44943

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
NATIONAL CENTER FOR ENVIRONMENTAL ASSESSMENT
WASHINGTON, DC 20460

JAN - 8 1999

OFFICE OF
RESEARCH AND DEVELOPMENT

MEMORANDUM

SUBJECT: Transmittal of External Review Draft of Document *Perchlorate Environmental Contamination: Toxicology Review and Risk Characterization Based on Emerging Information*

FROM: William H. Farland, Ph.D.
Director
National Center for Environmental Assessment

TO: Timothy Fields, Jr.
Acting Assistant Administrator
Office of Solid Waste and Emergency Response

Attached please find a copy of the external review draft of the document entitled *Perchlorate Environmental Contamination: Toxicology Review and Risk Characterization Based on Emerging Information*. Although the document was completed on the December 31, 1998, we regret that we were not able to transmit the document until today because the PDF conversion was problematic, owing to the large number of graphics and tables. As you know, this is the document that was developed based on data from expedited studies, as part of the EPA partnership with the Department of Defense to target a testing strategy at the suspected effects of perchlorate relevant to human health and to provide a screening ecological assessment. It features some unique approaches to risk assessment, notably a harmonized noncancer and cancer oral human health risk benchmark.

We appreciate OSWER taking the lead on the logistics for the external peer review and look forward to having the benefit of this additional input on the assessment. Because data remain pending in a number of critical areas (reproductive, immunotoxicity, and physiologically-based pharmacokinetic modeling), the document should be considered a work in progress. This is consistent with Dorothy Canter's expressed views. The external peer review will allow us the opportunity to have others, including the public, comment as to whether we appear to be on the correct path and perhaps offer suggestions on how to integrate the remainder of incoming data in context with what has been accomplished with this draft.

Recycled/Recyclable • Printed with Vegetable Oil Based Inks on 100% Recycled Paper (40% Postconsumer)

The draft document is concurrently being directly mailed to Region 9, the Office of Water, and the Department of Defense. We will ensure that full sets of supporting materials will also be available at various locations, including the Superfund Resource Centers in Regions 9, 8 and 6, the National Center for Environmental Assessment offices in Cincinnati and Research Triangle Park, Wright-Patterson Air Force Base in Dayton, OH, the Californian Department of Health Services, and the California Environmental Protection Agency's Office of Environmental Health Hazard Assessment. The PDF version will be located at the following URL on NCEA's web (http://www.epa.gov/ncea/perch.htm) site with a link to the perchlorate web site maintained by the Office of Water (http://www.epa.gov/ogwd/ccl/perclorperchl.html).

The success of this project is due to the close working relationship that each of the involved offices (Region 9, NCEA/ORD, NHEERL/ORD, NRML/ORD, OSWER, OW), as well as DoD has been able to maintain during this rather intense period. We look forward to continued contribution in that same spirit as we push toward the external peer review and subsequent revision of the assessment. Do not hesitate to contact NCEA for any additional support.

Attachment

cc: Norine Noonan (ORD)
Lt. Col. Dan Rogers (DoD)
Gary Foley (NERL)
Tim Oppelt (NRMRL)
Larry Reiter (NHEERL)
Les Grant (NCEA-RTP)
Terry Harvey (NCEA-Cin)
Michael Callahan (NCEA-W)
Dorothy Canter (OSWER)
Peter Grevatt (OSWER)
Mike Osinksi (OW)
Keith-Takata (Region 9)
Kevin Mayer (Region 9)
Edward Urbansky (NRML)
Annie Jarabek (NCEA-RTP)
Linda Tuxen (NCEA IO)

United States
Environmental Protection
Agency

Office of Research
and Development
Washington, DC 20460

NCEA-1-0503
December 31, 1998
External Review Draft

EPA

Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization Based on Emerging Information

Review Draft (Do Not Cite or Quote)

Notice

This document is a preliminary draft. It has not been formally released by EPA and should not at this stage be construed to represent Agency policy. It is being circulated for comment on its technical accuracy and policy implications.

NCEA-1-0503
December 31, 1998
External Review Draft

Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization Based on Emerging Information

Notice

This document is a preliminary draft. It has not been formally released by EPA and should not at this stage be construed to represent Agency policy. It is being circulated for comment on its technical accuracy and policy implications.

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

Disclaimer

This document is an external draft for review purposes only and does not constitute U.S. Environmental Protection Agency policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Table of Contents

Page

List of Tables viii
List of Figures xiii
Contributors and Reviewers xxi
Preface xxiii
Acknowledgments xxv

EXECUTIVE SUMMARY E-1

1. INTRODUCTION 1-1
   1.1 PRODUCTION USES AND SOURCES OF PERCHLORATE CONTAMINATION 1-1
   1.2 OCCURRENCE AND HISTORICAL HUMAN HEALTH RISK CHARACTERIZATION 1-3
   1.3 ECOTOXICOLOGY ASSESSMENT 1-12
   1.4 FUTURE REGULATORY PLANS 1-13
      1.4.1 U.S. Environmental Protection Agency Regulatory Plans 1-14
      1.4.2 State Regulatory Plans 1-15
   1.5 SUMMARY 1-15

2. PHYSICOCHEMICAL CHARACTERISTICS 2-1

3. HISTORICAL HAZARD IDENTIFICATION 3-1
   3.1 HUMAN DATA 3-1
      3.1.1 Epidemiological Data 3-1
      3.1.2 Studies in Patients with Graves' Disease 3-1
      3.1.3 Studies in Healthy Human Subjects 3-4
      3.1.4 Hematological Effects 3-5
   3.2 LABORATORY ANIMAL BIOASSAYS 3-6
      3.2.1 Short-Term and Subchronic Studies 3-6
      3.2.2 Long-Term Studies 3-9
   3.3 DEVELOPMENTAL AND REPRODUCTIVE STUDIES 3-10
   3.4 ABSORPTION, DISTRIBUTION, METABOLISM, AND ELIMINATION STUDIES 3-12
      3.4.1 Human Studies 3-13
      3.4.2 Laboratory Animal Studies 3-13
   3.5 SUMMARY 3-17

4. TOXICOKINETICS/TOXICODYNAMICS AND MODE-OF-ACTION TESTING STRATEGY 4-1
   4.1 ABSORPTION, DISTRIBUTION, METABOLISM, AND ELIMINATION OF PERCHLORATE 4-1
   4.2 IODINE METABOLISM AND THYROID PHYSIOLOGY 4-2

Table of Contents
(cont'd)

Page

4.3 TOXICOKINETICS OF PERCHLORATE 4-7
4.4 TOXICODYNAMICS OF THYROID HORMONE PERTURBATIONS 4-9
4.4.1 Carcinogenic Effects 4-9
4.4.2 Other Potential Adverse Effects Resulting from Thyroid Disruption 4-13
4.5 DEVELOPMENT OF A TOXICITY TESTING STRATEGY BASED ON MODE OF ACTION 4-18

5. RESULTS OF MODE-OF-ACTION TESTING STRATEGY AND RECENT STUDIES 5-1
5.1 STUDIES IN HUMANS 5-1
5.2 LABORATORY ANIMAL BIOASSAYS 5-7
5.2.1 Caldwell et al. (1995) 14-Day Study 5-7
5.2.1.1 Thyroid Histology Data 5-7
5.2.1.2 Thyroid and Pituitary Hormone Analyses 5-9
5.2.2 The 90-Day Testing Strategy Bioassay in Rats 5-11
5.2.2.1 General Toxicity and Histology Results 5-15
5.2.2.2 Thyroid and Pituitary Hormone Analyses 5-17
5.2.3 Neurodevelopmental Toxicity Study in Rats 5-22
5.2.3.1 Results of General Toxicity Measures, Neurohistology, and Morphology 5-25
5.2.3.2 Evaluation of Thyroid Histology 5-27
5.2.3.3 Thyroid and Pituitary Hormone Analyses 5-31
5.2.3.4 Behavioral Evaluations 5-32
5.2.4 Two-Generation Reproductive Toxicity Study in Rats 5-38
5.2.4.1 General Toxicity Results and Evaluation of Reproductive Parameters 5-40
5.2.4.2 Evaluation of Thyroid Histology 5-42
5.2.4.3 Thyroid and Pituitary Hormone Analyses 5-42
5.2.5 Segment II Developmental Toxicity Study in Rabbits 5-42
5.2.5.1 Results of Maternal Examinations 5-43
5.2.5.2 Developmental Endpoints 5-44
5.2.5.3 Maternal Thyroid and Pituitary Hormone Analyses 5-44
5.2.6 Immunotoxicity Study in Mice 5-46
5.2.6.1 Results for General Toxicity, Organ Weight, and Cellularity Measures 5-50
5.2.6.2 Evaluation of Thyroid Histology 5-51
5.2.6.3 Thyroid and Pituitary Hormone Analyses 5-51
5.2.6.4 Results of Immune Function Assays 5-52
5.2.6.5 Results for Evaluations of Hematological Parameters 5-55
5.2.6.6 Results Summary with a Discussion of Parameters of Experiments in Progress 5-55

Table of Contents
(cont'd)

Page

5.3 GENOTOXICITY ASSAYS 5-57
5.3.1 In Vitro Assays 5-58
5.3.2 In Vivo Assays. 5-59
5.3.3 Summary of Genotoxicity Battery Results 5-61
5.4 ABSORPTION, DISTRIBUTION, METABOLISM, AND ELIMINATION AND MECHANISTIC STUDIES 5-61

6. DOSE-RESPONSE ASSESSMENTS FOR HUMAN HEALTH 6-1
6.1 ORAL REFERENCE DOSE 6-1
6.1.1 Choice of Critical Effect and Principal Study 6-2
6.1.1.1 Correlation Analyses of Hormone and Thyroid Histopathology . . . 6-12
6.1.1.2 Weight-of-Evidence Summary 6-30
6.1.1.3 Possible Susceptibility 6-32
6.1.2 Point-of-Departure Analysis 6-32
6.1.2.1 Benchmark Dose Estimates Submitted to the U.S. Environmental Protection Agency 6-33
6.1.2.2 U.S. Environmental Protection Agency Benchmark Dose Estimates for Standard Thyroid Histopathology 6-34
6.1.2.3 U.S. Environmental Protection Agency Benchmark Dose Estimates for Triiodothyronine, Thyroxine, and Thyroid Stimulating Hormones 6-40
6.1.2.4 U.S. Environmental Protection Agency Benchmark Dose Estimates for Motor Activity 6-42
6.1.2.5 Summary of U.S. Environmental Protection Agency Benchmark Dose Analyses 6-45
6.1.3 Application of Uncertainty Factors 6-45
6.1.4 Designation of Confidence Levels 6-49
6.1.5 Operational Derivation 6-50
6.2 INHALATION REFERENCE CONCENTRATION 6-50
6.3 CANCER ASSESSMENT 6-51
Appendix 6A: Correlation Tables for Figures 6-3 Through 6-16 . . . . . 6A-1
Appendix 6B: Benchmark Dose Statisitics 6B-1

7. SCREENING ECOLOGICAL RISK ASSESSMENT FOR PERCHLORATE 7-1
7.1 INTRODUCTION 7-1
7.1.1 Management Goals and Decisions 7-1
7.1.2 Scope, Complexity, and Focus 7-2
7.2 PROBLEM FORMULATION 7-3
7.2.1 Assessment Endpoints 7-3
7.2.1.1 Fish Community Richness and Productivity 7-3
7.2.1.2 Aquatic Invertebrate Community Richness and Productivity 7-4

Table of Contents
(cont'd)

Page

7.2.1.3 Aquatic Plant Productivity 7-4
7.2.1.4 Soil Invertebrate Community Richness and Productivity 7-4
7.2.1.5 Terrestrial Plant Productivity 7-4
7.2.1.6 Population Productivity of Herbivorous Wildlife 7-5
7.2.2 Conceptual Models 7-5
7.2.3 Analysis Plan 7-7
7.3 ANALYSIS 7-7
7.3.1 Characterization of Exposure 7-7
7.3.1.1 Water Concentrations 7-7
7.3.1.2 Aquatic Bioaccumulation 7-8
7.3.1.3 Soil Levels 7-8
7.3.1.4 Uptake by Vegetation 7-9
7.3.1.5 Herbivore Exposure 7-9
7.3.2 Characterization of Effects 7-10
7.3.2.1 Aquatic Organisms 7-10
7.3.2.2 Terrestrial Organisms 7-13

8. MAJOR CONCLUSIONS IN RISK CHARACTERIZATION OF HAZARD AND DOSE RESPONSE 8-1
8.1 HUMAN HEALTH 8-1
8.1.1 Hazard Potential 8-1
8.1.2 Dose Response 8-2
8.1.3 Risk Characterization 8-3
8.1.3.1 Direct Exposures 8-3
8.1.3.2 Indirect Exposures 8-4
8.1.4 Major Uncertainties and Research Needs 8-5
8.2 ECOTOXICOLOGY 8-5
8.2.1 Aquatic Life 8-5
8.2.2 Risks to Consumers of Aquatic Life 8-6
8.2.3 Terrestrial Life 8-7
8.2.3.1 Plants 8-7
8.2.3.2 Soil Invertebrates 8-7
8.2.3.3 Herbivores 8-8
8.2.4 Uncertainties 8-8
8.2.4.1 Uncertainties Concerning Aquatic Risks 8-8
8.2.4.2 Uncertainties Concerning Terrestrial Risks 8-9
8.2.5 Research Needs 8-10
8.2.5.1 Exposure 8-10
8.2.5.2 Effects 8-11

9. REFERENCES 9-1

Table of Contents
(cont'd)

Page

APPENDIX A: Schematics of Study Designs for Neurodevelopmental, Two-Generation Reproductive and Developmental Studies A-1

APPENDIX B: List of Abbreviations and Acronyms B-1

List of Tables

Number		Page
1-1	Occurrence and Potential Sources of Perchlorate in Drinking Water Systems in U.S. Environmental Protection Agency Region 9 (1997-1998)	1-11
2-1	Gibbs Free Energies of Formation for Selected Anions in Aqueous Solution	2-2
2-2	Physicochemical Properties of Ammonium and Alkali Metal Perchlorates at 25 °C	2-3
4-1	Interspecies and Intraspecies Differences in Thyroid Structure and Triiodothyronine, Thyroxine, and Thyroid Stimulating Hormones	4-7
4-2	Mechanisms of Antithyroid-Mediated Neoplasia in Rodents	4-11
4-3	Main Symptoms and Effects of Hypothyroidism	4-16
4-4	Minimum Database for Derivation of an Oral Reference Dose	4-19
4-5	Factors for Uncertainties in Applied Extrapolations Used To Derive Reference Doses	4-19
4-6	Perchlorate Peer Review Recommended Studies Summary	4-22
5-1	Thyroid Disorders and Their Approximate Prevalences in the Human Neonatal Period	5-3
5-2	Summary of No-Observed-Adverse-Effect Levels for the Caldwell et al. (1995) 14-Day Study	5-14
5-3	Combined Incidence Data and Average Severity Scores for Male and Female Postnatal Day 5 Rat Pups for Follicular Epithelial Cell Hypertrophy and Decrease in Follicular Lumen Size Based on Standard Histology	5-29
5-4	Ratio of All Rats with Any Evidence of Follicular Epithelial Cell Hypertrophy of the Thyroid Gland to Total Number of Rat Pups Examined Based on Standard Histology	5-30
5-5	Percent Inhibition of Iodide Uptake in the Thyroid Gland of Sprague-Dawley Rats Dosed with Perchlorate	5-64
6-1A	Summary of Triiodothyronine Thyroid Hormone Effects	6-3
6-1B	Summary of Thyroxine Thyroid Hormone Effects	6-4

List of Tables
(cont'd)

Number		Page
6-1C	Summary of Thyroid Stimulating Hormone Pituitary Hormone Effects	6-5
6-1D	Summary of Thyroid Histopathology Effects	6-6
6-2	Summary of Hormone and Histology Effects	6-7
6-3	Default Dose-Response Procedures for Thyroid Carcinogens	6-51
6-4	Data Demonstrating Antithyroid Activity	6-52
6A-1	Pearson's r Correlations Between Thyroid Hormones and Thyroid Stimulating Hormones in Rats of the Caldwell et al. (1995) 14-Day Study	6A-1
6A-2	Spearman's r_S Correlations Between the Rank Order of Hormone Levels and the Standard Histological Severity Rating of Follicular Epithelial Cell Hypertrophy or Decrease in Follicular Lumen Size in Rats of the Caldwell et al. (1995) 14-Day Study	6A-1
6A-3	Pearson's r Correlations Between Thyroid Hormones and Thyroid Stimulating Hormones in Rats for the Combined 14- and 90-Day Data of the Springborn Laboratories, Inc. (1998) Subchronic Rat Study	6A-2
6A-4	Spearman's r_S Correlations Between the Rank Order of Hormone Levels and the Standard Histological Severity Rating of Follicular Epithelial Cell Hypertrophy/Hyperplasia or Decrease in Follicular Lumen Size for the Combined 14- and 90-day Data of the Springborn Laboratories, Inc. (1998) Subchronic Rat Study	6A-2
6A-5	Pearson's r Correlations Between Thyroid Hormones and Thyroid Stimulating Hormones for the 14-Day Data of the Springborn Laboratories, Inc. (1998) Subchronic Rat Study	6A-2
6A-6	Spearman's r_S Correlations Between the Rank Order of Hormone Levels and the Standard Histological Severity Rating of Follicular Epithelial Cell Hypertrophy/Hyperplasia for the 14-Day Data of the Springborn Laboratories, Inc. (1998) Subchronic Rat Study	6A-3
6A-7	Pearson's r Correlations Between Thyroid Hormones and Thyroid Stimulating Hormones of the 90-Day Data of the Springborn Laboratories, Inc. (1998) Subchronic Rat Study	6A-3

List of Tables
(cont'd)

Number		Page
6A-8	Spearman's r_S Correlations Between the Rank Order of Hormone Levels and the Standard Histological Severity Rating of Follicular Epithelial Cell Hypertrophy/Hyperplasia for the 90-Day Data of the Springborn Laboratories, Inc. (1998) Subchronic Rat Study	6A-3
6A-9	Pearson's r Correlations Between Thyroid Hormones and Thyroid Stimulating Hormones for the First-Generation Rat Pups on Postnatal Day 5 in the Developmental Neurotoxicity Study (Argus Research Laboratories, Inc., 1998a)	6A-4
6A-10	Spearman's r_S Correlations Between the Rank Order of Hormone Levels and the Standard Histological Severity Rating of Follicular Epithelial Cell Hypertrophy for the First-Generation Rat Pups on Postnatal Day 5 in the Developmental Neurotoxicity Study (Argus Research Laboratories, Inc., 1998a)	6A-4
6A-11	Pearson's r Correlations Between Thyroid Hormones and Thyroid Stimulating Hormones in Rabbits on Gestation Day 29 of the Developmental Study (Argus Research Laboratories, Inc., 1998c)	6A-4
6A-12	Spearman's r_S Correlations Between the Rank Order of Hormone Levels and the Standard Histological Severity Rating of Follicular Epithelial Cell Hypertrophy for Rabbits on Gestation Day 29 of the Developmental Study (Argus Research Laboratories, Inc., 1998c)	6A-5
6B-1	Functions Used in Benchmark Dose Modeling	6B-1
6B-2	Benchmark Dose Estimates for Male Hormone Data of Caldwell et al. (1995) 14-Day Rat Study, Using Kodell-West Algorithm	6B-2
6B-3	Coefficients and Goodness-Of-Fit Statistics of Kodell-West Model Fits to Male Hormone Data of Caldwell et al. (1995) 14-Day Rat Study	6B-3
6B-4	Benchmark Dose and Benchmark Dose 95% Lower Limit Estimates of the Incidence of Follicular Epithelial Cell Hypertrophy in the Caldwell et al. (1995) 14-Day Study	6B-3

List of Tables
(cont'd)

Number | Page

6B-5 Benchmark Dose and Benchmark Dose 95% Lower Limit Estimates of the Incidence of Follicular Epithelial Cell Hypertrophy/Hyperplasia for the 14-Day Time Point of the Springborn Laboratories, Inc. (1998) Subchronic Study 6B-4

6B-6 Benchmark Dose and Benchmark Dose 95% Lower Limit Estimates of the Incidence of Follicular Epithelial Cell Hypertrophy/Hyperplasia for the 90-Day Time Point of the Springborn Laboratories, Inc. (1998) Subchronic Study 6B-4

6B-7 Benchmark Dose and Benchmark Dose 95% Lower Limit Estimates of the Incidence of Follicular Epithelial Cell Hypertrophy in the First-Generation Pups on Postnatal Day 5 from the Developmental Neurotoxicity Study 6B-5

6B-8 Benchmark Dose Estimates Using Power Function Fit to Combined Male and Female Hormone Data of Caldwell et al. (1995) 14-Day Rat Study 6B-6

6B-9 Benchmark Dose and Benchmark Dose 95% Lower Limit Estimates Using Power Function Fit to Combined Male and Female Hormone Data of Caldwell et al. (1995) 14-Day Rat Study 6B-7

6B-10 Benchmark Dose and Benchmark Dose 95% Lower Limit Estimates for Combined Male and Female Hormone Data of 14-Day Time Point in the Springborn Laboratories, Inc. (1998) Subchronic Study 6B-8

6B-11 Benchmark Dose and Benchmark Dose 95% Lower Limit Estimates for Combined Male and Female Hormone Data of 14-Day Time Point in the Springborn Laboratories, Inc. (1998) Subchronic Study 6B-9

6B-12 Benchmark Dose Estimates for Combined Male and Female Hormone Data of 90-Day Time Point in the Springborn Laboratories, Inc. (1998) Subchronic Study 6B-10

6B-13 Benchmark Dose and Benchmark Dose 95% Lower Limit Estimates for Combined Male and Female Hormone Data of 90-Day Time Point in the Springborn Laboratories, Inc. (1998) Subchronic Study 6B-10

6B-14 Benchmark Dose and Benchmark Dose 95% Lower Limit Estimates for Hormone and Thyroid Morphometry Data of First-Generation Pups at Postnatal Day 5 in the Developmental Neurotoxicity Study 6B-11

List of Tables
(cont'd)

Number		Page
6B-15	Benchmark Dose and Benchmark Dose 95% Lower Limit Estimates for Hormone Data of First-Generation Pups at Postnatal Day 5 in the Developmental Neurotoxicity Study	6B-12
6B-16	Benchmark Dose and Benchmark Dose 95% Lower Limit Estimates Using the Linear Model Fit to the Motor Activity Data of First-Generation Pups at Postnatal Day 14 in the Developmental Neurotoxicity Study	6B-12
6B-17	Benchmark Dose and Benchmark Dose 95% Lower Limit Estimates Using the Power Model Fit to the Hormone Data of Female Rabbits on Gestation Day 29 in the Developmental Study	6B-13
6B-18	Benchmark Dose and Benchmark Dose 95% Lower Limit Estimates Using the Power Model Fit to the Hormone Data of Female Rabbits on Gestation Day 29 in the Developmental Study (Argus, 1998c)	6B-13
7-1	Results of Perchlorate Toxicity Tests in Aquatic Species	7-11
7-2	Procedure for Deriving Tier II Water Quality Values for Sodium Perchlorate	7-12

List of Figures

Number		Page
1-1	Sources and pathways of groundwater contamination for perchlorate	1-4
1-2	States indicated as having confirmed perchlorate manufacturers or users are based on U.S. Environmental Protection Agency Information Request responses from current manufacturers	1-7
1-3	States with confirmed releases, in which facilities have directly measured perchlorate in groundwater or surface water	1-8
1-4	Locations of facilities in U.S. Environmental Protection Agency Region 9 and in Magna, UT (Region 8), at which perchlorate has been released to groundwater or surface water	1-9
1-5	Considerations for comprehensive characterization of perchlorate contamination	1-17
1-6	Structure and membership of the executive committee, subcommittee areas, and co-chairs of the Interagency Perchlorate Steering Committee	1-19
2-1	Chemical structure of perchlorate	2-1
4-1	Schematic representation of thyroid hormone biosynthesis and secretion in a single thyroid follicular cell	4-4
4-2	Schematic of the hypothalamic-pituitary-thyroid axis and feedback mechanisms	4-6
4-3	Schematic of antithyroid effects that influence thyroid carcinogenesis	4-10
4-4	Proliferative changes involved in the multistage characterization of thyroid follicular cell neoplasia in rodents represent a morphologic continuum	4-12
4-5	Possible molecular events in human thyroid follicular carcinogenesis	4-14
4-6	Schematic representation of the role of the placenta in thyroid hormone metabolism during human pregnancy	4-15
4-7	Schematic illustrating that a high confidence oral reference dose is based on data that address all potentially critical stages over a lifetime	4-19
4-8	Schematic characterization of comprehensive exposure-dose-response continuum and the evolution of protective to predictive dose-response estimates	4-20

List of Figures
(cont'd)

Number		Page
5-1	Effects in the Caldwell et al. (1995) study of 14-day drinking water administration of ammonium perchlorate to Sprague-Dawley rats on serum total triiodothyronine concentrations	5-10
5-2	Effects in the Caldwell et al. (1995) study of 14-day drinking water administration of ammonium perchlorate to Sprague-Dawley rats on serum total thyroxine concentrations	5-11
5-3	Effects in the Caldwell et al. (1995) study of 14-day drinking water administration of ammonium perchlorate to Sprague-Dawley rats on serum thyroid stimulating hormone concentrations	5-12
5-4	Effects in the Caldwell et al. (1995) study of 14-day drinking water administration of ammonium perchlorate to Sprague-Dawley rats on serum reverse triiodothyronine concentrations	5-13
5-5	Effects in the Caldwell et al. (1995) study of 14-day drinking water administration of ammonium perchlorate to Sprague-Dawley rats on serum thyroglobulin concentrations	5-14
5-6	Effects from 90-day drinking water administration of ammonium perchlorate to Sprague-Dawley rats on serum total triiodothyronine concentrations	5-19
5-7	Effects from 90-day drinking water administration of ammonium perchlorate to Sprague-Dawley rats on serum total thyroxine concentrations	5-20
5-8	Effects from 90-day drinking water administration of ammonium perchlorate to Sprague-Dawley rats on serum total thyroxine	5-21
5-9	Effects from 90-day drinking water administration to ammonium perchlorate to Sprague-Dawley rats on serum total thyroid stimulating hormone	5-23
5-10	Effects from maternal drinking water administration of ammonium perchlorate to Sprague-Dawley rats on thyroid gland follicular lumen size in first-generation offspring on Postnatal Day 5	5-31
5-11	Effects from maternal drinking water administration of ammonium perchlorate to Sprague-Dawley rats on serum total triiodothyronine concentrations in first-generation offspring on Postnatal Day 5	5-33

List of Figures
(cont'd)

Number		Page
5-12	Effects from maternal administration of ammonium perchlorate to Sprague-Dawley rats on serum total thyroxine concentrations in first-generation offspring on Postnatal Day 5	5-34
5-13	Effects from maternal drinking water administration of ammonium perchlorate to Sprague-Dawley rats on serum thyroid stimulating hormone concentrations in first-generation offspring on Postnatal Day 5	5-35
5-14	The effects of developmental exposure to perchlorate on motor activity in male rats on Postnatal Day 14	5-36
5-15	Lack of effects from ammonium perchlorate drinking water administration in pregnant New Zealand rabbits during Gestation Days 6 to 28 on triiodothyronine	5-46
5-16	Effects from ammonium perchlorate drinking water administration in pregnant New Zealand rabbits during Gestation Days 6 to 28 on thyroxine	5-47
5-17	Lack of effects from ammonium perchlorate drinking water administration in pregnant New Zealand rabbits during Gestation Days 6 to 28 on thyroid stimulating hormones	5-48
5-18	Effects from drinking water administration of ammonium perchlorate in mice on total serum thyroxine	5-52
5-19	Effects from drinking water administration of ammonium perchlorate exposure in mice on total serum thyroid stimulating hormones	5-53
5-20	Inhibition by perchlorate of iodide uptake in the thyroid of rats given a single iv injection	5-63
6-1	Schematic of the exposure-dose-response continuum considered in the context of biomarkers and level of organization at which toxicity is observed	6-11
6-2	Pattern of change in fetal and neonatal thyroid function parameters during pregnancy and extrauterine adaptation in the human	6-13
6-3	Correlations between triiodothyronine versus thyroxine and thyroxine versus thyroid stimulating hormone in rats of the 14-day study of Caldwell et al. (1995)	6-15

List of Figures
(cont'd)

Number		Page
6-4	Correlations between the rank order of thyroxine versus standard histological severity rating of follicular epithelial cell hypertrophy or of decrease in follicular lumen size for the rats of the 14-day study of Caldwell et al. (1995)	6-16
6-5	Correlations between the rank order of thyroid stimulating hormones versus standard histological severity rating of follicular epithelial cell hypertrophy or of decrease in follicular lumen size for the rats of the 14-day study of Caldwell et al. (1995)	6-17
6-6	Correlations between triiodothyronine versus thyroxine and thyroxine versus thyroid stimulating hormones for the combined data of the 14-day and 90-day time points from the subchronic study in rats	6-18
6-7	Correlation between the rank order of thyroxine or thyroid stimulating hormones versus standard histopathology severity rating of thyroid hypertrophy/hyperplasia for the combined data of the 14-day and 90-day time points from the subchronic study in rats	6-19
6-8	Correlations between triiodothyronine versus thyroxine and thyroxine versus thyroid stimulating hormones for the combined data of the 14-day time point from the subchronic study in rats	6-20
6-9	Correlations between the rank order of thyroxine or thyroid stimulating hormones versus standard histopathology severity rating of thyroid hypertrophy/hyperplasia for the 14-day time point from the subchronic study in rats	6-21
6-10	Correlations between triiodothyronine versus thyroxine and thyroxine versus thyroid stimulating hormones for the combined data of the 90-day time point from the subchronic study in rats	6-22
6-11	Correlations between the rank order of thyroxine or thyroid stimulating hormones versus standard histopathology severity rating of thyroid hypertrophy/hyperplasia for the 90-day time point from the subchronic study in rats	6-23
6-12	Correlations between triiodothyronine versus thyroxine and thyroxine versus thyroid stimulating hormones for the first-generation rat pups on Postnatal Day 5 in the developmental neurotoxicity study	6-24

List of Figures
(cont'd)

Number | Page

6-13 Correlations between the rank order of thyroxine versus standard histopathology severity rating of thyroid follicular epithelial cell hypertrophy rating or for standard histopathology severity rating of the decrease in follicular lumen size for the Postnatal Day 5 pups in the neurodevelopmental study 6-25

6-14 Correlations between the rank order of thyroid stimulating hormones versus standard histopathology rating of thyroid follicular epithelial cell hypertrophy rating or for standard histopathology severity rating of the decrease in follicular lumen size for the Postnatal Day 5 pups in the neurodevelopmental study 6-26

6-15 Correlations between triiodothyronine versus thyroxine and thyroxine versus thyroid stimulating hormones for the data in the parent generation on Gestation Day 29 rabbits from the developmental study 6-27

6-16 Correlations between the rank order of thyroxine or thyroid stimulating hormones and the standard histopathological severity rating of follicular hypertrophy in the parent generation on Gestation Day 29 rabbits from the developmental study 6-28

6-17 Benchmark dose estimates derived from fits of various model functions to the dose-response of incidence of standard histopathological detection in rats of follicular epithelial cell hypertrophy or hypertrophy/hyperplasia 6-35

6-18 Benchmark dose 95% lower confidence limit estimates derived from fits of various model functions to the dose-response of incidence of standard histopathological detection in rats of follicular epithelial cell hypertrophy or hypertrophy/hyperplasia 6-36

6-19 Box and whisker plots of benchmark dose estimates derived from the fits of various model functions to the dose-response of incidence of standard histopathological detection in rats of follicular epithelial cell hypertrophy or hypertrophy/hyperplasia 6-37

6-20 Box and whisker plots of benchmark dose 95% lower confidence limit estimates derived from the fits of various model functions to the dose-response of incidence of standard histopathological detection in rats of follicular epithelial cell hypertrophy or hypertrophy/hyperplasia 6-38

List of Figures
(cont'd)

Number		Page
6-21	Model fit of the Quantal Linear/Weibull/Gamma functions to the litter-by-litter incidence of standard histopathological detection of follicular epithelial cell hypertrophy for the first-generation rat pups on Postnatal Day 5 in the developmental neurotoxicity study	6-39
6-22	Frequency of occurrence per litter by dose group of each standard histopathological severity rating for follicular epithelial cell hypertrophy in the first-generation rat pups on Postnatal Day 5 of the developmental neurotoxicity study	6-40
6-23	Frequency of occurrence by dose group of each standard histopathological severity rating for follicular epithelial cell hypertrophy for the rats in the 14-day study of Caldwell et al. (1995)	6-41
6-24	Benchmark dose and benchmark dose 95% lower confidence limit estimates derived from fits of various model functions to the dose-response data for hormone, thyroid morphometry, and motor activity endpoints from the various studies in the mode-of-action testing strategy	6-43
6-25	Box and whisker plot of benchmark dose and benchmark dose 95% lower confidence limit estimates derived from the fits of various model functions to the dose-response data for hormone, thyroid morphometry, and motor activity endpoints from the various studies in the mode-of-action testing strategy	6-44
6-26	Consideration of uncertainty and variability influence interspecies and intrahuman extrapolation	6-47
6-27	Schematic of uncertainty factor components incorporated into exposure-dose-response characterization for interspecies and intrahuman extrapolations	6-47
6-28	Schematic of physiologically based pharmacokinetc model that is under development to provide mechanistic data and improve quantitative interspecies extrapolation and possibly intrahuman variability	6-49
7-1	A conceptual model of exposure to ecological endpoint receptors to perchlorate	7-6
A-1	Schematic of the neurobehavioral developmental study of ammonium perchlorate administered orally in drinking water to Sprague-Dawley rats	A-2

List of Figures
(cont'd)

Number | Page

A-2 Schematic of the oral, two-generation reproduction study of ammonium perchlorate in Sprague-Dawley rats A-3

A-3 Schematic of the oral developmental toxicity study of ammonium perchlorate in New Zealand rabbits A-4

Contributors and Reviewers

Chemical Manager/Assessment Author

Annie M. Jarabek
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Research Triangle Park, NC

Contributing Assessment Authors

Members of the U.S. Environmental Protection Agency perchlorate risk assessment review team are listed below in alphabetical order.

Randy Bruins, Ph.D.
National Center for Environmental Assessment
Cincinnati, OH

Harlal Choudhury, Ph.D.
National Center for Environmental Assessment
Cincinnati, OH

Eric Clegg, Ph.D.
National Center for Environmental Assessment
Washington, DC

Kevin Crofton, Ph.D.
National Health and Environmental Effects Laboratory
Research Triangle Park, NC

Vicki Dellarco, Ph.D.
Office of Water
Washington, DC

Andrew Geller, Ph.D.
National Health and Environmental Effects Laboratory
Research Triangle Park, NC

Gary Kimmel, Ph.D.
National Center for Environmental Assessment
Washington, DC

Mary Manibusan
Office of Water
Washington, DC

Ralph Smialowicz, Ph.D.
National Health and Environmental Effects Laboratory
Research Triangle Park, NC

Glenn Suter, Ph.D.
National Center for Environmental Assessment
Cincinnati, OH

Contributors and Reviewers
(cont'd)

Internal EPA Reviewers

Dorothy Canter, Ph.D.
Office of Solid Waste and Emergency Response
Washington, DC

Christopher Cubbison, Ph.D.
National Center for Environmental Assessment
Cincinnati, OH

Joyce Donohue, Ph.D.
Office of Water
Washington, DC

Richard Hill, Ph.D.
Office of Prevention, Pesticides, and Toxic Substances
Washington, DC

Carole Kimmel, Ph.D.
National Center for Environmental Assessment
Washington, DC

Chris Lau, Ph.D.
National Human and Ecological Effects Research Laboratory
Research Triangle Park, NC

Susan Makris, Ph.D.
Office of Prevention, Pesticides, and Toxic Substances
Washington, DC

Vince Nabholz, Ph.D.
Office of Prevention, Pesticides, and Toxic Substances
Washington, DC

William van der Schalie, Ph.D.
National Center for Environmental Assessment
Washington, DC

Jennifer Seed, Ph.D.
Office of Prevention, Pesticides, and Toxic Substances
Washington, DC

Sherry Selevan, Ph.D.
National Center for Environmental Assessment
Washington, DC

MaryJane Selgrade, Ph.D.
National Human and Ecological Effects Research Laboratory
Research Triangle Park, NC

Carolyn Smallwood
National Center for Environmental Assessment
Cincinnati, OH

Stan Smucker, Ph.D.
U.S. EPA Region 9, Superfund Division
San Francisco, CA

Dan Stralka, Ph.D.
U.S. EPA Region 9, Superfund Division
San Francisco, CA

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 Section 7, 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). 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, 1996), 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, 1994a), 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), and The Risk Assessment Guidelines of 1986 (U.S. Environmental Protection Agency, 1987).

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 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). 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 (extrarespiratory 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.

Acknowledgments

The authors are indebted to the following individuals who imparted their insights, data analysis, and expertise to improve specific areas of the report, including Dr. Allan Marcus (EPA, National Center for Environmental Assessment [NCEA]), Dr. Edward Urbansky (EPA, National Risk Management Research Laboratory), Kevin Mayer (EPA, Region 9), and Dr. Martha Moore (EPA, National Health and Environmental Effects Laboratory).

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 and every one 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. 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).

Other individuals at AFRL/HEST at Wright-Patterson Air Force Base should be noted for their invaluable technical contributions: Dr. William Baker, for all the histopathology analysis and reports that he generated in a short period of time; Latha Narayanan, for her expert and reliable analyses of thyroid and pituitary hormone data; and Drs. Jeff Fisher and Kyung Yu for their work on iodide and perchlorate kinetics.

The Perchlorate Study Group (PSG), particularly Michael Girard, also is recognized for its aid in sponsoring studies and ensuring timely data delivery in appropriate formats for EPA analyses. Toxicology Excellence for Risk Assessment also was very responsive in this regard on behalf of the PSG.

Additional gratitude is expressed to those individuals who submitted unpublished reports and literature summaries for consideration by the EPA review team. Notable contributions were provided by Dr. Steven Lamm of Consultants in Epidemiology and Occupational Health, Inc., and Drs. Gay Goodman and Richard Pleus of Intertox.

The authors also are indebted to the dedication and expertise of the following individuals of OAO Corporation for their roles in document production: John Barton, for project coordination and technical editing; Carolyn Perry, Bettye Kirkland, and Yvonne Harrison, for word processing; Dave Leonhard and Veda Williams, for graphic arts; and David Belton, for reference retrieval and editing.

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

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, to evaluate the potential for perchlorate carcinogenicity, and to provide a screening ecological risk assessment for perchlorate based on toxicity data that recently have become available. 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, and, thus, it can act as a thrust booster. Perchlorate salts also are used on a large scale as a component of air bag inflators.
- Other uses of perchlorate salts include their use 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 also has been reported to be a potential source of perchlorate contamination.
- 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 a low-level (4 ppb) detection method. There are 14 states with confirmed releases in ground or surface water. There are 44 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.
- At this time, there has not been a systematic national survey of perchlorate occurrence. Identification of the magnitude and extent of perchlorate occurrence in the environment is important in assessing the routes of exposure to humans and to determining the different types of organisms and ecosystems that may be affected.

An Integrated Approach to Comprehensive Risk Characterization

- Perchlorate is of concern because of existing uncertainties in the toxicological database available to adequately address the potential for perchlorate to produce human health effects at low levels in drinking water; the actual extent of the occurrence of perchlorate in ground and surface waters, which is compounded by some uncertainty in the validation of the analytical detection method; the efficacy of different treatment technologies for various water uses, such as drinking water or agricultural application; and the extent and nature of ecological impact or transport and transformation phenomena in various environmental media.
- To adequately and comprehensively characterize the risk of perchlorate contamination to provide scientific input to decision making regarding management strategies to mitigate potential risk, a number of key pieces of information are necessary. Accurate characterization of exposures relies on reliable analytical methods. 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 also includes other governmental representatives from the Agency for Toxic Substances and Disease Registry and the National Institute for Environmental Health Sciences and affected state, tribal, and local governments.
- The charter 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 and emerging data.
- There is currently no National Primary Drinking Water Regulation for perchlorate. Perchlorate was placed on the Contaminant Candidate List in March 1998. The list serves as the source for priority contaminants, defined as either known or anticipated to occur in public water systems, for research, guidance development, and selection of contaminants for making 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.

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. 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.
- 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, thereby causing a reduction in the hormones thyroxine (T3) and triiodothyronine (T4). When these hormones enter the blood circulation, they are bound to plasma proteins. Differences in plasma protein binding between rats and humans account for differences in the circulating half-life of the hormones and in thyroid structure between the species. There may be other locations of inhibition of iodide transport in the gland, but perchlorate itself is not metabolized in the thyroid or peripheral tissues.
- Control of the circulating concentrations of these hormones is regulated primarily by a negative feedback involving three organs (1) the thyroid, which produces T4 and T3, and (2) the pituitary gland and (3) the hypothalamus, which respond to and help maintain optimal T4 and T3 levels by what is known as the hypothalamic-pituitary-thyroid axis or feedback system. 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.
- Potential effects of perchlorate, given its mode of action as an inhibitor of iodide uptake that results in disturbances of the hypothalamic-pituitary-thyroid axis, included concerns for carcinogenic, neurodevelopmental, developmental, reproductive, and immunotoxic effects.

Additionally, no study had ever evaluated the potential for other systemic effects. Further, there was concern for ecotoxicology effects on various aquatic and terrestrial plants and animals.

- The human health testing strategy included eight different recommended studies to address data gaps and enhance the mechanistic information on the mode of action to provide a comprehensive database on which to arrive at a revised human health risk assessment with greater confidence than previous provisional values. These studies are described 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) Genotoxicity assays to evaluate the potential for carcinogenicity by evaluating the potential for direct effects on deoxyribonucleic acid.
  (8) Immunotoxicity studies to evaluate the potential for perchlorate to disrupt immune function.

- A battery of ecological screening tests was conducted 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 for 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, the following species were selected for the first round of testing. Lettuce was substituted for duckweed because of tribal concerns regarding the sizable lettuce crop along the Colorado river.
  (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
  (7) Frog Embryo Teratogenesis Assay: *Xenopus*
  (8) Phytoremediation study to examine uptake, distribution, and degradation in experimental systems with rooted cuttings of woody plants, including willow, Eastern Cottonwood, and eucalyptus.

Human Health Assessment

- The testing strategy confirmed that the target tissue for perchlorate toxicity was the thyroid gland, as indicated by both perturbations of T3, T4, and TSH hormones and by thyroid histopathology in both adult and postnatal rats. The hormone effects occurred at the lower range of exposures tested, from 0.01 to 1.0 mg/kg-day, whereas the histopathology typically occurred at higher doses, with the exception of follicular epithelial cell hyperplasia observed in rat pups on Postnatal Day 5 (PND5) and in a 14-day study of young rats. Neurobehavioral effects and effects in the brains of offspring occurred at higher concentrations. Preliminary data on reproductive parameters and immunotoxicity indicate potential for an effect. No effects were observed in rabbits of the developmental study.
- Thyroid tumors were observed in previous studies in rats exposed in long-term bioassays at high doses. Perchlorate was not found to be genotoxic in any assay of the genotoxicity battery,

although repeated experiments have been requested for two assays. The preliminary data on these repeated studies confirm the lack of genotoxicity by perchlorate.

- Because of strong correlations between changes in 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 on thyroid hyperplasia 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 lesion will preclude any subsequent sequelae at higher doses.
- 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, as described, so that rats appear to be more sensitive to thyroid cancer caused by thyroid-pituitary disruption. This approach requires demonstration that the indirect disruption is the only mode of action, and that the chemical is not genotoxic. Adverse noncancer thyroid effects, such as thyroid enlargement and histopathology, are presumed to pose a human noncancer health hazard. Perchlorate was demonstrated to be nongenotoxic in the testing battery employed, suggesting the indirect mode of action for potential tumor formation.
- The revised RfD, assumed also to be protective on potential carcinogencity was derived using effects in thyroid histopathology observed in pups on PND5 in the neurodevelopmental study at 0.1 mg/kg-day. The effects in the thyroids of the rat pups at lower levels than in the mother were corroborated by effects in pups of previous studies of guinea pigs and rabbits.

A composite uncertainty factor of 100 was used to address uncertainties resulting from data gaps because of pending studies and for extrapolation of a minimal lowest-observed-adverse-effect level (LOAEL) and intrahuman pharmocodynamic differences and for interspecies differences. Because the test article was ammonium perchlorate, an adjustment factor of 0.85 also 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. The resultant revised RfD value for perchlorate is 0.0009 mg/kg-day. Confidence in the RfD was designated as medium.

- Pending data on the results of the two-generation reproductive study, immunotoxicity studies, and characterization of perchlorate kinetics and iodide inhibition are expected to impact this assessment. Any risk assessment is an iterative process, and incorporation of new data may require additional evaluation and consideration.

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 (0.1 mg/kg-day) to obtain a screening benchmark of 0.01 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.
- No bioaccumulation data are available to indicate whether perchlorate accumulates in animal tissues. Limited data suggest that perchlorate is taken up and concentrated in aerial plant parts, especially leaves. In addition, these studies were phytoremediation studies, so that concentration factors that may result from steady-state could not be estimated.

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.
- Human health risk research needs include a more accurate linkage between the biologically effective internal dose (e.g., characterization of the dose response for perchlorate inhibition of iodide uptake) in both adult and fetus. More definitive studies of the degree of perturbation of the hypothalamic-pituitary-thyroid axis (i.e., changes in T3, T4, and TSH levels associated with thyroid histopathology), and neurobehavioral effects especially, would improve dramatically the confidence in the assessment. Quantitative interspecies extrapolation requires acute and steady-state characterization of perchlorate toxicokinetics and toxicodynamics.
- 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 March 1999.
- Perchlorate has caused tumors in rodents only at high exposures for long periods. 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 chapter is to provide background information on the current status of perchlorate (ClO_4^-) contamination and a historical perspective on how certain issues have evolved to prominence, and to place the scope of this current assessment in context with the overall integrated approach to addressing perchlorate contamination.

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 ammonium, potassium, magnesium, or sodium salts. Of these, all are extremely soluble, except for potassium perchlorate, which generally is regarded as sparingly soluble. However, it does dissolve completely given the conditions under which the contamination has occurred. 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 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, 1979).

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

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,

chemicals, 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). Chemical fertilizer also has been reported to be a potential source of perchlorate contamination (TRC Environmental Corporation, 1998). 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. Wet ashing organic matter with perchloric acid still is performed today as a means of preparation for certain samples. Anhydrous magnesium perchlorate 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 analysis.

The large-scale production of perchlorate-containing chemicals in the United States began in the mid-1940s. The approximate percentage for end use of production sold was 92% as an oxidizer, 7% as an explosive, and 1% other uses. The typical volume of production ranged from 1 to 15 million lb per year (Rogers, 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. 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 primarily in areas associated

with development, testing, or manufacture of aerospace materials. Perchlorate contamination also may occur where mining activities use explosives extensively (Siddiqui et al., 1998).

Although ammonium perchlorate is released initially, the salt is highly soluble and dissociates completely to ammonium (NH_4) and perchlorate ions on dissolving in water:

$$NH_4ClO(s) \xrightarrow{H_2O} NH_4^+(aq) + ClO_4^-(aq) . \qquad (1\text{-}2)$$

The 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 at least some ammonium ion (Urbansky, 1998). 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 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 groundwater and surface water conditions. Figure 1-1 summarizes the various pathways through which perchlorate can reach ground and surface water sources.

1.2 OCCURRENCE AND HISTORICAL HUMAN HEALTH RISK CHARACTERIZATION

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

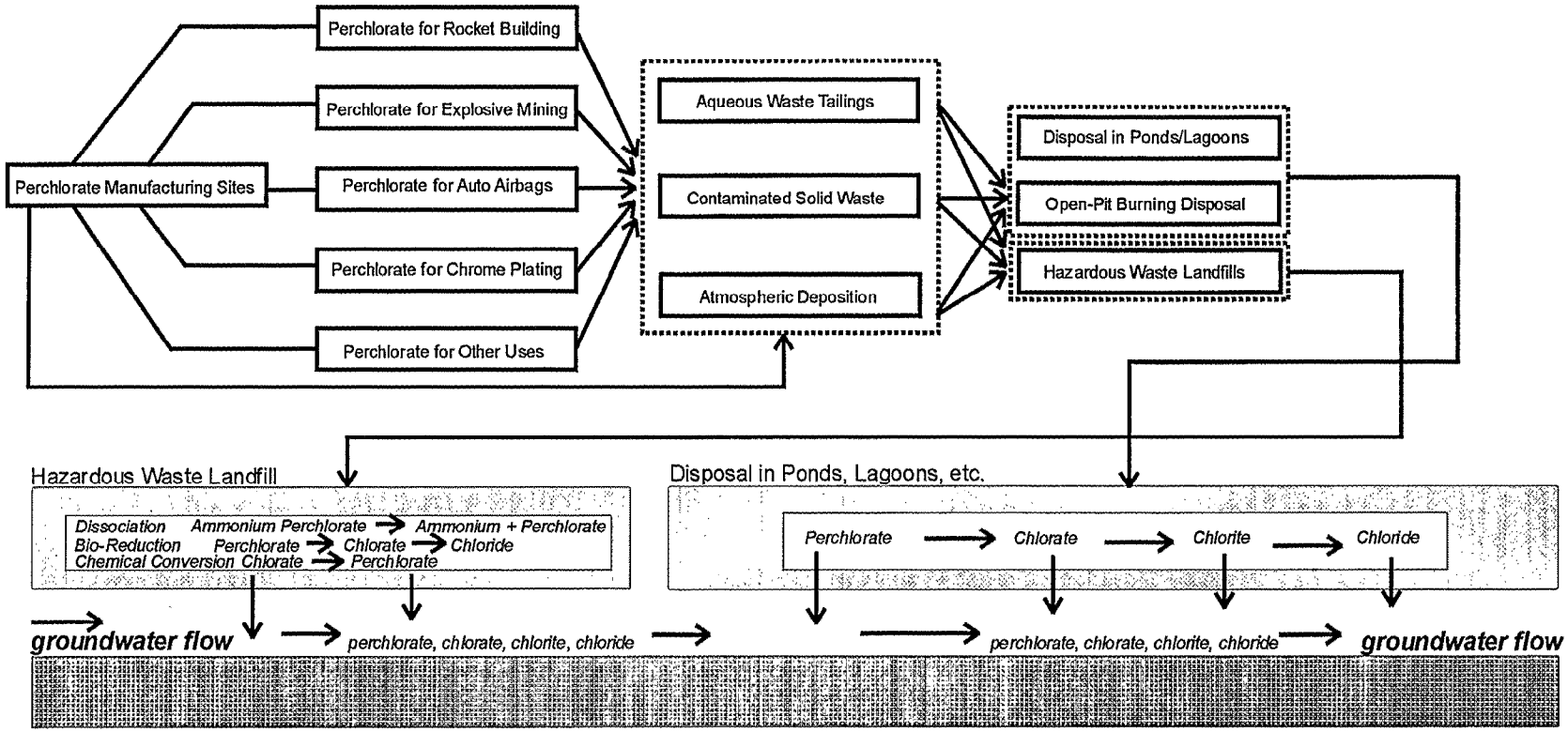


Figure 1-1. Sources and pathways of groundwater contamination for perchlorate.

Source: Siddiqui et al. (1998).

and Prevention (CDC) to evaluate the potential health effects of these levels of perchlorate (Takata, 1985). The CDC recommended in response validation of the colorimetric measures but could not answer with respect to the potential for toxicity of the chemical because of toxicity data insufficiencies (Margolis, 1986). Additional testing, particularly to determine potential target tissues and the effects from long-term, low-level exposures was recommended. The absence of a valid analytical method for low concentrations of perchlorate and of data 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) were 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.

The EPA Region 9 office then 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. The California Department of Health Services (CA DHS) adopted 18 ppb as its provisional action level in 1997 after perchlorate was discovered in a number of California water supplies.

In January 1997, the California Department of Health Services' Division of Drinking Water and Environmental Management requested the Sanitation and Radiation Laboratory Branch (SRLB) to 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. No procedures were available for measuring perchlorate at such low levels. 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 detection method, perchlorate had been discovered at various manufacturing sites and in well water and drinking water supplies in California, Nevada, and Utah. At this time, there has not been a systematic national survey of perchlorate occurrence. Only a relatively small number of water supplies have been monitored using the more sensitive method, primarily in the western states, with a few sample results now available in the South.

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. Figure 1-2 indicates states with confirmed perchlorate manufacturers or users and Figure 1-3 indicates those

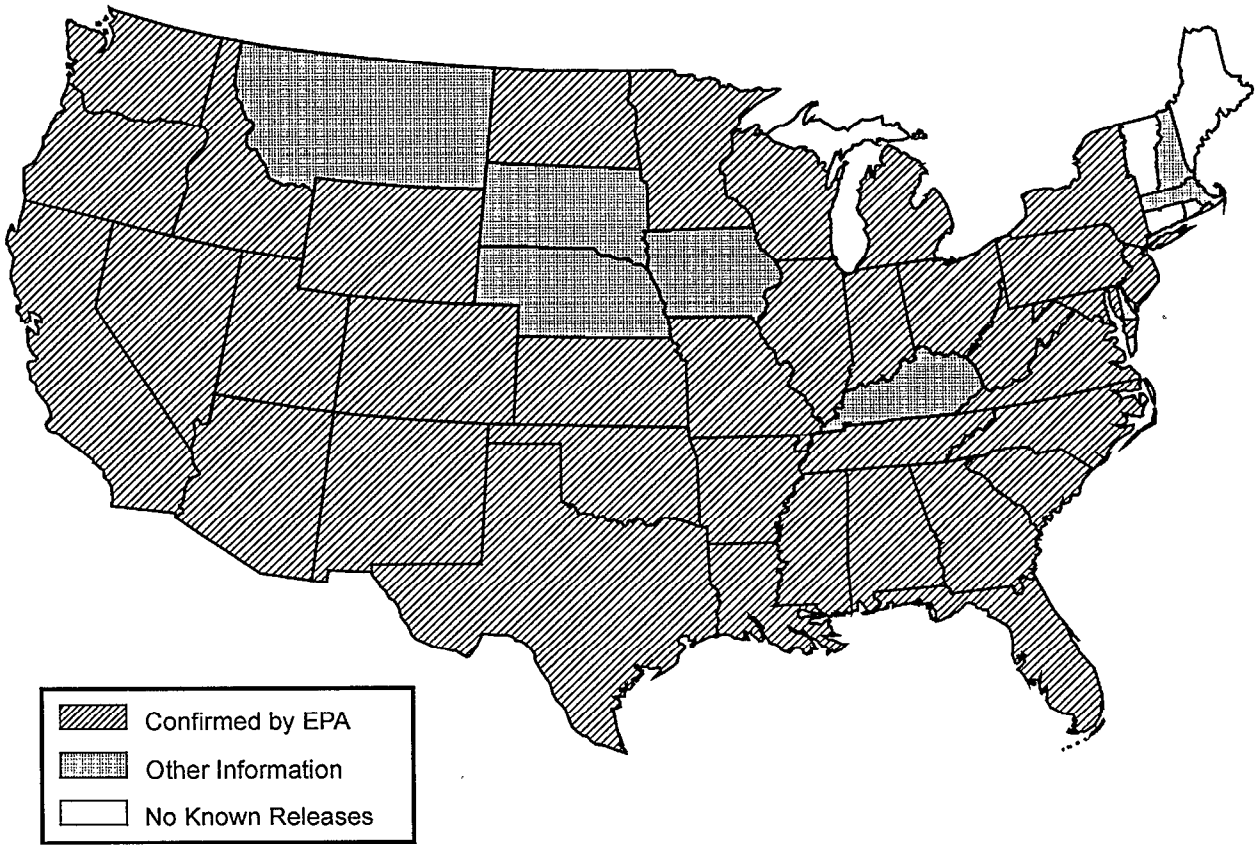


Figure 1-2. States indicated as having confirmed perchlorate manufacturers or users (hatch marks) are based on EPA Information Request responses from current manufacturers (identifying shipments of at least 500 pounds in any year). States noted by shading resulted from database searches for types of facilities where releases have occurred in California (rocket manufacturing and testing and explosives manufacturing). No facilities have been identified in Alaska, Hawaii, Maine, Vermont, Connecticut, or Rhode Island.

states with confirmed releases in which facilities have directly measured perchlorate in groundwater or surface water.

In California, most of the 14 separate detections are associated with 12 facilities that have manufactured or tested solid rocket fuels for DoD or NASA. Two facilities that manufactured ammonium perchlorate in Nevada were found to have released perchlorate to groundwater that is the source for low levels (4 to 16 ppb) in Lake Mead and the Colorado River. This water is used for drinking water, irrigation, and recreation for millions of people in Nevada, California,

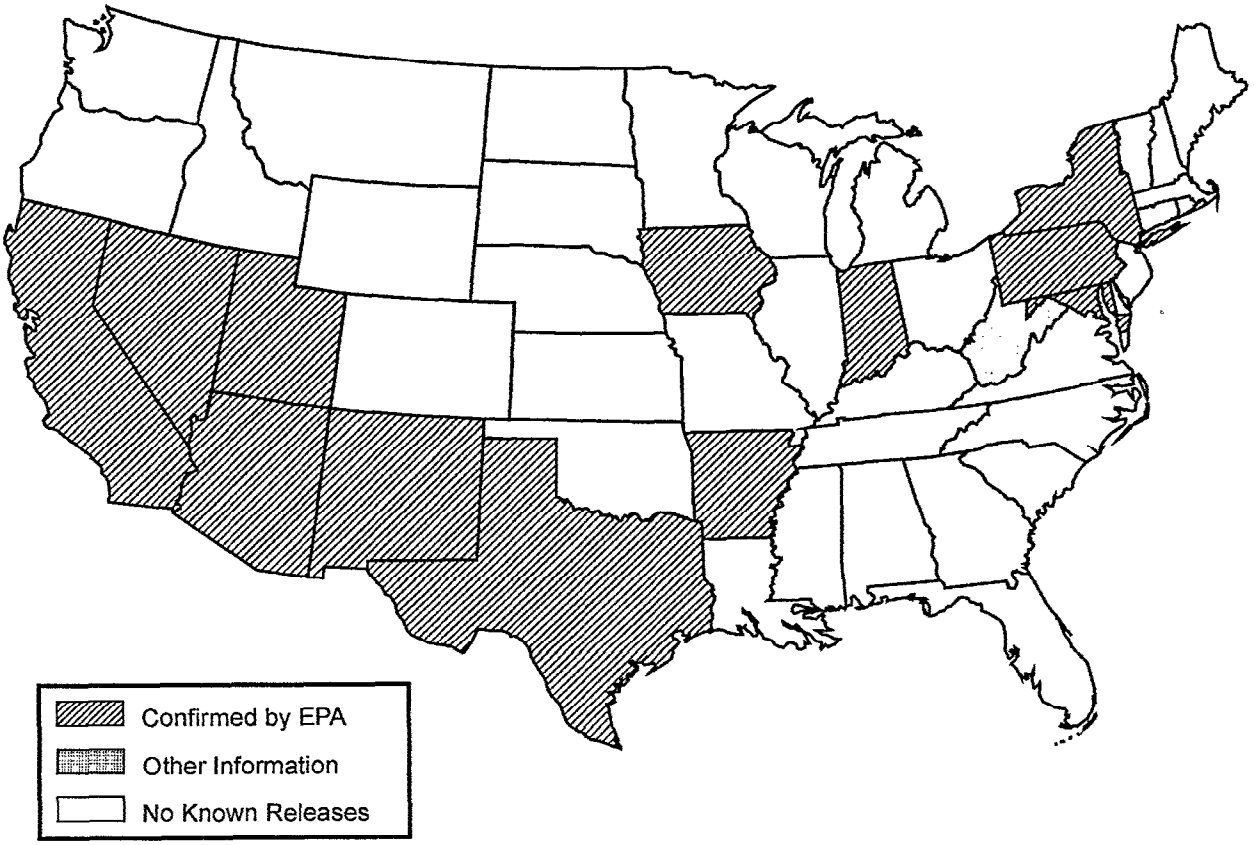


Figure 1-3. States with confirmed releases (hatch marks), in which facilities have directly measured perchlorate in groundwater or surface water. Perchlorate measured in water in West Virginia for a confidential client has been reported at a public conference but has not been confirmed independently by EPA. Monitoring for perchlorate releases in most states is very limited or nonexistent.

Arizona, and Native American tribes (see Figure 1-4). 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). Water suppliers in both northern and southern California have detected perchlorate in 144 public water supply wells, with 38 of these above the provisional action level in California of 18 ppb. The highest level of perchlorate reported in any public water supply well was 280 ppb, with few others greater than 100 ppb.

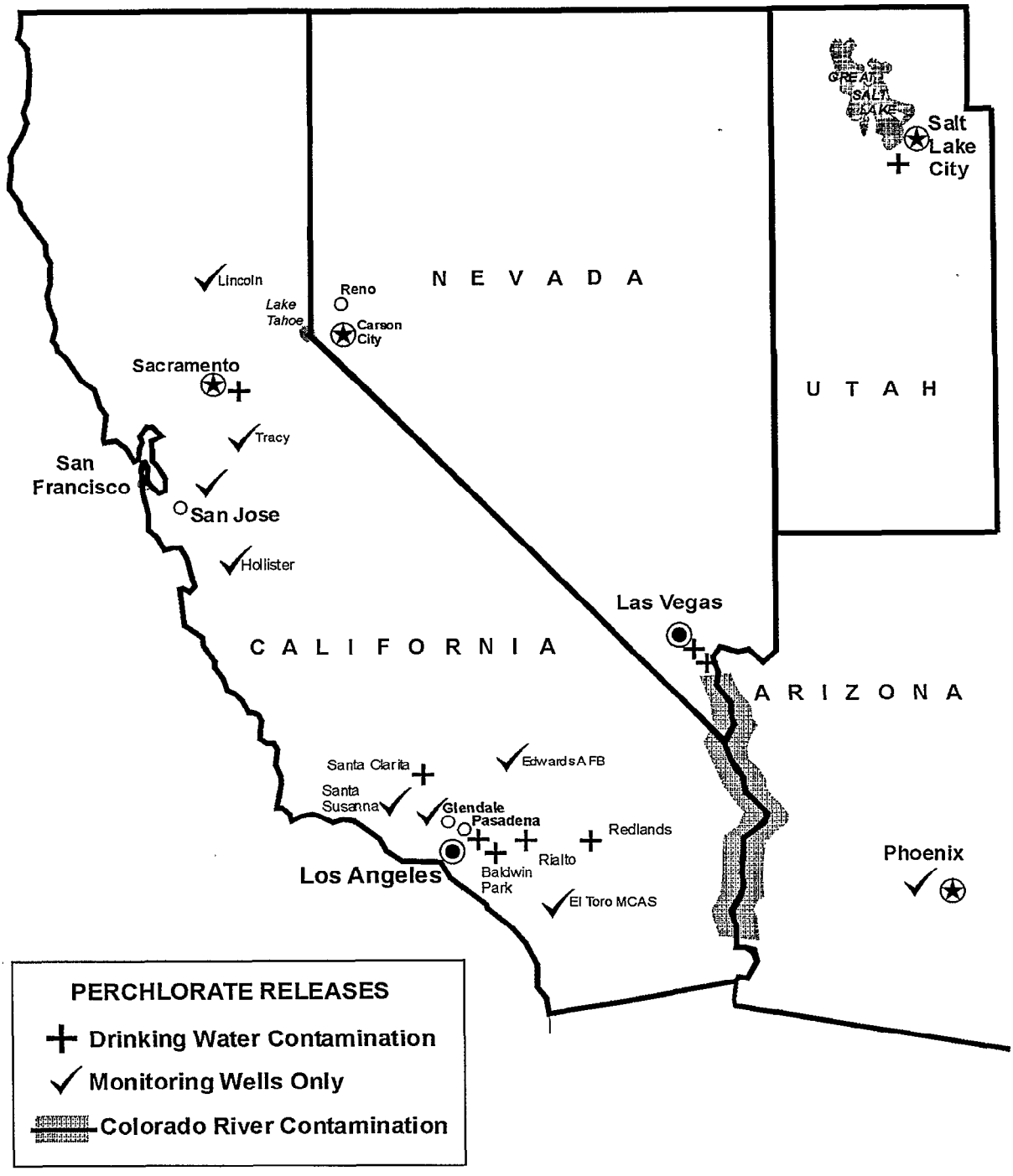


Figure 1-4. Locations of facilities in EPA Region 9 and in Magna, UT (Region 8), at which perchlorate has been released to groundwater or surface water. Facilities that have resulted in perchlorate entering public water supplies are identified with a ✚. In Nevada, two facilities are noted, although it is not certain that perchlorate from one of these sites has yet reached the Colorado River. Additional potential releases in the region are still under investigation.

Table 1-1 provides information on the occurrence and potential sources of perchlorate in the drinking water systems in EPA Region 9. Perchlorate also has entered a private water supply well in Utah from contamination on the property of a rocket motor manufacturer near Magna, west of Salt Lake City. McGregor Naval Weapons Industrial Reserve Plant in north Texas was discovered to have released detectable levels of perchlorate to off-site springs and streams. Perchlorate has been confirmed in monitoring wells near McGregor, TX, and East Camden, AR. Releases of perchlorate to surface water or groundwater have been identified in Utah and Maryland near rocket manufacture and testing facilities. Perchlorate was found in a number of water supply wells on Long Island, NY. 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). Perchlorate has been reported in groundwater or surface water in West Virginia, New Mexico, Iowa, Indiana, and Pennsylvania (Sidiqqui et al., 1998).

Motivated by the anticipated impact of the reduced analytical detection method, a reevaluation of the provisional 1992 and 1995 RfDs that serve as the basis of the provisional action level was warranted. The outcome of an external peer review convened in March 1997 of an analogous RfD derivation by an independent organization (Toxicology Excellence for Risk Assessment, 1997) was the determination 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 a 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 reduce uncertainties in various extrapolations (Toxicology Excellence for Risk Assessment, 1998b). The objective of the new studies is to provide a comprehensive database that provides for development of a robust RfD estimate that reduces the uncertainties inherent in the provisional values. The strategy that

TABLE 1-1. OCCURRENCE AND POTENTIAL SOURCES OF PERCHLORATE IN DRINKING WATER SYSTEMS IN U.S. ENVIRONMENTAL PROTECTION AGENCY REGION 9 (1997-1998)[a]

Type	Location	Suspected Source	Water System Affected	Max. ppb
Groundwater	Sacramento Rancho Cordova, CA	Aerojet General Rocket manufacturer	Arden Cordova Sacramento County WC Mather AFB (not in use)	280
Groundwater	Upper Santa Ana Valley Redlands, CA	Lockheed Propulsion Rocket manufacturer	Victoria Farms City of Loma Linda City of Redlands City of Riverside Loma Linda University	140
Groundwater	Raymond Basin Pasadena, CA	NASA-Jet Propulsion Lab Rocket research	Cal-American City of Pasadena Las Flores WC Lincoln Ave. WC Rubio Canyon Valley WC	50
Groundwater	San Gabriel Valley Baldwin Park, CA	Aerojet General Rocket manufacturer	Azusa Light and Power La Puente Valley WD San Gabriel Valley WC Suburban Water System Valley County WD	160
Groundwater	Santa Clarita Valley, CA	Whittaker Bermite Ordinance and rockets	Newhall CWD Santa Clarita WC Valencia WC	30
Groundwater	Rialto, CA	B.F. Goodrich Rocket manufacturer	City of Rialto WD West San Bernardino Co.	30 (270)
Groundwater	Santa Susanna, CA	Rocketdyne Rocket manufacturer	Monitoring wells only	NA
Groundwater	Hollister, CA	Whittaker Ordinance	One private well and agricultural. and monitoring wells	800
Groundwater	San Jose, CA	UTC (United Tech.) Rocket research	Monitoring wells and seasonal surface water	NA
Groundwater	San Fernando Valley Glendale, CA	Grand Central Rocket Rocket manufacturer	Monitoring well only	NA
Groundwater	Edwards AFB Edwards, CA	Jet Propulsion Lab, North Base Rocket research	Soil and monitoring wells	NA
Groundwater	Lawrence Livermore National Laboratory Site 300 Tracy, CA	U.S. Dept. of Energy Explosives research	Monitoring wells only	NA
Groundwater	El Toro MCAS Orange Co., CA	Marine Corps Air Station Unknown source	Monitoring wells only	NA

TABLE 1-1 (cont'd). OCCURRENCE AND POTENTIAL SOURCES OF PERCHLORATE IN DRINKING WATER SYSTEMS IN U.S. ENVIRONMENTAL PROTECTION AGENCY REGION 9 (1997-1998)[a]

Type	Location	Suspected Source	Water System Affected	Max. ppb
Surface Water	Coon Creek Lincoln, CA	Alpha Explosives Explosives	Coon Creek	NA
Surface Water	Henderson, NV Las Vegas Wash Lake Mead Lower Colorado River	Kerr-McGee/Pacific Engineering and Production Company (PEPCON) Perchlorate manufacturer	Southern NV Water Authority Metropolitan WD of Southern California Central Arizona Project Multiple tribes and cities	14
Groundwater	Phoenix-Goodyear Airport Goodyear, AZ	Unidynamics Explosives/Ordinance	Monitoring and agricultural wells only	NA

[a]WC = Water Commission, WD = Water Department/, NA = not available.

Source: Mayer (1998).

formed the basis of the new battery of toxicity studies is discussed in Chapter 4. These data feature prominently in this assessment to recommend a revised human health risk characterization, including a revised RfD for perchlorate.

1.3 ECOTOXICOLOGY ASSESSMENT

The mobility and persistence of perchlorate that is discussed above 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. Currently, there are very limited data to evaluate the effects of perchlorate on ecological systems nor are there data about the possible uptake of perchlorate into agricultural products through irrigation of the food crops. Analytical tests have been derived to detect perchlorate in water, but little is known about testing food crops for perchlorate.

Searches of available databases have revealed minimal information on the ecological effects of ammonium perchlorate or any of perchlorate's other salts. Little data exist to describe perchlorate's effects on various soil, sediment, or aquatic receptors, including aquatic vertebrates, aquatic or sediment invertebrates, and bacteria or plants. The data that are available suggest

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, in recent studies, has been shown to completely block the metamorphosis of tadpoles. Effects on development and population growth also have been indicated in the freshwater sea 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 (see IPSC below in 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 ecological receptors, across 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 are screening-level tests and will provide only an idea of gross toxicity, they will provide needed dose-response information to make decisions on the need for a next tier of tests to be completed (e.g., bioavailability, bioaccumulation, histopathology). These tests will not measure the amount of perchlorate in the tissues of the species being tested. Testing of biological tissues is currently being considered by the Analytical Subcommittee of the IPSC. Chapter 7 provides the ecotoxicology assessment based on these new screening data and the IPSC report.

1.4 FUTURE REGULATORY PLANS

This section briefly describes pending regulatory activities that are anticipated to be impacted by this evaluation and characterization of perchlorate contamination, notably 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, 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 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), will be the source of priority contaminants for research, guidance development, and selection of contaminants for making regulatory determinations or monitoring by the states. The SDWA requires EPA to make a determination of whether or not to regulate not less than five contaminants from the CCL by 2001. The CCL also must be reviewed and updated every 5 years (next in 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 its occurrence in drinking water supplies at the time of publication. As a result of the public comments and additional occurrence information obtained, EPA determined that sufficient information exists to raise concern over perchlorate's potential public health impact, and it was added 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 by 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 needs for additional research in the areas of health effects, treatment technologies, and analytical methods and more complete occurrence data.

1.4.2 State Regulatory Plans

As discussed above, the CA DHS and the California EPA Office of Environmental Health Hazard Assessment 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 that is provided by the utility exceeds the action level, CA DHS will advise the utility to arrange for public notification to its customers. On August 1, 1997, CA DHS informed drinking water utilities of its intention to develop a regulation to require monitoring for perchlorate as an unregulated chemical. Legislative action to establish a state drinking water standard for perchlorate by January 2000 (California Senate Bill 1033) was vetoed by the governor after passage by both houses. The governor supported the prioritization of regulating perchlorate in drinking water but objected to the strict time schedule required.

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

The perchlorate contamination is of concern because of the existing uncertainties in the toxicological database available to adequately address perchlorate's potential to produce human health effects at low levels in drinking water; the actual extent of the occurrence of perchlorate in ground and surface waters, which is compounded by some uncertainty in the validation of the analytical detection method; the efficacy of different treatment technologies for various water

uses, such as drinking water or agricultural application; and the extent and nature of ecological impact or transport and transformation phenomena in various environmental media.

Thus, a number of key pieces of information are necessary to adequately characterize the risk of perchlorate contamination in order to provide scientific input to decision making regarding management strategies to mitigate potential risk. Accurate characterization of exposures relies on reliable analytical detection methods. The exposure estimates can not 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 apprised of accurate and reliable information that is up to date with the evolving state-of-the-science.

The National Center for Environmental Assessment (NCEA) in the Office of Research and Development (ORD) of EPA has evaluated the emerging information and new human health/toxicity and ecotoxicity data from the testing strategy (see Chapter 4) or other sources that were available by early November 1998. The purpose is to determine revised risk characterizations to serve in this integrative approach as more robust risk estimates than those that exist provisionally to better gauge the potential human health and ecological impact in a comprehensive fashion (Figure 1-5). As with any risk assessment, incorporation of new data is an iterative process. In this case, some of the data from the originally proposed strategies will not be available until January and February 1999. Because of regulatory schedule constraints, this assessment has gone forward with the recognition that additional data may always warrant further revision. Data that will be arriving in the period between the issuance of the external peer review draft and the external peer review workshop are identified herein as "to be determined" and may be presented at the meeting.

Independent, external peer review of the study protocols, toxicity studies, and revised RfD/health 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 EPA Office of Solid Waste and Emergency Response (OSWER) has tasked a qualified contractor to manage peer review of technical issues related to the development of the human health and ecotoxicology assessments, including study design, conduct of toxicity studies, statistical treatment of data, selection of critical effect and uncertainty factors, and risk characterization. The peer review will be conducted by a panel of technical experts in

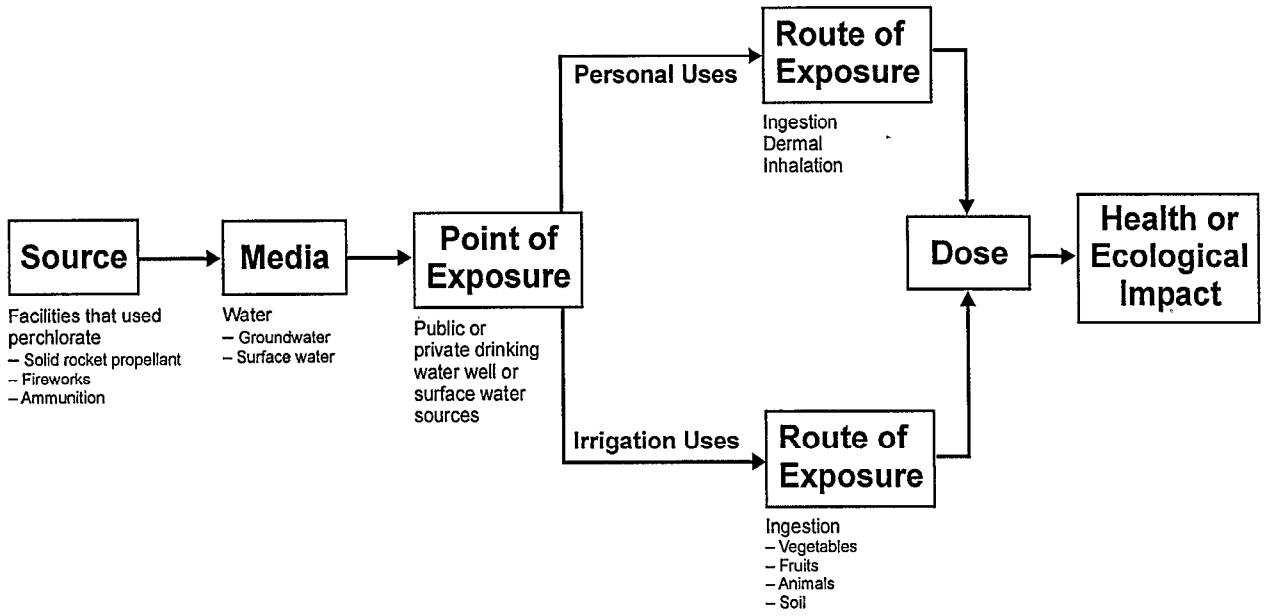


Figure 1-5. Considerations for comprehensive characterization of perchlorate contamination.

Source: Modified from Underwood (1998).

ecotoxicology; neurotoxicology; developmental, reproductive, genetic, and general toxicology; pathology; biostatistics; dose-response modeling; and risk assessment. Peer reviewers will be selected from a pool of candidates nominated by stakeholders in the perchlorate issues. 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. Peer reviewers will review independently the risk assessment package and supporting studies and will submit their written comments to OSWER's contractor prior to the peer review meeting. The peer reviewers' comments will be compiled by OSWER's contractor and will be distributed to all of the peer reviewers and the public in advance of the meeting. The peer reviewers will gather for a 2-day meeting in a location selected for its accessibility to stakeholders and peer reviewers. The public will be invited to attend and observe the peer review meeting. Following the peer review meeting, the peer review panel will generate a report detailing their comments on the reference dose package and supporting studies. The NCEA then will generate a responsiveness

summary report that will discuss in detail how comments made by the peer reviewers have been addressed. The revised risk characterization will be issued subsequently by EPA.

It should be noted that this assessment effort was accomplished in an extraordinarily 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 Agency for Toxic Substances and Disease Registry (ATSDR); the National Institute for Environmental Health Sciences; and affected state, tribal, and local governments. Participation in the IPSC also has been solicited from other governmental entities. The charter 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 provides 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, which are identified in the comprehensive characterization framework in Figure 1-5. Research to obtain additional data and development of new methods or 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 on addressing the unique issues of the perchlorate contamination. The IPSC recently collaborated with EPA ORD on a 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, 1998c). Updates on activities of the IPSC can be found on the EPA Office of Water (OW) web site at the following address: http://www.epa.gov./ogwdooo/ccl/perchlor/indexkeys.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.

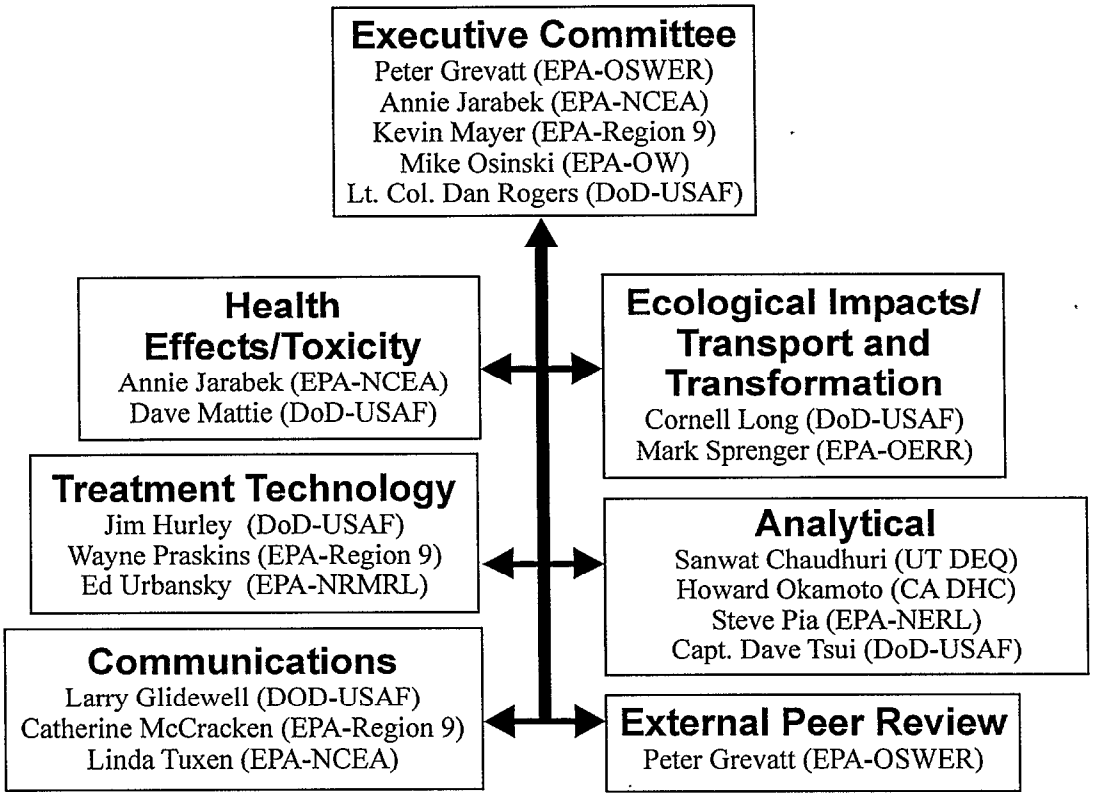


Figure 1-6. Structure and membership of the executive committee, subcommittees areas, and co-chairs of IPSC. The IPSC is designed to ensure an integrated approach to addressing the perchlorate contamination challenge and to informing stakeholders with accurate accounts of technical issues. (OERR = Office of Emergency Response and Remediation, NRMRL = National Risk Management Research Laboratory, UT DEP = Utah Department of Environmental Quality)

2. PHYSICOCHEMICAL CHARACTERISTICS

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, 1979), 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.

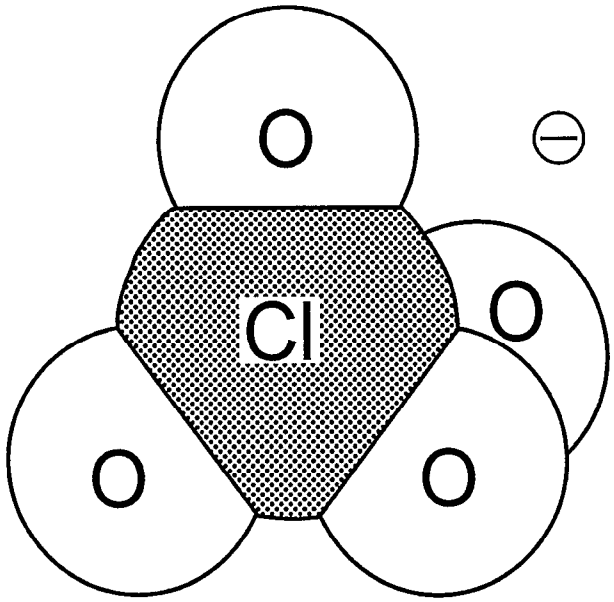


Figure 2-1. Chemical structure of perchlorate.

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 use, it is comparable with other noncomplexing or weakly ligating anions (e.g.,

trifluoromethanesulfonate [triflate, $CF_3SO_3^-$, tetrafluoroborate [BF_4^-], and, to a lesser extent, nitrate [NO_3^-]). Some exceptions are known, but rare. All of these anions have a highly delocalized (NO_3^-, ClO_4^-, $CF_3SO_3^-$) 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

Anion	ΔG_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

Source: Urbansky (1998).

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^+]), and quaternary ammonium salts are less soluble still. The outstanding example is sodium

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

TABLE 2-2. PHYSICOCHEMICAL PROPERTIES OF AMMONIUM AND ALKALI METAL PERCHLORATES AT 25 °C

	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						
ΔH_f°, kJ mol^{-1}	-290.4	-384.0	-385.7	-435.5	-434.7	-434.7
ΔG_f°, kJ mol^{-1}	-88.9[b]	-254[c]	-255[b]	-304	-306	-307
ΔS_f°, kJ mol^{-1}	186[b]	130[c]	142[b]	151	161	175
ΔH_{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).

Source: Schilt (1979).

Because of their large solubilities, the health risk assessment herein for perchlorate anion (ClO_4^-) would be 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 toxicity results more from the irritating action on skin and mucous membranes of the hydrogen ion.

Perchlorate is a strong oxidizing agent 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 (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),

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

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

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

The effect of pH can be explained in terms of Le Châtelier's principle. In Reaction 2-1, hydrogen ion is plentiful (1 M) and acts a reactant; this drives the reaction forwards. In Reaction 2-2, however, hydroxide ion is a product of the reaction and 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. This is analogous to a car with a very strong parking brake parked on a very steep hill. The

driving force (gravity) for the car to roll down the hill is very large, but the car does not roll down the hill because the parking brake is also very strong. 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 point out 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.

Another 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, such as the decomposition of ammonium perchlorate in Reaction 1-1, where 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

---

[1]This is the case with reactions occurring exposed to the open air, for instance, 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 of the thermodynamic relationships herein still apply at other conditions, but reference tables exist only for standard conditions. To use other conditions, appropriate correction must be made. All thermodynamic data are for a temperature of 298 K.

[2]Expansion work 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: $W_{exp} = -P\Delta V = \Delta nRT$ (T, P, *and* R 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 even approach reversibility. For example, explosions are so irreversible, and so much internal energy is lost as heat that the nonexpansion work is much smaller than ΔG°_{rxn}.

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.

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

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 great deal 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 up 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 does not suddenly explode. The free energy is not released because the reaction kinetics are too slow at room temperature—only an infinitesimal fraction of the

[4]Readers who have studied thermodynamics will undoubtedly recall that the true 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.

molecules possesses enough energy to reach the activation energy of the transition state. The activation energy for the reaction between an ammonium cation and a perchlorate anion is too great for any reaction to be observed.

The important 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 are close to the activation energy required to form the transition state. In a similar case, the kinetic barrier (activation energy) is responsible for the fact that 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:

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

Reaction 2-6 proceeds through an associative mechanism where the incoming water molecule attacks the central chlorine atom. Consider the simplest example, hypochlorous acid, where the following mechanism is the accepted explanation.

$$HOCl + H_2Ø \rightleftharpoons [HO\cdots Cl\cdots ØH]^{\ddagger} \rightleftharpoons H_2O + ClØH \qquad (2\text{-}7)$$

$$\ddots \quad /$$

$$H$$

The species $[HOClØH{\bullet}H]^{\ddagger}$ represents the activated complex, and it is the transition state of Reaction 2-7. This species may go back to reactants or on to products.[5] As the number of oxygen atoms increases, the water has greater difficulty entering. The oxidation state of the

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

chlorine increases +2 with each additional oxygen atom; accordingly, the chlorine becomes more and more electron-poor and tries to hold the oxygen atoms closer to share their electrons. This factor will be expanded on further when perchlorate is examined specifically.

With perchlorate, which contains chlorine(VII), the central chlorine atom is blocked sterically from the attack of an incoming reducing agent by the tetrahedrally oriented oxygen atoms. Consequently, perchlorate reduction is constrained to occur by oxygen atom abstraction as the first step. As the oxidation state of the central chlorine atom increases, the strength of the chlorine-oxygen bonds also increases. The electron-deficient chlorine(VII) tries to draw electron density back from the oxygen ligands, which results in increased $O(p\pi) \rightarrow Cl(d\pi)$ 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 more difficult.

Perchloric acid normally exhibits its oxidizing behavior when hot and concentrated. Under these conditions, hydrogen ions can act as oxide-ion (but not oxygen-atom) acceptors to produce water, but thermal stimulation is still required. 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 do react with perchlorate are all sensitive to atmospheric oxygen because they are such 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 (e.g., tetrahydridoborate [III] or tetrahydridoaluminate [III], which are both excellent sources of hydride ion [Urbansky, 1998]) would be so violent that deoxyribonucleic acid (DNA) would be attacked by it. Thus, 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, avoidance of elevated temperatures, and neutral pH). This is supported by absorption, distribution, metabolism, and elimination studies that show perchlorate is excreted virtually unchanged after absorption (see Section 4.1).

Earlier, a comparison was made between activation energy and a parking brake. Suppose grease were applied to the brake pad. This weakens the parking brake, and the car rolls down the

hill. This is exactly how a catalyst works. A catalyst speeds up a chemical reaction by reducing the activation energy, increasing the number of collisions, or orienting chemical reactants to promote reaction. Many catalysts reduce the activation energy, and 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 it is the transition state where they can still separate and go back to the original reactants. If they have sufficient internal energy (the activation energy), they can react. For perchlorate, this means an oxygen atom is being transferred to the reductant. If a catalyst is involved, it can act as an intermediary, taking oxygen atoms from the perchlorate and giving them to the reductant. Some bacteria have catalysts that do just that. These biological catalysts (enzymes) called *reductases* allow the microbes to use perchlorate as an oxidant (electron acceptor) in anaerobic metabolic pathways. Although many of them prefer oxygen to perchlorate, they will consume the perchlorate under low-oxygen conditions. Very little is known about the chemical make-up of these reductases. Nonetheless, perchlorate-reducing monera use these enzymes under conditions where conventional inorganic chemistry suggests that reaction should be imperceptibly slow (Urbansky, 1998; Logan, 1998).

3. HISTORICAL HAZARD IDENTIFICATION

This chapter briefly summarizes the perchlorate database existing prior to the initiation of the testing strategy described in Chapter 4. Many of these have been excerpted from the Toxicology Excellence for Risk Assessment (TERA) 1997 database and Allred (1998) reviews. The majority of studies evaluated only thyroid parameters, with the exception of some hematological effects that were observed in Graves' disease patients. Most of the studies were performed with potassium perchlorate, which, as discussed in Chapter 2, is not as readily soluble as some of the other salts. However, potassium perchlorate is sufficiently soluble for clinical use, and the effects observed can be attributed to those of the anion.

The results of the studies are listed as "suggestive" of effect levels because each suffered from experimental design limitations that precluded their use in quantitative dose-response assessment, as determined by an external peer review in March 1997 (Toxicology Excellence for Risk Assessment, 1998). Nevertheless, the studies are useful to hazard identification, provide some human data, and were the basis of the testing strategy discussed in Chapter 4.

3.1 HUMAN DATA

3.1.1 Epidemiological Data

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.

3.1.2 Studies in Patients with Graves' Disease

Potassium perchlorate has been used to treat Graves' disease in humans, and most of the prior data on perchlorate effects on humans are in patients with this disease. Graves' disease is an autoimmune disorder in which patients carry immunoglobulins in their blood that bind to the thyroid stimulating hormone (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 loss of weight. Perchlorate inhibits the excessive synthesis and secretion of thyroid hormones (TH) 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). The MMIA prevents the oxidation of iodide ion to iodine and its attachment to tyrosyl groups (see Chapter 4), so that the MMIA was given to cause accumulation of ^{131}I in the thyroid. A single dose of 10 mg perchlorate appeared to cause about 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 h when 100 mg of potassium perchlorate was given orally 1 h prior to administration of the tracer. Beyond 6 h, accumulation (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 of these patients who were given on more than three doses for a total of not more than 600 mg potassium perchlorate. This study was used to identify a lowest-observed-adverse-effect level (LOAEL) of 1.4 mg/kg-day[6] 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

[6]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.

was not designated for this study. This study later was determined by an expert peer review panel to be inadequate for RfD derivation (Toxicology Excellence for Risk Assessment, 1998).

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 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 patient, 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 (2%) 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 followed for 22 weeks. The authors report the "time to cure" Graves' disease for perchlorate of approximately 9 weeks. The authors also report 1 of 12 infants born of mothers given 600 to 1,000 mg/day, was born with a very slightly enlarged thyroid, which returned to normal size in 6 weeks; no other abnormalities were noted. This study suggested a LOAEL between 9 and 14 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.

3.1.3 Studies in Healthy Human Subjects

Far less data are available to demonstrate the effects of perchlorate in healthy individuals. In the available studies, exposure to perchlorate was 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) perchlorate 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 a further increase in the secretion of nonthyroxine iodide, suggesting that perchlorate causes only a partial release of endogenous iodide. This study suggests a 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 orally 900 mg/day of potassium perchlorate 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 (Brabant et al., 1992) with perchlorate treatment longer than 4 weeks. As a result of the longer treatment, thyroid volumes increased in all subjects, although TSH levels did not increase.

3.1.4 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 with 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 out 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, where 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 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 partly, 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 pechlorate may cause the 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 healthy normal humans. The underlying abnormal immunologic function in Graves' disease make these patients more sensitive to immunologic challenges. Immunoreactivity to antithyroid drugs is another expression of the compromised immune system in these patients (Wall et al., 1984; Wing and Fantus, 1987); thus, they are expected to have drug allergies with increased frequency (Wall et al., 1984).

3.2 LABORATORY ANIMAL BIOASSAYS

3.2.1 Short-Term and Subchronic Studies

Mannisto et al. (1979) measured serum levels of TSH, T3, and T4 by radioimmunassays in groups of 5 to 6 male Sprague-Dawley (SD) 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.

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 authors calculated the corresponding doses to be 0, 0.11, 0.44, 1.11, 2.26, 4.32, 11.44, or 22.16 mg/kg-day for males and 0, 0.12, 0.47, 1.23, 3.06, 4.91, 11.47, or 24.86 mg/kg-day for females. Thyroids were weighed, and thyroid hormone levels were measured with a radioimmune assay technique. Relative thyroid weights were statistically significantly increased in the two highest dose groups compared with controls. Thyroglobulin levels and TSH increased in both male and female rats in a dose-dependent manner. The TSH increase was statistically significant at the 0.47-mg/kg-day dose for females and at the 1.11-mg/kg-day dose for males. Both T3 and T4 showed statistically significant decreases; however, the T4 effect did not show a dose relationship. For T3, the decrease was statistically significant at the lowest dose, 0.12 mg/kg-day, in females and at the 0.44-mg/kg-day dose level in males. This study suggested that female rats are more sensitive than male rats to the effects of perchlorate. This study suggests a LOAEL in females of 0.12 mg/kg-day, and the same dose in males is a NOAEL. Reanalyses of these data and effect levels are described in Section 5.2.2.

Shigan (1963) administered 190 mg/kg-day in water to rabbits and white rats (number, sex, and strain not identified) for 3 mo. The author does 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. The author does not indicate that these changes were observed in both rabbits and rats. Perchlorate had no effect in the remaining tests. This study suggests a LOAEL of 190 mg/kg-day, although the study translation is reported incompletely, which limits 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, in addition, 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 suggests 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 can be calculated. Absolute and relative thyroid weights were significantly increased compared with 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 with controls. The TSH levels were increased statistically significantly compared with 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 can be 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 with controls. The English translation summary of the histological examinations described 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 hyperplasia and hypertrophy 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.

3.2.2 Long-Term Studies

Kessler and Krunkemper (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. 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 with 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 for 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 displayed benign tumor 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 Kalisnik (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, although 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 increased total volume of the thyroid and 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 LOAEL of 2,147 mg/kg-day for thyroid effects.

3.3 DEVELOPMENTAL AND REPRODUCTIVE 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) and 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%-dose group.

A related study was performed by Brown-Grant and Sherwood (1971). Wistar rats were mated shortly postpartum, 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 16 pregnant guinea pigs 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 with 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 felt these results demonstrated that the placenta is permeable to perchlorate. Because fetal thyroids were more enlarged relative to maternal glands, the fetal thyroid system is independent of the maternal system and more sensitive to changes in iodine availability.

3.4 ABSORPTION, DISTRIBUTION, METABOLISM, AND ELIMINATION STUDIES

Limited absorption, distribution, metabolism, and elimination (ADME) studies were in existence prior to the testing strategy discussed in Chapter 4 to characterize the pharmacokinetics of perchlorate. Although experimental studies in laboratory species and humans have been performed using radiolabeling techniques, most have been at high concentrations, and the published data are expressed simply as thyroid:blood ratios of radioactivity counts that provide

no information on internal dose to biological tissues. Oral administration, the most relevant to the contamination issue, was not the norm. Time-course studies are very limited and essentially completely lacking for repeated administration. More importantly, no data exist on the co-administration of iodide and perchlorate, and such data are necessary to develop a physiologically based pharmacokinetic model (Fisher, 1998a). This section describes the limited pharmacokinetic information that was considered when the data gap was highlighted during the development of protocols for the testing strategy.

3.4.1 Human Studies

The majority of the human data on perchlorate ADME is comprised of the therapeutic case and clinical studies of Graves' disease patients previously described in Section 3.1. 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 $K^{36}Cl^{18}O_4$. 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 divided out among ^{36}Cl , $^{36}Cl^{18}O_4^-$, and $^{36}ClO_4^- + {}^{36}Cl^-$ and indicated that perchlorate was excreted unchanged in the urine. Thus, no human data exist to adequately characterize the pharmacokinetics of perchlorate during steady-state, low-dose, repeated administration.

3.4.2 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 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 is not how most of the human data are expressed, and the time points to measure uptake also are highly dissimilar to those measured in humans.

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 h, and 12 h. The activity was greatest in the thryoid 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 the one of the only co-administration studies, Anbar et al. (1959) administered $^{131}I^-$ and $^{36}ClO_4^-$ in equimolar concentrations at the same time. 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 with the use of sodium perchlorate. The rats were first administered 6 mg of 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, together with 5 to 50 μCi $^{131}I^-$. The rats were sacrificed 1.0 to 1.5 h after the iodide administration. Perchlorate reduced the thryoid:blood ratio from 22.7 to 0.45; radioiodide was found to take up 30% of the thyroid gland volume when entering 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 different tissues also was examined. Perchlorate did not affect the organ:serum iodide ratios of the following tissues: submaxillary gland, parotid, pituitary, adrenals, testes, spleen, kidney, 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 ratios for controls administered sodium chloride (1.45 and 15.8, respectively), suggesting a gastric iodide pump subject to inhibition by perchlorate.

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 fell to approximately 5% of this peak value by 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 has been raised as to whether there is some curvilinearity to the urinary excretion, suggesting some 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 $^{36}Cl^-$ was 25.2 μCi/mmol. Thyroid impairment was effected by pretreatment with TSH (1 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 concentrate in the rat thyroid compared with the plasma, and the concentration was related inversely to dose. The high dose level did not result in concentration of perchlorate in the thryoid. Rats pretreated with TSH or PTU also concentrated perchlorate at the lower dose levels. Hypophysectomized rats were not able to concentrate perchlorate compared with intact rats at the two lower levels, but the thyroid perchlorate concentration at the high dose level was not different for intact versus 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. Concentration 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 with 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 (see Chapter 4) to actively sequester perchlorate is exceeded at higher doses. Male Sprague-Dawley rats were functionally neprhectomized 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 postdosing 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 by perchlorate of iodide uptake 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 h, 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 h, 93.4 to 97.4% of the administered dose was recovered, again suggesting no metabolism of perchlorate.

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 h. The second portion of the curve accounts for only 4% of the dose, and the half-life was 72 to 80 h. Calves have a faster overall rate of elimination, but the initial elimination is slower. The first-phase half-life was found to be approximately 2.0 to 2.5 h, and the second-phase half-life ranged from 23 to 27 h.

3.5 SUMMARY

The available database prior to initiation of the testing strategy (see Chapter 4) 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 basis for the effect on thyroid hormone function is the competitive inhibition of iodide anion uptake into the thyroid by ClO_4^-, which then results in reduced thyroid hormone production. Perchlorate also causes an efflux (discharge) of stored iodide in 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 the effect of the disease, 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 iodine 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.

The thyroid hormone deficiencies, such as those induced by perchlorate, can affect normal metabolism, growth, and development. No robust data existed to evaluate potential target tissues or effects other than those in the thyroid. The data on the thyroid effects were insufficient for quantitative dose-response assessment. There were no data to evaluate the effects of perchlorate in potentially susceptible populations, such as developing fetuses, nor were there data on the effects of perchlorate on reproductive capacity of male or female laboratory animals.

Benign tumors have 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. 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). The EPA has adopted the policy that an assumption of a threshold for carcinogenicity based on these precursor lesions along the progression is appropriate for the dose-response of chemicals that cause this type of disruption in the thyroid when they do not have genotoxic activity (i.e., cause damage to DNA or show other genetic disruption [U.S. Environmental Protection Agency, 1998a]). This assessment will explore the possibility of establishing a dose-response estimate using the NOAEL for hormone (T3, T4, and TSH) and initial thyroid histopathology as the precursor lesions to be an estimate also protective for potential benign tumor development. Existing shorter term studies indicate that perchlorate causes changes in the thyroid typical of the progression described, and genotoxic studies are required to establish that perchlorate does not have any activity relevant to carcinogenicity.

4. TOXICOKINETICS/TOXICODYNAMICS AND MODE-OF-ACTION TESTING STRATEGY

Based on the hazard characterization of Chapter 3 and the recommendations of the 1997 TERA external review panel, this chapter explains the rationale that was the basis underlying the testing strategy to evaluate the potential critical targets for perchlorate 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 6).

4.1 ABSORPTION, DISTRIBUTION, METABOLISM, AND ELIMINATION OF PERCHLORATE

As discussed in Chapter 2, perchlorate salts are dissolved readily in water. The resultant anion is easily absorbed from the gastrointestinal tract. However, because of its high charge, perchlorate does not pass readily through the skin. Electrolytes applied from aqueous solutions do not penetrate the skin readily (Schueplein and Bronaugh, 1983). Uptake of inorganic ions such as perchlorate is typically less than 10% and frequently less than 1% through the skin. Exposure via inhalation to fumes or vapors is expected to be negligible because the vapor pressure of perchlorate salts and acids is expected to be low at room temperatures. Exposure to particles would depend on the particle size (aerodynamic diameter) distribution.

Perchlorate appears to be eliminated rapidly, primarily in the urine (>90%), and virtually unchanged both in the rat (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 h and 95% within 48 h. Half-lives have been reported for the rat from <8 h (95% in 60 h) to ≈20 h (Wolff, 1998). Stanbury and Wyngaarden (1952) reported that perchlorate appears in the urine within 10 to 15 min of oral dosing, and peak plasma levels occur within 3 h. 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 h. 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 h, and the second 23 to 27 h (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 4.3, following discussions of iodine metabolism and thyroid physiology in Section 4.2.

4.2 IODINE METABOLISM AND THYROID PHYSIOLOGY

Iodine plays a central role in thyroid physiology, being both a constituent of thyroid hormones and a regulator of thyroid gland function. Like perchlorate, iodine is absorbed efficiently in the gastrointestinal tract. Iodine in organic form is converted mostly to iodide (I^-) 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 only about 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, avoidance of elevated 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/iodide symporter*. 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 will be of 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, 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 effects of the thyroid hormones on protein synthesis (Hill et al., 1989).

Figure 4-1 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. The follicular cell in situ 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 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 T4, which is still 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 resolved into vesicles that fuse with lysosomes (LY, dark circle). Lysosome proteolysis (striated circle) breaks down Tg to amino acids, T4, T3, diiodotyrosine (DIT) and

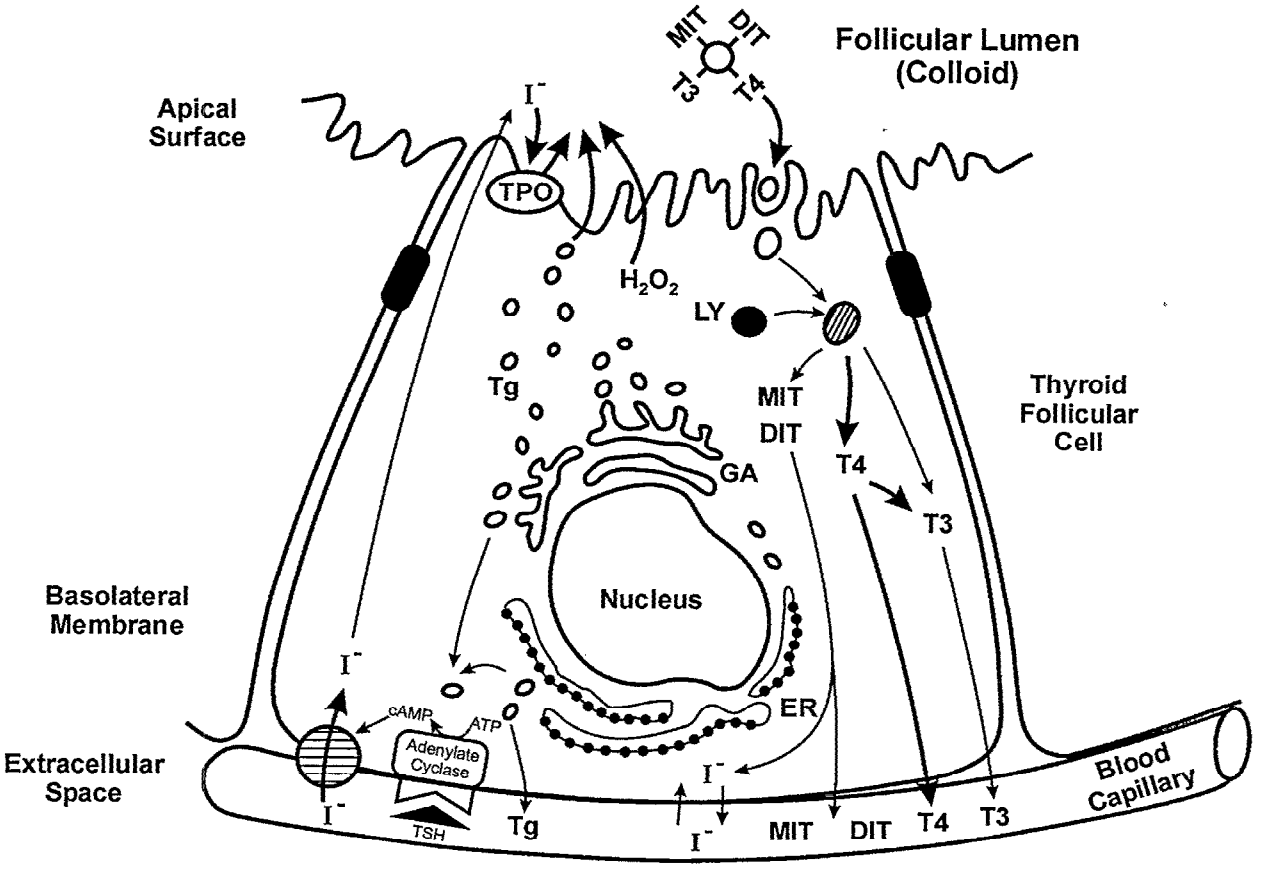


Figure 4-1. Schematic representation of thyroid hormone biosynthesis and secretion in a single thyroid follicular cell.

Source: Modified from Hill et al. (1989), Cavalieri (1997), and Fisher (1996).

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 and in certain thyroid disorders. Type I iodothyronine deiodinase converts some of the free T4 into 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 8 to 10 times the rate of T3), and it can vary as a function of the iodine intake, T4 is considered to be a

prohormone. Thus, T3 is about fourfold more potent than T4, and about 33% of the T4 secreted undergoes 5'-deiodination to T3 in the peripheral tissues; another 40% undergoes deiodination of the inner ring to yield the inactive material, reverse triiodothyronine (rT3), which recently has been thought to play an inhibitory role on the conversion of T4 to T3. On entering the circulation, both T4 and T3 are bound and transported in strong, but not covalent, association with plasma proteins. The major 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 or albumin with a less strong 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 4-2 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 then prompts the thyroid to eventually 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 and albumin [PA] 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 the 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.

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 reduced, the pituitary is prompted to deliver more TSH to the thyroid to increase the output of T4 and T3. This negative feedback loop helps the body to respond to varying demands for thyroid hormone and to maintain hormone homeostasis. Circulating T4, T3, and TSH thus are monitored readily in experimental animals and humans to serve as biomarkers of exposure and effect of agents that disrupt the status of the hypothalamic-pituitary-thyroid axis (U.S. Environmental Protection Agency, 1998a).

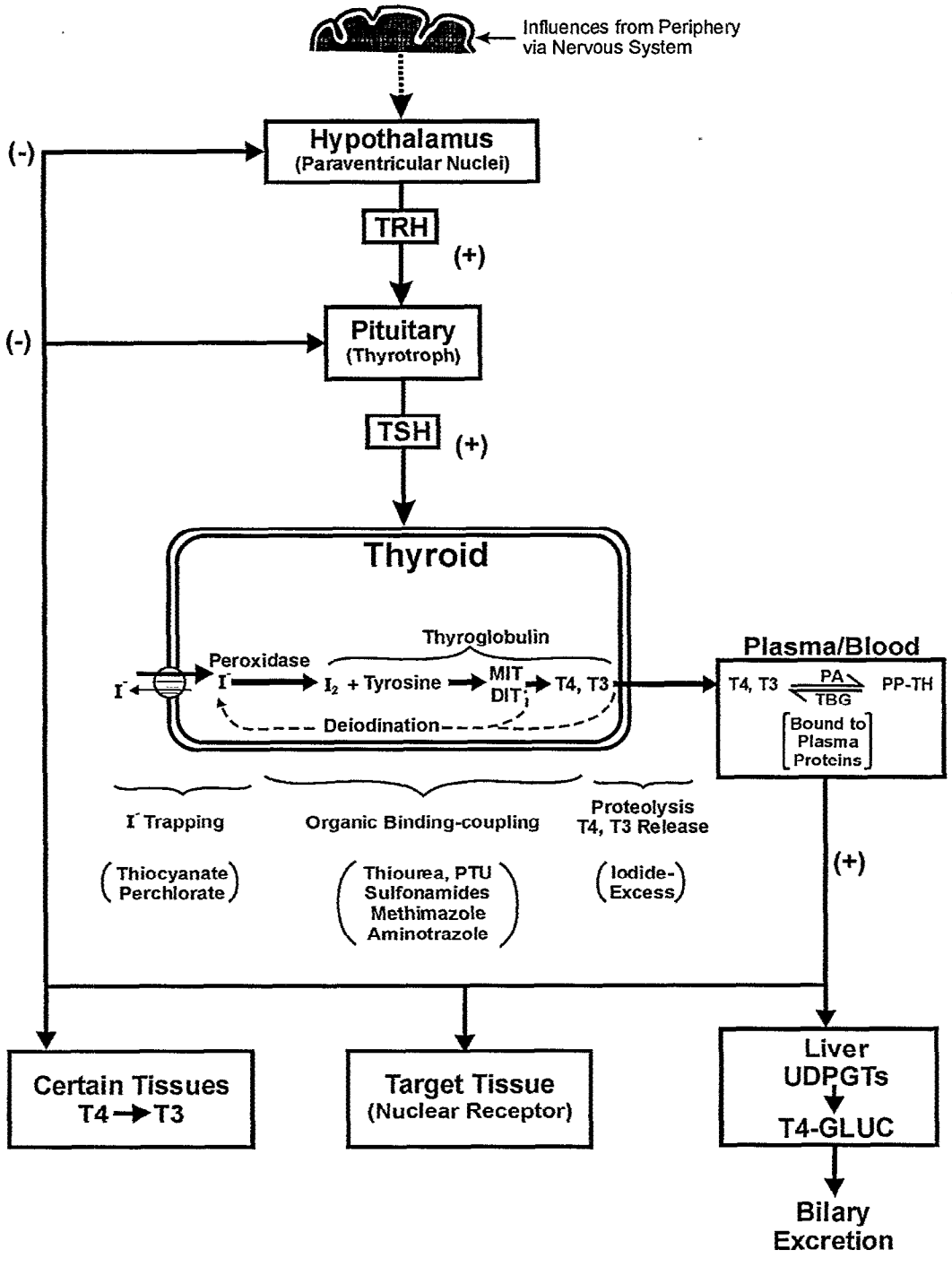


Figure 4-2. Schematic of the hypothalamic-pituitary-thyroid axis and feedback mechanisms (PP-TH = plasma protein-thyroid hormone, PTU = propylthiouracil, UDPGT = uridine diphosphyl glucuronosyl transferase, T4 GLUC = T4-glucuronide conjugate).

Source: Modified from U.S. Environmental Protection Agency (1998a), Hill et al. (1998), and Capen (1997).

In the absence of thyroid-binding globulin, as in the rat and mouse, more 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 h, in contrast to 6 to 7 days in humans. Rats compensate for the increased turnover rate by secreting more TSH from the pituitary gland. Table 4-1 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 4.4.

TABLE 4-1. INTERSPECIES AND INTRASPECIES DIFFERENCES IN THYROID STRUCTURE AND T3, T4, AND TSH HORMONES[a]

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 = F	$M^d \leq 2 \times F^e$
Cancer sensitivity	F = 2.5 × M	M > F

[a]M = male, F = female.

Source: U.S. Environmental Protection Agency (1998a).

4.3 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). It is established as a competitive inhibitor of iodide uptake across the basolateral membrane (i.e., of the symporter). Perchlorate and several related monovalent anions are selected by the symporter. 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 necessary mechanistic relation to what goes on at low concentrations in the thyroid. It is important to determine which solution properties of the anions determined this series (e.g., crystal radius, hydrated radius, hydration enthalpy, charge density). Strong base anion-exchange resins (usually a large cation with 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 thyroid may be based on an anion-exchange mechanism using a large cation such as a quaternarny amine (e.g., arginine) (Wolff, 1989).

Perchlorate also has been used to stimulate 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 could 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, which suggests that additional control points may exist.

Thus, perchlorate appears to have no effect on the iodination process itself but, rather, displaces the iodide by the competitive uptake at the symporter. 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, would provide useful data 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 4.4.

4.4 TOXICODYNAMICS OF THYROID HORMONE PERTURBATIONS

4.4.1 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, eventually 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 4-3 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 4-2 lists mechanisms of antithyroid-mediated neoplasia in rodents. The potential for an indirect effect of perchlorate has been established, but genotoxicity information would be required to evaluate its potential for direct effects. As will be discussed in Section 4.5, a battery of such assays was included in the testing strategy.

Long-term perturbations in the hypothalamic-pituitary-thyroid axis by various influences listed in Table 4-2 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 deals with the influence of protein carriers of thyroid hormones in the blood (Table 4-1). 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 a result, 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

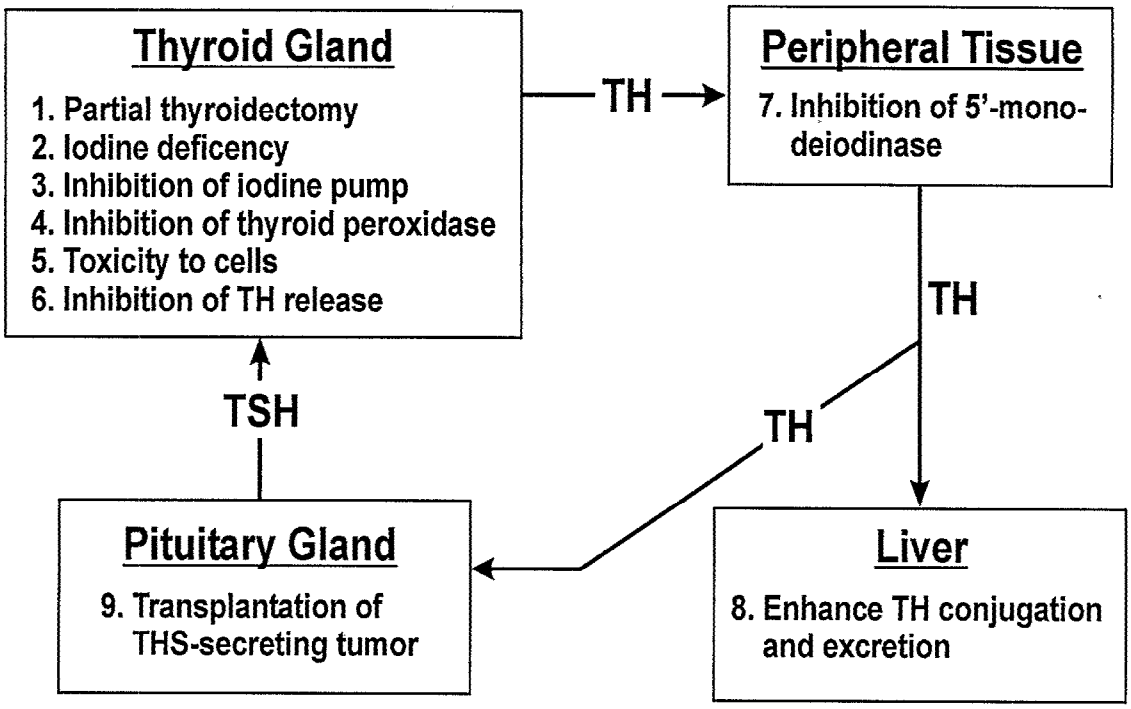


Figure 4-3. Schematic of antithyroid effects that influence thyroid carcinogenesis.

Source: U.S. Environmental Protection Agency (1998a) and Hill et al. (1998).

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 h, whereas in humans, it is about 24 h. High thyroid hormone synthetic activity is demonstrated in thyroid 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

TABLE 4-2. MECHANISMS OF ANTITHYROID-MEDIATED NEOPLASIA IN RODENTS[a]

- **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

Source: U.S. Environmental Protection Agency (1998a).

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 also has been reported to produce a high incidence. 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 a malignant tumor (Figure 4-4). The precise molecular steps in the carcinogenic process leading to thyroid follicular

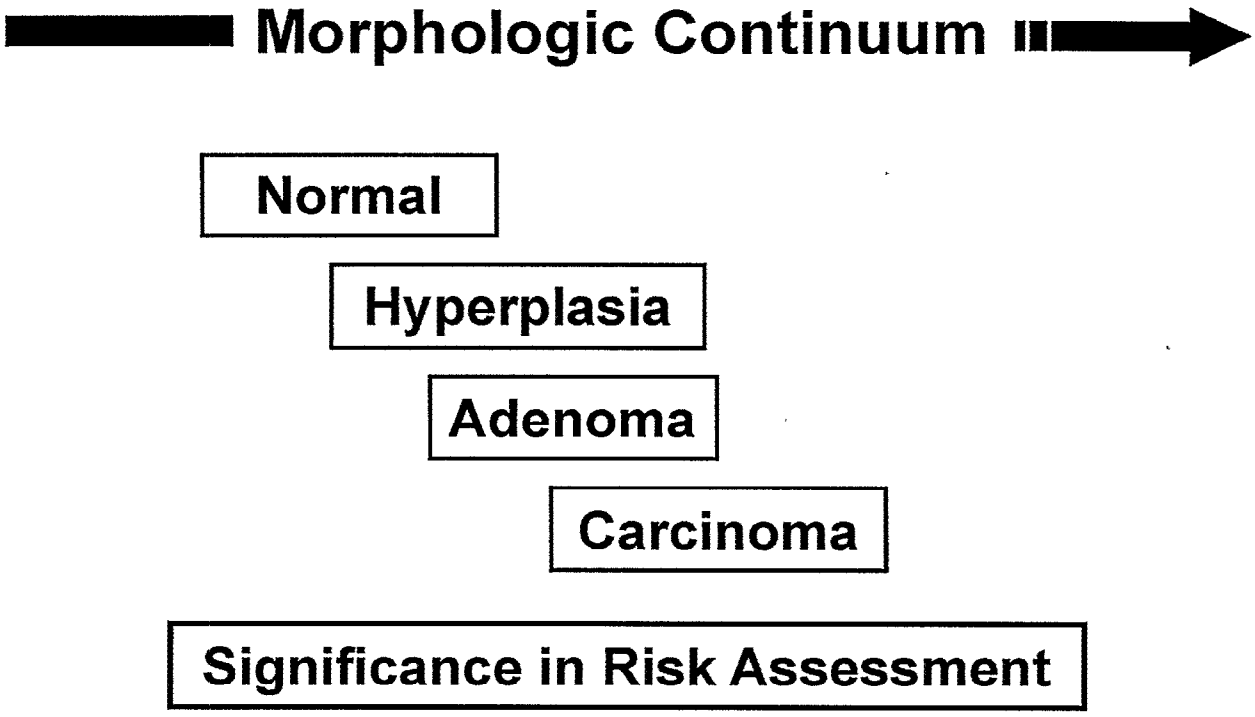


Figure 4-4. Proliferative changes involved in the multistage characterization of thyroid follicular cell neoplasia in rodents represent a morphologic continuum. Although these lesions typically are classified as discrete entities, the overlap in morphologic features should be emphasized because only 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 conservative (protective).

Source: Capen (1997).

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). Still other factors, such as transforming growth factor β, certain interferons, and interleukin 1, may inhibit growth.

Figure 4-5 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; 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. The EPA has adopted the following science 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 6 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.

4.4.2 Other Potential Adverse Effects Resulting from Thyroid Disruption

As expressed by the 1997 TERA external review panel, concern existed for other potential adverse effects of perchlorate-induced hypothyroidism. For instance, thyroid hormone is critical

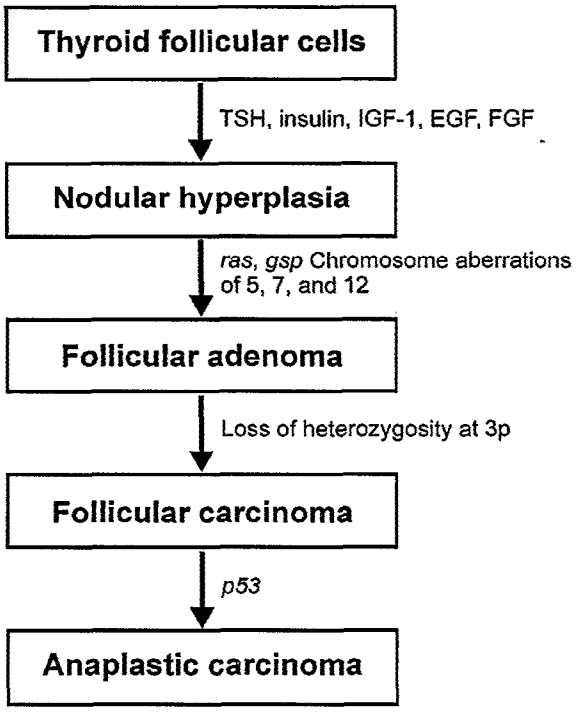


Figure 4-5. Possible molecular events in human thyroid follicular carcinogenesis (*ras* = *ras* protooncogene, *gsp* = GTP-binding protein mutation, *p53* = *p53* tumor suppressor gene).

Source: U.S. Environmental Protection Agency (1998a) and Hill et al. (1998).

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 4-6. The fetus is dependent on maternal hormone levels for some time. Once the fetal thyroid begins to produce on its own, because perchlorate can cross the placenta the potential for disruption of fetal hormone production remains. 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 4-3). It is important to emphasize that even transient disruption may lead to permanent effects in the developing organism.

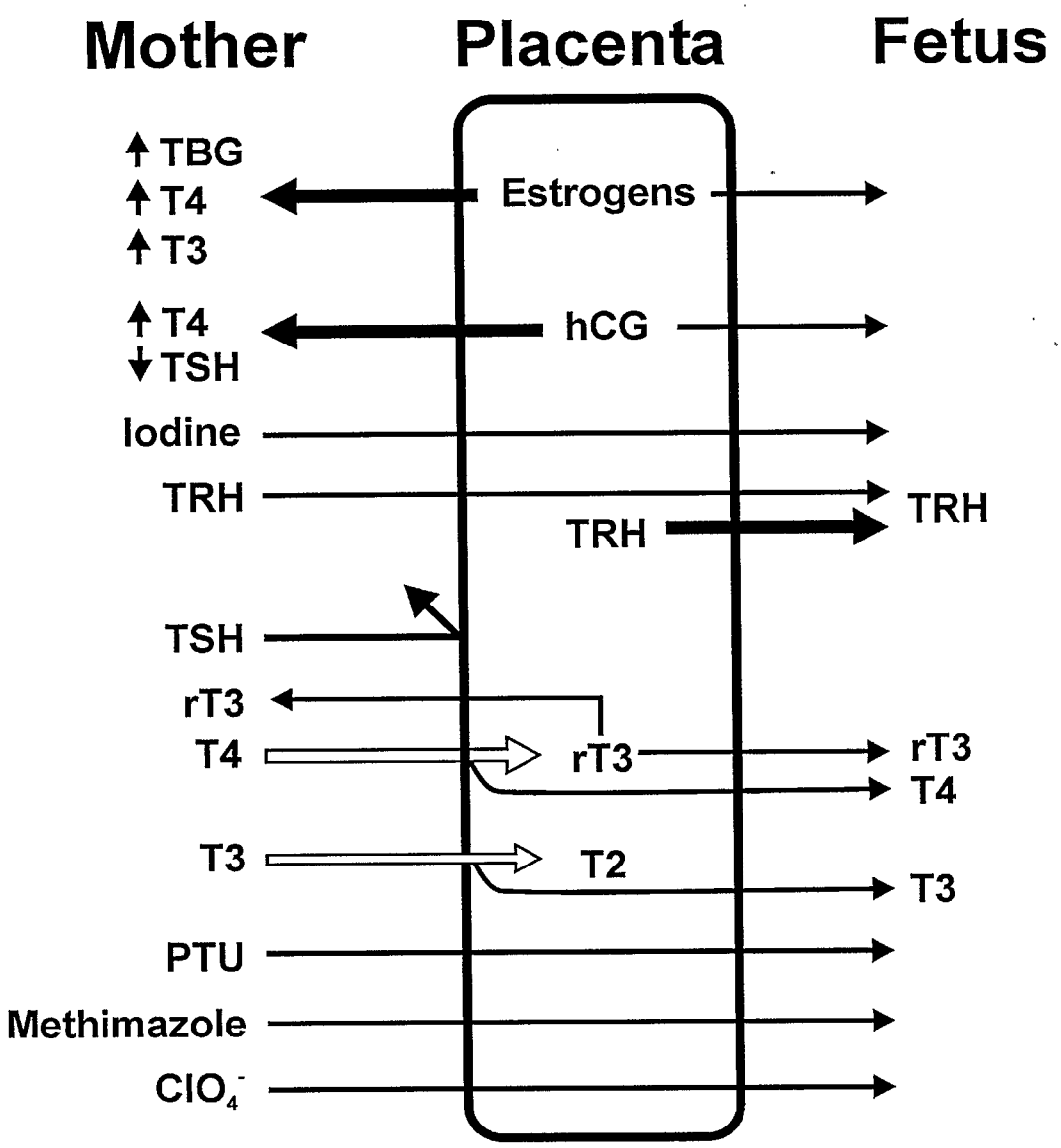


Figure 4-6. Schematic representation of the role of the placenta in thyroid hormone metabolism during human pregnancy. The placenta produces estrogens and hCG that increase maternal TBG levels and stimulate maternal thyroid hormone production, respectively. Both activities tend to increase maternal T4 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.

Source: Modified from Fisher (1996) and Underwood (1998).

TABLE 4-3. MAIN SYMPTOMS AND EFFECTS OF HYPOTHYROIDISM

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

The adverse consequences of hypothyroidism and hypothyroxenemia during development may result in entirely different outcomes compared with adult exposure. Chemical-induced alterations in thyroid hormone homeostasis are known to adversely impact 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 et al., 1998). These effects are caused by a lack of thyroid hormones, rather than by tumor development or thyroid hypertrophy/hyperplasia. During development, thyroid hormones regulate cell proliferation, migration, and differentiation. Intracellularly, THs bind to thyroid hormone receptors that then interact with thyroid response elements to alter expression of 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.

As mentioned above, reproductive toxicity was also a concern. 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. Obviously, prolactin is an important hormone in lactation. Also, 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, thereby 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, 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, which may be linked to immune mechanisms. A small number of patients undergoing perchlorate therapy have been reported to develop aplastic anemia, agranulocytosis, lymphadenopathy, or leukopenia. In addition, skin rash has been reported to occur as a consequence of perchlorate therapy. 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.

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

Because the RfD is intended as 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 in which potential effects could occur (e.g., the developing fetus through adult) and for effects on reproductive capability (shown schematically in Figure 4-7). As discussed in the previous sections and in Chapter 3, 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 of effects caused by long-term exposures and no data to evaluate the effects of perchlorate in a potentially susceptible population such as developing fetuses, nor were there data on the effects of perchlorate on reproductive capacity of male or female laboratory animals. Table 4-4 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 4-5), either because of 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 (e.g., depending on the quality of the individual studies and whether the dose response for suspected endpoints is characterized well).

The objective of the new studies is to provide a comprehensive database that describes the mode-of-action-based pathogenesis in quantitative terms, so that the resultant estimate can be more predictive and ultimately provide for development of a robust RfD estimate that reduces the uncertainties inherent in the provisional, presumably protective values (see Figure 4-8). As illustrated in Figure 4-8, 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

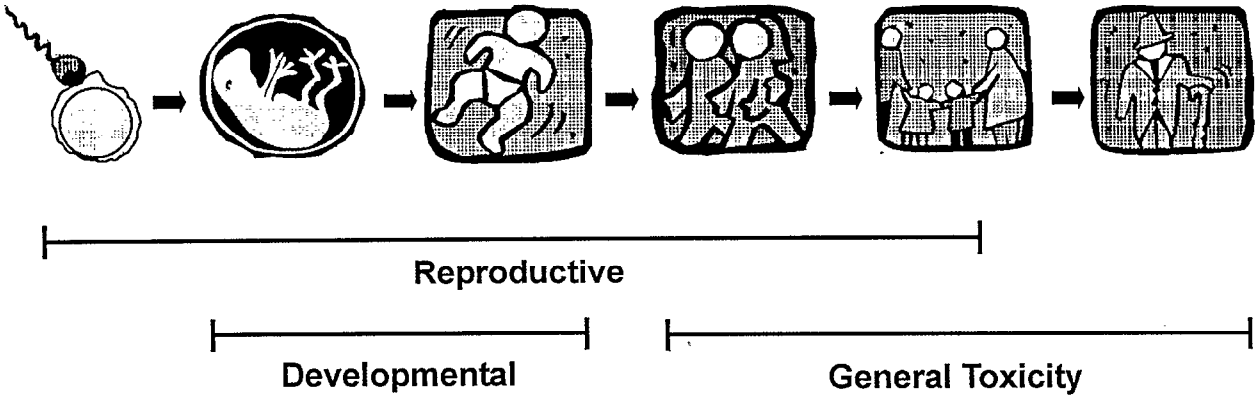


Figure 4-7. Schematic illustrating that a high confidence RfD is based on data that address all potentially critical stages over a lifetime.

TABLE 4-4. 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 4-5. 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(HED) to NOAEL(HED)
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]HED = human equivalent dose.

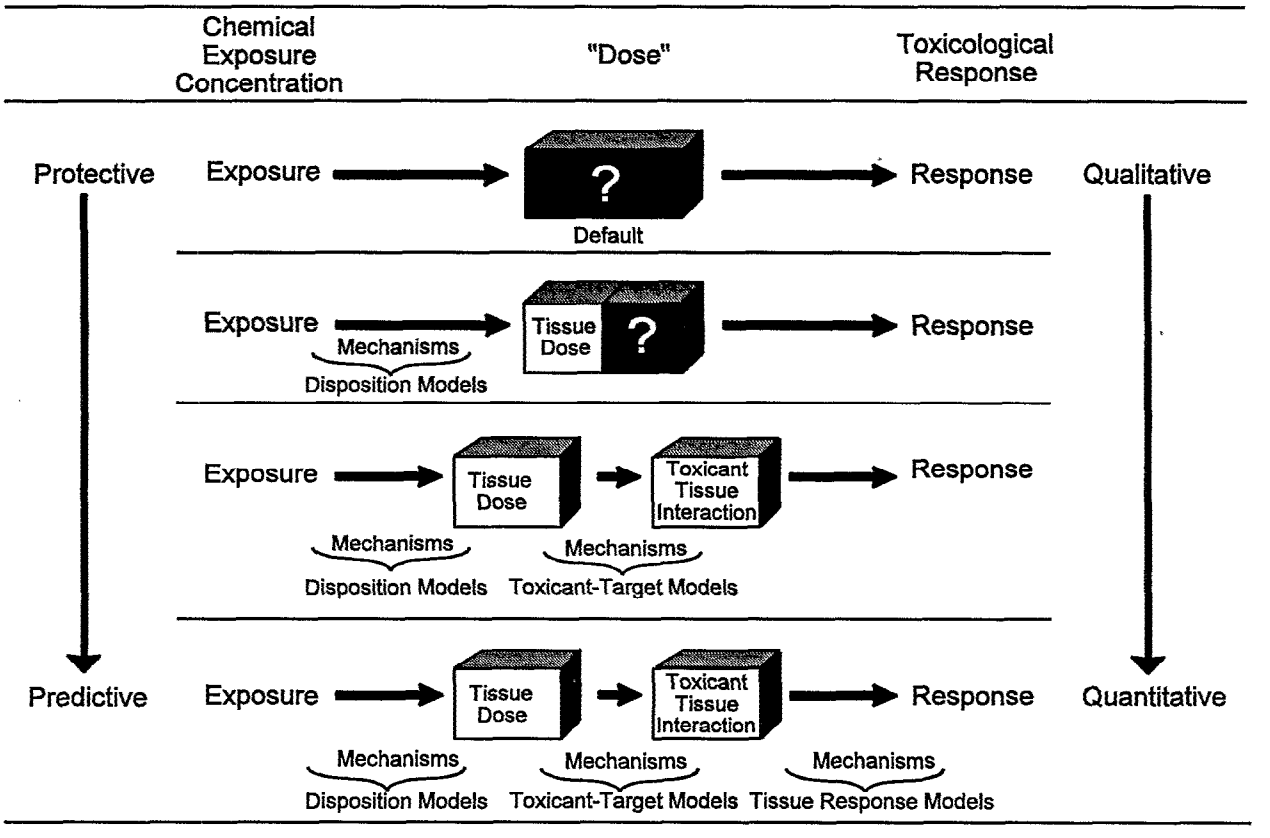


Figure 4-8. Schematic characterization of comprehensive exposure-dose-response continuum and the evolution of protective to predictive dose-response estimates.

Source: U.S. Environmental Protection Agency (1994) and Jarabek (1995b).

has been placed on the need to distinguish clearly between exposure concentration and 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 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 testing strategy to provide such a comprehensive array of endpoints. These studies are described below along with their anticipated role in informing the revised health risk assessment (see Table 4-6).

(1) 90-Day Subchronic Oral Bioassay Study. This study is considered the minimum data requirement for derivation of an oral RfD. The study will identify other target tissues, test young adult rats, and also provide data on the effect of repeated exposure to perchlorate on thyroid hormone levels. These data also may allow reduction of the uncertainty factor applied for database deficiencies.

(2) Neurobehavioral Developmental Study. This study will 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 may be a critical effect, and the developing organism a sensitive subpopulation. These data may allow reduction of the uncertainty factors applied for intrahuman variability and database deficiencies.

(3) Segment II Developmental Study. This study will evaluate the potential for perchlorate to cause birth defects in rabbits and may identify a potentially critical effect and subpopulation. This study also will provide data on the thyroid hormone effects in a second species

TABLE 4-6. PERCHLORATE PEER REVIEW RECOMMENDED STUDIES SUMMARY[a]

Study	Description	Potential Use in Assessment
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
90-Day subchronic bioassay + TH + 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 UF for database deficiencies
Genotoxicity assays	Test for toxicity to DNA	Mode of action information for thyroid neoplasia; may reduce UF for database deficiencies
Mechanistic studies	Evaluate mechanism of TH response and sensitivity in rats and humans	Interspecies extrapolation; determine susceptible subpopulation
ADME studies	Characterize absorption, distribution, metabolism, and elimination in rats and humans; iodine inhibition and perchlorate kinetices and hormone homeostasis	Interspecies extrapolation
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
Immunotoxicity	Evaluates immune system structure and function	May reduced UF for database deficiencies if not critical effects

(in addition to rats). These data may allow reduction of the uncertainty factor applied for database deficiencies.

(4) Two-Generation Reproductive Toxicity Study. This study will evaluate the potential for perchlorate to cause deficits in reproductive performance in adult rats and for toxicity in the

young offspring. This study may identify a potentially critical effect and allow for reduction of the uncertainty factor applied for database deficiencies.

(5) **Absorption, Distribution, Metabolism, and Elimination Studies.** These ADME studies will be performed to understand the pharmacokinetics (i.e., how perchlorate is absorbed, distributed, metabolized, and excreted) of perchlorate in test animals and humans. These data will provide information that will allow construction of quantitative extrapolation of dose across species (e.g., rat to human).

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

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

(8) **Immunotoxicity Studies.** These studies will evaluate the potential for perchlorate to disrupt immune function and identify a potentially critical effect. These data may 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.

The results of the studies in the testing strategy will now be reported together with EPA's interpretation and evaluation in Chapter 5.

5. RESULTS OF MODE-OF-ACTION TESTING STRATEGY AND RECENT STUDIES

5.1 STUDIES IN HUMANS

The Environmental Health Investigations Branch within the CA DHS, under a cooperative agreement with ATSDR, has 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). In an initial preliminary health review (California Department of Health Services, 1997), several statewide databases were reviewed for possible perchlorate-related outcomes during the suspected years of contamination and limited to the likely areas of exposure by zip code. In California, newborn thyroid hormone levels are drawn and maintained on file with the Genetic Disease Branch of the Centers for Disease Control and Prevention. Data for the period 1985 through 1996 were abstracted for relevant zip codes for a total of 11,814 thyroid hormone screens, and four cases of hypothyroidism were observed. An expected value based on the statewide rate would have been 3.76. The non-exposed areas found six cases of hypothyroidism with 6.41 cases expected. These data did not suggest an 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 from 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 the database can not differentiate this aspect, it was concluded that these data would not be useful in determining the prevalence of thyroid enlargement 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, which is less than the statewide rate of 41.6/year. The rate for aplastic anemia was 3.8 hospitalizations per 100,000 individuals per year, which is 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, which would seem to be the likely explanation for this outcome, and acquired immunodeficiency syndrome may be another. The registry also was searched for childhood leukemia (either acute lymphocytic leukemia or acute myelogenous leukemia) cases. 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, these data were also not informative because of the other likely causes for these conditions. No increase in incidence for other measures (decreased neonatal thyroid levels, hypothyroidism, or childhood leukemia rates) were observed. The CA DHS noted that the major limitation with studies of this nature is the limitation imposed by the absence of good exposure estimations and the absence of data on transport and transformation models to provide dose reconstruction for the affected population. It is unclear when the contaminated plume entered the drinking water supply, and 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, 1998c). Finally, the other difficulty with assessing these outcome surveys is that perchlorate is not specific for producing thyroid dysfunction or hematological abnormalities. Table 5-1 shows the approximate prevalence of these disorders in the neonatal period, in this case, ranging from 1:30,000 to 1:100,000, suggesting that studies with large numbers may be necessary to detect subtle effects.

Based on these results, the CA DHS conducted exposure investigations of several other water service areas (California Department of Health Services, 1998a,b,c,d,e) and ascertained that completed exposure pathways to perchlorate contaminated water exist in several of these areas. These studies reinforce the need for both better exposure estimates and a revised health risk estimate, the goal of this document, in order to perform a proper risk characterization.

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" (approximate average, 17) for eight homogenous exposure groups defined, based on similar job activities: control, maintenance/foreman, and six discrete operator job categories, using either

TABLE 5-1. THYROID DISORDERS AND THEIR APPROXIMATE PREVALENCES IN THE HUMAN NEONATAL PERIOD

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	

Source: Fisher (1996).

personal breathing zone samples (n = 119) for the work categories or full-shift area samples for the control group (n = 19). The control exposure was not zero but was several orders of magnitude below any exposure category. The 1997 analyses were based on the quantification of ammonium ion using National Institute for Occupational Safety and Health Method 6016, with a minimum reporting limit of 0.017 mg/m^3, and a large number of the samples were reported as nondetectable. The 1998 analyses were performed by using the modified EPA 300.0 methodology, which 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 postshift 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 diameter distribution data, an inhaled "dose" was calculated for a single shift as (Gibbs et al., 1998):

$$\begin{pmatrix}\text{respiratory}\\ \text{rate}\end{pmatrix} \times \begin{pmatrix}\text{inhalation}\\ \text{concentration}\end{pmatrix} \times \begin{pmatrix}\text{exposure}\\ \text{duration}\end{pmatrix} \times \begin{pmatrix}\text{fraction}\\ \text{absorbed}\end{pmatrix}. \tag{5-1}$$

Working lifetime exposure estimates were calculated as:

$$\sum(\text{mean group exposure}) \times (\text{years in exposure group}) \times 2{,}000, \tag{5-2}$$

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-h and 0.0068 m^3/kg-h 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 smaller 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, and current practice usually scales ventilation rate based on body weight, so that a higher ventilation rate would be expected.

The absence of particle size diameter and distribution data is perhaps one of the most significant limitations of the study. Its proper interpretation requires the particle distribution data that EPA scientists on several occasions recommended obtaining to gain better insight on 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 available to inhalation 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, certainly a consideration in dusty environments with large diameter particles, especially when it is mentioned that 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

deposition fraction, which would be approximately 0.3 at 5 μm (U.S. Environmental Protection Agency, 1996b).

The assumption with respect to solubility of the inhaled particles is also problematic because this is also particle diameter dependent. The particle diameter dictates where in the respiratory tract a particle deposits and the various regions (extrathoracic, tracheobronchial, pulmonary) with local milieu that would influence solubility. Solubility and clearance both have been shown to be size dependent (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 accounted for, and the hydroscopicity, which influences initial deposition site, may not be the same. The assumptions with respect to dose would have benefitted from some validation by evaluation of mass balance. Perchlorate could have been measured in the blood when samples were taken for thyroid hormone analyses, and, because it is excreted in the urine, this too could have been monitored for perchlorate concentration to afford 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 with standard tests from the complete blood count obtained during medical surveillance examinations, including 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, 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 evaluated included race, gender, age, hours awake prior to the preshift 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 if the measurement was from a routine physical in 1996 or for a preshift or a postshift 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 would be log-transformed, and whether any outliers (defined as a value corresponding to a residual larger in absolute value than three standard deviations) would 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. 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 international unit/mL increase across the shift. This was attributed to the influence of circadian changes in serum TSH.

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. 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. 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 EPA was reluctant to assign a NOAEL or LOAEL estimate from this study because of the considerable uncertainties in the exposure estimates, relatively small sample sizes, and the lack of correction for TSH circadian changes.

The EPA is also aware of an additional study on 58 employees in perchlorate production, Thyroid Health Status of Ammonium Perchlorate Workers: A Cross-Sectional Occupational Health Study, that has been submitted for publication by Dr. Steve Lamm and associates. After peer review and acceptance by the journal, these data will be formally considered by EPA. Despite its small sample size, the study did include perchlorate urinary analysis and attempted to address inhalability of the aerosols.

5.2 LABORATORY ANIMAL BIOASSAYS

This section presents new analyses for the Caldwell et al. (1995) 14-day study discussed in Chapter 3. It also evaluates the results of the 90-day study (which included a 14-day sacrifice) that was part of the testing strategy (Springborn Laboratories, Inc., 1998).

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

As part of this assessment, EPA requested from the Air Force Research Laboratory/Human Effectiveness Directorate (AFRL/HEST) the previously unpublished histopathology data from the 14-day oral dosing study performed by Caldwell et al. (1995) discussed in Chapter 3. 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). These histopathology data discussed herein were provided in a consultative letter from the AFRL/HEST (Channel, 1998a). 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 official publication of these data.

5.2.1.1 Thyroid Histology Data

The consultative letter of Channel (1998a) 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 AFRL/HEST and never officially published (Eggers, 1996, as cited in Channel, 1998a). The concentrations of ammonium perchlorate tested in Sprague-Dawley rats (6/sex/group) were 0, 1.25, 5.0, 12.5, 25, 50, 125, and 250 mg/L. 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 reading these reports because the conversion is sometimes not included (e.g., the

Channel [1998a]) consultative letter reports results in units of the test concentrations rather than the dose converted to milligrams per kilogram per day.

Channel (1998a) submits 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 is not provided, and Eggers (1996) apparently combined both sexes for the analyses. It is recommended in the report (Channel, 1998a), and EPA concurs, that a reanalysis is warranted. This is particularly so for a number of reasons: (1) there was a gender-by-treatment interaction observed in the thyroid hormone analyses (see Section 5.2.2.3); (2) 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; and (3) 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 on 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.

The EPA concludes that these histopathology data as presented do not identify a NOAEL but rather a LOAEL at 0.11/0.12 mg/kg-day by standard histology for follicular epithelial cell hypertrophy. The incidence of decrease in follicular lumen size as determined by standard histology identifies a NOAEL at the 0.11/0.12-mg/kg-day dose and a LOAEL at the 0.44/0.47-mg/kg-day dose; whereas the morphometric analysis was less sensitive for this same measure, identifying a NOAEL at the 0.44/047-mg/kg-day dose and a LOAEL at the 1.11/1.23-mg/kg-day dose. It is interesting to note the difference in sensitivity between standard histopathology and the computerized morphometry for decrease in follicular lumen size analysis. The EPA has exercised additional statistical models to evaluate the incidence data for these histology data and these are discussed in Chapter 6.

5.2.1.2 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. Mean contrasts were performed using Tukey's Studentized Range (HSD) Test. To correct for multiple comparisons (i.e., five separate two-way ANOVA tests), the acceptable alpha for significance (for all interaction main effects tests) was corrected to 0.0224 (alpha of 0.05 divided by the square root of the number of dependent variables). Results of these reanalyses are similar to those stated in the Caldwell et al. (1995) and King (1995) reports, with some notable exceptions (see below).

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 5-1 illustrates dose-dependent decreases in T3 for both genders. Females were slightly more sensitive compared with males. The overall gender-by-treatment interaction was not significant for T4, but there was a significant main effect of treatment. Therefore, data from males and females were combined for all subsequent analyses; these data are plotted in Figure 5-2. Figure 5-2 clearly indicates that perchlorate decreases 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; Figure 5-3 illustrates dose-dependent increases in TSH for both genders. Females were slightly more sensitive compared with 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 on rT3, but there was a significant main effect of treatment. Therefore, data from males and females were combined for all subsequent analyses and plotted in Figure 5-4. This figure 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

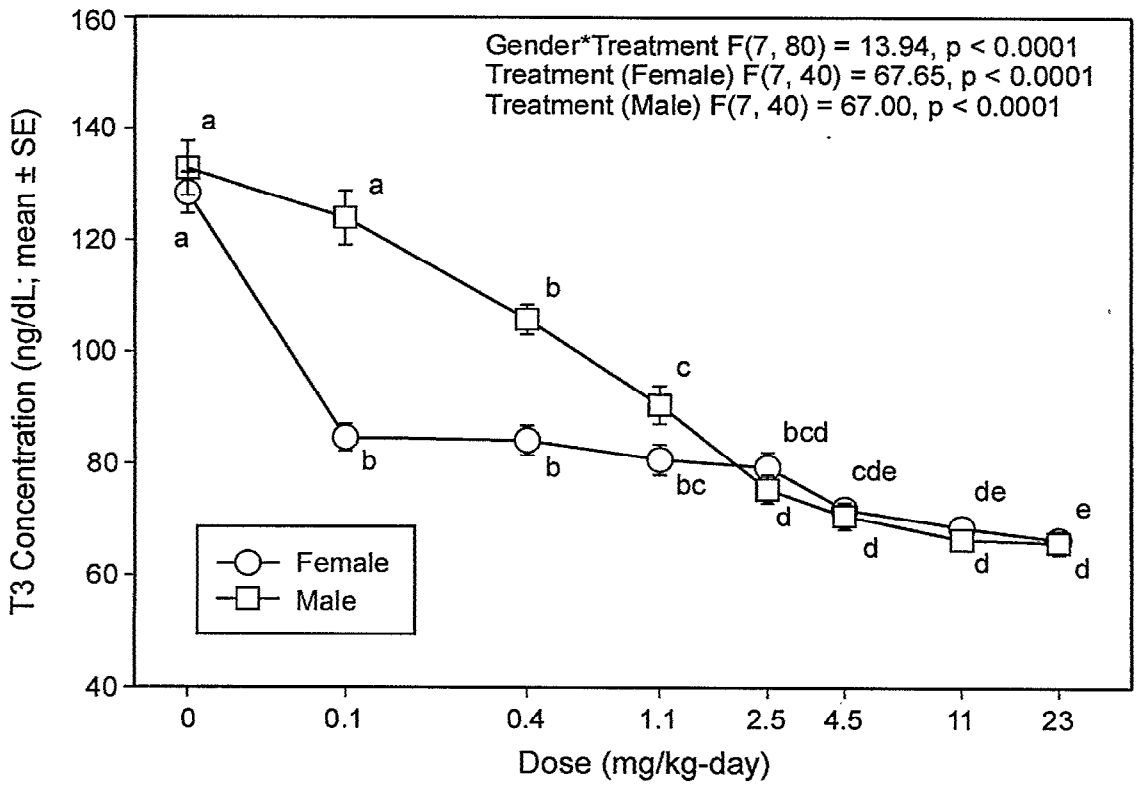


Figure 5-1. Effects in the Caldwell et al. (1995) study of 14-day drinking water administration of ammonium perchlorate to SD rats on serum total T3 concentrations. Data of Channel (1998a) and Crofton (1998a). There was a significant gender-by-treatment (gender*treatment) interaction and significant treatment effects for both genders; therefore, data were plotted separately by gender. Means with different letters were significantly different ($p < 0.05$, Tukey's Studentized Range (HSD) Test after significant main effect). Daily dose was estimated from water consumption data.

effects for both genders. Figure 5-5 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. There was no NOAEL established by this study.

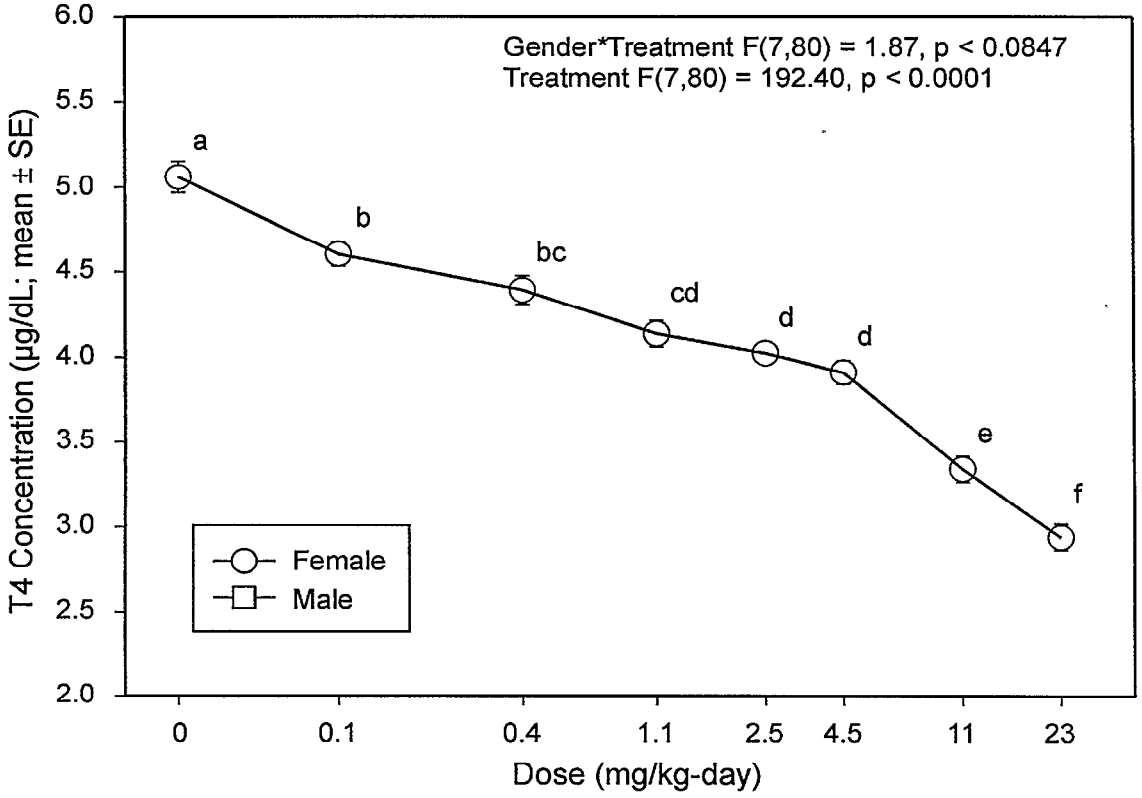


Figure 5-2. Effects in the Caldwell et al. (1995) study of 14-day drinking water administration of ammonium perchlorate to SD rats on serum total T4 concentrations. Data of Channel (1998a) and Crofton (1998a). There was no gender*treatment interaction, but there was a main treatment effect; therefore, data were collapsed across gender. Means with different letters were significantly different ($p < 0.05$, Tukey's Studentized Range (HSD) Test after significant main effect). Daily dose was estimated from water consumption data.

The lowest dosage of 0.11 mg/kg-day was a LOAEL for T4, T3, and hTG. Table 5-2 summarizes the results of these new EPA analyses.

5.2.2 The 90-Day Testing Strategy Bioassay in Rats

The 90-day study that was part of the strategy tested 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). A 14-day sacrifice also was

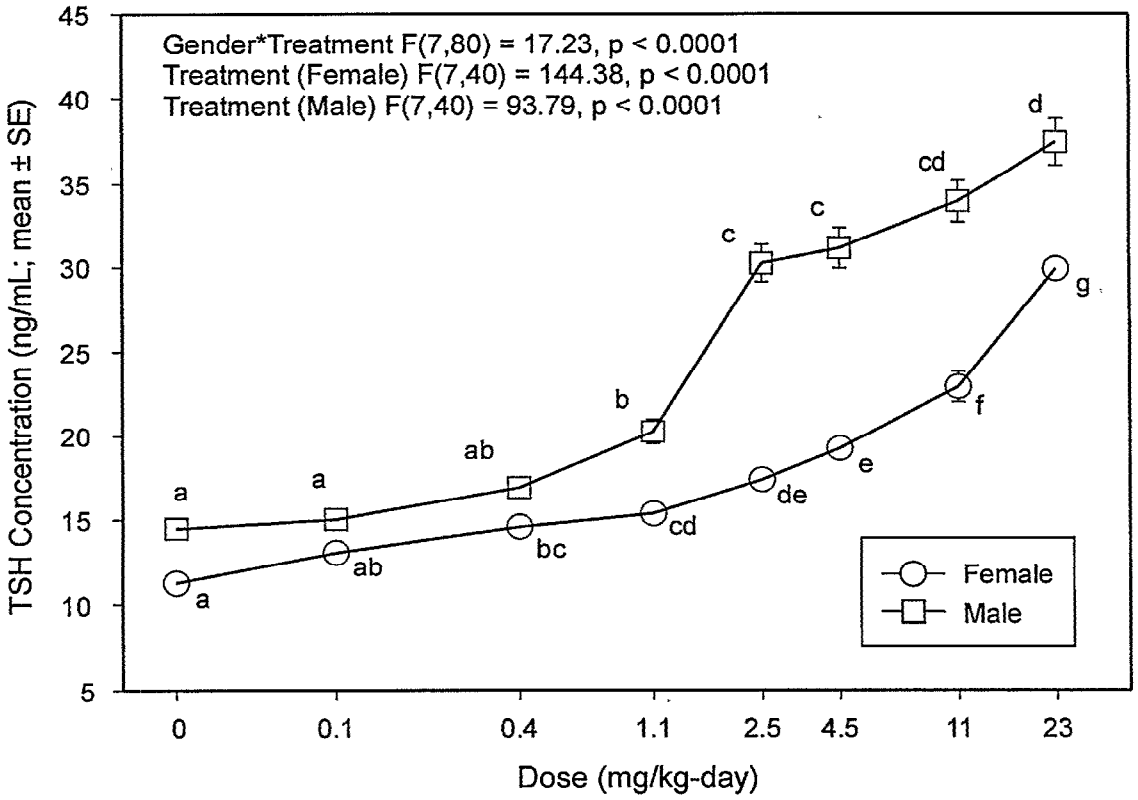


Figure 5-3. Effects in the Caldwell et al. (1995) study of 14-day drinking water administration of ammonium perchlorate to SD rats on serum TSH concentrations. Data of Channel (1998a) and Crofton (1998a). There was a significant gender*treatment interaction and significant treatment effects for both genders; therefore, data were plotted separately by gender. Means with different letters were significantly different ($p < 0.05$, Tukey's Studentized Range (HSD) Test after significant main effect). Daily dose was estimated from water consumption data.

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 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

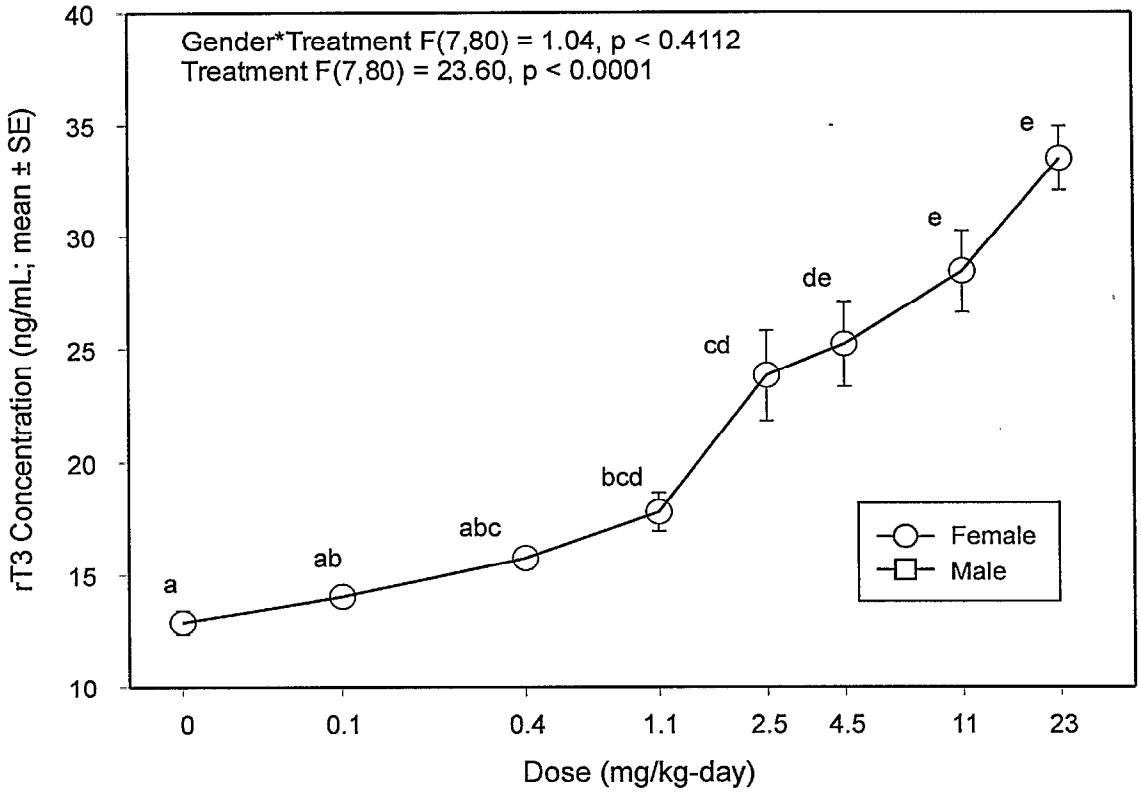


Figure 5-4. Effects in the Caldwell et al. (1995) study of 14-day drinking water administration of ammonium perchlorate to SD Rats on serum rT3 concentrations. Data of Channel (1998a) and Crofton (1998a). There was no gender*treatment interaction, but there was a main treatment effect; therefore, data were collapsed across gender. Means with different letters were significantly different ($p < 0.05$, Tukey's Studentized Range (HSD) Test after significant main effect). Daily dose was estimated from water consumption data.

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 acceptable range (Springborn Laboratories, Inc., 1998; Appendix B). Control drinking water solutions also 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.

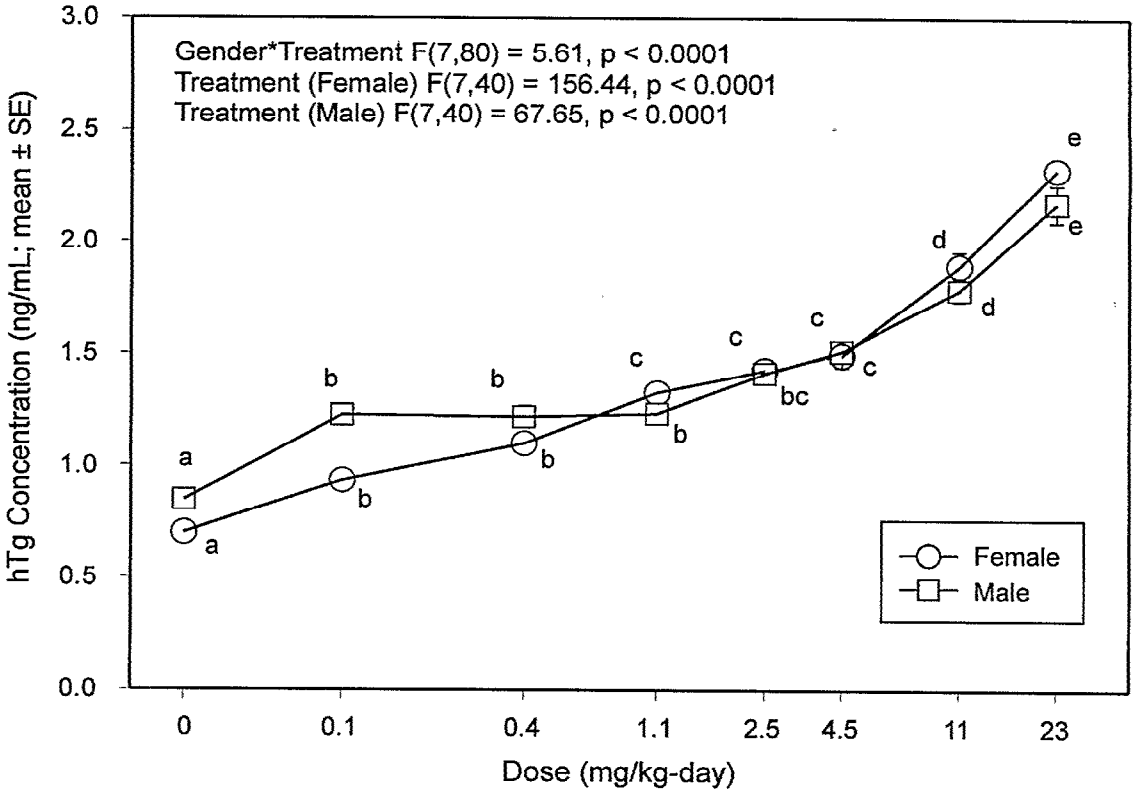


Figure 5-5. Effects in the Caldwell et al. (1995) study of 14-day drinking water administration of ammonium perchlorate to SD rats on serum hTg concentrations. Data of Channel (1998a) and Crofton (1998a). There was a significant gender*treatment interaction, and significant treatment effects for both genders; therefore, data were plotted separately by gender. Means with different letters were significantly different ($p < 0.05$, Tukey's Studentized Range (HSD) Test after significant main effect). Daily dose was estimated from water consumption data.

TABLE 5-2. SUMMARY OF NOAELS FOR THE CALDWELL et al. (1995) 14-DAY STUDY[a]

	T4	T3	TSH	rT3	hTG
Females	0.11[b]	0.12[b]	0.47	0.17	0.12[b]
Males		0.11	0.44		0.11[b]

[a] Ammonium perchlorate in milligrams per kilogram per day, estimated from water consumption data by the authors.
[b] = LOAEL, no NOAEL was defined.

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, 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. All hormone and tissue collection was balanced over time-of-day to control for circadian rhythms of hormones.

5.2.2.1 General Toxicity and Histology Results

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, but this 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 toxicology findings were observed among the groups with respect to clinical observations, body weights, food or water consumption, ophthalmology, hematology, or clinical chemistry. 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. 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. The only treatment-related lesions on gross necropsy were reddened thyroids, attributed to minimal congestion of the blood vessels. A treatment-related effect in the thyroid, diagnosed as follicular cell hyperplasia, also was observed, with histopathology in both sexes at the high dose. The hyperplasia was characterized by an increased number of small follicles in the central portion of the thyroid and an increase in the height of the follicular epithelium compared to that of controls. Occasional secondary, small follicles were noted within larger follicles. The degree of severity of the hyperplasia was reported as minimal in most thyroids. The incidence of the follicular cell hyperplasia in males at the 0-, 0.01-, 0.05-, 0.2-, 1.0-, and 10-mg/kg-day level was 1/8, 0/10, 1/10, 0/10, 0/10, and 10/10 or 2/10, 0/10, 0/10, 0/10,

1/10, and 8/10 for the 14-day or 90-day evaluations, respectively. The incidence in females at these same dose levels was 0/10, 0/10, 0/10, 0/10, 0/10, and 7/10 at the 14-day evaluation and 0/10, 0/10, 0/10, 0/10, 0/10, and 9/10 at the 90-day evaluation. No pathology was observed in the thyroids of either male or female rats at the 120-day (30-day recovery) evaluation, indicating recovery (reversibility) of the lesions. 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.

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 estrus 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%), 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) does not provide the level of statistical power that would be desired, this indication of an effect in a study with limited power is of concern and needs to be evaluated carefully when the results of the two-generation reproductive study become available.

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.

5.2.2.2 Thyroid and Pituitary Hormone Analyses

The assays for T4, T3, and TSH were performed using radioimmunoassay (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 (Days 14 and 90 and a Day 120 recovery time). As part of its 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). Data from each hormone were subjected to separate, three-way ANOVA tests, with day (Days 14, 90, and 120), gender (male and female), and treatment (dose) as independent between-subject variables. Dependent variables were T3, T4, and TSH. Step-down ANOVA tests were conducted as indicated by significant interactions. Mean contrasts were performed using Tukey's Studentized Range (HSD) Test. To correct for multiple comparisons (i.e., three separate three-way ANOVA tests) the acceptable alpha for significance was corrected to 0.0289 (alpha of 0.05 divided by the square root of the number of main comparisons).

Results of the EPA reanalyses 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, and this results from a slight difference in magnitude of effects between genders, but no differences in LOAELs between genders (with minor exceptions likely caused by small changes in variance between groups, which are probably not biologically significant [see below]). Second, the new EPA analyses failed to detect a significant effect of perchlorate on TSH at the 120-day time point. Results of the analyses for each thyroid hormone and TSH are discussed individually below.

There was a significant day-by-gender-by-treatment interaction for T3, and subsequent step-down ANOVA tests showed significant gender-by-treatment interactions for the 14- and 90-day time points. 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 5-6). There was no statistically significant effect of any dose of perchlorate on females at Day 14. The lack of effect 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 5-6 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 based on T3 for both males and females was 0.01 on Day 90. The LOAEL for Day 120 was 10 mg/kg-day, indicative of a recovery of T3 concentrations after cessation of treatment.

The overall day-by-gender-by-treatment interaction for T4 was not significant, but there were significant day-by-treatment and gender-by-treatment interactions. Thus, subsequent step-down ANOVAs were conducted as follows: main effects of treatment at each time point and main effects of treatment for each gender. These data are plotted in Figures 5-7 and 5-8. Figure 5-7 clearly indicates that perchlorate decreases T4 in a time- and dose-related manner. The effect at the 14-day time point was limited to the high dose only. The lack of effect at the lower doses is inconsistent with the previous 14-day study (Caldwell et al., 1995), where significant decreases were found at all doses tested (see Figure 5-2). The reason for this discrepancy remains to be determined. The potency increased at the 90-day time point, where all doses were significantly different from controls. There appears to be a lack of recovery at the 120-day time point; however, the lack of a 0.01-mg/kg-day group at this time point makes a definitive conclusion difficult. The apparent lack of recovery of T4 is not consistent with the recovery of T3 at all but the highest dose (compare lower panels of Figures 5-6 and 5-7). The biological plausibility of this difference is unknown. Figure 5-8 illustrates the significant gender-by-treatment interaction. Although the interaction was significant because of the slightly greater magnitude of the effect in males, the NOAELs were not different between the genders,

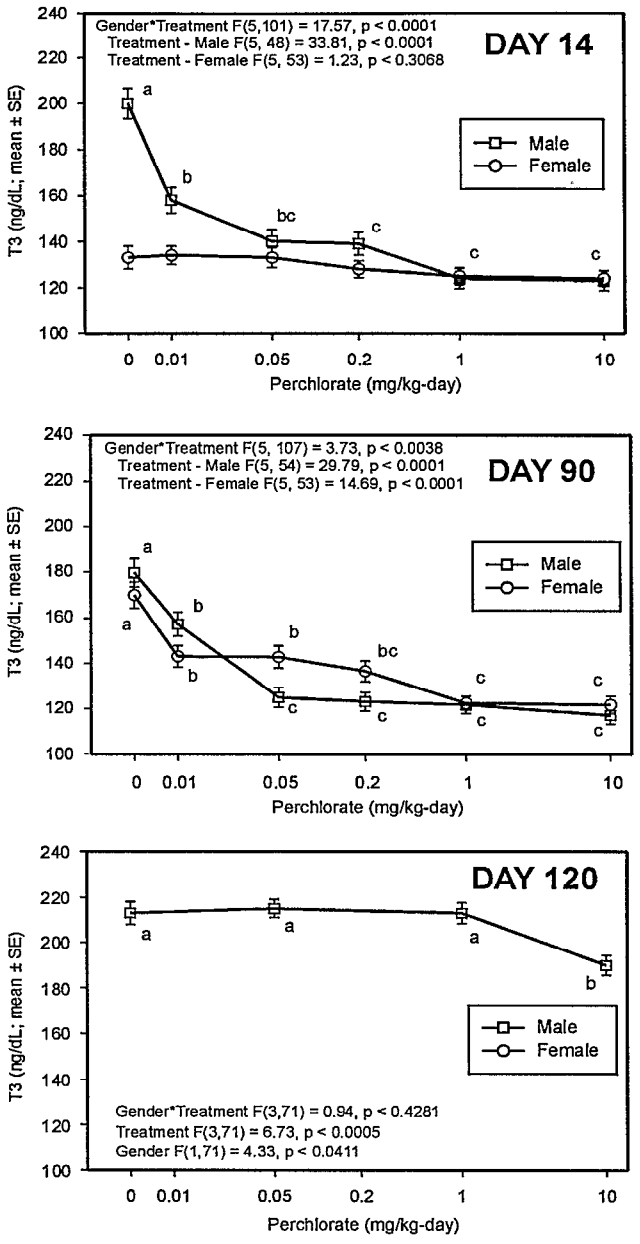


Figure 5-6. Effects from 90-day drinking water administration of ammonium perchlorate to SD rats on serum total T3 concentrations. Data of Springborn Laboratories, Inc. (1998). There was a significant day-by-gender-by-treatment (day*gender*treatment) interaction ($F[8,279] = 6.72$, $p < 0.0001$) and significant gender-by-treatment interactions for Day 14 and Day 90, but not for Day 120; therefore, data were plotted by gender for Day 14 and Day 90 and collapsed across gender and plotted by dose for Day 120. Means with different letters were significantly different ($p < 0.05$, Tukey's Studentized Range (HSD) Test after significant main effect). The 120-day time point is 30 days after cessation of exposure.

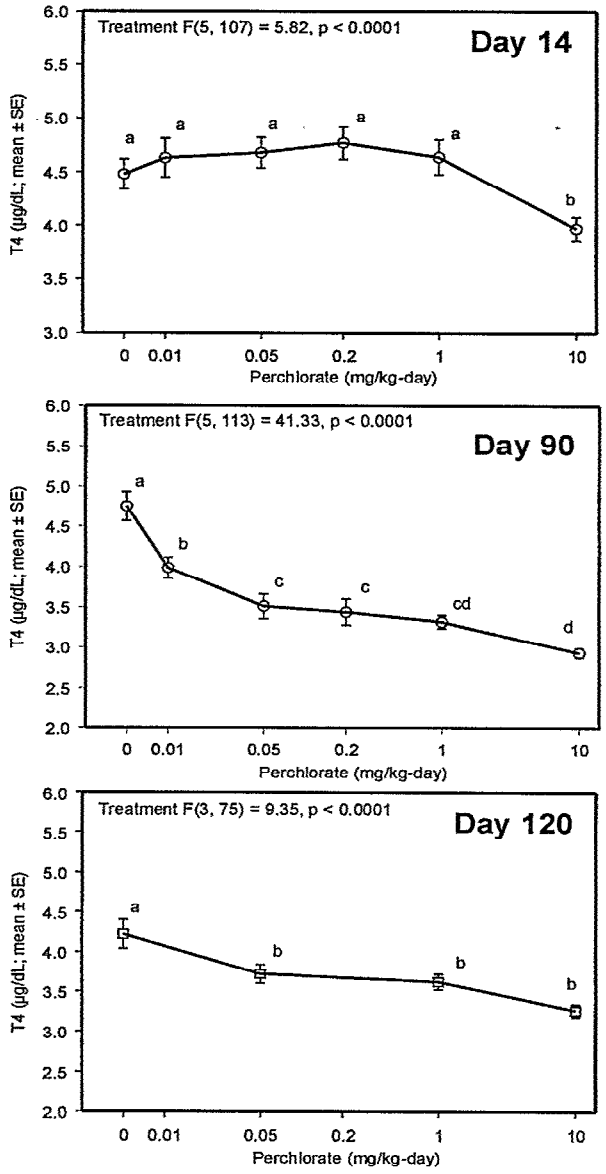


Figure 5-7. Effects from 90-day drinking water administration of ammonium perchlorate to SD rats on serum total T4 concentrations. Data of Springborn Laboratories, Inc. (1998). There was no day-by-gender-by-treatment interaction ($F[8,279] = 1.22$, $p < 0.2862$), but there was a main day*treatment interaction ($F[8,279] = 6.84$, $p < 0.0001$); therefore, data were collasped across gender and plotted by dose for each day. Means with different letters were significantly different ($p < 0.05$, Tukey's Studentized Range (HSD) Test after significant main effect). The 120-day time point is 30 days after cessation of exposure.

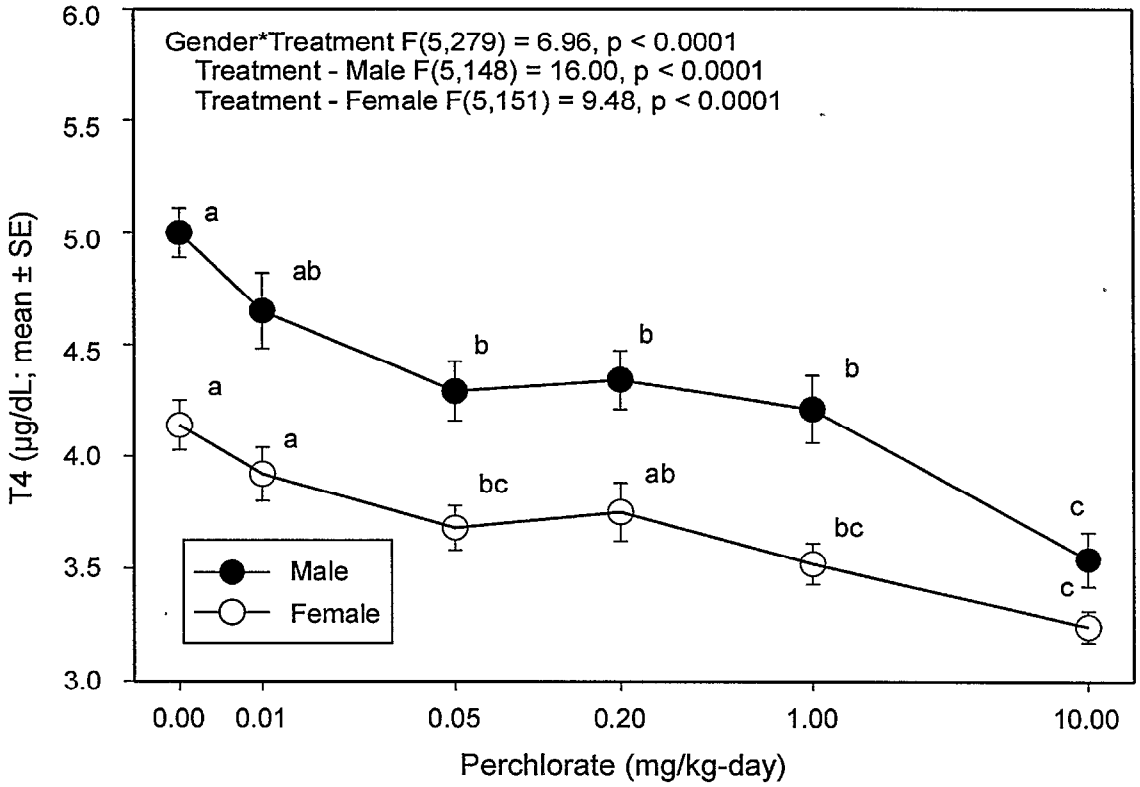


Figure 5-8. Effects from 90-day drinking water administration of ammonium perchlorate to SD rats on serum total T4. Data of Springborn Laboratories, Inc. (1998). There was a significant gender*treatment interaction; therefore, data was collapsed across days. Means with different letters are significantly different ($p < 0.05$, Tukey's Studentized Range (HSD) Test after significant main effect).

supporting the conclusion that both genders are equally susceptible to the hypothyroxenemic effects of perchlorate.

There was a significant day-by-gender-by-treatment interaction for TSH, and subsequent step-down ANOVA tests showed a significant gender-by-treatment interaction for the 14-day time point only. Therefore, separate ANOVA tests were conducted on each gender to test for a main effect of treatment for the 14-day 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 14- and 90-day time points (see Figure 5-9). The NOAEL for the Day 14 data was 0.01 mg/kg-day for the females and 0.05 mg/kg-day for the males. This 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 recovered to control values 30 days after cessation of treatment.

The data demonstrate a dose- and time-dependent effect of perchlorate on thyroid hormones and TSH. The LOAEL, based on decreases in T3 and T4 at 90 days, is 0.01 mg/kg-day. No NOAEL could be calculated for T3 and T4. The NOAEL for TSH is 0.05 mg/kg-day, based on significant increases in both genders on both Days 14 and 90. Partial recovery at the 120-day evaluation (30 days after cessation of treatment) also was demonstrated.

5.2.3 Neurodevelopmental Toxicity Study in Rats

The neurobehavioral developmental study of ammonium perchlorate that was part of the 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. Subsequent supplemental data submittals and additional analyses were requested by EPA and provided by Argus Laboratories pertaining to this 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 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

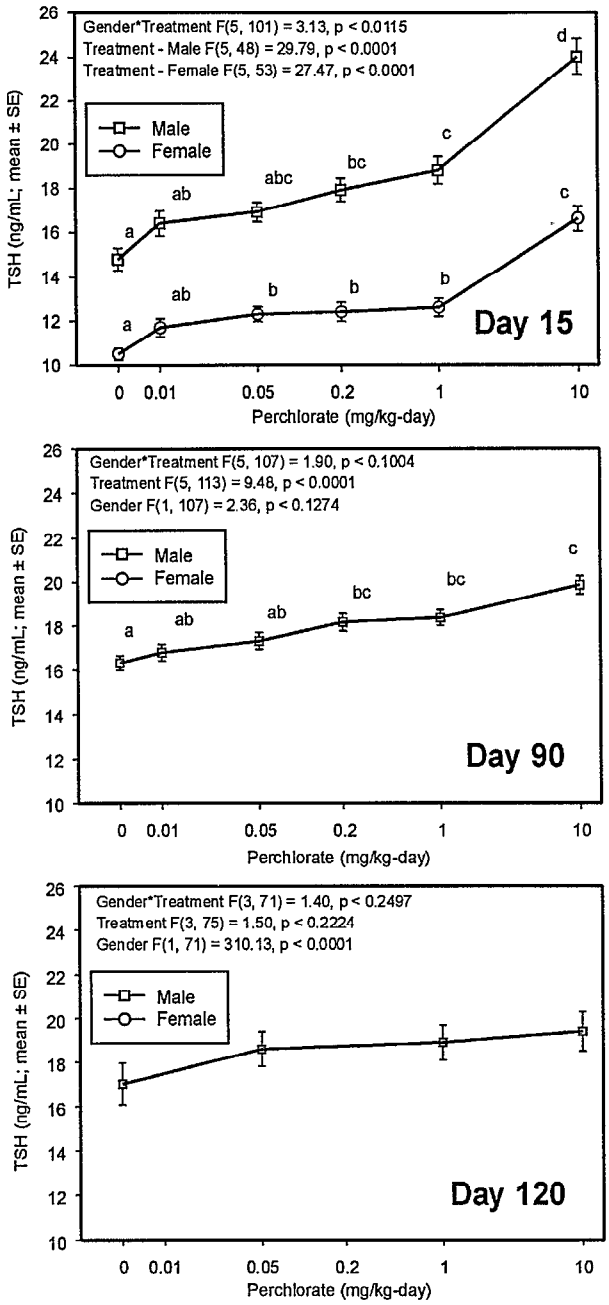


Figure 5-9. Effects from 90-day drinking water administration to ammonium perchlorate to SD rats on serum total TSH. Data of Springborn Laboratories, Inc. (1998). There was a significant day*gender*treatment interaction ($F[8,279] = 2.83$, $p < 0.0049$) and a main gender*treatment interaction for Day 1, 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 ($p < 0.05$, Tukey's Studentized Range (HSD) Test after significant main effect). The 120-day time point is 30 days after cessation of exposure.

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 postweaning period, and at sacrifice. The same set of signs as examined during exposure were evaluated on a weekly basis during postweaning. Thyroids from all F0-generation rats were weighed and evaluated histologically. Five dams per group were selected for sacrifice and blood collection on 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 5.2.3.2). 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 postweaning. Body weight was recorded on PNDs 1, 5, 8, 12, 14, 18, and 22 and then weekly during postweaning. Feed consumption values were recorded weekly during postweaning. 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. Postweaning 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 PND 28, and male pups were evaluated for the age of preputial separation beginning on PND 39. 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.

5.2.3.1 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 5.2.3.2 and 5.2.3.3.

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 5.2.3.4.

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 not be significant, but 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) for this review 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 for the controls and 3- and 10-mg/kg-day groups, respectively. Incorporation of historical control data from both PND10 and 12 (mean for controls = 264 for PNDs 10 and 265 for PND12; York, 1998a) supports the conclusion that the control values for corpus callosum size in the (York 1998a; see also Argus Research Laboratories, Inc., 1998a) data set are within the "normal" range. The EPA does not agree with the argument put forth in Argus Research Laboratories, Inc. (1998a) that these effects are "not suggestive of a neurotoxic effect" because of "an unknown biological significance." The EPA considers a 27% increase in the size of any brain region to be a potentially adverse effect (U.S. Environmental Protection Agency, 1998b). Therefore, the LOAEL is 10 mg/kg-day, and the NOAEL is 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) has been 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.

5.2.3.2 Evaluation of Thyroid Histology

Appendix O of the Argus Research Laboratories, Inc. (1998a) neurodevelopmental study presents thyroid histology data provided by the sponsor (AFRL/HEST). Additional morphometric data were provided by Dr. William H. Baker, AFRL/HEST, Wright-Patterson AFB, in Microsoft Excel® spreadsheets and subsequently formally transmitted to EPA by consultative letter (Channel, 1998b). Note that the data analyzed by EPA for PND5 F1 rats (pups) are from the final report for the PND5 time point (Channel, 1998b), which were first provided on September 25, 1998, but had to be revised by AFRL/HEST because of errors in treatment codes. The EPA reanalysis using additional statistical methods of the PND90 time point data in the F1-generation rats is pending until the external peer review because EPA did not receive these revised data until the October 27, 1998, consultative letter (Channel, 1998b), and assessment concern already was focused on data analyses for the pups at PND5. Channel (1998b) reports 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-WRST). These data suggest a recovery from the effects observed in the thyroids of the pups at PND5.

The histology data in Appendix O of the Argus Research Laboratories, Inc. (1998a) report contain measurements performed by Dr. William Baker of both follicular epithelial cell height and the follicular lumen diameter. For the final morphometric study (Channel, 1998b), an 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, 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. Also, as described in Section 5.2.1, the standard histopathology submitted for the 14-day "Caldwell Study" (Channel, 1998a) identifies a LOAEL for follicular epithelial cell hypertrophy at 0.11/0.12 mg/kg-day in males/females, whereas the LOAEL for lumen area was higher at 2.26/3.06 mg/kg-day in males/females (Channel, 1998a). This corroborates that the lumen area measurements may be underestimating the effects of perchlorate in the PND5 F1 animals. This conclusion also is supported by the histopathology seen in the developmental study of rabbits (Section 5.2.4) in which an effect on the follicle height was a more frequently noted parameter of hypertrophy than were decreases in lumen size. A difference between standard histology and morphometry to discern a decrease in follicular lumen size also is noted. The NOAEL and LOAEL for decrease in follicular lumen area using standard histopathology were 0.44/0.47 and 1.11/1.23 mg/kg-day in males/females of the Channel (1998a) submission, whereas the NOAEL and LOAEL identified by morphometry were 1.11/1.23 and 2.26/3.06 mg/kg-day for males/females.

The disparity between standard histopathology and morphometry is illustrated again in the data of Appendix O of the Argus Research Laboratories, Inc. (1998a) report for the thyroid histology in the pups on PND5. Table 5-3 presents the combined incidence and data and averaged severity scores for male and female rat pups for follicular cell hypertrophy and decrease in follicular lumen size. Scoring of follicular size ranged from 0 (normal) to 3. A similar assessment was made of follicular epithelial cells noting their general height. Scores for this measure ranged from 0 (normal) to 2, noting a mild increase in follicular epithelial height. The plots of the data are found in Appendix O (Argus Research Laboratories, Inc., 1998a; Tables 1 and 2, Figures 1 and 2). Dr. William Baker (AFRL/HEST) provided Excel® spreadsheets of the individual animal data and severity rating so that EPA could perform contingency table analyses that allowed severity and incidence to be considered together (Marcus, 1998). When data on both sexes were combined, the lowest dose, 0.1 mg/kg-day was significant at 0.012 (df = 8) for the follicular cell hypertrophy and for the lumen size at 0.008 (df = 12). Exact tests also indicated that this dose was significantly different than that of the controls. The data presented in the Argus Research Laboratories, Inc. (1998a) report did not combine the male and female data,

TABLE 5-3. COMBINED INCIDENCE DATA AND AVERAGE SEVERITY SCORES FOR MALE AND FEMALE PND5 RAT PUPS FOR FOLLICULAR EPITHELIAL CELL HYPERTROPHY AND DECREASE IN FOLLICULAR LUMEN SIZE BASED ON STANDARD HISTOLOGY

		Perchlorate (mg/kg-day)				
Measure		Control	0.1	1.0	3.0	10.0
Cell hypertrophy	Incidence[a]	3/12	8/12	9/12	8/12	12/12
	Severity[b]	0.33	0.84	1.08	0.83	1.42
Lumen size	Incidence	6/12	10/12	10/12	11/12	12/12
	Severity	0.66	1.17	1.25	1.75	2.16

[a]Number of animals observed with hypertrophy over total.
[b]Mean of scores for combined male and female data.

reported statistical significance only for the males at 10 and 3 mg/kg-day, and did not provide a reason for discounting the significance at 0.1 (but not at 1.0). The combining of the data gave better power to evaluate the effect, and the contingency analysis allowed severity to be factored directly. Therefore, based on the preceding discussion of relative sensitivity between follicular epithelial cell hypertrophy and desrease in lumen size, as well as on this analysis of differences between standard histology and morphometry, EPA designated 0.1 mg/kg-day a LOAEL based on standard histopathology changes in follicular epithelial cell hypertrophy and decrease in follicular lumen size observed in the PND5 pups. The EPA also decided to rely on the standard histology for correlating thyroid hormone concentrations to thyroid histopathology, as assessed by changes in follicular epithelial cell hypertrophy (see Section 6.1.1).

The morphometry data presented for these same measures in Appendix O (Argus Research Laboratories, Inc., 1998a) show statistical significance only for males at the 10-mg/kg-day group. An analysis of the combined morphometry data for the sexes was not performed.

There was also some evidence of thyroid hypertrophy at 3 and 10 mg/kg only in the PND10 group. This conclusion is based on increased incidence of minimally and moderately ranked thyroid (Argus Research Laboratories, Inc., 1998a; Appendix N, Table N3). The increase in total incidence ratio is shown in Table 5-4.

TABLE 5-4. RATIO OF ALL RAT PUPS WITH ANY EVIDENCE OF FOLLICULAR EPITHELIAL CELL HYPERTROPHY OF THE THYROID GLAND TO TOTAL NUMBER OF RAT PUPS EXAMINED BASED ON STANDARD HISTOLOGY

	Ratio				
Perchlorate (mg/kg-day)	Control	0.10	1.00	3.00	10.00
All	0.60	0.56	0.76	0.72	0.68
PND22[a]	0.52	0.48	0.68	0.52	0.48
PND10[b]	0.40	0.40	0.40	1.00	1.00

[a]Includes animals terminated on PND22 (dosing stopped on PND10).
[b]Includes only a limited number of animals terminated on PND10.

Data from the dependent measure (follicle lumen size), based on the morphometric analyses (Channel, 1998b), were subjected to three-way ANOVA tests, with gender (male and female), treatment (dose), and block (two separate analyses of separate blocks of data) as independent between-subjects variables (Crofton, 1998e). Step-down ANOVA tests were conducted as indicated by significant interactions. Mean contrasts were performed using Tukey's Studentized Range (HSD) Test. There was a significant main effect of treatment for the lumen size data for the 3- and 10-mg/kg-day groups compared to controls. The data are plotted in Figure 5-10.

Results of these EPA reanalyses are similar to those stated in the report (Argus Research Laboratories, Inc., 1998a), except that there were no gender-related effects detected. There was a significant decrease in the follicular lumen size measurement on PND5 in both the 3- and 10-mg/kg-day groups. Therefore, the NOAEL for thyroid histopathology, based on the morphometric assessments, is 1.0 mg/kg-day. These results are consistent with the known mechanism-of-action of perchlorate (i.e., competitive inhibition of iodine uptake and subsequent decreased synthesis and release of thyroid hormone). The resulting increase in TSH will result in increased utilization of stored thyroid hormones and, thereby, effect a decrease in the follicle lumen size.

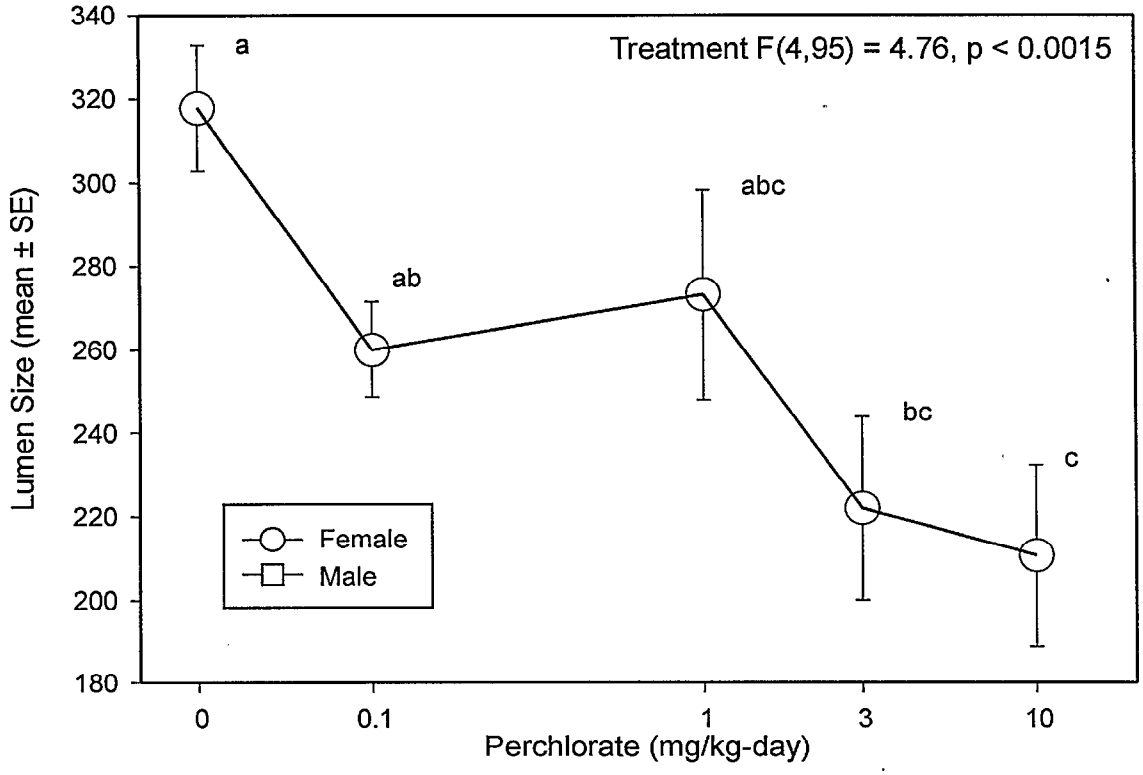


Figure 5-10. Effects from maternal drinking water administration of ammonium perchlorate to SD rats on thyroid gland follicular lumen size in F1-generation offspring on PND5. Data of Channel (1998b) and Argus Research Laboratories, Inc. (1998a). Means with different letters were significantly different ($p < 0.05$). Daily dose was estimated from water consumption data.

5.2.3.3 Thyroid and Pituitary Hormone Analyses

Serum was collected and thyroid hormone analyses performed as part of the neurodevelopmental study (Argus Research Laboratories, Inc., 1998a). The following is a statistical analysis of the thyroid and pituitary hormone data (T4, T3, and TSH) found in that report (Crofton, 1998f). 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 PP10. Only the F1 data were reanalyzed because of the very limited (n = 2 to 5/group) data for the parental F0.PP10 group.

The individual animal data analyzed herein were not submitted formally to EPA at the time of this assessment. 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 as the independent, between-subjects variable. Mean contrasts were performed using Tukey's Studentized Range (HSD) Test. To correct for multiple comparisons (i.e., separate analyses for T4 and TSH), the acceptable alpha for significance (for all interaction main effects tests) was corrected to 0.028 (alpha of 0.05 divided by the square root of the number of ANOVA tests).

There were significant main effects of treatment for all the hormones. The data are plotted in Figures 5-11 through 5-13. 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 as the expected increase in TSH. The NOAEL for the effects of perchlorate on T3, T4, and TSH are 0.1, 1.0, and 3.0 mg/kg-day, respectively. The difference between LOAELs for T3 and T4 likely are not biologically plausible. Both T3 and T4 were reduced approximately 10% at the 1.0 mg/kg-day dose; statistical significance was found with the T3 data because of a slightly lower variability at this dose. 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.

5.2.3.4 Behavioral Evaluations

The EPA review of the behavioral evaluations performed on Subset 3 pups agrees with the Argus Research Laboratories, Inc. (1998a) report with the exception of 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 disagrees with the Argus Research Laboratories, Inc. (1998a) report and subsequent submissions (York, 1998a,b,c,d,e) in regard to the significance of the motor activity changes.

The data originally were analyzed by 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

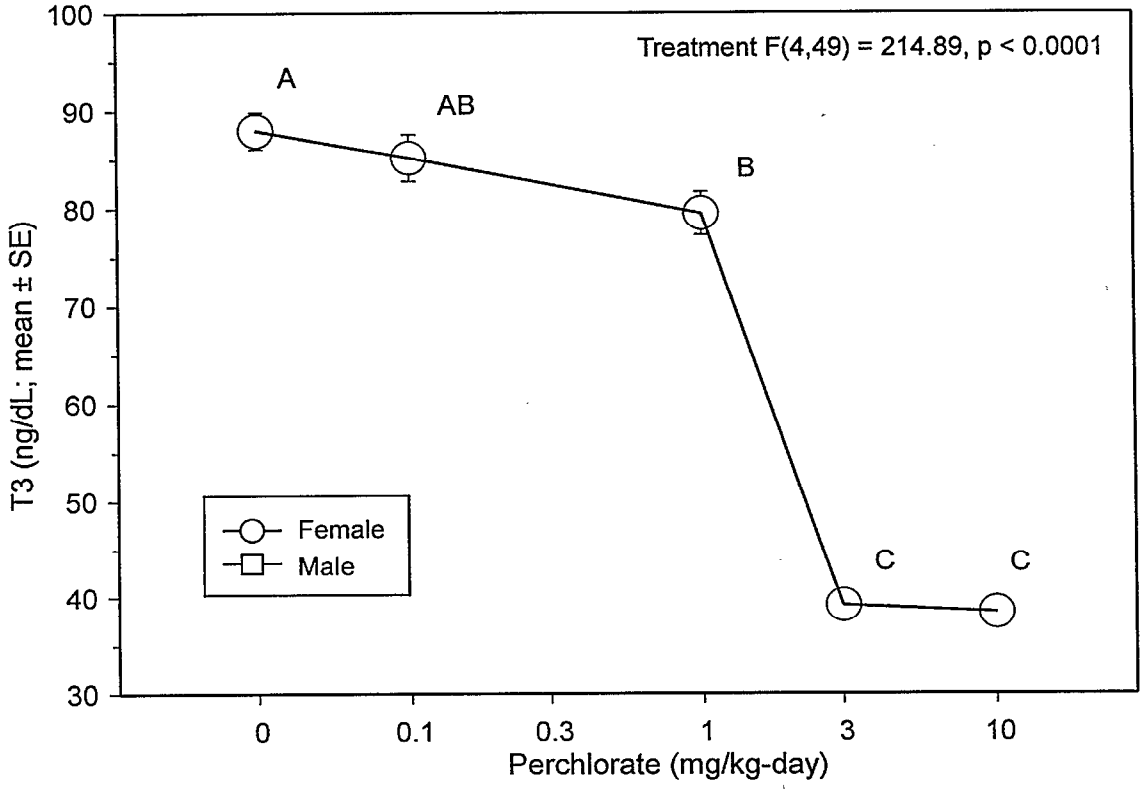


Figure 5-11. Effects from maternal drinking water administration of ammonium perchlorate to SD rats on serum total T3 concentrations in F1-generation offspring (pups) on PND5. Data of Argus Research Laboratories, Inc. (1998a). Means with different letters were significantly different ($p < 0.05$). Daily dose was estimated from water consumption data.

sponsor (PSG) that an additional analysis of the data be conducted using gender as a within-subject variable, or alternatively, using 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 any significance to the dose-dependent increase in total session counts that amounted to a 95% increase over controls in the highest dosage group (see Figure 5-14). 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. The EPA notes that the subsequent

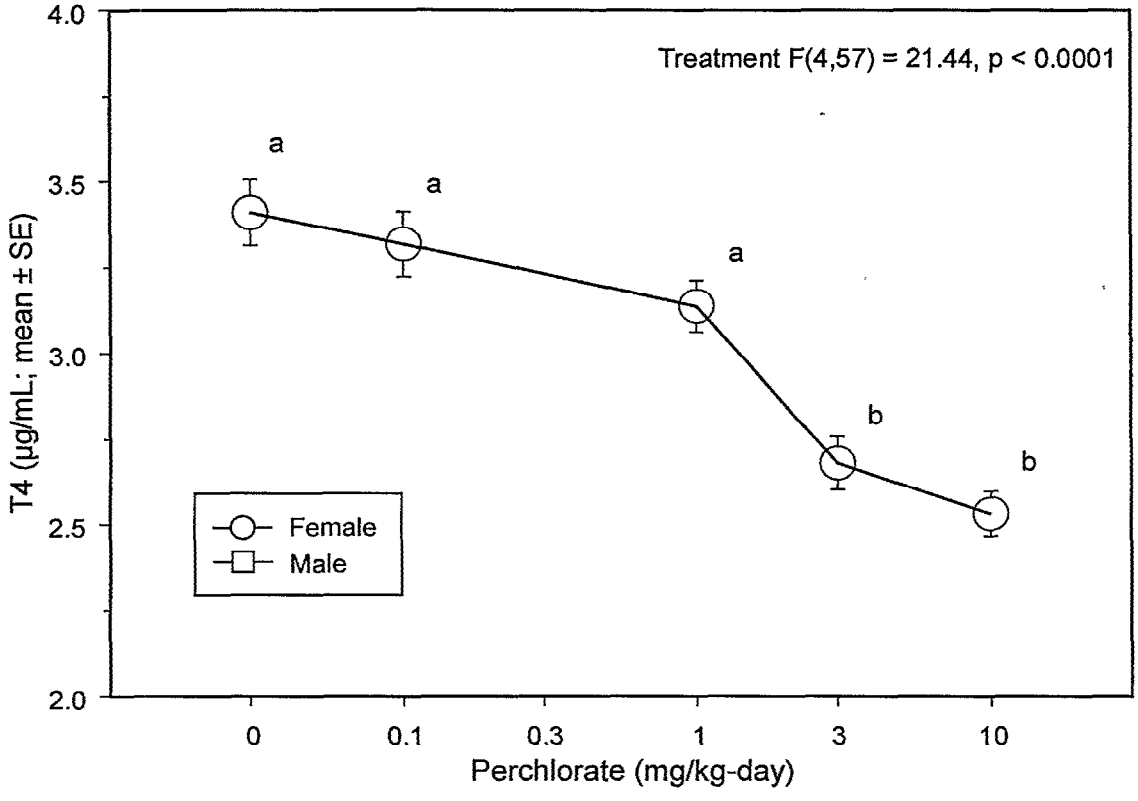


Figure 5-12. Effects from maternal administration of ammonium perchlorate to SD rats on serum total T4 concentrations in F1-generation offspring (pups) on PND5. Data of Argus Research Laboratories, Inc. (1998a). Means with different letters were significantly different ($p < 0.05$). Daily dose was estimated from water consumption data.

secondary analysis submitted is still not what EPA requested. It was requested that gender be nested under litter, rather than used as a between-subject variable.

Regardless of the adequacy of the analyses, Argus Laboratory and the sponsor (PSG) have 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 5-14 illustrates the clear dose-dependent increase in two different measurements of motor activity: (1) time-spent-in-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

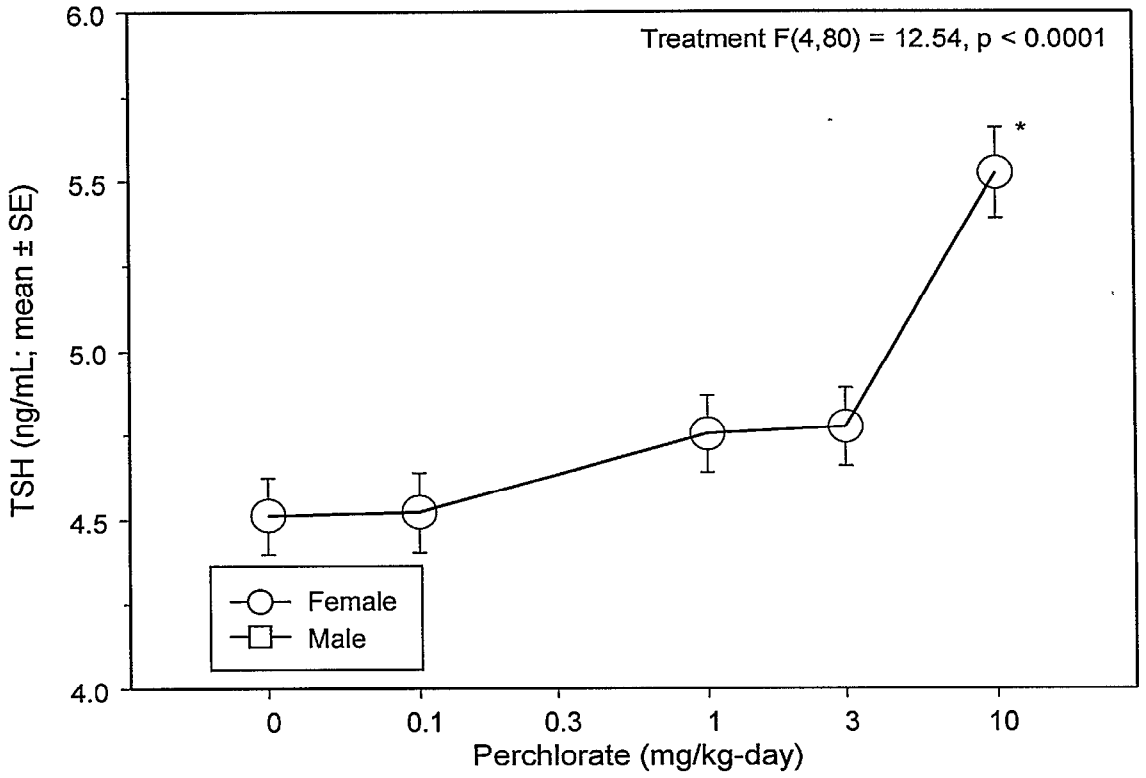


Figure 5-13. Effects from maternal drinking water administration of ammonium perchlorate to SD rats on serum TSH concentrations in F1-generation offspring (pups) on PND5. Data of Argus Research Laboratories, Inc. (1998a). *= Significantly different from control group, $p < 0.05$.

controls. Expert opinion of EPA neurotoxicologists was sought, and it is their opinion that increases in motor activity over 50%, especially in developing animals, are clearly of concern from a biological perspective (Crofton et. al., 1998). The critical issue for evaluation of these motor activity data is how to resolve the difference between what is a clearly biologically significant alteration in behavior with the 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.

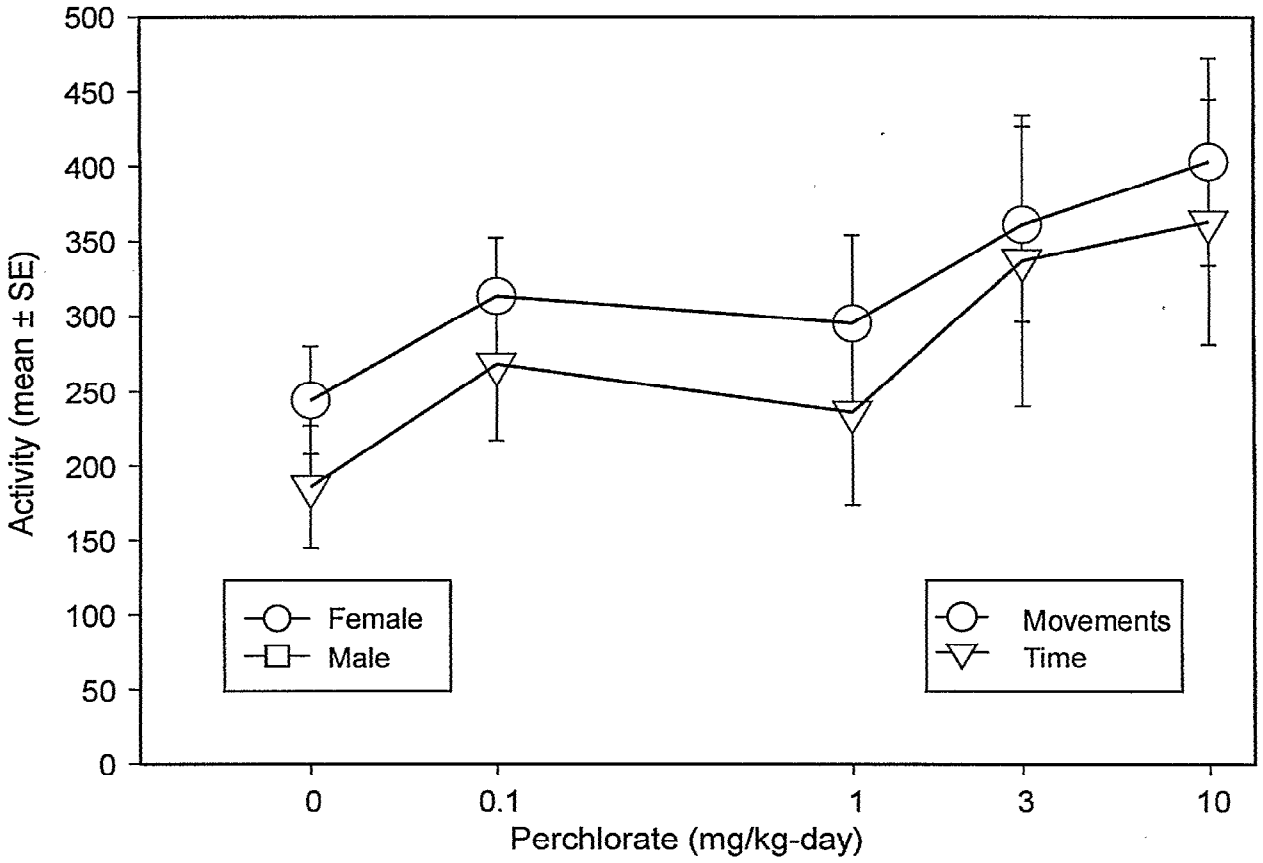


Figure 5-14. The effects of developmental exposure to perchlorate on motor activity in male rats on PND14. Data of Argus Research Laboratories, Inc. (1998a). The dose-dependent increases in both number of movements and time spent in movement were not statistically different, even though the increases were substantial at the higher dosages.

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 (≥32%) in activity. However, ability to detect decreases does not necessarily translate to increases.

The historical control data from PND14 rats was 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 has very little control over the behavior, or the data are from a different test apparatus or from a different usage of the same apparatus. In any case, the data are of no use in helping EPA to determine the historical profile of control animals behavior in this test apparatus.

In lieu of the absence of useful positive control and historical control data, EPA is still left with the issue of statistical versus biological significance. There are a number of reasons for the lack of statistical significance. The first reason is 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 is 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 is 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) will tend to wash out 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 is 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 would not be found again if this experiment was repeated.

The conclusion of a biological significance to the effect seen in this report is supported by both the underlying mode of action of perchlorate and the effects of other chemical and physical

insults on the motor activity of postnatal 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 exactly 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. 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 maintains that the increase in activity should be considered biologically significant until additional data can be marshaled to suggest or prove otherwise. The inadequacy of standard parametric statistics to detect a significant difference suggests 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) (see Section 6.1.2).

5.2.4 Two-Generation Reproductive Toxicity Study in Rats

At the time of this review, the report available on the two-generation (one litter per generation) study in Sprague-Dawley rats (Argus Research Laboratories, Inc., 1998b) was limited to the parental (P1) generation data and did not include tissue histopathology, thyroid hormone, or sperm morphology results. Data were not reported for the F1 generation beyond weaning. Therefore, these interim report data should not be construed as providing a complete evaluation of the effects of ammonium perchlorate on the rat reproductive system at this time. Additional results from this study will be presented at the external peer review. 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.

Generally, the study quality appears to acceptable. 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 on 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 to be prepared at least once, but the exact number was not reported. Stability of solutions was assumed based on determinations by AFRL/HEST for the 90-day bioassay, as discussed in Section 5.2.1.

On arrival, rats were assigned randomly to individual housing, and then consecutive order was used to assign the P1 generation rats to cohabitation (one male rat per female rat). Cohabitation period consisted of 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 and 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, postpartum) 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 postpartum 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 postpartum period, all surviving P1 rats were sacrificed. Gross necropsy was performed on all animals, and all gross lesions will be 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 will be 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.

5.2.4.1 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., 1998a; Tables B5 and B6). 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 occurred at 30 mg in both sexes (Argus Research Laboratories, Inc., 1998a; Tables B11 through B13 and C26 through C28). There was a significant increase in ovarian weight at the 0.3-mg/kg-day dose level only (Argus Research Laboratories, Inc., 1998a; 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., 1998a; 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 observation of persistent

diestrus and one with an observation of persistent estrus (Argus Research Laboratories, Inc., 1998a; 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 0.3mg/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 those females that were pregnant, litter size was slightly lower at 3.0- and 30-mg/kg-day dose levels, with the values being 15.0, 14.9, 14.1, and 14.0 with increasing dose level, with a similar trend seen in numbers of implantation sites (15.8, 15.8, 15.0, and 15.0). Although none of these results were statistically significant for the P1 generation, close attention should be given to these parameters in evaluating the results from the F1 generation, particularly when the results on estrous cycling are considered. Note should be made that female intake of perchlorate during the last week of gestation was higher (Argus Research Laboratories, Inc., 1998a; Table C1). Also, in many of the perchlorate intake and feed consumption summary data, observations were reported for low numbers of rats, apparently because of spillage. There were no significant differences or trends in the sperm data available at this time.

To summarize the results reported to date, a potential adverse effect was seen in thyroid weight, with a LOAEL for males of 3 mg/kg-day. For females, the LOAEL for the same effect is 30 mg/kg-day. This organ weight effect needs to be supported by the histopathology or hormone data that are not yet available for this study, but the effects appear to be consistent with the other studies in the testing strategy. There are hints of effects in the 0.3-mg/kg-day dose group in the mating, fertility, estrous cycle, and ovarian and pituitary weights, but the effects did not show a progressive dose-response. If similar observations are made with the F1 generation or in other studies involving endocrine-related effects, consideration should be given to the possibility of an inverted U dose-response behavior, as was suggested by the reproductive estrous cycling parameter results from the 90-day bioassay (Springborn Laboratories, Inc., 1998) in Section 5.2.1. These results may warrant a modification to the methods by which estrous cycle data are evaluated and the presentation of the sperm evaluation information for the final report. Argus laboratory has agreed to provide the sperm morphology and estrous cyclicity data to EPA in mid-January 1999, so that they may be analyzed and presented at the external peer review.

5.2.4.2 Evaluation of Thyroid Histology

Some thyroid histology data from this study may be available for analysis by EPA in time for presentation at the external peer review meeting.

5.2.4.3 Thyroid and Pituitary Hormone Analyses

Unaudited hormone data may be available to EPA in time for analysis for the external peer review meeting.

5.2.5 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 testing strategy (Argus Research Laboratories, Inc., 1998c). A schematic of the study design is provided in Figure A-3 of Appendix A to this document to aid understanding of terminology and the protocol. The study design meets the requirements of the 1998 EPA Office of Pollution Prevention and Toxic Substances 870.3700 guideline. A deviation from the use of double staining was noted in Appendix D of the Argus report, but EPA has determined that this should not have 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 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 at gross external examination. The 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 low number of animals (n = 5) used in a range-finding study, caused EPA to counsel the sponsor (PSG) to perform 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 go below those 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 5.2.1. 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.

5.2.5.1 Results of Maternal Examinations

Two does in the 1.0-mg/kg-day group aborted either dead pups or 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 incorrectly identified and shipped by the supplier because the pups appeared to be full-term (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, but none of these responses showed a dose-response with the current treatment regimen, and none were out of the range of normal occurrence. There was an apparent dose-related decrease in thyroid weight (not statistically significant). The only remarkable histopathology in the does was

observed in the thyroids. Hypertrophy of the follicular epithelium that consisted of an increased height or enlargement of the epithelium, was observed in 0/0, 0/0, 0/0, 7/25, 13/25, and 16/25 of the 0, 0.1, 1.0, 10, 30, and 100 mg/kg-day dose groups, respectively. The severity of the lesion was also dose dependent; ranging from 3 with minimal hypertrophy at the 10-mg/kg-day level to 10 with minimal, 2 with mild, and 4 with moderate at the 100-mg/kg-day level. The hypertrophy occasionally resulted in a decrease in the lumen of the follicles, which contained pale and occasionally vacuolated colloid. The maternal NOAEL and LOAEL, based on the thyroid histopathology, are designated at 1.0 mg/kg-day and 10.0 mg/kg-day, respectively.

5.2.5.2 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 reason for the statistically significant decrease in folded retina was attributed to be 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, "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.

5.2.5.3 Maternal Thyroid and Pituitary Hormone Analyses

The thyroid and pituitary hormone (T3, T4, and TSH) analyses were performed by AniLytics, Inc., for the does of 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, and, therefore, conducted reanalyses for these two groups separately (Crofton, 1998h). All data were taken from Appendix I of the report (Argus Research Laboratories, Inc.,1998c), and the analyses also 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. Mean contrasts were performed using Tukey's Studentized Range (HSD) Test. To correct for multiple comparisons (i.e., three separate ANOVA tests) the acceptable alpha for significance (for all interaction main effects tests) was corrected to 0.0289 (alpha of 0.05 divided by the square root of the number of dependent variables). Individual analyses for each hormone are discussed below.

The main effect of treatment was not significant for T3. The T3 data are plotted in Figure 5-15. 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 plotted in Figure 5-16. The main effect of treatment was not significant for TSH (Figure 5-17). 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 (Crofton, 1998h), excluding nonpregnant animals, demonstrates a NOAEL at 0.1 mg/kg-day for T4. There were statistically significant decreases in T4 demonstrated for the 1.0-, 10-, 30-, and 100-mg/kg-day groups. 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 hard to explain. One must note that these data are from rabbits (the majority of other data are from rats); the data were collected 1 day prior to birth (all other data were collected in adults or from postnatal day time points) and were collected from the maternal compartment. 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.

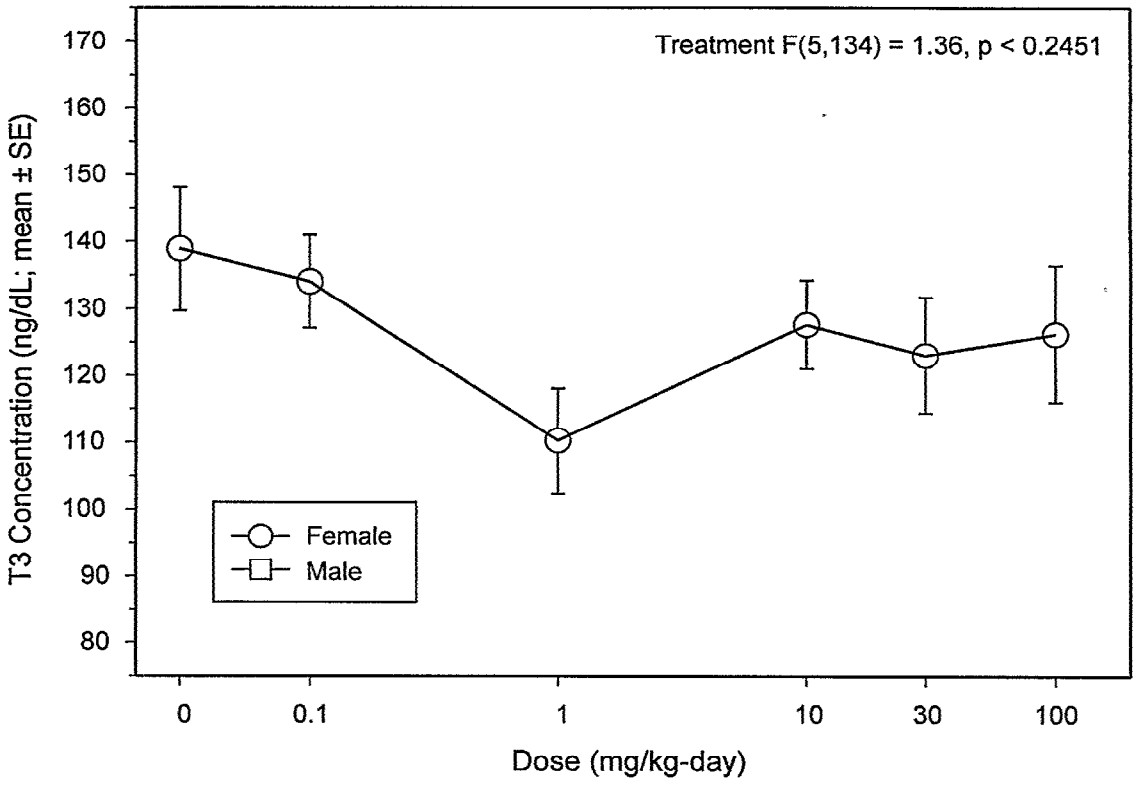


Figure 5-15. Lack of effects from ammonium perchlorate drinking water administration in pregnant New Zealand rabbits during GD6 to GD28 on T3. Data of Argus Research Laboratories, Inc. (1998c). Samples were obtained on GD28. There was no significant effect of treatment. Daily dose was estimated from water consumption data.

These data warrant caution when comparing effects of perchlorate in the maternal with the fetal/postnatal compartments.

5.2.6 Immunotoxicity Study in Mice

An array of 14- and 90-day experiments to evaluate the effects of drinking water administration of ammonium perchlorate on immunotoxicological and hematological parameters was performed using female B6C3F1 mice (Keil et al., 1998). Parameters also were evaluated 30 days after one 90-day study to assess the reversibility on any observed effect. The mouse is the typical experimental species for immunotoxicological studies. In addition, data were

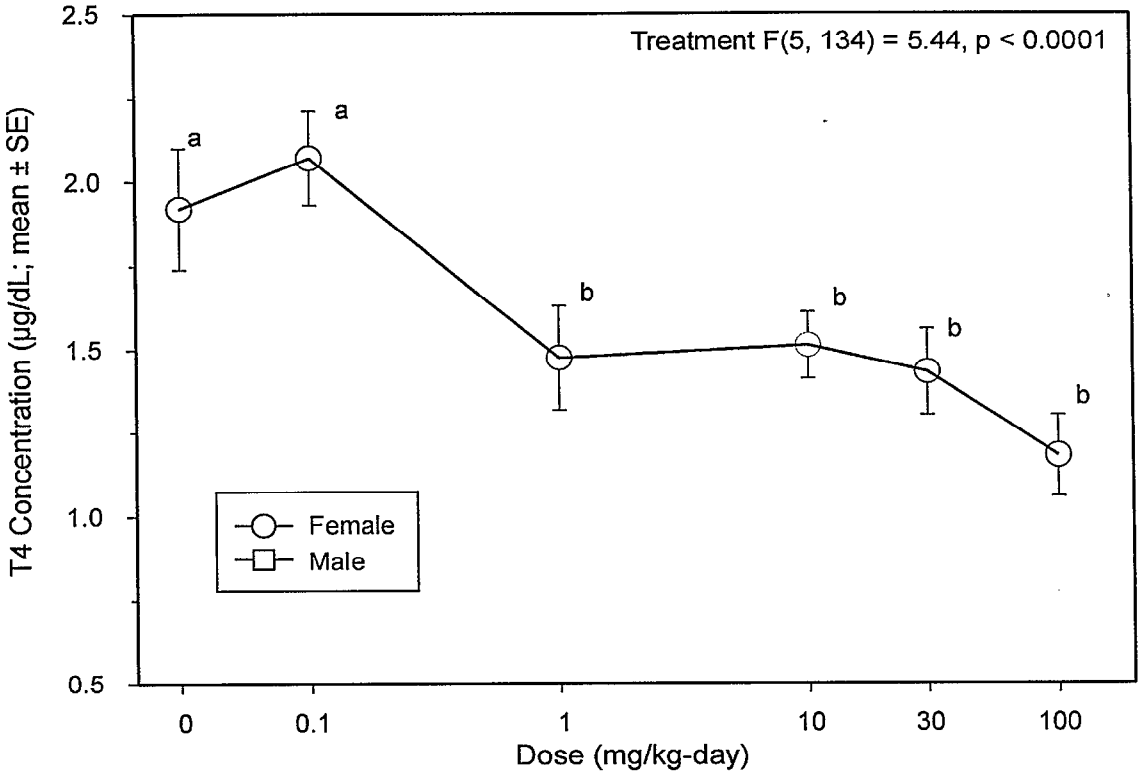


Figure 5-16. Effects from ammonium perchlorate drinking water administration in pregnant New Zealand rabbits during GD6 to GD28 on T4. Data of Argus Research Laboratories, Inc. (1998c). Samples were obtained on GD28. There was a main effect of treatment. Means with different letters were significantly different ($p < 0.05$, Tukey's Studentized Range (HSD) Test after significant main effect). Daily dose was estimated from water consumption data.

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 B6C3F1 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 the same lot was used for all studies. Primary stock solution was prepared approximately every 1 to 2 mo, and dosing solutions were prepared weekly. 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

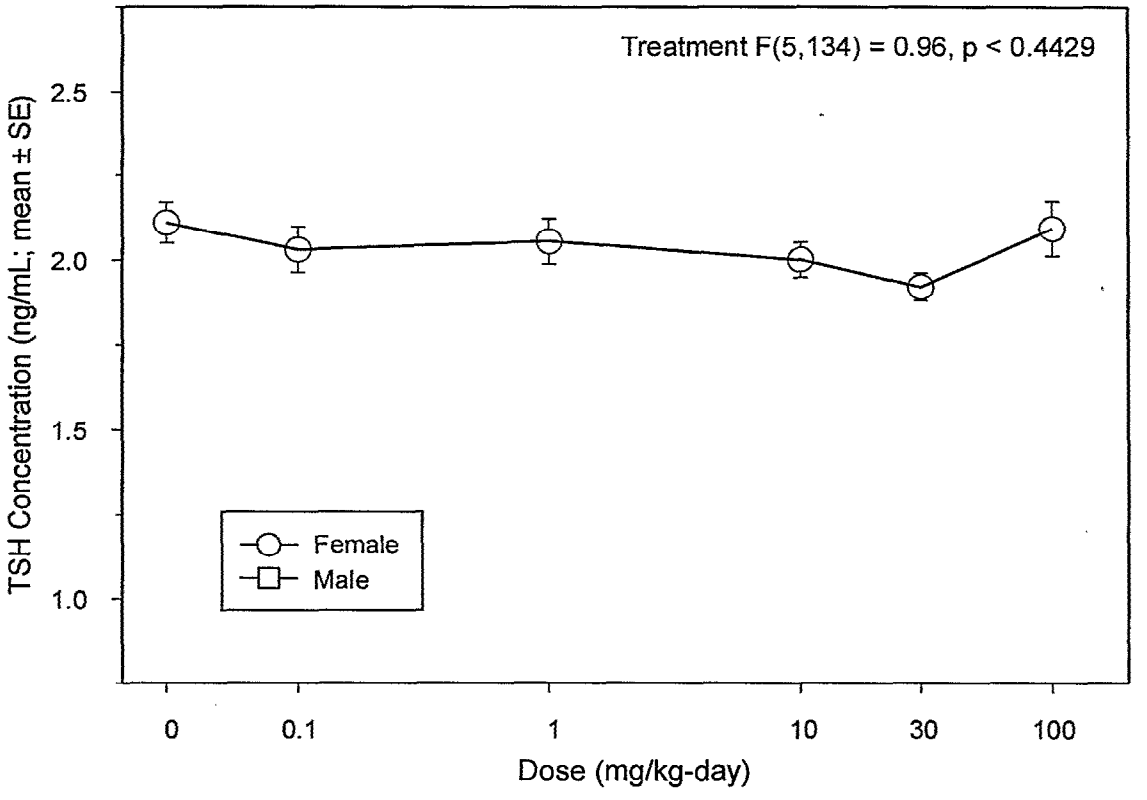


Figure 5-17. Lack of effects from ammonium perchlorate drinking water administration in pregnant New Zealand rabbits during GD6 to GD28 on TSH. Data of Argus Research Laboratories, Inc. (1998b). Samples were obtained on GD28. There was no significant effect of treatment. Daily dose was estimated from water consumption data.

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). The mice were exposed to levels of 0, 0.1, 1.0, 3.0, or 30 mg/kg-day. The doses were established based on the mean body weight for each treatment group per week. Each dose group consisted of 6 mice for a total of 30 per experiment.

A number of 14-day experiments were conducted. In Experiments "C", "G", "I", "J", "T", and "K", 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. In Experiments "U" and "V", 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. In experiments "H", "F", and "M", mice were challenged with P815 tumor cells by ip injection. At the 14-day terminal sacrifice, the spleens were removed for the cytotoxic T lymphocyte (CTL) activity assay.

A series of 90-day experiments also were conducted. In Experiments "A", "D", and "N", 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. In Experiments "B" and "E", these same endpoints were measured after a 30-day recovery period. In Experiment "P", mice were challenged with P815 tumor cells by ip injection on Day 76. Spleens were removed at terminal sacrifice for the CTL activity assay.

Two 90-day studies using host-resistance models also were conducted. Mice in Experiment "L" were challenged with *Listeria monocytogenes* by iv injection. At terminal (90-day) sacrifice, the spleens and livers were removed and cultured for *Listeria monocytogenes* growth. In Experiments "Q" and "O", mice were challenged with B16F10 tumor cells by intravenous injection on Day 76. At the 90-day sacrifice, the lungs were removed, and the number of tumor nodules in both lungs were enumerated.

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. Results for the general toxicity and organ weight measures will be discussed in Section 5.2.6.1. Thyroid histopathology evaluations will be reported in Section 5.2.6.2, and analyses of T3, T4, and TSH in Section 5.2.6.3. Results for the immunotoxicological and hematological parameters are discussed in Sections 5.2.6.4 and 5.2.6.5. A summary of the results and the potential significance of the parameters yet to be measured in experiments in progress will be presented in Section 5.2.6.6.

5.2.6.1 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. There was no consistent alteration in splenic cellularity observed in the 14–, 90-, or 120-day studies, nor in splenocyte CD4/CD8 subsets. A decrease in splenic cellularity was observed in the 14-day experiment "J" and the 90-day Experiment "N" at the 3- and 30-mg/kg-day ammonium perchlorate doses. Increased splenic cellularity was observed in the 14-day Experiment "C" at 1- and 3-mg/kg-day doses. The decreases or increases in total spleen cells, at random doses, in Experiments "J", "N", and "C" may have been caused by technical error, because, in each of these three experiments, the spleen weights or spleen-weight-to-body-weight ratios were not different from the controls, and the changes in cellularity were not dose dependent. Furthermore, splenic cellularity was not affected in two other 14-day studies ("G" and "I") or in two other 90-day studies ("A" and "D"). An increase in splenic cellularity is indicated for mice exposed to 30 mg/kg-day in the figure for the 120-day Experiment "B"; however, the statistics for these data were not found in the report (Keil et al., 1998). Similarly, no statistics were found for Experiment "E", another 120-day study presented as a figure with no effects indicated.

No consistent alteration in thymus total cellularity was observed in 14- and 90-day studies; no 120-day study data are presented. Thymus cellularity, however, was affected in the 14-day Experiment "C" at 3 mg/kg-day. Because this reduction occurred in the absence of a decrease in thymus weights, these results suggest that technical errors may have played a role in the development of these data. No consistent alteration in thymocyte subsets was observed in 14- and 90-day studies; no 120-day study data are presented. The CD4+CD8+ thymocytes were increased in mice exposed to 0.1-, 1.0-, and 30-mg/kg-day doses in the 14-day Experiment "C", whereas CD4+CD8- thymocytes were decreased at the 0.1-mg/kg-day dose. However, in another 14-day study ("G"), no change in thymocyte subpopulations was observed. An increase in the percentage of CD8+CD4+ thymocytes and a decrease in CD4+CD8- thymocytes were observed in mice exposed to 30 mg/kg-day in the 90-day Experiment "D"; however, in two other 90-day studies ("A" and "N"), no changes in thymocyte subpopulations were observed.

No consistent alteration in peritoneal macrophage cellularity was observed in 14-, 90-, and 120-day studies. A decrease in cellularity was noted in the 3-mg/kg-day group in the 90-day study ("D"), whereas an increase in cellularity, at this same dosage, was observed in the 90-day

study ("A"). In a repeat 90-day study ("N"), no changes in peritoneal macrophage cellularity were observed in any dosage group compared with the control group. No effects were observed on bone marrow cellularity in 14- and 90-day experiments. Because of the absence of effects in these studies, no 120-day study was performed.

5.2.6.2 Evaluation of Thyroid Histology

The EPA expects to receive thyroid histology data for these mice by January 15 and will present results of the analyses thereof at the external peer review meeting.

5.2.6.3 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 (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). Data for dependent measures (T4 and TSH) were subjected to separate analyses. The T4 data were analyzed with a two-way ANOVA, with duration (14, 90, and 120 days) and treatment (dose) as the independent between-subjects variables. The TSH data were analyzed with a two-way ANOVA with duration (90 and 120 days) and treatment (dose) as the independent between-subjects variables. Mean contrasts were performed using Tukey's Studentized Range (HSD) Test. To correct for multiple comparisons (i.e., separate analyses for T4 and TSH), the acceptable alpha for significance (for all interaction main effects tests) was corrected to 0.035 (alpha of 0.05 divided by the square root of the number of dependent variables).

There was a significant duration-by-treatment interaction on T4 and significant main effect of treatment for the 14- and 90-day data. There was no main effect of treatment for the 120-day data. The T4 data are plotted in Figure 5-18. There was no significant interaction of duration and treatment, nor was there a main effect of treatment on TSH. Data are plotted in Figure 5-19.

Results of these EPA reanalyses are different from those stated in the (Keil et al., 1998) report. The EPA reanalysis of the data indicates a statistically significant time- and dosage-dependent decrease in T4 following perchlorate exposure; after 14 days of exposure at

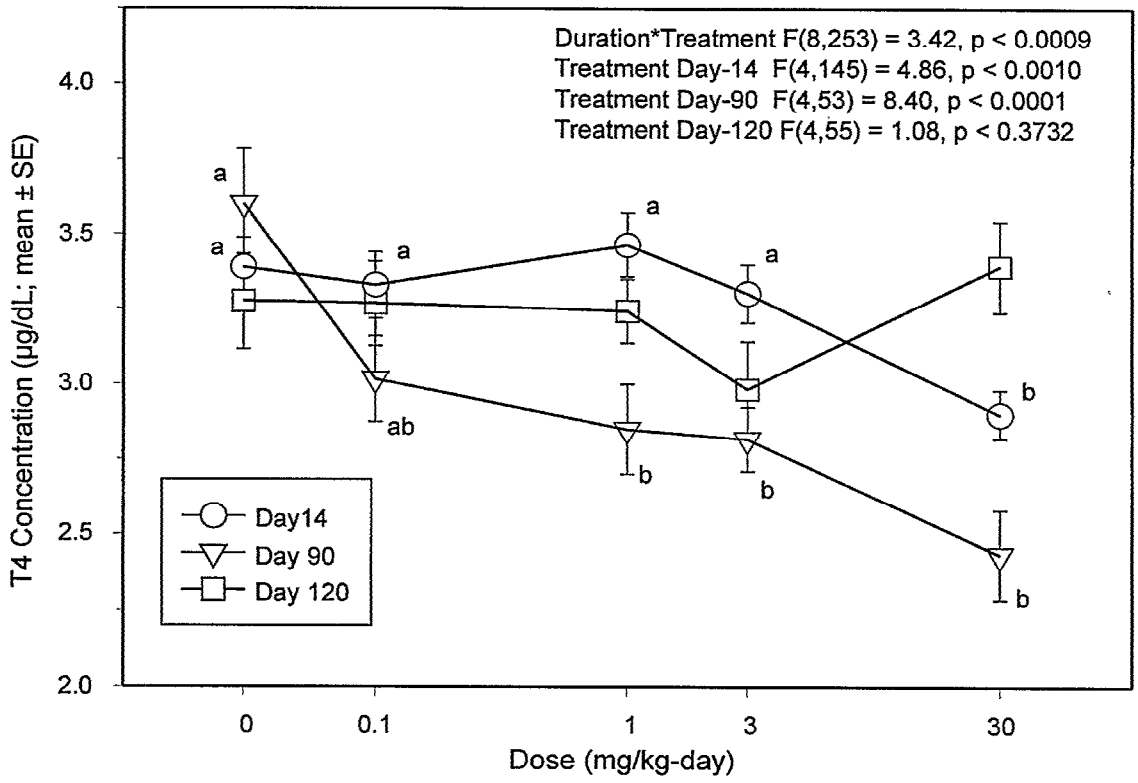


Figure 5-18. Effects from drinking water administration of ammonium perchlorate in mice on total serum T4. Data of Keil et al. (1998). Mice were exposed for 90 days. Samples were obtained on Days 14 and 90 and 30 days after cessation of exposure (Day 120). There was a significant interaction of duration and treatment, and main effects of treatment on Days 14 and 90. Means with different letters were significantly different ($p < 0.05$). Daily dose was estimated from water consumption data.

30 mg/kg-day and after 90 days of exposure at 1, 3, and 30 mg/kg-day. The decrease in T4 recovered to control values 30 days after cessation of exposure (Day 120). The NOAEL for the effects of perchlorate on T4 in the mouse was 0.1 mg/kg-day. There was no statistical significance of any dose of perchlorate on TSH.

5.2.6.4 Results of Immune Function Assays

No consistent alteration in CTL activity was observed in three 14-day studies ("M", "H", and "F"). No effects were observed on CTL activity in Experiments "M" and "H". However,

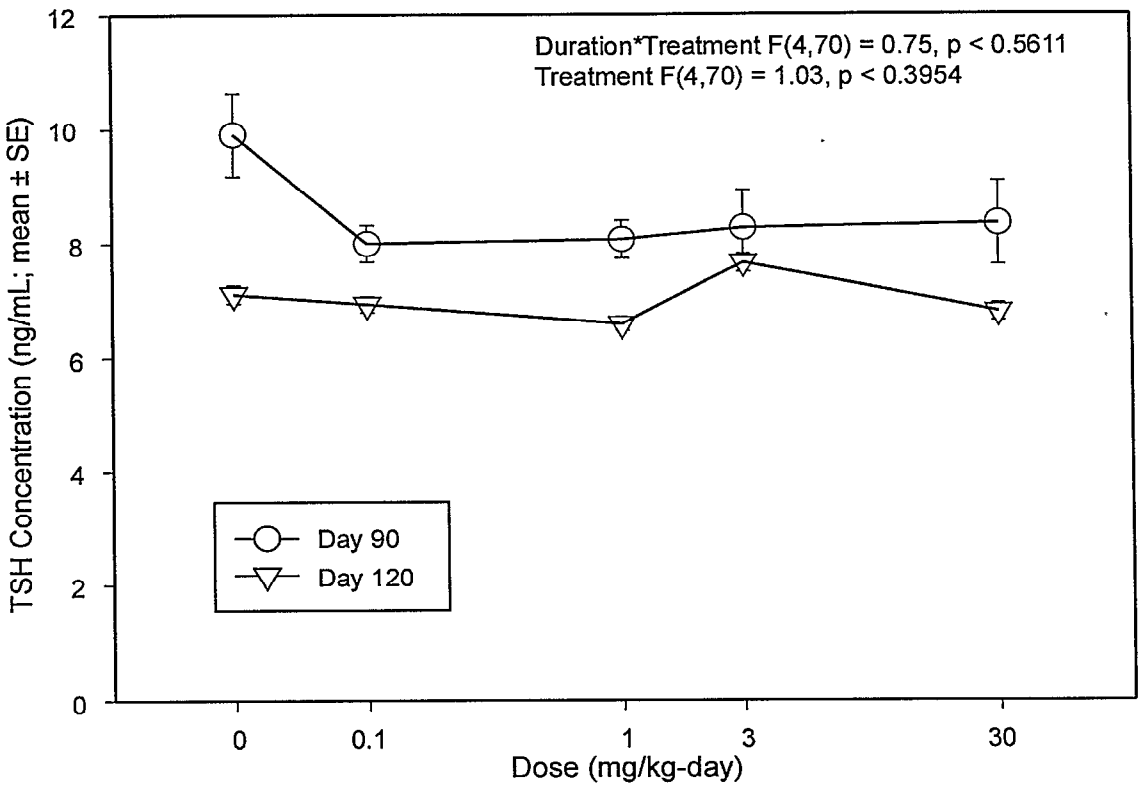


Figure 5-19. Effects from drinking water administration of ammonium perchlorate exposure in mice on total serum TSH. Data of Keil et al. (1998). Mice were exposed for 90 days. Samples were obtained at the end of exposure (Day 90) and 30 days after cessation of exposure on Day 120. There was no significant interaction of duration and treatment, nor was there a main effect of treatment. Daily dose was estimated from water consumption data.

in Experiment "F", increases in CTL activity were observed at the 0.1-mg/kg-day ammonium perchlorate dose for 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") there were no alterations in CTL activity at any dosages or E:T ratios. There was also no consistent alteration in the DTH response, as measured by the lymphoproliferation (LP) of splenocytes from *L. monocytogenes*-challenged mice incubated with soluble *Listeria* antigen (SLA), in two 14-day studies ("U" and "V"). The LP response was increased only in cultured splenocytes from mice in the 30-mg/kg-day group stimulated with 0.1 μg/mL SLA in Experiment "U" and in splenocyte cultures from mice in the

3-mg/kg-day group stimulated with 8 μg/mL SLA in Experiment "V". The "Results Summary and Status" page (Keil et al., 1998) indicates that a 90-day DTH study is planned.

No alteration in splenic natural killer (NK) cell activity was observed in two 14-day studies ("G" and "T"). 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") in which NK cell activity was increased at the 30-mg/kg-day ammonium perchlorate in Experiment "N", but 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". The lack of any change in the number of B16F10 tumor nodules in the lungs of mice from the 90-day "Q" study, 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 splenocytes, respectively.

Decreased phagocytosis was observed at 3 and 30 mg/kg-day of ammonium perchlorate in the 14-day "C" and 90-day "A" studies. In the 90-day "N" study, macrophage phagocytosis was decreased in all dosage 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. These data suggest that ammonium perchlorate suppresses the phagocytic capacity of peritoneal macrophages, but that this suppression may be reversed after a 30-day recovery period.
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 dosages 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. Also, increased nitrite production from macrophages cultured with IFN was observed at 3 mg/kg-day in the 90-day "N" study. 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. These data suggest a "trend" toward increased nitrite production at the higher dosages of ammonium perchlorate.

A single study (90-day Experiment "L") has been 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. Also, it was not possible to enumerate the number of CFU/g spleen in mice because of inadequate dilution of spleen suspensions. Additional assays are currently in progress (see Keil et al., 1998; "Results Summary and Status" page). No effects were observed in the single 90-day B6F10 tumor challenge host-resistance model (Experiment "Q"). Another experiment ("O") is in progress.

5.2.6.5 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 cell count and differential counts. 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") 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"). No other reticulocyte data are available because of "the minimal availability of blood obtained from each mouse" in other studies; however, a 14-day study is planned (see Keil et al., 1998; "Results Summary and Status" page).

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"). However, there was no effect of ammonium perchlorate exposure on the stem cell assay in a 90-day study ("D"). Repeat 14- and 90-day studies on the stem cell assay are in progress (see Keil et al., 1998; "Results Summary and Status" page).

5.2.6.6 Results Summary with a Discussion of Parameters of Experiments in Progress

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, EPA noted that humoral immunity (i.e., B cells and antibody response) was not, although suggested by an EPA reviewer on protocol review. The EPA suggested strongly that the antibody response to sheep red blood cell response (SRBC) 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 sponsor and investigators, Keil et al. (1998), have agreed to perform this assay, and the results will be available at the end of January 1999. The EPA will evaluate the results and present them at the external peer review meeting. Unfortunately, results of the two host-resistance models will not be available until June 1999. These data would provide a more comprehensive evaluation of the potential for immunosuppression. No official response for an EPA request to perform a sensitization test has been received at this date. Finally, the thyroid histology and thyroid and pituitary hormone data will provide additional insights on interspecies variability for this effect.

To date, the most "consistent" and potentially significant immune function parameter effected is the suppression of peritoneal macrophage phagocytosis of *L. monocytogenes*. Decreased phagocytosis of this bacterium was observed at 3- and 30-mg/kg-day doses of ammonium perchlorate in a 14-day study ("C") and in a 90-day study ("A"); whereas, in the 90-day "N" study, the phagocytosis was decreased in all dosage groups. However, in the 14-day study ("G") and 90-day study ("D"), no alteration in macrophage phagocytic activity was observed. In the two 120-day (30-day recovery) "B" and "E" studies, no effect on macrophage phagocytosis was observed. Taken together, these data suggest that ammonium perchloarte suppresses the 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, a "trend" toward increased resistance to *L. monocytogenes* infection was suggested by the only infectivity data available at this time (i.e., 90-day Experiment "L"). These

data, however, are suspect, given that technical difficulties were encountered. These included considerable variability in the number of *L. monocytogenes* CFU/g liver recovered from control mice, as well as the inability to enumerate the number of CFU/g spleen in mice because of inadequate dilution of spleen suspensions. Additional assays are in progress (see Keil et al., 1998; "Results Summary and Status" page), and perhaps these experiments will decrease the variability, and a consistent pattern will emerge. The EPA questioned why the phagocytic index (i.e., number of bacteria per macrophage) was not performed to better assess the phagocytic capacity of macrophages. This is routinely done with the percentage of macrophages that phagocytized bacteria as well. The EPA has requested that these indices be measured in the remaining experiments.

Other trends were suggested in various experiments (e.g., macrophage nitrite production, bone marrow stem cell assay), but the variability and issue of possible technical difficulties between studies or lack of statistical analyses precluded definitive conclusions. Some of the repeat assays planned may provide a more coherent picture. At this point, the 90-day Experiment "N" identifies a LOAEL for an effect on macrophage phagocytosis at 0.1 mg/kg-day, whereas two others (the 14- and 90-day "C" and "A" studies) identify the NOAEL at 1.0 mg/kg-day and the LOAEL at 3.0 mg/kg-day, and a concern for potential immunotoxicity remains to be resolved with more definitive testing.

5.3 GENOTOXICITY ASSAYS

ManTech Environmental Technology, Inc., 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 on its possible carcinogenicity (ManTech Environmental Technology, Inc., 1998).

Ammonium Perchlorate was evaluated in the Ames assay (*Salmonella typhimurium*/ microsome mutagenesis assay), which is a well-defined assay for detection of carcinogens/ mutagens. It measures the reversion from a his$^-$ (histidine independent) state 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., performed this assay in Swiss-CD-1 mice. The assay also was performed as part of the 90-day bioassay in Spraque-Dawley rats (Springborn Laboratories, Inc., 1998). This is 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.

5.3.1 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. The 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, using the plate incorporation assay. The EPA requested that the assay be repeated by the National Toxicology Program (NTP). The NTP evaluated ammonium perchlorate in the Salmonella/Ames assay in tester strains TA98, TA100, TA1535, TA97, TA102, and TA104 (Zeiger, 1998a). 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. It should be noted that the additional tester strains used by NTP, TA102 and TA104, are able to detect a variety of oxidative mutagens.

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 are not of sufficient quality to support a negative-response conclusion. The sponsor (PSG) has agreed to repeat the mouse lymphoma assay, and the results are expected to be reported at the external peer review meeting.

5.3.2 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 FIFRA/TSCA testing guidelines. No increase in the frequency of micronuclei were found for any dose group. The negative findings reported in this study are considered equivocal because it is uncertain 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 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 remains 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). 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. A preliminary communication of the results of this study (Zeiger, 1998b) indicates that 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 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. These results appear to be consistent with those of the ManTech Environmental Technology, Inc. (1998) study that used gavage drinking water administration, and suggest that perchlorate does not induce micronuclei. The final NTP report results will be available for the external review meeting, and a preliminary EPA analysis will be provided.

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, although 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).

5.3.3 Summary of Genotoxicity Battery Results

Negative results were reported in all genotoxicity assays conducted on ammonium perchlorate. Ammonium perchlorate was not mutagenic in the Ames assay (with or without S9 activation) when evaluated by two independent laboratories. Ammonium perchlorate was negative in the mouse lymphoma assay without S9 activation. Although the findings were equivocal with S9 activation in the mouse lymphoma assay, perchlorate is not expected to be metabolized to a mutagenic metabolite. It should be noted that the mouse lymphoma assay with S9 activation will be repeated to provide an adequate study. Results are expected to be provided by the sponsor (PSG) in late January 1999. The negative findings from the rat and mouse micronuclei assays are also equivocal because it appears that the highest doses tested did not produce some indication of overt toxicity, although the Spraque-Dawley rats from the 90-day study at the highest dose had both thyroid hormone perturbations and follicular cell hyperplasia. Although ammonium perchlorate is not likely to be a direct DNA reactive mutagen, and the in vitro studies discussed above provide support for that conclusion, EPA also has requested that the NTP repeat the rodent micronucleus test to address the limitations of the in vivo genotoxicity testing. The final report for these data also will be available at the external peer review meeting, but preliminary results confirm that perchlorate is negative in the micronuclei assay.

5.4 ABSORPTION, DISTRIBUTION, METABOLISM, AND ELIMINATION AND MECHANISTIC STUDIES

As discussed in Chapter 4, limited data exist on the disposition kinetics (absorption, distribution, metabolism, and elimination) of perchlorate, particularly at steady-state exposures, or on a comprehensive characterization of its mechanism of action in the thyroid. A number of

studies are underway as part of the mode-of-action testing strategy in both rats and humans that will help to characterize these deficiencies and preliminary, results are described herein.

A recently completed single-dose intravenous study in Sprague-Dawley rats with perchlorate to characterize its inhibition of iodide uptake supports that there is inhibition at low concentrations, with 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 mixed 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 will begin in January 1999. 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 h.

Figure 5-20 shows the inhibition of ^{125}I uptake by perchlorate into the thyroid as 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 h 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. Table 5-5 provides the percent of inhibition of ^{125}I uptake as measured by bound ^{125}I in the thyroid.

Recovery of ^{125}I in urine 24 h 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-h period. 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 with the control ^{125}I-dosed rats for up to 6 h in all dose groups, suggesting that thyroid function was altered by perchlorate, and a

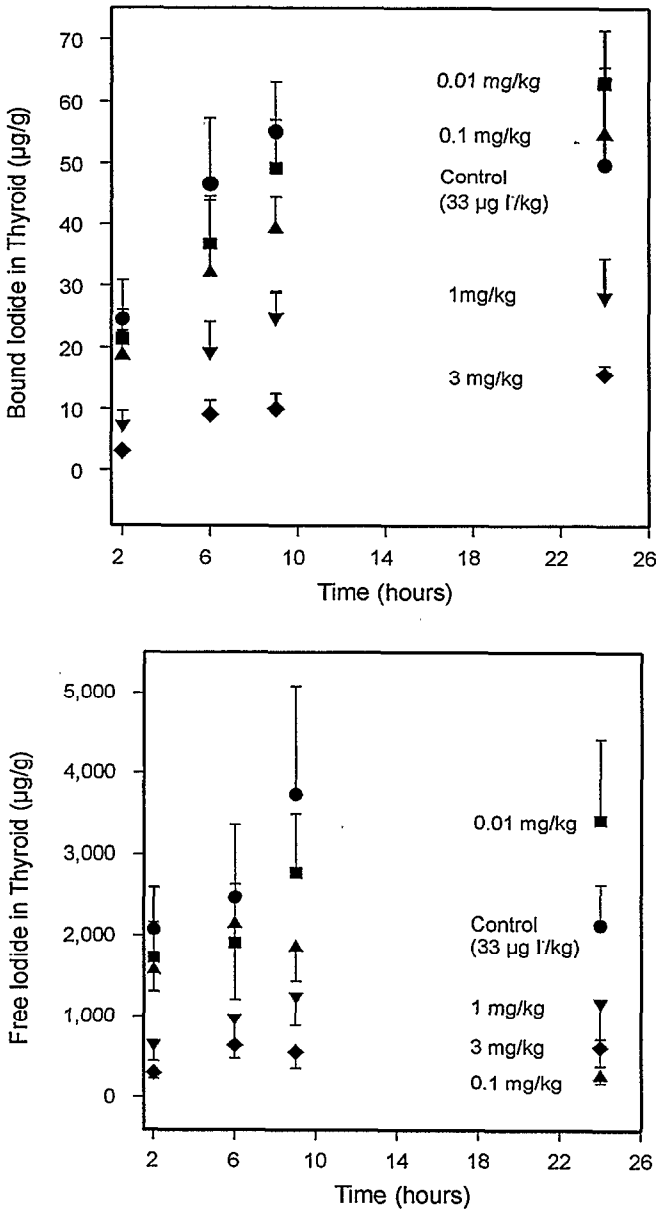


Figure 5-20. Inhibition by perchlorate of iodide uptake in the thyroid of rats given a single iv injection. Data of Meyer (1998). Dose is expressed as milligrams perchlorate per kilogram body weight.

transient "discharge" of organified ^{125}I occurred, as previously 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) discussed in Section 3.4.2.

TABLE 5-5. PERCENT INHIBITION OF IODIDE UPTAKE IN THE THYROID GLAND OF SD RATS DOSED WITH PERCHLORATE (Data of Meyer, 1998)

Time Points[a]	Dose (mg perchlorate/kg)	[Iodide] (μg/g)	Percentage of Inhibition
2 h	Control[b]	24.4	—
	0.01	21.3	13
	0.1	18.6	24
	1	7.4	70
	3	2.99	88
6 h	Control[b]	46.5	—
	0.01	36.7	21
	0.1	32.0	31
	1	19.2	59
	3	9.13	80
9 h	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 h after dosing with ammonium perchlorate.
[b]Dosed with only iodide (33 μg/kg).

Repeated dose studies also are planned in rats (Fisher, 1998a) and in humans (Channel, 1998c) to establish the kinetics of perchlorate at steady-state and to further characterize the inhibition of iodide uptake by perchlorate. These data will be used to develop a physiologically based pharmacokinetic (PBPK) model to describe the ADME of perchlorate and aid in interspecies extrapolation. These data also thereby likely will allow a better estimate of internal dose that may help to refine dose-response relationships (i.e., provide a different dose metric for use in dose-response analyses other than administered dose). 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. Obtaining data on the species differences (i.e., rat versus human in particular) in perchlorate inhibition of the symporter will provide a basis for evaluation of the degree of uncertainty that should be applied when utilizing laboratory animal data as the model (see Chapter 6).

6. DOSE-RESPONSE ASSESSMENTS FOR HUMAN HEALTH

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 in that the thyroid remained a principal target tissue. However, the data from the testing strategy allowed a more comprehensive evaluation of the possible sequelae of the thyroid-pituitary axis perturbations with respect to other endpoints, notably effects in the offspring of exposed dams and on reproductive and immunotoxicity parameters.

6.1 ORAL REFERENCE DOSE

This section will review the results of all the data from the testing strategy, together with other data that provide robust dose-response information, to ascertain the critical effect for perchlorate. The *critical effect* is defined as the effect that is adverse that first appears in the dose scale as it is increased or is a known precursor to the first adverse effect. The premise of this designation is that, if the critical effect is prevented, then all subsequent adverse effects at higher doses are prevented. The principal study is that study that best characterizes the dose response for the critical effect.

In this case for perchlorate, an overall model based on its mode of action is developed, both to support the hormone and thyroid histology data as precursor lesions to more severe effects and the harmonization of "noncancer" and "cancer" approaches. Choice of the point of departure for the assessment based on the critical effect then will be discussed in Section 6.1.2. Application of factors to account for uncertainty and variability in the extrapolations required to use the data will be discussed in Section 6.1.3, and the assignment of confidence levels will be discussed in Section 6.1.4. The overall operational derivation then is presented in Section 6.1.5.

Section 6.2 discusses the inhalation reference concentration. Section 6.3 presents a discussion of the cancer assessment in the context of the RfD. Susceptible population considerations are discussed in Section 6.1.1.3.

6.1.1 Choice of Critical Effect and Principal Study

Because no other target tissue for systemic toxicity was observed, the overwhelming weight of the evidence from these studies support the use of the hormone and thyroid histology evidence as the choice for critical effects. Although there are some concerns regarding potential immunotoxicity and reproductive parameters that will have to wait to be resolved until the final data are submitted, the results of the testing strategy predominantly indicate that these effects would likely be at higher concentrations than those at which perturbations in thyroid hormone economy is occurring. There was some question of a reproductive effect in rats occurring at the 0.3 mg/kg dose, where thyroid histopathology also was observed, and, in the immunotoxicity data, there was an indication of an effect on macrophage function at 3 mg/kg-day in mice. The likely NOAEL for effects on motor activity appears to be at 3 mg/kg-day. Brain weight changes also appear to have a NOAEL at 3 mg/kg-day.

The effects on thyroid-pituitary hormones were clearly the most sensitive, with no NOAEL identified in some studies as low as 0.01 mg/kg-day (Table 6-1A through 6-1C). The changes in T3 and T4 typically seemed to precede changes in TSH, but there were overlaps. As anticipated, however, as revealed by the light-shaded cells (NOAELs) versus dark-shaded cells (LOAELs), a pattern emerges of thyroid hormone perturbations preceding increases in TSH, with histopathology in the thyroid ensuing at the same dose or slightly above. Standard histopathology identified thyroid follicular cell hyperplasia as a LOAEL both in the rats of the 14-day Caldwell et al. (1995) study and in rat pups of the neurodevelopmental toxicity study on PND5 at 0.1 mg/kg-day for both follicular epithelial cell hyperplasia and decreases in follicular lumen size (Table 6-1D).

Table 6-2 summarizes these data together with those studies performed in rats preceding those two performed in other species, the rabbit developmental study and the immunotoxicity studies in mice. Although the rat was more sensitive, there did not appear to be a very significant interspecies effect on thyroid hormones between rabbits and rats, although the sample size was small for the thyroid and pituitary hormone analyses in the rabbit developmental study.

TABLE 6-1A. SUMMARY OF TRIIODOTHYRONINE (T3) THYROID HORMONE EFFECTS[a]

(Light-shaded cells designate NOAELs; dark cells LOAELs; ✓= dose tested)

Study/Species	Dose/ Duration Age Tested	Sex	Doses (mg/kg/day)												
			0	0.01	0.05	0.1	0.2	0.3	0.44	1.0	3.0	5.0	10.0	30.0	100
Caldwell 14-day Rat (Caldwell et al., 1995)	14 days	M&F	✓			F M	rT3 F, M		✓	✓	✓	✓	✓[2]	✓[3]	
Subchronic Rat (Springborn Laboratories, Inc., 1998)	14 days	M&F	✓	M	M		M			M			M		
	90 days		✓	✓	✓		✓			✓			✓		
	120 days		✓		✓					✓			✓		
Developmental Neurotoxicity Rat (Argus Research Laboratories, Inc., 1998a)	FO: PP10	F	✓			✓				✓	✓		✓		
	F1: PND5	M&F	✓			✓				✓	✓		✓		
	F1:PND90		ND			ND				ND	ND		ND		
Two-Generation Reproductive Rat (Argus Research Laboratories, Inc., 1998b)	F0		TBD					TBD			TBD			TBD	
	F1		TBD					TBD			TBD			TBD	
	F2		TBD					TBD			TBD			TBD	
Seg II Developmental Rabbit (Argus Research Laboratories, Inc., 1998c)	F0: 29 GD	F	✓			✓				✓			✓	✓	✓
Immunotoxicity Mouse (Keil et al., 1998)	14 days	F	TBD			TBD				TBD	TBD			TBD	
	90 days	F	TBD			TBD				TBD	TBD			TBD	
	120 days	F	ND			ND				ND	ND			ND	

[a]M = male; F = female; T3 = triiodothyronine; rT3 = reverse T3; F0: PP10 = parental generation, Postpartum Day 10; F1: PND5 = first generation, Postnatal Day 5; F1: PND90 = first generation, Postnatal Day 90; ND = Not Done; TBD = to be determined; F0: 29 GD = parental generation, 29th gestational day.

[1]4.32 and 4.91 mg/kg-day in males and females, respectively.
[2]11.44 and 11.47 mg/kg-day in males and females, respectively.
[3]22.16 and 24.86 mg/kg-day in males and females, respectively.

TABLE 6-1B. SUMMARY OF THYROXINE (T4) THYROID HORMONE EFFECTS[a]

(Light-shaded cells designate NOAELs; dark cells LOAELs; ✓= dose tested)

Study/Species	Dose/ Duration Age Tested	Sex	Doses (mg/kg/day)												
			0	0.01	0.05	0.1	0.2	0.3	0.44	1.0	3.0	5.0	10.0	30.0	100
Caldwell 14-day Rat (Caldwell et al., 1995)	14 days	M&F	✓			✓			✓	✓		✓	✓	✓	
						hTg									
Subchronic Rat (Springborn Laboratories, Inc., 1998)	14 days	M&F	✓	✓	✓		✓			✓			✓		
	90 days		✓	✓	✓		✓			✓			✓		
	120 days		✓		✓					✓			✓		
Developmental Neurotoxicity Rat (Argus Research Laboratories, Inc., 1998a)	FO: PP10	F	✓			T4				✓	✓		✓		
	F1: PND5	M&F	✓			✓				✓	✓		✓		
	F1:PND90		ND			ND				ND	ND		ND		
Two-Generation Reproductive Rat (Argus Research Laboratories, Inc., 1998b)	F0		TBD					TBD			TBD			TBD	TBD
	F1		TBD					TBD			TBD			TBD	TBD
	F2		TBD					TBD			TBD			TBD	TBD
Seg II Developmental Rabbit (Argus Research Laboratories, Inc., 1998c)	F0: 29 GD	F	✓			✓									
Immunotoxicity Mouse (Keil et al., 1998)	14 days	F	✓			✓				✓	✓			✓	
	90 days	F	✓			✓				✓	✓			✓	
	120 days		✓			✓				✓	✓			✓	

[a]M = male; F = female; F0: PP10 = parental generation, Postpartum Day 10; F1: PND5 = first generation, Postnatal Day 5; F1: PND90 = first generation, Postnatal Day 90; ND = Not done; TBD = to be determined; F0: 29 GD = parental generation, 29th gestational day.

[1]4.32 and 4.91 mg/kg-day in males and females, respectively.
[2]11.44 and 11.47 mg/kg-day in males and females, respectively.
[3]22.16 and 24.86 mg/kg-day in males and females, respectively.

TABLE 6-1C. SUMMARY OF THYROID STIMULATING HORMONE (TSH) PITUITARY HORMONE EFFECTS[a]

(Light-shaded cells designate NOAELs; dark cells LOAELs; ✓= dose tested)

Study/Species	Dose/ Duration Age Tested	Sex	Doses (mg/kg/day)												
			0	0.01	0.05	0.1	0.2	0.3	0.44	1.0	3.0	5.0	10.0	30.0	100
Caldwell 14-day Rat (Caldwell et al., 1995)	14 days	M&F	✓			✓		F M	F M	F M	✓	✓	✓	✓	
Subchronic Rat (Springborn Laboratories, Inc., 1998)	14 days	M&F	✓	F	F M		✓			✓			✓		
	90 days		✓	✓	✓		✓			✓			✓		
	120 days		✓		✓					✓			✓		
Developmental Neurotoxicity Rat (Argus Research Laboratories, Inc., 1998a)	FO: PP10	F	✓			✓				✓	✓		✓		
	F1: PND5	M&F	✓			✓				✓	✓		✓		
	F1: PND90		ND			ND				ND	ND		ND		
Two-Generation Reproductive Rat (Argus Research Laboratories, Inc., 1998b)	F0		TBD					TBD			TBD			TBD	
	F1		TBD					TBD			TBD			TBD	
	F2		TBD					TBD			TBD			TBD	
Seg II Developmental Rabbit (Argus Research Laboratories, Inc., 1998c)	F0: 29 GD	F	✓			✓				✓			✓	✓	✓
Immunotoxicity Mouse (Keil et al., 1998)	14 days	F	✓			✓				✓	✓			✓	
	90 days	F	✓			✓				✓	✓			✓	
	120 days	F	✓			✓				✓	✓			✓	

[a]M = male; F = female; TSH = thyroid stimulating hormone (thyrotropin); F0: PP10 = parental generation, Postpartum Day 10; F1: PND5 = first generation, Postnatal Day 5; F1: PND90 = first generation, Postnatal Day 90; ND = Not done; TBD = to be determined; F0: 29 GD = parental generation, 29th gestational day.

[1]4.32 and 4.91 mg/kg-day in males and females, respectively.
[2]11.44 and 11.47 mg/kg-day in males and females, respectively.
[3]22.16 and 24.86 mg/kg-day in males and females, respectively.

TABLE 6-1D. SUMMARY OF THYROID HISTOPATHOLOGY EFFECTS[a]

(Light-shaded cells designate NOAELs; dark cells LOAELs; ✓= dose tested)

Study/Species	Dose/ Duration Age Tested	Sex	Doses (mg/kg/day)												
			0	0.01	0.05	0.1	0.2	0.3	0.44	1.0	3.0	5.0	10.0	30.0	100
Caldwell 14-day Rat (Caldwell et al., 1995)	14 days	M&F	✓			SH-FCH			SH-FLS	SH-Lum	✓	✓	✓	✓	
						SH-FLS		MH-FLS	MH-FLS						
Subchronic Rat (Springborn Laboratories, Inc., 1998)	14 days	M&F	✓	✓	✓		✓			SH-FCH Thy-wt			✓		
	90 days		✓	✓	✓		✓			SH-Fch Thy-wt			✓		
	120 days		✓		✓					✓			✓		
Developmental Neurotoxicity Rat (Argus Research Laboratories, Inc., 1998a)	FO: PP10	F	✓			✓				SH-Thy	✓		✓		
	F1: PND5	M&F	✓			SH-FCH				MH-FLS	✓		✓		
	F1:PND90[4]		✓			✓				✓					
Two-Generation Reproductive Rat (Argus Research Laboratories, Inc., 1998b)	F0		TBD					TBD			TBD			TBD	
	F1		TBD					TBD			TBD			TBD	
	F2		TBD					TBD			TBD			TBD	
Seg II Developmental Rabbit (Argus Research Laboratories, Inc., 1998c)	F0: 29 GD	F	✓			✓				SH-FCH			✓	✓	✓
Immunotoxicity Mouse (Keil et al., 1998)	14 days	F	TBD			TBD				TBD	TBD			TBD	
	90 days	F	TBD			TBD				TBD	TBD			TBD	
	120 days	F	TBD			TBD				TBD	TBD			TBD	

[a]M = male; F = female; Histo = histology; SH-FCH = subjective histology based on follicular cell hyperplasia; SH-FLS = subjective histology based on decrease in follicular lumen size; SH-Thy = subjective histology based on thyroid; Thy-WT = thyroid weight; MH-FCH = morphometric histology based on follicular epithelial cell hyperplasia; MH-FLS = morphometric histology based on decrease in follicular lumen size; morphoF0: PP10 = parental generation, Postpartum Day 10; F1: PND5 = first generation, Postnatal Day 5; MH = morphometry based on lumen size; F1: PND90 = first generation, Postnatal Day 90; TBD = to be determined; F0: 29 GD = parental generation, 29th gestational day.

[1]4.32 and 4.91 mg/kg-day in males and females, respectively.
[2]11.44 and 11.47 mg/kg-day in males and females, respectively.
[3]22.16 and 24.86 mg/kg-day in males and females, respectively.
[4]Data have not been reanalyzed by EPA.

TABLE 6-2. SUMMARY OF HORMONE (T3, T4, and TSH) AND HISTOLOGY EFFECTS[a]

(Light-shaded cells designate NOAELs; dark cells LOAELs; ✓ = dose tested)

Study/Species	Dose Duration/ Age Tested	Sex	Effects	DOSES (mg/kg/day) 0	0.01	0.05	0.1	0.2	0.3	0.44	1.0	3.0	5.0	10.0	30.0	100
Caldwell 14-day Rat (Caldwell et al., 1995)	14 days	M&F	T3	✓			F	✓			✓		✓	✓	✓	
			rT3				M	✓					✓	✓	✓	
			T4 hTG	✓				✓		✓	✓		✓	✓	✓	
			TSH	✓			F	✓		M	✓	✓	✓	✓	✓	
			Hist	✓			SH-FCH			SH-FLS	SH-Thy	✓	✓	✓	✓	
							SH-FLS			MH-FLS	SH-Lum					
Subchronic Rat (Springborn Laboratories, Inc., 1998)	14 days	M&F	T3	✓	M	M		M						M		
			T4	✓	✓	✓		✓			✓			✓		
			TSH	✓	F	F		✓			✓			✓		
						M										
			Hist	✓	✓	✓		✓			SH-FCH Thy-wt			✓		
	90 days		T3	✓	✓	✓		✓			✓			✓		
			T4	✓	✓	✓		✓			✓					
			TSH	✓	✓	✓		✓			✓			✓		
			Hist	✓	✓	✓		✓			SH-FCH Thy-wt			✓		
	120 days		T3	✓							✓			✓		
			T4	✓		✓					✓			✓		
			TSH	✓		✓					✓			✓		
			Hist	✓		✓					✓			✓		

TABLE 6-2 (cont'd). SUMMARY OF HORMONE (T3, T4, and TSH) AND HISTOLOGY EFFECTS[a]

(Light-shaded cells designate NOAELs; dark cells LOAELs; ✓ = dose tested)

Study/Species	Dose Duration/ Age Tested	Sex	Effects	DOSES (mg/kg/day) 0	0.01	0.05	0.1	0.2	0.3	0.44	1.0	3.0	5.0	10.0	30.0	100
Developmental Neurotoxicity Rat (Argus Research Laboratories, Inc., 1998a)	FO: PP10	F	T3	✓			✓				✓	✓		✓		
			T4	✓			✓				✓	✓		✓		
			TSH	✓			✓				✓	✓		✓		
			Hist	✓			✓				SH-Thy	✓		✓		
	F1: PND5	M&F	T3	✓			✓				✓	✓		✓		
			T4	✓			✓				✓					
			TSH	✓			✓				✓	✓		✓		
			Hist	✓			SH-FCH				✓	✓		✓		
			Hist				✓				MH	✓		✓		
	F1: PND90	M&F	T3	ND							ND	ND		ND		
			T4	ND							ND	ND		ND		
			TSH	ND							ND	ND		ND		
			Hist[4]	✓							✓	20%		80%		
Two-Generation Reproductive Rat (Argus Research Laboratories, Inc., 1998b)	F0		T3	TBD					TBD			TBD			TBD	
			T4	TBD					TBD			TBD			TBD	
			TSH	TBD					TBD			TBD			TBD	
			Hist	TBD					TBD			TBD			TBD	
	F1		T3	TBD					TBD			TBD			TBD	
			T4	TBD					TBD			TBD			TBD	
			TSH	TBD					TBD			TBD			TBD	
			Hist	TBD					TBD			TBD			TBD	
	F2		T3	TBD					TBD			TBD			TBD	
			T4	TBD					TBD			TBD			TBD	
			TSH	TBD					TBD			TBD			TBD	
			Hist	TBD					TBD			TBD			TBD	

TABLE 6-2 (cont'd). SUMMARY OF HORMONE (T3, T4, and TSH) AND HISTOLOGY EFFECTS[a]

(Light-shaded cells designate NOAELs; dark cells LOAELs; ✓ = dose tested)

Study/Species	Dose Duration/ Age Tested	Sex	Effects	DOSES (mg/kg/day) 0	0.01	0.05	0.1	0.2	0.3	0.44	1.0	3.0	5.0	10.0	30.0	100
Seg. II Developmental Rabbit (Argus Research Laboratories, Inc., 1998c)	F0: 29 GD	F	T3	✓			✓				✓			✓	✓	✓
			T4				✓				✓			✓	✓	✓
			TSH	✓			✓				✓			✓	✓	✓
			Hist	✓			✓				SH-FCH			✓	✓	✓
Immunotox Mouse (Keil et al., 1998)	14 days	F	T3	TBD			TBD				TBD	TBD			TBD	
			T4	✓			✓				✓	✓			✓	
			TSH	✓			✓				✓	✓			✓	
			Hist	TBD			TBD				TBD	TBD			TBD	
	90 days	F	T3	TBD			TBD				TBD	TBD			TBD	
			T4	✓			✓				✓	✓			✓	
			TSH	✓			✓				✓	✓			✓	
			Hist	TBD			TBD				TBD	TBD			TBD	
	120 days	F	T3	ND			ND				TBD	ND			ND	
			T4	✓			✓				✓	✓			✓	
			TSH	✓			✓				✓	✓			✓	
			Hist	TBD			TBD				TBD	TBD			TBD	

[a]M = male; F = female; T3 = triiodothyronine; rT3 = reverse T3; T4 = thryoxine; hTG = thyroglobulin; TSH = thyroid stimulating hormone (thyrotropin); Hist = histology; SH-FCH = subjective histology based on follicular epithelial cell hyperplasia; SH-FLS = subjective histology based on decrease in follicular lumen size; SH-Thy = subjective histology based on thyroid; Thy-WT = thyroid weight; F0: PP10 = parental generation, Postpartum Day 10; F1: PND5 = first generation, Postnatal Day 5; MH = morphometric histology based on lumen size; ND = Not done; F1: PND90 = first generation, Postnatal Day 90; TBD = to be determined; F0: 29 GD = parental generation, 29th gestational day.

[1](4.32 and 4.91) mg/kg-day in males and females, respectively.
[2](11.44 and 11.47) mg/kg-day in males and females, respectively.
[3](22.16 and 24.86) mg/kg-day in males and females, respectively.
[4]Data have not been reanalyzed by EPA.

In addition, limited sample size and differences in dose spacing and in time (e.g., end of gestation versus postnatal) complicate these comparisons. Differences in pathologists across the studies may confound interpretation of the histology data. Although the laboratories were careful to collect samples from each treatment group at different times of the day, the mean values would thereby include durnal rhythms. This would contribute to "within group error" and reduce the statistical power to detect differences. With respect to gender differences, it is difficult to ascribe any biological significance to these findings when the data are viewed across the array of studies. Further, minimal differences between gender are generally associated with chemically induced hypothyroidism. Evaluation of the hormone data for mice awaits repeat assays and the completion of T3 data.

Based on the pattern that emerged from these data, a sequential mode of action model was proposed to map the relationships between external dose, internal dose, the biologically effective dose, and altered structural and functional parameters of established relevance to risk assessment (Figure 6-1). This scheme essentially fleshes out in finer detail the progressive steps along the exposure-dose-response continuum discussed in Chapter 4 (see Figure 4-8) and is couched in terms of establishing biomarkers of exposure and effect. Any "compartment" along the continuum may have prognostic significance to established outcomes of interest (e.g., thyroid histology) and be relevant to risk assessment without comprehensive quantification of mechanistic determinants. Note that the earliest biological effect, changes in thyroid and pituitary hormones, is the precursor lesion for both the potential carcinogenic and neurodevelopmental effects.

The difficulty in designating an effect level for these perturbations, however, was in the degree of change to designate as adverse. Screening neurodevelopmental studies may not have the power to ascertain neurological effects that might result from small changes in 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 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) suggest that measurements of nervous system development are less sensitive than measurements of T4. Two of reasons for this relationship are presented.

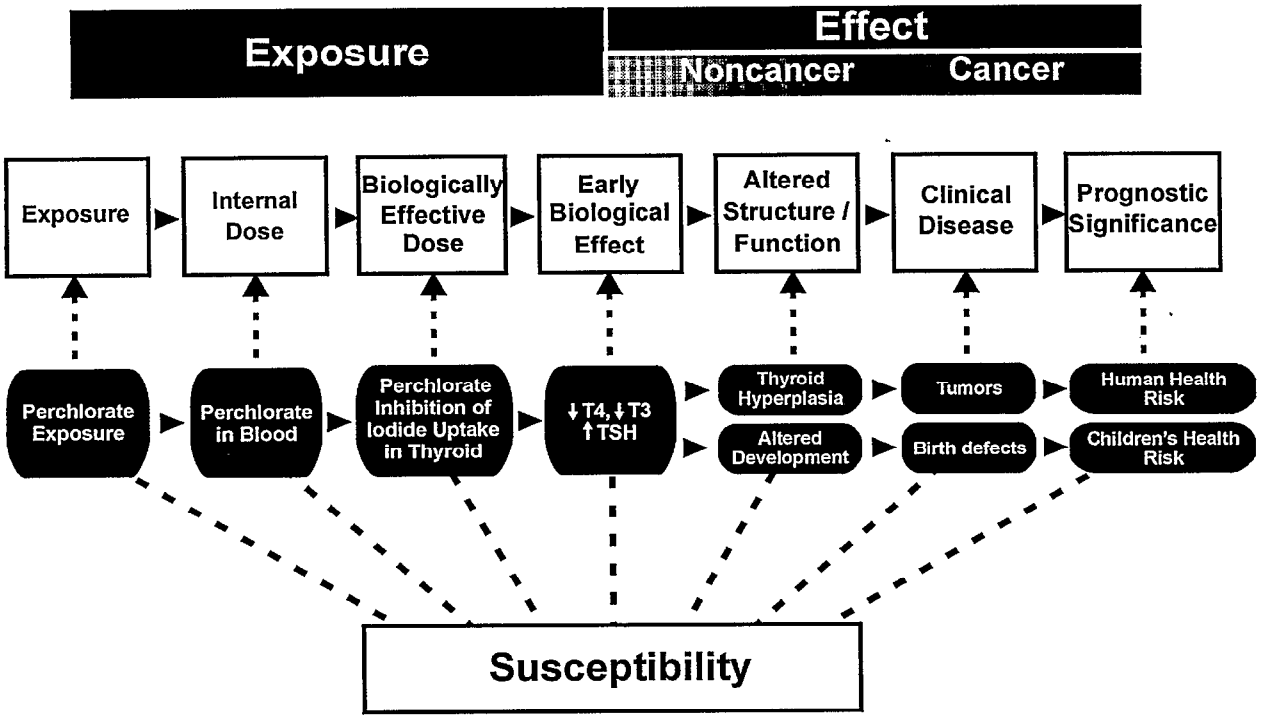


Figure 6-1. Schematic of the exposure-dose-response continuum considered in the context of biomarkers (classified as measures of exposure, effect, and susceptibility) and level of organization at which toxicity is observed (U.S. Environmental Protection Agency, 1994a; Schulte, 1989). An overall model of how to map the toxicity from perchlorate can be developed on this basis by establishing causal linkage or prognostic correlations of precursor lesions.

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 of this model development is that currently available testing methods, particularly screening methods, may not be very sensitive. Recent data suggest that the battery is insensitive to alterations in thyroid hormones during development (Goldey, 1995a,b). As noted in Chapter 5, the increased size of the corpus callosum (Section 5.2.3.1) and the alteration in the ontogeny of motor activity (Section 5.2.3.4) argue that there may, in fact, be an affect of perchlorate on the structure and function of the developing nervous system occurring at lower concentrations.

A particular concern was the thyroid follicular cell hyperplasia and the decrease in follicular lumen size observed at the 0.1-mg/kg-day dose in the pups of the neurodevelopmental study at PND5. Figure 6-2 shows the pattern of change in fetal and neonatal thryoid function during pregnancy in humans, and an analogous pattern is likely to exist in the rats. If the TSH was increased sufficiently to cause hypertrophy in these pups, there is a concern that it could have occurred in utero, because 5 days of postpartum exposure via lactation alone probably is not sufficient to develop that degree of change in the gland. Further, according to Fisher (1998b), who has worked with models of lactational transfer of ionized small molecules, (e.g., trichloroacetie formed by metabolism of trichloroethylene), approximately 0.5 to 1% of the administered dose was available over the course of 21 days of lactation. Fisher (1998b) estimated that less than 5% of the administered perchlorate dose would be transferred via lactation over the entire lactation period and that most would be excreted in the urine.

6.1.1.1 Correlation Analyses of Hormone and Thyroid Histopathology

To further support the mode-of-action mapping, a series of correlations were performed evaluating the relationships between the thyroid hormones, TSH, and thyroid histology (Geller, 1998a). Because of the controlling feedback mechanisms involved in the hypothalamic-pituitary-thyroid axis, as perchlorate exposures perturb thyroid economy, one would expect certain relationships in the correlations. The thyroid produces T4 in large quantities and T3 in smaller amounts; most T3 is produced by deiodination of T4 at target tissues. Low levels of circulating T4 and T3 lead to increased production and release of TSH by the pituitary. Long periods of elevated TSH can result in hypertrophy or hyperplasia of the follicular epithelial cells, as well as a decrease in the size of the follicular lumen. Thus, positive correlations between T3 and T4, whereas negative correlations between T3 or T4 and TSH are expected if these perturbations are affecting the thyroid economy. Positive correlations between TSH and thyroid histopathology are expected, whereas T3 or T4 would be correlated negatively (inversely) with thyroid histopathology.

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

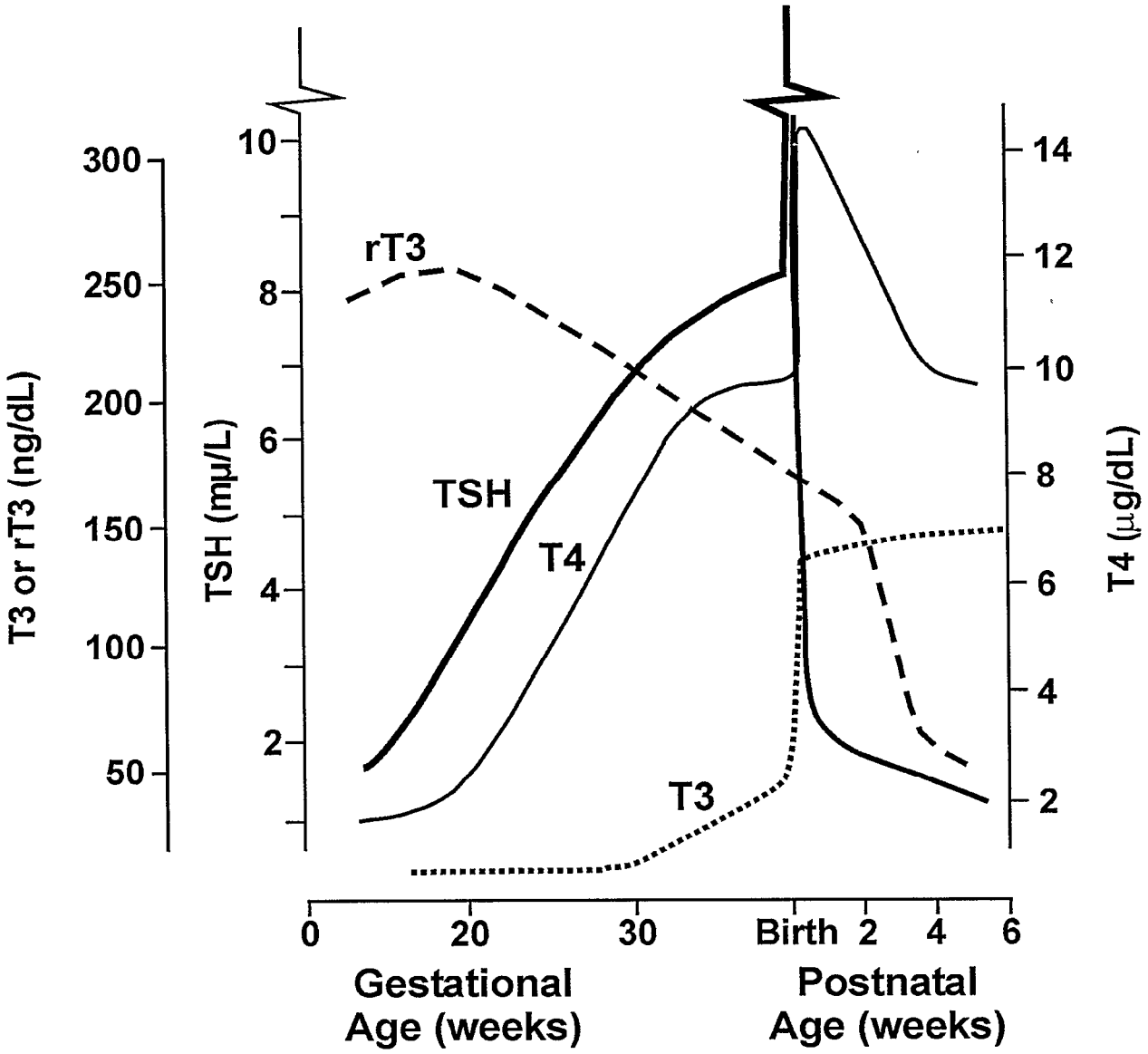


Figure 6-2. Pattern of change in fetal and neonatal thyroid function parameters during pregnancy and extrauterine adaptation in the human. A similar pattern is thought to exist in the rat (see text for further details).

Source: Fisher (1996).

simplest to recode the data by converting the variable values into rank scores. Spearman's rank order (r_s) correlation 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 6-3 through 6-16 can be found in Appendix 6A.

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 6-3 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 correlated highly with two standard histopathological ratings of thyroid pathology, follicular epithelial cell hypertrophy and decrease in follicular lumen size. Figure 6-4 shows the rank of T4 level versus the severity rating for these two standard histopathology measures to be highly correlated inversely. Figure 6-5 illustrates that the TSH levels also are strongly positively correlated with these same measures. Table 6-A2 in Appendix 6A shows that the severity ratings for the two standard histopathology measures (follicular epithelial cell hypertrophy and decrease in follicular lumen size) also are highly correlated with each other.

Figures 6-6 and 6-7 show the correlations for the 14-day and 90-day time points combined for the subchronic study performed in rats (Springborn Laboratories, Inc., 1998). As shown in Figure 6-6 (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), and both were negatively and positively correlated (Figure 6-7) with the standard histopathology measures of hyperplasia used in this study. After 14-days of dosing (Figure 6-8), T3 and T4 are highly associated (top panel), but there is an unexpected positive relation between T4 and TSH (bottom panel). However, as expected, T4 and TSH are associated negatively and

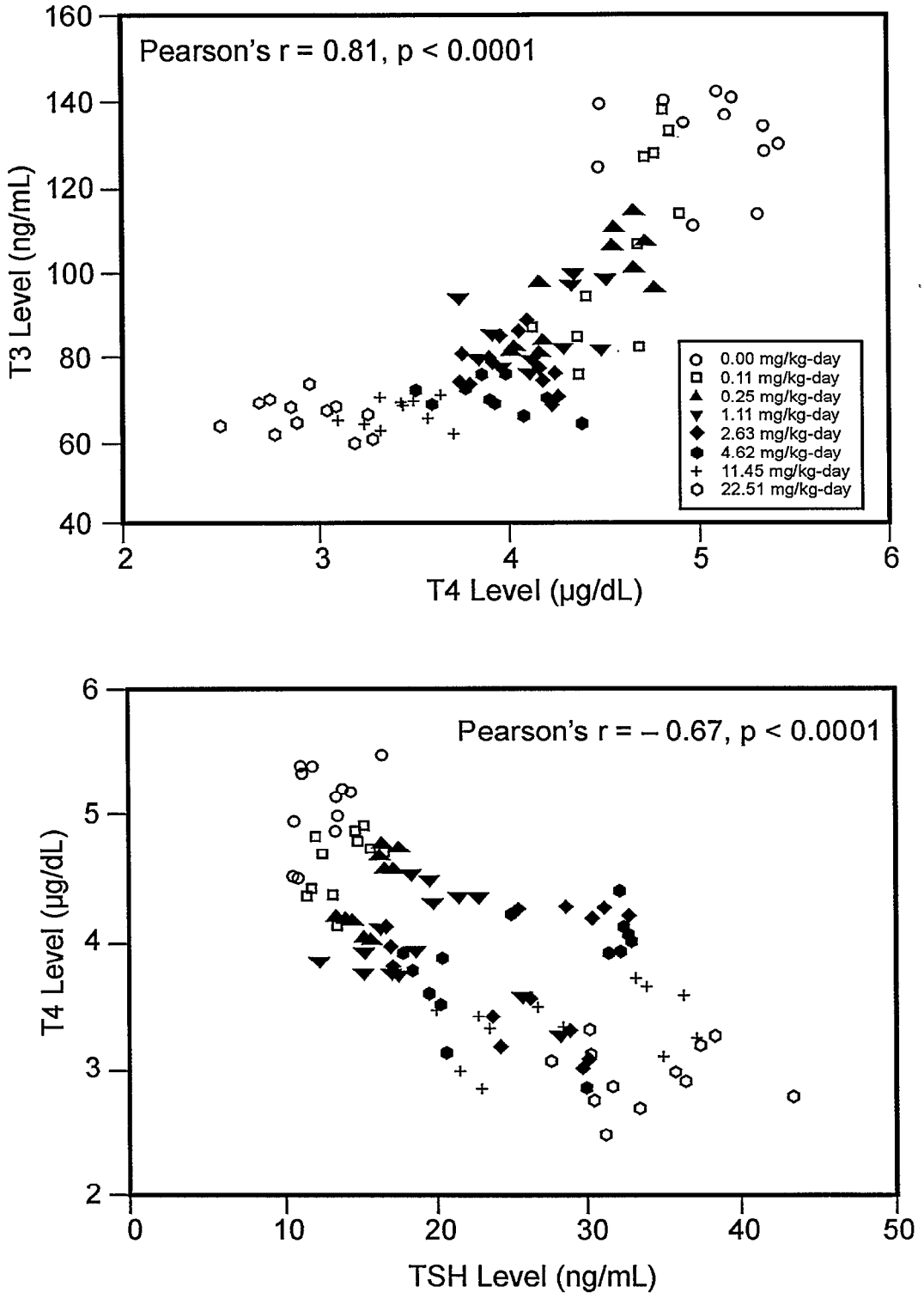


Figure 6-3. Correlations between T3 versus T4 (top panel) and T4 versus TSH (bottom panel) in rats of the 14-day study of Caldwell et al. (1995). Data of Channel (1998a) and Crofton (1998a).

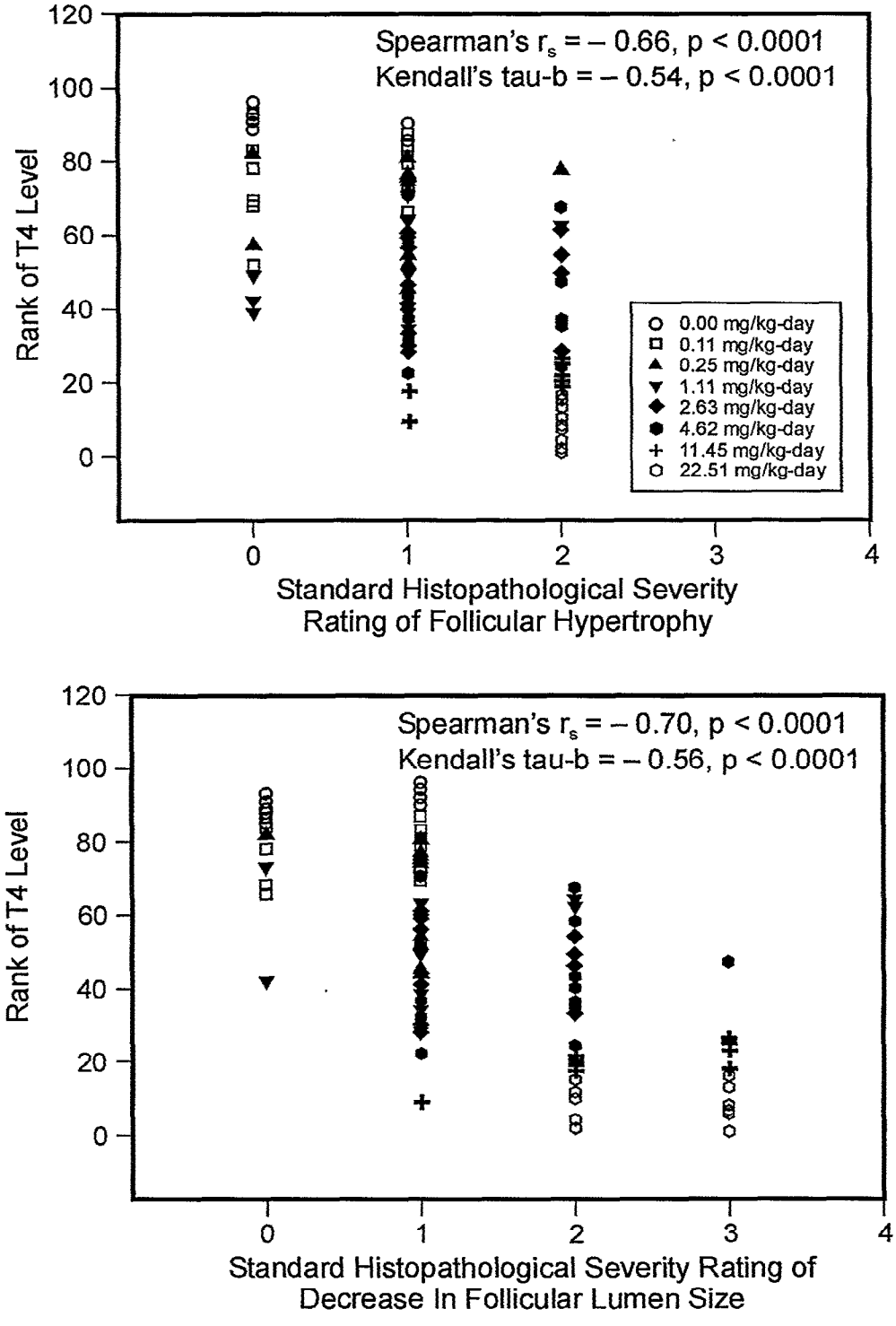
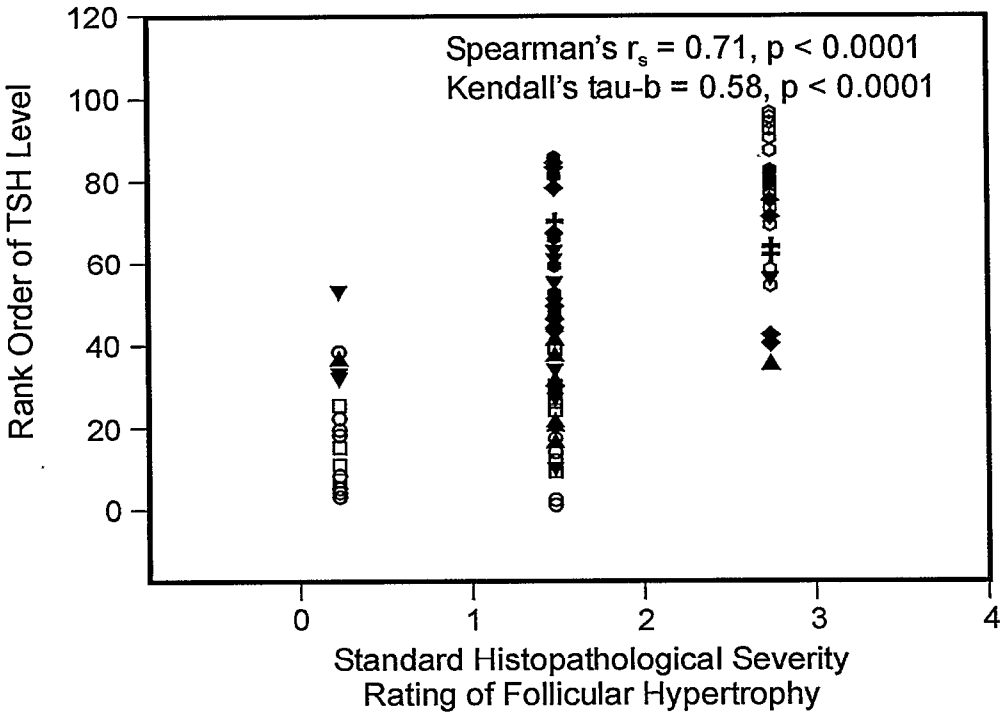


Figure 6-4. Correlations between the rank order of T4 versus standard histological severity rating of follicular epithelial cell hypertrophy (top panel) or of decrease in follicular lumen size (bottom panel) for the rats of the 14-day study of Caldwell et al. (1995). Data of Channel (1998a) and Crofton (1998a).



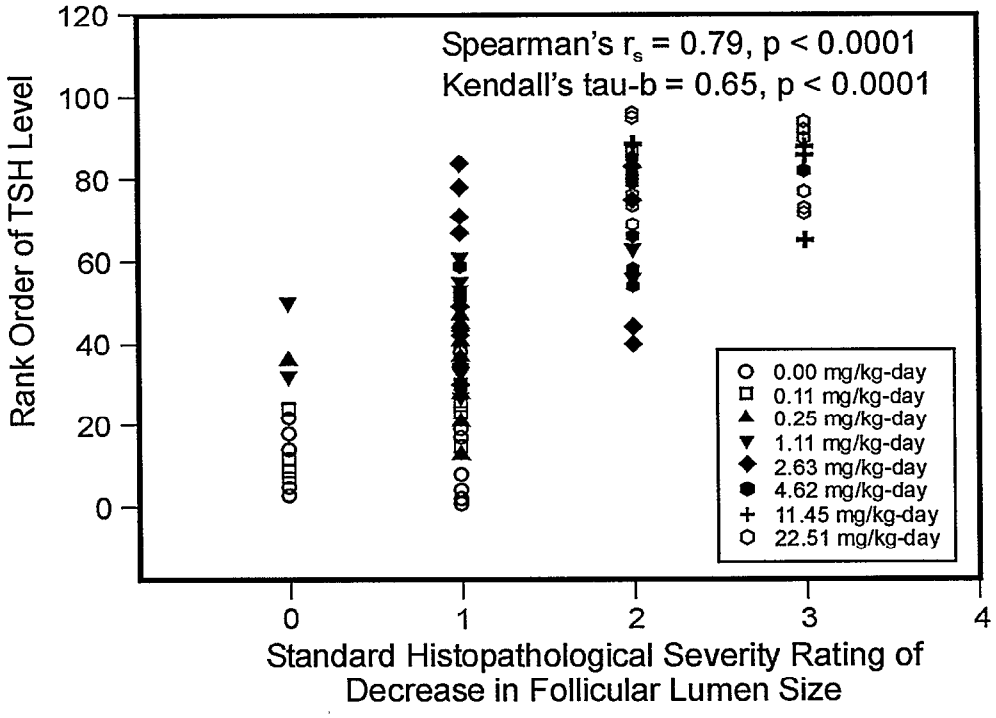


Figure 6-5. Correlations between the rank order of TSH versus standard histological severity rating of follicular epithelial cell hypertrophy (top panel) or of decrease in follicular lumen size (bottom panel) for the rats of the 14-day study of Caldwell et al. (1995). Data of Channel (1998a) and Crofton (1998a).

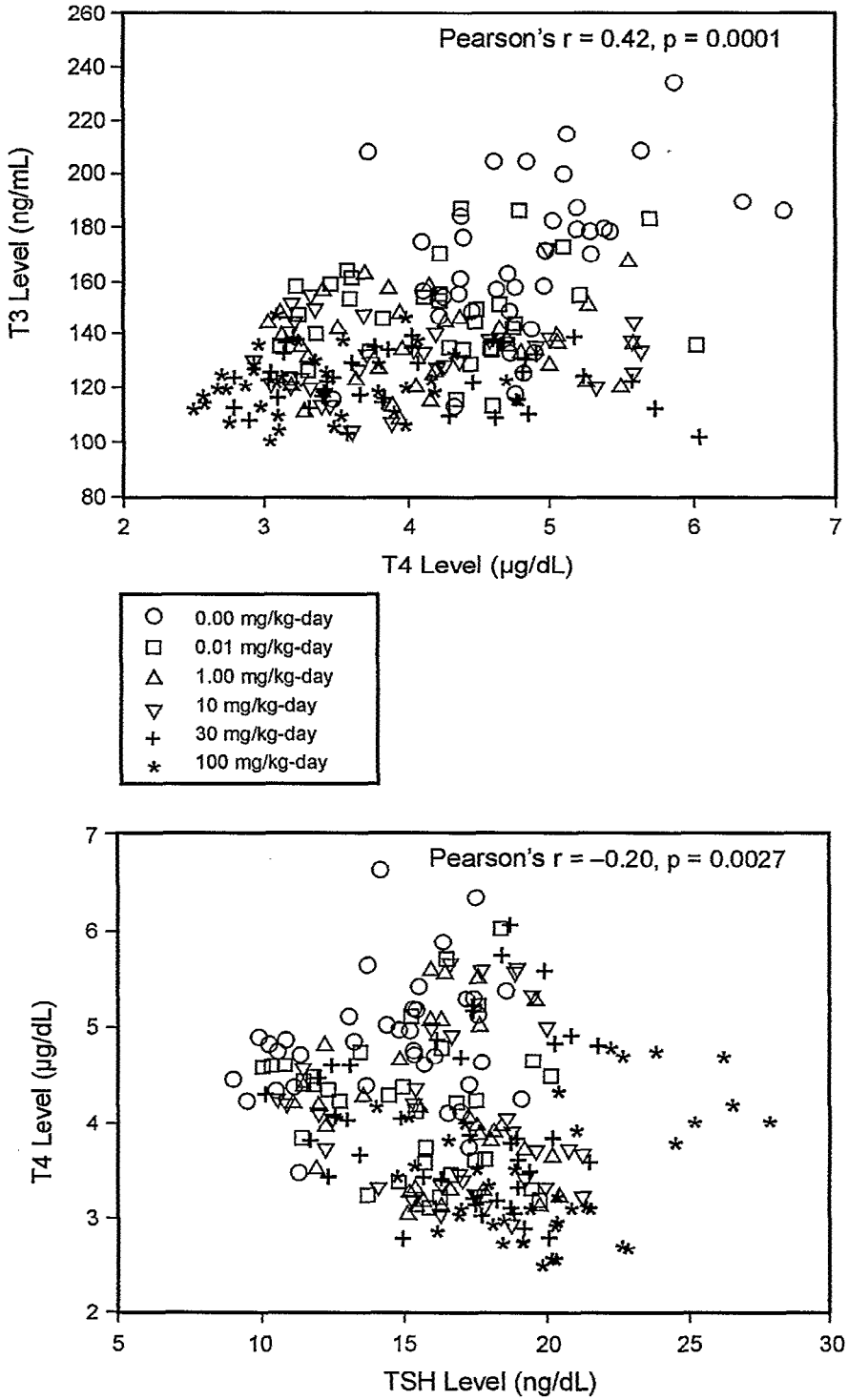


Figure 6-6. Correlations between T3 versus T4 (top panel) and T4 versus TSH (bottom panel) for the combined data of the 14-day and 90-day time points from the subchronic study in rats (Springborn Laboratories, Inc., 1998).

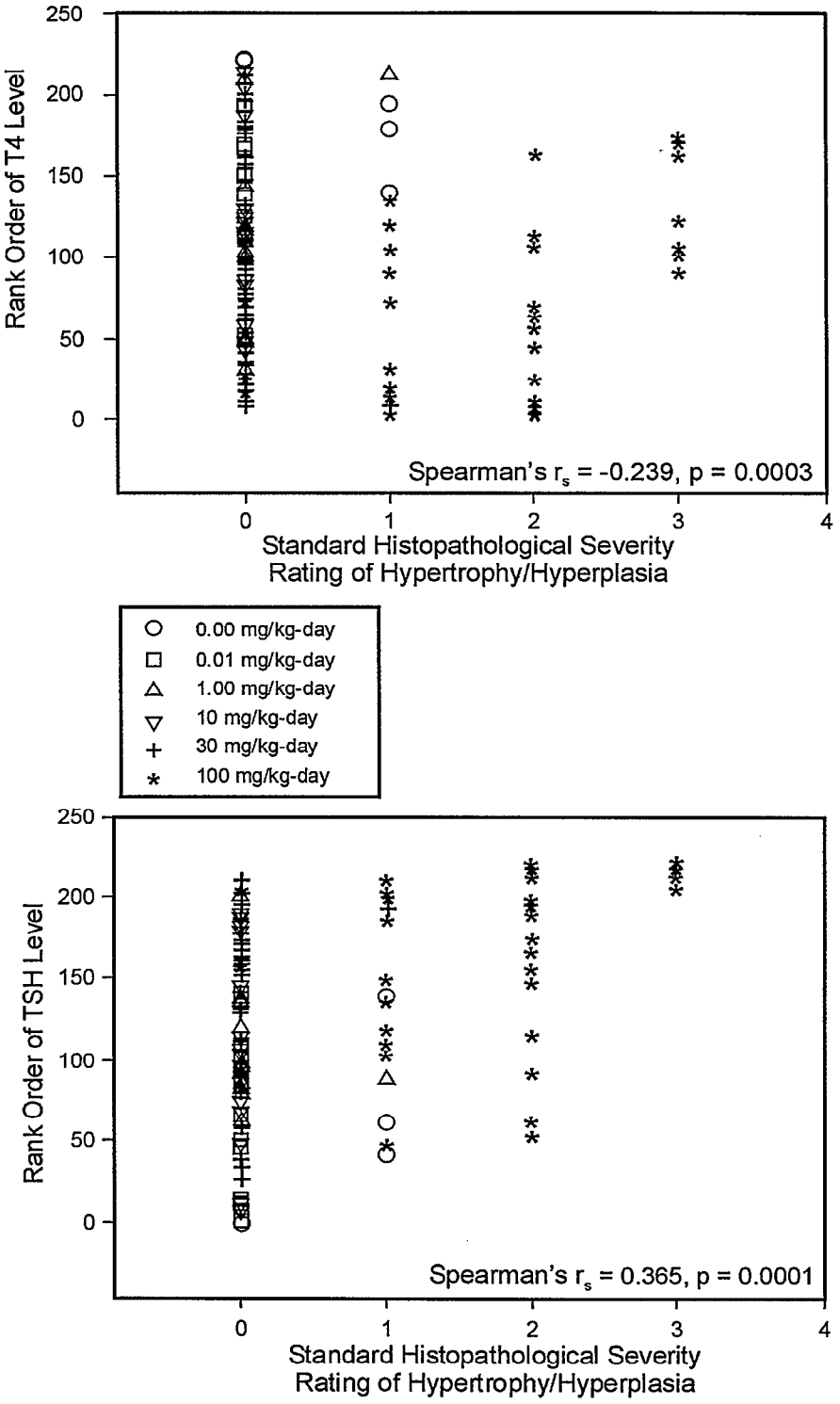


Figure 6-7. Correlation between the rank order of T4 (top panel) or TSH (bottom panel) versus standard histopathology severity rating of thyroid hypertrophy/ hyperplasia for the combined data of the 14-day and 90-day time points from the subchronic study in rats (Springborn Laboratories, Inc., 1998).

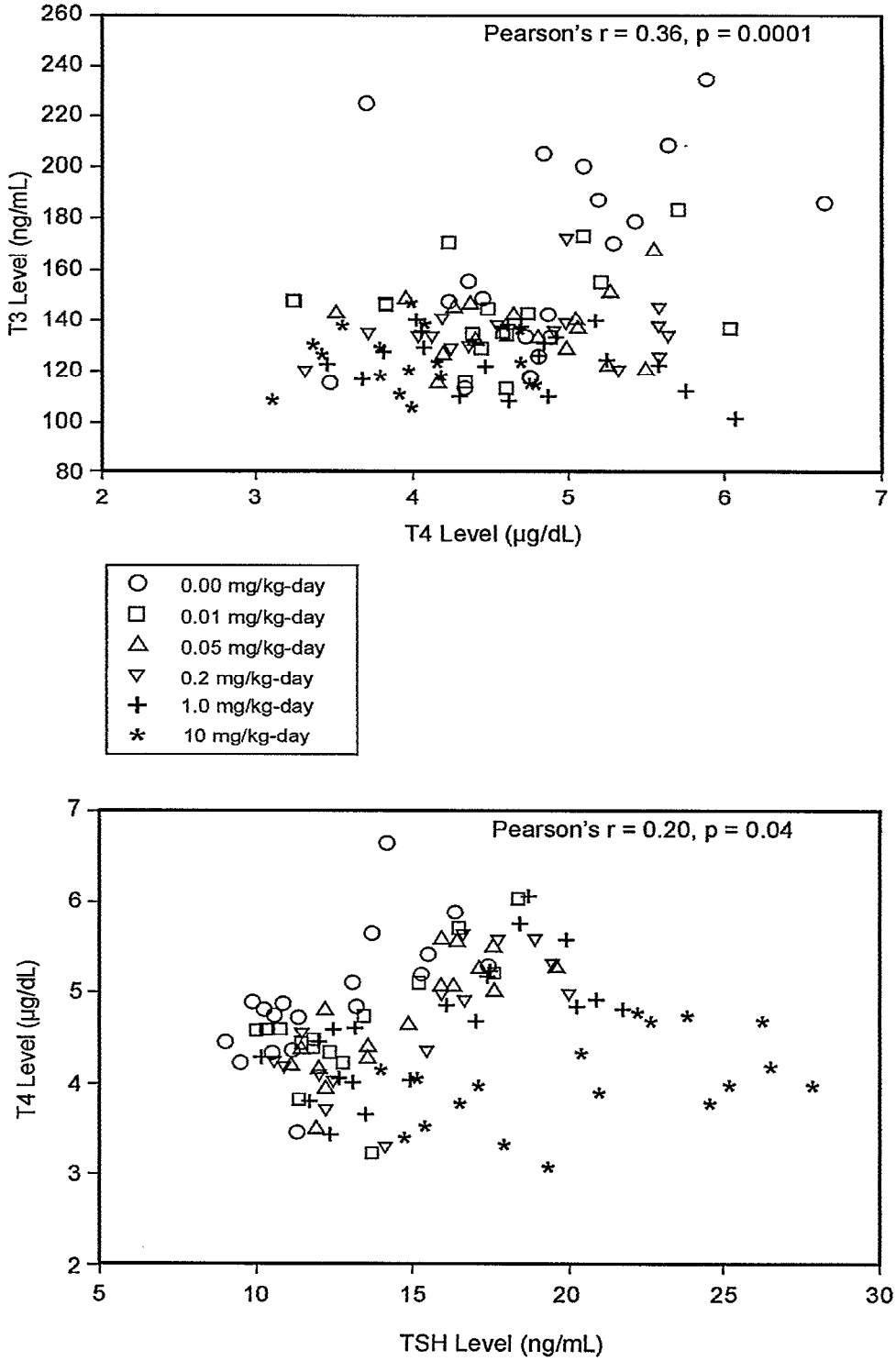


Figure 6-8. Correlations between T3 versus T4 (top panel) and T4 versus TSH (bottom panel) for the combined data of the 14-day time point from the subchronic study in rats (Springborn Laboratories, Inc., 1998).

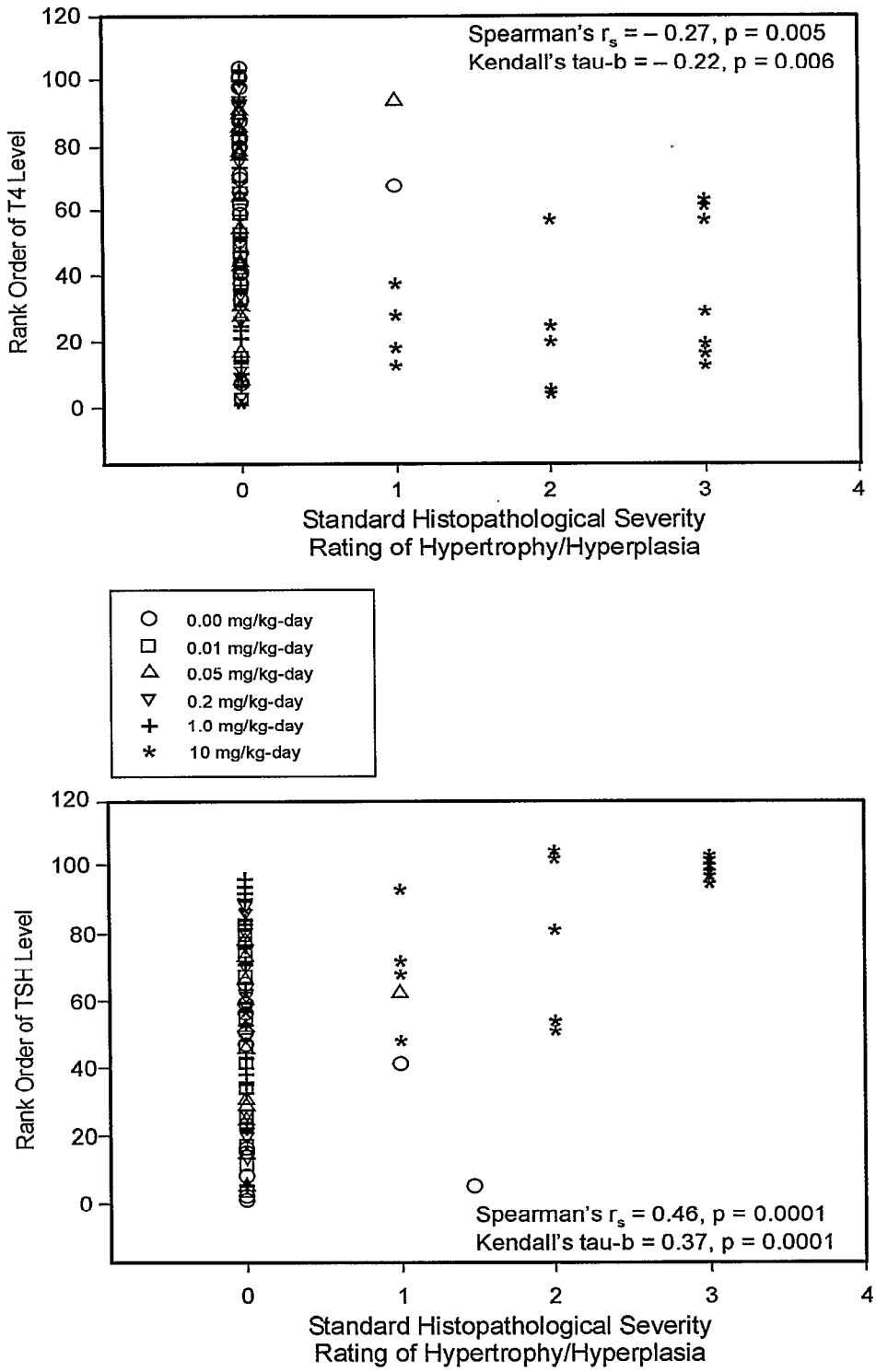


Figure 6-9. Correlations between the rank order of T4 (top panel) or TSH (bottom panel) versus standard histopathology severity rating of thyroid hypertrophy/ hyperplasia for the 14-day time point from the subchronic study in rats (Springborn Laboratories, Inc., 1998).

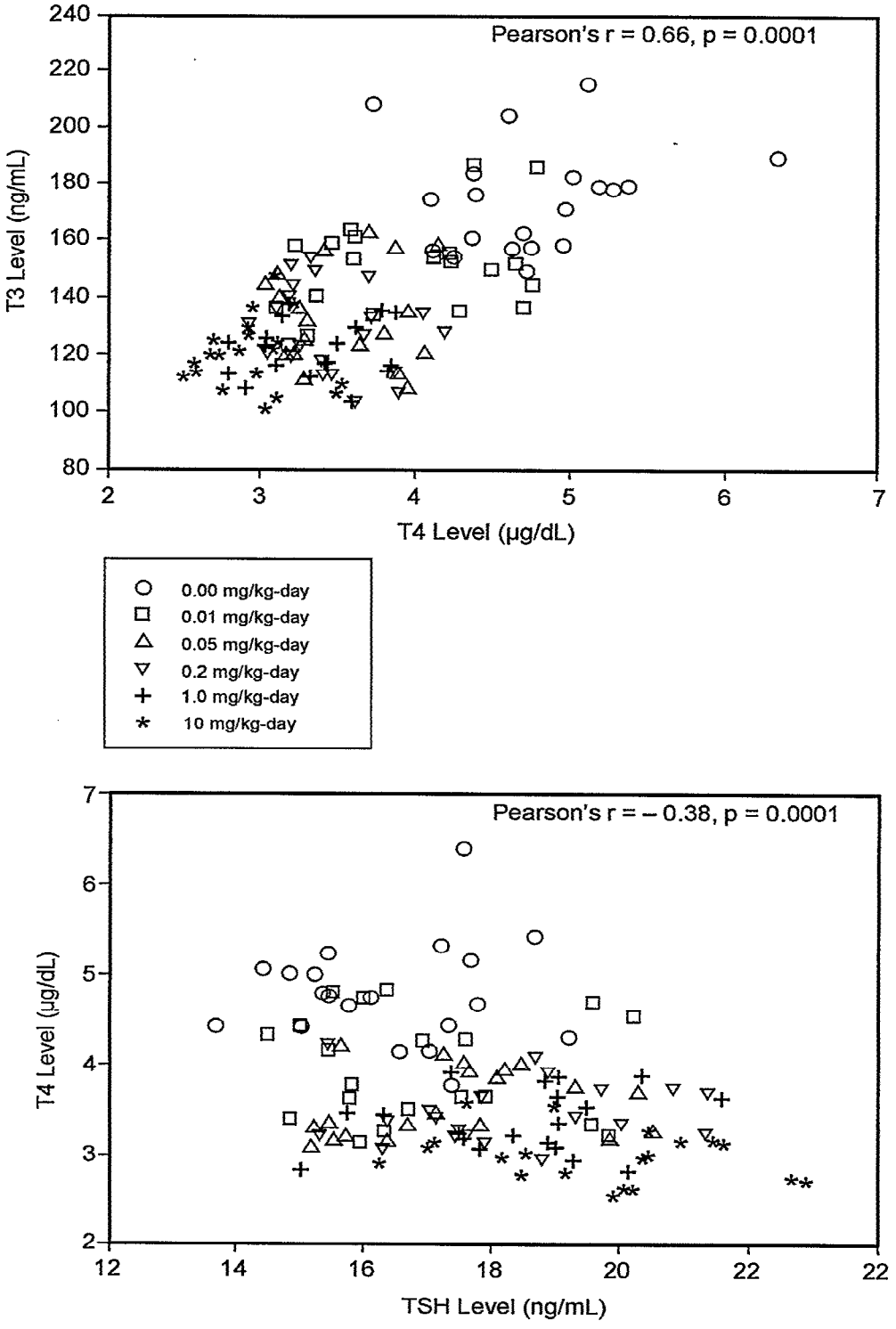


Figure 6-10. Correlations between T3 versus T4 (top panel) and T4 versus TSH (bottom panel) for the combined data of the 90-day time point from the subchronic study in rats (Springborn Laboratories, Inc., 1998).

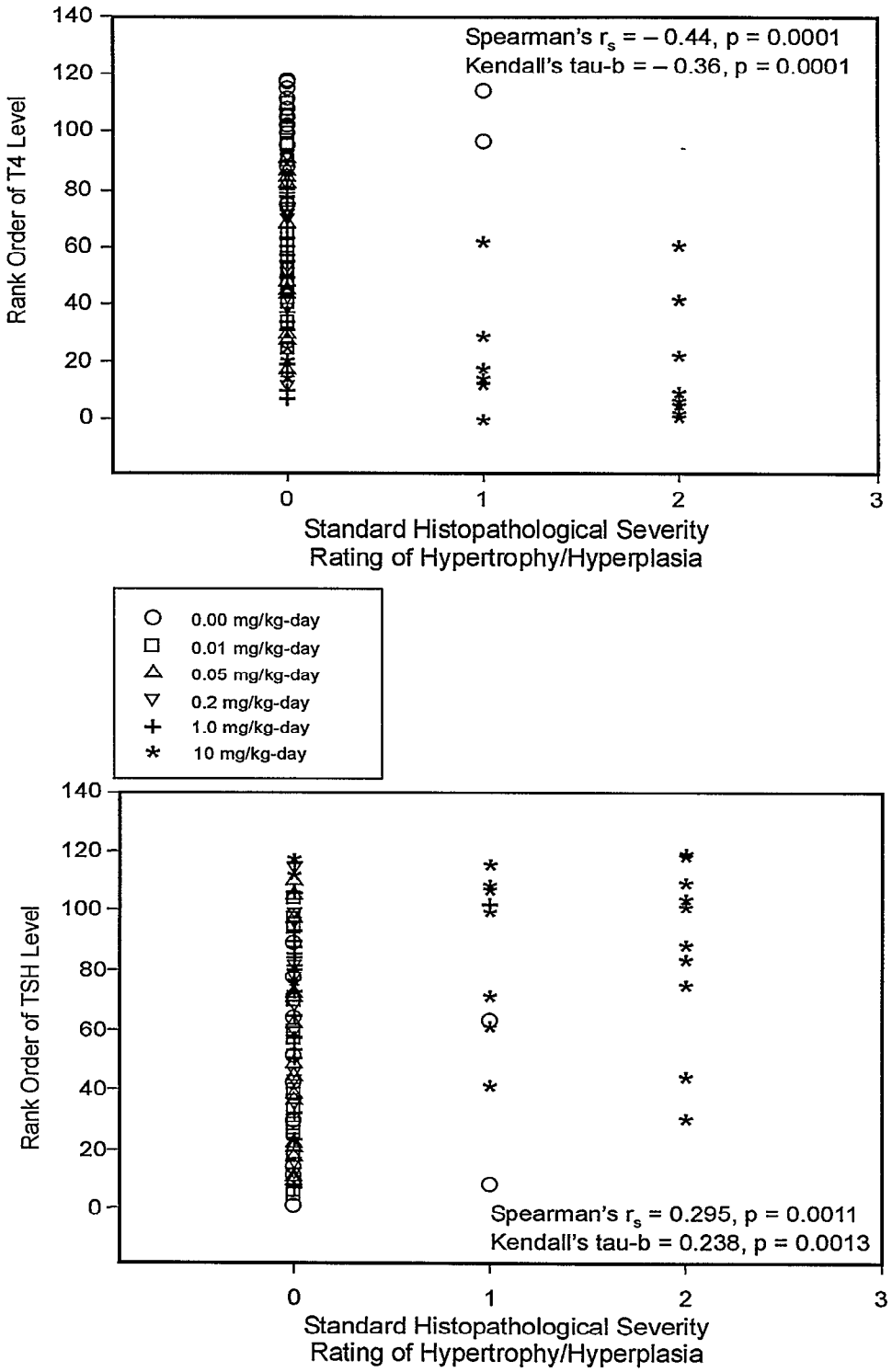


Figure 6-11. Correlations between the rank order of T4 (top panel) or TSH (bottom panel) versus standard histopathology severity rating of thyroid hypertrophy/ hyperplasia for the 90-day time point from the subchronic study in rats (Springborn Laboratories, Inc., 1998).

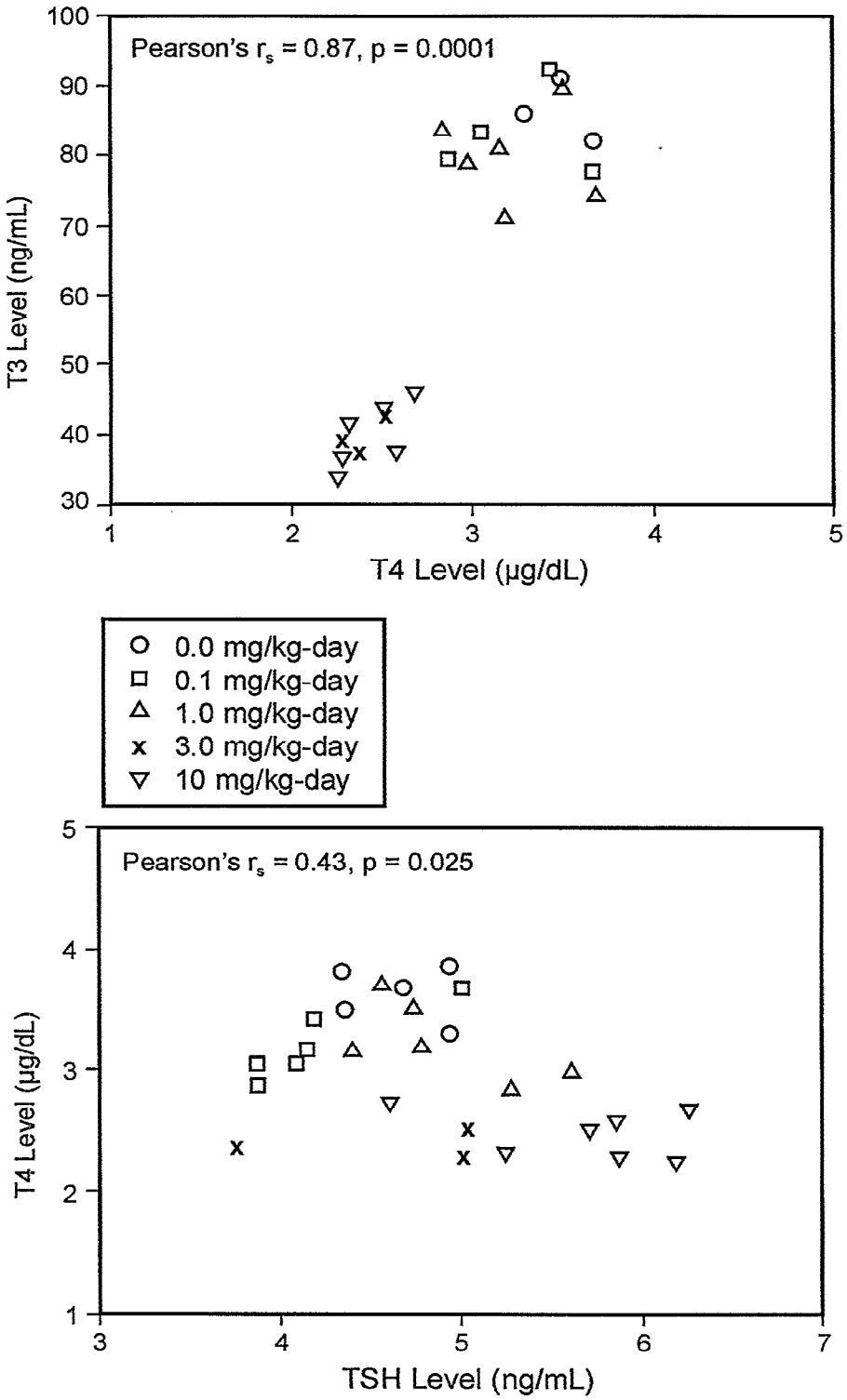


Figure 6-12. Correlations between T3 versus T4 (top panel) and T4 versus TSH (bottom panel) for the F1 rat pups on PND5 in the developmental neurotoxicity study. Data of Argus Research Laboratories, Inc. (1998a), York (1998c), Channel (1998b), and Crofton (1998f).

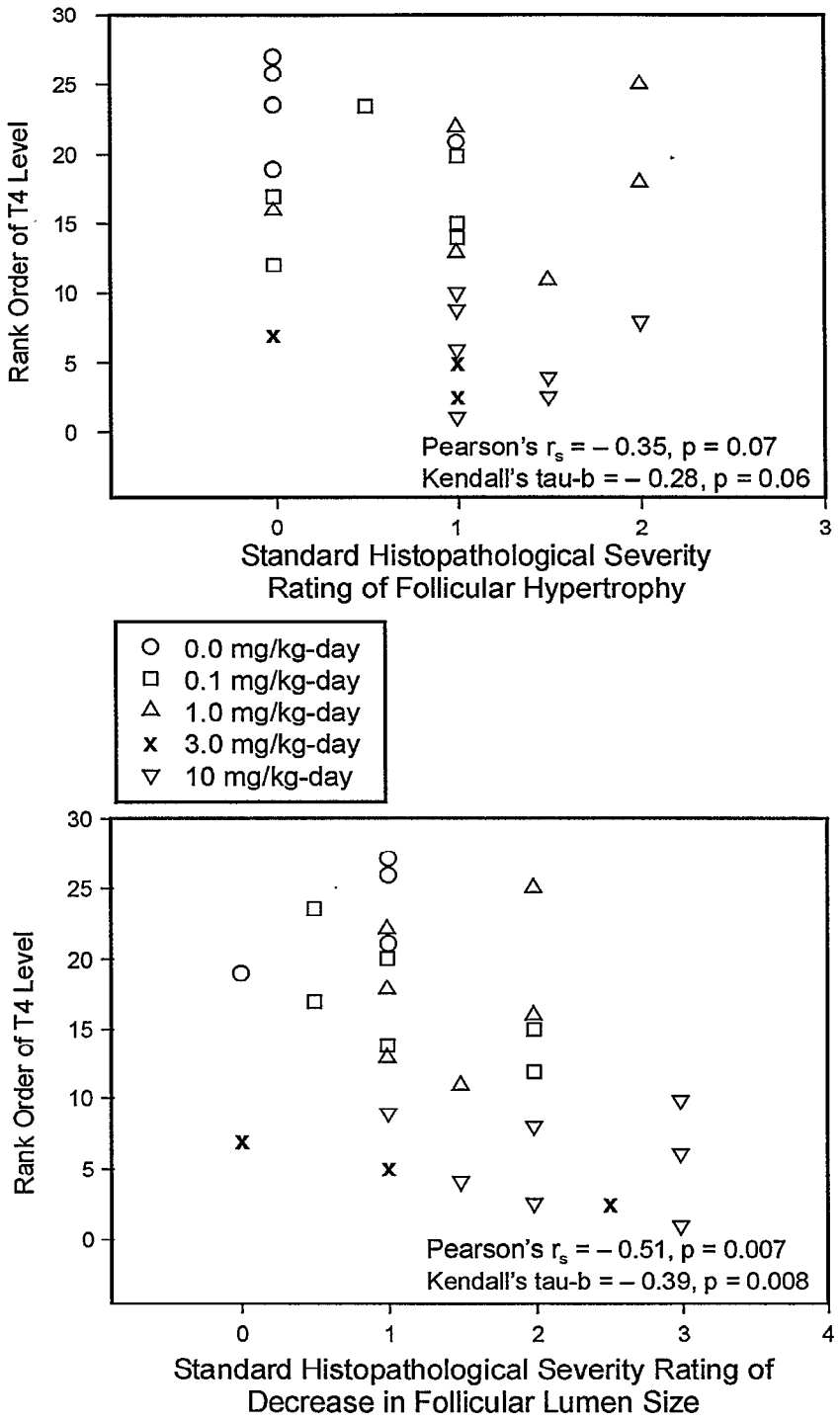


Figure 6-13. Correlations between the rank order of T4 versus standard histopathology severity rating of thyroid follicular epithelial cell hypertrophy rating (top panel) or for standard histopathology severity rating of the decrease in follicular lumen size (bottom panel) for the Postnatal Day 5 (PND5) pups in the neurodevelopmental study. Data of Argus Research Laboratories, Inc. (1998b), York (1998c), Channel (1998b), and Crofton (1998e,f).

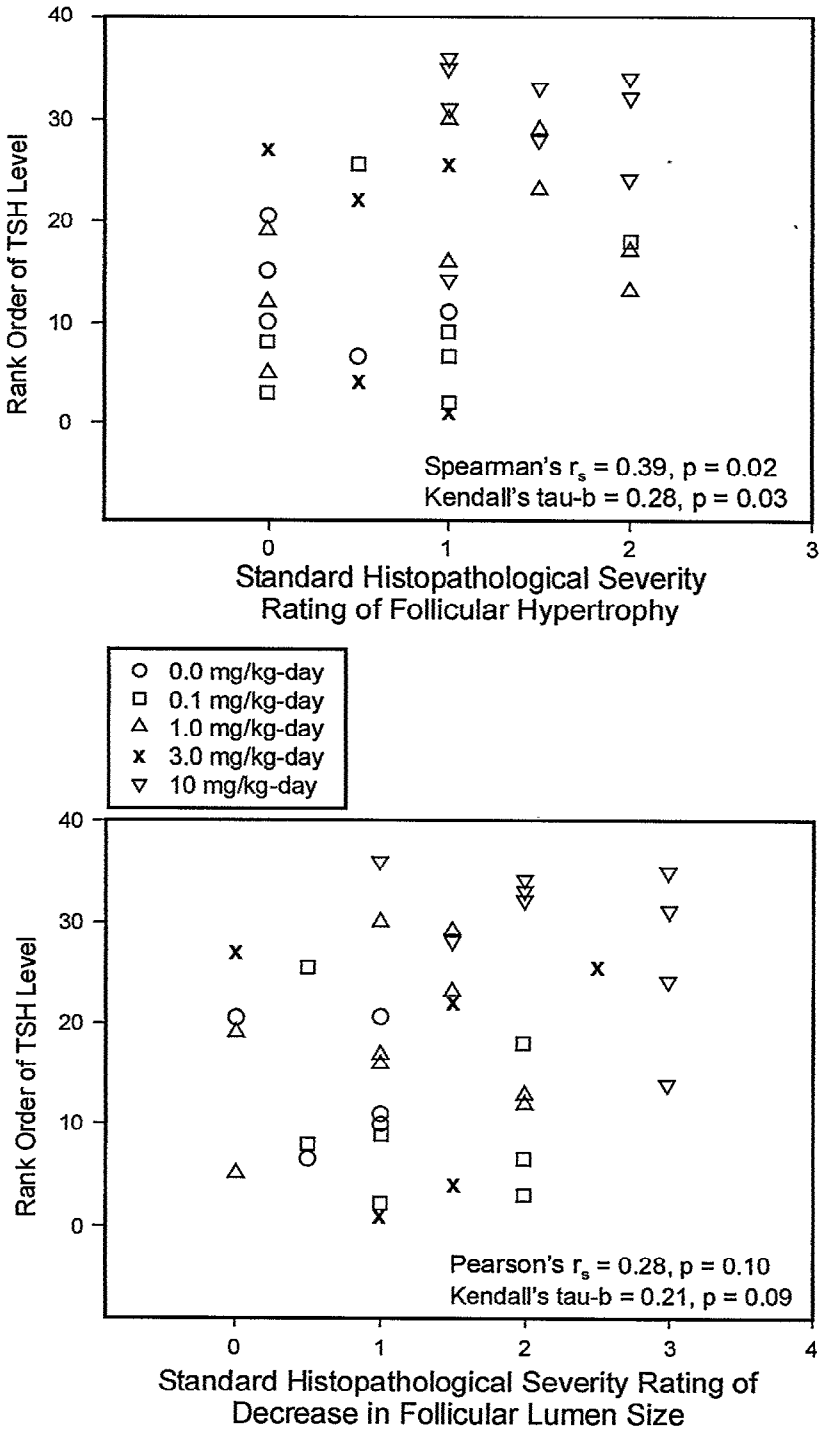


Figure 6-14. Correlations between the rank order of TSH versus standard histopathology rating of thyroid follicular epithelial cell hypertrophy rating (top panel) or for standard histopathology severity rating of the decrease in follicular lumen size (bottom panel) for the PND5 pups in the neurodevelopmental study. Data of Argus Research Laboratories, Inc. (1998a), York (1998c), Channel (1998b), and Crofton (1998e,f).

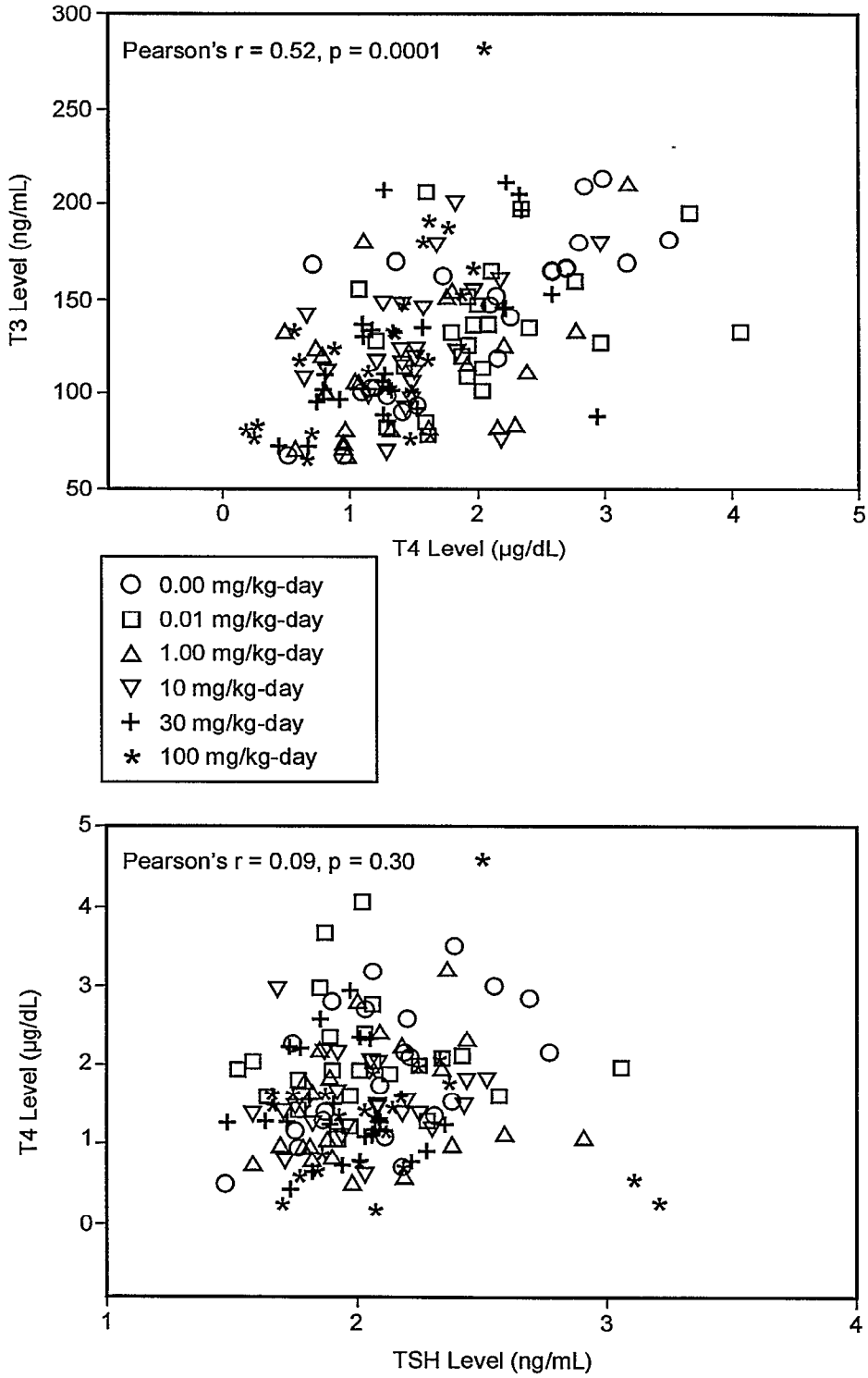


Figure 6-15. Correlations between T3 versus T4 (top panel) and T4 versus TSH (bottom panel) for the data in parent F0 generation on Gestation Day 29 (GD29) rabbits from the developmental study (Argus Research Laboratories, Inc., 1998c).

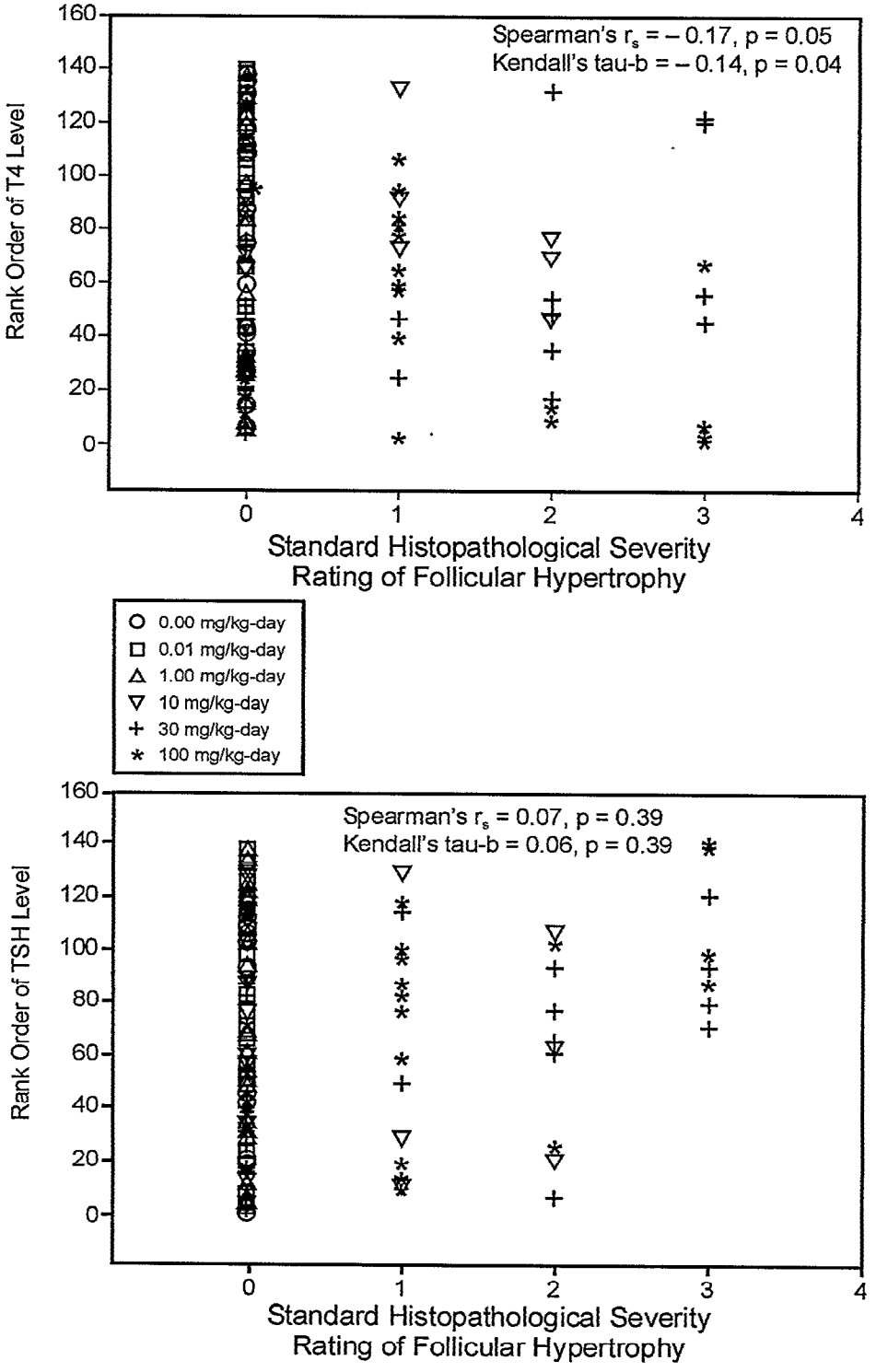


Figure 6-16. Correlations between the rank order of T4 (top panel) or TSH (bottom panel) and the standard histopathological severity rating of follicular hypertrophy in parent F0 generation on GD29 rabbits from the developmental study (Argus Research Laboratories, Inc., 1998c).

positively with follicular hyperplasia (Figure 6-9, top and bottom panels respectively). At the 90-day time point, there are the expected strong correlations between T3 and T4 (Figure 6-10, top panel), between T4 and TSH (bottom panel), and T4 or TSH with follicular cell hyperplasia (Figure 6-11, top and bottom panels, respectively.

Correlations also were performed on the data from the neurodevelopmental study for the PND5 pups; T3 and T4 were strongly positively correlated, and T4 and TSH were negatively correlated (Figure 6-12). Figure 6-13 (top panel) shows that T4 is negatively associated with both follicular cell hypertrophy and decrease in lumen area (bottom panel), but the correlation reaches significance only for decrease in lumen area. Figure 6-14 shows that TSH is positively correlated with follicular hypertrophy (top panel) and decrease in lumen size (bottom panel), but the correlation reaches a significance level only with the hyperplasia.

Correlations also were performed for the developmental toxicity study in rabbits (Argus Research Laboratories, Inc., 1998c); T3 and T4 are strongly positively correlated (Figure 6-15, top panel), but there was no significant correlation between T4 and TSH. Figure 6-16 shows that T4 is borderline significant with follicular cell hyperplasia (top panel), whereas TSH is not correlated with follicular cell hyperplasia (top panel).

In total, these correlations lended 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 standard ratings of thyroid histopathology. 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 two measures of thyroid histopathology. In the subchronic (Springborn Laboratories, Inc., 1998) study, correlations were established between hormone levels and follicular epithelial cell hypertrophy/hyperplasia 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 also found, along with significant correlations of these hormone levels with the severity ratings of thyroid histopathology.

Similar relationships were observed in pups on PND 5 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 one or the other of the thyroid histopathology measures. 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, which are thought to result directly from elevation of TSH.

The rabbit developmental study (Argus Research Laboratories, Inc., 1998c) yielded significant correlations between T3 and T4, and between T4 and histopathology. It did not, however, show the expected relationships between T4 and TSH or between TSH and histopathology seen in the rat studies. This may be because of sample size and the time at which the hormone levels were determined (GD 29).

6.1.1.2 Weight-of-Evidence Summary

Based on the historical hazard evaluation discussed in Chapter 3 and bolstered by the strong correlations for the mapping of the mode of action across the studies, especially in rats, the disturbances in thyroid homeostasis based on perturbations of the hypothalamic-pituitary-thyroid axis were focused on for choice of the critical effect. Changes in thyroid hormones and TSH typically precede thyroid histopathology, although the exact degree of change necessary to induce thyroid histopathology or to effect neurological development is unknown. As evidenced by the data in Chapter 3, as well as by the results of the new testing strategy, effects on developmental toxicity and reproductive and immune system function appear likely to occur at concentrations above those at which perturbations in thyroid hormone economy occurs.

Concern over the magnitude to consider meaningful in the hormone data remains a dilemma. In clinical studies, a normal range typically is defined by a control, healthy population. However, 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 fluctuation in the control population occurred as that in the exposed population at the time of measurement. A small shift in the mean of a population can have significant consequences to individuals in the tails of the distibrutions of those populations. Indeed, such an evaluation underlies the basis for the blood lead level used to regulate the National Ambient Air Quality Standard (Davis and Elias, 1996). 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 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, where 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 (see Section 6.2).

In light of this stance on the hormone ANOVA analyses and referring to the data in Table 6-2, it is noted that the NOAELs for the hormones in the pups on PND5 of the neurodevelopmental study (0.1, 1.0, and 3.0 mg/kg-day for T3, T4, and TSH , respectively), in this case, fall below (T3 and T4) or at about the same level (TSH) as those for motor activity. This may be because of the possible saturation effects at the symporter, with subsequent passive diffusion of perchlorate at higher concentrations, resulting in an initial steep slope for hormone changes, followed by a long shallow slope for changes at the higher concentrations, or may be, in part, because of differences in sensitivity among the measures, as discussed in Chapter 5. The pattern is consistent with that seen in the subchronic rat study (Springborn Laboratories, Inc., 1998) where there is a several-order-of-magnitude difference between the the initial changes in hormone status and the LOAEL for thyroid histology (10.0 mg/kg-day).

The exceptions to this pattern, however, were the standard histology measure of follicular epithelial cell hyperplasia in the Caldwell et al. (1995) 14-day study at 0.1 mg/kg-day and the same measure that also was observed in the pups on PND5 of the developmental neurotoxicity study (Argus Research Laboratories, Inc., 1998a). It could be argued that the Caldwell et al. (1995) data represent an initial response (14-day) in hormone homeostasis that comes to some level of tolerance by the 90-day time period, especially once the saturable aspect of the symporter is considered. However, the thyroid histopathology in the pups on PND5 likely is not caused by

this phenomenon and represents a cause for concern. The findings are consistent with those in guinea pigs of the Postel (1957) study and those of Lampe et al. (1967) in rabbits where the fetal changes in thyroid were shown to be independent and more sensitive than those of the dams. The thyroid histology LOAEL shifts to higher doses at PND10 and PND22, most likely because of decreases in dose via lactation, as discussed in Chapter 5.

The EPA thus chose to focus on the histology LOAEL at 0.1 mg/kg-day in the PND5 pups as the critical effect. In this case, this effect appears to precede the hormonal and neurobehavioral changes. The effect also occurs at doses lower than those associated with histology after longer term exposures in adults. Benchmark dose analyses, discussed in Section 6.2, were then attempted to refine this initial decision regarding the critical effect.

6.1.1.3 Possible Susceptibility

Based on the mode-of-action for perchlorate, the competitive inhibtion of iodide uptake, and the subsequent perturbation of thyroid hormone homeostastis, 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, although 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 and hypothyroid individuals or those anti-thyroid treated with drugs, may be others more susceptible than the general popultation to the effects of perchlorate.

6.1.2 Point-of-Departure Analysis

Because the EPA advocates the use of quantitative dose response modeling (Crump et al., 1995) and in the hopes of gaining more insight on how to integrate the thyroid hormone and histopathology data, a series of benchmark dose (BMD) analyses were performed on all the data from the studies in the testing strategy (Geller, 1998b). Table 6B-1 of Appendix 6B provides the functional forms used for the modeling of continuous data (hormone and motor activity analyses) and dichotomous data (thyroid histopathology). The BMD and the 95% lower limit (BMDL) were compared to NOAEL and LOAEL estimates derived by ANOVA.

For the continuous 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, 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.

The BMD and BMDL estimates were calculated for the incidence of standard thyroid histopathology measures using a BMR of a 10% incidence over control (i.e., BMD10 and BMDL10). The histopathology data were recoded to count any severity rating greater than zero as an incident of histopathology. Data were fit with the entire gamut of functions available through the EPA Benchmark Dose Software (Beta versions 0.96 and 1.1).

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, nonmonotonicities 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) is not reliable.

6.1.2.1 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 nonmonotonicity. 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, 1998b).

An alternative approach to calculating BMD estimates based on additional risk also was derived using the Kodell-West algorithm (Kodell-West, 1993). The 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, 1998b). The EPA estimates correspond to those previously reported, as shown in Table 6B-2 of Appendix 6B. The coefficients of the fits are provided in Table 6B-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.

6.1.2.2 U.S. Environmental Protection Agency Benchmark Dose Estimates for Standard Thyroid Histopathology

The BMD estimates were generated using a BMR of 10% increase in incidence over control. Figures 6-17 and 6-18 provide graphical illustration of the range of BMD and BMDL estimates fit to the standard histopathology of follicular epithelial cell hypertrophy/hyperplasia data for the Caldwell et al. (1995) 14-day study in rats, the subchronic study in rats (Springborn Laboratories, Inc., 1998), and the developmental neurotixicity study (Argus Research Laboratories, Inc., 1998a; York, a,b,c,d,e). Figures 6-19 and 6-20 summarize these same estimates as "box and whisker" plots.

As shown in Table 6B-4, all the functions provided adequate fits to the follicular epithelial cell hypertrophy data of Caldwell et al. (1995), and the BMD and BMDL were within a factor of 2 or 3 of one another.

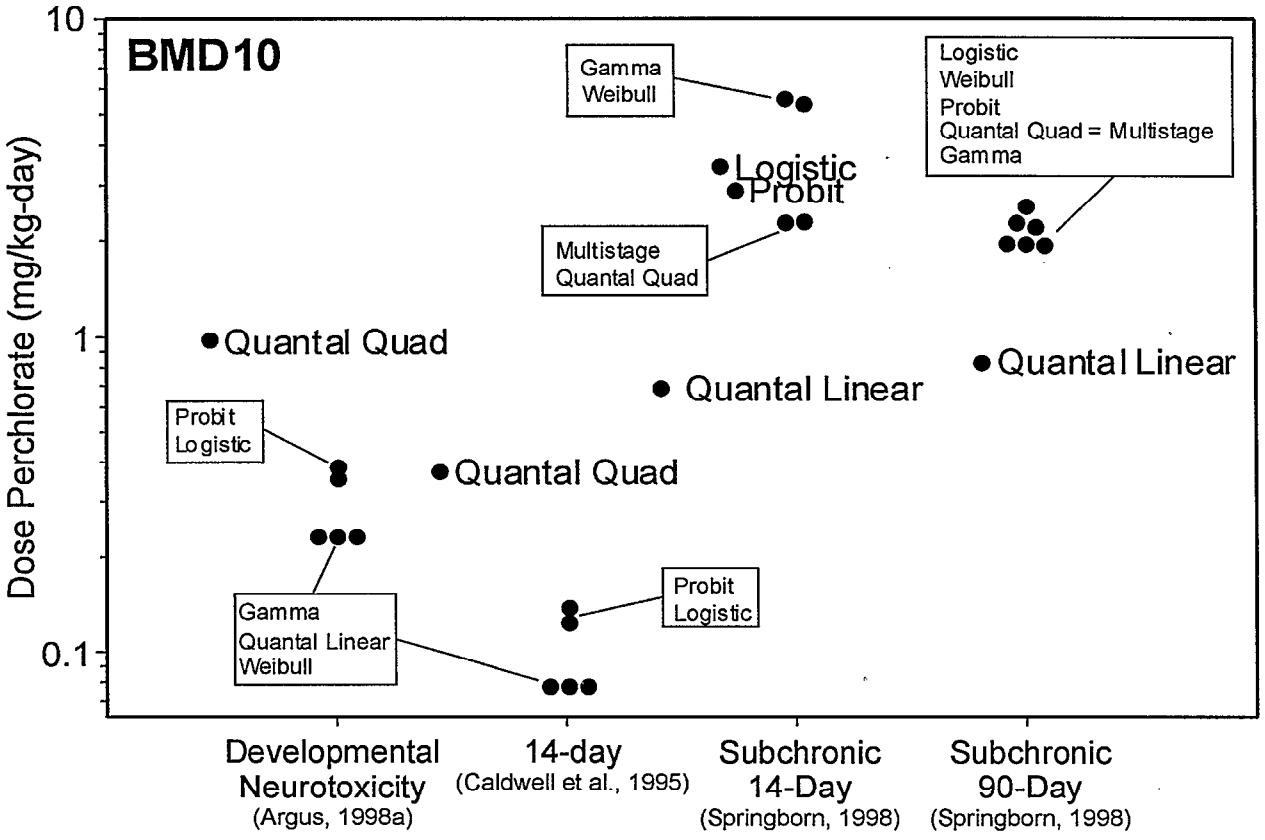


Figure 6-17. BMD estimates derived from fits of various model functions to the dose-response of incidence of standard histopathological detection in rats of follicular epithelial cell hypertrophy (Caldwell et al., 1995 14-day study) or hypertrophy/hyperplasia (Argus Research Laboratories, Inc., 1998a developmental neurotoxicity study; 14-day and 90-day data of Springborn Laboratories, Inc., 1998). A benchmark response level of 10% was used. Details on analyses and graphical displays can be found in Geller (1998b). Tables 6B-2 through 6B-7 in Appendix 6B provide statistical output.

Table 6B-5 shows that model fits yielded higher BMD and BMDL estimates for the data of the subchronic study (Springborn Laboratories, Inc., 1998) than for those of the other histopathology data, in keeping with the higher values noted previously for this study. For the 14-day time point, all of the BMD and BMDL estimates were within a factor of 5.5 of the NOAEL of 1.0 mg/kg-day. For the 90-day time point, the BMD and BMDL estimates were within a factor of 2.5 of the NOAEL.

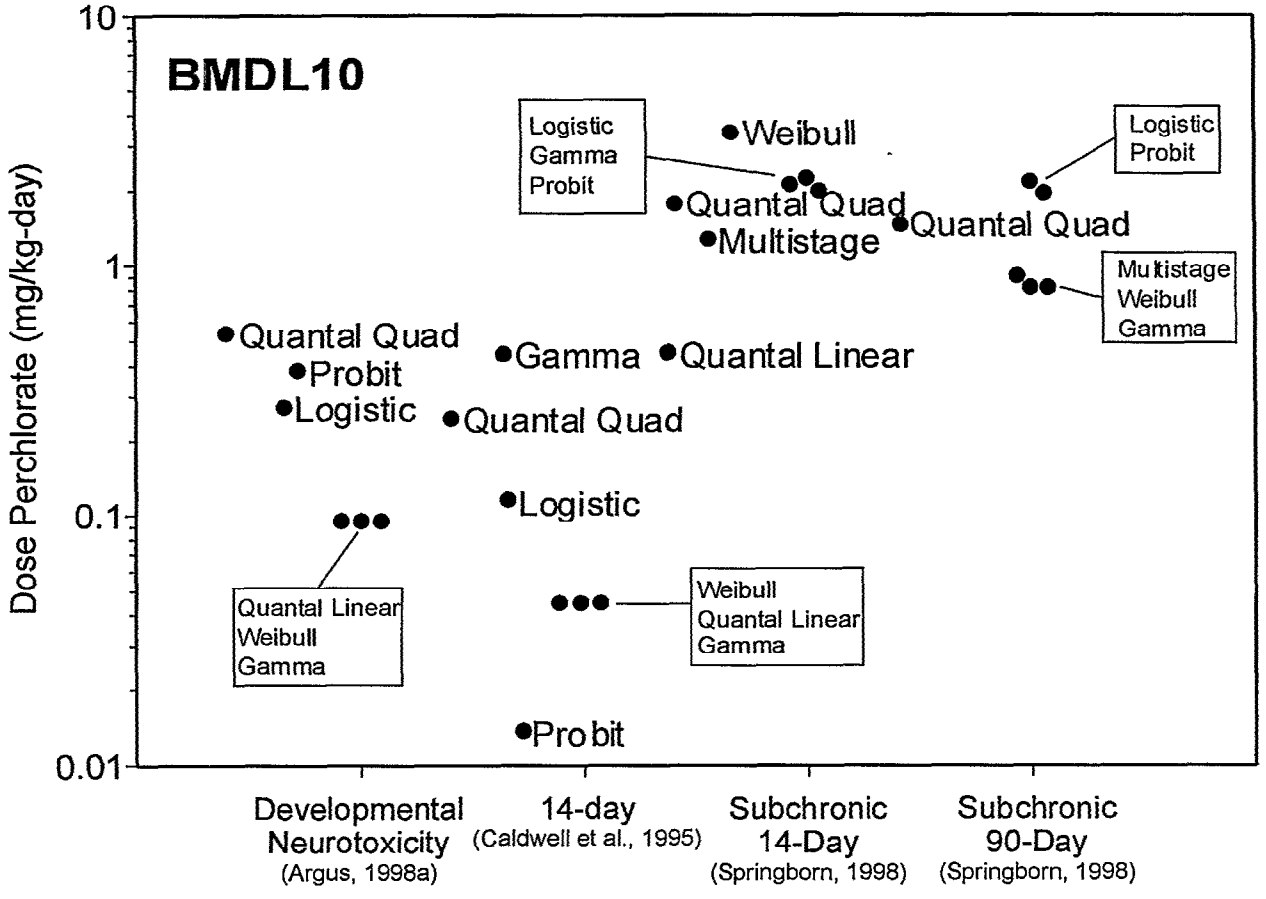


Figure 6-18. BMDL (95% lower confidence limit on BMD) estimates derived from fits of various model functions to the dose-response of incidence of standard histopathological detection in rats of follicular epithelial cell hypertrophy (Caldwell et al., 1995 14-day study) or hypertrophy/hyperplasia (Argus Research Laboratories, Inc., 1998a developmental neurotoxicity study; 14-day and 90-day data of Springborn Laboratories, Inc., 1998). A benchmark response level of 10% was used. Details on analyses and graphical displays can be found in Geller (1998b). Tables 6B-2 through 6B-7 in Appendix 6B provide statistical output.

The Quantal Linear, Weibull, and Gamma functions produced identical fits to the follicular epithelial cell hypertrophy data observed in the pups on PND5 of the neurodevelopmental study (Argus Research Laboratories, Inc., 1998a; York, a,b,c,d,e). Figure 6-21 provides the graphical display of the model fit. The BMD estimate was 0.234 mg/kg-day. The BMDL estimate was 0.1 mg/kg-day, identical to the LOAEL determined by ANOVA. The fits of the other functions

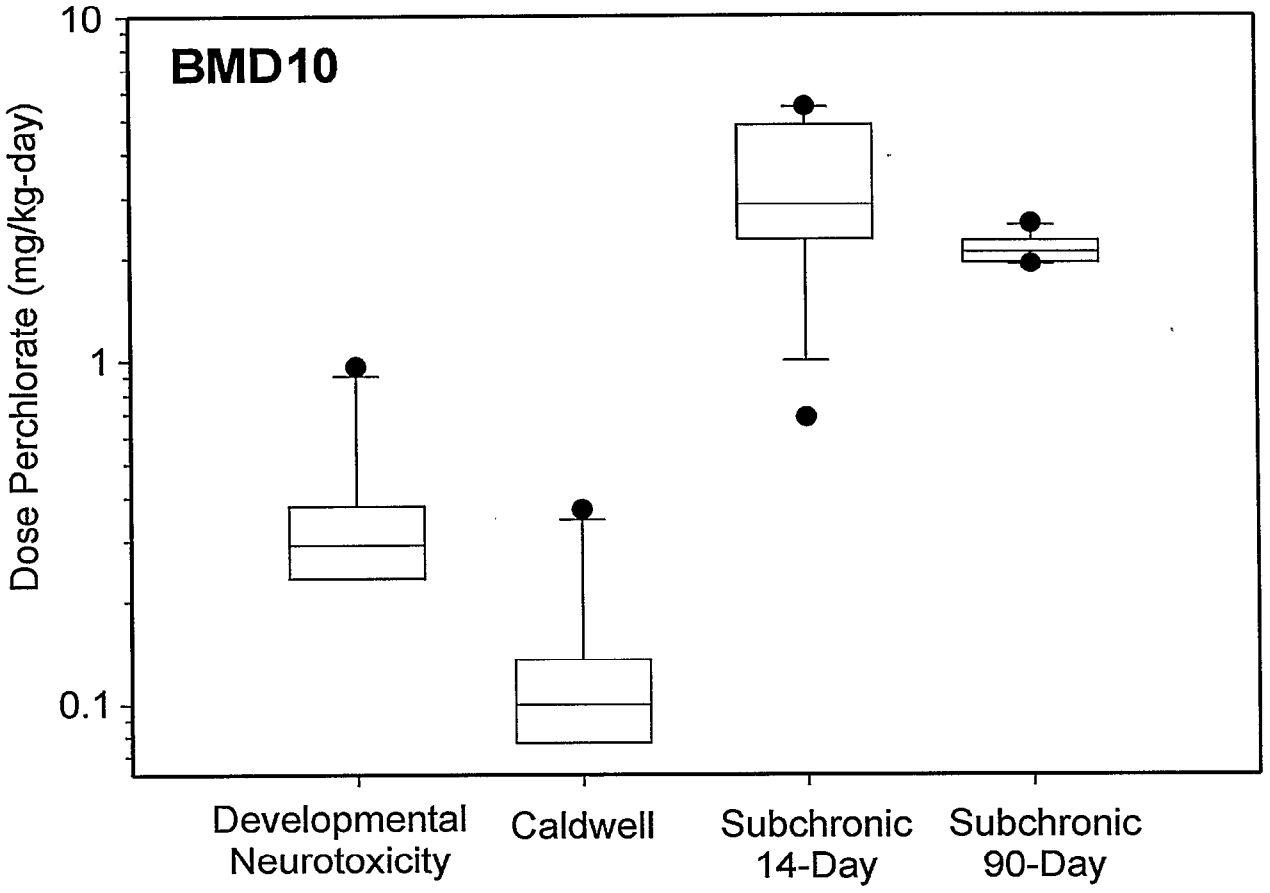


Figure 6-19. Box and whisker plots of BMD estimates derived from the fits of various model functions to the dose-response of incidence of standard histopathological detection in rats of follicular epithelial cell hypertrophy (Caldwell et al., 1995 14-day study) or hypertrophy/hyperplasia (Argus Research Laboratories, Inc., 1998a developmental neurotoxicity study; 14-day and 90-day data of Springborn Laboratories, Inc., 1998). A benchmark response level of 10% was used. Box outlines 25th and 75th percentiles with line at the median; whiskers illustrate the 10th and 90th percentiles and the points show outliers. Details on analyses and graphical displays can be found in Geller (1998b). Tables 6B-2 through 6B-7 in Appendix 6B provide statistical output.

yielded slightly higher values. Because the BMDL typically is used as a NOAEL surrogate for RfD derivation, the frequency of occurrence for each severity rating of the follicular epithelial cell hypertrophy was evaluated. Figure 6-22 shows this as a histogram for the developmental

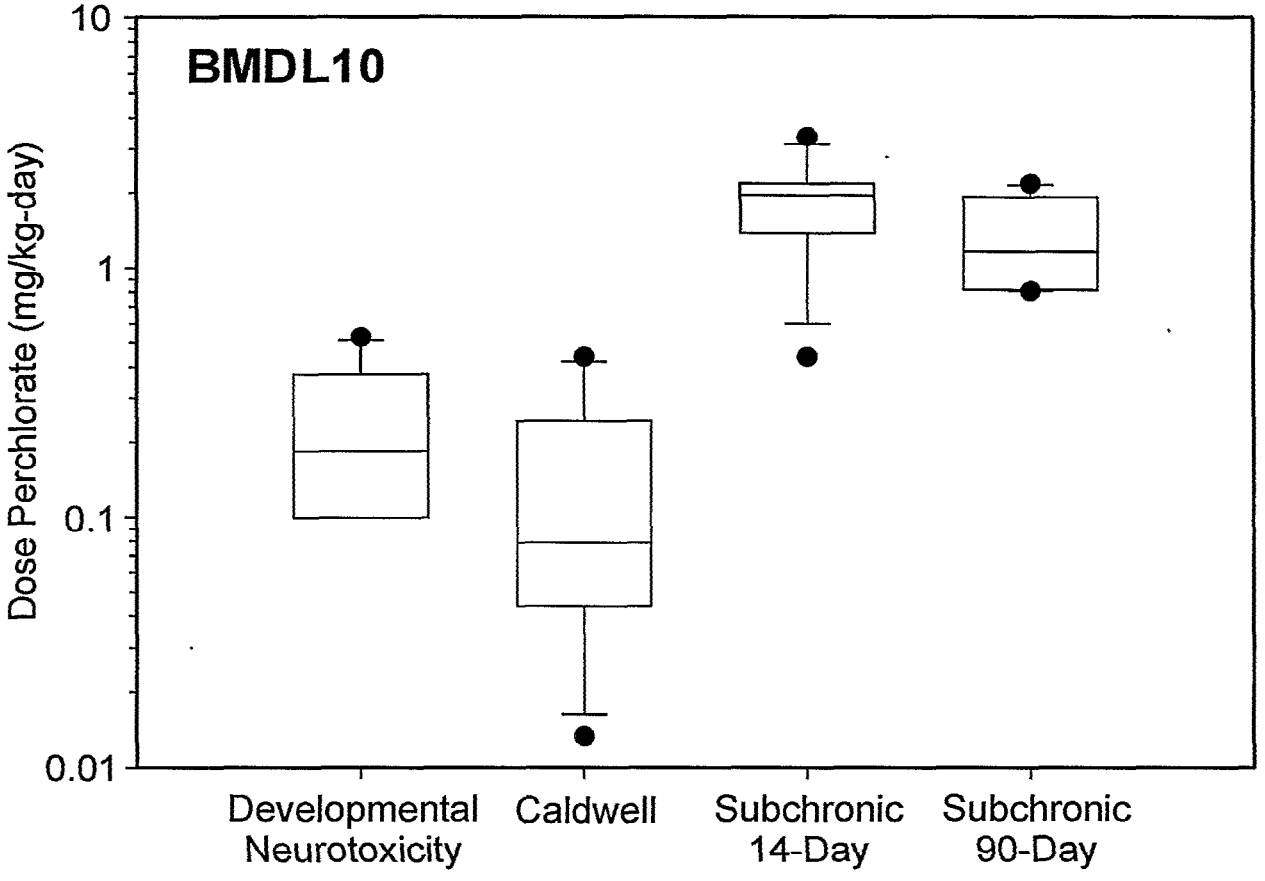


Figure 6-20. Box and whisker plots of BMDL (95% lower confidence limit on BMD) estimates derived from the fits of various model functions to the dose-response of incidence of standard histopathological detection in rats of follicular epithelial cell hypertrophy (Caldwell et al., 1995 14-day study) or hypertrophy/hyperplasia (Argus Research Laboratories, Inc., 1998a developmental neurotoxicity study; 14-day and 90-day data of Springborn Laboratories, Inc., 1998). Box outlines 25th and 75th percentiles with line at the median; whiskers illustrate the 10th and 90th percentiles and the points show outliers. Details on analyses and graphical displays can be found in Geller (1998b). Tables 6B-2 through 6B-7 in Appendix 6B provide statistical output.

neurotoxicity data. Note that, at the 0.1-mg/kg-day dose, most of the ratings are in the lower range, and that there is variability across the dose levels, although the latter may be because of the phenomenon of saturation of the symporter that has been suggested.

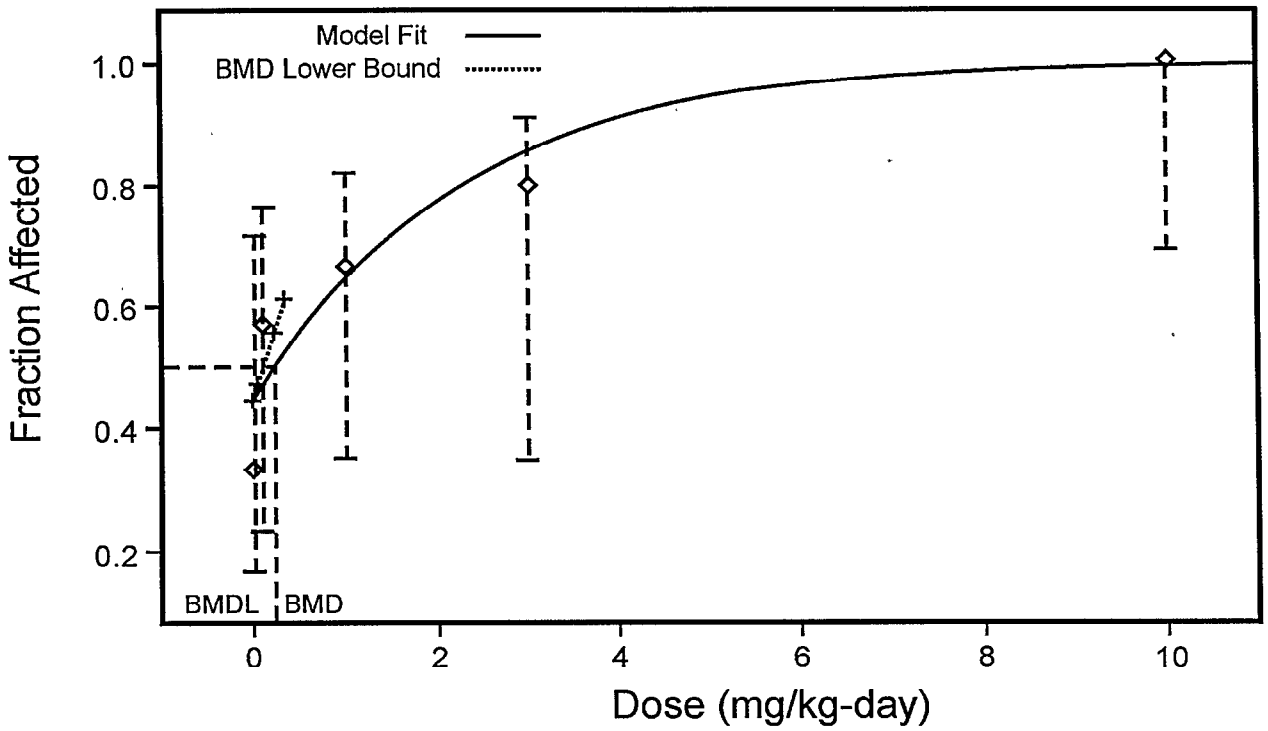


Figure 6-21. Model fit of the Quantal Linear/Weibull/Gamma functions to the litter-by-litter incidence of standard histopathological detection of follicular epithelial cell hypertrophy for the F1 rat pups on PND5 in the developmental neurotoxicity study. Data are shown by diamond symbols with 95% confidence limits. Model fit is solid line and lower limit is shown by dotted line. The BMD estimate is 0.234 and the BMDL is 0.10 mg/kg-day. Data of Argus Research Laboratories, Inc. (1998a) and York (1998a,b,c,d,e).

Figure 6-23 illustrates the same occurrence frequency analysis for the data of the Caldwell et al. (1995) study. Note that, at the 0.1-mg/kg-day dose, there are no ratings greater than 2, indicating a mild effect, and that the severity clearly is dose dependent, with 100% of the severity at the rating of 2 at the 22.53-mg/kg-day dose. This again emphasizes the apparent greater sensitivity of the fetus versus adult with respect to hormone perturbations and supports the 0.1-mg/kg-day value as one that is also protective for the development of subsequent neoplasia in adults.

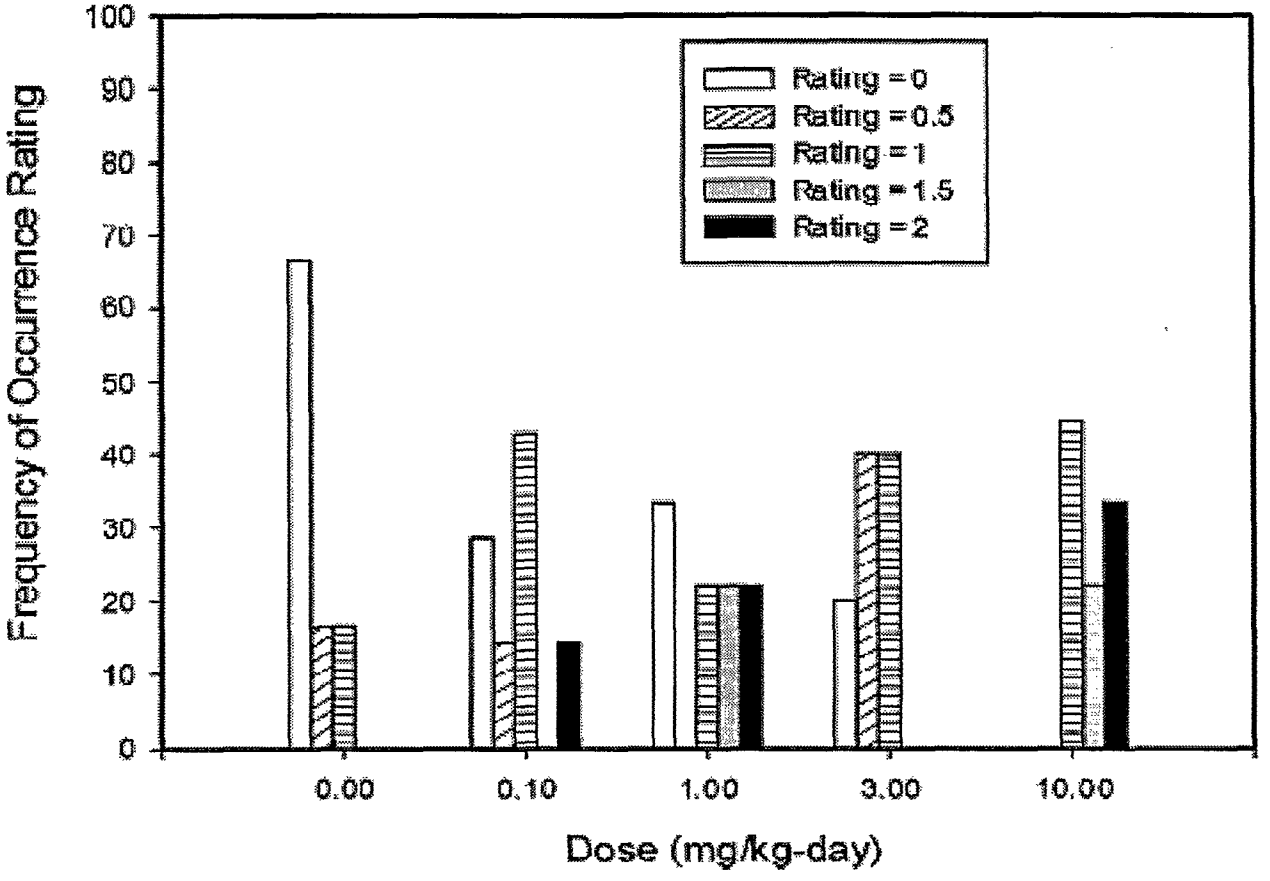


Figure 6-22. Frequency of occurrence per litter by dose group of each standard histopathological severity rating for follicular epithelial cell hypertrophy in the F1 rat pups on PND5 of the developmental neurotoxicity study. Data of Argus Research Laboratories, Inc. (1998a) and York (1998a,b,c,d,e).

6.1.2.3 U.S. Environmental Protection Agency Benchmark Dose Estimates for Triiodothyronine, Thyroxine, and Thyroid Stimulating 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

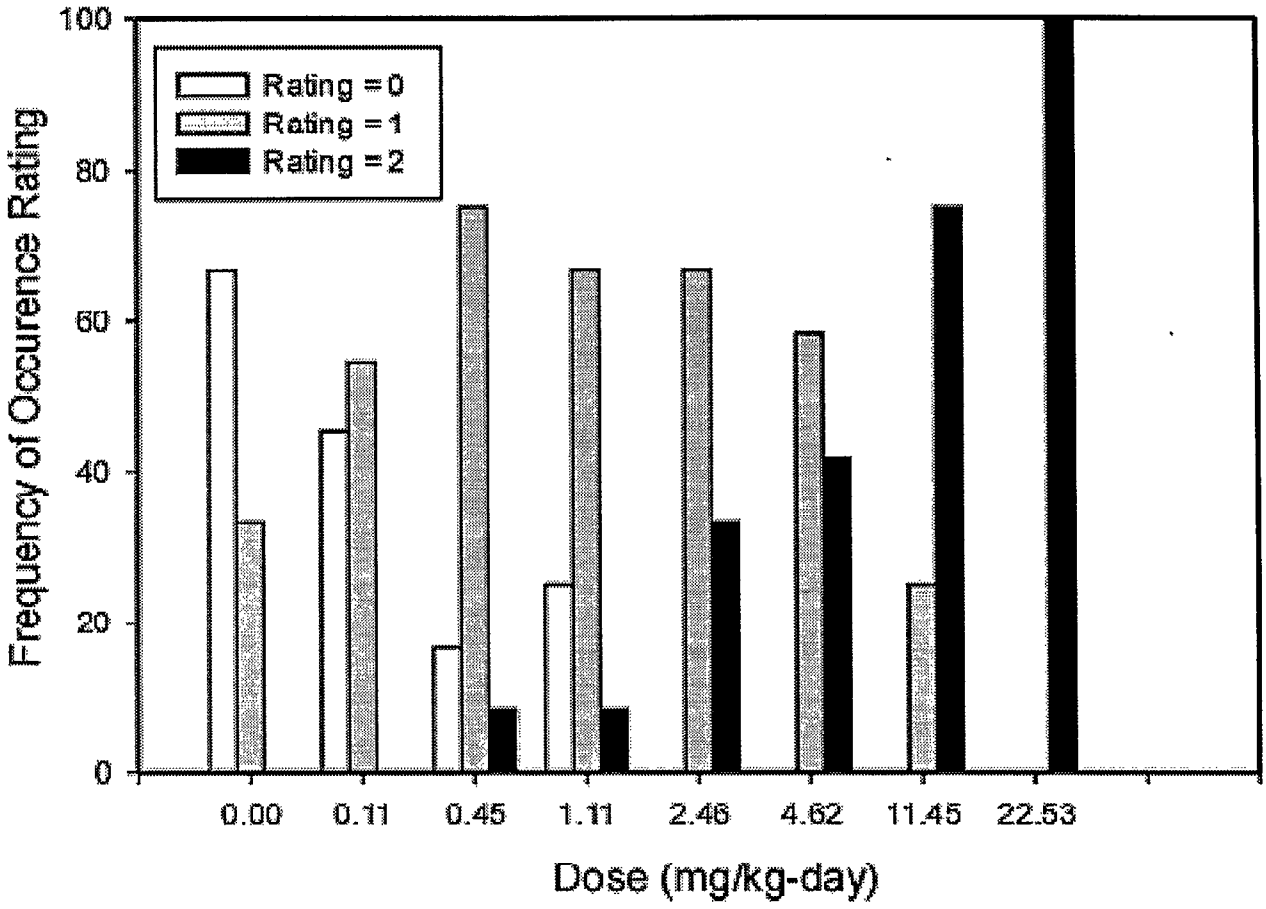


Figure 6-23. Frequency of occurrence by dose group of each standard histopathological severity rating for follicular epithelial cell hypertrophy for the rats in the 14-day study of Caldwell et al. (1995). Data of Channel (1998a).

approaches zero. 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) attempting 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 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 equisite sensitivity to very

low doses of perchlorate. This suggests that to fit models with nonsupralinear slopes, lower doses need to be tested.

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.

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 (Figures 6-24 and 6-25), 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 2 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 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 (Figure 6-25) 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.

6.1.2.4 U.S. Environmental Protection Agency Benchmark Dose Estimates for Motor Activity

There were no statistically significant effects in the motor activity data from PND14 pups in the developmental neurotoxicity study (Argus Research Laboratories, Inc., 1998a). 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

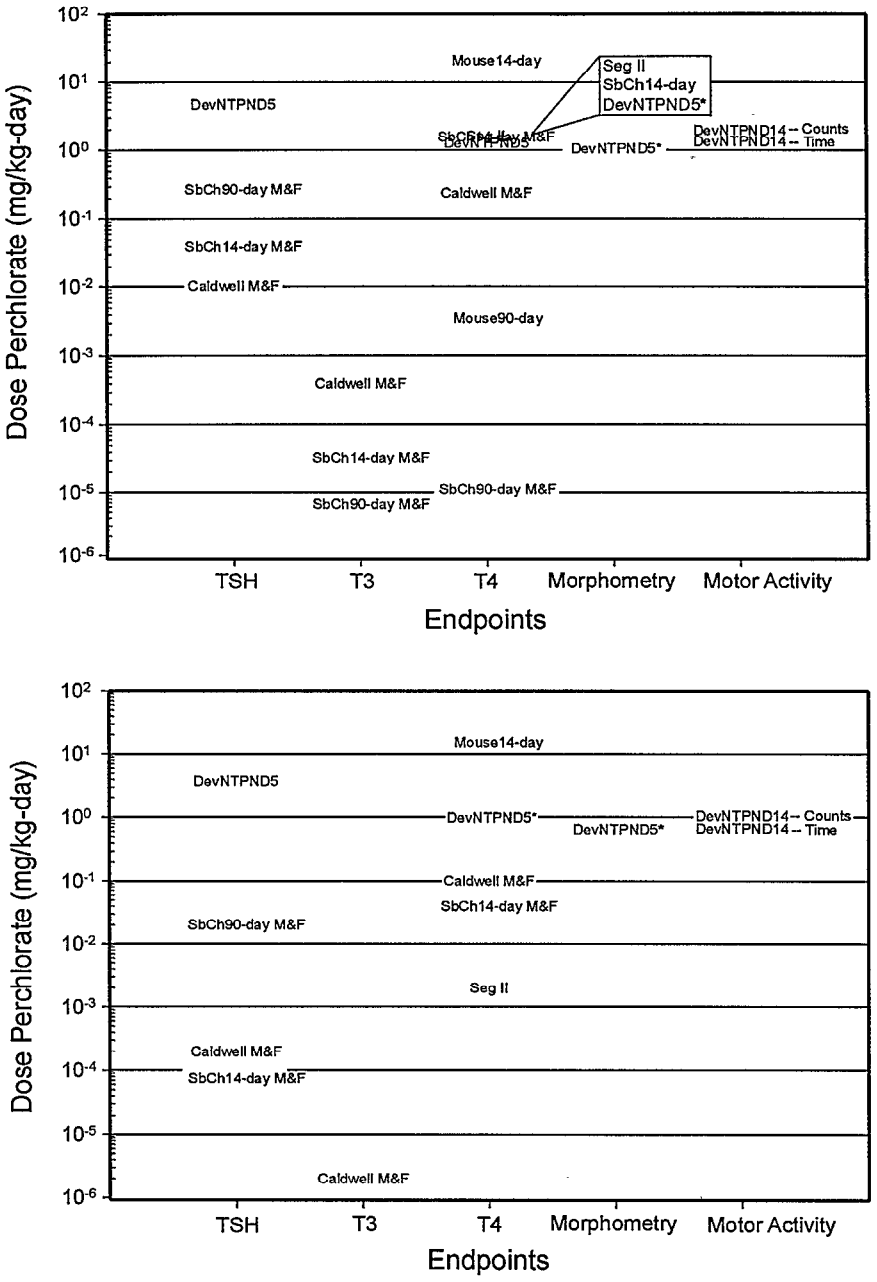


Figure 6-24. BMD (top panel) and lower 95% confidence limit BMDL (bottom panel) estimates derived from fits of various model functions to the dose-response data for hormone, thyroid morphometry, and motor activity endpoints from the various studies in the mode-of-action testing strategy. Data for rats include those of Caldwell et al. (1995), Channel (1998a), Crofton (1998a), Springborn Laboratories, Inc. (1998), Argus Research Laboratories, Inc. (1998a); for rabbits those of Argus Research Laboratories, Inc. (1998b) and for mice those of Keil et al. (1998). Details on analyses and graphical displays can be found in Geller (1998b). Tables 6B-8 through 6B-18 in Appendix 6B provide statistical output. *Indicates nonmontonic fit to the data.

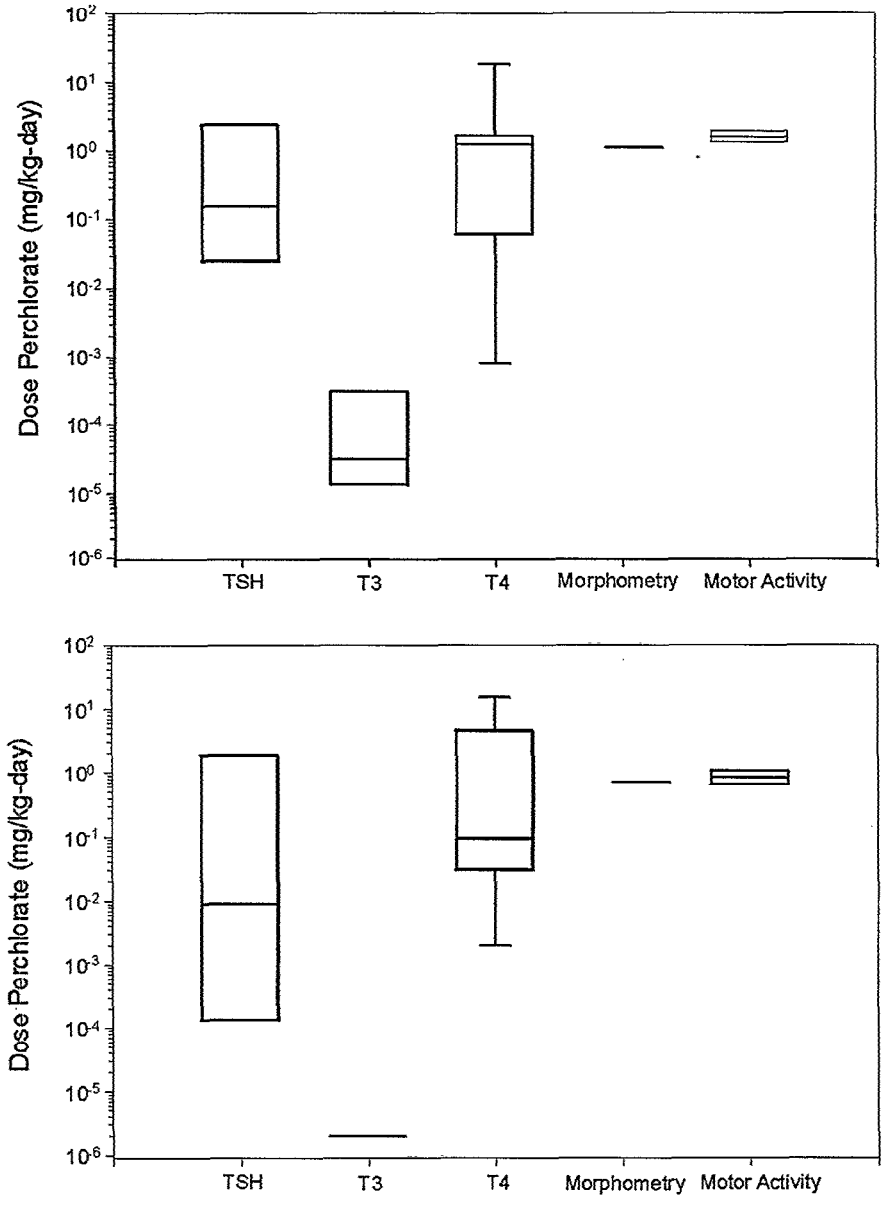


Figure 6-25. Box and whisker plot of BMD (top panel) and lower 95% confidence limit BMDL (bottom panel) estimates derived from the fits of various model functions to the dose-response data for hormone, thyroid morphometry, and motor activity endpoints from the various studies in the mode-of-action testing strategy. Data for rats include those of Caldwell et al. (1995), Channel (1998a), Crofton (1998a), Springborn (1998), Argus Research Laboratories, Inc. (1998a); for rabbits those of Argus Research Laboratories, Inc. (1998b) and for mice those of Keil et al. (1998). Box outlines 25th and 75th percentiles with line at the median; whiskers illustrate the 10th and 90th percentiles and the points show outliers. Details on analyses and graphical displays can be found in Geller (1998b). Tables 6B-8 through 6B-18 in Appendix 6B provide statistical output.

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 (see Section 5.2.3.4, Figure 5-14). These BMD analyses bring the motor activity NOAEL more within the range of the T3 and T4 NOAEL and below that for TSH.

6.1.2.5 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. EPA was able to successfully model the hormone data, however, and 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., 1999). These analyses are being considered, as well as Bayesian meta-analysis of the data sets.

Given that the BMDL estimate was 0.1 mg/kg-day, and that the contingency table analysis confirmed statistical significance of the 0.1-mg/kg-day level for the thyroid histology in the PND5 pups, EPA remained comfortable with using this as a point of departure. It was designated as a minimal LOAEL because of the uncertainty in modeling and because if fell above the LOAEL values based on ANOVA for the hormones.

6.1.3 Application of Uncertainty Factors

Much of the uncertainty surrounding the provisional RfD has been addressed by the data provided by the testing strategy. A partial uncertainty factor (UF) of 3 for database was retained, however, out of concern for the outstanding data from the two-generation reproductive study and the immunotoxicity data. This was warranted because effects were suggested in both of these studies. Although the dose spacing in these studies may be such that the impact of the new data will be at higher doses than that already chosen as the point of departure, the UF applied for the database is a factor applied for uncertainty (i.e., this can be rectified by providing the appropriate information).

The subchronic-to-chronic UF was not considered necessary because the mode of action of perchlorate indicates that, if the point of departure is based on levels below which significant precursor lesions occur, then it will be protective of "downstream" events. The level chosen in the pups (0.1 mg/kg-day) is supported by minimal thyroid histopathology effects seen in the Caldwell et al. (1995) study and is below that at which thyroid histopathology was seen in the subchronic (Springborn Laboratories, Inc., 1998) study. Both the thyroid lesions and the hormone changes have been shown to be reversible after cessation of exposure. The relatively short half-life of perchlorate also argues against the application of this factor.

A partial factor of 3 was applied to the minimal LOAEL because the histopathology seen was mild and the BMDL, typically used as a NOAEL surrogate for RfD derivation was at 0.1 mg/kg-day. The contingency analysis indicated small statistical significance. The concern was that the fetus may be a susceptible stage of development. The hormone analyses indicate a range of estimates, some below the 0.1-mg/kg level, but how to interpret these in light of the variability across the studies, and particularly to the sharp increase in slope at the lower doses. Some internal reviewers did not agree with the use of this factor and felt that the value should be designated a NOAEL. However, the majority of reviewers felt that a factor should be applied for intrahuman pharmacodynamics (see below). Based on these considerations, the factor was believed also to account for potential pharmacodynamic differences in intrahuman variability as well because it is the hormone data that mediate the observed histopathology. The issue will be addressed best by development of a PBPK model to provide alternative dose metrics for the adults (e.g., percent of symporter inhibition as opposed to administered dose) and an extended PBPK model that addresses thyroid hormone dosimetry in the pregnant rat, placental transfer, fetus, lactating rat, and nursing pup (e.g., percent of inhibition in the pup, as opposed to the dose administered to the doe).

Figure 6-26 illustrates schematically that the interspecies and intraspecies UFs embody attributes of both uncertainty and variability. Obviously there is uncertainty in the extrapolation from rat to human, but also variability in the parameters (e.g., dose) used to characterize the response. Variability across humans typically is applied to account for potentially susceptible portions of the population. As shown in Figure 6-27 (Jarabek, 1995b), both of these factors typically are broken into components of approximately three each for pharmacokinetics

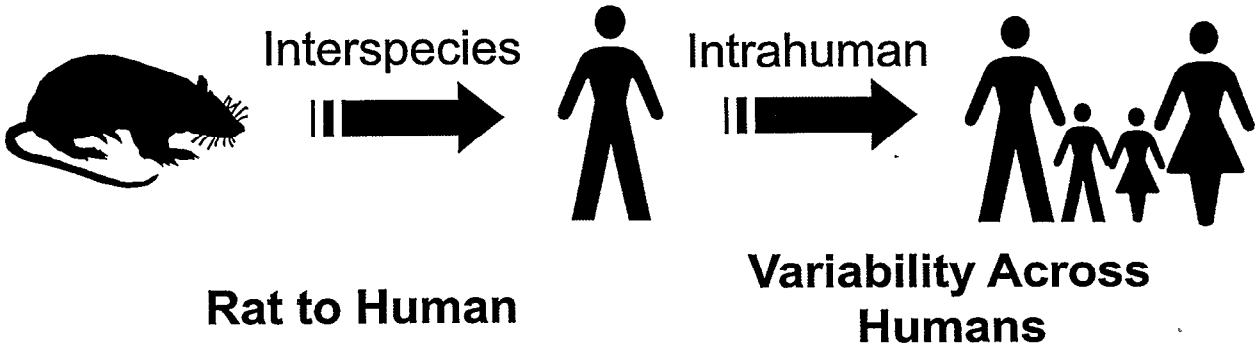


Figure 6-26. Consideration of uncertainty and variability influence interspecies and intrahuman extrapolation.

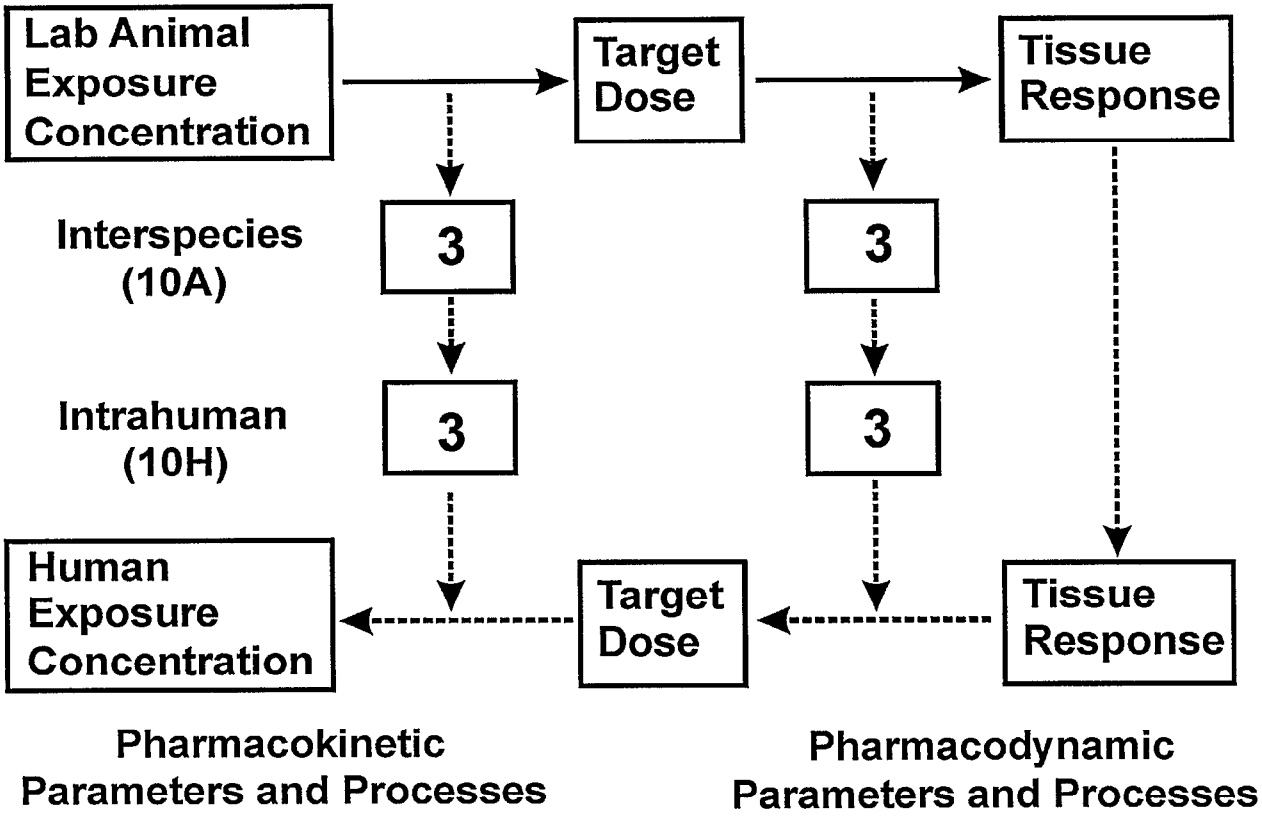


Figure 6-27. Schematic of uncertainty factor components incorporated into exposure-dose-response characterization for interspecies and intrahuman extrapolations.

Source: Jarabek (1995b).

(toxicokinetics) and pharmacodynamic (toxicodynamic) processes. This scheme is consistent with that used by the World Health Organization.

In the case of the proposed mode-of-action model, the component for the pharmacodynamic portion of the model was thought unnecessary in the interspecies UF because it is fairly well established that rats are more sensitive to thyroid-pituitary perturbations because of plasma protein binding differences of the thyroid hormones and TSH levels. A partial factor of 3 was retained, however, for the pharmacokinetic portion of the UF because there are extremely limited data on perchlorate kinetics. The key piece of information that would obviate this UF is to establish whether or not the rat symporter is more or less sensitive than that of the human to the competitive inhibition of iodide uptake by perchlorate. A partial factor of 3 also is retained for the pharmacokinetic portion of the intrahuman UF.

The pharmacodynamic portion was reduced because the animal model used is for a fetal effect, and it is likely that the human hormone homeostasis may have more stability. The factor is also addressed by the controversial application of the 3 to the minimal LOAEL in rat pups because some reviewers felt that this factor was not necessary. Overlap among the factors used in the RfD/RfC methods always has been acknowledged. In the overall assessment, a composite factor of 100 was viewed as approriate by the assessment team, although several internal reviewers argued for a factor of at least 300. Narrowing the range of this composite factor awaits the pending data and mechanistic information.

Parceling of the UF into these components (as shown in Figure 6-27) is limited when a database such as the one for perchlorate begins to amass a significant amount of mechanistic information. At some point, models can more accurately describe the mechanistic determinants (e.g., of pharmacokinetics) for which these factors are applied (Jarabek, 1995a). Figure 6-28 provides a schematic for a physiologically based pharmacokinetic (PBPK) model, which is under under development at AFRL/HEST, that likely will provide a more accurate description of the interspecies kinetics, including the species differences on perchlorate inhibition of iodide uptake (Fisher, 1998b). The model will modify previously published pregnancy and lactation models in the rat (Fisher et al., 1989,1990). No quantitative models of this type exist to describe iodide uptake and hormone formation, although Kohn et al. (1996) developed one for thyroid hormones only and the effects of dioxin. The model will be able to address the question raised in Chapter 4 regarding the diffusion of iodide into the lumen when there is significant iodide excess and that

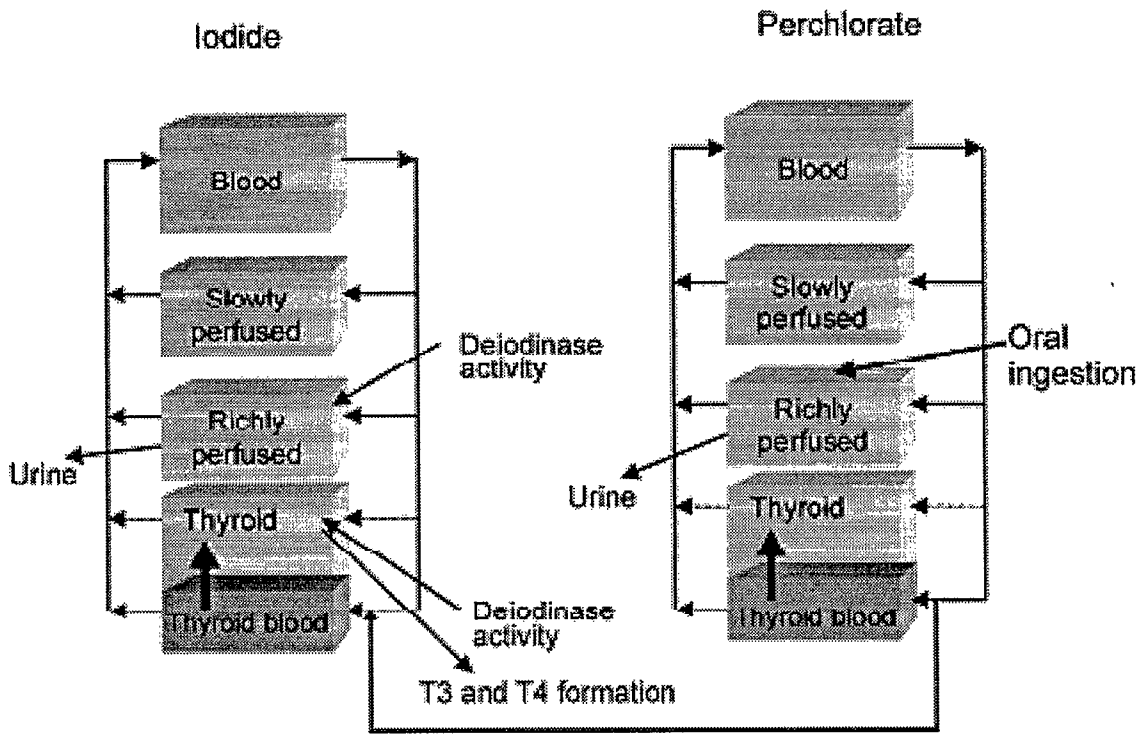


Figure 6-28. Schematic of PBPK model that is under development to provide mechanistic data and improve quantitative interspecies extrapolation and possibly intrahuman variability.

Source: Fisher (1998b).

of the perchlorate-induced efflux from the lumen. This will be an important aspect of describing any potential for nonlinearities and may help to map the outcome measures more meaningfully. Some preliminary data from this model will be available in mid-December, and results will be reported in the external review draft. A presentation at the workshop on the progress on model validation is anticipated.

6.1.4 Designation of Confidence Levels

Confidence in the principal study is medium. The dose level of 0.1 mg/kg-day was the lowest tested, and it was determined to be a minimal LOAEL (not NOAEL). The small sample

size for the critical effect also reduces the confidence in the study. The confidence at this time in the database is medium, given the pending two-generation reproductive and immunotoxicity data. Confidence is likely to be high once these deficiencies are rectified. Because confidence in the database takes precedence in setting the overall confidence in the RfD, the confidence in the RfD currently is medium.

6.1.5 Operational Derivation

The operational derivation of the RfD is as follows: the composite factor of 100 (partial factors of 3 each for database and extrapolations of a minimal LOAEL and interspecies and intrahuman variability) is applied to the LOAEL of 0.1 mg/kg-day for thyroid histology observed in the pups of the neurodevelopmental study on PND5 (Argus Research Laboratories, Inc., 1998a). According to Dollarhide (1998), who spoke with Argus laboratory on behalf of the sponsor (PSG), the reported doses were ammonium perchlorate and not the anion itself. Thus, an adjustment for percent of the molecular weight of the salt from ammonium (15.35%) also must be made. Further, because the analytical methods measure the anion concentration in environmental samples, this is the appropriate expression for the RfD to make valid comparisons for risk characterization. Thus, the derivation for an RfD for the perchlorate anion as itself is as follows:

$$0.1 \text{ mg/kg-day} \times 0.85 / 100 = 0.0009 \text{ mg/kg-day}. \qquad (6\text{-}1)$$

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.

6.2 INHALATION REFERENCE CONCENTRATION

Derivation of an inhalation reference concentration is precluded because there are no inhalation data available to characterize dose-response or the portal-of-entry modulation of internal dose. However, EPA has been questioned as to whether the potential for inhalation

exposure of perchlorate from showering with contaminated water is 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.

6.3 CANCER ASSESSMENT

The EPA 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 6-3 lists the default procedures for thyroid carcinogens that would be used. What has been proposed in this assessment is the harmonization of the "noncancer" and "cancer" approaches because the target tissue is the thyroid and the utilization of the point of departure for the oral RfD as a protective estimate of subsequent cancer development as well because it is based the follicular cell hypertrophy, which is one of the required lesions to demonstrate antithyroid activity. Table 6-4 shows the types of data required.

TABLE 6-3. DEFAULT DOSE-RESPONSE PROCEDURES FOR THYROID CARCINOGENS

	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

Source: U.S. Environmental Protection Agency (1998a).

TABLE 6-4. DATA DEMONSTRATING ANTITHYROID ACTIVITY

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	

Source: U.S. Environmental Protection Agency (1998a).

Perchlorate has demonstrated clearly an effect in both adult and fetal stages in thyroid follicular epithelial cell hypertrophy/hyperplasia, 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 to the efflux (discharge) phenomenon. Dose-correlations in this case were not with tumors but rather for precursor lesions (hypertrophy, hyperplasia, and decreased follicular lumen size). Reversibility has been demonstrated in weight, hypertrophy, hyperplasia, and thyroid and pituitary hormones in the 30-day recovery period after the 90-day inhalation study in rats and in T4 levels in 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. There was a progression within the 90-day study, however, between the 14- and 90-day time points.

Analyses of other anions have fairly well established that the mode of action of perchlorate is based on it being an anion that is recognized by the Na^+/I^- symporter.

The genotoxicity battery has fairly well established that perchlorate is not genotoxic, although EPA will remain only slightly equivocal on this issue until the repeat of the mouse lymphoma and micronuclei assays. Perchlorate is not likely to be genotoxic.

Thus, the RfD derived herein also should afford protection for any potential carcinogenicity of ingested perchlorate, and the estimate should be considered to harmonize noncancer and cancer estimates for oral exposures.

APPENDIX 6A

Correlation Tables for Figures 6-3 Through 6-16

TABLE 6A-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 6A-2. SPEARMAN'S r_S CORRELATIONS (n = 95) BETWEEN THE RANK ORDER OF HORMONE LEVELS AND THE STANDARD HISTOLOGICAL SEVERITY RATING OF FOLLICULAR EPITHELIAL CELL HYPERTROPHY (FH) OR DECREASE IN FOLLICULAR LUMEN SIZE (LS) IN RATS OF THE CALDWELL et al. (1995) 14-DAY STUDY

	FH	LS
T3	−0.67 p = 0.0001	−0.74 p = 0.0001
T4	−0.66 p = 0.0001	−0.70 p = 0.0001
TSH	0.71 p = 0.0001	0.79 p = 0.0001
FH	1.00 p = 0.00	0.75 p = 0.0001

TABLE 6A-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 6A-4. SPEARMAN'S r_S CORRELATIONS (n = 223) BETWEEN THE RANK ORDER OF HORMONE LEVELS AND THE STANDARD HISTOLOGICAL SEVERITY RATING OF FOLLICULAR EPITHELIAL CELL HYPERTROPHY/HYPERPLASIA (FH) OR DECREASE IN FOLLICULAR LUMEN SIZE (LS) FOR THE COMBINED 14- AND 90-DAY DATA OF THE SPRINGBORN LABORATORIES, INC. (1998) SUBCHRONIC RAT STUDY

	FH
T3	-0.256 p = 0.0001
T4	-0.239 p = 0.0003
TSH	0.365 p = 0.0001

TABLE 6A-5. 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 6A-6. SPEARMAN'S r_S CORRELATIONS (n = 104) BETWEEN THE RANK ORDER OF HORMONE LEVELS AND THE STANDARD HISTOLOGICAL SEVERITY RATING OF FOLLICULAR EPITHELIAL CELL HYPERTROPHY/HYPERPLASIA (FH) FOR THE 14-DAY DATA OF THE SPRINGBORN LABORATORIES, INC. (1998) SUBCHRONIC RAT STUDY

	FH
T3	−0.24 p = 0.01
T4	−0.27 p = 0.005
TSH	0.463 p = 0.0001

TABLE 6A-7. 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 6A-8. SPEARMAN'S r_S CORRELATIONS (n = 119) BETWEEN THE RANK ORDER OF HORMONE LEVELS AND THE STANDARD HISTOLOGICAL SEVERITY RATING OF FOLLICULAR EPITHELIAL CELL HYPERTROPHY/HYPERPLASIA (FH) FOR THE 90-DAY DATA OF THE SPRINGBORN LABORATORIES, INC. (1998) SUBCHRONIC RAT STUDY

	FH
T3	−0.27 p = 0.003
T4	−0.44 p = 0.0001
TSH	0.295 p = 0.001

TABLE 6A-9. 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

TABLE 6A-10. SPEARMAN'S r_S CORRELATIONS (n = 22 to 27) BETWEEN THE RANK ORDER OF HORMONE LEVELS AND THE STANDARD HISTOLOGICAL SEVERITY RATING OF FOLLICULAR EPITHELIAL CELL HYPERTROPHY (FH) FOR THE F1 RAT PUPS ON PND5 IN THE DEVELOPMENTAL NEUROTOXICITY STUDY
(Argus Research Laboratories, Inc., 1998a)

	FH	LA
T3 (n = 23)	-0.33 p = 0.12	-0.44 p = 0.03
T4 (n = 27)	-0.35 p = 0.07	-0.505 p = 0.007
TSH (n = 36)	0.39 p = 0.019	0.28 p = 0.10

TABLE 6A-11. PEARSON'S r CORRELATIONS (n = 140) BETWEEN THYROID HORMONES AND TSH IN RABBITS ON GESTATION DAY 29 OF THE DEVELOPMENTAL STUDY
(Argus Research Laboratories, Inc., 1998c)

	T3	T4	TSH
T3	1.00 p = 0.00	0.52 p = 0.0001	0.20 p = 0.016
T4		1.00 p = 0.00	0.088 p = 0.302
TSH			1.00 p = 0.00

TABLE 6A-12. SPEARMAN'S r_S CORRELATIONS (n = 140) BETWEEN THE RANK ORDER OF HORMONE LEVELS AND THE STANDARD HISTOLOGICAL SEVERITY RATING OF FOLLICULAR EPITHELIAL CELL HYPERTROPHY (FH) FOR RABBITS ON GESTATION DAY 29 OF THE DEVELOPMENTAL STUDY
(Argus Research Laboratories, Inc., 1998c)

	FH
T3	0.034 p = 0.685
T4	-0.166 p = 0.05
TSH	0.074 p = 0.385

APPENDIX 6B

Benchmark Dose Statistics

TABLE 6B-1. FUNCTIONS USED IN BENCHMARK DOSE (BMD) MODELING

Models for Continuous Data		
	Power function	$f(dose) = control + slope * dose^{power}$
	Polynomial function (includes linear and quadratic)	$f(dose) = \beta 0 + \beta 1 * dose + \beta 2 * dose^2 + \ldots$
Models for Dichotomous Data		
	Gamma	$P(response) = bckgrd + (1\text{-}bckgrd) * CumGamma\ (slope * dose^{power})$
	Logistic	$P(response) = 1/(1+e^{(-\ intercept\ -\ slope*dose)})$
	Probit	$P(response) = CumNorm(intercept + slope*dose)$
	Quantal Linear	$P(response) = bckgrd + (1 - bckgrd)*(1-e^{(-slope*dose)})$
	Quantal Quadratic	$P(response) = bckgrd + (1 - bckgrd)*(1-e^{(-slope*dose^{2})})$
	Weibull	$P(response) = bckgrd + (1 - bckgrd)*(1-e^{(-slope*dose\ power)})$
	Multistage	$P(response) = bckgrd + (1 - bckgrd)*(1-e^{(-(\beta 1*dose + \beta 2*dose^{2} + \ldots)})$

TABLE 6B-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, 1998b).
[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 6B-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 Fit[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, 1998b). Identical coefficients were generated by using EPA BMD software.
[a]p > 0.05 denotes significant fit. Goodness-of-fit derived using -2 log (likelihood ratio) test from EPA BMD software (see Geller, 1998b).

TABLE 6B-4. BENCHMARK DOSE (BMD) AND BMD 95% LOWER LIMIT (BMDL) ESTIMATES OF THE INCIDENCE OF FOLLICULAR EPITHELIAL CELL HYPERTROPHY IN THE CALDWELL et al. (1995) 14-DAY STUDY (Benchmark response based on 10% extra risk.)

Model	p of fit,df	BMD	BMDL	LOAEL	BMD: LOAEL	BMDL: LOAEL
Gamma	0.75, 6	0.077	0.044	0.1	0.77	0.44
Logistic	0.70, 6	0.123	0.115	0.1	1.23	1.15
Probit	0.71, 6	0.135	0.0134	0.1	1.35	0.134
Quantal Linear	0.75, 6	0.077	0.044	0.1	0.77	0.44
Quantal Quadratic	0.54, 6	0.37	0.243	0.1	3.70	2.43
Weibull	0.746, 6	0.077	0.044	0.1	0.77	0.44

TABLE 6B-5. BENCHMARK DOSE (BMD) AND BMD 95% LOWER LIMIT (BMDL) ESTIMATES OF THE INCIDENCE OF FOLLICULAR EPITHELIAL CELL HYPERTROPHY/HYPERPLASIA FOR THE 14-DAY TIME POINT OF THE SPRINGBORN LABORATORIES, INC. (1998) SUBCHRONIC STUDY
(Benchmark response based on 10% extra risk.)

Model	p of fit,df	BMD	BMDL	NOAEL	BMD: NOAEL	BMDL: NOAEL
Gamma	0.39, 3	5.32	2.10	1.0	5.32	2.10
Logistic	0.43, 4	3.37	2.21	1.0	3.37	2.21
Probit	0.40, 4	2.85	1.96	1.0	2.85	1.96
Quantal Linear	0.10, 4	0.683	0.44	1.0	0.683	0.44
Quantal Quadratic	0.49, 4	2.25	1.76	1.0	2.25	1.76
Weibull	0.39, 3	5.45	3.37	1.0	5.45	3.37
Multistage	0.33, 3	2.25	1.25	1.0	2.25	1.25

TABLE 6B-6. BENCHMARK DOSE (BMD) AND BMD 95% LOWER LIMIT (BMDL) ESTIMATES OF THE INCIDENCE OF FOLLICULAR EPITHELIAL CELL HYPERTROPHY/HYPERPLASIA FOR THE 90-DAY TIME POINT OF THE SPRINGBORN LABORATORIES, INC. (1998) SUBCHRONIC STUDY
(Benchmark response based on 10% extra risk.)

Model	p of fit,df	BMD	BMDL	NOAEL	BMD: NOAEL	BMDL: NOAEL
Gamma	0.14, 3	1.91	0.81	1.0	1.91	0.81
Logistic	0.21, 4	2.50	2.18	1.0	2.50	2.18
Probit	0.18, 4	2.20	1.93	1.0	2.20	1.93
Quantal Linear	0.02, 4	0.55	0.36	1.0	0.55	0.36
Quantal Quadratic	0.22, 4	1.93	1.46	1.0	1.93	1.46
Weibull	0.14, 3	2.23	0.82	1.0	2.23	0.82
Multistage	0.13, 3	1.93	0.87	1.0	1.93	0.87

TABLE 6B-7. BENCHMARK DOSE (BMD) AND BMD 95% LOWER LIMIT (BMDL) ESTIMATES OF THE INCIDENCE OF FOLLICULAR EPITHELIAL CELL HYPERTROPHY IN THE F1 PUPS ON PND5 FROM THE DEVELOPMENTAL NEUROTOXICITY STUDY (Argus Research Laboratories, Inc., 1998a) (Benchmark response based on 10% extra risk.)

Model	p of fit,df	BMD	BMDL	LOAEL	BMD: LOAEL	BMDL: LOAEL
Gamma	0.85, 3	0.234	0.10	0.1	2.34	1.0
Logistic	0.84, 3	0.35	0.27	0.1	3.5	2.7
Probit	0.84, 3	0.379	0.376	0.1	3.79	3.76
Quantal Linear	0.85, 3	0.234	0.10	0.1	2.34	1.0
Quantal Quadratic	0.74, 3	0.96	0.53	0.1	9.6	5.3
Weibull	0.85, 3	0.234	0.10	0.1	2.34	1.0

TABLE 6B-8. 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% CTL 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.

TABLE 6B-9. 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.

TABLE 6B-10. 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% CTL 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 6B-11. 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 6B-12. 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% CTL 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 6B-13. 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 6B-14. 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, 1998b)[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% CTL SD
TSH	Linear	0.50	4.64	3.77	3.0	1.55	1.26	0.45 0.465
	Power	0.31	4.48	1.43	3.0	1.49	0.48	
ln TSH	Linear	0.48	5.51	4.43	3.0	1.84	0.54	-0.1054
	Power	0.30	5.03	2.11	3.0	1.68	0.70	
T3	Neither linear, quadratic, or power FCNS fit data	<0.00001 for all	No fit	No fit	0.1	NA	NA	
lnT3	Neither linear, quadratic, or power FCNS fit data	<0.00001 for all	No fit	No fit	0.1	NA	NA	
T4	Nonmonotonic quadratic significant fit	0.50 min = 7.45 mg/kg	*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	CTL-10% CTL (=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)	CTL-10% CTL (= 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, CTL = control, and SD = standard deviation.

TABLE 6B-15. 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, 1998b) (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 6B-16. 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% CTL 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 6B-17. 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 6B-18. 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

7. SCREENING ECOLOGICAL RISK ASSESSMENT FOR PERCHLORATE

7.1 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 1-2). In some areas involved with the manufacture, use, or disposal of perchlorate salts, the perchlorate, as the anion dissociated from these salts, has contaminated soils or ground or surface waters (Figure 1-3). These releases have been confirmed in 14 states, especially those located in the southwestern United States, where the majority of sampling has occurred (Figures 1-3 and 1-4). There is a need to determine the likelihood that the perchlorate ion is causing effects on ecosystems or ecosystem components. This chapter presents a screening-level ecological risk assessment of environmental contamination by the perchlorate ion. In organization, it will follow the outline of the Guidelines for Ecological Risk Assessment (U.S. Environmental Protection Agency, 1998d).

7.1.1 Management Goals and Decisions

The discovery that perchlorate has contaminated ground and surface waters in certain locations has raised public and regulatory agency concerns. Most of these concerns have focused on potential public exposures through drinking water and the possible need to improve analytical and treatment methods and to develop drinking water regulations (Section 1.4), and an extensive scientific assessment effort is underway to address those concerns (Section 1.5). A balanced approach requires the assessment of ecological effects as well. The goals of this screening-level ecological risk assessment are therefore to provide an indication of 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 will be used to address the following questions.

•Are ecological exposures below levels of concern, or are management actions needed to reduce those exposures?

•Are analytical detection methods for perchlorate sufficient, or is there a likelihood of adverse ecological effects occurring at levels below detection limits?

•Is the available ecotoxicological information on perchlorate sufficient, or are additional studies needed?

7.1.2 Scope, Complexity, and Focus

This screening-level assessment is very limited in scope in that it relies on a limited set of source materials. These materials 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 (few studies were available), and a rationale for the selection of a battery of ecotoxicology tests to be 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 Report. 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.

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.

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.

Phytotransformation Study. The study, Laboratory Characterization of Phyto-transformation Products of Perchloroethylene (PCE), Trichloroethylene (TCE) and Perchlorate (Nzengung, n.d.) examined perchlorate distribution and degradation in experimental systems containing sand, aqueous perchlorate solution, and rooted cuttings of woody plant species. The study also examined systems containing chopped leaves or microbial mats and aqueous perchlorate solution.

7.2 PROBLEM FORMULATION

The characteristics of perchlorate and its sources are described earlier in this document (Chapters 1 and 2), and this assessment is site independent. Therefore, this problem formulation focuses on the selection of assessment endpoints, derivation of the conceptual model, and the analysis plan.

7.2.1 Assessment Endpoints

Assessment endpoints are operational definitions of the environmental values to be protected. For ecological risk assessments, they are chosen based on policy goals and societal values, their ecological relevance, and their susceptibility to the stressor. They are defined in terms of an entity and a property of that entity. The endpoints for this assessment are described in the following five subsections.

7.2.1.1 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 the policy 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.

7.2.1.2 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 indirectly important in terms of trophic support of fisheries and of some terrestrial insectivores. Species richness is important in terms of the policy 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.

7.2.1.3 Aquatic Plant Productivity

Algae and other aquatic plants have little direct societal value but are important to energy and nutrient dynamics in aquatic ecosystems. Because of their importance to the trophic support of fisheries and other aquatic consumers, productivity is the endpoint property for this assemblage.

7.2.1.4 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 indirectly important in terms of trophic support of some terrestrial insectivores. Species richness is important in terms of the policy of maintaining biodiversity.

7.2.1.5 Terrestrial Plant 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. Because there are insufficient data for estimating the sensitivity of a plant species, and methods for estimating the distribution of sensitivity comparable to those for aquatic species do not exist, no endpoint species is specified, and species richness of the plant community is not used as an endpoint property.

7.2.1.6 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.

7.2.2 Conceptual Models

The conceptual model describes the relationships between sources of perchlorate and the endpoint receptors (Figure 7-1). One source is spills to soil of perchlorate solutions from flushing rockets; combustion of rocket fuel; or improper disposal of rocket fuel, explosives, or manufacturing wastes. The other is aqueous discharge of waste water from manufacturing, or possibly fertilizer use. The spills contaminate the soil at the site and, through leaching and run-off, contaminate the surface water and groundwater. 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. Herbivorous terrestrial wildlife consume plants that have bioconcentrated perchlorate.

The relative simplicity of this conceptual model results from the exclusion of some potential routes and receptors. Dietary exposures are excluded from aquatic systems because perchlorate is not believed to bioconcentrate or bioaccumulate to any significant extent. Wildlife are assumed to have negligible exposure from air or 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 in both cases 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 is highly soluble and is unlikely to adsorb to particles 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.

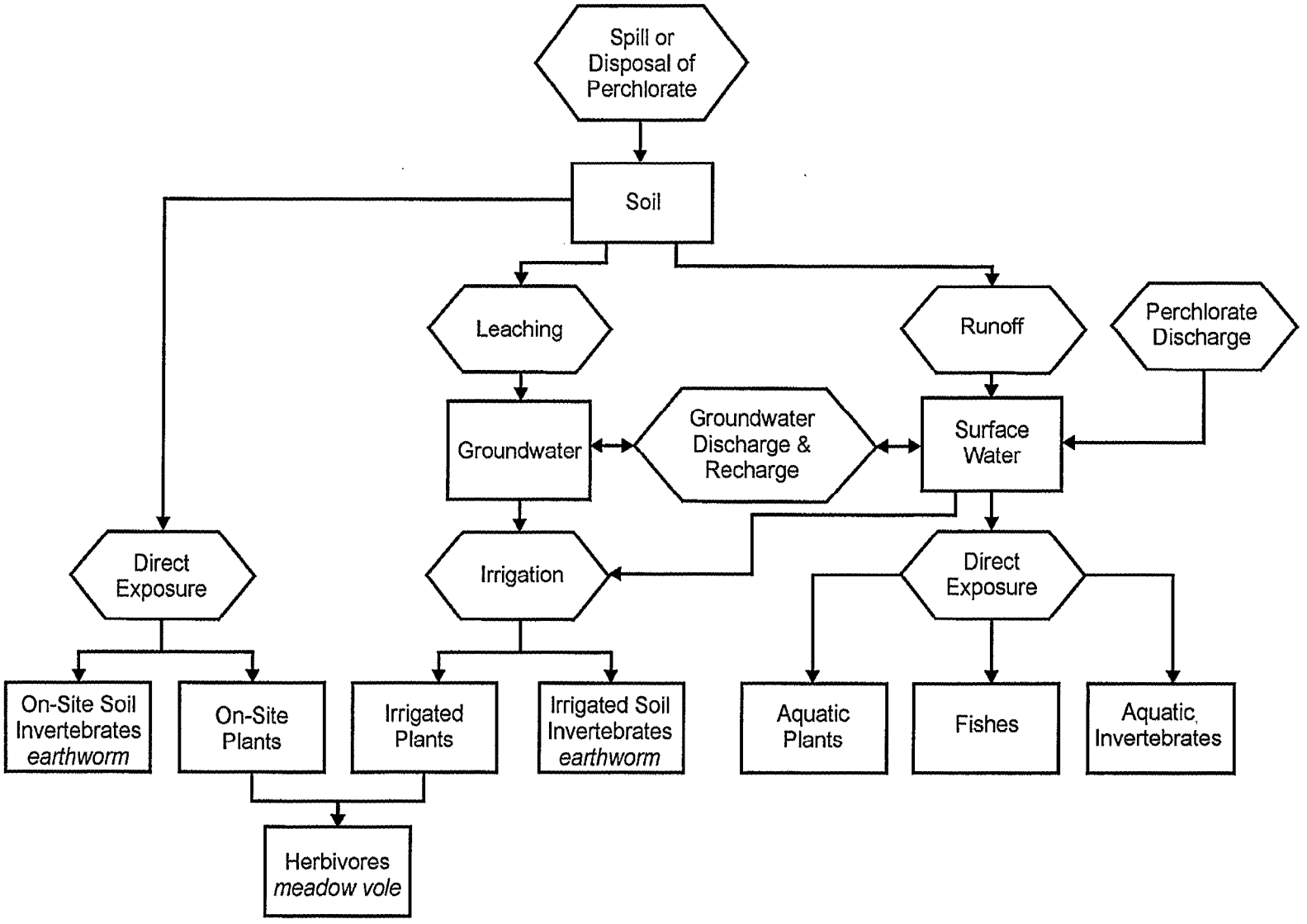


Figure 7-1. A conceptual model of exposure of ecological endpoint receptors to perchlorate. Specific endpoint taxa are identified in italic font; all other endpoints are defined at the community level. Processes are designated by hexagonal boxes, compartments by rectangular boxes.

7.2.3 Analysis Plan

This screening assessment uses existing information to determine whether the existing environmental contamination by perchlorate poses a clearly significant risk, poses a clearly insignificant risk, or poses an ambiguous risk. Hence, the risk assessment does not include any original research or testing. The analysis of effects will consist of the derivation of screening benchmarks through the application of conservative extrapolation models. The analysis of exposure for aqueous endpoints consists of measured concentrations reported in Chapter 1. Soil exposure estimates are based on exposure to perchlorate in irrigation water.

7.3 ANALYSIS

7.3.1 Characterization of Exposure

7.3.1.1 Water Concentrations

Fishes and aquatic invertebrates 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 7-1).

Perchlorate salts are dissolved readily given the conditions under which the contamination has occurred, resulting in perchlorate and the associated cation. Because 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, perchlorate is both very mobile in aqueous systems and persistent for many decades under typical ground and surface water conditions (Section 1.1).

Little 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 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 dominated 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. Sufficient information is not available to characterize those water bodies and concentrations. It is also possible that, within large water bodies, there are locally elevated concentrations at sites of groundwater discharge.

It is assumed that irrigation waters pumped from Lake Mead or the Colorado River are in the range given above. 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.

7.3.1.2 Aquatic Bioaccumulation

As discussed above, no information is available to indicate that the perchlorate ion accumulates in animal tissues.

7.3.1.3 Soil Levels

Off-site soils may be contaminated via irrigation (Figure 7-1). Because of its high water solubility, perchlorate is unlikely to accumulate via adsorption to irrigated soils; aqueous perchlorate was found not to adsorb to sand in laboratory reactors (Nzengung, n.d.). Further, rooted cuttings of woody plants placed in these reactors were found to readily degrade perchlorate (Nzengung, n.d.), suggesting that perchlorate in ambient soils may be subject to phytodegradation. By gross approximation, then, soil concentrations (expressed as milligrams per kilogram) would be unlikely to exceed concentrations (expressed as milligrams per liter) in irrigation water. However, the concentration of perchlorate salts in irrigated soils with high evaporation rates cannot be ruled out. 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.

7.3.1.4 Uptake by Vegetation

An experiment with plants that may be candidates for use in phytoremediation of perchlorate-contaminated sites showed that perchlorate may concentrate in vegetation (Nzengung, n.d.). 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, took up perchlorate into the aerial plant parts and degraded a fraction of the compound to chloride. Fraction degraded varied with species and also seemed to be confounded by changes in ionic strength of the nutrient solution during the experiments. In each case, however, perchlorate was taken up and concentrated in aerial plant parts, especially leaves. Concentration factors, expressed as the ratio of leaf concentration (milligrams per kilogram, wet weight) to initial solution concentration (milligrams per liter), ranged from 7.5 to 25.

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 or degraded, 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. Therefore, a simple, 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.

7.3.1.5 Herbivore Exposure

The representative herbivore selected for this assessment, *M. pennsylvanicus*, has a diet consisting mainly of monocot and dicot shoots; it 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.

7.3.2 Characterization of Effects

7.3.2.1 Aquatic Organisms

Effects on the richness and productivity of fish and aquatic invertebrate 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 four aquatic species (Table 7-1). In acute tests (48 and 96 h) with sodium perchlorate, using the water flea *Daphnia magna* and the fathead minnow *Pimephales promelas*, respectively, the endpoint studied was lethality. In 7-day tests with a different water flea (*Ceriodaphnia dubia*) and with *P. promelas*, acute lethality was studied in addition to more sensitive endpoints. The latter included the number of offspring per female (*C. dubia*) and growth (i.e., body weight; *P. promelas*). A 7-day test with *C. dubia* generally is considered a chronic test because test organisms produce three broods during the test; a 7-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).

Steps followed in the derivation of the Tier II value for sodium perchlorate are presented in Table 7-2. 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. This observation raises the possibility that the SCV may be lower than is necessary to protect against perchlorate ion toxicity if it were associated with a less toxic cation.

Similar chronic (or subchronic) tests were conducted with ammonium perchlorate (Table 7-1). Results (expressed as ClO_4^-) were very similar for *C. dubia*, but *P. promelas* was

TABLE 7-1. RESULTS OF PERCHLORATE TOXICITY TESTS IN AQUATIC SPECIES

Test Species	Test Description		Endpoints (as mg/L ClO_4)[a]				
	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 h	Acute (48-h)	490 (406 - 591)	—	—	—	—
Pimephales promelas	12 - 13 days	Acute (96-h)	1,655 (1,507 - 1,817)	—	—	—	—
Ceriodaphnia dubia	<24 h	Chronic (7-day)	66 (40-144) [48-h]	10	33	18.2	17 (8.1 - 20.5)
Pimephales promelas	<24 h	Subchronic (7-day)	614 (540 - 714) [96-h]	155	280[c]	208[c]	212[c] (175 - 231)[c]
Ammonium perchlorate (NH_4ClO_4)[d] tests (Block Environmental Services, Inc., 1998)							
Ceriodaphnia dubia	<24 h[e]	Chronic (6-day)	77.8 [6-days]	9.6	24	15	24
Pimephales promelas	<24 h[e]	Subchronic (7-day)	270 [7-days]	9.6	96	30	114
Ammonium perchlorate (NH_4ClO_4)[d] tests (Dumont and Bantle, 1998)							
Xenopus	Embryo	96-h	420	—	—	—	—
Xenopus	Embryo	96-h	336[f]	—	—	—	—

[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]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.
[e]Not reported; assumed based on standard protocols.
[f]IC_{50} for malformations.

TABLE 7-2. 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, 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: ACR = 1,655 ÷ 208 = 8.0 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	The SACR is the geometric mean of the ACRs.
Derive the secondary chronic value (SCV)	0.6	SCV = SAV ÷ SACR 5.0 ÷ 8.0

more sensitive to ammonium perchlorate than to sodium perchlorate. Tier II values for ammonium perchlorate are not presented, however, for several reasons, including the lack of ammonium controls, making it difficult to determine whether 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, 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 7-1) 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, 1998e), the former value is probably too low to be responsible for the observed effects[7]. On the other hand, the LOEC observed with *P. promelas* at 96 mg/L (Table 7-1) 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, 1998e). 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, 1998e).

7.3.2.2 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. The exposure was to sodium perchlorate solution added to the solid media. The results may be expressed as milligrams per kilogram soil or sand, or as milligrams per liter of irrigation solution. Although the exposure was to sodium perchlorate solution added to the solid medium, the results are reported as milligrams per kilograms of soil or sand. Growth was a more sensitive response

[7]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, but, 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.

than germination or survival. The quartile inhibitory 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.

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. 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 7.3.2.1) to obtain a conservative estimate of a threshold for soil community effects, are as follows:

$$\begin{aligned} \text{Threshold} &= LC_{50} \div (\text{factor for interspecies variance} \times \text{acute-chronic ratio}) \\ &= 4{,}450 \text{ mg/kg} \div (242 \times 18) \\ &= 1 \text{ mg/kg.} \end{aligned}$$

The equivalent aqueous phase benchmark is 2.8 mg/L. This approach requires the assumptions that the variance among soil species is approximately the same as among aquatic species, and that the distribution of acute-chronic ratios across chemicals is approximately the same for both communities. The interspecies variance factor is the one for a test species that has not been demonstrated to be highly sensitive.

Herbivores. The human health risk assessment for perchlorate uses 0.1 mg/kg-day as the LOAEL from which the RfD is derived (Chapter 6). That value is based on thyroid histopathology in F1-generation rat pups on PND5. Because the representative species for the herbivore endpoint (meadow vole) is a rodent, that value is used as well. 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. To account for interspecies variance and LOAEL to NOAEL extrapolation, an uncertainty factor of 10 is applied to obtain a screening benchmark of 0.01 mg/kg-day.

8. MAJOR CONCLUSIONS IN RISK CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

8.1 HUMAN HEALTH

8.1.1 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, and 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 primarily in areas associated with development, testing, or manufacturing of aerospace materials. Another potential source of perchlorate contamination occurs when mining activities use explosives extensively.

Perchlorate is rapidly absorbed from the gastrointestinal tract, but dermal and inhalation exposures are not expected to be significant exposure routes. The known mode of action for perchlorate is that it acts as a competitive inhibitor of active iodide uptake by the symporter in most mammals, including human and laboratory test species. This decrease in intrathyroidal iodide results in a decreased production of T3 and T4 thyroid hormones. This decrease can potentially perturb the hypothalamic-pituitary-thyroid axis to increase TSH from the pituitary to stimulate production of thyroid hormone. Prolonged stimulation 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.

The target tissue for systemic effects of perchlorate is the thyroid. Changes in the thyroid hormone homestasis are the initial harbingers for the subsequent initial histopathological

changes, including follicular hypertrophy and decrease in follicular lumen size. If perchlorate exposure is stopped, the thyroid effects have been shown to be reversible after exposures as long as 90-days in rats. There are also some case studies in humans treated therapeutically with perchlorate indicating 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 growth, particularly of the nervous system if the pregnant mother was hypothyroid. The potential for major disturbances in thyroid hormone homeostasis to disturb reproductive capacity or to induce immune effects also exists.

8.1.2 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 these laboratory animal data because there are no good dose-response data in human subjects exposed at low levels for long periods of time. An approach was proposed whereby, if the precursor lesion to the potential tumors was used as the point of departure, such as an increase in hyperplasia caused by thyroid-pituitary axis disturbance, then the estimate derived using that point of departure would be protective of cancer development as well. The critical effect was determined to be the dose level that caused thyroid follicular cell hypertrophy in rat pups on PND5 of a neurodevelopmental toxicity study that exposed the mothers during gestation. A composite uncertainty factor of 100 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. Partial uncertainty factors were applied for interspecies and intrahuman extrapolation, the use of a minimal LOAEL, and database deficiencies. It should be noted that some of these database deficiencies are likely to be reconciled by the time of the external peer review. Confidence in the study, the database, and the RfD is rated as medium. The major areas of uncertainty are interspecies differences in pharmacokinetics and the level of hormone perturbation to designate as adverse, particularly vis à vis potential neurodevelopmental effects.

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 0.0009 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. Because of the application of uncertainty factors, this dose is approximately 1/100 of the dose that resulted in thyroid follicular hypertrophy in rat pups exposed in utero and examined on PND5 (Argus Research Laboratories Inc, 1998a).

8.1.3 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 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 also should be considered whenever attempting risk characterization of a specific human population exposed to a particular scenario.

8.1.3.1 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. This is especially important for perchlorate because the thyroid histopathology effects have been shown to be reversible. Caution is warranted following transient exposures with respect to potentially permanent effects caused by decrements in thyroid hormones in the developing fetus, however. 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. Also, the estimate was based on extrapolation from laboratory animals using default

approaches. The derivation has not yet had the benefit of taking internal dosimetry into account to derive an estimate based on PBPK models of the toxicokinetics and toxicodynamics of perchlorate. Nevertheless, many useful RfD estimates are derived without using PBPK models, and this estimate is considered to be based on sound science approaches to the newly available database. 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 typically translates into a range of threefold below to threefold above the RfD.

8.1.3.2 Indirect Exposures

Where crops are irrigated with perchlorate-contaminated water, 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, which 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 of perchlorate's 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 of perchlorate behavior and fate in plant-soil-water systems, including studies that simulate leaf crop irrigation.

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 by March 1999. If the needed information can be obtained on perchlorate behavior and fate, these methods can be used to develop estimates of human exposure and risk.

8.1.4 Major Uncertainties and Research Needs

The need for accurate exposure estimates already has been highlighted as necessary for accurate and comprehensive characterization of the risk of perchlorate contamination. This section will summarize briefly research needs associated with aspects of uncertainty for the human health risk dose-response estimate that were highlighted in Chapter 6.

The greatest need for improving the dose response is a more accurate characterization of the linkage between the biologically effective internal dose (e.g., the dose response for perchlorate inhibition of iodide uptake in the thyroid gland). This need must be addressed in the fetal compartment as well, so 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 the thyroid histology, and neurobehavioral deficits especially, would improve dramatically the confidence that the characterization of the exposure-dose-response continuum is accurate. The current studies may warrant repeating with larger sample sizes and lower doses, as well as evaluation of fetal hormone levels and more specific neurobehavioral assays. Finally, mechanistic determinants of these toxicokinetic and toxicodynamic parameters and processes should be characterized in both laboratory animals and humans.

8.2 ECOTOXICOLOGY

8.2.1 Aquatic Life

Procedures for deriving Tier II water quality values were used in Section 7.3.2.1 to jointly characterize potential effects of the perchlorate ion on the richness and productivity of fish and 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. 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 and aquatic invertebrate and plant communities appears to be low. However, because much higher perchlorate concentrations (37×10^6 μg/L) have been reported in monitoring wells at rocket motor manufacture or testing sites, there is a likelihood that smaller surface water systems close to sites of contamination, especially systems that are groundwater dominated, may exist that have perchlorate concentrations high enough to cause toxicity to aquatic life. 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 not a potent teratogen.

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.

8.2.2 Risks to Consumers of Aquatic Life

No information was available to indicate that perchlorate is bioconcentrated by aquatic life, and, therefore, there currently is no indication that aquatic life consumers are at risk from perchlorate.

8.2.3 Terrestrial Life

8.2.3.1 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 are unknown but are likely to be higher than the screening benchmark of 4 mg/kg and even the lethal concentrations ($\geq$180 mg/kg). In the absence of information concerning accumulation of perchlorate in irrigated soils, it is assumed that soil concentrations equal irrigation water concentrations (Section 7.3.1.3). Reported surface water concentrations in the Colorado River, 4 to 16 μg/L, would translate to 0.004 to 0.016 mg/kg. The higher concentration is a factor of 250 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. Alternatively, the test results converted to concentrations in added solutions can be used, and exposure to that solution can be assumed equivalent to irrigation water. The aqueous benchmark (16 mg/L) is a factor of 1,000 less than the Colorado River water and a factor of 57 less than the public well water. 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.

8.2.3.2 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 concentrations at disposal sites are unknown but are likely to be higher than the soil screening benchmark of 1 mg/kg and may exceed the acute lethal concentrations (4,450 mg/kg). In the absence of information concerning accumulation of perchlorate in irrigated soils, it is assumed that soil concentrations equal irrigation water concentrations (Section 7.3.1.3). Reported surface water concentrations in the Colorado River, 4 to 16 μg/L, would translate to 0.004 to 0.016 mg/kg. The higher concentration is a factor of 62 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. Alternatively, the test results converted to concentrations in added solutions can be used, and exposure to that solution can be assumed equivalent to irrigation water. The aqueous screening benchmark (2.8 mg/L) is a factor of

175 less than Colorado River water and a factor of 10 less than the public well water. 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.

8.2.3.3 Herbivores

Estimated exposures of voles on sites irrigated with surface water (0.18 mg/kg-day) and groundwater (3.2 mg/kg-day) exceed the LOAEL of 0.1 mg/kg-day as well as the screening benchmark of 0.01 mg/kg-day. Hence, there is a potential hazard to all herbivorous wildlife occurring 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.

8.2.4 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.

8.2.4.1 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 that 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.

Because perchlorate has been shown to be bioconcentrated by plants, concentration of perchlorate from water and bioaccumulation from aquatic plants are previously unanticipated

concerns. Because of the absence of this information concerning perchlorate accumulation by aquatic biota, exposure of organisms that feed on fish and other aquatic organisms could not be assessed.

Aquatic Effects. The effects of perchlorate on algae and aquatic macrophytes are unknown. As a result, risks to aquatic primary producers could not be estimated.

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 may accumulate in aquatic plants as well, 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.

Effects of perchlorate on fish were estimated using a subchronic test of one life stage. Because of the potential for chronic effects caused by thyroid dysfunction, chronic (i.e., life cycle) effects may be underestimated by at least a factor of 10.

The uncertainty factors in the secondary chronic value are high because of the lack of test results for aquatic organisms other than fathead minnows and daphnids.

8.2.4.2 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 is 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.

Perchlorate levels in directly and indirectly contaminated soils have not been reported. As a result, risks at directly contaminated sites could not be assessed.

The fate of perchlorate in soil, including its tendency for evaporative concentration, is unknown. As a result, soil concentrations were assumed to be equal to irrigation water concentrations. This sort of assumption could be low by multiple orders of magnitude if evaporative concentration occurs with perchlorate, as it does with metals.

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.

The bioconcentration of perchlorate by plants suggests that accumulation in other terrestrial organisms may be possible, but it is unknown. As a result, food chain exposures were not assessed.

Terrestrial Effects. The toxicity of perchlorate to nonmammalian vertebrate wildlife is unknown. As a result, risks to birds, reptiles, and amphibians were not assessed.

The toxicity of perchlorate to terrestrial invertebrates, other than acute lethality to earthworms, is unknown. As a result, risks to terrestrial invertebrates were inadequately assessed.

8.2.5 Research Needs

The following major research needs for exposure and effects analysis are listed in approximate priority order.

8.2.5.1 Exposure

•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.

•Because the available information on accumulation in terrestrial vascular plants is from a study that was not designed to quantify accumulation factors, the accumulation of perchlorate in terrestrial plants should be further investigated.

•Because of the potential for evaporative concentration, the fate of perchlorate in irrigated soils should be investigated.

8.2.5.2 Effects

•The effects of exposure of aquatic plants should be determined.

•The effects of exposure of nondaphnid invertebrates should be determined.

•The effects of chronic exposure of fish should be determined.

•The effects of dietary exposure to perchlorate should be determined in birds and in herbivorous or litter-feeding invertebrates.

•If perchlorate occurs at significant levels in estuarine systems, its toxicity in saline waters should be determined.

9. REFERENCES

Allred, M. (1998) Chemical specific consultation for perchlorate. Atlanta, GA: U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry; October 8.

Anbar, M.; Guttmann, S.; Lewitus, Z. (1959) The mode of action of perchlorate ions on the iodine uptake of the thyroid gland. Int. J. Appl. Radiat. Isot. 7: 87-96.

Andersen, M. E.; Krishnan, K.; Conolly, R. B.; McClellan, R. O. (1992) Mechanistic toxicology reserach and biologically-based modeling: partners for improving quantitative risk assessments. CIIT Activities 12(1): 1-7.

Argus Research Laboratories, Inc. (1998a) A neurobehavioral developmental study of ammonium perchlorate administered orally in drinking water to rats [report amendment: July 27]. Horsham, PA: Argus Research Laboratories, Inc.; protocol no. 1613-002.

Argus Research Laboratories, Inc. (1998b) Oral (drinking water) two-generation (one litter per generation) reproduction study of ammonium perchlorate in rats. Horsham, PA: Argus Research Laboratories, Inc.; protocol no. 1416-001.

Argus Research Laboratories, Inc. (1998c) Oral (drinking water) developmental toxicity study of ammonium perchlorate in rabbits [report amendment: September 10]. Horsham, PA: Argus Research Laboratories, Inc.; protocol no. 1416-002.

Argus Research Laboratories, Inc. (1998d) Oral (drinking water) dosage-range developmental toxicity study of ammonium perchlorate in rabbits. Final pilot report. Horsham, PA: Argus Research Laboratories, Inc.; protocol no. 1416-002P; December 10.

Atterwill, C. K.; Collins, P.; Brown, C. G.; Harland, R. F. (1987) The perchlorate discharge test for examining thyroid function in rats. J. Pharmacol. Methods 18: 199-203.

Barton, H. A.; Andersen, M. E.; Allen, B. (1999) Dose response characteristics of uterine responses in rats exposed to estrogen agonists. Regul. Toxicol. Pharmacol. [in press].

Barzilai, D.; Sheinfeld, M. (1966) Fatal complications following use of potassium perchlorate in thyrotoxicosis: report of two cases and a review of the literature. Israel J. Med. 2: 453-456.

Beals, J. A. J.; Funk, L. M.; Fountain, R.; Sedman, R. (1996) Quantifying the distribution of inhalation exposure in human populations: distribution of minute volumes in adults and children. Environ. Health Perspect. 104: 974-979.

Block Environmental Services, Inc. (1998) LC50 aquatic toxicity test results for ammonium perchlorate—a two-species chronic definitive bioassay. San Jose, CA: Santa Clara Valley Water District; August 21.

Brabant, G. (1994) Personal communication with Dr. G. Brabant concerning ongoing perchlorate work in humans by Drs. Donald Tocco and Bruce Mulholt in March and April 1994 [as cited in Toxicology Excellence for Risk Assessment, 1997].

Brabant, G.; Bergmann, P.; Kirsch, C. M.; Kohrle, J.; Hesch, R. D.; Von Zur Muhlen, A. (1992) Early adaptation of thyrotropin and thyroglobulin secretion to experimentally decreased iodine supply in man. Metabolism 41: 1093-1096.

Braverman, L. E.; Utiger, R. D. (1991) Introduction to thyrotoxicosis. In: Werner and Ingbar's the thyroid: a fundamental and clinical text. 6th ed. Philadelphia, PA: J.B. Lippincott Co.; pp. 645-647.

Brown-Grant, K. (1966) Failure of orally administered perchlorate to affect deciduoma formation or pregnancy in the rat. J. Reprod. Fertil. 12: 353-357.

Brown-Grant, K.; Sherwood, M. R. (1971) Viability of the rat blastocyst following the oral administration of potassium perchlorate or potassium iodide to the mother. J. Reprod. Fertil. 27: 265-267.

Burgi, H.; Benguerel, M.; Knopp, J.; Kohler, H.; Studer, H. (1974) Influence of perchlorate on the secretion on non-thyroxine iodine by the normal human thyroid gland. Eur. J. Clin. Invest. 4: 65-69.

Caldwell, D. J.; Mattie, D. R. (1995) Study design for the toxicity evaluation of ammonium perchlorate. In: Proceedings of the 1995 JANNAF safety and environmental protection subcommittee joint workshop, environmentally sound processing technology; July; Tampa, FL. Columbia, MD: Chemical Propulsion Information Agency; Joint Army, Navy, NASA, Air Force (JANNAF) interagency propulsion committee publication 626.

Caldwell, D. J.; King, J. H., Jr.; Kinkead, E. R.; Wolfe, R. E.; Narayanan, L.; Mattie, D. R. (1995) Results of a fourteen day oral-dosing toxicity study of ammonium perchlorate. In: Proceedings of the 1995 JANNAF safety and environmental protection subcommittee meeting: volume 1; December; Tampa, FL. Columbia, MD: Chemical Propulsion Information Agency; Joint Army, Navy, NASA, Air Force (JANNAF) interagency propulsion committee publication 634.

California Department of Health Services. (1997) Preliminary health reviews in Rancho Cordova, Sacramento County, California. Atlanta, GA: U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry; CERCLIS no. CAD980358832, October 16.

California Department of Health Services. (1998a) Perchlorate contamination in the Citizens Utilities' suburban and Security Park water service areas. Atlanta, GA: U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry; health consultation, March 18.

California Department of Health Services. (1998b) Perchlorate contamination in the Arden Cordova water service area. Atlanta, GA: U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry; health consultation, April 21.

California Department of Health Services. (1998c) Perchlorate contamination in the Mather Air Force Base water service area. Atlanta, GA: U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry; health consultation, May 6.

California Department of Health Services. (1998d) Perchlorate contamination in the Fair Oaks water district. Atlanta, GA: U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry; health consultation, June 5.

California Department of Health Services. (1998e) Perchlorate contamination in the Sunrise district of the Sacramento County water service. Atlanta, GA: U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry; health consultation, June 18.

Campbell, B. A.; Lytle, L. D.; Fibiger, H. C. (1969) Ontogeny of adrenergic arousal and cholinergic inhibitory mechanisms in the rat. Science (Washington, DC) 166: 635-637.

Cao, X. Y.; Jiang, X. M.; Dou, Z. H.; Rakeman, M. A.; Zhang, M. L.; O'Donnel, K.; Ma, T.; Amette, K.; DeLong, N.; DeLong, G. R. (1994) Timing of vulnerability of the brain to iodine deficiency in endemic cretinism. N. Engl. J. Med. 331: 1739-1744.

Capen, C. C. (1997) Mechanistic data and risk assessment of selected toxic end points of the thyroid gland. Toxicol. Pathol. 25: 39-48.

Cavalieri, R. R. (1997) Iodine metabolism and thyroid physiology: current concepts. Thyroid 7: 177-181.

Channel, S. R., Maj. (1998a) Consultative letter, AFRL-HE-WP-CL-1998-0027, morphometric analysis report - thyroid: a neurobehavioral developmental study of ammonium perchlorate administered orally in drinking water to rats [memorandum with attachments to Annie Jarabek]. Wright-Patterson Air Force Base, OH: Human Effectiveness Directorate, Operational Toxicology Branch; October 26.

Channel, S. R., Maj. (1998b) Consultative letter, AFRL-HE-WP-CL-1998-0026, histopathology report for thyroids from a fourteen-day oral dosing toxicity study of ammonium perchlorate [memorandum with attachments to Annie Jarabek]. Wright-Patterson Air Force Base, OH: Human Effectiveness Directorate, Operational Toxicology Branch; October 27.

Channel, S. R., Maj. (1998c) Consultative letter, AFRL-HE-WP-CL-1998-0031, pharmacokinetic study of perchlorate administered orally to humans [memorandum to Annie Jarabek]. Wright-Patterson Air Force Base, OH: Air Force Research Laboratory, Human Effectiveness Directorate; December 18.

Chow, S. Y.; Woodbury, D. M. (1970) Kinetics of distribution of radioactive perchlorate in rat and guinea-pig thyroid glands. J. Endocrinol. 47: 207-218.

Chow, S. Y.; Chang, L. R.; Yen, M. S. (1969) A comparison between the uptakes of radioactive perchlorate and iodide by rat and guinea-pig thyroid glands. J. Endocrinol. 45: 1-8.

Cohen, J. (1987) Statistical power analysis for the behavioral sciences. Hillsdale, NJ: Lawrence Erlbaum Associates, Publishers.

Comer, C. P.; Norton, S. (1982) Effects of perinatal methimazole exposure on a developmental test battery for neurobehavioral toxicity in rats. Toxicol. Appl. Pharmacol. 63: 133-141.

Connell, J. M. (1981) Long-term use of potassium perchlorate. Postgrad. Med. J. 57: 516-517.

Cox, C. (1994) Statistical issues for animal studies of developmental neurotoxicity. In: Weiss, B.; O'Donoghue, J. L., eds. Neurobehavioral toxicity: analysis and interpretation. New York, NY: Raven Press; pp. 93-101.

Crofton, K. M. (1998a) Analysis and graphics of thyroid hormone data from the rat 14-day "Caldwell" perchlorate study [memorandum with attachment to Annie Jarabek]. Research Triangle Park, NC: U.S. Environmental Protection Agency, National Health and Environmental Effects Research Laboratory; October 18 (revised November 21).

Crofton, K. M. (1998b) Re-analysis of thyroid hormone data from the subchronic perchlorate study submitted by Springborn Laboratories (SLI study no. 3455.1) [memorandum with attachment to Annie Jarabek]. Research Triangle Park, NC: U.S. Environmental Protection Agency, National Health and Environmental Effects Research Laboratory; July 21 (revised October 12 and November 18).

Crofton, K. M. (1998c) Preliminary analysis of the postnatal day 12 neuromorphology data from the rat developmental neurotoxicology study [memorandum to Annie Jarabek]. Research Triangle Park, NC: U.S. Environmental Protection Agency, National Health and Environmental Effects Research Laboratory; December 29.

Crofton, K. M. (1998d) Personal communication [with Charles Capen of Ohio State University concerning the comparative sensitivity of standard histology versus morphometric measurements and of follicular epithelial cell height versus lumen area regarding chemically-induced changes in thyroid gland structure]. Research Triangle Park, NC: U.S. Environmental Protection Agency, National Health and Environmental Effects Research Laboratory; November 6.

Crofton, K. M. (1998e) Analysis of the thyroid histology data from the rat developmental neurotoxicology study [memorandum with attachment to Annie Jarabek]. Research Triangle Park, NC: U.S. Environmental Protection Agency, National Health and Environmental Effects Research Laboratory; October 22 (revised November 6).

Crofton, K. M. (1998f) Analysis of the thyroid hormone data from the rat developmental neurotoxicology study [memorandum with attachment to Annie Jarabek]. Research Triangle Park, NC: U.S. Environmental Protection Agency, National Health and Environmental Effects Research Laboratory; September 24 (revised November 6).

Crofton, K. M. (1998g) Personal communication. [With Dr. Simon Mats, Primedica, Worcester, MA, concerning the lack of use of litter as dependent variable in the reanalysis of the motor activity data from the Argus developmental neurotoxicity study]. Research Triangle Park, NC: U.S. Environmental Protection Agency, National Health and Environmental Effects Research Laboratory; November 4.

Crofton, K. M. (1998h) Analysis and graphics of thyroid hormone data from the rabbit developmental perchlorate study [memorandum with attachment to Annie Jarabek]. Research Triangle Park, NC: U.S. Environmental Protection Agency, National Health and Environmental Effects Research Laboratory; October 10 (revised October 28).

Crofton, K. M. (1998i) Analysis and graphics of thyroid hormone data from the mouse immunotoxicology study [memorandum with attachment to Annie Jarabek]. Research Triangle Park, NC: U.S. Environmental Protection Agency, National Health and Environmental Effects Research Laboratory; October 18 (revised November 10 and 23).

Crofton, K. M. (1998j) Sensitivity of neurodevelopmental tests versus thyroid hormone concentrations [memorandum to Annie Jarabek]. Research Triangle Park, NC: U.S. Environmental Protection Agency, National Health and Environmental Effects Research Laboratory; December 29.

Crofton, K. M.; MacPhail, R. C.; Tilson, H. A. (1998) Analysis of the motor activity data from the rat developmental neurotoxicology study [memorandum to Annie Jarabek]. Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development; November 5.

Crooks, J.; Wayne, E. J. (1960) A comparison of potassium perchlorate, methylthiouracil, and carbimazole in the treatment of thyrotoxicosis. Lancet (February 20): 401-404.

Crump, K. S.; Allen, B. C.; Faustman, E. M. (1995) The use of the benchmark dose approach in health risk assessment. Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum; report no. EPA/630/R-94/007. Available from: NTIS, Springfield, VA; PB95-213765/XAB.

Dean, J. A., ed. (1985) Lange's handbook of chemistry. 13th ed. New York, NY: McGraw Hill Book Company.

Dollarhide, J. S. (1992) Provisional non-cancer and cancer toxicity values for potassium perchlorate (CASRN 7778-74-7) (Aerojet General Corp./CA) [memorandum with attachment to Dan Strakla]. Cincinnati, OH: U.S. Environmental Protection Agency, Environmental Criteria and Assessment Office; December 2.

Dollarhide, J. S. (1995) Review of proposed RfD for perchlorate [memorandum with attachment to Mike Girard]. Cincinnati, OH: U.S. Environmental Protection Agency, National Center for Environmental Assessment; October 23.

Dollarhide, J. S. (1998) Personal communication [regarding dosing solutions of ammonium perchlorate versus anion only]. Cincinnati, OH: Toxicology Excellence for Risk Assessment; November 30.

Dourson, M. (1998) Mouse micronucleus test for perchlorate & literature review of relevant mouse studies [report with memorandum from Tara M. Anderson attached]. Cincinnati, OH: Toxicology Excellence for Risk Assessment; October 10.

Dumont, J. N.; Bantle, J. A. (1998) FETAX analysis of ammonium perchlorate. Stillwater, OK: Oklahoma State University, Department of Zoology; May 22.

Durand, J. (1938) Recherches sur l'elimination des perchlorates, sur leur repartition dans les organes et sur leur toxicite. Bull. Soc. Chim. Biol. 20: 423-433.

EA Engineering, Science and Technology, Inc. (1998) Results of acute and chronic toxicity testing with sodium perchlorate. Final report. Brooks Air Force Base, TX: Armstrong Laboratory; November.

Eichler, O.; Hackenthal, E. (1962) Uber Ausscheidung und Stoffwechsel von Perchlorat gemessen mit $^{36}ClO_4^-$ [Secretion and metabolism of perchlorate measured with $^{36}ClO_4^-$]. Naunyn-Schmidedberg's Arch. Exp. Pathol. Pharmakol. 243: 554-565.

Environmental Resources Management, Inc. (1995) Extended literature review concerning NOAEL and LOAEL values for perchlorate. Written for the Perchlorate Study Group. Exton, PA: Environmental Resources Management, Inc.

Farid, N. R.; Shi, Y.; Zou, M. (1994) Molecular basis of thyroid cancer. Endocr. Rev. 15: 202-232.

Fawcett, J. W.; Clarke, C. W. F. (1961) Aplastic anaemia due to potassium perchlorate. Br. Med. J. (May 27): 1537.

Federal Register. (1991) Guidelines for developmental toxicity risk assessment. F. R. (December 5) 56: 63798-63826.

Federal Register. (1996) Proposed guidelines for carcinogen risk assessment: notice of availability and opportunity to comment. F. R. (April 23) 61: 17960-18011.

Fisher, D. A. (1996) Disorders of the thyroid in the newborn and infant. In: Sperling, M. A., ed. Pediatric endocrinology. Philadelphia, PA: W. B. Saunders Company; pp. 51-70.

Fisher, J. W. (1998a) Consultative letter, AFRL-HE-WP-CL-1998-0022, pharmacokinetics of iodide uptake inhibition in the thyroid by perchlorate [memorandum with attachments to Annie Jarabek]. Wright-Patterson Air Force Base, OH: Air Force Research Laboratory, Human Effectiveness Directorate, Operational Toxicology Branch (AFRL/HEST); October 1.

Fisher, J. W. (1998b) Personal communication to Annie Jarabek [concerning percentage of perchlorate likely to transfer to pups during lactation]. Wright-Patterson Air Force Base, OH: Air Force Research Laboratory, Human Effectiveness Directorate, Operational Toxicology (AFRL/HEST) Branch; December 22.

Fisher, J. W.; Whittaker, T. A.; Taylor, D. H.; Clewell, H. J., III; Andersen, M. E. (1989) Physiologically based pharmacokinetic modeling of the pregnant rat: a multiroute exposure model for trichloroethylene and its metabolite, trichloroacetic acid. Toxicol. Appl. Pharmacol. 99: 395-414.

Fisher, J. W.; Whittaker, T. A.; Taylor, D. H.; Clewell, H. J., III; Andersen, M. E. (1990) Physiologically based pharmacokinetic modeling of the lactating rat and nursing pup: a multiroute exposure model for trichloroethylene and its metabolite, trichloroacetic acid. Toxicol. Appl. Pharmacol. 102: 497-513.

Gauss, W. (1972) Das verhalten einiger physiologischer und histologischer kriterien der schilddrusenfunktion bei einmaliger oder langerer verabreichung von kaliumperchlorat an adulte mause I. Langzeitversuche. Z. Mikrosk. Anat. Forsch. 4: 469-500.

Geller, A. M. (1998a) Non-parametric correlations run on thyroid data from studies submitted for evaluation of perchlorate [memorandum with attached report to Annie Jarabek]. Research Triangle Park, NC: U.S. Environmental Protection Agency, National Health and Environmental Effects Research Laboratory; December 21.

Geller, A. M. (1998b) Benchmark dose calculations on thyroid data from studies submitted for evaluation of perchlorate [memorandum with attachments to Annie Jarabek]. Research Triangle Park, NC: U.S. Environmental Protection Agency, National Health and Environmental Effects Research Laboratory; December 29.

Giardino, N. J.; Eamen, N. A.; Andelman, J. B. (1992) Modeling volatilization of trichloroethylene from a domestic shower spray: the role of drop-size distribution. Environ. Sci. Technol. 26: 1602-1606.

Gibbs, J. P.; Ahmad, R.; Crump, K. S.; Houck, D. P.; Leveille, T. S.; Findley, J. E.; Francis, M. (1998) Evaluation of a population with occupational exposure to airborne ammonium perchlorate for possible acute or chronic effects on thyroid function. J. Occup. Environ. Med. 40:1072-1082.

Gjemdal, N. (1963) Fatal aplastic anaemia following use of potassium perchlorate in thyrotoxicosis. Acta Med. Scand. 174: 129-131.

Godley, A. F.; Stanbury, J. B. (1954) Preliminary experience in the treatment of hyperthyroidism with potassium perchlorate. J. Clin. Endocrinol. 14: 70-78.

Goldey, E. S.; Kehn, L. S.; Rehnberg, G. L.; Crofton, K. M. (1995a) Effects of developmental hypothyroidism on auditory and motor function in the rat. Toxicol. Appl. Pharmacol. 135: 67-76.

Goldey, E. S.; Kehn, L. S.; Lau, C.; Rhenberg, G. L.; Crofton, K. M. (1995b) Developmental exposure to polychlorinated biphenyls (Arochlor 1254) reduces circulating thyroid hormone concentrations and causes hearing deficits in rats. Toxicol. Appl. Pharmacol. 135: 77-88.

Goldman, S. J.; Stanbury, J. B. (1973) The metabolism of perchlorate in the rat. Endocrinology 92: 1536-1538.

Halmi, N. S.; Stuelke, R. G.; Schnell, M. D. (1956) Radioiodide in the thyroid and in other organs of rats treated with large doses of perchlorate. Endocrinology 58: 634-650.

Hancock, P. V. (1998) Information on ammonium perchlorate [memorandum to Annie Jarabek concerning size of ammonium perchlorate in processing and manufacturing plants].

Hard, G. C. (1998) Recent developments in the investigation of thyroid regulation and thyroid carcinogenesis. Environ. Health Perspect. 106: 427-436.

Hasselblad, V.; Jarabek, A. M.; Svendsgaard, D. J.; Davis, J. M. (1995) Restricted versus unrestricted models: Why is one the magic number? In: Abstracts of the 34th annual meeting of the Society of Toxicology; March; Baltimore, MD. Toxicologist 15: 178.

Hiasa, Y.; Kitahori, Y.; Kato, Y.; Ohshima, M.; Konishi, N.; Shimoyama, T.; Sakaguchi, Y.; Hsahimoto, H.; Minami, S.; Murata, Y. (1987) Potassium perchlorate, potassium iodide, and propylthiouracil: promoting effect on the development of thyroid tumors in rats treated with N-bis(2-hydrowypropyl)-nitrosamine. Jpn. J. Cancer Res. 78: 1335-1340.

Hill, R. N.; Erdreich, L. S.; Paynter, O.; Roberts, P. A.; Rosenthal, S. L.; Wilkinson, C. F. (1989) Thyroid follicular cell carcinogenesis. Fundam. Appl. Toxicol. 12: 629-697.

Hill, R. N.; Crisp, T. M.; Hurley, P. M.; Rosenthal, S. L.; Singh, D. V. (1998) Risk assessment of thyroid follicular cell tumors. Environ. Health Perspect. 106: 447-457.

Hobson, Q. J. G. (1961) Aplastic anaemia due to treatment with potassium perchlorate [letter]. Br. Med. J. (May 13): 1368-1369.

Holson, R. R.; Pearce, B. (1992) Principles and pitfalls in the analysis of prenatal treatment effects in multiparous species. Neurotoxicol. Teratol. 14: 221-228.

Hurley, P. M.; Hill, R. N.; Whiting, R. J. (1998) Mode of carcinogenic action of pesticides inducing thyroid follicular cell tumors in rodents. Environ. Health Perspect. 106: 437-445.

Interagency Perchlorate Steering Committee. (1998) Perchlorate ecological risk studies. Washington, DC: Interagency Perchlorate Steering Committee, Ecological Risk/Transport and Transformation Subcommittee; November 13.

International Commission on Radiological Protection. (1994) Human respiratory tract model for radiological protection: a report of a task group of the International Commission on Radiological Protection. Oxford, United Kingdom: Elsevier Science Ltd. (ICRP publication 66; Annals of the ICRP: v. 24, nos. 1-3).

Jannini, E. A.; Ulisse, S.; D'Armiento, M. (1995) Thyroid hormone and male gonadal function. Endocr. Rev. 16: 443-459.

Jarabek, A. M. (1995a) The application of dosimetry models to identify key processes and parameters for default dose-response assessment approaches. Toxicol. Lett. 79: 171-184.

Jarabek, A. M. (1995b) Interspecies extrapolation based on mechanistic determinants of chemical disposition. Hum. Ecol. Risk Assess. 1: 641-662.

Jarabek, A. M. (1995c) Consideration of temporal toxicity challenges current default assumptions. Inhalation Toxicol. 7: 927-946.

Jarabek, A. M. (1998) Personal communication. [Final consult letter. Re: morphology for neurodevelopmental..., specifically (1) measurement of mean follicular lumen area, and (2) arbitrary choice of this measure versus follicular height]. Research Triangle Park, NC: U.S. Environmental Protection Agency, National Center for Environmental Assessment; October 23.

Johnson, R. S.; Moore, W. G. (1961) Fatal aplastic anaemia after treatment of thyrotoxicosis with potassium perchlorate. Br. Med. J. (May 13): 1369-1371.

Kammüller, M. E. (1995) Drug-induced autoimmunity. Hum. Exp. Toxicol. 14: 117-119.

Kavlock, R. J.; Allen, B. C.; Faustman, E. M.; Kimmel, C. A. (1995) Dose-response assessments for developmental toxicity: IV. benchmark doses for fetal weight changes. Fundam. Appl. Toxicol. 26: 211-222.

Keil, D.; Warren, A.; Bullard-Dillard, R.; Jenny, M.; EuDaly, J. (1998) Effects of ammonium perchlorate on immunological, hematological, and thyroid parameters. Charleston, SC: Medical University of South Carolina, Department of Medical Laboratory Sciences; report no. DSWA01-97-1-008.

Kessler, F. J.; Krunkemper, H. J. (1966) Experimental thyroid tumors caused by many years of potassium perchlorate administration. Klin. Wochenschr. 44: 1154-1156.

King, J. H., Jr. (1995) Effects of ammonium perchlorate on the thyroid hormone levels of the Sprague-Dawley rat [thesis]. Air Force Institute of Technology; AFIT/GEE/ENV/95D-09.

Kodell, R. L.; West, R. W. (1993) Upper confidence limits on excess risk for quantitative responses. Risk Anal. 13: 177-182.

Kohn, M. C.; Sewall, C. H.; Lucier, G. W.; Portier, C. J. (1996) A mechanistic model of effects of dioxin on thyroid hormones in the rat. Toxicol. Appl. Pharmacol. 165: 29-48.

Krevans, J. R.; Asper, S. P., Jr.; Rienhoff, W. F, Jr. (1962) Fatal aplastic anemia following use of potassium perchlorate in thyrotoxicosis. JAMA J. Am. Med. Assoc. 181: 162-164.

Lampe, L.; Modis, L.; Gehl, A. (1967) Effect of potassium perchlorate on the foetal rabbit thyroid. Acta Med. Acd. Sci. Hung. 23 (3): 223-232.

Logan, B. E. (1998) A review of chlorate- and perchlorate-respiring microorganisms. Biorem. J. 2: 69-79.

Luster, M. I.; Munson, A. E.; Thomas, P. T.; Holsapple, M. P.; Fenters, J. D.; White, K. L., Jr.; Lauer, L. D.; Germolec, D. R.; Rosenthal, G. J.; Dean, J. H. (1988) Development of a testing battery to assess chemical-induced immunotoxicity: National Toxicology Program's guidelines for immunotoxicity evaluation in mice. Fundam. Appl. Toxicol. 10: 2-19.

ManTech Environmental Technology, Inc. (1998) Genotoxicity assays for ammonium perchlorate. Final report. Cincinnati, OH: ManTech Environmental Technology, Inc.; study no. 6100-001.

Männistö, P. T.; Ranta, T.; Leppäluoto, J. (1979) Effects of methylmercaptoimidazole (MMI), propylthiouracil (PTU), potassium perchlorate ($KClO_4$) and potassium iodide (KI) on the serum concentrations of thyrotrophin (TSH) and thyroid hormones in the rat. Acta Endocrinol. 91: 271-281.

Mantel, N.; Bryan, W. R. (1961) "Safety" testing of carcinogenic agents. J. Natl. Cancer Inst. 27: 455-470.

Marcus, A. H. (1998) Statistical analyses of standard histopathological measures of thyroid hypertrophy and follicular lumen size decrease in PND5 rats [memorandum to Annie Jarabek]. Research Triangle Park, NC: U.S. Environmental Protection Agency, National Center for Environmental Assessment; December 28.

Margolis, S. (1986) Health assessment, San Gabriel Valley, Los Angeles, California [memorandum to Donald W. Hawkins concerning detection of perchlorate in residential wells]. Washington, DC: U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry; January 21.

Mayer, K. (1998) Perchlorate: occurrence data to replace Table 1 from Siddiqui et al. [letter to Annie Jarabek]. San Francisco, CA: U.S. Environmental Protection Agency, Region IX - SFD-7-2; November 24.

McClain, R. M. (1992) Thyroid gland neoplasia: non-genotoxic mechanisms. Toxicol. Lett. 64/65: 397-408.

Meyer, G. D., Maj. (1998) Consultative letter, AFRL-HE-WP-CL-1998-0035, pharmacokinetic data for iodide uptake inhibition in the thyroid by perchlorate [memorandum to Annie Jarabek]. Wright-Patterson Air Force Base, OH: Air Force Research Laboratory, Human Effectiveness Directorate; December 23.

Morgans, M. E.; Trotter, W. R. (1960) Potassium perchlorate in thyrotoxicosis [letter]. Br. Med. J. (October 8): 1086-1087.

Murrell, J. A.; Portier, C. J.; Morris, R. W. (1998) Characterizing dose-response I: critical assessment of the benchmark dose concept. Risk Anal. 18: 13-26.

National Research Council. (1983) Risk assessment in the federal government: managing the process. Washington, DC: National Academy Press.

Norberg-King, T. J. (1990) Seven-day tests and chronic tests [author's reply]. Environ. Toxicol. Chem. 9: 1435-1436.

Nzengung, V. A. (n.d.) Laboratory characterization of phyto-transformation products of perchloroethylene (PCE), trichloroethylene (TCE) and perchlorate. Final report. Athens, GA: University of Georgia, Department of Geology.

Pajcr, Z.; Kališnik, M. (1991) The effect of sodium perchlorate and ionizing irradiation on the thyroid parenchymal and pituitary thyrotropic cells. Oncology 48: 317-320.

Porterfield, S. P. (1994) Vulnerability of the developing brain to thyroid abnormalities: environmental insults to the thyroid system. Environ. Health Perspect. Suppl. 102(2): 125-130.

Postel, S. (1957) Placental transfer of perchlorate and triiodothyronine in the guinea pig. Endocrinology 60: 53-66.

Rockette, H. E.; Arena, V. C. (1983) Mortality patterns of workers in the Niagra plant. Occidental Chemical Corporation [submitted to U.S. Environmental Protection Agency under the Toxic Substances Control Act]; microfiche no. OTS05370001.

Rogers, D. E., Lt. Col. (1998) Perchlorate user and production information [memorandum with attachments to Annie M. Jarabek]. Wright-Patterson Air Force Base, OH: Department of the Air Force, Air Force Materiel Command Law Office; October 30.

Ruppert, P. H.; Dean, K. F.; Reiter, L. W. (1985a) Development of locomotor activity of rat pups in figure-eight mazes. Dev. Psychobiol. 18: 247-260.

Ruppert, P. H.; Dean, K. F.; Reiter, L. W. (1985b) Development of locomotor activity of rat pups exposed to heavy metals. Toxicol. Appl. Pharmacol. 78: 69-77.

Said, S.; Schlumberger, M.; Suarez, H. G. (1994) Oncogenes and anti-oncogenes in human epithelial thyroid tumors. J. Endocrinol. Invest. 17: 371-379.

Sample, B. E.; Suter, G. W., II. (1994) Estimating exposure of terrestrial wildlife to contaminants. Oak Ridge, TN: Oak Ridge National Laboratories; ES/ER/TM-125.

Scheuplein, R. J.; Bronaugh, R. L. (1983) Percutaneous absorption. In: Goldsmith, L. A., ed. Biochemistry and physiology of the skin. Volume 2. New York, NY: Oxford University Press; p. 1279.

Schilt, A. A. (1979) Introduction. In: Perchloric acid and perchlorates. Columbus, OH: G. Frederick Smith Chemical Co.; pp. 8-63.

Schneider, B. F.; Golden, W. L. (1986) Acquisition of acoustic startle shows a dose-response to serum free T_4. Int. J. Dev. Neurosci. 4: 397-400.

Schulte, P. A. (1989) A conceptual framework for the validation and use of biologic markers. Environ. Res. 48: 129-144.

Selivanova, L. N.; Boltromeyuk, L. P.; Aref'eva, Z. S.; Vavilova, L. N. (1986) Dynamics of the absorption and elimination of perchloric acid salts in laboratory animals and livestock. Khim. Sel'sk. Khoz. (1963-1987) (5): 43-45.

Sher, E. S.; Xu, X. M.; Adams, P. M.; Craft, C. M.; Stein, S. A. (1998) The effects of thyroid hormone level and action in developing brain: are these targets for the actions of polychlorinated biphenyls and dioxins? Toxicol. Ind. Health 14: 121-158.

Shigan, S. A. (1963) Substantiation of the maxximum permissable concentration of ammonium perchlorate in water of reservoirs. Gig. Sanit. 28: 8-14.

Siddiqui, M.; LeChevallier, M. W.; Ban, J.; Phillips, T.; Pivinski, J. (1998) Occurrence of perchlorate and methyl tertiary butyl ether (MTBE) in groundwater of the American water system. Vorhees, NJ: American Water Works Service Company, Inc.; September 30.

Snipes, M. B.; James, A. C.; Jarabek, A. M. (1997) The 1994 ICRP66 human respiratory tract dosimetry model as a tool for predicting lung burdens from exposures to environmental aerosols. Appl. Occup. Environ. Hyg. 12: 547-554.

Southwell, N.; Randall, K. (1960) Potassium perchlorate in thyrotoxicosis. Lancet (March 19): 653-654.

Springborn Laboratories, Inc. (1998) A 90-day drinking water toxicity study in rats with ammonium perchlorate: amended final report [amended study completion date: June 3]. Spencerville, OH: Springborn Laboratories, Inc.; study no. 3455.1.

Stanbury, J. B.; Wyngaarden, J. B. (1952) Effect of perchlorate on the human thyroid gland. Metabolism 1: 533-539.

Stralka, D. (1992) Toxicity assessment request on perchlorate salts [memorandum with attachment to Mindy Henson, Superfund Technical Support Center]. San Francisco, CA: U.S. Environmental Protection Agency; October 9.

Sunar, O. (1963) Case report—agranulocytosis associated with potassium perchlorate treatment. J. Laryng. 77: 353-355.

Suter, G. W., II. (1990) Seven-day tests and chronic tests [letter]. Environ. Toxicol. Chem. 9: 1435.

Suter, G. W., II; Tsao, C.L. (1996) Toxicological benchmarks for screening potential contaminants of concern for effects on aquatic biota: 1996 revision. Oak Ridge, TN: Oak Ridge National Laboratories; ES/ER/TM-96/R2; June 26.

TRC Environmental Corporation. (1998) Chemical fertilizer as a potential source of perchlorate. Burbank, CA: Lockheed Martin Corporation; November.

Takata, K. (1985) Request for CDC assistance regarding potential health effects of perchlorate contamination at the San Gabriel Valley superfund sites [memorandum with attachments to Don Hawkins]. San Francisco, CA: U.S. Environmental Protection Agency, Superfund Records Center, Region 9; December 23.

Tamasy, V.; Meisami, E.; Vallerga, A.; Timiras, P. S. (1986) Rehabilitation from neonatal hypothyroidism: spontaneous motor activity, exploratory behavior, avoidance learning and responses of pituitary-thyroid axis to stress in male rats. Psychoneuroendocrinology 11: 91-103.

Toxicology Excellence for Risk Assessment. (1997) Proposed perchlorate reference dose (RfD). Cincinnati, OH: Toxicology Excellence for Risk Assessment; prepared for the Perchlorate Study Group.

Toxicology Excellence for Risk Assessment. (1998a) Notes from the March 1997 *ITER* peer review meeting. Cincinnati, OH: Toxicology Excellence for Risk Assessment. Available online at: www.tera.org/peer/.

Toxicology Excellence for Risk Assessment. (1998b) Results of the perchlorate study protocol review meeting: perchlorate study protocol peer review May 20, 1997 summary meeting notes. Cincinnati, OH: Toxicology Excellence for Risk Assessment. Available online at: www.tera.org.

Tsui, D. (1998) Oxidizing potential of perchlorate under physiological conditions [letter to Annie Jarabek]. Wright-Patterson Air Force Base, OH: Air Force Research Laboratory, Human Effectiveness Directorate, Operational Toxicology Branch; July 17.

Tsui, D. T.; Mattie, D. R.; Narayanan, L. (1998) Stability and concentration verification of ammonium perchlorate dosing solutions. Wright-Patterson Air Force Base, OH: Air Force Research Laboratory, Human Effectiveness Directorate; AFRL-HE-WP-TR-1998-0068.

U.S. Environmental Protection Agency. (1987) The risk assessment guidelines of 1986. Washington, DC: Office of Health and Environmental Assessment; report no. EPA/600/8-87/045. Available from: NTIS, Springfield, VA; PB88-123997/XAB.

U.S. Environmental Protection Agency. (1988) Recommendations for and documentation of biological values for use in risk assessment. Cincinnati, OH: Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office; EPA report no. EPA-600/6-87-008. Available from: NTIS, Springfield, VA; PB88-179874.

U.S. Environmental Protection Agency. (1993) Water quality guidance for the Great Lakes System and correction; proposed rules. F. R. 58(72): 20,802-21,047.

U.S. Environmental Protection Agency. (1994) Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry [draft final]. Research Triangle Park, NC: Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office; report no. EPA/600/8-88/066F.

U.S. Environmental Protection Agency. (1995) Guidance on risk characterization [memorandum of U.S. EPA Administrator, Carol M. Browner]. March 21.

U.S. Environmental Protection Agency. (1996a) Guidelines for reproductive toxicity risk assessment. Washington, DC: Office of Research and Development; report no. EPA/630/R-96/009. Available online at: www.epa.gov/ORD/WebPubs/repro/.

U.S. Environmental Protection Agency. (1996b) Air quality criteria for particulate matter. Research Triangle Park, NC: National Center for Environmental Assessment-RTP Office; report nos. EPA/600/P-95/001aF-cF. 3v. Available from: NTIS, Springfield, VA; PB96-168224.

U.S. Environmental Protection Agency. (1998a) Assessment of thyroid follicular cell tumors. Washington, DC: Office of Research and Development; report no. EPA/630/R-97/002. Available from: NTIS, Springfield, VA; PB98-133119. Available online at: www.epa.gov/ncea/thyroid.htm.

U.S. Environmental Protection Agency. (1998b) Guidelines for neurotoxicity risk assessment. Washington, DC: National Center for Environmental Assessment; report no. EPA/630/R-95/001Fa. Available online at: http://www.epa.gov/ncea/nurotox.htm.

U.S. Environmental Protection Agency. (1998c) Perchlorate: assessment of the state of the science. Report to the Congress of the United States. Washington, DC: Office of Research and Development; September.

U.S. Environmental Protection Agency. (1998d) Guidelines for ecological risk assessment. Washington, DC: Risk Assessment Forum; report no. EPA/630/R-95/002F. Available online at: www.epa.gov/ncea/ecorsk.htm.

U.S. Environmental Protection Agency. (1998e) 1998 update of ambient water quality criteria for ammonia. Washington, DC: Office of Water; report no. EPA 822-R-98-008. Available online at: www.epa.gov/ost/standards/amonsub.html.

Underwood, M. (1998) Exposure concerns and health implications of perchlorate. Presented at: The southeast focused ground water conference: discussing the issue of MTBE and perchlorate in groundwater; June; Anaheim, CA. Westerville, OH: National Ground Water Association.

Urbansky, E. T. (1998) Perchlorate chemistry: implications for analysis and remediation. Biorem. J. 2: 81-95.

Von Burg, R. (1995) Toxicology update: perchlorates. J. Appl. Toxicol. 15: 237-241.

Wall, J. R.; Fang, S. L.; Kuroki, T.; Ingbar, S. H.; Braverman, L. E. (1984) In vitro immunoreactivity to propylthiouracil, methimazole, and carbimazole in patients with Graves' disease: a possible cause of antithyroid drug-induced agranulocytosis. J. Clin. Endocrinol. Metab. 58: 868-872.

Weast, R. C., ed. (1989) CRC handbook of chemistry and physics. 69th ed. Boca Raton, FL: CRC Press, Inc.

Wenzel, K. W.; Lente, J. R. (1984) Similar effects of thionamide drugs and perchlorate on thyroid-stimulating immunoglobulins in Graves' disease: evidence against an immunosuppressive action on thionamide drugs. J. Clin. Endocrinol. Metab. 58: 62-69.

Whalan, J. E.; Redden, J. C. (1994) Interim policy for particle size and limit concentration issues in inhalation toxicity studies. U.S. Environmental Protection Agency, Office of Pesticide Products; February 1.

Wiltse, J.; Dellarco, V. L. (1996) U.S. Environmental Protection Agency guidelines for carcinogen risk assessment: past and future. Mutat. Res. 365: 3-15.

Wing, S. S.; Fantus, I. G. (1987) Adverse immunologic effects of antithyroid drugs. Can. Med. Assoc. J. 136: 121-125, 127.

Wolff, J. (1998) Perchlorate and the thyroid gland. Pharmacol. Rev. 50: 89-105.

Wolff, J.; Maurey, J. R. (1962) Thyroidal iodide transport. III. Comparison of iodide with anions of periodic group VIIA. Biochim. Biophys. Acta 57: 422-426.

York, R. G. (1998a) Update on additional items for 1613-002 [letter to Darol E. Dodd]. Horsham, PA: Argus Research Laboratories, Inc.; September 25.

York, R. G. (1998b) Protocol 1613-002 - A neurobehavioral developmental study of ammonium perchlorate administered orally in drinking water to rats. Sponsor's study number: 7757A210-1096-25F [letter to Annie Jarabek]. Horsham, PA: Argus Research Laboratories, Inc.; October 2.

York, R. G. (1998c) [Letter to Darol E. Dodd concerning geneology of the F1 generation rats]. Horsham, PA: Argus Research Laboratories, Inc.; October 26.

York, R. G. (1998d) Study 1613-002 - A neurobehavioral developmental study of ammonium perchlorate administered orally in drinking water to rats. Sponsor's study number: 7757A210-1096-25F [letter with attachments to Annie Jarabek]. Horsham, PA: Argus Research Laboratories, Inc.; November 5.

York, R. G. (1998e) 1416-002 - Oral (drinking water) developmental toxicity study of ammonium perchlorate in rabbits [letter with attachments to Annie Jarabek]. Horsham, PA: Argus Research Laboratories, Inc.; October 7.

Zeiger, E. (1998a) Salmonella mutagenicity testing of ammonium perchlorate [memorandum with attachment to Annie Jarabek]. Research Triangle Park, NC: U.S. Department of Health and Human Services, National Institute of Environmental Health Sciences; September 29.

Zeiger, E. (1998b) Ammonium perchlorate MN test results [memorandum to Annie Jarabek]. Research Triangle Park, NC: U.S. Department of Health and Human Services, National Institute of Environmental Health Sciences; December 23.

APPENDIX A

Schematics of Study Designs for Neurodevelopmental, Two-Generation Reproductive and Developmental Studies

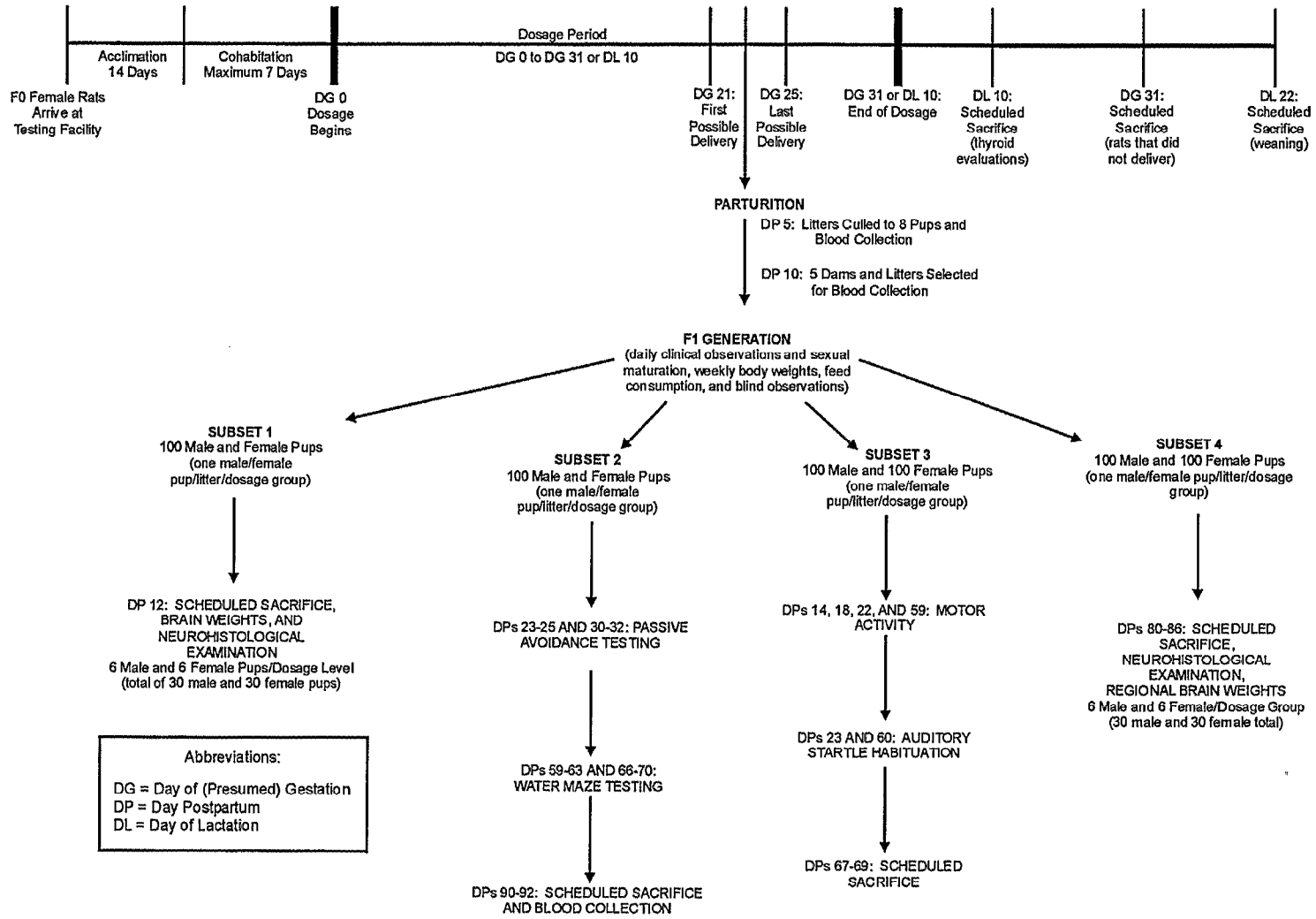


Figure A-1. Schematic of the neurobehavioral developmental study of ammonium perchlorate administered orally in drinking water to SD rats (Argus Research Laboratories, Inc., 1998a).

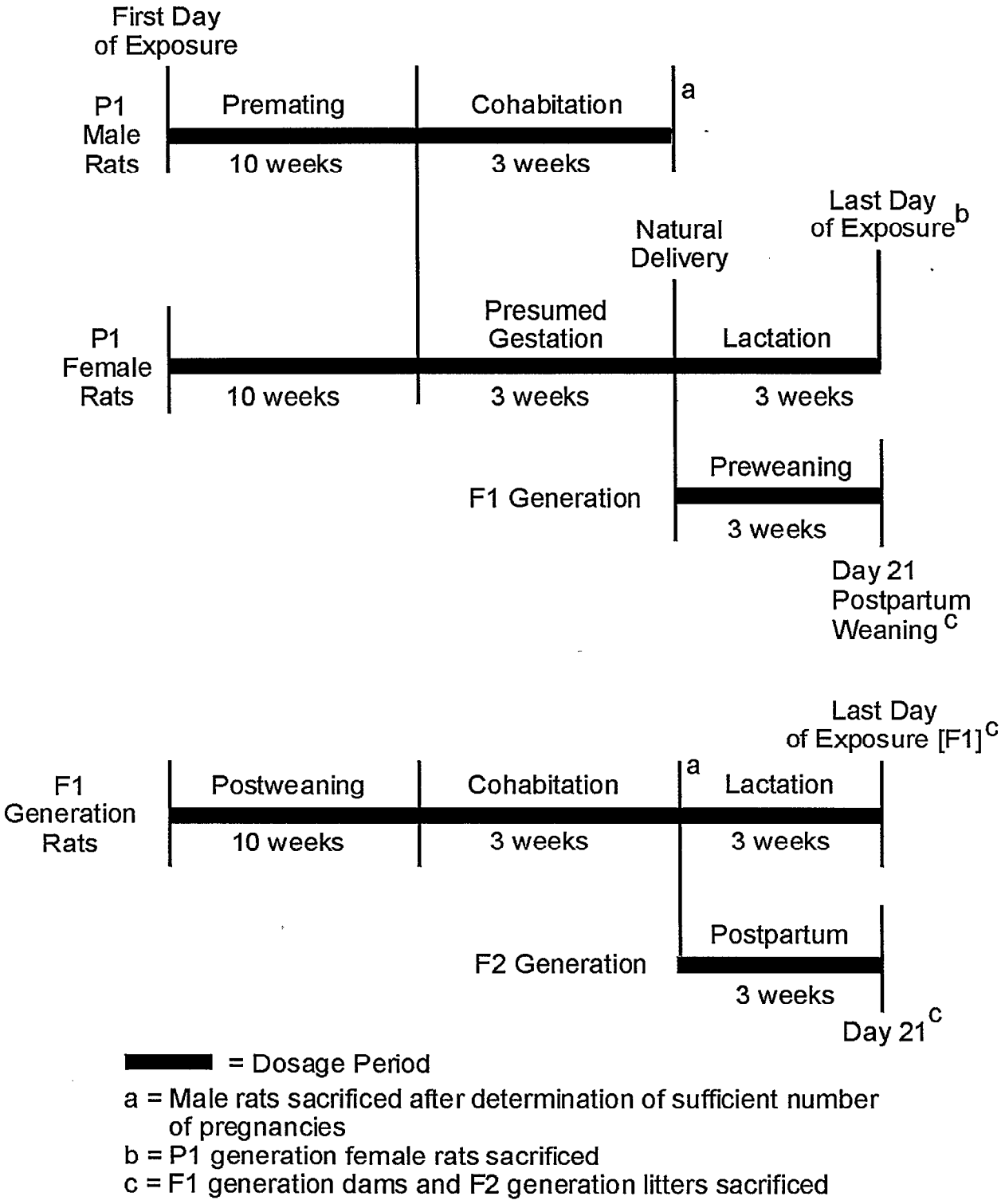
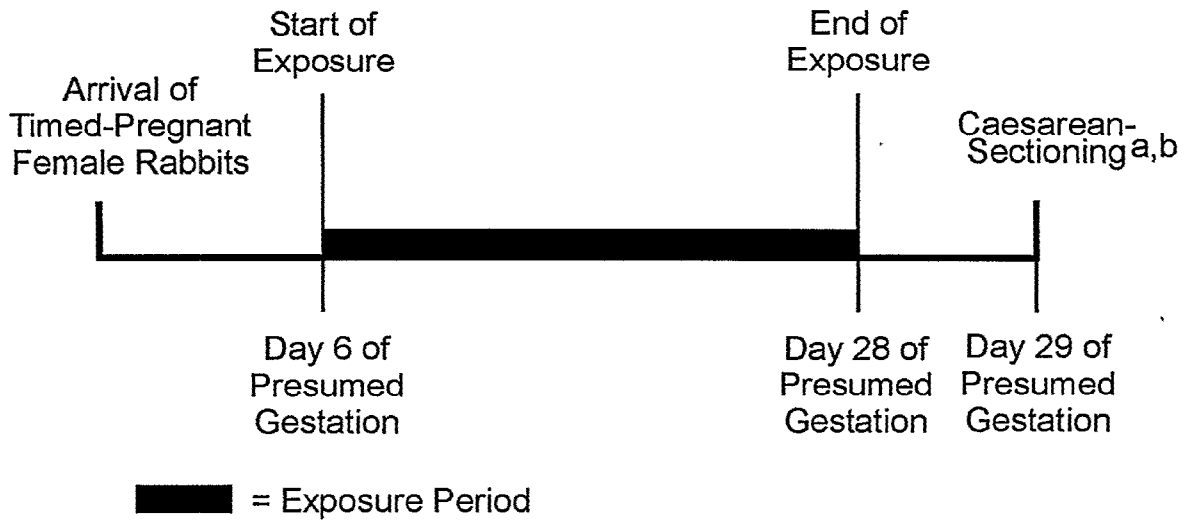


Figure A-2. Schematic of the oral (drinking water), two-generation (one litter per generation) reproduction study of ammonium perchlorate in SD rats (Argus Research Laboratories, Inc., 1998b).



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

ACR	Acute chronic ratio
ADME	Absorption, distribution, metabolism, and elimination
AFB	Air Force Base
AFRL/HEST	Air Force Research Laboratory/Human Effectiveness Directorate
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
ATSDR	Agency for Toxic Substances and Disease Registry
AV	Acute value
BMD	Benchmark dose
BMDL	Benchmark dose level
C'	Complement
CA DHS	California Department of Health Services
cAMP	Cyclic adenosine monophosphate
CCL	Contaminant candidate list
CFU	Colony forming units
ChV	Chronic value
ClO_4^-	Perchlorate
CsCl	Cesium chloride
CTL	Cytotoxic T lymphocyte
CV	Coefficients of variation
df	Degrees of freedom
DIT	Diiodotyrosine
DNA	Deoxyribonucleic acid
DoD	Department of Defense

DTH	Delayed type hypersensitivity
EGF	Epidermal growth factor
EPA	U.S. Environmental Protection Agency
ER	Endoplasmic reticulum
F1	First generation
FAVF	Final acute value factor
FETAX	Frog Embryo Teratogenesis Assay: *Xenopus*
FGF	Fibroblast growth factor
FH	Follicular epithelial cell hypertrophy or hyperplasia
F0	Parental generation
GA	Golgi apparatus
GD	Gestation day
GGTP	g-glutamyl transpeptidase
GMAV	Genus mean acute value
Gy	Gray (equal to 100 rads)
H_2O_2	Hydrogen peroxide
hCG	Human chorionic gonadotropin
HED	Human equivalent dose
HSD	Studentized Range Test
hTG	Thyroglobulin
I^-	Iodide
IC	Inhibitory concentration
IC	Ion chromatographic
IFN	Interferon
IGF-1	Insulin-like growth factor
IPSC	Interagency Perchlorate Steering Committee
IRIS	Integrated Risk Information System
LC	Lethal concentration

LD	Lactation day
LOAEL	Lowest-observed-adverse-effect level
LOEC	Lowest-observed-effect concentration
LP	Lymphoproliferation
LS	Lumen size
LY	Lysosomes
MCAS	Marine Corps Air Station
MF	Modifying factor
MIT	Monoiodotyrosine
MMIA	1-methyl-2-mercaptoimidazole
MTD	Maximum tolerated dose
n	Sample size number
Na^+	Sodium ion
NASA	National Aeronautics and Space Administration
NCEA	National Center for Environmental Assessment
NDEP	Nevada Division of Environmental Protection
NH_4	Ammonium
NH_4ClO_4	Ammonium perchlorate
NK	Natural killer
NOAEL	No-observed-adverse-effect level
NOEC	No-observed-effect concentration
NPDWR	National Primary Drinking Water Regulations
NRMRL	National Risk Management Research Laboratory
NTP	National Toxicology Program
OERR	Office of Emergency Response and Remediation
ORD	Office of Research and Development
OSWER	Office of Solid Waste and Emergency Response
OW	Office of Water

p	Probability
ppb	Parts per billion
ppm	Parts per million
P1	Parental generation
PA	Prealbumin and albumin
PBPK	Physiologically based pharmacokinetic
PCE	Polychromatic erythrocyte
PND	Postnatal day
PSG	Perchlorate Study Group
PTU	Propylthiouracil
RfC	Inhalation reference concentration
RfD	Oral reference dose
RIA	Radioimmunoassay
RO	Reverse osmosis
rT3	Reverse triiodothyronine
SACR	Secondary acute-chronic ratio
SAV	Secondary acute value
SD	Standard deviation
SDWA	Safe Drinking Water Act
SGOT	Serum glutamyl oxaloacetic transaminase
SLA	Soluble *Listeria* antigen
SRLB	Sanitation and Radiation Laboratory Branch
$T_{1/2}$	Half-life
T3	Triiodothyronine
T4	Thyroxine
TBG	Thyroid-binding globulin
TERA	Toxicological Excellence for Risk Assessment
Tg	Thyroglobulin

TPO	Thyroid peroxidase
TRH	Thyrotropin-releasing hormone
TSH	Thyroid stimulating hormone
UF	Uncertainty factor
USAF	U.S. Air Force
UT DEQ	Utah Department of Environmental Quality