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Provisional Peer-Reviewed Toxicity Values for

2,6-Dinitrotoluene
(CASRN 606-20-2)

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

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COMMONLY USED ABBREVIATIONS

BMC	benchmark concentration
BMCL	benchmark concentration lower 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 2,6-DINITROTOLUENE (CASRN 606-20-2)

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

2,6-Dinitrotoluene (also called 2,6-DNT) has several names, including toluene, 2,6-dinitro-; 2-methyl-1,3-dinitrobenzene or benzene, 2-methyl-1,3-dinitro; and 1-methyl-2,6-dinitrobenzene (U.S. EPA, 1990; IARC, 1996; NLM, 2011). The isomer 2,6-dinitrotoluene often occurs in a mixture with the isomer 2,4-dinitrotoluene (2,4-DNT), made by combining toluene with mixed nitric and sulfuric acids. The isomeric composition of dinitrotoluene (DNT) may vary, but technical grade DNT (CASRN 25321-14-6) refers to a mixture of approximately 76% 2,4-DNT and 19% 2,6-DNT. The remaining 5% is a combination of the remaining four dinitrotoluene isomers: 2,3-DNT, 2,5-DNT, 3,4-DNT, and 3,5-DNT. In the literature, this mixture is also called dinitrotoluene (isomers mixture), DNT or DNT 80/20. In this document, the name technical grade DNT (tgDNT) is used as a representative of this mixture composition (76% 2,4-DNT and 19% 2,6-DNT). DNT is used to make flexible polyurethane foams used in the bedding and furniture industries, and as a chemical intermediate in the production of toluene diamines and diisocyanates. DNT is also used to produce dyes, explosives, and propellants (IARC, 1996; ATSDR, 1998). The empirical formula for 2,6-DNT is $C_7H_6N_2O_4$, and its structure is shown in Figure 1. A table of the physicochemical properties of 2,6-DNT is provided in Table 1.

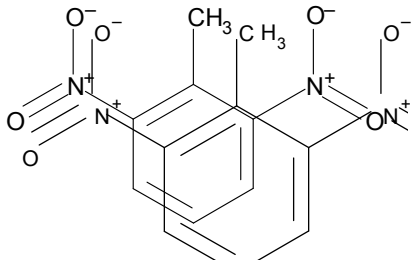

Figure 1. 2,6-Dinitrotoluene Structure

Table 1. Physicochemical Properties for 2,6-Dinitrotoluene (CASRN 606-20-2)[a]

Property (unit)	Value
Boiling point (ºC)	285
Melting point (ºC)	66
Density (g/cm^3)	1.2833 at 111ºC
Vapor pressure (mm Hg at 25ºC)	5.67×10^{-4}
pH (unitless)	ND
Solubility in water (mg/L at 20ºC)	180
Relative vapor density (air = 1)	6.28
Molecular weight (g/mol)	182.14

[a]Values from NLM (2011) and IARC (1996).

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 tgDNT (approximated as 76% 2,4-DNT and 19% 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. A previous PPRTV for 2,6-DNT was posted in 2004 (U.S. EPA, 2004; see Table 2). The purpose of this PPRTV is to review and update the toxicity of 2,6-DNT (approximately 99% 2,6-DNT).

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 (various compositions of DNTs) 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 (various compositions of DNTs) 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 $\mu g/m^3$	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

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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
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
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	4.5×10^{-1} per mg/kg-d (screening p-OSF)	NV	NV	NV	Based on a $BMDL_{10HED}$ of 0.224 from a 104-wk study in rats with increased incidence of liver hepatocellular carcinomas, liver neoplastic nodules, mammary fibroadenomas and subcutaneous fibromas in males	U.S. EPA (2013)	4-3-2013

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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
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-DNT based 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
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

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
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
Health effects assessment	NV	NV	NV	NV	NA	U.S. EPA (1987)	2-6-2013

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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
PPRTV	5×10^{-3} mg/kg-d (screening subchronic p-RfD)[i] 9×10^{-4} mg/kg-d (screening chronic p-RfD)[j]	NV	1×10^{-2} mg/kg-d (subchronic p-RfD)[k] 1×10^{-3} mg/kg-d (chronic p-RfD)[k]	NV	[i]Based on a $BMDL_{10HED}$ of 0.52 mg/kg-d for hepatic necrosis in a 26-week oral study in male rats; [j]Based on a $BMDL_{10HED}$ of 0.087 mg/kg-d for hepatic necrosis in a 104-week oral study in male rats; [k]Based on a NOAEL of 4 mg/kg-d for numerous health effects in a 13-wk oral study in male and female dogs	tgDNT is U.S. EPA (2013); 2,6-DNT is U.S. EPA (2004)	4-3-2013 and 2-6-2013
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–k]See notes column for corresponding information.

NA = not applicable; NV = not available.

Literature searches were conducted on sources published from 1900 through February 2013 for studies relevant to the derivation of provisional toxicity values for 2,6-DNT CAS Number (606-20-2). The following databases were searched by chemical name, synonyms, or CASRN: ACGIH, ANEUPL, ATSDR, BIOSIS, Cal EPA, CCRIS, CDAT, ChemIDplus, CIS, CRISP, DART, EMIC, EPIDEM, ETICBACK, FEDRIP, GENE-TOX, HAPAB, HERO, HMTC, HSDB, IARC, INCHEM IPCS, IPA, ITER, IUCLID, LactMed, NIOSH, NTIS, NTP, OSHA, OPP/RED, PESTAB, PPBIB, PPRTV, PubMed (toxicology subset), RISKLINE, RTECS, TOXLINE, TRI, U.S. EPA IRIS, U.S. EPA HEAST, U.S. EPA HEEP, U.S. EPA OW, and U.S. EPA TSCATS/TSCATS2. The following databases were searched for toxicity values or exposure limits: ACGIH, ATSDR, Cal EPA, U.S. EPA IRIS, U.S. EPA HEAST, U.S. EPA HEEP, U.S. EPA OW, U.S. EPA TSCATS/TSCATS2, NIOSH, NTP, OSHA, and RTECS.

REVIEW OF POTENTIALLY RELEVANT DATA (CANCER AND NONCANCER)

Table 3 provides an overview of the relevant database for 2,6-DNT and includes all potentially relevant repeated short-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 2,6-Dinitrotoluene (CASRN 606-20-2)

Category	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[a]	LOAEL[a]	Reference (Comments)	Notes[b]
Human								
1. Oral (mg/kg-d)[a]								
Acute[c]	ND							
Short-term[d]	ND							
Long-term[e]	ND							
Chronic[f]	ND							
2. Inhalation (mg/m^3)[a]								
Acute[c]	ND							
Short-term[d]	ND							
Long-term[e]	ND							
Chronic[f]	ND							
Animal								
1. Oral (mg/kg-d)[a]								
Short-term	6/0, Sprague-Dawley rat, gavage, 14 d	0, 4, 7, 14, 35, 68, 134 mg/kg-d	Mild anemia at 14 mg/kg-d, increased relative kidney weight at ≥7 mg/kg-d, decreased body weight at ≥35 mg/kg-d, increased ALT activity at ≥68 mg/kg-d, increased absolute and relative spleen weight at ≥68 mg/kg-d; decreased absolute and relative testes weight and absolute epididymides weight at 134 mg/kg-d; histopathological changes in various organs at ≥35 mg/kg-d	7	DU	14	Lent et al. (2012a)	PR

Table 3. Summary of Potentially Relevant Data for 2,6-Dinitrotoluene (CASRN 606-20-2)

Category	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[a]	LOAEL[a]	Reference (Comments)	Notes[b]
Subchronic	16/16, CD rat, dietary, 4 or 13 wk	0, 7, 35, 145 (M); 0, 7, 37, 155 (F) (Adjusted)	Lesions in the liver and spleen at ≥35 mg/kg-d, testicular atrophy and aspermatogenesis in males at 145 mg/kg-d	7	DU	35	Lee et al. (1976a)	NPR
Subchronic	16/16, Albino Swiss mouse, dietary, up to 13 wk	0, 11, 51, 289 (M); 0, 11, 55, 299 (F) (Adjusted)	Mortality at ≥51 mg/kg-d, decreased relative liver weight at ≥11 mg/kg-d in males, decreased relative kidney weight at 51 mg/kg-d in males	NDr	DU	11	Lee et al. (1976b)	NPR
Subchronic	**4/4, Beagle dog, oral (gelatin capsules), d/wk not reported, 4 or 13 wk**	**0, 4, 20, 100 (Adjusted)**	**Mortality, neurotoxicity, hematological effects, histopathology of liver, kidney, and testes, anemia, Heinz bodies, and methemoglobinemia at ≥20 mg/kg-d, splenic extramedullary hematopoiesis at ≥4 mg/kg-d**	**NDr**	**DU**	**4**	**Lee et al. (1976c)**	**PS, NPR**
Chronic	28/0, F344/Cr1BR rat, dietary, 26 or 52 wk	0, 7, 14 (Adjusted)	Decreased body weight, increased relative liver weight, increased ALT activity; all at ≥7 mg/kg-d	NDr	0.69 for increased relative liver weight at 52 wk	7	Leonard et al. (1987)	PR
Developmental	ND							
Reproductive	ND							
Carcinogenicity	30/0, F344 rat, diet (high in pectin, pectin-free, or 5% pectin), up to 12 mo	ADD: 0, 0.6−0.7, 3.0−3.5 HED: 0, 0.13–0.15, 0.63–0.74	Increase in hepatocellular carcinomas and neoplastic nodules in rats fed diet high in pectin content at 3.0−3.5 mg/kg-d	NA	NA	NA	Goldsworthy et al. (1986); Tumors were only observed in rats fed 2,6-DNT in diet high in pectin content	PR

Table 3. Summary of Potentially Relevant Data for 2,6-Dinitrotoluene (CASRN 606-20-2)

Category	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[a]	LOAEL[a]	Reference (Comments)	Notes[b]
Carcinogenicity	**28/0, F344/CrlBR rat, dietary, 52 wk**	**ADD: 0, 7, 14** **HED: 0, 1.9, 3.6**	**Increase in neoplastic nodules and hepatocellular carcinomas at 7 and 14 mg/kg-d**	**NA**	**0.25 for increased hepatocellular carcinomas**	**NA**	**Leonard et al. (1987)**	**PS, PR**
Carcinogenicity	26/26, A/J mouse, gavage, 2 d/wk, 12 wk	ADD: 0, 343.9, 857.1, 1714 HED: 0, 79, 198, 396	No increase in lung tumor incidence	NA	NA	NA	Stoner et al. (1984)	PR
2. Inhalation (mg/m^3)[a]								
Subchronic	ND							
Chronic	ND							
Developmental	ND							
Reproductive	ND							
Carcinogenicity	ND							

[a]Dosimetry: NOAEL, BMDL/BMCL, and LOAEL values are converted to an adjusted daily dose (ADD in mg/kg-d) for oral noncancer effects. 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. Values from animal developmental studies are not adjusted to a continuous exposure.
[b]Notes: IRIS = Utilized by IRIS, date of last update; PS = Principal study; PR = Peer reviewed; NPR = Not peer reviewed.
[c]Acute = Exposure for 24 hr or less (U.S. EPA, 2002).
[d]Subchronic = Repeated exposure for >24 hr ≤30 d (U.S. EPA, 2002).
[e]Long-term = Repeated exposure for >30 d ≤10% lifespan (based on 70 yr typical lifespan) (U.S. EPA, 2002).
[f]Chronic = Repeated exposure for ≥10% lifespan (U.S. EPA, 2002).

DU = Data unsuitable, NA = Not applicable, NV = Not available, ND = No data, NDr = Not determined, NI = Not identified, NP = Not provided, NR = Not reported, NR/Dr = Not reported but determined from data, NS = Not selected, FEL = Frank effect level.

HUMAN STUDIES

Oral Exposures

No studies investigating the effects of oral exposure to 2,6-DNT in humans have been identified.

Inhalation Exposures

No data on the effects of pure 2,6-DNT in humans following inhalation exposure are identified.

ANIMAL STUDIES

Oral Exposures

The effects of oral exposure of animals to 2,6-DNT have been evaluated in one short-term (Lent et al. 2012a), one subchronic (Lee et al., 1976), one chronic (Leonard et al., 1987), and three carcinogenicity studies (Goldsworthy et al., 1986; Leonard et al., 1987; Stoner et al., 1984).

Short-term Studies

Lent et al., 2012a

In a peer-reviewed study, Lent et al. (2012a) investigated the effects of short-term oral administration of various DNT isomers including 2,6-DNT in male rats. Groups of Sprague-Dawley rats (6/males/dose level) were gavaged with 2,6-DNT (>99% pure) at doses of 0, 4, 7, 14, 35, 68, 134 mg/kg-day for 14 days. All animals were observed twice a day for clinical signs of toxicity and morbidity. Body weight and food consumption were measured on Days 0, 1, 3, 7, and 14. Blood samples were collected for hematology and clinical chemistry tests at study termination prior to necropsy. Weights of the liver, spleen, kidneys, heart, brain, testes, and epididymides were recorded and relative organ weights were calculated. Various tissues underwent histopathological examination.

The study authors observed no clinical signs of toxicity. At study termination, absolute body weight exhibited a biologically relevant decrease (>10%) at 35, 68, and 134 mg/kg-day. This decrease in body weight was statistically significant at 134 mg/kg-day. Food consumption was also statistically significantly decreased at 134 mg/kg-day from Days 0 to 7. The study authors noted the following statistically significant changes in blood parameters: decreased hemoglobin and hematocrit in all dose groups, increased total and percent neutrophils at 68 and 134 mg/kg-day, increased monocytes and percent lymphocytes at 134 mg/kg-day, decreased albumin and total protein in all dose groups, decreased chlorine at 134 mg/kg-day, and increased ALT and AST at 68 and 134 mg/kg-day. Mild anemia was reported at 14 mg/kg-day. Relative kidney weight was statistically significantly increased at 14, 68, and 134 mg/kg-day. Relative spleen weight was statistically significantly increased at 68 and 134 mg/kg-day, and absolute spleen weight was statistically significantly increased at 134 mg/kg-day. Absolute and relative testes and absolute epididymides weights were statistically significantly decreased at 134 mg/kg-day. With respect to histopathological examination, the following changes were observed: tubular degeneration, multinucleated giant cell formation, and interstitial atrophy in the testes at 68 and 134 mg/kg-day and hepatocellular hyperplasia, oval cell hyperplasia, and hepatocellular hypertrophy were observed in the liver at ≥35 mg/kg-day. Mitotic activity, cell necrosis, and karyocytomegaly were also noted in the liver at ≥68 mg/kg-day. Proximal tubule degeneration and renal tubular basophilia were observed in the kidney at 134 mg/kg-day, as well as lymphoid hyperplasia at ≥68 mg/kg-day. Splenic extramedullary hematopoiesis was reported

at 68 and 134 mg/kg-day along with lymphoid depletion of the spleen at 134 mg/kg-day. The study authors did not report statistical analyses for any of the histopathological changes. Based on mild anemia and increased relative kidney weight, a LOAEL of 14 mg/kg-day is determined with a corresponding NOAEL of 7 mg/kg-day.

Subchronic Studies

Lee et al., 1976

Lee et al. (1976) conducted a series of tests investigating the subchronic oral toxicity of 2,6-DNT in rats, mice, and dogs. For the sake of clarity, in this document, the study is divided into three separate summaries (Lee et al., 1976) based on the species tested. These studies are not considered to be peer reviewed.

Lee et al., 1976a

Groups of CD rats (16/sex/dose level) were fed diets containing 2,6-DNT (>99% pure) at 0, 0.01, 0.05, or 0.25% for 4 or 13 weeks. These doses were calculated by the study authors to be equivalent to 0, 7, 35, or 145 mg/kg-day for males and 0, 7, 37, or 155 mg/kg-day for females. All animals were observed for clinical signs of toxicity and behavioral changes. Body weight was recorded weekly while food consumption was determined throughout the study. At 4, 8, 13, and/or 17 weeks, blood samples were collected for hematology and clinical chemistry tests. Of the 16 rats/sex/group, 4 were sacrificed at 4 weeks, and 4 were sacrificed at 13 weeks. Additionally, the treatment of 4 rats/sex/group was discontinued at the end of 4 and 13 weeks. These rats were kept for observation for 4 weeks and necropsied for examination at 8 and 17 weeks, respectively, to study the reversibility of any adverse effects and were examined for gross lesions. Weights of the liver, spleen, kidneys, heart, and brain were recorded. Relative organ weights were calculated. Various tissues were removed and stained for microscopic examination of lesions.

Lee et al. (1976a) reported that treatment with 2,6-DNT did not cause overt neuromuscular signs, but rats in the high-dose group (145 mg/kg-day [males], 155 mg/kg-day [females]) were less active and had rough coats and signs of malnutrition. There were no deaths during the study. Tables B.1 and B.2 provide treatment-related effects on body weights and absolute and relative organ weights. Body weights were markedly and consistently reduced at the mid- and high-doses of 35 and 145 mg/kg-day in males and 37 and 155 mg/kg-day in females throughout the exposure period. At 13 weeks, absolute body weights were 17 and 25% lower than controls in mid-dose males and females, respectively, and 53 and 38% lower than controls in high-dose males and females, respectively. Absolute body weights were also reduced in males and females at the low dose of 7 mg/kg-day for much of the study, but the difference from controls was less than 10% (9.8% in males and 5.5% in females).

Lee et al. (1976a) found that after 13 weeks, there were several statistically significant organ weight changes. Statistically significant increases in relative liver weight in males were observed in the liver at 7 mg/kg-day and at 145 mg/kg-day (but not at 35 mg/kg-day) and in females at 155 mg/kg-day. Absolute liver weight was statistically significantly increased in males at 35 and 145 mg/kg-day. Relative organ weights (spleen, kidneys, heart, and brain) were statistically significantly increased in males at 145 mg/kg-day. There were also statistically significant increases in absolute spleen, kidney, and heart weights in male rats at 145 mg/kg-day. Relative spleen weights were statistically significantly increased in females at 155 mg/kg-day,

and relative brain weights were statistically significantly increased at 37 and 155 mg/kg-day. Study authors also reported a statistically significant decrease in absolute heart weight in females at 155 mg/kg-day.

Lee et al. (1976a) observed no significant and/or consistent treatment-related hematological effects in the low-dose rats (7 mg/kg-day). A statistically significant decrease in reticulocyte count from baseline (baseline refers to data obtained from rats during pretreatment) was observed in control males and males at 7 and 35 mg/kg-day at 4 and 13 weeks (see Table B.3). A statistically significant increase in leukocyte count was observed in males at 35 mg/kg-day at Week 13. In addition, a statistically significant increase in erythrocyte count was observed in males at this dose group (35 mg/kg-day) at Weeks 4 and 13. This increase in erythrocyte count was considered as mild by the study authors and was not seen in male controls or males fed the low or high level of 2,6-DNT. Males at 145 mg/kg-day showed a decrease in erythrocyte count with a compensatory reticulocytosis and an increase in leukocyte count at 4 weeks; these parameters partially recovered at 8 (data not shown) or 13 weeks. Females fed 7 and 37 mg/kg-day had a statistically significant decrease in reticulocyte count at 4 and 13 weeks when compared to baseline. Females fed 37 mg/kg-day had a statistically significant increase in leukocytes at 13 weeks (see Table B.3). Females at 155 mg/kg-day showed an increase in leukocyte and reticulocyte count at 4 weeks; these parameters partially recovered at 13 weeks. A statistically significant increase in methemoglobin was seen in males at 145 mg/kg-day and in females at 155 mg/kg-day after 8 (data not shown) or 13 weeks; however, after recovery for an additional 4 weeks, this increase was no longer observed (data not shown). In summary, the only significant hematological changes from control were increased leukocytes (males), increased reticulocytes (males and females), and decreased erythrocytes (males) following 4 weeks of exposure to the high dose of 2,6-DNT (145 mg/kg-day [males] and 155 mg/kg-day [females]).

Lee et al. (1976a) reported extramedullary hematopoiesis in the spleen and liver; also, bile duct hyperplasia in the liver was observed in males at 35 mg/kg-day and in females at 37 mg/kg-day following 13 weeks of exposure (see Tables B.4 and B.5). Hemosiderosis was also observed in the spleen in males at 145 mg/kg-day and in females at 155 mg/kg-day at 13 weeks. Focal atrophy of the testes was observed in control and low dose males at 13 weeks. Retardation of spermatogenesis was observed at 13 weeks in mid-dose males, and atrophy of the testes and aspermatogenesis were observed in males at 145 mg/kg-day. In general, the effects on the testes, spleen, and liver were more frequent and more severe in the high-dose rats than in the mid-dose rats. Only partial recovery of the tissue lesions was seen after the 4-week recovery periods (data not shown).

Lee et al. (1976a) observed several effects in animals exposed to 2,6-DNT. Decreases in body weights (greater than 10%) were biologically significant in both males and females; however, study authors suggested that the decreases in body weight could be due to decreased food consumption. Therefore, decreased body weight could be due to the effect of 2,6-DNT on food palatability and not necessarily a systemic toxicological effect of the chemical. Additionally, hematological effects are noted but are only significantly different from control at the high-dose in animals exposed for 4 weeks, with no significant effects, as compared to control, at 12 weeks. Histopathology revealed lesions in the liver (i.e., hematopoiesis and bile duct hyperplasia) that were statistically significantly increased in males exposed to 35 mg/kg-day for 13 weeks and in females exposed to 37 mg/kg-day; incidence of bile duct hyperplasia was

statistically significant at 145 mg/kg-day in males and 155 mg/kg-day in females. Incidence of splenic hemosiderosis was statistically significantly increased as compared to controls in males exposed to 7 mg/kg-day for 13 weeks. However, at the next dose tested of 35 mg/kg-day, splenic hemosiderosis was not seen in any of the rats examined. While effects were also noted in the testes, atrophy observed in the control and low-dose group makes these effects difficult to interpret. Thus, based on the increased incidences of splenic and liver hematopoiesis in male rats, a LOAEL of 35 mg/kg-day is identified from this study with a corresponding NOAEL of 7 mg/kg-day.

Lee et al., 1976b

Lee et al. (1976b) fed groups of 16 male and 16 female albino Swiss mice a diet containing 0, 0.01, 0.05, or 0.25% 2,6-DNT (>99% pure) for up to 13 weeks. According to the authors, the corresponding intakes of test material were 0, 11, 51, or 289 mg/kg-day for males and 0, 11, 55, or 299 mg/kg-day for females. The basic design and procedure for this study were the same as that described for rats (Lee et al., 1976a); however, blood clinical chemistry tests in mice were not performed.

Lee et al. (1976b) reported no compound-related effects in mice at 11 mg/kg-day. However, several deaths occurred during the study, including 3 males in the control group (Week 12), 2 males in the low-dose group of 11 mg/kg-day (Weeks 1 and 3), 8 males and 1 female in the mid-dose group of 51 mg/kg-day (males) and 55 mg/kg-day (females), and 8 males and 6 females in the high-dose group of 289 mg/kg-day (males) and 299 mg/kg-day (females). Furthermore, all males in the high-dose group died before Week 9, while two of eight females in the high-dose group survived the full 13 weeks of feeding. The authors stated that in the mid- and high-dose groups, most of the deaths could be contributed to 2,6-DNT administration. The exact cause of death was not discussed, but most mice that died had low body weight, frequently with significant weight loss a week or two before death. In the mid- and high-dose groups, food consumption was lower than in controls. Blood analyses in the mid- and high-dose groups revealed a number of statistically significant changes relative to the controls at the respective time intervals; however, the authors stated that these changes were mild, inconsistent, and not related to 2,6-DNT exposure. The study authors also observed decreased absolute and relative liver weight (≥10%) at 11 mg/kg-day in male mice treated for 13 weeks. Absolute kidney weight in male mice was increased (≥10%) at 11 mg/kg-day and then decreased at 51 mg/kg-day. The biological significance of the observed decrease in absolute liver and kidney weights is unknown due to the accompanied increases in body weight that were biologically significant at 11 and 51 mg/kg-day in mice treated for 13 weeks. Relative kidney weight was biologically and statistically significantly decreased at 51 mg/kg-day in male mice treated for 13 weeks.

Lee et al. (1976b) noted marked aspermatogenesis in all males at 289 mg/kg-day, and depressed spermatogenesis was seen in one male from the mid-dose group (51 mg/kg-day) treated for 4 weeks and in two males from the low-dose group (11 mg/kg-day) treated for 4 or 13 weeks. Bile duct hyperplasia occurred in the only mouse that survived treatment with the high dose of 2,6-DNT for 13 weeks and in two mice fed the mid-dose for 13 weeks. Bile duct hyperplasia was also observed in two high-dose mice treated for 4 weeks and allowed to recover for 4 weeks, suggesting that this lesion developed slowly. The investigators indicated that extramedullary hematopoiesis in the liver and spleen was seen more often in mice treated with 2,6-DNT than in the controls and that generally, the incidence and severity were dose related.

No testicular lesions were observed in mice treated for 4 weeks and allowed to recover for 4 weeks. Because no high-dose males survived longer then Week 9, it is not known whether testicular lesions would have occurred in this dose group following 13 weeks of treatment. There was partial recovery of the bile duct hyperplasia after the 4-week recovery period, but extramedullary hematopoiesis continued to be observed in the liver and/or spleen. A LOAEL of 11 mg/kg-day is identified based on decreased relative liver weight. Because 11 mg/kg-day is the lowest dose tested, a NOAEL could not be determined.

Lee et al., 1976c

Lee et al. (1976c) is selected as the principal study for deriving the screening subchronic and chronic p-RfDs. Lee et al. (1976c) dosed groups of beagle dogs (4/sex/dose level) with 2,6-DNT (>99% pure) in gelatin capsules at doses of 0, 4, 20, or 100 mg/kg-day for 4 or 13 weeks. The dogs were evaluated in the same manner as in the rat study (Lee et al., 1976a) with the following exceptions: at the end of 4 and 13 weeks, one male and one female dog from each group were euthanized for necropsy; liver, spleen, kidneys, brain, adrenals, thyroid and gonads were examined and the weights recorded; the treatment for one male and one female dog from each group was discontinued at the end of 4 and 13 weeks and observed until necropsy at the end of 8 and 17 weeks, respectively; food consumption was recorded daily.

Lee et al. (1976c) observed the deaths of all dogs receiving 100 mg/kg-day of 2,6-DNT were between Weeks 2 and 8 (one dog died during the second week, and the last dog on this treatment died in the eighth week). The signs exhibited by these dogs consisted of listlessness, incoordination, lack of balance, pale gums, dark urine, and weakness (particularly of the hind limbs); tremors were seen occasionally. Terminal signs seen in some dogs included yellow gums and darkened sclera. Because of the severity of symptoms observed in the dogs exposed to 100 mg/kg-day of 2,6-DNT, they were placed on the reversibility study after 4 weeks and continued for 19 weeks (23 weeks total) before they were sacrificed. Similar symptoms were observed in dogs at 20 mg/kg-day but were less severe. Signs of toxicity in the mid-dose group (20 mg/kg-day) were not seen until Week 4. Two female dogs at 20 mg/kg-day died during Week 9. A Fisher's exact test comparing death in the control and mid-dose groups yielded a *p*-value of 0.233, indicating a nonstatistically significant difference. However, group sizes were too small for the statistical test to have much power to detect an effect and the deaths may have been compound-related, as gross necropsy showed emaciation and jaundice. No significant treatment-related effects occurred in dogs at 4 mg/kg-day (other than mild splenic extramedullary hematopoiesis in some dogs). However, animals at both 20 and 100 mg/kg-day showed clear signs of toxicity (neurological, hematological, and liver histopathology), and the incidence and severity of the effects were dose related. Extramedullary hematopoiesis in the spleen was observed at 4 mg/kg-day and appeared to be reversible depending upon the length of exposure and postexposure recovery period even at the higher doses.

Lee et al. (1976c) found no significant alterations in body weight in animals receiving 2,6-DNT at 4 mg/kg-day, but dogs at 20 mg/kg-day began to lose weight during Weeks 4 and 5, which correlated with the adverse effects previously noted. Dogs at 100 mg/kg-day lost weight from the first week of treatment. Food consumption correlated with weight changes. During the reversibility studies, the affected dogs quickly returned to normal food consumption rates.

Due to the mortality observed in one dog in the high-dose group during Week 2, the study authors collected blood samples from all surviving dogs in all dose groups at the end of Week 2, in addition to the scheduled analyses (Lee et al., 1976c). Control dogs and dogs at 4 mg/kg-day showed mild fluctuations in hematology and clinical chemistry parameters, which were not considered to be biologically significant by the study authors. However, significant effects were observed at 2 weeks in dogs exposed to 20 mg/kg-day, including anemia characterized by decreases in hematocrit and hemoglobin with a compensatory reticulocytosis. Small amounts of methemoglobin were seen at Week 8, and Heinz bodies were seen at Week 13. Serum alanine aminotransferase (ALT) activity was increased at Weeks 8 and 13. One of the females that died in Week 9 was severely anemic, with large amounts of Heinz bodies and methemoglobin, and elevated serum ALT, aspartate aminotransferase (AST), and alkaline phosphatase (AP). Blood analysis done on Week 2 in dogs at 100 mg/kg-day showed severe effects, including a 66% reduction in RBC and signs of immature erythrocytes. Also evident was leukocytosis, with an increased percentage of neutrophils and decreased percentage of lymphocytes, and increased serum AP and ALT activities. Laboratory data from dogs in the mid-dose group (20 mg/kg-day) treated for 4 or 13 weeks showed recovery after 4 weeks, but high-dose dogs (100 mg/kg-day) treated for 4 weeks did not recover until Week 19.

No significant alterations in organ weights were seen in dogs at 4 mg/kg-day compared to the control group (Lee et al., 1976c). Treatment-related histological alterations in dogs at both 20 and 100 mg/kg-day after 4 weeks of treatment included extramedullary hematopoiesis in the liver and spleen, bile duct hyperplasia, degeneration and/or subacute inflammation in the liver, and degeneration and/or depression of spermatogenesis in the testes. The incidence and severity of these lesions were generally dose related. Lymphoid depletion in the spleen and lymph node, and involution of the thymus were also seen in high-dose animals. A female dog from the low-dose group (4 mg/kg-day) had several graafian follicles but no corpora lutea. This female also had mild extramedullary hematopoiesis in the spleen. Because this effect was observed in dogs given this dose for 13 weeks and in dogs given higher doses, these alterations were considered to be compound related. Treatment up to 13 weeks with the mid- or high-dose of 2,6-DNT caused similar lesions in the liver and spleen. It also caused kidney effects consisting of dilated tubules, foci of inflammation, degeneration, yellow pigment, and/or casts in the tubules. The high-dose caused lesions in the testes, lymph nodes, and thymus. The effects observed in dogs treated with 20 mg/kg-day of 2,6-DNT at 13 weeks were usually more numerous and more severe than those seen at 4 weeks. Mild extramedullary hematopoiesis and lymphoid depletion in the spleen of some dogs at 4 mg/kg-day were also considered compound related by the study authors. Splenic extramedullary hematopoiesis was observed in all dogs treated at 4, 20, and 100 mg/kg-day for 13 weeks. In dogs treated for 4 weeks and allowed to recover, extramedullary hematopoiesis and adverse testicular effects were milder. Two dogs receiving 2,6-DNT at 100 mg/kg-day and allowed to recover for 19 weeks showed complete recovery. Dogs treated for 13 weeks did not show full recovery, as one dog in the mid-dose group still had various lesions in the liver, kidney, and testes, and a low-dose female dog still had minimal bile duct hyperplasia. In dogs treated for 13 weeks and allowed to recover for 4 weeks, splenic extramedullary hematopoiesis was not observed in any dose group. To determine whether treatment with 2,6-DNT causes an allergic reaction, the study authors measured its effects on serum IgE levels. The results revealed no apparent change in serum IgE concentration.

A LOAEL of 4 mg/kg-day is identified based on splenic extramedullary hematopoiesis. However, the biological significance of this endpoint is indefinite. Because 4 mg/kg-day is the lowest dose tested, a NOAEL cannot be identified.

Chronic Studies

Leonard et al., 1987

Leonard conducted a 1-year chronic toxicity and carcinogenicity study on pure 2,6-DNT and technical grade DNT. The unpublished study was designed to compare the hepatic carcinogenic potential of technical grade DNT, 2,6-DNT, and 2,4-DNT. The tumor data reported in this study are discussed separately under the carcinogenicity studies subheading below.

In a peer-reviewed study, Leonard et al. (1987) fed groups of 28 male Fischer (F344)/CrlBR rats a diet containing 2,6-DNT at doses of 0, 7, or 14 mg/kg-day for 52 weeks. The authors stated that purified 2,6-DNT was used, but the actual purity was not specified. Concentrations of 2,6-DNT were adjusted in each diet batch based on food consumption and average body weight to maintain the 2,6-DNT doses at the target levels. Rats were housed as four per cage, and average dietary consumption for each cage was determined weekly. Body weights were measured every 2 weeks throughout the study. The study authors sacrificed 4 animals in each group after 6 and 26 weeks of feeding and measured hepatic microsomal epoxide hydrolase (EH) and cytosolic DT-diaphorase (DTD) activities (these are considered to be phenotypic markers of neoplastic lesions). At the end of the treatment period, all surviving animals were sacrificed and necropsied, selected organs were weighed (liver and lungs), histopathological examination was performed on liver and lung tissue, and hepatic EH and DTD activities were measured. Serum enzyme activities (ALT and glutamyl transferase [GGT]) were also determined. Statistical evaluations were done using the F-test and Dunnett's test ($p \leq 0.05$). Hematology and clinical chemistry were not evaluated in rats sacrificed at 1 year.

Leonard et al. (1987) reported no treatment-related deaths. Statistically significant changes were seen in body weight, organ weight, and serum chemistry in rats that received 7 or 14 mg/kg-day 2,6-DNT at 26 or 52 weeks, including decreased body weight, increased absolute and relative liver weights and increased serum GGT activity (see Table B.11). These effects, however, were more pronounced at 52 weeks. For example, terminal body weights of the 7 and 14 mg/kg-day rats were decreased relative to the controls by 5 and 18% and 20 and 32% at Weeks 26 and 52, respectively. It is unclear from the study report if the observed decreased body weight was due to a systemic effect of 2,6-DNT or an effect of the chemical on food palatability and consumption. At 52 weeks, statistically significantly increased serum ALT activity was seen in both the 7- and 14-mg/kg-day dose groups, and statistically significantly increased serum gamma-glutamyl transpeptidase activity was seen at 14 mg/kg-day (see Table B.11). The study authors did not report the effects of 2,6-DNT exposure for 6 weeks on body weight, liver weight, or serum enzyme activities. Microscopic evaluation of the liver sections from animals that had received 52 weeks of dietary treatment revealed hepatocyte degeneration and vacuolation in the majority of these treated animals at all doses. These effects, however, did not appear to be dose-related and were also seen in controls. Over 90% of the treated animals had acidophilic and basophilic hepatocyte foci. Neither type of foci was apparent in the controls. The study authors also noted bile duct hyperplasia in most animals fed 2,6-DNT. No specific mention was made of nonneoplastic lesions in the lungs. A LOAEL of 7 mg/kg-day based on a biological (≥10% change) and statistically significant increase in relative liver weight observed at 12 months is identified from this study. Because 7 mg/kg-day is the

lowest dose tested, a NOAEL cannot be identified from this study. It is unclear if the noncancer liver effects observed in this study were due to the presence of hepatocellular tumors caused by 2,6-DNT treatment.

Developmental Studies

No studies were identified.

Reproductive Studies

The studies by Lee et al. (1976) report limited reproductive toxicological endpoints (e.g., aspermatogenesis). These studies are summarized in the *Subchronic Studies* section.

Carcinogenicity Studies

Goldsworthy et al., 1986

Goldsworthy et al. (1986) conducted a study to evaluate the effect of diets varying in pectin content on the induction of foci and hepatic tumor by 2,6-DNT. Six groups of 30 male F344 (CDF/CrlBR) rats were placed on one of three diets containing sufficient quantities of 2,6-DNT (purity of 99.9%) to produce daily doses of 0.6−0.7 or 3−3.5 mg/kg-day. The diets used were NIH-07, an open formula cereal-based diet high in pectin content; AIN-76A, a purified pectin-free diet; or AP, which is AlN-76A supplemented with 5% pectin. These three diets served as the control diets for the addition of 2,6-DNT (see Table B.12). The study authors incorporated 2,6-DNT into the diets by premixing the 2,6-DNT into 100 grams of the test diet followed by blending the mixture. Body weight and food consumption were recorded monthly. The study authors screened the rats for the absence of viral titers throughout the study. Ten animals from each group were sacrificed at 3, 6, and 12 months, and the livers were evaluated histopathologically. Quantitative stereology was used to assess the number of hepatic foci per liver. Three markers commonly used to detect hepatic preneoplasia—GGT, ATP, G6P—were used to score and quantitate foci. The study authors performed statistical analysis on the number of foci per cm^3 liver using a Newman-Keuls multiple comparison test ($p \leq 0.05$).

Goldsworthy et al. (1986) reported no deaths in the control or treatment groups. Body weight was increased in all rats fed all three diets (all animals in the 6 groups) for 3, 6, and 12 months. All groups receiving the high-dose of 2,6-DNT (3.0−3.5 mg/kg-day) gained approximately 10% less weight than their respective controls at 12 months (data on body and liver weight were presented in a graphical format by the study authors and not in tables). Liver weights were not significantly altered throughout the study compared to the control groups, except for the high-dose NIH-DNT (3−3.5 mg/kg-day) treated group, which showed a marked increase in liver weights at 12 months. In the other dose groups, the liver weights remained constant, and the liver/body-weight ratio was decreased in rats throughout the treatment periods. The study authors reported that no changes in monthly food consumption were observed in treated rats during the treatment period (data were not shown in the study). No further information was provided regarding nonneoplastic effects.

Goldsworthy et al. (1986) examined the effect of the control and DNT-diets on the fraction of animals with foci (see Table B.13). The fraction of animals with hepatocyte foci (i.e., GGT, ATP, G6P) was increased in a dose- and time-dependent manner in animals administered 2,6-DNT in the test diet with NIH > AP > AIN. Animals fed AIN and AP diets, with or without 2,6-DNT, had few or no GGT foci throughout the study. At 12 months, the number of ATP and G6P foci was approximately equal in all DNT-treated groups, and the number of GGT foci in the

high dose NIH-DNT group (3−3.5 mg/kg-day) was impossible to quantitate accurately, which was explained by the study authors to be due to the presence of neoplastic nodules and hepatocellular carcinomas.

Hepatocellular carcinomas and neoplastic nodules were observed only in rats fed the NIH diet containing 2,6-DNT (statistical analysis was only done on the number of foci per cm^3 in the liver and not on the hepatocellular carcinomas and neoplastic nodules). At 12 months, the treatment group on the NIH diet that received the high dose of 2,6-DNT (3−3.5 mg/kg-day) exhibited a 100% incidence of hepatic foci, including 6/10 rats with hepatocellular carcinomas and 6/10 with neoplastic nodules; the low dose of 2,6-DNT (0.6−0.7 mg/kg-day) exhibited 3/10 rats with neoplastic nodules, each rat with a single nodule; and the control diet had no tumors or neoplastic nodules present in 10 rats. No tumors or neoplastic nodules were observed in rats receiving the control AIN diet with or without added pectin or added 2,6-DNT in these diets.

Based on these results, the study authors concluded that 2,6-DNT is a potent hepatocarcinogen in male F344 rats, and its carcinogenic potency differs depending on whether rats are fed an NIH or AIN (with or without pectin) diet. They stated that the carcinogenicity of 2,6-DNT would not have been observed in the study if tested only in the pectin purified diets and scored by the GGT marker alone.

Leonard et al., 1987

The carcinogenic study by Leonard et al. (1987) is selected as the principal study for deriving the oral slope factor (p-OSF). In a peer-reviewed study, Leonard et al. (1987) fed groups of 28 male Fischer (F344)/CrlBR rats a diet containing 2,6-DNT at doses of 0, 7, or 14 mg/kg-day for 52 weeks. Other details concerning the study methodology are presented in the *Chronic Studies* section.

Leonard et al. (1987) noted that administration of 2,6-DNT at doses of 7 and 14 mg/kg-day to male rats in the diet for 52 weeks resulted in an increased incidence of neoplastic lesions in the liver (see Table B.14). Neoplastic nodules were found in 18/20 rats at the low-dose (7 mg/kg-day) and 15/19 rats at the high-dose (14 mg/kg-day). Hepatocellular carcinomas, described as trabecular, occurred in 17/20 rats at 7 mg/kg-day and 19/19 rats at 14 mg/kg-day, and one tumor described as an adenocarcinoma was found in a rat at 7 mg/kg-day. Cholangiocarcinomas occurred in 2/20 rats at 7 mg/kg-day. Liver tumors metastasized to the lung in 3/20 rats at 7 mg/kg-day and 11/19 at 14 mg/kg-day. These results indicated that overall neoplastic nodule incidence paralleled tumor incidence, with the exception of the high-dose 2,6-DNT-treated group. According to the study authors, this difference is due to the extensive tumor involvement in the livers from the high-dose group.

Hepatic microsomal EH and cytosolic DTD activity are induced following treatment with a number of hepatocarcinogens and are considered to be phenotypic markers of neoplastic nodules (Leonard et al., 1987). The study authors noted a dose-related increase in EH activity in rats treated with 7 mg/kg-day and 14 mg/kg-day for 26 weeks to 380% and 520% of controls, respectively. This increase was sustained at similar levels from 6 weeks to 1 year (data in the study on hepatic microsomal EH and cytosolic DTD activity were presented in graphical format and not in a table). At 1 year, EH activity in the high-dose group was lower than that observed at 6 weeks and 26 weeks (220% of controls). The study authors attributed this change to the

extensive tumor burden in the livers from these animals. They also stated that this observation is consistent with the lower nodule incidence observed in these animals, and this provides indirect evidence to support the suggestion that EH elevations occur in the nodules but not the tumors. In contrast to EH activities, DTD activity was increased in the high-dose 2,6-DNT-treated rats at 6 weeks. This increase was enhanced to a somewhat greater extent in both 2,6-DNT-treated groups at 26 weeks. At 12 months, DTD activities were maximally enhanced in the low- and high-dose 2,6-DNT-treated animals to 420 and 650% of controls, respectively. The study authors stated that the increase in DTD activity appeared to be linked to the presence of nodules and tumors, particularly at 12 months.

In summary, administration of 2,6-DNT at oral doses of 7 and 14 mg/kg-day produced hepatocellular carcinomas in 85% and 100% of male rats, respectively. The majority of tumors had a trabecular pattern, and pulmonary metastases. Similar to the 2,6-DNT results, rats fed a diet containing 35 mg/kg-day technical grade DNT (equivalent to 7 mg/kg-day 2,6-DNT) yielded a positive hepatocarcinogenic response. The 2,6-DNT isomer induced hepatocellular carcinomas in twice as many animals as did the technical grade DNT, and 2,4-DNT was not hepatocarcinogenic when fed to rats at twice the high dose of 2,6-DNT (2 mg/kg-day) over the same time period. The study authors concluded that 2,6-DNT is a complete carcinogen, capable of both initiation and promotion, and the hepatocarcinogenicity of technical grade DNT is mainly due to 2,6-DNT.

Stoner et al., 1984

In a 12-week study, Stoner et al. (1984) administered 0, 1200, 3000, or 6000 mg/kg 2,6-DNT (98% purity) to A/J mice (26 mice/sex/dose group) for 2 days/week for 12 weeks by gavage. The corresponding duration-adjusted doses are 0, 342.9, 857.1, or 1714 mg/kg-day. The animals were sacrificed after 30 weeks, and the lungs were examined. Lung tumors, which appeared as white nodules on the surface, were counted and randomly sampled for histopathological evaluation and confirmation of adenoma. In addition, liver, kidneys, spleen, intestines, thymus, stomach, and endocrine glands were examined grossly. If gross lesions were observed, the organs were examined histologically for the presence of neoplasms. The lung tumor response (percentage of mice that developed lung tumors and the number of lung tumors per mouse) in experimental and control groups was compared by Student's t-test. No increase in lung tumor incidence or in the number of lung tumors per mouse, as compared to controls, was observed.

Ellis et al., 1979

Ellis et al. (1979) performed a 2 year carcinogenicity study in which Sprague Dawley rats (38/sex/dose), CD-1 Swiss mice (58/sex/dose), and beagle dogs (6/sex/dose) were treated with 2,4-DNT (approximately 98% 2,4-DNT and 2% 2,6-DNT) in the diet. The rat portion of the study was used as the principal study by IRIS to derive the p-OSF for the 2,4-/2,6-DNT mixture (various compositions of DNTs; U.S. EPA, 1990). Because the focus of this PPRTV is to review the toxicity of only 2,6-DNT, the Ellis et al. (1979) study is not considered as a potential principal study because of the low amount (2%) of 2,6-DNT that was in the test compound.

Inhalation Exposures

No studies were identified.

OTHER DATA (SHORT-TERM TESTS, OTHER EXAMINATIONS)

Studies on the genotoxicity, carcinogenicity by routes other than oral or inhalation, short-term toxicity, toxicokinetics, and mode of action/mechanism of 2,6-DNT are available. These are summarized in Tables 4A and 4B.

Table 4A. Summary of 2,6-Dinitrotoluene Genotoxicity Studies

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, TA100, TA1535, TA1538)	+ (TA98, TA100, TA1535, TA1537, TA1538)		Couch et al. (1981)
Reverse mutation	*S. typhimurium* strains TA98, TA1537, TA1538	NR	− (TA98, 1537) + (TA1538)	− (TA98, TA1537, TA1538)		Ellis et al. (1978, as cited in ATSDR, 1998)
Reverse mutation	*S. typhimurium* strain TA100	50−1000 µg/plate	+	ND		Simmon et al. (1977)
Reverse mutation	*S. typhimurium* strains TA98, TA100	5−2000 µg/plate	−	−		Sayama et al. (1989, 1992)
Reverse mutation	*S. typhimurium* strains TA98, TA100, YG1021, YG1024, YG1026, YG1029, YG1041, YG1042	0−5 µM	− (TA98, TA100, YG1021) + (YG1024, YG1026, YG1029, YG1041, YG1042)	ND	Highest degree of mutagenicity in YG1041 and YG1042	Sayama et al. (1998)
Reverse mutation	*S. typhimurium* strains TA98, TA100	NR	+ (TA98, TA100)	+ (TA98) − (TA100)		Tokiwa et al. (1981)

Table 4A. Summary of 2,6-Dinitrotoluene Genotoxicity Studies

Endpoint	Test System	Dose Concentration[a]	Results[b]		Comments	References
			Without Activation	With Activation		
Reverse mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538, TA100NR3	10−5000 µg	+ (TA100) − (TA98, TA1535, TA1537, TA1538, TA100NR3)	+ (TA100) − (TA98, TA1535, TA1537, TA1538, TA100NR3)		Spanggord et al. (1982)
Reverse mutation	*S. typhimurium* strains TA98, TA100	0.1−10 µmol/plate	− (TA98, TA100)	+ (TA98) − (TA100)	In TA98, it was negative with rat liver activation and positive with hamster liver activation and in TA100 it was negative with both rat and hamster liver activation	Dellarco and Prival (1989)
Reverse mutation	*S. typhimurium* strains TA98, TA1538	1−1000 nmol/plate	+ (weakly positive at highest doses)	+ (weakly positive at highest doses)		Whong and Edwards (1984)
Reverse mutation	*S. typhimurium* strains TA98, TA98NR, TA98/1,8-DNP_6, YG1021, YG1024	NR	+ (TA98, YG1021, YG1024, TA98/1,8-DNP_6) − (TA98NR)	ND		Einistoe et al. (1991)
Reverse mutation	*S. typhimurium* strains NR	NR	NT	+		Pearson et al. (1979, as cited by ATSDR, 1998)
Reverse mutation	S. typhimurium strains TA98, YG1021, YG1024, YG1041	10−100 µg/plate	+ (TA98, (YG1021 YG1024, YG1041)	ND		Hagiwara et al. (1993)

Table 4A. Summary of 2,6-Dinitrotoluene Genotoxicity Studies

Endpoint	Test System	Dose Concentration[a]	Results[b]		Comments	References
			Without Activation	With Activation		
Reverse mutation	*S. typhimurium* strains TA98, TA100	50−500 µg/plate	− (TA98) + (TA100)	− (TA98) + (TA100)		George et al. (2001)
Reverse mutation	*S. typhimurium* strain TA100	1−150 µg/mL	−	ND		Padda et al. (2003)
Forward mutation	TM 677	500 µg/mL	+	+	2,6-DNT and technical-grade DNT	Couch et al. (1981)
SOS repair induction	ND					
Genotoxicity studies in nonmammalian eukaryotic organisms						
Mutation	ND					
Recombination induction	ND					
Chromosomal aberration	ND					
Chromosomal malsegregation	ND					
Mitotic arrest	ND					
Genotoxicity studies in mammalian cells—in vitro						
Mutation	Chinese hamster ovary/HGPRT	2.5 mM	−	−		Abernathy and Couch (1982)
Mutation	Chinese hamster ovary	NR	−	ND		Lee et al. (1976)
Mutation	P388 mouse lymphoma cells	1.6−1000 µg/mL	−	−	2,6-DNT and technical grade DNT	Styles and Cross (1983)
Morphological transformation	Syrian hamster embryo cells	up to 100 µg/mL	−	ND	2,6-DNT and technical grade DNT	Holen et al. (1990)

Table 4A. Summary of 2,6-Dinitrotoluene Genotoxicity Studies

Endpoint	Test System	Dose Concentration[a]	Results[b]		Comments	References
			Without Activation	With Activation		
Chromosomal aberrations	Human peripheral lymphocytes	0.002−0.10 mmol/L	+	ND		Huang et al. (1995)
Sister chromatid exchange (SCE)	ND					
DNA damage	Rat germ cells	0.000032−0.02 mmol/L	+	ND	DNA strand breaks showed a dose-response relationship	Yang et al. (2005)
DNA adducts	ND					
DNA repair	Primary rat hepatocytes	1×10^{-4}, 1×10^{-3} M	−	ND		Bermudez et al. (1979)
DNA repair	Primary rat hepatocytes	0.1, 1.0 mM	−	−		Butterworth et al. (1989)
DNA repair	Human hepatocytes	0.01−1.0 mM	−	−		Butterworth et al. (1989)
Unscheduled DNA synthesis	Rat spermatocytes	10−1000 µM	−	ND		Working and Butterworth (1984)
DNA and protein synthesis	Chinese hamster ovary cells	10−1000 µg/mL	+ (weak)	ND		Garrett and Lewtas (1983)
Genotoxicity studies in mammals—in vivo						
Chromosomal aberrations	CD rats (sex not reported)	35−37 mg/kg-d	+	ND		Lee et al. (1976)
Sister chromatid exchange (SCE)	ND					
DNA damage	Male Sprague-Dawley rats	35, 68, and 134 mg/kg-d (gavage)	+ (liver)	ND		Lent et al. (2012b)

Table 4A. Summary of 2,6-Dinitrotoluene Genotoxicity Studies

Endpoint	Test System	Dose Concentration[a]	Results[b]		Comments	References
			Without Activation	With Activation		
DNA adducts	Male F344 rats	1.2 mmol/kg i.p.	+	ND		La and Froines (1993)
Mouse biochemical or visible specific locus test	ND					
Dominant lethal	ND					
Unscheduled DNA synthesis	Male F344 rats	5−100 mg/kg (gavage)	+	ND		Mirsalis and Butterworth (1982)
Unscheduled DNA synthesis	Male F344 rats	20 mg/kg (gavage)	−	ND		Working and Butterworth (1984)
Micronucleus test	Male F344 rats	125, 250 mg/kg (gavage)	+ (liver) − (peripheral blood)	ND		Takasawa et al. (2010)
Micronucleus test	Male Sprague-Dawley rats	35, 68, and 134 mg/kg-d (gavage)	− (peripheral blood)	ND		Lent et al. (2012b)
Genotoxicity studies in subcellular systems						
DNA binding	ND					

[a]Lowest effective dose for positive results, highest dose tested for negative results.
[b]+ = Positive, ± = Equivocal or weakly positive, − = Negative, T = Cytotoxicity, NA = Not applicable, ND = No data, NDr = Not determined, NR = Not reported, NR/Dr = Not reported, but determined from data.

Table 4B. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Carcinogenicity other than oral/inhalation	26/26 A/J mice were administered 2,4-DNT (92−95% pure, with the major impurity being 2,6-dinitrotoluene), 2,6-DNT (98% pure), and 2:l mixture of 2,4-DNT and 2,6-DNT by i.p. injection at doses of MTD (maximal tolerated dose), 0.5 MTD, and 0.2 MTD three times per week for 8 wk. The total doses were 0, 600, 1500, and 3000 (maximum tolerated dose, MTD) mg/kg bw for 2,4- and 2,6-DNT. For the 2:l mixture of 2,4-DNT and 2,6-DNT, the total doses were 0, 960, 2400, and 4800 (MTD) mg/kg bw. Animals were killed after 30 wk, and the lungs, liver, kidneys, spleen, intestines, thymus, stomach, salivary, and endocrine glands were examined grossly. If gross lesions were observed, they were examined histologically for the presence of neoplasms.	No increase in lung tumor incidence or in the number of lung tumors per mouse was observed compared to controls in all three compounds. No lesions were observed in any other organ site.	2,6-DNT, 2,4-DNT, and the 2:l mixture of 2,4-DNT and 2,6-DNT did not induce lung tumors in A/J mice when given by i.p. injection. According to the study authors, the inability of these dinitrotoluenes to induce lung tumors in A/J mice is expected because it is known that hepatocarcinogens are either inactive or only weakly active for lung tumor induction in strain A mice.	Stoner et al. (1984)

Table 4B. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Carcinogenicity other than oral/inhalation	Study authors performed a number of initiation-promotion liver foci assays in CDF (F344)/CrlBR rats using 4 initiation-promotion protocols. The study aimed to evaluate the relative initiating potential of each DNT isomer (2,4 and 2,6) and compare it with the initiating potential of technical grade DNT. A group of 8−10 male (F344)/CrlBR rats were administered by gavage a single dose of 75 mg/kg bw in corn oil of either technical grade DNT or 2,6-DNT (purity >99.4%), or 2,4-DNT (purity >99.4%) at 12 hr post partial hepatectomy. The numbers of gamma glutamyltranspeptidase-positive (GGT) foci were quantified.	Technical grade DNT was a weak initiator when administered as a single oral dose (75 mg/kg) at 12 hr post hepatectomy. Significant dose-related increases in the number of GGT^+ foci were observed compared with control rats administered 2,6-DNT. No initiating activity was demonstrated with 2,4-DNT.	2,6-DNT and technical grade DNT were weak tumor initiators with comparable initiating activity. Thus, the initiating activity of 2,6-DNT likely accounts for the initiating activity in technical grade DNT. 2,4-DNT was not a tumor initiator.	Leonard et al. (1983)

Table 4B. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Carcinogenicity other than oral/inhalation	A rat hepatic initiation-promotion protocol, 8−10 male CDF (F344)/CrlBR rats were initiated with a single dose of 150 mg/kg bw diethylnitrosamine by i.p. injection and permitted to recover for 2 wk. After the recovery period, the animals were placed on diets containing: • 27 mg/kg-d of 2,4-DNT (99.4% pure) or • 2.8, 7 or 14 mg/kg-d 2,6-DNT (99.4% pure) or • 14 or 35 mg/kg-d technical grade DNT Rats receiving technical-grade DNT were killed after 3 or 6 wk of feeding and those receiving the purified isomers after 6 or 12 wk of feeding. Sections from three liver lobes of each animal were stained for gamma glutamyl transferase (GGT), and the number of GGT^+ foci per cm^3 was calculated.	Initiation-promotion assay: 1) Technical grade DNT: at 3 wk, dose-dependent increase in number of GGT^+ foci and foci volume; at 6 wk, time-dependent increase in the number of foci and foci volume relative to 3 wk treatment. 2) 2,6-DNT: time- and dose-dependent increase in the number of GGT^+ foci and foci volume at 6 wk 3) 2,4-DNT: time-dependent increase in the number of GGT^+ foci at 6 wk. Technical grade DNT, 2,4-DNT, and 2,6-DNT had hepatocyte foci-promoting activity.	2,6-DNT is a complete hepatocarcinogen while 2,4-DNT was a pure promoter.	Leonard et al. (1986)
LD_{50} studies	Rat (gavage) Mouse (gavage)	LD_{50} (rat) = 795 mg/kg to180 mg/kg LD_{50} (mouse) = 621 mg/kg to 807 mg/kg	LD_{50} (rat) = 795 to 180 mg/kg-d LD_{50} (mouse) = 621 to 807 mg/kg	Lee et al. (1975, as cited in ATSDR, 1998); Ellis et al. (1978, as cited in ATSDR, 1998); Vernot et al. (1977, as cited in ATSDR, 1998)

Table 4B. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Metabolism/ toxicokinetic	Male and female F344 rats were administered by gavage (^{14}C)-ring-labeled 2,6-DNT (99% radio chemically pure) at a dose of 10 mg/kg-bw. Urine and fecal samples were collected and analyzed for metabolites.	The major route of excretion of ^{14}C after a single dose was via the urine (males, 53.6 ± 2.6%; females, 54.0 ± 4.8%). Fecal excretion accounted for 17.9% (males) and 19.8% (females) of the dose. The urinary metabolites of 2,6-DNT identified were • 2,6-dinitrobenzyl (21.7%); • 2,6-dinitrobenzoic acid (21.1%); and • 2-amino-6-nitrobenzoic acid (14.0%). Results were compared with to those found after administration of 10 mg/kg bw 2,4-DNT to male and female F344 rats. The only major difference in the disposition of the two isomers was that no *N*-acetylaminonitrobenzoic acid was found after administration of 2,6-DNT in vitro. This may reflect steric hindrance to *N*-acetylation of an amino group adjacent to a methyl group.	Urine is the major route of 2,6-DNT excretion. Disposition of 2,4-DNT and 2,6-DNT is not the same	Long and Rickert (1982, as cited in IARC, 1996)
Metabolism/ toxicokinetic	Six male F344 rats received an i.p. injection of either sulfotransferase inhibitor: DCNP (2,6-dichloro-4-nitrophenol) or PCP (pentachlorophenol) (40 µmol/ kg). After 45 min, three of these animals were administered orally [3-^{3}H]-2,6-DNT at a dose of 28 mg/kg and were euthanized 12 hr later. Urine was collected for analysis of metabolites.	Prior administration of PCP had no significant effect on the excretion of the benzyl glucuronide or benzoic acid metabolites of 2,6-DNT. (The effects of DCNP on 2,6-DNT excretion were not tested.)	Prior administration of PCP had no significant effect on the excretion of urinary metabolites of 2,6-DNT.	Kedderis et al. (1984)

Table 4B. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Metabolism/ toxicokinetic	The study authors examined metabolites formed by anaerobic incubation of 2,6-DNT or 2,4-DNT with intestinal microflora of male Wistar rats in vitro.	The metabolites formed with the incubation of 2,6-DNT were • 2-nitroso-6-nitrotoluene (reached peak at 2 hr of the anaerobic incubation); • 2-hydroxyl amino-6-nitrotoluene (reached peak at 5 hr); • 2-amino-6-nitrotoluene (reached peak at 6 hr); and • 2,6-diaminotoluene (reached peak at 12 hr). Two nitroazoxy compounds: 2,2'-dimethyl-5,5'-dinitroazoxybenzene and 4,4'-dimethyl-3,3'-dinitroazoxybenzene, in addition to known metabolites (nitrosonitrotoluenes, hydroxylaminonitrotoluenes, aminonitrotoluenes, and diaminotoluene), were detected in the incubation of 2,4-DNT with intestinal microflora.	2,6-diaminotoluene is the terminal intestinal metabolite of 2,6-DNT.	Sayama et al. (1993, as cited in ATSDR, 1998)
Metabolism/ toxicokinetic	Groups of six male F344 rats were pretreated with Aroclor 1254 at a dose of 25 mg/kg for 1 week and then administered 75 mg/kg 2,6-DNT in DMSO by gavage for 5 wk. Urine was collected for analysis. Interim sacrifices were carried out at 2 and 4 wk of treatment, and the liver, small intestine, large intestine, and cecum of each rat was excised at autopsy for analysis. Gastrointestinal enzyme activities were measured, and DNA adducts from liver were also determined.	A significant increase in the excretion of mutagenic urinary DNT metabolites was observed after the first week of Aroclor 1254 treatment, peaked at Wk 2 and then declined by nearly 25% at Wk 4. However, at the end of the treatment, a 4-fold increase in the formation of hepatic DNA adducts was observed. This increase in DNA adducts and decrease in urinary mutagens was due to the significant elevation in hepatic metabolism and to the increase in ß-glucuronidase activity in the small intestine and cecum at 4 wk.	Pretreatment of F344 rats with Aroclor 1254 significantly altered select intestinal enzyme activity, stimulated hepatic enzyme activity, accelerated the biotransformation and bioactivation of 2,6-DNT, and potentiated the formation of 2,6-DNT-derived DNA adducts in the liver. The authors noted that hepatic metabolism alone likely did not account for the potentiated bioactivation of 2,6-DNT.	Chadwick et al. (1993)

Table 4B. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Metabolism/ toxicokinetic	2,6-DNT metabolism by human liver and male F344 rat liver subcellular fractions under aerobic (100% oxygen) and anaerobic (100% nitrogen) incubations conditions was examined.	Under aerobic conditions, • The major 2,6-DNT metabolite formed by hepatic microsomes was 2,6-dinitrobenzyl alcohol (2,6-DNBalc); and • Rates of 2,6-DNBalc formation by human and rat liver microsomes under aerobic conditions were 247 and 132 pmol/min per mg protein, respectively. Under anaerobic conditions, • 2-amino-6-nitrotoluene (2Am6NT) was the major metabolite; and • Rates of 2Am6NT formation by human and rat liver microsomes were 292 and 285 pmol/min per mg protein, respectively. Anaerobic reduction of 2,6-DNT and 2Am6NT by rat and human liver microsomes is mediated by cytochrome P-450. Liver cytosolic fractions also metabolized 2,6-DNT to 2Am6NT under anaerobic conditions.	The major metabolites isolated from microsomal fractions of human and rat liver preparations incubated with 2,6-DNT were 2,6-dinitrobenzyl alcohol and 2-amino-6-nitrotoluene.	Chapman et al. (1992, 1993)

Table 4B. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Metabolism/ toxicokinetic	Male Wistar rats (n = 6) were administered orally a single dose of 2,6-DNT or 2,4-DNT (75 mg/kg). Urine samples were collected after 24 hr and were analyzed for conjugated and unconjugated metabolites using high performance liquid chromatography.	The major urinary metabolite identified after oral administration of 2,6-DNT was 2,6-dinitrobenzyl glucuronide, which accounted for about 17.4% of the administered dose. Other metabolites identified were • 2,6-dinitrobenzyl alcohol (0.53%); • 2-amino-6-nitrotoluene (0.44%); and • 2,6-dinitrobenzoic acid (0.17%). Urinary excretion of oxidized and *N*-acetylated derivatives for 2,4-DNT was observed but not after administration of 2,6-DNT, demonstrating metabolic differences in male Wistar rats for the two isomers.	2,6-dinitrobenzyl glucuronide is the major urinary metabolite of 2,6-DNT. Metabolic differences for 2,4-DNT and 2,6-DNT exist in male Wistar rats. Metabolism of 2,6-DNT differs between two strains of rat (Wistar and F344 [Rickert et al., 1983]).	Mori et al. (1996)

Table 4B. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Metabolism/ toxicokinetic	Ninety-nine Chinese workers exposed to dinitrotoluenes and 61 nonmatched, nonexposed controls working in the same factory manufacturing TNT were examined and subjected to questionnaires inquiring about exposure history and other health and lifestyle factors. Blood samples were collected, and the levels of hydrolyzable Hb adducts were determined. Female Wistar rats (n = 3) were administered a single dose of 0.5 mmol/kg of 2,6-DNT or 2,4-DNT by gavage. The rats were killed after 24 hr, and hydrolyzable hemoglobin (Hb) adducts were determined for each of the compounds investigated. The Hb adduct profile in rats was compared to those in Chinese workers.	Hydrolysis of Hb from rats dosed with 2,4-DNT yielded 4-amino-2-nitrotoluene (4A2NT), 24TDA, and 4-acetylamino-2-aminotoluene (4AA2AT). Hydrolysis of Hb from rats dosed with 2,6-DNT yielded three amines, 2-amino-6-nitrotoluene (2A6NT), 26TDA and 2-acetylamino-6-aminotoluene (2AA6AT) with 2A6NT being the predominant adduct. Hb adduct levels in rats dosed with 2,4-DNT were higher than adduct levels in rats dosed with 2,6-DNT. A similar Hb adduct pattern was found in Chinese workers exposed to dinitrotoluenes, although 2-acetylamino-6-aminotoluene (2AA6AT) was not found in the workers. 2A6NT was the predominant adduct in 2,6-DNT exposed workers, and 4A2NT was the predominant adduct in 2,4-DNT exposed workers.	Quantification of DNT-Hb adducts provided an effective biomarker of toxicity (inertia, somnolence, nausea, and dizziness) and could be used to estimate the risk associated with a particular exposure to DNT.	Jones et al. (2005)
Distribution and absorption/ toxicokinetic	Male F344 rats (CDF (F344)/CrlBR) (36/group) were administered gavage doses of 10 or 35 mg/kg of radiolabeled 2,6-DNT. Three rats from each group were killed at 1, 2, 4, 8, 12, 24, 48, 96, 192, and 384 hr after the dose. Livers and intestine were removed and analyzed for metabolites.	Increase in hepatic concentrations of radioactivity in male rats in 2 stages, with the first peak occurring l−2 hr and a second peak occurring 8−12 hr after the dose. The second peak was followed by a gradual decline up to 16 d and was thought to be the result of enterohepatic cycling.	The rapid disappearance of radioactivity from the first quarter of the small intestine of rats following the oral administration of uniformly [14C]-ring-labeled 2,4- or 2,6-DNT indicates rapid and fairly complete absorption.	Rickert et al. (1983)

Table 4B. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Distribution and excretion/ toxicokinetic	Male A/J mice were given 1-, 10-, or 100-mg/kg doses of the radiolabeled [^{3}H] 2,6-DNT (2.5 μCi/mouse) by oral or i.p. exposure. The amount of ^{3}H in blood, liver, kidney, lung, and intestine was measured.	Distribution of radioactivity in the blood, liver, kidneys, lungs, and intestines was the same 8 hr after dosing, with very low levels of radioactivity in brain, heart, and spleen. Orally administered 2,6-DNT was eliminated primarily via urine (approximately 50% of the administered dose after 8 hr).	2,6-DNT was rapidly and extensively metabolized following both routes of administration. The liver and intestines appear to be the primary organ sites for metabolism.	Schut et al. (1983, as cited in U.S. EPA, 2011a, ORNL, 1995)
Mode of action/ mechanistic	Male F344 rats (CDF (F344)/CrlBR) (36/group) were administered by gavage doses of 10 or 35 mg/kg of radiolabeled 2,4- or 2,6-DNT. Three rats from each group were killed at 1, 2, 4, 8, 12, 24, 48, 96, 192, and 384 hr after the dose. Livers and intestine were removed and analyzed for metabolites. Hepatic covalent binding to RNA, DNA, and protein were measured. Also, intestinal disposition of [14C] dinitrotoluenes was determined.	DNT-related material is covalently bound to hepatic DNA, RNA, and protein after administration of either isomer. Covalent binding to each macromolecular species was proportional to dose. Terminal half-lives of radioactivity indicated that macromolecular damage produced by 2,6-DNT was no more persistent than that produced by 2,4-DNT. However, 2,6-DNT was 10 times more potent than 2,4-DNT in producing unscheduled DNA synthesis after 12 hr.	Results indicate that DNT-related material is covalently bound to hepatic DNA, RNA, and protein after administration of either isomer, but that the degree of binding after 2,6-DNT is greater than after 2,4-DNT.	Rickert et al. (1983)
Mode of action/ mechanistic	Six male F344 rats received an i.p. injection of either sulfotransferase inhibitor: DCNP (2,6-dichloro-4-nitrophenol) or PCP (pentachlorophenol). After 45 min, three of these animals were administered orally [3-^{3}H]-2,6-DNT at a dose of 28 mg/kg and killed 12 hr later. Livers were excised and minced, and covalently bound radiolabel to hepatic macromolecular and hepatic DNA was determined.	No signs of toxicity were observed in any of the animals receiving 2,6-DNT or sulfotransferase inhibitors at the doses administered. Prior administration of the sulfotransferase inhibitors DCNP or PCP resulted in a significant decrease in the hepatic macromolecular covalent binding of 2,6-DNT by 65 to 70%. Prior administration of the sulfotransferase inhibitors DCNP or PCP decreased the binding of 2,6-DNT to DNA by 95%.	These results suggest that a sulfotransferase-dependent pathway is responsible for the majority of the covalent binding of 2,6-DNT to hepatic DNA.	Kedderis et al. (1984)

Table 4B. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Mode of action/ mechanistic	Male F344 rats and Male A/J mice were administered 150 mg/kg of [3-^{3}H]-2,6-DNT and 2,4-DNT by i.p. The animals were killed by cervical dislocation after 12 or 24 hr (two animals/time point), and their liver, lungs, and small and large intestines were removed. Covalently bound radiolabel to DNA from these tissues was determined.	Treatment in F344 rat resulted in • a covalent binding of 2,6-DNT and 2,4-DNT to DNA of the liver; and • lower binding to DNA of the lungs and the intestine. Treatment of A/J mice resulted in • lower binding in the liver; • no detectable binding of 2,6-DNT in extrahepatic tissues; and • low amounts of binding of 2,4-DNT to lung and intestinal DNA.	Binding of 2,6-DNT to liver DNA requires its prior reductive metabolism, probably by intestinal microorganisms, and that the higher binding of 2,6-DNT in the F344 rat than in the A/J mouse may, in part, be responsible for the high susceptibility of the F344 rat to 2,6-DNT carcinogenesis.	Dixit et al. (1986)
Mode of action/ mechanistic	F344 rats were given 219 mg/kg of 2,6-DNT or 2,6-diaminotoluene by single i.p. injection. In another experiment, 2,6-DNT was also given to the animals by gavage. In both experiments, DNA adduct formation in the liver was determined.	Four adducts were detected following administration of 2,6-DNT. No adducts were observed following administration of 2,6-diaminotoluene. 2,6-DNT produced extensive hemorrhagic necrosis in the liver, whereas no evidence of hepatocellular necrosis was detected following administration of 2,6-diaminotoluene. No quantitative or qualitative differences in adduct formation were found when treatment occurred by gavage or i.p. injection.	The differences between the two compounds in both DNA binding and cytotoxicity were consistent with the differences in their carcinogenicity: 2,6-DNT is a potent hepatocarcinogen while 2,6-diaminotoluene is not carcinogenic.	La and Froines (1993); La and Froines (1992, as cited in ATSDR, 1998)

Table 4B. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Mode of action/ mechanistic	Male B6C3F$_1$ mice (n = 5) were dosed orally with 50 mg/kg 2,6-DNT daily for 3 consecutive days. CD-1 mice (n = 4) were given a single oral dose of 75 mg/kg 2,6-DNT. F344 rats (n = 6) were treated orally with 75 mg/kg 2,6-DNT three times at biweekly intervals. DNA adduct formation in the liver was determined.	Two distinct hepatic DNA adducts were detected in B6C3F$_1$, which differed from the four adducts observed in hepatic DNA from 2,6-DNT treated F344 rats. This difference in the number of adducts in B6C3F$_1$ mice in comparison with F344 rats was explained to be due to • the differences in dosing regimen; and • 80% of the dose administered to B6C3F$_1$ mice is excreted in feces.	Different number of adducts observed in mice compared to rats	George et al. (1996)
Immunotoxicity	ND			
Neurotoxicity	ND			

ND = No data

Tests Evaluating Carcinogenicity, Genotoxicity, and/or Mutagenicity

The in vitro mutagenicity or genotoxicity of 2,6-DNT has been evaluated in studies with bacterial and mammalian cell systems. It has shown mixed results in the *Salmonella typhimurium* Ames assay using several strains with and without metabolic activation, see Table 4A. It was negative in most studies in mammalian cell systems, including studies for mutations in Chinese hamster ovary cells (Abernathy and Couch, 1982; Lee et al., 1976), only weakly positive in a study by Garrett and Lewtas (1983) and negative in mouse lymphoma cells (Styles and Cross, 1983) and Syrian hamster embryo cells (Holen et al., 1990). It was also negative for DNA repair in rat and human hepatocytes (Bermudez et al., 1979; Butterworth et al., 1989) and for unscheduled DNA synthesis in rat spermatocytes (Working and Butterworth, 1984). Positive results were reported for chromosomal aberrations in human peripheral lymphocytes (Huang et al., 1995) and DNA strand breaks in rat germ cells (Yang et al., 2005).

2,6-DNT has also been tested in vivo in mutagenicity and genotoxicity studies, with mixed results. In rats, 2,6-DNT induced positive results for chromosomal aberrations (Lee et al., 1976), DNA adducts (La and Froines, 1993), DNA damage (Lent et al., 2012b), and micronuclei (Takasawa et al., 2010) in the liver, ,but was negative for unscheduled DNA synthesis (Working and Butterworth, 1984) and micronuclei in peripheral blood (Takasawa et al., 2010, Lent et al., 2012b).

In terms of tests evaluating the carcinogenicity of 2,6-DNT, Stoner et al. (1984) did not report an increase in lung tumor induction in mice exposed intraperitoneally (i.p.) to 2,6-DNT, 2,4-DNT, or a mixture of 2,4-DNT and 2,6-DNT. In hepatic tumor initiation-promotion protocols, both 2,6-DNT and technical grade DNT were reported to have tumor promoting and tumor initiating activity (Leonard et al., 1983). In contrast, 2,4-DNT was a hepatic tumor promoter but not a tumor initiator in the same in vivo hepatic initiation-promotion protocol. Leonard et al. (1986) reported that 2,6-DNT, technical grade DNT, and 2,4-DNT have hepatocyte foci promoting activity by the i.p. route. 2,6-DNT was approximately 10 times more potent than 2,4-DNT.

LD_{50} Toxicity Studies

Characteristic signs of 2,6-DNT toxicity in animals include central nervous system depression, respiratory depression, and ataxia (U.S. EPA, 1986, as cited in U.S. EPA, 2004). The following LD_{50} values were identified for 2,6-DNT (Lee et al., 1975; Ellis et al., 1978; Vernot et al., 1977, all as cited in ATSDR, 1998): 535 and 795 mg/kg for male and female CD rats, respectively; 180 mg/kg for male Sprague-Dawley rats; 621 and 807 mg/kg for male and female CD mice, respectively; and 1000 mg/kg for CF-mice.

Metabolism/Toxicokinetic Studies

Information on the toxicokinetics of 2,6-DNT is available in several reviews (ATSDR, 1998; U.S. EPA, 2004, 1987; OECD, 2004; Rickert et al., 1984, as cited in ATSDR, 1998) and is described in Table 4B. Results of the available studies indicate that DNT, including the isomer 2,6-DNT and technical grade DNT, is absorbed through the gastrointestinal tract, respiratory tract, and skin in most species. Metabolism of 2,6-DNT is believed to occur in the liver and the intestine. Urine appears to be the major route of 2,6-DNT excretion. The main urinary metabolites of 2,6-DNT are the corresponding dinitrobenzyl alcohol glucuronide, dinitrobenzoic acid, and aminonitrobenzoic acid (Rickert and Long, 1982, as cited in Mori et al., 1996). Figure 2 illustrates the proposed pathway for the metabolism of 2,6-DNT in rats from gastric absorption

to urinary excretion (Rickert et al., 1984, as cited in ATSDR, 1998; Sayama et al., 1993, as cited in ATSDR, 1998; Chapman et al., 1993; La and Froines, 1993). After 2,6-DNT is absorbed from the gastrointestinal tract, it is metabolized by the following steps: (1) Oxidation of the aliphatic methyl group of 2,6-DNT by hepatic cytochrome P-450 to form dinitrobenzyl alcohol, which is then conjugated with glucuronic acid, partially excreted in the bile and subsequently transferred to the intestine; (2) In the intestine, hydrolyzation of the glucuronide and reduction of one nitro group occur by intestinal microflora to form aminonitrobenzyl alcohol; (3) A portion of this metabolite is reabsorbed from the intestine and circulated back to the liver by enterohepatic circulation; and (4) In the liver, the amine group is *N*-hydroxylated by cytochrome P450 to form an unstable sulfate conjugate (Kedderis et al., 1984). The sulfate conjugate can decompose and form carbonium or nitrenium ions, which then can bind to hepatic macromolecules, leading to mutations and subsequently to liver tumors.

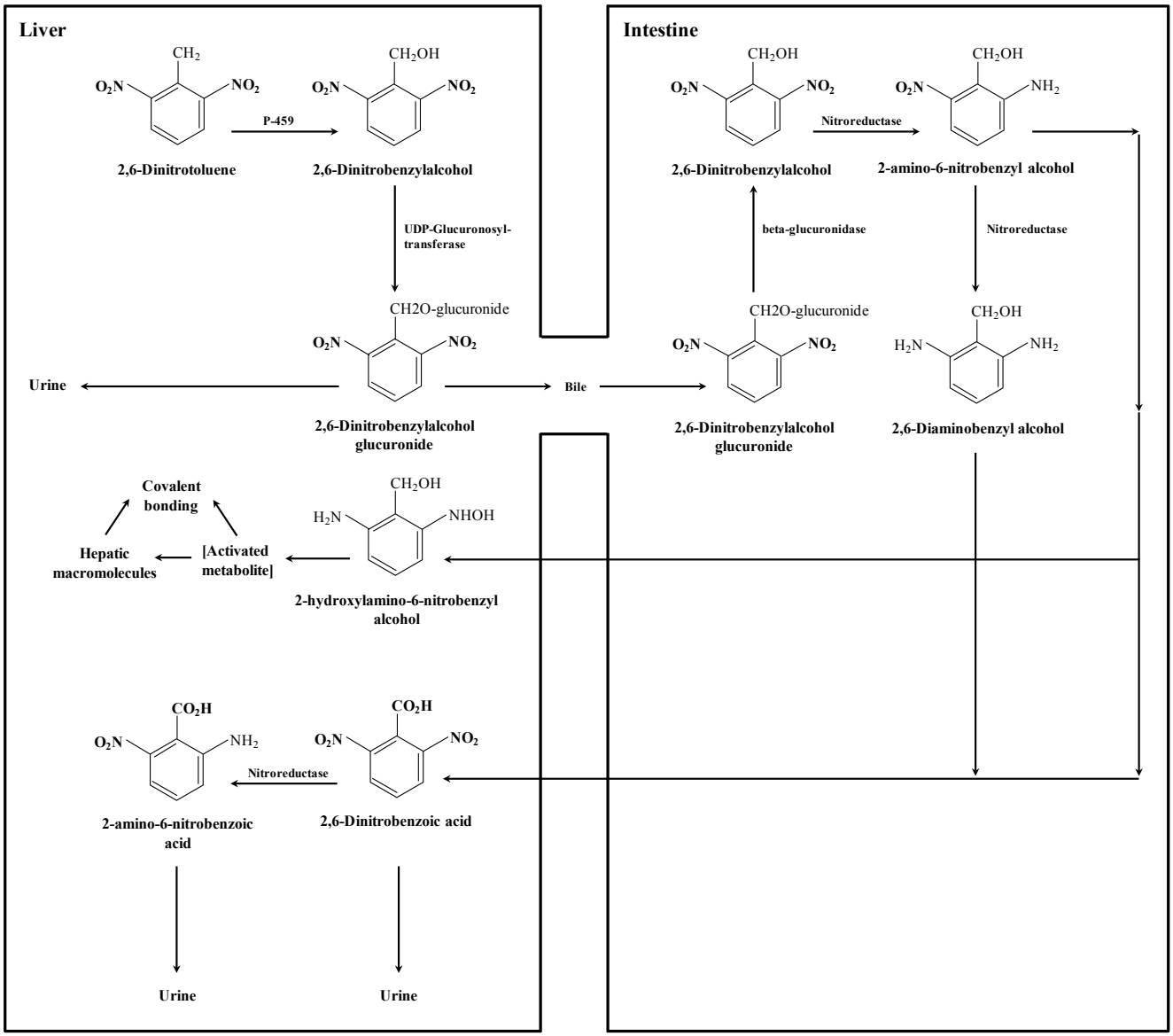

Figure 2. Metabolism Pathway of 2,6-DNT

Mode-of-Action/Mechanistic Studies

Ellis et al. (1979, as cited in ATSDR, 1998; U.S. EPA, 2004) described the mechanism by which DNT (in general) induced hematotoxicity in animals. DNT compounds or their metabolites can oxidize the ferrous ion in hemoglobin and produce methemoglobin. Hydroxylamine is probably the oxidizing species, because it is an intermediate in the reduction of nitrogen compounds to amines. Methemoglobin can form aggregates of hemoglobin degradation products called Heinz bodies, which are sensitive indicators of hemoglobin destruction. High levels of methemoglobin lead to the development of anemia, which is compensated by reticulocytosis. When reticulocytosis cannot compensate adequately, then frank anemia develops. This hematotoxic syndrome is a common effect of exposure to aromatic amines and most organic and inorganic nitrates.

The principal mechanism that is thought to be responsible for the genotoxicity of 2,6-DNT involves the bioactivation of 2,6-DNT to reactive metabolites, which are capable of covalent binding to hepatic macromolecules. As illustrated in Figure 2, conjugation, biliary excretion, microbial metabolism in the gut, and intestinal reabsorption are prerequisites to hepatic binding of DNT. Swenberg et al. (1983) demonstrated covalent binding of 2,6-DNT to rat hepatocyte RNA following oral dosing with 2,6-DNT, with hepatocytes of female rats showing slightly less binding than male rats. Rickert et al. (1983) reported similar hepatic binding of 2,6-DNT to protein, RNA, and DNA of rats. Hepatic binding may be greater for 2,6-DNT than for 2,4-DNT (Rickert et al., 1983). Diet (i.e., as it affects microbial activity and number) also may influence the degree to which binding of DNT metabolites occurs. Hepatic DNA adducts have been detected by ^{32}P-postlabeling technique in 2,6-DNT-treated male B6C3F_1 and CD-1 mice and F344 rats (George et al., 1996). Further information on these studies is provided in Table 4B.

Neurotoxicity

In animal studies, 2,6-DNT has been shown to affect the nervous system of mice and dogs (Lee et al., 1976b,c). Clinical signs in dogs have included incoordination and stiffness of the hind legs leading to complete paralysis, cerebellar vacuolation, hypertrophy, and focal gliosis, and cerebellar and brain stem hemorrhage. In mice, depression and hyperexcitability were observed, while some rats administered 2,6-DNT showed neuromuscular symptoms. Further details on this study are presented in Table 3 and in the *Subchronic Studies* section.

There are no data on the biochemical events involved in the toxicity of the nervous system.

DERIVATION OF PROVISIONAL VALUES

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

Table 5. Summary of Reference Values for 2,6 Dinitrotoluene (CASRN 606-20-2)

Toxicity Type (Units)	Species/Sex	Critical Effect	p-Reference Value	POD Method	POD	UF_C	Principal Study
Screening Subchronic p-RfD (mg/kg-d)	Dog/M and F	Increased incidence of splenic extramedullary hematopoiesis	3×10^{-3}	$LOAEL_{HED}$	3	1,000	Lee et al. (1976c)
Screening Chronic p-RfD (mg/kg-d)	Dog/M and F	Increased incidence of splenic extramedullary hematopoiesis	3×10^{-4}	$LOAEL_{HED}$	3	10,000	Lee et al. (1976c)
Subchronic p-RfC (mg/m^3)	NDr						
Chronic p-RfC (mg/m^3)	NDr						

NDr = Not determined.

Table 6. Summary of Cancer Values for 2,6 Dinitrotoluene (CASRN 606-20-2)

Toxicity type	Species/Sex	Tumor type	Cancer value	Principal study
p-OSF	F344 Rat/M	Hepatocellular carcinomas	1.5×10^{0} $(mg/kg\text{-}d)^{-1}$	Leonard et al. (1987)
p-IUR	NDr			

NDr = Not determined.

DERIVATION OF ORAL REFERENCE DOSES

Derivation of Subchronic Provisional RfD (Subchronic p-RfD)

There are three subchronic-duration studies (Lee et al. 1976) presented in one report on 2,6-DNT in rats, mice, and dogs (see Table 3). Lee et al. (1976) is considered inadequate for p-RfD derivation because it is a nonpeer-reviewed and unpublished report. However, the Lee et al. (1976c) study is suitable for the derivation of a screening subchronic toxicity value. Appendix A provides details on the screening subchronic p-RfD.

Derivation of Chronic Provisional RfD (Chronic p-RfD)

One chronic oral study in rats is available (Leonard et al., 1987), but it is not a comprehensive study and only investigated effects in the liver. Leonard et al. (1987) is not considered to derive the chronic p-RfD because it is unclear if the limited noncancer effects in the liver could be attributed to the carcinogenic effects of 2,6-DNT. The subchronic study in dogs by Lee et al. (1976c) (see discussion in the derivation of the subchronic p-RfD section above), is not used to derive the chronic p-RfD because it is a nonpeer-reviewed and unpublished report. However, the Lee et al. (1976c) dog study is used to derive the screening chronic p-RfD. Details are provided in Appendix A.

DERIVATION OF INHALATION REFERENCE CONCENTRATIONS

No studies were identified that could be used to derive provisional inhalation RfCs for 2,6-DNT. Available epidemiological studies consist primarily of occupational studies in which workers were exposed to the technical grade DNT mixture, which consists primarily of the 2,4-DNT isomer. In these studies, dermal as well as inhalation exposure was investigated, and exposure to 2,6-DNT was not quantified. No animal inhalation studies are available for 2,6-DNT.

Derivation of Subchronic Provisional RfC (Subchronic p-RfC)

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

Derivation of Chronic Provisional RfC (Chronic p-RfC)

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

CANCER WEIGHT-OF-EVIDENCE DESCRIPTOR

Table 7 identifies the cancer weight-of-evidence (WOE) descriptor for 2,6-DNT.

Table 7. Cancer WOE Descriptor for 2,6-DNT

Possible WOE Descriptor	Designation	Route of Entry (Oral, Inhalation, or Both)	Comments
"Carcinogenic to Humans"	ND	ND	No human cancer studies on pure 2,6-DNT are available by any route of exposure.
"Likely to Be Carcinogenic to Humans"	NA	NA	The cancer weight of evidence does not meet the examples to be considered *"Likely to be Carcinogenic to Humans."*
"Suggestive Evidence of Carcinogenic Potential"	**Selected**	**Both[a]**	**As described below, 2,6-DNT is considered to have *"Suggestive Evidence of Carcinogenic Potential."***
"Inadequate Information to Assess Carcinogenic Potential"	NA	NA	There is evidence to assess the carcinogenic potential of 2,6-DNT.
"Not Likely to Be Carcinogenic to Humans"	NA	NA	Evidence of the carcinogenic potential of 2,6-DNT is available in animals.

[a]Although data on the carcinogenic effects of 2,6-DNT via the inhalation route are limited to human exposures to a DNT mixture, 2,6-DNT is considered to have *suggestive evidence of carcinogenic potential* by all routes of exposure based on EPA's *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 descriptor may apply to all routes of exposure that have not been adequately tested at sufficient doses.

NA = Not applicable, ND = No data.

No human cancer studies of pure 2,6-DNT are available. Results from experimental animal studies showed that 2,6-DNT: (1) increased the incidence of hepatocellular neoplastic nodules and carcinomas in a chronic dietary exposure bioassay with male F344 rats (Leonard et al., 1987); (2) is a tumor initiator and promoter in rat liver using the in vivo hepatic initiation-promotion assay (Leonard et al., 1983, 1986); (3) is mutagenic in bacteria and induces DNA damage and mutations in mammalian cells in culture (Rickert et al., 1984, as cited in ATSDR, 1998; Sayama et al., 1998).

As stated in the EPA's cancer guidelines (U.S. EPA, 2005), one of the examples for a chemical to be considered to have *suggestive evidence of carcinogenic potential* is*:* "*a small, and possibly not statistically significant, increase in tumor incidence observed in a single animal or human study that does not reach the weight of evidence for the descriptor 'Likely to Be Carcinogenic to Humans'.*" Based on these guidelines and the carcinogenicity data from available animal studies, the WOE descriptor of *suggestive evidence of carcinogenic potential* is appropriate for 2,6-DNT.

EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005) indicates that for tumors occurring at a site other than the initial point of contact, the cancer 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 2,6-DNT demonstrates that tumors occur in tissues remote from the site of absorption. 2,6-DNT has been shown to be a hepatocarcinogen in rats in bioassays of various experimental designs by oral exposure. An excess of hepatobiliary cancer was found among munition workers exposed to dinitrotoluenes in which exposures are presumed to be predominantly inhalation with contributions from the dermal route. Information on the carcinogenic effects of 2,6-DNT via the dermal route in humans and animals is limited or absent. Data on the absorption of 2,6-DNT show that the chemical is readily absorbed via all routes of exposure, including oral, inhalation, and dermal. Therefore, based on the observance of liver tumors following oral exposure and absorption by all routes of exposure, it is assumed that an internal dose will be achieved regardless of the route of exposure. Therefore, 2,6-DNT is considered to have *suggestive evidence of carcinogenic potential* 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" (p. 1−10). 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" (p. 1−10).

The potential mode of action for 2,6-DNT is unclear. Table 4A summarizes the studies examining genotoxicity (e.g., clastogenicity, mutagenicity) of 2,6-DNT. 2,6-DNT was shown to be both positive and negative for mutagenicity in *S. typhimurium* strains. 2,6-DNT was not mutagenic in mammalian cell systems (i.e., chinese hamster ovary (CHO) cells and p388 mouse lymphoma cells). 2,6-DNT did not cause morphological transformations in Syrian hamster embryo cells but did induce chromosomal aberrations in human peripheral lymphocytes in vitro. 2,6-DNT also caused DNA damage in rats both in vitro (germ cells) and in vivo (liver) but was negative for DNA repair in rat and human hepatocytes. Assays of unscheduled DNA synthesis in rat spermatocytes and CHO cells showed a negative response under in vitro conditions. 2,6-DNT induced both chromosomal aberrations and DNA adducts in rats in vivo. Assays of unscheduled DNA synthesis were both positive and negative in rats. 2,6-DNT caused micronuclei formation in the liver of rats but not in the peripheral blood. Taken together, the available data do not provide a definitive conclusion regarding the mode-of-action for 2,6-DNT-induced carcinogenicity. Therefore, a detailed mode-of-action discussion for 2,6-DNT is precluded and a linear approach is applied as recommended by the U.S. EPA (2005).

DERIVATION OF PROVISIONAL CANCER POTENCY VALUES

Derivation of Provisional Oral Slope Factor (p-OSF)

As noted in Table 7, EPA concluded that there is *suggestive evidence of carcinogenic potential* for 2,6-DNT. The *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005) state: "When there is suggestive evidence, the Agency generally would not attempt a dose-response assessment, as the nature of the data generally would not support one; however, when the evidence includes a well-conducted study, quantitative analyses may be useful for some purposes, for example, providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities. In each case, the rationale for the

quantitative analysis is explained, considering the uncertainty in the data and the suggestive nature of the weight of evidence. These analyses generally would not be considered Agency consensus estimates."

In this case, although there are no epidemiologic studies that have evaluated the carcinogenicity of 2,6-DNT in humans, its carcinogenicity has been evaluated in studies in both rats and mice. As highlighted in Table 3, these studies indicate that there are differing results regarding the carcinogenic potential of 2,6-DNT. However, the study by Leonard et al. (1987) is a well-conducted study showing evidence of increased incidence of hepatic carcinomas in male rats at multiple treatment levels. The data from this study are adequate to support a quantitative cancer dose-response assessment. Considering these data and the uncertainty associated with the suggestive nature of the tumorigenic response, EPA concluded that quantitative analyses may be useful for providing a sense of the magnitude of potential carcinogenic risk. Based on the weight of evidence, a dose-response assessment of the carcinogenicity of 2,6-DNT is deemed appropriate.

The study by Leonard et al. (1987) is selected as the principal study for deriving the p-OSF, with a cancer endpoint of hepatocellular carcinomas in male rats. This study is peer reviewed, examined a sufficient number of animals (28 per dose group), was well conducted, and was of sufficient duration (52 weeks). It was not stated whether the study was performed under GLP standards, but the study appears scientifically sound. Details are provided in the *Carcinogenicity Studies* section. The study by Goldsworthy et al. (1986) was not selected as the principal study because the reported carcinogenicity of 2,6-DNT was enhanced by the content of pectin in diet and was not solely related to pure 2,6-DNT. In this study, 2,6-DNT was provided in diets with varying pectin content, which is believed to promote or enhance 2,6-DNT-induced carcinogenesis. The hepatocellular carcinomas and neoplastic nodules were observed only in rats fed 2,6-DNT in diets high in pectin content (NIH-2,6-DNT), and, therefore, the reported liver tumor incidences cannot be used to derive a p-OSF for 2,6-DNT. The Stoner et al. (1984) study was not selected as the principal study because it was of insufficient duration (12 weeks) to determine carcinogenic effects.

In the study by Leonard et al. (1987), 28 male F344 rats were fed 2,6-DNT (purity unknown) in the diet for 1 year, and the results were compared with an untreated control group of 28 rats. 2,6-DNT induced hepatocellular carcinomas in 100% (19/19) of the high-dose rats (14 mg/kg-day) and 85% (17/20) of the low-dose rats (7 mg/kg-day), compared to no incidence (0/20) in controls. Statistical significance tests conducted by the EPA indicated that incidence of hepatocellular carcinomas was statistically significant at both the high- and low-dose groups compared to controls. The dose-response data for hepatocellular carcinomas in male rats (see Table 8 and B.14) can be used to derive a p-OSF for 2,6-DNT. Statistical analyses performed for these data were done by Fisher's Exact test.

Table 8 presents BMD input data for incidence of hepatocellular carcinomas in male rats exposed to 2,6-DNT orally for 1 year. The model result and BMD output text are provided in Appendix D.

Table 8. BMD Input for Incidence of Hepatocellular Carcinomas in the Male (F344) Crlbr Rat Exposed to 2,6-DNT Orally for 1 Year[a]

$Dose_{ADJ}$ (mg/kg-day)	Number of Subjects	Response (Hepatocellular Carcinoma)
0	20	0
7	20	17*
14	19	19*

[a]Leonard et al. (1987).
*$p < 0.001$ by Fisher's Exact Test performed by EPA.

Table 9 shows the BMD modeling results. Adequate model fit is obtained for the hepatocellular carcinoma incidence data using the multistage-cancer model. The modeling results yield a BMD_{10} of 2.7 mg/kg-day and a $BMDL_{10}$ of 0.25 mg/kg-day. 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 2,6-DNT.

Table 9. Goodness-of-Fit Statistics and BMD_{10} and $BMDL_{10}$ Values for Dichotomous Model for Hepatocellular Carcinoma in the Male (F344)/Crlbr Rat Exposed to 2,6-DNT Orally for 1 Year[a]

Multistage Cancer Model	Goodness-of-fit *p*-value[b]	AIC	BMD_{10} (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)
Hepatocellular carcinoma	1.0	18.91	2.7	0.25

[a]Leonard et al. (1987).
[b]Values >0.1 meet conventional goodness-of-fit criteria.

$$\begin{aligned} POD_{HED} &= BMDL_{10}\ (\text{mg/kg-day}) \times DAF \\ &= BMDL_{10}\ (\text{mg/kg-day}) \times (BW_a^{1/4} \div BW_h^{1/4}) \\ &= BMDL_{10}\ (\text{mg/kg-day}) \times (0.376^{1/4} \div 70^{1/4}) \\ &= 0.25\ \text{mg/kg-day} \times 0.27 \\ &= 0.068\ \text{mg/kg-day} \end{aligned}$$

Note: The BW_a of 0.376 kg is the mean body weight from the low-dose male group at Week 104 (see Table B.11).

$$\begin{aligned} \textbf{p-OSF} &= \mathbf{0.1 \div BMDL_{10HED}} \\ &= 0.1 \div 0.068\ \text{mg/kg-day} \\ &= \mathbf{1.5 \times 10^0\ (mg/kg\text{-}day)^{-1}} \end{aligned}$$

The p-OSF is 1.5×10^0 $(\text{mg/kg-day})^{-1}$, as calculated based on BMD modeling from Leonard et al. (1987).

Derivation of Provisional Inhalation Unit Risk (p-IUR)

No human and animal studies examining the carcinogenicity of 2,6-DNT following inhalation exposure are available located. Therefore, derivation of a p-IUR is precluded.

APPENDIX A. PROVISIONAL SCREENING VALUES

For the reasons noted in the main PPRTV document, it is inappropriate to derive a provisional subchronic or chronic p-RfD for 2,6-DNT. 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 PPRTV documents to ensure their appropriateness within the limitations detailed in the document. Users of screening toxicity values in an appendix to a PPRTV assessment should understand that there is considerably more uncertainty 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 PROVISIONAL ORAL REFERENCES DOSES

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

The 13-week toxicity study in dogs (Lee et al., 1976c) is selected as the principal study for the derivation of the screening subchronic p-RfD. The critical effect is increased incidence of splenic extramedullary hematopoiesis in male and female dogs. No human studies are available on oral exposure to 2,6-DNT. One subchronic animal oral study is available (Lee et al., 1976), which, for the sake of clarity in this document, is divided into three separate study summaries based on the species tested: Lee et al. (1976a) in rats, (1976b) in mice, and (1976c) in dogs. Lee et al. (1976) is an unpublished and nonpeer-reviewed study. It is unclear if the study was performed according to Good Laboratory Practice (GLP) guidelines. The dog study (Lee et al. 1976c) is considered to have a small sample size as only one dog/sex/dose level were treated for 13 weeks in the control and low-dose group. At the mid-dose level, only two females and one male were treated for 13 weeks and two dogs/sex/dose level at the high dose. However, this study examined an adequate number of endpoints and has been previously used by both EPA and ATSDR to develop reference values (see Table 2) and is considered to be adequate for the derivation of screening oral reference values. Lee et al. (1976c) (dogs) is selected as the principal study because treatment-related effects observed in the dogs were more sensitive than effects observed in the rats (Lee et al. 1976a) and mice (Lee et al. 1976b). Study details are provided in the "Review of Potentially Relevant Data" section.

The most sensitive treatment-related effect observed in the Lee et al. (1976c) study was increased incidence of splenic extramedullary hematopoiesis in male and female beagle dogs. Splenic extramedullary hematopoiesis was observed in every dog treated at 4 (2/2), 20 (3/3), and 100 (4/4) mg/kg-day for 13 weeks compared to zero incidence in the controls (see Table B.8). These data could not be modeled by Benchmark Dose Software (BMDS version 2.1.2) due to the lack of a dose-response. Because these data were not amenable to BMD modeling, a NOAEL/LOAEL approach was employed to identify a potential point of departure (POD). For increased incidence of splenic extramedullary hematopoiesis in male and female beagle dogs, there was an increase at the low-dose group, identifying a LOAEL of 4 mg/kg-day. A Fisher's exact test comparing splenic extramedullary hematopoiesis in the control and treated groups indicated a nonstatistically significant difference. However, group sizes were too small for the statistical test to have much power to detect an effect.

Dogs are considered to be the most sensitive species to the toxicological effects of 2,6-DNT compared to rats and mice. For rats, the most sensitive potential POD is increased incidences of liver and splenic extramedullary hematopoiesis in male rats with a LOAEL of 35 mg/kg-day and a corresponding NOAEL of 7 mg/kg-day. In mice, decreased relative liver weight that was biologically significant (≥10%) is the most sensitive effect with a LOAEL of 11 mg/kg-day. Effects in dogs, rats, and mice were modeled by BMDS (version 2.1.2) for consideration of a potential POD when data were amenable to BMD modeling. Details of the modeling methods are provided in Appendix C. Potential PODs for dogs, rats, and mice are listed in Table A.1.

Of the toxicological effects observed in dogs, rats, and mice in the subchronic study by Lee et al. (1976), the most sensitive is increased incidence of splenic extramedullary hematopoiesis in male and female beagle dogs with a LOAEL of 4 mg/kg-day. The selection of increased incidence of splenic extramedullary hematopoiesis is supported by the observation that the severities of this effect increased with dose in dogs (see Table B.8). At 4 mg/kg-day in male and female dogs, the severity of splenic extramedullary hematopoiesis ranged from minimal to mild; at 20 mg/kg-day, the severity ranged from minimal to moderate; at 100 mg/kg-day, the severity ranged from marked to markedly severe. Furthermore, splenic extramedullary hematopoiesis was not present in male and female dogs that were treated with 2,6-DNT for 13 weeks and allowed to recover for 4 weeks, suggesting that this effect is treatment-related. Splenic extramedullary hematopoiesis was reported in rats gavaged with 2,6-DNT for 14 days at doses of 68 (1/6) and 134 (6/6) mg/kg-day, the incidence of this lesion in controls was not reported by the study authors (Lent et al., 2012a). Splenic extramedullary hematopoiesis was also observed in male and female dogs treated at 4 (1/2), 20 (2/2), and 100 (2/2) mg/kg-day for 4 weeks compared to zero incidence in controls. Splenic extramedullary hematopoiesis was also statistically significantly increased in both male and female rats treated for 13 weeks (see Tables B.4 and B.5). Further support for splenic extramedullary hematopoiesis as a critical effect following 2,6-DNT treatment is provided by hematological data in Tables B.3. 2,6-DNT statistically significantly increased reticulocytes (an indicator of hematopoiesis) in male and female dogs at 100 mg/kg-day following 2 and 4 weeks of treatment. Additionally, 2,6-DNT statistically significantly increased the amount of reticulocytes in male and female rats compared to controls at the highest dose tested following 4 weeks of exposure. **Therefore, the LOAEL of 4 mg/kg-day based on increased incidence of splenic extramedullary hematopoiesis in male and female dogs is chosen as the POD to derive a screening subchronic p-RfD.**

It is important to note that the selection of the LOAEL of 4 mg/kg-day for increased incidence of splenic extramedullary hematopoiesis in male and female beagle dogs as the POD would also be protective against the 2,6-DNT-induced mortality that was observed in mice and dogs. In mice, 8 of 16 males at the high-dose (289 mg/kg-day) died before Weeks 9, and 6 of 8 females died at the high-dose (299 mg/kg-day) before the end of the study. In addition, 8 of 16 males and 1 of 16 females died at the mid-dose (51 and 55 mg/kg-day, respectively), and 2 of 16 males died at the low-dose (11 mg/kg-day). The study authors stated that in the mid- and high-dose groups, most of the deaths could be contributed to 2,6-DNT administration. These data suggest an FEL of 51 mg/kg-day for the mouse study (Lee et al. 1976b). In dogs, all animals (2 males and 2 females) in the high dose group (100 mg/kg-day) died between Weeks 2 and 8, and 2 of 3 dogs (both females) in the mid-dose group (20 mg/kg-day) died during Week 9. These data suggest an FEL of 20 mg/kg-day for the dog study (Lee et al. 1976c).

Table A.1. Potential Subchronic PODs in Animals Following 13 Weeks of Treatment to 2,6-DNT

Effect	Sex/Species	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)	Comment
Decreased Body Weight	Males/Rats	7	35	13	Due to decreased palatability possibly due to chemical exposure
Increased Relative Liver Weight	Males/Rats	Not determined	7	Not run	No dose-response, effect most likely due to decreased body weight
Increased Relative Spleen Weight	Males/Rats	35	145	No fit	Effect most likely due to decreased body weight
Decreased Relative Kidney Weight	Males/Rats	Not determined	7	Not run	No dose-response, effect reverses at higher doses
Increased Relative Heart Weight	Males/Rats	35	145	Not run	No dose-response, effect most likely due to decreased body weight
Increased Relative Brain Weight	Males/Rats	35	145	No fit	Not a valid toxicological endpoint
Splenic Hemosiderosis	Males/Rats	Not determined	7	Not run	No dose-response
Splenic Hematopoiesis	Males/Rats	7	35	Not run	Data not suitable for BMD modeling
Liver Hematopoiesis	Males/Rats	7	35	Not run	Data not suitable for BMD modeling
Decreased Body Weight	Females/ Rats	37	155	6.4	Due to decreased palatability possibly due to chemical exposure
Increased Relative Liver Weight	Females/ Rats	Not determined	7	No fit	Effect most likely due to decreased body weight
Increased Relative Spleen Weight	Females/ Rats	37	155	48	Effect most likely due to decreased body weight
Increased Relative Kidney Weight	Females/ Rats	7	37	Not run	No dose-response, effect most likely due to decreased body weight
Decreased Relative Heart Weight	Females/ Rats	155	Not determined	Not Run	No dose-response
Increased Relative Brain Weight	Females/ Rats	7	37	3.1	Not a valid toxicological endpoint
Splenic Hemosiderosis	Females/ Rats	37	155	Not run	Data not suitable for BMD modeling
Splenic Hematopoiesis	Females/ Rats	7	37	Not run	Data not suitable for BMD modeling
Liver Hematopoiesis	Females/ Rats	37	155	Not run	Data not suitable for BMD modeling

Table A.1. Potential Subchronic PODs in Animals Following 13 Weeks of Treatment to 2,6-DNT

Effect	Sex/Species	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)	Comment
Decreased Relative Liver Weight	Males/Mice	Not determined	11	No fit	
Decreased Relative Kidney Weight	Males/Mice	11	51	Not run	LOAEL is 10-fold higher than LOAEL for splenic extramedullary hematopoiesis in dogs
Mortality	Males/Mice	11	51 (FEL)	Not run	
Mortality	Females/ Mice	55	299 (FEL)	Not run	
Liver Hematopoiesis	Both/Dogs	4	20	Not run	Data not suitable for BMD modeling
Liver bile duct hyperplasia	Both/Dogs	4	20	Not run	Data not suitable for BMD modeling
Liver degeneration	Both/Dogs	4	20	Not run	Data not suitable for BMD modeling
Splenic Hematopoiesis	Both/Dogs	Not determined	4	Not run	Data not suitable for BMD modeling
Mortality	Both/Dogs	4	20 (FEL)	Not run	

Dosimetric adjustment for daily exposure:

The following dosimetric adjustments were made for each dose in the principal study for dietary treatment to adjust for daily exposure. Dosimetric adjustment for 4 mg/kg-day is presented below.

$$\begin{aligned}(DOSE_{ADJ}) &= DOSE_{\text{Lee et al., 1976c}} \times [\text{conversion to daily dose}] \\ &= 4 \text{ mg/kg-day} \times (\text{days of week dosed} \div 7) \\ &= 4 \text{ mg/kg-day} \times (7 \div 7) \\ &= 4 \text{ mg/kg-day}\end{aligned}$$

In EPA's *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 PBPK model for 2,6-DNT is not available for use in extrapolating doses from animals to humans. The selected critical effect of splenic extramedullary hematopoiesis was associated with the parent compound or a stable metabolite. Furthermore, this splenic effect is not a portal-of-entry or developmental effect. Therefore, scaling by $BW^{3/4}$ is relevant for deriving human equivalent doses (HEDs) for these effects.

Following U.S. EPA (2011b) guidance, the POD for splenic extramedullary hematopoiesis in adult animals is converted to a HED through application of a dosimetric adjustment factor (DAF[1]) derived as follows:

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

where

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

[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 $DAF = BW_a^{1/4} \div BW_h^{1/4}$.

Using a BW_a of 12 kg for dogs and a BW_h of 70 kg for humans (U.S. EPA, 1988), the resulting DAF is 0.63. Applying this DAF to the LOAEL identified for the critical effect in mature dogs yields a $LOAEL_{HED}$ as follows:

$$\begin{aligned} LOAEL_{HED} &= 4 \text{ mg/kg-day} \times DAF \\ &= 4 \text{ mg/kg-day} \times 0.63 \\ &= 3 \text{ mg/kg-day} \end{aligned}$$

The screening subchronic p-RfD for 2,6-DNT is derived as follows:

$$\begin{aligned} \textbf{Screening Subchronic p-RfD} &= \textbf{LOAEL}_{\textbf{HED}} \div \textbf{UF}_{\textbf{C}} \\ &= 3 \text{ mg/kg-day} \div 1{,}000 \\ &= \textbf{3} \times \textbf{10}^{\textbf{-3}} \textbf{ mg/kg-day} \end{aligned}$$

The UF_C of 1,000 is presented in Table A.2.

Table A.2 summarizes the UFs for the screening subchronic p-RfD for 2,6-DNT. Confidence in the screening value is by definition, low.

Table A.2. UFs for Screening Subchronic p-RfD of 2,6-DNT[a]

UF	Value	Justification
UF_A	3	For the POD based on an increased incidence of splenic extramedullary hematopoiesis (Lee et al., 1976c), a UF_A of 3 ($10^{0.5}$) has been applied to account for uncertainty in characterizing the toxicodynamic differences between dogs and humans following oral 2,6-DNT 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	10	A UF_D of 10 has been applied because there are no acceptable two-generation reproductive toxicity or developmental toxicity studies via the oral route.
UF_H	10	A UF_H of 10 has been applied for inter-individual variability to account for human-to-human variability in susceptibility in the absence of quantitative information to assess the toxicokinetics and toxicodynamics of 2,6-DNT in humans.
UF_L	3	A UF_L of 3 has been applied for LOAEL-to-NOAEL extrapolation because the POD is a LOAEL based on splenic extramedullary hepatopoiesis, for which the biological significance is not entirely clear.
UF_S	1	A UF_S of 1 has been applied because a subchronic-duration study was selected as the principal study.
UF_C	1,000	

[a]Lee et al. (1976c).

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

The 13-week toxicity study in dogs (Lee et al., 1976c) is selected as the principal study for the derivation of the screening chronic p-RfD. The critical effect is increased incidence of splenic extramedullary hematopoiesis in male and female dogs. No human chronic studies are available for 2,6-DNT. There is an available chronic oral study in male rats by

Leonard et al. (1987) that investigated the carcinogenic and noncancer effects of only 2,6-DNT on the liver. The carcinogenic effects are described in the "Derivation of Provisional Oral Slope Factor" section. Regarding the noncancer effects, Leonard et al. (1987) reported increased absolute and relative liver weight, decreased body weight, and increased ALT activity (only at 12 months) in male rats at both 6 and 12 months of exposure to 2,6-DNT. Decreased body weight cannot be considered as a critical effect because it is not clear if this effect is due to direct treatment with 2,6-DNT or to a reduction in food consumption. Decreased food consumption was observed in rats in the subchronic study by Lee et al. (1976a), suggesting that decreased body weight in rats reported in the Leonard et al. (1987) study could be due to reduced food consumption. Because there were statistically and biologically significant changes in body weight, changes in absolute organ weights are not considered for potential POD selection. The data for increased relative liver weight were analyzed by the EPA's BMDS (version 2.1.2) continuous-variable models. For increased relative liver weight at 6 months, a LOAEL of 7 mg/kg-day based on a biologically (≥10% change) and statistically significant change is a potential POD. Following 12 months of treatment with 2,6-DNT, BMDS calculated a $BMDL_{10}$ of 0.69 mg/kg-day for increased relative liver weight. For increased ALT activity at 12 months, a LOAEL of 7 mg/kg-day based on a statistically significant change is a potential POD. Complete modeling methods and results are in presented Appendix C. The study authors also reported nonneoplastic lesions (e.g., bile duct hyperplasia, basophilic foci, etc) in the liver but presented no quantitative data for these effects that could be used to derive a chronic p-RfD.

Based on the BMD modeling results, the most sensitive effect following chronic exposure to 2,6-DNT appears to be increased relative liver weight (BMDL = 0.69 mg/kg-day). However, the chronic study by Leonard et al. (1987) is not a comprehensive study and only reports carcinogenic and noncancer effects in the liver, as well as evaluation of pulmonary metastases. It is also unclear if the reported noncancer effects in the liver may be due to the hepatocarcinogenic effects of 2,6-DNT because increased relative liver weight and increased ALT activity were observed at the same doses (7 and 14 mg/kg-day) as hepatocellular carcinomas following 12 months of exposure. Whereas increased relative liver weight was also observed at 6 months, the study authors did not report pathology results for this time period so it is possible that hepatocellular carcinomas may have been present. From the subchronic-duration study by Lee et al. (1976), it is clear that the spleen is a target organ for 2,6-DNT toxicity; the most sensitive subchronic effect from that study was increased incidence of splenic extramedullary hematopoiesis in dogs with a NOAEL of 4 mg/kg-day, which is more sensitive than splenic and liver effects observed in rats. Because the chronic-duration study by Leonard et al. (1987) did not investigate splenic effects in any species, the sensitivity of spleen toxicity following chronic 2,6-DNT exposure is unknown. Therefore, to protect against potential splenic effects from chronic 2,6-DNT exposure, the LOAEL of 4 mg/kg-day based on increased incidence of splenic extramedullary hematopoiesis in dogs from the subchronic-duration study by Lee et al. (1976c) is used as the POD to derive a screening chronic p-RfD. For the same reasons listed in the screening subchronic p-RfD discussion above, the study by Lee et al. (1976c) meets standards of study design and performance. Details are provided in the "Review of Potentially Relevant Data" section.

Dosimetric adjustment for daily exposure:

The following dosimetric adjustments were made for each dose in the principal study for dietary treatment to adjust for daily exposure. Dosimetric adjustment for 4 mg/kg-day is presented below.

$$\begin{aligned}(DOSE_{ADJ}) &= DOSE_{\text{Lee et al., 1976c}} \times [\text{conversion to daily dose}] \\ &= 4 \text{ mg/kg-day} \times (\text{days of week dosed} \div 7) \\ &= 4 \text{ mg/kg-day} \times (7 \div 7) \\ &= 4 \text{ mg/kg-day}\end{aligned}$$

Following U.S. EPA (2011b) guidance, the POD for splenic extramedullary hematopoiesis in adult animals is converted to a HED through application of a dosimetric adjustment factor (DAF^1) derived as follows:

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

where

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

Using a BW_a of 12 kg for dogs and a BW_h of 70 kg for humans (U.S. EPA, 1988), the resulting DAF is 0.63. Applying this DAF to the LOAEL identified for the critical effect in mature dogs yields a $LOAEL_{HED}$ as follows:

$$\begin{aligned}LOAEL_{HED} &= 4 \text{ mg/kg-day} \times DAF \\ &= 4 \text{ mg/kg-day} \times 0.63 \\ &= 3 \text{ mg/kg-day}\end{aligned}$$

The screening chronic p-RfD for 2,6-DNT based on a $LOAEL_{HED}$ of 3 mg/kg-day for splenic extramedullary hematopoiesis in male and female dogs, is derived as follows:

$$\begin{aligned}\textbf{Screening Chronic p-RfD} &= \textbf{LOAEL}_{\textbf{HED}} \div \textbf{UF}_{\textbf{C}} \\ &= 3 \text{ mg/kg-day} \div 10{,}000 \\ &= \mathbf{3 \times 10^{-4} \text{ mg/kg-day}}\end{aligned}$$

The UF_C of 10,000 is presented in Table A.3.

Table A.3 summarizes the UFs for the screening chronic p-RfD for 2,6-DNT. Confidence in the screening value is by definition, low.

Table A.3. UFs for Screening Chronic p-RfD of 2,6-DNT[a]

UF	Value	Justification
UF_A	3	For the POD based on an increased incidence of splenic extramedullary hematopoiesis (Lee et al., 1976c), a UF_A of 3 ($10^{0.5}$) has been applied to account for uncertainty in characterizing the toxicodynamic differences between dogs and humans following oral 2,6-DNT 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	10	A UF_D of 10 has been applied because there are no acceptable two-generation reproductive toxicity or developmental toxicity studies via the oral route.
UF_H	10	A UF_H of 10 has been applied for inter-individual variability to account for human-to-human variability in susceptibility in the absence of quantitative information to assess the toxicokinetics and toxicodynamics of 2,6-DNT in humans.
UF_L	3	A UF_L of 3 has been applied for LOAEL-to-NOAEL extrapolation because the POD is a LOAEL based on splenic extramedullary hepatopoiesis, for which the biological significance is not entirely clear.
UF_S	10	A UF_S of 10 has been applied to account for the extrapolation from less than chronic exposure.
UF_C	10,000	

[a]Lee et al. (1976c).

APPENDIX B. DATA TABLES

Table B.1. Body Weight and Absolute and Relative Organ Weight in Male CD Rats Fed 2,6-DNT for 4 or 13 Weeks[a]

Parameter	Exposure Group, mg/kg-d			
	0	7	35	145
Male Rat—4 Wks				
Sample size	4	4	4	4
Body weight[b] (g)	391 ± 8	364 ± 18	306 ± 9*	225 ± 52*
Absolute liver[b] (g)	12.6 ± 0.6	12.7 ± 0.6	11.7 ± 0.5	9.2 ± 0.6*
Relative liver[b] (g organ/100 g body wt)	3.2 ± 0.1	3.5 ± 0.3	3.8 ± 0.1	4.1 ± 0.2*
Absolute spleen[b] (g)	0.81 ± 0.03	0.72 ± 0.03	0.64 ± 0.03	0.97 ± 0.09
Relative spleen[b] (g organ/100 g body wt)	0.21 ± 0.01	0.20 ± 0.02	0.21 ± 0.01	0.43 ± 0.03*
Absolute kidneys[b] (g)	3.26 ± 0.08	3.01 ± 0.12	3.04 ± 0.21	2.29 ± 0.20*
Relative kidneys[b] (g organ/100 g body wt)	0.83 ± 0.01	0.83 ± 0.05	0.97 ± 0.05	0.02 ± 0.08
Absolute heart[b] (g)	1.34 ± 0.10	1.26 ± 0.03	1.06 ± 0.02*	0.79 ± 0.05*
Relative heart[b] (g organ/100 g body wt)	0.34 ± 0.02	0.35 ± 0.02	0.35 ± 0.00	0.35 ± 0.02
Absolute brain[b] (g)	2.08 ± 0.04	2.02 ± 0.09	2.05 ± 0.04	1.94 ± 0.07
Relative brain[b] (g organ/ 100 g body wt)	0.53 ± 0.02	0.56 ± 0.03	0.67 ± 0.02*	0.86 ± 0.02*
Male Rat—13Wks				
Sample size	4	4	4	4
Body weight[b] (g)	545 ± 21	486 ± 24	451 ± 13*	256 ± 12*
Absolute liver[b] (g)	15.4 ± 0.5	17.0 ± 1.3	10.7 ± 0.2*	8.9 ± 0.3*
Relative liver[b] (g organ/100 g body wt)	2.8 ± 0.0	3.5 ± 0.3*	2.4 ± 0.1	3.5 ± 0.1*
Absolute spleen[b] (g)	0.95 ± 0.06	1.02 ± 0.03	0.90 ± 0.10	0.65 ± 0.02*
Relative spleen[b] (g organ/100 g body wt)	0.17 ± 0.00	0.21 ± 0.01	0.20 ± 0.02	0.26 ± 0.02*
Absolute kidneys[b] (g)	3.09 ± 0.10	2.33 ± 0.18	3.05 ± 0.02	2.11 ± 0.07
Relative kidneys[b] (g organ/100 g body wt)	0.57 ± 0.03	0.48 ± 0.03	0.68 ± 0.02	0.83 ± 0.06*
Absolute heart[b] (g)	1.56 ± 0.09	1.60 ± 0.15	1.27 ± 0.01	0.99 ± 0.08*
Relative heart[b] (g organ/100 g body wt)	0.29 ± 0.01	0.33 ± 0.03	0.29 ± 0.00	0.39 ± 0.02*

Table B.1. Body Weight and Absolute and Relative Organ Weight in Male CD Rats Fed 2,6-DNT for 4 or 13 Weeks[a]

Parameter	Exposure Group, mg/kg-d			
	0	**7**	**35**	**145**
Absolute brain[b] (g)	2.09 ± 0.10	2.17 ± 0.07	2.l8 ± 0.07	1.97 ± 0.05
Relative brain[b] (g organ/ 100 g body wt)	0.39 ± 0.03	0.45 ± 0.02	0.48 ± 0.00	0.78 ± 0.05[*]

[a]Lee et al. (1976a).
[b]Means ± SE.
[*]Significantly different from corresponding control values at $p < 0.05$ (Dunnett's multiple comparison procedure).

Table B.2. Body Weight and Absolute and Relative Organ Weight in Female CD Rats Fed 2,6-DNT for 4 or 13 Weeks[a]

Parameter	Exposure Group, mg/kg-d			
	0	7	37	155
Female Rat—4 Wks				
Sample size	4	4	4	4
Body weight[b] (g)	232 ± 5	210 ± 7	194 ± 8*	157 ± 10*
Absolute liver[b] (g)	6.9 ± 0.3	6.5 ± 0.3	7.1 ± 0.2	6.0 ± 0.6*
Relative liver[b] (g organ/100 g body wt)	3.0 ± 0.1	3.1 ± 0.2	3.7 ± 0.1*	4.2 ± 0.2*
Absolute spleen[b] (g)	0.51 ± 0.04	0.54 ± 0.04	0.70 ± 0.08	0.46 ± 0.02
Relative spleen[b] (g organ/100 g body wt)	0.22 ± 0.02	0.26 ± 0.02	0.36 ± 0.05*	0.29 ± 0.02
Absolute kidneys[b] (g)	1.78 ± 0.04	1.62 ± 0.07	1.58 ± 0.10	1.44 ± 0.10*
Relative kidneys[b] (g organ/100 g body wt)	0.77 ± 0.03	0.77 ± 0.02	0.81 ± 0.04	0.91 ± 0.03*
Absolute heart[b] (g)	0.92 ± 0.08	0.86 ± 0.07	0.75 ± 0.04	0.58 ± 0.07*
Relative heart[b] (g organ/100 g body wt)	0.40 ± 0.04	0.41 ± 0.04	0.39 ± 0.03	0.37 ± 0.04
Absolute brain[b] (g)	1.88 ± 0.05	1.73 ± 0.03	1.82 ± 0.07*	1.86 ± 0.06
Relative brain[b] (g organ/ 100 g body wt)	0.81 ± 0.01	0.83 ± 0.02	0.94 ± 0.04	1.20 ± 0.07*
Female Rat—13Wks				
Sample size	4	4	4	4
Body weight[b] (g)	286 ± 11	270 ± 8	214 ± 17*	176 ± 9*
Absolute liver[b] (g)	7.9 ± 0.2	8.3 ± 0.6	7.1 ± 0.4	7.2 ± 0.2
Relative liver[b] (g organ/100 g body wt)	2.8 ± 0.0	3.1 ± 0.2	3.4 ± 0.3	4.1 ± 0.1*
Absolute spleen[b] (g)	0.58 ± 0.04	0.69 ± 0.09	0.54 ± 0.05	0.57 ± 0.06
Relative spleen[b] (g organ/100 g body wt)	0.20 ± 0.01	0.25 ± 0.03	0.26 ± 0.04	0.32 ± 0.02*
Absolute kidneys[b] (g)	1.65 ± 0.15	1.93 ± 0.07	1.71 ± 0.04	1.60 ± 0.08
Relative kidneys[b] (g organ/100 g body wt)	0.66 ± 0.01	0.62 ± 0.11	0.81 ± 0.06	0.91 ± 0.06*
Absolute heart[b] (g)	0.94 ± 0.03	0.86 ± 0.06	0.87 ± 0.06	0.66 ± 0.05*
Relative heart[b] (g organ/100 g body wt)	0.33 ± 0.01	0.32 ± 0.02	0.41 ± 0.03	0.38 ± 0.03
Absolute brain[b] (g)	1.88 ± 0.06	2.08 ± 0.07	2.00 ± 0.04	1.86 ± 0.06
Relative brain[b] (g organ/ 100 g body wt)	0.66 ± 0.03	0.77 ± 0.05	0.95 ± 0.06*	1.06 ± 0.03*

[a]Lee et al. (1976a).
[b]Means ± SE.
*Significantly different from corresponding control values at $p < 0.05$ (Dunnett's multiple comparison procedure).

Table B.3. Selected Hematology Parameters in the CD Rats Fed 2,6-DNT for 4 or 13 Weeks[a]

Parameter	Male Exposure Group, mg/kg-d			
	0	**7**	**35**	**145**
Male Rat—4 Wks				
Leukocytes[b] ($\times 10^3$/MM^3)	17.4 ± 1.7	19.5 ± 1.1	22.2 ± 1.2	31.5 ± 2.0*,**
Reticulocytes [b] (%)	1.39 ± 0.23*	1.73 ± 0.13*	0.85 ± 0.09*	9.33 ± 1.27*,**
Erythrocytes[b] ($\times 10^6$/MM^3)	7.15 ± 0.17	6.94 ± 0.03	7.78 ± 0.18*	5.80 ± 0.35**
Methemoglobin[b] (%)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Male Rat—13 Wks				
Leukocytes[b] ($\times 10^3$/MM^3)	20.7 ± 1.9	25.2 ± 4.9	27.4 ± 3.1*	25.0 ± 4.0
Reticulocytes[b] (%)	1.00 ± 0.12*	0.85 ± 0.20*	0.81 ± 0.16*	0.82 ± 0.26
Erythrocytes[b] ($\times 10^6$/MM^3)	6.81 ± 0.20	6.88 ± 0.57	7.76 ± 0.15*	7.40 ± 0.14*
Methemoglobin[b] (%)	0.6 ± 0.6	0.0 ± 0.0	0.0 ± 0.0	3.0 ± 1.2*
Parameter	**Female Exposure Group, mg/kg-d**			
	0	**7**	**37**	**155**
Female Rat—4 Wks				
Leukocytes[b] ($\times 10^3$/MM^3)	19.3 ± 1.7	17.6 ± 1.6	22.7 ± 2.1	24.0 ± 3.1*
Reticulocytes[b] (%)	1.18 ± 0.20	1.07 ± 0.15*	0.85 ± 0.26*	5.24 ± 1.12**
Erythrocytes [b] ($\times 10^6$/MM^3)	6.92 ± 0.18	6.35 ± 0.31	6.78 ± 0.20	6.91 ± 0.22
Methemoglobin [b] (%)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 0.6
Female Rat—13 Wks				
Leukocytes[b] ($\times 10^3$/MM^3)	17.5 ± 2.7	20.1 ± 2.0	26.4 ± 2.7*	20.5 ± 1.7
Reticulocytes[b] (%)	1.38 ± 0.16	1.24 ± 0.40*	1.13 ± 0.15*	0.89 ± 0.10
Erythrocytes[b] ($\times 10^6$/MM^3)	6.49 ± 0.44	6.35 ± 0.28	6.44 ± 0.24	7.06 ± 0.26
Methemoglobin[b] (%)	0.6 ± 0.6	0.0 ± 0.0	0.0 ± 0.0	2.4 ± 1.0*

[a]Lee et al. (1976a).
[b]Means ± SE.
*Significantly different from baseline (Dunnett's multiple comparison procedure).
**Significantly different from the controls at the respective time interval (Dunnett's multiple comparison procedure).

Table B.4. Tissue Lesions in Male Rats Fed 2,6-DNT for 13 Weeks[a]

Lesion Type[b]		Exposure group, mg/kg-d			
		0	**7**	**35**	**145**
Sample size		4	4	4	4
Heart	Focal myocarditis	1[c]	0	0	0
Trachea	Tracheitis	1[d]	0	0	0
Lung	Chronic murine pneumonia	1[c]	0	0	0
Submaxillary Salivary gland	Sialadenitis	0	1[d]	0	0
Liver	Focal mononuclear infiltration	2[c]	2[c]	1[c]	1[c]
	Extramedullary hematopoiesis	0	0	4[c, *]	4[c, *]
	Bile duct hyperplasia	0	0	3[c]	4[c, *]
	Focal degeneration	0	0	0	1[c]
Kidney	Dilatation of pelvis and/or tubules	0	0	0	2[c]
Testis	Focal atrophy	1[d]	1[d]	0	0
	Degeneration, retardation of spermatogenesis	0	0	1[c]	0
	Atrophy and aspermatogenesis	0	0	0	4[d, *]
Spleen	Hemosiderosis	0	4[c, *]	0	4[d, *]
	Extramedullary hematopoiesis	0	0	4[c, *]	4[c, *]
Bone marrow	M/E ratio	- to 1.5	-	-	1.3−1.5

[a]Lee et al. (1976a).
[b]Four rats examined per dose group; data are expressed as number of rats exhibiting each type of lesion, except for bone marrow ratio in which the ratio in all four rats is provided as a range.
[c]Lesions were graded by the author as "present, minimal, or mild."
[d]Lesions were graded by the author as "moderate, marked, or severe."
[*]$p < 0.05$ by Fisher's Exact Test performed by EPA.

Table B.5. Tissue Lesions in Female Rats Fed 2,6-DNT for 13 Weeks[a]

Lesion Type[b]		Exposure group, mg/kg-d			
		0	**7**	**37**	**155**
Sample size		4	4	4	4
Liver	Focal mononuclear infiltration	2[c]	2[c]	2[c]	1[c]
	Extramedullary hematopoiesis	0	0	2[c]	4[c, *]
	Bile duct hyperplasia	0	0	3[c]	4[c, *]
	Focal degeneration	0	0	0	1[c]
Kidney	Microcalculi	1[c]	0	0	1[c]
	Focal mononuclear infiltration	1[c]	0	0	1[c]
Uterus	Infiltration of eosinophils	2[c]	0	0	0
Spleen	Hemosiderosis	2[c]	2[c], 2[d]	0	4[d, *]
	Extramedullary hematopoiesis	0	0	4[c, *]	4[c, *]
Bone marrow	M/E ratio	- to 1.6	-	-	- to 1.5

[a]Lee et al. (1976a).
[b]Four rats examined per dose group; data are expressed as number of rats exhibiting each type of lesion, except for bone marrow ratio in which the ratio in all four rats is provided as a range.
[c]Lesions were graded by the author as "present, minimal, or mild."
[d]Lesions were graded by the author as "moderate, marked, or severe."

Table B.6. Body Weight and Absolute and Relative Liver Weight in Male Albino Swiss Mice Fed 2,6-DNT for 13 Weeks[a]

Parameter	Exposure Group, mg/kg-d			
	0	11	51	289
Sample size	2	4	3	0
Body weight[b] (g)	27.5 ± 2.5	32.5±1.6	31.3±2.2	NA
Absolute liver[b] (g)	1.75 ± 0.46	1.51 ± 0.08	1.54 ± 0.08	NA
Relative liver[b] (g organ/100 g body wt)	6.3 ± 1.1	4.6 ± 0.1	4.9 ± 0.3	NA
Absolute kidneys[b] (g)	0.87 ± 0.01	1.01 ± 0.10	0.54 ± 0.06	NA
Relative kidneys[b] (g organ/100 g body wt)	3.19 ± 0.25	3.11 ± 0.22	1.73 ± 0.13*	NA

[a]Lee et al. (1976b).
[b]Means ± SE.
*Significantly different from corresponding control values at $p < 0.05$ (Dunnett's multiple comparison procedure).
NA=Not applicable.

Table B.7. Mortality in Male Albino Swiss Mice Treated with 2,6-DNT in Diet for 4 or 13 Weeks

Exposure Group, mg/kg-day	No. of Animals	No. of Mice Dying During Specified Weeks[a,b]				
		0− 4	5−8	9−13	14−17	Total[c]
Males						
0	16	0	0	3	0	3
11	16	2	0	0	0	2
51	16	6	0	1	1	8
289	16	0	6	2	0	8
Females						
0	16	0	0	0	0	0
11	16	0	0	0	0	0
55	16	1	0	0	0	1
299	16	1	3	2	0	6

[a]Number of dead animals.
[b]Number of animals includes mice treated for 4 and 13 weeks and necropsied and mice allowed to recover for 4 weeks.
[c]Calculated for this review from data reported in the study.

Source: Lee et al. (1976b)

Table B.8. Tissue Lesions in Male and Female Dogs Treated with 2,6-DNT for 4 or 13 Weeks[a]

Lesion Type[b]		Exposure group, mg/kg-d			
		0	4	20	100
4 weeks					
Sample Size		2	2	2	2
Lung	Focal inflammation	2	0	1	1
Liver	Extramedullary hematopoiesis	0	0	1	2
	Bile duct hyperplasia	0	0	1	0
	Degeneration	0	0	0	1
	Subacute inflammation	0	0	1	1
Testis	Degeneration and/or retardation of spermatogenesis	0	0	1	1
Ovary	Several graafian follicles, but no corpea lutea		1	1	1
Thyroid	Increase in parafollicular cells	0	1	2	1
Spleen	Extramedullary hematopoiesis	0	1	2	2
	Lymphoid depletion				1
Tonsil	Inflammation	0	1	0	1
Lymph Node	Lymphoid degeneration and depletion	0	0	0	1
Thymus	Involution	0	0	0	1
	Focal hyalinization of the corpuscles				1
Bone marrow	M/E ratio	1.3 to 1.4	1.1 to 1.3	1.1	- to 0.9
13 weeks					
Sample size		2	2	3	4
Liver	Extramedullary hematopoiesis	0	0	3	4
	Bile duct hyperplasia	0	0	2	2
	Focal degeneration	0	0	3	4
Kidney	Dilatation of pelvis and/or tubules	0	0	0	2
Testis	Atrophy and aspermatogenesis	0	0	0	2
Spleen	Extramedullary hematopoiesis	0	2[c]	3[d]	4[e]
Bone marrow	M/E ratio	1.3 to 1.5	1.3 to 1.5	- to 1.3	0.9 to 1.0

[a]Lee et al. (1976c).
[b]Data are expressed as number of dogs exhibiting each type of lesion, except for bone marrow ratio in which the ratio in dogs is provided as a range.
[c]Lesions were graded by the author as "minimal or mild."
[d]Lesions were graded by the author as "minimal, mild, or moderate."
[e]Lesions were graded by the author as "marked or markedly severe."

Table B.9. Mortality in Male and Female Dogs Treated with 2,6-DNT in Diet for 13 Weeks

Exposure Group, mg/kg-day	No. of Animals	No. of Dogs Dying During Specified Weeks[a]			
		0−4	5−8	9−13	Total[b]
Males/Females					
0	2	0	0	0	0
4	2	0	0	0	0
20	3	0	0	2	2
100	4	0	4	0	4

[a]Number of dead animals.
[b]Calculated for this review from data reported in the study.

Source: Lee et al. (1976c).

Table B.10. Reticulocyte Data in Dogs Treated with 2,6-DNT for up to 13 Weeks[a]

Parameter	Male/Female Exposure Group, mg/kg-day			
	0	4	20	100
2 Wks				
Reticulocytes [b] (%)	0.76 ± 0.07	1.10 ± 0.13	1.43 ± 0.32	16.99 ± 3.33[*,**]
4 Wks				
Reticulocytes [b] (%)	0.59 ± 0.08	0.98 ± 0.10	1.67 ± 0.43	6.23 ± 1.60[**]
8 Wks				
Reticulocytes[b] (%)	1.01 ± 0.24	0.88 ± 0.08	0.84 ± 0.30	No data
13 Wks				
Reticulocytes [b] (%)	0.56 ± 0.11	0.62 ± 0.0	1.91	No data

[a]Lee et al. (1976c).
[b]Means ± SE.
[*]Significantly different from baseline (Dunnett's multiple comparison procedure).
[**]Significantly different from the controls at the respective time interval (Dunnett's multiple comparison procedure).

Table B.11. Body Weight, Liver Weight, and Serum Enzyme Activities in the Male (F344)/CrlBR Rat Exposed to Oral 2,6-DNT for 26 and 52 Weeks[a]

Parameter	Exposure Group, mg/kg-d		
	0	7	14
Male Rat—6 Mo			
Sample size	4	4	4
Terminal body wt[b] (g)	395 ± 2	376 ± 9	316 ± 9*
Liver wt[b] (g)	9.62 ± 0.25	10.52 ± 0.47	11.42 ± 0.59*
Liver/body wt × 100[b]	2.43 ± 0.07	2.80 ± 0.07*	3.62 ± 0.08*
Serum activity			
ALT[b] (IU/liter)	69 ± 5	58 ± 3	48 ± 5
GGT[b] (IU/liter)	0.2 ± 0.2	1.2 ± 0.5	3.7 ± 0.6*
Male Rat—12 Mo			
Sample size	20	20	20
Terminal body wt[b] (g)	434 ± 3	356 ± 5*	297 ± 7*
Liver wt[b] (g)	10.30 ± 0.16	21.09 ± 0.74*	38.20 ± 2.14*
Liver/body wt × 100[b]	2.38 ± 0.04	5.99 ± 0.29*	13.19 ± 0.97*
Serum activity			
ALT[b] (IU/liter)	133 ± 14	230 ± 75*	1,044 ± 163*
GGT[b] (IU/liter)	3.8 ± 0.5	43 ± 18	205 ± 46*

[a]Leonard et al. (1987).
[b]Means ± SEM.
*Significantly different from corresponding control values at $p < 0.05$.

Table B.12. Treatment Protocol Adapted from Goldsworthy et al., 1986

Treatment Group[a]	No of Animals	Diet	Concentration of 2,6-DNT in the Diet, mg/kg-d[b]
NIH	30	NIH-07	0
NIH-HD DNT	30	NIH-07	3−3.5
NIH-LD DNT	30	NIH-07	0.6−0.7
AIN	30	AIN-76A	0
AIN-HD DNT	30	AIN-76A	3−3.5
AIN-LD DNT	30	AIN-76A	0.6−0.7
AP	30	AIN-76A + 5% pectin	0
AP-HD DNT	30	AIN-76A + 5% pectin	3−3.5
AP-LD DNT	30	AIN-76A + 5% pectin	0.6−0.7

[a]The diets used were NIH-07, an open formula cereal-based diet high in pectin content; AIN-76A, a purified pectin-free diet; or AP, which is AlN-76A supplemented with 5% pectin. These three diets served as the control diets for the addition of 2.6-DNT at either a high-dose (HD DNT) or low-dose (LD DNT).
[b]HED are 0, 0.13−0.15, and 0.63−0.74 mg/kg-day for the control, low-dose, and high-dose, respectively.

Table B.13. Effect of the Control and DNT-Diets on the Fraction of Animals with Hepatic Foci Identified by Three Phenotypic Markers[a]

Treatment Group[b]	Hepatic Phenotypic Markers		
	GGT	ATP	G6P
At 3 months			
NIH	2/10	6/10	5/10
NIH-LD DNT	6/10	10/10	9/10
NIH-HD DNT	9/10	10/10	8/10
AIN	0/10	4/10	1/10
AIN-LD DNT	0/10	7/10	5/10
AIN-HD DNT	0/10	7/10	7/10
AP	0/10	4/10	5/10
AP-LD DNT	0/10	8/10	7/10
AP-HD DNT	0/10	9/10	10/10
At 6 months			
NIH	2/10	5/10	5/10
NIH-LD DNT	9/10	8/10	10/10
NIH-HD DNT	8/10	10/10	10/10
AIN	0/10	4/10	3/10
AIN-LD DNT	0/10	7/10	4/10
AIN-HD DNT	3/10	10/10	10/10
AP	0/10	4/10	3/10
AP-LD DNT	0/10	9/10	6/10
AP-HD DNT	3/10	10/10	10/10
At 12 months			
NIH	10/10	10/10	9/10
NIH-LD DNT	10/10	10/10	10/10
NIH-HD DNT	10/10[c]	10/10[c]	10/10[c]
AIN	0/10	9/10	6/10
AIN-LD DNT	5/10	10/10	8/10
AIN-HD DNT	10/10	10/10	10/10
AP	3/10	9/10	9/10
AP-LD DNT	7/10	10/10	10/10
AP-HD DNT	10/10	10/10	10/10

[a]Goldsworthy et al. (1986).
[b]The diets used were NIH-07, an open formula cereal-based diet high in pectin content; AIN-76A, a purified pectin-free diet; or AP, which is AlN-76A supplemented with 5% pectin. These three diets served as the control diets for the addition of 2,6-DNT at either a high-dose (HD DNT) or low-dose (LD DNT).
[c]Livers exhibited multi foci, neoplastic nodules (6/10), and hepatocellular carcinomas (6/10). The presence of these lesions did not allow for the accurate quantitation of the foci in these livers.

Table B.14. Incidence of Neoplastic Lesions and Metastases in a 1-Year Oral Study of 2,6-DNT in the Male (F344)/Crlbr Rat[a]

Lesion Type	Exposure Group, mg/kg-d		
	0 (0)	7	14
Neoplastic nodules	0/20	18/20*	15/19*
Hepatocellular carcinoma:Trabecular	0/20	17/20*	19/19*
Adenocarcinoma	0/20	1/20	0/19
Cholangiocarcinoma	0/20	2/20	0/19
Pulmonary metastases	NA	3/20	11/19

[a]Leonard et al. (1987).
NA = not applicable (no primary tumors present).
**p* < 0.001 by Fisher's Exact Test performed by EPA.

APPENDIX C. BMD OUTPUTS FOR THE SUBCHRONIC AND CHRONIC p-RfDs

MODELING PROCEDURE FOR CONTINUOUS DATA

The BMD modeling of continuous data was conducted with EPA's BMDS (version 2.1.2). For these data, all continuous models available within the software were fit using a BMR of 10% relative risk or 1 standard deviation. 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 in 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 screening subchronic and chronic p-RfD.

APPENDIX D. BENCHMARK DOSE CALCULATIONS FOR THE ORAL SLOPE FACTOR

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 benchmark dose software (BMDS) 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: goodness-of-fit p-value ($p > 0.1$), visual inspection of the dose-response curve, and 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 point of departure. In accordance with EPA (2000) guidance, BMDs and BMDLs associated with an extra risk of 10% are calculated.

MODEL-FITTING RESULTS FOR HEPATOCELLULAR CARCINOMAS IN MALE F344 RATS (Leonard et al., 1987)

Table B.14 shows the dose-response data on hepatocellular tumors in male F344 rats administered 2,6-DNT via the diet for 12 months (Leonard et al., 1987). Modeling was performed according to the procedure outlined above using BMDS version 2.1.2 with parameter restrictions for rats based on the duration-adjusted animal doses shown in Table 3. Model predictions are shown in Table 9. For incidence of hepatocellular carcinomas in both male rats, the multistage-cancer model provided an adequate fit (goodness-of-fit p-value >0.1). The 3-degree polynomial model yielded a BMD_{10} value of 2.7 mg/kg-day with an associated 95% lower confidence limit ($BMDL_{10}$) of 0.25 mg/kg-day for male rats. The fit of the multistage-cancer models to the hepatocellular carcinoma incidence data for male rats is shown in Table 9 and Figure D.1.

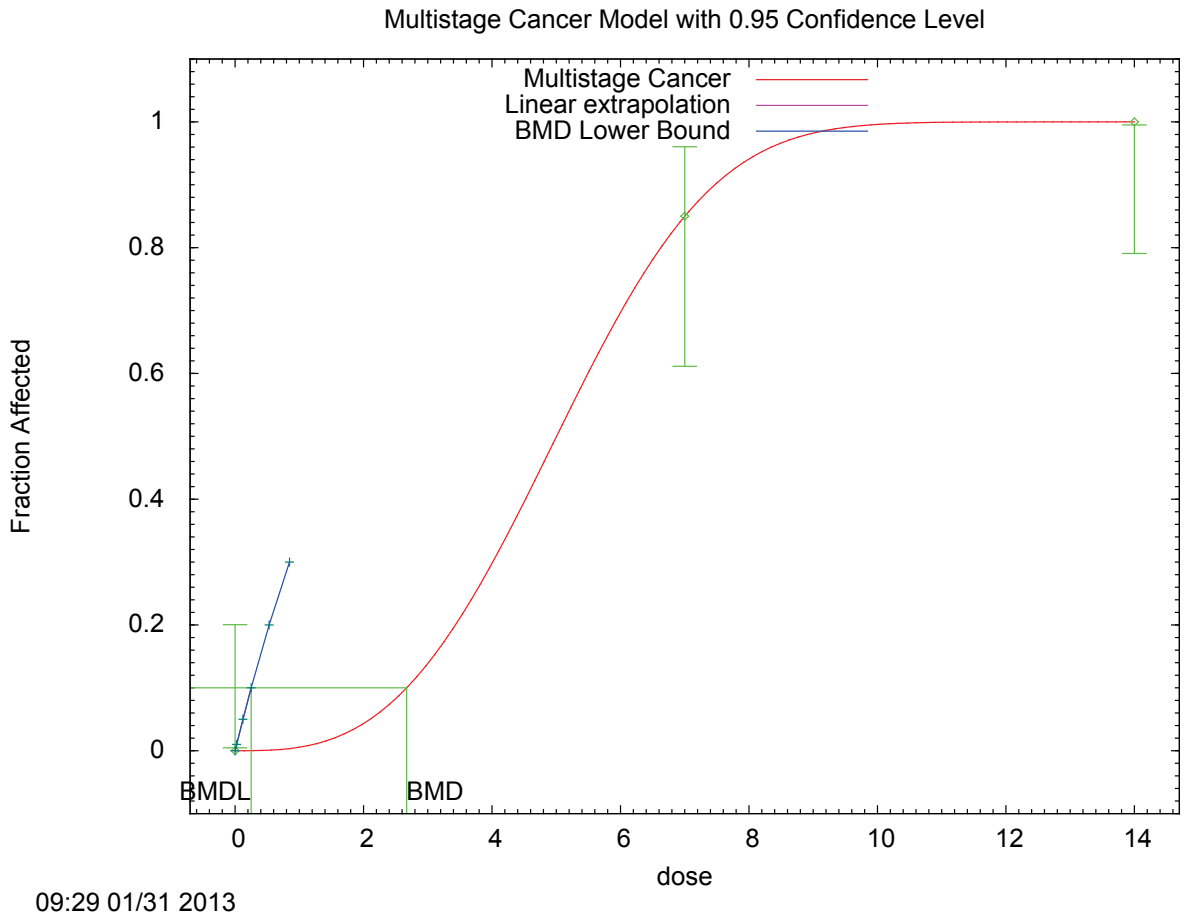

Figure D.1. Multistage Cancer Model for Hepatocellular Carcinomas in Male Rats (Leonard et al., 1987)

Text Output for Multistage Cancer Model for Hepatocellular Carcinomas in Male Rats (Leonard et al., 1987)

```
 ======================================================================
        Multistage Cancer Model. (Version: 1.9;  Date: 05/26/2010)
        Input Data File: C:/Documents and Settings/JKaiser/Desktop/modeling
results/msc_26dnt_hcar_m_Msc3-BMR10.(d)
        Gnuplot Plotting File:  C:/Documents and Settings/JKaiser/Desktop/modeling
results/msc_26dnt_hcar_m_Msc3-BMR10.plt
                                                Thu Jan 31 09:29:24 2013
 ======================================================================

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

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

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1-beta2*dose^2-beta3*dose^3)]

   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 = 4
 Total number of specified parameters = 0
 Degree of polynomial = 3


 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) =            0
                        Beta(3) = 3.92465e+016


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Background    -Beta(1)    -Beta(2)
                 have been estimated at a boundary point, or have been specified by
the user,
                 and do not appear in the correlation matrix )

                Beta(3)

   Beta(3)            1



                                Parameter Estimates
```

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0	*	*	*
Beta(2)	0	*	*	*
Beta(3)	0.00553091	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-8.45418	3			
Fitted model	-8.45419	1	9.74156e-006	2	1
Reduced model	-39.4517	1	61.995	2	<.0001

AIC: 18.9084

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	20	-0.000
7.0000	0.8500	17.000	17.000	20	0.000
14.0000	1.0000	19.000	19.000	19	0.002

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

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 2.67071

BMDL = 0.250402

BMDU = 3.13339

Taken together, (0.250402, 3.13339) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.399358

APPENDIX E. REFERENCES

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