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Provisional Peer-Reviewed Toxicity Values for

Trinitrophenylmethylnitramine
(CASRN 479-45-8)

Superfund Health Risk Technical Support Center
National Center for Environmental Assessment
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Questions regarding the contents of this document may be directed to the U.S. EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300).

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COMMONLY USED ABBREVIATIONS

BMC	benchmark concentration
BMCL	benchmark concentration lower confidence limit
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
HEC	human equivalent concentration
HED	human equivalent dose
IUR	inhalation unit risk
LOAEL	lowest-observed-adverse-effect level
$LOAEL_{ADJ}$	LOAEL adjusted to continuous exposure duration
$LOAEL_{HEC}$	LOAEL adjusted for dosimetric differences across species to a human
NOAEL	no-observed-adverse-effect level
$NOAEL_{ADJ}$	NOAEL adjusted to continuous exposure duration
$NOAEL_{HEC}$	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	provisional inhalation unit risk
POD	point of departure
p-OSF	provisional oral slope factor
p-RfC	provisional inhalation reference concentration
p-RfD	provisional oral reference dose
RfC	inhalation reference concentration
RfD	oral reference dose
UF	uncertainty factor
UF_A	interspecies uncertainty factor
UF_C	composite uncertainty factor
UF_D	database uncertainty factor
UF_H	intraspecies uncertainty factor
UF_L	LOAEL-to-NOAEL uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
WOE	weight of evidence

PROVISIONAL PEER-REVIEWED TOXICITY VALUES FOR TRINITROPHENYLMETHYLNITRAMINE (CASRN 479-45-8)

BACKGROUND

A Provisional Peer-Reviewed Toxicity Value (PPRTV) is defined as a toxicity value derived for use in the Superfund Program. PPRTVs are derived after a review of the relevant scientific literature using established Agency guidance on human health toxicity value derivations. All PPRTV assessments receive internal review by a standing panel of National Center for Environment Assessment (NCEA) scientists and an independent external peer review by three scientific experts.

The purpose of this document is to provide support for the hazard and dose-response assessment pertaining to chronic and subchronic exposures to substances of concern, to present the major conclusions reached in the hazard identification and derivation of the PPRTVs, and to characterize the overall confidence in these conclusions and toxicity values. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of this substance.

The PPRTV review process provides needed toxicity values in a quick turnaround timeframe while maintaining scientific quality. PPRTV assessments are updated approximately on a 5-year cycle for new data or methodologies that might impact the toxicity values or characterization of potential for adverse human health effects and are revised as appropriate. It is important to utilize the PPRTV database (http://hhpprtv.ornl.gov) to obtain the current information available. When a final Integrated Risk Information System (IRIS) assessment is made publicly available on the Internet (http://www.epa.gov/iris), the respective PPRTVs are removed from the database.

DISCLAIMERS

The PPRTV document provides toxicity values and information about the adverse effects of the chemical and the evidence on which the value is based, including the strengths and limitations of the data. All users are advised to review the information provided in this document to ensure that the PPRTV used is appropriate for the types of exposures and circumstances at the site in question and the risk management decision that would be supported by the risk assessment.

Other U.S. Environmental Protection Agency (EPA) programs or external parties who may choose to use PPRTVs are advised that Superfund resources will not generally be used to respond to challenges, if any, of PPRTVs used in a context outside of the Superfund program.

QUESTIONS REGARDING PPRTVs

Questions regarding the contents and appropriate use of this PPRTV assessment should be directed to the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300).

INTRODUCTION

Trinitrophenylmethylnitramine (also known as tetryl) has many synonyms, including: aniline, *N*-methyl-*N*,2,4,6-tetranitro-; benzeneamine, *N*-methyl-*N*,2,4,6-tetranitro-; CE; *N*-methyl-*N*-2,4,6-tetranitrobenzeneamine; nitramine; picrylmethylnitramine; *N*-picryl-*N*-methylnitramine; picrylnitromethylamine; tetralit; tetralite; tetril; *N*-methyl-*N*,2,4,6-tetranitroaniline; 2,4,6-tetryl; 2,4,6-trinitrophenyl-*n*-methylnitramine; 2,4,6-trinitrophenylmethylnitramine; *N*-2,4,6-tetranitro-*N*-methylaniline; and methylpicrylnitramine (HSDB, 2005) (see Figure 1). Tetryl was formerly used as a military explosive, but is no longer used or manufactured in the United States (ATSDR, 1995). It is explosive when exposed to heat or flame (ATSDR, 1995; IPCS, 1997). Table 1 provides a list of tetryl's physicochemical properties.

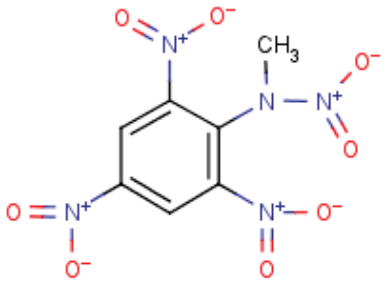


Figure 1. Trinitrophenylmethylnitramine Structure

Table 1. Physicochemical Properties of Trinitrophenylmethylnitramine (CASRN 479-45-8)[a]

Property (unit)	Value
Boiling point (ºC)	187
Melting point (ºC)	130−132
Density (g/cm^3) (at 19ºC)	1.57
Vapor pressure (Pa at 25ºC)	1.2×10^{-7}[b]
pH (unitless)	ND
Solubility in fresh water (mg/L at 20ºC) Solubility in salt water (mg/L at 25ºC)	75 26
Relative vapor density (air = 1)	ND
Molecular weight (g/mol)	287.14

[a]Values from ATSDR (1995) and HSDB (2005).
[b]Estimated value—model not provided.

ND = no data.

A summary of available toxicity values for trinitrophenylmethylnitramine from U.S. EPA and other agencies/organizations is provided in Table 2.

Table 2. Summary of Available Toxicity Values for Trinitrophenylmethylnitramine (CASRN 479-45-8)

Source/Parameter[a]	Value (Applicability)	Notes	Reference	Date Accessed
Noncancer				
ACGIH	8-hr TLV-TWA: 1.5 mg/m^3	TLV-TWA based on upper respiratory tract irritation	ACGIH (2013)	NA
ATSDR	NV	Developed a toxicological profile for tetryl but did not recommend any minimal risk level values for oral or inhalation exposure	ATSDR (1995)	NA
Cal/EPA	NV	NA	Cal/EPA (2012)[b]	8-13-2013[b]
NIOSH	REL-TWA: 1.5 mg/m^3 IDLH: 750 mg/m^3	Skin designation	NIOSH (2010)	NA
OSHA	8-hr PEL-TWA: 1.5 mg/m^3	Skin designation	OSHA (2006, 2011)	NA
IRIS	NV	NA	U.S. EPA	8-13-2013
Drinking Water Standards and Health Advisories List	NV	NA	U.S. EPA (2011a)	NA
HEAST	Subchronic RfD: 0.1 mg/kg-d Chronic RfD: 0.01 mg/kg-d	Based on a LOAEL of 125 mg/kg-d for liver, kidney, and spleen lesions (in rabbits) over 9-month period	U.S. EPA (2003)	1-5-2011
	NV	NA	U.S. EPA (2011b)	NA
CARA HEEP	NV	NA	U.S. EPA (1994)	NA
WHO	NV	NA	WHO	8-13-2013
Cancer				
ACGIH	NV	NA	ACGIH (2013)	NA
IRIS	NV	NA	U.S. EPA	8-13-2013
HEAST	NV	NA	U.S. EPA (2011b)	NA
IARC	NV	NA	IARC (2013)	NA
NTP	NV	NA	NTP (2011)	NA
Cal/EPA	NV	NA	Cal/EPA (2009)	NA

[a]Sources: Integrated Risk Information System (IRIS) database; Health Effects Assessment Summary Tables (HEAST); International Agency for Research on Cancer (IARC); National Toxicology Program (NTP); California Environmental Protection Agency (Cal/EPA); American Conference of Governmental Industrial Hygienists (ACGIH); Agency for Toxic Substances and Disease Registry (ATSDR); National Institute for Occupational Safety and Health (NIOSH); Occupational Safety and Health Administration (OSHA); Chemical Assessments and Related Activities (CARA) list; Health and Environmental Effects Profile (HEEP); World Health Organization (WHO).

IDLH= immediately dangerous to life or health; NA = not applicable; NV = not available; PEL-TWA = permissible exposure level-time weighted average; REL-TWA = recommended exposure level-time weighted average; TLV-TWA = threshold limit value-time weighted average.

Literature searches were conducted on sources published from 1900 through August 2013, for studies relevant to the derivation of provisional toxicity values for tetryl, CASRN 479-45-8. The following databases were searched by chemical name, synonyms (trinitrophenylmethylnitramine; aniline, *N*-methyl-*N*,2,4,6-tetranitro-; benzeneamine; *N*-methyl-*N*,2,4,6-tetranitro-; CE; *N*-methyl-*N*-2,4,6-tetranitrobenzeneamine; nitramine; picrylmethylnitramine; *N*-picryl-*N*-methylnitramine; picrylnitromethylamine; tetralit; tetralite; tetril; *M*-methyl-*N*,2,4,6-tetranitroaniline; 2,4,6-tetryl; 2,4,6-trinitrophenyl-*n*-methylnitramine; 2,4,6-trinitrophenylmethylnitramine; *N*-2,4,6-tetranitro-*N*-methylaniline; and methylpicrylnitramine), or CASRN: ACGIH, ANEUPL, ATSDR, BIOSIS, Cal/EPA, CCRIS, CDAT, ChemIDplus, CIS, CRISP, DART, EMIC, EPIDEM, ETICBACK, FEDRIP, GENE-TOX, HAPAB, HERO, HMTC, HSDB, IARC, INCHEM IPCS, IPA, ITER, IUCLID, LactMed, NIOSH, NTIS, NTP, OSHA, OPP/RED, PESTAB, PPBIB, PPRTV, PubMed (toxicology subset), RISKLINE, RTECS, TOXLINE, TRI, U.S. EPA IRIS, U.S. EPA HEAST, U.S. EPA HEEP, U.S. EPA OW, and U.S. EPA TSCATS/TSCATS2. The following databases were searched for toxicity values or exposure limits: ACGIH, ATSDR, Cal/EPA, U.S. EPA IRIS, U.S. EPA HEAST, U.S. EPA HEEP, U.S. EPA OW, U.S. EPA TSCATS/TSCATS2, NIOSH, NTP, OSHA, and RTECS.

REVIEW OF POTENTIALLY RELEVANT DATA (CANCER AND NONCANCER)

Table 3 provides an overview of the relevant database for tetryl and includes all potentially relevant repeated short-term, subchronic-, and chronic-duration studies. Principal studies are identified. The phrase "statistical significance" as used throughout the document indicates a *p*-value of <0.05.

Table 3. Summary of Potentially Relevant Data for Trinitrophenylmethylnitramine (CASRN 479-45-8)								
Category	**Number of Male/Female, Strain, Species, Study Type, Study Duration**	**Dosimetry[a]**	**Critical effects**	**NOAEL[a]**	**BMDL/ BMCL[a]**	**LOAEL[a]**	**Reference (Comments)**	**Notes[b]**
Human								
None								
Animal								
			1. Oral (mg/kg-d)[a]					
Short-Term	5/5, F344 rat, diet, 14 d	0, 31.8, 80, 121, 170.5, 349.7 (M); 0, 32.1, 82.5, 130.3, 178.9, 374.4 (F)	Increased methemoglobin at 121 mg/kg-d (M) and increased reticulocytes and total bilirubin at 130 mg/kg-d (F).	80.0 (M)	DU	121.0 (M)	Reddy et al. (1994a, 1999)	PR (1999)
	4, rat (sex, strain not reported), gavage, d/wk not reported, once or daily until death	0; 1,000; 2,000	Rats died within 10 to 18 d at 1,000 mg/kg-d; effects on the liver, kidney, and spleen at 2,000 mg/kg-d.	NDr	DU	NDr	Parmeggiani et al. (1956, as cited in ATSDR, 1995)	PR
	4, rat (sex, strain not reported), gavage, d/wk not reported, 15 d	0, 250	Kidney lesions at 250 mg/kg-d	NDr	DU	250		
Subchronic	**15/15, F344, rat, diet, 90 d**	**0, 13.0, 62.4, 179.6 (M); 0, 14.2, 68.8, 199.0 (F)**	**Erythrocyte effects including decreased hemoglobin, decreased hematocrit, increased reticulocyte count and increased methemoglobin. Methemoglobin chosen as critical effect: significantly increased at mid and high dose in males and females at 90 d.**	**NDr**	**25.5 (M), 31.1 (F)**		**Reddy et al. (1994b, 1999)**	**PR, PS**
	20/0, rabbit, gavage, d/wk not reported, 3 mo	25	Death in 18/20 rabbits; lung, liver, kidney, spleen congestion	NDr	DU	NDr	Guarino and Zambrano (1957, as cited in ATSDR, 1995)	PR, no control group

Table 3. Summary of Potentially Relevant Data for Trinitrophenylmethylnitramine (CASRN 479-45-8)

Category	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[a]	Critical effects	NOAEL[a]	BMDL/ BMCL[a]	LOAEL[a]	Reference (Comments)	Notes[b]
Subchronic	12, sex not reported, rabbit, gavage, d/wk not reported, 120 days	0, 125	Decreased blood coagulability	NDr	DU	125	Daniele (1964, as cited in ATSDR, 1995)	PR, study only evaluated blood coagulability
	12/0, rabbit, gavage, d/wk not reported, 6−9 mo (3 rabbits—6 mo; 9 rabbits—9 mo)	125	Effects on the liver, kidney, and spleen	NDr	DU	NDr	Fati and Daniele (1965, as cited in ATSDR, 1995)	PR, no control group
Chronic	ND							
Developmental	ND							
Reproductive	ND							
Carcinogenicity	0/20, rat, gavage, every 3 d for 30 d	0, 196	Stomach adenomas and mammary hyperplasia in one rat from each group (not statistically significant)	NDr	DU	NDr	Griswold et al. (1968)	PR
2. Inhalation (mg/m^3)[a]								
Subchronic	ND							
Chronic	ND							
Developmental	ND							
Reproductive	ND							
Carcinogenicity	ND							

[a]Dosimetry: NOAEL, BMDL/BMCL, and LOAEL values are converted to an adjusted daily dose (ADD in mg/kg-d) for oral noncancer effects and a human equivalent dose (HED in mg/kg-d) for oral carcinogenic effects. All long-term exposure values (4 wk and longer) are converted from a discontinuous to a continuous (weekly) exposure. Values from animal developmental studies are not adjusted to a continuous exposure. Values for inhalation (cancer and noncancer), and oral (cancer only) are further converted to an HEC/D. Values from animal developmental studies are not adjusted to a continuous exposure.
HED = avg. mg test article ÷ avg. kg body weight ÷ Number daily doses.

[b]Notes: IRIS = Utilized by IRIS, date of last update; PS = principal study, PR = peer reviewed, NPR = not peer reviewed.

DU = data unsuitable, NV = not available, ND = no data, NDr = not determinable, NI = not identified, NP = not provided, NR = not reported, NR/Dr = not reported but determined from data, NS = not selected.

HUMAN STUDIES

Oral Exposures

No studies investigating the effects of oral exposure to tetryl in humans have been identified.

Inhalation Exposures

The effects of inhalation exposure of humans to tetryl are limited to case studies and reports of workers exposed in occupational settings. According to numerous secondary sources (HSDB, 2005; ATSDR, 1995; Talmage, 1999) and primary sources (Troup, 1946; Hardy and Maloof, 1950; Bergman, 1952; Goh, 1984), these studies are lacking adequate quantitative exposure estimates and consist of both inhalation and dermal exposures. During occupational exposure to tetryl, workers are most commonly exposed to dusts via inhalation and direct skin contact (Bergman, 1952; Goh, 1984; Hardy and Maloof, 1950; Troup, 1946). Dermal and ocular irritation and dermal sensitization are the most common effects reported from tetryl exposure. Tetryl also reacts with amino acids (Brownlie and Cumming, 1946) glutathione and hemoglobin (Maroziene et al., 2001). In several case reports, workers developed rashes on the face, neck, shoulders, forearms, and hands (Bergman, 1952; Goh, 1984; Hardy and Maloof, 1950; Troup, 1946). Swelling of the lips and hands were noted in one case (Goh, 1984), and discoloration of the skin and hair in other cases (Goh, 1984; Bergman, 1952; Troup, 1946). A popular eruption accompanied by gross edema and exfoliation have been noted (Hilton and Swanson, 1941; Smith, 1916). In a case study of 1,258 workers exposed to tetryl, 944 workers (75%) experienced dermatitis (Witkowski et al., 1942, as cited in Myers and Spinnato, 2007b). Yellow staining on the hands, face, neck, and hair was also reported in workers exposed to tetryl (Hilton and Swantson, 1941, as cited in Myers and Spinnato, 2007b). Respiratory effects including asthma, tracheitis, burning and itching of the respiratory tract, sneezing, and inflammation of the mucous membranes were reported due to inhalation exposure (Troup, 1946; Bergman, 1952). In addition, clinical signs of toxicity were noted to the hematopoietic system including anemia, malformed and variably sized red blood cells, decreased hemoglobin concentration, leukocytosis, and leukopenia (Ruxton, 1917; Brabham., 1943; Probst et al., 1944; Hardy and Maloof, 1950). Other reports of toxicity included menstrual changes, irritability, headache, general malaise, lassitude, and sleeplessness (Hardy and Maloof, 1950; Bergman, 1952). As noted above, the available studies did not report estimated exposure concentrations, and several cases involved coexposure to multiple chemicals. Thus, these studies are not considered appropriate for deriving a provisional inhalation reference concentration for tetryl.

ANIMAL STUDIES

Oral Exposures

The effects of oral exposure of animals to tetryl have been evaluated in two short-term studies (Reddy et al., 1994a, 1999; Parmeggiani et al., 1956, as cited in ATSDR, 1995); four subchronic-duration studies (Reddy et al., 1994b, 1999; Guarino and Zambrano, 1957, as cited in ATSDR, 1995; Daniele, 1964, as cited in ATSDR, 1995; Fati and Daniele, 1965, as cited in ATSDR, 1995), and one carcinogenicity study (a short-term screening assay of 30 days) (Griswold et al., 1968).

Short-Term Studies

Reddy et al., 1994a, 1999

Reddy et al. (1999) conducted a published peer-reviewed short-term toxicity study in rats. Groups of 5 male and 5 female F344 rats were fed 0; 500; 1,250; 2,000; 2,500; or 5,000 ppm tetryl (99.45% purity) in the diet for 14 days (Reddy et al., 1994a, 1999). These doses were calculated by the study authors to be equivalent to 0, 31.8, 80.0 121.0, 170.5, and 349.7 mg/kg-day for males and 0, 32.1, 82.5, 130.3, 178.9, and 374.4 mg/kg-day for females. The results of this study were originally compiled in an unpublished report by Reddy et al. (1994a). All rats were observed daily for clinical signs, behavioral responses, and for mortality and morbidity. Animals were weighed on days 0, 7, and 14, and food and water consumption were measured twice weekly. Blood samples were collected at sacrifice and the following hematology endpoints were measured: red and white blood cell counts, differential leukocyte count, packed cell volume, platelet count, hemoglobin, and methemoglobin. Clinical chemistry endpoints included sodium, potassium, total protein, albumin, calcium, phosphorus, total bilirubin, blood urea nitrogen, creatinine, alanine aminotransferase, aspartate aminotransferase, glucose, alkaline phosphatase, triglycerides, and cholesterol. Organs weights were determined for spleen, kidneys, testes with epididymides, brain, liver, adrenals, lungs, thymus, ovaries, and heart, and various other tissues (unspecified) were also examined. Histopathology was performed on all tissues and organs in the control and high dose animals; and on spleen, kidney, and testes in all dose groups. Histopathology was examined by a certified pathologist and inflammatory and degenerative lesions were graded according to a scale of 1 to 4 (minimal, mild, moderate, or marked). Statistical evaluations were performed using ANOVA, F-test ($p \leq 0.05$) and Dunnett's test.

No mortality, clinical signs of toxicity, or reduced food or water consumption were observed (Reddy et al., 1994a, 1999). In males, a statistically significant decrease in body weights and a statistically significant increase in relative kidney weights were reported at 349.7 mg/kg-day. In females, statistically significant increases in relative liver weights at 178.9 and 374.4 mg/kg-day, and increases in spleen weights were reported at 374.4 mg/kg-day. No other significant changes in organ weights were noted. Analysis of hematological parameters in males showed a statistically significant increase in reticulocytes at 121.0 mg/kg-day and in methemoglobin at 121.0, 170.5, and 349.7 mg/kg-day. In females, a statistically significant decrease in hemoglobin at 178.9 and 374.4 mg/kg-day, a decrease in hematocrit at 130.3 mg/kg-day, an increase in reticulocytes at 130.3 and 178.9 mg/kg-day, and an increase in methemoglobin at 374.4 mg/kg-day were reported. Clinical chemistry results in males consisted of statistically significant increases in total protein at 31.8, 80.0, 121.0, and 349.7 mg/kg-day, increases in albumin at all doses, increases in calcium at 31.8 and 80.0 mg/kg-day, decreases in alkaline phosphatase at 80.0, 121.0, 170.5, and 349.7 mg/kg-day, and increases in glucose at 170.5 mg/kg-day. In females, statistically significant increases in total protein and albumin were observed at 82.5, 130.3, 178.9, and 374.4 mg/kg-day, increases in total bilirubin at 130.3, 178.9, and 374.4 mg/kg-day, and decreases in sodium at 374.4 mg/kg-day. Histopathological analysis of the tissues revealed an increased incidence of cytoplasmic droplets in the proximal renal cortical tubular epithelial cells of male rats at doses of 80 mg/kg-day and higher. A LOAEL of 121.0 mg/kg-day is identified for this study based on increased methemoglobin in males. The NOAEL is 80.0 mg/kg-day.

Parmeggiani et al., 1956, as cited in ATSDR, 1995

Parmeggiani et al. (1956, as cited in ATSDR, 1995; in Italian) administered a single dose of 1,000 or 2,000 mg/kg tetryl to groups of four rats, daily doses of 1,000 or 2,000 mg/kg-day to groups of four rats until death, or daily doses of 250 mg/kg to a group of four rats for 15 days. Groups of four rats were used as controls. The strain and sex of the rats were not reported. According to ATSDR (1995), exposure was via gavage. No adverse effects were noted in the rats administered a single dose of 1,000 mg/kg. The rats administered a single dose of 2,000 mg/kg showed effects on the liver, renal tubular epithelia swelling, and hemosiderosis and atrophy of the spleen. Animals administered daily doses of 1,000 or 2,000 mg/kg died within 10 to 18 days. Weight loss, dyspnea, rough coat, and yellow pigmentation in the tail, ears and nose, and limb paralysis and convulsions were observed in these rats. Histopathological examination of the liver revealed changes in the hepatocytes and activation of Kupffer cells, and examination of the kidney revealed swollen tubular epithelium, cytoplasmic changes, and nuclear pyknosis. Moderate spleen hemosiderosis with lymphatic follicle atrophy was observed in rats at 2,000 mg/kg-day. The lower dose of 250 mg/kg-day also produced degenerative kidney lesions, and may be considered a 15-day LOAEL.

Reddy et al., 1997

Reddy et al. (1997) administered tetryl by gavage in corn oil at 0; 500; or 1,000 mg/kg to male F344 rats. After 24 hours, changes in enzymes, hematology, and histopathology were studied. Ethoxy and pentoxy *O*-dealkylase activities showed a significant decrease at both doses, and there was an increase in methemoglobin and a decrease in lymphocyte counts at both doses, and an increase in glucose and serum urea nitrogen at 1,000 mg/kg only. Glycogen accumulated in the livers and focal coagulative necrosis of the gastric mucosa was noted at both doses.

Wells et al. 1920, as cited in ATSDR, 1995

Wells et al. (1920, as cited in ATSDR, 1995) reported that 1 to 3 daily oral doses of tetryl at 1,000 mg/kg by gavage in milk were lethal to rabbits. No information was reported on the number, sex, or species of rabbits. Swelling and degeneration of the epithelium of the kidneys, edema of the lungs and bronchi, and accumulation of hematic pigment in the spleen were reported. No effects on the liver were observed. In another study by Wells et al. (1920, as cited in Myers and Spinnato, 2007b), dogs were administered 100 mg/kg-day tetryl subcutaneously. Hepatic lesions consisting of necrosis and fatty degeneration and kidney effects consisting of swelling of the tubular epithelium, fatty deposits, necrosis, and albuminuria were reported.

Subchronic Studies

Reddy et al., 1994b, 1999

Reddy et al. (1994b, 1999) is considered the principal study for derivation of the provisional subchronic and chronic p-RfDs. In a Good Laboratory Practice (GLP)-compliant peer-reviewed, published study, groups of 15 male and 15 female F344 rats were fed 0; 200; 1,000; or 3,000 ppm tetryl (99.45% purity) in the diet for 90 days (Reddy et al., 1999). These doses were calculated by the study authors to be equivalent to 0, 13.0, 62.4, and 179.6 mg/kg-day for males and 0, 14.2, 68.8, and 199.0 mg/kg-day for females. Many of the results from Reddy et al. (1999) were originally presented in an unpublished report by Reddy et al. (1994b).

The animals were housed in temperature (20−22°C) and humidity (40−60%) controlled rooms on a 12:12 hour light:dark cycle and were housed individually in polycarbonate cages and food and water were provided ad libitum. Five rats per group were killed on Day 45 and their

hematology and clinical chemistry endpoints were measured, and 10 rats per group were sacrificed at the end of the study (Day 90). Rats were observed daily for clinical signs and for morbidity and mortality. Food and water consumption were measured twice weekly. Blood samples were collected at Day 45 and Day 90. Hematology endpoints included red and white blood cell counts, differential leukocyte count, packed cell volume, platelet count, hemoglobin, and methemoglobin. Clinical chemistry endpoints included sodium, potassium, total protein, albumin, calcium, phosphorus, total bilirubin, blood urea nitrogen, creatinine, alanine aminotransferase, aspartate aminotransferase, glucose, alkaline phosphatase, triglycerides, and cholesterol. Body weight was recorded, as were weights for brain, liver, spleen, kidneys, adrenals, lungs, thymus, testes with epididymides, ovaries, and heart, as well as various other unspecified organs. Histopathology was performed by a certified pathologist on all tissues and organs in the control and high dose animals; histopathology of the spleen, kidneys, and testes were carried out in all animals. Inflammatory and degenerative lesions were graded according to a scale of 1 to 4 (minimal, mild, moderate, or marked). All animals were also examined for ophthalmologic changes. Statistical evaluations were done using ANOVA, F-test ($p \leq 0.05$) and Dunnett's test.

No deaths or clinical signs of toxicity were observed in the rats (Reddy et al., 1994b, 1999). Statistically significant decreases in food consumption were observed at all dose levels in both males and females, and a statistically significant increase in water consumption was noted only in the 199.0 mg/kg-day females. At the end of Week 6 (42 days), no significant changes were noted in body weights in males and a significant decrease was noted in the 199.0 mg/kg-day females. At the end of the study, statistically significant decreases in body weight were observed in the 179.6 mg/kg-day males and in the 68.8 and 199.0 mg/kg-day females (see Tables B.1 and B.2).

Relative organ weight changes after 90 days are shown in Tables B.1 and B.2. Organ weight changes were not evaluated at 45 days. The only statistically significant change in organ weight at the lowest dose was an increase in relative kidney weights at 14.2 mg/kg-day in females (5.4%); however, this change is not considered biologically significant because it is <10%. Statistically significant increases were observed in relative liver weights of males at 62.4 and 179.6 mg/kg-day (10.4% and 28.3%, respectively) and of females at 68.8 and 199.0 mg/kg-day (10.1% and 21.4%, respectively). Statistically significant increases were also observed in relative kidney weights of males at 62.4 and 179.6 mg/kg-day (12.5% and 19.4%, respectively) and of females at 68.8 and 199.0 mg/kg-day (5.4% and 17.8%, respectively).

Absolute changes in organ weights (as presented in Reddy et al., 1994b) were as follows: statistically significant increases in spleen weights in males at 179.6 mg/kg-day, statistically significant decreases in brain weights in males at 179.6 mg/kg-day and in females at 68.8 and 199.0 mg/kg-day, statistically significant decreases in adrenal weights in females at 68.8 mg/kg-day, and statistically significant decreases in adrenal and thymus weights in males at 179.6 mg/kg-day and in females at 199.0 mg/kg-day. No changes were noted in absolute kidney weights of either males or females at any dose and absolute liver weights were increased in males at 179.6 mg/kg-day and in females at 199.0 mg/kg-day.

A number of effects of tetryl on hematological parameters were noted after 45 and 90 days in males (see Tables B.3 and B.4). These data demonstrate a pattern of dose-dependent changes indicative of methemoglobinemia and hemolytic anemia including decreased

hemoglobin and hematocrit and increased reticulocyte content. Similar effects were observed after 45 days in females, however, increases in methemoglobin were observed at 45 days in females at all doses and at 90 days at 68.8 and 199.0 mg/kg-day only.

Effects of tetryl on clinical chemistry parameters were noted after 45 and 90 days (see Tables B.5 and B.6). After 90 days in males, statistically significant changes consisted of: increases in blood urea nitrogen (BUN) at 62.4 mg/kg-day, decreases in alkaline phosphatase at 13.0, 62.4, and 179.6 mg/kg-day, increases in albumin and calcium at 62.4 and 179.6 mg/kg-day, increases in cholesterol and total bilirubin at 13.0, 62.4, and 179.6 mg/kg-day, and increases in total protein at 62.4 and 179.6 mg/kg-day. After 45 days in males, some of the same effects were observed (increases in albumin, calcium, and total protein). However, at 45 days (and not at 90 days), a significant increase in potassium was noted in males at 179.6 mg/kg-day. After 90 days in females, statistically significant changes were as follows: increases in glucose at 68.8 mg/kg-day, decreases in alkaline phosphatase at 199.0 mg/kg-day, increases in aspartate aminotransferase (AST) at 14.2 mg/kg-day, increases in albumin at 14.2, 68.8, and 199.0 mg/kg-day, decreases in triglycerides at 199.0 mg/kg-day, increases in cholesterol at 14.2, 68.8, and 199.0 mg/kg-day, increases in total bilirubin at 14.2 and 199.0 mg/kg-day, and increases in total protein at 68.8 and 199.0 mg/kg-day. The only significant effect that was also observed at 45 days in females was an increase in total bilirubin (which occurred at 199.0 mg/kg-day only). Increased creatinine was noted at 45 days (and not at 90 days) in females at 68.8 and 199.0 mg/kg-day. According to the study authors, all other clinical chemistry parameters were within normal limits (Reddy et al., 1999).

Histopathology at 90 days revealed that the spleens of both males and females were characterized by prominent deposition of intra- and extracellular pigment (which was characterized by the study authors as "probable hemosiderin") at 179.6 mg/kg-day in males and 199.0 mg/kg-day in females. At 179.6 mg/kg-day, males also exhibited excessive erythroid cell hyperplasia. In the kidneys, pigment deposition was noted in the renal cortical epithelium at 62.4 and 179.6 mg/kg-day in males and 68.8 and 199.0 mg/kg-day in females. In males, a dose-related increase in the severity of tubular degeneration and regeneration, hyaline casts, and cytoplasmic droplets in proximal cortical tubular epithelial cells was noted. Cytoplasmic droplets were observed in male rats at doses of 62.4 and 179.6 mg/kg-day. The study authors reported that the immunohistochemical staining properties of the droplets were consistent with alpha-2-u-globulin and were observed by the study authors to be morphologically similar to the droplets observed in the 14-day (Reddy et al., 1994a, 1999) study, except for a diminished intensity of eosinophilic staining.

The study authors did not consider the changes in the clinical chemistry parameters at 13.0 mg/kg-day in males (decreases in alkaline phosphatase, increases in cholesterol and total bilirubin) or 14.2 mg/kg-day in females (increases in albumin, cholesterol, and bilirubin) to be biologically relevant because the values were within an accepted normal reference range (Reddy et al., 1994b, 1999). The deposition of cytoplasmic droplets in the kidneys at 62.4 and 179.6 mg/kg-day in males and 68.8 and 199.0 mg/kg-day in females was not considered by the study authors to be significantly detrimental to the kidney since they cleared with time. However, the increased relative liver weights at 62.4 and 179.6 mg/kg-day in males and 68.8 and 199.0 mg/kg-day in females, the decrease in alkaline phosphatase in all dose groups in males and at 199.0 mg/kg-day in females, the increase in serum cholesterol in all dose groups in males and females, and the increase in total bilirubin in all dose groups in males and in the 14.2 and

199.0 mg/kg-day dose groups in females represent sensitive markers of liver damage. The increase in methemoglobin at 14.2 mg/kg-day in females at 45 days is also indicative of toxicity in the context of the other adverse hematological effects seen at the higher doses and after the longer 90-day duration. Since the effects seen at the low dose would not be considered adverse without additional supporting evidence of blood and liver effects at the two higher doses, the lowest doses of 13.0 mg/kg-day in males and 14.2 mg/kg-day in females are considered to be "minimal" LOAELs, based on elevated serum total bilirubin and cholesterol in males and females and increased serum methemoglobin in females and decreased alkaline phosphatase in males. A NOAEL cannot be identified since the LOAEL was the lowest dose tested.

Guarino and Zambrano, 1957, as cited in ATSDR, 1995

Guarino and Zambrano (1957, as cited in ATSDR, 1995; in Italian) administered tetryl by gavage at 25 mg/kg-day to 20 male rabbits for up to 3 months. No controls were used and no information was provided regarding the strain of the rabbits or dosing regimen sufficient to perform a duration adjustment. The mean survival time of the rabbits was 2 months, with 25 mg/kg-day lethal to 18 out of 20 rabbits. No information was provided regarding the cause of death. Gross and microscopic signs of lung congestion were observed. Congestion and yellowish color in the liver were noted and microscopic examination revealed epithelial swelling, fatty infiltration, and necrotic foci. The kidneys were visibly congested, with lesions to the parenchymal tissue and swelling and vacuolar degeneration of the convoluted tubules. The spleens were also congested, with free erythrocytes in the splenic sinuses. A NOAEL or LOAEL is not identified from this study since only one dose was administered, with no control group.

Daniele, 1964, as cited in ATSDR, 1995

A study of blood coagulability in rabbits administered tetryl by gavage at 0 (n = 3) and 125 mg/kg-day (n = 12) for 120 days was reported by Daniele (1964, as cited in ATSDR, 1995; in Italian). No information was given to inform a duration adjustment. Blood coagulability was evaluated through the measurement of several components of the coagulation pathway (e.g., platelets). Although the study authors do report some significant differences between treated and control animals, experimental variability and the small number of animals tested make interpretation difficult.

Fati and Daniele, 1965, as cited in ATSDR, 1995

Fati and Daniele (1965, as cited in ATSDR, 1995) administered tetryl by gavage at 125 mg/kg-day to 12 male rabbits for 6−9 months. Effects on the liver noted in the rabbits exposed for 6 months consisted of hepatocyte changes, characterized by swelling, vacuolization, cytoplasmic opacity and slight granularity, and focal inflammation without necrosis. In the animals exposed for 9 months, additional hepatocyte changes, consisting of diffuse turbid swelling, highly granular cytoplasm, polymorphic nuclei, and hyperchromia and pyknosis, parenchymal necrosis, Kupffer cell hyperplasia, and vascular congestion were observed. No effects on the kidneys were observed in rabbits exposed for 6 months. In rabbits exposed for 9 months, mild renal congestion was observed, and microscopic examination of the kidneys found turbid swelling, vacuolar degeneration, narrowed and poorly distinguishable tubular lumen, and cellular hypertrophy. This study provides suggestive evidence that the liver, kidney, and spleen are target organs of tetryl toxicity in rabbits following subchronic oral exposure. However, neither a NOAEL nor a LOAEL can be identified from this study since no control group was employed.

Chronic Studies
No chronic-duration studies on oral exposure to tetryl have been identified.

Developmental Studies
No developmental studies on oral exposure to tetryl have been identified.

Reproductive Studies
No reproductive studies on oral exposure to tetryl have been identified.

Carcinogenicity Studies
Griswold et al., 1968
Griswold et al. (1968) is a short-term screening assay in which groups of 20 female Sprague-Dawley rats were administered 0 or 40 mg tetryl by gavage in sesame oil every 3 days for 30 days (equivalent to 196 mg/kg per dose; duration adjusted to 65 mg/kg-day). Treatment began when the rats were 40 days of age and the rats were weighed and inspected weekly for tumors. The rats were killed after 9 months of observation but it was unclear whether the 30-day dosing period was included in this time period. Histopathology of the mammary tissue, ovaries, liver, intestinal tract, pituitary, and adrenal glands was performed. Adenoma of the stomach and mammary hyperplasia were seen in one animal from each group, but these results were not biologically or statistically significant.

Inhalation Exposures
Gell, 1944, as cited in ATSDR, 1995
A single short-term study of the effects of exposure to tetryl smoke was reported by Gell et al. (1944). In this study, 8 guinea pigs were exposed to a smoke of tetryl particles 30 minutes per day for 6 days (Gell, 1944, as cited in ATSDR, 1995). According to ATSDR (1995), the tetryl particle smoke was generated by blowing air over a 10% solution of tetryl in acetone. The study authors estimated the tetryl concentration in the chamber to be about 400 mg/m^3 and total absorption to be about 7−10 mg per animal. The guinea pigs were then exposed intravenously to picryl protein antigens prepared from the sera of rabbits exposed to analogs or metabolites of tetryl. One of these animals died and 6 out of 8 developed anaphylactic sensitivity.

No other studies investigating the effects of inhalation exposure to tetryl in animals have been identified.

OTHER DATA (SHORT-TERM TESTS, OTHER EXAMINATIONS)
Maroziene et al. (2001) report the results of in vitro studies with human erythrocytes in which they compared the effectiveness of several nitro aromatics in the production of methemoglobinemia. Intact and lysed erythrocytes were incubated with nitro aromatic agents at concentrations up to 300 μM. Tetryl produced a significant increase in methemoglobinemia in both intact and lysed erythrocytes, but the increase was more marked in lysed erythrocytes, which the study authors attributed to protective mechanisms operative in intact erythrocytes. After 2 hours of incubation, tetryl produced three- to five-times more methemoglobin than trinitrotoluene or *m*-dinitrobenzene. While conjugation with glutathione was demonstrated, the reaction product also demonstrated the ability to induce methemoglobin formation, though at a potency of about 20% that of the parent compound.

A few studies on genotoxicity, short-term toxicity and toxicokinetics of tetryl are available. These are summarized in Tables 4A and 4B. No studies were identified regarding chromosomal aberrations or malsegregation in prokaryotes; any effects in mammalian cells in vitro; mammals in vivo; or genotoxicity in subcellular systems.

Table 4A. Summary of Tetryl Genotoxicity

Endpoint	Test System	Dose Concentration[a]	Results[b]		Comments	References
			Without Activation	With Activation		
Genotoxicity studies in prokaryotic organisms						
Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537, TA1538	1.0−333.3 µg/plate	+ (TA98, TA100, TA1537, TA1538) − (TA1535)	+	Reduced mutagenicity in all activated strains	McGregor et al. (1980)
Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537, TA1538	2.5−30 µg/plate	+ (TA100, TA1535) − (TA98, TA1537)	+	Reduced mutagenicity in activated TA100	Whong et al. (1980)
Reverse mutation	*Salmonella typhimurium* TA98, TA100,	40−200 µg/plate	+	+	Reduced mutagenicity in both activated strains	Tan et al. (1992)
Reverse mutation	*Salmonella typhimurium* TA98, TA100	0−100 µg/plate	+	+	Reduced mutagenicity in both activated strains	George et al. (2001)
SOS repair induction	*E.Coli* W3310/polA$^+$, p3478/polA$^-$	100 µg/plate−10 mg/plate	−	−	NA	McGregor et al. (1980)
Genotoxicity studies in nonmammalian eukaryotic organisms						
Mutation	*Neurospora crassa* N23, 12-9-17	5−80 µg/plate	+	ND	Increased *ad-3*$^+$ reversions in the N23 strain in a dose-dependent manner. No tetryl-induced frame shift mutations in the 12-9-17 strain.	Whong et al. (1980)
Recombination induction	*Saccharomyces cerevisiae* D_5	ND	+	−	NA	McGregor et al. (1980)
Mitotic gene conversion	*Saccharomyces cerevisiae* D_4	5−30 µg/plate	+	ND	Increased conversions were noted in both *ade*$^+$ and *trp*$^+$ with increased tetryl concentrations	Whong et al. (1980)

[a]Lowest effective dose for positive results, highest dose tested for negative results.
[b]+ = positive; ± = equivocal or weakly positive; − = negative; T = cytotoxicity; NA = not applicable; ND = no data; NDr = not determinable; NR = not reported; NR/Dr = not reported but determined from data.

ND = no data.

Table 4B. Other Studies

Test	Materials and Methods	Results	References
Short-term studies	Rats exposed to 0; 500;1,000 mg/kg by gavage and effects observed 24 hrs later.	Enzyme changes, increase in methemoglobin, serum urea nitrogen, decrease in lymphocytes.	Reddy et al. (1997, as cited in Myers and Spinnato, 2007b)
	Rabbits exposed 1−3 times per day to 1,000 mg/kg by gavage in milk Dogs exposed to 100 mg/kg-d subcutaneously.	Mortality; kidney, lung, and spleen effects. Liver and kidney effects.	Wells et al. (1920, as cited in ATSDR, 1995); Wells et al. (1920, as cited in Myers and Spinnato, 2007b)
	Guinea pigs (n = 8) exposed to smoke of tetryl particles 30 minutes per day for 6 days. Subsequently exposed to picryl protein antigens.	One animal died, 6/8 showed anaphylactic sensitivity.	Gell (1944, as cited in ATSDR, 1995)
Metabolism/ toxicokinetic	5 male Sprague-Dawley rats administered 25, 100, 300 mg/kg subcutaneously.	Highest amounts of tetryl found in blood, liver, muscle; highest tissue:blood ratios found in brain, kidney, liver. *N*-methyl-2,4,6-trinitroaniline identified as major metabolite. Picric acid and picramic acid identified as urinary metabolites. Elimination in both the urine and feces.	Myers and Spinnato (2007a,b)

Tests Evaluating Genotoxicity and/or Mutagenicity

McGregor et al., 1980; Whong et al., 1980; Tan et al., 1992; George et al., 2001

The genotoxic effects of tetryl were assessed in vitro in multiple Ames Reverse Mutation assays utilizing *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538, both with and without metabolic activation systems (McGregor et al., 1980; Whong et al., 1980; Tan et al., 1992; George et al., 2001). Tetryl tested positive for mutagenicity showing an increase in histidine revertants in all tested strains without metabolic activation. Studies indicate that metabolic activation diminishes the mutagenic response of tetryl in all *Salmonella typhimurium* tester strains.

No differential toxicity was noted between *E. Coli* $polA^+$ and $polA^-$ tester strains with or without metabolic activation following a DNA repair assay (McGregor et al., 1980). Results indicate that tetryl does not induce DNA repair under the tested conditions.

In a forward mutation assay utilizing *Neurospora crassa* tester strains N23 and 12-9-17 (frameshift mutations), an increase in *ad-3*$^+$ reversions (base pair) was reported in the N23 strains in a dose-dependent manner, but frameshift mutations were not observed (Whong et al., 1980).

Tetryl was assessed for genotoxicity following mitotic gene conversion and recombination induction assays in *Saccharomyces cerevisiae* tester strains D_4 and D_5, respectively (Whong et al., 1980; McGregor et al., 1980). Increased conversions were noted in both *ade* and *trp* in a dose-dependent manner in the D_4 strains (Whong et al., 1980). Clear indications of mitotic recombination and other aberrations were also reported in the D_5 strain (McGregor et al., 1980).

Metabolism/Toxicokinetic Studies

Myers and Spinnato, 2007a,b

Myers and Spinnato (2007a,b) administered 25, 100, or 300 mg/kg tetryl dissolved in dimethyl sulfoxide (DMSO) by subcutaneous injection to groups of 5 male Sprague-Dawley rats. Tissue distribution data showed that the highest amounts of tetryl were found in the blood, liver, and muscle. The brain, kidney, and liver had the highest tissue to blood ratios compared to the other tissues. The major urinary metabolites identified were picric acid and picramic acid. The major metabolite identified from microsomal fraction studies was *N*-methyl-2,4,6-trinitroaniline (NMPA), with two enzymes responsible for NMPA formation; one was a type of microsomal NAD(P)H: quinine oxidoreductase that was NADH-specific and the other appeared to be NADPH: cytochrome-P450 reductase (Myers and Spinnato, 2007a). Tetryl elimination occurred in equal amounts in the urine and feces. The existing data for metabolism and toxicokinetics are insufficient to support the development of a dosimetric model.

DERIVATION OF PROVISIONAL VALUES

Tables 5 and 6 present a summary of noncancer and cancer reference values, respectively. IRIS data are indicated in the table, if available.

Table 5. Summary of Noncancer Reference Values for Trinitrophenylmethylnitramine (CASRN 479-45-8)

Toxicity Type (units)	Species/Sex	Critical Effect	p-Reference Value	POD Method	POD_{HED}	UF_C	Principal Study
Subchronic p-RfD (mg/kg-d)	Rat/M	Methemoglobinemia	2×10^{-2}	BMDL	6.1	300	Reddy et al. (1994b, 1999)
Chronic p-RfD (mg/kg-d)	Rat/M	Methemoglobinemia	2×10^{-3}	BMDL	6.1	3,000	Reddy et al. (1994b, 1999)
Subchronic p-RfC (mg/m^3)	NDr						
Chronic p-RfC (mg/m^3)	NDr						

NDr = not determinable.

Table 6. Summary of Cancer Values for Trinitrophenylmethylnitramine (CASRN 479-45-8)

Toxicity Type	Species/Sex	Tumor Type	Cancer Value	Principal Study
p-OSF	NDr			
p-IUR	NDr			

NDr = not determinable.

DERIVATION OF ORAL REFERENCE DOSES

Derivation of Subchronic Provisional RfD (Subchronic p-RfD)

No human studies are available on oral exposure to tetryl. Four subchronic animal oral studies are available (Reddy et al., 1994b, 1999; Guarino and Zambrano, 1957, as cited in ATSDR, 1995; Daniele, 1964, as cited in ATSDR, 1995; Fati and Daniele, 1965, as cited in ATSDR, 1995). Reddy et al. (1994b, 1999) was chosen as the principal study since this study was both GLP-compliant and subsequently published in the peer-reviewed literature. Study design included a control group and three doses tested in both sexes, and the investigators examined a large number of endpoints comprising weight changes, histopathology, clinical chemistry, and hematology. The other three studies were older studies in Italian and cited in a secondary source.

In Reddy et al. (1994b, 1999), groups of 10 male and 10 female F344 rats were fed 0; 200; 1,000; or 3,000 ppm tetryl in the diet for 90 days; initial body weights were reported to be approximately 125 grams. These dietary concentrations were calculated by the study authors to be equivalent to daily doses of 0, 13.0, 62.4, and 179.6 mg/kg-day for males and 0, 14.2, 68.8, and 199.0 mg/kg-day for females. Several hematology and clinical chemistry endpoints were evaluated at 45 and 90 days. Organ weights and histopathology were evaluated only at 90 days. The brain, liver, spleen, kidneys, adrenals, lungs, thymus, testes with epididymides, ovaries, and heart were weighed, and various other tissues were also examined. Histopathology was performed on all tissues and organs in the 0 and 179.6 mg/kg-day dose groups in males and in the 199.0 mg/kg-day dose group in females, with histopathology of the spleen, kidneys, and testes carried out in the remaining dose groups as well.

Effects were observed on the blood, kidney, spleen, and liver (Reddy et al., 1994b, 1999). The effects at 90 days in the middle- and high-dose groups (62.4 and 179.6 mg/kg-day in males and 68.8 and 199.0 mg/kg-day in females) included hematological effects (decreased red blood cell count, hemoglobin, and hematocrit and increased methemoglobin and reticulocytes), pigment deposition in the renal cortical epithelium, increased relative spleen weights, and increased liver weights and decreased alkaline phosphatase. In the low-dose groups (13.0 mg/kg-day in males and 14.2 mg/kg-day in females), decreases in alkaline phosphatase in males and increases in total bilirubin in males and females were observed. The pigment deposition in the renal cortical epithelium and spleen may be related to erythrocyte damage and hemolysis; and were consistent with methemoglobin induction.

The study authors did not consider the changes in the clinical chemistry parameters at 13.0 mg/kg-day in males (decreases in alkaline phosphatase, increases in cholesterol and total bilirubin) or 14.2 mg/kg-day in females (increases in albumin, cholesterol, and total bilirubin) to be biologically meaningful because the values were within an accepted normal reference range (Reddy et al., 1994b, 1999). Given the decrease in feed intake and body weight, changes in relative organ weights were not considered. However, absolute brain weight was increased and absolute adrenal weight was decreased in females at 68.8 mg/kg-day. The increase in methemoglobin was observed in both sexes, but was more pronounced in females. Methemoglobin formation demonstrated a clear dose dependent increase with statistically significant three-fold increases over controls evident at the lowest dose of 14.2 mg/kg-day in females at 45 days. This effect is also indicative of toxicity in the context of the other adverse

hematological effects seen at the higher doses and after the longer 90-day duration (increased methemoglobin, decreased RBC count, decreased hemoglobin, decreased hematocrit, increased reticulocytes, increased spleen weight).

Benchmark dose (BMD) analysis of the principal study was carried out on a number of endpoints, including increased body weights, organ weights and increased methemoglobin in both males and females and compared to other potential point of departure values. The EPA Benchmark Dose Software (BMDS version 2.1.2) continuous-variable models with constant variance (Hill, linear, power and polynomial) was fit to the data for each of these endpoints. The majority of the modeled endpoints had goodness of fit values (*p*-values) <0.1, which indicates that these models do not adequately fit these data. The only endpoints that had models with *p*-values >0.1 were body weights in males and methemoglobinemia. The male body weight data are not used as the point-of-departure (POD) for the subchronic p-RfD because of the potential contribution of decreased food intake. BMD modeling was successfully applied to 90 day and 45 day data describing methemoglobinemia in male and female rats. Several models had *p*-values >0.1 for methemoglobinemia in male and female rats; of these models the linear models (linear, polynomial, power models) all had the lowest BMDL of 25.5 mg/kg-day, observed in male rats exposed for 90 days (see Appendix C). This value was lower than the LOAEL observed for absolute organ weight changes in females (68.8 mg/kg-day). Thus, the BMDL of 25.5 mg/kg-day for males is selected as the POD for derivation of the subchronic p-RfD. The subchronic p-RfD for tetryl is derived as follows:

In EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011c), the Agency endorses a hierarchy of approaches to derive human equivalent oral exposures from data from laboratory animal species, with the preferred approach being physiologically based toxicokinetic modeling. Other approaches may include using some chemical-specific information, without a complete physiologically based toxicokinetic model. In lieu of chemical-specific models or data to inform the derivation of human equivalent oral exposures, EPA endorses body weight scaling to the 3/4 power (i.e., $BW^{3/4}$) as a default to extrapolate doses of orally administered agents from all laboratory animals to humans for the purpose of deriving a RfD under certain exposure conditions. More specifically, the use of $BW^{3/4}$ scaling for deriving a RfD is recommended when the observed effects are associated with the parent compound or a stable metabolite, but not for portal-of-entry effects or developmental endpoints.

A validated human PBPK model for tetryl is not available for use in extrapolating doses from animals to humans. The selected critical effect of erythrocyte changes, exemplified by increased methemoglobin content, was associated with the parent compound. Furthermore, these erythrocyte effects are not portal-of-entry or developmental effects. Therefore, scaling by $BW^{3/4}$ is relevant for deriving human equivalent doses (HEDs) for these effects.

Following U.S. EPA (2011c) guidance, the POD for methemoglobinemia (BMDL 25.5 mg/kg-day) in adult male rats is converted to a HED through application of a dosimetric adjustment factor (DAF)[1] derived as follows:

$$DAF = (BW_a^{1/4} \div BW_h^{1/4})$$

where

DAF = dosimetric adjustment factor
BW_a = animal body weight
BW_h = human body weight

Using a BW_a of 0.25 kg for rats and a BW_h of 70 kg for humans (U.S. EPA, 1988), the resulting DAF is 0.24. Applying this DAF to the BMDL identified for the methemoglobinemia in mature rats yields a $BMDL_{HED}$ as follows:

$$BMDL_{HED} = BMDL\ (mg/kg\text{-}day) \times DAF$$
$$= 25.5\ mg/kg\text{-}day \times 0.24$$
$$= 6.1\ mg/kg\text{-}day$$

The subchronic p-RfD for tetryl is derived as follows:

$$\textbf{Subchronic p-RfD} = BMDL_{HED} \div UF$$
$$= 6.1\ mg/kg\text{-}day \div 300$$
$$= \mathbf{2 \times 10^{-2}\ mg/kg\text{-}day}$$

The UF_C for the subchronic p-RfD for tetryl is 300, as summarized in Table 7.

[1]As described in detail in *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011c), rate-related processes scale across species in a manner related to both the direct ($BW^{1/1}$) and allometric scaling ($BW^{3/4}$) aspects such that $BW^{3/4} \div BW^{1/1} = BW^{-1/4}$, converted to a $DAF = BW_a^{1/4} \div BW_h^{1/4}$.

Table 7. Uncertainty Factors for Subchronic p-RfD of Trinitrophenylmethylnitramine[a]

UF	Value	Justification
UF_A	3	A UF_A of 3 ($10^{0.5}$) has been applied to account for uncertainty in characterizing the toxicodynamic differences between rats and humans following oral tetryl exposure. The toxicokinetic uncertainty has been accounted for by calculation of a human equivalent dose (HED) through application of a dosimetric adjustment factor (DAF) as outlined in the EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011c).
UF_D	10	A UF_D of 10 has been applied because there are no acceptable two-generation reproductive toxicity or developmental toxicity studies.
UF_H	10	A UF_H of 10 has been applied for inter-individual variability to account for human-to-human variability in susceptibility in the absence of quantitative information to assess the toxicokinetics and toxicodynamics of tetryl in humans.
UF_L	1	A UF_L of 1 has been applied for LOAEL-to-NOAEL extrapolation because the POD is a BMDL.
UF_S	1	A UF_S of 1 has been applied because a subchronic-duration study was selected as the principal study.
UF_C	300	

[a]Reddy et al. (1999).

The confidence in the subchronic p-RfD for tetryl is low as explained in Table 8 below.

Table 8. Confidence Descriptors for Subchronic p-RfD for Trinitrophenylmethylnitramine

Confidence Categories	Designation[a]	Discussion
Confidence in study	H	The study by Reddy et al. (1994b, 1999) is a well-conducted study, with a sufficient number of animals and examined many endpoints
Confidence in database	L	The database is lacking two-generation reproductive and developmental toxicity studies
Confidence in subchronic p-RfD[b]	L	The overall confidence is low

[a]L = low, M = medium, H = high.
[b]The overall confidence cannot be greater than lowest entry in table.

Derivation of Chronic Provisional RfD (Chronic p-RfD)

The principal study used to identify the critical effect of methemoglobinemia in male rats was reported by Reddy et al. (1994b, 1999). Results from this GLP-compliant 90-day study supported a BMDL value of 25.5 mg/kg-day, which was converted to a $BMDL_{HED}$ of 6.1 mg/kg-day by body weight scaling as described above, and serves as the POD for derivation of the chronic p-RfD. The chronic p-RfD for tetryl is derived as follows:

$$\textbf{Chronic p-RfD} = BMDL_{HED} \div UF$$
$$= 6.1 \text{ mg/kg-day} \div 3{,}000$$
$$= \mathbf{2 \times 10^{-3} \text{ mg/kg-day}}$$

The UF_C for the chronic p-RfD for tetryl is 3,000, as summarized in Table 9.

Table 9. Uncertainty Factors for Chronic p-RfD of Trinitrophenylmethylnitramine[a]

UF	Value	Justification
UF_A	3	A UF_A of 3 ($10^{0.5}$) has been applied to account for uncertainty in characterizing the toxicodynamic differences between rats and humans following oral tetryl exposure. The toxicokinetic uncertainty has been accounted for by calculation of a human equivalent dose (HED) through application of a dosimetric adjustment factor (DAF) as outlined in the EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011c).
UF_D	10	A UF_D of 10 has been applied because there are no acceptable two-generation reproductive toxicity or developmental toxicity studies.
UF_H	10	A UF_H of 10 has been applied for inter-individual variability to account for human-to-human variability in susceptibility in the absence of quantitative information to assess the toxicokinetics and toxicodynamics of tetryl in humans.
UF_L	1	A UF_L of 1 has been applied for LOAEL-to-NOAEL extrapolation because the POD is a BMDL.
UF_S	10	A UF_S of 10 has been applied because a subchronic-duration study was selected as the principal study.
UF_C	3,000	

[a]Reddy et al. (1999).

The confidence in the chronic p-RfD for tetryl is low as explained in Table 10 below.

Table 10. Confidence Descriptors for Chronic p-RfD for Trinitrophenylmethylnitramine

Confidence Categories	Designation[a]	Discussion
Confidence in study	H	The study by Reddy et al. (1994b, 1999) is a well-conducted study, with a sufficient number of animals and examined many endpoints
Confidence in database	L	The database is lacking two-generation reproductive and developmental toxicity studies
Confidence in chronic p-RfD[b]	L	The overall confidence is low

[a]L = low, M = medium, H = high.
[b]The overall confidence cannot be greater than lowest entry in table.

DERIVATION OF INHALATION REFERENCE CONCENTRATIONS

The available data concerning health effects in humans following inhalation exposure to tetryl are limited to case studies and reports of workers exposed in occupational settings, which include exposures to multiple chemicals. These studies are lacking in adequate quantitative exposure estimates (Troup, 1946; Hardy and Maloof, 1950; Bergman, 1952; Goh, 1984; ACGIH, 2013; ATSDR, 1995; Talmage et al., 1999). Since levels of exposure to tetryl were not provided in these studies, NOAELS or LOAELs could not be established and health effects data in these workers are unsuitable for the derivation of an RfC. No subchronic or chronic animal studies on the toxicity of tetryl by inhalation exposure were identified.

Derivation of Subchronic Provisional RfC (Subchronic p-RfC)

No subchronic p-RfC can be derived because no inhalation studies on exposure to tetryl were identified.

Derivation of Chronic Provisional RfC (Chronic p-RfC)

No chronic p-RfC can be derived because no inhalation studies on exposure to tetryl were identified.

CANCER WEIGHT-OF-EVIDENCE DESCRIPTOR

There are no human data available on the carcinogenicity of tetryl. There is only one short-term screening assay (Griswold et al., 1968) in which rats were exposed for 30 days and then evaluated 10 months later. The EPA has not classified tetryl for carcinogenicity, and no other agencies have reviewed or classified the carcinogenic potential of the chemical (IARC, 2013; NTP, 2011; Cal/EPA, 2009). While there are no data on the carcinogenicity of tetryl from in vivo bioassays, it is important to note that there are in vitro data that demonstrate mutagenic activity (McGregor et al., 1980; Whong et al., 1980; Tan et al., 1992; George et al., 2001). Thus, the data taken together indicate the cancer weight-of-evidence descriptor for tetryl as "*inadequate information to assess carcinogenic potential*".

Table 11 identifies the cancer weight-of-evidence descriptor for tetryl.

Table 11. Cancer WOE Descriptor for Trinitrophenylmethylnitramine

Possible WOE Descriptor	Designation	Route of Entry (Oral, Inhalation, or Both)	Comments
"Carcinogenic to Humans"	NA	NA	There are no human data available.
"Likely to Be Carcinogenic to Humans"	NA	NA	There is not enough evidence to support this statement.
"Suggestive Evidence of Carcinogenic Potential"	NA	NA	There is not enough evidence to support this statement.
"Inadequate Information to Assess Carcinogenic Potential"	**Selected**	**Both**	**There are no human or animal carcinogenicity data available.**
"Not Likely to Be Carcinogenic to Humans"	NA	NA	There is not enough evidence to support this statement.

DERIVATION OF PROVISIONAL CANCER POTENCY VALUES

Derivation of Provisional Oral Slope Factor (p-OSF)

The available data do not support derivation of any oral cancer values.

Derivation of Provisional Inhalation Unit Risk (p-IUR)

The available data do not support derivation of any inhalation cancer values.

APPENDIX A. PROVISIONAL SCREENING VALUES

Appendix A is not applicable.

APPENDIX B. DATA TABLES

Table B1. Effect of Tetryl on Body Weights and Organ-to-Body Weight Ratios (Relative Organ Weights) After 90 Days on Males[a]

Concentration (ppm) diet	0	200	1,000	3,000
(mg/kg-d)	0	13.0	62.4	179.6
Body weight (g)	304.19 ± 4.78	305.04 ± 4.12	297.37 ± 3.89	279.95 ± 4.71*
Kidneys (%)	0.72 ± 0.01	0.75 ± 0.01	0.81 ± 0.04*	0.86 ± 0.01*
Lungs (%)	0.47 ± 0.01	0.48 ± 0.03	0.48 ± 0.02	0.51 ± 0.01
Liver (%)	3.07 ± 0.04	3.06 ± 0.05	3.39 ± 0.04*	3.94 ± 0.03*
Heart (%)	0.33 ± 0.01	0.35 ± 0.01	0.32 ± 0.01	0.33 ± 0.01
Brain (%)	0.63 ± 0.01	0.63 ± 0.01	0.64 ± 0.01	0.66 ± 0.01
Spleen (%)	0.20 ± 0.00	0.20 ± 0.00	0.21 ± 0.00	0.25 ± 0.00*
Adrenals (%)	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.03 ± 0.00
Thymus (%)	0.09 ± 0.01	0.08 ± 0.00	0.09 ± 0.01	0.09 ± 0.01
Gonads (%)	1.67 ± 0.09	1.76 ± 0.08	1.67 ± 0.07	1.72 ± 0.06

[a]Reddy et al. (1999).
Data are mean ± standard deviation.
* Significantly different from the control group ($p \leq 0.05$) by the Dunnett's test.

Table B.2. Effect of Tetryl on Body Weights and Organ-to-Body Weight Ratios (Relative Organ Weights) After 90 Days on Females[a]

Concentration (ppm) diet	0	200	1,000	3,000
(mg/kg-d)	0	14.2	68.8	199.0
Body weight (g)	171.55 ± 2.13	168.44 ± 2.52	163.70 ± 2.49*	153.33 ± 1.00*
Kidneys (%)	0.73 ± 0.01	0.77 ± 0.01*	0.77 ± 0.01*	0.86 ± 0.01*
Lungs (%)	0.59 ± 0.02	0.57 ± 0.02	0.57 ± 0.01	0.62 ± 0.02
Liver (%)	2.76 ± 0.04	2.87 ± 0.04	3.04 ± 0.04*	3.35 ± 0.03*
Heart (%)	0.40 ± 0.01	0.38 ± 0.01	0.39 ± 0.01	0.43 ± 0.02
Brain (%)	1.07 ± 0.02	1.06 ± 0.04	1.05 ± 0.02	1.10 ± 0.01
Spleen (%)	0.26 ± 0.01	0.25 ± 0.00	0.27 ± 0.00	0.30 ± 0.01*
Adrenals (%)	0.05 ± 0.00	0.05 ± 0.00	0.04 ± 0.00	0.05 ± 0.00
Thymus (%)	0.14 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.01
Gonads (%)	0.10 ± 0.01	0.08 ± 0.00	0.13 ± 0.05	0.09 ± 0.00

[a]Reddy et al. (1999).
Data are mean ± standard deviation.
* Significantly different from the control group ($p \leq 0.05$) by the Dunnett's test.

Table B.3. Effect of Tetryl on Hematological Parameters after 45 or 90 Days on Males[a]

Concentration (ppm) diet	Sample at 45 or 90 days	0	200	1,000	3,000
(mg/kg-d)		0	13.0	62.4	179.6
RBC ($\times 10^6/\mu L$)	45 90	8.66 ± 0.12 9.34 ± 0.19	8.87 ± 0.30 9.31 ± 0.23	8.71 ± 0.15 9.27 ± 0.18	8.50 ± 0.20 8.94 ± 0.18*
Hemoglobin (g/dL)	45 90	15.44 ± 0.29 15.83 ± 0.23	15.40 ± 0.78 15.61 ± 0.41	14.90 ± 0.43 15.31 ± 0.23*	14.12 ± 0.48* 14.22 ± 0.25*
Hematocrit (%)	45 90	44.90 ± 0.97 48.37 ± 0.79	45.20 ± 1.71 48.05 ± 1.14	43.94 ± 1.19 47.15 ± 1.23*	43.14 ± 0.80 44.98 ± 0.95*
WBC ($\times 10^3/\mu L$)	45 90	4.58 ± 0.58 4.35 ± 0.64	4.20 ± 0.58 4.21 ± 0.68	4.20 ± 0.87 4.30 ± 0.63	4.10 ± 0.81 4.43 ± 0.58
Platelets ($\times 10^3/\mu L$)	45 90	700.60 ± 92.44 733.70 ± 87.65	789.00 ± 34.55 728.30 ± 43.06	844.20 ± 37.25* 745.10 ± 83.61	946.20 ± 73.40 856.70 ± 45.82*
Reticulocytes (%)	45 90	2.42 ± 0.29 1.96 ± 0.19	2.26 ± 0.34 1.88 ± 0.20	2.84 ± 0.26 2.05 ± 0.13	4.16 ± 0.15* 3.35 ± 0.18*
MetHb (%)	45 90	0.42 ± 0.31 0.50 ± 0.40	0.88 ± 0.48 0.58 ± 0.32	1.36 ± 0.33* 1.37 ± 0.27*	2.44 ± 0.46* 2.67 ± 0.54*

[a]Reddy et al. (1999).
n = 5 rats per dose group at 45 d; *n* = 10 rats per dose group at 90 d.
Data are mean ± standard deviation.
*Significantly different from the control group ($p \leq 0.05$) by the Dunnett's test.

RBC = red blood cells; WBC = white blood cells; MetHB = methemoglobin.

Table B.4. Effect of Tetryl on Hematological Parameters after 45 or 90 Days on Females[a]

Concentration (ppm) diet	Sample at 45 or 90 days	0	200	1,000	3,000
(mg/kg-d)		0	14.2	68.8	199.0
RBC ($\times 10^6/\mu L$)	45 90	8.15 ± 0.21 8.24 ± 0.21	7.99 ± 0.16 8.23 ± 0.27	7.94 ± 0.18 8.12 ± 0.19	7.80 ± 0.25* 7.70 ± 0.34*
Hemoglobin (g/dL)	45 90	15.66 ± 0.25 15.66 ± 0.35	14.96 ± 0.42 15.66 ± 0.58	14.66 ± 0.40* 15.16 ± 0.32*	14.36 ± 0.68* 14.53 ± 0.44*
Hematocrit (%)	45 90	43.72 ± 2.01 44.61 ± 1.58	42.42 ± 1.00 44.55 ± 1.41	43.72 ± 1.83 43.85 ± 1.41	42.88 ± 1.68 43.22 ± 1.49
WBC ($\times 10^3/\mu L$)	45 90	4.18 ± 0.49 4.14 ± 0.70	4.04 ± 0.96 3.41 ± 0.57*	4.70 ± 0.33 3.69 ± 0.57	4.78 ± 0.73 3.71 ± 0.56
Platelets ($\times 10^3/\mu L$)	45 90	778.60 ± 62.07 742.50 ± 32.03	778.40 ± 64.76 758.10 ± 60.50	716.6 ± 125.3 811.40 ± 71.07*	796.40 ± 82.38 853.40 ± 53.81*
Reticulocytes (%)	45 90	2.02 ± 0.25 1.71 ± 0.28	1.94 ± 0.27 1.77 ± 0.22	2.32 ± 0.11 2.06 ± 0.28*	3.68 ± 0.43* 2.63 ± 0.37*
MetHb	45 90	0.28 ± 0.25 0.59 ± 0.33	0.90 ± 0.31* 0.68 ± 0.33	1.10 ± 0.27* 1.09 ± 0.33*	1.96 ± 0.41* 2.23 ± 0.34*

[a]Reddy et al. (1999).
n = 5 rats per dose group at 45 d; *n* = 10 rats per dose group at 90 d.
Data are mean ± standard deviation.
* Significantly different from the control group ($p \leq 0.05$) by the Dunnett's test.

RBC = red blood cells; WBC = white blood cells; MetHB = methemoglobin.

Table B.5. Effect of Tetryl on Clinical Chemistry After 45 and 90 Days on Males[a]

Concentration (ppm) diet	Sample at 45 or 90 d	0	200	1,000	3,000
(mg/kg-d)		0	13.0	62.4	179.6
Glucose (mg/dL)	45 90	194.60 ± 21.85 185.60 ± 18.06	183.60 ± 15.88 187.50 ± 19.46	185.20 ± 19.61 187.50 ± 15.66	175.20 ± 9.65 180.00 ± 17.99
BUN (mg/dL)	45 90	19.60 ± 1.52 20.20 ± 0.92	18.40 ± 3.13 20.60 ± 2.22	20.40 ± 1.52 22.20 ± 1.03*	18.00 ± 1.22 21.0 ± 1.15
Creatinine (mg/dL)	45 90	0.60 ± 0.07 0.62 ± 0.04	0.56 ± 0.05 0.62 ± 0.04	0.62 ± 0.04 0.61 ± 0.03	0.60 ± 0.00 0.60 ± 0.05
Alkaline phosphatase (IU/L)	45 90	135.80 ± 8.78 104.90 ± 6.87	134.00 ± 14.61 93.70 ± 6.60*	123.80 ± 10.03 88.30 ± 8.71*	119.60 ± 12.56 80.90 ± 6.38*
AST (IU/L)	45 90	126.80 ± 16.18 157.0 ± 43.60	113.60 ± 12.22 150.30 ± 29.70	134.40 ± 39.30 164.60 ± 21.88	111.60 ± 19.83 175.50 ± 30.75
ALT (IU/L)	45 90	53.80 ± 12.68 85.10 ± 28.73	45.80 ± 6.80 83.80 ± 17.78	51.60 ± 23.20 80.40 ± 16.30	38.60 ± 8.56 66.50 ± 11.00
Potassium (mEq/L)	45 90	4.74 ± 0.51 4.65 ± 0.31	4.70 ± 0.59 4.80 ± 0.38	4.82 ± 0.44 4.62 ± 0.38	5.62 ± 0.54* 5.00 ± 0.24
Albumin (g/dL)	45 90	4.38 ± 0.08 4.72 ± 0.16	4.64 ± 0.26 4.82 ± 0.12	4.78 ± 0.16* 5.00 ± 0.11*	5.04 ± 0.09* 5.12 ± 0.21*
Calcium (mg/dL)	45 90	10.64 ± 0.15 10.47 ± 0.12	10.80 ± 0.07 10.60 ± 0.18	11.02 ± 0.16* 10.67 ± 0.18*	11.14 ± 0.15* 10.70 ± 0.21*
Phosphorus (mg/dL)	45 90	9.82 ± 0.52 9.05 ± 0.55	9.64 ± 0.60 8.95 ± 0.66	9.84 ± 0.43 8.59 ± 0.97	10.82 ± 1.01 8.97 ± 0.89
Triglycerides (mg/dL)	45 90	99.80 ± 23.55 104.40 ± 27.63	88.60 ± 44.81 103.40 ± 30.56	97.00 ± 28.22 119.40 ± 28.89	64.20 ± 24.80 89.30 ± 32.15
Cholesterol[b] (mg/dL)	90	58.60 ± 7.06	66.40 ± 4.77*	79.10 ± 4.65*	105.40 ± 7.57*
Sodium (mEq/L)	45 90	137.60 ± 1.52 143.00 ± 0.94	139.00 ± 1.00 143.50 ± 1.08	139.00 ± 1.22 143.00 ± 0.82	138.40 ± 0.55 142.30 ± 0.67
Total bilirubin (mg/dL)	45 90	0.14 ± 0.09 0.05 ± 0.05	0.14 ± 0.09 0.10 ± 0.00*	0.12 ± 0.04 0.09 ± 0.03*	0.18 ± 0.04 0.10 ± 0.00*
Total protein (g/dL)	45 90	6.30 ± 0.14 6.81 ± 0.21	6.60 ± 0.21* 6.88 ± 0.13	6.88 ± 0.13* 7.22 ± 0.23*	7.12 ± 0.18* 7.39 ± 0.30*

[a]Reddy et al. (1999, 1994b).
n = 5 rats per dose group at 45 d; n = 10 rats per dose group at 90 d.
[b]Cholesterol was only measured at Day 90.
Data are mean ± standard deviation.
* Significantly different from the control group ($p \leq 0.05$) by the Dunnett's test.

ALT = Alanine Aminotransferase; AST = Aspartate Aminotransferase.

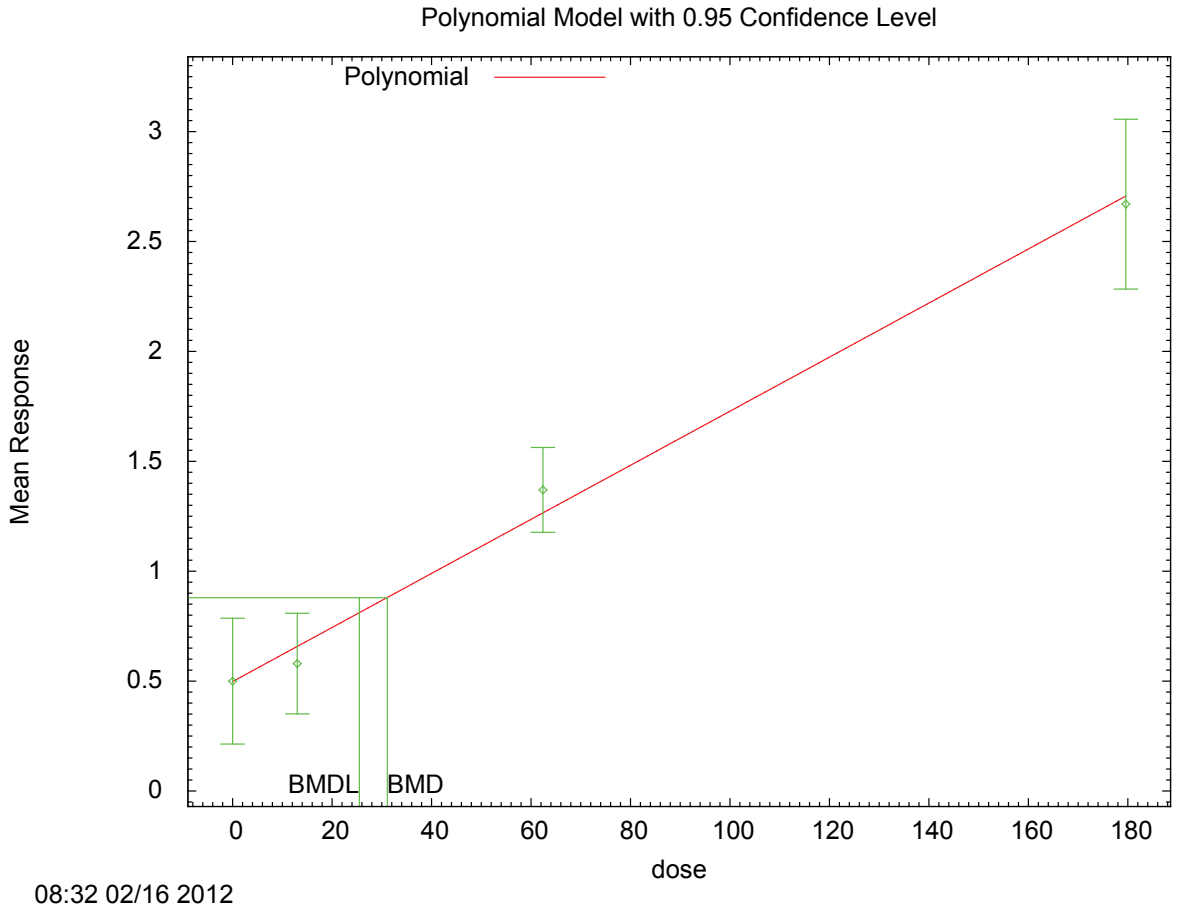
Table B.6. Effect of Tetryl on Clinical Chemistry After 45 and 90 Days on Females[a]

Concentration (ppm) diet	Sample at 45 or 90 d	0	200	1,000	3,000
(mg/kg-d)		0	14.2	68.8	199.0
Glucose (mg/dL)	45 90	154.80 ± 21.04 125.10 ± 17.64	166.00 ± 5.00 138.80 ± 15.42	161.40 ± 17.87 145.30 ±18.66*	130.20 ± 20.35 130.00 ± 17.31
BUN (mg/dL)	45 90	18.40 ± 3.21 18.30 ± 2.21	17.60 ± 1.67 19.00 ± 1.89	17.00 ± 1.00 20.20 ± 2.39	17.20 ± 1.92 20.20 ±2.97
Creatinine (mg/dL)	45 90	0.52 ± 0.04 0.54 ± 0.05	0.54 ± 0.05 0.55 ± 0.05	0.64 ± 0.05* 0.56 ± 0.05	0.60 ± 0.00* 0.57 ± 0.05
Alkaline phosphatase (IU/L)	45 90	123.20 ± 10.83 76.50 ± 11.40	122.50 ± 26.31 74.00 ± 15.32	122.40 ± 24.57 67.90 ± 8.02	116.00 ± 16.84 63.30 ± 9.35*
AST (IU/L)	45 90	106.20 ± 26.44 132.00 ± 27.94	95.20 ± 11.12 180.20 ± 65.81*	182.20 ± 92.88 160.70 ± 37.69	147.20 ± 33.94 159.30 ± 33.04
ALT (IU/L)	45 90	37.40 ± 4.28 55.20 ± 19.23	35.60 ± 5.68 85.40 ± 46.21	60.20 ± 35.15 79.80 ± 30.14	38.20 ± 6.38 59.00 ± 16.42
Potassium (mEq/L)	45 90	4.58 ± 0.37 4.03 ± 0.25	4.52 ± 0.49 4.49 ± 0.50	4.30 ± 0.16 4.48 ± 0.45	4.54 ± 0.45 4.56 ± 0.86
Albumin (g/dL)	45 90	4.38 ± 0.08 4.42 ± 0.15	4.22 ± 0.16 4.67 ± 0.23*	4.68 ± 0.13 4.72 ± 0.12*	4.58 ± 0.33 4.96 ± 0.21*
Calcium (mg/dL)	45 90	10.66 ± 0.21 10.04 ± 0.24	10.60 ± 0.20 10.23 ± 0.23	10.96 ± 0.13 10.18 ± 0.21	10.70 ± 0.21 10.34 ± 0.43
Phosphorus (mg/dL)	45 90	8.90 ± 1.27 8.31 ± 0.97	8.38 ± 0.52 8.63 ± 1.10	10.02 ± 1.42 8.57 ± 0.98	9.44 ± 1.69 9.12 ± 0.99
Triglycerides (mg/dL)	45 90	39.60 ± 8.82 40.60 ± 13.93	38.80 ± 10.85 44.60 ± 15.14	47.60 ± 14.54 36.20 ± 13.31	29.40 ± 3.58 25.40 ± 3.86*
Cholesterol[b] (mg/dL)	90	102.70 ± 6.80	112.90 ± 8.85*	123.00 ± 8.91*	131.50 ± 10.22*
Sodium (mEq/L)	45 90	138.40 ± 1.67 142.70 ± 0.82	138.60 ± 0.55 142.90 ± 0.74	139.60 ± 1.82 142.80 ± 1.14	139.60 ± 0.55 143.50 ± 1.08
Total bilirubin (mg/dL)	45 90	0.18 ± 0.04 0.11 ± 0.03	0.14 ± 0.05 0.19 ± 0.10*	0.20 ± 0.00 0.16 ± 0.07	0.26 ± 0.05* 0.22 ± 0.04*
Total protein (g/dL)	45 90	6.20 ± 0.32 6.18 ± 0.19	6.04 ± 0.15 6.37 ± 0.28	6.52 ± 0.08 6.52 ± 0.20*	6.28 ± 0.04 6.71 ± 0.33*

[a]Reddy et al. (1999, 1994b).
n = 5 rats per dose group at 45 d; n = 10 rats per dose group at 90 d.
[b]Cholesterol was only measured at Day 90.
Data are mean ± standard deviation.
* Significantly different from the control group ($p \leq 0.05$) by the Dunnett's test.

APPENDIX C. BMD OUTPUTS

Table C.1. Tetryl 90 Day Male Methemoglobinemia Data					
Continuous					
Model Name	**BMD (mg/kg-d)**	**BMDL (mg/kg-d)**	**p-Value Test 4**	**AIC**	**Scaled Residual of Interest**
Hill	33.9864	17.1635	NA	−28.344925	-3.10×10^{-07}
Linear	31.103	25.486	0.5277	−31.066295	−0.641
Polynomial	31.103	25.486	0.5277	−31.066295	−0.641
Power	31.103	25.486	0.5277	−31.066295	−0.641



```
 ================================================================
        Polynomial Model. (Version: 2.16;  Date: 05/26/2010)
        Input Data File: C:/Documents and
Settings/jlipscom/Desktop/Benchmark/BMDS220/Data/lin_tetrylmale90dmehb_Opt.(d)
        Gnuplot Plotting File:  C:/Documents and
Settings/jlipscom/Desktop/Benchmark/BMDS220/Data/lin_tetrylmale90dmehb_Opt.plt
                                                Thu Feb 16 09:16:18 2012
 ================================================================
 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    The form of the response function is:
   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...

   Dependent variable = Mean
   Independent variable = Dose
   rho is set to 0
   The polynomial coefficients are restricted to be positive
   A constant variance model is fit

   Total number of dose groups = 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
                          alpha =     0.156725
                            rho =            0   Specified
                         beta_0 =     0.497816
                         beta_1 =    0.0122696

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

                   alpha       beta_0       beta_1

     alpha             1     1.1e-010    -7.7e-011

    beta_0     1.1e-010            1        -0.67

    beta_1    -7.7e-011        -0.67            1

                                 Parameter Estimates
                                                          95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
          alpha         0.145634        0.0325648           0.0818084
0.20946
         beta_0         0.497816        0.0811846            0.338697
0.656935
         beta_1        0.0122696      0.000851996           0.0105997
0.0139394

     Table of Data and Estimated Values of Interest
 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------
    0    10        0.5        0.498          0.4        0.382         0.0181
   13    10       0.58        0.657         0.32        0.382         -0.641
 62.4    10       1.37         1.26         0.27        0.382          0.883
179.6    10       2.67          2.7         0.54        0.382          -0.26
```

```
 Model Descriptions for likelihoods calculated

 Model A1:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma(i)^2

 Model A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2
     Model A3 uses any fixed variance parameters that
     were specified by the user

 Model  R:         Yi = Mu + e(i)
           Var{e(i)} = Sigma^2

                     Likelihoods of Interest
            Model      Log(likelihood)   # Param's      AIC
             A1           19.172462           5     -28.344925
             A2           21.919655           8     -27.839310
             A3           19.172462           5     -28.344925
          fitted          18.533147           3     -31.066295
              R          -17.908395           2      39.816789

                  Explanation of Tests
 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                    Tests of Interest
   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              79.6561          6          <.0001
   Test 2              5.49439          3           0.139
   Test 3              5.49439          3           0.139
   Test 4              1.27863          2          0.5277

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1.  A homogeneous variance
model appears to be appropriate here

The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data

             Benchmark Dose Computation
Specified effect =             1
Risk Type        =     Estimated standard deviations from the control mean
Confidence level =          0.95
             BMD =        31.103
            BMDL =        25.486
```

Table C.2. Tetryl 90 Day Female Methemoglobinemia Data							
Model Name	**BMD (mg/kg-d)**	**BMDL (mg/kg-d)**	***p*-Value Test 1: Lack Dose Response?**	***p*-Value Test 3: Good Variance Model?**	***p*-Value for Fit: Does the Model for the Mean Fit?**	**AIC**	**Scaled Residual of Interest**
Exponential			<0.0001	0.9996	0.5198	Array	Array
Hill	45.6563	27.3945	<0.0001	0.9996	NA	−42.295529	−0.0122
Linear	38.1113	31.0666	<0.0001	0.9996	0.8649	−46.006495	0.00214
Polynomial	38.1113	31.0666	<0.0001	0.9996	0.8649	−46.006495	0.00214
Power	45.6278	31.3902	<0.0001	0.9996	0.9724	−44.295671	−0.0116

Table C.3. Tetryl 45 Day Male Methemoglobinemia Data					
Model Name	**BMD (mg/kg-day)**	**BMDL (mg/kg-day)**	***p*-Value for Fit: Does the Model for the Mean Fit?**	**AIC**	**Scaled Residual of Interest**
Exponential			0.06406	Array	Array
Hill	22.4966	10.9077	0.2656	−11.657626	0.861
Linear	36.5918	27.5312	0.3018	−12.501205	0.789
Polynomial	36.5918	27.5312	0.3018	−12.501205	0.789
Power	36.5918	27.5312	0.3018	−12.501205	0.789

Table C.4. Tetryl 45 Day Female Methemoglobinemia Data					
Model Name	**BMD (mg/kg-day)**	**BMDL (mg/kg-day)**	***p*-Value for Fit: Does the Model for the Mean Fit?**	**AIC**	**Scaled Residual of Interest**
Exponential			0.008342	Array	Array
Hill	23.6864	8.11893	0.01679	−16.816854	1.74
Linear	46.4202	34.5036	0.02789	−17.375309	0.346
Polynomial	46.4202	34.5036	0.02789	−17.375309	0.346
Power	46.4202	34.5036	0.02789	−17.375309	0.346

APPENDIX D. REFERENCES

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