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Provisional Peer-Reviewed Toxicity Values for

2-Amino-4,6-dinitrotoluene
(CASRN 35572-78-2)

U.S. EPA Office of Research and Development
Center for Public Health and Environmental Assessment

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Center for Public Health and Environmental Assessment
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Questions regarding the content of this PPRTV assessment should be directed to the U.S. EPA Office of Research and Development's Center for Public Health and Environmental Assessment.

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COMMONLY USED ABBREVIATIONS AND ACRONYMS

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

Abbreviations and acronyms not listed on this page are defined upon first use in the PPRTV document.

PROVISIONAL PEER-REVIEWED TOXICITY VALUES FOR 2-AMINO-4,6-DINITROTOLUENE (CASRN 35572-78-2)

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.

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.

Currently available PPRTV assessments can be accessed on the U.S. Environmental Protection Agency's (EPA's) PPRTV website at https://www.epa.gov/pprtv. PPRTV assessments are eligible to be updated on a 5-year cycle and revised as appropriate to incorporate new data or methodologies that might impact the toxicity values or affect the characterization of the chemical's potential for causing adverse human-health effects. Questions regarding nomination of chemicals for update can be sent to the appropriate U.S. EPA Superfund and Technology Liaison (https://www.epa.gov/research/fact-sheets-regional-science).

QUALITY ASSURANCE

This work was conducted under the U.S. EPA Quality Assurance (QA) program to ensure data are of known and acceptable quality to support their intended use. Surveillance of the work by the assessment managers and programmatic scientific leads ensured adherence to QA processes and criteria, as well as quick and effective resolution of any problems. The QA manager, assessment managers, and programmatic scientific leads have determined under the QA program that this work meets all U.S. EPA quality requirements. This PPRTV was written with guidance from the CPHEA Program Quality Assurance Project Plan (PQAPP), the QAPP titled *Program Quality Assurance Project Plan (PQAPP) for the Provisional Peer-Reviewed Toxicity Values (PPRTVs) and Related Assessments/Documents (L-CPAD-0032718-QP),* and the PPRTV development contractor QAPP titled *Quality Assurance Project Plan—Preparation of Provisional Toxicity Value (PTV) Documents (L-CPAD-0031971-QP)*. As part of the QA system, a quality product review is done prior to management clearance. A Technical Systems Audit may be performed at the discretion of the QA staff.

All PPRTV assessments receive internal peer review by at least two Center for Public Health and Environmental Assessment (CPHEA) scientists and an independent external peer review by at least three scientific experts. The reviews focus on whether all studies have been correctly selected, interpreted, and adequately described for the purposes of deriving a provisional reference value. The reviews also cover quantitative and qualitative aspects of the provisional value development and address whether uncertainties associated with the assessment have been adequately characterized.

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

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

QUESTIONS REGARDING PPRTVS

Questions regarding the content of this PPRTV assessment should be directed to the U.S. EPA Office of Research and Development (ORD) CPHEA website at https://www.epa.gov/pprtv/forms/contact-us-about-pprtvs.

INTRODUCTION

2-Amino-4,6-dinitrotoluene (2-ADNT), CASRN 35572-78-2, is a derivative of compounds known as nitroaromatics or nitroarenes. Nitroaromatics are used as explosive materials, pesticides, solvents, and intermediates in chemical synthesis (Haderlien et al., 1996). 2-ADNT is a primary biotransformation product of 2,4,6-trinitrotoluene (TNT) formed during biological degradation and reduction processes (Thorn and Kennedy, 2002; Wood and Tiller, 1996). The main human urinary metabolites of TNT are 2-ADNT and 4-amino-2,6-dinitrotoluene (4-ADNT). Neither compound is listed on U.S. EPA's Toxic Substances Control Act's public inventory (U.S. EPA, 2015), nor is it registered with Europe's Regulation on Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) program (ECHA, 2018).

The empirical formula for 2-ADNT is $C_7H_7N_3O_4$ (see Figure 1). Table 1 summarizes the compound's physicochemical properties. 2-ADNT is a solid at room temperature. Its estimated low vapor pressure and low Henry's law constant indicate that it is unlikely to volatilize from either dry or moist surfaces. The estimated moderate water solubility and moderate soil adsorption coefficient indicate that 2-ADNT may leach to groundwater or undergo runoff after a rain event. However, adsorption to soil is directly related to organic content of the soil (Wood and Tiller, 1996). Some studies have shown that reduced TNT amines, including, 2-ADNT, will form solvent nonextractable bound residue with organic matter in soil via covalent bonds. Once bonded, the long-term release from soils is negligible (Thorn and Kennedy, 2002).

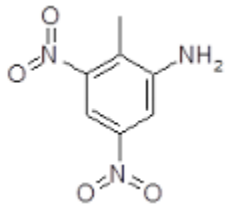


Figure 1. 2-ADNT (CASRN 35572-78-2) Structure

Table 1. Physicochemical Properties of 2-ADNT (CASRN 35572-78-2)

Property (unit)	Value
Physical state	Solid
Boiling point (°C)	352 (predicted average)
Melting point (°C)	173
Density (g/mL)	1.52 (predicted average)
Vapor pressure (mm Hg at 25°C)	4.6×10^{-6} (predicted average)
pH (unitless)	NV
pKa (unitless)	0.36[b]
Solubility in water (mg/L at 25°C)	6.12×10^{-3} (predicted average)
Octanol-water partition coefficient (log K_{ow})	1.94 (predicted average)
Henry's law constant (atm-m^3/mol at 25°C)	4.4×10^{-8} (predicted average)
Soil adsorption coefficient K_{oc} (L/kg)	196 (predicted average)
Atmospheric OH rate constant (cm^3/molecule-sec at 25°C)	1.49×10^{-12} (predicted average)
Atmospheric half-life (d)	9.4 (estimated)[b]
Relative vapor density (air = 1)	NV
Molecular weight (g/mol)	197.15
Flash point (closed cup in °C)	192 (predicted average)

[a]Unless otherwise noted, information is sourced from U.S. EPA CompTox Chemicals Dashboard (2-amino-4,6-dinitrotoluene; https://comptox.epa.gov/dashboard/DTXSID6044068; accessed April 2020). Values are experimental averages unless noted as predicted averages.
[b]Thorn and Kennedy (2002).

2-ADNT = 2-amino-4,6-dinitrotoluene; NA = not applicable; NV = not available.

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

Table 2. Summary of Available Toxicity Values for 2-ADNT (CASRN 35572-78-2)

Source[a]	Value	Notes	Reference(s)[b]
Noncancer			
IRIS	NV	NA	U.S. EPA (2018)
HEAST	NV	NA	U.S. EPA (2011b)
DWSHA	NV	NA	U.S. EPA (2012)
ATSDR	NV	NA	ATSDR (2018)
IPCS	NV	NA	IPCS (2018)
CalEPA	NV	NA	CalEPA (2016a); CalEPA (2016b); CalEPA (2018)
OSHA	NV	NA	OSHA (2006); OSHA (2011)
NIOSH	NV	NA	NIOSH (2016)
ACGIH	NV	NA	ACGIH (2018)
Cancer			
IRIS	NV	NA	U.S. EPA (2018)
HEAST	NV	NA	U.S. EPA (2011b)
DWSHA	NV	NA	U.S. EPA (2012)
NTP	NV	NA	NTP (2016)
IARC	NV	NA	IARC (2018)
CalEPA	NV	NA	CalEPA (2016a); CalEPA (2017); CalEPA (2018)
ACGIH	NV	NA	ACGIH (2018)

[a]Sources: ACGIH = American Conference of Governmental Industrial Hygienists; ATSDR = Agency for Toxic Substances and Disease Registry; CalEPA = California Environmental Protection Agency; DWSHA = Drinking Water Standards and Health Advisories; HEAST = Health Effects Assessment Summary Tables; IARC = International Agency for Research on Cancer; IPCS = International Programme on Chemical Safety; IRIS = Integrated Risk Information System; NIOSH = National Institute for Occupational Safety and Health; NTP = National Toxicology Program; OSHA = Occupational Safety and Health Administration.
[b]Reference date is the publication date for the database and not the date the source was accessed.

2-ADNT = 2-amino-4,6-dinitrotoluene; NA = not applicable; NV = not available.

Non-date-limited literature searches were conducted in June 2015 and updated in April 2020 for studies relevant to the derivation of provisional toxicity values for 2-amino-4,6-dinitrotoluene (CASRN 35572-78-2). Searches were conducted using U.S. EPA's Health and Environmental Research Online (HERO) database of scientific literature. HERO searches the following databases: PubMed, TOXLINE (including TSCATS1), and Web of Science. The following resources were searched outside of HERO for health-related values: American Conference of Governmental Industrial Hygienists (ACGIH), Agency for Toxic Substances and Disease Registry (ATSDR), California Environmental Protection Agency (CalEPA), Defense Technical Information Center (DTIC), European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC), European Chemicals Agency (ECHA), U.S. EPA Chemical Data Access Tool (CDAT), U.S. EPA ChemView, U.S. EPA Health Effects Assessment Summary Tables (HEAST), U.S. EPA Integrated Risk Information System (IRIS), U.S. EPA Office of Water (OW), International Agency for Research on Cancer (IARC), Japan Existing Chemical Data Base (JECDB), National Institute for Occupational Safety and Health (NIOSH), National Toxicology Program (NTP), Organisation for Economic Co-operation and Development (OECD) Existing Chemicals Database, OECD Screening Information Data Set (SIDS) high production volume (HPV) chemicals via International Programme on Chemical Safety (IPCS) INCHEM, Occupational Safety and Health Administration (OSHA), and World Health Organization (WHO).

REVIEW OF POTENTIALLY RELEVANT DATA (NONCANCER AND CANCER)

There are no potentially relevant short-term, subchronic, or chronic studies or developmental or reproductive toxicity studies in humans or animals for 2-ADNT as shown in Tables 3A and 3B.

Table 3A. Summary of Potentially Relevant Noncancer Data for 2-ADNT (CASRN 35572-78-2)							
Category	**Number of Male/Female, Strain, Species, Study Type, Reported Doses, Study Duration**	**Dosimetry**	**Critical Effects**	**NOAEL**	**LOAEL**	**Reference**	**Notes**
Human							
1. Oral (mg/kg-d)							
ND							
2. Inhalation (mg/m^3)							
ND							
Animal							
1. Oral (mg/kg-d)							
ND							
2. Inhalation (mg/m^3)							
ND							

2-ADNT = 2-amino-4,6-dinitrotoluene; LOAEL = lowest-observed-adverse-effect level; ND = no data; NOAEL = no-observed-adverse-effect level.

Table 3B. Summary of Potentially Relevant Cancer Data for 2-ADNT (CASRN 35572-78-2)							
Category	**Number of Male/Female, Strain, Species, Study Type, Reported Doses, Study Duration**	**Dosimetry**	**Critical Effects**	**NOAEL**	**LOAEL**	**Reference**	**Notes**
Human							
1. Oral (mg/kg-d)							
ND							
2. Inhalation (mg/m^3)							
ND							
Animal							
1. Oral (mg/kg-d)							
ND							
2. Inhalation (mg/m^3)							
ND							

2-ADNT = 2-amino-4,6-dinitrotoluene; LOAEL = lowest-observed-adverse-effect level; ND = no data; NOAEL = no-observed-adverse-effect level.

HUMAN STUDIES

Oral Exposures

No studies have been identified.

Inhalation Exposures

No studies have been identified.

ANIMAL STUDIES

Oral Exposures

No studies have been identified.

Inhalation Exposures

No studies have been identified.

OTHER DATA (SHORT-TERM TESTS, OTHER EXAMINATIONS)

Acute Animal Studies

Available data on the toxicity of 2-ADNT are limited to acute lethality studies, which report median lethal dose (LD_{50}) values of 1,394−2,240 and 1,522−1,722 mg/kg in the rat and mouse, respectively (Ellis et al., 1980; Ellis et al., 1978). 2-ADNT is a mild skin irritant in laboratory animals, but it is not an eye irritant or a skin sensitizer (Ellis et al., 1980; Ellis et al., 1978).

Genotoxicity

Available data on the genotoxicity of 2-ADNT are summarized in Table 4. In general, data indicate that 2-ADNT is mutagenic and has the capacity to cause deoxyribonucleic acid (DNA) damage in bacteria. Evidence in mammalian cells is limited and inconsistent but suggests that 2-ADNT may be mutagenic in mammalian cells. No studies evaluating clastogenic effects were identified. The potential for 2-ADNT to cause genotoxicity following in vivo exposure has not been evaluated.

The majority of available studies indicate that 2-ADNT is mutagenic in *Salmonella typhimurium* and *Vibrio fischeri* with and without metabolic activation [Neuwoehner et al. (2007); Karamova et al. (1994) as cited in Lachance et al. (1999); Honeycutt et al. (1996); Spanggord et al. (1995); Tan et al. (1992); Spanggord et al. (1982)], although it is not mutagenic in nitroreductase-deficient *S. typhimurium* strains (TA98 or TA100) (Spanggord et al., 1995; Spanggord et al., 1982), suggesting that reduction of the nitro to the amino group is accompanied by the generation of oxidative metabolites (oxidative stress), which directly or indirectly damages DNA. In the rat study, mutagenicity of the ADNT urinary fraction (containing both 2- and 4-ADNT) was dependent upon nitroreductase activity in *S. typhimurium* (Brooks et al., 1997). Urine from workers exposed to TNT, which contained 2-ADNT as one of the primary metabolites, also showed mutagenic activity in *S. typhimurium* strains (Brooks et al., 1997; Ahlborg et al., 1988). In this study, mutagenic activity was observed in both standard and nitroreductase-deficient strains, but mutagenicity was not significantly correlated with urinary 2-ADNT concentrations (Ahlborg et al., 1988).

In mammalian cells, 2-ADNT was not mutagenic in Chinese hamster ovary (CHO) or Chinese hamster V79 lung fibroblast cells with or without metabolic activation (Kennel et al., 2000; Lachance et al., 1999); however, 2-ADNT induced point mutations and loss of

heterozygosity in the *p53* gene of mouse NG108 neuroblastoma and human MCF7 breast cancer cells (Banerjee and Dutta, 1997).

2-ADNT caused DNA damage in *Salmonella choleraesuis* in the NM2009 assay (a strain of *Salmonella* overexpressing the *O*-acetyltransferase gene) without metabolic activation; it did not cause DNA damage in the NM2009 assay with metabolic activation or in the *umu* test with or without metabolic activation (Neuwoehner et al., 2007). 2-ADNT caused DNA damage in *Escherichia coli* without, but not with, metabolic activation (Neuwoehner et al., 2007).

Table 4. Summary of 2-ADNT (CASRN 35572-78-2) Genotoxicity						
Endpoint	**Test System**	**Dose/ Concentration**	**Results without Activation[a]**	**Results with Activation[a]**	**Comments**	**References**
Genotoxicity studies in prokaryotic organisms						
Mutation	*Salmonella typhimurium* strains TA98 and TA100	0−37.6 mg/L	−	+ (TA100) − (TA98)	Fluctuation test. Mutagenicity in TA100 was observed at ≥18.8 mg/L with S9.	Neuwoehner et al. (2007)
Mutation	*S. typhimurium* strains TA98 and TA100	0−44.1 µM (TA98) 0−39.6 µM (TA100)	+	+	Fluctuation test. Mutagenicity in TA98 was observed at ≥8.1 µM with or without S9. Mutagenicity in TA100 was observed at ≥6.6 µM without S9 and ≥13.2 µM with S9.	Lachance et al. (1999)
Mutation	*S. typhimurium* strains TA98 and TA100	5 concentrations (not specified)	+	+ (TA100) − (TA98)	Plate incorporation assay.	Honeycutt et al. (1996)
Mutation	*S. typhimurium* strains TA100 and its nitroreductase-deficient mutant, TA100 NR	0, 31.2, 62.5, 125, 250, 500, 1,000 µg/plate	+ (TA100) − (TA100 NR)	NT	Plate incorporation assay. A 2.9- to 10.5-fold increase in the number of TA100 revertants was observed at ≥250 µg/plate. Data suggest that bacterial nitroreductase activity is responsible for mutagenic activity in TA100 strain.	Spanggord et al. (1995)
Mutation	*S. typhimurium* strains TA98 and TA100	NR	+	+ (TA100) − (TA98)	NA	Karamova et al. (1994) as cited in Lachance et al. (1999) [Russian study]
Mutation	*S. typhimurium* strains TA98 and TA100	0, 100, 200, 300, 400 µg/plate	+	+	Plate incorporation assay. Concentration-dependent increases were observed in the number of revertants with and without S9; mutagenicity was less with S9 activation in both strains.	Tan et al. (1992)

Table 4. Summary of 2-ADNT (CASRN 35572-78-2) Genotoxicity

Endpoint	Test System	Dose/ Concentration	Results without Activation[a]	Results with Activation[a]	Comments	References
Mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA98, TA100, and nitroreductase-deficient strain TA100 NR3	10−5,000 µg/plate	+ (TA1537, TA1538, TA98, TA100) − (TA 1535, TA100 NR3)	+ (TA1535, TA1527, TA1538, TA98, TA100) − (TA100 NR3)	Plate incorporation assay. Data suggest that bacterial nitroreductase activity is responsible for mutagenic activity in TA100 strain.	Spanggord et al. (1982)
Mutation	*S. typhimurium* strains TA98 and TA100	NR	−	−	NA	Won et al. (1976)
Mutation	*Vibrio fischeri*	10 serial dilutions from saturated stock solutions in DMSO	+	+	Mutatox bioluminescence assay. Mutagenic responses were observed at 1−3.9 µg/cuvette with S9 and 3.9−15.6 µg/cuvette without S9.	Honeycutt et al. (1996)
Mutation	*S. typhimurium* strains TA98 and its nitroreductase-deficient mutant, TA98 NR, were exposed to urine samples collected from 41 TNT-exposed munitions workers collected preshift on Monday (after not working over the weekend) or immediately following a work shift. Urinary levels of TNT, 2-ADNT, and 4-ADNT were quantified.	Mean urinary 2-ADNT levels were 1.28, 3.73, and 16.65 µmol/mol creatine in workers exposed to no/low, medium, and high exposure levels of TNT, respectively.	+	NT	Increased mutagenic activity was observed in both tester strains following exposure to urine collected at either time point in exposed compared with unexposed workers; postwork urine was significantly more mutagenic than prework urine in high exposure group only. Mutagenic activity was not significantly correlated with urinary 2-ADNT levels. Data do not support that bacterial nitroreductase activity is responsible for mutagenicity.	Ahlborg et al. (1988)

Table 4. Summary of 2-ADNT (CASRN 35572-78-2) Genotoxicity

Endpoint	Test System	Dose/ Concentration	Results without Activation[a]	Results with Activation[a]	Comments	References
Mutation	*S. typhimurium* strains TA98, TA100, and their nitroreductase-deficient mutants, TA98 NR and TA100 NR, were exposed to F344 rat urine sample fractions containing 2- and 4-ADNT.	Rats were exposed to 0 and 75 mg TNT/kg via gavage; urinary levels of ADNTs were not reported	+ (TA98) − (TA98 NR, TA100, TA100 NR)	NT	A significant (≥threefold) increase in revertants was observed in the TA98 strain exposed to the ADNT fraction. Data suggest that bacterial nitroreductase activity is responsible for mutagenic activity in TA98 strain.	Brooks et al. (1997)
DNA damage (SOS chromotest)	*Escherichia coli* PQ37	0−12.4 mg/L	+	−	A significant (>1.5-fold) induction was observed at ≥6.3 mg/L without activation.	Neuwoehner et al. (2007)
DNA damage (*umu* test)	*Salmonella choleraesuis* subsp. *chol.* (prior *S. typhimurium*) TA1535/pSK1002	0−12.4 mg/L	−	−	NA	Neuwoehner et al. (2007)
DNA damage (NM2009 test)	*S. choleraesuis* subsp. *chol.* NM2009 (TA1535/pSK1002/ pNM12)	0−12.4 mg/L	+	−	A significant (>1.5-fold) induction was observed at ≥4.7 mg/L without activation.	Neuwoehner et al. (2007)
Genotoxicity studies in mammalian cells—in vitro						
Mutation (HGPRT assay)	CHO cells (K-1-BH4 subclone)	0, 20, 35, 50 ppm	−	−	Survival was >90% at all concentrations.	Kennel et al. (2000)
Mutation (HGPRT locus)	Chinese hamster V79 lung fibroblast cells	0−300 µM	−	−	Cytotoxicity was observed at ≥178 µM without S9.	Lachance et al. (1999)

Table 4. Summary of 2-ADNT (CASRN 35572-78-2) Genotoxicity						
Endpoint	**Test System**	**Dose/ Concentration**	**Results without Activation[a]**	**Results with Activation[a]**	**Comments**	**References**
Mutation	Mouse NG108 neuroblastoma cells and human MCF7 breast cancer cells	NR	+		2-ADNT induced point mutations and loss of heterozygosity at the *p53* locus as well as apoptosis. It is unclear if metabolic activation was used.	Banerjee and Dutta (1997) [abstract]

[a]+ = positive; − = negative.

2-ADNT = 2-amino-4,6-dinitrotoluene; 4-ADNT = 4-amino-2,6-dinitrotoluene; ADNT(s) = amino dinitrotoluene(s); CHO = Chinese hamster ovary; DMSO = dimethylsulfoxide; DNA = deoxyribonucleic acid; HGPRT = hypoxanthine-guanine phosphoribosyltransferase; NA = not applicable; NR = not reported; NT = not tested; TNT = 2,4,6-trinitrotoluene.

Carcinogenicity Studies

While there are no specific carcinogenicity data for 2-ADNT, there is relevant information in an unpublished 2-year study reported in 1984 by the IIT Research Institute using the related chemical TNT (a metabolic precursor; see below).

Metabolism/Toxicokinetic Studies

There are no available data regarding toxicokinetics following exposure to 2-ADNT; however, some information regarding 2-ADNT metabolism can be gleaned from TNT and 4-ADNT metabolism studies because 2-ADNT and 4-ADNT have been identified as major urinary metabolites of TNT in humans (see Figure C-2) (Kongtip et al., 2012; Sabbioni and Rumler, 2007; Sabbioni et al., 2007; Sabbioni et al., 2005). In an oral study in rats, approximately 50% of an administered radiolabeled dose of 4-ADNT was absorbed within 24 hours (Ellis et al., 1978). Absorbed 4-ADNT was widely distributed throughout the body, with the highest distribution to the liver, kidney, and skeletal muscle before being rapidly excreted in the urine and feces (approximately 75% of the administered dose was excreted within 24 hours; see Table 5) (Ellis et al., 1980; Ellis et al., 1978). Very little parent compound was present in the urine, suggesting extensive metabolism, but metabolites recovered were not identified (Ellis et al., 1980; Ellis et al., 1978). Fecal and gastrointestinal (GI) tract recovery, including both biliary excretion and unabsorbed compound, accounted for ~50% of the administered dose (see Table 5). A separate study indicated that approximately 20% of the administered oral dose in rats is excreted in the bile within 24 hours (parent compound and metabolite levels in the bile were not reported), with an additional 40% of radiolabeled compound remaining in the GI tract (Ellis et al., 1978). In humans exposed to TNT, the formation of hemoglobin adducts of the amino dinitrotoluenes is in general concordance with the ratio of urinary excretion (Ellis et al., 1978). The variations in quantities of excreted metabolites among the different occupational cohorts studied are likely explained by the different routes of exposure to TNT, including dermal uptake. Most studies show that urinary excretion of the amino adinitrotoluenes (4-ADNT plus 2-ADNT) in a range of 1−10 mg/L are typical—for instance, in persons employed with the disposal of military waste (note that these concentrations overlap the concentrations tested in the bacterial genotoxicity studies). After TNT is metabolized into 2-ADNT, further nitro reduction produces 4,6-diamino-2-nitrotoluene and 2,6-diamino-4-nitrotoluene (ATSDR, 1995). Subsequently, the amino group undergoes conjugation with sulfate, glucuronide, and acetyl moieties, and the conjugated metabolites are excreted in the urine (Kongtip et al., 2012; Sabbioni and Rumler, 2007; Sabbioni et al., 2007; Sabbioni et al., 2005; ATSDR, 1995). These studies indicate that 4-ADNT is also well absorbed, widely distributed, extensively metabolized, and rapidly excreted via feces and urine.

Table 5. Distribution and Excretion of Radioactivity in Rats Receiving ^{14}C-4-ADNT (CASRN 19406-51-0)[a]

Compartment	% Administered Dose	
	4 hr	**24 hr**
GI tract plus contents	70.1 ± 4.1[b]	5.5 ± 1.1
Feces	0.6 ± 0.1	44.4 ± 12.4
Whole blood[c]	0.6 ± 0.2	0.2 ± 0.0
Expired air	NDr	0.2 ± 0.1
Urine	11.1 ± 3.5	30.1 ± 9.5
Spleen	<0.1	<0.1
Liver	0.9 ± 0.2	0.5 ± 0.1
Kidneys	0.4 ± 0.1	0.1 ± 0.0
Brain	0.1 ± 0.0	<0.1
Lungs	0.1 ± 0.0	<0.1
Muscle[d]	2.4 ± 0.6	0.3 ± 0.0
Total recovery	86.4 ± 8.1	81.2 ± 4.5

[a]Ellis et al. (1978).
[b]Mean ± SE for three rats.
[c]Based on 7% of the body weight.
[d]Based on 40% of the body weight.

4-ADNT = 4-amino-2,6-dinitrotoluene; GI = gastrointestinal; NDr = not determined; SE = standard error.

DERIVATION OF PROVISIONAL VALUES

DERIVATION OF ORAL REFERENCE DOSES

No studies have been identified regarding toxicity of 2-ADNT to humans by oral exposure. Animal studies of 2-ADNT are limited to acute lethality studies, which are of inadequate duration and scope to support derivation of a subchronic or chronic provisional reference dose (p-RfD). Because of the limitations of the available data for 2-ADNT, subchronic and chronic p-RfDs were not derived directly. Instead, screening p-RfDs are derived in Appendix A using an alternative analogue approach. Based on the overall analogue approach presented in Appendix A, TNT was selected as the most appropriate analogue for 2-ADNT for deriving a screening subchronic and chronic p-RfD.

DERIVATION OF INHALATION REFERENCE CONCENTRATIONS

The absence of relevant inhalation data precludes derivation of provisional reference concentrations (p-RfCs) for 2-ADNT directly. An alternative analogue approach was pursued, but screening p-RfCs could not be derived because inhalation toxicity values for potential analogues are lacking (see Appendix A).

Table 6 presents a summary of noncancer reference values from Appendix A.

Table 6. Summary of Noncancer Reference Values for 2-ADNT (CASRN 35572-78-2)

Toxicity Type (units)	Species/ Sex	Critical Effect	p-Reference Value	POD Method	POD (HED)	UF_C	Principal Study
Screening subchronic p-RfD (mg/kg-d)	Dog/ Both	Mild hepatocyte swelling	3×10^{-4}	LOAEL	0.3 (based on analogue POD)	1,000	U.S. DOD (1983) as cited in U.S. EPA (2002a)
Screening chronic p-RfD (mg/kg-d)	Dog/ Both	Mild hepatocyte swelling	1×10^{-4}	LOAEL	0.3 (based on analogue POD)	3,000	U.S. DOD (1983) as cited in U.S. EPA (2002a)
Subchronic p-RfC (mg/m^3)	NDr						
Chronic p-RfC (mg/m^3)	NDr						

2-ADNT = 2-amino-4,6-dinitrotoluene; HED = human equivalent dose; LOAEL = lowest-observed-adverse-effect level; NDr = not determined; POD = point of departure; p-RfC = provisional reference concentration; p-RfD = provisional reference dose; UF_C = composite uncertainty factor.

CANCER WEIGHT-OF-EVIDENCE DESCRIPTOR

Under the U.S. EPA Cancer Guidelines (U.S. EPA, 2005), there is *"Inadequate Information to Assess the Carcinogenic Potential"* of 2-ADNT (see Table 7) because no relevant studies are available in humans or animals. Within the current U.S. EPA Cancer Guidelines (U.S. EPA, 2005), there is no standard methodology to support the identification of a weight-of-evidence (WOE) descriptor and derivation of provisional cancer risk estimates for

data-poor chemicals using an analogue approach. In the absence of an established framework, a screening evaluation of potential carcinogenicity is provided using the methodology described in Appendix B. This evaluation determined that there was a concern for potential carcinogenicity of 2-ADNT (see Appendix C).

Table 7. Cancer WOE Descriptor for 2-ADNT (CASRN 35572-78-2)

Possible WOE Descriptor	Designation	Route of Entry (oral, inhalation, or both)	Comments
"*Carcinogenic to Humans*"	NS	NA	There are no human carcinogenicity data identified to support this descriptor.
"*Likely to be Carcinogenic to Humans*"	NS	NA	There are no animal carcinogenicity studies identified to support this descriptor.
"*Suggestive Evidence of Carcinogenic Potential*"	NS	NA	There are no animal carcinogenicity studies identified to support this descriptor.
"Inadequate Information to Assess Carcinogenic Potential"	**Selected**	**Both**	**This descriptor is selected because of the lack of adequate data in humans or animals to evaluate the carcinogenic potential of 2-ADNT.**
"*Not Likely to be Carcinogenic to Humans*"	NS	NA	No evidence of noncarcinogenicity is available.

2-ADNT = 2-amino-4,6-dinitrotoluene; NA = not applicable; NS = not selected; WOE = weight of evidence.

DERIVATION OF PROVISIONAL CANCER RISK ESTIMATES

The absence of data precludes development of cancer risk estimates for 2-ADNT (see Table 8).

Table 8. Summary of Cancer Risk Estimates for 2-ADNT (CASRN 35572-78-2)

Toxicity Type (units)	Species/Sex	Tumor Type	Cancer Value	Principal Study
p-OSF $(mg/kg\text{-}d)^{-1}$	NDr			
p-IUR $(mg/m^3)^{-1}$	NDr			

2-ADNT = 2-amino-4,6-dinitrotoluene; NDr = not determined; p-IUR = provisional inhalation unit risk; p-OSF = provisional oral slope factor.

APPENDIX A. SCREENING NONCANCER PROVISIONAL VALUES

For reasons noted in the main Provisional Peer-Reviewed Toxicity Value (PPRTV) document, it is inappropriate to derive provisional toxicity values for 2-amino-4,6-dinitrotoluene (2-ADNT) because of a paucity of chemical-specific information. However, information is available for this chemical, which although insufficient to support deriving a provisional toxicity value under current guidelines, may be of use to risk assessors. In such cases, the Center for Public Health and Environmental Assessment (CPHEA) 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 provisional reference values 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 could be 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 CPHEA.

APPLICATION OF AN ALTERNATIVE ANALOGUE APPROACH

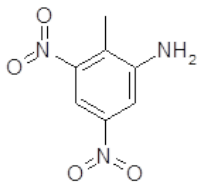
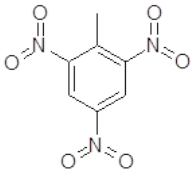
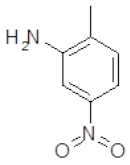
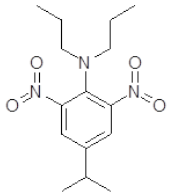
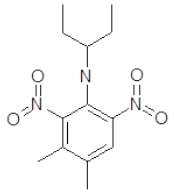
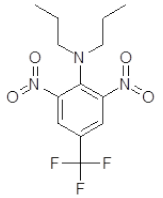
The analogue approach allows for the use of data from related compounds to calculate screening values when data for the compound of interest are limited or unavailable. Details regarding searches and methods for analogue analysis are presented in Wang et al. (2012). Three types of potential analogues (structural, metabolic, and toxicity-like) are identified to facilitate the final analogue chemical selection. The analogue approach may or may not be route-specific or applicable to multiple routes of exposure. In this section of the document, it is limited to oral noncancer effects only, based on the available toxicity data. All information was considered together as part of the final weight-of-evidence (WOE) approach to select the most suitable analogue both toxicologically and chemically.

Structural Analogues

An initial analogue search focused on identifying structurally similar chemicals with toxicity values from the Integrated Risk Information System (IRIS), PPRTV Program, Agency for Toxic Substances and Disease Registry (ATSDR), or California Environmental Protection Agency (CalEPA) databases to take advantage of the well-characterized chemical-class information. This was accomplished by electronically searching U.S. EPA's Distributed Structure-Searchable Toxicity (DSSTox) database (DSSTox, 2016) and the National Library of Medicine's (NLM's) ChemIDplus database (ChemIDplus, 2018). Five structural analogues to 2-ADNT that have oral noncancer toxicity values were identified: 2,4,6-trinitrotoluene (TNT) (U.S. EPA, 2002a), 2-methyl-5-nitroaniline (U.S. EPA, 2011c), isopropalin (U.S. EPA, 2002b), pendimethalin (U.S. EPA, 1988), and trifluralin (U.S. EPA, 2002c). 4-Amino-2,6-dinitrotoluene (4-ADNT) was also identified as a structural analogue (95% structural similarity), but this analogue did not have oral noncancer toxicity values. Data is included from this chemical because it informs the interpretation of data for 2-ADNT. Table A-1 summarizes the analogues' physicochemical properties and structural similarity scores. The DSSTox similarity score for 2-methyl-5-nitroaniline was 71%, while the similarity scores for isopropalin, pendimethalin, and trifluralin were between 33−39%. No data were available in DSSTox for TNT. The ChemIDplus similarity score was highest for TNT (87%), followed by pendimethalin (72%), isopropalin (65%), 2-methyl-5-nitroaniline (58%), and trifluralin (53%). Physicochemical

properties of the potential analogues suggest that TNT and 2-methyl-5-nitroaniline are more hydrophilic than the other potential analogues (i.e., increased water solubility, decreased log K_{ow}); however, these materials are all expected to be bioavailable. Unlike 2-ADNT and 2-methyl-5-nitroaniline, which are primary aromatic amines and have basic pKa values, TNT is not ionizable although its metabolites with amino groups are.

Table A-1. Physicochemical Properties of 2-ADNT (CASRN 35572-78-2) and Candidate Structural Analogues[a]

	2-ADNT	TNT	2-Methyl-5-nitroaniline	Isopropalin	Pendimethalin	Trifluralin
Structure						
CASRN	35572-78-2	118-96-7	99-55-8	33820-53-0	40487-42-1	1582-09-8
Molecular weight	197.15	227.13	152.15	309.37	281.31	335.29
DSSTox similarity score (%)[b]	100	ND	71	34	39	33
ChemIDplus similarity score (%)[c]	100	87	58	65	72	53
Melting point (°C)	174.5	80.1	107	91.2 (predicted average)	56.3	49.0
Boiling point (°C)	347 (predicted average)	240	329	372 (predicted average)	330	367 (predicted average)
Vapor pressure (mm Hg at 25°C)	8.01×10^{-6} (predicted average)	8.02×10^{-6}	2.87×10^{-4} (predicted average)	3.00×10^{-5}	3.00×10^{-5}	4.58×10^{-5}
Henry's law constant (atm-m^3/mole at 25°C)	4.4×10^{-8} (predicted average)	4.99×10^{-7} (predicted average)	5.28×10^{-8} (predicted average)	9.58×10^{-7} (predicted average)	1.68×10^{-6} (predicted average)	1.03×10^{-4}
Water solubility (mg/L)	6.12×10^{-3} (predicted average)	5.72×10^{-4} (predicted average)	7.94×10^{-3} (predicted average)	3.39×10^{-7}	1.07×10^{-6}	7.01×10^{-7}
Log K_{ow}	1.79 (predicted average)	1.60	1.87	5.07 (predicted average)	5.20	5.34
pKa	0.36	ND	2.345	ND	ND	ND

[a]Unless otherwise noted, information for each respective compound is sourced from U.S. EPA CompTox Chemicals Dashboard (2-amino-4,6-dinitrotoluene; https://comptox.epa.gov/dashboard/DTXSID6044068; accessed April 2020). Values are experimental averages unless noted as predicted averages.
[b]DSSTox (2016).
[c]ChemIDplus Advanced, similarity scores (ChemIDplus, 2018).

2-ADNT = 2-amino-4,6-dinitrotoluene; NA = not applicable; ND = no data; TNT = 2,4,6-trinitrotoluene.

One key structural distinction between the target compound and isopropalin, pendimethalin, and trifluralin is the presence of the alkyl groups attached to the amine group. This is expected to influence the potential of these analogues to ionize, and because the log K_{ow} differs significantly for those analogues with larger alkyl-groups, the fat solubility and toxicokinetics would be expected to differ significantly as well. As indicated in the metabolism section below, the proximity of these alkyl groups with the ortho-nitro substituent is expected to sterically hinder the metabolic pathways used by these chemicals. Analogues lacking bulky alkyl-groups would be expected to be more similar.

Metabolic Analogues

Table A-2 summarizes the available toxicokinetics data for 2-ADNT and the structurally similar compounds identified as potential analogues.

Table A-2. Comparison of Available Toxicokinetic Data for 2-ADNT (CASRN 35572-78-2) and Candidate Analogues

Compound	Absorption, Distribution, Excretion	Metabolism	References
2-ADNT	• No direct data • Based on excretion patterns following TNT exposure; the primary route of excretion is expected to be urine and feces	• Primary metabolite of TNT • May undergo further nitro reduction to produce 4,6-diamino-2-nitrotoluene and 2,6-diamino-4-nitrotoluene • The amino group undergoes conjugation with sulfate, glucuronide, and acetyl moieties	Kongtip et al. (2012); Sabbioni and Rumler (2007); Sabbioni et al. (2007); Sabbioni et al. (2005); ATSDR (1995)
4-ADNT (while no toxicity value exists for this analogue, information is included because of expected similarity with 2-ADNT)	• Rapid oral absorption with approximately 30% of the dose excreted in the urine and 20% excreted in the bile • Extensive distribution; highest percentage of dose in liver, kidney, and skeletal muscle • Urine and feces are primary routes of excretion	• Primary metabolite of TNT • May undergo further nitro reduction to produce 4,6-diamino-2-nitrotoluene and ring hydroxylation to form 3-hydroxy-4-amino-2,6-dinitrotoluene • The amino group undergoes conjugation with sulfate, glucuronide, and acetyl moieties	Kongtip et al. (2012); Sabbioni and Rumler (2007); Sabbioni et al. (2007); Sabbioni et al. (2005); ATSDR (1995); Ellis et al. (1980); Ellis et al. (1978)
TNT	• Rapid oral absorption with approximately 60% recovery in urine of rats, mice, and dogs; biliary excretion also occurs (quantitative results not available) • Extensive distribution; highest distribution to liver, skeletal muscle, blood, and fat • Urine is the primary route of excretion	• Metabolic pathways include oxidation of the methyl group, benzene ring oxidation, and reduction of the nitro group • Primary metabolites identified in human urine include 2-ADNT, 4-ADNT, 2,4-diamino-6-nitrotoluene, 4-hydroxylamino-2,6-dintrotoluene, and 3-hydroxy-4-amino-2,6-dinitrotoluene • Similar metabolites were identified in rat, mouse, rabbit, and dog urine	ATSDR (1995); Midwest Research Institute (1981); Dilley et al. (1982)
2-Methyl-5-nitroaniline	• No data on absorption or distribution • Urine is the primary route of excretion	• May undergo further oxidative and reductive degradation to yield the corresponding amino, hydroxylamino, and nitroso derivatives	MAK-Commission (2012); IARC (1990a); Mori et al. (1981)
Isopropalin	• ND	• ND	NA

Table A-2. Comparison of Available Toxicokinetic Data for 2-ADNT (CASRN 35572-78-2) and Candidate Analogues

Compound	Absorption, Distribution, Excretion	Metabolism	References
Pendimethalin	• Limited oral absorption, as suggested by excretion of parent compound in the feces (70−90% of administered dose) • Extensive distribution; highest concentration in fat • Feces is the primary route of excretion	• Major metabolic pathways in the rat include hydroxylation of the 4-methyl and the *N*-1-ethyl group, oxidation of these alkyl groups to carboxylic acids, nitro reduction, cyclization, and conjugation • *N*-dealkylation of the isopentyl group has not been found to be significant • In the liver and kidney, cyclization reactions result in methylbenzimidazole carboxylic acids	HSDB (2011); Zulalian (1990)
Trifluralin	• In rats, 80% of an oral dose was excreted in feces (only 8% unchanged) • Incomplete absorption was indicated by 11−14% recovery of radioactivity in bile. • No data on distribution were reported • Feces is the primary route of excretion	• Extensive nitro reduction occurred in the GI tract, presumably by gut microflora • The absorbed fraction was extensively metabolized by nitro reduction and *N*-dealkylation of one or both propyl groups • In vitro studies using rat liver microsomes demonstrated side chain hydroxylation and benzimidazole formation	HSDB (2012); IARC (1991)

2-ADNT = 2-amino-4,6-dinitrotoluene; 4-ADNT = 4-amino-2,6-dinitrotoluene; GI = gastrointestinal; NA = not applicable; ND = no data; TNT = 2,4,6-trinitrotoluene.

2-ADNT is a major metabolite of TNT, appearing in the urine of TNT-exposed workers (Kongtip et al., 2012; Sabbioni and Rumler, 2007; Sabbioni et al., 2007; Sabbioni et al., 2005; Ahlborg et al., 1988; Yinon and Hwang, 1987; Woollen et al., 1986; Yinon and Hwang, 1986a; Almog et al., 1983; Channon et al., 1944; Lemberg and Callaghan, 1944), rats (Yinon and Hwang, 1985), rabbits (Yinon and Hwang, 1986b), and dogs (Snyder, 1946). Common downstream metabolites resulting from further nitro reduction of these compounds include 4,6-diamino-2-nitrotoluene and 2,6-diamino-4-nitrotoluene (ATSDR, 1995). Before excretion, the amino group may undergo conjugation with sulfate, glucuronide, and acetyl moieties (ATSDR, 1995).

Based on these data, TNT is considered a metabolic analogue for 2-ADNT because (1) 2-ADNT is a primary metabolite of TNT and (2) TNT and 2-ADNT share common downstream metabolites that are excreted in the urine. TNT and the related compound, 4-ADNT, show similar rate and extent of absorption, distribution, and excretion, suggesting that 2-ADNT may be similar (ATSDR, 1995; Ellis et al., 1980; Ellis et al., 1978). Available information for trifluralin and pendimethalin indicates that they might be less appropriate metabolic analogues for 2-ADNT based on demonstrated or expected utilization of different metabolic pathways (cyclization reactions in addition to nitro reduction; see Table A-2) and/or different primary route of excretion (i.e., feces rather than the urine) than TNT and 4-ADNT (HSDB, 2012, 2011; IARC, 1991; Zulalian, 1990). Data regarding toxicokinetics for 2-methyl-5-nitroaniline and isopropalin were too limited to determine their suitability as metabolic analogues.

Toxicity-Like Analogues

Table A-3 summarizes available toxicity data for 2-ADNT and the structurally similar compounds identified as potential analogues. Available toxicity data for 2-ADNT are limited to acute oral lethality studies, skin/eye irritation and sensitization studies, and in vitro genotoxicity studies. No repeated-dose toxicity studies are available for 2-ADNT.

Table A-3. Comparison of Available Human Health Assessment Values and Acute Toxicity Data for 2-ADNT (CASRN 35572-78-2) and Potential Analogues

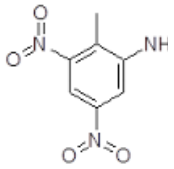
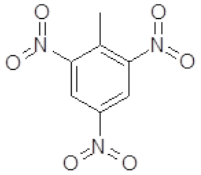
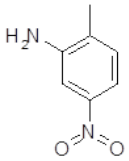
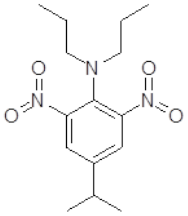
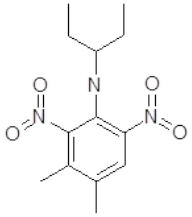
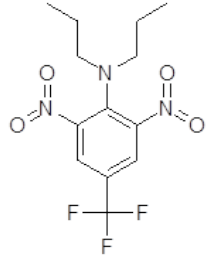
	2-ADNT	TNT	2-Methyl-5-nitroaniline	Isopropalin	Pendimethalin	Trifluralin
Structure						
CASRN	35572-78-2	118-96-7	99-55-8	33820-53-0	40487-42-1	1582-09-8
Repeated-dose toxicity—oral, subchronic						
Subchronic p-RfD (mg/kg-d)	NV	NV	NV	NV	NV	NV
Notes	NV	The IRIS RfD is based on a 26-wk feeding study in dogs (see below)	NV	The IRIS RfD is based on a 13-wk dietary study in rats (see below)	NV	NV
Source	NV	U.S. EPA (2002a)	U.S. EPA (2011c)	U.S. EPA (2002b)	U.S. EPA (1988)	U.S. EPA (2002c)
Repeated-dose toxicity—oral, chronic						
Chronic p-RfD/ IRIS RfD (mg/kg-d)	NV	5×10^{-4}	2×10^{-2} (screening)	1.5×10^{-2}	4×10^{-2}	7.5×10^{-3}
Critical effects	NV	Hepatocyte swelling (trace to mild severity) and other hepatic effects	Reduced body weight (~20% decrease in females)	Reduced hemoglobin and hematocrit, and altered organ weights (not further described)	Increased serum ALP and liver weight, liver inflammation and hemosiderosis	Increased liver weight and methemoglobin

Table A-3. Comparison of Available Human Health Assessment Values and Acute Toxicity Data for 2-ADNT (CASRN 35572-78-2) and Potential Analogues

	2-ADNT	TNT	2-Methyl-5-nitroaniline	Isopropalin	Pendimethalin	Trifluralin
Other effects	NV	Increased absolute and relative liver weight, cirrhosis, and hemosiderosis of the liver at higher doses	Hepatocellular carcinomas in mice; no other non-neoplastic effects reported	NV	NV	Decreased weight gain and increased liver and spleen weight were observed at higher doses; total serum lipids, triglycerides, and cholesterol were also increased
Species	NV	Dog	Mouse	Rat	Dog	Dog
Duration	NV	26 wk (6 mo)	78 wk + 20 wk observation 2 yr	13 wk	2 yr	12 mo
Route	NV	Oral (gelatin capsule)	Oral (diet)	Oral (diet)	Oral (diet)	Oral (diet)
Notes	NV	Anemia and hepatomegaly were also seen in mice. Urinary bladder papilloma and carcinoma were observed in female F344 rats. Reported toxic effects in humans include cataracts, aplastic anemia, hepatitis, and hepatomegaly (ATSDR, 1995)	Methemoglobinemia and liver failure were reported in humans	NV	Decreased food consumption, body weight, hemoglobin, and hematocrit, liver hypertrophy, and increased weight were observed in a 90-d rat study. Reduced litter size, survival index, and pup weight were observed in a 3-generation reproductive toxicity study in rats	Systemic effects seen at higher doses include increased globulin excretion in rats, increased relative liver weight in rats and mice, and enlarged livers, discolored kidneys, corneal vascularization, hemolytic anemia, and increased serum ALP in dogs. Effects seen in reproductive and developmental studies in rats and rabbits include reduced litter size, decreased fetal body weight, reduced skeletal maturity, and increased vascular fragility
Source	NV	U.S. EPA (2002a)	U.S. EPA (2011c)	U.S. EPA (2002b)	U.S. EPA (1988)	U.S. EPA (2002c)

Table A-3. Comparison of Available Human Health Assessment Values and Acute Toxicity Data for 2-ADNT (CASRN 35572-78-2) and Potential Analogues

	2-ADNT	TNT	2-Methyl-5-nitroaniline	Isopropalin	Pendimethalin	Trifluralin
Acute toxicity						
Rat oral LD_{50} (mg/kg)	1,394	607	NV	5,000	1,050	1,930
Toxic effects	Excitement, depressed activity	Respiratory stimulation; inflammation, necrosis, or scarring of the bladder; changes in urinalysis parameters	NV	NV	NV	Depressed activity, pupillary dilation
Mouse oral LD_{50} (mg/kg)	1,522	660	NV	>5,000	1,340	3,197
Toxic effects	Depressed activity	Depressed activity, tremor, convulsions, or effect on seizure threshold	NV	NV	NV	NV
Genotoxicity in vitro	Yes	Yes	NV	NV	NV	NV
Carcinogenicity in vivo	NV	Yes	NV	NV	NV	NV
Source	ChemIDplus (2018)	ChemIDplus (2018)	ChemIDplus (2018)	ChemIDplus (2018)	ChemIDplus (2018)	ChemIDplus (2018)

2-ADNT = 2-amino-4,6-dinitrotoluene; ALP = alkaline phosphatase; IRIS = Integrated Risk Information System; LD_{50} = median lethal dose; NA = not applicable; NV = not available; p-RfD = provisional reference dose; RfD = oral reference dose; TNT = 2,4,6-trinitrotoluene.

The liver was a common toxicity target for the analogues, with reports of altered biochemistry (electrolytes, enzymes), increased organ weight, cirrhosis, hepatitis, and hepatic tumors in animal and/or human studies. For isopropalin, altered hematological and organ-weight changes were noted, but the individual organ (presumably liver) was not specifically reported. All the candidate analogues also produced adverse blood effects in exposed animals and/or humans; these effects included anemia, reduced hemoglobin and hematocrit, and methemoglobinemia. Reproductive and developmental effects in animals were associated with exposure to pendimethalin and trifluralin, but at doses higher than those causing liver and blood effects (U.S. EPA, 2011c, 2002a, b, c, 1988).

Liver toxicity was the critical effect for TNT. The oral reference dose (RfD) of 5×10^{-4} mg/kg-day is based on mild hepatocyte swelling (a precursor to further histopathologic lesions) in dogs following exposure to 0.5 mg/kg-day for 6 months (U.S. EPA, 2002a). Additional liver effects were observed in dogs at higher doses, including increased organ weight, cirrhosis, and hemosiderosis (indicative of a hematological effect as well) (U.S. EPA, 2002a). Hepatomegaly, along with anemia, was also observed in mice exposed to higher doses, indicating that dogs are a more sensitive laboratory species. Adverse effects reported in TNT-exposed humans are primarily hematologic and hepatologic including aplastic anemia, hepatitis, hepatomegaly, and also cataracts (ATSDR, 1995).

Liver toxicity was also the critical effect for pendimethalin for oral exposure in animal studies, and dogs appear to be the most sensitive laboratory species (U.S. EPA, 1988). The RfD of 4×10^{-2} mg/kg-day for pendimethalin is based on increased serum alkaline phosphatase (ALP), increased liver weight, and liver inflammation and hemosiderosis in dogs exposed to 50 mg/kg-day for 2 years. Critical effects in rats exposed to pendimethalin at 250 mg/kg-day for 90 days included decreased hemoglobin and hematocrit, liver hypertrophy, increased liver weight, and decreased body weight and food consumption. Reduced litter size, survival index, and pup weight were observed in a three-generation reproductive toxicity study in rats exposed to 250 mg/kg-day.

Liver and blood effects were cocritical effects for oral exposure to trifluralin in animal studies. The RfD of 7.5×10^{-3} mg/kg-day for trifluralin is based on increased liver weight and methemoglobinemia in dogs exposed to 3.75 mg/kg-day for 12 months (U.S. EPA, 2002c). Additional effects observed in dogs at higher doses included increased serum lipids, triglycerides, and cholesterol, increased liver and spleen weight, corneal vascularization, and hemolytic anemia. Effects observed in other laboratory species included increased globulin excretion and decreased body weight in rats and increased liver weight in rats and mice at ≥10 mg/kg-day, indicating that dogs are also the most sensitive laboratory species for trifluralin toxicity. Reproductive and developmental effects were observed at doses much higher than those causing liver and blood effects (≥100 mg/kg-day), including reduced litter size, decreased fetal body weight, reduced skeletal maturity, and increased vascular fragility in rats and rabbits.

For isopropalin, reduced hemoglobin and hematocrit and unspecified organ weight changes in rats fed 48 mg/kg-day for 13 weeks were cocritical effects for the chronic RfD of 1.5×10^{-2} mg/kg-day (U.S. EPA, 2002b). Based on the general constellation of toxic effects of other potential analogues, "unspecified organ weight changes" likely includes elevated liver

weight. No adverse effects were observed in dogs fed up to 56 mg/kg-day for 90 days, suggesting that the dog is not the most sensitive laboratory species to isopropalin toxicity.

Decreased body weight was the critical effect for the screening chronic p-RfD for 2-methyl-5-nitroaniline (U.S. EPA, 2011c). The screening chronic p-RfD of 2×10^{-2} mg/kg-day was based on ~20% decrease in body weight in female mice exposed to ≥207 mg/kg-day for 78 weeks; body-weight effects were not observed in male mice or male or female rats in this chronic study, but decreased body weight was observed in male and female rats and mice at higher doses in the associated dose range-finding studies. Hepatocellular carcinomas were reported in male and female mice after chronic exposure. Reported effects in humans exposed to 2-methyl-5-nitroaniline included methemoglobinemia and liver failure (U.S. EPA, 2011c).

In summary, toxicity data on the potential analogues demonstrate similar adverse hepatic and hematological effects across chemicals, studies, and animal species (see Table A-3). Hepatic and hematological effects have also been associated with human exposure to TNT and 2-methyl-5-nitroaniline. While the potency may differ between potential analogues, the available data show clear commonalities (liver and blood) in the critical effects for all five potential analogues. Available data regarding 2-ADNT toxicity are inadequate to identify toxicity targets; thus, it is not possible to select, or to rule out, any of the candidate analogues based on a direct comparison of the toxic effects of 2-ADNT and candidate analogues. However, based on commonalities in hepatic endpoints across all candidate analogues, and concordance within the category, it is reasonably expected that 2-ADNT would also exhibit similar toxic effects.

Weight-of-Evidence Approach

A WOE approach is used to evaluate information from potential candidate analogues as described by Wang et al. (2012). In this procedure, commonalities in structural/physicochemical properties, toxicokinetics, metabolism, toxicity, or mode of action (MOA) between potential analogues and chemical(s) of concern are identified. Emphasis is given to toxicological and/or toxicokinetic similarity over structural similarity. Analogue candidates are excluded if they do not have commonality or demonstrate different physicochemical properties and toxicokinetic profiles that set them apart from the pool of potential analogues and/or chemical(s) of concern. From the remaining potential analogues, the most appropriate analogue (most biologically or toxicologically relevant analogue chemical, based on expert judgement) with the highest structural similarity and/or the more sensitive toxicity value is selected.

Across the analogues, there are structural, metabolic, and toxicity-like similarities. As discussed previously, when considering structural similarity, the analogues with bulkier alkyl side chains had lower percent similarity scores, and TNT appeared to be the closest structural analogue. When considering metabolism, TNT was identified as the most appropriate metabolic analogue as one of its primary metabolites is the target chemical, 2-ADNT, and both compounds share downstream metabolites. The herbicidal analogues (isopropalin, pendimethalin) have larger alkyl side chains and are likely to be toxicokinetically different, and thus, less appropriate analogues. The herbicides also do not share metabolites with 2-ADNT. The other analogue, 2-methyl-5-nitroaniline, has lower structural similarity, lower metabolic similarity, and weaker toxicological similarity. The overlap between TNT and 2-ADNT in metabolic pathways is expected to result in a concomitant overlap in target organs and toxicological effects. There is

strong toxicity-like concordance in the health effects of all the chemically similar analogues, in that all the potential analogues with available data had the liver and blood as target organs, supporting the use of analogue chemicals with similar structural and toxicological properties. Here, the analogue was chosen primarily based on metabolic similarity, structural similarity, and shared metabolites. In conclusion, TNT was the most appropriate analogue structurally and metabolically. The similarity of toxicological outcomes across the analogues builds confidence in the toxicologic read-across for 2-ADNT. TNT is also the most health-protective analogue because its point of departure (POD) and corresponding RfD value are lower than the other potential analogue chemicals.

ORAL NONCANCER TOXICITY VALUES

Derivation of a Screening Subchronic Provisional Reference Dose

Based on the overall analogue approach presented in this PPRTV assessment, TNT was selected as the most appropriate analogue for 2-ADNT for deriving a screening subchronic p-RfD. While the U.S. EPA's IRIS program does not have a subchronic RfD value for TNT, the chronic RfD value is based on an unpublished subchronic oral study in dogs by the U.S. Department of Defense [U.S. DOD (1983) as cited in U.S. EPA (2002a)]. The IRIS summary report for TNT described this study as follows:

> *The U.S. Department of Defense (U.S. DOD, 1983) commissioned a study to determine the effects of TNT (approximately 99% pure) administered daily by gelatin capsule, containing a mix of TNT with Purina Certified Rodent* [sic] *Chow, to groups of six beagle dogs/sex at 0, 0.5, 2, 8, or 32 mg/kg/day for 25 weeks. Animals were approximately 6.5 months old at the start of the TNT dosing schedule. Animals were observed several times daily, before and after dosing, for toxic signs and were examined weekly by palpation for detectable masses. Body weight and food intakes were recorded weekly. Other toxicologic endpoints included a comprehensive clinical chemistry and hematological evaluation, urinalyses, and periodic electrocardiography (ECG) and ophthalmic examinations. During week 27 all animals were fasted for 16 to 18 hours and were sacrificed by injection of intravenous pentobarbital sodium. Major organs were weighed, and all organs were collected and fixed for microscopic examination. Statistical analyses were performed.*
>
> *Several indications of liver injury were observed upon gross and histologic examination. Male (8 and 32 mg/kg/day) and female (32 mg/kg/day) dogs had significant increases in relative and/or absolute liver weight accompanied by moderate to marked hepatocytic cloudy swelling and hepatocytomegaly. The hepatic swelling and hepatocytomegaly was observed at all dose levels, but to a greater degree in the high-dose group; lesions at the low dose (0.5 mg/kg/day) were described as trace to mild. No such lesions were seen in the control animals. Microscopic evidence of cirrhosis was seen, primarily in males, at the 8 and 32 mg/kg/day dose levels. Hemosiderosis of the liver was seen in the majority of dogs at 2 and 8 mg/kg/day (the two highest levels) as well as in one female at the 2 mg/kg/day level. None of these microscopic lesions were seen in the two females necropsied prior to termination of this study. The 0.5 mg/kg/day test level is the LOAEL for liver effects. The histopathology at this*

level is trace to mild and is unsupported by effects on the liver enzymes and organ weight.

The critical effect for the subchronic-duration dog feeding study was trace to mild hepatocyte swelling; this lowest-observed-adverse-effect level (LOAEL) of 0.5 mg/kg-day was used as the POD. The chronic RfD for TNT was derived using a composite uncertainty factor (UF_C) of 1,000 (see Table A-4) to account for uncertainties due to interspecies extrapolation (UF_A), intraspecies variability (UF_H), subchronic-to-chronic extrapolation (UF_S), and LOAEL-to-NOAEL (no-observed-adverse-effect level) extrapolation (UF_L) (U.S. EPA, 2002a). However, because the U.S. EPA endorses body-weight scaling to the 3/4 power (i.e., $BW^{3/4}$) as a default to extrapolate toxicologically equivalent doses of orally administered agents from all laboratory animals to humans for the purpose of deriving an RfD from effects that are not portal-of-entry effects (U.S. EPA, 2011d), the POD was converted into a human equivalent dose (HED) resulting in the UF_A being reduced to 3. Additionally, the UF_S was decreased to 1 because the study is the appropriate duration for a subchronic derivation, and the database uncertainty factor (UF_D) for database uncertainties was increased to 10 to account for the absence of any repeated-dose toxicity information for 2-ADNT. Thus, the screening subchronic p-RfD for 2-ADNT was derived using a composite UF_C of 1,000 reflecting a UF_H of 10, a UF_D of 10, a UF_L of 3 (for use of a LOAEL), and a UF_A of 3.

Following U.S. EPA (2011d) guidance, the POD for hepatocyte swelling in male and female dogs is converted to an HED by applying a dosimetric adjustment factor (DAF) 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 reference BW_a of 12 kg for dogs and a reference BW_h of 70 kg for humans, the resulting DAF is 0.63 (U.S. EPA, 2011d). Applying this DAF to the LOAEL of 0.5 mg/kg-day yields a POD (HED) as follows:

$$\begin{aligned} \text{POD (HED)} &= \text{LOAEL (mg/kg-day)} \times \text{DAF} \\ &= 0.5 \text{ mg/kg-day} \times 0.64 \\ &= 0.3 \text{ mg/kg-day} \end{aligned}$$

Using the POD (HED), the screening subchronic p-RfD for 2-ADNT is derived as follows:

$$\begin{aligned} \textbf{Screening Subchronic p-RfD} &= \text{Analogue POD (HED)} \div UF_C \\ &= 0.3 \text{ mg/kg-day} \div 1{,}000 \\ &= \mathbf{3 \times 10^{-4} \text{ mg/kg-day}} \end{aligned}$$

Table A-4 summarizes the uncertainty factors for the screening subchronic p-RfD for 2-ADNT.

Table A-4. Uncertainty Factors for the Screening Subchronic p-RfD for 2-ADNT (CASRN 35572-78-2)

UF	Value	Justification
UF_A	3	A UF_A of 3 ($10^{0.5}$) is applied to account for uncertainty associated with extrapolating from animals to humans when cross-species dosimetric adjustment (HED calculation) is performed.
UF_D	10	A UF_D of 10 is applied to account for the absence of repeated-dose toxicity data for 2-ADNT, using TNT as the most appropriate analogue.
UF_H	10	A UF_H of 10 is applied for interindividual variability to account for human-to-human variability in susceptibility in the absence of quantitative information to assess the toxicokinetics and toxicodynamics of 2-ADNT in humans.
UF_L	3	A UF_L of 3 ($10^{0.5}$) is applied because the POD is a LOAEL with trace to mild severity.
UF_S	1	A UF_S of 1 is applied because a subchronic study was selected as the principal study.
UF_C	1,000	Composite UF = $UF_A \times UF_D \times UF_H \times UF_L \times UF_S$.

2-ADNT = 2-amino-4,6-dinitrotoluene; HED = human equivalent dose; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; POD = point of departure; p-RfD = provisional reference dose; TNT = 2,4,6-trinitrotoluene; UF = uncertainty factor; UF_A = interspecies uncertainty factor; UF_C = composite uncertainty factor; UF_D = database uncertainty factor; UF_H = intraspecies uncertainty factor; UF_L = LOAEL-to-NOAEL uncertainty factor; UF_S = subchronic-to-chronic uncertainty factor.

Derivation of a Screening Chronic Provisional Reference Dose

Based on the overall analogue approach presented in this PPRTV assessment, TNT was selected as the most appropriate analogue for 2-ADNT for deriving a screening chronic p-RfD. The study used for the U.S. EPA (2002a) chronic RfD for TNT was the unpublished subchronic-duration dog study described above [U.S. DOD (1983) as cited in U.S. EPA (2002a)]. The screening chronic p-RfD for 2-ADNT was derived as described above for the screening subchronic p-RfD, with an additional UF_S of 3 for extrapolation from a subchronic-to-chronic duration because the principal study was a 25-week study performed in dogs. A default uncertainty factor of 10 was not applied as there is evidence in the nitroarene literature (see below), that methemoglobinemia, and the subsequent constellation of sequelae including splenic and hepatic effects do not seem to significantly increase in incidence and/or severity with chronic exposures. The following examples in the nitroarene literature provide evidence to support reducing the default UF_S.

1,3,5-Trinitrobenzene

In the IRIS assessment (U.S. EPA, 1997), discussion of the principal study (Reddy et al., 1996) included the following passage:

> *Relative organ weight changes for the brain (increase), spleen (increase), liver (increase) and testes (decrease in 90- and 180-day periods) were reported for all treated animals dosed with [1,3,5-trinitrobenzene] (TNB) at levels higher than 3 mg/kg-day; adverse hematological findings (decreased hematocrit and hemoglobin) and increased methemoglobulin* [sic]*) were consistently reported in all animals treated at these levels. Histopathological findings in the 1-year study revealed extramedullary hematopoiesis in rats treated with TNB at doses of*

3 mg/kg-day or higher. In the 2-year study, these effects were seen only in rats dosed with TNB at the high dosage level (13.23 mg/kg-day). The adverse effects, such as increased methemoglobin, erythroid cell hyperplasia, and increased relative organ weights, observed during interim sacrifices in rats receiving 60 ppm TNB did not persist and were not detected in rats fed 60 ppm TNB for 2 years, suggesting that an adaptive mechanism has taken place in order to compensate adverse effects observed during interim sacrifices.

In this case, the 90-day LOAEL was 2.6 mg/kg-day, while the 2-year LOAEL was 13 mg/kg-day, which would lead to a subchronic-to-chronic extrapolation factor of less than 1, and thus the default UF_S of 10 would be excessive.

Tetranitroaniline (Tetryl)

As described by Reddy et al. (1998), the LOAEL for methemoglobin changes with time, from 83 mg/kg-day at 14 days, dropping to 14 mg/kg-day at 45 days, but back up to 69 mg/kg-day at 90 days. Thus, the peak in methemoglobin, the downstream events of which include splenic and hepatic effects, peaks at 45 days, but by 90 days an adaptive response had begun, again suggesting that a UF_S is unnecessary.

Nitrobenzene

As described in the IRIS assessment of nitrobenzene (U.S. EPA, 2009), percent methemoglobinemia was determined in several species of animals exposed to nitrobenzene (Cattley et al., 1994). In this study, the dose-response to nitrobenzene was observed at 15 and 24 months. In CD rats, for example, the percent methemoglobin was 1.18/2.75 in control, 4.08/2.87 at 1 mg/kg-day, 6.22/2.35 at 5 mg/kg-day, and 5.85/4.60 at 25 mg/kg-day (at 15/24 months, respectively). Thus, at 24 months the percent methemoglobinemia was lower than that at 15 months at all doses except control, again suggesting that a UF_S would be unnecessary.

In summary, when looking across the health effects in the previous examples, the liver and hematological effects seem to reach a plateau suggesting that an increase in duration of exposure will lead to some increases in incidence and/or severity but not to the extent to warrant the application of a 10-fold UF_S. A threefold UF_S is thus applied to cover any remaining uncertainty.

The screening chronic p-RfD for 2-ADNT is then derived as follows:

$$\begin{aligned} \textbf{Screening Chronic p-RfD} &= \text{POD (HED)} \div UF_C \\ &= 0.3 \text{ mg/kg-day} \div 3{,}000 \\ &= \mathbf{1 \times 10^{-4} \text{ mg/kg-day}} \end{aligned}$$

Table A-5 summarizes the uncertainty factors for the screening chronic p-RfD for 2-ADNT.

Table A-5. Uncertainty Factors for the Screening Chronic p-RfD for 2-ADNT (CASRN 35572-78-2)

UF	Value	Justification
UF_A	3	A UF_A of 3 ($10^{0.5}$) is applied to account for uncertainty associated with extrapolating from animals to humans when cross-species dosimetric adjustment (HED calculation) is performed.
UF_D	10	A UF_D of 10 is applied to account for the absence of repeated-dose toxicity data for 2-ADNT, using TNT as the most appropriate analogue.
UF_H	10	A UF_H of 10 is applied for interindividual variability to account for human-to-human variability in susceptibility in the absence of quantitative information to assess the toxicokinetics and toxicodynamics of 2-ADNT in humans.
UF_L	3	A UF_L of 3 is applied because the POD is a LOAEL with trace to mild severity.
UF_S	3	A UF_S of 3 is applied because while the principal study is a subchronic study, the health effects of methemoglobinemia and the subsequent constellation of sequelae including splenic and hepatic effects do not seem to significantly increase in incidence and/or severity with chronic exposure.
UF_C	3,000	Composite UF = $UF_A \times UF_D \times UF_H \times UF_L \times UF_S$.

2-ADNT = 2-amino-4,6-dinitrotoluene; HED = human equivalent dose; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; POD = point of departure; p-RfD = provisional reference dose; TNT = 2,4,6-trinitrotoluene; UF = uncertainty factor; UF_A = interspecies uncertainty factor; UF_C = composite uncertainty factor; UF_D = database uncertainty factor; UF_H = intraspecies uncertainty factor; UF_L = LOAEL-to-NOAEL uncertainty factor; UF_S = subchronic-to-chronic uncertainty factor.

INHALATION NONCANCER TOXICITY VALUES

Derivation of Screening Provisional Reference Concentrations

No subchronic or chronic inhalation reference values have been located for candidate analogues for 2-ADNT (U.S. EPA, 2011c, 2002a, b, c, 1988), precluding derivation of provisional reference concentration (p-RfC) values for 2-ADNT based on an alternative analogue approach.

APPENDIX B. BACKGROUND AND METHODOLOGY FOR THE SCREENING EVALUATION OF POTENTIAL CARCINOGENICITY

For reasons noted in the main Provisional Peer-Reviewed Toxicity Value (PPRTV) document, there is inadequate information to assess the carcinogenic potential of 2-amino-4,6-dinitrotoluene (2-ADNT). However, information is available for this chemical which, although insufficient to support a weight-of-evidence (WOE) descriptor and derivation of provisional cancer risk estimates under current guidelines, may be of use to risk assessors. In such cases, the Center for Public Health and Environmental Assessment (CPHEA) summarizes available information in an appendix and develops a "screening evaluation of potential carcinogenicity." Appendices receive the same level of internal and external scientific peer review as the provisional cancer assessments in PPRTVs to ensure their appropriateness within the limitations detailed in the document. Users of the information regarding potential carcinogenicity in this appendix should understand that there could be more uncertainty associated with this evaluation than for the cancer WOE descriptors presented in the body of the assessment. Questions or concerns about the appropriate use of the screening evaluation of potential carcinogenicity should be directed to the CPHEA.

The screening evaluation of potential carcinogenicity includes the general steps shown in Figure B-1. The methods for Steps 1−8 apply to any target chemical and are described in this appendix. Chemical-specific data for all steps in this process are summarized in Appendix C.

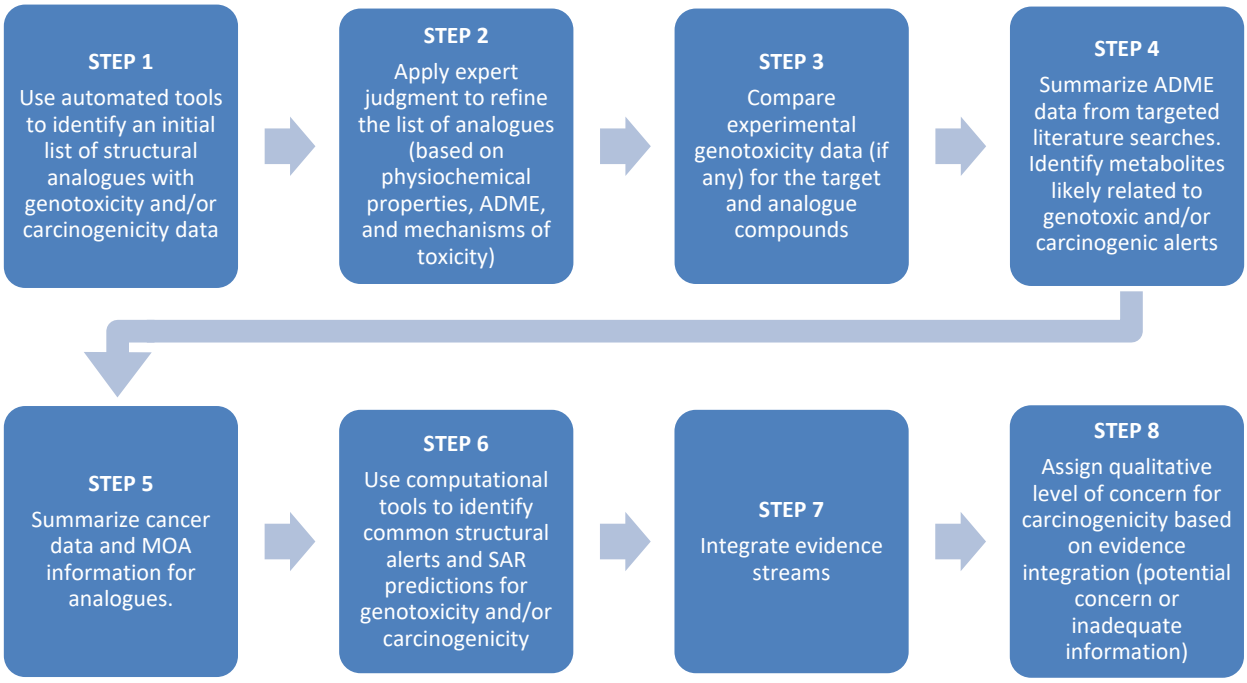


Figure B-1. Steps Used in the Screening Evaluation of Potential Carcinogenicity

STEP 1. USE OF AUTOMATED TOOLS TO IDENTIFY STRUCTURAL ANALOGUES WITH CARCINOGENICITY AND/OR GENOTOXICITY DATA

ChemACE Clustering

The U.S. EPA's Chemical Assessment Clustering Engine (ChemACE) (U.S. EPA, 2011a) is an automated tool that groups (or clusters) a user-defined list of chemicals based on chemical structure fragments. The methodology used to develop ChemACE was derived from U.S. EPA's Analog Identification Methodology (AIM) tool, which identifies structural analogues for a chemical based on common structural fragments. ChemACE uses the AIM structural fragment recognition approach for analogue identification and applies advanced queries and user-defined rules to create the chemical clusters. The ChemACE outputs are available in several formats and layouts (i.e., Microsoft Excel, Adobe PDF) to allow rapid evaluation of structures, properties, mechanisms, and other parameters which are customizable based on an individual user's needs. ChemACE grouping has been successfully used with chemical inventories for identifying trends within a series of structurally similar chemicals, demonstrating structural diversity in a chemical inventory, and for detecting structural analogues to fill data gaps and/or perform read-across.

For this project, ChemACE is used to identify potential structural analogues of the target compound that have available carcinogenicity assessments and/or carcinogenicity data. An overview of the ChemACE process in shown in Figure B-2.

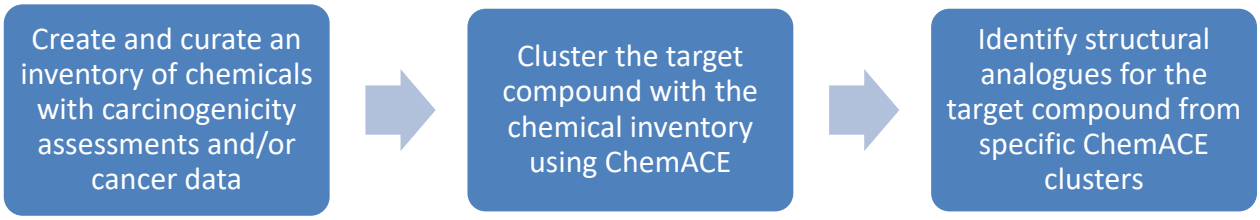


Figure B-2. Overview of ChemACE Process

The chemical inventory was populated with chemicals that have available carcinogenicity assessments and/or carcinogenicity data from the following databases and lists:

- Carcinogenic Potency Database (CPDB) (CPDB, 2011)
- Agents classified by the International Agency for Research on Cancer (IARC) monographs (IARC, 2018)
- National Toxicology Program (NTP) Report on Carcinogens (ROC) (NTP, 2016)
- NTP technical reports (NTP, 2017)
- Integrated Risk Information (IRIS) carcinogens (U.S. EPA, 2017)
- California EPA Prop 65 list (CalEPA, 2017)
- European Chemicals Agency (ECHA) carcinogenicity data available in the Organisation for Economic Co-operation and Development (OECD) Quantitative Structure-Activity Relationship (QSAR) Toolbox (OECD, 2018)
- PPRTVs for Superfund (U.S. EPA, 2019)

In total, 2,123 distinct substances were identified from the sources above. For the purpose of ChemACE grouping each individual substance needed to meet the following criteria:

1) Substance is not a polymer, metal, inorganic, or complex salt because ChemACE is not designed to accommodate these substances;
2) Substance has a CASRN or unambiguous chemical identification; and
3) Substance has a unique Simplified Molecular Input Line Entry System (SMILES) notation (encoded molecular structure format used in ChemACE) that can be identified from one of these sources:
    a. Syracuse Research Corporation (SRC) and Distributed Structure-Searchable Toxicity (DSSTox) database lists of known SMILES associated with unique CASRNs (the combined lists contained >200,000 SMILES) or
    b. ChemIDplus, U.S. EPA Chemicals Dashboard, or internet searches.

Of the initial list of 2,123 substances, 201 were removed because they did not meet one of the first two criteria, and 155 were removed because they did not meet the third. The final inventory of substances contained 1,767 unique compounds.

Two separate ChemACE approaches were compared for clustering of the chemical inventory. The restrictive clustering approach, in which all compounds in a cluster contain all of the same fragments and no different fragments, yielding 208 clusters. The less restrictive approach included the following rules for remapping the chemical inventory:

- treat adjacent halogens as equivalent, allowing fluorine (F) to be substituted for chlorine (Cl), Cl for bromine (Br), Br for iodine (I);
- allow methyl, methylene, and methane to be equivalent;
- allow primary, secondary, and tertiary amines to be equivalent; and
- exclude aromatic thiols (removes thiols from consideration).

Clustering using the less restrictive approach (pass 2) resulted in 284 clusters. ChemACE results for clustering of the target chemical within the clusters of the chemical inventory are described in Appendix C.

Analogue Searches in the OECD QSAR Toolbox (DICE Method)

The OECD QSAR Toolbox (Version 4.1) is used to search for additional structural analogues of the target compound. There are several structural similarity score equations available in the toolbox (DICE, Tanimoto, Kulczynski-2, Ochiai/Cosine, and Yule). DICE is considered the default equation. The specific options that are selected for performing this search include a comparison of molecular features (atom-centered fragments) and atom characteristics (atom type, count hydrogens attached and hybridization). Chemicals identified in these similarity searches are selected if their similarity scores exceed 50%.

The OECD QSAR Toolbox Profiler is used to identify those structural analogues from the DICE search that have carcinogenicity and/or genotoxicity data. Nine databases in the OECD QSAR Toolbox (Version 4.1) provide data for carcinogenicity or genotoxicity (see Table B-1).

Analogue search results for the target chemical are described in Appendix C.

Table B-1. Databases Providing Carcinogenicity and Genotoxicity Data in the OECD QSAR Toolbox (Version 4.1)

Database Name	Toolbox Database Description[a]
CPDB	The CPDB provides access to bioassay literature with qualitative and quantitative analysis of published experiments from the general literature (through 2001) and from the NCI/NTP (through 2004). Reported results include bioassays in rats, mice, hamsters, dogs, and nonhuman primates. A calculated carcinogenic potency (TD_{50}) is provided to standardize quantitative measures for comparison across chemicals. The CPDB contains 1,531 chemicals and 3,501 data points.
ISSCAN	The ISSCAN database provides information on carcinogenicity bioassays in rats and mice reported in sources that include NTP, CPDB, CCRIS, and IARC. This database reports a carcinogenicity TD_{50}. There are 1,149 chemicals and 4,518 data points included in the ISSCAN database.
ECHA CHEM	The ECHA CHEM database provides information on chemicals manufactured or imported in Europe from registration dossiers submitted by companies to ECHA to comply with the REACH Regulation framework. The ECHA database includes 9,229 chemicals with almost 430,000 data points for a variety of endpoints including carcinogenicity and genotoxicity. ECHA does not verify the information provided by the submitters.
ECVAM Genotoxicity and Carcinogenicity	The ECVAM Genotoxicity and Carcinogenicity database provides genotoxicity and carcinogenicity data for Ames positive chemicals in a harmonized format. ECVAM contains in vitro and in vivo bacteria mutagenicity, carcinogenicity, CA, CA/aneuploidy, DNA damage, DNA damage and repair, mammalian culture cell mutagenicity, and rodent gene mutation data for 744 chemicals and 9,186 data points.
Cell transformation assay ISSCTA	ISSCTA provides results of four types of in vitro cell transformation assays including Syrian hamster embryo cells, mouse BALB/c 3T3, mouse C3H/10T1/2, and mouse Bhas 42 assays that inform nongenotoxic carcinogenicity. ISSCTA consists of 352 chemicals and 760 data points.
Bacterial mutagenicity ISSSTY	The ISSSTY database provides data on in vitro *Salmonella typhimurium* Ames test mutagenicity (positive and negative) taken from the CCRIS database in TOXNET. The ISSSTY database provides data for 7,367 chemicals and 41,634 data points.
Genotoxicity OASIS	The Genotoxicity OASIS database provides experimental results for mutagenicity results from "Ames tests (with and without metabolic activation), in vitro chromosomal aberrations and MN and MLA evaluated in vivo and in vitro, respectively." The Genotoxicity OASIS database consists of 7,920 chemicals with 29,940 data points from 7 sources.

Table B-1. Databases Providing Carcinogenicity and Genotoxicity Data in the OECD QSAR Toolbox (Version 4.1)

Database Name	Toolbox Database Description[a]
Micronucleus OASIS	The Micronucleus OASIS database provides experimental results for in vivo bone marrow and peripheral blood MNT CA studies in blood erythrocytes, bone marrow cells, and polychromatic erythrocytes of humans, mice, rabbits, and rats for 557 chemicals.
Micronucleus ISSMIC	The ISSMIC database provides data on the results of in vivo MN mutagenicity assay to detect CAs in bone marrow cells, peripheral blood cells, and splenocytes in mice and rats. Sources include TOXNET, NTP, and the Leadscope FDA CRADA toxicity database. The ISSMIC database includes data for 563 chemicals and 1,022 data points.

[a]Descriptions were obtained from the OECD QSAR Toolbox documentation (Version 4.1) (OECD, 2018).

CA = chromosomal aberration; CCRIS = Chemical Carcinogenesis Research Information System; CPBD = Carcinogenic Potency Database; CRADA = cooperative research and development agreement; DNA = deoxyribonucleic acid; ECHA = European Chemicals Agency; ECVAM = European Centre for the Validation of Alternative Methods; FDA = Food and Drug Administration; IARC = International Agency for Research on Cancer; ISSCAN = Istituto Superiore di Sanità Chemical Carcinogen Database; ISSCTA = Istituto Superiore di Sanità Cell Transformation Assay Database; ISSMIC = Istituto Superiore di Sanità Micronucleus Database; ISSSTY = Istituto Superiore di Sanità *Salmonella typhimurium* Database; MLA = mouse lymphoma gene mutation assay; MN = micronuclei; MNT = micronucleus test; NCI = National Cancer Institute; NTP = National Toxicology Program; OECD = Organisation for Economic Co-operation and Development; QSAR = quantitative structure-activity relationship; REACH = Registration, Evaluation, Authorisation and Restriction of Chemicals; TD_{50} = median toxic dose.

STEPS 2−5. ANALOGUE REFINEMENT AND SUMMARY OF EXPERIMENTAL DATA FOR GENOTOXICITY, TOXICOKINETICS, CARCINOGENICITY, AND MODE OF ACTION

The outcome of the Step 1 analogue identification process using ChemACE and the OECD QSAR Toolbox is an initial list of structural analogues with genotoxicity and/or carcinogenicity data. Expert judgment is applied in Step 2 to refine the list of analogues based on physiochemical properties, absorption, distribution, metabolism, and excretion (ADME), and mechanisms of toxicity. The analogue refinement process is chemical-specific and is described in Appendix C. Steps 3, 4, and 5 (summary of experimental data for genotoxicity, toxicokinetics, carcinogenicity, and mode of action [MOA]) are also chemical specific (see Appendix C for further details).

STEP 6. STRUCTURAL ALERTS AND STRUCTURE-ACTIVITY RELATIONSHIP PREDICTIONS FOR 2-ADNT AND ANALOGUES

Structural alerts (SAs) and predictions for genotoxicity and carcinogenicity are identified using six freely available structure-based tools (described in Table B-2). The tool results for the target and analogue compounds are provided in Appendix C.

Table B-2. Tools Used to Identify SAs and the Prediction of Carcinogenicity and Genotoxicity

Name	Description[a]
OECD QSAR Toolbox (Version 4.1)	Seven OECD QSAR Toolbox profiling methods were used, including: • Carcinogenicity (genotox and nongenotox) alerts by ISS (Version 2.3); updated version of the module originally implemented in Toxtree. Toxtree is a decision tree for estimating carcinogenicity based on 55 SAs (35 from the Toxtree module and 20 newly derived). • DNA alerts for Ames by OASIS (Version 1.4); based on the Ames mutagenicity TIMES model; uses 85 SAs responsible for interaction of chemicals with DNA. • DNA alerts for CA and MNT by OASIS (Version 1.1); based on the DNA reactivity of the CAs TIMES model; uses 85 SAs for interaction of chemicals with DNA. • In vitro mutagenicity (Ames test) alerts by ISS (Version 2.3); based on the Mutagenicity module in Toxtree. ISS s a decision tree for estimating in vitro (Ames test) mutagenicity, based on a list of 43 SAs relevant for the investigation of chemical genotoxicity via DNA adduct formation. • In vivo mutagenicity (MN) alerts by ISS (Version 2.3); based on the ToxMic rulebase in Toxtree. The rulebase has 35 SAs for in vivo MN assays in rodents. • OncoLogic Primary Classification (Version 4.0); "developed by LMC and OECD to mimic the structural criteria of chemical classes of potential carcinogens covered by the U.S. EPA's OncoLogic Cancer Expert System for Predicting the Carcinogenicity Potential" for categorization purposes only, not for predicting carcinogenicity. This tool is applicable to organic chemicals with at least one of the 48 alerts specified. • Protein binding alerts for CAs by OASIS (Version 1.3); based on 33 SAs for interactions with specific proteins including topoisomerases, cellular protein adducts, etc.
OncoLogic (Version 7)	OncoLogic is a tool for predicting the potential carcinogenicity of chemicals based on the application of rules for SAR analysis, developed by experts. Results may range from "low" to "high" concern level.

Table B-2. Tools Used to Identify SAs and the Prediction of Carcinogenicity and Genotoxicity

Name	Description[a]
ToxAlerts	ToxAlerts is a platform for screening chemical compounds against SAs, developed as an extension to the OCHEM system (https://ochem.eu). Only "approved alerts" were selected, which means a moderator approving the submitted data. A list of the ToxAlerts found for the chemicals screened in the preliminary batch is below: • Genotoxic carcinogenicity, mutagenicity: o Aliphatic halide (general) o Aliphatic halide (specific) o Aliphatic halogens o Aromatic amine (general) o Aromatic amine (specific) o Aromatic amines o Aromatic and aliphatic substituted primary alkyl halides o Aromatic nitro (general) o Aromatic nitro (specific) o Aromatic nitro groups o Nitroarenes o Nitro-aromatic o Primary and secondary aromatic amines o Primary aromatic amine, hydroxyl amine, and its derived esters or amine generating group • Nongenotoxic carcinogenicity o Aliphatic halogens
ToxRead (Version 0.9)	ToxRead is a tool designed to assist in making read-across evaluations reproducible. SAs for mutagenicity are extracted from similar molecules with available experimental data in its database. Five similar compounds were selected for this project. The rule sets included: • Benigni/Bossa as available in Toxtree (Version 1) • SARpy rules extracted by Politecnico di Milano, with the automatic tool SARpy • IRFMN rules extracted by human experts at Istituto di Ricerche Farmacologiche Mario Negri • CRS4 rules extracted by CRS4 Institute with automatic tools
Toxtree (Version 2.6.13)	Toxtree estimates toxic hazard by applying a decision tree approach. Chemicals were queried in Toxtree using the Benigni/Bossa rulebase for mutagenicity and carcinogenicity. If a potential carcinogenic alert based on any QSAR model or if any SA for genotoxic and nongenotoxic carcinogenicity was reported, then the prediction was recorded as a positive carcinogenicity prediction for the test chemical. The output definitions from the tool manual are listed below: • SA for genotoxic carcinogenicity (recognizes the presence of one or more SAs and specifies a genotoxic mechanism) • SA for nongenotoxic carcinogenicity (recognizes the presence of one or more SAs and specifies a nongenotoxic mechanism) • Potential *Salmonella typhimurium* TA100 mutagen based on QSAR • Unlikely to be a *S. typhimurium* TA100 mutagen based on QSAR • Potential carcinogen based on QSAR (assigned according to the output of QSAR8 aromatic amines) • Unlikely to be a carcinogen based on QSAR (assigned according to the output of QSAR8 aromatic amines) • Negative for genotoxic carcinogenicity (no alert for genotoxic carcinogenicity) • Negative for nongenotoxic carcinogenicity (no alert for nongenotoxic carcinogenicity)

Table B-2. Tools Used to Identify SAs and the Prediction of Carcinogenicity and Genotoxicity

Name	Description[a]
VEGA	VEGA applies several QSARs to a given chemical, as described below: • Mutagenicity (Ames test) CONSENSUS model: a consensus assessment is performed based on predictions of the VEGA mutagenicity models (CAESAR, SARpy, ISS, and *k*-NN) • Mutagenicity (Ames test) model (CAESAR): integrates 2 models, one is a trained SVM classifier, and the other is for FN removal based on SAs matching • Mutagenicity (Ames test) model (SARpy/IRFMN): rule-based approach with 112 rules for mutagenicity and 93 for nonmutagenicity, extracted with SARpy software from the original training set from the CAESAR model; includes rules for both mutagenicity and nonmutagenicity • Mutagenicity (Ames test) model (ISS): rule-based approach based on the work of Benigni and Bossa (ISS) as implemented in the software Toxtree (Version 2.6) • Mutagenicity (Ames test) model (*k*-NN/read-across): performs a read-across and provides a qualitative prediction of mutagenicity on *S. typhimurium* (Ames test) • Carcinogenicity model (CAESAR): Counter Propagation Artificial neural network developed using data for carcinogenicity in rats extracted from the CPDB • Carcinogenicity model (ISS): built implementing the same alerts Benigni and Bossa (ISS) implemented in the software Toxtree (Version 2.6) • Carcinogenicity model (IRFMN/ANTARES): a set of rules (127 SAs), extracted with the SARpy software from a data set of 1,543 chemicals obtained from the carcinogenicity database of EU-funded project ANTARES • Carcinogenicity model (IRFMN/ISSCAN-CGX): based on a set of rules (43 SAs) extracted with the SARpy software from a data set of 986 compounds; the data set of carcinogenicity of different species was provided by Kirkland et al. (2005).

[a]There is some overlap between the tools. For example, OncoLogic classification is provided by the QSAR Toolbox, but the prediction is available only through OncoLogic, and alerts or decision trees were used in or adapted from several models (e.g., Benigni and Bossa alerts and Toxtree decision tree) (OECD, 2017).

ANTARES = Alternative Nontesting Methods Assessed for REACH Substances; CA = chromosomal aberration; CAESAR = Computer Assisted Evaluation of Industrial Chemical Substances According to Regulations; CONSENSUS = Consensus Assessment based on multiple models (CAESAR, SARpy, ISS, and *k*-NN); CPDB = Carcinogenic Potency Database; DNA = deoxyribonucleic acid; EU = European Union; FN = false negative; IRFMN = Istituto di Ricerche Farmacologiche Mario Negri; ISS = Istituto Superiore di Sanità; ISSCAN-CGX = Istituto Superiore di Sanità Chemical Carcinogen; *k*-NN = *k*-nearest neighbor; LMC = Laboratory for Mathematical Chemistry; MN = micronucleus; MNT = micronucleus test; OCHEM = Online Chemical Monitoring Environment; OECD = Organisation for Economic Co-operation and Development; QSAR = quantitative structure-activity relationship; REACH = Registration, Evaluation, Authorisation and Restriction of Chemicals; SA = structural alert; SAR = structure-activity relationship; SVM = support vector machine; TIMES = The Integrated MARKEL-EFOM System; VEGA = Virtual models for property Evaluation of chemicals within a Global Architecture.

STEP 7. WEIGHT-OF-EVIDENCE INTEGRATION FOR SCREENING EVALUATION OF 2-ADNT CARCINOGENICITY

Data identified across multiple lines of evidence from Steps 1−6 (outlined above) are integrated to determine the qualitative level of concern for the potential carcinogenicity of the target compound (Step 8). In the absence of information supporting carcinogenic portal-of-entry effects, the qualitative level of concern for the target chemical should be considered applicable to all routes of exposure.

Evidence integration for the target compound is provided in Appendix C.

APPENDIX C. RESULTS OF THE SCREENING EVALUATION OF POTENTIAL CARCINOGENICITY

STEP 1. USE OF AUTOMATED TOOLS TO IDENTIFY STRUCTURAL ANALOGUES WITH CARCINOGENICITY AND/OR GENOTOXICITY DATA

U.S. EPA's Chemical Assessment Clustering Engine (ChemACE) grouping was performed as described in Appendix B. The cluster containing 2-amino-4,6-dinitrotoluene (2-ADNT) (less restrictive approach; Cluster 86) also contained 4-amino-2,6-dinitrotoluene (4-ADNT) (an additional target compound being concurrently evaluated in a separate provisional toxicity document) and four structural analogues (2-methyl-5-nitroaniline, isopropalin, pendimethalin, and trifluralin; see structures in Appendix A). The six cluster members all contain a benzene backbone and one or more nitro groups, an amine group, and an alkyl group (see Figure C-1). 2,4,6-Trinitrotolulene (TNT), the metabolic precursor of 2-ADNT, was included in ChemACE inventory for clustering (Cluster 83) but was not included in Cluster 86 because it lacks an amine group.

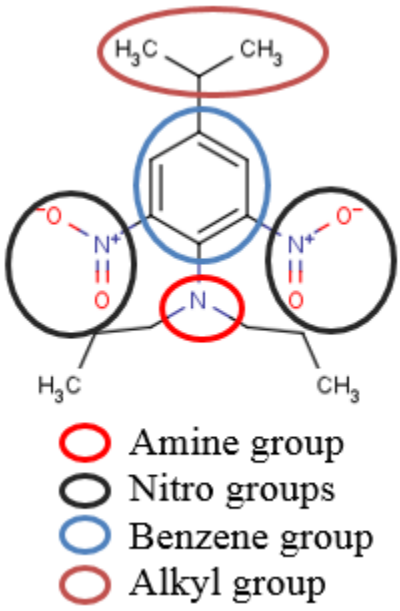


Figure C-1. Illustration of Common Fragments in Cluster 86

The Organisation for Economic Co-operation and Development (OECD) Quantitative Structure-Activity Relationship (QSAR) Toolbox Profiler was used to identify structural analogues from the DICE analogue search with carcinogenicity and/or genotoxicity data (see Step 1 methods in Appendix B). This process identified an additional 77 compounds to be considered as potential analogues for 2-ADNT, including the parent compound TNT. 2-Methyl-5-nitroaniline was identified by both ChemACE and the DICE search. Isopropalin and pendimethalin were identified as potential analogues by ChemACE but were not included in the OECD QSAR Toolbox databases (see Table B-1) and were, therefore, not available for

consideration by the DICE search. Refinement of selection of final analogues is described below.

STEP 2. ANALOGUE REFINEMENT USING EXPERT JUDGMENT

Expert judgment was applied to refine the initial list of 80 potential analogues based on physiochemical properties, absorption, distribution, metabolism, and excretion (ADME), and mechanisms of toxicity.

2-ADNT is a metabolite of TNT. TNT and nitro-group reduction metabolites of TNT containing at least one nitro group and one or more additional nitro groups, hydroxylamines, nitrosamines, and/or primary amines on the aromatic ring were preferred as potential analogues. Other potential analogues had (1) one aromatic ring (benzene) substituted with (2) one nitro group and one other nitro, amine, hydroxylamine, or nitroso functional group on the ring, in a meta-position relative to the first nitro group; (3) a methyl group on the ring; and (4) no other functional group. Based on the characteristics of the functional groups mentioned above, it is expected that such compounds would have similar bioavailability, bioactivity, and available metabolic pathways as the target compound.

Of the 80 chemicals identified as potential analogues by ChemACE and the OECD Toolbox analogue selection tool (DICE), 62 were not selected for further review. Common rationales for not selecting these chemicals included the presence of polycylic aromatics or ring systems other than toluene, occurrence of functional groups not present in 2-ADNT or its nitro-reduction products (e.g., phenols, halogens, carboxylic acids), or *N*-alkyl substituted amines (e.g., isopropalin, pendimethalin) and acetamide derivatives of aromatic amines. In addition, ortho- and/or para-substituted diamines, nitro amines, or dinitro compounds were not selected. Each of these attributes introduce significant differences in bioavailability, reactivity, and applicable metabolic pathways relative to 2-ADNT.

The remaining 18 potential analogues for 2-ADNT are listed in Table C-1. The existence of a cancer risk estimate and/or a weight-of-evidence (WOE) determination for cancer is indicated for each analogue.

Table C-1. Cancer Assessment Information for Analogues of 2-ADNT (CASRN 35572-78-2)

Analogue Name (CASRN)	Cancer Risk Estimates	WOE Determinations
2,4,6-Trinitrotolulene (118-96-7)[a]	U.S. EPA (2002a) – OSF	U.S. EPA (2002a) – *"Possible"* IARC (1996) – *"Not Classifiable"* CalEPA (2016b) – *"Known"*
2,6-Diamino-4-nitrotoluene (59229-75-3)[a]	None	None
4-Hydroxylamino-2,6-dinitrotoluene (59283-75-9)[a]	None	None
N-Hydroxy-2-methyl-3,5-dinitrobenzenamine (59283-76-0)[a]	None	None
2,4-Diamino-6-nitrotoluene (6629-29-4)[a]	None	None
2,4-Dihydroxyamino-6-nitrotoluene (185376-54-9)[a]	None	None
2-Methyl-5-nitroaniline (99-55-8)[a, b]	U.S. EPA (2011c) – p-OSF	U.S. EPA (2011c) – *"Suggestive"* IARC (1990b) – *"Not Classifiable"*
2,4-Dinitrotoluene (121-14-2)[a]	U.S. EPA (1987) – OSF	U.S. EPA (1987) – *"Probably"* IARC (1996) – *"Possibly"*
2,6-Dinitrotoluene (606-20-2)[a]	U.S. EPA (2013) – p-OSF	U.S. EPA (2013) – *"Suggestive"* IARC (1996) – *"Possibly"* CalEPA (2016b) – *"Known"*
2,4-Dinitro-*m*-xylene (603-02-1)[a]	None	None
1,2-Dimethyl-3,5-dinitrobenzene (616-69-3)[a]	None	None
4,6-Dinitro-*m*-xylene (616-72-8)[a]	None	None
4-Methyl-3-nitrobenzenamine (119-32-4)[a]	None	None
2-Methyl-1-nitro-3-nitrosobenzene (143922-95-6) [a]	None	None
3-Methyl-2,4,6-trinitrotoluene (22603-58-3)[a]	None	None
3-Nitro-*o*-toluidine (603-83-8)[a]	None	None
4-Nitro-2-nitrosotoluene (82414-02-6)[a]	None	None
4-Nitroso-2-nitrotoluene (82414-03-7)[a]	None	None

[a]Found by DICE.
[b]Found by ChemACE.

2-ADNT = 2-amino-4,6-dinitrotoluene; OSF = oral slope factor; p-OSF = provisional oral slope factor; WOE = weight of evidence.

The 14 analogues that lack cancer risk estimate and/or a WOE determination for cancer (highlighted in gray in Table C-1) were excluded as potential analogues for the screening evaluation of potential carcinogenicity. Compounds selected for further consideration were TNT, 2-methyl-5-nitroaniline, 2,4-dinitrotoluene (2,4-DNT), and 2,6-DNT.

STEP 3. COMPARISON OF THE EXPERIMENTAL GENOTOXICITY DATA FOR 2-ADNT AND ANALOGUES

The limited genotoxicity data available for 2-ADNT are described in the "Other Data" section in the main body of this report (see Table 4). Data indicate that 2-ADNT is mutagenic in *Salmonella* and has the capacity to cause deoxyribonucleic acid (DNA) damage in bacteria. 2-ADNT may also be mutagenic in mammalian cells; however, evidence in mammalian cells is limited and inconsistent. No studies evaluating clastogenic effects were identified, and the potential for 2-ADNT to cause genotoxicity following in vivo exposure has not been evaluated. A summary of the genotoxicity data for analogue compounds is provided below. Although there are inconsistencies in findings for analogue compounds across genotoxicity study types, the data demonstrate that each analogue produces some toxicity in DNA or chromosomes (e.g., bacterial mutagenicity, increased sister chromatid exchange [SCE] and chromosomal aberrations [CAs], or DNA adducts and damage).

TNT was mutagenic in *Salmonella* in the presence and absence of metabolic activation (Bolt et al., 2006; ATSDR, 1995). 2-Methyl-5-aniline was weakly mutagenic in *Salmonella* in both the presence and absence of metabolic activation at high millimolar concentrations (U.S. EPA, 2011c). 2,4-DNT and 2,6-DNT have been shown to induce gene mutations in *Salmonella* test systems in the presence or absence of metabolic activation in some assays, but to produce negative or equivocal results in others (ATSDR, 2018). Mutagenicity in *Salmonella* for 2-ADNT and its analogues appears to be related to the endogenous level of nitroreductase activity in the test strain (ATSDR, 2018; U.S. EPA, 2011c).

Limited mammalian cell mutagenicity data for TNT produced inconsistent findings (i.e., positive in the mouse lymphoma assay without metabolic activation; negative in Chinese hamster V79 cells, with and without metabolic activation) (Bolt et al., 2006; ATSDR, 1995). No data were available for mutagenicity in mammalian cells following exposure to 2-methyl-5-aniline (U.S. EPA, 2011c). In general, both 2,4-DNT and 2,6-DNT failed to induce gene mutations in mammalian cells (ATSDR, 2018).

TNT did not induce CAs or increase micronuclei (MN) frequency in in vivo studies in rats (no in vitro studies were located) (Bolt et al., 2006; ATSDR, 1995). 2-Methyl-5-aniline induced CAs and SCEs in vitro with and without metabolic activation, although CA results without metabolic activation were inconsistent [no in vivo studies were located; U.S. EPA (2011c)]. 2,4-DNT induced SCE in in vivo and in vitro (with metabolic activation) and produced mixed findings in in vivo MN assays and in vitro CA assays (no in vivo studies were located) (ATSDR, 2018). 2,6-DNT induced CAs in vitro (no in vivo studies were located) (ATSDR, 2018).

Both 2,4-DNT and 2,6-DNT were demonstrated to bind to rat liver DNA following in vivo exposure and produce DNA damage in vitro. 2,6-DNT also induced DNA damage in rats treated via gavage (ATSDR, 2018). 2-Methyl-5-aniline induced significant morphological transformations in the in vitro Syrian hamster embryo (SHE) cell transformation assay (U.S. EPA, 2011c). Inconsistent results were observed for 2,4-DNT and 2,6-DNT in the SHE cell transformation assay (ATSDR, 2018). 2,4-DNT did not induce dominant lethal mutations in rats (ATSDR, 2018).

In summary, 2-ADNT and each of the analogue compounds exhibit substantial evidence of genotoxicity.

STEP 4. TOXICOKINETICS OF 2-ADNT AND ANALOGUES

The toxicokinetics of 2-ADNT, TNT, 2-methyl-5-nitroaniline, 2,4-DNT, and 2,6-DNT are briefly described in Table C-2. Metabolic pathways relevant to this review are shown in Figure C-2.

Table C-2. Summary of Toxicokinetic Data for 2-ADNT (CASRN 35572-78-2) and Analogues

Compound	Absorption, Distribution, Excretion	Metabolism	References
2-ADNT	• No direct data • Based on excretion patterns following TNT exposure, the primary route of excretion is expected to be urine	• Primary metabolite of TNT • May undergo further nitro reduction to produce 4,6-diamino-2-nitrotoluene and 2,6-diamino-4-nitrotoluene • The amino group undergoes conjugation with sulfate, glucuronide, and acetyl moieties	Kongtip et al. (2012); Sabbioni and Rumler (2007); Sabbioni et al. (2007); Sabbioni et al. (2005); ATSDR (1995)
TNT	• Rapid oral absorption with approximately 60% recovery in urine of rats, mice, and dogs; biliary excretion also occurs (quantitative results not available) • Extensive distribution; highest distribution to liver, skeletal muscle, blood, and fat • Urine is the primary route of excretion	• Metabolic pathways include oxidation of the methyl group, benzene ring oxidation, and reduction of the nitro group • Primary metabolites identified in human urine include 2-ADNT, 4-ADNT, 2,4-diamino-6-nitrotoluene, 4-hydroxylamino-2,6-dintrotoluene, and 3-hydroxy-4-amino-2,6-dinitrotoluene • Similar metabolites were identified in rat, mouse, rabbit, and dog urine	ATSDR (1995)
2-Methyl-5-nitroaniline	• No data on absorption or distribution • Urine is the primary route of excretion	• May undergo further oxidative and reductive degradation to yield the corresponding amino, hydroxylamino, and nitroso derivatives	MAK-Commission (2012); IARC (1990a); Mori et al. (1981)
2,6-DNT and 2,4-DNT	• Well absorbed following oral, inhalation, and dermal exposure • Extensive distribution with early preferential uptake to liver, kidney, and lungs • Metabolites excreted in urine and bile (enterohepatic cycling)	• Metabolism occurs in the liver and GI tract (microflora) • Metabolic pathways include CYP oxidation and nitro reduction • Primary urinary metabolites include dinitrobenzyl alcohol glucuronide, dinitrobenzoic acid, and aminonitrobenzoic acid	ATSDR (2018); U.S. EPA (2013)

2-ADNT = 2-amino-4,6-dinitrotoluene; 4-ADNT = 4-amino-2,6-dinitrotoluene; CYP = cytochrome P; DNT = dinitrotoluene; GI = gastrointestinal; TNT = 2,4,6-trinitrotoluene.

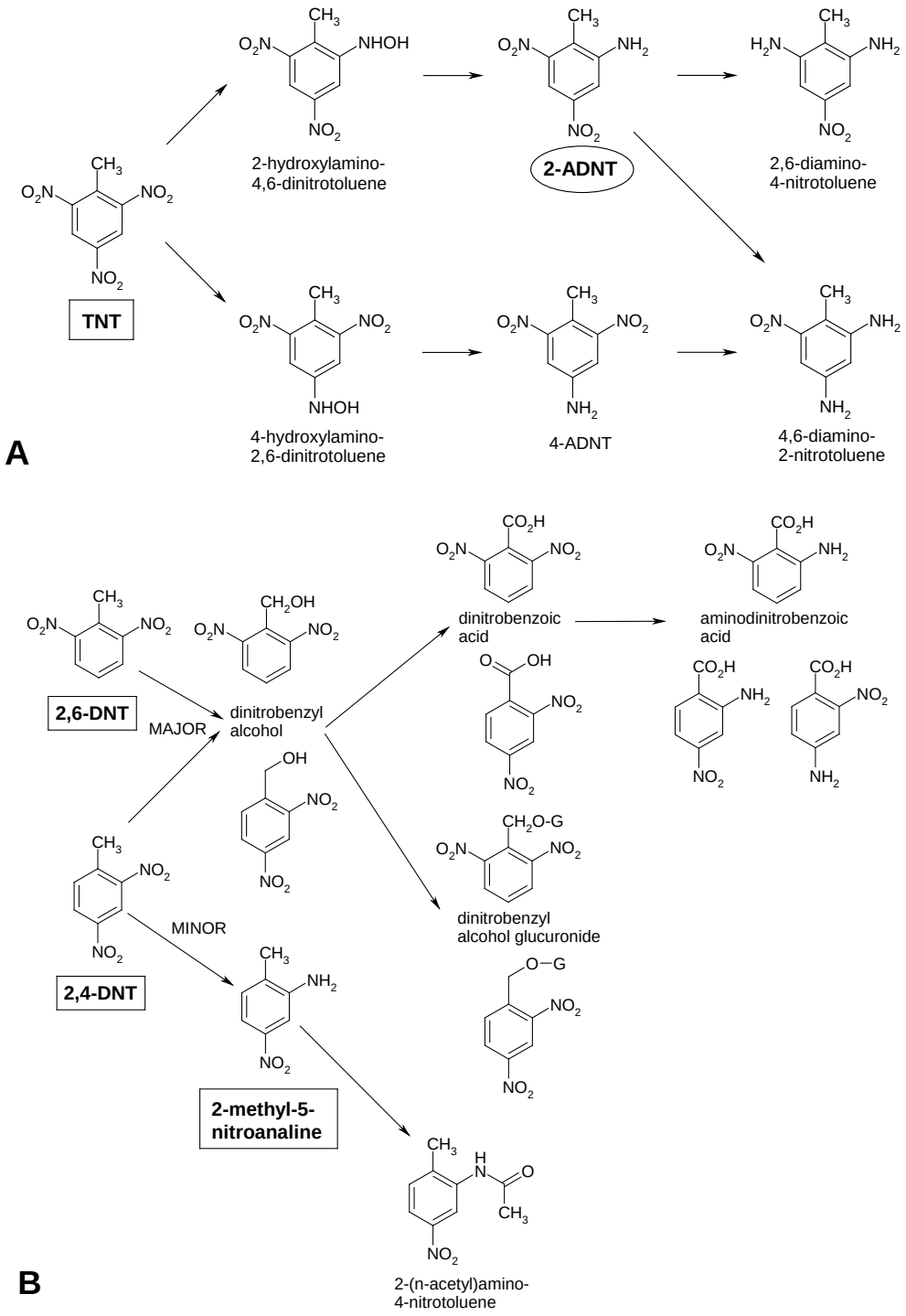


Figure C-2. Metabolic Pathways of 2-ADNT (CASRN 35572-78-2) and Analogues[1]

[1]The target compound is circled, and the analogues are shown in boxes. **Panel A:** TNT metabolic pathway including 2-ADNT. **Panel B:** Metabolic pathways for 2,4-DNT and 2,6-DNT. Sources: ATSDR (2018); U.S. EPA (2011c); ATSDR (1995).

2-ADNT is a major metabolite of TNT, appearing in the urine of TNT-exposed workers (Kongtip et al., 2012; Sabbioni and Rumler, 2007; Sabbioni et al., 2007; Sabbioni et al., 2005; Ahlborg et al., 1988; Yinon and Hwang, 1987; Woollen et al., 1986; Yinon and Hwang, 1986a; Almog et al., 1983; Channon et al., 1944; Lemberg and Callaghan, 1944), rats (Yinon and Hwang, 1985), rabbits (Yinon and Hwang, 1986b), and dogs (Snyder, 1946). Common downstream metabolites that result from further nitro reduction of these compounds include 4,6-diamino-2-nitrotoluene and 2,6-diamino-4-nitrotoluene (ATSDR, 1995).

2-Methyl-5-nitroaniline is one of several urinary metabolites of 2,4-DNT (U.S. EPA, 2011c). Limited toxicokinetic data suggest that it may undergo further oxidative and reductive degradation to amino, hydroxyamino, and nitroso derivatives (MAK-Commission, 2012; IARC, 1990a; Mori et al., 1981). 2,4-DNT and 2,6-DNT exhibit similar toxicokinetics (see Table C-2), and both compounds are metabolized in the liver and gastrointestinal tract of rodents (ATSDR, 2018; U.S. EPA, 2013, 2008). CYP oxidation predominates in the liver, leading to the formation of dinitrobenzyl alcohol, which is further metabolized to dinitrobenzoic acid. Dinitrobenzyl alcohol is also conjugated with glucuronide, which is partially excreted into the bile where hydrolysis and reductive metabolism by gut microflora occurs, followed by enterohepatic cycling. Common metabolic pathways for 2-ADNT and all analogue compounds include oxidation of the methyl group and reduction of nitro groups. Evaluation of the primary urinary metabolite data for each compound suggests that nitro reduction predominates for 2-ADNT and TNT, while oxidation of the toluene methyl group may occur more readily for the 2,4-DNT and 2,6-DNT. The evidence suggests that reduction of the nitro groups results in generation of reactive oxidant species (ROS), which produces the downstream effects.

STEP 5. CARCINOGENICITY OF 2-ADNT ANALOGUES AND MODE-OF-ACTION DISCUSSION

U.S. EPA cancer WOE descriptors for 2-ADNT and its analogue compounds are shown in Table C-3. As noted in the main Provisional Peer-Reviewed Toxicity Value (PPRTV) document, there is *"Inadequate Information to Assess the Carcinogenic Potential"* of 2-ADNT. Each of the analogue compounds is characterized as having evidence of carcinogenic potential. Under the 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), there is *"Suggestive Evidence of Carcinogenic Potential"* for 2-methyl-5-nitroaniline and 2,6-DNT. U.S. EPA carcinogenicity assessments for TNT and 2,4-DNT were written under previous guidelines (U.S. EPA, 1986) and the WOE descriptors were *"Possible Human Carcinogen (Group C)"* for TNT and *"Probably Carcinogenic in Humans (Group B2)"* for 2,4-DNT (U.S. EPA, 1986). Available carcinogenicity bioassay data were for the oral route only. Oral slope factor (OSF) values varied across several orders of magnitude (1.5×10^{0} to 9×10^{-3}), with the highest potency value calculated for 2,6-DNT and the lowest potency value derived for 2-methyl-5-nitroaniline. Urinary bladder tumors were observed in rats following dietary exposure to TNT (U.S. EPA, 2002a). Liver tumors were observed in mice exposed to 2-methyl-5-nitroaniline in the diet and rats exposed to 2,4-DNT or 2,6-DNT in the diet. The carcinogenic mode of action (MOA) has not been established for any of the analogue compounds, although they all exhibit some evidence of genotoxicity (see Step 3, and Table C-3), thus a mutagenic MOA cannot be ruled out. In hepatic initiation promotion studies, 2,6-DNT was reported to be a both an initiator and promoter of carcinogenesis, while 2,4-DNT showed tumor-promoting activity only (ATSDR, 2018; U.S. EPA, 2013).

Table C-3. Comparison of Available Carcinogenicity Data for 2-ADNT (CASRN 35572-78-2) and Potential Analogues

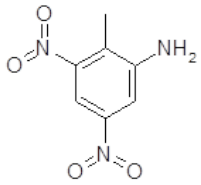
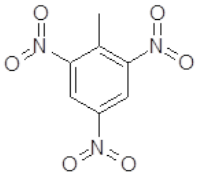
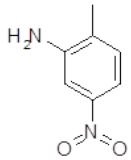
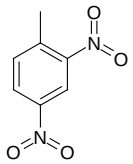
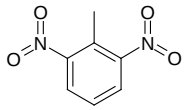
	2-ADNT CASRN 35572-78-2	**TNT CASRN 118-96-7**	**2-Methyl-5-nitroaniline CASRN 99-55-8**	**2,4-DNT CASRN 121-14-2**	**2,6-DNT CASRN 606-20-2**
Structure					
WOE characterization	*"Inadequate Information to Assess Carcinogenic Potential"* (see Table 7)	*"Possible Human Carcinogen (Group C)"*	*"Suggestive Evidence of Carcinogenic Potential"*	*"Probably Carcinogenic in Humans (Group B2)"*	*"Suggestive Evidence of Carcinogenic Potential"*
OSF $(mg/kg\text{-}d)^{-1}$	NA	3×10^{-2}	9×10^{-3} (provisional)	6.8×10^{-1}	1.5×10^{0} (provisional)
Data set(s) used for OSF derivation	NA	Urinary bladder tumors in F344 rats (F only; transitional cell papilloma and transitional squamous cell carcinomas)	Hepatocellular carcinomas in B6C3F1 mice (M and F)	Combined incidence of liver (hepatocellular carcinoma, neoplastic nodule) and mammary gland (adenoma, fibroadenoma, fibroma, adenocarcinoma, carcinoma) tumors in female S-D rats	Hepatic carcinomas in F344 rats (M only)
Other tumors observed in animal bioassays	NA	Leukemia and/or malignant lymphoma of the spleen in B6C3F1 mice (F only)	Hemangioma or hemangiosarcoma in B6C3F1 mice (M and F)	Renal tumors in CD-1 mice (M only)	None
Study doses (mg/kg-d)	NA	0, 0.4, 2, 10, 50 (HEDs: 0, 0.065, 0.325, 1.62, 8.12)	M: 0, 206, 394 (HEDs: 0, 25, 48); F: 0, 207, 397 (HEDs: 0, 25, 47)	F: 0, 0.706, 5.14, 45.3 (HEDs: 0, 0.129, 0.927, 7.557)	0, 7, 14; note: HEDs were not reported for individual doses (POD only)
Route (method)	NA	Diet	Diet	Diet	Diet
Duration	NA	24 mo	18 mo	24 mo	12 mo

Table C-3. Comparison of Available Carcinogenicity Data for 2-ADNT (CASRN 35572-78-2) and Potential Analogues

	2-ADNT CASRN 35572-78-2	TNT CASRN 118-96-7	2-Methyl-5-nitroaniline CASRN 99-55-8	2,4-DNT CASRN 121-14-2	2,6-DNT CASRN 606-20-2
POD type	NA	BMDL (linearized multistage procedure, extra risk; no further details reported)	$BMDL_{10}$ (HED)	NA	$BMDL_{10}$ (HED)
Source	NA	U.S. EPA (2002a)	U.S. EPA (2011c)	U.S. EPA (1987)	U.S. EPA (2013)

2-ADNT = 2-amino-4,6-dinitrotoluene; BMDL = benchmark dose lower confidence limit; DNT = dinitrotoluene; F = female(s); HED = human equivalent dose; M = male(s); NA = not applicable; OSF = oral slope factor; POD = point of departure; S-D = Sprague-Dawley; TNT = 2,4,6-trinitrotoluene; WOE = weight of evidence.

STEP 6. STRUCTURAL ALERTS AND STRUCTURE-ACTIVITY RELATIONSHIP PREDICTIONS FOR 2-ADNT AND ANALOGUES

Structural alerts (SAs) and predictions for genotoxicity and carcinogenicity were identified using computational tools as described in Appendix B. The model results for 2-ADNT and its analogue compounds are shown in Table C-4. Concerns for carcinogenicity and/or mutagenicity of 2-ADNT and its analogues were indicated by several models within each predictive tool (see Table C-4). Table C-5 provides a list of the specific SAs that underlie the findings of a concern for carcinogenicity or mutagenicity in Table C-4.

OECD QSAR Toolbox models showed a concern for mutagenicity for 2-ADNT and all analogues based on SAs (see Table C-5), as well as a concern for CAs for 2-methyl-5-nitroaniline based on protein binding alerts (no results were reported for protein binding for 2-ADNT or other analogues). The ToxRead and Virtual models for the Evaluation of chemicals within a Global Architecture (VEGA) models also indicated a concern for mutagenicity for 2-ADNT and all analogues. The Toxtree tool indicated no concern for 2-ADNT or 2-methyl-5-nitroaniline mutagenicity in *Salmonella* TA100 (no data for TNT, 2,4-DNT, or 2,6-DNT). The Toxtree results for 2-ADNT and 2-methyl-5-nitroaniline are inconsistent with positive experimental data (see Step 3), as well as the results of the other QSAR models.

OECD QSAR Toolbox models showed a concern for carcinogenicity for 2-ADNT and all analogues based on SAs (see Table C-5). The level of carcinogenicity concern in OncoLogic for 2-ADNT was "moderate" based on structure-activity relationship (SAR) analysis only (aromatic amine and amine-generating groups). OncoLogic indicated the level of concern for carcinogenicity as "high−moderate" for 2,6-DNT and 2-methyl-5-nitroaniline based on carcinogenicity data and the presence of amine-generating groups (SAR analysis). OncoLogic reported a "low−moderate" level of concern (shown as no data in the heat map) for TNT and 2,4-DNT based on limitations in the available carcinogenicity data. Carcinogenicity models in VEGA produced inconsistent results (e.g., no concern for carcinogenicity of 2-ADNT using the Computer Assisted Evaluation of Industrial Chemical Substances According to Regulations [CAESAR] model; concern for carcinogenicity by the Istituto Superiore di Sanità [ISS], Istituto di Ricerche Farmacologiche Mario Negri [IRFMN]/Alternative Nontesting Methods Assessed for REACH Substances [ANTARES] and the IRFMN/Istituto Superiore di Sanità Chemical Carcinogen [ISSCAN-CGX] models). Similarly, carcinogenicity models in VEGA produced inconsistent results (e.g., ISS and the IRFMN/ISSCAN-CGX models showed concern for carcinogenicity for all analogues; Computer Assisted Evaluation of Industrial Chemical Substances According to Regulations [CAESAR] showed no concern for carcinogenicity of TNT and 2-methyl-5-nitroaniline, but concern for 2,6-DNT; IRFMN/ANTARES showed concern for carcinogenicity of all analogues, except for 2-methyl-5-nitroaniline). The Toxtree tool indicated that 2-ADNT and 2-methyl-5-nitroaniline were potential carcinogens based on QSAR (no data for TNT, 2,4-DNT, or 2,6-DNT). Using this tool, there was no concern for nongenotoxic carcinogenicity for 2-ADNT or any of its analogues.

The ToxAlerts tool showed a concern for genotoxic carcinogenicity and/or mutagenicity for 2-ADNT and all analogues based on various SAs (see Table C-5). The ToxAlerts models

that rely on an SA for aromatic amines showed no results for TNT, 2,4-DNT, and 2,6-DNT because they lack an amine functional group. However, nitro reduction to amines is a common metabolic pathway for these compounds (see Step 5 above). The Toxtree models suggest a concern for genotoxic carcinogenicity for 2-ADNT and all analogues based on SAs (see Table C-5).

Table C-4. Heat Map Illustrating the Structural Alert and SAR Prediction Results for 2-ADNT (CASRN 35572-78-2) and Analogues[a]

Tool	Model[b]	2-ADNT	TNT	2-Methyl-5-nitroaniline	2,6-Dintrotoluene	2,4-Dintrotoluene
Mutagenicity/genotoxicity alerts						
OECD QSAR Toolbox	DNA alerts for Ames by OASIS					
	In vitro mutagenicity (Ames test) alerts by ISS					
	In vivo mutagenicity (micronucleus) alerts by ISS					
	Protein binding alerts for chromosomal aberration by OASIS					
ToxRead	ToxRead (mutagenicity)					
VEGA	Mutagenicity (Ames test) CONSENSUS model—assessment					
	Mutagenicity (Ames test) model (CAESAR)—assessment					
	Mutagenicity (Ames test) model (SARpy/IRFMN)—assessment					
	Mutagenicity (Ames test) model (ISS)—assessment					
	Mutagenicity (Ames test) model (*k*-NN/read-across)—assessment					
Toxtree	Potential *Salmonella typhimurium* TA100 mutagen based on QSAR					
Carcinogenicity alerts						
OECD QSAR Toolbox	Carcinogenicity (genotoxicity and nongenotoxicity) alerts by ISS					
OncoLogic	OncoLogic (prediction of the carcinogenic potential of the chemical)					
VEGA	Carcinogenicity model (CAESAR)—assessment					
	Carcinogenicity model (ISS)—assessment					
	Carcinogenicity model (IRFMN/ANTARES)—assessment					
	Carcinogenicity model (IRFMN/ISSCAN-CGX)—assessment					
Toxtree	Potential carcinogen based on QSAR					
	Nongenotoxic carcinogenicity					

Table C-4. Heat Map Illustrating the Structural Alert and SAR Prediction Results for 2-ADNT (CASRN 35572-78-2) and Analogues[a]

Tool	Model[b]	2-ADNT	TNT	2-Methyl-5-nitroaniline	2,6-Dintrotoluene	2,4-Dintrotoluene
Combined alerts						
ToxAlerts	Aromatic amine (general) (for genotoxic carcinogenicity, mutagenicity)					
	Aromatic amine (specific) (for genotoxic carcinogenicity, mutagenicity)					
	Aromatic amines (for genotoxic carcinogenicity, mutagenicity)					
	Aromatic nitro (general) (for genotoxic carcinogenicity, mutagenicity)					
	Aromatic nitro (specific) (for genotoxic carcinogenicity, mutagenicity)					
	Aromatic nitro groups (for genotoxic carcinogenicity, mutagenicity)					
	Nitroarenes (for genotoxic carcinogenicity, mutagenicity)					
	Nitro-aromatic (for genotoxic carcinogenicity, mutagenicity)					
	Primary and secondary aromatic amines (for genotoxic carcinogenicity, mutagenicity)					
	Primary ar. amine, hydroxyl amine and its derived esters or amine generating group (genotoxicity. carcinogenicity., mutagenicity.)					
Toxtree	Structural alert for genotoxic carcinogenicity					
	[b]Model results or alerts indicating no concern for carcinogenicity/mutagenicity.					
	[b]Model results outside the applicability domain for carcinogenicity/mutagenicity.					
	[b]Model results or alerts indicating concern for carcinogenicity/mutagenicity.					

[a]All tools and models described in Appendix B were used. Models with results are presented in the heat map (models without results were omitted).

4-ADNT = 4-amino-2,6-dinitrotoluene; ANTARES = Alternative Nontesting Methods Assessed for REACH Substances; CAESAR = Computer Assisted Evaluation of Industrial Chemical Substances According to Regulations; CONSENSUS = Consensus Assessment based on multiple models (CAESAR, SARpy, ISS, and *k*-NN); DNA = deoxyribonucleic acid; IRFMN = Istituto di Ricerche Farmacologiche Mario Negri; ISS = Istituto Superiore di Sanità; ISSCAN-CGX = Istituto Superiore di Sanità Chemical Carcinogen; *k*-NN = *k*-nearest neighbor; OECD = Organisation for Economic Co-operation and Development; SAR = structure-activity relationship; QSAR = quantitative structure-analysis relationship.

Table C-5. Structural Alerts for 2-ADNT (CASRN 35572-78-2) and Analogues

SA	Tools	Compounds
Aromatic amine (primary and secondary)	OncoLogic (includes compounds with amine-generating groups)	2-ADNT 2-Methyl-5-nitroaniline 2,4-DNT 2,6-DNT
	ToxAlerts	2-ADNT 2-Methyl-5-nitroaniline
Aromatic nitro (also nitro aromatic and nitroarenes)	OECD QSAR Toolbox	2-ADNT TNT 2-Methyl-5-nitroaniline 2,4-DNT 2,6-DNT
	ToxAlerts	
	Toxtree	
	OncoLogic	TNT
Primary aromatic amine, hydroxyl amine, and its derived esters or amine-generating group	OECD QSAR Toolbox	2-ADNT 2-Methyl-5-nitroaniline
	ToxAlerts	
	Toxtree	
Substituted anilines	OECD QSAR Toolbox	2-Methyl-5-nitroaniline
Nitroaniline derivatives	OECD QSAR Toolbox	2-ADNT 2-Methyl-5-nitroaniline
Polynitroarenes	OECD QSAR Toolbox	2-ADNT TNT 2,4-DNT 2,6-DNT

2-ADNT = 2-amino-4,6-dinitrotoluene; DNT = dinitrotoluene; OECD = Organisation for Economic Co-operation and Development; QSAR = quantitative structure-activity relationship; SA = structural alert; TNT = 2,4,6-trinitrotoluene.

STEP 7. WEIGHT-OF-EVIDENCE INTEGRATION FOR SCREENING EVALUATION OF 2-ADNT CARCINOGENICITY

Table C-6 presents the data for multiple lines of evidence pertinent to the screening evaluation of the carcinogenic potential of 2-ADNT. TNT was included as an analogue for 2-ADNT because it is a metabolic precursor with common downstream metabolites. Common metabolic pathways also exist between 2-ADNT and other analogue compounds. 2-Methyl-5-nitroaniline, 2,4-DNT, 2,6-DNT and TNT are structural categorical analogues because they are nitro- and/or amine-substituted toluene compounds with meta positioning of nitro/amine groups. A comparison of the experimental genotoxicity data for 2-ADNT and its analogues shows some inconsistencies in findings for analogue compounds across genotoxicity study types. However, the data demonstrate that each analogue produces toxicity in DNA or chromosomal damage (e.g., bacterial mutagenicity, increased SCE and CAs, or DNA adducts and damage). Additionally, each of the analogue compounds exhibits carcinogenic potential based on urinary bladder tumors (TNT) or liver tumors (2-methyl-5-nitroaniline, 2,4-DNT, and 2,6-DNT) observed in rodent studies (see Table C-2). The MOA for carcinogenicity has not

been established for the analogue compounds; however, because genotoxicity is a common feature of the analogues in the category and because these analogues have nitro groups that are reduced to amines, oxidation products (ROS) would be expected. Computational tools demonstrated common structural alerts for 2-ADNT and categorical analogue compounds (e.g., aromatic amine and nitro groups) and similar SAR predictions showing concern for carcinogenicity/genotoxicity.

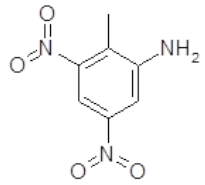
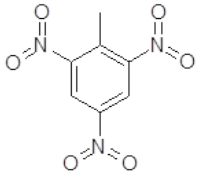
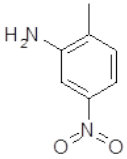
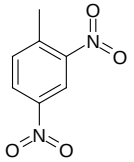
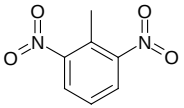
Table C-6. Integration of Evidence for 2-ADNT (CASRN 35572-78-2) and Analogues					
Evidence Streams	**2-ADNT CASRN 35572-78-2**	**TNT CASRN 118-96-7**	**2-Methyl-5-nitroaniline CASRN 99-55-8**	**2,4-DNT CASRN 121-14-2**	**2,6-DNT CASRN 606-20-2**
Structure					
Analogue selection and evaluation (see Steps 1 and 2)	NA; target compound	Metabolic precursor to the target; contains (1) 1 aromatic ring (benzene) substituted with (2) 3 nitro groups, (3) a methyl group on the ring, and (4) no other functional group	Contains (1) 1 aromatic ring (benzene) substituted with (2) 1 nitro group and 1 other nitro-, amine-, hydroxylamine-, or nitroso-functional group on the ring, in a meta-position relative to the first nitro group, (3) a methyl group on the ring, and (4) no other functional group	Contains (1) 1 aromatic ring (benzene) substituted with (2) 1 nitro group and 1 other nitro, amine, hydroxylamine, or nitroso functional group on the ring, in a meta-position relative to the first nitro group, (3) a methyl group on the ring, and (4) no other functional group	Contains (1) 1 aromatic ring (benzene) substituted with (2) 1 nitro group and one other nitro-, amine-, hydroxylamine-, or nitroso-functional group on the ring, in a meta-position relative to the first nitro group, (3) a methyl group on the ring, and (4) no other functional group

Table C-6. Integration of Evidence for 2-ADNT (CASRN 35572-78-2) and Analogues

Evidence Streams	2-ADNT CASRN 35572-78-2	TNT CASRN 118-96-7	2-Methyl-5-nitroaniline CASRN 99-55-8	2,4-DNT CASRN 121-14-2	2,6-DNT CASRN 606-20-2
Experimental genotoxicity data (see Step 3)	Positive for mutagenicity and DNA damage in *Salmonella*; limited and inconsistent findings for mutagenicity in mammalian cells; no studies evaluating clastogenic effects (see Table 4)	Mutagenic in *Salmonella*; limited and inconsistent findings for mutagenicity in mammalian cells; negative in rodents (in vivo) for CA and increased MN frequency (Bolt et al., 2006; U.S. EPA, 1988)	Mixed findings (positive and negative) for mutagenicity in *Salmonella*; induced CAs, SCE, and cell transformation in mammalian cells in vitro (U.S. EPA, 2011c)	Mixed findings (positive and negative) for mutagenicity in *Salmonella*; generally negative for mutagenicity in mammalian systems in vitro; induced SCE in vivo and in vitro (with metabolic activation) and produced mixed findings in in vivo MN assays and in vitro CA assays (no in vivo studies were located); in vivo formation of DNA adducts in rat liver; caused DNA damage in vitro; mixed results in cell transformation assays; negative in the dominant lethal assay (ATSDR, 2018)	Mixed findings (positive and negative) for mutagenicity in *Salmonella*; generally negative for mutagenicity in mammalian systems in vitro; induced CAs in vitro (no in vivo studies were located); in vivo formation of DNA adducts in rat liver; caused DNA damage in vitro and in vivo; mixed results in cell transformation assays. (ATSDR, 2018; U.S. EPA, 2013)
ADME evaluation (see Step 4)	2-ADNT is a primary metabolite of TNT	2-ADNT and TNT have common downstream metabolites; the nitroreductase pathway predominates	Common metabolic pathways with 2-ADNT include oxidation of the methyl group and reduction of nitro groups; predominant pathway is not known	Common metabolic pathways with 2-ADNT include oxidation of the methyl group and reduction of nitro groups; hepatic oxidation predominates	Common metabolic pathways with 2-ADNT include oxidation of the methyl group and reduction of nitro groups; hepatic oxidation predominates
Cancer data and MOA (see Step 5)	ND	Urinary bladder tumors in rats; MOA not established	Liver tumors in mice; MOA not established	Liver and mammary gland tumors in rats; MOA not established; tumor initiator and promoter	Liver tumors in rats; MOA not established; tumor promoter

Table C-6. Integration of Evidence for 2-ADNT (CASRN 35572-78-2) and Analogues

Evidence Streams	2-ADNT CASRN 35572-78-2	TNT CASRN 118-96-7	2-Methyl-5-nitroaniline CASRN 99-55-8	2,4-DNT CASRN 121-14-2	2,6-DNT CASRN 606-20-2
Common SAs and SAR predictions (see Step 6)	SAs: • Aromatic amine (primary and secondary) • Aromatic nitro (also nitro aromatic and nitroarenes) • Primary aromatic amine, hydroxyl amine, and its derived esters or amine generating group • Nitroaniline derivatives • Polynitroarenes SAR predictions: • Concerns for mutagenicity and carcinogenicity in most models; no concern for carcinogenicity in 1/4 VEGA models and for nongenotoxic carcinogenicity in Toxtree	SAs: • Aromatic nitro (also nitro aromatic and nitroarenes) • Polynitroarenes SAR predictions: • Concerns for mutagenicity and carcinogenicity in most models; no concern for carcinogenicity in 1/4 VEGA models and for nongenotoxic carcinogenicity in Toxtree	SAs: • Aromatic amine (primary and secondary) • Aromatic nitro (also nitro aromatic and nitroarenes) • Primary aromatic amine, hydroxyl amine, and its derived esters or amine generating group • Substituted anilines • Nitroaniline derivatives SAR predictions: • Concerns for mutagenicity and carcinogenicity in most models; no concern for carcinogenicity in 2/4 VEGA models and for nongenotoxic carcinogenicity in Toxtree	SAs: • Aromatic amine (primary and secondary) • Aromatic nitro (also nitro aromatic and nitroarenes) • Polynitroarenes SAR predictions: • Concerns for mutagenicity and carcinogenicity in most models; no concern for nongenotoxic carcinogenicity in Toxtree	SAs: • Aromatic amine (primary and secondary) • Aromatic nitro (also nitro aromatic and nitroarenes) • Polynitroarenes SAR predictions: • Concerns for mutagenicity and carcinogenicity in most models; no concern for nongenotoxic carcinogenicity in Toxtree

2-ADNT = 2-amino-4,6-dinitrotoluene; ADME = absorption, distribution, metabolism, and excretion; CA = chromosomal aberration; DNA = deoxyribonucleic acid; DNT = dinitrotoluene; MOA = mode of action; MN = micronuclei; NA = not applicable; ND = no data; SA = structural alert; SAR = structure-activity relationship; SCE = sister chromatid exchange; TNT = 2,4,6-trinitrotoluene; VEGA = Virtual models for property Evaluation of chemicals within a Global Architecture.

STEP 8. QUALITATIVE LEVEL OF CONCERN FOR 2-ADNT POTENTIAL CARCINOGENICITY

Table C-7 identifies the qualitative level of concern for potential carcinogenicity of 2-ADNT based on the multiple lines of evidence described above. Due to lack of information supporting carcinogenic portal-of-entry effects, the qualitative level of concern for this chemical is considered applicable to all routes of exposure.

Table C-7. Qualitative Level of Concern for Carcinogenicity of 2-ADNT (CASRN 35572-78-2)

Level of Concern	Designation	Comments
Concern for Potential Carcinogenicity	**Selected**	**All 4 analogues of 2-ADNT have carcinogenic potential based on urinary bladder or liver tumors observed in rodent studies. Each analogue produces some evidence of genotoxicity; however, the carcinogenic MOA for analogue compounds is not known, but a mutagenic MOA cannot be ruled out. Common metabolic pathways exist between 2-ADNT and analogue compounds, and 2-ADNT is a primary metabolite of TNT. 2-ADNT and its analogues have common SAs (e.g., aromatic amine and nitro groups) and SAR predictions showing concern for carcinogenicity/genotoxicity.**
Inadequate Information for Assigning Qualitative Level of Concern	NS	NA

2-ADNT = 2-amino-4,6-dinitrotoluene; MOA = mode of action; NA = not applicable; NS = not selected; SA = structural alert; SAR = structure-activity relationship; TNT = 2,4,6-trinitrotoluene.

APPENDIX D. REFERENCES

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