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EPA

Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization

External Review Draft (Do Not Cite or Quote)

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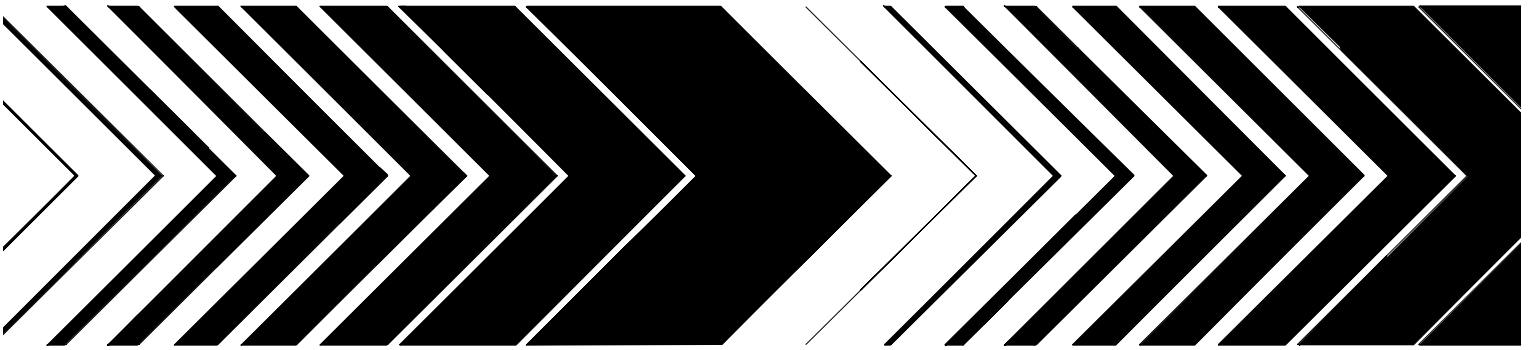

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National Center for Environmental Assessment
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Table of Contents

Table of Contents
(cont'd)

Table of Contents
(cont'd)

Table of Contents
(cont'd)

Table of Contents
(cont'd)

Page

Table of Contents
(cont'd)

Table of Contents
(cont'd)

Table of Contents
(cont'd)

List of Tables

List of Tables
(cont'd)

List of Tables
(cont'd)

List of Tables
(cont'd)

List of Tables
(cont'd)

List of Figures

List of Figures
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1998 External Peer Review Draft

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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).

Acknowledgments

The authors are indebted to the following individuals who imparted their insights, data, experimental analysis, and expertise to improve specific areas of the report or to facilitate the process of its development and review.

As noted in the introduction (Chapter 1), this assessment could not have been accomplished without the cooperation of individuals who work for the governmental entities represented in the Interagency Perchlorate Steering Committee. Each of the subcommittee members contributed to discussions as the process evolved, via stakeholder forums or meetings, and the integrated approach to the overall risk characterization framework began to materialize. Special acknowledgment for oversight of the testing strategy endeavor, notably communication with the contract labs, expediting data delivery, and writing reports goes to Lt. Col. Dan Rogers (U.S. Air Force Materiel Command); Dr. Richard Stotts, Dr. Dave Mattie, and Capt. David Tsui (Air Force Research Laboratory/Human Effectiveness Directorate [AFRL/HEST], Operational Toxicology Branch); and Cornell Long and Dr. Ron Porter (AFRL/HEST, Human Systems Center).

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Richard Wilson (EPA, NCEA), as always, is applauded for his tireless and cheerful dedication to the task of photocopying both drafts of the document and its supporting materials throughout the years.

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

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

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

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

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

An Integrated Approach to Comprehensive Risk Characterization

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

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

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

Physicochemical Characteristics

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

Hazard Identification and Mode of Action Testing Strategy

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

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

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

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

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

(6) Mechanistic studies that characterize the effects of perchlorate on the iodide uptake mechanism across species as a link with the ADME studies to aid in the quantitative extrapolation of dose across species.

(7) A battery of genotoxicity assays to evaluate the potential for carcinogenicity by evaluating the potential for direct effects on deoxyribonucleic acid (DNA).

(8) Immunotoxicity studies to evaluate the potential for perchlorate to disrupt immune function, including cell-mediated and humoral toxicity.

- After the External Peer Review in 1999, additional studies were performed to replicate the neurodevelopmental study (i.e., changes in brain morphometry and motor activity); determine the developmental toxicity potential in rats versus rabbits; investigate additional aspects of immunotoxicity; and develop a consistent nomenclature and scoring system for the histopathological lesions in the thyroid gland. Additional pharmacokinetic data was also developed into physiologically-based pharmacokinetic (PBPK) models of perchlorate and iodide distribution.
- A battery of ecological screening tests as part of the 1997 testing strategy was conducted as part of the 1997 testing strategy in laboratory organisms representative of ecological receptors across soil, sediment, and water to evaluate dose-response relationships. These were considered to be a tier of tests to give an idea of gross toxicity that would determine the need and types of tests to be performed in the next tier. The tests did not measure the amount of perchlorate in the tissues of the species being tested. Based on stakeholder input and the need for a more focused battery of tests, lettuce was substituted for duckweed because of Tribal concerns regarding the sizable lettuce crop along the Colorado river. The following species were selected for the first round of testing:

  (1) *Daphnia magna* (water flea) to represent an aquatic invertebrate

  (2) *Ceriodaphnia magna* (water flea) to represent an aquatic invertebrate

  (3) *Lactuca sativa* (lettuce) to represent a vascular plant

  (4) *Pimephales promelas* (fathead minnow) to represent an aquatic invertebrate

  (5) *Eisenia foetida* (earthworm) to represent a soil invertebrate

  (6) *Microtus pennsylvanicus* (meadow vole) to represent an herbivore
- Other studies in the set of tests included the Frog Embryo Teratogenesis Assay: *Xenopus* (FETAX) and a phytoremediation study to examine uptake, distribution, and degradation in

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

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

Human Health Assessment

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

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

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

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

Screening Ecological Risk Assessment

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

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

Uncertainties and Assessment Research Needs

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

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

Risk Characterization

- As noted above, the lack of exposure information precludes comparison with the human health and ecological toxicity assessment for accurate characterization of risk. Indirect human exposure pathways can be addressed best by a new EPA document, Methodology for Assessing Health Risks Associated with Multiple Pathway of Exposure to Combustor Emissions, which is scheduled for final release in January 2002.
- Noncancer neurobehavioral effects have been shown at lower doses. The estimate for perchlorate has been based on precursor effects considered protective for both the thyroid neoplasia and neurodevelopmental effects. It is appropriate for comparison against direct oral exposures. The frequency and magnitude of exposure are key attributes for characterization compared with those assumptions of continuous lifetime exposure assumed in the derivation. The degree to which the particular suspected population at risk fits with the assumptions used in the RfD derivation should be kept in mind when performing any risk characterization. Further, RfD estimates are not intended to serve as a “bright line” because, by definition, there is an order-of-magnitude uncertainty around the estimate. This typically translates into a range of threefold below to threefold above the RfD.
- Ecological risk could not be precluded nor accurately characterized because of the significant 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 (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 (NH_4ClO_4) makes up 69.7% of the propellant for the space shuttle rocket motors and 65 to75% 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).

$$4\ NH_4ClO_4(s) \rightarrow 2\ Cl_2(g) + 3\ O_2(g) + 2\ N_2O(g) + 8\ H_2O(g) \qquad (1\text{-}1)$$

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

Specific uses of various perchlorate salts include: solid rocket fuel oxidizer, flares, and pyrotechnics (potassium); solid rocket fuel oxidizer, explosives, chemical processes and pyrotechnics (ammonium); precursor to potassium and ammonium perchlorate and in explosives (sodium); and military batteries (magnesium) (Rogers, 1998). Perchlorate salts also are used on a large scale as a component of air bag inflators. Other industrial or commercial applications of perchlorate salts include their use in nuclear reactors and electronic tubes; as additives in lubricating oils; in tanning and finishing leathers; as a mordant for fabrics and dyes; in electroplating, aluminum refining, and rubber manufacture; and in the production of paints and enamels (Siddiqui et al., 1998). A 1998 report raised the concern that chemical fertilizer is a potential source of perchlorate contamination (TRC Environmental Corporation, 1998). More recent studies limit concern to certain types of fertilizer containing Chilean caliche (Urbansky, 2000; U.S. Environmental Protection Agency, 2001a,b; Urbansky and Collette, 2001); however, production practices have been changed to address that issue. Besides their large-scale commercial uses, perchlorate salts often are employed on a small scale in laboratory chemical studies as ionic strength adjustors or as noncomplexing counterions. Some still are used in medical diagnostics in thyroid function tests. Perchloric acid is used for various laboratory applications requiring a strong acid. Wet ashing organic matter with perchloric acid still is performed today as a means of preparation for certain samples. Anhydrous magnesium perchlorate ($Mg(ClO_4)_2$) is a strong desiccant; however, historically, Anhydrone®, a slightly hydrated form of $Mg(ClO_4)_2$, has been used to collect the water formed in combustion analyses.

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

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

When ammonium perchlorate is released to water, the salt is highly soluble and dissociates completely releasing ammonium (NH_4) and perchlorate (ClO_4^-):

$$NH_4ClO_4(s) \xrightarrow{H_2O} NH_4^+(aq) + ClO_4^-(aq). \quad (1\text{-}2)$$

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

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

1.2 EVOLUTION OF ANALYTICAL DETECTION METHODS AND EMERGING OCCURRENCE DATA

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

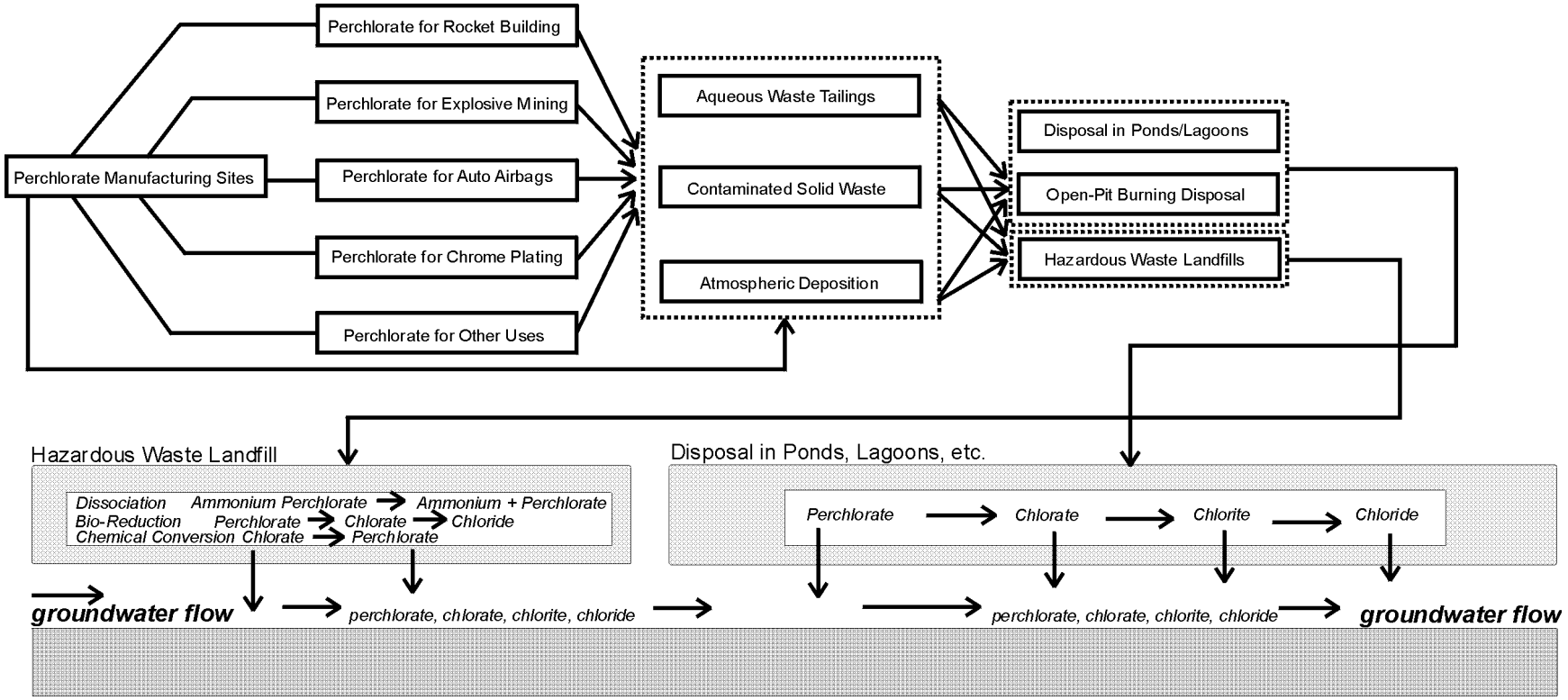

Figure 1-1. Sources and pathways of groundwater contamination for perchlorate. (Modified from Siddiqui et al., 1998.)

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

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

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

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

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

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

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

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

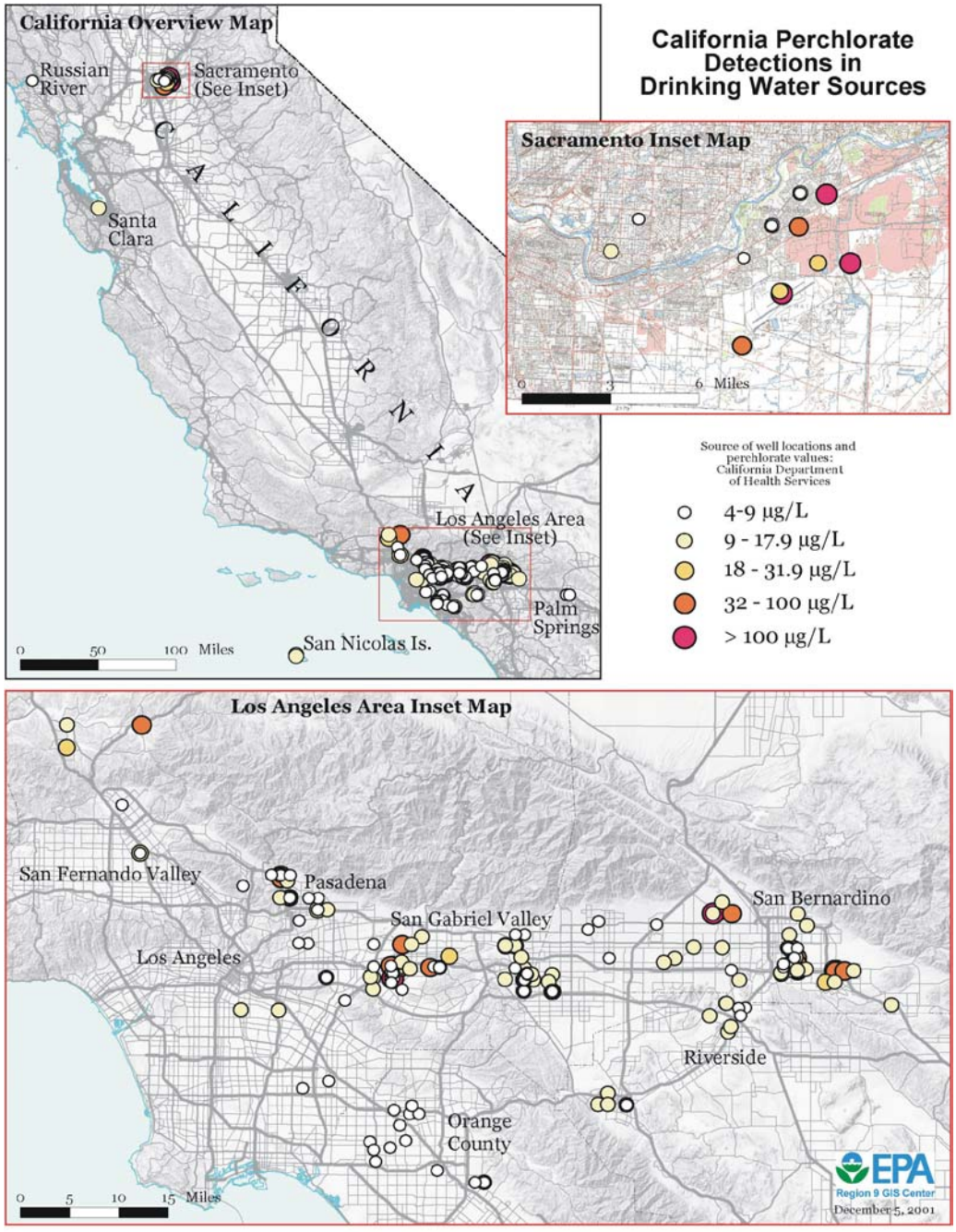

Figure 1-2. Distribution of perchlorate detected in public water supply sources in California. Also noted are several large sites of groundwater contamination that include perchlorate releases (Mayer, 2001).

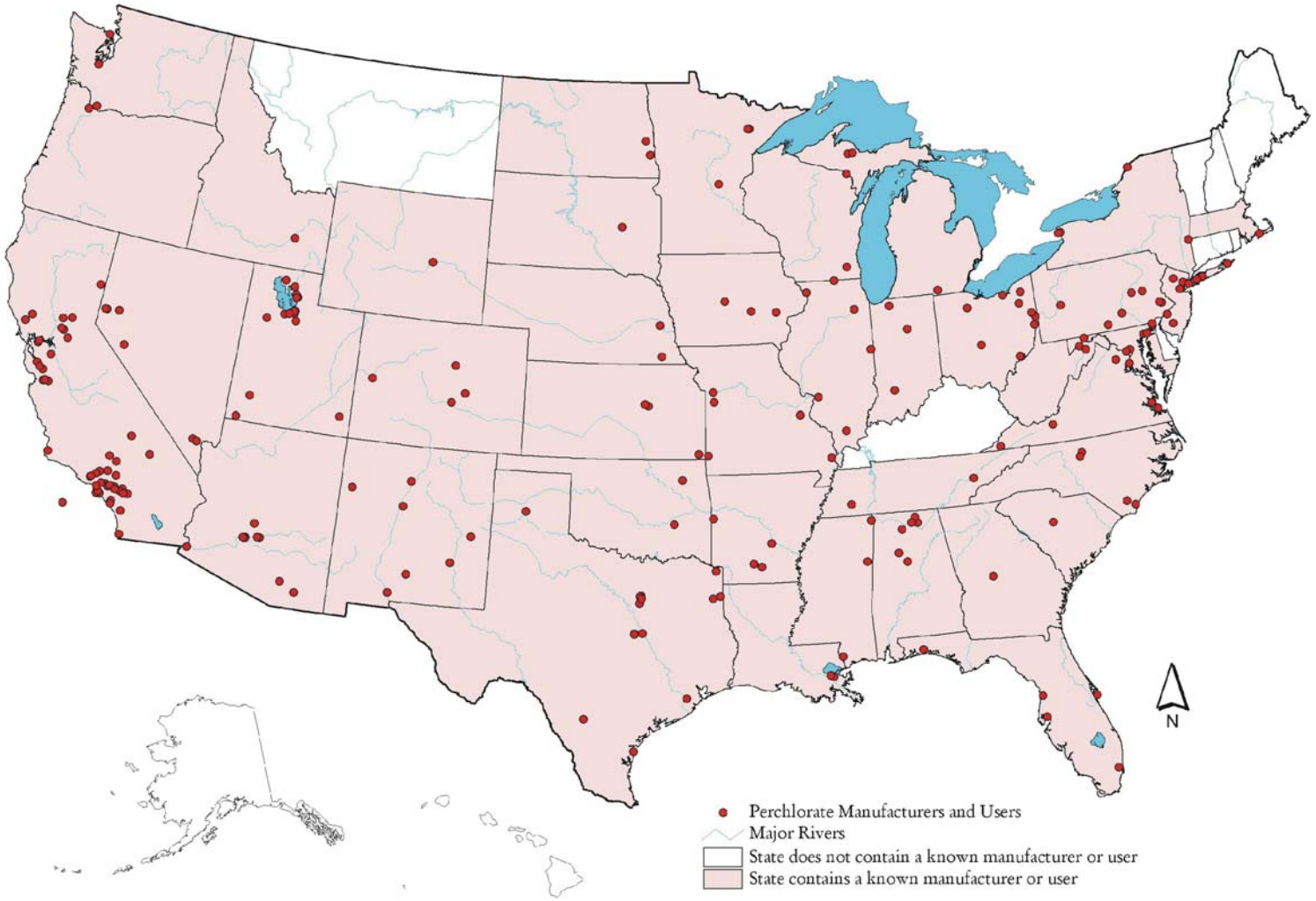

Figure 1-3. Locations of specific perchlorate manufacturers or users identified through responses to EPA Information Requests from current manufacturers (identifying shipments of at least 500 pounds in any year) and through investigations by state and local authorities (Mayer, 2001).

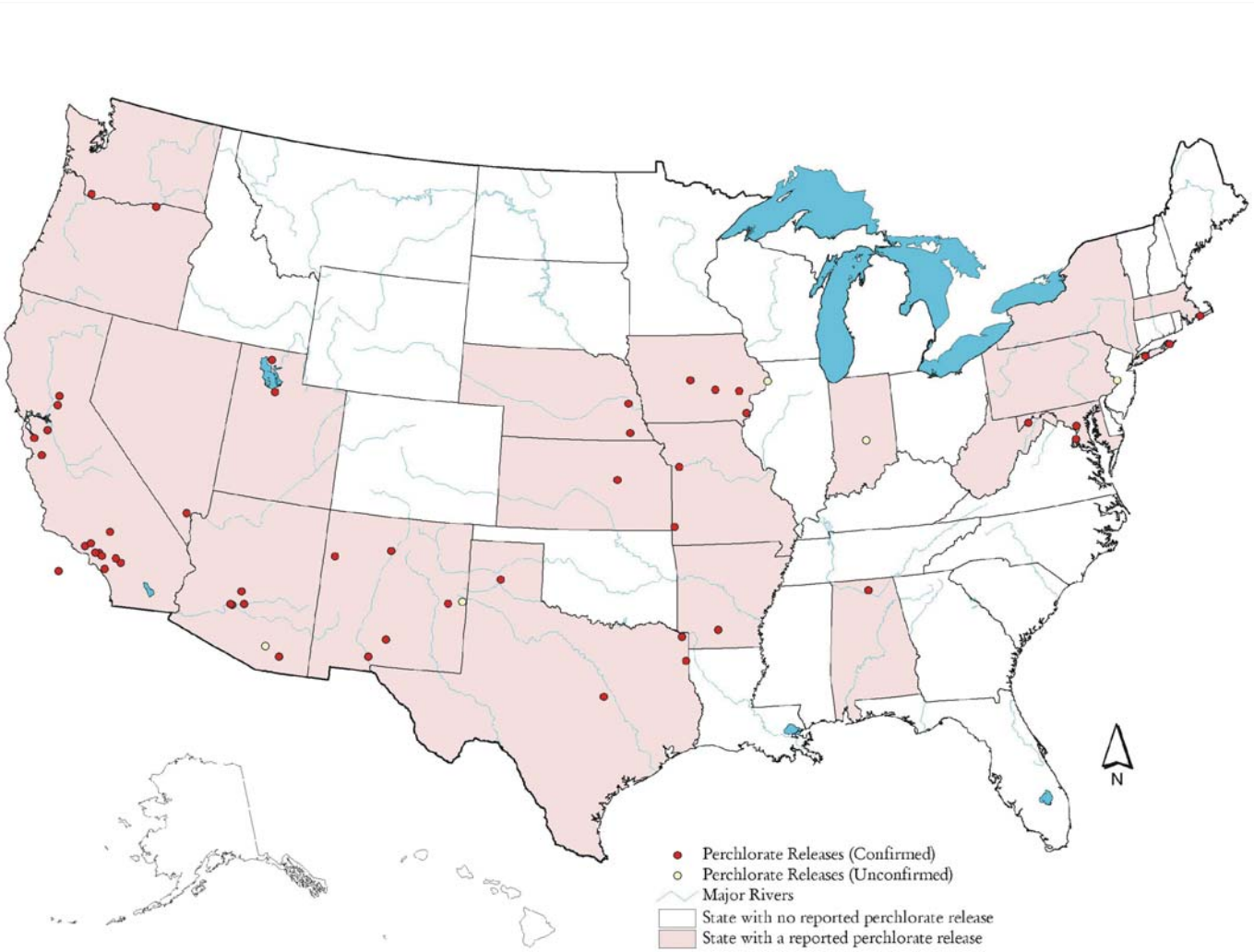

Figure 1-4. Locations of reported environmental releases of perchlorate to groundwater, surface water, or soil. Perchlorate measured in four water supplies in New Mexico, Iowa, Indiana, and Pennsylvania has been published in Siddiqui et al., 1998, but has not been confirmed independently by EPA or state 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, 2OO1[a] (Mayer, 2001)

State	Location	Suspected Source	Type of Contamination	Max. Conc. ppb
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, 2OO1[a] (Mayer, 2001)

State	Location	Suspected Source	Type of Contamination	Max. Conc. ppb
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, 2OO1[a] (Mayer, 2001)

State	Location	Suspected Source	Type of Contamination	Max. Conc. ppb
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, 2OO1[a] (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

[a]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.

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

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

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

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

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

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

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

1.3 HEALTH AND ECOTOXICOLOGY RISK ASSESSMENTS—HISTORICAL OVERVIEW

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

1.3.1 Overview of Perchlorate Health Risk Assessment

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

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

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

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

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

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

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

1.3.2 Overview of Ecotoxicology Screening Level Assessment

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

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

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

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

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

1.4 RISK CHARACTERIZATION AND REGULATORY AGENDA

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

1.4.1 U.S. Environmental Protection Agency Regulatory Plans

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

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

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

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

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

1.4.2 State Regulatory Plans

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

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

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

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

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

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

1.5 SUMMARY

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

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

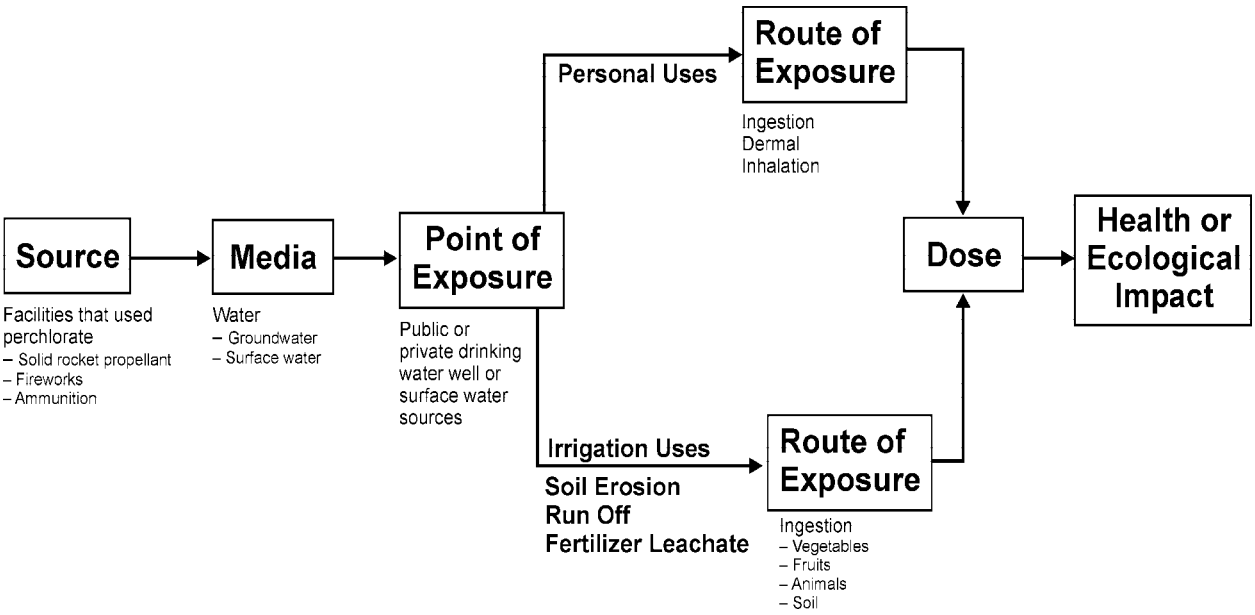

Figure 1-5. Considerations for comprehensive characterization of perchlorate contamination. (Modified from Underwood, 1998.)

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

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

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

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

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

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

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

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

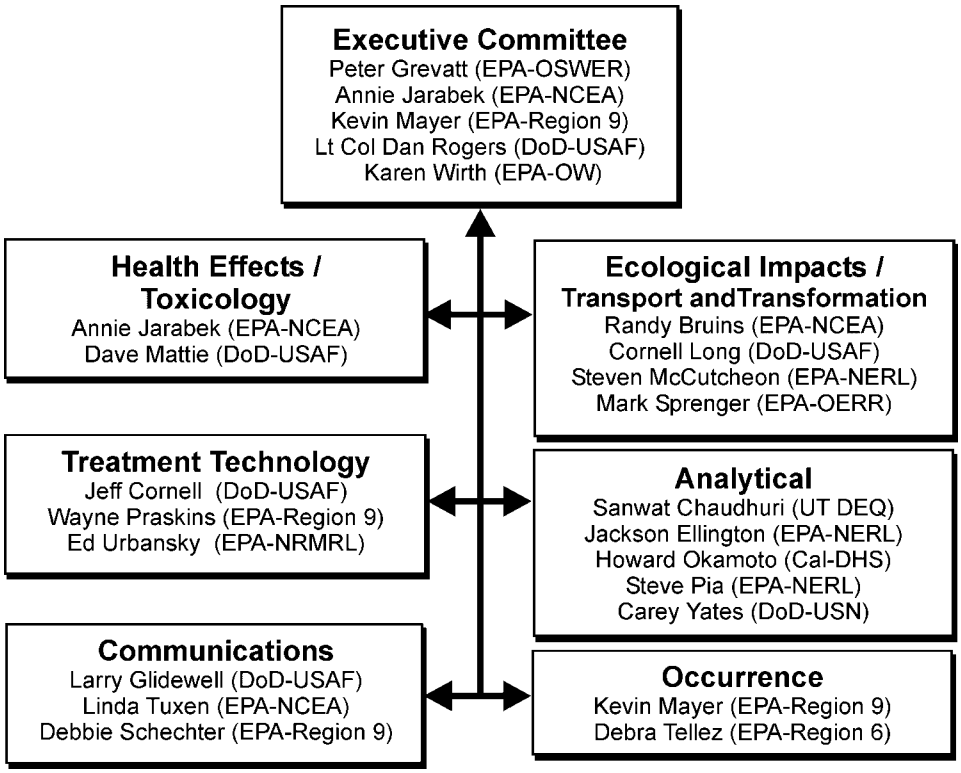

Figure 1-6. Structure and membership of the executive committee, subcommittees areas, and co-chairs of IPSC. The IPSC is designed to ensure an integrated approach to addressing the perchlorate contamination challenge and to provide accurate accounts of technical issues to stakeholders. (OSWER = Office 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^3/mol at 25 °C, compared with 36.7 for iodide.

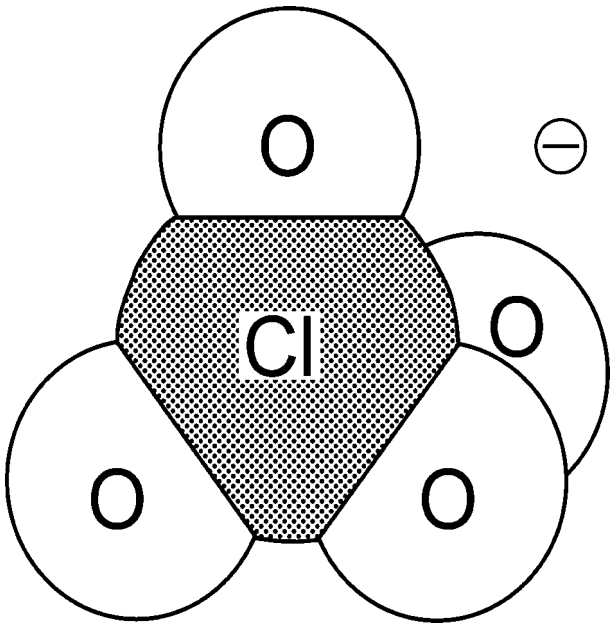

Figure 2-1. Chemical structure of perchlorate.

Perchlorate is widely known to be a very poor complexing agent and is used extensively as a counter anion in studies of metal cation chemistry, especially in nonaquous solution (Urbansky, 1998). In this application, it is comparable with other noncomplexing or weakly ligating anions, such as trifluoromethanesulfonate (triflate [$CF_3SO_3^-$]), tetrafluoroborate (BF_4^-), and, to a lesser extent, nitrate (NO_3^-). Some exceptions are known, but are rare, such as some copper compounds (Burke et al., 1982). All of these anions have a highly delocalized ($CF_3SO_3^-$, NO_3^-, ClO_4^-) or sterically blocked (BF_4^-) monovalent anionic charge and large volume. The low charge 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	$\Delta G_f^{"}$, kJ Mol^{-1}
BF_4^-	−1,490
PO_4^{3-}	−1,019
SO_4^{2-}	−744
HCO_3^-	−587
OH^-	−157
Cl^-	−131
NO_3^-	−109
Br^-	−104
ClO_4^-	−8.5
ClO_3^-	−8.0

This low association with cations is responsible for the extremely high solubilities of perchlorate salts in aqueous and nonaqueous media. As noted, the ammonium and the alkali metal salts of perchlorate generally are readily soluble in water. Salts of the smaller univalent cations (i.e., ammonium [NH_4^+], lithium [Li^+], and sodium [Na^+]) are very soluble; whereas, those of the larger univalent cations are less so (i.e., potassium [K^+], rubidium [Rb^+], and cesium [Cs^+]). Quaternary ammonium salts are less soluble still. The outstanding example is sodium

perchlorate, which is extremely soluble (>8 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).

	Magnitude of Physicochemical Property of Perchlorate					
Physical Property	NH_4	Li	Na	K	Rb	Cs
Molecular Weight (g mol^{-1})	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
n-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						
$\Delta H_f^{"}$, kJ mol^{-1}	−290.4	−384.0	−385.7	−435.5	−434.7	−434.7
$\Delta G_f^{"}$, kJ mol^{-1}	−88.9[b]	−254[c]	−255[b]	−304	−306	−307
$\Delta S_f^{"}$, kJ mol^{-1}	186[b]	130[c]	142[b]	151	161	175
$\Delta H_{soln}^{"}$, kJ mol^{-1}	−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	—	—

[a]Thermochemical data converted from kcal/mol using 1,000 cal = 4.184 J.
[b]Weast (1989).
[c]Dean (1985).

Because of their large solubilities, the health risk assessment for perchlorate anion (ClO_4^-) 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

lithium perchlorate *[CASRN* 7791-03-9*]*. The estimate is not appropriate to characterize the risk of effects of perchloric acid ($HClO_4$) *[CASRN* 7601-90-3*]* because it is a strong acid, and the dominant mode of toxicity is the irritating action of the hydrogen ion on skin and mucous membranes.

Perchlorate can be a strong oxidizing agent under certain conditions as indicated by its high reduction potential; therefore, the question has arisen as to whether or not it has the potential to behave as an oxidant in biological systems. The thermodynamics of the halogen oxoanions and oxoacids to participate in redox reactions are well understood. Under standard conditions in 1 M acid, where the species is reduced to chloride, the oxidizing strength and standard reduction potential ($E°$) increase as follows: $Cl_2 < HOCl < HClO_2 < ClO_3^- < ClO_4^-$. The reduction potentials for the oxoanions increase with increasing acidity or decreasing pH (i.e., they are stronger oxidizing agents in acidic solution). Consider, for example, the reduction of chlorine(VII) to chlorine(V) under both acidic and alkaline conditions. In 1.0 M $H^+(aq)$ solution (pH = 0),

$$ClO_4^- + 2\ H^+ + 2\ e^- \rightarrow ClO_3^- + H_2O, \qquad E° = 1.20\ V. \tag{2-1}$$

In 1.0 M $OH^-(aq)$ solution (pH = 14),

$$ClO_4^- + H_2O + 2\ e^- \rightarrow ClO_3^- + 2\ OH^-, \qquad E° = 0.37\ V. \tag{2-2}$$

The effect of pH can be explained in terms of Le Châtelier's principle. In Reaction 2-1, hydrogen ion is plentiful and acts a reactant; this drives the reaction forwards. In Reaction 2-2, hydroxide ion is a product of the reaction and is already present at 1 M. This reduces the driving force for this reaction to take place. The reaction is still spontaneous, as shown by the positive value of $E°$; nonetheless, the driving force is considerably smaller for this case. Thermodynamically, perchlorate is a stronger oxidant in the chlorine oxoanion series at the extremes of the pH scale; however, such extremes are difficult to achieve in vivo (Tsui, 1998).

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

That is, the most stable species, hypochlorite (ClO^-), reacts fastest; whereas, the least stable species, perchlorate (ClO_4^-), reacts the slowest. It is important to note that the activation energy required for the reduction of perchlorate to take place is a function not only of the perchlorate, but also of the chemical nature of the reductant. With common reducing agents (e.g., thiosulfate, sulfite, or ferrous ions), the activation energy is too high for any reaction to be observed. In fact, this property (lack of lability) is exploited routinely in chemical studies where perchlorate salts are used to control the ionic medium and strength, but do not themselves react.

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

[1]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 ΔA°_{rxn}, is used instead. The superscript circle indicates standard state conditions (i.e., solution concentrations of 1 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.

[2]Expansion work (W_{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: $W_{exp} = -P\Delta V = \Delta nRT$ (where P = pressure (atm), V = volume (L), n = number of moles, R = ideal gas constant (L atm k^{-1}), and T = temperature (K) and T and P are constant). For reactions occurring in the condensed phases, $W_{exp} \approx 0$.

[3]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 ΔG°_{rxn}.

$$\Delta G^\circ_{rxn} = -w_{max} = -nFE^\circ \qquad (T, P \text{ constant}) \tag{2-3}$$

The negative sign is necessary because the work done on the environment represents a loss of free energy from the chemical system. Nonexpansion work includes, but is not limited to, causing an electric current to flow or lifting an object against gravity. Whenever a chemical reaction has the ability to do work on the surroundings, it will take place spontaneously.[4] ΔG°_{rxn} is calculated as follows using Hess's law:

$$\Delta G^\circ_{rxn} = \Sigma\, \Delta G^\circ_f \text{ (all products)} - \Sigma\, \Delta G^\circ_f \text{ (all reactants)}. \tag{2-4}$$

The Gibbs free energy of formation, ΔG°_f, is calculated for the formation of a compound from its standard state as an element; consequently, $\Delta G^\circ_f = 0$ for $Cl_2(g)$ and $O_2(g)$. For Reaction 1-1,

$$\begin{aligned} \Delta G^\circ_{rxn} &= 2\Delta G^\circ_f\ [N_2O(g)] + 8\Delta G^\circ_f\ [H_2O(g)] - 4\Delta G^\circ_f\ [NH_4ClO_4(s)] \\ &= 2(104) + 8(-229) - 4(-89)\ \text{kJ} = -1{,}268\ \text{kJ}. \end{aligned} \tag{2-5}$$

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

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[4]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., $\Delta S^\circ_{univ} > 0$). It can be shown that $\Delta G^\circ_{rxn} = -T\Delta S^\circ_{univ}$; therefore, $\Delta S^\circ_{univ} > 0$ means $\Delta G^\circ_{rxn} < 0$, and $\Delta S^\circ_{univ} > 0$ means $\Delta G^\circ_{rxn} < 0$ (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.

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

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

$$OClO_n^- + H_2Ø \rightleftharpoons ØClO_n^- + H_2O, \quad Ø \text{ a labeled oxygen atom; } 0 \le n \le 3. \tag{2-6}$$

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

$$HOCl + H_2Ø \rightleftharpoons [HO\cdots Cl\cdots ØH_2]^\ddagger \rightleftharpoons OH^- + ClØH + H^+ \overset{H_2O}{\rightleftharpoons} H_2O + ClØH \tag{2-7}$$

The aquated species $[HO\cdots Cl\cdots ØH_2]^\ddagger$ represents the activated complex and is the transition state of Reaction 2-7; the proton is not directly transferred from the labeled water molecule to the hydroxide that is part of the HOCl molecule. Rather, a proton is lost to the bulk water of the solution form the activated complex, and another proton is gained. This activated complex may revert back to reactants or proceed to products.[5] As the number of oxygen atoms increases, the water has greater difficulty accessing the reaction site. The oxidation state of the chlorine increases by 2 with each additional oxygen atom; accordingly, the chlorine becomes more and

[5]Note that $\Delta G^\circ_{rxn} = 0$ because the reactants and products are chemically identical. This suggests a process at equilibrium in which the forward and reverse rates are balanced.

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

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

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

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

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

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

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

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

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

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

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

3.1.1 Human Studies

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

Anbar et al. (1959) demonstrated that perchlorate was not metabolized in humans. Four patients were administered 200 mg (approximately 2.9 mg/kg using a default body weight of 70 kg) double-labeled $K^{36}Cl^{18}O_4$, and urine was collected 3 h after dosing. Perchlorate was found to be excreted at approximately 200 μg/min in the urine. Total urine radioactivity was distributed between ^{36}Cl , $^{36}Cl^{18}O_4^-$, $^{36}ClO_4^-$ and $^{36}Cl^-$ and indicated that perchlorate was excreted unchanged in the urine. No human data existed with which to adequately characterize the pharmacokinetics of perchlorate during steady-state, low-dose, repeated administration.

3.1.2 Laboratory Animal Studies

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

Anbar et al. (1959) also attempted to confirm the lack of perchlorate accumulation and lack of metabolism in the thyroid in rats. White rats were injected ip with $^{36}KClO_4$, and the specific activity per gram of tissue was measured at 30 min, 4 hr, and 12 hr. The activity was greatest in the thyroid and peaked at 4 h. The salivary and adrenal glands also had high activity levels. Rabbits also were tested; the thyroid activity levels were again the highest of any tissue and peaked at 2 h. Rabbit testes had the next highest specific activities.

In one of the only co-administration studies, Anbar et al. (1959) simultaneously administered $^{131}I^-$ and $^{36}ClO_4^-$ in equimolar concentrations. The thyroid:blood specific activity for iodide was slightly higher than the ratio for perchlorate (1.80 and 1.69, respectively).

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

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

Goldman and Stanbury (1973) administered 0.1 μCi of the potassium salt of ^{36}Cl-labeled perchlorate ($K^{36}ClO_4$) by ip injection to male Sprague-Dawley rats that had been maintained on a low-iodine diet for 4.5 to 5.0 weeks prior to dosing (approximately 40 μg stable perchlorate per injection). The radionucleide retention in the thyroid, expressed as percent of dose per gram of 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 (5 rats). The peak was reported to appear around 4 h and then to fall to approximately 5% of this peak value after approximately 96 h. An exponential function was used to estimate a half-life of 20 h. Urinary excretion data indicated that the disappearance rate from the plasma and thyroid and the appearance rate in the urine corresponded closely although the question was raised as to whether there is some curvilinearity to the urinary excretion, which may suggest limited saturation. The retained dose and its standard deviation in tissues at 96 h were reported as 0.142 ± 0.1, 0.125 ± 0.09, 0.098 ± 0.03, 0.048 ± 0.04, and background for the thyroid, kidney, spleen, liver, and brain, respectively.

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

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

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

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

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

Eichler and Hackenthal (1962) presented perchlorate elimination data for male and female Wistar rats dosed subcutaneously with 0.2, 1.0, or 6.0 of the $^{36}Cl^-$ sodium perchlorate salt ($Na^{36}ClO_4$) per 100 g body weight (2, 10, or 60 mg/kg). The elimination curves showed nearly linear, rapid excretion of perchlorate until 6 hr, at which time the curve slope started to decrease. The rate of excretion increased with dose. The elimination rates of the different doses prior to 24 h were significantly different from each other but were similar after 24 h. Over 60 hr, 93.4 to 97.4% of the administered dose was recovered, again suggesting that perchlorate was not metabolized.

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

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

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

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

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

Repeated dose studies in rats (Fisher, 1998a) and in humans (Channel, 1998a) to establish the kinetics of perchlorate at steady-state performed by AFRL/HEST to further characterize the 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[a]	Dose (mg perchlorate/kg)	[Iodide] (μg/g)	Percentage of Inhibition
2 hr	Control[b]	24.4	—
	0.01	21.3	13
	0.1	18.6	24
	1	7.4	70
	3	2.99	88
6 hr	Control[b]	46.5	—
	0.01	36.7	21
	0.1	32.0	31
	1	19.2	59
	3	9.13	80
9 hr	Control[b]	55	—
	0.01	49.2	11
	0.1	39.2	29
	1	24.7	55
	3	10.0	82

[a]Time points correspond to dosing with ^{125}I and to 4, 6, and 11 hr after dosing with ammonium perchlorate.
[b]Dosed with only iodide (33 μg/kg).

3.2 IODINE METABOLISM AND THYROID PHYSIOLOGY

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

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

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

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

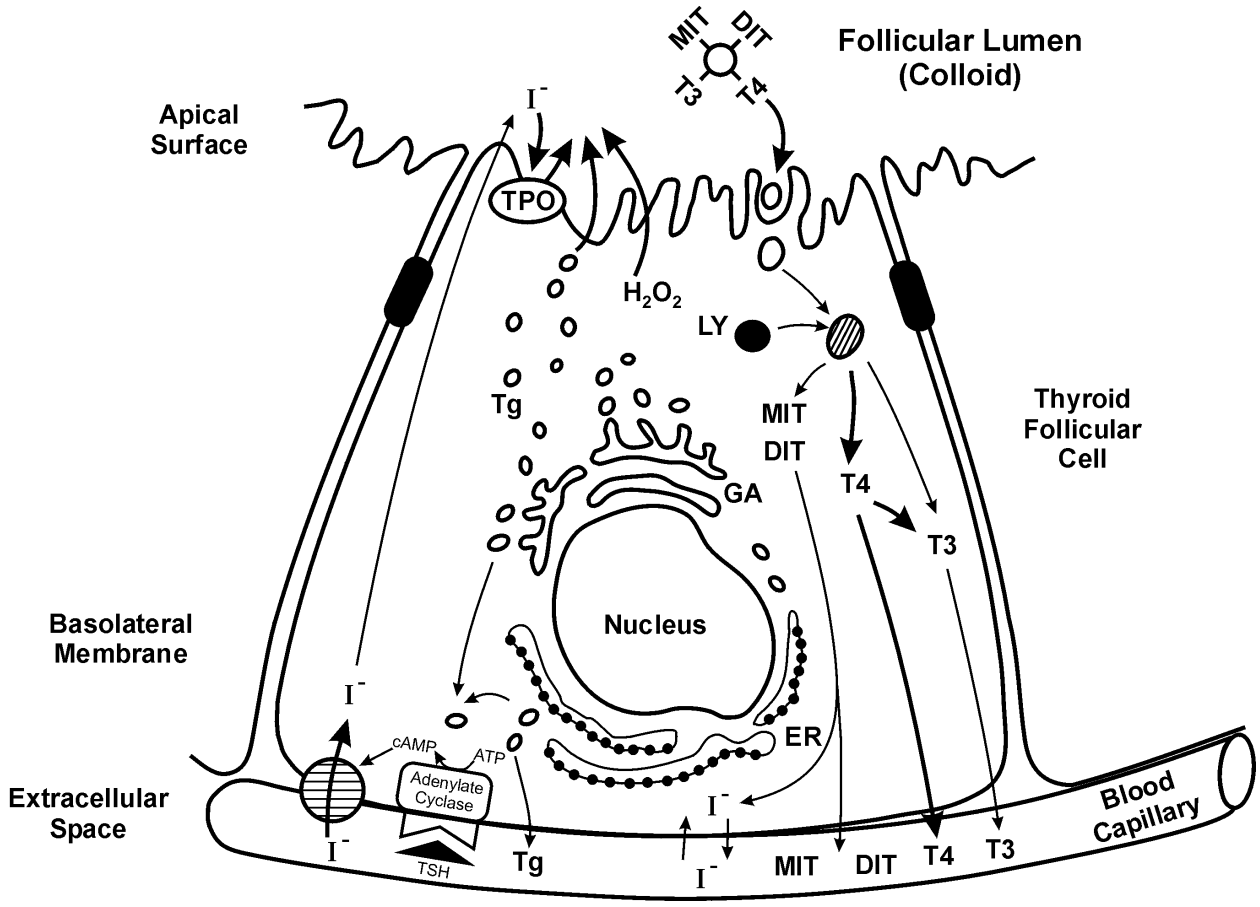

Figure 3-1. Schematic representation of thyroid hormone biosynthesis and secretion in a single thyroid follicular cell. (Modified from Hill et al., 1989; Cavalieri, 1997; and Fisher, 1996.)

fuse with the cell membrane and discharge their contents into the follicular lumen. Iodide enters the cell by active transport, and then, at the apical surface, is oxidized by thyroid peroxidase (TPO). The hydrogen-peroxide-generating system is represented by hydrogen peroxide (H_2O_2). Organification occurs at or near this apical cell-colloid interface; the oxidized iodide is incorporated into tyrosine residues in peptide linkage in Tg. Two iodinated tyrosyl groups couple in ether linkage to form tetraiodothyronine (T4), which initially remains trapped in Tg. Hormone secretion first involves pinocytosis of colloid-containing iodinated Tg (large open circle) at the apical border of the follicular lumen and resolution into vesicles that fuse with lysosomes (LY, dark circle). Lysosome proteolysis (striated circle) then converts Tg to amino acids, T4,

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

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

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

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

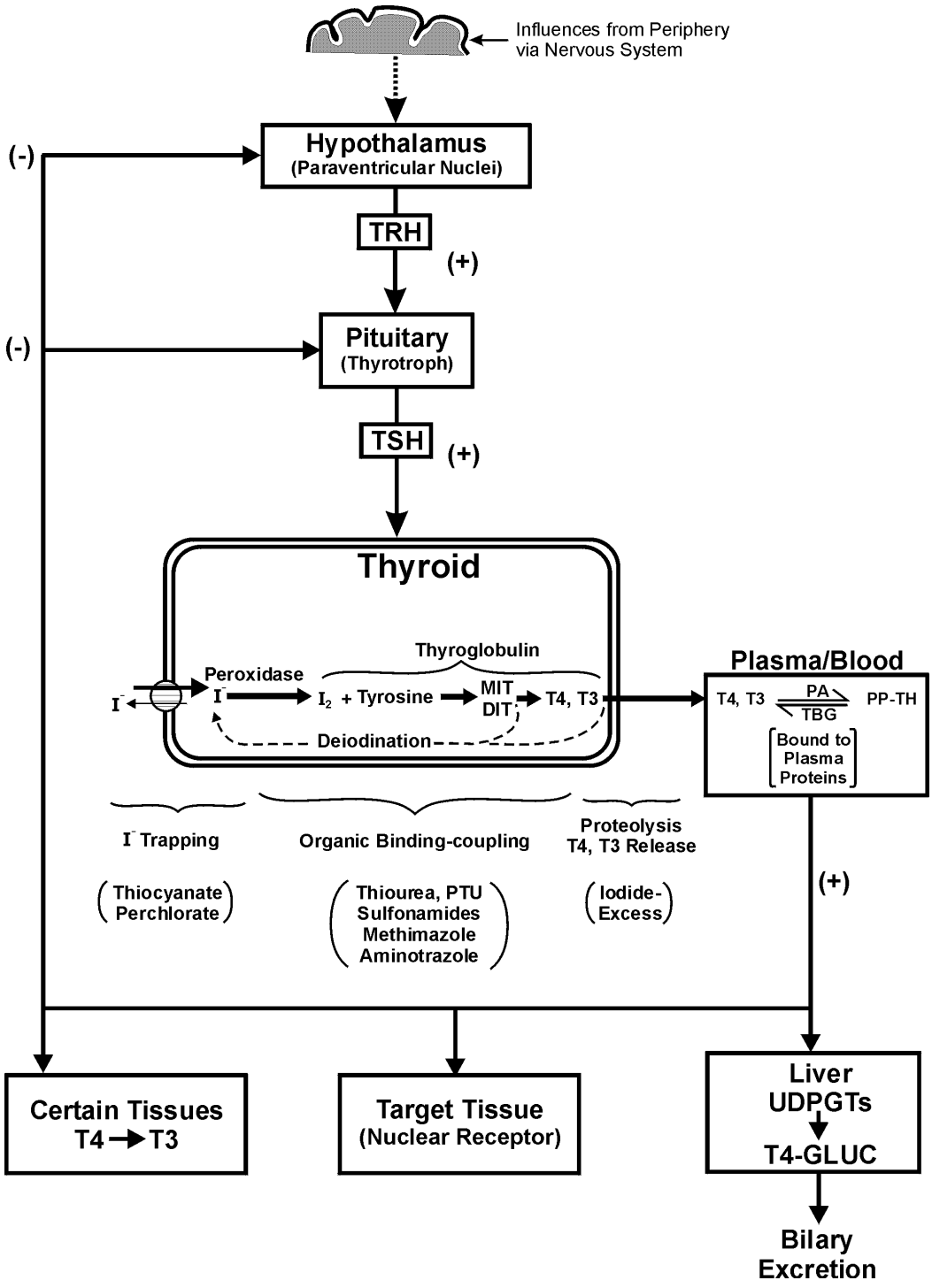

Figure 3-2. Schematic of the hypothalamic-pituitary-thyroid axis and feedback mechanisms (PP-TH = plasma protein-thyroid hormone, PTU = propylthiouracil, UDPGT = uridine diphosphyl glucuronosyl transferase, T4 GLUC = T4-glucuronide conjugate). (Modified from U.S. Environmental Protection Agency, 1998a;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

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

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

3.3 TOXICOKINETICS OF PERCHLORATE

Because of the complex anatomy of the thyroid follicle, all of the locations where perchlorate inhibition is exerted remain to be established (Wolff, 1998). Perchlorate has been established as a competitive inhibitor of iodide uptake across the basolateral membrane (i.e., acts by the inhibition at NIS). Figure 3-3 shows a comparison of the molecular dimensions of perchlorate and iodide. The following potency series was constructed for monovalent anion-based inhibition of iodide transport in thyroid slices: $TcO_4^- \geq ClO_4^- > ReO_4^- > SCN^- > BF_4^- > I^- > NO_3^- > Br^- > Cl^-$ (Wolff, 1998). However, it is not clear whether this anion sequence, measured at very high concentrations, has any mechanistic relation to what occurs at low concentrations in the thyroid. It is important to determine which solution properties of the anions determine this sequence (e.g., crystal radius, hydrated radius, hydration enthalpy, charge density). Strong base anion-exchange resins (usually a large cation with a weak field) exhibit a marked preference for ClO_4^- (e.g., compared to Cl^-); thus, it seems likely that selectivity for iodide or perchlorate in the

TABLE 3-2. INTERSPECIES AND INTRASPECIES DIFFERENCES IN THYROID STRUCTURE AND T3, T4, AND TSH HORMONES (U.S. Environmental Protection Agency, 1998a)

Parameter	Human	Rat
Thyroxine-binding globulin	Present	Essentially absent
T_4 Half-life	5 to 6 Days	0.5 to 1 Day
T_3 Half-life	1 Day	0.25 Day
T_4 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	$M^a = F^a$	$M \leq 2 \times F$
Cancer sensitivity	$F = 2.5 \times M$	$M > F$

[a]M = male, F = female.

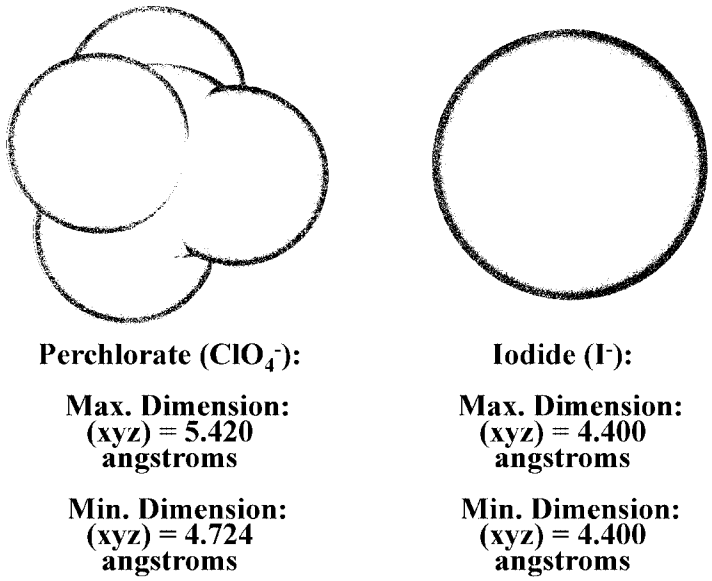

Figure 3-3. Comparison of the molecular dimensions for the perchlorate (left) and iodide (right) anions.

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

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

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

3.4 TOXICODYNAMICS OF THYROID HORMONE PERTURBATIONS

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

3.4.1 Carcinogenic Effects

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

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

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

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

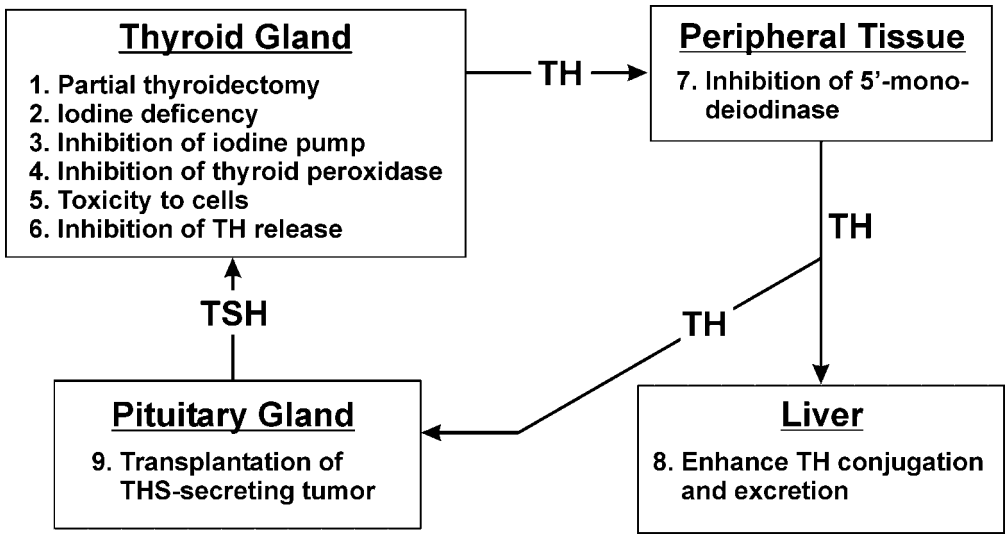

Figure 3-4. Schematic of antithyroid effects that influence thyroid carcinogenesis. (U. S. Environmental Protection Agency, 1998a; and Hill et al., 1998).

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

- **DNA Directed**
  - – X rays
  - – ^{131}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

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

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

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

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

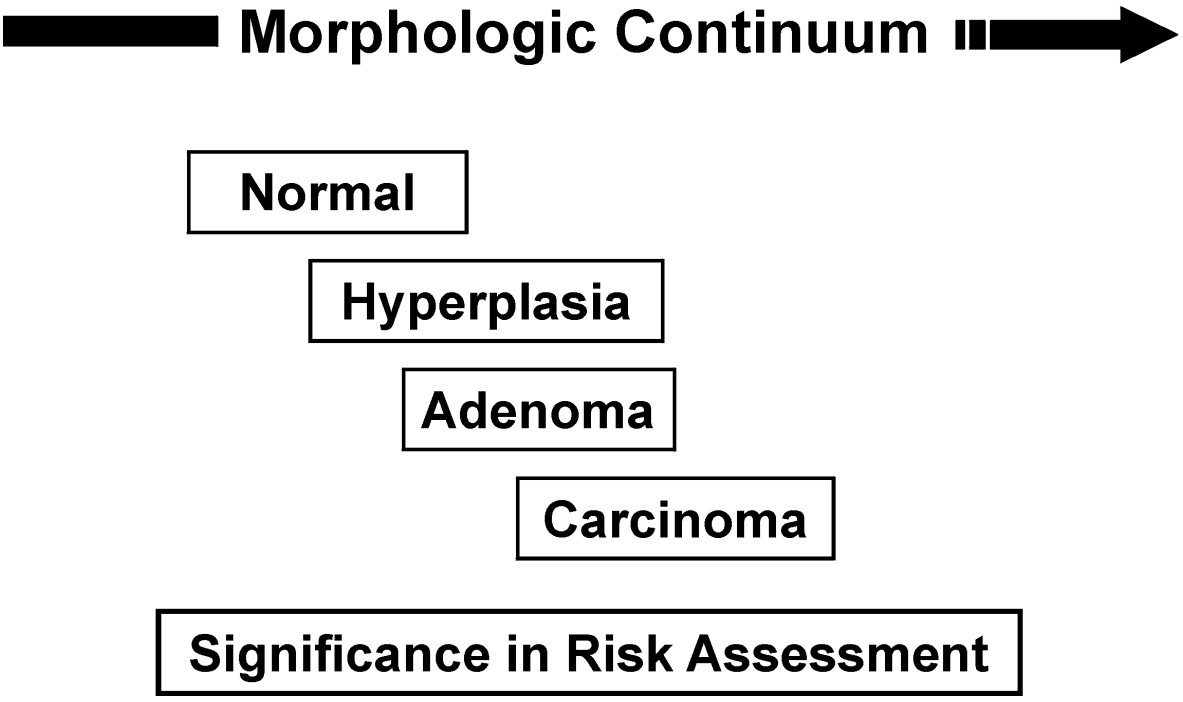

Figure 3-5. Proliferative changes involved in the multistage characterization of thyroid follicular cell neoplasia in rodents represent a morphologic continuum. Although these lesions typically are classified as discrete entities, the overlap in morphologic features should be emphasized because only 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).

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

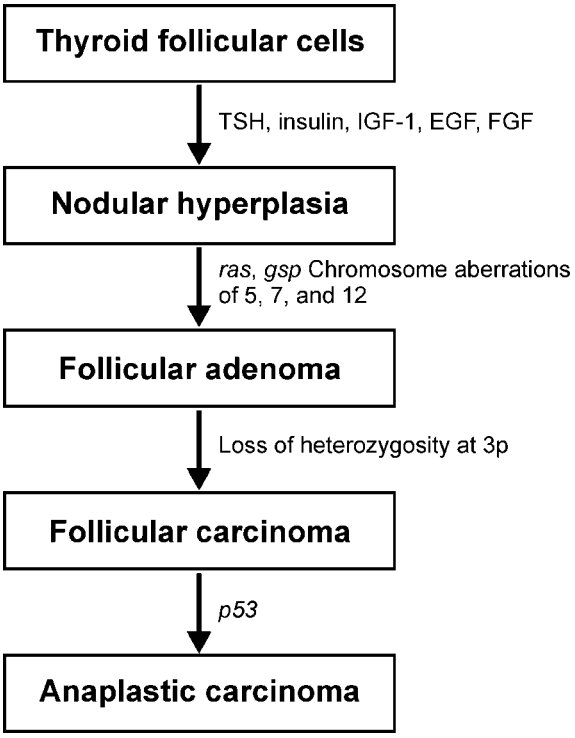

Figure 3-6. Possible molecular events in human thyroid follicular carcinogenesis (*ras* = *ras* protooncogene, *gsp* = GTP-binding protein mutation, *p*53 = *p*53 tumor suppressor gene) (U.S. Environmental Protection Agency, 1998a and Hill et al., 1998).

policy that recognizes the role of mode-of-action information regarding thyroid-pituitary disruption and mutagenesis to potential thyroid carcinogenesis (U.S. Environmental Protection Agency, 1998a).

- It is presumed that chemicals that produce rodent thyroid tumors may pose a carcinogenic hazard for the human thyroid.
- In the absence of chemical-specific data, humans and rodents are presumed to be equally sensitive to thyroid cancer caused by thyroid-pituitary disruption. This is a conservative position when thyroid-pituitary disruption is the sole mode of action because rodents appear to be more sensitive to this carcinogenic mode-of-action than are humans. When the thyroid carcinogen is a mutagenic chemical, the possibility that children may be more sensitive than adults needs to be evaluated on a case-by-case basis.

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

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

3.4.2 Neurodevelopmental Deficits and Other Potential Adverse Effects Resulting from Thyroid Hormone Disruption

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

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

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

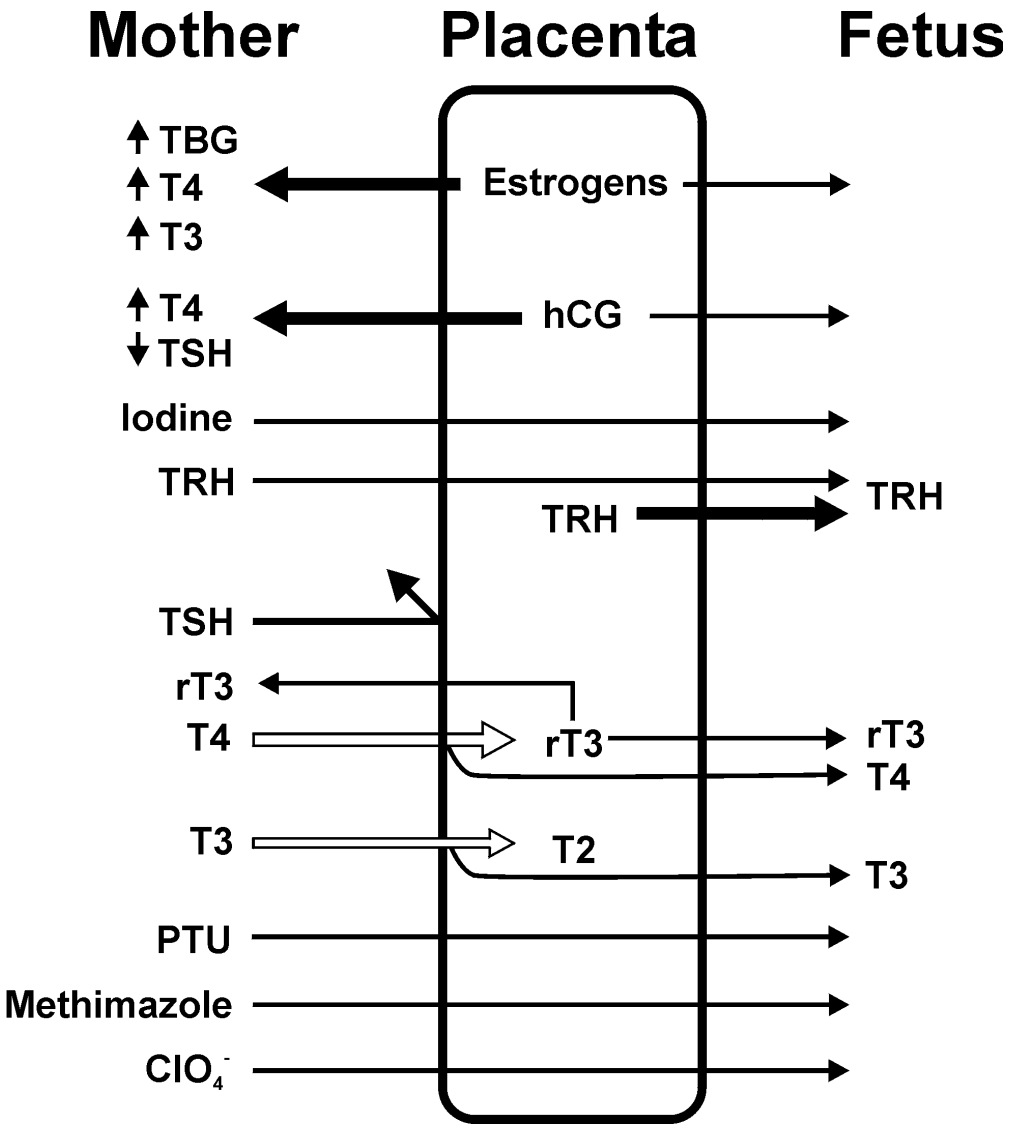

Figure 3-7. Schematic representation of the role of the placenta in thyroid hormone metabolism during human pregnancy. The placenta produces estrogens and hCG that increase maternal TBG levels and stimulate maternal thyroid hormone production, respectively. Both activities tend to increase maternal T4 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

Developmental *(Transient disruption leads to permanent effects.)*	**Adult** *(Transient disruption leads to transient effects.)*
• Delayed reflex ontogeny • Impaired fine motor skills • Deaf-mutism, spasticity • Gait disturbances • Mental retardation • Speech impairments	• Run down, slow, depressed • Sluggish, cold, tired • Dryness and brittleness of hair • Dry and itchy skin, constipation • Muscle cramps • Increased menstrual flow • Thyroid tumors in rodents

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

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

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

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

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

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

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

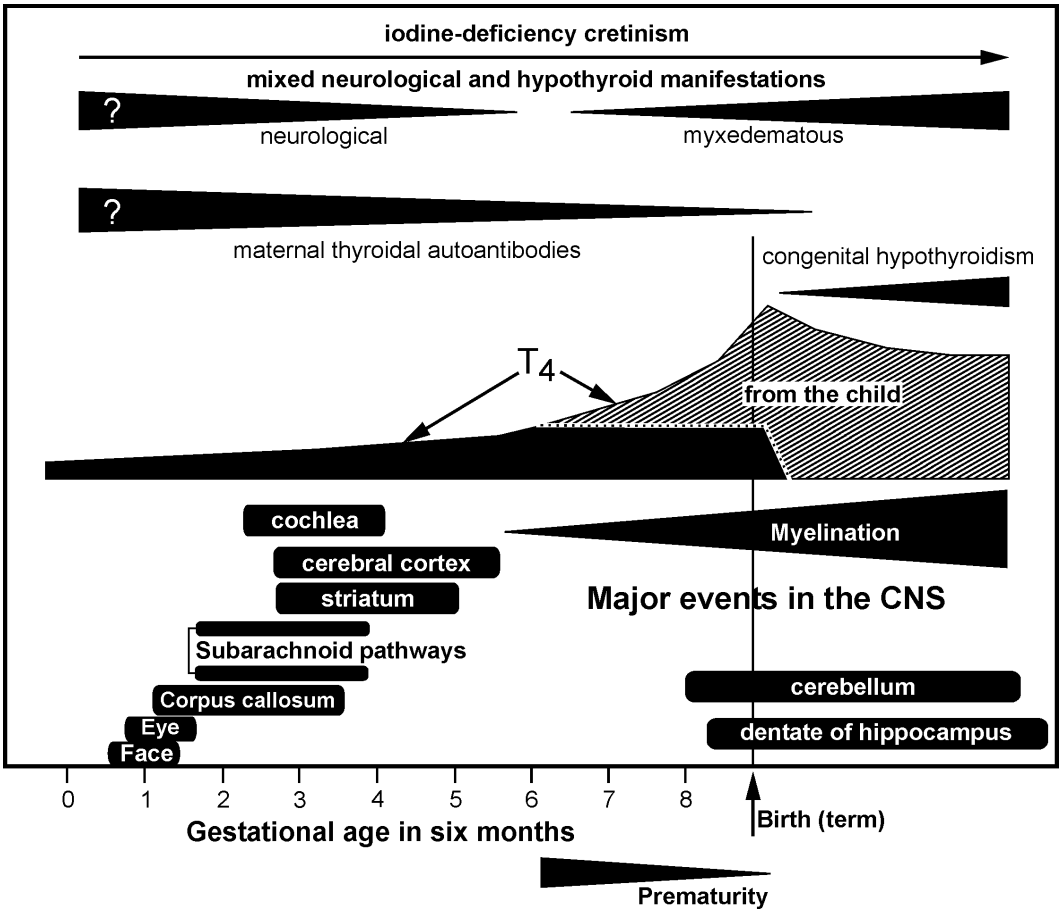

Figure 3-8. Approximate timing of major insults to the brain resulting from hypothyroxinemia, superimposed on major neurodevelopmental events in humans. Conditions resulting in early maternal hypothyroxinemia, combined to later impairment of the fetal thyroid, are the most damaging, with central nervous 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).

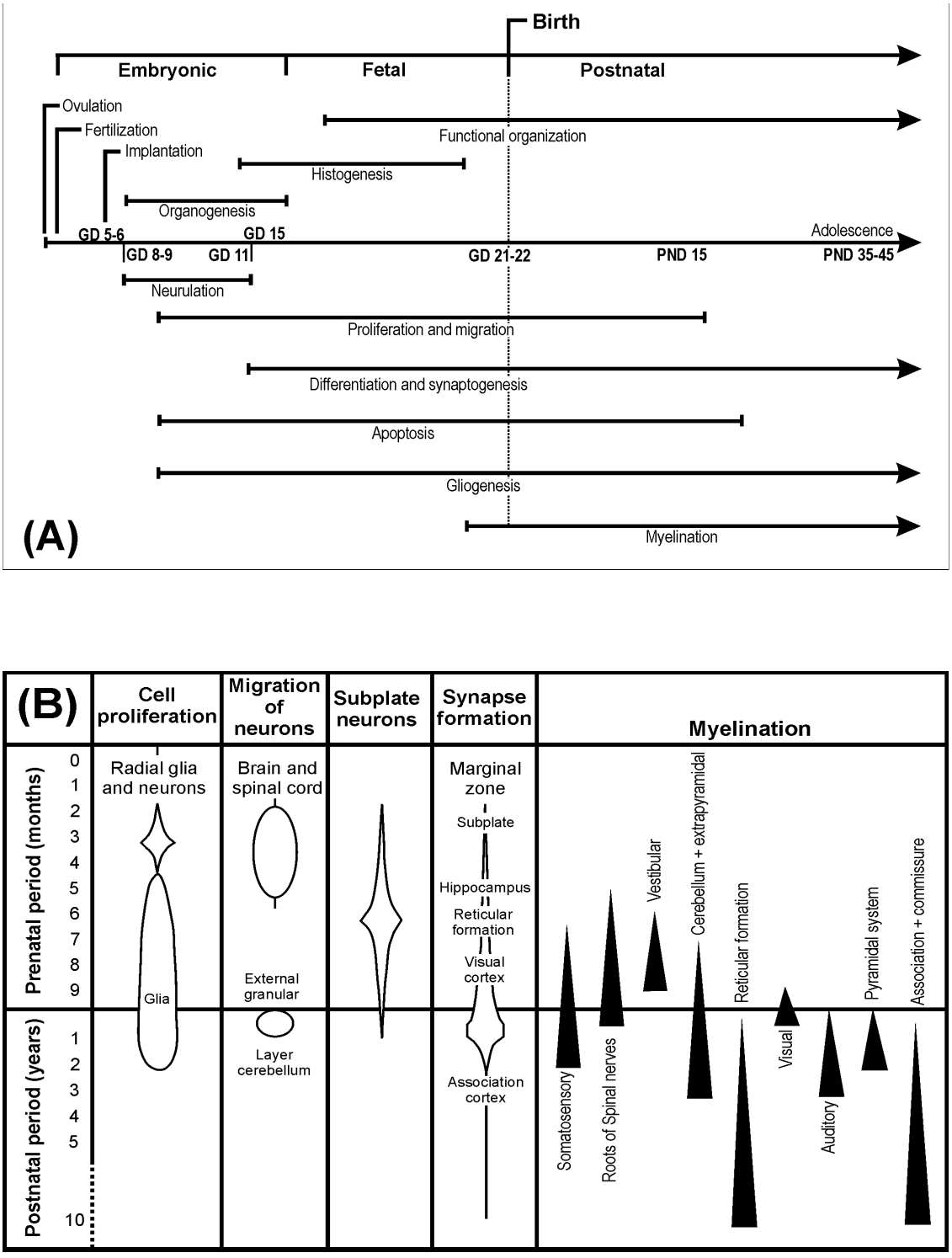

Figure 3-9. Timelines of developmental processes in the nervous system of rats (a) and humans (b). Rat timeline is compared to timing of fertilization, organogenesis, and histogenesis. Human perinatal period is scaled in months and the postnatal development is scaled in years (Rice and Barone, 2000).

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

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

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

Age or Status	Adequate Intake (AI) μg/day	Estimated Average Requirement (EAR) μg/day	Recommended Dietary Allowance (RDA) μg/day
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

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

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

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

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

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

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

3.5 DEVELOPMENT OF A TOXICITY TESTING STRATEGY BASED ON MODE OF ACTION

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

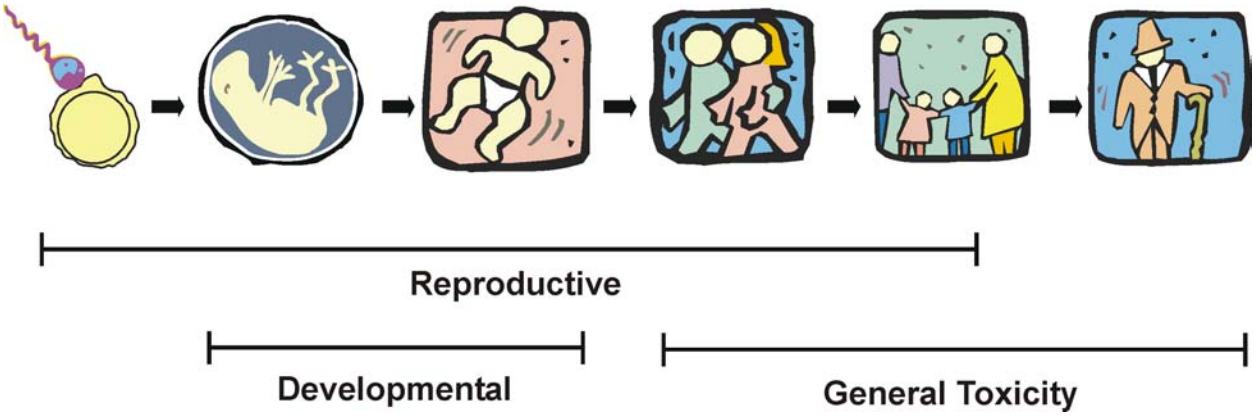

Figure 3-10. Schematic illustrating that a high confidence RfD is based on data that address all potentially critical stages over a lifetime.

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

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

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

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

[a]Rationale is to use different species to evaluate variability in species sensitivity unless a particular laboratory animal model is more appropriate.
[b]Rationale is to address all potentially critical life stages.

TABLE 3-7. FACTORS FOR UNCERTAINTIES IN APPLIED EXTRAPOLATIONS USED TO DERIVE REFERENCE DOSES[a]

10_H	–	Human to sensitive human
10_A	–	Experimental animal to human
10_S	–	Subchronic to chronic duration
10_L	–	LOAEL(HEE)[a] to NOAEL(HEE)[a]
10_D	–	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).

[a]HEE = human equivalent exposure.

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

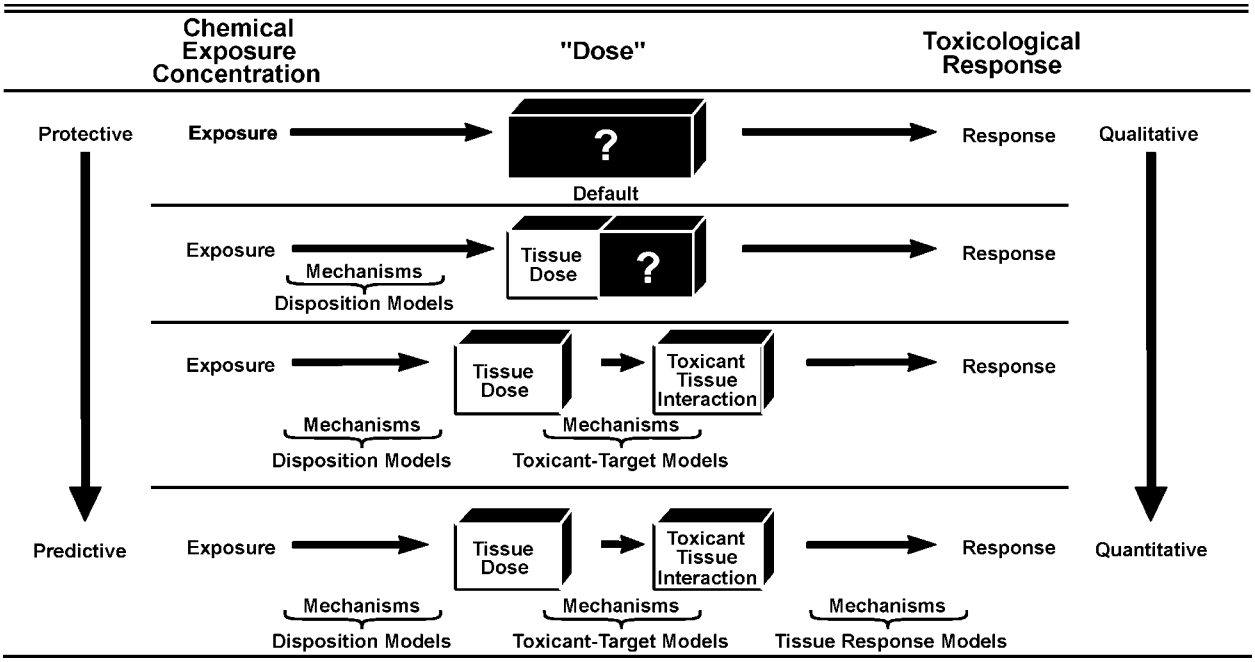

Figure 3-11. Schematic characterization of comprehensive exposure-dose-response continuum and the evolution of protective to predictive dose-response estimates (U.S. Environmental Protection Agency, 1994 and Jarabek 1995b).

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

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

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

TABLE 3-8. PERCHLORATE PEER REVIEW RECOMMENDED STUDIES SUMMARY

Study	Description	Potential Use in Assessment
90-Day subchronic bioassay + TH[a] + 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 kinetices 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

[a]Thyroid hormones (T4 and T3); Thyroid stimulating hormone (TSH), a pituitary hormone, was also assayed in those studies.

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

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

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

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

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

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

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

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

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

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

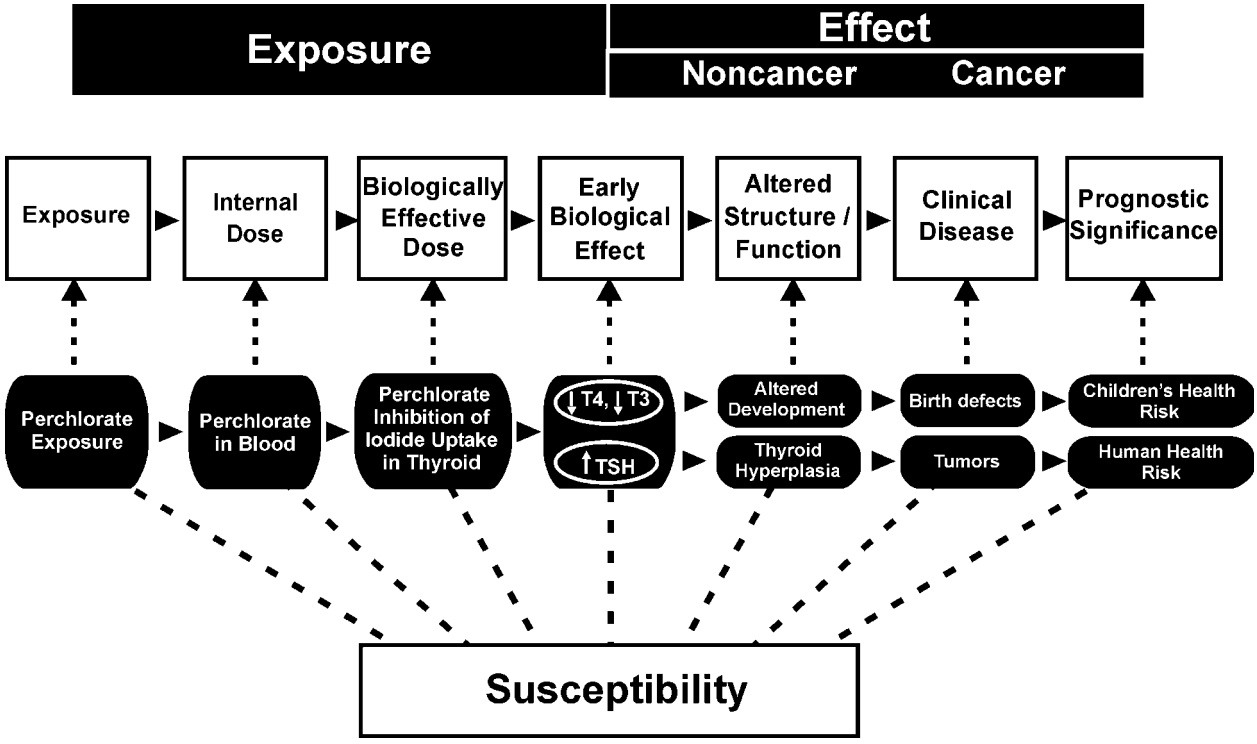

Figure 3-12. Mode-of-action model for perchlorate toxicity proposed by the U.S. EPA (U.S. Environmental Protection Agency, 1998d). Schematic shows the exposure-dose-response continuum considered in the context of biomarkers (classified as measures of exposure, effect, and susceptibility) and level of 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

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

4.1 EPIDEMIOLOGICAL DATA

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

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

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

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

4.1.1 Ecological Studies

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

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

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

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

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

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

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

In a study of the general population, Li et al. (2001) investigated physician-generated medical insurance claims for thyroid problems in a Medicaid insured population in Nevada, comparing all counties that were known not to have perchlorate contaminated drinking water with the one county that had contamination at approximately 10 μg/L. This was a study of period-prevalence, i.e., the proportion of the population that had claims for thyroid-related disorders anytime during a two-year period. Incident cases could not be identified within this database. Thyroid patients were defined as having one or more of the following diagnoses of thyroid disease according to the International Classification of Diseases, 9th Revision (ICD-9): (1) simple and unspecified goiter (ICD-9 Code 240); (2) non-toxic nodular goiter (ICD-9 Code 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).

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

4.1.2 Occupational Studies

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

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

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

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

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

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

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

Working lifetime exposure estimates were calculated as:

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

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

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

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

calculate the deposition fraction, approximately 0.3 at 5 μm (U.S. Environmental Protection Agency, 1996b).

The assumption about the solubility of the inhaled particles is also problematic because this parameter is particle-diameter dependent. The particle diameter dictates the location (extrathoracic, tracheobronchial, pulmonary) where the particle deposits and the local milieu and clearance vary with location also influence solubility (U.S. Environmental Protection Agency, 1996b; Snipes et al., 1997). The solubility of cesium chloride (CsCl) in beagles was used to estimate a fraction absorbed. Although CsCl and NH_4ClO_4 may have similar solubilities, additional uncertainty is introduced because the CsCl particle diameter or inhalability function for the beagles was not taken into account; and the hydroscopicity, which influences the initial deposition site, may not be the same. The assumptions about dose could have been validated with a mass balance approach. For example, perchlorate could have been measured in the blood when samples were taken for thyroid hormone analyses. Additionally, urine samples could have been monitored for perchlorate because it is excreted in the urine. These additional measurements would have afforded some confidence that the inhaled dose estimates were reasonable.

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

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

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

Estimated doses for the single shift-study ranged from 0.0002 to 0.436 mg/kg-day with a mean of 0.036 mg/kg-day and median of 0.013 mg/kg-day. The dose estimate was not a significant predictor of thyroid function parameters measured in 83 control (65 male, 18 female) or 18 exposed (15 male, 3 female) individuals. Working lifetime exposure estimates ranged from 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 the high-dose group. The duration of exposure ranged from 1 to 27 years (mean 8.3).

No significant correlations were detected in any measures of thyroid, bone marrow, liver, or kidney function; however, significant gender and race differences were apparent in the clinical tests of bone marrow, liver, and kidney functions. Females were slightly lower in hemoglobin, hematocrit, SGPT, GGTP, and creatinine than males; black workers were slightly lower than whites in hemoglobin and hematocrit and slightly higher in creatinine.

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

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

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

$$D = k[Ei - 0.354\ E0]/0.646. \qquad (4\text{-}3)$$

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

12 hours x 60 minutes / hour x 0.001 g/mg x 1 mg creatinine/min x [post-shift]/0.646. (4-4)

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

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

4.2 CLINICAL STUDIES

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

4.2.1 Studies in Healthy Human Subjects

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

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

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

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

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

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

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

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

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

TABLE 4-2. URINE AND SERUM PERCHLORATE (ClO_4^-) VALUES BEFORE, DURING, AND AFTER THE INGESTION OF 10 mg OF ClO_4^- DAILY FOR 14 DAYS (Lawrence et al., 2000)

Time	Urine Perchlorate[a] (mg/24 hr)	Serum Perchlorate[a] (μg/mL)
Baseline	< 0.5	0
7 Days ClO_4^-	7.7 ± 0.8[a]	0.61 ± 0.02
14 Days ClO_4^-	7.5 ± 1.0	0.59 ± 0.02
14 Days After ClO_4^-	< 0.5	0

[a]Mean ± SE.

TABLE 4-3. URINE AND SERUM IODINE VALUES BEFORE, DURING, AND AFTER THE INGESTION OF 10 mg OF ClO_4^- DAILY FOR 14 DAYS (Lawrence et al., 2000)

Time	Urine Iodine[a] (μg/24 hr)	Serum Iodine[a] (μg/dL)
Baseline	254 ± 69	6.5 ± 0.42[a]
7 Days ClO_4^-	233 ± 49	6.2 ± 0.34
14 Days ClO_4^-	385 ± 123	6.4 ± 0.37
14 Days After ClO_4^-	208 ± 42	6.3 ± 0.57

[a]Mean ± SE.

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

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

TABLE 4-4. THYROID ^{123}I UPTAKES BEFORE, DURING, AND AFTER THE INGESTION OF 10 mg ClO_4^- DAILY FOR 14 DAYS (Lawrence et al., 2000)

	Thyroid ^{123}I Uptake[a] (% Dose)		
Time	Baseline	14 days on ClO_4^-	14 days after ClO_4^-
4 Hours	12.5 ± 1.3	8.2 ± 0.7[b]	16.6 ± 2.4[c]
8 Hours	17.3 ± 1.9	10.6 ± 1.0[b]	21.9 ± 2.8[c]
24 Hours	23.6 ± 2.6	14.0 ± 1.6[b]	27.1 ± 3.3[d]

[a]mean ± S.E.
[b]$p < 0.01$ vs. baseline and after ClO_4^-.
[c]$p < 0.01$ vs. baseline.
[d]$p < 0.05$ vs. baseline.

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

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

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

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

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

The EPA has also been made aware of another human clinical study being performed at Loma Linda and funded by Lockheed Martin (Beck, 2001). The study is not yet completed because the objective sample size for each dose group has not yet been attained. Human euthyroid volunteers (male and non-pregnant females) have been dosed with perchlorate in gel caps at 0.007, 0.014, and 09.04 mg/kg-day. Measurements were made at baseline, 3-months, 6-months, and after recovery from exposure for RAIU, T3, T4, and TSH levels. These dosages are the same as already tested so the added value to the human database, especially with respect to the now prominent concern for neurodevelopmental effects secondary to hypothyroxinemia or even transient decrements in T4, is not readily apparent. The additional data may potentially reduce the variability and low power due to the small sample sizes of the previous studies if 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 (≈80%) of ^{131}I from the thyroids of Graves' disease patients previously treated with tracer amounts of ^{131}I and 1-methyl-2-mercaptoimidazole (MMIA). MMIA was given to cause accumulation of ^{131}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 ~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}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}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

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

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

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

[1]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.

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

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

4.2.2.1 Hematological Effects

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

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

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

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

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

4.3 SUMMARY OF CONCLUSIONS REGARDING HUMAN HEALTH EFFECTS STUDIES

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

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

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

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

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

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

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

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

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

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

	Publication	Study Population	ClO_4^- Source and Levels	Duration	Outcomes studied	Findings	Problems/Comment
1	Gibbs JP, Ahmad R, Crump KS, et al JOEM 1998; 40:1072-1082. *Evaluation of a population with occupational exposure to airborne ammonium perchlorate for possible acute or chronic effects on thyroid function.*	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 $\mu m/m^3$ using closed face cassettes	1 day 1-27 yr.	T3U, T4, FTI, TSH, liver, kidney and hematol fcn T4: 7.5 μg/dL TSH: 2.0 μIU/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. **Indications of chronic effects.**
2	Lamm SH, Braverman LE, Li FX, et al. JOEM 1999; 41:248-260. *Thyroid health status of ammonium perchlorate workers: a Cross-sectional occupational health study.*	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. *The use of perchlorate for the prevention of thyrotoxicosis in patients given iodine rich contrast agents.*	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_4^- Source and Levels	Duration	Outcomes studied	Findings	Problems/Comment
4	Li FX, Squartsoff L, Lamm SH. JOEM 2001; 43:630-634, *Prevalence of thyroid diseases in Nevada counties with respect to perchlorate in drinking water.*	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). **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.** 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. *Does perchlorate in drinking water affect thyroid function in newborns or school-age children?*	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, hematol, liver, kidney, prev:goiter, prev:family H_x thy disease T4:10.0 μg/dL TSH: 3.0 μIU/mL	Did comparisons across cities. Urinary I/creatine 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_x 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. **Significant paradoxical effects indicate uncontrolled confounding and inappropriate thy fcn model in relation to P in this population**. Possible role of ambient temperatures.
6	Lawrence JE, Lamm SH, Pino S, Richman K, Braverman LE. Thyroid 2000; 10:659-663. *The effect of short-term low-dose perchlorate on various aspects of thyroid function.*	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.	Hematol, liver test results clinically "normal" but no data presented. **Inappropriate assessment: clinical rather than epidemiological**. T3 effect not addressed; dietary I not controlled or reported. **Suggests long term iodine depletion.**

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

	Publication	Study Population	ClO_4^- Source and Levels	Duration	Outcomes studied	Findings	Problems/Comment
7	Lawrence JE, Lamm S, Braverman LE. Thyroid 2001. 11:295 (letter) *Low dose perchlorate (3 mg daily) and thyroid function.*	8 healthy volunteers	Potassium perchlorate 3 mg/day	14 days	T3, T4, FTI, TSH, THBR, RAIU, liver, hematol	No signif changes (data not presented) except for depressed I-uptake at 14 days (10%) with significant rebound (22%) at 28 days;	**Implies some I depletion over 2 weeks at 3 mg/day** (seen by other investigators at 1.4 mg/day).
8	Lamm SH, Doemland M. JOEM 1999; 41:409-411. *Has perchlorate in drinking water increased the rate of congenital hypothyroidism?*	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= $35/10^5$) —	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. **Identification of cases is limited by screening procedure that dues not consider age at screen, ethnicity and birthweight.** Unable to address transient developmental sequelae.
9	Li Z, Li FX, Byrd D, et al. and Lamm. JOEM 2000; 42:200-205. *Neonatal thyroxine level and perchlorate in drinking water.*	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.	**These T4 levels are much higher than in other neonate studies (7-10).** 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. *Neonatal thyroid-stimulating hormone level and perchlorate in drinking water.*	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. **Insensitive to developmental issues and short-term time variability of P exposure.**

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

	Publication	Study Population	ClO_4^- Source and Levels	Duration	Outcomes studied	Findings	Problems/Comment
11	Brechner RJ, Parkhurst GD, Humble WO et al. JOEM 2000; 42:777-782. *Ammonium perchlorate contamination of Colorado River drinking water is associated with abnormal thyroid function in newborns in Arizona.*	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. *Gestational exposure to perchlorate is associated with measures of decreased thyroid function in a population of California neonates.*	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. **This study presents strong evidence of perchlorate health effects in neonates from drinking water contamination with perchlorate.**

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

	Publication	Study Population	ClO_4^- Source and Levels	Duration	Outcomes studied	Findings	Problems/Comment
13	Soldin OP, Braverman LE, Lamm SH. Therapeutic Drug Monitoring 2001; 23:316-331. *Perchlorate clinical pharmacology and human health: a review.*	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_x = 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^+)-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

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

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

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

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

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

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

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

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

TABLE 5-1. BENCHMARK DOSE (BMD)[a] AND BENCHMARK DOSE LOWER CONFIDENCE LIMIT (BMDL)[a] 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	χ^2 [b]	Exp[c]	BMD	BMDL	χ^2 [b]	Exp[c]	BMD	BMDL	χ^2 [b]	Exp[c]
1. Caldwell Tbls. 1 and 2	0, 1.25, 5, 12.5, 25, 50, 125, 250	13.29	0.72	0.97	4.37	Not done[d]				35.29	0.78	0.20	0.88
2. Subchonic, 14-day Tbls. 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[e]			
3. Subchronic, 90-day Tbls. 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 Tbls. 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 Tbls. 10 and 11	0, 0.1, 1, 3, 10	0.45 0.53	0.009 0.33	0.46 0.67[f]	0.94 1.0	0.92 1.27	0.24 0.88	.024 0.26[f]	0.81 1.0	15.18 11.02	1.86 3.62	0.70 0.32[f]	0.36 1.0
7. Neurobehav., adult Tbls. 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 Tbls. 14 and 15	0, 0.3, 3, 30	1.97	0.11	0.68	3.84	Poor fit[g]				7.89	2.44	0.41	0.72
9. 2-gen., P2 Tbls. 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 Tbls. 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 Tbls. 20 and 21	0, 0.3, 3, 30	Poor fit				1.19	0.32	0.25	0.52	NOE			
BMDL Range: Rat Studies		**0.009 - 0.90**				**0.008 - 0.74**				**0.0004 - 3.62**			
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

[a] Units of mg/kg-day.
[b] χ^2 p-value.
[c] Exponent in Weibull model fit not restrained to ≥ 1.0 unless indicated.
[d] Not done: Because of non-routine staining, cytological characteristics were not adequate to make determination of hypertrophy on these samples (Wolf, personal communication).
[e] No observed effect (NOE): Either no incidence of endpoint noted in animals tested or no notable difference between dosed and controls.
[f] Exponent in Weibull model fit restrained to ≥ 1.
[g] Poor fit: $p < 0.05$ for χ^2 test.

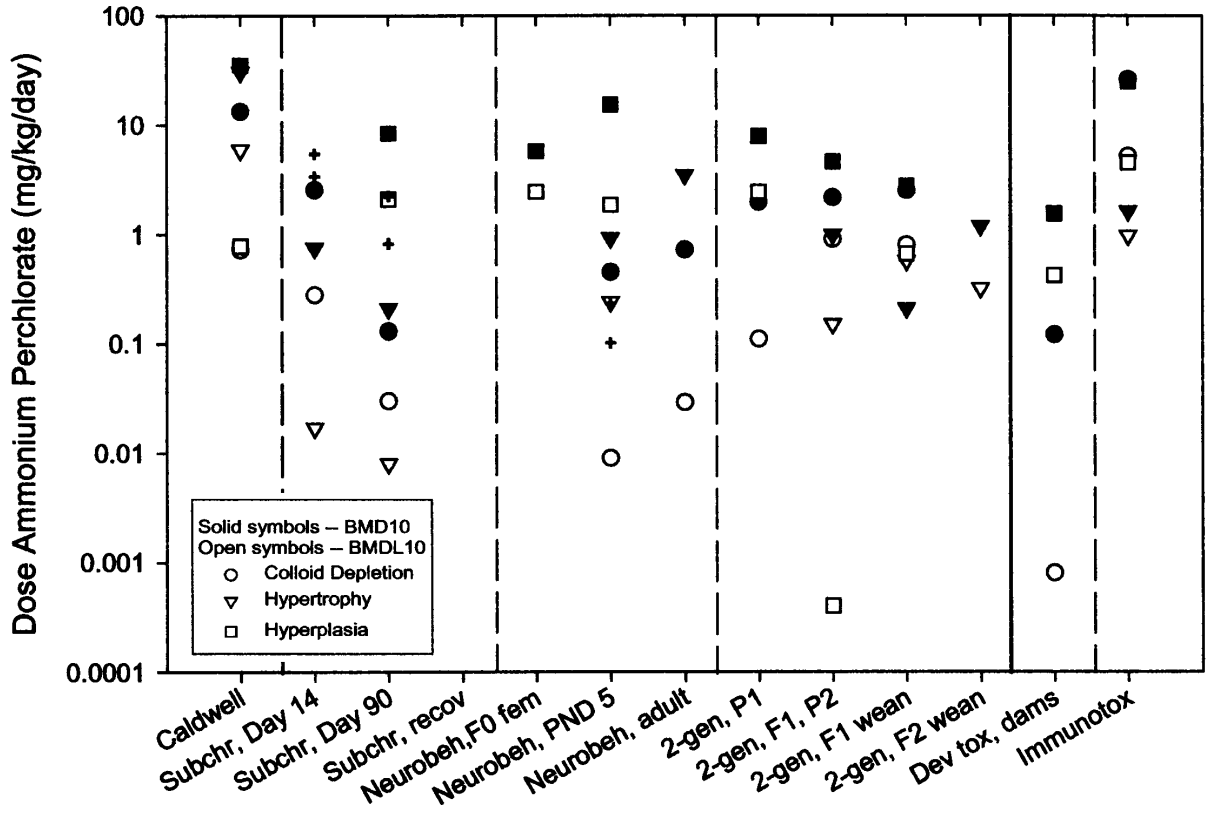

Figure 5-1. Benchmark dose (BMD) and benchmark dose lower limit (BMDL) estimates recalculated for thyroid histopathology based on 2000 Pathology Working Group review (Wolf, 2000; 2001). Data on incidence of colloid depletion, thyroid hypertrophy and thyroid hyperplasia were submitted to the EPA for the 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).

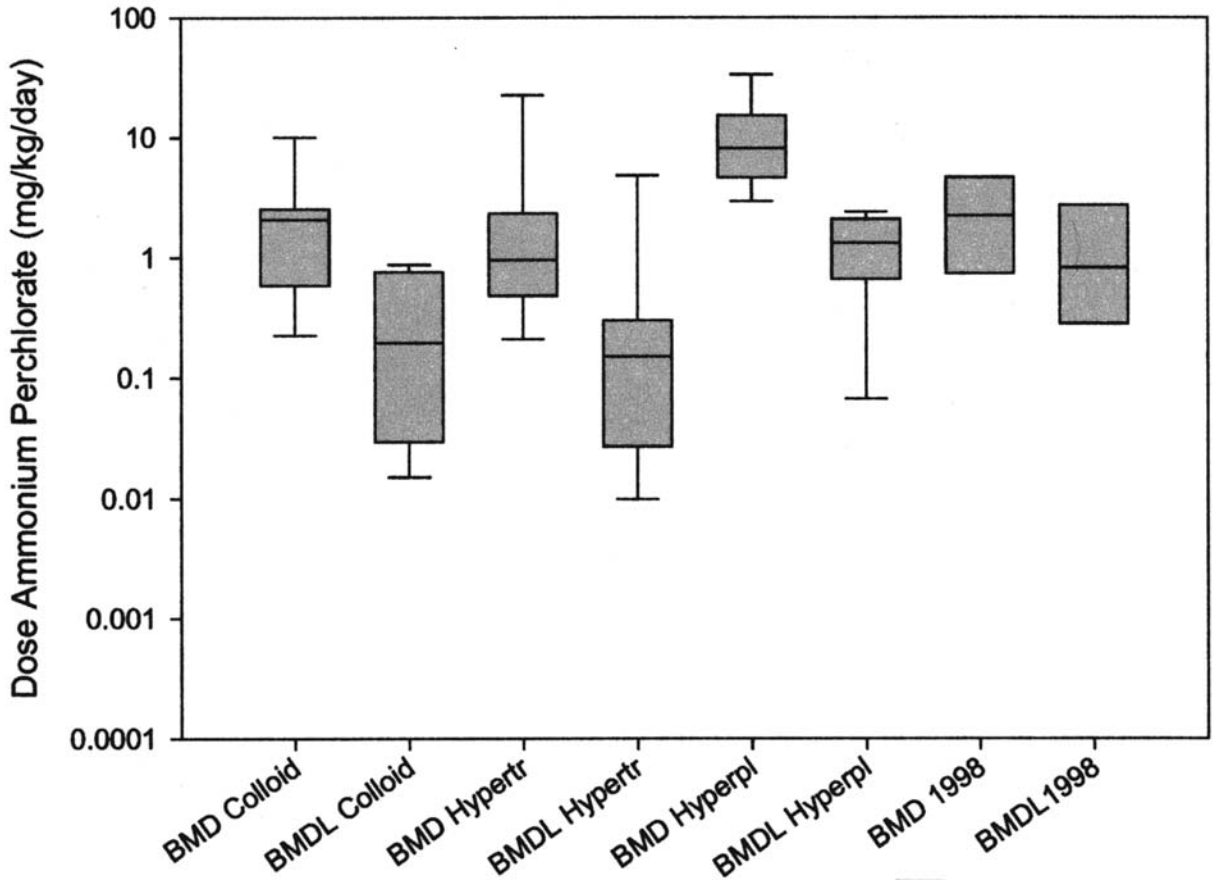

Figure 5-2. Distribution of BMD and BMDL estimates shown by "box and whisker" plots of colloid depletion (colloid), hypertrophy (hyptry), and hyperplasia (hyppls) from rat studies recalculated for thyroid histopathology based on 2000 Pathology Working Group review (Wolf, 2000; 2001). Values are presented 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 25th percentile, a line within the box denotes the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers above and below the box indicate the 90th and 10th 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).

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

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

5.1 CHRONIC STUDIES AND GENOTOXICITY ASSAYS

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

It should be noted that perchlorate exposure also caused a statistically-significant increase 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 NOAEL	Original Analyses LOAEL	Re-Analyses[a,b] NOAEL	Re-Analyses[a,b] LOAEL
Rat 14-Day (Caldwell et al., 1995)	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	**—**	**0.12**
		hTg	M	—	0.11	—	0.11
			F	—	0.12	—	0.12
		rT3	M	0.44	1.11	**0.11**	**0.44**
			F	0.47	1.23	**0.12**	**0.47**
Rat Subchronic Study (Springborn, 1998)	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	**—**	**0.05**
			F				
		TSH	M	0.05	0.2	**0.01**	**0.05**
			F	0.01	0.05	**—**	**0.01**

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[a,b]	
				NOAEL	LOAEL	NOAEL	LOAEL
Rat Subchronic Study (Springborn, 1998) (cont'd)	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
		TSH	M	0.05	0.2	0.05	0.2
			F				
Rat Subchronic Study (Springborn, 1998)	120-Day *(0, 0.05,1.0, 10.0)*	T3	M	1.0	10.0	1.0	10.0
			F				
		T4	M	—	0.05	**—**	**0.05**
			F			**1.0**	**10.0**
		TSH	M	10.0	—	**—**	**0.05**
			F				
Rat Developmental Neurotoxicity Study (Argus, 1998a)	PND5 *(0, 0.1, 1.0, 3.0, 10.0)*	Lumen size	M	1	3	**0.3**	**3**
			F				
	PND90 *(0, 0.1, 1.0, 3.0, 10.0)*	Lumen size	M	Data not available for original analyses		**10**	**—**
			F			**10**	**—**
	PND5 *(0, 0.1, 1.0, 3.0, 10.0)*	T4		1.0	3.0	**0.1**	**1.0**
		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[a,b]	
				NOAEL	LOAEL	NOAEL	LOAEL
Mouse Hormone and Immunotoxicity (Keil et al., 1998)	14-Day *(0.0, 0.1, 1.0, 3.0, 30)*	T4	M	3.0	30.0	—	**0.1**
		T3	M	Data not available at time of 1998 analysis		—	**0.1**[c]
		TSH	M	No data			
	90-Day *(0.0, 0.1, 1.0, 3.0, 30)*	T4	M	0.1	3.0	—	0.1[c]
		T3	M	Data not available at time of 1998 analysis		—	**0.1**[d]
		TSH	M	30.0	—	—	**0.1**[d]
	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	—	—	—
Rabbit Developmental Toxicity (Argus, 1998b)	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	—

[a]Bold indicates where 2001 analyses is different than 1998 analyses.
[b]Results from the liberal and conservative statistical approaches were the same.
[c]No dose response - 0.1 and 1.0 differ from control; 0.3 and 30.0 do not differ from control.
[d]No dose response - 0.1 and 1.0 differ from control; 0.3 and 30.0 do not differ from control.

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

5.1.1 Cancer Studies

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

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

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

5.1.2 Genotoxicity Assays

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

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

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

5.1.2.1 *In Vitro* Assays

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

333, and 100 μg/plate, with and without metabolic activation from Arochlor-induced rat and hamster livers. Ammonium perchlorate was neither toxic nor mutagenic under the conditions of the NTP assay.

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

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

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

5.1.2.2 *In Vivo* Assays

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

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

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

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

5.1.2.3 Summary of Genotoxicity Battery Results

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

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

5.2 GENERAL TOXICITY: SHORT-TERM AND SUBCHRONIC TESTING

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

5.2.1 Historical Data

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

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

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

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

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

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

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

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

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

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

5.2.2.1 Thyroid Histology Data

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

group. A separate computerized morphometric analysis of follicular lumen size was performed 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, and a statistically significant difference in the incidence of decrease in lumen size was evident in the males at the 1.11 mg/kg-day dose and, in females, at the 4.91 mg/kg-day dose; however, the gender-by-treatment effect was not taken into account. Relative thyroid weights were significantly increased in 11.44/11.47 and the 22.16/24.86 mg/kg-day dose groups compared to controls.

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

5.2.2.2 Thyroid and Pituitary Hormone Analyses

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

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

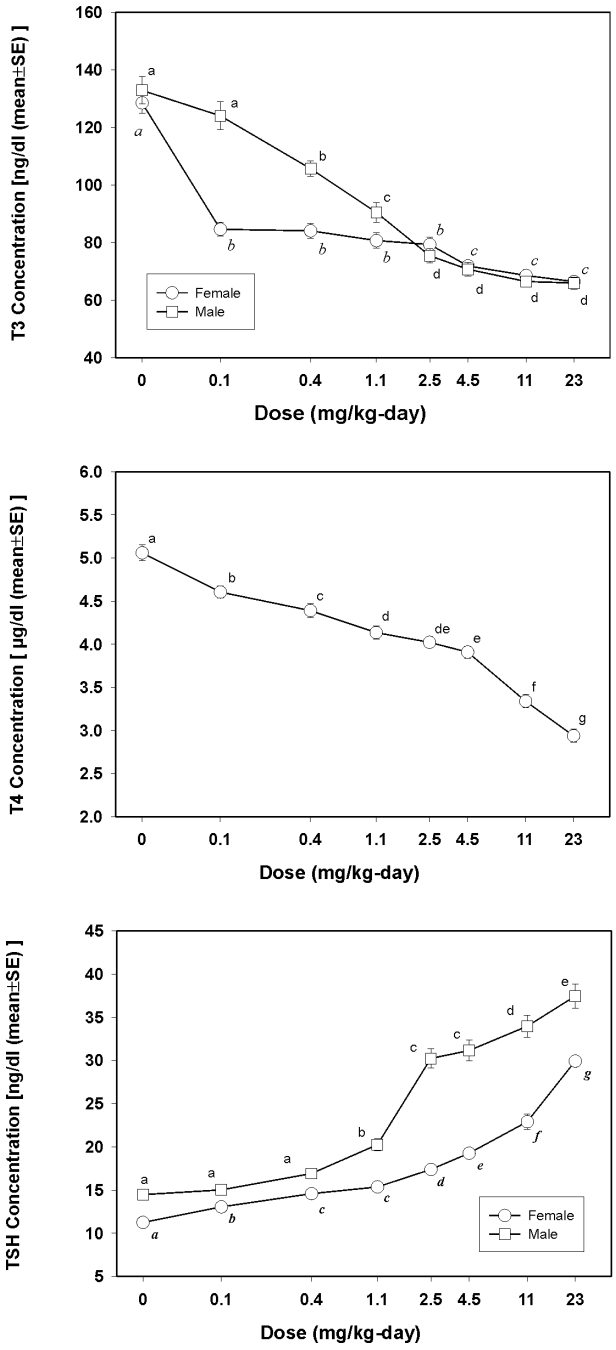

Figure 5-3. Effects in the Caldwell et al. (1995) study of 14-day drinking water administration of ammonium perchlorate to SD rats on serum total T3 (A), T4 (B), and TSH (C) concentrations (ng/mL; mean ± SE) as recalculated in Table 5-2 (Crofton and Marcus, 2001). Means with different letters were significantly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

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

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

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

5.2.3 The 90-Day Testing Strategy Bioassay in Rats

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

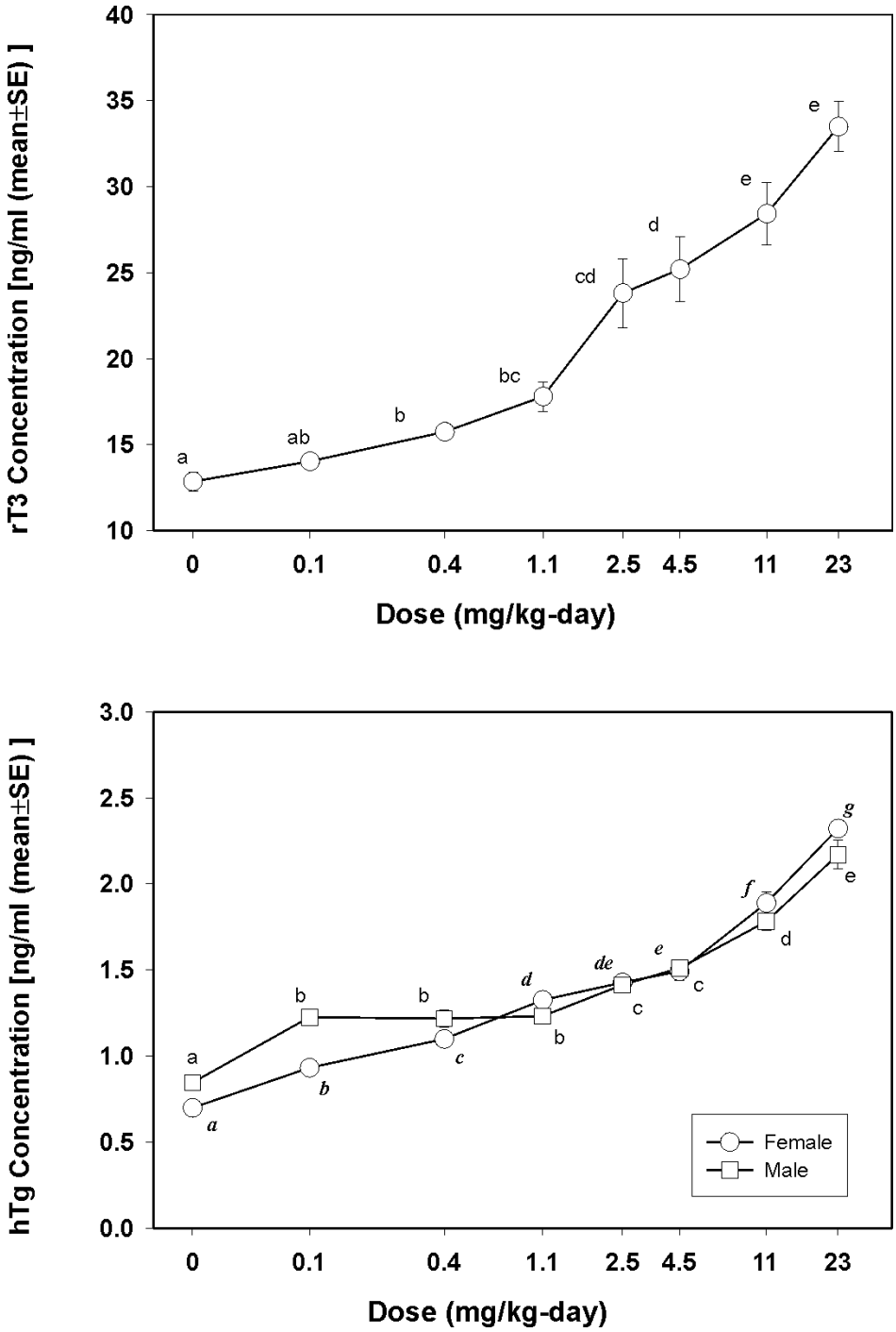

Figure 5-4. Effects in the Caldwell et al. (1995) study of 14-day drinking water administration of ammonium perchlorate to SD rats on serum rT3 (A) and hTg (B) concentrations (ng/mL; mean ± SE) as recalculated in Table 5-2 (Crofton and Marcus, 2001). Data of Channel (1998b) and Crofton (1998a). Means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

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

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

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

5.2.3.1 General Toxicity, Thyroid Histopathology Results, and Satellite Reproductive Assay

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

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

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

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

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

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

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

5.2.3.2 Thyroid and Pituitary Hormone Analyses

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

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

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

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

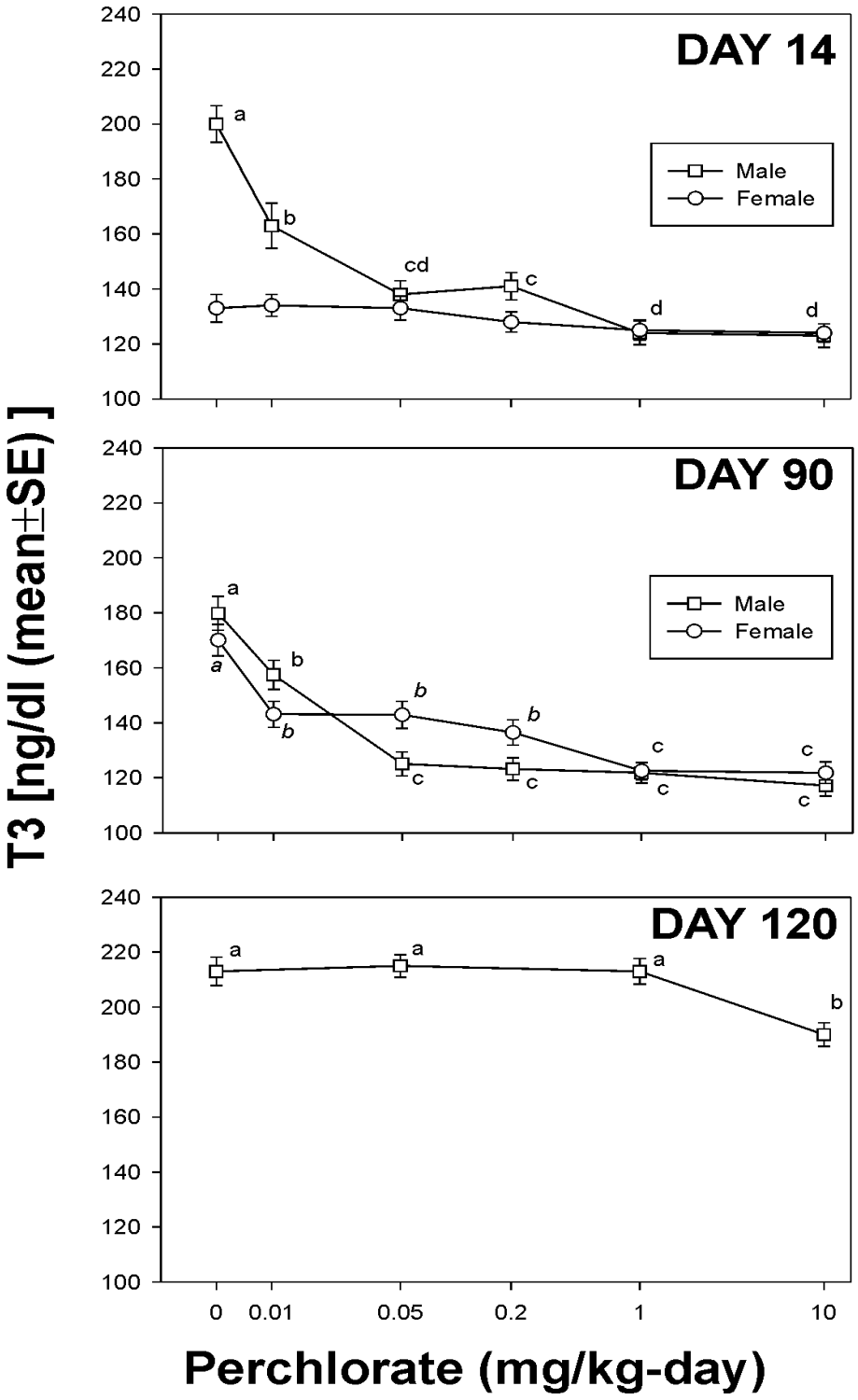

Figure 5-5. Effects from 90-day drinking water administration of ammonium perchlorate to SD rats on serum total T3 concentrations as recalculated in Table 5-2 (Crofton and Marcus, 2001). Means with different letters were statistically different ($p < 0.05$). The 120-day time point is 30 days after cessation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

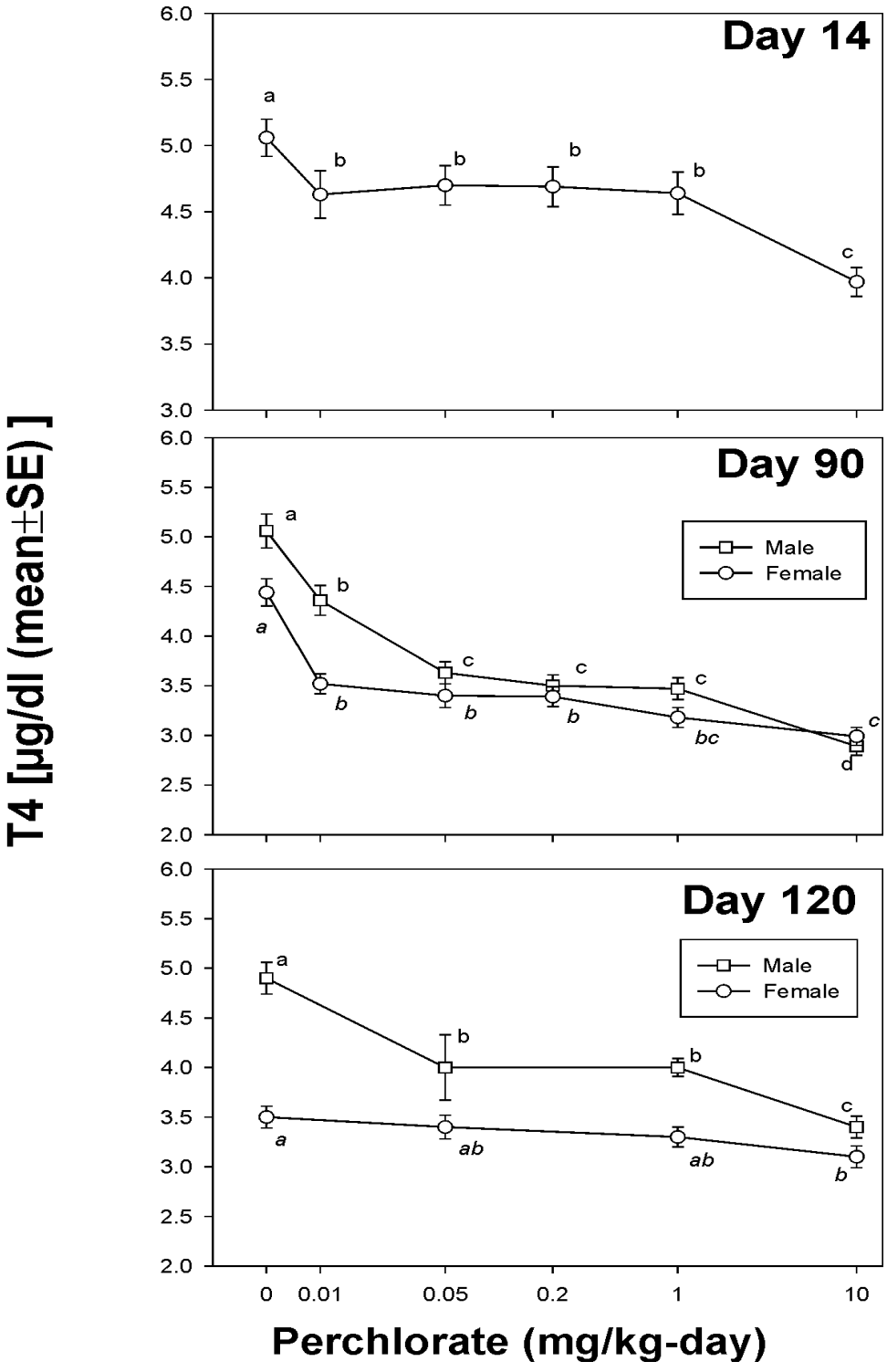

Figure 5-6. Effects from 90-day drinking water administration of ammonium perchlorate to SD rats on serum total T4 concentrations as recalculated in Table 5-2 (Crofton and Marcus, 2001). Means with different letters were significantly different ($p < 0.05$). The 120-day time point is 30 days after cessation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

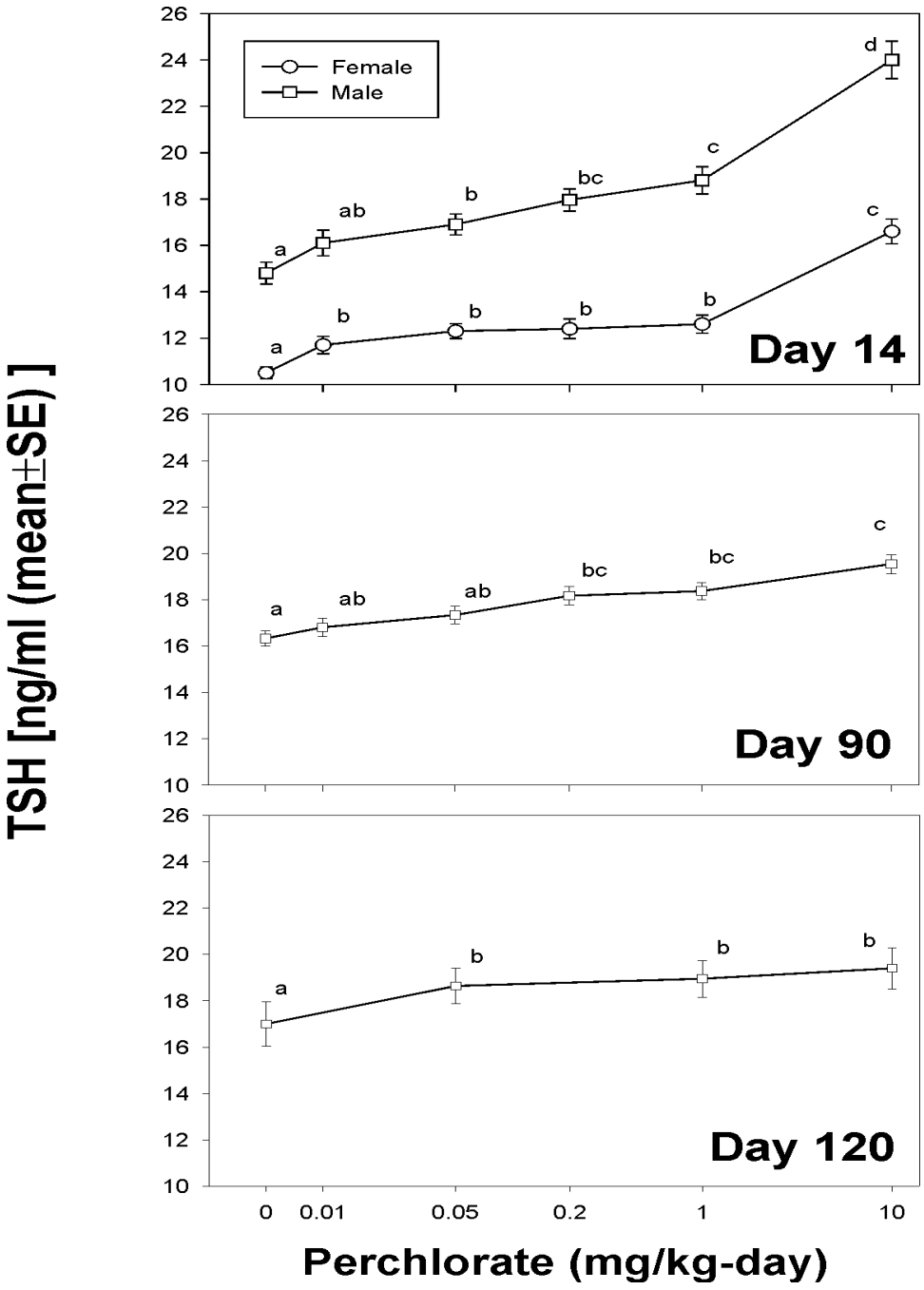

Figure 5-7. Effects from 90-day drinking water administration of ammonium perchlorate to SD rats on serum total TSH as recalculated in Table 5-2 (Crofton and Marcus, 2001). Data of Springborn Laboratories, Inc. (1998). A main gender-by-treatment interaction was observed for Day 14, but not Days 90 and 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.

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

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

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

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

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

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

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

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

were assigned randomly to Subset 1 for brain weight and neurohistological examination (including morphometric measurements). All pups were selected for fixed brain weights on PND12; 6/sex/dose (total of 30 male and 30 female pups) were selected for neurohistological examination. The second male and female pup (1/sex/dose; total of 100 male and 100 female pups) were assigned randomly to Subset 2 for passive avoidance testing on PNDs 23 to 25 and PNDs 30 to 32; water maze testing on PNDs 59 to 63 and PNDs 66 to70; and scheduled sacrifice at PNDs 90 to 92, with blood collection for thyroid and pituitary hormone analysis. The third male and female pup (1/sex/dose; total of 100 male and 100 female pups) were assigned randomly to Subset 3 for motor activity evaluation on PNDs 14, 18, 22, and 59; auditory startle habituation on PNDs 23 and 60; and scheduled sacrifice on PNDs 67 to 69. The fourth male and female pup (1/sex/dose; total of 100 male and 100 female pups) were assigned randomly to Subset 4 for regional brain weight evaluation on PNDs 81 to 86 (6/sex/dose; total of 30 male and 30 female rats) and neurohistological examination on PNDs 82 to 85 (6/sex/dose; total of 30 male and 30 female rats). Female pups also were evaluated for the age of vaginal patency beginning on PND28, and male pups were evaluated for the age of preputial separation beginning on PND39. A few of these measurements inadvertently went unrecorded, but the laboratory asserted that this did not affect the results because a sufficient amount of data on other rats was recorded.

5.3.1.1 Results of General Toxicity Measures, Neurohistology, and Morphology

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

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

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

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

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

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

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

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

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

5.3.1.2 Evaluation of Thyroid Histopathology

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

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

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

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

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

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

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

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

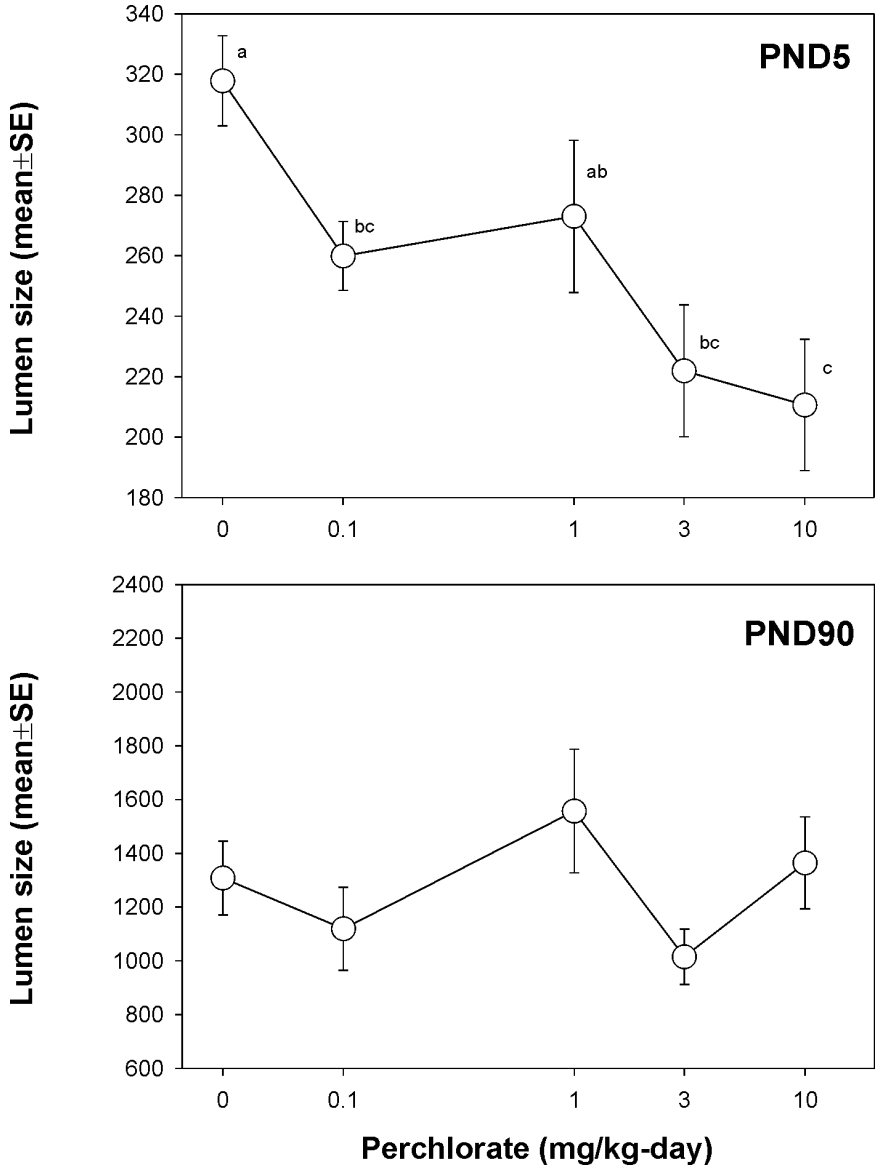

Figure 5-8. Effects from maternal drinking water administration of ammonium perchlorate to SD rats on thyroid gland follicular lumen size in F1-generation offspring on PND5 as recalculated in Crofton and Marcus (2001). Data of Channel (1998c) and Argus Research Laboratories, Inc. (1998a). Means with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

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

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

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

5.3.1.3 Thyroid and Pituitary Hormone Analyses

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

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

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

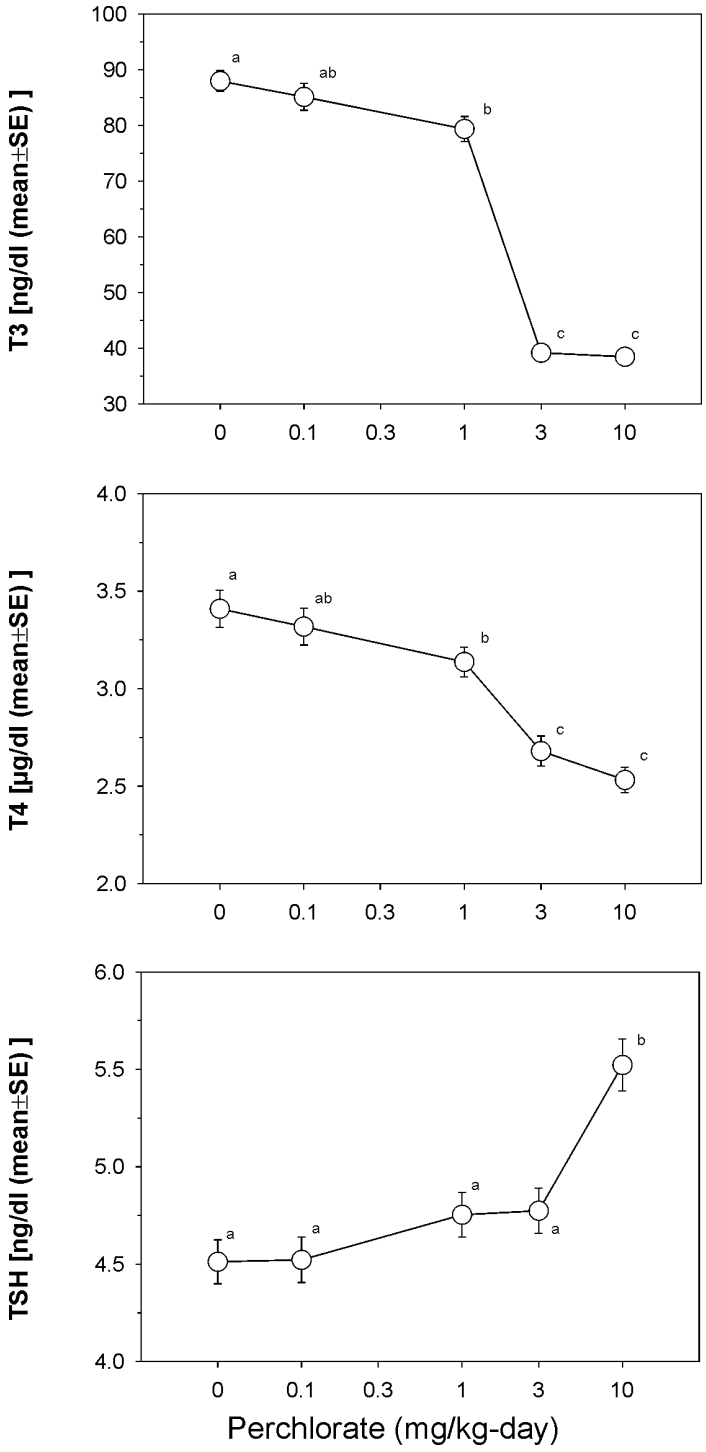

Figure 5-9. Effects from maternal drinking water administration of ammonium perchlorate to SD rat F1-generation pups on serum total T3 (A), T4 (B) and TSH (C) concentrations (ng/dL; mean ± SE) as recalculated in Table 5-2 (Crofton and Marcus, 2001). Data of Argus Research Laboratories, Inc. (1998a).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

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

5.3.1.4 Behavioral Evaluations

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

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

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

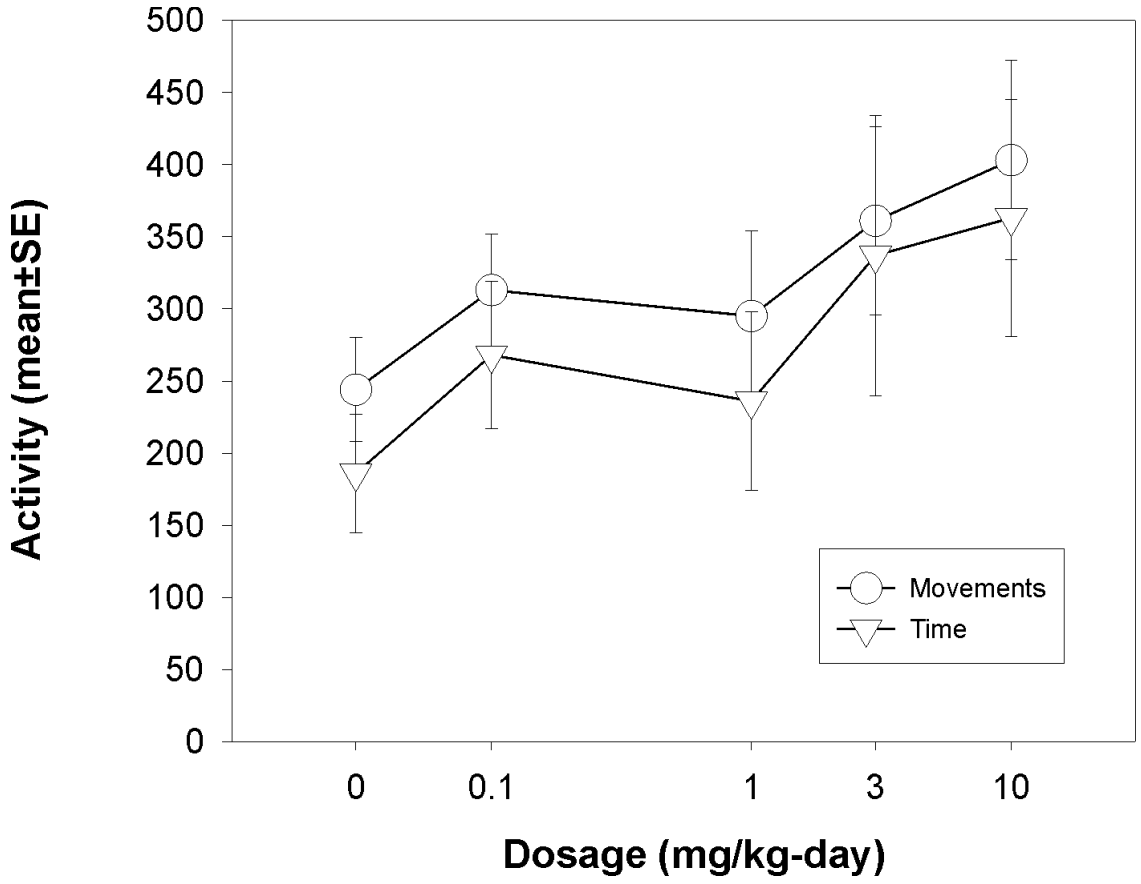

Figure 5-10. The effects of developmental exposure to perchlorate on motor activity in male rats on PND14. Data of Argus Research Laboratories, Inc. (1998a). The dose-dependent increases in both number of movements and time spent in movement were not statistically different, even though the increases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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

5.3.2.1 EPA and NIEHS Statistical Analyses of Motor Activity Effects

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

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

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

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

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

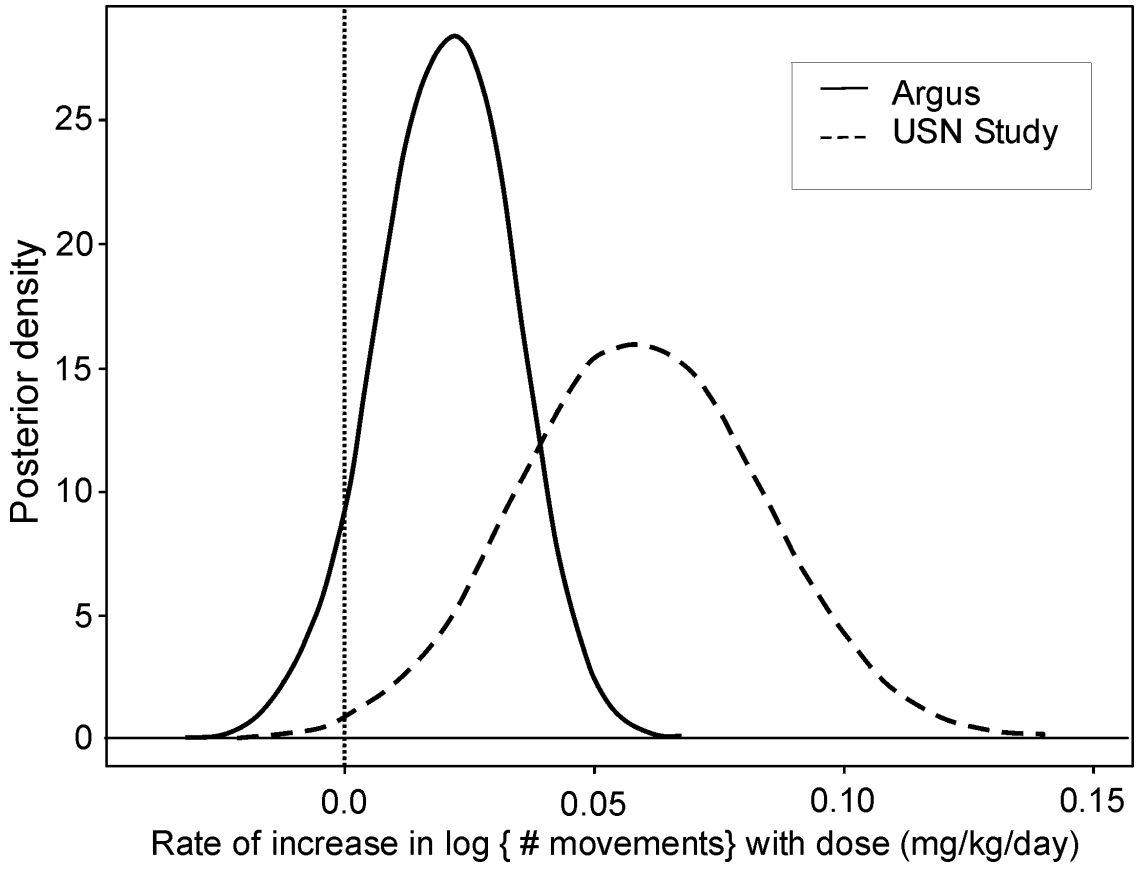

Figure 5-11. Bayesian estimates of the posterior densities for the expected increase in the logarithm of the number of ambulatory movements at the final habituation time per unit dose (mg/kg-day) increase of ammonium perchlorate (Dunson, 2001a). A separate analysis for the Argus Research Laboratories,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

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

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

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

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

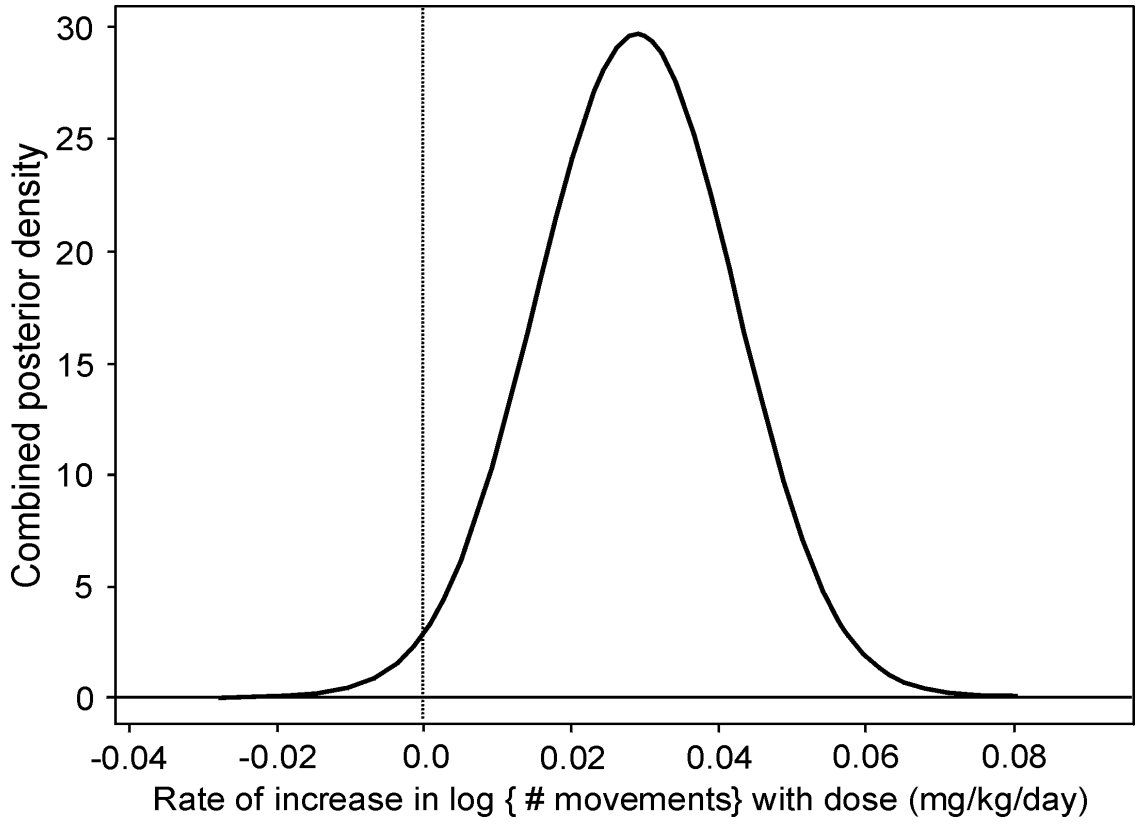

Figure 5-12. Bayesian estimate of the posterior density for the expected increase in the logarithm of the number of ambulatory movements at the final habituation time per unit dose (mg/kg-day) increase of ammonium perchlorate for the combined data from the two studies of motor activity effects shown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

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

5.3.3 The 2001 "Effects Study"

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

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

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

5.3.3.1 Results of General Toxicity Measures

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

5.3.3.2 Evaluation of Thyroid Histopathology

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

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

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

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

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

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

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

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

5.3.3.2.1 Benchmark Dose Analyses of Thyroid Histopathology

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

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

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

TABLE 5-3. BENCHMARK DOSE (BMD)[a] AND BENCHMARK DOSE LOWER CONFIDENCE LIMIT (BMDL)[a] 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	χ^2 [b]	Exp [c]	BMD	BMDL	χ^2 [b]	Exp [c]	BMD	BMDL	χ^2 [b]	Exp [c]
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 [4]	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

[a] Units of mg/kg-day.
[b] χ^2 goodness of fit criterion
[c] Exponent in log-logistic function restricted to be ≥ 1.0.
[d] NOE = No observed effect.

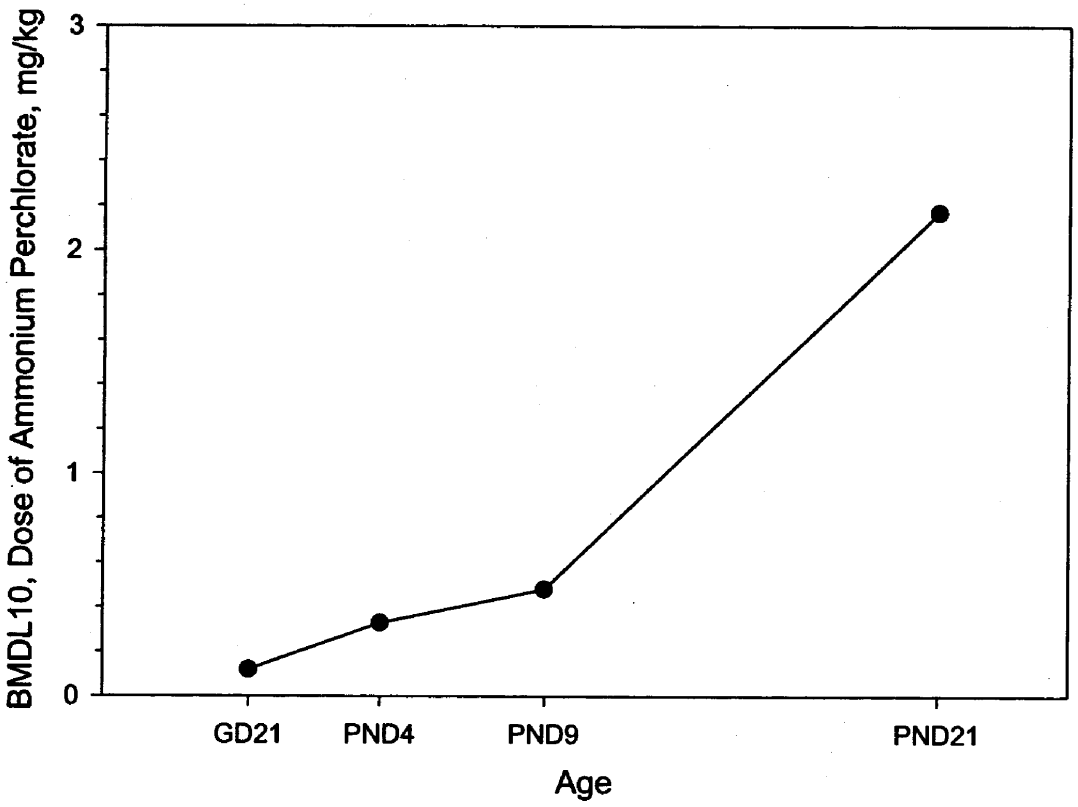

Figure 5-13. Lower confidence limit on the dose of ammonium perchlorate in drinking water that produced a 10% increase in the incidence of colloid depletion in the thyroid gland as a function of post-natal age of rat pups. Data of Argus Laboratories, Inc. (2001). Male and female data combined (Geller,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

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

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

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

5.3.3.3 Thyroid and Pituitary Hormone Analyses

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

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

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

				Effect Level Designation	
Generation	**Hormone**	**Age**	**Sex**	**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	1.0
			M		
	T4	GD21	Pooled	0.01	0.1
		PND5	Pooled		
		PND10	Pooled		
		PND22	F	no significant effects	
			M	—	0.01
	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

[a]Dosages of 0, 0.01, 0.1, 1.0, and 30 mg/kg-day.

5.3.3.3.1 Maternal Hormone Analyses

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

5.3.3.3.2 Fetal and Neonatal Hormone Analyses

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

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

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

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

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

5.3.3.4 Brain Morphometry Effects

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

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

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

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

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

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

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

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

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

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

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

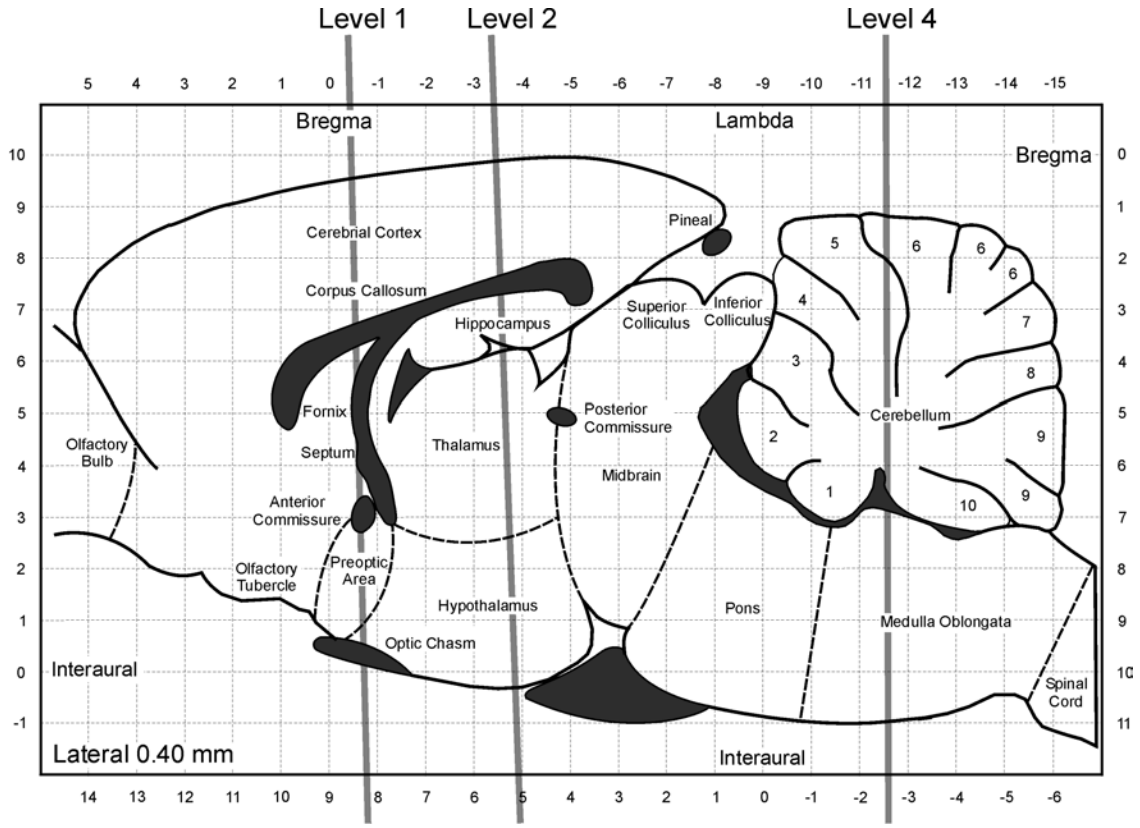

Figure 5-14. Topograph of the approximate anatomical landmarks on the ventral and dorsal surfaces of the brain used for making the morphometry measurements (Garman, 2001c). The topograph provided is for an adult brain, but the same landmarks are used for PND9 and PND21 brains although the sections at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

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

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

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

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

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

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

5.3.3.4.1 Description of EPA Profile Analysis of Brain Morphometry Effects

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

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

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

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

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

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

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

5.3.3.4.2 Results of EPA Profile Analysis of Brain Morphometry Effects

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

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

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

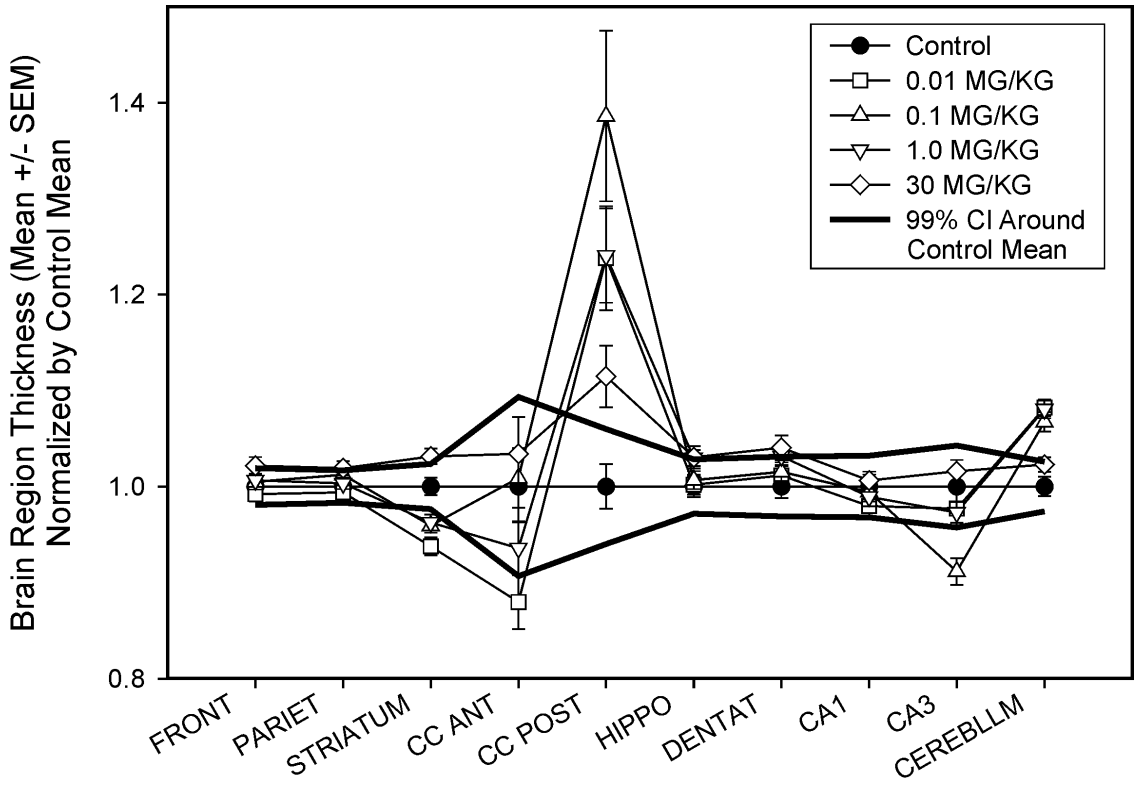

Figure 5-15. Profile analysis of brain morphometry measurements for PND21 rat pup brain regions. The male and female data on linear thickness measurements were combined and normalized by the control mean of each region. The control data are represented by the horizontal line at 1.0. Profile analysis 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 ± 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).

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

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

5.3.3.4.2.1 Univariate analyses of brain morphometry

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

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

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

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

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

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

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

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

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

5.3.3.4.3 Conclusions of EPA Brain Morphometry Analyses of Brain Morphometry Effects

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

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

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

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

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

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

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

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

5.4 DEVELOPMENTAL STUDIES

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

5.4.1 Historical Studies

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

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

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

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

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

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

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

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

5.4.2 Segment II Developmental Toxicity Study in Rabbits

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

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

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

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

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

5.4.2.1 Results of Maternal Examinations and Thyroid Histopathology

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

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

5.4.2.2 Developmental Endpoints

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

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

5.4.2.3 Maternal Thyroid and Pituitary Hormone Analyses

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

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

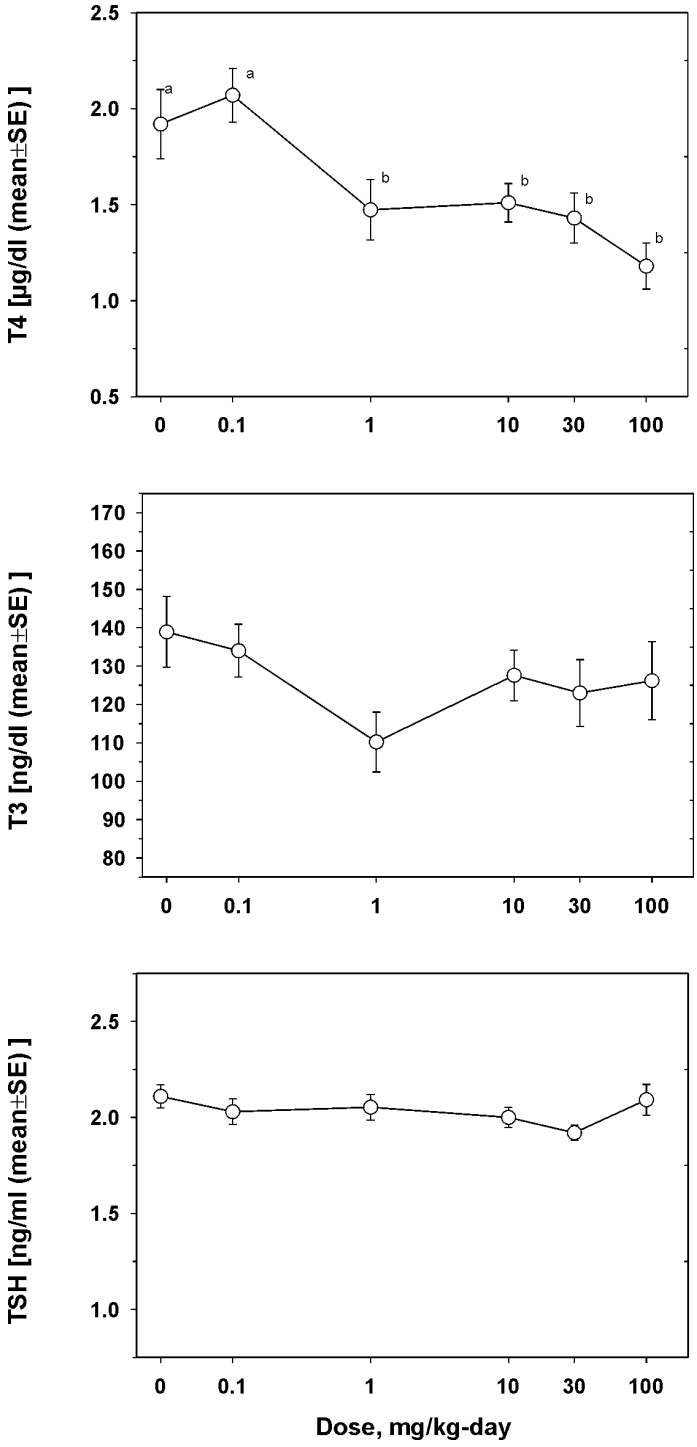

Figure 5-16. Effects from ammonium perchlorate in drinking water administration in pregnant New Zealand rabbits during GD6 to GD28 on T3 (A), T4 (B) and TSH (C) concentrations (ng/dL; mean ± SE) as recalculated in Table 5-2 (Crofton and Marcus, 2001). Data of Argus Research Laboratories, Inc. (1998c).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

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

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

5.4.3 Segment II Developmental Study in Rats

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

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

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

5.4.3.1 Results of Maternal Examinations

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

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

5.4.3.2 Developmental Endpoints

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

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

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

5.4.3.3 Conclusions Regarding Developmental Toxicity in Rats

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

5.5 TWO-GENERATION REPRODUCTIVE TOXICITY STUDY

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

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

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

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

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

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

5.5.1 General Toxicity Results and Evaluation of Reproductive Parameters

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

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

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

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

5.5.2 Evaluation of Thyroid Histology

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

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

5.5.2.2 Bayesian Analysis of Tumor Incidence

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

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

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

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

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

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

5.5.2.2.1 Choosing prior distributions based on historical controls

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

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

5.5.2.2.2 Results of NIEHS analysis

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

5.5.2.2.3 Summary of NIEHS analysis of tumor data

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

5.5.3 Thyroid and Pituitary Hormone Analyses

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

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

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

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

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

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

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

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

5.6 IMMUNOTOXICITY STUDIES

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

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

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

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

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

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

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

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

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

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

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

The results of the BRT-Burleson Research Technologies, Inc. (2000a,b,c) studies were analyzed as follows. Data from each treatment group were compared by first performing a Bartlett's Chi-Square test for variance of homogeneity. If found to be non-significant, ANOVA was employed using dose. If significant, then Dunnett's *t*-test was performed, with $p<0.05$ being significant. On the other hand, if Bartlett's Chi-square was significant, the non-parametric Kruskal-Wallis test was performed, which if significant was followed by a Jonckheere's-Terpera test for dose-dependent trends. The parametric ANOVA and the non-parametric extended Cochran-Mantel-Haenszel test were performed to determine whether the data could be pooled.

Results for the general toxicity and organ weight measures will be discussed in Section 5.6.1. Thyroid histopathology evaluations will be reported in Section 5.6.2, and analyses

of T3, T4, and TSH in Section 5.6.3. Results for the immunotoxicological and hematological parameters are discussed in Sections 5.6.4 and 5.6.5. A summary of the results and their potential significance is presented in Section 5.6.6.

5.6.1 Results for General Toxicity, Organ Weight, and Cellularity Measures

There were no effects observed on body, thymus, spleen, liver, or kidney weights in the 14-, 90-, or 120-day studies (Keil et al., 1999: Tables 6-8). Earlier interim reports indicated considerable variability in the splenic and thymic cellularity of ammonium perchlorate-exposed mice. This variability was due, in large part, to technical errors. Recognizing this, the contractor performed additional studies (i.e., "on at least two or more occasions") in which "no significant changes in cellularity were observed." (Keil et al., 1999). As such, in the "Final Report" no consistent alteration in splenic or thymic cellularity was observed in the 14–, 90-, or 120-day studies (Keil et al., 1999: Tables 9-11), nor in splenic lymphocyte CD4/CD8 subsets (Keil et al., 1999: Tables 14 and 15). With the exception that CD4-CD8+ thymic lymphocytes were increased in mice exposed to 0.1- and 1.0-mg/kg-day doses in the 14-day experiment, there were no other alterations in thymocyte subsets observed in the 14- or 90-day studies (Keil et al., 1999: Table 12). Furthermore, there were no alterations in the number of peritoneal macrophages obtained from mice exposed to any doses of ammonium perchlorate in the 14-, 90-, and 120-day studies (Keil et al., 1999: Tables 9-11), nor in bone marrow cellularity in the 14- and 90-day studies (Keil et al., 1999: Tables 9 and 10). Due to the absence of effects in the latter studies, no 120-day study was performed.

5.6.2 Evaluation of Thyroid Histology

Thyroid histopathology evaluation was performed for two experiments (A and D) in the Keil et al. (1998) study and eventually published in the final report (Keil et al., 1999). These data were transmitted by Warren (1999), and a preliminary review by EPA was presented at the 1999 external peer review (Jarabek, 1999). The materials were provided to the PWG review, and the results are found in Wolf (2000, 2001; Table 23). These results corroborate the preliminary analyses that showed decreased colloid, follicular hypertrophy and hyperplasia to occur at the 30 mg/kg-day dose. Congestion in the intrafollicular capillaries and the nuclear to cytoplasmic

ratio of the follicular cells were not recorded by the PWG but were both noted in the Warren (1999) report at 30 mg/kg-day (Jarabek, 1999). Hypertrophy was additionally observed in the lower doses of experiment "A", and the reason for the disparity between the two studies is unclear. These results support the assertion that the hypothalamic-pituitary-thyroid feedback regulatory mechanism is conserved across species (rats, rabbits, mice and humans) and suggest a NOAEL of 3 mg/kg-day in this strain of mouse.

5.6.3 Thyroid and Pituitary Hormone Analyses

The report (Keil et al., 1998) contains thyroid hormone and thyrotrophin (TSH) data from 14- and 90-day exposures to ammonium perchlorate in B6C3F1 mice. The following is a statistical analysis of the thyroid and pituitary hormone data (T4 and TSH) found in that report. There were no data for T3 reported in the original study submitted to EPA (Keil et al., 1998). The EPA reanalyzed the data that were supplied in Excel® spreadsheets to EPA by Dr. Deborah Keil, and the data are published therein (Crofton, 1998i). Subsequent submission of additional data files also containing data for T3 were included in reanalyses (Crofton, 2001a). Data for dependent measures (T4 and TSH) were subjected to separate analyses. The T4 and TSH data were analyzed with a two-way ANOVA, with duration (14, 90, and 120 days) and treatment (dose) as the independent between-subjects variables as reanalyzed by Crofton and Marcus (2001) and Crofton (2001a) as reported in Table 5-2. Mean contrasts were performed using Duncan's Multiple Range Test.

Results of these EPA reanalyses are different from those stated in the Keil et al. (1998) report. The EPA reanalysis of the T3 data (Crofton, 2001a) found main effects of time and treatment, but no time-by-treatment interaction. Mean contrast testing showed a LOAEL of 0.1 mg/kg-day; however, the dose-related decrease was not linear. The 0.1 and 3.0 mg/kg-day doses differed from controls but the 1.0 and 30.0 mg/kg-day doses did not. There was a significant time-by-treatment interaction for T4. After 14 days of exposure there was no effect with a NOAEL at 30 mg/kg-day; whereas, after 90 days of exposure the LOAEL was 0.1 mg/kg-day. T4 recovered after 30 days postexposure. There was no effect of perchlorate on TSH concentration contrary to the changes in histopathology discussed in Section 5.6.2.

These effects are of interest in that they demonstrate effects in mice comparable in nature to that in rats and indicate that the hypothalamic-pituitary-thyroid feedback system is conserved across species.

5.6.4 Results of Immune Function Assays

No consistent alteration in CTL activity was observed in three 14-day studies ("M", "H", and "F", Keil et al., 1998). No effects were observed on CTL activity in Experiments "M" and "H". However, in Experiment "F", increases in CTL activity were observed at the 0.1-mg/kg-day ammonium perchlorate dose for effector to target cell (E:T) ratios of 100:1, 30:1, and 10:1, and, 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., 1998) there were no alterations in CTL activity at any dosages or E:T ratios. The variability and inconsistencies observed in the early interim reports were ascribed to "technical issues" that were consequently "corrected". In fact, the data presented in Tables 23 and 24 (Keil et al., 1999) which includes data for dexamethasone, a potent immunosuppressant and positive control, indicates that there were no effects of ammonium perchlorate AP exposure on CTL activity.

There was also no consistent alteration in the DTH response, as measured by the lymphoproliferation (LP) of splenic lymphocytes from *L. monocytogenes*-challenged mice incubated with soluble *Listeria* antigen (SLA) in two 14-day studies ("U" and "V", Keil et al., 1998). The LP response was increased only in cultured splenic lymphocytes from mice in the 30-mg/kg-day group stimulated with 0.1 μg/mL SLA in Experiment "U" and in cultures of splenic lymphocytes from mice in the 3-mg/kg-day group stimulated with 8 μg/mL SLA in Experiment "V" (Keil et al., 1998). The "Results Summary and Status" page of Keil et al. (1998) indicates that a 90-day DTH study was planned. These 90-day data and a summary of the 14-day data are presented in Tables 32 and 31 respectively, of the "Final Report" (Keil et al., 1999). The data indicated an enhanced LP response in mice dosed at 30-mg/kg-day in both the 14-day and 90-day studies.

No alteration in splenic natural killer (NK) cell activity was observed in two 14-day studies ("G" and "T", Keil et al., 1998). The 14-day Experiment "T" data are presented in a table; however, the raw data and statistics for this study were not found in the submission. Inconsistent results were obtained in two 90-day studies ("D" and "N", Keil et al., 1998) in which NK cell activity was increased at the 30-mg/kg-day ammonium perchlorate in Experiment "N"; however,

no effects were observed at any doses in Experiment "D". A similar increase in NK cell activity at the 30-mg/kg-day dose was observed in the 120-day Experiment "E" (see also the data in Tables 21-22, Keil et al., 1999, in which the positive control dexamethasone was employed). The lack of any change in the number of B16F10 tumor nodules in the lungs of mice from the 90-day "Q" study (Keil et al., 1998; see also Table 33, Keil et al., 1999), particularly at 30 mg/kg-day, suggests that the increased NK-activity does not reflect a significant biological effect (see below). The EPA notes that there is a good deal of variation in NK activity data for the controls in the 14-day "G" study, the 90-day "D" and "N" studies, and the 120-day "E" study, which were 34, 6.4, 13.6, and 18.4 lytic units/10^7 splenic lymphocytes, respectively. Also, the 14-day "G" study was not included in Table 33 (Keil et al., 1999).

Decreased *in vitro* phagocytosis of *L. monocytogenes* was observed at 3 and 30 mg/kg-day of ammonium perchlorate in the 14-day "C" and 90-day "A" studies (Keil et al., 1998). In the 90-day "N" study, macrophage phagocytosis was decreased in all dose groups. However, in the 14-day "G" and 90-day "D" studies and in two 120-day studies ("B" and "E"), no effect on macrophage phagocytosis was observed (Keil et al., 1998). In the "Final Report" (Keil et al., 1999), these alterations were confirmed (i.e., decreased phagocytosis at 1.0 and 30.0 mg/kg-day in the 14-day study (Keil et al., 1999: Table 27) and decreased phagocytosis at 0.1, 1.0, 3.0, and 30.0 mg/kg-day in the 90-day study (Keil et al., 1999: Table 28). However, after a 30 day recovery period (i.e., 120-day study, Keil et al., 1999: Table 29) phagocytic function was comparable across control and treated mice. These data suggest that ammonium perchlorate suppresses the phagocytic capacity of peritoneal macrophages *in vitro*, but that this suppression may be reversed after a 30-day recovery period. Criticism of the use of an *in vitro* rather than an *in vivo* assessment of macrophage function was raised in the 1998 EPA ERD document and at the 1999 external peer review by Dr. Kimber White (Research Triangle Institute, 1999).

No consistent alteration in peritoneal macrophage nitrite production was observed in 14-, 90-, and 120-day studies. Increased nitrite production from macrophages cultured with interferon (IFN) occurred at doses of 3 and 30 mg/kg-day and from macrophages cultured with IFN and lipopolysaccharide for the 30-mg/kg-day dose in the 90-day "D" study (Keil et al., 1998). Also, increased nitrite production from macrophages cultured with IFN was observed at 3 mg/kg-day in the 90-day "N" study (Keil et al., 1998). An increase in nitrite production for macrophages cultured with IFN or LPS alone also occurred for the 30-mg/kg-day group in the 120-day "B"

study (Keil et al., 1998). These data suggest a "trend" toward increased nitrite production at the higher doses of ammonium perchlorate.

A subsequent analysis of these data, as presented in Tables 25 and 26 (Keil et al., 1999), demonstrates "no significant difference in nitrite production of peritoneal macrophages" (Keil et al., 1999).

A 90-day study ("L", Keil et al., 1998) was performed to determine if exposure of mice to ammonium perchlorate results in alterations in resistance to infection with *L. monocytogenes*. A trend toward increased resistance was suggested by the data; however, technical difficulties were encountered. For example, there was variability in the number of *L. monocytogenes* CFU/g liver recovered from control mice. It was not possible to enumerate the number of CFU/g spleen in mice due to the high concentration of bacteria injected and also to an inadequate dilution of spleen cell suspensions. In a subsequent 90-day study, mice were challenged with a lower concentration of bacteria such that both the CFU/g liver and spleen could be determined. These results, presented in Table 34 (Keil et. al., 1999), indicate that ammonium perchlorate exposure does not alter resistance to infectious challenge to *L. monocytogenes*.

No effects were observed in an initial 90-day B6F10 tumor challenge host-resistance model experiment ("Q", Keil et al., 1998). Another 90-day B6F10 tumor challenge experiment (i.e., "O") was performed, and the combined results of these two experiments are presented in Table 33 (Keil et al., 1999). These data indicate that there were no differences in the number of tumors present in the lungs of ammonium perchlorate-exposed mice compared with control mice.

Two separate groups of studies examining the effect that ammonium perchlorate has on the antibody response to SRBCs were performed by independent contractors (Keil et al, 1999; BRT-Burleson Research Technologies, Inc, 2000a,b,c). Initial studies were performed by Keil et al. (1999), in which the IgM and IgG antibody responses were determined using ELISAs. As indicated in the figures on page 59 (Keil et al., 1999), no change in the IgM levels in a 14-day study, nor in the IgM and IgG levels in a 90-day study, was observed between control and any ammonium perchlorate treated mice .

In the second set of studies, the anti-IgM SRBC PFC assay was employed (BRT-Burleson Research Technologies, Inc, 2000a,b,c), using CP as a positive immunosuppressant control. In the 14-day study there were no differences in the PFC response between control and treated mice when expressed either as the number of PFC/spleen or PFC/10^6 spleen cells (BRT-Burleson

Research Technologies, Inc, 2000a,b,c: Figures 3 and 4). On the other hand, in the 90-day study the PFC response was increased in the 2.0 and 50.0 mg/kg-day groups when expressed as the PFC/spleen and increased only in the 50.0 mg/kg-day group expressed as PFC/10^6 spleen cells (BRT-Burleson Research Technologies, Inc., 2000a,b,c: Figures 5 and 6). This disparity was not due to any difference in splenic cellularity between the control and treated mice. In both the 14- and 90-day studies, CP significantly inhibited the PFC response, expressed either as PFC/spleen or PFC/10^6 spleen cells compared to the controls.

The results of the effect that 14- and 90-day exposure to ammonium perchlorate has on the development of a contact hypersensitivity response to DNCB, as determined by the LLNA, indicate that an ammonium perchlorate dose as low as 0.06 mg/kg-day enhances this response. In the 14-day study, the LLNA was increased at doses of 0.06, 0.2, and 50.0 mg/kg-day, but not 2.0 mg/kg-day (BRT-Research Technologies, Inc., 2000a,b,c: Figure 8). The results of the 90-day study were somewhat different in that, while the LLNA was enhanced at 0.06 and 0.2 mg/kg-day, it was suppressed at 50 mg/kg-day (BRT-Research Technologies, Inc., 2000a,b,c: Figure 9). Another disparity between these two studies was that while CP suppressed the LLNA in the 14-day study, it did not suppress this response in the 90-day study.

5.6.5 Results for Evaluations of Hematological Parameters

There were no differences observed between control and dosed mice in 14- or 90-day experiments for erythrocyte cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration; nor in leukocyte differential counts of neutrophils, monocytes, and lymphocytes. Because of the absence of effects in these studies, no 120-day study was performed. No effects were observed in a single 14-day study (Experiment "T", Keil et al., 1998) on platelet counts. An increase in the percentage of reticulocytes was observed in the peripheral blood of mice exposed to 3 mg/kg-day of ammonium perchlorate in a 90-day study ("N", Keil et al., 1998). No other reticulocyte data are available because of "the minimal availability of blood obtained from each mouse" in other studies (Keil et al., 1998). In a subsequent 14-day study, there were no changes in the hematological parameters examined between control and ammonium-perchlorate-treated mice (Keil, et al., 1999: Table 16).

No consistent alteration in the bone marrow stem cell assay was observed. An increase in the number of colony-forming units was observed in bone marrow cell cultures from mice dosed at 30 mg/kg-day in a 14-day study ("K", Keil et al., 1998). However, there was no effect of ammonium perchlorate exposure on the stem cell assay in a 90-day study ("D", Keil et al., 1998). In a subsequent 90-day study, while no alteration in the stem cell assay was observed between control and ammonium perchlorate-treated mice, exposure to the positive control dexamethasone resulted in suppression of the stem cell assay (Keil, et al., 1999: Table 20).

5.6.6 Results Summary

The results of the various studies of immue function are summarized in Table 5-5. Although innate (i.e., macrophage and NK cell function) and cell-mediated (i.e., cytotoxic T lymphocytes [CTL], CD4, and CD8) immune functions were evaluated in the initial studies by Keil et al, (1998), EPA noted that humoral immunity (i.e., B cells and antibody response) was not (Smialowicz, 1999). The EPA suggested strongly that the antibody response to SRBCs is one of the most commonly effected functional parameters in animals exposed to chemical immunosuppressants (Luster et al., 1988). In fact, it is one of the assays required by EPA for test rules. The EPA also requested that an additional 90-day *L. monocytogenes* host-resistance study be undertaken consequent to technical problems associated with the initial 90-day study (Keil et al., 1998). As such, the EPA felt that these data would provide a more comprehensive evaluation of the potential for immunosuppression by ammonium perchlorate. In addition, the EPA requested that thyroid histology and thyroid and pituitary hormone data be obtained in order to provide additional insights on interspecies variability for this effect.

Consequently, the sponsor and investigators, Keil et al. (1998), agreed to perform these assays, the results of which are presented in the "Final Report" (Keil et al., 1999).

Subsequent to receipt of the results of the antibody response to SRBCs (Keil et al., 1999), in which antibody titers were expressed as the O.D. 50 or midpoint titer, rather than the more conventional titer to achieve a 0.5 O.D., a second request to determine the potential effects of ammonium perchlorate on the response to SRBCs was issued. In this same solicitation, the EPA also requested that a sensitization test be performed. The results of these studies are found in 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
Mouse/B6C3F1/Female (Keil et al., 1998;1999)	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 (*in vitro*)	Increased at 0.1, 1.0 and 3.0; no effect in subsequent "corrected" study.
		L. monocytogenes challenge	Not Done
		DTH to *L. monocytogenes* antigen	Increased at 30. NOAEL = 3.0, LOAEL = 30
		Macrophage phagocytosis (*in vitro*)	Decreased at 1.0 and 30. NOAEL = 0.1, LOAEL = 1.0
		Macrophage nitrate (*in vitro* + IFN or LPS)	None
		IgM ELISA to SRBCs	None
	90-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
Mouse/B6C3F1/Female (Kiel et al., 1998; 1999)	90-days 0, 0.1, 1.0, 3.0, or 30	NK cell activity/B16F10 tumor challenge	Increase NK activity at 30 in one experiment and at 30 in 120-day study: NOAEL = 3, LOAEL = 30. No effect on B16F10 tumor challenge.
		CTL to P815 cells (*in vitro*)	None
		L. monocytogenes challenge	None
		DTH to *L. monocytogenes* antigen	Increase at 30. NOAEL = 3.0, LOAEL = 30
		Macrophage phagocytosis (*in vitro*)	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
		Macrophage nitrate (*in vitro* + IFN or LPS)	None
		IgM ELISA to SRBCs	None
Mouse/CBA/JHsd/Female (BRT-Burleson Research Technologies, Inc., 2000a,b,c)	14-days 0, 0.02, 0.06, 0.2, 2.0, or 50	anti-SRBC PFC/10^6 cells	None
		anti-PFC/spleen	None
		LLNA to DNCB	Increased at 0.06, 0.2, and 50, but not at 2.0. NOAEL = .02, LOAEL = 0.06
	90-days 0, 0.02, 0.06, 0.2, 2.0, or 50	anti-SRBC PFC/10^6 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.

The three immune function parameters altered by ammonium perchlorate exposure were the following: suppression of *in vitro* peritoneal macrophage phagocytosis of *L. monocytogenes*, enhancement of the PFC response to SRBCs, and enhancement of the LLNA to DNCB. These results are summarized and discussed below.

Decreased *in vitro* phagocytosis of *L. monocytogenes* by peritoneal macrophages obtained from mice dosed for 14 days at 1- or 3- and 30-mg ammonium perchlorate/kg-day was observed (Keil et al, 1998, 1999). In mice exposed for 90-days, phagocytosis was decreased in all dosage groups (Keil, 1998, 1999). However, in the 120-day (i.e., 90-day ammonium perchlorate exposure followed by 30-day recovery) studies, no effect on macrophage phagocytosis of *L. monocytogenes* was observed (Keil et al., 1998, 1999). Taken together, these data suggest that

ammonium perchlorate suppresses the *in vitro* phagocytic capacity of peritoneal macrophages, but that this suppression is reversed after a 30 day recovery period.

This decrease in macrophage phagocytic activity could be expected to be reflected in the results of the *L. monocytogenes* infectivity data because, along with other immune system components, macrophages play a pivotal role in resistance to infection by this bacterium. For example, the pathogenesis of *L. monocytogenes* is associated with its ability to grow within mononuclear phagocytes. Complement (C') plays an important role in *L. monocytogenes* infections, as demonstrated by the fact that C'-deficient mice have impaired host resistance to this bacterium. This impairment in C'-deficient mice is caused by the absence of macrophage-associated C'. The T-lymphocytes also play a major role in defense against *L. monocytogenes* because complete elimination of bacteria from infected tissue is accomplished by macrophages activated by T-cell dependent mechanisms.

However, the *L. monocytogenes* host-resistance studies indicate that ammonium perchlorate exposure of mice does not alter the ability to combat this bacterial infection. With the exception that clearance of *L. monocytogenes* from the liver of mice given a 5360 CFU challenge following dosing at 3.0 mg AP/kg/day for 90 days was reduced, no other effect was observed (Keil et a., 1999: Table 43). These data imply that while in vitro phagocytosis by peritoneal macrophages of this bacterium was reduced following ammonium perchlorate exposure, the ability of macrophages from other *in situ* sites (e.g., spleen, liver) to clear *L. monocytogenes* was not altered.

Exposure of mice to 2.0 or 50.0 mg ammonium perchlorate/kg/day for 90, but not 14, days resulted in enhancement of the antibody response to SRBCs as determined by the PFC assay (BRI-Burleson Research Technologies, Inc., 2000a,b,c). In both the 14- and 90-day studies, the PFC response was suppressed by dosing mice with the immunosuppressive positive control CP. The PFC assay is routinely used for identifying chemicals that are immunosuppressive. The reason why the highest dose(s) of ammonium perchlorate, given over 90 days, enhanced this response is not known. It is possible that under these dosing conditions ammonium perchlorate may have an adjuvant-like or enhancing effect on the antibody response to SRBCs. The ELISA data for mice exposed to up to 30.0 mg ammonium perchlorate/kg/day, for 14 or 90 days (Keil et al., 1999), do not corroborate this enhanced response to SRBCs as determined by the PFC

assay. However, taken together, the PFC and ELISA data indicate that ammonium perchlorate does not suppress the immune response to SRBCs.

The LLNA is an accepted approach for identifying chemicals with the potential of causing dermal contact hypersensitivity (CHS) reactions in humans. In this assay the test substance, 2,4-dinitrochlorobenzene (DNCB) was topically applied on three consecutive days to both ears of the mouse. Two days later the mice were injected iv with radioactive uridine (e.g., ^{125}IUDR). Five hours later, the lymph nodes draining the ears, referred to as the "auricular" lymph nodes, were removed and ^{125}IUDR incorporation by the lymph node cells determined. Since the nodes draining the ear (i.e., "auricular" nodes) have no standard anatomical nomenclature, experience in identifying these nodes as well as appropriate and consistent excision of these nodes from control and test mice is critical. The LLNA evaluates the induction phase of the CHS reaction by assessing the influx of lymphoid cells and the differential argumentation of lymphocyte proliferation elicited by exposure to the test chemical relative to that of a vehicle control.

The data from BRT-Burleson Research Technologies, Inc. (2000a,b,c) report that exposure to ammonium perchlorate enhances/exacerbates the LLNA response to DNCB at doses of 0.06, 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 enhanced this response, a dose of 2.0 mg/kg-day did not. Similarly, a dose of 2.0 mg/kg-day in the 90-day study did not enhance the LLNA response to DNCB. In contrast to the 14-day study, exposure of mice to 50.0 mg ammonium perchlorate/kg/day in the 90-day study resulted in suppression of the LLNA response. In the 90-day study, the positive control CP did not suppress the LLNA response to DNCB. The failure of CP to suppress this response in the 90-day vs. 14-day study is disquieting because CP was administered similarly (i.e., 15 mg/kg-day for 5 consecutive days prior to the LLNA) in both studies. The only difference between these two studies was that the mice in the 90-day study were 11 weeks older. This difference in age, however, should not influence the ability of CP to suppress this response. The fact that CP did not suppress the LLNA response in the 90-day study calls into question the performance of this and perhaps the 14-day study.

Application of the LLNA for identification of chemicals that are contact sensitizers routinely involves a demonstration of a dose-related increase in the LLNA using, at a minimum, three increasing concentrations of the test agent. Neither the 14- nor 90-day ammonium perchlorate LLNA data demonstrate a dose-response relationship, which would be expected if

ammonium perchlorate was acting additively or synergistically with DNCB to increase the LLNA response. While higher concentrations of a contact sensitizing agent will increase the LLNA response, there is no information in the literature that indicates such an increase results in a more serious or potentially detrimental effect on the host. Consequently, the physiologic significance of the observed increase in the LLNA response to DNCB in ammonium perchlorate-exposed mice is unknown. This is unlike the situation with immunosuppressive agents where suppression of specific immune function(s) can be linked to a biological detrimental effect (i.e., decreased host resistence to an infectious agent or tumor).

It is interesting to note that there are published reports in which non-sensitizing agents have been employed to improve the sensitivity of the LLNA to detect sensitizers. For example, Vitamin A acetate dietary supplementation enhances the detection of weak sensitizers, and at low concentrations of moderate sensitizers, assessed by the LLNA. The mechanism(s) for this increased detection of contact sensitizers is not known. However, topically applied retinol causes epidermal hyperplasia which may lead to increased numbers of antigen-presenting cells in the epidermis. Retinoids also up regulate the sensitization phase of DTH induction through direct or indirect stimulation of T cells. Non-sensitized mice, fed a diet supplemented with retinol, display somewhat higher LLNA responses compared to control mice on a normal diet. This suggests that dietary retinol itself causes cellular infiltration and/or proliferation in the absence of a contact sensitizer as measured by the LLNA. It may be that ammonium perchlorate, in the absence of DNCB, has the capability of raising the baseline LLNA response compared to water control mice. Unfortunately, there were no negative controls in the Burleson et al. (2000) studies. Appropriate negative controls would have included the following: (1) ammonium perchlorate-dosed non-sensitized mice; (2) ammonium perchlorate-dosed and ammonium perchlorate-challenged mice; and (3) water control mice dermally exposed to ammonium perchlorate on the ear pinna. Another group of appropriate and informative studies would involve ammonium perchlorate-dosed mice that would be challenged with a series of low to moderate concentrations of DNCB, for comparison with the current LLNA "optimal DNCB" response concentration data.

Enhancement of the LLNA to DNCB in mice exposed to 0.06 mg ammonium perchlorate/kg-day for 14 or 90 days represents the Lowest Observed Effect Level (LOEL) for all of the immune function tests performed. While this is the LOEL it is unknown if this is the

Lowest-Observed-Adverse-Effect- Level (LOAEL) because it is not clear that enhancement of the LLNA is a physiologically relevant adverse effect. Studies are needed to determine if ammonium perchlorate itself is a contact sensitizer as determined by the LLNA, as described above, and whether the degree of the LLNA response to ammonium perchlorate itself or to a known contact sensitizer can be linked to a quantifiable adverse outcome.

It is important to note that clinical studies in the 1960s reported that some patients suffering from Graves' disease and treated with potassium perchlorate presented with agranulocytosis and/or skin rashes. While the studies reported by Keil et al. (1998, 1999) indicated that there were no alterations in the proportion of peripheral blood leukocytes of mice dosed with ammonium perchlorate for 14- or 90-days, the work of BRI-Burleson Research Technologies, Inc. (2000a,b,c) suggests that ammonium perchlorate appears to exacerbate the contact sensitizing potential of the known skin sensitizer DNCB. However, due to the uncertainties associated with any attempt to extrapolate from the incomplete database of the mouse LLNA performed by BRI-Burleson Research Technologies, Inc. (2000a,b,c) to the clinical observations of skin rash and agranulocytosis in Graves' disease patients treated with potassium perchlorate, an uncertainty factor based on deficiencies in the database is recommended to be applied to this 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

studies in the perchlorate testing strategy, together with experimental data and parameter values available in the existing literature. The subsequent model structures for the human and various life stages of the rat were similarly developed based largely on the adult male rat structure through scaling and optimization of parameters to available data.

It should be noted that the original motivation for including human studies (as discussed in Chapter 3) in the perchlorate testing strategy was to support such interspecies extrapolation and not to derive NOAEL estimates for thyroid effects in the human population. As discussed in Chapter 4, the EPA feels that both the observational epidemiological and the human clinical studies have significant scientific and technical limitations that preclude their use as the basis for a quantitative dose-response assessment. In addition, some of the clinical studies contained in this database fall in the category of studies not to be considered under EPA's Dec. 14, 2001 interim policy on the use of third-party human studies (U.S. Environmental Protection Agency, 2001c). 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 an RfD. The clinical study subject attributes (euthyroid adults) and study design issues (sample size, RAIU time points, etc.) made these data less reliable than the laboratory animal toxicological data to ascertain effect levels for the basis of an RfD derivation. Models of perchlorate distribution for human pregnancy and lactation have not been developed.

More detailed discussion can be found for each model structure in the accompanying references provided for each in the sections that follow. The adult male rat and human model (Merrill, 2001c,d) will be discussed in Section 6.2. Section 6.3 discusses the pregnant dam and fetal rat PBPK model (Clewell, 2001a), and the lactating dam and neonate model (Clewell, 2001b) is discussed in Section 6.4. The purpose of providing these model descriptions and a discussion of the data used to develop and validate their structures is to provide the external peer reviewers an opportunity to critically evaluate the model structures, the use of the data in model development or validation exercises, and the model applications.

The simultaneous ordinary differential equations used in the proposed PBPK models to simulate radioiodide and perchlorate distribution were written and solved using advanced continuous simulation language (ACSL) software (AEqis Technologies, Austin, TX).

6.1 MODE-OF-ACTION FRAMEWORK AND UNDERLYING MODELING APPROACH

The mode-of-action model proposal by the EPA for the previous perchlorate assessment and discussed in Chapter 3 served as the conceptual construct for the development of the PBPK models. Shown again in Figure 6-1, the model lays out the biomarkers of exposure and effect in a continuum from ingestion of perchlorate in drinking water and uptake into the blood, the key event of iodide uptake inhibition at the NIS in the thyroid gland, and subsequent effects on thyroid hormone economy leading to neurodevelopmental and neoplastic sequelae.

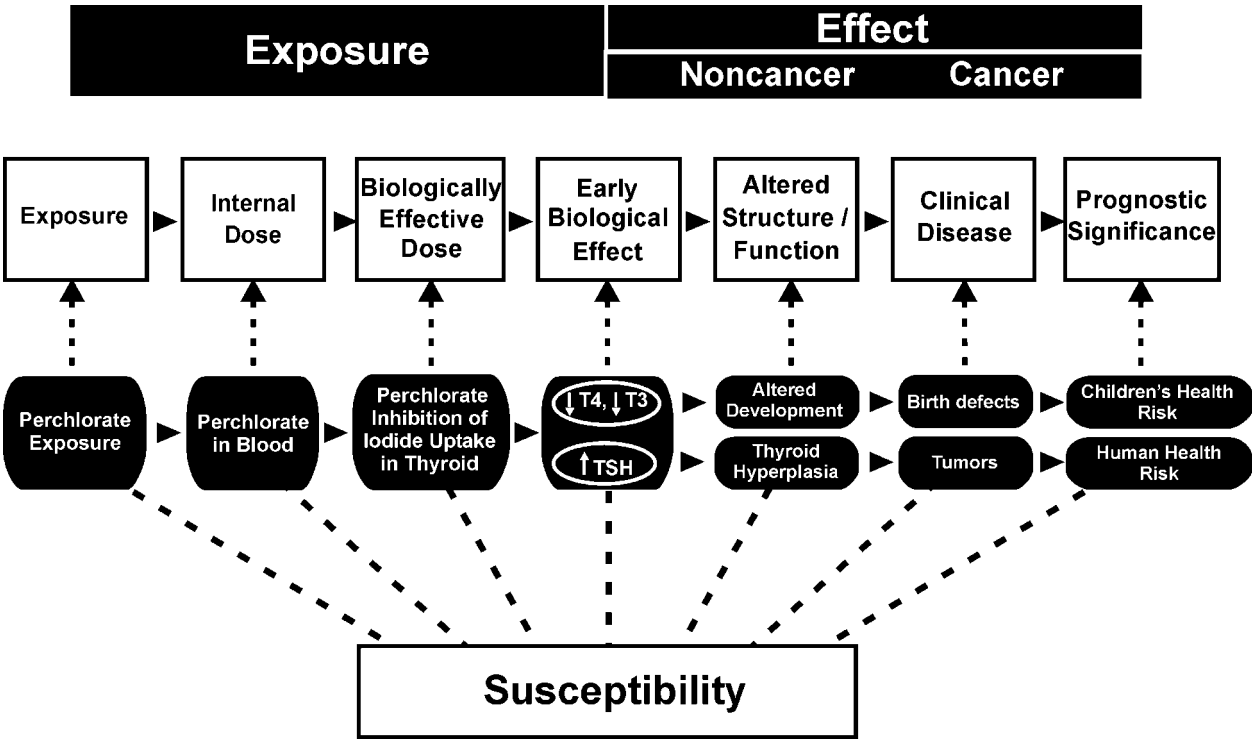

Figure 6-1. Mode-of-action model for perchlorate toxicity proposed by the U.S. EPA (U.S. Environmental Protection Agency, 1998d). Schematic shows the exposure-dose-response continuum considered in the context of biomarkers (classified as measures of exposure, effect, and susceptibility) and level of 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 casual linkage or prognostic correlations of precursor lesions.

The temporal pathological and serum hormone changes that accompany exposure to perchlorate corresponding to this continuum are represented in Figure 6-2. The inhibition of iodide uptake at the NIS results in a transient decrease in serum T4 and T3. This transient phase of thyroid hormone deficit is of concern during pregnancy and development due to the critical role that these hormones play in preventing adverse neurodevelopmental sequelae as described in Chapters 3 and 5. The hypothalamic-pituitary-thyroid feedback system is designed to regulate the circulating levels of thyroid hormone and will respond to the thyroid hormone decreases by upregulating TSH production in order to stimulate the thyroid to increase its production of thyroid hormones to compensate. Represented as the "chronic phase" in Figure 6-2, the upregulation of TSH would bring the system back into apparent homeostasis. As depicted in the figure, however, this apparent homeostasis may actually represent subclinical disease in that the system is only maintaining homeostasis by upregulation and can be considered a stressed system with respect to its ability to compensate for additional insults caused by other chemicals or diseases that might impact the thyroid. Further, it should be emphasized that recent epidemiological investigations have indicated concern about decrements in T4, i.e., thyroxinemia without concomitant upregulation of TSH that would constitute hypothyroidism (Morreal de Escobar, 2000; Haddow et al., 1999; Pop et al., 1999).

In order to adequately characterize the transient phase of events, evaluation of the initial effect of perchlorate at the NIS is necessary. This can be accomplished by determining perchlorate inhibition with radioactive iodide uptake (RAIU) studies. The timing and route of administration are important considerations in evaluating these types of studies. Studies of RAIU that occur during the chronic phase, such as longer-term studies of hormones, offer little insight to the critical decrements in T4 that may occur during the transient phase due to iodine inhibition. Likewise, longer-term studies of hormones often represent the upregulated system and may not be especially informative.

6.1.1 Parallelogram Approach to Interspecies Extrapolation

PBPK models have proven to be very useful tools for performing interspecies extrapolation of dose for applications in risk analysis. Interspecies extrapolation is often necessary because, as in this case of perchlorate, critical effects at levels of organization below that of the population (e.g., thyroid histopathology or brain morphometry) can not be evaluated easily or ethically in

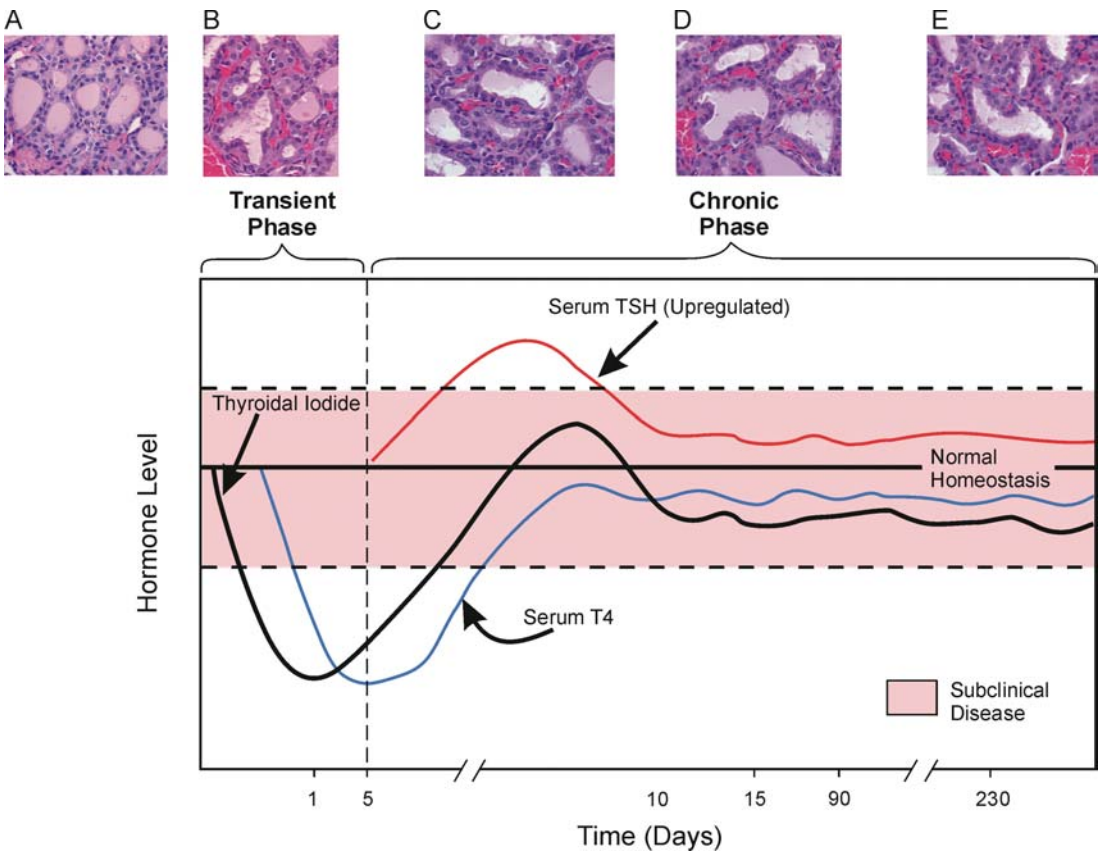

Figure 6-2. Schematic of thyroid and pituitary hormone levels with associated pathology after acute versus chronic dosing with perchlorate. The transient phase is represented by decreases in thyroidal iodide due to the inhibition by perchlorate at the NIS with subsequent drop in T4. The transient drops 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.

humans. A basic tenet of molecular epidemiology is that these precursor lesions are often more closely related to the exposure than are the traditional outcome measures of morbidity and mortality (U.S. Environmental Protection Agency, 1994).

A parallelogram approach as shown in Figure 6-3 is used to predict the dose-response relationship for humans based on the dose-response in laboratory animals. Because these critical

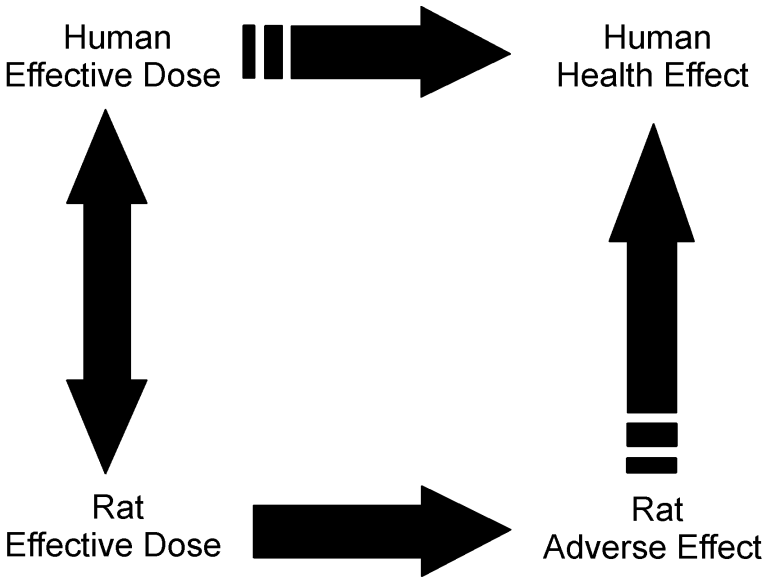

Figure 6-3. Schematic of parallelogram approach used for interspecies extrapolation (U.S. Environmental Protection Agency, 1994). Dose and adverse effect in rat can be used to predict human effective dose and response.

effects cannot be accurately measured in humans, the dose associated with an observed critical effect in the laboratory animal is scaled to the human by adjusting the PBPK model with human physiological parameters and variables. The human model is typically constructed by allometrically scaling some parameters in the laboratory animal model based on body weight, and some parameters such as partition coefficients can be measured *in vitro*. An administered dose associated with the critical effect is determined based on an appropriate internal dose metric. The internal dose is scaled to an equivalent exposure (HEE) in humans by exercising the human model with human parameters and exposure assumptions. Thus, the HEE represents the human exposure that would result in the same amount of internal dose metric in a human as that which caused the effect in the laboratory animal.

The dose-response relationship is considered to be the same as that in the laboratory animal as the default or more biologically-based models may contain additional parameters that also account for species-specific determinants of toxicant-target interaction. Figure 6-4 illustrates the use of the laboratory animal and human PBPK models to arrive at the HEE. Simulations used to

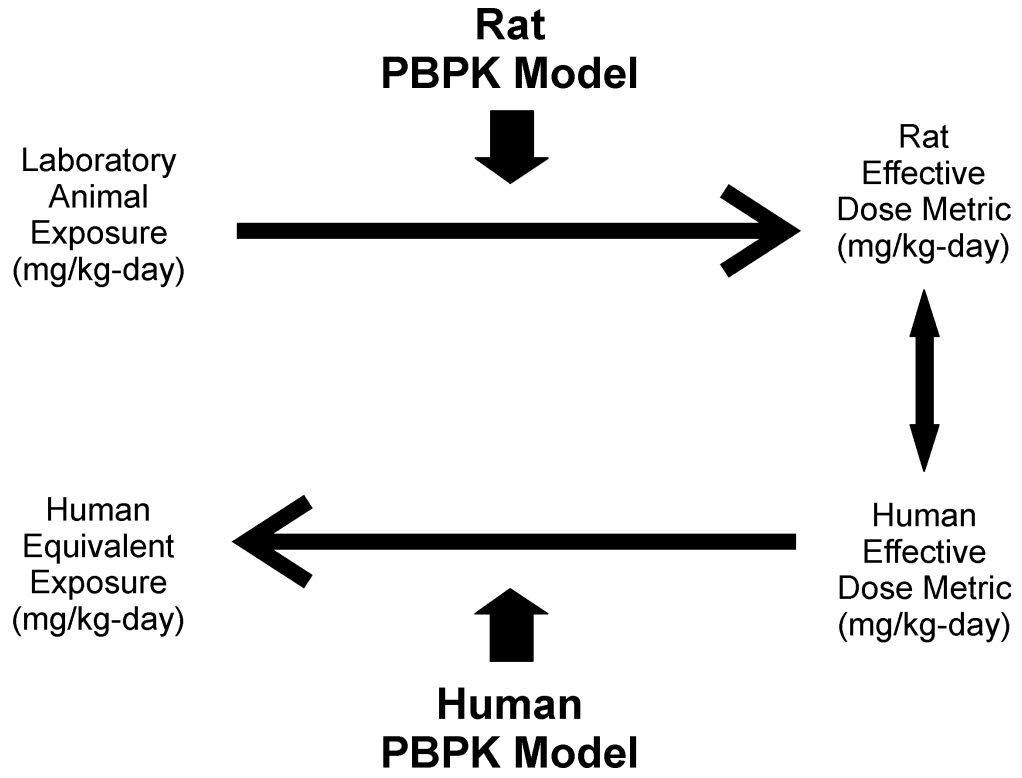

Figure 6-4. Illustration of how human equivalent exposure (HEE) is calculated using PBPK models. An effective internal dose associated with a critical health effect at an administered dose (mg/kg-day) is calculated by simulating the experimental exposure regimen (e.g., 5 days/week) for a relevant metric (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.

arrive at HEE for different internal dose metrics and a sensitivity analysis of the adult model structure will be discussed in Section 6.5.

The parallelogram approach has also been used to predict effective doses for structurally related chemicals (Jarabek et al., 1994). Disposition of one chemical associated with an effect can be predicted for another after appropriate adjustments for chemical structure and activity are made. In the case of these models, it should be appreciated that the accurate modeling of iodide in addition to that of perchlorate represents such a validation.

6.1.2 Extending the Parallelogram Approach to Various Experimental Life Stages

Because effects at various life stages (adult, pregnant dam, fetus, lactating dam, and neonate) were evaluated in the perchlorate laboratory animal studies, the parallelogram approach had to be extended as shown in Figure 6-5. There are no human models of perchlorate disposition for pregnant women, lactating women, fetuses, or children, so the relationships to the adult human HEE had to rely on the relationships determined in the laboratory animal species. This approach assumes that the relationships, expressed as ratios between one life stage and another, will be comparable in humans.

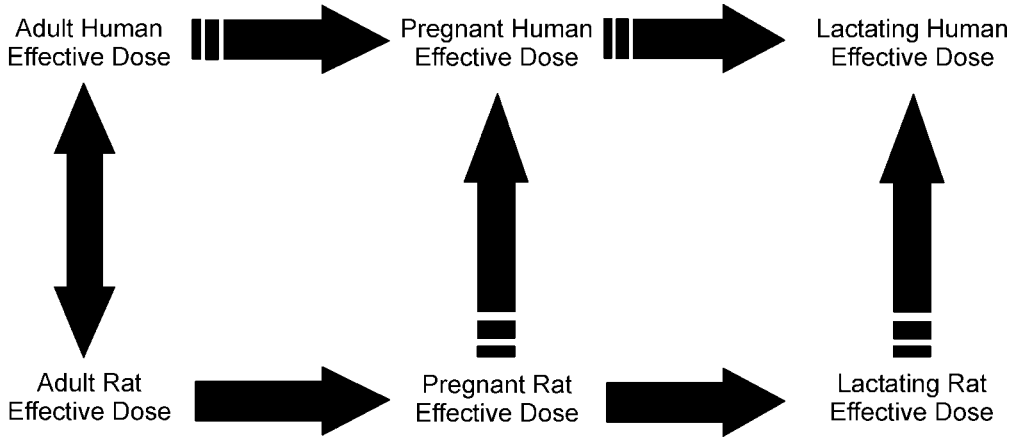

Figure 6-5. Schematic of extended parallelogram approach used for perchlorate due to effects at different life stages. Doses in the pregnant rat and fetus are related back to the adult male rat, likewise, the effects in lactating rats and neonates.

The various PBPK models are used to predict equivalent effective doses at the various administered doses used in the experiments; e.g., 1.0 mg/kg-day ammonium perchlorate given in drinking water to both the adult male rat and the pregnant dam. Each PBPK model is exercised (adult rat and pregnant rat) to predict the amount of internal dose metric achieved at each life stage. The ratio of the effective internal dose metrics of the life stage in question is then used to

adjust the HEE based on the adult male rat. For example, the HEE for the pregnant dam would be found by adjusting the HEE for the adult male rat by the ratio of the male rat:pregnant rat as:

$$\text{Pregnant HEE (mg/kg-day)} = \text{Adult male rat HEE (mg/kg-day)} \times \frac{\text{Adult male rat internal effective dose metric}}{\text{Pregnant rat internal effective dose metric}} \quad (6\text{-}1)$$

This ratio is unitless and accounts for the differences between the two life stages in question in an analogous fashion to the dosimetric adjustment factor (DAF) used in the EPA's inhalation reference concentration methods to extrapolate respiratory tract doses in different regions of the laboratory animal to human equivalent concentrations (U.S. Environmental Protection Agency, 1994). The same ratio approach is used to extend the model predictions to HEE estimates for the fetus, lactating dam, and neonate. Development of the ratios for two internal effective dose metrics, perchlorate area-under-curve (AUC) concentrations in serum and iodide uptake inhibition, will be discussed in Section 6.5.

6.2 ADULT RAT AND HUMAN MODEL STRUCTURES

Because the same model structure is used to describe perchlorate and iodide disposition (absorption, distribution, and elimination) for both the adult male rat and human, this section will describe the development of both of these models together. Data supporting development and validation of the structures will be summarized in this section while additional detail, including some of the governing equations, can be found in the consultative letters from the AFRL/HEST (Merrill, 2001c,d).

As discussed in Chapter 2, the perchlorate anion (ClO_4^-) is very similar in ionic size, shape, and charge to that of iodide (I^-). These shared properties allow perchlorate to interfere with the first stage of thyroid hormone synthesis by competitively inhibiting the active transfer of iodide into the thyroid by the sodium (Na^+)-iodide (I^-) symporter or NIS. The NIS is a protein that resides in the basolateral membrane of thyroid epithelial cells (Spitweg et al., 2000). NIS simultaneously transports both sodium and iodide ions from extracellular plasma into the thyroid epithelial cell via an active process. Energy is provided by the electrochemical gradient across

the cell membrane. The low intracellular concentration of sodium is maintained by sodium-potassium pumps (Ajjan et al., 1998). The kinetics of perchlorate and iodide anions differ mainly in that iodide is organified in the thyroid (thyroid hormone production); whereas, perchlorate is thought to be unreactive and eventually diffuses from the thyroid into systemic circulation.

The proposed PBPK model structure for the adult male rat (Merrill, 2001c) and human (Merrill, 2001d) describes active uptake of iodide and perchlorate in gastric juice, thyroid, and skin, and competitive inhibition of iodide uptake by perchlorate in NIS-containing tissues, as well as venous equilibration with slowly and richly perfused tissues as shown in Figure 6-6. Tissues that exhibited evidence of sodium iodide symporter and were found to concentrate either anion were depicted as compartments with nonlinear uptake (Merrill, 2001c,d). Tissues with active uptake include the thyroid, skin, and gastric mucosa (Wolff, 1998; Chow et al., 1969; Kotani et al., 1998). Although other tissues have been known to sequester iodide and similar anions (e.g., salivary glands, choroid plexus, ovaries, mammary glands, placenta) (Brown-Grant, 1961, Honour et al., 1952; Spitzweg et al., 1998), the iodide and perchlorate pools of these tissues was expected to be too small to significantly affect plasma levels. These tissues were lumped with slowly and richly perfused tissues.

The model also includes separate compartments for plasma, kidney, liver, and fat. These compartments do not maintain concentrations greater than the plasma at steady state, and therefore, were not described with terms for active uptake. The rapid urinary clearance of perchlorate (Yu, 2000) mandated the inclusion of a kidney compartment in the model. A liver compartment was also utilized due to its significant impact on iodide homeostasis. The majority of extrathyroidal deiodination takes place within the liver. Fat was primarily added as an exclusionary compartment. Due to its significant percentage of body weight, the skin represents an important pool for slow iodide turnover.

The modelers at AFRL/HEST found that a separate skin compartment was necessary. Experiments performed with radioiodide in rats resulted in skin:serum iodide ratios of close to one (Yu, 2000). Other researchers have reported higher ratios in rats, but results have not been consistent. Similar observations during dialysis with pertechnate of slow uptake and retention in human skin was observed by Hays and Green (1973) and the skin was therefore maintained as a separate compartment in the model. The skin contains two sub-compartments representing the

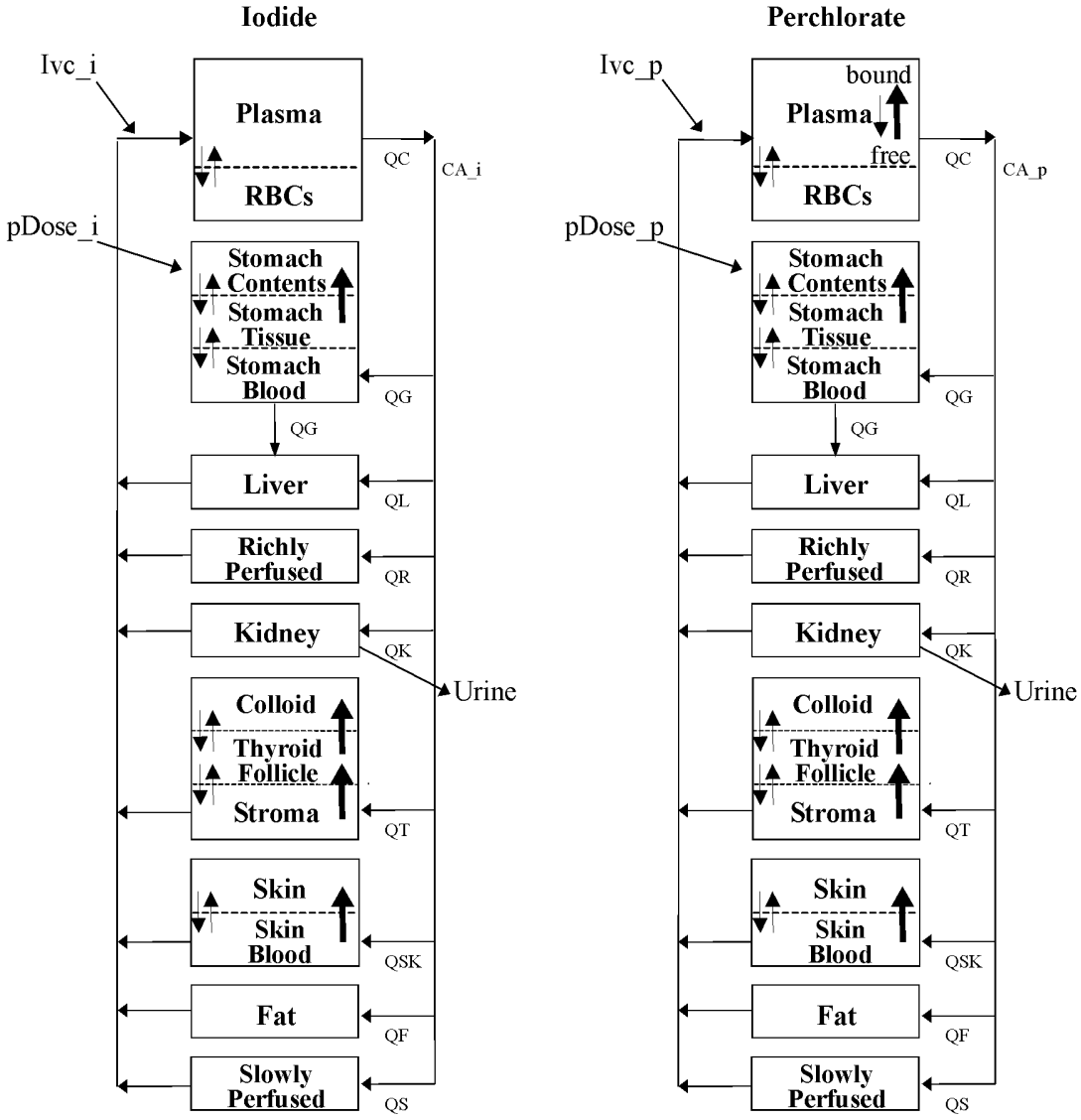

Figure 6-6. Schematic for the adult male rat and human PBPK models of perchlorate and iodide distribution (Merrill, 2001c,d). Bold arrows indicate active uptake (except for plasma binding) at NIS sites in thyroid, gut, and skin. Plasma binding was also described with Michaelis-Menten terms for the association 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 (ClO_4^-) and iodide (I^-) anions into deeper subcompartments.

capillary bed and the skin tissue. The thyroid and stomach consist of three sub-compartments: the stroma, the follicle, and the colloid in the thyroid and the capillary bed, stomach wall, and contents in the case of the stomach.

Active uptake into the thyroid colloid, stomach contents, and skin were described using Michaelis-Menten kinetics for nonlinear processes (Figure 6-6, bold arrows). Permeability area cross products and partition coefficients were used to describe the first order movement of the anions (ClO_4^- and I^-) between the capillary bed, tissue, and inner (deep) compartments (Figure 6-6, small arrows) that results from the inherent electrochemical gradient within the tissues. Passive diffusion through the kidney, liver, and fat compartments were described with partitions and blood flows. Plasma binding of perchlorate was described with Michaelis-Menten terms for the association of the perchlorate anions to plasma binding sites and a first order clearance rate for the dissociation. First-order clearance rates from the kidney were also used to describe urinary clearance of the anions.

The blood compartment differs between the perchlorate and iodide models. The perchlorate blood compartment is composed of plasma and plasma proteins to simulate binding. Plasma binding was required to simulate serum perchlorate concentrations at lower doses. Iodinated hormones bind to plasma proteins, but free iodide apparently does not. Therefore, a single compartment for plasma iodide was used. The free anions in plasma are available for diffusion and active uptake into tissues.

The presence of NIS is an indicator of active uptake for iodide. NIS is highly expressed in thyroid epithelial cells. Lower levels of expression have been detected in the mammary gland, salivary gland, skin, stomach, and colon (Ajjan et al., 1998; Spitzweg et al., 1998). However, only the thyroid has been found to organify iodide (Ajjan et al., 1998). The most important regulator of symporter gene and protein expression is thyroid-stimulating hormone (TSH). This is also the case for other important thyroid proteins such as thyroglobulin and thyroid peroxidase (Spitzweg et al., 1998).

The parameters used in the adult male rat and human model for the various compartments are provided in Table 6-1. The parameters were based on literature values or fitted to data using the model as described in the table. It is important to note that the model structure for both species is the same. The difference, per typical for PBPK models, is that there are species- and 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				
Tissue Volumes	**Male Rat**	**Source**	**Human**	**Source**
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_4^- kinetics (Yu et al., 2000)	1.7	Brown et al., 1997
Gastric Juice VGJc (%BW)	1.68	In house male rat ClO_4^- 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 Vttotc (%BW)	0.0077	Malendowicz, 1977	0.03	Yokoyama et al., 1986
Thyroid Follicle VTc (%Vttot)	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 Tissue Volumes	Male Rat	Source	Human	Source
Blood Flows				
Cardiac Output QCc (L/hr-kg)	14.0	Brown et al., 1997, Hanwell & Linzell, 1973	16.5	Brown et al., 1997; Hanwell & Linzell, 1973
Slowly Perfused QSc (%QC)	24.0	Brown et al., 1997	5.2	Brown et al., 1997
Richly Perfused QRc (%QC)	76.0	Brown et al., 1997	17.5	Brown et al., 1997
Fat QFc (%QC)	6.9	Brown et al., 1997	22.0	Brown et al., 1997
Kidney QKc (%QC)	14.0	Brown et al., 1997	1.0	Leggett & Williams, 1995; Malik et al., 1976
Liver QLc (%QC)	17.0	Brown et al., 1997	1.6	Brown et al., 1997
Stomach QGc (%QC)	1.61	Malik et al., 1976	13.0	Calculated, using 24% QC as flow to all slowly perfused tissues (Brown et al., 1997)
Skin QSkc (%QC)	5.8	Brown et al., 1997	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		

body weight), the maximum capacity of thyroid iodide or perchlorate uptake, and plasma binding of perchlorate. The chemical-specific parameter for each model for both perchlorate and iodide are provided in Table 6-2.

In order to simulate the daily dosing regimen of the drinking water experiment, the rats were assumed to drink at constant rate for 12 of the 24 hours per day (1800 to 0600 hours). A pulse function in ACSL was used to introduce drinking water to the gastrointestinal (GI) compartment of the rat for the first 12 hours of each 24-hour period and to stop dosing while the rat was presumably sleeping. Intravenous (iv) dosing was introduced into the venous blood compartment of the model. Intraperitoneal (ip) injection was introduced into the model in the same manner as the iv dosing.

6.2.1 Data and Methods

This section summarizes the AFRL/HEST data and data available in the literature that were used for model development. Details on experimental methods, including protocol design, exposure regimen, chemical source and purity, animals (housing, feeding, surgical procedures, etc), and the analytical methods for measurement of RAIU; of perchlorate in plasma, urine and tissues; and of thyroid hormones and TSH can be found in the associated consultative letters from AFRL/HEST (Merrill, 2001c,d; Yu, 2000, 2001, 2002; Yu et al., 2000).

6.2.1.1 Studies in Laboratory Rats

The studies performed at AFRL/HEST included both "acute" iv experiments to measure radiolabled iodide or perchlorate as well as measurements of the same after drinking water administration. These two different regimens provided a better characterization of the transient ("acute") and chronic behavior necessary for an accurate description of the disposition of the anions. Adult male Sprague-Dawley rats (330 ± 35 g; n = 6 rats per group) that were purchased from Charles River Laboratory (Raleigh, NC) were used in the experiments.

In these experiments, the term total iodine includes bound iodine plus fee inorganic iodide. Carrier doses included tracer doses of carrier free radiolabled iodide ($^{125}I^-$) along with non-radiolabeled iodide. Free $^{125}I^-$ radioactivity was determined by subtracting the bound from total 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)[a]

Partition Coefficients (unitless)	Male Rat			Human		
	Perchlorate	Iodide	Source	Perchlorate	Iodide	Source
Slowly Perfused/Plasma PS_	0.31	0.21	Yu et al., 2000; Halmi et al., 1956	0.31	0.21	Halmi et al., 1956;Yu et al., 2000
Richly Perfused/Plasma PR_	0.56	0.40	Yu *et al.*, 2000; Halmi *et al.*, 1956	0.56	0.40	Halmi *et al.*, 1956;Yu *et al.*, 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
Max Capacity, Vmaxc (ng/hr-kg)						
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)[a]

Partition Coefficients (unitless)	Male Rat			Human		
	Perchlorate	Iodide	Source	Perchlorate	Iodide	Source
Affinity Constants, Km (ng/L)						
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
Permeability Area Cross Products (L/hr-kg)						
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
Clearance Values (L/hr-kg)						
Urinary excretion CLUc_	0.07	0.05	Fitted	0.126	0.1	Fitted
Plasma unbinding Clunbc__	0.1	—	Fitted	0.025	—	Fitted

[a]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_T*i*, Vmaxc_T*p*, etc.).

6.2.1.1.1 Acute iv Experiments in Rats

Radiolabeled iodide ($^{125}I^-$) kinetics. Male rats were administered a single iv tail-vein injection with physiological saline (control group) or 33 mg/kg $^{125}I^-$ (with carrier) in physiological saline. Rats were euthanized by 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 $^{125}I^-$ (with carrier) and euthanized at 0.5, 2 and 6 hours post dosing. Total, bound, and free $^{125}I^-$ were analyzed in thyroid and serum, and total $^{125}I^-$ was measured in skin and gastric contents (Yu, 2001).

Radiolabeled $^{36}ClO_4^-$ kinetics. Naïve adult male rats (300 ± 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 μCi. Rats were euthanized by 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°C until analysis of $^{36}ClO_4^-$. 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.

$^{125}I^-$ Kinetics and Inhibition from Acute iv Dosing with 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 $^{125}I^-$ 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 $^{125}I^-$. Rats were euthanized at 15 min, 1, 2, and 4 hours after they were dosed with iodide. Levels of perchlorate and $^{125}I^-$ 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 $^{125}I^-$ with carrier and euthanized at 2 hours post iodide dosing. Blood and thyroid gland were collected for ClO_4^- and $^{125}I^-$ 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 Hoschschule, 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 μCi radiolabeled iodide ($^{131}I^-$), approximately 3.44×10^{-3} ng $^{131}I^-$/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

distribution, the authors ran a control session on the same subjects without aspirating gastric secretions. Aspirated gastric secretions accounted for 23% of the $^{131}I^-$ administered.

6.2.1.2.2 Perchlorate Kinetics and Inhibition of Thyroid Iodide Uptake (Greer et al., 2000)

Perchlorate data. As described in Chapter 4, Greer et al. (2000) recently studied the effects of repeated low level exposure to perchlorate on humans. Subjects received 0.5, 0.1, 0.02, or 0.007 mg/kg-day perchlorate in drinking water over a two week period. Each dose group consisted of eight healthy volunteers (four males and four females) with no signs or symptoms of thyroid disorders (euthyroid). The daily dose was dissolved in 400 mL water and divided into four 100 mL servings that were ingested at approximately 0800, 1200, 1600, and 2000 hours.

Baseline serum and urine samples were collected before the first perchlorate treatment. During perchlorate exposure, serum samples were collected at the following approximate times: day 1 at 1200 and 1600, day 2 at 0800, 1200, and 1700, day 3 at 0900, day 4 at 0800 and 1200, day 8 between 0800 and 0900 and day 14 at 0800 and 1700. Serum samples were also collected on post-exposure days 1, 2, 3, and 14. Twenty-four hour urine collections were taken on exposure days 1, 2, 14 and post-exposure days 1 through 3. Serum and 24-hour urine samples from the study were provided to AFRL/HEST compliments of Dr. Monte Greer of Oregon Health Science University (OHSU), Portland, OR, and Dr. Gay Goodman of Intertox, Seattle, WA. The samples were analyzed for perchlorate at the Operational Toxicology Branch, Human Effectiveness Directorate at the Air Force Research Laboratory (AFRL/HEST), Wright Patterson Air Force Base (WPAFB), OH, using the analytical methods described in Merrill (2001d).

Iodide Inhibition Data. Eight and 24 hour thyroid $^{123}I^-$ uptakes (radioiodine uptake or RAIU) were measured one to two days prior to perchlorate treatment (baseline) on days 2 and 14 of perchlorate exposure and 14 days after perchlorate exposure was discontinued. A gelatin capsule containing 100 mCi of $^{123}I^-$ was administered orally at 0800, before the first perchlorate solution for that day was drunk. Thyroid scans were then taken 8 and 24 hours later.

Thyroid and Pituitary Hormone Data. The serum samples were also analyzed for TSH, T4, T3, and free T4 at OHSU. However, these hormone data were not used in the PBPK model described below. Statistical analysis of the data is described in Attachment 2 of Merrill (2001d).

In summary, there was little effect of perchlorate on levels of T4, free T4, or T3. TSH decreased significantly from baseline by Exposure Day 3. On Post-Exposure Day 1, the TSH

levels of the subjects in the 0.5 mg/kg-day group had decreased by an average of 35% from baseline (ranging from 17% to 52%). Therefore, it appears that TSH was dropping while inhibition remained the same. It is possible that there is an increase in thyroid sensitivity to TSH as an early response to inhibition (Brabant et al., 1992). This increased sensitivity (possibly an increase affinity of the TSH receptor) could possibly decrease circulating TSH levels while T4 has not decreased sufficiently yet to stimulate the hypothalamus to increased TRH secretions. After perchlorate was discontinued, between Post-Exposure Days 1 and 15, the mean TSH level increased significantly over baseline (23% greater than baseline), with TSH of one subject remaining below baseline. The drop in TSH during perchlorate exposure and the rise above baseline measurements after perchlorate seem counter-intuitive to the TSH regulation expected but may be part of a rebound phenomenon as the NIS begins to upregulate.

In addition, the data by Greer et al. (2000) showed an increase in radioiodide uptake in excess of baseline measurements 14 days after perchlorate exposure. An increase in radioiodide uptake is expected due to the rise in TSH mentioned above. This rebound effect has been noted in other human inhibition studies (using both iodide and perchlorate as inhibitors). Saxena et al. (1962) evaluated the prophylactic doses of iodide required to suppress thyroid uptake of $^{131}I^-$ in euthyroid mentally defective children. They found a minimal effective oral dose of 1500 to 2000 μg iodide per square meter of body surface per day was required to completely suppress $^{131}I^-$ uptake. Within a week after iodide administration was stopped, a rebound of uptake was noted. In some instances these uptakes were even higher in subsequent weeks.

6.2.1.2.3 Supporting Kinetic Studies

Both urine and serum perchlorate concentrations for a validation exercise were provided from a recent unpublished study by Drs. Brabant and Leitolf of Medizinische Hochschule, Hanover, Germany. In their study, seven healthy males ingested 12.0 mg/kg perchlorate dissolved in 1 liter of drinking water every day for two weeks. The daily perchlorate dose was divided equally in three portions and ingested three times per day (approximately between 0600 and 0800, 1100 and 1300 and 1800, and 2000 hours). Blood specimens were collected on days 1, 7, and 14 of perchlorate treatment and on the two mornings after perchlorate administration was discontinued. Samples were analyzed for perchlorate at AFRL/HEST.

Three published studies reported cumulative urine concentrations collected from healthy males after receiving a high oral dose of perchlorate (Durand, 1938; Kamm and Drescher, 1973; Eichler, 1929). Oral doses administered in these studies were 784 mg $NaClO_4$ (635 mg ClO_4^-; Durand, 1938); 1000 mg $NaClO_4$ (765 mg ClO_4^-; Kamm and Drescher, 1973), and 2000 mg $KClO_4$ (1400 mg ClO_4^-; Eichler, 1929). The studies did not report serum perchlorate levels but could be used to validate the model.

Stanbury and Wyngaarden (1952) measured radioiodide uptake in a patient with Grave's disease. The patient received a tracer dose of $^{131}I^-$ as a control before perchlorate dosing and again one hour after administration of 100 mg $KClO_4$. Thyroid scans of radioiodide uptake were performed both after the control and perchlorate sessions to determine the level of inhibition.

6.2.2 Adult Male Rat Model Development

This section summarizes some key features necessary to the development of the adult male rat model structure and shows results of predictions made with simulations against experimental data used to parameterize and validate the model.

6.2.2.1 Physiologic Parameters and Tissue Partition Coefficients

The adult male rat volumes and blood flows were obtained from the literature or the AFRL/HEST studies as described in Table 6-1. Allometric scaling was used to account for parameter differences due differences in body weights between rats and humans. Because no steady-state values from infusion studies were available, the partition coefficients for iodide and perchlorate were estimated from the various studies listed in Table 6-2. The liver:serum and muscle:serum ratios of 0.56 and 0.31 were obtained in the AFRL/HEST radiolabled perchlorate ($^{36}ClO_4^-$) iv study described above. The liver:serum partition value was used to represent partitioning to the liver and richly perfused compartments and the muscle:serum value to represent the slowly perfused compartment.

For compartments with nonlinear uptake of the anions, effective partition coefficients were used that represented either approximate tissue:serum concentration ratios or electrical potential gradients. Chow and Woodbury (1970) measured electrochemical potentials within the thyroid stroma, follicular membrane, and colloid at three different doses of perchlorate. The measured difference in electrical potential between the thyroid stroma and follicle was interpreted by

Merrill (2001c) as an effective partition coefficient for the perchlorate and iodide anions, hindering the entry of negatively charged ions into the follicle. The equal and opposite potential from the follicle to the colloid enhances passage of negatively charged species into the colloid and indicates an effective partition coefficient of greater than one. The equivalence between electrical potential differences $\phi_i - \phi_f$ and effective partition coefficients for the thyroid subcompartments (stroma:follicle and follicle:colloid) were estimated in the manner of Kotyk and Janacek (1977) based on the Chow and Woodbury (1970) data as described in Merrill (2001c).

6.2.2.2 Chemical-Specific Parameters

The various active transport processes, tissue permeabilities, and clearance rates (excretion) are described in PBPK models for each species on a chemical-specific basis. This section outlines how the values for perchlorate and iodide used in the adult male rat model were derived. The values can be found in Table 6-2 and details on derivation in Merrill (2001c).

6.2.2.2.1 Affinity Constants and Maximum Velocities for Active Transport Processes

Kinetic values for the saturable (Michaelis-Menten) active uptake process of perchlorate, the affinity constant and maximum velocity capacity (Km_*p* and Vmaxc_*p*), were not available in the literature nor were they determined experimentally at AFRL/HEST. Only the affinity of iodide for NIS was available in the literature. The Merrill (2001c) adult rat model uses a Michaelis-Menten affinity constant (Km) value of 4.0×10^6 ng/L to describe the affinity of iodide (Km_*i*) across compartments involving active transport by NIS (e.g., in the thyroid and gastric juices). This was based on the mean value of 3.96×10^6 ng/L for iodide derived by Gluzman and Niepomniszcze (1983) from thyroid slices of 5 normal individuals. The thyroid slices were incubated with several medium iodide concentrations. The experimentally determined Km values for iodide are similar across species (Gluzman and Niepomniszcze, 1983) and across different tissues (Wolff, 1998). This average literature value was therefore used for iodide in tissues described with active uptake.

The values for perchlorate affinity were originally assumed to be the same as those for the Km of iodide, due to the similar mechanism in which the two anions are transported into the tissues. Thus, the iodide values were adjusted for the difference in mass to give an estimated

value for the affinity of perchlorate. The molar equivalent of iodide's Km for perchlorate is 3.1×10^6 ng/L. However, these values were not adequate for use in the models. Several literature sources suggest that perchlorate may have a significantly higher affinity for NIS than iodide. In his 1963 paper (Wolff and Maurey, 1963) and his 1998 review, Wolff concluded that perchlorate has a greater affinity than iodide for the NIS. This assumption was based upon his work with iodide, perchlorate, and several other anions actively sequestered in the thyroid. Wolff measured the Km of a few of the anions and inhibition constants (Ki's) for several ions, including perchlorate. As noted in Chapter 2, Wolff found that the relative potency of inhibition by the various anions could be described with the following series: TcO_4^->ClO_4^->ReO_4^->SCN^- >BF_4^-> I^->NO_3^->Br^->Cl^-. Wolff reported that the measured Km values for several of these inhibiting anions were not the same as those measured for iodide. In fact, measured values for Km and Ki for several of the inhibiting anions revealed that affinity increased with increased inhibitory potency.

Several studies suggest perchlorate is a more potent inhibitor than iodide. In the rat thyroid, Wyngaarden et al. (1952) have shown that perchlorate was a more powerful inhibitor of the iodide trap than thiocyanate. Halmi and Stuelke (1959) showed that perchlorate was ten times as effective as iodide in depressing tissue to blood ratios in the rat thyroid and gut. Similarly, Harden et al. (1968) compared human saliva to plasma radioiodide concentration ratios after equimolar doses of perchlorate and iodide. The saliva:plasma iodide ratios during resting conditions were approximately seven times lower after a molar equivalent dose of perchlorate versus iodide. Lazarus et al. (1974) also demonstrated that perchlorate was taken up to greater extent in mice salivary glands than iodide. These studies, in addition to the work of Chow et al. (1969), support the use of a lower Km for perchlorate uptake in the tissues with sodium iodide symporter. Based on this information, a value of 2.5×10^5 ng/L for the thyroid (Km_T*p*) and 2.0×10^5 ng/L for skin (Km_S*p*) or gut (Km_G*p*), approximately 10 times lower than that of iodide, was estimated by Merrill (2001c,d) to represent perchlorate's affinity for transport by the NIS.

The apical follicular membrane (between the thyroid follicle and colloid) also exhibits a selective iodide uptake mechanism. Golstein et al. (1992) measured a Km value of approximately 4.0×10^9 ng/L for the transport of iodide between the thyroid follicle and colloid (Km_DT*p*) in bovine thyroid. This iodide channel also appears to be very sensitive to

perchlorate inhibition and shares a similar permeability to perchlorate as to iodide. The ability of perchlorate to inhibit iodide uptake at the apical follicular membrane suggests that the Km of perchlorate at the apical follicular membrane (Km_Dt*p*) is also lower than that of iodide. Model simulations of thyroid inhibition supported a value of 1.0×10^8 ng/L, approximately ten times less than that of iodide.

Whereas the Km is similar across tissues containing NIS, the maximum velocity term (Vmaxc) does vary between tissues and species (Wolff, 1998), being lower in humans than other species (Gluzman and Niepomniszcze, 1983; Wolff and Maurey, 1961). Maximum velocities or capacities (Vmaxc) were not found in the literature and were estimated for a given compartment by fitting the simulation to the data at varying doses.

6.2.2.2.1 Effective Partitions, Permeability Area Cross Products and Clearance Values

Permeability area cross products and partition coefficients were used to describe diffusion limited uptake in tissues requiring subcompartments. The permeability area values in the Merrill (2001c) model were fitted by setting the partition coefficients to the literature values in Table 6-2. Fitted clearance values were used to describe first-order urinary excretion rates and reversible plasma binding to serum. Equations for these representations are provided in Merrill (2001c).

6.2.2.3 Adult Male Rat Model Simulation Results and Validation

The simulations shown in this section result from exercising the model with the physiological and chemical-specific parameters provided in Tables 6-1 and 6-2. Figure 6-7 illustrates the model predictions versus data time course for the iv radiolabeled perchlorate study described in Section 6.2.1.1.1. The model produced good simulations for the trend of the data but slightly over predicts the thyroid concentrations at later time points (Panel A). Model predictions fit the data well for perchlorate concentrations in the serum (Panel B) and kidney (Panel C), as well as the amount excreted in the urine (Panel D). Other tissue concentrations not shown herein also were predicted well by the model (Merrill, 2001c).

Figure 6-8 shows that plasma binding of perchlorate was necessary to provide adequate model predictions. Thyroid, serum, and urine were collected from the iv studies described in 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.

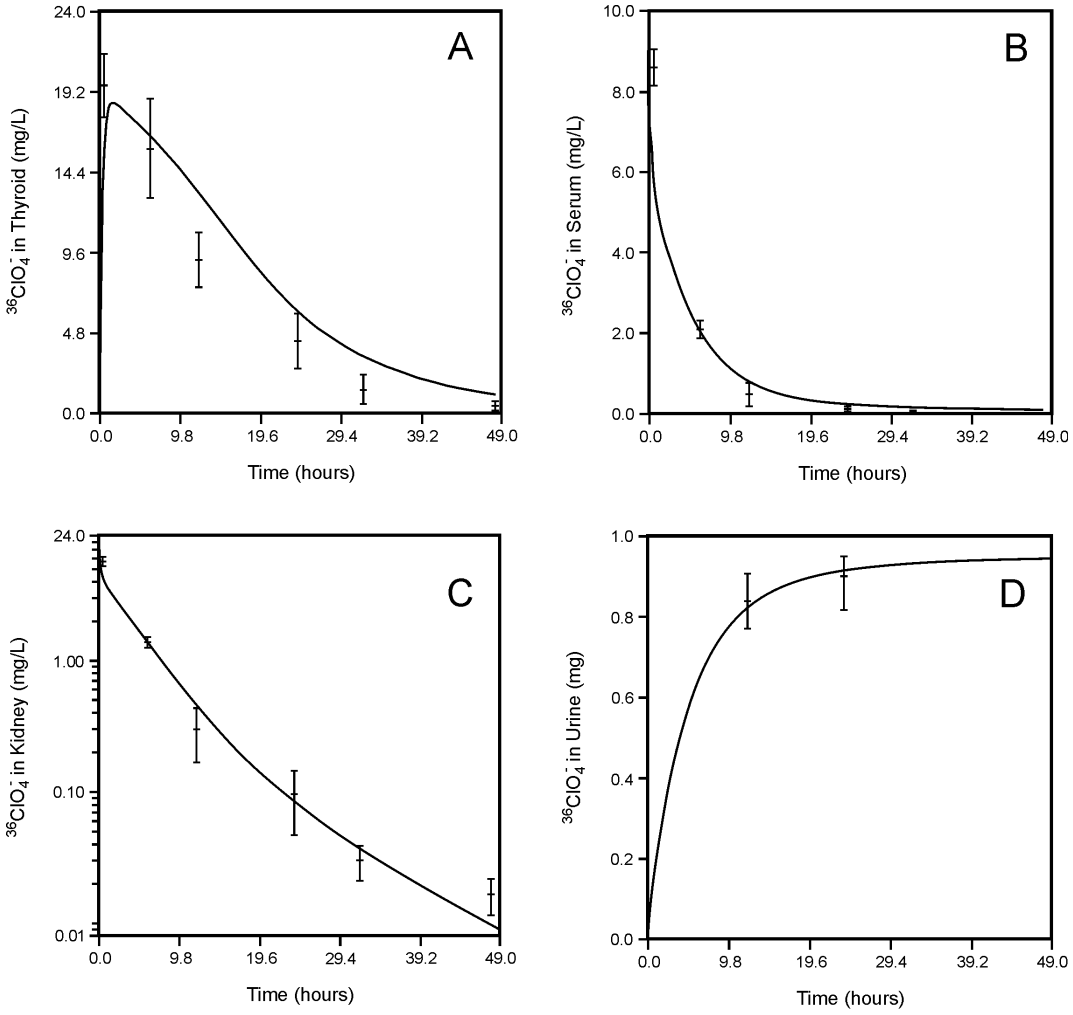

Figure 6-7. Adult male rat PBPK model predictions after an acute iv dosing with radiolabeled perchlorate ($^{36}ClO_4^-$). Panels A and B show model predictions (lines) versus data time course (mean ± SD) of labeled perchlorate (mg/L) in the thyroid and serum. Panel C shows model predictions versus data time course of labeled perchlorate (mg/L) in the kidney. Panel D shows cumulative excretion (mg) of labeled perchlorate in the urine (Merrill, 2001c).

Model predictions without plasma binding (Panel A, left) resulted in an underestimation of serum perchlorate concentrations at the 1 mg/kg-day dosage level and below. Low serum predictions suggested either greater uptake into other tissues or protein binding. To provide better estimates of perchlorate serum concentrations at the 0.01 and 0.1 mg/kg doses, Merrill (2001c) added protein binding to the venous blood compartment of the model. An affinity

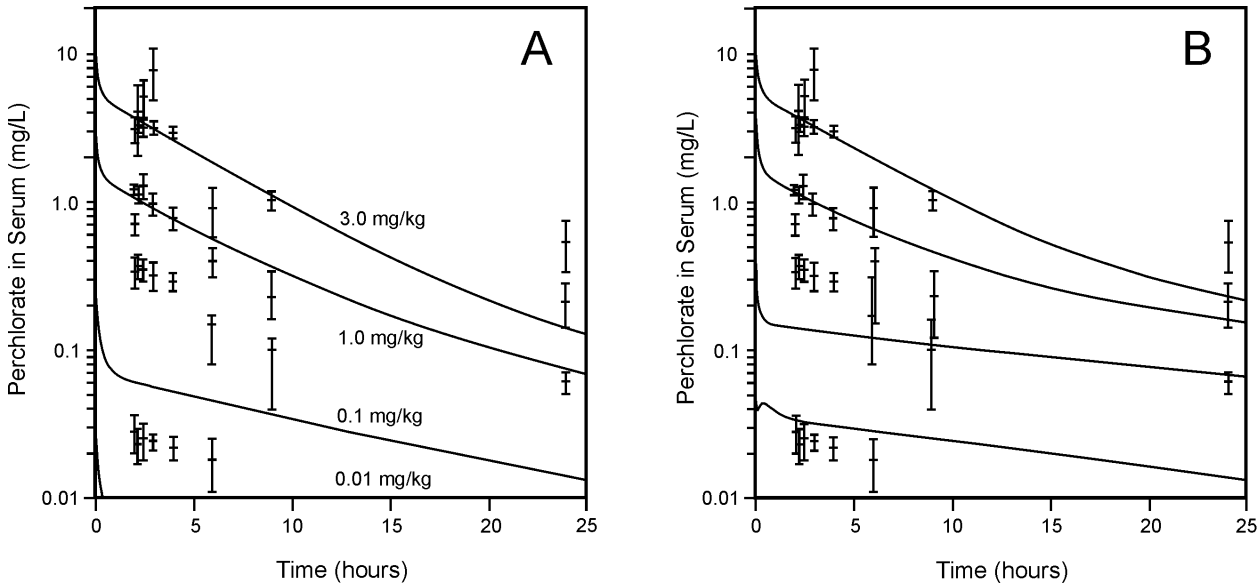

Figure 6-8. Simulations illustrating the necessity of including plasma binding in the adult male rat PBPK model structure (Merrill, 2001c). Model predictions (lines) versus data time course (mean ± SD) of perchlorate concentration (mg/L) in serum after doses of 3.0, 1.0, 0.1 and 0.01 mg/kg-day are shown 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).

constant for this binding of perchlorate in the blood (Km_B*p*) of 1.1E6 ng/L and a maximum velocity capacity for this blood binding (Vmaxc_B*p*) of 9.3E3 ng/h/kg was fitted to serum levels from doses ranging 0.01 to 3.0 mg/kg (Panel B, right). The model underpredicts serum perchlorate from the 0.1 mg/kg dose group; but it fits serum at 0.01 mg/kg and cumulative urine across the doses. Interestingly, the urinary excretion at 0.01 mg/kg was lower than the other doses, accounting for elevated serum concentrations. Mean 24 hour urinary excretions (± SD) of perchlorate were approximately 97% (± 2), 72% (± 1), 87% (± 17), and 91% (± 13) of the administered iv dose for the 0.01, 0.1, 1.0, and 3.0 mg/kg dose groups, respectively.

The literature discussed in Chapter 3 and in Merrill (2001c) suggests that serum albumin is the major binding protein; however, it does not confirm that albumin is the only binding site. Merrill (2001c) notes that no studies were found that evaluated whether perchlorate or similar anions bind to thyroglobulin. However, Yamada (1967) studied the effects of perchlorate and

other anions on T4 metabolism and noted a significant decrease in serum protein-bound iodide (PBI) in thyroidectomized T4-maintained perchlorate-fed rats. In a 1968 in vitro study, Yamada and Jones reported that T4 was displaced from plasma protein as indicated by an uptake of T4 by muscle in the presence of plasma taken from perchlorate-fed rats. This suggested, but did not demonstrate directly, that perchlorate interferes with T4 binding with plasma proteins.

Pertechnetate is known to bind to plasma proteins. Hays and Green (1973) studied the blocking of pertechnetate binding with human serum proteins by other anions. Perchlorate was found to be one of the most effective, while iodide was ineffective. In dialysis studies, inorganic iodide did not bind to plasma proteins. The pertechnetate binding appeared to be reversible in serum.

Simulations of thyroid perchlorate concentrations and of the amount of perchlorate excreted in the urine from the four dose groups are shown in Figure 6-9. It was noted that the thyroid concentrations resulting from the 3.0 mg/kg cold perchlorate study were slightly higher than those from the radiolabeled perchlorate ($^{36}ClO_4^-$) study at 3.3 mg/kg (Figures 6-9A and 6-7A, respectively). This may reflect the analytical differences in measuring cold versus radiolabeled perchlorate. The model slightly underpredicts the thyroid concentrations at 3.0 mg/kg, based on the cold perchlorate data (Figure 6-9A), and slightly overpredicts the $^{36}ClO_4^-$ thyroid concentration at 3.3 mg/kg (Figure 6-7A).

The model is able to adequately predict data from studies that were not used in the development process. Figure 6-10 shows the model predictions versus the data of Chow and Woodbury (1970) and Eichler (1929). Model predictions fit the data well for radiolabeled perchlorate concentration in the thyroid (A); whereas, the serum (B) is underpredicted. Merrill (2001c) notes the difference and provides some plausible explanations. The rats in the Chow and Woodbury (1970) study were functionally nephrectomized by ligating the renal pedicle of both kidneys and given the radiolabeled perchlorate ip. Analytical differences between AFRL/HEST and Chow and Woodbury could exist, and it is also possible that the nephrectomization creates physiological changes that can not be accounted for sufficiently by "turning off" urinary excretion in the model simulations. One hypothesis is that saturation in NIS-containing tissues occurs to a lesser extent as a result of increased extracellular sodium cation (Na^+) and possibly other competitive anions when renal clearance is blocked, thereby increasing the arterial radiolabeled perchlorate. While the underprediction in serum would suggest the need for an

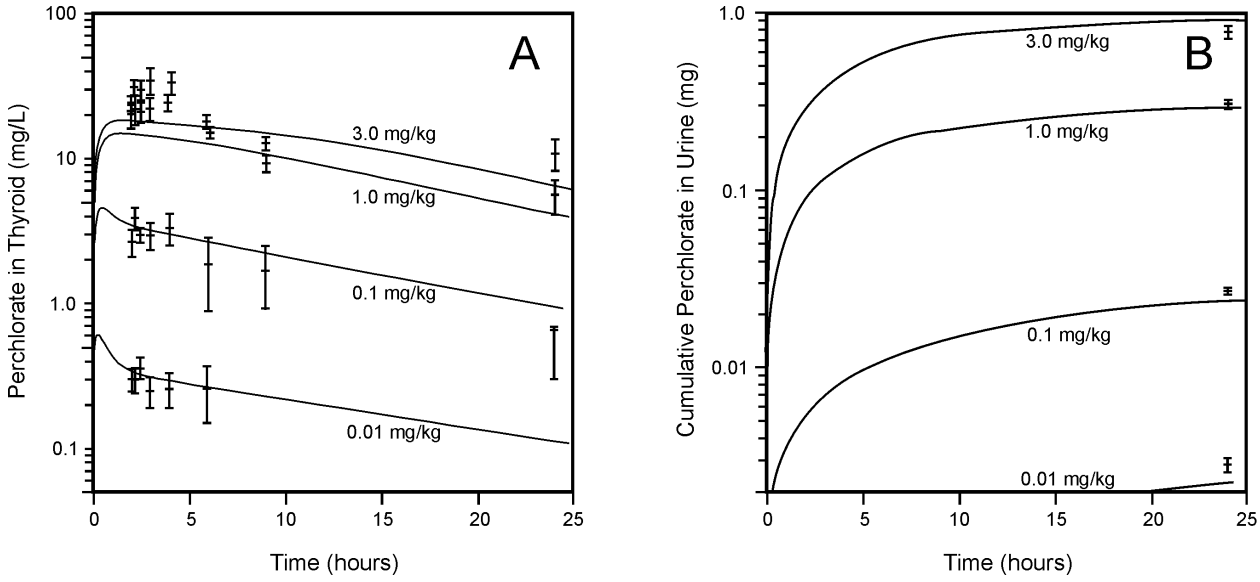

Figure 6-9. Adult male rat PBPK model predictions (lines) versus data time course (mean ± SD) of perchlorate concentrations in the thyroid (mg/L) in Panel A or cumulative excreted perchlorate in the urine (mg) in Panel B (Merrill, 2001c). Male rats were dosed iv with 3.0, 1.0, 0.1 or 0.01 mg/kg-day perchlorate (Yu, 2000).

increased binding constant for perchlorate, this was not consistent with the data from AFRL/HEST for studies at lower doses (Merrill, 2001c). Panel C in Figure 6-10 shows the model predictions versus the data of Eichler (1929) for cumulative perchlorate excreted in the urine. These rats were given perchlorate subcutaneously (sc) at doses of 1.6, 8.0, and 49 mg/kg.

The adult male rat model (Merrill, 2001c) is also able to predict iodide distribution. Figure 6-11 shows the model predictions versus a time course for radiolabeled iodide data from the AFRL/HEST experiments outlined in Section 6.2.1.1.1. Adequate fit is demonstrated for both the thyroid and serum concentrations at doses of radiolabeled iodide differing by an order of magnitude (0.033 and 0.33 mg/kg).

Figure 6-12 demonstrates the fit of the model simulations of perchlorate thyroid concentration (mg/L) after drinking water exposures to perchlorate. The model was coded to simulate oral dosing for 12 hours per day, assuming that rats drink fairly continuously during their waking hours. The same perchlorate parameters used to describe the "acute" (iv) kinetics also adequately described serum concentrations from these "chronic" drinking water exposures

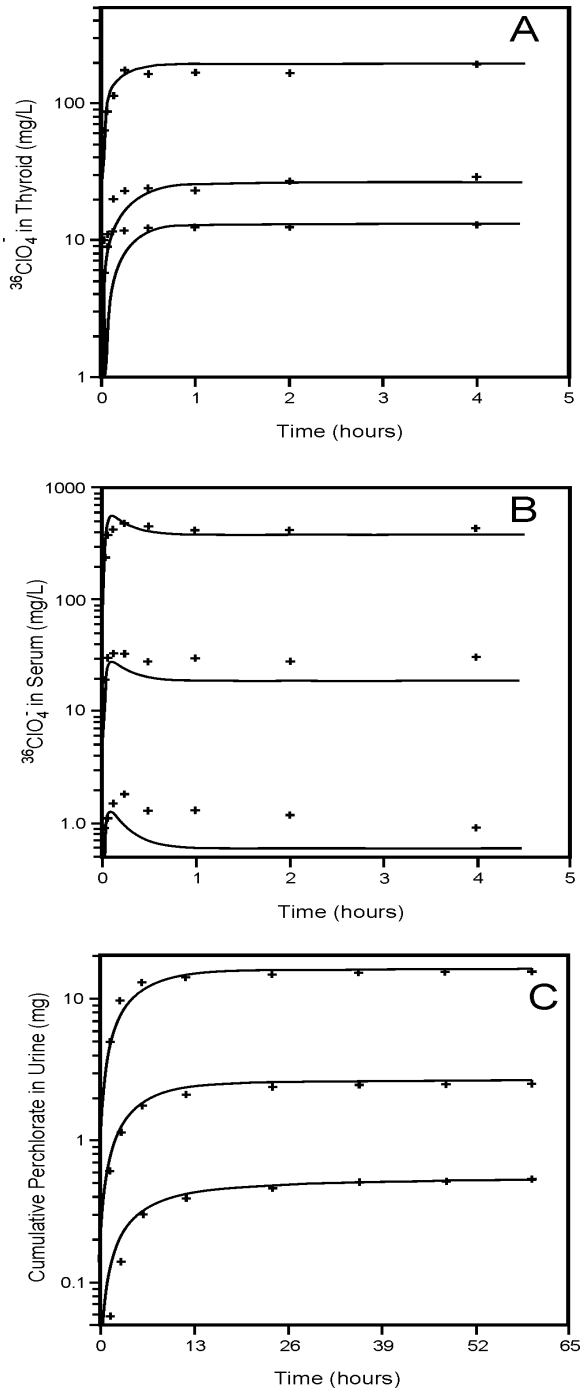

Figure 6-10. Validation for male rat PBPK model of perchlorate disposition (Merrill, 2001c). Model predictions (lines) versus data time course for concentrations (mg/L) in the thyroid (A) and serum (B) for ip administration in rats of 200, 10, and 0.5 mg/kg $^{36}ClO_4^-$ (data of Chow and Woodbury, 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).

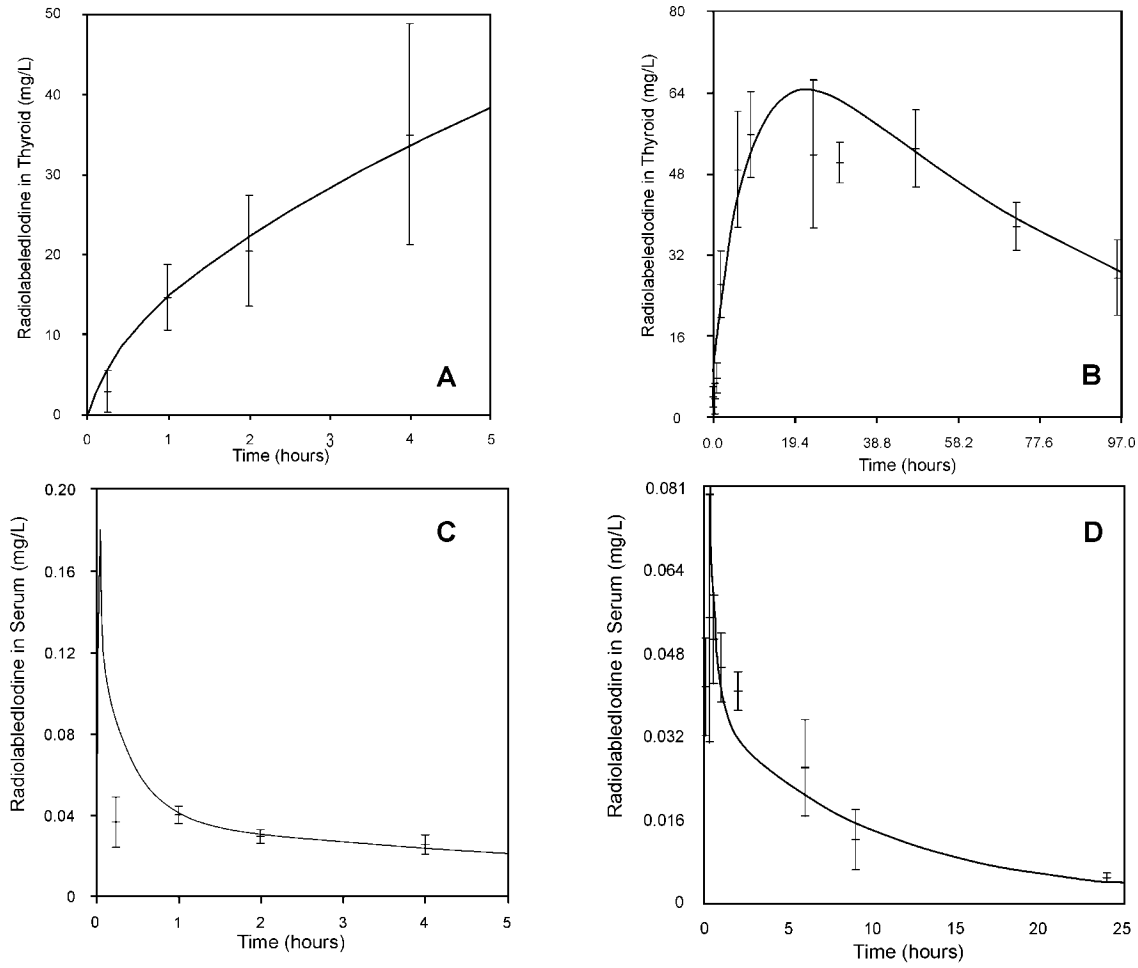

Figure 6-11. Male rat PBPK model (Merrill, 2001c) predictions (lines) versus data time course (mean ± SD) of iodide concentrations (mg/L) at two doses of $^{125}I^-$ with carrier, 0.033 mg/kg or 0.33 mg/kg, in the thyroid (A) or (B) and in the serum (C) or (D). Data of Yu (2001).

(data shown in Merrill, 2001c) but failed to predict thyroid concentrations from the 3.0 mg/kg-day dose and higher. TSH in these same studies was increased during drinking water exposure across all doses so that Merrill (2001c) accounted for the TSH-induced upregulation in the NIS by fitting an increased effective thyroid follicle:stroma partition coefficient (PT_*p*) at these higher doses. Merrill (2001c) noted that TSH is not expected to increase NIS in tissues other than the thyroid (Brown-Grant, 1961**)** and that these simulations agree. Given the small size of the thyroid, its upregulation would not decrease serum concentrations significantly. This explains

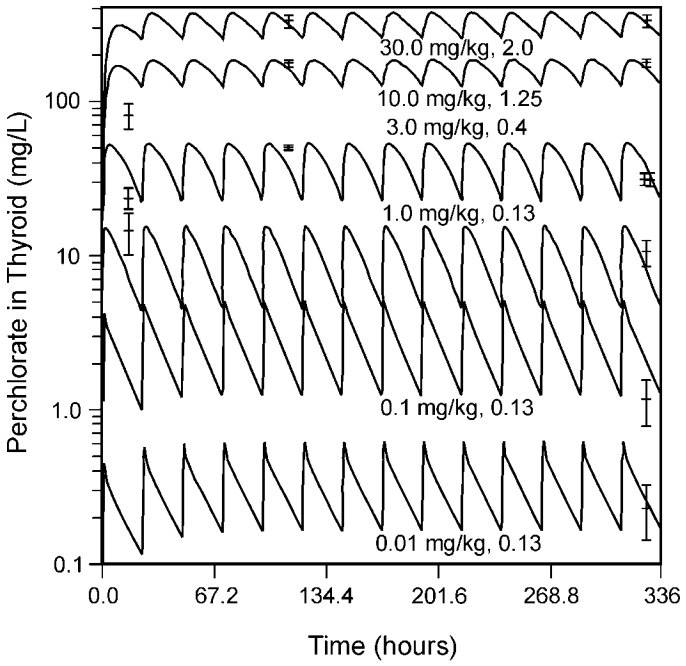

Figure 6-12. Male rat PBPK model predictions (lines) versus data time course (mean ± SD) of thyroid perchlorate concentrations (mg/L) in male rats during ingestion of 30, 10, 3.0, 1.0, 0.1, or 0.01 mg/kg-day in drinking water for 14 days (Merrill, 2001c). Data across the doses were fit by increasing 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.

why the model successfully predicted serum perchlorate concentrations across drinking water doses with the same parameters used to describe acute exposures and why it could not predict thyroid concentrations above 3 mg/kg-day.

It could be expected that other parameters (e.g., follicle size and follicular Vmaxc) would also increase with TSH stimulation. There is an increase in percent of thyroid volume attributed to the follicle cells (Conde et al., 1991; Ginda et al., 2000), total protein, RNA and DNA content, and the incorporation of labeled amino acids into protein (Pisarev and Kleiman de Pisarev, 1980). However, Merrill (2001c) notes that adequate predictions could be achieved by adjusting additional parameters; although, without incorporation of regulation by the hypothalamic-pituitary-thyroid axis, such adjustments provide little additional insight.

The ability of the adult male rat model to predict iodide uptake inhibition in the thyroid is demonstrated in Figure 6-13 for a single iv dose of perchlorate (right) or for a 14-day drinking

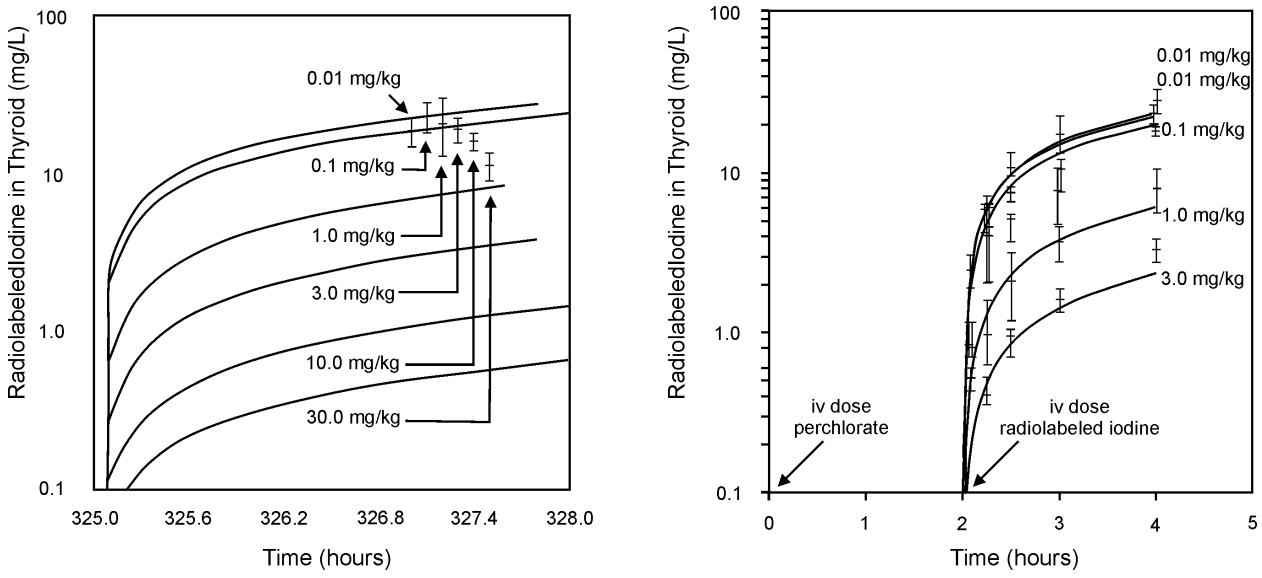

Figure 6-13. Male rat PBPK model predictions (lines) versus data time course (mean ± SD) of iodide uptake inhibition in male rats administered perchlorate either by a single iv dose (right) or in drinking water for 14 days (left), followed by an iv dose of 33 ug/kg $^{125}I^-$ with carrier (Merrill, 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).

water exposure (left). Perchlorate-induced inhibition of $^{125}I^-$ uptake in the thyroid was 13, 24, 70, and 88% at 2 hours and 11, 29, 55, and 82% at 9 hours after iv dosing with $^{125}I^-$ with carrier for the 0.01, 0.1, 1.0, and 3.0 mg/kg dose groups. Good simulations were achieved across doses. However, at 3.0 mg/kg, the model slightly overpredicts inhibition 6 hrs after the perchlorate dose (4 hours after $^{125}I^-$ administration). TSH was measured from the highest dose level (3.0 mg/kg) between 8 and 48 hours post dosing and was found to increase between 8 and 12 hrs. It is possible that TSH was already elevated at 6 hrs, allowing upregulation of the thyroid to compensate for inhibition at that time point, which the model would not predict. Yu (2000) provides greater details on hormone fluctuations resulting from the AFRL/HEST experiments.

With respect to iodide inhibition after 14 days of drinking water exposure to perchlorate at 0.01, 0.1, 1.0, 3.0, 10.0, and 30.0 mg/kg-day (Figure 6-13, left), the model overpredicts inhibition at the 1.0 mg/kg-day dosage and greater. TSH-induced upregulation of the thyroid compensates for competitive inhibition, resulting in little or no inhibition of radioiodide uptake on Day 14 of

exposure in all dose groups except 30 mg/kg-day. In all treated groups, TSH levels were already increased after the first day. Serum T4 initially decreased in all dose groups except the 0.01 mg/kg-day group. By day 14, T4 levels had increased to control values in the 0.1 and 1.0 mg/kg-day dose groups. FT4 increased in all dose groups on day 1, returned to normal values by day 5, and were significantly elevated across all dose groups by day 14 (except the 0.1 mg/kg-day group).

6.2.3 Human Model Development

The adult human PBPK model (Merrill, 2001d) was developed concurrently with that for the adult male rate (Merrill, 2001c) and updates the preliminary structure provided to EPA (Merrill, 2000). Much of the early development was based upon generalizations from previous AFRL/HEST work on perchlorate (Fisher, 1998a; 2000) and the work of Hays and Wegner (1965) describing iodide kinetics. As discussed above and shown in Figure 6-1, a nearly identical model structure to that of the adult male rat was used for the adult human. The human physiological parameters will of course be different as these should be species-specific. This section will only highlight notable differences in parameter sources between the two models.

6.2.3.1 Physiologic Parameters and Tissue Partition Coefficients

Human tissue volumes and blood flows were obtained from the literature as shown in Table 6-1. Merrill (2001d) notes that considerable variability was reported for some parameters. For example, blood flow to the gastrointestinal (GI) tract can increase ten-fold in response to enhanced functional activity (secretion and digestion) (Granger et al., 1985). Blood flows used in the model represent estimates of resting values. Human data on the volume of the gut capillary bed (VGBc) were not found in the published literature. Therefore, Merrill (2001d) used a value derived from rat stomach data (Altman and Dittmer, 1971a) for the volume of the gastrointestinal blood (VGBc) in the human model.

Thyroid volume was obtained from ultrasound measurements on 57 healthy volunteers with no thyroid disorders (37 to 74 years of age) in a study conducted by Yokoyama et al. (1986). The mean thyroid volume was 13.4 ± 4.1 mL and mean thyroid volume to body weight ratio was 0.251 ± 0.074 mL/kg (mean ± SD), approximately 0.03% of body weight. Yokoyama et al. (1986) found a positive correlation between thyroid volume and both body weight and age, with

weight having the most pronounced influence. The percent of total thyroid volume attributed to the thyroid follicular epithelium, colloid, and stroma were estimated from histometric measurements of patients at necropsy by Brown et al. (1986). Their findings on the histological features of thyroids of men and women showed overlapping distributions without evidence of a significant difference between sexes. However, a significant sex difference in total fat mass is reported in humans, with women having approximately 10% more fat than men (Brown et al., 1997). Based on these data, Merrill (2001d) used a gender-specific value for this parameter.

6.2.3.2 Chemical-Specific Parameters

The various active transport processes, tissue permeabilities, and clearance rates (excretion) are described in PBPK models for each species on a chemical-specific basis. This section outlines how the values for perchlorate and iodide used in the human model were derived. The values can be found in Table 6-2, and the details on derivation are in Merrill (2001d).

6.2.3.2.1 Affinity Constants and Maximum Velocities

The Michaelis-Menten affinity constant (Km) estimates for perchlorate and iodide in the various tissues with active transport were developed in the human in an analogous fashion to that in the rat, as described above in Section 6.2.2.2., based on Golstein et al. (1992), Gluzman and Niepomniszcze (1983), and Wolff (1998). The maximum velocity capacity (Vmaxc) values were estimated for the various compartments by fitting the simulations to available data at various doses (Merrill, 2001d).

6.2.3.2.2 Effective Partitions, Permeability Area Cross Products, and Clearance Values

Permeability area cross products and clearance values for perchlorate and iodide were developed by fitting to literature values in an analogous fashion to that for the rat described in Section 6.2.2.3 (Merrill, 2001d).

6.2.3.3 Adult Human Model Parameterization and Validation

The human PBPK model for iodide was developed based on the data of Hays and Solomon (1965) described in Section 6.2.1.2.1. Model predictions versus the data are shown in Figure 6-14 for iodide concentrations (ng/L) in the serum (A), thyroid (B), and gastric juice (C);

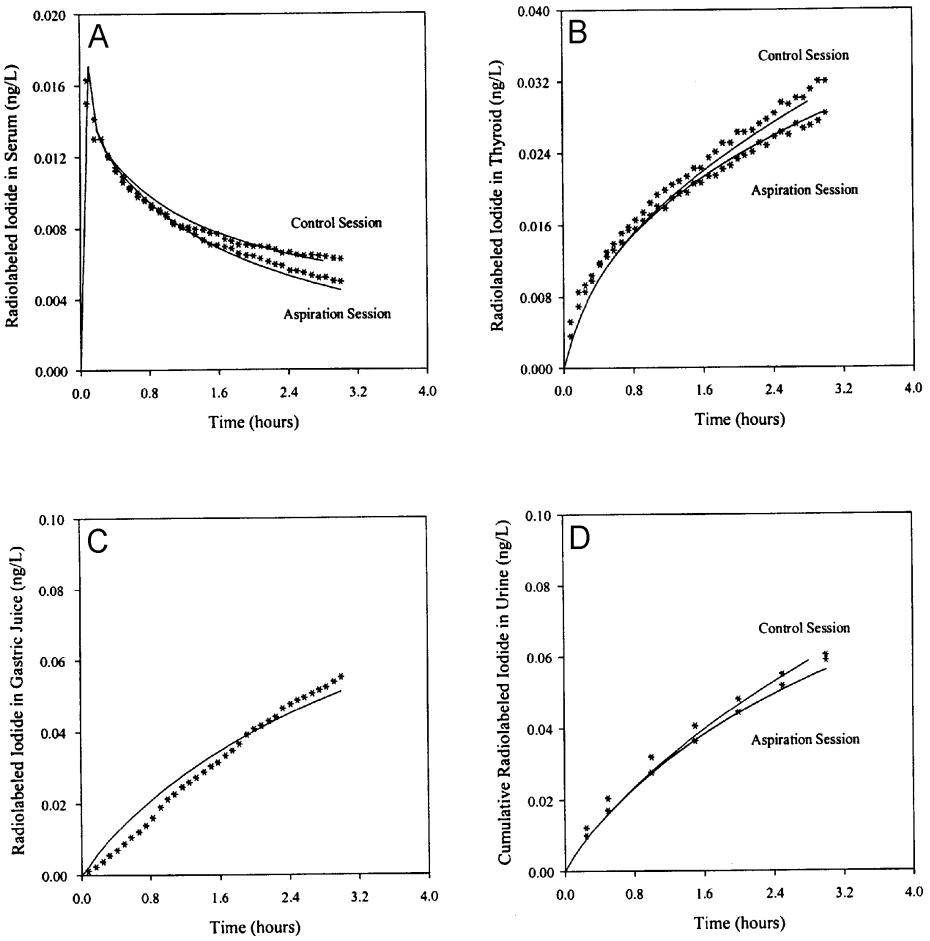

Figure 6-14. Human PBPK model (Merrill, 2001d) predictions (lines) versus mean $^{131}I^-$ concentration (mg/L) time course (asterisks) in serum (A), thyroid (B), gastric juice (C), and urine (D). Data of Hays and Solomon (1965) are for nine healthy males dosed with 10 μCi $^{131}I^-$ (approximately 3.44 ng/kg).

cumulative iodide excreted in the urine (ng) is shown in D. In this study, aspirated gastric juice accounted for an average of 23% of the iv dose within 3 hours after iv injection with radiolabeled iodide ($^{131}I^-$) (Merrill, 2001d). Simulation of the gastric juice removed during the aspiration session (Figure 6-14, C) required mathematically removing the amount of $^{131}I^-$ reabsorbed by the stomach wall. This was accomplished by adjusting the rate of reabsorption of $^{131}I^-$ from gastric juice to gastric tissue during the aspiration session as described in Merrill (2001d). The Vmaxc values for the gut and thyroid were then obtained by fitting values of $^{131}I^-$ uptake into gastric juice

from the aspiration session (lower lines in Figures 6-14; B and C). The urinary clearance value was fitted to simulate both cumulative urine content and serum iodide concentration from the aspiration session data (lower lines in Figures 6-14; A and D). Once parameters were established using the aspiration session, the rate of change in the gastric juice and partitioning back into the gastric juice from the systemic circulation was fitted to predict the corresponding increase in $^{131}I^-$ in plasma, thyroid, and urine seen in the control session versus the aspiration session (upper lines in Figures 6-14; A, B and D).

Figure 6-15 illustrates that, as for the adult male rat model, plasma binding of perchlorate was necessary to fit the serum concentration data of the 14-day study by Greer et al. (2000). The model indicates that humans have a lower binding capacity for perchlorate than rats. For example, the Vmaxc value for perchlorate is 9.310^3 ng/hr-kg in the male rat versus 5.0×10^2 ng/hr-kg in the human. Merrill (2001d) noted that while the effect of the plasma binding is subtle at 0.5 mg/kg-day dose, including the plasma binding improved the fit for uptake and clearance at the 0.1 and 0.02 mg/kg-day dosage levels.

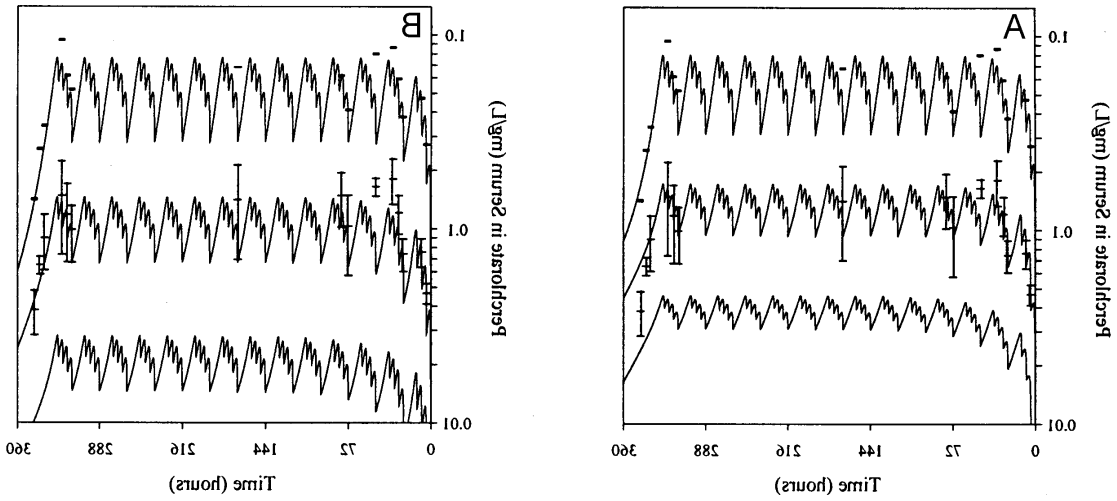

Figure 6-15. Simulations illustrating the necessity of including plasma binding in the human PBPK model structure (Merrill, 2001d). Model predictions (lines) versus data time course (mean ± SD) are shown with (A) and without (B) plasma binding for serum concentrations (mg/L) from 4 male subjects dosed with perchlorate at 0.5, 0.1, or 0.02 mg/kg-day for 14 days (data of Greer et al., 2000).

Cumulative urinary perchlorate excretion (mg) predictions versus the data (mean ± SD) at each dosage level are shown in Figure 6-16. Merrill (2001d) also simulated serum concentration (mg/L) and cumulative urinary perchlorate levels (mg) for each individual in the 0.5, 0.1, and 0.02 mg/kg-day dose groups of the Greer et al. (2000) study. An average value for urinary clearance of perchlorate (ClUc_*p*) of 0.126 L/hr-kg (± 0.050) was calculated from the individually fitted values. Figures 6-17 and 6-18 show a representative plot of model prediction versus individual subject data at the 0.5 and 0.1 mg/kg-day dosage. Additional plots provided in Merrill (2001d) provide an appreciation for the high degree of variability in the data.

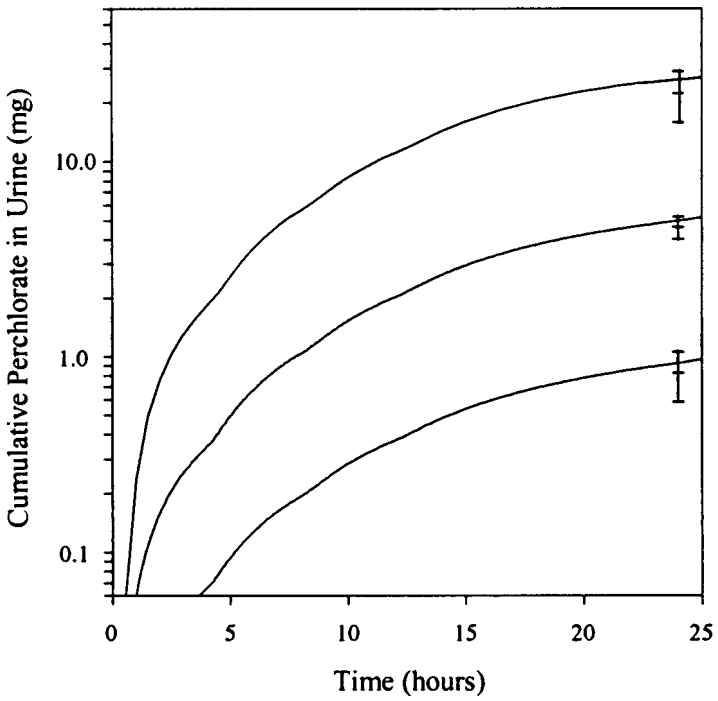

Figure 6-16. Human PBPK model predictions (lines) versus data (mean ± SD) of the observed cumulative urine excretion (mg) in male subjects dosed with perchlorate 0.5, 0.1, or 0.02 mg/kg-day for 14 days. Model of Merrill (2001d) and data of Greer et al. (2000).

Serum perchlorate levels were not available for the 0.02 mg/kg-day dose group, but cumulative urinary excretion amounts (mg) for this group were fitted using the average

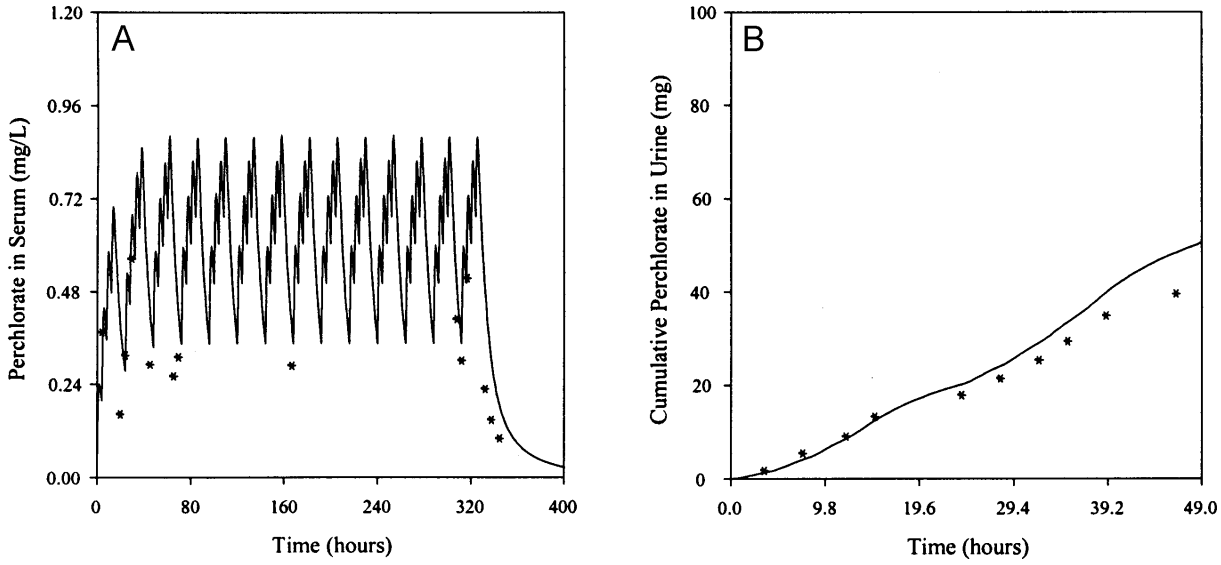

Figure 6-17. Human PBPK model predictions (lines) versus data of one subject's serum perchlorate concentration (mg/L) shown in (A) and corresponding 48-hour cumulative urine perchlorate (mg) shown in (B). Subject consumed 0.5 mg/kg-day perchlorate in drinking water, 4 times per day, for 14 days. Model predictions for the individual obtained by using study average value of all subjects for urinary clearance of perchlorate (ClUc_*p*). Model of Merrill (2001d) and data of Greer et al. (2000).

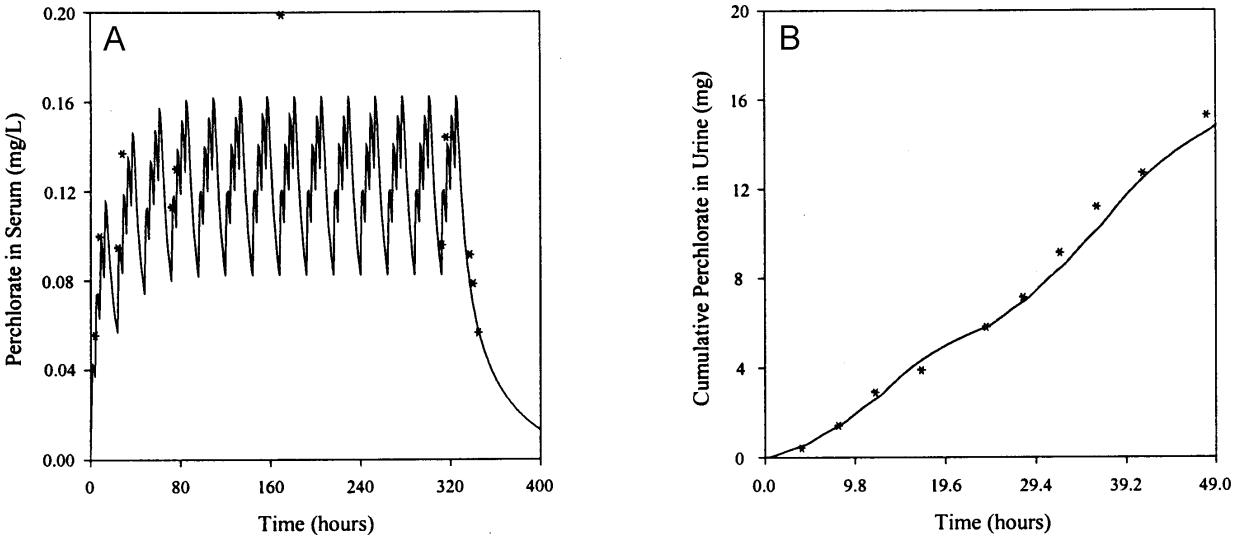

Figure 6-18. Human PBPK model predictions (lines) versus data of one subject's serum perchlorate concentration (mg/L) shown in (A) and corresponding 48-hour cumulative urine perchlorate (mg) shown in (B). Subject consumed 0.1 mg/kg-day perchlorate in drinking water, 4 times per day, for 14 days. Model predictions for the individual obtained by using study average value of all subjects for urinary clearance of perchlorate (ClUc_*p*). Model of Merrill (2001d) and data of Greer et al. (2000).

perchlorate urinary clearance (ClUC_*p*) value of 0.126 L/hr-kg calculated from the individual fits for the 0.1 and 0.5 mg/kg-day groups. Figure 6-19 shows the model predictions versus 48-hour cumulative urine perchlorate (mg) for two different subjects.

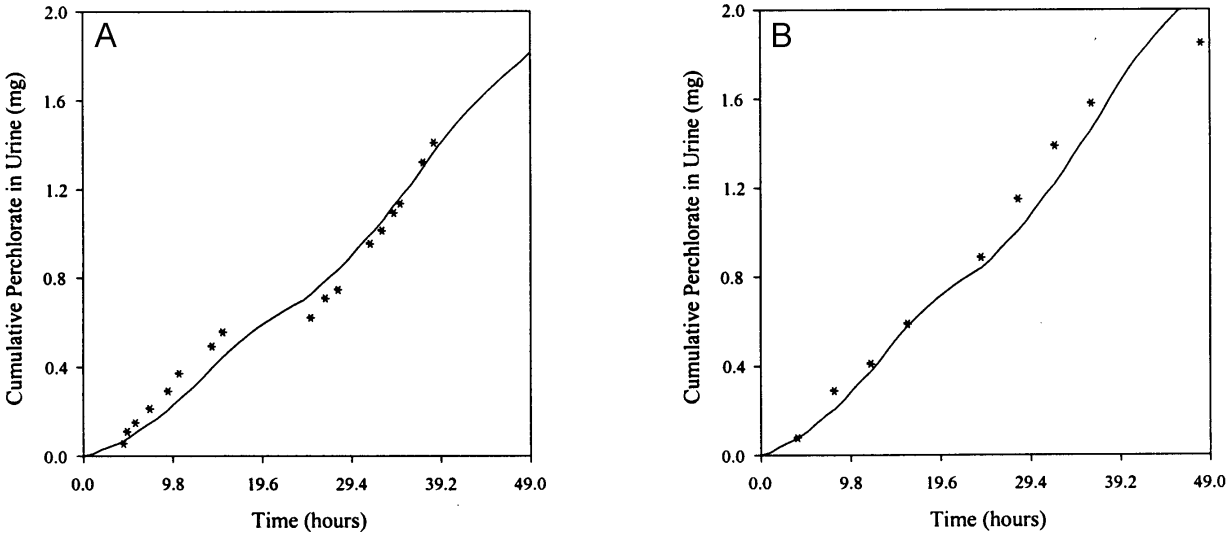

Figure 6-19. Human PBPK model predictions (lines) versus data of 48-hour cumulative urine perchlorate (mg) shown for two different subjects. Subject consumed 0.02 mg/kg-day perchlorate in drinking water, 4 times per day, for 14 days. Model predictions for the individual obtained by using study average value of all subjects for urinary clearance of perchlorate (ClUc_*p*). Model of Merrill (2001d) and data of Greer et al. (2000).

Due to its small size, variations in the thyroid parameters have little effect on serum concentrations of both iodide and perchlorate. As described for Figure 6-14, Merrill (2001d) estimated parameters for iodide disposition, including those of the thyroid, from fits to the data of Hays and Solomon (1965). Using these same iodide parameters, baseline thyroid RAIU measurements performed by Greer et al. (2000) were fit with the model by adjusting the Vmaxc for the thyroid follicular epithelium (Vmaxc_T*i*). Figures 6-20, 6-21, 6-22, and 6-23 illustrate the model predictions of thyroid RAIU versus data for subjects in the 0.5, 0.1, 0.02, and 0.007 mg/kg-day dosage groups, using either the individual's Vmaxc_T*i* (left) or an average value (right). The average Vmaxc_T*i* (1.5×10^5 ng/hr-kg) was obtained from fitting baseline

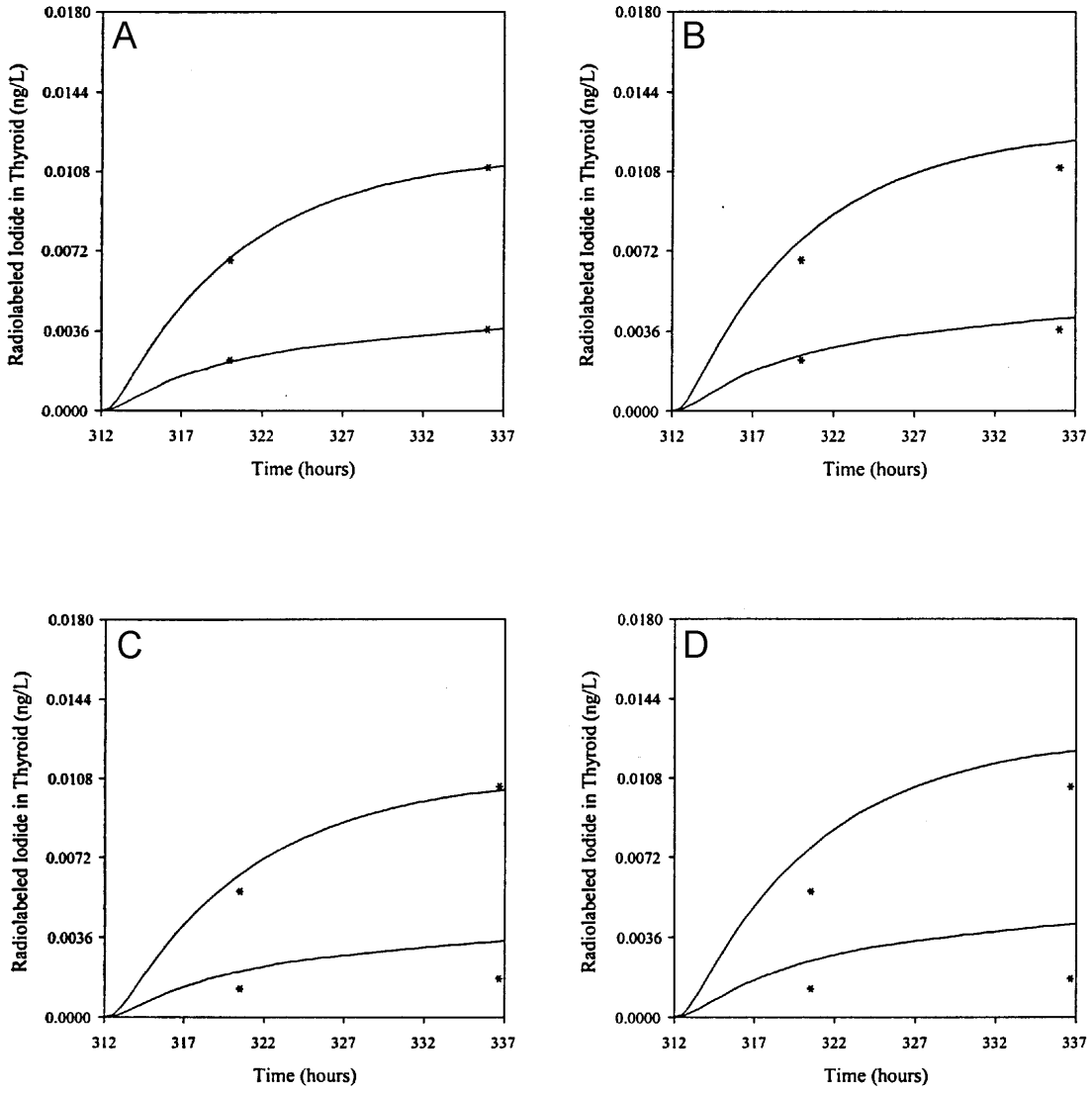

Figure 6-20. Human PBPK model predictions (lines) versus data (asterisks) for thyroid RAIU (ng/L) on day 14 of perchlorate exposure at 0.5 mg/kg-day for a healthy female (top panel) and male (bottom panel). Prediction on left for female (A) obtained by using individually fitted maximum capacity (ng/hr-kg) for active transport of iodide into the thyroid follicular epithelium (Vmaxc_T*i*) of 1.3×10^5 and on right (B) by using an average Vmaxc_T*i*. Prediction on left for male (C) obtained by using individually fitted Vmaxc_T*i* of 1.24×10^5 and on right (D) by using an average Vmaxc_T*i* of 1.5×10^5. Model of Merrill (2001d) and data of Greer et al. (2000).

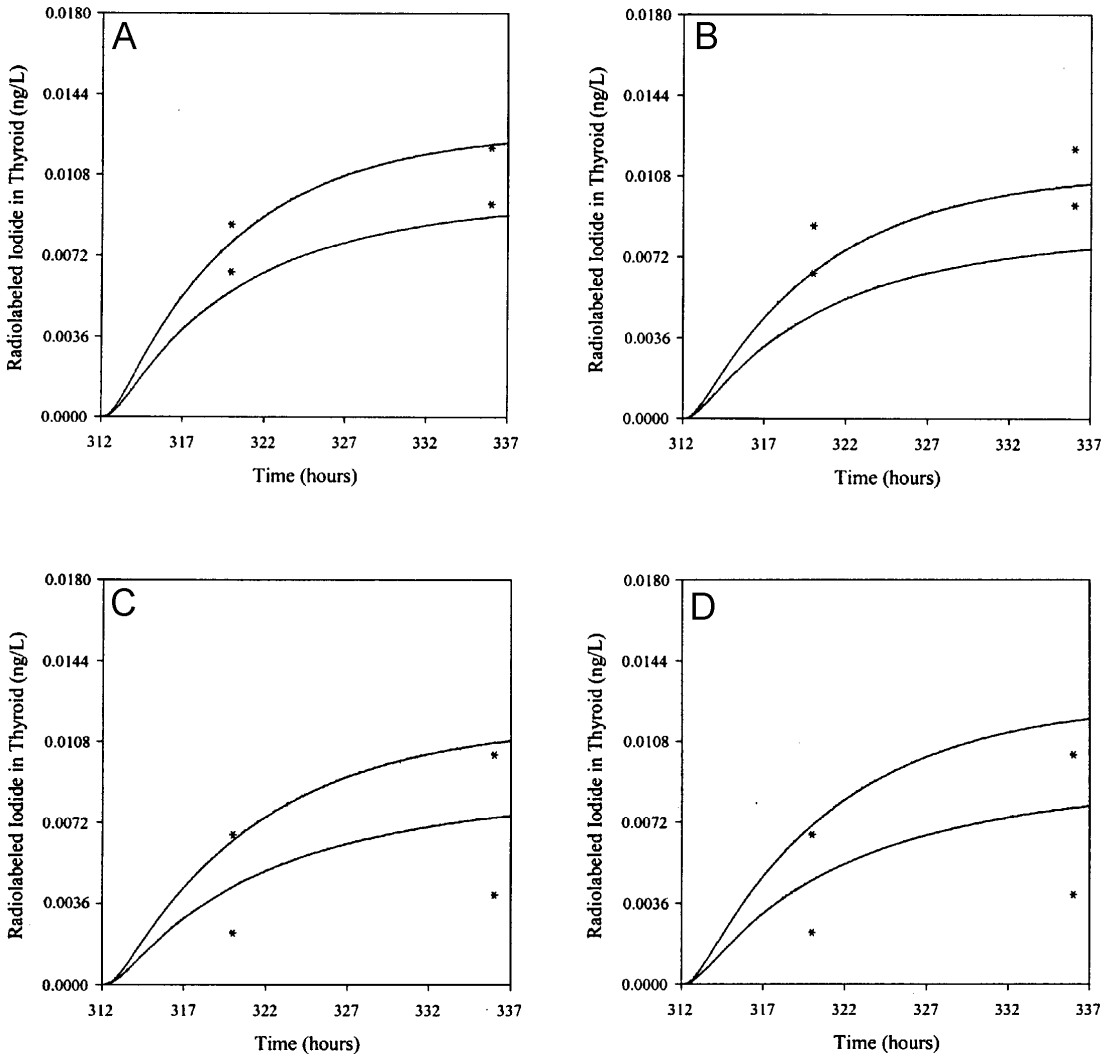

Figure 6-21. Human PBPK model predictions (lines) versus data (asterisks) for thyroid RAIU (ng/L) on day 14 of perchlorate exposure at 0.1 mg/kg-day for a healthy female (top panel) and male (bottom panel). Prediction on left for female (A) obtained by using individually fitted maximum capacity (ng/hr-kg) for active transport of iodide into the thyroid follicular epithelium (Vmaxc_T*i*) of 1.65×10^5 and on right (B) by using an average Vmaxc_T*i*. Prediction on left for male (C) obtained by using individually fitted Vmaxc_T*i* of 1.2×10^5 and on right (D) by using an average Vmaxc_T*i* of 1.5×10^5. Model of Merrill (2001d) and data of Greer et al. (2000).

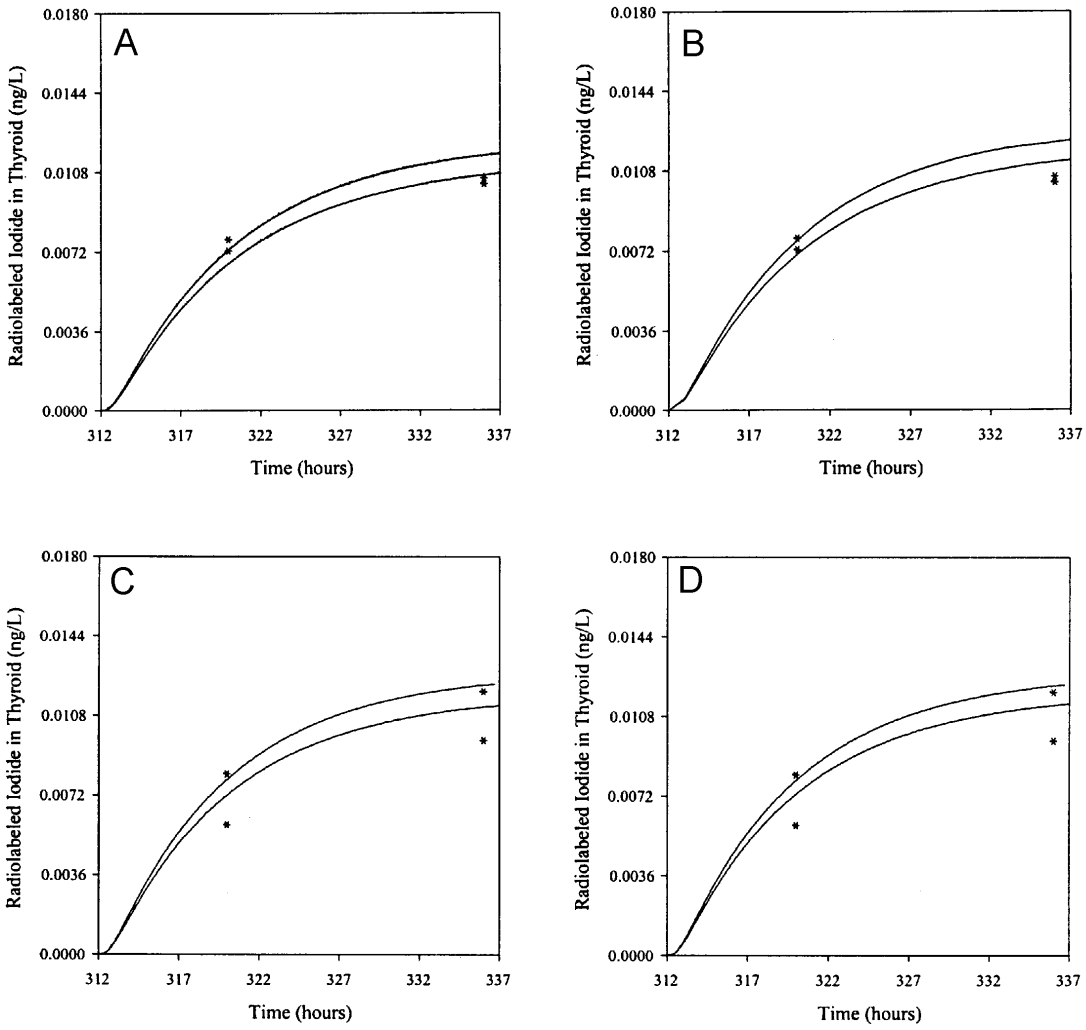

Figure 6-22. Human PBPK model predictions (lines) versus data (asterisks) for thyroid RAIU (ng/L) on day 14 of perchlorate exposure at 0.02 mg/kg-day for a healthy female (top panel) and male (bottom panel). Prediction on left for female (A) obtained by using individually fitted maximum capacity (ng/hr-kg) for active transport of iodide into the thyroid follicular epithelium (Vmaxc_T*i*) of 1.4×10^5 and on right (B) by using an average Vmaxc_T*i*. Prediction on left for male (C) obtained by using individually fitted Vmaxc_T*i* of 1.5×10^5 and on right (D) by using an average Vmaxc_T*i* of 1.5×10^5. Model of Merrill (2001d) and data of Greer et al. (2000).

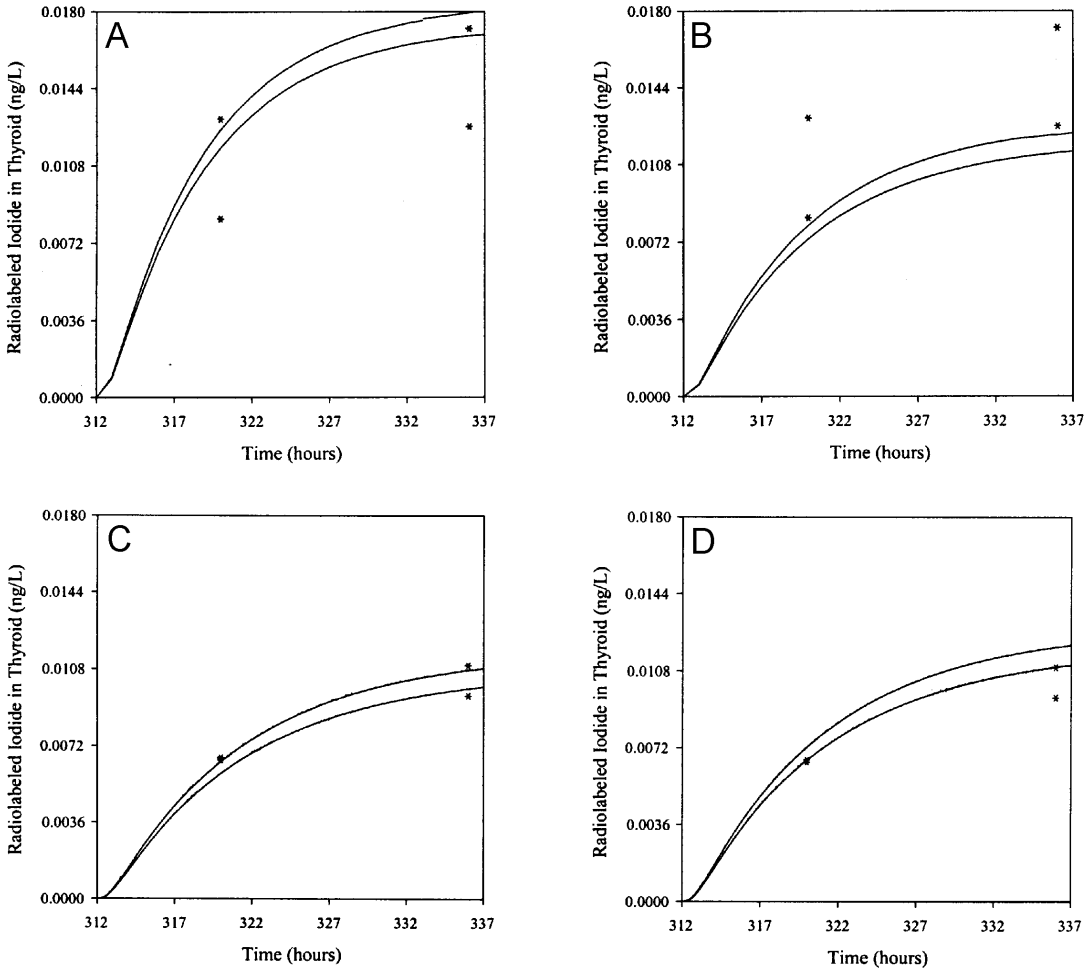

Figure 6-23. Human PBPK model predictions (lines) versus data (asterisks) for thyroid RAIU (ng/L) on day 14 of perchlorate exposure at 0.007 mg/kg-day for a healthy female (top panel) and male (bottom panel). Prediction on left for female (A) obtained by using individually fitted maximum capacity (ng/hr-kg) for active transport of iodide into the thyroid follicular epithelium (Vmaxc_T*i*) of 2.8×10^5 and on right (B) by using an average Vmaxc_T*i*. Prediction on left for male (C) obtained by using individually fitted Vmaxc_T*i* of 1.24×10^5 and on right (D) by using an average Vmaxc_T*i* of 1.35×10^5. Model of Merrill (2001d) and data of Greer et al. (2000).

radioiodide uptake measurements provided by Greer et al. (2000) across doses (see Merrill, 2001d; Table 3). Merrill (2001d) hypothesized that the large variability in Vmaxc_T*i*, ranging from 5.0×10^4 to 5.0×10^5 ng/hr-kg, may be attributed to variability in endogenous iodide levels, as dietary iodide was not controlled. Merrill (2001d) estimated these values from best visual fits of baseline 8- and 24-hour thyroid RAIU data. Inhibition data restricted to each time point (i.e., 8- versus 24-hour time points) and from higher dose groups would be useful to test the robustness of the model to predict inhibition of uptake of iodide in the thyroid.

The ability of the human model to predict data from other independent experiments not used to develop the model is illustrated in Figure 6-24. The model adequately predicts cumulative perchlorate in urine (mg) reported in three published studies using therapeutic perchlorate dose levels (Merrill, 2001d). Oral doses administered in these studies were approximately 9.07 mg/kg (Durand, 1938), 9.56 mg/kg (Kamm and Drescher, 1973), and 20 mg/kg (Eichler, 1929). It is worth noting that the previously determined urinary clearance value (ClUc_*p*) of 0.126 L/hr-kg was used with all validation data and that an adequate fit was observed.

The ability of the model to predict cumulative perchlorate in urine from three different studies at three different doses with the same set of parameters, established from the studies by Hays and Solomon (1965) and Greer et al. (2000), demonstrates the usefulness of the model and provides validation for the model structure and the physiological and chemical parameters used.

The model also predicts serum perchlorate concentrations at 12 mg/kg-day from an unpublished study performed by Dr. Georg Brabant at the Medizinische Hochschule, Hanover, Germany (Figure 6-25). Subjects received 12 mg/kg-day perchlorate in drinking water near meal times. Variability in the observed serum measurements is believed to reflect variability in the dosing regimen, as the experimental protocol was less fixed than that used in Greer et al. (2000). Again the usefulness of the model is demonstrated by its ability to successfully predict serum concentrations from a dose 24 times higher than the high dose used to establish perchlorate parameters (0.5 mg/kg-day).

The model is also able to successfully predict the thyroidal iodide uptake in a subject from the Stanbury and Wyngaarden (1952) study with patients with Grave's disease. The maximum velocity capacity in the follicular epithelium (Vmaxc_T*i*) had to be increased to 5.0E6 ng/hr-kg, a factor of ten times higher than in normal subjects, in order to achieve this fit (upper line in

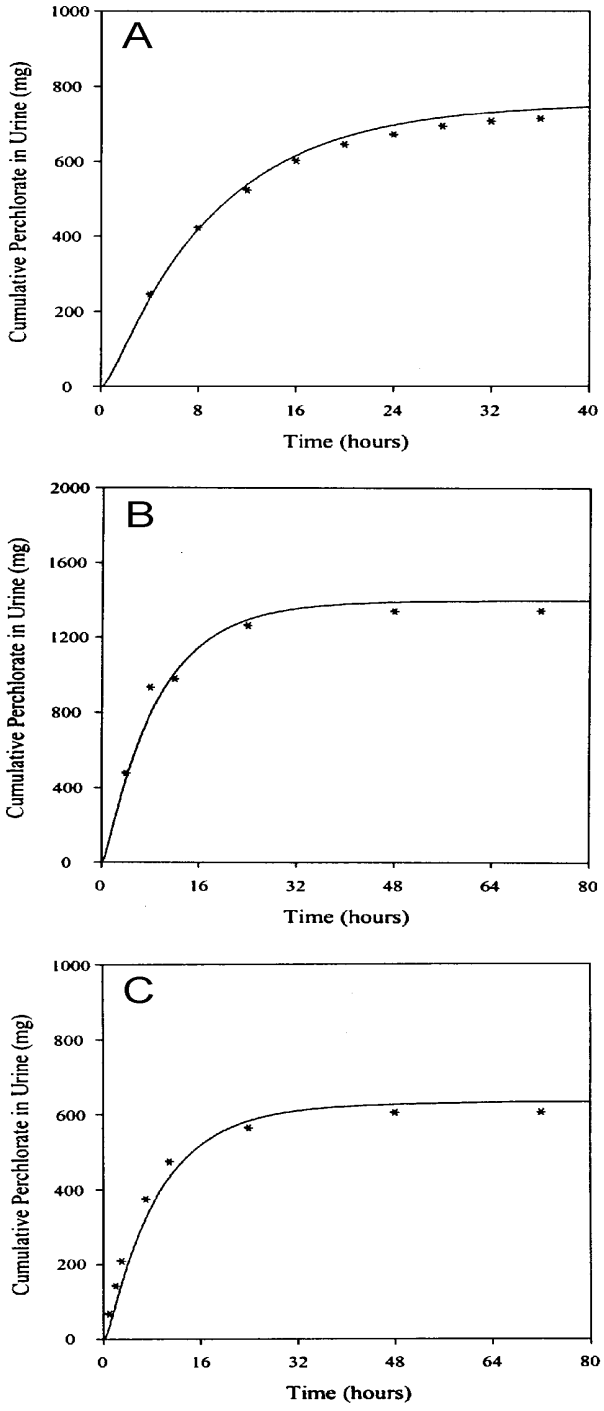

Figure 6-24. Validation for human PBPK model (Merrill, 2001d). Model predictions (lines) versus data (asterisks) for cumulative perchlorate excretion in urine (mg) in a healthy male after an oral dose of 9.56 mg (A), 20 mg (B) or 9.07 mg (C). Data are from three different studies. Data of Kamm and Drescher (1973) for (A), Eichler (1929) for (B) and Durand (1938) for (C).

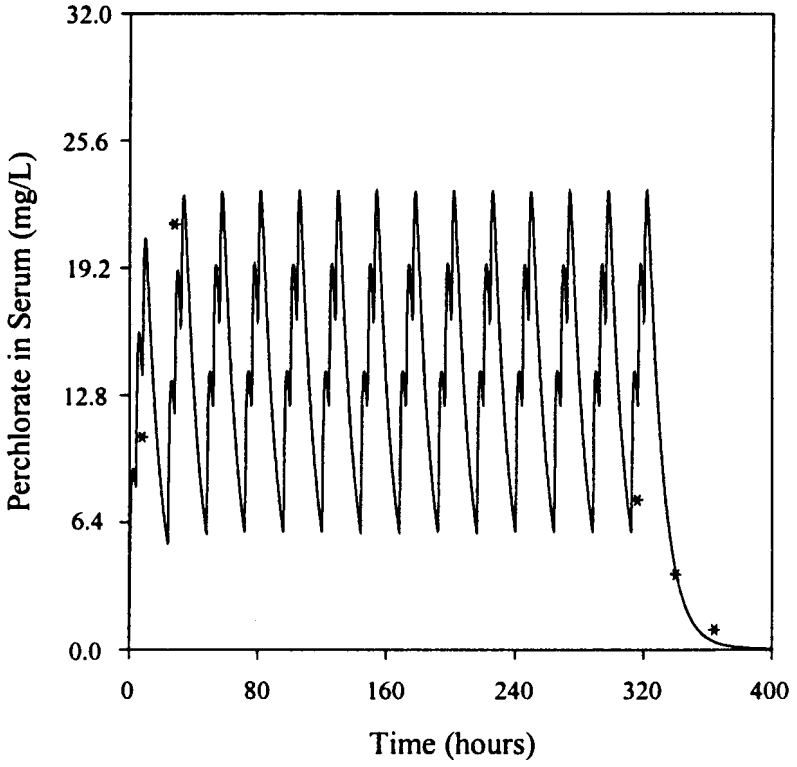

Figure 6-25. Validation for human PBPK model (Merrill, 2001d). Model predictions (lines) versus data (asterisks) for serum perchlorate concentrations (mg/L) in 5 subjects received 12 mg/kg-day in drinking water (data of Brabant and Letiolf, 2000 as cited in Merrill, 2001d). Subjects were instructed to 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.

Figure 6-26). This increase in Vmaxc_T*i* is supported in the literature, as Gluzman and Niepomniszcze (1983) measured elevated Vmaxc(s) in thyroid specimens from subjects with Grave's disease. However, the model underpredicts the degree of inhibition caused by perchlorate in this subject (Figure 6-26, lower line). It would appear that the increased inhibition could be attributed to a lower Km value. However, Gluzman and Niepomniszcze (1983) noted that the Km did not differ greatly between thyroid specimens from hyperthyroid subjects and

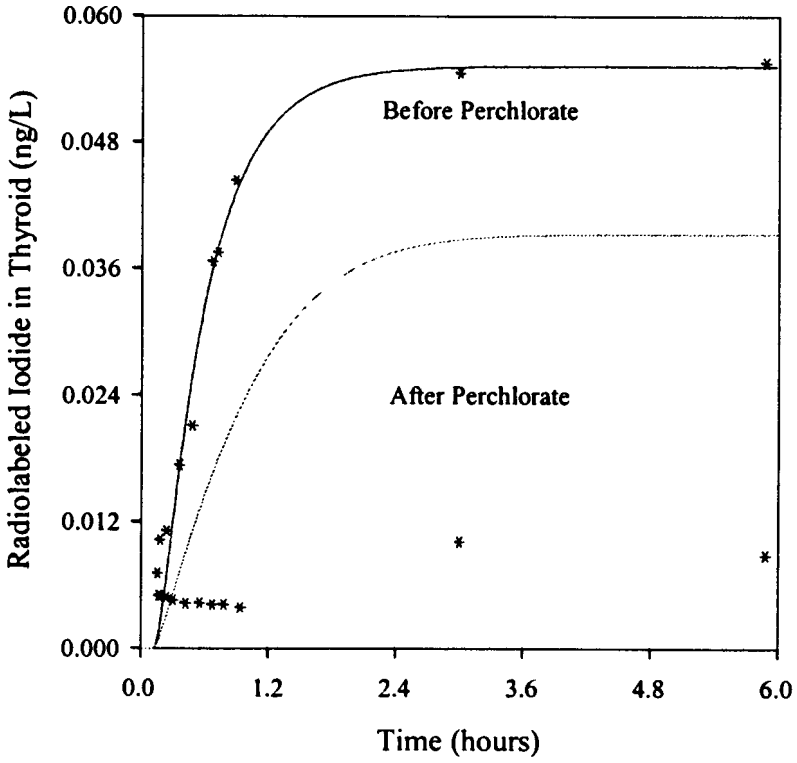

Figure 6-26. Validation for human PBPK model (Merrill, 2001d). Model predictions (lines) versus data (asterisks) for RAIU in the thyroid ($^{131}I^-$ ng/L) of a male with Graves' disease after an iv dose of 10 μCi $^{131}I^-$ before and after a 100 mg dose of potassium perchlorate. Data of Stanbury and Wyngaarden (1952).

normal subject. This suggests that the increased inhibition by perchlorate seen in Grave's disease may be attributed to a mechanism other than NIS affinity (Merrill, 2001d).

6.2.4 Summary

The proposed model structures for the adult male rat (Merrill, 2001c) and adult human (Merrill, 2001d) have been shown to adequately describe both perchlorate and iodide disposition by demonstrating good correspondence between predicted tissue compartment concentrations and measured values in the thyroid, serum, red blood cells, urine, liver, muscle, skin, and stomach in the rat and by adequately predicting serum concentrations and cumulative urine after drinking water exposure to perchlorate spanning four orders of magnitude (0.02 to 12.0 mg/kg-day) in the human. Serum perchlorate levels for human subjects were not available at

0.02 mg/kg-day; however, the model did predict the cumulative urine from that dose group (Figure 6-19).

The model structure of the thyroid requires three compartments (stroma, follicle, and colloid) to quantify rapid organification in the gland. Differences in model parameters between iodide and perchlorate indicate that iodide kinetics are very similar to perchlorate kinetics, but cannot be applied directly. The main differences involve the saturable kinetics of the thyroid, skin, and stomach, with perchlorate exhibiting higher Vmaxc's except in the skin. Because organification of iodide occurs in both the thyroid follicle and colloid, their respective Vmaxc's are over 1,000 and 10 times higher than those for perchlorate, which is discharged unchanged. Perchlorate affinity for the symporters into the follicle and colloid were approximately an order of magnitude greater (lower Km) than those of iodide.

The thyroid perchlorate concentrations from high drinking water exposures in the rat were fitted by increasing the effective follicle:stroma partition coefficient (PT_*p*) to account for TSH stimulation and upregulation of NIS. Since these values were not supported by additional data, thyroid concentrations may not be as reliable. Further, the toxic effects of perchlorate are most likely due to secondary effects on thyroid hormones due to its action at the NIS.

The model, however, could simulate serum concentrations from drinking water exposures using parameters established from the acute data. The thyroid, given it's small size, would not be expected to significantly alter serum concentrations, even during hyperstimulation. Although TSH has not been shown to increase the NIS in other tissues, NIS-containing tissues were not obtained from the AFRL/HEST studies to support this.

The models support plasma protein binding of perchlorate in both species; a saturable term is required to simulate serum concentrations at lower doses. It is possible that perchlorate competes with thyroxine for the same binding sites of plasma proteins, as the work of Yamada and Jones (1968) suggests. Urinary clearance values of 0.05 L/hr for iodide and 0.07 L/hr for perchlorate were used across data sets in the rats, and average urinary clearance values were found to be 0.1 L/hr-kg for iodide and 0.126 L/hr-kg in humans. Excretion constants were highest among the 0.1 mg/kg-day group. With the urinary excretion rates fitted to cumulative urine data, the model tends to slightly underestimate serum perchlorate levels at repeated low doses. Elevated serum concentrations may indicate plasma binding of perchlorate. Yamada and Jones (1967) studied effects of different anions on plasma binding to thyroxine and noted that

some of the thyroxine had been displaced after perchlorate was introduced. Thus, it is possible that perchlorate competes with thyroxine for the same binding sites of plasma proteins (Merrill, 2001c,d; Clewell, 2001a).

While there are limited data suggesting iodide and perchlorate uptake through the skin, the models and the kinetic studies required this assumption in the models for both rats and humans. Without the skin compartment, the models overestimated circulating plasma inorganic iodide and perchlorate in both species. Due to its large size, skin appears to be an important pool for slow turnover of these anions. Brown-Grant (1961) noted that the uptake of iodide was higher in the male rat and pup than in the female. The findings of Merrill (2001c) agree, with the rat model requiring a higher Vmaxc in the skin for the male rat than that reported for the pregnant rat (Clewell, 2001a) discussed in the next section. Cutaneous uptake of iodide and perchlorate in mice and rats has been reported (Brown-Grant and Pethes, 1959; Zeghal et al., 1995). The lack of reported iodide in human skin from clinical radioiodide scans may be due to the difficulty in differentiating it from background radioactivity.

Merrill (2001d) notes that GI clearance of iodide is rapid and plays an important role in radioiodide conservation. Further, Merrill (2001d) suggests that the appearance of time-course radioiodine in stomach contents of any species is complicated by the fact that it reflects more than sequestration of radioiodide by NIS. Its appearance also reflects radioiodide contributed through the gradual accumulation of iodide in saliva that is swallowed involuntarily throughout the study. Several studies that examined sequestration of these anions in digestive juices have all shown high variability in the concentrations measured over time (Honour et al., 1952; Hays and Solomon, 1965; Merrill, 2001d). There is a tendency for the gastric juice to plasma ratio to be low when the rate of secretion of juice is high (Honour et al., 1952). Fluctuations in the secretion rate are probably the most important factor in determining the pattern of the concentration ratios in individuals. Therefore, variability in stomach or GI tract parameters between models is expected. However, the early rise in the gastric juice:plasma ratio mentioned earlier is a constant feature across these data sets, whether or not an attempt was made to eliminate contamination of gastric juices by dietary contents or saliva. The human model successfully predicted this same trend.

Merrill (2001d) also noted dietary iodine and endogenous inorganic iodide levels to be clearly important in modeling iodide and perchlorate kinetics, because excessive iodide levels

cause the ion to inhibit its own uptake. Plasma inorganic iodide (PII) is rarely reported in the literature due to analytical difficulties, and it was not available in any of the studies presented in this paper. While measurements of tracer radioiodide can be fitted to predict transfer rates, its use is limited when attempting to predict the saturation of nonlinear compartments, such as the thyroid that are dependent upon the existing amount of iodide already present. Subsequent modeling efforts on predicting subsequent effects of iodide inhibition on thyroid hormone synthesis and regulation in humans will require the capability of the model to predict PII.

6.3 PREGNANT RAT AND FETAL MODEL STRUCTURE

This section describes the model developed by AFRL/HEST in response to concerns about interspecies extrapolation of effects due to perchlorate exposure during gestation (Clewell, 2001a). The model predicts the distribution of perchlorate within the pregnant and fetal rat through gestation and at birth and predicts the short-term effect of acute perchlorate exposure on iodide kinetics, including iodide uptake into the maternal thyroid. The general model structure relied on the adult male rat model (Merrill, 2001c) described in Section 6.2 and approaches to gestational growth of the dam and fetus were based on the work of O'Flaherty et al. (1992) and Fisher et al. (1989) with weak acids.

The model structure is shown in Figure 6-27. Table 6-3 provides the physiological parameters for the pregnant rat and fetus PBPK models. Table 6-4 provides the perchlorate-specific parameters, and Table 6-5 provides the iodide-specific parameters for each.

The compartments shared with the adult male rat were developed as described in Section 6.2. The pregnant rat model also includes a mammary gland and placenta compartment. The mammary gland consists of two subcompartments that represent the capillary bed and the tissue. The mammary gland has been shown to concentrate both perchlorate and iodide during lactation. However, the mammary NIS is regulated by hormones produced during lactation and has been found to increase at the onset of lactation (Tazebay et al., 2000). This concentrating mechanism does not appear to be as established during pregnancy. Studies reported by Yu (2000) showed mammary gland:plasma ratios of less than one for perchlorate. However, mammary gland perchlorate levels are slowly built up and remain high well into the clearance phase of the serum. This behavior suggested a very slow diffusion between the mammary gland

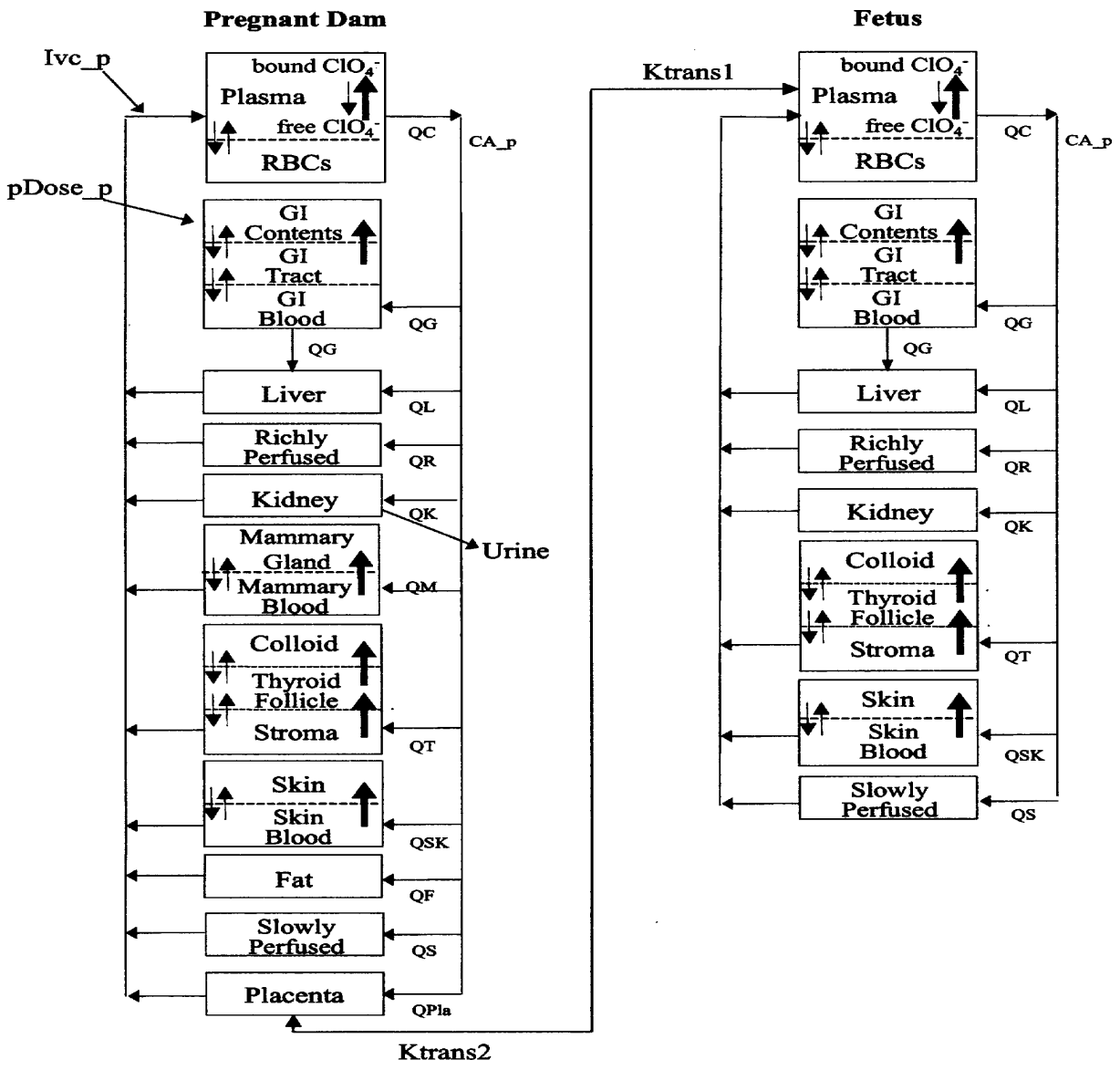

Figure 6-27. Schematic for the pregnant dam and fetal rat PBPK model of perchlorate and iodide distribution (Clewell, 2001a). Bold arrows indicate (except for plasma binding) active uptake at NIS sites into the thyroid, GI contents, and skin. Plasma binding was also described with Michaelis-Menten terms 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 (ClO_4^-) and iodide (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		
Tissue Volumes (%BW)	**Dam**	**Fetus**	**Source**
Body Weight *BW* and *V1fet* (kg)	0.280 - 0.361	0.0 - .0045	O'Flaherty et al., 1992
Slowly Perfused VSc (%BW)	74.6	74.6	Brown et al., 1997
Richly Perfused VRc (%BW)	11	11	Brown et al., 1997
Fat *VFc* (%BW)	10.0 - 11.0	0.0	Naismith et al., 1982
Kidney *VKc* (%BW)	1.7	1.7	Brown et al., 1997
Liver *VLc* (%BW)	3.4	3.4	Brown et al., 1997
GI Tract *VGc* (%BW)	3.60	3.60	Brown et al., 1997
GI Contents *VGJc* (%BW)	7.20	7.20	Yu et al., 2000
GI Blood *VGBc* (%VG)	2.9	2.9	Altman and Dittmer, 1971
Skin Tissue *VSkc* (%BW)	19.0	19.0	Brown et al., 1997
Skin Blood *VSkBc* (%VSk)	2.0	2.0	Brown et al., 1997
Thyroid Total *VTtotc* (%BW)	0.0105	0.0234	Malendowicz and Bednarek, 1986; Florsheim et al., 1966
Thyroid Follicle *VTc* (%BW)	45.9	61.4	Malendowicz and Bednarek, 1986; Conde et al., 1991
Thyroid Colloid *VDTc* (%BW)	45	18.3	Malendowicz and Bednarek, 1986; Conde et al., 1991
Thyroid Blood *VTBc* (%VT)	9.1	20.3	Malendowicz and Bednarek, 1986; Conde et al., 1991
Plasma *VPlasc* (%BW)	4.7	4.7	Brown et al., 1997; Altman and Dittmer, 1971
Red Blood Cells *VRBCc* (%BW)	2.74	2.74	Brown et al., 1997; Altman and Dittmer, 1971
Placenta *VPl ac* (%BW)	0.0 - 2.57	—	O'Flaherty et al., 1992
Mammary Tissue *VMc* (%BW)	1.0 - 5.5	—	Knight et al., 1984; O'Flaherty et al., 1992
Blood Flows (%QC)			
Cardiac Output *QCc* (L/hr-kg)	14	14.0	Buelke-Sam, 1982a & b; O'Flaherty et al., 1992
Slowly Perfused *QSc* (%QC)	24.0	24.0	Brown et al., 1997
Richly Perfused *QRc* (%QC)	76.0	76.0	Brown et al., 1997
Fat *QFc* (%QC)	7 - 8.1	0.0	Brown et al., 1997
Kidney *QKc* (%QC)	14.0	14.0	Brown et al., 1997
Liver *QLc* (%QC)	18.0	18.0	Brown et al., 1997
GI *QGc* (%QC)	13.60	13.60	Brown et al., 1997
Thyroid *QTc* (%QC)	1.6	1.6	Brown et al., 1997
Mammary *QMc* (%QC)	0.2 - 1.2	—	Hanwell and Linzell, 1973
Placenta *QPlc* (%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)[a]

Pregnancy Parameters	Perchlorate Values		
Partition Coefficients (unitless)	**Dam**	**Fetus**	**Source**
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	—	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_	0.50	1.80	Yu et al., 2000
GI Contents/GI Tissue PGJ_	1.30	2.30	Yu, 2000
Skin Tissue/Skin Blood PSk_	1.15	1.15	Yu, 2000
Thyroid Tissue/Thyroid Blood PT_	0.13 / 2.25	0.13 / 2.25	Chow and Woodbury, 1970[b]
Thyroid Lumen/Thyroid Tissue PDT_	7.00	7.00	Chow and Woodbury, 1970
Red Blood Cells/Plasma	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
Max Capacity, Vmaxc (ng/hr-kg)			
Thyroid Follicle Vmaxc_T	1.80E+03	1.80E+03	Fitted[c]
Thyroid Colloid Vmaxc_DT	1.00E+04	1.00E+04	Fitted[c]
Skin Vmaxc_S	6.00E+05	4.00E+05	Fitted
Gut Vmaxc_G	8.00E+05	8.00E+05	Fitted
Mammary Gland Vmaxc_M	3.90E+04	---	Molar equivalent to Vmaxc_M*i*
Plasma Binding Vmaxc_Bp	5.00E+03	1.50E+03	Fitted
Affinity Constants, Km (mg/L)			
Thyroid Follicle Km_T	1.00E+05	1.00E+05	Wolff, 1998
Thyroid Colloid Km_DT	1.00E+08	1.00E+08	Golstein et al., 1992; Wolff, 1998
Skin Km_S	1.00E+05	1.00E+05	Wolff, 1998
Gut Km_G	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	Fitted
Permeability Area Cross Products, (L/hr-kg)			
GI Blood to GI Tissue PAGc_	1.00	1.00	Fitted
GI Tissue to GI Contents PAGJc_	1.00	1.00	Fitted
Thyroid Blood to Thyroid Tissue PATc_	4.0E-5 / 6.0E-4	4.0E-5 / 6.0E-4	Fitted[b]
Thyroid Tissue to Thyroid Lumen PADTc_	0.01	0.01	Fitted
Skin Blood to Skin Tissue PASkc_	1.00	1.00	Fitted
Plasma to Red Blood Cells PRBCc_	1.00	1.00	Fitted
Clearance Values, (L/hr-kg)			
Urinary Excretion CLUc_	0.07	—	Yu et al., 2000
Transfer from Placenta to Fetus Cltrans1c_	0.10	0.10	Yu, 2000
Transfer from Fetus to Placenta Cltrans2c_	0.19	0.19	Yu, 2000
Dissociation from Plasma Binding Sites Clunbc_p	0.034	0.010	Yu, 2000

[a]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_T*i*, etc.)
[b]Parameters with two values indicate acute and drinking water parameters, respectively.
[c]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)[a]

Pregnancy Parameters	Iodide Values		
Partition Coefficients (unitless)	**Dam**	**Fetus**	**Iodide Source**
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_4^-)
Max Capacity, Vmaxc (ng/hr-kg)			
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
Affinity Constants, Km (mg/L)			
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 Gland Km_M	4.00E+06	—	Gluzman and Niepomniszcze, 1983
Permeability Area Cross Products, (L/hr-kg)			
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
Clearance Values, (L/hr-kg)			
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

[a]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_T*i*, etc.)

and blood, so that Clewell (2001a) described the two-subpartment mammary gland with both diffusion of iodide and active uptake by the NIS.

Although it has been suggested that the placenta may contain the capability for active uptake in the rat, AFRL/HEST data did not indicate placenta:plasma levels greater than one for perchlorate or iodide (Yu, 2000), and unpublished iodide time course data indicate that the behavior of iodide in the placenta closely mirrors that of the plasma (Clewell, 2001a). Thus, the placenta was simulated with a single, flow-limited compartment.

Partitioning into the mammary, placenta, and other diffusion-limited compartments was based on effective partitioning. This effective partitioning is probably very similar to that in the thyroid where an electrochemical gradient is responsible for allowing the ClO_4^- anion to move between the serum and the tissue (Chow and Woodbury, 1970). Urinary clearance and placental-fetal transfer of the anions were represented by first order clearance rates.

The structure of the fetal perchlorate model is similar to that of the pregnant rat, with the exception of the mammary gland and placenta compartments. In order to simplify the model, all of the fetuses from a single litter were combined in the structure of the model, essentially viewing the individual fetuses as one entity, or one large fetus. The dose to the fetus is based on the transfer of perchlorate from the maternal placenta to the serum of the fetus, rather than through direct exposure to the drinking water. Though a kidney is included in the fetal model, urinary excretion is not used to identify the loss of perchlorate for the fetus. Since the ability to produce urine is not well developed until after parturition, the loss from the fetus is described as first order clearance from the fetal arterial blood to the placenta (Clewell, 2001a).

The pregnancy model attempts to describe perchlorate distribution in a highly dynamic system. In addition to total body weight changes in the dam and fetus, maternal mammary tissue and blood flow, cardiac output, fractional body fat, placenta and fetus body weight, and fractional body fat are also changing with respect to time. Growth equations, based on O'Flaherty et al. (1992) were used to account for these changes (Clewell, 2001a). All tissue volume and blood flow values were adjusted with respect to the changing parameters.

6.3.1 Data and Methods

This section summarizes the data that Clewell (2001a) used for development and validation of the pregnant and fetal rat model structures. Details on experimental methods, including:

protocol design, exposure regimen, chemical source and purity, animals (housing, feeding, surgical procedures, etc.), and the analytical methods can be found in the consultative letter and associated reports from AFRL/HEST or cited papers therein.

6.3.1.1 AFRL/HEST Experiments in Laboratory Rats

These studies are described in the consultative letters and reports of Clewell (2001a), Yu (2000, 2001, 2002) and Yu et al. (2000).

6.3.1.1.1 Drinking Water Study

Perchlorate drinking water experiments used in model development were performed at AFRL/HEST and described in detail in the report Yu (2000). Pregnant dams of the Sprague-Dawley strain were exposed to drinking water treated with perchlorate from gestational day (GD) 2 through 20, at perchlorate doses of 0.0, 0.01, 0.1, 1.0 and 10.0 mg/kg-day. GD0 was determined by the presence of a vaginal plug. Both dams and fetuses were sacrificed on GD20 and maternal and fetal serum analyzed for free and total thyroxine (fT4 and tT4), triiodothyronine (T3), and TSH. Maternal serum, thyroid, skin, GI contents, placenta, and amniotic fluid were analyzed for perchlorate at all of the above doses. Fetal serum, skin and GI tract were also analyzed for perchlorate at all of the doses. Two hours before sacrifice, the dams were given iv doses of 33 mg/kg radiolabeled iodide ($^{125}I^-$) with carrier. Tissue concentrations of iodide were measured in order to determine the inhibition in the various tissues after long-term exposure to perchlorate.

6.3.1.1.2 Preliminary Iodide Kinetics Study

A preliminary study of radiolabeled ($^{125}I^-$) kinetics was performed by AFRL/HEST in which timed-pregnant dams of the Sprague-Dawley strain were exposed via tail-vein injection to a tracer dose (average dose = 2.19 ng/kg body weight) of the radiolabeled anion on GD20. Dams (n=6) were sacrificed at 0.5, 2, 4, and 8 hours post-dosing. Maternal serum, thyroids, skin, GI contents, placenta and mammary gland tissue, as well as fetal serum, skin, and GI tract were collected and analyzed for iodide content at each time point. Serum was pooled for all fetuses 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 $^{125}I^-$ 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 $^{125}I^-$ 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 ± 5 g) were given an injection of 10 μCi carrier free $^{125}I^-$ 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 ($^{131}I^-$) 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

favorably with those seen on GD20 in the Sprague-Dawley fetus. As a result, Clewell (2001a) used the GD20 time point as a means of validating GD20 parameters for iodide across different data sets and doses. Placental and whole body fetal $^{131}I^-$ were measured in a well-type scintillation detector.

6.3.1.2.3 Feldman et al., 1961

Feldman and coauthors measured the uptake of iodide into the fetal thyroid and rest of body carcass on GDs 16, 17, 18, and 19 in pregnant female Holtzman rats. A single subcutaneous injection was given to the dam, containing 50 μCi of $^{131}I^-$ on each of the days mentioned above. Fetal thyroid and carcasses were measured at 24 hours post dosing.

6.3.2 Pregnant Rat and Fetus Model Development

This section summarizes only the key features that were different than the adult male rat model previously described in Section 6.2.

6.3.2.1 Physiological Parameters and Tissue Partition Coefficients

Maternal parameters were scaled allometrically based on body weight as previously described for the male rat. Fetal values were scaled in the same manner as the maternal parameters. However, since the model actually represents several fetuses, it was necessary to first scale the values for the individual fetus and then adjust for the total number of fetuses in the litter (Clewell, 2001a).

Clewell (2001a) based the physiological description of the maternal and fetal rat during gestation on O'Flaherty et al. (1992). However, growth descriptions, body weights, and organ descriptions were optimized for use within this particular model structure. The model is able to account for differences in gestation time, pup birth weight, and litter size between experiments and strains of rats. Growth equations and parameters that change over time were described with mathematical descriptions of available literature and experimental data. Details and equations 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_{init}). 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

by two separate processes: the exponential decline in yolk sac volume and the increase in chorioallantoic placenta (Clewell, 2001a).

O'Flaherty et al. (1992) also described the growth of the uterus and liver during gestation. However, Clewell (2001a) did not include a specific description of growth in these organs because the liver is not believed to have a major role in perchlorate kinetics. Further, because the iodide model does not describe deiodination, the description of liver growth was deemed unnecessary. The use of a uterine compartment was also not included in the Clewell (2001a) model due to the lack of available perchlorate and iodide data. The uterus was considered to be part of the lumped richly perfused tissue. EPA agrees that adding a description of liver growth would only bring additional complexity to the model structure without providing a real benefit to the description of perchlorate and total iodide kinetics and that the uterine compartment would be purely hypothetical and could not be validated without pertinent data.

6.3.2.1.2 Maternal Blood Flow

Clewell (2001a) described temporal changes in maternal cardiac output during gestation as the sum of the initial cardiac output, given in Brown et al. (1997) for a non-pregnant rat, and the change in blood flow to the placenta, mammary, and fat tissues. The approach of O'Flaherty et al. (1992) to changing blood flows was utilized in placental, mammary, and fat blood flows. The fraction of cardiac output to the mammary gland and fat tissues are described as proportional to the change in volume of the tissue. The change in blood flow to the yolk sac placenta is approximately proportional to the change in volume of the yolk sac. However, the blood flow to the chorioallantoic placenta increases at a faster rate than the change in volume, so three different equations were used to describe the blood flow for each different stage of placental growth (GD1 to GD6, GD7 to GD10, GD11 to GD12, and GD13 to GD21).

6.3.2.1.3 Fetal Tissues

A three stage description of fetal growth was also described in O'Flaherty et al. (1992) in order to mathematically reproduce data obtained from Beaton et al. (1954), Sikov and Thomas (1970), Goedbloed (1972), and Buelke-Sam et al. (1982a). Because data are not available for fetal volume between gestational days 1 through 11, an exponential growth curve was used as a reasonable approximation of fetal growth and was fit to the first available data for fetal volume

(Clewell, 2001a). The second stage of growth describes a slower increase in fetal volume, beginning on GD11, based on the same data. Clewell (2001a) described the third stage of fetal growth as a linear increase between days 18 and the day of parturition. The equation is dependent on the weight of the pup at the time of birth so that the model can account for the differences in birth weight encountered when simulating different data sets.

Individual fetal organ weights were assumed to increase linearly with respect to change in fetal body weight and were therefore scaled allometrically to account for changes in tissue volumes. Values for tissue volumes were taken from the literature and from experimental data for the fetus when available. However, most volumes were taken from adult rat data and scaled allometrically for the fetus due to the lack of tissue data in fetuses.

Florsheim et al. (1966) measured thyroid and body weight of the rat fetus and pup from GD18 through PND22 and reported a linear relationship between the thyroid weight and body weight throughout the time period. The value given for the thyroid of the fetus in %fetal body weight for GD19 was used in the Clewell (2001a) model. On the other hand, the physiology of the developing thyroid was found by Conde et al. (1991) to change significantly between birth and PND120. Conde reported a decrease in follicle volume from 61.4% to 37.2% of the total volume of the thyroid from birth to 120 days. An increase in colloid volume from 18.3% of the total thyroid volume at birth to 32.5% at 120 days was also reported. In the absence of histometric data in the fetal thyroid, the follicle, colloid, and stroma volumes for the fetus were described using the thyroid fractions measured at birth. The value for thyroid stroma was calculated within the model by subtracting the colloid and follicle volumes from the total thyroid volume.

The fetal body fat content was assumed to be zero in the Clewell (2001a) model. This assumption is reasonable in light of the data given in Naismith et al. (1982). Naismith et al. (1982) measured values for the body fat of PND2 and 16 rat pups, corresponding to 0.16% and 3.7% of the body weight. Given that body fat quickly increases in the neonatal period, it is not unreasonable to assume that body fat in the fetus is negligible. The volume is certainly not large enough to interfere with iodide or perchlorate kinetics. All other parameters were scaled allometrically by fetal weight from the adult male rat. The male rat physiological parameters were used rather than female parameters for several reasons. First, the male rat pups have been shown to be more sensitive to perturbation of hormone homeostasis by perchlorate, and therefore

are considered the sensitive endpoint (Yu, 2000). Additionally, Clewell (2001a) asserts that sufficient evidence was not found to indicate that physiological parameters between male and female rats were present in the fetus.

6.3.2.1.4 Fetal Blood Flow

Fetal blood flow was assumed to operate independently from the mother. The transfer of the chemical was accomplished via diffusion between the placenta and fetal blood. Therefore, the fetal cardiac output and blood flow to organs (as % cardiac output) were scaled allometrically from the male rat values relative to the fetal volume.

6.2.2.2 Chemical-Specific Parameters

The various active transport processes, tissue permeabilities, and clearance rates (excretion) are described in PBPK models for each species on a chemical-specific basis. This section outlines how the values for perchlorate and iodide used in the pregnant and fetal rat model were derived. The values can be found in Tables 6-4 and 6-5; details on the derivation can be found in Clewell (2001a).

6.3.2.2.1 Affinity Constants and Maximum Velocities for Active Uptake Processes

These were developed as described previously for the adult male rat model (Merrill, 2001c) in Section 6.2. The chemical specific parameters were kept the same in male, female, neonatal and fetal rats, and humans whenever possible. However, it was necessary to change a few of the parameters, including the maximum velocities (Vmaxc's) in the Clewell (2001a) model for pregnant rat and fetus. The Km values were similar between tissues and between female and male rat and human models. However, the maximum velocity or capacity differs between tissues (Wolff and Maurey, 1961). Since Vmaxc values for perchlorate were not given in literature, the values were estimated with the model. In order to determine Vmaxc using the model, the simulation for the tissue of interest was compared to various data sets with several different perchlorate dose levels. The value for Vmaxc within a given compartment was then determined 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 (Clewell, 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 μCi or 3 sequential doses of 10 μCi 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). Clewell (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 $KClO_4$), and they reported an effective partition of 0.66 for the rat mammary gland. This value is in general agreement with that chosen by Clewell (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 (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. 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 (ClUc_*i*) was determined primarily by fitting the model simulation to the iodide

serum data, as blood levels were more dependent on excretion than on the amount of iodide in other tissues. The clearance of both iodide and perchlorate between the fetal serum and maternal placenta were based on the fit of the model simulation to the fetal and maternal blood levels and to the placenta concentration.

6.3.2.3 Pregnant Rat and Fetus Model Parameterization and Validation

This section summarizes how Clewell (2001a) used the various data sets to parameterize the model and the validation exercises performed.

6.3.2.3.1 Perchlorate Model Parameterization

Clewell (2001a) performed model parameterization for perchlorate using the data obtained from the AFRL/HEST drinking water studies on GD20. Optimized kinetic parameters (Vmax and permeability area values) were determined by fitting the model simulation to the experimental data. As for the adult male rat and human, it was necessary to account for the serum binding of perchlorate in order to adequately describe the blood perchlorate concentrations at the lower doses (0.01 and 0.1 mg/kg-day). Figure 6-28 illustrates the importance of binding in the model simulations of both maternal (A) and fetal (B) serum at 0.01 (left) versus the 10.0 (right) mg/kg-day dose. Binding does not have a noticeable effect on the plasma concentrations in the highest dose. However, as the perchlorate dose decreases, the effect of binding is more pronounced. Therefore, at lower levels, a larger percent of the injected dose will be bound. As the amount consumed is increased, the binding process is saturated and eventually the amount of perchlorate that is bound is negligible in contrast to the large amount of free perchlorate in the plasma. This is to be expected because the number of binding sites is limited.

Figure 6-29 shows the fit of the model to the maternal serum (left) and thyroid (right) perchlorate concentration (mg/L) in the dam on GD20. Since saturation of the symporter occurs between the 1.0 and 10.0 mg/kg-day dose groups, the influence of Vmaxc in the tissues was primarily in the 0.01 to 1.0 mg/kg-day doses. Thus, the fit of the model simulation to the data in the lower three doses was used to determine the values for Vmaxc in the tissues. On the other hand, the Vmaxc did not have a significant effect on the highest dose. The model fits to the 10 mg/kg-day dose group were primarily affected by the partition coefficients and permeability area values. Clewell (2001a) obtained the permeability area values in the tissues by fitting the

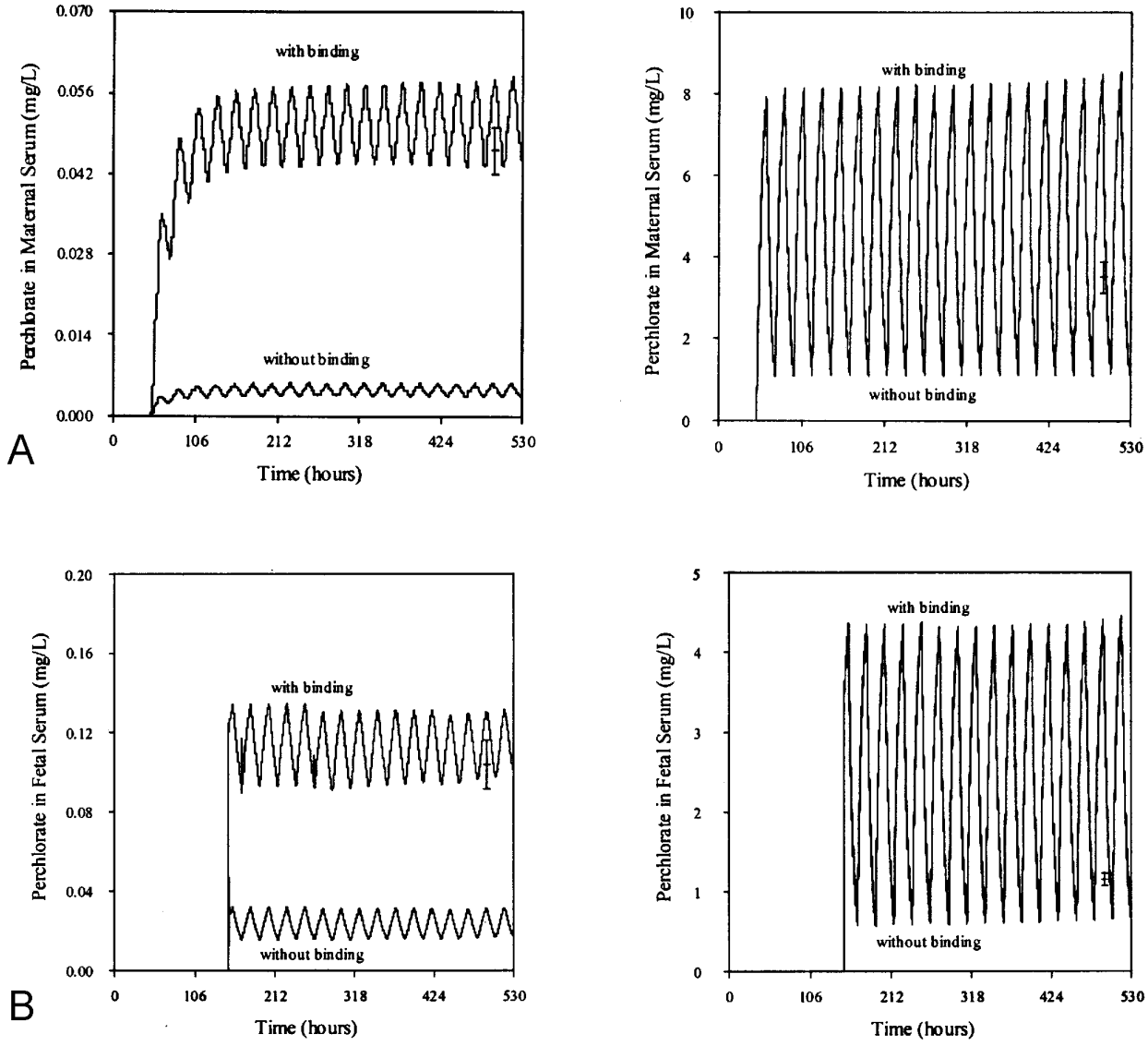

Figure 6-28. Simulations illustrating the necessity of including plasma binding in the pregnant dam and fetal rat PBPK model structure (Clewell, 2001a). Model predictions (lines) versus data time course (mean ± SD) are shown with and without plasma binding for maternal (A) and fetal (B) serum concentrations (mg/L) at two different doses, 0.01 mg/kg-day (left) and 10.0 mg/kg-day (right).

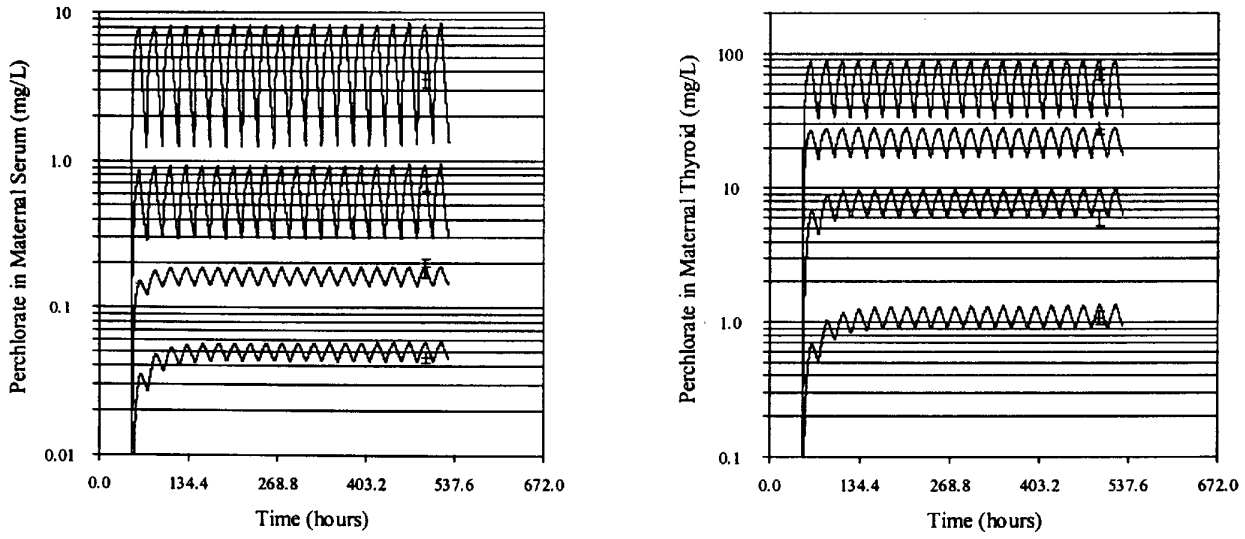

Figure 6-29. Pregnant dam and fetal rat PBPK model predictions (lines) versus data time course (mean ± SD) of perchlorate concentrations (mg/L) in maternal serum (left) and thyroid (right) on GD20 (Clewell, 2001a). Pregnant rats were dosed in drinking water with 10.0, 1.0, 0.1, or 0.01 mg/kg-day perchlorate. Data of Yu (2000).

highest dose to the 10 mg/kg-day data in the tissues. Maternal placenta, mammary gland, and GI tract concentrations were available at the 10 mg/kg dose only. These tissues were used to verify the applicability of the assigned partition coefficients to the model. Since mammary glands were not available for the 0.01 through 1.0 mg/kg-day dose groups, it was not possible to fit the Vmaxc value to data at which the symporter has a significant effect. Therefore, the Vmaxc in the mammary gland was assigned the molar equivalent of the iodide Vmaxc. This is probably a reasonable value in the non-lactating gland. Clewell (2001a) provides additional figures that demonstrate the fit of the model to the GI tract, mammary glands, and placenta in the pregnant dam.

Fewer data were available for perchlorate distribution in the fetus than in the dam due to the experimental difficulty involved in sampling the small fetal tissues. Figure 6-30 depicts the model simulation of the fetal serum concentration (mg/L) compared to the data obtained in the drinking water study. Fetal serum and skin were pooled by litter. Fits to additional compartments are provided in Clewell (2001a).

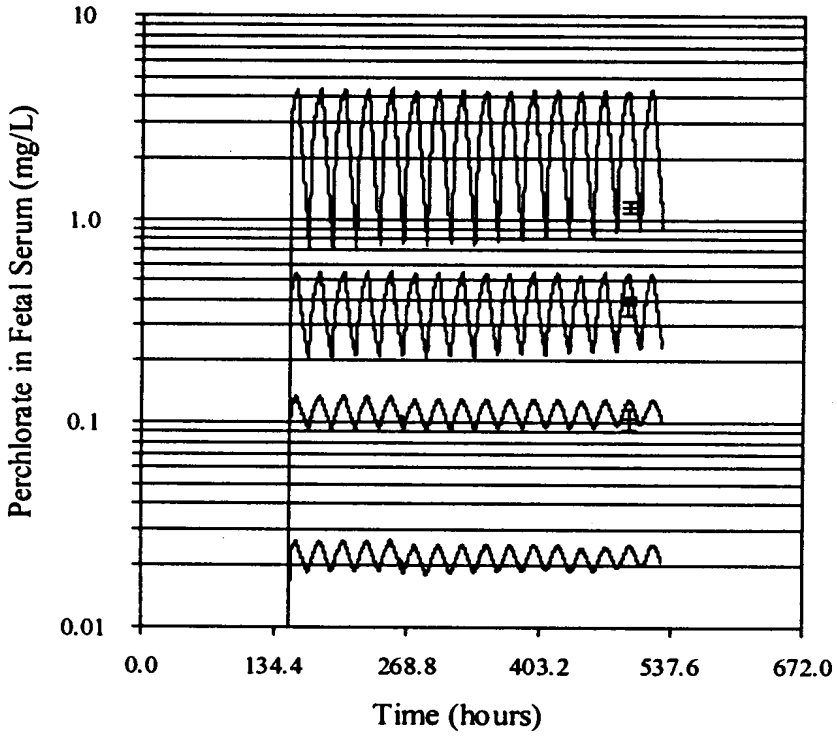

Figure 6-30. Pregnant dam and fetal rat PBPK model predictions (lines) versus data time course (mean ± SD) of perchlorate concentrations (mg/L) in pooled fetal serum on GD20 (Clewell, 2001a). Pregnant rats were dosed in drinking water with 10.0, 1.0, 0.1, or 0.01 mg/kg-day perchlorate. Data of Yu (2000).

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 Vmaxc 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 $^{125}I^-$ 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

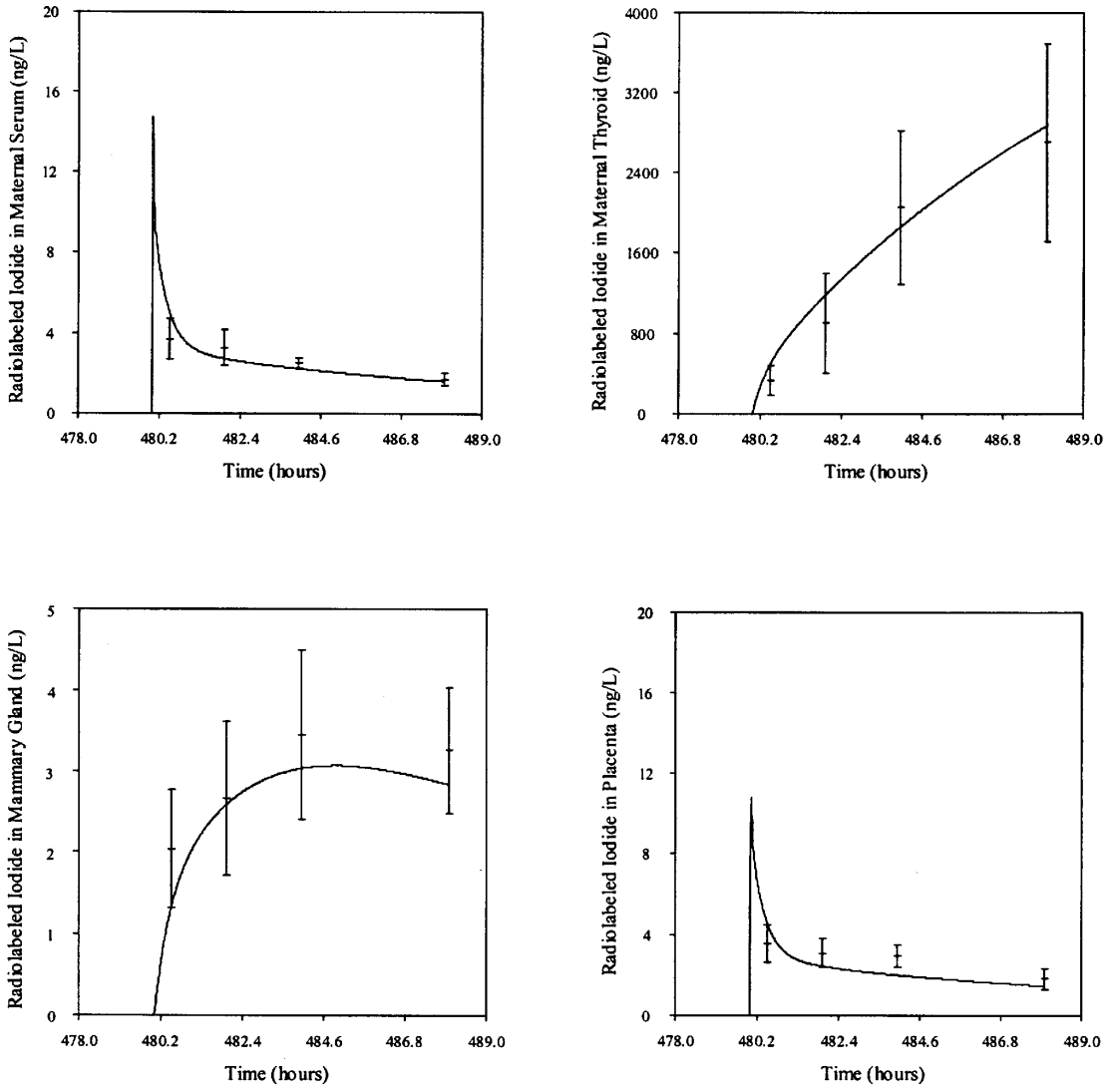

Figure 6-31. Pregnant dam and fetal rat PBPK model predictions (lines) versus data time course (mean ± SD) of $^{125}I^-$ radiolabeled iodide concentrations (ng/L) in maternal serum (top left), thyroid (top right), mammary gland (bottom left), and placenta (bottom right) on GD20 (Clewell, 2001a) Pregnant rats were dosed by iv injection with 2.19 ng/kg $^{125}I^-$ on GD20. Data of Yu (2002).

model. The behavior of these mammary tissue data indicate that iodide is maintained in the mammary gland well into the clearance phase of the serum. In order to simulate this behavior, it was necessary to use a low permeability area value (0.02 L/hr-kg) in the mammary gland

(Clewell, 2001a). The mammary:plasma ratios of greater than one were fit with the Vmaxc for mammary NIS.

Clearance values for the transfer of iodide between the placenta and fetal blood were determined by optimizing the fit of the fetal serum to the data points while maintaining the fit of the simulations of the maternal blood and fetal tissue data. Figure 6-32 shows the model simulation versus the fetal data in the preliminary iodide time course study for radiolabeled iodide in fetal serum (ng/L). Clewell (2001a) shows additional simulations for fetal skin and fetal GI tract.

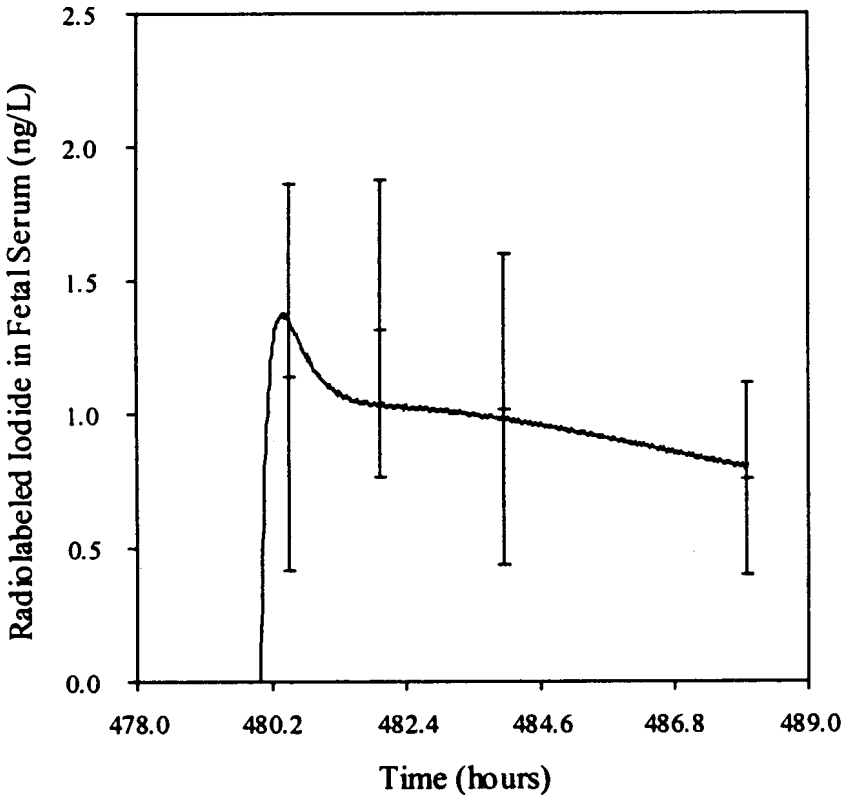

Figure 6-32. Pregnant dam and fetal rat PBPK model predictions (lines) versus data time course (mean ± SD) of $^{125}I^-$ radiolabeled iodide concentrations (ng/L) in fetal serum on GD20 after an iv injection to the dam with 2.19 ng/kg $^{125}I^-$ (Clewell, 2001a). Data of Yu (2002).

The data of Feldman et al. (1961) were used by Clewell (2001a) to determine the values for maximum velocity of iodide uptake in the fetal thyroid. An exponential function was fit to the experimental values and time points where time was gestation in hours. This equation was then used in the model to account for the increasing ability of the fetal thyroid to incorporate iodide. Iodide levels were negligible on GD16 but increased dramatically from GD17 to GD19 (see Clewell, 2001a; Figure 25).

6.3.3 Model Validation

The Clewell (2001a) model predictions for the inhibition of iodide uptake into the thyroid and the resulting effect on the maternal and fetal serum was validated against the data collected by AFRL/HEST during the inhibition study on GD20. The kinetic parameters derived from the perchlorate drinking water and preliminary iodide data sets were used in the model. Because the inhibition study was performed with an acute perchlorate dose, it was necessary to make some slight changes in the parameters describing thyroid perchlorate kinetics. The long-term exposure to perchlorate in the drinking water studies (18 days) that were used to determine the perchlorate parameters is sufficient to induce up-regulation in the thyroid (Yu, 2000). Thus, it was determined that the thyroid parameters in the dam at this point would be different from those seen in an acute situation. The only parameters altered in order to model the acute perchlorate were the partition coefficient (from 2.25 to 0.13) and permeability area value (from 6.0E-4 to 4.0E-5) into the thyroid at the basolateral membrane (thyroid follicle). The value for the partitioning into the follicle in a naive thyroid was calculated as described previously from Chow and Woodbury (1970). The permeability area value in the naive thyroid follicle was determined with the lactation model, which is described in another consultative letter describing model development for the lactating rat (Clewell, 2001b).

The model simulation was fit to the available kinetic data in the thyroid while keeping all other thyroid parameters identical to those in the pregnancy model. Figure 6-33 illustrates the model prediction of thyroidal iodide uptake with and without perchlorate inhibition, utilizing these pre-set parameters. The model prediction of inhibition in the thyroid gland at 0.5, 1., 2, 4, 8, 12, and 24 hours after dosing with iodine shows an excellent fit to the data. The use of parameters derived from the drinking water perchlorate data for acute iodide uptake kinetics is well supported by the inhibition of iodide because inhibition is highly dependent on the

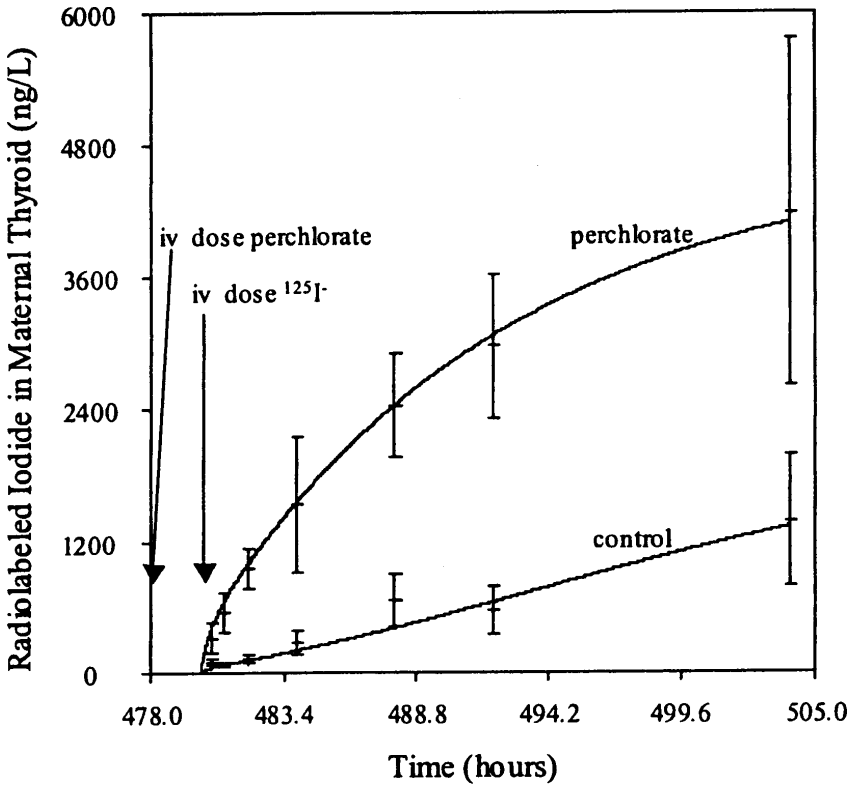

Figure 6-33. Validation for pregnant dam and fetal rat PBPK model (Clewell, 2001a). Model predictions (lines) versus data time course (mean ± SD) of $^{125}I^-$ radiolabeled iodide concentrations (ng/L) in maternal thyroid with and without 1.0 mg/kg perchlorate administered by iv injection to the dam 2 hours prior to an iv injection with 1.87 ng/kg $^{125}I^-$ (Clewell, 2001a). The top simulation represents the control thyroid and the lower indicates the inhibited thyroid. Data of Yu (2000, 2002).

perchlorate concentration in the thyroid and the perchlorate affinity constants in the apical and basolateral membranes of the thyroid. Figure 6-34 illustrates the effect of perchlorate thyroid inhibition on the maternal (top) and fetal (bottom) blood iodide levels. Significant differences were found in the maternal serum iodide concentrations collected at the 1, 4, and 24 hour time points. Fetal serum, however, did not show any significant differences in the total serum iodide between the control and inhibited groups. Additional statistical analysis of these data are provided as Attachment #2 in Clewell (2001a).

Clewell (2001a) performed a model simulation of data presented by Versloot et al. (1997) in order to test the ability of the model to predict diverse data sets collected under different

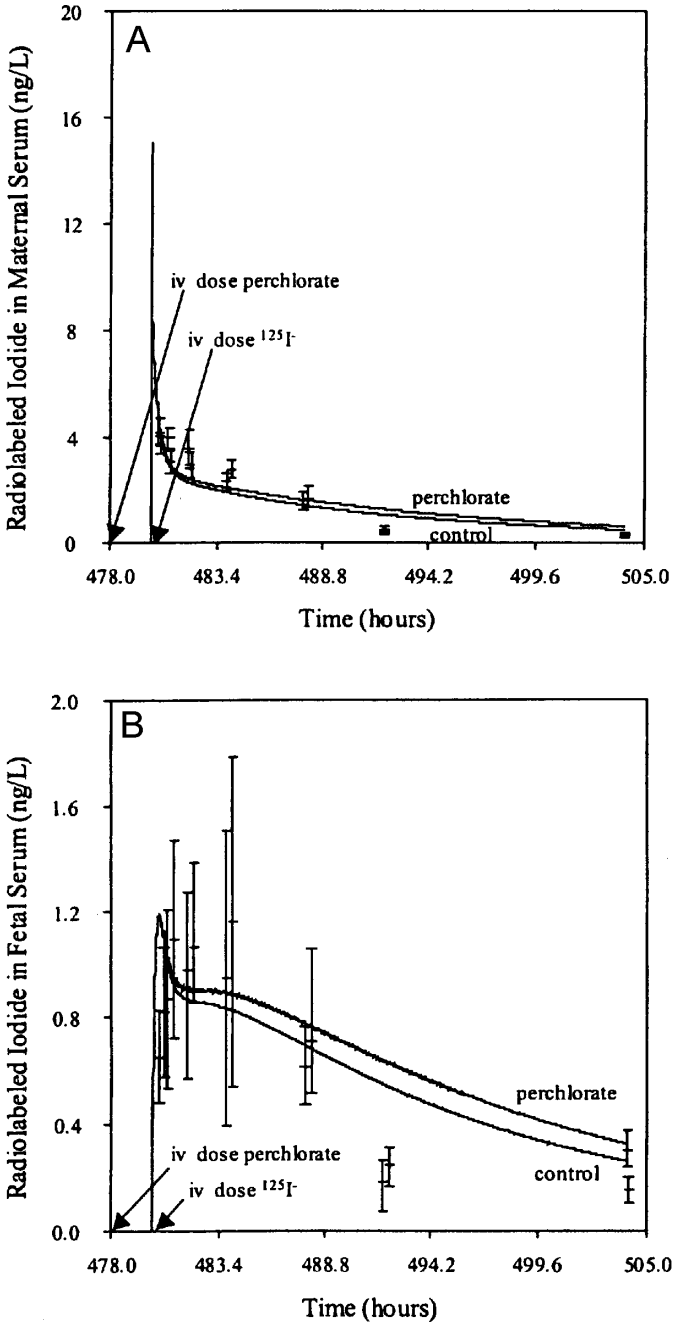

Figure 6-34. Validation for pregnant dam and fetal rat PBPK model (Clewell, 2001a). Model predictions (lines) versus data time course (mean ± SD) of $^{125}I^-$ radiolabeled iodide concentrations (ng/L) in maternal (A) and fetal (B) serum with and without a 1.0 mg/kg perchlorate dose administered by iv injection to the dam 2 hours prior to an iv injection with 1.87 ng/kg $^{125}I^-$ (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).

conditions. This data set provided an additional time point for the iodide model validation (GD19). Dams were exposed by iv injection to 10 μCi (1.74 ng/kg) carrier-free radiolabeled iodide ($^{125}I^-$) on GD19. Figure 6-35 shows the model predictions versus data (mean ± SD) for the amount (ng) of iodide taken up in maternal thyroid (A), mammary gland (B), and placenta (C), or fetal thyroid (D). The model is able to accurately describe these tissues of interest and fits other compartments (data shown in Clewell, 2001a) within a two-fold factor without changing any parameters. This illustrates its predictive power and usefulness to the extrapolations required.

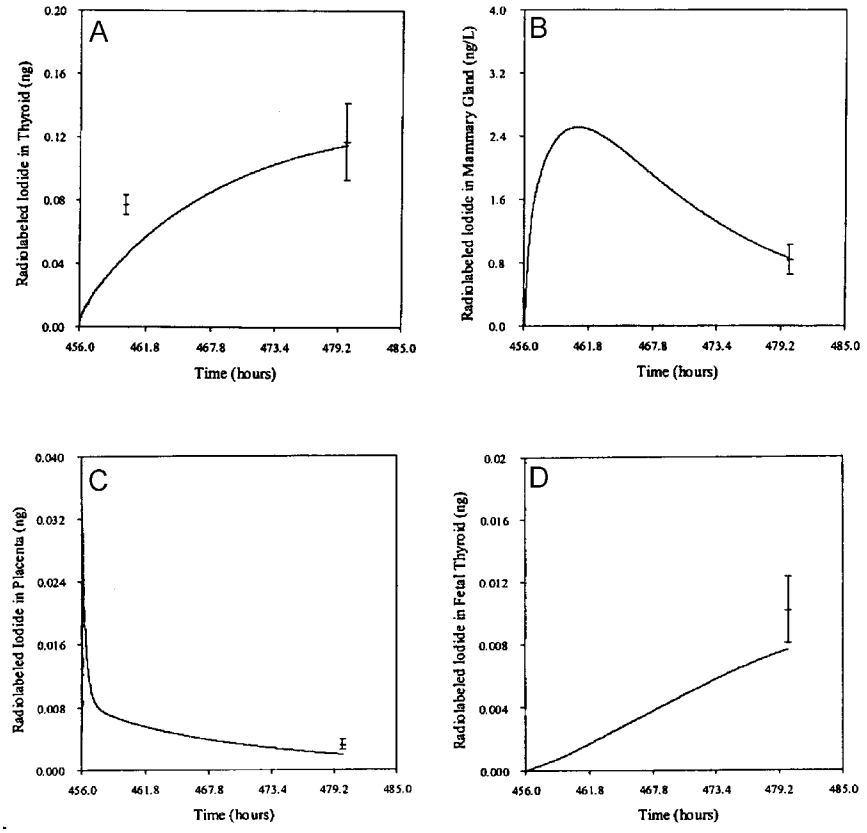

Figure 6-35. Validation for pregnant dam and fetal rat PBPK model (Clewell, 2001a). Model predictions (lines) versus data time course (mean ± SD) of total $^{125}I^-$ radiolabeled iodide in the maternal thyroid (A), mammary gland (B), placenta (C), or fetal thyroid (D) at 24 hours afer exposure to the dam by iv injection of 10 μCi (1.74 ng/kg carrier-free) $^{125}I^-$ in GD19 dams. Data of Versloot et al. (1997).

Model predictions were also shown to be in good agreement with another unrelated data set, that of Sztanyik and Turai (1988), who measured carrier-free radiolabeled iodide ($^{131}I^-$) in GD20 dams and in the total (whole body) fetuses afer an iv injection (Clewell, 2001a). This validation illustrated adequate model fit to another time point and radioactive species of iodide. The model was additionally validated against AFRL/HEST data for dams and fetuses after administration of radiolabeled iodide ($^{125}I^-$) with carrier at doses four orders of magnitude greater than that used to parameterize the model (33000 ng/kg versus 2.19 ng/kg). These validation simulations are shown in Clewell (2001a).

As a final validation exercise, the model was used to predict radiolabeled iodide uptake inhibition after perchlorate exposures in drinking water for 18 days at 0.0, 0.01, 1.0, and 10 mg/kg-day (Yu, 2000). It was apparent that even at the lowest dose, the hormonal system had experienced a perturbation and was attempting to compensate for the interruption caused by the perchlorate exposure (Clewell, 2001a). Maternal T4 decreased in a dose-dependent manner, while TSH increased. The maternal total T4 and TSH changes were statistically significant at all doses. Free T4 was significantly increased at the 0.1, 1.0, and 1.0 mg/kg-day doses and total T3 was significantly decreased at the 1.0 and 10.0 mg/kg-day doses. The fetus appeared to follow the same trends as those seen in the dam. However, only the 1.0 and 10.0 mg/kg-day dose groups show significant decreases in total T4 and the 0.01, 1.0, and 10.0 mg/kg-day doses resulted in significantly increases in fetal free T4 and TSH. No significant decrease was seen in fetal T3. The statistical analysis of the hormone data is provided as Attachment #3 in Clewell (2001a).

From the perspective of iodide kinetics, these hormone changes are important indicators of thyroid up-regulation. When TSH is increased, the thyroid is stimulated to increase iodide uptake. It is evident, then, that after exposure to perchlorate in drinking water for 18 days, the thyroid of the pregnant dam has experienced both inhibition and up-regulation and has successfully compensated for the competition of perchlorate for binding sites of NIS. Therefore, it is not surprising that no inhibition was reported on GD20. It is not that the inhibition is not taking place, but rather that the system has compensated for the effect.

None of the models is currently equipped with the capability to account for up-regulation of the thyroid. Therefore, when a simulation of the inhibition is performed with the model, the concentration of iodide is under-predicted in a perchlorate-dose dependent manner (Clewell,

2001a). Figure 6-36 shows the model prediction of iodide in the thyroid of the dam at drinking water doses of 0.0, 0.1, 1.0, and 10.0 mg/kg-day. The Vmaxc for iodide was decreased to 2.5×10^4 to fit the mean from the control data with the control simulation in order to make the comparison of the inhibition data and simulations clearer. All experimental data were actually taken two hours post dosing. However, the data points were separated slightly by time on the plot in order to make them more visible. The prediction of thyroid perchlorate levels from this same study can be seen in Figure 6-29 (right).

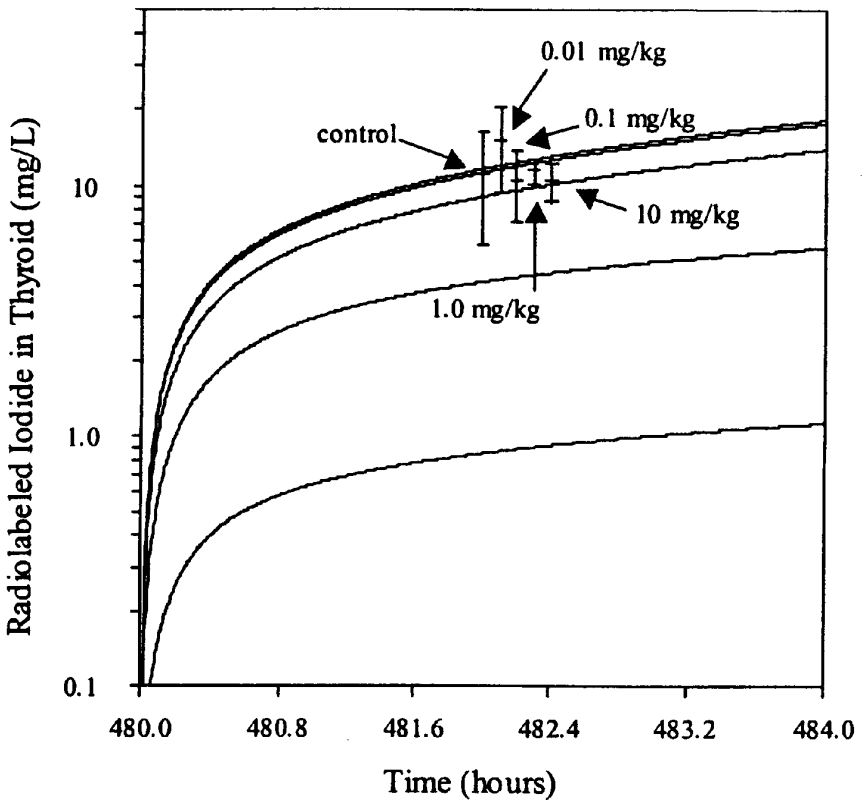

Figure 6-36. Validation for pregnant dam and fetal rat PBPK model (Clewell, 2001a). Model predictions (lines) versus data time course (mean ± SD) of radiolabeled iodide in the maternal thyroid of the dam at doses of perchlorate in drinking water at 0.0, 0.01, 1.0, and 10.0 mg/kg-day for 18 days. Data of Yu (2000).

6.3.4 Summary

The proposed model for the pregnant rat and fetus developed by Clewell (2001a) appears to adequately describe perchlorate and iodide distribution in a highly dynamic, changing system, by accounting for growth with age-specific functions. The model predicts the transfer of perchlorate to the fetus and is also able to describe the uptake into fetal tissues of interest, such as the serum and thyroid. Fetal and dam tissues were predicted well by fitting data that spans three orders of magnitude (i.e., 0.01 to 10.0 mg/kg-day).

In addition to the requisite compartments for pregnancy (mammary gland, placenta, fetus), some differences exist that affect the kinetics of both perchlorate and iodide. The thyroidal maximum capacities are lower in the pregnant dam than in the male rat. Model parameterization in the male rat indicated the need for Vmax values for uptake into the follicle of the thyroid of 2.2×10^3 L/hr-kgr for perchlorate and 5.5×10^4 L/hr-kg for iodide, while the gestation model required values of 1.8×10^3 L/hr-kg and 4.0×10^4 L/hr-kg for the same parameters. This difference is supported in the literature. Versloot et al. (1997) suggest that the pregnant rat may have a lowered reserve of iodide in the thyroid toward the end of pregnancy, causing increased activity in the thyroid. The increased response of the pregnant rat was also seen in the studies performed by Yu (2000) and Yu et al. (2000) that reported a greater than average inhibition in the thyroid of the pregnant dam than in the male rat at the same perchlorate dose (78% vs. 70% over 8 hours). The skin of the pregnant dam also required a smaller value for Vmaxc than the male rat. This is supported by the work of Brown-Grant and Petes (1959), which reported higher levels of iodide in the skin male rats than in female rats. Skin, therefore, appears to be a more important iodide reserve in the male rat than the female. It is reassuring that the model is able to account for the majority of differences in the uptake, distribution, and excretion between the male and pregnant female by incorporating known differences in physiology.

Clewell (2001a) notes that at this time the amount of data concerning perchlorate kinetics in the pregnant rat is very limited. Although perchlorate has been used extensively in literature to study the thyroidal uptake of iodide, it has not been commonly used in rat gestation studies. As such, the perchlorate model was limited to utilizing the drinking water studies for parameterization. However, acute kinetic data were available for perchlorate in the lactating dam and were utilized in the development of the rat lactation model (Clewell, 2001b; see Section 6.4). This system is similar to that of the pregnant dam. Consequently, it was possible to simulate the

perchlorate kinetics of the dam with the same general model structure, changing only the physiological parameters. Therefore, it seemed reasonable to use the acute perchlorate parameters from the lactation model. The use of the described parameters for acute perchlorate kinetics is also supported by the ability of the model to predict inhibition in the pregnant dam. Clewell (2001a) discusses that acute perchlorate kinetic data to further verify the model are currently being analyzed by AFRL/HEST, and these were provided to the EPA too late for evaluation (Yu, 2002). In these studies, tissues were collected from pregnant dams and fetuses at various time points after iv injections of perchlorate. The use of these data in the modeling effort may be described in draft manuscripts provided to the external peer review.

The kinetic behavior of iodide was also accurately simulated with a range of doses that spans nearly five orders of magnitude (0.36 to 33,000 ng/kg). The active sequestration of iodide in maternal and fetal tissues and the transfer of iodide between mother and fetus was described kinetically with the model, and data have been simulated at a variety of doses and at various time points up to 24 hours post exposure. The fact that the model was able to simulate data from other laboratories under a variety of different conditions attests to the validity of the model structure and its applicability to other studies. The ability of the model to predict iodide was indicative of the usefulness of the model for predictive purposes. It was possible to predict inhibition out to 24 hours while simulating the serum and thyroid perchlorate and iodide levels with satisfactory accuracy. This provides support for the chosen model structure, as well as validation for the physiological and chemical descriptions used.

Clewell (2001a) notes that the inability of the model to respond to this auto-regulation presents a considerable need for further model development since drinking water scenarios would allow time for the hypothalamic-pituitary-feedback system to upregulate. Given that the temporal windows of developmental susceptibility are not well established across species, this issue may have to wait for further fundamental neurodevelopmental research.

The EPA has also become aware of a recent human biokinetic model for iodine and radionuclides at various ages (fetus, children, mothers) that may provide some additional information with which to validate the iodide kinetic components of the proposed models from 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

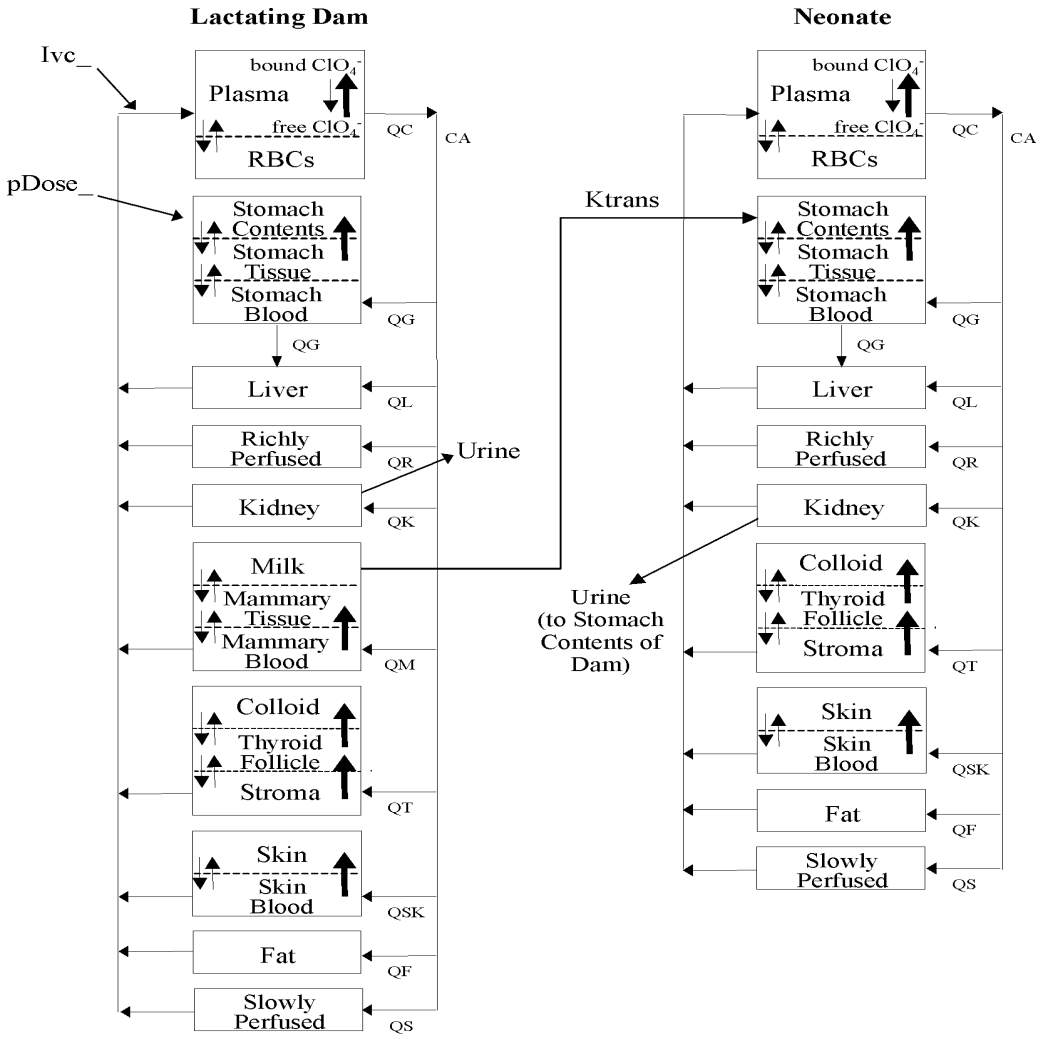

Figure 6-37. Schematic for the lactating dam and neonatal rat PBPK model of perchlorate and iodide distribution (Clewell, 2001b). Boxes represent specific compartments in the model structure. The thyroid consists of the stroma, the follicle, and the colloid, and the stomach consists of the capillary 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 (ClO_4^-) and iodide (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 (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		
Tissue Volumes[a]	**Dam**	**Neonate**	**Source**
Body Weight *BW* (kg)	0.277 - 0.310	0.0075 - 0.1985	Yu, 2000
Slowly Perfused *VSc* (%BW)	37.07-40.42	53.92-49.31	Brown et al., 1997
Richly Perfused *VRc* (%BW)	5.35	5.36	Brown et al., 1997
Fat *VFc* (%BW)	12.45 - 6.9	0.0 - 4.61	Naismith et al., 1982
Kidney *VKc* (%BW)	1.7	1.7	Brown et al., 1997
Liver *VLc* (%BW)	3.4	3.4	Brown et al., 1997
Stomach Tissue *VGc* (%BW)	0.54	0.54	male rat ClO_4^- kinetics
Gastric Juice *VGJc* (%BW)	1.68	1.68	Yu, 2000
Stomach Blood *VGBc* (%VG)	2.9	2.9	Altman & Dittmer, 1971
Skin Tissue *VSkc* (%BW)	19.0	19.0	Brown et al., 1997
Skin Blood *VSkBc* (%VSkc)	2.0	2.0	Brown et al., 1997
Thyroid Total *VTtotc* (%BW)	0.0105	0.0125	Malendowicz & Bednarek, 1986; Florsheim et al., 1966
Thyroid Follicle *VTc* (%Vttot)	45.89	37.2	Malendowicz & Bednarek, 1986; Conde et al.,1991
Thyroid Colloid *VDTc* (%VTtot)	45	13.8	Malendowicz & Bednarek, 1986; Conde et al.,1991
Thyroid Blood *VTBc* (%VTtot)	9.1	49.0	Malendowicz & Bednarek, 1986; Conde et al.,1991
Plasma *VPlasc* (%BW)	4.7	4.7	Brown et al., 1997, Altman & Dittmer, 1971
Red Blood Cells *VRBCc* (%BW)	2.74	2.74	Brown et al., 1997, Altman & Dittmer, 1971
Mammary Tissue *VMc* (%BW)	4.4 - 6.6	—	Knight et al., 1984
Mammary Blood *VMBc* (%VM)	18.1	—	Assume same % as Thyroid Blood
Milk *VMk* (L)	0.002	—	Fisher et al., 1990
Blood Flows			
Cardiac Output *QCc* (L/hr-kg)	14.0 - 21.0	14.0	Hanwell & Linzell, 1973; Brown et al., 1997
Slowly Perfused *QSc* (%QC)	7.9-1.9	16.9	Brown et al., 1997
Richly Perfused *QRc* (%QC)	40.8	40.8	Brown et al., 1997
Fat *QFc* (%QC)	7.0	7.0	Brown et al., 1997
Kidney *QKc* (%QC)	14.0	14.0	Brown et al., 1997
Liver *QLc* (%QC)	18.0	18.0	Brown et al., 1997
GI *QGc* (%QC)	1.61	1.61	Brown et al., 1997
Skin QSkc (%QC)	0.058	0.058	Brown et al., 1997
Thyroid *QTc* (%QC)	1.6	1.6	Brown et al., 1997
Mammary *QMc* (%QC)	9.0 - 15.0	—	Hanwell & Linzell, 1973

[a]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)[a]

Perchlorate Parameters	Lactation Values		
Partition Coefficients (unitless)	**Dam**	**Neonate**	**Source**
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[b]
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
Max Capacity, Vmaxc (ng/hr-kg BW)			
Thyroid Follicle Vmaxc_T	1.50E+03	1.50E+03	Fitted[c]
Thyroid Colloid Vmaxc_DT	1.00E+04	1.00E+04	Fitted[c]
Skin Vmaxc_S	8.00E+05	8.00E+05	Fitted
Gut Vmaxc_G	1.00E+06	1.00+06	Fitted
Mammary Tissue Vmaxc_M	2.0E+5/2.0E+4	—	Fitted[b,c]
Milk Vmaxc_Mk	2.00E+04	—	Fitted
Plasma Binding Vmaxc_B	9.00E+03	1.00E+03	Fitted
Affinity Constants, Km (ng/L)			
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)[a]

	Lactation Values		
Perchlorate Parameters	**Dam**	**Neonate**	**Source**
Permeability Area Cross Products, (L/hr-kg)			
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[b,c]
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
Clearance Values, (L/hr-kg)			
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

[a]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_T*i*, Vmaxc_T*p*, etc.).
[b]Neonate was given maternal values for Vmax (scaled by body weight) in the absence of data.
[c]Parameters with two values indicate acute and drinking water parameters, respectively.

obtain these initial values for tissue loading at birth, the pregnancy model had to include all of the compartments contained in the lactation model (Clewell, 2001a). The pregnancy model was then allowed to run until the day of birth (GD22), and the average tissue concentrations of perchlorate or iodide for the final day of gestation were used as the starting values for the respective tissues in the lactation model (Clewell, 2001b).

As discussed, the mammary tissue has been shown to concentrate both perchlorate and iodide during lactation via the NIS symporter. Additionally, hormones produced during lactation such as prolactin which stimulates milk production, have been shown to regulate the mammary NIS. Suckling of the neonatal rats has also been shown to stimulate mammary NIS activity (Tazebay et al., 2000). An additional symporter has been identified in the experiments of Shennan (2001). *In vitro* studies of iodide transport into the mammary gland and the resulting efflux of sulfate from the cells in the absence of sodium cation ($Na^{+)}$, indicates that another form 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)[a]

Iodide Parameters	Lactation Values		
Partition Coefficients (unitless)	**Dam**	**Neonate**	**Source**
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
Max Capacity, Vmaxc (ng/hr-kg BW)			
Thyroid Follicle Vmaxc_T	4.00E+04	4.00E+04	Fitted[b]
Thyroid Colloid Vmaxc_DT	6.00E+07	6.00E+07	Fitted[b]
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
Affinity Constants, Km (ng/L)			
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
Permeability Area Cross Products, (L/hr-kg)			
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[b]
Thyroid Tissue to Thyroid Colloid PADTc_	1.00E-04	1.00E-04	Fitted[b]
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
Clearance Values, (L/hr-kg)			
Urinary excretion CLUc_	0.03	0.02	Fitted
Transfer from Milk to Pup Ktransc	6.4E-04 - 1.04E-03		Sampson & Jansen, 1984

[a]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_T*i*, Vmaxc_T*p*, etc.).
[b]Neonate was given maternal values for Vmax (scaled by body weight) in the absence of data.

that this anion transport mechanism is able to transfer perchlorate and iodide into the secretory cells against a concentration gradient. Since the secretory cells are responsible for secreting their contents into the milk, the anion transport mechanism was included in the milk compartment of the Clewell (2001b) model.

The structure of the Clewell (2001b) neonatal model is similar to that of the pregnant and fetal rat model, with the exception of the mammary gland compartment as will be described in 6.4.2.1.1. In order to simplify the model, all neonates from a single litter were combined in the structure of the model, essentially viewing the entire litter as one entity, or one large neonate. The dose to the neonate is based on the transfer of perchlorate from the maternal milk to the GI contents of the neonate rather than through direct exposure to the drinking water. The 60% of urinary excretion of the neonate is then entered back into the GI contents of the dam in order to account for maternal ingestion of the pup's urine during cleaning, based on the work of Samuel and Caputa (1965).

The same challenge posed by the pregnancy model (i.e., to describe perchlorate and iodide distribution in a highly dynamic system) was the objective of the lactating and neonatal rat model (Clewell, 2001b). In addition to total body weight changes in the dam and neonate, maternal mammary tissue and blood flow, cardiac output, fractional body fat and neonatal body weight, and fractional body fat change with respect to time. All tissue volume and blood flow values were adjusted with respect to the changing parameters.

Clewell (2001b) assumed the neonate to be nursing at a constant rate, 24 hours a day. This assumption is based on the fact that young nursing rats are unable to go for long periods of time without suckling. The loss through suckling was then described with a first order clearance rate from the mother's milk to the gastric juice of the neonate, based on the experiments of Sampson and Jansen (1984). The milk production rate was assumed to be equal to the amount of milk ingested by the litter.

6.4.1 Data and Methods

This section summarizes the data that Clewell (2001b) used for development and validation of the lactating and neonatal rat model structures. Details on experimental methods, including: protocol design, exposure regimen, chemical source and purity, animals (housing, feeding,

surgical procedures, etc.), and the analytical methods can be found in the consultative letter and associated reports from AFRL/HEST or papers cited therein.

6.4.1.1 AFRL/HEST Experiments in Laboratory Rats

These studies are described in the consultative letter and reports of Clewell (2001b), Yu (2000, 2002), Yu et al. (2000), and Mahle (2000; 2001).

6.4.1.1.1 Drinking Water Study

Perchlorate drinking water experiments used in development of the Clewell (2001b) model included this study in which pregnant Sprague-Dawley dams were exposed to drinking water treated with perchlorate from GD 2 through PND5 or PND10 at perchlorate doses of 0.0, 0.01, 0.1, 1.0, and 10.0 mg/kg-day. GD0 was determined by the presence of a vaginal plug. Litters were standardized to eight pups (four male and four female, when possible) on PND2. Dams and their litters were euthanized on either PND5 or PND10; maternal and neonatal serum was analyzed for fT4, tT4, T3, and TSH. Maternal serum, thyroid, skin, and gastric contents were analyzed for perchlorate at all doses. Neonatal serum, skin, and GI contents were also analyzed for perchlorate at all doses. Milk was analyzed only on PND10 at all doses. Perchlorate analysis was performed only on maternal gastric tract, mammary tissue, and neonatal gastric tract samples from the PND5 study at the 10.0 mg/kg-day dose. Two hours before euthanization, the dams were given iv doses of 33 mg/kg radiolabeled iodide ($^{125}I^-$) with carrier. Tissue concentrations of iodide were measured in order to determine the inhibition in the various tissues after long-term exposure to perchlorate. This study is described in detail in the consultative letter (Yu, 2000).

6.4.1.1.2 Cross-fostering Study

The cross-fostering study involved four groups of rats with varied experimental conditions: true control, control, exposed, and true exposed. True control rats were never dosed with perchlorate. Neonates remained with the dam after birth. In the control group, dams were never exposed to perchlorate in drinking water. However, at the time of birth, the neonates were replaced with pups (less than 24 hours old) that had been exposed to perchlorate throughout gestation (1.0 mg/kg-day to mother through drinking water). In the exposed group, the dams were dosed with 1.0 mg/kg-day perchlorate in drinking water from GD2 to PND10. At the time

of birth, the neonates were replaced with pups (less than 24 hours old) that had never been exposed to perchlorate. The true exposed dams were dosed with 1.0 mg/kg-day perchlorate from GD2 to PND10. Neonates remained with their mother after birth. All dams and pups were euthanized on PND10. The skin, GI contents, and serum from the neonates and dam were analyzed for perchlorate. Results indicated that both true control and control (exposed neonates with control dams) showed no perchlorate present on PND10. True exposed and exposed (exposed dams with control litters) showed comparable perchlorate levels on PND10. This study is described in detail in the consultative letters (Mahle, 2000; 2001).

6.4.1.1.3 Perchlorate Kinetics Study

In order to evaluate the acute kinetics of perchlorate in the lactating dam and neonate, AFRL/HEST performed a study of the kinetic behavior of perchlorate after the administration of an acute dose. PND10 Sprague-Dawley dams were given 0.1 mg/kg perchlorate by tail-vein injection. The dams were left with their neonates until the time of euthanization at 0.5, 1, 2, 4, 8, or 12 hours post-dosing. Maternal serum, thyroid, stomach contents, skin, and mammary gland were collected and analyzed for perchlorate content at all time points. Neonate serum, stomach contents, and skin were also collected for perchlorate analysis at all time points. Fat, liver, kidney and bladder tissues were also collected from the dam at the eight hour time point. Perchlorate analysis was performed on the serum of the dam and neonates and the maternal thyroid, mammary gland, GI contents, and skin.

6.4.1.1.4 Iodide Inhibition Kinetics Study

A study of iodide time course and inhibition kinetics 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 PND10. The perchlorate dose was followed at two hours post-dosing with a tail-vein injection of carrier free radiolabeled iodide ($^{125}I^-$) at an average dose of 2.10 ng/kg. Dams (n=6) were euthanized after 0.5, 1, 2, 4, 8, and 24 hours. Maternal and neonatal serum, skin, GI contents and tract, as well as the maternal thyroid and mammary gland tissue, were collected and analyzed for total iodide content at each time point. Neonatal serum was pooled by 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 ($^{131}I^-$) 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 μCi of carrier-free radiolabeled iodide ($^{131}I^-$). 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

the maternal body weight on days 2, 7, 14, and 21, respectively. The residual milk was assumed to be 0.002 L based on the model of Fisher et al. (1990). Naismith et al. (1982) examined the change in body fat content of the lactating rat. They reported values for the volume of maternal body fat of 15.2 and 6.9% of the body weight on PND 2 and 16, respectively. The body fat composition of the dam on PND1 was calculated to be 12.4% from the PBPK model for perchlorate and iodide kinetics in the pregnant rat model described in Section 6.3 (Clewell, 2001a).

In order to describe the changes in the physiology of the lactating rat, it was not sufficient to simply scale some of the parameters allometrically. As opposed to the typical growth scenario, some of the tissues in the lactating rat cannot be assumed to increase at the same rate in this dynamic system. Rather, a few tissues, such as the mammary gland and fat, are changing at an accelerated rate in comparison to the other organs. These parameters required additional descriptions for their growth beyond the previously described allometric scaling by body weight. Clewell (2001b) based the approach to modeling these changing parameters on the work of Fisher et al. (1990) with trichloroethylene.

Additionally, the thyroid of the female rat was found by investigators to be significantly larger than that of the male rat (Malendowicz and Bednarek, 1986). Clewell (2001b) assigned values to these parameters based on these data and relevant to the gender and condition (i.e., lactation) of the animal. A value of 1.05% of the maternal body weight was used for the thyroid in the lactation model. The volume fractions of the colloid, follicle, and stroma were given values of 45, 46, and 9% of the thyroid volume. These are significantly different from the values given for the male rat. The volume of the colloid in particular is much greater in the female than the male rat (46 vs. 24% of the thyroid volume). Parameters that were not available specifically for the female were described by adjusting the values for the male rat by body weight.

In the PND10 drinking water study performed by AFRL/HEST (see Attachment #2; Clewell, 2001b), the body weight of the dam showed an average increase of 12% between PND1 and PND10, but did not show a significant difference in weight between dose groups. As a result, Clewell (2001b) calculated the average body weight of the dams for all dose groups for each day of the study and then programmed this changing body weight into the model as a table 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 is 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.

6.4.2.1.3 Blood Flows

All maternal and neonatal blood flows that were not directly affected by the changes induced by lactation were scaled by weight from the adult male rat parameters. For those blood flow parameters that change in response to lactation, some additional description was required (Clewell, 2001b). Cardiac output has been shown to increase during lactation (Hanwell and Linzell, 1973). The values given by Hanwell and Linzell (1973) of 14.0, 18.6, 19.0, and 21.0 L/hr-kg for days 3, 8, 13, and 23 of lactation were used in the model as a table function to describe the change in cardiac output over time (Clewell, 2001b). Additionally, the blood flow to the mammary tissue was also found to increase during lactation. Reported fractional blood flows to the mammary tissue of 9, 10, 11, 14, 14, and 15% of the cardiac output on PNDs 1, 5, 10, 15, 17, and 21, again from Hanwell and Linzell (1973), were used.

6.4.2.2 Chemical-Specific Parameters

The various active transport processes, tissue permeabilities and clearance rate (excretion) are described in PBPK models for each species on a chemical-specific basis. This section outlines how the values for perchlorate and iodide used in the lactating and neonatal rat model were derived. The values can be found in Tables 6-7 and 6-8. Details on the derivation can be found in Clewell (2001b).

6.4.2.2.1 Affinity Constants and Maximum Velocities for Active Uptake Processes

Whenever possible, chemical specific parameters were kept the same in human and in male, female, neonatal, and fetal rats. However, it was necessary to change a few of the parameters, including the maximum velocity capacity (Vmaxc). The Km values were similar between tissues and between female and male rat and human models. However, the maximum velocity capacity differs between tissues (Wolff and Maurey, 1961). Since values for the tissue maximum velocity capacity for perchlorate (Vmaxc-*p*) were not given in literature, the values were estimated with the model. In order to determine Vmax with the model, the simulation for the tissue of interest was compared to various data sets with several different perchlorate dose levels. The value for Vmaxc within a given compartment was then determined by the best fit of 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 ($^{36}ClO_4^-$) 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 (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 (Vmaxc 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,

pregnant rat and fetus), it was necessary to account for the serum binding of perchlorate in order to adequately describe the serum perchlorate concentrations at the lower doses (0.01 and 0.1 mg/kg-day). Figure 6-38 illustrates the importance of binding in the model simulations in the dam on these days.

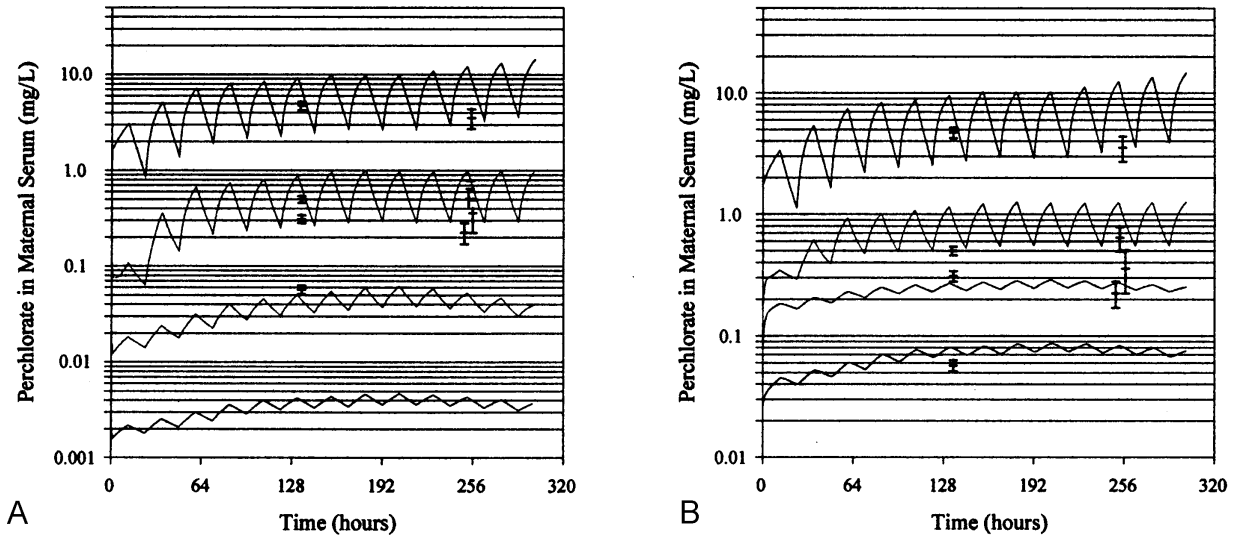

Figure 6-38. Simulations illustrating the necessity of including plasma binding in the lactating dam and neonatal rat PBPK model structure (Clewell, 2001b). Model predictions (lines) versus data time course (mean ± SD) for maternal serum perchlorate concentrations (mg/L) on PND5 and PND10 at doses to the dam of 10.0, 1.0, 0.1, and 0.01 mg/kg-day are shown with (A) and without (B) plasma binding.

Figure 6-39 shows the perchlorate tissue concentrations (mg/L) in the lactating dam thyroid (A) and in maternal milk (B) at PND5 and PND10 for the 0.01, 0.1, 1.0 and 10.0 mg/kg-day doses. It was noticed that during the drinking water studies, the daily dose to the dams varied somewhat due to their changing water intake. Therefore, all of the model simulations of the drinking water studies reflect the actual daily dose to the dam, which Clewell (2001b) calculated from the daily water consumption and body weight measurements.

Figure 6-40 shows the model simulations of the male and female neonate plasma levels compared to the data obtained in the AFRL/HEST drinking water study. Plasma concentrations

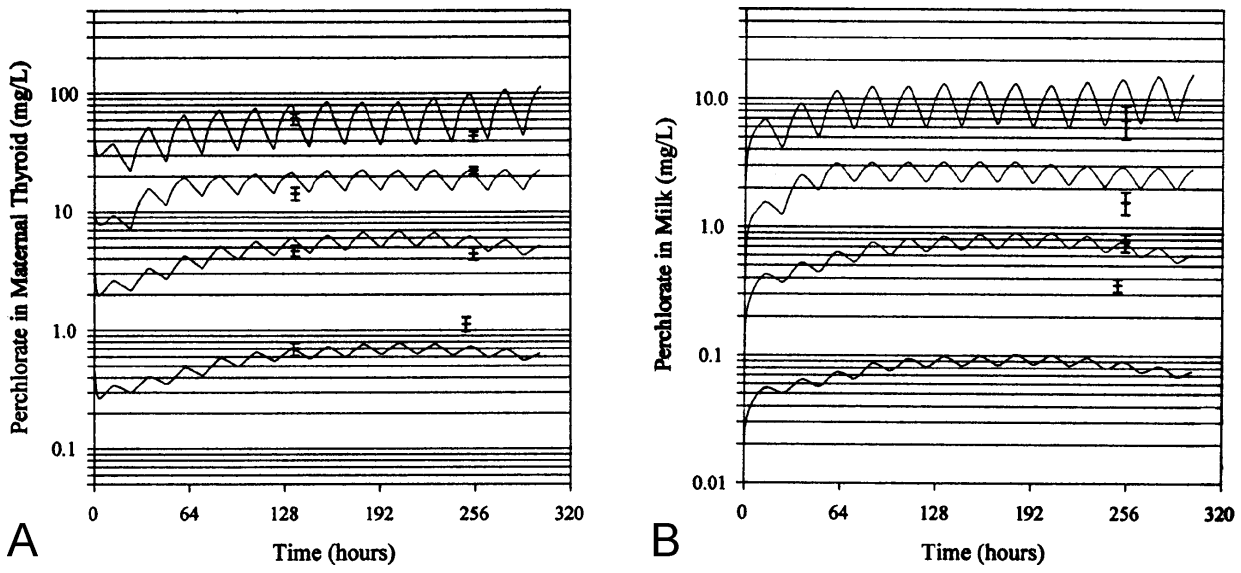

Figure 6-39. Lactating dam and neonatal rat PBPK model predictions (lines) versus data time course (mean ± SD) of perchlorate concentrations (mg/L) in the maternal thyroid (A) and milk (B) on PND5 and PND10 at doses in drinking water to the dam of 10.0, 1.0, 0.1, or 0.01 mg/kg-day perchlorate (Clewell, 2001b).

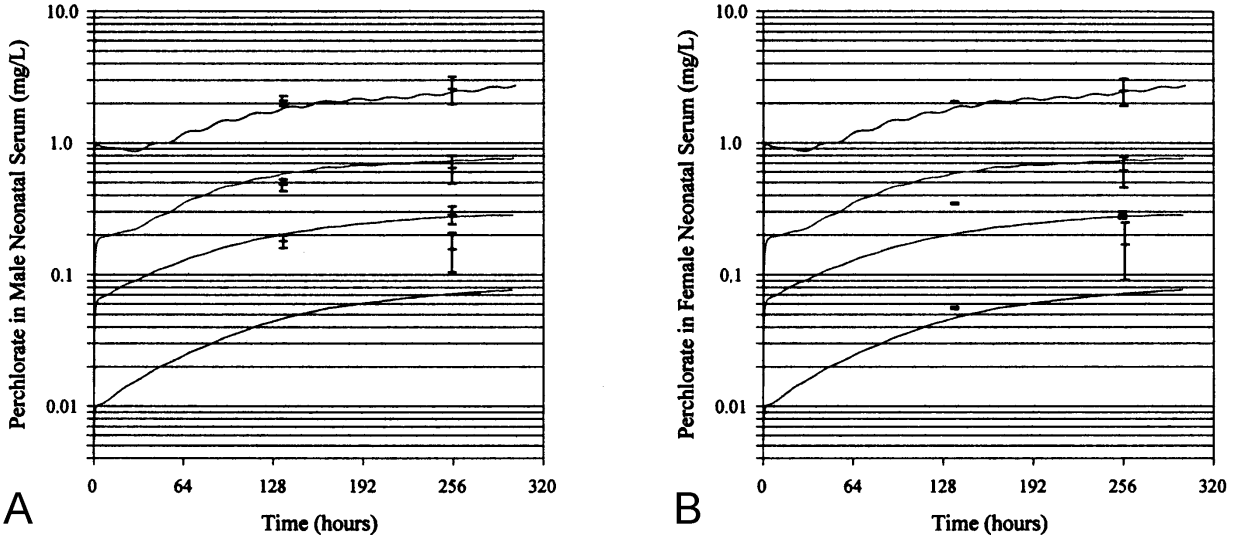

Figure 6-40. Lactating dam and neonatal rat PBPK model predictions (lines) versus data time course (mean ± SD) of perchlorate concentrations (mg/L) in the serum of male (A) and female (B) neonates on PND5 and PND10 at doses in drinking water to the dam of 10.0, 1.0, 0.1, or 0.01 mg/kg-day perchlorate (Clewell, 2001b).

varied significantly between the male and female neonates, and Clewell (2001b) noted that the difference appears to be a function of age. At PND5, the male neonatal plasma concentrations were nearly 4 times higher than those of the female neonates in the 0.1 mg/kg-day dose group. By PND10, however, no significant sex difference was found in the plasma perchlorate concentrations at the same dose.

Clewell (2001b) fit the male neonatal serum data because the male pups showed higher perchlorate concentrations in the serum than the female pups (Yu, 2000). The neonatal serum was under-predicted by the model in the 0.01 mg/kg-day dose group. Clewell (2001b) strongly asserts that this was due to the fact that the milk concentration was also under-predicted in that same dose group. The three higher doses are well described in the male neonate. The female pups also show acceptable fits at PND10. However, since the PND5 data were much lower in the female than male neonates, the model over-predicts the PND5 time-points in the 0.1 and 1.0 mg/kg-day doses. Fits of the model to neonatal skin and GI tract are discussed in Clewell (2001b).

As in the maternal model, the clearance value for urinary excretion was determined by the fit of the model to the serum from the 10 mg/kg-day dose, while the lower doses were used to determine the kinetic parameters for the binding in the neonate. Both binding and urinary clearance were considerably lower in the pup than in the dam (Table 6-7).

6.4.2.3.2 Iodide Model Parameterization

Clewell (2001b) developed the iodide aspect of the model by visually fitting the model to measured tissue concentrations in the dam and neonate from the control group of the inhibition kinetic study. Only the values for Vmax and permeability area needed to be fit with the model. As shown in Figure 6-41, the model simulations of iodide concentrations (ng/L) after an iv injection of 2.10 ng/kg radioalabeled iodide ($^{125}I^-$) on PND10 versus the experimental data in the lactating dam are shown in the dam serum (A) and thyroid (B) and in male (C) and fetal (D) neonatal serum.

The model simulations describe the data well with the exception of the longest time point in the neonates. 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 maternal skin, GI, and mammary gland (Clewell, 2001b). Permeability area values were adjusted to

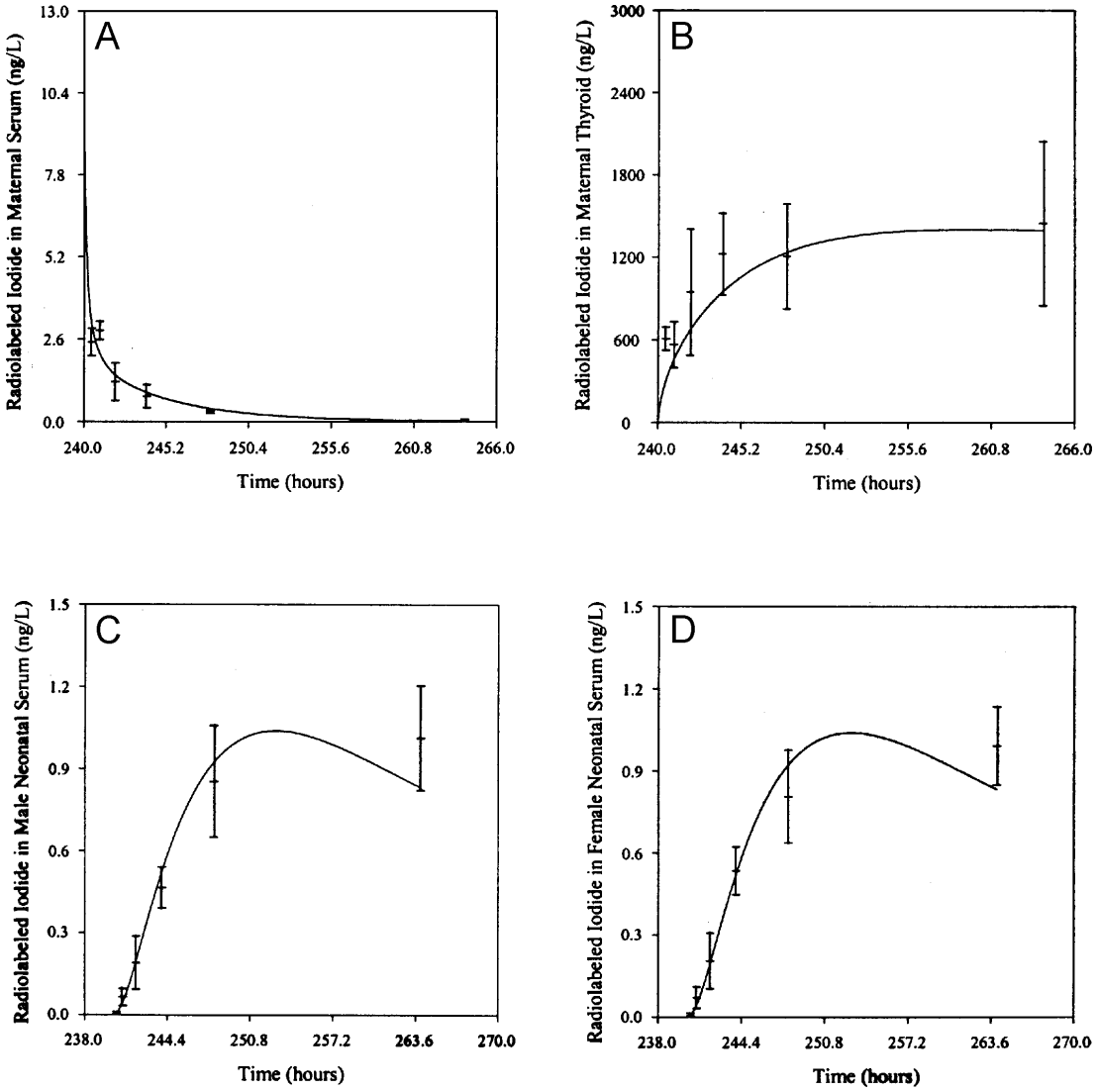

Figure 6-41. Lactating dam and neonatal rat PBPK model predictions (lines) versus data time course (mean ± SD) of iodide concentrations (mg/L) in the maternal serum (A) or thyroid (B) and in male (C) or female (D) neonatal pups on PND10 after an iv dose to the lactating dams of 2.10 ng/kg $^{125}I^-$ (Clewell, 2001b). Data of Yu (2000, 2002).

describe the behavior of the iodide data; 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; decreasing permeability area slowed the uptake and clearance.

The behavior of the iodide in the neonatal skin and GI tract and contents appeared to be different from the dam. The iodide tended to stay in the tissue of the neonate longer, requiring a slower clearance in the fetal tissues than was used in the corresponding maternal tissue. As a result, permeability area values used for the GI and skin in the neonate were lower than those used in the dam (Table 6-8). For example, the permeability area value in the skin was determined to be 0.5 L/hr-kg in the dam, but was decreased to 0.02 L/hr-kg in the neonate. However, these values correspond well to the values used for the fetus in the pregnancy model (Clewell, 2001a).

The neonatal urinary clearance value was determined to be 0.02 L/hr-kg in the neonate, which is very similar to the maternal value (0.03 L/hr-kg of the dam). This was a surprise, because the neonate was expected to have a much lower rate of excretion than the more mature dam; however, Clewell (2001b) notes that this trend is supported in the literature. Capek and Jelinek (1956) measured the amount excreted by pups at various ages. The neonates required external stimulation by the mother in order to release the urine from their bladders. However, when that stimulation was supplied, the neonates were able to excrete urine at the same rate as an adult rat. Therefore, it is reasonable that the urinary excretion rate is similar between the pup and adult. The amount of iodide lost to urine is then dependent on both the urinary clearance value and the concentration of the ion in the kidney (Clewell, 2001b).

6.4.3 Model Validation

The ability of the model to simulate the kinetics of perchlorate in the lactating dam and neonate was tested against the perchlorate time course data collected *in vivo* by AFRL/HEST. Since the study was performed with an acute perchlorate dose, it was necessary to make minor changes in the thyroid perchlorate parameters. The long-term exposure to perchlorate in the drinking water studies that were used to determine the perchlorate parameters is sufficient to induce up-regulation in the thyroid (Yu, 2000). Therefore, the thyroid parameters in the dam at this point would be different from those seen in an acute situation. Clewell (2001b) achieved the model fits to the acute data by altering the partition coefficient (from 2.25 in the drinking water to 0.13 in the acute exposure) and permeability area value (from 6.0E-4 to 4.0E-5) into the thyroid at the basolateral membrane (thyroid follicle). The value for the partitioning into the follicle in a naïve thyroid was calculated as described previously from Chow and Woodbury

(1970). The permeability area value in the naïve thyroid follicle was determined by fitting the model prediction to the thyroid data, while keeping good fits in the serum and other tissues. Figure 6-42 shows the model predictions versus the data time course of perchlorate concentrations in maternal serum (A), thyroid (B), or mammary gland (C) and in neonatal serum.

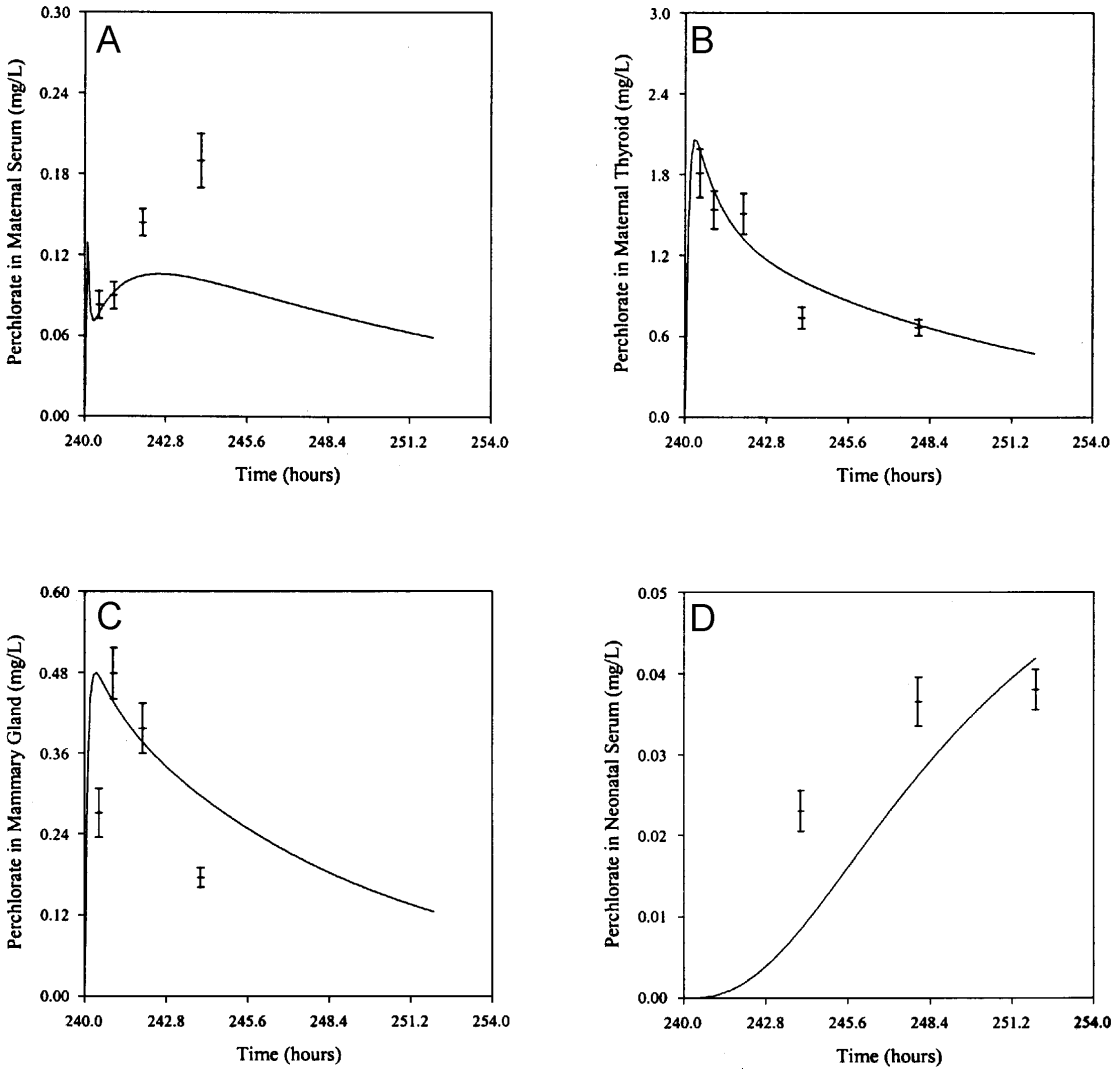

Figure 6-42. Validation for lactating dam and neonatal rat PBPK model (Clewell, 2001b). Model predictions (lines) versus data time course (mean ± SD) of perchlorate in the maternal serum (A), thyroid (B), or mammary gland (C) and in neonatal serum (D) after an iv dose of 1.0×10^6 mg/kg perchlorate on PND10. Data of Yu (2000, 2002) and Yu et al. (2000).

The maternal serum is not fit particularly well and the neonatal serum fit could also be improved. Clewell (2001b) notes the difficulties may be due to the use of the iv kinetic data as well as some additional challenges not yet met by the model with respect to the mammary gland. Clewell (2001b) increased the transfer of perchlorate through the milk in the acute studies in order to fit the model derived on drinking water studies to these acute (iv) data. That is, the value for the Vmax into the mammary tissue was increased in order to allow more perchlorate into the mammary compartment, and the permeability area into the milk was decreased in order to minimize the back flow of perchlorate into the mammary from the milk. This essentially forced the perchlorate in the milk to be passed to the neonate rather than return to the mammary tissue of the mother. The Vmaxc for the binding in the neonate was decreased slightly from the value used in the drinking water simulations. This may have been due to increased transfer of iodide in the acute simulations. When the same parameters were used in the mammary compartment that were determined with the drinking water studies, the amount in the mammary tissue was low and the clearance of the mammary was too slow. As a result, acute neonatal serum levels were under-predicted. By adjusting the Vmaxc, the model was able to achieve reasonable fits to the available data in the maternal and neonatal tissues. Clewell (2001b) suggests that different fractions of the dose are transferred through the milk during an acute (iv) exposure versus a drinking water scenario.

Figure 6-43 shows the model predictions against the data obtained in the AFRL/HEST cross-fostering study described in Section 6.4.1.1.2. Perchlorate concentrations (mg/L) in the maternal thyroid of dams exposed during gestation (A) or only during lactation (B) show similar results. Perchlorate concentrations (mg/L) in neonatal serum exposed only during gestation (C) or only during lactation (D) also contained similar levels. Because the data were taken on PND10, the sex difference seen at the earlier time points was not present and the simulation is shown for the average of all pups.

The model is able to predict the data from the cross-fostering study very well. It is apparent from the data and from the model prediction of the cross-fostering data that the gestational exposure to perchlorate does not affect the perchlorate concentrations of the maternal serum and thyroid or the neonatal serum. This is in agreement with other studies that indicate the rapid clearance of perchlorate in the urine (Yu et al., 2000), but not in agreement with the toxicological

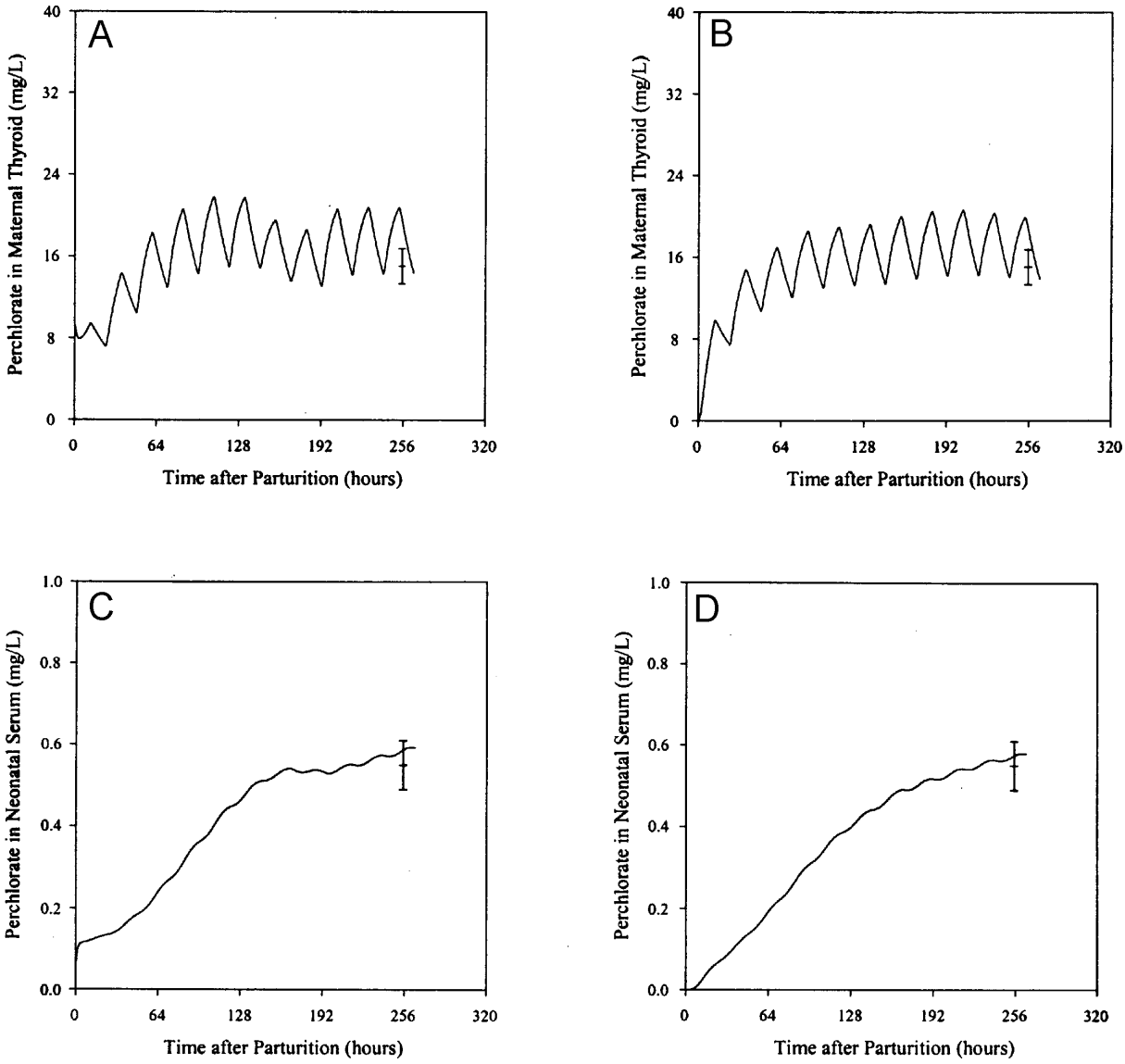

Figure 6-43. Validation for lactating dam and neonatal rat PBPK model (Clewell, 2001b). Model predictions (lines) versus data time course (mean ± SD) of perchlorate in the maternal thyroid during gestation (A) or during lactation only (B) and in the neonatal serum during gestation (C) or during lactation only (D) after an iv dose of 1.0×10^6 mg/kg perchlorate on PND10. Data of Mahle (2001).

observations between the 1998 and 2001 developmental neurotoxicological studies performed by Argus Research Laboratories, Inc. (1998; 2001). Differences in the hormone data are discussed in Clewell (2001c) and other differences may be due to strain differences (Fail et al., 1999). From the model, even though the neonatal urinary excretion is much lower than that of the dam

(0.005 vs. 0.07 L/hr-kg), the prenatal exposure does not affect the serum levels of the neonate past PND2. This is in accord with the observations made of the BMDL estimates for the post-natal thyroid discussed in Chapter 5.

Additional validation exercises were performed by Clewell (2001b), showing reasonably adequate model fits to the data of Potter et al. (1959) and that of Sztanyik and Turai (1988) as shown in Clewell (2001b). Maternal radiolabeled iodide concentrations were overpredicted in the thyroid on PND18. The maternal milk concentrations were also overpredicted for the earlier time point, but were within the range at the later. The model predicted the radiolabeled iodide data obtained in the litters of Sztanyik and Turai (1988) quite well. This indicates that the lactation and neonatal kinetics are characterized accurately.

Figure 6-44 shows that the Clewell (2001b) model is able to predict the radiolabeled iodide ($^{125}I^-$) uptake-inhibition data in maternal thyroids on PND10 from the AFRL/HEST "acute" (iv) studies with perchlorate. The inhibition was described well by the model across the range of time points from 0.5 to 24 hours postdosing. The top line indicates the prediction for the control thyroid, and the bottom line shows the effect of perchlorate. The model is able to describe the kinetics of iodide under both conditions.

The Clewell (2001b) model is also able to predict the radiolabeled iodide uptake inhibition data from AFRL/HEST obtained after "chronic" drinking water exposures. Figure 6-45 shows the radiolabeled iodide ($^{125}I^-$) concentrations (mg/L) in the maternal thyroids at PND5 after 23 days of dosing with perchlorate at 0.0, 0.01, 1.0, and 10.0 mg/kg-day.

6.4.4 Summary

Clewell (2001b) highlights some important differences in the lactating dam and neonatal rat model structure that were necessary in order to adequately describe the distribution kinetics of perchlorate and iodide. The loss of iodide and perchlorate in the milk results in much faster clearance rates of the anions from the dam. Studies also suggest that the loss of iodide to the mammary gland and milk decreases the iodide available for the maternal thyroid (Brown-Grant, 1961; Yu, 2000; Yu et al., 2000). The thyroidal maximum capacities are lower in the lactating and pregnant dam than in the male rat. Model parameterization in the male rat indicated the need for Vmaxc values for uptake into the follicle of the thyroid of 2.2×10^3 L/hr-kg for perchlorate and 5.5×10^4 L/hr-kg for iodide while the gestation model required values of 1.5×10^3 L/hr-kg

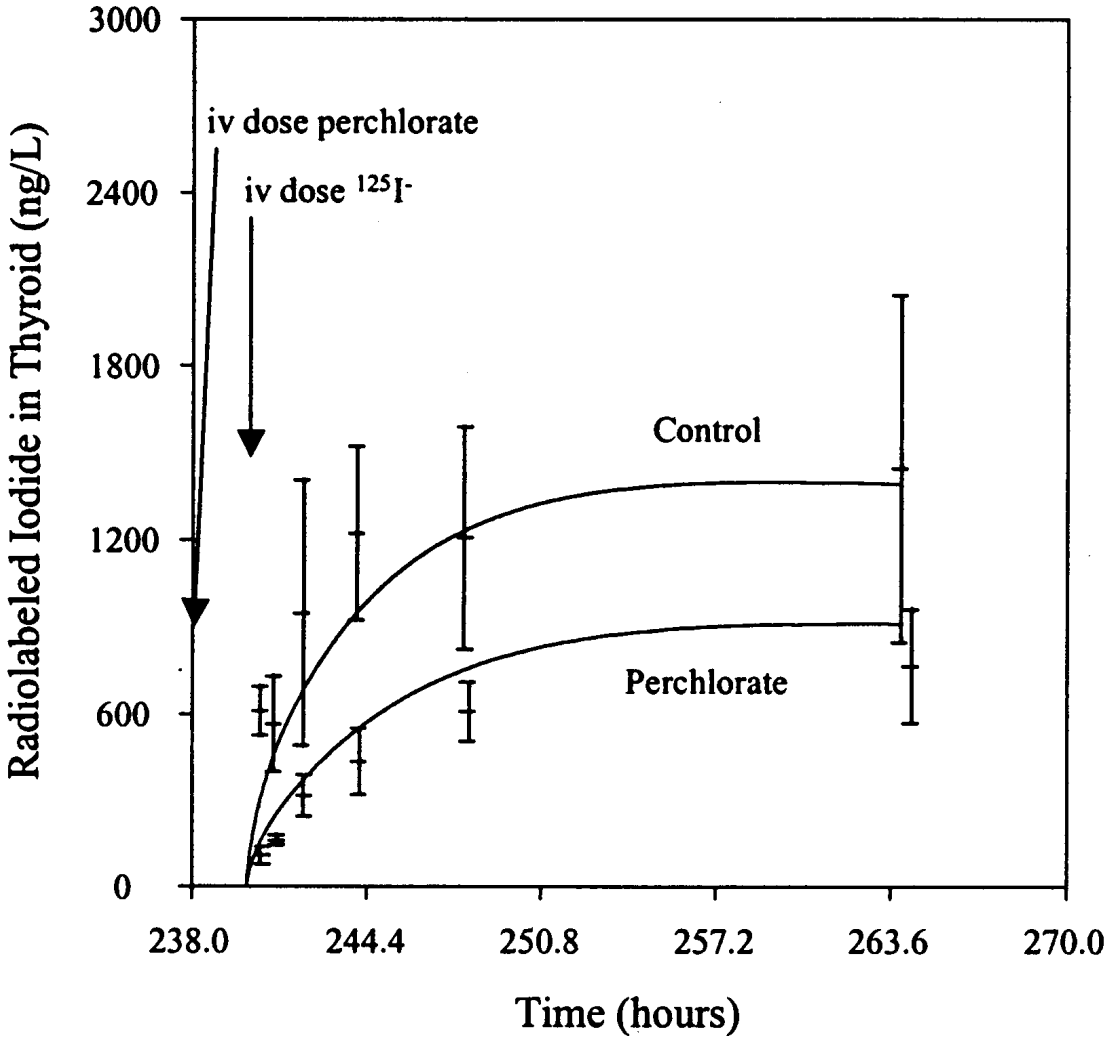

Figure 6-44. Validation for lactating dam and neonatal rat PBPK model (Clewell, 2001b). Model predictions (lines) versus data time course (mean ± SD) of $^{125}I^-$ radiolabeled iodide in the maternal thyroid with and without an iv dose of perchlorate at 1.0 mg/kg perchlorate 2 hours prior to an iv dose of 2.10 ng/kg $^{125}I^-$ 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).

and 4.0×10^4 L/hr-kg for the same parameters. This difference is supported in the literature. Versloot et al. (1997) suggest that the pregnant rat may have a lowered reserve of iodide in the thyroid toward the end of pregnancy, causing increased activity in the thyroid. This may also be true in the lactating rat. The skin of the lactating dam also required a smaller value for Vmaxc

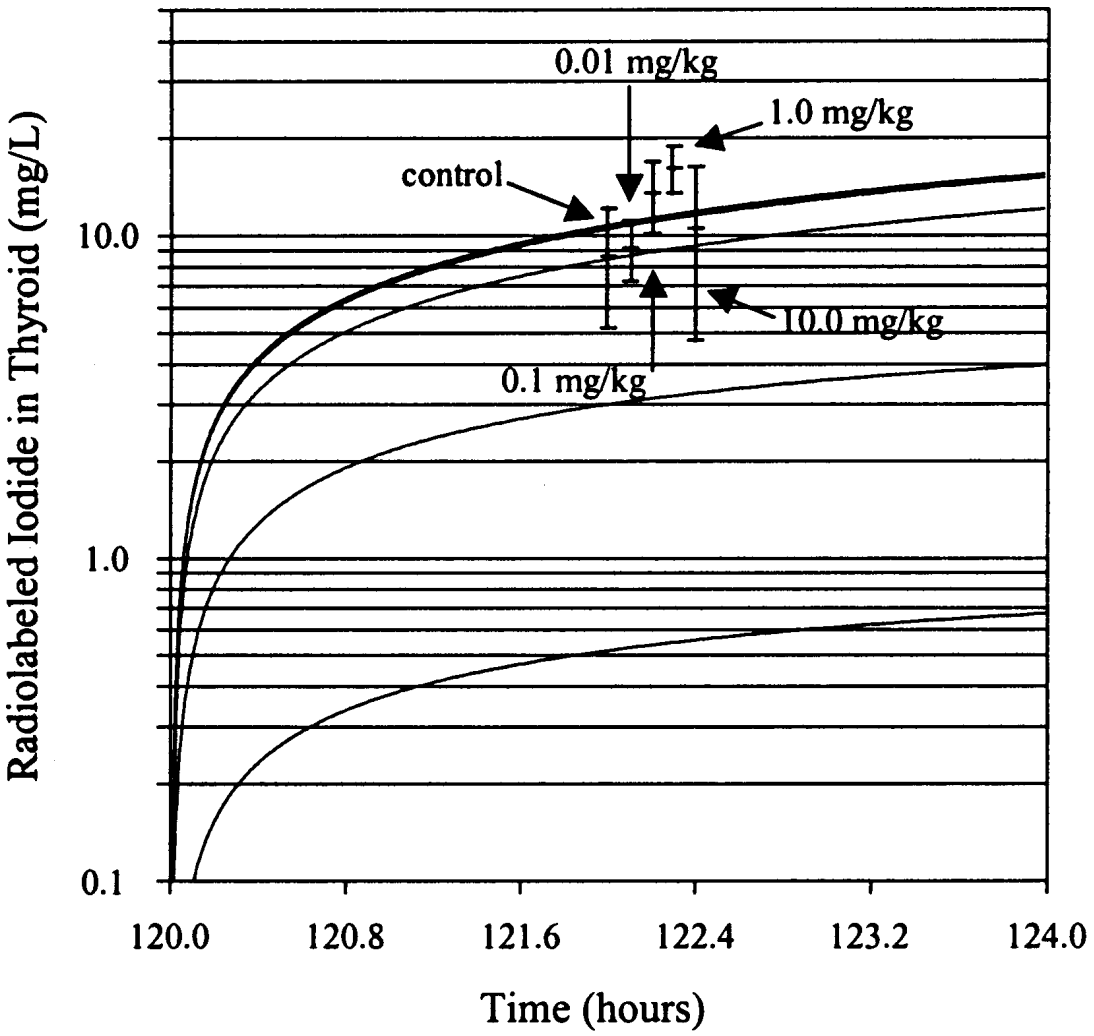

Figure 6-45. Validation for lactating dam and neonatal rat PBPK model (Clewell, 2001b). Model predictions (lines) versus data time course (mean ± SD) of $^{125}I^-$ radiolabeled iodide in the maternal thyroid on PND5 after 23 days dosing with perchlorate in drinking water at 0.0, 0.1, 1.0, and 10.0 mg/kg-day. All experimental data were taken two hours post-dosing. Data of Yu et al. (2000).

than the male rat. This is supported by the work of Brown-Grant and Pethes (1959), who reported higher levels of iodide in the skin of male rats than in female rats. Skin, therefore, appears to be a more important iodide reserve in the male rat than the female.

The described PBPK lactation model is able to predict the distribution of perchlorate in the tissues of active uptake and serum of the lactating dam and neonate on PND5 and PND10 after exposure to perchlorate in drinking water. Perchlorate distribution in this dynamic system is

described utilizing a pharmacokinetic approach to the modeling and accounting mathematically or physiological changes, such as changing tissue volumes and maternal and neonatal growth. The model predicts the transfer of perchlorate to the neonate and is also able to describe the uptake into tissues of interest in the neonate, such as the GI contents and skin; however, the EPA believes that both the maternal and neonatal serum fits could be improved. This may already be accomplished with the additional data to which Clewell (2001b) alludes or, as noted previously, the radionuclide modeling efforts of the ICRP (2001, 1989) may be informative.

The kinetic behavior of iodide is well described with the existing model, in spite of the physiological complexity of the described system. The dam and neonate were accurately simulated at a range of doses that spans four orders of magnitude (2.10 to 33,000 ng/kg) between days 1 and 18 of lactation. The active sequestration of iodide in maternal and neonatal tissues and the transfer of iodide between mother and neonate was described kinetically with the model; data have been simulated at a variety of doses and at various time points up to 14 days after exposure. The fact that the model was able to simulate data from other laboratories under a variety of different conditions attests to the validity of the model structure and its applicability to other studies. This also provides greater confidence in the model structure.

The clear differences between the perchlorate data from iv and drinking water studies draw attention to unresolved issues in the transfer kinetics of perchlorate. Although lactational transfer has long been studied, the transport mechanisms of this ion have yet to be elucidated in the literature. A second transporter has been identified in the mammary gland, which actively transports anions against the chemical gradient. However, the relationship of this transporter and the anion concentration resulting from prolonged exposure to the high doses of perchlorate used in these studies is not known. Clewell (2001b) suggests that it is possible that the high anion load resulting from the long-term exposure to perchlorate may have resulted in decreased transport of the ion. It is feasible that the movement of iodide may be regulated in the mammary tissue, because the ion is vital to the development of the newborn. The data obtained between the acute and drinking water studies suggest that a feedback mechanism is in place, because the model over-predicts the milk transfer in the drinking water data when the acute parameters are used. Clewell (2001b) notes in-house experiments that may help resolve these issues are currently underway. Additional data were provided by Yu (2002), but is not clear that all these 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

concentrations, were used based on the assumption that these dose metrics would represent an averaging of the serum and thyroid perchlorate concentrations and would be better correlated with the inhibition effect on iodide uptake. The correlation was shown to be good between the AUC and the degree of inhibition (see Section 6.5.2). Further, due to the rapid phase of distribution after an iv dose, measurements of concentrations are very difficult to attain experimentally and are more variable. Using simulated peak concentrations after iv injections is potentially problematic due to the inexact modeling of the actual distribution of dose in the tail-vein volume and the exact time of mixing in the whole blood compartment (Merrill, 2001e). It was also observed by EPA that the ratios for peak perchlorate serum values (Merrill, 2001e; Table 6) were in good agreement with those for the perchlorate serum AUC and that the serum AUC were slightly more conservative if really different at all at the lower doses of concern to the risk assessment.

The perchlorate AUC concentration in the thyroid was also considered, but the EPA and AFRL/HEST agreed that this was a less satisfactory dose metric based on a number of considerations. These included the following: that the thyroid Vmaxc estimates had to be adjusted to account for upregulation of the NIS, but that this adjustment was more an empirical exercise than a true biological model (since the hormone changes discussed above regulate the NIS); that the thyroid concentrations were not actually measured in the fetus and neonate so that verification of the parameters was not possible; and that the effects of perchlorate are related to its effects on the NIS and secondary impact on thyroid hormone economy rather than to the concentrations in the gland itself. Results of a sensitivity analysis on the adult male rat model structure supported these conclusions (Merrill, 2001e). The results of the sensitivity analysis will be discussed in Section 6.5.1. Thus, the models were exercised to develop human equivalent exposure (HEE) estimates based on internal perchlorate concentration and iodide uptake inhibition, both components of exposure in the proposed EPA model (Merrill, 2001e). The purpose of Section 6.5.2 is to describe the modeling exercises underlying the HEE estimates that are used in Chapter 7.

6.5.1 Sensitivity Analysis of Proposed Adult Male Rat Model

A sensitivity analysis was performed on the adult male rat model of Merrill (2001c) in order to determine which parameters had the most significant impact on serum and thyroid AUC

perchlorate concentrations. All chemical specific kinetic parameters were increased individually by 1% from the original, optimized values. The model-predicted dosimetrics were recalculated after each change to determine the effect on the AUC estimates. This exercise was performed at the four-hour time point after iv dosing for the 0.1 and 1.0 mg/kg-day doses. The equation describing the calculation of the Sensitivity Coefficient value for each PBPK perchlorate parameter tested is (Merrill, 2001e):

$$\text{Sensitivity Coefficient} = \frac{(A - B)/B}{(C - D)/D}, \qquad (6\text{-}2)$$

where:

A = AUC for either serum or thyroid perchlorate with 1% increased parameter value,
B = AUC for either serum or thyroid perchlorate at initial parameter value,
C = Parameter value increase 1% over initial parameter value, and
D = Original initial starting parameter value.

Results are presented for the physiological parameters and chemical specific parameters separately. Tables 6-9 and 6-10 provide the results for the 0.1 mg/kg-day dose, and Tables 6-11 and 6-12 provide the results for the 1.0 mg/kg-day dose. The sensitivity coefficients for the AUC estimates in both the thyroid and serum are provided and the changes in predicted AUC estimates for the thyroid and serum are presented in the final two columns (Merrill, 2001e).

The sensitivity of serum and thyroid concentrations to model parameters is not linear. At an iv dose level of 1.0 mg/kg, the model prediction of the AUC for serum ClO_4^- concentration is most sensitive to urinary clearance (ClUc_*p*). A one percent increase in this value, from 0.07 to 0.0707 ng/hr-kg, causes a decrease in AUC serum ClO_4^- concentration from 4.69×10^5 to 4.63×10^5 ng, with a sensitivity coefficient of −1.271 (Table 6-12). Serum concentration is next most sensitive to the rate ClO_4^- unbinds from plasma proteins (Clunbc_*p*), with a sensitivity coefficient of -0.869 (Table 6-12).

TABLE 6-9. SENSITIVITY ANALYSIS FOR PHYSIOLOGICAL PARAMETERS IN THE ADULT MALE RAT MODEL AT 0.1 mg/kg PERCHLORATE (ClO_4^-) DOSE (Merrill, 2001e)

Parameter[a]	Original Parameter Value	1% Increase in Parameter Value	AUC Thyroid Sensitivity Coefficient	AUC Serum Sensitivity Coefficient	Increase in AUC Thyroid ClO_4^- (ng)[b]	Increase in AUC Serum ClO_4^- (ng)[c]
BW	3.00E-01	3.03E-01	0.315	0.182	1.88E+06	9.95E+04
Blood Flows (fraction of cardiac output, QCc [L/hr])						
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[d]	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
Tissue Volumes (fraction of body weight)						
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

[a]Parameters as defined in Tables 6-1 and 6-2.
[b]AUC Thyroid Concentration using original parameters = 1.88E+06 ng ClO_4^-.
[c]AUC Serum Concentration using original parameters = 9.94E+04 ng ClO.
[d]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_4^-) DOSE (Merrill, 2001e)

Parameter[a]	Original Parameter Value	1% Increase in Parameter Value	AUC Thyroid Sensitivity Coefficient	AUC Serum Sensitivity Coefficient	Increase in AUC Thyroid ClO_4^- (ng)[b]	Increase in AUC Serum ClO_4^- (ng)[c]
Iodide Tissue/Blood Partition Coefficients						
PS_*p*	3.10E-01	3.13E-01	0.149	0.085	1.88E+06	9.94E+04
PR_*p*	5.60E-01	5.66E-01	0.192	0.111	1.88E+06	9.95E+04
PK_*P*	9.90E-01	1.00E+00	0.192	0.111	1.88E+06	9.95E+04
PL_*p*	5.60E-01	5.66E-01	0.187	0.108	1.88E+06	9.95E+04
PG_*p*	1.80E+00	1.82E+00	0.160	0.088	1.88E+06	9.94E+04
PGJ_*p*	2.30E+00	2.32E+00	0.165	0.090	1.88E+06	9.94E+04
PT_*p*	1.30E-01	1.31E-01	1.184	0.113	1.90E+06	9.95E+04
PDT_*p*	7.00E+00	7.07E+00	0.928	0.114	1.89E+06	9.95E+04
PF_*p*	5.00E-02	5.05E-02	0.197	0.114	1.88E+06	9.95E+04
PSk_*p*	7.00E-01	7.07E-01	11.154	6.024	2.08E+06	1.05E+05
PRBC_*p*	8.00E-01	8.08E-01	11.324	6.112	2.09E+06	1.05E+05
PS_*p*	3.10E-01	3.13E-01	0.149	0.085	1.88E+06	9.94E+04
PR_*p*	5.60E-01	5.66E-01	0.192	0.111	1.88E+06	9.95E+04
PK_*P*	9.90E-01	1.00E+00	0.192	0.111	1.88E+06	9.95E+04
PL_*p*	5.60E-01	5.66E-01	0.187	0.108	1.88E+06	9.95E+04
PG_*p*	1.80E+00	1.82E+00	0.160	0.088	1.88E+06	9.94E+04
PGJ_*p*	2.30E+00	2.32E+00	0.165	0.090	1.88E+06	9.94E+04
PT_*p*	1.30E-01	1.31E-01	1.184	0.113	1.90E+06	9.95E+04
PDT_*p*	7.00E+00	7.07E+00	0.928	0.114	1.89E+06	9.95E+04
PF_*p*	5.00E-02	5.05E-02	0.197	0.114	1.88E+06	9.95E+04
PSk_*p*	7.00E-01	7.07E-01	11.154	6.024	2.08E+06	1.05E+05
PRBC_*p*	8.00E-01	8.08E-01	11.324	6.112	2.09E+06	1.05E+05
Perchlorate Active Uptake Parameters - Vmaxc (ng/hr-kg BW) Km (ng/L)						
Vmaxc_T*p*	2.90E+03	2.93E+03	47.830	6.088	2.77E+06	1.05E+05
Km_T*p*	2.50E+05	2.53E+05	45.154	6.090	2.72E+06	1.05E+05
Vmaxc_DT*p*	1.00E+05	1.01E+05	55.875	6.081	2.92E+06	1.05E+05
Km_DT*p*	1.00E+08	1.01E+08	55.673	6.081	2.92E+06	1.05E+05
Vmaxc_G*p*	1.00E+04	1.01E+04	55.769	6.080	2.92E+06	1.05E+05
Km_G*p*	2.00E+05	2.02E+05	55.774	6.081	2.92E+06	1.05E+05
Vmaxc_S*p*	6.50E+05	6.57E+05	54.713	5.678	2.90E+06	1.05E+05
Km_S*p*	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_4^-) DOSE (Merrill, 2001e)

Parameter[a]	Original Parameter Value	1% Increase in Parameter Value	AUC Thyroid Sensitivity Coefficient	AUC Serum Sensitivity Coefficient	Increase in AUC Thyroid ClO_4^- (ng)[b]	Increase in AUC Serum ClO_4^- (ng)[c]
Perchlorate Plasma Binding Parameters						
Vmaxc_B*p*	9.50E+03	9.60E+03	54.857	6.417	2.90E+06	1.06E+05
km_B*p*	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
Perchlorate Urinary Clearance and Permeability Area Cross Products (L/hr-kg)						
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

[a]Parameters as defined in Tables 6-1 and 6-2.
[b]AUC Thyroid concentration using original parameters = 1.88E+06 ng ClO_4^-.
[c]AUC Serum concentration using original parameters = 9.94E+04 ng ClO_4^-.

The predicted AUC for total thyroid concentration at a dose level of 1.0 mg/kg-day is most sensitive to changes in the maximum capacity of the thyroid colloid (Vmaxc_DTp). A one percent increase in this value from 1.00×10^5 to 1.0110^5 ng/hr-kg results in an increase in AUC thyroid concentration from 9.84×10^6 to 1.04×10^7 ng (Table 6-12). However, the AUC thyroid concentration is almost equally sensitive to other parameters of saturable processes, including Vmaxc, Km, and the permeability area cross product values of other saturable tissues.

With a lower iv dose of 0.1 mg/kg, the blood serum concentration remains sensitive to changes in urinary clearance, but demonstrates increased sensitivity to the parameters of saturable compartments and effective partitioning with skin (PSk_*p*) and red blood cells (PRBC_*p*). Serum concentration is most sensitive to the maximum capacity for plasma binding (Vmaxc_B*p*) at this dose level (Table 6-10).

At the lower dose level of 0.1 mg/kg, thyroid concentrations show a similar sensitivity to 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_4^-) DOSE (Merrill, 2001e)

Parameter[a]	Original Parameter Value	1% Increase in Parameter Value	AUC Thyroid Sensitivity Coefficient	AUC Serum Sensitivity Coefficient	Increase in AUC Thyroid ClO_4^- (ng)[b]	Increase in AUC Serum ClO_4^- (ng)[c]
BW	3.00E-01	3.03E-01	−5.944	−0.534	9.81E+06	4.67E+05
Blood Flows [fraction of cardiac output, QCc (L/hr)]						
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[b]	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
Tissue Volumes (fraction of bodyweight)						
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

[a]Parameters as defined in Tables 6-1 and 6-2.
[b]Original AUC Thyroid concentration = 9.84E+06 ng ClO_4^-.
[c]Original AUC Serum concentration = 4.69E+05 ng ClO_4^-.
[d]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_4^-) DOSE (Merrill, 2001e)

Parameter[a]	Original Parameter Value	1% Increase in Parameter Value	AUC Thyroid Sensitivity Coefficient	AUC Serum Sensitivity Coefficient	Increase in AUC Thyroid ClO_4^- (ng)[b]	Increase in AUC Serum ClO_4^- (ng)[c]
Perchlorate Tissue/Blood Partition Coefficients						
PS_*p*	3.10E-01	3.13E-01	−7.862	−0.728	9.80E+06	4.66E+05
PR_*p*	5.60E-01	5.66E-01	−7.649	−0.688	9.80E+06	4.66E+05
PK_*P*	9.90E-01	1.00E+00	−7.649	−0.688	9.80E+06	4.66E+05
PL_*p*	5.60E-01	5.66E-01	−7.670	−0.692	9.80E+06	4.66E+05
PG_*p*	1.80E+00	1.82E+00	−7.905	−0.714	9.80E+06	4.66E+05
PGJ_*p*	2.30E+00	2.32E+00	−7.883	−0.711	9.80E+06	4.66E+05
PT_*p*	1.30E-01	1.31E-01	12.911	−0.683	9.90E+06	4.66E+05
PDT_*p*	7.00E+00	7.07E+00	7.606	−0.683	9.87E+06	4.66E+05
PF_*p*	5.00E-02	5.05E-02	−7.628	−0.684	9.80E+06	4.66E+05
PSk_*p*	7.00E-01	7.07E-01	−8.885	−0.846	9.80E+06	4.65E+05
PRBC_*p*	8.00E-01	8.08E-01	−7.649	−0.691	9.80E+06	4.66E+05
Perchlorate Active Uptake Parameters - Vmaxc (ng/hr-kg BW), Km (ng/L)						
Vmaxc_T*p*	2.90E+03	2.93E+03	12.890	−0.683	9.90E+06	4.66E+05
Km_T*p*	2.50E+05	2.53E+05	−15.745	−0.682	9.76E+06	4.66E+05
Vmaxc_DT*p*	1.00E+05	1.01E+05	123.554	−0.687	1.04E+07	4.66E+05
Km_DT*p*	1.00E+08	1.01E+08	120.784	−0.687	1.04E+07	4.66E+05
Vmaxc_G*p*	1.00E+04	1.01E+04	122.062	−0.687	1.04E+07	4.66E+05
Km_G*p*	2.00E+05	2.02E+05	122.062	−0.687	1.04E+07	4.66E+05
Vmaxc_S*p*	6.50E+05	6.57E+05	120.997	−0.806	1.04E+07	4.66E+05
Km_S*p*	2.00E+05	2.02E+05	122.914	−0.641	1.04E+07	4.66E+05
Perchlorate Plasma Binding Parameters - Vmaxc (ng/hr-kg BW), Km (ng/L)						
Vmaxc_B*p*	9.50E+03	9.60E+03	122.062	−0.500	1.04E+07	4.67E+05
km_B*p*	1.10E+04	1.11E+04	122.062	−0.694	1.04E+07	4.66E+05
Kunbc_*p*	1.00E-01	1.01E-01	122.275	−0.869	1.04E+07	4.65E+05
Perchlorate Urinary Clearance and Permeability Area Cross Products (L/hr-kg)						
ClUc_*p*	7.00E-02	7.07E-02	115.031	−1.271	1.04E+07	4.63E+05
PAGc_*p*	8.00E-01	8.08E-01	122.275	−0.685	1.04E+07	4.66E+05
PAGJc_*p*	8.00E-01	8.08E-01	122.275	−0.686	1.04E+07	4.66E+05
PATc_*p*	5.00E-05	5.05E-05	100.969	−0.686	1.03E+07	4.66E+05
PADTc_*p*	1.00E-02	1.01E-02	120.784	−0.687	1.04E+07	4.66E+05
PASKc_*p*	4.00E-01	4.04E-01	123.341	−0.567	1.04E+07	4.67E+05
PARBCc_*p*	1.00E-01	1.01E-01	122.062	−0.687	1.04E+07	4.66E+05

[a]Parameters as defined in Tables 6-1 and 6-2.
[b]Original AUC Thyroid concentration = 9.84E+06 ng ClO_4^-.
[c]Original AUC Serum concentration = 4.69E+05 ng ClO_4^-.

and urinary clearance. However, the predicted thyroid concentrations at both dose levels (1.0 and 0.1 mg/kg) are most sensitive to a change in Vmax_DT*p*. The Vmax values of the thyroid were established by empirically fitting thyroid radioiodide and perchlorate uptake from several data sets ranging in three orders of magnitude

6.5.2 Derivation of Human Equivalent Exposure Estimates

As discussed, the following internal dosimetrics were chosen to represent output from each of the PBPK models: area under the curve (AUC) perchlorate concentrations in serum and thyroid; peak serum and thyroid perchlorate concentrations; the total amount of perchlorate excreted in the urine; the AUC for the lactational and placental transfer of perchlorate; and the percent inhibition of iodide uptake into the thyroid. In order to explore the dose-response elationship of these values, the target dosimetrics were evaluated across several doses in both acute and sub-chronic exposure scenarios using previously developed PBPK models at the AFRL/HEST; i.e., the models for the adult male rat (Merrill, 2001c) and human (Merrill, 2001d) described in Section 6.2, the pregnant and fetal rat model (Clewell, 2001a) and the lactating and neonatal rat model (Clewell, 2001b).

Acute (iv) pharmacokinetic studies in the adult male rat were used as the basis for this dose-response analysis because iodide uptake inhibition could be correlated to perchlorate levels. Further, as discussed in Section 6.1, the initial inhibition of iodide is viewed in the conceptual model as the important step in the transient phase (Figure 6-2). Transient decrements in T4 can result in permanent neurodevelopmental sequelae. In drinking water studies, upregulation of NIS in the rat is so rapid that it resulted in no measurable thyroid iodide inhibition, so the iv doses were used to estimate this initial insult. The target internal dosimetrics were first calculated in each of the rat models for acute exposure to perchlorate (single *iv* administration) at doses of 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 to data-validated inhibition, the 2 to 4 hr time-frame was used for all acute calculations. The AUC for thyroid and serum were calculated by integrating predicted tissue concentrations from 2 to 4 hrs post dosing.

These same dosimetrics calculated for acute exposures were also determined for subchronic (drinking water) perchlorate exposures at doses of 0.01, 0.1, 1.0, 3.0, 5.0, 10.0, 30.0, and 100.0 mg/kg-day. In order to achieve steady state concentrations, the models were run until the

predicted peak and trough heights did not change from one day to the next (Merrill, 2001e). Serum and thyroid perchlorate AUC concentrations were then determined over a 24 hr period (240-264 hrs in male, lactating, and neonatal rats; 480-504 hrs in pregnant and fetal rats). Although the tissues reach steady state perchlorate concentrations within one week, the above time-points were chosen in the lactation and gestation models for their ability to be verified with data (Clewell, 2001a,b). The male rat model was run at the same time as lactation for the sake of consistency with the other models. The total perchlorate AUC in the serum and thyroid were determined from each the models at 240 and 264 hrs (or 480 and 504 hrs). The difference in the two values was then divided by 24 hrs to give the AUC in units of ng/L-hr.

The AFRL/HEST experiments (Yu, 2000; Yu et al., 2000) have shown upregulation of the NIS to be both time and dose-dependent. Thus, at lower doses, the rat thyroid was completely upregulated after only a few days of drinking water exposure. Iodide uptake in the thyroid at higher perchlorate doses ($\geq$10 mg/kg-day) was completely restored by the 18th day of exposure, the time of data collection in the pregnant and fetal rats (Clewell, 2001).

Drinking water studies in the adult male rats showed elevated perchlorate uptake in the thyroid at drinking water doses of 3.0 mg/kg-day and higher (Yu et al., 2000; Merrill et al., 2001c). Increased perchlorate uptake also results from upregulation of NIS. Since perchlorate is transferred into the thyroid via NIS, the inhibiting anion is "upregulated" along with iodide. In order to simulate increased perchlorate concentrations in thyroids of the 3.0, 10.0, and 30.0 mg/kg-day dose groups, the original value for follicular Vmaxc (Vmaxc_T*p*) was adjusted to obtain the best fit of the model simulation to experimental data (Table 6-13). Since there were no pharmacokinetic data available for the 5.0 and 100.0 mg/kg-day dose groups, values for Vmaxc_T*p* were estimated from a Michaelis-Menten fit to the adjusted Vmaxc's at 3.0, 10.0, and 30.0 mg/kg-day doses (Figure 6-46). Target dosimetrics in the male rat were calculated for both originally optimized parameters and these adjusted ("upregulated") parameters.

This process of adjusting ("upregulating") the Vmaxc_T*p* values was not necessary in the gestation, lactation, or human models, as they were able to successfully describe perchlorate concentrations in serum and thyroid at all measured doses (0.01 – 10.0 mg/kg-day in gestation and lactation; 0.02 – 12 mg/kg-day in human) using one set of model parameters (Clewell, 2001a,b; Merrill, 2000). Merrill (2001e) posits that it was not necessary because it is likely that a 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_T*p*[a] AFTER PERCHLORATE DRINKING WATER EXPOSURE IN THE ADULT MALE RAT MODEL (Merrill, 2001e)

Drinking Water Dose (mg/kg-day)	Adjusted Vmaxc_T*p* (ng/hr-kg)
0.01	2900
0.1	2900
1	2900
3	9000
5	17500[b]
10	32000
30	55000
100	79000[b]

[a]Maximum velocity capacity of active transport in the thyroid follicle.
[b]Data not available for these dose levels.

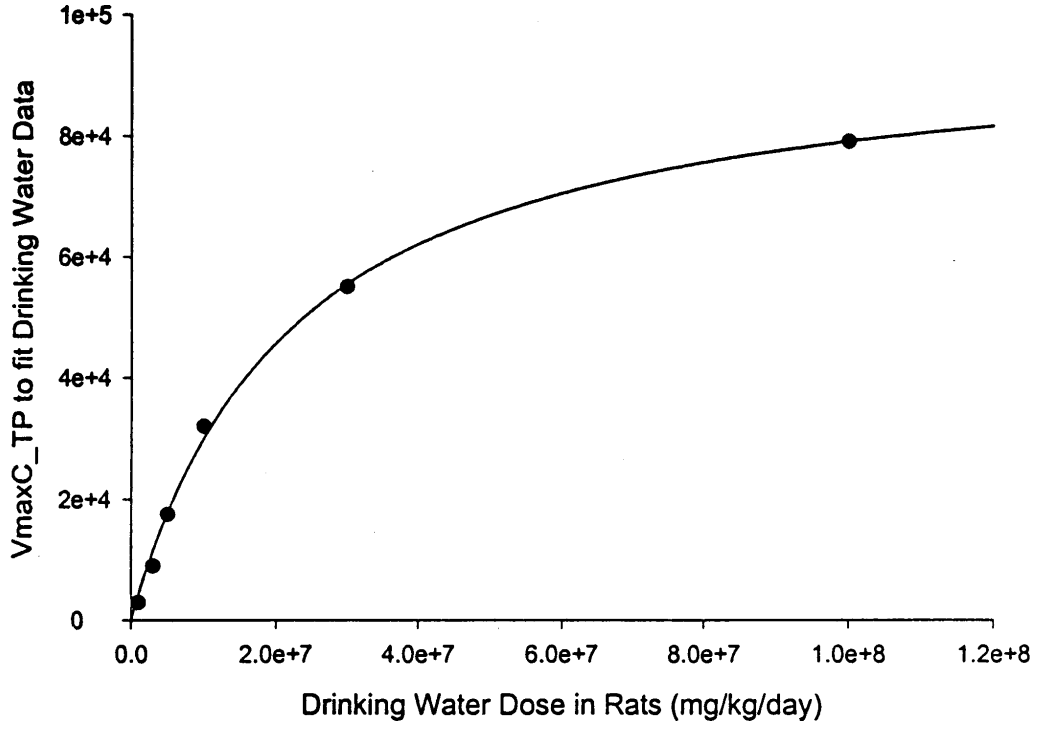

Figure 6-46. Upregulation of maximal capacity (ng/kg-hr) of active transport into the thyroid follicle for perchlorate (Vmaxc_T*p*) optimized by fitting to drinking water data in the rat. Upregulation is first noted in the 3.0 mg/kg-day dose group.

thyroidal up-regulation. As a result, the effect of perchlorate on the thyroid was less dramatic than in the male rat where a completely naïve system is perturbed by an inhibiting chemical. Thus, the PBPK models for gestation and lactation were able to describe thyroid perchlorate levels at drinking water doses from 0.01 to 10.0 mg/kg-day without adjusting the follicular Vmaxc (Vmaxc_T*p*) values with dose.

Increased follicular Vmaxc values were not needed to fit the human data likely due to the larger size of the human thyroid colloid versus that of the rat and to the differences in plasma protein binding discussed in Chapter 3.

The human PBPK model (Merrill, 2001d) was used to calculate all target dose metrics in both acute and two-week drinking water perchlorate exposures in a 70 kg adult at doses of 0.01, 0.1, 1.0, 3.0, 5.0, 10.0, 30.0, and 100.0 mg/kg-day. Acute serum and thyroid perchlorate AUC concentration estimates were calculated with the model over an eight hr time period (from 24 to 32 hrs post-exposure) in order to correlate perchlorate parameters to data-validated iodide inhibition. For two-week drinking water exposures, the thyroid and serum perchlorate AUC concentration estimates were calculated over a 24 hr period after serum and thyroid concentrations reached steady state. The 240 to 264 hr time period was chosen for consistency with the male rat model (Merrill, 2001c).

The adult human model (Merrill, 2001d) was also used to predict dosimetry in a 15 kg child. The same dosimetrics were run in the model for the child and adult. However, since an average child drinks less water than an adult (approximately 1 L/d as opposed to 2 L/d in the adult), the actual exposures of a child and adult from the same water source would be different. For example, a 15kg child consuming 1 L of contaminated water would receive a daily dose (per kg bodyweight) that was 2.3 times that of a 70 kg adult consuming 2 L of water. Table 2 shows the concentration of the drinking water required to deliver the same dose to a 15 kg child and a 70 kg adult. For the purpose of this paper, dosimetric comparisons were calculated using the same dose (mg/kg-day) in the adult and child.

Figure 6-47 shows the curve generated from plotting the experimentally-determined percent inhibition versus the corresponding PBPK-derived serum (A) and thyroid (B) perchlorate AUC concentration estimates after acute (iv) exposure in rats. Thyroidal radiolabeled iodide ($^{125}I^-$) uptake measurements were taken two hours after iv administration of perchlorate. The solid line

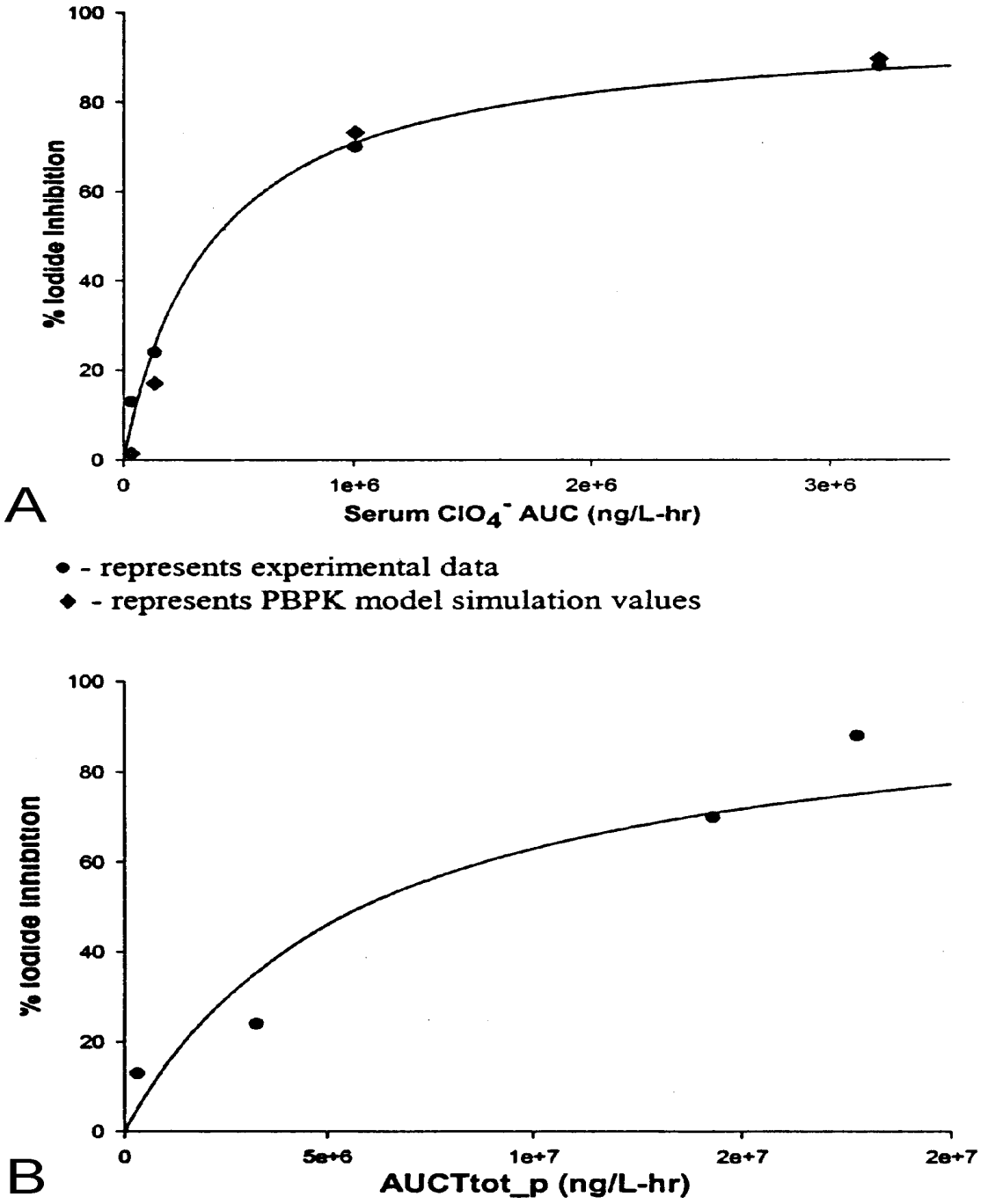

Figure 6-47. Michaelis-Menten fit of the "acute" male rat area under the curve (AUC) for serum (A) and thyroidal perchlorate (AUCTtot_*p*) in ng/L-hr. Model predictions and actual data shown for percent radiolabeled iodide uptake inhibition after iv injection of perchlorate.

represents a fit (not a PBPK model simulation) using the Michaelis-Menten type equation given below:

$$Y = (A \times AUC_{dose})/(AUC_{dose} + B) \quad (6\text{-}3)$$

Where 'Y' represents the predicted percent inhibition of radioiodide uptake, 'A' represents the maximal percent inhibition of radioiodide uptake, 'B' is related to the affinity of iodide uptake based on serum concentration, and AUC_{dose} represents the AUC at each specific dose of perchlorate. The above equation was also used to derive the dose-response relationship in subsequent figures. The correlation coefficient (r^2) greater than 0.91 in all cases indicated excellent fit for all (see Merrill, 2001e; Table 3).

Figure 6-48 shows the PBPK-derived AUC perchlorate concentration estimates for drinking water exposure to the adult male rat versus the calculated percent inhibition of radioiodide in the serum (A) and thyroid (B). The values for AUC of perchlorate concentration in the serum were determined by running the adult male rat model (Merrill, 2001c) across doses. Corresponding percent inhibitions were calculated by putting serum AUC perchlorate concentration values into the equation from Figure 6-47. Human response (thyroid inhibition) to subchronic exposure is similar to that of an acute exposure in the rat. This approach allows the sub-chronic serum levels in the rat be related to iodide uptake in the native thyroid. The values for AUC of thyroid perchlorate concentration (B) were determined by running the male rat model (Merrill, 2001c) at steady state (between 240 and 264 hours of drinking water exposure) across the doses shown. Corresponding percent inhibitions were calculated by putting thyroid AUC values in the equation from Figure 6-47.

The actual human iodide inhibition data (Greer et al., 2000) were plotted as a function of the perchlorate AUC concentration estimates for serum and thyroid calculated with the PBPK model in Figure 6-49. The measured percent inhibition of radiolabeled iodide uptake in the serum and thyroid on Day 2 of drinking water exposure to perchlorate is shown versus the PBPK-derived estimates for human volunteers (both male and female). Inhibition data from time points earlier than Day 2 of perchlorate in the human drinking water (Greer et al., 2000) and inhibition data from acute perchlorate dosing in humans were not available. Therefore, the inhibition measurements on Day 2 of perchlorate drinking water exposure were the closest-

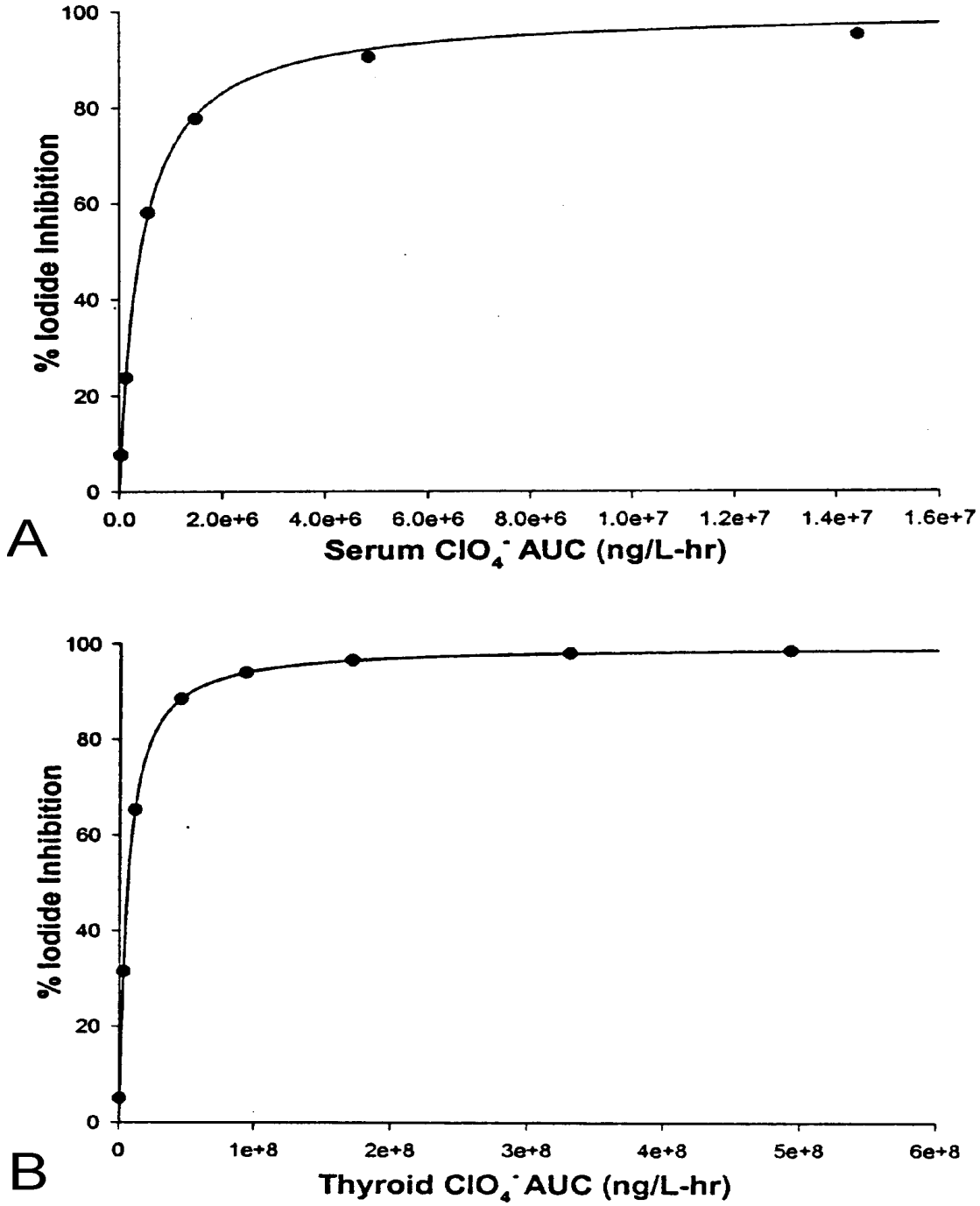

Figure 6-48. Michaelis-Menten fit of the "chronic" male rat area under the curve (AUC) for serum (A) and thyroidal (B) perchlorate (ng/L-hr). Model predictions and actual data shown for percent radiolabeled iodide uptake inhibition after drinking water exposure of perchlorate. Fit for serum calculated percent inhibition of radioiodide uptake calculated from equation used in Figure 6-47 (A) and for thyroid from Figure 6-47(B).

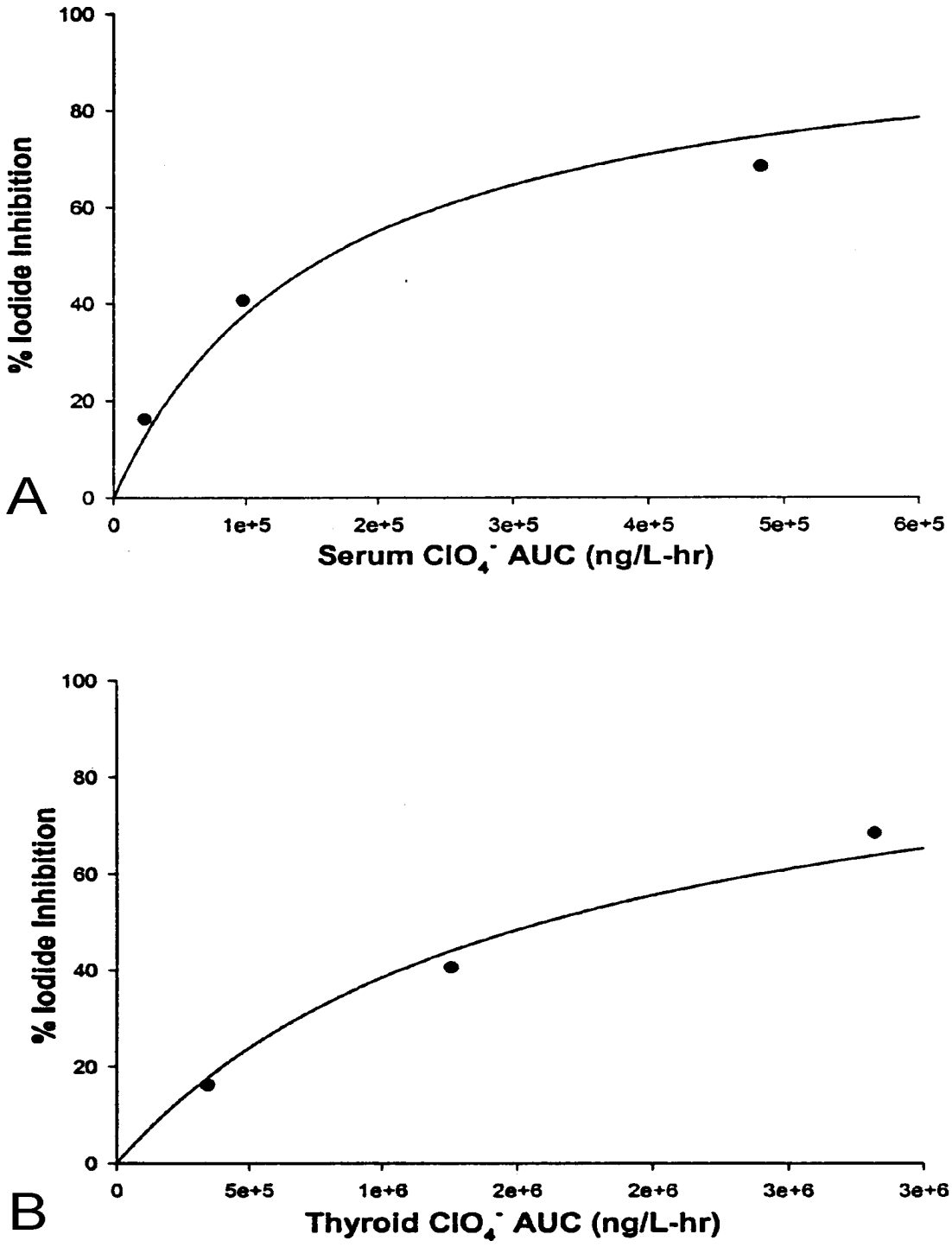

Figure 6-49. Michaelis-Menten fit of the human area under the curve (AUC) for serum (A) and thyroidal (B) perchlorate (ng/L-hr) on exposure Day 2. Model predictions and actual data shown for percent radiolabeled iodide uptake inhibition after drinking water exposure of perchlorate.

available representation of an acute human dose. Measured serum TSH and thyroid hormones indicated that thyroids were in normal homoeostatic state in human volunteers during the entire two week study (Merrill, 2001d).

The HEE estimates were calculated using the models as described in Section 6.1 (Figure 6-4). The HEE that would result in the same perchlorate AUC concentration estimates for serum (A) and thyroid (B) in the human and rat and the corresponding percent inhibition of iodide uptake is presented in Figure 6-50. Values for percent inhibition were determined from the rat serum AUC during drinking water exposures to perchlorate using the Michaelis-Menten equations from Figure 6-47. The correlation coefficient for both the serum and thyroid AUC versus percent iodide uptake inhibition relationship was 0.99.

6.5.3 Summary

The correlation coefficients for the dose-response relationships using the PBPK-model generated HEE estimates between serum and thyroid perchlorate AUC concentration versus iodide inhibition indicated good fits. Tables of the actual estimates and their ratios can be found in Merrill (2001e).

The rat serum ratios (AUC and peak concentrations) change significantly between 0.1 and 3.0 mg/kg-day due to binding of perchlorate by plasma proteins. Plasma binding is saturated at doses greater than 1.0 mg/kg-day. Male rat to human ratios are notably lower than those ratios between rats, as plasma binding of perchlorate occurs to a much lesser extent in humans.

HEE estimates were calculated for both a 15 and 70 kg human. The differences between the 15 and 70 kg human HEE estimates were never greater than 75%, indicating that body weight doesn't significantly affect the target dose metrics. Interestingly, the HEE estimates were greater in the 15 kg child. One might expect the adult and child HEE estimates to be nearly equal, given no parameters were changed in the human model except body weight. However, physiological parameters within the model are linearly scaled by body weight; whereas, chemical-specific parameters are scaled nonlinearly (e.g., as a multiple of body weight to a power of ¾). As indicated later in the sensitivity analysis, the internal dose metrics presented are more sensitive to chemical-specific parameters, especially those describing saturable kinetics. Therefore, the chemical-specific parameter values for the 15 kg child are proportionally greater

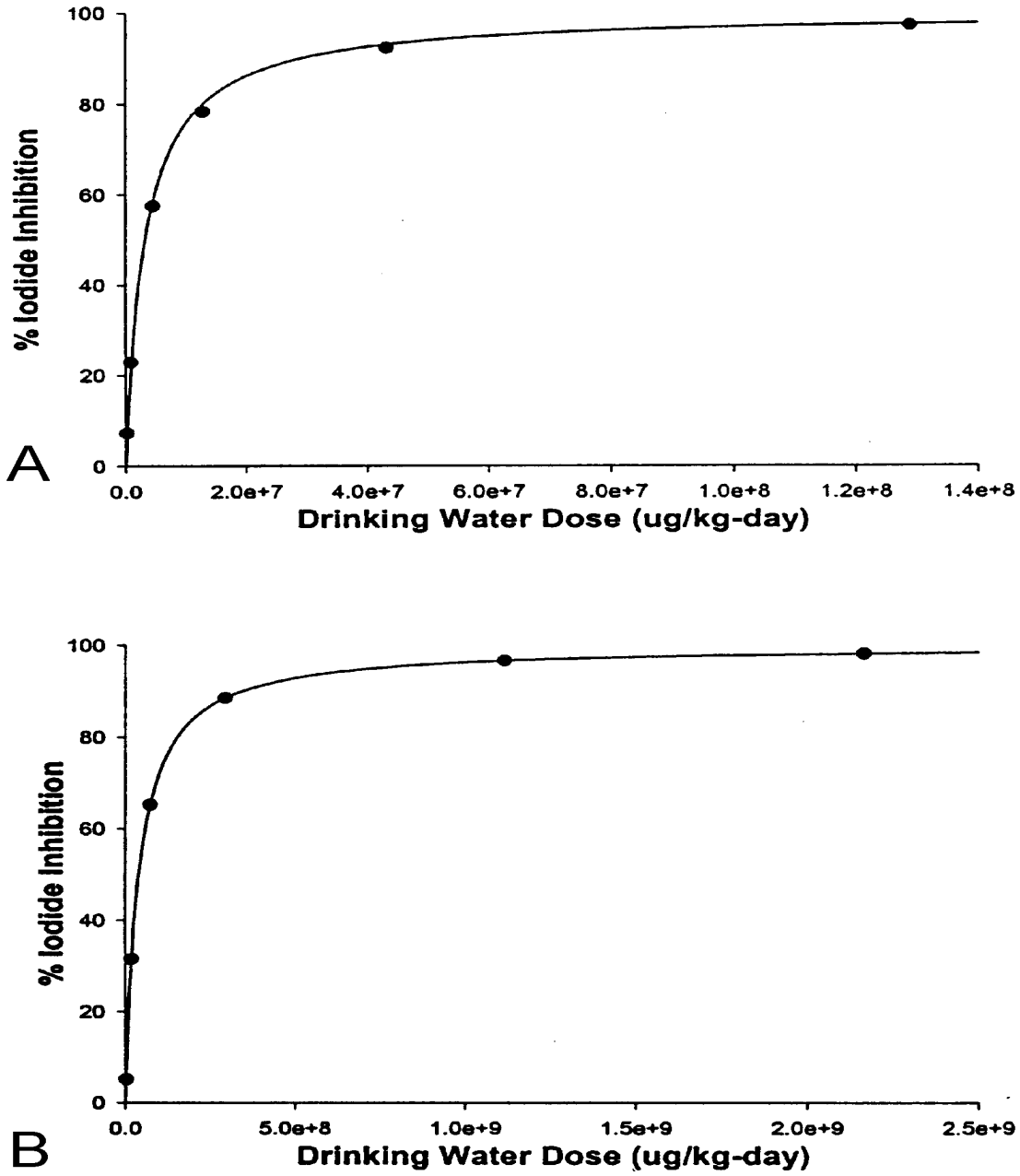

Figure 6-50. Michaelis-Menten fit of the human equivalent exposure (HEE) of perchlorate in drinking water derived from the area under the curve (AUC) for serum (A) or thyroid (B) versus percent predicted inhibition in the rat after an "acute" (iv) dose.

(in terms of body weight) than those of the adult. As a result, a slightly higher dose is required to saturate these tissues in a child.

When comparing the dose metrics for serum versus thyroid, the HEE estimates calculated from the thyroid were less than the HEE estimates calculated from the serum by a factor of 100 at a 0.01 mg/kg-day dose level. This difference became a factor of 10 starting at the 5.0 mg/kg-day concentration for the 15 kg child and at 10.0 mg/kg-day for the adult.

These considerations will be explored in Chapter 7 to develop dosimetric adjustment factors for the observed effect levels.

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^+)-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

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

Thyroid hormone deficiencies, such as those induced by perchlorate, can affect normal metabolism, growth, and development. However, no robust data existed previously with which to evaluate potential target tissues or effects other than those in the thyroid. The data on the thyroid effects were also insufficient for quantitative dose-response assessment. Additionally, there were no data with which to evaluate the effects of perchlorate in potentially susceptible populations, such as developing fetuses; nor were there data on the effects of perchlorate on the reproductive capacity of male or female laboratory animals.

Benign tumors had been reported in the thyroids of male Wistar rats and female BALB/c mice treated with repeated, high-dose exposures (2 years at 1,339 mg/kg-day and 46 weeks at 2,147 mg/kg-day, respectively) of potassium perchlorate in drinking water, establishing perchlorate as a carcinogen. Benign tumors in the thyroid have been established to be the result of a series of progressive changes that occur in the thyroid in response to interference with thyroid-pituitary homeostasis (i.e., perturbation of the normal stable state of the hormones and functions shared between these two related glands). This progression is similar regardless of the cause of the thyroid hormone interference (Hill et al., 1989; Capen, 1997; Hurley et al., 1998). EPA has adopted the policy that for the dose-response of chemicals that cause disruption in the thyroid but that do not have genotoxic activity (i.e., cause damage to DNA or show other genetic disruption) a threshold for carcinogenicity is to be based on precursor lesions (U.S. Environmental Protection Agency, 1998e).

In the case of perchlorate, an overall model based on its mode of action has been developed as shown in Figure 7-1. The model supports iodide inhibition as the key event that precedes the hormone and thyroid changes with subsequent neurodevelopmental and neoplastic sequelae. Focusing on the key event of iodide uptake inhibition allows a harmonized approach to both the "noncancer" and "cancer" toxicity that occurs downstream along the continuum. Thus, one harmonized risk estimate is derived for both sequelae based on their common mode of action.

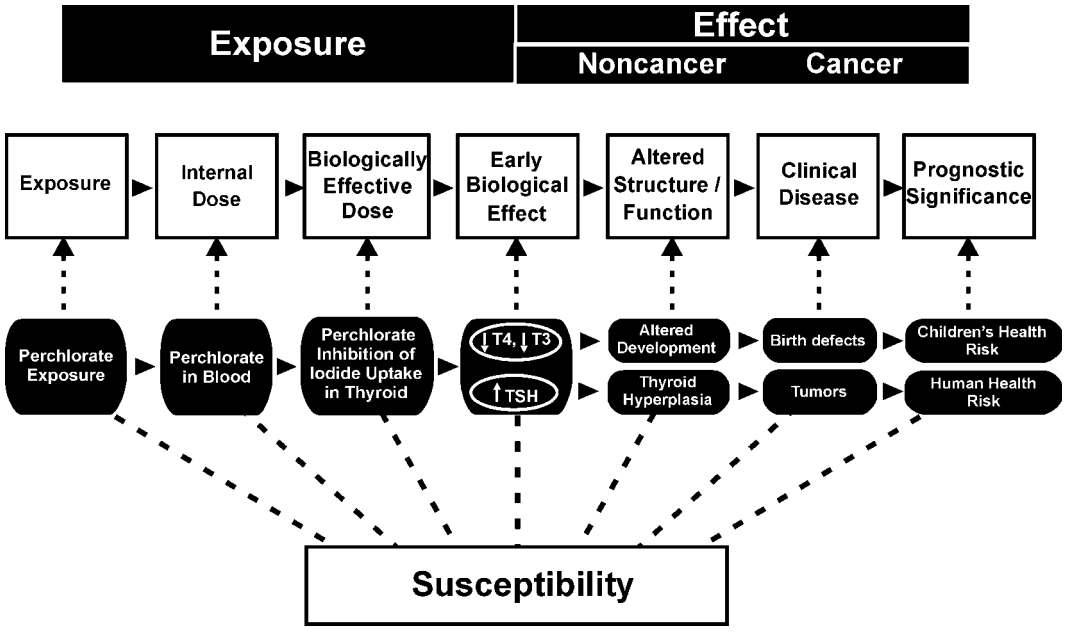

Figure 7-1. Mode-of-action model for perchlorate toxicity proposed by the U.S. EPA (U.S. Environmental Protection Agency, 1998d). Schematic shows the exposure-dose-response continuum considered in the context of biomarkers (classified as measures of exposure, effect, and susceptibility) and level of 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.

This chapter presents the synthesis of the most relevant data for deriving a revised quantitative assessment of human health risk for perchlorate. The new data were consistent with the limited historical characterization and the 1998 EPA assessment in that the anti-thyroid

effects remain the focus of concern and the key event of its mode of action remained identified as the inhibition of iodide uptake at the NIS. However, data from the testing strategy allowed a more comprehensive evaluation of the possible sequelae of the iodide uptake inhibition and its thyroid-pituitary axis perturbations with respect to other endpoints, notably effects in dams and their offspring and on nerurodevelopmental, reproductive, and immunotoxicity parameters.

The key event is defined as an empirically observable precursor step that is a necessary element of the mode of action or is a marker for such an element. This will be discussed in Section 7.1.1. Section 7.1.2 discusses dosimetric adjustment of effect levels observed in the laboratory animals to human equivalent exposures (HEE). Choice of the point of departure for the assessment based on a quantitative consideration of the key event, observed effects, and weight of the evidence is discussed in Section 7.1.3. Application of factors to account for uncertainty and variability in the extrapolations required to use the data is discussed in Section 7.1.4. The overall operational derivation is presented in Section 7.1.5, and the assignment of confidence levels is discussed in Section 7.1.6. Section 7.1.5 also presents a discussion of the cancer assessment in the context of the RfD. Section 7.2 discusses the inhalation reference concentration. Susceptible population considerations are discussed in Section 7.1.5.3. Section 7.3 presents a brief summary of the findings.

7.1.1 Key Events and Weight of the Evidence

Results of the testing strategy have established that the critical target tissue for perchlorate is the thyroid gland, with some remaining concern for adequate characterization of its potential for immunotoxicity, notably contact hypersensitivity. Changes in thyroid weights, three response indices of thyroid histopathology (colloid depletion, hypertrophy and hyperplasia), and thyroid and pituitary hormones were consistently altered across the array of experimental designs represented by the data base. The developmental and reproductive NOAEL and LOAEL values were higher than those associated with thyroid toxicity per se.

Figure 7-2 highlights the temporal considerations that have to be superimposed on evaluation of the data from the various studies in laboratory animals and humans in order to characterize the anti-thyroid effects from perchlorate exposure. Conceptually, competitive inhibition of iodide uptake at the NIS by perchlorate is the key event leading to both potential neurodevelopmental and neoplastic sequelae. The decrement in iodide uptake leads to

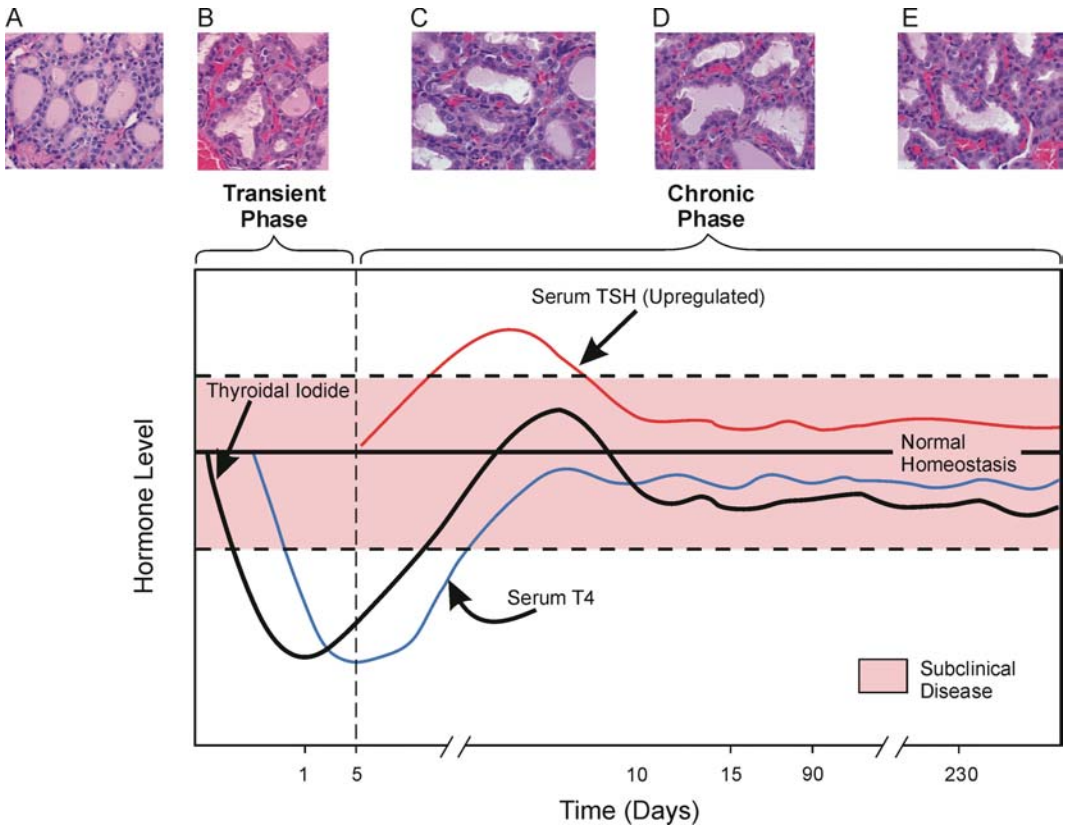

Figure 7-2. Schematic of thyroid and pituitary hormone levels with associated pathology after acute versus chronic dosing with perchlorate. The transient phase is represented by decreases in thyroidal iodide due to the inhibition by perchlorate at the NIS with subsequent drop in T4. The transient drops 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.

subsequent drops in T4 (and T3) that can lead to permanent neurodevelopmental deficits. Corroborating evidence for this likely outcome given the mode of action of perchlorate comes from the iodide deficiency literature and recent studies showing that maternal hypothyroxinemia (i.e., decrements in T4 with or without concomitant increases in TSH) is linked to poor developmental, neuropsychological and cognitive outcomes (Haddow, et al., 1999; Pop et al.,

1999; Morreale de Escobar, et al., 2000). It should be noted that medical concern for hypothyroxinemia remains in the "chronic phase"; i.e., once TSH upregulates to attempt to regulate the hypothalamic-pituitary-thyroid feedback system back to an apparent homeostasis, because this stress on the system essentially represents a "subclinical" disease state. Indeed, adverse outcome in women with hypothyroxinemia per se has been demonstrated because adversity includes the inability of an organism to respond to additional stressors. The system in this case, particularly when considered on a population level, would present a diminished capacity to compensate for other anti-thyroid insults. Since a large percentage of women are believed to already be hypothyroid, the importance of this effect to women in general, pregnant women, and fetuses on a population level can not be discounted. Weiss (2000) has noted that even if the magnitude of effect may be relatively small for most environmental levels, such neurotoxicity is extremely significant for public health.

Of notable concern, as previously discussed in Chapter 3, is that the developing fetus is dependent on the mother for its T4 and T3 through parturition, as illustrated in Figure 7-3 for humans with a similar pattern in rats. During the period illustrated in Figure 7-3, a number of critical stages in neural development take place, some of which depend on thyroid hormones. The cell precursors of the brain and spinal cord which compose the central nervous system (CNS) begin to develop early in embryogenesis through the process called neurulation. Beginning early in the second week of gestation in rodents (GD9.5 in rats) and the first month of gestation in humans, specific areas of the CNS begin to form with the neurogenesis and migration of cells in the forebrain, midbrain, and hindbrain. This sequence of developmental processes includes proliferation, migration, differentiation, synaptogenesis, apoptosis, and myelination (Rice and Barone, 2000). As discussed in Chapter 3, thyroid hormones play a role throughout this process, regulating proliferation, migration, and differentiation. Alterations in these processes can result in abnormalities of the brain and developmental delays.

The upregulation in TSH in the "chronic phase" (see Figure 6-2) also presents an increased potential for neoplasia because stimulation of the thyroid to produce more T4 and T3 can result in hyperplasia. Both the decrement in T4 and T3 and increase in TSH is shown in Figure 7-1 at the same step along the continuum. Which of these thyroid responses is the most sensitive to hormone changes has not specifically been studied in the perchlorate testing strategy. As noted in the analyses of the studies in Chapter 5, there is a considerable degree of overlap among the

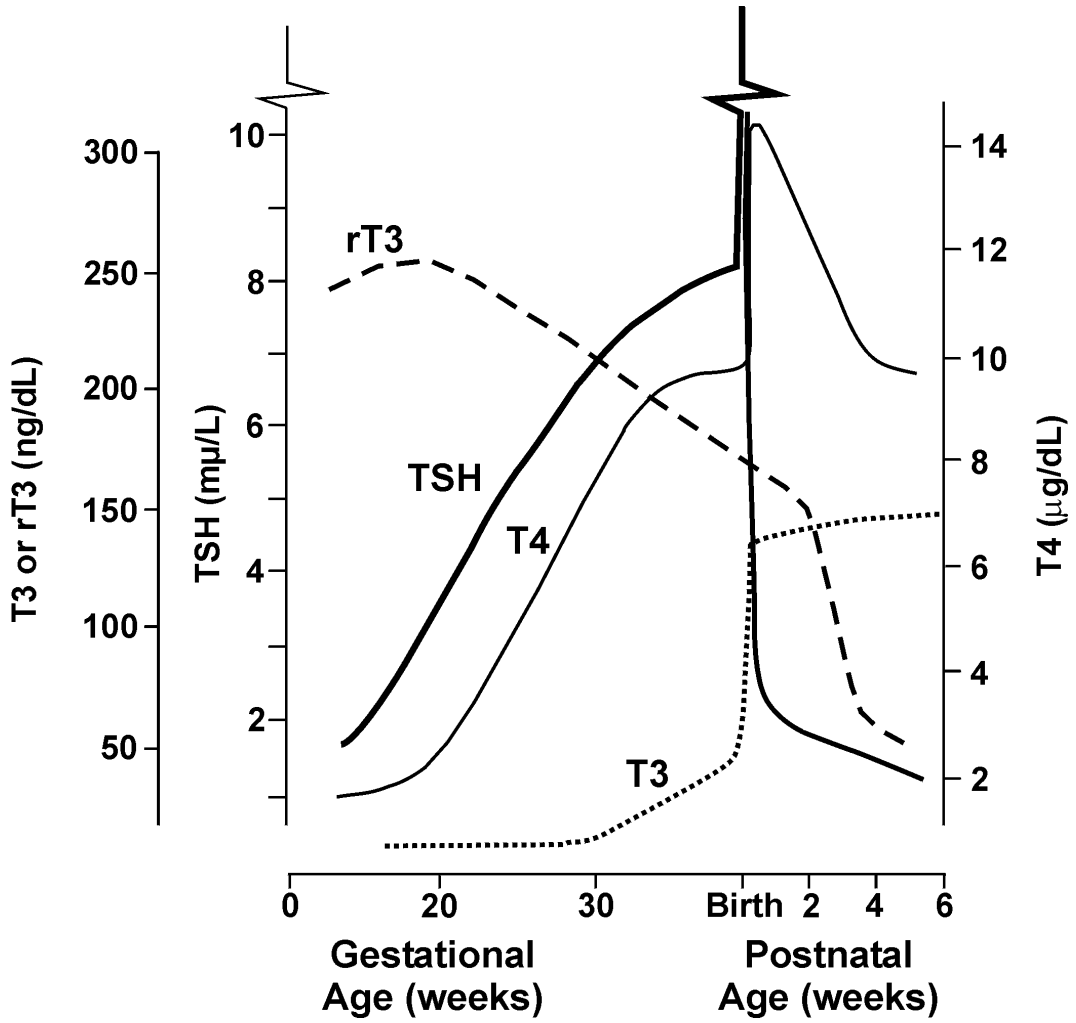

Figure 7-3. Pattern of change in fetal and neonatal thyroid function parameters during pregnancy and extrauterine adaptation in the human (from Fisher, 1996). A similar pattern is thought to exist in the rat (see text for further details).

three different diagnoses of thyroid histopathology: colloid depletion, hypertrophy, and hyperplasia.

Colloid depletion does appear to be slightly more sensitive across the perchlorate studies. The fact that thyroid follicular colloid depletion is a consistent finding not only across this study, but in rodents in general, would suggest that it is a good indicator of sufficient exposure to inhibit thyroid hormone synthesis. From a physiologic point of view this may be logical and supports the mode-of-action model. If there is any reserve thyroid hormone in the colloid, it is depleted

before serum hormones are altered. Once serum levels are altered, TSH is upregulated and hypertrophy and hyperplasia are initiated in an attempt by the gland to restore circulating levels of T4 and T3. The diagnosis of colloid depletion has been reported with a similar compound, sodium chlorate, in the rat (Hooth et al., 2001), with many other chemicals in the rat, and with numerous goitrogens and pharmaceutical agents in the mouse. Colloid depletion in association with hypertrophy and hyperplasia suggests sufficient dose of the compound to inhibit colloid synthesis and decreases of circulating serum thyroid hormone levels sufficient to stimulate TSH.

Colloid depletion as the most sensitive indicator is most notable in the pups of the 2001 "Effects Study" on GD21 and then immediately post parturition on PND4. Alternatively, as discussed in Chapter 5, it may have been harder to diagnose hypertrophy and hyperplasia in the younger (smaller) and growing glands. The BMDL for colloid depletion increased with post-natal age and by PND21, hyperplasia was also present. In contrast, all three thyroid indices were present in the PND4 pups of the previous Argus Laboratories, Inc. (1998a) study. This may be due to the difference in dosing of the dams. The dams in the 1998 study were only dosed during gestation and, therefore, likely had a greater decrement in thyroid hormones. The dams in the 2001 study were dosed for two weeks during cohabitation, sufficient time as evidenced in the data described in Chapter 6, for upregulation of NIS to compensate.

Other studies indicate that whichever index is most sensitive could be dependent on dose spacing in the study, age of animals on test, and sacrifice time point. For example, hyperplasia was the most sensitive of the three in the P2-generation adults (19 week F1-generation pups) and these same pups developed thyroid adenomas.

The proposed mode of action mapped in Figure 7-1 is supported by correlations between thyroid hormones and TSH and between thyroid hormones or TSH and an objective measure of lumen size from laboratory animals exposed to ammonium perchlorate. There were positive correlations between T3 and T4, and negative correlations between either T3 and T4 and TSH, as expected based on the mode of action model (Appendix 7A). The positive correlation between TSH and decreased follicular lumen size and negative correlation between T4 or T3 and decreased follicular lumen size similarly support the proposed model (Appendix 7A). Some of the correlations used in the 1998 assessment were precluded due to the limited severity scoring system used by the PWG.

Additional support for the mode of action comes from data that now allow the linkage of both neurodevelopmental and neoplastic sequelae into the model. These definitive data were not available prior to the 1997 perchlorate testing strategy and especially not before the most recent studies recommended by the 1999 external peer review. The repeat of observed effects on the motor activity and brain morphometry results by new studies allowed definitive determination that perchlorate exposure poses a neurodevelopmental hazard.

Repeatability and variability in statistics, sometimes a concern for evaluation of behavioral assays (Cory-Slechta et al., 2001) were addressed by the Bayesian approach employed for the motor activity analysis (Dunson, 2001a) that showed remarkable reproducibility between the two studies despite the deficits previously noted for the Argus Research Laboratories, Inc. (1998a) study. The effects on the size of the corpus callosum measurements were also repeated, and effects on additional brain regions identified. The new data were subject to a more rigorous statistical analysis than in 1998. The profile analysis described in Chapter 5 required that all areas of the brain measured were altered in a dose-dependent fashion and effects were again demonstrated not only in the corpus callosum but other brain regions as well (Geller, 2001d).

Likewise the neoplastic potential for perchlorate that had been demonstrated only at high doses in historical studies was confirmed at lower doses by the thyroid adenomas reported by the PWG (Wolf, 2000; 2001) for the F1-generation pups at 19 weeks (P2 parents) from the two-generation reproductive study (Argus Laboratories, Inc., 1999). Consistent with the proposed mode-of-action model, the anti-thyroid effects leading to neoplasia are likely to be via the non-linear mechanism described above. The genotoxicity battery established that perchlorate is not directly damaging to DNA.

Thus, the key event for the anti-thyroid effects of perchlorate is its perturbation of the hypothalamic-pituitary-thyroid axis by competitive inhibition of iodide uptake at the NIS. The evidence for this effect is built upon the observation of consistent changes across a range of experimental designs, including various species. These changes demonstrate effects on thyroid and pituitary hormones, increases in thyroid weight, and increases in three different diagnoses of thyroid histopathology (colloid depletion, hypertrophy, and hyperplasia). In addition, corresponding neurodevelopmental (motor activity and brain morphometry) and neoplastic outcomes were observed in special assays; these outcomes are also consistent with the proposed

mode of action and provide further evidence to confirm that the perturbation of the thyroid hormone economy should be viewed as adverse.

Due to the age and time-dependent nature of the critical effect, no one principal study is being chosen for this derivation. Instead, a weight-of-the-evidence approach will be taken to arrive at a point of departure in Section 7.1.3.

7.1.2 Dosimetric Adjustment of Exposures Associated with Effect Levels

Adjustments for interspecies differences in the internal dose delivered to target tissues should be made before an evaluation of the data array for valid comparisons across endpoints (U.S. Environmental Protection Agency, 1994). Based on the mode of action and the available PBPK model structures, two dose metrics were considered to describe the biologically effective dose for perchlorate: (1) the area under the curve (AUC) for perchlorate in the serum associated with drinking water exposures and (2) the percent of iodide uptake inhibition in the thyroid. These correspond to the different exposure components along the exposure-dose-response continuum in the mode-of-action model (Figure 7-1).

As described in Chapter 6, the serum perchlorate AUC was developed as the first dose metric based on data in rats and humans after drinking water exposures. To predict the "transient" phase of initial iodide inhibition in the rat, i.e., before upregulation of the NIS or increases in TSH, the second dose metric was based on RAIU measurements made in adult male rats dosed with perchlorate by iv two hours prior to an iv dose of radiolabled iodide. Table 7-1 presents the human equivalent exposures (HEE) estimates calculated using the PBPK models for serum perchlorate AUC as the dose metric. Table 7-2 shows the ratios for this same dose metric that can be applied in the parallelogram approach to arrive at estimates for different life stages used to observe effects in the different experimental endpoints. Fetal rat predictions were based on data developed for GD21. Neonatal rat predictions were based on data for PND10. This approach was taken since PBPK models for human pregnancy and lactation do not exist for perchlorate distribution. The calculation using the ratios approach is described in Chapter 6. The resultant adult HEE values for the different life stages of the rat experiments are shown in Table 7-3.

It can be observed in the tables in Merrill (2001e) that the pregnant and lactating rats have 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)

Adult Male Rat DW[a] Dose (mg/kg-day)	Human 15 kg HEE (mg/kg-day) based on serum[b] AUC	Human 70 kg HEE (mg/kg-day) based on serum[b] AUC	Human 15 kg HEE (mg/kg-day) based thyroid[b] AUC	Human 70 kg HEE (mg/kg-day) based on thyroid[b] AUC
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

[a]DW = drinking water.
[b]Calculated from PBPK-derived rat AUC(s) at steady state between 240 and 264 hrs during DW exposure, using upregulated Vmaxv_T_P 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)

Rat DW[a] Dose (mg/kg-day)	Male Rat: Pregnant Rat	Male Rat: Lactating Rat	Male Rat: Fetal Rat	Male Rat: Neonate Rat	Pregnant Rat: Fetal Rat	Lactating Rat: Neonate Rat
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

[a]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[a] (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

[a]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 7-1and 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

Rat iv Dose (mg/kg)	Adult male rat inhibition at 2-hr post iv dose	Human 15 kg HEE (mg/kg-day)	Human 70 kg HEE (mg/kg-day)
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[a]

Rat DW [b] Dose (mg/kg-day)	Adult Male Rat	Pregnant Rat	Fetal Rat[c]	Lactating Rat[d]	Neonate Rat[c,d]	70 kg Human
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%

[a]Based on iv administration to rat and drinking water in human.
[b]DW = drinking water
[c]Values for these tissues not validated versus data.
[d]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[a]

Rat DW[b] Dose (mg/kg-day)	Male Rat: Pregnant Rat	Male Rat: Lactating Rat	Male Rat: Fetal Rat[c]	Male Rat: Neonate Rat[c]	Pregnant Rat: Fetal Rat	Lactating Rat: Neonate Rat[c,d]
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

[a]Inhibition in human was PBPK-derived from 2 wks ClO_4^--exposure in drinking water (DW); all rat values simulated from an iv dose.
[b]DW = drinking water
[c]Model predicted in fetal and neonate rats not validated with data.
[d]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[a] (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

[a]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.

concentrations, were used based on the assumption that these dose metrics would represent an averaging of the serum and thyroid perchlorate concentrations and would be better correlated with the inhibition effect on iodide uptake. The correlation was shown to be good between the AUC and the degree of inhibition (Figures 6-47 through 6-50). Further, due to the rapid phase of distribution after an iv dose, measurement of concentrations are very difficult to attain experimentally and are more variable. Using simulated peak concentrations after iv injections is potentially problematic due to the inexact modeling of the actual distribution of dose in the tail-vein volume and the exact time of mixing in the whole blood compartment (Merrill, 2001e). It was also observed by EPA that the ratios for peak perchlorate serum values (Merrill, 2001e: Table 6) were in good agreement with those for the perchlorate serum AUC and that the serum AUC were slightly more conservative if different at all.

Merrill (2001e) expressed concern regarding the thyroid values in neonates and fetuses because these values were not validated against experimental data. Fetal and neonatal thyroid were never actually analyzed for perchlorate concentration. In the case of the fetus, kinetic parameters were determined by fitting model simulations of fetal thyroid concentration to available iodide data and assuming that the perchlorate:iodide ratio would be similar to that of the mother. In the case of the neonatal rat, no data were available for thyroid concentrations for either perchlorate or iodide. Thus, model predictions were based on allometrically scaling maternal parameters for thyroid uptake. It was the opinion of the AFRL/HEST authors that while the thyroid parameters in the fetus and neonatal rat were highly informative, they should not be used in the formal risk assessment (Merrill, 2001e). EPA concurs with these considerations and recommendation.

In general, the models were believed to provide a good description of perchlorate and iodide disposition in the blood. Using the models to describe dose metrics in the thyroid was viewed as less reliable due to assumptions regarding parameters and the lack of experimental data for validation. The models were able to successfully describe serum perchlorate and iodide concentrations for both acute (based on iv doses) and chronic drinking water in the adult male, pregnant, neonatal and fetal rat, and greater confidence can be afforded these predictions (Merrill, 2001e).

Tables 7-3 and 7-7 demonstrate good correspondence in the HEE estimates predicted for both dose metrics at the lower doses for the lactating and neonatal rats, but not for the male adult,

pregnant or fetal rats where there is an order of magnitude difference. The iodide inhibition metric predicts a 10-fold lower HEE in both the adult male and pregnant dam when compared to the HEE estimated based on the serum AUC. The fetal rat value for iodide inhibition was viewed as unreliable for the reasons stated above. All of the factors influencing this disparity are not fully appreciated at this time but can reasonably be ascribed to uncertainty in the thyroid descriptions that were not validated with experimental data, and will require additional studies to characterize accurately. For these reasons, the adjustment factor to arrive at an HEE estimate was based on perchlorate serum AUC as the dose metric.

7.1.3 Point-of-Departure Analysis

Various statistical procedures were used for each of the different outcome measures for the various endpoints described in Chapter 5. The weight-of-evidence approach herein relies on the results, and the details on the statistical analyses are provided in Chapter 5 and associated memoranda from EPA and NIEHS scientists. In general, benchmark dose analysis was used for the thyroid histopathology because the EPA advocates the use of quantitative dose-response modeling to diminish the influence of dose-spacing, sample size, and variability on the NOAEL designation (Crump et al., 1995). Likewise, ANOVA was used to evaluate the thyroid and pituitary hormone data (Crofton and Marcus, 2001) although benchmark analyses were also performed as a comparison (Geller, 2001c). The 1998 benchmark analyses for the hormone data from the previous set of studies (Geller, 1998a) is provided in Appendix 7B.

Specific Bayesian statistical analyses were employed for the motor activity data and for evaluating the significance of the tumors in the 19-week old F1-generation adult rats (Dunson, 2001a,b). Another specific statistical approach, profile analysis, was used to evaluate the brain morphometry effects (Geller, 2001d).

Several studies suggest 0.01 mg/kg-day as the exposure dose that is a level of concern for the adverse effects of perchlorate. The first is the profile analysis on brain morphometry effects in PND21 pups in the "Effects Study" (Argus Research Laboratories, Inc., 2001) which demonstrated a dose-dependent and significant effect on the size of the corpus callosum and other brain regions. Statistically significant changes were also demonstrated in the PND9 pups. This effect repeated effects on brain morphometry observed in the previous neurodevelopmental study (Argus Research Laboratories, Inc. 1998a) that were a noted concern to the EPA in the

1998 risk assessment. Changes in the corpus callosum at a later time point on PND82 were also observed in that previous study.

An increase in the corpus callosum plausibly represents a delay in developing brain structures since this area is known to increase in size and then decrease later during development. Neurodevelopmental toxicity suggestive of delays was also demonstrated by effects on motor activity in both the Argus Research Laboratories, Inc. (1998a) and repeated in the Bekkedal et al. (2000) study. The increases in motor activity represent activity that should have subsided by these test dates. A type of hyperactivity has been noted in monkeys exposed to PCBs (Rice, 2000).

These effects on brain morphometry and motor activity are of particular concern because the relative sensitivity of laboratory animal assays to adequately characterize the types of deficits related to maternal hypothyroxinemia in large population studies is unknown (Morreale de Escobar, 2000; Haddow et al., 1999; Pop, 1999). Screening neurodevelopmental studies may not have the power to ascertain neurological effects that might result from small changes in the thyroid-pituitary hormone economy. As pointed out by Crofton (1998j), the sensitivity of animal models used to explore the role of thyroid hormones in neural development is currently equivocal. Most of the data collected and published to date were with high doses of thyrotoxic chemicals (e.a., methimazole, propylthiouracil) or with thyroidectomy. It is not known whether the available tests are capable of detecting more subtle changes in nervous system development. An analysis presented by Crofton (1998j) suggested that measurements of nervous system development are less sensitive than measurements of T4. Two reasons for this relationship were presented. First, the brain may be protected from perturbations in circulating concentrations of T4, as demonstrated by upregulation of deiodinases in brain tissue that compensate for very large decreases in circulating T4. The second reason, and one for concern in the context development of this model, is that currently available testing methods, particularly screening methods, may not be sufficiently sensitive. Recent data suggest that the battery is insensitive to alterations in thyroid hormones during development (Goldey, 1995a,b).

The 0.01 mg/kg-day dosage as a level for concern was also supported by thyroid histopathology in the database. Changes in colloid depletion observed on PND4 in both the 1998 neurodevelopmental study (Argus Research Laboratories, Inc., 1998a) and the newer 2001 "Effects Study" (Argus Research Laboratories, Inc. 2001) were demonstrated. The BMDL

estimated for those studies on PND4 was 0.33 mg/kg-day, but an estimate of 0.009 mg/kg-day is also obtained with a model demonstrating adequate fit to the data. The BMDL for colloid depletion in pups on GD21 was 0.12 mg/kg-day, but for female pups alone on GD21 was 0.04 mg/kg-day. The BMDL estimated for thyroid hypertrophy in weanling pups from the two-generation study (Argus Research Laboratories, Inc., 1999) was 0.06 mg/kg-day. Of notable concern to this consideration was that the BMDL estimates decreased with duration in the 90-day study. The BMDL estimates for colloid depletion were 0.28 and 0.03 mg/kg-day at the 14-day and 90-day time points in the Springborn Laboratories, Inc. (1998) study. The BMDL estimates for hypertrophy were 0.017 and 0.008 mg/kg-day at the 14-day and 90-day time points. This effect of duration was of concern as it was also evident by the observation of tumors in the F1-generation adults at 19 weeks. Both observations suggest concern that duration may recalibrate either the homeostatic interactions of the hypothalamic-pituitary-feedback system or the cellular sensitivity and demand for the thyroid hormones.

The thyroid hormone data in a number of studies also designated 0.01 mg/kg-day as a LOAEL. Levels of T4 were significantly decreased and TSH levels statistically increased at this dosage in the dams on GD21 in the same study as the significant brain morphometry measurements in the PND21 pups (Argus Research Laboratories, Inc. 2001), revealing no NOAEL for hypothyroidism in the dams. The pups in that study were also affected at 0.01 mg/kg-day. Effects on T3 occurred at GD21, PND5, and PND9 at this dosage. The 0.01 mg/kg-day dose was the LOAEL for effects on T4 and TSH at PND21 in the male pups and for TSH in both sexes at PND9 as well. This same dose (0.01 mg/kg-day) was also the LOAEL for decreases in T4 and increases in TSH at the 14-day and 90-day time points in the 90-day study (Springborn Laboratories, Inc., 1998).

The ANOVA estimates for hormone data were used to characterize this effect after serious consideration. While in clinical studies a normal range typically is defined by a control healthy population, the ANOVA approach is an equally valid approach in that a statistically significant value represents a shift in the mean for the population. The control group defines the range for the unexposed, presumably healthy population, and statistically significant differences indicate that the mean for an exposed group is outside of that normal range. Circadian fluctuations are addressed because the same fluctuations in the control population occur as in the exposed population. A small shift in the mean of a population can have significant consequences to

individuals in the tails of the distributions of those populations. Indeed, such an evaluation underlies the basis for the blood lead level used in the National Ambient Air Quality Standard (Davis and Elias, 1996) and has been noted as an important consideration for neurotoxicity (Weiss, 2000).

The notion that continuous data should be considered in the context of the specific dose-response rather than to *a prioro* categories defined outside of the data under analysis is supported in the benchmark dose literature. Murrell et al. (1998) point out that a continuous quantity measurement such as the hormone data should be scaled by the range from background response level to maximum response level (for increasing response functions). The authors go on to note that it is a biological reality that, whatever the mechanism of effect of the toxicant, there is some dose level beyond which no further change in response is seen or is theoretically feasible. In general, there is some type of limitation or saturation phenomenon that occurs at high enough doses (e.g., in the saturation of the symporter capacity, as suggested by the modeling effort in Chapter 6 and the data of Chow and Woodbury [1970] and of Meyer [1998]).

An analogy to the case of quantal data for which an effect is defined as a probability metric in which the response reaches a maximum at one, is, that for continuous measures, the extra effect can be defined as the change in effect from background standardized by the total range of response (Murrell et al., 1998). The total response range is not necessarily the response range of the observed responses in a study; rather, it is defined by a determination of the minimum and maximum possible responses according to, for example, a model equation fitted to the data as in the case of benchmark analyses. In all BMD analyses, however, the hormone BMDL estimates were shown to be extremely low (Geller, 1998a; Geller, 2001c). This may not necessarily be surprising given that hormones are operative at low doses by definition, but corresponding changes in thyroid histopathology were more consistent with the ANOVA estimates.

Finally, the NOAEL for immunotoxicity suggested by the dermal contact hypersensitivity assay at 0.02 mg/kg-day can be viewed as supportive, especially since deficiencies in this study raise concern for the characterization and because a LOAEL for the effect was demonstrated at 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).

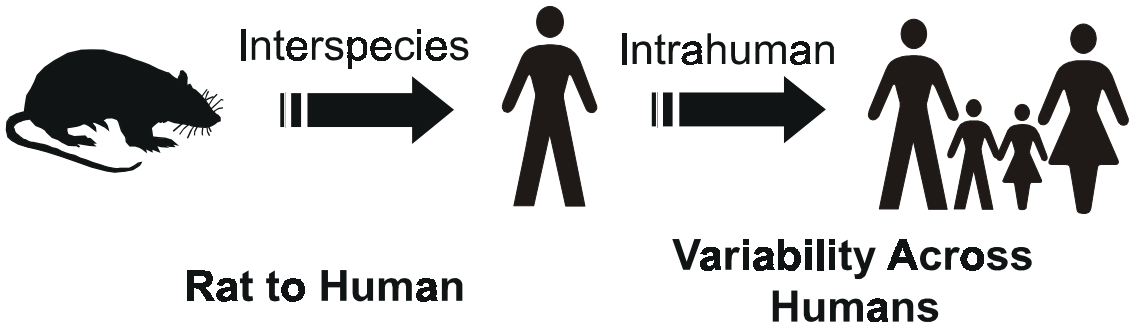

Figure 7-4. Consideration of uncertainty and variability influence interspecies and intrahuman extrapolation.

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

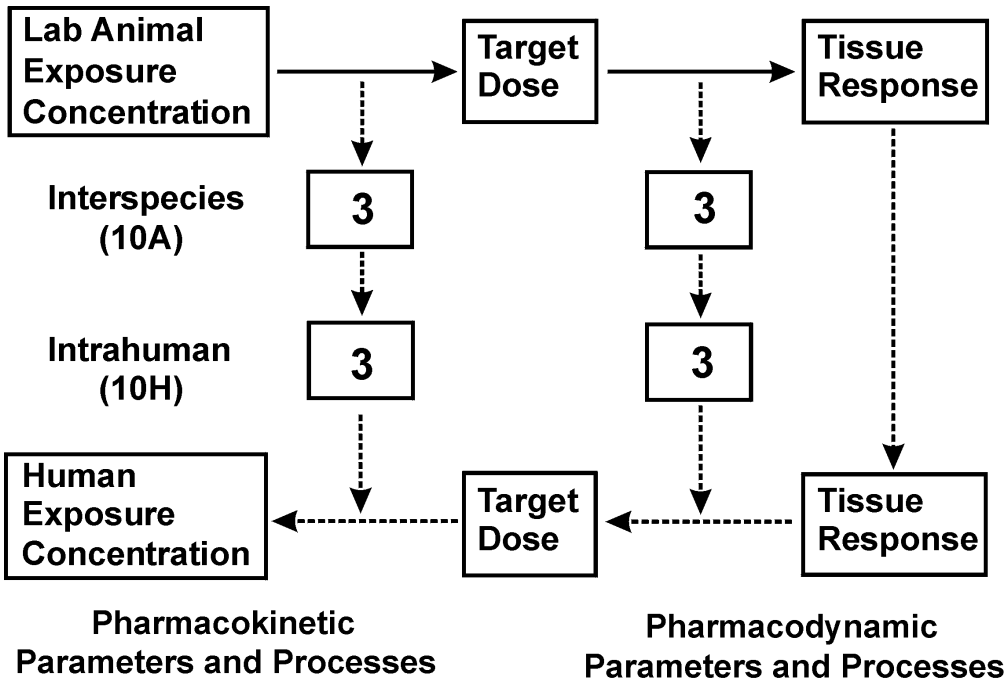

Figure 7-5. Schematic of uncertainty factor components incorporated into exposure-dose-response characterization for interspecies and intrahuman extrapolations (Jarabek, 1995b).

mitigated by further development of pregnancy and lactation models or the models might be further validated with radionucleide data using a parallelogram approach between perchlorate and iodide as described in Chapter 6. This reduced factor was a point of considerable debate, especially given the concern over the animal neurodevelopmental assays for adequately characterizing neuropsychological development deficits in susceptible populations. However, it was also discussed that the UF values are not entirely independent; e.g., aspects underlying the duration extrapolation also might underlay the intrahuman UF (Jarabek, 1995b).

The interspecies factor was omitted due to general confidence that the extrapolation based on perchlorate distribution (and on iodide inhibition by perchlorate at lower doses) was accurately characterized by the PBPK modeling effort described in Chapter 6. Concern for eliminating this factor was again considered in the context of the lack of independence with other applied UF. The concern that the HEE was not based on iodide inhibition but rather the serum

perchlorate AUC was assuaged somewhat by the correlations that demonstrated a close relationship between these two measures.

A full 10-fold factor was applied to extrapolate the LOAEL for the brain morphometry, thyroid histopathology, and hormone changes observed at the 0.01 mg/kg-day level. Designating these changes to be adverse is consistent with the proposed mode of action and existing Agency guidance and procedures. The neurotoxicity assessment guidelines (U.S. EPA, 1998a) specify changes in brain structure as adverse. The OPPTS has used thyroid hormone changes to designate effect levels. Finally, the shallow slope of the response curve at these lower levels suggested that a full factor should be applied.

A 3-fold factor for duration was applied due to the concern for the biological importance of the statistically significant increase in tumors in the F1-generation pups at 19 weeks (P2, second parental generation). The occurrence of these tumors with a dramatically reduced latency and with a significance in incidence greater than the NTP historical data (Dunson, 2001b) for thyroid tumors in this strain of rat was reason for concern. As discussed earlier, the concerns were that this observation represented the potential for *in utero* programming; and that the decrease in the NOAEL/LOAEL estimates for hormone perturbations and histopathology between the 14-day and 90-day time points represented a recalibration of the regulatory feedback system or changes in cellular sensitivity and demand for thyroid hormones with extended exposures. This factor can also be viewed as part of a data base deficiency because there are no long-term bioassays of perchlorate with contemporary design and data quality. While the original strategy aimed at determining a NOAEL for thyroid histopathology as a precursor lesion to tumors in the 90-day study, this finding in the F1-generation cannot be ignored, especially in light of an emerging appreciation of findings suggesting a phenomenon known as *in utero* imprinting with endocrine disruption (Prins et al., 2001; Phillips et al., 1998; Seckl, 1997). Thus, *in utero* disruption of thyroid hormones in the developing fetus may predispose the developing neonate and adult to future environmental insults to the thyroid gland by making the fetus more sensitive. Weiss (2000) has noted that changes in brain functions occur throughout life and some consequences of early damage may not even emerge until advanced age. This could be exacerbated if environmental insults to the thyroid were to be continued throughout life.

The potential for perchlorate to cause immunotoxicity remains a concern so that a 3-fold factor was applied for the database insufficiency. New studies based on recommendations at the

1999 external peer review had some deficiencies and reinforced concern about the lack of an accurate characterization of this endpoint.

7.1.5 Operational Derivation of the Reference Dose

The HEE for the neonatal rat corresponding to brain morphometry and hormone changes observed in the PND21 pups (also the PND9 pups) at the 0.01 mg/kg-day dosage would be 0.02 mg/kg-day (Table 7-3). However, because the dams on GD21 were shown to be hypothyroid (with statistically-significant decreases in T4 and increases in TSH) at this same dosage, and because the temporal windows underlying the neonatal brain morphometry effects are unknown, and because the brain morphometry effects may have occurred *in utero* due to the dams' hormone deficiency, the HEE estimate for dams of 0.01 mg/kg-day was chosen as the operational derivation. It was noted that this choice was not as conservative as using the HEE for iodide inhibition in the dams (0.002 mg/kg-day), but it was viewed as more accurate given the concerns for the reliability of the thyroid estimates.

According to Dollarhide (1998), who spoke with Argus laboratory on behalf of the sponsor (PSG), the reported doses were of ammonium perchlorate and not the anion itself. Thus, an adjustment for percent of the molecular weight of the salt from ammonium (15.35%) must also be made. Further, because the analytical methods measure the anion concentration in environmental samples, this is the appropriate expression for the RfD to use while making valid comparisons for risk characterization. Thus, the derivation for an RfD for the perchlorate anion as itself is as follows:

$$0.01 \text{ mg/kg-day} \times 0.85 / 300 = 0.00003 \text{ mg/kg-day}. \qquad (7\text{-}1)$$

Note that the appropriate adjustment for any salt of perchlorate (e.g., adjustment by a factor of 0.72 for potassium perchlorate) should be made when evaluating toxicity data for similar assessment activities.

It is critically important to distinguish the proposed RfD from any guidance value that may result. An RfD would be only one step in the future regulatory process of determining, based on a variety of elements, whether a drinking water standard for perchlorate is appropriate. As with any draft EPA assessment containing a quantitative risk value, that risk estimate is also draft and

should be construed at this stage to represent Agency policy. The units for an RfD are mg/kg-day. Conversion of an RfD to a drinking water equivalent level (DWEL) is based on adjusting by body weight (kg) and drinking water consumption (L) to arrive at a level expressed in units of mg/L (ppb). Derivation of a maximum contaminant level goal (MCLG) from a DWEL by the OW typically involves the use of a relative source contribution (RSC) factor to account for non-water sources of exposures such as those discussed in Chapters 8 and 9.

Because the effect is viewed to be the result of neurodevelopmental deficits resulting from the hypothyroid or hypothyroxinemic state induced by the mother's exposure, and because developmental neurotoxicity may emerge later in the life or be exacerbated later in life, conversion factors for the adult of 70 kg body weight and 2 L of water per day are considered appropriate. Recent guidance from the OW in its Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health (U.S. Environmental Protection Agency, 2000) provides a decision flow chart for derivation of the RSC and recommends 80% as a ceiling and 20% as the floor for this factor when data are adequate to estimate sources of exposure. When data are not adequate to estimate other anticipated exposures, OW recommends a default RSC of 20%. (U.S. Environmental Protection Agency, 2000: Chapter 4, Section 4.2.2.4 on apportionment decisions). EPA does not recommend that high-end intakes be assumed for every exposure source since the combination may not be representative of any actually exposed population or individual.

A hypothetical adjustment of the 0.00003 mg/kg-day RfD by 70 kg and 2 L would thereby result in a DWEL of 1 ug/L (ppb) and application of an RSC between 0.2 to 0.8 would thereby result in an MCLG in the range of 0.2 to 0.8 ug/L (ppb). These values are in the range of current analytical capabilities. As discussed in Chapter 1, improvements to the analytical methods on the near horizon or expected to be published this spring could result in minimum reporting limits in this range and lower (Yates, 2001).

Concern is often expressed in the regulatory arena for the potential added susceptibility of children in developing DWEL estimates based on different conversion factors (15 kg and 1 L). Consequently, the EPA asked for additional PBPK simulations to help inform this dialogue. As shown in Table 7-1, the HEE estimates for a 15 kg human for serum perchlorate AUC can be as great as two-fold higher than those predicted for the 70 kg human due to differences in distribution volumes and excretion. Thus, if the 15 kg and 1 L values are used to convert this 2-

fold higher HEE value in an analogous derivation to the adult RfD derivation and DWEL calculation above, an estimate of 1 ppb that is equivalent to the adult conversion results.

7.1.5.1 Comparison with Derivation Considering Human Data

It is important to evaluate this derivation in context with the evidence from the available and relevant human data. As described in Chapter 4, the EPA felt that both the observational epidemiological and the human clinical studies have significant scientific and technical limitations that preclude their use as the basis for a quantitative dose-response assessment. The clinical study subject attributes (euthyroid adults) and study design issues (sample size, RAIU time points, etc.) made these data less reliable than the laboratory animal toxicological data to ascertain effect levels for the basis of an RfD derivation. 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 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 an RfD. The ethical issues surrounding the conduct of these studies or their use for regulatory purposes in light of the Agency’s interim policy will not be discussed in this document. The Agency is requesting that the National Academy of Sciences conduct an expeditious review of the complex scientific and ethical issues posed by EPA’s possible use of third-party studies which intentionally dose human subjects with toxicants to identify or quantify their effects.

These issues not withstanding, a dose of 0.007 mg/kg-day has been suggested by some authors in an abstract (Greer et al., 2000) to be a NOAEL estimate. This was based on an

average 6.2 % decrease relative to baseline of RAIU measured on Day 14 of exposure to seven subjects at the 8-hour time point (unpublished data presented in Merrill, 2001a; Attachment #7). The values for RAIU ranged from a 38.6% decrease in a 34-year old female to a 27.9% increase in a 49-year old female at that dosage.

Prior to the articulation of the Agency's interim policy, the Agency had conducted a comparison of its reference dose derivation considering the results of the study described above, which falls within the category of a "third-party study" described by the authors as demonstrating a NOAEL in humans. If this study were to be considered in lieu of the laboratory animal data and PBPK modeling, the following would need to be considered. The seven subjects (six females and one male) were euthyroid and ranged in age from 18 to 49. Because this is a limited data set (small sample size), with noted variability and because of relevance to the elderly woman, cardiac risk patient, hypothyroid or hypothyroxinemic pregnant woman, or fetus as the susceptible population is difficult to ascertain, an uncertainty factor of 3-fold for this iodide uptake inhibition level as a minimal LOAEL as well as a 3-fold factor for intrahuman variability would be warranted. This is particularly relevant if this value is viewed in context with the neurodevelopmental effects in laboratory animal data. At a minimum each factor should be 3-fold, and discussion with respect to the meaning of these factors with respect to population effects again entertained. None of the human studies of perchlorate reviewed in Chapter 4 have adequately investigated neurodevelopmental outcomes. The concern for duration of exposure was at least a 3-fold factor per the above laboratory animal data discussion and should also be applied, as well as the 3-fold factor for database deficiencies because these considerations and deficiencies are not obviated by the use of human data.

Thus, a derivation based on the available human data would estimate the RfD at a maximum of 0.00007 mg/kg-day, an estimate in rather good agreement with that proposed based on the laboratory animal data (0.00003 mg/kg-day). If a larger UF were to be applied to the human data, as could be justified for the intrahuman factor, the resultant estimate would be essentially equivalent to that proposed using the laboratory animal data.

The consistency between the estimates based on the laboratory animal versus the human data is likely due, at least in apart, to the use of AFRL/HEST PBPK modeling (Merrill, 2001c,d; Clewell, 2001a,b) to perform the interspecies extrapolation rather than the use of default factors. It should be noted that the original motivation for performing these human studies (as discussed

in Chapter 3) in the perchlorate testing strategy was to support such interspecies pharmacokinetic extrapolation and not to derive NOAEL estimates for thyroid effects in the human population. In addition, as noted in Chapter 4, the EPA felt that both the observational epidemiological and the human clinical studies have significant scientific and technical limitations that precluded their use as the basis for a quantitative dose-response assessment. As mentioned previously, under the interim policy articulated on December 14, 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. Nonetheless, the use of both previously published and newly-derived human data by the Air Force in its modeling efforts was important. The AFRL/HEST PBPK model approach allowed EPA to confirm that humans were as sensitive as rats to the iodide uptake inhibition effects of perchlorate at the NIS, the key event for the proposed mode-of action of perchlorate on the thyroid. In addition, the PBPK models increased the accuracy of interspecies extrapolation by allowing the incorporation and integration of ADME data to describe perchlorate and iodide disposition relative to the key event. These two outcomes from the integration of human and animal data in the AFRL/HEST models provide greater confidence than would the laboratory animal data alone that the reference dose that is derived will be protective of human health.

7.1.5.2 Comparison with Derivation Based on Tumor Data

To address neoplasia as the other potential adverse endpoint, this section will discuss how an estimate could be derived based on the recently acquired tumor data.

7.1.5.2.1 Choice of Dose-Response Procedure

As discussed in Chapter 5, the genotoxicity assays included in the testing strategy determined that perchlorate was not likely to be mutagenic. This was one of the critical determinants in deciding on a dose-response approach for a cancer derivation. The EPA guidance on assessment of thyroid follicular cell tumors (U.S. Environmental Protection Agency, 1998a) sets forth data needs to establish the default dose-response procedure that should be used to establish that a chemical has antithyroid activity (i.e., that it is disrupting the thyroid-pituitary 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)

	Array of Effects		
Example	Mutagenic	Antithyroid	Dose-Response Methodology
1	Either or both unknown		Linear
2	Yes	No	Linear
3	No	Yes	Margin of exposure
4	Yes	Yes	Linear and margin of exposure

used. The thyroid lesions observed (colloid depletion, hypertrophy, and hyperplasia) are among the required lesions to demonstrate antithyroid activity. Table 7-9 shows the types of data 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	

What has been proposed in this assessment is the harmonization of the "noncancer" and "cancer" assessment approaches because the target tissue is the thyroid and the mode of action is the same for both the neurodevelopmental and neoplastic sequelae. The proposed RfD based on precursor lesions is analogous to a nonlinear approach and viewed as a protective for thyroid tumors.

Perchlorate has clearly demonstrated an effect in both adult, fetal, and neonatal stages in thyroid histopathology, as well as a decrease in lumen size in a dose-dependent fashion. Thyroid and pituitary hormone changes and expected correlations all have been demonstrated for T3, T4,

and TSH across an array of studies at different time points. The site of action has been established as competitive inhibition of the iodide symporter although there remains some uncertainty as to whether that is the only locus for the effect (e.g., evidence for intrathyroidal activity) because of the efflux (discharge) phenomenon. Dose-correlations in this case were not with tumors, but rather for precursor lesions (colloid depletion, hypertrophy, hyperplasia, and decreased follicular lumen size). Reversibility has been demonstrated in thyroid weight, colloid depletion, hypertrophy, hyperplasia, and thyroid and pituitary hormones in the 30-day recovery period after the 90-day study in rats and in T4 levels of the various immunotoxicity experiments in mice.

Lesion progression was difficult to determine because of dose-spacing and differences in sample size and histological methods among the studies. However, there was a progression within the 90-day study between the 14- and 90-day time points.

Analyses of other anions have fairly well established that the mode of action of perchlorate arises from it being an anion that is recognized by the NIS (see Chapter 3).

Thus, the appropriate dose-response procedure for perchlorate would be a nonlinear margin-of exposure approach based on demonstration that it is not genotoxic and that its anti-thyroid effects are consistent with a mode of action leading from inhibition of iodide uptake at the NIS through precursor lesions of perturbation of thyroid hormone economy and resultant histopathological changes in the thyroid gland.

7.1.5.2.2 Dose-response Assessment for Thyroid Neoplasia

Thyroid adenomas were statistically increased in the high dose (30 mg/kg-day) group of F1-generation animals sacrificed as adults (P2-generation) at 19 weeks in the Argus Research Laboratories, Inc. (1999) two-generation reproductive study. Both the latency and incidence of these tumors were remarkable relative to the entirety of the NTP data base for this type of tumor in this strain of rat (Dunson, 2001b). Colloid depletion, hypertrophy, and hyperplasia were all observed at dosages of 0.3 mg/kg-day and above with BMDL estimates of 0.9, 0.15, and 0.0004 mg/kg-day. This last estimate is outside the range of possible dosimetric adjustment so it will not be carried forward, but consideration of the overlap among colloid depletion, hypertrophy, and hyperplasia should be superimposed on the derivation. The HEE values for adult versus neonatal rats are comparable at these dosages. Using the adult male rat dosimetric

adjustment factor to correspond to sacrifice date results in HEE estimates of 0.45 and 0.02 for colloid depletion and hypertrophy.

Using the nonlinear approach and applying a composite factor of 100 to the HEE estimates to account as above for uncertainty in intrahuman variability, duration, and database deficiencies; and with factor for a minimal LOAEL of 3 to account for the fact that hyperplasia occurred at over an order of magnitude lower than these two thyroid histopathology estimates, results in an RfD derivation in the range of 0.005 to 0.0002 mg/kg-day. Applying a larger uncertainty factor for intrahuman variability would result in a range of 0.002 to 0.00007 mg/kg-day. Thus, the derivation based on tumor outcome data supports the mode-of-action concept and corroborates that the proposed RfD that as derived would be protective of both neurodevelopmental and neoplastic sequelae.

7.1.5.3 Possible Susceptibility

Based on the mode-of-action for perchlorate, the competitive inhibition of iodide uptake, and the subsequent perturbation of thyroid hormone homeostasis, a number of factors potentially could cause an increase in susceptibility of a population to perchlorate toxicity. As already indicated by the choice of critical effect, the fetus, and perhaps the developing child, may represent susceptible populations. However, critical data on the steady-state pharmacokinetics and placental dosimetry are lacking to definitively state whether or not there is an inherent pharmacodynamic component to the apparent sensitivity of pups versus dams in the laboratory animal models. Individuals that are iodine deficient may be another susceptible population. The elderly, especially women, and hypothyroid and hypothyroxinemic individuals or those treated with anti-thyroid drugs, may be others more susceptible than the general population to the effects of perchlorate. Patients with cardiac dysfunction or elevated levels of cholesterol may also be at increased risk.

7.1.6 Designation of Confidence Levels

Confidence in the principal study is medium. The dose level of 0.01 mg/kg-day was the lowest tested, and it was determined to be a LOAEL (not NOAEL). The small sample size for the critical effect also reduces confidence in the study. Despite the new data, the confidence in the database at this time remains medium because the sensitivity of these animal assays versus

evaluation of neuropsychological development in human population studies is not known, and because a concern for potential immunotoxicity remains. Based on confidence in the study and on the database together in setting the overall confidence in the RfD, the confidence in the RfD currently is also medium.

7.2 INHALATION REFERENCE CONCENTRATION

Derivation of an inhalation reference concentration is precluded because there are no inhalation data available with which to characterize dose-response or the portal-of-entry modulation of internal dose. However, the EPA has been questioned as to whether the potential for inhalation exposure of perchlorate from showering with contaminated water poses a health risk. Given the low vapor pressure of perchlorate, it is not likely that it would come out of solution. Further, Giardino et al. (1992) characterized shower particle droplet size as ranging from 200 to 3,000 μm. Thus, there is minimal chance for inhalation or deposition of perchlorate-laden droplets in the respiratory tract.

7.3 SUMMARY

The model based on mode of action for perchlorate served as a useful construct for the integration of a diverse set of data. Results of studies in the testing strategy confirmed that the target tissue for perchlorate is the thyroid and that the key event for its antithyroid effects is the inhibition of iodide uptake at the NIS with corresponding perturbations of thyroid hormone economy. Disturbances in thyroid hormone economy were confirmed to result in thyroid histology as diagnosed by decreases in colloid depletion or follicular lumen size and increases in hypertrophy and hyperplasia. Effects on both neurodevelopmental indices (brain morphometry and motor activity) and neoplasia that could be expected based on the mode of action were also demonstrated. Other developmental and reproductive effects were not observed to be as sensitive as the neurodevelopmental and thyroid histopathological changes. Accurate characterization of the immunotoxicity of perchlorate, notably its potential to cause contact

hypersensitivity, either secondarily to these hormone effects or possibly via a direct effect of the anion itself, remains a concern.

APPENDIX 7A

CORRELATION ANALYSES

The correlation analyses were of two types. Hormone levels are continuous, ratio-scaled values, so correlations were computed using the conventional Pearson's r statistic. Correlations between ratio-scaled hormone levels and ordinally-scaled standard histology ratings must be computed using nonparametric correlations. To compare variables from the different scales, it is simplest to recode the data by converting the variable values into rank scores. Spearman's rank order (r_s) was used to compute the correlation between the rankings of two variables. When there were ties in the ranks, as there were in this data set, each value was assigned the mean of the ranks that they would otherwise occupy. A correlation coefficient was then computed for the rankings of the variables of interest.

An alternative statistic used for comparing the data sets was Kendall's tau, best thought of as a measure of agreement or concordance between two sets of ranked data. It searches for the number of inversions in two sets of ranked data (i.e., observations are ranked according to the first variable, then reranked according to the second, and the number of interchanges that occur is used to compute the statistic). The Spearman and Kendall statistics produced nearly identical results. Statistics were computed using SAS® software (PROC RANK and PROC CORR, SAS Institute, Cary, NC). All statistics corresponding to Figures 7A-1 through 7A-7 can be found in Tables 7A-1 through 7A-6.

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

In general, positive correlations were expected between T3 and T4 and between TSH and the histopathology rating. Negative correlations were expected between T4 and TSH and between T4 and histopathology.

Figure 7A-1 shows the correlations between T3 and T4 and between T4 and TSH levels from the 14-day Caldwell et al. (1995) study in rats. Robust relationships are illustrated: a positive correlation is shown between T3 and T4; whereas, the T4 and TSH varied inversely. Hormone levels also correlated highly with decrease in follicular lumen size. Figure 7A-2 shows the rank of T4 level and TSH level versus the severity rating for follicular lumen size to be highly correlated inversely. Figure 7A-3 shows the correlations for the combined 14-day and 90-day time points (male and female) from the subchronic study performed in rats (Springborn

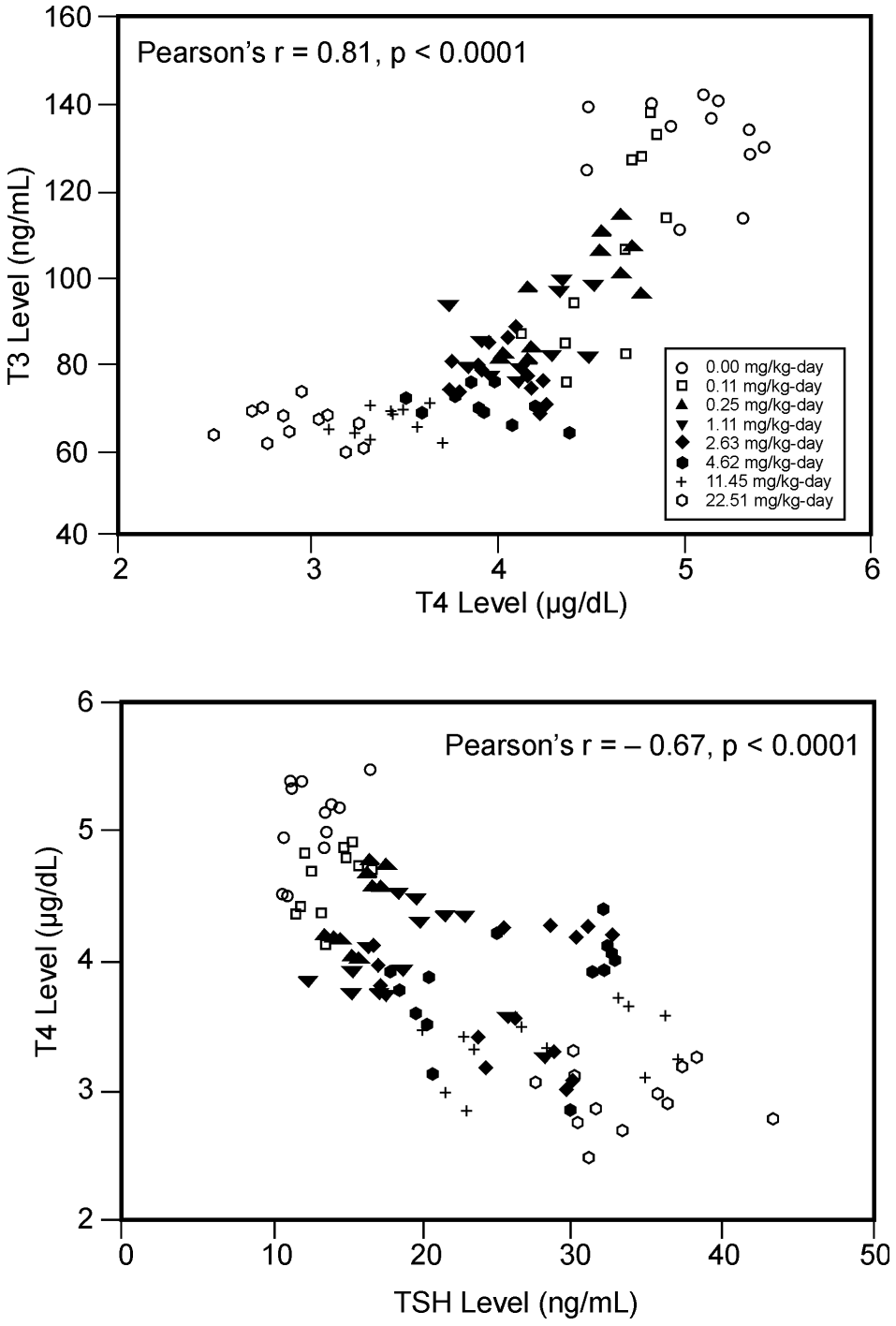

Figure 7A-1. Correlations between T3 versus T4 (top panel) and T4 versus TSH (bottom panel) in rats of the 14-day Caldwell et al. (1995) study (Geller, 1998a). Data of Channel (1998a) and Crofton (1998a).

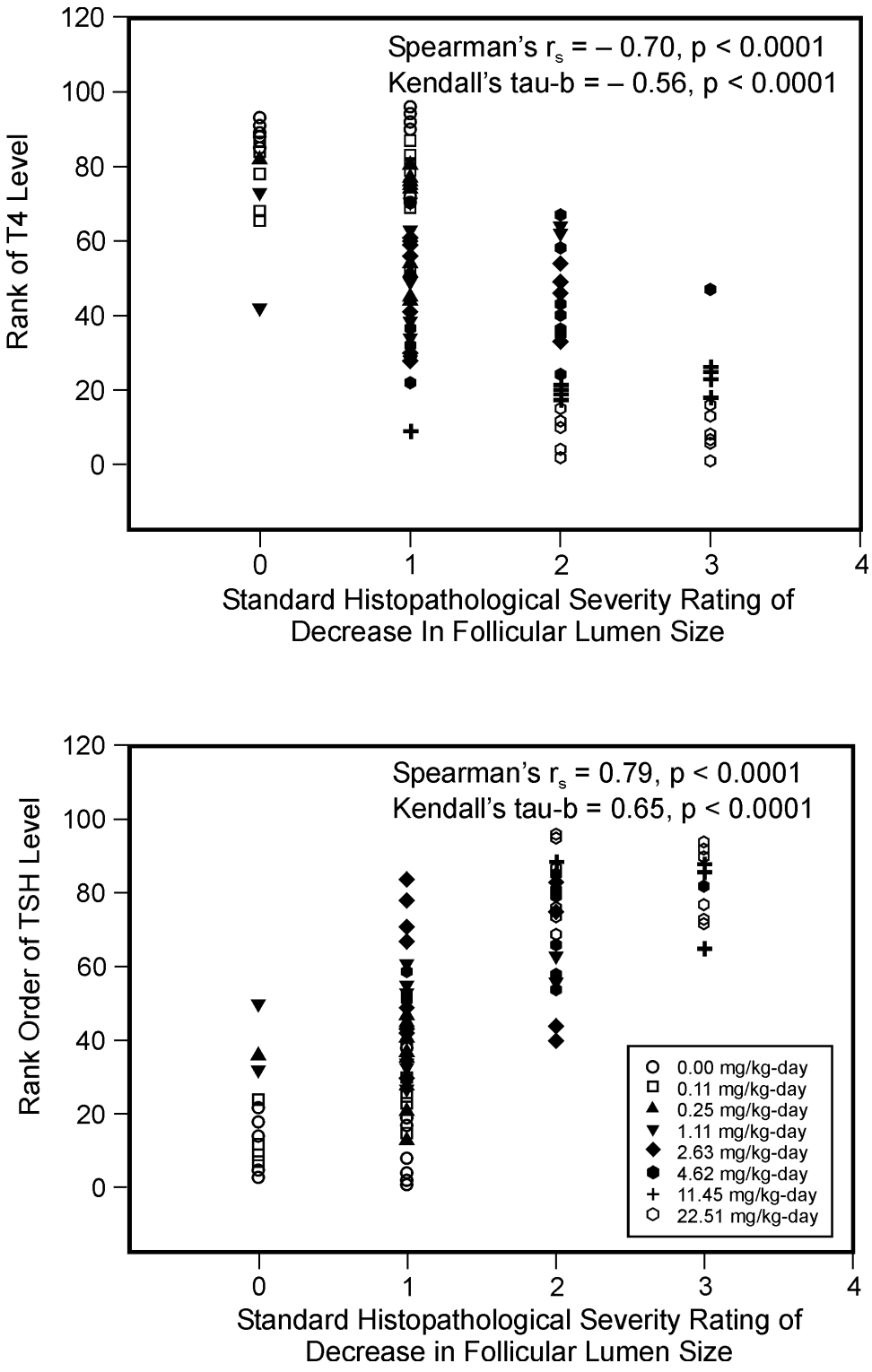

Figure 7A-2. Correlations between the rank order of T4 (top panel) and TSH (bottom panel) versus decrease in follicular lumen size in rats of the 14-day Caldwell et al. (1995) study (Geller, 1998a). Data of Channel (1998a) and Crofton (1998a).

Laboratories, Inc., 1998). As shown in Figure 7A-3 (top panel), T3 and T4 were highly significantly correlated, with low levels of T3 and T4 associated with high doses. Both T4 and TSH were significantly negatively correlated (bottom panel). After 14-days of dosing (Figure 7A-4), T3 and T4 are highly associated (top panel), but there is an unexpected positive relation between T4 and TSH (bottom panel). At the 90-day time point, there are the expected strong correlations between T3 and T4 (Figure 7A-5, top panel) and between T4 and TSH (bottom panel).

Correlations also were performed on the data from the neurodevelopmental study for the PND5 pups (Argus Research Laboratories, Inc., 1998a). T3 and T4 were strongly positively correlated, and T4 and TSH were negatively correlated (Figure 7A-6). Figure 7A-7 (top panel) shows that T4 is negatively associated with a significant decrease in lumen area. Figure 7A-7 (bottom panel) also shows that TSH is positively correlated with a decrease in lumen size.

In total, these correlations lent strong support to the mapping model proposed. Strong correlations were observed between T3 and T4 levels, T3 or T4, and TSH levels, and hormone levels and a decrease in thyroid lumen size. These relationships were most definitive in the Caldwell et al. (1995) study, in which strong correlations existed between the elements of the thyroid hormone homeostasis feedback loop and between hormone levels and severity ratings for lumen size decrease as a measure of thyroid histopathology. In the subchronic (Springborn Laboratories, Inc., 1998) study, correlations were established between hormone levels across both the 14- and 90-day dosing points and for each time point individually. At 14 days of dosing, the expected inverse relationship between T4 and TSH was not found. At the 90-day dosing point, the inverse relationships between T3 or T4 and TSH were found.

Similar relationships were observed in pups on PND5 of the developmental neurotoxicity study (Argus Research Laboratories, Inc., 1998a; York, 1998c). The T4 and TSH were significantly correlated negatively, as expected. The T3, T4, and TSH were all significantly correlated with decrease in lumen size. The correlations in the rat studies support the model that manipulations resulting in decreased levels of circulating thyroid hormone are linked to thyroid histopathological changes that are thought to result directly from elevation of TSH.

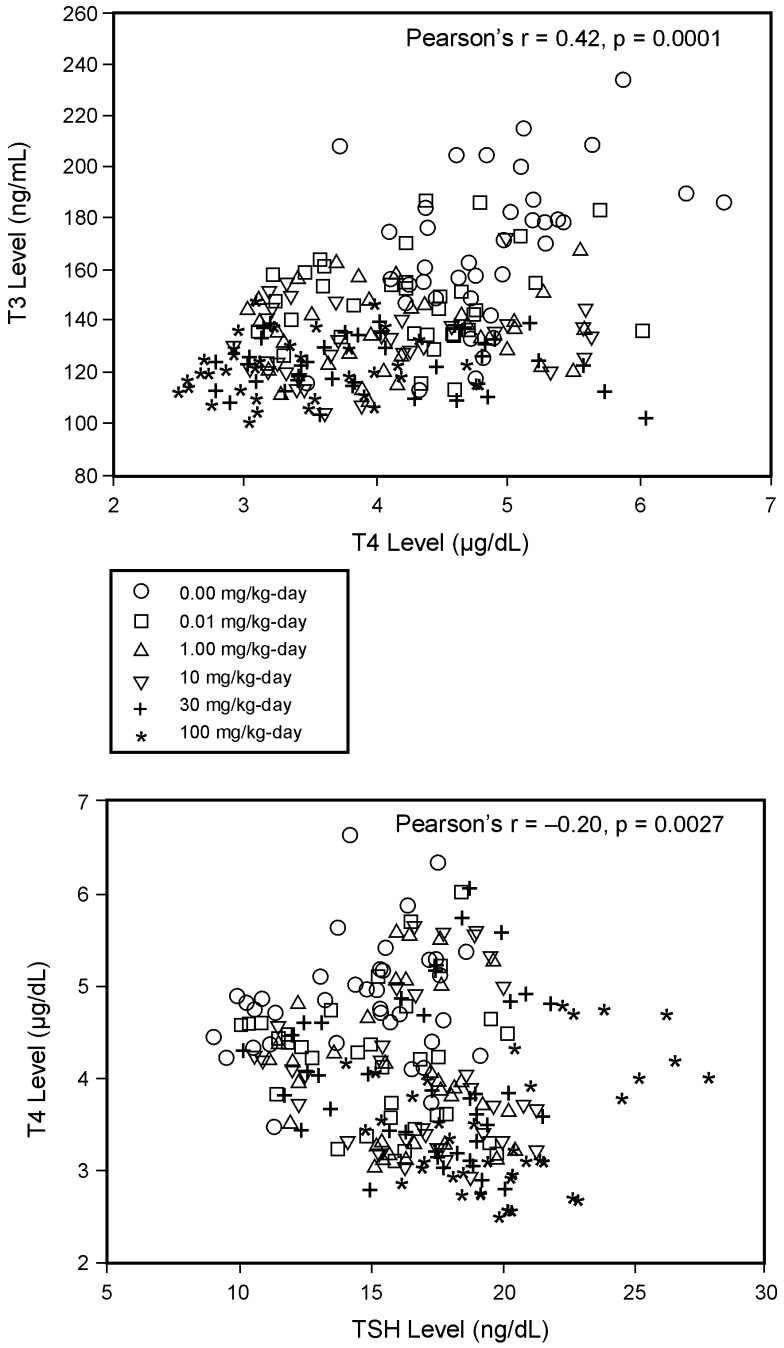

Figure 7A-3. Correlations between T3 versus T4 (top panel) and T4 versus TSH (bottom panel) for the combined male and female data of the 14-day and 90-day time points from the Springborn Laboratories Inc. (1998) subchronic study (Geller, 1998a).

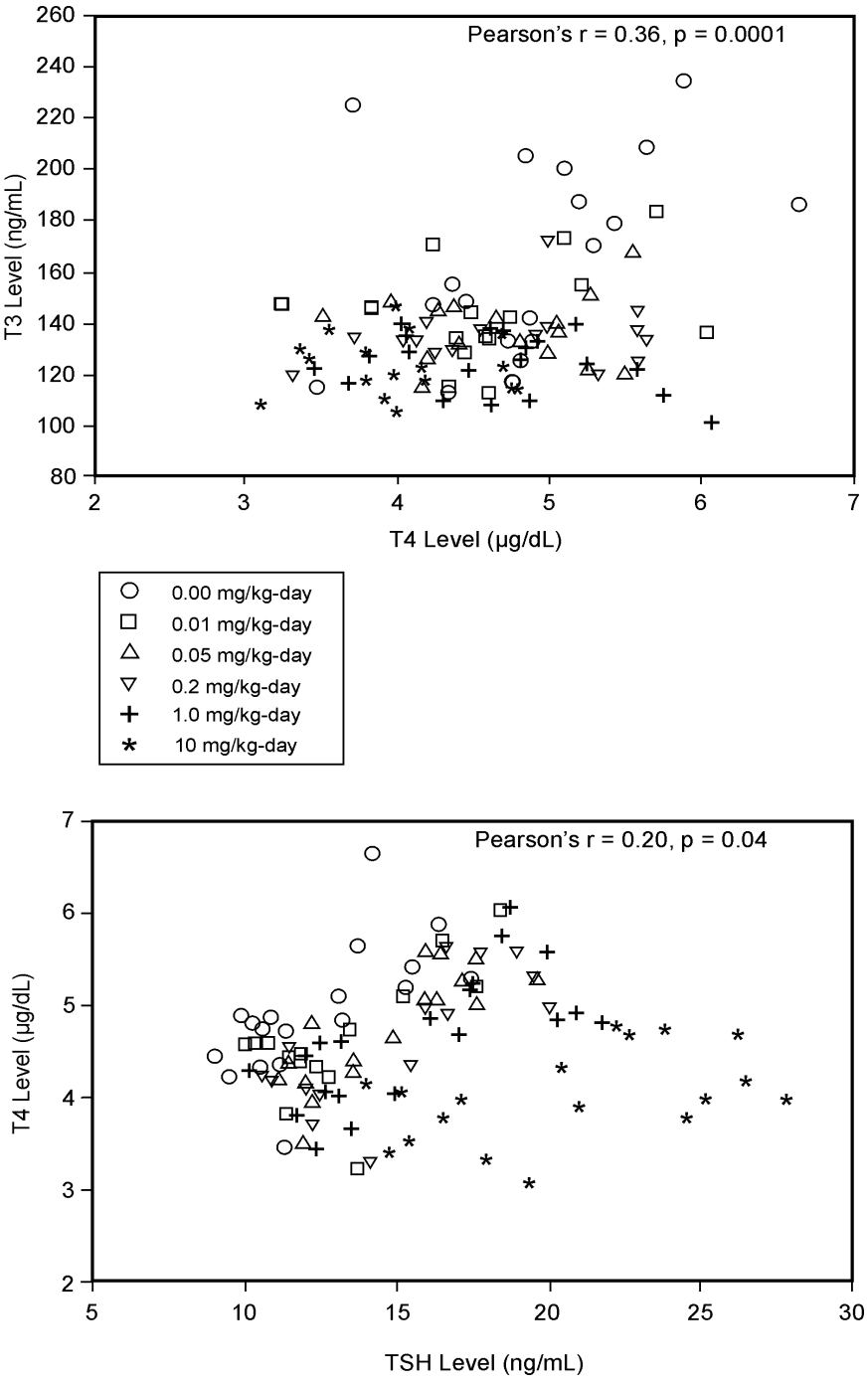

Figure 7A-4. Correlations between T3 versus T4 (top panel) and T4 versus TSH (bottom panel) for the combined male and female data of the 14-day time point from the Springborn Laboratories Inc. (1998) subchronic study in rats (Geller, 1998b).

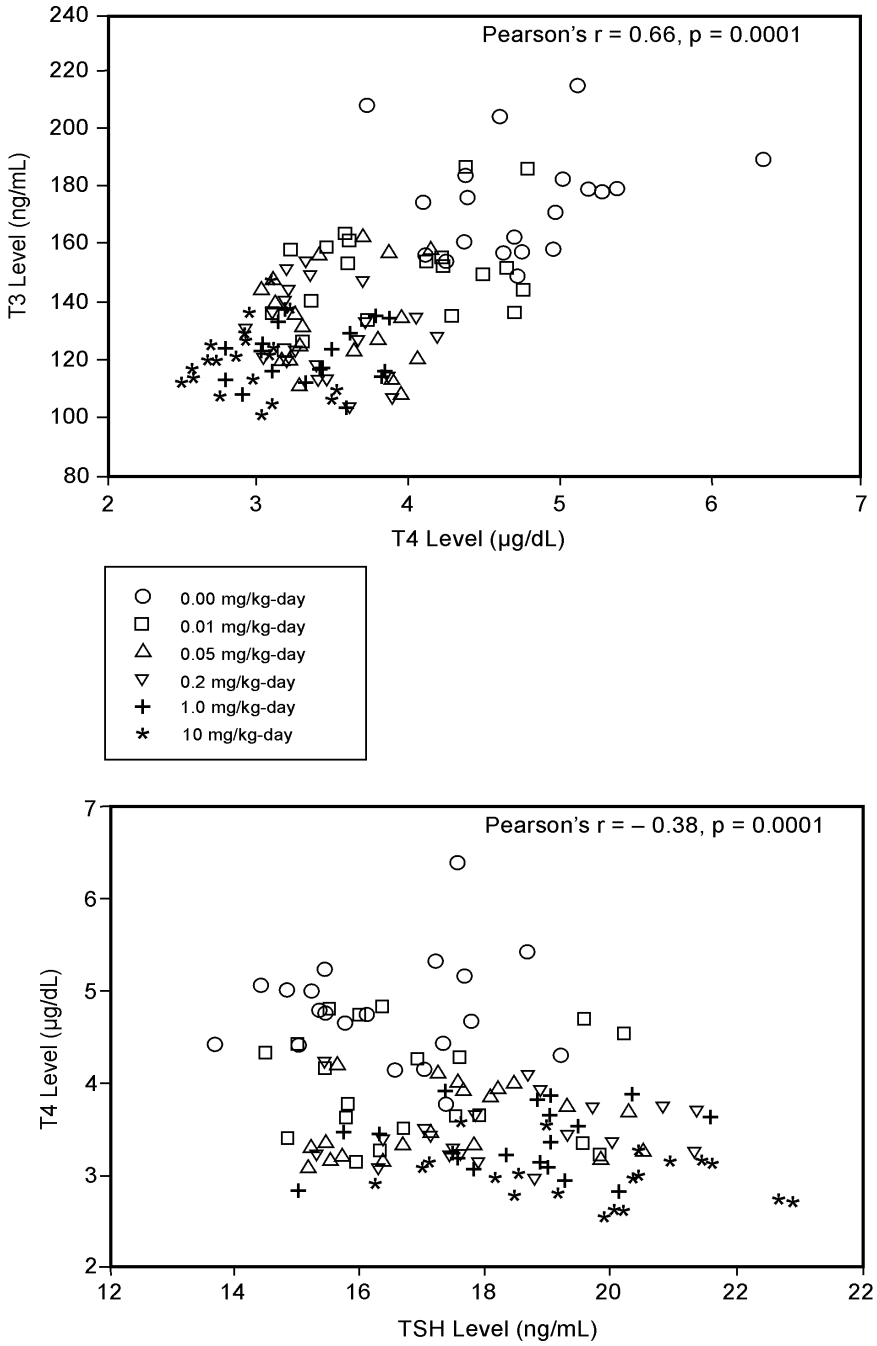

Figure 7A-5. Correlations between T3 versus T4 (top panel) and T4 versus TSH (bottom panel) for the combined male and female data of the 90-day time point from the Springborn Laboratories Inc. (1998) subchronic study in rats (Geller, 1998b).

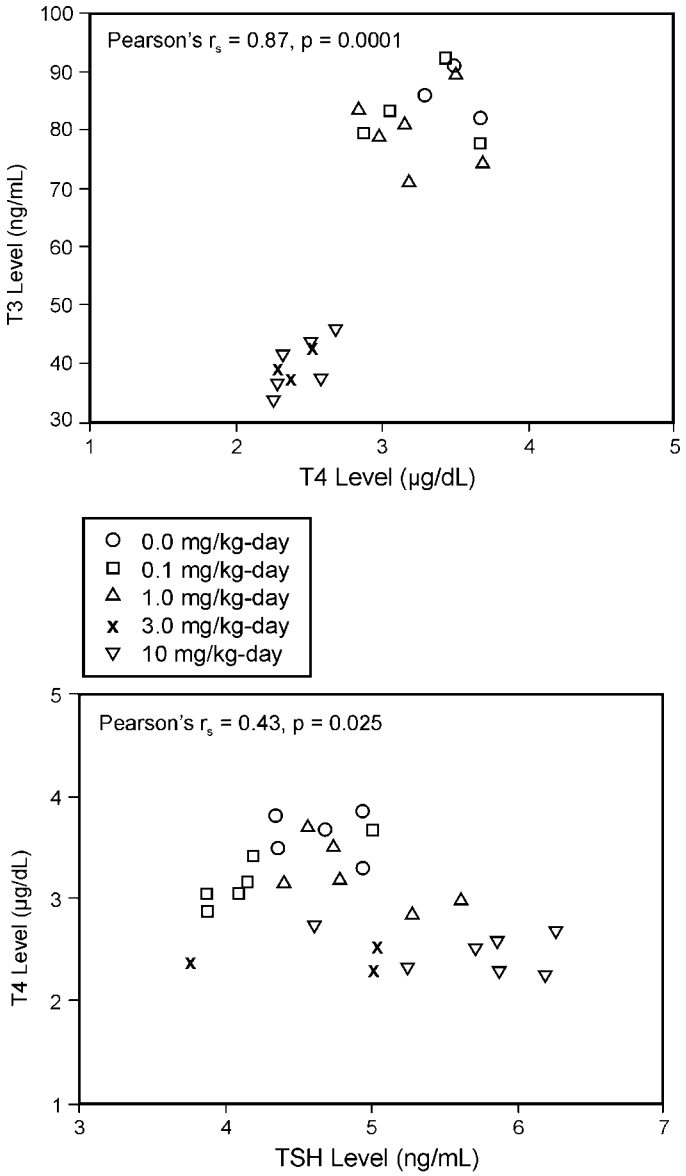

Figure 7A-6. Correlations between T3 versus T4 (top panel) and T4 versus TSH (bottom panel) for the F1-generation rat pups on PND5 in the developmental neurotoxicity study (Geller, 1998b). Data of Argus Research Laboratories, Inc. (1998a), York (1998c), Channel (1998c), and Crofton (1998f).

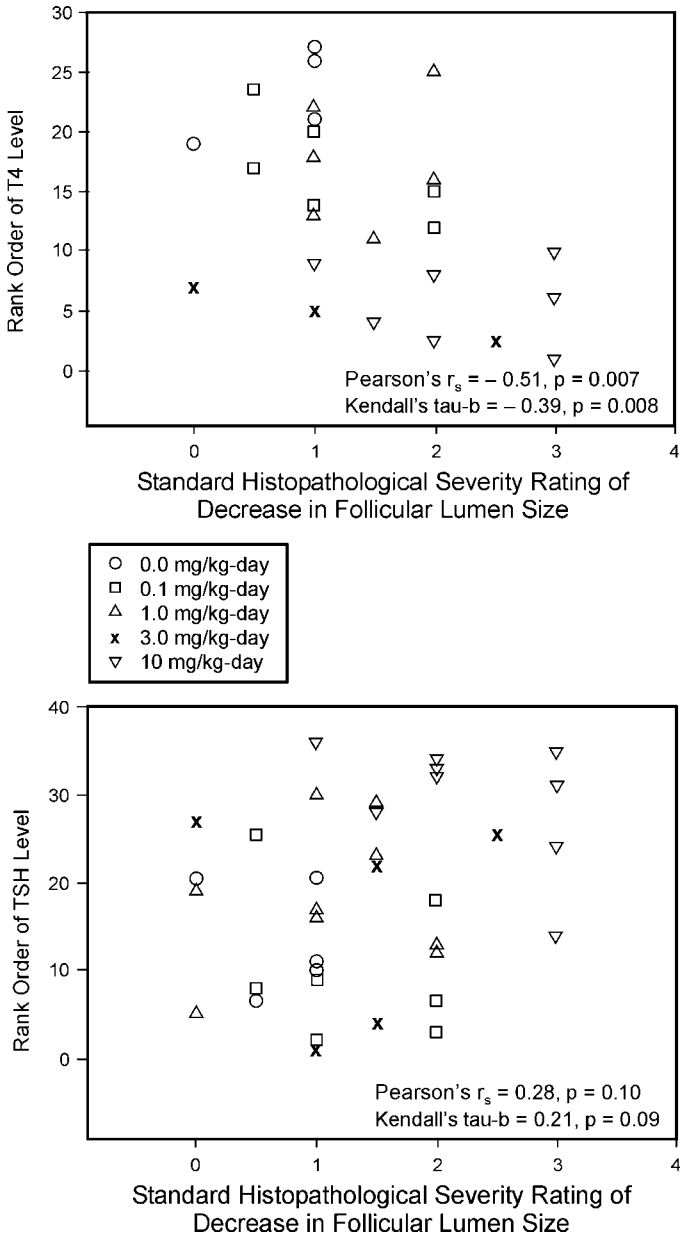

Figure 7A-7. Correlations between the rank order of T4 (top panel) and TSH (bottom panel) versus histopathology severity rating of the decrease in follicular lumen size for the postnatal day 5 (PND5) pups in the 1998 neurodevelopmental study (Geller, 1998b). Data of Argus Research Laboratories, Inc. (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	$f(dose) = control + slope * dose^{power}$
Polynomial function (includes linear and quadratic)	$f(dose) = \beta 0 + \beta 1 * dose + \beta 2 * dose^2 + ...$

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,

and whether the fit was biologically plausible. The latter criterion in most cases, non-monotonicities in the function fit to the data, precluded a fit from consideration. In general, the second order quadratic fits suffered from minima or maxima between the data points from the two highest data points in a given experiment. This consideration also precluded the use of polynomials of higher than second order because these higher order polynomials generally had a local maxima or minima between data points (dose levels) and did not model the data plausibly. It should be noted that the interpretation of the test for constant variance included in the output of the version of the BMD software (version 0.96) was not reliable.

7B.1 Benchmark Dose Estimates Submitted to U.S. Environmental Protection Agency

Two sets of BMD calculations were derived from the Caldwell et al. (1995) 14-day study and submitted to the EPA (Dollarhide and Dourson, 1997). One set was calculated for TSH and T4 levels for males and females separately using the THC (polynomial fit) module of the Crump software, and the model coefficients were restricted to be nonnegative to prevent non-monotonicity. This resulted in linear fits to curvilinear data, and the fits were judged to be poor by both visual inspection and statistical goodness-of-fit criteria (Geller, 1998a).

An alternative approach to calculating BMD estimates based on additional risk also was derived using the Kodell-West algorithm (Kodell-West, 1993). This model generates a quadratic fit to the dose-response data using a maximum likelihood estimator, defines an adverse effect level based on the variability present in the data, and then calculates additional risk. The EPA recalculated these fits using Kodell's SAS® program (Geller, 1998a). The EPA estimates correspond to those previously reported, as shown in Table 7B-2 of Appendix 7B. The coefficients of the fits are provided in Table 7B-3. None of the fits to the data reached statistical significance, and all contain minima (T3 and T4) or maxima (TSH) within the dose range tested. Again, the lack of fit raises difficulties with interpretation and suggests that these estimates should not be used as the basis for risk assessment. The EPA also calculated BMD estimates on ln-transformed data because the Kodell-West algorithm assumes constant variance, and the transformed data is more likely to fit this assumption. The BMD estimates calculated with the ln 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%
TSH	EPA[a]	D&D, 1997[b]	EPA[a]	D&D, 1997[b]	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
ln TSH					1.11
k = 3		0.845		2.115	0.76; 1.91
k = 2		0.181		0.987	0.16; 0.89
T3	EPA[a]	D&D, 1997[b]	EPA[a]	D&D, 1997[b]	0.11[c,d]
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
lnT3					0.11[c,d]
k = 3		0.891		2.244	8.1; 20.4
k = 2		0.190		1.042	1.73; 9.47
T4	EPA[a]	D&D, 1997[b]	EPA[a]	D&D, 1997[b]	0.11[c,d]
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
ln (T4)					0.11[c,d]
k = 3		1.002		2.490	9.11; 22.64
k = 2		0.215		1.169	1.95; 10.63

[a]EPA refers to BMD estimates calculated using SAS® software received from Dr. Ralph Kodell for Kodell-West calculations (Geller, 1998a).
[b]D&D refers to BMDs included in Dollarhide and Dourson (1997).
[c]LOAEL; otherwise, value indicates NOAEL.
[d]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[a]

Responders	B0	B1	B2	Dose (mg/kg-day) of Global Max/Min	p of Fit2[b]
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

[a]Coefficients generated by using SAS software received from Dr. Ralph Kodell (Geller, 1998a). Identical coefficients were generated by using EPA BMD software.
[b]$p > 0.05$ 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[a]	0.272	0.014	0.0002	0.44	0.032	4.55e-4	1.29 1.88
ln TSH[a]	0.099	0.017	0.002	0.44	0.039	4.55e-3	−0.1053
Female TSH[b]	0.077	0.19	0.032	0.1	1.90	0.32	1.125 0.48
Female ln(TSH)[a]	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[a]	0.107	0.00035	0.00	0.1[c]	0.0035	NA	13.07 10.21
lnT3[a]	0.091	0.0004	2e-6	0.1[c]	0.004	2.00e-5	−0.1053
T4[a]	0.303	0.243	0.096	0.1[c]	2.43	0.96[c]	0.506 0.321
ln (T4)[d]	0.172	0.340	0.0997	0.1[c]	3.40	1.00[c]	−0.1053

[a]Unrestricted quadratic: fit nonmonotonic, not significant. Restricted polynomial (linear): fit not significant.
[b]Unrestricted quadratic: fit monotonic but not significant. Restricted polynomial (linear): fit not significant.
[c]LOAEL; otherwise, value is NOAEL.
[d]Unrestricted quadratic: fit not significant, global minimum at approximate high dose. Restricted polynomial (linear): fit not significant.

The BMD estimates calculated with a benchmark response of 10% less than control on the TSH hormone dose-response data are spread over 2.5 orders of magnitude, a similar range to that seen in the distribution of NOAELs calculated for TSH. The BMDL estimates are distributed more widely, over 5 orders of magnitude. These reflect the steepness of the confidence limits on the slope at low doses.

The T3 BMD estimates are spread over approximately two orders of magnitude, similar to the variability seen across studies in the LOAEL and NOAEL estimates. The T3 BMD estimates 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)[a]	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[c]	130.69	0.10[b]
ln(T3)[a]	0.091	0.000002	0.000642	0.478		0.10[b]
T4	0.303	0.243 0.096	2.28 1.299	21.44 16.78	5.06	0.10[b]
ln(T4)[a]	0.172	0.100	1.213	16.89		0.10[b]

[a]For ln-transformed data, only BMDL estimates are displayed.
[b]LOAEL, not NOAEL.
[c]BMDL calculation failed at some values. This means BMDL value may not be accurate.

for only one of the data sets, and this value was approximately 10,000 times lower than the LOAEL. The BMD estimates comprising the 25th to 75th percentiles for T4 cover the same 2.5 orders of magnitude as those covered by the NOAEL and LOAEL estimates for T4. The BMDL estimates for this same percentile range are distributed a little more widely, but do include the range of T4 NOAEL and LOAEL estimates.

7B.3 Summary of U.S. Environmental Protection Agency Benchmark Dose Analyses

The BMD analyses of previously reported estimates for the hormone data of Caldwell et al., (1995)14-day study in rats (Dollarhide and Dourson, 1997) were shown to be based on inadequate model fits. The EPA was able to successfully model the hormone data. However, these estimates raised a number of issues with respect to approaches for these types of data. An alternative may be to pursue a model form of the Hill equation which recently has been used 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 2.52
	Quadratic	0.069	Fit significant, but not monotonic		0.01			
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[a]	NA	0.0033	16.65 38.51
	Quadratic	Fit not significant, nonmonotonic			0.01[a]			
lnT3	Power	0.35	0.000168	Lower limit includes 0	0.01[a]	NA	0.0168	−0.1053
	Quadratic	Fit not significant, nonmonotonic			0.01[a]			
T4	Power	0.203	1.16	0.0035	1.0	0.0035	1.16	0.506 0.603
	Quadratic[b]	0.12	3.27	1.09	1.0	1.09	3.27	
ln (T4)	Power	0.22	1.64	0.04	1.0	0.04	1.64	−0.1053
	Quadratic[b]	0.16	3.25	1.06	1.0	1.06	3.25	

[a]LOAEL; otherwise, value is NOAEL.
[b]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[a]	166.5	0.01[b]
ln(T3)	Power	0.35	Lower limit includes 0	0.000054[a]	43.16[a]		0.01[b]
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

[a]BMDL calculation failed at a number of values. This means BMDL value may not be accurate.
[b]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)OAEL	BMDL: N(L)OAEL	BMR: 10% Control SD
TSH[a]	0.42	0.269	0.018	0.05	5.38	0.36	1.633 1.464
ln TSH[a]	0.40	0.492	0.0796	0.05	9.84	1.6	−0.1053
T3[a]	0.01	No fit	No fit	0.01[b]	NA	NA	17.50 18.924
lnT3[a]	0.01	No fit	No fit	0.01[b]	NA	NA	NA
T4[a]	0.14	6e-6	Lower limit includes 0	0.01[b]	6e-4	NA	0.475 0.576
ln (T4)[a]	0.17	1.10e-5	0.00	0.01[b]	1.1e-3	∞	−0.1053

[a]Unrestricted quadratic: fit nonmonotonic, not significant. Restricted polynomial (linear): fit not significant.
[b]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[a]	4.75	0.01[b]
ln(T4)	Power	0.165	0.00	0.004	4.87		0.01[b]
T3	Power	0.01		No significant fit		174.96	0.01[b]
ln(T3)	Power	0.01		No significant fit			0.01[b]
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

[a]BMDL calculation failed at a number of values. This means BMDL value may not be accurate.
[b]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)[a]

(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
	Power	0.31	4.48	1.43	3.0	1.49	0.48	0.465
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	*1.26*	*0.98*	*1.0*	*1.26*	*0.98*	0.341 0.370
ln (T4)	Nonmonotonic quadratic significant fit	0.50 min = 7.14 mg/kg	*1.18*	*0.92*	*1.0*	*1.18*	*0.92*	
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	*1.053*	*0.644*	*1.00*	*1.053*	*0.644*	
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	*0.822*	*0.538*	*1.00*	*0.822*	*0.538*	

[a]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	0.50[a]	1.26[a] 0.973[a]	2.89[a] 2.16[a]	BMD set to[a] 1,000[a]	3.41	1.0
ln(T4)	0.50[a]	0.92[a]	NC[a]	NC[a]		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

[a]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[a]	0.72	1.94	1.04	None	NA	NA	24.45 162.75
Time[b]	0.69	1.33	0.66	None	NA	NA	18.60 184.78

[a]Number of movements.
[b]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:

- Are ecological risks best characterized as *de minimis* (exposures clearly are below levels of concern), *de manifestis* (risks are clearly significant and require management action to reduce exposures); or somewhere in between and requiring further characterization?
- Are analytical detection methods for determining levels of perchlorate in the environment sufficient, or is it likely that adverse ecological effects occur at levels below current detection limits?
- Is the available ecotoxicological information on perchlorate sufficient, or are additional studies needed?

8.1.2 Scope, Complexity, and Focus

In the previous ERD version of this document (U.S. EPA, 1998d), the available information for this ecological risk assessment was characterized as "very limited" and the assessment was characterized as "screening-level." Information about the environmental levels of perchlorate to which organisms were exposed and about its effects on diverse taxonomic groups was practically nonexistent. Since then, additional information has become available that improves the database in some respects. Most significantly, additional data are available on effect levels in aquatic animals, an aquatic plant, a terrestrial plant, and a soil invertebrate; some of these data are for chronic exposures. Effect levels in rodents have been reevaluated as part of the human health risk assessment for perchlorate, and the ecological implications of those changes are reflected herein. In addition, surveys have been conducted at several sites of known or suspected perchlorate contamination, and environmental and biological materials have been analyzed for perchlorate. Nonetheless, the level of knowledge on this issue must still be characterized as limited because the number of species tested is still quite minimal, and the site surveys focused only on the range of exposures at those sites. This ecological risk assessment is therefore still a screening-level, rather than definitive, assessment. The materials used in the 1998 ERD and those that are new to this present draft, are described in this section.

Interagency Perchlorate Steering Committee Report. Perchlorate Ecological Risk Studies is a report of the IPSC's Ecological Risk/Transport and Transformation Subcommittee, dated November 13, 1998 (Interagency Perchlorate Steering Committee, 1998). This report presents a literature review on perchlorate toxicity to nonmammalian organisms, recognizing that few published studies exist, and a rationale for the selection of a battery of ecotoxicology tests

conducted for the USAF Armstrong Laboratory by EA Engineering, Science and Technology, Inc. It then summarizes those test results, discusses the findings in the context of observed exposures, discusses uncertainties, and makes recommendations for further study. The present report constitutes a reevaluation of much of the same information from EPA's perspective, except that EPA did not examine the open literature studies reviewed by the IPSC subcommittee.

Test Battery Reports. The EA Engineering, Science and Technology, Inc. (1998) final report, *Results of Acute and Chronic Toxicity Testing with Sodium Perchlorate*, dated November 1998, details the test methods and results of the ecotoxicology battery. A follow-up report (EA Engineering, Science and Technology, Inc., 2000) details the test methods and results from additional chronic toxicity testing with the freshwater amphipod *Hyalella azteca* and the fathead minnow *Pimephales promelas*.

Block Environmental Services, Inc., Report. The report, *LC_{50} Aquatic Toxicity Test Results for Ammonium Perchlorate—A Two-Species Chronic Definitive Bioassay* (Block Environmental Services, Inc., 1998) presents additional bioassay results that were not included in the IPSC report.

Algal Toxicity Testing. The EA Engineering, Science and Technology, Inc. (1999) final report, *Results of Algal Toxicity Testing with Sodium Perchlorate*, dated September 1999, details the test methods and results of the ecotoxicological testing with the algae, *Selenastrum capricornutum*.

Frog Embryo Teratogenesis Assay: *Xenopus* (FETAX) Study. The report, FETAX Analysis of Ammonium Perchlorate (Dumont and Bantle, 1998), prepared by the Department of Zoology, Oklahoma State University, and dated May 22, 1998, presents results of the Frog Embryo Teratogenesis Assay: *Xenopus* (FETAX) conducted with ammonium perchlorate. Recent data received by the EPA that the Agency has not yet fully reviewed indicate effects on thyroid function, metamorphosis and sex ratio in developing *Xenopus laevis* (Goleman et al., 2002). These data are made available with this document to the external peers for their review.

Phytotransformation Study. Two sets of studies report on the accumulation and potential degradation of perchlorate by plants. The study, *Laboratory Characterization of Phyto-transformation Products of Perchloroethylene (PCE), Trichloroethylene (TCE) and Perchlorate* (Nzengung, n.d.; Nzengung et al., 1999), examined perchlorate distribution and degradation in experimental systems containing sand, aqueous perchlorate solution, and rooted

cuttings of woody plant species. This study also examined systems containing chopped leaves or microbial mats and aqueous perchlorate solution. A second study, *Potential Species for Phytoremediation of Perchlorate* (Susarla et al., 1999a; Susarla et al., 2000a), reported perchlorate depletion from test media over a ten day period by 13 vascular plant species and their potential for phytoremediation of perchlorate contaminated sites.

Biotransport Investigation Studies. These studies assess the potential for bioaccumulation of perchlorate in food webs by answering the question of whether perchlorate is present in biological receptors. The report *Scientific and Technical Report for Perchlorate Biotransport Investigation: A Study of Perchlorate Occurrence in Selected Ecosystems* (Parsons, 2001) examined perchlorate concentrations in site media and in various ecological receptors at six sites with known or suspected perchlorate contamination: (1) sites associated with withdraw of irrigation water from the Colorado River in the vicinity of Yuma, Arizona; (2) Las Vegas Wash and Lake Mead near Las Vegas, Nevada; (3) Allegany Ballistics Laboratory, Rocket Center, West Virginia; (4) Holloman Air Force Base in Otero County, New Mexico; (5) Naval Surface Warfare Center, Indian Head, Maryland; and (6) Longhorn Army Ammunition Plant, Karnack, Texas. Additional data are available for one of these sites, Longhorn Army Ammunition Plant (LHAAP), Texas, in a paper published by Smith et al. (2001). In both studies, ion chromatography with an AS-16 analytical column was used to measure for perchlorate concentrations. Analyses with this analytical column have been shown to be superior than other columns for detecting and quantifying perchlorate (Ellington and Evans, 2000; Susarla et al., 2000b).

All these sites, except for those in the vicinity of Yuma, are associated with localized contamination related to the manufacture, handling, or use of perchlorate in solid propellants. The Yuma sites are approximately 250 miles downstream along the Colorado River from the Las Vegas Wash and Lake Mead sites; the report suggests that there is no localized source of the perchlorate; therefore, the most likely potential source of any perchlorate contamination in these soils is believed to be Colorado River irrigation water. However, portions of the Yuma Proving Grounds are drained by washes that pass near some of the agricultural locations sampled, and the information provided in the report was not sufficient for ruling out the possibility of contamination from the Yuma Proving Grounds.

8.2 PROBLEM FORMULATION

The characteristics of perchlorate and its sources are described earlier in this document (Chapters 1 and 2). Because this assessment is site independent, this problem formulation focuses on the selection of assessment endpoints, derivation of the conceptual model, and the analysis plan.

8.2.1 Assessment Endpoints

In ecological risk assessment, assessment endpoints are operational definitions of the environmental values to be protected. They are chosen based on policy goals and societal values, their ecological relevance, and their susceptibility to the stressor and are defined in terms of an entity and a property of that entity. The assessment endpoints for this ecological risk assessment are described in the following five subsections.

8.2.1.1 Fish Community Richness and Productivity

Fish communities are valued societally and are ecologically important. The productivity of these communities is important in terms of the support of fisheries. Species richness is important in terms of maintaining biodiversity. This importance is reflected by the use of species sensitivity distributions in the derivation of national ambient water quality criteria and the use of fish species richness as an important component of bioassessment procedures for enforcement of the Clean Water Act.

8.2.1.2 Aquatic Invertebrate Community Richness and Productivity

Aquatic invertebrate communities have little direct societal value but are important to energy and nutrient dynamics in aquatic ecosystems. The productivity of these communities is important in terms of trophic support of fisheries, of other groups of aquatic species, and of some terrestrial insectivores. Species richness is important in terms of maintaining biodiversity. This importance is reflected by the use of species sensitivity distributions in the derivation of national ambient water quality criteria and the use of invertebrate species richness as an important component of bioassessment procedures for enforcement of the Clean Water Act.

8.2.1.3 Aquatic Plant Richness and Productivity

Algae and other aquatic plants have little direct societal value but are important to energy and nutrient dynamics in aquatic ecosystems. Species richness is important in terms of maintaining biodiversity. Because of their importance to the trophic support of fisheries and other aquatic consumers, productivity is an important endpoint for this assemblage.

8.2.1.4 Soil Invertebrate Community Richness and Productivity

Soil invertebrate communities have little direct societal value, but, in nearly all terrestrial ecosystems, they are important to energy and nutrient dynamics and to maintenance of soil structure. The productivity of these communities is also important in terms of trophic support of some terrestrial insectivores. Species richness is important in terms of the policy of maintaining biodiversity.

8.2.1.5 Terrestrial Plant Richness and Productivity

Terrestrial plants are valued highly by society for production of food, fiber, and timber, as well as their aesthetic value. The primary valued property of terrestrial plants is their productivity. As autotrophs, plants are the basis of energy and nutrient dynamics in most terrestrial or aquatic food webs. Moreover, species richness is important in terms of the policy of maintaining biodiversity.

8.2.1.6 Population Productivity of Herbivorous Wildlife

Herbivorous wildlife are included as an endpoint entity because of the apparent bioconcentration of perchlorate in plant foliage. The meadow vole (*Microtus pennsylvanicus*) is used as a representative species for this group. Population productivity is used as the endpoint property because growth and reproduction are generally sensitive properties and because herbivores are valued for their production of food for human and nonhuman carnivores.

8.2.2 Conceptual Models

The conceptual model describes the relationships between sources of perchlorate and the endpoint receptors (Figure 8-1). Sources include spills during the flushing of rockets; the combustion of rocket fuel; the improper disposal of rocket fuel, open burn or open detonation

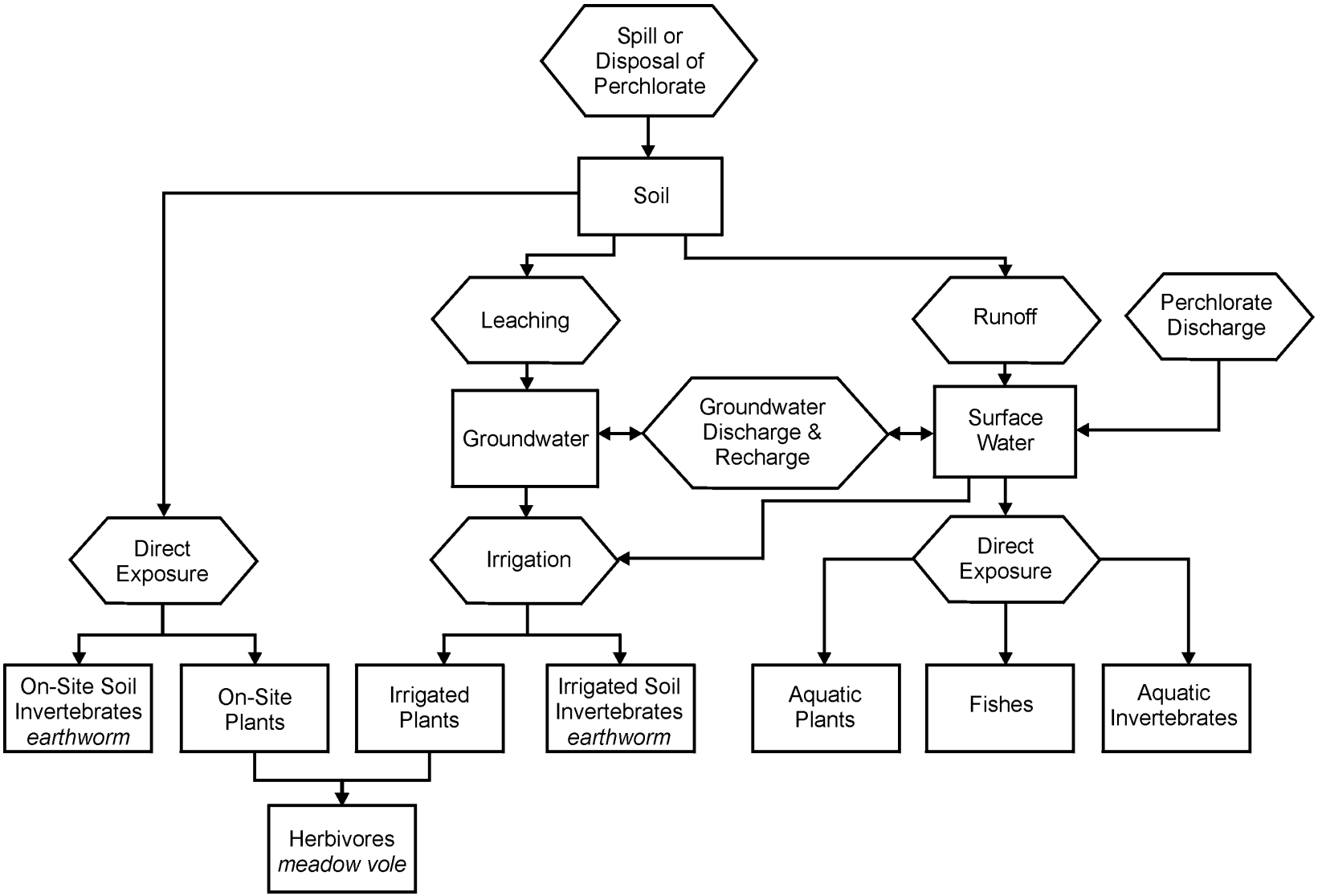

Figure 8-1. A conceptual model of exposure of ecological endpoint receptors to perchlorate. Specific endpoint taxa are identified in italics; all other endpoints are defined at the community level. Processes are designated by hexagonal boxes, compartments by rectangular boxes.

operations, explosives, or manufacturing wastes; and the aqueous discharge of waste water from manufacturing of perchlorate. The most recent information on perchlorate content in fertilizers demonstrates that fertilizer use is unlikely to constitute an environmentally significant source of perchlorate contamination, and ecological risks from this source are not considered further (see Chapter 9). Spills contaminate the soil at the site and, through leaching and run-off, contaminate the surface water and groundwater. The discharge of groundwater to surface water may result in locally high levels of perchlorate in surface waters. Aquatic communities are exposed directly to contaminated surface water; soil invertebrate and plant communities are exposed to perchlorate in soil at the spill site and through irrigation with either surface or groundwater; and herbivorous terrestrial wildlife are exposed through their consumption of plants that have bioconcentrated perchlorate. However, the potential for transfer of perchlorate further up the terrestrial food web is currently unknown.

This conceptual model is relatively simple because it excludes some potential routes and receptors. Dietary exposures are excluded from aquatic systems because, as of this writing, available data have not shown perchlorate to bioconcentrate to any significant extent. Information newly received form the U.S. Army Corps of Engineers (Condike, 2001) report on the analysis of environmental samples from perchlorate-contaminated water bodies near McGregor Naval Weapons Industrial Reserve Plant (NWIRP), TX, and purports to show fish tissue concentrations that exceed comparable water concentrations. These data suggest that perchlorate not only accumulates but is bioconcentrated. This information, which has not yet been fully reviewed by the U.S. EPA, is herewith made available with this document to external peers for their review.

Wildlife are assumed to have negligible exposure from air or from direct exposure to soil. Exposures of wetlands to groundwater or surface water are not included explicitly because their exposures and effects are assumed to be equivalent to irrigation exposures. That is, plants and invertebrates are assumed to be exposed to pore-water concentrations equal to surface or groundwater concentrations. Exposures to contaminated sediments also are not included explicitly because they are believed to be equivalent to surface water exposures. Perchlorate salts are highly water soluble and the anion is unlikely to adsorb to anionic particles, such as soils or humic substances, to a significant extent. Therefore, sediment exposures are expected to be dominated by exposure to pore water, which is assumed to be equal to surface water.

8.2.3 Analysis Plan

This screening assessment uses existing information to determine whether the existing environmental contamination by perchlorate poses a clearly significant risk, insignificant risk, or an ambiguous risk. The analysis of effects will consist of the derivation of screening benchmarks through the application of conservative extrapolation models. The analysis of exposure for ecological endpoints consists of measured concentrations reported in Chapter 1 or derived from Parsons (2001) or Smith et al. (2001). Soil exposure estimates are based on exposure to perchlorate in irrigation water.

8.3 ANALYSIS

8.3.1 Characterization of Exposure

8.3.1.1 Water Concentrations

As previously described, fishes, aquatic invertebrates, and aquatic plants may be exposed directly to concentrations of perchlorate in surface waters. These concentrations may result from surface run-off from perchlorate-contaminated soil, from leaching of perchlorate from contaminated soil via shallow groundwater, or from direct discharge of aqueous wastes. Surface or groundwater may be used for irrigation, resulting in direct exposure of soil invertebrates or plants (Figure 8-1).

Perchlorate salts are dissolved readily given the conditions under which the contamination has occurred, releasing the perchlorate anion and the associated cation. Sorption is not expected to attenuate perchlorate because it absorbs weakly to most soil minerals, and natural chemical reduction in the environment is not expected to be significant. Consequently, perchlorate is both very mobile in aqueous systems and persistent for many decades under typical ground and surface water conditions (Section 1.1).

Limited information is available on perchlorate concentrations in surface waters. Perchlorate from an ammonium perchlorate manufacturing area has been detected at 4 to 16 μg/L downstream in Lake Mead and the Colorado River (Section 1.2). Information on the frequency or central tendency (mean or median) of perchlorate detection in those water bodies was not available for this review, but it is assumed that some aquatic organisms are exposed chronically

to concentrations as high as 16 μg/L. On the other hand, perchlorate concentrations have been measured as high as 0.37% (37×10^6 μg/L) in groundwater-monitoring wells at facilities that manufacture or test rocket motors and at 280 μg/L in public water supply wells (Section 1.2) Smaller surface water bodies, including some that are supplied primarily by groundwater, are likely to exist near sites of soil contamination and to have perchlorate concentrations much higher than those reported for Lake Mead and the Colorado River. A spring associated with the Las Vegas Wash site had concentrations of 1.0 to 1.3×10^5 μg/L in surface water (Parsons, 2001). Perchlorate concentrations in a pond (INF Pond) that receives water from the pump and treat system at the Longhorn Army Ammunition Plant near Karnack, TX ranged from 30,776 to 31,438 μg/L in November 1999 (Smith et al., 2001) and ranged from 3500 to 3800 μg/L in September 2000 (Parsons, 2001). It is also possible that, within large water bodies, there are locally elevated concentrations at sites of groundwater discharge. In the vicinity of a sediment delta created by the Las Vegas Wash in Las Vegas Bay of Lake Mead, Parsons (2001) documents a maximum perchlorate concentration of 68 μg/L in surface water. At the Allegany Ballistics Laboratory in Rocket Center, WV, discharge water from a Comprehensive Environmental Response Compensation, and Liability Act (CERCLA) groundwater pump and treat facility to the North Branch Potomac River contained 250 to 280 μg/L perchlorate (Parsons 2001). Surface water concentrations in Town Gut Marsh adjacent to the Naval Surface Warfare Center at Indian Head, MD ranged from not detected (reporting limit = 4.0 μg/L) to 25 μg/L. It should be noted that the groundwater pump and treat facilities either at Longhorn Army Ammunition Plant or Allegany Ballistics Laboratory were not equipped with facilities to treat perchlorate in water.

Surface water concentrations in Harrison Bayou below the discharge point for the INF pond at LHAAP also ranged from undetectable (reporting limit = 4.0 μg/L) to 4.0 μg/L (Parsons, 2001; Smith et al., 2001). However, Smith et al. (2001) point out that water from the pond is discharged to Harrison Bayou only during periods when Harrison Bayou is flowing, and neither study apparently sampled Harrison Bayou when water was being discharged from the pond. Therefore, higher concentrations of perchlorate in surface water of Harrison Bayou are likely to be measured at other times.

It is assumed that irrigation waters pumped from Lake Mead or the Colorado River are in the range of downstream concentrations given above (i.e., 4 - 16 μg/L). Groundwater irrigation

may be contaminated at levels similar to those observed in public water supplies (≤280 μg/L), unless the well is appreciably nearer a perchlorate-contaminated site.

8.3.1.2 Aquatic Bioaccumulation

As discussed above, little information has been previously available on the potential for the perchlorate ion to accumulate in animal tissues. The studies outlined in the Parsons (2001) report sought to answer the question whether perchlorate is present in ecological receptors. In these studies, concentrations of perchlorate in aquatic vegetation, fish, amphibians, aquatic invertebrates, and birds were compared to surface-water, pore-water, and sediment concentrations from the same water body. This information is supplemented by the additional studies conducted at LHAAP by Smith et al. (2001).

When perchlorate concentrations in physical media (i.e., surface water or sediment) were greater than the reporting limits for biological media (≥300 ppb [μg/L or μg/kg] in Parsons [2001]), concentrations in aquatic vegetation were similar to or greater than the concentrations in surface water or pore water; but concentrations in fish, amphibians, or invertebrates were less. In Smith et al. (2001) reported the detection of high concentrations of perchlorate in the INF Pond and lower concentrations in aquatic vegetation and in animals than in surface water or sediments.

In Parsons (2001), when perchlorate concentrations in the physical media were lower, concentrations in aquatic vegetation or amphibians were in a few cases greater than the concentrations in surface water or sediment; but in most cases, perchlorate was not detected in aquatic receptors. However, our understanding of bioaccumulation of perchlorate in this lower concentration range is limited because the reporting limits in the Parsons (2001) studies for perchlorate in animal tissues (i.e., 300-400 μg/kg) were greater then the reporting limits for surface water or pore water (i.e., ≈ 4 μg/L) or for sediments (i.e., ≈ 80 μg/L).

Although Smith et al. (2001) do not identify their reporting limits, their reporting limits for biological tissues appear to be less (i.e., ≈70 μg/kg based on their lowest detected concentration) than those of Parsons (2001). In the Smith et al. (2001) study of LHAAP, detected perchlorate concentrations were similar in surface water (44-85 μg/L), sediments (78 μg/kg), and fish tissues (83-131 μg/kg) at Goose Prairie Creek. In Harrison Bayou, the single detected concentration in surface water (4 μg/L) was less than detected concentrations in animal tissues (86-356 μg/kg).

However, as the authors discuss, the measured concentration in surface water in Harrison Bayou is likely less than when water is being discharged from the INF Pond (Smith et al., 2001). In addition, the study did not collect sufficient samples from any one site and medium or species for any significant statistical comparisons to be made.

Information newly received form the U.S. Army Corps of Engineers (Condike, 2001) report on the analysis of environmental samples from perchlorate-contaminated water bodies near McGregor Naval Weapons Industrial Reserve plant (NWIRP), TX, and purports to show fish tissue concentrations that exceed comparable water concentrations. These data suggest that perchlorate not only accumulates but is bioconcentrated. This information, which has not yet been fully reviewed by the U.S. EPA, is herewith made available with this document to external peers for their review.

The above information indicates that perchlorate may bioaccumulate in aquatic organisms living in contaminated waters, but it does not resolve the question of whether perchlorate may bioconcentrate in the tissues of aquatic organisms to levels exceeding the surface water concentrations. The existing data are also insufficient to determine whether there is further trophic transfer of perchlorate within aquatic food webs.

8.3.1.3 Soil Levels

On-site soils may be contaminated by direct spills of perchlorate solutions from flushing rockets, combustion of rocket fuel, improper disposal of rocket fuel, open burn/open detonation operations, explosives, or manufacturing wastes. Perchlorate concentration measurements at disposal sites range from less than 1 to 1470 mg/kg (Parsons, 2001). Off-site soils may be contaminated via irrigation (Figure 8-1). Because of the high water solubility of perchlorate salts, perchlorate is unlikely to accumulate via adsorption to irrigated soils, and aqueous perchlorate was not found to adsorb to sand in laboratory reactors (Nzengung, n.d.). By gross approximation, then, soil concentrations (expressed as milligrams per kilogram) would be unlikely to exceed the concentrations (expressed as milligrams per liter) in irrigation water. Similarly, concentrations of perchlorate in soil pore water may be assumed to be equal to the concentration in irrigation water, both in the field and in soil toxicity tests. However, the concentration of perchlorate salts in irrigated soils with high evaporation rates cannot be ruled out. At the Yuma site, soils are irrigated with water from the Colorado River, and concentrations

of perchlorate in surface-water samples collected near the irrigation intake locations ranged from 0.003 to 0.006 mg/L. In surface soil, the single detection (0.090 mg/kg) was well above the concurrently-measured water concentrations, as were the perchlorate detection limits in soil (0.079 to 0.080 mg/kg). The relatively higher detection limits in soil, the limited nature of the sampling in soil and water, and the lack of information about potential sources other than irrigation water (see Section 8.1.2) complicate the interpretation of the presence and fate of perchlorate in irrigated soils.

8.3.1.4 Uptake by Vegetation

Several laboratory experiments have examined plant uptake of perchlorate from solution culture. Experiments with candidate plants for use in the phytoremediation of perchlorate-contaminated sites showed that perchlorate may concentrate in vegetation (Nzengung, n.d.; Susarla et al., 2000a). Nzengung (n.d.) used rooted cuttings of woody plants, willow (*Salix* spp.), Eastern Cottonwood (further identified only as "poplar"), and eucalyptus (*Eucalyptus cineria*) planted in sand with nutrient solution containing perchlorate at 20 or 100 mg/L for 24 to 42 days. In each case, perchlorate was taken up and concentrated in aerial plant parts, especially leaves. Concentration factors, expressed as the ratio of leaf concentration (mg/kg wet weight) to initial solution concentration (mg/L), ranged from 7.5 to 25.

Susarla et al. (2000a) used seedlings or rooted cuttings of 13 vascular plant species, planted in sand with nutrients, and exposed for ten days to 0.2, 2.0 or 20 mg/L perchlorate. These researchers also reported depletion of perchlorate from test media. Qualitative analyses suggested accumulation of perchlorate in the aerial tissues of most of the species analyzed. Using their data and the approach reported by Nzengung above, we calculated concentration factors ranging from 0 to 330.

Nzengung (n.d.) and Susarla et al. (2000a) reported that perchlorate accumulated primarily in the leaves, followed by stems, then roots. Predicted perchlorate breakdown products, chlorate, chlorite, and chloride were detected in plant tissues in both studies, but quantitative evidence was not presented. In addition to this lack of quantitative data, there are other concerns related to the potential for plants to degrade perchlorate. First, information concerning accumulation and potential transformation is limited to a few studies by these two laboratories. Second, the method used for perchlorate analysis yielded estimates of perchlorate in fertilizer that were

subsequently found to be overestimated by 30 to 150% (Susarla et al., 2000b). Third, no physiological explanation has been suggested for why plants should accumulate this salt far in excess of concentrations in water or groundwater, though it appears this may be simply a function of water uptake rates to meet transpirational losses. Fourth, these two studies were short-term, material depletion studies, a type of study we believe will overestimate long-term accumulation rates because some of the "response" is likely the result of factors not related to the chemical in question. There is ample evidence from salt accumulation studies of plants to suggest that the initial increases in perchlorate accumulation by plants may be due to a salt effect; that is, nutrient salts are initially indistinguishable from perchlorate salts in that they all represent an ionic imbalance across the cell wall. One of the confounding issues that can only be determined with longer-term studies is the effect of increased cell sap salinity on additional perchlorate uptake. As sap salinity increases, there should be an increase in H_2O uptake, further increasing the perchlorate concentrations. This will continue only until a certain concentration of salts, including perchlorate, is reached, at which time the plant will close its stomata and shunt sap salts to vacuoles.

In addition to the above stated concerns, there is no reason to expect that these are steady-state concentration factors. These experiments were designed to quantify phytotransformation of an initially introduced perchlorate quantity, rather than bioconcentration, with respect to an ambient perchlorate concentration. As the perchlorate-amended nutrient solution was transpired, and some perchlorate was taken up, it was replenished by solution, without added perchlorate; thus, perchlorate in the test chamber diminished throughout the experiment. Concentration factors that would be observed at steady state, such as may result from continual irrigation with perchlorate-contaminated water, cannot be estimated from this study. Because of the uncertainties associated with both perchlorate accumulation and degradation by plants, a simple, conservative, screening-level assumption that concentrations in leaves can exceed water concentrations by a factor of 100 was made.

If irrigation is from surface water sources similar to the Colorado River or Lake Mead, with concentrations as high as 16 μg/L, then plant concentrations are assumed to be as high as 1.6 mg/kg. If irrigation is from groundwater sources similar to potable water supplies, with concentrations as high as 280 μg/L, then plant concentrations will be assumed to be as high as 28 mg/kg.

Concentrations in plant tissues and soils also have been measured in the field. Ellington et al. (2001) measured perchlorate concentrations in leaves of tobacco, *Nicotiana tabacum* var. K326, field-grown in soil amended with Chilean saltpeter, which is naturally high in perchlorate. Perchlorate concentrations (± SD) in leaf lamina from the 1999 crop were 96.0 ± 0.6 mg/kg dry weight and 14.6 ± 0.1 mg/kg wet weight; concentration in a composite soil sample collected in December 1999 was 0.34 ± <0.01 mg/kg dry weight. The concentration factor in this study was approximately 43, on the basis of wet weight in leaf lamina and dry weight in soil.

The field studies by Parsons (2001) found that, for various sites, wet-weight perchlorate concentrations in terrestrial vegetation samples were 1.5 to 80 times the wet-weight concentrations in soil samples. The data from one site (i.e., Building 25C) at LHAAP (Smith et al., 2001) seem to indicate greater concentration factors, but the soil and plant samples were taken at different times of the year (i.e., January and October, respectively) and only one sample each of three plant species was analyzed.

Soil-to-vegetation concentration factors derived from the above field studies were similar in magnitude, but when using them for risk assessment care should be taken to note the different bases; exposure concentration was variously reported as mg/kg wet weight in soil or mg/kg dry weight in soil. The maximum measured concentration in vegetation at irrigated sites in the vicinity of Yuma, Arizona was 1.0 mg/kg wet weight. At sites with soil contamination related to the manufacture, handling, or use of perchlorate in solid propellants, maximum plant concentrations were 428 mg/kg wet weight at a spring; 99.2 mg/kg wet weight at a site upstream from Lake Las Vegas in the Las Vegas Wash area of the Lake Mead Recreational Area, Nevada; and 300 mg/kg wet weight at the Burn Area of Allegany Ballistics Laboratory, West Virginia. In most cases, detection limits were ~0.4 mg/kg wet weight.

8.3.1.5 Herbivore Exposure

The representative herbivore selected for this assessment, *M. pennsylvanicus*, has a diet consisting mainly of monocot and dicot shoots, has an estimated food consumption rate of 0.005 kg/day wet weight, and a body weight of 0.044 kg (Sample and Suter, 1994). Using the assumptions stated above, daily exposures resulting from surface water and groundwater irrigation may be as high as 0.18 mg/kg-day and 3.2 mg/kg-day, respectively. Daily exposures resulting from maximum measured concentrations in plants range from 0.11 mg/kg-day at the

irrigated sites in the vicinity of Yuma to 49 mg/kg-day for the sites with direct soil contamination.

In the Parsons (2001) studies, except when concentrations in surface soils were high (i.e., ≥9000 μg/kg), perchlorate was not detected in terrestrial birds, mammals, or insects with reporting limits of 300 to 400 μg/kg. The vertebrates collected varied substantially between sites, but the birds collected include the mourning dove (*Zenaida macroura*), tree swallow (*Tachycineta bicolor*), roughwing swallow (*Stelgidopteryx serripennis*), lesser nighthawk (*Chordeiles acutipennis*), nighthawk (*C. minor*); Gambel's quail (Callipepla gambelii), starling (*Sturnus vulgaris*); American crow (*Corvus brachyrhynchus*), eastern bluebird (*Sialia sialis*), eastern phoebe (*Sayornis phoebe*), and blue grosbeak (*Guiraca caerulea*). The mammals collected include the cactus mouse (*Peromyscus eremicus*), rock pocket mouse (*Chaetodipus intermeius*), Audubon's cottontail (*Sylvilagus audubonii*), deer mouse (*P. maniculatus*), long-tailed pocket mouse (*Perognathus formosus*), western pipestrelle (*Pipistrellus hesperus*), house mouse (*Mus musculus*), white-footed mouse (*Peromyscus leucopus*) meadow vole (*Microtus pennsylvanicus*), Merriam's kangaroo rat (*Dipodomys merriami*), desert pocket mouse (*C. penicillatus*), hispid cotton rat (*Sigmodon hispidus*), western harvest mouse (*Reithrodontomys megalotis*), marsh rice rat (*Oryzomys palustris*); northern short-tailed shrew (*Blarina brevicauda*), racoon (*Procyon lotor*), eastern harvest mouse (*R. fulvescens*), little brown bat (*Myotis lucifugus*), eastern cottontail (*S. floridanus*). At those sites where perchlorate concentrations in surface soils were high, perchlorate concentrations in potential herbivore tissues were generally an order of magnitude or more less than that in vegetation. At one site, Yuma, with lower perchlorate concentrations in soil (i.e., mean of all results = 81 μg/kg), perchlorate was detected in a single terrestrial reptile sample (brush lizard, *Urosaurus graciosus*), but this detection was lower than the mean perchlorate concentration in vegetation. Although detected soil concentrations were lower (i.e., 50 to 322 μg/kg) in Smith et al. (2001), the concentrations of perchlorate in two composite samples of livers from harvest mice (*Reithrodontomys fulvescens*) were several orders of magnitude less than the detected 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 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 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 Description		Endpoints (as mg/L perchlorate in water or mg/kg in soil or sand)[a]				
Test Species	**Age**	**Duration**	**Acute LC_{50} (95% CL)**	**NOEC**	**LOEC**	**ChV**	**IC_{25} (95% CL)**
Sodium perchlorate ($NaClO_4$)[b] tests (EA Engineering, Science and Technology, Inc., 1998)							
Daphnia magna	<24 hr	Acute (48-hr)	490 (406 - 591)	—	—	—	—
Pimephales promelas	12 - 13 days	Acute (96-hr)	1,655 (1,507 - 1,817)	—	—	—	—
Ceriodaphnia dubia	<24 hr	Chronic (7-day)	66 (40-144) [48-h]	10	33	18.2	17 (8.1 - 20.5)
Pimephales promelas	<24 hr	Subchronic (7-day)	614 (540 - 714) [96-h]	155	280[c]	208[c]	212[c] (175 - 231)[c]
Lactuca sativa	<24 hr	Subchronic (7-day)	614 (540 - 714) [96-h]	155	280[c]	208[c]	212[c] (175 - 231)[c]
Lactuca sativa		Chronic definitive (28-d, sand)		<80	80	<80	41
Lactuca sativa		Chronic definitive (28-d, soil)		40	40	56.6	30
Lactuca sativa		Chronic definitive (28-d, sand)		20	40	28.3	34.3
Eisenia foetida		Acute definitive (7 day/14 day, soil)	4,450/4,450	—	—	—	—
Sodium perchlorate ($NaClO_4$)[b] tests (EA Engineering, Science and Technology, Inc., 2000)							
Pimephales promelas	Embryo	Chronic (35-day, early life stage)	> 490 [96-hr]	> 490[d] <28[e]	> 490[d] 28[e]	> 490[d]	> 490[d] <28[e]
Hyalella azteca	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 Description		Endpoints (as mg/L perchlorate in water or mg/kg in soil or sand)[a]				
Test Species	**Age**	**Duration**	**Acute LC_{50} (95% CL)**	**NOEC**	**LOEC**	**ChV**	**IC_{25} (95% CL)**
Sodium perchlorate ($NaClO_4$)[b] tests (EA Engineering, Science, and Technology, 1999)							
Selenastrum capriconutum	7 days	Acute (96-hr)	—	500	1,200	775	615 (149-1,126)
Ammonium perchlorate (NH_4ClO_4)[f] tests (Block Environmental Services, Inc., 1998)							
Ceriodaphnia dubia	<24 hr [g]	Chronic (6-day)	77.8 [6-days]	9.6	24	15	24
Pimephales promelas	<24 hr [g]	Subchronic (7-day)	270 [7-days]	9.6	96	30	114
Ammonium perchlorate (NH_4ClO_4)[f] tests (Dumont and Bantle, 1998)							
Xenopus	Embryo	96-hr	420[h]	—	—	—	—
Xenopus	Embryo	96-hr	336[h]	—	—	—	—

[a]Notation: LC_{50} = Concentration lethal to 50% of individuals; NOEC = No-observed-effect concentration; LOEC = Lowest-observed-effect concentration; ChV = Chronic value; IC_{25} = Concentration inhibiting a process (e.g., growth, reproduction) by 25%; CL = confidence limits.
[b]Sodium chloride control showed no adverse effects of sodium ion except as indicated. Reported values are based on nominal concentrations.
[c]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.
[d]Standard endpoints: survival, growth, biomass
[e]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.
[f]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.
[g]Not reported; assumed based on standard protocols.
[h]IC_{50} for malformations.

TABLE 8-2. PROCEDURE FOR DERIVING TIER II WATER QUALITY VALUES FOR SODIUM PERCHLORATE

Step	Value (mg/L ClO_4^-)	Rationale
Identify the lowest genus mean acute value (GMAV)	66	Lowest GMAV is for genus *Ceriodaphnia* (based on *C. dubia*)
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 *C. dubia*: ACR=AV ÷ CV = 66 ÷ 18.2 = 3.6 For *P. promelas*, 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 *H. azteca* 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 *P. promelas* 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 *P. promelas* value is carried through and shown in parentheses.)

the possibility that if perchlorate were associated with a less toxic cation, the SCV may be lower than is necessary to protect against perchlorate ion toxicity. Further tests with perchlorate may be needed to assess potentially less toxic cations.

Similar chronic (or subchronic) tests were conducted with ammonium perchlorate (Table 8-1). Results, expressed as ClO_4^-, were very similar for *C. dubia*, but *P. promelas* was more sensitive to ammonium perchlorate than to sodium perchlorate. However, Tier II values for ammonium perchlorate are not presented for several reasons, including the lack of ammonium controls which makes it difficult to determine whether the observed effects were caused by the perchlorate anion; the lack of acute values for *C. dubia* and *P. Pimephales*; and the fact that the FETAX (*Xenopus*) test is designed to detect teratogenic potential, and the embryo is not a particularly sensitive life stage for toxicity. When perchlorate is administered as the ammonium salt, the ammonium ion concentration expressed on an ammonia-nitrogen (in milligrams of NH_3-N/L) basis is 14% of the respective perchlorate ion concentration. A Lowest-Observed-Effect-Concentration (LOEC) for *C. dubia* of 24 mg/L perchlorate (Table 8-1) thus corresponds to 3.4 mg NH_3-N/L. Based on a species mean chronic value (SMCV) of 13 mg NH_3-N/L for *C. dubia* exposed to ammonia alone (U.S. Environmental Protection Agency, 1998h), the former value is probably too low to be responsible for the observed effects[1]. On the other hand, the LOEC observed with *P. promelas* at 96 mg/L (Table 8-1) corresponds to 14 mg NH_3-N/L, which exceeds a SMCV of 3.09 mg NH_3-N/L (U.S. Environmental Protection Agency, 1998h). Therefore, ammonium exposure alone could have been responsible for the effects of ammonium perchlorate observed in *P. promelas*.

The SAV and SCV derived above based on sodium perchlorate are probably protective even if ammonium perchlorate is the form released, however. Calculated NH_3-N concentrations corresponding to those values are below the acute and chronic ambient water quality criteria for ammonia, regardless of pH (U.S. Environmental Protection Agency, 1998h). While SAV and SCV are not calculated for plants, it appears that there is little perchlorate or ammonium toxicity to the alga *Selenastrum* in toxicity studies (Table 8-1).

[1]Ammonia/ammonium toxicity increases as test-water pH increases (U.S. Environmental Protection Agency, 1998e). The value of 13 mg NH_3-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 NH_3-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_{25}s) 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_{25} 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_{50} 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_{50} 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_{50} (see Section 8.3.2.1) to obtain a conservative estimate of a soil screening benchmark for soil community effects, are as follows:

Threshold = LC_{50} ÷ (factor for interspecies variance × acute-chronic ratio)
= 4,450 mg/kg ÷ (242 × 18)
= 1 mg/kg as a wet-weight concentration in soil.

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

dams on GD21 and F1-generation rat pups on PND5, PND10, and PND22. Because the representative species for the herbivore endpoint (meadow vole) is a rodent, that value is used here as well. The population-level implications of this effect are unknown; however, it seems likely that such effects on the thyroid, pituitary, and brain could diminish survivorship and fecundity and diminish population production. To account for interspecies variance and LOAEL to NOAEL extrapolation, an uncertainty factor of 10 is applied to obtain a dietary screening benchmark for herbivores of 0.001 mg/kg body weight-day, or ~0.01 mg/kg as a wet-weight concentration in plant tissue (see exposure assumptions in Section 8.3.1.5).

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.

While much of the perchlorate problem can be traced to specific sites, a few reports have suggested that fertilizers could represent another source of perchlorate in the environment (TRC Environmental Corporation, 1998). These will be addressed in further detail in Section 9.1.1. Sporadic detection of perchlorate in fertilizers was initially alarming because of the widespread use of fertilizers in production farming. In addition to the ecological impact, this raised the issue of assigning responsibility for clean-up costs. Because of the dependence of U.S. agriculture on chemical commodity fertilizers, it was clear that assessment of any possible role of fertilizers would require investigation.

This chapter summarizes the available data on the potential for exposure through runoff, erosion, fertilizer, and groundwater movement. Evidence concerning the potential of perchlorate to contaminate soil, sediment, vegetation, livestock and wildlife is also evaluated.

9.1 FERTILIZERS AS SOURCES OF PERCHLORATE SALTS

9.1.1 The Potential Role of Fertilizers

Recently, attention has been drawn to the possible roles of fertilizers as a source of perchlorate contamination for two reasons. First, perchlorate-tainted agricultural runoff could lead to pollution of natural waterways used as drinking water sources. Second, there is a potential for food plants to take up and retain any soluble compounds—including perchlorate salts—and thus provide an alternate route of exposure. It has long been known that Chile possesses caliche ores rich in sodium nitrate ($NaNO_3$) that coincidentally are also a natural source of perchlorate (Schilt, 1979; Ericksen, 1983). The origin of the sodium perchlorate ($NaClO_4$) in the caliche deposits remains an area of debate, but perchlorate is present and can be incorporated into any products made from the caliche.

An examination of two manufacturing lots found perchlorate concentrations below 2 mg/g, (i.e., < 0.2% w/w) with some lot-to-lot variability (Urbansky et al., 2001). Presently, the caliche-derived products are sold in the U.S. only by Sociedad Quimíca y Minera (SQM), but other companies have mineral rights to some Chilean deposits and mines (U.S. Environmental Protection Agency 2001b) and are potential sources of caliche-derived products. SQM has now modified its refining process to produce a fertilizer that contains less than 0.1 mg/g (<100 µg/g)

of perchlorate, further reducing any environmental release (Lauterbach, 2001). Because nitrate salts (saltpeters) find use as fertilizers, these natural resources have been mined and refined to produce commercial fertilizers for domestic use or for export. Chilean nitrates make up about 0.1% of the U.S. market. Most U.S. fertilizers are derived from other raw materials other than sodium nitrate and ammonium nitrate (NH_4NO_3), which is often used for purposes similar to $NaNO_3$, is manufactured from methane, nitrogen, and oxygen. There is no evidence that any ammonium nitrate is derived from Chilean caliche. On account of its low usage, perchlorate from Chilean nitrates cannot represent a continuing, significant anthropogenic source of perchlorate nationwide, especially with its lowered perchlorate content.

9.1.2 Raw Material Use

As with many commodity chemicals, large scale purchases are dictated by cost of raw materials, which are in turn influenced by transportation costs. Consequently, proximate (rather than distant) sources of agricultural chemicals are likely to play the greatest roles in production farming. Additionally, processing aids (e.g., clays) are likely to be derived from the nearest sources.

Commodity chemicals used as agricultural fertilizers contain fairly high concentrations of one, or sometimes two, of the primary plant nutrients, expressed as nitrogen (N), phosphorous (as the oxide P_2O_5), or potassium (as the oxide K_2O). Trace metals (e.g., copper) can be applied separately or along with these primary nutrients on a farm site. The primary phosphorus sources are ammonium phosphates and triplesuperphosphate (a hydrous calcium phosphate). The primary potassium source is potassium chloride. A mixture of synthetic and natural components are used in fertilizer manufacture, described in detail elsewhere (U.S. Environmental Protection Agency, 2001b).

Fertilizer application in production farming is highly dependent on the crop and the native soil. Agriculture is influenced by climate, weather, topography, soil type, and other factors that are generally similar within a geographical region; therefore, crops and fertilizer use are also similar within such a region. For example, the Corn Belt relies heavily on urea and anhydrous ammonia as nitrogen sources. Ammonium nitrate finds greater use in tobacco farming, and potassium magnesium sulfate finds more use in milk-producing states. Because all plants require the same primary nutrients, there is some fertilizer usage to provide these regardless of crop.

Local soil conditions also dictate what nutrients should be augmented, causing there to be large regional variations.

Consumer fertilizer (specialty) products can be distributed over large geographical regions because of the nature of the market. For example, major manufacturers have a limited number of sites dedicated to blending multiple-nutrient formulations. These products are often sold as bagged fertilizers through home-improvement centers, nurseries, florists, horticulturists, and department (or other retail) stores. Unlike agricultural fertilizers, consumer products are usually multi-nutrient formulations. In addition, trace metals are sometimes incorporated directly into them. Because the average user will apply only a very small amount of trace metals (or even primary nutrients) relative to a production farm, it is more economical, more practical, and more convenient to use multiple-nutrient formulations. Moreover, the average consumer does not have the wherewithal to disperse careful doses of several single-component fertilizers at the appropriate times of the growing season.

Because fertilizer application on production farms is geographically delimited, there is considerable interest in knowing which commodity chemicals might contain perchlorate—at least in terms of dosing. Such information might suggest regions which should be investigated for perchlorate contamination. Moreover, it will be important to know what crops might be affected—if any.

9.1.3 Fertilizer Analysis Studies

Aside from the analyses of Chilean caliche, there were no studies to suggest that any other processed fertilizer or raw material might contain perchlorate prior to 1998. That year, the Ecosystems Research Division of the EPA's National Exposure Research Laboratory (NERL-ERD) found perchlorate in several samples that were not derived from Chile saltpeter (Susarla, 1999a). This finding was later duplicated by other investigators from the North Carolina State University College of Agriculture. However, the presence of perchlorate could only be confirmed in consumer products, not in agricultural fertilizers. Moreover, subsequent analyses of bags of the same materials acquired at a later date (likely from different manufacturing lots) did not show perchlorate (Susarla et al., 2000). The choice of fertilizers did not account for the possibility that the same raw materials must have been used in a variety of products at a point in time. Additionally, a few major companies are responsible for making a large number of

products under several brand names. Furthermore, some companies rely on toll manufacturing so that the products are actually made by another company to meet a specific formulation. Accordingly, an error or contamination associated with one raw material could affect a variety of products without regard to company or application.

Perchlorate was found in six of eight lawn and garden fertilizers tested, according to a report provided to the EPA by the U.S. Air Force Materiel Command (TRC, 1998). However, the report's authors were careful to point out that the results were obtained from a single sampling event and that raw material usage was variable; therefore, no general conclusions could be drawn. These qualifiers are consistent with the limitations enumerated above, but they do point towards a temporal contamination of some products.

This study helped bring to light a number of important issues for trace analysis of fertilizers. First, most of the research on determining perchlorate to that time had been focused on either finished potable water or raw source water (Urbansky, 2000). Second, fertilizers are considerably more complicated matrixes than dilute water solutions. Third, a solid fertilizer is not a homogeneous substance. In particular, multi-component formulations used as lawn and garden fertilizers are macroscopically heterogeneous and it is possible to sort out the particles visually. Thus, representative subsampling becomes a key issue. Fourth, the effectiveness of leaching out any perchlorate ion into an aqueous phase was unknown. Fifth, the products chosen did not reflect the chemical fertilizers used for production farming, but rather the ingredients used for lawn and garden fertilizers during a specific time period.

Around the same time, the U.S. Air Force Research Laboratories (AFRL) performed a study to assess interlaboratory corroboration; that is, the ability of different labs to analyze the same sample and get the same result (AFRL, 1999; Eldridge, 2000). A variety of techniques performed by multiple laboratories showed acceptable agreement on the concentrations of perchlorate in solutions prepared from the purchased products. Several limitations (such as product choice and sampling difficulties with heterogeneous solid products) made it impossible to gain an understanding of agricultural fertilizer use, and the AFRL intentionally restricted its use of the data to evaluating interlaboratory agreement. However, data from the AFRL study was sufficient to confirm independently that some lawn and garden fertilizer products did contain perchlorate during a certain period of time.

Subsequently, the Water Supply and Water Resources Division of the EPA's National Risk Management Research Laboratory (NRMRL) conducted its own survey of fertilizers in a collaboration with the Oak Ridge National Laboratory (Urbansky et al., 2000a; Urbansky et al., 2000b). In addition to a variety of products purchased from retailers, products were purchased from farming supply stores (e.g., 50-lb bags of urea or ammonium nitrate) in Indiana, Ohio, Kentucky, Pennsylvania, and Tennessee. In addition, commodity chemical samples were collected from local distributors in Ohio and Indiana. These included urea, potassium chloride, ammonium monohydrogen phosphate, and granular triplesuperphosphate, among others. Samples were leached or dissolved and subjected to complexation electrospray ionization mass spectrometry (cESI-MS) or ion chromatography (IC). Of 45 tested products, the only ones that were found to contain any perchlorate were those based on Chile saltpeter. While this study was the first to include the same products used on agricultural production farms, it did not address the issues of sampling, product inhomogeneity, or geographical source variation.

In an effort to better address sampling, raw material usage, and other issues, the EPA undertook an additional study of fertilizers. The project was divided into two phases, the first part of which evaluated the testing laboratories for their ability to identify and quantitate perchlorate in a fertilizer matrix. In the second phase, samples gathered under the supervision of state agricultural agents were homogenized and sent to the laboratories for analysis using a method established by the EPA (U.S. Environmental Protection Agency, 2001a). This investigation was the most thorough in terms of including agriculturally relevant materials used to manufacture a wide variety of specialty products or sold directly to farmers. It also spanned all major national suppliers of these products. Although it reflected only a temporal snapshot, as had all of the other studies, the survey of fertilizers incoporated the greatest number of unique samples, quality control tests, and standardized practices, as well as other design improvements. Four laboratories analyzed all of the materials, and some samples were analyzed by additional laboratories. No other materials were found to contain perchlorate at measurable concentrations, and the EPA concluded that the only clearly identifiable fertilizer source of perchlorate was caliche. The data collected in this endeavor were additionally used to evaluate laboratory performance and further validate the method (Urbansky and Collette, 2001). A set of archived samples of all the Phase 2 materials was analyzed while evaluating an alternate ion

chromatographic column and independently verified all of the results reported in U.S. Environmental Protection Agency (2001a) (DeBorba and Urbansky, 2001).

The findings reported in U.S. Environmental Protection Agency (2001a) are the most comprehensive in terms of the types of materials tested, the manufacturers, the number of laboratories analyzing each field sample of material, and the quality control checks. In these regards, it represents our best understanding of fertilizers in terms of perchlorate content. While the presence of perchlorate in the materials gathered in late 1998 through early 1999 remains enigmatic, there is no evidence to support the concern that there is ongoing or routine perchlorate contamination in the U.S. fertilizer supply. Reports in 1999 may have reflected the temporal contamination of one or more raw materials or merely an error in manufacture. Based on the studies reported to date (Collette and Williams, 2000; Gu et al., 2000; Urbansky et al., 2000a; Urbansky et al., 2000b; Robarge et al., 2000; EPA, 2001b; Williams et al., 2001; DeBorba and Urbansky, 2001), there is a consensus among researchers from the EPA, the fertilizer industry, and other federal and state laboratories that currently used fertilizers are negligible contributors to environmental perchlorate contamination. Even imported Chile saltpeter or products derived from it contribute minimally due to their low use and low perchlorate content. Consequently, the EPA has concluded that further investigation is unwarranted (U.S. Environmental Protection Agency, 2001b).

IMC-Agrico, a major North American fertilizer manufacturer, has instituted its own monitoring program for its raw materials and products as a result of continuing interest among the scientific, industrial, and regulatory communities. These products include various potassium ores (langbeinite, sylvinite), potash-based products (potassium chloride, potassium sulfate and potassium magnesium sulfate), and phosphate products (ammonium monohydrogen phosphate, ammonium dihydrogen phosphate and granular triplesuperphosphate). After more than 100 analyses using the latest method (EPA, 2001a), IMC reported to the EPA that no perchlorate was detected in any of the materials it tested during a period spanning nearly three years. In addition, IMC states that it has analyzed Magruder check samples for perchlorate. The Magruder check sample program is jointly administered by the Association of American Plant Food Control Officials and The Fertilizer Institute; it bears the name of a chemist from the F. S. Royster Guano Company named E. W. Magruder, who initiated the program in 1922. The program selects, prepares, and distributes samples of various materials and finished products to subscribing

laboratories and then collects and analyzes the data. Magruder samples reflect monthly snapshots taken from the entire fertilizer industry. Perchlorate has not been detected in any IMC product or any of 16 Magruder samples, according to IMC (personal communication from William L. Hall).

9.1.4 Complicating Factors

It is worth pointing out at the U.S. Geological Survey (USGS) and Air Force Research Laboratories have found perchlorate in isolated samples of sylvite taken from New Mexico (Harvey et al., 1999). The USGS is engaged in additional sampling of North American mining sites in order to assess whether there are natural mineral deposits of potassium perchlorate in sylvite or sylvinite. Because little is known about the mechanisms of perchlorate formation in the natural environment (which are assumed to be meteorological in nature), it is not clear whether these findings represent a low-level background to be expected in evaporite mineral deposits or not. Nonetheless, perchlorate has not been detected in any samples of agricultural grade potassium chloride (0-0-62 or 0-0-60) taken under the direction of the EPA or by IMC-Agrico. Accordingly, it appears that this mineral commodity does not suffer from inclusions of perchlorate salts to any environmentally relevant extent.

Decades ago, ammonium nitrate was prepared from Chilean sodium nitrate by ion exchange rather than by gaseous reactants. It appears that cost began to prohibit this practice for fertilizer-grade ammonium nitrate. Nonetheless, some facilities appear to have continued the practice for explosives-grade ammonium nitrate that was used for blasting in mining operations throughout the American Southwest. It is unlikely that reliable data can be obtained from more than the past 10 years or so. Prior to the establishment of nitric acid and ammonia factories, natural saltpeters played significant roles in American agriculture. Thus, there may be contamination of groundwater in regions where these materials were used historically. The lack of information concerning natural attenuation, as well as a limited knowledge of hydrogeology, makes it difficult to determine where and how such problem sites might be found. For this reason, monitoring for perchlorate under the EPA's Unregulated Contaminant Monitoring Rule can be expected to provide some of the most useful information.

Even though perchlorate was identified in some fertilizer products and was presumably introduced through a contaminated raw material, this incident appears to have been entirely

isolated. Furthermore, awareness within the fertilizer industry and the environmental community is now heightened to the point that it appears unlikely to happen again.

9.2 MONITORING FATE AND TRANSPORT IN LIVING PLANTS

Due to the reported occurrence of perchlorate in certain water resources and in certain fertilizer products, several groups have begun to address the extent and significance of perchlorate uptake by plants. For example, if produce is grown using irrigation water tainted with perchlorate, or if agricultural soil is amended with perchlorate-tainted fertilizer, this might constitute a route of human exposure if perchlorate is taken up and retained in the edible parts of produce plants. The possibility of a relevant exposure route would be increased if perchlorate was found to bioaccumulate and if it was shown to survive the various processes that edible plants undergo before being consumed. Unfortunately, experimental results that definitively gauge the extent of risk from this route of exposure have not yet been published. However, some progress toward this goal has been made.

9.2.1 Difficulties in Analyzing Plant Tissues and Other Environmental Samples for Perchlorate

One problem that has delayed accurate and definitive studies of perchlorate uptake by edible plants is the difficulty of analyzing for perchlorate in plant materials. Ion chromatography is currently the recommended method for routine analysis of inorganic ions such as perchlorate. It is a sensitive, reliable, and easily-implemented technique when perchlorate occurs in a matrix that has a relatively low level of total dissolved solids (TDS). Unfortunately, extracts of plant materials contain high concentrations of TDS, inorganic ions, amino acids, sugars, fatty acids, and nucleotides—all of which contribute to the ionic strength of the sample (Ellington and Evans, 2000). In such matrices with high TDS/ionic strength, other ions can overwhelm the conductivity detector and effectively mask the signal from perchlorate. Ion chromatography is not unique in this regard. Other techniques and methods suitable for reasonably dilute drinking water matrices (Urbansky et al., 2000c; Magnuson et al., 2000a, b; Urbansky et al., 1999; Urbansky and Magunson, 2000) cannot be readily applied to fertilizers or botanical and physiological fluids. The problems of trace ionic analysis have led to the development of other

methods that rely on expensive instrumentation, but are not generally available, such as asymmetric waveform ion mobility mass spectrometry (Handy et al., 2000; Ells et al., 2000) or tandem mass-spec (MS-MS) systems (Koester et al., 2000).

Recently Ellington and Evans (2000) have reported an IC-based method using an enhanced clean-up procedure for the quantitation of perchlorate in plant materials that greatly reduces interferences from dissolved matter. The minimum reporting level (MRL) of perchlorate in lettuce and tomato was found to be approximately 250 μg/g on a wet mass basis. Lettuce and tomato were chosen as representative plants because they are considered high priority candidates for screening foodstuffs (Ellington and Evans, 2000). Perchlorate was spiked into the extraction water for one half of the duplicate freeze-dried samples, while one half were extracted with pure water. In the absence of other ions, some perchlorate is lost to the alumina used for the clean-up; however, this should not impact application of the method to plant material because most extracts have sufficient ionic strength. Note that perchlorate was not detected in any produce, nor was the method applied to any edible plants that were grown with intentional exposure to perchlorate.

9.2.2 Ecological Transport

In the laboratory setting, some plant species will absorb perchlorate when exposed to contaminated irrigation water. Uptake by plants has been explored for possible use in phytoremediation (Nzengung, 1999; 2000). Some investigators have speculated that bacteria are responsible for this phenomenon in plants. Perchlorate-reducing monera have been identified by several laboratories, and cultured from a variety of sources (including Las Vegas Wash sediments, food processing sludge, soils, and sewage sludge); (Logan, 1998; Coates et al., 1999; Coates et al., 2000; Kim and Logan, 2001; Wu et al., 2001; Logan, 2001). Recent work showing perchlorate reduction in saline solution suggests that attenuation may be possible even in briny locations (e.g., the Las Vegas Wash) or in fertilizer-laden farm runoff (Logan et al., 2001; Okeke et al., 2001). This suggests that perchlorate-reducing bacteria are present at significant levels in the environment. On the other hand, the bacteria isolated thus far prefer oxygen over nitrate over perchlorate. In order to for perchlorate reduction to occur, the water must be anoxic and all of the nitrate must have been consumed. Moreover, these bacterial cultures require a suitably moist environment; arid soils or regions with low rainfall may not sustain their growth. Natural

attenuation probably varies around the nation, depending on local factors. Accordingly, it is not possible to draw any meaningful conclusions about the ecological impact of fertilizers that contain perchlorate, for they may or may not be applied in areas where this type of bacterial degradation can occur.

Another factor that has prevented the early materialization of definitive data on risk from perchlorate in edible plants is that many researchers who have addressed plant uptake of perchlorate are primarily interested in other aspects of the problem. For example, Ellington et al. (2001) have applied the optimized IC-based method described above first to the analysis of perchlorate in tobacco plants and tobacco products. Tobacco was chosen because it is grown in some locations in soils amended with Chile saltpeter.

Ellington and Evans (2000) obtained green (uncured) tobacco leaves from the Coastal Plain Experiment Station (CPES) in Tifton, GA in late July 1999. The plants grew in soil that had been amended with two fertilizer products, one of which was Chile saltpeter. The perchlorate level in the Chile saltpeter was 1.5 mg/g, consistent with contemporaneous reports (Urbansky et al., 2001; personal communication from W.P. Robarge). Perchlorate was also found in a 6-6-18 plant food that had been applied to the same soil. While 3% of the nitrogen was from nitrate, the perchlorate concentration was only 36 μg/g; whereas, based on the typical perchlorate content in Chile saltpeter, it should have been about eight times larger if all of the nitrate were from Chile saltpeter. This suggests that synthetic nitrates were also part of the fertilizer's constitution. Perchlorate concentrations in the dried tissue varied from 12.5 to 165 μg/g, depending on the portion of the leaf examined and the curing process employed. Soil samples leached with deionized water contained 0.3 μg/g on a dry weight basis. EPA researchers also analyzed several off-the-shelf cigarettes (2 brands), cigars (1 brand), and chewing tobacco (7 brands) and found perchlorate concentrations ranging from 0.4 to 21.5 μg/g (undried), and only one product that contained none (Wolfe et al., 1999; Ellington et al., 2001). They confirmed the IC results by chlorine NMR spectrometry and capillary electrophoresis. Collectively, these observations argue that tobacco plants can take up perchlorate from perchlorate-contaminated fertilizers via the soil. Furthermore, they indicate the importance of investigating whether crop plants can accumulate perchlorate in their edible portions and whether any contamination can persist through the processing that precedes consumption.

Several groups have looked at the accumulation of perchlorate in various inedible plants as a potential means of fate and remediation. Perchlorate-tainted water from the Las Vegas Wash enters Lake Mead and the Colorado River and therefore has the potential to affect the potable water of many people as well as the irrigation water used for much of the lettuce produced in the U.S. Salt cedar (*Tamarix ramosissima*) is an invasive woody plant that grows prolifically in and around the Las Vegas Wash. Salt cedar consumes and transpires an enormous amount of water when it is actively growing. Furthermore, it accumulates and secretes salt. For these reasons, Urbansky et al., (2000d) have analyzed samples of salt cedar that were taken from the Las Vegas Wash. They found perchlorate at 5-6 μg/g in dry twigs extending above the water and 300 μg/g in stalks immersed in the water from a plant growing in a contaminated stream, suggesting that salt cedar plays a role in the ecological distribution of perchlorate.

Still others have investigated plant uptake with the specific goal of identifying remediation strategies for perchlorate. The biodegradation of perchlorate in woody plants has been investigated as a means of phytoremediation (Nzengung et al., 1999; Nzengung and Wang, 2000). Nzengung et al. (1999) and Nzengung and Wang (2000) found that willow trees (genus *Salix*) were able to decontaminate aqueous solutions containing 10–100 mg/L of perchlorate to below the method detection limit of 2 μg/L and suggest that two distinct phytoprocesses were at work in their studies. Specifically, they observe evidence for rhizodegradation from the exudates released from the plant, and—more importantly from the standpoint of relevance for food safety issues—they see accumulation in branches and leaves. Only about 11% of the perchlorate spiked into the water in which the trees were grown was found to phytodegrade in 26 days. The majority of perchlorate that was removed from solution after 26 days was found in the leafs. Longer term experiments suggest that the perchlorate did not accumulate in the leaves, but was very slowly transformed there as well. Generally, the perchlorate level in the leaves increased to a maximum before decreasing to undetectable levels after perchlorate was completely removed from solution. Nzengung et al. assumed that the phytodegradation pathway of perchlorate leads to chloride. Moreover, Nzengung et al. explored the role of other anions in the removal of perchlorate in solution. They found that the perchlorate removal rate was decreased as the NO_3^- level was increased. This was attributed to competing reactions in which both anions were utilized as electron acceptors. Clearly this has relevance for the food safety issue and should be investigated further. For example, the type of fertilizer used in food crop production may have

an effect on the degree to which perchlorate is taken up, depending on the major components of the fertilizer.

Susarla and coworkers have published results of their investigations on transformation of perchlorate by a wide range of plant types. For example, Susarla et al. (1999b,c) have performed screening studies to determine what species might show potential for further investigations of perchlorate phytoremediation. Thirteen vascular plant species were selected for evaluation in these preliminary experiments. This included four tree species, four herbaceous wetland species, four aquatic species, and one herbaceous upland species. Laboratory-scale experiments were conducted in order to, among other things, evaluate the ability of these plants to remove perchlorate from solution, evaluate the role of nutrients on perchlorate removal, and determine the fate of perchlorate removed form solution (e.g., plant tissue distribution, accumulation versus breakdown). Each of these topics is indirectly relevant to the issue of uptake by edible plants.

For all of these experiments, perchlorate concentrations of 0.2, 2.0 and 20 mg/L were tested in aqueous and sand treatments for ten-day periods. Perchlorate was found to be depleted from solution in the presence of all but two species. Susarla et al. (1999a,c) used a system of five categories to classify the performance of the species based on the degree to which they depleted the solution. None of the trees tested were included in the highest category of performance, but some of the wetland and aquatic plants were. Plant tissue (e.g., roots, stems, leaves) were analyzed from samples that demonstrated the maximum drop in perchlorate concentration. Susarla et al. (1999a,c) report perchlorate, or some transformation metabolite (chlorate, chlorite, chloride), in all tissues analyzed. Results of these studies suggested significant influences on depletion of perchlorate from, among other things, growth substrate (sand versus aqueous treatment), the level of nutrients, stage of plant maturity, and the presence of other ions. All of these influences should prove to be valuable insights when considering the uptake of perchlorate by edible plants. Based on screening studies, additional studies focused on the phytotransformation of perchlorate by the aquatic plant parrot-feather (*Myriophyllum aquaticum*); (Susarla et al., 1999b; Susarla et al., 1999c).

Tobacco is one crop for which the use of Chilean nitrate salts can be documented in some locations. In northern Kentucky, these products are primarily used for seedling beddings rather than fertilizing fields; for various reasons, ammonium nitrate is preferred by many farmers in Kentucky. Such preferences vary throughout tobacco-producing states and regions, however.

Data on application of Chile saltpeter is sparse, and it is not possible to estimate the ecological impact in any meaningful way. There can be no question that at least some vascular plants absorb perchlorate from their local environments. Furthermore, perchlorate has been found in a number of plants and animals living in contaminated environs (Smith et al., 2001). An obvious concern raised by finding measurable perchlorate concentrations in plant tissues is whether this ion can affect food crops and what factors might influence its uptake and accumulations. These issues shall be considered next.

9.2.3 Extrapolating to Food Plants

Because so much U.S. produce is fertilized with perchlorate-free chemical commodities, the risk from exposures via fertilizers is small. Some crops (e.g., corn, wheat, and rice) are fertilized with materials that are unquestionably perchlorate-free. Additionally, there is no reason to suspect any perchlorate associated with growing grains. However, the risk of exposure resulting from irrigation with perchlorate-tainted water in the American Southwest is unknown. At present, there are no efforts to test fruits and vegetables for perchlorate. Many of the studies on uptake by plants have been based on concentrations higher than those encountered in irrigation water. Furthermore, some products derived from Chile saltpeter are known to be among those used on California citrus crops.

One of the few studies of perchlorate uptake by edible plants is the ongoing work of Hutchinson and coworkers with lettuce grown in a greenhouse with perchlorate-tainted irrigation water. Lettuce is of particular importance for assessing the risk of perchlorate to the food supply since much of the lettuce produced in the U.S. is irrigated by water that is fed by the Las Vegas Wash, which is contaminated with perchlorate. Also, lettuce has a high water content and virtually the entire above-ground plant is consumed without cooking or processing. These characteristics would present a potential risk if lettuce efficiently accumulates perchlorate. Hutchinson and coworkers are irrigating lettuce plants with five different concentrations of perchlorate (0.1, 0.5, 1.0, 5.0, and 10.0 μg/L) for a period of 90 days following planting. At various intervals of time they divide the plants into green tissue and root samples and analyze each sample for perchlorate using an analytical method adapted from Ellington and Evans (2000). Their results show an accumulation of perchlorate into the green tissue. The level of perchlorate built up steadily over the first 50–60 days of the experiments, then generally leveled

off. At about 50 days into the experiment, the lettuce irrigated with 10.0 ppm perchlorate exhibits a perchlorate content of about 3 mg/g on a lettuce dry matter basis. Since lettuce is about 90% water, this would amount to about 0.300 mg/g on a wet weight basis. The amount of perchlorate detected in the leaves is generally linear with dosing level for a given day. Experiments are underway to determine whether lettuce has the capability to degrade perchlorate if the supply of the contaminant is stopped. However, this determination is frustrated by the fact that lettuce continues to grow. Therefore, a decline in concentration (e.g., expressed in mg/g) does not adequately reflect the situation. The preliminary results from these studies (Hutchinson et al., 2000) suggest that, when complete, they will constitute considerable progress on the issue of exposure to perchlorate from edible plants.

Even if many food plants can be shown to absorb and retain perchlorate, the primary source of the contaminant is irrigation water polluted from defense-related activities. Because these activities are reasonably localized geographically, most of the country's agricultural products should be perchlorate-free, e.g., corn, wheat, rice, milk. On the other hand, some types of produce are supplied almost entirely by regions dependent on contaminated irrigation water. Therefore, these sites represent possible exposure routes for most of the nation via foods such as lettuce.

Historically, much of the emphasis on fertilizer pollution from agricultural runoff has been on fertilizers applied to the soil. However, potassium nitrate is usually applied to the leaves of citrus trees when a potassium deficiency is found by analyzing leaf tissue. Such foliar application would not necessarily contribute significantly to runoff type pollution of waterways, but could lead to the absorption of contaminants through the leaves and wood. There are no reliable data on the sources of potassium nitrate used for citrus crops. While it is known that absorption of anions similar to perchlorate (e.g., pertechnetate) are affected by the ionic strength and compostion of the surrounding solution, little is known about the factors that influence perchlorate influx via roots or leaves. In addition, the fate of absorbed perchlorate in the plants is also unknown. It may be that xylem-supplied tissues, such as leaves, are the final repository rather than phloem-supplied tissues, such as fruits.

These issues and more have begun to be examined by the EPA, but there are many unknowns (U.S. Environmental Protection Agency, 2001b). Until such time as quantitative studies are performed on various species to determine what factors influence the absorption,

accumulation, and distribution of perchlorate in plants, it is not possible to estimate whether foods can serve as meaningful contribution to the body burden or to the risk posed to humans from perchlorate contamination. Even if they do, there is considerable peace of mind in knowing that fertilizers and water supplies are generally not providing any perchlorate to the plants in the first place. Consequently, only a small number of foods are worth considering for further study. On the other hand, it is not known to what extent other countries rely on natural saltpeters to fertilize food crops. Moreover, it is not known whether fruits and vegetables absorb and retain the perchlorate ion. Therefore, it is not possible to say whether fruits and vegetables grown outside the U.S. serve as a possible exposure routes at this time. Depending on the season, imported oranges, apples, and grapes and their juices are consumed throughout the U.S.

Because there are no data on perchlorate in imported produce, no data on perchlorate in U.S. produce, and no data from controlled laboratory experiments on uptake in fruit crops, it is impossible to assess whether these foods can contribute to perchlorate consumption in humans or whether drinking water constitutes the entire body burden. However, the available information on fertilizers and irrigation water suggests that foods do not contribute to the body burden. At the present time, the available data point towards drinking water as the principal exposure pathway for humans.

9.3 SUMMARY

Despite some initial findings implicating fertilizers as a source of perchlorate, more thorough and better designed studies that were conducted subsequently have not found this to be the case. Current fertilizer manufacturing practices and raw material sources make it unlikely that perchlorate contamination could occur widely and without discovery. While some plants may absorb or even accumulate perchlorate in specific tissues, there are many unknowns with regard to the edible portions of nutritionally and agriculturally important crops. Many factors influence transport of ions, and current understandings of plant physiology and botany suggest perchlorate uptake would be reduced as a result of such factors. Even if perchlorate uptake occurred in some food crops, perchlorate contamination is localized geographically outside of major agricultural regions, minimizing possibility of uptake in edible produce. While perchlorate-tainted irrigation water may be a source available for uptake of perchlorate by plants,

this is again localized, and has not been proven to occur at the concentrations of perchlorate that are observed environmentally. Difficulties in analyzing many plant or animal tissues originally were obstacles to executing appropriate studies, but these problems have generally been solved. Ideally, more data would be available on food plants, but current evidence suggests that drinking water is the primary exposure pathway to perchlorate for humans.

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^+)-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

of thyroid hormone in an attempt to compensate. Prolonged stimulation of the thyroid by TSH may result in thyroid neoplasia, particularly in rodents known to be sensitive. Tumors have occurred in rats dosed with high levels of perchlorate for long periods and at much lower doses in relatively young adult animals (19 weeks) dosed *in utero* and during development. These findings have raised concerns about the *in utero* imprinting of the regulatory system responsible for controlling thyroid hormone economy.

The target tissue for systemic effects of perchlorate has been identified as the thyroid. The key event of its mode of action is iodide uptake inhibition at the NIS. Changes in the thyroid hormone homeostasis result in histopathological changes in the thyroid, including: colloid depletion, follicular hypertrophy, follicular hyperplasia, and decrease in follicular lumen size. If perchlorate exposure is stopped, the thyroid histopathological effects have been shown to be reversible after exposures as long as 90-days in rats, but incomplete recovery of thyroid hormones occurs in this same time period. There are also some case studies in humans treated therapeutically with perchlorate that indicate reversibility of thyroid hormone changes after years of exposure.

Other potentially adverse and permanent effects from decreased thyroid hormone include effects during development *in utero* and early growth, particularly effects on the nervous system if the pregnant mother was hypothyroxinemic or hypothyroid. Laboratory animal assays performed in response to recommendations made at the peer review in 1999 and as part of the perchlorate testing strategy confirmed neurodevelopmental effects observed in previous studies. Changes in brain morphometry and motor activity were observed. The potential for major disturbances in thyroid hormone homeostasis to disturb reproductive capacity or to induce immune effects also exists. The ability of perchlorate to cause contact hypersensitivity is suggested but remains not well characterized. Finally, a remarkable conservation of the thyroid hormone regulatory system has been demonstrated across species. Inhibition of iodide uptake by the NIS has been shown in pharmacokinetic studies to be very similar across species, including humans.

10.1.2 Dose Response

The revised RfD is based on an assessment that reviewed a set of studies that were developed to explicitly evaluate these potential toxicities. The quantitative estimate of risk is

based on laboratory animal data because there are no good observational epidemiological data concerning human subjects representative of the critical sensitive populations (hypothyroxinemic pregnant women or children) or that have evaluated neurodevelopmental outcomes; nor have adequate clinical studies been performed. A harmonized approach was proposed based on the key event of iodide inhibition and its relationship to disturbances in the hypothalamic-pituitary-thyroid axis as evidenced by effects on thyroid and pituitary hormones, thyroid histopathology, and brain morphometry. Using these precursor lesions as the basis for the point-of-departure is considered to be protective for cancer development as well as for neurodevelopmental sequelae.

The database supported a point-of-departure for the RfD deviation at 0.01 mg/kg-day based on changes in maternal thyroid and pituitary hormones and on changes in the brain morphometry and thyroid and pituitary hormones of fetal and neonatal pups. A composite uncertainty factor of 300 was applied in the derivation. An adjustment also was made for administration of perchlorate as ammonium perchlorate. The RfD is for perchlorate as the anion because that is what is sampled and analyzed in environmental media and because the salts of perchlorate readily dissolve. Uncertainty factors were applied for intrahuman extrapolation, the use of a LOAEL, concern regarding the lack of studies of longer duration and database deficiencies. Confidence in the study, the database, and the RfD is rated as medium. A major uncertainty is the sensitivity that the screening neurodevelopmental studies provide to protect against neuropsychological deficits of exposures that might occur within critical developmental windows or in susceptible human populations.

The daily perchlorate exposure to the human population that is likely to be without appreciable risk of either cancer or noncancer toxicity during a lifetime is 0.00003 mg perchlorate/kg-day. It again is noted that this RfD is specific for the anion because that is what is detected in most environmental samples and because most salts of perchlorate readily dissolve. Because of the application of uncertainty factors, this dose is approximately 1/300 of the dose that resulted in brain morphometry and thyroid changes in pups and hypothyroid status (decreased T4 and increased TSH) in rat mothers (Argus Research Laboratories Inc, 2001) and in their pups both during gestation (GD21) and in the post-natal period (PND4 through PND21).

10.1.3 Risk Characterization

Comprehensive risk characterization for the perchlorate contamination issue, as discussed in Chapter 1 (see Figure 1-5), requires accurate information on exposure levels determined by a validated analytical method. Dose-response estimates such as the value derived herein can then be used to gauge the potential toxicity of those exposures. Exposure can be either direct, most likely by ingestion, or indirect, such as by consumption of contaminated food. When using the dose-response assessment derived herein to compare with exposure estimates, one should remain keenly aware that many of these exposure aspects have not yet been characterized accurately for perchlorate. Fate and transport information do not exist to track the spatial and temporal distribution of perchlorate; the potential for evaporative concentration in soils has not been characterized, nor has its uptake in plants or herbivores. In addition, there are uncertainties remaining in the dose-response estimate itself. These concerns also should be considered whenever attempting to characterize the risk to a specific human population exposed to a particular scenario.

10.1.3.1 Direct Exposures

Typically the RfD is used as a comparison for oral ingestion, such as by drinking water. The RfD is compared with an exposure estimate of the drinking water concentration to characterize potential toxicity. When making this comparison, the assumptions underlying derivation of the RfD must be kept in mind. The RfD is intended to be protective of susceptible populations exposed daily. The frequency and magnitude of exposure is a key attribute of accurate dose-response characterization (Jarabek, 1995c) and an equally important component of risk characterization. Transient decreases in T4 can cause permanent neurodevelopmental deficits. Thus, the degree to which the particular suspected population at risk fits with the underlying assumptions of the RfD derivation should be kept in mind. Finally, the degree of imprecision in the derivation of an RfD should be taken into account. The RfD estimates are not intended to serve as “bright line” estimates. By definition, there is an order of magnitude uncertainty around the estimate. This generally translates into a range of approximately three-fold below to three-fold above the RfD, but also depends on the nature of the effects used as the basis.

10.1.3.2 Indirect Exposures

Where crops are irrigated with perchlorate-contaminated water, indirect human exposures may result. A number of factors need to be considered in estimating human exposure through crops.

Concentration in plant parts as a result of root uptake normally is calculated using a soil-to-plant transfer factor that is expressed as the ratio of plant to soil concentration. If perchlorate is subject to evaporative concentration in irrigated soils, then soil concentration, and therefore uptake, may be higher than that expected simply based on concentration in irrigation water. If a leaf crop such as lettuce is spray-irrigated, perchlorate could be concentrated evaporatively on external leaf surfaces. Because perchlorate salts have high water solubility, this contamination probably would be removed largely by washing. On the other hand, if perchlorate is phytodegraded, as one study has suggested (Nzengung, n.d.), soil or plant concentrations may be lower than otherwise expected. Studies are needed to determine the behavior and fate of perchlorate in plant-soil-water systems, including studies that simulate leaf crop irrigation and that account for full life cycles of crops.

Besides estimates of perchlorate concentrations in crops, the calculation of human daily intake depends on the number of crop types that are contaminated, the extent to which a particular individual obtains the crops from a contaminated source, and the individual's daily consumption of the crops. These factors may vary widely in the exposed population, and methods for accounting for the combined variability should be used in characterizing these exposures.

Methods for estimating human exposures resulting from crop uptake of soil-deposited contaminants are presented in Chapters 6 (Determining Exposure Through the Terrestrial Food Chain) and 10 (Risk Assessment) of the EPA document, "Methodology for Assessing Health Risks Associated with Multiple Pathways of Exposure to Combustor Emissions (EPA 600/ R-98/137)." That document currently is undergoing revision and is scheduled for final release in January 2002. If the needed information can be obtained on perchlorate behavior and fate, the methods described therein can be used to develop estimates of human exposure and risk.

10.1.4 Major Uncertainties and Research Needs

Reliable exposure estimates are required to accurately and comprehensively characterize the risk of perchlorate contamination. This section will briefly summarize research needs associated with aspects of uncertainty about the human health risk dose-response estimate that were highlighted in Chapter 7.

The greatest need for continued improvement in the dose-response assessment is a more accurate characterization of the linkage between the key event of the mode of action (i.e., inhibition of iodide uptake in the thyroid gland), subsequent changes in thyroid hormones, and the correlation to outcome measures in hypothyroxinemic pregnant animals and their pups. Because this need must be addressed in the fetal compartment as well, accurate characterization of toxicokinetics during pregnancy and lactation also are required. More definitive studies of the degree of change in perturbation of the hypothalamic-pituitary-thyroid axis (i.e., change in hormone levels) that is associated with thyroid histology, and with neurobehavioral deficits especially, would improve the confidence in the accuracy of the exposure-dose-response continuum. The current studies may need to be repeated with larger sample sizes and lower doses, and new studies may be needed to evaluate effects on fetal hormone levels and neurodevelopmental measures both in the laboratory and in a survey of the human population. Research on potential factors influencing sensitivity is also critically requisite. Animal models of thyroid impairment such as iodide deficiency and "womb to tomb" exposure designs should be explored. Finally, mechanistic determinants of these toxicokinetic and toxicodynamic parameters and processes should be further characterized.

10.2 ECOTOXICOLOGY

10.2.1 Aquatic Life

Procedures for deriving Tier II water quality values were used in Section 8.3.2.1 to jointly characterize the potential effects of the perchlorate ion on the richness and productivity of fish, aquatic invertebrate, and plant communities. Tier II values are derived when data are not sufficient for deriving ambient water quality criteria. The Tier II value derivation procedures account for missing information with approximately 80% confidence. In this case, the Tier II

values derived, termed secondary acute and chronic values, were 5 and 0.6 mg/L (i.e., 5,000 and 600 μg/L), respectively; difficulties associated with the interpretation of one test result in an uncertainty range for the secondary chronic value of < 0.32 to > 0.83 mg/L (< 320 to > 830 μg/L). Perchlorate levels reported for large surface waters (as high as 16 μg/L) and ground waters (as high as 280 μg/L in public supply wells) are well below the secondary acute and chronic values. Thus, at these exposure levels, the likelihood of effects on the richness and productivity of fish, aquatic invertebrate, and plant communities appears to be low. However, because much higher perchlorate concentrations have been reported in monitoring wells at rocket motor manufacturing or testing sites (37×10^6 μg/L) and in groundwater-dominated surface water systems close to sites of contamination (3500 to 1.3×10^5 μg/L), sites clearly exist that have perchlorate concentrations high enough to cause toxicity to aquatic life. These sites include springs, such as that sampled along Las Vegas Wash in Nevada (Parsons, 2001) and the INF Pond at Longhorn Army Ammunition Plant in Texas (Parsons, 2001; Smith et al., 2001). On the other hand, concentrations below the Tier II values were detected in larger water bodies immediately adjacent to sites of contaminations, such as in Lake Mead immediately adjacent to the mouth of the Las Vegas Wash (less than 4 to 68 μg/L). Water discharged from a CERCLA groundwater pump-and-treat facility that was not equipped to treat perchlorate at Allegany Ballistics Laboratory to the North Branch Potomac River contained 250 to 280 μg/L perchlorate (Parsons, 2001).

Where high levels of contamination exist, sensitive aquatic organisms such as daphnids may be the most likely to experience effects; in the reported tests, effects were seen on both survival and reproduction (neonates per organism). A teratogenicity assay, FETAX, showed malformations in frog embryos occurring at only slightly lower concentrations than lethality, indicating that perchlorate is probably not a potent developmental toxicant. Tier II values are not estimated for plants, but results from algal toxicity tests suggest that even at the higher perchlorate concentrations associated with rocket motor manufacturing, risk of toxicity to aquatic plants is low.

The perchlorate anion can be associated with various cations including sodium, ammonium, and potassium. When sodium perchlorate was tested, the sodium cation was not toxic to daphnids in sodium chloride control tests but did show toxicity to minnows. Ammonium controls were not used in tests with ammonium perchlorate, but ammonium ion is a

known toxicant with toxicity that varies according to water temperature and pH. In any aquatic system where perchlorate is present, attention should be given to determining the concentrations of potentially toxic cations that may contribute to ecological effects.

Based on a secondary chronic value of 600 μg/L (uncertainty range, < 320 to > 830 μg/L) for perchlorate, the analytical detection methods for perchlorate in water are sufficient. The detection limit achieved for perchlorate in water was 4 μg/L (Parsons, 2001; Smith et al., 2001), which is much less than the secondary chronic value. Thus, the likelihood that adverse ecological effects will occur below detection limits is low.

10.2.2 Risks to Consumers of Aquatic Life

Information from Parsons (2001) and Smith et al. (2001) indicate that perchlorate may bioaccumulate in aquatic invertebrates and fish in contaminated waters, but perchlorate is not expected to bioconcentrate in these organisms to levels exceeding the surface water concentrations. Therefore, there currently is no indication that consumers of aquatic invertebrates or fish are at increased risk of effects from bioconcentration in areas where perchlorate concentrations in surface water occur. However, there is some uncertainty about the potential for bioaccumulation of perchlorate at low concentrations (i.e., 4 to 300 μg/L in water) because of the higher detection limits for perchlorate in animal tissues, which were 300 to 400 μg/kg in Parsons (2001) and about 70 μg/kg in Smith et al. (2001). Furthermore, perchlorate may bioconcentrate (i.e., to levels exceeding those in water) in aquatic plants; therefore, consumers of aquatic plants may be at greater risk than consumers of aquatic invertebrates or fish, but information is not available concerning effect levels in aquatic herbivores.

10.2.3 Terrestrial Life

10.2.3.1 Plants

Terrestrial plants may be exposed to perchlorate in soil at disposal sites and at sites irrigated with contaminated surface water or groundwater. Perchlorate concentrations in soil at disposal sites range from less than 1 to 1470 mg/kg (Parsons, 2001) and can be higher than the screening benchmark of 4 mg/kg and even higher than the lethal concentrations ($\geq$180 mg/kg; EA Engineering, Science and Technology, Inc., 1998).

In the absence of reliable information concerning the accumulation of perchlorate in irrigated soils, it may be assumed that soil concentrations equal irrigation-water concentrations (Section 8.3.1.3). Reported surface-water concentrations in the Colorado River, 4 to 16 μg/L, would translate to 0.004 to 0.016 mg/kg. At the Yuma site, there was a single detection in surface soil of 0.090 mg/kg; all other measurements were below the detection limits of 0.079 to 0.080 mg/kg (Parsons, 2001). Even the single detected concentration is a factor of 44 lower than the benchmark value. The reported groundwater concentration in public wells of 280 μg/L would translate to 0.28 mg/kg, which is a factor of 14 lower than the benchmark value. Hence, perchlorate does not appear to constitute a hazard to plants irrigated with surface water. However, given the large uncertainties concerning exposure, a hazard from groundwater irrigation cannot be precluded.

Based on this screening benchmark of 4 mg/kg for perchlorate, the analytical detection methods for perchlorate in soil are sufficient for determining whether soils will cause toxicity to plants, and there is little likelihood of adverse ecological effects occurring at levels below detection limits. The detection limit achieved for perchlorate in soils was generally 75-80 μg/kg (Parsons, 2001), but there was at least one soil sample where the reporting limit was 803 μg/kg. However, all of these limits are less than the screening benchmark.

10.2.3.2 Soil Invertebrates

Soil invertebrates may be exposed to perchlorate in soil at disposal sites and at sites irrigated with contaminated surface water or groundwater. Perchlorate concentration measurements at disposal sites range from less than 1 to 1470 mg/kg (Parsons, 2001) and, therefore, can exceed the soil screening benchmark of 1 mg/kg. In the absence of reliable information concerning the accumulation of perchlorate in irrigated soils, it may be assumed that soil concentrations equal irrigation water concentrations (Section 8.3.1.3). Reported surface water concentrations in the Colorado River, 4 to 16 μg/L, would translate to 0.004 to 0.016 mg/kg in soils. At the Yuma site, the single detection in surface soil was 0.090 mg/kg with detection limits of 0.079 to 0.080 mg/kg. This detected concentration is a factor of 11 lower than the soil screening benchmark value (1 mg/kg). The reported groundwater concentration in public wells of 280 μg/L would translate to 0.28 mg/kg, which is a factor of 4 lower than the benchmark value. Hence, perchlorate does not appear to constitute a hazard to soil invertebrates

in soil irrigated with surface water. However, given the large uncertainties concerning exposure, a hazard from groundwater irrigation cannot be precluded.

Based on this screening benchmark of 1 mg/kg for perchlorate, the analytical detection methods for perchlorate in soil are sufficient, and there is little likelihood of adverse ecological effects occurring at levels below detection limits. The detection limit achieved for perchlorate in soils was generally 75-80 μg/kg (Parsons, 2001), but there was at least one soil sample where the reporting limit was 803 μg/kg. However, all of these limits are less than this screening benchmark.

10.2.3.3 Herbivores

Exposures of voles to perchlorate based on measured plant concentrations at rocket motor manufacturing or testing sites (0.11 mg/kg day to a maximum of 49 mg/kg day) exceed both the LOAEL of 0.01 mg/kg/day and the screening benchmark of 0.001 mg/kg day**.** Estimated exposures of voles consuming plants on sites irrigated with surface water (0.18 mg/kg day) and groundwater (3.2 mg/kg day) also exceed the LOAEL and the screening benchmark. Hence, there is a potential hazard to all herbivorous wildlife living in areas that may be irrigated with contaminated water. At disposal sites, wildlife would be at risk from the effects of loss of food and habitat from toxic effects on plants, as well as the potential for direct toxic effects via consumption of perchlorate-tainted food or water.

Assuming a water ingestion rate of 0.21 g/g-day (U.S. EPA, 1993a,b), the screening benchmark for herbivores is equivalent to a water concentration of 4.8 μg/L. Perchlorate levels reported for large surface waters (as high as 16 μg/L) are greater than this concentration. Much higher perchlorate concentrations have been reported in monitoring wells at rocket motor manufacturing or testing sites (37×10^6 μg/L) and in groundwater-dominated surface water systems close to sites of contamination (3500 to 1.3×10^5 μg/L), and rodent exposures via drinking water at these sites would exceed the rodent NOAEL.

Based on screening level benchmarks for herbivores, the analytical detection methods for perchlorate in plant tissues may not be sufficient for the detection of concentrations potentially toxic to herbivores even though the analytical detection methods for perchlorate in water are sufficient. The detection limits achieved for perchlorate in water and in plant tissues were 4 μg/L and 0.4 mg/kg, respectively (Parsons, 2001; Smith et al., 2001).

10.2.3.4 Carnivores

Available evidence indicates that concentrations in terrestrial invertebrates are less than the concentrations in plants and similar to that in soils. As a result, there currently is no indication that terrestrial carnivores are at additional risk from perchlorate. Risks of direct toxic effects are therefore lower for carnivores than herbivores. In locations where perchlorate levels are sufficient to significantly affect herbivores, carnivores are more likely to be affected by loss of prey than by perclorate toxicity. Therefore toxic effects are not quantified.

10.2.4 Uncertainties

This discussion of uncertainties is limited to qualitative uncertainties associated with major gaps in the data available for ecological risk assessment of perchlorate. This is because, as with other screening assessments, quantitative uncertainties are treated through the use of conservative assumptions. It is also because the data gaps are the major sources of uncertainty, not imprecision or inaccuracy of the available data.

10.2.4.1 Uncertainties Concerning Aquatic Risks

Aquatic Exposures. The primary uncertainty associated with this assessment of aquatic risks is the paucity of data on perchlorate occurrence in surface waters. For lack of systematic sampling and analysis, the spatial and temporal distribution of perchlorate in water is unknown. It is not certain whether the reported concentrations in water represent the highest existing levels. This is not a large source of uncertainty for this screening assessment if it is assumed that sampling has been biased to areas of highest likely contamination. However, it would be a major source of uncertainty in any subsequent definitive assessment.

Aquatic Effects. While the effects of perchlorate on some species of algae are known, the effects on aquatic macrophytes are unknown. As a result, risks to aquatic primary producers are estimated using only the chronic toxicity test results for the alga *Selenastrum*. Because of physiological differences between algae and vascular plants, effects on aquatic primary producers are not adequately assessed. In addition, it us unknown how or if physiological variations among various species of algae or plants may affect their susceptibility to perchlorate.

Algae, aquatic macrophytes, and terrestrial leaf litter are the bases of food chains in many aquatic ecosystems. Because perchlorate has been shown to concentrate in leaves of terrestrial

plants and aquatic plants, the potential for direct impacts to primary consumers (i.e., planktonic and benthic invertebrate communities) is a concern that could not be addressed in this assessment.

A 35-day, early-life stage (ELS) test with *Pimephales*, generally regarded as a chronic test but short of a full-life-cycle test, showed no significant effects on any standard endpoint (survival, growth or biomass) at the highest concentration tested (490 mg/L). However, 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. This finding suggests the presence of subtle effects that could be ecologically significant and raises doubt about whether a chronic NOEC has been adequately determined for this species. This uncertainty is displayed as a range surrounding the secondary chronic value (i.e., < 0.32 to > 0.83 mg/L). Because of the inequality signs, even the width of the range is uncertain. For this reason, and because of the potential for chronic effects caused by thyroid dysfunction, chronic effects should be investigated in a full life cycle test.

The uncertainty factors in the secondary chronic value are high because of the lack of test results for aquatic organisms other than fathead minnows, amphipods, and daphnids.

10.2.4.2 Uncertainties Concerning Terrestrial Risks

Terrestrial Exposure. The available data concerning aqueous perchlorate levels is sparse and has not been collected systematically. As a result, the spatial and temporal distribution of perchlorate in irrigation water is unknown. It is not clear that the reported concentrations in water represent the highest existing levels. This is not a major source of uncertainty for this screening assessment if it is assumed that sampling has been biased to areas of highest likely contamination. However, it would be a major source of uncertainty in any subsequent definitive assessment.

The fate of perchlorate in soil, including its tendency for evaporative concentration, is not well characterized. As a result, soil concentrations were assumed to be equal to irrigation water concentrations. This assumption could be low by multiple orders of magnitude if evaporative concentration occurs with perchlorate, as it does with metals. The limited data for irrigated soils near Yuma (Parsons, 2001) do not support the occurrence of such a high degree of evaporative

concentration, but neither are they sufficient to rule out concentration by up to a factor of 10 or so. More information on the fate of perchlorate in irrigated soils is needed.

The bioconcentration of perchlorate by plants suggests that perchlorate may be elevated in leaves and leaf litter to levels that may affect invertebrate herbivores and soil invertebrate communities. For lack of data concerning dietary toxicity, risks to invertebrates by this route were not assessed.

Available toxicity data for rodents suggest that vertebrate herbivores may be sensitive to low levels of perchlorate in plant tissues; concentrations potentially causing toxicity are calculated to be lower than those currently detectable by chemical analyses of plants. In Parsons (2001), detection limits for plants were generally about 0.4 mg/kg wet weight; similar detection limits were achieved by Ellington and Ellis (2000) and Ellington et al. (2001), as compared to an exposure benchmark of 0.01 mg/kg in plant tissue for a representative herbivore (see Section 8.3.2.2). Therefore, lower detection limits for perchlorate in plant tissues may be needed to completely assess the risks to vertebrate herbivores.

Terrestrial Effects. The toxicity of perchlorate to nonmammalian vertebrate wildlife is unknown. As a result, risks to birds, reptiles, and amphibians could not be assessed.

The toxicity of perchlorate to terrestrial invertebrates, other than acute lethality to earthworms, is unknown. As a result, risks to other terrestrial invertebrates were inadequately assessed.

10.2.5 Research Needs

Three questions were asked of the screening ecological risk assessment for perchlorate:

- Are ecological risks best characterized as *de minimis* (exposures clearly are below levels of concern), *de manifestis* (risks are clearly significant and require management action to reduce exposures); or somewhere in between and requiring further characterization?
- Are analytical detection methods for determining levels of perchlorate in the environment sufficient, or is there a likelihood of adverse ecological effects occurring at levels below current detection limits?
- Is the available ecotoxicological information on perchlorate sufficient, or are additional studies needed?

In the immediate vicinity of facilities that were involved in the manufacture, use, or disposal of perchlorate salts, particularly facilities involved in handling of solid rocket propellents, ecological exposure can exceed levels of concern and management actions may be needed to reduce these exposures. Site-specific risk assessments would be needed to guide remediation of such locally contaminated sites. Farther from such facilities, ecological exposures appear to be below levels of concern.

The analytical detection methods for perchlorate are generally sufficient, and there appears to be no indication of adverse ecological effects occurring at levels below detection limits, except that detection limits in plant tissues are not low enough to ensure that risks to herbivores are detected. Additionally, there is some uncertainty about the potential for bioaccumulation at low concentrations of perchlorate in surface water, because of differences in the analytical detection limits between water and animal tissues.

The available ecotoxicological information on perchlorate is sufficient for this screening-level ecological risk assessment. However, additional ecotoxicological studies could reduce the uncertainties about the toxicity of perchlorate to other potential ecological receptors.

While the available information may yield an adequate screening level ecological risk assessment, the following research needs for exposure and effects analysis deserve mention.

10.2.5.1 Exposure

Concerning exposure, at least three important issues remain unresolved:

- Because the available data on accumulation in terrestrial and aquatic vascular plants are from studies that were not designed to quantify accumulation factors, the accumulation of perchlorate in terrestrial and aquatic plants should be further investigated.
- Because of the potential for evaporative concentration, the fate of perchlorate in irrigated soils should be investigated.
- Because the concentrations that have potential for dietary toxicity to vertebrate herbivores are less than the limits of detection currently achievable by chemical analysis of plants, analytical methods for plant tissues that could lower the limits of detection should be investigated.

10.2.5.2 Effects

Also requiring further attention are issues related to the effects of potential perchlorate exposure:

- The effects of exposure of aquatic plants should be determined.
- The effects of exposure of noncrustacean invertebrates should be determined.
- The effects of dietary exposure to perchlorate should be determined in birds and in herbivorous or litter-feeding invertebrates.
- The effects of dietary and cutaneous exposure to perchlorate should be determined for adult amphibians and aquatic reptiles.
- If perchlorate occurs at significant levels in estuarine systems, its toxicity in saline waters should be determined.

10.2.5.3 Site-Specific Investigations

Some of the research needs that were listed in the previous ERD of this document have been met by the research conducted by the US Air Force IERA (Parsons, 2001) in which perchlorate concentrations in environmental media (i.e., surface soils, surface water, sediments, and pore water) and biological tissues (i.e., terrestrial plants, invertebrates, reptiles, birds, and mammals and aquatic vegetation, invertebrates, fish, amphibians, reptiles, and birds) were surveyed at six sites with known perchlorate contamination. These data are supplemented by additional sampling at one of the sites, Longhorn Army Ammunition Plant in Texas, by Smith et al. (2001). These studies do address some questions about exposure that were expressed in the previous ERD of this document (U.S. EPA, 1998d), i.e:

- Because concentrations of perchlorate in water are poorly known, and concentrations in soil and biota are unknown, a survey of perchlorate contamination should be conducted.
- Because, contrary to expectations, perchlorate accumulates to high concentrations in terrestrial vascular plants, the accumulation of perchlorate in aquatic plants and in animals should be investigated.

However, these studies were screening-level surveys that took small numbers of samples during limited periods of time. In addition, the studies were not designed to address questions about the effects of exposure. In some locations, concentrations in environmental media were high enough

that toxicity to ecological receptors was highly likely (i.e., the risks were *de manifestis*), and in other locations toxicity could not be ruled out (i.e., the risks could not be termed *de minimus*). Therefore, systematic sampling is needed in these locations to more definitively quantify exposures and effects, so that the likelihood, nature and extent of ecological risks may be quantified, appropriate remedial alternatives may be designed, and effectiveness of site cleanup may be judged. In addition, site surveys may be required in other locations where perchlorate contamination is suspected.

10.3 CHARACTERIZATION PROGRESS SUMMARY

Despite the fact that the appreciation of widespread perchlorate contamination emerged only five years ago, considerable progress has been made in hazard identification and quantitative dose-response characterization for both the human health and ecotoxicological risks of potential perchlorate exposures. The thyroid has been confirmed as the target tissue in humans, laboratory animals, and wildlife. The key event of the mode of action for perchlorate is iodide uptake inhibition at the NIS with the potential for both subsequent neurodevelopmental and neoplastic sequelae. A harmonized human health reference dose has been proposed to be protective for both sequelae based on a mode of action model. Data insufficiencies for various ecotoxicological receptors and for accurate exposure estimates precludes other than a screening-level assessment at this time. Additional research is needed to determine the contribution of exposure sources other than drinking water. This requires more progress in the area of analytical methods to extend current approaches to other media.

As with any risk assessment, additional insights and new research will continue to change our understanding as the knowledge base is informed with new data and as the scientific and technical areas relevant to the particular risk characterization mature and evolve. Work dedicated to the areas defined in this chapter should allow continued improvement of the risk characterizations for perchlorate in the future.

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APPENDIX A

Schematics of Study Designs for Neurodevelopmental, Two-Generation Reproductive and Developmental Studies

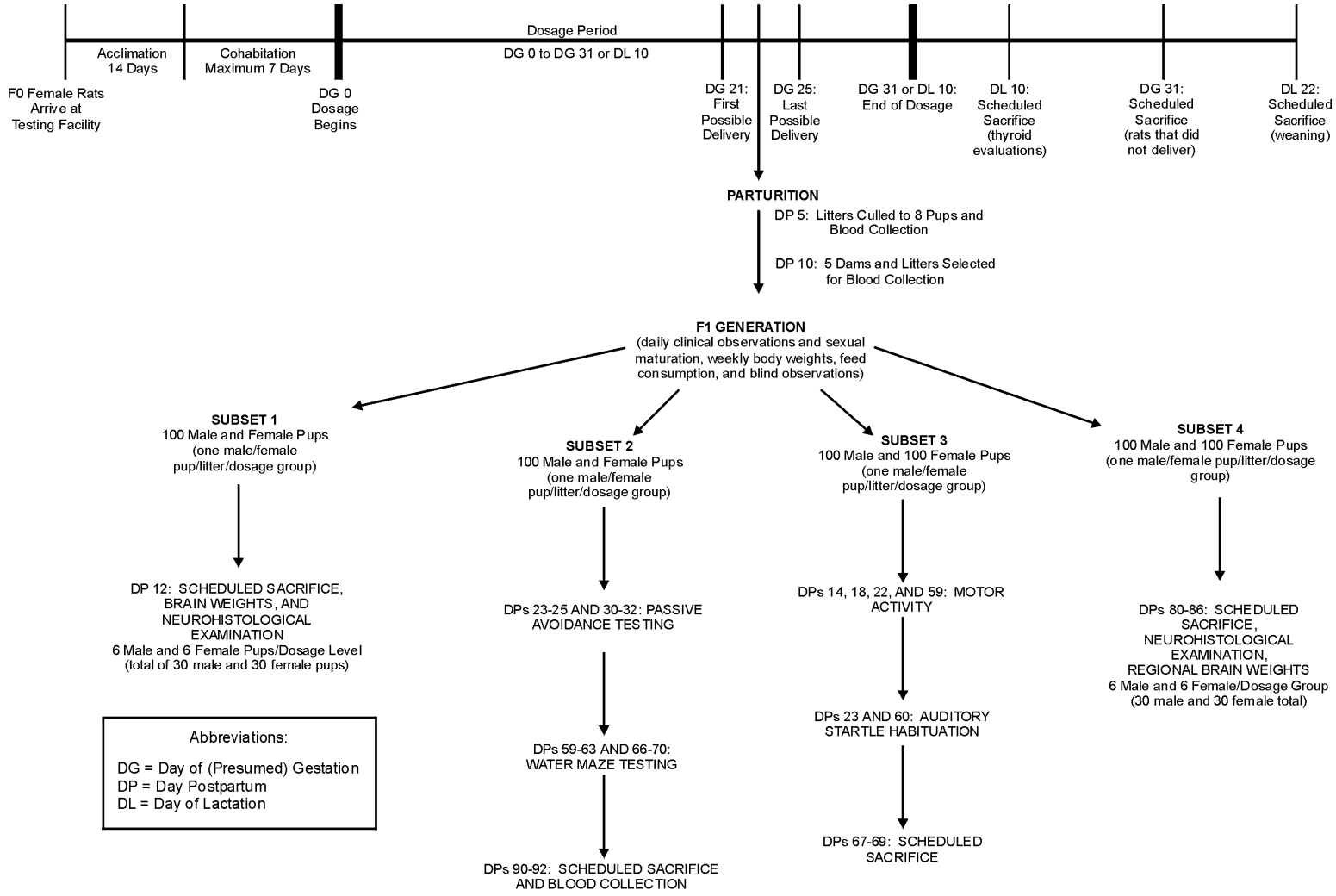


Figure A-1. Schematic of the neurobehavioral developmental study of ammonium perchlorate administered orally in drinking water to SD rats (Argus Research Laboratories, Inc., 1998a).

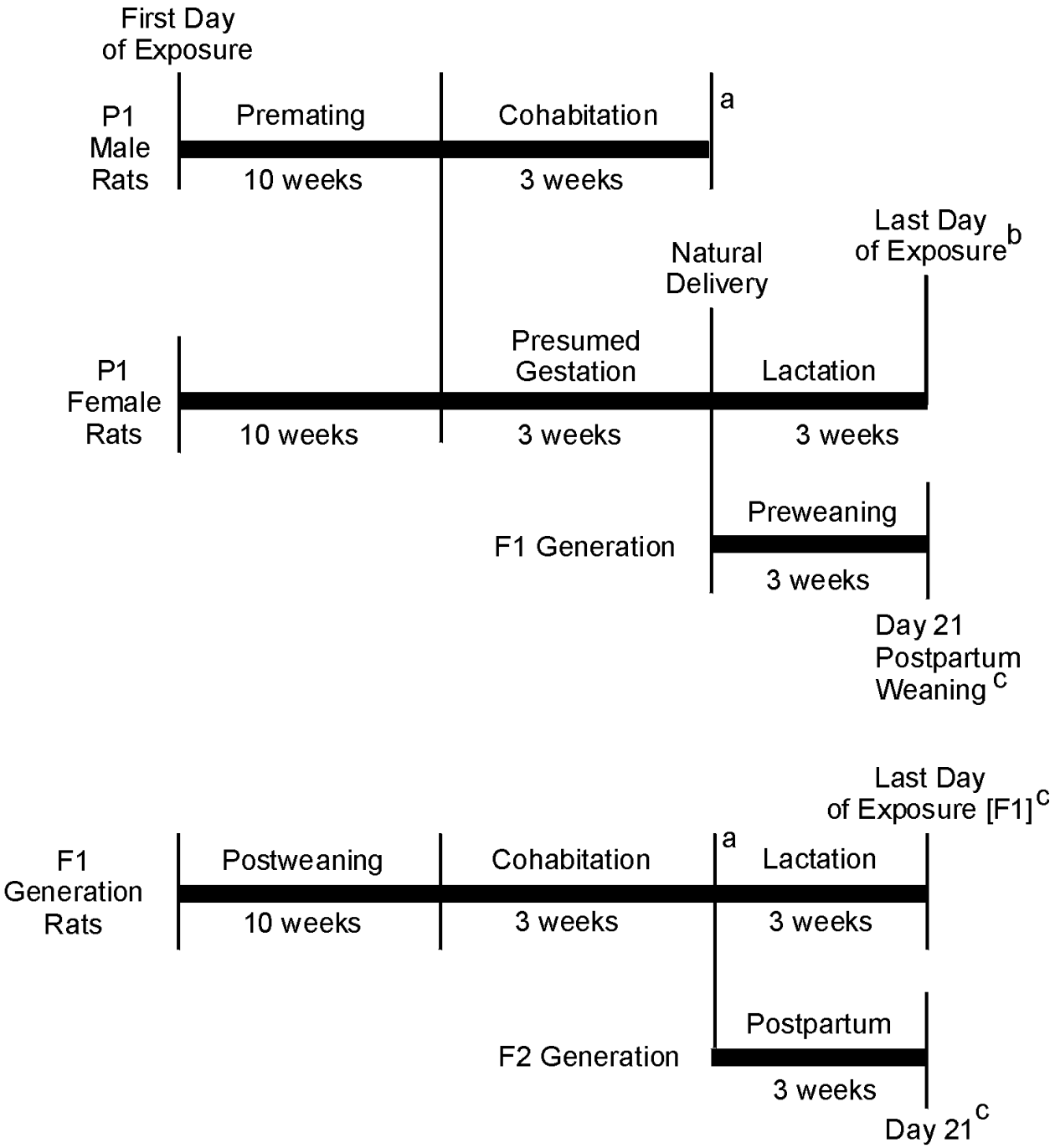
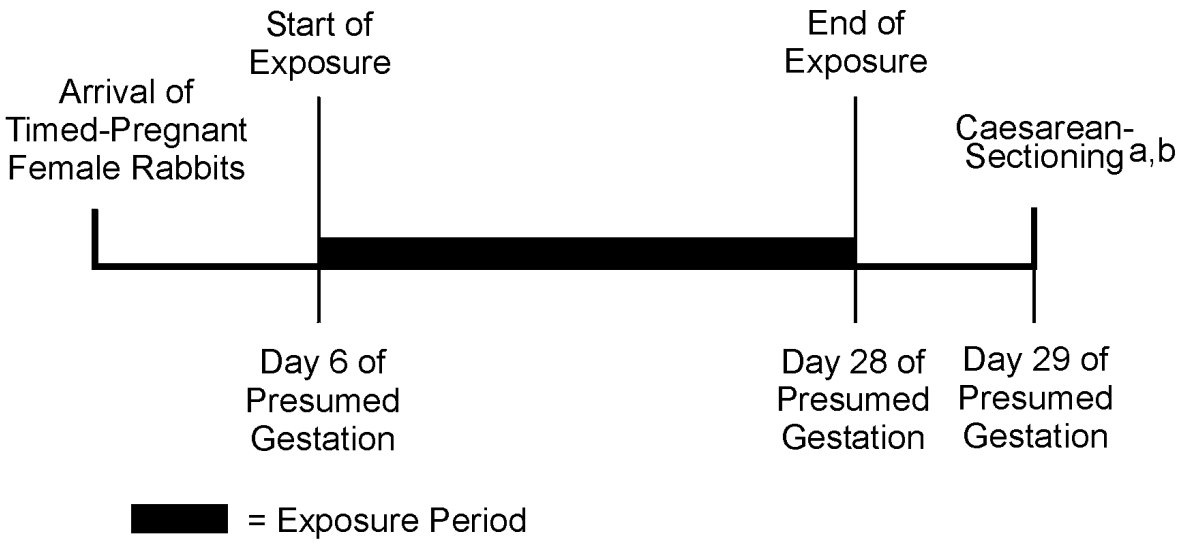


Figure A-2. Schematic of the oral (drinking water), two-generation (one litter per generation) reproduction study of ammonium perchlorate in SD rats (Argus Research Laboratories, Inc., 1998b).



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

Acronym	Definition
$\Delta A°_{rxn}$	Helmholtz free energy of reaction
$\Delta G°_{f}$	Gibbs free energy of formation
$\Delta G°_{rxn}$	Gibbs free energy of reaction
$\Delta S°_{univ}$	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
BF_4^-	tetrafluoroborate
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
BMR	benchmark response

Acronym	Definition
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_2	chlorine
CI	confidence interval
ClO^-	hypochlorite
ClO_4^-	perchlorate
ClUC-p	perchlorate urinary clearance
CNS	central nervous system
CP	cyclophosphamide
CPES	Coastal Plain Experiment Station
CPM	counts per minute
Cs^+	cesium
CsCl	cesium chloride
CTL	cytotoxic T-lymphocyte
CV	coefficient of variation
DAF	dosimetric adjustment factor

Acronym	Definition
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
$E°$	standard electric potential
F	Faraday constant
F1	first generation
F2	second generation
FAVF	Final acute value factor
FCN	function
FETAX	Frog Embryo Teratogenesis Assay: *Xenopus*
FGF	fibroblast growth factor
FH	follicular epithelial cell hypertrophy or hyperplasia
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
fT4	free thyroxine

Acronym	Definition
GA	Golgi apparatus
GD	gestation day
GGTP	g-glutamyl transpeptidase
GI	gastrointestinal
GMAV	Genus mean acute value
gsp	GTP-binding protein mutation
Gy	Gray (equal to 100 rads)
H^+	hydrogen
H_2O_2	hydrogen peroxide
hCG	human chorionic gonadotropin
$HClO_4$	perchloric acid
HEE	human equivalent exposure
HOCl	hypochlorous
I^-	iodide
IC	ion chromatography
IC_{25}	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

Acronym	Definition
iv	intravenously
K^+	potassium
K_2O	potassium oxide
Km	Michaelis-Menten affinity constant
KNO_3	potassium nitrate
LC_{50}	concentration lethal to 50% of population
LD	lactation day
LHAAP	Longhorn Army Ammunition Plant
Li^+	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
$Mg(ClO_4)_2$	magnesium perchlorate
MIT	monoiodotyrosine
MMIA	1-methyl-2-mercaptoimidazole
MANOVA	multiple analysis of variance
MCLG	maximum contaminant level goal

Acronym	Definition
MRL	minimum reporting limit
mRNA	messenger ribonucleic acid
MS-MS	mass spec — mass spec
MTD	maximum tolerated dose
n	number of electrons or number of moles
n.d.	no date
N-P-K ratio	nitrogen-phosphorous-potassium ratio
Na^+	sodium
$NaClO_4$	sodium perchlorate
$NaNO_3$	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_4^+	ammonium
NH_4ClO_4	ammonium perchlorate
NH_4NO_3	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 resinance
NO_3^-	nitrate
NOAEL	No-Observed-Adverse-Effect Level
NOEC	No-Observed-Effect Concentration

Acronym	Definition
NPDWR	National Primary Drinking Water Regulations
NRMRL	National Risk Management Research Laboratory
NTP	National Toxicology Program
O_2	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_2O_5	disphosphorus pentoxide
p53	*p53* 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

Acronym	Definition
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
ras	*ras* protooncogene
Rb^+	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_3	reverse triiodothyronine
SACR	secondary acute-chronic ratio
SAV	secondary acute value
sc	subcutaneously
SCN	thiocyanate
SCV	secondary chronic value
SD	standard deviation

Acronym	Definition
SD rats	Spraque-Dawley strain
SDWA	Safe Drinking Water Act
SE	standard error of the mean
SGOT	serum glutamyl oxacetic transsaminase
SGPT	serum glutamyl pyruvic transaminase
SLA	soluble *Listeria* 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

Acronym	Definition
UF	uncertainty factor
USAF	United States Air Force
USGS	United States Geological Survey
USN	United States Navy
V	volume
Vmaxc	Michaelis-Menten maximum velocity capacity
W_{exp}	expansion work
WHO	World Health Organization
WPAFB	Wright Patterson Air Force Base
WSWRD	Water Supply and Water Resources Division