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Provisional Peer-Reviewed Toxicity Values for

Technical Grade Dinitrotoluene
(CASRN 25321-14-6)

Superfund Health Risk Technical Support Center
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

AUTHORS, CONTRIBUTORS, AND REVIEWERS

CHEMICAL MANAGER

Zhongyu (June) Yan, PhD
National Center for Environmental Assessment, Cincinnati, OH

Q. Jay Zhao, PhD, DABT
National Center for Environmental Assessment, Cincinnati, OH

PRIMARY INTERNAL REVIEWERS

Ghazi Dannan, PhD
National Center for Environmental Assessment, Washington, DC

Paul G. Reinhart, PhD, DABT
National Center for Environmental Assessment, Research Triangle Park, NC

This document was externally peer reviewed under contract to
Eastern Research Group, Inc.
110 Hartwell Avenue
Lexington, MA 02421-3136

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 bound 95% confidence interval
BMD	benchmark dose
BMDL	benchmark dose lower bound 95% confidence interval
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 reference concentration (inhalation)
p-RfD	provisional reference dose (oral)
RfC	reference concentration (inhalation)
RfD	reference dose (oral)
UF	uncertainty factor
UF_A	animal-to-human uncertainty factor
UF_C	composite uncertainty factor
UF_D	incomplete-to-complete database uncertainty factor
UF_H	interhuman 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 TECHNICAL GRADE DINITROTOLUENE (CASRN 25321-14-6)

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

Technical grade dinitrotoluene (tgDNT), CAS No. 25321-14-6, is a mixture of dinitrotoluene (DNT) isomers with the molecular formula $C_7H_6N_2O_4$ (NLM, 2011). tgDNT comprises predominantly 2,4-DNT and 2,6-DNT (approximated as 76% and 19%, respectively). The remaining 5% is a combination of the four other DNT isomers: 2,3-, 2,5-, 3,4-, and 3,5-DNT. tgDNT may also contain trace amounts of trinitrotoluene, cresols, mononitrobenzene, and mononitrotoluenes and is used in the production of toluene diisocyanate, which is used in the preparation of polyurethane products and in the manufacture of explosives (NLM, 2011). Table 1 provides physicochemical properties of tgDNT.

Table 1. Physicochemical Properties of tgDNT (CASRN 25321-14-6)[a]

Property (unit)	Value
Boiling point (ºC)	250
Melting point (ºC)	ND
Density (g/cm^3 at 71ºC)	1.32
Vapor pressure (mm Hg at 25ºC)	3.97×10^{-4}
pH (unitless)	ND
Solubility in water (mg/L at 22ºC)	2.7×10^{2}
Relative vapor density (air = 1)	6.27
Molecular weight (g/mol)	182.134

[a]NLM (2011).

ND = no data.

IRIS has developed assessments for 2,4-DNT (approximately 98% 2,4-DNT and 2% 2,6-DNT; U.S. EPA,1993) and for a 2,4-/2,6-DNT mixture (various compositions of DNTs; U.S. EPA, 1990). There is also a PPRTV assessment for 2,6-DNT (approximately 99% 2,6-DNT; U.S. EPA, 2013). Table 2 provides a summary of available toxicity values from the U.S. Environmental Protection Agency (U.S. EPA) and other agencies/organizations for tgDNT, the 2,4-DNT and 2,6-DNT isomers, and the 2,4-/2,6-DNT mixture. For the purpose of this PPRTV, only the toxicity of tgDNT (approximated as 76% 2,4-DNT and 19% 2,6-DNT) is evaluated.

Table 2. Summary of Available Toxicity Values for tgDNT (CASRN 25321-14-6), 2,4-DNT (CASRN 121-14-2), 2,6-DNT (CASRN 606-20-2), and 2,4-/2,6-DNT Mixture (no CASRN)[a]

Source/ Parameter[b,c]	tgDNT Value (approximately 76% 2,4-DNT and 19% 2,6-DNT)	2,4-DNT Value (approximately 98% 2,4-DNT and 2% 2,6-DNT)	2,6-DNT Value (approximately 99% 2,6-DNT)	2,4-/2,6-DNT Mixture Value (various compositions of DNTs)	Notes	Reference	Date Accessed
Cancer							
IRIS/OSF	NV	NV	NV	6.8×10^{-1} per mg/kg-d	IRIS entry is for 2,4-/2,6-DNT mixture with no CASRN; principal study used rats dosed with a mixture of 98% 2,4-DNT and 2% 2,6-DNT to determine OSF	U.S. EPA (1990)	9-13-2012
IRIS/drinking water unit risk	NV	NV	NV	1.9×10^{-5} per µg/L	IRIS entry is for 2,4-/2,6-DNT mixture with no CASRN; principal study used rats dosed with a mixture of 98% 2,4-DNT and 2% 2,6-DNT to determine OSF	U.S. EPA (1990)	9-13-2012
HEAST	NV	NV	NV	NV	None	U.S. EPA (2003)	9-13-2012
IARC/cancer WOE	NV	NV	NV	NV	Group 2B—Possibly carcinogenic to humans for 2,4- and 2,6-DNT	IARC (1996)	9-13-2012
NTP	NV	NV	NV	NV	None	NTP (2011)	9-13-2012
Cal EPA/unit risk	NV	8.9×10^{-5} per µg/m^3	NV	NV	Data source was RCHAS-S	Cal EPA (2009)	9-13-2012
Cal EPA/OSF	NV	3.1×10^{-1} per mg/kg-d	NV	NV	Data source was RCHAS-S	Cal EPA (2009)	9-13-2012
ACGIH (cited in NLM, 2011)	NV	NV	NV	NV	Group A3—Confirmed animal carcinogen with unknown relevance to humans for tgDNT, 2,4- and 2,6-DNT	NLM (2011)	9-13-2012
Drinking Water/ cancer risk health advisory	5×10^{-3} mg/L	5×10^{-3} mg/L	5×10^{-3} mg/L	NV	None	U.S. EPA (2011a)	9-13-2012

Table 2. Summary of Available Toxicity Values for tgDNT (CASRN 25321-14-6), 2,4-DNT (CASRN 121-14-2), 2,6-DNT (CASRN 606-20-2), and 2,4-/2,6-DNT Mixture (no CASRN)[a]

Source/ Parameter[b,c]	tgDNT Value (approximately 76% 2,4-DNT and 19% 2,6-DNT)	2,4-DNT Value (approximately 98% 2,4-DNT and 2% 2,6-DNT)	2,6-DNT Value (approximately 99% 2,6-DNT)	2,4-/2,6-DNT Mixture Value (various compositions of DNTs)	Notes	Reference	Date Accessed
Health effect assessment	2.3×10^{-1} per mg/kg-d[d] and 2.1×10^{-1} per mg/kg-d[e]	6.8×10^{-1} per mg/kg-d[f]	NV	NV	[d]Based on a 104-wk study in rats with increased incidence of liver tumors in males; [e]Based on a 104-wk study in rats with increased incidence of liver tumors in females; [f]Based on a 2-yr study in rats with increased incidence of combined mammary/hepatic tumors;	U.S. EPA (1987)	2-6-2013
PPRTV	NV	NV	1.5×10^{0} per mg/kg-d	NV	Based on a $BMDL_{10HED}$ of 0.25 from a 52-wk study in rats with increased incidence of liver tumors in males	U.S. EPA (2013)	4-3-2013
Noncancer							
ACGIH/TLV	0.2 mg/m^3	NV	NV	NV	NA	NLM (2011)	9-13-2012
ATSDR/acute oral MRL	NV	5×10^{-2} mg/kg-d	NV	NV	Toxicological profile for 2,4-DNT; based on neurotoxicity in dogs	ATSDR (1998)	11-21-2012
ATSDR/chronic or intermediate-duration oral MRL	NV	2×10^{-3} mg/kg-d[g]	4×10^{-3} mg/kg-d[h]	NV	[g]Chronic oral MRL for 2,4-DNT; based on neurotoxicity, Heinz bodies, and biliary tract hyperplasia in dogs; [h]Intermediate-duration oral MRLfor 2,6-DNTbased on hematological effects of splenic extramedullary erythropoiesis and lymphoid depletion in dogs	ATSDR (1998)	11-21-2012
Cal EPA/REL	NV	NV	NV	NV	NA	Cal EPA (2012a, b)	8-1-2012

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Source/ Parameter[b,c]	tgDNT Value (approximately 76% 2,4-DNT and 19% 2,6-DNT)	2,4-DNT Value (approximately 98% 2,4-DNT and 2% 2,6-DNT)	2,6-DNT Value (approximately 99% 2,6-DNT)	2,4-/2,6-DNT Mixture Value (various compositions of DNTs)	Notes	Reference	Date Accessed
Drinking water	NV	2×10^{-3} mg/kg-d (1-d Health advisory) 1×10^{-1} mg/L (Drinking water equivalent level) 1×10^{0} mg/L (1- and 10-d Health advisory for a 10-kg child)	1×10^{-3} mg/kg-d (1-d Health advisory) 4×10^{-2} mg/L (Drinking water equivalent level) 4×10^{-1} and 4×10^{-2} mg/L (1- and 10-d Health advisory for a 10-kg child)	NV	NA	U.S. EPA (2011a)	2-6-2013
NIOSH/REL	1.5 mg/m^3	NV	NV	NV	TWA for 10-hr workday; document specifies CASRN for tgDNT but notes that various isomers of DNT exist	NIOSH (2007)	9-13-2012
OSHA/PEL	1.5 mg/m^3	NV	NV	NV	TWA for 8-hr workday	OSHA (2006)	9-13-2012
IRIS/Oral RfD	NV	2×10^{-3} mg/kg-d	NV	NV	Based on a 2-yr study in dogs dosed with 98 2,4-DNT and 2% 2,6-DNT; critical effect of CNS neurotoxicity, Heinz bodies in erythrocytes, and hyperplasia of biliary tract	U.S. EPA (1993)	9-13-2012
IRIS/Inhalation RfC	NV	NV	NV	NV	None	U.S. EPA (1990)	9-13-2012
HEAST/ subchronic Oral RfD	NV	2×10^{-3} mg/kg-d	NV	NV	Based on a 2-yr study in dogs dosed with a mixture of 98% 2,4-DNT and 2% 2,6-DNT; critical effect of CNS neurotoxicity, Heinz bodies in erythrocytes, and hyperplasia of biliary tract	U.S. EPA (2003)	9-13-2012

Table 2. Summary of Available Toxicity Values for tgDNT (CASRN 25321-14-6), 2,4-DNT (CASRN 121-14-2), 2,6-DNT (CASRN 606-20-2), and 2,4-/2,6-DNT Mixture (no CASRN)[a]

Source/ Parameter[b,c]	tgDNT Value (approximately 76% 2,4-DNT and 19% 2,6-DNT)	2,4-DNT Value (approximately 98% 2,4-DNT and 2% 2,6-DNT)	2,6-DNT Value (approximately 99% 2,6-DNT)	2,4-/2,6-DNT Mixture Value (various compositions of DNTs)	Notes	Reference	Date Accessed
Health effects assessment	NV	NV	NV	NV	NA	U.S. EPA (1987)	2-6-2013
PPRTV	NV	NV	3×10^{-3} mg/kg-d (screening subchronic p-RfD) 3×10^{-4} mg/kg-d (screening chronic p-RfD)	NV	Based on a $LOAEL_{HED}$ of 3 mg/kg-d for splenic extramedullary hematopoiesis in male and female dogs in a 13-wk oral study	U.S. EPA (2013)	4-3-2013
CARA HEEP	NV	NV	NV	NV	None	U.S. EPA (1994)	9-13-2012
WHO	NV	NV	NV	NV	None	WHO (2012)	8-1-2012

[a]No information was available from any source for 2,3-, 2,5-, 3,4-, and 3,5-DNT.
[b]Sources: Integrated Risk Information System (IRIS); 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); Health and Environmental Effects Profile (HEEP); World Health Organization (WHO).
[c]Parameters: weight of evidence (WOE); reference dose (RfD); reference concentration (RfC); oral slope factor (OSF); minimum risk level (MRL); time-weighted average (TWA); reference exposure level (REL); permissible exposure limit (PEL); Reproductive and Cancer Hazard Assessment Section (RCHAS).
[d–h]See notes column for corresponding information.

NA = not applicable; NV = not available.

Literature searches were conducted on sources published from 1900 through July 10, 2012 for studies relevant to the derivation of provisional toxicity values for tgDNT. The following databases were searched by chemical name, synonyms, or CAS No.: 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 relevant health information: 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 tgDNT and includes potentially relevant repeated long-term-, subchronic-, and chronic-duration studies. The phrase "statistical significance," used throughout the document, indicates a *p*-value of <0.05.

Table 3. Summary of Potentially Relevant Data for tgDNT (CASRN 25321-14-6)

Category	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL	BMDL/ BMCL	LOAEL	Reference	Comments	Notes
Human									
1. Oral									
Acute[b]	ND								
Short-term[c]	ND								
Long-term[d]	ND								
Chronic[e]	ND								
2. Inhalation									
Acute[b]	ND								
Short-term[c]	ND								
Long-term[d]	154/0 workers, occupational survey, 12 mo, mixed isomers of DNT (unknown composition of DNT)	NV	Complaints such as unpleasant taste, weakness, headache, loss of appetite, dizziness, nausea, insomnia, pain in extremities, vomiting, and numbness and tinging; clinical signs included low-grade anemia, and cyanosis	NV	DU	NV	McGee et al. (1942)		PR

Table 3. Summary of Potentially Relevant Data for tgDNT (CASRN 25321-14-6)

Category	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL	BMDL/ BMCL	LOAEL	Reference	Comments	Notes
Long-term[d]	714 workers (sex not reported), occupational survey, 3 yr, mixed isomers of DNT (unknown composition of DNT)	<1 mg/m^3	Fewer complaints of unpleasant taste, weakness, headache, loss of appetite, dizziness, nausea, insomnia, pain in extremities, vomiting, and numbness and tinging; reduced incidences of low-grade anemia, and cyanosis compared to McGee et al. (1942) study	NV	DU	NV	McGee et al. (1947)	A follow-up study to McGee et al. (1942)	PR
	30/0 workers, occupational survey, exposure duration (varies), mixed isomers of DNT (unknown composition of DNT) and coexposed to toluene diamine (TDA)	undetectable-0.23 mg/m^3 (personal sample); undetectable-0.42 mg/m^3 (area sample)	Significant[f] reduction in sperm count	NC	NC	NC	Ahrenholz (1980)		NPR

Table 3. Summary of Potentially Relevant Data for tgDNT (CASRN 25321-14-6)

Category	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL	BMDL/ BMCL	LOAEL	Reference	Comments	Notes
Long-term[d]	50/0 workers (nonsemen study) and 41/0 workers (semen study); occupational survey, exposure duration (varies), mixed isomers of DNT (approximately 80% 2,4-DNT and 20% 2,6-DNT) and coexposed to TDA	0.00929−0.318 mg/m^3 (time-weighted average, TWA)	No significant[f] difference in serum enzymes, sperm volume, sperm counts and morphological changes in workers in exposed group, and spontaneous abortions in their wives	0.0739 mg/m^3 (mean, TWA)	DU	NV	Ahrenholz and Meyer (1982)		NPR
	203/0 workers, occupational survey, ≥6 mo, coexposure to DNT and TDA, mixed isomers of DNT (unknown composition of DNT)	<1.5 mg/m^3 (Permissible Exposure Limit, PEL)	No significant differences in work history, medical history, physical examination, reproductive history, fertility, laboratory serum (follicle-stimulating hormone), mean sperm count, and sperm morphology in exposed group	NV	DU	NV	Hamill et al. (1982)	Investigated only male reproductive and fertility endpoints	PR
	208/10 workers, occupational study, no specified exposure duration, DNT/TDA, mixed isomers of DNT (unknown composition of DNT)	NV	No significant associations found for male reproductive toxicity	NV	DU	NV	Levine (1983)	Exposure-related information on fertility among female employees was insufficient to analyze	NPR Reproductive study

Table 3. Summary of Potentially Relevant Data for tgDNT (CASRN 25321-14-6)

Category	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL	BMDL/ BMCL	LOAEL	Reference	Comments	Notes
Long-term[d]	586 workers (sex not reported), retrospective survey, DNT/TDA mixed isomers of DNT (unknown composition of DNT)		No significant difference was found between the fertility of workers exposed to DNT in the three U.S. chemical plants and the fertility of unexposed workers	NV	DU	NV	Levine et al. (1985)		PR
	156/0 and 301/0 (two cohorts from two different plants), occupational cohort study, exposure at least 30 d during 1950s for the first cohort; exposure for ≥30 d during the 1940s and 1950s for the second cohort. 76% 2,4-DNT, 19% 2,6-DNT, and 5% other isomers for the first cohort; 98% 2,4-DNT and 1% 2,6-DNT for the second cohort	NV	For total workers in both plants, no significant increases in death from any specific types of cancer were observed. Excess mortality from ischemic heart disease (IHD) at both plants with standardized mortality ratios (SMRs) of 131 and 143 (95% confidence intervals [CI]s: 65−234, and 107−187, respectively)	NV	NU	NA	Levine et al. (1986)	Additional analyses revealed a15-yr latent period and suggested a relationship between duration and intensity of exposure	PR

Table 3. Summary of Potentially Relevant Data for tgDNT (CASRN 25321-14-6)

Category	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL	BMDL/ BMCL	LOAEL	Reference	Comments	Notes
Long-term[d]	4989 exposed (M)/5636 unexposed workers (M), occupational cardiovascular mortality study, participants exposed for ≥5 mo, (unknown composition of DNT)	NV	No significant association for an increased risk of either IHD or cerebrovascular disease	NV	NU	NA	Stayner et al. (1992)		PR
	4989 exposed (M)/7436 unexposed (M), occupational carcinogenicity study, participants exposed for ≥5 mo, (unknown composition of DNT)	NV	Excess of hepatobiliary cancer in exposed workers; standardized mortality ratio (SMR) of 2.67 (95% confidence interval [CI]: 0.98−5.83) when compared with U.S. population; standard rate ratio (SRR) of 3.88 (95% CI: 1.04−14.41) when compared with unexposed group	NV	NU	NV	Stayner et al. (1993)	Failed to demonstrate an exposure-response relationship between duration of exposure and hepatobiliary cancer mortality; limited by small number of participants with long exposure durations.	PR The study subjects were selected from the second plant examined by Levine et al. (1986)
Chronic[e]	500 workers (sex not reported), occupational carcinogenicity study, exposed for 7 to 37 yr, 30% tgDNT, which consisted of 75% 2,4-DNT and 20% 2,6-DNT.	NV	High exposure to DNT related to urothelial cancer	NV	NU	NV	Bruning et al. (1999)		PR Retrospective study

Table 3. Summary of Potentially Relevant Data for tgDNT (CASRN 25321-14-6)

Category	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL	BMDL/ BMCL	LOAEL	Reference	Comments	Notes
Chronic[e]	180 workers exposed for 7 to 37 yr (sex not reported), 30% tgDNT, which consisted of 75% 2,4-DNT and 20% 2,6-DNT	NV	A straight dose-dependent pathological (tubular and/or glomerular damage) pattern; nephrotoxic effect toward the proximal tubule under the exposure conditions	NV	NU	NV	Bruning et al. (2001)		PR
	Three case studies(sex not reported); >7 yr, exposure to DNT and possible exposure to nitrobenzene, mixed isomers of DNT (unknown composition of DNT)		High exposure to DNT associated with human urothelial cancer	NV	NU	NV	Harth et al. (2005)		PR
Animal									
1. Oral									
Subchronic	10/10, albino Fischer 344 (F344), rat, diet, 4 wk	M: 0, 31.9, 61.9, or 134 F: 0, 32.0, 63.6, or 120	Increases in hematological parameters, including methemoglobin (MetHb), reticulocytes, and Heinz bodies in males	NDr	NV	31.9	CIIT (1983)		NPR

Table 3. Summary of Potentially Relevant Data for tgDNT (CASRN 25321-14-6)

Category	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL	BMDL/ BMCL	LOAEL	Reference	Comments	Notes
Chronic	**10/10 (Study initial 130/130), F344, rat, diet, 26 wk**	**M: 0, 3.47, 13.6, or 34.6 F: 0, 3.22, 13.9, or 34.9**	**Increased absolute and relative liver weights and increased hepatotoxicity in males**	**3.47**	**2.16 based on increased incidence of hepatocyte necrosis in males**	**13.6**	**CIIT (1982a)**		**NPR, PS**
	10/10, F344, rat, diet, 52 wk	M: 0, 3.47, 13.9, or 34.9 F: 0, 3.46, 13.9, or 35.1	Increased absolute and relative liver weight and hepatotoxicity in males	NDr	NU	3.47	CIIT (1982a)		NPR
	20/20, F344, rat, diet, 55 wk	M: 34.9 F: 35.1	Hepatotoxicity in both males and females	NDr	NV	NDr	CIIT (1982a)	All surviving high dose rats were terminated at 55 wk due to severe toxicity.	NPR
	20/20, F344, rat, diet, 78 wk	M: 0, 3.49, or 14.0 F: 0, 3.45, or 14.0	Increased relative liver weight and hepatotoxicity in females	NDr	NU	3.45	CIIT (1982a)		NPR
	75−87/84−87, F344, rat, diet, 104 wk	**M: 0, 3.51, or 14.0 F: 0, 3.46, or 14.0**	**Increased absolute and relative liver weights and hepatotoxicity in females**	**NDr**	**0.363 based on increased incidence of hepatocyte necrosis in males**	**3.46**	**CIIT (1982a)**		**NPR, PS**

Table 3. Summary of Potentially Relevant Data for tgDNT (CASRN 25321-14-6)

Category	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL	BMDL/ BMCL	LOAEL	Reference	Comments	Notes
Chronic	28/0, F344, rat, diet, 52 wk	0, 35	Decreased body weight, increased absolute and relative liver weights, accompanied by pathological findings in the liver and bile duct	NDr	NU	35	Leonard et al. (1987)		PR
Developmental	Female, 22 control, 13, 7, 13, 7, 13, 6 for treatment groups, respectively, F344 rat, gavage, GDs 7−20	0, 14, 35, 37.5, 75, 100, or 150	Increased maternal relative liver weight (maternal effects); increased resorption at 150 mg/kg-d (fetal effects)	Maternal: 37.5 Fetal: 100	NU	Maternal:75 Fetal: 150	Price et al. (1985) CIIT (1982b)		PR NPR
Reproductive	ND								
Carcinogenic	28/0, F344 rat, diet, 52 wk	ADD: 0, 35 HED: 0, 9.3	47% increase in incidence of hepatocellular carcinomas compared with controls	NV	NV	NV	Leonard et al. (1987)		PR
	75−87/84−87, F344, rat, diet, 104 wk	**ADD: 0, 3.51, or 14 (M); 0, 3.46, or 14.03 (F)** **HED: 0, 0.922, or 3.45 (M); 0, 0.851, or 3.23 (F)**	**Dose-dependent increase in hepatocellular carcinomas and neoplastic nodules, mammary fibroadenomas, and subcutaneous fibromas**	**NV**	**$BMDL_{10HED}$: 0.224**	**NV**	**CIIT (1982a)**		**NPR, PS**

Table 3. Summary of Potentially Relevant Data for tgDNT (CASRN 25321-14-6)

Category	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL	BMDL/ BMCL	LOAEL	Reference	Comments	Notes
2. Inhalation									
Subchronic	ND								
Chronic	ND								
Developmental	ND								
Reproductive	ND								
Carcinogenic	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. Values are also presented as 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.
[b]Acute = exposure for ≤24 hr (U.S. EPA, 2002).
[c]Short-term = repeated exposure for >24 hr ≤30 d (U.S. EPA, 2002).
[d]Long-term = repeated exposure for >30 d ≤10% lifespan (based on 70-yr typical lifespan) (U.S. EPA, 2002).
[e]Chronic = repeated exposure for >10% lifespan (U.S. EPA, 2002).
[f]Significant (increase/decrease/difference) means either statistically or biologically significant (increase/decrease/difference) in this document.

GD = gestation day, ND = no data, NA = not applicable, NV = not available, NDr = not determinable, NC = not calculated, NPR = not peer-reviewed, PR = peer-reviewed, PS = principal study.

HUMAN STUDIES

Oral Exposures

No studies were identified.

Inhalation Exposures

Relevant data are available from epidemiological studies on the effects in humans of inhalation exposure to 2,4/2,6-DNT mixtures of various compositions. These effects have been evaluated in several occupational studies of workers in DNT manufacturing plants in which exposures were identified by the study authors to be predominantly via the inhalation route with contributions from the dermal route. No details on the exposure concentrations to DNT are given in these studies, and, therefore, they can only be used as qualitative descriptions of symptoms reported upon exposure. Also, in some of the studies, the isomer composition of the DNT mixture was not specified. The identified studies from occupational exposure to DNT have examined the clinical long-term effects (McGee et al., 1947; McGee et al., 1942), the potential for adverse reproductive effects (Levine et al., 1985; Levine, 1983; Ahrenholz and Meyer, 1982; Hamill et al., 1982; Ahrenholz, 1980), adverse effects on the cardiovascular system and carcinogenic risk (Harth et al., 2005; Bruning et al., 2001; Bruning et al., 1999; Stayner et al., 1993; Stayner et al., 1992; Levine et al., 1986).

Epidemiological Studies of General Toxicity

McGee et al. (1942) and McGee et al. (1947)

In an epidemiological study performed by McGee et al. (1942), 154 male workers in a military plant that manufactured powder containing a DNT mixture (primarily 2,4-DNT) were observed for 12 months. In the 12-month period, 96 individuals reported complaints of unpleasant taste (62%), 78 reported weakness (51%), 76 reported headache (49%), 72 reported loss of appetite (47%), 68 reported dizziness (44%), 57 reported nausea (37%), 57 reported insomnia (37%), 40 reported pain in extremities (26%), 35 reported vomiting (23%), and 29 reported numbness and tinging (19%). Additionally, 84 individuals exhibited clinical signs of sickness, which included pallor (36%), cyanosis (34%), and anemia (23%). These symptoms are consistent with methemoglobinemia and disappeared 2 to 3 days after exposure to the powder was terminated. The study authors also reported two instances of acute toxic hepatitis with jaundice.

After an effort was initiated to reduce the exposure between 1942 and 1945, a follow-up study by the same investigators in the same plant (McGee et al., 1947) evaluated 714 workers (sex not reported) who were exposed to less than 1 mg/m^3 DNT. Each of the individuals received medical examinations at intervals of 2 to 4 weeks. From these examinations, the study authors reported that signs and symptoms of illness were noticeably decreased when compared to the signs and symptoms experienced by the 154 workers from the 12-month study in 1942. Only 13.2% and 8.7% of men from the follow up study reported weakness and headaches, respectively, while around 50% of the men from the 1942 study reported the same effects. The reports of loss of appetite, nausea and vomiting, vertigo, pain, or tingling/numbing in the extremities were also reduced in this follow-up study as compared to the initial study. Pallor was rarely observed in the follow-up study, no hepatitis was observed, and a marked reduction in cyanosis (8.7%) and anemia (10.2%) was reported as well. The study authors (McGee et al., 1947; McGee et al., 1942) did not provide detailed DNT compositions or exposure data, and no unexposed control groups were used as a basis for comparison.

Epidemiological Studies of Reproductive Effects

Ahrenholz (1980) and Ahrenholz and Meyer (1982)

Ahrenholz (1980) reported on the potential for reproductive effects in male workers exposed to mixed isomers of DNT (composition of DNT was not reported, and exposure duration varied) and toluene diamine (TDA) in a TDA plant at Olin Chemical Company in Brandenburg, KY. TDA was produced through catalytic hydrogenation of DNT. The study authors conducted environmental and medical surveys in September 1979, and a follow-up investigation was conducted in January 1980. During both the initial and follow-up surveys, personal air samples were taken by mounting sample collection media in the operators' breathing zones. Area air sampling was also conducted. Medical evaluations consisting of a detailed questionnaire were used to acquire information on a range of potentially toxic effects. In addition, tobacco and alcohol consumption and medical history were recorded. A reproductive history was also elicited. The wives of workers were given a different, more detailed reproductive questionnaire in an attempt to validate the information given by the workers themselves. A physical examination with a special emphasis on the male reproductive system and secondary sex characteristics was performed. Blood specimens were obtained for analyses of blood urea nitrogen (BUN), creatinine, bilirubin, alkaline phosphatase, serum glutamic oxalic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), serum testosterone, serum luteinizing hormone, and serum follicle stimulating hormone. A semen specimen was also collected and analyzed for volume, sperm count, and morphologic pattern. The male workers that participated in the surveys were divided into three exposure groups: (1) exposed, (2) intermediate exposed (contact with DNT for one to several years on an intermittent basis but with no exposure for the last 2 years), and (3) unexposed (no exposure during their employment). The exposure groupings were determined by reviewing job descriptions and by discussing exposures with the individuals, the company, and the local union representatives.

The study group consisted of 30 male workers (9 from the exposed group, 12 from the intermediate exposed group, and 9 from the unexposed group). However, only seven total personal samples and three area samples were collected with the concentration ranging from not detectable to 0.23 mg/m^3 (personal samples) and from not detectable to 0.42 mg/m^3 (area samples). The author stated that the concentrations of DNT in the plant were below the OSHA standard PEL of 1.5 mg/m^3 over an 8-hour workday. Serum examinations of renal and hepatic profiles indicated no significant difference between any of the tested groups. A slight increase in miscarriages among the wives of the exposed workers was found but could not be conclusively related to the exposures. There were no significant differences in congenital defects and total pregnancies between the groups. However, sperm counts in the exposed group were significantly lower compared to the unexposed group. No significant differences were reported between unexposed and intermediate exposed groups or between intermediate exposed and exposed groups. The study author concluded that the results were strongly suggestive of a reproductive problem but more workers needed to be evaluated.

Ahrenholz and colleagues (Ahrenholz and Meyer, 1982) also conducted a similar study at Olin Chemical Company, in Moundsville, WV where male workers were exposed to both DNT and TDA. DNT was reported in an approximate 80:20 ratio of 2,4-DNT and 2,6-DNT. Fifty males (in the nonsemen portion of the study) and 41 of these 50 workers (in the semen portion of the study) were divided into three groups based on the same criteria in Ahrenholz (1980). The exposure duration ranged from 3 to 27.5 years. Due to laboratory error in preparation of the sampling media, personal exposure data were determined to be invalid. Seven DNT area

samples ranged in concentration from 0.026 mg/m^3 (0.00929 mg/m^3, TWA) to 0.89 mg/m^3 (0.318 mg/m^3, TWA) with a mean of 0.207 mg/m^3 (0.0739 mg/m^3, TWA). The study authors reported no significant difference between the exposed and unexposed groups in serum enzymes, sperm volume, sperm counts, and morphological changes. The questionnaire data on the employees' reproductive history did not show statistically significant differences in the number of spontaneous abortions in wives of workers employed in the DNT area when compared with non-DNT exposed workers in the plant. A NOAEL of 0.0739 mg/m^3 is identified based on no significant differences in serum enzymes, sperm volume, sperm counts and morphological changes in workers in the exposed group, and spontaneous abortions in their wives.

The studies conducted by Ahrenholz (1980) and Ahrenholz and Meyer (1982) were limited by small sample size, limited exposure data, problematic grouping, coexposure with TDA and other unknown chemicals, and unknown composition of DNT (Ahrenholz, 1980).

Hamill et al. (1982)

Hamill et al. (1982) conducted a study to determine the reproductive effects of occupational DNT and/or TDA exposure among 203 male workers from a chemical complex in Lake Charles, LA. Of the 203 employees in the cohort, 84 were exposed to DNT and/or TDA (exposure level within the OSHA PEL of 1.5 mg/m^3 within an 8-hour workday, detailed exposure data and composition of DNT were not reported), and 119 were not exposed. Each worker was subjected to a semen analysis for sperm count and morphology, blood testing (for serum follicle-stimulating hormone [FSH] measurement), a urogenital examination, an estimation of testicular volume, and an interview. Information was also acquired on reproductive history, medical and surgical history, past and present work history, assessment of exposure, exposure to other chemicals with potential reproductive toxicity, smoking habits, alcohol consumption, and recent medication. Data were collected between January and June 1981. Based on exposure history (intensity, frequency, and how recent exposure to DNT and/or TDA occurred), the participants were classified into four groups: (1) none to minimal, (2) low to high, (3) low to moderate, and (4) high. The duration was at least 6 months.

No significant differences were observed between the exposure groups with respect to work history, medical history, or physical examination characteristics. Additionally, there were no significant differences in reproductive histories, or decreases in fertility related to DNT and/or TDA exposure. Finally, no significant differences were discovered in the laboratory findings including serum FSH, mean sperm count, and sperm morphology. The study authors concluded that both TDA and DNT did not present a detectable reproductive hazard to the workers. However, the study is limited due to lack of detailed exposure data, exposure to mixed chemicals, and unknown composition of DNT.

Levine (1983)

Levine (1983) investigated the effect of DNT exposure on the fertility of workers occupationally exposed to a DNT mixture (composition not further specified; exposure duration not reported) in a U.S. toluene diisocyanate (TDI) plant. DNT and TDA are intermediates in the manufacturing of TDI. DNT was used to manufacture TDA in the plants and data were collected in 1981. Due to the job rotation, it was assumed that there was a coexposure to both DNT and TDA. A total of 208 male (166 white, 42 nonwhite) and 10 married female workers were interviewed. Exposure-related fertility among female employees was insufficient to analyze. The fertility analysis for male employees revealed that their wives exhibited no evidence of an

abnormal aggregation of miscarriages, stillbirths, neonatal deaths, or birth defects. This study revealed little to suggest that occupational exposure to DNT may have affected reproduction adversely and is limited due to lack of comparison with unexposed male employees, lack of data regarding quantitative exposure, exposure to mixed chemicals and other unknown chemicals, and unknown composition of DNT.

Levine et al. (1985)

Levine et al. (1985) investigated the effect of DNT exposure on the fertility of workers occupationally exposed to a DNT mixture (composition not further specified) in three U.S. chemical plants. The plants manufactured TDA, DNT, and/or TDI. Data were collected between 1979 and 1981, several years after the exposure period (between 1973 and 1976). A total of 586 workers (144 from Plant A, 207 from Plant B, and 235 from Plant C; sex not reported) were interviewed. No significant difference was found between the fertility of workers who were exposed to DNT in the three U.S. chemical plants and the fertility of unexposed workers. The study is limited due to the lack of quantitative exposure data provided, exposure to mixed and other unknown chemicals, and unknown composition of DNT.

Epidemiological Studies of Carcinogenicity or Cardiovascular Diseases

Levine et al. (1986)

Levine et al. (1986) evaluated workers at two army ammunition plants to assess the relationship between exposure to DNT and carcinogenicity. In the first plant, located in Joliet, IL, tgDNT (approximately 76% 2,4-DNT, 19% 2,6-DNT, and 5% other isomers) was manufactured and purified to at least 98% 2,4-DNT and about 1% 2,6-DNT. From the first plant, a total of 156 men, who worked in the DNT production line for at least 30 days during the 1950s, participated in the study. At the second plant, located in Radford, VA, the purified DNT (98% 2,4-DNT and approximately 1% 2,6-DNT) was used in certain single-based propellant formulations. This cohort consisted of 301 men who had worked for 30 days or more during the 1940s and 1950s in specific jobs that had potential for DNT exposure. Workers from both plants were presumed white males and considered exposed to DNT via the inhalation and dermal routes. The exposure levels from the first plant were judged by the study authors to be high. Jobs at the second plant were categorized by plant technical personnel according to opportunity for exposure: high, moderate, low or none. Cohort mortality was followed from enrollment through the end of 1980. Numbers of observed and expected deaths were recorded for each underlying cause and the standardized mortality ratio (SMR) was computed using mortality rates of U.S. white males as the standard.

Of the 457 men in both plants, 164 had died compared to 127 expected deaths using mortality rates of U.S. white males as the standard. The combined SMR of 129 ($p = 0.001$) for all causes of death was significantly high, and it increased to an SMR of 140 ($p = 0.00007$) after 15 years elapsed since entry into the study. This increase in overall mortality was attributed to increased death from disease of the circulatory system (SMR: 140, $p = 0.002$) or due to death from accidents, poisonings, and violence (SMR: 191, $p = 0.0007$). Death as a result of malignant neoplasms was less than the expected mortality rate (SMR: 87); however, this decrease was not significant. No significant increases in death from any specific types of cancer were observed. Increased mortality from disease of the circulatory system was determined to be primarily based on an increase in mortality from ischemic heart disease (IHD) (SMRs of 131 and 143 for the first and the second plant, respectively; 95% confidence intervals of 65−234 and 107−187 for the first

and the second plant, respectively). Deaths from IHD remained high even when compared with expected numbers derived using mortality rates of the counties in which the plants were located.

The relationship between mortality from IHD to duration and intensity of DNT exposure was analyzed at 15 years from entry into the study onward. The results are suggestive of a dose- and duration-response relationship for DNT and mortality from IHD. The study authors suggested that the increase in mortality from heart disease was a result of damage to the coronary arteries from exposure to DNT. The study is limited due to lack of exposure data, exposure to other unknown chemicals, and lack of unexposed controls.

Stayner et al. (1992)

Stayner et al. (1992) performed a retrospective cohort study using current and former white male workers from a propellant production facility in Radford, VA. The study aimed to determine the possible relationship between exposure to DNT (composition of DNT is not reported) and the risk of death as a result of cardiovascular disease including ischemic heart IHD and cerebrovascular disease. A total of 4989 workers with probable exposure to DNT and 5636 unexposed workers were selected for the study (workers' operations were rated concerning the probability of exposure to DNT). All exposed and unexposed workers who participated in the study had been employed for at least 5 months at the study plant between January 1949 and January 1980. The difference in mortality between the cohorts (exposed and unexposed groups) and the U.S. population was evaluated using SMRs. SMRs were also used to evaluate specific causes of death in addition to standardized rate ratios (SRRs), which are ratios of observed deaths in the exposed groups to the observed deaths in the unexposed groups. Death from all causes (including cardiovascular and noncardiovascular causes) was similar for DNT-exposed (SMR: 1.00) and unexposed (SMR: 0.99) groups when compared to the U.S. population. Mortality from cerebrovascular disease in the DNT-exposed group was less than that in the unexposed group (SMR: 0.95, SRR: 0.89). IHD mortality in the DNT-exposed group was similar to that of the unexposed group (SMR: 0.98, SRR: 0.99). Hypertension without heart disease (SMR: 1.17) and other myocardial degeneration (SMR: 1.41) were slightly elevated in the DNT-exposed group. The study authors concluded that DNT exposure did not appear to be associated with an increased risk of either IHD or cerebrovascular disease. The study authors also concluded that potential biases related to the company's medical screening program for workers exposed to DNT may have limited the ability to detect these effects. However, the study authors did not provide exposure or DNT composition data, and the definition of DNT-exposed groups was not clear.

Stayner et al. (1993)

Stayner et al. (1993) investigated the relationship between workers exposed to DNT and cancer of the liver and biliary tract. The cohort was selected from the second plant examined by Levine et al. (1986), but with more subjects who were exposed to DNT. A total of 4989 male workers exposed to DNT and 7436 unexposed male workers were included in this investigation. All the enrolled workers had worked at least 5 months at the study facility between January 1949 and January 1980. The vital status (i.e., whether dead or alive) of the workers at the end of 1982 was collected and used to develop SMRs and SRRs to analyze the various relationships between DNT exposure and mortality.

Mortality for the study cohort was compared to the expected mortality for the U.S. population. Death as a result of cancer was less than expected for the DNT exposed and unexposed groups (SMRs of 0.84 and 0.78, respectively). An increase in hepatobiliary cancers (defined as biliary, liver, and gall bladder cancers combined) was observed in the DNT-exposed cohort as compared to the U.S. population (SMR: 2.67, 95% CI = 0.98−5.83) (but this was identified as borderline statistically significant [$p = 0.052$]). When compared to unexposed workers of the same facility, however, the SRR for hepatobiliary cancers in the DNT-exposed group was significantly increased (SRR: 3.88, 95% CI = 1.04−14.41, $p = 0.04$). No exposure-response relationship was detected between the duration of exposure to DNT and hepatobiliary cancer mortality.

According to the investigators, the study had several limitations, mainly that it was originally designed to evaluate the risk associated with exposure to nitroglycerin (which was also manufactured at the plant) rather than DNT. Other limitations included the small number of hepatobiliary cancer cases (six), the small number of workers with a long exposure period to DNT, and the lack of quantitative DNT exposure data. Worker exposure was classified qualitatively, and those workers that were "probably" exposed were included in the exposed group. Individuals in this group may have had minimal contact with DNT, as the group was defined only by having contact with materials containing DNT as opposed to being exposed to DNT directly. Therefore, inclusion of these individuals could have introduced bias by preventing the detection of the true level of excess risk for hepatobiliary cancer following exposure to DNT. Another limitation identified by the study authors was the possibility of exposure to chemicals other than DNT. Nevertheless, the study authors concluded that the excess in hepatobiliary cancer mortality observed among DNT-exposed workers in this study added some support to the hypothesis that occupational exposure to DNT may be carcinogenic. The authors noted that this study investigated more subjects, and hence, it has more statistical power to detect an excess of hepatobiliary cancer than the Levine et al. (1986) study.

Bruning et al. (1999)

Bruning et al. (1999) performed a retrospective survey on underground miners who were formerly exposed to an explosive (Donarit) containing 30% tgDNT, which consisted of approximately 75% 2,4-DNT and 20% 2,6-DNT (the remaining 5% was unknown). The cohort was selected from a mining area in Mansfeld, which is located in the former German Democratic Republic (GDR). Health records were used to identify miners with former exposures to DNT ($n = 500$) and their incidence of urogenital malignant diseases. Of these 500 subjects (sex not reported), a group of 340 miners with available information on malignant urogenital tract disease was asked to participate. Among the 340 miners, 183 gave their consent and were subjected to a standard medical examination and a retrospective occupational exposure assessment. Additional information was obtained on occupational histories, including exposures to any type of hazardous chemicals, smoking histories, history of former kidney and renal diseases, as well as history of cancers within families.

The study authors reported that the miners had been exposed through two routes: inhalation of the smoke after explosions and skin contact with DNT-containing explosive sticks. The exposures were ranked into low, medium, high, and very high exposure categories. Between 1984 and 1997, 14 cases of renal cell cancer and 6 cases of urothelial cancer were identified in the group of 500 underground miners with former exposure to DNT. Exposure duration ranged from 7−37 years, and latency periods ranged from 21−46 years. The incidences of urothelial and

renal cancer in this group were 4.5 and 14.3 times higher than anticipated, respectively, on the basis of the cancer registers of the GDR. The exposure categorization of the 14 renal cell cancer cases revealed a distribution (i.e., number of workers in low, medium, high and very high exposure categories) similar to that of the 183 exposed miners without cancer. However, the six cases of urothelial cancer were predominantly confined to the high exposure category. The study authors concluded that high exposure to DNT might be associated with urothelial tumor formation. The study was limited due to the lack of quantitative exposure data and coexposure to other unknown chemicals.

Bruning et al. (2001)

In another study, Bruning et al. (2001) investigated signs of subclinical renal damage in the same subjects that were reported in Bruning et al. (1999), consisting of a group of 161 no cancer miners and 19 cases with renal (n = 14) or urothelial cancer (n = 5), all of whom had been exposed to explosives containing tgDNT [the same exposure duration and DNT composition information as presented in Bruning et al. (1999)]. The exposures were categorized semiquantitatively, according to the type and duration of contact with DNT, into low, medium, high, and very high. Evaluation of urinary protein excretion patterns indicated that there was a straight dose-dependent relationship in the pathology of tubular and/or glomerular damage, indicating that DNT-induced damage is directed toward the renal tubular system. In addition, there was a dose-dependent increase in the biomarkers of alpha1-microglobulin and glutathione S-transferase alpha, indicating a nephrotoxic effect toward the proximal tubule under the exposure conditions. By contrast, there was no similar change in glutathione S-transferase pi, indicating no nephrotoxicity to the distal tubule. The study was limited due to the lack of exposure data and coexposure to other unknown chemicals.

Harth et al. (2005)

In this case report, Harth et al. (2005) reported a cluster of three cases of urothelial cancer (sex not reported) among a group of about approximately 60 workers who were exposed to DNTs during manufacture of a DNT explosive (Donarit) at a factory in the former GDR. The cases occurred within a period of 12 years (1990−2002) leading to a 15.9-fold higher incidence of cancer of the urinary bladder than that of the federal state where the chemical factory was located, even though no adjustments were made for age and smoking. From 1970 until 1974, the production of DNT and nitrobenzene was located in one building, raising the possibility of coexposure to nitrobenzene during this period. The exposure durations for the three cases were longer than 7 years. The observation of the cluster of urothelial cancer in people highly exposed to DNTs underlines the possibility of human carcinogenicity of DNTs, with the human urothelium as a relevant target tissue. The study was limited due to the lack of exposure data, unknown composition of DNT, and coexposure to other unknown chemicals.

ANIMAL STUDIES

Oral Exposures

The effects of oral exposure of animals to tgDNT have been evaluated in one subchronic (CIIT, 1983), two chronic, (Leonard et al., 1987; CIIT, 1982a) one developmental (Price et al., 1985; CIIT, 1982b), and two carcinogenicity studies (Leonard et al., 1987; CIIT, 1982a).

Subchronic Studies

CIIT (1983)

In an unpublished, nonpeer-reviewed study, CIIT (1983) administered tgDNT (consisting of 76.49% 2,4-DNT, 18.83% 2,6-DNT, 2.43% 3,4-DNT, 1.54% 2,3-DNT, 0.65% 2,5-DNT, and 0.040% 3,5-DNT) via diet to groups of 10 albino F344 rats/sex/group for 4 weeks. The nominal doses were 0, 37.5, 75, or 150 mg/kg-day per group. Based on weekly tgDNT consumption provided by the study authors, the adjusted daily doses (ADDs, calculated based on TWA doses) are 0, 31.9, 61.9, or 134 mg/kg-day for males and 0, 32.0, 63.6, or 120 mg/kg-day for females. Fresh diets were prepared weekly and adjusted based on body weight and food consumption data. No certificate of good laboratory practice (GLP) was included in the study report.

The study authors observed animals for signs of morbidity and mortality twice daily and recorded signs of gross toxicity and/or pharmacologic effects, food consumption, and individual body weights weekly. Blood samples were collected from nonfasted females and males on Days 27 and 28, respectively. Blood samples were analyzed for methemoglobin (MetHb), reticulocytes, and Heinz bodies. After sacrifice, gross pathological examinations were performed on the lungs, liver, spleen, kidneys, ovaries, and vagina. Organ weights were not measured.

No treatment-related mortalities occurred during the study period. The only clinical signs noted by the study authors were alopecia around the right eye of two females in the low-dose group and urine stains on the fur of four high-dose females at Week 3 and two high-dose females at Week 4. At Weeks 3 and 4, body weights of females treated with 120 mg/kg-day were significantly decreased by 17% and 21%, respectively. All the high-dose treated males experienced a significant reduction in mean body weight in a time-dependent manner compared with controls (10%, 26%, 36%, and 38% reductions compared to controls at Weeks 1, 2, 3, and 4, respectively). In addition, body weights of the mid-dose males were significantly decreased by 11% and 17% compared with controls at Weeks 3 and 4, respectively (see Table B.1). Food consumption was reduced in all treatment groups compared with controls in a dose-dependent manner (data not shown). Therefore, the decreased body weights were likely related to the reduction in food consumption in addition to tgDNT treatment.

Dose-dependent, significant increases in mean reticulocytes and Heinz bodies were reported in all treated animals (see Table B.2). MetHb was significantly increased in the low and high-dose females and in the high-dose males (see Table B.2). The study authors noted the following gross pathological observations in high-dose females and in low- and high-dose males: discoloration (yellow), mottled appearance, and/or rough or granular surface of the liver. Discoloration (green) of the kidneys was observed in high-dose males and females. Dark yellow vaginal stains were also noted in two high-dose females, and an ovarian cyst was found in one mid-dose female. Table B.3 presents the gross pathology results.

Based on the significant hematological changes in male rats, a LOAEL of 31.9 mg/kg-day is identified. Data preclude identification of a NOAEL.

Chronic Studies

CIIT (1982a)

CIIT (1982a) is selected as the principal study for deriving the screening subchronic and chronic p-RfDs. **CIIT (1982a)** administered tgDNT (consisting of 76.5% 2,4-DNT,

18.8% 2,6-DNT, 2.4% 3,4-DNT, and <2.3% 2,3-, 2,5-, and 3,5-DNT) to groups of 130 F344 rats/sex/group (Charles River Breeding Laboratories, Wilmington, MA) via diet at target dose levels of 0, 3.5, or 14.0 mg/kg-day for 2 years, or 35 mg/kg-day for 55 weeks (sacrificed early due to treatment-related incidence of liver tumors). The control group rats received a basal diet only. The study authors conducted interim sacrifices at 26 weeks, 52 weeks, 55 weeks (for high-dose group only), 78 weeks, and 104 weeks at termination. For each dose group, a ADD was calculated based on tgDNT concentration in food, food consumption, and time periods (calculated based on TWA) provided by the study authors. The calculated ADDs are shown in each data summary table (see Tables B.4 to B.15). The test diets were prepared weekly based on measured body weight and food consumption data to ensure constant intake of tgDNT on a mg/kg body-weight basis. The study authors did not provide a statement confirming GLP status.

Animals were examined twice daily for general physical appearance, mortality, and morbidity. The study authors recorded food consumption in each cage and individual body weights every week for the first 14 weeks, biweekly for the following 12 weeks, and every fourth week for the remainder of the test period. Mean daily tgDNT consumption was calculated for each dose level weekly from Weeks 1 through 14, biweekly through Week 26, and monthly for the remainder of the treatment period.

Hematology, clinical chemistry, and urinalyses were performed on 10 animals/sex/group at Weeks 26 and 52, and on 20 animals/sex/group at Weeks 78 and 104. An additional 20 animals from the high-dose group were tested for hematology and clinical chemistry at Week 55. The measured hematological parameters included hematocrit, hemoglobin (Hb), and MetHb; red blood cell (RBC), reticulocyte, Heinz body, total white blood cell (WBC), and differential leukocyte counts; and mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC). Serum samples were also analyzed for clinical chemistry, including alkaline phosphatase (ALP), BUN, and SGPT. Urinalysis measured appearance, pH, specific gravity, glucose (GLU), ketone, total protein (TPR), occult blood, and sediment. Ophthalmologic examinations were performed on both eyes of each animal using an indirect ophthalmoscope 1 or 2 days prior to sacrifice at 26, 52, 78, and 104 weeks.

Ten rats/sex/group were sacrificed at Weeks 26 and 52. At Week 55, all surviving high-dose rats were sacrificed due to severe toxicity (high incidence of tumors). At Week 78, 20 rats/sex/ group were sacrificed, and at Week 104, all surviving rats were sacrificed. The study authors performed examinations for gross signs of toxicity and incidence and recorded the location of tumors at identical intervals. At necropsy, the following organs were excised and weighed: brain, heart, liver, kidneys, lungs, and testes with epididymides. Ovaries were not weighed until after fixation. The following tissues were examined histopathologically from animals in the control and high-dose groups at Weeks 26 and 52, in the control and mid-dose groups at Weeks 78 and 104, and in the high-dose groups at Week 55: brain (cerebellum, cerebrum, brain stem), eyes, testes with epididymides, thoracic spinal cord, pituitary, thyroid, parathyroid, adrenal, heart, aorta, lungs, spleen, liver, kidneys, stomach, small intestine (duodenum, jejunum, ileum), large intestine (upper and lower colon, rectum), pancreas, ovary with oviduct, uterus, prostate, thymus, esophagus, trachea, nasal turbinate, adipose tissue, submaxillary salivary gland, lymph nodes (mesenteric and thoracic), urinary bladder, thigh skeletal muscle with sciatic nerve, bone marrow (sternum), skin (flank), mammary gland, and

unusual lesions. Histopathological examination was also performed on the livers from the low-dose groups at Weeks 26, 52, 78, and 104 and from the mid-dose group at Weeks 26 and 52.

The study authors reported a decrease in survival in male rats in the mid-dose group at termination and in the high-dose group at Week 55. All surviving rats in the high dose group were sacrificed at Week 55 due to severe liver toxicity and tumor formation noted at the Week 52 interim sacrifice. Survival in the remaining dose groups was similar to controls. The study authors reported treatment-related hunched, thin, and/or bloated appearance in all animals.

The study summaries are presented below in the order of the sacrifice. The study authors reported that the results of urinalysis were unremarkable throughout the 2-year period.

26-week study

The ADDs at this interim sacrifice were calculated to be as follows: 0, 3.47, 13.6, or 34.6 mg/kg-day for males, and 0, 3.22, 13.9, or 34.9 mg/kg-day for females. At Week 26, 10 animals/sex/group were sacrificed. Hematology, clinical chemistry, urinalyses, gross pathology, organ/body-weight ratios, and histopathology evaluations were conducted. Selected hematology results are provided in Table B.5. There was a 31% increase in WBC count in the mid-dose males and dose-related increases in MetHb levels (by up to 400%), reticulocyte count (by up to 83%), as well as a decrease in RBC count in high-dose males (8%). The hematology results for females are not summarized in this document because no biological significance was found. RBC and reticulocyte counts were not significantly changed, while MetHb was significantly decreased in all treatment groups. Clinical chemistry results indicated that BUN levels in high-dose males and females were significantly increased by 17% and 27%, respectively (see Table B.6). Both males and females experienced dose-related decreases in body weight compared to control animals (see Tables B.4 and B.7). However, the decreased body weight may be partially due to the reduction of food intake (see Table B.4).

The study authors reported significant dose-dependent increases in both absolute (12% and 50%) and relative (25% and 95%) liver weight in the mid- and high-dose males, respectively (see Table B.8). A similar liver weight increase was also reported in tgDNT-treated females (see Table B.9). In addition, there were dose-dependent increases in relative kidney weights with significant changes observed in the high dose group (38% in males and 24% in females; see Tables B.8 and B.9). Other observed significant organ-weight changes included increased relative heart, relative lung weight in high-dose males and females, and increased relative testis weight in high-dose males. Gross pathology findings revealed tgDNT-treatment-related gross alterations in the livers. Histopathology findings indicated hepatotoxicity in the mid- and high-dose males and females (see Table B.10). Indicators of hepatotoxicity consisted of minimally to moderately severe nonsuppurative pericholangitis in high-dose males and in mid- and high-dose females; necrosis of hepatocytes in mid- and high-dose males and females; vacuolated hepatocytes in high-dose males and females; and slightly to moderately severe biliary hyperplasia, periportal fibrosis, and necrosis of bile duct epithelium in high-dose males. Other signs of histopathology were found in the heart, spleen, and kidney (incidence data are not displayed in this document). These findings included an increase in the incidence and severity of chronic myocarditis in high-dose males. Considering 4/10 control males also had minimal chronic myocarditis, the study authors concluded that this probably represents a treatment-related exacerbation of spontaneous disease. Also, an increase in spleen hemosiderin and extramedullary hematopoiesis was observed in the high-dose males and females. In the kidney,

slightly increased incidence of chronic interstitial nephritis was noted in the high-dose males. In addition, there was increased incidence and amount of tubular pigment in the mid- and high-dose males and females. Although moderately severe testicular degeneration was observed in high-dose males, the study authors suggested that the unilateral change may represent a spontaneous lesion.

Two high-dose males had hepatocellular carcinomas, which were not observed in the control group (see Table B.14), suggesting these tumors may have been induced by tgDNT.

Based on increased absolute and relative liver weight and hepatotoxicity in mid-dose males, a LOAEL of 13.6 mg/kg-day and a NOAEL of 3.47 mg/kg-day are identified.

52-week study

The ADDs at this interim sacrifice were calculated as follows: 0, 3.47, 13.9, or 34.9 mg/kg-day for males, and 0, 3.46, 13.9, or 35.1 mg/kg-day for females. At Week 52, 10 animals/sex/group were sacrificed. Hematology, clinical chemistry, urinalyses, gross pathology, organ/body-weight ratio, and histopathology evaluations were conducted. Compared to controls, there were dose-related increases in MetHb levels (not significant) and reticulocyte count (significant in high-dose group), and decreases in RBC count (significant in the mid-and high-dose groups) and Hb (significant in the high-dose group; see Table B.5). Also, WBC counts in the high-dose group were increased. In female rats, no treatment-related hematological alterations were observed.

Body-weight changes were similar to the findings at Week 26 (see Table B.7). Significant increases in both absolute and relative liver weights were reported in the low-dose males and mid- and high-dose males and females. There were dose dependent increases in male (significant in the mid- and high-dose groups) and female relative kidney weight (significant in the high-dose group) and in male relative heart weight (significant in the high-dose group) (see Tables B.8 and B.9). Gross pathology findings revealed distinct gross alterations of the livers, namely, focal discolorations in the mid- and high-dose males and females. Also, liver nodular lesions were noted in 8/10 males and 4/10 females in the high-dose group. The liver histopathology findings included hyperbasophilia, megalocytosis of hepatocytes, and vacuolation and necrosis of individual hepatocytes (see Table B.11). Other treatment-related lesions in the high-dose males consisted of exacerbation of chronic interstitial nephritis and renal tubular pigment, increased incidence and severity of testicular degeneration, and increased proliferation of hematopoietic cells in the splenic red pulp and sternal marrow, suggesting an increased RBC turnover rate. Also, the study authors noted that the cardiomyopathy observed in the high-dose males at 26 weeks was not obvious in this group at 52 weeks.

A dose-dependent increase in hepatocellular carcinomas (3/10 in mid-dose and 10/10 in high-dose) was observed in males (see Table B.14). Hepatocellular carcinomas were observed in 4/10 females in the high-dose group. Neoplastic nodules were noted in the livers of 4/10 mid-dose and 3/10 high-dose males, as well as 8/10 high-dose females. Cholangiocarcinomas were observed in 2/10 high-dose males and 2/10 high-dose females. One of 10 mid-dose males had biliary hyperplasia with atypia of the bile duct epithelium, which the study authors believed to be a precursor lesion of cholangiocarcinoma.

Based on increased absolute and relative liver weight and hepatotoxicity in low-dose males, a LOAEL of 3.47 mg/kg-day is identified. Data preclude identification of a NOAEL.

55-week study

The ADDs at this interim sacrifice were calculated as follows: 34.9 mg/kg-day for males and 35.1 mg/kg-day for females (only high-dose group). At Week 55, all surviving high-dose animals were sacrificed. Hematology, clinical chemistry, urinalyses, and histopathology evaluations were conducted on 20 rats/sex, but the results on hematology and clinical chemistry were only reported on 10 rats/sex. Because there were no significant hematological findings in the treated females, Table B.5 displays male results only. Tables B.6, B.8, and B.9 present clinical chemistry and organ/body-weight ratios. Histopathological findings (not displayed in tables) in high-dose animals indicated that hepatocellular carcinomas occurred in 20/20 males and in 11/20 females. Two male rats had hepatocellular-cholangiocarcinomas and 3/20 males had cholangiocarcinomas. Biliary hyperplasia with atypia of bile duct epithelium was also observed in 2/20 males. Due to lack of a control group, data preclude identification of a NOAEL or a LOAEL.

78-week study

The ADDs at this interim sacrifice were calculated as follows: 0, 3.49, or 14.0 mg/kg-day for males, and 0, 3.45, or 14.0 mg/kg-day for females. At Week 78, 20 animals/sex from each dose level were sacrificed (only control, low-dose, and mid-dose groups were available; the high-dose males and females were all sacrificed at Week 55). Hematology, clinical chemistry, urinalyses, gross pathology, organ/body-weight ratio, and histopathology evaluations were conducted. Among the hematology findings were decreased hematocrit and RBC counts in the mid-dose males and dose-dependent increases of 13% and 112% in reticulocyte counts in the low-and mid-dose males, respectively (see Table B.5). In treated female rats, significantly increased WBC counts were observed in mid- and high-dose groups, and no other significant hematological changes were observed. Clinical chemistry indicated significantly higher mean SGPT values in mid-dose males (93% higher than controls) (see Table B.6). Body-weight changes in the mid-dose males and females were similar to those observed in the 26-week treatment. Absolute and relative liver weights were significantly increased in the low- and mid-dose males and in the mid-dose females (all the high-dose rats were sacrificed at Week 55; see Tables B.8 and B.9). In low- and mid-dose males, the study authors also reported significantly dose-dependent increases in the relative weights of testes (27% and 37%, respectively), lungs (14% and 30%, respectively), and kidneys (12% and 55%, respectively; see Table B.8). Also, the relative kidney weight was increased by 32% and the absolute brain weight was decreased by 11% in the mid-dose females see Table B.9). Hepatotoxicity including cystic degeneration, necrosis of individual hepatocytes and fatty metamorphosis were observed in low- and mid-dose males and females (see Table B.12). An increase in the severity of chronic interstitial nephritis was observed in mid-dose males and females. Treatment-related testicular pathology observed at the Week 52 interim sacrifice could not be determined because all control and mid-dose males had interstitial cell testicular tumors. Hepatocellular carcinomas were observed in 19/20 mid-dose males (see Table B.14); neoplastic nodules were observed as follows: 1/20 in low-dose males, 11/20 in mid-dose males, 2/20 in low-dose females, and 10/20 in mid-dose females. Four cholangiocarcinomas were recognized in mid-dose males in addition to 2/20 incidence of biliary hyperplasia with atypia of bile ductal epithelium in the same

group. In this interim sacrifice, the study authors observed increases in relatively common benign neoplasms, such as mammary fibroadenomas, subcutaneous fibromas, pituitary chromophobe adenomas, and pulmonary carcinomas.

Based on increased relative liver weight and hepatotoxicity in low-dose females, a LOAEL of 3.45 mg/kg-day is identified. Data preclude identification of a NOAEL.

104-week study

The ADDs doses at this terminal sacrifice were calculated as follows: 0, 3.51, or 14 mg/kg-day for males, and 0, 3.46, or 14 mg/kg-day for females. At Week 104, all surviving animals were sacrificed. Hematological results indicated that in males there were dose-dependent increases in MetHb (not significant); a significant increase in reticulocyte counts in the mid-dose group; and significant decreases in hematocrit, Hb, and RBC counts in the mid-dose group (see Table B.5). No remarkable hematological changes were observed in treated female rats (data not shown). Evaluation of clinical chemistry revealed significantly increased SGPT and BUN in the mid-dose males (see Table B.6). Similar body weight changes were observed in mid-dose males and females as those observed in the 52 or 78-week treatments (see Table B.7). Dose-dependent, significantly increased organ weights in the low- and mid-dose groups included relative heart weights in males and females, both absolute and relative liver weights in males and females, relative testis weight in males, and relative kidney and ovary weights in females (see Table B.8 and Table B.9). Gross pathology findings revealed apparent treatment-related liver lesions and nodular and/or mass formation in low- and mid-dose males and females. Histopathology indicated that hepatotoxicity occurred in the livers of treated animals and consisted of fatty metamorphosis, necrosis, cystic degeneration, and megalocytosis in low- and mid-dose males and females (see Table B.13). An increase in the severity of chronic interstitial nephritis occurred in the kidneys of mid-dose males and females. The study authors did not observe treatment-related histopathological changes in the ovaries or in the hearts from either sexes; therefore, the biological significance of the increased relative heart and ovary weights in the low- and mid-dose groups is unclear. Testicular pathological changes were not determined as observed in Week 78. Therefore, the biological significance of the increased relative testes weight is also unclear.

There is a clear dose-dependent increased incidence of hepatocellular carcinomas and neoplastic nodules in both males and females (see Table B.15). "Neoplastic nodule" is a term used in rodent liver pathology. Because neoplastic nodules are believed to progress to hepatocellular carcinomas (Bannasch et al., 1982; Bannasch, 1976), this endpoint is included in the dose-response analysis. Hepatocholangiocarcinomas were observed in 1/68 mid-dose female, and cholangiocarcinomas were observed in 2/23 mid-dose males. In addition, 2/23 mid-dose males had biliary hyperplasia with atypia of bile duct epithelium. Parathyroid hyperplasia was also observed in mid-dose males and females, and 2/23 mid-dose males had parathyroid adenomas. Increased relatively common benign neoplasms were observed in both males and females (see Table B.15).

Based on increased absolute and relative liver weight and hepatotoxicity in low-dose females, a LOAEL of 3.46 mg/kg-day was identified. Data preclude identification of a NOAEL.

Leonard et al. (1987)

Leonard et al. (1987) administered 0 or 35 mg/kg-day tgDNT (final composition of 76.5% 2,4-DNT, 18.8% 2,6-DNT, 2.43% 3,4-DNT, 1.54% 2,3-DNT, 0.69% 2,5-DNT, and 0.04% 3,5-DNT) via diet to groups of 28 male F344/CrlBR rats (Charles River Breeding Laboratories, Kingston, NY) for 1 year. New food batches were prepared monthly and the tgDNT concentration was adjusted in each batch based on food consumption and average body weight in order to maintain target dose levels. It is unclear if this study was conducted under GLP.

Rats were housed four per cage, and average dietary consumption for each cage was determined weekly. Body weights were measured every 2 weeks throughout the study. Four animals in each group were sacrificed after 6 and 26 weeks of feeding. At the end of the 52-week treatment period, all surviving animals were sacrificed and necropsied, the liver and lungs were weighed, histopathological examination was performed, and hepatic microsomal epoxide hydrolase (EH) and cytosolic DT-diaphorase (DTD) activities were measured. Serum enzyme activities (SGPT) and glutamyl transferase (GGT) were also determined. Other clinical chemistry, hematology, and pathology examinations besides liver and lung were not conducted.

The study authors reported that body weight was significantly reduced 11% and 26% in rats treated with tgDNT compared with controls at 26 and 52 weeks, respectively (see Table B.16). Relative liver weight was significantly increased at both time periods (see Table B.16) as well. At the end of 52 weeks, absolute liver weight was 89% more and relative liver weight was 155% more than that of controls (see Table B.16). After 52 weeks of treatment, the study authors noted nonneoplastic lesions consisting of hepatocytic degeneration and vacuolation in the majority of animals, as well as acidophilic and basophilic cell foci in over 90% of the animals. Bile duct hyperplasia and a highly variable incidence of cholangiofibrosis were also noted.

Based on significant decreases in body weight, and increases in absolute liver and relative liver weight accompanied by pathological findings in the liver and bile duct, a LOAEL of 35 mg/kg-day is identified from this study. Data preclude identification of a NOAEL.

Table B.17 summarizes neoplastic lesions of the liver. Neoplastic nodules were found in the livers in 53% of animals treated with tgDNT. Hepatocellular carcinomas were seen in 47% of the tgDNT-treated animals; all lesions had a typical trabecular pattern. Cholangiocarcinomas were also reported in 11% of the treated animals. The study authors concluded that tgDNT is a potent, complete hepatocarcinogen in male F344 rats. The study is limited by the use of only one dose of tgDNT, which precludes examining dose-response relationships. In addition, the study authors did not provide quantitative data for nonneoplastic lesions and only reported general findings.

Developmental Studies

Price et al. (1985) and CIIT (1982b)

Research Triangle Institute (RTI) performed a study investigating the potential developmental toxicity of tgDNT following maternal gestational exposure in F344 rats. The study was performed in 1980, and a Final Report was submitted under the Toxic Substances Control Act (TSCA) to EPA's Office of Toxic Substances by CIIT (1982b). Additionally, Price and colleagues, the study authors from RTI, reported on the maternal and fetal toxicity in a

peer-reviewed, published study (Price et al., 1985). The tgDNT used in the study contained the following composition of isomers: 76% 2,4-DNT; 19% 2,6-DNT; 2.4% 3,4-DNT; 1.5% 2,3-DNT; <1%; 2,5-DNT; and <1%; 3,5-DNT. The rats were administered the tgDNT by gavage, with laboratory-grade corn oil used as the vehicle. The study authors administered tgDNT at doses of 0 (vehicle control), 35, 75, or 150 mg/kg-day (first breeding round) to groups of 13 pregnant F344 rats on gestation days (GDs) 7 through 20. Due to high mortality rates observed in the 150 mg/kg-day dose group, the doses used in the second and third breeding rounds were reduced to 14, 37.5, and 100 mg/kg-day. Table B.18 shows the numbers of females in each dose group.

The study authors examined dams daily for clinical signs of toxicity and recorded body weights on GDs 0 and 7–20. On GD 20, 13, 7, 13, 7, 13, and 6 dams from each dose group (14, 35, 37.5, 75, 100, and 150 mg/kg-day, respectively) and 22 dams from the control group were sacrificed and evaluated for implantations, resorptions, and dead or live fetuses. In addition, body weight, liver weight, spleen weight, number of corpora lutea, and gravid uterine weight for each dam were recorded. The pregnancy rates in mated dams from the control and low- through high-dose groups were 20 (91%), 10 (77%), 7 (100%), 12 (92%), 6 (86%), 12 (92%), and 5 (83%), respectively. No histopathological examinations were conducted on the dams. Live fetuses were examined for uterine position, body weight, crown-rump length, placenta weight, sex, and gross morphological abnormalities. Maternal and fetal blood samples from the 100-mg/kg-day treatment group were analyzed for MetHb content. In addition, blood samples from dams and one male and one female fetus per litter from the 100-mg/kg-day treatment group were evaluated for RBC count, WBC count, hematocrit, MCV, RBC distribution width (RDW), and platelet count. The study authors also examined 50% of the fetuses in each litter for visceral and skeletal malformations, malformations of the head, and liver and spleen weights.

A high mortality rate was observed in dams from the first breeding date exposed to 150 mg/kg-day tgDNT, as 46.2% of rats (6/13) in this treatment group died between GDs 11 and 18. Therefore, in the second and third breedings, the study authors reduced the tgDNT doses. Treatment with tgDNT at 14, 35, and 100 mg/kg-day also resulted in mortality rates of 4.5% (1/22), 7.7% (1/13), and 4.3% (1/23), respectively, through GD 20 (see Table B.18). No deaths occurred in females treated with the vehicle control (corn oil). The study authors stated that the cause of death of one rat/group from the 14-, 35-, and 100-mg/kg/day-DNT groups was initially suspected to be related to gavage error. Gavage error was also suspected to be the cause of death in 2/6 rats from the highest dose group (150 mg/kg); however, the study authors concluded that the cause of death in the remaining 4 rats appeared to be treatment related as death was preceded by clinical signs of toxicity. Clinical signs related to tgDNT treatment included rough coat, lethargy, and hind-limb weakness, and were observed in 7/13 females in the 150-mg/kg-day dose group beginning on the fifth to eighth day of dosing (GDs 11−14) and continuing until death (GDs 12−18) or scheduled sacrifice (GD 20).

On GD 20, significant increases in MetHb, reticulocyte count, MCV (RBC size), RDW, and platelet count were observed in dams treated with 100-mg/kg-day tgDNT. Significant decreases in RBC count and hematocrit were also observed in this group (see Table B.19). Because the study authors did not analyze low-dose blood samples, it is not clear if hematological parameters were adversely affected at lower doses.

A significant dose-related decrease in absolute maternal weight gain (maternal weight gain during treatment minus gravid uterine weight) was observed in the 14, 100, and 150-mg/kg-day tgDNT dose groups (see Table B.20). However, no decreases in absolute maternal weight gain was observed at 35, 37.5, and 75 mg/kg-day, suggesting the reduction of weight gain at 14 mg/kg-day was not treatment related, at least at dose levels <100 mg/kg-day. Significant dose-related increases in relative maternal liver weight were observed in the 75- (11%), 100- (12%), and 150-mg/kg-day (17%) treatment groups (see Table B.20). A significant increase in maternal relative spleen weight was observed at all doses of tgDNT ≥35 mg/kg-day (see Table B.20). Due to lack of histopathological data, the biological significance of the increased spleen weight is not clear. There was an increased resorption rate (46.0%) in the high-dose group compared to the control (16.8%); however, this change was not significant. No other significant effects on measures related to reproduction (incidence of live or dead fetuses per dam, see Table B.20) or fetal morphology (see Table B.21) were observed. The study authors, therefore, concluded that tgDNT was not observed to be teratogenic in F344 rats even at dose levels that produced significant maternal toxicity.

In litters with live fetuses, no significant difference was observed in the proportion of male fetuses per litter, average fetal body weight per litter, average fetal crown-rump length per litter, or average placental weight per litter (see Table B.22). Changes in liver/body and spleen/body-weight ratios were observed in some treatment groups, but no dose-response relationship was apparent (see Table B.22). Fetuses from the 100-mg/kg-day group exposed to tgDNT exhibited decreased reticulocyte count, decreased RBC count, and increased MCV (see Table B.19). Although the decreased RBC count and increased MCV were significant, the 1% RBC count decrease (2.15×10^6 compared to 2.17×10^6) and 2% RBC size increase (160.61 μm^3 compared to 156.54 μm^3) were not considered biologically significant.

The Final Report submissions (CIIT, 1982b) include the teratological study [the same information included in Price et al. (1985)] as well as a postnatal developmental evaluation. In the postnatal developmental evaluation, the remaining female rats that were not sacrificed on GD 20, including 8, 5, 15, 6, 9, and 1 pregnant females from the low- through high-dose groups, respectively, and 15 controls were observed through parturition, death, or GD 24, whichever came first. One female in the 14-mg/kg-day group died on GD 22, and 1 female in the 150-mg/kg-day group died on GD 23 (the females from this group were not available for postnatal developmental evaluation). Twelve females failed to deliver by GD 24 and were sacrificed; 11 of these females were determined not to be pregnant. In total, pups were observed from 5−14 litters per treatment group from birth (postnatal day [PND] 0) to PND 60.

Body weight and crown-rump length of each live pup were recorded on PND 0, then litters were culled to no more than eight live pups (four male and four female, as possible). The study authors recorded body weight daily and noted age of appearance of physical landmarks (pinna detachment, pilation, incisor eruption, eye opening, testes descent, vaginal opening), neurobehavioral landmarks (surface righting, cliff avoidance, auditory startle, wire grasping, and mid-air righting), and open field behavior on PND 30. A limited number of pups were sacrificed from each treatment group on PNDs 0, 10, 25, and 50; the remaining pups were sacrificed on PND 60. Body weight, liver weight, and spleen weight were recorded at each sacrifice date; testis weights were recorded from the sacrifice at PND 60. All dams were sacrificed on PND 30,

and maternal body weight, liver weight, and spleen weight at sacrifice were recorded. Blood samples were collected from dams at PND 30 and pups on PNDs 0, 10, 25, and 50 from the 75- and 100-mg/kg-day groups and the corresponding controls.

The study author (CIIT, 1982b) reported that dams in the 100-mg/kg-day group exhibited decreased body weight on PND 15, and dams in the 75-mg/kg-day group had reduced reticulocyte counts on PND 30; no other signs of maternal toxicity were observed. The study authors state that significant differences in some observations from vehicle controls were observed in the litters, but these differences were not dose-related, including elevated litter size on PND 0 in the 75-mg/kg-day group; elevated female crown-rump length on PND 0 in the 14- and 37.5-mg/kg-day groups; increased male body weight on PND 0 in the 14-mg/kg-day group; increased reticulocyte count in the 75-mg/kg-day group or decreased reticulocyte count in the 100-mg/kg-day group on PND 50; and either early or delayed appearance of eye opening in the14-mg/kg-day group or the 35- and 75-mg/kg-day groups, respectively. There was a dose-dependent increase in relative liver weight for pups in all treatment groups on PND 0, but no difference was observed on PND 60 in any group, indicating that tgDNT toxicity was reversed by PND 60. The study authors stated that a dose-related decrease in rearing behavior in the open field was observed at 100 mg/kg-day in female pups, suggestive of sex-specific neuromotor deficits (see Table B.23).

The Price et al. (1985) study stated that tgDNT was not found to be teratogenic following oral administration and concluded that there was no evidence for selective sensitivity of the developing conceptus to tgDNT because prenatal viability was reduced only at the dose near the maternal LD_{50}. The CIIT (1982b) studies concluded that while various dosages of tgDNT could produce facilitation or retardation of growth or development, dose-response relationships for these changes do not exist.

Based on significantly increased relative liver weight in pregnant F344 dams at GD 20, a LOAEL of 75 mg/kg-day and a NOAEL of 37.5 mg/kg-day are identified for maternal toxicity. Considering a 29.2% increase in resorption rate accompanied by an increase in dead fetuses and a decrease in live fetuses in the high-dose group, a LOAEL of 150 mg/kg-day and a NOAEL of 100 mg/kg-day are established for developmental toxicity. Further, a LOAEL of 100 mg/kg-day and a NOAEL of 75 mg/kg-day were identified for postnatal toxicity based on decreased rearing behavior.

Reproductive Studies

No studies were identified.

Carcinogenicity Studies

Carcinogenicity studies by CIIT (1982a) and Leonard et al. (1987) were summarized in the *Chronic Studies* section. **The carcinogenicity study by CIIT (1982a) is selected as the principal study for deriving the screening provisional oral slope factor (p-OSF).**

Inhalation Exposures

No studies were identified.

Other Data (Other Examinations)

Mutagenicity or genotoxicity of tgDNT has been evaluated in in vitro and in vivo test systems, and Table 4 summarizes the study results. Mixed results were reported from both in vitro and in vivo test systems. Mutagenicity tests were positive in *Salmonella typhimurium* Ames assays, with and without metabolic activation, while they were negative in mammalian cell systems (e.g., HGPRT gene mutations in Chinese hamster ovary [CHO] cells and TK mutations in mouse lymphoma cells). Similar to the in vitro test systems, mixed results were also seen in in vivo studies. While unscheduled DNA synthesis showed a positive response in most rat hepatocytes (Mirsalis et al., 1989; Hamilton and Mirsalis, 1987; Ashby et al., 1985; Mirsalis and Butterworth, 1982; Mirsalis et al., 1982) and in rat lymphocytes (Kligerman et al., 1982), a mouse bone marrow micronucleus test (Ashby et al., 1985) and dominant lethal assay (Soares and Lock, 1980) indicated negative responses.

Table 4. Summary of tgDNT Genotoxicity

Endpoint	Test System	Dose Concentration[a]	Results[b]		Comments	References
			Without Activation	With Activation		
Genotoxicity studies in prokaryotic organisms						
Reverse mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538	1000 µg/plate	+ (TA98, TA1538)	+ (TA98, TA1538)	NA	Couch et al. (1981)
Forward mutation	*S. typhimurium* strain TM 677	500 µg/mL	+	+	NA	Couch et al. (1981)
Genotoxicity studies in mammalian cells—in vitro						
HGPRT Mutation	Chinese hamster ovary (CHO)	≤2 mM	–	–	NA	Abernethy and Couch (1982)
TK+/–	P388 mouse lymphoma cells	1.6–1000 µg/mL	–	–	NA	Styles and Cross (1983)
Unscheduled DNA synthesis (UDS)	Primary hepatocyte cultures from adult male F344 rats	0, 1×10^{-4}, or 1×10^{-5} M	–	ND	Evaluated	Bermudez et al. (1979)
Genotoxicity studies in mammals—in vivo						
Sister chromatid exchange (SCE)	Rat lymphocyte culture from male F344 rats	0 or 100 mg/kg (oral)	+	ND	NA	Kligerman et al. (1982)
UDS	Hepatocytes/Male Alderley Park rats	0, 100, or 200 mg/kg (oral)	+	ND	NA	Ashby et al. (1985)
UDS	Hepatocytes/Male F344 rats	0, 25, 100, 150, or 200 mg/kg (oral)	+	ND	NA	Ashby et al. (1985)
UDS	Hepatocytes/Male F344 rats	0 or 100 mg/kg (oral)	+	ND	Mixture of DNTs, with dissimilar composition as tgDNT	Hamilton and Mirsalis (1987)
UDS	Hepatocytes/Male and female F344 rats	≤200 mg/kg (oral)	+	ND	NA	Mirsalis and Butterworth (1982)
UDS	Hepatocytes/Male germ-free (axenic) F344 rats or Charles River Altered Schaedler Flora rats (CRASF; similar to normal gut microflora)	0 or 100 mg/kg (oral)	Axenix – CRASF +	ND	Results indicate that gut flora is necessary for tgDNT to induce UDS	Mirsalis et al. (1982)
UDS	Hepatocytes/Male F344 rats	0, 35, 125, or 250 mg/kg (oral)	+ (at doses ≥125 mg/kg)	ND	NA	Mirsalis et al. (1989)

Table 4. Summary of tgDNT Genotoxicity

Endpoint	Test System	Dose Concentration[a]	Results[b]		Comments	References
			Without Activation	With Activation		
Mouse biochemical or visible specific locus test	T stock male and C57BL/6J female mice; C57BL/6 J male and C57BLBL/6J female mice	0 or 100 mg/kg	–	ND	Recessive spot test; different matings were tested; treated on a day designated as 1/4 of pregnancy	Soares and Lock (1980)
Micronucleus test	Bone marrow/Male (CBA × BalbC)F1 mice	0, 200, 400 mg/kg (IP)	–	ND	NA	Ashby et al. (1985)
Dominant lethal	Male DBA/2J mice	0 or 250 mg/kg (IP or oral)	–	ND	Treated on two consecutive days	Soares and Lock (1980)

[a]Lowest effective dose for positive results or highest dose tested for negative results.
[b]+ = positive; IP= intraperitoneal injection; NA = not applicable; ND = no data.

DERIVATION OF PROVISIONAL REFERENCE DOSES

Tables 5 and 6 present a summary of noncancer reference values and cancer values, respectively.

Table 5. Summary of Noncancer Reference Values for Technical Grade Dinitrotoluene (CASRN 25321-14-6)

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)	F344 Rat/M	Increased hepatocyte necrosis at week 26	5×10^{-3}	$BMDL_{10}$	0.52	100	CIIT (1982a)
Screening Chronic p-RfD (mg/kg-d)	F344 Rat/M	Increased hepatocyte necrosis at week 104	9×10^{-4}	$BMDL_{10}$	0.087	100	CIIT (1982a)
Subchronic p-RfC (mg/m^3)	NDr						
Chronic p-RfC (mg/m^3)	NDr						

NDr = not determined.

Table 6. Summary of Cancer Values for Technical Grade Dinitrotoluene (CASRN 25321-14-6)

Toxicity Type	Species/Sex	Tumor Type	Cancer value	Principal Study
Screening p-OSF	F344 Rat/M	Combined tumor incidence for hepatocellular carcinomas, liver neoplastic nodules, mammary fibroadenomas and subcutaneous fibromas	4.5×10^{-1} $(mg/kg\text{-}d)^{-1}$	CIIT (1982a)
p-IUR	NDr			

NDr = not determined.

DERIVATION OF ORAL REFERENCE DOSES

Derivation of Subchronic Provisional RfD (Subchronic p-RfD)

A subchronic p-RfD cannot be derived for tgDNT because the only potential principal study by CIIT (CIIT, 1983) is limited and is not suitable to derive a subchronic p-RfD (see Appendix A for details). However, Appendix A provides a "screening level" value for subchronic oral exposure based on a comprehensive unpublished study (CIIT, 1982a).

Derivation of Chronic Provisional RfD (Chronic p-RfD)

A chronic p-RfD cannot be derived for tgDNT because no peer-reviewed studies are suitable to derive a chronic p-RfD (see Appendix A for details). However, Appendix A provides a "screening level" value for chronic oral exposure based on a comprehensive unpublished chronic study (CIIT, 1982a).

DERIVATION OF INHALATION REFERENCE CONCENTRATIONS

No studies were identified that could be used to derive provisional inhalation RfCs for tgDNT. Available epidemiological studies consist primarily of occupational studies in which workers were exposed to a tgDNT mixture, and/or other known and unknown chemicals. In addition, none of the epidemiological studies provided quality exposure information. No animal inhalation studies for tgDNT were identified.

CANCER WEIGHT-OF-EVIDENCE DESCRIPTOR

Table 7 identifies the cancer weight-of-evidence (WOE) descriptor for tgDNT.

Table 7. Cancer WOE Descriptor for tgDNT

Possible WOE Descriptor	Designation	Route of Entry (Oral, Inhalation, or Both)	Comments
"Carcinogenic to Humans"	NA	Inhalation	Relevant human cancer studies on tgDNT are available on the effects of human inhalation/dermal exposure to mixed DNT.
"Likely to Be Carcinogenic to Humans"	**Selected**	**Both[a]**	**In the cancer study by CIIT (1982a), oral exposure to tgDNT caused an increased incidence of hepatocellular tumors, liver neoplastic nodules, mammary fibroadenomas, and subcutaneous fibromas in both male rats and an increased incidence of hepatocellular tumors, liver neoplastic nodules, and subcutaneous fibromas in female F344 rats. In another cancer study by Leonard et al. (1987), tgDNT caused an increased incidence of hepatocellular carcinomas and liver neoplastic nodules in F344 male rats after 1 year of oral exposure.**
"Suggestive Evidence of Carcinogenic Potential"	NA	NA	The evidence from animal and human data is more than suggestive of carcinogenicity, which raises a concern for carcinogenic effects and is judged sufficient for a stronger conclusion.
"Inadequate Information to Assess Carcinogenic Potential"	NA	NA	There is evidence to assess the carcinogenic potential of tgDNT.
"Not Likely to Be Carcinogenic to Humans"	NA	NA	Evidence of the carcinogenic potential of tgDNT is available in animals and humans.

[a]tgDNT is considered "*Likely to be Carcinogenic to Humans*" by all routes of exposure based on *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), which indicates that for tumors occurring at a site other than the initial point of contact, the cancer WOE descriptor may apply to all routes of exposure that have not been adequately tested at sufficient doses.

NA = not applicable.

Under the 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), the WOE descriptor for tgDNT is "*Likely to Be Carcinogenic to Humans*" by all routes of exposure (see Table 7). This descriptor is based on (1) suggestive evidence of carcinogenicity in humans and (2) strong evidence in animals by oral exposure.

There is some evidence for an increased risk of certain types of cancer in occupational populations exposed to tgDNT. An association between tgDNT exposure and an increased risk of hepatobiliary cancer was found in a retrospective mortality study involving workers at a U.S. Army munitions facility (Stayner et al., 1993). A study of underground mining workers exposed to tgDNT as an explosive (Bruning et al., 1999) also indicated that tgDNT might be associated with urothelial tumor formation. The workers in this study were believed to be

exposed to tgDNT via dermal or inhalation exposure. However, these studies were limited by a variety of factors, including inadequate exposure information (i.e., concentration and duration), and therefore, do not permit a definitive conclusion on the carcinogenicity of tgDNT in humans.

Results from experimental animal studies showed that tgDNT increased the incidence of multiple tumor types in F344 rats in two separate studies (Leonard et al., 1987; CIIT, 1982a). Significant increases in hepatocellular neoplastic nodules and carcinomas (males and females), subcutaneous fibromas (males and females), and mammary fibroadenomas (males only) were observed in F344 rats in chronic-duration dietary exposure bioassays (CIIT, 1982a), and increases in hepatocellular carcinoma were also observed in male F344 rats in a 1-year dietary study (Leonard et al., 1987). In addition, tgDNT caused hepatocellular tumors in rats as early as 26 weeks; therefore, the positive tumor results observed in these studies can be considered an early onset of carcinogenicity. As stated in the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), examples for a chemical to be considered "*Likely to Be Carcinogenic to Humans*" are (1) "an agent that has tested positive in animal experiments in more than one species, sex, strain, site, or exposure route, with or without evidence of carcinogenicity in humans;" (2) "a positive tumor study that raises additional biological concerns beyond that of a statistically significant result, for example, a high degree of malignancy, or an early age at onset." Based on these examples from the cancer guidelines and the carcinogenicity data from available human and animal studies, the WOE descriptor of "*Likely to Be Carcinogenic to Humans*" is appropriate for tgDNT.

The *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005) indicate that for tumors occurring at a site other than the initial point of contact, the cancer WOE descriptor may apply to all routes of exposure that have not been adequately tested at sufficient doses. An exception occurs when there are convincing toxicokinetic data that absorption does not occur by other routes. Information available on the carcinogenic effects of tgDNT demonstrates that tumors occur in tissues remote from the site of absorption. tgDNT has been shown to be a hepatocarcinogen in rats in two bioassays of various experimental designs by oral exposure. Increased hepatocellular carcinoma in munition workers and urothelial cancer in mining workers are presumed to have been exposed predominantly through the inhalation route with a contribution from the dermal route. Information on the carcinogenic effects of tgDNT via the dermal route in humans and animals is limited or absent. There are no toxicokinetic data indicating absorption does not occur by other routes. Therefore, based on the observation of liver tumors in animals following oral exposure and in humans following occupational inhalation and dermal exposure, it is assumed that an internal effective dose will be achieved regardless of the route of exposure. Thus, tgDNT is considered "*Likely to be Carcinogenic to Humans*" by all routes of exposure.

MODE-OF-ACTION DISCUSSION

The *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005) define mode-of-action "as a sequence of key events and processes, starting with the interaction of an agent with a cell, proceeding through operational and anatomical changes, and resulting in cancer formation". Examples of possible modes of carcinogenic action for any given chemical include "mutagenicity, mitogenesis, inhibition of cell death, cytotoxicity with reparative cell proliferation, and immune suppression".

The potential mode of action for tgDNT is unclear. Table 4 summarizes the studies examining genotoxicity (e.g., clastogenicity, mutagenicity) of tgDNT. tgDNT was shown to be positive for mutagenicity in *S. typhimurium* strains (Couch et al., 1981) but was not mutagenic in mammalian cell systems [e.g., HGPRT mutation in CHO cells and TK mutation in mouse lymphoma cells (Styles and Cross, 1983; Abernethy and Couch, 1982). While most assays of unscheduled DNA synthesis in rat hepatocytes showed a positive response following oral dosing (Mirsalis et al., 1989; Hamilton and Mirsalis, 1987; Ashby et al., 1985; Mirsalis and Butterworth, 1982; Mirsalis et al., 1982), mouse bone marrow micronuclei (Ashby et al., 1985) and dominant lethal assays (Soares and Lock, 1980) were negative.

DERIVATION OF PROVISIONAL CANCER POTENCY VALUES

Derivation of Provisional Oral Slope Factor (p-OSF)

A p-OSF cannot be derived for tgDNT because no peer-reviewed studies are suitable to derive a p-OSF. However, Appendix A provides a "screening level" value for a p-OSF based on a comprehensive unpublished carcinogenicity study (CIIT, 1982a).

Derivation of Provisional Inhalation Unit Risk (p-IUR)

No human or animal studies examining the carcinogenicity of tgDNT following inhalation exposure were identified. Therefore, derivation of a p-IUR is precluded.

APPENDIX A. PROVISIONAL SCREENING VALUES

For the reasons noted in the main document, subchronic and chronic p-RfDs for tgDNT could not be derived. However, information is available for this chemical which, although insufficient to support derivation of a provisional toxicity value, 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 an appendix and develops a "screening value". Appendices receive the same level of internal and external scientific peer review as the main 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 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.

DERIVATION OF SCREENING SUBCHRONIC PROVISIONAL RfD (SCREENING SUBCHRONIC p-RfD)

No human oral studies were identified. The database for oral tgDNT toxicity in animals includes one subchronic-duration study (CIIT, 1983), two chronic-duration studies (Leonard et al., 1987; CIIT, 1982a), and one developmental study (Price et al., 1985; CIIT, 1982b). The chronic-duration F344 rat study by CIIT (1982a) is composed of five interim evaluations at Weeks 26, 52, 55, 78, and 104. CIIT (1983) (4 weeks exposure duration) reported significant treatment-related hematological effects. As a result, a LOAEL of 31.9 mg/kg-day is identified based on the significant hematological changes in male rats. However, this study was not selected as the principal study because it only focused on hematological and gross pathological examinations, and no clinical chemistry, organ weight, and histopathology endpoints were examined. The 26-week interim study within the chronic-duration study (CIIT, 1982a) provided a comprehensive toxicity evaluation and is the closest exposure duration to the standard 13 weeks of a subchronic-duration study. Thus, in the absence of a comprehensive evaluation following a shorter exposure duration, the 26-week study from CIIT (1982a) is selected as the principal study in lieu of the 4-week study by CIIT (1983) and is protective of subchronic-duration exposure. In this 26-week study, hematology, clinical chemistry, urinalyses, gross pathology, and histopathology were all conducted to evaluate tgDNT toxicity. The study authors reported toxicity of tgDNT on organ weight (e.g., liver and kidneys) and hematological (e.g., MetHb levels, reticulocyte count, MCV, and RBC count), and histopathological end points (e.g., liver and spleen). A NOAEL of 3.47 mg/kg-day and a LOAEL of 13.6 mg/kg-day were identified based on increased absolute and relative liver weight and hepatotoxicity in male rats. For comparison purposes, Table A.1 summarizes all the potential critical effects from the 26-week study. All the endpoints shown in the table were modeled with benchmark dose software (BMDS) (version 2.2.2), and the estimated $BMDL_{10}$s are also summarized in the table. Among all the candidate endpoints for potential critical effect, the increased incidence of hepatocyte necrosis in male rats resulted in the lowest $BMDL_{10}$ of 2.16 mg/kg-day, which is followed by a $BMDL_{10}$ of 2.27 mg/kg-day for periportal hyperbasophilic hepatocytes in females. Therefore, increased hepatocyte necrosis is considered the critical effect, and using the $BMDL_{10}$ for this endpoint as the point of departure (POD) would protect all the sensitive effects observed in rats after 26 weeks of oral exposure. In addition to the subchronic-duration studies mentioned above, there is a developmental study (Price et al., 1985) that is also considered as part of the

database for derivation of a screening subchronic p-RfD. In this study, a NOAEL of 37.5 mg/kg-day and LOAEL of 75 mg/kg-day were established based on maternal toxicity (i.e., increased relative liver weight). A NOAEL of 100 mg/kg-day and a LOAEL of 150 mg/kg-day were established for developmental toxicity based on increased resorption rate accompanied by an increase in dead fetuses and a decrease in live fetuses. Among all available subchronic data (including subchronic-duration and developmental studies), the $BMDL_{10}$ of 2.16 mg/kg-day for increased incidence of hepatocyte necrosis in male rats is the most sensitive and is considered protective for all potential tgDNT-induced effects including developmental toxicity. Therefore, the $BMDL_{10}$ of 2.16 mg/kg-day is selected as the POD for derivation of the screening subchronic p-RfD. The NOAEL of 3.47 mg/kg-day for increased absolute and relative liver weights in males in the same 26-week interim sacrifice is considered supportive.

Table A.1. Potential Critical Effects in Male and Female F344 Rats After Dietary Exposure to tgDNT for 26 Weeks

End points	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	$BMDL_{10}$ (mg/kg-d)	POD (mg/kg-d)
Males				
Relative liver weight	3.47	13.6	No fit	3.47
Relative kidney weight	13.6	34.6	No fit	13.6
Hepatocyte necrosis	3.47	13.6	2.16	2.16
Females				
Relative liver weight	3.22	13.9	5.60	5.60
Relative kidney weight	13.9	34.9	No fit	13.9
Periportal hyperbasophilic hepatocytes	3.22	13.9	2.27	2.27

In *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011b), 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 toxicologically equivalent 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 physiologically based pharmacokinetic (PBPK) model for tgDNT is not available for use in extrapolating doses from animals to humans. In addition, the selected POD of 2.16 mg/kg-day is based on increased incidence of hepatocyte necrosis, which is not a portal-of-entry or developmental effect. Therefore, scaling by $BW^{3/4}$ is relevant for deriving human equivalent doses (HEDs) for this effect.

Following U.S. EPA (2011b) guidance, the POD for the 26-week rat study is converted to a HED through the 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 default BW_h of 70 kg for humans (U.S. EPA, 1988), the resulting DAF is 0.24. Applying this DAF to the $BMDL_{10}$ identified in the 26-week rat study yields a $BMDL_{10HED}$ as follows:

$$\begin{aligned} POD_{HED} &= BMDL_{10}\ (mg/kg\text{-}day) \times DAF \\ &= BMDL_{10}\ (mg/kg\text{-}day) \times 0.24 \\ &= 2.16\ (mg/kg\text{-}day) \times 0.24 \\ &= 0.52\ mg/kg\text{-}day \end{aligned}$$

$$\begin{aligned} \textbf{Screening Subchronic p-RfD} &= POD_{HED} \div UF_C \\ &= 0.52\ mg/kg\text{-}day \div 100 \\ &= \mathbf{5 \times 10^{-3}\ mg/kg\text{-}day} \end{aligned}$$

Table A.2. Uncertainty Factors for the Screening Subchronic p-RfD for tgDNT

UF	Value	Justification
UF_A	3	For the POD based on an increased incidence of hepatocyte necrosis (CIIT, 1982a), an 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 tgDNT exposure. The toxicokinetic uncertainty has been accounted for by calculation of a HED through application of a DAF as outlined in the *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011b).
UF_D	3	An UF_D of 3 has been applied because there is one developmental toxicity study (Price et al., 1985) in addition to subchronic- and chronic-duration studies, but there are no two-generation reproductive toxicity studies.
UF_H	10	An 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 tgDNT in humans.
UF_L	1	An UF_L of 1 has been applied for LOAEL-to-NOAEL extrapolation because the POD is a $BMDL_{10}$.
UF_S	1	An UF_S of 1 has been applied because a subchronic-duration study was selected as the principal study.
UF_C	100	

[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, 2011b), 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 $DAF = BW_a^{1/4} \div BW_h^{1/4}$.

DERIVATION OF SCREENING CHRONIC PROVISIONAL RfD (SCREENING CHRONIC p-RfD)

The chronic-duration study database for tgDNT includes two studies (Leonard et al., 1987; CIIT, 1982a). Leonard et al. (1987) conducted a 1-year study with two doses of tgDNT (0 and 35 mg/kg-day) administered to male F344 rats. The study authors reported significantly decreased body weight, increased absolute and relative liver weights, and liver pathological changes. A LOAEL of 35 mg/kg-day is established based on the observed effects; no NOAEL could be identified. The chronic-duration study by CIIT (1982a) is a comprehensive study composed of three interim chronic-duration evaluations (i.e., 52-,78-, and 104-week interim sacrifices). Table A.3 summarizes all potential critical effects and corresponding NOAELs and LOAELs for each interim study. All the endpoints shown in this table were modeled with BMDS (version 2.2.2), and the estimated BMDLs are also summarized. As shown in Table A.3, the interim sacrifices consistently indicated that the liver and kidneys are the target organs of tgDNT, and the liver is a more sensitive target organ than the kidneys. During all three interim sacrifices, the responses observed in male rats were consistently more sensitive than in the female rats (e.g., relatively lower PODs for hepatocyte necrosis ranged from 0.059 to 0.5 mg/kg-day in males vs. 1.31 to 3.64 [LOAEL] in females). Among the responses observed in male rats, hepatocyte necrosis is consistently shown as the most sensitive response. Therefore, male rat hepatocyte necrosis data have been further evaluated (see Table A.4) to find the most sensitive POD. Although, the necrosis incidences in male rats at 52 weeks appeared to be more sensitive than the 78- and 104-week groups, the results might be questionable because (1) the 52-week high-dose group showed a much lower response (50%) compared to 70% and 90% incidence at the low- and mid-doses, respectively; and (2) the 78- and 104-week studies, which had a longer exposure duration and larger sample sizes, showed less response (35−60%) at low- and mid-doses compared to those (70% and 90%, respectively) at 52-week sacrifice. At 104 weeks, male hepatocyte necrosis incidence at the low-dose (54%) was higher than that at the mid dose (48%); therefore, BMD modeling cannot adequately model the full data set (see Appendix C for details). In order to provide a best estimate for this data set, the mid-dose data point (48%) was dropped and two data points (control and low dose) were modeled with BMDS (version 2.2.2), with an estimated $BMDL_{10}$ of 0.363 mg/kg-day (see Appendix C for details). As part of the database to derive a chronic RfD, the developmental study by Price et al. (1985) identified higher NOAELs for maternal toxicity (37.5 mg/kg-day) and developmental toxicity (100 mg/kg-day). Therefore, the $BMDL_{10}$ of 0.363 mg/kg-day for hepatocyte necrosis at 104 weeks in the CIIT (1982a) study is the most sensitive and is considered protective for all the observed effects and is chosen as the POD for derivation of the screening chronic p-RfD.

Table A.3. Potential Critical Effects in Male and Female F344 Rats After Dietary Exposure to tgDNT for 52, 78, and 104 Weeks

Endpoints	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	$BMDL_{10}$ (mg/kg-d)	POD (mg/kg-d)	POD Selection (mg/kg-d)
52-Week Males					
Relative liver weight	NA	3.47	1.66	1.66	
Relative kidney weight	3.47	13.9	No fit	3.47	
Hepatocyte necrosis	NA	3.47	0.059	0.059	0.059
Hyperbasophilic hepatocyte	3.47	13.9	No fit	3.47	
Vacuolation	NA	3.47	No fit	3.47 (LOAEL)	
52-Week Females					
Relative liver weight	3.46	13.9	No fit	3.46	
Relative kidney weight	13.9	35.1	No fit	13.9	
Hepatocyte necrosis	13.9	35.1	3.64	3.64	
Hepatocyte megalocytosis	13.9	35.1	6.86	6.86	
Hyperbasophilic hepatocyte	3.46	13.9	2.20	2.20	2.20
78-Week Males					
Relative liver weight	NA	3.49	1.34	1.34	
Relative kidney weight	NA	3.49	1.07	2.72	
Hepatocyte necrosis	NA	3.49	0.50	0.5	0.5
Cystic degeneration	3.49	14	No fit	3.49	
78-Week Females					
Relative liver weight	NA	3.45	2.15[a]	2.15	
Relative kidney weight	3.45	14	3.56	3.67	
Hepatocyte necrosis	3.45	14	1.31	1.31	1.31
104-Week Males					
Relative liver weight	NA	3.51	1.36[a]	1.36	
Relative kidney weight	Original data illegible				
Hepatocyte necrosis	NA	3.51	0.363[a]	0.363	0.363
Cystic degeneration	3.51	14.0	No fit	3.51	
Hepatocyte megalocytosis	NA	3.51	No fit	3.51 (LOAEL)	
104-Week Females					
Relative liver weight	NA	3.46	Control SD from original data is illegible	3.46 (LOAEL)	3.46 (LOAEL)
Relative kidney weight	3.46	14.0	No fit	3.46	
Hepatocyte necrosis	NA	3.46	No fit	3.46 (LOAEL)	
Hepatocyte megalocytosis	NA	3.46	2.09	2.09	

[a]BMD modeling was performed with two data points (control and low dose).

A.4. Hepatocyte Necrosis in Male F344 Rats After Dietary Exposure to tgDNT for 52, 78 and 104 Weeks[a]

Week	Control	Low-dose	Mid-dose	High-dose
52 (n = 10)	0/10	7/10 (70[c])	9/10 (90)	5/10 (50)
78 (n = 20)	0/20	7/20 (35)	12/20 (60)	NA[b]
104 (n = 61, 70, and 23 for control, low-, and mid-dose groups, respectively)	0/61	38/70 (54)	11/23 (48)	NA[b]

[a]CIIT (1982a).
[b]All the high-dose rats were sacrificed at Week 55.
[c]Percent animals with necrosis

Following U.S. EPA (2011b) guidance, the POD for the rat 104-week study is converted to a HED through an application of a DAF[2] 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 default BW_h of 70 kg for humans (U.S. EPA, 1988), the resulting DAF is 0.24. Applying this DAF to the $BMDL_{10}$ identified in the rat 104-week study yields a $BMDL_{10HED}$ as follows:

$$\begin{aligned} POD_{HED} &= BMDL_{10} \text{ (mg/kg-day)} \times DAF \\ &= BMDL_{10} \text{ (mg/kg-day)} \times 0.24 \\ &= 0.363 \text{ (mg/kg-day)} \times 0.24 \\ &= 0.087 \text{ mg/kg-day} \end{aligned}$$

$$\begin{aligned} \textbf{Screening Chronic p-RfD} &= POD_{HED} \div UF_C \\ &= 0.087 \text{ mg/kg-day} \div 100 \\ &= \mathbf{9 \times 10^{-4} \text{ mg/kg-day}} \end{aligned}$$

[2]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, 2011b), 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 A.5. Uncertainty Factors for the Screening Chronic p-RfD for tgDNT

UF	Value	Justification
UF_A	3	For the POD based on an increased incidence of hepatocyte necrosis (CIIT, 1982a), 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 tgDNT exposure. The toxicokinetic uncertainty has been accounted for by calculation of a HED through application of a dosimetric adjustment factor (DAF) as outlined in *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011b).
UF_D	3	An UF_D of 3 has been applied because there is one developmental toxicity study (Price et al., 1985) in addition to subchronic- and chronic-duration studies, but there are no two-generation reproductive toxicity studies.
UF_H	10	An UF_H of 10 has been applied for interindividual variability to account for human-to-human variability in susceptibility in the absence of quantitative information to assess the toxicokinetics and toxicodynamics of tgDNT in humans.
UF_L	1	An UF_L of 1 has been applied for LOAEL-to-NOAEL extrapolation because the POD is a $BMDL_{10}$.
UF_S	1	An UF_S of 1 has been applied because a chronic-duration study was selected as the principal study.
UF_C	100	

DERIVATION OF PROVISIONAL CANCER POTENCY VALUES

Derivation of Screening Provisional Oral Slope Factor (p-OSF)

There are two oral carcinogenicity studies in rats (Leonard et al., 1987; CIIT, 1982a). Leonard et al. (1987) indicated that exposure to tgDNT (35 mg/kg-day) for 1 year caused a 47% increase in the incidence of hepatocellular tumors in male rats compared to the control (only one treatment dose). In the study conducted by CIIT (1982a), the incidence of hepatocellular carcinoma was observed beginning at Week 26, and the highest incidence occurred at Week 104. In addition, increased liver neoplastic nodules, mammary fibroadenomas, and subcutaneous fibromas were observed in males at Week 104. Appendix B summarizes these carcinogenicity results. The overall tumor response is relatively higher in male rats than in female rats at 104 weeks from the CIIT (1982a) study, and, therefore, Table A.6 summarizes only the male incidence data.

Table A.6 shows BMD dose-response modeling was conducted for various tumor types. All these tumor incidence data successfully fit to the Multistage-Cancer model in BMDS (version 2.2.2l see Appendix D for details), and Table A.7 summarizes the modeling results. Based on the BMD modeling results, the calculated cancer slope factors (oral slope factor or OSF) for hepatocellular carcinomas, liver neoplastic nodules, combined hepatocellular carcinoma and/or neoplastic nodules, mammary fibroadenomas, and subcutaneous fibromas are 0.047, 0.037, 0.060, 0.027, and 0.072 $(mg/kg\text{-}day)^{-1}$, respectively. Because treatment with tgDNT produced multiple types of tumors in male rats in three different tissues in the CIIT bioassay (CIIT, 1982a), the overall oral cancer slope factor for tgDNT exposure was derived based on the male incidence data for combined hepatocellular carcinoma and/or neoplastic nodules, mammary fibroadenomas, and subcutaneous fibromas by assuming that different tumor types are independent from each other. The overall tumor incidence was fit with the MS_Combo multiple tumor model (BMDS version 2.2.2; see Appendix D for details), and the estimated

$BMDL_{10}$ is 0.852 mg/kg-day. Similar to the screening subchronic and chronic p-RfDs, this $BMDL_{10}$ was further converted from an animal dose to an HED, and then used as the POD_{HED} to derive the p-OSF for tgDNT.

Table A.6. Incidences of Hepatocellular Carcinomas, Liver Neoplastic Nodules, Mammary Fibroadenomas, and Subcutaneous Fibromas in Male Rats at Week 104[a]

Parameter	Exposure Group, ADD mg/kg-d[b]		
	Control (0)	Low-dose (3.51)	High-dose (14.0)
Number examined	61	70	23
Hepatocellular carcinoma	1 (2)	9 (13)*	21 (91)*
Neoplastic nodules	9 (15)	11 (16)	15 (65)*
Hepatocellular carcinomas and/or neoplastic nodules	10 (16)	19 (27)	23 (100)*
Mammary fibroadenomas	3 (5)	7 (10)	5 (22)*
Subcutaneous fibromas	5 (8)	14 (20)	14 (61)*

[a]CIIT (1982a).
[b]Values expressed as number of animals (% of animals with lesion/effect); % calculated by EPA.

*Statistically different from controls, $p < 0.05$.

Table A.7. Goodness-of-Fit Statistics and BMD_{10} and $BMDL_{10}$ Values for Dichotomous Model for Four Types of Tumors and Combined Tumors in Male F344 Rats Exposed to tgDNT Orally for 104 Weeks[a]

Multistage-Cancer Model	Goodness-of-fit *p*-Value[b]	BMD_{10} (mg/kg-d)	$BMDL_{10}$ (mg/kg-d)	Cancer Slope Factor $(mg/kg\text{-}d)^{-1}$ (Animal Dose)
Hepatocellular carcinoma	0.6332	3.04	2.15	0.047
Liver neoplastic nodules	0.5454	4.86	2.69	0.037
Hepatocellular carcinoma and/or neoplastic nodule(s)	0.2389	2.42	1.68	0.060
Mammary fibroadenomas	0.9081	7.37	3.73	0.027
Subcutaneous fibromas	0.4138	2.01	1.38	0.072
Combined tumors	NA	1.20	0.852	

[a]CIIT (1982a).
[b]Values >0.1 meet conventional goodness-of-fit criteria.

$$
\begin{aligned}
POD_{HED} &= BMDL_{10}(mg/kg\text{-}day) \times DAF \\
&= BMDL_{10}\ (mg/kg\text{-}day) \times (BW_a^{1/4} \div BW_h^{1/4}) \\
&= BMDL_{10}\ (mg/kg\text{-}day) \times (0.333^{1/4} \div 70^{1/4}) \\
&= 0.852\ (mg/kg\text{-}day) \times 0.263 \\
&= 0.224\ mg/kg\text{-}day
\end{aligned}
$$

Note: The BW_a of 0.333 kg is the mean body weight from the low-dose male group at Week 104 (see Table B.7).

$$
\begin{aligned}
\textbf{Screening p-OSF}_{\textbf{Human}} &= BMR \div BMDL_{10HED}\ (mg/kg\text{-}day) \\
&= 0.1 \div 0.224 \\
&= \mathbf{4.5 \times 10^{-1}\ (mg/kg\text{-}day)^{-1}}
\end{aligned}
$$

The p-OSF is 4.5×10^{-1} $(mg/kg\text{-}day)^{-1}$ based on combined tumor incidences in male rats from CIIT (1982a).

APPENDIX B. DATA TABLES

Table B.1. Average Body Weights (g) of Male and Female F344 Rats After Dietary Exposure to tgDNT for 4 Weeks[a]

Number of animals/group	10	10	10	10
Males				
Time period	**Exposure Group (ADD, mg/kg-d)[b]**			
	0 (Control)	**31.9**	**61.9**	**134**
Initial	176.5 ± 11.0	177.9 ± 11.1 (101)	177.1 ± 10.8 (100)	177.9 ± 11.7 (101)
Week 1	207.1 ± 7.4	205.9 ± 10.4 (99)	201.8 ± 9.4 (97)	186.6 ± 10.5* (90)
Week 2	228.0 ± 8.4	221.4 ± 11.8 (97)	207.8 ± 9.8* (91)	167.6 ± 10.7* (74)
Week 3	236.7 ± 9.6	231.4 ± 13.0 (98)	209.6 ± 10.1* (89)	152.2 ± 10.2* (64)
Week 4	251.8 ± 13.2	236.9 ± 13.2* (94)	208.8 ± 11.9* (83)	155.8 ± 10.9* (62)
Females				
Time period	**Exposure Group (ADD, mg/kg-d)[b]**			
	0 (Control)	**32.0**	**63.6**	**120**
Initial	125.8 ± 3.2	127.7 ± 3.4 (102)	128.4 ± 3.7 (102)	126.3 ± 6.7 (100)
Week 1	136.2 ± 2.7	138.0 ± 5.5 (101)	137.2 ± 4.3 (101)	139.1 ± 7.5 (102)
Week 2	146.0 ± 3.2	147.3 ± 5.8 (101)	142.3 ± 4.8 (97)	135.6 ± 9.5* (93)
Week 3	153.8 ± 6.0	151.9 ± 7.7 (99)	149.4 ± 6.6 (97)	127.7 ± 11.0* (83)
Week 4	158.4 ± 4.3	155.1 ± 7.5 (98)	149.4 ± 7.4* (94)	124.5 ± 10.1* (79)

[a]CIIT (1983).
[b]Values expressed as mean ± SD (% of control); % calculated by EPA.

*Statistically different from controls, $p < 0.05$.

Table B.2. Hematology Results for Male and Female F344 Rats After Dietary Exposure to tgDNT for 4 Weeks[a]

Number of animals/group	10	10	10	10
Males				
Parameter	**Exposure Group (ADD, mg/kg-d)[b]**			
	0 (Control)	**31.9**	**61.9**	**134**
MetHb (%)	1.12 ± 0.61	1.20 ± 0.40 (107)	1.38 ± 0.71 (123)	2.63 ± 1.03* (235)
Reticulocytes (%)	1.11 ± 0.31	1.99 ± 0.82* (179)	2.81 ± 1.55* (253)	6.84 ± 3.05* (616)
Heinz bodies (%)	0.00 ± 0.00	0.04 ± 0.05*	0.06 ± 0.07*	5.25 ± 3.28*
Females				
	Exposure Group (ADD, mg/kg-d)[b]			
	0 (Control)	**32.0**	**63.6**	**120**
MetHb (%)	0.57 ± 0.48	1.46 ± 0.76* (256)	1.04 ± 0.77 (182)	1.99 ± 0.88* (349)
Reticulocytes (%)	1.45 ± 0.27	3.27 ± 0.94* (226)	2.95 ± 0.81* (203)	4.05 ± 0.91* (279)
Heinz bodies (%)[c]	0.00 ± 0.00	0.07 ± 0.07*	0.09 ± 0.06*	0.14 ± 0.11*

[a]CIIT (1983).
[b]Values expressed as mean ± SD (% of control); % calculated by EPA.

*Statistically different from controls, $p < 0.05$.

Table B.3. Gross Pathology for Male and Female F344 Rats After Dietary Exposure to tgDNT for 4 Weeks[a]

Number of animals/group	10	10	10	10
Parameter	**Exposure Group (ADD, mg/kg-d)[b]**			
	0 (Control)	**31.9**	**61.9**	**134**
Males				
Mottled lungs (dark-red or brown areas)	0	6 (60)	10 (100)	3 (30)
Liver				
Surface rough or granular	0	5 (50)	10 (100)	0 (0)
Mottled appearance	0	5 (50)	0 (0)	3 (30)
Yellowish tinge	0	3 (30)	0 (0)	6 (60)
Spleen				
Dark or black	0	1 (10)	0 (0)	10 (100)
Surface rough or granular	0	1 (10)	0 (0)	0 (0)
Thickened	0	0 (0)	0 (0)	3 (30)
Enlarged	0	0 (0)	0 (0)	1 (10)
Kidneys				
Greenish tinge	0	0 (0)	0 (0)	3 (30)
Dark zone at cortico-medullary junction	0	1(10)	0 (0)	1 (10)
Small white raised nodule on ventral surface	0	0 (0)	0 (0)	1 (10)
Parameter	**Exposure Group (ADD, mg/kg-d)[b]**			
	0 (Control)	**32.0**	**63.6**	**120**
Females				
Mottled lungs	0	0 (0)	0 (0)	1 (10)
Liver				
Yellowish tinge	0	0 (0)	0 (0)	4 (40)
Spleen				
Dark or black	0	0 (0)	0 (0)	5 (50)
Thickened	0	0 (0)	0 (0)	7 (70)
Enlarged	0	0 (0)	0 (0)	2 (20)
Kidneys				
Greenish tinge	0	0 (0)	0 (0)	1 (10)
Water cyst in ovary	0	0 (0)	1 (10)	0 (0)
Dark yellow stains around vagina	0	0 (0)	0 (0)	2 (20)

[a]CIIT (1983).
[b]Values expressed as number of animals affected (% affected); % calculated by EPA.

Table B.4. Selected Average Body Weights (g) and Food Consumption (g/week/rat) of Male and Female F344 Rats After Dietary Exposure to tgDNT for up to 2 Years[a]

Time Period	Exposure Group (ADD, mg/kg-d)			
	Control (0)	**Low-dose (3.51)**	**Mid-dose (14.0)**	**High-dose (34.9)**
Average body weight[b] (Males)				
Week 0 (*n* = 130)	154 ± 14.5	153 ± 14.2 (99)	151 ± 14.2 (98)	153 ± 14.6 (99)
Week 26 (*n* = 130)	351 ± 22	338 ± 21.6 (96)	301 ± 19.2 (86)	265 ± 17.6 (75)
Week 50 (*n* = 113−119)[c]	387 ± 23.9	370 ± 20.3 (96)	316 ± 18.3 (82)	270 ± 18 (70)
Week 78 (*n* = 95−106)	396 ± 22.3	373 ± 21.7 (94)	309± 25.8 (78)	NA
Week 102 (*n* = 25−75)	370 ± 31	351 ± 30.8 (95)	293 ± 39.3 (80)	NA
Average food consumption (Males) (g/week/rat)				
Week 0 (*n* = 130)	0	0	0	0
Week 26 (*n* = 130)	108	111(103)	101 (94)	101 (94)
Week 50 (*n* = 113−119)[c]	112	109 (97)	110 (98)	114 (102)
Week 78 (*n* = 95−106)	107	107 (100)	106 (99)	NA
Week 102 (*n* = 25−75)	105	104 (99)	92 (88)	NA
Time Period	**Exposure Group (ADD, mg/kg-d)**			
	Control (0)	**Low-dose (3.46)**	**Mid-dose (14.0)**	**High-dose (35.1)**
Average body weight [b] (Females)				
Week 0 (*n* = 130)	118 ± 8.4	121 ± 8.2 (103)	121 ± 7.7 (103)	118 ± 8.4 (100)
Week 26 (*n* = 130)	198 ± 10.7	193 ± 11.6 (97)	187 ± 11.2 (94)	172 ± 11.2 (87)
Week 50 (*n* = 120)[c]	226 ± 14.5	220 ± 14.9 (97)	197 ± 13.5 (90)	180 ± 11.1 (80)
Week 78 (*n* = 104−107)	272 ± 20	251 ± 24.1 (92)	213 ± 12.8 (78)	NA
Week 104 (*n* = 61−69)	288 ± 29	267 ± 25.1 (93)	213 ± 18.8 (74)	NA
Average food consumption (Females) (g/week/rat)				
Week 0 (*n* = 130)	0	0	0	0
Week 26 (*n* = 130)	79	78 (99)	73 (92)	73 (92)
Week 50 (*n* = 120)[c]	81	79 (98)	72 (89)	71 (88)
Week 78 (*n* = 104−107)	87	85 (98)	80 (92)	NA
Week 104 (*n* = 61−69)	88	89 (101)	91(103)	NA

[a]CIIT (1982a).
[b]Body-weight values expressed as mean ± SD (% of control); % calculated by EPA.
[c]Body weight and food consumption data were collected on Week 50, not Week 52.

Table B.5. Selected Hematology Results for Male F344 Rats After Dietary Exposure to tgDNT for up to 2 Years[a]

Parameter	Exposure Group (ADD, mg/kg-d)[b]			
	Control (0)	**Low-dose (3.47)**	**Mid-dose (13.6)**	**High-dose (34.6)**
Week 26 (*n* = 10)				
Hb (g/dL)	17.12 ± 0.42	17.36 ± 0.48 (101)	17.26 ± 1.12 (101)	16.48 ± 0.44 (96)
Hematocrit (100%)	48.70 ± 2.21	50.00 ± 2.71 (103)	52.05 ± 4.37 (107)	49.30 ± 2.21 (101)
RBC ($\times 10^6$/mm^3)	8.967 ± 0.554	9.209 ± 0.428 (103)	8.921 ± 0.525 (99)	8.272 ± 0.392* (92)
Reticulocyte (%)	1.97 ± 0.80	2.23 ± 0.70 (113)	2.83 ± 0.85 (144)	3.60 ± 0.84* (183)
MetHb (%)	0.36 ± 0.36	0.97 ± 0.71 (269)	0.74 ± 0.55 (205)	1.80 ± 1.18* (500)
WBC ($\times 10^3$/mm^3)	12.31 ± 1.89	14.60 ± 3.15 (119)	16.13 ± 2.11* (131)	15.04 ± 2.73 (122)
Parameter	**Exposure Group (ADD, mg/kg-d)[b]**			
	Control (0)	**Low-dose (3.47)**	**Mid-dose (13.9)**	**High-dose (34.9)**
Week 52 (*n* = 10)				
Hb (g/dL)	16.05 ± 0.59	16.11 ± 0.61 (100)	16.29 ± 0.66 (101)	14.07 ± 1.16* (88)
Hematocrit (100%)	46.70 ± 1.70	46.45 ± 1.57 (99)	46.90 ± 1.91 (100)	47.30 ± 2.89 (101)
RBC ($\times 10^6$/mm^3)	8.507 ± 0.778	8.572 ± 0.570 (101)	7.585 ± 0.647* (89)	5.546 ± 0.728* (65)
Reticulocyte (%)	2.08 ± 0.73	2.42 ± 0.79 (116)	2.01 ± 0.74 (97)	3.87 ± 1.90* (186)
MetHb (%)	0.87 ± 0.56	1.37 ± 0.61 (157)	1.49 ± 0.96 (171)	1.55 ± 0.92 (178)
WBC ($\times 10^3$/mm^3)	13.29 ± 3.23	11.60 ± 4.13 (87)	14.45 ± 3.60 (109)	19.30 ± 3.47* (145)
Parameter	**Exposure Group (ADD, mg/kg-d)[b]**			
	NA	**NA**	**NA**	**High-dose (34.9)**
Week 55 (*n* = 10)				
Hb (g/dL)	NA	NA	NA	12.82 ± 0.78
Hematocrit (100%)	NA	NA	NA	38.37 ± 2.08
RBC ($\times 10^6$/mm^3)	NA	NA	NA	4.129 ± 1.245
Reticulocyte (%)	NA	NA	NA	1.61 ± 0.77
MetHb (%)	NA	NA	NA	1.42 ± 1.33
WBC ($\times 10^3$/mm^3)	NA	NA	NA	4.33 ± 0.69

Table B.5. Selected Hematology Results for Male F344 Rats After Dietary Exposure to tgDNT for up to 2 Years[a]

Parameter	Exposure Group (ADD, mg/kg-d)[b]			
	Control (0)	**Low-dose (3.49)**	**Mid-dose (14.0)**	**NA**
Week 78 (*n* = 20)				
Hb (g/dL)	16.81 ± 1.60	18.25 ± 2.16 (109)	15.34 ± 2.79 (91)	NA
Hematocrit (100%)	51.37 ± 3.55	55.60 ± 5.58* (108)	44.80 ± 7.11* (87)	NA
RBC (× 10^6/mm^3)	9.303 ± 0.897	9.608 ± 0.859 (103)	7.998 ± 1.328* (86)	NA
Reticulocyte (%)	2.02 ± 1.04	2.28 ± 1.25* (113)	4.29 ± 2.64* (212)	NA
MetHb (%)	1.77 ± 1.16	1.56 ± 0.46 (88)	1.31 ± 1.01(74)	NA
WBC (× 10^3/mm^3)	10.36 ± 2.26	9.71 ± 2.86 (94)	10.76 ± 3.89 (104)	NA
Parameter	**Exposure Group (ADD, mg/kg-d)[b]**			
	Control (0)	**Low-dose (3.51)**	**Mid-dose (14.0)**	**NA**
Week 104 (*n* = 19−20)				
Hb (g/dL)	18.48 ± 3.13	19.40 ± 2.86 (105)	12.08 ± 2.72* (65)	NA
Hematocrit (100%)	54.60 ± 9.38	56.42 ± 8.09 (103)	36.95 ± 7.21* (68)	NA
RBC (× 10^6/mm^3)	9.227 ± 1.602	9.543 ± 1.112 (103)	6.819 ± 1.807* (74)	NA
Reticulocyte (%)	3.53 ± 3.53	2.99 ± 1.89 (85)	6.72 ± 2.93* (190)	NA
MetHb (%)	1.16 ± 1.01	1.32 ± 0.99 (114)	1.46 ± 0.86 (126)	NA
WBC (× 10^3/mm^3)	11.22 ± 4.06	12.85 ± 3.62 (115)	9.35 ± 10.16 (83)	NA

[a]CIIT (1982a).
[b]Values expressed as mean ± SD (% of control); % calculated by EPA.

*Statistically different from controls, $p < 0.05$.

Table B.6. Selected Clinical Chemistry Results for Male and Female F344 Rats After Dietary Exposure to tgDNT for up to 2 Years[a]

Males				
Parameter	**Exposure Group (ADD, mg/kg-d)[b]**			
Week 26 (*n* = 10)	**Control (0)**	**Low-dose (3.47)**	**Mid-dose (13.6)**	**High-dose (34.6)**
SGPT (IU/L)	43.0 ± 13.6	24.7 ± 9.3* (57)	25.8 ± 4.9* (60)	39.1 ± 8.3 (91)
ALP (IU/L)	77.60 ± 7.90	67.70 ± 7.24* (87)	63.10 ± 3.90* (81)	78.56 ± 6.17 (101)
BUN (mg/dL)	21.03 ± 2.09	17.98 ± 1.37* (85)	18.66 ± 1.55 (89)	24.56 ± 2.63* (117)
Week 52 (*n* = 10)	**0 (Control)**	**Low-dose (3.47)**	**Mid-dose (13.9)**	**High-dose (34.9)**
SGPT (IU/L)	41.5 ± 6.5	22.2 ± 5.8 (53)	25.1 ± 5.7 (60)	138.7 ± 72.4* (334)
ALP (IU/L)	68.70 ± 10.95	53.50 ± 4.25 (78)	57.60 ± 8.67 (84)	134.20 ± 65.45* (195)
BUN (mg/dL)	18.00 ± 1.08	17.30 ± 0.96 (96)	16.77 ± 1.87 (93)	29.91 ± 5.83* (166)
Week 55 (*n* = 10)	**NA**	**NA**	**NA**	**High-dose (34.9)**
SGPT (IU/L)	NA	NA	NA	136.8 ± 96.1
ALP (IU/L)	NA	NA	NA	161.55 ± 66.89
BUN (mg/dL)	NA	NA	NA	28.92 ± 3.37
Week 78 (*n* = 20)	**Control (0)**	**Low-dose (3.49)**	**Mid-dose (14.0)**	**NA**
SGPT (IU/L)	28.7 ± 6.5	24.1 ± 16.2 (84)	55.3 ± 56.3* (193)	NA
ALP (IU/L)	74.80 ± 10.35	64.95 ± 29.20 (87)	79.95 ± 47.52 (107)	NA
BUN (mg/dL)	21.55 ± 1.73	17.09 ± 2.22* (79)	25.14 ± 6.52 (117)	NA
Week 104 (*n* = 19–20)	**0**	**Low-dose (3.51)**	**Mid-dose (14.0)**	**NA**
SGPT (IU/L)	22.5 ± 4.6	21.7 ± 5.9 (96)	78.7 ± 42.7* (350)	NA
ALP (IU/L)	65.10 ± 11.54	45.05 ± 9.59* (69)	89.58 ± 51.09 (138)	NA
BUN (mg/dL)	27.54 ± 7.53	19.33 ± 3.08* (70)	77.49 ± 47.03* (281)	NA
Females				
Parameter	**Exposure Group (ADD, mg/kg-d)[b]**			
Week 26 (*n* = 10)	**Control (0)**	**Low-dose (3.22)**	**Mid-dose (13.9)**	**High-dose (34.9)**
BUN (mg/dL)	18.82 ± 1.43	19.20 ± 3.04 (102)	18.90 ± 1.49 (100)	23.88 ± 4.31*(127)

[a]CIIT (1982a).
[b]Values expressed as mean ± SD (% of control); % calculated by EPA.

*Statistically different from controls, $p < 0.05$.

Table B.7. Terminal Body Weights (g) of Male and Female F344 Rats After Dietary Exposure to tgDNT for 2 Years[a]

Average body weight—interim and terminal sacrifices (males)				
Time Period	**Exposure Group (ADD, mg/kg-d)[b]**			
Week 26 (*n* = 10)	**Control (0)**	**Low-dose (3.47)**	**Mid-dose (13.6)**	**High-dose (34.6)**
	328 ± 23	311 ± 14* (95)	297 ± 31* (91)	253 ± 13* (77)
Week 52 (*n* = 10)	**Control (0)**	**Low-dose (3.47)**	**Mid-dose (13.9)**	**High-dose (34.9)**
	363 ± 22	335 ± 21 (92)	287 ± 21* (79)	245 ± 14* (67)
Week 55 (*n* = 101)	**NA**	**NA**	**NA**	**High-dose (34.9)**
	NA	NA	NA	259 ± 18
Week 78 (*n* = 20)	**Control (0)**	**Low-dose (3.49)**	**Mid-dose (14.0)**	**NA**
	374.30 ± 16.936	358.20 ± 15.531* (96)	286.00 ± 20.261* (76)	NA
Week 104 (*n* = 58, 68, and 19 for 0, 3.5, and 14 mg/kg-d, respectively)	**0**	**Low-dose (3.51)**	**Mid-dose (14.0)**	**NA**
	352.03 ± 28.836	333.19 ± 20.806* (95)	257.79 ± 30.608* (73)	NA
Average body weight—interim and terminal sacrifices (females)				
Time Period	**Exposure Group (ADD, mg/kg-d)[b]**			
Week 26 (*n* = 10)	**Control (0)**	**Low-dose (3.22)**	**Mid-dose (14.0)**	**High-dose (34.9)**
	184 ± 8	182 ± 12* (99)	182 ± 13* (99)	158 ± 6* (86)
Week 52 (*n* = 10)	**Control (0)**	**Low-dose (3.46)**	**Mid-dose (13.9)**	**High-dose (35.1)**
	214 ± 14	207 ± 15 (97)	190 ± 12* (89)	169 ± 11* (79)
Week 55 (*n* = 109)	**NA**	**NA**	**NA**	**High-dose (35.1)**
	NA	NA	NA	170 ± 13
Week 78 (*n* = 20)	**Control (0)**	**Low-dose (3.45)**	**Mid-dose (14.0)**	**NA**
	259.65 ± 14.651	238.80 ± 11.551* (92)	201.35 ± 11.815* (78)	NA
Week 104 (*n* = 55, 59, and 59 for 0, 3.5, and 14 mg/kg-d, respectively)	**Control (0)**	**Low-dose (3.46)**	**Mid-dose (14.0)**	**NA**
	271.88 ± 28.15	256.22 ± 22.627* (94)	198.84 ± 19.27* (73)	NA

[a]CIIT (1982a).
[b]Values expressed as mean ± SD (% of control); % calculated by EPA.

*Statistically different from controls, $p < 0.05$.

Table B.8. Selected Organ Weights (g) of Male F344 Rats After Dietary Exposure to tgDNT for 2 Years[a]

Time Period	Exposure Group (ADD, mg/kg-d)[b]			
Week 26 (*n* = 10)	**Control (0)**	**Low-dose (3.47)**	**Mid-dose (13.6)**	**High-dose (34.6)**
Absolute liver weight	8.78 ± 0.87	8.80 ± 0.56 (100)	9.87 ± 0.80* (112)	13.19 ± 1.01* (150)
Relative liver weight	2.68 ± 0.23	2.83 ± 0.07 (105)	3.35 ± 0.36* (125)	5.23 ± 0.48* (195)
Absolute brain weight	1.97 ± 0.05	1.96 ± 0.06 (99)	1.95 ± 0.05 (99)	1.95 ± 0.04 (99)
Relative heart weight	0.32 ± 0.03	0.32 ± 0.03 (100)	0.30 ± 0.03 (94)	0.37 ± 0.05* (116)
Relative kidney weight	0.685 ± 0.04	0.687 ± 0.025 (100)	0.736 ± 0.066 (107)	0.942 ± 0.044* (138)
Relative lung weight	0.415 ± 0.049	0.407 ± 0.022 (98)	0.416 ± 0.045 (100)	0.496 ± 0.031* (120)
Relative testis weight	1.32 ± 0.11	1. 35 ± 0.07 (102)	1.34 ± 0.08 (102)	1.67 ± 0.18* (127)
Week 52 (*n* = 10)	**Control (0)**	**Low-dose (3.47)**	**Mid-dose (13.9)**	**High-dose (34.9)**
Absolute liver weight	9.11 ± 0.66	10.15 ± 0.54 (111)	15.46 ± 1.23* (170)	28.73 ± 7.86* (315)
Relative liver weight	2.51 ± 0.12	3.00 ± 0.20 (120)	5.41 ± 0.54* (216)	11.81 ± 3.54* (471)
Absolute brain weight	2.13 ± 0.16	2.01 ± 0.08 (94)	1.99 ± 0.07 (93)	2.00 ± 0.13 (94)
Relative heart weight	0.296 ± 0.02	0.335 ± 0.05 (113)	0.355 ± 0.035 (120)	0.455 ± 0.25* (154)
Relative kidney weight	0.704 ± 0.083	0.718 ± 0.066 (102)	0.909 ± 0.035* (129)	1.160 ± 0.190* (165)
Relative lung weight	0.417 ± 0.055	0.400 ± 0.054 (96)	0.476 ± 0.060 (114)	0.591 ± 0.130* (142)
Relative testis weight	1.42 ± 0.21	1.46 ± 0.19 (103)	1.71 ± 0.10 (120)	1.59 ± 0.40 (112)
Week 55 (*n* = 101)	**NA**	**NA**	**NA**	**High-dose (34.9)**
Absolute liver weight	NA	NA	NA	29.88 ± 5.77
Relative liver weight	NA	NA	NA	11.65 ± 2.45
Absolute brain weight	NA	NA	NA	1.97 ± 0.08
Relative heart weight	NA	NA	NA	0.39 ± 0.04
Relative kidney weight	NA	NA	NA	1.15 ± 0.28
Relative lung weight	NA	NA	NA	0.620 ± 0.471
Relative testis weight	NA	NA	NA	1.48 ± 0.45
Week 78 (*n* = 20)	**Control (0)**	**Low-dose (3.49)**	**Mid-dose (14.0)**	**NA**
Absolute liver weight	9.422 ± 0.6001	11.363 ± 0.9594* (121)	18.580 ± 3.8101*[c] (197)	NA
Relative liver weight	2.518 ± 0.1279	3.175 ± 0.2682* (126)	6.259 ± 1.0671* (249)	NA
Absolute brain weight	2.1010 ± 0.11026	2.1180 ± 0.06661 (101)	2.0150 ± 0.12089 (96)	NA
Relative heart weight	0.290 ± 0.03	0.302 ± 0.02 (104)	0.400 ± 0.60* (138)	NA

Table B.8. Selected Organ Weights (g) of Male F344 Rats After Dietary Exposure to tgDNT for 2 Years[a]

Relative kidney weight	0.665 ± 0.0282	0.744 ± 0.0419* (112)	1.029 ± 0.1003* (155)	NA
Relative lung weight	0.365 ± 0.262	0.416 ± 0.819* (114)	0.476 ± 0.0372* (130)	NA
Relative testis weight	1.450 ± 0.3114	1.845 ± 0.2662* (127)	1.981 ± 0.3586* (137)	NA
Week 104 (*n* = various)	**Control (0)**	**Low-dose (3.51)**	**Mid-dose (14.0)**	**NA**
Absolute liver weight	10.615 ± 1.7036	12.126 ± 1.5875* (114)	17.349 ± 2.112* (163)	NA
Relative liver weight	3.024 ± 0.4842	3.644 ± 0.4394* (121)	6.901 ± 1.1686* (228)	NA
Absolute brain weight	2.0791 ± 0.08113	2.0926± 0.10566 (101)	2.0542 ± 0.10089 (99)	NA
Relative heart weight	0.321 ± 0.31	0.337 ± 0.38* (105)	0.389 ± 0.08* (121)	NA
Relative kidney weight	c	0.642 ± 0.0661	1.227 ± 0.2115*	NA
Relative lung weight	0.490 ± 0.116	0.519 ± 0.112 (106)	0.588 ± 0.170* (120)	NA
Relative testis weight	2.150 ± 0.4924	2.581 ± 0.5747* (120)	2.978 ± 1.316* (139)	NA

[a]CIIT (1982a).
[b]Values expressed as mean ± SD (% of control); % calculated by EPA.
[c]Data illegible.

*Statistically different from controls, $p < 0.05$.

Table B.9. Selected Organ Weights (g) of Female F344 Rats After Dietary Exposure to tgDNT for up to 2 Years[a]

Parameter	Exposure Group (ADD, mg/kg-d)[b]			
Week 26 (*n* = 10)	**Control (0)**	**Low-dose (3.22)**	**Mid-dose (13.9)**	**High-dose (34.9)**
Absolute liver weight	4.81 ± 0.17	4.98 ± 0.35 (103)	5.89 ± 0.44* (118)	7.26 ± 0.56* (151)
Relative liver weight	2.62 ± 0.10	2.74 ± 0.08 (105)	3.24 ± 0.11* (124)	4.60 ± 0.34* (176)
Absolute brain weight	1.85 ± 0.07	1.84 ± 0.06 (99)	1.80 ± 0.03 (97)	1.80 ± 0.04 (97)
Relative heart weight	0.36 ± 0.02	0.36 ± 0.03 (100)	0.37 ± 0.03 (103)	0.41 ± 0.04* (114)
Relative kidney weight	0.689 ± 0.017	0.725 ± 0.027 (105)	0.738 ± 0.027* (107)	0.853 ± 0.051* (124)
Relative lung weight	0.53 ± 0.04	0.50 ± 0.01 (94)	0.53 ± 0.05 (100)	0.59 ± 0.05* (111)
Relative ovary weight	0.040 ± 0.008	0.041 ± 0.007 (103)	0.043 ± 0.007 (108)	0.048 ± 0.007 (120)
Week 52 (*n* = 10)	**Control (0)**	**Low-dose (3.46)**	**Mid-dose (13.9)**	**High-dose (35.1)**
Absolute liver weight	5.74 ± 0.30	5.80 ± 0.50 (101)	7.06 ± 0.41* (123)	8.90 ± 0.61* (155)
Relative liver weight	2.69 ± 0.18	2.82 ± 0.27 (105)	3.73 ± 0.14* (139)	5.28 ± 0.48* (196)
Absolute brain weight	1.94 ± 0.16	1.85 ± 0.06 (95)	1.83 ± 0.08 (94)	1.85 ± 0.07 (95)
Relative heart weight	0.43 ± 0.10	0.34 ± 0.03* (79)	0.38 ± 0.03 (88)	0.46 ± 0.03 (107)
Relative kidney weight	0.727 ± 0.074	0.720 ± 0.047 (99)	0.762 ± 0.105 (105)	0.949 ± 0.044* (131)
Relative lung weight	0.538 ± 0.113	0.480 ± 0.050 (89)	0.500 ± 0.040 (93)	0.570 ± 0.020 (106)
Relative ovary weight	0.045 ± 0.008	0.051 ± 0.0125 (113)	0.0740 ± 0.0699 (164)	0.057 ± 0.015 (127)
Week 55 (*n* = various)	**NA**	**NA**	**NA**	**High-dose (35.1)**
Absolute liver weight	NA	NA	NA	9.92 ± 1.21
Relative liver weight	NA	NA	NA	5.87 ± 0.93
Absolute brain weight	NA	NA	NA	1.84 ± 0.11
Relative heart weight	NA	NA	NA	0.44 ± 0.04
Relative kidney weight	NA	NA	NA	0.964 ± 0.080
Relative lung weight	NA	NA	NA	0.616 ± 0.114
Relative ovary weight	NA	NA	NA	0.058 ± 0.032
Week 78 (*n* = 20 for control and low-dose, *n* = 19 for mid-dose)	**Control (0)**	**Low-dose (3.45)**	**Mid-dose (14.0)**	**NA**
Absolute liver weight	6.639 ± 0.4462	6.731 ± 0.7549 (101)	9.074 ± 0.4790* (137)	NA
Relative liver weight	2.560 ± 0.155	2.826 ± 0.334* (110)	4.543 ± 0.2875* (177)	NA
Absolute brain weight	2.1035 ± 0.15936	2.0290 ± 0.18547 (96)	1.8730 ± 0.13483* (89)	NA
Relative heart weight	0.378 ± 0.0626	0.374 ± 0.0803 (99)	0.430 ± 0.0619 (114)	NA
Relative kidney weight	0.718 ± 0.0741	0.731 ± 0.0906 (102)	0.951 ± 0.2626* (132)	NA

Table B.9. Selected Organ Weights (g) of Female F344 Rats After Dietary Exposure to tgDNT for up to 2 Years[a]

Parameter	Exposure Group (ADD, mg/kg-d)[b]			
Relative lung weight	0.475 ± 0.0598	0.491 ± 0.0798 (103)	0.508 ± 0.826 (107)	NA
Relative ovary weight	0.0852 ± 0.17849	0.1293 ± 0.17126 (152)	0.0573 ± 0.01170 (67)	NA
Week 104 (*n* = various)	**Control (0)**	**Low-dose (3.46)**	**Mid-dose (14.0)**	**NA**
Absolute liver weight	7.462 ± 1.0733	8.702 ± 1.1791* (117)	12.309 ± 1.7879* (165)	NA
Relative liver weight	2.783 ± [c]	3.486 ± 0.4438 * (125)	6.159 ± 0.8252* (221)	NA
Absolute brain weight	1.9211 ± 0.18071	1.9129 ± 0.08483 (100)	1.9085 ± 0.11577 (99)	NA
Relative heart weight	0.342 ± 0.0512	0.373 ± 0.0456* (109)	0.449 ± 0.0501* (131)	NA
Relative kidney weight	0.732 ± 0.0971	0.793 ± 0.0732* (108)	1.157 ± 0.1445* (128)	NA
Relative lung weight	0.496 ± 0.1963	0.488 ± 0.0934 (98)	0.647 ± 0.3783*[c] (130)	NA
Relative ovary weight	0.0392 ± 0.01892[c]	0.0460 ± 0.01491* (117)	0.0823 ± 0.1136* (210)	NA

[a]CIIT (1982a).
[b]Values expressed as mean ± SD (% of control); % calculated by EPA.
[c]Data were illegible.

*Statistically different from controls, $p < 0.05$.

Table B.10. Selected Hepatotoxicity Incidences for Male and Female F344 Rats After Dietary Exposure to tgDNT for 26 Weeks[a]

Parameter	Exposure Group (ADD, mg/kg-d)[b]			
Males (10/group)	**Control (0)**	**Low-dose (3.47)**	**Mid-dose (13.6)**	**High-dose (34.6)**
Fatty metamorphosis	0	4 (40)	4 (40)	0
Hepatocyte necrosis	0	0	7 (70)*	9 (90)*
Megalocytosis	0	0	0	4 (40)
Periportal hyperbasophilic hepatocytes	0	0	8 (80)*	0
Vacuolation	0	1 (10)	0	4 (40)
Parameter	**Exposure Group (ADD, mg/kg-d)[b]**			
Females (10/group)	**Control (0)**	**Low-dose (3.22)**	**Mid-dose (13.9)**	**High-dose (34.9)**
Fatty metamorphosis	0	0	0	0
Hepatocyte necrosis	0	0	2 (20)	4 (40)
Megalocytosis	0	0	0	0
Periportal hyperbasophilic hepatocytes	1 (10)	2 (20)	6 (60)	10 (100)*
Vacuolation	0		0	2 (20)

[a]CIIT (1982a).
[b]Values expressed as number of animals with lesions (% of animals with lesion/effect); % calculated by EPA.

*Statistically different from controls, $p < 0.05$.

Table B.11. Selected Hepatotoxicity Incidences for Male and Female F344 Rats After Dietary Exposure to tgDNT for 52 Weeks[a]

Parameter	Exposure Group (ADD, mg/kg-d)[b]			
Males (10/group)	**Control (0)**	**Low-dose (3.47)**	**Mid-dose (13.9)**	**High-dose (34.9)**
Hepatocyte necrosis	0	7 (70)*	9 (90)*	5 (50)*
Megalocytosis	0	1 (10)	7 (70)*	3 (30)
Periportal hyperbasophilic hepatocytes	0	2 (20)	9 (90)*	6 (60)*
Vacuolation	0	7 (70)*	7 (70)*	6 (60)*
Parameter	**Exposure Group (ADD, mg/kg-d)[b]**			
Females (10/group)	**Control (0)**	**Low-dose (3.46)**	**Mid-dose (13.9)**	**High-dose (35.1)**
Hepatocyte necrosis	0	0	2 (20)*	7 (70)*
Megalocytosis	0	0	2 (20)	4 (40)
Periportal hyperbasophilic hepatocytes	0	1 (10)	6 (60)*	5 (50)*
Vacuolation	0	0	0	1 (10)

[a]CIIT (1982a).
[b]Values expressed as number of animals with lesions (% of animals with lesion/effect); % calculated by EPA.

*Statistically different from controls, $p < 0.05$.

Table B.12. Selected Hepatotoxicity Incidences at Week 78[a]

Fatty Metamorphosis Incidence			
Males (20/group)	**Exposure Group (ADD, mg/kg-d)[b]**		
Grading of Finding	**Control (0)**	**Low-dose (3.49)**	**Mid-dose (14.0)**
Minimal	0	7 (35)	1 (5)
Slight	0	6 (30)	13 (65)
Moderate	0	0	3 (15)
Moderately severe	0	0	0
Females (20/group)	**Exposure Group (ADD, mg/kg-d)[b]**		
Grading of Finding	**Control (0)**	**Low-dose (3.45)**	**Mid-dose (14.0)**
Minimal	0	0	5 (25)
Slight	0	1 (5)	11 (55)
Moderate	0	0	0
Moderately severe	0	0	1 (5)
Other Hepatotoxicity Incidence			
Males (20/group)	**Exposure Group (ADD, mg/kg-d)[b]**		
Grading of Finding	**Control (0)**	**Low-dose (3.49)**	**Mid-dose (14.0)**
Cystic degeneration	0	0	8 (40)*
Necrosis of individual hepatocytes	0	7 (35)*	12 (60)*
Females (20/group)	**Exposure Group (ADD, mg/kg-d)[b]**		
Grading of Finding	**Control (0)**	**Low-dose (3.45)**	**Mid-dose (14.0)**
Necrosis of individual hepatocytes	0	3 (15)	20 (100)*
Megalocytosis of hepatocytes	0	0	2 (10)

[a]CIIT (1982a).
[b]Values expressed as number of animals with lesions, (% of animals with lesion/effect), % calculated by EPA.

*Statistically different from controls, $p < 0.05$.

Table B.13. Selected Hepatotoxicity Incidences at Week 104[a]

Fatty Metamorphosis Incidence			
Males	**Exposure Group (ADD, mg/kg-d)[b]**		
Grading of Finding	**Control (0) (*n* = 61)**	**Low-dose (3.51) (*n* = 70)**	**Mid-dose (14.0) (*n* = 23)**
Minimal	8 (13)	17 (24)	3 (13)
Slight	1 (2)	33 (47)	5 (22)
Moderate	0 (0)	1 (1)	5 (22)
Moderately severe	0 (0)	0 (0)	0 (0)
Females	**Exposure Group (ADD, mg/kg-d)[b]**		
Grading of Finding	**Control (0) (*n* = 57)**	**Low-dose (3.46) (*n* = 61)**	**Mid-dose (14.0) (*n* = 68)**
Minimal	2 (4)	6 (10)	1 (1)
Slight	3 (5)	6 (10)	24 (35)
Moderate	2 (4)	1 (2)	38 (56)
Moderately severe	0 (0)	0 (0)	2 (3)
Other Hepatotoxicity Incidence			
Males	**Exposure Group (ADD, mg/kg-d)[b]**		
Grading of Finding	**Control (0) (*n* = 61)**	**Low-dose (3.51) (*n* = 70)**	**Mid-dose (14.0) (*n* = 23)**
Cystic degeneration	2 (3)	2 (3)	16 (70)*
Necrosis of individual hepatocytes	0 (0)	38 (54)*	11 (48)*
Megalocytosis of hepatocytes	0 (0)	34 (49)*	8 (35)*
Females	**Exposure Group (ADD, mg/kg-d)[b]**		
Grading of Finding	**Control (0) (*n* = 57)**	**Low-dose (3.46) (*n* = 61)**	**Mid-dose (14.0) (*n* = 68)**
Number examined	57	61	68
Cystic degeneration	3 (5)	0 (0)	6 (9)
Necrosis of individual hepatocytes	1 (2)	18 (30)*	22 (32)*
Megalocytosis of hepatocytes	1 (2)	22 (36)*	37 (54)*

[a]CIIT (1982a).
[b]Values expressed as number of animals with lesions, (% of animals with lesion/effect), % calculated by EPA.

*Statistically different from controls, $p < 0.05$.

Table B.14. Incidences of Hepatocellular Carcinomas in Male F344/CrlBR Rats at Weeks 26, 52, and 78[a]

Parameter	Exposure Group (ADD, mg/kg-d)[b]			
26 Week (*n* = 10)	**Control (0)**	**Low-dose (3.47 mg/kg-d)**	**Mid-dose (13.6 mg/kg-d)**	**High-dose (34.6 mg/kg-d)**
Hepatocellular carcinoma	0	0	0	2 (20)
52 Week (*n* = 10)	**Control (0)**	**Low-dose (3.47 mg/kg-d)**	**Mid-dose (13.9 mg/kg-d)**	**High-dose (34.9 mg/kg-d**
Hepatocellular carcinoma	0	0	3 (30)	10 (100)*
78 Week (*n* = 20)	**Control (0)**	**Low-dose (3.49 mg/kg-d)**	**Mid-dose (14.0 mg/kg-d)**	**NA**
Hepatocellular carcinoma	0	0	19 (95)*	NA

[a]CIIT (1982a).
[b]Values expressed as number of animals (% of animals with lesion/effect); % calculated by EPA.

*Statistically different from controls, $p < 0.05$.

Table B.15. Incidences of Hepatocellular Carcinomas, Liver Neoplastic Nodules, and Other Tumors in Male and Female F344 Rats at Week-104[a]

Parameter	Exposure Group (ADD, mg/kg-d)[b]		
Males	**Control (0)**	**Low-dose (3.51 mg/kg-d)**	**Mid-dose (14.0 mg/kg-d)**
Number examined	61	70	23
Hepatocellular carcinoma	1 (2)	9 (13)*	21 (91) *
Neoplastic nodule(s)	9 (15)	11 (16)	15 (65)*
Hepatocellular carcinoma and /or neoplastic nodule(s)	10 (16)	19 (27)	23 (100)*
Mammary fibroadenomas	3 (5)	7 (10)	5 (22)*
Subcutaneous fibromas	5 (8)	14 (20)	14 (61)*
Parameter	**Exposure Group, (ADD, mg/kg-d)[b]**		
Females	**Control (0)**	**Low-dose (3.46 mg/kg-d)**	**Mid-dose (14.0 mg/kg-d)**
Number examined	57	61	68
Hepatocellular carcinoma	0	0	40 (59)*
Neoplastic nodule(s)	5 (9)	12 (20)	53 (78)*
Hepatocellular carcinoma and /or neoplastic nodule(s)	5 (9)	12 (20)	66 (97)*
Mammary fibroadenomas	15 (26)	12 (20)	24 (35)
Subcutaneous fibromas	0	2 (3)	7 (10)*

[a]CIIT (1982a) .
[b]Values expressed as number of animals (% of animals with lesion/effect); % calculated by EPA.

*Statistically different from controls, $p < 0.05$.

Table B.16. Body and Liver Weights of Male F344 Rats After Dietary Exposure to tgDNT for 1 Year[a]

Parameter	Exposure Group (Adjusted Daily Dose, mg/kg-d)[b]	
	0 (Control)	35
26 Week		
Number of animals	4	4
Body weight (g)	395 ± 2	353 ± 8 (89)*
Liver/body weight	2.43 ± 0.07	2.82 ± 0.07 (116)*
52 Weeks		
Number of animals	20	19
Terminal body weight (g)	434 ± 3	321 ± 4 (74)*
Liver weight (g)	10.30 ± 0.16	19.49 ± 0.35 (189)*
Liver/body weight (relative liver weight)	2.38 ± 0.04	6.08 ± 0.10 (255)*

[a]Leonard et al. (1987).
[b]Values expressed as mean ± SEM (% of control); % calculated by EPA.

*Statistically different from controls, $p < 0.05$.

Table B.17. Incidence of Hepatic Neoplastic Lesions and Hepatic Metastases in Male F344 Rats After Dietary Exposure to tgDNT for 1 Year[a]

Parameter	Exposure Group (Adjusted Daily Dose, mg/kg-d)[b]	
	0 (Control)	35
Total number of animals	20	19
Neoplastic nodules	0	10 (53)*
Hepatocellular carcinoma		
Trabecular	0	9 (47)*
Adenocarcinoma	0	0
Cholangiocarcinoma	0	2 (11)

[a]Leonard et al. (1987).
[b]Values expressed as number of animals (% with lesion).

*Statistically different from controls, $p < 0.05$.

Table B.18. Distribution of Experimental Subjects Across Dose Groups and Breeding Dates in the Developmental Study of tgDNT in F344 Rats[a]

Parameter	Exposure Group (Adjusted Daily Dose, mg/kg-d)[b]						
		Technical DNT					
	Vehicle Control	14	35	37.5	75	100	150
Mortality for all treated females							
Total females treated	37	22	13	22	13	23	13
No. deaths (GDs 0−20) (%)	0 (0)	1 (4.5)	1 (7.7)	0 (0)	0 (0)	1 (4.3)	6 (46.2)
Assignment of surviving females for maternal and developmental evaluation							
First breeding	9	0	7	0	7	0	6
Second breeding	7	6	0	6	0	6	0
Third breeding	6	7	0	7	0	7	0
Total No. assigned	22 (91)	13	7	13	7	13	6
No. of Pregnancy (% pregnant[d])	20 (91)	10 (77)	7 (100)	12 (92)	6 (86)	12 (92)	5 (83)

[a]Price et al. (1985).
[b]Values expressed as number of animals (% of animals death).
[c]Number of dams scarified at GD 20.
[d]Percent of pregnant dams.

Table B.19. Hematology Parameters in F344 Dams and Fetuses on GD 20 Following Maternal Exposure to tgDNT from GDs 7–20[a]

Parameter	Exposure Group (Adjusted Daily Dose, mg/kg-d)[b]			
	Dams		Fetuses	
	Vehicle Control	tgDNT (100)	Vehicle Control	tgDNT (100)
% MetHb	3.8 ± 0.5 (16)	7.7 ± 0.6** (11)	13.4 ± 1.6 (14)	8.9 ± 1.4 (10)
% Reticulocytes	2.32 ± 0.57 (12)	6.31 ± 1.51* (11)	99.67 ± 0.06 (23)	98.98 ± 0.22* (21)
WBC ($\times 10^3/mm^3$)	5.34 ± 0.32 (5)	6.06 ± 0.64 (5)	0.93 ± 0.19 (8)	0.83 ± 0.12 (8)
RBC ($\times 10^6 mm^3$)	6.24 ± 0.20 (5)	4.88 ± 0.18* (5)	2.17 ± 0.08 (10)	2.15 ± 0.06** (9)
Hematocrit (%)	32.5 ± 1.00 (5)	26.0 ± 0.98* (5)	34.08 ± 1.24 (10)	32.17 ± 1.97 (9)
MCV (μm^3)	52.14 ± 0.29 (5)	54.54 ± 0.55* (5)	156.54 ± 0.88 (10)	160.61 ± 1.17* (9)
RDW	7.96 ± 0.24 (5)	9.68 ± 0.41* (5)	14.71 ± 0.12 (10)	14.71 ± 0.19 (9)
Platelets ($\times 10^3/mm^3$)	1063.00 ± 20.92 (4)	1625.00 ± 97.50* (5)	406.86 ± 27.90 (7)	412.2 ± 35.76 (5)

[a]Price et al. (1985).
[b]Data are presented as x ± SE. Number of individual maternal or fetal blood samples evaluated is shown in parentheses, except for fetal MetHb, which represents the number of litters evaluated after pooling individual fetal blood within each litter.

*$p < 0.05$ t-test (two-tailed).
**$p < 0.01$ t-test (two-tailed).

Table B.20. Summary of Select Organ Weights and Production Endpoints in Pregnant F344 Dams Exposed to tgDNT from GDs 7−20[a]

Parameter	Exposure Group (Adjusted Daily Dose, mg/kg-d)[b]						
		tgDNT					
	Vehicle Control	14	35	37.5	75	100	150
Number sacrificed	20	10	7	12	6	12	5
Weight gain, GDs 0−20 (g)[c]	61.83 ± 3.61†	64.94 ± 4.63 (105)	66.09 ± 6.01 (107)	55.81 ± 5.76 (90)	64.75 ± 6.78 (105)	52.67 ± 4.54 (85)	8.08 ± 20.13 (13)**
Gravid uterine weight (g)	37.70 ± 3.22	47.75 ± 2.39 (127)	39.39 ± 3.25(104)	33.64 ± 5.16 (89)	41.69 ± 5.90 (111)	37.43 ± 3.84 (99)	22.09 ± 9.82 (59)
Absolute weight gain (g)[d]	24.14 ± 2.09§§††	17.19 ± 3.88 (71)*	26.70 ± 4.31 (111)	22.17 ± 2.10 (92)	23.06 ± 2.31 (95)	15.24 ± 1.94 (63)**	14.01 ± 13.38 (58)**
Liver weight (% body weight)	4.09 ± 0.08§§††	3.91 ± 0.10 (96)*	4.12 ± 0.09 (101)	3.96 ± 0.09 (97)	4.55 ± 0.10 (111)**	4.58 ± 0.08 (112)**	4.79 ± 0.36 (117)
Spleen weight (% body weight)	0.197 ± 0.003††	0.185 ± 0.007 (94)*	0.223 ± 0.011 (113)*	0.215 ± 0.006 (109)*	0.246 ± 0.010 (125)**	0.320 ± 0.027 (162)**	0.284 ± 0.059 (144)*
% Resorptions[e]	16.8 ± 5.4	2.3 ± 1.5	4.1 ± 4.1	14.6 ± 5.2	11.0 ± 9.3	12.7 ± 5.4	46.0 ± 22.3
% Dead fetuses[e]	0.0	2.4 ± 1.6	0.0	0.0	1.3 ± 1.3	0.0	3.6 ± 3.6
% Live fetuses[e]	83.2 ± 5.4	95.4 ± 1.9	95.9 ± 4.1	85 ± 5.2	87.7 ± 9.1	87.3 ± 5.4	50.4 ± 20.6

[a]Price et al. (1985).
[b]Data are presented as $x \pm$ SE (% compared to control). Number using dam or average litter value as the experimental unit.
[c]Includes gravid uterine weight.
[d]Weight gain during gestation minus gravid uterine weight.
[e]Expressed as the percentage of total implants per dam (compared to controls).

*$p < 0.05$ Mann-Whitney *U* test (two-tailed).
**$p < 0.01$ Mann-Whitney *U* test (two-tailed).
†$p < 0.05$ Kruskal-Wallis one-way ANOVA.
††$p < 0.01$ Kruskal-Wallis one-way ANOVA.
§§$p < 0.01$ Jonckheere's test.

Table B.21. Teratological Defects in F344 Rat Fetuses Following Maternal Exposure to tgDNT or Vehicle Control on GDs 7–20[a]

Parameter	Exposure Group (Adjusted Daily Dose, mg/kg-d)[b]						
		tgDNT					
	Vehicle Control	14	35	37.5	75	100	150
Number of live litters examined	20	10	7	12	6	12	3
No. of live fetuses examined[c]	146	92	63	77	50	88	22
Gross malformations							
Anophthalmia (bilateral or right side)	0	1	2	0	0	0	0
Agnathia	0	1	0	0	0	0	0
Umbilical hernia	0	1	0	0	0	0	0
Visceral malformations							
Hydronephrosis (bilateral)	0	1	0	0	0	0	0
Skeletal malformations							
Abnormal skull fusion	4	2	0	1	0	3	0
Fused thoracic arches	0	1	0	0	0	1	0
Thoracic centra off center	0	0	0	0	0	1	0
Lumbar centra off center	0	0	0	0	0	1	0
Ribs fused to each other	0	1	0	0	0	0	0
Short rib	0	1	0	0	0	1	0
Variations	0	0	0	0	0	0	0
Hematoma (back)	0	0	0	2	0	0	0
Misaligned sternebrae	3	4	0	0	0	6	0
Doubled centra	2	1	8	1	3	1	0
Clubbed limb without bone change	2	0	0	0	0	0	0

[a]Price et al. (1985).
[b]Data are expressed as the number of fetuses exhibiting each type of defect. Thus, a single fetus may be represented more than once in this table.
[c]Only 50% of the fetuses were examined for visceral malformations and internal malformations of the head.

Table B.22. Status of Live Fetuses from F344 Rats Following Maternal Exposure to tgDNT or Vehicle Control on GDs 7–20[a]

Parameter	Exposure Group (Adjusted Daily Dose, mg/kg-d)[b]						
	Vehicle Control	tgDNT					
		14	35	37.5	75	100	150
No. litters with live fetuses	20	10	7	12	6	12	3
Live litter size	7.3 ± 0.7	9.2 ± 0.7	9.0 ± 0.9	6.4 ± 1.1	8.3 ± 1.3	7.3 ± 0.9	7.3 ± 1.7
Male/live × 100 (%)	48.8 ± 5.0	53.0 ± 6.3	46.4 ± 4.5	44.4 ± 8.0	52.7 ± 4.9	43.0 ± 5.9	57.4 ± 22.8
Body weight (g)	3.21 ± 0.05	3.39 ± 0.07 (106)	3.29 ± 0.07 (100)	3.34 ± 0.06 (104)	3.29 ± 0.13 (102)	3.17 ± 0.08 (98)	3.14 ± 0.18 (98)
Crown-rump length (cm)	3.55 ± 0.03	3.51 ± 0.07 (99)	3.57 ± 0.07 (101)	3.58 ± 0.07 (101)	3.53 ± 0.05 (99)	3.46 ± 0.07 (97)	3.53 ± 0.15 (99)
Liver weight (% body weight)	8.09 ± 0.11††	7.38 ± 0.12 (91)**	8.35 ± 0.14 (103)*	7.82 ± 0.10 (96)	8.44 ± 0.29 (104)	8.12 ± 0.08 (100)	8.50 ± 0.30 (105)
Spleen weight (% body weight)	0.097 ± 0.0005††	0.081 ± 0.008 (83)	0.131 ± 0.006 (135)**	0.084 ± 0.004 (87)	0.119 ± 0.003 (123)*	0.085 ± 0.004 (88)	0.128 ± 0.012 (132)
Placental weight (g)	0.494 ± 0.022	0.539 ± 0.054 (109)	0.440 ± 0.009 (89)	0.536 ± 0.046 (108)	0.453 ± 0.018 (92)	0.51 ± 0.028 (103)	0.458 ± 0.057 (93)

[a]Price et al. (1985).
[b]Data are presented as x ± SE. Number using dam or average litter value as the experimental unit.

*$p < 0.05$ Mann-Whitney U test (two-tailed).
**$p < 0.01$ Mann-Whitney U test (two-tailed).
††$p < 0.01$ Kruskal-Wallis one-way ANOVA.

Table B.23. Rearing Behavior in Postnatal F344 Rat Female Pups when Dams were Exposed to tgDNT from GDs 7–20[a]

End point	Exposure Group (Adjusted Daily Dose, mg/kg-d)[b]					
	tgDNT					
	0 (control)	14	35	37.5	75	100
PND 30						
Rearing behavior	20.2 ± 2.2	14.5 ± 2.4	24.6 ± 2.2	17.0 ± 0.8	16.7 ± 1.5	12.7 ± 1.8*

[a]CIIT (1982b).
[b]Data are presented as x ± SE.

*Statistically different from controls, $p < 0.05$.

APPENDIX C. BMD MODELING RESULTS

MODELING PROCEDURE FOR DICHOTOMOUS DATA

The BMD modeling of dichotomous data was conducted with EPA's BMDS (version 2.2.2). For these data, all of the dichotomous models (i.e., Gamma, Multistage, Logistic, Log-logistic, Probit, Log-probit, and Weibull) available within the software were fit using a default benchmark response (BMR) of 10% extra risk. Adequacy of model fit was judged based on the χ^2 goodness-of-fit *p*-value ($p > 0.1$), magnitude of scaled residuals in the vicinity of the BMR, and visual inspection of the model fit. Among all models providing adequate fit, the lowest BMDL was selected if the BMDLs estimated from different models varied greater than 3-fold; otherwise, the BMDL from the model with the lowest Akaike Information Criterion (AIC) was selected as a potential POD from which to derive the RfD.

In addition, in the absence of a mechanistic understanding of the biological response to a toxic agent, data from exposures much higher than the study LOAEL do not provide reliable information regarding the shape of the response at low doses. Such exposures, however, can have a strong effect on the shape of the fitted model in the low-dose region of the dose-response curve. Thus, if lack of fit is due to characteristics of the dose-response data for high doses, then the *Benchmark Dose Technical Guidance* document allows for data to be adjusted by eliminating the high-dose group (U.S. EPA, 2012). Because the focus of BMD analysis is on the low-dose region of the response curve, elimination of the high-dose group is deemed reasonable.

MODELING PROCEDURE FOR CONTINUOUS DATA

The BMD modeling of continuous data was conducted with EPA's BMDS (version 2.2.2). For these data, all continuous models available within the software were fit using a default BMR of 1 standard deviation relative risk. For liver-, body-, and kidney-weight changes, a BMR of 10% relative risk was also used. An adequate fit was judged based on the χ^2 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 homogeneous. If a homogeneous variance model was deemed appropriate based on the statistical test provided by BMDS (i.e., Test 2), the final BMD results were estimated from a homogeneous 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 nonhomogeneous variance. If this nonhomogeneous variance model did not adequately fit the data (i.e., Test 3; *p*-value < 0.1), the data set was considered unsuitable for BMD modeling. Among all models providing adequate fit, the lowest BMDL was selected if the BMDLs estimated from different models varied greater than 3-fold; otherwise, the BMDL from the model with the lowest AIC was selected as a potential POD from which to derive the RfD.

INCREASED INCIDENCE OF HEPATOCYTE NECROSIS IN MALE RATS TREATED WITH tgDNT FOR 26 WEEKS

The procedure outlined above was applied to the data for increased hepatocyte necrosis (see Table C.1) in male rats exposed to tgDNT via diet (CIIT, 1982a) for 26 weeks. Table C.2 summarizes the BMD modeling results. All the models fit except the Logistic and Probit models. Among the fitting models, the LogLogistic model has the lowest AIC. Thus, the

$BMDL_{10}$ of 2.16 mg/kg-day from this model is selected for this end point (see Figure C.1 and the BMD text output for details).

Table C.1. Hepatocyte Necrosis in Male F344 Rats After Dietary Exposure to tgDNT for 26 Weeks[a]

	Control	Low-dose (3.47 mg/kg-d)	Mid-dose (13.6 mg/kg-d)	High-dose (34.6 mg/kg-d)
Sample size	10	10	10	10
Incidence	0	0	7	9

[a]CIIT (1982a).

Table C.2. BMD Modeling Results on Hepatocyte Necrosis in Male F344 Rats After Dietary Exposure to tgDNT for 26 Weeks

Model Name	AIC	*p*-value	BMD	$BMDL_{10}$	Scaled Residual of Interest
Gamma	25.6203	0.2905	4.54802	1.36	−0.794
Logistic	30.1827	0.0174	5.63874	3.47	−1.058
LogLogistic	**24.262**	**0.5178**	**5.17793**	**2.16**	−0.61
LogProbit	24.2957	0.5089	5.07807	2.32	−0.569
Multistage	27.0948	0.1987	2.96349	1.14	−1.159
Probit	30.5661	0.029	5.67662	3.59	−1.058
Weibull	26.3218	0.2588	3.60128	1.23	−1.024

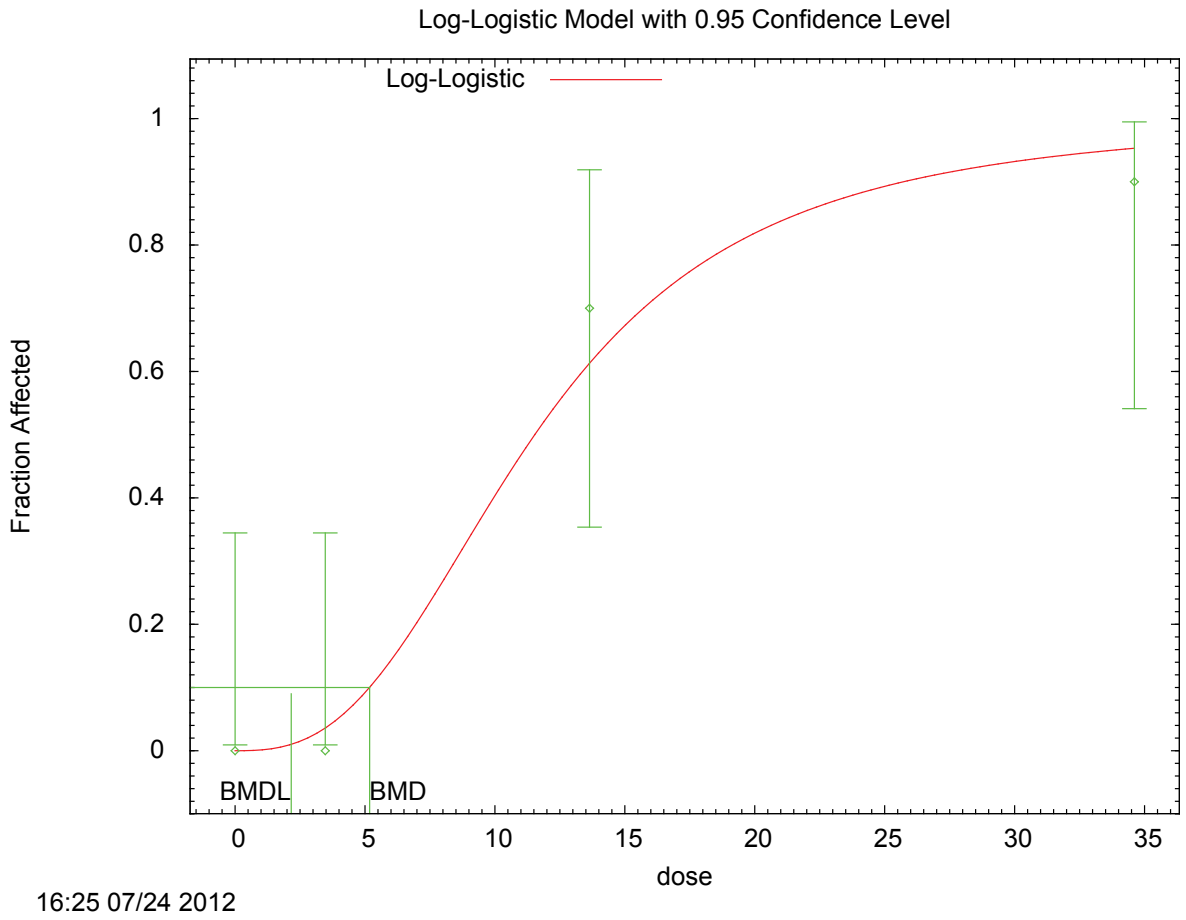

Figure C.1. LogLogistic Model for Increased Hepatocyte Necrosis in Male F344 Rats After Dietary Exposure to tgDNT for 26 Weeks

Text Output for LogLogistic Model for Increased Hepatocyte Necrosis in Male Rats After Dietary Exposure to tgDNT for 26 weeks (CIIT, 1982a)

```
 ================================================================================
        Logistic Model
 ================================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

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


   Dependent variable = Effect
   Independent variable = Dose
   Slope parameter is restricted as slope >= 1

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

User has chosen the log transformed model

Default Initial Parameter Values
background = 0
intercept = -5.72644
slope = 2.32112

Asymptotic Correlation Matrix of Parameter Estimates

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

	intercept	slope
intercept	1	-0.98
slope	-0.98	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
background	0	*	*	*
intercept	-6.69767	*	*	*
slope	2.73682	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-9.35947	4			
Fitted model	-10.131	2	1.54303	2	0.4623
Reduced model	-26.9205	1	35.122	3	<.0001

AIC: 24.262

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	10	0.000
3.4700	0.0358	0.358	0.000	10	-0.610
13.6400	0.6115	6.115	7.000	10	0.574
34.6100	0.9527	9.527	9.000	10	-0.784

Chi^2 = 1.32 d.f. = 2 P-value = 0.5178

Benchmark Dose Computation

```
Specified effect =            0.1
Risk Type        =      Extra risk
Confidence level =           0.95
             BMD =        5.17793
            BMDL =        2.16115
```

INCREASED INCIDENCE OF HEPATOCYTE NECROSIS IN MALES TREATED WITH tgDNT FOR 104 WEEKS (CIIT, 1982a)

The procedure outlined above was applied to the data for increased hepatocyte necrosis (see Table C.3) in male rats exposed to tgDNT via diet (CIIT, 1982a) for 104 weeks. Table C.4 summarizes the BMD modeling results. As assessed by the χ^2 goodness-of-fit *p*-values, all the models failed to model this data set (see Table C.4).

To further attempt to calculate a BMDL based on this data set, BMD models were run with the control and low-dose data points after dropping the mid-dose data point. Table C.5 presents the BMD modeling results. All the models available from the BMDS except the Weibull model successfully fit the data set. The estimated $BMDL_{10}$s from these models differed by more than 3-fold, so the lowest BMDL of 0.363 mg/kg-day (from the Multistage 2, 3) is selected for this endpoint (see Figure C.2 and the BMD text output for Multistage 2 model).

Table C.3. Hepatocyte Necrosis in Male F344 Rats After Dietary Exposure to tgDNT for 104 Weeks[a]

	Control	Low-dose (3.51mg/kg-d)	Mid-dose (14 mg/kg-d)
Sample size	61	70	23
Incidence	0	38	11

[a]CIIT (1982a).

Table C.4. BMD Results for Hepatocyte Necrosis in Male F344 Rats After Dietary Exposure to tgDNT for 104 Weeks

Model Name	AIC	*p*-value	BMD_{10}	$BMDL_{10}$	Scaled Residual of Interest
Gamma	154.943	0	0.856072	0.677433	0
Logistic	181.412	0	2.98469	2.21859	4.388
LogLogistic	141.63	0.0015	0.479011	0.335441	0
LogProbit	156.474	0	1.27286	1.02557	0
Multistage 2	154.943	0	0.856072	0.677433	0
Multistage 3	154.943	0	0.856072	0.677433	0
Probit	180.738	0	2.85092	2.17342	4.457
Weibull	154.943	0	0.85607	0.677433	0

Table C.5. BMD Results After Dropping Mid-dose Point for Hepatocyte Necrosis in Male F344 Rats After Dietary Exposure to tgDNT for 104 Weeks

Model Name	AIC	*p*-value	BMD_{10}	$BMDL_{10}$	Scaled Residual of Interest
Logistic	100.526	NA	3.07144	1.576	0
Multistage 2	100.526	NA	0.774156	0.363194	0
Multistage 3	102.526	NA	1.02805	0.363194	0
Probit	100.526	NA	2.66223	1.36408	0

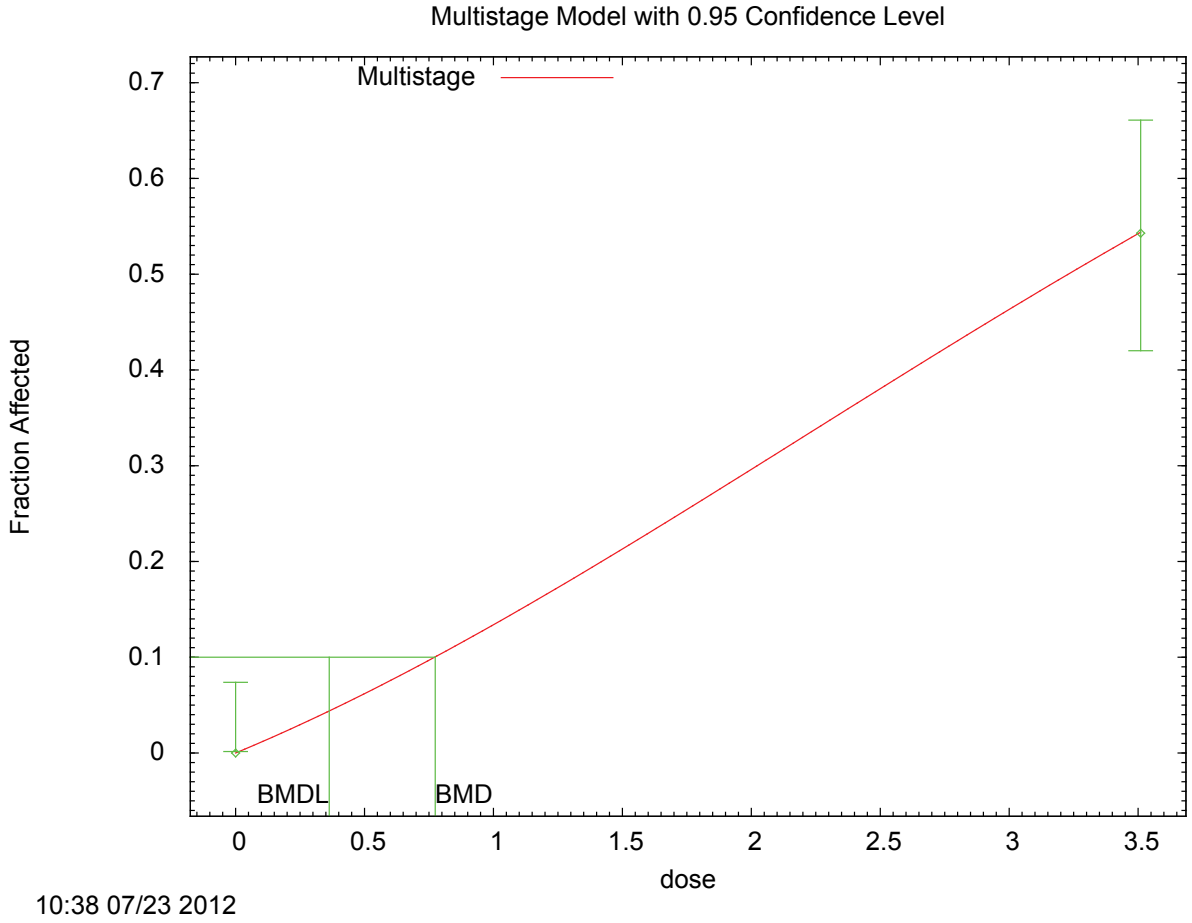

Figure C.2. Multistage 2 BMD Model for Increased Hepatocyte Necrosis in Male Rats After Dietary Exposure to tgDNT for 104 Weeks (CIIT, 1982a)

Text Output for Multistage 2 BMD Model for Increased Hepatocyte Necrosis in Male Rats After Dietary Exposure to tgDNT at 104 weeks (CIIT, 1982a)

```
  Multistage Model. (Version: 3.2;  Date: 05/26/2010)

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
```

```
Observation # < parameter # for Multistage model.
   The form of the probability function is:

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

   The parameter betas are restricted to be positive


   Dependent variable = Effect
   Independent variable = Dose

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


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



                  Default Initial Parameter Values
                     Background =     0.542857
                        Beta(1) =     0.223008
                        Beta(2) =    0.0635351


           Asymptotic Correlation Matrix of Parameter Estimates

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

                Beta(1)      Beta(2)

   Beta(1)      NA             NA

   Beta(2)      NA             NA


NA - This parameter's variance has been estimated as zero or less.
THE MODEL HAS PROBABLY NOT CONVERGED!!!



                                Parameter Estimates

                                                          95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background                0            *               *                   *
        Beta(1)         0.111504            *               *                   *
        Beta(2)        0.0317676            *               *                   *

* - Indicates that this value is not calculated.

At least some variance estimates are negative.
```

```
THIS USUALLY MEANS THE MODEL HAS NOT CONVERGED!
Try again from another starting point.

Error in computing chi-square; returning 2
```

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-48.2628	2			
Fitted model	-48.2628	2	0	0	NA
Reduced model	-78.8908	1	61.256	1	<.0001

AIC: 100.526

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	61	0.000
3.5100	0.5429	38.000	38.000	70	0.000

Chi^2 = 0.00 d.f. = 0 P-value = NA

Benchmark Dose Computation

```
Specified effect =           0.1

Risk Type        =      Extra risk

Confidence level =          0.95

             BMD =       0.774156

            BMDL =       0.363194

            BMDU =        1.48533

Taken together, (0.363194, 1.48533) is a 90     % two-sided confidence
       interval for the BMD
```

APPENDIX D. BENCHMARK DOSE CALCULATIONS FOR THE SCREENING p-OSF

MODEL-FITTING PROCEDURE FOR CANCER INCIDENCE DATA

The model-fitting procedure for dichotomous cancer incidence data is as follows. The Multistage-Cancer model in the EPA's BMDS (version 2.2.2) is fit to the incidence data using the extra risk option. The Multistage-Cancer model is run for all polynomial degrees up to $n - 1$ (where n is the number of dose groups including control). An adequate model fit is judged by three criteria: (1) goodness-of-fit p-value ($p > 0.1$), (2) visual inspection of the dose-response curve, and (3) scaled residual at the data point (except the control) closest to the predefined benchmark response (BMR). Among all the models providing adequate fit to the data, the BMDL from the best fitting Multistage-Cancer model as judged by the goodness-of-fit p-value, is selected as the POD. In accordance with U.S. EPA (2012) guidance, BMDs and BMDLs associated with an extra risk of 10% are calculated.

HEPATOCELLULAR CARCINOMAS IN MALE F344 RATS AFTER DIETARY EXPOSURE TO tgDNT FOR 104 WEEKS (CIIT, 1982a)

Table A.6 shows the dose-response data on hepatocellular carcinomas, liver neoplastic nodules, hepatocellular carcinomas and/or neoplastic nodules, mammary fibroadenomas, and subcutaneous fibromas in male F344 rats administered tgDNT via the diet for 104 weeks (CIIT, 1982a). Modeling was performed according to the procedure outlined above using BMDS for each individual tumor type based on the ADDs, and Table D.1 summarizes the results. For incidence of hepatocellular carcinomas in male rats, the 2-degree Multistage-Cancer model provided an adequate fit (goodness-of-fit p-value >0.1; see Table D.1 and Figure D.1). The estimated BMD_{10} value is 3.04 mg/kg-day with a $BMDL_{10}$ of 2.15 mg/kg-day.

Table D.1. BMD Results for Hepatocyte Carcinomas in Male F344 Rats After Dietary Exposure to tgDNT for 104 Weeks

Model Name	Degree of polynomial	AIC	p-value	BMD	$BMDL_{10}$	Scaled Residual of Interest
Multistage-Cancer	1	95.5511	0.0006	1.3531	0.998	0.35
Multistage-Cancer	2	81.7442	0.6332	**3.03847**	**2.15**	−0.366

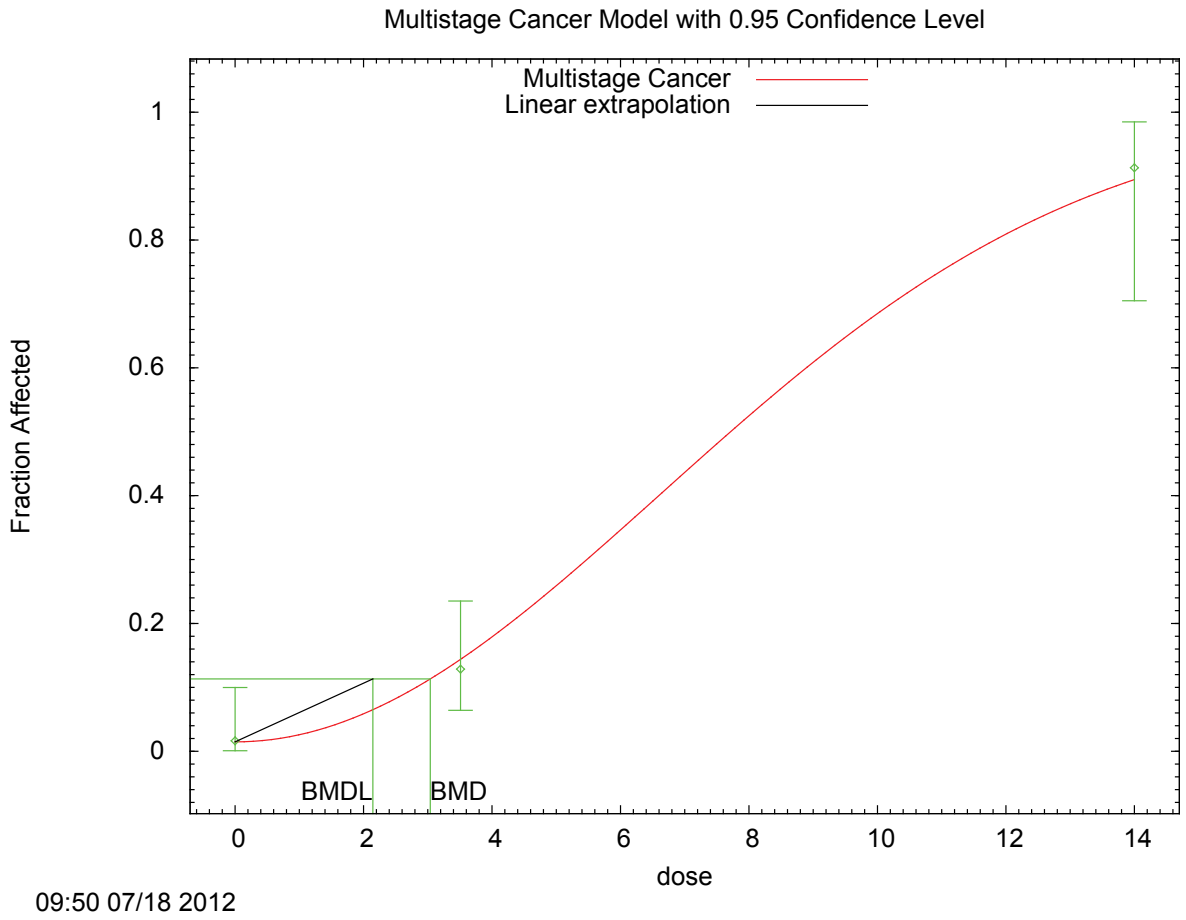

Figure D.1. Multistage-Cancer BMD Model for Increased Hepatocellular Carcinomas in Male Rats at 104 Weeks (CIIT, 1982a)

Text Output for Multistage-Cancer BMD Model for Increased Hepatocellular Carcinomas in Male Rats (CIIT, 1982a)

```
=============================================================================
        Multistage Cancer Model
 ============================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

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

   The parameter betas are restricted to be positive


   Dependent variable = Effect
   Independent variable = Dose

 Total number of observations = 3
```

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

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

Default Initial Parameter Values
Background = 0.000900222
Beta(1) = 0
Beta(2) = 0.012451

Asymptotic Correlation Matrix of Parameter Estimates

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

	Background	Beta(2)
Background	1	-0.32
Beta(2)	-0.32	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0147051	*	*	*
Beta(1)	0	*	*	*
Beta(2)	0.0114121	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-38.7541	3			
Fitted model	-38.8721	2	0.236087	1	0.627
Reduced model	-77.3384	1	77.1687	2	<.0001

AIC: 81.7442

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0147	0.897	1.000	61	0.110
3.5100	0.1439	10.076	9.000	70	-0.366
14.0000	0.8948	20.580	21.000	23	0.286

```
 Chi^2 = 0.23      d.f. = 1        P-value = 0.6332


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        3.03847

            BMDL =        2.14507

            BMDU =        3.67911

Taken together, (2.14507, 3.67911) is a 90     % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor =     0.0466186
```

LIVER NEOPLASTIC NODULES IN MALE F344 RATS AFTER DIETARY EXPOSURE TO tgDNT FOR 104 WEEKS (CIIT, 1982a)

Modeling was performed according to the procedure outlined above using BMDS based on the ADDs, and Table D.2 summarizes the results. For incidence of liver neoplastic nodules in male rats, the 2-degree Multistage-Cancer model provided an adequate fit (goodness-of-fit *p*-value >0.1; see Table D.2 and Figure D.2). The estimated BMD_{10} value is 4.86 mg/kg-day with a $BMDL_{10}$ of 2.69 mg/kg-day.

Table D.2. BMD Results for Liver Neoplastic Nodules in Male F344 Rats After Dietary Exposure to tgDNT for 104 Weeks

Model Name	Degree of polynomial	AIC	*p*-value	BMD	$BMDL_{10}$	Scaled Residual of Interest
Multistage-Cancer	1	150.829	0.0262	2.43085	1.58	−1.606
Multistage-Cancer	2	146.021	0.5454	**4.85647**	**2.69**	−0.45

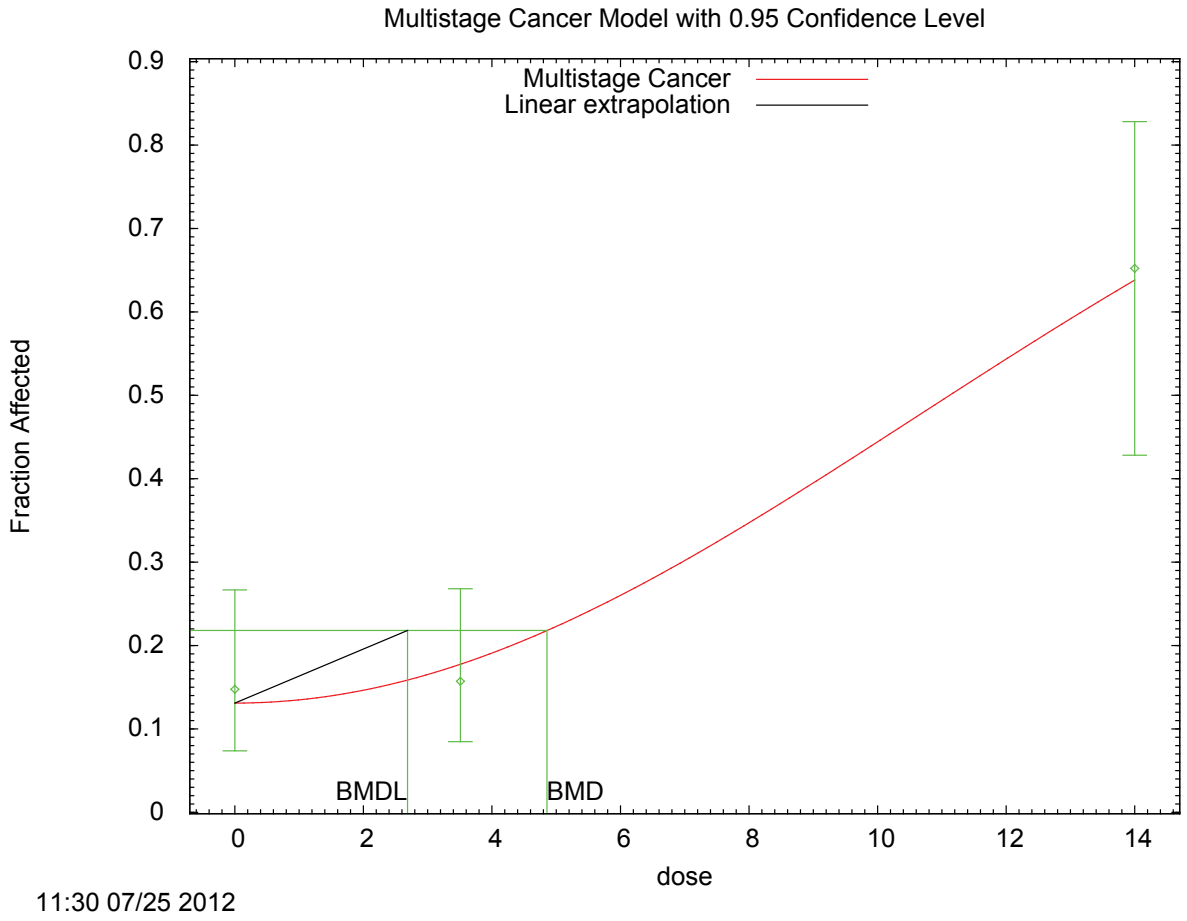

Figure D.2. Multistage-Cancer BMD Model for Increased Neoplastic Nodules in Male Rats at 104 Weeks (CIIT, 1982a)

Text Output for Multistage-Cancer BMD Model for Increased Neoplastic Nodules in Male Rats (CIIT, 1982a)

```
=================================================================
        Multistage Cancer Model. (Version: 1.9;  Date: 05/26/2010)
        Input Data File: C:/USEPA/BMDS220/Data/104 week/msc_104 week male neoplastic
nodules_Opt.(d)
        Gnuplot Plotting File:  C:/USEPA/BMDS220/Data/104 week/msc_104 week male
neoplastic nodules_Opt.plt
                                                Wed Jul 25 11:30:27 2012
 =================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

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

   The parameter betas are restricted to be positive


   Dependent variable = Effect
   Independent variable = Dose

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


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



                  Default Initial Parameter Values
                     Background =     0.128217
                        Beta(1) =            0
                        Beta(2) =   0.00468028


           Asymptotic Correlation Matrix of Parameter Estimates

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

             Background      Beta(2)

Background            1        -0.36

   Beta(2)        -0.36            1


                                Parameter Estimates
```

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.131201	*	*	*
Beta(1)	0	*	*	*
Beta(2)	0.00446721	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-70.8268	3			
Fitted model	-71.0106	2	0.367614	1	0.5443
Reduced model	-82.5378	1	23.422	2	<.0001

AIC: 146.021

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.1312	8.003	9.000	61	0.378
3.5100	0.1777	12.441	11.000	70	-0.450
14.0000	0.6380	14.675	15.000	23	0.141

Chi^2 = 0.37 d.f. = 1 P-value = 0.5454

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 4.85647

BMDL = 2.68723

BMDU = 6.53922

Taken together, (2.68723, 6.53922) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.037213

COMBINED HEPATOCELLULAR CARCINOMAS AND LIVER NEOPLASTIC NODULES IN MALE F344 RATS AFTER DIETARY EXPOSURE TO tgDNT FOR 104 WEEKS (CIIT, 1982a)

Modeling was performed according to the procedure outlined above using BMDS based on the ADDs, and Table D.3 summarizes the results. For incidence of combined hepatocellular carcinomas and liver neoplastic nodules in male rats, the 2-degree Multistage-Cancer model provided an adequate fit (goodness-of-fit *p*-value >0.1; see Table D.3 and Figure D.3). The estimated BMD_{10} value is 2.42 mg/kg-day with a $BMDL_{10}$ of 1.68 mg/kg-day.

Table D.3. BMD Results for Combined Hepatocellular Carcinomas and Liver Neoplastic Nodules in Male F344 Rats After Dietary Exposure to tgDNT for 104 Weeks

Model Name	Degree of polynomial	AIC	*p*-value	BMD	BMDL	Scaled Residual of Interest
Multistage-Cancer	1	156.06	0.0008	0.991314	0.721042	0.828
Multistage-Cancer	2	142.255	0.2389	**2.41883**	**1.67831**	−0.762

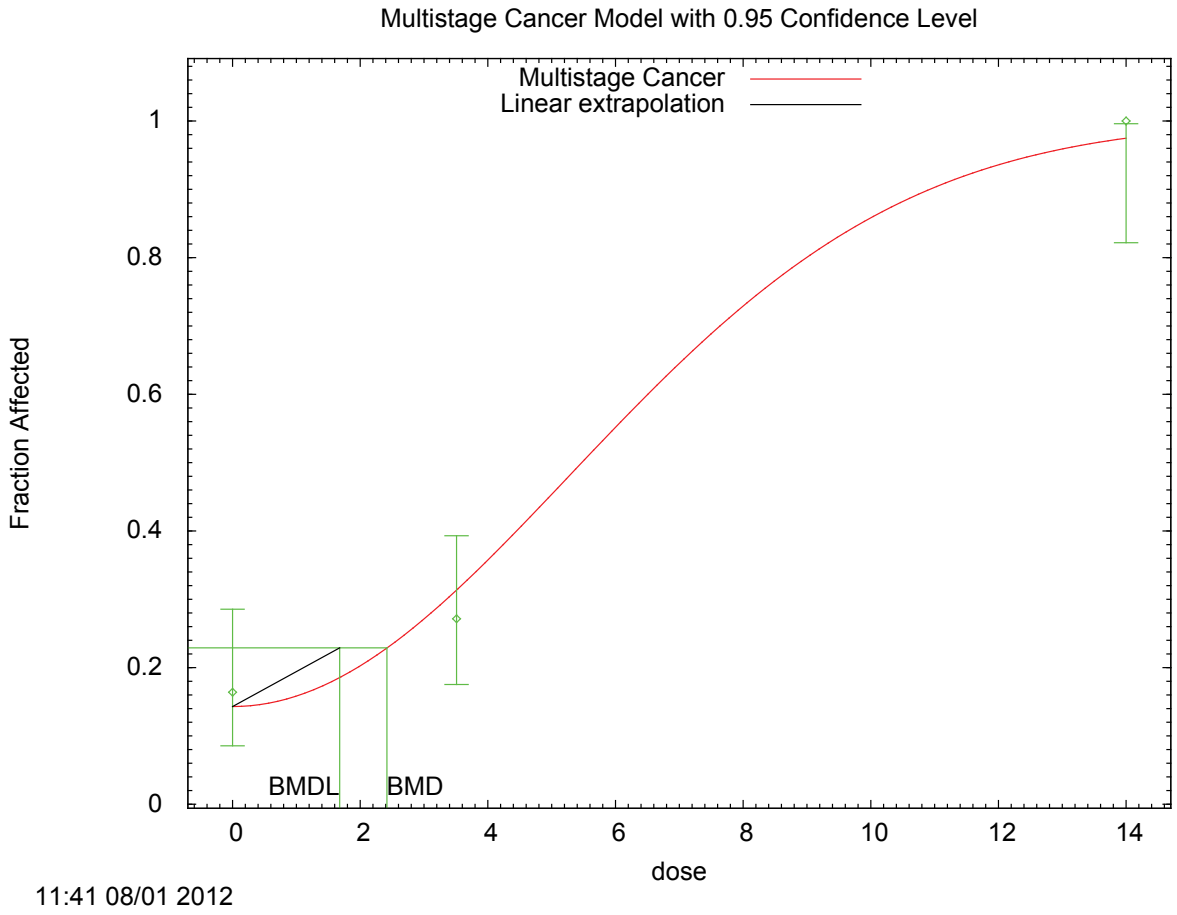

Figure D.3. Multistage-Cancer BMD Model for Increased Combined Hepatocellular Carcinomas and Neoplastic Nodule in Male Rats at 104 Weeks (CIIT, 1982a)

Text Output for Multistage-Cancer BMD Model for Increased Combined Hepatocellular Carcinomas and Neoplastic Nodule in Male Rats (CIIT, 1982a)

```
 ======================================================================
        Multistage Cancer Model. (Version: 1.9;  Date: 05/26/2010)
        Input Data File: C:/USEPA/BMDS220/Data/msc_livercombined_Msc2-BMR10.(d)
        Gnuplot Plotting File:  C:/USEPA/BMDS220/Data/msc_livercombined_Msc2-
BMR10.plt
                                            Wed Aug 01 11:41:18 2012
 ======================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

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

   The parameter betas are restricted to be positive
```

```
   Dependent variable = Response
   Independent variable = Dose

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


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



                  Default Initial Parameter Values
                     Background =            0
                        Beta(1) =            0
                        Beta(2) = 5.25101e+017


           Asymptotic Correlation Matrix of Parameter Estimates

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

             Background      Beta(2)

Background            1        -0.36

   Beta(2)        -0.36            1


                                Parameter Estimates

                                                          95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background         0.143194            *               *                  *
        Beta(1)                0            *               *                  *
        Beta(2)        0.0180081            *               *                  *

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -68.1416         3
   Fitted model        -69.1276         2       1.97201      1          0.1602
  Reduced model        -98.4788         1       60.6745      2         <.0001

           AIC:         142.255


                                Goodness  of  Fit
                                                        Scaled
```

Dose	Est._Prob.	Expected	Observed	Size	Residual
14.0000	0.9749	22.422	23.000	23	0.770
3.5100	0.3137	21.957	19.000	70	-0.762
0.0000	0.1432	8.735	10.000	61	0.462

Chi^2 = 1.39 d.f. = 1 P-value = 0.2389

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 2.41883

BMDL = 1.67831

BMDU = 3.09631

Taken together, (1.67831, 3.09631) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.0595837

MAMMARY FIBROADENOMAS IN MALE F344 RATS AFTER DIETARY EXPOSURE TO tgDNT FOR 104 WEEKS (CIIT, 1982a)

Modeling was performed according to the procedure outlined above using BMDS based on the ADDs, and Table D.4 summarizes the results. For incidence of mammary fibroadenomas in male rats, both 1- and 2-degree Multistage-Cancer models provided an identical fit (goodness-of-fit *p*-value >0.1; see Table D.4 and Figure D.4). The estimated BMD_{10} value is 7.37 mg/kg-day with a $BMDL_{10}$ of 3.73 mg/kg-day.

Table D.4. BMD Results for Mammary Fibroadenomas in Male F344 Rats After Dietary Exposure to tgDNT for 104 Weeks

Model Name	Degree of polynomial	AIC	*p*-value	BMD	$BMDL_{10}$	Scaled Residual of Interest
Multistage-Cancer	1	97.5334	0.9081	**7.37334**	**3.73**	0.085
Multistage-Cancer	2	97.5334	0.9081	7.37334	3.73	0.085

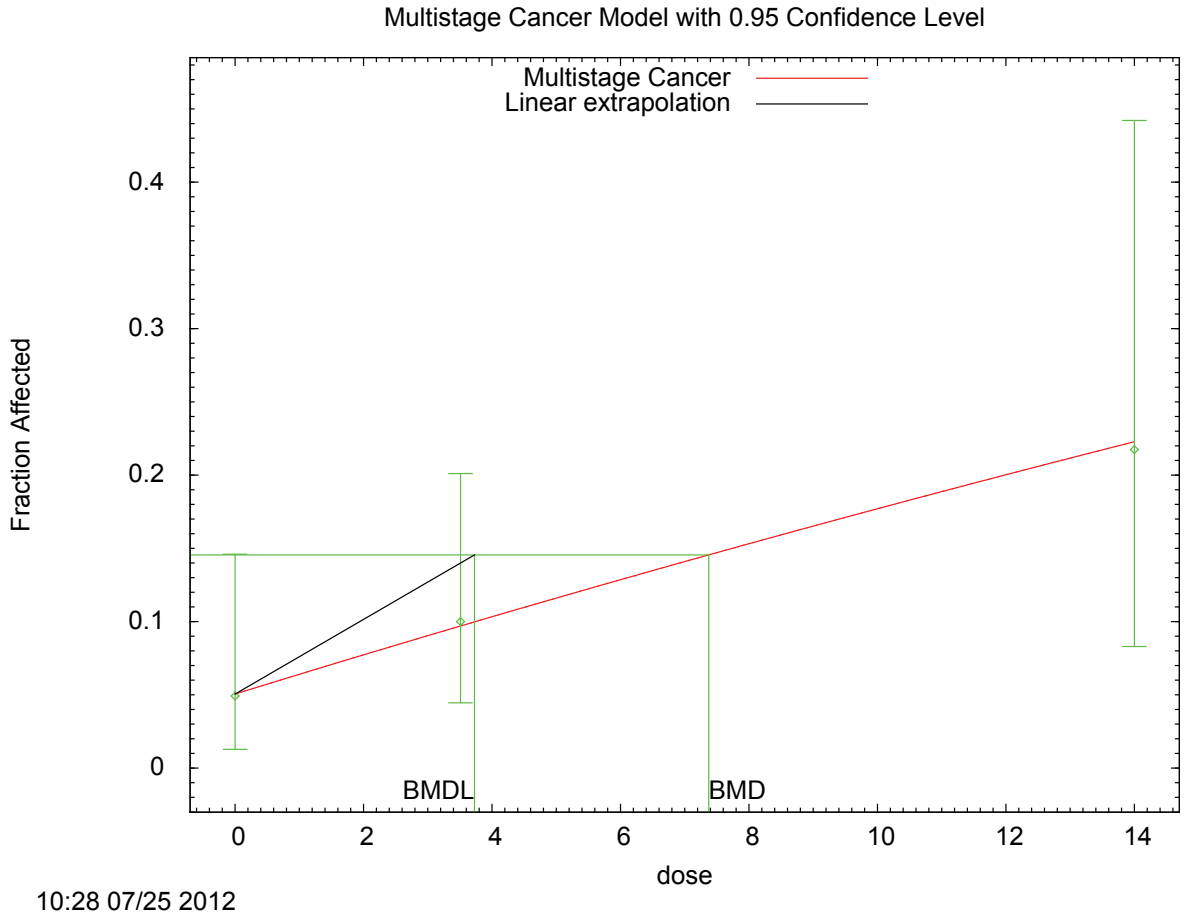

Figure D.4. Multistage-Cancer BMD Model for Increased Mammary Fibroadenomas in Male Rats at 104 Weeks (CIIT, 1982a)

Text Output for Multistage-Cancer BMD Model for Increased Mammary Fibroadenomas in Male Rats (CIIT, 1982a)

```
=================================================================
        Multistage Cancer Model. (Version: 1.9;  Date: 05/26/2010)
        Input Data File: C:/USEPA/BMDS220/Data/msc_Mammary_Msc1-BMR10.(d)
        Gnuplot Plotting File:  C:/USEPA/BMDS220/Data/msc_Mammary_Msc1-BMR10.plt
                                          Wed Aug 01 15:18:00 2012
 =================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

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

   The parameter betas are restricted to be positive


   Dependent variable = Response
   Independent variable = Dose

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


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


                  Default Initial Parameter Values
                     Background =    0.0518599
                        Beta(1) =    0.0137724


           Asymptotic Correlation Matrix of Parameter Estimates

             Background      Beta(1)

Background            1        -0.62

   Beta(1)        -0.62            1


                                Parameter Estimates

                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background        0.0505334            *               *                   *
        Beta(1)        0.0142894            *               *                   *

* - Indicates that this value is not calculated.
```

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-46.7601	3			
Fitted model	-46.7667	2	0.0133121	1	0.9081
Reduced model	-49.1781	1	4.83601	2	0.0891

AIC: 97.5334

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0505	3.083	3.000	61	-0.048
3.5100	0.0970	6.789	7.000	70	0.085
14.0000	0.2227	5.122	5.000	23	-0.061

Chi^2 = 0.01 d.f. = 1 P-value = 0.9081

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 7.37334

BMDL = 3.72678

BMDU = 33.6009

Taken together, (3.72678, 33.6009) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.0268328

SUBCUTANEOUS FIBROMAS IN MALE F344 RATS AFTER DIETARY EXPOSURE TO tgDNT FOR 104 WEEKS (CIIT, 1982a)

Modeling was performed according to the procedure outlined above using BMDS based on theADDs, and Table D.5 summarizes the results. For incidence of subcutaneous fibromas in male rats, the 1-degree Multistage-Cancer model provided an adequate fit (goodness-of-fit *p*-value >0.1; see Table D.5 and Figure D.5). The estimated BMD_{10} value is 2.01 mg/kg-day with a $BMDL_{10}$ of 1.38 mg/kg-day.

Table D.5. BMD Results for Subcutaneous Fibromas in Male F344 Rats After Dietary Exposure to tgDNT for 104 Weeks

Model Name	Degree of polynomial	AIC	*p*-value	BMD	$BMDL_{10}$	Scaled Residual of Interest
Multistage-Cancer	1	140.118	0.4138	**2.01413**	**1.38**	−0.582
Multistage-Cancer	2	141.438	NA	2.79287	1.45	0

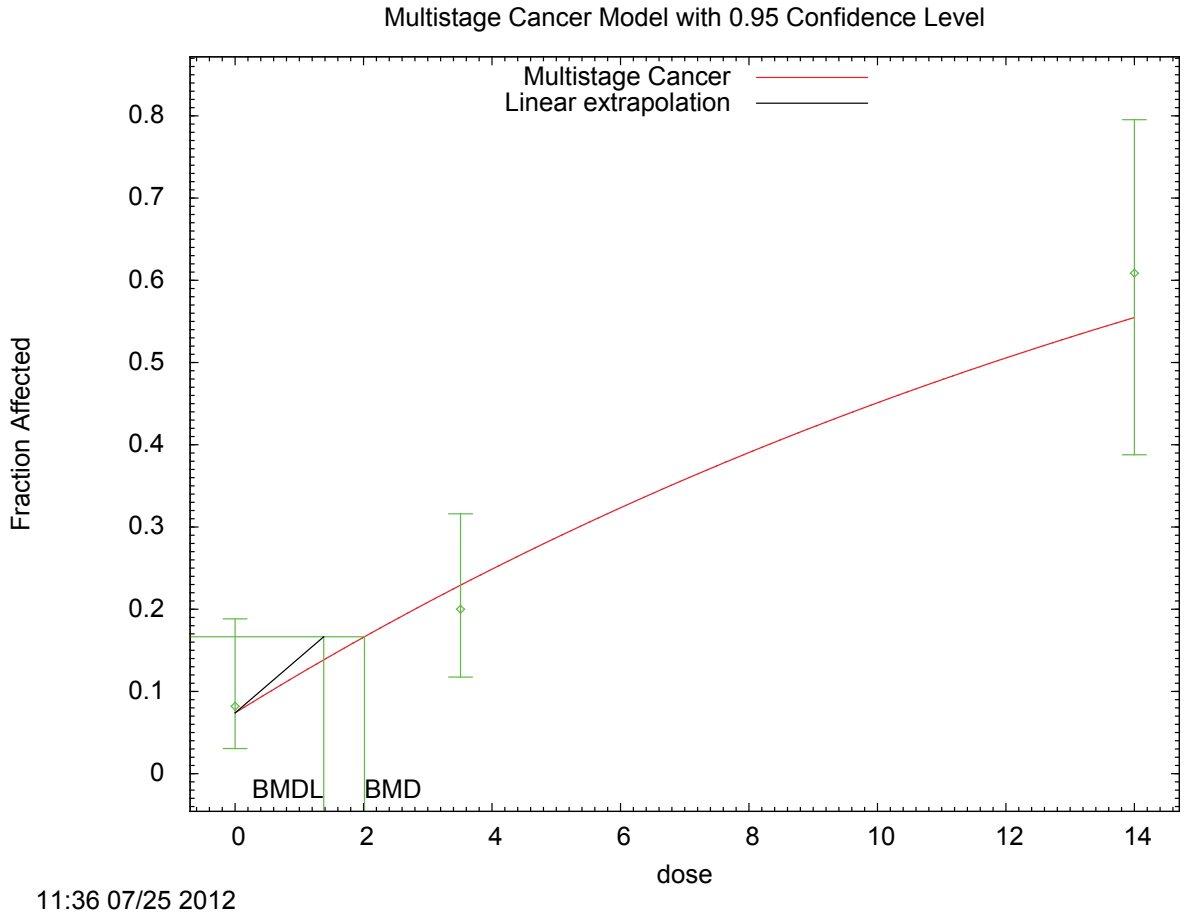

Figure D.5. Multistage-Cancer BMD Model for Increased Subcutaneous Fibromas in Male Rats at 104 Weeks (CIIT, 1982a)

Text Output for Multistage-Cancer BMD Model for Increased Subcutaneous Fibromas in Male Rats (CIIT, 1982a)

```
================================================================
        Multistage Cancer Model. (Version: 1.9;  Date: 05/26/2010)
        Input Data File: C:/USEPA/BMDS220/Data/104 week/msc_Subcutaneous
fibromas_Opt.(d)
        Gnuplot Plotting File:  C:/USEPA/BMDS220/Data/104 week/msc_Subcutaneous
fibromas_Opt.plt
                                                Wed Jul 25 10:38:29 2012
 ================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

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

   The parameter betas are restricted to be positive


   Dependent variable = Effect
   Independent variable = Dose

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


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



                  Default Initial Parameter Values
                     Background =    0.0491346
                        Beta(1) =    0.0625806


           Asymptotic Correlation Matrix of Parameter Estimates

             Background      Beta(1)

Background            1        -0.56

   Beta(1)        -0.56            1


                                Parameter Estimates

                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background        0.0738705            *               *                   *
        Beta(1)        0.0523107            *               *                   *
```

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-67.7191	3			
Fitted model	-68.0589	2	0.679587	1	0.4097
Reduced model	-80.0153	1	24.5923	2	<.0001

AIC: 140.118

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0739	4.506	5.000	61	0.242
3.5100	0.2292	16.045	14.000	70	-0.582
14.0000	0.5547	12.759	14.000	23	0.521

Chi^2 = 0.67 d.f. = 1 P-value = 0.4138

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 2.01413

BMDL = 1.37981

BMDU = 3.29703

Taken together, (1.37981, 3.29703) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.0724738

MS_COMBO-MULTIPLE TUMOR BMD MODEL FOR ALL TUMOR TYPES IN MALE F344 RATS AFTER DIETARY EXPOSURE TO tgDNT FOR 104 WEEKS (CIIT, 1982a)

MS_Combo-multiple tumor BMD modeling was used to combine tumor incidence data for combined hepatocellular carcinomas and/or neoplastic nodules, mammary fibroadenomas, and subcutaneous fibromas in male rats. For each tumor type, the best-fitting multistage model (i.e., the degree of Poly setting) was maintained in the MS_Combo model run. The calculated combined tumor $BMDL_{10}$ based on the MS_Combo model is 0.852 mg/kg-day (see MS_Combo text output for details). This $BMDL_{10}$ is used as the POD to derive the p-OSF.

Text Output for MS_COMBO Multiple Tumor Model for Combined Tumors in Male Rats

```
================================================================
         MS_COMBO. (Version: 1.5 Beta;  Date: 01/25/2011)
         Input Data File: C:\USEPA\BMDS220\Data\New.(d)
         Gnuplot Plotting File:  C:\USEPA\BMDS220\Data\New.plt
                                              Wed Aug 01 15:35:40 2012
 ================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

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

   The parameter betas are restricted to be positive


   Dependent variable = Response
   Independent variable = Dose

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


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



                  Default Initial Parameter Values
                     Background =            0
                        Beta(1) =            0
                        Beta(2) = 5.25101e+017


           Asymptotic Correlation Matrix of Parameter Estimates

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

             Background      Beta(2)
```

```
Background            1        -0.36

   Beta(2)        -0.36            1


                              Parameter Estimates

                                                          95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background         0.143194            *               *                   *
        Beta(1)                0            *               *                   *
        Beta(2)        0.0180081            *               *                   *

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model         -68.1416         3
   Fitted model         -69.1276         2       1.97201      1          0.1602
  Reduced model         -98.4788         1       60.6745      2         <.0001

           AIC:          142.255

 Log-likelihood Constant             63.914608924352244


                                  Goodness  of  Fit
                                                                        Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------------
   14.0000     0.9749        22.422    23.000          23        0.770
    3.5100     0.3137        21.957    19.000          70       -0.762
    0.0000     0.1432         8.735    10.000          61        0.462

 Chi^2 = 1.39      d.f. = 1        P-value = 0.2389


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        2.41883

            BMDL =        1.67831

            BMDU =        3.09631

Taken together, (1.67831, 3.09631) is a 90     % two-sided confidence
interval for the BMD


 ===================================================================
```

```
        MS_COMBO. (Version: 1.5 Beta;  Date: 01/25/2011)
        Input Data File: C:\USEPA\BMDS220\Data\New.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS220\Data\New.plt
                                              Wed Aug 01 15:35:40 2012
 ========================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

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

   The parameter betas are restricted to be positive


   Dependent variable = Response
   Independent variable = Dose

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


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



                  Default Initial Parameter Values
                     Background =    0.0518599
                        Beta(1) =    0.0137724


           Asymptotic Correlation Matrix of Parameter Estimates

             Background      Beta(1)

Background            1        -0.62

   Beta(1)        -0.62            1



                                Parameter Estimates

                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background        0.0505334            *                *                  *
        Beta(1)        0.0142894            *                *                  *

* - Indicates that this value is not calculated.



                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
```

```
     Full model        -46.7601         3
   Fitted model        -46.7667         2     0.0133121      1          0.9081
  Reduced model        -49.1781         1       4.83601      2          0.0891

           AIC:         97.5334

 Log-likelihood Constant             41.819300823452593


                                 Goodness  of  Fit
                                                                      Scaled
    Dose     Est._Prob.    Expected    Observed     Size       Residual
 ---------------------------------------------------------------------------
    0.0000     0.0505         3.083     3.000          61       -0.048
    3.5100     0.0970         6.789     7.000          70        0.085
   14.0000     0.2227         5.122     5.000          23       -0.061

 Chi^2 = 0.01      d.f. = 1        P-value = 0.9081


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        7.37334

            BMDL =        3.72678

            BMDU =        33.6009

Taken together, (3.72678, 33.6009) is a 90     % two-sided confidence
interval for the BMD



 ======================================================================
        MS_COMBO. (Version: 1.5 Beta;  Date: 01/25/2011)
        Input Data File: C:\USEPA\BMDS220\Data\New.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS220\Data\New.plt
                                              Wed Aug 01 15:35:40 2012
 ======================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

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

   The parameter betas are restricted to be positive


   Dependent variable = Response
   Independent variable = Dose

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

Degree of polynomial = 1

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

Default Initial Parameter Values
Background = 0.0491346
Beta(1) = 0.0625806

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.56
Beta(1)	-0.56	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0738705	*	*	*
Beta(1)	0.0523107	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-67.7191	3			
Fitted model	-68.0589	2	0.679587	1	0.4097
Reduced model	-80.0153	1	24.5923	2	<.0001

AIC: 140.118

Log-likelihood Constant 62.107410554719884

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0739	4.506	5.000	61	0.242
3.5100	0.2292	16.045	14.000	70	-0.582
14.0000	0.5547	12.759	14.000	23	0.521

Chi^2 = 0.67 d.f. = 1 P-value = 0.4138

Benchmark Dose Computation

Specified effect = 0.1

```
Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        2.01413

            BMDL =        1.37981

            BMDU =        3.29703

Taken together, (1.37981, 3.29703) is a 90     % two-sided confidence
interval for the BMD


**** Start of combined BMD and BMDL Calculations.****

  Combined Log-Likelihood                     -183.95322559798808

  Combined Log-likelihood Constant             167.84132030252471


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        1.19552

            BMDL =       0.852494
```

APPENDIX E. REFERENCES

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