of soil invertebrates or  
19 plants (Figure 8-1).

20 Perchlorate salts are dissolved readily given the conditions under which the contamination  
21 has occurred, releasing the perchlorate anion and the associated cation. Sorption is not expected  
22 to attenuate perchlorate because it absorbs weakly to most soil minerals, and natural chemical  
23 reduction in the environment is not expected to be significant. Consequently, perchlorate is both  
24 very mobile in aqueous systems and persistent for many decades under typical ground and  
25 surface water conditions (Section 1.1).

26 Limited information is available on perchlorate concentrations in surface waters.  
27 Perchlorate from an ammonium perchlorate manufacturing area has been detected at 4 to 16  $\mu\text{g/L}$   
28 downstream in Lake Mead and the Colorado River (Section 1.2). Information on the frequency  
29 or central tendency (mean or median) of perchlorate detection in those water bodies was not  
30 available for this review, but it is assumed that some aquatic organisms are exposed chronically

1 to concentrations as high as 16  $\mu\text{g/L}$ . On the other hand, perchlorate concentrations have been  
2 measured as high as 0.37% ( $37 \times 10^6 \mu\text{g/L}$ ) in groundwater-monitoring wells at facilities that  
3 manufacture or test rocket motors and at 280  $\mu\text{g/L}$  in public water supply wells (Section 1.2)  
4 Smaller surface water bodies, including some that are supplied primarily by groundwater, are  
5 likely to exist near sites of soil contamination and to have perchlorate concentrations much  
6 higher than those reported for Lake Mead and the Colorado River. A spring associated with the  
7 Las Vegas Wash site had concentrations of 1.0 to  $1.3 \times 10^5 \mu\text{g/L}$  in surface water (Parsons,  
8 2001). Perchlorate concentrations in a pond (INF Pond) that receives water from the pump and  
9 treat system at the Longhorn Army Ammunition Plant near Karnack, TX ranged from 30,776 to  
10 31,438  $\mu\text{g/L}$  in November 1999 (Smith et al., 2001) and ranged from 3500 to 3800  $\mu\text{g/L}$  in  
11 September 2000 (Parsons, 2001). It is also possible that, within large water bodies, there are  
12 locally elevated concentrations at sites of groundwater discharge. In the vicinity of a sediment  
13 delta created by the Las Vegas Wash in Las Vegas Bay of Lake Mead, Parsons (2001) documents  
14 a maximum perchlorate concentration of 68  $\mu\text{g/L}$  in surface water. At the Allegany Ballistics  
15 Laboratory in Rocket Center, WV, discharge water from a Comprehensive Environmental  
16 Response Compensation, and Liability Act (CERCLA) groundwater pump and treat facility to  
17 the North Branch Potomac River contained 250 to 280  $\mu\text{g/L}$  perchlorate (Parsons 2001). Surface  
18 water concentrations in Town Gut Marsh adjacent to the Naval Surface Warfare Center at Indian  
19 Head, MD ranged from not detected (reporting limit = 4.0  $\mu\text{g/L}$ ) to 25  $\mu\text{g/L}$ . It should be noted  
20 that the groundwater pump and treat facilities either at Longhorn Army Ammunition Plant or  
21 Allegany Ballistics Laboratory were not equipped with facilities to treat perchlorate in water.

22 Surface water concentrations in Harrison Bayou below the discharge point for the INF pond  
23 at LHAAP also ranged from undetectable (reporting limit = 4.0  $\mu\text{g/L}$ ) to 4.0  $\mu\text{g/L}$  (Parsons,  
24 2001; Smith et al., 2001). However, Smith et al. (2001) point out that water from the pond is  
25 discharged to Harrison Bayou only during periods when Harrison Bayou is flowing, and neither  
26 study apparently sampled Harrison Bayou when water was being discharged from the pond.  
27 Therefore, higher concentrations of perchlorate in surface water of Harrison Bayou are likely to  
28 be measured at other times.

29 It is assumed that irrigation waters pumped from Lake Mead or the Colorado River are in  
30 the range of downstream concentrations given above (i.e., 4 - 16  $\mu\text{g/L}$ ). Groundwater irrigation

1 may be contaminated at levels similar to those observed in public water supplies ( $\leq 280 \mu\text{g/L}$ ),  
2 unless the well is appreciably nearer a perchlorate-contaminated site.

### 3 4 **8.3.1.2 Aquatic Bioaccumulation**

5 As discussed above, little information has been previously available on the potential for the  
6 perchlorate ion to accumulate in animal tissues. The studies outlined in the Parsons (2001)  
7 report sought to answer the question whether perchlorate is present in ecological receptors.  
8 In these studies, concentrations of perchlorate in aquatic vegetation, fish, amphibians, aquatic  
9 invertebrates, and birds were compared to surface-water, pore-water, and sediment  
10 concentrations from the same water body. This information is supplemented by the additional  
11 studies conducted at LHAAP by Smith et al. (2001).

12 When perchlorate concentrations in physical media (i.e., surface water or sediment) were  
13 greater than the reporting limits for biological media ( $\geq 300$  ppb [ $\mu\text{g/L}$  or  $\mu\text{g/kg}$ ] in Parsons  
14 [2001]), concentrations in aquatic vegetation were similar to or greater than the concentrations in  
15 surface water or pore water; but concentrations in fish, amphibians, or invertebrates were less.  
16 In Smith et al. (2001) reported the detection of high concentrations of perchlorate in the INF  
17 Pond and lower concentrations in aquatic vegetation and in animals than in surface water or  
18 sediments.

19 In Parsons (2001), when perchlorate concentrations in the physical media were lower,  
20 concentrations in aquatic vegetation or amphibians were in a few cases greater than the  
21 concentrations in surface water or sediment; but in most cases, perchlorate was not detected in  
22 aquatic receptors. However, our understanding of bioaccumulation of perchlorate in this lower  
23 concentration range is limited because the reporting limits in the Parsons (2001) studies for  
24 perchlorate in animal tissues (i.e., 300-400  $\mu\text{g/kg}$ ) were greater than the reporting limits for  
25 surface water or pore water (i.e.,  $\approx 4 \mu\text{g/L}$ ) or for sediments (i.e.,  $\approx 80 \mu\text{g/L}$ ).

26 Although Smith et al. (2001) do not identify their reporting limits, their reporting limits for  
27 biological tissues appear to be less (i.e.,  $\approx 70 \mu\text{g/kg}$  based on their lowest detected concentration)  
28 than those of Parsons (2001). In the Smith et al. (2001) study of LHAAP, detected perchlorate  
29 concentrations were similar in surface water (44-85  $\mu\text{g/L}$ ), sediments (78  $\mu\text{g/kg}$ ), and fish tissues  
30 (83-131  $\mu\text{g/kg}$ ) at Goose Prairie Creek. In Harrison Bayou, the single detected concentration in  
31 surface water (4  $\mu\text{g/L}$ ) was less than detected concentrations in animal tissues (86-356  $\mu\text{g/kg}$ ).

1 However, as the authors discuss, the measured concentration in surface water in Harrison Bayou  
2 is likely less than when water is being discharged from the INF Pond (Smith et al., 2001). In  
3 addition, the study did not collect sufficient samples from any one site and medium or species for  
4 any significant statistical comparisons to be made.

5 Information newly received from the U.S. Army Corps of Engineers (Condike, 2001) report  
6 on the analysis of environmental samples from perchlorate-contaminated water bodies near  
7 McGregor Naval Weapons Industrial Reserve plant (NWIRP), TX, and purports to show fish  
8 tissue concentrations that exceed comparable water concentrations. These data suggest that  
9 perchlorate not only accumulates but is bioconcentrated. This information, which has not yet  
10 been fully reviewed by the U.S. EPA, is herewith made available with this document to external  
11 peers for their review.

12 The above information indicates that perchlorate may bioaccumulate in aquatic organisms  
13 living in contaminated waters, but it does not resolve the question of whether perchlorate may  
14 bioconcentrate in the tissues of aquatic organisms to levels exceeding the surface water  
15 concentrations. The existing data are also insufficient to determine whether there is further  
16 trophic transfer of perchlorate within aquatic food webs.

### 17 18 **8.3.1.3 Soil Levels**

19 On-site soils may be contaminated by direct spills of perchlorate solutions from flushing  
20 rockets, combustion of rocket fuel, improper disposal of rocket fuel, open burn/open detonation  
21 operations, explosives, or manufacturing wastes. Perchlorate concentration measurements at  
22 disposal sites range from less than 1 to 1470 mg/kg (Parsons, 2001). Off-site soils may be  
23 contaminated via irrigation (Figure 8-1). Because of the high water solubility of perchlorate  
24 salts, perchlorate is unlikely to accumulate via adsorption to irrigated soils, and aqueous  
25 perchlorate was not found to adsorb to sand in laboratory reactors (Nzengung, n.d.). By gross  
26 approximation, then, soil concentrations (expressed as milligrams per kilogram) would be  
27 unlikely to exceed the concentrations (expressed as milligrams per liter) in irrigation water.  
28 Similarly, concentrations of perchlorate in soil pore water may be assumed to be equal to the  
29 concentration in irrigation water, both in the field and in soil toxicity tests. However, the  
30 concentration of perchlorate salts in irrigated soils with high evaporation rates cannot be ruled  
31 out. At the Yuma site, soils are irrigated with water from the Colorado River, and concentrations

1 of perchlorate in surface-water samples collected near the irrigation intake locations ranged from  
2 0.003 to 0.006 mg/L. In surface soil, the single detection (0.090 mg/kg) was well above the  
3 concurrently-measured water concentrations, as were the perchlorate detection limits in soil  
4 (0.079 to 0.080 mg/kg). The relatively higher detection limits in soil, the limited nature of the  
5 sampling in soil and water, and the lack of information about potential sources other than  
6 irrigation water (see Section 8.1.2) complicate the interpretation of the presence and fate of  
7 perchlorate in irrigated soils.

#### 8 9 **8.3.1.4 Uptake by Vegetation**

10 Several laboratory experiments have examined plant uptake of perchlorate from solution  
11 culture. Experiments with candidate plants for use in the phytoremediation of perchlorate-  
12 contaminated sites showed that perchlorate may concentrate in vegetation (Nzengung, n.d.;  
13 Susarla et al., 2000a). Nzengung (n.d.) used rooted cuttings of woody plants, willow (*Salix* spp.),  
14 Eastern Cottonwood (further identified only as “poplar”), and eucalyptus (*Eucalyptus cineria*)  
15 planted in sand with nutrient solution containing perchlorate at 20 or 100 mg/L for 24 to 42 days.  
16 In each case, perchlorate was taken up and concentrated in aerial plant parts, especially leaves.  
17 Concentration factors, expressed as the ratio of leaf concentration (mg/kg wet weight) to initial  
18 solution concentration (mg/L), ranged from 7.5 to 25.

19 Susarla et al. (2000a) used seedlings or rooted cuttings of 13 vascular plant species, planted  
20 in sand with nutrients, and exposed for ten days to 0.2, 2.0 or 20 mg/L perchlorate. These  
21 researchers also reported depletion of perchlorate from test media. Qualitative analyses  
22 suggested accumulation of perchlorate in the aerial tissues of most of the species analyzed.  
23 Using their data and the approach reported by Nzengung above, we calculated concentration  
24 factors ranging from 0 to 330.

25 Nzengung (n.d.) and Susarla et al. (2000a) reported that perchlorate accumulated primarily  
26 in the leaves, followed by stems, then roots. Predicted perchlorate breakdown products, chlorate,  
27 chlorite, and chloride were detected in plant tissues in both studies, but quantitative evidence was  
28 not presented. In addition to this lack of quantitative data, there are other concerns related to the  
29 potential for plants to degrade perchlorate. First, information concerning accumulation and  
30 potential transformation is limited to a few studies by these two laboratories. Second, the  
31 method used for perchlorate analysis yielded estimates of perchlorate in fertilizer that were

1 subsequently found to be overestimated by 30 to 150% (Susarla et al., 2000b). Third, no  
2 physiological explanation has been suggested for why plants should accumulate this salt far in  
3 excess of concentrations in water or groundwater, though it appears this may be simply a  
4 function of water uptake rates to meet transpirational losses. Fourth, these two studies were  
5 short-term, material depletion studies, a type of study we believe will overestimate long-term  
6 accumulation rates because some of the “response” is likely the result of factors not related to the  
7 chemical in question. There is ample evidence from salt accumulation studies of plants to  
8 suggest that the initial increases in perchlorate accumulation by plants may be due to a salt effect;  
9 that is, nutrient salts are initially indistinguishable from perchlorate salts in that they all represent  
10 an ionic imbalance across the cell wall. One of the confounding issues that can only be  
11 determined with longer-term studies is the effect of increased cell sap salinity on additional  
12 perchlorate uptake. As sap salinity increases, there should be an increase in H<sub>2</sub>O uptake, further  
13 increasing the perchlorate concentrations. This will continue only until a certain concentration of  
14 salts, including perchlorate, is reached, at which time the plant will close its stomata and shunt  
15 sap salts to vacuoles.

16 In addition to the above stated concerns, there is no reason to expect that these are steady-  
17 state concentration factors. These experiments were designed to quantify phytotransformation of  
18 an initially introduced perchlorate quantity, rather than bioconcentration, with respect to an  
19 ambient perchlorate concentration. As the perchlorate-amended nutrient solution was transpired,  
20 and some perchlorate was taken up, it was replenished by solution, without added perchlorate;  
21 thus, perchlorate in the test chamber diminished throughout the experiment. Concentration  
22 factors that would be observed at steady state, such as may result from continual irrigation with  
23 perchlorate-contaminated water, cannot be estimated from this study. Because of the  
24 uncertainties associated with both perchlorate accumulation and degradation by plants, a simple,  
25 conservative, screening-level assumption that concentrations in leaves can exceed water  
26 concentrations by a factor of 100 was made.

27 If irrigation is from surface water sources similar to the Colorado River or Lake Mead, with  
28 concentrations as high as 16  $\mu\text{g/L}$ , then plant concentrations are assumed to be as high as  
29 1.6 mg/kg. If irrigation is from groundwater sources similar to potable water supplies, with  
30 concentrations as high as 280  $\mu\text{g/L}$ , then plant concentrations will be assumed to be as high as  
31 28 mg/kg.

1 Concentrations in plant tissues and soils also have been measured in the field. Ellington  
2 et al. (2001) measured perchlorate concentrations in leaves of tobacco, *Nicotiana tabacum* var.  
3 K326, field-grown in soil amended with Chilean saltpeter, which is naturally high in perchlorate.  
4 Perchlorate concentrations ( $\pm$  SD) in leaf lamina from the 1999 crop were  $96.0 \pm 0.6$  mg/kg dry  
5 weight and  $14.6 \pm 0.1$  mg/kg wet weight; concentration in a composite soil sample collected in  
6 December 1999 was  $0.34 \pm <0.01$  mg/kg dry weight. The concentration factor in this study was  
7 approximately 43, on the basis of wet weight in leaf lamina and dry weight in soil.

8 The field studies by Parsons (2001) found that, for various sites, wet-weight perchlorate  
9 concentrations in terrestrial vegetation samples were 1.5 to 80 times the wet-weight  
10 concentrations in soil samples. The data from one site (i.e., Building 25C) at LHAAP (Smith  
11 et al., 2001) seem to indicate greater concentration factors, but the soil and plant samples were  
12 taken at different times of the year (i.e., January and October, respectively) and only one sample  
13 each of three plant species was analyzed.

14 Soil-to-vegetation concentration factors derived from the above field studies were similar  
15 in magnitude, but when using them for risk assessment care should be taken to note the different  
16 bases; exposure concentration was variously reported as mg/kg wet weight in soil or mg/kg dry  
17 weight in soil. The maximum measured concentration in vegetation at irrigated sites in the  
18 vicinity of Yuma, Arizona was 1.0 mg/kg wet weight. At sites with soil contamination related to  
19 the manufacture, handling, or use of perchlorate in solid propellants, maximum plant  
20 concentrations were 428 mg/kg wet weight at a spring; 99.2 mg/kg wet weight at a site upstream  
21 from Lake Las Vegas in the Las Vegas Wash area of the Lake Mead Recreational Area, Nevada;  
22 and 300 mg/kg wet weight at the Burn Area of Allegany Ballistics Laboratory, West Virginia.  
23 In most cases, detection limits were  $\sim 0.4$  mg/kg wet weight.

#### 24 25 **8.3.1.5 Herbivore Exposure**

26 The representative herbivore selected for this assessment, *M. pennsylvanicus*, has a diet  
27 consisting mainly of monocot and dicot shoots, has an estimated food consumption rate of  
28 0.005 kg/day wet weight, and a body weight of 0.044 kg (Sample and Suter, 1994). Using the  
29 assumptions stated above, daily exposures resulting from surface water and groundwater  
30 irrigation may be as high as 0.18 mg/kg-day and 3.2 mg/kg-day, respectively. Daily exposures  
31 resulting from maximum measured concentrations in plants range from 0.11 mg/kg-day at the

1 irrigated sites in the vicinity of Yuma to 49 mg/kg-day for the sites with direct soil  
2 contamination.

3 In the Parsons (2001) studies, except when concentrations in surface soils were high (i.e.,  
4  $\geq 9000 \mu\text{g}/\text{kg}$ ), perchlorate was not detected in terrestrial birds, mammals, or insects with  
5 reporting limits of 300 to 400  $\mu\text{g}/\text{kg}$ . The vertebrates collected varied substantially between  
6 sites, but the birds collected include the mourning dove (*Zenaida macroura*), tree swallow  
7 (*Tachycineta bicolor*), roughwing swallow (*Stelgidopteryx serripennis*), lesser nighthawk  
8 (*Chordeiles acutipennis*), nighthawk (*C. minor*); Gambel's quail (*Callipepla gambelii*), starling  
9 (*Sturnus vulgaris*); American crow (*Corvus brachyrhynchus*), eastern bluebird (*Sialia sialis*),  
10 eastern phoebe (*Sayornis phoebe*), and blue grosbeak (*Guiraca caerulea*). The mammals  
11 collected include the cactus mouse (*Peromyscus eremicus*), rock pocket mouse (*Chaetodipus*  
12 *intermeius*), Audubon's cottontail (*Sylvilagus audubonii*), deer mouse (*P. maniculatus*), long-  
13 tailed pocket mouse (*Perognathus formosus*), western pipistrelle (*Pipistrellus hesperus*), house  
14 mouse (*Mus musculus*), white-footed mouse (*Peromyscus leucopus*) meadow vole (*Microtus*  
15 *pennsylvanicus*), Merriam's kangaroo rat (*Dipodomys merriami*), desert pocket mouse  
16 (*C. penicillatus*), hispid cotton rat (*Sigmodon hispidus*), western harvest mouse (*Reithrodontomys*  
17 *megalotis*), marsh rice rat (*Oryzomys palustris*); northern short-tailed shrew (*Blarina*  
18 *brevicauda*), racoon (*Procyon lotor*), eastern harvest mouse (*R. fulvescens*), little brown bat  
19 (*Myotis lucifugus*), eastern cottontail (*S. floridanus*). At those sites where perchlorate  
20 concentrations in surface soils were high, perchlorate concentrations in potential herbivore  
21 tissues were generally an order of magnitude or more less than that in vegetation. At one site,  
22 Yuma, with lower perchlorate concentrations in soil (i.e., mean of all results = 81  $\mu\text{g}/\text{kg}$ ),  
23 perchlorate was detected in a single terrestrial reptile sample (brush lizard, *Urosaurus graciosus*),  
24 but this detection was lower than the mean perchlorate concentration in vegetation. Although  
25 detected soil concentrations were lower (i.e., 50 to 322  $\mu\text{g}/\text{kg}$ ) in Smith et al. (2001), the  
26 concentrations of perchlorate in two composite samples of livers from harvest mice  
27 (*Reithrodontomys fulvescens*) were several orders of magnitude less than the detected  
28 concentrations in their potential food, plant leaves or seeds.

## 8.3.2 Characterization of Effects

### 8.3.2.1 Aquatic Organisms

Effects on the richness and productivity of fish, aquatic invertebrate, and aquatic plant communities are jointly characterized using the procedures for deriving Tier II water quality values (U.S. Environmental Protection Agency, 1993; Suter and Tsao, 1996). Tier II values are derived where data are not sufficient for deriving ambient water quality criteria (AWQC). The Tier II value derivation procedures account for missing information with approximately 80% confidence.

Test results potentially useful for deriving Tier II values were available for five aquatic species (Table 8-1). In acute tests (48 and 96 h) with sodium perchlorate, using the water flea *Daphnia magna*, the amphipod *Hyalella azteca*, and the fathead minnow *Pimephales promelas*, the endpoints lethality and inhibition were studied. In seven-day tests with a different water flea (*Ceriodaphnia dubia*) and with *P. promelas*, acute lethality was studied in addition to more sensitive endpoints, including the number of offspring per female (*C. dubia*) and growth rate (i.e., body weight; *P. promelas*). A seven-day test with *C. dubia* generally is here used in place of a chronic (i.e., twenty-one day) test because test organisms produce three broods during the test; a seven-day test with *P. promelas* is arguably subchronic because of the test's short duration relative to the organism's lifespan (Suter, 1990; Norberg-King, 1990). A 35-day, early-life-stage (ELS) test with *Pimephales*, here used in place of a chronic test, showed no significant effects on any standard endpoint (survival, growth or biomass) at the highest concentration tested. However, all larvae exposed to perchlorate concentrations, including the lowest concentration of 28 mg/L, exhibited redness and swelling that was not observed in the larvae exposed to the control water. This finding suggests the presence of subtle effects that could be ecologically significant and raises doubt about whether a chronic No-Observed-Effect-Concentration (NOEC) has been adequately determined for this species.

Steps followed in the derivation of the Tier II value for sodium perchlorate are presented in Table 8-2. The secondary acute value (SAV), 5 mg/L (as  $\text{ClO}_4^-$ ), is derived to be protective of 95% of species during short-term exposures with 80% confidence. The secondary chronic value (SCV), 0.6 mg/L (as  $\text{ClO}_4^-$ ), likewise is derived to be protective of 95% of species during long-term exposures with 80% confidence. A sodium chloride control test showed that some of the toxicity to *P. promelas* was potentially attributable to the sodium cation. These tests suggest

**TABLE 8-1. RESULTS OF PERCHLORATE TOXICITY TESTS IN AQUATIC AND TERRESTRIAL SPECIES**

Test Species	Test Description		Endpoints (as mg/L perchlorate in water or mg/kg in soil or sand) <sup>a</sup>				
	Age	Duration	Acute LC <sub>50</sub> (95% CL)	NOEC	LOEC	ChV	IC <sub>25</sub> (95% CL)
Sodium perchlorate (NaClO <sub>4</sub> ) <sup>b</sup> tests (EA Engineering, Science and Technology, Inc., 1998)							
<i>Daphnia magna</i>	<24 hr	Acute (48-hr)	490 (406 - 591)	—	—	—	—
<i>Pimephales promelas</i>	12 - 13 days	Acute (96-hr)	1,655 (1,507 - 1,817)	—	—	—	—
<i>Ceriodaphnia dubia</i>	<24 hr	Chronic (7-day)	66 (40-144) [48-h]	10	33	18.2	17 (8.1 - 20.5)
<i>Pimephales promelas</i>	<24 hr	Subchronic (7-day)	614 (540 - 714) [96-h]	155	280 <sup>c</sup>	208 <sup>c</sup>	212 <sup>c</sup> (175 - 231) <sup>c</sup>
<i>Lactuca sativa</i>	<24 hr	Subchronic (7-day)	614 (540 - 714) [96-h]	155	280 <sup>c</sup>	208 <sup>c</sup>	212 <sup>c</sup> (175 - 231) <sup>c</sup>
<i>Lactuca sativa</i>		Chronic definitive (28-d, sand)		<80	80	<80	41
<i>Lactuca sativa</i>		Chronic definitive (28-d, soil)		40	40	56.6	30
<i>Lactuca sativa</i>		Chronic definitive (28-d, sand)		20	40	28.3	34.3
<i>Eisenia foetida</i>		Acute definitive (7 day/14 day, soil)	4,450/4,450	—	—	—	—
Sodium perchlorate (NaClO <sub>4</sub> ) <sup>b</sup> tests (EA Engineering, Science and Technology, Inc., 2000)							
<i>Pimephales promelas</i>	Embryo	Chronic (35-day, early life stage)	> 490 [96-hr]	> 490 <sup>d</sup> <28 <sup>c</sup>	> 490 <sup>d</sup> 28 <sup>c</sup>	> 490 <sup>d</sup>	> 490 <sup>d</sup> <28 <sup>c</sup>
<i>Hyalella azteca</i>	7 - 14 days	Chronic definitive (28-day)		> 1000	> 1000	> 1000	> 1000

**TABLE 8-1 (cont'd). RESULTS OF PERCHLORATE TOXICITY TESTS IN AQUATIC AND TERRESTRIAL SPECIES**

Test Species	Test Description		Endpoints (as mg/L perchlorate in water or mg/kg in soil or sand) <sup>a</sup>				
	Age	Duration	Acute LC <sub>50</sub> (95% CL)	NOEC	LOEC	ChV	IC <sub>25</sub> (95% CL)
<i>Selenastrum capricornutum</i>	7 days	Acute (96-hr)	—	500	1,200	775	615 (149-1,126)
Ammonium perchlorate (NH <sub>4</sub> ClO <sub>4</sub> ) <sup>f</sup> tests (Block Environmental Services, Inc., 1998)							
<i>Ceriodaphnia dubia</i>	<24 hr <sup>g</sup>	Chronic (6-day)	77.8 [6-days]	9.6	24	15	24
<i>Pimephales promelas</i>	<24 hr <sup>g</sup>	Subchronic (7-day)	270 [7-days]	9.6	96	30	114
Ammonium perchlorate (NH <sub>4</sub> ClO <sub>4</sub> ) <sup>f</sup> tests (Dumont and Bantle, 1998)							
<i>Xenopus</i>	Embryo	96-hr	420 <sup>h</sup>	—	—	—	—
<i>Xenopus</i>	Embryo	96-hr	336 <sup>h</sup>	—	—	—	—

<sup>a</sup>Notation: LC<sub>50</sub> = Concentration lethal to 50% of individuals; NOEC = No-observed-effect concentration; LOEC = Lowest-observed-effect concentration; ChV = Chronic value; IC<sub>25</sub> = Concentration inhibiting a process (e.g., growth, reproduction) by 25%; CL = confidence limits.

<sup>b</sup>Sodium chloride control showed no adverse effects of sodium ion except as indicated. Reported values are based on nominal concentrations.

<sup>c</sup>Sodium chloride control showed significant adverse effects attributable to sodium cation at highest test concentration. Effects observed at this perchlorate concentration may have been caused in part by sodium ion toxicity.

<sup>d</sup>Standard endpoints: survival, growth, biomass

<sup>e</sup>Although there were no effects on standard endpoints at any tested concentration, the investigators reported that all larvae exposed to perchlorate concentrations, including the lowest concentration of 28 mg/L, exhibited redness and swelling, which was not observed in the larvae exposed to the control water.

<sup>f</sup>Ammonium control was not used; adverse effects of ammonium ion cannot be ruled out at all effect concentrations. *C. dubia* and *P. promelas* results are based on measured concentrations. *Xenopus* results are based on nominal concentrations. Confidence limits are not reported.

<sup>g</sup>Not reported; assumed based on standard protocols.

<sup>h</sup>IC<sub>50</sub> for malformations.

**TABLE 8-2. PROCEDURE FOR DERIVING TIER II WATER QUALITY VALUES FOR SODIUM PERCHLORATE**

Step	Value (mg/L ClO <sub>4</sub> <sup>-</sup> )	Rationale
Identify the lowest genus mean acute value (GMAV)	66	Lowest GMAV is for genus <i>Ceriodaphnia</i> (based on <i>C. dubia</i> )
Determine the final acute value factor (FAVF), a factor that compensates for lack of data on a sufficient number of taxonomic groups	13.2 (unitless)	The FAVF varies according to the number of specified taxonomic groups for which GMAVs were available. In this case, two specified values were available (a nonsalmonid fish and a planktonic crustacean), of which one is a daphnid; the value selected from the FAVF table (U.S. Environmental Protection Agency, 1993; Suter and Tsao, 1996) is 13.2.
Calculate the secondary acute value (SAV)	5.0	SAV=GMAV ÷ FAVF = 66 ÷ 13.2
Identify three or more acute-chronic ratios (ACRs), which are ratios of acute value (AV) to chronic value (CV) for a given species (but ratios must be geometrically averaged within any single genus)	3.6, 8.0 (range, <3.4 - >59), 17.9	ACRs can be derived for two species in different genera. For <i>C. dubia</i> : ACR=AV ÷ CV = 66 ÷ 18.2 = 3.6 For <i>P. promelas</i> , two AVs are available. The lower (614) is thrown out because the larval stage is not standard for acute tests; the higher (1,655) is used. Three CVs are available: >490 for standard endpoints, and <28 for redness and swelling, in the 35-d ELS test; and 208 for survival in the 7-d test. There is uncertainty as to the interpretation of the ELS test results; the 7-d result is used and the two results from the ELS are used to determine a range, shown in parentheses: ACR = 1,655 ÷ 208 (range, >490 - <28) = 8.0 (range, <3.4 - >59) No ratio is possible for <i>H. azteca</i> because we are unable to calculate CV due to no acute toxicity. Because a third value is not available, a default value of 17.9 (which provides 80% confidence based on other toxicants) is substituted, according to the Tier II method.
Derive the secondary acute-chronic ratio (SACR)	8.0 (range, <6.0 - >15.6)	The SACR is the geometric mean of the ACRs. (The uncertainty range associated with the <i>P. promelas</i> value is carried through and shown in parentheses.)
Derive the secondary chronic value (SCV)	0.60 (range, <0.32 - >0.83)	SCV=SAV ÷ SACR, 5.0 ÷ 8.0 (The uncertainty range associated with the <i>P. promelas</i> value is carried through and shown in parentheses.)

1 the possibility that if perchlorate were associated with a less toxic cation, the SCV may be lower  
2 than is necessary to protect against perchlorate ion toxicity. Further tests with perchlorate may be  
3 needed to assess potentially less toxic cations.

4 Similar chronic (or subchronic) tests were conducted with ammonium perchlorate  
5 (Table 8-1). Results, expressed as  $\text{ClO}_4^-$ , were very similar for *C. dubia*, but *P. promelas* was  
6 more sensitive to ammonium perchlorate than to sodium perchlorate. However, Tier II values for  
7 ammonium perchlorate are not presented for several reasons, including the lack of ammonium  
8 controls which makes it difficult to determine whether the observed effects were caused by the  
9 perchlorate anion; the lack of acute values for *C. dubia* and *P. Pimephales*; and the fact that the  
10 FETAX (*Xenopus*) test is designed to detect teratogenic potential, and the embryo is not a  
11 particularly sensitive life stage for toxicity. When perchlorate is administered as the ammonium  
12 salt, the ammonium ion concentration expressed on an ammonia-nitrogen (in milligrams of  
13  $\text{NH}_3\text{-N/L}$ ) basis is 14% of the respective perchlorate ion concentration. A Lowest-Observed-  
14 Effect-Concentration (LOEC) for *C. dubia* of 24 mg/L perchlorate (Table 8-1) thus corresponds  
15 to 3.4 mg  $\text{NH}_3\text{-N/L}$ . Based on a species mean chronic value (SMCV) of 13 mg  $\text{NH}_3\text{-N/L}$  for  
16 *C. dubia* exposed to ammonia alone (U.S. Environmental Protection Agency, 1998h), the former  
17 value is probably too low to be responsible for the observed effects<sup>1</sup>. On the other hand, the  
18 LOEC observed with *P. promelas* at 96 mg/L (Table 8-1) corresponds to 14 mg  $\text{NH}_3\text{-N/L}$ , which  
19 exceeds a SMCV of 3.09 mg  $\text{NH}_3\text{-N/L}$  (U.S. Environmental Protection Agency, 1998h).  
20 Therefore, ammonium exposure alone could have been responsible for the effects of ammonium  
21 perchlorate observed in *P. promelas*.

22 The SAV and SCV derived above based on sodium perchlorate are probably protective  
23 even if ammonium perchlorate is the form released, however. Calculated  $\text{NH}_3\text{-N}$  concentrations  
24 corresponding to those values are below the acute and chronic ambient water quality criteria for  
25 ammonia, regardless of pH (U.S. Environmental Protection Agency, 1998h). While SAV and  
26 SCV are not calculated for plants, it appears that there is little perchlorate or ammonium toxicity  
27 to the alga *Selenastrum* in toxicity studies (Table 8-1).

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<sup>1</sup>Ammonia/ammonium toxicity increases as test-water pH increases (U.S. Environmental Protection Agency, 1998e). The value of 13 mg  $\text{NH}_3\text{-N/L}$  corresponds to a pH of 8.0; however, unless the test water pH had exceeded 8.8, it is doubtful that 3.4 mg  $\text{NH}_3\text{-N/L}$  was responsible for the observed effects.

### 8.3.2.2 Terrestrial Organisms

**Plants.** The only available phytotoxicity information comes from 28-day seedling growth tests of lettuce (*Lactuca sativa*) performed in soil and sand cultures with sodium perchlorate (EA Engineering, Science and Technology, Inc., 1998). Although the exposure was to sodium perchlorate solution added to the solid media, the results may be expressed as milligrams per kilogram soil or sand, wet weight, or as milligrams per liter of irrigation solution. Growth was a more sensitive response than germination or survival. The quartile inhibitory wet-weight concentrations (IC<sub>25s</sub>) for growth in soil and sand were 78 mg/kg (293 mg/L) and 41mg/kg (160 mg/L), respectively. Survival was reduced 26% at 420 mg/kg (2,520 mg/L) in soil and 39% at 180 mg/kg (840 mg/L) in sand. To account for interspecies variance, a factor of 10 is applied to the lowest IC<sub>25</sub> to obtain a screening benchmark of 4 mg/kg as a wet-weight concentration in soil (or 16 mg/L as a concentration in irrigation solution).

**Soil Invertebrates.** The only available toxicity data for soil invertebrates is a 14-day acute lethality test of the earthworm (*Eisenia foetida*) performed in artificial soil irrigated with sodium perchlorate. The LC<sub>50</sub> at both 7 and 14 days was 4,450 mg/kg as a wet-weight concentration in soil. No factors or other models are available to extrapolate from that LC<sub>50</sub> to chronic effects on survival, growth, or fecundity or to extrapolate from this species to the soil invertebrate community as a whole. Therefore, the factors applied for aquatic communities in cases where there is only one LC<sub>50</sub> (see Section 8.3.2.1) to obtain a conservative estimate of a soil screening benchmark for soil community effects, are as follows:

$$\begin{aligned}\text{Threshold} &= \text{LC}_{50} \div (\text{factor for interspecies variance} \times \text{acute-chronic ratio}) \\ &= 4,450 \text{ mg/kg} \div (242 \times 18) \\ &= 1 \text{ mg/kg as a wet-weight concentration in soil.}\end{aligned}$$

The equivalent aqueous phase benchmark is 2.8 mg/L. This approach requires the assumptions that the variance among soil species is approximately the same as among aquatic species, and that the distribution of acute-chronic ratios across chemicals is approximately the same for both communities. The interspecies variance factor is the one for a test species that has not been demonstrated to be highly sensitive.

**Herbivores.** The human health risk assessment for perchlorate uses 0.01 mg/kg-day as the LOAEL from which the RfD is derived (Chapter 7). That value is based on perturbations in thyroid and pituitary hormones, thyroid histopathology, and changes in brain morphometry in P0

1 dams on GD21 and F1-generation rat pups on PND5, PND10, and PND22. Because the  
2 representative species for the herbivore endpoint (meadow vole) is a rodent, that value is used  
3 here as well. The population-level implications of this effect are unknown; however, it seems  
4 likely that such effects on the thyroid, pituitary, and brain could diminish survivorship and  
5 fecundity and diminish population production. To account for interspecies variance and LOAEL  
6 to NOAEL extrapolation, an uncertainty factor of 10 is applied to obtain a dietary screening  
7 benchmark for herbivores of 0.001 mg/kg body weight-day, or ~0.01 mg/kg as a wet-weight  
8 concentration in plant tissue (see exposure assumptions in Section 8.3.1.5).  
9

## CHAPTER 9. EVALUATION OF EVIDENCE FOR INDIRECT EXPOSURES

The primary purpose of this document is to derive human and ecological risk estimates for perchlorate. As indicated in Chapter 1, pollution of drinking water supplies is the major concern. Most perchlorate salts are used as solid oxidants or energy boosters in rockets or ordnance; therefore, much of the perchlorate-tainted waterways in the U.S. can be traced to military operations, defense contracting, or associated manufacturing facilities. Figure 1-5 shows that the perchlorate anion could potentially be found in many natural waterways that are used for irrigation or consumed by livestock or wildlife. Thus, it is logical to question whether there are means through which humans might consume perchlorate other than drinking water. This question is compounded by the chemical nature of perchlorate, which grants it long life under typical environmental conditions (Urbansky, 1998; Urbansky and Schock, 1999; Espenson, 2000).

As discussed in Section 7.1.5, once a reference dose for perchlorate is established, any burden posed by exposure routes other than potable water necessarily requires that the contaminant's concentration in a water supply be lowered by an equivalent amount if it is determined to calculate a maximum contaminant level goal (MCLG). A relative source contribution (RSC) of between 20% to 80% is used to adjust the RfD according to the decision framework presented in the EPA's methodology for deriving ambient water quality criteria (U.S. Environmental Protection Agency, 2000).

Because polluted waters are used for irrigation, there are also questions concerning absorption, elimination, and retention in food plants. However, this issue becomes considerably less important if it can be demonstrated that the irrigation water is perchlorate-free. Likewise, there are concerns that animals raised for food would consume plants that had received perchlorate-tainted water. As described in Chapter 8, studies are being conducted to assess the occurrence of perchlorate in biological fluids and tissues of animals and plants in affected regions in recognition of the inter-connectedness of the food chain/food web continuum.

1 While much of the perchlorate problem can be traced to specific sites, a few reports have  
2 suggested that fertilizers could represent another source of perchlorate in the environment (TRC  
3 Environmental Corporation, 1998). These will be addressed in further detail in Section 9.1.1.  
4 Sporadic detection of perchlorate in fertilizers was initially alarming because of the widespread  
5 use of fertilizers in production farming. In addition to the ecological impact, this raised the issue  
6 of assigning responsibility for clean-up costs. Because of the dependence of U.S. agriculture on  
7 chemical commodity fertilizers, it was clear that assessment of any possible role of fertilizers  
8 would require investigation.

9 This chapter summarizes the available data on the potential for exposure through runoff,  
10 erosion, fertilizer, and groundwater movement. Evidence concerning the potential of perchlorate  
11 to contaminate soil, sediment, vegetation, livestock and wildlife is also evaluated.  
12  
13

## 14 **9.1 FERTILIZERS AS SOURCES OF PERCHLORATE SALTS**

### 15 **9.1.1 The Potential Role of Fertilizers**

16 Recently, attention has been drawn to the possible roles of fertilizers as a source of  
17 perchlorate contamination for two reasons. First, perchlorate-tainted agricultural runoff could  
18 lead to pollution of natural waterways used as drinking water sources. Second, there is a  
19 potential for food plants to take up and retain any soluble compounds—including perchlorate  
20 salts—and thus provide an alternate route of exposure. It has long been known that Chile  
21 possesses caliche ores rich in sodium nitrate ( $\text{NaNO}_3$ ) that coincidentally are also a natural source  
22 of perchlorate (Schilt, 1979; Ericksen, 1983). The origin of the sodium perchlorate ( $\text{NaClO}_4$ ) in  
23 the caliche deposits remains an area of debate, but perchlorate is present and can be incorporated  
24 into any products made from the caliche.

25 An examination of two manufacturing lots found perchlorate concentrations below 2 mg/g,  
26 (i.e., < 0.2% w/w) with some lot-to-lot variability (Urbansky et al., 2001). Presently, the caliche-  
27 derived products are sold in the U.S. only by Sociedad Química y Minera (SQM), but other  
28 companies have mineral rights to some Chilean deposits and mines (U.S. Environmental  
29 Protection Agency 2001b) and are potential sources of caliche-derived products. SQM has now  
30 modified its refining process to produce a fertilizer that contains less than 0.1 mg/g (<100  $\mu\text{g/g}$ )

1 of perchlorate, further reducing any environmental release (Lauterbach, 2001). Because nitrate  
2 salts (saltpeters) find use as fertilizers, these natural resources have been mined and refined to  
3 produce commercial fertilizers for domestic use or for export. Chilean nitrates make up about  
4 0.1% of the U.S. market. Most U.S. fertilizers are derived from other raw materials other than  
5 sodium nitrate and ammonium nitrate ( $\text{NH}_4\text{NO}_3$ ), which is often used for purposes similar to  
6  $\text{NaNO}_3$ , is manufactured from methane, nitrogen, and oxygen. There is no evidence that any  
7 ammonium nitrate is derived from Chilean caliche. On account of its low usage, perchlorate  
8 from Chilean nitrates cannot represent a continuing, significant anthropogenic source of  
9 perchlorate nationwide, especially with its lowered perchlorate content.

### 11 **9.1.2 Raw Material Use**

12 As with many commodity chemicals, large scale purchases are dictated by cost of raw  
13 materials, which are in turn influenced by transportation costs. Consequently, proximate (rather  
14 than distant) sources of agricultural chemicals are likely to play the greatest roles in production  
15 farming. Additionally, processing aids (e.g., clays) are likely to be derived from the nearest  
16 sources.

17 Commodity chemicals used as agricultural fertilizers contain fairly high concentrations of  
18 one, or sometimes two, of the primary plant nutrients, expressed as nitrogen (N), phosphorous (as  
19 the oxide  $\text{P}_2\text{O}_5$ ), or potassium (as the oxide  $\text{K}_2\text{O}$ ). Trace metals (e.g., copper) can be applied  
20 separately or along with these primary nutrients on a farm site. The primary phosphorus sources  
21 are ammonium phosphates and triplesuperphosphate (a hydrous calcium phosphate). The  
22 primary potassium source is potassium chloride. A mixture of synthetic and natural components  
23 are used in fertilizer manufacture, described in detail elsewhere (U.S. Environmental Protection  
24 Agency, 2001b).

25 Fertilizer application in production farming is highly dependent on the crop and the native  
26 soil. Agriculture is influenced by climate, weather, topography, soil type, and other factors that  
27 are generally similar within a geographical region; therefore, crops and fertilizer use are also  
28 similar within such a region. For example, the Corn Belt relies heavily on urea and anhydrous  
29 ammonia as nitrogen sources. Ammonium nitrate finds greater use in tobacco farming, and  
30 potassium magnesium sulfate finds more use in milk-producing states. Because all plants require  
31 the same primary nutrients, there is some fertilizer usage to provide these regardless of crop.

1 Local soil conditions also dictate what nutrients should be augmented, causing there to be large  
2 regional variations.

3 Consumer fertilizer (specialty) products can be distributed over large geographical regions  
4 because of the nature of the market. For example, major manufacturers have a limited number of  
5 sites dedicated to blending multiple-nutrient formulations. These products are often sold as  
6 bagged fertilizers through home-improvement centers, nurseries, florists, horticulturists, and  
7 department (or other retail) stores. Unlike agricultural fertilizers, consumer products are usually  
8 multi-nutrient formulations. In addition, trace metals are sometimes incorporated directly into  
9 them. Because the average user will apply only a very small amount of trace metals (or even  
10 primary nutrients) relative to a production farm, it is more economical, more practical, and more  
11 convenient to use multiple-nutrient formulations. Moreover, the average consumer does not have  
12 the wherewithal to disperse careful doses of several single-component fertilizers at the  
13 appropriate times of the growing season.

14 Because fertilizer application on production farms is geographically delimited, there is  
15 considerable interest in knowing which commodity chemicals might contain perchlorate—at  
16 least in terms of dosing. Such information might suggest regions which should be investigated  
17 for perchlorate contamination. Moreover, it will be important to know what crops might be  
18 affected—if any.

### 20 **9.1.3 Fertilizer Analysis Studies**

21 Aside from the analyses of Chilean caliche, there were no studies to suggest that any other  
22 processed fertilizer or raw material might contain perchlorate prior to 1998. That year, the  
23 Ecosystems Research Division of the EPA's National Exposure Research Laboratory (NERL-  
24 ERD) found perchlorate in several samples that were not derived from Chile saltpeter (Susarla,  
25 1999a). This finding was later duplicated by other investigators from the North Carolina State  
26 University College of Agriculture. However, the presence of perchlorate could only be  
27 confirmed in consumer products, not in agricultural fertilizers. Moreover, subsequent analyses  
28 of bags of the same materials acquired at a later date (likely from different manufacturing lots)  
29 did not show perchlorate (Susarla et al., 2000). The choice of fertilizers did not account for the  
30 possibility that the same raw materials must have been used in a variety of products at a point in  
31 time. Additionally, a few major companies are responsible for making a large number of

1 products under several brand names. Furthermore, some companies rely on toll manufacturing  
2 so that the products are actually made by another company to meet a specific formulation.  
3 Accordingly, an error or contamination associated with one raw material could affect a variety of  
4 products without regard to company or application.

5 Perchlorate was found in six of eight lawn and garden fertilizers tested, according to a  
6 report provided to the EPA by the U.S. Air Force Materiel Command (TRC, 1998). However,  
7 the report's authors were careful to point out that the results were obtained from a single  
8 sampling event and that raw material usage was variable; therefore, no general conclusions could  
9 be drawn. These qualifiers are consistent with the limitations enumerated above, but they do  
10 point towards a temporal contamination of some products.

11 This study helped bring to light a number of important issues for trace analysis of  
12 fertilizers. First, most of the research on determining perchlorate to that time had been focused  
13 on either finished potable water or raw source water (Urbansky, 2000). Second, fertilizers are  
14 considerably more complicated matrixes than dilute water solutions. Third, a solid fertilizer is  
15 not a homogeneous substance. In particular, multi-component formulations used as lawn and  
16 garden fertilizers are macroscopically heterogeneous and it is possible to sort out the particles  
17 visually. Thus, representative subsampling becomes a key issue. Fourth, the effectiveness of  
18 leaching out any perchlorate ion into an aqueous phase was unknown. Fifth, the products chosen  
19 did not reflect the chemical fertilizers used for production farming, but rather the ingredients  
20 used for lawn and garden fertilizers during a specific time period.

21 Around the same time, the U.S. Air Force Research Laboratories (AFRL) performed a  
22 study to assess interlaboratory corroboration; that is, the ability of different labs to analyze the  
23 same sample and get the same result (AFRL, 1999; Eldridge, 2000). A variety of techniques  
24 performed by multiple laboratories showed acceptable agreement on the concentrations of  
25 perchlorate in solutions prepared from the purchased products. Several limitations (such as  
26 product choice and sampling difficulties with heterogeneous solid products) made it impossible  
27 to gain an understanding of agricultural fertilizer use, and the AFRL intentionally restricted its  
28 use of the data to evaluating interlaboratory agreement. However, data from the AFRL study was  
29 sufficient to confirm independently that some lawn and garden fertilizer products did contain  
30 perchlorate during a certain period of time.

1 Subsequently, the Water Supply and Water Resources Division of the EPA's National Risk  
2 Management Research Laboratory (NRMRL) conducted its own survey of fertilizers in a  
3 collaboration with the Oak Ridge National Laboratory (Urbansky et al., 2000a; Urbansky et al.,  
4 2000b). In addition to a variety of products purchased from retailers, products were purchased  
5 from farming supply stores (e.g., 50-lb bags of urea or ammonium nitrate) in Indiana, Ohio,  
6 Kentucky, Pennsylvania, and Tennessee. In addition, commodity chemical samples were  
7 collected from local distributors in Ohio and Indiana. These included urea, potassium chloride,  
8 ammonium monohydrogen phosphate, and granular triplesuperphosphate, among others.  
9 Samples were leached or dissolved and subjected to complexation electrospray ionization mass  
10 spectrometry (cESI-MS) or ion chromatography (IC). Of 45 tested products, the only ones that  
11 were found to contain any perchlorate were those based on Chile saltpeter. While this study was  
12 the first to include the same products used on agricultural production farms, it did not address the  
13 issues of sampling, product inhomogeneity, or geographical source variation.

14 In an effort to better address sampling, raw material usage, and other issues, the EPA  
15 undertook an additional study of fertilizers. The project was divided into two phases, the first  
16 part of which evaluated the testing laboratories for their ability to identify and quantitate  
17 perchlorate in a fertilizer matrix. In the second phase, samples gathered under the supervision of  
18 state agricultural agents were homogenized and sent to the laboratories for analysis using a  
19 method established by the EPA (U.S. Environmental Protection Agency, 2001a). This  
20 investigation was the most thorough in terms of including agriculturally relevant materials used  
21 to manufacture a wide variety of specialty products or sold directly to farmers. It also spanned all  
22 major national suppliers of these products. Although it reflected only a temporal snapshot, as  
23 had all of the other studies, the survey of fertilizers incorporated the greatest number of unique  
24 samples, quality control tests, and standardized practices, as well as other design improvements.  
25 Four laboratories analyzed all of the materials, and some samples were analyzed by additional  
26 laboratories. No other materials were found to contain perchlorate at measurable concentrations,  
27 and the EPA concluded that the only clearly identifiable fertilizer source of perchlorate was  
28 caliche. The data collected in this endeavor were additionally used to evaluate laboratory  
29 performance and further validate the method (Urbansky and Collette, 2001). A set of archived  
30 samples of all the Phase 2 materials was analyzed while evaluating an alternate ion

1 chromatographic column and independently verified all of the results reported in U.S.  
2 Environmental Protection Agency (2001a) (DeBorba and Urbansky, 2001).

3 The findings reported in U.S. Environmental Protection Agency (2001a) are the most  
4 comprehensive in terms of the types of materials tested, the manufacturers, the number of  
5 laboratories analyzing each field sample of material, and the quality control checks. In these  
6 regards, it represents our best understanding of fertilizers in terms of perchlorate content. While  
7 the presence of perchlorate in the materials gathered in late 1998 through early 1999 remains  
8 enigmatic, there is no evidence to support the concern that there is ongoing or routine perchlorate  
9 contamination in the U.S. fertilizer supply. Reports in 1999 may have reflected the temporal  
10 contamination of one or more raw materials or merely an error in manufacture. Based on the  
11 studies reported to date (Collette and Williams, 2000; Gu et al., 2000; Urbansky et al., 2000a;  
12 Urbansky et al., 2000b; Robarge et al., 2000; EPA, 2001b; Williams et al., 2001; DeBorba and  
13 Urbansky, 2001), there is a consensus among researchers from the EPA, the fertilizer industry,  
14 and other federal and state laboratories that currently used fertilizers are negligible contributors  
15 to environmental perchlorate contamination. Even imported Chile saltpeter or products derived  
16 from it contribute minimally due to their low use and low perchlorate content. Consequently, the  
17 EPA has concluded that further investigation is unwarranted (U.S. Environmental Protection  
18 Agency, 2001b).

19 IMC-Agrico, a major North American fertilizer manufacturer, has instituted its own  
20 monitoring program for its raw materials and products as a result of continuing interest among  
21 the scientific, industrial, and regulatory communities. These products include various potassium  
22 ores (langbeinite, sylvinit), potash-based products (potassium chloride, potassium sulfate and  
23 potassium magnesium sulfate), and phosphate products (ammonium monohydrogen phosphate,  
24 ammonium dihydrogen phosphate and granular triplesuperphosphate). After more than 100  
25 analyses using the latest method (EPA, 2001a), IMC reported to the EPA that no perchlorate was  
26 detected in any of the materials it tested during a period spanning nearly three years. In addition,  
27 IMC states that it has analyzed Magruder check samples for perchlorate. The Magruder check  
28 sample program is jointly administered by the Association of American Plant Food Control  
29 Officials and The Fertilizer Institute; it bears the name of a chemist from the F. S. Royster Guano  
30 Company named E. W. Magruder, who initiated the program in 1922. The program selects,  
31 prepares, and distributes samples of various materials and finished products to subscribing

1 laboratories and then collects and analyzes the data. Magruder samples reflect monthly  
2 snapshots taken from the entire fertilizer industry. Perchlorate has not been detected in any IMC  
3 product or any of 16 Magruder samples, according to IMC (personal communication from  
4 William L. Hall).

#### 6 **9.1.4 Complicating Factors**

7 It is worth pointing out at the U.S. Geological Survey (USGS) and Air Force Research  
8 Laboratories have found perchlorate in isolated samples of sylvite taken from New Mexico  
9 (Harvey et al., 1999). The USGS is engaged in additional sampling of North American mining  
10 sites in order to assess whether there are natural mineral deposits of potassium perchlorate in  
11 sylvite or sylvinite. Because little is known about the mechanisms of perchlorate formation in  
12 the natural environment (which are assumed to be meteorological in nature), it is not clear  
13 whether these findings represent a low-level background to be expected in evaporite mineral  
14 deposits or not. Nonetheless, perchlorate has not been detected in any samples of agricultural  
15 grade potassium chloride (0-0-62 or 0-0-60) taken under the direction of the EPA or by IMC-  
16 Agrico. Accordingly, it appears that this mineral commodity does not suffer from inclusions of  
17 perchlorate salts to any environmentally relevant extent.

18 Decades ago, ammonium nitrate was prepared from Chilean sodium nitrate by ion  
19 exchange rather than by gaseous reactants. It appears that cost began to prohibit this practice for  
20 fertilizer-grade ammonium nitrate. Nonetheless, some facilities appear to have continued the  
21 practice for explosives-grade ammonium nitrate that was used for blasting in mining operations  
22 throughout the American Southwest. It is unlikely that reliable data can be obtained from more  
23 than the past 10 years or so. Prior to the establishment of nitric acid and ammonia factories,  
24 natural saltpeters played significant roles in American agriculture. Thus, there may be  
25 contamination of groundwater in regions where these materials were used historically. The lack  
26 of information concerning natural attenuation, as well as a limited knowledge of hydrogeology,  
27 makes it difficult to determine where and how such problem sites might be found. For this  
28 reason, monitoring for perchlorate under the EPA's Unregulated Contaminant Monitoring Rule  
29 can be expected to provide some of the most useful information.

30 Even though perchlorate was identified in some fertilizer products and was presumably  
31 introduced through a contaminated raw material, this incident appears to have been entirely

1 isolated. Furthermore, awareness within the fertilizer industry and the environmental community  
2 is now heightened to the point that it appears unlikely to happen again.

## 3 4 5 **9.2 MONITORING FATE AND TRANSPORT IN LIVING PLANTS**

6 Due to the reported occurrence of perchlorate in certain water resources and in certain  
7 fertilizer products, several groups have begun to address the extent and significance of  
8 perchlorate uptake by plants. For example, if produce is grown using irrigation water tainted  
9 with perchlorate, or if agricultural soil is amended with perchlorate-tainted fertilizer, this might  
10 constitute a route of human exposure if perchlorate is taken up and retained in the edible parts of  
11 produce plants. The possibility of a relevant exposure route would be increased if perchlorate  
12 was found to bioaccumulate and if it was shown to survive the various processes that edible  
13 plants undergo before being consumed. Unfortunately, experimental results that definitively  
14 gauge the extent of risk from this route of exposure have not yet been published. However, some  
15 progress toward this goal has been made.

### 16 17 **9.2.1 Difficulties in Analyzing Plant Tissues and Other Environmental** 18 **Samples for Perchlorate**

19 One problem that has delayed accurate and definitive studies of perchlorate uptake by  
20 edible plants is the difficulty of analyzing for perchlorate in plant materials. Ion chromatography  
21 is currently the recommended method for routine analysis of inorganic ions such as perchlorate.  
22 It is a sensitive, reliable, and easily-implemented technique when perchlorate occurs in a matrix  
23 that has a relatively low level of total dissolved solids (TDS). Unfortunately, extracts of plant  
24 materials contain high concentrations of TDS, inorganic ions, amino acids, sugars, fatty acids,  
25 and nucleotides—all of which contribute to the ionic strength of the sample (Ellington and  
26 Evans, 2000). In such matrices with high TDS/ionic strength, other ions can overwhelm the  
27 conductivity detector and effectively mask the signal from perchlorate. Ion chromatography is  
28 not unique in this regard. Other techniques and methods suitable for reasonably dilute drinking  
29 water matrices (Urbansky et al., 2000c; Magnuson et al., 2000a, b; Urbansky et al., 1999;  
30 Urbansky and Magunson, 2000) cannot be readily applied to fertilizers or botanical and  
31 physiological fluids. The problems of trace ionic analysis have led to the development of other

1 methods that rely on expensive instrumentation, but are not generally available, such as  
2 asymmetric waveform ion mobility mass spectrometry (Handy et al., 2000; Ells et al., 2000) or  
3 tandem mass-spec (MS-MS) systems (Koester et al., 2000).

4 Recently Ellington and Evans (2000) have reported an IC-based method using an enhanced  
5 clean-up procedure for the quantitation of perchlorate in plant materials that greatly reduces  
6 interferences from dissolved matter. The minimum reporting level (MRL) of perchlorate in  
7 lettuce and tomato was found to be approximately 250  $\mu\text{g/g}$  on a wet mass basis. Lettuce and  
8 tomato were chosen as representative plants because they are considered high priority candidates  
9 for screening foodstuffs (Ellington and Evans, 2000). Perchlorate was spiked into the extraction  
10 water for one half of the duplicate freeze-dried samples, while one half were extracted with pure  
11 water. In the absence of other ions, some perchlorate is lost to the alumina used for the clean-up;  
12 however, this should not impact application of the method to plant material because most  
13 extracts have sufficient ionic strength. Note that perchlorate was not detected in any produce,  
14 nor was the method applied to any edible plants that were grown with intentional exposure to  
15 perchlorate.

## 16 17 **9.2.2 Ecological Transport**

18 In the laboratory setting, some plant species will absorb perchlorate when exposed to  
19 contaminated irrigation water. Uptake by plants has been explored for possible use in  
20 phytoremediation (Nzengung, 1999; 2000). Some investigators have speculated that bacteria are  
21 responsible for this phenomenon in plants. Perchlorate-reducing monera have been identified by  
22 several laboratories, and cultured from a variety of sources (including Las Vegas Wash  
23 sediments, food processing sludge, soils, and sewage sludge); (Logan, 1998; Coates et al., 1999;  
24 Coates et al., 2000; Kim and Logan, 2001; Wu et al., 2001; Logan, 2001). Recent work showing  
25 perchlorate reduction in saline solution suggests that attenuation may be possible even in briny  
26 locations (e.g., the Las Vegas Wash) or in fertilizer-laden farm runoff (Logan et al., 2001; Okeke  
27 et al., 2001). This suggests that perchlorate-reducing bacteria are present at significant levels in  
28 the environment. On the other hand, the bacteria isolated thus far prefer oxygen over nitrate over  
29 perchlorate. In order to for perchlorate reduction to occur, the water must be anoxic and all of  
30 the nitrate must have been consumed. Moreover, these bacterial cultures require a suitably moist  
31 environment; arid soils or regions with low rainfall may not sustain their growth. Natural

1 attenuation probably varies around the nation, depending on local factors. Accordingly, it is not  
2 possible to draw any meaningful conclusions about the ecological impact of fertilizers that  
3 contain perchlorate, for they may or may not be applied in areas where this type of bacterial  
4 degradation can occur.

5 Another factor that has prevented the early materialization of definitive data on risk from  
6 perchlorate in edible plants is that many researchers who have addressed plant uptake of  
7 perchlorate are primarily interested in other aspects of the problem. For example, Ellington et al.  
8 (2001) have applied the optimized IC-based method described above first to the analysis of  
9 perchlorate in tobacco plants and tobacco products. Tobacco was chosen because it is grown in  
10 some locations in soils amended with Chile saltpeter.

11 Ellington and Evans (2000) obtained green (uncured) tobacco leaves from the Coastal Plain  
12 Experiment Station (CPES) in Tifton, GA in late July 1999. The plants grew in soil that had  
13 been amended with two fertilizer products, one of which was Chile saltpeter. The perchlorate  
14 level in the Chile saltpeter was 1.5 mg/g, consistent with contemporaneous reports (Urbansky  
15 et al., 2001; personal communication from W.P. Robarge). Perchlorate was also found in a  
16 6-6-18 plant food that had been applied to the same soil. While 3% of the nitrogen was from  
17 nitrate, the perchlorate concentration was only 36  $\mu\text{g/g}$ ; whereas, based on the typical perchlorate  
18 content in Chile saltpeter, it should have been about eight times larger if all of the nitrate were  
19 from Chile saltpeter. This suggests that synthetic nitrates were also part of the fertilizer's  
20 constitution. Perchlorate concentrations in the dried tissue varied from 12.5 to 165  $\mu\text{g/g}$ ,  
21 depending on the portion of the leaf examined and the curing process employed. Soil samples  
22 leached with deionized water contained 0.3  $\mu\text{g/g}$  on a dry weight basis. EPA researchers also  
23 analyzed several off-the-shelf cigarettes (2 brands), cigars (1 brand), and chewing tobacco  
24 (7 brands) and found perchlorate concentrations ranging from 0.4 to 21.5  $\mu\text{g/g}$  (undried), and  
25 only one product that contained none (Wolfe et al., 1999; Ellington et al., 2001). They confirmed  
26 the IC results by chlorine NMR spectrometry and capillary electrophoresis. Collectively, these  
27 observations argue that tobacco plants can take up perchlorate from perchlorate-contaminated  
28 fertilizers via the soil. Furthermore, they indicate the importance of investigating whether crop  
29 plants can accumulate perchlorate in their edible portions and whether any contamination can  
30 persist through the processing that precedes consumption.

1           Several groups have looked at the accumulation of perchlorate in various inedible plants as  
2 a potential means of fate and remediation. Perchlorate-tainted water from the Las Vegas Wash  
3 enters Lake Mead and the Colorado River and therefore has the potential to affect the potable  
4 water of many people as well as the irrigation water used for much of the lettuce produced in the  
5 U.S. Salt cedar (*Tamarix ramosissima*) is an invasive woody plant that grows prolifically in and  
6 around the Las Vegas Wash. Salt cedar consumes and transpires an enormous amount of water  
7 when it is actively growing. Furthermore, it accumulates and secretes salt. For these reasons,  
8 Urbansky et al., (2000d) have analyzed samples of salt cedar that were taken from the Las Vegas  
9 Wash. They found perchlorate at 5-6  $\mu\text{g/g}$  in dry twigs extending above the water and 300  $\mu\text{g/g}$   
10 in stalks immersed in the water from a plant growing in a contaminated stream, suggesting that  
11 salt cedar plays a role in the ecological distribution of perchlorate.

12           Still others have investigated plant uptake with the specific goal of identifying remediation  
13 strategies for perchlorate. The biodegradation of perchlorate in woody plants has been  
14 investigated as a means of phytoremediation (Nzengung et al., 1999; Nzengung and Wang,  
15 2000). Nzengung et al. (1999) and Nzengung and Wang (2000) found that willow trees (genus  
16 *Salix*) were able to decontaminate aqueous solutions containing 10–100 mg/L of perchlorate to  
17 below the method detection limit of 2  $\mu\text{g/L}$  and suggest that two distinct phytoprocesses were at  
18 work in their studies. Specifically, they observe evidence for rhizodegradation from the exudates  
19 released from the plant, and—more importantly from the standpoint of relevance for food safety  
20 issues—they see accumulation in branches and leaves. Only about 11% of the perchlorate spiked  
21 into the water in which the trees were grown was found to phytodegrade in 26 days. The  
22 majority of perchlorate that was removed from solution after 26 days was found in the leaves.  
23 Longer term experiments suggest that the perchlorate did not accumulate in the leaves, but was  
24 very slowly transformed there as well. Generally, the perchlorate level in the leaves increased to  
25 a maximum before decreasing to undetectable levels after perchlorate was completely removed  
26 from solution. Nzengung et al. assumed that the phytodegradation pathway of perchlorate leads  
27 to chloride. Moreover, Nzengung et al. explored the role of other anions in the removal of  
28 perchlorate in solution. They found that the perchlorate removal rate was decreased as the  $\text{NO}_3^-$   
29 level was increased. This was attributed to competing reactions in which both anions were  
30 utilized as electron acceptors. Clearly this has relevance for the food safety issue and should be  
31 investigated further. For example, the type of fertilizer used in food crop production may have

1 an effect on the degree to which perchlorate is taken up, depending on the major components of  
2 the fertilizer.

3 Susarla and coworkers have published results of their investigations on transformation of  
4 perchlorate by a wide range of plant types. For example, Susarla et al. (1999b,c) have performed  
5 screening studies to determine what species might show potential for further investigations of  
6 perchlorate phytoremediation. Thirteen vascular plant species were selected for evaluation in  
7 these preliminary experiments. This included four tree species, four herbaceous wetland species,  
8 four aquatic species, and one herbaceous upland species. Laboratory-scale experiments were  
9 conducted in order to, among other things, evaluate the ability of these plants to remove  
10 perchlorate from solution, evaluate the role of nutrients on perchlorate removal, and determine  
11 the fate of perchlorate removed from solution (e.g., plant tissue distribution, accumulation versus  
12 breakdown). Each of these topics is indirectly relevant to the issue of uptake by edible plants.

13 For all of these experiments, perchlorate concentrations of 0.2, 2.0 and 20 mg/L were tested  
14 in aqueous and sand treatments for ten-day periods. Perchlorate was found to be depleted from  
15 solution in the presence of all but two species. Susarla et al. (1999a,c) used a system of five  
16 categories to classify the performance of the species based on the degree to which they depleted  
17 the solution. None of the trees tested were included in the highest category of performance, but  
18 some of the wetland and aquatic plants were. Plant tissue (e.g., roots, stems, leaves) were  
19 analyzed from samples that demonstrated the maximum drop in perchlorate concentration.  
20 Susarla et al. (1999a,c) report perchlorate, or some transformation metabolite (chlorate, chlorite,  
21 chloride), in all tissues analyzed. Results of these studies suggested significant influences on  
22 depletion of perchlorate from, among other things, growth substrate (sand versus aqueous  
23 treatment), the level of nutrients, stage of plant maturity, and the presence of other ions. All of  
24 these influences should prove to be valuable insights when considering the uptake of perchlorate  
25 by edible plants. Based on screening studies, additional studies focused on the  
26 phytotransformation of perchlorate by the aquatic plant parrot-feather (*Myriophyllum*  
27 *aquaticum*); (Susarla et al., 1999b; Susarla et al., 1999c).

28 Tobacco is one crop for which the use of Chilean nitrate salts can be documented in some  
29 locations. In northern Kentucky, these products are primarily used for seedling beddings rather  
30 than fertilizing fields; for various reasons, ammonium nitrate is preferred by many farmers in  
31 Kentucky. Such preferences vary throughout tobacco-producing states and regions, however.

1 Data on application of Chile saltpeter is sparse, and it is not possible to estimate the ecological  
2 impact in any meaningful way. There can be no question that at least some vascular plants  
3 absorb perchlorate from their local environments. Furthermore, perchlorate has been found in a  
4 number of plants and animals living in contaminated environs (Smith et al., 2001). An obvious  
5 concern raised by finding measurable perchlorate concentrations in plant tissues is whether this  
6 ion can affect food crops and what factors might influence its uptake and accumulations. These  
7 issues shall be considered next.

### 9 **9.2.3 Extrapolating to Food Plants**

10 Because so much U.S. produce is fertilized with perchlorate-free chemical commodities,  
11 the risk from exposures via fertilizers is small. Some crops (e.g., corn, wheat, and rice) are  
12 fertilized with materials that are unquestionably perchlorate-free. Additionally, there is no reason  
13 to suspect any perchlorate associated with growing grains. However, the risk of exposure  
14 resulting from irrigation with perchlorate-tainted water in the American Southwest is unknown.  
15 At present, there are no efforts to test fruits and vegetables for perchlorate. Many of the studies  
16 on uptake by plants have been based on concentrations higher than those encountered in  
17 irrigation water. Furthermore, some products derived from Chile saltpeter are known to be  
18 among those used on California citrus crops.

19 One of the few studies of perchlorate uptake by edible plants is the ongoing work of  
20 Hutchinson and coworkers with lettuce grown in a greenhouse with perchlorate-tainted irrigation  
21 water. Lettuce is of particular importance for assessing the risk of perchlorate to the food supply  
22 since much of the lettuce produced in the U.S. is irrigated by water that is fed by the Las Vegas  
23 Wash, which is contaminated with perchlorate. Also, lettuce has a high water content and  
24 virtually the entire above-ground plant is consumed without cooking or processing. These  
25 characteristics would present a potential risk if lettuce efficiently accumulates perchlorate.  
26 Hutchinson and coworkers are irrigating lettuce plants with five different concentrations of  
27 perchlorate (0.1, 0.5, 1.0, 5.0, and 10.0  $\mu\text{g/L}$ ) for a period of 90 days following planting.  
28 At various intervals of time they divide the plants into green tissue and root samples and analyze  
29 each sample for perchlorate using an analytical method adapted from Ellington and Evans  
30 (2000). Their results show an accumulation of perchlorate into the green tissue. The level of  
31 perchlorate built up steadily over the first 50–60 days of the experiments, then generally leveled

1 off. At about 50 days into the experiment, the lettuce irrigated with 10.0 ppm perchlorate  
2 exhibits a perchlorate content of about 3 mg/g on a lettuce dry matter basis. Since lettuce is  
3 about 90% water, this would amount to about 0.300 mg/g on a wet weight basis. The amount of  
4 perchlorate detected in the leaves is generally linear with dosing level for a given day.  
5 Experiments are underway to determine whether lettuce has the capability to degrade perchlorate  
6 if the supply of the contaminant is stopped. However, this determination is frustrated by the fact  
7 that lettuce continues to grow. Therefore, a decline in concentration (e.g., expressed in mg/g)  
8 does not adequately reflect the situation. The preliminary results from these studies (Hutchinson  
9 et al., 2000) suggest that, when complete, they will constitute considerable progress on the issue  
10 of exposure to perchlorate from edible plants.

11 Even if many food plants can be shown to absorb and retain perchlorate, the primary source  
12 of the contaminant is irrigation water polluted from defense-related activities. Because these  
13 activities are reasonably localized geographically, most of the country's agricultural products  
14 should be perchlorate-free, e.g., corn, wheat, rice, milk. On the other hand, some types of  
15 produce are supplied almost entirely by regions dependent on contaminated irrigation water.  
16 Therefore, these sites represent possible exposure routes for most of the nation via foods such as  
17 lettuce.

18 Historically, much of the emphasis on fertilizer pollution from agricultural runoff has been  
19 on fertilizers applied to the soil. However, potassium nitrate is usually applied to the leaves of  
20 citrus trees when a potassium deficiency is found by analyzing leaf tissue. Such foliar  
21 application would not necessarily contribute significantly to runoff type pollution of waterways,  
22 but could lead to the absorption of contaminants through the leaves and wood. There are no  
23 reliable data on the sources of potassium nitrate used for citrus crops. While it is known that  
24 absorption of anions similar to perchlorate (e.g., pertechnetate) are affected by the ionic strength  
25 and composition of the surrounding solution, little is known about the factors that influence  
26 perchlorate influx via roots or leaves. In addition, the fate of absorbed perchlorate in the plants is  
27 also unknown. It may be that xylem-supplied tissues, such as leaves, are the final repository  
28 rather than phloem-supplied tissues, such as fruits.

29 These issues and more have begun to be examined by the EPA, but there are many  
30 unknowns (U.S. Environmental Protection Agency, 2001b). Until such time as quantitative  
31 studies are performed on various species to determine what factors influence the absorption,

1 accumulation, and distribution of perchlorate in plants, it is not possible to estimate whether  
2 foods can serve as meaningful contribution to the body burden or to the risk posed to humans  
3 from perchlorate contamination. Even if they do, there is considerable peace of mind in knowing  
4 that fertilizers and water supplies are generally not providing any perchlorate to the plants in the  
5 first place. Consequently, only a small number of foods are worth considering for further study.  
6 On the other hand, it is not known to what extent other countries rely on natural saltpeters to  
7 fertilize food crops. Moreover, it is not known whether fruits and vegetables absorb and retain  
8 the perchlorate ion. Therefore, it is not possible to say whether fruits and vegetables grown  
9 outside the U.S. serve as a possible exposure routes at this time. Depending on the season,  
10 imported oranges, apples, and grapes and their juices are consumed throughout the U.S.

11 Because there are no data on perchlorate in imported produce, no data on perchlorate in  
12 U.S. produce, and no data from controlled laboratory experiments on uptake in fruit crops, it is  
13 impossible to assess whether these foods can contribute to perchlorate consumption in humans or  
14 whether drinking water constitutes the entire body burden. However, the available information  
15 on fertilizers and irrigation water suggests that foods do not contribute to the body burden. At  
16 the present time, the available data point towards drinking water as the principal exposure  
17 pathway for humans.

### 18 19 20 **9.3 SUMMARY**

21 Despite some initial findings implicating fertilizers as a source of perchlorate, more  
22 thorough and better designed studies that were conducted subsequently have not found this to be  
23 the case. Current fertilizer manufacturing practices and raw material sources make it unlikely  
24 that perchlorate contamination could occur widely and without discovery. While some plants  
25 may absorb or even accumulate perchlorate in specific tissues, there are many unknowns with  
26 regard to the edible portions of nutritionally and agriculturally important crops. Many factors  
27 influence transport of ions, and current understandings of plant physiology and botany suggest  
28 perchlorate uptake would be reduced as a result of such factors. Even if perchlorate uptake  
29 occurred in some food crops, perchlorate contamination is localized geographically outside of  
30 major agricultural regions, minimizing possibility of uptake in edible produce. While  
31 perchlorate-tainted irrigation water may be a source available for uptake of perchlorate by plants,

1 this is again localized, and has not been proven to occur at the concentrations of perchlorate that  
2 are observed environmentally. Difficulties in analyzing many plant or animal tissues originally  
3 were obstacles to executing appropriate studies, but these problems have generally been solved.  
4 Ideally, more data would be available on food plants, but current evidence suggests that drinking  
5 water is the primary exposure pathway to perchlorate for humans.  
6

# 10. MAJOR RISK CHARACTERIZATION CONCLUSIONS

## 10.1 HUMAN HEALTH

This section summarizes major findings regarding human health presented in Chapters 1, 2, 3, 4, 5, and 7.

### 10.1.1 Hazard Potential

Perchlorate is an anion that originates as a contaminant in ground and surface waters from the dissolution of ammonium, potassium, magnesium, or sodium salts. Ammonium perchlorate is the oxidizer and primary ingredient in solid propellant for rocket motors. Perchlorate salts also are used on a large scale as a component of air bag inflators and in the manufacture of pyrotechnics and explosives. Solid rocket inventories are growing at a significant rate as systems reach the end of their service life: the solid rocket disposal inventory is expected to be over 164 million lb by the year 2005. Because the accepted method for removal and recovery of solid rocket propellant is high-pressure water washout, a large amount of aqueous solution containing ammonium perchlorate is generated. A number of locations where perchlorate has been detected in groundwater or surface waters are in areas associated with the development, testing, or manufacturing of aerospace materials. Perchlorate contamination also occurs when explosives are used extensively, e.g., open burn/open detonation operations and some mining activities.

Perchlorate is rapidly absorbed from the gastrointestinal tract, whereas dermal and inhalation exposures are not expected to be significant exposure routes for the general public. The known mode of action for perchlorate is that it acts as a competitive inhibitor of active iodide uptake by the *sodium (Na<sup>+</sup>)-iodide (I) symporter (NIS)* in most mammals, including humans, laboratory test species, and wildlife. This decrease in intrathyroidal iodide results in a decreased production of T3 and T4 thyroid hormones. Decrements in thyroid hormones can cause permanent neurodevelopmental deficits and impair adult organisms as well. A decrease in thyroid hormones can also potentially perturb the hypothalamic-pituitary-thyroid axis to increase the pituitary's production of TSH and, consequently, stimulate the thyroid to increase production

1 of thyroid hormone in an attempt to compensate. Prolonged stimulation of the thyroid by TSH  
2 may result in thyroid neoplasia, particularly in rodents known to be sensitive. Tumors have  
3 occurred in rats dosed with high levels of perchlorate for long periods and at much lower doses in  
4 relatively young adult animals (19 weeks) dosed *in utero* and during development. These  
5 findings have raised concerns about the *in utero* imprinting of the regulatory system responsible  
6 for controlling thyroid hormone economy.

7 The target tissue for systemic effects of perchlorate has been identified as the thyroid. The  
8 key event of its mode of action is iodide uptake inhibition at the NIS. Changes in the thyroid  
9 hormone homeostasis result in histopathological changes in the thyroid, including: colloid  
10 depletion, follicular hypertrophy, follicular hyperplasia, and decrease in follicular lumen size.  
11 If perchlorate exposure is stopped, the thyroid histopathological effects have been shown to be  
12 reversible after exposures as long as 90-days in rats, but incomplete recovery of thyroid  
13 hormones occurs in this same time period. There are also some case studies in humans treated  
14 therapeutically with perchlorate that indicate reversibility of thyroid hormone changes after years  
15 of exposure.

16 Other potentially adverse and permanent effects from decreased thyroid hormone include  
17 effects during development *in utero* and early growth, particularly effects on the nervous system  
18 if the pregnant mother was hypothyroxinemic or hypothyroid. Laboratory animal assays  
19 performed in response to recommendations made at the peer review in 1999 and as part of the  
20 perchlorate testing strategy confirmed neurodevelopmental effects observed in previous studies.  
21 Changes in brain morphometry and motor activity were observed. The potential for major  
22 disturbances in thyroid hormone homeostasis to disturb reproductive capacity or to induce  
23 immune effects also exists. The ability of perchlorate to cause contact hypersensitivity is  
24 suggested but remains not well characterized. Finally, a remarkable conservation of the thyroid  
25 hormone regulatory system has been demonstrated across species. Inhibition of iodide uptake by  
26 the NIS has been shown in pharmacokinetic studies to be very similar across species, including  
27 humans.

### 29 **10.1.2 Dose Response**

30 The revised RfD is based on an assessment that reviewed a set of studies that were  
31 developed to explicitly evaluate these potential toxicities. The quantitative estimate of risk is

1 based on laboratory animal data because there are no good observational epidemiological data  
2 concerning human subjects representative of the critical sensitive populations (hypothyroxinemic  
3 pregnant women or children) or that have evaluated neurodevelopmental outcomes; nor have  
4 adequate clinical studies been performed. A harmonized approach was proposed based on the  
5 key event of iodide inhibition and its relationship to disturbances in the hypothalamic-pituitary-  
6 thyroid axis as evidenced by effects on thyroid and pituitary hormones, thyroid histopathology,  
7 and brain morphometry. Using these precursor lesions as the basis for the point-of-departure is  
8 considered to be protective for cancer development as well as for neurodevelopmental sequelae.

9 The database supported a point-of-departure for the RfD deviation at 0.01 mg/kg-day based  
10 on changes in maternal thyroid and pituitary hormones and on changes in the brain morphometry  
11 and thyroid and pituitary hormones of fetal and neonatal pups. A composite uncertainty factor of  
12 300 was applied in the derivation. An adjustment also was made for administration of  
13 perchlorate as ammonium perchlorate. The RfD is for perchlorate as the anion because that is  
14 what is sampled and analyzed in environmental media and because the salts of perchlorate  
15 readily dissolve. Uncertainty factors were applied for intrahuman extrapolation, the use of a  
16 LOAEL, concern regarding the lack of studies of longer duration and database deficiencies.  
17 Confidence in the study, the database, and the RfD is rated as medium. A major uncertainty is  
18 the sensitivity that the screening neurodevelopmental studies provide to protect against  
19 neuropsychological deficits of exposures that might occur within critical developmental windows  
20 or in susceptible human populations.

21 The daily perchlorate exposure to the human population that is likely to be without  
22 appreciable risk of either cancer or noncancer toxicity during a lifetime is 0.00003 mg  
23 perchlorate/kg-day. It again is noted that this RfD is specific for the anion because that is what is  
24 detected in most environmental samples and because most salts of perchlorate readily dissolve.  
25 Because of the application of uncertainty factors, this dose is approximately 1/300 of the dose  
26 that resulted in brain morphometry and thyroid changes in pups and hypothyroid status  
27 (decreased T4 and increased TSH) in rat mothers (Argus Research Laboratories Inc, 2001) and in  
28 their pups both during gestation (GD21) and in the post-natal period (PND4 through PND21).

1       **10.1.3 Risk Characterization**

2           Comprehensive risk characterization for the perchlorate contamination issue, as discussed  
3 in Chapter 1 (see Figure 1-5), requires accurate information on exposure levels determined by a  
4 validated analytical method. Dose-response estimates such as the value derived herein can then  
5 be used to gauge the potential toxicity of those exposures. Exposure can be either direct, most  
6 likely by ingestion, or indirect, such as by consumption of contaminated food. When using the  
7 dose-response assessment derived herein to compare with exposure estimates, one should remain  
8 keenly aware that many of these exposure aspects have not yet been characterized accurately for  
9 perchlorate. Fate and transport information do not exist to track the spatial and temporal  
10 distribution of perchlorate; the potential for evaporative concentration in soils has not been  
11 characterized, nor has its uptake in plants or herbivores. In addition, there are uncertainties  
12 remaining in the dose-response estimate itself. These concerns also should be considered  
13 whenever attempting to characterize the risk to a specific human population exposed to a  
14 particular scenario.

15  
16       **10.1.3.1 Direct Exposures**

17           Typically the RfD is used as a comparison for oral ingestion, such as by drinking water.  
18 The RfD is compared with an exposure estimate of the drinking water concentration to  
19 characterize potential toxicity. When making this comparison, the assumptions underlying  
20 derivation of the RfD must be kept in mind. The RfD is intended to be protective of susceptible  
21 populations exposed daily. The frequency and magnitude of exposure is a key attribute of  
22 accurate dose-response characterization (Jarabek, 1995c) and an equally important component of  
23 risk characterization. Transient decreases in T4 can cause permanent neurodevelopmental  
24 deficits. Thus, the degree to which the particular suspected population at risk fits with the  
25 underlying assumptions of the RfD derivation should be kept in mind. Finally, the degree of  
26 imprecision in the derivation of an RfD should be taken into account. The RfD estimates are not  
27 intended to serve as “bright line” estimates. By definition, there is an order of magnitude  
28 uncertainty around the estimate. This generally translates into a range of approximately  
29 three-fold below to three-fold above the RfD, but also depends on the nature of the effects used  
30 as the basis.

1 **10.1.3.2 Indirect Exposures**

2 Where crops are irrigated with perchlorate-contaminated water, indirect human exposures  
3 may result. A number of factors need to be considered in estimating human exposure through  
4 crops.

5 Concentration in plant parts as a result of root uptake normally is calculated using a soil-to-  
6 plant transfer factor that is expressed as the ratio of plant to soil concentration. If perchlorate is  
7 subject to evaporative concentration in irrigated soils, then soil concentration, and therefore  
8 uptake, may be higher than that expected simply based on concentration in irrigation water. If a  
9 leaf crop such as lettuce is spray-irrigated, perchlorate could be concentrated evaporatively on  
10 external leaf surfaces. Because perchlorate salts have high water solubility, this contamination  
11 probably would be removed largely by washing. On the other hand, if perchlorate is  
12 phytodegraded, as one study has suggested (Nzengung, n.d.), soil or plant concentrations may be  
13 lower than otherwise expected. Studies are needed to determine the behavior and fate of  
14 perchlorate in plant-soil-water systems, including studies that simulate leaf crop irrigation and  
15 that account for full life cycles of crops.

16 Besides estimates of perchlorate concentrations in crops, the calculation of human daily  
17 intake depends on the number of crop types that are contaminated, the extent to which a  
18 particular individual obtains the crops from a contaminated source, and the individual's daily  
19 consumption of the crops. These factors may vary widely in the exposed population, and  
20 methods for accounting for the combined variability should be used in characterizing these  
21 exposures.

22 Methods for estimating human exposures resulting from crop uptake of soil-deposited  
23 contaminants are presented in Chapters 6 (Determining Exposure Through the Terrestrial Food  
24 Chain) and 10 (Risk Assessment) of the EPA document, "Methodology for Assessing Health  
25 Risks Associated with Multiple Pathways of Exposure to Combustor Emissions (EPA 600/  
26 R-98/137)." That document currently is undergoing revision and is scheduled for final release in  
27 January 2002. If the needed information can be obtained on perchlorate behavior and fate, the  
28 methods described therein can be used to develop estimates of human exposure and risk.

1     **10.1.4 Major Uncertainties and Research Needs**

2             Reliable exposure estimates are required to accurately and comprehensively characterize  
3 the risk of perchlorate contamination. This section will briefly summarize research needs  
4 associated with aspects of uncertainty about the human health risk dose-response estimate that  
5 were highlighted in Chapter 7.

6             The greatest need for continued improvement in the dose-response assessment is a more  
7 accurate characterization of the linkage between the key event of the mode of action (i.e.,  
8 inhibition of iodide uptake in the thyroid gland), subsequent changes in thyroid hormones, and  
9 the correlation to outcome measures in hypothyroxinemic pregnant animals and their pups.  
10 Because this need must be addressed in the fetal compartment as well, accurate characterization  
11 of toxicokinetics during pregnancy and lactation also are required. More definitive studies of the  
12 degree of change in perturbation of the hypothalamic-pituitary-thyroid axis (i.e., change in  
13 hormone levels) that is associated with thyroid histology, and with neurobehavioral deficits  
14 especially, would improve the confidence in the accuracy of the exposure-dose-response  
15 continuum. The current studies may need to be repeated with larger sample sizes and lower  
16 doses, and new studies may be needed to evaluate effects on fetal hormone levels and  
17 neurodevelopmental measures both in the laboratory and in a survey of the human population.  
18 Research on potential factors influencing sensitivity is also critically requisite. Animal models of  
19 thyroid impairment such as iodide deficiency and “womb to tomb” exposure designs should be  
20 explored. Finally, mechanistic determinants of these toxicokinetic and toxicodynamic  
21 parameters and processes should be further characterized.

22  
23  
24     **10.2 ECOTOXICOLOGY**

25     **10.2.1 Aquatic Life**

26             Procedures for deriving Tier II water quality values were used in Section 8.3.2.1 to jointly  
27 characterize the potential effects of the perchlorate ion on the richness and productivity of fish,  
28 aquatic invertebrate, and plant communities. Tier II values are derived when data are not  
29 sufficient for deriving ambient water quality criteria. The Tier II value derivation procedures  
30 account for missing information with approximately 80% confidence. In this case, the Tier II

1 values derived, termed secondary acute and chronic values, were 5 and 0.6 mg/L (i.e., 5,000 and  
2 600  $\mu\text{g/L}$ ), respectively; difficulties associated with the interpretation of one test result in an  
3 uncertainty range for the secondary chronic value of  $< 0.32$  to  $> 0.83$  mg/L ( $< 320$  to  
4  $> 830$   $\mu\text{g/L}$ ). Perchlorate levels reported for large surface waters (as high as 16  $\mu\text{g/L}$ ) and ground  
5 waters (as high as 280  $\mu\text{g/L}$  in public supply wells) are well below the secondary acute and  
6 chronic values. Thus, at these exposure levels, the likelihood of effects on the richness and  
7 productivity of fish, aquatic invertebrate, and plant communities appears to be low. However,  
8 because much higher perchlorate concentrations have been reported in monitoring wells at rocket  
9 motor manufacturing or testing sites ( $37 \times 10^6$   $\mu\text{g/L}$ ) and in groundwater-dominated surface  
10 water systems close to sites of contamination (3500 to  $1.3 \times 10^5$   $\mu\text{g/L}$ ), sites clearly exist that  
11 have perchlorate concentrations high enough to cause toxicity to aquatic life. These sites include  
12 springs, such as that sampled along Las Vegas Wash in Nevada (Parsons, 2001) and the INF  
13 Pond at Longhorn Army Ammunition Plant in Texas (Parsons, 2001; Smith et al., 2001). On the  
14 other hand, concentrations below the Tier II values were detected in larger water bodies  
15 immediately adjacent to sites of contaminations, such as in Lake Mead immediately adjacent to  
16 the mouth of the Las Vegas Wash (less than 4 to 68  $\mu\text{g/L}$ ). Water discharged from a CERCLA  
17 groundwater pump-and-treat facility that was not equipped to treat perchlorate at Allegany  
18 Ballistics Laboratory to the North Branch Potomac River contained 250 to 280  $\mu\text{g/L}$  perchlorate  
19 (Parsons, 2001).

20 Where high levels of contamination exist, sensitive aquatic organisms such as daphnids  
21 may be the most likely to experience effects; in the reported tests, effects were seen on both  
22 survival and reproduction (neonates per organism). A teratogenicity assay, FETAX, showed  
23 malformations in frog embryos occurring at only slightly lower concentrations than lethality,  
24 indicating that perchlorate is probably not a potent developmental toxicant. Tier II values are not  
25 estimated for plants, but results from algal toxicity tests suggest that even at the higher  
26 perchlorate concentrations associated with rocket motor manufacturing, risk of toxicity to aquatic  
27 plants is low.

28 The perchlorate anion can be associated with various cations including sodium,  
29 ammonium, and potassium. When sodium perchlorate was tested, the sodium cation was not  
30 toxic to daphnids in sodium chloride control tests but did show toxicity to minnows.

31 Ammonium controls were not used in tests with ammonium perchlorate, but ammonium ion is a

1 known toxicant with toxicity that varies according to water temperature and pH. In any aquatic  
2 system where perchlorate is present, attention should be given to determining the concentrations  
3 of potentially toxic cations that may contribute to ecological effects.

4 Based on a secondary chronic value of 600  $\mu\text{g/L}$  (uncertainty range, < 320 to > 830  $\mu\text{g/L}$ )  
5 for perchlorate, the analytical detection methods for perchlorate in water are sufficient. The  
6 detection limit achieved for perchlorate in water was 4  $\mu\text{g/L}$  (Parsons, 2001; Smith et al., 2001),  
7 which is much less than the secondary chronic value. Thus, the likelihood that adverse  
8 ecological effects will occur below detection limits is low.

## 10 10.2.2 Risks to Consumers of Aquatic Life

11 Information from Parsons (2001) and Smith et al. (2001) indicate that perchlorate may  
12 bioaccumulate in aquatic invertebrates and fish in contaminated waters, but perchlorate is not  
13 expected to bioconcentrate in these organisms to levels exceeding the surface water  
14 concentrations. Therefore, there currently is no indication that consumers of aquatic  
15 invertebrates or fish are at increased risk of effects from bioconcentration in areas where  
16 perchlorate concentrations in surface water occur. However, there is some uncertainty about the  
17 potential for bioaccumulation of perchlorate at low concentrations (i.e., 4 to 300  $\mu\text{g/L}$  in water)  
18 because of the higher detection limits for perchlorate in animal tissues, which were 300 to 400  
19  $\mu\text{g/kg}$  in Parsons (2001) and about 70  $\mu\text{g/kg}$  in Smith et al. (2001). Furthermore, perchlorate  
20 may bioconcentrate (i.e., to levels exceeding those in water) in aquatic plants; therefore,  
21 consumers of aquatic plants may be at greater risk than consumers of aquatic invertebrates or  
22 fish, but information is not available concerning effect levels in aquatic herbivores.

## 24 10.2.3 Terrestrial Life

### 25 10.2.3.1 Plants

26 Terrestrial plants may be exposed to perchlorate in soil at disposal sites and at sites  
27 irrigated with contaminated surface water or groundwater. Perchlorate concentrations in soil at  
28 disposal sites range from less than 1 to 1470  $\text{mg/kg}$  (Parsons, 2001) and can be higher than the  
29 screening benchmark of 4  $\text{mg/kg}$  and even higher than the lethal concentrations ( $\geq 180 \text{ mg/kg}$ ;  
30 EA Engineering, Science and Technology, Inc., 1998).

1 In the absence of reliable information concerning the accumulation of perchlorate in  
2 irrigated soils, it may be assumed that soil concentrations equal irrigation-water concentrations  
3 (Section 8.3.1.3). Reported surface-water concentrations in the Colorado River, 4 to 16  $\mu\text{g/L}$ ,  
4 would translate to 0.004 to 0.016 mg/kg. At the Yuma site, there was a single detection in  
5 surface soil of 0.090 mg/kg; all other measurements were below the detection limits of 0.079 to  
6 0.080 mg/kg (Parsons, 2001). Even the single detected concentration is a factor of 44 lower than  
7 the benchmark value. The reported groundwater concentration in public wells of 280  $\mu\text{g/L}$   
8 would translate to 0.28 mg/kg, which is a factor of 14 lower than the benchmark value. Hence,  
9 perchlorate does not appear to constitute a hazard to plants irrigated with surface water.  
10 However, given the large uncertainties concerning exposure, a hazard from groundwater  
11 irrigation cannot be precluded.

12 Based on this screening benchmark of 4 mg/kg for perchlorate, the analytical detection  
13 methods for perchlorate in soil are sufficient for determining whether soils will cause toxicity to  
14 plants, and there is little likelihood of adverse ecological effects occurring at levels below  
15 detection limits. The detection limit achieved for perchlorate in soils was generally 75-80  $\mu\text{g/kg}$   
16 (Parsons, 2001), but there was at least one soil sample where the reporting limit was 803  $\mu\text{g/kg}$ .  
17 However, all of these limits are less than the screening benchmark.  
18

### 19 **10.2.3.2 Soil Invertebrates**

20 Soil invertebrates may be exposed to perchlorate in soil at disposal sites and at sites  
21 irrigated with contaminated surface water or groundwater. Perchlorate concentration  
22 measurements at disposal sites range from less than 1 to 1470 mg/kg (Parsons, 2001) and,  
23 therefore, can exceed the soil screening benchmark of 1 mg/kg. In the absence of reliable  
24 information concerning the accumulation of perchlorate in irrigated soils, it may be assumed that  
25 soil concentrations equal irrigation water concentrations (Section 8.3.1.3). Reported surface  
26 water concentrations in the Colorado River, 4 to 16  $\mu\text{g/L}$ , would translate to 0.004 to  
27 0.016 mg/kg in soils. At the Yuma site, the single detection in surface soil was 0.090 mg/kg with  
28 detection limits of 0.079 to 0.080 mg/kg. This detected concentration is a factor of 11 lower than  
29 the soil screening benchmark value (1 mg/kg). The reported groundwater concentration in public  
30 wells of 280  $\mu\text{g/L}$  would translate to 0.28 mg/kg, which is a factor of 4 lower than the  
31 benchmark value. Hence, perchlorate does not appear to constitute a hazard to soil invertebrates

1 in soil irrigated with surface water. However, given the large uncertainties concerning exposure,  
2 a hazard from groundwater irrigation cannot be precluded.

3 Based on this screening benchmark of 1 mg/kg for perchlorate, the analytical detection  
4 methods for perchlorate in soil are sufficient, and there is little likelihood of adverse ecological  
5 effects occurring at levels below detection limits. The detection limit achieved for perchlorate in  
6 soils was generally 75-80  $\mu\text{g}/\text{kg}$  (Parsons, 2001), but there was at least one soil sample where the  
7 reporting limit was 803  $\mu\text{g}/\text{kg}$ . However, all of these limits are less than this screening  
8 benchmark.

### 9 10 **10.2.3.3 Herbivores**

11 Exposures of voles to perchlorate based on measured plant concentrations at rocket motor  
12 manufacturing or testing sites (0.11 mg/kg day to a maximum of 49 mg/kg day) exceed both the  
13 LOAEL of 0.01 mg/kg/day and the screening benchmark of 0.001 mg/kg day. Estimated  
14 exposures of voles consuming plants on sites irrigated with surface water (0.18 mg/kg day) and  
15 groundwater (3.2 mg/kg day) also exceed the LOAEL and the screening benchmark. Hence,  
16 there is a potential hazard to all herbivorous wildlife living in areas that may be irrigated with  
17 contaminated water. At disposal sites, wildlife would be at risk from the effects of loss of food  
18 and habitat from toxic effects on plants, as well as the potential for direct toxic effects via  
19 consumption of perchlorate-tainted food or water.

20 Assuming a water ingestion rate of 0.21 g/g-day (U.S. EPA, 1993a,b), the screening  
21 benchmark for herbivores is equivalent to a water concentration of 4.8  $\mu\text{g}/\text{L}$ . Perchlorate levels  
22 reported for large surface waters (as high as 16  $\mu\text{g}/\text{L}$ ) are greater than this concentration. Much  
23 higher perchlorate concentrations have been reported in monitoring wells at rocket motor  
24 manufacturing or testing sites ( $37 \times 10^6 \mu\text{g}/\text{L}$ ) and in groundwater-dominated surface water  
25 systems close to sites of contamination ( $3500$  to  $1.3 \times 10^5 \mu\text{g}/\text{L}$ ), and rodent exposures via  
26 drinking water at these sites would exceed the rodent NOAEL.

27 Based on screening level benchmarks for herbivores, the analytical detection methods for  
28 perchlorate in plant tissues may not be sufficient for the detection of concentrations potentially  
29 toxic to herbivores even though the analytical detection methods for perchlorate in water are  
30 sufficient. The detection limits achieved for perchlorate in water and in plant tissues were 4  $\mu\text{g}/\text{L}$   
31 and 0.4 mg/kg, respectively (Parsons, 2001; Smith et al., 2001).

1 **10.2.3.4 Carnivores**

2 Available evidence indicates that concentrations in terrestrial invertebrates are less than the  
3 concentrations in plants and similar to that in soils. As a result, there currently is no indication  
4 that terrestrial carnivores are at additional risk from perchlorate. Risks of direct toxic effects are  
5 therefore lower for carnivores than herbivores. In locations where perchlorate levels are  
6 sufficient to significantly affect herbivores, carnivores are more likely to be affected by loss of  
7 prey than by perchlorate toxicity. Therefore toxic effects are not quantified.

8  
9 **10.2.4 Uncertainties**

10 This discussion of uncertainties is limited to qualitative uncertainties associated with major  
11 gaps in the data available for ecological risk assessment of perchlorate. This is because, as with  
12 other screening assessments, quantitative uncertainties are treated through the use of conservative  
13 assumptions. It is also because the data gaps are the major sources of uncertainty, not  
14 imprecision or inaccuracy of the available data.

15  
16 **10.2.4.1 Uncertainties Concerning Aquatic Risks**

17 **Aquatic Exposures.** The primary uncertainty associated with this assessment of aquatic  
18 risks is the paucity of data on perchlorate occurrence in surface waters. For lack of systematic  
19 sampling and analysis, the spatial and temporal distribution of perchlorate in water is unknown.  
20 It is not certain whether the reported concentrations in water represent the highest existing levels.  
21 This is not a large source of uncertainty for this screening assessment if it is assumed that  
22 sampling has been biased to areas of highest likely contamination. However, it would be a major  
23 source of uncertainty in any subsequent definitive assessment.

24 **Aquatic Effects.** While the effects of perchlorate on some species of algae are known, the  
25 effects on aquatic macrophytes are unknown. As a result, risks to aquatic primary producers are  
26 estimated using only the chronic toxicity test results for the alga *Selenastrum*. Because of  
27 physiological differences between algae and vascular plants, effects on aquatic primary producers  
28 are not adequately assessed. In addition, it is unknown how or if physiological variations among  
29 various species of algae or plants may affect their susceptibility to perchlorate.

30 Algae, aquatic macrophytes, and terrestrial leaf litter are the bases of food chains in many  
31 aquatic ecosystems. Because perchlorate has been shown to concentrate in leaves of terrestrial

1 plants and aquatic plants, the potential for direct impacts to primary consumers (i.e., planktonic  
2 and benthic invertebrate communities) is a concern that could not be addressed in this  
3 assessment.

4 A 35-day, early-life stage (ELS) test with *Pimephales*, generally regarded as a chronic test  
5 but short of a full-life-cycle test, showed no significant effects on any standard endpoint  
6 (survival, growth or biomass) at the highest concentration tested (490 mg/L). However, all  
7 larvae exposed to perchlorate concentrations, including the lowest concentration of 28 mg/L,  
8 exhibited redness and swelling, which was not observed in the larvae exposed to the control  
9 water. This finding suggests the presence of subtle effects that could be ecologically significant  
10 and raises doubt about whether a chronic NOEC has been adequately determined for this species.  
11 This uncertainty is displayed as a range surrounding the secondary chronic value (i.e., < 0.32 to  
12 > 0.83 mg/L). Because of the inequality signs, even the width of the range is uncertain. For this  
13 reason, and because of the potential for chronic effects caused by thyroid dysfunction, chronic  
14 effects should be investigated in a full life cycle test.

15 The uncertainty factors in the secondary chronic value are high because of the lack of test  
16 results for aquatic organisms other than fathead minnows, amphipods, and daphnids.

#### 18 **10.2.4.2 Uncertainties Concerning Terrestrial Risks**

19 **Terrestrial Exposure.** The available data concerning aqueous perchlorate levels is sparse  
20 and has not been collected systematically. As a result, the spatial and temporal distribution of  
21 perchlorate in irrigation water is unknown. It is not clear that the reported concentrations in  
22 water represent the highest existing levels. This is not a major source of uncertainty for this  
23 screening assessment if it is assumed that sampling has been biased to areas of highest likely  
24 contamination. However, it would be a major source of uncertainty in any subsequent definitive  
25 assessment.

26 The fate of perchlorate in soil, including its tendency for evaporative concentration, is not  
27 well characterized. As a result, soil concentrations were assumed to be equal to irrigation water  
28 concentrations. This assumption could be low by multiple orders of magnitude if evaporative  
29 concentration occurs with perchlorate, as it does with metals. The limited data for irrigated soils  
30 near Yuma (Parsons, 2001) do not support the occurrence of such a high degree of evaporative

1 concentration, but neither are they sufficient to rule out concentration by up to a factor of 10 or  
2 so. More information on the fate of perchlorate in irrigated soils is needed.

3 The bioconcentration of perchlorate by plants suggests that perchlorate may be elevated in  
4 leaves and leaf litter to levels that may affect invertebrate herbivores and soil invertebrate  
5 communities. For lack of data concerning dietary toxicity, risks to invertebrates by this route  
6 were not assessed.

7 Available toxicity data for rodents suggest that vertebrate herbivores may be sensitive to  
8 low levels of perchlorate in plant tissues; concentrations potentially causing toxicity are  
9 calculated to be lower than those currently detectable by chemical analyses of plants. In Parsons  
10 (2001), detection limits for plants were generally about 0.4 mg/kg wet weight; similar detection  
11 limits were achieved by Ellington and Ellis (2000) and Ellington et al. (2001), as compared to an  
12 exposure benchmark of 0.01 mg/kg in plant tissue for a representative herbivore (see Section  
13 8.3.2.2). Therefore, lower detection limits for perchlorate in plant tissues may be needed to  
14 completely assess the risks to vertebrate herbivores.

15 **Terrestrial Effects.** The toxicity of perchlorate to nonmammalian vertebrate wildlife is  
16 unknown. As a result, risks to birds, reptiles, and amphibians could not be assessed.

17 The toxicity of perchlorate to terrestrial invertebrates, other than acute lethality to  
18 earthworms, is unknown. As a result, risks to other terrestrial invertebrates were inadequately  
19 assessed.

## 20 21 **10.2.5 Research Needs**

22 Three questions were asked of the screening ecological risk assessment for perchlorate:

- 23 • Are ecological risks best characterized as *de minimis* (exposures clearly are below levels of  
24 concern), *de manifestis* (risks are clearly significant and require management action to reduce  
25 exposures); or somewhere in between and requiring further characterization?
- 26 • Are analytical detection methods for determining levels of perchlorate in the environment  
27 sufficient, or is there a likelihood of adverse ecological effects occurring at levels below current  
28 detection limits?
- 29 • Is the available ecotoxicological information on perchlorate sufficient, or are additional studies  
30 needed?

1 In the immediate vicinity of facilities that were involved in the manufacture, use, or  
2 disposal of perchlorate salts, particularly facilities involved in handling of solid rocket  
3 propellents, ecological exposure can exceed levels of concern and management actions may be  
4 needed to reduce these exposures. Site-specific risk assessments would be needed to guide  
5 remediation of such locally contaminated sites. Farther from such facilities, ecological exposures  
6 appear to be below levels of concern.

7 The analytical detection methods for perchlorate are generally sufficient, and there appears  
8 to be no indication of adverse ecological effects occurring at levels below detection limits, except  
9 that detection limits in plant tissues are not low enough to ensure that risks to herbivores are  
10 detected. Additionally, there is some uncertainty about the potential for bioaccumulation at low  
11 concentrations of perchlorate in surface water, because of differences in the analytical detection  
12 limits between water and animal tissues.

13 The available ecotoxicological information on perchlorate is sufficient for this screening-  
14 level ecological risk assessment. However, additional ecotoxicological studies could reduce the  
15 uncertainties about the toxicity of perchlorate to other potential ecological receptors.

16 While the available information may yield an adequate screening level ecological risk  
17 assessment, the following research needs for exposure and effects analysis deserve mention.

#### 18 19 **10.2.5.1 Exposure**

20 Concerning exposure, at least three important issues remain unresolved:

- 21 • Because the available data on accumulation in terrestrial and aquatic vascular plants are from  
22 studies that were not designed to quantify accumulation factors, the accumulation of  
23 perchlorate in terrestrial and aquatic plants should be further investigated.
- 24 • Because of the potential for evaporative concentration, the fate of perchlorate in irrigated soils  
25 should be investigated.
- 26 • Because the concentrations that have potential for dietary toxicity to vertebrate herbivores are  
27 less than the limits of detection currently achievable by chemical analysis of plants, analytical  
28 methods for plant tissues that could lower the limits of detection should be investigated.

1 **10.2.5.2 Effects**

2 Also requiring further attention are issues related to the effects of potential perchlorate  
3 exposure:

- 4 • The effects of exposure of aquatic plants should be determined.
- 5 • The effects of exposure of noncrustacean invertebrates should be determined.
- 6 • The effects of dietary exposure to perchlorate should be determined in birds and in herbivorous  
7 or litter-feeding invertebrates.
- 8 • The effects of dietary and cutaneous exposure to perchlorate should be determined for adult  
9 amphibians and aquatic reptiles.
- 10 • If perchlorate occurs at significant levels in estuarine systems, its toxicity in saline waters  
11 should be determined.

12  
13 **10.2.5.3 Site-Specific Investigations**

14 Some of the research needs that were listed in the previous ERD of this document have  
15 been met by the research conducted by the US Air Force IERA (Parsons, 2001) in which  
16 perchlorate concentrations in environmental media (i.e., surface soils, surface water, sediments,  
17 and pore water) and biological tissues (i.e., terrestrial plants, invertebrates, reptiles, birds, and  
18 mammals and aquatic vegetation, invertebrates, fish, amphibians, reptiles, and birds) were  
19 surveyed at six sites with known perchlorate contamination. These data are supplemented by  
20 additional sampling at one of the sites, Longhorn Army Ammunition Plant in Texas, by Smith  
21 et al. (2001). These studies do address some questions about exposure that were expressed in the  
22 previous ERD of this document (U.S. EPA, 1998d), i.e:

- 23 • Because concentrations of perchlorate in water are poorly known, and  
24 concentrations in soil and biota are unknown, a survey of perchlorate contamination  
25 should be conducted.
- 26 • Because, contrary to expectations, perchlorate accumulates to high concentrations in  
27 terrestrial vascular plants, the accumulation of perchlorate in aquatic plants and in  
28 animals should be investigated.

29 However, these studies were screening-level surveys that took small numbers of samples during  
30 limited periods of time. In addition, the studies were not designed to address questions about the  
31 effects of exposure. In some locations, concentrations in environmental media were high enough

1 that toxicity to ecological receptors was highly likely (i.e., the risks were *de manifestis*), and in  
2 other locations toxicity could not be ruled out (i.e., the risks could not be termed *de minimus*).  
3 Therefore, systematic sampling is needed in these locations to more definitively quantify  
4 exposures and effects, so that the likelihood, nature and extent of ecological risks may be  
5 quantified, appropriate remedial alternatives may be designed, and effectiveness of site cleanup  
6 may be judged. In addition, site surveys may be required in other locations where perchlorate  
7 contamination is suspected.  
8  
9

### 10 **10.3 CHARACTERIZATION PROGRESS SUMMARY**

11 Despite the fact that the appreciation of widespread perchlorate contamination emerged  
12 only five years ago, considerable progress has been made in hazard identification and  
13 quantitative dose-response characterization for both the human health and ecotoxicological risks  
14 of potential perchlorate exposures. The thyroid has been confirmed as the target tissue in  
15 humans, laboratory animals, and wildlife. The key event of the mode of action for perchlorate is  
16 iodide uptake inhibition at the NIS with the potential for both subsequent neurodevelopmental  
17 and neoplastic sequelae. A harmonized human health reference dose has been proposed to be  
18 protective for both sequelae based on a mode of action model. Data insufficiencies for various  
19 ecotoxicological receptors and for accurate exposure estimates precludes other than a screening-  
20 level assessment at this time. Additional research is needed to determine the contribution of  
21 exposure sources other than drinking water. This requires more progress in the area of analytical  
22 methods to extend current approaches to other media.

23 As with any risk assessment, additional insights and new research will continue to change  
24 our understanding as the knowledge base is informed with new data and as the scientific and  
25 technical areas relevant to the particular risk characterization mature and evolve. Work  
26 dedicated to the areas defined in this chapter should allow continued improvement of the risk  
27 characterizations for perchlorate in the future.  
28

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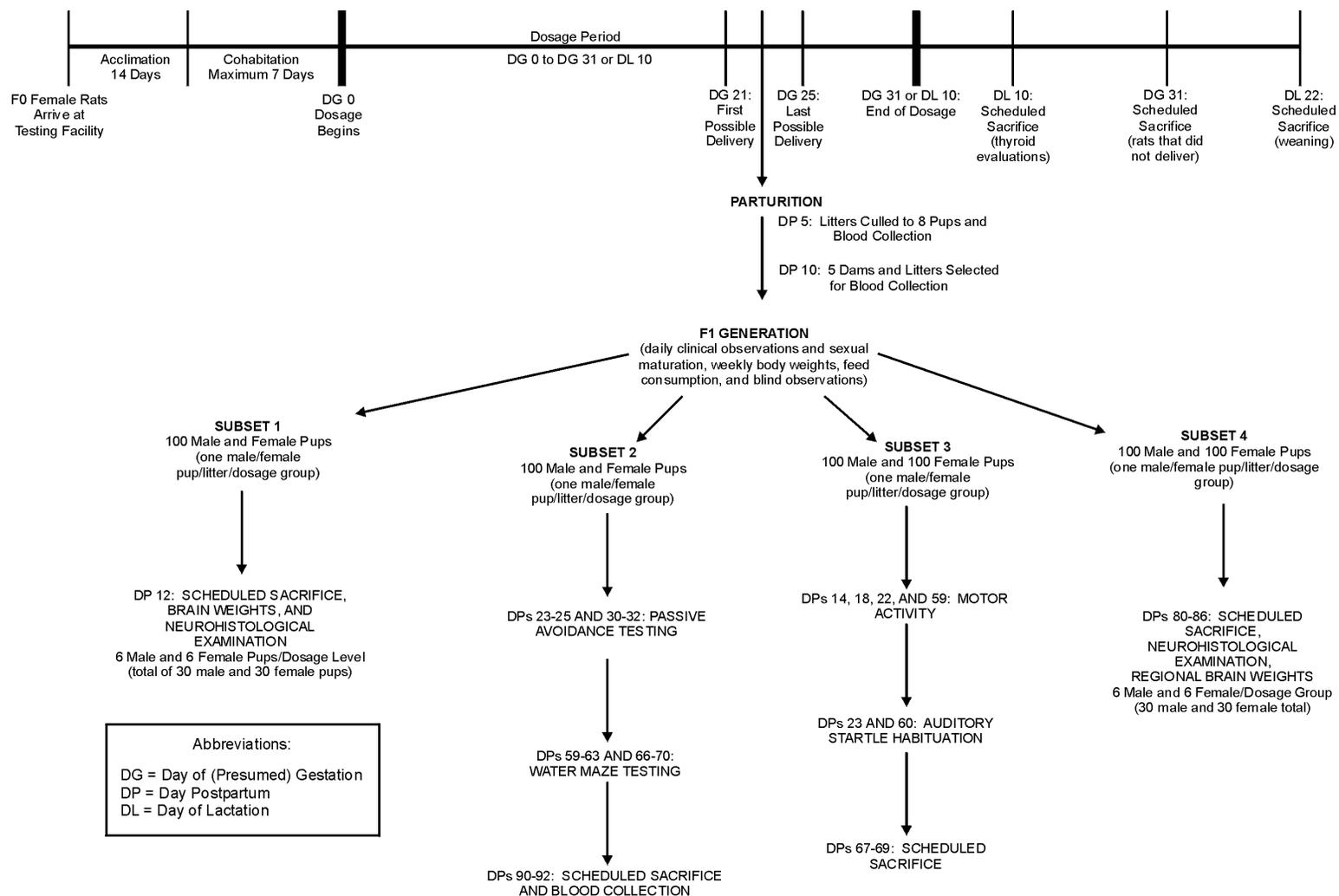
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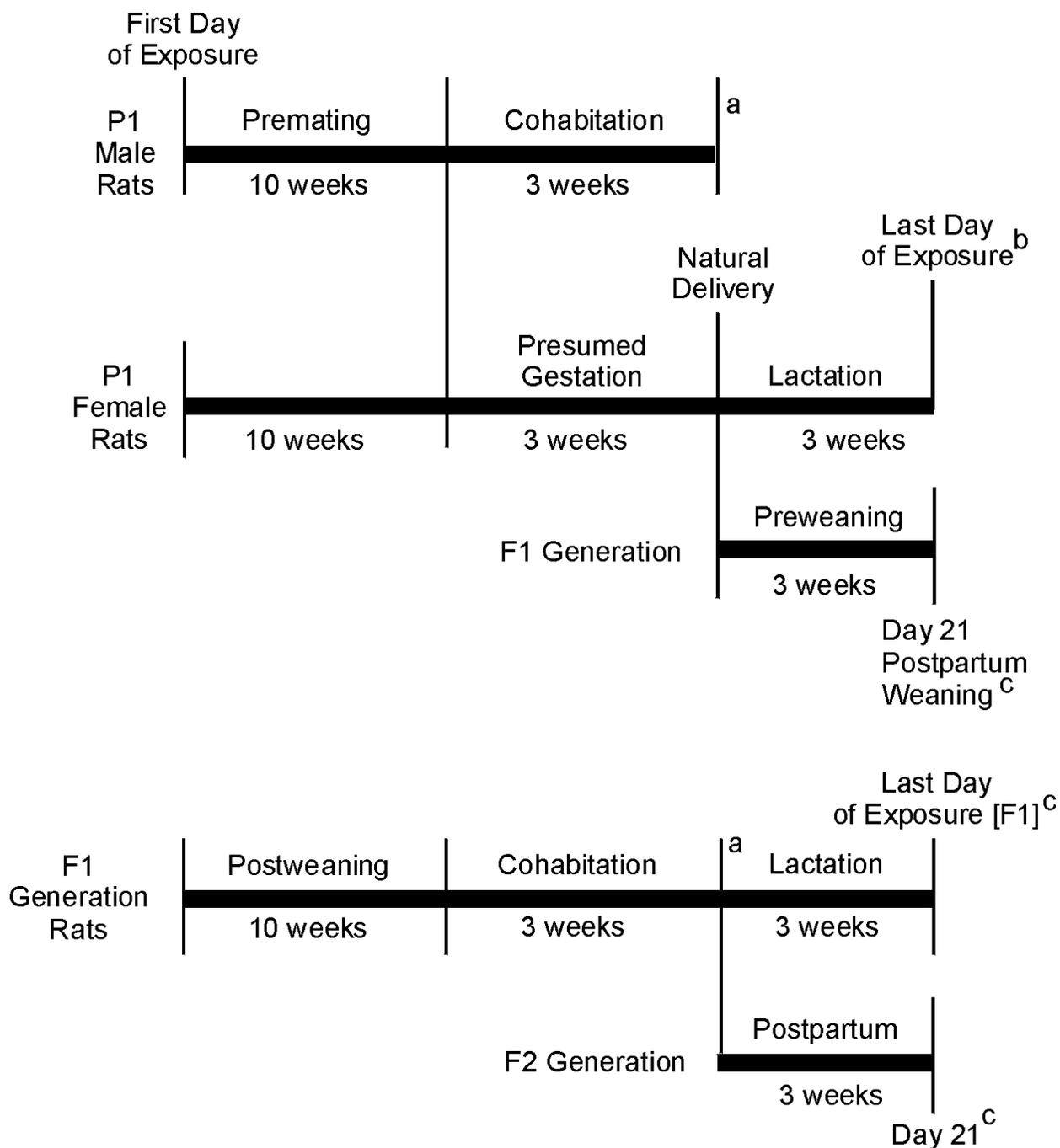
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## **APPENDIX A**

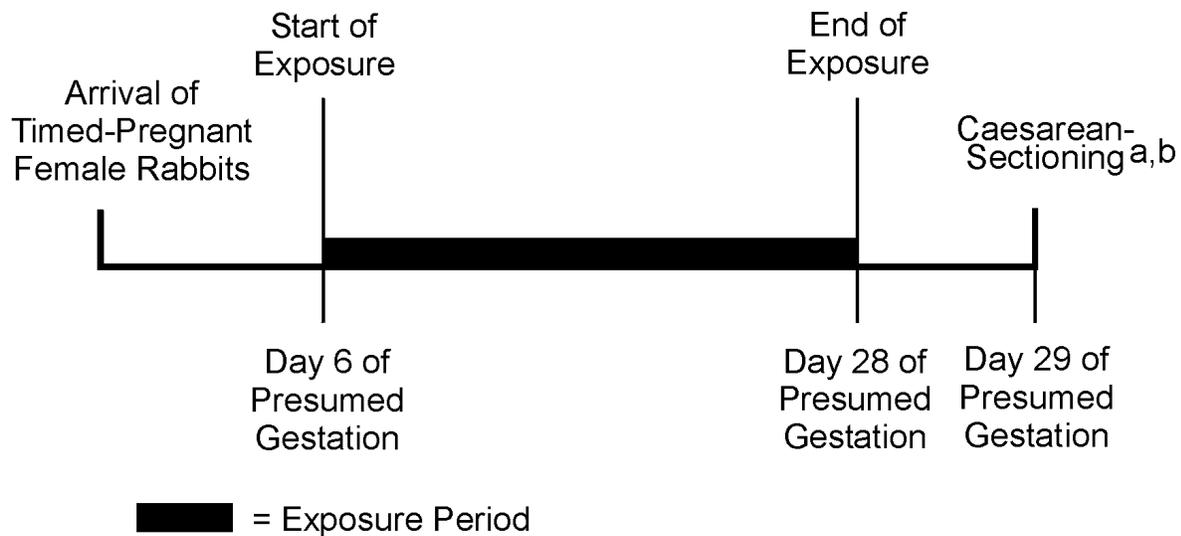
# **Schematics of Study Designs for Neurodevelopmental, Two-Generation Reproductive and Developmental Studies**



**Figure A-1. Schematic of the neurobehavioral developmental study of ammonium perchlorate administered orally in drinking water to SD rats (Argus Research Laboratories, Inc., 1998a).**



**Figure A-2. Schematic of the oral (drinking water), two-generation (one litter per generation) reproduction study of ammonium perchlorate in SD rats (Argus Research Laboratories, Inc., 1998b).**



a = Blood samples taken from does for thyroid and pituitary hormone (T3, T4, TSH) analyses.  
 b = Fetal evaluations (external examinations and soft tissue and skeletal examinations).

**Figure A-3. Schematic of the oral (drinking water) developmental toxicity study of ammonium perchlorate in New Zealand rabbits (Argus Research Laboratories, Inc., 1998c).**

## APPENDIX B

### List of Acronyms and Abbreviations

<b>Acronym</b>	<b>Definition</b>
$\Delta A^\circ_{\text{rxn}}$	Helmholtz free energy of reaction
$\Delta G^\circ_f$	Gibbs free energy of formation
$\Delta G^\circ_{\text{rxn}}$	Gibbs free energy of reaction
$\Delta S^\circ_{\text{univ}}$	net entropy of the universe
a-p	anterior-posterior
Ab	antibody
ACSL	advanced continuous simulation language
ADHD	attention deficit hyperactivity disorder
ADME	absorption, distribution, metabolism, and elimination
AFB	air force base
AFRL	U.S. Air Force Research Laboratories
AFRL/HEST	Air Force Research Laboratory/Human Effectiveness Directorate
AIDS	acquired immunodeficiency syndrome
AITD	autoimmune thyroid disease
ANCOVA	analysis of covariance
AP	ammonium perchlorate
ATP	adenosine triphosphate
AUC	area-under-the-curve
AV	acute value
AWQC	ambient water quality criteria
$\text{BF}_4^-$	tetrafluoroborate
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
BMR	benchmark response

<b>Acronym</b>	<b>Definition</b>
BW	body weight
C'	complement
CA DHS	California Department of Health Services
cAMP	cyclic adenosine monophosphate
CBC	complete blood count
CCL	Contaminant Candidate List
CD4/CD8	cluster of differentiation — cellular markers 4 and 8
CDC	Centers for Disease Control and Prevention
CERCLA	Comprehensive Environmental Response Compensation Liability Act
cESI-MS	complexation electrospray ionization mass spectrometry
CFU	colony-forming units
CHS	contact hypersensitivity
ChV	chronic value
Cl <sub>2</sub>	chlorine
CI	confidence interval
ClO <sup>-</sup>	hypochlorite
ClO <sub>4</sub> <sup>-</sup>	perchlorate
CIUC-p	perchlorate urinary clearance
CNS	central nervous system
CP	cyclophosphamide
CPES	Coastal Plain Experiment Station
CPM	counts per minute
Cs <sup>+</sup>	cesium
CsCl	cesium chloride
CTL	cytotoxic T-lymphocyte
CV	coefficient of variation
DAF	dosimetric adjustment factor

<b>Acronym</b>	<b>Definition</b>
DEQ	Department of Environmental Quality
DIT	diiodotyrosine
DNA	deoxyribonucleic acid
DNCB	dinitrochlorobenzene
DoD	Department of Defense
DoE	Department of Energy
DTH	delayed-type hypersensitivity
DWEL	drinking water equivalent level
E:T	effector to target cell
EAR	estimated average requirement
EGF	epidermal growth factor
ELISA	enzyme linked immunosorbant assay
ELS	early-life stage
EPA	U.S. Environmental Protection Agency
EPL	Experimental Pathology Laboratories, Inc.
ER	endoplasmic reticulum
$E^\circ$	standard electric potential
$F$	Faraday constant
F1	first generation
F2	second generation
FAVF	Final acute value factor
FCN	function
FETAX	Frog Embryo Teratogenesis Assay: <i>Xenopus</i>
FGF	fibroblast growth factor
FH	follicular epithelial cell hypertrophy or hyperplasia
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
ft4	free thyroxine

<b>Acronym</b>	<b>Definition</b>
GA	Golgi apparatus
GD	gestation day
GGTP	g-glutamyl transpeptidase
GI	gastrointestinal
GMAV	Genus mean acute value
<i>gsp</i>	GTP-binding protein mutation
Gy	Gray (equal to 100 rads)
H <sup>+</sup>	hydrogen
H <sub>2</sub> O <sub>2</sub>	hydrogen peroxide
hCG	human chorionic gonadotropin
HClO <sub>4</sub>	perchloric acid
HEE	human equivalent exposure
HOCl	hypochlorous
I <sup>-</sup>	iodide
IC	ion chromatography
IC <sub>25</sub>	quartile inhibitory concentration
ICD-9	International Classification of Diseases, 9th Revision
ID	iodine deficiency
IFN	interferon
IGF-1	insulin-like growth factor
IgG	immunoglobulin G
IgM	immunoglobulin M
ip	intraperitoneally
IPSC	Interagency Perchlorate Steering Committee
IRIS	Integrated Risk Information System
IU	international unit
IUDR	uridine

<b>Acronym</b>	<b>Definition</b>
iv	intravenously
K <sup>+</sup>	potassium
K <sub>2</sub> O	potassium oxide
K <sub>m</sub>	Michaelis-Menten affinity constant
KNO <sub>3</sub>	potassium nitrate
LC <sub>50</sub>	concentration lethal to 50% of population
LD	lactation day
LHAAP	Longhorn Army Ammunition Plant
Li <sup>+</sup>	lithium
LLNA	local lymph node assay
ln	natural log
LOAEL	lowest-observed-adverse-effect level
LOEC	lowest-observed-effect concentration
LOEL	lowest-observed effect level
LP	lymphoproliferation
LS	Lumen size
LY	lysosomes
M-W RST	Mann-Whitney Rank Sum Test
MCA	3-methyl cholanthrene
MCL	maximum contaminant level
MDL	minimum detection limit
MF	modifying factor
Mg(ClO <sub>4</sub> ) <sub>2</sub>	magnesium perchlorate
MIT	monoiodotyrosine
MMIA	1-methyl-2-mercaptoimidazole
MANOVA	multiple analysis of variance
MCLG	maximum contaminant level goal

<b>Acronym</b>	<b>Definition</b>
MRL	minimum reporting limit
mRNA	messenger ribonucleic acid
MS-MS	mass spec — mass spec
MTD	maximum tolerated dose
<i>n</i>	number of electrons or number of moles
n.d.	no date
N-P-K ratio	nitrogen-phosphorous-potassium ratio
Na <sup>+</sup>	sodium
NaClO <sub>4</sub>	sodium perchlorate
NaNO <sub>3</sub>	sodium nitrate
NAS	National Academy of Sciences
NASA	National Aeronautics and Space Administration
NCE	Normochromatic erythrocyte
NCEA	National Center for Environmental Assessment
NDEP	Nevada Division of Environmental Protection
NERL-ERD	Natural Exposure Research Laboratory's Ecosystems Research Division
NH <sub>4</sub> <sup>+</sup>	ammonium
NH <sub>4</sub> ClO <sub>4</sub>	ammonium perchlorate
NH <sub>4</sub> NO <sub>3</sub>	ammonium nitrate
NHEERL	National Health and Environmental Effects Research Laboratory
NIEHS	National Institute for Environmental Health Sciences
NIS	sodium iodide symporter
NK	natural killer
NMR	nuclear magnetic resonance
NO <sub>3</sub> <sup>-</sup>	nitrate
NOAEL	No-Observed-Adverse-Effect Level
NOEC	No-Observed-Effect Concentration

<b>Acronym</b>	<b>Definition</b>
NPDWR	National Primary Drinking Water Regulations
NRMRL	National Risk Management Research Laboratory
NTP	National Toxicology Program
O <sub>2</sub>	oxygen
OEHHA	Office of Environmental Health Hazard Assessment
OEPP	Office of Emergency Response and Remediation
OPPTS	Office of Prevention, Pesticides, and Toxic Substances
ORD	Office of Research and Development
OSWER	Office of Solid Waste and Emergency Response
OW	Office of Water
p	probability
P	pressure
P1	parental generation
P <sub>2</sub> O <sub>5</sub>	disphosphorus pentoxide
<i>p53</i>	<i>p53</i> tumor suppressor gene
PA	prealbumin
PAS	periodic acid shift
PBI	protein-bound iodide
PBPK	physiologically based pharmacokinetic
PCE	polychromatic erythrocyte
PCB	polychlorinated biphenyl
PFC	plaque-forming cell
PHG	public health goal
PII	plasma inorganic iodide
PND	post-natal day
PP	post partum
PP-TH	plasma protein-thyroid hormone

<b>Acronym</b>	<b>Definition</b>
ppb	parts per billion
ppm	parts per million
PQL	practical quantitation limit
PSG	Perchlorate Study Group — consortium of defense contractors
PT-p	thyroid follicle:stroma partition coefficient
PTU	propylthiouracil
PWG	Pathology Work Group
QA/QC	quality assurance/quality control
R	ideal gas constant
RAIU	radioactive iodine uptake
<i>ras</i>	<i>ras</i> protooncogene
Rb <sup>+</sup>	rubidium
RDA	recommended dietary allowance
RfC	inhalation reference concentration
RfD	oral reference dose
RIA	radioimmunoassay
RL	reproducibility limits
RO	reverse osmosis
$r_s$	Spearman's rank order
RSC	relative source contribution
rT <sub>3</sub>	reverse triiodothyronine
SACR	secondary acute-chronic ratio
SAV	secondary acute value
sc	subcutaneously
SCN	thiocyanate
SCV	secondary chronic value
SD	standard deviation

<b>Acronym</b>	<b>Definition</b>
SD rats	Sprague-Dawley strain
SDWA	Safe Drinking Water Act
SE	standard error of the mean
SGOT	serum glutamyl oxacetic transaminase
SGPT	serum glutamyl pyruvic transaminase
SLA	soluble <i>Listeria</i> antigen
SMCV	species mean chronic value
SNK	Student Newman Keuls
SRBC	sheep red blood cell
SRLB	Sanitation and Radiation Laboratory Branch
T	temperature
T2	diiodothyronine
T3	triiodothyronine
T4	thyroxine or tetraiodothyronine
T4 GLUC	T4-glucuronide conjugate
TBG	thyroid-binding globulin
TCE	trichloroethylene
TDS	total dissolved solids
Tg	thyroglobulin
TH	thyroid hormone
TPO	thyroid peroxidase
TRH	thyrotropin-releasing hormone
TSCA	Toxic Substances Control Act
TSH	thyroid-stimulating hormone
tT4	total thyroxine
UCMR	Unregulated Contaminant Monitoring Rule
UDPGTs	uridine diphosphyl glucuronosyl transferases

<b>Acronym</b>	<b>Definition</b>
UF	uncertainty factor
USAF	United States Air Force
USGS	United States Geological Survey
USN	United States Navy
V	volume
V <sub>maxc</sub>	Michaelis-Menten maximum velocity capacity
W <sub>exp</sub>	expansion work
WHO	World Health Organization
WPAFB	Wright Patterson Air Force Base
WSWRD	Water Supply and Water Resources Division