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Provisional Peer-Reviewed Toxicity Values for

2,4,4-Trimethylpentene
(CASRN 25167-70-8)

Superfund Health Risk Technical Support Center
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COMMONLY USED ABBREVIATIONS AND ACRONYMS

α2u-g	alpha 2u-globulin
ACGIH	American Conference of Governmental Industrial Hygienists
AIC	Akaike's information criterion
ALD	approximate lethal dosage
ALT	alanine aminotransferase
AST	aspartate aminotransferase
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
BMDS	Benchmark Dose Software
BMR	benchmark response
BUN	blood urea nitrogen
BW	body weight
CA	chromosomal aberration
CAS	Chemical Abstracts Service
CASRN	Chemical Abstracts Service Registry Number
CBI	covalent binding index
CHO	Chinese hamster ovary (cell line cells)
CL	confidence limit
CNS	central nervous system
CPN	chronic progressive nephropathy
CYP450	cytochrome P450
DAF	dosimetric adjustment factor
DEN	diethylnitrosamine
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
FEV_1	forced expiratory volume of 1 second
GD	gestation day
GDH	glutamate dehydrogenase
GGT	γ-glutamyl transferase
GSH	glutathione
GST	glutathione-S-transferase
Hb/g-A	animal blood-gas partition coefficient
Hb/g-H	human blood-gas partition coefficient
HEC	human equivalent concentration
HED	human equivalent dose
i.p.	intraperitoneal
IRIS	Integrated Risk Information System
IVF	in vitro fertilization
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LOAEL	lowest-observed-adverse-effect level
MN	micronuclei
MNPCE	micronucleated polychromatic erythrocyte
MOA	mode of action
MTD	maximum tolerated dose
NAG	N-acetyl-β-D-glucosaminidase
NCEA	National Center for Environmental Assessment
NCI	National Cancer Institute
NOAEL	no-observed-adverse-effect level
NTP	National Toxicology Program
NZW	New Zealand white (rabbit breed)
OCT	ornithine carbamoyl transferase
ORD	Office of Research and Development
PBPK	physiologically based pharmacokinetic
PCNA	proliferating cell nuclear antigen
PND	postnatal day
POD	point of departure
POD_{ADJ}	duration-adjusted POD
QSAR	quantitative structure-activity relationship
RBC	red blood cell
RDS	replicative DNA synthesis
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	regional gas dose ratio
RNA	ribonucleic acid
SAR	structure activity relationship
SCE	sister chromatid exchange
SD	standard deviation
SDH	sorbitol dehydrogenase
SE	standard error
SGOT	glutamic oxaloacetic transaminase, also known as AST
SGPT	glutamic pyruvic transaminase, also known as ALT
SSD	systemic scleroderma
TCA	trichloroacetic acid
TCE	trichloroethylene
TWA	time-weighted average
UF	uncertainty factor
UF_A	interspecies uncertainty factor
UF_H	intraspecies uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
UF_D	database uncertainty factor
U.S.	United States of America
WBC	white blood cell

PROVISIONAL PEER-REVIEWED TOXICITY VALUES FOR 2,4,4-TRIMETHYLPENTENE (CASRN 25167-70-8)

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.

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

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

2,4,4-Trimethylpentene, CASRN 25167-70-8, is a mixture of isomers 2,4,4-trimethylpent-1-ene (CASRN 107-39-1) and 2,4,4-trimethylpent-2-ene (CASRN 107-40-4); 75% consists of the 1-ene and 25% of the 2-ene. The mixture is commonly referred to as "diisobutylene" (Sigma-Aldrich). In this document the mixture is referred to as 2,4,4-trimethylpentene which is the common usage. OECD (2008) also indicates that a synonym is diisobutylene and indicates that the 1-ene is 70−80% and the 2-ene is 15−25% of the mixture. This chemical is listed as a high-production-volume chemical by the Organisation for Economic Cooperation and Development (OECD, 2004). It is primarily used as a chemical intermediate for the production of other industrial chemicals, but also used as a solvent for paints, lacquers, and varnishes (OECD, 2008). The annual production volume in 2002 was 40,000−50,000 tonnes (metric) (OECD, 2008). There is an indication of bioaccumulation potential (OECD, 2008). The empirical formula for 2,4,4-trimethylpentene is C_8H_{16} (see Figure 1). A table of physicochemical properties for a commercial mixture of 75% 2,4,4-trimethylpent-1-ene and 25% 2,4,4-trimethylpent-2-ene is provided below (see Table 1). The isomeric mixture has high vapor pressure, indicating that it will predominantly exist in the atmosphere as a vapor (HSDB, 2002a, b); further, for humans in the environment, the main intake route in regional scenario is inhalation. The mixture is moderately soluble in water, but it is expected to display high volatility from water surfaces (OECD, 2008).

Figure 1. 2,4,4-Trimethylpent-1-ene (Left) and 2,4,4-Trimethylpent-2-ene (Right) Structures

Table 1. Physicochemical Properties of 2,4,4-Trimethylpentene (Mixture) (CASRN 25167-70-8)[a]

Property (unit)	Value
Boiling point (°C)	97–107
Melting point (°C)	−101 to −106
Density (g/cm^3)	0.72
Vapor pressure (mmHg at 38°C)	75–103 (100–137 hPa)
pH (unitless)	ND
Solubility in water (g/L at 20°C)	1.8×10^{-3}[b]
Relative vapor density (air = 1)	ND
K_{ow}	4.4 (OECD, 2008)
Molecular weight (g/mol)	112.22

[a]ECB (2000)
[b]EU (2008)

ND = no data.

A summary of available toxicity values for 2,4,4-trimethylpentene from U.S. EPA and other agencies/organizations is provided in Table 2.

Table 2. Summary of Available Toxicity Values for 2,4,4-Trimethylpentene (Mixture) (CASRN 25167-70-8)

Source/Parameter[a,b]	Value (applicability)	Notes	Reference
Noncancer			
ACGIH	NV	NA	ACGIH (2015)
ATSDR	NV	NA	ATSDR (2015)
Cal/EPA	NV	NA	Cal/EPA (2014); Cal/EPA (2015a); Cal/EPA (2015b)
NIOSH	NV	NA	NIOSH (2015)
OSHA	NV	NA	OSHA (2011); OSHA (2006)
IRIS	NV	NA	U.S. EPA (2015)
DWSHA	NV	NA	U.S. EPA (2012)
HEAST	NV	NA	U.S. EPA (2011a)
CARA HEEP	NV	NA	U.S. EPA (1994)
WHO	NV	NA	WHO (2015)
AIHA (WEEL)	75 ppm (344 mg/m^3)	8-hr TWA	AIHA (2007)
Cancer			
IRIS	NV	NA	U.S. EPA (2015)
HEAST	NV	NA	U.S. EPA (2011a)
DWSHA	NV	NA	U.S. EPA (2012)
IARC	NV	NA	IARC (2013)
NTP	NV	NA	NTP (2014)
Cal/EPA	NV	NA	Cal/EPA (2015a); Cal/EPA (2011); Cal/EPA (2015b)
ACGIH	NV	NA	ACGIH (2015)

[a]Sources: ACGIH = American Conference of Governmental Industrial Hygienists; AIHA = American Industrial Hygiene Association; ATSDR = Agency for Toxic Substances and Disease Registry; Cal/EPA = California Environmental Protection Agency; CARA = Chemical Assessments and Related Activities; DWSHA = Drinking Water Standards and Health Advisories; HEAST = Health Effects Assessment Summary Tables; HEEP = Health and Environmental Effects Profile; IARC = Institute for Occupational Safety and Health; IRIS = Integrated Risk Information Systems; NIOSH = National Institute for Occupational Safety and Health; NTP = National Toxicology Program; OSHA = Occupational Safety and Health Administration; WHO = World Health Organization.
[b]Parameters: TWA = time-weighted average; WEEL = workplace environmental exposure level.

NA = not applicable; NV = not available

Literature searches were conducted in July 2013 covering years back to 1900 and in March 2015 for studies relevant to the derivation of provisional toxicity values for 2,4,4-trimethylpentene, CASRN 25167-70-8. In March 2015 the literature search was updated but no new relevant information was found. Searches were conducted using U.S. EPA's Health and Environmental Research Online (HERO) database of scientific literature. HERO searches the following databases: PubMed, TOXLINE (including TSCATS1), and Web of Science. The following databases were searched outside of HERO for health-related values: ACGIH, ATSDR, Cal/EPA, U.S. EPA IRIS, U.S. EPA HEAST, U.S. EPA Office of Water, U.S. EPA TSCATS2/TSCATS8e, NIOSH, NTP, and OSHA.

REVIEW OF POTENTIALLY RELEVANT DATA (NONCANCER AND CANCER)

Tables 3A and 3B provide an overview of the relevant databases for 2,4,4-trimethylpentene and include all potentially relevant and repeated short-term-, subchronic-, and chronic-duration studies. Principal studies are identified in bold. Reference can be made to details provided in Tables 3A and 3B. The phrase "statistical significance," used throughout the document, indicates a p-value of < 0.05 unless otherwise noted.

Table 3A. Summary of Potentially Relevant Noncancer Data for 2,4,4-Trimethylpentene (CASRN 25167-70-8)

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								
1. Oral (mg/kg-d)[a]								
ND								
2. Inhalation (mg/m^3)								
ND								
Animal								
1. Oral (mg/kg-d)[a]								
Short-term	5 M/5 F, S-D CD-1 rat, gavage, 28 consecutive d.	0, 100, 300, 1,000 ADD: 0, 100, 300, 1,000	Increased absolute and relative liver-weights in both sexes; increased serum albumin and total protein levels in males; increased absolute kidney weight in females, and decreased glucose levels in females at 1,000 mg/kg-d	300	ND	1,000	Huntingdon Life Sciences (1997b) (increased kidney weights in male rats at 1,000 mg/kg-d likely associated with α2u-g accumulation (demonstrated in the reproduction study); not relevant to humans)	NPR

Table 3A. Summary of Potentially Relevant Noncancer Data for 2,4,4-Trimethylpentene (CASRN 25167-70-8)

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]
Reproductive/ developmental	10 M/10 F, S-D CD-1 rat, gavage, 15 d prior to pairing, through mating, gestation, and lactation to LD 3 (44−46 doses for males; 40−45 doses for females). Parental animals were treated for 6 wk.	0, 100, 300, 1,000 ADD: 0, 100, 300, 1,000	Systemic: Increased absolute and relative liver weights in males at 300 and 1,000 mg/kg-d; increased absolute and relative liver weights in females at 1,000 mg/kg-d; increased absolute and relative kidney weight in females at 1,000 mg/kg-d; low incidences of minimal liver pathology in both sexes at 1,000 mg/kg-d; Basophilic cortical tubules at 100 mg/kg-d Reproductive: No effects observed. Developmental: No effects observed.	100 (systemic) 1,000 (reproductive) 1,000 (developmental)	173 for increased relative liver weight in males	300 (systemic) ND (reproductive) ND (developmental)	Huntingdon Life Sciences (1997a) Swenberg and Schoonhoven (2004) (nephrotoxicity in male rats at ≥100 mg/kg-d associated with α2u-g accumulation; not relevant to humans)	PS; NPR

Table 3A. Summary of Potentially Relevant Noncancer Data for 2,4,4-Trimethylpentene (CASRN 25167-70-8)

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]
2. Inhalation (mg/m^3)								
ND								

[a]Dosimetry: Values presented as adjusted daily dose (ADD) in mg/kg-day for oral exposure.
[b]Notes: NPR = not peer reviewed; PS = principal study.

Treatment/exposure duration: unless otherwise noted: short-term = repeated exposure for >24 hours ≤30 days (U.S. EPA, 2002); long-term (subchronic) = repeated exposure for >30 days ≤10% lifespan for humans (more than 30 days up to approximately 90 days in typically used laboratory animal species) (U.S. EPA, 2002); chronic = repeated exposure for >10% lifespan for humans (more than approximately 90 days to 2 years in typically used laboratory animal species) (U.S. EPA, 2002).

F = female(s); M = male(s); LD = Lactation Day; ND = not determined; S-D = Sprague-Dawley.

Table 3B. Summary of Potentially Relevant Cancer Data for 2,4,4-Trimethylpentene (CASRN 25167-70-8)						
Category	**Number of Male/Female, Strain, Species, Study Type, Study Duration**	**Dosimetry**	**Critical Effects**	**BMDL/ BMCL**	**Reference (comments)**	**Notes**
Human						
1. Oral (mg/kg-d)						
ND						
2. Inhalation (mg/m^3)						
ND						
Animal						
1. Oral (mg/kg-d)						
ND						
2. Inhalation (mg/m^3)						
ND						

ND = no data.

HUMAN STUDIES

Oral Exposures

No studies have been identified.

Inhalation Exposures

No studies have been identified.

ANIMAL STUDIES

Oral Exposures

Overview of Animal Oral Exposure Studies

Potentially relevant data for noncancer effects come from two unpublished gavage studies conducted in rats: a 28-day study (Huntingdon Life Sciences, 1997b) and a 6-week exposure study designed to evaluate reproductive effects as well as systemic effects on target organs in parental animals (Swenberg and Schoonhoven, 2004; Huntingdon Life Sciences, 1997a). Note that the rats in the 28-day study were 35−42 days old at commencement of dosing and were thus sexually mature during a large portion of the dosing period. The Huntingdon Life Sciences studies (1997a, b) do not specify the relative amounts of the 1-ene and 2-ene components. Both these studies identified the liver and kidneys as target organs. No treatment-related reproductive effects were observed in the 6-week study. In the 28-day study, increased liver weight among rats at the highest dose tested (1,000 mg/kg-day) was associated with variations in plasma protein (males) and glucose (females) concentrations in the absence of corresponding pathological changes. Increased liver weights were also observed in male rats at 300 mg/kg-day and in both male and female rats at 1,000 mg/kg-day in the reproductive toxicity study.

Increased kidney weight in the presence of renal lesions among male rats was observed in the reproductive toxicity study at doses ≥100 mg/kg-day (Huntingdon Life Sciences, 1997a). Females receiving 1,000 mg/kg-day in this study had slightly elevated kidney weights without microscopic changes. Increased kidney weights were also observed in male rats at 1,000 mg/kg-day in the 28-day study, although no corresponding pathological changes were observed (Huntingdon Life Sciences, 1997b). Immunohistochemistry staining using mouse anti-alpha-2u-globulin monoclonal antibodies confirmed the accumulation of alpha 2u-globulin (α2u-g), a low molecular weight protein almost exclusively produced by the male rat (Swenberg and Schoonhoven, 2004). The significance of this is discussed in the derivation section of this document.

Short-Term-Duration Studies

Huntingdon Life Sciences (1997b); gavage study, 4 weeks

Groups of 10 (35–42 days of age) Sprague-Dawley (S-D) CD-1 rats (5/sex) received daily doses of 2,4,4-trimethylpentene (50:50 mixture of 2 original batches of 2,4,4-trimethylpentene; purity 99.1%) at 0, 100, 300, or 1,000 mg/kg-day via gavage in maize oil for 28 consecutive days. Survival and clinical signs were monitored twice daily, and body weight and food consumption were measured weekly. Each animal was subjected to a functional observation battery that evaluated open-field observations weekly (activity, alertness, behavior, convulsions, defecations, exophthalmos, fur appearance, gait, grooming frequency, lacrimation, palpebral closure, piloerection, posture, pupil size, ease of cage removal, respiration rate, salivation, tremors, and amount of urination), and sensory reactivity tests (auditory pinna reflex,

auditory startle reflex, body temperature, flexor responses, landing foot splay, pain response, pupil closure response, reaction to handling, and righting reflex) followed by evaluations of grip strength and motor activity administered during Week 4 of treatment. Each animal was starved overnight following the neurobehavioral tests, and blood samples were collected for hematology (packed cell volume [PCV], hemoglobin concentration [Hb], counts of red blood cells [RBCs], total and differential white blood cells [WBCs], platelets, mean cell hemoglobin concentration [MCHC], mean cell hemoglobin [MCH], mean cell volume [MCV], and prothrombin time [PT]) and serum chemistry (alkaline phosphatase [ALP], alanine aminotransferase [ALT], aspartate aminotransferase [AST], gamma-glutamyl transpeptidase [GGT], urea, glucose [GLUC], cholesterol [CHOL], creatinine [CREA], plasma protein, albumin, albumin/globulin ration [A/G], sodium, and potassium). At sacrifice, all animals were subjected to a detailed necropsy that included gross pathology, organ weights (adrenals, brain, epididymides, heart, kidneys, liver, spleen, testes, and thymus), histopathology, and collection of bone marrow samples for evaluation of composition. The organ tissues evaluated microscopically for the control and high-dose groups were adrenals, brain, caecum, colon, duodenum, epididymides, heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes, ovaries, prostate, sciatic nerve, spinal cord, spleen, stomach, testes, thymus, thyroid, trachea, urinary bladder, and uterus with cervix. Organ tissue evaluated from the low- and mid-dose groups consisted of kidneys, liver, and lungs only.

No deaths were observed in the study (Huntingdon Life Sciences, 1997b). Slight increases in weight gain, food consumption, and food conversion efficiency were noted for high-dose females. A slight increase in food intake was also observed among high-dose males and a slightly increased body-weight gain, and food conversion efficiency was observed for mid-dose females (see Table B-1). High-dose animals exhibited brown staining of the dorsal and ventral fur (see Table B-1). Open-field observations showed that staining was first observed in males during Week 2 and only at the end of treatment in females. Females in this dose group also showed an ungroomed appearance. Salivation after administration, a common finding in gavage studies, was observed on isolated occasions in some high-dose animals. It is unclear whether the cause of the brown staining of the coat in high-dose animals was related to salivation. No treatment-related changes were observed based on the sensory reactivity tests. There were no statistically significant differences in grip strength or motor activity. Changes in hematology and blood chemistry parameters are shown in Table B-1. Hematological changes were restricted to slightly higher (statistically significant) MCH and MCV in high-dose females. In the absence of other hematological changes and the absence of similar findings in males, the toxicological significance of these effects is uncertain. Changes in blood chemistry parameters included statistically significantly decreased plasma glucose concentrations in high-dose females, and statistically significantly increased plasma protein and albumin concentrations in high-dose males. Changes in plasma urea concentrations differed by sex, whereby statistically significant increases were observed in high-dose males and statistically significant decreases were observed in mid- and high-dose females. No treatment-related effects were observed on cellularity or composition of the bone marrow. Organ weights are shown in Table B-2. The absolute and relative liver weights were statistically and biologically significantly increased in high-dose animals (46 and 44%, respectively, in males; 30 and 21%, respectively, in females) than in controls. The absolute kidney weights were statistically (not females) and biologically significantly increased in high-dose males (39%) and females (14%); relative kidney weights were statistically and biologically significantly increased in mid-dose (not statistically significant) and high dose males (11% and 35%, respectively) than in controls. No corresponding changes in pathology were observed. A lowest-observed-adverse-effect level

(LOAEL) of 1,000 mg/kg-day is identified for increased liver and kidney weights. The no-observed-adverse-effect level (NOAEL) is 300 mg/kg-day.

Subchronic-Duration Studies
No studies have been identified.

Chronic-Duration Studies
No studies have been identified.

Reproductive/Developmental Studies
Huntingdon Life Sciences (1997a), Swenberg and Schoonhoven (2004)
Groups of 20 S-D CD-1 rats (10/sex) received daily doses of 2,4,4-trimethylpentene (50:50 mixture of 2 original batches of 2,4,4-trimethylpentene; purity 99.1%) at 0, 100, 300, or 1,000 mg/kg-day via gavage in maize oil for 15 days prior to mating. Treatment was continued in females through mating, gestation, and lactation to Day 3 of lactation, and to termination following approximately 6 weeks (between 44 and 46 dose administrations) of treatment in males. Survival and clinical signs were monitored twice daily. Male body weights were measured weekly; female body weights were measured weekly until mating was detected, and then on Gestation Days (GDs) 0, 7, 14, and 20 and Lactation Days (LDs) 1 and 4. Food consumption was recorded weekly until mating for both males and females, and then for females on GDs 0–3, 4–6, 7–10, 11–13, 14–16, and 17−19 and LDs 1−3. Vaginal smears were taken from females 10 days prior to mating to evaluate the regularity and duration of the estrus cycle. Gestation lengths were calculated and the offspring evaluated for litter size, survival (number live and dead), weight (live offspring only), sex ratio, clinical signs, and gross pathology. At termination, all adult animals were subjected to a complete gross necropsy. The numbers of corpora lutea and uterine implantation sites were recorded for all females. Organ weights (epididymides, kidneys, liver, ovaries, prostate, seminal vesicles, testes, and uterus with cervix) were measured, and histopathology was performed on all parental animals. The tissues evaluated microscopically for abnormalities in the control and high-dose groups were epididymides, kidneys, liver, ovaries, and testes. Tissues evaluated for abnormalities in the low- and mid-dose groups were kidneys and liver only.

One high-dose female that displayed underactive behavior, hunched posture, piloerection, slow respiration, brown-colored urine, and brown perigenital staining was sacrificed on LD 2 (Huntingdon Life Sciences, 1997a). Necropsy of this animal revealed signs of poor feeding, a swollen liver, dark urinary bladder contents, and pale, mottled kidneys. Microscopic kidney changes included moderate cortical tubule degeneration and moderate proteinaceous casts in the collecting ducts. Similar signs or findings were not observed in other animals; thus, the study authors considered the death of this animal as incidental. High-dose animals exhibited staining of the dorsal and ventral fur (see Table B-3). Staining was first observed in males during Week 2 and in females during Week 4. Transient salivation after administration was observed among high-dose animals and as single occurrences for three mid-dose males and one low-dose male. Salivation is a common finding in gavage studies, and it is unclear if the cause of the brown staining of the coat in high-dose animals was related to salivation. No significant treatment-related effects were observed on body weight gain, food consumption, estrous cycle regularity or duration, mating performance, fertility, gestation length, parturition, or gestation index. The numbers of corpora lutea, implantation sites, litter sizes, offspring survival, and offspring body weights and weight gains during the first 4 days of age were not affected by

treatment. One litter showed low offspring survival resulting from overnight flooding of the cage on LDs 2–3 that was not treatment-related. Necropsy of offspring revealed no findings that could clearly be ascribed to parental treatment to 2,4,4 trimethylpentene. Organ weights and incidences of pathological findings in parent rats are shown in Table B-3. No significant treatment-related effects were observed on reproductive organs of parental animals.

Absolute liver weights were statistically and biologically significantly increased in mid- and high-dose males (17 and 61%, respectively) and high-dose females (22%); in addition, the relative liver weights were also statistically and biologically significantly increased in mid- and high-dose males (15 and 62%, respectively) and high-dose females (26%). Similarly, absolute kidney weights were statistically and biologically significantly increased in mid- and high-dose males (23 and 29%, respectively) and high-dose females (12%) than in controls; relative kidney weights were statistically (not females) and biologically significantly increased in mid- and high-dose males (21 and 29%, respectively) and high-dose females (17%). Macroscopic evaluation of high-dose parental animals revealed swollen livers or liver lobes among all males and four females, and enlarged kidneys among two males. Microscopic evaluation of the livers from treated animals revealed centriacinar fatty vacuolation in two high-dose females (one of these animals was the early decedent), as well as arteritis and biliary fibrosis in one high-dose male, and focal inflammation with associated hepatocytic degeneration in one high-dose male. Microscopic evaluation of the kidneys from treated animals revealed basophilic cortical tubules in males of all treatment groups, as well as proteinaceous casts and interstitial inflammatory cells in mid- and high-dose males. The incidence of basophilic cortical tubules was statistically significantly increased at ≥100 mg/kg-day in male rats. The incidences of proteinaceous casts and interstitial inflammatory were statistically significantly increased only at 300 mg/kg-day in males.

Kidney sections from the rats evaluated in the Huntingdon Life Sciences (1997a) study were subsequently analyzed by immunostaining using mouse anti-α2u-g monoclonal antibodies for the presence of α2u-g (Swenberg and Schoonhoven, 2004) (see Table B-4). Renal lesions ascribed to α2u-g-associated nephropathy with the involvement of hyaline droplet accumulation are considered a species- and sex-specific effect for male rats (U.S. EPA, 1991). These findings are discussed in more detail in the derivation section. Formalin-fixed kidney tissue samples (from males [10/dose] at 0, 100, 300, and 1,000 mg/kg-day and females [10/dose] at 0 and 1,000 mg/kg-day) were blocked in paraffin and sectioned for α2u-g staining. Kidney sections from male F344 rats gavaged with 150 mg/kg-day d-limonene for 4 days were also included in the study as positive controls. All positive controls gave the expected response. None of the kidney sections from female rats exhibited any positive staining for α2u-g. In contrast, all slides from male animals exhibited some positive staining for α2u-g. Control males demonstrated minimal staining with an average score of 1.2. Average scores increased for male rats with dose, whereby low-dose males demonstrated mild staining (average score 2.2) and mid- and high-dose males demonstrated moderate to severe staining (average scores of 3.6 and 3.8, respectively). Most of the male mid- and high-dose rats exhibited severe staining with numerous specific positive staining droplets. Tissue samples from all but two of the high-dose males exhibited positive staining granular casts. Swenberg and Schoonhoven (2004) concluded that α2u-g disease was present in the male rat kidneys.

The reproductive/developmental NOAEL for the Huntingdon Life Sciences (1997a) study is 1,000 mg/kg-day, and no reproductive/developmental LOAEL is identified based on the

lack of any significant treatment-related effects. The study authors of the Huntingdon Life Sciences (1997a) study also identified a LOAEL of 100 mg/kg-day for nephrotoxic effects based on the pathological renal changes observed in male rats at this dose level and higher. However, based on the subsequent evaluation of all the relevant information (see details in Appendix A), the observed renal lesions in male rats were considered to be a consequence of formation of α2u-g, a known species-specific effect in male rats that is not predictive of similar effects in humans. Thus, the systemic LOAEL for this study is 300 mg/kg-day based on statistically and biologically significantly increased absolute and relative liver weights in males with a corresponding NOAEL of 100 mg/kg-day

Secondary Source Publications

EU (2008), EU (2009)

This report summarizes relevant physical, chemical, and toxicological information on 2,4,4-trimethylpentene with an emphasis is on fate and transport and health and safety measures. The Huntingdon Life Sciences (1997a, b) and Swenberg and Schoonhoven (2004) papers are summarized but no tables are provided. There is a detailed discussion of α2u-g disease, which is unique to the male rat kidney and not relevant to human toxicity. The authors agreed with Swenberg and Schoonhoven (2004) that α2u-g disease is present in the Huntingdon Life Sciences (1997a) study. A paper from the European Commission (SCHER, 2006) entitled *Opinion on Risk Assessment Report on 2,4,4-Trimethylpentene, Human Health Part*, states agreement with the opinions in EU (2008). However, the European Union (EU) authors did not apply all of the criteria required in the EPA guidance for verification of α2u-g disease.

Inhalation Exposures

No studies examining the effects of 2,4,4-trimethylpentene in animals exposed via inhalation have been identified.

OTHER DATA (SHORT-TERM TESTS, OTHER EXAMINATIONS)

Genotoxicity

A secondary source (EU, 2008) summarized the findings from two unpublished in vitro mutagenicity studies that were not available to review at the time of preparing this PPRTV assessment. 2,4,4-Trimethylpentene was negative for bacterial mutation in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* WP2uvrA in the presence and absence of Aroclor-1254-induced rat liver S9 at concentrations ranging up to 5,000 µg/plate [Shell (1996) as cited in EU (2008)]. Results were also negative in an assay for chromosomal aberrations in human lymphocytes in the presence or absence of S9-mix [Shell (1997) as cited in EU (2008)].

Acute Toxicity Studies

EU (2008) also summarized the findings from three industry studies that evaluated the acute toxicity of 2,4,4-trimethylpentene that were not available to review at the time of preparing this PPRTV assessment.

Huntingdon Life Sciences (1996) as cited in EU (2008) administered commercial 2,4,4-trimethylpentene (purity 95.19%) to groups of rats (5/sex; strain not specified) at a dosage of 2,000 mg/kg via gavage in maize oil as a single treatment. There were no effects on survival,

clinical signs, or body-weight gain, and necropsy findings were unremarkable (endpoints not specified).

Bayer AG (1972) as cited in EU (2008) treated groups of male Wistar rats (15/dose) orally (not further specified) with a mixture of C8 olefins (approximately 75% 2,4,4-trimethylpentene-1 and 15% 2,4,4-trimethylpentene-2) at 250, 500, 1,000, or 2,500 mg/kg without inducing lethality at any dose. Additionally, a group of 30 male Wistar rats was treated daily for 5 days with increasing doses of the same substance as follows: 200 mg/kg at Day 1, 300 mg/kg at Day 2, 450 mg/kg at Day 3, 675 mg/kg at Day 4, and 1,015 mg/kg at Day 5. There were no mortalities over the 7-day observation period, but clinical signs (not specified) were noted after Day 3.

Bayer AG (1972) as cited in EU (2008) exposed groups of Wistar rats (20/sex/concentration) via whole-body inhalation to a mixture of C8 olefins (approximately 75% 2,4,4-trimethylpentene-1 and 15% 2,4,4-trimethylpentene-2) as a mist at concentrations ranging from 7.6–44.0 mg/L for 4 hours. Mortality was observed starting at concentrations of approximately 21–25 mg/L; the median lethal concentration (LC_{50}) values for male and female rats were 31.5 and 30.0 mg/L, respectively. Subsequently, groups of Wistar rats (10/sex) were exposed to the same substance at 21.6 mg/L for 4 hours/day on 5 consecutive days. Three males and 2 females died within 24 hours. Clinical signs in the surviving animals included convulsions, followed by sedation and respiratory distress.

DERIVATION OF PROVISIONAL VALUES

The reproductive/developmental gavage study in rats by Huntingdon Life Sciences (1997a) is considered inadequate for p-RfD derivation because it is a non-peer-reviewed and unpublished report. However, the Huntingdon Life Sciences (1997a) study is suitable for the derivation of screening toxicity values. Appendix A provides details on the screening subchronic and chronic p-RfD.

DERIVATION OF INHALATION REFERENCE CONCENTRATIONS

No subchronic- or chronic-duration studies of humans or animals exposed to 2,4,4-trimethylpentene via inhalation were identified in the available literature, precluding derivation of inhalation RfCs.

CANCER WEIGHT-OF-EVIDENCE DESCRIPTOR

Table 4 identifies the cancer weight-of-evidence (WOE) descriptor for 2,4,4-trimethylpentene.

Table 4. Cancer Weight-of-Evidence Descriptor for 2,4,4-Trimethylpentene

Possible WOE Descriptor	Designation	Route of Entry (oral, inhalation, or both)	Comments
"Carcinogenic to Humans"	NS	NA	There are no human data to support this.
"Likely to Be Carcinogenic to Humans"	NS	NA	There are no suitable animal studies to support this.
"Suggestive Evidence of Carcinogenic Potential"	NS	NA	There are no suitable animal studies to support this.
"Inadequate Information to Assess Carcinogenic Potential"	**Selected**	**Both**	**This descriptor is selected due to the lack of any information on the carcinogenicity of 2,4,4-trimethylpentene.**
"Not Likely to Be Carcinogenic to Humans"	NS	NA	There are no suitable animal studies to support this.

NA = not applicable; NS = not selected

DERIVATION OF PROVISIONAL CANCER POTENCY VALUES

The lack of data on the carcinogenicity of 2,4,4-trimethylpentene precludes the derivation of quantitative estimates for either oral (p-OSF) or inhalation (p-IUR) exposure (see Table 5).

Table 5. Summary of Cancer Values for 2,4,4-Trimethylpentene (CASRN 25167-70-8)

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

NDr = not determined.

APPENDIX A. SCREENING PROVISIONAL VALUES

SCREENING PROVISIONAL VALUES

For reasons noted in the main provisional peer-reviewed toxicity value (PPRTV) document, it is inappropriate to derive provisional toxicity values for 2,4,4-trimethylpentene. However, "screening" toxicity values may be developed from these studies which, under current guidelines, may be of limited use to risk assessors. In such cases, the Superfund Health Risk Technical Support Center summarizes available information in this Appendix and develops "screening" values. Appendices receive the same level of internal and external scientific peer review as the PPRTV documents to ensure their appropriateness within the limitations detailed in the document. Users of screening toxicity values in an appendix to a PPRTV assessment should understand that there is considerably more uncertainty or lack of verification processes associated with the derivation of an appendix screening toxicity value than for a value presented in the body of the assessment. Questions or concerns about the appropriate use of screening values should be directed to the Superfund Health Risk Technical Support Center. Table A-1 presents a summary of screening noncancer reference values.

Table A-1. Summary of Screening Noncancer Reference Values for 2,4,4-Trimethylpentene (CASRN 25167-70-8)

Toxicity Type (units)	Species/Sex	Critical Effect	p-Reference Value	POD Method	POD_{HED}	UF_C	Principal Study
Screening subchronic p-RfD (mg/kg-d)	Rat/male	Increased relative liver weight	1×10^{-1}	$BMDL_{10}$	$BMDL_{10HED} = 41.5$	300	Huntingdon Life Sciences (1997a)
Screening chronic p-RfD (mg/kg-d)	Rat/male	Increased relative liver weight	1×10^{-2}	$BMDL_{10}$	$BMDL_{10HED} = 41.5$	3,000	Huntingdon Life Sciences (1997a)
Subchronic p-RfC (mg/m^3)	NDr						
Chronic p-RfC (mg/m^3)	NDr						

NDr = not determined.

DERIVATION OF ORAL REFERENCE DOSES

Derivation of Screening Subchronic Provisional RfD (Subchronic p-RfD)

The reproductive toxicity study in rats by Huntingdon Life Sciences (1997a) is selected as the principal study for derivation of the screening subchronic provisional oral reference dose (p-RfD). Increased relative liver weight in male rats is selected as the critical effect.

Justification for the Critical Effect

Increased relative liver weight was chosen as the critical effect for deriving the screening subchronic p-RfD for 2,4,4-trimethylpentene because it is the most clearly identified effect relevant to human health in the available information on oral effects from 2,4,4-trimethylpentene exposure in rats. Increased liver weights (absolute and relative) were observed in rats at the following lowest-observed-adverse-effect levels (LOAELs):

- 300 mg/kg-day (and no-observed-adverse-effect level [NOAEL] of 100 mg/kg-day) in male rats exposed via gavage for 6 weeks during a reproductive toxicity study (Huntingdon Life Sciences, 1997a).
- 1,000 mg/kg-day (and NOAEL of 300 mg/kg-day) in female rats exposed via gavage for approximately 6 weeks during a reproductive toxicity study (Huntingdon Life Sciences, 1997a).
- 1,000 mg/kg-day (and NOAEL of 300 mg/kg-day) in male and female rats exposed via gavage for 28 days (Huntingdon Life Sciences, 1997b).

Increases in liver weight (both absolute and relative) were relatively large in magnitude and demonstrated a clear dose-response. Among male rats in the reproductive toxicity study, the increases were ≥15% at the LOAEL of 300 mg/kg-day and >60% at 1,000 mg/kg-day. Increases in liver weights among female rats at 1,000 mg/kg-day in this study were >20%. Additionally, minimal histopathological changes were noted in a small number of animals at 1,000 mg/kg-day that included arteritis and biliary fibrosis in one male, focal inflammation with associated hepatocytic degeneration in one male, and centriacinar hepatocytic fatty vacuolation in two females (one was an early decedent). Increases in liver weights (>20%) among rats in the 28-day study were accompanied by changes in some serum chemistry parameters, including decreased glucose in females and increased total protein and albumin levels in males, although treatment-related pathological changes were not seen in the livers of treated rats in this study.

Justification of the Principal Study

The reproductive/developmental toxicity study of rats (Huntingdon Life Sciences, 1997a) was selected as the principal study for the following reasons:

1) The study was performed in accordance with OECD Test Guideline 421 and meets the standards of study design and performance with regard to numbers of animals, examination of potential toxicity endpoints, and presentation of information. Details of the study are provided in the "Review of Potentially Relevant Data" section.
2) The study identified a LOAEL for increased liver weight in male rats that was lower than the LOAEL for the same effect in the 28-day study (Huntingdon Life Sciences, 1997b).
3) The study used a larger sample size (10/sex/dose) and a longer exposure duration (6 weeks) than the 28-day study (sample size of 5/sex/dose).

Discussion of Male Rat Kidney Findings

The only other target organ identified in the available studies was the kidney. Increases in kidney weight and renal lesions were observed in male rats in the reproductive toxicity study at doses ≥100 mg/kg-day of 2,4,4-trimethylpentene (Huntingdon Life Sciences, 1997a). Increased kidney weights were also observed in male rats at ≥300 mg/kg-day in the 28-day study, although no corresponding pathological changes were observed (Huntingdon Life Sciences, 1997b). Kidneys of male and female rats from the 6-week study (Huntingdon Life Sciences, 1997a) were examined by Swenberg and Schoonhoven (2004) for possible alpha-2u-globulin (α2u-g) disease. None of the kidney sections from female rats exhibited any positive staining for α2u-g. In contrast, all slides from male animals exhibited dose-dependent increases in positive staining for α2u-g. Most of the male mid- and high-dose rats exhibited severe α2u-g staining with numerous specific positive staining droplets. Tissue samples from all but two of the high-dose males exhibited positive staining granular casts. Swenberg and Schoonhoven (2004) concluded that α2u-g disease was present in the kidneys of male rats treated with 2,4,4-trimethylpentene.

The EPA criteria for α2u-g verification requires observation of an increase in number and size of hyaline droplets only in male kidneys, identification of the protein contained in the hyaline droplets as α2u-g, and some observation of the sequelae of events (i.e., single cell necrosis, exfoliation of epithelial cells into tubular lumen, and granular casts) in the development of α2u-g disease. Verification of increase in the number and size of hyaline droplets only in male kidneys was presented by Swenberg and Schoonhoven (2004) as were the immunological test results for identification of α2u-g in the droplets (see Table B-4). Granular casts were reported in the Huntingdon Life Sciences (1997a) 6-week study in males only with 7/10 and 2/10 rats at 300- and 1,000-mg/kg-day groups, respectively, as shown in Table B-3.

Additionally, basophilic cortical tubules were reported in the Huntingdon Life Sciences (1997a) 6-week study at an occurrence of 0/10, 7/10, 9/10, and 7/10 at 0, 100-, 300-, and 1,000-mg/kg-day dose groups, respectively while being reported in only one female in the 100-mg/kg-day group (see Table B-3). According to Hard and Khan (2004), basophilic tubules, along with thickened basement membranes, hyaline cast formation, and glomerulosclerosis are histologic hallmarks of early chronic progressive nephropathy (CPN). Although CPN is characterized as a disease of aging male rats, basophilic tubules may be observed in male rats as young as 2-months-old. The authors indicate that basophilic tubules have a high rate of cell proliferation and also that enlarged kidneys are often observed with terminal CPN. Thus, the observed increase in kidney weight in male rats from the Huntingdon Life Sciences (1997a) 6-week study could be related to CPN. Hard and Khan (2004) report that CPN and α2u-g disease are frequently observed together, and conclude that CPN is not relevant to humans under these conditions.

Based on the information, the observed renal lesions and increased kidney weight in male rats in the 6-week study (Huntingdon Life Sciences, 1997a) were considered to be a consequence of formation of α2u-g, a known species-specific effect in male rats that is not predictive of similar effects in humans.

Female kidney weight change was observed in both the 4-week and 6-week studies (see Table B-2 and B-3) but is relatively small and occurred at higher doses vs. male kidneys and no pathological change associated with α2u-g was observed in the female kidneys. EPA guidance on alpha α2u-g, with regard to chemicals inducing α2u-g (CIGA), states [U.S. EPA

(1991), page 4]: "In cases where nephrotoxicity was observed in mice or female rats, it was less severe or qualitatively different from that in male rats and did not involve the spectrum of discrete lesions associated with α2u-g accumulation in the male rat." This is consistent with the observation in the female rats from the 4-week and 6-week studies. Thus, the findings related to the female kidney weight change do not alter the conclusion that α2u-g disease is occurring in the male rat.

Approach for Deriving the Screening Subchronic p-RfD

After discounting kidney effects in male rats as described above, the LOAEL of 300 mg/kg-day based on increased liver weights in male rats from the Huntingdon Life Sciences (1997a) reproductive and developmental toxicity study is the lowest LOAEL of the available studies of 2,4,4-trimethylpentene. The mean absolute and relative liver weights in male rats as observed in the principal study were selected to derive potential point of departures (PODs) via benchmark dose (BMD) modeling (see Table A-2). For comparative purposes, increased absolute and relative kidney and liver weights in females were also selected for BMD modeling (see Table A-2). Of the endpoints modeled, the lowest POD is a benchmark dose lower confidence limit of 10 ($BMDL_{10}$) of 41.5 mg/kg-day for increased relative liver weight in male rats. Liver and kidney weight data from males and female rats from the 28-day study were not modeled, due to the higher LOAEL of 1,000 mg/kg-day, smaller magnitude of change, smaller sample size, and shorter exposure duration for that study.

Table A-2. Potential PODs in Rats Exposed via Gavage to 2,4,4-Trimethylpentene for 6 Weeks[a]

Endpoint	Animal POD[a] (mg/kg-d)	POD_{HED} (mg/kg-d)
Huntingdon Life Sciences (1997a)		
Increased absolute liver weight (M)	$BMDL_{10}$ = 175	42.0
Increased relative liver weight (M)	**$BMDL_{10}$ = 173**	**41.5**
Increased absolute liver weight (F)	$BMDL_{10}$ = 254	61.0
Increased relative liver weight (F)	$BMDL_{10}$ = 520	125
Increased absolute kidney weight (F)	NOAEL = 300	72.0
Increased relative kidney weight (F)	NOAEL = 300	72.0

[a]BMD modeling results are described in more detail in Appendix C.
[b]HED calculated by multiplying animal POD by a DAF of 0.24 for rats (U.S. EPA, 2011b).

F = female; M = male.

Application of Dosimetric Adjustment Factor to Obtain a Human Equivalent Dose (HED)

In *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011b), the U.S. EPA 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. Another approach may use chemical-specific information, including what is known about the toxicokinetics and toxicodynamics of the chemical, to derive chemical-specific adjustments. In lieu of

chemical-specific information to derive human equivalent oral exposures, U.S. EPA endorses body-weight scaling to the 3/4 power (i.e., $BW^{3/4}$) as a default to extrapolate toxicologically equivalent doses of orally administered agents from all laboratory animals to humans for the purpose of deriving an RfD under certain exposure conditions. More specifically, the use of $BW^{3/4}$ scaling for deriving an 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. Following EPA guidance, the $BMDL_{10}$ obtained from modeling the relative liver weight for male rats in the Huntingdon Life Sciences (1997a) study is converted to a human equivalent dose (HED) through an application of a dosimetric adjustment factor (DAF) derived as follows:

Relative liver weight in male rats is selected as the critical effect because it provides a lower BMDL from the data set.

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

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 standard BW_h of 70 kg for humans, the resulting DAF is 0.24 (U.S. EPA, 2011b). Applying this DAF to the $BMDL_{10}$ obtained from modeling the increased relative liver weight data for adult male rats yields a $BMDL_{10HED}$ as follows:

$$\begin{aligned} BMDL_{10HED} &= BMDL_{10} \times DAF \\ &= 173 \text{ mg/kg-day} \times 0.24 \\ &= 41.5 \text{ mg/kg-day} \end{aligned}$$

The screening subchronic p-RfD for 2,4,4-trimethylpentene for increased relative liver weight in male rats, is derived as follows:

$$\begin{aligned} \textbf{Screening Subchronic p-RfD} &= BMDL_{10HED} \div UF_c \\ &= 41.5 \text{ mg/kg-day} \div 300 \\ &= \mathbf{1 \times 10^{-1} \text{ mg/kg-day}} \end{aligned}$$

Table A-3 summarizes the UFs for the screening subchronic p-RfD for 2,4,4-trimethylpentene.

Table A-3. Uncertainty Factors for the Screening Subchronic p-RfD for 2,4,4-Trimethylpentene

UF	Value	Justification
UF_A	3	A UF_A of 3 is applied to account for remaining uncertainty such as the toxicodynamic differences between rats and humans following oral 2,4,4-trimethylpentene exposure. The calculation of the HED through application of a DAF accounted for the toxicokinetic uncertainty 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, 2011b).
UF_H	10	A UF_H of 10 is applied to account for human variability in susceptibility, in the absence of information to assess variability of 2,4,4-trimethylpentene in humans.
UF_D	10	A UF_D of 10 is applied to account for deficiencies and uncertainties in the database; lack of a 2-generation reproduction study or a comprehensive developmental study.
UF_L	1	A UF_L of 1 is applied because the POD is a BMDL.
UF_S	1	A UF_S of 1 is applied because the POD is based on a reproductive developmental toxicity study whereby parental rats were exposed for 6 weeks.
UF_C	300	Composite UF = $UF_A \times UF_H \times UF_D \times UF_L \times UF_S$.

Derivation of Screening Chronic Provisional RfD (Chronic p-RfD)

There are no chronic-duration studies of humans or animals orally exposed to 2,4,4-trimethylpentene. In the absence of additional data, the subchronic p-RfD based on increased relative liver weights in male rats following a 6-week exposure during a reproductive developmental study (Huntingdon Life Sciences, 1997a) is used as the basis of a screening chronic p-RfD.

The screening chronic p-RfD for 2,4,4-trimethylpentene, based on the subchronic $BMDL_{10HED}$ for increased relative liver weight in male rats, is derived as follows:

$$\textbf{Screening Chronic p-RfD} = BMDL_{10HED} \div UF_c$$
$$= 41.5 \text{ mg/kg-day} \div 3{,}000$$
$$= \mathbf{1 \times 10^{-2} \text{ mg/kg-day}}$$

Table A-4 summarizes the UFs for the screening chronic p-RfD for 2,4,4-trimethylpentene.

Table A-4. Uncertainty Factors for the Screening Chronic p-RfD for 2,4,4-Trimethylpentene

UF	Value	Justification
UF_A	3	A UF_A of 3 is applied to account for remaining uncertainty such as the toxicodynamic differences between rats and humans following oral 2,4,4-trimethylpentene exposure. The calculation of the HED through application of a DAF accounted for the toxicokinetic uncertainty 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, 2011b).
UF_H	10	A UF_H of 10 is applied to account for human variability in susceptibility, in the absence of information to assess variability of 2,4,4-trimethylpentene in humans.
UF_D	10	A UF_D of 10 is applied to account for deficiencies and uncertainties in the database; lack of suitable 2-generation reproduction study or comprehensive development study.
UF_L	1	A UF_L of 1 is applied because the POD is a BMDL, not a LOAEL.
UF_S	10	A UF_S of 10 is applied to account for the lack of chronic data for 2,4,4-trimethylpentene.
UF_C	3,000	Composite UF = $UF_A \times UF_H \times UF_D \times UF_L \times UF_S$.

APPENDIX B. DATA TABLES

Table B-1. Selected Effects on Male and Female Sprague-Dawley CD-1 Rats Exposed to 2,4,4-Trimethylpentene via Gavage for 4 Weeks[a]

Endpoint	Exposure Group (mg/kg-d)			
	0	100	300	1,000
Males				
Number of animals	5	5	5	5
Body weight gain (g)	173 ± 24.4[b]	190 ± 26.3	174 ± 22.2	180 ± 33.6
Food consumption (g/animal)	678[c]	709	709	731
Food conversion efficiency (%)	25.5	26.8	24.5	24.6
Brown staining of fur	0/5[d]	0/5	1/5	4/5
Ungroomed coat	0/5	0/5	0/5	0/5
MCH (pg)	19.5 ± 0.6	19.8 ± 0.6	19.9 ± 0.7	19.7 ± 0.5
MCV (fl)	56.2 ± 1.6	56.9 ± 1.5	57.0 ± 2.4	56.7 ± 1.6
Plasma glucose (mmol/L)	6.0 ± 0.8	5.6 ± 0.5	4.9 ± 0.4*	5.6 ± 1.1
Plasma protein (g/L)	60 ± 3	62 ± 2	61 ± 1	65 ± 4*
Albumin (g/L)	37 ± 2	37 ± 1	37 ± 1	39 ± 3*
Plasma urea (mmol/L)	3.6 ± 0.5	4.0 ± 0.5	3.8 ± 0.4	4.8 ± 1.0*
Females				
Number of animals	5	5	5	5
Body weight gain (g)	73 ± 13.6	63 ± 9.4	80 ± 12.5	89 ± 10.9
Food consumption (g/animal)	478	459	477	526
Food conversion efficiency (%)	15.3	13.7	16.8	16.9
Brown staining of fur	1/5	1/5	1/5	4/5
Ungroomed coat	0/5	0/5	0/5	4/5
MCH (pg)	19.1 ± 0.4	19.5 ±0.6	19.5 ± 0.3	19.9 ± 0.4*
MCV (fl)	54.0 ± 0.9	54.0 ± 1.6	54.5 ± 0.6	56.1 ± 1.3*
Plasma glucose (mmol/L)	6.0 ± 0.7	5.7 ± 0.4	5.3 ± 0.5	5.0 ± 0.4**
Plasma protein (g/L)	62 ± 3	64 ± 4	62 ± 2	65 ± 2
Chemical albumin (g/L)	40 ± 3	42 ± 4	40 ± 1	41 ± 3
Plasma urea (mmol/L)	5.1 ± 0.7	5.1 ± 1.2	3.9 ± 0.4*	3.7 ± 0.3**

[a]Huntingdon Life Sciences (1997b).
[b]Mean ± SD.
[c]Mean.
[d]Number affected/number examined.
*Significantly different from controls ($p < 0.05$) based on Student's t-test, as reported by study authors.
**Significantly different from controls ($p < 0.01$) based on Student's t-test, as reported by study authors.

Table B-2. Selected Effects on Organ Weights in Male and Female Sprague-Dawley CD Rats Exposed to 2,4,4-Trimethylpentene via Gavage for 4 Weeks[a]

Endpoint	Exposure Group (mg/kg-d)			
	0	100	300	1,000
Males				
Number of animals	5	5	5	5
Terminal body weight (g)	347.0 ± 35.2[b]	353.9 ± 31.4 (+2.0%)	338.8 ± 27.1 (−2.4%)	353.5 ± 42.1 (+1.9%)
Absolute liver weight (g)	18.5 ± 2.3	17.1 ± 2.1 (−5.5%)	16.5 ± 1.7 (−11%)	27.1 ± 3.2** (+46%)**
Relative liver weight (%)	5.34 ± 0.29	4.84 ± 0.29 (−9.5%)	4.88 ± 0.38 (−8.6%)	7.71 ± 0.79** (+44%)**
Absolute kidney weight (g)	2.63 ± 0.21	2.84 ± 0.35 (+8.0%)	2.87 ± 0.27 (+9.1%)	3.65 ± 0.67** (+39%)**
Relative kidney weight (%)	0.761 ± 0.059	0.802 ± 0.041 (+5.4%)	0.846 ± 0.042 (+11%)	1.03 ± 0.084** (+35%)**
Females				
Number of animals	5	5	5	5
Terminal body weight (g)	212.1 ± 11.8	204.5 ± 14.6 (−3.6%)	218.2 ± 17.1 (+2.9%)	232.3 ± 21.1 (+9.5%)
Absolute liver weight (g)	9.9 ± 0.8	9.9 ± 1.3 (0%)	10.1 ± 0.8 (+2.0%)	12.9 ± 0.8** (+30%)**
Relative liver weight (%)	4.64 ± 0.16	4.86 ± 0.48 (+4.7%)	4.66 ± 0.44 (+0.4%)	5.61 ± 0.62* (+21%)*
Absolute kidney weight (g)	1.75 ± 0.22	1.82 ± 0.24 (+4.0%)	1.88 ± 0.16 (+7.4%)	2.00 ± 0.18 (+14%)
Relative kidney weight (%)	0.822 ± 0.080	0.89 ± 0.107 (+8.3%)	0.863 ± 0.038 (+5.0%)	0.862 ± 0.088 (+4.9%)

[a]Huntingdon Life Sciences (1997b).
[b]Mean ± SD.
*Significantly different from controls ($p < 0.05$) based on Dunnett's test, as reported by study authors.
**Significantly different from controls ($p < 0.01$) based on Dunnett's test, as reported by study authors.

Table B-3. Selected Effects on Parental Sprague-Dawley CD Rats Exposed to 2,4,4-Trimethylpentene via Gavage for Approximately 6 Weeks[a]

Endpoint	Exposure Group (mg/kg-d)			
	0	100	300	1,000
Males				
Number of animals	10	10	10	10
Brown staining of fur	0/10[b]	0/10	1/10	6/10
Yellow staining of fur	0/10	0/10	0/10	10/10
Terminal body weight (g)	509.1 ± 21.8[c]	519.9 ± 52.9 (+2.1%)	517.6 ± 16.6 (+1.7%)	506.5 ± 27.5 (−0.5%)
Absolute liver weight of liver (g)	20.9 ± 3.4	21.7 ± 3.1 (+3.8%)	24.4 ± 1.6* (+17%)	33.7 ± 3.1** (+61%)
Relative liver weight (g)	4.10 ± 0.60	4.17 ± 0.34 (+1.7%)	4.72 ± 0.31* (+15%)	6.65 ± 0.51** (+62%)
Absolute kidney weight (g)	3.86 ± 0.30	4.13 ± 0.59 (+7.0%)	4.75 ± 0.56** (+23%)	4.97 ± 0.45** (+29%)
Relative kidney weight (g)	0.758 ± 0.045	0.794 ± 0.069 (+4.7%)	0.918 ± 0.094** (+21%)**	0.981 ± 0.072** (+29%)**
Basophilic cortical tubules	0/10	7/10**	9/10***	7/10**
Proteinaceous casts in collecting ducts	0/10	0/10	7/10**	2/10
Interstitial inflammatory cells	0/10	0/10	5/10*	1/10
Females				
Number of animals	10	10	10	9
Brown staining of fur	0/10	2/10	1/10	4/10
Yellow staining of fur	0/10	0/10	1/10	6/10
Terminal body weight (g)	320.0 ± 19.9	316.4 ± 15.9 (−1.1%)	336.2 ± 15.8 (+5.1%)	311.9 ± 33.5 (−2.5%)
Absolute liver weight (g)	17.4 ± 2.0	16.8 ± 1.6 (+9.7%)	18.6 ± 2.9 (+6.9%)	21.3 ± 3.7** (+22%)
Relative liver weight (g)	5.44 ± 0.74	5.34 ± 0.66 (−1.8%)	5.53 ± 0.92 (+1.7%)	6.83 ± 0.87** (+26%)
Absolute kidney weight (g)	2.31 ± 0.19	2.51 ± 0.19 (+8.7%)	2.50 ± 0.18 (+8.2%)	2.59 ± 0.18** (+12%)**
Relative kidney weight (g)	0.723 ± 0.051	0.794 ± 0.063[†] (+9.8%)	0.745 ± 0.056 (+3%)	0.845 ± 0.151 (+17%)
Basophilic cortical tubules	0/10	1/10	0/10	0/9
Proteinaceous casts in collecting ducts	0/10	0/10	0/10	0/9
Interstitial inflammatory cells	0/10	0/10	0/10	0/9

[a]Huntingdon Life Sciences (1997a).
[b]Number affected/number examined.
[c]Mean ± SD.
*Significantly different from controls ($p < 0.05$) based on Dunnett's test, as reported by study authors.
**Significantly different from controls ($p < 0.01$) based on Dunnett's test, as reported by study authors.
***Significantly different from controls ($p < 0.001$) based on Dunnett's test, as reported by study authors.
[†]Significantly different from controls ($p < 0.05$) using Behren's-Fisher's test, as reported by study authors.

Table B-4. Immunohistochemical Staining of alpha 2u-globulin in Kidney Sections from Parental Sprague-Dawley CD Rats Exposed to 2,4,4-Trimethylpentene via Gavage[a]

Endpoint	Exposure Group (mg/kg-d)			
	0	**100**	**300**	**1,000**
Males				
Number of animals	10	10	10	10
Immunohistochemical grading[b]				
Negative	0/10[c]	0/10	0/10	0/10
Grade 1 (minimal)	8/10	0/10	0/10	0/10
Grade 2 (mild)	2/10	8/10	1/10	0/10
Grade 3 (moderate)	0/10	2/10	2/10	2/10
Grade 4 (strong)	0/10	0/10	7/10	8/10
Average grade	1.2	2.2	3.6	3.8
Females				
Number of animals	10	10	10	9
Immunohistochemical grading				
Negative	10/10	NA	NA	10/10
Grade 1 (minimal)	0/10	NA	NA	0/10
Grade 2 (mild)	0/10	NA	NA	0/10
Grade 3 (moderate)	0/10	NA	NA	0/10
Grade 4 (strong)	0/10	NA	NA	0/10
Average grade	0	NA	NA	0

[a]Swenberg and Schoonhoven (2004).
[b]Grading criteria for immunostaining for α2u-g. Specific staining of protein droplets enhanced the grading score by 0.2–0.4 depending on the number of immunostained droplets; Grade 4 indicates strong positive staining of protein, protein droplets, and/or granular casts.
[c]Number affected/number examined.

NA = not applicable.

APPENDIX C. BENCHMARK DOSE MODELING RESULTS

MODELING PROCEDURE FOR CONTINUOUS DATA

The benchmark dose (BMD) modeling of liver and kidney weight data was conducted with the U.S. EPA's Benchmark Dose Software (BMDS, Version 2.5). For these data, all continuous models available within the software were fit using a benchmark response (BMR) of 10% increase from control mean for this endpoint. An adequate fit was judged based on the goodness-of-fit *p*-value ($p > 0.1$), magnitude of the scaled residuals in the vicinity of the BMR, and visual inspection of the model fit. In addition to these three criteria for judging adequacy of model fit, a determination was made as to whether the variance across dose groups was constant. If a constant variance model was deemed appropriate based on the statistical test provided in BMDS (i.e., Test 2), the final BMD results were estimated from a constant variance model. If the test for homogeneity of variance was rejected ($p < 0.1$), the model was run again while modeling the variance as a power function of the mean to account for this nonconstant variance. If this nonconstant variance model did not adequately fit the variance data (i.e., Test 3; $p < 0.1$), the data set was considered unsuitable for BMD modeling. Among all models providing adequate fit, the lowest benchmark dose lower confidence limit (BMDL) was selected if the BMDLs estimated from different models varied greater than threefold; otherwise, the BMDL from the model with the lowest Akaike's Information Criterion (AIC) was selected as a potential point of departure (POD) from which to derive a provisional oral reference dose (p-RfD).

For increased relative liver weight in male rats, all constant variance models provided adequate fits to the variance and all, except the Exponential5 Model and the Hill Model, provided adequate fit to the means (see Table C-1). For each data set, BMDLs from adequately fitting models were sufficiently close, so the models with the lowest AIC were selected. The Exponential2 Model was selected as the best fitting model for increased relative liver weight in male rats.

Table C-1. Relative Liver Weights in Male Rats Exposed by Gavage for 6 Weeks[a]							
Model Name	**BMD**	**BMDL**	***p*-Value Test 2**	***p*-Value Test 3**	***p*-Value For Fit**	**AIC**	**Scaled Residual**
Exponential2	**191.636**	**173.174**	**0.1184**	**0.1184**	**0.7627**	**−20.49093**	**−0.5841**
Exponential3	205.769	173.294	0.1184	0.1184	0.4775	−18.52819	0.3146
Exponential4	151.146	131.817	0.1184	0.1184	0.2679	−17.80501	−0.58
Exponential5	209.883	119.307	0.1184	0.1184	NDr	−16.69944	0.2358
Hill	239.276	123.061	0.1184	0.1184	NDr	−17.032725	-3.03×10^{-7}
Linear	151.147	131.817	0.1184	0.1184	0.5413	−19.80505	−0.58
Polynomial	198.506	134.375	0.1184	0.1184	0.4714	−18.513931	−0.578
Polynomial	198.506	134.375	0.1184	0.1184	0.4714	−18.513931	−0.578
Power	209.772	135.081	0.1184	0.1184	0.5576	−18.688842	0.243

[a]Huntingdon Life Sciences (1997b)

AIC = Akaike's Information Criteria; BMD = benchmark dose; BMDL = lower confidence limit (95%) on the benchmark dose; NDr = not determined.

The BMDS output for the selected model (Exponential2) follows.

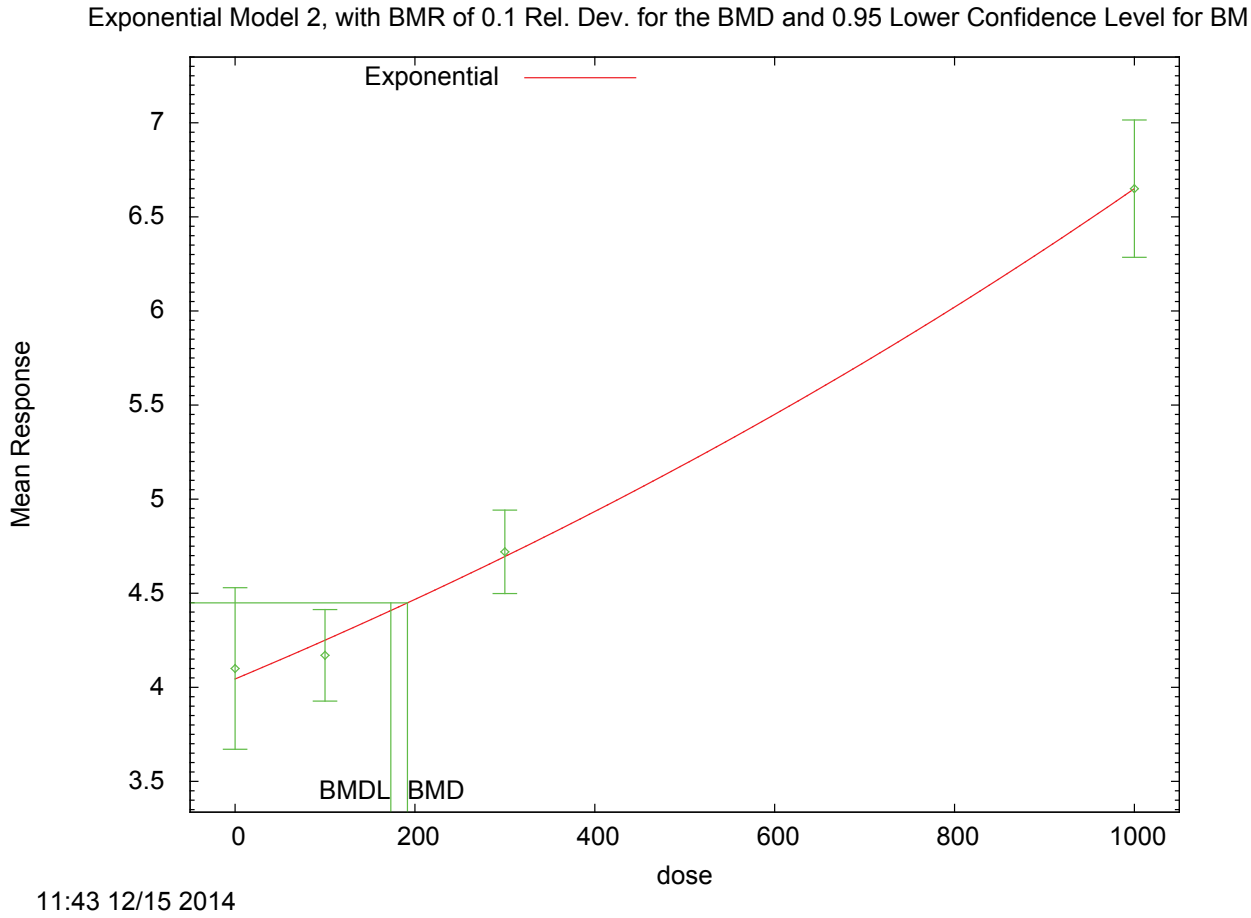

Figure D-1. Exponential2 (Increased Relative Liver Weight, Male Rat)

```
Exponential Model. (Version: 1.9;  Date: 01/29/2013)
         Input Data File: M:/14 trimethylpentene PPRTV new/exp_Male rel
liver_Exp-ConstantVariance-BMR10-Up.(d)
         Gnuplot Plotting File:
                                                  Mon Dec 15 11:43:08 2014
 ================================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
          sign = -1 for decreasing trend.

      Model 2 is nested within Models 3 and 4.
      Model 3 is nested within Model 5.
      Model 4 is nested within Model 5.


   Dependent variable = Mean
   Independent variable = Dose
```

Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
rho is set to 0.
A constant variance model is fit.

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact

Initial Parameter Values

Variable	Model 2
lnalpha	-1.67582
rho(S)	0
a	4.04479
b	0.000496862
c	0
d	1

(S) = Specified

Parameter Estimates

Variable	Model 2
lnalpha	-1.66227
rho	0
a	4.04423
b	0.00049735
c	0
d	1

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	10	4.1	0.6
100	10	4.17	0.34
300	10	4.72	0.31
1000	10	6.65	0.51

Estimated Values of Interest

Dose	Est Mean	Est Std	Scaled Residual
0	4.044	0.4356	0.4049
100	4.25	0.4356	-0.5841
300	4.695	0.4356	0.1816
1000	6.65	0.4356	-0.00113

Other models for which likelihoods are 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)} = exp(lalpha + log(mean(i)) * rho)

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

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	13.51636	5	-17.03273
A2	16.44884	8	-16.89768
A3	13.51636	5	-17.03273
R	-24.52685	2	53.0537
2	13.24547	3	-20.49093

Additive constant for all log-likelihoods = -36.76. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A2 vs. A1)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does Model 2 fit the data? (A3 vs. 2)

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	81.95	6	< 0.0001
Test 2	5.865	3	0.1184
Test 3	5.865	3	0.1184
Test 4	0.5418	2	0.7627

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. Model 2 seems to adequately describe the data.

Benchmark Dose Computations:

Specified Effect = 0.100000

```
       Risk Type = Relative deviation

Confidence Level = 0.950000

             BMD =      191.636

            BMDL =      173.174
```

For increased absolute liver weight in male rats, all constant variance models provided adequate fits to the variance and all, except the Exponential5 Model and the Hill Model, provided adequate fit to the means (see Table C-2). For each data set, BMDLs from adequately fitting models were sufficiently close, so the models with the lowest AIC were selected. The Exponential2 and 3 Models were selected as the best fitting model for increased absolute liver weight in male rats.

Table C-2. Absolute Liver Weights in Male Rats Exposed by Gavage for 6 Weeks[a]

Model Name	BMD	BMDL	*p*-Value Test 2	*p*-Value Test 3	*p*-Value For Fit	AIC	Scaled Residual
Exponential2	**199.012**	**175.156**	**0.1279**	**0.1279**	**0.9199**	**126.7712**	**−0.2621**
Exponential3	**199.012**	**175.156**	**0.1279**	**0.1279**	**0.9199**	**126.7712**	**−0.2621**
Exponential4	158.27	133.561	0.1279	0.1279	0.6581	128.8	−0.2521
Exponential5	202.245	98.1559	0.1279	0.1279	NDr	130.6041	4.96×10^{-7}
Hill	201.864	97.0004	0.1279	0.1279	NDr	130.60412	-3.44×10^{-7}
Linear	158.288	133.578	0.1279	0.1279	0.9068	126.799776	−0.252
Polynomial	179.168	133.994	0.1279	0.1279	0.7563	128.700454	−0.25
Polynomial	179.167	133.994	0.1279	0.1279	0.7563	128.700454	−0.25
Power	186.682	134.14	0.1279	0.1279	0.8033	128.666187	−0.198

[a]Huntingdon Life Sciences (1997a)

AIC = Akaike's Information Criteria; BMD = benchmark dose; BMDL = lower confidence limit (95%) on the benchmark dose; NDr = not determined.

The BMDS output for the selected model (Exponential2) follows.

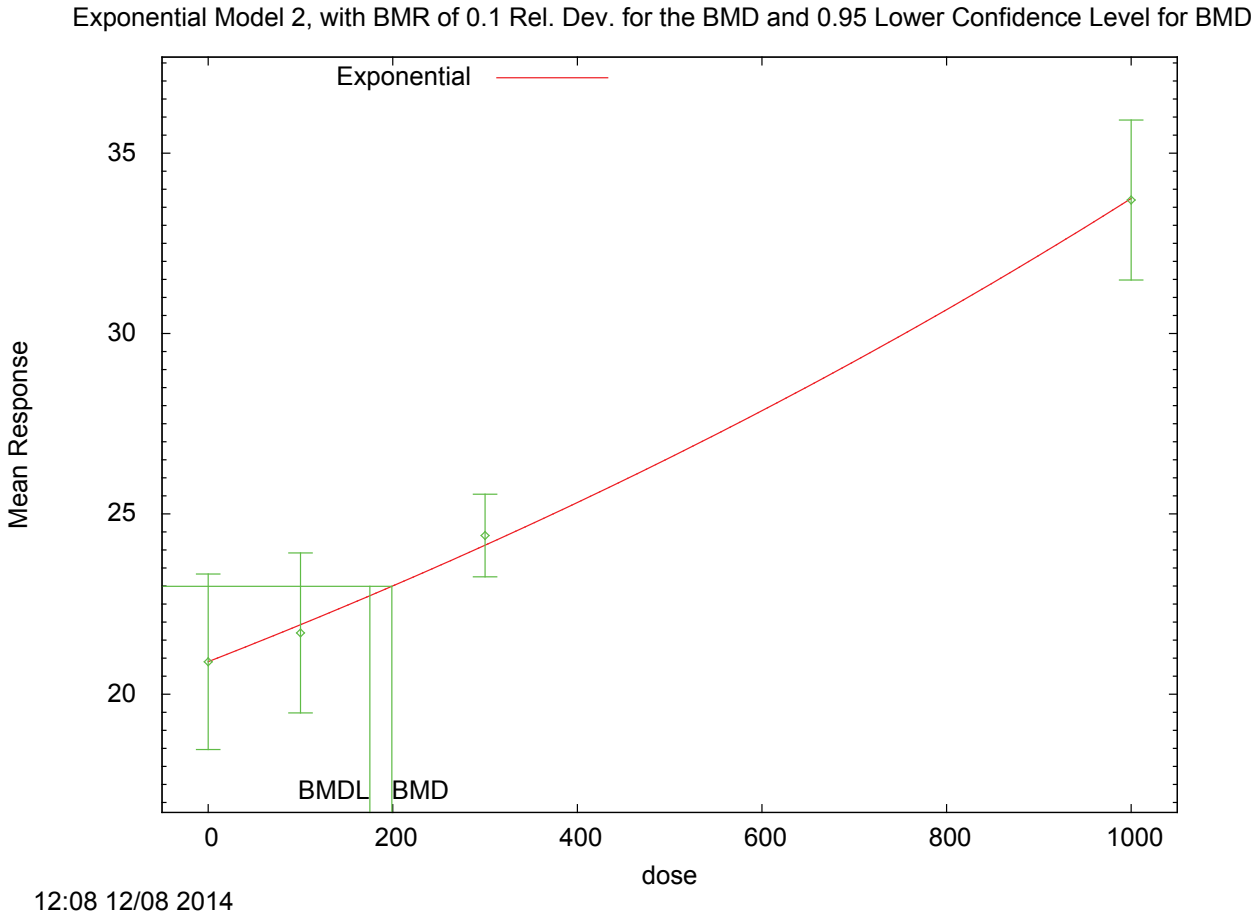

Figure C-2. Absolute Liver Weights in Male Rats Exposed by Gavage for 6 Weeks (Huntingdon Life Sciences, 1997a)

```
Exponential Model. (Version: 1.9;  Date: 01/29/2013)
        Input Data File: M:/14 trimethylpentene PPRTV new/exp_male abs liver wt_Exp-
ConstantVariance-BMR10-Up.(d)
        Gnuplot Plotting File:
                                                  Mon Dec 08 12:08:38 2014
 ================================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
          sign = -1 for decreasing trend.

      Model 2 is nested within Models 3 and 4.
      Model 3 is nested within Model 5.
      Model 4 is nested within Model 5.
```

```
Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
rho is set to 0.
A constant variance model is fit.

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact
```

Initial Parameter Values

Variable	Model 2
lnalpha	2.0151
rho(S)	0
a	20.8831
b	0.00048099
c	0
d	1

(S) = Specified

Parameter Estimates

Variable	Model 2
lnalpha	2.01928
rho	0
a	20.9021
b	0.000478918
c	0
d	1

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	10	20.9	3.4
100	10	21.7	3.1
300	10	24.4	1.6
1000	10	33.7	3.1

Estimated Values of Interest

Dose	Est Mean	Est Std	Scaled Residual
0	20.9	2.745	-0.002405
100	21.93	2.745	-0.2621
300	24.13	2.745	0.3092
1000	33.74	2.745	-0.0493

Other models for which likelihoods are 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)} = exp(lalpha + log(mean(i)) * rho)

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

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-60.30206	5	130.6041
A2	-57.45862	8	130.9172
A3	-60.30206	5	130.6041
R	-90.17613	2	184.3523
2	-60.3856	3	126.7712

Additive constant for all log-likelihoods = -36.76. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A2 vs. A1)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does Model 2 fit the data? (A3 vs. 2)

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	65.44	6	< 0.0001
Test 2	5.687	3	0.1279
Test 3	5.687	3	0.1279
Test 4	0.1671	2	0.9199

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. Model 2 seems to adequately describe the data.

```
Benchmark Dose Computations:

  Specified Effect = 0.100000

         Risk Type = Relative deviation

  Confidence Level = 0.950000

               BMD =      199.012

              BMDL =      175.156
```

For increased absolute liver weight in female rats, no constant variance models provided adequate fit. Of the modeled variance models, all models provided adequate to the variance and all models, except the Exponential5 Model and the Hill Model, provided adequate fit to the means (see Table C-3). For each data set, BMDLs from adequately fitting models were sufficiently close, so the models with the lowest AIC were selected. The Linear and Polynomials Models were selected as the best fitting model for increased absolute liver weight in female rats.

Table C-3. Absolute Liver Weights in Female Rats Exposed by Gavage for 6 Weeks[a]

Model Name	BMD	BMDL	*p*-Value Test 2	*p*-Value Test 3	*p*-Value For Fit	AIC	Scaled Residual
Exponential2	423.641	285.983	0.05349	0.7723	0.205	114.5728	0.4784
Exponential3	423.641	285.983	0.05349	0.7723	0.205	114.5728	0.4784
Exponential4	370.497	158.357	0.05349	0.7723	0.07942	116.4798	0.2959
Exponential5	299.096	202.404	0.05349	0.7723	NDr	116.3688	−0.2949
Hill	298.828	193.177	0.05349	0.7723	NDr	116.368769	−0.295
Linear	**396.663**	**254.484**	**0.05349**	**0.7723**	**0.2119**	**114.506154**	**0.391**
Polynomial	**396.663**	**254.484**	**0.05349**	**0.7723**	**0.2119**	**114.506154**	**0.391**
Polynomial	**396.663**	**254.484**	**0.05349**	**0.7723**	**0.2119**	**114.506154**	**0.391**
Power	412.928	254.793	0.05349	0.7723	0.07895	116.489422	0.451

[a]Huntingdon Life Sciences (1997a)

AIC = Akaike's Information Criteria; BMD = benchmark dose; BMDL = lower confidence limit (95%) on the benchmark dose; ND = not determined.

The BMDS output for the selected model (Linear) follows.

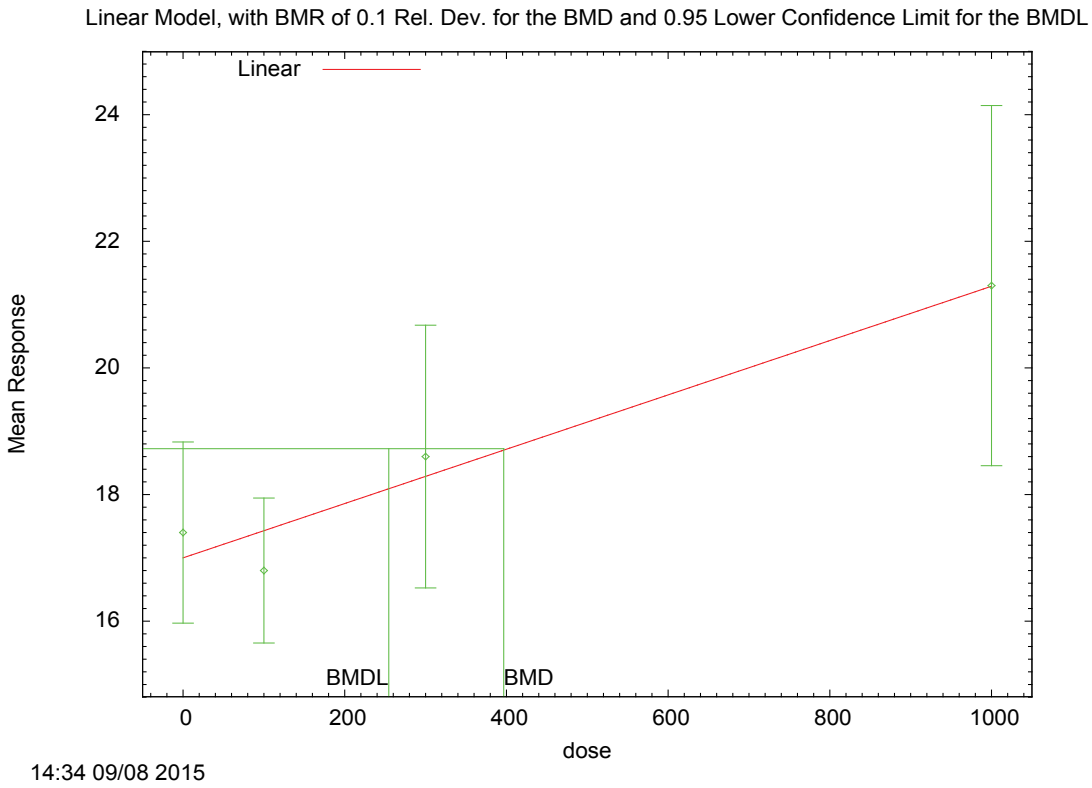

Figure C-3. Absolute Liver Weights in Female Rats Exposed by Gavage for 6 Weeks (Huntingdon Life Sciences, 1997a)

For increased relative liver weight in female rats, all constant variance models provided adequate fits to the variance and all, except the Exponential5 Model and the Hill Model, provided adequate fit to the means (see Table C-4). For each data set, BMDLs from adequately fitting models were sufficiently close, so the models with the lowest AIC were selected. The Polynomial Models were selected as the best fitting model for increased relative liver weight in female rats.

Table C-4. Relative Liver Weights in Female Rats Exposed by Gavage for 6 Weeks[a]

Model Name	BMD	BMDL	*p*-Value Test 2	*p*-Value Test 3	*p*-Value For Fit	AIC	Scaled Residual
Exponential2	377.148	281.449	0.726	0.726	0.6043	24.59303	−0.6258
Exponential3	624.253	295.213	0.726	0.726	0.7296	25.70508	0.06
Exponential4	348.599	246.776	0.726	0.726	0.2613	26.84753	−0.7286
Exponential5	613.743	223.092	0.726	0.726	NDr	27.70224	0.05497
Hill	337.118	223.824	0.726	0.726	NDr	27.672103	-2.38×10^{-6}
Linear	348.6	246.777	0.726	0.726	0.5321	24.847527	−0.729
Polynomial	**611.289**	**519.905**	**0.726**	**0.726**	**0.9433**	**23.702306**	**0.0489**
Polynomial	**611.289**	**519.905**	**0.726**	**0.726**	**0.9433**	**23.702306**	**0.0489**
Power	613.746	266.529	0.726	0.726	0.7327	25.702242	0.055

[a]Huntingdon Life Sciences (1997a)

AIC = Akaike's Information Criteria; BMD = benchmark dose; BMDL = lower confidence limit (95%) on the benchmark dose; ND = not determined.

The BMDS output for the selected model (Polynomial) follows.

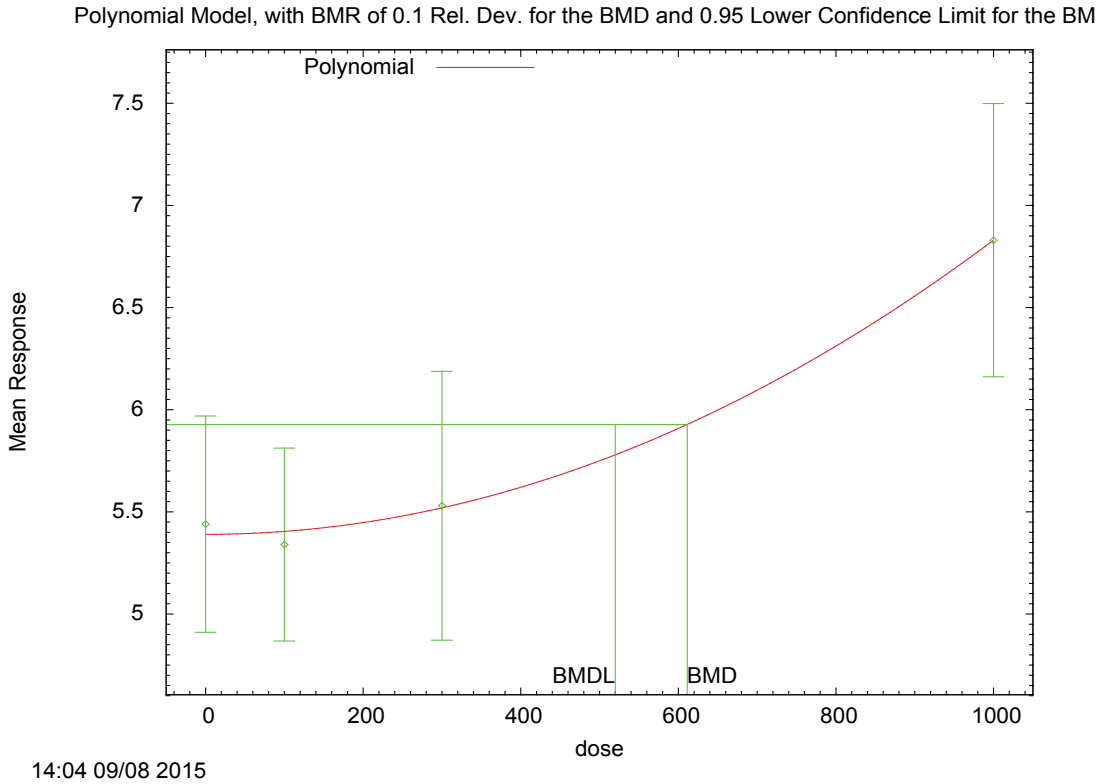

Figure C-4. Relative Liver Weights in Female Rats Exposed by Gavage for 6 Weeks

For increased absolute kidney weight in female rats, no constant or modeled variance models provided adequate fit to the data (see Table C-5).

Table C-5. Absolute Kidney Weights in Female Rats Exposed by Gavage for 6 Weeks[a]

Model Name	BMD	BMDL	*p*-Value Test 2	*p*-Value Test 3	*p*-Value For Fit	AIC	Scaled Residual
Exponential2	1,238.65	746.102	0.9958	0.9822	0.07833	−83.68289	−0.2535
Exponential3	1,238.65	746.102	0.9958	0.9822	0.07833	−83.68289	−0.2535
Exponential4	234.103	0.324251	0.9958	0.9822	0.2679	−85.54913	−0.7826
Exponential5	234.103	0.371548	0.9958	0.9822	0.2679	−85.54913	−0.7826
Hill	321.532	0.0059102	0.9958	0.9822	0.3609	−85.941752	−0.723
Linear	1,236.2	724.915	0.9958	0.9822	0.08074	−83.743478	−0.267
Polynomial	1,236.2	724.915	0.9958	0.9822	0.08074	−83.743478	−0.267
Polynomial	1,236.2	724.915	0.9958	0.9822	0.08074	−83.743478	−0.267
Power	1,236.2	724.915	0.9958	0.9822	0.08074	−83.743478	−0.267

[a]Huntingdon Life Sciences (1997a)

AIC = Akaike's Information Criteria; BMD = benchmark dose; BMDL = lower confidence limit (95%) on the benchmark dose.

For increased relative kidney weight in female rats, no constant or modeled variance models provided adequate fit to the data (see Table C-6).

Table C-6. Relative Kidney Weights in Female Rats Exposed by Gavage for 6 Weeks[a]

Model Name	BMD	BMDL	*p*-Value Test 2	*p*-Value Test 3	*p*-Value For Fit	AIC	Scaled Residual
Exponential2	315.596	217.829	<0.0001	<0.0001	NDr	−118.4772	−1.661
Exponential3	927.373	ND	<0.0001	<0.0001	NDr	−121.9865	−0.587
Exponential4	283.092	193.262	<0.0001	<0.0001	NDr	−115.6189	−1.719
Exponential5	901.374	326.142	<0.0001	<0.0001	NDr	−119.9865	−0.587
Hill	893.088	328.212	<0.0001	0.3372	0.01048	−121.986497	−0.587
Linear	283.424	193.504	<0.0001	0.3372	0.001574	−117.628728	−1.72
Polynomial	550.47	411.155	<0.0001	0.3372	0.01085	−121.489747	−1.03
Polynomial	672.018	395.7	<0.0001	0.3372	0.02626	−123.258174	−0.585
Power	924.869	515.745	<0.0001	<0.0001	<0.0001	−123.986497	−0.587

[a]Huntingdon Life Sciences (1997a)

AIC = Akaike's Information Criteria; BMD = benchmark dose; BMDL = lower confidence limit (95%) on the benchmark dose; NDr = not determined.

APPENDIX D. REFERENCES

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