EPA/635/R-08/001F
www.epa.gov/iris

TOXICOLOGICAL REVIEW

OF

THALLIUM AND COMPOUNDS

(CAS No. 7440-28-0)

In Support of Summary Information on the Integrated Risk Information System (IRIS)

September 2009

U.S. Environmental Protection Agency
Washington, DC

DISCLAIMER

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

CONTENTS—TOXICOLOGICAL REVIEW OF THALLIUM AND COMPOUNDS (CAS No. 7440-28-0)

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND ACRONYMS

AchE	acetyl cholinesterase
AIC	Akaike Information Criterion
ALA	aminolevulinic acid
ALT	alanine aminotransferase
AST	aspartate aminotransferase
ATSDR	Agency for Toxic Substance and Disease Registry
BMD	benchmark dose
BMD_{10}	benchmark dose corresponding to a 10% extra risk
$BMDL_{10}$	95% lower bound on the benchmark dose corresponding to a 10% extra risk
BMDS	benchmark dose software
BMR	benchmark response
BUN	blood urea nitrogen
CASRN	Chemical Abstracts Service Registry Number
ChAT	choline acetyltransferase
CHO	Chinese hamster ovary
EC_{50}	effective concentration necessary to produce a 50% response
EPA	Environmental Protection Agency
GI	gastrointestinal
GLP	good laboratory practice
GSH	reduced glutathione
5-HT	5-hydroxytryptamine
i.p.	intraperitoneal
IPCS	International Programme on Chemical Safety
i.v.	intravenous
IRIS	Integrated Risk Information System
LD_{50}	median lethal dose
LDH	lactate dehydrogenase
LOAEL	lowest-observed-adverse-effect level
MAO	monoamine oxidase
MDA	malondialdehyde
MEPP	miniature endplate potential
MRI	Midwest Research Institute
NA	nucleus accumbens
NHANES	National Health and Nutrition Examination Survey
NLM	National Library of Medicine
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
OSHA	Occupational Safety and Health Administration
PAD	peripheral arterial disease
POD	point of departure
PBPK	physiologically based pharmacokinetic
RfC	inhalation reference concentration
RfD	oral reference dose
s.c.	subcutaneous
SCE	sister chromatid exchange
Tl	thallium
UF	uncertainty factor

FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard and dose-response assessment in IRIS pertaining to chronic exposure to thallium and compounds. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of thallium and compounds.

The intent of Section 6, *Major Conclusions in the Characterization of Hazard and Dose Response*, is to present the major conclusions reached in the derivation of the reference dose, reference concentration, and cancer assessment, where applicable, and to characterize the overall confidence in the quantitative and qualitative aspects of hazard and dose response by addressing the quality of the data and related uncertainties. The discussion is intended to convey the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's IRIS Hotline at (202) 566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (email address).

AUTHORS, CONTRIBUTORS, AND REVIEWERS

CHEMICAL MANAGER/AUTHOR

Octavia Conerly, M.S.P.H.
Office of Water
U.S. Environmental Protection Agency
Washington, DC

AUTHORS

Susan Rieth, M.P.H.
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

CONTRACTOR SUPPORT

Robyn B. Blain, Ph.D.
ICF Consulting
Fairfax, VA

OFFICE OF RESEARCH AND DEVELOPMENT CO-LEAD

Susan Rieth, M.P.H.
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

REVIEWERS

This document has been reviewed by EPA scientists, interagency reviewers from other federal agencies and White House offices, and the public, and peer reviewed by independent scientists external to EPA. A summary and EPA's disposition of the comments received from independent external peer reviewers and from the public is included in Appendix A.

INTERNAL EPA REVIEWERS

Joyce Donohue, Ph.D.
Office of Water

Elizabeth Doyle, Ph.D.
Office of Water

Steven Kueberuwa, Ph.D.
Office of Water

Mike Hughes, Ph.D.
Office of Research and Development

Amal Mahfouz, Ph.D.
Office of Water

David Thomas, Ph.D.
Office of Research and Development

EXTERNAL PEER REVIEWERS

Ronald Baynes, DVM, Ph.D.
North Carolina State University

George Cherian, Ph.D.
University of Western Ontario

Lucio G. Costa, Ph.D. (chair)
University of Washington

George Daston, Ph.D.
The Procter & Gamble Company

Robert Hoffman, M.D.
New York University

Deborah Rice, Ph.D.
Maine Center for Disease Control

1. INTRODUCTION

This document presents background information and justification for the Integrated Risk Information System (IRIS) Summary of the hazard and dose-response assessment of thallium and compounds. IRIS Summaries may include oral reference dose (RfD) and inhalation reference concentration (RfC) values for chronic and other exposure durations, and a carcinogenicity assessment.

The RfD and RfC, if derived, provide quantitative information for use in risk assessments for health effects known or assumed to be produced through a nonlinear (presumed threshold) mode of action. The RfD (expressed in units of mg/kg-day) is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The inhalation RfC (expressed in units of mg/m^3) is analogous to the oral RfD, but provides a continuous inhalation exposure estimate. The inhalation RfC considers toxic effects for both the respiratory system (portal of entry) and for effects peripheral to the respiratory system (extrarespiratory or systemic effects). Reference values are generally derived for chronic exposures (up to a lifetime), but may also be derived for acute (≤24 hours), short-term (>24 hours up to 30 days), and subchronic (>30 days up to 10% of lifetime) exposure durations, all of which are derived based on an assumption of continuous exposure throughout the duration specified. Unless specified otherwise, the RfD and RfC are derived for chronic exposure duration.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral and inhalation exposure may be derived. The information includes 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. Quantitative risk estimates may be derived from the application of a low-dose extrapolation procedure. If derived, the oral slope factor is a plausible upper bound on the estimate of risk per mg/kg-day of oral exposure. Similarly, an inhalation unit risk is a plausible upper bound on the estimate of risk per $\mu g/m^3$ air breathed.

Development of these hazard identification and dose-response assessments for thallium and compounds has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). EPA guidelines and Risk Assessment Forum Technical Panel Reports that may have been used in the development of this assessment include the following: *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986a), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986b), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991a), *Interim Policy for Particle Size*

and Limit Concentration Issues in Inhalation Toxicity (U.S. EPA, 1994a), *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998), *Science Policy Council Handbook*: *Risk Characterization* (U.S. EPA, 2000a), *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000b), *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 2000c), *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002), *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA., 2005b), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 2006a), and *A Framework for Assessing Health Risks of Environmental Exposures to Children* (U.S. EPA, 2006b).

The literature search strategy employed for this compound was based on the Chemical Abstracts Service Registry Number (CASRN) and at least one common name. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document. The relevant literature was reviewed through May 2009.

2. CHEMICAL AND PHYSICAL INFORMATION

Metallic thallium (Tl) is bluish white or grey, very soft, malleable, and insoluble in water. Thallium is a Group IIIA metal, one whose salts do not hydrolyze at pH ≥ 7 to form insoluble hydroxides. According to Mulkey and Oehme (1993), this is a physical property that contributes to thallium's marked toxicity. Thallium exists in monovalent (thallous, thallium (I), Tl^{+1}) and trivalent (thallic, thallium (III), Tl^{+3}) states. Monovalent thallium is favored in the standard potential of Tl^{+3}/Tl^{+1}, coupling with a redox potential of +1.25V ($Tl^{+3} + 2e^-$ becomes Tl^{+1}). According to Pearson (1963), monovalent thallium is a Lewis acid (electron pair receiver) that prefers to interact with inorganic and organic sulfur, carbon, phosphorous, and arsenic moieties as the electron pair donor (Lewis base). Monovalent thallium ions also are more stable in aqueous solution, but trivalent thallium (Tl^{+3}) can be stabilized by complexing agents (Sabbioni et al., 1980a). Trivalent thallium forms more stable organic compounds than monovalent thallium.

Monovalent thallium is similar to potassium (K^+) in ionic radius and electrical charge, which contribute to its toxic nature. Many of the thallium salts are soluble in water with the exception of thallium (III) oxide, which is insoluble. Thallium compounds and their chemical and physical properties are listed in Table 2-1.

Thallium occurs naturally in the earth's crust, with a crustal abundance of approximately 1 mg/kg. In soil, thallium concentrations are on the order of 0.1 to 1 mg/kg; higher concentrations occur in the vicinity of metallic ore deposits. Measureable concentrations of thallium are also found in marine water, freshwater, and air. Thallium is taken up by vegetation, with the extent of uptake determined by soil acidity and plant species (Kazantzis, 2007).

According to the International Programme on Chemical Safety (IPCS) (1996), thallium is used only in small amounts by industry, and thus worldwide production of pure thallium is low. Sources for the production of thallium are zinc, lead, and sometimes copper or iron smelters and sulfuric acid plants as well as a by-product of cadmium production. In 1981 the production of thallium in the U.S. was discontinued. Thallium is released to the environment through the combustion of fossil fuels (in particular from coal-fired power-generating plants), refinement of oil fractions, smelting of ferrous and non-ferrous ores (including lead, copper, and zinc), and by some other industrial processes such as cement production and brickworks (Kazantzis, 2007; IPCS, 1996).

Due to its ability to remove hair, thallium (I) sulfate was used in the past as a depilatory agent. Thallium (I) sulfate was once used in medicine to treat infections, such as venereal diseases, ringworm of the scalp, typhus, tuberculosis, and malaria. It was also used in the past as a pesticide for various rodents and insects but has been banned for this use in the U.S. since 1972. Currently, thallium is used in the semiconductor industry and the manufacture of optic

lenses. When thallium is alloyed with mercury, it is used on switches and closures, which can operate at subzero temperatures. Thallium compounds are also used to manufacture low-melting glass, low-temperature thermometers, alloys, electronic devices, mercury lamps, fireworks, and imitation gems. Thallium radioisotopes are used in medicine for scintigraphy of certain tissues and the diagnosis of melanoma (Ibrahim et al., 2006; National Library of Medicine [NLM], 1998; IPCS, 1996; Agency for Toxic Substance and Disease Registry [ATSDR], 1992; U.S. EPA, 1991b).

Table 2-1. Chemical and physical properties of thallium and selected thallium compounds

Name	CASRN	Chemical formula	Molecular weight	Melting point (°C)	Boiling point (°C)	Solubility in water (g/L)
Metallic thallium	7440-28-0	Tl	204.38	303.5	1,457	Insoluble
Thallium (I) acetate	563-68-8	$TlC_2H_3O_2$	263.43	131	No data	Very soluble
Thallium (I) carbonate	6533-73-9	Tl_2CO_3	468.78	273	No data	40.3 (15.5°C)
Thallium (I) chloride	7791-12-0	TlCl	239.84	430	720	Very soluble (20°C)
Thallium (I) nitrate	10102-45-1	$TlNO_3$	266.39	206	430	95.5 (20°C)
Thallium (I) oxide	1314-12-1	Tl_2O	424.77	596	No data	Soluble (as TlOH)
Thallium (III) oxide	1314-32-5	Tl_2O_3	456.76	717	875	Insoluble
Thallium (I) selenite	12039-52-0	Tl_2SeO_3	535.72	No data	No data	No data
Thallium (I) sulfate	7446-18-6	Tl_2SO_4	504.82	632	Decomposes	48.7 (20°C)

Sources: IPCS (1996); Downs (1993); ATSDR (1992).

3. TOXICOKINETICS

3.1. ABSORPTION

Studies in humans and animals indicate that thallium compounds are readily absorbed through various routes of exposure, but few studies provide quantitative measures of absorption. Mulkey and Oehme (1993) reported that water soluble salts are rapidly and completely absorbed from the respiratory tract, gastrointestinal (GI) tract, or skin but did not provide data or cite references to support this conclusion. Thallium ions have been detected in the urine of exposed humans (Ludolph et al., 1986; Davis et al., 1981; Schaller et al., 1980; Cavanagh et al., 1974; Gefel et al., 1970) and animals (Thomas and McKeever, 1993; Waters et al., 1992; Leloux et al., 1987; Talas and Wellhöner, 1983), which implies absorption from environmental sources.

Shaw (1933) determined that 61.6% of an oral dose of thallium (I) sulfate (25 mg/kg Tl) was absorbed by a dog. Lie et al. (1960) determined that thallium was completely absorbed via the GI tract, following oral administration of 767 µg/kg ^{204}Tl as thallium (I) nitrate. This was based on observations in male Wistar-derived rats where the body burden decreased exponentially and extrapolated to 100% absorption. The same results were obtained when thallium (as thallium nitrate) was administered by other routes of exposure (intravenous [i.v.], 38 µg/kg; intramuscular, 96 µg/kg; subcutaneous [s.c.], 96 µg/kg; intratracheal, 123 µg/kg; and intraperitoneal [i.p.], 146 µg/kg). Eighty percent of a single dose of 10 nmol of thallium, as thallium (I) sulfate, was absorbed within 1 hour from tied-off jejunal segments in anesthetized rats (Forth and Rummel, 1975; Leopold et al., 1968).

No information was found regarding the absorption of thallium salts via inhalation. There are a few case reports (Hirata et al., 1998; Ludolph et al., 1986) in which occupational exposure has been associated with toxicity, but it could not be determined if exposure occurred via inhalation or another route (e.g., oral or dermal).

The use of thallium salts in the past as depilatory agents, treatment for ringworm of the scalp, and treatment for night sweats associated with tuberculosis suggests dermal absorption (Léonard and Gerber, 1997; Reed et al., 1963; Lie et al., 1960).

3.2. DISTRIBUTION

Thallium ions are rapidly distributed (as early as 1 hour after exposure) throughout the body in both experimental animals (Careaga-Olivares and Gonzalez-Ramirez, 1995; Galván-Arzate and Rios, 1994; Aoyama, 1989; Rios et al., 1989; Talas and Wellhöner, 1983; Sabbioni et al., 1980a, b; Lameijer and van Zwieten, 1977; Andre et al., 1960; Downs et al., 1960; Lie et al., 1960; Lund, 1956) and humans (Talas et al., 1983; Davis et al., 1981; Cavanagh et al., 1974; Barclay et al., 1953), regardless of the route of exposure, dose, and length of exposure (Sabbioni et al., 1980a, b; Lameijer and van Zwieten, 1977). The highest thallium concentrations have

typically been found in the kidney and the lowest concentrations in the brain, with none being detected in fat tissue. Thallium also has been demonstrated to cross the placenta in humans (Hoffman, 2000) and experimental animals (Gibson and Becker, 1970).

The distribution of thallium in newborn Wistar rats differed from that in adult Wistar rats. Newborns administered an i.p. dose of 16 mg/kg thallium (I) acetate (12.4 mg/kg Tl) had the highest levels of thallium in the testis, heart, and kidneys, in that order, 24 hours after administration (Galván-Arzate and Rios, 1994). Levels in the liver and brain were approximately three- to fourfold lower. In adult rats, the level of thallium in the kidney 24 hours after an i.p. dose of 16 mg/kg thallium (I) sulfate was approximately twofold higher than the level present in the testis (Rios et al., 1989). Galván-Arzate and Rios (1994) also demonstrated age-related differences in the regional distribution of thallium in the brain. Twenty-four hours after i.p. injection of 16 mg/kg thallium (I) acetate, the thallium content among all regions of the brain of newborn rats was homogeneous, whereas the thallium content in the brains of 5- to 20-day-old rats showed a region-dependent distribution, with thallium levels in the cortex significantly lower than levels in the hypothalamus.

3.3. METABOLISM

Because thallium is an element, it is not metabolized. It is not known if thallium is transformed from one valence state to another in vivo.

3.4. ELIMINATION

Thallium salts are eliminated mainly via urine and feces, but the amount excreted via each route varies depending on the species. Thallium also has been found to be excreted in breast milk, sweat, saliva, and tears. Thallium deposition into hair and nails also is considered an important route of elimination (Kazantzis, 2007; IPCS, 1996).

A study of a human cancer patient orally administered thallium (I) sulfate and radiolabeled thallium (I) nitrate ($^{204}TlNO_3$) demonstrated that thallium was mainly excreted in the urine; 15.3% of the thallium salts were recovered in the urine over 5.5 days with 0.4% recovered in the feces over 3 days (Barclay et al., 1953).

In a survey of 776 members of the general population (≥40 years of age) that participated in the 1999–2000 National Health and Nutrition Examination Survey (NHANES), the geometric mean level of thallium in the urine was 0.16 µg/L, with a maximum of 0.86 µg/L (Navas-Acien et al., 2005).

The *Third National Report on Human Exposure to Environmental Chemicals* (Centers for Disease Control and Prevention [CDC], 2005) provides ongoing biomonitoring data for the U.S. population for environmental chemicals over the periods 1999–2000 and 2001–2002 collected from NHANES participants. Selected urinary thallium level data from this survey are provided in Table 3-1. For the U.S. population (ages 6 and older), the geometric mean urinary thallium

concentration for survey years 2001–2002 was 0.165 µg/L, and the 95th percentile concentration was 0.440 µg/L.

Table 3-1. Urine concentrations of thallium for the U.S. population from NHANES, 1999–2002

	Survey years	Sample size	Urinary concentration of thallium in µg/L urine [µg/g creatinine][a]		
			Selected percentiles of the U.S. population		
			Geometric mean	50th	95th
Total, ages 6 and older	99–00	2413	0.176 (0.162–0.192) [0.166 (0.159–0.173)]	0.200 (0.180–0.210) [0.168 (0.162–0.176)]	0.450 (0.420–0.470) [0.366 (0.338–0.387)]
	01–02	2653	0.165 (0.154–0.177) [0.156 (0.151–0.162)]	0.180 (0.170–0.200) [0.156 (0.148–0.164)]	0.440 (0.410–0.470) [0.348 (0.337–0.365)]
Age group					
6–11 years	99–00	336	0.201 (0.167–0.243) [0.221 (0.197–0.248)]	0.200 (0.150–0.260) [0.221 (0.196–0.236)]	0.440 (0.350–0.590) [0.424 (0.356–0.600)]
	01–02	362	0.172 (0.147–0.202) [0.211 (0.198–0.226)]	0.200 (0.160–0.220) [0.207 (0.198–0.221)]	0.380 (0.360–0.420) [0.411 (0.389–0.456)]
12–19 years	99–00	697	0.202 (0.181–0.225) [0.153 (0.146–0.160)]	0.210 (0.200–0.240) [0.154 (0.146–0.162)]	0.460 (0.430–0.510) [0.321 (0.265–0.364)]
	01–02	746	0.200 (0.182–0.220) [0.143 (0.137–0.150)]	0.210 (0.190–0.240) [0.145 (0.135–0.152)]	0.460 (0.400–0.500) [0.307 (0.299–0.333)]
20 years & older	99–00	1380	0.170 (0.157–0.183) [0.162 (0.153–0.171)]	0.180 (0.170–0.200) [0.167 (0.154–0.176)]	0.450 (0.420–0.470) [0.364 (0.325–0.389)]
	01–02	1545	0.159 (0.147–0.173) [0.153 (0.147–0.159)]	0.190 (0.170–0.200) [0.152 (0.144–0.161)]	0.440 (0.400–0.490) [(0.342 (0.313–0.362)]
Gender					
Males	99–00	1200	0.197 (0.179–0.217) [0.154 (0.147–0.161)]	0.220 (0.190–0.240) [0.156 (0.149–0.164)]	0.440 (0.420–0.480) [0.338 (0.300–0.364)]
	01–02	1313	0.184 (0.173–0.196) [0.146 (0.140–0.153)]	0.200 (0.190–0.220) [0.148 (0.141–0.156)]	0.420 (0.390–0.460) [0.307 (0.291–0.342)]
Females	99–00	1213	0.159 (0.145–0.175) [0.178 (0.167–0.189)]	0.180 (0.150–0.200) [(0.182 (0.169–0.196)]	0.450 (0.410–0.490) [0.380 (0.333–0.462)]
	01–02	1340	0.149 (0.137–0.163) [0.167 (0.158–0.176)]	0.150 (0.150–0.170) [0.167 (0.153–0.179)]	0.430 (0.400–0.500) [(0.375 (0.348–0.402)]

[a]95th percentile confidence interval in parentheses.

Source: CDC (2005).

As noted above, thallium elimination is not limited to renal excretion. IPCS (1996) estimated that in humans renal excretion accounts for approximately 70% of total daily excretion of thallium. This estimate is based on limited human data.

In contrast to humans, thallium is excreted to a greater extent in the feces than in the urine of rats and rabbits. IPCS (1996) estimated that in rats about 2/3 of the intake of thallium

was excreted via the GI tract and about 1/3 via the kidney. Lund (1956) determined that, after 26 days, 51.4% of an i.p. dose of 10 mg/kg thallium (I) sulfate in the rat was eliminated via the feces, while 26.4% was excreted in the urine. Talas and Wellhöner (1983) demonstrated that thallium (I) acetate administered to rabbits via i.v. injection (as a radioactive tracer) was excreted mainly in the feces. Both studies found that, although the feces was the major route of excretion in the rat and rabbit, neither species had high levels in the bile, suggesting that excretion via the liver was relatively low. Lund (1956) determined that thallium was mainly excreted in the feces through gastric and intestinal secretions, which is likely associated with potassium excretion. Lund (1956) demonstrated that rabbits excreted thallium through the kidneys by glomerular filtration, but approximately one-half the dose filtered was reabsorbed in the tubuli.

In Syrian golden hamsters, thallium (I) sulfate was mainly excreted in the feces after i.p. administration but was excreted at an equal rate in the feces and urine after an oral dose (Aoyama, 1989). Shaw (1933) demonstrated that 32 and 61.6% of a single oral dose of 25 mg/kg Tl as thallium (I) sulfate administered to a dog was excreted in the urine at 3 and 36 days after dosing, respectively.

Sabbioni et al. (1980b) determined that thallium (I) sulfate administered at doses of 0.00004–2,000 μg/rat was persistent in the kidneys for 8 days (192 hours) after dosing with 2.5% of the dose still present at that time (suggesting a half-life of approximately 1.5 days). Lehmann and Favari (1985) and Lie et al. (1960) estimated the biological half-life of thallium in rats to range from 3–8 days. The biological half-life in humans has been estimated to be approximately 10 days, with values up to 30 days reported (IPCS, 1996).

3.5. PHYSIOLOGICALLY BASED TOXICOKINETIC MODELS

There are no physiologically based toxicokinetic models for thallium compounds.

4. HAZARD IDENTIFICATION

4.1. STUDIES IN HUMANS—EPIDEMIOLOGY, CASE REPORTS, CLINICAL CONTROLS

Studies of thallium toxicity in humans are comprised of clinical reports, case studies, and medical surveys. Because case reports largely involved accidental ingestion, intentional poisoning, or suicide attempts, they do not provide useful information on thallium toxicity associated with chronic exposure. Available epidemiology studies involving long-term exposure to thallium are limited by small study populations and insufficient characterization of long-term exposure. Health effects information was based on self-reporting (via questionnaire) or medical histories/physical examinations of uncertain scope.

In adults, the average lethal oral dose has been estimated to range from 10 to 15 mg/kg (Gosselin et al., 1984; Schoer, 1984). Without treatment, death typically follows in about 10–12 days, but death as soon as 8–10 hours also has been documented (IPCS, 1996).

4.1.1. Incident/Case Reports

As indicated by case reports, the acute toxicity of thallium is characterized by alopecia (hair loss), severe pain in the extremities, lethargy, ataxia, abdominal pain or vomiting, back pain, abnormal reflexes, neuropathy, muscle weakness, coma, convulsion, other neurological symptoms (i.e., mental abnormalities, tremors, abnormal movements, abnormal vision, and headache), and death (Lu et al., 2007; Tsai et al., 2006; Saha et al., 2004: Sharma et al., 2004; Rusyniak et al., 2002; Atsmon et al., 2000; Hirata et al., 1998; Feldman and Levisohn, 1993; Yokoyama et al., 1990; Heyl and Barlow, 1989; Roby et al., 1984; Limos et al., 1982; Davis et al., 1981; Cavanagh et al., 1974; Gefel et al., 1970; Reed et al., 1963). Symptoms were observable within 14 hours after a high dose (i.e., 5–10 g of thallium (I) nitrate), with death occurring 8 days later (Davis et al., 1981). The lowest known single dose of thallium associated with adverse effects was reported to be 0.31 g of thallium (I) acetate (3.4 mg/kg Tl, assuming a 70 kg body weight) (Cavanagh et al., 1974). This dose caused paresthesia, pain, weakness, vomiting, and alopecia in a 26-year-old male. Approximately 1 month after the onset of symptoms, complete recovery occurred following treatment. In adults, doses ranging from 6 to 40 mg/kg have been reported to be lethal (IPCS, 1996). Table 4-1 summarizes the individual case reports.

Table 4-1. Thallium toxicity in humans following oral exposure

Reference	Sex	Age	Dose	Symptoms[a]	Final outcome
Males—adult					
Gefel et al. (1970)	Male	41 years	Unknown but chronic; urine thallium 0.15 mg/100 mL	High blood pressure; lower back pain; vomiting; severe pain in the feet; weakness of the calf muscle; alopecia; slurred speech; atrophic lower limbs; limited vision	Death
Cavanagh et al. (1974)	Male	60 years	0.93 g thallium (I) acetate in 2 divided doses	Diarrhea; vomiting; dizziness; back pain; paresthesia of the feet and lower legs; high blood pressure; facial weakness; dysphagia; difficulty breathing	Death within a week of symptoms
Cavanagh et al. (1974)	Male	56 years	0.93 g thallium (I) acetate in 3 divided doses	Abdominal pain; diarrhea; vomiting; paresthesia; photophobia, nystagmus, visual impairment; facial weakness; bilateral ptosis	Death within 3 weeks of symptoms
Cavanagh et al. (1974)	Male	26 years	0.31 g thallium (I) acetate	Paresthesia in both feet; chest pain; tenderness over the sternum; vomiting, weakness, pain in the knees and ankles that inhibited walking; alopecia	Recovery
Davis et al. (1981)	Male	19 years	5–10 g thallium (I) nitrate	Nausea; vomiting; slurred speech; paresthesia of hands and feet; respiratory weakness	Death
Limos et al. (1982)	Male	56 years	Unknown	Visual disturbances; alopecia; elevated AST and ALT; high blood glucose and creatine kinase; decreased myelinated fibers; denervated Schwann cell clusters	Bedridden; could not speak
Limos et al. (1982)	Male	26 years	Unknown	Visual disturbances; alopecia; elevated AST and ALT; high blood glucose and creatine kinase; decreased myelinated fibers; denervated Schwann cell clusters	Residual tremors of the extremities and muscle weakness of the lower limbs
Roby et al. (1984)	Male	45 years	Unknown; urine thallium: 2,000 µg/L	Burning pain in feet; inability to walk; alopecia; acute fibrillation	Continued neurological dysfunction

Table 4-1. Thallium toxicity in humans following oral exposure

Reference	Sex	Age	Dose	Symptoms[a]	Final outcome
Heyl and Barlow (1989)	Male	"Five young men"	Unknown	Follicular plugging of the skin (nose, cheeks, and nasolabial folds) by keratinous material; crusted eczematous lesions and acneiform eruptions on the face; dry scaling on palms and soles; and alopecia (scalp, eyelashes, lateral eyebrows, arms, and legs). Skin biopsies (scalp and cheek): disintegrating hair shafts, gross follicular plugging, and eosinophilic keratohyalin granules in the epidermis; necrotic sebaceous glands; pustular lesions on the face: folliculitis and necrosis of the follicles; (feet) marked hyperkeratosis and hypergranulosis.	4/5 recovered; 1/5 experienced permanent neurological damage
Yokoyama et al. (1990)	Male	31 years	Unknown; urine thallium: 3.5 mg/L	Nausea, vomiting; leg pain; alopecia; abnormal behavior; decreased conduction velocity of fast nerve fibers	Recovery
Hantson et al. (1997)	Male	48 years	200 mg thallium (I) sulfate	No overt symptoms within 24 hours; increase in binucleated cells with micronuclei 15 days after exposure	Recovery
Hirata et al. (1998)	Male	29 years	Unknown; hair thallium: 20 ng/g (32 months after possible exposure)	Alopecia; abdominal pain; diarrhea; tingling in extremities; neuropathy	Recovery
Atsmon et al. (2000)	Male	40 years	Unknown; urine thallium: 7 mg	Weakness of the limbs; vomiting; severe neurological symptoms; alopecia; high blood pressure; increased ALT and AST; Mees lines; decreased visual acuity; bilateral foot drop	Recovery
Sharma et al. (2004)	Male	48 years	Unknown; serum thallium: 870 µg/100 mL urine thallium: 5,000 µg/mL	Painful peripheral neuropathy, decreased consciousness	Death

Table 4-1. Thallium toxicity in humans following oral exposure

Reference	Sex	Age	Dose	Symptoms[a]	Final outcome
Females—adult					
Roby et al. (1984)	Female	51 years	Unknown; serum thallium: 50 µg/100 mL; urine thallium: 5,000 µg/L	Numbness and weakness of the legs and hands; alopecia; fluctuating pulse and blood pressure; bradycardia; hypotension	Persistent ventricular ectopy and neurological dysfunction, necessitating placement at a nursing home
Roby et al. (1984)	Female	61 years	Unknown; serum thallium: 740 µg/100 mL	Burning chest pain; paresthesia; difficulty speaking and swallowing; inability to walk; hypotension; acute respiratory distress syndrome (ARDS)	Death
Roby et al. (1984)	Female	80 years	Unknown; serum thallium: 422 µg/100 mL; urine thallium: 21,600 µg/L	ARDS	Death
Hoffman (2000)	Female	Pregnant; ages not specified	150–1,350 mg thallium (I) sulfate	Paresthesia; abdominal pain; muscle weakness; lethargy; alopecia; Mees lines	None specified
Saha et al. (2004)	Female	26 years	Unknown; serum thallium: 12 µg/100 mL	Headache, lethargy, abdominal pain, muscle cramps, joint pain, backache, numbness of fingers, alopecia, erosion of nails	Not specified
Both sexes—adult					
Brockhaus et al. (1981)	Both	Not reported	Unknown	Sleep disorders; tiredness; weakness; nervousness; headache; other psychic alterations; neurological and muscular symptoms	Not reported
Schoer (1984); Gosselin et al. (1984)	Both	Adult	10–15 mg/kg thallium	None specified	Death (average lethal dose)
Rusyniak et al. (2002)	Both	Various	Unknown; various levels were detected in urine	Myalgia; arthralgia; paresthesia; dysesthesia; joint stiffness; insomnia; alopecia; abdominal pain	Recovery in 7 adults; 5 had ongoing psychiatric problems

Table 4-1. Thallium toxicity in humans following oral exposure

Reference	Sex	Age	Dose	Symptoms[a]	Final outcome
Tsai et al. (2006)	Both	48-year old female; 52-year old male	1.5–2 .4 g	Confusion, disorientation, hallucination, anxiety, depression, memory impairment, peripheral neuropathy, erythematous skin rashes, diarrhea, tachycardia, alopecia	Impairment of memory and verbal fluency remained at 6 months; neuropsychological impairment persisted at 9 months
Lu et al. (2007); Kuo et al. (2005)	Both	48 and 52 years	1.5 and 2.3 g/person (estimated); Serum thallium: 950–2,056 µg/L Urine thallium: 11,325–14,520 µg/L	Nausea, vomiting; general aching muscle pain; numbness of tongue and mouth within a few hours; severe paresthesia and dysesthesia in hands and feet (one day postexposure); erythematous rash; diarrhea; urine retention; hyporeflexia; muscle weakness; hypoesthesia; acneiform eruptions; alopecia (1–3 weeks); Mees lines (2–3 months). Skin biopsy: parakeratosis; dilated hair follicles filled with keratin and necrotic sebaceous materials; mild epidermal atrophy; vacuolar degeneration of the basal layer. Cutaneous nerve biopsy: axonal degeneration; loss of epidermal nerves indicating involvement of the small sensory nerves (2 months).	At 1-year follow-up, persistent paresthesia, dysesthesia, and impairment of small sensory nerve fibers in skin
Children					
Reed et al. (1963)	Both	1–11 years	Unknown	Alopecia; lethargy; ataxia; abdominal pain; vomiting; abnormal reflexes; neuropathy; muscle weakness; coma; convulsion	Neurological abnormalities; retardation; psychosis; death
Feldman and Levisohn (1993)	Male	10 years	Unknown; serum thallium: 296 µg/L; urine thallium: 322 µg/24 hours	Alopecia; leg paresthesia; abdominal pain; seizures	Recovery
Hoffman (2000)	Both	Transplacental	Unknown	Premature birth; low birth weight; alopecia	None specified

Table 4-1. Thallium toxicity in humans following oral exposure

Reference	Sex	Age	Dose	Symptoms[a]	Final outcome
Ammendola et al. (2007)	Male	16 years	1.3 g thallium sulfate; urine thallium: 3,400 μg/L	Acute stage: GI disturbances, alopecia, and clinical and electrodiagnostic signs of severe polyneuropathy.	3 years post-poisoning: neurological symptoms making progress; electrophysiological signs of peripheral neuropathy mainly confined to lower limbs. 6 years post-poisoning: persistent weakness and sensory disturbances of distal lower extremities; neurological and electrodiagnostic abnormalities affecting mainly the feet.

[a]ALT = alanine aminotransferase; AST = aspartate aminotransferase.

High blood pressure or fluctuating blood pressure was noted upon hospital admission in several cases (Roby et al., 1984; Cavanagh et al., 1974; Gefel et al., 1970). Elevated serum aspartate aminotransferase (AST) (formerly referred to as serum glutamic oxaloacetic transferase) and serum alanine aminotransferase (ALT) (formerly referred to as serum glutamate pyruvate transaminase), high blood glucose, and creatine kinase values also have been noted in case reports of thallium exposure (Atsmon et al., 2000; Limos et al., 1982). The same symptoms were noted across age and sex groupings. Retardation and psychosis were the most common findings in children (1–11 years old) after nonlethal thallium exposure. Several cases were so severe that institutionalization was necessary (Reed et al., 1963). Thallium significantly decreased the conduction velocities of faster nerve fibers in a 31-year-old male, who ingested a thallium-containing rodenticide, compared with baseline levels recorded following recovery.

In most case-study reports, thallium was detectable in the urine or tissues. In some cases, thallium could not be definitively associated with the symptoms because other heavy metals were also found in the blood or urine of the subject.

Hantson et al. (1997) evaluated cytogenetic changes in blood from a 48-year-old man who accidentally ingested 200 mg of thallium (I) sulfate intended for rodenticide use. Despite the lack of overt symptoms 24 hours after ingesting the thallium, the man was admitted to the emergency room and Prussian blue treatments were commenced. Blood samples were obtained on days 1 and 15 for cytogenetic analysis. Slight increases in mean sister chromatid exchange (SCE) numbers on days 1 and 15 were not considered related to thallium exposure. A 3.5-fold increase in binucleated cells with micronuclei (35% versus 10% in the historical controls) was noted on day 15. The thallium level was determined to be 14.4 μg/dL in blood at the time of hospital admission, and the concentration in urine was 3,804 μg/g Tl creatinine (reference value, <1 μg/g Tl).

Fifty-one case histories of women treated for thallium poisoning following external application of a 3 to 8% thallium (I) acetate ointment were reviewed for signs of possible thallium intoxication (Munch, 1934). Neurological and GI symptoms were observed in 29 cases after an unspecified number of applications with 2–24 ounces of the ointment. This was approximately equivalent to a dose of 53–636 mg/kg Tl per application using a 5.5% ointment on a 50 kg woman. Alopecia followed several weeks after beginning treatment.

Hoffman (2000) provided case reports and a comprehensive literature review of thallium poisoning that occurred during pregnancy. Exposures were primarily oral, but some of the cases involved dermal exposure. The majority of the doses were unreported, but those doses that were documented ranged from 150–1,350 mg thallium (I) sulfate. Of the 18 cases that met Hoffman's criteria (cases were excluded if maternal or fetal outcomes were not provided), 5 women were exposed during the first trimester and 5 during the second trimester; the remaining 8 were exposed during the third trimester. The ages of the women were not reported. While the mothers developed the classic symptoms of thallium poisoning, including paresthesia, abdominal

pain, muscle weakness, lethargy, alopecia, and Mees lines (single transverse white bands occurring on the nails), the only consistent finding in their offspring was a trend toward prematurity and low birth weight. Several of the children had alopecia, particularly those exposed during the third trimester.

4.1.2. Population Surveys

Several published studies have surveyed populations living near a cement plant in Lengerich, a small city in northwest Germany. These populations were studied because of their potential to experience exposure to thallium as a result of its presence as an impurity in pyrite and its release during the roasting of pyrite for use in making some types of cement. Thallium was discharged to outdoor air, deposited in soils, and taken up by local crops and indigenous plants. People who lived near the plant and consumed home-grown foods thus were exposed to thallium through their diets. Prior to 1979, the concentration of thallium in the pyrite was 400 ppm. After 1979, a pyrite with lower levels of thallium (2 ppm) was used.

Brockhaus et al. (1981) conducted an epidemiologic study of a group of 1,200 people living near the cement plant in Lengerich. Urinary thallium data were also collected from two reference populations without increased thallium intake—one group consisting of 31 persons living in a small (rural) city in northwest Germany and a second group consisting of 10 persons living in an urban area in Dusseldorf, Germany. The study investigators did not perform specific tests for toxicity but surveyed for the presence of certain symptoms by using questionnaires. Thallium exposure was assessed by measurements in urine and hair. The thallium body burden of the study population was increased over the reference populations, as indicated by a mean urinary thallium level of 5.2 ± 8.3 μg/L (range: <0.1–76.5 μg/L) in the study population compared to the reference population means of 0.4 ± 0.2 μg/L (rural) and 0.3 ± 0.2 μg/L (urban) (range: 0.1–1.2 μg/L). The predominant contributing factor to the thallium burden was consumption of homegrown fruits and vegetables. When the consumption of homegrown foods was restricted, thallium exposure was reduced, as indicated by decreased thallium in the urine. No correlation between dermal or GI symptoms and thallium level was observed. There was a negative correlation between urinary thallium and hair loss (13.6% with urine levels <2 μg/L, 6.6% with urine levels 2–20 μg/L, and 5.9% with urine levels >20 μg/L; 10.7% with hair levels <10 ng/g, 9.6% with hair levels 10–50 ng/g, and 2.3% with hair levels >50 ng/g). These data appear to conflict with other reports that indicate hair loss increases with increasing thallium exposure. A positive association was observed among thallium levels in urine or hair and the following self-reported symptoms: sleep disorders, tiredness, weakness, nervousness, headache, and psychological alterations as well as neurological and muscular symptoms (Brockhaus et al., 1981).

Dolgner et al. (1983) examined the potential developmental effects of thallium in this same German population. Of 300 births registered in Lengerich between January 1, 1978, and

August 31, 1979, questionnaires on health status and maternal risk factors were completed by the mothers of 297 infants. One hundred fifty-four urine and 164 hair samples were analyzed for thallium content. All children with suspected congenital malformations or other abnormalities were examined physically, and medical histories of mothers were taken. Eleven out of the 297 births were identified as exhibiting congenital malformations or abnormalities (confirmed by a pediatrician) with five major malformations noted. Two of the five major malformations in the study population were determined by the authors to likely be due to hereditary factors.

The observed rate of congenital malformations in the study population (5/297) was compared to the expected rate of 0.8/297 births based on annual statistics from the North Rhine-Westphalia region of Germany for 1974–1978. Congenital malformations in the reference population were thought to be underreported because reporting of birth defects is not required on birth certificates in that area of Germany. The study authors noted that other investigations reported an incidence of 2–3% of birth defects among live births, a value that is consistent with 1.7% incidence of birth defects in the study population (5/297 for major malformations) and 3.7% (11/297) for all malformations. The study authors concluded that a causal relationship between thallium exposure and congenital malformations in this population was unlikely. However, study deficiencies, including lack of information on exposure to thallium at the time of pregnancy, limit the strength of this study.

Navas-Acien et al. (2005) examined the association between urinary levels of various metals, including thallium, with peripheral arterial disease (PAD) in a cross-sectional analysis of 790 participants in NHANES 1999–2000. Thallium was not associated with PAD in this sample of the U.S. population.

4.1.3. Occupational Exposure

Schaller et al. (1980) examined 128 men (ages 16–62 years) who were exposed to thallium for 1–42 years in three cement manufacturing plants in the Franconia region of Germany. Health effects were determined through medical histories and a physical examination for symptoms. Information on the scope of the physical examinations was not provided. Analyses of roasted pyrites and electro-filter dust confirmed the presence of thallium in various production areas in the plants. The median concentration of thallium in the urine in exposed workers was 0.8 μg/g Tl creatinine with a range of <0.3–6.3 μg/g Tl creatinine. The range in 20 individuals without known occupational exposure was <0.3–1.1 μg/g Tl creatinine (median concentration not reported). Medical histories and physical examinations did not indicate thallium poisoning. The health status of exposed workers, however, was not compared with an unexposed reference population, and a single measurement of urinary thallium did not provide a measure of past exposures.

Thirty-six cement plant workers (presumably in Germany) were examined for clinical and electrophysiological parameters (Ludolph et al., 1986). Thallium levels were found to be

elevated in the blood of 16 workers, urine of 5 workers, and hair of 5 workers. It was not noted if these were all separate cases or if elevations in all three parameters occurred in the same individuals. The study determined that 28–39% of the individuals had some form of peripheral and central motor and sensory impairment. The neurological impairments could not conclusively be attributed to thallium exposure because half the patients suffered from concurrent diseases (including peptic ulcer, diabetes, disorders of joints and connective tissue, and hypertensive vascular disease), which could possibly cause neuromuscular impairment. No controls were employed, and no correlations were made with the levels of thallium in individuals and their disease states.

In another occupational study, Marcus (1985) examined medical records for 86 workers (sex not reported) occupationally exposed to thallium at a magnesium seawater battery factory. Exposure was determined by measuring thallium in urine samples. Marcus also examined the records of 79 unexposed workers matched for age, length of employment, shift pattern, and type of work. Exposed workers did not have an increase in incidence of benign neoplasms or any other clinical diagnoses when compared with unexposed workers. This study is limited by lack of exposure quantitation, the size of the cohort, and unknown length of follow-up.

Although there are many case reports of thallium poisoning in the literature, the doses were largely unknown because ingestion was accidental or occurred through criminal poisoning. Given the severity of reported symptoms, most of the exposures were likely to have been relatively large. The few epidemiology studies that looked at populations surrounding a cement factory that released thallium only attempted to compare thallium exposure with congenital malformations or surveyed symptoms. None of the studies specifically studied cancer as an endpoint. Overall, the available epidemiology literature is considered limited and inconclusive.

4.2. LESS-THAN-LIFETIME AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS—ORAL AND INHALATION

4.2.1. Oral Exposure

4.2.1.1. *Acute and Subchronic Studies*

4.2.1.1.1. *Rats.* In a study performed by Midwest Research Institute (MRI) (1988) for EPA's Office of Solid Waste, male and female Sprague-Dawley rats (45 days old, 20/sex/group) were administered 0 (untreated and vehicle controls), 0.01, 0.05, or 0.25 mg/kg-day of an aqueous solution of thallium (I) sulfate (approximately 0, 0.008, 0.04, or 0.20 mg/kg-day Tl) by gavage for 90 days. The study was conducted in compliance with EPA good laboratory practice (GLP) mandates. The MRI (1988) study is an unpublished study; accordingly, an external peer review was initiated by EPA in November 2006. Body weight, food consumption, hematologic and clinical chemistry parameters, ophthalmologic examinations, gross pathological observations, and organ weights (liver, kidneys, brain, gonads, spleen, heart, and adrenals) were recorded for all animals. Neurotoxicological examinations (three times/week) were performed on six

rats/sex/group; these examinations were apparently observational (further details were not provided in the study report). Tissues from three rats/sex/group were prepared for neuropathologic examination. Complete histopathologic examinations (including neuropathologic examinations) were conducted for the vehicle control and 0.2 mg/kg-day Tl groups only; for the other three groups, only the livers, lungs, kidneys and gross lesions were examined histopathologically. Neuropathologic examinations included the following: dorsal and ventral root fibers of the spinal nerves, dorsal root ganglia, spinal cord at C3–C6 and L1–L4, and six sections of the brain.

There were no statistically significant differences in body weight, food consumption, or absolute and relative organ weights among control groups and groups receiving thallium (I) sulfate. The study authors concluded that the histopathologic examination did not reveal any treatment-related effects.

Lacrimation (secretion of tears), exophthalmos (abnormal protrusion of the eyeball), and miosis (contraction of the pupil) were observed at higher incidences in the treated male and female rats compared with both untreated and vehicle controls (Table 4-2). Ophthalmologic examination and gross and histopathologic examination of the eyes, however, revealed no treatment-related abnormalities. The incidence of clinical observations related to the coats (including rough coat, piloerection, shedding, and alopecia) and behavior (including aggression, tension/agitation, hyperactivity, vocalization, and self-mutilation) were also elevated in male and female rats at the higher doses (Table 4-2).

Table 4-2. Selected clinical observations in Sprague-Dawley rats treated with thallium sulfate for 90 days

Observation[a]	Untreated control	Vehicle control	0.008 mg/kg-day	0.04 mg/kg-day	0.2 mg/kg-day
Male					
Coat/skin					
Rough coat	1/20	3/20	11/20	16/20	19/20
Piloerection	0/20	0/20	1/20	4/20	13/20
Shedding	0/20	0/20	4/20	10/20	8/20
Alopecia	2/20	1/20	4/20	9/20	4/20
Eyes					
Lacrimation	1/20	6/20	19/20	20/20	20/20
Exophthalmos	1/20	5/20	12/20	20/20	20/20
Miosis	0/20	1/20	5/20	7/20	15/20
Behavior[b]	3/20	0/20	7/20	6/20	7/20
Female					
Coat/skin					
Rough coat	1/20	0/20	1/20	5/20	11/20
Piloerection	0/20	0/20	0/20	3/20	8/20
Shedding	0/20	0/20	2/20	3/20	13/20
Alopecia	4/20	1/20	4/20	9/20	12/20
Eyes					
Lacrimation	7/20	6/20	20/20	20/20	20/20
Exophthalmos	5/20	6/20	19/20	20/20	20/20
Miosis	2/20	3/20	1/20	11/20	8/20
Behavior[b]	2/20	2/20	0/20	1/20	7/20

[a]Listed as number of animals with the sign observed at least once during the 90-day study.
[b]Animals exhibiting one or more behavioral observations at least once during the 90-day study, including the following: aggression, tension/agitation, hyperactivity, vocalization, self-mutilation.

Source: MRI (1988).

As noted above, the incidence of alopecia was increased, particularly in female rats (see Table 4-3). Examination of individual animal clinical observation data for female rats from the MRI (1988) study showed that alopecia was first observed in control and treated groups anywhere from study day 44 to 60. Based on a statistical analysis performed by the U.S. EPA[1], the incidence of alopecia (total number of cases in each dose group) was statistically

[1] A statistical analysis of the incidence of alopecia (based on the total number of cases of alopecia in each dose group) was performed by EPA by using Fisher's exact test. Incidence in the treated groups was compared with incidence in the untreated control, vehicle control, and pooled control.

significantly elevated relative to controls in mid-dose males and mid- and high-dose females. Most instances of alopecia in females were attributed to barbering behavior (where fur was present but cropped short). Of the 12 high-dose females with alopecia, 5 instances were not totally attributed to barbering behavior. Histopathologic examination revealed atrophy of the hair follicles in two high-dose female rats. Because the skin was examined for histopathologic changes only in the vehicle control and high-dose groups, no information on dermal histopathology was available for the low- and mid-dose groups. The two high-dose females with atrophy of the hair follicles also had alopecia; whether the hair follicle atrophy and alopecia occurred at the same location on the rats could not be determined from the study report. The study authors concluded that the alopecia was attributable to the cyclic pattern of hair growth in rodents. Consequently, the authors did not consider these findings to be toxicologically significant.

Table 4-3. Incidence of alopecia in rats

Dose (mg/kg-day Tl)	Males		Females	
	Alopecia[a, b]	Hair follicle atrophy[c]	Alopecia[a, b]	Hair follicle atrophy[c]
0 (untreated control)	2/20	--[d]	4/20	--[d]
0 (vehicle control)	1/20	0/20	1/20	0/20
0.008	4/20	--[d]	4/20	--[d]
0.04	9/20[e]	--[d]	9/20[f]	--[d]
0.2	4/20	0/20	12/20[e]	2/20

[a]Number of animals with alopecia at least once during the 90-day study based on clinical observations.
[b]Of the animals with alopecia, the following are the numbers of cases in each dose group that the study authors stated were "not totally attributed to barbering behavior":
Males: untreated control, 1; vehicle control, 0; 0.008 mg/kg-day, 2; 0.04 mg/kg-day, 4; 0.2 mg/kg-day, 1.
Females: untreated control, 0; vehicle control, 0; 0.008 mg/kg-day, 1; 0.04 mg/kg-day, 3; 0.2 mg/kg-day, 5.
[c]Based on histopathologic observation.
[d]Skin was not examined for histopathologic lesions.
[e]Incidence of alopecia (total number of cases) was statistically significantly elevated ($p < 0.05$) relative to incidence in vehicle control, incidence in untreated control, and pooled incidence of vehicle and untreated control, based on Fisher's exact test performed by EPA.
[f]Incidence of alopecia (total number of cases) was statistically significantly elevated ($p < 0.05$) relative to incidence in vehicle control and pooled incidence of vehicle and untreated control, based on Fisher's exact test performed by EPA.

Source: MRI (1988).

Subtle but statistically significant changes were observed in several blood chemistry parameters that the investigators considered probably treatment related. Specifically, dose-related increases in AST, lactate dehydrogenase (LDH), and sodium levels and decreases in blood sugar levels were detected in male and female rats after 30 and 90 days of exposure. Reported values for the selected blood chemistry parameters are summarized in Table 4-4. Other

changes in blood chemistry parameters were less consistent across species, dose groups, and exposure durations.

At 90 days, the differences in AST, LDH, sodium, and blood sugar levels in dosed male and female rats were no greater than +31, +38, +4, and –21%, respectively, of the vehicle control group values. The investigators observed that the increases in AST and LDH levels could indicate a possible effect of treatment on cardiac function, that increases in LDH coupled with subtle changes in electrolytes could indicate an effect on renal function, and that, in rare instances, a decrease in blood sugar coupled with an increase in sodium occurs as a defense mechanism for maintaining cellular integrity. The investigators concluded that none of the changes observed in the blood chemistries of male or female rats during the study were of sufficient magnitude to significantly affect the health status of the animals. Further, histopathologic evaluation did not confirm any cellular damage suggested by the clinical chemistry findings.

Table 4-4. Selected blood chemistry values

Endpoint	Study day	Untreated control	Vehicle control	0.008 mg/kg-day	0.04 mg/kg-day	0.2 mg/kg-day
Males[a]						
AST (I.U.)	30	91 ± 26.5	108 ± 18.6	128 ± 24.5[b]	134 ± 29.0[b,c]	152 ± 20.1[b,c]
	90	77 ± 19.7	87 ± 17.8	99 ± 20.4	113 ± 27.0[b,c]	114 ± 31.1[b,c]
LDH (I.U.)	30	795 ± 322	1206 ± 424[b]	1333 ± 340[b]	1396 ± 407[b]	1802 ± 341[b,c]
	90	587 ± 305	856 ± 385	1003 ± 363[b]	1071 ± 507[b]	1119 ± 477[b]
Na (meq/L)	30	148 ± 1.3	149 ± 2.4	152 ± 4.0[b]	154 ± 2.5[b,c]	153 ± 2.1[b,c]
	90	144 ± 1.6	147 ± 2.0[b]	147 ± 1.9[b]	149 ± 2.0[b,c]	151 ± 2.2[b,c]
Blood sugar (mg/100 mL)	30	100 ± 22.1	97 ± 18.1	93 ± 10.0	90 ± 18.3	62 ± 14.8[b,c]
	90	158 ± 15.6	138 ± 16.8[b]	131 ± 17.6[b]	121 ± 15.7[b]	113 ± 22.4[b,c]
Females[a]						
AST (I.U.)	30	95 ± 22.8	115 ± 30.3	127 ± 27.8[b]	149 ± 26.8[b,c]	154 ± 18.2[b,c]
	90	77 ± 19.2	90 ± 19.1	93 ± 33.1	111 ± 30.7[b]	112 ± 31.0[b]
LDH (I.U.)	30	1047 ± 335	1277 ± 495	1402 ± 501	1763 ± 370[b,c]	1764 ± 361[b,c]
	90	745 ± 320	881 ± 273	823 ± 354	1044 ± 436	1219 ± 338[b]
Na (meq/L)	30	148 ± 1.7	150 ± 1.9	153 ± 4.1[b,c]	154 ± 2.8[b,c]	155 ± 2.5[b,c]
	90	146 ± 1.8	146 ± 1.0	148 ± 1.8[b,c]	150 ± 2.0[b,c]	152 ± 1.0[b,c]
Blood sugar (mg/100 mL)	30	103 ± 23.9	80 ± 13.3[b]	80 ± 9.0[b]	67 ± 20.0[b]	50 ± 11.8[b,c]
	90	110 ± 28.7	89 ± 15.9	103 ± 19.9	88 ± 20.4	70 ± 18.0[b]

[a]Mean ± standard deviation of 7–10 rats.
[b]Significantly different ($p < 0.05$) from the untreated control group.
[c]Significantly different ($p < 0.05$) from the vehicle control group.

Source: MRI (1988).

The authors concluded that the minor dose-related changes in this study did not affect the health status of the treated animals and therefore were not toxicologically significant and

identified the highest dose, 0.25 mg/kg-day thallium (I) sulfate (0.20 mg/kg-day Tl), as a no-observed-effect level (NOEL). However, upon further analysis by EPA of the MRI (1988) findings as part of this health assessment, a different determination was reached regarding the no-observed-adverse-effect level (NOAEL) and lowest-observed-adverse-effect level (LOAEL) (see the discussion in Section 5.1.1).

Manzo et al. (1983) administered drinking water containing thallium (I) sulfate at a concentration of 10 mg/L Tl (approximately equivalent to a dose of 1.4 mg/kg-day Tl based on reported thallium intakes and an assumption that the rats weighed 200 g) to 80 female Sprague-Dawley rats for 36 weeks. Mortality was 15 and 21% after 40 and 240 days of treatment, respectively. After 4+ weeks (32 days) of treatment, hair loss appeared and involved about 20% of the animals thereafter. Functional and histopathologic changes were observed in the peripheral nerves, including changes in motor and sensory action potentials and histopathologic changes in the sciatic myelin sheath and axonal destruction characterized by Wallerian degeneration (degeneration of the axon and its myelin sheath distal to a site of injury), mitochondrial degeneration, neurofilamentous clustering, and elevated lysosomal activity.

Ten adult male albino rats were administered 0.8 mg/kg (1/20th of the LD_{50}) of thallium (I) sulfate orally (presumably via gavage) for 3 months (El-Garawany et al., 1990). Blood samples were obtained initially and at monthly intervals. At all three monthly intervals, the treated group had statistically significantly ($p < 0.001$) increased levels of blood urea, serum creatinine, serum bilirubin, and serum ALT. The largest increase (<90%) in these parameters occurred in the first month, with smaller increases (>15%) occurring for each additional month.

Mourelle et al. (1988) examined the effects of silymarin, an antioxidant, on various biochemical indicators of liver damage in male Wistar rats (200–250 g) induced by oral administration (gavage) of thallium (I) sulfate (10 mg/kg) dissolved in water. The controls were given vehicle only. Ten rats per group were sacrificed at 0, 24, 48, 72, and 96 hours and 5, 10, and 20 days after treatment. Without silymarin administration, thallium administration produced a statistically significant ($p < 0.05$) decrease in the content of glycogen and reduced glutathione (GSH) and caused a statistically significant ($p < 0.05$) increase in malondialdehyde (MDA) production and triglycerides in the liver 48 hours after treatment. (MDA production and GSH content in the liver served as indicators of lipid peroxidation.) Levels of serum alkaline phosphatase were increased and liver cell membrane alkaline phosphatase activity was decreased after 24 hours and remained unchanged for 5 days. Furthermore, Na^+/K^+-ATPase activity in the liver cell membranes was rapidly reduced within 24 hours of thallium treatment; the decrease persisted through day 5 and began to rebound by day 10, with values similar to the control by day 20. Serum and liver cell membrane gamma-glutamyl transpeptidase and serum ALT were significantly ($p < 0.001$) elevated by 24 hours and remained elevated through day 5. Administration of silymarin (100 mg/kg i.p.) completely prevented these biochemical changes. The authors suggested that silymarin acted by stabilizing membranes via some antioxidant

property. During the 20 days, none of the rats treated with thallium alone died, but the rats exhibited signs of toxicity that included hypomotility and piloerection.

Downs et al. (1960) fed groups of Wistar-derived albino rats (5/sex/dose) diets containing nominal concentrations of 0, 5, 15, or 50 mg thallium (I) acetate/kg (or ppm) in the diet (corresponding to approximately 0, 0.4, 1.2, or 3.9 mg/kg-day Tl for 100 g rats, assuming food consumption of 10 g/day). Animals were allowed ad libitum access to these diets for 15 weeks. At the 50 ppm dose level, mortality was 100% by week 5 in males and by week 13 in females. By week 15, 4/10 control animals died (2/sex), making interpretation of survival in the remaining dose groups difficult (15 ppm, 3/5 males and 1/5 females died; 5 ppm, 2/6 males and 0/4 females died). An additional treatment group (30 ppm) and control group (corresponding to 0 and 2.4 mg/kg-day Tl) were added 6 weeks after the study had been initiated and were maintained on the diet for 9 weeks. At the end of the 9 weeks, 2/5 male and 1/5 female controls were dead and 4/5 males and 3/5 females at 30 ppm were dead. At termination, the only gross finding was alopecia in the 15 and 30 ppm groups. The alopecia was noted beginning 2 weeks after commencement of the diet, with the rats nearly free of hair at termination. The authors reported a slight increase in kidney weight (doses not specified; data not shown). The authors also reported that histopathologic evaluations did not indicate treatment-related pathology, but they did not prepare skin sections. The study findings for alopecia suggest a NOAEL and LOAEL of 0.4 mg/kg-day Tl and 1.2 mg/kg-day Tl, respectively, for this endpoint. Because mortality occurred in rats in both the control and treated groups, it is not possible to determine whether the deaths in low-dose (5 ppm) male rats were related to thallium exposure. Therefore, a NOAEL and LOAEL cannot be reliably established for this study.

Downs et al. (1960) also examined the effects of thallium (III) oxide on weanling Wistar-derived albino rats (five rats/sex/treatment). Rats received 0, 20, 35, 50, 100, or 500 mg/kg (or ppm) thallium (III) oxide in the diet for 15 weeks. This was equivalent to doses of 0, 1.8, 3.1, 4.5, 9.0, or 44.8 mg/kg-day Tl, respectively. All rats (males and females) treated with 50 ppm and greater in the diet died before 8 weeks. The mortality rates in the remaining groups at 15 weeks were as follows: 1/5 control males, 0/5 males treated with 20 ppm, and 4/5 males treated with 35 ppm; 0/5 control females, 2/5 females treated with 20 ppm, and 2/5 females treated with 35 ppm. Thallium (III) oxide caused a dose-related decrease in body weight at 15 weeks. Body weight reductions relative to the control were 50 and 180 g in males treated with 20 and 35 ppm dietary doses, respectively, and 50 g in females treated with 35 ppm in the diet. Males treated with either 20 or 35 ppm in the diet had marked hair loss beginning around 4 weeks, with near complete hair loss after 6 weeks; females were less affected.

There was a statistically significant ($p \leq 0.05$) increase in absolute kidney weights in males and females treated with 20 ppm and females treated with 35 ppm and a dose-response trend in kidney to body weight ratio. Histopathologic examination did not reveal any alterations in the kidney related to thallium treatment. Histopathologic evaluation of the skin revealed a

decrease in the number of hair follicles and hair shafts, atrophy of the remaining follicles, decrease in the size of the sebaceous glands, and hyperkeratinized epidermis. However, the incidence by dose was not presented. The lowest level tested, 1.8 mg/kg-day Tl (20 ppm thallium (III) oxide in the diet), is considered to be a LOAEL based on findings of alopecia and significant elevations in kidney weights for male and female rats. A NOAEL was not identified for this study.

Leloux et al. (1987) investigated the acute toxicity of oral exposure to thallium (I) nitrate in the adult Wistar rat. In the first experiment, a single dose of 20 mg/kg thallium (I) nitrate was administered via gavage to male and female rats (three per sex); all males and females were found dead within 40 and 54 hours post-dosing, respectively. Increases in absolute kidney (36%, females; 61%, males) and adrenal (47%, females; 100%, males) weights were observed following the single exposure. The second experiment involved administering four daily gavage doses of 1 mg/kg-day thallium (I) nitrate to 20 animals of each sex. Male rats treated with four doses began to lose their hair 96 hours after the first exposure. All treated animals had diarrhea. After the fourth gavage dose, 2/20 males and 2/20 females died. Two more females died within 126 hours, and 11 females and 15 males died within 168 hours. Three rats of each sex were sacrificed at 126 hours post-dosing for gross pathological examination and organ weight changes. The remaining two females were sacrificed at 192 hours post-dosing. Treated animals weighed less than the untreated controls. The tissues did not demonstrate any macroscopic degenerative changes, but there was an increase in the absolute weights of the kidneys (33%, females; 48%, males) and eyes (54%, females; 34%, males). Histopathology was not performed.

4.2.1.1.2. ***Dogs.*** Reports of thallium toxicity in dogs are limited to a few cases in the literature of accidental exposure. A 9-month-old Doberman pinscher accidentally consumed mole bait containing 1% thallium (Waters et al., 1992). Two days later the dog was lethargic, vomited blood, and had bloody feces. The dog had moderate hypoproteinemia and a slight prolonged activated clotting time. The dog's condition was improved by the third day following supportive care, including treatment with activated charcoal.

Thomas and McKeever (1993) reported a case of a 1-year-old neutered male miniature schnauzer that had ingested an unknown amount of bread soaked in thallium (the level in one piece of bread was 1.6 ppm). Beginning symptoms were lethargy, followed 2 weeks later by severe, rapidly progressing alopecia. No abnormalities were found in a complete blood count test, serum chemistry profile, urinalysis, or abdominal radiographs. Diphenylthiocarbazone treatments (40 mg/kg three times daily) were started upon establishing thallium toxicity. On the second day of veterinary treatment, the dog showed signs of respiratory distress and was euthanized due to its poor condition. An autopsy revealed severely congested and edematous lungs, congestion of the liver and kidneys, and areas of congestion and hemorrhage in the

pancreas. Histologic evaluations demonstrated abnormalities in the lungs, kidneys, liver, and pancreas. Thallium was detected in the liver (11 ppm), kidneys (12 ppm), and spleen (7 ppm).

Histopathology of the skin from 13 cases of thallium poisoning in dogs revealed dyskeratotic and necrolytic changes in the skin and hair follicles (Schwartzman and Kirschbaum, 1961). The most prominent features were massive parakeratosis, spongiform abscess formation, and induction of telogen follicles.

4.2.1.2. *Chronic Studies and Cancer Bioassays*

There are no chronic animal studies or cancer bioassays for thallium reported in the literature.

4.2.2. Inhalation Exposure

No studies were identified that examined the effects of inhaled thallium in animal models.

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES

4.3.1. Reproductive Toxicity

Effects of thallium on male reproduction have been investigated in rats (Formigli et al., 1986; Gregotti et al., 1985; Zasukhina et al., 1983) and mice (Wei, 1987). These studies suggest that thallium exposure can produce effects on the testes and sperm. None of the available reproductive toxicity studies, however, used standard protocols for evaluating reproductive endpoints. No studies of the potential reproductive toxicity of thallium in female experimental animals were identified.

Male Wistar rats (10/group) were administered drinking water containing 10 ppm thallium (I) sulfate (approximately 0.7 mg/kg-day Tl based on reported daily thallium consumption [270 µg Tl/rat] and initial body weights [350–380 g]) (Formigli et al., 1986). The compound was administered for 30 and 60 days. Although the study authors stated that the controls were pair-fed, they also stated that food was available ad libitum and that thallium did not affect food consumption. No abnormalities were observed after 30 days of treatment. However, after 60 days of treatment, the following testicular effects were observed: disarrangement of the tubular epithelium, cytoplasmic vacuolation and distention of smooth endoplasmic reticulum of the Sertoli cells, reduced testicular β-glucuronidase activities (an enzyme primarily located in the Sertoli cell and spermatogonia), high concentrations of thallium in the testes, and reduced sperm motility. Plasma testosterone levels were within normal limits. β-Glucuronidase activity was also affected after 60 days of treatment and ultrastructural changes were observed in the Sertoli cells. From these results, a LOAEL of 0.7 mg/kg-day Tl (10 mg/L) was identified.

Gregotti et al. (1985) also reported β-glucuronidase activity and ultrastructural changes in the Sertoli cells after 60 days of treatment. Gregotti et al. (1992) further examined this effect in vitro and demonstrated that thallium (even at the lowest concentration) causes a dose- and time-dependent detachment of germ cells from Sertoli cells when testicular cells were treated with thallium concentrations corresponding to 1.4, 7, and 35 μg/g testis, estimated from protein content of cultures.

Zasukhina et al. (1983) performed a dominant lethal test with male rats that were given daily oral doses of thallium (I) carbonate (0.005, 0.05, and 0.5 μg/kg-day) for 8 months and subsequently mated with untreated females. The authors concluded that thallium carbonate produced a treatment-related enhancement of embryonic mortality. The authors' conclusion was not, however, supported by the data presented in the paper. The number of implantation sites (10.11 ± 0.48 to 11.05 ± 0.49), number of "yellow bodies" in pregnant rats (11.11 ± 0.35 to 11.75 ± 0.33), and number of live embryos (9.77 ± 0.48 to 10.87 ± 0.37) were similar across the control and treated groups. ("Yellow bodies" were not further defined in the paper but may mean corpora lutea.) Because the paper lacked statistical analysis, it was unclear whether the differences were statistically significant. Only the number of resorptions in the control group (0.87 ± 0.13) was appreciably higher than that in the treated groups (0.22 ± 0.12 to 0.33 ± 0.13). In addition, the authors' calculation of total embryonic deaths could not be reproduced from the data provided. Finally, overall confidence in the reported findings was low because of inadequate reporting (e.g., the number of male rats exposed and the rat strain were not reported), the use of nonstandard terminology, the relatively small number of pregnant females (16–18 per group), and lack of statistical analysis.

Wei (1987) administered 0, 0.001, 0.01, 0.1, 1.0, or 10.0 mg/L thallium (I) carbonate in drinking water for 6 months to groups of male Kunming mice (20/group) weighing 15–20 g at study initiation. (Assuming an average body weight of 20 g over the exposure period and drinking water ingestion rate of 6 mL/day [Derelanko and Hollinger, 1995], these exposure levels are approximately equivalent to doses of 0.0003, 0.003, 0.03, 0.3, and 3 mg/kg-day Tl. These doses are approximate only because the age of animals at study initiation, terminal body weights, and water consumption were not reported.) At the end of the exposure period, half of the male mice (10/group) were sacrificed for epididymal sperm examination. The remaining males (10/group) were housed with untreated females (1:2 ratio) for 1 week to evaluate male reproductive function. At the end of the 1-week mating period, these male mice were also sacrificed for epididymal sperm examination. Untreated female mice were sacrificed on gestation day 20 and evaluated for the following measures of reproductive function: number of pregnant female mice, number of live and dead fetuses, number of implantations, and number of early resorptions. Water intake, body weights, behavior, and animal health were reportedly assessed; however, this information was not provided in the study report. The author reported that sperm motility (rapid speed, sperm immobility) was affected at the lowest drinking water

concentration (0.001 mg/L) tested. Effects were shown to increase with increasing concentration, thus indicating a dose-response relationship. At 0.01 mg/L and higher, the number of dead sperm was statistically significantly increased. Sperm count was statistically significantly reduced and the percent of deformed sperm was increased at concentrations of 0.1 mg/L and higher. The author observed that there was an adverse effect on sperm quality (motility) at low doses, and, as the dose increased, there was an accompanying decrease in sperm count in addition to the motility change. Examination of reproductive function in the group of mice housed for 1 week with untreated females revealed that the reproductive index (number of pregnant female mice/number of mated female mice) and the number of implantations were not statistically different between treated and control animals. The mean number of live fetuses was statistically significantly increased at concentrations of 0.01 mg/L and above. The percent of dead fetuses was significantly lower than in the control group at concentrations of 0.001, 0.01, and 0.1 mg/L but was increased at the two highest concentrations (1 and 10 mg/L). Review of the reported results reveals that a number of male mice were not accounted for at study termination. Of the initial 20 male mice/group, sperm results were provided for only 12 mice in the 0.1 mg/L group, 10 mice in the 10 mg/L group, and 16 mice in the remaining groups. No explanation is provided for the loss of animals over the 6-month study. The author concluded that the lowest dose tested (0.001 mg/L thallium (I) carbonate) was a LOAEL, causing reproductive effects in male mice.

Table 4-5 summarizes thallium toxicity in animals following oral exposure.

Table 4-5. Thallium toxicity in animals following oral exposure

Reference	Species	Age	Sex	Route	Dose and duration	NOAEL	LOAEL	Effect
Acute studies								
Leloux et al. (1987)	Rat 3/sex	Adult	Both	Oral (gavage)	20 mg/kg thallium (I) nitrate; single dose	NI[a]	15 mg/kg Tl	Difficulty breathing; rough coat; increased absolute kidney, adrenal weights; death
Leloux et al. (1987)	Rat 10/sex/ group	Adult	Both	Oral (gavage)	0, 1 mg/kg thallium (I) nitrate; once daily for 4 days	NI	0.77 mg/kg Tl	Alopecia; diarrhea; increased absolute kidney, eye weights; death
Mourelle et al. (1988)	Rat 10/group	NS[b]	Male	Oral (gavage)	0, 10 mg/kg thallium (I) sulfate; single dose. Sacrificed at 24 hours to 2 days after dosing	NI	8.1 mg/kg Tl	Liver changes: increased triglycerides and lipid peroxidation; decreased glutathione and glycogen; increased alkaline phosphatase in serum and liver cell membranes
Subchronic studies								
Downs et al. (1960)	Rat/ 5/sex/ group	NS	Both	Oral (feed)	0, 5, 15, or 50 ppm thallium (I) acetate (corresponding to 0, 0.4, 1.2, or 3.9 mg/kg-day Tl); 15 weeks 0 or 30 ppm (corresponding to 0 or 2.4 mg/kg-day Tl); 9 weeks	0.4 mg/kg-day Tl*	1.2 mg/kg-day Tl*	Alopecia; increased kidney weight; mortality in treated and control groups. *The NOAEL and LOAEL are for alopecia. Because of reported mortality in the control and treated groups, a study NOAEL and LOAEL cannot be reliably determined.
Downs et al. (1960)	Rat 5/sex/ group	Weanling	Both	Oral (feed)	0, 20, 35, 50, 100, and 500 ppm thallium (III) oxide (corresponding to 0, 1.8, 3.1, 4.5, 9.0, and 44.8 mg/kg-day Tl); 15 weeks	NI	1.8 mg/kg-day Tl (20 ppm)	Reduced body weight; alopecia; increased mortality; increased absolute and relative kidney weights
El-Garawany et al. (1990)	Rat n = 10	NS	Male	Oral[c]	0.8 mg/kg thallium (I) sulfate; 90 days	NI	0.65 mg/kg-day Tl	Increased blood urea; serum creatinine; serum bilirubin; serum ALT
Manzo et al. (1983)	Rat n = 80	NS	Female	Oral (DW[d])	10 mg/L Tl as thallium (I) sulfate; 36 weeks	NI	1.4 mg/kg-day Tl	Nerve histopathology; alopecia; mortality

Table 4-5. Thallium toxicity in animals following oral exposure

Reference	Species	Age	Sex	Route	Dose and duration	NOAEL	LOAEL	Effect
MRI (1988)	Rat 20/sex/ group	45 days	Both	Oral (gavage)	0, 0.01, 0.05, or 0.25 mg thallium (I) sulfate/kg (corresponding to 0, 0.008, 0.04, or 0.20 mg/kg-day Tl); 90 days	NI	0.008 mg/kg-day Tl[e]	Increased incidence of alopecia and other observations related to coat (rough coat, piloerection, shedding); lacrimation, exophthalmos, and miosis; and various behavioral observations; statistically significant increases in AST, LDH, and sodium levels; decreased blood sugar levels. The study authors identified 0.2 mg/kg-day Tl as the NOAEL.
Reproductive and developmental toxicity								
Formigli et al. (1986); Gregotti et al. (1985)	Rat 10/group	Adult	Male	Oral (DW)	0, 10 ppm thallium (I) sulfate; 30 or 60 days	NI	0.7 mg/kg-day Tl	Testicular effects: tubular epithelium disarrangement; cytoplasmic vacuolation; reduced sperm motility; distention of smooth endoplasmic reticulum of Sertoli cells; reduced β-glucuronidase activity
Wei (1987)	Mouse	NS	Male	Oral (DW)	0, 0.001, 0.01, 0.1, 1.0, and 10 mg/L thallium (I) carbonate (corresponding to 0, 0.0003, 0.003, 0.03, 0.3, and 3 mg/kg-day Tl); 6 months	NI	0.0003 mg/kg -day Tl	Decreased sperm motility and counts; increase in deformed sperm; decrease in live fetuses. Dose estimated from an assumed average body weight of 20 g and drinking water ingestion rate of 6 mL/day.
Rossi et al. (1988)	Rat	Perinatal	Both	Mother's, then pup's DW (DW)	0, 1 mg/dL of thallium (I) sulfate Day 1 of gestation to weaning then thru 60 days	NI	NI	Prenatal and postnatal exposure caused a delay in the development of the pilus apparatus by 50 days; reduction of the α- and β-adrenergic and muscarinic vasomotor reactivity noted.

[a]NI = not identified.
[b]NS = not specified.
[c]Presumably via gavage.
[d]DW = drinking water.
[e]See discussion of the NOAEL and LOAEL determination in Section 5.1.1.

4.3.2. Developmental Toxicity

Developmental toxicity studies in the rat (Barroso-Moguel et al., 1992; Rossi et al., 1988; Gibson and Becker, 1970) and chicken embryo (Hall, 1985, 1972; Karnofsky et al., 1950) provide evidence that thallium exposure during development can produce abnormalities (including effects on the developing vascular autonomic nervous system and bones) and reduced fetal body weight. Of the studies in rats, only one involved oral drinking water exposure to thallium (Rossi et al., 1988); in other developmental rat studies, dams were exposed by i.p. injection.

A group of NOS albino male and female rats was administered 1 mg/dL of thallium (I) sulfate from day 1 of gestation to weaning (22 days after birth) via the dams' drinking water then through their own drinking water until 60 days of age (Rossi et al., 1988). These rats were considered prenatally exposed. Another group of NOS albino male and female rats was exposed to 1 mg/dL of thallium (I) sulfate via the dams' drinking water from birth until weaning (22 days after birth) then through their own drinking water until 60 days of age. These rats were considered postnatally exposed. Both situations (pre- and postnatal exposure) caused a delay in the development of the pilus apparatus by 50 days. A reduction of the α- and β-adrenergic and muscarinic vasomotor reactivity also was noted. Authors noted that this reduction may be due to one of the following mechanisms: probable reduction in the number and/or sensitivity of both α- and β-adrenergic and muscarinic receptors or a change of cell membrane in relation to a possible modification of potassium cell concentration.

Gibson and Becker (1970) administered thallium (I) sulfate (i.p.) to pregnant Simonsen Sprague-Dawley rats during early (2.5 mg/kg on days 8, 9, and 10) or late (2.5 or 10 mg/kg on days 12, 13, and 14) gestation. Fetuses were examined for abnormalities. All three thallium treatments caused a statistically significant ($p < 0.05$) reduction in fetal body weight. Thallium treatment (2.5 mg/kg) during early gestation caused a slight (not statistically significant) increase in the incidence of hydronephrosis (29% in treated versus 16% in control) and missing or non-ossified vertebral bodies (36% in treated versus 17% in controls). The 2.5 mg/kg thallium (I) sulfate treatment administered during late gestation caused a statistically significant ($p < 0.05$) increase in the incidence of hydronephrosis (47% in treated versus 16% in control) and missing or non-ossified vertebral bodies (60% in treated versus 17% in controls). Increasing the dose to 10 mg/kg thallium (I) sulfate during late gestation did not increase the incidence of developmental abnormalities. In fact, 10 mg/kg thallium (I) sulfate administered during late gestation had no effect on the incidence of hydronephrosis and was comparable to the 2.5 mg/kg dose administered during late gestation in the induction of missing or non-ossified vertebral bodies (i.e., 60% in treated versus 17% in controls). Maternal toxicity (diarrhea, lethargy, irritability, poor hair luster, and hair loss) was noted.

Barroso-Moguel et al. (1992) administered a single i.p. injection of 32 mg/kg aqueous thallium (I) acetate to 20 newborn (24-hour-old) Wistar rats. Results were compared with those

of five vehicle controls. Rats (four treated and one control per time point) were sacrificed at 24, 48, and 72 hours and at 7 and 50 days. Cartilaginous and osseous tissue alterations were noted. Diarrhea was observed through 72 hours postinjection. Two rats surviving to 50 days postinjection had persisting alopecia (one irreversible and one with discrete recovery). Although skeletal images of 72-hour-old animals did not show any differences when compared with the control, microscopic images of the distal third of the tibia showed disorganization and edema of the fibroblasts of the fibrous layer. By day 7, delays in ossification in the right forelimb were noted. Microscopic examination demonstrated a majority of pyknotic chondrocytes and the lack of bone trabeculae calcification. Profound skeletal alterations were noticeable 50 days after injection. Many of the cartilaginous cells were altered or dead, leading to a decrease of the growth cartilage and scanty bone trabeculae with few osteoblasts. The bone marrow also had few myeloblasts and megakaryocytes.

Hall (1972) incubated chick embryos in a forced-draft Humidaire incubator and injected 0.6 mg thallium (I) sulfate/0.5 mL saline into each embryo via the chorioallantoic membrane at 7 days of incubation. This dose caused a minimal lethal effect with survival varying from 94 to 100%. Treated embryos were smaller than controls from 10 days of incubation onward and by 18 days were 26% smaller than controls. Treated embryos failed to commence ossification or had not progressed to similar developmental stages observed in the control. The long bones of treated embryos were smaller (the tibia to a greater degree than the femur), contained less organic material, and contained more water (as a percent of dry weight) than the untreated control embryos. An abnormal distribution of the acid mucopolysaccharides and necrotic areas in maturing hypertrophic chondrocytes were detected histologically. In addition, biochemical assays verified the reduced acid mucopolysaccharide activity. Hall (1985) further demonstrated that the critical period of susceptibility ended at 8 2/3 days of incubation. Tibial growth was inhibited by thallium sulfate in 8-day-old embryos but not in 9-day-old embryos.

Achondroplasia (a birth defect characterized by imperfect bone formation) was also induced in embryonic chicks via in vitro cultures with injection into the chorioallantoic membrane (Hall, 1985, 1972) or injection into the yolk sac (Karnofsky et al., 1950). Karnofsky et al. (1950) determined that thallium (I) sulfate was lethal to 2-day embryos at a dose lower than would be necessary to induce achondroplasia. Treatment of 4-day-old chick embryos with 0.2, 0.5, 1.0, or 2.0 mg/egg induced 0, 45, 92, and 100% incidence of achondroplasia, respectively. Although the data were not presented, the study authors reported that thallium (I) nitrate produced achondroplasia at similar doses.

4.4. OTHER ENDPOINT-SPECIFIC STUDIES

A number of investigators have specifically examined the effect of thallium compounds administered to experimental animals by injection (s.c., i.p., or i.v.) and reported effects on the liver, kidneys, heart, and nervous system.

4.4.1. Liver and Kidney Toxicity

Liver and kidney were among the organs affected when male and female Sprague-Dawley rats were given s.c. injection of thallium (I) acetate as acute (single dose of 20–50 mg/kg), subacute (2–3 weekly injections of 10–15 mg/kg), or chronic (10–20 mg/kg, followed by weekly injections of 5 mg/kg or occasionally 2.5 mg/kg for up to 26 weeks) exposures (Herman and Bensch, 1967). Toxicity was observed in all treatment groups. Animals dosed acutely displayed the symptoms earlier than those on subacute or chronic dosing schedules. Animals were sacrificed when signs of toxicity became apparent.

Acutely exposed animals had the following changes observed via light microscopy: eosinophilic granular casts in 50–75% of the renal proximal and distal tubules, mild to moderate enteritis, moderate to severe colitis, and dense infiltration of polymorphonuclear leukocytes and lymphocytes that extended through all layers of the wall of the large intestine. Electron microscopy revealed severe degenerative changes in the mitochondria of the renal tubular cells and hepatocytes. In rats that received subacute injections of thallium acetate, eosinophilic granular casts occurred in one-third of the proximal and distal tubules. Electron microscopy revealed moderately prominent mitochondrial granules in the kidney, dense bodies in the cytoplasm of the cells in the loops of Henle, and distal convoluted tubules. Mitochondrial granules of hepatocytes were slightly enlarged and lacked electron-lucent cores.

In the chronically exposed rats, electron microscopy revealed increased size of mitochondrial granules in the proximal convoluted tubules and an increase in cup-shaped mitochondria in the distal convoluted tubules. Hepatocytes had increased numbers of large complex residual bodies and lipid droplets, and the mitochondria were swollen with enlargement of mitochondrial granules.

Yoshida et al. (1997) administered a single i.p. injection of thallium (I) sulfate (25 mg/kg) to ICR mice (30–35 g). As was observed in Mourelle et al. (1988) after a single oral dose of thallium (I) sulfate (10 mg/kg) to Wistar rats, liver Na^+/K^+-ATPase was statistically significantly decreased by 12 hours. However, the rebound occurred by 24 hours instead of the 5 days observed in the Mourelle et al. (1988) study. While the Na^+/K^+-ATPase activity was decreased, the ATP levels were increased and returned to control values by 12 hours post-dosing. The effects were slightly different in the kidney. At 6 hours Na^+/K^+-ATPase activity was statistically significantly decreased but had rebounded by 12 hours. The ATP levels were significantly ($p < 0.01$) increased through 12 hours then decreased to levels significantly ($p < 0.01$) lower than controls by 24 hours; ATP levels did not rebound until 240 hours after thallium administration.

Male albino rats (210–260 g) receiving a single i.p. injection of 30, 60, or 120 mg/kg thallium (I) sulfate had statistically significant ($p < 0.05$) increased levels of AST and ALT above controls (0.5 mL of 0.9% saline) 16 hours after treatment, regardless of dose (Leung and Ooi, 2000). There was a dose-dependent increase in ALT and AST between 30 and 60 mg/kg,

but the levels did not increase from 60 to 120 mg/kg. Some 30 mg/kg animals exhibited weakness, sluggishness, loss of hair, ptosis of the eyelids, diarrhea, and respiratory difficulty; they were sacrificed 4 days post-dosing. The ALT and AST levels in these animals were still elevated over the controls by a factor of 2–2.5. Serum creatinine levels also were elevated by approximately 2.5 times over the control (0.5 mg/dL control versus 1.33 mg/dL treated). Histologic evaluation confirmed damage to both the kidney and the liver. The damage in the liver consisted of necrosis and swollen and vacuolated cells, which appeared to reduce the sinusoidal space. Kidney tubules were atrophied and vacuolated with cell outlines less distinct and cells containing many pyknotic nuclei. Amorphous material was apparent in the lumen of the proximal tubules, and the brush borders were disorganized.

Appenroth et al. (1995) examined the effects of a single i.p. dose of thallium (I) sulfate (5, 10, 15, or 20 mg/kg) on renal function and morphology in adult female Wistar rats. Low doses (i.e., 5 and 10 mg/kg) of thallium (I) sulfate increased the volume of urine (measured on day 2) but did not affect the protein level. Higher doses (i.e., 15 and 20 mg/kg) caused a reduction in urinary volume along with an increase in the urinary protein concentration. The glomerular filtration rate was statistically significantly ($p \leq 0.05$) reduced at 2 days after treatment with 20 mg/kg but had returned to control levels by day 10. Blood urea nitrogen (BUN) levels were significantly ($p \leq 0.05$) increased 2 days after treatment but had returned to normal values by day 10. Histopathology showed a thickening of ascending limb of the loop of Henle notable on day 2 after treatment and resolved by day 10. Na^+/K^+-ATPase activity was significantly ($p \leq 0.05$) increased in the medulla on day 2 but was significantly ($p \leq 0.05$) reduced by day 5. No changes in Na^+/K^+-ATPase activity were noted in the cortex.

In follow-up studies, Appenroth et al. (1996) and Fleck and Appenroth (1996) examined the age-related nephrotoxicity of thallium (I) sulfate. Both studies demonstrated that nephrotoxicity was more severe in the adult rat (Wistar) than in young rats (10 or 20 days old) after a single i.p. injection of 20 mg/kg thallium (I) sulfate. Appenroth et al. (1996) determined that there were several biochemical differences in kidney function between 10- and 20-day-old rats (Wistar) administered 20 mg/kg thallium (I) sulfate, but there were no structural changes indicating kidney damage in either group of young rats. In comparison, the thick ascending limb of loop of Henle in 55-day-old rats showed damage. Thallium did not affect the glomerular filtration rate in either 10- or 20-day-old rats but caused a significant reduction in the rate in 55-day-old rats (Appenroth et al., 1996; Fleck and Appenroth, 1996). Thallium caused a statistically significant ($p < 0.05$) increase in the fractional excretion of a few amino acids (i.e., ß-alanine, taurine, and 1-methylhistidine), a statistically significant ($p < 0.05$) decrease in the fractional excretion of glycine in 10-day-old rats, and a statistically significant ($p < 0.05$) increase in the fractional excretion of 13 amino acids in 55-day-old rats. Fleck and Appenroth (1996) determined that thallium affects renal tubular amino acid resorption and causes kidney damage only when mature kidney function is present.

Woods and Fowler (1986) examined the effects of a single i.p. dose of thallium (III) chloride ($TlCl_3$) at doses of 50, 100, or 200 mg/kg on liver structure and function in male Sprague-Dawley rats (CD strain; 150–200 g) 16 hours after treatment. They determined a dose-related effect on the volume density of mitochondria (increased), rough endoplasmic reticulum (increased), lysosomes (increased), and cytoplasm (decreased). The surface densities of the inner cristae of mitochondria and the rough endoplasmic reticulum also were determined to increase in a dose-dependent manner. Statistically significant ($p < 0.05$) increases occurred in monoamine oxidase (MAO) (100 and 200 mg/kg) and ferrochelatase (50, 100, and 200 mg/kg). Statistically significant ($p < 0.05$) decreases occurred in aminolevulinic acid (ALA) synthetase (50, 100, and 200 mg/kg), aminopyrine demethylase (200 mg/kg), aniline hydroxylase (50, 100, and 200 mg/kg), and NADPH cytochrome c (P450) reductase (50, 100, and 200 mg/kg). In addition, in vitro studies using 50, 100, or 200 μg/mL thallium (III) chloride demonstrated a significant ($p < 0.05$) reduction in ALA synthetase, ferrochelatase, aniline hydroxylase, ALA dehydratase, and NADPH cytochrome c (P450) reductase for all dose concentrations. A dose-related loss of ribosomes from the smooth endoplasmic reticulum and proliferation of the rough endoplasmic reticulum were observed through ultrastructural examination. Also observed were generalized mitochondrial swelling and increased numbers of electron-dense autophagic lysosomes.

Table 4-6 summarizes toxicity data from animal studies involving i.p. or i.v. injection.

Table 4-6. Thallium toxicity in animals via injection

Reference	Species	Age or weight	Sex	Route/exposure period	Doses	NOAEL/LOAEL	Study type/effect
Acute studies (single dose)							
Ali et al. (1990)	Rat	Adult	Male	i.p./single injection	20 mg/kg Tl as thallium (I) acetate	LOAEL = 20 mg/kg Tl	Neurological toxicity: neurochemical changes in the brain that were resolved within 24 hrs
Appenroth et al. (1995)	Rat	Adult	Female	i.p./single injection	5, 10, 15, and 20 mg/kg thallium (I) sulfate	LOAEL = 12 mg/kg Tl	Kidney toxicity: decreased urine volume; increased urine protein; thickened ascending limb of the loop of Henle; changes in brain Na^+/K^+-ATPase
Barroso-Moguel et al. (1990)	Rat	Newborn	Both	i.p./single injection	32 mg/kg thallium (I) acetate	LOAEL = 25 mg/kg Tl	Neurological toxicity: neuronal and vascular damage in the brain
Barroso-Moguel et al. (1992)	Rat	Newborn	Both	i.p./single injection	32 mg/kg thallium (I) acetate	LOAEL = 25 mg/kg Tl	Developmental toxicity: diarrhea; alopecia; cartilaginous and osseous tissue alterations; profound skeletal alterations
Barroso-Moguel et al. (1996)	Rat	Newborn	Both	i.p./single injection	16 mg/kg thallium (I) acetate	LOAEL = 12 mg/kg Tl	Developmental toxicity: diarrhea; muscle atrophy; small size; alopecia; death; interstitial edema between myelin sheaths; thinner muscle fibers; nerve fiber damage
Kuperberg et al. (1998)	Rat	250–300 g	Male	i.p./single injection	25 mg/kg thallium (I) acetate	LOAEL = 19 mg/kg Tl	Bladder and neurological toxicity: distended bladder; reduced acetyl cholinesterase activity in the brain and bladder; increased choline acetyltransferase activity in the brain and bladder
Lameijer and van Zwieten (1976)	Rat	Young adult	Male	i.v./single injection	3–100 mg/kg thallium (I) sulfate	LOAEL = 24 mg/kg Tl (30 mg/kg thallium (I) sulfate)	Cardiotoxicity: hypertension

Table 4-6. Thallium toxicity in animals via injection

Reference	Species	Age or weight	Sex	Route/exposure period	Doses	NOAEL/LOAEL	Study type/effect
Leung and Ooi (2000)	Rat	210–260 g	Male	i.p./single injection	30, 60, 120 mg/kg thallium (I) sulfate	LOAEL = 24 mg/kg Tl	General toxicity: increased AST and ALT; weakness; sluggishness; alopecia; ptosis of the eyelids; diarrhea; respiratory difficulty; liver necrosis; kidney damage
Osorio-Rico et al. (1995)	Rat	200–250 g	Male	i.p./single injection	30 or 50 mg/kg thallium (I) acetate	LOAEL = 23 mg/kg Tl	Neurological toxicity: increase in MAO and 5-hydroxytryptamine in the brain
Woods and Fowler (1986)	Rat	Young adult	Male	i.p./single injection	50, 100, and 200 mg/kg thallium (III) chloride	LOAEL = 42 mg/kg Tl	Liver toxicity: increased volume density of mitochondria, lysosomes, and rough endoplasmic reticulum of the liver; decreased cytoplasm in the liver; increased MAO and ferrochelatase; changes in several liver enzymes
Acute studies (3–10 doses)							
Brown et al. (1985)	Rat	250 g	Male	i.p./6 days	4 or 8 mg/kg-day thallium (I) acetate	LOAEL = 3.1 mg/kg-day Tl	Neurological toxicity: increased lipid peroxidation in the brain; increased β-galactosidase activity in the brain; behavioral changes
Gibson and Becker (1970)	Rat	Fetus; 8, 9, and 10 or 12, 13, and 14 days of gestation	Both	Transplacental via i.p. injection to dam/3 days	2.5 or 10 mg/kg-day thallium (I) sulfate	LOAEL = 1.9 mg/kg-day Tl	Developmental toxicity: reduced fetal body weight; increase in hydronephrosis; increase in missing or non-ossified vertebral bodies
Gibson and Becker (1970)	Rat	Pregnant	Female	i.p./3 days	2.5 or 10 mg/kg-day thallium (I) sulfate	LOAEL = 1.9 mg/kg-day Tl	General toxicity: diarrhea; lethargy; irritability; poor hair luster; alopecia

Table 4-6. Thallium toxicity in animals via injection

Reference	Species	Age or weight	Sex	Route/exposure period	Doses	NOAEL/LOAEL	Study type/effect
Hasan et al. (1977)	Rat	~150 g	Male	i.p./7 days	5 mg/kg thallium (I) acetate	LOAEL = 3.9 mg/kg-day Tl	General toxicity: anorexia; poor hair luster; diarrhea; difficulty walking; abnormal head rotation; lethargy; death; changes in Golgi complexes and smooth cisternae/ vesicles of hypothalamic neurons; decreased succinic dehydrogenase and guanine deaminase activities in the brain
Hasan et al. (1978)	Rat	~150 g	Male	i.p./7 days	5 mg/kg thallium (I) acetate	LOAEL = 3.9 mg/kg-day Tl	Neurological toxicity: decreased dopamine, norepinephrine, and 5-hydroxytryptamine in the brain
Hasan and Ali (1981)	Rat	~150 g	Male	i.p./7 days	5 mg/kg thallium (I) acetate	LOAEL = 3.9 mg/kg-day Tl	General toxicity: anorexia; poor hair luster; diarrhea; difficulty walking; abnormal head rotation; lethargy; increased lipid peroxidation; aggregation of lipofuscin granules in the perikarya of cerebellar neurons
Hasan and Haider (1989)	Rat	~150 g	Male	i.p./6 days	5 mg/kg thallium (I) acetate	LOAEL = 3.9 mg/kg-day Tl	Neurological toxicity: GSH
Kuperberg et al. (1998)	Rat	250–300 g	Male	i.p./5 days	0, 0.1, 1.0, or 5.0 mg/kg thallium (I) acetate	LOAEL = 0.08 mg/kg-day Tl	Neurological toxicity: difficulty walking and maintaining pressure on the hind paws; loss of coordination in motor activity; lethargy; reduced food consumption; distended bladder; decreased acetyl cholinesterase activity in the bladder
Subchronic studies							
Galván-Arzate et al. (2000)	Rat	200–250 g	Male	i.p./30 days	0.8 or 1.6 mg/kg-day thallium (I) acetate	LOAEL = 0.6 mg/kg-day Tl	Neurological toxicity: increased lipid peroxidation in the brain

Table 4-6. Thallium toxicity in animals via injection

Reference	Species	Age or weight	Sex	Route/exposure period	Doses	NOAEL/LOAEL	Study type/effect
Kennedy and Cavanagh (1977)	Cat	Not specified	Female	s.c./4–26 weeks	2.3–4.5 mg/kg per week thallous acetate (unclear if dose was reported as thallous acetate or as thallium)	0.32 mg/kg-day (unclear if dose was reported as thallous acetate or as thallium)	Dying back of central and peripheral sensory neurons; no motor nerve fiber degeneration

4.4.2. Cardiotoxicity

Male Wistar rats (180 to 220 g) injected i.v. with 3 to 100 mg/kg thallium (I) sulfate, while under pentobarbital anesthesia, rapidly developed hypotension with the lowest blood pressures reached within 3 to 5 minutes (Lameijer and van Zwieten, 1976). Blood pressures dropped in a dose-dependent manner with doses of 30–100 mg/kg causing a drop of 20–40% from initial values and a maximum effect achieved in the 50 to 100 mg/kg range. Thallium had a greater effect on the diastolic pressure. After 10 minutes, animals injected with doses ranging from 3–40 mg/kg had blood pressures resembling those prior to thallium injection. The higher doses had a more permanent effect on blood pressure. In addition to lower blood pressure, the rats had a dose-dependent decrease in heart rate with no maximum achieved. Rats treated with 100 mg/kg had heart rates that were one-third their pre-injection rates. The same effects were observed in anesthetized cats injected i.v. with monovalent thallium but not when the thallium was infused into the left vertebral artery (Lameijer and van Zwieten, 1976).

4.4.3. Neurotoxicity

No studies of thallium neurotoxicity following exposures by the inhalation or dermal routes of exposure were identified. Manzo et al. (1983) demonstrated functional and histopathologic changes in peripheral nerves in rats that received thallium sulfate in drinking water at a dose equivalent to approximately 1.4 mg/kg-day Tl (see Section 4.2.1.1). Study findings reported below involved administration of thallium compounds by injection (i.p., s.c., i.v.).

A single i.p. injection of 20 mg/kg thallium (I) acetate in 8–12 (exact number per group not specified) adult male Sprague-Dawley rats (~300 g) resulted in a statistically significant ($p < 0.02$) decrease in aspartate and taurine in the hippocampus 6 hours after treatment that was resolved by 24 hours (Ali et al., 1990). Glutamine and taurine levels were statistically significantly increased ($p < 0.05$) in the frontal cortex at 6 hours. While the glutamine returned to control levels by 24 hours, the taurine was still significantly elevated. A dose-dependent decrease in dopamine and muscarinic cholinergic receptor binding in caudate nucleus was not reported in these treated rats but was observed in caudate nucleus incubated in vitro with thallium. The study report indicated that the effect was not observed 24 hours after the last subacute dose (5 mg/kg daily for 10 days, i.p.) of thallium (I) acetate in a separate study, but the results were not presented. The subacute study demonstrated a statistically significant ($p < 0.05$) increase in dopamine, 3,4-dihydroxyphenylacetic acid, and 5-hydroxytryptamine (5-HT [serotonin]) levels in the amygdala nucleus, as well as an increase in 5-HT in the hypothalamus. Dopamine, 3,4-dihydroxyphenylacetic acid, and 5-HT remained similar to control concentrations in the caudate nucleus, frontal cortex, and hippocampus.

Kennedy and Cavanagh (1977) examined the neurotoxic properties of thallous acetate in 16 female cats. Cats were injected subcutaneously with thallous acetate in distilled water once per week (or in some cases every other week or every few weeks) at mean weekly doses of 2.3–4.5 mg/kg per week for 4–26 weeks. (It was unclear if doses were reported as thallous acetate or thallium). Six cats died during the study without opportunity for neurological assessment. Remaining animals were observed daily for clinical signs. At intervals from 4–12 weeks after exposure was initiated, nine animals were sacrificed and nervous system tissues were subject to perfusion fixation; one cat was exposed weekly for 26 weeks. Ataxia and hypotonia were observed in nearly all the cats. Histopathologic examination revealed a distal degeneration confined to the central and peripheral axons of primary sensory neurons. No motor nerve fiber degeneration was found. The investigators proposed that muscle tissue, which has the largest reservoir of K^+ ions in the body, may serve as a sink for thallium ions such that thallium ions never reach a concentration in the extracellular space sufficient to damage nerve fibers. Experimental evidence for this hypothesis, however, was not available.

A set of companion studies in newborn Wistar rats (Barroso-Moguel et al., 1996, 1990) demonstrated severe and progressive lesions in nerve fibers following i.p. administration of thallium (I) acetate. In the first study (Barroso-Moguel et al., 1990), 32 mg/kg thallium (I) acetate was given to 15 newborn Wistar rats. Equal numbers were sacrificed at 24, 48, and 72 hours and on days 7 and 51 (three rats/time point). Alterations in the capillary vessel walls of the brain, observed within the first hours after thallium injection, progressed to irregular thickened walls and fibrotic sclerosis, which obstructed the lumen by 51 days postexposure. Other changes in the brain began in a diffuse manner with all sections affected. Cortical neurons developed the first and most intense lesions; by 51 days, the cortical neurons had mostly disappeared and lesions were apparent in the central grey nuclei.

In a follow-up study (Barroso-Moguel et al., 1996), 16 mg/kg thallium (I) acetate was administered via i.p. injection to 20 newborn Wistar rats (10 each sacrificed at 8 and 50 days of age). General toxicity was manifested through diarrhea, progressive muscular atrophy, small body size, and persistent alopecia. Eight of the 20 thallium-treated rats died during the study. By 8 days of age, thallium-treated rats had interstitial edema between the myelin sheaths (causing separation of the nerve fibers), edema around some axons and within the myelin, and, in some cases, initial damage and degeneration of the myelin sheaths. In addition, muscle fibers were thinner and showed signs of beginning progressive muscular atrophy. Hemorrhage, necrosis, and destruction of the striation were present in some areas of the muscle. By 50 days of age, the rats developed nerve damage with progressive disappearance of nerve fibers and granular, filiform, and amorphous inclusions in abnormal axons and collapsing myelin sheaths. At this time, the muscle fibers lost their transverse striation. Other muscle fibers were observed

to be atrophic, fragmented, and exhibiting hyaline degeneration and initial fibroblast reaction; further, some were infiltrated with phagocytic macrophages.

Osorio-Rico et al. (1995) measured MAO activity and 5-HT turnover rates in different regions of the brain in 127 male Wistar rats (200–250 g) 24 hours after an i.p. administration of 30 or 50 mg/kg aqueous thallium (I) acetate. Results demonstrated MAO was significantly increased ($p < 0.05$) at 30 mg/kg in the midbrain (27.7% over controls) and pons (37% over controls) sections. MAO increases also were observed at 50 mg/kg in the midbrain (48% over controls) and pons (47%). 5-HT turnover was significantly increased in the pons (172% over controls; $p < 0.001$) after 30 mg/kg treatment and in the pons (166.7% over controls; $p < 0.001$) and midbrain (56% over controls; $p < 0.01$) after 50 mg/kg treatment. No significant changes were observed in the dopamine turnover rate.

Subcutaneous injection of thallium (I) acetate as acute (single dose of 20–50 mg/kg), subacute (2–3 weekly injections of 10–15 mg/kg), or chronic doses (10–20 mg/kg, followed by weekly injections of 5 mg/kg or occasionally 2.5 mg/kg for up to 26 weeks) to male and female Sprague-Dawley rats (250–500 g) caused toxicity in all treatment groups (Herman and Bensch, 1967). Symptoms reported in all groups included diarrhea, marked weight loss, anorexia, and lethargy. Animals dosed acutely displayed the symptoms earlier than those on subacute or chronic dosing schedules. Chronically exposed animals also had hair loss (maximal at 2–4 weeks after initial injection), irritability, and dragging of the hind limbs. Animals were sacrificed when signs of toxicity became apparent.

In acutely exposed animals, the mitochondria of the brain were frequently filled with an overabundance of stacked mitochondrial cristae. Three of four animals administered thallium (I) acetate subacutely had changes in the brain, including occasional foci of perivascular cuffing with lymphocytes and hemosiderin-filled macrophage, acute necrosis, and swollen histiocyte-like cells. Peripheral nerves had occasional dense bodies in an unmyelinated nerve plexus. Brain neurons had numerous lipofuscin bodies as did the neurons of the chronically exposed animals.

Twenty-four hours after a single dose of 25 mg/kg thallium (I) acetate, acetyl cholinesterase (AchE) activity was reduced in the hypothalamus and nucleus accumbens (NA) regions of the brain and the activity of choline acetyltransferase (ChAT) activity was significantly ($p \leq 0.05$) increased (Kuperberg et al., 1998). At 48 hours, the AchE in the hypothalamus was still significantly ($p \leq 0.05$) reduced, and the AchE in the NA region of the brain was back to control levels. AchE activity also was reduced in the duodenum and the sphincter-trigon region of the bladder following this single high dose, while ChAT activity was significantly ($p \leq 0.05$) increased in the ileum, duodenum, and both regions of the bladder. After 48 hours, the AchE levels in the duodenum and the sphincter-trigon and detrusor regions of the bladder were still significantly ($p \leq 0.05$) reduced.

Adult male Sprague-Dawley rats (250–300 g) administered 0.1, 1.0, or 5.0 mg/kg thallium (I) acetate (i.p.) daily for 5 days exhibited difficulty walking and maintaining pressure on their hind paws, loss of coordination in motor activity, lethargy, and reduced food consumption (Kuperberg et al., 1998). Most of the rats (6/8) treated at the high dose died by 48 hours posttreatment. The only significant changes observed 24 hours after the last dose were a reduction of AchE levels in the NA region of the brain with the 1.0 mg/kg dose and an increase in the AchE levels in the striatum and midbrain of rats treated with 5 mg/kg. There was a decrease in AchE activity in the sphincter-trigon region of the bladder at 24 hours in all of the repeat-dose groups (i.e., 0.1, 1.0, and 5.0), which returned to control values by 48 hours. Decreased bladder AchE levels were also observed in the detrusor region of the bladder 24 hours after 1.0 or 5.0 mg/kg doses and 48 hours after the 0.1 mg/kg dose. The bladders of these rats were distended and contained twice the amount of urine seen in the controls.

Brown et al. (1985) examined lipid peroxidation in the brain after thallium exposure. In this study, groups of male Sprague-Dawley rats (250 g) were administered daily i.p. injections of 4 or 8 mg/kg thallium (I) acetate in water for 6 days. Controls received saline. Twenty-four hours after the final injection behavioral analysis was performed and the following morning, rats were sacrificed. A dose-dependent increase in lipid peroxidation was observed in the cerebellum, brain stem, and striatum but not in the midbrain and hippocampus. The 8 mg/kg dose also caused a statistically significant ($p < 0.05$) increase in the lipid peroxidation of the cortex. Beta-galactosidase activity followed a dose-dependent increase in the cerebellum, brain stem, and cortex. The 8 mg/kg dose also caused a statistically significant ($p < 0.05$) increase in beta-galactosidase activity in the midbrain and hippocampus. The beta-galactosidase activity in the striatum was not statistically significantly changed by either dose. In general, thallium decreased the frequency of grooming behavior while the frequency of exploratory and attention behaviors was increased. However, the changes were not dose dependent.

Hasan et al. (1977) administered 5 mg/kg thallium (I) acetate i.p. for 7 days to albino male rats (weighing approximately 150 g). Controls received sodium acetate solution in equal volumes with the same molar concentration. Clinical symptoms included anorexia, failure to gain weight, irritability, tenderness during handling, poor hair luster, lethargy, diarrhea, dragging hind limbs, and fits of abnormal rotation of head and neck, curving the body. Eight of 55 treated rats died by day 7. Electron microscopy showed an increased incidence of well-developed Golgi complexes and curved conformation of smooth cisternae and vesicles of the neurons in the hypothalamus. More significantly, there was a peculiar isolation of axonal endings of the anterior hypothalamus by membranous circumferential lamellae, which appeared to have arisen from the neighboring astrocytic processes. In addition, the succinic dehydrogenase and guanine deaminase activities in the cerebrum were significantly decreased in thallium-treated rats. Protein levels, MAO, adenosine triphosphate, and protease levels in the cerebrum were

unaffected. Mitochondrial succinic dehydrogenase also was decreased in the cerebrum of thallium-treated rats.

In a study by the same group of investigators (Hasan and Ali, 1981), male Charles Foster rats (approximately 150 g) injected with 5 mg/kg thallium (I) acetate daily for 7 days showed clinical symptoms similar to those reported by Hasan et al. (1977). All rats were lethargic after 4–5 days of treatment. Rats were sacrificed the day after the final dose (day 8), and their brains were removed and separated into sections. A statistically significant ($p < 0.001$) increase in lipid peroxidation was reported in the cerebral hemisphere, cerebellum, and brain stem by 49, 142, and 116%, respectively. Electron microscopy demonstrated prominent aggregation of lipofuscin granules in the perikarya cerebellar neurons (cell body of neurons in the brain) in thallium-treated rats that were hardly discernible in control rats. Comparisons made with nickel- and cobalt-treated rats demonstrated differences in the areas of increased lipid peroxidation. Nickel and cobalt both had the greatest impact on the brain stem, whereas thallium had a greater effect on the cerebellum. Although Hasan and Ali (1981) could not relate this to the differences in behavioral observations, they did note that nickel- and cobalt-treated rats were irritable and restless; these symptoms were not observed in the thallium-treated rats.

Hasan and colleagues also examined the effects of thallium (I) acetate on neurotransmitter levels and sulfhydryl groups in the brain. Charles Foster rats (approximately 150 g) were administered 5 mg/kg thallium (I) acetate i.p. for 6 days (Hasan and Haider, 1989) or 7 days (Hasan et al., 1978). Dopamine, norepinephrine, and 5-HT were reduced in the four sections of the brain examined (hypothalamus and limbic area, corpus striatum, cerebellum, and brain stem), but not all reductions were statistically significant. Dopamine was significantly reduced in the hypothalamus and limbic area (50%; $p < 0.05$) and corpus striatum (64%; $p < 0.01$). Norepinephrine was reduced by 9–33%, depending on the brain region, but none of these reductions were statistically significant. 5-HT was significantly reduced in the corpus striatum (53%; $p < 0.001$), cerebellum (36%; $p < 0.05$), and brain stem (66%; $p < 0.001$) (Hasan et al., 1978). Glutathione was significantly ($p < 0.001$) reduced in the cerebrum (56%), cerebellum (62%), and brain stem (74%), and sulfhydryl radicals were significantly ($p < 0.05$) reduced in the cerebellum (25%) and brain stem (32%) (Hasan and Haider, 1989).

Galván-Arzate et al. (2000) administered 0.8 mg/kg (considered 1/40 of the median lethal dose [LD_{50}]) or 1.6 mg/kg (considered 1/20 of the LD_{50}) thallium (I) acetate in deionized water via i.p. injection for 30 days to male Wistar rats (200–250 g). Three days after treatments ended, rats were sacrificed and their brains were dissected into five different regions (hypothalamus, cerebellum, frontal cortex, hippocampus, and corpus striatum). In each region, with the exception of the cerebellum, significant ($p < 0.01$) increases in thallium content were observed after administration of 1.6 mg/kg compared to administration of 0.8 mg/kg. There were no statistically significant differences in the deposition of thallium within each region of the brain

for each dose. The rate of lipid peroxidation, a marker of oxidative stress, was increased significantly ($p < 0.01$) in the corpus striatum (182%) and cerebellum (130%) after treatment with 0.8 mg/kg. At 1.6 mg/kg, all five regions exhibited statistically significant increases in lipid peroxidation over controls (corpus striatum, 161% increase, $p < 0.05$; hippocampus, 114% increase, $p < 0.01$; hypothalamus, 100% increase, $p < 0.01$; cerebellum, 81% increase, $p < 0.01$; and frontal cortex, 80% increase, $p < 0.05$). The two regions affected at 0.8 mg/kg (i.e., corpus striatum and cerebellum) were not affected to a greater extent at the higher dose. Lipid peroxidation was measured to determine if oxidative stress plays a role in thallium's toxicity. The study authors concluded that additional studies need to be performed to establish the precise mechanism of neurotoxicity.

4.5. MECHANISTIC DATA AND OTHER STUDIES IN SUPPORT OF THE MODE OF ACTION

Several, possibly related, mechanisms have been postulated for the toxic action of thallium; however, the exact mechanism or mechanisms of toxicity are unknown.

4.5.1. Interference with Potassium Transport

Monovalent thallium is similar to potassium (K^+) in ionic radius and electrical charge, which may contribute to its toxic properties.

Monovalent thallium has been demonstrated to have a 10-fold higher affinity for rabbit kidney Na^+/K^+-ATPase than does potassium (Britten and Blank, 1968). Barrera and Gómez-Puyou (1975) reported that monovalent thallium also inhibits the influx and efflux of potassium in rat liver mitochondria at concentrations (10–15 nmol bound monovalent thallium per mg of mitochondrial protein) that do not affect oxidative phosphorylation. This inhibitory effect of thallium seemed to be specific for potassium since it did not affect the movement of sodium**.**

Monovalent thallium was completely equilibrated by human red blood cells in 30 minutes in a high (140.5 mM)-sodium (Na^+) medium (Cavieres and Ellory, 1974) but was equilibrated even faster when the medium contained a low concentration (5 mM Na^+). In the high-Na^+ medium containing 1 mM external potassium, monovalent thallium caused a dose-dependent decrease in the ouabain-sensitive potassium influx. Monovalent thallium had a different effect in a medium containing 0.17 mM potassium; low (0.2 mM or less) monovalent thallium ion concentrations stimulated the ouabain-sensitive potassium influx but inhibited it at higher concentrations. Monovalent thallium also had an inhibitory effect on the ouabain-sensitive sodium efflux. It was suggested that the effects on ouabain-sensitive sodium efflux and potassium influx are related to thallium's high-affinity substitution of potassium at the external potassium sites of the sodium pump, which is actively transporting monovalent thallium ions in while pumping sodium ions out.

Tao et al. (2008) reported that thallium (I) could functionally replace potassium ion when applied in an in vitro system and that glutamate transporters could interact with thallium (I).

4.5.2. Disturbance of Mitochondrial Function and Energy Generation

Thallium may exert toxicity by disturbing mitochondrial function. Thallium (I) acetate caused an uncoupling of oxidative phosphorylation and swelling of isolated mitochondria and induced an increase in oxygen consumption and lactic acid production in ascites tumor cells in vitro (IPCS, 1996).

There is evidence that thallium reduces the available energy within peripheral nerves (resulting in a "dying back" type of neuronal degeneration) and other metabolically highly demanding cells (Kazantzis, 2007; Cavanagh, 1991). Cavanagh (1991) proposed that thallium in tissues produces a tissue deficiency of available riboflavin, leading to disturbances of metabolic reactions dependent on flavoproteins. Among these reactions would be steps in the passage of a number of important intermediate metabolites in the electron transport chain. This metabolic disruption could lead to substantial impairment of energy production in the cells. Cavanagh (1991) noted that support for this hypothesis includes the following: (1) riboflavin deficiency in the rat will lead to peripheral neuropathy and in the primate to loss of hair with circumoral skin lesions, and thallium toxicity in the primate produces a similar clinical picture to riboflavin deficiency; (2) the testis, which is peculiarly dependent on glucose as a substrate for energy metabolism, is sensitive to thallium toxicity; and (3) heart muscle, also known for high energy utilization, is also damaged as a result of chronic thiamine deficiency.

4.5.3. Induction of Oxidative Stress

Other research suggests that thallium may trigger toxicity through induction of oxidative stress. Hanzel et al. (2005) investigated effects of thallium (III) hydroxide on metabolism of GSH, which plays a key role in the regulation of cell redox state, in an in vitro system using rat brain cytosolic fractions. Thallium hydroxide decreased the content of GSH and inhibited glutathione peroxidase and glutathione reductase activity, suggesting that thallium impairs the glutathione-dependent antioxidant defense system. Using rat pheochromocytoma (P12) cells in vitro incubated with both thallium (I) nitrate and thallium (III) nitrate, Hanzel and Verstraeten (2006) found a concentration- and time-dependent decrease in cell viability, decreased mitochondrial membrane potential, increased steady-state levels of mitochondrial hydrogen peroxide (H_2O_2, a product of partial reduction of molecular oxygen whose generation is enhanced when electron transport is impaired), and GSH content. These investigators postulated that both ionic species of thallium enhance reactive oxygen species production in the cell, decreasing mitochondrial functionality and cell viability. In a follow-up study with P12 cells in vitro, Hanzel and Verstraeten (2009) observed that thallium (I) and (III) do not cause cell

necrosis, but significantly increased the number of cells with apoptotic features. The oxidatation state (i.e., I vs III) appeared to influence the apoptotic pathways are involved. Thallium (I)-mediated cell apoptosis was mainly associated with mitochondrial damage, whereas thallium (III) showed a mixed effect triggering both the intrinsic and extrinsic pathways of apoptosis.

Galván-Arzate et al. (2005) investigated the effects of a single dose (8 or 16 mg/kg, i.p.) of thallium acetate on lipid peroxidation in different brain regions of Wistar rats (as an indicator of oxidative damage) and alterations in endogenous antioxidant systems. Lipid peroxidation was increased in three of five brain regions from animals that received 16 mg/kg thallium acetate at day 7 postexposure (but not at days 1 and 3); antioxidants GSH and superoxide dismutase showed only a modest depletion in only one or two brain regions.

4.5.4. Reaction with Thiol Groups

The capacity of thallium to react with thiol groups, thereby interfering with a variety of processes, is postulated as another mechanism of toxicity, although interference with the metabolism of sulfur-containing amino acids does not seem to be directly involved in toxicity (IPCS, 1996). Thallium (I) chloride formed complexes with a number of sulfur-containing amino acids (i.e., L-cysteine, DL-penicillamine, N-acetyl-L-cysteine, and N-acetyl-DL-penicillamine) in aqueous solution (Bugarin et al., 1989). Because the thallium (I) complexes formed were weaker than those formed using dimethylthallium (III), the study authors concluded that this was unlikely to be the major mechanism of toxicity.

4.5.5. Other Endpoint-specific Mechanistic Data

4.5.5.1. *Cardiotoxicity*

Monovalent thallium ions caused a dose-dependent decrease in the heart rate and contractile force of spontaneously beating atria of guinea pigs (Lameijer and van Zwieten, 1976). At a concentration of 10^{-3} M, monovalent thallium reduced the heart rate by approximately 60%. When the isolated atria were electrically driven, monovalent thallium ions at doses up to 10^{-3} M were not able to significantly decrease the amplitude of contraction. Neither potassium (ranging from 2.4 to 9.4 mM) nor cocaine (10^{-6} or 10^{-5} M) were able to influence the reduction in heart rate or contractile force of spontaneously beating guinea pig atria caused by monovalent thallium ions (0.5 mM) (Lameijer and van Zwieten, 1976).

Isolated rat heart (from albino rats of both sexes) perfused with a nitrate-Krebs solution containing monovalent thallium ions (as thallium (I) nitrate) in place of potassium had a rapid decrease in beat frequency (Hughes et al., 1978). The heart stopped completely in an average of 7 minutes. Placing the hearts in normal or nitrate-Krebs saline allowed for some recovery in approximately 10 minutes. The monovalent thallium ions were still present in the heart tissue (9 mmol Tl^+/kg wet tissue) after 30 minutes in thallium-free solution; this residual thallium

caused the heartbeat frequency and amplitude to remain low. When only a portion of the potassium was replaced with monovalent thallium ions, there was a concentration ratio and time dependency on the reduction of the heart rate. In a separate experiment, it was noted that injecting thallium (I) nitrate into the perfusion stream close to the heart caused an initial acceleration of the heartbeat followed by a reduction in amplitude until at certain concentrations the heart stopped. The heartbeat could recover somewhat following nitrate-Krebs perfusion, but the heartbeat frequency and amplitude did not return to initial values and were instead comparable to those values noted prior to the heart stopping. There was a dose-dependent effect on the length of the cardiac paralysis (5–25 μmol monovalent thallium ions injected). The use of potassium nitrate instead of thallium (I) nitrate also caused a dose-dependent increase in the length of cardiac paralysis, but the length of paralysis was shorter than that with thallium (I) nitrate. Furthermore, potassium nitrate-treated hearts recovered completely when washed with nitrate-Krebs solution (Hughes et al., 1978).

4.5.5.2. *Neurotoxicity*

Wiegand et al. (1984) recorded the frequencies (reflects presynaptic processes) and amplitudes (reflects postsynaptic processes) of miniature endplate potentials (MEPPs) from neuromuscular junctions of rat (strain not specified) phrenic nerve (of the diaphragm) preparations. Investigators reported a gradual increase in the frequency of MEPPs by a factor of 10 within 30 and 180 minutes at concentrations of 1×10^{-3} and 5×10^{-4} M thallium (I) acetate, respectively, which was reversible. The amplitude was unchanged. Therefore, it was concluded that thallium interfered presynaptically with spontaneous transmitter release. A follow-up experiment using triangularis sterni muscles of adult mice (strain not specified) demonstrated that, although thallium disturbed the presynaptic transmission in a manner similar to divalent metal cations, thallium (monovalent; compound used not specified) acted via a different mechanism than either divalent cobalt or cadmium (Wiegand et al., 1990). This study also demonstrated that thallium did not influence the presynaptic potassium or calcium channels.

Windeback (1986) used an in vitro model system to compare the inhibitory effects of four metals on neurite outgrowth. Metals were added to cultures of E15 rat embryo dorsal root ganglion neurons; neurite outgrowth was measured during the linear phase of growth after 24 and 40–80 hours. Compared to mercury and arsenic, which inhibited neurite outgrowth at very low concentrations (50% inhibition at 3.9 and 9.6×10^{-6} M, respectively), neurons were relatively resistant to thallium (50% inhibition at 1.3×10^{-4} M), suggesting that populations of neurons have different susceptibilities to various metals. The basis for these different sensitivities was not explored in this study.

Hippocampal slices from adult guinea pigs or rats (strain not specified) were used to examine the effects of thallium on central neuronal activity (Lohmann et al., 1989). The study

authors did not note any differences between the guinea pig and rat results and appear to have combined the results. Light microscopy did not show any morphologic changes in thallium-treated hippocampal slices even with a high concentration (1–1.2 mM) for 6 hours. Thallium was determined to reversibly reduce the amplitudes of the compound action potential of CA1 pyramidal cells in a dose- and time-related manner. Thallium did not alter intracellular response parameters, indicating that membrane potential and input resistance were not affected, but postsynaptic potentials were inhibited. The study authors concluded that in the hippocampal slices thallium reacts mainly with postsynaptic target sites and exerts an unknown influence on intracellular metabolism of CA1 pyramidal cells (Lohmann and Wiegand, 1996; Lohmann et al., 1989).

Diaphragms from male and female albino rats perfused with a nitrate-Krebs solution containing monovalent thallium (as thallium (I) nitrate) in place of potassium (K^+) had an initial (1–2 minute) increase in the contraction amplitude, followed by a steady decline in response (Hughes et al., 1978). Four experiments were performed with the sequence taking 20–40 minutes to block indirect (nerve) stimulation and 70–100 minutes to block direct (muscle) stimulation. After returning the diaphragms to normal nitrate-saline, the block in response was reversed, but contraction amplitudes were 30% of the original response for both nerve and muscle after a 75-minute perfusion. The presence of monovalent thallium in the solution, whether as a replacement or an addition to potassium, caused a dose dependency to the response.

Diaz and Monreal (1994) examined the effects of thallium compounds (thallium (III) chloride, thallium (III) nitrate, and thallium (I) acetate) on proton and chloride permeabilities through myelin lipid bilayers using an in vitro system of liposomes prepared with lipids from brain myelin. Trivalent thallium, but not monovalent thallium, mediated a rapid chloride/hydroxyl ion exchange through the lipid bilayers. Trivalent thallium in the presence of reducing agents did not have the same reaction. The ion exchange was faster with trivalent thallium than with mercury (Hg^{+2}). In addition, the reaction occurred with a 10-fold lower concentration of trivalent thallium than of mercury.

4.5.5.3. *Dermal Toxicity*

Arbiser et al. (1997) examined the effects of thallium acetate on three types of skin cells, human keratinocytes, primary endothelial cells, and melanoma cells, to determine whether thallium affected cell growth and differentiation in vitro. Inhibition of proliferation of all three cell types was observed. In melanoma cells, thallium caused dose-dependent decreases in cell dendricity and shape but not cellular motility. In normal human keratinocytes, thallium appeared to interfere with the normal program of cutaneous keratinization. In an in vivo study by the same investigators using piebald LPJ mice with both melanin-rich and -poor areas in the same animal, one week administration of thallium acetate (5 mg/kg daily) by i.p. injection produced evidence

of lipid peroxidation in skin in a perifollicular distribution (Arbiser et al., 1997). (The investigators noted that lipid peroxidation in vivo results in oxidation of lipid membranes, resulting in increased concentrations of aldehydes, which can react with the Schiff reagent, thereby producing a colored product.) It was suggested that lipid peroxidation may result in cell death due to membrane damage and may partly account for thallium-induced alopecia.

4.5.6. Genotoxicity

Positive results were obtained for thallium (I) nitrate (1 mM) in the recombination-repair (Rec) assay using *Bacillus subtilis* strains H17 and M45; whether or not hepatic homogenates were used was not specified (Kada et al, 1980; Kanematsu et al., 1980). These positive results were obtained using "cold incubation," which increases the sensitivity of the assay by 20–50 times for many drugs. In this test, plates containing the bacteria with a 10 mm filter paper disk containing the metal solution (0.05 mL) were incubated at 4°C for 24 hours prior to being incubated at 37°C overnight. The differences in the inhibition of growth between the Rec^+ strain and the Rec^- strain were measured. Thallium (I) nitrate was not mutagenic in reverse mutation assays using *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 (histidine reversions) and *Escherichia coli* strains B/r WP2 tr^- and WP2 her^- tr^-; whether or not hepatic homogenates were used was not specified (Kanematsu et al., 1980). Negative results were obtained in a screening assay for the induction of mitogenic gene conversion and reverse mutation in the yeast *Saccharomyces cerevisiae* at a 0.1 M concentration of thallium (I) nitrate (Singh, 1983).

Thallium (I) nitrate did not affect cell division in *S. cerevisiae* (isolated from baker's yeast) and *E. coli* (strain B) but proved toxic to the aerobic growth processes of *S. cerevisiae* (Loveless et al., 1954). A dose of 250 µg/mL thallium (I) nitrate caused a 50% reduction in aerobic growth processes. The report did not identify specific doses of thallium (I) nitrate tested. The organisms were treated under conditions of logarithmic phase growth. *S. cerevisiae* was incubated for 4 hours with thallium (I) nitrate, and *E. coli* was incubated for 1.5 hours. The study authors noted that several of the other compounds tested (e.g., iodoacetamide) that were specific inhibitors of sulfhydryl groups also reduced the growth processes with no effect on cellular division.

A concentration of 1,000 µg/mL thallium (I) acetate reduced viability of Chinese hamster ovary (CHO) cells in culture to 20% with a concomitant decrease in DNA synthesis (i.e., 1% of control values) (Garrett and Lewtas, 1983). The EC_{50} (effective concentration necessary to produce a 50% response) values were 307 µg/mL for viability and 18 µg/mL for DNA synthesis. Thallium acetate also depressed ATP and protein synthesis in culture.

Single-strand DNA breaks occurred in cell cultures of C57BL/6 mouse and rat embryo fibroblasts exposed to thallium (I) carbonate at both concentrations tested in mouse fibroblasts

(i.e., 10^{-5} and 10^{-4} M) and all three concentrations tested in rat fibroblasts (i.e., 10^{-6}, 10^{-5}, and 10^{-4} M) (Zasukhina et al., 1983). However, thallium (I) carbonate did not induce single-strand DNA breaks in CBA mouse fibroblasts after treatment with 10^{-4}–10^{-6} M concentrations.

Zasukhina et al. (1983) performed a dominant lethal test on male white rats that received daily oral doses of thallium (I) carbonate (0.005–0.5 μg/kg-day) for 8 months and were subsequently mated with untreated females. Female rats were sacrificed on day 20, and mutagenic potential was evaluated based on evidence of embryotoxicity. The investigators reported an increase in embryonic death, suggestive of a dominant lethal effect. As reported previously (Section 4.3), however, confidence in this study is low. The number of resorptions was highest in the control group. In addition, methods were not adequately reported and results were not analyzed statistically.

4.6. SYNTHESIS OF MAJOR NONCANCER EFFECTS

4.6.1. Oral

Thallium is readily absorbed through the GI tract and distributed throughout the organs and tissues of the body. Although thallium is not metabolized, it occurs in two valence states. If or how the body modifies the valence state of thallium is unknown, but orally administered monovalent thallium and trivalent thallium appear to be distributed in a similar manner throughout the body (Sabbioni et al., 1980a, b). Once thallium is distributed, elimination occurs mainly in the urine and feces with the amounts in each varying by species.

Most of the available human case reports are the results of poisonings, suicide attempts, or accidental ingestion of rodenticides. The lowest known dose to cause symptoms is a single dose of 0.31 g; the patient recovered after treatment (Cavanagh et al., 1974). The only studies of repeated oral exposure to thallium were two surveys of populations exposed to thallium through contaminated homegrown foods (Dolgner et al., 1983; Brockhaus et al., 1981). Limitations in these epidemiology studies included the lack of objective tests for toxicity, reliance on the incidence of symptoms obtained from questionnaires, and characterization of chronic thallium exposure by measuring the levels in urine and hair at a single point in time. Three studies of occupationally exposed populations (Ludolph et al., 1986; Marcus, 1985; Schaller et al., 1980) did not conclusively establish an association between thallium exposure and impaired health status; however, all three studies were limited in terms of size of the study population and study design.

Symptoms of thallium toxicity are diverse in both humans and animals. The triad of gastroenteritis, polyneuropathy, and alopecia has been regarded as the classic syndrome of thallium poisoning (IPCS, 1996), although not all three of these effects are observed in all poisoning cases, and other symptoms develop in varying sequence depending on the magnitude and duration of thallium exposure.

The nervous system as a target organ of thallium is supported by observations from human case reports and animal studies. Relatively high doses of thallium cause neurological symptoms in humans (e.g., paresthesia of the hands and feet, weakness, tremors, coma, and convulsions) (see Table 4-1). Some of these neurological symptoms (e.g., paresthesia and weakness) were reversible, although recovery was slow. Other effects, including mental and/or psychological problems, were more persistent. Neurological symptoms have also been associated with chronic exposure to thallium in humans. These symptoms include sleep disorders, tiredness, weakness, nervousness, headache, psychological alterations, and neurological and muscular problems.

Although paresthesia of the hands and feet are trademark symptoms of thallium toxicity, it is generally alopecia that leads to a diagnosis of thallium poisoning in humans. Alopecia occurs about 2 weeks after exposure and is reversible after exposure to thallium is discontinued. Alopecia has also been repeatedly observed in experimental animals exposed to thallium compounds.

Thallium exposure in humans has been associated with respiratory effects and GI effects, including diarrhea and vomiting. Other toxic effects associated with oral thallium exposure in humans and animals are changes in blood pressure (high, low, and fluctuating values have all been noted) and liver and kidney damage (kidney damage is age dependent and occurs only in mature kidneys), all of which appear to be reversible with the removal of thallium exposure. Doses that do not affect survival have been shown to affect clinical chemistry parameters such as ALT, AST, BUN, blood glucose, and blood sodium levels, indicating liver and kidney damage with subchronic exposures (Leung and Ooi, 2000; Appenroth et al., 1996, 1995; Fleck and Appenroth, 1996; El-Garawany et al., 1990; Mourelle et al., 1988).

In experimental animal studies, thallium exposure has been associated with biochemical changes, lipid peroxidation, and histopathologic changes in the brain and functional and histopathologic changes in peripheral nerves (see Section 4.4.3). The areas affected in the brain differ with the age of the treated animal; nevertheless, all measured endpoints (symptoms, biochemical measurements, and histopathology) indicate that high doses (close to lethal doses) of thallium induce significant degradation of the nervous system. Results from in vitro studies further confirm these observations.

Despite the fact that the nervous system is a known target of thallium toxicity, studies using standard measures of neurobehavioral toxicity have not been performed. In a 90-day oral gavage study, MRI (1988) observed rats daily for clinical signs. Rats exposed to thallium sulfate showed consistently increased incidences of clinical observations related to the coat (rough coat, piloerection, shedding, and alopecia), eyes (lacrimation, exophthalmos, and miosis), and behavioral signs compared with untreated or vehicle controls. The underlying mode of action for these clinical observations is not known. Collectively, however, these observations suggest a

treatment-related effect of thallium on the rat and possibly an indirect measure of stress or other effects on the nervous system. For example, it has been suggested that barbering (or overgrooming) in rodents may represent a stress-evoked behavioral response or other nervous system dysfunction (Welch et al., 2007; Kalueff et al., 2006; Kalueff and Tuohimaa, 2005; Greer and Capecchi, 2002).

Thallium salts have been shown to affect reproductive function. A dose as low as 0.7 mg/kg-day Tl (10 ppm of thallium (I) sulfate) resulted in testicular damage and reduced sperm motility in male Wistar rats within 60 days (Formigli et al., 1986). Wei (1987) reported that doses as low as 0.001 mg/L in the drinking water for 6 months in Kunming mice reduced sperm motility (rapid speed only), and 0.01 mg/L reduced overall sperm motility and sperm counts and caused a reduction of live offspring. However, confidence in this study is low due to the non-reporting of several observations (water intake, body weights, behavior, and animal health). In addition, the numbers of animals examined for sperm and reproductive endpoints were ambiguously reported. Half of the animals were sacrificed at the end of the exposure period, while the other half were allowed to mate for 1 week and then sacrificed. The study results for male mice used for analysis of sperm and reproductive endpoints were reported together. That is, results for sperm count, sperm mobility, and all other epididymal sperm analysis for males sacrificed at the end of the exposure period were combined with the results for males that were allowed to mate and thus permitted a one-week recovery period prior to sacrifice. In addition, at the initiation of the study each group consisted of 20 animals. In the reporting of the results, however, sperm data were missing for 4 mice from each of the control, 0.001, 0.01, and 1 mg/L groups, for 8 mice from the 0.1 mg/L group, and for 10 mice from the 10 mg/L group. No explanation for the loss of male mice (ranging from 20 to 50%) is provided.

Limited data in humans and experimental animals suggest that thallium may produce developmental toxicity. A review of case studies of women exposed orally to high levels (approximately 120–1,100 mg) of thallium during pregnancy suggested a trend toward premature and low-birth-weight infants, especially if exposure took place in the last trimester; no other developmental abnormalities were identified (Hoffman, 2000). Dolgner et al. (1983) examined birth defects in a German population living near a cement plant emitting thallium dusts during the mid-1970s and found a higher incidence of congenital malformations than the incidence documented in the government birth records from the area. The association between the number of birth defects and thallium exposure was weak, however, because two of the malformations were considered hereditary and the incidence of birth defects, although greater than that determined from civil records, was consistent with that reported in the literature. Confidence in this study was limited by lack of exposure data during pregnancy and possible underreporting in controls. In vivo data in rats support an association between i.p. thallium exposure and low birth

weight, although such an association has not been reported with orally administered thallium. In vitro data demonstrated an increase in bone malformations in both rat and chick embryos.

4.6.2. Inhalation

There are currently no studies that examine the effects of inhaled thallium. A few case reports (Hirata et al., 1998; Ludolph et al., 1986) suggest an association between occupational exposure and toxicity (including alopecia, GI symptoms, and neuropathy), but the route or routes of exposure in these workplace setting could not be established. A study of a population living near a cement factory emitting thallium (Dolgner et al., 1983) determined that thallium exposure occurred via consumption of plants grown in thallium-contaminated soil to a greater extent than via inhalation.

4.6.3. Mode-of-Action Information

The underlying mode of action for clinically observed effects of thallium has been studied. Verstraeten (2006) determined that Tl^{1+} can oxidize membrane fatty acids, causing an increased fluidity in the membrane and an increased concentration of cellular oxidants associated with thallium toxicity. Ensuing axonal degeneration characterized by a decrease in density of large myelinated fibers and loss of epidermal nerves, indicating small sensory nerve involvement in thallium toxicity, has been reported (Kuo et al., 2005). The skin experiences parakeratosis and vacuolar degeneration of the basal layer, resulting in a loss of epidermal nerves and persistent damage to sensory nerve endings (Lu et al., 2007). Collectively, these observations suggest a treatment-related effect of thallium on the rats and direct effects on the nervous system.

Both potassium and thallium are monovalent cations with similar atomic radii (Tl^+: 1.50 Å; K^+: 1.38 Å) (Ibrahim et al., 2006). Thallium has been shown to replace potassium in the reaction of Na^+/K^+-ATPase (Barrera and Gómez-Puyou, 1975; Britten and Blank, 1968) and to mimic the biological actions of potassium. Monovalent thallium has been shown to have a 10-fold higher affinity than potassium for Na^+/K^+-ATPase and thus replaces potassium as a substrate for this enzyme (Barrera and Gómez-Puyou, 1975; Britten and Blank, 1968). In other studies it caused a decrease in Na^+/K^+-ATPase activity in the liver and kidney (Yoshida et al., 1997; Mourelle et al., 1988). After a single oral or i.p. dose of thallium (10 mg/kg orally or 25 mg/kg i.p.), the disruption of Na^+/K^+-ATPase activity was found to be reversible.

In addition to effects related to interference with potassium transport and function, there is evidence that thallium uncouples oxidative phosphorylation, adversely affects protein synthesis, reduces the available energy within peripheral nerve axons, and inhibits a number of enzymes, including alkaline phosphatase and succinic dehydrogenase (Kazantzis, 2007).

Thallium's activity as a Lewis acid with an affinity for organosulfur compounds (Lewis bases) may account for its adverse effect on hair production. Keratin is the primary protein

found in hair. It is rich in the amino acid cysteine and its low solubility is, in large part, the product of the formation of inter-polypeptide cysteine-cysteine cross-links during posttranslational modification of the nascent polypeptides. Thallium prevents keratinization of hair proteins by binding with cysteine and preventing the formation of the cross-linking bonds (Mulkey and Oehme, 1993), a property that may be related to the alopecia observed in humans and animals following thallium exposure. Binding to cysteine may also account for inhibition of enzymes with active site cysteine residues and increases oxidative stress as a result of GSH modification (Mulkey and Oehme, 1993).

4.7. EVALUATION OF CARCINOGENICITY

Under EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), there is "inadequate information to assess the carcinogenic potential" for thallium and thallium compounds. There are presently no studies that evaluate the carcinogenic potential of thallium in animals and no adequate studies of humans chronically exposed to thallium.

Two studies of chronic health effects in workers exposed to thallium are available (Marcus, 1985; Schaller et al., 1980), but these studies are inadequate for the assessment of carcinogenicity. The study by Marcus (1985) is limited by the examination of medical records only, lack of exposure quantitation, small cohort size, and the unknown length of observation. Schaller et al. (1980) identified health effects in a worker population at a single time point through medical histories and physical examinations for unspecified symptoms. Worker exposures to thallium were limited to a single measure of urinary thallium, which would not provide an adequate measure of past exposure. This health evaluation was not adequate to detect any carcinogenic response.

Relatively few studies have examined the genotoxicity of thallium compounds; these studies provide inconsistent evidence for genotoxicity. Positive results were obtained at 0.001M for thallium (I) nitrate in the Rec assay using *B. subtilis* strains H17 and M45 (Kanematsu et al., 1980). However, negative results were obtained in reverse mutation assays using several *S. typhimurium* and *E. coli* strains and mitogenic gene conversion and reverse mutation tests in yeast. Cytotoxic levels (1,000 μg/mL) of thallium (I) acetate caused depressed DNA synthesis in CHO cells (Garrett and Lewtas, 1983). Single-strand DNA breaks occurred in C57Bl/6 mouse and rat embryo fibroblasts exposed to thallium carbonate but not in similarly exposed CBA mouse fibroblasts (Zasukhina et al., 1983). A dose of 200 mg thallium sulfate caused a slight increase in SCEs in peripheral blood lymphocytes taken from a 48-year-old man on day 1 and day 15 postexposure and caused a 3.5-fold increase in binucleated cells with micronuclei (Hantson et al., 1997).

4.8. SUSCEPTIBLE POPULATIONS AND LIFE STAGES

There are little or no data available to establish a particular subpopulation as being particularly susceptible to the toxic effects of thallium compounds.

4.8.1. Possible Childhood Susceptibility

Exposures in human case reports generally are poorly characterized. Therefore, no comparison can be made between children and adults with regard to susceptibility. In rats, doses of thallium that caused maternal toxicity have been demonstrated to affect the developing fetus (Gibson and Becker, 1970). The only studies that examined the toxic effects of thallium at different ages were those of Appenroth et al. (1996) and Fleck and Appenroth (1996), which focused on the age-related effects on nephrotoxicity. In these studies, mature rats were determined to be more susceptible to kidney damage than young (10- or 20-day-old) rats, because mature kidney function appeared necessary for thallium to adversely affect the kidney.

4.8.2. Possible Gender Differences

Leloux et al. (1987), MRI (1988), and Downs et al. (1960) are the only available toxicity studies of thallium compounds that used both male and female rats. Leloux et al. (1987) administered only a single lethal dose of thallium (I) nitrate to rats and thus did not provide findings useful for discerning possible gender differences. Downs et al. (1960) reported slight differences in thallium (III) oxide toxicity between the sexes, with males dying earlier, exhibiting greater and more severe alopecia, and having more profound decreases in body weight than females. No sex-related differences in response were noted in rats treated with thallium (I) acetate (Downs et al., 1960). No marked differences in response were noted in male and female rats after exposure to thallium (I) sulfate (MRI, 1988); however, high-dose female rats exhibited a higher incidence of alopecia than males, and hair follicle atrophy was observed in females only. Overall, the limited data do not identify any consistent pattern of gender-related differences in response to thallium exposure.

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

5.1.1. Choice of Principal Study and Critical Effect—with Rationale and Justification

As discussed in Section 4.6.1., most information on thallium toxicity in humans comes from poisonings, suicide attempts, or accidental exposures. Epidemiologic studies of either the general population or occupationally exposed groups are limited by inadequate study design and/or insufficient exposure characterization. Thus, available human studies do not provide data useful for dose-response analysis.

There are only four repeat-dose oral toxicity studies of thallium that used more than one dose level: MRI (1988), Wei (1987), Zasukhina et al. (1983), and Downs et al. (1960). Of these studies, the subchronic (90-day) toxicity study of thallium (I) sulfate in Sprague-Dawley rats by MRI (1988) is the most comprehensive study of thallium toxicity. In the Downs et al. (1960) study of thallium (I) acetate, mortality in two control groups was 30–40%, complicating interpretation of the findings in treated rats (mortality and alopecia). A study of thallium (III) oxide by the same investigators (Downs et al., 1960) failed to identify a NOAEL. Further, death in 2/5 female rats at the lowest dose tested in this study was observed and may have been treatment related. The repeat-dose dominant lethal study of Zasukhina et al. (1983) suffered from critical reporting deficiencies and internal inconsistencies (see Section 4.3). Wei (1987) reported effects on sperm count, motility, and viability in Kunming mice exposed to drinking water concentrations of thallium (I) carbonate as low as 0.001 mg/L; however, when the treated males were mated with untreated females, the reproductive index and number of implantations were not affected at drinking water concentrations up to 10 mg/L (10,000-fold higher than the concentration identified by the study investigators as the LOAEL for sperm effects). The percent of dead fetuses was significantly lower than in the control group at concentrations of 0.001, 0.01, and 0.1 mg/L but was increased at the two highest concentrations (1 and 10 mg/L). Although the effects on sperm appeared to be dose related, some uncertainty is associated with the reporting of study findings (see Sections 4.3 and 4.6.1). Further, the unexplained absence of reported sperm results for 20–50% of the male mice in each of the treatment groups lowers confidence in these findings. Supporting literature on the reproductive toxicity of thallium is limited. Formigli et al. (1986) reported adverse effects on the testes and sperm of rats exposed to thallium sulfate in drinking water but at a drinking water concentration 10,000-fold higher than the LOAEL reported by Wei (1987). In the absence of confirmatory findings of sperm effects at the low drinking water concentrations used in the Wei (1987) study, the study findings were not considered sufficiently reliable to serve as the basis for RfD derivation.

Other repeat-dose studies of thallium oral toxicity used study designs that included only a single-dose group and thus did not provide data useful for dose-response analysis (El-Garawany et al., 1990; Rossi et al., 1988; Formigli et al., 1986; Gregotti et al., 1985; Manzo et al., 1983). Manzo et al. (1983) reported mortality at the only dose tested, making this study unsuitable for RfD derivation. Finally, doses in mg/kg-day could not be estimated from the study information provided in Rossi et al. (1988).

Based on the above considerations, the 90-day MRI (1988) study was selected as the principal study for derivation of the RfD. In the MRI (1988) study, rats (20/sex/group) were treated by gavage daily for 90 consecutive days with 0, 0.01, 0.05, or 0.25 mg/kg-day of an aqueous solution of thallium (I) sulfate (approximately 0, 0.008, 0.04, or 0.20 mg/kg-day Tl). No differences in body weight, body weight gains, food consumption, or absolute and relative organ weights were observed among control groups and groups receiving thallium sulfate. Lacrimation, exophthalmos, and miosis were observed at higher incidences in the treated male and female rats at all doses compared with both untreated and vehicle controls (see Table 4-2); however, ophthalmologic examination and gross and histopathologic examination of the eyes revealed no treatment-related abnormalities. The incidence of clinical observations related to the coat and skin (including rough coat, piloerection, shedding, and alopecia) and behavioral changes (including combined incidences of aggression, tense/agitated behaviors, hyperactivity, vocalization, and self-mutilation) were also elevated in treated male and female rats at all doses (see Table 4-2). The investigators attributed most, but not all, instances of alopecia to barbering behavior. For example, of the 12 high-dose females with alopecia, 5 instances were not totally attributed to barbering. Review of individual animal data revealed no discernable differences in either the severity or distribution pattern of alopecia across control and treated groups. Histopathologic examination did not reveal any statistically significant treatment-related effects, although it was noted that atrophy of the hair follicles occurred in two high-dose female rats. Tissue samples of the skin from low- and mid-dose groups, however, were not examined for histopathologic changes. Subtle, but statistically significant, changes were observed in several blood chemistry parameters (AST, LDH, sodium and blood sugar levels) (see Table 4-4). These changes were not confirmed by any histopathologic findings and were not considered by the investigators to be toxicologically significant.

Although the study authors identified the highest dose (0.20 mg/kg-day Tl) as a NOAEL based on lack of biological significance of the observed effects, EPA reached the determination that histopathologic findings in the skin of female rats and dose-related clinical findings, when considered collectively, may reflect an adverse effect on the health of the rats.

As noted above, histologic examination of skin samples from two high-dose females showed atrophy of hair follicles. These two animals also exhibited alopecia. Hair loss (alopecia) is characteristic of thallium poisoning in humans and experimental animals (Ibrahim et al., 2006;

Galván-Arzate and Santamaría, 1998), and typically occurs in humans within two weeks of exposure. It is hypothesized that thallium's affinity for sulfhydryl groups may be responsible for alopecia; thallium prevents keratinization of hair proteins by binding with cysteine. Skin biopsies have been taken from a limited number of patients with alopecia and other symptoms of thallium poisoning; these biopsies have revealed atrophic and necrotic changes of the skin (Lu et al., 2007; Heyl and Barlow, 1989; Saddique and Peterson, 1983). For example, skin biopsy findings from two patients who ingested water that contained thallium included parakeratosis, dilated hair follicles filled with keratin and necrotic sebaceous materials, mild epidermal atrophy, and vacuolar degeneration of the basal layer (Lu et al., 2007). Thus, the finding of two cases of atrophy of the hair follicles in high-dose female rats with alopecia is consistent with the atrophic changes observed in cases of human thallium poisoning, and suggests that alopecia at the high-dose (0.2 mg/kg-day Tl) may be related to thallium exposure. This endpoint was considered a candidate critical effect for derivation of the RfD.

The clinical observations from the MRI (1988) study were also considered as candidate critical effects for derivation of the RfD. As discussed in Section 4.6.1, dose-related increases in the incidence of lacrimation, exophthalomos, and miosis, coat-related findings, and behavioral observations suggest a possible effect on the health of the exposed rats from exposure to thallium, although the underlying basis for these observations is unknown. Increased incidences of barbering and behavioral changes are consistent with possible effects on the nervous system, a known target of thallium.

Clinical chemistry changes were not considered as the basis for determining the point of departure (POD) for the RfD. At 90 days, AST, LDH, sodium, and sugar levels in the blood of high-dose male and female rats differed by +31, +38, +4, and -21%, respectively, from vehicle control group values. Even with these changes, these clinical chemistry parameters are all well within two standard deviations of the mean for control Sprague-Dawley rats as reported by Petterino and Argentino-Storino (2006) and Matsuzawa et al. (1993). In the low-dose group, none of the blood chemistry parameters was statistically significantly different from the vehicle control group with the exception of the sodium level in female rats, which was increased over control values by only 1.4%. In light of these modest changes in blood chemistry parameters and the lack of other findings in exposed animals to confirm the biological significance of these changes, clinical chemistry parameter data were not selected for dose-response modeling.

In summary, two endpoints were considered as potential critical effects for determination of the POD for the RfD: (1) hair follicle atrophy in female rats that also had alopecia, and (2) clinical observations, including those related to animal coat (rough coat, piloerection, shedding, and alopecia), eyes (including lacrimation, exophthalmos, and miosis), and behavior.

5.1.2. Methods of Analysis

Hair Follicle Atrophy

The NOAEL-LOAEL approach was used for dose-response analysis of hair follicle atrophy in rats with alopecia. A benchmark dose (BMD) analysis was not conducted because the incidence of histopathologically-determined hair follicle atrophy was not considered amenable to BMD methods. Histopathological examination of the skin was performed for two groups only—the high-dose group and vehicle control. Two of 20 female rats in the high-dose group (10%) had hair follicle atrophy and alopecia that is consistent with thallium toxicity in both animals and humans and was thus characterized by EPA as possibly treatment-related. The high dose (0.2 mg/kg-day Tl) was therefore characterized as a LOAEL. Because skin tissue from rats in the low- and mid-dose groups was not examined for histopathologic changes, the NOAEL for this endpoint cannot be determined with certainty. Given the low incidence of hair follicle atrophy in females in the high-dose group and absence of cases of hair follicle atrophy in male rats, the mid-dose can reasonably be assumed to approximate a NOAEL for skin histopathology. Thus, an estimated NOAEL of 0.04 mg/kg-day Tl was used as the POD for hair follicle atrophy from the MRI (1988) study.

Clinical Observations

BMD modeling (U.S. EPA, 2000b) was used to analyze the clinical observation data from MRI (1988). Incidence data for the selected clinical observations in male and female rats are summarized in Table 5-1. All of the available dichotomous models in U.S. EPA's BMDS (version 1.4.1) (U.S. EPA, 2007) were fit to these incidence data.

Table 5-1. Incidence data and BMD modeling results for selected clinical observations in Sprague-Dawley rats treated with thallium sulfate for 90 days[a]

Observation	Dose (mg/kg-day)					BMD_{10}[b] (mg/kg-day)	$BMDL_{10}$[b] (mg/kg-day)
	Untreated control	Vehicle control	0.008	0.04	0.2		
Male							
Coat/skin							
Rough coat	1/20	3/20	11/20	16/20	19/20	BMD and BMDL well below experimental range.[d]	
Piloerection	0/20	0/20	1/20	4/20	13/20	0.020	0.013
Shedding	0/20	0/20	4/20	10/20	8/20	All dichotomous models in BMDS exhibited statistically significant lack of fit.[e]	
Alopecia	2/20	1/20	4/20	9/20	4/20	All dichotomous models in BMDS exhibited statistically significant lack of fit.[e]	
Eyes							
Lacrimation	1/20	6/20	19/20	20/20	20/20	BMD and BMDL well below experimental range.[d]	
Exophthalmos	1/20	5/20	12/20	20/20	20/20	BMD and BMDL well below experimental range.[d]	
Miosis	0/20	1/20	5/20	7/20	15/20	0.0069	0.0039
Behavior[c]	3/20	0/20	7/20	6/20	7/20	All dichotomous models in BMDS exhibited statistically significant lack of fit.[e]	
Female							
Coat/skin							
Rough coat	1/20	0/20	1/20	5/20	11/20	0.021[f]	0.013[f]
Piloerection	0/20	0/20	0/20	3/20	8/20	0.043[f]	0.026[f]
Shedding	0/20	0/20	2/20	3/20	13/20	0.020	0.013
Alopecia	4/20	1/20	4/20	9/20	12/20	0.018[f]	0.010[f]
Eyes							
Lacrimation	7/20	6/20	20/20	20/20	20/20	All dichotomous models in BMDS exhibited statistically significant lack of fit.[e]	
Exophthalmos	5/20	6/20	19/20	20/20	20/20	BMD and BMDL well below experimental range.[d]	
Miosis	2/20	3/20	1/20	11/20	8/20	All dichotomous models in BMDS exhibited statistically significant lack of fit.[e]	
Behavior[c]	2/20	2/20	0/20	1/20	7/20	0.14[f]	0.054[f]

Table 5-1. Incidence data and BMD modeling results for selected clinical observations in Sprague-Dawley rats treated with thallium sulfate for 90 days[a]

Observation	Dose (mg/kg-day)					BMD_{10}[b] (mg/kg-day)	$BMDL_{10}$[b] (mg/kg-day)
	Untreated control	Vehicle control	0.008	0.04	0.2		

[a] Listed as number of animals with the sign observed at least once during the 90-day study.
[b] BMD_{10} = Benchmark dose corresponding to a 10% extra risk; $BMDL_{10}$ = 95% lower bound on the dose corresponding to a 10% extra risk.
[c] Animals exhibiting one or more behavioral observations at least once during the 90-day study, including the following: aggressive, tense/agitated, hyperactive, vocalization, self-mutilation.
[d] Data set not amenable to modeling because of steep slope of the dose-response curve in low-dose region; BMD and BMDL well below experimental range.
[e] Some data sets shown in this table exhibited a statistically significant lack of fit with the models available in BMDS. Where lack of fit is due to characteristics of the dose-response data for high doses, one option sometime used is to adjust the data set by eliminating the high-dose group(s) (U.S. EPA, 2000b). In the absence of a mechanistic understanding of the biological response or other observed toxicity in the thallium-exposed animals that could explain the dose response at the higher doses, dose-response assessment with adjusted data sets (i.e., removing the high-dose group(s)) was not performed.
[f] Reported BMD_{10} and $BMDL_{10}$ represent averages of results from several similar model fits. See Appendix B for more details.

Source: MRI (1988).

Consistent with EPA (2000b) BMD technical guidance, consideration was given to identifying biologically relevant response levels for developing the RfD. A BMR of 10% is generally used to facilitate a consistent basis of comparison across assessments and was used for these endpoints in the absence of information regarding the level of change considered to be biologically significant. Doses (i.e., BMD_{10} [BMD corresponding to a 10% extra risk] and $BMDL_{10}$ [95% lower bound on the BMD corresponding to a 10% extra risk]) associated with a BMR of 10% extra risk are presented in Table 5-1.

Details of the BMD modeling conducted for each endpoint presented in Table 5-1 are provided in Appendix B. In general, model fit was assessed by a chi-square goodness-of-fit test (i.e., models with $p < 0.1$ failed to meet goodness-of-fit criterion) and visual inspection of the respective plots of observed versus predicted values from the various models. $BMDL_{10}$ estimates from these models that were within a factor of three of each other suggested no appreciable model dependence. Fitted models exhibiting adequate fit (i.e., $p \geq 0.1$) with Akaike Information Criterion (AIC) values within two units of the lowest AIC were considered indistinguishable from one another (Burnham and Anderson, 2002), and thus BMD_{10} and $BMDL_{10}$ values from these models were averaged. Model fits that yielded the same mathematical model were counted as a single model for averaging purposes (see Appendix B for details).

As Table 5-1 shows, clinical observation data sets for female rats were generally more amenable to BMD modeling than the male rat data sets. The $BMDL_{10}$ values for endpoints

related to the coat/skin in female rats ranged from 0.010 to 0.026 mg/kg-day, a range of only 2.6-fold. The lowest $BMDL_{10}$ value for female rats (0.010 mg/kg-day) comes from averaging the $BMDL_{10}$ values from several similar model fits of incidence data for alopecia. As discussed in Section 5.1.1, alopecia is a hallmark of thallium poisoning. To the extent that instances of alopecia resulted from barbering, the occurrence of alopecia could also be consistent with possible effects of thallium on the nervous system, although no direct evidence for neurotoxicity was provided.

In male rats, the smallest $BMDL_{10}$ (0.0039 mg/kg-day) is based on data for miosis. While elevated in thallium-treated animals, the biological significance of miosis is less clear than the collection of effects related to the rats coat and skin. Review of individual animal data from the MRI (1988) study revealed that at the high dose, miosis was observed between days 6 to 10 only, and in the majority of animals (approximately 70%) miosis was observed on only one or two days.

5.1.3. RfD Derivation—Including Application of Uncertainty Factors (UFs)

5.1.3.1. *Soluble Thallium Salts: Acetate, Carbonate, Chloride, Nitrate, and Sulfate*

The principal study considered for RfD derivation – MRI (1988) – involved administration of thallium (I) sulfate. There are no studies of thallium (I) acetate, thallium (I) carbonate, thallium (I) chloride, or thallium (I) nitrate that are appropriate to use as the basis for an RfD. For the following reasons, it was considered appropriate to treat these monovalent thallium salts as toxicologically equivalent to thallium (I) sulfate when expressed in terms of thallium. It is likely that the mechanism of toxicity is the same for these salts due to the fact that they all contain monovalent thallium ions and are water soluble. Only small differences in the toxicity of various water-soluble thallium (I) salts exist in mice, rats, rabbits, and dogs. In general, for most laboratory species at an observation period of approximately 1–2 weeks, the LD_{50} or minimum effective dose values range between 10 and 30 mg/kg body weight for thallium (I) salts, independent of the exposure route (IPCS, 1996). Therefore, the use of thallium (I) sulfate as a surrogate for the other thallium salts is considered appropriate.

Candidate RfD values for thallium were derived using PODs for two endpoints from the MRI (1988) study – a NOAEL of 0.04 mg/kg-day Tl for hair follicle atrophy and an average $BMDL_{10}$ of 0.01 mg/kg-day Tl for alopecia (as representative of clinical observations more generally).

Some degree of uncertainty is associated with both PODs. Hair follicle atrophy was reported in the MRI (1988) study only in high-dose female rats. Because skin tissue from the low- and mid-dose groups was not examined histopathologically, the NOAEL for this endpoint could only be estimated. The biological significance of the dose-related clinical observations in rats in the MRI (1988) study is unclear. In general, the collection of clinical observations data is

relatively subjective and less rigorously measured than endpoints such as clinical chemistry or histopathology. While clinical observations reported in the MRI (1988) study could represent an indirect measure of stress or possible effects on the nervous system, no direct support for these etiologies is available. The increased incidence of alopecia in female rats was dose-related (less clearly so in male rats); however, the background incidence of alopecia in control animals and attribution of some cases of alopecia by the study authors to barbering behavior introduce uncertainty in dose-response analysis of this endpoint. Furthermore, examination of individual animal data from the MRI (1988) study revealed no discernable difference in the severity or distribution of alopecia across control and treated groups. Effects on the eyes based on clinical observations (in particular lacrimation and exophthalmos) were not supported by ophthalmic examination or by gross or histopathological examination of the eyes.

A total uncertainty factor (UF) of 3,000 (10 for interspecies extrapolation, 10 for intraspecies extrapolation, 3 for extrapolation from a subchronic to a chronic study, and 10 for database deficiencies) was applied to both PODs to estimate candidate oral RfD values for soluble thallium salts.

- A default interspecies UF of 10 was applied for extrapolation from laboratory animals to humans. No information was available to characterize the toxicokinetic or toxicodynamic differences between experimental animals and humans.

- A default intraspecies UF of 10 was applied to account for variation in human susceptibility in the absence of information on the variability of response to thallium in the human population.

  Because no chronic toxicity studies for thallium are available, an UF of 3 was applied to account for extrapolation from subchronic to chronic exposure duration. Oral toxicity data for thallium suggest that an UF of 10 would overestimate the difference in response following subchronic and chronic oral exposures. Effects on the coat/skin as well as other clinical observations occur within weeks of exposure to thallium (i.e., these sensitive effects do not require chronic exposure in order to manifest).

- An UF for LOAEL to NOAEL extrapolation was not needed for hair follicle atrophy in rats with alopecia because a NOAEL was estimated from data provided in the principal study.

  Similarly, a UF to account for extrapolation from a LOAEL to NOAEL was not used for alopecia (as representative of clinical observations) because BMD methods were

used for dose-response analysis of these endpoints. The current approach is to address this extrapolation as one of the considerations in selecting a BMR for BMD modeling. In this case, a BMR of 10% increase in the incidence of alopecia was selected under the assumption that it represents a minimally biologically significant change.

- The thallium database includes several subchronic oral toxicity studies in rats. Studies of reproductive and developmental toxicity of thallium compounds in rats and mice are available; however, these studies used nontraditional study designs that did not provide adequate testing of reproductive or developmental endpoints. In reproductive toxicity studies by Gregotti et al. (1985) and Formigli et al. (1986), male rats only were exposed for periods up to 60 days and evaluation of reproductive toxicity was limited to examination of male reproductive organs. In two other reproductive toxicity studies, male rats (Zasukhina et al., 1983) or mice (Wei, 1987) only were exposed to thallium compounds for 6 to 8 months and mated with untreated females. Confidence in these latter two studies was low (see Sections 4.3 and 4.6.1). No studies of reproductive toxicity of thallium in females or multigeneration reproductive toxicity studies were identified. Developmental toxicity by the oral route was limited to Rossi et al. (1988) in which rats were exposed to thallium sulfate from gestation day 1 through 60 days of age or from birth through 60 days of age. No investigation of developmental endpoints at the end of the gestation period was performed. Despite the limitations in the available reproductive and developmental toxicity studies, these studies provide suggestive evidence that thallium compounds can adversely affect male reproductive organs and the developing fetus and highlight the deficiencies in the current thallium database. Limited neuropathologic examinations were included in the subchronic toxicity studies by MRI (1988) and Manzo et al. (1983), but no standard neurobehavioral studies were identified. Because the nervous system is a sensitive target of thallium toxicity, the limited investigation of thallium neurotoxicity represents a data deficiency. Thus, a database UF of 10 was applied to account for a lack of adequate developmental toxicity studies and a two-generation reproductive toxicity study, and additional uncertainty associated with the limited data available on neurotoxicity in light of the potential for neurotoxicity to represent a sensitive endpoint for thallium exposure.

Thus, the candidate RfDs for thallium (I) are calculated as the POD (NOAEL or BMDL) divided by the composite UF, as follows:

Derivation of candidate RfD based on hair follicle atrophy in animals with alopecia:

0.04 mg/kg-day Tl ÷ 3000 = 1 x 10^{-5} mg/kg-day Tl

Derivation of candidate RfD based on clinical observations/alopecia:

0.01 mg/kg-day Tl ÷ 3000 = 3 x 10^{-6} mg/kg-day Tl

The available toxicity database for thallium contains studies that are generally of poor quality. The MRI (1988) study that was selected as a candidate principal study suffers from certain critical limitations (e.g., high background incidence of alopecia, lack of histopathological examination of skin tissue in low- and mid-dose groups, and inadequate examination of objective measures of neurotoxicity), and there are particular difficulties in the selection of appropriate endpoints. Therefore, even though an RfD would generally be derived with a combined uncertainty factor of 3000, an RfD for soluble thallium salts is not derived in this specific case.

5.1.3.2. ***Insoluble Thallium Salts: Thallium (III) Oxide***

No oral studies of thallium (III) oxide were found that are adequate to support derivation of an RfD. Downs et al. (1960) administered thallium (III) oxide to Wistar-derived albino rats via the diet for 15 weeks at concentrations of 0, 20, 35, 50, 100, and 500 ppm. The study reported only body weights, mortality, kidney weights, and limited histopathology. Because all relevant endpoints were not evaluated, treatment group sizes were small (five per group per sex), and few animals survived to termination, this study could not be used to derive an RfD.

Because of differences in the physical-chemical properties of thallium (I) sulfate and thallium (III) oxide, toxicity information for thallium (I) sulfate cannot be used to inform the toxicity of thallium (III) oxide. Unlike thallium (I) sulfate, thallium (III) oxide is insoluble in water. Thallium (I) sulfate contains monovalent thallium (Tl^{+1}), while thallium (III) oxide contains trivalent thallium (Tl^{+3}). Although the gastric environment may increase the solubility of thallium (III) oxide, no studies are available that examine the effects of the gastric pH and gastric environment on the valence state of thallium (III) oxide.

Limited evidence from the toxicology literature suggests that the distribution of thallium (I) and thallium (III) compounds and the lethality of these two compounds may be comparable. Sabbioni et al. (1980a) determined that, following oral administration of either inorganic monovalent thallium (Tl^{+1}) as thallium (I) sulfate or trivalent thallium (Tl^{+3}) as thallium (III) chloride, a similar distribution of thallium in the tissues was seen (valence state could not be determined) at 16 hours and 8 days after administration, indicating that the valence state of thallium did not affect tissue distribution (and presumably uptake). Downs et al. (1960) demonstrated similar oral 7-day LD_{50} values for thallium (I) acetate (32 mg/kg Tl) and thallium (III) oxide (39 mg/kg Tl) in female rats, indicating that lethality may be independent of valence

state. Whether other endpoints would respond similarly to different valence states of thallium is unknown. Monovalent thallium compounds have been determined to behave similarly to potassium (K^+), thus disrupting $Na^{+/}K^+$-ATPase and the systems dependent on this transporter (e.g., liver and kidney). Trivalent thallium has not been demonstrated to behave in the same manner. A single in vitro study of mono- and trivalent thallium compounds (Diaz and Monreal, 1994) suggested that biological responses to thallium in the (I) and (III) valence states may differ. Using an in vitro system with liposomes prepared with lipid from brain myelin, these investigators reported that trivalent thallium, but not monovalent thallium, mediated a rapid chloride/hydroxyl ion exchange through the lipid bilayers.

Overall, the available toxicity information for thallium (III) oxide specifically and thallium compounds more generally is insufficient to support derivation of an RfD for thallium (III) oxide.

5.1.3.3. *Thallium (I) Selenite*

No toxicity studies of thallium (I) selenite are available. Thallium (I) selenite contains monovalent thallium as does thallium (I) sulfate. No information could be found in the literature, however, on the water solubility of thallium (I) selenite. In the absence of solubility information, toxicity information for thallium (I) sulfate cannot be used to inform the toxicity of thallium (I) selenite. Accordingly, the available data do not support derivation of an RfD for thallium (I) selenite.

5.1.4. Candidate RfD Comparison Information

PODs and candidate RfD values based on selected studies included in Table 4-5 are arrayed in Figures 5-1 to 5-5 and provide perspective on the magnitude of PODs and candidate RfD values associated with different toxicity endpoints. These figures should be interpreted with caution because the PODs across studies are not necessarily comparable, nor is the confidence in the data sets from which the PODs were derived the same. PODs in these figures may be based on a LOAEL or a BMDL, and the nature, severity, and incidence of effects occurring at a LOAEL are likely to vary. To some extent, the confidence associated with the data sets is reflected in the magnitude of the total UF applied to the POD (i.e., the size of the bar); however, the text of Sections 5.1.1 and 5.1.2 should be consulted for a more complete understanding of the issues associated with each data set.

Thallium causes toxicity in a wide range of target organs, including the nervous system, kidney, cardiovascular system, liver, skin, reproductive system, and possibly the developing fetus. Figure 5-1 provides a graphical display of dose-response information for skin histopathology (hair follicle atrophy in female rats with alopecia) based on the 90-day MRI (1988) study. Uncertainties in this data set are discussed in Section 5.1.3.1.

Figure 5-2 provides a graphical display of dose-response information for clinical observations from the 90-day study conducted by MRI (1988). Collectively, the dose-related increased incidences of effects on the coat, eyes, and behavior suggest a potential treatment-related effect of thallium, possibly including some measure of stress or other effect on the nervous system; however, the underlying basis for these clinical observations is unknown. It should be noted that Figure 5-2 includes PODs derived using $BMDL_{10}$ values as the POD for those data sets for which a model fit could be obtained from the models in BMDS, as well as a reference value derived using the LOAEL as the POD (since some data sets were not fit by the models in BMDS). Uncertainties in these data sets are discussed in Section 5.1.3.1.

In some studies, doses of thallium that do not affect survival have been shown to affect clinical chemistry parameters (e.g., BUN, serum creatinine, bilirubin, and ALT) that may be indicative of effects on the kidney and liver. Changes in clinical chemistry parameters observed in El-Garawany et al. (1990) are plotted in Figure 5-3. It should be noted that application of a composite UF of 10,000, reflecting five areas of uncertainty, indicates that derivation of a reference value using such a data set is highly uncertain. In such cases, U.S. EPA (2002) recommends that an RfD not be derived.

Evidence from experimental animal studies suggests that thallium may produce effects on reproductive function. Figure 5-4 includes plots of LOAELs and associated UFs for data sets from Formigli et al. (1986) and Wei (1987). Formigli et al. (1986) reported testicular effects in rats exposed to thallium sulfate for 60 days but used only a single dose level of thallium. Therefore, this study has limited utility for dose-response analysis. Wei (1987) found effects on sperm in mice exposed to thallium carbonate at drinking water concentrations as low as 0.001 mg/L. As discussed in Sections 4.6.1 and 5.1.1, confidence in this study is low because of uncertainties associated with estimates of exposure, reporting inconsistencies, and unexplained absence of sperm data for male mice in all dose groups (ranging from 20 to 50%). Composite UFs of 100,000 and 10,000 were applied to the LOAELs from the Formigli et al. (1986) and Wei (1987) data sets. As with the data from El-Garawany et al. (1990), the magnitude of the uncertainty associated with these data sets indicates that they are insufficient to support derivation of a reference value.

In humans, numerous case reports of neuropathy following thallium poisoning have been reported. In experimental animals, effects on the nervous system have been most clearly observed following oral exposure at levels also associated with increased mortality (e.g., Manzo et al. [1983]) or following injection exposure (see Section 4.4.3). Therefore, although the nervous system is known to be a target of thallium toxicity, the available studies do not provide adequate dose-response information on standard measures of nervous system toxicity at nonlethal doses that are appropriate for defining a POD.

Figure 5-5 displays PODs for the major targets of toxicity (from Figures 5-1 to 5-4) associated with oral exposure to thallium, including skin histopathology, alopecia and other

general clinical observations, changes in clinical chemistry possibly indicative of the kidney and liver as targets of thallium toxicity, and the reproductive system, along with the UFs that would be applied for candidate RfD derivation.

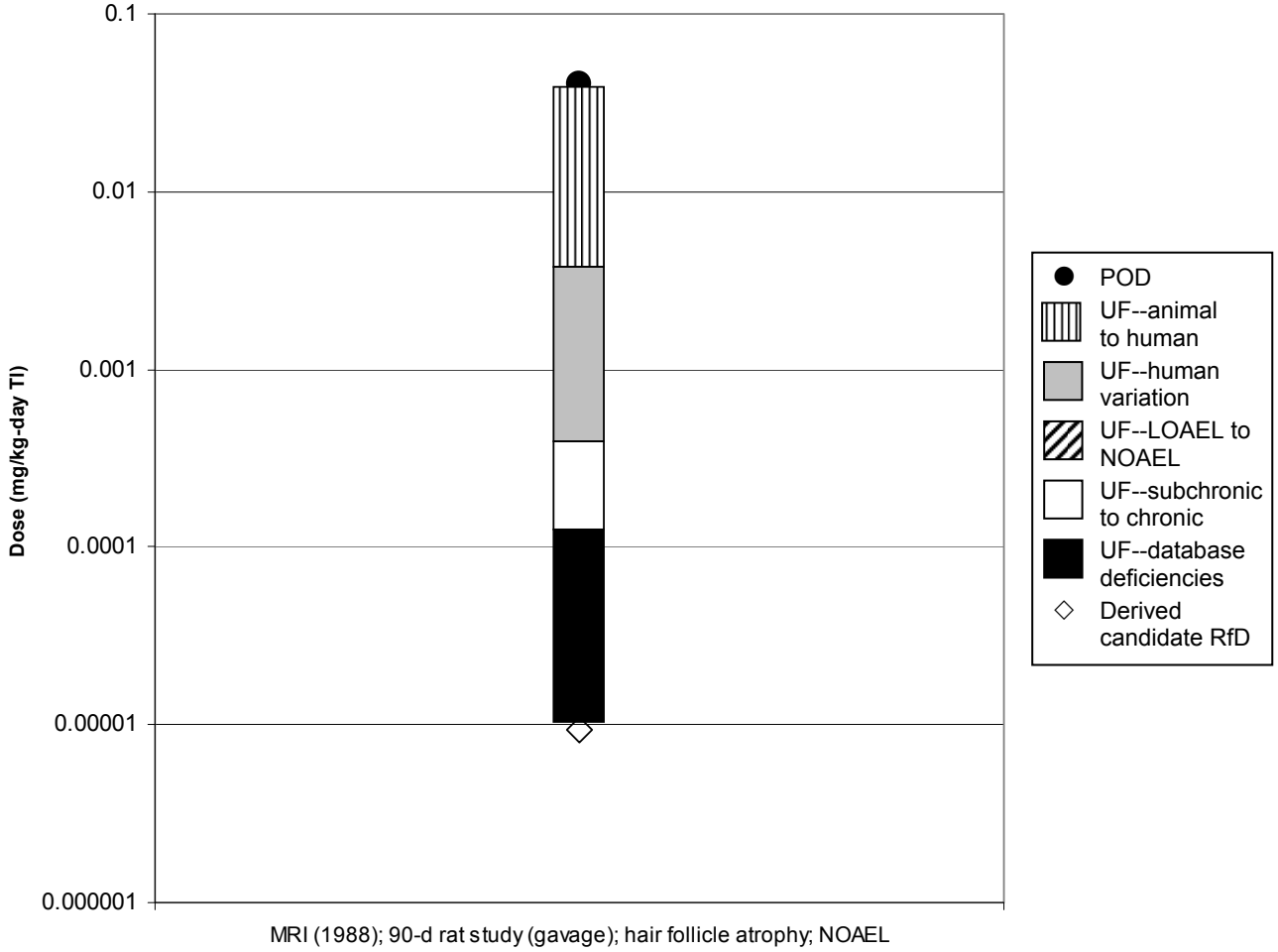


Figure 5-1. POD (mg/kg-day) with corresponding derived candidate reference value that would result if histopathologic changes of the skin (hair follicle atrophy) were used as the critical effect.

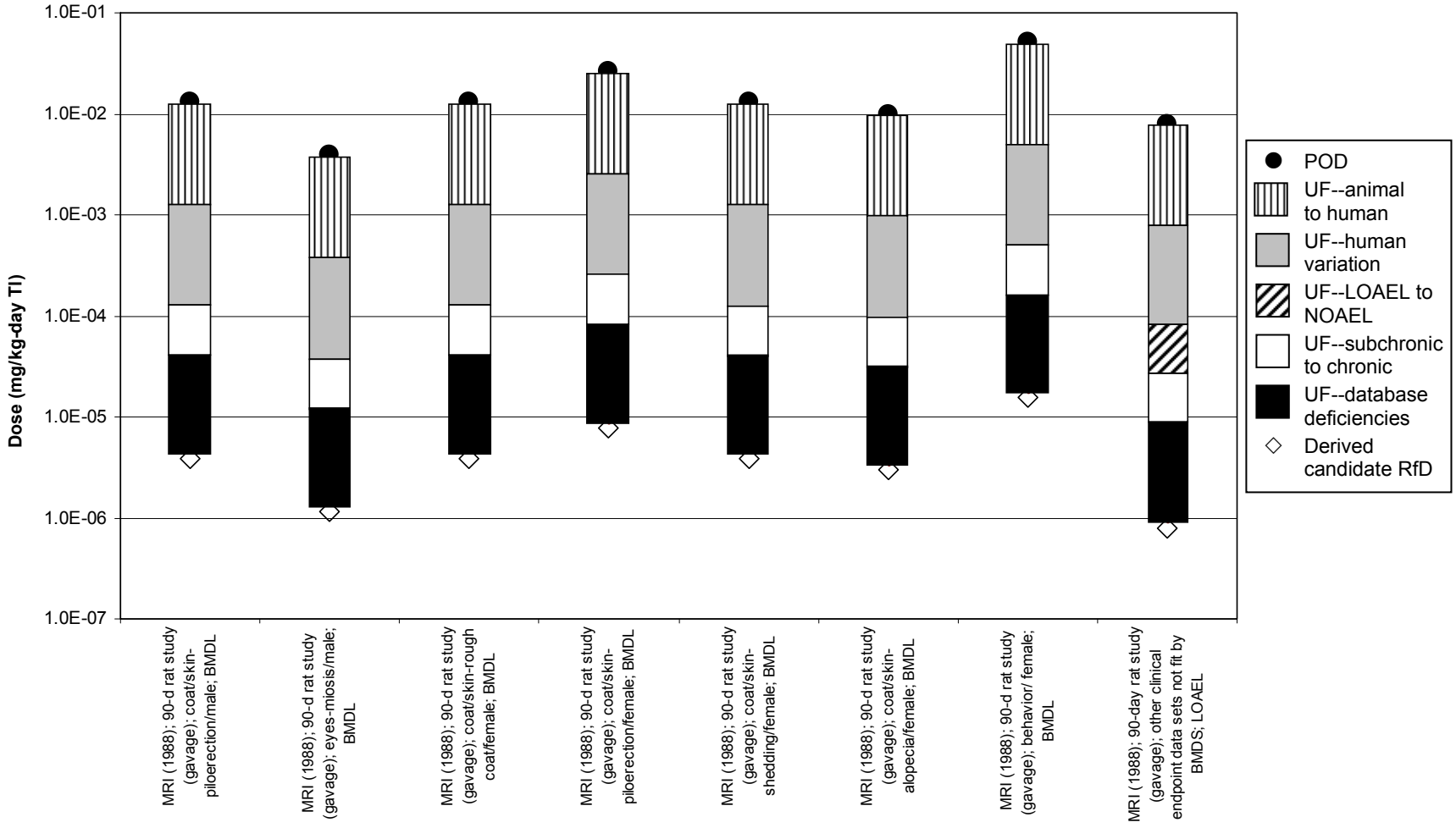


Figure 5-2. PODs (mg/kg-day) with corresponding derived candidate reference values that would result if clinical observations from MRI (1988) were used as the critical effect.

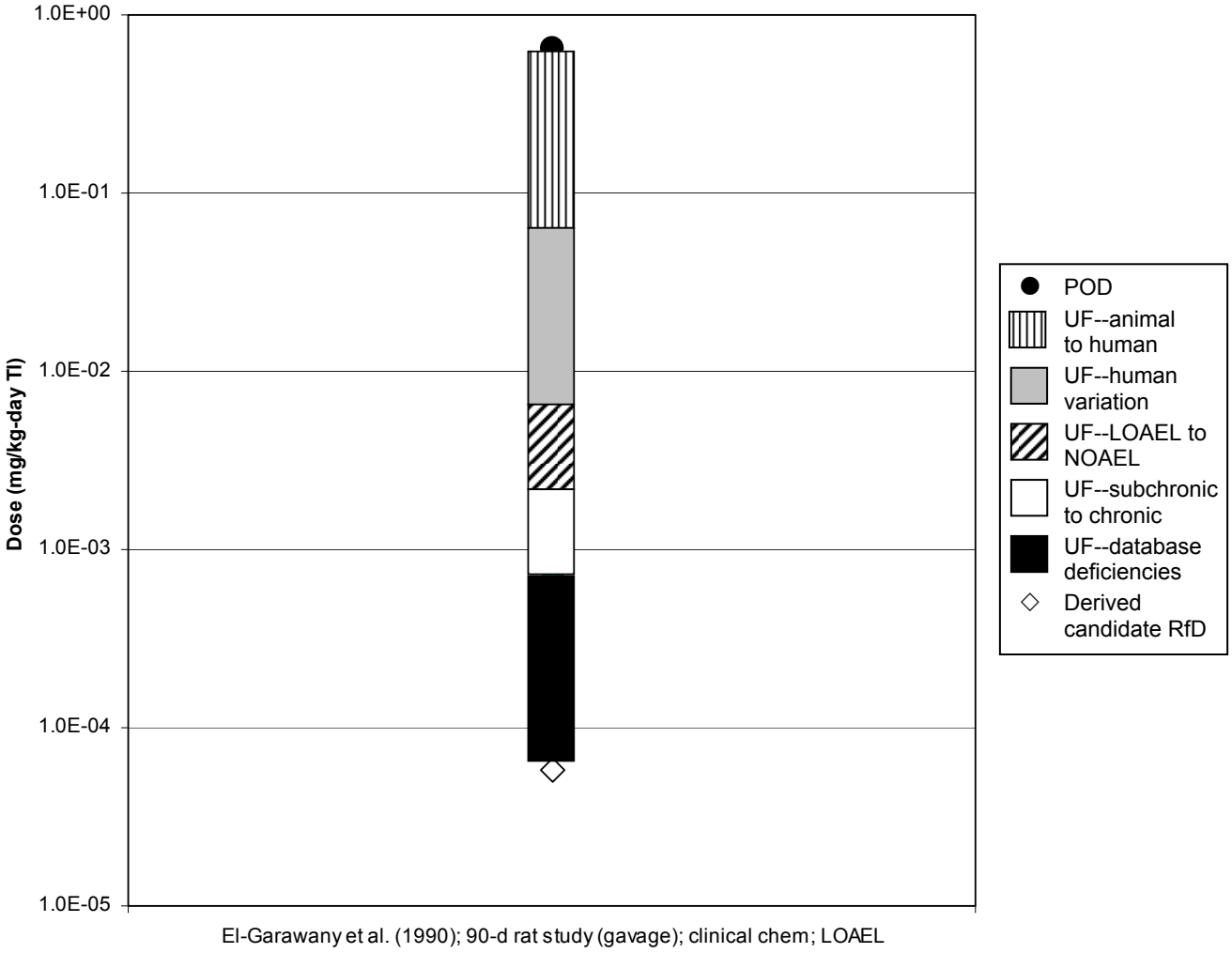


Figure 5-3. POD (mg/kg-day) with corresponding derived candidate reference value that would result if clinical chemistry changes (suggesting the liver or kidney as a target) were used as the critical effect.

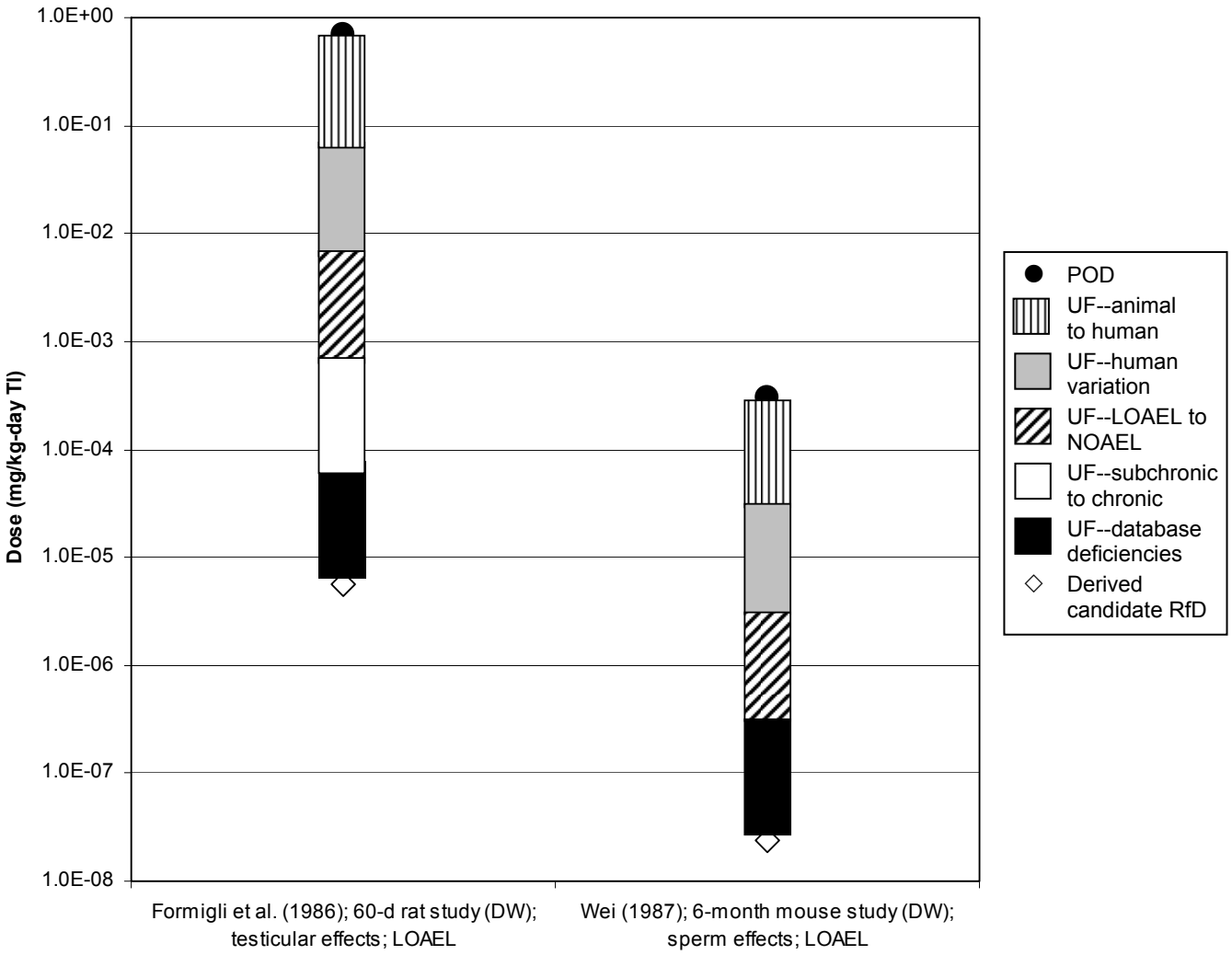


Figure 5-4. PODs (mg/kg-day) with corresponding derived candidate reference values that would result if reproductive toxicity endpoints were used as the critical effect.

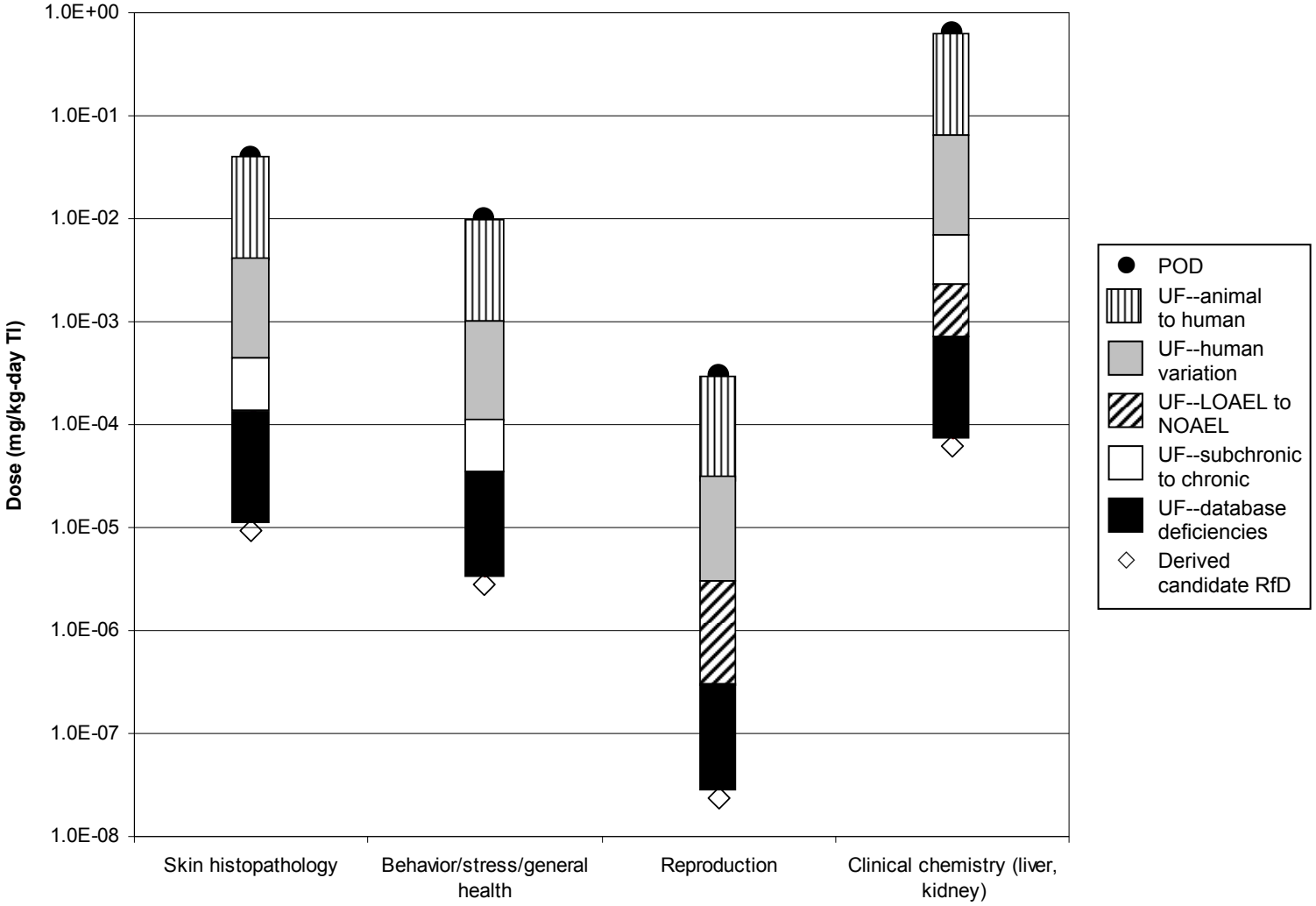


Figure 5-5. PODs (mg/kg-day) with corresponding derived candidate reference values that would result if alternative endpoints were used as the critical effect.

5.1.5. Previous RfD Assessment

The previous IRIS RfD values for thallium (I) acetate, thallium (I) carbonate, thallium (I) chloride, thallium (I) nitrate, and thallium (I) sulfate were posted to the IRIS database in September 1988. These assessments were based on the same principal study considered for the principal study in the current assessment (MRI, 1988). (The principal study was previously cited as U.S. EPA [1986c]. MRI subsequently issued a revised final report in 1988, which is the basis for the candidate RfD values developed in the current assessment. There are no substantive differences in the findings and conclusions between the 1986 and 1988 versions of the MRI report.) Previous RfD values (adjusted for differences in molecular weight) ranged from 8×10^{-5} to 9×10^{-5} mg/kg-day. These RfD values were based on a NOAEL of 0.25 mg/kg-day thallium sulfate, the highest dose tested by MRI (1988), and application of a composite UF of 3,000 (10 to extrapolate from subchronic to chronic data, 10 for intraspecies extrapolation, 10 to account for interspecies variability, and 3 to account for lack of reproductive and chronic toxicity data). Although both the previous and current assessments for soluble thallium salts (acetate, carbonate, chloride, nitrate, and sulfide) identified the same study as principal, the assessments differed in terms of the interpretation of the MRI (1988) findings and method for dose-response analysis. Most significantly, the current assessment does not recommend a value for the RfD.

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

Information on the inhalation toxicity of thallium is insufficient to derive an inhalation RfC. No studies of inhaled thallium in experimental animals were identified, and occupational epidemiology studies involving possible inhalation exposures to thallium were limited and inconclusive.

5.3. CANCER ASSESSMENT

There are no human or animal studies available that are adequate to assess the carcinogenic potential of thallium salts.

6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

6.1. HUMAN HAZARD POTENTIAL

Thallium is well absorbed from the GI tract, skin, and respiratory tract and is distributed throughout the body. Thallium, as an element, is not metabolized, but the extent to which it may be converted from one valence state to another (i.e., Tl^{+1} and Tl^{+3}) in the body is not known. Excretion of thallium occurs mainly through the urine and feces, but the amounts are species dependent. Humans mainly excrete thallium through the urine, but it also has been detected in the hair, sweat, tears, and breast milk.

Thallium salts cause a wide spectrum of adverse effects in humans and animals. Alopecia is an effect that is characteristic of thallium exposure. Alopecia generally occurs within 2 weeks of exposure and is reversible when thallium exposure is removed. Only one epidemiologic study reported a negative correlation between thallium exposure and hair loss (Brockhaus et al., 1981); however, this study lacked measures of chronic exposure to thallium and was limited by reliance on questionnaires to determine symptomology in thallium-exposed individuals.

Some study observations suggest that the nervous system may be the primary target organ for thallium salt toxicity. Neurotoxicity has been observed after a single dose of 0.31 g thallium acetate in an adult male or 1 mg/kg thallium (I) nitrate for 4 days in adult rats. A variety of neurological effects have been reported in humans, including lethargy, back pain, paresthesia of the hands and feet, weakness (including facial weakness), inability to walk, and prolonged mental defects. Some of the effects are reversible depending on the severity, while others are irreversible and may require long-term care. Animal studies support these findings in humans.

Kidney damage in humans has been noted by increases in BUN levels and serum creatinine and is reversible with treatment and/or discontinued exposure to thallium. Data from animal studies support those in humans with regard to kidney damage; thallium-related effects include increased or decreased urine output (depending on dose), protein in the urine, and changes in electrolyte balance. Histopathologic examination of thallium-exposed animals revealed changes in kidney morphology, including atrophied and vacuolated kidney tubules, amorphous material in the lumen of the proximal tubules, disorganized brush borders, and thickening ascending limb of the loop of Henle. Na^+/K^+-ATPase activity in the medulla also was significantly reduced. None of these changes were dose related and were generally seen with large doses of thallium. Animal studies also suggest that kidney toxicity requires mature kidney function. The subchronic (90-day) toxicity study in rats used to derive the RfD showed moderate increases in AST and LDH, which are general indicators of tissue damage (MRI, 1988).

However, a specific relation to possible kidney damage was not indicated due to the lack of changes in other clinical chemistry parameters (i.e., BUN and creatinine) and histopathologic changes in the kidney.

Cardiotoxicity findings in thallium-exposed humans are variable. Many case reports indicate hypertension, while a few reported hypotension. Animal studies indicate that thallium affects heart rate and causes a decrease in blood pressure, which also can be related to kidney effects and is supported by in vitro results.

Many of the case reports in humans reported increased ALT and/or AST levels, indicating liver toxicity. These effects returned to normal after patients received medical care for thallium exposure. Various indicators of liver damage, including increases in ALT, AST, serum bilirubin, lipid peroxidation, triglycerides, and serum alkaline phosphatase and decreases in glycogen, glutathione, and liver Na^+/K^+-ATPase, have been reported in animal studies. In addition, histopathology revealed swollen and vacuolated cells and swollen mitochondria. Many of these effects were reversible in animals after a single dose of thallium. Statistically significant increases in AST and LDH were observed in the 90-day MRI (1988) toxicity study in rats; however, the increases remained within the range reported in control rats and were not accompanied by histopathologic changes of the liver or changes in related clinical chemistry parameters (i.e., ALT).

Low birth weight is a likely adverse effect of thallium exposure in females (humans and animals) exposed during pregnancy. Male mice exposed to thallium were reported to have low sperm counts, low sperm motility, and increases in the number of deformed sperm. Testicular effects observed in animals included disarrangement of the tubular epithelium, cytoplasmic vacuolation and distention of smooth endoplasmic reticulum of the Sertoli cells, and reduced beta-glucuronidase activities. Mating exposed male mice to unexposed female mice appeared to cause a decrease in the number of live fetuses and an increase in the overall rate of dead fetuses; however, critical information concerning the doses of thallium administered makes it difficult to assess these results.

There are no human studies relating thallium exposure to developmental toxicity. A literature review of pregnant women exposed to thallium during various stages of pregnancy reported an association with low birth weight. A survey of children born near a cement plant emitting thallium found an increase in congenital malformations over those reported to the government; however, the study authors did not consider these malformations to be related to thallium exposure because two of the cases were considered attributable to hereditary factors and the incidence was similar to that reported in the literature. Because of various study limitations, the findings are considered inconclusive. In rats exposed transplacentally and/or via mother's milk, then via the drinking water until 60 days of age, thallium (1 mg/dL in the drinking water of dams' then offspring) affected bone development and vasomotor reactivity. Chick embryos

exposed to thallium developed achondroplasia; these data further support the potential role of thallium in the disruption of bone development.

There are no studies available to determine the carcinogenic potential of thallium in animals and no adequately conducted studies in humans. The limited number of studies of the genotoxicity of thallium compounds provides inconsistent evidence of genotoxic potential. Under EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), the Agency concluded that there is "inadequate information to assess the carcinogenic potential."

6.2. DOSE RESPONSE

The 90-day oral toxicity study of thallium (I) sulfate in Sprague-Dawley rats (MRI, 1988) was selected as a potential principal study. PODs for two alternative endpoints from the MRI (1988) study were selected for dose-response analysis – (1) hair follicle atrophy in female rats with alopecia, and (2) clinical observations, including those related to animal coat (rough coat, piloerection, shedding, and alopecia), eyes (including lacrimation, exophthalmos, and miosis), and behavior. Using hair follicle atrophy, the high dose (0.2 mg/kg-day Tl) was identified as a LOAEL; the mid dose (0.04 mg/kg-day Tl) was considered a NOAEL and was used as a candidate POD. For clinical observations, an average $BMDL_{10}$ of 0.01 mg/kg-day Tl was derived using BMD modeling methods.

A total uncertainty factor of 3,000 (10 for interspecies extrapolation, 10 for intraspecies extrapolation, 3 for extrapolation from a subchronic to chronic study, and 10 for database deficiencies) was applied to the PODs to yield candidate RfD values for thallium in the form of soluble thallium salts of 1×10^{-5} mg/kg-day Tl (for hair follicle atrophy) or 3×10^{-6} mg/kg-day Tl (for clinical observations). The available toxicity database for thallium contains studies that are generally of poor quality. The MRI (1988) study that was selected as a candidate principal study suffers from certain critical limitations (e.g., high background incidence of alopecia, lack of histopathological examination of skin tissue in low- and mid-dose groups, and inadequate examination of objective measures of neurotoxicity), and there are particular difficulties in the selection of appropriate endpoints. Therefore, even though an RfD would generally be derived with a combined uncertainty factor of 3000, an RfD for soluble thallium salts is not derived in this specific case.

The available data are not adequate to derive an RfD for thallium (III) oxide or thallium (I) selenite. Inhalation toxicity studies are not available to support derivation of RfCs for any thallium compounds.

7. REFERENCES

Ali, SF; Jairaj, K; Newport, GD; et al. (1990) Thallium intoxication produces neurochemical alterations in rat brain. Neurotoxicology 11:381–390.

Ammendola, A; Ammendola, E; Argenzio, F; et al. (2007) Clinical and electrodiagnostic follow-up of an adolescent poisoned with thallium. Neurol Sci 28:205–208.

Andre, T; Ullberg, S; Winqvist, G. (1960) The accumulation and retention of thallium in tissues of the mouse. Acta Pharmacol Toxicol 16:229–234. (As cited in CalEPA, 1999).

Appenroth, D; Gambaryan, S; Winnefeld, K; et al. (1995) Functional and morphological aspects of thallium-induced nephrotoxicity in rats. Toxicology 96(3):203–215.

Appenroth, D; Tiller, S; Gambaryan, S; et al. (1996) Ontogenetic aspects of thallium-induced nephrotoxicity in rats. J Appl Toxicol 16(3):235–243.

Aoyama, H. (1989) Distribution and excretion of thallium after oral and intraperitoneal administration of thallous malonate and thallous sulfate in hamsters. Bull Environ Contam Toxicol 42:456–463.

Arbiser, JL; Alani, R; Flynn, E; et al. (1997) Effects of thallium ion on cellular components of the skin. J Dermatol 24:147–155.

ATSDR (Agency for Toxic Substance and Disease Registry). (1992) Toxicological profile for thallium. Public Health Service, U.S. Department of Health and Human Services, Atlanta, GA. Available online at http://www.atsdr.cdc.gov/toxpro2.html.

Atsmon, J; Taliansky, E; Landau, M; et al. (2000) Thallium poisoning in Israel. Am J Med Sci 320(5):327–330.

Barclay, RK; Peacock, WC; Karnofsky, DA. (1953) Distribution and excretion of radioactive thallium in the chick embryo, rat, and man. J Pharmacol Exp Ther 107:178–187. (As cited in CalEPA, 1999).

Barrera, H; Gómez-Puyou, A. (1975) Characteristics of the movement of K^+ across the mitochondrial membrane and the inhibitory action of Tl^+. J Biol Chem 250(14):5370–5374.

Barroso-Moguel, R; Ríos-Castañeda, C; Villeda-Hernández, J; et al. (1990) Neurotoxicity of thallium biochemical and morphological study of organic lesions. Arch Invest Med 21:115–122.

Barroso-Moguel, R; Villeda-Hernández, J; Méndez-Armenta, M; et al. (1992) Osteochrondric lesions in developing rats intoxicated with thallium twenty-four hours after birth. Arch Med Res 23(3):129–133.

Barroso-Moguel, R; Méndez-Armenta, M; Villeda-Hernández, J; et al. (1996) Experimental neuromyopathy induced by thallium in rats. J Appl Toxicol 16(5):385–389.

Britten, JS; Blank, M. (1968) Thallium activation of (Na^+-K^+)-activated ATPase of rabbit kidney. Biochim Biophys Acta 159:160–166.

Brockhaus, A; Dolgner, R; Ewers, U; et al. (1981) Intake and health effects of thallium among a population living in the vicinity of a cement plant emitting thallium containing dust. Int Arch Occup Environ Health 48:375–389.

Brown, DR; Callahan, BG; Cleaves, MA; et al. (1985) Thallium-induced changes in behavioral patterns: correlation with altered lipid peroxidation and lysosomal enzyme activity in brain regions of male rats. Toxicol Ind Health 1(1):81–98.

Bugarin, MG; Casa, JS; Sordo, J; et al. (1989) Thallium (I) interactions in biological fluids: a potentiometric investigation of thallium (I) complex equilibria with some sulphur-containing amino acids. J Inorg Biochem 35:95–105.

Burnham, KP; Anderson, DR. (2002) Model selection and multimodel interference: a practical information-theoretic approach. 2nd edition. New York, NY: Springer-Verlag.

Careaga-Olivares, J; Gonzalez-Ramirez, D. (1995) Penicillamine produces changes in the acute blood elimination and tissue accumulation of thallium. Arch Med Res 26(4):427–430.

Cavanagh, JB. (1991) What have we learnt from Graham Frederick Young? Reflections on the mechanism of thallium neurotoxicity. Neuropathol Appl Neurobiol 17:3–9.

Cavanagh, JB; Fuller, NH; Johnson, HRM; et al. (1974) The effects of thallium salts, with particular reference to the nervous system changes. Q J Med 43:293–319.

Cavieres, JD; Ellory, JC. (1974) Thallium and the sodium pump in human red cells. J Physiol 243:243–266.

CalEPA (California Environmental Protection Agency) (1999) Public health goal for thallium in drinking water. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, Sacramento, CA. Available online at http://oehha.ca.gov/water/phg/pdf/thal_f.pdf.

CDC (Centers for Disease Control and Prevention). (2005) Third national report on human exposure to environmental chemicals. National Center for Environmental Health, Public Health Service, Department of Health and Human Services, Atlanta, GA; NCEH Pub. No. 05-0570. Available online at http://www.cdc.gov/ExposureReport/pdf/thirdreport.pdf.

Davis, LE; Standefer, JC; Kornfeld, M; et al. (1981) Acute thallium poisoning: toxicological and morphological studies of the nervous system. Ann Neurol 10:38–44.

Derelanko, MJ; Hollinger, MA; eds. (1995) CRC handbook of toxicology. Boca Raton, FL: CRC Press.

Diaz, RS; Monreal, J. (1994) Thallium mediates a rapid chloride/hydroxyl ion exchange through myelin lipid bilayers. Mol Pharmacol 46:1210–1216.

Dolgner, R; Brockhaus, A; Ewers, U; et al. (1983) Repeated surveillance of exposure to thallium in a population living in the vicinity of a cement plant emitting dust containing thallium. Int Arch Occup Environ Health 52:79–94.

Downs, AJ, ed. (1993) Chemistry of aluminium, gallium, indium, and thallium. London, UK: Blackie Academic & Professional.

Downs, WL; Scott, JK; Steadman, LT; et al. (1960) Acute and sub-acute toxicity studies of thallium compounds. Industr Hygiene J 21:399–406.

Elsenhans, B; Schumann, K; Forth, W. (1991) Toxic metals: interactions with essential metals. In: Rowland, IR; ed. Nutrition, toxicity, and cancer. Boca Raton: CRC Press; pp. 223–258.

El-Garawany, AA; Samaan, HA; Sadek, M. (1990) Comparative hepatorenal toxicity of some commonly used chemical environmental pollutants. Egypt J Pharm Sci 31(1–4):331–336.

Feldman, J; Levisohn, DR. (1993) Acute alopecia: clue to thallium toxicity. Pediatr Dermatol 10(1):29–31.

Fleck, C; Appenroth, D. (1996) Renal amino acid transport in immature and adult rats during thallium-induced nephrotoxicity. Toxicology 106:229–236.

Formigli, L; Scelsi, R; Poggi, P; et al. (1986) Thallium-induced testicular toxicity in the rat. Environ Res 40:531–539.

Forth, W; Rummel, W. (1975) Gastrointestinal absorption of heavy metals. In: Forth, W; Rummel, W; Aguiar, AJ; eds. International encyclopedia of pharmacology and therapeutics: Section 39B. Pharmacology of intestinal absorption: gastrointestinal absorption of drugs. Vol. II. Oxford, New York, NY: Pergamon Press; p. 647.

Galván-Arzate, S; Rios, C. (1994) Thallium distribution in organs and brain regions of developing rats. Toxicology 90:63–69.

Galván-Arzate, S; Santamaría, A. (1998) Thallium toxicity. Toxicol Lett 99:1–13.

Galván-Arzate, S; Martínez, A; Medina, E; et al. (2000) Subchronic administration of sublethal doses of thallium to rats: effects on distribution and lipid peroxidation in brain regions. Toxicol Lett 116:37–43.

Galván-Arzate, S; Pedraza-Chaverri, J; Medina-Campos, ON; et al. (2005) Delayed effects of thallium in the rat brain: regional changes in lipid peroxidation and behavioral markers, but moderate alterations in antioxidants, after a single administration. Food Chem Toxicol 43:1037–1045.

Garrett, NE; Lewtas, J. (1983) Cellular toxicity in Chinese hamster ovary cell cultures. I. Analysis of cytotoxicity endpoints for twenty-nine priority pollutants. Environ Res 32:455–465.

Gefel, A; Liron, M; Hirsch, W. (1970) Chronic thallium poisoning. Israel J Med Sci 6:380–382.

Gibson, JE; Becker, BA. (1970) Placental transfer, embryotoxicity, and teratogenicity of thallium sulfate in normal and potassium-deficient rats. Toxicol Appl Pharmacol 16:120–132.

Gosselin, RE; Smith, RP; Hodge, HC. (1984) Clinical toxicology of commercial products. 5th edition. Baltimore, MD: Williams and Wilkins; pp. III-379–III-383. (As cited in CalEPA, 1999).

Greer, JM; Capecchi, MR. (2002) Hoxb8 is required for normal grooming behavior in mice. Neuron 33:23–34.

Gregotti, C; Di Nucci, A; Formigli, L; et al. (1985) Altered testicular enzyme patterns in rats after long-term exposure to thallium sulphate. J Toxicol Clin Exp 5(4):265–271.

Gregotti, C; Di Nucci, A; Costa, LG; et al. (1992) Effects of thallium on primary cultures of testicular cells. J Toxicol Environ Health 36:59–69.

Hall, BK. (1972) Thallium-induced achondroplasia in the embryonic chick. Dev Biol 28:47–60.

Hall, BK. (1985) Critical periods during development as assessed by thallium-induced inhibition of growth of embryonic chick tibiae in vitro. Teratology 31:353–361.

Hantson, P; Desoir, R; Leonard, ED; et al. (1997) Cytogenetic observations following thallium poisoning. J Toxicol Environ Health 50:97–100.

Hanzel, CE; Verstraeten, SV. (2006) Thallium induces hydrogen peroxide generation by impairing mitochondrial function. Toxicol Appl Pharmacol 216:485–492.

Hanzel, CE; Verstraeten, SV. (2009) Tl(I) and Tl(III) activate both mitochondrial and extrinsic pathways of apoptosis in rat pheochromocytoma (PC12) cells. Toxicol Appl Pharmacol 236:59-70.

Hanzel, CE; Villarverde, MS; Verstraeten, SV. (2005) Glutathione metabolism is impaired in vitro by thallium(III) hydroxide. Toxicol 207:501–510.

Hasan, M; Ali, SF. (1981) Effects of thallium, nickel and cobalt administration on the lipid peroxidation in different regions of the rat brain. Toxicol Appl Pharmacol 57:8–13.

Hasan, M; Haider, SS. (1989) Acetyl-homocysteine thiolactone protects against some neurotoxic effects of thallium. Neurotoxicology 10:257–262.

Hasan, M; Chandra, SV; Bajpai, VK; et al. (1977) Electron microscopic effects of thallium poisoning on the rat hypothalamus and hippocampus: biochemical changes in the cerebrum. Brain Res Bull 2:255–261.

Hasan, M; Ali, SF; Tariq, M. (1978) Levels of dopamine, norepinephrine and 5-hydroxytryptamine in different regions of the rat brain in thallium toxicosis. Acta Pharmacol Toxicol 43:169–173.

Herman, MM; Bensch, KG. (1967) Light and electron microscopic studies of acute and chronic thallium intoxication in rats. Toxicol Appl Pharmacol 10:199–222.

Heyl, T; Barlow, RJ. (1989) Thallium poisoning: a dermatological perspective. Br J Dermatol 121:787–791.

Hirata, M; Taoda, M; Ono-Ogasawara, M; et al. (1998) A probable case of chronic occupational thallium poisoning in a glass factory. Ind Health 36:300–303.

Hoffman, RS. (2000) Thallium poisoning during pregnancy: a case report and comprehensive literature review. Clin Toxicol 38(7):767–775.

Hughes, MN; Man, WK; Whaler, BC. (1978) The toxicity of thallium (I) to cardiac and skeletal muscle. Chem Biol Interact 23:85–97.

Ibrahim, D; Frobert, B; Wolf, A; et al. (2006) Heavy metal poisoning: clinical presentations and pathophysiology. Clin Lab Med 26:67–97.

IPCS (International Programme on Chemical Safety). (1996) Thallium. Environmental health criteria. Vol. 182. World Health Organization, Geneva, Switzerland. Available online at http://www.inchem.org/ documents/ehc/ehc/ehc182.htm.

Kada, T; Hirano, K; Shirasu, Y. (1980) Screening of environmental chemical mutagens by the Rec-assay system with *Bacillus subtilis*. In: de Serres, FJ; Hollaender, A; eds. Chemical mutagens: principles and methods for their detection. New York, NY: Plenum Press; pp. 149–173.

Kalueff, AV; Tuohimaa, P. (2005) Contrasting grooming phenotypes in three mouse strains markedly different in anxiety and activity (129S1, BALB/c and NMRI). Behav Brain Res 160:1–10.

Kalueff, AV; Minasyan, A; Keisala, T; et al. (2006) Hair barbering in mice: implications for neurobehavioral research. Behav Proc 71:8–15.

Kanematsu, N; Hara, M; Kada, T. (1980) REC assay and mutagenicity studies on metal compounds. Mutat Res 77:109–116.

Karnofsky, DA; Ridgway, LP; Patterson, PA. (1950) Production of achondroplasia in the chick embryo with thallium. Proc Soc Exp Biol Med 73:255–259.

Kazantzis, G. (2007) Thallium. In: Nordberg, GF; Fowler, BA; Nordberg, M; et al.; eds. Handbook on the toxicology of metals. New York, NY: Elsevier; pp. 827–837.

Kennedy, P, Cavanagh, JB. (1977) Sensory neuropathy produced in the cat with thallous acetate. Acta Neuropathol (Berl) 39:81–88.

Kuo, HC; Huang, CC; Tsai, YT; et al. (2005) Acute painful neuropathy in thallium poisoning. Neurology 65:302–304.

Kuperberg, JM; Ngong, JM; Rutledge, LP; et al. (1998) Central and peripheral alteration of the cholinergic enzyme activities of the rat in response to repeated and acute thallium exposure. Toxic Subst Mech 17:285–298.

Lameijer, W; van Zwieten, PA. (1976) Acute cardiovascular toxicity of thallium (I) ions. Arch Toxicol 35:49–61.

Lameijer, W; van Zwieten, PA. (1977) Kinetic behavior of thallium in the rat: accelerated elimination of thallium owing to treatment with potent diuretic agents. Arch Toxicol 37:265–273.

Lehmann, FPA; Favari, L. (1985) Acute thallium intoxication: kinetic study of the relative efficacy of several antidotal treatments in rats. Arch Toxicol 57:56–60. (As cited in CalEPA, 1999).

Leloux, M-S; Lich, NP; Claude, J-R. (1987) Experimental studies on thallium toxicity in rats. I. Localization and elimination of thallium after oral acute and sub-acute intoxication. J Toxicol Clin Exp 7(4):247–257.

Léonard, A; Gerber, GB. (1997) Mutagenicity, carcinogenicity and teratogenicity of thallium compounds. Mutation Research 387:47–53.

Leopold, G; Furukawa, E; Forth, W; et al. (1968) [Comparative studies of the absorption of heavy metals in vivo and in vitro]. Naunyn Schmiedebergs Arch Pharmacol 263(1):275–276. (As cited in Elsenhans et al., 1991).

Leung, KM; Ooi, VEC. (2000) Studies on thallium toxicity, its tissue distribution and histopathological effects in rats. Chemosphere 41:155–159.

Lie, R; Thomas, RG; Scott, JK. (1960) The distribution and excretion of thallium-204 in the rat, with suggested MPCs and a bio-assay procedure. Health Phys 2:334–340. (As cited in U.S. EPA, 1991b).

Limos, CL; Ohnishi, A; Suzuki , N; et al. (1982) Axonal degeneration and focal muscle fiber necrosis in human thallotoxicosis: histopathological studies of nerve and muscle. Muscle Nerve 5:598–706.

Lohmann, H; Wiegand, H. (1996) Reduced probability of orthodromically evoked action potential firing in CA1 pyramidal cells of guinea pig hippocampal slices after acute thallium exposure. Arch Toxicol 70:430–439.

Lohmann, H; Csicsaky, M; Wiegand, H. (1989) The action of thallium on the excitability of CA1 pyramidal cells in hippocampal slices. Neurotoxicol Teratol 11:545–549.

Loveless, LE; Spoerl, E; Weisman, TH. (1954) A survey of effects of chemicals on division and growth of yeast and *Escherichia coli*. J Bacteriol 68:637–644.

Lu, CI; Huang, CC; Chang, YC; et al. (2007) Short-term thallium intoxication. Arch Dermatol 143:93–98.

Ludolph, A; Elger, CE; Sennhenn, R; et al. (1986) Chronic thallium exposure in cement plant workers: clinical and electrophysiological data. Trace Elem Med 3:121–125.

Lund, A. (1956) Distribution of thallium in the organism and its elimination. Acta Pharmacol Et Toxicol 12:251–259.

Manzo, L; Scelsi, R; Moglia, A; et al. (1983) Long-term toxicity of thallium in the rat. In: Brown, SS; Savory, J; eds. Chemical toxicology and clinical chemistry of metals. London: London Academy Press; pp. 401–405.

Marcus, RL. (1985) Investigation of a working population exposed to thallium. J Soc Occup Med 35:4–9.

Matsuzawa, T; Nomura, M; Unno, T. (1993) Clinical pathology reference ranges of laboratory animals. J Vet Med Sci 55:351–362.

Mourelle, M; Favari, L; Amezcua, JL. (1988) Protection against thallium hepatotoxicity by silymarin. J Appl Toxicol 8(5):351–354.

MRI (Midwest Research Institute). (1988) Toxicity of thallium (I) sulfate (CAS No. 7446-18-6) in Sprague-Dawley rats. Vol. 2. Subchronic (90-day) study [revised final report]. Prepared by Dynamac Corporation, Rockville, MD, for the Office of Solid Waste, Washington, DC; Project No. 8702-L(18); Work Assignment No. 111148-008. [An external peer review was conducted by EPA in November 2006 to evaluate the accuracy of experimental procedures, results, and interpretation and discussion of the findings presented. A report of this peer review is available through the EPA's IRIS Hotline, at (202) 566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (e-mail address) and at www.epa.gov/iris.]

Mulkey, JP; Oehme, FW. (1993) A review of thallium toxicity. Vet Human Toxicol 35(5):445–454.

Munch, JC. (1934) Human thallotoxicosis. J Am Med Assoc 102:1929–1934.

Navas-Acien, A; Silbergeld, EK; Sharrett, AR; et al. (2005) Metals in urine and peripheral arterial disease. Environ Health Perspect 113:164–169.

NLM (National Library of Medicine). (1998) Thallium. HSDB (Hazardous Substances Data Bank). National Institutes of Health, U.S. Department of Health and Human Services, Bethesda, MD. Available online at http://toxnet.nlm.nih.gov.

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

Oh, HS; Smart, RC. (1996) An estrogen receptor pathway regulates the telogen-anagen hair follicle transition and influences epidermal cell proliferation. Proc Natl Acad Sci USA 93(22):12525–12530.

Osorio-Rico, L; Galván-Arzate, S; Ríos, C. (1995) Thallium increases monoamine oxidase activity and serotonin turnover rate in rat brain regions. Neurotoxicol Teratol 17(1):1–5.

Pearson, RG. (1963) Hard and soft acids and bases. J Amer Chem Soc 85:3533–3539.

Petterino, C; Argentino-Storino, A. (2006) Clinical chemistry and haematology historical data in control Sprague-Dawley rats from pre-clinical toxicity studies. Exp Toxicol Pathol 57:213–219.

Reed, D; Crawley, J; Faro, SN; et al. (1963) Thallotoxicosis. JAMA 183(7):516–522.

Rios, C; Galván-Arzate, S; Tapia, R. (1989) Brain regional thallium distribution in rats acutely intoxicated with Tl_2SO_4. Arch Toxicol 63:34–37.

Roby, DS; Fein, AM; Bennet, RH; et al. (1984) Cardiopulmonary effects of acute thallium poisoning. Chest 85:236–240.

Rossi, F; Marrazzo, R; Berrino, L; et al. (1988) Prenatal and postnatal thallium exposure in rats: effect on development of vasomotor reactivity in pups. Teratog Carcinog Mutagen 8:13–23.

Rusyniak, DE; Furbee, RB; Kirk, MA. (2002) Thallium and arsenic poisoning in a small Midwestern town. Ann Emerg Med 39(3):307–311.

Sabbioni, E; Goetz, L; Marafante, E. (1980a) Metabolic fate of different inorganic and organic species of thallium in the rat. Sci Total Environ 15:123–135.

Sabbioni, E; Marafante, E; Rade, J; et al. (1980b) Metabolic patterns of low and toxic doses of thallium in the rat. Dev Toxicol Environ Sci 8:559–564.

Saddique, A; Peterson, CD. (1983) Thallium poisoning: a review. Vet Hum Toxicol 25:16–22.

Saha, A; Sadhu, HG; Karnik, AB; et al. (2004) Erosion of nails following thallium poisoning: a case report. Occup Environ Med 61:640–642.

Schaller, KH; Manke, G; Raithel, HJ; et al. (1980) Investigation of thallium-exposed workers in cement factories. Int Arch Occup Environ Health 47:223–231.

Schoer, J. (1984) Thallium. In: Hutzinger, O; ed. The handbook of environmental chemistry. Vol. 3. Anthropogenic compounds. Part C. Berlin: Springer-Verlag; pp. 143–214. (As cited in CalEPA, 1999).

Schwartzman, RM; Kirschbaum, JO. (1961) The cutaneous histopathology of thallium poisoning. J Invest Dermatol 39:169–173.

Sharma, AN; Nelson, LS; Hoffman, RS. (2004) Cerebrospinal fluid analysis in fatal thallium poisoning: evidence for delayed distribution into the central nervous system. Am J Forensic Med Pathol 25:156–158.

Shaw, PA. (1933) Toxicity and deposition of thallium in certain game birds. J Pharmacol Exp Ther 48:478–487. (As cited in U.S. EPA, 1991b)

Singh, I. (1983) Induction of reverse mutation and mitotic gene conversion by some metal compounds in *Saccharomyces cerevisiae*. Mutat Res 117:149–152.

Talas, A; Wellhöner, HH. (1983) Dose-dependency of Tl kinetics as studied in rabbits. Arch Toxicol 53:9–16.

Talas, A; Pretschner, DP; Wellhöner, HH. (1983) Pharmacokinetic parameters for thallium (I) ions in man. Arch Toxicol 53:1–7.

Tao, Z; Gameiro, A; Grewer, C. (2008) Thallium ions can replace both sodium and potassium ions in the glutamate transporter excitatory amino acid carrier I. Biochemistry 47:12923-30.

Thomas, ML; McKeever, PJ. (1993) Chronic thallium toxicosis in a dog. J Am Anim Hosp Assoc 29:211–215.

Tsai, Y-T; Huang, C-C; Kuo, H-C; et al. (2006) Central nervous system effects in acute thallium poisoning. Neurotoxicology 27:291–295.

U.S. EPA (Environmental Protection Agency). (1986a) Guidelines for the health risk assessment of chemical mixtures. Federal Register 51(185):34014–34025. Available online at http://www.epa.gov/ncea/raf/rafguid.htm.

U.S. EPA (1986b) Guidelines for mutagenicity risk assessment. Federal Register 51(185):34006–34012. Available online at http://www.epa.gov/ncea/raf/rafguid.htm.

U.S. EPA (1986c) Subchronic (90-day) toxicity of thallium (I) sulfate in Sprague-Dawley rats. Prepared by the Midwest Research Institute, Kansas City, MO for the Office of Solid Waste, Washington, DC.

U.S. EPA (1988) Recommendations for and documentation of biological values for use in risk assessment. Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment, Cincinnati, OH; EPA/600/6-87/008. Available from the National Technical Information Service, Springfield, VA, PB88-179874/AS, and online at http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=34855.

U.S. EPA (1991a) Guidelines for developmental toxicity risk assessment. Federal Register 56(234):63798–63826. Available online at http://www.epa.gov/ncea/raf/rafguid.htm.

U.S. EPA (1991b) Drinking water health advisory for thallium. Office of Water, Washington, DC. Available from the National Technical Information Service, Springfield, VA; PB92-135524.

U.S. EPA (1994a) Interim policy for particle size and limit concentration issues in inhalation toxicity: notice of availability. Federal Register 59(206):53799. Available online at http://www.epa.gov/EPA-PEST/1994/October/Day-26/pr-11.html.

U.S. EPA (1994b) Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry. Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment, Cincinnati, OH; EPA/600/8-90/066F. Available from the National Technical Information Service, Springfield, VA, PB2000-500023, and online at http://cfpub.epa.gov/ncea/raf/recordisplay.cfm?deid=71993.

U.S. EPA (1995) Use of the benchmark dose approach in health risk assessment. Risk Assessment Forum, Washington, DC; EPA/630/R-94/007. Available from the National Technical Information Service, Springfield, VA, PB95-213765, and online at http://cfpub.epa.gov/ncea/raf/raf_pubtitles.cfm?detype=document&excCol=archive.

U.S. EPA (1996) Guidelines for reproductive toxicity risk assessment. Federal Register 61(212):56274–56322. Available online at http://www.epa.gov/ncea/raf/rafguid.htm.

U.S. EPA (1997) Exposure factors handbook. National Center for Environmental Assessment, Office of Research and Development, Washington, DC; EPA/600-P-95-002Fa,b,c. Available online at http://www.epa.gov/ncea/pdfs/efh/front.pdf.

U.S. EPA (1998) Guidelines for neurotoxicity risk assessment. Federal Register 63(93):26926–26954. Available online at http://www.epa.gov/ncea/raf/rafguid.htm.

U.S. EPA (2000a) Science policy council handbook: risk characterization. Office of Science Policy, Office of Research and Development, Washington, DC; EPA/100-B-00-002. Available online at http://www.epa.gov/OSA/spc/pdfs/prhandbk.pdf.

U.S. EPA (2000b) Benchmark dose technical guidance document [external review draft]. Risk Assessment Forum, Washington, DC; EPA/630/R-00/001. Available online at http://cfpub.epa.gov/ncea/cfm/nceapublication.cfm?ActType=PublicationTopics&detype=DOCUMENT&subject=BENCHMARK+DOSE&subjtype=TITLE&excCol=Archive.

U.S. EPA (2000c) Supplementary guidance for conducting health risk assessment of chemical mixtures. Risk Assessment Forum, Washington, DC; EPA/630/R-00/002. Available online at http://cfpub.epa.gov/ncea/raf/chem_mix.cfm.

U.S. EPA (2002) A review of the reference dose concentration and reference concentration processess. Risk Assessment Forum, Washington, DC; EPA/630/P-02/002F. Available online at http://cfpub.epa.gov/ncea/raf/raf_pubtitles.cfm?detype=document&excCol=archive.

U.S. EPA (2005a) Guidelines for carcinogen risk assessment. Federal Register 70(66):17765–18717. Available online at http://www.epa.gov/cancerguidelines.

U.S. EPA (2005b) Supplemental guidance for assessing susceptibility from early-life exposure to carcinogens. Risk Assessment Forum, Washington, DC; EPA/630/R-03/003F. Available online at http://www.epa.gov/cancerguidelines.

U.S. EPA (2006a) Science policy council handbook: peer review. 3rd edition. Office of Science Policy, Office of Research and Development, Washington, DC; EPA/100/B-06/002. Available online at http://www.epa.gov/OSA/spc/2peerrev.htm.

U.S. EPA (2006b) A framework for assessing health risk of environmental exposures to children. National Center for Environmental Assessment, Washington, DC; EPA/600/R-05/093F. Available online at http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=158363.

U.S. EPA (2007) Benchmark dose software (BMDS) version 1.4.1. (last modified February 2007). Available online at http://www.epa.gov/ncea/bmds/index.html.

Valentin, J; ed. (2003) Basic anatomical and physiological data for use in radiological protection: reference values. ICRP publication 89. Oxford; New York, NY: Pergamon Press.

Verstraeten, SV. (2006) Relationship between thallium(I)-mediated plasma membrane fluidification and cell oxidants production in Jurkat T cells. Toxicology 222(1–2):95–102.

Waters, CB; Hawkins, EC; Knapp, DW. (1992) Acute thallium toxicosis in a dog. JAMA 201(6):883–885.

Wei, Q. (1987) Studies on spermotoxicity of thallium carbonate in drinking water and its effect on reproductive function of mice. Zhonghua Yu Fang Yi Xue Za Zhi 21(3):141–143.

Welch, JM; Lu, J; Rodriguiz, RM; et al. (2007) Cortico-striatal synaptic defects and OCD-like behaviours in Sapap3-mutant mice. Nature 448:894–900.

Wiegand, H; Papadopoulos, R; Csicsaky, M; et al. (1984) The action of thallium acetate on spontaneous transmitter release in the rat neuromuscular junction. Arch Toxicol 55:253–257.

Wiegand, H; Uhlig, S; Gotzsch, U; et al. (1990) The action of cobalt, cadmium and thallium on presynaptic currents in mouse motor nerve endings. Neurotoxicol Teratol 12:313–318.

Windebank, AJ. (1986) Specific inhibition of myelination of lead in vitro; comparison with arsenic, thallium and mercury. Exper Neurol 94:203–212.

Woods, JS; Fowler, BA. (1986) Alteration of hepatocellular structure and function by thallium chloride: Ultrastructural, morphometric, and biochemical studies. Toxicol Appl Pharmacol 83:218–229.

Yokoyama, K; Araki, S; Abe, H. (1990) Distribution of nerve conduction velocities in acute thallium poisoning. Muscle Nerve 13:117–120.

Yoshida, M; Igeta, S; Kawashima, R; et al. (1997) Changes in adenosine triphosphate (ATP) concentration and its activity in murine tissues after thallium administration. Bull Environ Contam Toxicol 59(2):268–273.

Zasukhina, GD; Vasilyeva, IM; Sdirkova, NI; et al. (1983) Mutagenic effect of thallium and mercury salts on rodent cells with different repair activities. Mutat Res 124(2):163–173.

APPENDIX A

Summary of External Peer Review and Public Comments and Disposition

The *Toxicological Review of Thallium and Compounds* (U.S. EPA, 2009) has undergone a formal external peer review performed by scientists in accordance with EPA guidance on peer review (U.S. EPA, 2006a, 2000a). The external peer reviewers were tasked with providing written answers to general questions on the overall assessment and on chemical-specific questions in areas of scientific controversy or uncertainty. A summary of significant comments made by the external reviewers and EPA's responses to these comments arranged by charge question follow. In many cases the comments of the individual reviewers have been synthesized and paraphrased in development of Appendix A. EPA also received scientific comments from the public. These comments and EPA's responses are included in a separate section of this appendix.

EXTERNAL PEER REVIEW PANEL COMMENTS

EPA revised the *Toxicological Review* to present dose-response analyses for two data sets from MRI (1988), hair follicle atrophy and clinical observations, and derived candidate RfDs taking the peer reviewers recommendations into account. However, as pointed out by the peer reviewers, the MRI (1988) study that was selected as a principal study suffers from certain critical limitations (e.g., high background incidence of alopecia, lack of histopathological examination of skin tissue in low- and mid-dose groups, and inadequate examination of objective measures of neurotoxicity), and there are particular difficulties in the selection of appropriate endpoints. For these reasons, an RfD for soluble thallium salts was not derived in this specific case. Text explaining the basis for EPA's decision was added to Section 5.1.3.1. This decision is considered consistent with the peer reviewers observations that the toxicity database for thallium is weak. EPA has provided responses to the reviewers' comments in detail below, because the derivations of the candidate RfDs were retained in the assessment.

The reviewers made several editorial suggestions to clarify specific portions of the text. These changes were incorporated in the document as appropriate and are not discussed further.

I. General Comments

1. Is the *Toxicological Review* logical, clear, and concise? Has EPA accurately, clearly, and objectively represented and synthesized the scientific evidence for noncancer and cancer hazard?

Comments: Four reviewers considered the *Toxicological Review* to be clearly written and/or to present a critical evaluation of the published literature. One of these reviewers stated that despite

considerable shortcomings in the overall database for thallium (which are acknowledged in the report), the authors of the review did a good job at synthesizing the available information and presenting it in a logical fashion. One reviewer observed that the synthesis was somewhat less than ideal in that it was interjected with editorial comments (some from original authors) and that it might have been more desirable to present the facts and leave the interpretation to the reader. This reviewer also believed that the *Toxicological Review* did not provide a sufficient review of the foreign language material or of environmental surveys. One reviewer stated that the reasons for EPA's reevaluation of the data used as the basis for the RfD currently on IRIS and the different conclusion regarding the NOAEL and LOAEL from the MRI (1988) study were not fully explained. This reviewer did not consider the comparison of the LOAEL in the MRI study with LOAELs from other studies that were three- to ninefold higher to be a "logical argument."

One reviewer noted that EPA's case for thallium-induced alopecia could have been strengthened if the *Toxicological Review* had included a discussion of the hair growth cycle in rats and how a clinical condition such as alopecia can be distinguishable from hair loss associated with normal growth cycle in rats. One reviewer stated that the MRI (1988) study could have been better described and evaluated. Two reviewers criticized the *Toxicological Review* for rejection of studies (including Zasukhina et al. [1983] and Wei [1987]) that may have provided useful information for reasons that were inadequate. One reviewer noted that the title *Toxicological Review of Thallium and Compounds* was a bit unclear and confusing and suggested as potential alternatives: *Toxicological Review of Thallium and Its Salts* or *Toxicological Review of Thallium and Thallium Compounds*.

Other comments were provided in response to this general charge question that were repeated in response to other more specific charge questions. In these cases, the peer reviewer comments (and EPA's responses) were summarized under the more specific charge question.

Response: The general structure of a toxicological review is to present a factual summary of toxicity studies in Section 4 and critical interpretation/synthesis in Section 5. The study authors' conclusions were presented in Sections 4 and 5 only where necessary (e.g., where it was necessary to document that EPA's interpretation of the study findings differed from the authors'.) The comment regarding insufficient consideration of the foreign language literature is addressed under general charge question 2.

The health effects of thallium exposure were reevaluated in light of the availability of new data and the need for an up-to-date assessment for regulatory purposes. The comment related to determination of the NOAEL and LOAEL from MRI (1988) is considered further under charge question A3. Comparison to LOAELs from other studies was removed.

Alopecia was one of a number of potential critical effects considered for dose-response analysis. The POD derived from alopecia using BMD modeling was consistent with the PODs derived from other related clinical effects. EPA did not consider a discussion of the hair growth

cycle in rats and how alopecia can be distinguished from normal cyclical hair loss to be necessary.

The *Toxicological Review* was revised to include an expanded discussion of the findings from MRI (1988) (see Section 4.2.1.1).

The studies by Zasukhina et al. (1983) and Wei (1987) were critically reevaluated. EPA again reached the conclusion that the Zasukhina et al. (1983) study suffered from critical deficiencies and did not provide a scientifically defensible basis for the oral RfD. More thorough justification for this determination was added to Section 4.3. The discussion of the Wei (1987) study was revised (Section 4.3 and entry in Table 4-5). In light of the uncertainties associated with incomplete reporting and reporting inconsistencies and lack of confirmation of these low-exposure findings on sperm, the Wei (1987) study was not used as the principal study (see discussions in Sections 4.6.1 and 5.1.1). Nevertheless, the study was included in Section 5.1.5, Candidate RfD Comparison Information. See also the response to charge question A.1 for additional response to this comment related to consideration of other studies.

The title, *Toxicological Review of Thallium and Compounds*, was retained for consistency with other similar assessments on the IRIS database.

2. Please identify any additional studies that should be considered in the assessment of the noncancer and cancer health effects of thallium and thallium compounds.

Comments: Three reviewers did not identify any additional studies that should be included in the assessment. One of these reviewers noted that a rapid Medline search identified several articles dealing with thallium toxicity that were not cited in the *Toxicological Review*; however, this reviewer did not review the studies to determine whether they provided important new additional information. Other reviewers identified the following thallium studies that were not cited in the *Toxicological Review*:

- Cavanagh, JB. (1988) Book chapter in Recent Advances in Nervous System Toxicology, Vol. 100, pp. 177–202 Galli, GL; Manzo, L; Spencer, PS, eds. New York: Plenum Press.
- Kazantzis, G. (2007) Thallium. In: Nordberg, GF; Fowler, BA; Nordberg, M; et al.; eds. Handbook on the toxicology of metals. New York, NY: Elsevier; pp. 827–837.
- Granero, S; Domingo, JL. (2002) Levels of metals in soils of Alcalá de Henares, Spain: human health risks. Environ Int 28(3):159–164.
- Kennedy, P; Cavanagh, JB. (1977) Sensory neuropathy produced in the cat with thallous acetate. Acta Neuropathol 39:81–88.
- Windebank, AJ. (1986) Specific inhibition of myelination of lead in vitro; comparison with arsenic, thallium and mercury. Exp Neurol 94:203–212.

One of the reviewers noted that the foreign literature may be very significant. This reviewer identified the following papers and suggested these and other foreign language literature be translated and included in the review:

- Kamil'dzhanov, AKh. (1993) [Experimental substantiation of maximum permissible concentration of thallium carbonate in environmental air]. Gig Sanit 5:8–10.

- Gerasimova, IL. (1991) [Establishment of MPEL for thallium iodide activated cesium iodide in the working zone air]. Gig Tr Prof Zabol 1:31.
- Viereck, L; Kramer, M; Eikmann, T; et al. (1990) [Determining guidelines for metals in children's playgrounds in North Rhine-Westphalia]. Öffentl Gesundheitswes 53(1):7–15.
- Krasovskiĭ, GN; Kenesariev, UI. (1984) [Methodological outline for the experimental substantiation of a system of indices of the adverse effect of metals on the health status of the population (the example of thallium)]. Gig Sanit 2:22–25.
- Zasukhina, GD; Vasil'eva, IM; Sdirkova, NI.(1980) [Approach to the determination of the mutagenic potential of environmental pollutants with the example of detecting the mutagenic action of thallium carbonate]. Dokl Akad Nauk SSSR 250(3):766–768.

One reviewer noted that "over-grooming" in any species may be a sign of stress, pain, or other changes in the central or peripheral nervous systems and provided the following references that address this issue:

- Greer, JM; Capecchi, MR. (2002) Hoxb8 is required for normal grooming behavior in mice. Neuron 33:23–34.
- Kalueff, AV; Tuohimaa, P. (2005) Contrasting grooming phenotypes in three mouse strains markedly different in anxiety and activity (129S1, BALB/c and NMRI). Behav Brain Res 160:1–10.
- Kalueff, AV; Minasyan, A; Keisala, T; et al. (2006) Hair barbering in mice: implications for neurobehavioral research. Behav Proc 71:8–15.
- Welch, JM; Lu, J; Rodriguiz, RM; et al. (2007) Cortico-striatal synaptic defects and OCD-like behaviours in Sapap3-mutant mice. Nature 448:894–900.

In addition, one reviewer noted that some of the cited references that include experimental studies using more than the LD_{50} values have little significance to the toxicity of thallium because of the high dose and suggested deleting most of them from the *Toxicological Review*.

Response: Information from the papers on thallium toxicity by Cavanagh and colleagues, Windebank, and Kazantzis was added to relevant sections of the *Toxicological Review*. A discussion of the evidence that over-grooming may be indicative of effects on the nervous system was also added, including citations to the papers identified above.

The paper by Granero and Domingo (2002) presented a risk assessment for 12 metals in soil in a region of Spain. For thallium, the EPA RfD previously on IRIS was used in the risk assessment. No further discussion of health effects information for thallium was presented.

Three of the studies from the foreign literature identified by one reviewer appear to address the derivation of regulatory limits (Kamil'dzhanov, 1993; Gerasimova, 1991; Viereck et al., 1990); limits developed by other regulatory agencies are generally not included in IRIS toxicological reviews. Therefore, these papers from the foreign language literature were not considered useful additions to the thallium toxicological review. The paper (in Russian) by Zasukhina et al. (1980) appears to be the same as the Zasukhina et al. (1983) paper published in English.

In general, a toxicological review is not intended to be a comprehensive treatise (see Foreword to the thallium *Toxicological Review*). EPA acknowledges that additional literature on

thallium may be available; however, EPA considers all literature that has a bearing on the derivation of toxicity reference values to have been included in the *Toxicological Review*.

3. Please discuss research that you think would be likely to increase confidence in the database for future assessments of thallium and compounds.

Comments: Several reviewers noted that the toxicity database for thallium is very limited. Research suggested by the reviewers to increase the confidence in the thallium database included the following study types and suggestions for investigation of specific endpoints:

- Acute dose-range finding studies and, if needed, a chronic inhalation study to identify the hazard of inhaled thallium.
- A chronic bioassay that would provide information on chronic noncancer effects and cancer endpoints. (One reviewer noted that such studies are expensive and time-consuming, and sources of funding may be difficult to find.)
- Studies of general reproductive toxicity (in particular to improve the ability to interpret the results in Kunming mice observed by Wei).
- Studies of developmental toxicity and developmental neurotoxicity.
- A modern in vivo genotoxicity evaluation. Depending on the outcome of a genotoxicity assessment, the value of conducting a lifetime chronic bioassay could be weighed.
- A much larger human investigation of an exposed population. (The reviewer noted that a human investigation would be very costly and that an animal model might be more practical.)
- Endpoints that the various reviewers suggested would be better characterized in studies of subchronic or chronic duration included alopecia and its sequelae and neurological, reproductive, endocrine, and cardiovascular endpoints.
- Additional clinical chemistry, functional, and histopathologic assessments to determine the source and intensity of the clinical chemistry changes observed in the MRI (1988) subchronic study.
- Studies that more accurately define dose-response relationship for males and females with appropriate dose ranges that capture the alopecia endpoint, including supportive histopathologic examination of alopecia skin and apparently unaffected skin areas of all treated and untreated animals and supportive in vivo and in vitro experiments demonstrating thallium interaction with hair follicles at various stages of the hair cycle in both males and females.
- More information on the interaction of thallium with trace elements such as selenium and potassium.

- More studies on the potential mechanisms of action of thallium, including alopecia and neurotoxicity (as perhaps the most economical way of addressing uncertainties about the target organs for thallium and the plausibility of some of the responses that have been observed).
- Studies to establish whether absorption, distribution, and elimination of thallium are linear following oral and dermal absorption of thallium salts.
- Studies on metabolism of thallium that address the question as to whether the body has the capability to convert thallium from one valence state to the other to determine the extent to which studies on monovalent thallium are relevant to the toxicity of trivalent thallium. (The reviewer noted that if there is little or no conversion, then it will be important to characterize the toxicity of trivalent thallium separately.)
- Pharmacokinetic studies that would support the development of a physiologically based pharmacokinetic model for thallium (to better understand thallium dosimetry) as well as relative toxicity and pharmacokinetics of the various thallium salts.

Response: No response required.

4. Please comment on the identification and characterization of sources of uncertainty in Sections 5 and 6 of the assessment document. Please comment on whether the key sources of uncertainty have been adequately discussed. Have the choices and assumptions made in the discussion of uncertainty been transparently and objectively described? Has the impact of the uncertainty on the assessment been transparently and objectively described?

Comments: Three reviewers observed that the sources of uncertainty were adequately addressed and that the discussion of uncertainty was comprehensive. One reviewer considered alopecia to be an acceptable marker of thallium toxicity until better markers become available, at least for female rats, but further noted that it may be difficult to use alopecia as a reliable marker in male rats in the absence of a statistically significant dose response effect for alopecia in males.

One reviewer observed that the very limited data set for thallium can be further appreciated by comparing the proposed RfD of 1×10^{-5} mg/kg-day (0.01 µg/kg-day) to background exposure to environmental levels of thallium. This RfD suggests that a 70 kg adult can consume 0.7 µg/day. The 90th percentile adult urinary thallium elimination from NHANES is 0.380–0.390 µg/L, which is about 0.760–0.780 µg/day in the urine alone. Because thallium has substantial fecal elimination, this suggests that, if the RfD is adopted, greater than 10% of Americans, and in reality probably close to 50% of Americans, would ingest more than the RfD. Because there is no evidence that thallium in the current U.S. diet poses any threat and because there is no possible remediation, this reviewer noted that adoption of this RfD would produce unnecessary concern if the above calculation were correct. This reviewer further noted that a

more thorough use of the MRI (1988) study would probably further lower the RfD, suggesting possibly that the entire U.S. population is exposed above threshold. This reviewer considered such an analysis to be an example of how poor the existing data are and further questioned the validity of the analysis.

Five reviewers agreed that the *Toxicological Review* correctly characterized confidence in the RfD for thallium as low and/or that the thallium database is weak. In light of this low confidence, one of these reviews offered the opinion that it may be better to suggest a range of RfD values for thallium by using various UFs because of uncertain endpoint, inadequate scientific data, and high UFs. A second reviewer stated that, in light of the weakness of the data set, it is important to openly discuss in the document the pros and cons of calculating an IRIS RfD for thallium. A third reviewer noted that the available data are so poor that it was questionable whether such an analysis is even valid. A fourth reviewer considered the low confidence to be a reflection of the limited database, including the lack of studies addressing the known toxic effects of thallium, particularly neurotoxicity, developmental toxicity, and endocrine effects, failure of the MRI (1988) study to identify a NOAEL if all relevant endpoints are considered, and consideration of other studies rejected by EPA that suggest that effects may occur at lower doses than in the MRI study.

Other comments offered on specific UFs are summarized under charge question A5 along with EPA's response.

Response: In response to the reviewer who suggested that different UFs be used for males and females, EPA observes that the overall thallium database does not suggest appreciable gender differences in response to thallium (see Section 4.8.2) and that the development of gender-specific reference values is not supported.

EPA disagrees with the suggestion that a range of RfD values be developed by using various UFs because of uncertain endpoint, inadequate scientific data, and high UFs. Comments from the peer reviewers in response to charge questions A5 and A6 provide little agreement on alternative UFs. EPA considers the limitations in the database for thallium not to support an UF smaller than 3,000, and therefore a range of RfD values based on different composite UFs is not scientifically justified.

A summary of NHANES biomonitoring data was added to Section 3.4. Because an assessment of potential exposure is generally outside the scope of an IRIS assessment, a discussion of estimated intakes from background sources of thallium was not included in the *Toxicological Review*.

II. Chemical-Specific Charge Questions

A. Oral Reference Dose (RfD) for Thallium

A1. The 90-day oral gavage study by MRI (1988) was selected as the basis for the RfD. Please comment on whether the selection of this study as the principal study has been scientifically justified. Has this study been transparently and objectively described in the document? Please identify and provide the rationale for any other studies that should be selected as the principal study.

Comments: Four reviewers generally agreed with the selection of the MRI (1988) study as the most appropriate basis for the RfD. One reviewer noted that this study may be an acceptable basis for the RfD, although this was not clear without further analysis of other studies. One other reviewer raised significant concerns about the study, including the fact that the choice of animal model, the doses given, and the duration of investigation were not well substantiated; sample size, statistical analysis, and calculations were not presented; and there was lack of publication in a peer-reviewed journal. This reviewer did not identify an alternative study to use as the basis for the RfD.

Several reviewers recommended that the summary of the MRI (1988) study be expanded to include additional study details. One reviewer suggested that the *Toxicological Review* provide a better time line for the appearance of alopecia as well as hair loss associated with the normal hair cycle in the rat and a better description of the distribution pattern of alopecia. Four reviewers indicated that other toxicological endpoints (including LDH activity, exophthalmos, lacrimation, and miosis) needed to be better presented/explained.

One reviewer noted that the *Toxicological Review* considered one or two other studies for use as the principal study and that a good rationale was provided as to why they were not as good a choice as the MRI study. A second reviewer stated that he was not aware of any other study that could have been used as a primary study to derive the POD and the RfD but, given the paucity of data available, suggested that other studies could be analyzed more in depth and information compared to that obtained from the MRI study. Three reviewers indicated that EPA's justification for excluding other in vivo studies as the principal studies were not sufficient. One of these reviewers recommended that RfDs be derived from the other endpoints identified in the MRI study and from the Wei (1987) and Zasukhina et al. (1983) studies and the results compared to determine where the MRI study lies with respect to sensitivity. One reviewer observed that epidemiologic studies would be ideal, but all available epidemiologic studies provided limited evidence of cause and effect associated with exposure in food or inhalation.

Response: Consistent with the feedback from the majority of reviewers, the MRI (1988) study was retained as a potential principal study. This study was subject to an independent peer review (as noted in Section 4.2.1.1). None of these independent reviewers considered the study to be inadequate as the basis for the RfD.

The summary of the MRI (1988) findings, including LDH activity, exophthalmos, lacrimation, and miosis, was expanded in the *Toxicological Review*, Section 4.2.1.1. Information on the time that alopecia was first observed was also added to this section based on examination of individual animal observational findings in the MRI (1988) study. Across control and treated groups, alopecia was first observed somewhere between study days 44 and 60. Severity scores across control and treated groups ranged from 1 to 5. There were no discernable differences in either the severity or distribution pattern of alopecia across control and treated groups.

Further consideration was given to the use of other in vivo studies as the basis for the RfD (see Sections 5.1.1 and 5.1.5). Where certain in vivo studies were not considered by EPA to be sufficiently reliable as the basis for the POD, more thorough justification was provided (see Section 5.1.1). Expanded discussion of the limitations of the Zasukhina et al. (1983) study was added to Section 4.3. As noted in response to general charge question 1, the discussion of the Wei (1987) study was revised (Section 4.3), and the study findings were further considered as the potential basis for the POD in Sections 5.1.1 and 5.1.5. For the reasons discussed in Sections 4.6.1 and 5.1.1, the Wei (1987) study was not considered sufficiently reliable as the basis for the RfD.

A2. Alopecia (hair loss) was selected as the most appropriate critical effect for the RfD. EPA characterizes alopecia as being an adverse effect. Please comment on whether the science and mode-of-action information supports alopecia as an adverse effect. EPA has stated: "Whether alopecia is itself an adverse effect merits consideration. In humans, alopecia is generally reversible upon cessation of thallium exposure. Alopecia, however, appears to be a part of a continuum of dermal changes observed following thallium exposure, as well as one of a spectrum of effects on target organs that include the nervous and gastrointestinal systems. For these reasons, alopecia supported by two cases of hair follicle atrophy is considered an adverse effect." Please comment on whether the selection of this critical effect has been scientifically justified. Is EPA's choice transparently and objectively described in the document? Please provide a detailed explanation. Please identify and provide the rationale for any other endpoints that should be used instead of alopecia to develop the RfD.

Comments: Three reviewers agreed that alopecia should be considered an adverse effect in and of itself. One reviewer considered alopecia to be undesirable. Another reviewer considered alopecia with histopathology of the hair follicle to be adverse. A final reviewer stated that use of

alopecia "as a biomarker" is justified until a better marker for adverse effect such as neurotoxic effects is identified. Several reviewers observed that the fact that alopecia may be reversible does not influence its adversity, whereas one review stated that a drawback to the use of alopecia as an endpoint for RfD is that it is a reversible effect. Reviewers pointed to other limitations in the use of alopecia as the critical effect. One reviewer considered limitations to the alopecia data set to be the facts that histopathology was only noted in the assigned LOAEL dose group and was not examined in the assigned NOAEL and lower dose groups and that there is no evidence that the skin sections examined were from the alopecia areas of the skin. A second reviewer considered a drawback to be that the MRI (1988) study did not show a statistically significant dose-response effect for alopecia in male rats. One reviewer suggested that the *Toxicological Review* could provide a more detailed discussion of mechanisms of alopecia, particularly with regard to thallium.

Two reviewers disagreed with the use of alopecia as the critical effect for RfD determination for thallium. One reviewer based this opinion on the fact that it was not clear to this reviewer from either the description of the study or the peer review of the study as to whether this effect was really treatment related. This reviewer noted that there was a high background incidence of alopecia in the study population and that the observation in the study of "barbering behavior" in the animals may have accounted for much of the alopecia. It was this reviewer's experience with lab animals that there can be any number of reasons for hair loss, including changes in caging or husbandry, and the fact that there was such a high background incidence of alopecia suggested that the effect could have been unrelated to treatment. A second reviewer disagreed with the choice of alopecia in the MRI (1988) study because several findings (changes in biochemical parameters, exophthalmos, miosis, and changes in coat) appear to show better dose-response effects than alopecia.

In response to this and charge question A3, four reviewers suggested that other effects be considered as the basis for the RfD, including clinical chemistry, exophthalmos, miosis, changes in coat, lacrimation, and behavioral effects that appear to show dose-response effects and could be more defensible than alopecia as the critical effect.

One reviewer stated that data from several limited subchronic studies (Wei, 1987; Formigli et al., 1986) and in vitro studies (Gregotti et al., 1992) suggested that reproductive (especially male) endpoints are worthy of further investigation. This reviewer noted that these studies were poorly reported by the authors, and a dose-response relationship was difficult to ascertain, but that this was "however a poor excuse for not attempting to determine a dose-response relationship and providing NOAELs and BMDLs." This reviewer pointed to the study by Galván-Arzate et al. (2005) that describes a dose-related increase in thallium deposition in the brain and lipid peroxidation in male Wistar rats after 30 day exposure to 0.8 mg/kg or 1.6 mg/kg doses. This reviewer also suggested that EPA provide a more critical review of the 36-week oral

study by Manzo et al. (1983) that described in some detail functional and histopathologic changes in the peripheral nervous system.

Response: Consistent with the position of three reviewers who agreed that alopecia is an adverse effect, alopecia was considered along with other clinical observations that showed an increased incidence with dose (see Table 5-1) as a candidate critical effect for establishing the POD for the RfD and as part of a spectrum of clinical observations in thallium-exposed rats. The limitations in the skin histopathology data were recognized (see Sections 4.2.1.1 and 5.1.1). EPA acknowledges that the incidence of alopecia in male rats does not increase monotonically with dose, although the incidence is higher in all groups of treated male rats compared with untreated and vehicle controls. Further, the incidences of other clinical observations were increased in treated male rats over the control groups, consistent with a treatment-related effect in male rats. Available information on the mechanism by which thallium induces alopecia is presented in Sections 4.5.5 and 4.6.3.

Consistent with the input from the reviewers, EPA expanded the analysis of the critical effect to include other clinical observations that showed a dose-response effect, including observations related to coat, lacrimation, exophthalmos, miosis, and behavior. For the reasons presented in Section 5.1.1, changes in clinical chemistry parameters were not considered as the basis for the POD. EPA acknowledges that other factors such as caging and husbandry can cause alopecia in laboratory rodents; however, the incidence was clearly elevated in both male and female rats over controls. Further, to the extent that alopecia was due to barbering, research has shown that barbering in rodents can reflect a stress-evoked behavioral response (see Section 4.6.1). Accordingly, EPA retained alopecia as one of a set of clinical observations that were indicative of a potential treatment-related effect in rats. Limitations in clinical observation data were further discussed in Section 5.1.3.1.

EPA reconsidered the findings from other studies, including Galván-Arzate et al. (2000), Gregotti et al. (1992, 1985), Wei (1987), Formigli et al. (1986), and Manzo et al. (1983), as the basis for the critical effect. As discussed in Section 5.1.1, Formigli et al. (1986), Gregotti et al. (1985), and Manzo et al. (1983) were all single-dose studies and thus did not provide data useful for dose-response analysis. In addition, these studies administered doses higher than those used in the MRI (1988) study and therefore provided less information on low-dose toxicity. In vitro studies such as Gregotti et al. (1992) and single administration studies such as Galván-Arzate et al. (2005) do not provide information appropriate for characterization of long-term oral exposure to thallium. The Wei (1987) study was considered further as the basis for the POD for the oral RfD (see Section 5.1.5). In response to one reviewer comment, EPA reexamined the discussion of the Manzo et al. (1983) study in Section 4.2.1.1 and determined it to be adequate. Given the increased mortality in thallium-exposed rats at 40 and 240 days after treatment and the fact that

only a single dose level was used in this study, Manzo et al. (1983) was not considered appropriate as a principal study for RfD derivation.

A3. At the high dose in the MRI (1988) study, two female rats exhibited moderate to severe alopecia that could not be attributed to self-barbering or normal cyclic hair growth. Histologic examination of skin samples from these high-dose females showed atrophy of hair follicles. EPA considered these findings to be adverse and thus the high dose in this study (0.25 mg/kg-day thallium sulfate) to be the lowest-observed-adverse-effect level (LOAEL). The mid-dose group (0.05 mg/kg-day thallium sulfate) was identified as the no-observed-adverse-effect level (NOAEL). Is EPA's interpretation of the study findings scientifically justified? Has this interpretation of the findings been transparently and objectively described in the document?

As part of the evaluation of alopecia as a critical effect for the RfD, EPA performed a series of Fisher's exact tests to determine if the incidence of alopecia in any of the three dose groups was statistically significantly elevated above controls by using all cases of alopecia reported by MRI (1988). Please comment on whether EPA chose the appropriate data set and the appropriate statistical test for this analysis.

The study investigators reached a different interpretation of the study findings than did EPA. The investigators considered alopecia to be attributable to the cyclic pattern of hair growth in rodents and, consequently, did not consider these findings to be biologically significant. The high dose (0.25 mg/kg-day thallium sulfate) was identified in the MRI (1988) study as the NOAEL. Is the study authors' conclusion that the high dose (0.25 mg/kg-day thallium sulfate) represents a NOAEL justified and supported by the study data?

Comments: One reviewer offered the opinion that EPA correctly identified an adverse effect that cannot be "attributable to the cyclic pattern of hair growth in rodents." This reviewer stated that EPA needs to clearly define what a NOAEL is versus a LOAEL as it pertains to alopecia; the *Toxicological Review* needs to make clear whether a dose of 0.04 mg/kg that caused significant incidence of alopecia with no evidence of histopathologic change in skin/hair follicle is a NOAEL. A second reviewer could not fully agree with lowering the NOAEL value to 0.05 mg/kg because it was based on a change in interpretation of the data rather than any new scientific evidence and because male rats did not show a statistically significant dose response for alopecia.

One reviewer considered the high dose of thallium (0.2 mg/kg-day), and possibly the middle dose (0.04 mg/kg), to be a LOAEL rather than a NOAEL. This reviewer also considered data on the incidence of alopecia not attributable to barbering to be amenable to BMD modeling.

One reviewer found it difficult to conclude definitively that the alopecia in two animals with hair follicle atrophy was in fact treatment related. This reviewer noted that hair follicles go through a natural cycle of activity and inactivity; it was not clear from the descriptions of atrophy that what was being observed was anything more than an observation of a normal condition in these animals; there was no systematic evaluation of dermal tissue from other areas in the same animals; and there was no evaluation of hair follicle status in all dose groups. Because this is not a standard assessment in subchronic studies, it was not possible to know whether what was observed is within the range of normal.

One reviewer found the use of alopecia findings to be questionable, noting that the mid-dose group also had alopecia and, although it may have been a result of barbering, barbering is not a normal behavior and suggests that the animals were under stress, possibly in pain; thus, this dose level could not be a NOAEL. This reviewer also stated that it was not valid to discount a sex difference because sex may affect sensitivity to a given toxin. Finally, this reviewer noted that other parameters that show a dose-response relationship might be amenable to BMD analysis.

One reviewer observed that the incidences of alopecia in middle- and high-dose groups were both significantly different from controls, whereas EPA considered the middle dose to represent a NOAEL with no explanation. It appeared to this reviewer that EPA considers hair follicle atrophy to be the adverse effect. Because histopathologically observed atrophy was examined only at the high dose, this reviewer believed that the high dose should be considered a LOAEL for hair follicle atrophy and a UF of 10 applied. More importantly, this reviewer believed that barbering represents a change in behavior (e.g., caused by pain, an increased arousal level, or stress) and should be considered an adverse effect. This reviewer noted that barbering increased in a dose-dependent manner. This reviewer considered many of the endpoints in the rat study to be consistent with neurological effects in humans and that they should not have been dismissed. This reviewer considered the lowest dose to be a LOAEL.

One reviewer suggested that EPA recognize in their review that there are possible estrogen receptor pathways within the dermal papilla that regulate the telogen-anagen follicle transition and that diffusible factors associated with the anagen follicle influence cell proliferation in the epidermis (Oh and Smart, 1996). This may explain male versus female differences observed in the MRI study.

Response: In response to peer reviewer comments, EPA broadened the evaluation of the MRI (1988) study findings beyond alopecia in the presence of hair follicle atrophy to include all clinical observations in male and female rats and presented the possibility that the dose-related increase in the incidence of clinical effects indicated a possible treatment-related effect. EPA identified two possible critical effects from the MRI (1988) study – hair follicle atrophy and clinical observations – reflecting different interpretations of MRI study findings. With respect to

hair follicle atrophy, EPA in Section 5.1.2 clarified that skin tissue was not examined for histopathological changes in the low- and mid-dose groups. The mid-dose group was assumed to approximate a NOAEL for this endpoint in light of the low incidence of hair follicle atrophy in high-dose female rats and absence in male rats at all dose levels. EPA acknowledges this to be a change in the interpretation of the data that was made in light of all relevant literature on thallium toxicity and considered this to be an appropriate basis for reaching a different determination of the NOAEL and LOAEL from the authors of the MRI (1988) study.

In the revised discussion of alopecia and other clinical observations, EPA considered the possibility that barbering may indicate that animals were under stress or that barbering (and other clinical observations) may reflect some other change in behavior (e.g., see Section 4.6.1).

As suggested by several reviewers, BMD methodology was applied to the various clinical observation data sets and was used to derive the potential PODs, including the POD for alopecia.

EPA appreciated one reviewer's suggestion of a possible estrogen receptor pathway as an underlying basis for alopecia; however, there is no evidence in the thallium toxicity literature for such a mode of action. Because of the lack of data to support this hypothesis, it was not added to the *Toxicological Review*.

In Section 4.8.1, EPA concluded that the available thallium toxicity literature as a whole showed no consistent pattern of gender-related difference in toxicity.

Comments: Two reviewers considered the nonparametric statistical analysis using Fisher's exact test to be appropriate. One of the reviewers, however, noted that the choice to include all animals that had alopecia is problematic, especially given that the study investigators did not attribute all instances of alopecia to thallium treatment. A third reviewer stated that a Fisher's exact test is a standard procedure for pair-wise comparisons but fails to take advantage of the fact that there are three dose groups. This reviewer suggested that a more appropriate analysis would be a trend analysis. A fourth reviewer stated that, whereas a Fisher's exact test is specifically designed for a simple comparison, a more complex analysis is generally performed in a dose-response relationship and it is quite likely that there would be no significance had that approach been taken.

Response: As noted previously, EPA revised the dose-response analysis to include consideration of a range of clinical observations and used a BMD approach in place of a NOAEL/LOAEL approach for identifying a POD. Therefore, less importance was placed on pair-wise comparison as the basis for identifying a NOAEL and LOAEL from the MRI (1988) study.

Comments: Two reviewers specifically commented on the study authors' identification of the NOAEL. One reviewer stated that the conclusion as to whether the observation of alopecia is an adverse one is a matter of judgment and that the investigators who wrote the original study report

had a different interpretation of the alopecia; however, it was less clear to this reviewer why they concluded that the clinical chemistry observations or exophthalmos were not adverse. A second reviewer noted that the presence of follicle changes suggests that this was truly a toxic effect and that barbering represented stress and possibly pain. This reviewer further noted that the study investigators' interpretation of these data added considerable doubt on their ability to interpret the other findings in their study and further calls into question the choice of this study as the critical data for determining the RfD.

Response: EPA disagrees with the reviewer who called into question the choice of the MRI (1988) study as a potential principal study because of doubt about the ability of the study authors to interpret study findings. EPA notes that this study was subject to an independent peer review. The peer reviewers of the MRI (1988) study generally found the study to have been conducted according to guideline protocols in place at the time. Further, EPA reached its own conclusions based on an independent review of study findings and did not rely only on the authors' interpretation of the data. Accordingly, EPA considers the selection of the MRI (1988) study as a principal study to be supported, although it suffers from some limitations. Limitations in study design and uncertainties in data sets from MRI (1988) were addressed in Section 5.1.3.1.

Additional comments in response to this charge question, related to the consideration of other endpoints as critical effects, are summarized under charge question A2.

A4. The traditional NOAEL-LOAEL approach was used to define the point of departure (POD) for the RfD. A benchmark dose (BMD) analysis was considered but was not conducted because of the nature of the data set for alopecia. Please provide comments with regards to whether a NOAEL-LOAEL approach is the best approach for determining the POD. Has the approach been scientifically justified? Is it transparently and objectively described? Please identify and provide a rationale for any alternative approaches for the determination of the POD and if such approaches are preferred to EPA's approach.

Comments: Five reviewers agreed that the NOAEL-LOAEL approach is the most appropriate method to define the POD if it is derived using data for alopecia. One reviewer stated that, if the LOAEL-NOAEL approach from the MRI (1988) study is used, the lowest dose should be the POD as a LOAEL.

Five reviewers noted that there are data sets for other endpoints from the MRI (1988) study (e.g., exophthalmos, lacrimation, blood chemistry) for which BMD modeling should be considered. One of these reviewers also noted that the BMD approach should be tried for each of the endpoints that exhibits a dose-response relationship in the Wei (1987) and Zasukhina et al. (1983) studies. In this case, the POD used to derive the RfD may be the lowest BMDL, the one that EPA thinks is the most reliable or most orderly under analysis, or some kind of average.

One reviewer suggested deriving a range of RfD values rather than a single RfD value.

Response: BMD modeling of all appropriate data sets from the MRI (1988) study was conducted and presented in Appendix B of the *Toxicological Review*. BMD modeling was not performed for the Zasukhina et al. (1983) study because, as discussed in the response to charge question A1 comments, the study results were not considered scientifically reliable. The Wei (1987) study was included in Section 5.1.5, Candidate RfD Comparison Information, and the LOAEL was considered as a potential POD. Given the uncertainties associated with incomplete reporting and the lack of confirmatory findings at dose levels close to those used in the Wei (1987) study, data sets from this study were not analyzed using BMD methods.

As noted in response to general charge question 4 comments, it is EPA's general practice in developing IRIS reference values to derive a single value and not a range.

A5. Please comment on the selection of the uncertainty factors applied to the POD for the derivation of the RfD. For instance, are they scientifically justified and transparently and objectively described in the document? If changes to the selected uncertainty factors are proposed, please identify and provide a rationale(s).

Comments: None of the reviewers disagreed with the UF applied for interspecies or LOAEL to NOAEL extrapolation. Two reviewers questioned the need for application of an intraspecies UF. (One of these comments was offered in response to general charge question 4 but is summarized here.) In one case, this opinion was based on the fact that metals such as thallium are not metabolized and that differences in the sensitivity to thallium in humans will therefore be insignificant. In the second case, the reviewer identified limited dose-response data for human dermal exposure (resulting from the routine practice of dermal administration of thallium salts to children with ringworm) and, in particular, 1930s papers from Munch (1934) that demonstrated the distinction between epilation and toxicity and suggested a possible range for which animal effects can be correlated with human effects. This reviewer noted that it was unclear whether the addition of these data would improve uncertainty.

Only one reviewer questioned the subchronic to chronic UF of 3. It was this reviewer's opinion that there is little or no uncertainty when alopecia occurs following oral exposure to thallium. The effect occurs in less than a subchronic time frame, and therefore the UF for subchronic to chronic extrapolation may not be necessary. This reviewer also stated that EPA needs to counter this argument if they are concerned about toxic effects other than alopecia following chronic exposure.

Input on the database UF is discussed under charge question A6 below.

Response: Consistent with the input from the peer reviewers, the UFs for interspecies and LOAEL to NOAEL extrapolation were retained.

The paper by Munch (1934) was reviewed for information that would support reducing the uncertainty associated with intraspecies variability. This paper, which generally reported symptoms of overt toxicity, including death, was determined not to be useful for characterizing variation in intraspecies sensitivity to environmental exposures to thallium. Accordingly, the intraspecies UF of 10 was retained.

Consistent with the majority of reviewers, a subchronic to chronic UF of 3 was retained. One reviewer suggested that a subchronic to chronic UF might not be necessary; however, the UF of 3 was retained because, in the absence of any chronic toxicity information, it is unknown whether effects other than alopecia and other clinical observations could manifest at low doses.

A6. Please comment specifically on the database uncertainty factor of 10 applied in the RfD derivation. Please comment on the use of the database uncertainty factor specifically for the lack of adequate developmental toxicity studies and a two-generation reproductive toxicity study and additional uncertainty associated with the limited data available on neurotoxicity in light of the potential for neurotoxicity to represent a sensitive endpoint for thallium exposure. Please comment on whether the selection of the database uncertainty factor for the RfD has been scientifically justified. Has this selection been transparently and objectively described in the document?

Comments: Three reviewers considered EPA's database UF to be appropriate. One of these reviewers stated that the absence of specific dose-response studies addressing the issues of neurotoxicity, reproductive toxicity, and developmental toxicity of thallium suggests that a UF to account for an incomplete database is appropriate. Use of a database UF was supported by evidence that neurotoxicity is seen in humans upon (high) exposure to thallium and in animal studies (e.g., Manzo et al., 1983) and that reproductive toxicity is also seen in animal studies (Wei, 1987) at doses below those used in the MRI (1988) study. A second reviewer similarly noted that neurotoxic effects and reproductive effects were not adequately evaluated in the available animal studies and that available developmental toxicity studies, although not state-of-the-art, appear adequate to conclude that developmental toxicity would not drive the risk assessment for thallium. A third reviewer considered the database to be so weak that a UF of 10 must be utilized and that this choice was clearly described and appropriate.

One reviewer stated that a UF of 10 for deficiencies in the thallium toxicity database may be deemed to be too conservative. Another reviewer noted that a database UF of 10 may not be necessary if all the endpoints in the MRI (1988) study are considered, some of which may be indicative of neurotoxicity. This reviewer noted that the lack of a robust database on developmental toxicity is problematic, that developmental neurotoxicity and endocrine studies

have apparently not been performed, and that there are some data providing evidence for reproductive toxicity, but only for one sex. This reviewer suggested that the three studies should be used to generate sample RfDs from the endpoints that exhibit a dose-effect function; in this case, a database UF of 3 may be more appropriate.

One reviewer did not take issue with the database UF but proposed changing the single RfD to a range of RfDs.

Response: Consistent with the opinion of three of the external peer reviewers that the database UF of 10 is appropriate, the opinion of essentially all of the reviewers that the limited database for thallium toxicity results in an RfD of low confidence, the lack of adequate developmental toxicity studies and a two-generation reproductive toxicity study, and the uncertainty associated with the limited neurotoxicity data in light of the potential for neurotoxicity to represent a sensitive endpoint for thallium exposure, the full default database UF of 10 was retained. One reviewer observed that, based on the available studies, developmental toxicity would not likely drive the risk assessment for thallium. EPA does not believe that this observation should change the size of the database UF since the database deficiencies associated with reproductive toxicity and neurotoxicity also support a database UF of 10.

B. Inhalation Reference Concentration (RfC) for Thallium

B1. Has the rationale and justification for not deriving an RfC for thallium been transparently described in the document?

Comments: Three reviewers agreed that the available data on inhalation exposure to thallium were insufficient to support derivation of an RfC. One reviewer noted that an Occupational Safety and Health Administration (OSHA) threshold limit value of 0.10 mg/m^3 Tl is available and should be reported in the *Toxicological Review*.

Response: IRIS toxicological reviews generally do not include a summary of other Agency regulatory guidelines and standards. Regulatory values included in an IRIS assessment could become irrelevant with time as they are updated. Further, OSHA occupational limits take into consideration technological achievability and economic costs in addition to toxicity and therefore are not comparable to IRIS reference values. Therefore, the OSHA occupational limit was not included in the thallium *Toxicological Review*.

C. Carcinogenicity of Thallium and Compounds

C1. Under the EPA's (2005a) *Guidelines for Carcinogen Risk Assessment* (www.epa.gov/iris/backgr-d.htm), the Agency concluded that there is "inadequate information to assess the carcinogenic potential" of thallium and compounds. Please comment on the scientific justification for the cancer weight-of-evidence characterization. A quantitative cancer assessment was not derived for thallium. Has the scientific justification for not deriving a quantitative cancer assessment been transparently and objectively described?

Comments: All six reviewers agreed that the available literature does not support a characterization of the carcinogenicity of thallium and that the rationale for not performing a quantitative risk assessment for cancer was appropriate.

Response: No response needed.

PUBLIC COMMENTS

Two submissions from the public were received. The submissions received during the official public comment period were distributed to the external peer review panel prior to the public meeting and discussion of the assessment. Submissions offered some editorial comments and suggestions for clarification of specific portions of the text. Changes were incorporated in the *Toxicological Review* as appropriate and are not discussed further in this appendix.

A. General Comments

Comment: One public commenter considered thallium to be a very dangerous substance and urged EPA to retain the IRIS assessment for thallium on the IRIS database.

Response: EPA intends to retain an assessment of the health effects of thallium and compounds on the IRIS database. The draft *Toxicological Review* provided for public comment and external peer review represented an effort to update the health assessment for thallium and thallium compounds to reflect the current available science on this compound. However, EPA has not recommended an RfD for thallium compounds in this specific case. Justification for this decision is provided in Section 5.1.3.1.

B. Oral Reference Dose (RfD) for Thallium

Comment: One public commenter did not consider the 0.25 mg/kg-day dose to be a biologically significant LOAEL because there is inadequate evidence to link the high dose alopecia with biologically significant effects. Specific reasons for failing to establish this link included the following: (1) complete histopathologic examinations were conducted only in vehicle control and high-dose groups; therefore, the dose-response relationship of this effect was not established; (2) background occurrence of alopecia in study animals and the potential for misclassification adds uncertainty regarding the incidence of treatment-related alopecia in study animals; (3) patterns of alopecia and hair follicle atrophy were inconsistent between male and female rats; increases in the incidence of alopecia in male rats were not dose related; and atrophy of the hair follicles in male rats was absent; and (4) there is incomplete information on, and inconsistencies in, anatomical patterns of effects.

Response: As discussed in response to peer reviewer comments on charge question A2, EPA expanded the analysis of potential critical effects to include other clinical observations that showed a dose-response effect, including observations related to coat, lacrimation, exophthalmos, miosis, and behavior. Collectively, these effects were considered evidence of a possible treatment-related effect and to be biologically significant. Uncertainties associated with these endpoints were also discussed (Section 5.1.3.1).

Comment: One public commenter stated that EPA failed to adequately support its determination that the study dose selected as the POD constitutes a biologically significant endpoint. This commenter observed that alopecia should not be considered adverse because it is reversible after exposure ceases and noted that one reviewer of the MRI (1988) study described alopecia as an "early (low grade) lesion" and added that "it is not a lesion that could be considered as an adverse health effect at that stage, and it certainly is not life threatening."

Response: External peer reviewers generally considered alopecia to be an adverse effect. Several reviewers strongly recommended, however, that EPA consider other effects from the MRI (1988) study in addition to (or in place of) alopecia because these other effects were either more sensitive than alopecia, displayed a better dose response, and/or were more clearly treatment related. A more rigorous dose-response analysis of data sets for these other effects was added to the *Toxicological Review*.

Comment: One public commenter observed that available human data do not provide support for the choice of the LOAEL or NOAEL established by EPA, noting that, although limited in number and methodology, population-based surveys do not provide evidence for alopecia in

thallium-exposed populations. This commenter also noted that the draft RfD is inconsistent with the World Health Organization evaluation of thallium toxicity (presented in IPCS [1996]) that considers urinary concentrations <5 μg/L (assumed to be associated with a daily intake of 10 μg thallium) unlikely to cause adverse health effects based on the Brockhaus et al. (1981) study of residents living near a thallium-emitting cement plant in Germany. The commenter further observed that thallium exposures up to 100 times greater than the draft RfD did not include effects consistent with the draft RfD.

Response: EPA considered the use of the Brockhaus et al. (1981) and other population-based surveys as a basis for the RfD; however, the available studies were not considered sufficiently rigorous for reference value derivation.

Comment: One commenter considered the draft RfD (0.7 μg/day Tl for a 70 kg adult) to be of questionable relevance for protection of public health because intake of thallium from background sources (according to ATSDR [1992] as high as 5 μg/day from food and 2 μg/day from drinking water) exceeds levels of thallium oral exposure corresponding with the draft RfD. The commenter noted that, although some of this intake may be in less soluble and less well absorbed forms of thallium than the soluble salts upon which the draft RfD is based, these forms may better represent the forms of thallium present in soil and produce at many contaminated sites.

Response: Because the scope of an IRIS assessment does not generally include an analysis of potential exposure, a discussion of intake of thallium compounds from background sources was not included in the *Toxicological Review*. As noted in response to general charge question 4, EPA is not recommending an RfD value for thallium compounds.

Comment: One public commenter observed that the draft RfD applies only to soluble forms of thallium and strongly urged EPA to restrict application of the draft RfD for soluble salts to only those cases where these forms of thallium are known to be present.

Response: EPA is not recommending an RfD for soluble thallium salts. Therefore, restrictions on the application of an RfD is not an issue at this time.

Comment: One public commenter observed that, although human biomonitoring studies provide direct evidence of widespread, low-level thallium exposures in the general population, biomonitoring estimates from these studies describe uptake rather than intake, do not account for the unabsorbed fraction of the ingested dose, and need to be coupled with a better understanding of the absorption fraction for dietary thallium and fraction excreted in urine in order to estimate daily intakes more reliably.

Response: A summary of NHANES biomonitoring data was added to Section 3.4. Because an assessment of potential exposure is generally outside the scope of an IRIS assessment, an analysis of thallium intake based on available biomonitoring data was not included in the *Toxicological Review*.

APPENDIX B

Documentation of Benchmark Dose Modeling

B.1. Summary of BMDS Modeling Results for Clinical Observations in the Male Rat (MRI, 1988)

Table B-1. A summary of BMDS (version 1.4.1) modeling results based on incidence of rough coat in male Sprague-Dawley rats exposed to thallium sulfate via gavage for 90 days

Model	Chi-square *p* value[a]	AIC	BMD_{10} (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)
Gamma	0.0000	95.1404	0.003737	0.002460
Logistic	0.0000	104.547	0.009731	0.006365
Log-logistic	0.8762	85.7472	0.001063	0.0005675
Multistage (1°)	0.0000	95.1404	0.003737	0.002460
Probit	0.0000	106.954	0.01361	0.009597
Log-probit	0.0008	92.4736	0.004137	0.002679
Weibull	0.0000	95.1404	0.003737	0.002460

[a]Chi-square *p* value = *p* value from the chi-square test for lack of fit. Values <0.1 fail to meet conventional goodness-of-fit criteria.

Data source: MRI (1988).

Only the log-logistic model had a $p \geq 0.1$; however, the data set was not considered amenable to modeling because of the steep slope of the dose-response curve in the low-dose region and because the BMD_{10} and $BMDL_{10}$ were well outside experimental range of the data. BMDS output from the log-logistic model is provided below.

```
 ================================================================
Logistic Model. (Version: 2.9; Date: 02/20/2007)
Input Data File: M:\IRIS CHEMICALS\THALLIUM\BMD\POST EPR 6-2008\MALE\ROUGH_COAT-MALE.(d)
Gnuplot Plotting File:  M:\IRIS CHEMICALS\THALLIUM\BMD\POST EPR 6-2008\MALE\ROUGH_COAT-MALE.plt
                                                  Fri Jun 13 08:32:57 2008
 ================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]

   Dependent variable = #RoughCoat
   Independent variable = Dose(mg/kg-d)
   Slope parameter is restricted as slope >= 1

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

   User has chosen the log transformed model
```

Default Initial Parameter Values
background = 0.1
intercept = 4.47108
slope = 1

Asymptotic Correlation Matrix of Parameter Estimates

*** The model parameter(s) -slope
have been estimated at a boundary point, or have been specified by the user, and do not appear in the correlation matrix)

	background	intercept
background	1	-0.26
intercept	-0.26	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
background	0.101788	*	*	*
intercept	4.64934	*	*	*
slope	1	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-40.7444	4			
Fitted model	-40.8736	2	0.258303	2	0.8788
Reduced model	-69.3147	1	57.1405	3	<.0001

AIC: 85.7472

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.1018	4.072	4	40	-0.037
0.0080	0.5108	10.216	11	20	0.351
0.0400	0.8266	16.532	16	20	-0.314
0.2000	0.9590	19.180	19	20	-0.203

Chi^2 = 0.26 d.f. = 2 P-value = 0.8762

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.00106311

BMDL = 0.000567469

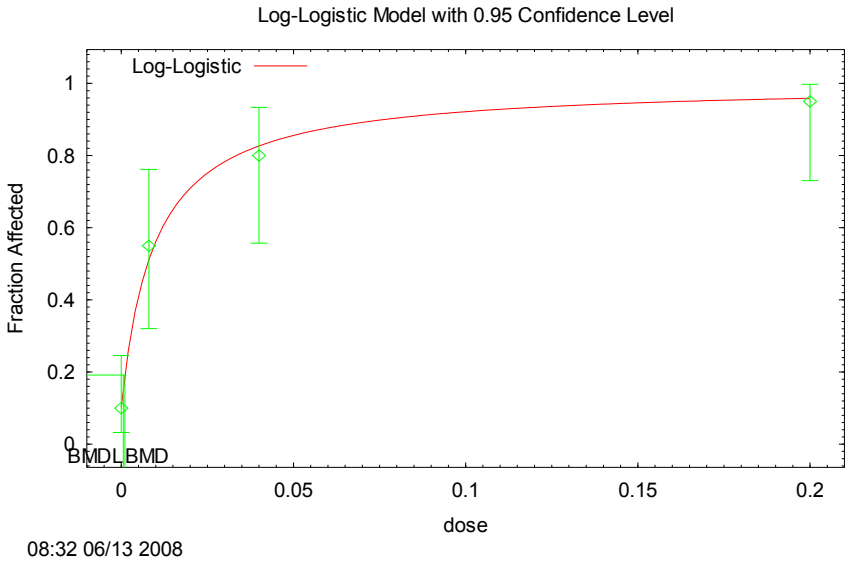


Table B-2. A summary of BMDS (version 1.4.1) modeling results based on incidence of piloerection in male Sprague-Dawley rats exposed to thallium sulfate via gavage for 90 days

Model	Chi-square *p*-value[a]	AIC	BMD_{10} (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)
Gamma	**0.9976**	**55.896**	**0.01958**	**0.01339**
Logistic	0.0868	63.7889	0.06642	0.04852
Log-logistic	0.9609	57.9327	0.01813	0.008735
Multistage (1°)	**0.9976**	**55.896**	**0.01958**	**0.01339**
Probit	0.1124	63.1776	0.0596	0.04436
Log-probit	0.3004	60.4202	0.03242	0.02167
Weibull	**0.9976**	**55.896**	**0.01958**	**0.01339**

[a]Chi-square *p* value = *p* value from the chi-square test for lack of fit. Values <0.1 fail to meet conventional goodness-of-fit criteria.

Data source: MRI (1988).

All models with the exception of the logistic model provided adequate fits of the data as assessed by a chi-square goodness-of-fit test ($p \geq 0.1$) and visual inspection of the respective plots of observed versus predicted values from the various models. The gamma, multistage, and Weibull models provided the same fit of the data and were judged to provide the best model fit based on the lowest AIC value. The BMDS output for the gamma model is provided below.

```
================================================================
Gamma Model. (Version: 2.8;  Date: 02/20/2007)
Input Data File: M:\IRIS CHEMICALS\THALLIUM\BMD\POST EPR 6-2008\MALE\PILOERECTION-MALE.(d)
Gnuplot Plotting File:  M:\IRIS CHEMICALS\THALLIUM\BMD\POST EPR 6-2008\MALE\PILOERECTION-MALE.plt
                                                    Fri Jun 13 09:33:49 2008
 ================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response]= background+(1-background)*CumGamma[slope*dose,power],
   where CumGamma(.) is the cummulative Gamma distribution function

   Dependent variable = #Piloerection
   Independent variable = Dose(mg/kg-d)
   Power parameter is restricted as power >=1

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                 Default Initial (and Specified) Parameter Values
                    Background =    0.0121951
                         Slope =      6.76558
                         Power =      1.26625

          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -Background    -Power
                have been estimated at a boundary point, or have been specified by the user,
                and do not appear in the correlation matrix )

                 Slope

    Slope            1


                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
    Background                0               NA
         Slope          5.38237          1.31322              2.8085             7.95624
         Power                1               NA

NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus
     has no standard error.


                       Analysis of Deviance Table

      Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
    Full model         -26.9273         4
   Fitted model         -26.948         1     0.0414636      3          0.9978
  Reduced model        -47.1393         1       40.4241      3         <.0001

          AIC:          55.896


                                Goodness  of  Fit
                                                                    Scaled
    Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------
    0.0000     0.0000         0.000           0          40         0.000
    0.0080     0.0421         0.843           1          20         0.175
    0.0400     0.1937         3.874           4          20         0.071
    0.2000     0.6592        13.184          13          20        -0.087

 Chi^2 = 0.04      d.f. = 3        P-value = 0.9976
```

```
   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =       0.0195751

            BMDL =      0.0133909
```

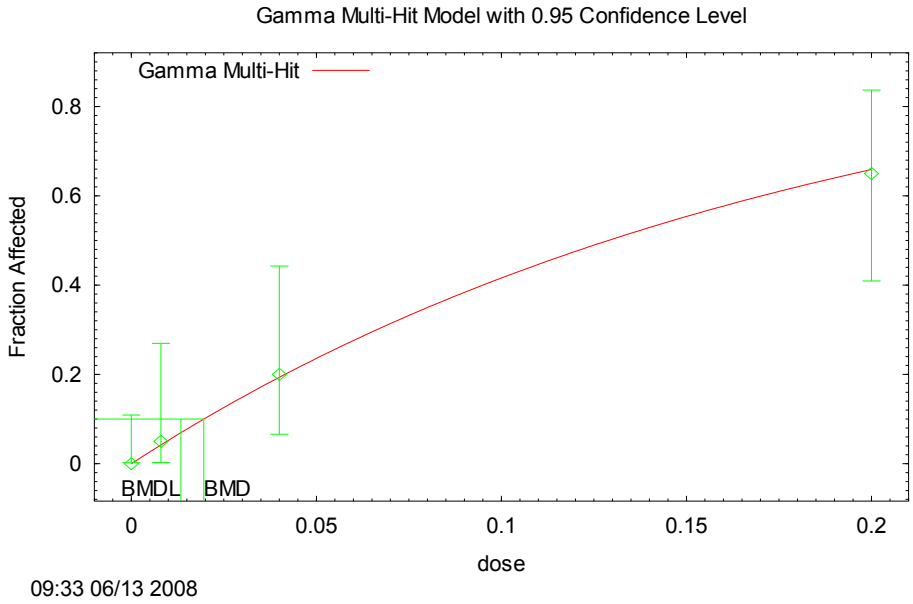


Table B-3. A summary of BMDS (version 1.4.1) modeling results based on incidence of shedding in male Sprague-Dawley rats exposed to thallium sulfate via gavage for 90 days

Model	Chi-square *p* value[a]	AIC	BMD_{10} (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)
Gamma	0.0001	97.3226	0.02650	0.01557
Logistic	0.0000	101.522	0.06984	0.04768
Log-logistic	0.0018	89.706	0.009591	0.005740
Multistage (1°)	0.0001	97.3226	0.02650	0.01557
Probit	0.0001	101.233	0.06524	0.04472
Log-probit	0.0000	103.483	0.06468	0.02975
Weibull	0.0001	97.3226	0.02650	0.01557

[a]Chi-square *p*-value = *p* value from the Chi-square test for lack of fit. Values <0.1 fail to meet conventional goodness-of-fit criteria.

Data source: MRI (1988).

This data set was not fit by any of the models in BMDS.

Table B-4. A summary of BMDS (version 1.4.1) modeling results based on incidence of alopecia in male Sprague-Dawley rats exposed to thallium sulfate via gavage for 90 days

Model	Chi-square *p* value[a]	AIC	BMD_{10} (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)
Gamma	0.0006	106.08	201851	0.04072
Logistic	0.0029	103.671	0.2356	0.07835
Log-logistic	0.0032	103.436	0.1419	0.02992
Multistage (1°)	0.0031	103.514	0.1701	0.04426
Probit	0.0030	103.655	0.2283	0.07445
Log-probit	0.0006	106.08	0.8226	0.1092
Weibull	0.0031	103.514	0.1701	0.04426

[a]Chi-square *p* value = *p* value from the chi-square test for lack of fit. Values <0.1 fail to meet conventional goodness-of-fit criteria.

Data source: MRI (1988).

This data set was not fit by any of the models in BMDS.

Table B-5. A summary of BMDS (version 1.4.1) modeling results based on incidence of lacrimation in male Sprague-Dawley rats exposed to thallium sulfate via gavage for 90 day

Model	Chi-square *p*-value[a]	AIC	BMD_{10} (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)
Gamma	0.9986	51.0387	0.0004624	0.0001676
Logistic	1.0000	49.0387	0.0008749	0.0005251
Log-logistic	0.9996	51.0387	0.004725	6.03×10^{-6}
Multistage (2°)	1.0000	51.0387	0.000374	0.0001676
Probit	1.0000	49.0387	0.000879	0.0005970
Log-probit	0.9990	51.0387	0.002302	0.0001857
Weibull	1.0000	49.0388	0.0003007	0.0001676

[a]Chi-square *p* value = *p* value from the chi-square test for lack of fit. Values <0.1 fail to meet conventional goodness-of-fit criteria.

Data source: MRI (1988).

This data set was not considered amenable to modeling because of the steep slope of the dose-response curve in the low-dose region and because the BMD_{10} and $BMDL_{10}$ values were well outside the experimental range of the data. The BMDS output for the logistic model is presented below.

```
 ================================================================
Logistic Model. (Version: 2.9; Date: 02/20/2007)
Input Data File: M:\IRIS CHEMICALS\THALLIUM\BMD\POST EPR 6-2008\MALE\LACRIMATION-MALE.(d)
Gnuplot Plotting File:  M:\IRIS CHEMICALS\THALLIUM\BMD\POST EPR 6-2008\MALE\LACRIMATION-MALE.plt
                                                  Fri Jun 13 09:43:31 2008
 ================================================================

 BMDS MODEL RUN
```

~~~~~~

The form of the probability function is:

P[response] = 1/[1+EXP(-intercept-slope*dose)]

Dependent variable = #Lacrimation
Independent variable = Dose(mg/kg-d)
Slope parameter is not restricted

Total number of observations = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
background = 0 Specified
intercept = 1.2157
slope = 14.6477

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -background
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	intercept	slope
intercept	1	-0.38
slope	-0.38	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
intercept	-1.5506	0.416124	-2.36618	-0.735007
slope	561.879	138.394	290.632	833.127

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-22.5194	4			
Fitted model	-22.5194	2	3.27387e-008	2	1
Reduced model	-64.1035	1	83.1684	3	<.0001

AIC: 49.0387

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.1750	7.000	7	40	-0.000
0.0080	0.9500	19.000	19	20	-0.000
0.0400	1.0000	20.000	20	20	0.000
0.2000	1.0000	20.000	20	20	0.000

Chi^2 = 0.00 d.f. = 2 P-value = 1.0000

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.000874909

BMDL = 0.000525133
~~~~~~

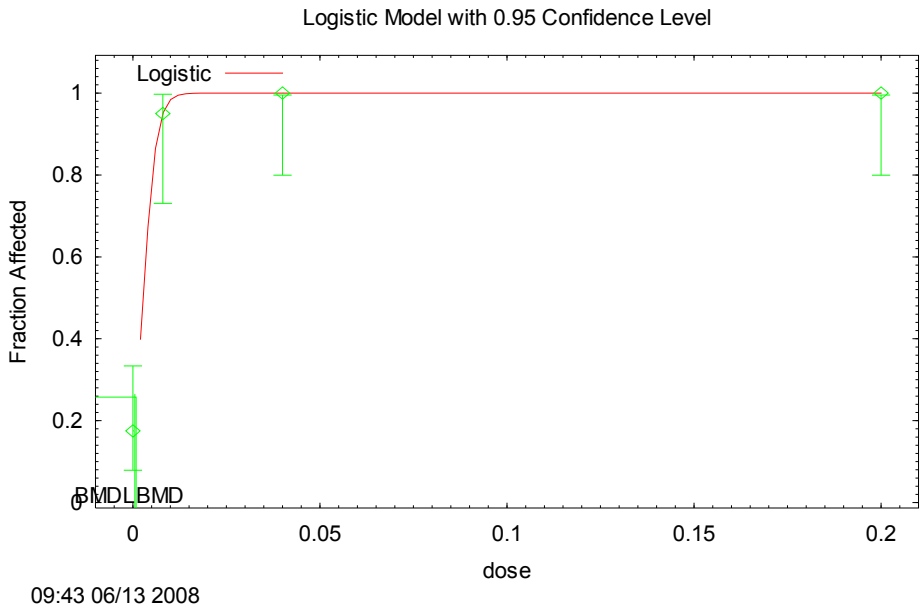


Table B-6. A summary of BMDS (version 1.4.1) modeling results based on incidence of exophthalmos in male Sprague-Dawley rats exposed to thallium sulfate via gavage for 90 days

Model	Chi-square *p* value[a]	AIC	BMD_{10} (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)
Gamma	0.9995	66.7372	0.004381	0.0006528
Logistic	0.9987	64.7422	0.002070	0.001434
Log-logistic	0.9996	66.7372	0.006493	0.001206
Multistage (2°)	1.0000	64.7372	0.002991	0.0006528
Probit	1.0000	64.7372	0.001947	0.001373
Log-probit	0.9995	66.7372	0.005341	0.001283
Weibull	0.9980	66.7372	0.002766	0.0006528

[a]Chi-square *p* value = *p* value from the chi-square test for lack of fit. Values <0.1 fail to meet conventional goodness-of-fit criteria.

Data source: MRI (1988).

This data set was not considered amenable to modeling because of the steep slope of the dose-response curve in the low-dose region and because the BMD_{10} and $BMDL_{10}$ values were well outside the experimental range of the data. Of the models with the lowest AIC values, the model with the lowest $BMDL_{10}$ (i.e., the two-stage multistage model) is presented below.

```
 ================================================================
Multistage Model. (Version: 2.8;  Date: 02/20/2007)
Input Data File: M:\IRIS CHEMICALS\THALLIUM\BMD\POST EPR 6-2008\MALE\EXOPHTHALMOS-MALE.(d)
Gnuplot Plotting File:  M:\IRIS CHEMICALS\THALLIUM\BMD\POST EPR 6-2008\MALE\EXOPHTHALMOS-MALE.plt
                                                Fri Jun 13 09:56:05 2008
 ================================================================

 BMDS MODEL RUN
```

~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1-beta2*dose^2)]

   The parameter betas are restricted to be positive

   Dependent variable = #Exophthalmos
   Independent variable = Dose(mg/kg-d)

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2

 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008

                  Default Initial Parameter Values
                     Background =   1
                        Beta(1) = 4.41266e+020
                        Beta(2) =   0

           Asymptotic Correlation Matrix of Parameter Estimates
           (*** The model parameter(s) -Beta(1)
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix)

             Background   Beta(2)

Background   1   -0.44

   Beta(2)   -0.44   1

                                Parameter Estimates
                                                        95.0% Wald Confidence Interval
      Variable   Estimate   Std. Err.   Lower Conf. Limit   Upper Conf. Limit
     Background   0.15   *   *   *
        Beta(1)   0   *   *   *
        Beta(2)   11777.7   *   *   *

* - Indicates that this value is not calculated.

                       Analysis of Deviance Table
       Model   Log(likelihood) # Param's Deviance Test d.f.   P-value
     Full model   -30.3686   4
   Fitted model   -30.3686   2   2.2259e-007   2   1
  Reduced model   -68.0292   1   75.3212   3   <.0001

           AIC: 64.7372

                                Goodness of Fit
                                                                    Scaled
     Dose   Est._Prob.   Expected   Observed   Size   Residual
  ----
    0.0000   0.1500   6.000   6   40   0.000
    0.0080   0.6000   12.000   12   20   -0.000
    0.0400   1.0000   20.000   20   20   0.000
    0.2000   1.0000   20.000   20   20   0.000

 Chi^2 = 0.00   d.f. = 2   P-value = 1.0000

   Benchmark Dose Computation

Specified effect =   0.1

Risk Type   =   Extra risk

Confidence level =   0.95
~~~~~~

```
BMD =      0.00299095

BMDL =     0.000652821

BMDU =      0.00435446

Taken together, (0.000652821, 0.00435446) is a 90      % two-sided confidence
interval for the BMD
```

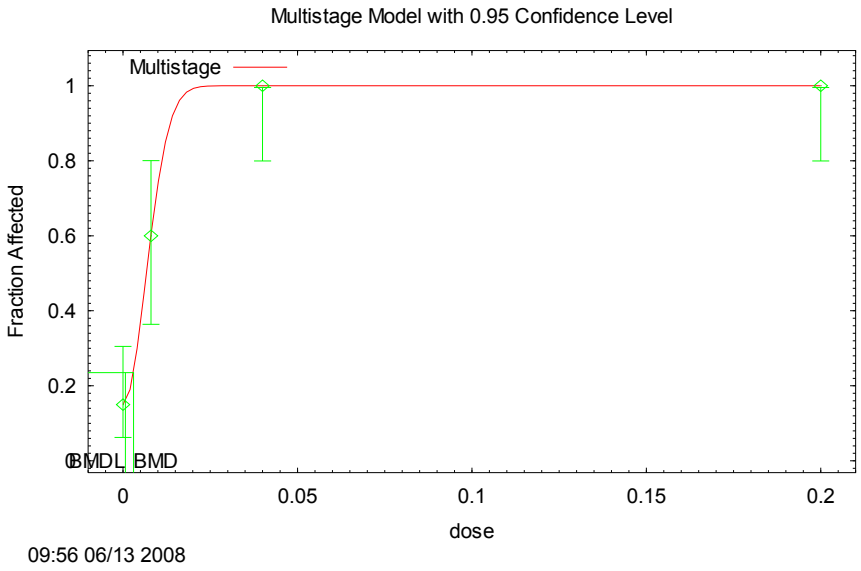


Table B-7. A summary of BMDS (version 1.4.1) modeling results based on incidence of miosis in male Sprague-Dawley rats exposed to thallium sulfate via gavage for 90 days

Model	Chi-square *p* value[a]	AIC	BMD_{10} (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)
Gamma	0.0924	88.4982	0.01341	0.009005
Logistic	0.0201	93.2144	0.03912	0.02853
Log-logistic	**0.3209**	**86.2645**	**0.006854**	**0.003910**
Multistage (1°)	0.0924	88.4982	0.01341	0.009005
Probit	0.0218	92.9002	0.03674	0.02777
Log-probit	0.0214	91.7394	0.02461	0.01547
Weibull	0.0924	88.4982	0.01341	0.009005

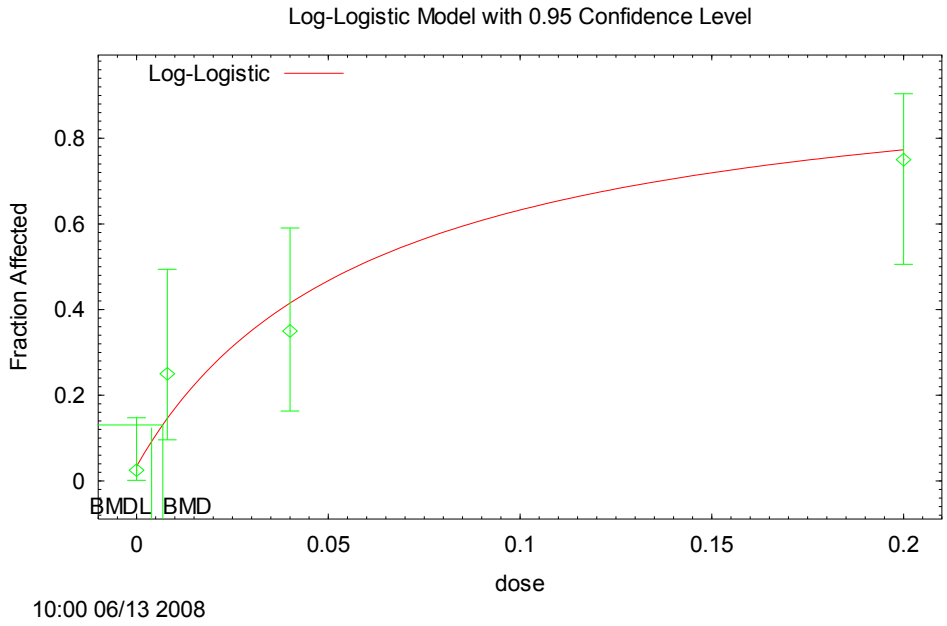
[a]Chi-square *p* value = *p* value from the chi-square test for lack of fit. Values <0.1 fail to meet conventional goodness-of-fit criteria.

Data source: MRI (1988).

The log-logistic model was the only model that did not exhibit statistically significant lack of fit. The BMDS output from this model is provided below.

```
 ================================================================
Logistic Model. (Version: 2.9; Date: 02/20/2007)
Input Data File: M:\IRIS CHEMICALS\THALLIUM\BMD\POST EPR 6-2008\MALE\MIOSIS-MALE.(d)
Gnuplot Plotting File:  M:\IRIS CHEMICALS\THALLIUM\BMD\POST EPR 6-2008\MALE\MIOSIS-MALE.plt
                                                    Fri Jun 13 10:00:04 2008
 ================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


   Dependent variable = #Miosis
   Independent variable = Dose(mg/kg-d)
   Slope parameter is restricted as slope >= 1

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


   User has chosen the log transformed model

                Default Initial Parameter Values
                   background =        0.025
                    intercept =      2.67092
                        slope =            1

          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -slope
                have been estimated at a boundary point, or have been specified by the user,
                and do not appear in the correlation matrix )

            background    intercept

background            1        -0.38

 intercept        -0.38            1

                                Parameter Estimates
                                                        95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
    background        0.0342297            *                *                   *
     intercept          2.78563            *                *                   *
         slope                1            *                *                   *

* - Indicates that this value is not calculated.

                       Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model         -40.1186         4
   Fitted model         -41.1323         2       2.02732      2          0.3629
  Reduced model         -59.2953         1       38.3534      3         <.0001

           AIC:          86.2645

                                Goodness  of  Fit
                                                                    Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------
    0.0000     0.0342         1.369         1          40       -0.321
    0.0080     0.1451         2.902         5          20        1.332
    0.0400     0.4141         8.282         7          20       -0.582
    0.2000     0.7723        15.447        15          20       -0.238

 Chi^2 = 2.27      d.f. = 2        P-value = 0.3209

   Benchmark Dose Computation
```

```
Specified effect =           0.1

Risk Type        =     Extra risk

Confidence level =          0.95

             BMD =     0.0068545

            BMDL =    0.00391034
```



\

Table B-8. A summary of BMDS (version 1.4.1) modeling results based on incidence of behavioral findings in male Sprague-Dawley rats exposed to thallium sulfate via gavage for 90 days

Model	Chi-square *p* value[a]	AIC	BMD_{10} (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)
Gamma	0.0271	108.74	0.06920	0.03038
Logistic	0.0251	109.105	0.09757	0.05538
Log-logistic	0.0283	108.549	0.05662	0.02007
Multistage (1°)	0.0271	108.74	0.06920	0.03038
Probit	0.0253	109.067	0.09430	0.05269
Log-probit	0.0191	109.827	0.1271	0.06223
Weibull	0.0271	108.74	0.06920	0.03038

[a]Chi-square *p* value = *p* value from the chi-square test for lack of fit. Values <0.1 fail to meet conventional goodness-of-fit criteria.

Data source: MRI (1988).

This data set was not fit by any of the models in BMDS.

B.2. Summary of BMDS Modeling Results for Clinical Observations in the Female Rat (MRI, 1988)

Table B-9. A summary of BMDS (version 1.4.1) modeling results based on incidence of rough coat in female Sprague-Dawley rats exposed to thallium sulfate via gavage for 90 days

Model	Chi-square *p* value[a]	AIC	BMD_{10} (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)
Gamma	**0.6681**	**72.0684**	**0.02434**	**0.01597**
Logistic	0.0831	75.8559	0.06646	0.04900
Log-logistic	**0.8772**	**71.5823**	**0.01778**	**0.01008**
Multistage (1°)	**0.6681**	**72.0684**	**0.02434**	**0.01597**
Probit	0.1033	75.4298	0.06033	0.04503
Log-probit	0.2042	74.1826	0.03935	0.02596
Weibull	**0.6681**	**72.0684**	**0.02434**	**0.01597**

[a]Chi-square *p* value = *p* value from the chi-square test for lack of fit. Values <0.1 fail to meet conventional goodness-of-fit criteria.

Data source: MRI (1988).

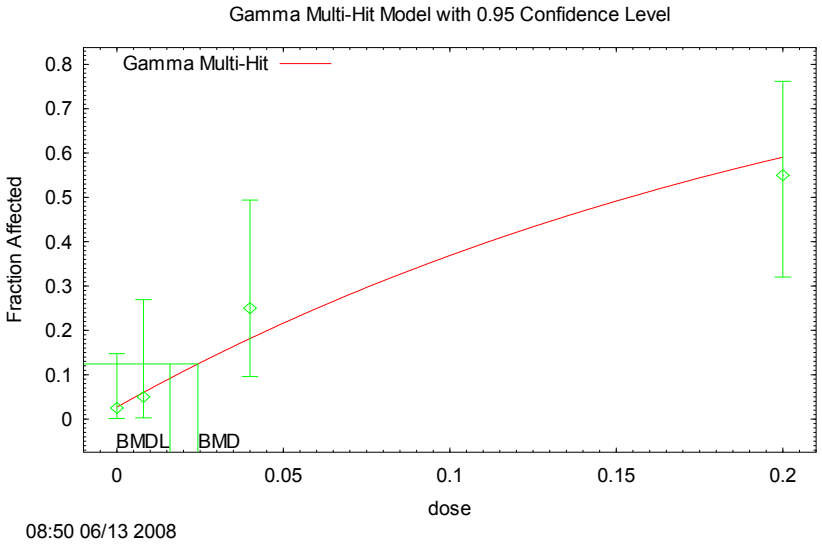
All models with the exception of the logistic model provided adequate fits to the data as assessed by a chi-square goodness-of-fit test ($p \geq 0.1$) and visual inspection of the respective plots of observed versus predicted values from the various models. $BMDL_{10}$ estimates from these models were within a factor of three of each other, suggesting no appreciable model dependence. Fitted models exhibiting adequate fit with AIC values within two units of the lowest AIC were considered indistinguishable from one another; thus, BMD_{10} and $BMDL_{10}$ values from these models were averaged to derive the POD. Model fits that yielded the same mathematical model were counted as a single model for averaging purposes (these models included gamma, multistage, and Weibull). Therefore, the BMD_{10} and $BMDL_{10}$ values for these models were averaged as follows:

Average BMD_{10} = (0.02434 + 0.01778) ÷ 2 = 0.02106 mg/kg-day
Average $BMDL_{10}$ = (0.01597 + 0.01008) ÷ 2 = 0.01303 mg/kg-day

BMDS outputs from the gamma and log-logistic models are presented below.

```
 ================================================================
Gamma Model. (Version: 2.8;  Date: 02/20/2007)
Input Data File: M:\IRIS CHEMICALS\THALLIUM\BMD\POST EPR 6-2008\FEMALE\ROUGH_COAT-FEMALE.(d)
Gnuplot Plotting File:  M:\IRIS CHEMICALS\THALLIUM\BMD\POST EPR 6-2008\FEMALE\ROUGH_COAT-
FEMALE.plt
                                                    Fri Jun 13 08:50:07 2008
 ================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response]= background+(1-background)*CumGamma[slope*dose,power],
   where CumGamma(.) is the cummulative Gamma distribution function


   Dependent variable = #RoughCoat
   Independent variable = Dose(mg/kg-d)
   Power parameter is restricted as power >=1

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                 Default Initial (and Specified) Parameter Values
                    Background =    0.0365854
                         Slope =      8.19903
                         Power =          1.3

          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -Power
                have been estimated at a boundary point, or have been specified by the user,
                and do not appear in the correlation matrix )

            Background        Slope

Background           1        -0.27

     Slope       -0.27            1


                                Parameter Estimates
                                                      95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
    Background        0.0268981        0.0242052          -0.0205432           0.0743394
         Slope          4.32831          1.21395             1.94901             6.70761
         Power                1               NA

NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus
     has no standard error.


                       Analysis of Deviance Table

      Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -33.6561         4
   Fitted model        -34.0342         2      0.756278      2          0.6851
  Reduced model        -47.1393         1       26.9666      3         <.0001

          AIC:         72.0684


                                Goodness  of  Fit
                                                                    Scaled
    Dose     Est._Prob.    Expected    Observed     Size        Residual
  ---------------------------------------------------------------------
    0.0000     0.0269         1.076         1          40       -0.074
    0.0080     0.0600         1.200         1          20       -0.189
    0.0400     0.1816         3.632         5          20        0.794
    0.2000     0.5905        11.811        11          20       -0.369
```

```
 Chi^2 = 0.81      d.f. = 2        P-value = 0.6681

   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =      0.0243422

            BMDL =     0.0159664
```




```
 ======================================================================
Logistic Model. (Version: 2.9; Date: 02/20/2007)
Input Data File: M:\IRIS CHEMICALS\THALLIUM\BMD\POST EPR 6-2008\FEMALE\ROUGH_COAT-FEMALE.(d)
Gnuplot Plotting File:  M:\IRIS CHEMICALS\THALLIUM\BMD\POST EPR 6-2008\FEMALE\ROUGH_COAT-
FEMALE.plt
                                                    Fri Jun 13 08:52:08 2008
 ======================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


   Dependent variable = #RoughCoat
   Independent variable = Dose(mg/kg-d)
   Slope parameter is restricted as slope >= 1

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


   User has chosen the log transformed model

                Default Initial Parameter Values
                   background =        0.025
                    intercept =      2.22927
                        slope =      1.17797

          Asymptotic Correlation Matrix of Parameter Estimates
```

(*** The model parameter(s) -slope
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	background	intercept
background	1	-0.26
intercept	-0.26	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
background	0.0230944	*	*	*
intercept	1.83236	*	*	*
slope	1	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-33.6561	4			
Fitted model	-33.7911	2	0.270147	2	0.8737
Reduced model	-47.1393	1	26.9666	3	<.0001

AIC: 71.5823

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0231	0.924	1	40	0.080
0.0080	0.0696	1.392	1	20	-0.345
0.0400	0.2184	4.369	5	20	0.342
0.2000	0.5658	11.315	11	20	-0.142

Chi^2 = 0.26 d.f. = 2 P-value = 0.8772

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.0177817

BMDL = 0.0100819

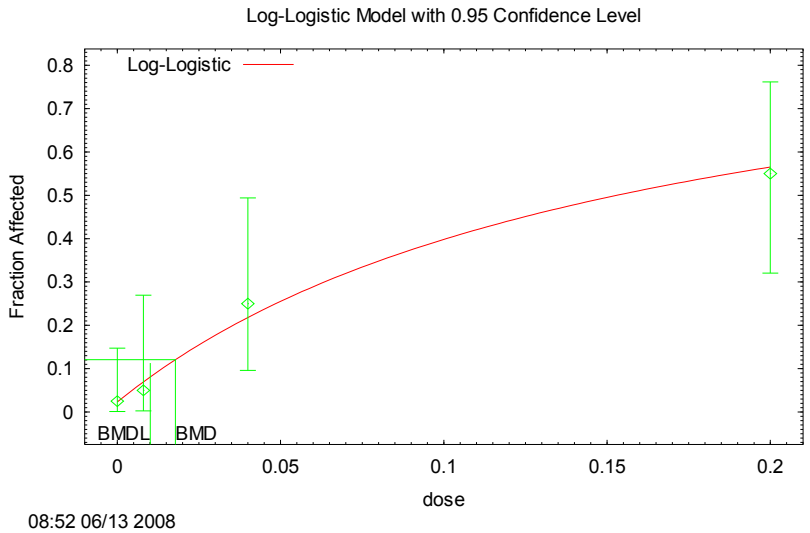


Table B-10. A summary of BMDS (version 1.4.1) modeling results based on incidence of piloerection in female Sprague-Dawley rats exposed to thallium sulfate via gavage for 90 days

Model	Chi-square *p* value[a]	AIC	BMD_{10} (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)
Gamma	**0.8149**	**47.1544**	**0.03857**	**0.02426**
Logistic	0.0484	54.0549	0.1008	0.07462
Log-logistic	**0.6738**	**48.9941**	**0.03656**	**0.01845**
Multistage (1°)	**0.8149**	**47.1544**	**0.03857**	**0.02426**
Probit	0.0600	53.5635	0.09134	0.06737
Log-probit	**0.2492**	**49.1272**	**0.05296**	**0.03658**
Weibull	**0.8149**	**47.1544**	**0.03857**	**0.02426**

[a]Chi-square *p* value = *p* value from the chi-square test for lack of fit. Values <0.1 fail to meet conventional goodness-of-fit criteria.

Data source: MRI (1988).

All models with the exception of the logistic and probit models provided adequate fits to the data as assessed by a chi-square goodness-of-fit test ($p \geq 0.1$) and visual inspection of the respective plots of observed versus predicted values from the various models. $BMDL_{10}$ estimates from these models were within a factor of three of each other, suggesting no appreciable model dependence. Fitted models exhibiting adequate fit with AIC values within two units of the lowest AIC were considered indistinguishable from one another; thus BMD_{10} and $BMDL_{10}$ values from these models (gamma, log-logistic, multistage, log-probit, and Weibull) were averaged to derive the POD. Model fits that yielded the same mathematical model were counted as a single model for averaging purposes (these models included gamma, multistage, and Weibull). Therefore, the BMD_{10} and $BMDL_{10}$ values for these models were averaged as follows:

Average BMD_{10} = (0.03857 + 0.03656 + 0.05296) ÷ 3 = 0.04270 mg/kg-day
Average $BMDL_{10}$ = (0.02426 + 0.0184 + 0.03658) ÷ 3 = 0.02641 mg/kg-day

BMDS outputs from the gamma, log-logistic, and log-probit models are presented below.

```
 ================================================================
Gamma Model. (Version: 2.8;  Date: 02/20/2007)
Input Data File: M:\IRIS CHEMICALS\THALLIUM\BMD\POST EPR 6-2008\FEMALE\PILOERECTION-FEMALE.(d)
Gnuplot Plotting File:  M:\IRIS CHEMICALS\THALLIUM\BMD\POST EPR 6-2008\FEMALE\PILOERECTION-
FEMALE.plt
                                                  Fri Jun 13 08:56:08 2008
 ================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response]= background+(1-background)*CumGamma[slope*dose,power],
```

where CumGamma(.) is the cummulative Gamma distribution function

Dependent variable = #Piloerection
Independent variable = Dose(mg/kg-d)
Power parameter is restricted as power >=1

Total number of observations = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial (and Specified) Parameter Values
Background = 0.0121951
Slope = 5.0954
Power = 1.3

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Background -Power
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Slope
Slope	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	NA		
Slope	2.73196	0.831275	1.10269	4.36123
Power	1	NA		

NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus has no standard error.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-21.9144	4			
Fitted model	-22.5772	1	1.32556	3	0.7231
Reduced model	-34.6515	1	25.4742	3	<.0001

AIC: 47.1544

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0	40	0.000
0.0080	0.0216	0.432	0	20	-0.665
0.0400	0.1035	2.070	3	20	0.682
0.2000	0.4210	8.419	8	20	-0.190

Chi^2 = 0.94 d.f. = 3 P-value = 0.8149

Benchmark Dose Computation

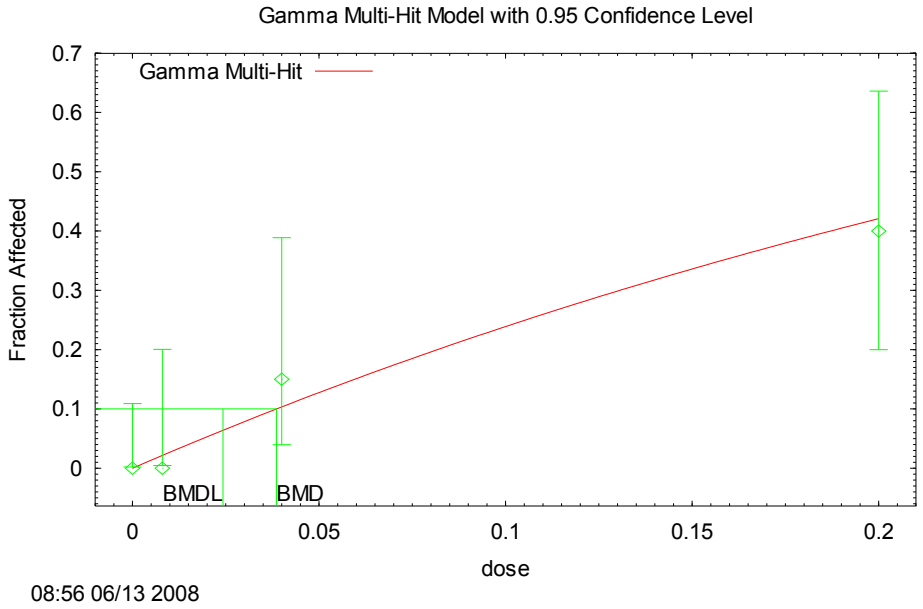
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.0385659

BMDL = 0.024262




```
=================================================================
Logistic Model. (Version: 2.9; Date: 02/20/2007)
Input Data File: M:\IRIS CHEMICALS\THALLIUM\BMD\POST EPR 6-2008\FEMALE\PILOERECTION-FEMALE.(d)
Gnuplot Plotting File:  M:\IRIS CHEMICALS\THALLIUM\BMD\POST EPR 6-2008\FEMALE\PILOERECTION-
FEMALE.plt
                                                    Fri Jun 13 08:56:57 2008
 =================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]

   Dependent variable = #Piloerection
   Independent variable = Dose(mg/kg-d)
   Slope parameter is restricted as slope >= 1

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


   User has chosen the log transformed model


                 Default Initial Parameter Values
                    background =            0
                     intercept =      1.35689
                         slope =      1.02772


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -background
                have been estimated at a boundary point, or have been specified by the user,
                and do not appear in the correlation matrix )

              intercept        slope

 intercept            1         0.92

     slope         0.92            1
```

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
background	0	*	*	*
intercept	1.45513	*	*	*
slope	1.10379	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-21.9144	4			
Fitted model	-22.497	2	1.16526	2	0.5584
Reduced model	-34.6515	1	25.4742	3	<.0001

AIC: 48.9941

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0	40	0.000
0.0080	0.0203	0.407	0	20	-0.644
0.0400	0.1093	2.186	3	20	0.583
0.2000	0.4203	8.407	8	20	-0.184

Chi^2 = 0.79 d.f. = 2 P-value = 0.6738

Benchmark Dose Computation

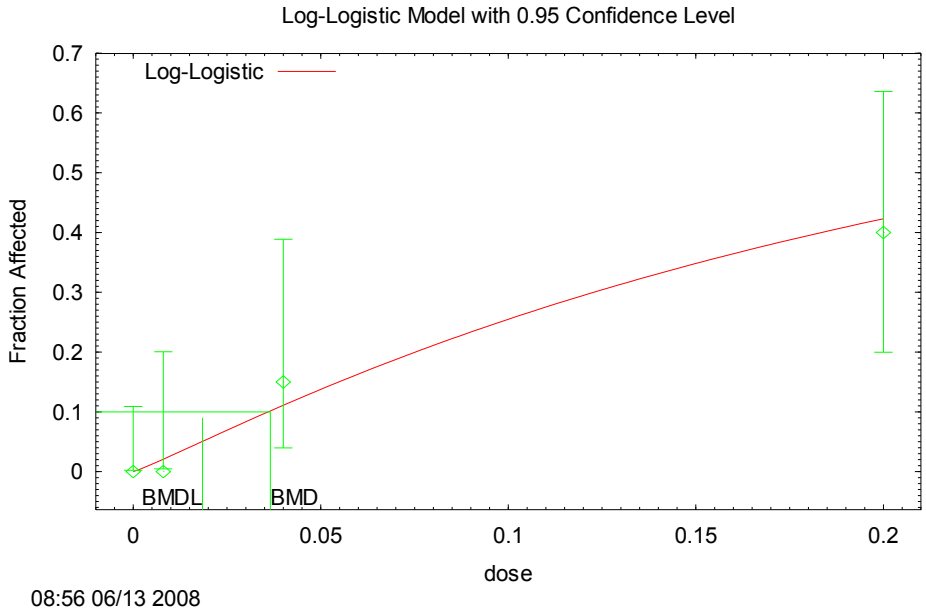
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.0365553

BMDL = 0.018448



```
================================================================
Probit Model. (Version: 2.8;  Date: 02/20/2007)
Input Data File: M:\IRIS CHEMICALS\THALLIUM\BMD\POST EPR 6-2008\FEMALE\PILOERECTION-FEMALE.(d)
Gnuplot Plotting File:  M:\IRIS CHEMICALS\THALLIUM\BMD\POST EPR 6-2008\FEMALE\PILOERECTION-
FEMALE.plt
                                                  Fri Jun 13 08:59:17 2008
 ================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = Background
               + (1-Background) * CumNorm(Intercept+Slope*Log(Dose)),

   where CumNorm(.) is the cumulative normal distribution function


   Dependent variable = #Piloerection
   Independent variable = Dose(mg/kg-d)
   Slope parameter is restricted as slope >= 1

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


   User has chosen the log transformed model


                 Default Initial (and Specified) Parameter Values
                    background =           0
                     intercept =     1.70413
                         slope =           1


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -background    -slope
                have been estimated at a boundary point, or have been specified by the user,
                and do not appear in the correlation matrix )


 intercept           1


                               Parameter Estimates

                                                        95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
    background                0              NA
     intercept          1.65675         0.228852            1.20821             2.10529
         slope                1              NA

NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus
     has no standard error.


                       Analysis of Deviance Table

      Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
    Full model         -21.9144         4
   Fitted model        -23.5636         1       3.29839      3          0.3479
  Reduced model        -34.6515         1       25.4742      3         <.0001

          AIC:          49.1272


                                  Goodness  of  Fit
                                                                    Scaled
    Dose     Est._Prob.    Expected    Observed     Size       Residual
  -----------------------------------------------------------------------
```

```
    0.0000      0.0000          0.000          0          40         0.000
    0.0080      0.0008          0.015          0          20        -0.123
    0.0400      0.0591          1.183          3          20         1.723
    0.2000      0.5189         10.377          8          20        -1.064

 Chi^2 = 4.12      d.f. = 3        P-value = 0.2492


   Benchmark Dose Computation

Specified effect =           0.1

Risk Type        =      Extra risk

Confidence level =          0.95

             BMD =      0.0529554

            BMDL =      0.0365797
```

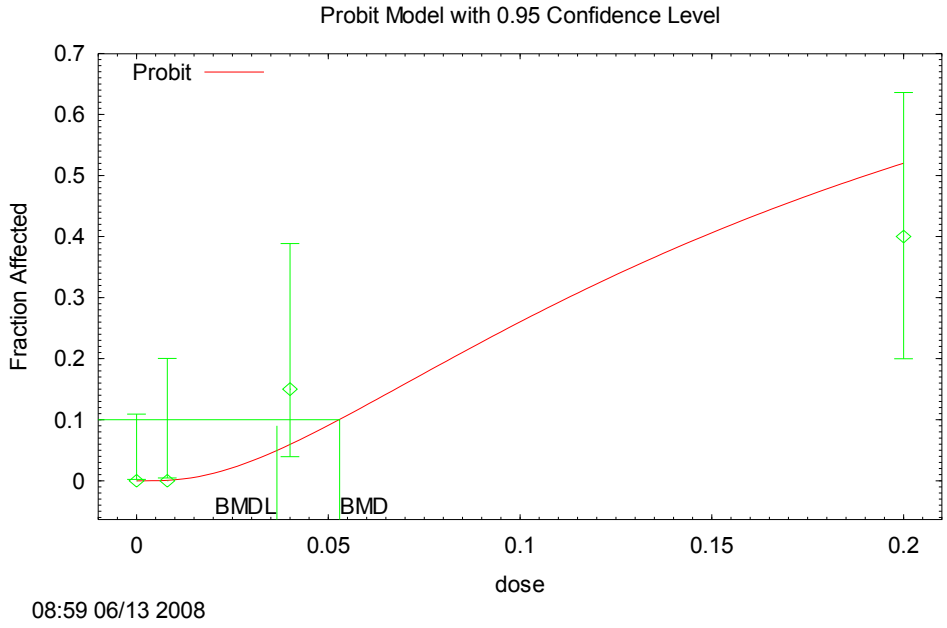


Table B-11. A summary of BMDS (version 1.4.1) modeling results based on incidence of shedding in female Sprague-Dawley rats exposed to thallium sulfate via gavage for 90 days

Model	Chi-square *p* value[a]	AIC	BMD_{10} (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)
Gamma	**0.5894**	**59.2904**	**0.01968**	**0.01347**
Logistic	0.1620	64.9908	0.06624	0.04847
Log-logistic	0.4442	61.4053	0.01453	0.008519
Multistage (1°)	**0.5894**	**59.2904**	**0.01968**	**0.01347**
Probit	0.1783	64.6269	0.05923	0.04416
Log-probit	0.1332	64.1872	0.03710	0.02449
Weibull	**0.5894**	**59.2904**	**0.01968**	**0.01347**

[a]Chi-square *p* value = *p* value from the chi-square test for lack of fit. Values <0.1 fail to meet conventional goodness-of-fit criteria.

Data source: MRI (1988).

All models provided adequate fits of the data as assessed by a chi-square goodness-of-fit test ($p \geq 0.1$) and visual inspection of the respective plots of observed versus predicted values from the various models. The gamma, multistage, and Weibull models provided the same fit of the data and were judged to provide the best model fit based on the lowest AIC value. The BMDS output for the gamma model is provided below.

```
================================================================
Gamma Model. (Version: 2.8;  Date: 02/20/2007)
Input Data File: M:\IRIS CHEMICALS\THALLIUM\BMD\POST EPR 6-2008\FEMALE\SHEDDING-FEMALE.(d)
Gnuplot Plotting File:  M:\IRIS CHEMICALS\THALLIUM\BMD\POST EPR 6-2008\FEMALE\SHEDDING-FEMALE.plt
                                                    Fri Jun 13 09:02:02 2008
 ================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response]= background+(1-background)*CumGamma[slope*dose,power],
   where CumGamma(.) is the cummulative Gamma distribution function

   Dependent variable = #Shedding
   Independent variable = Dose(mg/kg-d)
   Power parameter is restricted as power >=1

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

                  Default Initial (and Specified) Parameter Values
                     Background =    0.0121951
                          Slope =       8.9948
                          Power =      1.64923

           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Background    -Power
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )
                  Slope
     Slope            1


                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     Background                0              NA
          Slope          5.35376          1.30563             2.79477             7.91275
          Power                1              NA

NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus
     has no standard error.


                         Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model         -27.9048         4
   Fitted model         -28.6452         1       1.48083      3          0.6867
  Reduced model         -47.1393         1       38.4691      3         <.0001

           AIC:          59.2904


                                  Goodness  of  Fit
                                                                    Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
```

```
-------------------------------------------------------------------------
   0.0000     0.0000         0.000         0          40         0.000
   0.0080     0.0419         0.839         2          20         1.296
   0.0400     0.1928         3.855         3          20        -0.485
   0.2000     0.6572        13.145        13          20        -0.068

 Chi^2 = 1.92      d.f. = 3        P-value = 0.5894


   Benchmark Dose Computation

Specified effect =           0.1

Risk Type        =      Extra risk

Confidence level =          0.95

             BMD =       0.0196797

            BMDL =      0.0134653
```

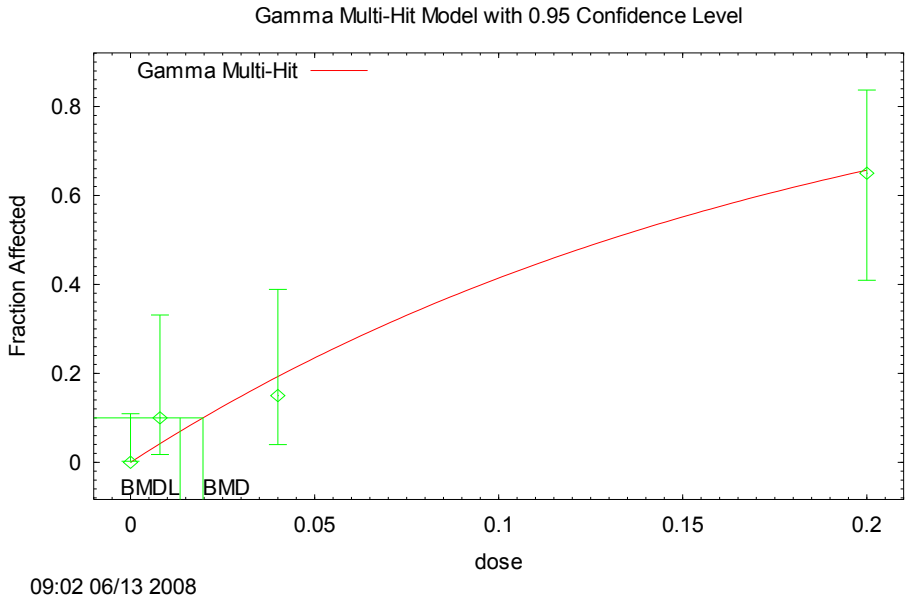


Table B-12. A summary of BMDS (version 1.4.1) modeling results based on incidence of alopecia in female Sprague-Dawley rats exposed to thallium sulfate via gavage for 90 days

Model	Chi-square *p* value[a]	AIC	BMD_{10} (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)
Gamma	**0.2392**	**111.335**	**0.02280**	**0.01389**
Logistic	0.0863	113.282	0.04556	0.03215
Log-logistic	**0.4922**	**109.978**	**0.01338**	**0.00665**
Multistage (1°)	**0.2392**	**111.335**	**0.02280**	**0.01389**
Probit	0.0924	113.141	0.04351	0.03125
Log-probit	0.0695	113.624	0.04057	0.02328
Weibull	**0.2392**	**111.335**	**0.02280**	**0.01389**

[a]Chi-square *p* value = *p* value from the chi-square test for lack of fit. Values <0.1 fail to meet conventional goodness-of-fit criteria.

Data source: MRI (1988).

The gamma, log-logistic, multistage, and Weibull models provided adequate fits to the data as assessed by a chi-square goodness-of-fit test ($p \geq 0.1$) and visual inspection of the respective plots of observed versus predicted values from the various models. $BMDL_{10}$ estimates from these models were within a factor of three of each other, suggesting no appreciable model dependence. Fitted models exhibiting adequate fit with AIC values within two units of the lowest AIC were considered indistinguishable from one another; thus, BMD_{10} and $BMDL_{10}$ values from these models (gamma, log-logistic, multistage, and Weibull) were averaged to derive the POD. Model fits that yielded the same mathematical model were counted as a single model for averaging purposes (these models included gamma, multistage, and Weibull). Therefore, the BMD_{10} and $BMDL_{10}$ values for these models were averaged as follows:

Average BMD_{10} = (0.02280 + 0.01338) ÷ 2 = 0.01809 mg/kg-day
Average $BMDL_{10}$ = (0.01389 + 0.00665) ÷ 2 = 0.01027 mg/kg-day

BMDS outputs from the gamma and log-logistic models are provided below.

```
================================================================
          Gamma Model. (Version: 2.8;  Date: 02/20/2007)
          Input Data File: M:\IRIS CHEMICALS\THALLIUM\BMD\POST EPR 6-2008\FEMALE\ALOPECIA-
FEMALE.(d)
          Gnuplot Plotting File:  M:\IRIS CHEMICALS\THALLIUM\BMD\POST EPR 6-2008\FEMALE\ALOPECIA-
FEMALE.plt
                                                    Fri Jun 13 09:06:01 2008
 ================================================================


 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response]= background+(1-background)*CumGamma[slope*dose,power],
   where CumGamma(.) is the cummulative Gamma distribution function
   Dependent variable = #Alopecia
   Independent variable = Dose(mg/kg-d)
   Power parameter is restricted as power >=1

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                  Default Initial (and Specified) Parameter Values
                     Background =     0.134146
                          Slope =       17.289
                          Power =          1.3


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Power
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

             Background        Slope

Background            1        -0.42
```

Slope -0.42 1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.160192	0.0508961	0.0604373	0.259946
Slope	4.62078	1.59736	1.49	7.75156
Power	1	NA		

NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus has no standard error.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-52.3019	4			
Fitted model	-53.6677	2	2.73165	2	0.2552
Reduced model	-61.0864	1	17.5691	3	0.0005397

AIC: 111.335

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.1602	6.408	5	40	-0.607
0.0080	0.1907	3.813	4	20	0.106
0.0400	0.3019	6.038	9	20	1.443
0.2000	0.6667	13.334	12	20	-0.633

Chi^2 = 2.86 d.f. = 2 P-value = 0.2392

Benchmark Dose Computation

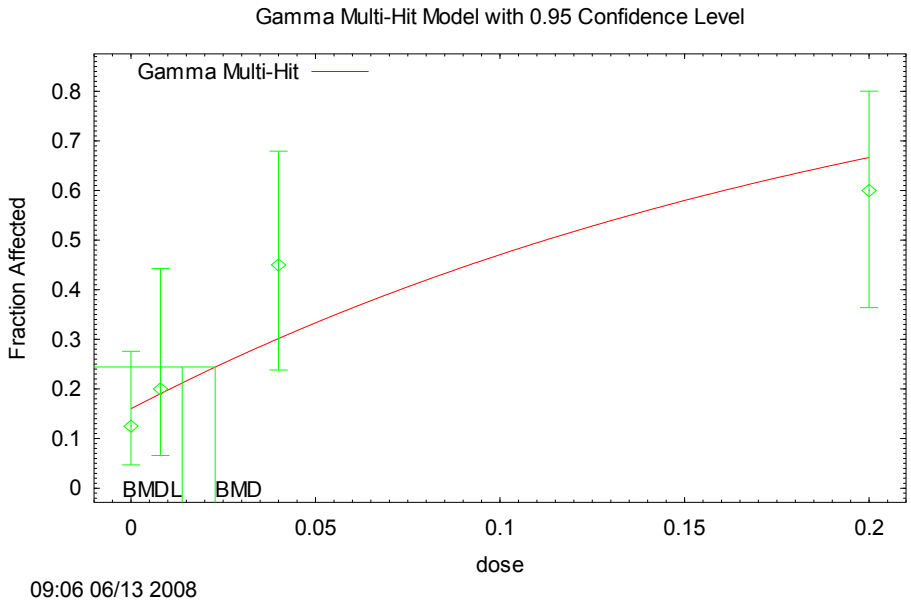
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.0228015

BMDL = 0.0138895



```
 ================================================================
Logistic Model. (Version: 2.9; Date: 02/20/2007)
Input Data File: M:\IRIS CHEMICALS\THALLIUM\BMD\POST EPR 6-2008\FEMALE\ALOPECIA-FEMALE.(d)
Gnuplot Plotting File:  M:\IRIS CHEMICALS\THALLIUM\BMD\POST EPR 6-2008\FEMALE\ALOPECIA-FEMALE.plt
                                                    Fri Jun 13 09:07:14 2008
 ================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]

   Dependent variable = #Alopecia
   Independent variable = Dose(mg/kg-d)
   Slope parameter is restricted as slope >= 1

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

   User has chosen the log transformed model

                 Default Initial Parameter Values
                    background =        0.125
                     intercept =      2.11821
                         slope =            1

           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -slope
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

             background    intercept

background            1        -0.48

 intercept        -0.48            1


                                Parameter Estimates
                                                        95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
    background         0.138817            *                *                   *
     intercept          2.11678            *                *                   *
         slope                1            *                *                   *

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -52.3019         4
   Fitted model        -52.9891         2        1.3744      2           0.503
  Reduced model        -61.0864         1       17.5691      3       0.0005397

           AIC:         109.978

                                Goodness  of  Fit
                                                                    Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------
    0.0000     0.1388         5.553         5          40       -0.253
    0.0080     0.1925         3.849         4          20        0.085
    0.0400     0.3536         7.071         9          20        0.902
    0.2000     0.6764        13.527        12          20       -0.730

 Chi^2 = 1.42      d.f. = 2        P-value = 0.4922

   Benchmark Dose Computation
```

```
Specified effect =           0.1

Risk Type        =      Extra risk

Confidence level =          0.95

             BMD =     0.0133799

            BMDL =     0.0066454
```

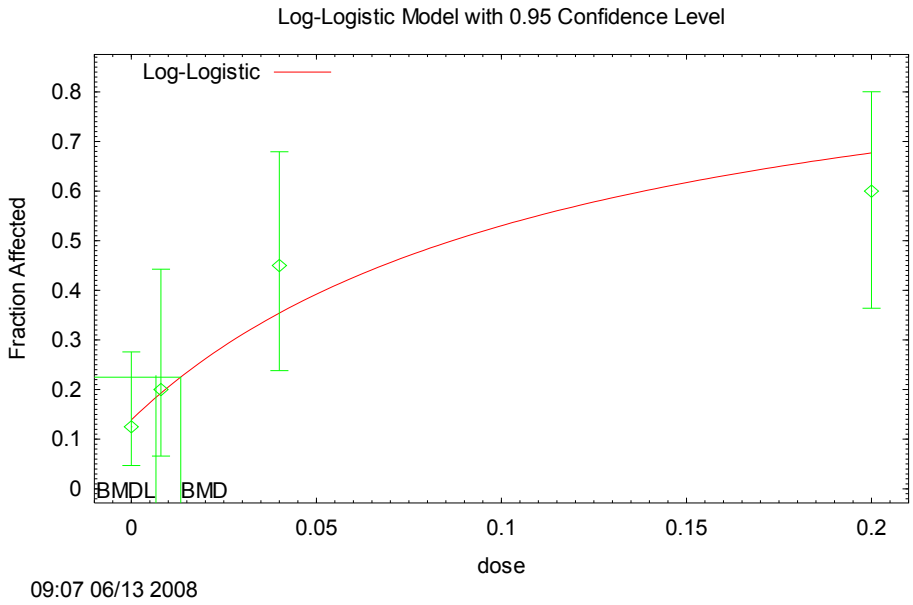


Table B-13. A summary of BMDS (version 1.4.1) modeling results based on incidence of lacrimation in female Sprague-Dawley rats exposed to thallium sulfate via gavage for 90 days

Model	Chi-square *p* value[a]	AIC	BMD_{10} (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)
Gamma	1.0000	54.4465	4.808×10^{-5}	BMD computation failed. Lower limit includes zero.
Logistic	1.0000	54.4465	0.0001258	
Log-logistic	1.0000	54.4465	1.517×10^{-11}	
Multistage (2°)	0.9996	56.4465	4.882×10^{-5}	8.1310×10^{-9}
Probit	1.0000	54.4465	0.00024051	BMD computation failed. Lower limit includes zero.
Log-probit	1.0000	54.4465	9.098×10^{-6}	
Weibull	1.0000	54.4465	4.72×10^{-5}	

[a]Chi-square *p* value = *p* value from the chi-square test for lack of fit. Values <0.1 fail to meet conventional goodness-of-fit criteria.

Data source: MRI (1988).

All models in BMDS failed to estimate useful BMD and BMDL values without excessive extrapolation.

Table B-14. A summary of BMDS (version 1.4.1) modeling results based on incidence of exophthalmos in female Sprague-Dawley rats exposed to thallium sulfate via gavage for 90 days

Model	Chi-square *p* value[a]	AIC	BMD_{10} (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)
Gamma	0.9983	60.9941	0.0005049	0.0001719
Logistic	1.0000	58.9941	0.0006936	0.0004144
Log-logistic	0.9996	60.9941	0.004856	6.86×10^{-6}
Multistage (2°)	1.0000	60.9941	0.001588	0.0001719
Probit	1.0000	58.9941	0.0007337	0.0004976
Log-probit	0.9969	60.9941	0.002044	0.0001944
Weibull	0.9962	60.9942	0.0003155	0.0001719

[a]Chi-square *p* value = *p* value from the chi-square test for lack of fit. Values <0.1 fail to meet conventional goodness-of-fit criteria.

Data source: MRI (1988).

The data set was not considered amenable to modeling because of the steep slope of the dose-response curve in the low-dose region and because the BMD_{10} and $BMDL_{10}$ values were well outside the experimental range of the data. Of the models with the lowest AIC values, the one with the lowest $BMDL_{10}$ (i.e., the logistic model) is presented below.

```
 ========================================================================
Logistic Model. (Version: 2.9; Date: 02/20/2007)
Input Data File: M:\IRIS CHEMICALS\THALLIUM\BMD\POST EPR 6-2008\FEMALE\EXOPHTHALMOS-FEMALE.(d)
Gnuplot Plotting File:  M:\IRIS CHEMICALS\THALLIUM\BMD\POST EPR 6-2008\FEMALE\EXOPHTHALMOS-
FEMALE.plt
                                                    Fri Jun 13 09:15:35 2008
 ========================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = 1/[1+EXP(-intercept-slope*dose)]

   Dependent variable = #Exophthalmos
   Independent variable = Dose(mg/kg-d)
   Slope parameter is not restricted

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                 Default Initial Parameter Values
                    background =            0   Specified
                     intercept =      1.43545
                         slope =      13.3397


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -background
                have been estimated at a boundary point, or have been specified by the user,
                and do not appear in the correlation matrix )
```

	intercept	slope
intercept	1	-0.33
slope	-0.33	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
intercept	-0.96942	0.354104	-1.66345	-0.275389
slope	489.249	135.678	223.326	755.172

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-27.4971	4			
Fitted model	-27.4971	2	3.53613e-007	2	1
Reduced model	-61.0864	1	67.1787	3	<.0001

AIC: 58.9941

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.2750	11.000	11	40	0.000
0.0080	0.9500	19.000	19	20	-0.000
0.0400	1.0000	20.000	20	20	0.000
0.2000	1.0000	20.000	20	20	0.000

Chi^2 = 0.00 d.f. = 2 P-value = 1.0000

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.000693631

BMDL = 0.000414359

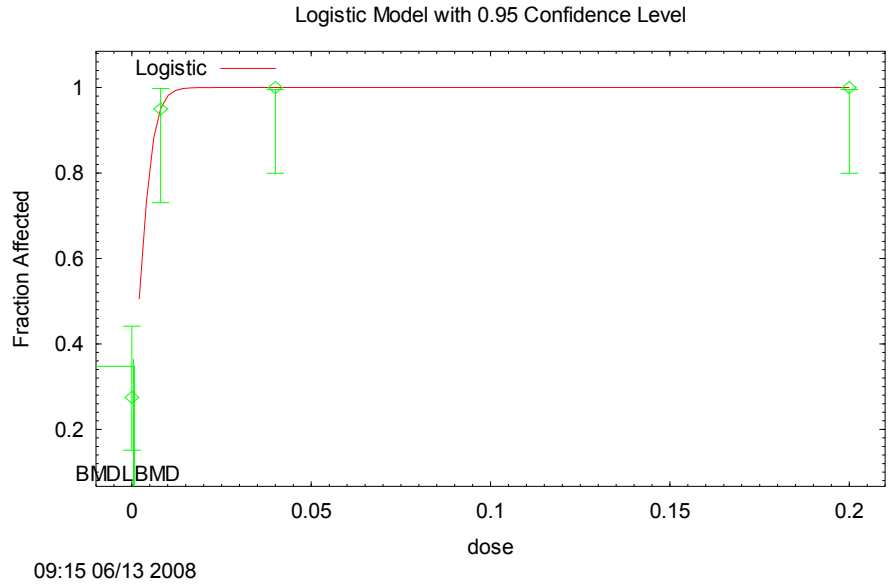


Table B-15. A summary of BMDS (version 1.4.1) modeling results based on incidence of miosis in female Sprague-Dawley rats exposed to thallium sulfate via gavage for 90 days

Model	Chi-square *p* value[a]	AIC	BMD_{10} (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)
Gamma	0.0010	109.237	0.03729	0.02024
Logistic	0.0004	111.015	0.07143	0.04590
Log-logistic	0.0028	107.854	0.02252	0.01048
Multistage (1°)	0.0010	109.237	0.03729	0.02024
Probit	0.0004	110.867	0.06780	0.04360
Log-probit	0.0001	112.733	0.08222	0.03640
Weibull	0.0010	109.237	0.03729	0.02024

[a]Chi-square *p* value = *p* value from the chi-square test for lack of fit. Values <0.1 fail to meet conventional goodness-of-fit criteria.

Data source: MRI (1988).

This data set was not fit by any of the models in BMDS.

Table B-16. A summary of BMDS (version 1.4.1) modeling results based on incidence of behavioral findings in female Sprague-Dawley rats exposed to thallium sulfate via gavage for 90 days

Model	Chi-square *p* value[a]	AIC	BMD_{10} (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)
Gamma	0.1256	69.3045	0.1594	0.04585
Logistic	0.2197	67.905	0.1009	0.07207
Log-logistic	0.1256	69.3045	0.1736	0.04377
Multistage (2°)	0.2766	67.482	0.1081	0.04445
Probit	0.2100	68.0129	0.09473	0.06621
Log-probit	0.1256	69.3045	0.1541	0.05892
Weibull	0.1256	69.3045	0.1760	0.04585

[a]Chi-square *p* value = *p* value from the chi-square test for lack of fit. Values <0.1 fail to meet conventional goodness-of-fit criteria.

Data source: MRI (1988).

All of the models provided adequate fits of the data as assessed by a chi-square goodness-of-fit test ($p \geq 0.1$) and visual inspection of the respective plots of observed versus predicted values from the various models. $BMDL_{10}$ estimates from these models were within a factor of three of each other, suggesting no appreciable model dependence. Fitted models exhibiting adequate fit with AIC values within two units of the lowest AIC were considered indistinguishable from one another; thus BMD_{10} and $BMDL_{10}$ values from all seven models were averaged to derive the POD. Therefore, the BMD_{10} and $BMDL_{10}$ values for these models were averaged as follows:

Average BMD_{10} = (0.1594 + 0.1009 + 0.1736 + 0.1081 + 0.09473 + 0.1541 + 0.1760) ÷ 7 = 0.1381 mg/kg-day

Average $BMDL_{10}$ = (0.04585 + 0.07207 + 0.04377 + 0.04445 + 0.06621 + 0.05892 + 0.04585) ÷ 7 = 0.05387 mg/kg-day

BMDS outputs from each model are provided below.

```
 ======================================================================
Gamma Model. (Version: 2.8;  Date: 02/20/2007)
Input Data File: M:\IRIS CHEMICALS\THALLIUM\BMD\POST EPR 6-2008\FEMALE\BEHAVIOR-FEMALE.(d)
Gnuplot Plotting File:  M:\IRIS CHEMICALS\THALLIUM\BMD\POST EPR 6-2008\FEMALE\BEHAVIOR-FEMALE.plt
                                                  Fri Jun 13 09:23:45 2008
 ======================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response]= background+(1-background)*CumGamma[slope*dose,power],
   where CumGamma(.) is the cummulative Gamma distribution function

   Dependent variable = #Behavior
   Independent variable = Dose(mg/kg-d)
   Power parameter is restricted as power >=1

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                  Default Initial (and Specified) Parameter Values
                     Background =     0.109756
                          Slope =      7.56372
                          Power =      2.63978


           Asymptotic Correlation Matrix of Parameter Estimates

             Background        Slope        Power

Background            1      -0.0024      -0.0022

     Slope      -0.0024            1            1

     Power      -0.0022            1            1


                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
    Background           0.0625       0.0270635          0.00945649            0.115543
         Slope          59.7049          4193.5            -8159.41             8278.82
         Power          14.0574         900.278            -1750.45             1778.57


                        Analysis of Deviance Table

      Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -29.9226         4
   Fitted model        -31.6523         3       3.45942      1         0.06289
  Reduced model        -36.6925         1       13.5399      3        0.003603

          AIC:         69.3045
```

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0625	2.500	4	40	0.980
0.0080	0.0625	1.250	0	20	-1.155
0.0400	0.0625	1.250	1	20	-0.231
0.2000	0.3500	7.000	7	20	0.000

Chi^2 = 2.35 d.f. = 1 P-value = 0.1256

Benchmark Dose Computation

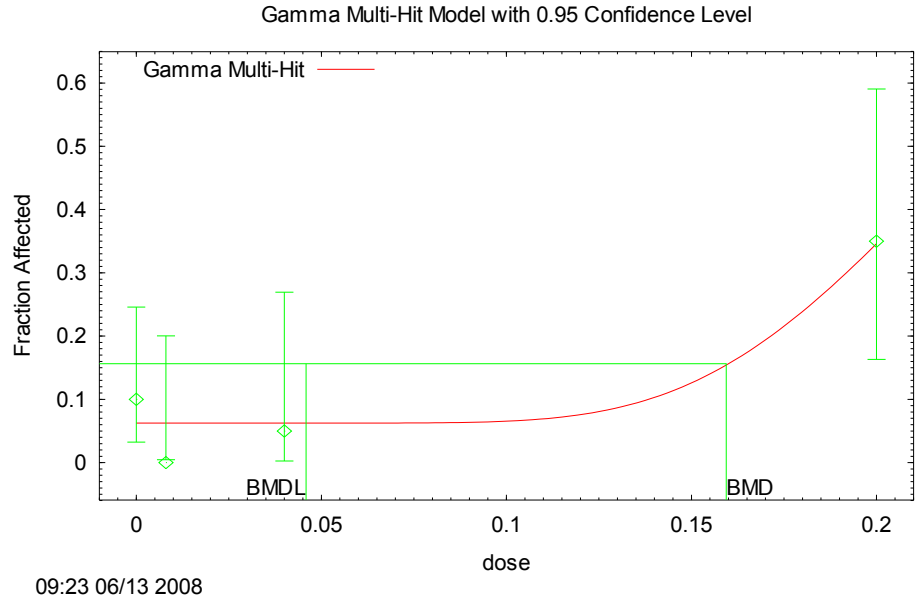
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.159402

BMDL = 0.0458492



==========

Logistic Model. (Version: 2.9; Date: 02/20/2007)
Input Data File: M:\IRIS CHEMICALS\THALLIUM\BMD\POST EPR 6-2008\FEMALE\BEHAVIOR-FEMALE.(d)
Gnuplot Plotting File: M:\IRIS CHEMICALS\THALLIUM\BMD\POST EPR 6-2008\FEMALE\BEHAVIOR-FEMALE.plt
Fri Jun 13 09:24:08 2008

==========

BMDS MODEL RUN

~~~~~~~~~~

The form of the probability function is:

P[response] = 1/[1+EXP(-intercept-slope*dose)]

Dependent variable = #Behavior
Independent variable = Dose(mg/kg-d)
Slope parameter is not restricted

Total number of observations = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
~~~~~~~~~~

Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
background = 0 Specified
intercept = -2.96071
slope = 11.6261

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -background
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	intercept	slope
intercept	1	-0.74
slope	-0.74	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
intercept	-2.81211	0.489479	-3.77147	-1.85275
slope	10.758	3.50284	3.89255	17.6234

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-29.9226	4			
Fitted model	-31.9525	2	4.05988	2	0.1313
Reduced model	-36.6925	1	13.5399	3	0.003603

AIC: 67.905

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0567	2.267	4	40	1.185
0.0080	0.0615	1.229	0	20	-1.144
0.0400	0.0846	1.691	1	20	-0.556
0.2000	0.3406	6.813	7	20	0.088

Chi^2 = 3.03 d.f. = 2 P-value = 0.2197

Benchmark Dose Computation

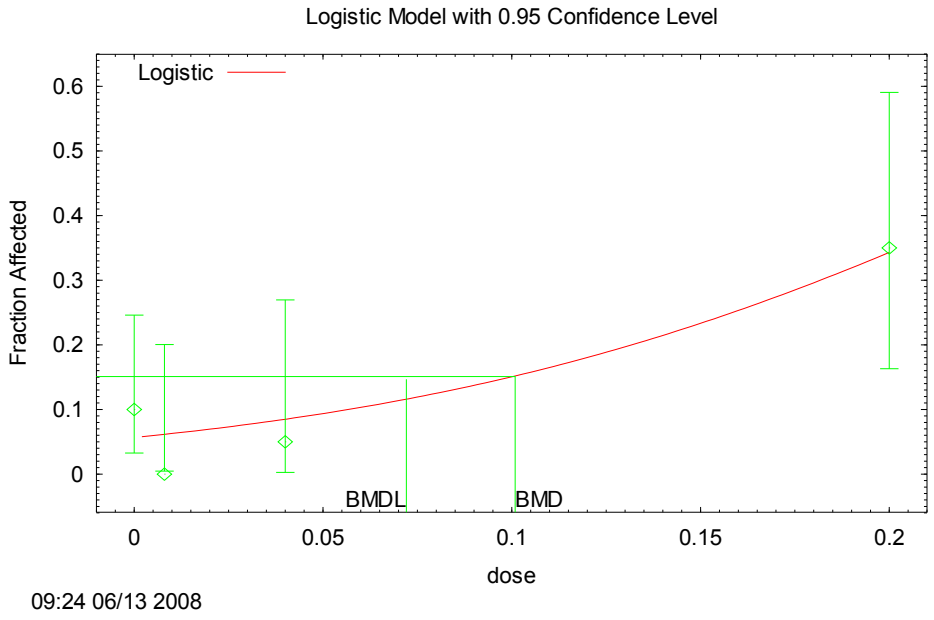
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.10089

BMDL = 0.0720676




```
 ======================================================================
Logistic Model. (Version: 2.9; Date: 02/20/2007)
Input Data File: M:\IRIS CHEMICALS\THALLIUM\BMD\POST EPR 6-2008\FEMALE\BEHAVIOR-FEMALE.(d)
Gnuplot Plotting File:  M:\IRIS CHEMICALS\THALLIUM\BMD\POST EPR 6-2008\FEMALE\BEHAVIOR-FEMALE.plt
                                                    Fri Jun 13 09:24:29 2008
 ======================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]

   Dependent variable = #Behavior
   Independent variable = Dose(mg/kg-d)
   Slope parameter is restricted as slope >= 1

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


   User has chosen the log transformed model


                  Default Initial Parameter Values
                     background =          0.1
                      intercept =     0.293481
                          slope =            1


           Asymptotic Correlation Matrix of Parameter Estimates

             background    intercept        slope

background            1       0.0045       0.0046

 intercept       0.0045            1            1

     slope       0.0046            1            1


                                Parameter Estimates
```

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
background	0.0625	*	*	*
intercept	14.8768	*	*	*
slope	9.75033	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-29.9226	4			
Fitted model	-31.6523	3	3.45942	1	0.06289
Reduced model	-36.6925	1	13.5399	3	0.003603

AIC: 69.3045

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0625	2.500	4	40	0.980
0.0080	0.0625	1.250	0	20	-1.155
0.0400	0.0625	1.250	1	20	-0.231
0.2000	0.3500	7.000	7	20	-0.000

Chi^2 = 2.35 d.f. = 1 P-value = 0.1256

Benchmark Dose Computation

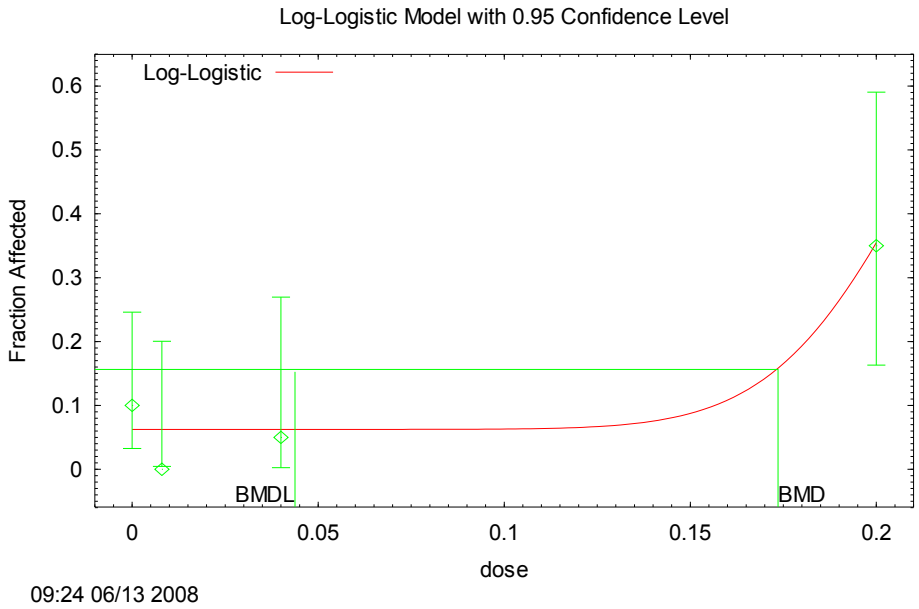
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

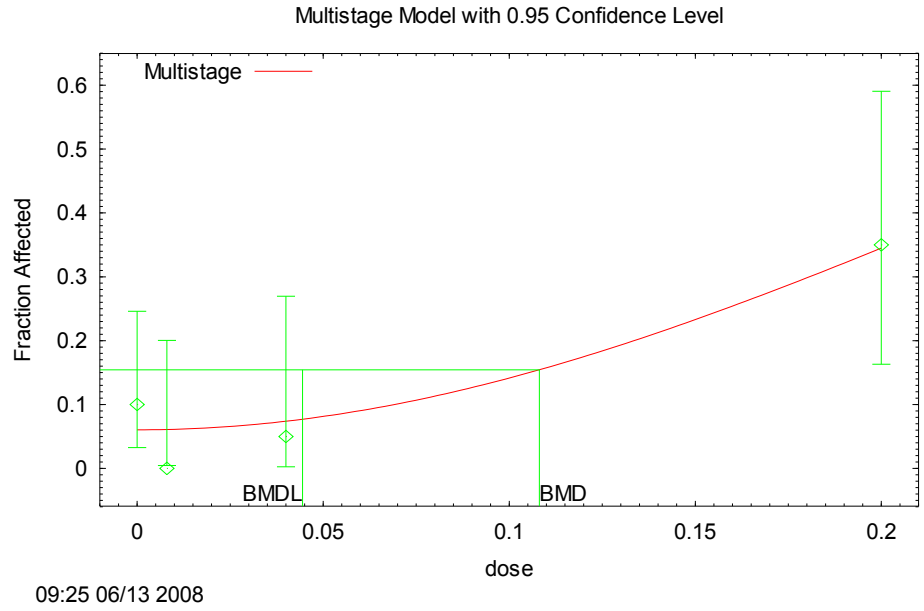
BMD = 0.173579

BMDL = 0.0437732



```
 ======================================================================
Multistage Model. (Version: 2.8;  Date: 02/20/2007)
Input Data File: M:\IRIS CHEMICALS\THALLIUM\BMD\POST EPR 6-2008\FEMALE\BEHAVIOR-FEMALE.(d)
Gnuplot Plotting File:  M:\IRIS CHEMICALS\THALLIUM\BMD\POST EPR 6-2008\FEMALE\BEHAVIOR-FEMALE.plt
                                                    Fri Jun 13 09:25:07 2008
 ======================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                -beta1*dose^1-beta2*dose^2)]

   The parameter betas are restricted to be positive

   Dependent variable = #Behavior
   Independent variable = Dose(mg/kg-d)

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008


                  Default Initial Parameter Values
                     Background =    0.0459871
                        Beta(1) =            0
                        Beta(2) =       9.5796


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Beta(1)
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

             Background      Beta(2)

Background            1         -0.4

   Beta(2)         -0.4            1


                                Parameter Estimates

                                                        95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
    Background         0.060443            *                *                   *
       Beta(1)                0            *                *                   *
       Beta(2)          9.01875            *                *                   *

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model         -29.9226         4
   Fitted model          -31.741         2       3.63685      2          0.1623
  Reduced model         -36.6925         1       13.5399      3        0.003603

           AIC:           67.482


                                 Goodness  of  Fit
                                                                      Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
```

```
 ------------------------------------------------------------------------
   0.0000     0.0604         2.418          4          40         1.050
   0.0080     0.0610         1.220          0          20        -1.140
   0.0400     0.0739         1.478          1          20        -0.409
   0.2000     0.3450         6.900          7          20         0.047

 Chi^2 = 2.57      d.f. = 2        P-value = 0.2766


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =       0.108085

            BMDL =      0.0444457

            BMDU =       0.173737

Taken together, (0.0444457, 0.173737) is a 90     % two-sided confidence
interval for the BMD
```




```
 ========================================================================
Probit Model. (Version: 2.8;  Date: 02/20/2007)
Input Data File: M:\IRIS CHEMICALS\THALLIUM\BMD\POST EPR 6-2008\FEMALE\BEHAVIOR-FEMALE.(d)
Gnuplot Plotting File:  M:\IRIS CHEMICALS\THALLIUM\BMD\POST EPR 6-2008\FEMALE\BEHAVIOR-FEMALE.plt
                                                    Fri Jun 13 09:25:44 2008
 ========================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = CumNorm(Intercept+Slope*Dose),

   where CumNorm(.) is the cumulative normal distribution function


   Dependent variable = #Behavior
   Independent variable = Dose(mg/kg-d)
   Slope parameter is not restricted
```

Total number of observations = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial (and Specified) Parameter Values
background = 0 Specified
intercept = -1.68929
slope = 6.25307

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -background
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	intercept	slope
intercept	1	-0.66
slope	-0.66	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
intercept	-1.58535	0.232401	-2.04084	-1.12985
slope	5.83053	1.91096	2.08512	9.57595

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-29.9226	4			
Fitted model	-32.0065	2	4.16782	2	0.1244
Reduced model	-36.6925	1	13.5399	3	0.003603

AIC: 68.0129

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0564	2.258	4	40	1.194
0.0080	0.0619	1.239	0	20	-1.149
0.0400	0.0882	1.763	1	20	-0.602
0.2000	0.3375	6.750	7	20	0.118

Chi^2 = 3.12 d.f. = 2 P-value = 0.2100

Benchmark Dose Computation

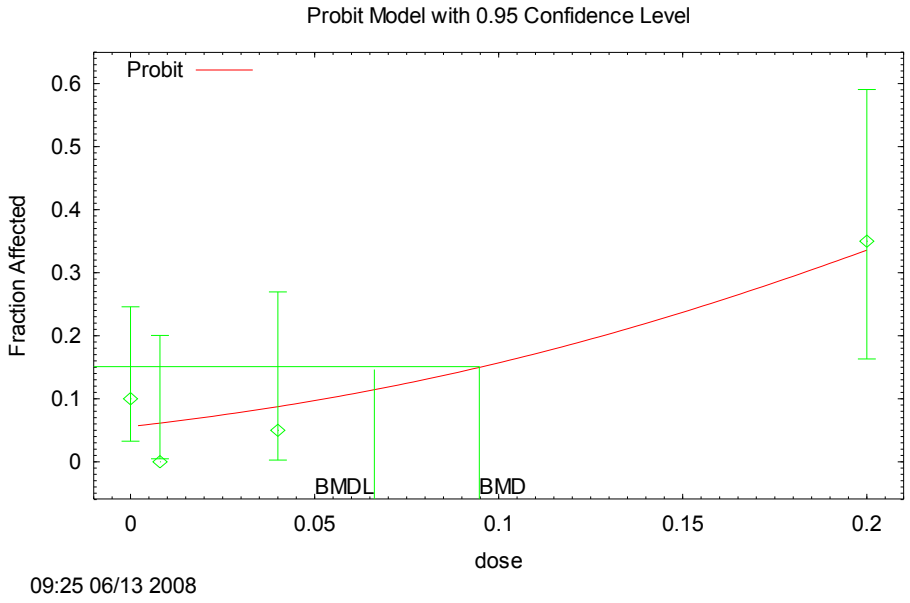
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.0947316

BMDL = 0.0662085




```
 ========================================================================
Probit Model. (Version: 2.8;  Date: 02/20/2007)
Input Data File: M:\IRIS CHEMICALS\THALLIUM\BMD\POST EPR 6-2008\FEMALE\BEHAVIOR-FEMALE.(d)
Gnuplot Plotting File:  M:\IRIS CHEMICALS\THALLIUM\BMD\POST EPR 6-2008\FEMALE\BEHAVIOR-FEMALE.plt
                                                    Fri Jun 13 09:26:03 2008
 ========================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = Background
               + (1-Background) * CumNorm(Intercept+Slope*Log(Dose)),

   where CumNorm(.) is the cumulative normal distribution function

   Dependent variable = #Behavior
   Independent variable = Dose(mg/kg-d)
   Slope parameter is restricted as slope >= 1

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


   User has chosen the log transformed model


                  Default Initial (and Specified) Parameter Values
                     background =          0.1
                      intercept =      1.18165
                          slope =            1


           Asymptotic Correlation Matrix of Parameter Estimates

             background    intercept        slope

background            1       -0.001     -0.00087

 intercept       -0.001            1            1

     slope     -0.00087            1            1
```

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
background	0.0625	0.0270635	0.00945646	0.115544
intercept	4.28948	360.951	-703.162	711.741
slope	2.97918	224.271	-436.585	442.543

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-29.9226	4			
Fitted model	-31.6523	3	3.45942	1	0.06289
Reduced model	-36.6925	1	13.5399	3	0.003603

AIC: 69.3045

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0625	2.500	4	40	0.980
0.0080	0.0625	1.250	0	20	-1.155
0.0400	0.0625	1.250	1	20	-0.231
0.2000	0.3500	7.000	7	20	0.000

Chi^2 = 2.35 d.f. = 1 P-value = 0.1256

Benchmark Dose Computation

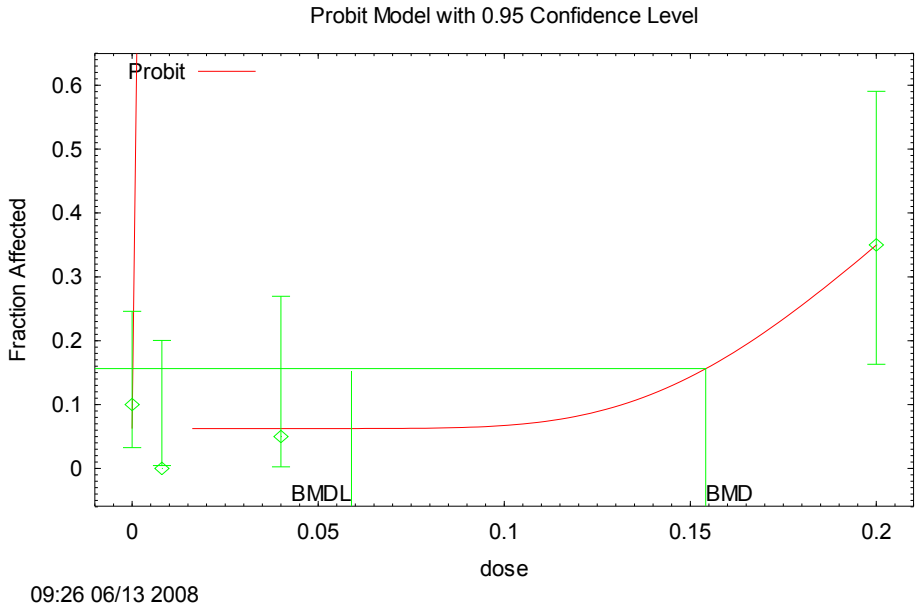
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.154125

BMDL = 0.0589218



```
 ================================================================
Weibull Model using Weibull Model (Version: 2.7;  Date: 2/20/2007)
Input Data File: M:\IRIS CHEMICALS\THALLIUM\BMD\POST EPR 6-2008\FEMALE\BEHAVIOR-FEMALE.(d)
Gnuplot Plotting File:  M:\IRIS CHEMICALS\THALLIUM\BMD\POST EPR 6-2008\FEMALE\BEHAVIOR-FEMALE.plt
                                                  Fri Jun 13 09:26:52 2008
 ================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(-slope*dose^power)]

   Dependent variable = #Behavior
   Independent variable = Dose(mg/kg-d)
   Power parameter is restricted as power >=1

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                 Default Initial (and Specified) Parameter Values
                    Background =     0.109756
                         Slope =      9.64766
                         Power =      2.10563

          Asymptotic Correlation Matrix of Parameter Estimates

            Background        Slope        Power

Background            1        0.017        0.017

     Slope        0.017            1            1

     Power        0.017            1            1

                               Parameter Estimates

                                                        95.0% Wald Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
    Background           0.0625        0.0270671          0.00944955             0.11555
         Slope     2.30426e+006     1.04698e+011       -2.05203e+011        2.05207e+011
         Power          9.72683          28231.5              -55323             55342.4

                         Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model         -29.9226         4
   Fitted model         -31.6523         3       3.45942      1         0.06289
  Reduced model         -36.6925         1       13.5399      3        0.003603

           AIC:          69.3045

                                 Goodness  of  Fit
                                                                      Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------
    0.0000     0.0625         2.500         4          40        0.980
    0.0080     0.0625         1.250         0          20       -1.155
    0.0400     0.0625         1.250         1          20       -0.231
    0.2000     0.3500         7.000         7          20        0.000

 Chi^2 = 2.35      d.f. = 1        P-value = 0.1256


   Benchmark Dose Computation

Specified effect =           0.1

Risk Type        =      Extra risk
```

```
Confidence level =           0.95

             BMD =       0.175955

            BMDL =     0.0458492
```